UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE, HEALTH AND LIFE SCIENCES

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DISPERSAL AND CONNECTIVITY OF NORTHEASTERN ATLANTIC PATELLID LIMPETS: A MULTIDISCIPLINARY APPROACH

PEDRO MIGUEL DE AZEVEDO RIBEIRO

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ABSTRACT

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DISPERSAL AND CONNECTIVITY OF NORTHEASTERN ATLANTIC PATELLID LIMPETS: A MULTIDISCIPLINARY APPROACH

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Dispersal and connectivity of patellid limpets (*Patella* spp.) in the eastern North Atlantic have been examined by addressing reproductive biology, larval development, population genetics and physical modelling of dispersal. The reproductive cycles of four limpet species were assessed on the northern and central Portuguese coast, to determine spawning periods. This information was incorporated into dispersal models. The results showed that *P. depressa* and *P. ulyssiponensis* have almost year-round breeding, with a brief resting phase in the early summer. Conversely, the two other species displayed much shorter spawning periods, with gamete release taking place between December and March in *P. vulgata* and between September and December in *P. rustica*.

The relationship between temperature and planktonic periods in *P. depressa*, *P. ulyssiponensis*, and *P. vulgata* was investigated with laboratory rearing experiments. Average duration of precompetent periods varied inversely with temperature, ranging between 3.7-14.0 days in *P. depressa*, 2.8-13.7 days in *P. ulyssiponensis* and 5.7-14.6 days in *P. vulgata*, whilst delay periods ranged between 15.8-25.4 days in *P. depressa*, 14.5-27 days in *P. ulyssiponensis* and 16.5-25 days in *P. vulgata*.

Population genetic structure was examined on a range-wide scale in *P. depressa* and along the Iberian coast in *P. rustica* using microsatellite markers, plus one mtDNA locus in *P. rustica*. Results suggested high levels of gene flow throughout the study areas and widespread lack of population differentiation in both species.

A biophysical model of dispersal has been developed to assess the degree of demographic connectivity over ecological and evolutionary time frames, and to identify possible barriers to dispersal for *P. depressa* and *P. rustica*. The model predicted high levels of connectivity through most of the study area in both species, but in *P. depressa* simulations identified two large extensions of adult habitat discontinuity as barriers to larval dispersal. The model also showed that despite the potential for long-distance dispersal, most of the larvae released at one given location settle within much shorter distances. These results illustrate the need to view the study of marine dispersal as a multidisciplinary task, and suggest that relying on just one line of evidence may produce misleading results.

Declaration of Authorship

I, Pedro Miguel de Azevedo Ribeiro, declare that the thesis entitled 'Dispersal and connectivity of northeastern Atlantic patellid limpets: a multidisciplinary approach' and the work presented in it are my own. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;

- where I have consulted the published work of others, this is always clearly attributed;

- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;

- all the work presented in this thesis was done entirely by me, except for the cloning of microsatellites (described in chapter 4) which was done by Montse Pérez at the Department of Biochemistry, Genetics and Immunology of the University of Vigo, and the computer programming required for the implementation of the dispersal model (described in chapter 7), which was done by António Múrias dos Santos, from Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO, University of Porto);

- parts of this work have been published as:

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Contents

Chapter 1

General Introduction

In marine invertebrate species with a pelagic early life stage, dispersal can be defined as the spread of larvae from a spawning to a settlement site. Population connectivity, a consequence of dispersal, is the exchange of individuals among geographically separated subpopulations (Levin 2006; Pineda *et al.* 2007). The scale and pattern of dispersal can have important consequences for population dynamics (Roughgarden *et al.* 1988; Gaines and Bertness 1993; Alexander and Roughgarden 1996; Eckman 1996), species interactions (Gaines and Lafferty 1995; Gaines *et al.* 2007), geographical distributions (Gaylord and Gaines 2000), population genetics (Bohonak 1999; Grosberg and Cunningham 2001; Palumbi 2003), as well as evolution and speciation (Hedgecock 1986; Valentine and Jablonski 1986; Palumbi 1994).

The assessment of spatial and temporal dispersal scales and connectivity patterns of marine organisms has been one of the key issues in marine ecology over recent decades. This area of research has recently experienced a great boost: there is increasing evidence that range limits of marine species are moving in response to climate warming, and abundance is changing (Southward *et al.* 1995; Holbrook *et al.* 1997; Sagarin *et al.* 1999; Garrabou *et al.* 2002; Wethey 2002; Beaugrand and Reid 2003; Borges *et al.* 2003; Hawkins *et al.* 2003; Zacherl *et al.* 2003; Genner *et al.* 2004; Hiscock *et al.* 2004; Paine and Trimble 2004; Lima *et al.* 2007a, b; Wethey and Woodin 2008); many marine species are being over-exploited (Jackson *et al.* 2001; Pauly *et al.* 2002; Myers and Worm 2003); habitat loss is accelerating (Steneck *et al.* 2002; Airoldi and Beck 2007); pollution has led to local extinctions (e.g. Harding *et al.* 1997; Crothers 1998). Understanding the influence of dispersal potential on the stability of species range boundaries provides predictive power on range shifts and a measure of population resilience and recovery potential under varying environmental conditions (Levin 2006). The need to establish protective measures towards overexploited species to ensure continual recruitment over large areas requires detailed knowledge on dispersal scales and mechanisms (Levin 2006; Jones *et al.* 2007a). Also, advection-diffusion models of dispersal are important to provide a theoretical framework for the configuration of marine reserves (Botsford *et al.* 2001; Gaines *et al.* 2003; Largier 2003; Siegel *et al.* 2003; Kaplan and Botsford 2005; Kaplan 2006).

The general concept that marine populations are open (that recruitment of new individuals does not depend on local production of offspring) has recently been challenged, and growing evidence suggests that populations may be more closed (dependent on self-seeding) than expected (Knowlton and Keller 1986; Jones *et al.* 1999; Cowen *et al.* 2000; Taylor and Hellberg 2003; Jones *et al.* 2005; Almany *et al.* 2007). An obvious consequence is that populations may become more vulnerable to phenomena acting locally, and that larval import may be insufficient to dampen severe fluctuations in abundance (Eckert 2003). As it becomes clear that dispersal is very heterogeneous in space, time, and among species, more studies are needed in order to provide a more complete picture of its scales and mechanisms (Kinlan and Gaines 2003).

In this thesis I combine studies on reproductive biology, larval rearing, population genetics using microsatellite DNA markers, plus modelling to examine connectivity between populations of various patellid species, in the general context of recent range expansion driven by climate change. Limpets (*Patella* spp.) are ideal models to study dispersal in the northeast Atlantic, for several reasons. They have extensive biogeographical ranges and are very common on intertidal rocky shores, allowing easy sampling for population genetics studies. In addition, their population dynamics and ecology have been extensively studied. Finally, because they possess a bipartite life-cycle, characterised by a planktonic larval period and a benthic adult stage, large-scale movement in these species can only be accomplished by larvae. Therefore, genetic structure patterns can be related to factors affecting larval transport, and other possible types of movements can be ruled out.

In the following sections of this introductory chapter I review the literature pertinent to this thesis. I start by reviewing current knowledge on larval ecology of benthic marine invertebrates, where I outline the diversity of larval forms in respect to aspects such as development mode, feeding, locomotion and behaviour. Next, I review studies on dispersal of marine organisms and the methodology that has been used to address this subject. I then provide a short account of northeastern Atlantic oceanography. In the next section I give a brief review on patellids limpet taxonomy, ecology, reproductive biology and phylogeny. Finally, I outline the rationale, structure and aims of this thesis.

1.1 Ecology of marine invertebrate larvae

1.1.1 Development modes

Numerous classification schemes have been proposed for larvae based on their modes of development (reviewed in Levin and Bridges 1995). Some emphasize larval nutrition, while others are based on type of habitat or time spent in the plankton. Thorson (1946, 1950) provided the first large-scale synthesis on larval development types from an ecological perspective, which is still widely used. This first classification of larval developmental modes was primarily based on nutritional mode and length of the planktonic phase. Free-swimming and feeding larvae were termed planktotrophic, and larvae feeding on egg reserves are known as lecithotrophic. Among these, there was a distinction between larvae developing in the water column from brooded or encapsulated larvae lacking a planktonic stage (direct developers). Brooded lecithotrophs included viviparous and adelphophagic forms (those feeding on nurse eggs).

Other classification systems have since then been proposed. Mileikovsky (1971) introduced a classification which included an additional type of development besides those advanced by Thorson (1950), the demersal type, consisting of free, non pelagic larvae developing near or on the bottom. Subsequently, Mileikovsky (1974), described seven subtypes of pelagic development, addressing the timing and duration of the pelagic phase within the complete life cycle, and the feeding mode. Chia (1974) distinguished direct development, in which a larval stage is lacking, from indirect development, for which he recognized ten larval types and patterns integrating nutritional mode with habitat. In a review of reproductive patterns of marine invertebrates, Grahame and Branch (1985) discussed the importance of 'mixed' life cycles, in which larvae are initially benthic and lecithotrophic (developing in egg capsules), and then go through a planktotrophic phase (Caswell 1981; Bouchet 1989).

A classification of larval forms explicitly addressing dispersal potential was firstly proposed by Scheltema (1971, 1989) and later modified by other authors (Levin and Bridges 1995). According to the time spent in the water column, larvae range from aplanic (direct developers) to teleplanic in which planktonic periods last for several months, possibly more than one year (Scheltema 1971). Larvae with very short planktonic periods, lasting from a few hours to a few days, are anchiplanic and those that remain in the water column for periods of one week to two months are termed actaeplanic (Scheltema 1989).

1.1.2 Trends in larval development

Largely based on observations in prosobranch gastropods, several authors have tried to explain variations in pattern of direct and planktotrophic developers with latitude. It is argued that most marine invertebrate species at high latitudes possess direct development, and that the frequency of species with pelagic larvae (planktotrophic and lecithotrophic) increases gradually towards the equator, where they become the predominant form (Thorson's rule, Mileikovsky 1971). Although numerous studies supported this hypothesis in a variety of taxonomic groups (Thorson 1950; Dell 1972; Curtis 1977; Picken 1979, 1980; Clarke 1992; Poulin and Féral 1996), recent work with polar species added many exceptions to the pattern, showing that a considerable number of species living at high latitudes have pelagic larvae, although a large proportion are lecithotrophic (Bosch and Pearse 1990; Pearse *et al.* 1991; Clarke 1992; Hain and Arnaud 1992; Pearse 1994; Pearse and Lockhart 2004).

Phylogenetic constraints, linked to the particular history of a certain taxon, are possibly more important in determining the developmental modes in marine gastropods than latitude. For example, in echinoids and bivalves, latitudinal gradients in development occur mainly because brooding taxa dominate in the southern oceans (Clarke 1992; Pearse 1994). On the other hand, the southern Atlantic coast shows a clear predominance of clades with non-pelagic larvae (Gallardo and Penchaszadeh 2001).

Within most phyla a broad range of larval modes can be found. However, progressing down the taxonomic hierarchy, more taxa are found with restricted developmental patterns (Levin and Bridges 1995). A considerable body of evidence indicates that the non-feeding larvae of contemporary species probably evolved from feeding larvae by means of progressive loss of feeding structures (Strathmann 1993; Nielsen 1998), which, once lost are rarely re-acquired (Strathmann 1978, 1985). Although some authors contest this theory, there is a general agreement that the ancestral larva was pelagic (Havenhand 1995). Conversely, Chaffe and Lindberg (1986) argue that the ancestral molluscan larva was non-feeding and non-pelagic, based on observations of early Cambrian molluscs suggesting they were of small adult size.

Also underlying the distribution of larval types in marine organisms is the relationship between body size and mode of development. It has been suggested that parental size may be important in determining reproductive mode of a species by influencing the number of eggs which can be produced (Underwood 1997). Because of the high larval mortality associated with planktotrophy (Rumrill 1990; Morgan 1995), species with this mode of development must produce a considerable number of eggs. As body size becomes small, species may be unable to produce sufficient planktotrophic larvae to ensure the maintenance of the population. There seems to be

a clear association between brooding and small body size in most invertebrate groups (Strathmann and Strathmann 1982; Chaffe and Lindberg 1986). The most common explanation proposed for this trend is that the variability of recruitment associated with species possessing a planktonic stage is incompatible with small and short-lived animals and with the relatively low number of eggs they produce. On the other hand, for large and long-lived animals, the advantages of dispersal could outweigh the risks involved (Strathmann and Strathmann 1982). Several examples are available from the literature which address and seem to support this hypothesis (Menge 1975; Todd 1979; Strathmann and Strathmann 1982; Jablonski and Lutz 1983; Grahame and Branch 1985; Morgan 1995; Underwood 1979; Pechenik 1999).

The role of adult body size in constraining certain developmental modes is not fully understood (Strathmann and Strathmann 1982). Underwood (1979) suggested there may be two size thresholds in prosobranch developmental patterns. First is a smaller size below which non-planktonic larvae must be produced, due to insufficient capacity to produce enough small eggs for a complete pelagic development. Below a second larger size limit, species are constrained to planktotrophy or brooding since they cannot produce enough lecithotrophic eggs. Above this second size threshold, any mode of reproduction is possible whereas the smallest species are restricted to non-dispersal lecithotrophy (Underwood 1979).

1.1.3 Larval behaviour

Marine larvae exhibit a behavioural repertoire which can deeply influence dispersal patterns at two distinct spatial scales. Over broad spatial scales, vertical migration, horizontal swimming and sensory capabilities, as well as ontogenetic changes in behaviour allow larvae to change the dispersal path by taking advantage of oceanographic conditions. At small spatial scales, differential response to environmental stimuli at the vicinity of settlement sites can determine the choice of larvae for a specific habitat.

1.1.3.1 Swimming ability and vertical migration

The interaction of larval behaviour and physical processes may influence dispersal patterns and contribute to departures from expected passive drift trajectories, either increasing advection from the natal population or enhancing self-recruitment (Sponaugle *et al.* 2002; Fiksen *et al.* 2007). Until recently, marine invertebrate larvae were considered weak swimmers (Chia *et al.* 1984; Young 1995). A growing body of evidence, however, suggests that at least decapod (Lutchembach and Orth 1992) and fish (Fischer 2005; Fisher *et al.* 2005) larvae can reach considerable horizontal

swimming speeds, which may allow them to compensate for displacement by currents (Armsworth 2000). Fast swimming is not always necessary, given that limited horizontal movements can take larvae to areas with contrasting current regimes (Vikebø *et al.* 2005).

Slow-swimming larvae may also influence dispersal distances with short and directed cross-shore movements. Dispersal of coastal larvae is often subject to the effects of a coastal boundary layer, which acts like a retention zone along the shoreline ('sticky waters', Largier 2003). Due to strong shear in alongshore flow and low cross-shore exchange, larvae released near the coastline can experience limited transport, despite stronger alongshore flow further offshore. Thus, by making short cross-shore movements, planktonic larvae can leave the retention zone and increase alongshore drift (Largier 2003).

Vertical migration of larvae is mainly affected by light, gravity and water motion, and may occur at a variety of periods (e.g. daily or tidal) or change over the course of development (reviewed in Kingsford *et al.* 2002). Simple vertical migrations may influence larval dispersal distances and trajectories, due to vertical shear. A number of studies in estuaries and coastal lagoons showed that upstream transport and retention of larvae may be mediated by selective tidal stream transport due to vertical migrations synchronized with tidal cycles (Dittel and Epifanio 1982; Queiroga 1998; Forward and Tankersley 2001; Arnold *et al.* 2005; Hare *et al.* 2005a; Simons *et al.* 2007). Changes in vertical positioning of larvae with tides have also been proposed as a mechanism promoting onshore transport of mussel larvae (Knights *et al.* 2006).

Diel vertical migrations may be responsible for the retention of larvae in upwelling systems. For example, Marta-Almeida *et al.* (2006) used a 3-dimensional model simulating dispersal of the littoral crab *Carcinus maenas* off the northwestern Iberian coast during periods of upwelling to show that diel vertical migration of larvae could enhance retention in nearshore waters. This was accomplished by a larger proportion of time spent in the onshore underflow than in the offshore surface flow (Marta-Almeida *et al.* 2006). A different mechanism, based on depth-keeping behaviour, was proposed by Shanks and Brink (2005) to explain larval retention of a variety of bivalve species on the inner shelf during periods of strong upwelling and downwelling currents.

The response of larvae to environmental stimuli often changes during the course of development, and can alter their vertical distribution. For instance, ontogenetic migration has been shown to promote onshore transport of larval fishes and invertebrates by moving larvae into onshore flowing layers (e.g. Shanks 1986; Manuel *et al.* 1996; Pineda 1999; Shanks *et al.* 2000; Dobretsov and Miron 2001). A particularly good example is provided by Paris and Cowen (2004) who suggested a mechanism of retention in a coral reef fish supported by robust empirical evidence. Surface currents

transported young larvae away from the parental site, but throughout ontogeny larvae migrated to deeper waters, where they found currents in the opposite direction that carried them back to their natal reef (Paris and Cowen 2004).

1.1.3.2 Behavioural response to settlement cues

Planktonic larval development of benthic marine invertebrates can be divided in two periods. The first one is termed precompetent period, during which larvae undergo rapid growth and morphological development. At the end of this stage, planktonic larvae acquire metamorphic competence, meaning that they possess developmental and physiological capabilities that allow them to settle into benthic habitat. The second stage of planktonic life is the competent period, which is the time over which larvae preserve the ability to settle (Jackson and Strathmann 1981).

The planktonic period is usually terminated by a settlement response. In the absence of suitable habitat for settlement, many marine invertebrate larvae remain competent to metamorphose for considerable periods of time (Bayne 1965; Kempf 1981; Sebens 1983; Pechenik 1990; Hadfield and Strathmann 1996; Pechenik 1999). Delay of metamorphosis has been shown to increase the risk of larval mortality, and can incur post-metamorphic costs in a variety of marine invertebrate species, especially those with lecithotrophic larvae, possibly because larval swimming can deplete energy reserves that might otherwise be allocated to post-metamorphic development (Highsmith and Emlet 1986; Woollacott *et al.* 1989; Pechenik 1990; Qian and Pechenik 1998; Wendt 1998; Maldonado and Young 1999; Wendt 2000; Pechenik and Rice 2001; Roberts and Lapworth 2001; Pechenik *et al.* 2002; Marshall and Keough 2003; Bennett and Marshall 2005). With age, larvae can thus become less specific and settle in the presence of a wide range of cues or become more sensitive to positive cues or less sensitive to settlement inhibitors (Knight-Jones 1953a; Rumrill 1989; Gibson 1994; Botello and Krug 2006; Gribben *et al.* 2006). A conceptual model has been proposed to explain this decreased selectivity of larvae over time based on depletion of energy reserves ('desperate larva hypothesis', Toonen and Pawlik 1994, 2001; Marshall and Keough 2003). On the other hand, extending the planktonic period in species with feeding larvae does not necessarily result in reduced adult performance, presumably because energy reserves are maintained by larval feeding (Pechenik and Eyster 1989; Rumrill 1989). This has led to the suggestion that, unlike non-feeding larvae, planktotrophic larvae are more likely to exhibit delayed metamorphosis in the absence of an appropriate settlement cue, and will not show decreasing selectivity (Toonen and Pawlik 2001; Elkin and Marshall 2007).

Settlement is probably under the influence of physical processes and active larval behaviour (reviewed by Butman 1987; Eckman *et al.* 1994; Abelson and Denny

1997). The hydrodynamic regime influences the probability of a larva finding suitable substrate, whereas behavioural traits determine whether settlement occurs (Mullineaux and Butman 1991; Pawlik *et al.* 1991; Mullineaux and Garland 1993; Pawlik and Butman 1993; Harvey *et al.* 1995; Toonen and Pawlik 1996; Harvey and Bourget 1997; Wright and Boxshall 1999; Boxshall 2000). In a variety of invertebrate species, final settlement results from an active exploration and selection of habitats in relation to the boundary layer hydrodynamics (Mullineaux and Butman 1991; Mullineaux and Garland 1993; Eckman *et al.* 1994; Qian *et al.* 1999; Pernet *et al.* 2003).

Larvae of many benthic species display active habitat selection and settle in response to particular physical or chemical cues associated with sites having appropriate conditions for benthic juveniles and adults (Thorson 1950; Burke 1983; Hadfield 1986; Pawlik 1990, 1992; Kingsford *et al.* 2002). Physical properties of settlement surfaces that influence larval choice include chemistry (e.g. Scheltema 1961; Roberts *et al.* 1991) and topography (e.g. Wethey 1986; Raimondi 1988; Walters 1992). Chemical cues may be waterborne or surface-associated, and include compounds emanating from prey species (e.g. Hadfield and Scheuer 1985; Lambert *et al.* 1997; Hadfield and Kohel 2004; Kohel *et al.* 2007), host plants (Williamson *et al.* 2000), microbial biofilms (Knight-Jones 1951; Wilson 1955; Johnson *et al.* 1991; Thompson *et al.* 1998; Wieczorek and Todd 1998; Zhao and Qian 2002; Swanson *et al.* 2006) and conspecific adults or juveniles (Knight-Jones 1953b; Burke 1984; Jensen and Morse 1984; Raimondi 1988; Gotelli 1990; Pearce and Scheibling 1990; Toonen and Pawlik 1994, 1996; Kay 2002; Zhao and Qian 2002). Larvae may also respond to negative biotic cues which help them avoid dominant spatial competitors (e.g. Grosberg 1981; Young and Chia 1981) and predators (Johnson and Strathmann 1989). Although numerous settlement inducers have been partially characterised (e.g. Morse *et al.* 1979; 1984; Jensen and Morse 1990; Zimmer-Faust and Tamburri 1994; Lambert *et al.* 1997; Krug and Manzi 1999; Takahashi *et al.* 2002), their relevance for settlement of larvae in the field has only been demonstrated in a few cases (Steinberg *et al.* 2002; Swanson *et al.* 2006).

1.1.4 Recruitment and population dynamics

Numerous manipulative experiments have demonstrated the importance of physical factors and biological interactions in shaping marine community pattern. For many years they provided convincing evidence that physical disturbances (Dayton 1971; Shanks and Wright 1986), competition (Connell 1961a, 1961b) and predation (Connell 1961a, 1961b; Paine 1971, 1974) were the primary forces regulating species composition, distribution and abundance. As increasing evidence demonstrated that the patterns embodied in such generalisations were absent or at least not evident in many

intertidal communities around the world (Lewin 1986), it became clear that too much emphasis had been placed on events within the assemblages, and that experimental analyses of patterns and processes in benthic assemblages should take into account the timing, magnitude and frequency of recruitment (Hawkins and Hartnoll 1982; Underwood and Denley 1984).

In species with complex life-cycles, variation in the supply of larvae and recruitment to adult habitat are fundamental processes in the determination of marine population structure and community dynamics (Roughgarden *et al.* 1988; Menge 1991; Grosberg and Levitan 1992; Underwood and Keough 2001). Recruitment to nearshore habitats of species with larvae that spend considerable time in the plankton are mostly dependent on stochastic events, such as hydrodynamic processes (Hawkins and Hartnoll 1982; Shanks 1986; Roughgarden *et al.* 1988; Pineda 1991; Gaines and Bertness 1992; Le Fèvre and Bourget 1992; Gaines and Bertness 1993; Alexander and Roughgarden 1996; Shkedy and Roughgarden 1997), wind direction (Bertness *et al.* 1996) and the availability of larval food (Underwood 1979; Hawkins and Hartnoll 1982; Olson and Olson 1989).

The role of larval supply as a determinant of important processes in benthic marine systems is not a new concept (Young 1987; Underwood and Fairweather 1989; Young 1990), and has been discussed for considerable time (e.g. Nelson 1925; Burkenroad 1946; Thorson 1946). Fisheries researchers have long been concerned with the recruitment dynamics of fish, realizing that this could explain the enormous temporal variation of fished stocks (Underwood and Fairweather 1989; Caley *et al.* 1996). Some authors have documented high regional variations in the recruitment of marine species (Thorson 1946, 1950; Caffey 1985; Connell 1985) and several studies have also addressed the influence of larval supply on the geographical distribution and relative abundance of several intertidal organisms (e.g. Southward and Crisp 1954; Lewis 1964, 1976; Kendall *et al.* 1985). Research on this subject (supply-side ecology, Lewin 1986) received a great boost after extensive studies demonstrated that the input and spatial distribution of larvae from the plankton to the shore, not post-settlement interactions, were key determinants of the distribution of adult populations of the barnacle *Balanus glandula* (Gaines *et al.* 1985; Gaines and Roughgarden 1985). Further advances were achieved by Roughgarden *et al.* (1985) with the development of a theoretical demographic model for an open marine population of sessile marine organisms with a pelagic larval phase. Those authors concluded that settlement rates and their fluctuations have a major influence on population structure and dynamics. If settlement rate is limited by space, demography is determined by processes such as competition and predation. On the other hand, if settlement is low, interactions will be much less severe and variable recruitment will dictate population fluctuations. The same predictions were independently obtained by Menge and Sutherland (1987), who

modelled the influence of recruitment intensity on levels of disturbance, competition and predation. Therefore, it seems clear that spatial and temporal variation in larval settlement have the potential to modify or even override more deterministic processes, thus increasing the complexity of community structure (Hartnoll and Hawkins 1985; Robles 1997) and usually contributing to the maintenance of a high diversity mosaic (Menge *et al.* 1993).

1.2 Assessment of dispersal scales and marine population connectivity

Many marine species have a planktonic stage that can last from days to months. Thus, it has long been assumed that larval transport was extensive and that local populations were demographically open, with recruitment probably dependent on the arrival of larvae from external sources (Roughgarden *et al.* 1985, 1988; Caley *et al.* 1996; Roberts 1997; Swearer *et al.* 2002). A growing number of studies, however, provided evidence that larvae of some species were capable of returning to their source population thus increasing self-recruitment and limiting population connectivity (Jones *et al.* 1999; Cowen *et al.* 2000; Mora and Sale 2002; Swearer *et al.* 2002; Taylor and Hellberg 2003; Jones *et al.* 2005). Knowledge of the levels of connectivity among marine populations can offer important insights on a variety of topics, such as population dynamics (Gaines and Lafferty 1995; Alexander and Roughgarden 1996; Caley *et al.* 1996; Eckman 1996; Gaines *et al.* 2007), metapopulation dynamics (Armsworth 2002; James *et al.* 2002; Hastings and Botsford 2006), species invasions (Neubert and Caswell 2000; Dunstan and Bax 2007), selection and adaptation (Warner 1997; Hare *et al.* 2005b), species response to climate change (Harley *et al.* 2006), and configuration of marine protected areas (Botsford *et al.* 2001; Gaines *et al.* 2003; Lubchenco *et al.* 2003; Palumbi 2004; Sale *et al.* 2005).

In species with a planktonic stage, reliable estimates of the extent to which populations are connected by exchange of offspring require knowing the origin and trajectories of dispersing larvae among subpopulations. Direct estimates of marine larval dispersal have rarely been obtained (Olson 1985; Davis and Butler 1989; Willis and Oliver 1990; Stoner 1992), mainly due to the small size of planktonic larvae and the potentially large scales of transport involved (Levin 1990; Thorrold *et al.* 2002; Levin 2006; Thorrold *et al.* 2007). Instead, a variety of indirect approaches have been used to assess the extent of larval dispersal or infer levels of connectivity. A brief account of the most relevant ones will be provided in the following sections.

1.2.1 Artificial and natural tags to track marine larvae

Considerable effort has been put into the development of methods to tag marine invertebrate and fish larvae with the purpose of following their movement from source to settlement sites. Tagging of larvae has relied on the analysis of the chemical composition of calcified structures, such as bones, shells, otoliths and statoliths, and followed two distinct approaches: the use of artificial chemical compounds, and natural geochemical signatures (reviewed in Levin 1990; Thorrold *et al.* 2002, 2007). The first approach requires marking a large number of larvae, releasing them from a source location and recapturing at potential settlement sites. Tracking of marine larvae using mark-recapture procedures face two major challenges. First, the small size of most larvae makes them very difficult to tag without introducing significant handling effects. Second, the strong diffusion that quickly dilutes larval concentrations coupled with the high mortality rates during the planktonic stage severely reduce chances of recovering tagged individuals (Thorrold *et al.* 2002, 2007).

A variety of compounds are used as markers, including fluorescent chemicals, trace elements and radioactive isotopes (e.g. Moran 2000; Twining *et al.* 2000; Thorrold *et al.* 2002; Moran and Marko 2005; Thorrold *et al.* 2006). Although most attempts at tracking larvae using artificial tags have not been successful, given the extremely low probability of recapture (e.g. Levin *et al.* 1993; Anastasia *et al.* 1998), a few tagging studies revealed high levels of retention in several coral reef fish species. For example, Jones *et al.* (1999) mass-marked the otoliths of developing embryos of the yellow damselfish (*Pomacentrus amboinensis*) with tetracycline, a fluorescent compound. Based on recapture data, those authors estimated that 15 to 60% of the larvae recruiting to Lizard Island (Great Barrier Reef) were born in the area. Using a similar approach, Jones *et al.* (2005) estimated self-recruitment to be as high as 42% in the panda clownfish (*Amphiprion polymnus*) at Schumann Island (Papua New Guinea). Almany et al (2007) used maternally-inherited stable isotopes to tag a high percentage of all fish larvae produced at a small island reserve in Papua New Guinea, and found that approximately 60% of the recruits were spawned locally.

The challenges of tag-recapture studies prompted for the development of marking techniques taking advantage of environmentally induced differences in elemental composition of calcified larval structures. Because these are formed at birth, they carry a natural signature of the natal source (Thorrold *et al.* 2002, 2007). The main advantage of these methods is that every larva is effectively tagged, and thus larval dilution and high mortality do not pose a problem (Thorrold *et al.* 2002, 2007). On the other hand, the use of natural tags requires that gradients in physicochemical conditions occur between study sites that can lead to measurable variations in tag composition. For this reason, most studies using natural tags focused on species that migrate between

estuarine and coastal habitats, where water properties are different (e.g. DiBacco and Levin 2000; DiBacco and Chadwick 2001; Thorrold *et al.* 2001). Searching for variation in natural tags at open-coast sites may be complicated by the fact that environmental gradients are usually more subtle. Thus, the use of elemental fingerprinting to assess connectivity patterns in the open-coast is limited by the extent of spatial variation that is reflected in the microchemistry of the structures being analysed (Gillanders *et al.* 2001; Becker *et al.* 2005; Zacherl 2005).

Although the use of geochemical signatures is still in its early steps, it holds great promise as a tool to study dispersal patterns in marine species. Trace elements present in the otoliths of marine fish have been used to track migration and dispersal patterns, and to identify spawning grounds, nursery habitats and self-recruitment incidence (Edmonds *et al.* 1991; Gillanders and Kingsford 1996; Thorrold *et al.* 1997; Campana 1999; Swearer *et al.* 1999; Gillanders and Kingsford 2000; Thorrold *et al.* 2001). In marine invertebrate larvae, enough spatial and temporal variation was found in statolith and protoconch trace element composition that could be examined to address questions concerning dispersal patterns (Zacherl *et al.* 2003; Becker *et al.* 2005; Zacherl 2005), and its potential has already been demonstrated. A recent study based on elemental fingerprinting of the protoconch found high levels of self-recruitment and contrasting connectivity patterns in two co-occurring, highly dispersive mussel species, *Mytilus galloprovincialis* and *Mytilus californianus*, along a 75-km stretch of coastline in southern California (Becker *et al.* 2007).

1.2.2 Pelagic larval duration

The time larvae spend in the plankton varies from species to species, and can range from minutes to months, or even years (e.g. Strathmann and Strathmann 2007). The distance they are transported by ocean currents should be roughly dependent on the duration of the pelagic period, since larvae that spend very little time in the water cannot be transported for long distances (Sponaugle *et al.* 2002). Conversely, larvae with extremely long planktonic residence times have been known to travel over very long distances (Scheltema 1971, 1986, 1988). Pelagic larval duration has always been a fundamental variable to take into account in predictive models of dispersal scales and connectivity patterns (e.g. Hill 1990; Roberts 1997; Cowen *et al.* 2000; Siegel *et al.* 2003; Cowen *et al.* 2006).

The relationship between propagule duration in the plankton and dispersal distance has been examined by Shanks *et al.* (2003). These authors compiled data on dispersal distance from direct observations, geographical distributions, experimental and genetic studies, and also from invasion rates for a variety of species including

algae, corals, tunicates, bryozoans, molluscs, crustaceans, fish and one vascular plant species. Information on the amount of time spent by propagules in the plankton was obtained from direct field observations (for one tunicate species) and from laboratory rearing experiments. Shanks *et al.* (2003) found a significant positive correlation between log-transformed values of the two variables, particularly in those species with short pelagic larval duration. On the other hand, a number of species with longer-lived larvae exhibited dispersal distances below the values predicted by the relationship. Similar results have been obtained by Siegel *et al.* (2003), who compared genetic estimates of dispersal compiled by Kinlan and Gaines (2003) for 32 species including 19 invertebrates, 12 fish and one macroalgae and planktonic larval durations estimated from otolith ageing and laboratory rearing assays.

Siegel *et al.* (2003) suggested that deviations from model predictions might be related to specific differences in life-history strategies of organisms with divergent planktonic larval durations. Thus, species with longer dispersal distances may be able to delay metamorphosis and remain in the plankton longer than in laboratory cultures. On the other hand, other factors such as temperature and food, mortality and active behaviour (Scheltema and Williams 1982; Rumrill 1990; Morgan 1995; Kingsford *et al.* 2002; Sponaugle *et al.* 2002; O' Connor *et al.* 2007) may contribute to significant deviations from expected magnitudes of dispersal, especially in species with long planktonic periods.

1.2.3 Population genetics

Genetic surveys can also provide an estimate of marine larval dispersal scale and pattern (Grosberg and Cunningham 2001; Hellberg *et al.* 2002; Palumbi 2003; Hedgecock *et al.* 2007). A direct relationship between dispersal ability and the level of population genetic differentiation has been found for most marine larval species (Bohonak 1999). Extensive planktonic dispersal was thought to promote extensive gene flow and thus decrease genetic differentiation, whilst reduced dispersal should have the reverse effect. Numerous examples can be found in the literature to support this assumption. For instance, many high dispersal species reveal little or no genetic differentiation (e.g. Doherty *et al.* 1995; Hellberg 1996; Pannacciulli *et al.* 1997; De Wolf *et al.* 2000; Kyle and Boulding 2000; Collin 2001; Duran *et al.* 2004a; Benzie and Smith-Keune 2006; Weber and Hawkins 2006; Quinteiro *et al.* 2007; Diz and Presa 2008), while other species with limited dispersal often show strong population structure over small spatial scales (Duran *et al.* 2004b; Calderón *et al.* 2007; Constantini *et al.* 2007). Many exceptions to this trend, however, have been documented, with highly dispersive species showing more genetic structure than expected in some cases (e.g.

Knowlton and Keller 1986; Todd *et al.* 1998; Staton and Rice 1999; Barber *et al.* 2000; Goldson *et al.* 2001; Barber *et al.* 2002b; Edmands and Harrison 2003; Taylor and Hellberg 2003; Bilodeau *et al.* 2005) and, conversely, species with limited dispersal ability exhibiting lower levels of genetic structure than expected (e.g. Sponer and Roy 2002; Colson and Hughes 2004; Holmes *et al.* 2004). From these few examples it seems clear that interpreting the relationship between dispersal potential and genetic heterogeneity is not straightforward.

Indeed, inferences of dispersal based on genetic variation may be potentially biased, because genetic structure may be caused by factors other than limited dispersal potential. For example, behavioural traits may act to promote local retention of larvae (e.g. Armsworth *et al.* 2001; Kingsford *et al.* 2002; Sponaugle *et al.* 2002; Paris and Cowen 2004; Gerlach *et al.* 2007), and alternative means of movement may enable species lacking planktonic dispersal to be transported for considerable distances (Highsmith 1985; Johannesson 1988; Helmuth *et al.* 1994; Grantham *et al.* 2003). Increased levels of genetic structure may also be caused by obstacles imposed to larval dispersal, such as hydrodynamic barriers (Barber *et al.* 2000, 2002b; Perrin *et al.* 2004; Sotka *et al.* 2004) and habitat discontinuities (Riginos and Nachman 2001; Johansson *et al.* 2008).

Small-scale genetic heterogeneity has often been linked to temporal genetic changes among adult cohorts or among recruits and adults (e.g. Johnson and Black 1984; Hedgecock 1994a; Edmands *et al.* 1996; Moberg and Burton 2000; Planes and Lenfant 2002; Turner *et al.* 2002; Selkoe *et al.* 2006). Possible explanations for this pattern, also known as 'chaotic genetic patchiness', include selection acting on larvae that may reduce the genetic diversity of recruits (Johnson and Black 1984), different sources of recruitment driven by variability in ocean flow (Kordos and Burton 1993) and the occurrence of high variance in reproductive success (sweepstakes reproduction hypothesis, Hedgecock 1994a, 1994b; Li and Hedgecock 1998). This hypothesis states that in highly fecund species reproducing under variable environmental conditions, it is possible that only a small part of adults reproduce each season, thus causing a drastic reduction in the effective population size and an increase in genetic drift (Hedrick 2005a).

Other factors intrinsic to genetic analyses can also lead to biased estimates of dispersal. Widely used measures of genetic structure, such as F_{ST} (Wright 1951) are based on models of population structure that make assumptions concerning inheritance, neutrality of markers, and equilibrium between migration, mutation and genetic drift (Waples 1998; Hellberg *et al.* 2002). In most of the cases, these assumptions are not met, and the inferred genetic differentiation may actually be due to selection or historical effects (e.g. Hilbish 1996; Cunningham and Collins 1998; Brown

et al. 2001; Wares 2001; Dufresne *et al.* 2002; Luttikhuizen *et al.* 2003; Uthicke and Benzie 2003; Duran *et al.* 2004a; Bilodeau *et al.* 2005; Hemmer-Hansen *et al.* 2007). Estimates of gene flow obtained from measures such as F_{ST} are thus likely to be biased, and may only provide a correct measure of migration rates within a few orders of magnitude (Whitlock and McCauley 1999).

The success of genetic assessments of connectivity depends on the degree of genetic structure. Usually, higher levels of genetic differentiation ensure better estimates of connectivity. Hellberg *et al.* (2002) outlined six patterns of genetic differentiation based on the interplay between migration rate and effective population size. These patterns form a continuum between completely closed (100% self-recruitment) and completely open populations (100% recruitment from other locations). Intermediate patterns include phylogeographic breaks, geographic clines driven by selection or secondary intergradation, stepping-stone gene flow determined by limited dispersal ability, and chaotic genetic patchiness (Hellberg *et al.* 2002). Most marine species with planktotrophic larvae exhibit high levels of gene flow and weak population differentiation (Waples 1998), and can thus be placed near the open end of this scale. Gene flow is typically expressed as the mean number of migrants per generation (Nm), which is estimated from measures of genetic structure (such as F_{ST} or G_{ST}). Because these two measures vary inversely, very low values of F_{ST} may lie within the range of sampling error, and therefore even moderate levels of gene flow cannot be differentiated from unlimited demographic exchanged (Palumbi 2003). Alternative methods to *F*-statistics for measuring population structure and estimating migration rates have been recently developed, based on the coalescent process (e.g. Beerli and Felsenstein 1999, 2001; Nielsen and Wakeley 2001). Although these approaches are more informative than F_{ST} and do not share the same limitations, estimation of migration rates can be biased by the effect of unsampled populations (Slatkin 2005).

Other analytical frameworks may provide better means of estimating dispersal in coastal marine species. Because these are usually distributed along a one-dimensional array (the coastline), dispersal seems to be better described by a stepping-stone model. In other words, there is a greater chance for exchange of individuals among adjacent populations and thus dispersal between localities is probably related to geographical distance (Hellberg 1994). Under restricted dispersal, a decrease in genetic correlation with increasing geographic distance, termed isolation by distance (Wright 1943), is commonly observed, and mean dispersal distance can be estimated from its regression slope. Palumbi (2003) simulated dispersal in a stepping-stone lattice to estimate the relationship between dispersal distance and isolation-by-distance regression slope for several marine invertebrate and fish species. Simulations showed that isolation by distance was most evident when comparing populations separated by 2-5 times the mean dispersal distance, and mean dispersal distances for the studied species

were in the range of 25-150 km. Kinlan and Gaines (2003) used a modified version of the model developed by Palumbi (2003) to estimate mean dispersal distances from isolation-by-distance slopes in a large number of marine species, comprising macroalgae, invertebrates and fish, and also compared these estimates to estimates of dispersal on terrestrial species. Their results showed differences on dispersal among marine taxonomic groups, and also a greater dispersal potential of marine species compared to terrestrial ones. It must be noted, however, that the isolation-by-distance relationship may not be linear at very short and long average dispersal distances, and that this non-linear pattern may be common in marine species, which limits the applicability of the method (Bradbury and Bentzen 2007).

Many marine species exhibit genetic clines, or geographic zones in which genetically differentiated populations interbreed (e.g. Hare and Avise 1996; Gardner 1997; Planes and Doherty 1997; Sotka *et al.* 2004). The geographic width of a cline is determined by a balance between the homogenizing effects of dispersal and the diversifying effects of selection (Slatkin 1973; Barton and Hewitt 1985). Thus, if clinal width and selection are quantified, average dispersal distances across the cline can be inferred. Dispersal may be quantified from the degree of linkage disequilibrium (the non-random gametic association of alleles between two or more loci), because linkage disequilibrium is generated when selection acts within the cline. Assuming equilibrium between migration and selection, the degree of linkage disequilibrium increases with increasing levels of gene flow (dispersal) along the cline (Slatkin 1973; Barton and Hewitt 1985). Sotka and Palumbi (2006) used this approach to infer dispersal distances from linkage disequilibrium and cline width reported in several published studies for marine species. Their estimates of dispersal distance matched expectations based on the life history of the species, and showed that, in the studied cases, average dispersal distance was 20% or less of the cline width.

One of the most important shortcomings of genetic estimates of connectivity using measures of differentiation based on *F*-statistics is that they only provide a long-term average view of dispersal, lacking the resolution to uncover ecologically relevant patterns of exchange (Palumbi 2003). This can be overcome with recent methods based on assignment of individuals to a natal source based on their multilocus genotypes at highly variable markers (Rannala and Mountain 1997; Pritchard *et al.* 2000; Falush *et al.* 2003; Manel *et al.* 2005). Assignment tests can be used to calculate the likelihood that a given individual originated from a particular source population or region, and even to estimate gene flow (Castric and Bernatchez 2004; Baums *et al.* 2005; Galindo *et al.* 2006; Lind *et al.* 2007; Underwood *et al.* 2007). The accuracy of these methods, however, can only be assured under conditions of high population structure and low to moderate levels of migration (Latch *et al.* 2006; Waples and Gaggiotti 2006).

1.2.4 Biophysical modelling

The wide range of spatial and temporal scales associated with the oceanic environment together with the variety of biological processes intrinsic to marine organisms, pose great challenges to the study of dispersal and marine population connectivity (Werner *et al.* 2007). Because sampling over all possible scales is not possible (Gawarkiewicz *et al.* 2007), modelling approaches integrating physical processes and biological traits provide a tractable means of investigating marine propagule dispersal and can potentially provide a mechanistic understanding from which testable hypothesis can be formulated (Werner *et al.* 2001; Levin 2006; Cowen *et al.* 2007; Werner *et al.* 2007).

Early models of dispersal in marine systems have relied either on simplified advection-diffusion models or on transport of passive particles subject to average flow fields (e.g. Hill 1990; Roberts 1997). Although these studies may provide theoretical frameworks for addressing applied issues related to larval dispersal, such as spacing of marine reserves, they do not provide realistic flow field scenarios or predict how biological factors may modulate the outcome of dispersal. To properly address these issues, more realistic biophysical models are required (Werner *et al.* 2001; Cowen *et al.* 2007; Werner *et al.* 2007).

Computer-assisted dispersal simulations integrating physical and biological models have witnessed great progress over the last decade (Levin 2006; Werner *et al.* 2007). With the huge increase of computational performance observed in recent years and the growing volume of ocean data available for model initialization and parameterization, spatio-temporal resolution of ocean circulation models (e.g. MICOM, HYCOM, GODAE, Mercator, ROMS) has improved significantly (Chassignet *et al.* 2006). These efforts have rendered particle transport simulations unprecedented realism, especially with the development of hybrid models which allow capturing a broad range of flow scales (e.g. Fringer *et al.* 2006). It is therefore possible to use these high-resolution circulation models to study the consequences of specific hydrographic features on dispersal. At the same time, there has been considerable effort devoted to research on larval biology and ecology, which allowed shedding light on the possible biological processes interfering with dispersal, such as mortality, sensory/locomotory capabilities and behaviour (Kingsford *et al.* 2002; Sponaugle *et al.* 2002; Levin 2006; Cowen *et al.* 2007; Pineda *et al.* 2007). Bringing both disciplines together gave origin to an invaluable tool for studying dispersal and connectivity.

The output of dispersal models is often expressed as dispersal curves, or dispersal kernels, which describe the probability distribution of settlement as a function of distance from natal source (Nathan 2001). Dispersal kernels can be used to explore interactions among the physical and biological factors determining dispersal (such as advective velocity, eddie diffusivity or planktonic larval duration) and to make

predictions about their relative importance for the outcome of dispersal. Most models describing dispersal kernels in marine systems report to coastal areas and were limited to 1-dimension (e.g. Largier 2003; Siegel *et al.* 2003; Aiken *et al.* 2007), although 2-dimensional probability functions have also been reported in cases where dispersal was not restricted to one dimension, like for example over a broad continental shelf (Edwards *et al.* 2007). The study of the implications of dispersal for the design of marine reserves and assessment of their efficacy has also relied on predictions of dispersal kernels using theoretical models (e.g. Botsford *et al.* 2001; Gaines *et al.* 2003; Largier 2003; Kaplan and Botsford 2005; Kaplan 2006).

Biophysical models of larval dispersal have provided valuable insights on the effect of a number of biological parameters on population connectivity. For example, Hohenlohe (2004) modelled larval exchange across a well-known oceanographic barrier (Point Conception, California) based on the life-history of two species of gastropod (*Littorina scutulata* and *Littorina plena*). His results suggested that timing and duration of spawning periods can have a major influence on levels of gene flow under seasonally variable oceanographic conditions, by temporarily eliminating putative barriers to dispersal.

The relative importance of larval mortality and flow on dispersal and recruitment patterns was investigated for the brittle-star *Ophiothrix fragilis* (Lefebvre *et al.* 2003) and for the polychaete *Pectinaria koreni* (Ellien *et al.* 2004), using an advection-diffusion circulation model of the English Channel. Both studies agreed that larval mortality could account for more loss of larvae than unfavourable hydrodynamic processes, and that retention was sufficient to ensure self-replenishment. On the other hand, several studies for the Caribbean region showed that when dispersal models included mortality, larval import from outer sources was very limited and local populations could not subsist without considerable levels of self-seeding (Cowen *et al.* 2000; Cowen *et al.* 2003, 2006).

When behaviour is incorporated into dispersal models, results usually show contrasting dispersal outcomes relative to passive scenarios. For example, Paris and Cowen (2004) reconstructed a 3-dimensional in situ larval fish transport model from field observations to study the effect of vertical swimming in larvae of a coral reef fish. Those authors found that ontogenetic vertical migration promoted retention of larvae due to stratified currents flowing in opposite directions. Dispersal simulations of cod larvae in the Northeast Arctic showed that larvae drifting at different depths (simulating vertical migration) ended up in very distant positions (Vikebø *et al.* 2005). In the same study, minor horizontal displacements achieved by constant swimming also resulted in considerable deviations from predicted trajectories under passive dispersal. The final example comes from a larval tracking model including 3-dimensional advection and turbulence in Banyuls Bay (Mediterranean French coast), and explicitly accounting for

ontogenetic and light-induced swimming behaviour in larvae of the polychaete *Owenia fusiformis* (Guizien *et al.* 2006). Model simulations showed that the final position and local retention of larvae depended on turbulent flow and larval swimming activity rates. Gerlach *et al.* (2007) combined dispersal modelling, genetics and behavioural experiments to investigate the role of sensorial capabilities (olfaction) in retention and homing behaviour of coral reef fish larvae. A hydrodynamic model of passive dispersal was developed to predict dispersal patterns among reefs. Model predictions indicated that under passive transport no genetic differentiation would be expected. Genetic analyses, however, revealed significant genetic substructure among reefs, suggesting that settling larvae were capable of discriminating the odours of water from nearby reefs and select the natal origin.

Other modelling studies aimed to uncover demographic connectivity patterns among multiple populations, by creating connectivity matrices. A connectivity matrix defines the probability of larval exchange among each pair of populations and the probability of retention at each population. This approach was adopted by James *et al.* (2002) in a 2-dimensional simulation of dispersal of reef fish larvae on the Great Barrier Reef. They simulated larval transport among 321 reefs and over a period of 20 years, combining mortality and age-dependent swimming behaviour (initially passive and later active) in an advective larval tracking model. The simulations predicted low levels of self-recruitment (less than 9% on average) and also indicated that a small number of populations were able to ensure the persistence of metapopulations over regional scales. A subsequent simulation encompassing a longer time series was used to generate connectivity matrices for the same section of the Great Barrier Reef by Bode *et al.* (2006). Based on the analysis of those matrices, the authors identified regional scale source-sink dynamics within the metapopulation. Contrasting results were obtained by a model of dispersal in coral reef fish for the Caribbean region. Cowen *et al.* (2006) generated the connectivity matrix among all available coral reef fish habitats (260 sites) from individual dispersal trajectories. Simulations obtained from a high-resolution hydrodynamic model and accounting for pelagic larval duration, behaviour (vertical and horizontal swimming), plus spawning season and frequency indicated high levels of retention and relatively short dispersal distances of ecological relevance. Coupled with basic population genetic models, connectivity matrices can also be used to predict broad scale genetic structure of marine populations resulting from larval dispersal. Model predictions can then be compared against empirical genetic data. This approach was followed by Galindo *et al.* (2006), who reported broad similarities on a regional scale among predicted and empirical genetic data in a Caribbean coral, *Acropora cervicornis*, suggesting that oceanographic simulations could provide a good picture of realised larval dispersal.

Understanding the mechanisms of biophysical barriers to dispersal may also

be achieved using modelling approaches. For example, Baums *et al.* (2006) simulated dispersal at two spatial scales to investigate possible causes for limited gene flow between western and eastern Caribbean populations of a reef-building coral (*Acropora palmata*). Integrating biological and physical parameters, the model indicated that an interaction between oceanographic features (small-scale eddies) and larval competency period could explain restricted dispersal across a narrow passage (Mona Strait) separating the two areas, thus promoting genetic differentiation between both population groups.

Besides the examples presented here, biophysical models have also been useful to address other issues, such as the stability of hybrid zones (Gilg and Hilbish 2003), species invasions and anthropogenic introductions (Dawson *et al.* 2005; Dunstan and Bax 2007; Dupont *et al.* 2007) and dispersal and dynamics of larval fish stocks (Bartsch and Coombs 2001, 2004; Santos *et al.* 2005; Kettle and Haines 2006). In the future, dispersal models may provide the starting point for developing testable hypotheses, means for refining existing ecological and evolutionary theories, and important insights into possible consequences of expected climatic changes (Werner *et al.* 2007).

1.3 Circulation patterns in the eastern North Atlantic

1.3.1 Large-scale circulation

The main large-scale currents associated with the northeast Atlantic region are the North Atlantic Current, the Azores Current and the Canary Current (Mason *et al.* 2006). The North Atlantic Current (to the north of Iberia) and the Azores Current (south of Iberia) are basin-scale currents, fed by the Gulf Stream. The North Atlantic Current splits into two branches, one flowing northeastward between Iceland and the British Isles, and the other one flowing eastward towards northern Europe (Mason *et al.* 2006). The Azores Current flows eastward between 34 and 35°N, and splits into two branches: the northern branch flows towards the Gulf of Cadiz, while the southern branch flows southeastward towards the Canary Islands (Johnson and Stevens 2000). Upon reaching the Gulf of Cadiz, the Azores Current turns south and flows along the northwest African coast (Johnson and Stevens 2000). The Canary Current is supplied by the northern branch of the Azores Current, and also receives a small input from the Portugal Current (Barton 2001). This current flows equatorward while interacting with the coastal upwelling waters, and it disconnects from the coast near Cape Blanco (21°N), flowing westward at the latitude of Cape Verde (Barton 2001; Arístegui *et al.* 2006).

1.3.2 Celtic Sea and the English Channel

North of the Bay of Biscay (48°N) the shelf widens considerably to the west, and the shelf edge establishes the western and southern boundaries of the Celtic Sea. To the north, the Celtic Sea is bounded by the Irish Sea and to the east by the English Channel, which provides a connection to the North Sea (Mason *et al.* 2006). Over the shelf region, where the residual circulation is slow, strong tidal currents generated from the Atlantic exert the main hydrodynamic influence. Tidal waves proceed from the deep ocean onto the shelf, where they are reflected and amplified to varying degrees by the coastal and bottom topography (Mason *et al.* 2006).

The Celtic Sea and the English Channel are subject to seasonal heating and cooling of the surface layers, and to strong westerly winds that persist for most of the year (Mason *et al.* 2006). Freshwater input from river runoff is generally low, with some outflow from rivers along the coast of Ireland and southwestern Great Britain, but especially from the Seine, at the French eastern English Channel coast (Salomon and Breton 1993). The English Channel, which does not exceed 100 m in depth over most of its extension, is characterised by a very strong tidal regime, which maintains a steady pattern of long-term circulation, regardless of the varying wind stress (Salomon and Breton 1991, 1993). This tidal residual circulation is characterized by an eastward flow at the central section of the Channel from the Atlantic to the North Sea, bordered with numerous gyres, some of them induced by coastal topography, the others being centered on islands which the periodical flow must get around (Ménesguen and Gohin 2006).

1.3.3 Bay of Biscay

Shelf currents in the Bay of Biscay are mainly driven by wind, water density, tides and coastal topography (Koutsikopoulos and Le Cann 1996; Planque *et al.* 2003). Over the Armorican shelf (northern part of the Bay), the residual current is weak and oriented towards the northwest (Pingree and Le Cann 1989). In the south, over the Aquitaine shelf, the residual circulation shows a marked seasonal variation, mainly due to the dynamics of dominant winds: in winter the current is oriented to the northwest and in the rest of the year towards the southeast (Koutsikopoulos and Le Cann 1996). More complexity is added to flow near the coast, which typically runs along the coastline, due to topographic features (Koutsikopoulos and Le Cann 1996). Northerly and easterly winds during spring and summer generate upwelling on the French and Spanish coasts, respectively. The French coast shows more intense upwelling than the Spanish coast, where downwelling is prevalent all year (Bosch and Pearse 1990). Near estuaries, especially of the rivers Loire and Gironde, freshwater outflows induce significant

surface currents. These plumes generally flow poleward due to the Earth's rotation, but their direction is frequently affected by the wind, which can sometimes transport them towards the southwest (Koutsikopoulos and Le Cann 1996; Puillat *et al.* 2006). The intensity of tidal currents is proportional to shelf width, so they are weak over the narrower southern shelf (Le Cann 1990). Surface flows over most of the Biscay shelf are weak, and therefore diffusive mechanisms may be important for larval dispersal (Koutsikopoulos *et al.* 1991).

1.3.4 North Atlantic Upwelling system

The area between 43°N and 10°N, extending from northwestern Iberia to the south of Senegal, is termed the North Atlantic Upwelling Region (Barton 1998; Relvas *et al.* 2007). This current system is characterised by a predominant equatorward wind direction during part of the year (permanent in some areas). Due to the coastline orientation (mostly from north to south), this wind generates upwelling, which establishes a southward flow. Within this system, the Iberian and Northwest African regions form two subsystems with distinctive characteristics. Off western Iberia, the circulation pattern shows a marked seasonal variability defined by the coastal wind regime, both in the western and northern coasts (Huthnance *et al.* 2002; van Aken 2002). The main upwelling season occurs between May and September (Fiúza *et al.* 1982); during winter, equatorward winds relax, and southerly, downwelling-favourable winds become predominant, the equatorward currents decrease in intensity and inshore northward circulation increases (Figueiras *et al.* 2002; Peliz *et al.* 2002; Sánchez and Relvas 2003). Other components of the Iberian current system include the Portugal Current, a slow, southward current flowing between continental Portugal and the Azores (Martins *et al.* 2002), and the subsurface poleward flowing Iberian Poleward Current, also termed Portugal Coastal Counter Current, which can reach the surface in winter (Frouin *et al.* 1990; Haynes and Barton 1990; Peliz *et al.* 2005), and extends into the Bay of Biscay (Martins *et al.* 2002).

Inner shelf circulation contrasts with the seasonal regime in the western Iberian subsystem (Relvas *et al.* 2007). Inshore countercurrents have been observed during the upwelling season (coincident with periods of relaxation), developing off southwest Iberia and progressing westward, often turning poleward around Cape São Vicente (Relvas and Barton 2002, 2005). A similar situation has been described by Sordo *et al.* (2001) off the Galician coast. During several years of observations, an inshore poleward flow was formed over the inner shelf after the cessation of upwelling, while on the middle and outer shelf, a tongue of cold water previously upwelled continued to move southward. Another important feature of the inner shelf circulation is the Western

Iberian Buoyant Plume, a low salinity surface water body resulting from winter-intensified river outflow on the northwest coast (Peliz *et al.* 2002, 2005). The plume can interact with wind forcing and develop into a narrow and strong poleward coastal current.

Separating the Iberian and Northwest African subsystems is the Gulf of Cadiz, where surface currents are dominated by an eastward flow entering the Mediterranean, and fed by the northern branch of the Azores Current (Johnson and Stevens 2000; Sánchez and Relvas 2003). Rapid current reversals have been reported for the southwestern Iberian shelf region (Sánchez *et al.* 2006). Also, a coastal countercurrent has frequently been identified flowing westward along the southern Iberian coast, then turning clockwise around Cape São Vicente and flowing poleward along the west coast (Relvas and Barton 2002, 2005).

The Northwest African coast is largely influenced by the general circulation of the North Atlantic subtropical gyre, particularly by its eastern branch, the Canary Current. The Canary Islands, act as a barrier to the Trade Winds and the Canary Current, introducing a strong variability in the atmospheric and oceanic flows (Barton 2001; Rodríguez *et al.* 2004). This produces mesoscale oceanographic features downwind of the archipelago, such as cyclonic and anticyclonic eddies and warm wakes (Arístegui *et al.* 1994; Barton 1998).

Apart from its northern and southern limits, the Northwest African subsystem is subject to year-round upwelling between 20°N and 30°N, peaking in July and August (Barton 2001). The north coast of Morocco (33-37°N) is oriented at a large angle to the Trade Winds, and so upwelling there is intermittent and short-lived. South of the 20°N upwelling starts in December and lasts until April or May (Nykjaer and van Camp 1994). Beneath the Canary Current, a subsurface slope current flows poleward. This current may extend to the surface during autumn, when the Trade Winds weaken or turn northward (Barton 2001).

1.4 Review of the taxonomy, reproductive biology and ecology of northeastern Atlantic and Mediterranean patellid limpets

1.4.1 Taxonomy, phylogeny and geographical distribution

Due to the substantial phenotypic variability of the characters used for identification, namely shell morphology and colour of the foot and pallial tentacles, the taxonomy of limpets has always been considered a controversial issue (Fischer-Piètte 1938;

Hawkins *et al.* 2000). The strong subjectivity that has always been associated with the identification of patellid limpets gave origin to a plethora of species and subspecies, and consequently to strong discrepancies among authors regarding limpet taxonomy and distribution (Fischer-Piètte 1938; Fischer-Piètte and Gaillard 1959). Advances in traditional taxonomic methods, in particular with the study of radular teeth morphology (e.g. Fischer-Piètte and Gaillard 1959), has enabled a better discrimination among species and led to a major review of the taxon. Based on shell and radular teeth morphology, Christiaens (1973) reduced the 240 names previously described in the literature to just 32 valid species in the genus *Patella*. Further studies have examined morphology supported by scanning electron microscopy, and analysed morphometrics with multivariate methods (e.g. Hodgson et al 1996; Ridgway et al 1998), often coupled with screening of allozyme loci (e.g. Côrte-Real *et al.* 1996a, b; Weber and Hawkins 2002; Mauro *et al.* 2003; Weber and Hawkins 2005, 2006). Molecular markers were also used to study phylogeny, phylogeography and population genetic structure in patellid limpets (Koufopanou *et al.* 1999; Sá-Pinto *et al.* 2005; Sá-Pinto *et al.* 2008).

The taxonomy of the family Patellidae (Order Patellogastropoda) has recently been reviewed by Ridgway *et al.* (1998), based on a cladistic analysis of morphological characters derived from shell shape and microstructure, headfoot and pallial complex, radula and sperm. The classification proposed by those authors divided the family in four monophyletic genera: *Helcion* (only present in southern Africa), *Scutellastra* (distributed throughout southern and southwestern Africa, Australia and the Pacific) *Cymbula* (occurring in southern Africa and the southern Indian Ocean), and *Patella* (restricted to the northeastern Atlantic and Mediterranean). Nine species have been recognized within the genus *Patella* by Ridgway *et al.* (1998): *Patella ulyssiponensis* Gmelin, 1791, *Patella depressa* Pennant, 1777, *Patella vulgata* Linnaeus, 1758, *Patella caerulea* Linnaeus, 1758, *Patella ferruginea* Gmelin, 1791, *Patella rustica* Linnaeus, 1758, *Patella candei* d'Orbigny, 1839, *Patella lugubris* Gmelin, 1791, and *Patella pellucida* Linnaeus, 1758.

Koufopanou *et al.* (1999) constructed a molecular phylogeny of the Patellidae, based on the survey of the mitochondrial rRNA genes 12S and 16S. These authors confirmed the monophily of the genus *Patella* and suggested that it is the sister-group of all the other Patellidae, and therefore its divergence probably dates back to the Upper Cretaceous. Estimates using the rate of molecular divergence for *Littorina* suggest an age between 69 and 169 million years before present for this taxon. The radiation of modern species, on the other hand, was estimated by Koufopanou *et al.* (1999) to have occurred between 5 and 20 million years before present, and could have been influenced by the opening and closing of the Mediterranean, speciation on recently-emerged Atlantic islands and possibly Plio-Pleistocene glaciations.

The phylogeny of the genus *Patella* was later reassessed by Sá-Pinto *et al.* (2005), based on sequences of the mitochondrial gene cytochrome *c* oxidase subunit I, and the mitochondrial rRNA 12S and 16S genes. Their analysis broadly confirmed the results obtained by Koufopanou *et al.* (1999), and identified five strongly supported clades: 1) *P. depressa* + *P. caerulea* + *P. lugubris* + *P. candei*, 2) *P. ulyssiponensis*, 3) *P. vulgata*, 4) *P. rustica* + *P. ferruginea*, and 5) *P. pellucida*. According to Sá-Pinto *et al.* (2005), the Mediterranean Sea and Macaronesian Islands seem to have played an important role in the speciation and divergence of the genus, although different clades showed different phylogeographic patterns.

Three species, *P. depressa*, *P. vulgata* and *P. pellucida* occur exclusively in continental Atlantic shores. The black-footed limpet *P. depressa* is a southern species, ranging from Senegal to North Wales (Fischer-Piètte and Gaillard 1959; Christiaens 1973; Southward *et al.* 1995), while *P. vulgata* has a northern distribution, extending from southern Portugal to northern Norway (Christiaens 1973; Southward *et al.* 1995). The blue-rayed limpet *P. pellucida* also ranges from Norway to Portugal (Weber *et al.* 1997), and is usually found in the low intertidal, attached to kelps.

Two species are endemic to the Mediterranean. While *P. caerulea* is common in both Eastern and Western Basins (Christiaens 1973), *P. ferruginea*, which once used to be widespread throughout Western Mediterranean rocky shores, as suggested by shell deposits (Fischer-Piètte 1935; Christiaens 1973), is now the most endangered invertebrate species of the region, and is restricted to a few areas (Guerra-Garcia *et al.* 2004; Casu *et al.* 2006).

Two endemic species occur in the Macaronesian archipelagos, one restricted to Cape Verde (*P. lugubris*) and the other one, *P. candei*, spread throughout the Azores, Madeira, Selvagens and the Canaries (Christiaens 1973). There has been considerable debate around the taxonomic status of *P. candei*. Christiaens (1973) recognized four subspecies on the basis of shell morphology: *P. c. gomesii* (mostly in the Azores), *P. c. ordinaria* (Madeira), *P. c. candei* and *P. c. crenata* (Madeira, Selvagens and Canary Islands). Côrte-Real *et al.* (1996a) compared genetic variability at allozyme loci in *P. c. gomesii*, *P. c. ordinaria* and *P. c. crenata*, and found sufficient differentiation to consider the former a separate species. Weber and Hawkins (2002) included samples of *P. c. candei* from Selvagens and found two differentiated groups, one including *P. c. candei* and *P. candei gomesii*, and the other one including *P. c. ordinaria* and *P. c. crenata*. The authors suggested that these two groups corresponded to two different subspecies, and that both human intervention and natural processes have played a major role in the evolution of these taxa. In a recent phylogeny published by Sá-Pinto *et al.* (2005), *P. candei* is represented as a paraphyletic group, and therefore neither of the previous studies was supported. Within the Macaronesian Islands, these authors recovered

two well-differentiated groups: one including *P. candei* from the Azores, Desertas and Madeira, and another one grouping *P. candei* from the Canaries and Selvagens with *P. lugubris* from Cape Verde. Several alternative explanations for the discrepancy between studies were advanced, such as introgression between *P. candei* from the Canaries and Selvagens and *P. lugubris*, or the occurrence of homoplasy or similar selective pressures within the groups found by Côrte-Real *et al.* (1996a) and Weber and Hawkins (2002). Further studies using nuclear markers and samples from continental African shores are required to elucidate this question (Sá-Pinto *et al.* 2005).

The lusitanian limpet, *P. rustica*, ranges from the Mediterranean to the Atlantic coasts of the Iberian Peninsula and North Africa (Christiaens 1973; Ridgway *et al.* 1998). Recent work suggests considerable differentiation between *P. rustica* and its sister taxon *Patella piperata* Gould, 1846, which occurs in all Macaronesian islands except the Azores (Côrte-Real *et al.* 1996a; Koufopanou *et al.* 1999; Sá-Pinto *et al.* 2005; Sá-Pinto *et al.* 2008). The southern limit of *P. rustica* in the Atlantic is located south of Mauritania, whilst the northern limit lies at Capbreton, on the French Basque coast (Fischer-Piètte and Gaillard 1959; Lima *et al.* 2007b). This species has recently undergone a range expansion, bridging an historical gap of approximately 280 km in northern Iberia (Lima *et al.* 2006).

The southern species *P. ulyssiponensis* has a more widespread distribution. It is present throughout the Mediterranean (and possibly the Black Sea, although this record has not been confirmed), the Macaronesian Islands (except Cape Verde), the North African coast, and in Europe, it occurs as far north as southern Norway and also in the North Sea coast of the British Isles (Christiaens 1973). In the Azores, this species has suffered heavy exploitation for human consumption, and populations have been severely reduced in many of the islands (Martins *et al.* 1987; Hawkins *et al.* 2000). The Macaronesian and continental forms of this species exhibit considerable differences in radular and shell morphology (Christiaens 1973; Weber and Hawkins 2005). Genetic differentiation was also found in mtDNA (Koufopanou *et al.* 1999; Sá-Pinto *et al.* 2005) and allozyme markers (Weber and Hawkins 2005), suggesting that they might be different species, with *Patella aspera* Röding, 1798 being the appropriate name for the Macaronesian form.

1.4.2 Reproductive biology

1.4.2.1 Fertilisation and early development

Patellid limpets are broadcast spawners and go through a planktonic larval stage in their life cycle. Fertilization success in *P. ulyssiponensis* and *P. vulgata* was found

to be a function of sperm concentration, gamete age and contact time. Laboratory experiments undertaken by Hodgson *et al.* (2007) showed that the rate of fertilization is positively correlated with sperm concentrations. Above a certain threshold, however, the normal development of larvae is compromised. The length of time that eggs and sperm remained in contact was also shown to influence fertilization in both species, with short contact times (a few minutes) resulting in highly variable fertilization rates, and contact periods of 15 minutes ensuring the majority of fertilizations. Finally, gamete longevity has a detrimental effect on levels of fertilization, with laboratory experiments showing that egg and sperm viability declined 12 and 6 hours after spawning, respectively (Hodgson *et al.* 2007). These findings suggest that dispersal in *Patella* should be made exclusively by planktonic larvae.

The first description of larval development in patellid limpets, and also the first one to accomplish artificial fertilisation in gastropods, was carried out by Patten (1886) for *P. caerulea*. His work, however, provided a largely inaccurate description of larval stages due to a high incidence of abnormal larvae, which according to Dodd (1957) was probably attributable to the use of unripe eggs in the trials and also to the occurrence of polyspermy. Smith (1935) reared larvae of *P. vulgata* to metamorphosis for the first time, although this has been observed in only one individual. The first detailed account of artificial fertilisation and timing of development from fertilization to larval metamorphosis was provided by Dodd (1957) , who reported a planktonic period of approximately 10 days at 12 °C for *P. vulgata*. This work still remains as the main reference concerning larval development times in the genus *Patella*, and no information is available on how this varies with parameters such as temperature, salinity or food availability.

1.4.2.2 Protandry

Hermaphroditism is commonly reported in marine animals, from fish (Warner 1975) to most invertebrate groups (Ghiselin 1987), including molluscs (Heller 1993). Protandry, a male to female sex change, has been suggested as the most common form of sequential hermaphroditism in molluscs (Heller 1993). It has long been suggested that some species of the genus *Patella* could be protandrous hermaphrodites, based on indirect evidence. Orton (1919, 1928) was the first researcher to link sex change in *P. vulgata* with a shift in sex-ratios with size (males being predominant at smaller size classes). Indirect evidence supporting the occurrence of protandry was later reported for *P. vulgata* (Dodd 1956; Orton *et al.* 1956), *P. ulyssiponensis* (Thompson 1979), *P. caerulea* (Bacci 1947, 1949), *P. ferruginea* (Frenkiel 1975) and *P. rustica* (Bacci 1975). In South Africa, Branch (1974) suggests the occurrence of sequential hermaphroditism in *Patella oculus* (now *Cymbula oculus*, Ridgway *et al.* 1998), and Creese *et al.*(1990)

reported the same phenomenon in the endemic limpet from the Kermadec Islands, *Patella kermadecensis* (now *Scutellastra kermadecensis*, Ridgway *et al.* 1998).

Despite the considerable body of literature dedicated to this subject, caution must be taken in the interpretation of the evidence. Shifts in sex-ratio with age do not imply the occurrence of protandrous sex change, because alternative explanations can be advanced: a) females having higher growth rates, b) males maturation occurring earlier or c) males having higher mortality (Orton 1928; Branch 1981). Thompson (1979) suggested that sex ratios in *P. ulyssiponensis* may be indicative of protandry, but also emphasized that conclusive evidence of sex change requires observing oogenesis in animals known to have been male. Individuals that appear to be functionally male and female simultaneously have been recorded in a number of species, including *P. vulgata*, *P. ulyssiponensis* and *P. depressa* (Dodd 1956), but these observations were considered to be unrelated to a change of sex (Branch 1981).

Experimental approaches to the study of sex change in limpets are not straightforward, mainly due to the difficulty of following this phenomenon through the life of an individual, and also the lack of visual external sexual characters in patellid limpets. Therefore, sex determination in limpets usually implies dissecting the animals. To overcome this drawback, Wright and Lindberg (1979) proposed a non-fatal method of sex determination by taking a gonad sample with the use of a hypodermic needle. The method was developed in the lottiid limpet *Lottia gigantea* and allowed following the same animals throughout the course of sex change (Wright and Lindberg 1982; Lindberg and Wright 1985). The same technique was recently applied by Le Quesne and Hawkins (2006) to investigate protandrous sex change in *P. vulgata*. These authors reported that 29% of the individuals initially sexed as males had become females after one year. Moreover, sex reversal in the opposite direction (protoginy) was also observed in one individual.

Protandry increases species vulnerability to harvesting. Because larger individuals have a greater probability of being collected, in heavily exploited populations there will be fewer large females, which can result in a decrease in reproductive output. Impacts of harvesting on *C. oculus* were investigated by Branch and Odendaal (2003), who compared populations between two South African marine protected areas and four exploited sites. Results revealed that in harvested sites, female:male ratio was much lower and reproductive output was drastically reduced. In the Azores, where limpets are heavily exploited, sex ratios in *P. aspera* (possibly a protandrous hermaphrodite) are heavily distorted, contrasting with *Patella candei*, which does not seem to change sex (Martins *et al.* 1987).

1.4.2.3 Hybridization

The hypothesis of hybridization within the genus *Patella* has long been considered to explain why some individuals exhibit intermediate morphological characteristics (Fretter and Graham 1976). On the other hand, such traits (e.g. shell morphology and foot colour), have been shown to be highly variable in function of the environment, and some authors argue that the intermediate forms are not hybrids but the result of morphological plasticity (Branch 1981; Titselaar 1998).

Genetic studies done so far to investigate hybridization in *Patella* seem to support this hypothesis, since they have failed to identify any genetic signatures of hybridization. Gaffney (1980) examined allozyme variation in *P. vulgata*, *P. ulyssiponensis* and *P. depressa* at seven locations in southwest England, and found high genetic differentiation among species, with no signs of hybridization. Recently, the occurrence of hybridization between *P. ulyssiponensis*, *P. depressa*, *P. vulgata* and *P. rustica* from the Atlantic coast of the Iberian Peninsula was investigated by Sá-Pinto *et al.* (2007) by screening ten allozyme loci. Based on genetic differentiation, the species were separated into four distinct clusters, and intermediate genotypes which could be assigned to hybrids were not found.

1.4.3 The structuring role of limpets in northeastern Atlantic rocky shore communities

Experimental studies have clearly established the importance of intertidal grazers in determining composition, distribution and dynamics of rocky shore algal communities (Lubchenco and Gaines 1981). Patellid limpets are amongst the most common and best studied herbivores on northern Atlantic rocky shores, and their role in structuring rocky shore communities has been widely demonstrated (reviewed in Branch 1981; Hawkins and Hartnoll 1983; Hawkins *et al.* 1992; Coleman *et al.* 2006). Pioneering limpet removal experiments carried out on the Isle of Man demonstrated that limpet grazing could prevent canopy-forming algae (in particular *Fucus vesiculosus*) from dominating wave-exposed shores (Jones 1946; Lodge 1948; Burrows and Lodge 1950; Southward 1964). The experiments resulted in the rapid development of ephemeral algae, followed by growth of a fucoid canopy which persisted for several years. This pattern was confirmed by Southward and Southward (1978), who observed an increase of low–shore red and brown algae after massive mortality of limpets following the Torrey Canyon oil spill in the British Isles. The importance of limpets in driving the dynamics of a patchy mosaic of *Fucus*, barnacles and bare rock on moderately-exposed shores in the British Isles and northern France was established in a number of manipulative experiments and modelling studies (Hawkins 1981; Hartnoll and Hawkins

1985; Johnson *et al.* 1997; Burrows and Hawkins 1998; Johnson *et al.* 1998).

The development of macroalgal canopies has important implications for community dynamics, because canopies can restrict recruitment of sessile species by sweeping or whiplash effects of the fronds (e.g. Hawkins 1983; Jenkins *et al.* 1999), or they can provide shelter from wave action and from the extreme effects of physical factors such as temperature (McCook and Chapman 1991; Leonard 2000). Limpets can also indirectly enhance or inhibit the establishment of animal species, because they are generalist grazers and may accidentally bulldoze or consume sessile invertebrates (Hawkins 1983).

The effects of grazing by limpets at regional and continental scales have recently been investigated. Jenkins *et al.* (2005) undertook limpet exclusion experiments at two sites separated by approximately 500 km (the Isle of Man and southwest England). Although the effect of limpet clearings was similar and deterministic at both sites, with initial growth of green ephemeral algae followed by development of a fucoid canopy, the abundance of fucoids in southwest England was significantly lower and more variable between plots. Similarly, Coleman *et al.* (2006) reported significant geographical differences in the effect of limpet removal at five locations spread across 17 degrees of latitude. Whilst at northern latitudes limpet removal on variability of algal cover had a strong effect (by enabling the growth of a fucoid canopy), at southern locations it had a lesser impact on spatial variability, by preventing the establishment of heterogeneous assemblages and thus leading to more unpredictable outcomes.

1.5 Rationale, aims and structure of thesis

My thesis addresses dispersal and population connectivity in patellid limpets. Using a dual approach, laboratory larval rearing experiments and genetic population analyses, I have sought to obtain potential and realized dispersal estimates for several species. I also developed a simulation model of dispersal integrating oceanographic data (sea temperature and currents) and larval development times, to: i) assess how well the model can predict observed levels of genetic structure and ii) investigate how parameters such as sea temperature, larval longevity, and habitat discontinuity impact connectivity among patellid populations.

Chapter 2 addresses the reproductive patterns of several patellid limpets (*P. depressa*, *P. vulgata*, *P. ulyssiponensis* and *P. rustica*) in Portugal (Ribeiro et al, in press). Determination of the reproductive periods was necessary to set the temporal window for the dispersal model. Reproductive data were also collected in the UK for *P. depressa*, *P. ulyssiponensis* and *P. vulgata*, which allowed pinpointing the optimal periods for the selection of ripe gonads, largely determining the success of artificial

fertilization techniques. Furthermore, knowing the periods during which limpets spawn also provided the range of sea water temperatures used in the experiments.

In chapter 3, I present the results of rearing experiments conducted to determine the duration of the pelagic phase in *P. ulyssiponensis*, *P. vulgata* and *P. depressa*. Experimental procedures were replicated at several temperature values to test the influence of this environmental variable on the duration of larval development. In addition, the influence of several substratum types as settlement cue was tested in all species.

In chapter 4, I describe the development of microsatellite markers for *P. depressa* and *P. rustica*, in order to enable fine-scale analysis of population genetic structure (published in Pérez *et al.* 2007, 2008).

In chapter 5, an analysis of the genetic population structure among populations of *P. depressa* along its northeastern Atlantic range has been done, using high-resolution microsatellite markers. The analysis of genetic polymorphism at microsatellite loci was made with the aim of identifying possible barriers to gene flow along the species range, and to estimate levels of gene flow among populations.

In chapter 6, mitochondrial and microsatellite markers have been used to investigate the genetic structure in *P. rustica*. This species has been shown to have undergone recent range extensions in northern Portugal, which have joined (or re-united) a previously disjunct biogeographic distribution (Lima *et al.* 2006, see Appendix D).

In chapter 7, a model of larval dispersal has been developed and applied to various patellid species, particularly exploring the sensitivity of temperature-dependent larval duration. By integrating information on realized dispersal potential assessed in the laboratory, together with available data on coastal current patterns, preliminary predictions of advection potential from selected point sources have been made. Ultimately, this will enable assessment of connectivity of adult populations by dispersal of larvae.

In chapter 8 a synthesis and general discussion of the thesis is presented. The implications of these findings for the phylogeography, biogeography, population dynamics plus conservation and management of patellids are discussed.

1.6 Submitted and published papers

The following manuscripts, which encompass a part of the results presented in this thesis have been submitted to or published in peer-reviewed journals:

Ribeiro, P.A., Xavier R., Santos A.M. and Hawkins S.J. (in press). Reproductive cycles of

four species of *Patella* on the northern and central Portuguese coast. *Journal of the Marine Biological Association of the United Kingdom*.

Pérez M., Branco M., Llavona A., Ribeiro P.A., Santos A.M., Hawkins S.J., Dávila J.A., Presa P. and Alexandrino P. (2007). Development of microsatellite loci for the black-footed limpet *Patella depressa*, and cross-amplification in two other *Patella* species. *Conservation Genetics* 8:739-742.

Pérez M., Llavona A., Presa P., Ribeiro P., Hawkins S.J., Santos A.M. and Alexandrino P. (2008). New polymorphic microsatellite markers for the limpet *Patella rustica* and cross-priming testing in four *Patella* species. *Molecular Ecology Resources* 8:926-929.

Chapter 2

Reproductive cycles of four species of *Patella* on the northern and central Portuguese coast

2.1 Introduction

Timing and extent of spawning periods can influence larval dispersal and population connectivity in benthic marine invertebrates in two ways. First, by determining the range of water temperatures that larvae will experience during development (Reitzel *et al.* 2004). If a species is a summer breeder, its planktonic larvae will be able to reach metamorphic competence and settle in a shorter period of time than in the case of a species which breeds in winter. Secondly, the duration of spawning periods will also determine properties and variability in ocean currents encountered by larvae during the dispersive stage (Hohenlohe 2004). Extended spawning or multiple spawning events will favour increased alongshore variability in larval transport trajectories and distances, thus promoting a greater exchange of propagules between populations (Byers and Pringle 2006; Pringle and Wares 2007). Breeding seasons with multiple spawning events also increase the chances of larval settlement, and therefore may play a very important role in assuring greater stability of marginal populations (Bowman and Lewis 1986). Therefore, the development of dispersal models that accurately simulate the timing of larval release into the water and their movement under realistic physical and hydrodynamic conditions requires that these two variables - time and duration of spawning - are known for the species in question. Thus, this chapter sets the scene for attempts later in this thesis to model larval dispersal of patellid limpets (see chapter 7).

Annual reproductive cycles and the underlying triggers for gonad development and spawning in limpets have been extensively investigated over many years (Orton *et al.* 1956) for several species (Branch, 1981). Reproductive cycles of *Patella* in the NE Atlantic region have been extensively studied (see chapter 3, Table 3.6), and are particularly well known in the British Isles for *Patella vulgata* Linnaeus, 1758 (e.g. Orton *et al.* 1956; Thompson 1980; Bowman and Lewis 1986; Davies *et al.* 1990; Delany *et al.* 2002), but also for *Patella depressa* Pennant, 1777 (Orton and Southward 1961;

Moore *et al.* 2007) and *Patella ulyssiponensis* Gmelin, 1791 (Evans 1953; Thompson 1979; McCarthy *et al.* 2008). To a lesser extent, northern France (Fischer-Piètte 1948; Choquet 1966) and Spain (Othaitz 1994) have also been covered. In contrast, reports on the reproductive cycles of *Patella* for Portugal are scarce, other than those by Guerra and Gaudêncio (1986) encompassing the entire Portuguese coast (at three locations) for *P. vulgata*, *P. depressa* and *P. ulyssiponensis* and Brazão *et al.* (2003), for *P. depressa* at two adjacent locations in central Portugal. Moreover, no published studies are available for *Patella rustica* Linnaeus, 1758. This warm-water species was until recently absent from northern Portugal, and is currently colonizing this region (Lima *et al.* 2006). Establishing the spawning period of *P. rustica* in Portugal would contribute to a better understanding of the processes driving this expansion.

The aims of this study were to gather updated information on the reproductive cycles of the most common limpet species on the northern Portuguese coast, and also to investigate for the first time the reproductive cycle of *P. rustica* in Portugal. Comparisons are made with studies in Portugal from the 1980s, as well as further north in Europe to understand the influence of climate change with time and differences with latitude. Also, knowledge on timing and extent of spawning periods will be necessary for setting up the dispersal model (chapter 7).

2.2 Material and Methods

The reproductive cycles of *P. depressa*, *P. ulyssiponensis* and *P. vulgata*, were investigated at the northernmost part of the Portuguese coast, within an area encompassing about 100 km of coastline, where five rocky shores were selected for sampling: Moledo do Minho (41°50'N, 8°53'W), Viana do Castelo (41°42'N, 8°52'W), Cabo do Mundo (41°13'N, 8°44'W), Homem do Leme (41°09'N, 8°42'W) and Aguda (41°02'N, 8°40'W). Monthly samples were taken between December 1997 and January 2001 for *P. depressa* and *P. ulyssiponensis* and between January 1999 and March 2001 in the case of *P. vulgata*. Samples of *P. depressa* were obtained from all five sites, whereas *P. ulyssiponensis* was collected from Moledo do Minho, Cabo do Mundo and Aguda and *P. vulgata* from Moledo do Minho, Viana do Castelo and Homem do Leme. Samples of *P. rustica* were obtained between October 2005 and October 2007 from Cabo Raso, located at the central Portuguese coast (38°42'N, 9°29'W), where their abundance was sufficient to enable regular collections.

Samples of 30-50 individuals of each species were collected haphazardly in each shore, within size ranges of 15-35 mm for *P. depressa*, 20-45 mm for *P. ulyssiponensis* and *P. vulgata*, and 20-35 mm for *P. rustica*. All samples were stored in a 5% formalin-sea water solution until gonad examination in the laboratory. The sex of all specimens was determined and the maturity of gonads was macroscopically assessed and

categorized according to the scale established by Orton *et al.* (1956) for *P. vulgata*. The reproductive cycle of each population was described by the monthly variation in the percentage of neuter or almost empty gonads (stages 0 and 1) and in the percentage of full gonads (stages 4 and 5). Spawning events were identified by a drop in the proportion of full gonads and a concomitant increase in the percentage of empty gonads.

Reproductive synchrony among sexes was investigated using standard correlation analyses (Pearson's correlation coefficient) on the proportion of gonads at an early development condition (stage 1) and the proportion of mature gonads (stages 4 and 5) for each location and species. Both variables were highly correlated among males and females (Pearson's r: $P < 0.001$ at every location and for all species), and therefore, subsequent analyses were performed on pooled data. Correlation analysis was also used to test for synchronisation among locations within each species, by calculating correlation matrices and testing the significance of correlation coefficients among each pair of locations.

2.3 Results

The average males:females ratio within sites over the study period showed a predominance of females in samples of *P. ulyssiponensis* (range 0.6:1 to 1.1:1), *P. vulgata* (0.6:1 to 1.0:1) and *P. rustica* (0.8:1), whilst in *P. depressa* males were collected consistently in higher numbers than females (1.3:1 to 1.5:1).

The reproductive cycles of *P. depressa* and *P. ulyssiponensis* displayed similar annual patterns (Figures 2.1 and 2.2), with gonad development being observed throughout the year and considerable proportions of undeveloped gonads occurring mainly between May and July. It is clear that both species went through multiple spawning events within each study year. Main spawning periods typically took place between September and January, followed by gonad re-ripening with another major spawning event between March and June. There was, however, considerable variation around the basic reproductive pattern among years and locations, particularly in *P. depressa*, as confirmed by the lack of a significant correlation in levels of gonad development among most pairs of locations (Table 2.1). An example of the variability displayed by *P. depressa* could be seen at Aguda in 1999 (Figure 2.1E), with 50-70% of the individuals remaining inactive from January to May, while in other locations this percentage was much lower. In *P. ulyssiponensis*, there was a significant correlation in the proportion of undeveloped gonads among all locations, but a weaker correlation was found when considering the proportion of ripe gonads (Table 2.2). In both species, results suggested that individuals within each population were poorly synchronised, since all stages of development were present in the majority of monthly samples. Higher

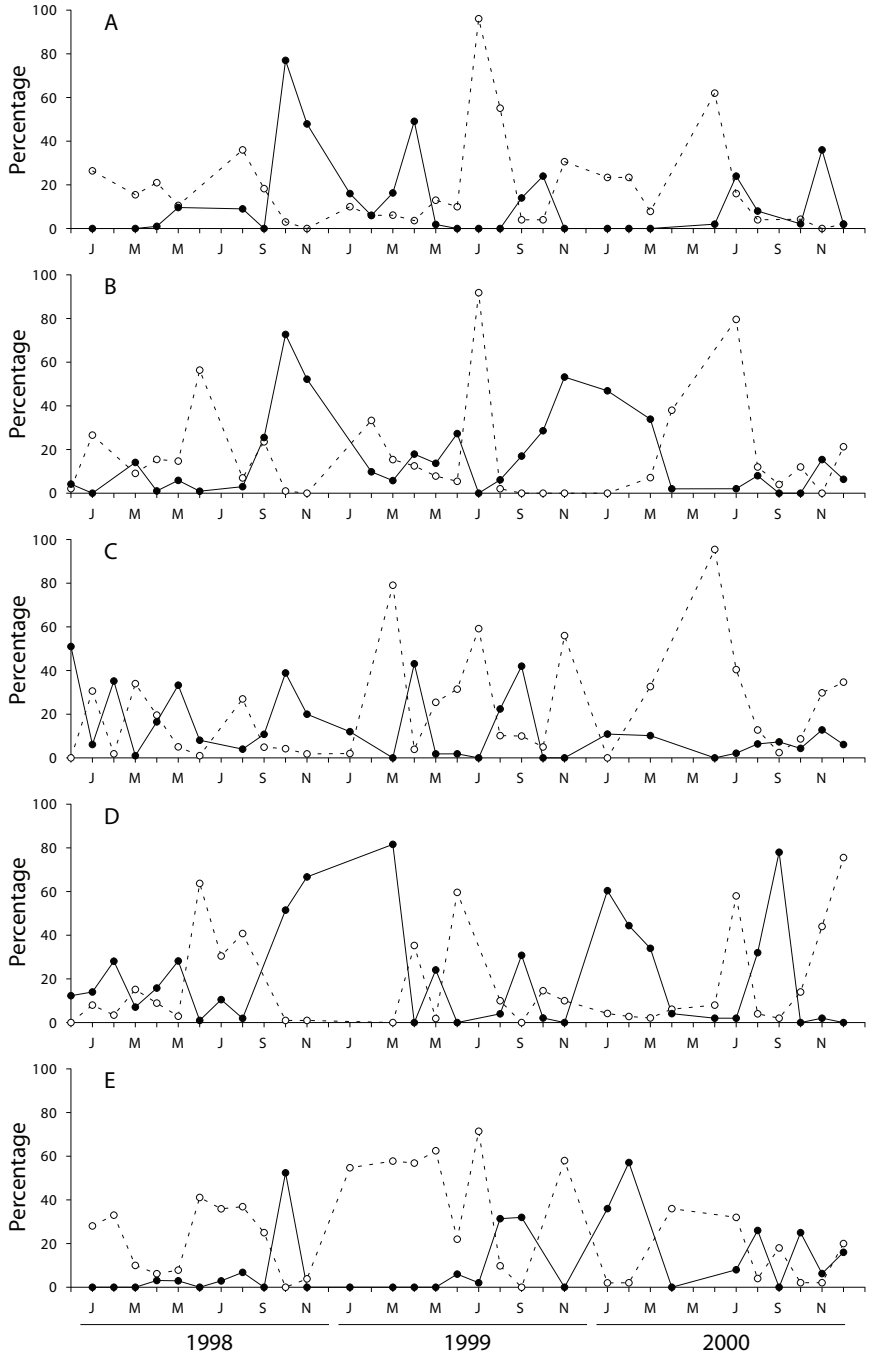

Figure 2.1 *Patella depressa*: percentage of gonads in undeveloped (neuter or stage 1, dashed line) and advanced (gonad stages 4 and 5, full line) maturation states, between December 1997 and December 2000. Location names are (A) Moledo do Minho, (B) Viana do Castelo, (C) Cabo do Mundo, (D) Homem do Leme, (E) Aguda.

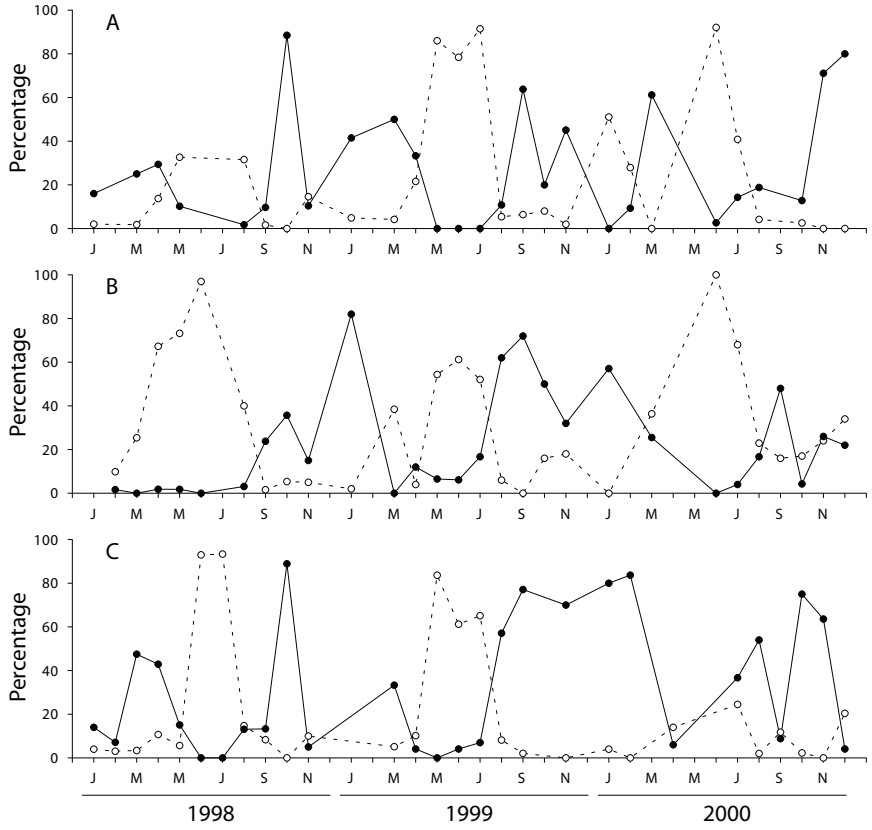

Figure 2.2 *Patella ulyssiponensis*: percentage of gonads in undeveloped (neuter or stage 1, dashed line) and advanced (gonad stages 4 and 5, full line) maturation states, between January 1998 and December 2000. Location names are (A) Moledo do Minho, (B) Cabo do mundo, (C) Aguda.

levels of within-population synchronisation were observed during the partial early summer pause and the subsequent development period.

In contrast, *P. vulgata* (Figure 2.3) and *P. rustica* (Figure 2.4) displayed highly synchronised seasonal breeding cycles, with smaller variation in gonad development within most samples, as well as minimal year to year differences. In *P. vulgata*, a significant correlation in the proportions of undifferentiated and full gonads was found among sampling sites throughout the study period (Table 2.3). Gonad development took place between September and November, with spawning occurring almost immediately. Gonad condition declined very abruptly between November and January (with a corresponding increase in the proportion of neuter gonads), and by the end of March all individuals were in the resting condition. Gamete release took place during just one annual spawning event, except at Homem do Leme in September 1998, where there was evidence of a minor initial spawning before the main event in November. Gonad development in *P. rustica* also exhibited marked synchronism, with 100% of the sampled individuals remaining reproductively inactive between December/January and June. Gametogenesis started in June, and sexual maturity steadily increased until

Table 2.1 *Patella depressa*: Pearson's correlation coefficient (*r*) among locations, using the proportion of undeveloped (neuters and stage 1, below diagonal) and full gonads (stages 4 and 5, above diagonal). Significant values are in bold.

	Moledo	V. Castelo	C. Mundo	H. Leme	Aguda
Moledo do Minho	-	**0.491**	**0.583**	0.283	0.106
Viana do Castelo	0.002	-	0.243	0.417	0.371
Cabo do Mundo	0.068	0.279	-	0.139	0.305
Homem do Leme	0.078	0.420	0.208	-	0.232
Aguda	0.175	0.206	**0.569**	0.155	-

Table 2.2 *Patella ulyssiponensis*: Pearson's correlation coefficient (*r*) among locations, using the proportion of undeveloped (neuters and stage 1, below diagonal) and full gonads (stages 4 and 5, above diagonal). Significant values are in bold.

	Moledo	C. Mundo	Aguda
Moledo do Minho	-	0.281	0.291
Cabo do Mundo	**0.502**	-	**0.46**
Aguda	**0.872**	**0.525**	-

Table 2.3 *Patella vulgata*: Pearson's correlation coefficient (*r*) among locations, using the proportion of undeveloped (neuters and stage 1, below diagonal) and full gonads (stages 4 and 5, above diagonal). Significant values are in bold.

	Moledo	V. Castelo	H. Leme
Moledo do Minho	-	**0.576**	**0.542**
Viana do Castelo	**0.904**	-	**0.737**
Homem do Leme	**0.891**	**0.943**	-

September/October, when spawning occurred. There was no sign of re-ripening during the study period, and all gonads were spent by December/January.

2.4 Discussion

The reproductive patterns obtained in the present study generally fit the latitudinal trend previously described for *Patella* and other intertidal species, consisting in progressively longer reproductive seasons and spawning occurring later in the year towards the south (Bowman and Lewis 1986; Lewis 1986). This trend is particularly noticeable in *P. depressa* and *P. ulyssiponensis*, two species that show markedly

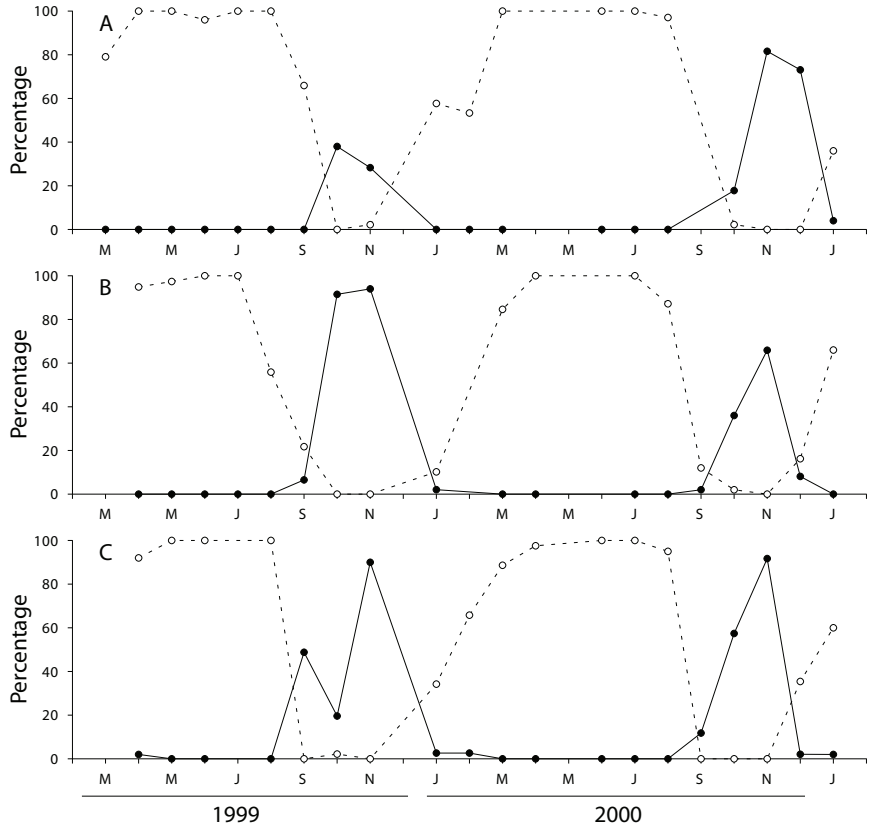

Figure 2.3 *Patella vulgata*: percentage of gonads in undeveloped (neuter or stage 1, dashed line) and advanced (gonad stages 4 and 5, full line) maturation states, between January 1999 and December 2000. Location names are (A) Moledo do Minho, (B) Viana do Castelo, (C) Homem do Leme.

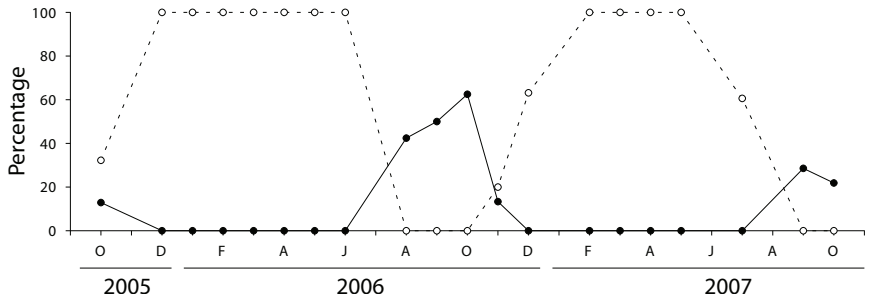

Figure 2.4 *Patella rustica*: percentage of gonads in undeveloped (neuter or stage 1, dashed line) and advanced (gonad stages 4 and 5, full line) maturation states, between October 2005 and October 2007, at Cabo Raso.

seasonal breeding cycles at the northern part of the range, with summer spawning periods (e.g. Dodd 1956; Orton and Southward 1961; Thompson 1979; Bowman 1985; Bowman and Lewis 1986; Delany *et al.* 2002; Moore *et al.* 2007; McCarthy *et al.* 2008), but on the other hand are capable of nearly continuous breeding in northern Portugal,

with loose synchrony in gonad development and spawning. Reproductive cycles of *P. vulgata* are much more synchronised throughout its range, and exhibit a marked seasonal trend, with well-defined breeding and resting periods. The most obvious change with latitude concerns the timing of spawning events, which tend to occur earlier in the northern part of the range. The main spawning period in *P. vulgata* starts in September in northern Scotland (Baxter 1982, 1983; Bowman and Lewis 1986) and also in northeastern England (Bowman and Lewis 1977; Bowman 1985; Bowman and Lewis 1986), October/November in southwestern Ireland (Thompson 1980; McCarthy *et al.* 2008), November in southwestern England (Evans 1953; Orton *et al.* 1956; Bowman and Lewis 1986) and northern France (Fischer-Piètte 1948; Choquet 1966), and November/December in northern Spain (Ibañez *et al.* 1986; Othaitz 1994) and northern Portugal (Guerra and Gaudêncio 1986; this study).

The present work provided the first description of the reproductive cycle of *P. rustica* on the Portuguese coast. However, since it lacked spatial replication and encompassed roughly two reproductive seasons, results must be interpreted with caution. During the study period, this species was reproductively active between July and December, and only one spawning event seemed to occur in each season. These results do not differ much from observations from the Basque coast of Spain, where this species displays a very similar breeding cycle, although with evidence of multiple spawning events (Othaitz 1994). In the Mediterranean, *P. rustica* has a longer breeding period, with less synchronous multiple spawnings occurring between August and November (Frenkiel 1975; Othaitz 1994).

The reproductive cycles of *P. depressa*, *P. ulyssiponensis* and *P. vulgata* showed general similarity with those previously reported by Guerra and Gaudêncio (1986) for the northern Portuguese coast, although there were important differences. Guerra and Gaudêncio (1986) suggested that *P. depressa* and *P. ulyssiponensis* displayed a bimodal breeding pattern, due to the occurrence of reproductive pauses in summer and winter. The present results did not support their assumption, since an obvious winter pause was never observed. On the other hand, in the case of *P. vulgata*, although the proportion of individuals with gonads in advanced stages of development remained similar, the breeding cycles in 1999-2000 were consistently shorter than the ones reported at Moledo do Minho in the early 1980s by Guerra and Gaudêncio (1986). These authors observed development starting in July/August, with minor spawning in September/October, the main spawning period taking place between November and January, and all gonads gradually emptying until May. In the present study, the onset of gonad activity was never observed before late August/September, whilst spawning took place between November and January, with all individuals entering the neuter state before the end of March. Another important difference resides in an apparently reduced ability of undergoing multiple spawning events during the breeding season of *P. vulgata*. Multiple

spawnings have been observed in *P. vulgata* in the UK (Blackmore 1969; Bowman and Lewis 1977, 1986) and also in northern Spain (Othaitz 1994). In northern Portugal, minor episodes of gamete release prior to the main spawning event were also recorded by Guerra and Gaudêncio (1986). However, the present study suggests that they no longer occur with the same frequency.

Sea temperature has long been considered one of the most influential factors in controlling breeding in marine invertebrates (Orton 1920; Olive 1995). The observed changes in the breeding cycles of *Patella* spp. from the early 1980s to the present might be related to the increase in sea water temperature estimated to occur along the Portuguese coast at a constant rate of 0.010 °C $year^{-1}$ (Lemos and Pires 2004). Moreover, average sea surface temperature during winter months (December to March) in northern Portugal increased from 13.7 °C for the period of 1981-1983 to 14.5 °C for 1998-2000 (values derived from in situ raw data obtained from the International Comprehensive Ocean-Atmosphere Data Set, Woodruff *et al.* 1998). A possible consequence of this increase could be the disappearance of a potential inhibitory effect of extreme low winter temperatures on gonad development, thus allowing for continuous breeding from autumn to spring in *P. depressa* and *P. ulyssiponensis*. In the case of *P. vulgata*, which is close to its southern range limit (Fischer-Piètte and Gaillard 1959; Southward *et al.* 1995), increased winter temperatures are likely to have the opposite effect on gametogenesis. There is strong evidence of changes in the reproductive cycles of *P. depressa* and *P. vulgata* as far north as Great Britain, possibly driven by climate warming. In recent years, *P. depressa* has been spawning earlier and for longer periods in southwest Britain, and there is also evidence of gonad re-ripening (Moore *et al.* 2007). This behaviour contrasts with the severe drop in spawning success of *P. vulgata* recently recorded at certain locations in that region, where only a small proportion of the populations reached advanced maturity stages at some locations near Plymouth (Moore *et al.* 2007). In the light of the present observations, it is likely that *P. vulgata* faces decreasing reproductive success also in Portugal. Further contraction of its annual spawning period could lead to growing decline in population size and connectivity at the southern end of the range. Similarly, *P. rustica* which is near its northern range limit (Fischer-Piètte and Gaillard 1959; Lima *et al.* 2007b) only spawns once, in contrast to the warmer Mediterranean and the Basque coast. However, the rising trend in sea water temperature along the Portuguese coast will favour the increase of the species reproductive potential, thus contributing to its expansion.

The sampling scheme adopted in this work does not allow investigating a possible role of environmental cues in the observed spawning patterns. However, the variability observed in the frequency and timing of spawning events in *P. depressa* and *P. ulyssiponensis*, both among years and locations, suggests a possible role of stochastic events in stimulating gamete release as soon as a certain level of gonad maturity is

reached at a local scale. The same type of cues may also be involved in the induction of spawning in *P. vulgata* and *P. rustica*. However the short time window suitable for breeding in these two species probably does not allow for gonad re-development in most seasons. A number of studies carried out in the UK (Orton *et al.* 1956; Orton and Southward 1961; Thompson 1979) suggest that spawning in *Patella* spp. is frequently associated with strong onshore winds and high wave action. Orton and Southward (1961) argued that air temperature was also a possible stimulus for spawning in *P. depressa*. Several studies conducted for *P. vulgata* suggested that spawning was not only associated with periods of rough seas, but also with decreases in sea temperature below 12 °C (Bowman and Lewis 1977, 1986; Bowman 1985; Delany *et al.* 2002). However, the large gaps between sampling dates did not allow the establishment of an unequivocal correspondence between periods of rough sea and spawning events. More recently, Le Quesne (2005) used high-frequency observations of gonad condition, wave action and water temperature to confirm this relationship by pinpointing main spawning events in *P. vulgata* to periods of high wave action, associated with a drop in sea temperature. Although lacking empirical evidence, it also appears to be the case in northern Portugal. However, the 12 °C threshold is probably not applicable throughout the geographical range of *P. vulgata* (Bowman 1985), and higher sea temperatures might be involved in stimulating spawning in northern Portuguese populations of *P. vulgata*.

Chapter 3

The effect of temperature on larval development of the limpets *Patella depressa*, *Patella ulyssiponensis* and *Patella vulgata*

3.1 Introduction

The majority of marine benthic invertebrate species exhibit a complex life cycle which includes a planktonic larval stage, followed by benthic juvenile and adult phases (Thorson 1950). The duration of the planktonic phase can be highly variable between species, ranging from minutes to months, or even years (Scheltema 1971, 1989; Kinlan and Gaines 2003; Shanks *et al.* 2003; Strathmann and Strathmann 2007). A number of factors, both physical (for example, oceanographic processes or coastline geomorphology) and biological (such as larval behaviour), may favour local retention or limit transport of larvae, thus influencing realised dispersal (Shanks 1995; Cowen *et al.* 2000; Gaylord and Gaines 2000; Kingsford *et al.* 2002; Sponaugle *et al.* 2002). It is the length of time spent by larvae in the water column that will ultimately determine how far they can potentially travel away from the source point. Recent studies have reported a positive relationship between planktonic larval duration and dispersal distance (Shanks *et al.* 2003; Siegel *et al.* 2003). Therefore, planktonic dispersal has a great influence on the distribution, population dynamics, connectivity and hence genetic structure of benthic species (Caley *et al.* 1996; Bohonak 1999; Grosberg and Cunningham 2001). Consequently, dispersal also has profound implications for conservation and the design of marine protected areas (Roberts 1997; Botsford *et al.* 2001; Lubchenco *et al.* 2003).

Temperature is considered to be one of the most influential factors affecting larval development (Hoegh-Guldberg and Pearse 1995). It is known to influence larval development rates of a large number of marine invertebrate species, such as echinoderms (Watts *et al.* 1982; McEdward 1985), barnacles (Burrows *et al.* 1999; Thiyagarajan *et al.* 2003; Desai and Anil 2004), and molluscs (Scheltema 1967; Pechenik 1984; Lima and Pechenik 1985). Recently, O' Connor *et al.* (2007) demonstrated that

there is a strong effect of temperature on planktonic larval duration. This relationship is quantitatively constant across nearly all species tested, encompassing six phyla and a range of body sizes and habitats.

For many marine taxa, larval development usually occurs faster in warmer waters (Hoegh-Guldberg and Pearse 1995). Given that development time is a predictor of dispersal potential, which in turn is positively correlated with population connectivity (Bohonak 1999), genetic structure is likely to be higher in warmer waters. A recent study comparing mitochondrial DNA sequence data for 28 chiton species by Kelly and Eernisse (2007) revealed a positive correlation between latitude and connectivity among species; thus, species occurring at lower latitudes tended to have more isolated populations. These authors argued that faster larval development would result in decreased larval duration, lower dispersal potential, and decreased connectivity among populations. This observation was not, however, extended to within species population comparisons, and does not provide support for intraspecific connectivity gradients driven by water temperature and changes in planktonic larval duration (Kelly and Eernisse 2007). Latitudinal variation in genetic divergence within species has, on the other hand, been found in 45 of 62 vertebrate taxa, including marine fish, turtle and mammal species (Martin and McKay 2004).

Limpets of the genus *Patella* are ubiquitous on the North Atlantic and Mediterranean rocky shores, where they are important grazers, structuring midshore communities (Southward 1964; Hawkins 1981; Hawkins and Hartnoll 1983; Boaventura *et al.* 2002; Jenkins *et al.* 2005; Coleman *et al.* 2006; Jonsson *et al.* 2006). Limpets are broadcast spawners and their life-cycle includes a planktonic larval stage. Estimates of planktonic larval duration in *Patella* are scarce and have only been accomplished at fixed water temperatures for *Patella caerulea* (Patten 1886; Dodd 1957; Wanninger *et al.* 1999) and *Patella vulgata* (Smith 1935; Dodd 1957). Therefore, the relationship between pelagic development and temperature within and among species remains unknown.

The main purpose of my work was to quantify the influence of water temperature on larval survival, settlement and development times of three of the most common NE Atlantic limpet species, which display broad latitudinal distribution ranges. The northern limpet *P. vulgata* Linnaeus, 1758 is present in the Atlantic European continental coast, from Southern Portugal to Northern Norway, and absent from the Mediterranean Sea (Christiaens 1973). *Patella depressa* Pennant, 1777 is a southern species restricted to the continental Atlantic coasts of Europe and North Africa, ranging from Senegal to North Wales (Fischer-Piètte and Gaillard 1959; Christiaens 1973; Southward *et al.* 1995). *Patella ulyssiponensis* Gmelin, 1791, also a southern species, has a more widespread distribution. It is present throughout the Mediterranean (and possibly the Black Sea, although this record is suspect, S.J. Hawkins, pers. comm.), the Macaronesian Islands, the North African coast, and in Europe, it occurs as far north as

southern Norway and also the North Sea coast of the British Isles (Christiaens 1973). Genetic differentiation between continental and Macaronesian forms of *P. ulyssiponensis* was found in mtDNA (Koufopanou *et al.* 1999; Sá-Pinto *et al.* 2005) and allozyme markers (Weber and Hawkins 2005), suggesting that they might be different species, with *Patella aspera* Röding, 1798 being the appropriate name for the Macaronesian form.

A preliminary choice experiment was conducted by exposing competent larvae to two types of substratum and quantifying their settlement response to each of them. Additionally, the occurrence and extent of delayed metamorphosis (i.e. the ability of larvae to postpone metamorphosis after becoming physiologically competent to do so) was investigated, because of its potential consequences for realized dispersal (Pechenik 1990).

Finally, to investigate if the planktonic period varies with latitude, due to differences in sea surface temperature (SST), estimates of planktonic larval duration were used to predict the effects of sea temperature on planktonic periods under realized SST scenarios during the species reproductive seasons obtained from the literature combined with oceanographic data on SST.

3.2 Material and Methods

3.2.1 Collection of specimens

All specimens used in this study were collected from Wembury (Devon, UK). Monthly gonad condition (following Orton *et al.* 1956) was assessed between June 2004 and August 2006 on samples of 30 individuals of each species (*P. depressa*, *P. ulyssiponensis* and *P. vulgata*) in order to detect peaks of gonad ripeness. Rearing experiments were carried out on different periods for each species, when gonads were ripening: August 2004, May 2005 and July-August 2006 for *P. depressa*; August-September 2004 and July-August 2006 for *P. ulyssiponensis*; November-December 2004 and November-December 2005 for *P. vulgata*.

Given that protandrous sex change occurs in limpets (Orton 1928; Dodd 1956; Orton *et al.* 1956; Branch 1981; Le Quesne and Hawkins 2006), large individuals were predominantly selected, thus increasing the probability of collecting females. For each round of trials, at least 20 (in the case of the larger species), or 30 (in the case of the smaller species *P. depressa*) individuals were collected and transported in capped containers partly filled with sea water to the laboratory at the Marine Biological Association, in Plymouth (UK). Limpets were kept in shallow tanks with flowing sea water at ambient temperature, until they were dissected for collection of gonad products

(usually for no more than a few hours to overnight).

3.2.2 Collection of gametes and fertilization procedure

The gamete collection and fertilization procedures adopted in this study were largely based on methods refined at the Marine Biological Association of the UK by Hodgson and Aquino-Souza (pers. comm.), based on Dodd (1957), described by Hodgson *et al.* (2007). Animals were dissected and gonads exposed for visual examination and assessment of the maturity stage (Orton *et al.* 1956). To ensure a high fertilization rate, and also to minimize the proportion of abnormal larvae, very unripe female gonads were discarded (Dodd 1957), and only those at stages 3, 4 or 5 (Orton *et al.* 1956) were used. Sea water used in all trials was UV-sterilized, filtered through a 0.2 μm mesh and stored in containers for later use.

For each fertilization trial, 3 to 5 male gonads (depending on their average size) were carefully dissected, placed in a Petri dish with a few drops of sea water, and cut into pieces with a scalpel. These were then transferred to a 50 ml Falcon tube, filled with filtered sea water at ambient temperature. The tube was then capped and gently inverted a few times, to release the sperm. This suspension was allowed to rest for 30 minutes, enabling the settlement of large pieces of gonad material and also the activation of sperm (Hodgson *et al.* 2007).

All available female gonads were carefully dissected and placed in a 200 ml crystallizing dish containing filtered sea water at ambient temperature. Gonads were partly pulled apart, while held with tweezers, and were agitated in the water to release the eggs. The water was then stirred and the eggs allowed to settle on the bottom. Since damaged eggs and debris take longer to settle than viable eggs, these were easily collected from the bottom with a Pasteur pipette and transferred to a 200 ml crystallizing dish with clean filtered sea water. This procedure was repeated twice in order to obtain clean batches of intact eggs.

Prior to fertilization, eggs were placed in 200 ml dishes containing alkaline sea water (pH 9.0 adjusted by adding NH_4OH) for 10 minutes. This technique has been previously used to increase fertilization rates in studies of embryonic and larval development in *P. vulgata* and *P. caerulea* (Wanninger *et al.* 1999; Gould *et al.* 2001), and its effect has been quantified for *P. depressa* and *P. ulyssiponensis* (Hodgson *et al.* 2007). Finally, the eggs were rinsed twice and placed in 500 ml beakers filled with clean filtered sea water for fertilization. A volume of 0.5 ml of the sperm suspension was pipetted to each beaker containing mature eggs. After one hour, cultures were passed through a 50 μm mesh where eggs were retained and evenly distributed into 500 ml beakers with fresh filtered sea water.

3.2.3 Maintenance of larval cultures

Cultures were left undisturbed at a constant temperature for at least 24 hours. Longer periods (48 hours) were allowed for cultures maintained between 5 °C and 12 °C, since at these lower temperatures swimming trochophores took longer to develop. Cultures were then slowly decanted through a partially submerged 50 μm mesh, where trochophores were retained. Only approximately 300 ml of sea water from each culture were decanted in order to minimize contamination by unfertilized eggs and debris settled on the bottom of the beaker. Given that the vast majority of morphologically normal trochophores aggregated near the water surface, larval loss was negligible. Larvae collected in the mesh were transferred to 500 ml beakers with clean filtered sea water kept at the initial temperature.

Cultures were changed to fresh filtered sea water every day until larvae reached competency. Feeding of veliger larvae was carried out once a day by adding 1ml of a pure culture of the diatom *Phaeodactylum tricornutum*, obtained from the Plymouth Culture Collection of Marine Algae, of the Marine Biological Association. This was done just 6 hours prior to a change of sea water, to prevent excessive deterioration of water quality, which might prove harmful to the larvae (Dodd 1957).

3.2.4 Effect of temperature on survival, percentage metamorphosis and development rate

The effect of water temperature on larval development and metamorphosis was tested at 5, 8, 12, 16, 20 and 24 °C (± 0.5 °C, for all the values). Cultures were reared in constant temperature rooms, except at 8 and 12 °C, for which a thermal bath and a temperature controlled cabinet (respectively) were required. The filtered sea water used in the trials was previously stored at the experimental temperature, so as to avoid temperature oscillations during water changes. The number of trials attempted for each combination of species and water temperature varied between 8 and 14. The goal was to obtain 8 replicate cultures reared to metamorphosis. If after 10 replicates no cultures had developed at a given temperature, the experiment at that temperature was terminated.

The average stage of development in each replicate culture was determined at twelve-hour intervals, by visual examination of 50 larvae under a stereo-microscope. Median development time of the major stages prior to metamorphosis (trochophore, pre-torsional, post-torsional and competent veliger) was estimated as the time required by at least 50% of the observed larvae to reach a given stage. Estimates of survival through development were based on average counts of living larvae in four 1-ml samples taken from each replicate culture with a pipette, and multiplied by the culture volume. Before collecting the samples, cultures were gently stirred to homogenize the

distribution of larvae in the water. Larval survival rate for each developmental stage was estimated by dividing the mean number of larvae that had reached that stage by the mean number of eggs ascertained at the start of the trial.

3.2.5 Larval settlement and metamorphosis

A choice experiment was conducted to induce settlement and metamorphosis in response to two types of substratum collected from the adult habitat. Small stones covered with red encrusting algae and bare stones were collected from the intertidal zone at Wembury and transported to the laboratory in damp plastic bags. Mobile animals and epibionts were removed and fragments of approximately 3 × 3 × 0.5 cm were cut with a mechanical saw. Rock fragments were then thoroughly scrubbed with a brush under flowing sea water and one piece of each type of rock was placed in 200 ml crystallizing dishes filled with filtered sea water. As soon as veligers started displaying behavioural competence (by remaining most of the time on the bottom and adhering to glass surfaces), 50 of them were transferred to each dish containing the choice substrata, in a total of eight replicates per temperature. From this moment on, water was not changed and larvae were fed just once with 0.2 ml of *P. tricornutum* culture.

Larval settlement was quantified by inspecting the crystallizing dishes under a stereo microscope, every 12 hours. Age at metamorphosis was considered to be the time at which settled veligers initiating metamorphosis (as indicated by velar loss) were first observed. Percentage metamorphosis was calculated for each replicate trial, as the number of larvae found attached to the substrata 24 hours after the first observation, divided by the initial number of larvae transferred to the dish.

3.2.6 Delay of metamorphosis

The capability of larvae to extend planktonic life was estimated by keeping 50 competent larvae in a 200 ml crystallizing dish in the absence of settlement substrata. Four replicates of this treatment were set up for each combination of species and temperature. Change of sea water and feeding with 0.2 ml of *P. tricornutum* took place every two days. Cultures were monitored twice a day for settled or dead larvae. Delay capability was ascertained by recording the time when at least 50% of the larvae were either dead or had metamorphosed onto the glass.

3.2.7 Estimating latitudinal variation of planktonic larval duration

To investigate a possible link between planktonic larval duration and latitude in the three limpet species, 24 studies reporting data on the reproductive cycles of *Patella* spp. in the N.E. Atlantic were reviewed. The studies encompassed information collected between 1946 and 2005, over a geographical area ranging in latitude between 37-58° N, which included Great Britain, Ireland, France, Spain and Portugal. Gonad development of *P. vulgata* was surveyed in 19 of the 24 studies, whilst for *P. depressa* and *P. ulyssiponensis* it was monitored in 11 and 9 studies, respectively.

In the great majority of the studies, sexual condition was ascertained by visual examination of the gonads, mostly using the staging scheme described by Orton *et al.* (1956), based not only on gonad size and external morphology, but also on the percentage of mature or empty gonads. Only two studies relied on the Gonad Somatic Index (based on the weight of the gonad relative to the remaining visceral mass) to assess gonad stages of development. Two studies also used oocyte diameter frequencies as a measure of female sexual maturity. Finally, one study monitored gonad development using a staging system based on histological techniques. Information on the spawning periods was gathered from all studies by species, sampling site and year.

Geographical coordinates of sampling sites were determined. This procedure was not always feasible (e.g. Evans 1953; Choquet 1966; Bowman 1985; Bowman and Lewis 1986), because the authors summarized results obtained at several sampling sites within regions. In these cases, the coordinates allocated to the data corresponded to the middle point of the respective sampling area or region. Sea surface temperature (SST) along the study area at the dates of interest was derived from in situ raw data obtained from the International Comprehensive Ocean-Atmosphere Data Set (ICOADS, Woodruff *et al.* 1998). To avoid bias due to different daytime measurements, only data from 12:00 were used. Point data were imported into GRASS 6.0 Geographical Information System (GRASS Development Team 2006) and transformed into raster maps with a spatial resolution of 4 km using a surface interpolation method. Each cell was derived from the 12 nearest data points using the distance squared weighting algorithm (Jarvis and Stuart 2001). The resulting set of maps was stored in GRASS GIS as monthly layers and sampled within a buffer circle of 0.5° radius around each study location. For every month comprised in a reported spawning season at that location, mean SST within each buffer area was calculated, considering that it would represent a good estimate of the average temperature regime endured by dispersing larvae during their development.

Extreme monthly SST values recorded during annual spawning periods were used to calculate putative minimum and maximum planktonic larval duration (PLD) estimates, by applying the regression equations obtained in the laboratory rearing

experiments. Surveys with incomplete years were treated with caution, since data might not document the whole spawning period and, therefore, the full range of sea temperatures experienced by planktonic larvae in those years. Depending on the proportion and distribution of missing data, such years were discarded from the analysis or only one of the larval duration estimates (minimum or maximum) was used.

3.2.8 Statistical analyses

One-way analyses of variance (Underwood 1997) were used to test for differences among temperatures in the percentage survival of competent larvae. Two-way analyses of variance were used to test for differences in the proportion of larvae initiating metamorphosis among choice substrata and water temperatures. Variance heterogeneity was tested with Cochran's test (Underwood 1997). To achieve homoscedasticity, and since observations were expressed as percentages or proportions, each value was transformed to the arcsine of its square root (Winer *et al.* 1991; Underwood 1997). Since all factors analysed were fixed, differences between factor treatments were compared with Student-Newman-Keuls (SNK) tests (Underwood 1997). The effect of water temperature on larval development rates was investigated by searching for the regression model that best fitted the relationship between age at metamorphosis and rearing temperature. This relationship was found to be better explained by an exponential model (see Results). Therefore, the obtained exponential relationships were linearised by ln-transformation of age at metamorphosis data; a single factor analysis of covariance was then performed to test for differences in those relationships among species (Underwood 1997), the covariate in the analysis being temperature. Homogeneity of deviations from individual regressions was tested with Bartlett's test (Zar 1984), because it is capable of handling unbalanced datasets (Underwood 1997). Finally, simple linear regressions were used to investigate the relationship between realized planktonic larval dispersal and latitude. Significance of all slopes was tested with ANOVA.

3.3 Results

3.3.1 Larval development

The sequence of developmental events observed was very similar in the three species, also not differing from what has been previously described by other authors for *Patella* (Patten 1886; Smith 1935; Dodd 1957; Wanninger *et al.* 1999) and other patellogastropod limpet species (Kessel 1964; Kay and Emlet 2002). Well-formed

trochophores aggregated near the water surface, actively swimming in the horizontal or upward direction and performing large spiral trajectories. Vertical downward movement was usually short and by passive sinking when larvae briefly stopped beating the prototrochal cilia. Abnormal (mostly asymmetrical) trochophores tended to remain near the bottom, as they seemed unable to sustain active swimming for extended periods of time and thus could not keep their position high in the water column. During their brief swimming spells, abnormal larvae showed a distinctive movement pattern, performing fast and tight loops.

Trochophore larvae developed into early veligers with the formation of the foot rudiment and the velum, and also the secretion of the larval shell, or protoconch. At this stage, larvae were unable to withdraw into their shells. Torsion (a 180° rotation of the visceral body portion relative to the cephalo-pedal region) was not followed in detail. Therefore, it was not possible to confirm its monophasic nature, as has been recently reported for *P. caerulea* (Wanninger *et al.* 2000). After completion of torsion, larvae were able to retract into their shells, sealing the opening with the operculum, which was fully formed at this point. Larvae exhibited this behaviour, sinking passively, especially when disturbed (for example, if touched with a steel micro-probe or after the water had been agitated with a pipette in close proximity).

Towards the end of development, just before reaching metamorphic competence, larvae seemed no longer capable of sustaining active swimming for extended periods of time. Instead, they spent increasingly more time resting at the bottom of the culture vessels, with the cephalic region facing upward. Such periods of inactivity were interspersed with bursts of fast upward/horizontal swimming and passive sinking. Larvae were classified as competent for settlement when they were found attached to the culture glassware, often using the foot to briefly crawl across the surface.

3.3.2 Influence of temperature on larval development

Water temperature ranges suitable for larval development varied with species (Figure 3.1). Larvae of *P. depressa* developed through metamorphosis at temperatures ranging between 8 and 20 °C, whilst at 5 °C eggs did not hatch and at 24 °C all larvae died at early developmental stages. The percentage of successful rearing trials was higher at 16 and 20 °C (100% and 80% of the trials reaching metamorphosis, respectively) than at lower temperatures (57% at 8 and 12 °C). Larvae of *P. ulyssiponensis* developed between 8 °C and 24 °C, and zygotes did not divide at 5 °C. A high rate of rearing success was achieved (66-100%), and, as with the previous species, success was higher at warmer temperatures. In contrast, for *P. vulgata*, completion of larval development was only achieved at temperatures ranging between 5 °C and 16 °C. The percentage of successful

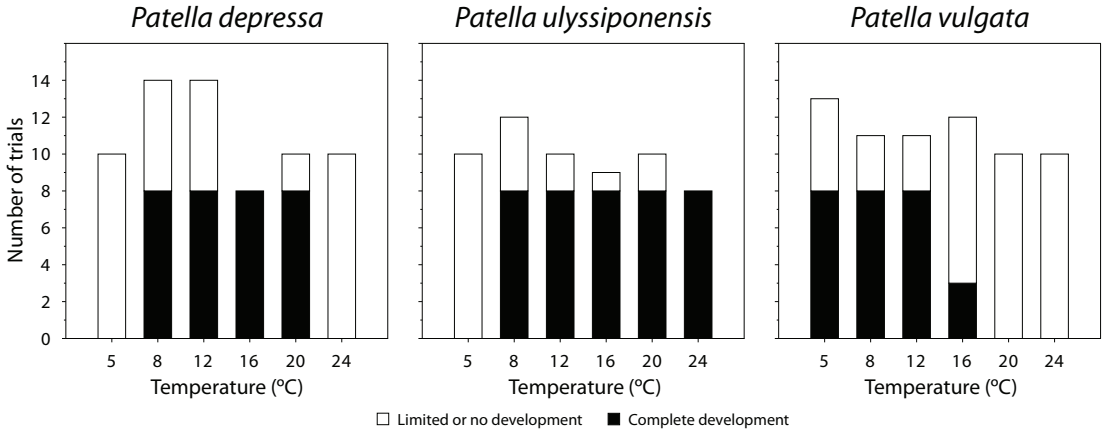

Figure 3.1 Number of successful and unsuccessful larval rearing trials, conducted for each combination of species and water temperature.

trials conducted at 16 °C was markedly lower than at colder temperatures; at that temperature, only 3 out of 12 independent rearing attempts reached metamorphosis.

3.3.3 Settlement and metamorphosis

Competent larvae usually settled within 24-48 hours after being put in contact with the settling substrata. In the beginning of the settlement process, larvae repeatedly swam upwards and sank onto the substrate. Many of them exhibited active exploratory behaviour, crawling across the substrate with the head oriented downwards. Start of metamorphosis, determined by shedding of the velum, usually took place shortly after settlement. Metamorphosed larvae exhibited great fidelity to the settlement site, since movements away from it were rare and short. Alternate clockwise and anti-clockwise rotation was the most frequent movement displayed by newly-metamorphosed larvae.

The metamorphic success of competent veligers is shown in Figure 3.2, as the proportion of larvae which settled onto each of the choice substrata, at all test temperatures. There was an obvious preference of larvae for 'lithothamnia'-covered stones in the three species. Settlement rates were very variable among replicates of treatment combinations. Average percentages of larvae that metamorphosed onto crustose red algae ranged between 8% and 22% in *P. depressa*, 13% and 33% in *P. ulyssiponensis* and 4% and 18% in *P. vulgata*. On the other hand, metamorphosis onto bare rock was rare, not exceeding 2.5%.

Results of the ANOVAs carried out to test for differences in settlement rates among temperatures and types of substratum are given in Table 3.1. All data were homoscedastic after arcsine transformation (Cochran's test: $C = 0.273$, $P_{(v=3,\ k=12)} = 0.162$ for *P. depressa*; $C = 0.198$, $P_{(v=3,\ k=15)} = 0.374$ for *P. ulyssiponensis*; $C = 0.297$,

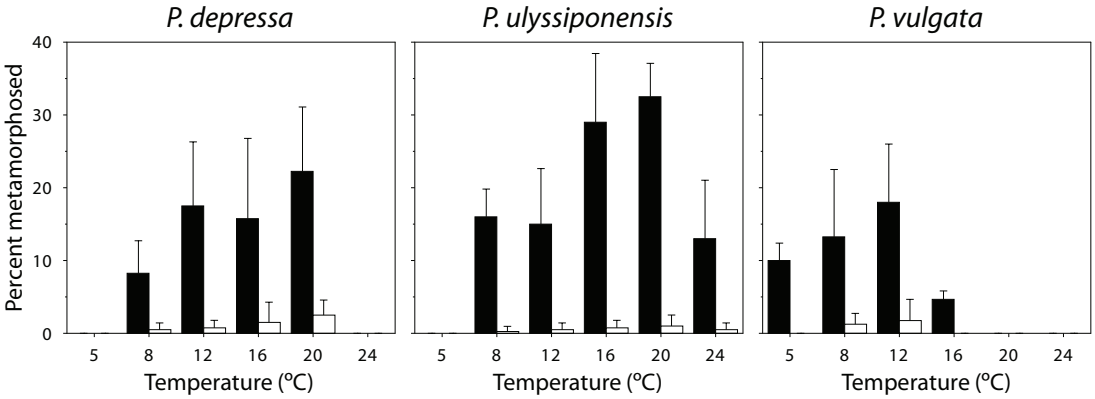

Figure 3.2 Proportion of larvae metamorphosed onto 'lithotamnia' (black bars) and bare rock (white bars), at different rearing temperatures. Data are means, error bars represent standard deviation, n=8 except for *P. vulgata* at 16 °C (n=3).

Table 3.1 ANOVA of settlement rate with temperature and choice substrata.

	Patella depressa			*Patella ulyssiponensis*			*Patella vulgata*		
Source	*df*	MS	*F*	*df*	MS	*F*	*df*	MS	*F*
Temperature (=T)	3	0.023	4.510 **	4	0.012	3.480 *	2	0.029	7.511 **
Substrate (=S)	2	0.909	177.149 ***	2	1.363	392.833 ***	2	0.568	147.584 ***
T x S	6	0.011	2.204 NS	8	0.006	1.721 NS	4	0.010	2.542 NS
Residual	36	0.005		45	0.003		27	0.004	
Total	47			59			35		

NS is not significant, * < 0.05; ** < 0.01; *** < 0.001.

$P_{(\nu=3,\ k=15)}$ = 0.313 for *P. vulgata*). For *P. vulgata*, data corresponding to 16 °C were removed from the analysis, due to insufficient replication. Results showed that there were significant differences among temperatures and settlement substrate, but no significant interaction, revealing that differences between treatments were consistent among factors. Multiple comparisons (Figure 3.3) showed a similar trend in the effect of temperature among species, with more larvae settling with increasing temperature. In *P. ulyssiponensis*, despite a steady increase of settlement rate with temperature, differences were only significant between 8 °C and 20 °C. Exceptionally, a decrease in settlement was observed from 20 to 24 °C. In *P. vulgata*, SNK tests detected a significantly lower settlement rate at 5 °C, but no difference between 8 °C and 12 °C. The outcome of the settlement choice assay was similar for all species. As already clearly indicated by results depicted in Figure 3.2, SNK tests revealed that crustose red algae prompted a significantly higher proportion of settled larvae, compared to bare rock and control treatments. In *P. vulgata*, bare rock was not significantly different from the control (without substrata), where no larvae had settled

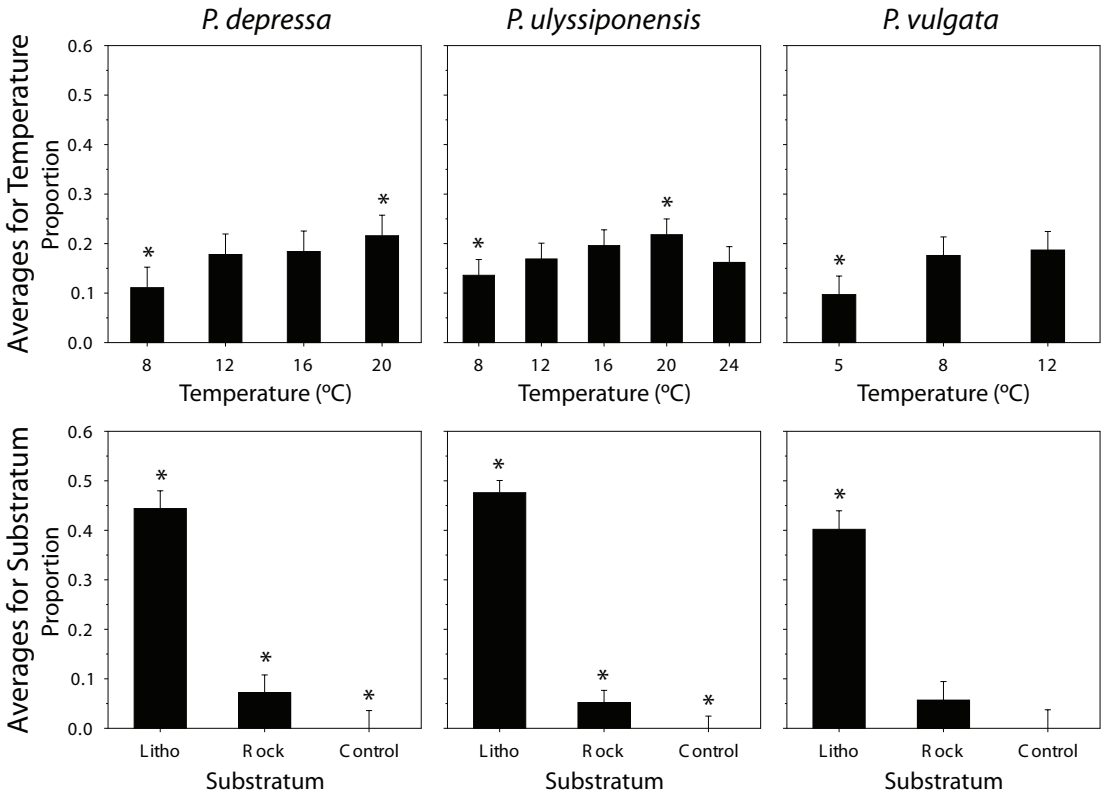

Figure 3.3 Multiple comparisons (SNK tests) after analysis of variance in Table 3.1. Graphics compare mean proportion of larvae settled among water temperatures and different types of settlement substratum (transformed data). Statistically significant differences are marked with *. Error bars measure $t_{(\alpha=0.05,\nu)} \times SE$.

at all (Figure 3.3).

3.3.4 Effect of water temperature on length of larval development

The timing of larval development in the studied species varied greatly with rearing temperature, development time decreasing considerably when water temperature increased (Table 3.2). This effect was obvious from the early larval stages, and more pronounced at lower temperature ranges. For example, development to trochophore took on average about 2 days to occur at the lowest experimental temperatures, and less than 0.5 days (slightly over in the case of *P. vulgata*) at the highest one. Average age at metamorphosis ranged between 3.7-14.0 days in *P. depressa*, 2.8-13.7 days in *P. ulyssiponensis* and 5.7-14.6 days in *P. vulgata*. These differences reflect the variation observed in suitable test temperature ranges between species. For similar temperature treatments, differences in development rates among species were slight. Exceptionally, development at 8 °C took considerably less time in *P. vulgata* than in the other two species. Furthermore, this was the only species developing at 5 °C, at rates comparable

Table 3.2 Larval development chronology, expressed in hours after fertilization (mean ± SD) of *P. depressa*, *P. ulyssiponensis*, and *P. vulgata*, at each of the experimental water temperatures. For all treatments, n = 8, except for *P. vulgata* at 16 °C (n = 3).

		Time since fertilization (Mean ± SD)					
Species	Stage or event	5 °C	8 °C	12 °C	16 °C	20 °C	24 °C
P. depressa	Swimming trochophore	-	47 ± 3	20 ± 2	13 ± 2	10 ± 1	8 ± 1
	Pre-torsional veliger	-	100 ± 5	75 ± 5	43 ± 7	28 ± 1	20 ± 1
	Post-torsional veliger	-	195 ± 9	133 ± 7	68 ± 11	40 ± 2	-
	Competence	-	307 ± 23	195 ± 15	115 ± 14	67 ± 4	-
	Metamorphosis	-	337 ± 19	224 ± 14	141 ± 14	89 ± 2	-
P. ulyssiponensis	Swimming trochophore	-	47 ± 1	25 ± 4	15 ± 2	11 ± 1	9 ± 2
	Pre-torsional veliger	-	120 ± 8	73 ± 9	43 ± 4	25 ± 1	21 ± 2
	Post-torsional veliger	-	200 ± 28	123 ± 26	68 ± 3	39 ± 1	37 ± 2
	Competence	-	285 ± 27	175 ± 17	99 ± 14	71 ± 1	50 ± 2
	Metamorphosis	-	328 ± 15	208 ± 24	133 ± 12	89 ± 2	67 ± 2
P. vulgata	Swimming trochophore	44 ± 3	22 ± 2	20 ± 2	15 ± 1	-	-
	Pre-torsional veliger	113 ± 2	50 ± 1	44 ± 2	33 ± 1	-	-
	Post-torsional veliger	211 ± 15	158 ± 10	125 ± 10	65 ± 3	-	-
	Competence	278 ± 13	209 ± 10	187 ± 5	95 ± 3	-	-
	Metamorphosis	351 ± 13	256 ± 13	217 ± 12	136 ± 2	-	-

to those of *P. depressa* and *P. ulyssiponensis* at 8 °C. Results showed that the temperature dependence of length of larval duration in the three limpet species can be appropriately described by exponential relationships with species-specific parameter values (Figure 3.4). Goodness-of-fit (measured by R^2) was very high in all cases, ranging between 0.932 (*P. vulgata*) and 0.983 (*P. depressa*).

After linearization of exponential relationships, an analysis of covariance of the regression lines was performed (Table 3.3). Deviations from individual regressions were homogenous (Bartlett's test $B = 1.799$, $P_{(\alpha=0.05,\ k=2)} = 0.407$), but slopes were significantly different ($F_{(2,\ 93)} = 21.645$, $P < 0.001$).

3.3.5 Effect of water temperature on larval survival

Results showed that survival at the earlier stages of development, although variable between replicates, was low among the three studied species (Figure 3.5). Excluding treatments at which successful fertilization has not been achieved, percentage survival to a fully developed trochophore stage was lowest in *P. depressa*, varying between 1.3-17.8%, whilst in *P. ulyssiponensis* and *P. vulgata* early survival rates were considerably higher, ranging between 4.7-27.6% and 6.0-30.3%, respectively. Dispersion

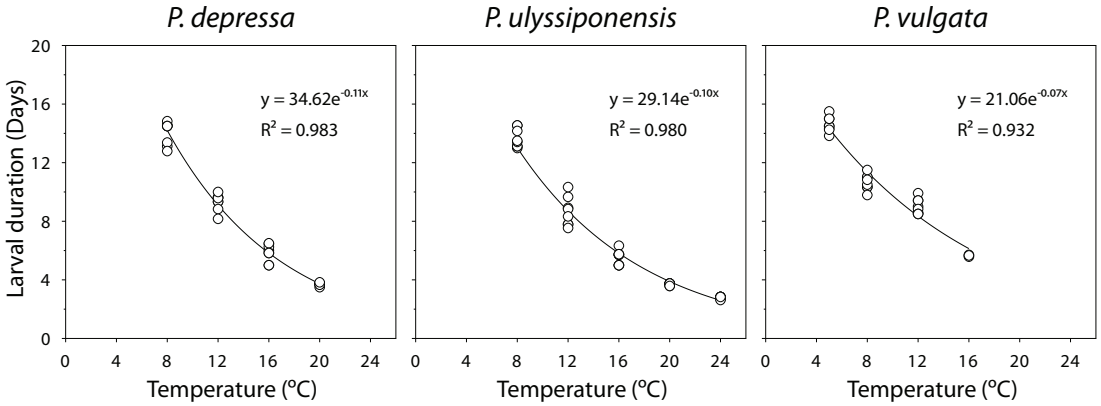

Figure 3.4 Relationship between water temperature and planktonic larval duration to metamorphosis in the presence of settlement substrata, for *P. depressa* (n=32), *P. ulyssiponensis* (n=40), and *P. vulgata* (n=27). Exponential regression lines were fitted to all datasets. Equations of the regression lines and coefficients of determination (R^2) are given in the graphs. Some data points overlap.

values (measured as standard deviation) were high, probably reflecting not only considerable variation in egg maturity and sperm quality among trials, but also differences in concentration of sperm stock solutions, all of which can have drastic consequences in fertilization rates (Dodd 1957; Hodgson *et al.* 2007).

In general, survival throughout development to metamorphic competence was low, since a pronounced decline in the number of larvae was observed across all species and temperatures. Survival rates at the monitored development stages were not strictly ordered according to temperature, which rather than suggesting solely an effect of time, indicated optimal temperature ranges for larval development. Thus, in *P. depressa* and *P. ulyssiponensis* there was a clear distinction in percent survival between 12-20 °C and lower temperatures. In the later species, the exceptionally high survival at 24 °C clearly stood above all other treatments. As for *P. vulgata*, 12 °C was clearly the optimal temperature for larval survival, followed by 5-8 °C.

Results of the ANOVAs of the effect of water temperature on percent survival of larvae at metamorphic competence are given in Table 3.4. Homoscedasticity was present in all species (Cochran's test: $C = 0.518$, $P_{(\nu=7,\ k=4)} = 0.069$ for *P. depressa*; $C = 0.394$, $P_{(\nu=7,\ k=5)} = 0.168$ for *P. ulyssiponensis*; $C = 0.409$, $P_{(\nu=7,\ k=4)} = 0.368$ for *P. vulgata*) after arcsine transformation. Given that only 3 replicates were available for *P. vulgata* at 16 °C, missing data were replaced with the average value of their respective cell, and the degrees of freedom were reduced accordingly (Underwood 1997). ANOVAs revealed a significant effect of water temperature on the percentage of planktonic larvae that survived to the competent stage. Multiple comparisons among temperature treatments (Figure 3.6) showed that these differences were statistically significant among extreme temperature values. In the case of *P. depressa* and *P. vulgata*, survival at the maximum

Table 3.3 Partial calculations of a single-factor ANCOVA for differences in the relationship between temperature and age at metamorphosis among the studied limpet species. Regressions slopes differed among species, and therefore differences among treatments could not be tested. Slope and intercept of the linearised exponential relationships are also given. *** $P < 0.001$.

Treatment	slope	intercept	SS	*df*	MS	*F*
P. depressa	-0.111	3.544	0.1390	30	0.0046	
P. ulyssiponensis	-0.101	3.372	0.2826	38	0.0074	
P. vulgata	-0.078	3.048	0.1537	25	0.0061	
Common deviations			0.8437	95	0.0089	
Sum of deviations			0.5753	93	0.0062	
Differences among slopes			0.2684	2	0.1342	21.6452 ***

temperature treatment was also significantly different from the others, suggesting that it was already beyond the optimal temperature for development.

3.3.6 Maximum length of larval life

Despite exhibiting behavioural competence, by repeatedly swimming upwards and sinking onto the bottom of the culture vessels, and also exploring the glass surface by crawling with the foot, larvae deprived of settlement cues did not spontaneously metamorphose. As larvae grew older, swimming activity progressively decreased and apart from the beating of the velar cilia, larvae became increasingly inactive. Towards the end of their lives, they remained at the bottom of the culture vessels, unattached and often withdrawn into their shell.

The three limpet species were capable of extending planktonic life after metamorphic competence (Figure 3.7). Average values across all test temperatures ranged between 15.8-25.4 days in *P. depressa*, 14.5-27 days in *P. ulyssiponensis* and 16.5-25 days in *P. vulgata*. Maximum longevity values for each species (by the same order) were 29.5, 31.5 and 29 days. Relative increases in planktonic larval duration were less pronounced at colder temperatures, varying between 21% (*P. vulgata* at 5 °C) and 42% (*P. ulyssiponensis* at 8 °C) beyond average planktonic duration of larvae that were induced to metamorphose. On the other hand, planktonic larval duration increased between 1.9 (*P. vulgata*) and 4.2 (*P. ulyssiponensis*) times in the absence of settlement cues at maximum test temperatures. In absolute terms, however, larval longevity in the absence of settlement cues was highest at the mid-temperature values. Results of the ANOVAs carried out for the effect of water temperature on the longevity of larvae in the absence of settlement cues are given in Table 3.5. Data were homoscedastic (Cochran's test: $C = 0.288$, $P_{(\nu=3,\ k=4)} = 0.441$ for *P. depressa*; $C = 0.377$,

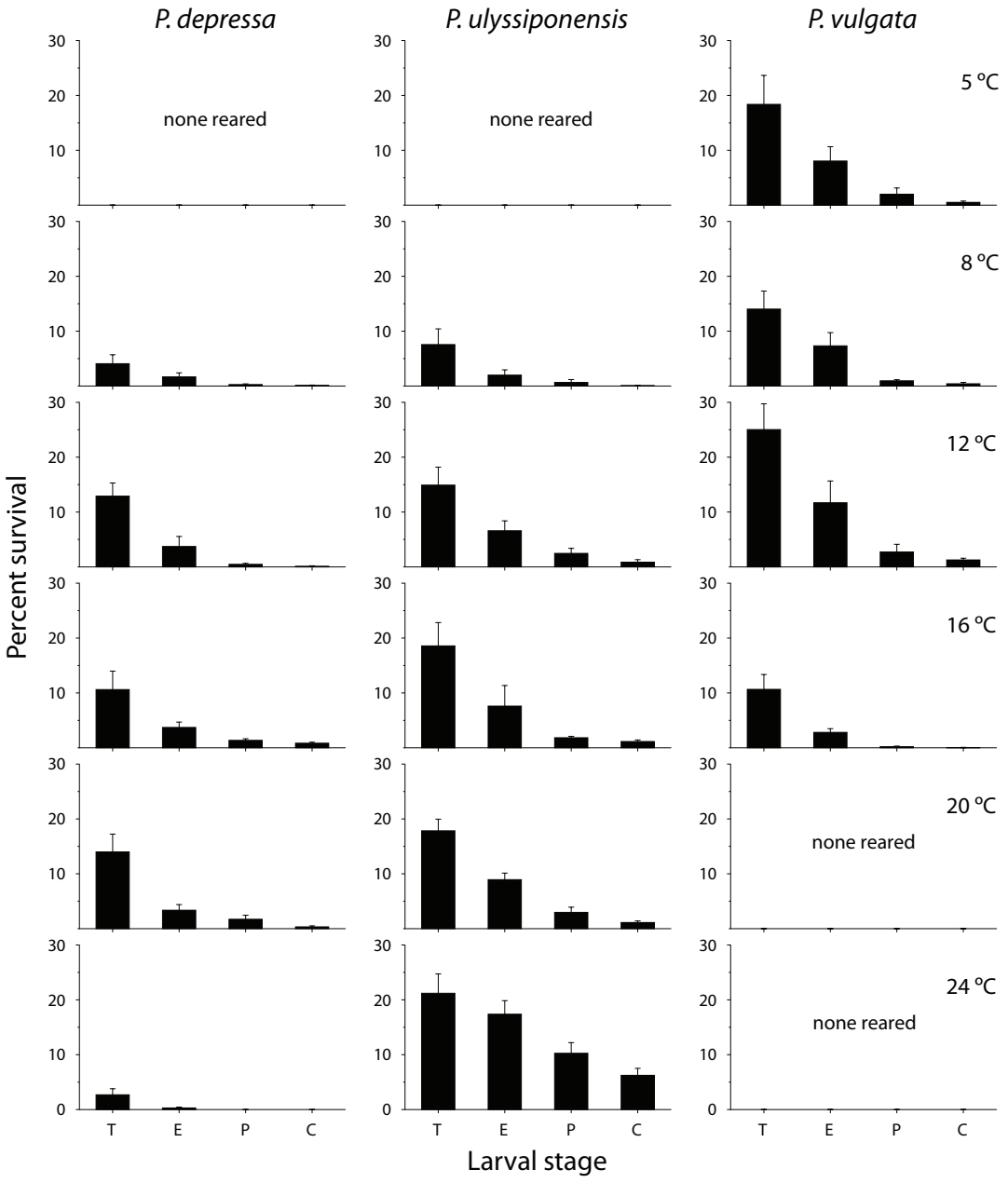

Figure 3.5 Percentage survival of larvae through developmental stages prior to metamorphosis. Stages are T- trochophore, E- early veliger, P- post-torsional veliger and C- competent veliger. Error bars represent standard deviation (n=8).

$P_{(v=3,\ k=5)}$ = 0.583 for *P. ulyssiponensis*; C = 0.608, $P_{(v=3,\ k=4)}$ = 0.126 for *P. vulgata*. Multiple comparisons (Figure 3.8) revealed statistically significant differences between mid- and extreme temperature values. In *P. depressa* and *P. ulyssiponensis*, maximum larval duration was significantly higher at 12 °C and 16 °C, while in *P. vulgata* larval longevity was significantly higher at 8 °C and 12 °C.

Table 3.4 ANOVA of percentage survival of larvae to metamorphic competence among test temperatures.

	P. depressa			*P. ulyssiponensis*			*P. vulgata*		
Source	*df*	MS	*F*	*df*	MS	*F*	*df*	MS	*F*
Temperature (=T)	3	0.576	42.961 ***	4	5.225	149.011 ***	3	0.895	34.240 ***
Residual	28	0.013		35	0.035		23	0.026	
Total	31			39			26		

*** < 0.001.

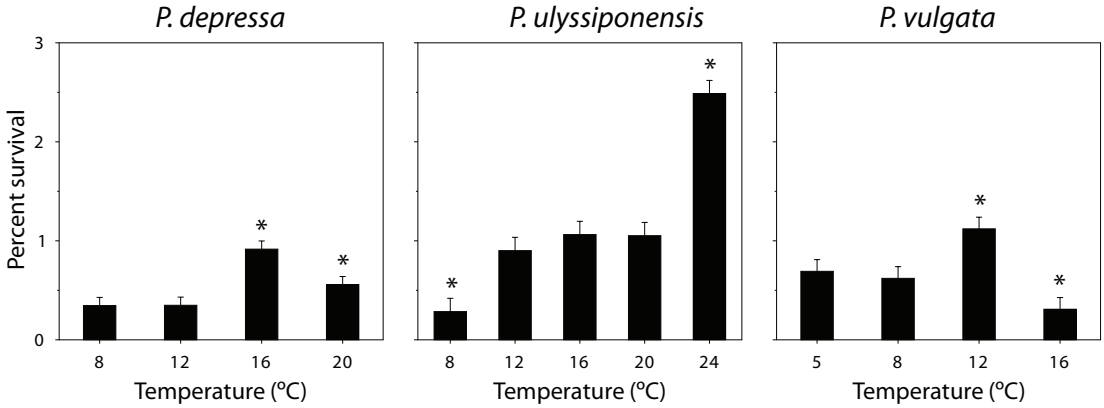

Figure 3.6 Multiple comparisons (SNK tests) after analyses of variance on average percentage survival of larvae at the competence stage (transformed data). Statistically significant differences are marked with *. Error bars measure $t_{(\alpha=0.05,\nu)} \times SE$.

3.3.7 Latitudinal variation of planktonic larval duration

Information regarding spawning seasons of *Patella* spp. along the NE Atlantic gathered since the 1940s were integrated with monthly mean SST values at the surveyed locations during the reported reproductive seasons (Table 3.6); these were then combined in the equations describing exponential temperature dependence of planktonic larval duration obtained in the present study. For each combination of location, species and survey year, estimates of minimum and maximum planktonic larval duration were calculated and results were plotted separately (Figure 3.9). In five out of six cases, an increasing trend of PLD with latitude was observed, the exception being maximum PLD in *P. depressa*, where a marked increasing trend was visible, but only at southern latitudes (below 45°N). Estimates of planktonic larval duration at higher latitudes did not follow the trend, being comparable to the ones obtained at southern locations.

Goodness-of-fit (R^2) of linear regressions between PLD and latitude was very

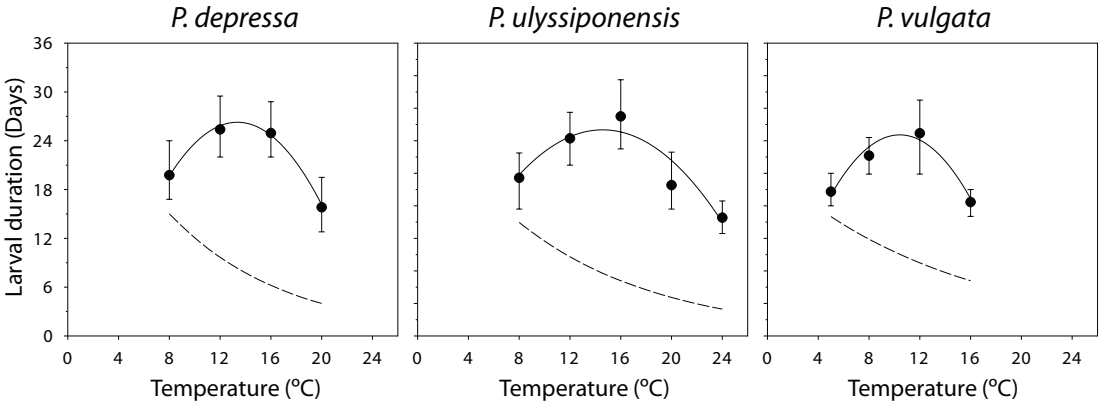

Figure 3.7 Mean larval duration of *P. depressa*, *P. ulyssiponensis*, and *Patella vulgata* in the absence of settlement substratum, as a function of water temperature. This relationship was better expressed by a second-order polynomial function, represented by solid lines. Number of replicate cultures per temperature is four, except for *P. vulgata* at 16 °C, for which only three replicates were available. Error bars represent extreme values, dashed lines correspond to exponential models of temperature dependence of larval duration in the presence of settlement substrata.

variable among datasets, but always higher for minimum PLD estimates. The greater dispersion of data points around the estimated regression lines for maximum PLD may have to do with the fact that the determination of the start of a spawning season (which usually occurs during warmer periods of the year) is more accurate than its end, which is often subjectively established. During visual assessment of gonad maturity stages, (the adopted method in the great majority of the reviewed studies) the later steady declines in gonad size may not be indicative of gradual release, but rather resorption of remaining gametes. On the other hand, abrupt falls in gonad size, like the ones observed at the beginning of spawning events, indicate gamete release (see chapter 2).

Minimum PLD increasing rates ranged from 0.109 and 0.137 days per degree of latitude, while maximum PLD increases ranged between 0.034 and 0.122 days per degree of latitude (Table 3.7). Slopes of all linear regressions were statistically significant, except for maximum PLD in *P. depressa*.

3.4 Discussion

3.4.1 Temperature dependence

This work demonstrated a clear effect of temperature on larval development, survival rates and planktonic duration in *P. depressa*, *P. ulyssiponensis*, and *P. vulgata*. As with many other marine invertebrate species (Hoegh-Guldberg and Pearse 1995; O' Connor

Table 3.5 ANOVA of maximum duration of larval life in the absence of settlement cues over temperature treatments.

	P. depressa			*P. ulyssiponensis*			*P. vulgata*		
Source	*df*	MS	*F*	*df*	MS	*F*	*df*	MS	*F*
Temperature (=T)	3	83.058	7.871 **	4	96.633	10.614 ***	3	61.466	9.548 **
Residual	12	10.552		15	9.105		11	6.437	
Total	15			19			14		

** $P < 0.01$; *** $P < 0.001$.

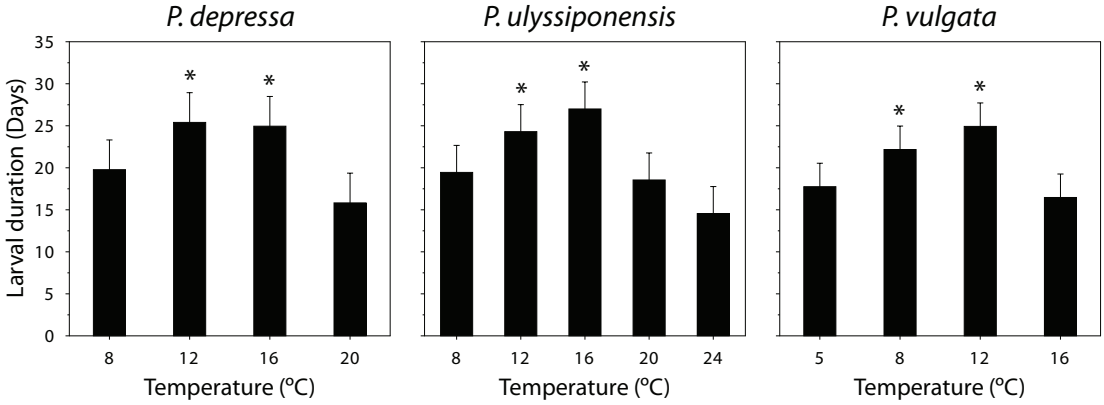

Figure 3.8 Multiple comparisons (SNK tests) after analyses of variance in Table 3.6. (average longevity of larvae in the absence of settlement cues). Statistically significant differences are marked with *. Error bars measure $t_{(\alpha=0.05,\nu)} \times SE$.

et al. 2007) temperature dependence of larval duration in *Patella* was adequately expressed as an exponential function with species-specific parameter values. Given the similarity in life-history traits that could explain observed discrepancies among species, such as development mode (Strathmann 1985; Emlet 1995) or egg size (Vance 1973; McEdward 1986; Levitan 2000), observed differences among the studied species could be explained mainly by variation of PLD among species at certain temperatures and by dispersion of measured PLD around the individual regression lines due to measurement error. Inter-specific variation in PLD at any given temperature may be explained by specific metabolic adaption to particular environmental conditions (O' Connor *et al.* 2007). Although lacking statistical support, slope and intercept parameter values are clearly more similar between *P. depressa* and *P. ulyssiponensis*, both southern species. In contrast, *P. vulgata* showed a better adaptation to low temperatures and decreased tolerance to warmer temperatures compared to the southern species.

Temperature affected not only length of larval development, but also survival. The three species displayed different levels of thermal tolerance during pelagic development. It is not possible to know if the observed rearing failures at upper extreme test temperatures reflect the physiological tolerance threshold of the species. Other factors

Table 3.6 Details of the studies used for compilation of data on the reproductive cycles of limpets in the northeast Atlantic. Methods of assessment of gonad development are: 1) visual, 2) gonado-somatic index, 3) oocyte size, 4) histological. *P.d.- Patella depressa*, *P.u.- Patella ulyssiponensis*, *P.v.- Patella vulgata*.

				SST range during spawning (°C)		
Source	Locations	Time span	Method	*P. d.*	*P. u.*	*P. v.*
Fischer-Piètte (1948)	Normandy (N France)	1946-1947	1	13.1-16.5	11.4-16.5	7.5-13.4
Evans (1953)	S England	1950	1	14.0-17.2	12.0-17.2	10.1-12.0
Orton et al. (1956)	Scotland, I. Man, SW England	1946-1949	1			6.7-14.5
Orton & Southward (1961)	SW England	1946-1949	1	13.4-17.0		
Choquet (1966)	Boulonnais (N France)	1963-1964	1			7.1-15.2
Blackmore (1969)	NE England	1964-1966	1			6.5-12.2
Lewis & Bowman (1975)	NE England	1967-1970	1			6.0-14.7
Bowman & Lewis (1977)	NE England	1969-1974	1			6.0-14.7
Hatch (1977)	S England	1974-1975	1	13.6-17.2		
Thompson (1979)	SW Ireland	1972-1974	1		12.5-15.9	
Thompson (1980)	SW Ireland	1972-1974	1			10.2-14.6
Bowman, 1981	SW England	1976-1979	1	13.5-18.0		
Baxter (1982)	Orkney (Scotland)	1979-1981	1			6.7-9.7
Baxter (1983)	Orkney (Scotland)	1979-1982	1			6.5-9.7
Bowman (1985)	British Isles	1973-1983	1			7.5-14.3
Bowman & Lewis (1986)	British Isles	1967-1984	1	13.5-16.4	10.7-16.9	7.5-14.7
Guerra & Gaudêncio (1986)	Portugal	1981-1984	1	13.1-20.2	13.1-20.2	13.1-16-7
Garwood (1987)	NE England	1983-1985	2, 3			7.2-11.5
Othaitz (1994)	Basque coast of Spain	1984-1989	1, 3	11.3-20.5	11.3-21.1	10-7-17.0
Delany et al (2002)	SW Ireland	1993-1995	1, 2		10.2-16.1	9.2-12.8
Brazão et al. (2003)	Central Portugal	2001-2002	1	14.8-18.7		
Moore et al (2007)	SW England	2003-2005	1	12.2-17.8		10.1-14.0
McCarthy et al. (2008)	SW Ireland	2003-2004	4		12.3-15.4	10.7-14.7
Ribeiro (this thesis)	N Portugal	1998-2000	1	13.7-20.5	13.7-19.8	13.0-15.8

such as contamination of cultures reared at higher temperatures by ciliates or bacteria should also be considered. However, the level of replication adopted in the present study does confer a reasonable degree of certainty to this assumption. Nonetheless, given that intervals between temperature treatments were considerable, lethal temperatures obtained in this study should serve only as rough estimates.

Within the limits of thermotolerance, survival throughout planktonic development was generally low, rarely exceeding 1% by the time larvae reached metamorphic competence (apart from *P. ulyssiponensis* at 24 °C, where survival was much higher). This is likely to be even lower in the sea, where there is probably less food available and predation will occur. Results showed that survival was higher at

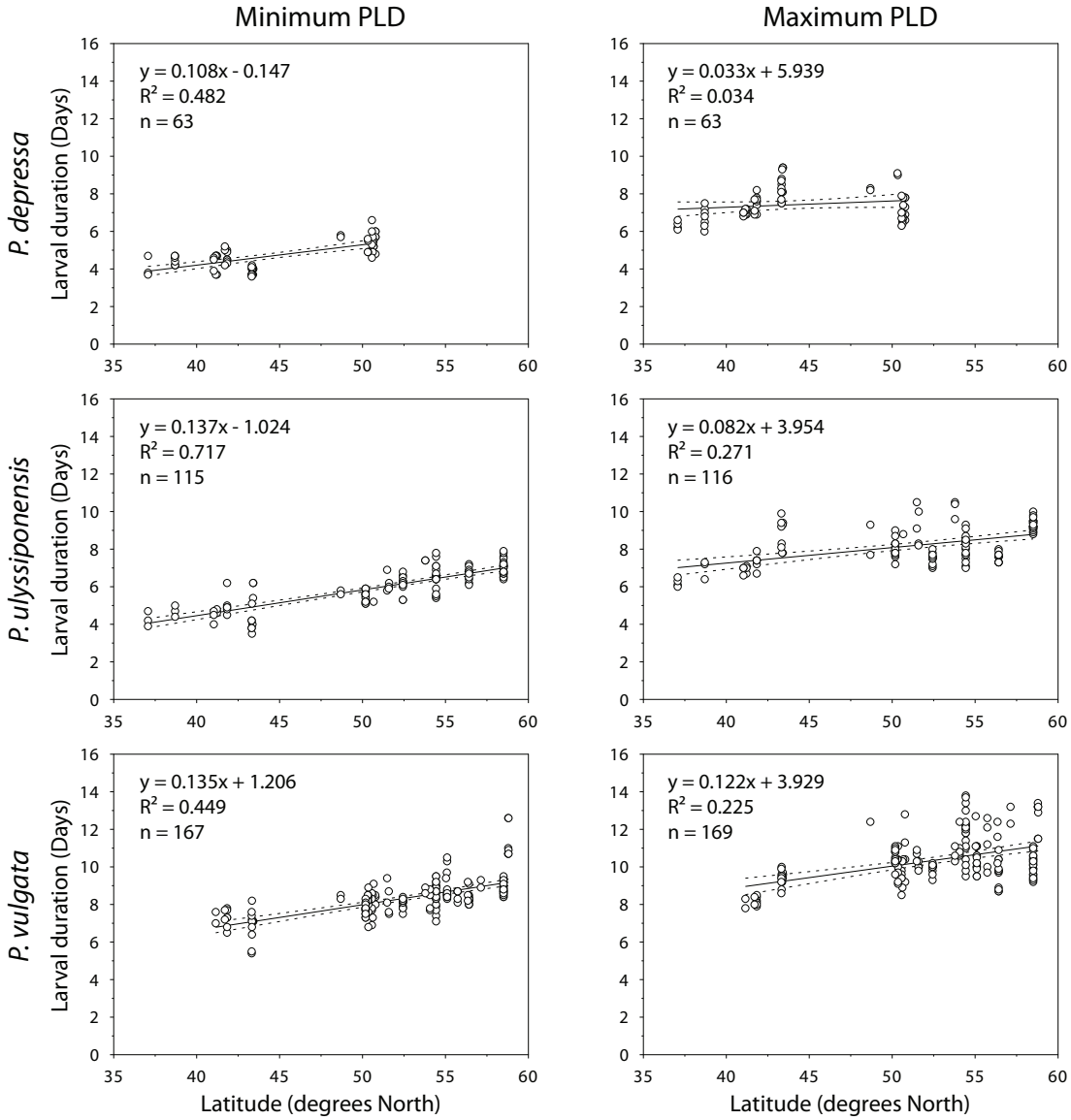

Figure 3.9 Relationship between latitude and planktonic larval duration (minimum and maximum). Estimates of PLD were based on results from published surveys on the reproductive cycles of *Patella* spp. in the northeast Atlantic and also SST records for the survey locations and reproductive seasons, applying the equations describing temperature dependence of PLD obtained in the present study. Linear regressions were fitted to all datasets. Coefficients of determination (R^2) and number of data points are given in the graphs. Dashed lines represent 95% confidence intervals.

certain temperatures or temperature ranges similar to those experienced by the species during their respective spawning seasons. Of the three species, *P. ulyssiponensis* showed the widest tolerance to temperature, and contrary to the remaining species, an upper lethal temperature was not reached. In relative terms, survival rates of *P. ulyssiponensis* were consistently high between 12-20 °C, and much higher at 24 °C. In *P. depressa*,

Table 3.7 Increasing trend (expressed by the slope of linear regressions) in planktonic larval duration (PLD) with latitude in the northeast Atlantic, for the three studied species. Statistically significant probability values are depicted in bold. NS - not significant.

		Slope	
Species	Range (lat.)	Minimum PLD	Maximum PLD
P. depressa	37.07 - 50.78° N	0.109 ***	0.109 NS
P. ulyssiponensis	37.07 - 58.50° N	0.137 ***	0.137 ***
P. vulgata	41.15 - 58.78° N	0.136 ***	0.136 ***

optimal survival rates were achieved at 16 °C, and declines were observed both below and above this temperature. As for *P. vulgata*, larval survival did not statistically differ between 5 °C and 8 °C, being significantly higher at 12 °C and lower at 16 °C. These data suggest a relationship between extent of geographical distribution and thermotolerance of planktonic larvae. For example, *P. ulyssiponensis* was the only species that developed at the highest temperature tested (24 °C), also being the only species of the three that extends into the Mediterranean, where water temperatures are warmer. The northern species *Patella vulgata* did not develop above 16 °C. Its southern range boundary lies in southern Portugal, where sea temperatures rarely decrease below that value. Furthermore, *P. vulgata*, which has the northernmost range limit of the three species, showed a better tolerance to low test temperatures. It is also interesting to note that *P. depressa*, which does not extend as far north as *P. ulyssiponensis*, also showed lower survival rates at 12 °C. Therefore, in the light of the present results, temperature-driven larval mortality may be a major determinant of range limits in these species. However, the correlative nature of the data cannot exclude other possible processes that have long been implicated in driving distributional limits, such as dispersal patterns and associated barriers (Gaylord and Gaines 2000; Gilg and Hilbish 2003; Zacherl *et al.* 2003; Lima *et al.* 2007b), increasing distance between populations at the range margin (Gilman 2006a), inadequate larval food supply (Gilman 2006b), reduction in reproductive output (Hutchins 1947; Mieszkowska *et al.* 2006), post-settlement mortality (Bowman and Lewis 1986) or adult mortality (Edwards and Hernández-Carmona 2005).

3.4.2 Larval selectivity during settlement

Larval recruitment onto suitable habitat is an essential step for the survival of juveniles and for the persistence of populations in benthic ecosystems (Underwood and Keough 2001). Therefore, it is beneficial for marine invertebrate larvae, and particularly those recruiting into patchy environments, to be able to explore different habitats and settle

where there are better chances for survival and post-settlement performance (Pawlik 1992; Elkin and Marshall 2007). Although preliminary, the settlement choice assays made in the present study demonstrated that limpet larvae were capable of active substratum selection. Settlement was much higher over crustose coralline algae than on bare rock, a consistent trend across all test temperatures.

Existing reports on rearing of patellid larvae are not very clear about the type of substratum used to achieve metamorphosis. Working with *P. vulgata*, Smith (1935) mentioned occasional use of rock chippings, but found it to be unnecessary, since larvae crawled on the glass surface of culture vessels. Metamorphosis was achieved in just one individual, however, which suggests that suitable settlement inducers were not present in the cultures. Dodd (1957) reared larvae of *P. caerulea* and *P. vulgata*, but metamorphosis was only observed and described in the latter. Despite having reared high numbers of post-metamorphic juveniles, this author did not provide any settlement substrata, arguing that metamorphosing individuals were obtained when *P. trycornutum* was added to the cultures. In this case it is possible that algal films formed on the glass walls provided the cue for settlement and metamorphosis of larvae (Strathmann *et al.* 1981; Thompson *et al.* 1998; Swanson *et al.* 2006). In a more recent study, Wanninger *et al.* (1999) reported spontaneous metamorphosis in larvae of *P. caerulea* and *P. vulgata*. In the case of *P. caerulea*, settlement was also observed on the walls of the culture vessels where metamorphosed individuals were feeding on algal films. In the present study, although crawling over the glass surface of containers was a commonly observed behaviour during the latter stages of planktonic development, spontaneous metamorphosis did not occur. Given that cultures were frequently changed to clean containers with filtered and sterilized seawater, it is possible that reared larvae were deprived of any stimulus that could trigger metamorphosis, such as the presence of algal films or any unknown inducer that might be dissolved in untreated seawater.

The results obtained in the settlement choice assays are in partial agreement with what was already known from field studies of recruitment in *Patella*. Small juveniles (1-8 mm) have been observed in damp crevices, small pits, low and mid-shore rock pools lined with crustose algae, and patches of small mussels among barnacles or bare rock (Orton 1929; Lewis and Bowman 1975; Bowman and Lewis 1977; Bowman 1981; Delany *et al.* 1998). Given the very small size of newly-settled larvae, these have never been observed on the shore, the exception being one case in southern Portugal, where newly-settled juveniles have been collected from artificial substrata (M.I. Seabra, pers. comm.). Therefore it is not possible to know whether the small juveniles occurring among mussel and barnacle patches actually settled there or rather migrated from settlement areas after reaching a certain size. The very low number of larvae which metamorphosed over bare rock indicates that it provided a weak cue for settlement. However, this may not be the case in the natural environment, especially if bare rock

is covered with an algal film. Additionally, mussels or barnacles may be effective settlement inducers of some species of limpet larvae (possibly *P. vulgata*, which seems to be less selective, S.J. Hawkins, pers. comm.). More studies will be required in order to unravel these and other questions concerning settlement behaviour of limpet larvae, preferably under realistic flow-field conditions – a factor that could limit the ability of larvae to swim in search of suitable settlement habitat (Butman 1987; Abelson and Denny 1997; Thompson *et al.* 1998; Boxshall 2000; Crimaldi *et al.* 2002).

3.4.3 Delayed metamorphosis

In the absence of settlement substrata, limpet larvae were able to extend the planktonic stage considerably. The experimental design adopted in this study did not allow determining for how long larvae remained capable of settling and metamorphosing, after reaching competence. Therefore, it is possible that delay periods have been overestimated, although the occurrence of extensive delay periods is not an uncommon feature in planktotrophic larvae (Sebens 1983; Pechenik 1990; Hadfield and Strathmann 1996). Observed differences between precompetent and competent periods with and without settlement induction were considerable and very variable with temperature, reaching as much as five-fold. Once again, there was a clear effect of temperature on maximum duration of larval life. Although extended larval duration was recorded at all experimental temperatures, it was optimal in a relatively narrow temperature range, 12-16 °C for *P. depressa* and *P. ulyssiponensis*, 8-12 °C for *P. vulgata*.

The observed delay capabilities reported here can hypothetically have a considerable positive effect on planktonic periods, and thus, on dispersal potential of limpet offspring. Maximum planktonic periods in the order of 30 days, like the ones recorded in the experiments, provide the potential for dispersal across very long distances. Additionally, the extended spawning periods of *P. depressa* and *P. ulyssiponensis* throughout most of their range will probably cause planktonic larvae to face added variability in speed and direction of ocean currents, which should favour high levels of gene flow among populations, leading to widespread panmixia and absence of genetic structure over large geographical areas (Hohenlohe 2002). However, delaying metamorphosis can also carry serious costs (Pechenik 1990), by reducing the number of larvae that eventually reach suitable habitat and also by compromising the likelihood of benthic juveniles to grow and reproduce. Increasing time spent in the plankton increases exposure of larvae to multiple sources of mortality, such as starvation or limited food resources (Olson and Olson 1989; Fenaux *et al.* 1994; Morgan 1995; Pechenik *et al.* 2002), offshore transport (Alexander and Roughgarden 1996) and predation (Morgan 1995; Pechenik 1999; Pechenik *et al.* 2004). Delaying metamorphosis has also been found to negatively affect rates of metamorphosis and

post-metamorphic growth, survival and reproductive output, particularly in species with lecithotrophic larvae (Woollacott *et al.* 1989; Wendt 1998; Maldonado and Young 1999; Pechenik 1999; Roberts and Lapworth 2001; Marshall *et al.* 2003; Bennett and Marshall 2005), but also feeding larvae (Sebens 1983; Qian and Pechenik 1998; Gebauer *et al.* 1999). Nevertheless, the magnitude of all these deleterious effects increases with time and therefore the ability to delay metamorphosis may have a tangible effect on realized dispersal and gene flow in *Patella*, but probably not to the full extent that can be predicted from laboratory estimates of larval longevity and competence periods.

Currently nothing is known about how often delayed metamorphosis occurs in nature, and what are the underlying causes that trigger it in the field. Although larvae can be tracked directly in the field under particular conditions (Olson 1985; Davis and Butler 1989; Willis and Oliver 1990), or tagged (Levin 1990; Levin *et al.* 1993; Thorrold *et al.* 2002; Zacherl 2005; Becker *et al.* 2007), empirical dispersal trajectories are hard to obtain, due to the very small size and high mortality rates of larvae (Levin 1990; Thorrold *et al.* 2002). How long-distance dispersal enabled by delayed metamorphosis impacts connectivity and genetic structure of marine invertebrate populations can be addressed by the use of dispersal/population models coupling ocean features such as dynamics and temperature, habitat availability and life-history parameters during both pelagic and benthic stages. Such models would allow testing of hypotheses on the relative importance of both medium- and long-range dispersal in setting geographical distributions of species and observed levels of genetic structure in natural populations (see chapter 7).

3.4.4 Latitudinal variation of planktonic larval duration

Estimates of planktonic larval duration in *Patella* based on reproductive surveys, SST and temperature dependence equations were found to vary significantly with latitude. Although the slopes of lines describing the PLD and latitude relationship varied among species, they were uniform in trend, and in all but one case (maximum PLD of *P. depressa*), statistically significant. The minimum amount of time required by larvae to complete development and eventually metamorphose will influence the probability of larval retention, since the less time larvae need to spend in the plankton, the more likely it is for them to recruit to local populations. On the other hand, maximum dispersal potential will determine the degree of connectivity among distant subpopulations. Thus, significant latitudinal variation in the estimated PLD ranges and, consequently, dispersal potential, could give origin to a latitudinal gradient in levels of gene flow. Genetic studies on *Patella* that would allow testing this hypothesis (using high-resolution genetic markers) are lacking. However, the few broad-scale genetic studies published so far report little or no differentiation over extensive areas of the

northeast Atlantic coastline, with no evidence of a gene flow gradient (Weber and Hawkins 2005, 2006). This pattern is recurrent in other marine species, such as two species of barnacles, *Chthamalus stellastus* and *Chthamalus montagui* (Pannaciulli *et al.* 1997), the oyster *Ostrea edulis* (Saavedra *et al.* 1993), the sea urchin *Paracentrotus lividus* (Duran *et al.* 2004a), the Norway lobster *Nephrops norvegicus* (Stamatis *et al.* 2004), the netted dog-whelk *Nassarius reticulatus* (Couceiro *et al.* 2007) and the stalked barnacle *Pollicipes pollicipes* (Quinteiro *et al.* 2007).

It is possible that temperature dependence of PLD within a species varies with latitude, due to local adaptations of geographically distant populations, a phenomenon that has been previously reported (Drent 2002). In the present case, it seems unlikely, though, because a pilot rearing experiment carried out with limpets from northern Portugal revealed similar larval development rates between 8 and 16 °C to those reported here (data not shown). On the other hand, delayed metamorphosis may have a homogenizing effect on patterns of gene flow throughout the species range, by allowing larvae to remain in the plankton after reaching metamorphic competence, thus masking temperature effects in the duration of larval life and consequently, dispersal potential.

In conclusion, there are differences due to the influence of temperature in length of larval life among species and among individuals of the same species. This will influence genetic interchange between populations, but panmixia is likely to be maintained by occasional interchange of individuals due to the ability to delay metamorphosis for considerable periods. Although these events may be significant in terms of evolution by reducing opportunities for allopatric speciation and also for population genetic structure, they are probably not important ecologically in terms of connectivity of populations. This is explained further in the general discussion (chapter 8).

Chapter 4

Development of microsatellite loci for the limpets *Patella depressa* and *Patella rustica*, and cross-amplification in other patellid species

4.1 Introduction

Microsatellites, also known as simple sequence repeats (SSR), variable number of tandem repeats (VNTR) or short tandem repeats (STR), consist of relatively small tandemly repeated DNA sequences that are found dispersed throughout the genome of all organisms so far analysed (Litt and Luty 1989; Tautz 1989; 2000). The definition of microsatellites in terms of the size of repeat units has been controversial: for example, Jarne and Lagoda (1996) consider microsatellite repeat unit to range between 1-5 base pairs (bp); Goldstein and Pollock (1997) and Zane *et al.* (2002) define them as 1-6 bp repeats; Chambers and MacAvoy (2000) as 2-6 bp repeats; and Armour *et al.* (1999) as 2-8 bp repeats. The most widely used types are di-, tri- and tetranucleotide microsatellites, and among these, CA and GA dinucleotides, and GATA and GACA tetranucleotides are the most studied repeat motifs (Jarne and Lagoda 1996; Hancock 1999).

With the widespread use of microsatellites as population genetic markers in recent years, understanding of their origin, evolution, functional properties and genomic distribution has increased (Li *et al.* 2002; Ellegren 2004; Cruz *et al.* 2005; Pérez *et al.* 2005). A large body of evidence shows that microsatellite genomic distribution is nonrandom, being more abundant in noncoding than in coding regions (Bachtrog *et al.* 1999; Katti *et al.* 2001; Li *et al.* 2002). Moreover, both absolute number of microsatellite loci and repeat preference vary significantly across taxa (Lagercrantz *et al.* 1993; Tóth *et al.* 2000; Ross *et al.* 2003), with concomitantly variable success rate in their isolation (Zane *et al.* 2002).

Most microsatellite loci are highly polymorphic owing to high rates of length

mutation (Hughes and Queller 1993; Amos *et al.* 1996), which typically range between 10^{-5} to 10^{-2} events per locus per generation (Bruford and Wayne 1993; Jarne and Lagoda 1996; Chakraborty and Kimmel 1999; Hancock 1999). Mutation rates can, however, vary with phyletic groups, repeat types, microsatellite types (perfect, compound or interrupted), with the nature of flanking sequences, the position on a chromosome and even allele size (Estoup and Cornuet 1999; Balloux and Lugon-Moulin 2002; Whittaker *et al.* 2003). Despite considerable debate, it is assumed that slipped-strand mispairing during DNA replication or repair is the predominant length mutation mechanism generating microsatellites (Levinson and Gutman 1987; Schlötterer and Tautz 1992; Eisen 1999), although other processes such as recombination between DNA strands may also be implicated (Hancock 1999; Li *et al.* 2002).

As with any other genetic marker type, the use of microsatellites is not free from drawbacks, which can significantly limit their application or confound data analysis. Disadvantages include the need for species-specific marker isolation, uncertainty regarding mutation models, size homoplasy of alleles and scoring errors. A brief account of each limitation is given below.

In most cases, microsatellites need to be isolated from the genome of target species, unless genome mapping or sequencing has been previously undertaken, and genomic databases can be scanned (Jarne and Lagoda 1996; Zane *et al.* 2002). This need arises because microsatellites usually occur at noncoding regions, where the mutation rate is higher than in coding regions. Therefore, flanking regions (where primers anneal during PCR amplification) are less conserved, which does not allow the use of universal primers, unlike with other marker types. For most species, the isolation and characterization of microsatellites involve a laborious (and costly) succession of procedures (cloning, detection of microsatellites and sequencing) in order to determine flanking sequences that can be used to design the primers (Zane *et al.* 2002). However, intrageneric microsatellite cross-amplification is common, although levels of polymorphism are usually lower.

The choice of which mutation model to apply in population genetics analysis is crucial, since estimation of several statistics based on allele frequencies, such as Wright's (1951) F_{ST} and Slatkin's (1995) analogue for microsatellite loci, R_{ST}, are dependent on the assumed mutation model (Estoup and Cornuet 1999; Selkoe and Toonen 2006). This dependence may be especially strong in the case of microsatellites, since sensitivity to the mutation model increases with the mutation rate (Estoup and Cornuet 1999). Three models of mutation are considered suitable for the study of microsatellites: the infinite allele model (IAM, Kimura and Crow 1964), the *K*-allele model (KAM, Crow and Kimura 1970) and the stepwise mutation model (SMM, Kimura and Ohta 1978). Under the IAM, mutation always gives origin to a new allele of any size, and the number of possible allele states has no limit. The KAM model predicts that there are exactly *K*

possible allele states, and any allele has a constant probability of mutating towards any of the other *K*-1 allele states. This model seems more realistic than the IAM model, due to size constraints which appears to act on microsatellite loci (Estoup and Cornuet 1999). Finally, under the SMM, mutation occurs by loss or gain (with equal probability) of one single tandem repeat, and therefore it is possible for an allele to mutate to an allele state already present in the population. Other models have been introduced, aiming to mirror the effects of different mutational mechanisms that are known to act on microsatellites (Selkoe and Toonen 2006). One example is the two phase model (TPM, Di Rienzo *et al.* 1994), under which a fixed proportion of mutations are single step changes (deletion or addition of one tandem repeat) and the remaining mutations, which are drawn from a geometric distribution, are larger than single steps.

Mutational processes of microsatellites can be very complex (Schlötterer 2000; Ellegren 2004). Statistical analyses of microsatellite population data have yielded contradictory or inconclusive results concerning which mutation model better predicts allelic distributions (Estoup and Cornuet 1999). There is growing evidence suggesting differences in mutational processes among loci (Estoup and Cornuet 1999; Balloux and Lugon-Moulin 2002), which might involve a potential influence of molecular properties of microsatellites, such as length and composition of the repeat motif (Estoup *et al.* 1995). Although it is assumed that microsatellite mutation follows the SMM in the majority of cases (Eisen 1999; Balloux and Lugon-Moulin 2002; Ellegren 2004), numerous exceptions have been reported (Pearse and Crandall 2004). Genetic distance and population structure metrics employing the SMM are highly sensitive to departures from assumptions of this mutational model, such as the occurrence of non-stepwise mutation or allele size constraints, and thus metrics using the IAM are considered more robust (Ruzzante 1998; Balloux and Lugon-Moulin 2002).

Microsatellite genotyping typically relies on allele sizing, in other words on the characterization of different allelic states by the length in base pairs of the amplified fragments. This is a very efficient genotyping method, especially when dealing with large sample sizes and high numbers of loci, and allows saving considerable time and money (Selkoe and Toonen 2006). There is a possibility, however, that two PCR products having the same size do not correspond to copies of the same allele, something that is termed size homoplasy, which can have several causes. Size homoplasy can be caused by point mutation which leaves the allele size unchanged, or by insertions and deletions in the flanking region that will create a new allele with the same size (Estoup *et al.* 2002). In these cases, size homoplasy can be detected by sequencing alleles (Estoup and Cornuet 1999; Estoup *et al.* 2002). However, another type of mutation may lead to undetectable size homoplasy, in cases where alleles are identical in state, but not identical by descent. This occurs when under the stepwise mutation process a back-mutation occurs to a previously existing allele size or when

two unrelated alleles converge in sequence by changing repeat number in two different places in the sequence (Selkoe and Toonen 2006).

Size homoplasy is linked with the underlying mutational model of the marker. It is expected to occur under any mutational model, except the IAM, since under this model any newly created allele is different from all the other alleles previously present in the population (Estoup and Cornuet 1999; Estoup *et al.* 2002). On the other hand, the frequency of size homoplasy will increase with mutation rate of the locus, effective population size, time of divergence between populations and strong allele size constraints (Nauta and Weissing 1996; Feldman *et al.* 1997; Estoup *et al.* 2002). Extensive size homoplasy is problematic in population genetics studies, since it can reduce the assessment of allelic diversity of populations, thus causing an increase in estimates of gene flow (Viard *et al.* 1998; Gaggiotti *et al.* 1999; Epperson 2005).

Another drawback of working with microsatellites is that genotyping can be very prone to errors, mostly due to amplification problems. Among the multiple sources of genotyping errors in microsatellites, which can be generated at any stage of the procedure, from sampling to data analysis, and can have a multitude of causes (Bonin *et al.* 2004; Hoffman and Amos 2005), three have been identified as main causes of concern. These are large allelic dropout, stuttering patterns and null alleles; all of these can bias data analyses by creating consistent allele and genotype frequency bias (DeWoody *et al.* 2006). Large allele dropout occurs in heterozygous genotypes, when the smaller allele is preferentially amplified by PCR (Wattier *et al.* 1998). Thus, the large allele may amplify much less than the small allele, and if template quality is low, it may fail to amplify altogether (Björklund 2005), resulting in inflation of homozygote frequency. Stutter bands are additional products that differ from the original template by multiples of the repeat unit length, and appear due to slippage of *Taq* polymerase during PCR amplification. Stutters can complicate interpretation of electrophoregrams, making it difficult to discriminate between homozygotes and heterozygotes, especially in the case of dinucleotide repeat motifs (van Oosterhout *et al.* 2004). Finally, null alleles result from mutation at primer binding sites; these are not amplified by PCR assays to detectable levels (Pemberton *et al.* 1995; Dakin and Avise 2004). Consequently, some heterozygotes may be scored as homozygotes and homozygous individuals for a null allele may fail to amplify any alleles. The presence of null alleles in a dataset will bias allele and genotype frequencies, causing heterozygote deficiency (Dakin and Avise 2004). Although not all genotyping errors are unavoidable, most of them can be prevented or mitigated with appropriate protocols, which involve implementing quality control procedures at various stages of a study (Bonin *et al.* 2004; Hoffman and Amos 2005; DeWoody *et al.* 2006; Miquel *et al.* 2006; Selkoe and Toonen 2006). Moreover, new statistical methods are constantly being developed for identifying genotyping errors and correcting allele and genotype frequencies (e.g. Brookfield 1996; van

Oosterhout *et al.* 2004; Björklund 2005; Kalinowski and Taper 2006; van Oosterhout *et al.* 2006; Chapuis and Estoup 2007; Johnson and Haydon 2007).

Because of their hypervariability, codominant inheritance and assumed neutrality (but see Kashi and Soller 1999; Li *et al.* 2002), microsatellites proved to be highly informative for population genetics studies (Bruford and Wayne 1993; Jarne and Lagoda 1996; Goldstein *et al.* 1999; Sunnucks 2000). In addition, microsatellite genotyping has become a relatively quick and inexpensive process (Selkoe and Toonen 2006), and the suite of statistical methods and software packages able to handle large microsatellite datasets expanded greatly (Luikart and England 1999; Manel *et al.* 2003; Pearse and Crandall 2004; Manel *et al.* 2005; Excoffier and Heckel 2006). Such progress, together with unprecedented and ever-increasing computational power have taken population genetic analyses far beyond the 'standard approach' coined by Neigel (1997), allowing a more detailed insight into contemporary structure and gene flow patterns, as well as a stronger inference about historical demography of natural populations, relying on fewer, more realistic, assumptions (Pearse and Crandall 2004). The use of microsatellites has thus become extremely common, as can be judged by the profusion of primer notes published in recent years. Microsatellites have become the markers of choice to address specific questions in areas like conservation and evolutionary biology and ecology (Beaumont and Bruford 1999; Balloux and Lugon-Moulin 2002; Zane *et al.* 2002).

Within the genus *Patella*, microsatellites have only been previously developed for the Mediterranean species *Patella caerulea* (Bertozzi *et al.*, unpublished, Genbank accession numbers AY727872 to AY727876) and cross-species tests are unknown. The aim of this work was to isolate high-resolution microsatellite markers from two limpet species, *Patella depressa* and *Patella rustica*, which will be used to investigate their fine-scale genetic population structure and contemporary dispersal patterns. In this chapter the isolation protocols of 11 microsatellite loci from *P. depressa* and 18 from *P. rustica* are described. Additionally, results of a preliminary screening assay on the target species and of cross-species testing of primer pairs on other *Patella* species are also provided. The methods and results described hereafter have already been published (Pérez *et al.* 2007, 2008).

4.2 Material and Methods

All the procedures described in this section were carried out at the Department of Biochemistry, Genetics and Immunology labs (University of Vigo, Spain), except for markers *Pde9 - Pde11* from *P. depressa*, which have been isolated at the Instituto de Investigación en Recursos Cinegéticos (Ciudad Real, Spain) and tested for variability at Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO-University of Porto, Portugal).

To isolate microsatellite regions a modified protocol of the enrichment technique FIASCO (Fast Isolation by Amplified fragment length polymorphism of Sequences COntaining repeats, Zane *et al.* 2002), was used. Genomic DNA was extracted from muscle tissue of one individual of *P. depressa* collected in Moledo do Minho and one individual of *P. rustica* from Viana do Castelo (both locations in Portugal), following a standard phenol-chloroform protocol (Sambrook *et al.* 1989). Genomic DNA (approximately 250 ng) was digested with MseI (New England Biolabs) at 37 °C for 3 hours. Digested DNA was ligated to 1 μM of MseI AFLP adaptor (5'-TACTCAGGACTCAT-3'/5'-GACGATGAGTCCTGAG-3') using Ready-to-go T4 DNA ligase (GE Healthcare). The ligation mixture was amplified in a total volume of 40 μL consisting of 20 μL of diluted DNA (1:10), 1× *Taq* DNA polymerase buffer (10 mM Tris-HCl, pH 9; 50 mM KCl, 0.1% Triton X-100, Promega), 200 μM of each dNTP, 4 U of *Taq* DNA Polymerase (Promega) and 240 ng of AFLP adaptor-specific primer (MseI-N). The polymerase chain reaction (PCR) conditions consisted of 26 cycles of 30s at 94 °C, 1min at 53 °C and 1min at 72 °C. About 750 ng (15 μL) of the amplified product was hybridized with 80 pmol of the 5'-biotinylated probes [for *P. depressa* $(CA)_{13}$ and $(AG)_{14}$, for *P. rustica* $(CA)_{13}$ $(GATA)_7$ and $(GACA)_7$], in a total volume of 100 μL containing 4.2× SSC and 0.07% SDS. After a denaturation step of 3min at 95 °C, annealing was performed at room temperature for 15min. DNA molecules hybridized to biotinylated oligonucleotides were selectively captured by streptavidin coated beads (Roche Diagnostics), the remaining protocol being identical to that described by Zane *et al.* (2002).

To minimize non-specific bindings of genomic DNA, an unrelated PCR product from *Mytilus galloprovincialis* and a cytochrome *b* amplicon of *Merluccius hernandezi* were used in *P. depressa* and *P. rustica* protocols, respectively. The PCR products of two elution steps were cloned into pGEM-T Easy Vector System II (Promega) following the protocol of the manufacturer. Inserts from 691 and 823 recombinant clones (for *P. depressa* and *P. rustica* respectively) were amplified by PCR in 20 μL containing 1 μL of an overnight grown colony, 1× *Taq* DNA polymerase buffer (10 mM Tris-HCl, pH 9; 50 mM KCl, 0.1% Triton X-100), 1.5 mM $MgCl_2$, 20 pmol of each primer (T7 and SP6) and 1 U of *Taq* DNA polymerase (Promega). Inserts ranging from 350 to 1100 bp were selected for Southern blot transfer and filter hybridization with the synthetic probes. A total of 206 (*P. depressa*) and 89 (*P. rustica*) positive clones were selected for sequencing. The clones were lysed, their plasmid purified using the GFX Micro Plasmid Prep Kit (GE Healthcare), and sequenced on both strands on an ABI PRISM 3100 Sequencer (Applied Biosystems) using the BigDye Terminator method version 3.0.

From all sequenced *P. depressa* clones, 7% contained microsatellites (14 dinucleotides and 1 trinucleotide), 37% consisted of mononucleotides and the remaining 56% did not contain microsatellites. PCR primers were selected in the

flanking regions of 14 microsatellites using Oligo 4.05 (Rychlick and Rhoads 1989) and amplification conditions were tested in *P. depressa*, *Patella candei* and *P. rustica* using a Mastercycler Gradient Thermocycler (Eppendorf). About 100 ng of total DNA from each individual was used as template in PCR reactions of 20 μL. PCR reaction mixture was as follows: 1 U *Taq* Polymerase in 1× reaction buffer (Promega), 20 pmol of each primer, 200 μM of each dNTP and $MgCl_2$ ranging from 1.0 to 2.0 mM. Two amplification reactions were assayed, one for microsatellites *Pde1*, *Pde2*, *Pde8*, *Pde9*, *Pde10*, and *Pde11* (Short method) and another one for *Pde3*, *Pde4*, *Pde5*, *Pde6*, and *Pde7* (Long method). The short method consisted of one cycle at 95 °C for 5 min, 35 cycles at the annealing temperature for 35s, 72 °C for 40s and 94 °C for 40s and a final extension at 72 °C for 10min. The long method consisted of 1min for each step and also a final extension at 72 °C for 10min. Allelic variation was assessed in 21–42 individuals of *P. depressa*, 15–34 of *P. candei* and 10–43 of *P. rustica*. The PCR products were visualized in 2% agarose gels and electrophoresed in 6% acrylamide:bisacrylamide gels (19:1), followed by silver staining and gel fixation (Promega). Allele sizes were characterized using a 20 bp ladder (Takara). Eleven out of 14 loci tested were amplified and polymorphic (*Pde6* was polymorphic only in *P. rustica*). All genetic parameters were estimated using GENEPOP version 3.4 (Raymond and Rousset 1995).

Sixty-seven clones from *P. rustica* (75.3%) contained repeated motifs [44 tetranucleotides: 37 $(GACA)_n$ and 7 $(GATA)_n$, 18 dinucleotides, 4 compound (di+tetra), and 1 mononucleotide]. Thirty-six sequences (54% of all microsatellite-containing sequences; 40.5% of the observed positives in Southern, and 4.37% of the initial plated cultures) were suitable for primer design using Oligo 4.05 (Rychlick and Rhoads 1989), and 27 microsatellite markers rendered an optimal PCR amplification. Allelic variation was assessed in 32 individuals of *P. rustica* from Viana do Castelo (Portugal). Cross-priming tests were carried out on 10 individuals of *P. depressa*, *P. caerulea* and *P. candei* and three individuals of *Patella vulgata*. About 100 ng of DNA per individual were used in a PCR of 20 μL consisting of 1 U *Taq* DNA polymerase in 1× reaction buffer (10 mM Tris-HCl, pH 9; 50 mM KCl, 0.1% Triton X 100, Promega), 20 pmol of each primer, 200 μM of each dNTP and $MgCl_2$ ranging from 1.3 to 1.9 mM. The amplification profile consisted of one cycle at 95 °C for 5min, followed by 35 cycles at the annealing temperature for 40s, 72 °C for 1min and 94 °C for 1min, ending with an extension step at 72 °C for 10min. PCR products were visualized in 2% agarose gels and electrophoresed in acrylamide gels using an ALF express II sequencer (GE Healthcare). Alleles were characterized using internal size standards (80–114–180–230–402 bp; these size standards are usually constructed with PCR products from specific fragments of phage M13), as well as external size controls from initial allele scorings of each marker. ALFWIN FRAGMENT ANALYSER version 1.01 (GE Healthcare) was used to score the genotypes. All genetic parameters were estimated using GENEPOP 3.4.

4.3 Results and Discussion

4.3.1 *Patella depressa*

Three estimators of intraspecific genetic diversity are provided in Table 4.1: observed heterozygosity (H_O ± SD = 0.51 ± 0.16), mean number of alleles per locus (N_a ± SD = 11 ± 5.12) and inbreeding coefficient (– 0.06 < F_{IS} < + 0.72). Genotypic frequencies conformed to Hardy–Weinberg expectations for four loci. The other six loci showed significant heterozygote deficit. Genotypic disequilibrium was not observed for any pair of loci (Fisher's method). The amplification of seven *P. depressa* primer pairs in *P. candei* (N_a ± SD = 3.14 ± 2.09) and five in *P. rustica* (N_a ± SD = 2 ± 1.26, Table 4.2) suggests a cross-species conservation of some sequences and their potential application in other species of the genus *Patella*, especially those more closely related with *P. depressa* (Koufopanou *et al.* 1999; Sá-Pinto *et al.* 2005).

4.3.2 *Patella rustica*

Fifteen out of 27 microsatellites were polymorphic in *P. rustica* (Table 4.3). Observed and expected heterozygosities were calculated using the GENETIX software (Belkhir *et al.* 2004). Test for Hardy–Weinberg were performed using GENEPOP 3.4. The number of alleles per locus ranged from 2 to 27, and the observed heterozygosity ranged from 0.056 to 0.871 (H_O ± SD = 0.48 ± 0.23). Six loci showed a significant deviation from Hardy–Weinberg expectations ($P \leq 0.001$). The influence of a small sample size or another biological phenomenon, such as a Wahlund effect, could be the cause of the Hardy–Weinberg departure. However, the presence of null alleles is a likely explanation since it is a widespread phenomenon in molluscan microsatellites (e.g. Foltz 1986; Li *et al.* 2003; Hedgecock *et al.* 2004; Astanei *et al.* 2005). The frequencies of putative null alleles in these six loci, as estimated by Brookfield's (1996) estimator 1 using MICRO-CHECKER (van Oosterhout *et al.* 2004) were as follows: *Pru5* = 0.1801; *Pru7* = 0.317; *Pru9* = 0.263; *Pru10* = 0.154; *Pru11* = 0.142; *Pru15* = 0.208. Also, loci *Pru9* and *Pru11* showed a high number of alleles with one repeat unit difference, indicating possible stuttering. In Pru11, the linkage of the main motif with a mononucleotide repeat $(GT)_9$-G_{12} can easily explain the existence of alleles with an impaired number of repeats. No cause for the Hardy–Weinberg departure can, however, be proposed with the present data. None of the 105 tests performed between locus pairs to test the genotypic disequilibrium were significant (Fisher's exact test, GENEPOP 3.4); therefore, the 15 polymorphic markers reported here seem to be unlinked. Eighteen loci (including three monomorphic in *P. rustica*) were tested in four other species of *Patella* (Table 3.4). The PCR conditions used were as those mentioned above except the $MgCl_2$

Table 4.1 Characterisation of 11 microsatellite loci from *Patella depressa*.

Locus	Repeat motif	Primer sequences (5'-3')	T (°C)	$MgCl_2$ (nM)	Size range (bp)	N_a	N_i	H_O	H_E	F_{IS}	GenBank accession no.
Pde1	$(GT)_{13}$ G_6	F: GGATTACCCGTAGAAACC	60	1.5	96-106	6	21	0.60	0.64	0.06	DQ822455
		R: ACCTGGTTAGCATTGAGC									
Pde2	$(GT)_{14}$	F: GTCCCATCCGAGCGACTA	60	1.5	89-93	5	21	0.45	0.43	-0.06	DQ822456
		R: ATCCAGAGACCCCCTCAAA									
Pde3	$(GT)_{13}$ G $(GT)_{16}$	F: GTATTATGTCGTCGCCCCTC	55	1.0	140-156	8	21	0.27	0.84	0.37*	DQ822457
		R: ATGCCCCAAAAACAATAGG									
Pde4	$(CA)_7$...$(AC)_6$...$(CA)_5$	F: CTGTAGGCGAACCACAGAC	60	1.5	193-205	5	24	0.50	0.87	0.19	DQ822458
		R: GCATCCCAGGTTAGTATGTAG									
Pde5	$(GT)_4$...$(GT)_6$ $(GT)_9$	F: CGGAGCAGAACAGTAGGAC	55	1.5	176-200	13	23	0.33	0.94	0.72*	DQ822459
		R: CAATCAAATGGGAATAAACAT									
Pde6	$(GTT)_5$	F: AACACGAACACATTACACG	N.D.								DQ822460
		R: AGCTTATACCCACAACAAA									
Pde7	$(GT)_9$	F: CAACAACCAGGGGCGTATT	55	1.5	173-185	12	24	0.64	0.90	0.27*	DQ822461
		R: AGAAATGCGGCCTGTAGGT									
Pde8	$(GT)_2$ A $(GT)_4$ TA	F: CCTGCGTCCACCATAGTT	55	2.0	82-102	12	22	0.59	0.93	0.37*	DQ822462
	$(TG)_6$ G_9	R: GCCTTCGGCCCTATCTC									
Pde9	$(GT)_{23}$	F: CCGGAGAGAATTTGTGCCTA	55	2.0	162-208	21	30	0.77	0.95	0.19	DQ822463
		R. ATCAAGTGGCCTGTGGTTTC									
Pde10	$(TC)_9$ C_2 $(TC)_{15}$	F: TTGCTTGCAGACGAAACATT	55	2.0	192-260	18	25	0.32	0.91	0.65*	DQ822464
		R: CTATGCGCGCAAGTGAATTA									
Pde11	$(TGTC)_3$ $(TC)_{11}$	F: GGAATTTTCCGGTTCCTAGC	55	2.0	242-262	10	42	0.64	0.85	0.59*	DQ822465
		R: ACGAAGTCATTTTGGGACGA									

T, annealing temperature; N_a, number of alleles; N_i, number of individuals successfully amplified; H_O, observed heterozigozity; H_E, expected heterozigozity; F_{IS} fixation index (Weir and Cockerham 1984), significance level * = $P \leq 0.01$

Table 4.2 Allele polymorphism and PCR conditions from cross-species amplification in *Patella candei* and *Patella rustica* using microsatellite primers developed for *Patella depressa*. N.A.- no amplification.

	Patella candei					*Patella rustica*				
Locus	T (°C)	$MgCl_2$ (mM)	Size range (bp)	N_a	N_i	T (°C)	$MgCl_2$ (mM)	Size range (bp)	N_a	N_i
Pde1	55	1.5	108-118	6	34	N.A.				
Pde2	N.A.					60	2.0	140	1	10
Pde3	55	1.0	126-152	6	24	55	1.3	194-208	3	17
Pde4	55	1.5	200-208	4	15	N.A.				
Pde5	55	1.7	220-224	3	24	50	2.0	174	1	10
Pde6	N.A.					55	1.5	114-120	4	16
Pde7	55	1.5	172	1	24	N.A.				
Pde8	N.A.					N.A.				
Pde9	55	2.0	138	1	20	55	2.0	142	1	43
Pde10	55	2.0	174	1	20	N.A.				
Pde11	N.A.					N.A.				

concentration, which was 1.5 mM and the annealing temperature was 50 °C. Since no optimization of the PCR conditions was made on the four species tested, the default figures given here represent the minimal rate of cross-species primer conservation. The cross-priming test across four species of limpets ranged from 13 loci amplifying in *P. depressa*, 11 of them polymorphic, to 10 loci in *P. vulgata*, 3 of which were polymorphic (Table 4.4).

Cross-priming assays were not by all means exhaustive, only comprising a few species. Nevertheless, results suggest cross-species conservation of sequences which is in agreement with inferred phylogenetic relationships within the genus *Patella* (Koufopanou *et al.* 1999; Sá-Pinto *et al.* 2005). However, homology should be confirmed by sequencing flanking regions. Ability to cross-amplify microsatellite markers and the proportion of polymorphic loci were fairly high, although they rapidly declined from more closely related to more distant species. Therefore, the potential utility of these markers for widespread use in limpets was only partly confirmed. In the case of *P. vulgata*, cross-priming tests resulted in poor amplification, with most of the loci being monomorphic or showing very low polymorphism. On the other hand, the microsatellite markers developed from *P. rustica* will likely be useful to address specific questions relevant for conservation of threatened species such as *P. candei* and *P. ulyssiponensis* in the Azores, which are subject to heavy commercial exploitation (Hawkins *et al.* 2000) and *P. ferruginea*, which is the most endangered marine invertebrate species in the western Mediterranean (Espinoza and Ozawa 2006).

In conclusion, most of the microsatellite markers developed in this work

Table 4.3 Characterisation of 18 microsatellite loci from *Patella rustica*. Pru16-18 are monomorphic in *Patella rustica* but polymorphic in other species. ξ, primer labeled with *Cy*5.

Locus	Repeat motif	Primer sequences (5'-3')	T (°C)	$MgCl_2$ (mM)	Size range (bp)	N_a	N_i	H_O	H_E	F_{IS}	GenBank accession no.
Pru1	$(GATA)_{23}$	F: TGTCTTCATGCTTATTAξ	52	1.5	142-242	21	32	0.87	0.85	-0.03	EU255238
		R: TTTTCATCCACTTTTAT									
Pru2	$(GACA)_6$	F: GTACGCTCTAACAAAATξ	52	1.5	175-189	5	32	0.26	0.31	0.19	EU255239
		R: TTCTATACAATCCGATT									
Pru3	$(CGGA)_{12}$	F: ACCCTACAAGTCCAAGAACCξ	55	1.5	128-178	9	32	0.58	0.84	0.32	EU255240
		R: TTTTATACGCCCACATTTTT									
Pru4	$(GACA)_7$	F: AATAAACACCTACACCAAGTξ	55	1.5	171-183	7	32	0.63	0.82	0.26	EU255241
		R: GTCACCCTCCTCTCCTAC									
Pru5	$(CTGT)_8$	F: TAGCTTCGACAGGCAACCξ	58	1.5	177-193	6	32	0.34	0.67	0.50*	EU255242
		R: CGTGTCCCAAAAAGTGTG									
Pru6	$(GT)_5$ AC $(GT)_{15}$ AC $(GT)_4$	F: TCAGATGTAACAGGTTTTAGGξ	52	1.8	97-146	20	32	0.71	0.91	0.24	EU255243
		R: TTGTGGATCAAAGAGGTACT									
Pru7	$(CT)_{18}$ N_{20} $(CT)_{17}$ N_{20} $(CTGT)_6$	F: AATTACTTCACTCTGGGξ	52	1.5	156-302	27	32	0.32	0.94	0.67*	EU255244
		R: GGTTAGTTCCTCTTGTG									
Pru8	$(TCTG)_6$	F: ATCTCAGCATTGGCTCξ	52	1.5	208-218	2	32	0.06	0.05	-0.01	EU255245
		R: AAGTTTGAACATCCTAGC									
Pru9	$(GACA)_{17}$	F: GACCTATATTTCAAGTTTCGξ	58	1.3	128-148	7	32	0.19	0.62	0.70*	EU255246
		R: TATACGCCCACATTTTTA									
Pru10	$(CT)_9$ GT $(CT)_5$... (CA) C_{11} $(CA)_5$	F: GTGCTGACGACCGAGACξ	58	1.5	172-204	10	32	0.45	0.72	0.39*	EU255247
		R: CTATTGCTGGTTTTTATTTTG									
Pru11	$(GT)_{19}$ G_{12}	F: CGACGACTTAGACAAACGAξ	50	1.9	226-236	7	32	0.43	0.67	0.37*	EU255248
		R: TACGGATCAGAAGATTCCAGG									

T, annealing temperature; N_a, number of alleles; N_i, number of individuals successfully amplified; H_O, observed heterozigozity; H_E, expected heterozigozity; F_{IS} fixation index (Weir and Cockerham 1984), significance level * = $P \leq 0.001$.

Table 4.3 Characterisation of 18 microsatellite loci from *Patella rustica* (continued).

Locus	Repeat motif	Primer sequences (5'-3')	T (°C)	$MgCl_2$ (mM)	Size range (bp)	N_a	N_i	H_O	H_E	F_{IS}	GenBank accession no.
Pru12	$(CA)_6$	F: CAGGAGAGGGCATTTTTξ	55	1.5	95-103	6	32	0.66	0.60	-0.09	EU255249
		R: ATGGTTGATAACGCTCG									
Pru13	$(GATA)_{18}$	F: ATAAAGACGGCAGTGAGATAξ	55	1.5	102-134	10	32	0.81	0.88	0.10	EU255250
		R: GTCTCCAAGTATTAGGATGAAT									
Pru14	$(CTGT)_{11}$	F: GTGAGTGGAATGTCTGTATGACξ	52	1.8	90-122	6	32	0.45	0.68	0.35	EU255251
		R: GCATTGACCTAATAAACACATA									
Pru15	$(GTCT)_{16}$	F: TAGCCAATGAAACGGTCξ	48	1.5	328-348	8	32	0.39	0.74	0.49*	EU255252
		R: TGTCCCAAAAAGTGTGA									
Pru16	$(GATA)_{39}$	F: GCGACATTGTTTTCACATξ	55	1.5	301	1	31	-	-	-	EU255253
		R: CCCCATTTCTCTCCCTAT									
Pru17	$(GACA)_9$	F: CGTTATAGTGTCCCAAAAAGTξ	58	1.5	247	1	32	-	-	-	EU255254
		R: ATTCTCTCAAACCCCATCCAG									
Pru18	$(TG)_{23}$	F: AGTCACCAAGGATAAATGξ	50	1.5	116	1	32	-	-	-	EU255255
		R: CCTGTAGGCGACACA									

T, annealing temperature; N_a, number of alleles; N_i, number of individuals successfully amplified; H_O, observed heterozigozity; H_E, expected heterozigozity; F_{IS} fixation index (Weir and Cockerham 1984), significance level * = $P \leq 0.001$.

Table 4.4 Locus polymorphisms from cross-species amplification of 18 microsatellites of *Patella rustica* in four limpet species. Na, number of alleles scored; N.A., no amplification; N.D., not determined.

	Patella depressa		*Patella candei*		*Patella caerulea*		*Patella vulgata*	
Locus	Size range (bp)	N_a	Size range (bp)	N_a	Size range (bp)	N_a	Size range (bp)	N_a
Pru1	194	1	149-155	2	153-161	4	188-194	3
Pru2	N.A.		N.A.		142-162	6	N.A.	
Pru3	157-165	3	155-165	5	N.D.		N.A.	
Pru4	174-184	4	N.D.		175/179	1	175/179	1
Pru5	194-206	4	190	1	161-195	7	186	1
Pru6	150	1	158-188	7	N.A.		N.A.	
Pru7	175-195	4	172-188	5	N.A.		N.A.	
Pru8	175-201	5	197	1	183-201	4	189-193	2
Pru9	N.A.		N.A.		N.A.		N.A.	
Pru10	178-202	7	156-180	5	156-182	6	190	1
Pru11	108-130	3	148	1	120-126	3	220	1
Pru12	110-116	3	N.A.		112-114	2	108	1
Pru13	N.A.		130	1	N.A.		N.A.	
Pru14	N.D.		N.D.		N.D.		105-119	3
Pru15	264-272	4	264	1	184-218	7	267	1
Pru16	N.A.		195-233	5	205-243	5	N.A.	
Pru17	223-229	3	217	1	198	1	194	1
Pru18	112-132	6	N.A.		114-134	5	N.A.	

represent a useful tool for population genetic studies of northeastern Atlantic and Mediterranean limpet species. These markers could be used to infer present day patterns of population connectivity and gene flow in natural populations. In particular, such high-resolution genetic markers would allow investigating detailed aspects of limpet population demography at their range limits, such as northward colonization potential of southern species (*P. depressa*, *P. rustica* and *P. ulyssiponensis*) or vulnerability of northern species (*P. vulgata*) to local extinction at the southern end of the range, under the present climate change scenario. Indirect estimates of spatial dispersal scales provided by microsatellite data could also be used to assess the efficiency of management strategies of marine species with planktonic dispersive stages (using limpets as model species) and would provide very important information which could be used in the design of marine reserves.

Chapter 5

Population genetic structure of *Patella depressa* throughout its geographic range as revealed by microsatellite polymorphism

5.1 Introduction

Knowledge of temporal and spatial scales of demographic connectivity remains a central issue in marine ecology. It has implications on diverse topics such as population dynamics (Roughgarden *et al.* 1988; Eckman 1996; Armsworth 2002; Kritzer and Sale 2004), biological invasions (Dupont *et al.* 2007; Herborg *et al.* 2007; Zardi *et al.* 2007), species response to climate change (Harley *et al.* 2006), and the design of marine protected areas (Sala *et al.* 2002; Palumbi 2004; Sale *et al.* 2005; Hastings and Botsford 2006). For many marine species possessing a sessile or sedentary adult phase, larval drift and recruitment are the key processes shaping patterns of population connectivity. Direct measurements of dispersal are difficult and have rarely been accomplished, because of the minute size of most marine larvae, difficulties in identification of non-calcified species via morphological characteristics, and the high mortality to which larvae are subject in the ocean (Levin 1990; Thorrold *et al.* 2002; Jones *et al.* 2005; Becker *et al.* 2007). Geographic patterns of genetic variation have often been used to provide indirect estimates of demographic exchange between populations (e.g. Palumbi 1995; Bossart and Prowell 1998; Palumbi 2003).

Many studies have addressed the link between population genetic connectivity and dispersal capability of larvae and propagules in marine systems (reviewed in Palumbi 1995; Grosberg and Cunningham 2001; Hellberg *et al.* 2002; Palumbi 2003). Marine species are typically described as showing less spatial genetic differentiation than terrestrial (Caley *et al.* 1996; Carr *et al.* 2003; Kinlan and Gaines 2003), freshwater and migratory species (Ward *et al.* 1994). Their large population sizes, the existence of a pelagic larval phase in the majority of life cycles ensuring high dispersal potential, even in species with sessile or sedentary adults, and the absence of geographical barriers in the ocean promote a high degree of connectivity among distant populations,

thus playing a key role in homogenising gene frequencies and precluding allopatric speciation (Palumbi 1992, 1994).

While many studies have confirmed the expectations of lack of population structure in species with potential for long-distance dispersal (reviewed in Palumbi 1995; Bohonak 1999; Kinlan *et al.* 2005), others reported more genetic differentiation than could be anticipated by the purported lack of barriers and high dispersal capability of many species (Burton and Lee 1994; Palumbi 1995; Palumbi *et al.* 1997; Todd 1998; Huvet *et al.* 2000; Goldson *et al.* 2001). The dispersive capacity of marine larvae is currently being questioned, as growing evidence suggests that a high proportion of offspring settles close to their natal location (Knowlton and Keller 1986; Jones *et al.* 1999; Swearer *et al.* 1999; Cowen *et al.* 2000; Swearer *et al.* 2002). Besides self-recruitment, other factors contribute to increased levels of population structure in the marine environment, by hampering larval movements. These include the existence of hydrodynamic barriers, such as currents or upwellings (Barber *et al.* 2000; Perrin *et al.* 2004; Sotka *et al.* 2004), physical gradients, like for example sea water temperature and salinity (Sponaugle *et al.* 2002) or habitat discontinuities (Riginos and Nachman 2001; Johansson *et al.* 2008). Finally, high dispersal rates do not always lead to reduced genetic differentiation, since high fecundities in many broadcast spawning species are often associated with a very high variance in reproductive success (Hedgecock 1994a, 1994b; Li and Hedgecock 1998), which can greatly reduce effective population size and promote local genetic drift (Hedrick 2005a). Acting isolated or in conjunction, all these factors define the level and directionality of gene flow and, together with historical demography and selection, shape the population genetic structure of marine species (Brown *et al.* 2001; Wares 2001; Dufresne *et al.* 2002; Duran *et al.* 2004a; Bilodeau *et al.* 2005; Hemmer-Hansen *et al.* 2007).

Microsatellites have proved useful in detecting weak levels of population structure in marine species characterized by large populations and high gene flow (Launey *et al.* 2002; Purcell *et al.* 2006; Ruzzante *et al.* 2006). Moreover, the use of these neutral, high-resolution markers allow the inference of levels of population differentiation driven solely by genetic drift and gene flow, disregarding selection and historical events, thus enabling the assessment of contemporary connectivity patterns between locations. This study focuses on the characterization of the population genetic structure of the limpet *Patella depressa*, based on microsatellite length polymorphism, aiming to assess levels of gene flow throughout most of the species range. There are several reasons to consider limpet species as adequate models to study dispersal and connectivity in the northeast Atlantic. They are broadcast spawners, with very large populations and broad geographic ranges. Additionally, large-scale movement is restricted to the planktonic larval stage, since adults are sedentary and there are no records of rafting in these species (except perhaps for the kelp-dwelling *Patella pellucida*), suggesting that gene

flow among locations is achieved by larval drift alone. The fact that this species has no commercial value is also an advantage, since it decreases the risk of genetic population structure being influenced by harvesting. Conversely, *P. depressa* can serve as proxy for commercially exploited or endangered species with similar life-history traits, including other patellid limpet species such as *Patella ulyssiponensis*, *Patella candei* and *Patella ferruginea* (Weber *et al.* 1998; Hawkins *et al.* 2000; Weber and Hawkins 2002, 2005; Casu *et al.* 2006; Espinoza and Ozawa 2006).

Specific aims of this study included investigating if gene flow is homogeneous across the study area, identifying areas where limited gene flow may occur and also inferring the factors which impose those restrictions to genetic connectivity. The geographical distribution of *P. depressa* spans approximately 40 degrees of latitude, from northern Wales to Senegal (Fischer-Piètte and Gaillard 1959; Christiaens 1973; Southward *et al.* 1995; Ridgway *et al.* 1998). It encompasses several oceanographic regions in the eastern North Atlantic, with distinct hydrodynamic properties and considerable coastline and topographic irregularities (reviewed by Mason *et al.* 2006). Moreover, extensive areas of unsuitable habitat are scattered throughout the species range, namely along the northwest coast of Africa, the Gulf of Cadiz, the central Portuguese coast and the southern French coast. All these features may represent effective barriers to larval dispersal and may thus impose limits to gene flow in *P. depressa,* detectable as changes in allele frequencies, which can be more or less sharp depending on the 'leakiness' of the barrier. On the other hand, if dispersal is homogeneous across space, with no barriers to gene flow within the distribution range, a large panmictic population or a pattern of isolation-by-distance are likely to occur, depending on the dispersal capabilities of the species relative to its distribution range (Wright 1943).

5.2 Material and Methods

5.2.1 Sample collection and DNA extraction

Specimens used in this analysis were collected between June 2003 and March 2005, from 19 sites distributed throughout the NE Atlantic, from SW England to Morocco (Figure 5.1 and Table 5.1), an area which practically covers the range of this species. Samples consisting of approximately 30 adult limpets were collected from mid-intertidal and stored in absolute ethanol until DNA extraction. To reduce the chance of sampling across different age cohorts, all collected individuals were of similar size, reflecting the dominant cohort of the populations. The identification of each individual was confirmed in the laboratory through the examination of morphological characters

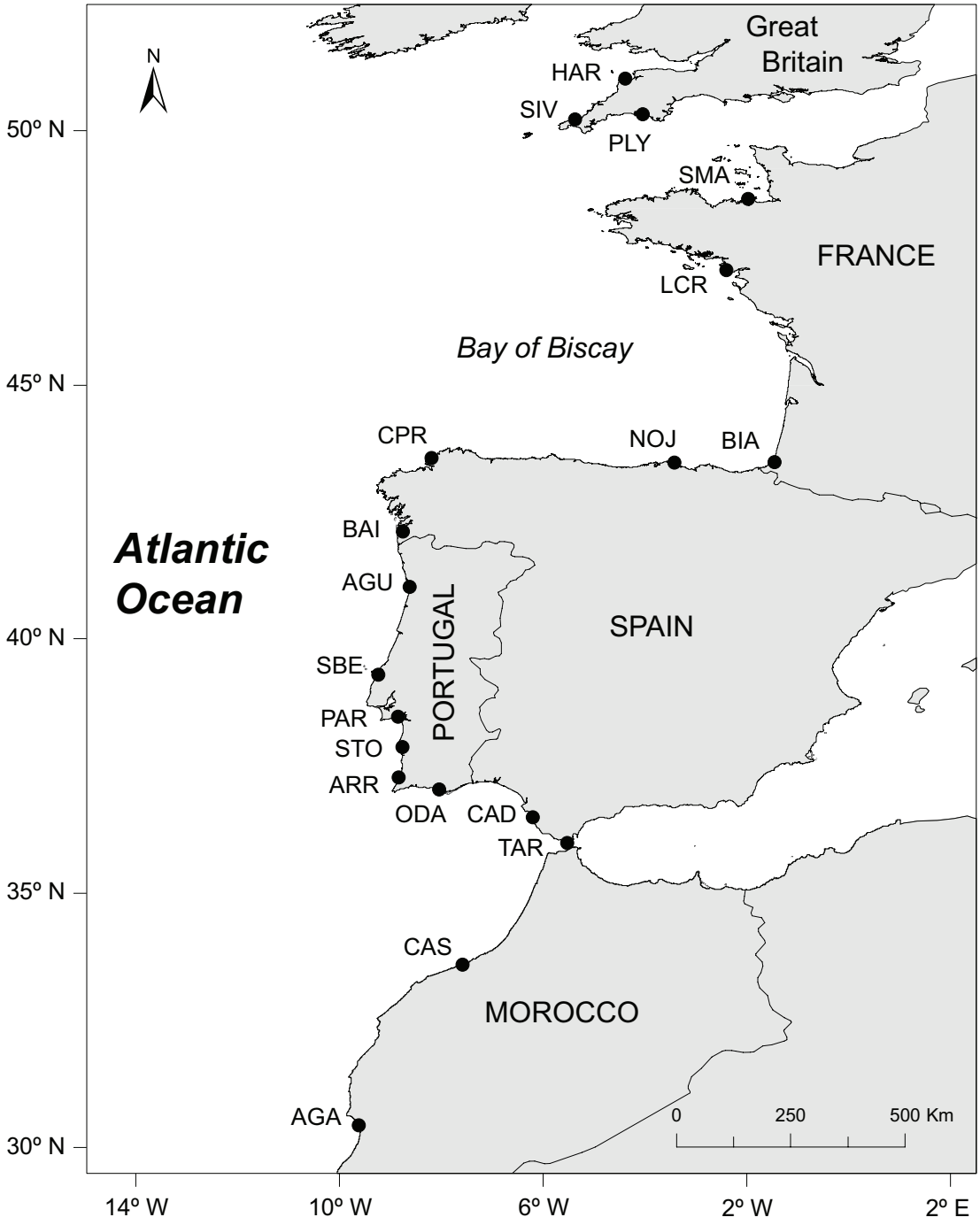

Figure 5.1 Geographical location of *Patella depressa* sampling sites. Sample codes are provided in Table 5.1.

(shell, foot colour, and in the most dubious cases, radular teeth following Fischer-Piètte and Gaillard (1959). After positive identification, each individual was dissected, and the foot was individually stored in absolute ethanol at room temperature. Total DNA was isolated from a small piece of foot muscle following standard extraction procedures (Sambrook *et al.* 1989), modified with the inclusion of a step for the precipitation of

Table 5.1 Location names, sample codes, geographical coordinates and number of individuals per sample.

Location	Sample code	Coordinates (latitude/longitude)	Sample size
Hartland	HAR	51° 02' N / 04° 30' W	32
St. Ives	SIV	50° 13' N / 05° 29' W	31
Plymouth	PLY	50° 21' N / 04° 09' W	29
St. Malo	SMA	48° 41' N / 02° 05' W	31
Le Croisic	LCR	47° 17' N / 02° 32' W	32
Biarritz	BIA	43° 29' N / 01° 34' W	31
Noja	NOJ	43° 30' N / 03° 32' W	31
Cabo Prior	CPR	43° 34' N / 08° 19' W	31
Baiona	BAI	42° 07' N / 08° 51' W	31
Aguda	AGU	41° 02' N / 08° 40' W	31
S. Bernardino	SBE	39° 18' N / 09° 21' W	31
Portinho da Arrábida	PAR	38° 29' N / 08° 58' W	31
S. Torpes	STO	37° 55' N / 08° 48' W	31
Arrifana	ARR	37° 17' N / 08° 52' W	29
Olhos d'Água	ODA	37° 05' N / 08° 11' W	30
Cádiz	CAD	36° 32' N / 06° 18' W	33
Tarifa	TAR	36° 00' N / 05° 36' W	31
Casablanca	CAS	33° 35' N / 07° 42' W	31
Agadir	AGA	30° 31' N / 09° 41' W	30

mucopolysaccharides (Sokolov 2000). After ethanol precipitation, the DNA was resuspended in 80 µL ultrapure water and stored at -20 °C. Extracted DNA was tested by electrophoresis of 2 µL of the DNA solution mixed with 2 µL loading buffer on a 0.8% agarose gel during 30min at 140 V, and visualization under UV light, after gel staining with ethidium bromide. Diluted aliquotes to be used in the assay were made from stock DNA solutions according to estimated individual concentrations.

5.2.2 Molecular analysis

Genetic variation within and among locations was assessed at five of the most polymorphic microsatellite loci developed for *P. depressa* (*Pde1*, *Pde3*, *Pde5*, *Pde7* and *Pde9*) selected among the set of available markers (see chapter 4). Selected loci included one compound di/mononucleotide repeat (*Pde1*), and four dinucleotide repeats, two of them interrupted (*Pde3* and *Pde5*) and two perfect (*Pde7* and *Pde9*). The remaining available loci were excluded from the assay due to severe amplification and screening difficulties.

Since the preliminary assay of microsatellites described in chapter 4 was conducted at a different laboratory, polymerase chain reaction (PCR) conditions for genotyping had to be once again optimized prior to this study (Table 5.2). Each 15-μL PCR reaction mixture contained 1 μL of template DNA, 0.5 U of *Taq* polymerase (Amplitaq Gold DNA Polymerase, Applied Biosystems), 10× PCR buffer at 1× concentration (GeneAmp PCR Gold Buffer- 15 mM Tris-HCl, 50 mM KCl; pH 8.0 at room temperature), 0.2 mM of each dNTP, 0.5 μM of each primer, $MgCl_2$ ranging from 1.5 to 2.0 mM and ultrapure water to adjust final volume. Thermal cycling conditions for PCR amplification consisted of one *Taq* polymerase activation step done at 95 °C for 10min, followed by 35 cycles of 30s at 95 °C, 40s at the primer-specific optimal annealing temperature and 40s at 72 °C, plus a final extension step of 10min at 72 °C.

The data collection and sizing of alleles procedure was not the same for all loci. This was because during the assay an automated sequencer with much higher output became available. The forward primers for loci Pde1, Pde3 and Pde7 were end-labelled with the fluorescent dye *Cy5*, visualized in 2% agarose gels and allele size was determined by electrophoresis in 6% acrylamide gels using an ALFexpress II automated sequencer (GE Healthcare). Although scoring errors can never be completely avoided, a few measures were adopted in order to minimize them. Thus, alleles were characterized using internal size standards (80–114–180–230–402 bp, constructed with PCR products from specific fragments of phage M13). Additionally, to ensure scoring consistency among gel runs, at least two control individuals of known genotype were included on each gel. Allele scoring used ALFwin Fragment Analyser version 1.02 software (GE Healthcare). Each gel was independently read by two people, and unequal readings were re-checked and corrected. Forward primers of loci *Pde5* and *Pde9* were labelled with NED and 6-FAM (Applied Biosystems), respectively. The PCR amplification products of these loci were visualized in 2% agarose gels and allele sizes were estimated by capillary electrophoresis on an ABI 3130XL automated sequencer (Applied Biosystems). To ensure accuracy of sizing, alleles were scored in comparison to an internal size standard (ROX 350, Applied Biosystems), using GeneMapper version 4.0 software (Applied Biosystems).

5.2.3 Statistical analyses

Genetic diversity was estimated per locus and per sampling site as allele frequency distributions, number of alleles, observed (H_O) and expected (H_E) heterozygosities, using the software GENEPOP 4.0 (Rousset 2008). To eliminate bias due to differences in sample size, allelic richness per locus by sample and overall was estimated for the loci in which no missing values were found, by rarefaction to the smallest sample size (Petit *et al.* 1998; Leberg 2002) using FSTAT 2.9.3.2 (Goudet 2001). The inbreeding

Table 5.2 Characteristics of five microsatellite loci used in the study, including primer sequences, PCR annealing temteratures (*T*), allele sizes, number of alleles (N_a), repeat motifs and GenBank accession numbers of the original sequenced clones. Allelic information was based on previous screening of a subset of individuals (see chapter 4). Labelled primers are indicated by ξ (*Cy5*), † (NED) or ‡ (FAM).

Locus	Primer sequences (5'-3')	*T* (°C)	$MgCl_2$ (mM)	Size (bp)	N_a	Repeat motif	GenBank Accession no.
Pde1	F: GGATTACCCGTAGAAACCξ	53	2.0	96-106	6	$(GT)_{13}$ $(G)_6$	DQ822455
	R: ACCTGGTTAGCATTGAGC						
Pde3	F: GTATTATGTCGTCGCCCCTCξ	56	1.5	140-156	8	$(GT)_{13}$ G $(GT)_{16}$	DQ822457
	R: ATGCCCCAAAAACAATAGG						
Pde5	F: CGGAGCAGAACAGTAGGAC†	55	1.8	176-200	13	$(GT)_4$…$(GT)_6(GT)_9$	DQ822459
	R: CAATCAAATGGGAATAAACAT						
Pde7	F: CAACAACCAGGGGCGTATTξ	53	2.0	173-185	12	$(GT)_9$	DQ822461
	R: AGAAATGCGGCCTGTAGGT						
Pde9	F: CCGGAGAGAATTTGTGCCTA‡	55	1.8	162-208	21	$(GT)_{23}$	DQ822463
	R: ATCAAGTGGCCTGTGGTTTC						

coefficient (F_{IS}, Wright 1951), estimated by *f* (Weir and Cockerham 1984) was also computed with GENEPOP 4.0. Deviations from Hardy-Weinberg expectations (HWE) and genotypic linkage disequilibrium between pairs of loci were assessed through exact tests, with significance determined by a Markov chain Monte Carlo method (Guo and Thompson 1992) as implemented in GENEPOP, with specific parameters of 10,000 dememorization steps followed by 500 batches of 5,000 iterations. To correct for increased type I error, significance values from multiple comparisons were adjusted using the sequential Bonferroni correction procedure with $\alpha = 0.05$ (Rice 1989).

Departures from HWE may be caused by technical issues, which can be minimised, but never completely avoided during genetic assays. The most relevant of these and that may bias results more significantly, include scoring errors caused by large allele drop-out, stuttering and the presence of null alleles. In cases where significant deviations from HWE occurred, the program MICRO-CHECKER version 2.2.3 (van Oosterhout *et al.* 2004) was used to check microsatellite data and infer most probable technical cause for these departures. Null allele frequencies were estimated according to Brookfield's (1996) estimator 1, a function implemented in MICRO-CHECKER. This estimator was selected because amplification failures were considered artifacts, rather than null allele homozygotes (Brookfield 1996).

Exact tests of allelic (genic) and genotype (genotypic) frequencies differentiation among pairs of samples were performed with GENEPOP 4.0 (Markov chain parameters: 10,000 dememorization steps followed by 500 batches of 5,000 iterations). The choice

of a suitable statistic for quantifying genetic differentiation among samples when using microsatellite loci is a matter of considerable debate (see chapter 4), because it depends on which mutational model is applied (e.g. Estoup *et al.* 1995; Estoup and Angers 1998; Pascual *et al.* 2000; 2001; Balloux and Lugon-Moulin 2002; Duran *et al.* 2004b). Methods assuming an infinite allele model of mutation (IAM, Kimura and Crow 1964) using F_{ST} (Wright 1951), or a stepwise mutation model (SMM, Kimura and Ohta 1978) using R_{ST} (Slatkin 1995) have been routinely employed. In view of this, and for purpose of comparison, genetic differentiation among samples was quantified using both measures, F_{ST} and R_{ST}, estimated respectively by θ (Weir and Cockerham 1984) and ρ_{ST} (Michalakis and Excoffier 1996). Moreover, size homoplasy may cause a decline in F_{ST}-based estimates of population differentiation, in the presence of large effective population sizes and high mutation rates (Estoup *et al.* 2002). The concomitant use of F_{ST} and R_{ST}, a measure which is less sensitive to homoplasy (O'Reilly *et al.* 2004), will provide greater consistency to estimates of population differentiation. Estimates of F_{ST} were calculated using MICROSATELLITE ANALYSER (Dieringer and Schlötterer 2003) and those of R_{ST} using ARLEQUIN 3.1 (Excoffier *et al.* 2005). Statistical significance of pairwise values was determined by 10,000 permutations of the data. For all multiple comparisons *P*-values were adjusted using sequential Bonferroni corrections (Rice 1989).

Genetic affinities among sampling locations were further assessed by reconstruction of a neighbour-joining tree (Saitou and Nei 1987) with topology based on pairwise chord distance (Cavalli-Sforza and Edwards 1967) computed from allele frequencies and statistical support of tree nodes provided by 1,000 bootstrapped data sets. This distance measure was selected because it makes no assumption regarding constant population size or mutation rates among loci, and therefore is particularly suitable to reconstruct tree topologies based on microsatellite data (Takezaki and Nei 1996). Analyses were performed using programs SEQBOOT, GENDIST, NEIGHBOR and CONSENSE included in the package PHYLIP 3.67 (Felsenstein 2004).

In addition to traditional *F*-statistics and genetic distance, recent Bayesian methods of identifying genetic structure were applied in an attempt to group genetically homogeneous clusters of samples. Among the several software programs available for clustering analysis, BAPS 5.1 (Corander and Marttinen 2006) was selected because it performs very well in identifying the number of subpopulations at low levels of differentiation, and also for its considerably higher computational efficiency in comparison to other programs (Latch *et al.* 2006). The software BAPS also allows clustering at different hierarchical levels, from the individual to sampling locations or populations, which increases statistical power, especially when the number of screened loci is low or genetic differentiation is weak (Corander *et al.* 2006). Moreover, it can incorporate geographical information provided by the sampling design. Three analyses

were performed with BAPS: 1) a spatial analysis at the individual level, 2) a spatial analysis at the sampling location level and 3) a non-spatial analysis at the sampling location level.

The relationship between genetic and geographical distance (isolation-by-distance, Wright 1943; Slatkin 1993) was verified using Mantel tests of correlation among matrices (Mantel 1967), implemented in the software IBD (Bohonak 2002). To assess whether this relationship was stronger under the IAM or the SMM, two sets of pairwise genetic distances were computed, $F_{ST} / (1 - F_{ST})$ and $R_{ST} / (1 - R_{ST})$ (Rousset 1997), and correlated with log-transformed geographical distances between sampling localities (measured as the shortest distance by sea, using GRASS 6.0 Geographical Information System, (GRASS Development Team 2006). The strength of the relationship was tested by regression analysis, also using the program IBD.

5.3 Results

5.3.1 Genetic diversity, Hardy-Weinberg equilibrium and null alleles

All microsatellite loci screened in this study showed high levels of polymorphism (Table 5.3). Over all samples, locus *Pde1* exhibited the lowest variation (13 alleles) and locus *Pde5* the highest (49 alleles). Among samples, the average number of alleles across loci was fairly even and with no visible spatial trend, ranging between 11.2 (at Tarifa, Spain) and 15.2 (at Cadiz, Spain). Average values of allelic richness (after rarefaction to a sample size of 13 diploid individuals, or 26 genes) ranged between 9.4 at Arrifana (Portugal) and 12.0 at Olhos d'Água (Portugal). Estimates of allelic richness across populations varied between 6.2 (for locus *Pde1*) and 16.5 (for locus *Pde5*). Overall, observed heterozygozities ranged from 0.22 in *Pde1* to 0.74 at locus *Pde9*, whilst within samples and across loci, estimates ranged between 0.45 (at Saint-Malo, France) and 0.58, at Casablanca (Morocco). Values of expected heterozygozity varied between 0.58 for locus *Pde1* and 0.93 for *Pde5,* and average values within samples ranged from 0.67 at Saint-Malo to 0.86, at Aguda, São Bernardino and Portinho d'Arrábida (all Portugal).

All loci can be considered independent estimates of genetic differentiation, since no linkage disequilibrium was detected among them. Of the 190 pairwise comparisons performed within samples, only 2 were significant at $P < 0.05$ (none of them remaining significant after Bonferroni correction for multiple comparisons), and none of the global tests was significant (all tests, $P > 0.05$, data not shown). Significant multilocus departures from HWE, indicated by significant inbreeding coefficients (F_{IS}) were detected in all samples (Table 5.3). Multilocus estimates of F_{IS} ranged between 0.30

Table 5.3 Genetic diversity estimates for five microsatellite loci in *Patella depressa*. *N* - number of genotypes; N_a - number of alleles; *A* - allelic richness; H_o - observed heterozigosity; H_E - expected heterozigosity; F_{IS} - inbreeding coefficient; *r* - null allele frequency estimate (estimator 1, Brookfiels 1996). Significant values after Bonferroni correction are depicted in bold type.

Locus	HAR	SIV	PLY	SMA	LCR	BIA	NOJ	CPR	BAI	AGU	SBE	PAR	STO	ARR	ODA	CAD	TAR	CAS	AGA	Global
Pde1																				
N	31	28	29	27	31	29	28	31	31	30	30	31	31	30	30	31	31	31	30	570
N a	6	5	8	4	3	5	5	7	9	9	6	7	10	6	6	8	6	8	7	13
A	4.886	3.882	5.995	3.419	2.894	4.276	4.623	5.477	6.756	7.314	5.757	5.902	7.273	4.815	5.038	5.925	3.839	6.257	5.653	6.205
H_O	0.129	0.179	0.379	0.074	0.000	0.345	0.107	0.323	0.355	0.200	0.333	0.161	0.161	0.267	0.233	0.258	0.129	0.290	0.267	0.221
H_E	0.480	0.421	0.711	0.299	0.415	0.438	0.576	0.555	0.759	0.692	0.720	0.647	0.727	0.698	0.573	0.695	0.242	0.701	0.690	0.581
F_{IS}	**0.735**	**0.580**	**0.471**	**0.756**	**1.000**	0.216	**0.817**	**0.423**	**0.537**	**0.714**	**0.542**	**0.754**	**0.781**	**0.622**	**0.597**	**0.633**	0.470	**0.590**	**0.617**	**0.625**
r	0.233	0.166	0.188	0.170	0.290	-	0.293	0.144	0.224	0.286	0.220	0.291	0.323	0.249	0.211	0.253	-	0.237	0.245	
Pde3																				
N	32	28	29	27	23	29	31	29	27	31	28	30	31	22	30	30	31	30	0	518
N a	9	10	9	8	8	8	9	10	7	12	10	10	10	8	10	10	9	7	n.d.	14
A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H_O	0.656	0.679	0.621	0.556	0.609	0.655	0.516	0.517	0.519	0.419	0.429	0.700	0.677	0.682	0.733	0.600	0.452	0.633	n.d.	0.592
H_E	0.772	0.786	0.791	0.629	0.830	0.733	0.787	0.769	0.759	0.836	0.851	0.819	0.810	0.823	0.805	0.816	0.742	0.814	n.d.	0.787
F_{IS}	0.152	0.139	0.218	0.119	0.271	0.108	0.348	0.331	**0.321**	**0.503**	**0.501**	0.148	0.166	0.175	0.091	0.268	**0.395**	0.224	n.d.	**0.253**
r	-	-	-	-	-	-	-	-	0.130	0.221	0.222	-	-	-	-	-	0.161	-	n.d.	
Pde5																				
N	25	26	20	21	30	29	29	17	28	31	26	19	29	30	25	30	13	15	27	470
N a	19	21	17	17	24	20	21	14	20	19	18	20	20	16	22	23	14	15	18	49
A	14.428	15.141	14.320	14.101	15.798	15.213	15.455	12.780	13.526	14.321	13.674	16.594	14.421	9.924	16.917	14.915	14.000	14.022	13.069	16.543
H_O	0.280	0.346	0.350	0.381	0.367	0.276	0.448	0.235	0.500	0.387	0.231	0.263	0.276	0.400	0.320	0.567	0.538	0.467	0.407	0.370
H_E	0.941	0.929	0.940	0.942	0.950	0.951	0.954	0.927	0.922	0.944	0.938	0.960	0.946	0.675	0.966	0.944	0.920	0.945	0.910	0.926
F_{IS}	**0.7068**	**0.6321**	**0.6336**	**0.6015**	**0.618**	**0.7136**	**0.5345**	**0.7519**	**0.4623**	**0.5939**	**0.7577**	**0.7313**	**0.7119**	0.4117	**0.6732**	**0.4039**	0.4247	**0.5149**	**0.5569**	**0.604**
r	0.334	0.296	0.296	0.281	0.293	0.341	0.253	0.345	0.213	0.281	0.359	0.347	0.339	-	0.322	0.188	-	0.233	0.257	

Table 5.3 Genetic diversity estimates for five microsatellite loci in *Patella depressa* (continued).

Locus	HAR	SIV	PLY	SMA	LCR	BIA	NOJ	CPR	BAI	AGU	SBE	PAR	STO	ARR	ODA	CAD	TAR	CAS	AGA	Global
Pde7																				
N	32	31	25	30	32	31	31	31	31	31	31	30	31	30	30	31	31	30	30	579
N a	10	12	12	12	14	14	12	12	11	12	14	14	14	11	13	11	12	12	11	23
A	8.501	9.664	10.217	7.796	9.558	10.274	9.636	8.892	8.379	9.557	10.221	11.031	10.386	8.818	10.367	8.817	10.186	8.734	8.317	9.655
H_O	0.500	0.548	0.640	0.467	0.625	0.645	0.581	0.613	0.613	0.677	0.581	0.700	0.613	0.667	0.600	0.516	0.839	0.600	0.567	0.610
H_E	0.869	0.874	0.871	0.572	0.860	0.896	0.889	0.876	0.830	0.886	0.857	0.910	0.893	0.874	0.892	0.863	0.901	0.839	0.853	0.858
F_{IS}	**0.429**	**0.377**	**0.269**	**0.186**	**0.276**	0.283	**0.351**	**0.304**	**0.265**	**0.239**	**0.326**	**0.234**	**0.317**	**0.240**	**0.331**	**0.406**	0.070	**0.288**	**0.339**	**0.293**
r	0.192	0.168	-	-	-	0.126	0.157	-	-	-	0.142	0.103	0.142	-	0.147	0.180	-	-	-	
Pde9																				
N	31	31	28	24	30	29	31	29	30	30	27	21	27	29	28	32	21	27	28	533
N a	21	15	17	17	21	22	20	19	19	22	24	16	21	17	21	24	15	20	18	32
A	14.56	11.355	12.475	13.336	15.32	15.559	14.161	14.075	14.066	14.732	16.198	13.353	14.579	14.028	15.535	15.826	12.395	15.007	14.195	14.774
H_O	0.710	0.677	0.750	0.792	0.700	0.862	0.871	0.690	0.800	0.667	0.889	0.619	0.926	0.655	0.714	0.625	0.524	0.926	0.607	0.737
H_E	0.945	0.904	0.918	0.929	0.947	0.955	0.939	0.941	0.936	0.940	0.953	0.935	0.934	0.946	0.955	0.953	0.929	0.950	0.940	0.939
F_{IS}	0.252	0.254	0.185	0.151	0.264	0.099	0.073	0.270	**0.150**	**0.294**	**0.069**	0.343	0.008	0.311	0.256	0.348	**0.442**	0.026	0.358	**0.217**
r	-	-	-	-	-	-	-	0.122	-	0.134	-	0.154	-	0.142	-	0.162	0.201	-	0.164	0.093
All loci																				
N	32	31	29	31	32	31	31	31	31	31	31	31	31	30	30	33	31	31	30	588
N a	13	13	13	12	14	14	13	12	13	15	14	13	15	12	14	15	11	12	14	26
A	10.594	10.011	10.752	9.663	10.893	11.331	10.969	10.306	10.682	11.481	11.463	11.720	11.665	9.396	11.964	11.371	10.105	11.005	10.309	11.794
H_O	0.455	0.486	0.548	0.454	0.460	0.557	0.505	0.476	0.557	0.470	0.493	0.489	0.531	0.534	0.520	0.513	0.496	0.583	0.462	0.506
H_E	0.801	0.783	0.846	0.674	0.800	0.795	0.829	0.814	0.841	0.860	0.864	0.854	0.862	0.803	0.838	0.854	0.747	0.850	0.848	0.818
F_{IS}	**0.4221**	**0.3764**	**0.340 €**	**0.3167**	**0.4371**	**0.3028**	**0.3874**	**0.385**	**0.3412**	**0.4571**	**0.4313**	**0.4132**	**0.3945**	0.349	**0.3739**	**0.4037**	0.3154	**0.3**	**0.4589**	**0.3792**

and 0.46, showing heterozygote deficiencies in all cases. The incidence of significant deviations from HWE was very different at each locus. On the one hand, loci *Pde3*, *Pde7* and *Pde9* showed significant heterozygote deficiencies in only some of the samples; on the other hand, loci *Pde1* and *Pde5* consistently exhibited significant departures from HWE across samples. Heterozygote deficiency is commonly the result of inbreeding, population substructure (Wahlund effect) and the presence of null alleles. Although MICRO-CHECKER inferred the presence of null alleles at all loci (Table 5.3), the variable pattern of heterozygote deficits across samples observed at loci *Pde3*, *Pde7* and *Pde9* seem to rule out this hypothesis. However, null alleles are the most probable cause for the widespread excess of homozygotes observed at loci *Pde1* and *Pde5* (inferred frequencies ranging between 0.14-0.32 and 0.19-0.36, respectively). Therefore, these two loci were excluded from further analyses.

5.3.2 Population structure

Pairwise exact tests of allelic differentiation among samples using Fisher's combined method resulted in 17 significant comparisons (out of 171), after sequential Bonferroni correction. Exact tests at the genotypic level showed very weak differentiation, with only 7 tests yielding significant results (Table 5.4). Estimates of population differentiation were low, as predicted given the high degree of variability of microsatellite loci (Hedrick 1999). The three loci contributed unequally to between-sample differentiation using estimates based on allele frequencies (F_{ST}). Global single locus F_{ST} estimates (and respective *P*-values) were 0.008 ($P = 0.019$) for *Pde3*, 0.015 ($P = 0.001$) for *Pde7* and 0.001 ($P = 0.226$) for *Pde9*. The multilocus estimate of F_{ST} was 0.008 ($P = 0.0001$). Pairwise multilocus F_{ST} values ranged between -0.012 and 0.100. Only 14 pairwise tests were significant after sequential Bonferroni correction, all of them involving comparisons with the sample from Saint-Malo (Table 5.5). Levels of population differentiation obtained with *R*-statistics (based on allele sizes) were even lower. Single locus estimates were -0.004 for *Pde3*, 0.019 for *Pde7* and -0.008 for *Pde9* and multilocus R_{ST} was -0.0011 (significant only for *Pde7,* with $P = 0.006$). None of the pairwise comparisons using R_{ST} was statistically significant after sequential Bonferroni correction, with values ranging between -0.122 and 0.115 (Table 5.5).

The neighbour-joining analysis based on chord distances (Figure 5.2) was consistent with the results obtained using F_{ST} and R_{ST} as measures of population structure. The small distance values among samples and the low bootstrap support of most tree nodes underscore the existence of shallow differentiation among samples and the lack of a consistent geographical pattern among locations. Likewise, Bayesian clustering analyses performed at all levels (individual with spatial information, sample without and with spatial information) recovered one single group, with a marginal

Table 5.4 Probability values for Fisher's combined test of pairwise genic (above diagonal) and genotypic (below diagonal) differentiation at 3 microsatellite loci. Values with * are significant at $P < 0.05$; values in bold are significant after Bonferroni correction for 171 multiple tests.

	HAR	SIV	PLY	SMA	LCR	BIA	NOJ	CPR	BAI	AGU	SBE	PAR	STO	ARR	ODA	CAD	TAR	CAS	AGA
HAR	-	0.013 *	0.104	**<0.001**	0.006 *	0.123	0.005 *	0.022 *	0.011 *	0.033 *	0.116	0.011 *	0.155	0.110	0.061	0.005 *	**<0.001**	0.001 *	0.063
SIV	0.150	-	0.012 *	<0.001 *	0.038 *	0.001 *	0.012 *	0.267	0.003 *	0.008 *	0.102	0.011 *	0.326	0.003 *	0.132	<0.001 *	0.001 *	**<0.001**	0.034 *
PLY	0.353	0.081	-	0.009 *	0.048 *	0.266	0.007 *	0.016 *	0.060	0.010 *	0.179	0.016 *	0.006 *	0.063	0.024 *	0.461	0.043 *	0.054	0.082
SMA	**<0.001**	0.014 *	0.056	-	**<0.001**	**<0.001**	**<0.001**	**<0.001**	<0.001 *	**<0.001**	0.011 *	**<0.001**	**<0.001**	**<0.001**	<0.001 *	**<0.001**	**<0.001**	**<0.001**	**<0.001**
LCR	0.114	0.343	0.279	**<0.001**	-	0.356	0.015 *	0.030 *	0.040 *	0.017 *	0.368	0.145	0.628	0.041 *	0.184	0.469	0.003 *	0.485	0.080
BIA	0.362	0.025 *	0.498	<0.001 *	0.722	-	0.005 *	0.084	0.029 *	0.005 *	0.424	0.539	0.279	0.206	0.564	0.492	0.186	0.105	0.296
NOJ	0.057	0.147	0.073	<0.001 *	0.154	0.045 *	-	0.001 *	0.021 *	0.009 *	0.108	0.029 *	0.866	0.014 *	0.616	0.022 *	0.056	0.006 *	0.080
CPR	0.264	0.696	0.109	<0.001 *	0.255	0.335	0.070	-	0.028 *	0.306	0.239	0.006 *	0.150	0.078	0.518	0.014 *	**<0.001**	0.007 *	0.219
BAI	0.128	0.052	0.229	0.009 *	0.236	0.154	0.172	0.224	-	0.008 *	0.207	0.091	0.045 *	0.314	0.012 *	0.070	0.014 *	0.373	0.014 *
AGU	0.348	0.173	0.154	**<0.001**	0.296	0.089	0.196	0.709	0.149	-	0.459	0.001 *	0.210	0.016 *	0.017 *	0.016 *	0.009 *	**<0.001**	0.015 *
SBE	0.565	0.420	0.471	0.119	0.872	0.748	0.456	0.665	0.600	0.856	-	0.132	0.933	0.564	0.991	0.285	0.074	0.511	0.060
PAR	0.137	0.203	0.157	0.004 *	0.622	0.830	0.164	0.122	0.423	0.072	0.502	-	0.499	0.041 *	0.275	0.006 *	0.024 *	0.156	0.021 *
STO	0.316	0.676	0.030 *	<0.001 *	0.869	0.379	0.942	0.485	0.154	0.533	0.975	0.747	-	0.352	0.966	0.211	0.070	0.121	0.276
ARR	0.443	0.077	0.275	**<0.001**	0.304	0.408	0.145	0.386	0.693	0.217	0.872	0.292	0.590	-	0.358	0.143	0.002	0.091	0.054
ODA	0.319	0.550	0.146	0.014 *	0.572	0.833	0.891	0.835	0.124	0.241	0.999	0.758	0.992	0.692	-	0.350	0.090	0.371	0.430
CAD	0.081	0.041 *	0.817	**<0.001**	0.862	0.790	0.239	0.207	0.382	0.229	0.777	0.160	0.456	0.549	0.712	-	0.082	0.126	0.726
TAR	0.006 *	0.053	0.226	<0.001	0.073	0.511	0.384	0.022 *	0.153	0.175	0.380	0.248	0.238	0.040 *	0.516	0.343	-	0.002 *	0.082
CAS	0.038 *	0.005 *	0.163	**<0.001**	0.803	0.246	0.049 *	0.060	0.660	0.011 *	0.821	0.363	0.218	0.291	0.628	0.443	0.027 *	-	0.070
AGA	0.418	0.282	0.315	**<0.001**	0.408	0.604	0.360	0.556	0.117	0.136	0.282	0.195	0.566	0.293	0.722	0.962	0.357	0.278	-

Table 5.5 Pairwise multilocus estimates of F_{ST} (θ, below diagonal) and R_{ST} (ρ_{ST}, above diagonal) between samples, obtained from 3 microsatellite loci. Values with * are significant at $P < 0.05$; values in bold are significant after Bonferroni correction for 171 multiple tests.

	HAR	SIV	PLY	SMA	LCR	BIA	NOJ	CPR	BAI	AGU	SBE	PAR	STO	ARR	ODA	CAD	TAR	CAS	AGA
HAR	-	0.013*	0.104	**<0.001**	0.006*	0.123	0.005*	0.022*	0.011*	0.033*	0.116	0.011*	0.155	0.110	0.061	0.005*	**<0.001**	0.001*	0.063
SIV	0.150	-	0.012*	<0.001*	0.038*	0.001*	0.012*	0.267	0.003*	0.008*	0.102	0.011*	0.326	0.003*	0.132	<0.001*	0.001*	**<0.001**	0.034*
PLY	0.353	0.081	-	0.009*	0.048*	0.266	0.007*	0.016*	0.060	0.010*	0.179	0.016*	0.006*	0.063	0.024*	0.461	0.043*	0.054	0.082
SMA	**<0.001**	0.014*	0.056	-	**<0.001**	**<0.001**	**<0.001**	**<0.001**	<0.001*	**<0.001**	0.011*	**<0.001**	**<0.001**	**<0.001**	<0.001*	**<0.001**	**<0.001**	**<0.001**	**<0.001**
LCR	0.114	0.343	0.279	**<0.001**	-	0.356	0.015*	0.030*	0.040*	0.017*	0.368	0.145	0.628	0.041*	0.184	0.469	0.003*	0.485	0.080
BIA	0.362	0.025*	0.498	<0.001*	0.722	-	0.005*	0.084	0.029*	0.005*	0.424	0.539	0.279	0.206	0.564	0.492	0.186	0.105	0.296
NOJ	0.057	0.147	0.073	<0.001*	0.154	0.045*	-	0.001*	0.021*	0.009*	0.108	0.029*	0.866	0.014*	0.616	0.022*	0.056	0.006*	0.080
CPR	0.264	0.696	0.109	<0.001*	0.255	0.335	0.070	-	0.028*	0.306	0.239	0.006*	0.150	0.078	0.518	0.014*	**<0.001**	0.007*	0.219
BAI	0.128	0.052	0.229	0.009*	0.236	0.154	0.172	0.224	-	0.008*	0.207	0.091	0.045*	0.314	0.012*	0.070	0.014*	0.373	0.014*
AGU	0.348	0.173	0.154	**<0.001**	0.296	0.089	0.196	0.709	0.149	-	0.459	0.001*	0.210	0.016*	0.017*	0.016*	0.009*	**<0.001**	0.015*
SBE	0.565	0.420	0.471	0.119	0.872	0.748	0.456	0.665	0.600	0.856	-	0.132	0.933	0.564	0.991	0.285	0.074	0.511	0.060
PAR	0.137	0.203	0.157	0.004*	0.622	0.830	0.164	0.122	0.423	0.072	0.502	-	0.499	0.041*	0.275	0.006*	0.024*	0.156	0.021*
STO	0.316	0.676	0.030*	<0.001*	0.869	0.379	0.942	0.485	0.154	0.533	0.975	0.747	-	0.352	0.966	0.211	0.070	0.121	0.276
ARR	0.443	0.077	0.275	**<0.001**	0.304	0.408	0.145	0.386	0.693	0.217	0.872	0.292	0.590	-	0.358	0.143	0.002	0.091	0.054
ODA	0.319	0.550	0.146	0.014*	0.572	0.833	0.891	0.835	0.124	0.241	0.999	0.758	0.992	0.692	-	0.350	0.090	0.371	0.430
CAD	0.081	0.041*	0.817	**<0.001**	0.862	0.790	0.239	0.207	0.382	0.229	0.777	0.160	0.456	0.549	0.712	-	0.082	0.126	0.726
TAR	0.006*	0.053	0.226	<0.001	0.073	0.511	0.384	0.022*	0.153	0.175	0.380	0.248	0.238	0.040*	0.516	0.343	-	0.002*	0.082
CAS	0.038*	0.005*	0.163	**<0.001**	0.803	0.246	0.049*	0.060	0.660	0.011*	0.821	0.363	0.218	0.291	0.628	0.443	0.027*	-	0.070
AGA	0.418	0.282	0.315	**<0.001**	0.408	0.604	0.360	0.556	0.117	0.136	0.282	0.195	0.566	0.293	0.722	0.962	0.357	0.278	-

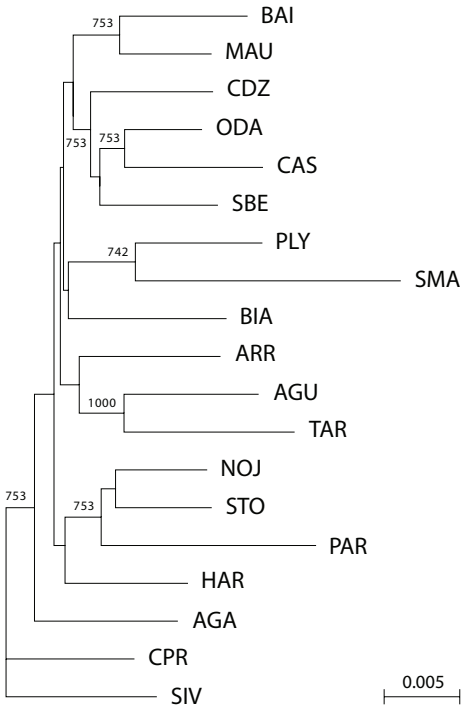

Figure 5.2 Neighbour-joining analysis of *Patella depressa* samples, based on chord distances (Cavalli-Sforza and Edwards 1967). Numbers next to branches are bootstrap support from 1000 bootstrapped data sets. Only bootstrap values > 50% are displayed.

posterior probability for the best clustering solution of 1.0 (marginal probability of other partitions was null).

Tests for isolation by distance were performed using genetic distances based both on allele frequencies and allele sizes (Figure 5.3). The Mantel test revealed a significant correlation between $F_{ST}/(1\text{-}F_{ST})$ genetic distance and log geographical distance matrices (Z = 4.614, r = 0.187, P = 0.0367). This was not a robust relationship, because reduced major axis regression recovered a positive relationship which only could only explain 3.5% of the variance. Conversely, there was no significant relationship between genetic distances calculated as $R_{ST}/(1\text{-}R_{ST})$ and log geographical distances among sampling locations (Z = -3.710, r = 0.088, P = 0.181).

5.4 Discussion

5.4.1 Genetic variability of microsatellite loci

This study encompassed 19 locations, distributed across most of the biogeographical range of *P. depressa*, and examined genetic polymorphism at five microsatellite loci. High levels of genetic diversity characterized all samples, from the northernmost

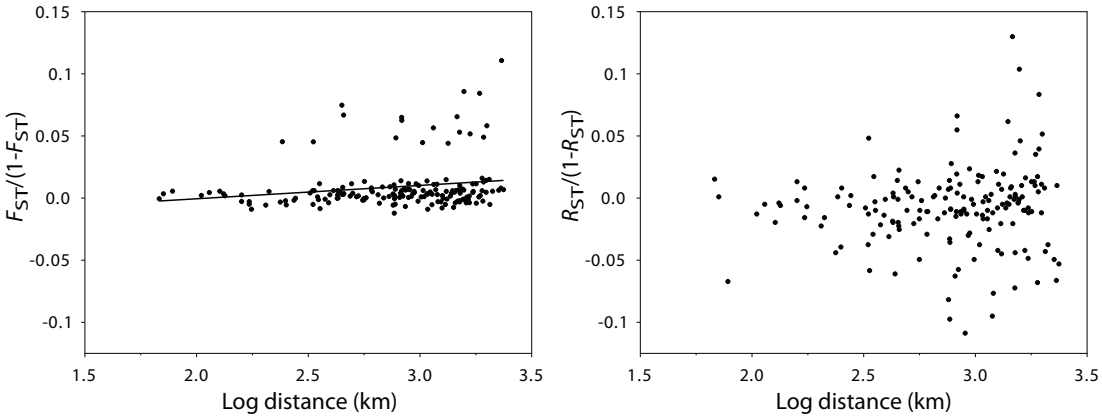

Figure 5.3 The relationship between geographic (log transformed) and genetic distance in *P. depressa*, among the 19 sampling sites. Genetic distances were based on IAM, $F_{ST}/(1 - F_{ST})$, and SMM, $R_{ST}/(1 - R_{ST})$. Geographic distance between any two locations was measured in kilometres as the shortest distance by sea.

(Hartland, U.K.) to the southernmost location (Agadir, Morocco). The levels of variability estimated for these markers were high and comparable to those obtained for other marine invertebrate species, such as sea urchins (Addison and Hart 2004), barnacles (Dufresne *et al.* 2002), crabs (Herborg *et al.* 2007), bivalves (Launey *et al.* 2002; Kenchington *et al.* 2006; Lind *et al.* 2007; Diz and Presa 2008) and gastropods (Sokolov *et al.* 2003; Viard *et al.* 2006; Weetman *et al.* 2006; Dupont *et al.* 2007; Gruenthal *et al.* 2007; Gruenthal and Burton 2008). The estimate of gene diversity across all loci (H_E = 0.818) was also consistent with the average value obtained for marine fish species by DeWoody and Avise (2000).

Most of the samples were in HWE for separate loci, but none of them was, when considering all loci together. All microsatellite markers showed heterozygote deficiency within samples, which was translated into significantly high positive F_{IS} values. Such heterozygote deficits were tested for the presence of null alleles, which was inferred at loci *Pde1* and *Pde5*. Excessive homozygosity due to the presence of null alleles is commonplace at microsatellite loci (e.g. Foltz 1986; Huvet *et al.* 2000 ; Hedgecock *et al.* 2004; Vadopalas *et al.* 2004; Astanei *et al.* 2005; Mariani *et al.* 2005; Peijnenburg *et al.* 2006), and may lead to overestimation of genetic differentiation statistics. Thus, several methods have been developed to circumvent this problem when analysing microsatellite data sets (e.g. van Oosterhout *et al.* 2004; Björklund 2005; van Oosterhout *et al.* 2006; Chapuis and Estoup 2007; Johnson and Haydon 2007). The solution adopted in this case was to exclude the two loci exhibiting null alleles, instead of adjusting allele frequencies, since correction procedures were not effective in eliminating excessive heterozygosity. Tests for significant departure from HWE with only the three remaining loci still showed homozygote excess in all samples, suggesting that biological factors could be responsible for these deviations.

Selection can also generate heterozygote deficiencies (Zouros and Foltz 1984). Although microsatellite loci are usually assumed to be neutral, a few studies suggest that they may be linked to genes under selection. For example, Bierne *et al.* (1998) discovered that microsatellite markers co-segregated with fitness-associated genes in oyster larvae (*Ostrea edulis*). A correlation between allele frequencies at two microsatellite loci and some allozyme alleles under selection has been reported by Dufresne *et al.* (2002) in the acorn barnacle (*Semibalanus balanoides*). Nielsen *et al.* (2006) found evidence of hitch-hiking selection at several microsatellite loci in the Atlantic cod (*Gadus morhua*), which potentially might have caused the inflation of previous estimates of genetic differentiation based on those markers. In the present case, selective forces that could act on linked loci in *P. depressa* throughout its wide geographic range are unknown, and they cannot explain the high F_{IS} values detected at all loci.

Alternatively, inbreeding can be advanced as a possible explanation for departure from HWE (e.g. Duran et al. 2004b; Le Goff-Vitry et al. 2004). In the case of *P. depressa*, this is unlikely to occur, since broadcast spawning, and especially the high dispersal capability of planktonic larvae, seems likely to result in sufficient mixing of offspring to ensure low probability of mating among close relatives.

Spatial or temporal cryptic population structure (Wahlund effects) can also explain departure from HWE (e.g. Duran *et al.* 2004b; Kenchington *et al.* 2006). A spatial Wahlund effect could be generated by pooling samples from an area exceeding larval dispersal potential. Considering the spatial scale of sampling, this scenario does not seem plausible. On the other hand, a temporal Wahlund effect may arise from the fact that populations of marine broadcast spawners experience large variance in reproductive success (sweepstakes reproduction, Hedgecock *et al.* 1992; Hedgecock 1994a, b). Because the sequence of events from spawning to larval recruitment is mainly governed by random processes, the number of adults effectively reproducing each year may vary greatly. This results in genetically differentiated generations, as well as low levels of genetic diversity within cohorts (Hedgecock 1994a; Li and Hedgecock 1998). Long spawning periods like the ones observed in *P. depressa* will likely enhance such genetic discrepancies, due to a greater magnitude of stochastic fluctuations in environmental conditions met during larval dispersal and recruitment. Although the sampling scheme adopted in this work took this phenomenon into account (by narrowing down the size range of sampled individuals), it is possible that some sampling across different cohorts has taken place. Comparison of population structure at several time periods or across age groups would be useful for clarifying if the widespread excess of homozygous individuals detected in this study is consistent with Wahlund effects caused by large variance in reproductive success.

5.4.2 Genetic structure

Significant differences were found in microsatellite allele frequencies among sampling locations, on 86 of the 171 pairwise tests performed at the 95% level and 51 tests at the 99% level, with 17 of them remaining significant after sequential Bonferroni correction. Furthermore, the global estimate of multilocus F_{ST} was low, but statistically significant ($P = 0.0001$). These results suggest that *P. depressa* populations are not panmictic and that either there is restriction to gene flow to some extent, or local variance in reproductive success causes strong genetic drift (or a combination of both).

Pairwise estimates of population differentiation were very low (excluding the highly divergent sample from Saint-Malo), either assuming an infinite allele model ($-0.009 < F_{ST} < 0.016$) or a stepwise mutation model ($-0.122 < R_{ST} < 0.058$). Low F_{ST} estimates are often expected from highly polymorphic markers, regardless of the underlying population structure. The extremely high heterozygosity of microsatellite loci implies that much of the genetic variation will occur within samples, and only a very small amount will be apportioned among populations, causing a deflation of F_{ST} values, and placing a relatively low maximum limit to them, even if no alleles are shared (Hedrick 1999). One important implication of this is that any genetic survey based on highly heterozygous markers will require large sample sizes, typically in the order of 50 individuals (Ruzzante 1998; Ryman *et al.* 2006) or even more when population differentiation is very low (Kalinowski 2005), to achieve sufficient statistical power for resolving population structure. Sample sizes in this study were considerably lower than that number, which may have prevented capturing the full variability of microsatellite loci.

In stark contrast with the large genetic homogeneity found throughout most of the study area, the sample from Saint-Malo was markedly differentiated from the rest. All pairwise F_{ST} estimates involving this sample were statistically significant at the 95% level, but only 14 of them remained so after sequential Bonferroni correction. Less differentiation was found with measures based on allele size (R_{ST}), but still most of the pairwise comparisons were significant at the 5% rejection level. Saint-Malo is located in the Bay of Mont Saint-Michel, on the north coast of France (English Channel). The present results seem to corroborate a series of studies conducted in this region, which suggested a crucial role of hydrodynamic features (such as tidal residual currents, buoyant plumes, eddies and gyres) and coastal geometry, in imposing restrictions to larval drift and gene flow. For example, Lefebvre *et al.* (2003) made use of a 2-dimensional model of ocean circulation in the English Channel, developed by Salomon and Breton (1991, 1993), to simulate larval dispersal of the brittle-star (*Ophiothrix fragilis*), a species with a planktonic period of up to 26 days. Simulations showed that only a few larvae originated in Brittany were able to drift

eastwards and settle in Normandy, and also that most of the larvae produced in the Bay of Saint-Michel were retained by permanent clockwise and anticlockwise eddies until the completion of larval development. Dupont *et al.* (2007) reported significant genetic differentiation among several bays (including the Bay of Saint-Michel), despite high levels of gene flow among populations within bays, in the gastropod *Crepidula fornicata* (a species with high dispersal potential). In that same study, larval dispersal simulations using the circulation model of Salomon and Breton (1991, 1993) indicated that local hydrographic conditions could largely explain the observed genetic patterns. Similar modelling studies have been performed with the polychaete species *Pectinaria koreni*, also a potential long-distance disperser, showing that high levels of larval retention within several bays along the French coast could be caused by hydrodynamic conditions (Ellien *et al.* 2000), although considerable variability in dispersal patterns was dictated by spawning time (Ellien *et al.* 2004). Furthermore, indirect evidence from genetic surveys also suggests restrictions to migration in *P. koreni* between populations from adjacent bays (Jolly *et al.* 2003) and, on a larger scale, between populations along the coasts of Brittany and the English Channel, where a sharp genetic break has been identified (Jolly *et al.* 2005).

Despite the weak levels of genetic differentiation inferred in this study, there was a low, but statistically significant pattern of isolation by distance, suggesting that dispersal is homogenous across the species biogeographical range, and that there are no effective barriers to gene flow (apart from the one described above, in the English Channel, causing higher differentiation of the population from Saint-Malo). Lack of panmixia can be explained by limited dispersal relative to the scale of sampling, which favours the buildup of large-scale genetic structure. These observations agree with the majority of the population genetic surveys carried out for species with planktonic dispersal capability in the northeast Atlantic. For instance, Pannacciulli *et al.* (1997) investigated population genetic structure of two barnacle species (*Chthamalus stellatus* and *Chthamalus* montagui) using allozyme electrophoresis. Both species showed genetic differentiation between the northeast Atlantic and Mediterranean basins, but little differentiation along the northeast Atlantic coast. The same pattern was inferred by Duran *et al.* (2004a) by analysing sequence variation in a fragment of the mitochondrial DNA gene cytochrome *c* oxidase subunit I (COI) in the sea urchin *Paracentrotus lividus*. Stamatis *et al.* (2004) analysed the genetic structure in Norway lobster (*Nephrops norvegicus*) populations at two mitochondrial DNA fragments and found low levels of population differentiation, but no evidence of isolation by distance across the species distribution, from the North Sea to the Mediterranean. Similar results were obtained by Roman and Palumbi (2004) for the European green crab (*Carcinus maenas*) along its Atlantic range, with only slight population structure being found between the central North sea and populations above, and clear differentiation

between Atlantic and Mediterranean basins. Ferguson *et al.* (2002) and Triantafyllidis *et al.* (2005) detected little differentiation and no correlation of genetic and geographic distances among Atlantic populations of the European lobster, *Homarus gammarus*, using allozyme and mtDNA markers. On the other hand, Ferguson *et al.* (2002) detected higher levels of heterogeneity in the Atlantic region among samples from adjacent areas, using microsatellites. Numerous studies on the flat oyster (*Ostrea edulis*) using different types of markers have revealed congruent structuring patterns, consisting of low levels of population differentiation along the northeast Atlantic basin, and a significant correlation between genetic and geographical distances (e.g. Saavedra *et al.* 1993; Launey *et al.* 2002; Diaz-Almela *et al.* 2004). Following the analysis of sequence variation at the COI mitochondrial DNA gene, Couceiro *et al.* (2007) detected a slight pattern of genetic isolation by distance among subpopulations of the netted dog whelk, *Nassarius reticulatus*, along the south European coastline. Quinteiro *et al.* (2007) analysed sequence variation at two different mitochondrial DNA genes, 12S ribosomal RNA and COI in the stalked barnacle (*Pollicipes pollicipes*), reporting not only a significant correlation between geographic and genetic distances throughout the entire range, but also a slightly higher genetic structure at some locations. Those authors found a genetic structure pattern which reflected constraints to planktonic dispersal imposed by coastal currents and mesoscale hydrodynamic features, more specifically the occurrence of eddies and gyres in the Bay of Biscay and the strong southward-flowing Canary current along the northwest coast of Africa.

The few published studies on genetics of patellid species at a comparable scale along the northeast Atlantic coast partly corroborate my results, but important differences arise. A recent survey of the genetic variability at allozyme loci carried out for *Patella vulgata* revealed moderate genetic subdivision over a geographical area ranging from the British Isles to southern Portugal (Weber and Hawkins 2006). However, an unexpected pattern of genetic differentiation was observed in the south of England, where south Devon and north Cornwall samples displayed distinctive allele frequencies, probably representing a transition zone between two areas of extensive gene flow. A similar segregation pattern had already been described in that area for two mussel species, *Mytilus galloprovincialis* and *Mytilus edulis*, where a stable hybrid zone is flanked by pure populations of *M. galloprovincialis* in north Cornwall and *M. edulis* in south Devon (Hilbish *et al.* 2002). This distribution was in part attributed to different selective forces acting on both sides of the Cornish peninsula and to asymmetric patterns of larval dispersal due to local currents (Gilg and Hilbish 2003). The lack of differentiation observed among the three U.K. samples of *P. depressa* seems to reinforce the idea that selection plays an important role in maintaining the genetic break described above, and that larval dispersal, although possibly restricted by ocean circulation patterns, may be sufficient to homogenise allele frequencies at neutral

microsatellite loci.

A large-scale genetic survey of *Patella ulyssiponensis* and *Patella rustica* covering the Mediterranean and part of the northeast Atlantic region was carried out by Sá-Pinto (2008), aiming to identify genetic discontinuities that might reveal the existence of barriers to gene flow. In both species, analyses of the genetic variation at several allozyme loci and a fragment of the mtDNA COI gene detected sharp breaks in the transition between the Atlantic and the Mediterranean, and between the Eastern and Western Mediterranean. Additional substructure was identified within the Atlantic basin, between a group composed of three samples, Estepona (Alboran Sea), Tarifa (at the southern tip of the Iberian Peninsula) and Agadir (North Africa) and the remaining Iberian samples. Sá-Pinto (2008) proposed two factors responsible for this observation. One was the occurrence of habitat discontinuities along the southern Iberian coast, which may be too large for planktonic larvae to bridge during the course of development, and would explain the genetic dissimilarity observed between Tarifa and the rest of the Iberian samples. A second factor involved in the establishment of the genetic structure inferred by Sá-Pinto (2008) concerns the prevailing ocean currents in the Gulf of Cadiz. As the eastward branch of the Azores current enters the Gulf, it turns southwards, flowing along the northwest African coast (Johnson and Stevens 2000; Barton 2001). This clockwise water motion is likely to promote asymmetric migration of larvae between southern Iberian and northwest African shores. It is, however, counterintuitive to explain the observed similarity between Estepona and Agadir based on ocean circulation, because surface currents flow from the Atlantic into the Mediterranean. It is possible that the genetic substructure uncovered by the survey of Sá-Pinto (2008) has been shaped by historical events affecting *P. ulyssiponensis* and *P. rustica* in a similar manner, instead of being the result of present-day demographic connectivity. On the other hand, the low spatial replication (especially in North Africa, where only one location has been sampled) raises questions about the robustness of those conclusions. Microsatellite data for *P. depressa* gathered with a more extensive sampling design seem to be compatible with this view, since no restrictions to gene flow were identified across the Gulf of Cadiz.

The reduced number of loci screened and the overall low genetic structure uncovered precluded from taking full advantage of recent methods that use multilocus genotypes to ascertain the probability of individual membership to a specific population (Manel *et al.* 2005; Corander and Marttinen 2006). Although F_{ST}-based methods remain useful for measuring average effects of gene flow in population studies (Neigel 2002), these novel alternative approaches can be very powerful at detecting population substructuring and, in certain cases, can even be used to estimate migration rates and gene flow among populations (e.g. Rannala and Mountain 1997; Pritchard *et al.* 2000; Falush *et al.* 2003). One important drawback lies in the fact that the application of

these analytical methods to highly variable markers requires larger datasets, both in the number of individuals and loci. This was not achieved in the present study, and therefore, conclusions drawn here should be considered cautiously. It is possible that subtle population differentiation and partial genetic breaks have been overlooked, due to lack of statistical power. Screening of more individuals per sample for a larger set of microsatellite loci are required to confirm the genetic structure of *P. depressa* described in this study or, conversely, to identify other putative barriers to gene flow throughout its extensive geographical range.

Chapter 6

Patterns of recent colonisation by *Patella rustica* investigated using mitochondrial and microsatellite DNA markers

6.1 Introduction

The lusitanian limpet *Patella rustica* ranges from the Mediterranean to the Atlantic coasts of the Iberian Peninsula and North Africa (Christiaens 1973; Ridgway *et al.* 1998). Recent work suggests that its sister taxon, *Patella piperata*, which occurs in Macaronesia (except the Azores Archipelago), could be a separate subspecies (Côrte-Real *et al.* 1996a; Koufopanou *et al.* 1999; Sá-Pinto *et al.* 2005). In the Atlantic, the southern limit of *P. rustica* is situated south of Mauritania, whilst its northern limit lies at Capbreton, on the French Basque coast (Fischer-Piètte and Gaillard 1959; Lima *et al.* 2007b).

In a recent paper, Lima *et al.* (2006) described the collapse of an historical distributional gap of *P. rustica* in northern Portugal, probably driven by climate warming. This gap was well documented and was first described in the early 1900s by Hidalgo (1917), who placed it between Nazaré (Portugal) in the south and La Coruña (Spain) in the north. According to the exhaustive surveys done by Fisher-Piètte and co-workers (Fischer-Piètte 1955; Fischer-Piètte and Gaillard 1959), *P. rustica* was absent from northern Portugal in the warm 1950s, but was probably expanding in the northern part of the gap (Galicia, northwest Spain). The colder period that occurred between the 1960s and the 1980s (Southward *et al.* 1995; Beaugrand and Reid 2003) might have contributed to maintain or even widen the gap. Multiple surveys carried out between 1993 and 1998 by Santos (2000) confirmed the southern limit of the distributional gap in the central Portuguese coast, more specifically at the location of São Martinho do Porto. Early records of *P. rustica* within this area are limited to a single individual found by Fisher-Piètte and Gaillard (1959), a few kilometres north of

Porto, and, more recently, to another individual spotted in 1995 at Homem do Leme, in Porto (S.J. Hawkins, pers. obs.). More frequent sightings have been made since 2002, with several adult individuals also being observed at Homem do Leme, and subsequent surveys revealed that the species was already present at very low densities in several other localities within the historical gap. Nowadays, *P. rustica* has a continuous distribution along the former gap, and reaches high densities at some locations, having also colonised coastal defences, which are present between areas of natural rocky shore.

Recent changes in the distribution and abundance of marine species have been widely documented on the eastern Atlantic and Pacific coasts (e.g. Southward *et al.* 1995; Holbrook *et al.* 1997; Sagarin *et al.* 1999; Garrabou *et al.* 2002; Wethey 2002; Beaugrand and Reid 2003; Borges *et al.* 2003; Hawkins *et al.* 2003; Zacherl *et al.* 2003; Genner *et al.* 2004; Hiscock *et al.* 2004; Paine and Trimble 2004; Lima *et al.* 2007a, b; Wethey and Woodin 2008). In all cases, recent climate warming was suggested to be driving such changes. Given the correlative nature of these observations, there remains some doubt as to whether statistical associations identify the true determinants of species' range shifts (Parmesan *et al.* 2005). The bridging of a distributional gap, as opposed to a simple unidirectional range expansion, provides a unique opportunity to test hypotheses concerning the role of complex climatic events in causing such biogeographical changes. Coastlines are roughly one-dimensional (Sagarin and Gaines 2002; Hiscock *et al.* 2004) and are particularly suitable for this research because there are two putative sources of colonisers. Hence, three possible colonisation routes can be predicted in *P. rustica*: (1) from the south; (2) from the north; (3) from both sides simultaneously.

Lima *et al.* (2006) suggested that the combination of several climatic events promoted the transport and settlement of colonisers from southern shores. This hypothesis may seem counterintuitive, because *P. rustica* has been known to occur in Galícia since the 1950s, this being the nearest putative source of colonisers. Assuming a spawning period between August and November (Ibañez *et al.* 1986; Othaitz 1994; see also chapter 2), larval drift would take place when equatorward currents are most intense (Santos *et al.* 2004). However, even though in the last decades hydrographic conditions remained stable, thus favouring larval dispersal from Galícia, there was no evidence of successful colonisation in northern Portugal. Sporadic observation of isolated individuals in the past may indicate that successful settlement took place occasionally, but not in sufficient numbers to allow these new populations to persist.

The hypothesis proposed by Lima *et al.* (2006) is founded on a combination of unusual climatic and hydrographic events that occurred during the 1990s, and in particular between 1997 and 1999. In addition to the general increase in average sea surface temperature observed along the Portuguese coast (Lemos and Pires 2004; Peliz *et al.* 2005), an analysis of sea surface temperature anomalies revealed a significant

warming during autumn/early winter, which might have provided temperature conditions within the limited range suitable for metamorphosis and growth, allowing limpets to attain a size large enough to survive the coming winter (Lima *et al.* 2006). On the other hand, a decrease in sea surface salinity off northern Portugal from 1995 onwards indicated an increase of the Western Iberian Buoyant Plume (WIPB). This consists of a low-salinity surface water layer fed by the winter-intensified runoff of rivers in the northwest coast of Portugal and Spain (Santos *et al.* 2004). Over the shelf, intensity and direction of surface currents are closely related to upwelling intensity and to the development of the WIBP. During non-upwelling periods, the predominant southward offshore currents decrease in intensity and inshore northward circulation increases (Figueiras *et al.* 2002; Peliz *et al.* 2002; Sánchez and Relvas 2003). The development of the WIBP results in isolation of inner-shelf waters and strong poleward transport (Peliz *et al.* 2002, 2005). During years of strong river discharge and weak upwelling, northward coastal transport is enhanced. Conversely, strong upwelling, reduced river runoff, or both, will have the opposite effect (Santos *et al.* 2004). Upwelling was permanently at a low phase from 1997 to 1999. Moreover, 1997 was an exceptionally mild year over Europe, with a winter season characterized by high temperatures and intense rainfall (Dong *et al.* 2000). The combination of these phenomena could have favoured the northward transport of *P. rustica* larvae from southern Portugal, and positive SST anomalies probably contributed to increased survival rates of planktonic larvae, thus allowing settlement and development of a large number of juveniles.

The first aim of this work was to investigate patterns of genetic substructuring of *P. rustica* along the Atlantic Iberian coast and to determine if populations living on both sides of the former distribution gap have been connected by larval dispersal. The second aim consisted in identifying the origin of the individuals that colonised the gap. Two types of genetic marker, mitochondrial DNA (mtDNA) and microsatellites, have been used. Mitochondrial DNA shows high mutation rates (with a few notable exceptions, such as in anthozoans, (e.g. Romano and Palumbi 1997; Shearer *et al.* 2002; Hellberg 2006), is maternally inherited (although biparental inheritance has been reported in bivalves, Skibinski *et al.* 1994; Zouros *et al.* 1994; Passamonti and Scali 2001) and lacks recombination, which makes it an ideal marker for phylogenetic and phylogeographic studies. Given that mtDNA is haploid, it is more susceptible to the effects of genetic drift. Its effective population size is one quarter that of nuclear markers (with balanced sex-ratios), which results in quicker fixation or loss of alleles, and higher population subdivision at mitochondrial loci (Birky *et al.* 1983; Buonaccorsi *et al.* 2001). Although mtDNA is very useful in providing information relative to historical demographic events, the inference drawn from it is limited to single-locus gene trees. Furthermore, despite having higher rates of nucleotide substitution than

nuclear markers (Brown *et al.* 1979), mtDNA is in part protein coding and is thus potentially under the influence of selective forces (Ballard and Kreitman 1995). The effects of selection may be confounded with demographic processes and may also cause decreased mutation rates, thus precluding the inference of contemporary levels of gene flow. In contrast, the assumed neutrality and high mutation rates of microsatellites markers make them more suited to resolve fine-scale genetic structure and patterns of gene flow at ecological time scales, but size homoplasy renders these markers less useful for estimating divergence among populations that have been isolated for longer periods of time (Estoup *et al.* 2002).

Comparative analyses of polymorphism in mtDNA and microsatellite markers should enable teasing apart the relative effects of historical and ecological factors that shaped present-day genetic structure of *P. rustica* on the Atlantic Iberian coast. Analysis of mtDNA provides an estimate of historical gene flow, to infer whether northern and southern Iberian populations have been historically isolated due to the inability of planktonic larvae to overcome the extension of unsuitable habitat located on the northern Portuguese coast. High-resolution microsatellite markers enable identification of the source, or sources, of colonists of northern Portuguese shores, as long as sufficient genetic differentiation exists between the two groups of historical populations.

6.2 Material and Methods

6.2.1 Sample collection and DNA extraction

Between 2004 and 2006, 12 locations along the Iberian Atlantic coast (Figure 6.1) were sampled. Eight of these were situated in the historical distribution range of the species; whereas the remaining four were located within the species former distribution gap in northern Portugal, having been recently colonised. Sample size at each location varied between 31 and 44 individuals (Table 6.1). To reduce the chance of sampling across different age cohorts, all the individuals collected were of similar size, reflecting the dominant cohort in the populations. Specimens were stored in absolute ethanol until DNA extraction.

Total genomic DNA was isolated from a portion of foot muscle following a phenol-chloroform extraction protocol (Sambrook *et al.* 1989), modified by adding a step for precipitation of mucopolysaccharides (Sokolov 2000). After ethanol precipitation, the DNA was resuspended in 80 µL ultrapure water and stored at -20 °C. Extracted DNA was tested by electrophoresis of 2 µL of the DNA solution mixed in 2 µL loading buffer, on a 0.8% agarose gel during 30min at 140 V, and visualization

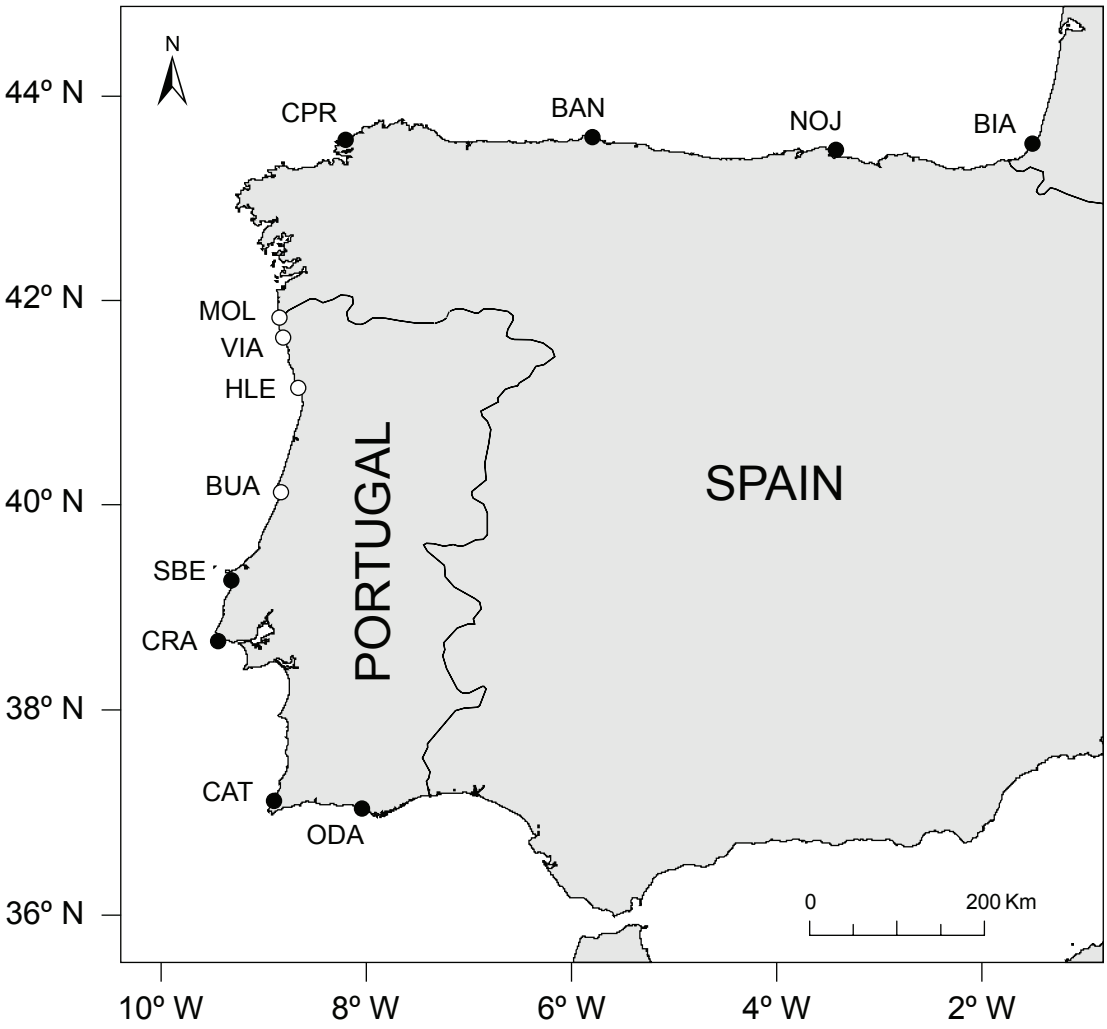

Figure 6.1 Geographical location of *Patella rustica* sampling sites. White dots correspond to recently colonised locations. Sample codes are provided in Table 6.1.

under UV light, after gel staining with ethidium bromide. Diluted aliquotes to be used in the assay were made from stock DNA solutions according to estimated individual concentrations.

6.2.2 Molecular analyses

6.2.2.1 Mitochondrial DNA

A fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was sequenced in 163 individuals belonging to the 12 sampling locations. Amplification of the COI sequence was done by polymerase chain reaction (PCR) using the universal

Table 6.1 Location names, sample codes, geographical coordinates and number of individuals per sample.

Location	Sample code	Coordinates (latitude/longitude)	Sample size
Biarritz	BIA	43° 29' N / 01° 34' W	44
Noja	NOJ	43° 30' N / 03° 32' W	35
Bañugues	BAN	43° 38' N / 05° 48' W	35
Cabo Prior	CPR	43° 34' N / 08° 19' W	35
Moledo do Minho	MOL	41° 50' N / 08° 53' W	32
Viana do Castelo	VIA	41° 42' N / 08° 52' W	31
Homem do Leme	HLE	41° 09' N / 08° 42' W	32
Buarcos	BUA	40° 11' N / 08° 55' W	32
São Bernardino	SBE	39° 18' N / 09° 21' W	35
Cabo Raso	CRA	38° 42' N / 09° 29' W	31
Castelejo	CAT	37° 06' N / 08° 57' W	35
Olhos d'Água	ODA	37° 05' N / 08° 11' W	35

primers LCO1490/HCO2198 (Folmer et al. 1994) in 20 µL reactions containing 1 µL of template DNA, 0.5 U of *Taq* polymerase (Platinum Taq DNA Polymerase, Invitrogen), 10× PCR buffer at 1× concentration, 0.2 mM of each dNTP, 0.5 µM of each primer, 2.5 mM $MgCl_2$ and ultrapure water to adjust final volume. Thermal cycling conditions consisted of 3min at 94 °C, followed by 35 cycles of 30s at 94 °C, 30s at 50 °C and 30s at 72 °C, and one final elongation step of 10min at 72 °C. Sequence reactions were performed on both directions and the service was provided by a commercial company. Sequences were checked and edited using CodonCode Aligner (CodonCode Corporation) and aligned with an online version of the software CLUSTALW2 (available at http://www.ebi.ac.uk/Tools/clustalw2). Missing or ambiguous end regions were trimmed so that all sequences had the same final length.

6.2.2.2 Microsatellites

Genetic variation was assessed in a total of 412 individuals at seven polymorphic microsatellite loci previously developed for *P. rustica* (see chapter 4). Five of the loci used consisted of perfect tetranucleotide repeats (*Pru1*, *Pru2*, *Pru5*, *Pru13* and *Pru14*) and the remaining two (*Pru6* and *Pru7*) were interrupted dinucleotide repeats (Table 6.2). Polymerase chain reaction (PCR) was performed in a 15-µL total volume reaction mixture containing 1 µL of template DNA, 0.5 U of *Taq* polymerase (Amplitaq Gold DNA Polymerase, Applied Biosystems), 10× PCR buffer at 1× concentration (GeneAmp PCR Gold Buffer- 15 mM Tris-HCl, 50 mM KCl; pH 8.0 at room temperature), 0.2

mM of each dNTP, 0.5 μM of each primer, $MgCl_2$ ranging from 1.2 to 2.0 mM (Table 6.2) and ultrapure water to adjust final volume. Thermal cycling conditions for PCR amplification consisted of one *Taq* polymerase activation step at 95 °C for 10min, followed by 35 cycles of 30s at 95 °C, 40s at the primer-specific optimal annealing temperature and 40s at 72 °C, and a final extension step of 10min at 72 °C. Reverse PCR primers were fluorescently labelled (Table 6.2) and PCR products were sized by capillary electrophoresis on an ABI 3130XL (Applied Biosystems) automated sequencer. To ensure sizing accuracy, alleles were scored in comparison to an internal size standard (ROX 350 Applied Biosystems) using GeneMapper 4.0 software (Applied Biosystems). In addition, around 10% of the samples were amplified twice at each locus, for estimation of genotyping consistency.

6.2.3 Statistical analysis

6.2.3.1 Mitochondrial DNA

The number of polymorphic sites, plus the number and types of mutations (indels, transitions and transversions) were determined using DnaSP 4.50.3 (Rozas *et al.* 2003). To represent haplotype genealogy and their relative frequencies in samples, a network was estimated using a median-joining algorithm as implemented in Network 4.5.0.1 (Bandelt *et al.* 1999). Measures of genetic diversity within samples and regions, more specifically haplotype frequency, haplotype and nucleotide diversity, h and π (Nei 1987) were computed with ARLEQUIN 3.1 (Excoffier *et al.* 2005).

Genetic differentiation between each pair of samples was estimated by computing pairwise Φ_{ST} values based on haplotype frequencies and molecular divergence, using ARLEQUIN 3.1. Significance was determined by performing 10,000 permutations and adjusted using sequential Bonferroni corrections for multiple comparisons (Rice 1989). With the software ARLEQUIN 3.1, a hierarchical analysis of molecular variance (AMOVA, Excoffier *et al.* 1992) framework was also used to estimate the partitioning of molecular variance components at three levels (between regions, among locations within regions and within locations), and investigate population subdivision. Two different analyses were performed: the first one comprised only the samples collected from the historical distribution range, arranged in two groups of four populations each (Northern Iberia: Biarritz, Noja, Bañugues, Cabo Prior; Southern Portugal: São Bernardino, Cabo Raso, Castelejo, Olhos d'Água); the second AMOVA encompassed these two groups, plus a third group made up of the four samples collected from the recently colonised locations (Northern Portugal: Moledo do Minho, Viana do Castelo, Homem do Leme and Buarcos). AMOVAs were based on haplotype frequency variance

Table 6.2 Microsatellite markers, fluorescent labels and PCR conditions used in population genotyping of *Patella rustica*. Labelled primers are indicated by ξ (NED), † (VIC) or ‡ (6-FAM).

Locus	Primer sequences (5'-3')	*T* (°C)	$MgCl_2$ (mM)	Sizes (bp)	N_a	Repeat motif	GenBank Accession no.
Pru1	F: TGTCTTCATGCTTATTA	50	1.8	142-247	20	$(GATA)_{23}$	EU255238
	R: TTTTCATCCACTTTTATξ						
Pru2	F: GTACGCTCTAACAAAAT	50	1.8	175-189	6	$(GACA)_6$	EU255239
	R: TTCTATACAATCCGATT†						
Pru5	F: TAGCTTCGACAGGCAACC	57	1.8	176-192	8	$(CTGT)_8$	EU255242
	R: CGTGTCCCAAAAAGTGTG‡						
Pru6	F: TCAGATGTAACAGGTTTTAGG	57	1.8	97-146	20	$GT)_5AC(GT)_{15}AC$	EU255243
	R: TTGTGGATCAAAGAGGTACT‡					$(GT)_4$	
Pru7	F: AATTACTTCACTCTGGG	52	2.0	156-302	34	$(CT)_{18}N20(CT)_{17}$	EU255244
	R: GGTTAGTTCCTCTTGTG‡					$N20(CTGT)_6$	
Pru13	F: ATAAAGACGGCAGTGAGATA	53	1.5	102-134	10	$(GATA)_{18}$	EU255250
	R: GTCTCCAAGTATTAGGATGAAT‡						
Pru14	F: GTGAGTGGAATGTCTGTATGAC	50	1.2	90-122	6	$(CTGT)_{11}$	EU255251
	R: GCATTGACCTAATAAACACATA‡						

and pairwise differences between haplotypes. Significance of Φ-statistics and associated variance components was tested using 20,000 permutations.

To assess evidence of recent population expansion, three approaches were followed. First, historical demographic changes in *P. rustica* at the sampled locations were inferred from mismatch distribution, which is the distribution of the number of pairwise sequence differences within a breeding population (Rogers and Harpending 1992). In samples taken from populations at demographic equilibrium, this distribution is usually multimodal, but it assumes a typically unimodal shape in samples drawn from recently expanded populations (Slatkin and Hudson 1991; Rogers and Harpending 1992). The validity of a model of demographic expansion was tested with ARLEQUIN 3.1, using the sum of squared deviations (SSD) between observed and expected mismatch distributions as a test statistic. Its significance was calculated by a parametric bootstrap with 1,000 replicates, and represents the probability of obtaining simulated SSD values larger than or equal to the observed one (Schneider and Excoffier 1999). The raggedness index of the observed distribution (Harpending 1994) was also computed using ARLEQUIN 3.1 and its significance tested as for *SSD*. This index takes larger values for multimodal distributions typical of stable populations than for unimodal and smoother distributions found in expanding populations.

Second, Fu's (1997) F_S neutrality test was also performed, since it is one of the most powerful statistical tests available for detecting population growth (Ramos-Onsins

and Rozas 2002). This test allows the detection of an excess of recent mutations, as is expected to occur after a significant population growth or a selective sweep, by comparing the value of θ estimated from π with that estimated from the number of alleles (Fu 1997). The significance of F_S statistics was tested by generating 10,000 random samples under a hypothesis of selective neutrality and population equilibrium, using a coalescent simulation algorithm, as implemented in ARLEQUIN 3.1. The *P*-value of the F_S statistic was obtained as the proportion of random values less than or equal to the observation. F_S statistic was considered as significant at the 5% level if its *P*-value was below 0.02 (Fu 1997).

A third analysis was performed to test for population expansion with the software package BEAST version 1.4.7 (Drummond and Rambaut 2007), which was used to estimate coalescence times for the pooled samples. This software assumes different demographic models as tree priors, using Bayesian inference for assessing the probability of model parameters, and a Markov chain Monte Carlo algorithm for drawing samples from a posterior distribution, so as to provide an estimate of that distribution. Analyses were run with coalescent tree priors, using two different demographic functions of population size through time: constant size and exponential growth. Two independent runs were performed under each scenario to check the consistency of results, by making sure that they both converged on the same distribution in the Markov chain Monte Carlo run. Given that BEAST co-estimates model parameters, the GTR substitution model (Rodríguez *et al.* 1990) was selected, with a proportion of invariable sites and a discreet approximation to the gamma-distributed rate of heterogeneity with four categories (Yang 1994), letting the program sample the values for all the parameters. A strict molecular clock model was assumed, with a fixed rate of 0.012 substitutions per site per million years, based on the estimate by Hellberg and Vacquier (1999) for the mitochondrial COI gene from the divergence observed between two gastropod (*Tegula* spp.) species that were separated by the emergence of the Isthmus of Panama. Each run consisted of a Markov chain Monte Carlo of 50×10^6 steps, sampled every 10,000 steps (after discarding the first 10% as burn-in). For the constant size coalescent analyses, a uniform prior for the population size (constant.popSize) parameter was assumed, with a lower limit of zero and an upper limit of 5,000. For the exponential growth coalescence analyses a uniform prior for the modern day population size parameter (exponential.popSize) with a lower limit of zero and an upper limit of 5,000, and a uniform prior for the exponential growth rate (G) with a lower limit of -1×10^6 and an upper limit of 1×10^6 were assumed.

The software TRACER 1.4 (Rambaut and Drummond 2007) was used to monitor the progress of each run and ensure that the chain has reached equilibrium. It also enabled diagnostic analysis of the output, especially to check for the effective sample size of each parameter (that is, the number of independent samples from

the marginal posterior distribution that the trace is equivalent to) and to obtain the 95% highest posterior density interval (i.e., the shortest interval containing 95% of the sampled values) for each parameter estimate. The same software was used to calculate the harmonic mean of likelihood (an estimator of the marginal likelihood) from the harmonic mean of sampled likelihoods of each run of BEAST, following the method described by Newton and Raftery (1994), with the modifications proposed by Suchard *et al.* (2001). To infer which of the two tree priors better conformed to the data, the value of the marginal likelihood obtained for the run using the coalescent exponential growth tree prior was compared with that obtained for the run with the coalescent constant size tree prior using a Bayes Factor approach (Nylander *et al.* 2004) implemented in TRACER 1.4.

Isolation by distance among all samples and among samples within regions was tested by correlation of $\Phi_{ST}/(1 - \Phi_{ST})$ genetic distances and log geographical distances matrices with a Mantel test (Mantel 1967), using the software IBD 1.52 (Bohonak 2002). Geographical distance between sampling locations was measured as the shortest distance by sea, using GRASS 6.0 Geographical Information System (GRASS Development Team 2006). Reduced major axis regression analysis was employed to assess the strength of the isolation by distance relationship instead of standard least-squares regression, since it is more adequate when the independent variable (in this case geographic distance) is measured with error (Sokal and Rohlf 1981; Hellberg 1994). A global isolation-by-distance analysis was conducted across all locations and separate analyses were performed within each geographical region (Northern Iberia, Northern Portugal and Southern Portugal. The significance of each test was assessed using 10,000 randomizations.

6.2.3.2 Microsatellites

Descriptive statistics (allele frequencies, observed number of alleles, observed and expected heterozygosities) were computed per sampling site and locus using GENEPOP 4.0 (Rousset 2008). To eliminate bias due to differences in sample size, allelic richness per locus by sample and overall was estimated by rarefaction to the smallest sample size (Petit *et al.* 1998; Leberg 2002) using FSTAT 2.9.3.2 (Goudet 2001).

Single and multilocus estimates of the inbreeding coefficient F_{IS} (f) were computed following Weir and Cockerham (1984) using GENEPOP 4.0. Deviations from Hardy-Weinberg equilibrium (HWE) and genotypic linkage disequilibrium between pairs of loci were tested using exact tests implemented in GENEPOP 4.0. Significance was determined by a Markov chain Monte Carlo method (Guo and Thompson 1992), with specific parameters of 10,000 dememorization steps followed by 500 batches of 5,000 iterations. To correct for increased type I error, significance values from multiple

comparisons were adjusted using the sequential Bonferroni correction procedure with a value of $\alpha = 0.05$ (Rice 1989).

The presence of null alleles and scoring errors caused by large allele dropout and stuttering was investigated with MICRO-CHECKER 2.2.3 (van Oosterhout *et al.* 2004). Null alleles were inferred when there was a consistent excess of homozygotes across allele size classes; large allele dropout was indicated if deviations were more prevalent in smallest allele size classes; stuttering was identified if there was a downward bias of heterozygous genotypes with alleles differing by one repeat unit. For samples in which observed genotype frequencies did not conform to HWE, null allele frequencies were estimated according to Brookfield's (1996) estimator 1, which does not take into account the presence of null allele homozygotes and amplification failures were considered artifacts. Because null alleles may inflate perceived levels of genetic differentiation by increasing the level of homozygotes and distorting allele frequencies (Chapuis and Estoup 2007), an alternative dataset was generated with adjusted allele and genotype frequencies to account for null alleles and each null allele recoded as missing data. All subsequent analyses to estimate genetic differentiation among samples were performed on both corrected and uncorrected datasets.

Exact tests of allelic (genic) and genotype (genotypic) frequencies differentiation among pairs of samples were performed with GENEPOP 4.0 (Markov chain parameters: 10,000 dememorisation steps followed by 500 batches of 5,000 iterations). Because most loci did not conform to the strict expectations of the stepwise mutation model, genetic differentiation among samples was quantified assuming an infinite allele model of mutation (Kimura and Crow 1964). Overall levels of population subdivision and differentiation among samples were assessed through estimates of F_{ST} (θ), calculated globally and between all sample pairs following Weir and Cockerham (1984). Their statistical significance was determined after 10000 permutations without assuming HWE within samples, with the software package MICROSATELITE ANALYSER (Dieringer and Schlötterer 2003). For all multiple comparisons *P*-values were adjusted using sequential Bonferroni corrections (Rice 1989).

Genetic affinities among sampling locations were further assessed by reconstruction of a neighbour-joining tree (Saitou and Nei 1987), with topology based on pairwise chord distance (Cavalli-Sforza and Edwards 1967) computed from allele frequencies and statistical support of tree nodes provided by 1,000 bootstrapped data sets. This distance measure was selected because it is particularly suitable for reconstructing tree topologies based on microsatellite data (Takezaki and Nei 1996). Analyses were performed using programs SEQBOOT, GENDIST, NEIGHBOR and CONSENSE included in the package PHYLIP 3.67 (Felsenstein 2004). The partitioning of total genetic variance based on allele frequencies was estimated using AMOVA as implemented in ARLEQUIN 3.1, with the same groupings as for mtDNA analyses.

In population genetic studies, the interpretation of genetic differentiation values based on F_{ST}-analogues, such as G_{ST} (Nei 1973), θ (Weir and Cockerham 1984), R_{ST} (Slatkin 1995) and Φ_{ST} (Excoffier *et al.* 1992), is often problematic because these measures are affected by the amount of genetic variation within samples (Meirmans 2006). This situation assumes special relevance for highly variable markers, such as microsatellites, which often have very high within-population heterozygosity, causing the magnitude of differentiation to be relatively small, even when no alleles are shared among subpopulations (Hedrick 1999). To overcome this situation, standardised measures of genetic differentiation have been proposed which have the same range for all loci (from 0 to 1) regardless of the level of subpopulation genetic variation; these allow the comparison between results obtained from markers with different degrees of polymorphism, such as microsatellite loci and mitochondrial DNA genes (Hedrick 2005b). Standardised measures of genetic divergence were calculated from microsatellite data dividing the observed measures by their maximum possible values, given the observed amount of genetic variation within samples. Maximum values were calculated following the pragmatic approach suggested by Meirmans (2006), which consists of recoding the data so that all samples have nonoverlapping sets of alleles. All negative F_{ST} values were converted to zero prior to standardisation, as also recommended by Meirmans (2006). Two data sets were constructed: one in which the original data were re-coded so that each location had unique alleles (used to calculate pairwise F_{ST} values among locations); another data set was re-coded so that each region had unique alleles (used to calculate standardised differentiation measures among regions).

Population subdivision was further investigated with Bayesian clustering methods, using the software BAPS 5.1 (Corander and Marttinen 2006) to group genetically homogeneous clusters of samples (see chapter 5 for details about the software). Three analyses were performed: (1) a spatial analysis at the individual level, (2) a spatial analysis at the sampling location level and (3) a non-spatial analysis at the sampling location level.

The relationship between pairwise multilocus estimates of genetic differentiation, computed as $F_{ST} / (1 - F_{ST})$ following (Rousset 1997), and log-transformed geographic distance between sites, was assessed with a Mantel test following the same methodology described above for mtDNA.

The program MIGRATE 3.0 (Beerli and Felsenstein 2001; Beerli 2003) was used to estimate population specific Θ (which for nuclear data equals $4N_e\mu$, where N_e is the effective population size and μ is the mutation rate per site) and pairwise scaled migration rates *M,* (i.e. m/μ, where m is the immigration rate per generation). This program uses a coalescence-based likelihood and Markov chain Monte Carlo method to estimate Θ and M values and allows for the assessment of asymmetric rates of migration (Beerli and Felsenstein 1999). The purpose of this analysis was not to use the

parameter estimates to reach estimates of actual effective population size and migration rate, as this would imply choosing an arbitrary mutation rate, but to compare patterns of directional migration among locations. The analysis was performed under the infinite allele model of mutation and starting parameters were generated from F_{ST} estimates. The Markov chain Monte Carlo run consisted of 10 short and 3 long chains, with 500 and 5,000 recorded genealogies respectively, after discarding the first 10,000 trees, to ensure parameter stability (burn-in). The reconstructed genealogies were sampled every 20 steps, for both short and long chains. An adaptive heating scheme was applied, which ran 4 parallel Markov chains at different temperatures (start temperatures were 1.00, 1.20, 1.50 and 3.00). A 'cold' chain sampled locally, while 'hotter' chains moved more freely and could therefore explore more of the genealogy space. This scheme should therefore allow for more efficient exploration of the genealogy space by swapping states between chains, and should be useful in situations with flat likelihood surfaces (Beerli 2004) such as would be expected in most marine species with a planktonic dispersive stage.

6.3 Results

6.3.1 Mitochondrial DNA variability

After alignment and trimming, sequence analysis of the 650bp COI gene fragment among the 163 specimens of *P. rustica* revealed 25 polymorphic sites, including 25 transitions and 1 transversion, which defined 30 haplotypes. Haplotype frequencies per sampling location are given in Appendix B (Table B.1). Three haplotypes (*Pr2*, *Pr4* and *Pr11*) were very common across samples (being present in all, 11 and 9 samples, respectively) and accounted for 57.7% of the sequences screened in the assay. Two haplotypes (*Pr1* and *Pr5*) were also found in the majority of samples (8 and 9, respectively), but only represented 18.4% of the individuals. Conversely, 20 haplotypes were represented by just one individual. The minimum number of base pair differences between any two haplotypes ranged from one to six. Nucleotide diversity was low overall (% π = 0.0035 ± 0.0005 SD), and very similar across regions, ranging from 0.0034 (± 0.0002) to 0.0038 (±0.0004), and across locations, varying between 0.0023 (± 0.0017) at São Bernardino and 0.0043 (± 0.0027) at Bañugues (Table 6.3). Overall haplotype diversity was high (h = 0.8730 ± 0.0122), with virtually similar regional values. Within-location haplotype diversities ranged from 0.7727 ± 0.1276 at São Bernardino to 0.9333 ± 0.0449 at Homem do Leme.

Table 6.3 Genetic diversity estimates and neutrality test for each sample, averaged over sample groups (Northern Iberia, Northern Portugal and Southern Portugal) and over all samples. *n* - sample size; *N* - number of haplotypes; *h* - haplotype diversity (Nei 1987); π - nucleotide diversity (Nei 1987); F_S – Test statistic for selective neutrality (Fu 1997). Significant values for tests of neutrality ($P < 0.05$) are in bold.

Location	*n*	*N*	*h* ± SD	π ± SD (%)	F_S
N. Iberia					
Biarritz	9	6	0.8333 ± 0.1265	0.0033 ± 0.0023	-1.9660
Noja	15	8	0.8667 ± 0.0673	0.0036 ± 0.0023	-2.5401
Bañugues	14	7	0.8571 ± 0.0652	0.0043 ± 0.0027	-1.0905
Cabo Prior	14	7	0.8791 ± 0.0576	0.0035 ± 0.0023	-1.6828
All	52	16	0.8793 ± 0.0245	0.0038 ± 0.0004	**-6.5053**
N. Portugal					
Moledo do Minho	15	6	0.8190 ± 0.0636	0.0032 ± 0.0021	-0.6973
Viana do Castelo	15	9	0.9048 ± 0.0544	0.0036 ± 0.0023	**-3.8316**
Homem do Leme	15	10	0.9333 ± 0.0449	0.0038 ± 0.0024	**-5.1054**
Buarcos	15	8	0.8952 ± 0.0525	0.0035 ± 0.0023	-2.5689
All	60	17	0.8751 ± 0.0245	0.0034 ± 0.0002	**-7.8591**
S. Portugal					
São Bernardino	12	7	0.7727 ± 0.1276	0.0023 ± 0.0017	**-3.4826**
Cabo Raso	13	5	0.7564 ± 0.0974	0.0028 ± 0.0019	-0.2912
Castelejo	13	7	0.8333 ± 0.0861	0.0041 ± 0.0026	-1.4379
Olhos d'Água	13	7	0.8974 ± 0.0537	0.0035 ± 0.0023	-1.9260
All	51	14	0.8573 ± 0.0249	0.0032 ± 0.0007	-5.1373
Total	163	30	0.8729 ± 0.0122	0.0035 ± 0.0005	**-19.6106**

6.3.2 Mitochondrial DNA gene genealogies and historical demography

The reconstructed haplotypic network showed no relevant geographic clustering of haplotypes (Figure 6.2). The most common haplotypes were distributed throughout the network, differing by only a few substitutions, and were present in the three geographic regions defined in the study. The remaining haplotypes were generally separated from the most common ones by 1 or 2 mutational steps, and a star-shaped genealogy was observed around haplotype *Pr11*, a pattern that is often associated with population expansion.

Contrasting with results from the haplotype network, population expansion was not confirmed by the observed mismatch distribution (Figure 6.3), which was significantly different from the expected distribution. This hypothesis was refuted by the

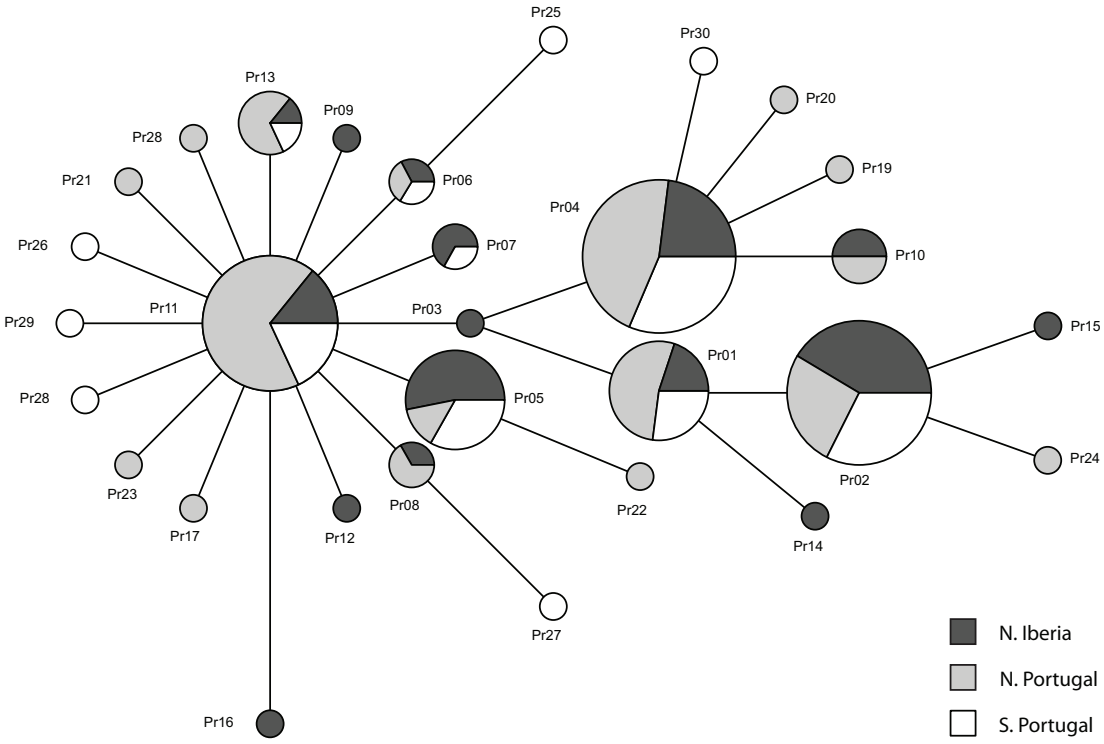

Figure 6.2 Median-joining network of COI haplotypes found in *Patella rustica* along the Atlantic coast of the Iberian Peninsula. Each circle represents one haplotype, and its area is proportional to its relative frequency, whereas line length is proportional to the number of mutational steps. Haplotypes shared among regions are represented as frequency diagrams, the area filled with a given colour being proportional to the number of individuals from each area.

SSD value and raggedness index, (both significant at $P < 0.05$).

The majority of samples showed non-significant values of Fu's F_S statistics (the exceptions being those from the recently colonised localities of Viana do Castelo and Homem do Leme, plus São Bernardino). In contrast, tests performed on regional samples, as well as with all data combined as a single group, yielded a significant negative result (for the total sample: $F_S = -19.611$, $P < 0.001$), indicating an excess of recent mutations, and thus supporting the occurrence of population growth or selective sweeps (Table 6.3).

For all the runs of BEAST, the Markov chain reached stability before the burn-in limit. All estimated parameters had effective sample sizes higher than 200, the threshold value which indicates adequate exploration of the sample space. The two independent runs performed for each tree prior converged on the same distribution, and their results could therefore be combined. The coalescent constant size tree prior was shown to fit the data significantly better than the coalescent exponential growth tree prior, since $2\log_e$ BF > 10 (Kass and Raftery 1995; Nylander *et al.* 2004), and thus only the results obtained with the latter are presented. Estimate of time to most recent common

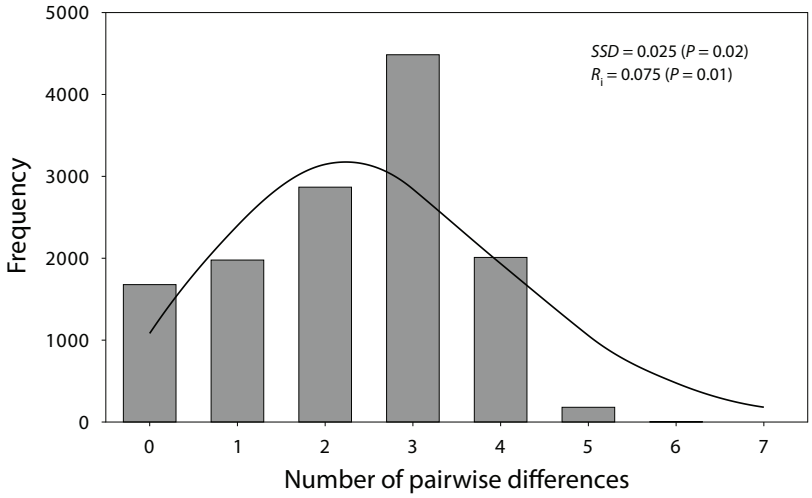

Figure 6.3 Mismatch distribution of mtDNA COI haplotypes for all samples combined. Bars represent the observed pairwise nucleotide mismatch distributions; the line represents the distribution predicted from a recent demographic expansion model. *SSD* – sum of squared deviations between the observed and expected mismatch distributions; R_i – raggedness index (Harpending 1994).

ancestor (TMRCA) under the scenario of constant population size was 395,000 years (95% highest posterior density interval 182,000 – 661,000 years).

6.3.3 Microsatellite DNA variability

Allele frequencies per locus and sampling location are listed in Appendix B (Table B.2). The seven microsatellite loci used in the assay were polymorphic in all samples (Table 6.4). Total number of alleles per locus amongst all samples ranged from 8 at locus *Pru5* to 51 at locus *Pru7*, with an average of 22. Among populations, the number of alleles averaged across all loci ranged between 9.3 at Cabo Prior and Olhos d'Água and 11.4 at Cabo Raso and Castelejo (average 10.5). These differences decreased slightly after standardisation by rarefaction to the smallest sample size (16 diploid individuals or 32 genes). Values of allelic richness across the seven loci varied between 7.6 at Olhos d'Água and 9.3 at Castelejo (average 8.6), with no identifiable geographic pattern, and across populations ranged between 3.7 at locus *Pru2* and 20.4 at locus *Pru7* (average 9.4). Expected heterozygozities across populations ranged between 0.253 for locus *Pru2* and 0.933 for *Pru7* (average 0.677) and observed heterozygozities ranged from 0.225 to 0.852 for *Pru2* and *Pru13* respectively (average 0.518). Within populations and over all loci, expected and observed heterozygozities ranged from 0.643 to 0.723 and from 0.473 to 0.598, respectively.

No evidence of genotypic linkage disequilibrium between any pair of loci has been found. Only 14 of the 252 pairwise comparisons within samples (all different

Table 6.4 Genetic diversity estimates for seven microsatellite loci screened in *Patella rustica*. Significant values after Bonferroni correction are depicted in bold type.

Location	*Pru1*	*Pru2*	*Pru5*	*Pru6*	*Pru7*	*Pru13*	*Pru14*	All loci
N. Iberia (NI)								
Biarritz								
N	36	36	26	21	32	32	21	44
Na	15	4	5	12	21	12	7	10.857
A	10.075	3.377	4.087	10.932	15.987	9.747	6.687	8.699
H_O	0.722	0.194	0.269	0.429	0.438	0.781	0.476	0.473
H_E	0.728	0.275	0.365	0.890	0.943	0.846	0.755	0.686
F_{IS}	0.009	0.296	0.266	**0.524**	**0.540**	0.078	0.375	**0.291**
r	-	-	-	0.235	0.255	-	-	
Noja								
N	30	31	34	29	26	30	30	35
Na	15	4	6	13	17	12	4	10.143
A	11.320	2.983	4.388	10.301	14.513	9.683	3.491	8.097
H_O	0.667	0.194	0.206	0.689	0.308	0.867	0.500	0.490
H_E	0.767	0.182	0.547	0.842	0.922	0.877	0.534	0.667
F_{IS}	0.133	-0.062	**0.627**	0.183	**0.671**	0.012	0.065	**0.264**
r	-	-	0.216	-	0.313	-	-	
Bañugues								
N	33	33	33	23	30	32	30	35
Na	16	5	6	11	19	11	7	10.714
A	10.606	3.897	5.185	9.576	14.791	9.875	5.550	8.497
H_O	0.727	0.273	0.272	0.739	0.567	0.938	0.667	0.598
H_E	0.662	0.252	0.479	0.830	0.927	0.886	0.571	0.658
F_{IS}	-0.101	-0.083	**0.434**	0.112	**0.393**	-0.059	-0.172	**0.091**
r	-	-	0.135	-	0.181	-	-	
Cabo Prior								
N	32	32	34	28	19	31	16	35
Na	11	4	5	11	15	10	9	9.286
A	8.993	3.482	4.185	9.906	14.118	8.999	9.000	8.383
H_O	0.594	0.281	0.206	0.643	0.579	0.871	0.563	0.534
H_E	0.670	0.375	0.475	0.854	0.929	0.847	0.649	0.686
F_{IS}	0.115	0.252	**0.571**	0.251	**0.383**	-0.029	0.137	**0.221**
r	-	-	0.179	-	0.171	-	-	
All within NI								
N	131	132	127	101	107	125	97	149
Na	24	7	8	19	34	16	11	17.000
A	10.249	3.435	4.461	10.179	14.852	9.576	6.182	7.379
H_O	0.678	0.236	0.238	0.625	0.473	0.864	0.552	0.524
H_E	0.707	0.271	0.467	0.854	0.930	0.864	0.627	0.674
F_{IS}	0.039	0.137	**0.504**	**0.261**	**0.503**	0.000	0.093	**0.224**

Table 6.4 Genetic diversity estimates for seven microsatellite loci screened in *Patella rustica* (continued).

Location	*Pru1*	*Pru2*	*Pru5*	*Pru6*	*Pru7*	*Pru13*	*Pru14*	All loci
N. Portugal (NP)								
Moledo do Minho								
N	31	31	31	29	26	19	30	32
N a	16	3	5	11	23	10	4	10.286
A	11.427	2.467	4.284	9.795	18.050	9.637	3.515	8.454
H_O	0.742	0.161	0.419	0.586	0.577	0.789	0.533	0.544
H_E	0.762	0.153	0.543	0.864	0.956	0.881	0.453	0.659
F_{IS}	0.027	-0.056	0.231	**0.326**	**0.401**	0.106	-0.181	**0.174**
r	-	-	-	0.142	0.186	-	-	
Viana do Castelo								
N	31	31	31	26	28	30	31	31
N a	15	5	5	15	25	9	4	11.143
A	11.610	3.903	4.493	13.534	18.933	7.830	3.768	9.153
H_O	0.871	0.258	0.290	0.769	0.321	0.767	0.323	0.514
H_E	0.825	0.292	0.667	0.931	0.960	0.798	0.588	0.723
F_{IS}	-0.057	0.118	**0.568**	**0.176**	**0.669**	0.040	**0.456**	**0.290**
r	-	-	0.221	0.075	0.320	-	0.162	
Homem do Leme								
N	32	32	32	32	25	32	30	32
N a	13	6	4	11	20	14	4	10.286
A	10.660	4.925	3.488	9.074	16.340	10.698	3.940	8.446
H_O	0.625	0.375	0.281	0.656	0.360	0.875	0.433	0.515
H_E	0.750	0.383	0.568	0.842	0.945	0.861	0.519	0.695
F_{IS}	0.169	0.022	**0.509**	**0.223**	**0.624**	-0.017	0.168	**0.247**
r	-	-	0.178	0.094	0.294	-	-	
Buarcos								
N	28	28	30	28	27	28	27	32
N a	15	3	6	11	23	14	5	11.000
A	11.180	2.542	3.530	9.580	18.457	11.293	4.123	8.672
H_O	0.679	0.179	0.233	0.642	0.519	0.786	0.370	0.487
H_E	0.750	0.168	0.476	0.846	0.958	0.871	0.519	0.655
F_{IS}	0.097	-0.063	**0.514**	0.244	**0.464**	0.100	0.290	**0.261**
r	-	-	0.160	-	0.217	-	-	
All within NP								
N	131	132	127	101	107	125	97	127
N a	26	9	6	16	47	17	6	18.143
A	11.219	3.459	3.949	10.496	17.945	9.865	3.837	7.161
H_O	0.729	0.243	0.306	0.663	0.444	0.804	0.415	0.515
H_E	0.772	0.249	0.564	0.871	0.955	0.853	0.520	0.683
F_{IS}	0.056	0.025	**0.461**	**0.242**	**0.541**	0.051	**0.205**	**0.249**

Table 6.4 Genetic diversity estimates for seven microsatellite loci screened in *Patella rustica* (continued).

Location	*Pru1*	*Pru2*	*Pru5*	*Pru6*	*Pru7*	*Pru13*	*Pru14*	All loci
S. Portugal (SP)								
São Bernardino								
N	34	34	35	33	24	34	28	35
N a	15	3	5	14	18	13	7	10.714
A	10.918	2.328	3.908	11.115	14.831	10.892	5.855	8.550
H_O	0.735	0.118	0.314	0.758	0.375	0.912	0.321	0.505
H_E	0.748	0.114	0.463	0.866	0.918	0.888	0.634	0.662
F_{IS}	0.018	-0.035	0.324	**0.127**	**0.597**	-0.027	0.498	**0.212**
r	-	-	-	-	0.276	-	0.186	
Cabo Raso								
N	31	31	31	23	25	31	24	31
N a	13	6	5	10	25	13	8	11.429
A	9.241	4.588	4.030	9.103	19.308	10.686	6.829	9.112
H_O	0.581	0.323	0.323	0.739	0.480	0.871	0.500	0.545
H_E	0.634	0.295	0.538	0.857	0.945	0.884	0.669	0.689
F_{IS}	0.085	-0.095	**0.404**	**0.14**	**0.497**	0.015	**0.257**	**0.202**
r	-	-	0.135	-	0.232	-	-	
Castelejo								
N	29	31	33	28	25	30	29	35
N a	13	6	6	14	20	13	8	11.429
A	9.795	4.550	5.152	11.158	15.954	11.578	6.640	9.261
H_O	0.621	0.161	0.367	0.750	0.280	0.800	0.552	0.504
H_E	0.611	0.319	0.593	0.836	0.928	0.902	0.697	0.698
F_{IS}	-0.016	0.499	**0.39**	**0.105**	**0.703**	0.115	0.211	**0.274**
r	-	0.116	0.139	-	0.330	-	-	
Olhos d'Água								
N	31	32	34	30	29	30	27	35
N a	11	4	3	14	16	11	6	9.286
A	7.630	3.228	2.928	11.038	12.879	9.421	5.898	7.575
H_O	0.516	0.188	0.323	0.667	0.276	0.967	0.630	0.510
H_E	0.513	0.231	0.463	0.842	0.864	0.838	0.749	0.643
F_{IS}	-0.005	0.19	**0.305**	0.211	**0.685**	-0.157	0.162	**0.209**
r	-	-	-	-	0.310	-	-	
All within (SP)								
N	125	128	133	114	103	125	108	136
N a	21	8	7	18	39	16	10	17
A	9.396	3.674	4.005	10.604	15.743	10.644	6.306	7.412
H_O	0.613	0.198	0.332	0.729	0.353	0.888	0.501	0.516
H_E	0.627	0.240	0.514	0.850	0.914	0.878	0.687	0.673
F_{IS}	0.022	0.177	**0.358**	**0.146**	**0.622**	-0.011	**0.277**	**0.237**

Table 6.4 Genetic diversity estimates for seven microsatellite loci screened in *Patella rustica* (continued).

Location	*Pru1*	*Pru2*	*Pru5*	*Pru6*	*Pru7*	*Pru13*	*Pru14*	All loci
All locations								
N	378	382	384	330	316	359	323	412
N a	31	12	8	23	51	20	12	22.429
A	10.612	3.746	4.330	11.093	20.380	10.303	5.511	9.425
H_O	0.673	0.225	0.292	0.672	0.423	0.852	0.489	0.518
H_E	0.702	0.253	0.515	0.858	0.933	0.865	0.611	0.677
F_{IS}	0.040	0.114	**0.439**	**0.215**	**0.554**	0.011	**0.198**	**0.236**

pairs, distributed over several samples) were significant at $P < 0.05$ and none remained significant after correction for multiple testing. Moreover, none of the global tests was significant ($P > 0.05$), and therefore loci can be considered independent variables. Significant departures from HWE indicated by F_{IS} values were detected by 27 of the 84 exact tests across loci and samples after sequential Bonferroni correction (initial $\alpha = 0.007$) and at three of the seven loci (*Pru5*, *Pru6* and *Pru7*) for all individuals combined. All deviations consisted of heterozygote deficiencies. Loci *Pru5* and *Pru7* were responsible for 74% of the total cases of significant heterozygote deficits (locus *Pru5* exhibited heterozygote deficits in 8 samples and *Pru7* in all 12 samples), whilst loci *Pru6* and *Pru14* showed significant heterozygote deficits in 4 and 3 populations, respectively. Loci *Pr1*, *Pr2* and *Pr13* were in agreement with HWE at all locations. Significant multilocus deviations from HWE were observed at all locations.

Heterozygote deficiency can result from inbreeding, population substructure and the presence of null alleles. However, in the present case, the patterns of deficits across loci and samples seem to rule out the first two possibilities, since in none of the samples a significant heterozygote deficit across loci was observed. The most probable causes for the excess of homozygotes observed at certain loci are technical artefacts generated during PCR amplification and the presence of null alleles. Scoring errors due to stuttering were inferred in nine samples for locus *Pru5*, six for locus *Pru6* and one for locus *Pru14*. Departures from HWE were identified as consistent with the presence of null alleles in nine samples at locus *Pru5* (inferred frequency range 0.135-0.221), four at *Pru6* (0.075-0.235), and in all samples at *Pru7* (0.171-0.330). Potential null alleles with significant frequencies were also identified at three other loci (*Pru1*, *Pru2* and *Pru14*), although with a much lower incidence (five samples in total). Correcting genotype frequencies for null alleles did not result in fewer departures from HWE in loci *Pru5* and *Pru7* (data not shown), and therefore these were excluded from further analyses.

6.3.4 Population structure

6.3.4.1 Mitochondrial DNA

Regional Φ_{ST} values were very low and statistically non-significant (Table 6.5). Estimates of pairwise Φ_{ST} among samples varied between -0.070 and 0.200 (Table 6.6), and only 8 out of the 66 values were statistical significance at $P < 0.05$ (seven of them involving the sample from São Bernardino). Results from hierarchical AMOVAs showed that almost all genetic variance could be attributed to differences within samples (Table 6.7). Moreover, measures of population subdivision were statistically non-significant at all levels of the analyses (among regions, among locations within regions and within locations). The global Mantel test revealed no significant correlation between pairwise genetic distance estimates, defined as $\Phi_{ST}/(1 - \Phi_{ST})$, and log-transformed geographical distances ($Z = 3.164$, $r = 0.015$, $P = 0.450$; Figure 6.4). Correlations between genetic and log-transformed geographical distances were also not significant within Northern Iberia ($Z = 0.280$, $r = -0.364$, $P = 0.794$), Northern Portugal ($Z = -0.425$, $r = 0.138$, $P = 0.410$) and Southern Portugal ($Z = 1.120$, $r = 0.095$, $P = 0.413$).

6.3.4.2 Microsatellites

Pairwise exact tests of allelic differentiation among samples using Fisher's combined method resulted in 12 significant comparisons (out of 66), after sequential Bonferroni correction. Exact tests at the genotypic level showed a weaker level of differentiation, with only 5 of them yielding a significant result (Table 6.8).

Estimates of population differentiation were predictably low, given the high degree of variability of microsatellite loci (Hedrick 1999). The global multilocus estimate of F_{ST} was low, but statistically significant ($F_{ST} = 0.007$, $P = 0.0008$), whilst global single-locus F_{ST} values ranged between 0.0034 for locus *Pru2* and 0.0096 for locus *Pru6*. At the regional level, only the pairwise F_{ST} between northern and southern Portuguese sample groups was significant (Table 6.5). Pairwise F_{ST} values among locations varied between -0.006 and 0.033, and 16 were significant at the 5% nominal level, but only 4 remained so after sequential Bonferroni correction (Table 6.6). Maximum differentiation was observed between the locations of Olhos d'Água and Moledo do Minho.

Further support for the lack of genetic structure was provided by the neighbour-joining tree constructed from chord distances among samples (Figure 6.5). The tree showed no clear partitioning into distinct groups, and bootstrap support values above 50% were rarely achieved. On the other hand, hierarchical AMOVAs indicated that almost all genetic variation was apportioned to the individual level (> 99%).

Table 6.5 Pairwise F_{ST}, standardised F_{ST} and Φ_{ST} values for regional differentiation in *Patella rustica*. Value in bold type was significant at $P < 0.05$. No values were significant after sequential Bonferroni correction.

Regional comparisons	F_{ST}	F (std)	Φ_{ST}
Northern Iberia vs. Northern Portugal	0.002	0.007	0.006
Southern Portugal vs. Northern Iberia	0.001	0.002	-0.011
Southern Portugal vs. Northern Portugal	**0.009**	0.027	-0.007

Table 6.6 Pairwise estimates of genetic differentiation between samples of *Patella rustica*. Above diagonal: F_{ST} incorporating genetic distances between haplotypes from sequence analysis of the mitochondrial COI gene (Φ_{ST}). Below diagonal: multilocus F_{ST} (θ) based on variation assessed at 5 microsatellite loci. * indicates significance at $P < 0.05$; values in bold were significant after sequential Bonferroni correction for 66 tests ($P < 0.0008$). See Table 6.1 for sample codes.

	BIA	NOJ	BAN	CPR	MOL	VIA	HLE	BUA	SBE	CRA	CAT	ODA
BIA	-	0.104	0.006	-0.041	0.064	0.063	0.005	-0.001	0.200*	0.163*	-0.070	-0.032
NOJ	0.009	-	-0.029	0.066	0.006	0.015	0.022	0.041	-0.014	0.023	0.058	0.003
BAN	0.015*	0.006	-	-0.001	-0.013	-0.005	-0.024	-0.005	0.054	0.009	-0.022	-0.040
CPR	0.002	-0.001	-0.001	-	-0.017	-0.017	-0.040	-0.057	0.158*	0.051	-0.058	-0.055
MOL	0.021*	-0.003	0.004	0.002	-	-0.047	-0.022	-0.042	0.089	-0.033	0.001	-0.040
VIA	0.010	0.016*	**0.027***	0.007	0.013*	-	-0.036	-0.041	0.100*	-0.035	0.010	-0.032
HLE	0.009	0.005	0.006	0.002	0.003	0.012*	-	-0.046	0.111*	0.001	-0.028	-0.052
BUA	0.011	-0.004	-0.001	-0.004	-0.006	0.014*	0.000	-	0.122*	0.001	-0.033	-0.051
SBE	0.000	-0.004	0.006	-0.002	0.004	0.013*	0.003	-0.003	-	0.149*	0.143*	0.090
CRA	0.002	0.001	-0.003	-0.005	0.008	0.019*	0.004	-0.004	-0.004	-	0.075	0.021
CAT	0.002	0.000	0.001	-0.006	0.008	0.024*	0.011	-0.001	-0.001	-0.005	-	-0.051
ODA	0.002	0.024*	0.018*	0.007	**0.033***	0.031*	**0.027***	**0.018***	0.011	0.002	0.006	-

Nevertheless, the AMOVA based on microsatellite genotypes and comprising all three geographical groups showed that small, but significant, amounts of the variance were partitioned among regions, and among locations within regions (Table 6.7).

The standardised multilocus global F_{ST} was 0.0197, only slightly higher than the estimate of global Φ_{ST} (0.0135). Similarly, standardised regional estimates of pairwise multilocus F_{ST} did not differ much from mtDNA Φ_{ST} estimates, except between Southern and Northern Portugal, where standardised F_{ST} was considerably higher (Table 6.5). Standardised pairwise F_{ST} values for comparisons between locations were lower than their mtDNA Φ_{ST} counterparts, varying between zero and 0.097 (Table 6.9).

Global Mantel tests showed a non-significant correlation between $F_{ST}/(1 - F_{ST})$

Table 6.7 Hierarchical AMOVA for *Patella rustica* from 12 locations in three regions defined in the species Atlantic Iberian range (Northern Iberia, Northern Portugal and Southern Portugal). Results are shown for multilocus AMOVA based on variance in allele frequencies at five microsatellite loci and in variance in haplotype frequencies at the mitochondrial DNA COI gene, taking into account sequence divergence. A) AMOVA among northern and southern groups within the historical distribution range. B) AMOVA including the three groups of locations. * indicates significance at $P < 0.05$; ** indicates significance at $P < 0.001$.

	mtDNA			Microsatellites		
Source of variation	Variance	%	Φ-statistics	Variance	%	*F*-statistics
A) N. Iberia vs. S. Portugal						
Among regions	-0.0242	-2.11	Φ_{CT} = -0.0211	0.0000	0.01	F_{CT} = -0.0001
Among samples within regions	0.0492	4.28	Φ_{SC} = 0.0420	0.0086	0.52	F_{SC} = 0.0052
Within samples	1.1227	97.83	Φ_{ST} = 0.0218	1.6471	99.48	F_{ST} = 0.0052
B) All groups						
Among regions	-0.0063	-0.55	Φ_{CT} = -0.0055	0.0054	0.32	F_{CT} = 0.0032 *
Among samples within regions	0.015	1.32	Φ_{SC} = 0.0131	0.0108	0.65	F_{SC} = 0.0066 *
Within samples	1.1315	99.23	Φ_{ST} = 0.0077	1.6390	99.02	F_{ST} = 0.0098 **

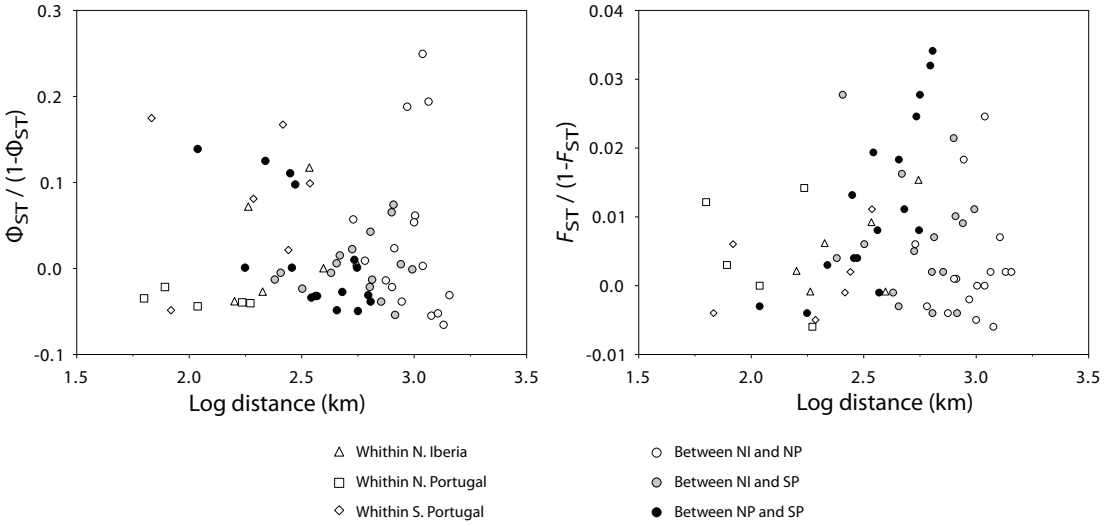

Figure 6.4 Relationships between geographic (log transformed) and genetic distance among the 12 sampling sites, inferred from mtDNA (left) and microsatellites (right). Geographic distance was measured in kilometres as the shortest alongshore distance among locations.

and log-transformed geographical distances ($Z = 1.256$, $r = 0.024$, $P = 0.428$; Figure 6.4). Isolation-by-distance was also not significant among samples from Northern Iberia ($Z = 0.072$, $r = -0.181$, $P = 0.630$), Northern Portugal ($Z = 0.093$, $r = -0.541$, $P = 0.901$) and Southern Portugal ($Z = 0.023$, $r = 0.339$, $P = 0.088$).

Bayesian clustering methods were used to infer the number of populations

Table 6.8 Probability values for Fisher's combined test of genic (above diagonal) and genotypic (below diagonal) differentiation at 5 microsatellite loci. * indicates significance at $P < 0.05$; values in bold were significant after sequential Bonferroni correction for 66 tests ($P < 0.0008$). See Table 6.1 for sample codes.

	BIA	NOJ	BAN	CPR	MOL	VIA	HLE	BUA	SBE	CRA	CAT	ODA
BIA	-	**<0.001***	0.052	0.006*	**<0.001***	**<0.001***	0.003*	**<0.001***	0.054	0.011*	0.030*	0.104
NOJ	0.010*	-	0.011*	0.032*	0.027*	**<0.001***	0.007	0.028*	0.213	0.017*	0.014*	**<0.001***
BAN	0.133	0.025*	-	0.135	0.008*	0.001*	0.075	0.141	0.211	0.621	0.388	0.032*
CPR	0.086	0.104	0.223	-	0.002*	0.083	0.019*	0.149	0.195	0.034*	0.381	0.022*
MOL	**<0.001***	0.076	0.016*	0.007*	-	**<0.001***	0.002*	0.056	0.020*	**<0.001***	0.011*	**<0.001***
VIA	0.015*	0.003*	0.005*	0.347	0.004*	-	**<0.001***	0.007*	0.008*	**<0.001***	0.002*	**<0.001***
HLE	0.036*	0.022*	0.134	0.095	0.005*	0.008*	-	0.122	0.075	0.006*	0.007*	0.001*
BUA	0.016*	0.061	0.217	0.234	0.091	0.053	0.166	-	0.305	0.387	0.294	0.038*
SBE	0.135	0.409	0.319	0.330	0.051	0.044*	0.129	0.425	-	0.474	0.428	0.053
CRA	0.044*	0.058	0.683	0.163	**<0.001***	**<0.001***	0.017*	0.540	0.534	-	0.315	0.036*
CAT	0.171	0.104	0.556	0.655	0.039*	0.024*	0.033*	0.593	0.677	0.526	-	0.057
ODA	0.239	**<0.001***	0.034*	0.071	**<0.001***	0.002*	0.002*	0.119	0.051	0.067	0.139	-

represented in the data. All runs performed (at the individual level with spatial information, at the sample level, without and with spatial information) grouped all samples together, and the marginal posterior probability for this clustering solution was 1.0 (marginal probability for the existence of other partitions was zero).

6.3.5 Scaled population sizes and patterns of gene flow

Estimates of population specific parameter Θ ($4N_e\mu$) did not differ much among samples, ranging from 0.93 to 1.28 (Table 6.10 for a summary, Table C.1, Appendix C for 95% confidence intervals). For these values to be interpreted, they would have to be scaled to mutation rates, which have not yet been measured for microsatellite loci in *P. rustica*. In addition, although mutation rates inferred in other species agree in being usually high, they may differ by several orders of magnitude, according to a variety of aspects such as species, microsatellite size, or repeat motif (Estoup and Cornuet 1999; Balloux and Lugon-Moulin 2002; Whittaker *et al.* 2003). Thus, any attempts to calculate effective population sizes from Θ assuming some arbitrarily chosen mutation rate were not deemed appropriate.

Estimates for migration rates (m/μ) ranged from 0.13 to 3.30 among the 12 sites for which microsatellite data were available, revealing some heterogeneity in the levels of connectivity along the study area (Table 6.10; see Table C.2 in Appendix C for 95% confidence intervals). Asymmetrical migrant interchange was not very marked, with

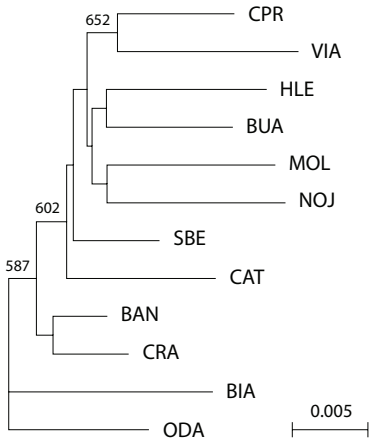

Figure 6.5 Unrooted neighbour-joining tree of *P. rustica* samples, based on chord distances (Cavalli-Sforza and Edwards 1967). Numbers next to branches indicate bootstrap support from 1000 bootstrapped data sets. Only bootstrap values > 50% are represented.

differences in opposite migration rates among sample pairs rarely reaching one order of magnitude. Nonetheless, although none of the populations sampled were consistently net donors or receivers, there was a predominance of northward/eastward migration, especially among the Asturian and Basque locations, and to a lesser extent, among northwestern Iberian ones.

6.4 Discussion

6.4.1 Genetic diversity

A combined analysis of mitochondrial and nuclear data from 12 samples of *P. rustica* collected along the Atlantic coast of the Iberian Peninsula was carried out to investigate historical demography and contemporary patterns of gene flow. A pattern of high haplotype diversity and low nucleotide diversity was congruent among all regions defined in the study (Northern Iberia, Northern Portugal and Southern Portugal) and also among locations within regions. Levels of haplotype diversity at the mtDNA COI locus were, in some cases, comparable to those obtained for other marine species with complex life-cycles inhabiting the northeast Atlantic margin (e.g. Roman and Palumbi 2004; Couceiro *et al.* 2007), and in other cases they were slightly lower (e.g. Wares 2001; Duran *et al.* 2004a; Quinteiro *et al.* 2007).

Microsatellite diversity was very different among loci ($0.253 < H_E < 0.933$), and apart from *Pru7*, generally lower than that observed in *Patella depressa* (see chapter 5). Only two loci showed allelic frequency distributions compatible with the presence of

Table 6.9 Standardised multilocus pairwise F_{st} estimates for 66 pairwise comparisons between 12 locations. See Table 6.1 for sample codes.

	BIA	NOJ	BAN	CPR	MOL	VIA	HLE	BUA	SBE	CRA	CAT	ODA
BIA	-											
NOJ	0.028	-										
BAN	0.046	0.015	-									
CPR	0.007	0.000	0.000	-								
MOL	0.062	0.000	0.012	0.005	-							
VIA	0.033	0.049	0.080	0.020	0.037	-						
HLE	0.027	0.015	0.017	0.007	0.008	0.039	-					
BUA	0.034	0.000	0.000	0.000	0.000	0.042	0.000	-				
SBE	0.001	0.000	0.016	0.000	0.011	0.041	0.009	0.000	-			
CRA	0.005	0.002	0.000	0.000	0.024	0.060	0.011	0.000	0.000	-		
CAT	0.007	0.000	0.004	0.000	0.022	0.076	0.034	0.000	0.000	0.000	-	
ODA	0.007	0.066	0.049	0.021	0.087	0.091	0.077	0.050	0.030	0.006	0.018	-

Table 6.10 Maximum likelihood estimates for population specific Θ and scaled directional migration rates (*M*) computed with MIGRATE. Results with 95% confidence intervals are given in Appendix C. See Table 6.1 for sample codes and location coordinates.

		M	Donor location										
Receiving	Θ	BIA	NOJ	BAN	CPR	MOL	VIA	HLE	BUA	SBE	CPR	CAT	ODA
BIA	0.963	-	0.954	1.680	2.330	1.562	1.344	0.954	1.138	1.868	0.694	1.344	1.387
NOJ	0.984	1.076	-	0.682	1.220	0.753	1.471	1.901	1.292	0.898	1.256	1.212	0.789
BAN	1.134	0.809	0.383	-	2.766	1.830	1.447	1.362	1.064	1.745	1.575	1.149	1.617
CPR	1.000	1.285	0.571	0.841	-	1.156	0.600	1.141	1.020	1.261	0.260	1.021	1.291
MOL	1.100	1.524	1.594	0.921	1.515	-	1.276	2.623	0.638	0.248	1.063	0.921	1.063
VIA	0.933	0.526	2.104	1.490	1.314	0.920	-	1.356	0.833	0.745	1.139	2.060	1.139
HLE	0.968	0.632	0.765	1.431	1.190	1.498	0.199	-	0.899	1.331	0.499	2.230	1.032
BUA	1.118	0.846	0.966	1.651	0.805	0.523	0.685	0.795	-	1.691	1.091	1.852	1.406
SBE	1.277	1.231	1.106	1.188	2.088	2.785	2.171	1.883	0.944	-	1.393	0.983	1.229
CPR	1.164	1.883	3.298	1.121	1.558	1.344	0.631	0.987	1.166	0.448	-	0.673	1.794
CAT	1.062	1.577	1.016	0.736	1.751	1.471	1.182	0.771	0.631	1.226	1.900	-	0.842
ODA	1.026	0.791	1.352	0.989	0.626	1.319	0.923	1.220	1.022	1.319	0.132	1.352	-

null alleles (*Pru5* and *Pru7*), and have been eliminated from the analyses. Significant deviations from HWE were not very frequent in the remaining loci, and consisted of heterozygote deficiencies. Several biological factors can be advanced to explain heterozygote deficiencies, including microsatellite hitch-hiking selection, inbreeding,

and spatial and temporal Wahlund effects (see the Discussion section of chapter 5). In the present case, a temporal Wahlund effect caused by variance in reproductive success is the most probable cause for the observed homozygote excess. However, the lower incidence of observed departures from HWE suggests that sweepstakes reproduction may not be so common in this species, probably because of the shorter reproductive seasons (comprising only one spawning event) and smaller effective population sizes in comparison with *P. depressa*.

All shores within the distributional gap were sampled in 2005. Quantitative data of the species abundance at several locations along the gap showed that in 2003, *P. rustica* had not yet been observed at two of the locations also sampled for this study - Moledo do Minho and Viana do Castelo (Lima *et al.* 2006) – and in 2005 only 109 and 92 individuals were spotted respectively at each location, following thorough surveys. It is therefore a reasonable conjecture that all the specimens collected at these sites and used in the genetic survey corresponded to first generation colonists, and that those collected from the other two shores within the former gap were also early colonists. This provided an excellent opportunity to gain some insights on the consequences of dispersal in a strongly advective environment on the genetic composition of range-expanded populations.

The establishment of new populations from a small number of individuals, often associated with episodes of species introductions and expansions, may result in founder effects. These are characterized by a drastic reduction of genetic diversity in colonists, which is typically a subset of that found in the source population (Hewitt 1996; Wares and Cunningham 2001). In the present case, despite the very low census sizes of recently established populations (Lima *et al.* 2006), genetic founder effects were not observed. In fact, mitochondrial and microsatellite diversity showed little difference among historical and recently colonised parts of the range: samples from both areas displayed high genetic polymorphism and low levels of heterozygote deficiency. Similar observations have been made in the marine environment, and they have previously been associated with very high levels of gene flow between founder and colonised locations over short to intermediate spatial and temporal scales (Holland 2001; Barber *et al.* 2002a). In the present case, highly diverse founded populations resulted from the settlement of a genetically diverse larval pool, which could have been originated in different ways. Given the short reproductive season of *P. rustica*, the most likely explanation is that these individuals resulted from one single larval settlement event, either of a mix from several sources, or from only one genetically diverse source.

6.4.2 Demographic history and coalescence time

The succession of Pleistocene glaciations and interglacial periods, and in particular

the Last Glacial Maximum (LGM), which started around 20,000 years ago (Frenzel *et al.* 2001), played a major role in shaping contemporary geographical distribution of northern hemisphere species and of their genetic diversity (Hewitt 1996). The effect of glaciations on marine populations was different on both margins of North Atlantic. On the North American coast, the glacier advance was responsible for the complete loss of rocky intertidal habitat, causing mass extinction of intertidal communities (Ingolfsson 1992; Wares and Cunningham 2001). On the eastern Atlantic margin, sea ice extended to the northern part of the Bay of Biscay (Frenzel *et al.* 2001), forcing the southward retreat of many species into refugial areas including the Macaronesian islands, the Iberian and North African coasts, and the Mediterranean (e.g.Luttikhuizen *et al.* 2003; Duran *et al.* 2004a; Gysels *et al.* 2004; Chevolot *et al.* 2006). As the sea-ice retreated, populations expanded northwards, and colonised areas that had been previously covered with ice. Several marine species, on the other hand, show evidence of persistence at northern latitudes (e.g. Coyer *et al.* 2003; Gysels *et al.* 2004; Olsen *et al.* 2004; Provan *et al.* 2005; Jolly *et al.* 2006; Hoarau *et al.* 2007).

Although the Iberian Peninsula remained ice-free during the LGM, sea temperatures suffered a severe drop. Reconstruction of sea surface temperatures during the maximum cooling of the last glaciation indicated that minimal negative deviations from present-day values may have reached 10 °C along the northern coast of Iberia, whilst in southwestern Iberia and the Gulf of Cadiz, those deviations did not exceed 4 °C (Frenzel *et al.* 2001; Pflaumann *et al.* 2003). Therefore, it is likely that during this period, a considerable part of the Atlantic Iberian coast was unsuitable for *P. rustica*.

Coastal shell midden deposits from the Asturian region (northern Spain) provide supporting evidence for this assumption. One of the largest and most important deposits is located at the cave site of La Riera, and covers a period from approximately 20,700 years before present (BP) to about 6,500 years BP (Ortea 1986). It comprises great numbers of edible mollusc shells, belonging mainly to rocky shore gastropods. The La Riera shell midden is dominated by two northern species, *Patella vulgata* and *Osilinus lineatus*, and two southern species, *P. depressa* and *Littorina littorea*. The variation in species representation throughout the sequence shows a striking congruence with their expected responses to the climatic conditions inferred for that period. Throughout the greater part of the sequence, corresponding to maximum glacial conditions, the northern species were dominant, being replaced by the two southern species during the late period of postglacial warming. Although alternative hypotheses have been advanced to explain this shift in species predominance, Bailey and Craighead (2003) provided convincing arguments towards the role of climate oscillations that have taken place during late Pleistocene and early Holocene. The lusitanian limpet *P. rustica* is represented in very small numbers at the La Riera deposits. Its presence is restricted to stratigraphic levels coincident with an interstadial of the glaciation period,

around 20,500 years BP, and also at the end of the sequence, when climate warming was ongoing (12,000 years BP and less).

In the present study, Bayesian analysis of sequence variation was done under two different models of demographic history: exponential growth and constant size. Model comparison by calculation of a Bayes Factor (BF) over marginal likelihoods clearly supported the model of constant population size ($2\log_e$ BF >10). Moreover, an analysis of the frequency distribution of pairwise differences between individual sequences (mismatch distribution, Rogers and Harpending 1992), also showed no evidence of a recent bottleneck or demographic expansion (Figure 6.3). The age of the population, based on coalescent estimates of TMRCA was 395,000 years (95% confidence interval ranged between 182,000 and 661,000 years). This estimate must be considered with caution though, because the strict molecular clock parameter used in the calculations was based on the substitution rate inferred for a different gastropod genus (*Tegula*), and calibrated using the split of two species, that have presumably been separated by the isthmus of Panama, about 3 million years ago (Hellberg and Vacquier 1999). The extrapolation of this rate to more recent time scales may cause an overestimation of TMRCA (Sá-Pinto 2008). Nevertheless, and despite the very broad confidence interval of the estimate, the age of the Iberian population of *P. rustica* predates by far the LGM. This is in agreement with a considerable body of evidence suggesting that many marine populations have survived the LGM (Wares and Cunningham 2001; Duran *et al.* 2004a; Calderón *et al.* 2008).

Assuming that *P. rustica* suffered a range retraction in response to a decrease in sea surface temperature during the LGM, it is possible that a few populations persisted in southern Iberia, and that the expansion after the end of the glacial period was too quick to allow sufficient time for differentiation by genetic drift on the founded populations. A similar assumption was made by Weber and Hawkins (2006), who found no significant differentiation at allozyme loci among distant populations of *P. vulgata*. Furthermore, the recent colonisation of northern Portuguese shores by *P. rustica* suggests that range expansion from southern refugial shores after favourable climatic conditions were met might have occurred very rapidly and with little or no founder effects associated.

Conversely, two lines of evidence point to a more recent demographic expansion of *P. rustica* in the Atlantic Iberian coast. Analysis of the mitochondrial data indicated an excess of recent mutations on the whole population, as detected by Fu's test of selective neutrality (Fu's F_S = -19.611; $P < 0.001$; Table 6.3). This result may point to population expansion, but on the other hand, may also be indicative of a selective sweep. Population expansion was also supported by the haplotype network (Figure 6.2), given that a star-shaped genealogy is expected to be observed in a species that has undergone recent demographic expansion (Slatkin and Hudson 1991). The disparate

results obtained in this study suggest that the mtDNA sequence data contain a signal that seems to reflect a relatively old population expansion. It is possible that the high dispersive capacity of *P. rustica* along the Iberian Atlantic coast caused a partial erosion of the genetic effects of more recent glaciations periods.

6.4.3 Genetic differentiation

The geographic patterns of genetic structure inferred in this study were broadly congruent among marker classes, both data sets showing weak levels of population subdivision across the study area. Nonetheless, microsatellites provided slightly better resolution than mtDNA, with statistically significant differentiation being recovered among historical and recent population groups by hierarchical AMOVA. Genetic heterogeneity was also detected by pairwise *F*-statistics based on microsatellite allele frequencies, among northern and southern Portuguese groups. This pattern of regional divergence was not confirmed by Bayesian clustering analysis, which failed to identify more than one population in the microsatellite data set. Although the software BAPS usually performs well at low levels of genetic differentiation, its efficiency to correctly infer the number of clusters drops significantly for differentiation values of $F_{ST} = 0.02$ or less (Latch *et al.* 2006).

Measures of population differentiation obtained with microsatellites are often deflated by hypervariability at microsatellite loci. One procedure that allows discerning to what degree this occurs consists in the standardisation of divergence measures against the maximum possible differentiation values, given the amount of polymorphism present in the data set. Standardised F_{ST} estimates computed from the present data were low, suggesting that the weak levels of population structure inferred in this study did not result from marker variability, but rather reflect the real levels of genetic connectivity among the studied locations.

Standardised measures of microsatellite divergence also allowed for a direct comparison among marker classes. The difference between estimates obtained with mitochondrial and nuclear markers did not conform to theoretical expectations. There was no consistent trend in the relationship between standardised F_{ST} and Φ-statistics, although standardised F_{ST} estimates were higher in the majority of pairwise comparisons and also globally. In gonochoric species with balanced sex-ratios, estimates of population differentiation obtained with mtDNA are expected to be four times higher than those obtained with nuclear markers, because of the fourfold difference in effective population size. Although sampling error cannot be ruled out, especially for mtDNA (Buonaccorsi *et al.* 2001), the present results may have been caused by strong deviations from balanced male to female proportions across the study area. The occurrence of protandrous hermaphroditism has long been proposed

in patellid limpets, based in most cases on indirect evidence from length-frequency distributions (e.g. Dodd 1956; Thompson 1979; Guerra and Gaudêncio 1986), and on direct observations (Le Quesne and Hawkins 2006). Given that mtDNA is maternally inherited, the sequential sex-change of individuals from male to female would cause an increase in the effective population size of mtDNA, and consequently contribute to a decrease in the fourfold discrepancy of differentiation measures expected among mitochondrial and nuclear marker classes. The confirmation or rejection of this hypothesis would require investigating the mechanisms of sex determination and the occurrence of sequential hermaphroditism in *P. rustica,* since no information is currently available on the subject.

Consistently higher genetic differentiation was found between São Bernardino and the remaining samples at the mtDNA COI locus. Similarly, microsatellite data produced greater values of divergence in the majority of comparisons involving samples from Viana do Castelo and Olhos d'Água. Such contrasting patterns across markers are inconclusive regarding the existence of population structure in the study area. Instead, insufficient sample sizes may have caused an overestimation of differentiation measures at those locations, which was enhanced against a background of low genetic structure. This most likely explains the exceptionally higher differentiation found in mtDNA data from São Bernardino, since no obvious barriers to gene flow exist at such a small spatial scale. On the other hand, the greater differentiation found at Olhos d'Água may reflect ocean circulation patterns off the southwest coast of Portugal. During the reproductive season of *P. rustica*, in late summer and early autumn, the predominant northeasterly winds blowing along the Western Iberian coast cause the formation of upwelling and an associated equatorward surface current along the continental shelf (Haynes *et al.* 1993). At the southern coast, upwelling events are not as intense and persistent, because favourable westerly winds are weaker and less common (Relvas and Barton 2002). During periods of locally induced or west coast upwelling, the surface current along the southern Iberian coast flows eastwards. Conversely, a frequent nearshore countercurrent flows westward, and often during periods of upwelling relaxation, it turns around Cape São Vicente, flowing poleward along the western coast (Relvas and Barton 2002, 2005). Restrictions to gene flow between west and south coast populations may occur when this circulation pattern is disrupted at the vicinity of Cabo São Vicente, due to rapid changes in current direction, which are frequent over that area (Sánchez *et al.* 2006), and also by the presence of meddies. These hydrodynamic features are common in the area around Cape São Vicente (Prater and Sanford 1994; Martins *et al.* 2002), and consist of eddies containing water from the Mediterranean outflow. Although meddies are typically formed along the southern shelf break at depths of at least several hundread meters, they may influence surface circulation (Pingree and Le Cann 1993).

The lack of a significant correlation between genetic and geographical distances

suggests low levels of larval retention, which is in agreement with the typical habitat required by *P. rustica*. This species inhabits steep or vertical wave-beaten surfaces on exposed rocky shores and coastal defences, where the retention of larvae during their planktonic phase is unlikely to occur. The absence of an isolation by distance pattern also suggests homogeneous gene flow throughout the study area. Assuming that the minimum time required for larval development in *P. rustica* is comparable to that of other patellid limpets, this species may be able to disperse for at least 4 to 6 days under realistic sea surface temperatures. This period is too short to enable extensive larval transport between distant locations, considering that average surface current velocities during the reproductive season of the species are in the order of 0.1-0.2 m s^{-1} (Frouin *et al.* 1990; Martins *et al.* 2002). Alternatively, a combination of exceptionally high current velocities and the putative ability of patellid larvae to delay metamorphosis (see chapter 3) may enable sporadic episodes of long distance dispersal, which would be sufficient to maintain genetic homogeneity over a large area, since a small amount of genetic exchange is enough to prevent the accumulation of genetic differentiation (Slatkin 1987).

The genetic similarity found among samples from opposite sides of the former distributional gap in northern Portugal indicates that this stretch of once inhospitable coastline did not represent an effective barrier to gene flow among the two areas. Two mutually nonexclusive explanations for the inferred pattern of genetic homogeneity can be advanced: (1) the gap was transient or suffered considerable expansions and retractions, and (2) planktonic larvae were capable of crossing it. Rapid changes in geographical distributions of many marine species in response to brief periods of natural climate warming and cooling have been reported in the northeast Atlantic (Southward *et al.* 1995). Likewise, the dynamics of the gap has certainly been influenced by past climatic oscillations. Historic records of sea surface temperature variation in the northeast Atlantic collected during the last century (reviewed in Hawkins *et al.* 2003) closely match the scarce documentation of changes in the distribution of *P. rustica* over northwestern Iberia. In the early 1900s, which corresponded to a cold spell in the northeast Atlantic, *P. rustica* was absent from northern Portugal (Hidalgo 1917). Between the 1920s and the 1950s there was a warming of sea surface temperatures, and by the end of this period *P. rustica* probably found conditions to settle and survive in part of the gap. This is congruent with reports of a range expansion in Galicia and an isolated observation in the middle of the gap by Fisher-Piètte and Gaillard (1959). During the colder period of the 1960s to the 1980s, just before the present expansion, the gap probably widened and gene flow between populations at both ends was at least severely restricted.

Connectivity among northern and southern historical populations may have also been maintained by long distance dispersal across the 280-km distributional gap.

Given that only very low levels of gene flow are necessary, infrequent long distance dispersal events taking place during periods of exceptionally strong flow could have been sufficient to preserve genetic homogeneity. This hypothesis assumes that larvae were able to survive the lower sea surface temperatures encountered off the northern Portuguese coast. Preliminary rearing experiments showed that *P. rustica* is capable of reaching the veliger stage at 12 °C (Ribeiro, unpublished data). Unfortunately, the rearing experiments could not be concluded, and therefore it was not possible to determine if larvae were able to achieve settlement at that temperature. Nevertheless, this result provides a good clue that average sea surface temperatures of northern Portuguese coastal waters during the species reproductive season did not preclude larval survival, and perhaps the absence of *P. rustica* from northern Portugal was driven by post-settlement juvenile mortality. This is in agreement with modelling studies done by Lima *et al.* (2007b) suggesting that sea and air temperature during the species reproductive season and early benthic life could largely explain the geographical distribution of *P. rustica* in northern Portugal.

Considering the short spawning season of *P. rustica*, a clear signal of asymmetrical migration would be expected in the genetic data. Analysis of microsatellite data using MIGRATE showed bidirectional migration among pairs of populations. Due to the lack of genetic differentiation among putative sources, the direction of colonisation of northern Portuguese shores could not be clearly established, even using high-resolution microsatellite markers. The analysis, however, detected a shallow trend for northern colonisation in shores located at the northern end of the gap (Moledo do Minho and Viana do Castelo), and conversely, a trend for colonisation from the south in shores located at the opposite side of the gap (Homem do Leme and Buarcos). It is worth noting that these two pairs of locations have almost certainly been colonised in different years (Lima *et al.* 2006), and therefore, the source of colonists could have been different. Spawning in *P. rustica* lasts from September/October to December (see chapter 2), and encompasses the transition between the upwelling season, with prevailing equatorward flow (Haynes *et al.* 1993), and the early winter oceanographic regime, dominated by the Iberian Poleward Current (Frouin *et al.* 1990; Haynes and Barton 1990) and the also poleward flowing WIBP (Peliz *et al.* 2002, 2005). Subtle variations in the interplay between the cessation of upwelling and the onset of spawning may be sufficient to determine opposite directions in alongshore dispersal among years.

Recent progress in the development of physical circulation models has provided a powerful tool to assess patterns and scales of larval movement. An integrated approach, combining nearshore physical oceanography and life-history traits of *P. rustica*, both in the plankton and during benthic life, should allow to test specific dispersal hypotheses raised by the genetic patterns uncovered in this study.

Chapter 7

Scales of population connectivity in *Patella depressa* and *Patella rustica* estimated with a biophysical model of larval dispersal

7.1 Introduction

Empirical estimates of marine larval dispersal are extremely difficult to achieve, due mainly to the small size of planktonic larvae and the potentially large scales of transport involved. Apart from a few exceptions, where realised dispersal was observed in the field (e.g. Olson 1985; Davis and Butler 1989; Willis and Oliver 1990; Stoner 1992), and tagging techniques allowed tracking of larvae from spawning to recruitment sites (e.g. Jones *et al.* 2005; Almany *et al.* 2007; Becker *et al.* 2007; reviewed in Thorrold *et al.* 2002; 2007), estimates of dispersal ability and demographic connectivity in the marine environment have relied essentially on indirect sources of evidence. These include the duration of the planktonic larval phase (e.g. Shanks *et al.* 2003), estimates of population genetic structure (e.g. Kinlan and Gaines 2003; Palumbi 2003; reviewed in Hedgecock *et al.* 2007), rates of invasion and colonization (e.g. Shanks *et al.* 2003; Kinlan *et al.* 2005), and dispersal simulations using advection / diffusion models (e.g. Roberts 1997; Cowen *et al.* 2000; Gaylord and Gaines 2000; Siegel *et al.* 2003).

Recently, numerical modelling of ocean circulation has undergone a huge progress, in part due to the development of better simulation algorithms, more detailed coastline and bathymetric data, plus an increment in the frequency and spatial coverage of observational data used for model forcing. Because of the rapid increase in computational power, it has been possible to integrate all these factors, enabling the performance of reliable simulations, with fine-scale temporal and spatial resolution (reviewed in Chassignet *et al.* 2006). Coupled with biological parameters such as spawning strategy, pelagic larval duration and swimming behaviour (e.g. James *et al.* 2002; Hohenlohe 2004; Cowen *et al.* 2006; Guizien *et al.* 2006), these models are becoming increasingly sophisticated, and represent invaluable tools for addressing the complex processes driving population connectivity in marine systems (reviewed in

Cowen 2002; Werner *et al.* 2007).

High-resolution biophysical models allow the determination of individual dispersal trajectories that result from the dynamics of oceanographic conditions and biological traits, thus providing a representation of the full range of variability in larval transport. The output of these models is often expressed in the form of dispersal kernels, which describe the spatial distribution of settlement locations in relation to larval origin and, conversely, the spatial distribution of origins of larvae that settle at a given site (Largier 2003). Following an appropriate framework, oceanographic simulations can be used to reliably predict the qualitative patterns of genetic structure of marine populations (e.g. Galindo *et al.* 2006). Further, dispersal models can make quantitative predictions of larval exchange at ecological time scales, and thus provide information that cannot be obtained using population genetics.

The genetic structure of two limpet species – *Patella depressa* and *Patella rustica* – has been described in previous chapters. The levels of geographic differentiation found for both species were very low, suggesting that substantial demographic connectivity occurs over broad spatial scales. These results may, however, be misleading because even very small rates of exchange, of no relevance to ecological processes such as recruitment, population dynamics and community structuring, would result in lack of genetic divergence among geographically isolated populations. Moreover, population genetics analysis provides a long-term average estimate of demographic exchange (Palumbi 2003), thus lacking resolution to quantify the extent to which populations are linked by dispersal within ecological time-frames. A dual approach, using models of the physical environment and of biological processes intrinsic to each species would allow overcoming these limitations, and it would enable to confirm the empirical patterns of genetic subdivision or to uncover putative restrictions to larval transport that were too weak to be detected by genetic techniques.

In this chapter I present results of a dispersal simulation model applied to *P. depressa* and *P. rustica*, integrating several physical and biological factors: hydrodynamic patterns, sea surface temperature (SST), habitat availability, spawning season, and planktonic period. The model domain is one-dimensional, following the coastline, and thus only alongshore transport was allowed. Given their weak swimming ability, limpet larvae were assumed to act as passive particles, being advected downstream from source locations by alongshore surface flows obtained from a basin-scale model of ocean circulation. Temperature-dependence of larval lifespan was explicitly addressed in the model, by combining information on development times assessed in the laboratory, together with available SST data for the NE Atlantic region. Dispersal was also modelled as a function of spawning season and the number of spawning events per season, which determined the variety of physical conditions met by planktonic larvae and the number of offspring produced. Other parameters were also considered

in the model, such as female fecundity, larval and adult mortality, Allee effects (negative population growth rates, (e.g. Stephens *et al.* 1999; Gascoigne and Lipcius 2004) and larval retention rates.

The main purpose of the present study was to simulate alongshore larval transport of *P. depressa* and *P. rustica* in the NE Atlantic coast, and to compare model predictions of population connectivity with empirical estimates inferred from genetic analyses. Although a close correspondence between both data sets was unlikely to occur, because this would require a higher level of detail in oceanographic simulations and additional biological data (such as accurate rates of recruitment and mortality), the model was expected to successfully predict broad-scale qualitative patterns of genetic structure.

Model predictions were also expected to shed some light onto the possible role of larval transport in establishing the northern range limit of *P. rustica*. In a recent paper, Lima *et al.* (2007b) argued that the boundary, placed at Capbreton (Basque coast of France), was probably set by the inability of planktonic larvae to overcome the large extension of unsuitable habitat separating that site and the next rocky shore located to the north. Because the model explicitly accounted for habitat suitability and temperature-dependent planktonic periods, it was also used to verify if this assumption was correct. Likewise, it will be possible to test if larvae of *P. rustica* were capable of transposing the former distributional gap located in northern Portugal, which could explain the lack of genetic differentiation inferred between populations from both sides of the gap (see chapter 6).

7.2 Material and Methods

7.2.1 Spatial domain and mapping of adult habitat

The model domain was linear and covered the northeastern Atlantic coastline bounded by 25-55°N latitude, ranging from North Africa to Southern England. The two major discontinuities found along the coast - the Strait of Gibraltar and the English Channel - were eliminated from the model by uniting both sides with a straight line along the shortest path in the Strait of Gibraltar and between the easternmost tip of the Cotentin Peninsula and the Isle of Wight (approximately at 1°W longitude), in the English Channel. The boundaries defined by those lines correspond roughly to eastern range boundaries of *P. depressa* in both areas.

The coastline was discretised into an array of points separated by 12 km, a value which is equivalent to the spatial resolution of the oceanographic model (see below). Each of the coastal points was defined as a potential spawning or settlement site,

depending on the existence of adult habitat within a 6-km radius. This information was obtained by superimposing the point array into Google Earth (http://earth.google.com) and using the satellite imagery to visually check for the presence of rocky shores in the vicinity of each point. In addition, average values of coastline orientation were also assigned to each point.

7.2.2 Ocean circulation model

Ocean current simulations were based on the Forecasting Ocean Assimilation Model (FOAM) provided by the National Centre for Ocean Forecasting (NCOF). FOAM is a 3D ocean model comprising 20 vertical layers, and assimilates *in situ* and remotely sensed observational data (SST, salinity, sea surface height) that are available in near real-time. The model is driven by six-hourly forecast surface-flux fields from the Met Office operational numerical weather prediction (NWP) system. High-resolution regional configurations of the model are able to resolve dynamic features like eddies, fronts and jets. A more complete description of the FOAM ocean model can be found at the Met Office web page (http://www.metoffice.gov.uk/research/ncof/foam/).

Hydrodynamic data used in this study came from the North Atlantic configuration of FOAM that has been running since July 2003, producing daily forecasts of velocity fields with 1/8° resolution. Archived data of daily zonal and meridional current velocities from the upper layer (5 m depth) and for the period between January 2004 and December 2006 were downloaded via the NERC Environmental Systems Science Centre GODIVA server (http://www.nerc-essc.ac.uk/godiva). Grid data were imported into GRASS 6.0 Geographical Information System (GRASS Development Team 2006) as daily raster maps, which were then sampled at the coastal points. Alongshore current values were computed as the product between current velocity and the cosine of the angular difference between the coastline orientation and the current vector at each point. Negative current values resulted in southward advection and positive values in northward transport.

7.2.3 Sea surface temperature

Monthly averaged SST data for the study area and the for the years 2004-2006 were obtained from the MODIS/Aqua Global Level 3 Mapped Mid-IR SST data set (http://podaac.jpl.nasa.gov/DATA_CATALOG/). The grid files were imported into GRASS GIS as raster maps with a spatial resolution of 4 km, which were sampled at the coast points.

7.2.4 Biological parameters

As with other patellid limpet species, the annual reproductive cycle of *P. depressa* varies according to latitude, with northern populations spawning mostly during late spring and summer, whilst southern populations breed throughout the year, and undergo multiple spawning events (see chapter 2). This latitudinal difference in reproductive seasonality has been expressed in the model: populations from Britain and northern France were set to spawn from May to October, while those located in Iberia and North Africa had an extensive breeding period, with a brief pause occurring during the months of July and August. Weekly spawning events were simulated throughout the range, to reflect the characteristic reproductive asynchrony found in *P. depressa* (see chapter 2).

The annual spawning season of *P. rustica* was simulated between September and December, at all sites. Multiple spawning events in this species have been reported for northern Iberian populations (Othaitz 1994), although the same phenomenon was not observed on the western Iberian coast during two years of assessment, where only one major spawning episode was recorded each year (see chapter 2). Nevertheless, in order to capture the whole range of oceanographic conditions hypothetically experienced by larvae, weekly spawning frequency has been set in the model.

Planktonic larval duration is the factor that ultimately determines scales of larval transport. Results from laboratory rearing experiments showed an inverse relationship between water temperature and the minimum time required for larval development in several limpet species. The length of the competent period also varied with temperature, and reflected a positive effect of optimal temperature conditions in extending larval life (see chapter 3). Because SST values may differ considerably throughout the geographical range during the species' reproductive seasons and also among seasons, there will be geographical and interannual differences in larval lifespan which translate into variation in distances travelled and, consequently, in demographic connectivity among populations. The model accounted for temperature dependence of planktonic larval duration by calculating larval precompetent and competent periods, given the value of SST at the natal site and spawning date. In *P. depressa*, the precompetent period (or planktonic larval duration, *PLD*) was calculated according to the relationship from Figure 3.4:

$$PLD = 34.62 \times \exp(-0.11T)$$

where T is the SST value in degrees Celsius. Maximum larval longevity (PLD_{max}), which coincides with the end of the competent period, was calculated according to the second-order polynomial relationship from Figure 3.7:

$$PLD_{max} = -0.231T^2 + 6.146T - 14.770$$

Both equations were valid only for SST values falling within the range of 8-20 °C. If during the course of development larvae were exposed to water temperatures lying outside this interval, they were all considered dead.

The effect of temperature on the duration of larval life has not been assessed in *P. rustica*. To overcome this limitation in the model, larval duration was estimated using data obtained for *P. ulyssiponensis* (Figures 3.4 and 3.7). Although larval development displayed essentially the same relationship with temperature among southern limpet species, *P. ulyssiponensis* showed greater tolerance to warmer temperatures than *P. depressa* (see chapter 3). Considering that the geographical distribution of *P. rustica* largely overlaps that of *P. ulyssiponensis* at the southern part of its range, the relationship between planktonic larval duration and temperature may be similar in both species, particularly at higher values. Thus, temperature-dependent planktonic larval duration in *P. rustica* was expressed by the equation:

$$PLD = 29.14 \times \exp(-0.10T)$$

Maximum larval longevity as a function of SST was calculated applying the relationship:

$$PLD_{max} = -0.129T^2 + 3.733T - 1.860$$

Because preliminary rearing assays indicated that *P. rustica* was able to develop to the competent stage at 12 °C, this was assumed as the lower threshold value, and thus predictions of larval duration were considered valid within the range 12-24 °C.

7.2.5 Dispersal model

The model was written in the programming language C++ and run on a standard PC with a LINUX operating system, from a console or terminal window. The parameters and data files required for the simulations were defined through a configuration file. This conferred great flexibility to the software, allowing to test different model setups, by tweaking only specific parameter values and leaving unchanged the main data files containing information about ocean currents, SST, habitat, spawning season and source populations.

At the start of each run, only a few locations were populated, corresponding to those that had been sampled for the genetic surveys (see Figures 5.1 and 6.1). Thus, initially there were 19 source populations in model runs for *P. depressa*, spread from Britain to Morocco, and 12 for *P. rustica*, distributed along the Iberian coast. The remaining coast was subsequently colonised by larval dispersal wherever suitable habitat was encountered.

To track the genetic effects of dispersal, each of the founder populations was composed of individuals carrying a unique genotype. The simulated genotypes

were haploid, maternally inherited, and non-recombinant, thereby behaving like mitochondrial markers. In addition, genotypes were selectively neutral and mutation was not considered in the model. Excluding males from the model implies the absence of sex-biased dispersal in these species.

The number of offspring produced by each female is a function of the number of eggs spawned, scaled by the fertilization rate. Estimates of fecundity in patellid limpets have only been obtained for *Patella vulgata*. Le Quesne (2005) suggested that the production of eggs by fully ripe (stage 5, following Orton *et al.* 1956) female *P. vulgata* varies exponentially with body length, ranging from a few thousand to several hundred thousand eggs. Given that population size structure was not simulated in the model, this relationship could not be used to calculate egg production. Fertilization success obtained in laboratory rearing experiments varied with water temperature, but was generally low, not exceeding 20% in *P. depressa* (see chapter 3). Other experiments have shown that high fertilization success in limpets requires high sperm concentrations and prolonged contact between gametes (Hodgson *et al.* 2007). The rapid gamete dilution and short contact time expected under natural conditions may drastically reduce zygote production. Thus, taking a conservative estimate, each spawning event was simulated to comprise the release of 100 larvae per female.

In each population, weekly spawning events released a larval cohort, which was advected along the coast according to the current velocity and direction at the birth location and date. Because only 3 years of data were available from FOAM, interannual variability throughout model runs was assured by multiplying daily alongshore velocities at each point by a random component obtained from a continuous uniform distribution bounded between 0.5 and 1.5. The advection velocity of the cohort was updated at daily time steps, or whenever it reached a contiguous coastal point.

Over the duration of the planktonic period, larval mortality occurred at a constant instantaneous rate of 0.2 day^{-1}, an average estimate from the range given by multiple monitoring studies of marine invertebrate larval cohorts in the plankton (Rumrill 1990; Morgan 1995). This value encompassed all sources of mortality, such as offshore transport, starvation and predation. Additionally, 50% of the cohort was retained in each location, where larvae continued to develop and eventually settled, provided that suitable habitat was present. Until completion of the pre-competent phase, larvae were unable to settle. After becoming competent, the cohort continued to drift, but larvae settled at a rate of 0.5 day^{-1} if suitable habitat was available. When maximum larval longevity was reached, all larvae that had not metamorphosed were considered dead. Because of lack of predation and dispersal in laboratory cultures the results from chapter 3 can only be used as indicative of the order of magnitude of larval mortality, which is similar to that set in the model.

After settlement, limpets only matured in their second year, as suggested by

Guerra and Gaudêncio (1986) for *P. depressa* on the Portuguese coast, and Othaitz (1994) for *P. rustica* on the northern Spanish coast. Estimates of adult lifespan in patellid limpets can be very disparate depending on habitat conditions (Fischer-Piètte 1941; Bowman 1981) and latitude (Lewis 1986). Guerra and Gaudêncio (1986) suggested a maximum longevity of 3 years for *P. depressa* on the Portuguese coast, but this species may live longer at higher latitudes, given the slower growth rates and larger sizes generally attained by northern limpet populations (Lewis 1986). Thus, a 5-year maximum lifespan was established for both species, because this information is lacking for *P. rustica*. Adult mortality was set at 0.45 $year^{-1}$, which results in over 95% mortality after 5 years, according to an exponential decay model. The maximum population size was set to 10,000 females at all coastline points.

7.2.6 Simulations

To address short- and long-term consequences of dispersal, three types of simulations were performed for each species. First, to obtain an estimate of annual scales of connectivity along the study area, dispersal from the selected point sources was simulated over a one-year period. Under this scenario, three replicate runs were performed, one for each year of ocean currents and temperature data (2004-2006), and the spatial distribution and number of settlers released from each location were averaged over the three years. A second simulation was run for 100 years, with the purpose of assessing the degree of connectedness by stepping-stone gene flow over long distances. Finally, the third simulation was performed under similar conditions as the previous one, only differing in the genetic information assigned to founder populations. Instead of a unique fixed allele, the pool of larvae produced by each population at the start of the run carried the same allele frequencies that have been empirically estimated using microsatellites (see chapters 5 and 6). Because microsatellite loci are diploid and the model produced haploid individuals, in practice this simulation dispersed alleles, not larvae.

7.3 Results

7.3.1 Simulation 1: annual dispersal kernels

The probability distribution of annual larval settlement, also known as dispersal kernel, exhibited substantial variability in shape among release locations, which reflects differences in the extent and predominant direction of dispersal (Figure 7.1). In southern England, larval transport occurred predominantly in the southward direction

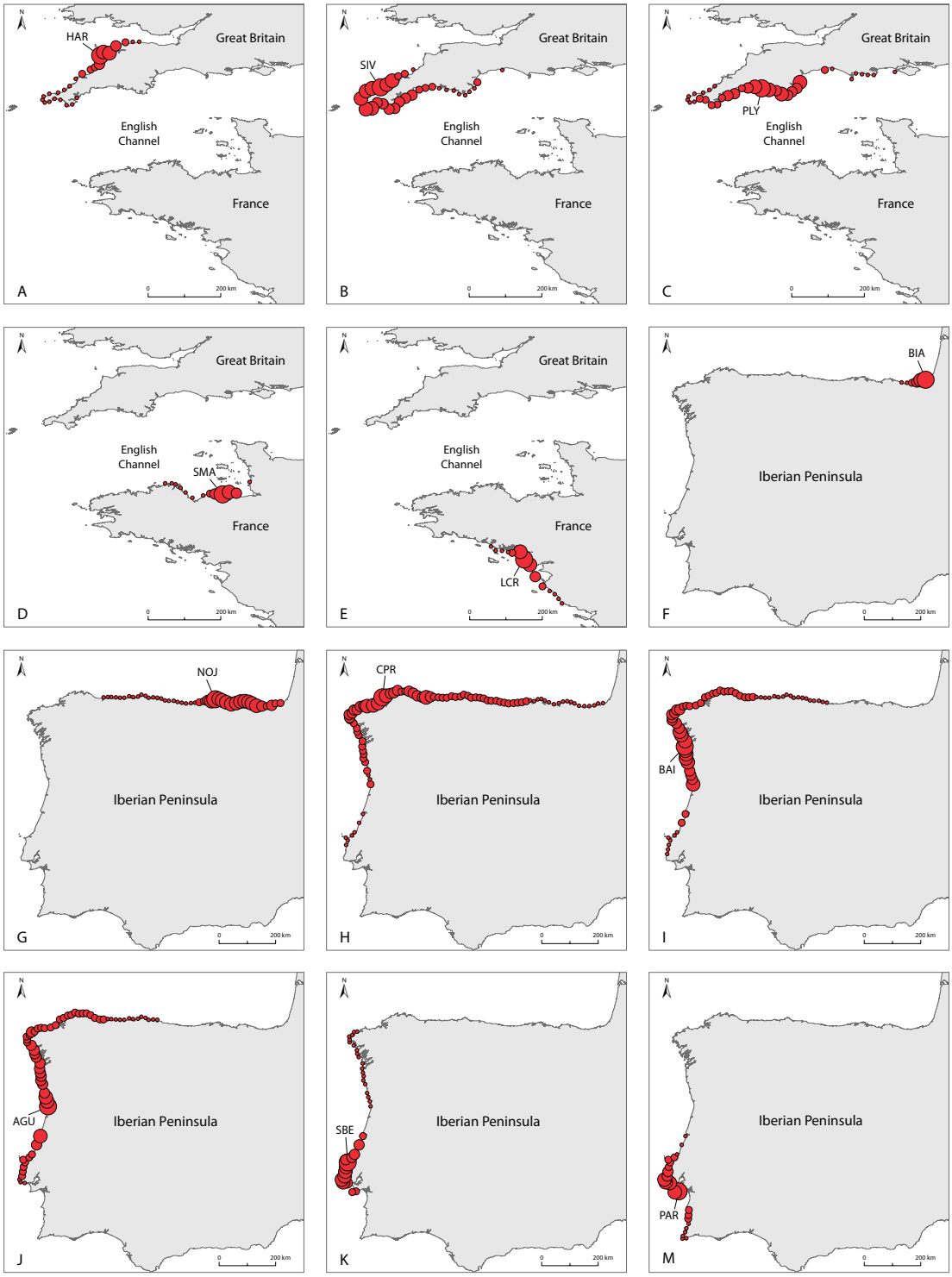

Figure 7.1 Dispersal simulations for *Patella depressa*. Maps show the distribution of settlers originating from each release location for the period of one year. Circle size is proportional to the total number of settled larvae. Location names: HAR – Hartland, SIV –St. Ives, PLY – Plymouth, SMA – Saint-Malo, LCR – Le Croisic, BIA – Biarritz, NOJ – Noja, CPR – Cabo Prior, BAI – Baiona, AGU – Aguda, SBE – São Bernardino, PAR – Portinho d'Arrábida, STO – São Torpes, ARR – Arrifana, ODA – Olhos d'Água, CAD – Cadiz, TAR – Tarifa, CAS – Casablanca, AGA – Agadir.

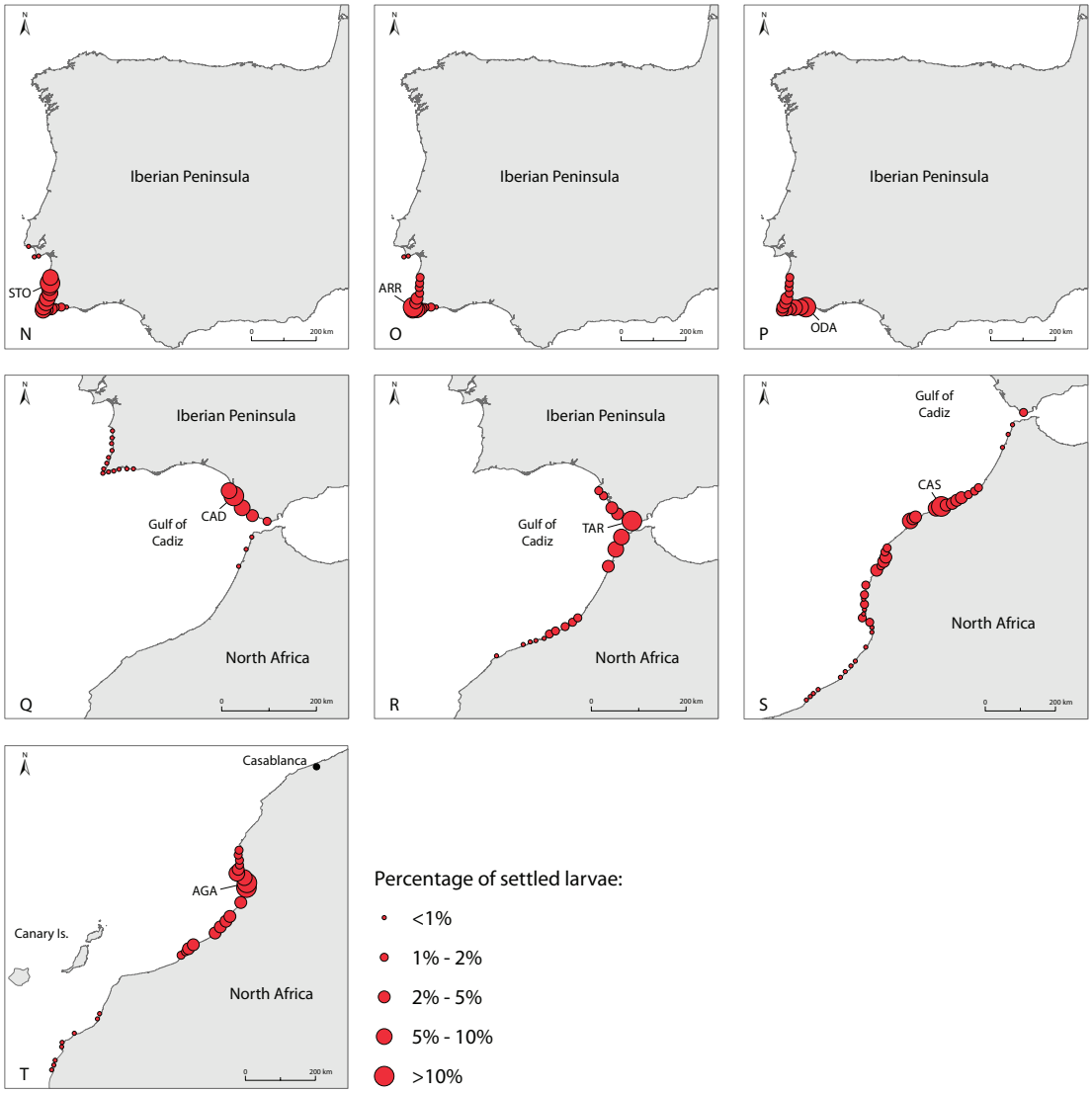

Figure 7.1 Dispersal simulations for *Patella depressa* (continued).

along north Cornwall (Figures 7.1A and 7.1B), and then eastward after larvae reached the southwestern tip of the Cornish peninsula. In this region, larval settlement to the north of the natal site took place within much shorter distances, indicating that northward drift was subject to slower currents. On the south coast of England, the distribution of settlers around the source locality was more symmetrical, with extensive dispersal taking place in both directions (Figure 7.1C).

Simulations suggested that the area around Saint-Malo (northern coast of France) favours retention, since most of the larvae released from that location settled in the vicinity of the home site, and only a small proportion of larvae drifted westwards and colonise coastal areas located outside the Gulf of Saint-Malo (Figure 7.1D). On the west coast of France, larvae originating from Le Croisic were mainly dispersed to the south, although northward transport also occurred to a lesser extent (Figure 7.1E).

Effective dispersal from Biarritz was short and unidirectional, only occurring westward along the Iberian coast (Figure 7.1F). The lack of northward dispersal from this location was possibly due to the inability of larvae to overcome the roughly 200-km stretch of unsuitable habitat in southwest France. Larvae released from most of the north and western Iberian populations were able to colonise large extensions of coastline, suggesting high levels of population connectivity in this region (Figures 7.1G to 7.1M). In comparison, dispersal from São Torpes, Arrifana and Olhos d'Água was much more limited in space (Figures 7.1N to 7.1P). The distribution of settlers from these three locations appeared to be hampered by extensive habitat discontinuities north of S. Torpes and west of Olhos d'Água. In the latter case, restrictions to dispersal only occurred towards the east, because larvae originating from Cadiz were able to cross the stretch of unsuitable habitat along the southern Iberian coast (Figure 7.1Q).

Model simulations predicted considerable larval transport from Cadiz and Tarifa across the Strait of Gibraltar and along the North African coast (Figures 7.1Q and 7.1R). Advected by strong southward-flowing currents typical of that region, the larvae were able to travel for considerable distances and colonise long stretches of coastline. A similar pattern of extensive southward drift was observed in cohorts released from Casablanca and Agadir, although northward transport was also enabled during periods of current reversal (Figures 7.1S and 7.1T).

Despite having a considerably shorter spawning period, *P. rustica* exhibited annual dispersal patterns along the Iberian coast similar to those of *P. depressa* (Figure 7.2). While in some cases distances travelled by *P. rustica* were slightly shorter (e.g. Cabo Prior (Figure 7.2D), or larvae reached the most distant settlement sites in smaller number (e.g. Olhos d'Água, Figure 7.2H), in others the opposite was observed (e.g. Biarritz and São Bernardino, Figures 7.2A and 7.2E). As with *P. depressa*, this species was also unable to bridge the habitat gap in southern France and settle at northern locations.

7.3.2 Simulation 2: long-term connectivity patterns

In this simulation, the entire coastline bearing suitable habitat was colonised in less than 10 years and adult population sizes stabilized within 20 years. Although stabilization of haplotype proportions was not achieved, conditions at 100 years were considered representative of a long-term state. There was no loss of haplotypes from the model domain and no haplotype fixation within populations, even for runs longer than 100 years (data not shown).

The patterns of gene flow along the study area can be seen in the number and proportion of haplotypes present at each population. The geographical distribution of haplotype frequencies for *P. depressa* after 100 years revealed 3 distinct areas of

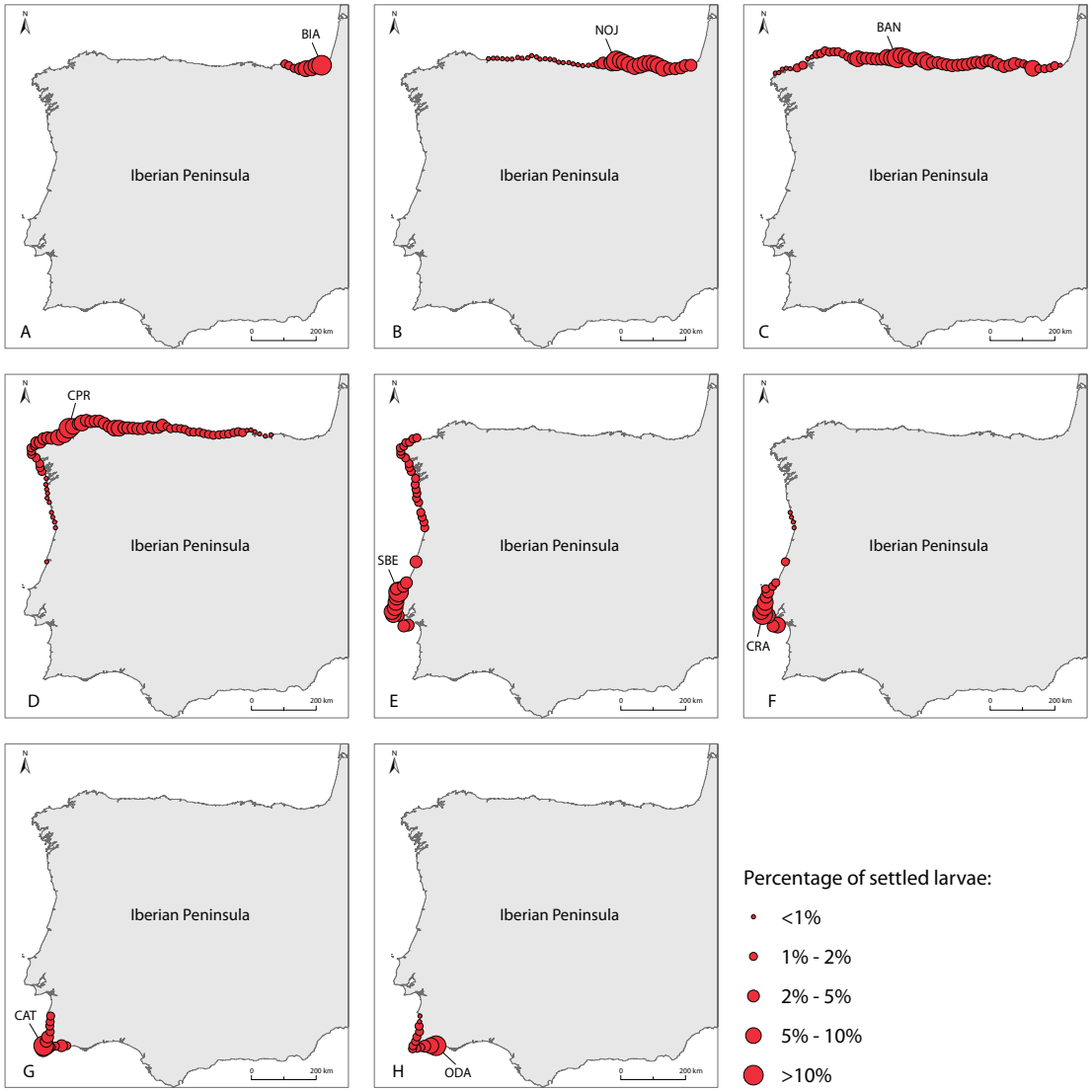

Figure 7.2 Dispersal simulations for *Patella rustica*. Maps show the distribution of settlers originating from each release location for the period of one year. Circle size is proportional to the total number of settled larvae. Location names: BIA – Biarritz, NOJ – Noja, BAN – Bañugues, CPR – Cabo Prior, SBE – São Bernardino, CRA – Cabo Raso, CAT – Castelejo, ODA – Olhos d'Água.

extensive connectivity, with restricted or no contact among them (Figure 7.3): (1) southern England and northern France, (2) the Iberian coast between Biarritz and Olhos d'Água, and (3) the Gulf of Cadiz and North Africa. The model produced a genetic break between Le Croisic and Biarritz, confirming the inability of limpet larvae to cross the long stretch of sandy coastline located between those two sites. Another sharp transition in haplotype frequencies was located on the southwestern Iberian coast, between Olhos d'Água and Cadiz, where gene flow was asymmetrical: haplotypes from populations located to the east and south of Olhos d'Água dispersed to the west

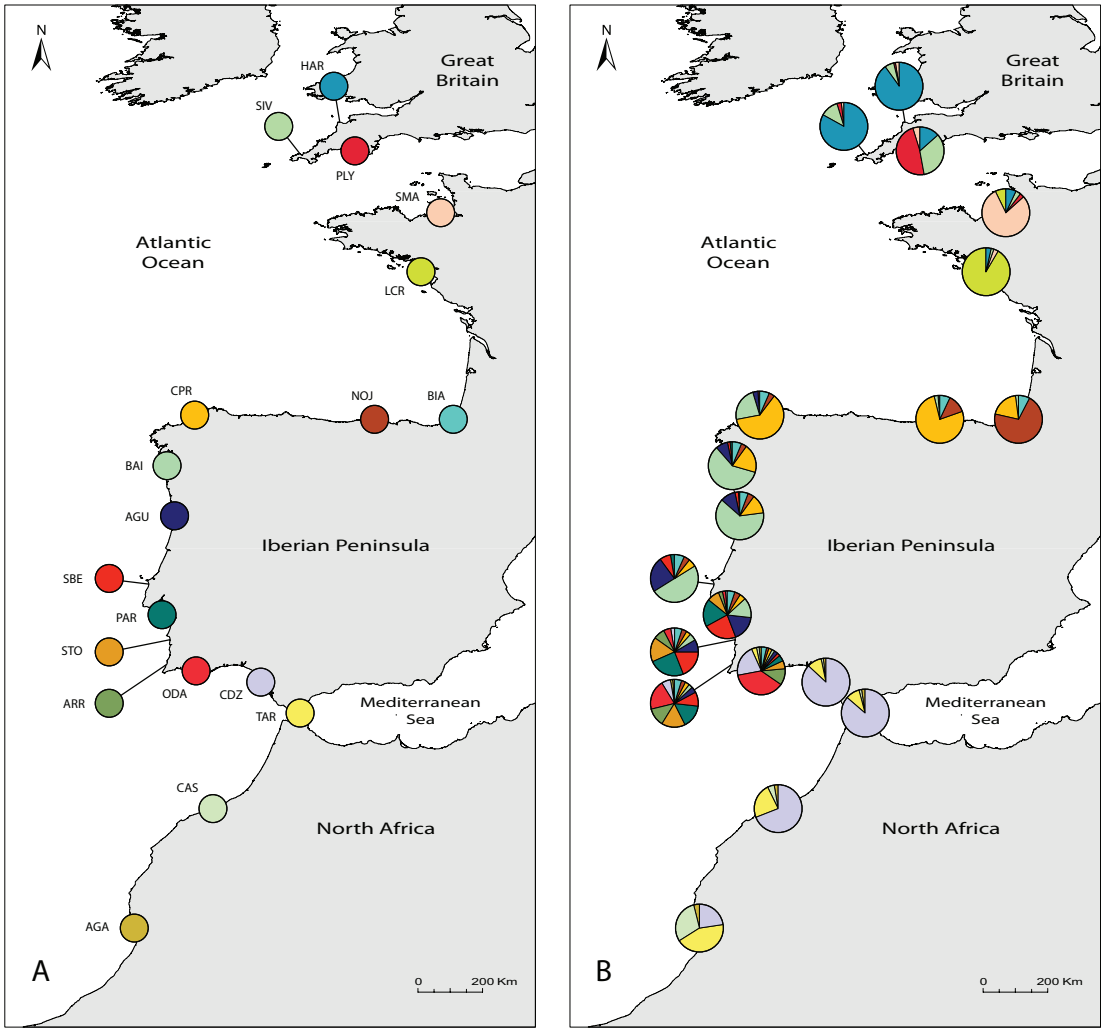

Figure 7.3 Haplotype frequencies at the start of the run (A) and after 100 years of simulated dispersal (B) for *Patella depressa*. Names of source locations are the same as in the caption of Figure 7.1.

and then northward along the western Iberian coast, whereas the opposite did not occur.

Model results for *P. rustica* followed a similar pattern. The model predicted a high degree of connectivity among Iberian populations, even across the former distributional gap of the species in northern Portugal (Figure 7.4). At a short temporal scale there was considerable annual variability in the alongshore direction of ocean currents, which translated into interannual northward- or southward-biased gene-flow (Figures 7.4B and 7.4C). In the long-run, however, the trend was for balanced levels of migration in both directions.

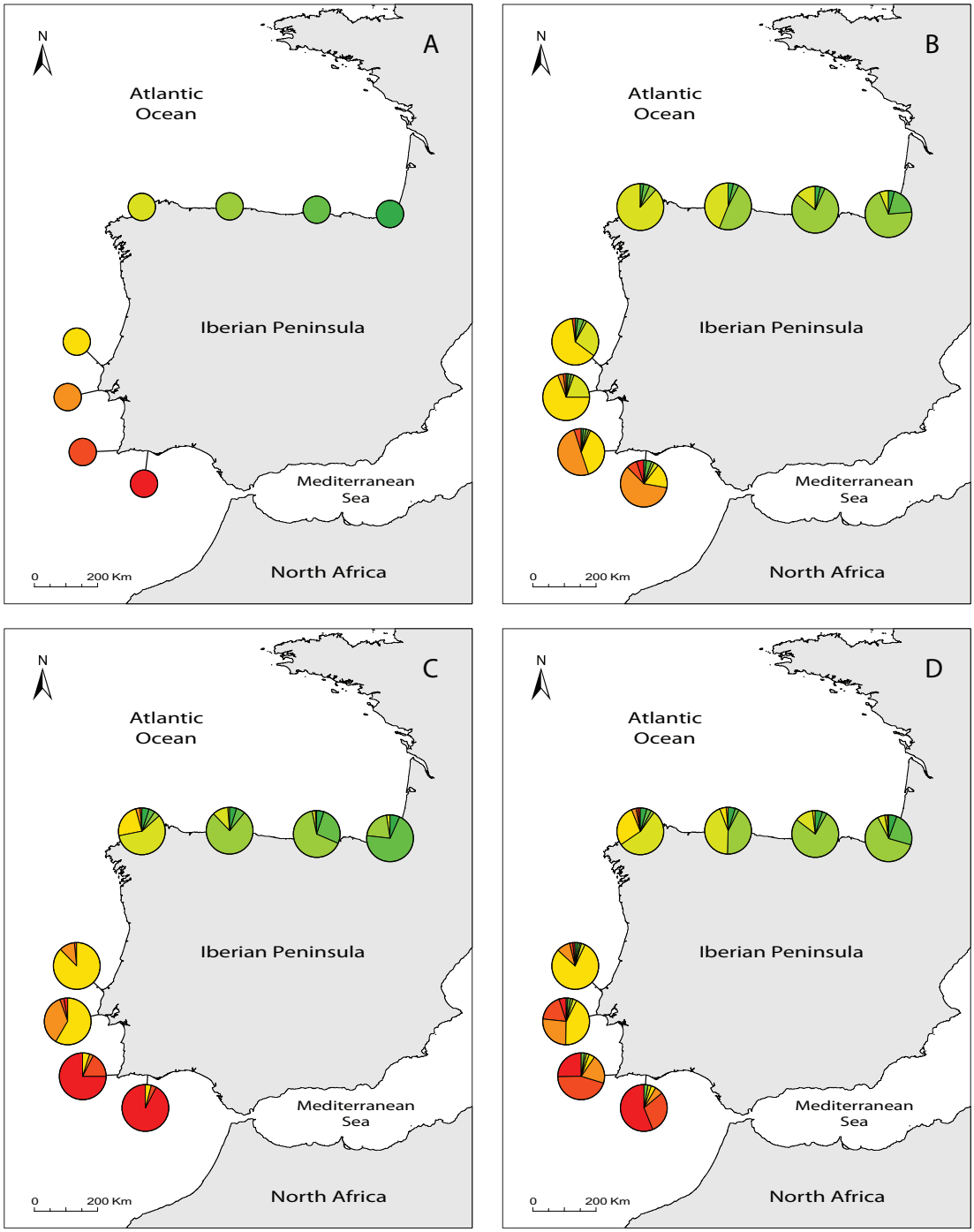

Figure 7.4 Genotype frequencies for *Patella rustica* at the start of the runs (A) and after 100 years of simulated dispersal (B-D). To show the influence of interannual variability of flow fields in the outcome of the simulations, the model was run using oceanographic data from 2004 (B), 2005 (C) and all years in succession (D).

7.3.3 Simulation 3: genetic structure

The geographical distribution of genetic variation after 100-year runs using empirical

Table 7.1 Hierarchical AMOVA of spatial genetic variation in *Patella depressa* at three microsatellite loci, after 100 years of simulated dispersal. Population groupings: (1) Hartland, St. Ives, Plymouth, Saint-Malo and Le Croisic; (2) Biarritz, Noja, Cabo Prior, Baiona, Aguda, São Bernardino, Portinho d'Arrábida, São Torpes, Arrifana and Olhos d'Água; (3) Cadiz, Tarifa, Casablanca and Agadir. * indicates significance at $P < 0.05$.

Source of variation	Variance	%	F-statistics
Among regions	0.0090	0.69	$F_{CT} = 0.0070$
Among samples within regions	0.0190	1.45	$F_{SC} = 0.0148$
Within samples	1.2818	97.86	$F_{ST} = 0.0217$

microsatellite data was investigated using hierarchical analysis of molecular variance (AMOVA). For *P. depressa*, populations were grouped according to the genetic breaks obtained in the previous model scenario. Thus, three groups have been defined: (1) Hartland, St. Ives, Plymouth, Saint-Malo and Le Croisic; (2) Biarritz, Noja, Cabo Prior, Baiona, Aguda, São Bernardino, Portinho d'Arrábida, São Torpes, Arrifana and Olhos d'Água; (3) Cadiz, Tarifa, Casablanca and Agadir. The hierarchical AMOVA (Table 7.1) showed that despite the existence of limited gene flow among population groups, most of the genetic variation (97.86%) was allocated to the individual level. Only very small, albeit significant, amounts were partitioned among groups (0.69%) and among populations within groups (1.45%).

For *P. rustica*, two population groups located on different sides of the historical distribution gap were defined: Northern Iberia: Biarritz, Noja, Bañugues and Cabo Prior; Southern Portugal: São Bernardino, Cabo Raso, Castelejo and Olhos d'Água. Results from the hierarchical AMOVA (Table 7.2) showed that only very small fractions of genetic variability were apportioned to the group level (0.58%) and to among populations within groups (0.54%). These values suggest that considerable levels of gene flow across northern Portugal prevented the buildup of relevant genetic differentiation among the two geographically separated groups.

7.4 Discussion

This study investigated alongshore larval dispersal potential of *P. depressa* and *P. rustica* in the northeast Atlantic, making use of a coupled physical-biological model. High levels of demographic exchange among populations have previously been inferred using genetic tools (see chapters 5 and 6). Yet, population genetics has limited application in identifying source and receiver populations and measuring the degree of these connections at ecological time scales (Largier 2003; Palumbi 2003). Therefore, the modelling approach provided complementary data on larval exchange, and thus

Table 7.2 Hierarchical AMOVA of spatial genetic variation in *Patella rustica* at five microsatellite loci, after 100 years of simulated dispersal. Population groupings: N. Iberia – Biarritz, Noja, Bañugues and Cabo Prior; S. Portugal – São Bernardino, Cabo Raso, Castelejo and Olhos d'Água. * indicates significance at $P < 0.05$.

Source of variation	Variance	%	F-statistics
Among regions	0.0097	0.58	$F_{CT} = 0.0060^{*}$
Among samples within regions	0.0090	0.54	$F_{SC} = 0.0048^{*}$
Within samples	1.6324	98.87	$F_{ST} = 0.0117^{*}$

contributed to a better insight into the processes driving contemporary patterns of connectivity in the two model species.

Despite possessing fairly short pre-competent periods, larvae were generally capable of travelling long distances and settling far from their release location. The average dispersal distance estimated for both species was generally in the order of 200 km, but in some regions (like North Africa and part of the northwestern Iberian coast) it reached 600 km. The bulk of the larval cohorts, however, settled within much shorter distances from the parental site, as soon as they attained metamorphic competence. Because of the wide competency window defined in the model (which was based on laboratory rearing experiments), larval cohorts were further advected downstream, while subjected to losses by mortality and retention at each coastal cell. As a consequence, most dispersal kernels were characterised by long tails made up of a small number of settlers at each cell, comprising only a minute proportion of the total amount of surviving larvae during one reproductive season. Genetic homogeneity between isolated populations can be preserved with a very low number of migrants per generation (Slatkin 1987). Therefore, occasional long-distance dispersal of only a few individuals within a cohort possibly has a meaningful impact over evolutionary time scales, by dampening the buildup of genetic substructure and countering local adaptations.

Conversely, even if such long-distance dispersal events take place frequently, they probably have little demographic relevance, because their contribution to local recruitment rates is largely insufficient to compensate annual mortality and sustain viable populations of limpets. Previous modelling studies suggested that the spatial extent of dispersal necessary to replenish populations is considerably more restricted (e.g. Cowen *et al.* 2003; Largier 2003; Cowen *et al.* 2006). This also seems to be the case for limpet populations in the northeast Atlantic region. Excluding the tails of predicted dispersal kernels, estimates of average alongshore distances travelled by successful settlers dropped to just over 90 km for *P. depressa* and 50 km for *P. rustica*.

The discrepancy found among the two species in average dispersal estimates is

most likely related to differences in timing and duration of spawning seasons. While *P. depressa* is reproductively active throughout the year, except during summer months, *P. rustica* only spawns between September and December (Guerra and Gaudêncio 1986; Othaitz 1994). Spawning periods determine the variability of ocean currents encountered by planktonic larvae, thus influencing dispersal trajectories. This assumes even more relevance off the Iberian coast, given the seasonal reversal displayed by surface circulation patterns: a persistent poleward current characterizes winter circulation (Frouin *et al.* 1990; Haynes and Barton 1990), whilst northerly winds blowing in spring and summer cause the formation of upwelling and an associated equatorward flow (Haynes *et al.* 1993).

Spawning date also influences sea temperatures experienced by planktonic larvae. Given that larval development rates of many marine invertebrate species, including limpets, are temperature-dependent (Hoegh-Guldberg and Pearse 1995; O' Connor *et al.* 2007; chapter 3, this thesis), time of spawning plays a very important role in determining larval life-span. Longer mean dispersal distances are expected for a species exhibiting almost year-round spawning, like *P. depressa*, in comparison to *P. rustica* which does not spawn in winter, because low winter sea temperatures will cause an increase in the duration of pre-competent periods.

Model results suggest that simple oceanography and a stepping-stone process of dispersal, together with a few biological parameters, can broadly explain connectivity patterns in *Patella*. The high levels of gene flow predicted over large extensions of coastline by the dispersal model are in partial agreement with empirical genetic data. One important difference between the two approaches was that the model predicted two barriers to dispersal for *P. depressa*, one located in southern France and the other one in southern Iberia. The characteristics of the sharp breaks in genetic composition showed that the first one coincided with an absolute barrier to gene flow, whilst the second one occurred in an area where one-way migration (from the Gulf of Cadiz and North Africa to the Portuguese coast) was observed.

Sharp genetic breaks are usually associated with circulation features such as fronts, convergent currents or areas of upwelling (e.g. Goldson *et al.* 2001; Barber *et al.* 2002b; Hohenlohe 2004; Sotka *et al.* 2004; Ayers and Waters 2005), and adult habitat gaps or environmental discontinuities (e.g. Johnson and Black 1995; Dawson 2001; Riginos and Nachman 2001). In the present study, the breaks were associated with stretches of coastline lacking settlement habitat, and thus reflected limitations in dispersal potential (rather than barriers to larval transport) under the flow fields and the estimated relationships between temperature and planktonic periods provided to the model.

These predictions are not incompatible with results from population genetics. The large effective population sizes and high fecundity associated with many marine species

are expected to reduce the effects of genetic drift, slowing the differentiation between populations, even in the absence of gene flow (Palumbi 1992). The results from the third model scenario seem to suggest just that. All source populations began with the same allele frequencies as the ones estimated using microsatellites, thus showing very low levels of genetic differentiation among them. Due to limited or no gene flow across the barriers, genetic drift would be expected to produce considerable amounts of large-scale genetic structure. Yet, the degree of genetic differentiation accumulated at the end of model runs among regions (i.e., the extensions of coastline separated by the barriers) was very low. Furthermore, it is possible that during episodes of exceptionally strong flows the barriers can be crossed and some genetic exchange occurs. The hydrographic data used in the dispersal model corresponded to just three years of real observations, and they can hardly be considered representative of the full range of oceanographic conditions found in the study area. Even though a certain degree of variability was added to the ocean circulation data, it is very unlikely that the fastest current speeds have been appropriately represented.

Larval dispersal of *P. rustica* along the Iberian coast was addressed primarily to investigate if populations located on both sides of the former distributional gap exchanged migrants, as this hypothesis could explain the lack of genetic differentiation found at mitochondrial DNA and microsatellite loci. My results showed that dispersal across the gap was probably frequent from populations located closer to the range edges, but not in ecologically meaningful numbers. On the other hand, it was sufficient to ensure a high degree of genetic homogeneity among the two isolated areas. It seems clear that the absence of *P. rustica* from northern Portugal until recently was not due to the lack of dispersal potential preventing larvae from crossing long stretches of unsuitable habitat (especially in the southern part of the gap), but rather to the inability of juveniles to survive through the early stages of benthic life. Results from the dispersal model thus seem to corroborate previous studies showing that changes in the distribution of *P. rustica* in the last few decades could be explained by an increase in sea and air temperatures coincident with reproductive and initial growth periods (Lima *et al.* 2007b).

The source of colonisation of northern Portuguese shores by *P. rustica*, which could not be established using molecular tools due to lack of genetic differentiation among historical populations (chapter 6), was also investigated. Model predictions were not congruent among the three years of real flow data, showing all three possible scenarios for larval transport across the gap: exclusively southward in 2004, exclusively northward in 2005 and in both directions in 2006. Given such interannual variability of dispersal patterns, and also the fact that the onset of colonisation could not be pinpointed (Lima *et al.* 2006), the source of newly-established populations remains unknown. On the other hand, model results clearly suggest that in subsequent years

recruits may have originated from either side of the gap.

The modelling approach taken in this study represented a first step to understand the factors determining larval dispersal patterns of *Patella* in the northeast Atlantic. The model relied on simplified assumptions to provide a basis from which it could be further developed. Model refinement can be achieved mainly by improving the circulation component and accounting for larval behaviour. An obvious consequence of the way transport was modelled (in a simple alongshore flow field solely with advective properties) is that directional flows persistent in time may cause populations to be swept downstream. Eddy diffusion, the nondirectional spreading of particles due to turbulent motions (Okubo 1971) acts to dampen this sweeping effect of directional flows (Cowen *et al.* 2000; Gaines *et al.* 2003; Largier 2003). Therefore, accounting for the diffusive effects on dispersal would enable a more accurate estimate of the degree of larval retention along a coastline. Because diffusion reflects individual differences in transport, including this parameter in the model would also require tracking individual larvae, instead of entire cohorts.

The view that larvae disperse passively has been challenged in recent years, with the discovery of sensorial capabilities and behavioural strategies that can influence the outcome of larval dispersal over a range of spatial scales (Metaxas 2001; Kingsford et al. 2002; Pineda et al. 2007). Changes in vertical position may expose larvae to different flow directions and speeds due to vertical shear (Paris and Cowen 2004; Arnold et al. 2005; Vikebø et al. 2005; Fiksen et al. 2007), tidal currents (Forward and Tankersley 2001; Simons et al. 2007) or upwelling (Shanks and Brink 2005; Marta-Almeida et al. 2006). Because flow speeds near the shore are usually slowed by bottom drag and coastline topography, small cross-shore movements by larvae may have important repercussions on alongshore transport (Largier 2003). With increasing understanding of larval behaviour, more sophisticated assumptions can be incorporated in biophysical models, and more accurate predictions of dispersal patterns can be obtained. There is, however, virtually no information on larval behaviour in *Patella*. If and how limpet larvae are able to influence their dispersal trajectories making use of sensorial and locomotory capabilities is not known. Yet, laboratory observations have shown that young larvae spend most of their time near the surface of culture vessels and older larvae tend to remain near the bottom, suggesting that vertical positioning and behavioural reactions may change throughout larval ontogeny. Therefore, advection of larvae as passive particles at a fixed shallow depth (5 m) through the course of development may be an unrealistic assumption.

Chapter 8

General Discussion

My thesis has examined dispersal and connectivity in the marine environment by addressing a number of aspects such as reproductive biology, larval development, population genetics and physical modelling of larval dispersal in several species of the genus *Patella* from the northeast Atlantic. The reproductive cycles of four limpet species (*Patella depressa*, *Patella ullyssiponensis*, *Patella vulgata*, and *Patella rustica*) were examined at several locations on the northern and central Portuguese coast, to determine spawning periods (Ribeiro et al, in press; see chapter 2). It is crucial to know the timing and extent of these periods to be able to develop dispersal models appropriately. The results showed that *P. depressa* and *P. ulyssiponensis* have almost year-round breeding, with a brief resting phase in the early summer. Conversely, the two other species displayed much shorter spawning periods, with gamete release taking place between December and March in *P. vulgata* and between September and December in *P. rustica*.

A series of laboratory rearing experiments established the relationship between temperature and precompetent and competent periods in planktonic stages of *P. depressa*, *P. ulyssiponensis*, and *P. vulgata* (chapter 3). Average duration of precompetent periods across test temperatures ranged between 3.7-14.0 days in *P. depressa* (for the temperature interval of 8-20 °C), 2.8-13.7 days in *P. ulyssiponensis* (8-24 °C) and 5.7-14.6 days in *P. vulgata* (5-16 °C), whilst delay periods ranged between 15.8-25.4 days in *P. depressa*, 14.5-27 days in *P. ulyssiponensis* and 16.5-25 days in *P. vulgata* (for the same experimental temperature intervals as above).

To estimate levels of population connectivity using genetics tools, two sets of microsatellite markers were for the first time successfully developed in *P. depressa* and *P. rustica* (Pérez *et al.* 2007; Pérez *et al.* 2008) see chapter 4). The genetic structure in these two species was examined on a range-wide scale in *P. depressa* (chapter 5) and along the Iberian coast in *P. rustica* (chapter 6) using those markers, plus one mtDNA locus in *P. rustica*, with results suggesting high levels of gene flow throughout the study areas and widespread lack of population differentiation in both species.

Finally, a biophysical model of dispersal has been developed to assess the degree of demographic connectivity over ecological and evolutionary time frames, and also

to identify possible barriers to dispersal for *P. depressa* and *P. rustica* (chapter 7). The model confirmed high levels of dispersal in both species, but in *P. depressa* simulations identified two barriers coincident with large extensions of adult habitat discontinuity. In this chapter, the different sections that make up the thesis are brought together in an integrated discussion focusing on the most important findings.

8.1 Temperature-driven larval development times, dispersal potential and geographical distribution limits

Before this work, little information was available concerning larval lifespan in *Patella* (Patten 1886; Smith 1935; Dodd 1957; Wanninger *et al.* 1999), and its relationship with water temperature was unknown. Temperature is probably the most influential factor on larval development rate (Hoegh-Guldberg and Pearse 1995), and therefore quantifying the relationship between this variable and development periods was crucial to predict spatial and temporal patterns of dispersal in the species studied. The set of experiments undertaken in the scope of this thesis showed that development time in *Patella* decreased exponentially with temperature, and that this relationship was essentially the same across species. These findings are in agreement with a number of studies that showed a similar response to temperature in a variety of marine invertebrate species, including barnacles (Scheltema and Williams 1982; Burrows *et al.* 1999; Thiyagarajan *et al.* 2003), echinoderms (Watts *et al.* 1982; McEdward 1985; Hoegh-Guldberg and Pearse 1995) and molluscs (Scheltema 1967; Pechenik 1984; Lima and Pechenik 1985; Drent 2002). Moreover, O' Connor *et al.* (2007) tested the generality of the temperature-dependence of planktonic larval duration for over 70 marine species by comparing results from laboratory rearing experiments, and found basically the same relationship across taxa. This implies that changes in ocean temperature are likely to have common repercussions on dispersal and survival of marine species with a planktonic larval stage (O' Connor *et al.* 2007).

Because the three species for which planktonic periods were assessed have large geographical distributions (Christiaens 1973; Southward *et al.* 1995), they would also be expected to display a wide tolerance to water temperature during larval development. Results were somewhat surprising in the sense that water temperature values that larvae withstood in the laboratory were within the range that they may encounter throughout the geographical distribution of the species. For example in *P. vulgata*, the upper temperature limit for development assessed in the laboratory was 16 °C, a value which is close to the minimum sea surface temperature observed during winter

at the southern distributional limit of the species (southern Portugal). Also, unlike *P. ulyssiponensis*, the other southern species, *P. depressa*, is absent from the Mediterranean, a fact that may reflect the lower level of tolerance to higher water temperatures verified in rearing trials. At the other end of the geographical range, *P. depressa* extends as far as North Wales, while *P. ulyssiponensis* (which achieved higher survival rates at lower temperatures than *P. depressa*) reaches the southern coast of Norway (Christiaens 1973; Southward *et al.* 1995). In view of these results, water temperature may have an important role in helping to set the species' geographical limit, particularly towards the equator where larval duration may be shorter (O' Connor *et al.* 2007), by indirectly imposing absolute restrictions to dispersal.

Changes in ocean temperature will therefore likely have an impact on population connectivity, with more immediate consequences for northern species at southern limits, which will be felt in two stages. First, an increase in temperature will cause a shortening of planktonic periods and consequently, a decrease in levels of larval exchange among populations. With less supply from other places, populations at the range border will become more dependent on local recruitment for replenishment, and thus more vulnerable to severe population fluctuations and extinction (Eckert 2003). Secondly, further rises in temperature may cause massive mortality in larval cohorts and eventually prevent dispersal. This may be the most likely scenario for populations of *P. vulgata* in southern Portugal. Recent surveys revealed that although this species has only retreated a few kilometres since the 1960s (Lima 2007), populations near the southern limit are at low densities and made up of few large individuals (P.A. Ribeiro and S.J. Hawkins, pers. obs.), an indication of poor recruitment (Lewis 1986). The decline in abundance towards the south may indicate a weakening of demographic connectivity, but may also be associated to a decrease in the reproductive output of the species. Compared to the 1980s, *P. vulgata* at present experiences shorter spawning seasons in northern Portugal, probably due to an inhibitory effect of recent warming of sea temperature on gametogenesis (see chapter 2). Although no data are available for populations further south, chances are that spawning periods become progressively shorter and the reproductive capability gradually disappears towards the south. Hence, southern populations may be largely dependent on larval import from locations to the north.

A logical prediction stemming from the inverse relationship between water temperature and pelagic larval duration is that dispersal distances at high latitudes should be greater than those at lower latitudes. This trend has been indirectly confirmed by the existence of latitudinal gradients in measures of genetic differentiation at mitochondrial DNA loci in chitons and several marine vertebrate taxa. A possible link between latitude and estimated planktonic larval duration in the most common patellid species of the northeastern Atlantic was investigated in my thesis. A comprehensive

review of the literature reporting surveys on the reproductive cycles of *P. depressa*, *P. ulyssiponensis* and *P. vulgata* covering 19 degrees latitude provided the time frame to assess the range of ocean temperatures to which larvae were exposed during transport. Estimates of precompetent periods calculated from temperature-dependence equations and based on real SST values increased significantly with latitude. Values of pelagic larval duration varied between 4-7 days (minimum estimate) and 7-9 days (maximum estimate) in *P. ulyssiponensis*, 7-11 and 8-14 days in *P. vulgata*, and 4-5 and 6-7 days in *P. depressa*. Although a corresponding decrease in population genetic differentiation at higher latitudes would be expected, this was not the case.

Several possible explanations can be advanced to explain this incongruence. First, the putative increase in connectivity arising from greater dispersal potential at northern latitudes may not be sufficient to increase gene flow to a level that could be detected by the genetic analysis. Assuming a constant rate of mortality with temperature, the exponential loss of larvae with increasing larval duration should lead to much lower cumulative rates of larval survival at low temperatures (O' Connor *et al.* 2007). Hence, the reduced survival over longer larval periods may result in little effective gain in the number of migrants over geographically distant populations. Given the usually very small values of genetic differentiation measures found in marine populations (Waples 1998; Palumbi 2003; Hedgecock *et al.* 2007), the detection of subtle genetic differences would require larger sample sizes of individuals or loci, or otherwise the signal would not be differentiated from sampling error (Waples 1998). Second, although average dispersal distances can be greater in cold water, the degree of genetic differentiation is mostly dictated by long-distance dispersal, which relates to the ability of competent larvae to delay settlement. Because delay periods in *Patella* do not vary exponentially with water temperature (instead they are higher at optimal temperature values, see chapter 3), long-range movements may be of similar magnitudes regardless of latitude. Finally, other factors besides larval lifespan may have a sizeable impact on dispersal distances. Model simulations showed that the greatest distances travelled by larvae of *P. depressa* were achieved in the northern Iberian and North African coasts, where alongshore currents were much stronger than elsewhere, despite the shorter residence periods of larvae in the plankton (Figure 8.1).

8.2 Ecological and evolutionary scales of connectivity

One of the key findings of my thesis was that in model simulations the abundance of recruits decreased very abruptly with distance from the source population (Figure 8.2). Although the model assessed dispersal from a number of origins along the study area subjected to different oceanographic regimes (especially in the case of *P. depressa*), simulated dispersal curves converged on a common shape of exponential decrease. As

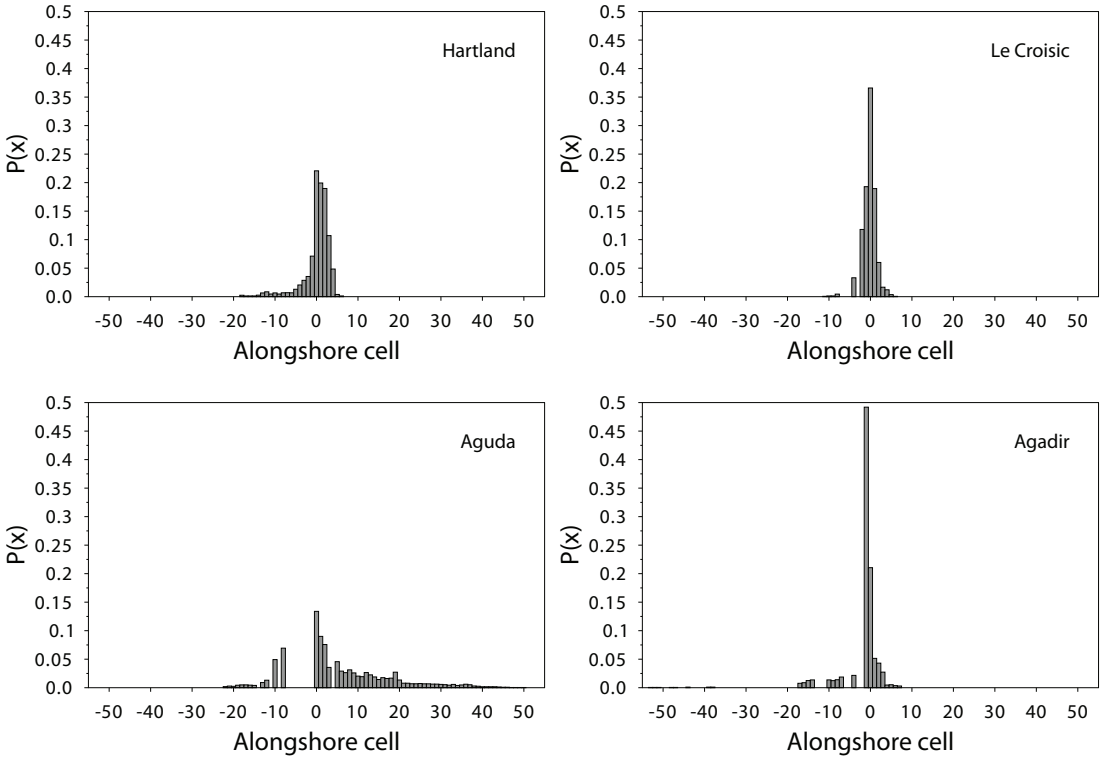

Figure 8.1 Example probability distributions of larval settlement as a function of alongshore distance from release location (dispersal kernels). Distance is expressed as the number of coastal cells (each cell being equivalent to 12 km of alongshore transport). Discontinuities in dispersal distributions reflect cells lacking settlement habitat.

a consequence, demographically relevant numbers of recruits are likely to come from relatively close source populations, separated by only a fraction of the distance that larvae can potentially travel (probably much less that 100 km). Above a certain critical distance, the supply of recruits may not be sufficient to compensate for losses in benthic populations due to mortality. Comparable results have been obtained in previous modelling efforts (Cowen *et al.* 2000; Cowen *et al.* 2006). For example, Cowen *et al.* (2006) used a high-resolution biophysical model for the Caribbean region, to investigate dispersal patterns in a variety of reef fish species. Despite the potential of larvae to drift for hundreds of kilometres, typical dispersal distances in ecologically relevant numbers were in the order of 10 to 100 km. Cowen *et al.* (2000; 2006) also argue that under realistic levels of diffusion and mortality, larval exchange even between closely located sites is not sufficient to sustain downstream populations over ecological time scales, and that these would have to rely in part on self-seeding. Similar evidence for limited demographically relevant dispersal was provided by McQuaid and Phillips (2000), while studying the invasive mussel *Mytilus galloprovincialis* in South Africa. They estimated dispersal distances between 12 and 97 km, depending on wind direction, but also found that 90% of the larvae recruited within 5 km of the home site.

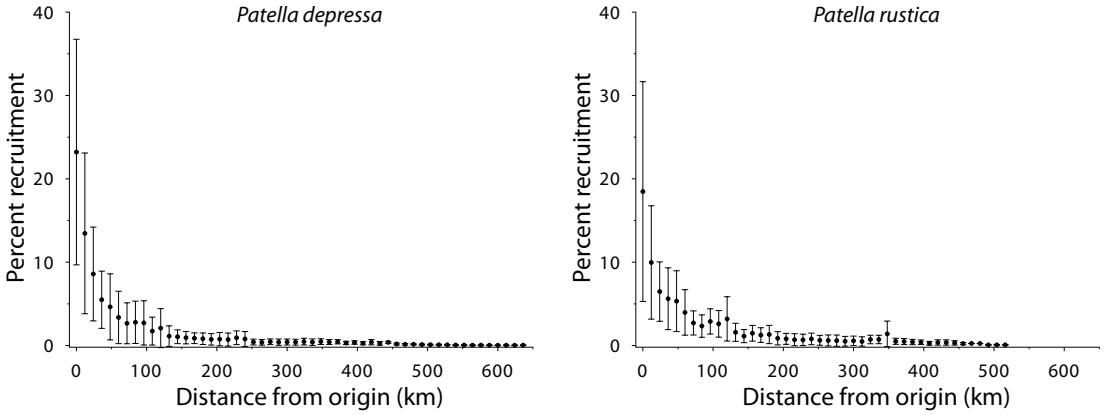

Figure 8.2 Percentage distribution of settled larvae as a function of dispersal distances, averaged over 19 (*P. depressa*) and 12 (*P. rustica*) source populations that were initially set in the model runs. Bars represent one standard deviation.

Evidence for limited extent of demographically meaningful dispersal in *P. depressa* seems to be provided by recent surveys in the Eastern Channel. The expansion of many benthic species in that region was made possible with the construction of coastal defences, which served as stepping-stones across areas of unsuitable habitat (Mieszkowska *et al.* 2005). The surveys showed that the eastern range limit of *P. depressa* in the English Channel has extended by 30 km since the 1980s, whereas other southern species with longer planktonic periods, such as *Melaraphe neritoides* and *Balanus perforatus*, experienced far greater range extensions (Mieszkowska *et al.* 2005). On the other hand, *P. ulyssiponensis*, which has a similar larval lifespan, but better tolerance to cold water during the planktonic stage, has extended 120 km eastwards in the same period of time. Because the spawning season of *P. ulyssiponensis* in southern England extends further into autumn than that of *P. depressa* (see chapter 3), this species may have benefited from colder water temperature and thus a slightly longer planktonic period which allowed larvae to cover greater distances. The range expansion of *P. rustica* in northern Portugal also suggests limited scales of dispersal for this species. During the early stages of colonisation, a sharp gradient in abundance towards the north was observed over a 60-km stretch of coastline (Lima *et al.* 2006), clearly indicating that demographically effective dispersal took place over distances comparable to the ones obtained in the model.

In contrast to the ecological scales of dispersal suggested by model simulations, genetic connectivity seems to occur over a much broader spatial extent (chapters 5 and 6). Weak levels of genetic differentiation obtained from microsatellite and mtDNA markers were congruent with the majority of population genetic surveys done for invertebrate species with planktonic dispersal capability in the northeast Atlantic region, including barnacles (Pannacciulli *et al.* 1997), stalked barnacles (Quinteiro *et*

al. 2007), sea urchins (Duran *et al.* 2004a), lobsters (Ferguson and *et al.* 2002; Stamatis *et al.* 2004), crabs (Roman and Palumbi 2004), oysters (Saavedra *et al.* 1993; Launey *et al.* 2002; Diaz-Almela *et al.* 2004), mussels (Diz and Presa 2008), whelks (Couceiro *et al.* 2007), and limpets (Weber and Hawkins 2002; Sá-Pinto 2008). This convergence of genetic structure patterns over a group of taxa with precompetent periods ranging from a few days to over one month (e.g. Lebour 1931; Bayne 1965; Burrows *et al.* 1999; chapter 3, this thesis) agrees with model predictions of dispersal and gene flow in *Littorina* (Hohenlohe 2004), suggesting that above a certain threshold, planktonic period has little effect on gene flow. Similarly, Dawson (2001) found no difference in phylogeographic patterns among species with intermediate (2 to 4 weeks) and long (8 weeks or more) pelagic larval duration.

Empirical estimates of gene flow were supported by model predictions (chapter 7). The general shape of dispersal curves suggests that the tails of long-distance dispersal may be the most important determinants of genetic connectivity in limpets and possibly in other marine invertebrate species with indirect life-cycles (Kinlan and Gaines 2003). Therefore, it may not be the precompetent period dictating the degree of genetic connectivity among marine populations, but rather the ability of species to extend larval life after reaching metamorphic competence. As demonstrated in this work, the dispersal potential granted by delayed metamorphosis may be sufficient to promote low genetic structure even in species with modest obligatory planktonic periods. A good example of the discrepancies between ecologically and genetically relevant scales of dispersal is provided by *P. rustica*. Populations on both sides of the gap were genetically homogeneous, despite the 280-km stretch of sandy coast separating them, which suggests that the exchange of individuals across this area may be sufficient to prevent the effects of genetic drift. On the other hand, the filling of the gap took several years, an indication that larvae did not arrive in sufficient numbers to ensure colonisation of the entire area during the course of one spawning season.

8.3 Implications for conservation and management of limpet populations

Limpets have been collected for human consumption since prehistoric times (Cunliffe and Hawkins 1988), and they are commonly found in large quantities in shell middens (e.g. Volman 1978; Ortea 1986; Bailey and Craighead 2003; Cabral and Silva 2003). At present, limpets are still collected for human consumption at several areas of the world, a practice that has contributed to severe reduction in abundance and even to the collapse of some populations (Branch 1975; Martins *et al.* 1987; Hawkins *et al.* 2000; Ferraz *et al.* 2001; Branch and Odendaal 2003). The main species under commercial

exploitation in the northeast Atlantic are *P. ulyssiponensis* in the European coast (particularly in the Iberian Peninsula), and *P. candei* and *P. aspera* in the Macaronesian archipelagos. Because the degree of demographic connectivity in islands is likely to be much lower than along continental coastlines, due to the reduced probability of larvae encountering suitable habitat for settlement, the consequences of overexploitation of limpet populations assumes special relevance in Macaronesia.

A recent study of the phylogeographical patterns and gene flow within the genus *Patella* in Macaronesia reported substantial levels of genetic homogeneity among islands in the Azores, with evidence of restricted gene flow only to two islands (Flores and Graciosa) in *P. candei*, and between the islands of Graciosa and Terceira in *P. aspera* (Sá-Pinto *et al.* 2008). Furthermore, these authors found no evidence of gene flow between insular and continental populations, or among archipelagos. As such, the replenishment of limpet populations in the Azores seems to be exclusively dependent on local recruitment. Although *P. candei* and *P. aspera* have not been examined in my thesis, they share a very similar biology and ecology with the other species within the genus, and therefore the conclusions drawn here can be broadly applied. Model simulations have shown that the spatial extent of dispersal in limpets is sufficient to ensure larval exchange among populations that are hundreds of kilometres apart. Although these results support a scenario of widespread gene flow among islands in the Azores, they also suggest that the magnitude of larval exchange may not be sufficient to sustain viable populations. Studies on population genetics are important for the elaboration of management plans, but these should be complemented with information on recruitment and modelling of dispersal using realistic flow fields. Disregarding one of these lines of evidence may pose a threat to conservation measures.

8.4 Limitations and further work

There are several limitations in the work of this thesis, but there also interesting opportunities for future research. One of the main limitations of the thesis concerned the development of good quality microsatellite markers in a sufficient number to allow the use of recent and more powerful analyses, such as assignment tests. Due to technical problems met during the various stages of genotyping (in particular for *P. depressa*), only a small subset of the initially available loci could be used. Screening of a higher number of loci would confer more power to the analyses and could thus reveal shallow levels of genetic differentiation, which may otherwise have remained undetected. Therefore, future work should focus on the development of more and better microsatellite markers for *P. depressa*, and on their use to confirm the patterns of population genetic structure indicated in my study.

The general validity of the relationship between temperature and planktonic

period has not been examined in this thesis. It is possible that local adaptations translate to different developmental responses to environmental characteristics which vary across the geographic range of the species (e.g. Palmer 1994; Drent 2002). On the other hand, the high levels of gene flow inferred for the studied species probably hamper the evolution of local adaptations, and therefore developmental plasticity in response to surrounding conditions might be expected to some extent. Flexible larval development has been documented in a variety of marine invertebrate species, and tested for several environmental parameters (e.g. Pawlik and Mense 1994; Bertram and Strathmann 1998). To test the hypothesis of local adaptation to temperature in *Patella* would require replicating larval rearing experiments with individuals from geographically distant populations, and checking for similarities in development, which would indicate phenotypic adjustment, or alternatively for differences suggesting local adaptation.

Due to logistic constraints it was not possible to successfully rear larvae of *P. rustica* during my thesis. For this reason, dispersal simulations done for this species were based on larval biology parameters assessed for *P. ulyssiponensis*. The planktonic periods probably do not differ much among these two species for the temperatures experienced during spawning seasons, given that comparable development rates have been observed for similar temperature values across the reared species. Nevertheless, the lack of empirical data for *P. rustica* limits the applicability of dispersal predictions, and it would therefore be important to determine larval lifespan of *P. rustica* and use these values to estimate scales of connectivity via model simulations.

The dispersal model developed in this thesis assumed that limpet larvae possess no behavioural traits that could modify their alongshore transport. Growing evidence, however, suggests that passive dispersal by marine invertebrate larvae is more the exception than the rule (e.g. Paris and Cowen 2004; Jones *et al.* 2005; Shanks and Brink 2005; Vikebø *et al.* 2005; Guizien *et al.* 2006; Fiksen *et al.* 2007; Gerlach *et al.* 2007) and that behaviour must be accounted for in order to adequately resolve larval dispersal curves and population connectivity (Cowen *et al.* 2006). Even though larvae generally have weak horizontal swimming capabilities (with the exception of decapod crustaceans and fish, Lutchembach and Orth 1992; Fischer 2005; Fisher *et al.* 2005), that do not allow them to counter the strength of most flow fields, they can change their vertical position and in this way be subjected to variable flow speed and direction (Paris and Cowen 2004; Vikebø *et al.* 2005; Marta-Almeida *et al.* 2006). Although preliminary observations have suggested changes in vertical positioning and swimming activity throughout larval development of *Patella* (see chapter 3), more detailed information is needed. This could be accomplished with studies on the response of larvae to environmental stimuli (e.g. light), the search for endogenous vertical migration rhythms and for ontogenic changes in behavioural reactions or in vertical positioning. In

addition, plankton surveys at various depths would complement data obtained from laboratory studies and provide information on the distribution of larvae along the water column throughout ontogeny and under real hydrodynamic conditions. Morphological identification of invertebrate larvae is challenging, however (Garland and Zimmer 2002), and the development of highly-sensitive and specific molecular methods (e.g. Hare *et al.* 2000; Morgan and Rogers 2001; Jones *et al.* 2007b; Pradillon *et al.* 2007) would be useful.

To be able to fully explore the possible effects of larval behaviour (such as vertical migrations and cross-shore movements) on the outcome of dispersal, further work will be needed with the use of three-dimensional circulation models accounting for both advection and eddy diffusion processes, coupled with an individual larva tracking model. In addition, a sensitivity analysis should be made to investigate how changes in biological parameters such as larval mortality and female fecundity influence the outcome of model simulations.

8.5 Concluding remarks

In recent decades, there has been growing awareness of the threats to marine populations, such as rapid changes in range and abundance of marine species, possibly related to climate warming (e.g. Southward *et al.* 1995; Beaugrand and Reid 2003; Hawkins *et al.* 2003; Genner *et al.* 2004; 2007a; Lima *et al.* 2007b), overharvesting (Jackson *et al.* 2001; Pauly *et al.* 2002; Myers and Worm 2003), habitat loss (Steneck *et al.* 2002; Airoldi and Beck 2007) and pollution (Crothers 1998; Lotze *et al.* 2006). All these issues have prompted the interest in studying dispersal, and made it one of the most prominent disciplines in the field of marine ecology (Levin 2006). Understanding the extent to which marine populations are connected by dispersal, knowing its mechanisms and identifying larval sources and sinks are essential goals that must be accomplished in order to produce reliable predictions about species range shifts and population resilience and recovery, as well as to devise the most adequate measures to protect commercially exploited species.

My thesis used a combination of approaches to explore some of the processes involved in dispersal and connectivity in the northeast Atlantic, using species of the genus *Patella* as model organisms. Results showed that planktonic larval development is markedly influenced by water temperature, and therefore factors such as spawning time and geographical location may have profound consequences in dispersal distances. Despite significant latitudinal differences in dispersal potential (driven by temperature), and the reduced residence times in the plankton at higher temperatures, the levels of realised dispersal (as estimated by genetic measures of population differentiation) were high and did not vary with

latitude. The dispersal model developed in this thesis showed that although there is considerable potential for long-distance dispersal, the great majority of larvae released at one given location settle within much shorter distances. These results illustrate the need to view the study of marine dispersal as a multidisciplinary task integrating several areas of research such as larval biology, genetics and biophysical modelling, and suggest that relying on just one line of evidence may produce misleading results.

References

Abelson A. and Denny M. (1997). Settlement of marine organisms in flow. *Annual Review of Ecology and Systematics*, 28: 317-339.

Addison J. A. and Hart M. W. (2004). Analysis of population genetic structure of the green sea urchin (*Strongylocentrotus droebachiensis*) using microsatellites. *Marine Biology*, 144: 243-251.

Aiken C. M., Navarrete S. A., Castillo M. I. and Castilla J. C. (2007). Along-shore larval dispersal kernels in a numerical ocean model of the central Chilean coast. *Marine Ecology Progress Series*, 339: 13-24.

Airoldi L. and Beck M. W. (2007). Loss, status and trends for coastal marine habitats of Europe. *Oceanography and Marine Biology: an Annual Review*, 45: 345-405.

Alexander S. E. and Roughgarden J. (1996). Larval transport and population dynamics of intertidal barnacles: a coupled benthic/oceanic model. *Ecological Monographs* 66: 259-275.

Almany G. R., Berumen M. L., Thorrold S. R., Planes S. and Jones G. P. (2007). Local replenishment of coral reef fish populations in a marine reserve. *Science*, 316: 742-744.

Amos W., Sawcer S. J., Feakes R. W. and Rubinztein D. C. (1996). Microsatellites show directional bias and heterozygote instability. *Nature Genetics* 13: 390-391.

Anastasia J. C., Morgan S. G. and N.S. F. (1998). Tagging crustacean larvae: assimilation and retention of trace elements. *Limnology and Oceanography*, 43: 362-368.

Arístegui J., Álvarez-Salgado X. A., Barton E. D., Figueiras F. G., Hernández-León S., Roy C. and Santos A. M. P. (2006). Oceanography and fisheries of the Canary Current/Iberian region of the eastern North Atlantic. In: *The Sea* (eds. A. R. Robinson and K. H. Brink). Harvard University Press, Cambridge, MS, pp. 877-931.

Arístegui J., Sangrá P., Hernández-León S., Cantón M., Hernández-Guerra A. and JKerling J. L. (1994). Island-induced eddies in the Canary islands. *Deep-Sea Research*, 41: 1509-1525.

Armour J. A. L., Alegre S. A., Miles S., Williams L. J. and Badge R. M. (1999). Minisatellites and mutation processes in tandemly repetitive DNA. In: *Microsatellites:*

evolution and applications. (eds. D. B. Goldstein and C. Schlötterer). Oxford University Press, Oxford, pp. 24-33.

Armsworth P. R. (2000). Modeling the swimming response of late stage larval reef fish to different stimuli. *Marine Ecology Progress Series*, 195: 231-247.

Armsworth P. R. (2002). Recruitment limitation, population regulation, and larval connectivity in reef fish metapopulations. *Ecology*, 83: 1092-1104.

Armsworth P. R., James M. K. and Bode L. (2001). When to press on or turn back: dispersal strategies for reef fish larvae. *American Naturalist*, 157: 434-450.

Arnold W. S., Hitchcock G. L., Frischer M. E., Wanninkhof R. and Sheng Y. P. (2005). Dispersal of an introduced larval cohort in a coastal lagoon. *Limnology and Oceanography*, 50: 587-597.

Astanei I., Gosling E., Wilson J. and Powell E. (2005). Genetic variability and phylogeography of the invasive zebra mussel, *Dreissena polymorpha* (Pallas). *Molecular Ecology*, 14: 1655-1666.

Ayers K. L. and Waters J. M. (2005). Marine biogeographic disjunction in central New Zealand. *Marine Biology*, 147: 1045-1052.

Bacci G. (1947). Sex reversal in *Patella coerulea* and *Diodora giberrula. Nature*, 160: 94-95.

Bacci G. (1949). Osservazioni statistiche sulla determinazione dei sessi in *Patella coerulea. Pubblicazioni della Stazione Zoologica di Napoli*, 22: 26-39.

Bacci G. (1975). Sex reversal in the genus *Patella* (Gastropoda Prosobranchiata). *Pubblicazioni della Stazione Zoologica di Napoli*, 39: 341-344.

Bachtrog D., Weiss S., Zangerl B., Brem G. and Schlötterer C. (1999). Distribution of dinucleotide microsatellites in the *Drosophila melanogaster* genome. *Molecular Biology and Evolution*, 16: 602-610.

Bailey G. N. and Craighead A. S. (2003). Late Pleistocene and Holocene coastal palaeoeconomies: a reconsideration of the molluscan evidence from northern Spain. *Geoarchaeology*, 18: 175-204.

Ballard J. W. O. and Kreitman M. (1995). Is mitochondrial DNA a strictly neutral marker? *Trends in Ecology and Evolution*, 10: 485-488.

Balloux F. and Lugon-Moulin N. (2002). The estimation of population differentiation with microsatellite markers. *Molecular Ecology*, 11: 155-165.

Bandelt H. J., Forster P. and Rohl A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, 16: 37-48.

Barber P. H., Moosa M. K. and Palumbi S. R. (2002a). Rapid recovery of genetic diversity of stomatopod populations on Krakatau: temporal and spatial scales of marine larval dispersal. *Proceedings of the Royal Society of London Series B, Biological Sciences*, 269: 1591-1597.

Barber P. H., Palumbi S. R., Erdmann M. V. and Moosa M. K. (2000). A marine Wallace's line? *Nature*, 406: 692-693.

Barber P. H., Palumbi S. R., Erdmann M. V. and Moosa M. K. (2002b). Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes and consequences. *Molecular Ecology*, 11: 659-674.

Barton E. D. (1998). Eastern boundary of the North Atlantic - northwest Africa and Iberia. In: *The Sea* (eds. A. R. Robinson and K. H. Brink). John Wiley & Sons, London, pp. 633-657.

Barton E. D. (2001). Canary and Portugal currents. In: *Encyclopaedia of Ocean Sciences.* (eds. J. H. Steele, K. K. Turekian and S. A. Thorpe). Academic Press, London, pp. 380-389.

Barton N. H. and Hewitt G. M. (1985). Analysis of hybrid zones. *Annual Review of Ecology and Systematics*, 16: 113-148.

Bartsch J. and Coombs S. H. (2001). An individual-based growth and transport model of the early life-history stages of mackerel (*Scomber scombrus*) in the eastern North Atlantic. *Ecological Modelling*, 138: 127-141.

Bartsch J. and Coombs S. H. (2004). An individual-based model of the early life history of mackerel (*Scomber scombrus*) in the eastern North Atlantic, simulating transport, growth and mortality. *Fisheries Oceanography*, 13: 365-379.

Baums I. B., Miller M. W. and Hellberg M. E. (2005). Regionally isolated populations of an imperiled Caribbean coral, *Acropora palmata*. *Molecular Ecology*, 14: 1377-1390.

Baums I. B., Paris C. B. and Chérubin L. M. (2006). A bio-oceanographic filter to larval dispersal in a reef-building coral. *Limnology and Oceanography*, 51: 1969-1981.

Baxter J. M. (1982). Population dynamics of *Patella vulgata* in Orkney. *Netherlands Journal of Sea Research*, 16: 96-104.

Baxter J. M. (1983). Annual variations in soft-body dry weight, reproductive cycle and sex ratios in populations of *Patella vulgata* at adjacent sites in the Orkney Islands. *Marine Biology*, 76: 149-157.

Bayne B. L. (1965). Growth and the delay of metamorphosis of the larvae of *Mytilus edulis* (L.). *Ophelia*: 1-47.

Beaugrand G. and Reid P. C. (2003). Long-term changes in phytoplankton, zooplankton

and salmon related to climate. *Global Change Biology*, 9: 801-817.

Beaumont M. A. and Bruford M. W. (1999). Microsatellites in conservation genetics. In: *Microsatellites: evolution and applications.* (eds. D. B. Goldstein and C. Schlötterer). Oxford University Press, Oxford, pp. 165-182.

Becker B. J., Fodrie F. J., McMillan P. A. and Levin L. A. (2005). Spatial and temporal variation in trace elemental fingerprints of mytillid mussel shells: a precursor to invertebrate larval tracking. *Limnology and Oceanography*, 50: 48-61.

Becker B. J., Levin L. A., Fodrie F. J. and McMillan P. A. (2007). Complex larval connectivity patterns among marine invertebrate populations. *Proceedings of the National Academy of Sciences of the United States of America*, 104: 3267-3272.

Beerli P. (2003). *MIGRATE- a maximum likelihood program to estimate gene flow using the coalescent,* Tallahassee/Seattle (http://evolution.gs.washington.edu/lamarc)

Beerli P. (2004). Effect of unsampled populations on the estimation of population sizes and migration rates between sampled populations. *Molecular Ecology*, 13: 827-836.

Beerli P. and Felsenstein J. (1999). Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. *Genetics*, 152: 763-773.

Beerli P. and Felsenstein J. (2001). Maximum-likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences of the United States of America*, 98: 4563-4568.

Belkhir K., Borsa P., Chikhi L., Pufaste N. and Bonhomme F. (2004). *GENETIX 4.03, Logiciel Sous Windows TM Pour la Génétique des Populations*. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II Montpellier, France.

Bennett C. E. and Marshall D. J. (2005). The relative energetic costs of the larval period, larval swimming and metamorphosis for the ascidian *Diplosoma listerianum*. *Marine and Freshwater Behaviour and Physiology*, 38: 21-29.

Benzie J. A. H. and Smith-Keune C. (2006). Microsatellite variation in Australian and Indonesian pearl oyster *Pinctada maxima* populations. *Marine Ecology Progress Series*, 314: 197-211.

Bertness M. D., Gaines S. D. and Wahle R. A. (1996). Wind-driven settlement patterns in the acorn barnacle *Semibalanus balanoides. Marine Ecology Progress Series*, 137: 103-110.

Bertram D. F. and Strathmann R. R. (1998). Effects of maternal and larval nutrition on

growth and form of planktotrophic larvae. *Ecology*, 79: 315-327.

Bierne N., Launey S., Naciri-Graven Y. and Bonhomme F. (1998). Early effects of inbreeding as revealed by microsatellite analysis on *Ostrea edulis* larvae. *Genetics*, 148: 1893-1906.

Bilodeau A. L., Felder D. L. and Neigel J. E. (2005). Population structure at two geographic scales in the burrowing crustacean *Callichirus islagrande* (Decapoda, Thalassinidea): historical and contemporary barriers to planktonic dispersal. *Evolution*, 59: 2125-2138.

Birky C. W., Maruyama T. and Fuerst P. (1983). An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. *Genetics*, 103: 513-527.

Björklund M. (2005). A method for adjusting allele frequencies in the case of microsatellite allele drop-out. *Molecular Ecology Notes*, 5: 676-679.

Blackmore D. T. (1969). Studies of *Patella vulgata* L. I. Growth, reproduction and zonal distribution. *Journal of Experimental Marine Biology and Ecology*, 3: 200-213.

Boaventura D., Alexander M., Della Santina P., Smith N. D., Ré P., Cancela da Fonseca L. and Hawkins S. J. (2002). The effects of grazing on the distribution and composition of low-shore algal communities on the central coast of Portugal and on the Southern coast of Britain. *Journal of Experimental Marine Biology and Ecology*, 267: 185-206.

Bode M., Bode L. and Armsworth P. R. (2006). Larval dispersal reveals regional sources and sinks in the Great Barrier Reef. *Marine Ecology Progress Series*, 308: 17-27.

Bohonak A. J. (1999). Dispersal, gene flow and population structure. *The Quarterly Review of Biology*, 74: 21-45.

Bohonak A. J. (2002). IBD (Isolation by Distance): a program for analyses of isolation by distance. *Journal of Heredity* 93: 153-154.

Bonin A., Bellemain E., Eidesen P. B., Pompanon F., Brochmann C. and Taberlet P. (2004). How to track and assess genotyping errors in population genetic studies. *Molecular Ecology*, 13: 3261-3273.

Borges M. F., Santos A. M. P., Crato N., Mendes H. and Mota B. (2003). Sardine regime shifts off Portugal: a time series analysis of catches and wind conditions. *Scientia Marina*, 67: 235-244.

Bosch I. and Pearse J. S. (1990). Developmental types of shallow-water asteroids of McMurdo Sound, Antarctica. *Marine Biology*, 104: 41-46.

Bossart J. L. and Prowell D. P. (1998). Genetic estimates of population structure and

gene flow: limitations, lessons and new directions. *Trends in Ecology and Evolution* 13: 202-206.

Botello G. and Krug P. J. (2006). 'Desperate larvae' revisited: age, energy and experience affect sensitivity to settlement cues in larvae of the gastropod *Alderia* sp. *Marine Ecology Progress Series*, 312: 149-159.

Botsford L. W., Hastings A. and Gaines S. D. (2001). Dependence of sustainability on the configuration of marine reserves and larval dispersal distance. *Ecology Letters*, 4: 144-150.

Bouchet P. (1989). A review of poecilogony in gastropods. *Journal of Molluscan Studies*, 55: 67-78.

Bowman R. S. (1981). The morphology of *Patella* spp. juveniles in Britain, and some phylogenetic inferences. *Journal of the Marine Biological Association of the United Kingdom*, 61: 647-666.

Bowman R. S. (1985). The biology of the limpet *Patella vulgata* L. in the British Isles: spawning time as a factor determining recruitment success. In: *The ecology of rocky coasts.* (eds. P. G. Moore and R. Seed). Columbia University Press, New York, pp. 178-193.

Bowman R. S. and Lewis J. R. (1977). Annual fluctuations in the recruitment of *Patella vulgata* L. *Journal of the Marine Biological Association of the United Kingdom*, 57: 793-815.

Bowman R. S. and Lewis J. R. (1986). Geographical variation in the breeding cycles and recruitment of *Patella* spp. *Hydrobiologia*, 142: 41-56.

Boxshall A. J. (2000). The importance of flow and settlement cues to larvae of the abalone, *Haliotis rufescens* Swainson. *Journal of Experimental Marine Biology and Ecology*, 254: 143-167.

Bradbury I. R. and Bentzen P. (2007). Non-linear genetic isolation by distance: implications for dispersal estimation in anadromous and marine fish populations. *Marine Ecology Progress Series*, 340: 245-257.

Branch G. M. (1974). The ecology of *Patella* Linnaeus from the Cape Peninsula, South Africa. 2. Reproductive cycles. *Transactions of the Royal Society of South Africa*, 41: 111-160.

Branch G. M. (1975). Notes on the ecology of *Patella concolor* and *Cellana capensis*, and the effects of human consumption on the limpet populations. *Zoologica Africana*, 10: 75-85.

Branch G. M. (1981). The biology of limpets: physical factors, energy flow and

ecological interactions. *Oceanography and Marine Biology: An Annual Review*, 19: 235-380.

Branch G. M. and Odendaal F. (2003). The effects of marine protected areas on the population dynamics of a South African limpet, *Cymbula oculus*, relative to the influence of wave action. *Biological Conservation*, 114: 255-269.

Brazão S., Boaventura D., Morais S., Narciso L. and Ré P. (2003). Reproduction of *Patella depressa* Pennant, 1777 on the central Portuguese coast. *Boletín del Instituto Español de Oceanografía*, 19: 453-460.

Brookfield J. F. Y. (1996). A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology*, 5: 453-455.

Brown A., Kann L. and Rand D. (2001). Gene flow versus local adaptation in the northern acorn barnacle *Semibalanus balanoides*: insights from mitochondrial DNA variation. *Evolution*, 55: 1972-1979.

Brown W. M., George M. and Wilson A. C. (1979). Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences of the United States of America*, 76: 1967-1971.

Bruford M. W. and Wayne R. K. (1993). Microsatellites and their application to population genetic studies. *Current Opinion in Genetics and Development*, 3: 939-943.

Buonaccorsi V. P., McDowell J. R. and Graves J. E. (2001). Reconciling patterns of inter-ocean molecular variance from four classes of molecular markers in blue marlin (*Makaira nigricans*). *Molecular Ecology*, 10: 1179-1196.

Burke R. D. (1983). The induction of metamorphosis of marine invertebrate larvae: stimulus and response. *Canadian Journal of Zoology*, 61: 1701-1719.

Burke R. D. (1984). Pheromonal control of metamorphosis in the Pacific sand dollar, *Dendraster excentricus. Science*, 225: 440-441.

Burkenroad D. M. (1946). Fluctuations in abundance of marine animals. *Science*, 103: 684-686.

Burrows E. M. and Lodge S. M. (1950). Note on the inter-relationships of *Patella*, *Balanus* and *Fucus* on a semi-exposed coast. *Reports of the Marine Biological Station, Port Erin*, 62: 30-34.

Burrows M. T. and Hawkins S. J. (1998). Modelling patch dynamics on rocky shores using deterministic cellular automata. *Marine Ecology Progress Series*, 167: 1-13.

Burrows M. T., Hawkins S. J. and Southward A. J. (1999). Larval development of the intertidal barnacles *Chthamalus stellatus* and *Chthamalus montagui. Journal of the*

Marine Biological Association of the United Kingdom, 79: 93-101.

Burton R. S. and Lee B. N. (1994). Nuclear and mitochondrial gene genealogies and allozyme polymorphism across a major phylogeographic break in the copepod *Tigriopus californianus. Proceedings of the National Academy of Sciences of the United States of America*, 91: 5197-5201.

Butman C. A. (1987). Larval settlement of soft-sediment invertebrates: the spatial scales of pattern explained by active habitat selection and the emerging role of hydrodynamical processes. *Oceanography and Marine Biology: An Annual Review*, 25: 113-165.

Byers J. E. and Pringle J. M. (2006). Going against the flow: retention, range limits and invasions in advective environments. *Marine Ecology Progress Series*, 313: 27-41.

Cabral J. P. and Silva A. C. F. (2003). Morphometric analysis of limpets from an Iron-Age shell midden found in northwest Portugal. *Journal of Archaeological Science*, 30: 817-829.

Caffey H. M. (1985). Spatial and temporal variation in settlement and recruitment of intertidal barnacles. *Ecological Monographs*, 55: 313-332.

Calderón I., Giribet G. and Turon X. (2008). Two markers and one history: phylogeography of the edible common sea urchin *Paracentrotus lividus* in the Lusitanian region. *Marine Biology*, 154: 137-151.

Calderón I., Ortega N., Duran S., Becerro M. and Pascual M. (2007). Finding the relevant scale: clonality and genetic structure in a marine invertebrate (*Crambe crambe*, Porifera). *Molecular Ecology*, 16: 1799-1810.

Caley M. J., Carr M. H., Hixon M. A., Hughes T. P., Jones G. P. and Menge B. A. (1996). Recruitment and the local dynamics of open marine populations. *Annual Review of Ecology and Systematics*, 27: 477-500.

Campana S. E. (1999). Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series*, 188: 263-297.

Carr M. H., Neigel J. E., Estes J. A., Andelman S., Warner R. R. and Largier J. L. (2003). Comparing marine and terrestrial ecosystems: implications for the design of coastal marine reserves. *Ecological Applications*, 13: S90-S107.

Castric V. and Bernatchez L. (2004). Individual assignment test reveals differential restriction to dispersal between two salmonids despite no increase of genetic differences with distance. *Molecular Ecology*, 13: 1299-1312.

Casu M., Casu D., Lai T., Cossu P. and Curini-Galletti M. (2006). Inter-simple sequence repeat markers reveal strong genetic differentiation among populations of the

endangered mollusc *Patella ferruginea* (Gastropoda: Patellidae) from two Sardinian marine protected areas. *Marine Biology*, 149: 1163-1174.

Caswell H. (1981). The evolution of 'mixed' life histories in marine invertebrates and elsewhere. *American Naturalist*, 117: 529-536.

Cavalli-Sforza L. L. and Edwards A. W. F. (1967). Phylogenetic analysis: models and estimation procedures. *American Journal of Human Genetics*, 19: 233-257.

Chaffe C. and Lindberg D. R. (1986). Larval biology of early Cambrian molluscs: the implications of small body size. *Bulletin of Marine Science*, 39: 536-549.

Chakraborty R. and Kimmel M. (1999). Statistics of microsatellite loci: estimation of mutation rate and pattern of population expansion. In: *Microsatellites: evolution and applications.* (eds. D. B. Goldstein and C. Schlötterer). Oxford University Press, Oxford, pp. 139-150.

Chambers G. K. and MacAvoy E. S. (2000). Microsatellites: consensus and controversy. *Comparative Biochemistry and Physiology Part B*, 126: 455-476.

Chapuis M.-P. and Estoup A. (2007). Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, 24: 621-631.

Chassignet E. P., Hurlburt H. E., Smedstad O. M., Halliwell G. R., Wallcraft A. J., Metzger E. J., Blanton B. O., Lozano C., Rao D. B., Hogan P. J. and Srinivasan A. (2006). Generalized vertical coordinates for eddy-resolving global and coastal ocean forecasts. *Oceanography*, 19: 118-129.

Chevolot M., Hoarau G., Rijnsdorp A. D., Stam W. T. and Olsen J. L. (2006). Phylogeography and population structure of thornback rays (*Raja clavata* L., Rajidae). *Molecular Ecology*, 15: 3693-3705.

Chia F., Buckland-Nicks S. J. and Young C. M. (1984). Locomotion of marine invertebrate larvae: a review. *Canadian Journal of Zoology*, 62: 1205-1222.

Chia F. S. (1974). Classification and adaptive significance of developmental patterns in marine invertebrates. *Thalassia Jugoslavica*, 10: 267-284.

Choquet M. (1966). Biologie de *Patella vulgata* L. dans le Boulonnais. *Cahiers de Biologie Marine*, 7: 1-22.

Christiaens J. (1973). Révision du genre *Patella* (Mollusca, Gastropoda). *Bulletin du Museum National d'Histoire Naturelle*, 182: 1305-1392.

Clarke A. (1992). Reproduction in the cold: Thorson revisited. *Invertebrate Reproduction and Development*, 22: 175-184.

Coleman R. A., Underwood A. J., Benedetti-Cecchi L., Aberg P., Arenas F., Arrontes

J., Castro J., Hartnoll R., Jenkins S. R., Paula J., Della Santina P. and Hawkins S. J. (2006). A continental scale evaluation of the role of limpet grazing on rocky shores. *Oecologia*, 147: 556-564.

Collin R. (2001). The effects of mode of development on phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae). *Molecular Ecology*, 10: 2249-2262.

Colson I. and Hughes R. N. (2004). Rapid recovery of genetic diversity of dogwhelk (*Nucella lapillus* L.) populations after local extinction and recolonization contradicts predictions from life-history characteristics. *Molecular Ecology*, 13: 2223-2233.

Connell J. H. (1961a). Effects of competition, predation by *Thais lapillus*, and other factors on natural populations of the barnacle *Balanus balanoides*. *Ecological Monographs*, 31: 61-104.

Connell J. H. (1961b). The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. *Ecology*, 42: 710-723.

Connell J. H. (1985). The consequences of variation in initial settlement vs. post settlement mortality in rocky intertidal communities. *Journal of Experimental Marine Biology and Ecology*, 93: 11-45.

Constantini F., Fauvelot C. and Abbiati M. (2007). Genetic structuring of the temperate gorgonian coral (*Corallium rubrum*) across the western Mediterranean Sea revealed by microsatellites and nuclear sequences. *Molecular Ecology*, 16: 5168-5182.

Corander J. and Marttinen P. (2006). Bayesian identification of admixture events using multilocus molecular markers. *Molecular Ecology*, 15: 2833-2843.

Corander J., Marttinen P. and Mäntyniemi S. (2006). A Bayesian method for identification of stock mixtures from molecular marker data. *Fishery Bulletin*, 104: 550-558.

Côrte-Real H. B. S. M., Hawkins S. J. and Thorpe J. P. (1996a). An interpretation of the taxonomic relationship between the limpets *Patella rustica* and *P. piperata*. *Journal of the Marine Biological Association of the United Kingdom*, 76: 717-732.

Côrte-Real H. B. S. M., Hawkins S. J. and Thorpe J. P. (1996b). Population differentiation and taxonomic status of the exploited limpet *Patella candei* in the Macaronesian islands (Azores, Madeira, Canaries). *Marine Biology*, 125: 141-152.

Couceiro L., Barreiro R., Ruiz J. M. and Sotka E. E. (2007). Genetic isolation by distance among populations of the netted dog whelk *Nassarius reticulatus* (L.) along the European Atlantic coastline. *Journal of Heredity*, 98: 603-610.

Cowen R. K. (2002). Larval dispersal and retention and consequences for population

connectivity. In: *Ecology of Coral Reef Fishes: Recent Advances.* (ed. P. F. Sale). Academic Press, San Diego, California, pp. 149-170.

Cowen R. K., Gawarkiewicz G., Pineda J., Thorrold S. R. and Werner F. E. (2007). Population connectivity in marine systems: an overview. *Oceanography*, 20: 14-21.

Cowen R. K., Lwiza K. M. M., Sponaugle S., Paris C. B. and Olson D. B. (2000). Connectivity of marine populations: open or closed? *Science*, 287: 857-859.

Cowen R. K., Paris C. B., Olson D. B. and Fortuna J. L. (2003). The role of long distance dispersal versus local retention in replenishing marine populations. *Gulf and Caribbean Research*, 14: 129-137.

Cowen R. K., Paris C. B. and Srinivasan A. (2006). Scaling of connectivity in marine populations. *Science*, 311: 522-527.

Coyer J. A., Peters A. F., Stam W. T. and Olsen J. L. (2003). Post-ice age recolonization and differentiation of *Fucus serratus* L. (Phaeophyceae; Fucaceae) populations in Northern Europe. *Marine Ecology*, 12: 1817-1829.

Creese R. G., Schiel D. R. and Kingsford M. J. (1990). Sex change in a giant endemic limpet, *Patella kermadecensis*, from the Kermadec Islands. *Marine Biology*, 104: 419-426.

Crothers J. H. (1998). The size and shape of dogwhelks, *Nucella lapillus* (L.) recolonising a site formerly polluted by tributyltin (TBT) in anti-fouling paint. *Journal of Molluscan Studies*, 64: 127-129.

Crow J. F. and Kimura M. (1970) *An introduction to population genetics theory.* Harper & Row, New York.

Cruz F., Pérez M. and Presa P. (2005). Distribution and abundance of microsatellites in the genome of bivalves. *Gene*, 346: 241-247.

Cunliffe B. and Hawkins S. J. (1988). The shell midden deposits in Mount Batten, Plymouth - a prehistoric Roman port. *Oxford University Committee for Archaeology Monograph*, 26: 35-38.

Cunningham C. W. and Collins T. M. (1998). Beyond area relationships: extinction and recolonization in molecular marine biogeography. In: *Molecular approaches to ecology and evolution.* (eds. R. DeSalle and B. Schierwater). Birkhäuser Verlag, Basel, pp. 297-321.

Curtis M. A. (1977). Life cycles and population dynamics of marine benthic polychaetes from the Disko Bay area of West Greenland. *Ophelia*, 16: 9-58.

Dakin E. E. and Avise J. C. (2004). Microsatellite null alleles in parentage analysis. *Heredity*, 93: 504-509.

Davies M. S., Jones H. D. and Hawkins S. J. (1990). Seasonal variation in the composition of pedal mucus from *Patella vulgata* L. *Journal of Experimental Marine Biology and Ecology*, 144: 101-112.

Davis A. R. and Butler A. J. (1989). Direct observations of larval dispersal in the colonial ascidian *Podoclavella moluccensis* Sluiter: evidence for closed populations. *Journal of Experimental Marine Biology and Ecology*, 127: 189-203.

Dawson M. N. (2001). Phylogeography in coastal marine animals: a solution from California? *Journal of Biogeography*, 28: 723-736.

Dawson M. N., Gupta A. S. and England M. H. (2005). Coupled biophysical global ocean model and molecular genetic analyses identify multiple introductions of cryptogenic species. *Proceedings of the National Academy of Sciences of the United States of America*, 102: 11968-11973.

Dayton P. K. (1971). Competition, disturbance and community organization: the provision and subsequent utilization of space in a rocky intertidal community. *Ecological Monographs*, 41: 357-389.

De Wolf H., Verhagen R. and Backeljau T. (2000). Large scale population structure and gene flow in the planktonic developing periwinkle, *Littorina striata*, in Macaronesia. *Journal of Experimental Marine Biology and Ecology*, 246: 69-83.

Delany J., McGrath D., O'Riordan R. and Myers A. (2002). Reproduction in the intertidal limpets *Patella vulgata* and *Patella ulyssiponensis*. In: *New survey of Clare Island.* (ed. A. Myers). Royal Irish Academy, Dublin, pp. 91-116.

Dell R. K. (1972). Antarctic benthos. *Advances in Marine Biology*, 10: 1-216.

DeWoody J., Nason J. D. and Hipkins V. D. (2006). Mitigating scoring errors in microsatellite data from wild populations. *Molecular Ecology Notes*, 6: 951-957.

DeWoody J. A. and Avise J. C. (2000). Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *Journal of Fish Biology*, 56: 461-473.

Di Rienzo A., Peterson A. C., Garza J. C., Valdès A. M., Slatkin M. and Freimer N. B. (1994). Mutational processes of simple-sequence repeat loci in human populations. *Proceedings of the National Academy of Sciences of the United States of America*, 91: 3166-3170.

Diaz-Almela E., Boudry P., Launey S., Bonhomme F. and Lapègue S. (2004). Reduced female gene flow in the European flat oyster *Ostrea edulis*. *Journal of Heredity*, 95: 510-516.

DiBacco C. and Chadwick D. B. (2001). Assessing the dispersal and exchange of

brachyuran larvae between regions of San Diego Bay, California and nearshore coastal habitats using elemental fingerprinting. *Journal of Marine Research*, 59: 53-78.

DiBacco C. and Levin L. A. (2000). Development and application of elemental fingerprinting to track the dispersal of marine invertebrate larvae. *Limnology and Oceanography*, 45: 871-880.

Dieringer D. and Schlötterer C. (2003). MICROSATELLITE ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes*, 3: 167-169.

Dittel A. I. and Epifanio C. E. (1982). Seasonal abundance and vertical distribution of crab larvae in Delaware Bay. *Estuaries*, 5: 197-202.

Diz A. P. and Presa P. (2008). Regional patterns of microsatellite variation in *Mytilus galloprovincialis* from the Iberian Peninsula. *Marine Biology*, 154: 277-286.

Dobretsov S. V. and Miron G. (2001). Larval and post-larval vertical distribution of the mussel *Mytilus edulis* in the White Sea. *Marine Ecology Progress Series*, 218: 179-187.

Dodd J. M. (1956). Studies on the biology of limpets III. Hermaphroditism in the three British species of *Patella*. *Journal of the Marine Biological Association of the United Kingdom*, 35: 327-340.

Dodd J. M. (1957). Artificial fertilisation, larval development and metamorphosis in *Patella vulgata* L. and *Patella caerulea* L. *Pubblicazioni della Stazione Zoologica di Napoli*, 29: 172-186.

Doherty P. J., Planes S. and Mather P. (1995). Gene flow and larval duration in seven species of fish from the Great Barrier Reef. *Ecology*, 76: 2373-2391.

Dong B. W., Sutton R. T., Jewson S. P., O'Neill A. and Slingo J. M. (2000). Predictable winter climate in the North Atlantic Sector during 1997-1999 ENSO cycle. *Geophysical Research Letters*, 27: 985-988.

Drent J. (2002). Temperature responses in larvae of *Macoma balthica* from a northerly and southerly population of the European distribution range. *Journal of Experimental Marine Biology and Ecology*, 275: 117-129.

Drummond A. J. and Rambaut A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Ecology*, 7: 214.

Dufresne F., Bourget E. and Bernatchez L. (2002). Differential patterns of spatial divergence in microsatellite and allozyme alleles: further evidence for locus-specific selection in the acorn barnacle, *Semibalanus balanoides*? *Molecular Ecology*, 11: 113-123.

Dunstan P. K. and Bax N. J. (2007). How far can marine species go? Influence of

population biology and larval movement on future range limits. *Marine Ecology Progress Series*, 344: 15-28.

Dupont L., Ellien C. and Viard F. (2007). Limits to gene flow in the slipper limpet *Crepidula fornicata* as revealed by microsatellite data and a larval dispersal model. *Marine Ecology Progress Series*, 349: 125-138.

Duran S., Palacín C., Becerro M. A., Turon X. and Giribets G. (2004a). Genetic diversity and population structure of the commercially harvested sea urchin *Paracentrotus lividus* (Echinodermata, Echinoidea). *Molecular Ecology*, 13: 3317-3328.

Duran S., Pascual M., Estoup A. and Turon X. (2004b). Strong population structure in the marine sponge *Crambe crambe* (Poecilosclerida) as revealed by microsatellite markers. *Molecular Ecology*, 13: 511-522.

Eckert G. L. (2003). Effects of the planktonic period on marine population fluctuations. *Ecology*, 84: 372-383.

Eckman J. E. (1996). Closing the larval loop: linking larval ecology to the population dynamics of marine benthic invertebrates. *Journal of Experimental Marine Biology and Ecology*, 200: 207-237.

Eckman J. E., Werner F. E. and Gross T. F. (1994). Modeling some effects of behavior on larval settlement in a turbulent boundary layer. *Deep-Sea Research II*, 41: 185-208.

Edmands S. and Harrison J. S. (2003). Molecular and quantitative trait variation within and among populations of the intertidal copepod *Tigriopus californicus*. *Evolution*, 57: 2277-2285.

Edmands S., Moberg P. E. and Burton R. S. (1996). Allozyme and mitochondrial DNA evidence of population subdivision in the purple sea urchin *Strongylocentrotus purpuratus*. *Marine Biology*, 126: 443-450.

Edmonds J. S., Caputi N. and Morita M. (1991). Stock discrimination by trace-element analysis of otoliths of orange roughy (*Hoplostethus atlanticus*), a deep-water marine teleost. *Australian Journal of Marine and Freshwater Research*, 42: 383-389.

Edwards K. P., Hare J. A., Werner F. E. and Seim H. (2007). Using 2-dimensional dispersal kernels to identify the dominant influences on larval dispersal on continental shelves. *Marine Ecology Progress Series*, 352: 77-87.

Edwards M. S. and Hernández-Carmona G. (2005). Delayed recovery of giant kelp near its southern range limit in the North Pacific following El Niño. *Marine Biology*, 147: 273-279.

Eisen J. A. (1999). Mechanistic basis for microsatellite instability. In: *Microsatellites: evolution and applications.* (eds. D. B. Goldstein and C. Schlötterer). Oxford

University Press, Oxford, pp. 34-48.

Elkin C. and Marshall D. J. (2007). Desperate larvae: influence of deferred costs and habitat requirements on habitat selection. *Marine Ecology Progress Series*, 335: 143-153.

Ellegren H. (2004). Microsatellites: simple sequences with complex evolution. *Nature Reviews Genetics*, 5: 435-445.

Ellien C., Thiébaut E., Barnay A. S., Dauvin J. C., Gentil F. and Salomon J. C. (2000). The influence of variability in larval dispersal on the dynamics of a marine metapopulation in the eastern Channel. *Oceanologica Acta*, 23: 423-442.

Ellien C., Thiébaut E., Dumas F., Salomon J. C. and Nival P. (2004). A modeling study of the respective role of hydrodynamic processes and larval mortality on larval dispersal and recruitment of benthic invertebrates: example of *Pectinaria koreni* (Annelida: Polychaeta) in the Bay of Seine (English Channel). *Journal of Plankton Research*, 26: 117-132.

Emlet R. B. (1995). Developmental mode and species geographic range in regular sea urchins (Echinodermata: Echinoidea). *Evolution*, 49: 476-489.

Epperson B. K. (2005). Mutation at high rates reduces spatial structure within populations. *Molecular Ecology*, 14: 703-710.

Espinoza F. and Ozawa T. (2006). Population genetics of the endangered limpet *Patella ferruginea* (Gastropoda: Patellidae): taxonomic, conservation and evolutionary considerations. *Journal of Zoological Systematics and Evolutionary Research*, 44: 8-16.

Estoup A. and Angers B. (1998). Microsatellites and minisatellites for molecular ecology: theoretical and empirical considerations. In: *Advances in Molecular Ecology* (ed. G. Carvalho). IOS Press, Amsterdam, pp. 55-86.

Estoup A. and Cornuet J.-M. (1999). Microsatellite evolution: inferences from population data. In: *Microsatellites: evolution and applications.* (eds. D. B. Goldstein and C. Schlötterer). Oxford University Press, Oxford, pp. 49-65.

Estoup A., Jarne P. and Cornuet J.-M. (2002). Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. *Molecular Ecology*, 11: 1591-1604.

Estoup A., Tailliez C., Cornuet J.-M. and Solignac M. (1995). Size homoplasy and mutational processes of interrupted microsatellites in two bee species, *Apis mellifera* and *Bombus terrestris* (Apidae). *Molecular Biology and Evolution*, 12: 1074-1084.

Evans R. G. (1953). Studies on the biology of the British limpets - the genus *Patella* on the South coast of England. *Proceedings of the Zoological Society of London*, 123: 357-

376.

Excoffier L. and Heckel G. (2006). Computer programs for population genetics data analysis: a survival guide. *Nature Reviews Genetics*, 7: 745-758.

Excoffier L., Laval G. and Schneider S. (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, 1: 47-50.

Excoffier L., Smouse P. E. and Quattro J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, 131: 479-491.

Falush D., Stephens M. and Pritchard J. K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, 164: 1567-1587.

Feldman M. W., Bergman A., Pollock D. D. and Goldstein D. B. (1997). Microsatellite genetic distances with range constraints: analytic description and problems of estimation. *Genetics*, 145: 207-216.

Felsenstein J. (2004). *PHYLIP (Phylogeny Inference Package) version 3.6*. Distributed by the author. Department of Genome Sciences, University of Washington Seattle.

Ferguson A. *et al.* (2002). Genetic diversity in the European lobster (*Homarus gammarus*): population structure and impacts of stock enhancement. *http://www.qub.ac.uk/bb/prodohl/GEL/gel.html.*

Ferraz R. R., Menezes G. M. and Santos R. S. (2001). Limpet (*Patella* spp.) (Molusca:Gastropoda) exploitation in the Azores, during the period 1993-1998. *Arquipelago. Life and Marine Sciences*, Supplement 2 (Part B): 59-65.

Figueiras F. G., Labarta U. and Fernández Reiriz M. J. (2002). Coastal upwelling, primary production and mussel growth in the Rías Baixas of Galicia. *Hydrobiologia*, 484: 121-131.

Fiksen Ø., Jørgensen C., Kristiansen T., Vikebø F. and Huse G. (2007). Linking behavioural ecology and oceanography: larval behaviour determines growth, mortality and dispersal. *Marine Ecology Progress Series*, 347: 195-205.

Fischer-Piètte E. (1935). Systématique et biogéographie - les patelles d'Europe et d'Afrique du nord. *Journal de Conchyliologie*, 79: 5-66.

Fischer-Piètte E. (1938). The concept of species anf geographical isolation in the case of north Atlantic *Patella*. *Journal of the Linnean Society of London*, 150: 268-275.

Fischer-Piètte E. (1941). Croissance, taille maxima et longévité possible de quelques animaux intercotidaux en fonction du milieu. *Annales de l'Institut Océanographique*

Paris 21: 1-28.

Fischer-Piètte E. (1948). Sur les éléments de prospérité des patelles et sur leur spécificité. *Journal de Conchyliologie*, 88: 45-96.

Fischer-Piètte E. (1955). Repartition le long des cotes septentrionales de l'Espagne, des principales éspèces peuplant les rochers intercotidaux. *Annales de l'Institut Océanographique Paris*, 31: 37-124.

Fischer-Piètte E. and Gaillard J.-M. (1959). Les Patelles, au long des côtes Atlantiques Ibériques et Nord-Marocaines. *Journal de Conchyliologie*, 99: 135-200.

Fischer R. (2005). Swimming speeds of larval coral reef fishes: impacts on self-recruitment and dispersal. *Marine Ecology Progress Series*, 285: 223-232.

Fisher R., Leis J. M., Clark D. L. and Wilson S. K. (2005). Critical swimming speeds of late-stage coral reef fish larvae: variation within species, among species and between locations. *Marine Biology*, 147: 1202-1212.

Fiúza A. F. G., Macedo M. E. and Guerreiro M. R. (1982). Climatological space and time variation of the Portuguese coastal upwelling. *Oceanologica Acta*, 5: 31-40.

Foltz D. W. (1986). Null alleles as a possible cause of heterozygote deficiency in the oyster *Crassostrea virginica* and other bivalves. *Evolution*, 40: 869-870.

Forward J., R.B. and Tankersley R. A. (2001). Selective tidal-stream transport of marine animals. *Oceanography and Marine Biology: An Annual Review*, 39: 305-353.

Frenkiel L. (1975). Contribution à l'étude des cycles de reproduction des Patellidae en Algèrie. *Pubblicazioni della Stazione Zoologica di Napoli*, 39: 153-189.

Frenzel B., Pécsi M. and Velichko A. A. (2001). Atlas of paleoclimates and paleoenvironments of the Northern hemisphere (digitized version). *Doi:10.1594/PANGAEA.58194.*

Fretter V. and Graham A. (1976). The prosobranch molluscs of Britain and Denmark. I. Pleurotomariacea, Fissurellacea and Patellacea. *Journal of Molluscan Studies*, Suppl. 1: 37.

Fringer O. B., McWilliams J. C. and Street R. L. (2006). A new hybrid model for coastal simulations. *Oceanography*, 19: 64-77.

Frouin R., Fiúza A. F. G., Ambar I. and Boyd T. J. (1990). Observations of a poleward surface current off the coasts of Portugal and Spain during winter. *Journal of Geophysical Research*, 95: 679-691.

Fu Y.-X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhicking and background selection. *Genetics*, 147: 915-925.

Gaffney P. (1980). On the number of *Patella* species in South-West England. *Journal of the Marine Biological Association of the United Kingdom*, 60: 565-574.

Gaggiotti O. E., Lange O., Rassmann K. and Gliddon C. (1999). A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. *Molecular Ecology*, 8: 1513-1520.

Gaines S., Brown S. and Roughgarden J. (1985). Spatial variation in larval concentration as a cause of spatial variation in settlement for the barnacle, *Balanus glandula*. *Oecologia*, 67: 267-272.

Gaines S. and Roughgarden J. (1985). Larval settlement rate: a leading determinant of structure in an ecological community of the marine intertidal zone. *Proceedings of the National Academy of Sciences of the United States of America*, 82: 3707-3711.

Gaines S. D. and Bertness M. (1992). Dispersal of juveniles and variable recruitment in sessile marine species. *Nature*, 360: 579-580.

Gaines S. D. and Bertness M. (1993). The dynamics of juvenile dispersal: why field ecologists must integrate. *Ecology*, 74: 2430-2435.

Gaines S. D., Gaylord B., Gerber L. R., Hastings A. and Kinlan B. P. (2007). Connecting places: the ecological consequences of dispersal in the sea. *Oceanography*, 20: 90-99.

Gaines S. D., Gaylord B. and Largier J. L. (2003). Avoiding current oversights in marine reserve design. *Ecological Applications*, 13: S32-S46.

Gaines S. D. and Lafferty K. D. (1995). Modelling the dynamics of marine species: the importance of incorporating larval dispersal. In: *Ecology of marine invertebrate larvae.* (ed. L. McEdward). CRC Press, Boca Raton, Florida.

Galindo H. M., Olson D. B. and Palumbi S. R. (2006). Seascape genetics: a coupled oceanographic-genetic model predicts population structure of Caribbean corals. *Current Biology*, 16: 1622-1626.

Gallardo C. S. and Penchaszadeh P. E. (2001). Hatching mode and latitude in marine gastropods: revisiting Thorson's paradigm in the southern hemisphere. *Marine Biology*, 138: 547-552.

Gardner J. P. A. (1997). Hybridization in the sea. *Advances in Marine Biology*, 31: 1-78.

Garland E. D. and Zimmer C. A. (2002). Techniques for the identification of bivalve larvae. *Marine Ecology Progress Series*, 225: 299-310.

Garrabou J., Laubier L. and Pérez T. (2002). Sessile benthic invertebrates as indicator of climate change: mass mortalities in the north-west Mediterranean in summer 1999. *Ocean Challenge*, 12: 17-23.

Garwood P. R. (1987). The cycle of gonad development in *Patella vulgata* (Mollusca: Gastropoda): the use of oocyte diameter measurements and gravimetric estimates of sexual maturity. *Sarsia*, 72: 29-35.

Gascoigne J. and Lipcius R. N. (2004). Allee effects in marine systems. *Marine Ecology Progress Series*, 269: 49-59.

Gawarkiewicz G., Monismith S. and Largier J. (2007). Observing larval transport processes affecting population connectivity: progress and challenges. *Oceanography*, 20: 40-53.

Gaylord B. and Gaines S. D. (2000). Temperature or transport? Range limits in marine species mediated solely by flow. *American Naturalist*, 155: 769-789.

Gebauer P., Paschke K. and Anger K. (1999). Costs of delayed metamorphosis: reduced growth and survival in early juveniles of an estuarine grapsid crab, *Chasmagnathus granulata*. *Journal of Experimental Marine Biology and Ecology*, 238: 271-281.

Genner M. J., Sims D. W., Wearmouth V. J., Southall E. J., Southward A. J., Henderson P. A. and Hawkins S. J. (2004). Regional climatic warming drives long-term community changes of British marine fish. *Proceedings of the Royal Society of London Series B, Biological Sciences*, 271: 655-661.

Gerlach G., Atema J., Kingsford M. J., Black K. P. and Miller-Sims V. (2007). Smelling home can prevent dispersal of reef fish larvae. *Proceedings of the National Academy of Sciences of the United States of America*, 104: 858-863.

Ghiselin M. T. (1987). Evolutionary aspects of marine invertebrate reproduction. In: *Reproduction of marine invertebrates.* (eds. A. C. Giese, J. S. Pearse and V. M. Pearse). Blackwell Scientific, Palo Alto, California.

Gibson G. (1994). Why be choosy - temporal changes in larval sensitivity to several naturally occurring metamorphic inducers in the opisthobranch *Haminaea callidegenita*. *Journal of Experimental Marine Biology and Ecology*, 194: 9-24.

Gilg M. R. and Hilbish T. J. (2003). Patterns of larval dispersal and their effect on the maintenance of a blue mussel hybrid zone in Southwestern England. *Evolution*, 57: 1061-1077.

Gillanders B. M. and Kingsford M. J. (1996). Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. *Marine Ecology Progress Series*, 141: 13-20.

Gillanders B. M. and Kingsford M. J. (2000). Elemental fingerprints of otoliths of fish may distinguish estuarine 'nursery' habitats. *Marine Ecology Progress Series*, 201: 273-286.

Gillanders B. M., Sanchez-Jerez P., Bayle-Sempere J. and Ramos-Espla A. (2001). Trace elements in otoliths of the two-banded bream from a coastal region in the south-west Mediterranean: are there differences among locations? *Journal of Fish Biology*, 59: 350-363.

Gilman S. E. (2006a). The northern geographic range limit of the intertidal limpet *Collisella scabra*: a test of performance, recruitment, and temperature hypotheses. *Ecography*, 29: 709-720.

Gilman S. E. (2006b). Life at the edge: an experimental study of a poleward range boundary. *Oecologia*, 148: 270-279.

Goldson A. J., Hughes R. N. and Gliddon C. J. (2001). Population genetic consequences of larval dispersal mode and hydrography: a case study with bryozoans. *Marine Biology*, 138: 1037-1042.

Goldstein D. B. and Pollock D. D. (1997). Launching microsatellites: a review of mutation processes and methods of phylogenetic inference. *Journal of Heredity*, 88: 335-342.

Goldstein D. B., Roemer G. W., Smith D. A., Reich D. E., Bergman A. and Wayne R. K. (1999). The use of microsatellite variation to infer population structure and demographic history in a natural model system. *Genetics*, 151: 797-801.

Gotelli N. J. (1990). Stochastic models of gregarious larval settlement. *Ophelia*, 32: 95-108.

Goudet J. (2001). *FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3).* http://unil.ch/izea/softwares/fstat.html.

Gould M. C., Stephano J. L., Ortiz-Barron B. J. and Perez-Quezada I. (2001). Maturation and fertilization in *Lottia gigantea* oocytes: intracellular pH, Ca2+, and electrophysiology. *Journal of Experimental Zoology*, 290: 411-420.

Grahame J. and Branch G. M. (1985). Reproductive patterns of marine invertebrates. *Oceanography and Marine Biology: An Annual Review*, 23: 373-398.

Grantham B. A., Eckert G. L. and Shanks A. L. (2003). Dispersal potential of marine invertebrates in diverse habitats. *Ecological Applications*, 13: S108-S116.

GRASS Development Team (2006). *Geographic Resources Analysis Support System (GRASS) Software*, ITC-irst Trento, Italy. http://grass.itc.it.

Gribben P. E., Marshall D. J. and Steinberg P. D. (2006). Less inhibited with age? Larval age modifies responses to natural settlement inhibitors. *Biofouling*, 22: 101-106.

Grosberg R. and Cunningham C. W. (2001). Genetic structure in the sea: from populations to communities. In: *Marine community ecology.* (eds. M. D. Bertness, S.

Gaines and M. E. Hay). Sinauer Associates, Sunderland, Massachussetts, pp. 61-84.

Grosberg R. K. (1981). Competitive ability influences habitat choice in marine environments. *Nature*, 290: 700-702.

Grosberg R. K. and Levitan D. R. (1992). For adults only? Supply-side ecology and the history of larval biology. *Trends in Ecology and Evolution*, 7: 130-133.

Gruenthal K. M., Acheson L. K. and Burton R. S. (2007). Genetic structure of natural populations of California red abalone (*Haliotis rufescens*) using multiple genetic markers. *Marine Biology*, 152: 1237-1248.

Gruenthal K. M. and Burton R. S. (2008). Genetic structure of natural populations of the California black abalone (*Haliotis cracherodii* Leach, 1814), a candidate for bendangered species status. *Journal of Experimental Marine Biology and Ecology*, 355: 47-58.

Guerra-Garcia J. M., Corzo J., Espinoza F. and García-Gómez J. C. (2004). Assessing habitat use of the endangered marine mollusc *Patella ferruginea* (Gastropoda, Patellidae) in northern Africa: preliminary results and implications for conservation. *Biological Conservation*, 116: 319-326.

Guerra M. T. and Gaudêncio M. J. (1986). Aspects of the ecology of *Patella* spp. on the Portuguese coast. *Hydrobiologia*, 142: 57-69.

Guizien K., Brochier T., Duchêne J.-C., Koh B.-S. and Marsaleix P. (2006). Dispersal of *Owenia fusiformis* larvae by wind-driven currents: turbulence, swimming behaviour and mortality in a three-dimensional stochastic model. *Marine Ecology Progress Series*, 311: 47-66.

Guo S. W. and Thompson E. A. (1992). Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics*, 48: 361-372.

Gysels E. S., Hellemans B., Pampoulie C. and Volckaert F. A. M. (2004). Phylogeography of the common goby, *Pomatoschistus microps*, with particular emphasis on the colonization of the Mediterranean and the North Sea. *Molecular Ecology*, 13: 403-417.

Hadfield M. G. (1986). Settlement and recruitment of marine invertebrates: a perspective and some proposals. *Bulletin of Marine Science*, 39: 418-425.

Hadfield M. G. and Kohel M. A. R. (2004). Rapid behavioral responses of an invertebrate larva to dissolved settlement cue. *Biological Bulletin*, 207: 28-43.

Hadfield M. G. and Scheuer D. (1985). Evidence for a soluble metamorphic inducer in *Phestilla*: ecological, chemical and biological data. *Bulletin of Marine Science*, 37: 556-566.

Hadfield M. G. and Strathmann M. F. (1996). Variability, flexibility and plasticity in life histories of marine invertebrates. *Oceanologica Acta*, 19: 323-334.

Hain S. and Arnaud P. M. (1992). Notes on the reproduction of high Antarctic molluscs from the Weddell Sea. *Polar Biology*, 12: 303-312.

Hancock J. M. (1999). Microsatellites and other simple sequences: genomic context and mutational mechanisms. In: *Microsatellites: evolution and applications.* (eds. D. B. Goldstein and C. Schlötterer). Oxford University Press, Oxford, pp. 1-9.

Harding M. J. C., Rodger G. K., Davies I. M. and Moore J. J. (1997). Partial recovery of the dogwhelk (*Nucella lapillus*) in Sullom Voe, Shetland from tributyltin contamination. *Marine Environmental Research*, 44: 285-304.

Hare J. A., Thorrold S., Walsh H., Reiss C., Valle-Levinson A. and Jones C. (2005a). Biophysical mechanisms of larval fish ingress into Chesapeake Bay. *Marine Ecology Progress Series*, 303: 295-310.

Hare M. P. and Avise J. C. (1996). Molecular genetic analysis of a stepped multilocus cline in the American oyster (*Crassostrea virginica*). *Evolution*, 50: 2305-2315.

Hare M. P., Guenther C. and W.F. F. (2005b). Nonrandom larval dispersal can steepen marine clines. *Evolution*, 59: 2509-2517.

Hare M. P., Palumbi S. R. and Butman C. A. (2000). Single-step species identification of bivalve larvae using multiplex polymerase chain reaction. *Marine Biology*, 137: 953-961.

Harley C. D. G., Hughes A. R., Hultgren K. M., Miner B. G., Sorte C. J. B., Thornber C. S., Rodriguez L. F., Tomanek L. and Williams S. L. (2006). The impacts of climate change in coastal marine systems. *Ecology Letters*, 9: 228-241.

Harpending R. C. (1994). Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology*, 66: 591-600.

Hartnoll R. G. and Hawkins S. J. (1985). Patchiness and fluctuations on moderately exposed rocky shores. *Ophelia*, 24: 53-63.

Harvey M. and Bourget E. (1997). Recruitment of marine invertebrates onto arborescent epibenthic structures: active and passive processes acting at different spatial scales. *Marine Ecology Progress Series*, 153: 203-215.

Harvey M., Bourget E. and Ingram R. G. (1995). Experimental evidence of passive accumulation of marine bivalve larvae on filamentous epibenthic structures. *Limnology and Oceanography*, 40: 94-104.

Hastings A. and Botsford L. W. (2006). Persistence of spatial populations depends on returning home. *Proceedings of the National Academy of Sciences of the United States*

of America, 103: 6067-6072.

Hatch D. J. (1977). *The inter-relationships of three Patella spp. on the South coast of Britain.* PhD thesis, University of Southampton.

Havenhand J. N. (1995). Evolutionary ecology of larval types. In: *Ecology of marine invertebrate larvae.* (ed. L. McEdward). CRC Press, Boca Raton, Florida.

Hawkins S. J. (1981). The influence of *Patella* grazing on the fucoid-barnacle mosaic in moderately exposed rocky shores. *Kieler Meeresforschungen*, 5: 537-543.

Hawkins S. J. (1983). Interactions of *Patella* and macroalgae with settling *Semibalanus balanoides* (L.). *Journal of Experimental Marine Biology and Ecology*, 71: 55-72.

Hawkins S. J., Côrte-Real H. B. S. M., Pannacciulli F. G., Weber L. C. and Bishop J. D. D. (2000). Thoughts on the ecology and evolution of the intertidal biota of the Azores and other Atlantic Islands. *Hydrobiologia*, 440: 3-17.

Hawkins S. J. and Hartnoll R. G. (1982). Settlement patterns of *Semibalanus balanoides* (L.) in the Isle of Man (1977-1981). *Journal of Experimental Marine Biology and Ecology*, 62: 271-283.

Hawkins S. J. and Hartnoll R. G. (1983). Grazing of intertidal algae by marine invertebrates. *Oceanography and Marine Biology: an Annual Review*, 21: 195-282.

Hawkins S. J., Hartnoll R. G., Kain J. M. and Norton T. A. (1992). Plant-animal interactions on hard substrata in the North-East Atlantic. In: *Plant-animal interactions in the marine benthos, systematics association special volume 46* (eds. D. M. John, S. J. Hawkins and J. H. Price). Clarendon Press, Oxford, pp. 1-32.

Hawkins S. J., Southward A. and Genner M. J. (2003). Detection of environmental change in a marine ecosystem - evidence from the western English Channel. *The Science of the Total Environment*, 310: 245-256.

Haynes R. and Barton E. D. (1990). A poleward flow along the Atlantic coast of the Iberian Peninsula. *Journal of Geophysical Research*, 95: 11425-11441.

Haynes R., Barton E. D. and Pilling I. (1993). Development, persistence, and variability of upwelling filaments off the Atlantic coast of the Iberian Peninsula. *Journal of Geophysical Research*, 98: 22681-22692.

Hedgecock D. (1986). Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? *Bulletin of Marine Science*, 39: 550-564.

Hedgecock D. (1994a). Does variance in reproductive success limit effective population sizes of marine organisms? In: *Genetics and evolution of aquatic organisms.* (ed. A. R. Beaumont). Chapman & Hall, London, pp. 122-134.

Hedgecock D. (1994b). Temporal and spatial genetic structure of marine animal populations in the California Current. *CalCOFI Report*, 35: 73-81.

Hedgecock D., Barber P. H. and Edmands S. (2007). Genetic approaches to measuring connectivity. *Oceanography*, 20: 70-79.

Hedgecock D., Chow V. and Waples R. S. (1992). Effective population numbers of shellfish broodstocks estimated from temporal variance in allelic frequencies. *Aquaculture*, 108: 215-232.

Hedgecock D., Li G., Hubert S., Bucklin K. and Ribes V. (2004). Widespread null alleles and poor cross-species amplification of microsatellite DNA loci cloned from the Pacific oyster, *Crassostrea gigas*. *Journal of Shellfish Research*, 23: 379-385.

Hedrick P. W. (1999). Perspective: highly variable loci and their interpretation in evolution and conservation. *Evolution*, 53: 313-318.

Hedrick P. W. (2005a). Large variance in reproductive success and the Ne/N ratio. *Evolution*, 59: 1596-1599.

Hedrick P. W. (2005b). A standardized genetic differentiation measure. *Evolution*, 59: 1633-1638.

Hellberg M. E. (1994). Relationships between inferred levels of gene flow and geographic distance in a philopatric coral, *Balanophyllia elegans*. *Evolution*, 48: 1829-1854.

Hellberg M. E. (1996). Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. *Evolution*, 50: 1167-1175.

Hellberg M. E. (2006). No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. *BMC Evolutionary Biology*, 6: 24.

Hellberg M. E., Burton R. S., Neigel J. E. and Palumbi S. R. (2002). Genetic assessment of connectivity among marine populations. *Bulletin of Marine Science*, 70: 273-290.

Hellberg M. E. and Vacquier V. D. (1999). Rapid evolution of fertilization selectivity and lysine cDNA sequences in Teguline gastropods. *Molecular Biology and Evolution*, 16: 839-848.

Heller J. (1993). Hermaphroditism in molluscs. *Biological Journal of the Linnean Society*, 48: 19-42.

Helmuth B., Veit R. R. and Holberton R. (1994). Long-distance dispersal of a sub-Antarctic brooding bivalve (*Gaimardia trapesina*) by kelp rafting. *Marine Biology*, 120: 421-426.

Hemmer-Hansen J., Nielsen E. E., Grønkjær P. and Loeschcke V. (2007). Evolutionary

mechanisms shaping the genetic population structure of marine fishes; lessons from the European flounder (*Platichthys flesus* L.). *Molecular Ecology*, 16: 3104-3118.

Herborg L.-M., Weetman D., van Oosterhout C. and Hänfling B. (2007). Genetic population structure and contemporary dispersal patterns of a recent European invader, the Chinese mitten crab, *Eriocheir sinensis*. *Molecular Ecology*, 16: 231-242.

Hewitt G. M. (1996). Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, 58: 247-276.

Hidalgo J. G. (1917). Fauna Malacológica de España, Portugal y las Baleares. Moluscos testaceos marinos.Trabajos del Museo Nacional de Ciencias Naturales. *Serie Zoológica*, 30: 1-752.

Highsmith R. C. (1985). Floating and algal rafting as potential dispersal mechanisms in brooding invertebrates. *Marine Ecology Progress Series*, 25: 169-179.

Highsmith R. C. and Emlet R. B. (1986). Delayed metamorphosis effect on growth and survival of juvenile sand dollars (Echinoidea: Clypeasteroida). *Bulletin of Marine Science*, 39: 347-361.

Hilbish T. J. (1996). Population genetics of marine species: the interaction of natural selection and historically differentiated populations. *Journal of Experimental Marine Biology and Ecology*, 200: 67-83.

Hilbish T. J., Carson E. W., Plante J. R., Weaver L. A. and Gilg M. R. (2002). Distribution of *Mytilus edulis*, *Mytilus galloprovinciallis*, and their hybrids in open-coast populations of mussels in southwestern England. *Marine Biology*, 140: 137-142.

Hill A. E. (1990). Pelagic dispersal of Norway lobster *Nephrops norvegicus* larvae examined using an advection-diffusion-mortality model. *Marine Ecology Progress Series*, 64: 217-226.

Hiscock K., Southward A., Tittley I. and Hawkins S. J. (2004). Effects of changing temperature on benthic marine life in Britain and Ireland. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 14: 333-362.

Hoarau G., Coyer A., Veldsink J. H., Stam W. T. and Olsen J. L. (2007). Glacial refugia and recolonization pathways in the brown seaweed *Fucus serratus*. *Molecular Ecology*, 16: 3606-3616.

Hodgson A. N., Le Quesne W. J. F., S.J. H. and Bishop J. D. D. (2007). Factors affecting fertilization success in two species of patellid limpet (Mollusca: Gastropoda) and development of fertilization kinetics models. *Marine Biology*, 150: 415-426.

Hoegh-Guldberg O. and Pearse J. S. (1995). Temperature, food availability, and the development of marine invertebrate larvae. *American Zoologist*, 35: 415-525.

Hoffman J. I. and Amos W. (2005). Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. *Molecular Ecology*, 14: 599-612.

Hohenlohe P. A. (2002). Life history of *Littorina scutulata* and *L. plena*, sibling gastropod species with planktonic larvae. *Invertebrate Biology*, 121: 25-37.

Hohenlohe P. A. (2004). Limits to gene flow in marine animals with planktonic larvae: models of *Littorina* species around Point Conception, California. *Biological Journal of the Linnean Society*, 82: 169-187.

Holbrook S. J., Schmitt R. J. and Stephens J. S. (1997). Changes in an assemblage of temperate reef fishes associated with a climate shift. *Ecological Applications*, 7: 1299-1310.

Holland B. S. (2001). Invasion without a bottleneck: microsatellite variation in natural and invasive populations of the brown mussel *Perna perna* (L). *Marine Biotechnology*, 3: 407-415.

Holmes S. P., Dekker R. and Williams I. D. (2004). Population dynamics and genetic differentiation in the bivalve mollusk *Abra tenuis*: aplanic dispersal. *Marine Ecology Progress Series*, 268: 131-140.

Hughes C. R. and Queller D. C. (1993). Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. *Molecular Ecology*, 2: 131-137.

Hutchins L. W. (1947). The bases for temperature zonation in geographical distribution. *Ecological Monographs*, 17: 325-335.

Huthnance J. M., van Aken H. M., White M., Barton E. D., Le Cann B., Coelho E. F., Fanjul E. A., Miller P. and Vitorino J. (2002). Ocean margin exchange - water flux estimates. *Journal of Marine Systems*, 32: 107-137.

Huvet A., Lapègue S., Magoulas A. and Boudry P. (2000). Mitochondrial and nuclear DNA phylogeography of *Crassostrea angulata*, the Portuguese oyster endangered in Europe. *Conservation Genetics*, 1: 251-262.

Ibañez M., Peña J. and Feliu J. (1986). Reproduction of *Patella* spp. on the Basque coast of Spain. *Hydrobiologia*, 142: 327.

Ingolfsson A. (1992). The origin of the rocky shore fauna of Iceland and the Canadian Maritimes. *Journal of Biogeography*, 19: 705-712.

Jablonski D. and Lutz R. A. (1983). Larval ecology of marine benthic invertebrates: paleobiological implications. *Biological Reviews*, 58: 21-89.

Jackson G. A. and Strathmann R. R. (1981). Larval mortality from offshore mixing as a link between precompetent and competent periods of development. *American*

Naturalist, 118: 16-26.

Jackson J. B. C., Kirby M. X., Berger W. H., Bjorndal K. A., Botsford L. W., Bourque B. J., Bradbury R. H., Cooke R., Erlandson J., Estes J. A., Hughes T. P., Kidwell S., Lange C. B., Lenihan H. S., Pandolfi J. M., Peterson C. H., Steneck R. S., Tegner M. J. and Warner R. R. (2001). Historical overfishing and the recent collapse of coastal ecosystems. *Science*, 293: 629-638.

James M. K., Armsworth P. R., Mason L. B. and Bode L. (2002). The structure of reef fish metapopulations: modelling larval dispersal and retention patterns. *Proceedings of the Royal Society of London Series B, Biological Sciences*, 269: 2079-2086.

Jarne P. and Lagoda P. J. L. (1996). Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution*, 11: 424-429.

Jarvis C. H. and Stuart N. (2001). A comparison among strategies for interpolating maximum and minimum daily air temperatures. Part II: The interaction between number of guiding variables and the type of interpolation method. *Journal of Applied Meteorology*, 40: 1075-1082.

Jenkins S. R., Coleman R. A., Della Santina P., Hawkins S. J., Burrows M. T. and Hartnoll R. G. (2005). Regional scale differences in the determinism of grazing effects in the rocky intertidal. *Marine Ecology Progress Series*, 287: 77-86.

Jenkins S. R., Norton T. A. and Hawkins S. J. (1999). Settlement and post-settlement interactions between *Semibalanus balanoides* (L.) (Crustacea: Cirripedia) and three species of fucoid canopy algae. *Journal of Experimental Marine Biology and Ecology*, 236: 49-67.

Jensen R. A. and Morse D. (1990). Chemically induced metamorphosis of polychaete larvae in both the laboratory and ocean environment. *Journal of Chemical Ecology*, 16: 911-930.

Jensen R. A. and Morse D. E. (1984). Intraspecific facilitation of larval recruitment: gregarious settlement of the polychaete *Phragmatopoma californica* (Fewkes). *Journal of Experimental Marine Biology and Ecology*, 83: 107-126.

Johannesson K. (1988). The paradox of Rockall: why is a brooding gastropod (*Littorina saxatilis*) more widespread than one having a planktonic larval dispersal stage (*L. littorea*)? *Marine Biology*, 99: 507-513.

Johansson M. L., Banks M. A., Glunt K. D., Hassel-Finnegan H. M. and Buonaccorsi V. P. (2008). Influence of habitat discontinuity, geographical distance, and oceanography on fine-scale population genetic structure of copper rockfish (*Sebastes caurinus*). *Molecular Ecology*, 17: 3051-3061.

Johnson C. R., Sutton D. C., Olson R. R. and Giddins R. (1991). Settlement of crown-of-thorns starfish: role of bacteria on surfaces of coralline algae and a hypothesis for deep-water recruitment. *Marine Ecology Progress Series*, 71: 143-162.

Johnson J. and Stevens I. (2000). A fine resolution model of the eastern North Atlantic between the Azores, the Canary Islands and the Gibraltar Strait. *Deep-Sea Research I*, 47: 875-899.

Johnson L. E. and Strathmann R. R. (1989). Settling barnacle larvae avoid substrata occupied by a mobile predator. *Journal of Experimental Marine Biology and Ecology*, 128: 87-103.

Johnson M. P., Burrows M. T., Hartnoll R. G. and Hawkins S. J. (1997). Spatial structure on moderately exposed rocky shores: patch scales and the interactions between limpets and algae. *Marine Ecology Progress Series*, 160: 209-215.

Johnson M. P., Burrows M. T. and Hawkins S. J. (1998). Individual based simulations of the direct and indirect effects of limpets on a rocky shore *Fucus* mosaic. *Marine Ecology Progress Series*, 169: 179-188.

Johnson M. S. and Black R. (1984). Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. *Evolution*, 38: 1371-1383.

Johnson M. S. and Black R. (1995). Neighbourhood size and the importance of barriers to gene flow in an intertidal snail. *Heredity*, 75: 142-154.

Johnson P. C. D. and Haydon D. T. (2007). Maximum-likelihood estimation of allelic dropout and false allele error rates from microsatellite genotypes in the absence of reference data. *Genetics*, 175: 827-842.

Jolly M. T., Jollivet D., Gentil F., Thiébaut E. and Viard F. (2005). Sharp genetic break between Atlantic and English Channel populations of the polychaete *Pectinaria koreni*, along the North coast of France. *Heredity*, 94: 23-32.

Jolly M. T., Viard F., Gentil F., Thiébaut E. and Jollivet D. (2006). Comparative phylogeography of two coastal polychaete tubeworms in the Northeast Atlantic supports shared history and vicariant events. *Molecular Ecology*, 15: 1841-1855.

Jolly M. T., Viard F., Weinmayr G., Gentil F., Thiébaut E. and Jollivet D. (2003). Does the genetic structure of *Pectinaria koreni* (Polychaeta: Pectinariidae) conform to a source-sink metapopulation model at the scale of the Baie de Seine? *Helgoland Marine Research*, 56: 238-246.

Jones G. P., Milicich M. J., Emslie M. J. and Lunow C. (1999). Self-recruitment in a coral reef fish population. *Nature*, 402: 802-804.

Jones G. P., Planes S. and Thorrold S. R. (2005). Coral reef fish settle close to home.

Current Biology, 15: 1314-1318.

Jones G. P., Srinivasan M. and Almany G. R. (2007a). Population connectivity and conservation of marine biodiversity. *Oceanography*, 20: 100-111.

Jones N. S. (1946). Browsing of *Patella*. *Nature*, 158: 557.

Jones W. J., Preston C. M., Marin III R., Scholin C. A. and Vrijenhoek R. C. (2007b). Molecular methods for *in situ* detection of marine invertebrate larvae. *Molecular Ecology Resources*, 8: 540-550.

Jonsson P. R., Granhag L., Moschella P. S., Aberg P., Hawkins S. J. and Thompson R. C. (2006). Interactions between wave action and grazing control the distribution of intertidal macroalgae. *Ecology*, 87: 1169-1178.

Kalinowski S. T. (2005). Do polymorphic loci require large sample sizes to estimate genetic distances? *Heredity*, 94: 33-36.

Kalinowski S. T. and Taper M. L. (2006). Maximum likelihood estimation of the frequency of null alleles at microsatellite loci. *Conservation Genetics*, 7: 991-995.

Kaplan D. M. (2006). Alongshore advection and marine reserves: consequences for modeling and management. *Marine Ecology Progress Series*, 309: 11-24.

Kaplan D. M. and Botsford L. W. (2005). Effects of variability in spacing of coastal marine reserves on fisheries yield and sustainability. *Canadian Journal of Fisheries and Aquatic Sciences*, 62: 905-912.

Kashi Y. and Soller M. (1999). Functional roles of microsatellites and minisatellites. In: *Microsatellites: evolution and applications.* (eds. D. B. Goldstein and C. Schlötterer). Oxford University Press, Oxford, pp. 10-23.

Kass R. E. and Raftery A. E. (1995). Bayes factors. *Journal of the American Statistical Association*, 90: 773-795.

Katti M. V., Ranjekar P. K. and Gupta V. S. (2001). Differential distribution of simple sequence repeats in eukaryotic genome sequences. *Molecular Biology and Evolution*, 18: 1161-1167.

Kay M. C. (2002). Recruitment in the intertidal limpet *Lottia digitalis* (Patellogastropoda: Lottiidae) may be driven by settlement cues associated with adult habitat. *Marine Biology*, 141: 467-477.

Kay M. C. and Emlet R. B. (2002). Laboratory spawning, larval development, and metamorphosis of the limpets *Lottia digitalis* and *Lottia asmi* (Patellogastropoda, Lottiidae). *Invertebrate Biology*, 121: 11-24.

Kelly R. P. and Eernisse D. J. (2007). Southern hospitality: a latitudinal gradient in gene

flow in the marine environment. *Evolution*, 61: 700-707.

Kempf S. C. (1981). Long-lived larvae of the gastropod *Aplysia juliana*: do they disperse and metamorphose or just slowly fade away? *Marine Ecology Progress Series*, 6: 61-65.

Kenchington E. L., Patwary M. U., Zouros E. and Bird C. J. (2006). Genetic differentiation in relation to marine landscape in a broadcast-spawning bivalve mollusk (*Placopecten magellanicus*). *Molecular Ecology*, 15: 1781-1796.

Kendall M. A., Bowman R. S., Williamson P. and Lewis J. R. (1985). Annual variations in the recruitment of *Semibalanus balanoides* on the North Yorkshire coast. *Journal of the Marine Biological Association of the United Kingdom*, 65: 1009-1030.

Kessel M. M. (1964). Reproduction and larval development of *Acmaea testudinalis* (Müller). *Biological Bulletin*, 127: 294-303.

Kettle A. J. and Haines K. (2006). Hoe does the European eel (*Anguilla anguilla*) retain its population structure during its larval migration across the North Atlantic Ocean? *Canadian Journal of Fisheries and Aquatic Sciences*, 63: 90-106.

Kimura M. and Crow J. F. (1964). The number of alleles that can be maintained in a finite population. *Genetics*, 49: 725-738.

Kimura M. and Ohta T. (1978). Stepwise mutation model and distribution of allele frequencies in a finite population. *Proceedings of the National Academy of Sciences of the United States of America*, 75: 2868-2872.

Kingsford M. J., Leis J. M., Shanks A., Lindeman K. C., Morgan S. J. and Pineda J. (2002). Sensory environments, larval abilities, and local self-recruitment. *Bulletin of Marine Science*, 70: 309-340.

Kinlan B. P. and Gaines S. D. (2003). Propagule dispersal in marine and terrestrial environments: a community perspective. *Ecology*, 84: 2007-2020.

Kinlan B. P., Gaines S. D. and Lester S. E. (2005). Propagule dispersal and the scales of marine community process. *Diversity and Distributions*, 11: 139-148.

Knight-Jones E. W. (1951). Gregariousness and some other aspects of settling behaviour of *Spirorbis*. *Journal of the Marine Biological Association of the United Kingdom*, 30: 201-222.

Knight-Jones E. W. (1953a). Decreased discrimination during settling after a prolonged planktonic life in larvae of *Spirorbis borealis* (Serpulidae). *Journal of the Marine Biological Association of the United Kingdom*, 32: 337-345.

Knight-Jones E. W. (1953b). Laboratory experiments on gregariousness during settling in *Balanus balanoides* and other barnacles. *Jounal of Experimental Biology*, 30: 584-

599.

Knights A. M., Crowe T. P. and Burnell G. (2006). Mechanisms of larval transport: vertical distribution of bivalve larvae varies with tidal conditions. *Marine Ecology Progress Series*, 326: 167-174.

Knowlton N. and Keller B. (1986). Larvae which fall short of their potential: highly localized recruitment in an alpheid shrimp with extended larval development. *Bulletin of Marine Science*, 39: 213-223.

Kohel M. A. R., Strother J. A., Reidenbach M. A., Koseff J. R. and Hadfield M. G. (2007). Individual-based model of larval transport to coral reefs in turbulent, wave-driven flow: behavioral responses to dissolved settlement inducer. *Marine Ecology Progress Series*, 335.

Kordos L. M. and Burton R. S. (1993). Genetic differentiation of Texas Gulf Coast populations of the blue crab *Callinectes sapidus*. *Marine Biology*, 117: 227-233.

Koufopanou V., Reid D. G., Ridgway S. A. and Thomas R. H. (1999). A molecular phylogeny of the patellid limpets (Gastropoda: Patellidae) and its implications for the origins of their antitropical distribution. *Molecular Phylogenetics and Evolution*, 11: 138-156.

Koutsikopoulos C., L. F. and Gagné J. A. (1991). Cross-shelf dispersion of Dover sole eggs and larvae (*Solea solea*) in Biscay Bay and recruitment to inshore nurseries. *Journal of Plankton Research*, 13: 923-945.

Koutsikopoulos C. and Le Cann B. (1996). Physical processes and hydrological structures related to the Bay of Biscay anchovy. *Scientia Marina*, 60 (Supl. 2): 9-19.

Kritzer J. P. and Sale P. F. (2004). Metapopulation ecology in the sea: from Levin's model to marine ecology and fisheries science. *Fish and Fisheries*, 5: 131-140.

Krug P. J. and Manzi A. E. (1999). Waterborne and surface-associated carbohydrates as settlement cues for larvae of the specialist marine herbivore *Alderia modesta*. *Biological Bulletin*, 197: 94-103.

Kyle C. J. and Boulding E. G. (2000). Comparative population genetic structure of marine gastropods (*Littorina* spp.) with and without pelagic larval dispersal. *Marine Biology*, 137: 835-845.

Lagercrantz U., Ellegren H. and Andersson L. (1993). The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. *Nucleic Acids Research*, 21: 1111-1115.

Lambert W. J., Todd C. D. and Hardege J. D. (1997). Partial characterization and biological activity of a metamorphic inducer of the dorid nudibranch *Adalaria*

proxima (Gastropoda: Nudibranchia). *Invertebrate Biology*, 116: 71-81.

Largier J. L. (2003). Considerations in estimating larval dispersal distances from oceanographic data. *Ecological Applications*, 13: S71-S89.

Latch E. K., Dharmarajan G., Glaubitz J. C. and Rhodes Jr. O. E. (2006). Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics*, 7: 295-302.

Launey S., Ledu C., Boudry P., Bonhomme F. and Naciri-Graven Y. (2002). Geographic structure in the European flat oyster (*Ostrea edulis* L.) as revealed by microsatellite polymorphism. *Journal of Heredity*, 93: 331-338.

Le Cann B. (1990). Barotropic tidal dynamics of the Bay of Biscay shelf: observations, numerical modelling and physical interpretation. *Continental Shelf Research*, 10: 723-758.

Le Fèvre J. L. and Bourget E. (1992). Hydrodynamics and behaviour: transport processes in marine invertebrate larvae. *Trends in Ecology and Evolution*, 7: 288-289.

Le Goff-Vitry M. C., Pybus O. G. and Rogers A. D. (2004). Genetic structure of the deep-sea coral *Lophelia pertusa* in the Northeast Atlantic revealed by microsatellites and internal transcribed spacer sequences. *Molecular Ecology*, 13: 537-549.

Le Quesne W. J. F. (2005). *The response of a protandrous species to exploitation, and the implications for management: a case study with patellid limpets.* PhD thesis, University of Southampton.

Le Quesne W. J. F. and Hawkins S. J. (2006). Direct observations of protandrous sex-change in the patellid limpet *Patella vulgata*. *Journal of the Marine Biological Association of the United Kingdom*, 86: 161-162.

Leberg P. L. (2002). Estimating allelic richness: effects of sample size and bottlenecks. *Molecular Ecology*, 11: 2445-2449.

Lebour M. V. (1931). The larval stages of *Nassarius reticulates* and *Nassarius incrassatus*. *Journal of the Marine Biological Association of the United Kingdom*, 17: 797-818.

Lefebvre A., Ellien C., Davoult D., Thiébaut E. and Salomon J. C. (2003). Pelagic dispersal of the brittle-star *Ophiothrix fragilis* larvae in a megatidal area (English Channel, France) examined using an advection/diffusion model. *Estuarine, Coastal and Shelf Science*, 57: 421-433.

Lemos R. T. and Pires H. O. (2004). The upwelling regime off the west Portuguese coast, 1941-2000. *International Journal of Climatology*, 24: 511-524.

Leonard G. M. (2000). Latitudinal variation in species interactions: a test in the New

England rocky intertidal zone. *Ecology*, 81: 1015-1030.

Levin L. A. (1990). A review of methods for labeling and tracking marine invertebrate larvae. *Ophelia*, 32: 115-144.

Levin L. A. (2006). Recent progress in understanding larval dispersal: new directions and digressions. *Integrative and Comparative Biology*, 46: 282-297.

Levin L. A. and Bridges T. S. (1995). Pattern and diversity in reproduction and development. In: *Ecology of marine invertebrate larvae.* (ed. L. McEdward). CRC Press, Boca Raton, Florida.

Levin L. A., Huggett D., Myers P., Bridges T. and Weaver J. (1993). Rare-earth tagging methods for the study of larval dispersal by marine invertebrates. *Limnology and Oceanography*, 38: 346-360.

Levinson G. and Gutman G. A. (1987). Slipped-strand mispairing: a major mechanism for DNA sequence evolution. *Molecular Biology and Evolution*, 4: 203-221.

Levitan D. R. (2000). Optimal egg size in marine invertebrates: theory and phylogenetic analysis of the critical relationship between egg size and development time in echinoids. *American Naturalist*, 156: 175-192.

Lewin R. (1986). Supply-side ecology. *Science*, 234: 25-27.

Lewis J. R. (1964) *The ecology of rocky shores.* English University Press Ltd, London.

Lewis J. R. (1976). Long term ecological surveillance: practical realities in the rocky littoral. *Oceanography and Marine Biology: An Annual Review*, 14: 371-390.

Lewis J. R. (1986). Latitudinal trends in reproduction, recruitment and population characteristics of some rocky littoral molluscs and cirripedes. *Hydrobiologia*, 142: 1-13.

Lewis J. R. and Bowman R. S. (1975). Local habitat-induced variations in the population dynamics of *Patella vulgata* L. *Journal of Experimental Marine Biology and Ecology*, 17: 165-203.

Li G. and Hedgecock D. (1998). Genetic heterogeneity, detected by PCR-SSCP, among samples of larval Pacific oysters (*Crassostrea gigas*) supports the hypothesis of large variance in reproductive success. *Canadian Journal of Fisheries and Aquatic Sciences*, 55: 1025-1033.

Li G., Hubert S., Bucklin K., Ribes V. and Hedgecock D. (2003). Characterization of 79 microsatellite DNA markers in the Pacific oyster *Crassostrea gigas*. *Molecular Ecology Notes*, 3: 228-232.

Li Y. C., Korol A. B., Fahima T., Beiles A. and Nevo E. (2002). Microsatellites: genomic

distribution, putative functions and mutational mechanisms. *Molecular Ecology*, 11: 2453-2465.

Lima F. P. (2007). *Biogeography of benthic invertebrate assemblages on the Portuguese coast. Relation with climatic and oceanographic patterns.* PhD thesis, University of Porto.

Lima F. P., Queiroz N., Ribeiro P. A., Hawkins S. J. and Santos A. M. (2006). Recent changes in the distribution of a marine gastropod, *Patella rustica* Linnaeus, 1758, and their relationship to unusual climatic events. *Journal of Biogeography*, 33: 812-822.

Lima F. P., Ribeiro P. A., Queiroz N., Hawkins S. J. and Santos A. M. (2007a). Do distributional shifts of northern and southern species of algae match the warming pattern? *Global Change Biology*, 13: 2592-2604.

Lima F. P., Ribeiro P. A., Queiroz N., Xavier R., Tarroso P., Hawkins S. J. and Santos A. M. (2007b). Modelling past and present geographical distribution of the marine gastropod *Patella rustica* as a tool for exploring responses to environmental change. *Global Change Biology*, 13: 2065-2077.

Lima G. M. and Pechenik J. A. (1985). The influence of temperature on growth rate and length of larval life of the gastropod, *Crepidula plana* Say. *Journal of Experimental Marine Biology and Ecology*, 90: 55-71.

Lind C. E., Evans B. S., Taylor J. J. U. and Jerry D. R. (2007). Population genetics of a marine bivalve, *Pinctada maxima*, throughout the Indo-Australian archipelago shows differentiation and decreased diversity at range limits. *Molecular Ecology*, 16: 5193-5203.

Lindberg D. R. and Wright W. G. (1985). Patterns of sex change of the protandric patellacean limpet *Lottia gigantea*. *Veliger*, 27: 261-265.

Litt M. and Luty J. M. (1989). A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics*, 44: 397-401.

Lodge S. M. (1948). Algal growth in the absence of *Patella* on an experimental strip of foreshore, Port St. Mary, Isle of Man. *Proceedings and Transactions of the Liverpool Biological Society*, 56: 78-85.

Lotze H. K., Lenihan H. S., Bourque B. J., Bradbury R. H., Cooke R. G., Kay M. C., Kidwell S. M., Kirby M. X., Peterson C. H. and Jackson J. B. C. (2006). Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science*, 312: 1806-1809.

Lubchenco J. and Gaines S. D. (1981). A unified approach to marine plant-herbivore interactions. I. Populations and communities. *Annual Review of Ecology and Systematics*, 12: 405-437.

Lubchenco J., Palumbi S. R., Gaines S. D. and Andelman S. (2003). Plugging a hole in the ocean: the emerging science of marine reserves. *Ecological Applications*, 13: S3-S7.

Luikart G. and England P. R. (1999). Statistical analysis of microsatellite DNA data. *Trends in Ecology and Evolution*, 14: 253-256.

Lutchembach M. W. and Orth R. J. (1992). Swimming velocities and behaviour of blue crab (*Callinectes sapidus* Rathbun) megalopae in still and flowing waters. *Estuaries*, 15: 186-192.

Luttikhuizen P. C., Drent J. and Baker A. J. (2003). Disjunct distribution of highly diverged mitochondrial lineage clade and population subdivision in a marine bivalve with pelagic larval dispersal. *Molecular Ecology*, 12: 2215-2229.

Maldonado M. and Young C. M. (1999). Effects of the duration of larval life on postlarval stages of the demosponge *Sigmadocia caerule*. *Journal of Experimental Marine Biology and Ecology*, 232: 9-21.

Manel S., Gaggiotti O. E. and Waples R. S. (2005). Assignment methods: matching biological questions with appropriate techniques. *Trends in Ecology and Evolution*, 20: 136-142.

Manel S., Schwartz M. K., Luikart G. and Taberlet P. (2003). Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology and Evolution*, 18: 189-197.

Mantel N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research*, 27: 209-220.

Manuel J. L., Gallager S. M., Pearce C. M., Manning D. A. and O'Dor R. K. (1996). Veligers from different populations of sea scallop *Placopecten magellanicus* have different vertical migration patterns. *Marine Ecology Progress Series*, 142: 147-163.

Mariani S., Hutchinson W. F., Hatfield E. M. C., Ruzzante D. E., Simmonds E. J., Dahlgren T. G., Andre C., Brigham J., Torstensen E. and Carvalho G. R. (2005). North Sea herring population structure revealed by microsatellite analysis. *Marine Ecology Progress Series*, 303: 245-257.

Marshall D. J. and Keough M. J. (2003). Variation in the dispersal potential of non-feeding invertebrate larvae: the desperate larva hypothesis and larval size. *Marine Ecology Progress Series*, 255: 145-153.

Marta-Almeida M., Dubert J., Peliz A. and Queiroga H. (2006). Influence of vertical migration pattern on retention of crab larvae in a seasonal upwelling system. *Marine Ecology Progress Series*, 307: 1-19.

Martin P. R. and McKay J. K. (2004). Latitudinal variation in genetic divergence of populations and the potential for future speciation. *Evolution*, 58: 938-945.

Martins C. S., Hamann M. and Fiúza A. F. G. (2002). Surface circulation in the eastern North Atlantic, from drifters and altimetry. *Journal of Geophysical Research*, 107: 3217.

Martins H. R., Santos R. S. and Hawkins S. J. (1987). Exploitation of limpets (*Patella* spp.) in the Azores with a preliminary analysis of the stocks. *ICES C.M.*, 1987/K53: 18.

Mason E., Coombs S. and Oliveira P. B. (2006). *An overview of the literature concerning the oceanography of the eastern North Atlantic region*. Relatórios Científicos e Técnicos IPIMAR Série Digital nº 33, IPIMAR, Lisboa.

Mauro A., Arculeo M. and Parrinello N. (2003). Morphological and molecular tools in identifying the Mediterranean limpets *Patella caerulea*, *Patella aspera* and *Patella rustica*. *Journal of Experimental Marine Biology and Ecology*, 295: 131-143.

McCarthy M., Woosnam P. and Culloty S. C. (2008). Histological investigation of the reproductive cycles of the limpets *Patella vulgata* and *Patella ulyssiponensis*. *Marine Biology*, 153: 871-877.

McCook L. J. and Chapman A. R. O. (1991). Community succession following massive ice scour on an exposed rocky shore: effects of *Fucus* canopy algae and of mussels during late succession. *Journal of Experimental Marine Biology and Ecology*, 154: 137-169.

McEdward L. R. (1985). Effects of temperature on the body form, growth, electron transport system activity, and development rate of an echinopluteus. *Journal of Experimental Marine Biology and Ecology*, 93: 169-181.

McEdward L. R. (1986). Comparative morphometrics of echinoderm larvae II. Larval size, shape, growth, and the scaling of feeding and metabolism in echinoplutei. *Journal of Experimental Marine Biology and Ecology*, 96: 267-286.

McQuaid C. D. and Phillips T. E. (2000). Limited wind-driven dispersal of the intertidal mussel larvae: *in situ* evidence from the plankton and the spread of the invasive species *Mytilus galloprovincialis* in South Africa. *Marine Ecology Progress Series*, 201: 211-220.

Meirmans P. G. (2006). Using the AMOVA framework to estimate a standardized

genetic differentiation measure. *Evolution*, 60: 2399-2402.

Ménesguen A. and Gohin F. (2006). Observation and modelling of natural retention structures in the English Channel. *Journal of Marine Systems*, 63: 244-256.

Menge B. A. (1975). Brood or broadcast? The adaptive significance of different reproductive strategies in the two intertidal sea-stars *Leptasterias hexactis* and *Pisaster ochraceus*. *Marine Biology*, 31: 87-100.

Menge B. A. (1991). Relative importance of recruitment and other causes of variation in rocky intertidal community structure. *Journal of Experimental Marine Biology and Ecology*, 146: 69-100.

Menge B. A., Farrell T. M., Olson A. M., van Tamelen P. and Turner T. (1993). Algal recruitment and the maintenance of a plant mosaic in the low intertidal region on the Oregon coast. *Journal of Experimental Marine Biology and Ecology*, 170: 91-116.

Menge B. A. and Sutherland J. P. (1987). Community regulation, variation in disturbance, competition and predation in relation to environmental stress and recruitment. *American Naturalist*, 130: 730-757.

Metaxas A. (2001). Behaviour in flow: perspectives on the distribution and dispersion of meroplanktonic larvae in the water column. *Canadian Journal of Fisheries and Aquatic Sciences*, 58: 86-98.

Michalakis Y. and Excoffier L. (1996). A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics*, 142: 1061-1064.

Mieszkowska N., Kendall M. A., Hawkins S. J., Leaper R., Williamson P., Hardman-Mountford N. J. and Southward A. J. (2006). Changes in the range of some common rocky shore species in Britain - a response to climate change? *Hydrobiologia*, 555: 241-251.

Mieszkowska N., Leaper R., Moore P., Kendall M. A., Burrows M. T., Lear D., Poloczanska E., Hiscock K., Moschella P. S., Thompson R. C., Herbert R. J., Laffoley D., Baxter J., Southward A. J. and Hawkins S. J. (2005) *Marine biodiversity and climate change: assessing and predicting the influence of climate change using intertidal rocky shore biota*. MarClim Final Report. Marine Biological Association of the United Kingdom, Plymouth.

Mileikovsky S. A. (1971). Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. *Marine Biology*, 10: 193-213.

Mileikovsky S. A. (1974). Types of larval development in marine bottom invertebrates:

An integrated ecological scheme. *Thalassia Jugoslavica*, 10: 171-179.

Miquel C., Bellemain E., Poillot C., Bessière J., Durand A. and Taberlet P. (2006). Quality indexes to assess the reliability of genotypes in studies using noninvasive sampling and multiple-tube approach. *Molecular Ecology Notes*, 6: 985-988.

Moberg P. E. and Burton R. S. (2000). Genetic heterogeneity among adult and recruit red sea urchins, *Strongylocentrotus franciscanus*. *Marine Biology*, 136: 773-784.

Moore P., Thompson R. C. and Hawkins S. J. (2007). Effects of grazer identity on the probability of escapes by a canopy-forming macroalga. *Journal of Experimental Marine Biology and Ecology*, 344: 170-180.

Mora M. and Sale P. F. (2002). Are populations of coral reef fish open or closed? *Trends in Ecology and Evolution*, 17: 422-428.

Moran A. L. (2000). Calcein as a marker in experimental studies of newly-hatched gastropods. *Marine Biology*, 137: 893-898.

Moran A. L. and Marko P. B. (2005). A simple technique for physical marking of larvae of marine bivalves. *Journal of Shellfish Research*, 24: 567-571.

Morgan S. G. (1995). Life and death in the plankton: larval mortality and adaptation. In: *Ecology of marine invertebrate larvae.* (ed. L. McEdward). CRC Press, Boca Raton, Florida, pp. 279-321.

Morgan T. S. and Rogers A. D. (2001). Specificity and sensitivity of microsatellite markers for the identification of larvae. *Marine Biology*, 139: 967-973.

Morse A. N. C., Froyd C. and Morse D. E. (1984). Molecules from cyanobacteria and red algae that induce larvae settlement and metamorphosis in the mollusc *Haliotis rufescens*. *Marine Biology*, 81: 293-298.

Morse D. E., Hooker N., Duncan H. and Jensen L. (1979). γ-aminobutyric acid, a neurotransmitter, induces planktonic abalone larvae to settle and begin metamorphosis. . *Science*, 204: 407-410.

Mullineaux L. S. and Butman C. A. (1991). Initial contact, exploration and attachment of barnacle (*Balanus amphitrite*) cyprids settling in flow. *Marine Biology*, 110: 93-103.

Mullineaux L. S. and Garland E. D. (1993). Larval recruitment in response to manipulated field flows. *Marine Biology*, 116: 667-683.

Myers R. A. and Worm B. (2003). Rapid worldwide depletion of predatory fish communities. *Nature*, 423: 280-283.

Nathan R. (2001). The challenges of studying dispersal. *Trends in Ecology and Evolution*, 16: 481-483.

Nauta M. J. and Weissing F. J. (1996). Constraints on allele size at microsatellite loci: implications for genetic differentiation. *Genetics*, 143: 1021-1032.

Nei M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America*, 70: 3321-3323.

Nei M. (1987) *Molecular Evolutionary Genetics.* Columbia University Press, New York.

Neigel J. E. (1997). A comparison of alternative strategies for estimating gene flow from genetic markers. *Annual Reviews in Ecology and Systematics*, 28: 105-128.

Neigel J. E. (2002). Is F_{ST} obsolete? *Conservation Genetics*, 3: 167-173.

Nelson T. C. (1925). On the occurrence and food habits of ctenophores in New Jersey inland coastal waters. *Biological Bulletin*, 48: 92-111.

Neubert M. G. and Caswell H. (2000). Demography and dispersal: calculation and sensitivity analysis of invasion speed for structured populations. *Ecology*, 81: 1613–1628.

Newton M. A. and Raftery A. E. (1994). Approximate Bayesian inference with the weighted likelihood bootstrap. *Journal of the Royal Statistical Society Series B*, 56: 3-58.

Nielsen C. (1998). Origin and evolution of animal life cycles. *Biological Reviews*, 73: 125-155.

Nielsen E. E., Hansen M. M. and Meldrup D. (2006). Evidence of microsatellite hitch-hiking selection in Atlantic cod (*Gadus morhua* L.): implications for inferring population structure in nonmodel organisms. *Molecular Ecology*, 15: 3219-3229.

Nielsen R. and Wakeley J. (2001). Distinguishing migration from isolation: a Markov chain Monte Carlo approach. *Genetics*, 158: 885-896.

Nykjaer L. and van Camp L. (1994). Seasonal and interannual variability of coastal upwelling along Northwest Africa and Portugal from 1981 to 1991. *Journal of Geophysical Research*, 99: 14197-14207.

Nylander J. A., Ronquist F., Huelsenbeck J. P. and Nieves-Aldrey J. L. (2004). Bayesian phylogenetic analysis of combined data. *Systematic Biology*, 53: 47-67.

O' Connor M. I., Bruno J. F., Gaines S. D., Halpern B. S., Lester S. E., Kinlan B. P. and Weiss J. M. (2007). Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. *Proceedings of the National Academy of Sciences of the United States of America*, 104: 1266-1271.

O'Reilly P. T., Canino M. F., Bailey K. M. and Bentzen P. (2004). Inverse relationship between F_{ST} and microsatellite polymorphism in the marine fish, walleye Pollock

(*Theragra chalcogramma*): implications for resolving weak population structure. *Molecular Ecology*, 13: 1799-1814.

Okubo A. (1971). Oceanic diffusion diagrams. *Deep-Sea Research*, 18: 789-802.

Olive P. J. W. (1995). Annual breeding cycles in marine invertebrates and environmental temperature: probing the proximate and ultimate causes of reproductive synchrony. *Journal of Thermal Biology*, 20: 79-90.

Olsen J. L., Stam W. T., Coyer J. A., Reusch T. B. H., Billingham M., Boström C., Calvert E., Christie H., Granger S., La Lumiére R., Milchakova N., Oudot-Le Secq M.-P., Procaccini G., Sanjabi B., Serrão E., Veldsink J., Widdicombe S. and Wyllie-Echeverria S. (2004). North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina* L. *Molecular Ecology*, 13: 1923-1941.

Olson R. R. (1985). The consequences of short-distance larval dispersal in a sessile marine invertebrate. *Ecology*, 66: 30-39.

Olson R. R. and Olson M. H. (1989). Food limitation of planktotrophic marine invertebrate larvae: does it control recruitment success? *Annual Reviews in Ecology and Systematics*, 20: 225-247.

Ortea J. (1986). The malacology of La Riera Cave. In: *La Riera Cave, Stone Age hunter-gatherer adaptations in Northern Spain. Anthropological Research Papers No. 36.* (eds. L. G. Straus and G. A. Clark). Arizona State University Press, Tempe, pp. 289-298.

Orton J. H. (1919). Sex-phenomena in the common limpet (*Patella vulgata*). *Nature*, 104: 373-374.

Orton J. H. (1920). Sea temperature, breeding and distribution in marine animals. *Journal of the Marine Biological Association of the United Kingdom*, 12: 339-366.

Orton J. H. (1928). Observations on *Patella vulgata*. Part I. Sex-phenomena, breeding and shell growth. *Journal of the Marine Biological Association of the United Kingdom*, 15: 851-862.

Orton J. H. and Southward A. J. (1961). Studies on the biology of limpets IV. The breeding of *Patella depressa* Pennant on the north Cornish coast. *Journal of the Marine Biological Association of the United Kingdom*, 41: 653-662.

Orton J. H., Southward A. J. and Dodd J. M. (1956). Studies on the biology of limpets II. The breeding of *Patella vulgata* L. in Britain. *Journal of the Marine Biological Association of the United Kingdom*, 35: 149-176.

Othaitz J. P. (1994). *Estudio de los ciclos reproductores de cuatro species de lapas y dos de tróquidos (Gastropoda, Prosobranchia) del piso intermareal de la costa Vasca.* PhD thesis, Universidad Autónoma de Madrid.

Paine R. T. (1971). A short term experimental investigation of resource partitioning in a New Zealand rocky intertidal habitat. *Ecology*, 52: 1096-1106.

Paine R. T. (1974). Intertidal community structure: experimental studies on the relationship between a dominant competitor and its principal predator. *Oecologia*, 15: 93-120.

Paine R. T. and Trimble A. C. (2004). Abrupt community change on a rocky shore - biological mechanisms contributing to the potential formation of an alternative state. *Ecology Letters*, 7: 441-445.

Palmer A. R. (1994). Temperature sensitivity, rate of development, and time to maturity: geographic variation in laboratory reared *Nucella* and cross-phyletic overview. In: *Reproduction and Development of Marine Invertebrates.* (eds. W. H. Wilson Jr., S. A. Stricker and G. L. Shinn). John Hopkins University Press, Baltimore, pp. 177-194.

Palumbi S. R. (1992). Marine speciation on a small planet. *Trends in Ecology and Evolution*, 7: 114-118.

Palumbi S. R. (1994). Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*, 25: 547-572.

Palumbi S. R. (1995). Using genetics as an indirect estimator of larval dispersal. In: *Ecology of marine invertebrate larvae.* (ed. L. McEdward). CRC Press, Boca Raton, Florida, pp. 369-386.

Palumbi S. R. (2003). Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications*, 13: S146-S158.

Palumbi S. R. (2004). Marine reserves and ocean neighborhoods: the spatial scale of marine populations and their management. *Annual Review of Environment and Resources*, 29: 31-68.

Palumbi S. R., Grabowski G., Duda T., Geyer L. and Tachino N. (1997). Speciation and population genetic structure in tropical Pacific sea urchins. *Evolution*, 51: 1506-1517.

Pannacciulli F. G., Bishop J. D. D. and Hawkins S. J. (1997). Genetic structure of populations of two species of *Chthamalus* (Crustacea: Cirripedia) in the North-east Atlantic and Mediterranean. *Marine Biology*, 128: 73-82.

Paris C. and Cowen R. K. (2004). Direct evidence of a biophysical retention mechanism for coral reef fish larvae. *Limnology and Oceanography*, 49: 1964-1979.

Parmesan C., Gaines S., Gonzalez L., Kaufman D. M., Kingsolver J., Peterson A. T. and Sagarin R. (2005). Empirical perspectives on species borders: from traditional biogeography to global change. *Oikos*, 108: 58-75.

Pascual M., Aquadro C. F., Soto V. and Serra L. (2001). Microsatellite variation in

colonizing and palearctic populations of *Drosophila subobscura. Molecular Biology and Evolution*, 18: 731-740.

Pascual M., Schug M. D. and Aquadro C. F. (2000). High density of long dinucleotide microsatellites in *Drosophila subobscura*. *Molecular Biology and Evolution*, 17: 1259-1267.

Passamonti M. and Scali V. (2001). Gender-associated mitochondrial DNA heteroplasmy in the venerid clam *Tapes philippinarum* (Mollusca Bivalvia). *Current Genetics*, 39: 117-124.

Patten W. (1886). The embriology of *Patella*. *Arbeiten aus dem Zoologischen Institute der Universitat Wien*, 6: 149-174.

Pauly D., Christensen V., Guenette S., Pitcher T. J., Sumaila U. R., Walters C. J., Watson R. and Zeller D. (2002). Towards sustainability in world fisheries. *Nature*, 418: 689-695.

Pawlik J., Butman C. A. and Starczak V. R. (1991). Hydrodynamic facilitation of gregarious settlement of a reef-building tube worm. *Science*, 251: 421-424.

Pawlik J. R. (1990). Natural and artificial induction of metamorphosis of *Phragmatopoma lapidosa californica* (Polychaeta: Sabellaridae) with a critical look at the effects of bioactive compounds on marine invertebrate larvae. *Bulletin of Marine Science*, 46: 512-536.

Pawlik J. R. (1992). Chemical ecology of the settlement of benthic marine invertebrates. *Oceanography and Marine Biology: An Annual Review*, 30: 273-335.

Pawlik J. R. and Butman C. A. (1993). Settlement of a marine tube worm as a function of current velocity: interacting effects of hydrodynamics and behavior. *Limnology and Oceanography*, 38: 1730-1740.

Pawlik J. R. and Mense D. J. (1994). Larval transport, food limitation, ontogenetic plasticity and the recruitment of Sabellariid polychaetes. In: *Reproduction and Development of Marine Invertebrates.* (eds. W. H. Wilson Jr., S. A. Stricker and G. L. Shinn). John Hopkins University Press, Baltimore, pp. 275-286.

Pearce C. M. and Scheibling R. E. (1990). Induction of settlement and metamorphosis in the sand dollar *Echinaracnius parma*: evidence for an adult-associated factor. *Marine Biology*, 107: 363-369.

Pearse D. E. and Crandall K. A. (2004). Beyond F_{ST}: analysis of population genetic data for conservation. *Conservation Genetics*, 5: 585-602.

Pearse J. S. (1994). Cold-water echinoderms break 'Thorson's rule'. In: *Reproduction, larval biology and recruitment of deep-sea benthos.* (eds. C. M. Young and K. J.

Eckelbarger). Columbia University Press, New York, pp. 26-43.

Pearse J. S. and Lockhart S. J. (2004). Reproduction in cold water: paradigm changes in the 20th century and a role for cidarid sea urchins. *Deep-Sea Research II*, 51: 1533-1549.

Pearse J. S., McClintock J. B. and Bosch I. (1991). Reproduction of Antarctic marine benthic invertebrates: tempos, modes and timing. *American Zoologist*, 31: 65-80.

Pechenik J. A. (1984). The relationship between temperature, growth rate, and duration of planktonic life for larvae of the gastropod *Crepidula fornicata* (L.). *Journal of Experimental Marine Biology and Ecology*, 74: 241-257.

Pechenik J. A. (1990). Delayed metamorphosis by larvae of benthic marine invertebrates: Does it occur? Is there a price to pay? *Ophelia*, 32: 63-94.

Pechenik J. A. (1999). On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. *Marine Ecology Progress Series*, 177: 269-297.

Pechenik J. A., Blanchard M. and Rotjan R. (2004). Susceptibility of larval *Crepidula fornicata* to predation by suspension-feeding adults. *Journal of Experimental Marine Biology and Ecology*, 306: 75-94.

Pechenik J. A. and Eyster L. S. (1989). Influence of delayed metamorphosis on the growth and metabolism of young *Crepidula fornicata* (Gastropoda) juveniles. *Biological Bulletin*, 176: 14-24.

Pechenik J. A., Jarrett J. N. and Rooney J. (2002). Relationships between larval nutritional experience, larval growth rates, juvenile growth rates, and juvenile feeding rates in the prosobranch gastropod *Crepidula fornicata*. *Journal of Experimental Marine Biology and Ecology*, 74: 241-257.

Pechenik J. A. and Rice M. E. (2001). Influence of delayed metamorphosis on postsettlement survival and growth in the sipunculan *Apionsoma misakianum*. *Invertebrate Biology*, 120: 50-57.

Peijnenburg K. T. C. A., Fauvelot C., Breeuwer A. J. and Menken B. J. (2006). Spatial and temporal genetic structure of the planktonic *Saggita setosa* (Chaetognatha) in European seas as revealed by mitochondrial and nuclear DNA markers. *Molecular Ecology*, 15: 3319-3338.

Peliz A., Dubert J., Santos A. M. P., Oliveira P. B. and Le Cann B. (2005). Winter upper ocean circulation in the Western Iberian Basin - fronts, eddies and poleward flows: an overview. *Deep-Sea Research Part I: Oceanographic Research Papers*, 52: 621-696.

Peliz A., Rosa T. L., Santos A. M. P. and Pissarra J. L. (2002). Fronts, jets, and counter-flows in the Western Iberian upwelling system. *Journal of Marine Systems*, 35: 61-77.

Pemberton J. M., Slate J., Bancroft D. R. and Barrett J. A. (1995). Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology*, 4: 249-252.

Pérez M., Branco M., Llavona A., Ribeiro P. A., Santos A. M., Hawkins S. J., Dávila J. A., Presa P. and Alexandrino P. (2007). Development of microsatellite loci for the black-footed limpet *Patella depressa*, and cross-amplification in two other *Patella* species. *Conservation Genetics*, 8: 739-742.

Pérez M., Cruz F. and Presa P. (2005). Distribution properties of polymononucleotide repeats in molluscan genomes. *Journal of Heredity*, 96: 40-51.

Pérez M., Llavona A., Presa P., Ribeiro P. A., Hawkins S. J., Santos A. M. and Alexandrino P. (2008). New polymorphic microsatellite markers for the limpet *Patella rustica* and cross-priming testing in four *Patella* species. *Molecular Ecology Resources*, 8: 926-929.

Pernet F., Tremblay R. and Bourget E. (2003). Settlement success, spatial pattern and behaviour of mussel larvae Mytilus spp. in experimental 'downwelling' systems of varying velocity and turbulence. *Marine Ecology Progress Series*, 260: 125-140.

Perrin C., Wing S. R. and Roy M. S. (2004). Effects of hydrographic barriers on population genetic structure of the sea star *Coscinasterias muricata* (Echinodermata, Asteroidea) in the New Zealand fiords. *Molecular Ecology*, 13: 2183-2195.

Petit R. J., El Mousadik A. and Pons O. (1998). Identifying populations for conservation on the basis of genetic markers. *Conservation Biology*, 12: 844-855.

Pflaumann U., Sarnthein M., Chapman M., d'Abreu L., Funnell B., Huels M., Kiefer T., Maslin M., Schulz H., Swallow J., van Kreveld S., Vautravers M., Vogelsang E. and Weinelt M. (2003). Glacial North Atlantic: sea-surface conditions reconstructed by GLAMAP 2000. *Paleoceanography*, 18: 1065.

Picken G. B. (1979). Nonpelagic reproduction of some Antarctic prosobranch gastropods from Signy Island, South Orkney Islands. *Malacologia*, 19: 109-128.

Picken G. B. (1980). Reproductive adaptations of Antarctic benthic invertebrates. *Biological Journal of the Linnean Society*, 14: 67-75.

Pineda J. (1991). Predictable upwelling and the shoreward transport of planktonic larvae by internal tidal bores. *Science*, 253: 548-551.

Pineda J. (1999). Circulation and larval distribution in internal tidal bore warm fronts. *Limnology and Oceanography*, 44: 1400-1414.

Pineda J., Hare J. A. and Sponaugle S. (2007). Larval transport and dispersal in the coastal ocean and consequences for population connectivity. *Oceanography*, 20: 22-

39.

Pingree R. and Le Cann B. (1989). Celtic and Armorican slope and shelf residual currents. *Progress in Oceanography*, 23: 303-338.

Pingree R. D. and Le Cann B. (1993). A shallow meddy (a smeddy) from the secondary Mediterranean salinity maximum. *Journal of Geophysical Research*, 98: 20169-20185.

Planes S. and Doherty P. J. (1997). Genetic and color interactions at a contact zone of *Acanthochromis polyacanthus*: a marine fish lacking pelagic larvae. *Evolution*, 51: 1232-1243.

Planes S. and Lenfant P. (2002). Temporal change in the genetic structure between and within cohorts of a marine fish, *Diplodus sargus*, induced by a large variance in individual reproductive success. *Molecular Ecology*, 11: 1515-1524.

Planque B., Beillois P., Jégou A.-M., Lazure P., Petitgas P. and Puillat I. (2003). Large-scale hydroclimatic variability in the Bay of Biscay: the 1990s in the context of interdecadal changes. *ICES Marine Science Symposia*, 219: 61-70.

Poulin E. and Féral J.-P. (1996). Why are there so many species of brooding Antarctic echinoids? *Evolution*, 50: 820-830.

Pradillon F., Schmidt A., Peplies J. and Dubilier N. (2007). Species identification of marine invertebrate early stages by whole-larvae *in situ* hybridization of 18S ribosomal RNA. *Marine Ecology Progress Series*, 333: 103-116.

Prater M. D. and Sanford T. B. (1994). A meddy off Cape St. Vincent. Part I: description. *Journal of Physical Oceanography*, 24: 1572-1586.

Pringle J. M. and Wares J. P. (2007). Going against the flow: maintenance of alongshore variation in allele frequency in a coastal ocean. *Marine Ecology Progress Series*, 335: 69-84.

Pritchard J. K., Stephens M. and Donnelly P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155: 945-959.

Provan J., Wattier R. A. and Maggs C. A. (2005). Phylogeographic analysis of the red seaweed *Palmaria palmata* reveals a Pleistocene marine glacial refugium in the English Channel. *Molecular Ecology*, 14: 793-803.

Puillat I., Lazure P., Jégou A.-M., Lampert L. and Miller P. (2006). Mesoscale hydrological variability induced by northwesterly wind on the French continental shelf of the Bay of Biscay. *Scientia Marina*, 70S1: 15-26.

Purcell J. F. H., Cowen R. K., Hughes C. R. and Williams D. A. (2006). Weak genetic structure indicates strong dispersal limits: a tale of two coral reef fish. *Proceedings of the Royal Society of London Series B, Biological Sciences*, 273: 1483-1490.

Qian P.-Y. and Pechenik J. A. (1998). Effects of larval starvation and delayed metamorphosis on juvenile survival and growth of the tube-dwelling polychaete *Hydroides elegans* (Haswell). *Journal of Experimental Marine Biology and Ecology*, 227: 169-185.

Qian P., Rittschof D., Sreedhar B. and Chia F. (1999). Macrofouling in unidirectional flow: miniature pipes as experimental models for studying the effects of hydrodynamics on invertebrate larval settlement. *Marine Ecology Progress Series*, 191: 141-151.

Queiroga H. (1998). Vertical migration and selective tidal stream transport in the megalopa of the crab *Carcinus maenas*. *Hydrobiologia*, 375/376: 137-149.

Quinteiro J., Rodríguez-Castro J. and Rey-Méndez M. (2007). Population genetic structure of the stalked barnacle *Pollicipes pollicipes* (Gmelin, 1789) in the northeastern Atlantic: influence of coastal currents and mesoscale hydrographic structures. *Marine Biology*, 153: 47-60.

Raimondi P. T. (1988). Settlement cues and determination of the vertical limit of an intertidal barnacle. *Ecology*, 69: 400-407.

Rambaut A. and Drummond A. J. (2007). *TRACER v1.4.* http://beast.bio.ed.ac.uk/Tracer.

Ramos-Onsins S. E. and Rozas J. (2002). Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution*, 19: 2092-2100.

Rannala B. and Mountain J. L. (1997). Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences of the United States of America*, 94: 9197-9201.

Raymond M. and Rousset F. (1995). GENEPOP (version 1.2): population genetic software for exact tests and ecumenicism. *Journal of Heredity*, 86: 248-249.

Reitzel A. M., Miner B. G. and McEdward L. R. (2004). Relationships between spawning date and larval development time for benthic marine invertebrates: a modeling approach. *Marine Ecology Progress Series*, 280: 13-23.

Relvas P. and Barton E. D. (2002). Mesoscale patterns in the Cape São Vicente (Iberian Peninsula) upwelling region. *Journal of Geophysical Research*, 107: 3164.

Relvas P. and Barton E. D. (2005). A separated jet and coastal counterflow during upwelling relaxation off Cape São Vicente (Iberian Peninsula). *Continental Shelf Research*, 25: 29-49.

Relvas P., Barton E. D., Dubert J., Oliveira P. B., Peliz A., Silva J. C. B. and Santos A. M. P. (2007). Physical oceanography of the western Iberian ecosystem: latest views and

challenges. *Progress in Oceanography*, 74: 174-171.

Rice W. R. (1989). Analyzing tables of statistical tests. *Evolution*, 43: 223-225.

Ridgway S. A., Reid D. G., Taylor J. D., Branch G. M. and Hodgson A. N. (1998). A cladistic phylogeny of the family Patellidae (Mollusca:Gastropoda). *Philosophical Transactions of the Royal Society of London B*, 353: 1645-1671.

Riginos C. and Nachman M. W. (2001). Population subdivision in marine environments: the contribution of biogeography, geographical distance and discontinuous habitat to genetic differentiation in a blennioid fish, *Axoclinus nigricaudus*. *Molecular Ecology*, 10: 1439-1453.

Roberts C. M. (1997). Connectivity and management of Caribbean coral reefs. *Science*, 278: 1454-1457.

Roberts D., Rittschof D., Holm E. and Schmidt A. R. (1991). Factors influencing initial larval settlement: temporal, spatial and surface molecular components. *Journal of Experimental Marine Biology and Ecology*, 150: 203-222.

Roberts R. D. and Lapworth C. (2001). Effect of delayed metamorphosis on larval competence, and post-larval survival and growth, in the abalone *Haliotis iris* Gmelin. *Journal of Experimental Marine Biology and Ecology*, 258: 1-13.

Robles C. D. (1997). Changing recruitment in constant species assemblages: implications for predation theory in intertidal communities. *Ecology*, 78: 1400-1414.

Rodríguez F., Oliver J. F., Marín A. and Medina J. R. (1990). The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology*, 142: 485-501.

Rodríguez J. M., Barton E. D., Hernández-León S. and Arístegui J. (2004). The influence of mesoscale physical processes on the larval fish community in the Canaries CTZ in summer. *Progress in Oceanography*, 62: 171-188.

Rogers A. R. and Harpending H. (1992). Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, 9: 552-569.

Roman J. and Palumbi S. R. (2004). A global invader at home: population structure of the green crab, *Carcinus maenas*, in Europe. *Molecular Ecology*, 13: 2891-2898.

Romano S. L. and Palumbi S. R. (1997). Molecular evolution of a portion of the mitochondrial 16S ribosomal gene region in scleractinian corals. *Journal of Molecular Evolution*, 45: 397-411.

Ross C. L., Dyer K. A., Erez T., Miller S. J., Jaenike J. and Markow T. A. (2003). Rapid divergence of microsatellite abundance among species of *Drosophila*. *Molecular Biology and Evolution*, 20: 1143-1157.

Roughgarden J., Gaines S. and Possingham H. (1988). Recruitment dynamics in complex life cycles. *Science*, 241: 1460-1466.

Roughgarden J., Iwasa Y. and Baxter C. (1985). Demographic theory for an open marine population with space limited recruitment. *Ecology*, 66: 54-67.

Rousset F. (1997). Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics*, 145: 1219-1228.

Rousset F. (2008). GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, 8: 103-106.

Rozas J., Sánchez-DelBarrio J. C., Messeguer X. and Rozas R. (2003). DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, 19: 2496-2497.

Rumrill S. S. (1989). Substratum selectivity, post-larval growth, and survival following extended competence in *Strongylocentrotus droebachiensis*. *American Zoologist*, 29: 29A.

Rumrill S. S. (1990). Natural mortality of marine invertebrate larvae. *Ophelia*, 32: 163-198.

Ruzzante D. E. (1998). A comparison of several measures of genetic distance and population structure with microsatellite data: bias and sampling variance. *Canadian Journal of Fisheries and Aquatic Sciences*, 55: 1-14.

Ruzzante D. E., Mariani S., Bekkevold D., André C., Mosegaard H., Clausen L. A. W., Dahlgren T. G., Hutchinson W. F., Hatfield E. M. C., Torstensen E., Brigham J., Simmonds E. J., Laikre L., Larsson L. C., Stet R. J. M., Ryman N. and Carvalho G. R. (2006). Biocomplexity in a highly migratory pelagic marine fish, Atlantic herring. *Proceedings of the National Academy of Sciences of the United States of America*, 273: 1459-1464.

Rychlick W. and Rhoads R. E. (1989). A computer program for choosing optimal oligonucleotides for filter hybridization, sequencing and in vitro amplification of DNA. *Nucleic Acids Research*, 17: 8543-8551.

Ryman N., Palm S., André C., Carvalho G. R., Dahlgren T. G., Jorde P. E., Laikre L., Larsson L. C., Palmé A. and Ruzzante D. E. (2006). Power for detecting genetic divergence: differences between statistical methods and marker loci. *Molecular Ecology*, 15: 2031-2045.

Sá-Pinto A., Alexandrino P. and Branco M. (2007). High genetic differentiation with no evidence of hybridisation between four limpet species (*Patella* spp.) revealed by allozyme loci. *Scientia Marina*, 71: 801-810.

Sá-Pinto A., Branco M., Harris D. J. and Alexandrino P. (2005). Phylogeny and phylogeography of the genus *Patella* based on mitochondrial DNA sequence data. *Journal of Experimental Marine Biology and Ecology*, 325: 95-110.

Sá-Pinto A., Branco M., Sayanda D. and Alexandrino P. (2008). Patterns of colonization, evolution and gene flow in species of the genus *Patella* in the Macaronesian Islands. *Molecular Ecology*, 17: 519-532.

Sá-Pinto A. I. (2008). *Evolution and speciation in the marine realm: the genus Patella as a case study.* PhD thesis, University of Porto.

Saavedra C., Zapata C., Guerra A. and Alvarez G. (1993). Allozyme variation in European populations of the oyster *Ostrea edulis*. *Marine Biology*, 115: 85-95.

Sagarin R. D., Barry J. P., Gilman S. E. and Baxter C. H. (1999). Climate-related changes in intertidal community over short and long time scales. *Ecological Monographs*, 69: 465-490.

Sagarin R. D. and Gaines S. D. (2002). Geographical abundance distributions of coastal invertebrates: using one-dimensional ranges to test biogeographic hypotheses. *Journal of Biogeography*, 29: 985-997.

Saitou N. and Nei M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4: 406-425.

Sala E., Aburto-Oropeza O., Paredes G., Parra I., Barrera J. C. and Dayton P. K. (2002). A general model for designing networks of marine reserves. *Science*, 298: 1991-1993.

Sale P. F., Cowen R. K., Danilowicz B. S., Jones G. P., Kritzer J. P., Lindeman K. C., Planes S., Polunin N. V. C., Russ G. R. and Sadovy Y. J. (2005). Critical science gaps impede use of no-take fishery reserves. *Trends in Ecology and Evolution*, 20: 74-80.

Salomon J. C. and Breton M. (1991). Courants résiduels de marée dans la Manche. *Oceanologica Acta*, 11: 47-53.

Salomon J. C. and Breton M. (1993). An atlas of long-term currents in the Channel. *Oceanologica Acta*, 16: 439-448.

Sambrook J., Fritsh E. F. and Maniatis T. (1989) *Molecular cloning. A laboratory manual.* Cold Spring Harbor Laboratory Press, New York.

Sánchez R. F., Mason E., Relvas P., da Silva A. J. and Peliz Á. (2006). On the inner-shelf circulation in the northern Gulf of Cádiz, southern Portuguese shelf. *Deep-Sea Research II*, 53: 1198-1218.

Sánchez R. F. and Relvas P. (2003). Spring-summer climatological circulation in the upper layer in the region of Cape St. Vincent, Southwest Portugal. *ICES Journal of Marine Science*, 60: 1232-1250.

Santos A. J. P., Nogueira J. and Martins H. (2005). Survival of sardine larvae off the Atlantic Portuguese coast: a preliminary numerical study. *ICES Journal of Marine Science*, 62: 634-644.

Santos A. M. (2000). *Intertidal ecology of northern Portuguese rocky shores.* PhD thesis, University of Southampton, UK.

Santos A. M. P., Peliz A., Dubert J., Oliveira P. B., Angelico M. M. and Re P. (2004). Impact of a winter upwelling event on the distribution and transport of sardine (*Sardina pilchardus*) eggs and larvae off western Iberia: a retention mechanism. *Continental Shelf Research*, 24: 149-165.

Scheltema R. S. (1961). Metamorphosis of the veliger larvae of *Nassarius obsoletus* (gastropoda) in response to bottom sediment. *Biological Bulletin*, 120: 92-109.

Scheltema R. S. (1967). The relationship of temperature to the larval development of *Nassarius obsoletus* (Gastropoda). *Biological Bulletin*, 132: 253-265.

Scheltema R. S. (1971). The dispersal of larvae of shoal-water benthic invertebrate species over long distances by ocean currents. In: *Proceedings of the Fourth European Marine Biology Symposium* (ed. D. Crisp). Cambridge University Press Cambridge, UK, pp. 7-28.

Scheltema R. S. (1986). Long-distance dispersal by planktonic larvae of shoal-water benthic invertebrates among central Pacific islands. *Bulletin of Marine Science*, 39: 241-256.

Scheltema R. S. (1988). Initial evidence for the transport of teleplanic larvae of benthic invertebrates across the East Pacific Barrier. *Biological Bulletin*, 174: 145-152.

Scheltema R. S. (1989). Planktonic and non-planktonic development among prosobranch gastropods and its relationship to the geographical range of species. In: *Reproduction, genetics and distributions of marine organisms.* (eds. J. S. Ryland and P. A. Tyler). Olsen and Olsen, Fredensborg, pp. 183-188.

Scheltema R. S. and Williams I. P. (1982). Significance of temperature to larval survival and length of development in *Balanus eburneus* (Crustacea: Cirripedia). *Marine Ecology Progress Series*, 9: 43-49.

Schlötterer C. (2000). Evolutionary dynamics of microsatellite DNA. *Chromosoma*, 109: 365-371.

Schlötterer C. and Tautz D. (1992). Slippage synthesis of simple sequence DNA. *Nucleic Acids Research*, 20: 211-215.

Schneider S. and Excoffier L. (1999). Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites:

application to human mitochondrial DNA. *Genetics*, 152: 1079-1089.

Sebens K. P. (1983). Settlement and metamorphosis of a temperate soft-coral larva (*Alcyonium siderium* Verrill): induction by crustose algae. *Biological Bulletin*, 165: 286-304.

Selkoe K. A., Gaines S. D., Caselle J. E. and Warner R. R. (2006). Current shifts and kin aggregation explain genetic patchiness in fish recruits. *Ecology*, 87: 3082-3094.

Selkoe K. A. and Toonen R. J. (2006). Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters*, 9: 615-629.

Shanks A., Largier J., Brink L., Brubaker J. and Hooff R. (2000). Demonstration of the onshore transport of larval invertebrates by the shoreward movement of an upwelling front. *Limnology and Oceanography*, 45: 230-236.

Shanks A. L. (1986). Tidal periodicity in the daily settlement of intertidal barnacle larvae and an hypothesized mechanism for the cross-shelf transport of cyprids. *Biological Bulletin*, 170: 429-440.

Shanks A. L. and Brink L. (2005). Upwelling, downwelling, and cross-shelf transport of bivalve larvae: test of a hypothesis. *Marine Ecology Progress Series*, 302: 1-12.

Shanks A. L., Grantham B. and Carr M. H. (2003). Propagule dispersal distance and the size and spacing of marine reserves. *Ecological Applications*, 13: S159-S169.

Shanks A. L. and Wright W. G. (1986). Adding teeth to wave action: the destructive effects of wave-borne rocks on intertidal organisms. *Oecologia*, 69: 420-428.

Shearer T. L., Van Oppen M. J. H., Romano S. L. and Wörheide G. (2002). Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). *Molecular Ecology*, 11: 2475-2487.

Shkedy Y. and Roughgarden J. (1997). Barnacle recruitment and population dynamics predicted from coastal upwelling. *Oikos*, 80: 487-498.

Siegel D. A., Kinlan B. P., Gaylord B. and Gaines S. D. (2003). Lagrangian descriptions of marine larval dispersion. *Marine Ecology Progress Series*, 260: 83-96.

Simons R. D., Monismith S. G., Johnson L. E., Winkler G. and Saucier F. J. (2007). Zooplankton retention in the estuarine transition zone of the St. Lawrence Estuary. *Limnology and Oceanography*, 51: 2621-2631.

Skibinski D. O. F., Gallagher C. and Beynon C. M. (1994). Sex-limited mitochondrial DNA transmission in the marine mussel *Mytilus edulis*. *Genetics*, 138: 801-809.

Slatkin M. (1973). Gene flow and selection in a cline. *Genetics*, 75: 733-756.

Slatkin M. (1987). Gene flow and the geographic structure of natural populations.

Science, 236: 787-792.

Slatkin M. (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, 47: 264-279.

Slatkin M. (1995). A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, 139: 457-462.

Slatkin M. (2005). Seeing ghosts: the effect of unsampled populations on migration rates estimated for sampled populations. *Molecular Ecology*, 14: 67-73.

Slatkin M. and Hudson R. R. (1991). Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, 129: 555-562.

Smith F. G. W. (1935). The development of *Patella vulgata*. *Philosophical Transactions of the Royal Society of London B*, 225: 95-125.

Sokal R. and Rohlf F. J. (1981) *Biometry.* W.H. Freeman and Co., San Francisco.

Sokolov E. P. (2000). An improved method for DNA isolation from mucopolysaccharide-rich molluscan tissues. *Journal of Molluscan Studies*, 66: 573-575.

Sokolov E. P., Pörtner H.-O., Lucassen M. and Sokolova I. M. (2003). Microscale genetic differentiation along the vertical shore gradient in White Sea snails *Littorina saxatillis* (Olivi) assessed by microsatellite markers. *Journal of Molluscan Studies*, 69: 388-391.

Sordo I., Barton E. D., Cotos J. M. and Pazos Y. (2001). An inshore poleward current in the NW of the Iberian Peninsula detected from satellite images, and its relation with *G. catenatum* and *D. acuminata* blooms in the Galician Rias. *Estuarine, Coastal and Shelf Science*, 53: 787-799.

Sotka E. E. and Palumbi S. R. (2006). The use of genetic clines to estimate dispersal distances of marine larvae. *Ecology*, 87: 1094-1103.

Sotka E. E., Wares J. P., Barth J. A., Grosberg R. K. and Palumbi S. R. (2004). Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle *Balanus glandula*. *Molecular Ecology*, 13: 2143-2156.

Southward A. J. (1964). Limpet grazing and the control of vegetation on rocky shores. In: *Grazing in terrestrial and marine environments.* (ed. D. J. Crisp). Blackwell, Oxford, pp. 265-273.

Southward A. J. and Crisp D. J. (1954). Recent changes in the distribution of intertidal barnacles *Chthamalus stellatus* (Poli) and *Balanus balanoides* (L.) in the British Isles. *Journal of Animal Ecology*, 23: 163-177.

Southward A. J., Hawkins S. J. and Burrows M. T. (1995). Seventy years' observations of changes in distribution and abundance of zooplankton and intertidal organisms in the western English Channel in relation to rising sea temperature. *Journal of Thermal Biology*, 20: 127-155.

Southward A. J. and Southward E. C. (1978). Recolonization of rocky shores in Cornwall after the use of toxic dispersants to clean up the *Torrey Canyon* oil spill. *Journal of the Fisheries Research Board of Canada*, 35: 682-706.

Sponaugle S., Cowen R. K., Shanks A., Morgan S. G., Leis J. M., Pineda J., Boehlert G. W., Kingsford M. J., Lindeman K., Grimes C. and Munro J. L. (2002). Predicting self-recruitment in marine populations: biophysical correlates and mechanisms. *Bulletin of Marine Science*, 70: 341-375.

Sponer R. and Roy M. S. (2002). Phylogeographic analysis of the brooding brittle star *Amphipholis squamata* (Echinodermata) along the coast of New Zealand reveals high cryptic genetic variation and cryptic dispersal potential. *Evolution*, 56: 1954-1967.

Stamatis C., Triantafyllidis A., Moutou K. A. and Mamuris Z. (2004). Mitochondrial DNA variation in Northeast Atlantic and Mediterranean populations of Norway lobster, *Nephrops norvegicus*. *Molecular Ecology*, 13: 1377-1390.

Staton J. L. and Rice M. E. (1999). Genetic differentiation despite teleplanic larval dispersal: allozyme variation in sipunculans of the *Apionsoma* species-complex. *Bulletin of Marine Science*, 65: 467-480.

Steinberg P. D., Nys R. and Kjelleberg S. (2002). Chemical cues for surface colonization. *Journal of Chemical Ecology*, 28: 1935-1951.

Steneck R. S., Graham M. H., Bourque B. J., Corbett D., Erlandson J. M., Estes J. A. and Tegner M. J. (2002). Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental Conservation*, 29: 436-459.

Stephens P. A., Sutherland W. J. and Freckleton R. P. (1999). What is the Allee effect? *Oikos*, 87: 185-190.

Stoner D. S. (1992). Vertical distribution of a colonial ascidian on a coral reef: the roles of larval dispersal and life-history variation. *American Naturalist*, 139: 802-824.

Strathmann M. F. and Strathmann R. R. (2007). An extraordinarily long larval duration of 4.5 years from hatching to metamorphosis for teleplanic veligers of *Fusitriton oregonensis*. *Biological Bulletin*, 213: 152-159.

Strathmann R. R. (1978). The evolution and loss of feeding larval stages of marine invertebrates. *Evolution*, 32: 894-906.

Strathmann R. R. (1985). Feeding and non-feeding larval development and life-history

evolution in marine invertebrates. *Annual Review of Ecology and Systematics*, 16: 339-361.

Strathmann R. R. (1993). Hypotheses on the origins of marine larvae. *Annual Review of Ecology and Systematics*, 24: 89-117.

Strathmann R. R. and Strathmann M. F. (1982). The relationship between adult size and brooding in marine invertebrates. *American Naturalist*, 119: 91-101.

Suchard M. A., Weiss R. E. and Sinsheiner J. S. (2001). Bayesian selection of continuous-time Markov chain evolutionary models. *Molecular Biology and Evolution*, 18: 1001-1013.

Sunnucks P. (2000). Efficient genetic markers for population biology. *Trends in Ecology and Evolution*, 15: 199-203.

Swanson L. R., de Nys R., Huggett M. J., Green J. K. and Steinberg P. (2006). *In situ* quantification of a natural settlement cue and recruitment of the Australian sea urchin *Holopneustes purpurascens*. *Marine Ecology Progress Series*, 314: 1-14.

Swearer S. E., Caselle J. E., Lea D. W. and Warner R. R. (1999). Larval retention and recruitment in an island population of a coral-reef fish. *Nature*, 402: 799-802.

Swearer S. E., Shima J. S., M.E. H., Thorrold S. R., Jones G. P., Robertson D. R., Morgan S. G., Selkoe K. A., Ruiz G. M. and Warner R. R. (2002). Evidence of self-recruitment in demersal marine populations. *Bulletin of Marine Science*, 70: 251-271.

Takahashi Y., Itoh K., Ishii M., Suzuki M. and Itabashi Y. (2002). Induction of larval settlement and metamorphosis of the sea urchin *Strongylocentrotus intermedius* by glycoglycerolipids from the green alga *Ulvella lens*. *Marine Biology*, 140: 763–771.

Takezaki N. and Nei M. (1996). Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics*, 144: 389-399.

Tautz D. (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research*, 17: 6463-6471.

Taylor M. S. and Hellberg M. E. (2003). Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. *Science*, 299: 107-109.

Thiyagarajan V., Harder T. and Qian P. Y. (2003). Combined effects of temperature and salinity on larval development and attachment of the subtidal barnacle *Balanus trigonus* Darwin. *Journal of Experimental Marine Biology and Ecology*, 287: 223-236.

Thompson G. B. (1979). Distribution and population dynamics of the limpet *Patella aspera* (Lamarck) in Bantry Bay. *Journal of Experimental Marine Biology and Ecology*, 40: 115-135.

Thompson G. B. (1980). Distribution and population dynamics of the limpet *Patella vulgata* in Bantry Bay. *Journal of Experimental Marine Biology and Ecology*, 45: 173-217.

Thompson R. C., Norton T. A. and Hawkins S. J. (1998). The influence of epilithic microbial films on the settlement of *Semibalanus balanoides* cyprids - a comparison between laboratory and field experiments. *Hydrobiologia*, 375/376: 203-216.

Thorrold S. R., Jones C. M. and Campana S. E. (1997). Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*). *Limnology and Oceanography*, 42: 102-111.

Thorrold S. R., Jones G. P., Hellberg M. E., Burton R. S., Swearer S. E., Neigel J. E., Morgan S. G. and Warner R. R. (2002). Quantifying larval retention and connectivity in marine populations with artificial and natural markers. *Bulletin of Marine Science*, 70: 291-308.

Thorrold S. R., Jones G. P., Planes S. and Hare J. A. (2006). Transgenerational marking of embryonic otoliths in marine fishes using barium stable isotopes. *Canadian Journal of Fisheries and Aquatic Sciences*, 63: 1193-1197.

Thorrold S. R., Latkoczy C., Swart P. and Jones C. M. (2001). Natal homing in a marine fish metapopulation. *Science*, 291: 297-299.

Thorrold S. R., Zacherl D. C. and Levin L. A. (2007). Population connectivity and larval dispersal using geochemical signatures in calcified structures. *Oceanography*, 20: 80-89.

Thorson G. (1946). Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae in the Sound (Øresund). *Meddelelser fra Kommissionen for Danmarks Fiskeri -og Havundersøgelser: Serie Plankton*, 4: 1-523.

Thorson G. (1950). Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews*, 25: 1-45.

Titselaar F. L. M. (1998). A review of the recent European patellids (Mollusca: Gastropoda). The patellids of the Azores, Madeira, the Selvagens and the Canary Islands. *Vita Marina*, 45: 21-62.

Todd C. D. (1979). Reproductive energetics of two species of dorid nudibranchs with planktotrophic and lecithotrophic larval strategies. *Marine Biology*, 53: 57-68.

Todd C. D. (1998). Larval supply and recruitment of benthic invertebrates: do larvae always disperse as much as we believe? *Hydrobiologia*, 375/376: 1-21.

Todd C. D., Lambert W. J. and Thorpe J. P. (1998). The genetic structure of intertidal populations of two species of nudibranch molluscs with planktotrophic and pelagic lecithotrophic larval stages: are pelagic larvae 'for' dispersal? *Journal of Experimental Marine Biology and Ecology*, 228: 1-28.

Toonen R. J. and Pawlik J. R. (1994). Foundations of gregariousness. *Nature*, 370: 511-512.

Toonen R. J. and Pawlik J. R. (1996). Settlement of the tube worm *Hydroides dianthus* (Polychaeta: Serpulidae): cues for gregarious settlement. *Marine Biology*, 126: 725–733.

Toonen R. J. and Pawlik J. R. (2001). Settlement of the gregarious tube worm *Hydroides dianthus* (Polychaeta: Serpulidae). II. Testing the desperate larva hypothesis. *Marine Ecology Progress Series*, 224: 115-131.

Tóth G., Gáspari Z. and Jurka J. (2000). Microsatellites in different eukaryotic genomes: survey and analysis. *Genome Research*, 10: 967-981.

Triantafyllidis A., Apostolidis A. P., Katsares V., Kelly E., Mercer J., Hughes M., Jørstad K. E., Tsolou A., Hynes R. and Triantaphyllidis C. (2005). Mitochondrial DNA variation in the European lobster (*Homarus gammarus*) throughout the range. *Marine Biology*, 146: 223-235.

Turner T. F., Wares J. P. and Gold J. R. (2002). Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish (*Sciaenops ocellatus*). *Genetics*, 162: 1329-1339.

Twining B. S., Gilbert J. J. and Fisher N. S. (2000). Evidence of homing behavior in the coral reef mysid Mysidium gracile. *Limnology and Oceanography*, 45: 1845-1849.

Underwood A. J. (1979). The ecology of intertidal gastropods. *Advances in Marine Biology*, 16: 111-210.

Underwood A. J. (1997) *Experiments in ecology: their logical design and interpretation using analysis of variance.* Cambridge University Press, Cambridge.

Underwood A. J. and Denley E. J. (1984). Paradigms, explanations and generalizations in models for the structure of intertidal communities on rocky shores. In: *Ecological communities, conceptual issues and the evidence* (eds. D. R. Strong, D. Simberloff, L. G. Abele and A. B. Thistle). Princeton University Press, Princeton, pp. 151-180.

Underwood A. J. and Fairweather P. G. (1989). Supply-side ecology and benthic marine assemblages. *Trends in Ecology and Evolution*, 4: 16-20.

Underwood A. J. and Keough M. J. (2001). Supply-side ecology-the nature and consequences of variations in recruitment of intertidal organisms. In: *Marine*

community ecology. (eds. M. D. Bertness, S. Gaines and M. E. Hay). Sinauer Associates, Sunderland, pp. 183-200.

Underwood J. N., Smith L. D., van Oppen J. H. and Gilmour J. P. (2007). Multiple scales of genetic connectivity in a brooding coral on isolated reefs following catastrophic bleaching. *Molecular Ecology*, 16: 771-784.

Uthicke S. and Benzie J. A. H. (2003). Gene flow and population history in high dispersal marine invertebrates: mitochondrial DNA analysis of *Holothuria nobilis* (Echinodermata: Holothuroidea) populations from the Indo-Pacific. *Molecular Ecology*, 12: 2635-2648.

Vadopalas B., Leclair L. L. and Bentzen P. (2004). Microsatellite and allozyme analyses reveal few genetic differences among spatially distinct aggregations of geoduck clams (*Panopea abrupta*, Conrad 1849). *Journal of Shellfish Research*, 23: 693-706.

Valentine J. W. and Jablonski D. (1986). Mass extinctions: sensitivity of marine larval types. *Proceedings of the National Academy of Sciences of the United States of America*, 83: 6912-6914.

van Aken H. M. (2002). Surface currents in the Bay of Biscay as observed with drifters between 1995 and 1999. *Deep-Sea Research I. Oceanographic research papers*, 49: 1071-1086.

van Oosterhout C., Hutchinson W. F., Wills D. P. M. and Shipley P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4: 535-538.

van Oosterhout C., Weetman D. and Hutchinson W. F. (2006). Estimation and adjustment of microsatellite null alleles in nonequilibrium populations. *Molecular Ecology Notes*, 6: 255-256.

Vance R. R. (1973). On reproductive strategies in marine bottom invertebrates. *American Naturalist*, 107: 339-352.

Viard F., Ellien C. and Dupont L. (2006). Dispersal ability and invasion success of *Crepidula fornicata* in a single gulf: insights from genetic markers and larval dispersal model. *Helgoland Marine Research*, 60: 144-152.

Viard F., Franck P., Dubois M.-P., Estoup A. and Jarne P. (1998). Variation of microsatellite size homoplasy across electromorphs, loci, and populations in three invertebrate species. *Journal of Molecular Evolution*, 47: 42-51.

Vikebø F., Sundby S., Adlandsvik B. and Fiksen Ø. (2005). The combined effect of transport and temperature on distribution and growth of larvae and pelagic juveniles of Arcto-Norwegian cod. *ICES Journal of Marine Science*, 62: 1375-1386.

Volman T. P. (1978). Early archeological evidence for shellfish collecting. *Science*, 201: 911-913.

Walters L. J. (1992). Field settlement locations on subtidal marine hard substrata: is active larval exploration involved? *Limnology and Oceanography*, 37: 1101-1107.

Wanninger A., Ruthensteiner B. and Haszprunar G. (2000). Torsion in *Patella caerulea* (Mollusca, Patellogastropoda): ontogenetic processes, timing, and mechanisms. *Invertebrate Biology*, 119: 177-187.

Wanninger A., Ruthensteiner B., Lobenwein S., Salvenmoser W., Dictus W. J. A. G. and Haszprunar G. (1999). Development of the musculature in the limpet *Patella* (Mollusca, Patellogastropoda). *Development Genes and Evolution*, 209: 226-238.

Waples R. (1998). Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, 89: 438-450.

Waples R. S. and Gaggiotti O. (2006). What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, 15: 1419-1439.

Ward R. D., Woodward M. and Skibinski D. O. F. (1994). A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. *Journal of Fish Biology*, 44: 213-232.

Wares J. P. (2001). Patterns of speciation inferred from mitochondrial DNA in North American *Chthamalus* (Cirripedia: Balanomorpha: Chthamaloidea). *Molecular Phylogenetics and Evolution*, 18: 104-116.

Wares J. P. and Cunningham C. W. (2001). Phylogeography and historical ecology of North Atlantic intertidal. *Evolution*, 55: 2455-2469.

Warner R. R. (1975). The adaptive significance of sequential hermaphroditism in animals. *American Naturalist*, 109: 61-82.

Warner R. R. (1997). Evolutionary ecology: how to reconcile pelagic dispersal with local adaptation. *Coral Reefs*, 16S: S115-S128.

Wattier R., Engel C. R., Saumitou-Laprade P. and Valero M. (1998). Short allele dominance as a source of heterozygote deficiency at microsatellite loci: experimental evidence at the dinucleotide locus Gv1CT in *Gracilaria gracilis* (Rhodophyta). *Molecular Ecology*, 7: 1569-1573.

Watts S. A., Scheibling R. E., Marsh A. G. and McClintock J. B. (1982). Effect of temperature and salinity on larval development of sibling species of *Echinaster* (Echinodermata: Asteroidea) and their hybrids. *Biological Bulletin*, 163: 348-354.

Weber L. I., Gray D. R., Hodgson A. N. and Hawkins S. J. (1997). Genetic divergence

between the South African *Helcion* species and North-East Atlantic *H. pellucidum* (Mollusca: Patellogastropoda). *Journal of the Marine Biological Association of the United Kingdom*, 77: 1139-1150.

Weber L. I. and Hawkins S. J. (2002). Evolution of the limpet *Patella candei* d'Orbigny (Mollusca, Patellidae) in the Atlantic archipelagos: human intervention and natural processes. *Biological Journal of the Linnean Society*, 77: 341-353.

Weber L. I. and Hawkins S. J. (2005). *Patella aspera* and *Patella ulyssiponensis*: genetic evidence of speciation in the North-east Atlantic. *Marine Biology*, 147: 153-162.

Weber L. I. and Hawkins S. J. (2006). Allozymic differentiation among geographically distant populations of *Patella vulgata* (Mollusca, Patellogastropoda). *Hydrobiologia*, 553: 267-275.

Weber L. I., Thorpe J. P., Santos R. S. and Hawkins S. J. (1998). Identification of stocks of the exploited limpets *Patella aspera* and *P. candei* at Madeira Archipelago by allozyme electrophoresis. *Journal of Shellfish Research*, 17: 945-954.

Weetman D., Hauser L., Bayes M. K., Ellis J. R. and Shaw P. W. (2006). Genetic population structure across a range of geographic scales in the commercially exploited marine gastropod *Buccinum undatum*. *Marine Ecology Progress Series*, 317: 157-169.

Weir B. S. and Cockerham C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, 38: 1358-1370.

Wendt D. E. (1998). Effect of larval swimming duration on growth and reproduction of *Bugula neritina* (Bryozoa) under field conditions. *Biological Bulletin*, 195: 126-135.

Wendt D. E. (2000). Energetics of larval swimming and metamorphosis in four species of *Bugula* (Bryozoa). *Biological Bulletin*, 198: 346-356.

Werner F. E., Cowen R. K. and Paris C. B. (2007). Coupled biological and physical models: present capabilities and necessary developments for future studies of population connectivity. *Oceanography*, 20: 54-69.

Werner F. E., Quinlan J. A., Lough R. G. and Lynch D. R. (2001). Spatially-explicit individual based modelling of marine populations: a review of the advances in the 1990's. *Sarsia*, 86: 411-421.

Wethey D. S. (1986). Ranking of settlement cues by barnacle larvae: influence of surface contour. *Bulletin of Marine Science*, 39:393-400.

Wethey D. S. (2002). Biogeography, competition, and microclimate: the barnacle *Chthamalus fragilis* in New England. *Integrative and Comparative Biology*, 42: 872-880.

Wethey D. S. and Woodin S. A. (2008). Ecological hindcasting of biogeographic responses to climate change in the European intertidal zone. *Hydrobiologia*, 606: 139-151.

Whitlock M. C. and McCauley D. E. (1999). Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4N_m+1)$. *Heredity*, 82: 117-125.

Whittaker J. C., Harbord R. M., Boxall N., Mackay I., Dawson G. and Sibly R. M. (2003). Likelihood-based estimation of microsatellite mutation rates. *Genetics*, 164: 781-787.

Wieczorek S. K. and Todd C. D. (1998). Inhibition and facilitation of settlement of epifaunal marine invertebrate larvae by microbial biofilm cues. *Biofouling*, 12: 81-118.

Williamson J. E., de Nys R., Kumar N., Carson D. G. and Steinberg P. D. (2000). Induction of metamorphosis in the sea urchin *Holopneustes purpurascens* by a metabolite complex from the algal host *Delisea pulchra. Biological Bulletin*, 198: 332.345.

Willis B. L. and Oliver J. K. (1990). Direct tracking of coral larvae: implications for dispersal studies of planktonic larvae in the central region of the Great Barrier Reef. *Coral Reefs*, 3: 229-236.

Wilson D. P. (1955). The role of micro-organisms in the settlement of *Ophelia bicornis* Savigny. *Journal of the Marine Biological Association of the United Kingdom*, 34: 531-543.

Winer B. J., Brown D. R. and Michels K. M. (1991) *Statistical principles in experimental design.* McGraw-Hill, New York.

Woodruff S. D., Diaz H. F., Elms J. D. and Worley S. J. (1998). COADS release 2 data and metadata enhancements for improvements of marine surface flux fields. *Physics and Chemistry of the Earth*, 23: 517-526.

Woollacott R. M., Pechenik J. A. and Imbalzano K. M. (1989). Effects of duration of larval swimming period on early colony development in *Bugula stolonifera* (Bryozoa: Cheilostomata). *Marine Biology*, 102: 57-63.

Wright J. and Boxshall A. (1999). The influence of small-scale flow and chemical cues on the settlement of two congeneric barnacle species. . *Marine Ecology Progress Series*, 183: 179-187.

Wright S. (1943). Isolation by distance. *Genetics*, 28: 114-138.

Wright S. (1951). The genetical structure of populations. *Annals of Eugenics*, 15: 323-354.

Wright W. G. and Lindberg D. R. (1979). A non-fatal method of sex determination for patellacean gastropods. *Journal of the Marine Biological Association of the United Kingdom*, 59: 803.

Wright W. G. and Lindberg D. R. (1982). Direct observation of sex change in the patellacean limpet *Lottia gigantea*. *Journal of the Marine Biological Association of the United Kingdom*, 62: 737-738.

Yang Z. (1994). Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *Journal of Molecular Evolution*, 39: 306-314.

Young C. M. (1987). Novelty of 'supply-side ecology'. *Science*, 235: 415-416.

Young C. M. (1990). Larval ecology of marine invertebrates: a sesquicentennial history. *Ophelia*, 32: 1-48.

Young C. M. (1995). Behavior and locomotion during the dispersal phase of larval life. In: *Ecology of Marine Invertebrate Larvae* (ed. L. McEdward). CRC Press, Boca Raton, Florida, pp. 249-278.

Young C. M. and Chia F. S. (1981). Laboratory evidence for delay of larval settlement in response to a dominant competitor. *International Journal of Invertebrate Reproduction*, 3: 221-226.

Zacherl D., Gaines S. D. and Lonhart S. I. (2003). The limits to biogeographical distributions: insights from the northward range extension of the marine snail, *Kelletia kelletii* (Forbes, 1852). *Journal of Biogeography*, 30: 913-924.

Zacherl D. C. (2005). Spatial and temporal variation in statolith and protoconch trace elements as natural tags to track larval dispersal. *Marine Ecology Progress Series*, 290: 145-163.

Zane L., Bargelloni L. and Patarnello T. (2002). Strategies for microsatellite isolation: a review. *Molecular Ecology*, 11: 1-16.

Zar J. H. (1984) *Biostatistical analysis.* Prentice-Hall International, New Jersey.

Zardi G. I., McQuaid C. D., Teske P. R. and Barker N. P. (2007). Unexpected genetic structure of mussel populations in South Africa: indigenous *Perna perna* and invasive *Mytilus galloprovincialis. Marine Ecology Progress Series*, 337: 135-144.

Zhao B. and Qian P. Y. (2002). Larval settlement and metamorphosis in the slipper limpet *Crepidula onyx* (Sowerby) in response to conspecific cues and the cues from biofilm. *Journal of Experimental Marine Biology and Ecology*, 269: 39-51.

Zimmer-Faust R. K. and Tamburri M. N. (1994). Chemical identity and ecological implications of a waterborne, larval settlement cue. *Limnology and Oceanography*, 39:

1075-1087.

Zouros E., Ball A. O., Saavedra C. and Freeman K. (1994). An unusual type of mitochondrial DNA inheritance in the blue mussel *Mytilus. Proceedings of the National Academy of Sciences of the United States of America*, 91: 7463-7467.

Zouros E. and Foltz D. W. (1984). Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacologia*, 25: 581-591.

Appendix A

Allele frequencies for microsatellite loci in each sample of *Patella depressa*

Table A1. Proportional allele frequencies per sampling location, for five microsatellite loci screened in *Patella depressa*. Sample codes as in Table 5.1.

Size	HAR	SIV	PLY	SMA	LCR	BIA	NOJ	CPR	BAI	AGU	SBE	PAR	STO	ARR	ODA	CAD	TAR	CAS	AGA
Pde1																			
96	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.048	0.000	0.000	0.000	0.000	0.000	0.000
97	0.032	0.000	0.034	0.000	0.000	0.000	0.107	0.032	0.032	0.050	0.050	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.033
99	0.000	0.000	0.017	0.000	0.000	0.000	0.036	0.000	0.000	0.050	0.100	0.048	0.000	0.017	0.033	0.016	0.000	0.016	0.017
100	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.032	0.016	0.100	0.000	0.000	0.016	0.000	0.000	0.048	0.032	0.032	0.000
101	0.032	0.018	0.034	0.093	0.000	0.000	0.071	0.032	0.081	0.033	0.000	0.097	0.032	0.017	0.083	0.032	0.016	0.032	0.033
102	0.000	0.125	0.000	0.019	0.194	0.069	0.000	0.032	0.145	0.067	0.133	0.161	0.290	0.233	0.117	0.339	0.032	0.306	0.467
103	0.710	0.750	0.466	0.833	0.742	0.741	0.625	0.645	0.419	0.533	0.483	0.565	0.435	0.467	0.633	0.435	0.871	0.452	0.300
104	0.065	0.018	0.017	0.056	0.000	0.000	0.000	0.048	0.065	0.000	0.133	0.065	0.048	0.083	0.117	0.000	0.000	0.081	0.083
105	0.129	0.089	0.224	0.000	0.065	0.086	0.161	0.177	0.210	0.117	0.100	0.048	0.048	0.183	0.017	0.081	0.032	0.048	0.067
106	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.000
107	0.000	0.000	0.172	0.000	0.000	0.000	0.000	0.000	0.016	0.017	0.000	0.016	0.032	0.000	0.000	0.016	0.016	0.032	0.000
108	0.032	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
109	0.000	0.000	0.034	0.000	0.000	0.086	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.000	0.000	0.000	0.000
Pde3																			
130	0.000	0.018	0.017	0.019	0.043	0.000	0.000	0.017	0.000	0.016	0.000	0.033	0.016	0.000	0.000	0.000	0.000	0.000	-
132	0.016	0.018	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.065	0.018	0.000	0.000	0.000	0.000	0.017	0.000	0.000	-
134	**0.016**	**0.089**	**0.000**	**0.037**	**0.000**	**0.000**	**0.161**	**0.034**	**0.037**	**0.048**	**0.071**	**0.017**	**0.065**	**0.023**	**0.100**	**0.017**	**0.065**	**0.000**	-
136	0.297	0.196	0.190	0.111	0.174	0.172	0.097	0.224	0.167	0.177	0.214	0.200	0.097	0.250	0.150	0.100	0.113	0.183	-
138	0.344	0.393	0.379	0.593	0.261	0.466	0.403	0.414	0.389	0.323	0.268	0.333	0.371	0.227	0.383	0.317	0.468	0.317	-
140	0.094	0.143	0.138	0.074	0.261	0.138	0.081	0.034	0.056	0.016	0.125	0.100	0.177	0.182	0.133	0.150	0.113	0.167	-
142	0.141	0.036	0.034	0.000	0.065	0.034	0.081	0.086	0.056	0.161	0.107	0.033	0.113	0.068	0.050	0.033	0.000	0.083	-
144	0.063	0.036	0.138	0.093	0.087	0.069	0.113	0.086	0.259	0.048	0.089	0.150	0.065	0.205	0.067	0.233	0.065	0.167	-
146	0.016	0.018	0.034	0.056	0.087	0.086	0.016	0.017	0.037	0.065	0.071	0.083	0.048	0.023	0.050	0.083	0.129	0.050	-
148	0.000	0.000	0.034	0.000	0.022	0.000	0.032	0.000	0.000	0.032	0.000	0.000	0.016	0.000	0.000	0.033	0.016	0.000	-
150	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.017	0.000	0.000	0.017	0.000	0.016	0.000	-
152	0.000	0.054	0.034	0.000	0.000	0.017	0.000	0.069	0.000	0.032	0.018	0.033	0.032	0.023	0.033	0.000	0.000	0.033	-
154	0.016	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.017	0.017	0.016	0.000	-
156	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-
Pde5																			
154	0.020	0.038	0.025	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
156	0.160	0.000	0.000	0.000	0.017	0.069	0.069	0.000	0.000	0.048	0.038	0.053	0.034	0.000	0.040	0.000	0.000	0.000	0.000
158	0.040	0.000	0.075	0.000	0.000	0.034	0.017	0.059	0.000	0.016	0.000	0.053	0.052	0.000	0.040	0.017	0.000	0.000	0.000
160	0.040	0.000	0.000	0.000	0.017	0.069	0.052	0.088	0.000	0.000	0.038	0.053	0.000	0.033	0.000	0.083	0.000	0.000	0.019
162	0.000	0.058	0.000	0.119	0.000	0.034	0.052	0.000	0.054	0.000	0.019	0.026	0.000	0.000	0.040	0.000	0.000	0.000	0.037
164	0.000	0.038	0.025	0.024	0.050	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
166	0.000	0.038	0.000	0.000	0.150	0.017	0.000	0.000	0.018	0.000	0.000	0.000	0.034	0.033	0.060	0.000	0.038	0.000	0.056
168	0.020	0.019	0.000	0.048	0.017	0.052	0.069	0.206	0.018	0.032	0.019	0.053	0.000	0.017	0.080	0.067	0.038	0.000	0.000
170	0.000	0.000	0.000	0.000	0.033	0.086	0.000	0.029	0.143	0.000	0.019	0.053	0.052	0.033	0.040	0.000	0.000	0.033	0.056
172	0.000	0.019	0.000	0.048	0.017	0.052	0.000	0.059	0.036	0.048	0.019	0.000	0.017	0.000	0.000	0.000	0.038	0.033	0.019

Table A1. Proportional allele frequencies per sampling location, for five microsatellite loci screened in *Patella depressa* (continued).

Size	HAR	SIV	PLY	SMA	LCR	BIA	NOJ	CPR	BAI	AGU	SBE	PAR	STO	ARR	ODA	CAD	TAR	CAS	AGA
174	0.000	0.038	0.050	0.119	0.083	0.034	0.000	0.029	0.000	0.016	0.038	0.026	0.000	0.000	0.020	0.017	0.077	0.067	0.000
176	0.000	0.000	0.050	0.000	0.083	0.034	0.034	0.088	0.000	0.000	0.115	0.000	0.034	0.017	0.020	0.017	0.000	0.067	0.056
178	0.040	0.000	0.000	0.048	0.000	0.034	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.080	0.000	0.038	0.067	0.019
180	0.040	0.058	0.050	0.071	0.050	0.034	0.034	0.059	0.036	0.048	0.019	0.053	0.000	0.017	0.040	0.100	0.038	0.100	0.037
182	0.020	0.038	0.075	0.143	0.067	0.069	0.052	0.029	0.018	0.097	0.000	0.105	0.103	0.050	0.020	0.033	0.000	0.133	0.000
184	0.100	0.077	0.125	0.048	0.033	0.052	0.017	0.059	0.071	0.113	0.096	0.000	0.052	0.033	0.060	0.133	0.000	0.100	0.037
186	0.060	0.058	0.050	0.071	0.083	0.000	0.034	0.000	0.179	0.032	0.115	0.026	0.121	0.567	0.040	0.100	0.115	0.033	0.241
188	0.060	0.058	0.175	0.024	0.033	0.138	0.103	0.000	0.054	0.065	0.096	0.000	0.086	0.050	0.060	0.017	0.154	0.133	0.093
190	0.080	0.231	0.050	0.000	0.000	0.017	0.103	0.000	0.018	0.129	0.115	0.079	0.069	0.050	0.060	0.033	0.231	0.033	0.000
191	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
192	0.100	0.058	0.075	0.048	0.050	0.069	0.086	0.000	0.054	0.065	0.096	0.132	0.086	0.017	0.000	0.100	0.038	0.000	0.000
194	0.000	0.000	0.000	0.000	0.050	0.000	0.017	0.000	0.143	0.000	0.038	0.000	0.017	0.033	0.080	0.033	0.000	0.100	0.130
196	0.020	0.038	0.000	0.000	0.000	0.000	0.034	0.000	0.000	0.048	0.000	0.000	0.086	0.000	0.040	0.000	0.000	0.000	0.037
198	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.040	0.033	0.000	0.033	0.019
200	0.020	0.000	0.000	0.024	0.000	0.000	0.069	0.118	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.033	0.000
202	0.020	0.019	0.000	0.000	0.000	0.000	0.000	0.059	0.018	0.048	0.000	0.026	0.000	0.000	0.000	0.017	0.000	0.000	0.000
204	0.000	0.000	0.000	0.000	0.033	0.052	0.000	0.000	0.000	0.081	0.000	0.026	0.017	0.000	0.060	0.050	0.038	0.000	0.037
206	**0.000**	**0.038**	**0.050**	**0.024**	**0.017**	**0.000**	**0.034**	**0.000**	**0.000**	**0.032**	**0.000**	**0.000**	**0.069**	**0.017**	**0.040**	**0.000**	**0.038**	**0.000**	**0.000**
208	0.000	0.000	0.050	0.071	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
210	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.088	0.018	0.000	0.038	0.000	0.000	0.000	0.000	0.017	0.000	0.033	0.000
212	0.040	0.019	0.000	0.000	0.017	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000
214	0.040	0.000	0.000	0.000	0.000	0.000	0.052	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000
216	0.000	0.019	0.000	0.000	0.000	0.017	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
218	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.017	0.000	0.000	0.000	0.000	0.000	0.000
220	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.000	0.000
222	0.000	0.000	0.000	0.000	0.033	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
224	0.000	0.019	0.000	0.000	0.000	0.000	0.034	0.000	0.000	0.000	0.038	0.053	0.000	0.017	0.000	0.000	0.000	0.000	0.000
226	0.080	0.000	0.025	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.077	0.000	0.000
228	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.016	0.000	0.053	0.000	0.000	0.000	0.050	0.038	0.000	0.074
230	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.032	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
232	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.017	0.000	0.000	0.000
234	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
238	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.053	0.000	0.000	0.000	0.000	0.000	0.000	0.000
240	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
242	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019
244	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
246	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000
248	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000
250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019

Table A1. Proportional allele frequencies per sampling location, for five microsatellite loci screened in *Patella depressa* (continued).

Size	HAR	SIV	PLY	SMA	LCR	BIA	NOJ	CPR	BAI	AGU	SBE	PAR	STO	ARR	ODA	CAD	TAR	CAS	AGA
Pde7																			
143	0.000	0.000	0.000	0.000	0.016	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
145	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000
147	0.000	0.000	0.000	0.017	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
149	0.000	0.016	0.040	0.050	0.000	0.016	0.000	0.065	0.000	0.000	0.016	0.017	0.000	0.000	0.033	0.016	0.000	0.000	0.000
151	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
153	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.032	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000
157	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000
159	0.125	0.065	0.060	0.017	0.031	0.097	0.065	0.032	0.016	0.032	0.048	0.100	0.065	0.050	0.083	0.065	0.081	0.017	0.100
161	0.188	0.258	0.260	0.650	0.250	0.129	0.226	0.177	0.306	0.226	0.306	0.167	0.210	0.167	0.217	0.145	0.194	0.283	0.133
163	0.047	0.081	0.040	0.017	0.031	0.016	0.016	0.032	0.048	0.097	0.016	0.050	0.065	0.000	0.033	0.065	0.065	0.017	0.033
165	0.219	0.177	0.220	0.083	0.047	0.129	0.081	0.161	0.145	0.081	0.113	0.050	0.113	0.217	0.100	0.145	0.113	0.050	0.200
167	0.047	0.032	0.040	0.017	0.109	0.113	0.081	0.145	0.226	0.145	0.065	0.150	0.081	0.167	0.083	0.065	0.161	0.200	0.117
169	0.156	0.113	0.080	0.000	0.172	0.081	0.129	0.177	0.048	0.129	0.032	0.050	0.081	0.067	0.083	0.129	0.081	0.067	0.083
171	0.063	0.097	0.080	0.033	0.203	0.210	0.129	0.145	0.081	0.113	0.177	0.117	0.177	0.133	0.183	0.274	0.081	0.200	0.267
173	0.109	0.016	0.060	0.033	0.031	0.097	0.113	0.016	0.065	0.081	0.081	0.083	0.097	0.100	0.083	0.065	0.081	0.033	0.017
175	0.031	0.065	0.040	0.033	0.047	0.032	0.097	0.000	0.032	0.000	0.032	0.117	0.032	0.000	0.033	0.000	0.065	0.067	0.017
177	**0.000**	**0.032**	**0.060**	**0.033**	**0.016**	**0.016**	**0.000**	**0.016**	**0.016**	**0.016**	**0.032**	**0.033**	**0.000**	**0.017**	**0.033**	**0.016**	**0.016**	**0.017**	**0.000**
179	0.016	0.048	0.000	0.017	0.016	0.000	0.016	0.000	0.016	0.048	0.016	0.017	0.016	0.050	0.017	0.016	0.016	0.033	0.000
181	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.016	0.000	0.016	0.032	0.000	0.016	0.000	0.000	0.000	0.048	0.000	0.017
183	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.000	0.000	0.017	0.016	0.017	0.017	0.000	0.000	0.000	0.000
185	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.017
191	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
197	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pde9																			
142	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000
148	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
152	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
154	0.000	0.000	0.018	0.000	0.017	0.000	0.032	0.034	0.017	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000
156	0.016	0.000	0.018	0.000	0.000	0.034	0.000	0.000	0.000	0.017	0.019	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000
158	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.019	0.054
160	0.032	0.000	0.000	0.000	0.017	0.034	0.016	0.000	0.000	0.000	0.019	0.000	0.019	0.000	0.054	0.031	0.000	0.056	0.000
162	0.048	0.016	0.018	0.042	0.050	0.052	0.000	0.034	0.017	0.033	0.019	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.018
164	0.000	0.000	0.000	0.000	0.050	0.086	0.000	0.052	0.050	0.000	0.037	0.024	0.019	0.069	0.054	0.031	0.024	0.019	0.000
166	0.016	0.000	0.036	0.021	0.017	0.017	0.048	0.000	0.033	0.017	0.037	0.095	0.019	0.017	0.036	0.047	0.071	0.093	0.036
168	0.032	0.065	0.071	0.042	0.033	0.052	0.097	0.052	0.083	0.017	0.037	0.048	0.037	0.052	0.071	0.109	0.119	0.130	0.125
170	0.081	0.016	0.000	0.083	0.033	0.069	0.048	0.017	0.083	0.033	0.000	0.095	0.074	0.069	0.018	0.000	0.024	0.037	0.036
172	0.016	0.000	0.054	0.000	0.050	0.017	0.048	0.034	0.000	0.050	0.037	0.000	0.019	0.034	0.089	0.031	0.048	0.056	0.054
174	0.032	0.081	0.018	0.083	0.033	0.017	0.032	0.121	0.000	0.100	0.056	0.048	0.037	0.069	0.107	0.031	0.000	0.019	0.054
176	0.129	0.065	0.071	0.083	0.067	0.086	0.032	0.069	0.067	0.017	0.019	0.167	0.037	0.052	0.054	0.016	0.000	0.056	0.054

Table A1. Proportional allele frequencies per sampling location, for five microsatellite loci screened in *Patella depressa* (continued).

Size	HAR	SIV	PLY	SMA	LCR	BIA	NOJ	CPR	BAI	AGU	SBE	PAR	STO	ARR	ODA	CAD	TAR	CAS	AGA
178	0.081	0.097	0.196	0.104	0.050	0.069	0.016	0.052	0.150	0.150	0.111	0.000	0.000	0.121	0.018	0.094	0.143	0.074	0.054
180	0.016	0.161	0.036	0.188	0.150	0.103	0.161	0.121	0.067	0.117	0.130	0.071	0.185	0.103	0.071	0.063	0.095	0.037	0.036
182	0.081	0.210	0.125	0.042	0.117	0.086	0.081	0.121	0.100	0.067	0.056	0.119	0.111	0.052	0.089	0.109	0.119	0.074	0.161
184	0.097	0.048	0.125	0.104	0.033	0.017	0.081	0.034	0.033	0.100	0.037	0.071	0.111	0.086	0.018	0.047	0.119	0.056	0.054
186	0.081	0.081	0.000	0.000	0.050	0.000	0.048	0.086	0.117	0.033	0.056	0.048	0.074	0.052	0.036	0.031	0.024	0.037	0.071
188	0.081	0.000	0.071	0.042	0.050	0.052	0.032	0.017	0.050	0.033	0.074	0.000	0.037	0.069	0.054	0.063	0.000	0.074	0.036
190	0.032	0.032	0.071	0.021	0.033	0.052	0.081	0.017	0.033	0.033	0.019	0.048	0.037	0.034	0.018	0.078	0.024	0.000	0.036
192	0.048	0.000	0.000	0.042	0.000	0.017	0.000	0.052	0.000	0.017	0.093	0.024	0.037	0.034	0.036	0.016	0.024	0.019	0.000
194	0.016	0.065	0.000	0.000	0.033	0.000	0.016	0.017	0.017	0.000	0.037	0.024	0.037	0.034	0.036	0.000	0.000	0.019	0.000
196	0.032	0.016	0.036	0.021	0.000	0.052	0.081	0.017	0.000	0.033	0.019	0.024	0.037	0.052	0.054	0.016	0.071	0.000	0.071
198	0.016	0.032	0.018	0.021	0.017	0.017	0.016	0.052	0.017	0.000	0.019	0.000	0.019	0.000	0.054	0.047	0.000	0.074	0.036
200	0.016	0.000	0.018	0.042	0.050	0.034	0.016	0.000	0.017	0.000	0.019	0.024	0.000	0.000	0.000	0.031	0.000	0.037	0.018
202	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.033	0.050	0.019	0.000	0.019	0.000	0.000	0.031	0.000	0.000	0.000
204	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.017	0.000	0.019	0.000	0.019	0.000	0.018	0.016	0.000	0.019	0.000
206	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.016	0.071	0.000	0.000
208	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000
212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000

Appendix B

Haplotype frequencies for mtDNA COI and allele frequencies for microsatellite loci in each sample of *Patella rustica*

Table B.1 Proportional haplotype frequencies per sampling location for the mtDNA COI gene fragment sequenced in *Patella rustica*. Sample codes as in Table 6.1.

Haplotype	BIA	NOJ	BAN	CPR	MOL	VIA	HLE	BUA	SBE	CRA	CAT	ODA
Pr1	0.111	0.000	0.000	0.143	0.000	0.200	0.200	0.133	0.083	0.000	0.077	0.154
Pr2	0.444	0.067	0.286	0.286	0.200	0.067	0.133	0.133	0.083	0.077	0.385	0.231
Pr3	0.111	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pr4	0.111	0.133	0.143	0.214	0.333	0.267	0.200	0.267	0.000	0.462	0.231	0.154
Pr5	0.111	0.200	0.286	0.000	0.000	0.067	0.067	0.000	0.083	0.154	0.077	0.077
Pr6	0.111	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.083	0.000	0.000	0.000
Pr7	0.000	0.067	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.077
Pr8	0.000	0.000	0.071	0.000	0.067	0.067	0.000	0.000	0.000	0.000	0.000	0.000
Pr9	0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pr10	0.000	0.000	0.071	0.071	0.000	0.000	0.067	0.067	0.000	0.000	0.000	0.000
Pr11	0.000	0.333	0.000	0.143	0.267	0.067	0.067	0.200	0.500	0.231	0.000	0.231
Pr12	0.000	0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pr13	0.000	0.000	0.000	0.071	0.067	0.133	0.000	0.067	0.000	0.077	0.000	0.000
Pr14	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pr15	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pr16	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pr17	0.000	0.000	0.000	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pr18	0.000	0.000	0.000	0.000	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000
Pr19	0.000	0.000	0.000	0.000	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000
Pr20	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.000	0.000	0.000	0.000	0.000
Pr21	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.000	0.000	0.000	0.000	0.000
Pr22	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.000	0.000	0.000	0.000	0.000
Pr23	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.000	0.000	0.000	0.000	0.000
Pr24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.000	0.000	0.000	0.000
Pr25	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000
Pr26	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000
Pr27	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.000
Pr28	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.000
Pr29	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.000
Pr30	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.077

Table B.2 Proportional allele frequencies per sampling location, for seven microsatellite loci screened in *Patella rustica*. Sample codes as in Table 6.1.

Allele size	BIA	NOJ	BAN	CPR	MOL	VIA	HLE	BUA	SBE	CPR	CAT	ODA
Pru1												
130	0.000	0.000	0.015	0.000	0.000	0.000	0.031	0.036	0.000	0.016	0.000	0.016
142	0.000	0.000	0.000	0.000	0.016	0.032	0.000	0.000	0.000	0.000	0.000	0.000
146	0.014	0.067	0.030	0.031	0.032	0.065	0.047	0.036	0.029	0.000	0.017	0.048
150	0.014	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000
154	0.069	0.067	0.030	0.047	0.129	0.113	0.031	0.018	0.044	0.000	0.034	0.081
160	0.014	0.017	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
164	0.028	0.033	0.015	0.078	0.048	0.081	0.063	0.071	0.088	0.097	0.034	0.016
168	0.000	0.050	0.030	0.000	0.000	0.016	0.000	0.018	0.044	0.065	0.034	0.000
172	0.000	0.017	0.000	0.031	0.048	0.000	0.047	0.036	0.029	0.000	0.017	0.016
176	0.111	0.033	0.076	0.031	0.065	0.065	0.094	0.125	0.044	0.016	0.034	0.048
180	0.000	0.000	0.015	0.000	0.016	0.000	0.000	0.000	0.015	0.032	0.017	0.000
184	0.014	0.000	0.045	0.031	0.000	0.032	0.031	0.018	0.015	0.016	0.000	0.000
188	0.000	0.017	0.000	0.031	0.032	0.016	0.000	0.018	0.000	0.000	0.034	0.000
192	0.028	0.033	0.015	0.094	0.000	0.048	0.031	0.018	0.015	0.032	0.000	0.000
196	0.500	0.467	0.576	0.563	0.468	0.387	0.484	0.482	0.485	0.597	0.621	0.694
200	0.097	0.067	0.061	0.047	0.016	0.065	0.063	0.036	0.088	0.016	0.069	0.032
204	0.028	0.000	0.000	0.000	0.000	0.000	0.031	0.018	0.000	0.000	0.000	0.000
208	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000
212	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000
216	0.000	0.000	0.030	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.016
224	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000
234	0.028	0.083	0.015	0.000	0.000	0.000	0.000	0.018	0.029	0.032	0.000	0.000
236	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
238	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000
240	0.000	0.000	0.000	0.000	0.032	0.016	0.031	0.000	0.000	0.016	0.034	0.000
242	0.028	0.017	0.015	0.000	0.000	0.016	0.000	0.054	0.044	0.048	0.034	0.016
244	0.014	0.000	0.015	0.000	0.000	0.032	0.000	0.000	0.015	0.000	0.000	0.000
246	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.016
248	0.014	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000
250	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.000	0.000	0.000	0.000	0.000
252	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pru2												
158	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.016	0.000
160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000
162	0.028	0.000	0.015	0.000	0.065	0.000	0.000	0.000	0.000	0.016	0.016	0.016
166	0.000	0.016	0.030	0.000	0.000	0.000	0.031	0.000	0.000	0.016	0.000	0.000
174	0.000	0.000	0.000	0.000	0.000	0.016	0.031	0.000	0.000	0.000	0.000	0.000
178	0.847	0.903	0.864	0.781	0.919	0.839	0.781	0.911	0.941	0.839	0.823	0.875
180	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000

Table B.2 Proportional allele frequencies per sampling location, for seven microsatellite loci screened in *Patella rustica* (continued).

Allele size	BIA	NOJ	BAN	CPR	MOL	VIA	HLE	BUA	SBE	CPR	CAT	ODA
182	0.097	0.065	0.030	0.094	0.016	0.048	0.063	0.000	0.044	0.048	0.032	0.031
186	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000
190	0.028	0.000	0.061	0.109	0.000	0.081	0.078	0.071	0.015	0.048	0.081	0.078
192	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
194	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000
Pru5												
170	0.000	0.029	0.045	0.000	0.016	0.000	0.000	0.017	0.014	0.016	0.030	0.000
174	0.019	0.015	0.000	0.000	0.032	0.016	0.016	0.000	0.000	0.016	0.000	0.000
178	0.038	0.029	0.076	0.103	0.177	0.258	0.094	0.117	0.114	0.194	0.182	0.250
182	0.135	0.309	0.106	0.147	0.129	0.145	0.313	0.167	0.143	0.129	0.106	0.059
186	0.788	0.603	0.712	0.706	0.645	0.500	0.578	0.700	0.714	0.645	0.606	0.691
190	0.000	0.000	0.045	0.029	0.000	0.081	0.000	0.000	0.000	0.000	0.015	0.000
198	0.019	0.000	0.015	0.015	0.000	0.000	0.000	0.000	0.014	0.000	0.061	0.000
200	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pru6												
95	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017
97	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
98	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000
99	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.030	0.000	0.000	0.017
101	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.036	0.000
103	0.119	0.017	0.022	0.054	0.000	0.038	0.000	0.018	0.030	0.000	0.036	0.000
105	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
107	0.119	0.000	0.043	0.000	0.000	0.000	0.016	0.000	0.000	0.022	0.000	0.017
109	0.000	0.052	0.000	0.054	0.000	0.038	0.016	0.036	0.045	0.022	0.018	0.033
111	0.024	0.103	0.130	0.107	0.052	0.077	0.047	0.054	0.045	0.152	0.054	0.033
113	0.024	0.000	0.000	0.054	0.052	0.058	0.094	0.071	0.076	0.043	0.018	0.050
115	0.000	0.052	0.022	0.089	0.086	0.077	0.047	0.036	0.045	0.000	0.089	0.017
117	0.143	0.121	0.217	0.214	0.121	0.058	0.156	0.179	0.167	0.239	0.143	0.317
119	0.119	0.172	0.087	0.036	0.121	0.077	0.281	0.161	0.167	0.152	0.107	0.100
121	0.238	0.328	0.326	0.286	0.293	0.058	0.219	0.304	0.273	0.217	0.357	0.217
123	0.024	0.052	0.022	0.018	0.069	0.096	0.047	0.018	0.045	0.065	0.018	0.050
125	0.000	0.034	0.000	0.054	0.052	0.154	0.016	0.071	0.015	0.000	0.036	0.000
127	0.048	0.000	0.022	0.000	0.017	0.058	0.063	0.000	0.030	0.000	0.018	0.067
129	0.071	0.017	0.065	0.036	0.121	0.135	0.000	0.054	0.015	0.043	0.054	0.033
131	0.000	0.000	0.043	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000
133	0.000	0.017	0.000	0.000	0.017	0.019	0.000	0.000	0.015	0.000	0.000	0.033
135	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
137	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pru7												
132	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000

Table B.2 Proportional allele frequencies per sampling location, for seven microsatellite loci screened in *Patella rustica* (continued).

Allele size	BIA	NOJ	BAN	CPR	MOL	VIA	HLE	BUA	SBE	CPR	CAT	ODA
156	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000
164	0.000	0.000	0.000	0.000	0.000	0.089	0.000	0.000	0.000	0.000	0.000	0.000
176	0.000	0.000	0.017	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000
178	0.031	0.000	0.050	0.000	0.058	0.000	0.060	0.037	0.000	0.060	0.140	0.121
180	0.016	0.000	0.000	0.000	0.019	0.000	0.020	0.000	0.000	0.020	0.020	0.000
182	0.000	0.000	0.033	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000
184	0.000	0.000	0.017	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000
186	0.000	0.000	0.033	0.000	0.000	0.000	0.020	0.111	0.000	0.020	0.020	0.000
188	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.060	0.000	0.000
190	0.000	0.038	0.067	0.026	0.019	0.018	0.060	0.019	0.000	0.020	0.020	0.000
192	0.000	0.038	0.017	0.026	0.019	0.000	0.060	0.000	0.000	0.060	0.020	0.000
194	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.074	0.000	0.000	0.000	0.052
196	0.000	0.000	0.000	0.000	0.019	0.107	0.020	0.037	0.000	0.000	0.040	0.017
198	0.016	0.019	0.033	0.000	0.019	0.054	0.020	0.000	0.021	0.020	0.040	0.017
200	0.016	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000
202	0.016	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.020	0.000	0.000
204	0.016	0.000	0.017	0.000	0.019	0.018	0.000	0.019	0.042	0.040	0.020	0.052
206	0.016	0.000	0.000	0.000	0.000	0.018	0.000	0.019	0.000	0.020	0.000	0.000
208	0.047	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.017
210	0.000	0.000	0.000	0.000	0.000	0.018	0.020	0.019	0.000	0.000	0.020	0.017
212	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.020	0.000	0.000
214	0.000	0.000	0.000	0.000	0.000	0.071	0.000	0.000	0.021	0.000	0.000	0.000
216	0.063	0.000	0.167	0.053	0.096	0.000	0.000	0.000	0.063	0.000	0.060	0.034
218	0.031	0.077	0.017	0.053	0.058	0.000	0.060	0.074	0.000	0.000	0.000	0.328
220	0.078	0.115	0.133	0.132	0.000	0.000	0.120	0.056	0.146	0.040	0.000	0.052
222	0.094	0.000	0.033	0.026	0.019	0.000	0.060	0.000	0.021	0.020	0.000	0.000
224	0.000	0.058	0.000	0.000	0.038	0.018	0.020	0.000	0.104	0.040	0.180	0.034
226	0.047	0.000	0.000	0.026	0.077	0.000	0.120	0.037	0.021	0.000	0.080	0.034
228	0.109	0.096	0.000	0.105	0.096	0.000	0.000	0.056	0.208	0.200	0.000	0.052
230	0.047	0.058	0.033	0.079	0.058	0.018	0.060	0.056	0.063	0.020	0.000	0.000
232	0.063	0.038	0.050	0.053	0.038	0.000	0.120	0.111	0.083	0.080	0.000	0.000
234	0.000	0.000	0.117	0.184	0.000	0.071	0.000	0.000	0.000	0.040	0.040	0.000
236	0.016	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.040	0.000	0.000
238	0.125	0.000	0.000	0.079	0.058	0.018	0.000	0.000	0.042	0.000	0.120	0.034
240	0.000	0.019	0.000	0.053	0.000	0.036	0.000	0.000	0.021	0.000	0.000	0.000
242	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.037	0.021	0.040	0.040	0.103
244	0.000	0.000	0.000	0.000	0.115	0.036	0.060	0.037	0.042	0.020	0.000	0.000
246	0.094	0.038	0.000	0.079	0.058	0.000	0.000	0.000	0.021	0.020	0.040	0.000
248	0.000	0.058	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
250	0.000	0.019	0.000	0.000	0.000	0.000	0.020	0.019	0.000	0.020	0.000	0.000

Table B.2 Proportional allele frequencies per sampling location, for seven microsatellite loci screened in *Patella rustica* (continued).

Allele size	BIA	NOJ	BAN	CPR	MOL	VIA	HLE	BUA	SBE	CPR	CAT	ODA
252	0.000	0.058	0.000	0.000	0.038	0.071	0.000	0.037	0.000	0.020	0.000	0.000
254	0.000	0.212	0.000	0.000	0.000	0.036	0.000	0.037	0.000	0.000	0.020	0.000
256	0.031	0.019	0.100	0.000	0.000	0.000	0.000	0.037	0.000	0.000	0.020	0.000
258	0.031	0.000	0.050	0.000	0.019	0.036	0.000	0.019	0.000	0.040	0.040	0.000
260	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.034
262	0.000	0.000	0.000	0.000	0.000	0.054	0.000	0.037	0.000	0.000	0.000	0.000
264	0.000	0.000	0.000	0.000	0.000	0.071	0.000	0.019	0.000	0.000	0.000	0.000
266	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000
268	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000
270	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000
Pru13												
98	0.000	0.033	0.031	0.000	0.026	0.000	0.016	0.018	0.015	0.000	0.033	0.000
102	0.000	0.017	0.063	0.048	0.053	0.050	0.031	0.036	0.059	0.048	0.033	0.017
106	0.031	0.150	0.047	0.097	0.079	0.133	0.031	0.036	0.074	0.032	0.083	0.033
110	0.109	0.133	0.047	0.113	0.184	0.133	0.078	0.125	0.103	0.129	0.083	0.150
114	0.313	0.200	0.172	0.306	0.211	0.383	0.297	0.214	0.235	0.210	0.167	0.333
118	0.172	0.183	0.172	0.177	0.184	0.150	0.156	0.250	0.103	0.194	0.200	0.100
122	0.094	0.117	0.156	0.032	0.079	0.067	0.078	0.071	0.044	0.113	0.117	0.133
126	0.047	0.033	0.156	0.065	0.053	0.050	0.109	0.054	0.162	0.097	0.050	0.050
128	0.047	0.017	0.016	0.016	0.000	0.000	0.016	0.018	0.015	0.016	0.000	0.050
130	0.109	0.083	0.078	0.097	0.105	0.017	0.109	0.000	0.074	0.032	0.067	0.000
134	0.016	0.000	0.063	0.048	0.026	0.017	0.000	0.054	0.059	0.065	0.067	0.083
136	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
138	0.031	0.000	0.000	0.000	0.000	0.000	0.016	0.018	0.000	0.016	0.050	0.033
140	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000
144	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.044	0.000	0.017	0.000
148	0.016	0.000	0.000	0.000	0.000	0.000	0.016	0.071	0.015	0.032	0.033	0.000
152	0.000	0.017	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000
156	0.000	0.017	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000
162	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000
170	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017
Pru14												
100	0.000	0.017	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.000
104	0.095	0.000	0.100	0.031	0.000	0.129	0.067	0.019	0.036	0.021	0.069	0.093
108	0.381	0.617	0.633	0.563	0.717	0.581	0.667	0.648	0.518	0.521	0.466	0.389
110	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
112	0.071	0.000	0.017	0.031	0.000	0.000	0.000	0.000	0.036	0.063	0.069	0.074
114	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
116	0.071	0.067	0.067	0.031	0.083	0.032	0.083	0.056	0.036	0.063	0.017	0.093
120	0.310	0.300	0.150	0.219	0.183	0.258	0.183	0.259	0.321	0.250	0.293	0.296

Table B.2 Proportional allele frequencies per sampling location, for seven microsatellite loci screened in *Patella rustica* (continued).

Allele size	BIA	NOJ	BAN	CPR	MOL	VIA	HLE	BUA	SBE	CPR	CAT	ODA
124	0.048	0.000	0.017	0.000	0.000	0.000	0.000	0.019	0.000	0.042	0.000	0.000
128	0.024	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.036	0.021	0.000	0.056
132	0.000	0.000	0.017	0.000	0.017	0.000	0.000	0.000	0.018	0.021	0.034	0.000
140	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000

Appendix C

Maximum-likelihood estimates of population genetic parameters obtained with MIGRATE for *Patella rustica*

Table C.1 Maximum likelihood estimates and 95% confidence intervals (CI) for Θ ($4N_e\mu$) computed with MIGRATE from microsatellite data for 12 samples of *Patella rustica*. Location coordinates are listed in Table 6.1, locations are mapped in Figure 6.1.

Sampling location	Code	Θ	CI (95%)
Biarritz	BIA	0.963	0.854 - 1.091
Noja	NOJ	0.984	0.881 - 1.104
Bañugues	BAN	1.134	1.007 - 1.284
Cabo Prior	CPR	1.000	0.903 - 1.113
Moledo do Minho	MOL	1.100	0.984 - 1.235
Viana do Castelo	VIA	0.933	0.835 - 1.047
Homem do Leme	HLE	0.968	0.875 - 1.075
Buarcos	BUA	1.118	0.989 - 1.270
São Bernardino	SBE	1.277	1.131 - 1.449
Cabo Raso	CRA	1.164	1.028 - 1.326
Castelejo	CAT	1.062	0.951 - 1.190
Olhos d'Água	ODA	1.026	0.919 - 1.150

Table C.2 Maximum likelihood estimates and 95% confidence intervals (CI) for directional migration rates M (m/μ) computed with MIGRATE from microsatellite data for 12 samples of *Patella rustica*. Location names and coordinates are listed in Table 6.1, locations are mapped in Figure 6.1.

Locations	*M*	CI (95%)	Locations	*M*	CI (95%)
BIA - NOJ	1.076	0.736 - 1.509	NOJ-BIA	0.954	0.608 - 1.410
BIA - BAN	0.809	0.497 - 1.229	BAN - BIA	1.680	1.205 - 2.266
BIA - CPR	1.285	0.937 - 1.710	CPR - BIA	2.330	1.761 - 3.010
BIA - MOL	1.524	1.112 - 2.026	MOL - BIA	1.562	1.106 - 2.130
BIA - VIA	0.526	0.282 - 0.882	VIA - BIA	1.344	0.924 - 1.874
BIA - HLE	0.632	0.389 - 0.961	HLE - BIA	0.954	0.609 - 1.410
BIA - BUA	0.846	0.534 - 1.261	BUA - BIA	1.138	0.754 - 1.634
BIA - SBE	1.231	0.842 - 1.726	SBE - BIA	1.868	1.363 - 2.485
BIA - CRA	1.883	1.370 - 2.512	CRA - BIA	0.694	0.407 - 1.091
BIA - CAT	1.577	1.160 - 2.083	CAT - BIA	1.344	0.924 - 1.874
BIA - ODA	0.791	0.515 - 1.151	ODA - BIA	1.387	0.960 - 1.925
NOJ - BAN	0.383	0.184 - 0.691	BAN - NOJ	0.682	0.420 - 1.038
NOJ - CPR	0.571	0.351 - 0.867	CPR - NOJ	1.220	0.854 - 1.677
NOJ - MOL	1.594	1.173 - 2.107	MOL - NOJ	0.753	0.475 - 1.123
NOJ - VIA	2.104	1.563 - 2.756	VIA - NOJ	1.471	1.065 - 1.968
NOJ - HLE	0.765	0.494 - 1.122	HLE - NOJ	1.901	1.434 - 2.460
NOJ - BUA	0.966	0.630 - 1.406	BUA - NOJ	1.292	0.914 - 1.761
NOJ - SBE	1.106	0.739 - 1.577	SBE - NOJ	0.898	0.591 - 1.298
NOJ - CRA	3.298	2.597 - 4.114	CRA - NOJ	1.256	0.884 - 1.719
NOJ - CAT	1.016	0.690 - 1.432	CAT - NOJ	1.212	0.854 - 1.677
NOJ - ODA	1.352	0.979 - 1.809	ODA - NOJ	0.789	0.504 - 1.167
BAN - CPR	0.841	0.567 - 1.192	CPR - BAN	2.766	2.147 - 3.495
BAN - MOL	0.921	0.611 - 1.322	MOL - BAN	1.830	1.336 - 2.433
BAN - VIA	1.490	1.044 - 2.049	VIA - BAN	1.447	1.014 - 1.990
BAN - HLE	1.431	1.045 - 1.902	HLE - BAN	1.362	0.943 - 1.890
BAN - BUA	1.651	1.196 - 2.209	BUA - BAN	1.064	0.700 - 1.537
BAN - SBE	1.188	0.806 - 1.674	SBE - BAN	1.745	1.264 - 2.335
BAN - CRA	1.121	0.737 - 1.620	CRA - BAN	1.575	1.120 - 2.138
BAN - CAT	0.736	0.464 - 1.097	CAT - BAN	1.149	0.768 - 1.639
BAN - ODA	0.989	0.676 - 1.387	ODA - BAN	1.617	1.156 - 2.187
CPR - MOL	1.515	1.104 - 2.017	MOL - CPR	1.156	0.825 - 1.564
CPR - VIA	1.314	0.898 - 1.842	VIA - CPR	0.600	0.374 - 0.903
CPR - HLE	1.190	0.840 - 1.625	HLE - CPR	1.141	0.816 - 1.543
CPR - BUA	0.805	0.502 - 1.212	BUA - CPR	1.020	0.714 - 1.403
CPR - SBE	2.088	1.566 - 2.715	SBE - CPR	1.261	0.917 - 1.682
CPR - CRA	1.558	1.094 - 2.137	CRA - CPR	0.260	0.193 - 0.604
CPR - CAT	1.751	1.310 - 2.283	CAT - CPR	1.021	0.715 - 1.403
CPR - ODA	0.626	0.385 - 0.952	ODA - CPR	1.291	0.943 - 1.716

Table C.2 Maximum likelihood estimates and 95% confidence intervals (CI) for directional migration rates *M* (*m*/μ) computed with MIGRATE from microsatellite data for 12 samples of *Patella rustica* (continued).

Locations	*M*	CI (95%)	Locations	*M*	CI (95%)
MOL - VIA	0.920	0.581 - 1.372	VIA - MOL	1.276	0.903 - 1.739
MOL - HLE	1.498	1.102 - 1.979	HLE - MOL	2.623	2.069 - 3.266
MOL - BUA	0.523	0.288 - 0.862	BUA - MOL	0.638	0.387 - 0.980
MOL - SBE	2.785	2.175 - 3.501	SBE - MOL	0.248	0.107 - 0.480
MOL - CRA	1.344	0.918 - 1.884	CRA - MOL	1.063	0.727 - 1.490
MOL - CAT	1.471	1.070 - 1.962	CAT - MOL	0.921	0.611 - 1.322
MOL - ODA	1.319	0.951 - 1.771	ODA - MOL	1.063	0.727 - 1.490
VIA - HLE	0.199	0.079 - 0.405	HLE - VIA	1.356	0.931 - 1.892
VIA - BUA	0.685	0.409 - 1.063	BUA - VIA	0.833	0.512 - 1.265
VIA - SBE	2.171	1.638 - 2.809	SBE - VIA	0.745	0.445 - 1.157
VIA - CRA	0.631	0.355 - 1.021	CRA - VIA	1.139	0.756 - 1.635
VIA - CAT	1.182	0.825 - 1.628	CAT - VIA	2.060	1.526 - 2.706
VIA - ODA	0.923	0.622 - 1.309	ODA - VIA	1.139	0.756 - 1.635
HLE - BUA	0.795	0.493 - 1.201	BUA - HLE	0.899	0.601 - 1.281
HLE - SBE	1.883	1.390 - 2.481	SBE - HLE	1.331	0.960 - 1.787
HLE - CRA	0.987	0.630 - 1.458	CRA - HLE	0.499	0.287 - 0.796
HLE - CAT	0.771	0.492 - 1.139	CAT - HLE	2.230	1.737 - 2.807
HLE - ODA	1.220	0.868 - 1.656	ODA - HLE	1.032	0.710 - 1.439
BUA - SBE	0.944	0.609 - 1.384	SBE - BUA	1.691	1.230 - 2.255
BUA - CRA	1.166	0.773 - 1.673	CRA - BUA	1.091	0.729 - 1.557
BUA- CAT	0.631	0.382 - 0.968	CAT - BUA	1.852	1.367 - 2.440
BUA - ODA	1.022	0.703 - 1.426	ODA - BUA	1.406	0.989 - 1.926
SBE - CRA	0.448	0.225 - 0.787	CRA - SBE	1.393	0.975 - 1.915
SBE - CAT	1.226	0.863 - 1.678	CAT - SBE	0.983	0.640 - 1.430
SBE - ODA	1.319	0.951 - 1.771	ODA - SBE	1.229	0.840 - 1.723
CRA - CAT	1.900	1.437 - 2.452	CAT - CRA	0.673	0.387 - 1.073
CRA - ODA	0.132	0.041 - 0.306	ODA - CRA	1.794	1.293 - 2.408
CAT - ODA	1.352	0.979 - 1.809	ODA - CAT	0.842	0.549 - 1.225

Appendix D

Joint publications produced during the time of the Ph.D. studentship relevant to this thesis

Journal of Biogeography (J. Biogeogr.) (2006) 33, 812–822

ORIGINAL ARTICLE

Recent changes in the distribution of a marine gastropod, *Patella rustica* Linnaeus, 1758, and their relationship to unusual climatic events

Fernando P. Lima[1,2,3]*, Nuno Queiroz[1], Pedro A. Ribeiro[1,3,4], Stephen J. Hawkins[3,4] and António M. Santos[1,2]

[1]*CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, Vairão, Portugal,* [2]*Departamento de Zoologia-Antropologia, Faculdade de Ciências da Universidade do Porto, Porto, Portugal,* [3]*Marine Biological Association of the United Kingdom, Plymouth, UK and* [4]*School of Biological Sciences, University of Southampton, Southampton, UK*

*Correspondence: Fernando P. Lima, CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, 4485-661 Vairão, Portugal.
E-mail: fplima@fc.up.pt

ABSTRACT

Aim Recent colonization of northern Portuguese shores by *Patella rustica* Linnaeus, 1758, led to the bridging of a historical gap in the distribution known since the 1900s. Long-term oceanographic data collected over the last half-century were examined in order to detect possible mechanisms for the observed change in its distribution.

Location This study was carried out along the entire Portuguese coastline, from 41°50′ to 37°06′ N. Time-series of hydrographical variables (sea surface temperature and salinity) were derived for the Atlantic coast of the Iberian Peninsula.

Methods Abundance and size-frequency distributions of the newly observed limpet populations were compared with those from well-established populations in southern Portugal. Anomalies were computed for sea surface temperature (1950–2000) and sea surface salinity (1958–2001) data, covering the whole Atlantic coast of the Iberian Peninsula. An upwelling index (1967–2005) was derived for a single location within the distributional gap of *P. rustica*. Split moving window analysis was performed to detect significant discontinuities in hydrographical data sets.

Results *Patella rustica* has gradually been expanding in northern Iberia, and in the late 1990s the historical gap in distribution in northern Portugal was bridged. Size-frequency distribution differed between historical and recent populations, the latter lacking small-sized individuals. At the same time, several anomalous oceanographic events occurred off the Portuguese coast and were probably related to this expansion.

Main conclusions Although sea surface temperature might be a major determinant of the reproductive success of *P. rustica* and hence its dispersal potential, it is more likely that a coincidence of several factors occurring in the late 1990s provided exceptional conditions that allowed the geographical expansion of this species.

Keywords
Biogeography, climate change, dispersal, *Patella rustica*, Portugal, sea salinity, sea temperature, upwelling.

INTRODUCTION

Recent ecological literature reflects concerns with anthropogenic global climate change (Southward *et al.*, 1995; Stensen *et al.*, 2002; Walther *et al.*, 2002; Parmesan & Yohe, 2003; Root *et al.*, 2003) and has provided insights into the causes of species range limits and shifts in response to changing climatic conditions (Sagarin *et al.*, 1999; Sagarin & Gaines, 2002a;

www.blackwellpublishing.com/jbi
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Zacherl *et al.*, 2003). Keeping records of alterations in the environment and creating predictive models that enable the detection of variations in climate is one of today's primary aims in ecology (Lewis, 1999; Hawkins *et al.*, 2003; Hiscock *et al.*, 2004).

Changes in the distribution and abundance of several marine species on the eastern coasts of the Atlantic and Pacific oceans have been particularly well documented over the last decades. Consistent responses to climate change have been described at different trophic levels for both sessile and pelagic assemblages (Southward *et al.*, 1995; Holbrook *et al.*, 1997; Sagarin *et al.*, 1999; Wethey, 2002; Beaugrand & Reid, 2003; Borges *et al.*, 2003; Hawkins *et al.*, 2003; Zacherl *et al.*, 2003; Genner *et al.*, 2004; Hiscock *et al.*, 2004; Paine & Trimble, 2004). In contrast to pelagic systems, intertidal communities are ideal for studying climate-driven community changes because the constituent species are typically long-lived, less influenced by short-term factors and respond more readily to multi-decadal periods of environmental variability (Barry *et al.*, 1995; Southward *et al.*, 1995). In addition, their ranges are roughly one-dimensional and easily defined as the coastal endpoints. Therefore, conspicuous, easily identified, slow moving or sedentary rocky shore species are some of the best candidates to monitor climate changes (Lewis, 1996, 1999; Sagarin & Gaines, 2002b; Hiscock *et al.*, 2004).

Because alterations of the environment affect a series of physiological factors that act on population-level processes, it is thought to be more important to understand the mechanisms that influence population dynamics and the synchrony between climatic oscillations and species life cycles, instead of studying only the physiological limitations of individuals (Holbrook *et al.*, 1997; Zacherl *et al.*, 2003; Hagberg *et al.*, 2004). Thus, quantitative approaches like studies of the populations dynamics of newly established populations are encouraged (Kolar & Lodge, 2001), and may indicate which processes determine the success or failure of a particular shift or invasion (Zacherl *et al.*, 2003; Genner *et al.*, 2004), allowing more successful prediction of the patterns of invasive species.

The Portuguese coast is particularly well suited for the study of the putative effect of climate warming on species distribution for several reasons. First, the clear north–south orientation of the coastline is the most appropriate for this type of analysis (Rivadeneira & Fernández, 2005). Second, it is characterized by particular hydrographical features, with a cooler northern region affected by both upwelling and rainfall leading to a higher volume of river run-off and a much warmer southern region with a strong Mediterranean influence (Sánchez & Relvas, 2003; Santos *et al.*, 2004; Peliz *et al.*, 2005). Third, this is a contact region between warm- and cold-water species, where both northern and southern boundaries of several organisms can be found (Fischer-Piètte, 1959, 1963; Fischer-Piètte & Gaillard, 1959; Ardré, 1970; Santos, 2000; Boaventura *et al.*, 2002). Finally, shifts in species distribution have been described since the 1950s, not only for this particular stretch of coastline but also for the Iberian Peninsula (Fischer-Piètte & Forest, 1951; Fischer-Piètte, 1957a,b, 1960; Fischer-Piètte & Kirsch, 1957; Ardré, 1971; Santos, 2000; Pereira *et al.*, 2006).

The geographical distribution of *Patella rustica* Linnaeus, 1758, ranges from the Mediterranean to the Atlantic coast of the Iberian Peninsula and northern Africa, including the Macaronesian Islands (Ridgway *et al.*, 1998). In the Atlantic, its southern limit is speculated to occur further south than Mauritania, whilst its northern limit is located at the French Basque Country (Fischer-Piètte, 1955; Crisp & Fischer-Piètte, 1959). Within this range there is a well-documented gap. Hidalgo (1917) placed it between the locations of Nazaré (Portugal, 39°36′16″ N, 9°05′08″ W) in the south, and La Coruña (NW Spain, 43°22′54″ N, 8°26′23″ W) in the north. Although the work of Nobre (1940) provides no information regarding the northern boundary of this species, he identified São Pedro de Moel (39°45′34″ N, 9°01′58″ W) as the northern limit of *P. rustica* in Portugal.

The works of Fischer-Piètte (1955) and Fischer-Piètte & Gaillard (1959) still remain the most comprehensive accounts of the distribution and abundance of patellid limpets on the Atlantic coast of the Iberian Peninsula. According to these authors, during the 1950s *P. rustica* was still largely absent from northern Portugal (surprisingly, one individual was found at Vila do Conde, 41°21′05″ N, 8°45′21″ W). From its northern geographical boundary in Biarritz (south-west France) toward La Coruña, densities were described as progressively decreasing. However, they observed new populations in Galicia (north-west Spain), with limpets occurring in low densities only at very exposed locations. After a detailed review of Hidalgo's (1917) work, Fischer-Piètte & Gaillard (1959) concluded that they were observing an on-going expansion of *P. rustica* in the northern part of the distributional gap.

More recent data, based on multiple surveys carried out between 1993 and 1998 over the entire Portuguese coast (Santos, 2000), confirmed the southern boundary of the distributional gap in central Portugal (São Martinho do Porto, 39°30′43″ N, 9°08′33″ W). In 1996, S. J. Hawkins (pers. obs.) spotted a single *P. rustica* at Moledo do Minho. Despite continuous sampling in the area since 1993, it was only in the winter of 2002 that several individuals (> 20) were recorded at Homem do Leme (Porto), within the historical gap. A subsequent survey revealed that the species was already present at very low densities in several shores north of Porto.

This study describes the recent bridging of the 280 km distributional gap of *P. rustica* in northern Portugal. Density and size-frequency distribution of the newly observed populations were compared with those from well-established southern populations, and long-term hydrographical data were used both to demonstrate the gap area as one with anomalous oceanographic conditions and to formulate alternative hypotheses to explain the recent change in the geographical distribution of this species. Plausible hypotheses, like changes in upwelling regimes, sea surface temperature or coastal current patterns are discussed, and a testable explanatory model is suggested.

MATERIAL AND METHODS

On the Atlantic coasts of Europe and North Africa, *P. rustica* is normally found in the upper eulittoral zone (usually above mean high water neap) of exposed rocky shores. Although other limpet species (*Patella vulgata* Linnaeus, 1758 and *Patella depressa* Pennant, 1777) also occur in this zone, *P. rustica* is easily identified by the characteristic black spots that ornament its shell. This feature can be observed even in small (*c.* 5 mm) animals, so juvenile identification does not pose any problems. In addition, *P. rustica* has a strong preference for vertical walls or very steep surfaces and, at least on the Portuguese coast, is seldom found outside this habitat.

During February 2003, density and size-frequency surveys of *P. rustica* where carried out in two different regions. The first encompassed recently colonized locations within the distributional gap of *P. rustica* in northern Portugal, whilst the second included historically inhabited areas in central and southern Portugal. In each region, six shores were visited (Fig. 1). Within the gap area, the southern half consists mainly of large stretches of sandy shores, interspersed with a few rocky shores that do not provide suitable habitat for *P. rustica* (no rocky substrate above mean tide level). An initial survey was done over the entire area, but the species was only found in the northern half of the gap, which is mainly rocky. Since the recent colonization of northern Portugal could be an on-going process rather than a single event, sampling was repeated in February 2005 for the northern shores.

On the southern shores, the density of limpets was estimated using several haphazardly placed 50 × 50 cm quadrats. Due to the high density of limpets, and to avoid bias toward larger size classes, size-frequency data were collected by measuring all limpets within each quadrat. The maximum length of each limpet was measured with digital callipers and rounded to the nearest millimetre. Because low-water spring tides occur in the early morning (07:00–09:00), sampling time was roughly limited to 3 h on each shore. At northern locations, in order to obtain comparable values of density, a 3-h survey was also undertaken. However, because densities were very low, all individuals observed were counted and measured. Length–frequency histograms were built for each sample using 2 mm size classes.

To test for non-independence between size structure and location a chi-square test was used. Limpets were divided into three length classes: < 13 mm, 13–28 mm and > 28 mm. The choice of the interval to represent non-reproductive (juvenile) stages was problematic because size at sexual maturity is unknown for *P. rustica*. The upper limit of 12 mm was based on the assumption that it would be similar to the value already known for *P. depressa* (Guerra & Gaudêncio, 1986; P. A.

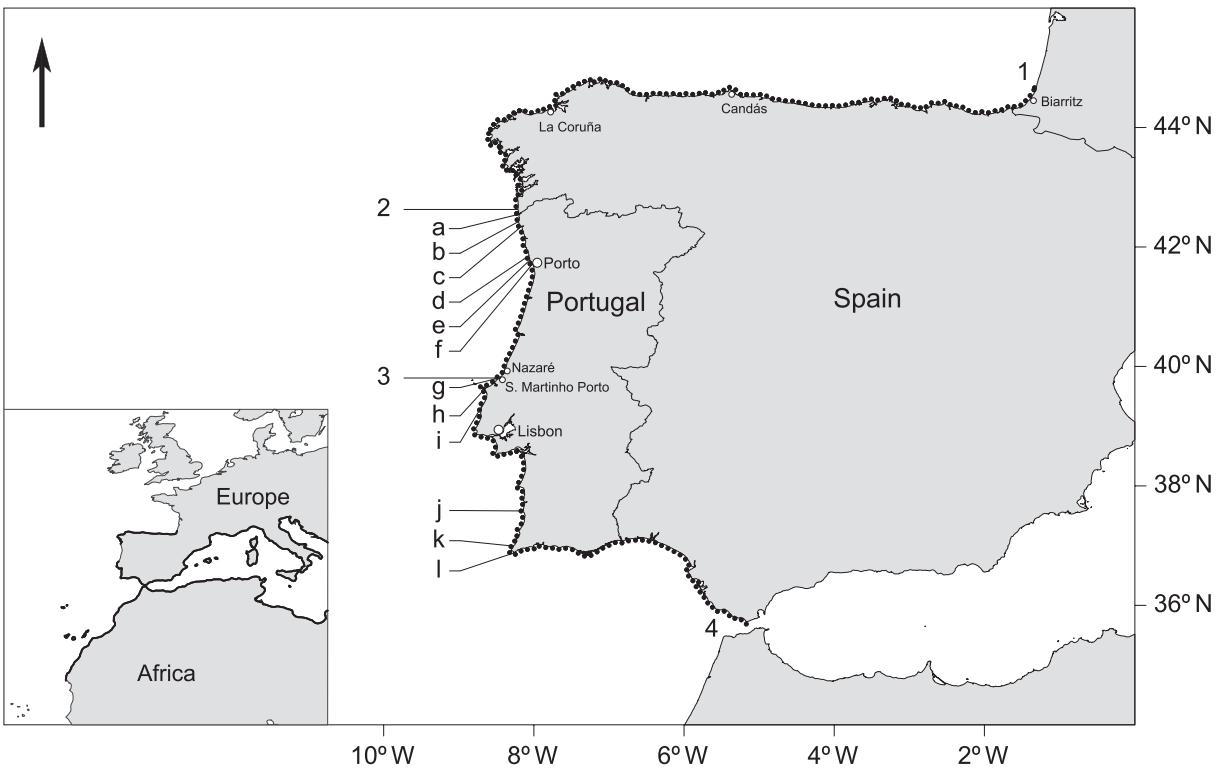

Figure 1 Study area. 1, Biarritz (northern limit of *P. rustica* distribution). The coastline between 2 (Baiona) and 3 (São Martinho do Porto) is the historical gap in northern Portugal. 4, Strait of Gibraltar. Locations visited: (a) Moledo do Minho, (b) Montedor, (c) Forte da Vigia, (d) Mindelo, (e) Cabo do Mundo, (f) Homem do Leme, (g) São Martinho do Porto, (h) Baleal, (i) Santa Cruz, (j) Amoreira, (k) Castelejo and (l) Beliche. Hydrographical coastal sampled points are represented as black circles. Inset figure shows known distribution of *P. rustica* prior to recent expansion.

Ribeiro, unpubl. data), which displays a largely overlapping Atlantic distribution.

Sea surface temperature (SST) along the Atlantic coast of the Iberian Peninsula, from January 1950 to December 2000, was derived from *in situ* raw data obtained from the International Comprehensive Ocean–Atmosphere Data Set (ICOADS; Woodruff *et al.*, 1988). To avoid bias due to different daytime measurements, only data from 12:00 were used. Point data were imported into the GRASS Geographical Information System (GRASS Development Team, 2005) and transformed into raster maps with a spatial resolution of 4 × 4 km using a surface interpolation method. Each cell was derived from the 12 nearest data points using the distance squared weighting algorithm (Jarvis & Stuart, 2001).

Sea surface salinity (SSS) data, with an average resolution of 0.5° latitude × 0.5° longitude cells, were extracted from the SODA-POP v1.2 model data set, available at the National Virtual Ocean Data System live server webpage (http://ferret.pmel.noaa.gov/NVODS/servlets/dataset) and were imported into GRASS GIS. Data were available for the period between January 1958 and December 2001.

The resulting set of 612 (51 years × 12 months) SST and 528 (44 years × 12 months) SSS maps was stored in GRASS GIS as monthly layers and sampled along the Iberian coastline at regularly spaced points, 10 km distant from each other and 2 km offshore. The resulting data for each physical parameter were combined to build a table with each row denoting a geographical location and each column a month/year combination. Monthly standardized anomalies were computed by subtracting from each value the monthly mean across all years and dividing it by the monthly standard deviation. To identify significant hydrographical time-series discontinuities on the recently colonized area, split moving window (SMW) boundary analysis (Ludwig & Cornelius, 1987; Cornelius & Reynolds, 1991) was carried out for SSS and SST anomalies from the area between 39°30′ and 41°54′ N. This technique has been successfully applied to time-series of both biological and physical variables (Beaugrand & Reid, 2003; Beaugrand, 2003, 2004). Because the SMW outcome may vary as a function of window size, three window sizes were used (12, 24 and 48 months). Only results simultaneously detected by the three windows were considered.

A monthly based upwelling index for the location of 41°00′ N, 10°00′ W (off Porto) and from January 1967 to March 2005 was provided by the Pacific Fisheries Environmental Laboratory (PFEL; http://las.pfel.noaa.gov/las/main.pl). A 4-month running mean was computed to remove small-scale variation.

RESULTS

In 2003, recently established northern populations consisted of sparsely distributed and isolated individuals (Table 1). Although the 3-h sampling sessions were enough to cover the entire rocky surface of the appropriate high shore at each location, a maximum of 148 limpets was found at Homem do Leme and two shores appeared to completely lack this species, as not one individual was found. A sharp gradient of abundance, with number of individuals decreasing from south to north, was observed. In contrast, on southern shores densities were always higher than 60 individuals per m^2.

By 2005, all six shores on the northern Portuguese coast had been colonized by *P. rustica*. The total number of limpets in each locality increased several fold after 2003. However, a clear gradient of abundance, with number of individuals decreasing toward the north, could still be observed.

Length–frequency histograms showed a marked difference between recently colonized locations in northern Portugal and southern historically inhabited areas (Fig. 2). A chi-square test revealed no independence between length classes and sampled

Table 1 Density (limpets m^{-2}) and modal class with minimum and maximum sizes (mm) of *P. rustica* sampled at each location during 2003 and 2005 surveys. – indicates the absence of this species in that site and year. Real counts are shown in parentheses. Locations not visited are indicated with n.a.

		2003		2005	
Site	Geographical location	Density	Modal class (min–max)	Density	Modal class (min–max)
Moledo do Minho	41°50′41″ N, 8°52′07″ W	(0)	–	(109)	19 (12–30)
Montedor	41°44′30″ N, 8°52′37″ W	(7)	24 (17–26)	(71)	21 (12–32)
Forte da Vigia	41°41′55″ N, 8°51′22″ W	(0)	–	(92)	22 (13–31)
Mindelo	41°18′37″ N, 8°44′32″ W	(46)	29 (17–43)	(246)	29 (16–42)
Cabo do Mundo	41°13′17″ N, 8°42′56″ W	(87)	24 (13–37)	(109)	22 (14–34)
Homem do Leme	41°09′22″ N, 8°40′58″ W	(148)	22 (13–37)	(279)	25 (12–39)
São Martinho do Porto	39°30′39″ N, 9°08′36″ W	83 (250)	23 (5–30)	n.a.	n.a.
Baleal	39°22′29″ N, 9°20′21″ W	98 (295)	20 (5–34)	n.a.	n.a.
Santa Cruz	39°08′02″ N, 9°23′00″ W	70 (211)	17 (7–25)	n.a.	n.a.
Amoreira	37°20′57″ N, 8°50′47″ W	65 (196)	20 (8–31)	n.a.	n.a.
Castelejo	37°06′01″ N, 8°56′43″ W	86 (259)	22 (8–42)	n.a.	n.a.
Beliche	37°01′30″ N, 8°57′48″ W	98 (295)	23 (7–34)	n.a.	n.a.

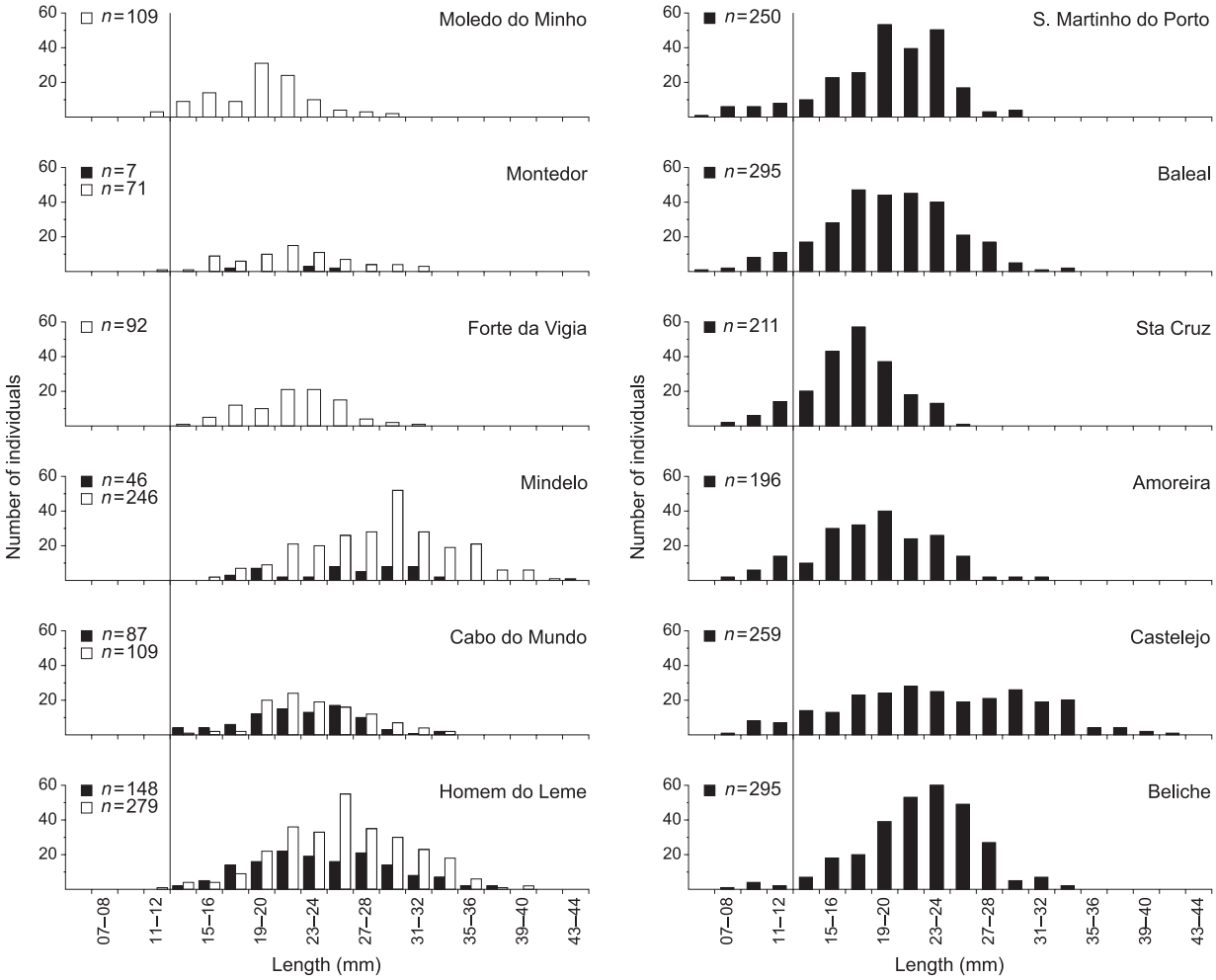

Figure 2 Length–frequency distributions for 2003 (black bars) and 2005 (white bars) surveys. Vertical lines denote a size of 13 mm, used to distinguish between juvenile and adult classes.

locations ($\chi^2 = 105.00$, d.f. = 30, $P < 0.05$), meaning that the population structure was significantly different between sites. All northern populations had fewer small individuals than expected. In 2003 they were composed solely of adults and in 2005 only 0.75% of the observed limpets belonged to the smaller size class. By contrast, populations in the historic portion of the range had a considerably higher frequency of juveniles (7.65%). In 2005, two different patterns in population structure were observed in the newly colonized sites: whilst populations settled before 2003 had a high proportion of adults of more than 28 mm (35.64%), in those populations established in subsequent years this percentage was only 4.41%.

ICOADS monthly mean SST showed a consistent annual pattern throughout the 51-year time-series, with evident seasonality. During winter and spring, a temperature gradient is established along the Iberian coast, from cold waters in Biarritz (south-west France) to the warmer zone in the Strait of Gibraltar (Fig. 3a). However, between summer and autumn the situation is not so linear. Although there is no change in the southern half of the gradient (3–4, Fig. 3a), the northern half experiences an inversion of the winter/spring pattern as surface waters become warmer (1–2, Fig. 3a). As a result, both extremes of the Iberian coast are warmer than northern Portugal (2–3, Fig. 3a). This colder region between these extremes matched the area from which *P. rustica* was traditionally absent.

SST anomalies were predominantly higher since 1990, with particularly warm sea water temperatures in the autumn, winter and spring during 1997 and 1998. Negative anomalies were almost absent for the last 15 years of the time-series. On the area between 39°30′ and 41°54′ N, SMW gradient analysis revealed one significant discontinuity in mid-1997 (Fig. 3b). This anomaly corresponds to the abrupt transition between one period of relative normality and another characterized by an unusual increase in SST.

POP-SODA sea surface salinity data also showed strong annual seasonality. Usually, SSS increases in warmer seasons and decreases during winter and spring (Fig. 3c). Even though salinity is generally inversely proportional to latitude, on the northern Portuguese coast it is lower than the surrounding

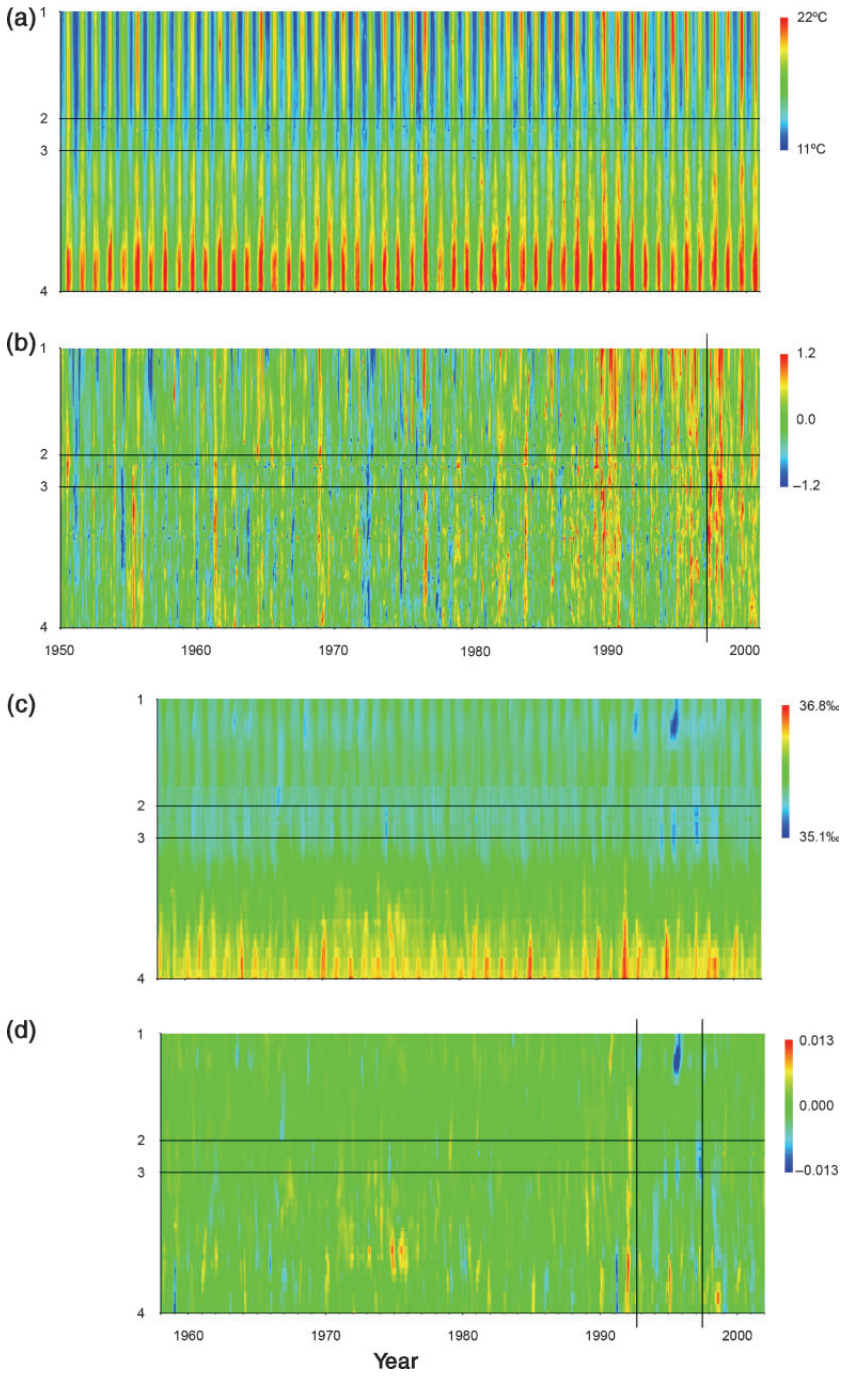

Figure 3 Monthly mean SST (a), SST normalized anomalies (b), SSS (c) and SSS anomalies (d) along the Atlantic coast of the Iberian Peninsula. Horizontal lines (2, 3) limit the historical gap in northern Portugal (see Fig. 1 for locations). Vertical lines correspond to significant SMW results.

areas, reaching values only comparable to those observed in the Bay of Biscay. For the area between 39°30′ and 41°54′ N, SMW gradient analysis revealed two significant discontinuities (Fig. 3d): one in the autumn of 1992 and another in the summer/autumn of 1997. The first anomaly corresponds to a situation where a high-salinity phase that took place in the spring of 1992 ended abruptly and the second denotes a step transition from a period of anomalous low salinity (during the spring of 1997) to a state of relative normality.

Upwelling off the northern Portuguese coast typically occurs from late spring to late autumn (Lemos & Pires, 2004; Peliz *et al.*, 2005). Although this pattern was relatively constant between 1967 and 2005, several anomalous episodes were identified (Fig. 4). The summer upwelling indexes of 1997, 1998 and 1999 were the lowest ever, reaching a historical minimum in 1997. Moreover, the lowest winter upwelling value over the entire time-series was recorded in 1998.

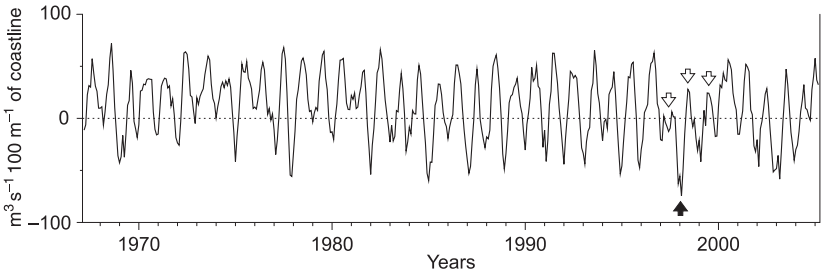

Figure 4 Four-month running mean computed on upwelling index (m^2 s^{-1} per 100 m of coastline) for the location of 41°00′ N 10°00′ W, between January 1967 and March 2005. The black arrow denotes the 1997/98 winter anomaly and white arrows indicate summer minimums from 1997 to 1999.

DISCUSSION

The present results show unequivocally that *P. rustica* has recently colonized the northern Portuguese coast, leading to the bridging of a historical gap in distribution known since the early 1900s. From 2003 onward, there was an increase in the number of colonized locations, and most notably in the number of individuals present at each location. The estimation of the minimum time of colonization based solely on length–frequency data would require knowledge of the growth rates of *P. rustica*. However, this parameter has not yet been assessed and results from growth studies of other *Patella* species are discrepant. For example, Guerra & Gaudêncio (1986) suggest a possible longevity of no more than 4 years for *Patella ulyssiponensis* Gmelin, 1791, *P. depressa* and *P. vulgata* on the Portuguese coast, based on length–frequency analysis. Since these species reach a maximum size of 3–4 cm in the region, growth rates must be relatively high, especially during the early stages of benthic life. In contrast, at higher latitudes (the UK and France) estimated growth rates fall into the range of 1–5 mm $year^{-1}$ for *P. vulgata* and *P. depressa*, with a maximum life span of 5–16 years, depending on habitat conditions (Fischer-Piètte, 1941; Bowman, 1981).

Taking a conservative estimate of 4–6 years for the maximum longevity of *P. rustica*, and given that the largest individuals were observed in some of the northern shores, data from the present work suggest that colonization of the northern Portuguese coast by this species started in the late 1990s. Additionally, the lack of small size classes, when compared with the size-frequency distributions of well-established southern populations (where breeding and recruitment are thought to be more regular and successful) may indicate that populations of *P. rustica* in northern Portugal resulted from sporadic settlement events (Lewis, 1986; Zacherl *et al.*, 2003).

High growth rates of early life stages can explain, in part, the absence of limpets on two northern shores (Moledo do Minho and Forte da Vigia) during the 2003 surveys and their subsequent occurrence in 2005. Yet, a 2-year period seems not to be enough to explain the presence of the largest limpets. It is possible that juvenile limpets use different nursery habitats on northern shores, thus being difficult to observe during their initial life stages. Because in the midshore zone extensive sampling of the mussel/barnacle mosaic and rock pools in the scope of environmental monitoring studies never revealed a single *P. rustica*, whilst other species of *Patella* were abundant, it is more likely that cryptic habits, like sheltering in crevices in the upper shore, may have rendered these individuals almost impossible to spot.

Finding the factors that maintained the distributional gap until the late 1990s is a first step to understanding the causes of the recent colonization of *P. rustica* in northern Portugal. As previously stated, between 39°30′ and 41°54′ N, the Portuguese coast displays oceanographic features that are distinct from the surrounding areas. Considering that the breeding period begins in July and spawning occurs between August and November/December (Ibanez *et al.*, 1986), low temperatures during this phase of the life cycle could have inhibited juvenile development, particularly because immature molluscs are very sensitive to severe conditions during settlement and early life stages (Lewis, 1986). Coincidently, an increase in average SST was observed on the Portuguese coast during recent decades (Lemos & Pires, 2004; Peliz *et al.*, 2005). Moreover, recent sea surface warming during autumn/early winter, as shown by the analysis of SST anomalies, might have provided temperatures within the limited range suitable for metamorphosis and fast growth, allowing limpets to attain a sufficient size to survive the coming winter. Northern Portuguese populations of *P. rustica* are characterized by low densities and predominance of large individuals, which is typical of populations that fail to reproduce successfully and hence have infrequent recruitment success owing to thermal stress at the northern boundary of their range, because of low temperatures (Lewis *et al.*, 1982; Lewis, 1986; Zacherl *et al.*, 2003).

Apparently, intolerance to low salinity alone is not a plausible explanation for the maintenance of such a long-term gap, since SSS in this area within the historical gap is similar to that observed in the Bay of Biscay, where *P. rustica* has been recorded since the early 1900s. Flow fields often observed in association with biogeographical boundaries have the potential to constrain a species' geographical range, even when suitable habitat is available elsewhere (Gaylord & Gaines, 2000). Therefore, another explanation for the maintenance of the gap would be a deficient larval input resulting from adverse sea currents. Although it was not possible to obtain detailed data on surface currents, it is known that over the continental shelf current intensity and direction are closely related to upwelling intensity and to the development of the Western Iberia Buoyant Plume (WIBP). During negative upwelling periods (downwelling), the predominant southward offshore currents

decrease in intensity and inshore poleward circulation rises in strength (Figueiras *et al.*, 2002; Peliz *et al.*, 2002; Sánchez & Relvas, 2003). The WIBP consists of a low-salinity surface water layer fed by the winter-intensified run-off of several rivers on the northwest coast of Portugal and Spain (Santos *et al.*, 2004). The development of the WIBP results in isolation of inner-shelf waters and strong poleward transport (Peliz *et al.*, 2002, 2005). In years with strong buoyant discharge and weak upwelling, northward coastal transport is enhanced. On the contrary, strong upwelling, reduced river run-off or both, will have the opposite effect (Santos *et al.*, 2004).

Assuming the reproductive period suggested by Ibanez *et al.* (1986), larval release and dispersal will occur when equatorward currents are at their maximum, because in late summer/autumn upwelling is usually strong and river run-off is at its minimum due to the drier summer season. Surprisingly, even though in the last decades hydrographical conditions remained stable, favouring larval drift from Galicia, there is no evidence of successful colonization. It is possible that southern Galician populations are not reproductive and subsist only through repopulation by larvae that arrive from north-west Spain every autumn. In this case, larval drift coupled with larval life span may have limited dispersal toward the south. On the other hand, sporadic observation of isolated individuals within the historical gap (see the Introduction) may indicate that settlement has indeed taken place but is intermittent, or somehow that individuals did not survive to reach adult stages. Although not easily identifiable, this phenomenon is probably more frequent than assumed (Sax & Brown, 2000; Zacherl *et al.*, 2003), and is a more likely explanation for the maintenance of the gap under conditions that favour larval transport from Galicia.

Lower SSS values on the northern Portuguese coast from 1995 onward indicate an increase of the WIBP. In addition, from 1997 to 1999 upwelling was consistently low. These two phenomena combined could have favoured larval transport from central and southern Portugal toward the north. Taking into account that during this period positive SST anomalies could have reduced the thermal pressure on early life stages, it is plausible to assume a scenario where a combination of several oceanographic variables allowed southern larvae to drift northward, settle and survive in such numbers that populations started to develop. The observed decrease in number of individuals toward the north, which was consistent in 2003 and 2005 surveys, also supports the hypothesis that colonization occurred in that direction.

The multi-factor explanation is more plausible than supposing that just one factor was responsible for the observed changes. In many cases, species range limits are determined by the interaction of several factors, including ocean currents, upwelling, salinity, fetch, sea temperature, as well as the type of planktonic development (Crisp & Southward, 1958; Menge, 2000; Zacharias & Roff, 2001; Rivadeneira & Fernández, 2005). By overlapping SST, SSS and upwelling time series it was possible to find a common time window, from the beginning of 1997 to the end of 1998, when it is more likely that the expansion has taken place. Moreover, 1997 was an exceptionally mild year over Europe, with a winter season characterized by high temperatures and intense rainfall (Dong *et al.*, 2000), and 1998 was the warmest year of the last millennium in the Northern Hemisphere (IPCC, 2001).

As proposed by Lewis (1986), occasional successful settlement may result in a sudden increase in numbers, which then persist conspicuously for several years. In the present case, however, several independent settlement episodes have to be considered, since between 2003 and 2005 the number of individuals increased several fold in colonized sites, and new populations were found. Two explanations are possible and not mutually exclusive: one or more new colonization episodes occurred with larvae that originated from historical locations, or reproduction of the newly established populations was successful, providing larvae that not only settled on the same shores but also expanded to the vicinity. Once critical population size was established self-recruitment would be possible.

The recent colonization described is most likely the corollary of the expansion process observed in Galicia during the 1950s by Fischer-Piètte & Gaillard (1959), which was also coincident with a warmer period in north-west Europe (see Hawkins *et al.*, 2003 for a review). However, the lack of detailed oceanographic data sets for that time frame in northern Spain make it impossible to correlate their findings objectively with changes in the environment.

A detailed knowledge of the population dynamics of this species, particularly in this geographical region, is essential for a better understanding of the colonization process. Determining the exact extent of the reproductive period, as well as the growth rate and life span of *P. rustica*, will reduce the uncertainties related to the time of colonization. The multi-factor hypothesis presented here describes a sequence of climatic and hydrographical events that ultimately led to the colonization of northern shores. It implicitly assumes that this colonization occurred from the south. Provided that there is a reasonable degree of genetic differentiation between northern and southern populations, this hypothesis can be tested using highly variable molecular markers. Thus, studies on population genetics should help to identify the source of newly established populations of *P. rustica* in northern Portugal and also to determine possible past expansion and/or retraction events in this region.

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REFERENCES

Ardré, F. (1970) Contribution à l'étude des algues marines du Portugal I. La Flore. *Portugaliae Acta Biologica*, **10,** 1–423.

Ardré, F. (1971) Contribution à l'étude des algues marines du Portugal II. Ecologie et Chorologie. *Bulletin du Centre d'Etudes et de Recherches Scientifiques, Biarritz*, **8,** 359–574.

Barry, J.P., Baxter, C.H., Sagarin, R.D. & Gilman, S.E. (1995) Climate-related, long-term faunal changes in a California rocky intertidal community. *Science*, **267,** 672–675.

Beaugrand, G. (2003) Long-term changes in copepod abundance and diversity in the north-east Atlantic in relation to fluctuations in the hydroclimatic environment. *Fisheries Oceanography*, **12,** 270–283.

Beaugrand, G. (2004) The North Sea regime shift: evidence, causes, mechanisms and consequences. *Progress in Oceanography*, **60,** 245–262.

Beaugrand, G. & Reid, P.C. (2003) Long-term changes in phytoplankton, zooplankton and salmon related to climate. *Global Change Biology*, **9,** 801–817.

Boaventura, D., Ré, P., Fonseca, L.C. & Hawkins, S.J. (2002) Intertidal rocky shore communities of the continental Portuguese coast: analysis of distribution patterns. *PSZN Marine Ecology*, **23,** 69–90.

Borges, M.F., Santos, A.M.P., Crato, N., Mendes, H. & Mota, B. (2003) Sardine regime shifts off Portugal: a time series analysis of catches and wind conditions. *Scientia Marina*, **67,** 235–244.

Bowman, R.S. (1981) The morphology of *Patella* spp. juveniles in Britain and some phylogenetic inferences. *Journal of the Marine Biological Association of the United Kingdom*, **61,** 647–666.

Cornelius, J.M. & Reynolds, J.F. (1991) On determining the statistical significance of discontinuities within ordered ecological data. *Ecology*, **72,** 2057–2070.

Crisp, D.J. & Fischer-Piètte, E. (1959) Répartition des principales espèces intercotidales de la côte atlantique française en 1954–1955. *Annales de l'Institut Océanographique Paris*, **36,** 275–388.

Crisp, D.J. & Southward, A.J. (1958) The distribution of intertidal organisms along the coasts of English Channel. *Journal of the Marine Biological Association of the United Kingdom*, **37,** 1031–1048.

Dong, B.W., Sutton, R.T., Jewson, S.P., O'Neill, A. & Slingo, J.M. (2000) Predictable winter climate in the North Atlantic Sector during 1997–1999 ENSO cycle. *Geophysical Research Letters*, **27,** 985–988.

Figueiras, F.G., Labarta, U. & Fernández Reiriz, M.J. (2002) Coastal upwelling, primary production and mussel growth in the Rías Baixas of Galicia. *Hydrobiologia*, **484,** 121–131.

Fischer-Piètte, E. (1941) Croissance, taille maxima et longevite possible de quelques animaux intercotidaux en fonction du milieu. *Annales de l'Institut Océanographique Paris*, **21,** 1–28.

Fischer-Piètte, E. (1955) Repartition le long des cotes septentrionales de l'Espagne, des principales éspèces peuplant les rochers intercotidaux. *Annales de l'Institut Océanographique Paris*, **31,** 37–124.

Fischer-Piètte, E. (1957a) Sur les déplacements des frontières biogéographiques, observés au long des côtes ibériques dans le domain intercotidal. *Publicaciones del Instituto de Biologia Aplicada*, **26,** 35–40.

Fischer-Piètte, E. (1957b) Sur les progrès des espèces septentrionales dans le bios intercotidal ibérique: situation en 1956–1957. *Comptes Rendus des Scéances de l'Académie des Sciences*, **245,** 373–375.

Fischer-Piètte, E. (1959) Pelvetia canaliculata examinée de proche en proche de la Manche au Portugal. *Colloques Internationaux du Centre National de la Recherche Scientifique*, pp. 65–73, Dinard, 20–28 Septembre, 1957. Centre National de la Recherche Scientifique. Paris, France.

Fischer-Piètte, E. (1960) Nouvelle avance de mollusques intercotidaux septentrionaux vers le Sud du Portugal. *Comptes Rendus des Scéances de l'Académie des Sciences*, **251,** 1–2.

Fischer-Piètte, E. (1963) La distribution des principaux organisms nord-ibériques en 1954–55. *Annales de l'Institut Océanographique Paris*, **40,** 165–311.

Fischer-Piètte, E. & Forest, J. (1951) Noveau progress du cirripede austral *Elminius modestus* Darwin sur les cotes Atlantiques Françaises et Iberiques. *Chemosphere*, **2,** 293–299.

Fischer-Piètte, E. & Gaillard, J.-M. (1959) Les patelles au long des côtes atlantiques Ibériques et Nord-Marocaines. *Journal of Conchology*, **99,** 135–200.

Fischer-Piètte, E. & Kirsch, B.S. (1957) Sur la distribution de *Monodonta colubrina* Gould. Espèce nouvelle pour la faune Française. *Journal of Conchology*, **92,** 25–32.

Gaylord, B. & Gaines, S. (2000) Temperature or transport? Range limits in marine species mediated solely by flow. *The American Naturalist*, **155,** 769–783.

Genner, M.J., Sims, D.W., Wearmouth, V.J., Southall, E.J., Southward, A.J., Henderson, P.A. & Hawkins, S.J. (2004) Regional climatic warming drives long-term community changes of British marine fish. *Proceedings of the Royal Society of London Series B, Biological Sciences*, **271,** 655–661.

GRASS Development Team (2005) *Geographic resources analysis support system (GRASS)*. GNU General Public License, ITC-irst, Trento, Italy (http://grass.itc.it).

Guerra, M.T. & Gaudêncio, M.J. (1986) Aspects of the ecology of *Patella* spp. on the Portuguese coast. *Hydrobiologia*, **142,** 57–69.

Hagberg, J., Tunberg, B.G., Wieking, G., Krönkle, I. & Belgrano, A. (2004) Effects of climate variability on benthic

communities. *The North Atlantic: a comparative perspective. Marine ecosystems and climate variation* (ed. by N.C. Stenseth and G. Ottersen), pp. 115–121. Oxford University Press, Oxford.

Hawkins, S.J., Southward, A. & Genner, M.J. (2003) Detection of environmental change in a marine ecosystem – evidence from the western English Channel. *The Science of the Total Environment*, **310,** 245–256.

Hidalgo, J.G. (1917) *Fauna Malacológica de España, Portugal y las Baleares. Moluscos testaceos marinos*. Trabajos del Museo Nacional de Ciencias Naturales, Serie Zoológica, 30.

Hiscock, K., Southward, A., Tittley, I. & Hawkins, S.J. (2004) Effects of changing temperature on benthic marine life in Britain and Ireland. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **14,** 333–362.

Holbrook, S.J., Schmitt, R.J. & Stephens, J.S. (1997) Changes in an assemblage of temperate reef fishes associated with a climate shift. *Ecological Applications*, **7,** 1299–1310.

Ibanez, M., Pena, J. & Feliu, J. (1986) Reproduction of *Patella* spp. on the Basque coast of Spain. *Hydrobiologia*, **142,** 327.

IPCC (2001) *Climate change 2001: the scientific basis. Contribution of working group I to the third assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge.

Jarvis, C.H. & Stuart, N. (2001) A comparison among strategies for interpolating maximum and minimum daily air temperatures. Part II: The interaction between number of guiding variables and the type of interpolation method. *Journal of Applied Meteorology*, **40,** 1075–1082.

Kolar, C.S. & Lodge, D.M. (2001) Progress in invasion biology: predicting invaders. *Trends in Ecology and Evolution*, **16,** 199–204.

Lemos, R.T. & Pires, H.O. (2004) The upwelling regime off the west Portuguese coast, 1941–2000. *International Journal of Climatology*, **24,** 511–524.

Lewis, J.R. (1986) Latitudinal trends in reproduction, recruitment and population characteristics of some rocky littoral molluscs and cirripedes. *Hydrobiologia*, **142,** 1–13.

Lewis, J.R. (1996) Coastal benthos and global warming: strategies and problems. *Marine Pollution Bulletin*, **32,** 698–700.

Lewis, J.R. (1999) Coastal zone conservation and management: a biological indicator of climatic influences. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **9,** 401–405.

Lewis, J.R., Bowman, R.S., Kendall, M.A. & Williamson, P. (1982) Some geographical components in population dynamics: possibilities and realities in some littoral species. *Netherlands Journal of Sea Research*, **16,** 18–28.

Ludwig, J.A. & Cornelius, J.M. (1987) Locating discontinuities along ecological gradients. *Ecology*, **68,** 448–450.

Menge, B.A. (2000) Top-down and bottom-up community regulation in marine rocky intertidal habitats. *Journal of Experimental Marine Biology and Ecology*, **250,** 257–289.

Nobre, A. (1940) *Fauna malacológica de Portugal, I: Moluscos marinhos e de águas salobras*. Imprensa Portuguesa, Porto.

Paine, R.T. & Trimble, A.C. (2004) Abrupt community change on a rocky shore - biological mechanisms contributing to the potential formation of an alternative state. *Ecology Letters*, **7,** 441–445.

Parmesan, C. & Yohe, G. (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, **421,** 37–42.

Peliz, A., Rosa, T.L., Santos, A.M.P. & Pissarra, J.L. (2002) Fronts, jets, and counter-flows in the Western Iberian upwelling system. *Journal of Marine Systems*, **35,** 61–77.

Peliz, A., Dubert, J., Santos, A.M.P., Oliveira, P.B. & Le Cann, B. (2005) Winter upper ocean circulation in the Western Iberian Basin – fronts, eddies and poleward flows: an overview. *Deep Sea Research Part I: Oceanographic Research Papers*, **52,** 621–696.

Pereira, S.G., Lima, F.P., Queiroz, N.C., Ribeiro, P.A. & Santos, A.M. (2006) Biogeographic patterns of intertidal macroinvertebrates and their association with macroalgae distribution along the Portuguese rocky coast. *Hydrobiologia*, **555,** 185–192.

Ridgway, S.A., Reid, D.G., Taylor, J.D., Branch, G.M. & Hodgson, A.N. (1998) A cladistic phylogeny of the family Patellidae (Mollusca: Gastropoda). *Philosophical Transactions of the Royal Society of London B*, **353,** 1645–1671.

Rivadeneira, M. & Fernández, M. (2005) Shifts in southern endpoints of distribution in rocky intertidal species along the south-eastern Pacific coast. *Journal of Biogeography*, **32,** 203–209.

Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C. & Pounds, J.A. (2003) Fingerprints of global warming on wild animals and plants. *Nature*, **421,** 57–60.

Sagarin, R.D. & Gaines, S.D. (2002a) The 'abundant centre' distribution: to what extent is it a biogeographical rule? *Ecology*, **5,** 137–147.

Sagarin, R.D. & Gaines, S.D. (2002b) Geographical abundance distributions of coastal invertebrates: using one-dimensional ranges to test biogeographic hypotheses. *Journal of Biogeography*, **29,** 985–997.

Sagarin, R.D., Barry, J.P., Gilman, S.E. & Baxter, C.H. (1999) Climate-related changes in intertidal community over short and long time scales. *Ecological Monographs*, **69,** 465–490.

Sánchez, R.F. & Relvas, P. (2003) Spring-summer climatological circulation in the upper layer in the region of Cape St. Vincent, Southwest Portugal. *ICES Journal of Marine Science*, **60,** 1232–1250.

Santos, A.M. (2000) *Intertidal ecology of northern Portuguese rocky shores*. School of Biological Science, University of Southampton, UK.

Santos, A.M.P., Peliz, A., Dubert, J., Oliveira, P.B., Angelico, M.M. & Re, P. (2004) Impact of a winter upwelling event on the distribution and transport of sardine (*Sardina pilchardus*) eggs and larvae off western Iberia: a retention mechanism. *Continental Shelf Research*, **24,** 149–165.

Sax, D.F. & Brown, J.H. (2000) The paradox of invasion. *Global Ecology and Biogeography*, **9,** 363–371.

Southward, A.J., Hawkins, S.J. & Burrows, M.T. (1995) Seventy years' observations of changes in distributions and abundance of zooplankton and intertidal organisms in the western English Channel in relation to rising sea temperature. *Journal of Thermal Biology*, **20,** 127–155.

Stensen, N.C., Mysterud, A., Ottersen, G., Hurrel, J.W., Chan, K. & Lima, M. (2002) Ecological effects of climate fluctuations. *Science*, **297,** 1292–1296.

Walther, G., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J., Hoeg-Guldberg, O. & Bairlein, F. (2002) Ecological responses to recent climate change. *Nature*, **416,** 389–395.

Wethey, D.S. (2002) Biogeography, competition, and microclimate: the barnacle *Chthamalus fragilis* in New England. *Integrative and Comparative Biology*, **42,** 872–880.

Woodruff, S.D., Diaz, H.F., Elms, J.D. & Worley, S.J. (1988) COADS release 2 data and metadata enhancements for improvements of marine surface flux fields. *Physics and Chemistry of the Earth*, **23,** 517–526.

Zacharias, M.A. & Roff, J.C. (2001) Explanations of patterns of intertidal diversity at regional scales. *Journal of Biogeography*, **28,** 471–483.

Zacherl, D., Gaines, S.D. & Lonhart, S.I. (2003) The limits to biogeographical distributions: insights from the northward range extension of the marine snail, *Kelletia kelletii* (Forbes, 1852). *Journal of Biogeography*, **30,** 913–924.

BIOSKETCHES

Fernando P. Lima is a PhD student at the University of Porto, Portugal. He is particularly interested in the study of diversity and biogeography of marine organisms. His current research focuses on changes in species distribution and their relation with climate change. He is also interested in the biology of elasmobranchs, experimental ecology and coastal environmental impact assessment.

Nuno Queiroz is a researcher in the Research Center in Biodiversity and Genetic Resources (University of Porto, Portugal) and the current chairman of the Portuguese Association for the Study and Conservation of Elasmobranchs. His research focuses on the study of elasmobranch population dynamics and genetics, shark movement patterns and their relation with oceanographic parameters. He is also interested in fisheries impact/management and the responses of marine species to climate change.

Pedro A. Ribeiro is a PhD student at the University of Southampton, UK. His current research addresses the effects of climate change on dispersal and connectivity in marine populations. Other interests include marine biodiversity and biogeography, experimental rocky shore community ecology and environmental impact assessment on coastal areas.

Stephen J. Hawkins is the Director of the Marine Biological Association of the United Kingdom. His research interests include marine ecology, long-term changes in rocky shore communities in relation to climate, behavioural ecology of intertidal grazers and taxonomy and phylogeography of north-east Atlantic and Mediterranean patellids, cirripedes and trochids.

António M. Santos is an Assistant Professor at the Department of Zoology and Anthropology at the Faculty of Sciences of the University of Porto. His research interests include biodiversity and biogeography of intertidal rocky shore organisms, effects of climate change and human-induced disturbances on coastal ecosystems.

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Modelling past and present geographical distribution of the marine gastropod *Patella rustica* as a tool for exploring responses to environmental change

FERNANDO P. LIMA*†‡, PEDRO A. RIBEIRO†‡, NUNO QUEIROZ†‡, RAQUEL XAVIER†, PEDRO TARROSO†, STEPHEN J. HAWKINS‡§ and ANTÓNIO M. SANTOS*†

Departamento de Zoologia-Antropologia, Faculdade de Ciências da Universidade do Porto, 4099-002 Porto, Portugal, †CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, 4485-661 Vairão, Portugal, ‡Marine Biological Association of the United Kingdom, Plymouth, UK, §School of Oceanographic Sciences, University of Wales, Bangor, Menai Bridge, Anglesey, UK

Abstract

A climate envelope approach was used to model the distributions of the intertidal gastropod *Patella rustica*, to test the robustness of forecast responses to climate change. The model incorporated variables that were likely to determine the abundance and the northern range limit of this species in the NE Atlantic. The model was built using classification and regression tree analysis (CART) trained with historical distribution data from the mid 1950s and a set of corresponding climatic and oceanographic variables. Results indicated air and sea temperature, in particular during the reproductive and settlement periods, as the main determinants of the Atlantic distribution of *P. rustica*. The model was subsequently fed with contemporary climatic data and its output was compared with the current distribution and abundance of *P. rustica*, assessed during a 2002–2003 survey. The model correctly hindcasted the recent collapse of a distributional gap in northern Portugal, as well as an increase in abundance at locations within its range. The predicted northward expansion of the northern range limit did not occur because the absence of the species was confirmed in a survey encompassing the whole Atlantic French coast up to Brest. Stretches of unsuitable habitat too long to be overcome by dispersal are the likely mechanism controlling the northern limit of the distribution of this intertidal species.

Keywords: biogeography, classification and regression trees (CART), climate change, intertidal, marine gastropod, modelling, *Patella rustica*

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Introduction

The application of species distribution models has considerably increased in the last two decades, mainly driven by the need to predict the potential impacts of climate change on the distribution of organisms (Guisan & Thuiller, 2005). From the vast array of methods currently available, single-species bioclimatic envelope models (BEMs) have been widely used (Heikkinen *et al*., 2006). These models use the current geographic distribution of a species to infer its environmental requirements, and then to predict its geographic distribution for the current, or for past or future climates (Hijmans & Graham, 2006). Yet, given their correlative nature, the validity of such approaches has been progressively questioned (see Araújo & Guisan, 2006). The problem is twofold. First, BEMs seldom account for the effects of biotic interactions, adaptive change and dispersal (Pearson & Dawson, 2003). This results in highly biased models that tend to produce inaccurate scenarios (Davies *et al*., 1998; Hampe, 2004). Second, independent validation of models is often not possible, because the events being predicted have not yet occurred or are poorly known (Araújo & Guisan, 2006). Nonindependent validation (resubstitution, data splitting) usually ends up in unrealistically optimistic estimates of their predictive ability (Araújo *et al*., 2005).

Correspondence: Present address: Fernando Pádua Silva e Lima, School of Biological Sciences, University of South Carolina, SC 29208, USA, tel. +1 803 777 3936, fax +1 803 777 4002, e-mail: fplima@biol.sc.edu

Despite being highly deterministic, BEMs have some advantages over the more complex rule-based mechanistic models (see Guisan & Zimmermann, 2000), provided that they are used in the appropriate context (Araújo *et al*., 2005), that the choice of the model is well grounded (Hijmans & Graham, 2006) and that results are interpreted with caution (Hodkinson, 1999). BEMs can present a fast and useful tool for the identification of key relationships between species and the factors controlling their distribution (Huntley *et al*., 2004), especially at large scales (e.g. continental and global) where the importance of climatic forcing is thought to be higher (Pearson *et al*., 2002; Pearson & Dawson, 2003). When range-limiting physiological factors for a given species are poorly known, these methods may represent the best possible approach (Crumpacker *et al*., 2001).

In the marine environment, BEMs have been sparsely applied to model species distributions (but see De'ath & Fabricius, 2000; Clark *et al*., 2003; Friedlaender *et al*., 2006; Kaschner *et al*., 2006). This is unfortunate because there are many well-documented cases of recent changes (either expansions or retractions) of species' ranges which are thought to be a direct consequence of global warming (for a review see Helmuth *et al*., 2006b). Rocky intertidal communities, in particular, are well suited for studying climate-driven community changes (Herbert *et al*., 2003; Simkanin *et al*., 2005) because their ecologies are well-known (Southward *et al*., 1995) from decades of experimental studying of recruitment dynamics and biological interactions (see Raffaelli & Hawkins, 1996; Bertness *et al*., 2001 for reviews). Moreover, their inhabitants are often exposed to temperature and weather extremes, living close to their thermal tolerance (Wolcott, 1973; Tomanek & Helmuth, 2002). Also, intertidal organisms can be easily surveyed because they are generally conspicuous, slow moving or sedentary, and their ranges are roughly one-dimensional, conveniently defined by the coastal end points (Sagarin & Gaines, 2002). Thus, the seashore provides both a trackable testing ground for scientific debate on the utility and applicability of BEMs to model and predict species distributions under climate change and a wealth of insight into the probable complexity of the phenomena being modelled (see Helmuth *et al*., 2006b).

In this work, a single-species bioclimatic envelope approach was used to gain further insights into the factors driving the distribution of a marine gastropod, *Patella rustica* Linnaeus 1758, in the northeast Atlantic. In a recent paper, Lima *et al*. (2006) described the collapse of an historical distributional gap, in northern Portugal, which occurred in the late 1990s and coincided with a combination of unusual climatic and hydrographic events that were particularly pronounced between 1997 and 1999. In this period, a significant warming of sea-surface temperature (SST) during autumn/early winter was observed in addition to the general increase in average SST off the Portuguese coast (Lemos & Pires, 2004; Peliz *et al*., 2005; Lemos & Sansó, 2006). This, combined with strong river runoff and weak upwelling (Dong *et al*., 2000; Santos *et al*., 2004), may have facilitated the bridging of the gap.

The present work focused only on the northern part of *P. rustica* distribution in the Atlantic, mainly because empirical evidence showed that recent changes have already occurred in this area, and a northwards expansion was expected. *P. rustica* ranges from the Mediterranean to the Atlantic coast of the Iberian Peninsula and Northern Africa, including the Macaronesian Islands, except the Azores (Ridgway *et al*., 1998). In the Atlantic, its southern limit is speculated to occur further south than Mauritania (Christiaens, 1973). However, apart from this reference, no reliable data exist that could have been used for modelling purposes. Thanks to the exhaustive work of Fischer-Piétte and colleagues (Crisp & Fischer-Piétte, 1959; Fischer-Piétte & Gaillard, 1959) the distribution of *P. rustica* along the Atlantic European coast is well-known for the late 1950s. These data were used together with several climatic and oceanographic variables to build a BEM. The model was subsequently evaluated by feeding it with climatic and oceanographic data from the 1990s, when the distributional gap was bridged, hindcasting the present day distribution of the species. The output was then compared with the extant distribution of *P. rustica* surveyed in 2002–2003. Thus, the model was implemented to 'understand' the likely mechanisms governing the distribution of this limpet rather than to 'predict' its future distribution and model evaluation was used to assess the 'robustness' of inferred mechanisms rather than to estimate its predictive accuracy (Araújo & Guisan, 2006). More importantly, evaluation was done with an independent data set (apart from spatial and temporal autocorrelation), which is highly recommended but rarely used in BEMs (Araújo *et al*., 2005).

Materials and methods

Biological data

Data on the distribution and abundance of *P. rustica* along the European coastline (from the English Channel to Morocco) were gathered for the 1950s from the comprehensive works of Fischer-Piétte (1953, 1955, 1958, 1963), Crisp & Southward (1958), Crisp & Fischer-Piétte (1959) and Fischer-Piétte & Gaillard (1959). Because in each work, density of limpets was expressed by different abundance scales, the number of abundance categories was reduced to three: absent, rare and

abundant. This caused some information loss (in some cases true densities were available), but avoided wrong categorization and provided comparable data for the Atlantic range of *P. rustica*. Contemporary abundances were recorded by thoroughly resurveying historical localities between 2002 and 2003 and from Biarritz towards the north in 2006 (see Fig. 1). Density of limpets was estimated using several randomly placed $50 \times 50\,cm^2$ quadrats, and then converted to the three-class abundance scale.

Environmental data

Two climatic data sets were assembled and used in the model. Because climatic and oceanographic factors such as temperature and salinity are highly variable over the years (Levitus *et al*., 2000), a time window large enough to encompass a significant amount of variability but simultaneously representative of the sampling years and correlated with changes in distribution of *P. rustica* was selected (Lima *et al*., 2006). The first set included data from the 1950s (January 1950–December 1959) and the second one incorporated data from the decade of 1990 (January 1991–December 2000). The environmental conditions were described using six main climatic and oceanographic variables: near surface air temperature (NSAT), cloud cover (CC), precipitation (PP), SST, sea surface salinity (SSS) and wave action (WA).

NSAT, CC and PP monthly averaged data covering European land surface at a 10 min resolution were obtained from the CRU TS 1.2 dataset, available from the Tyndall Centre for Climate Change Research and Climate Research Unit (http://www.cru.uea.ac.uk/, Mitchell *et al*., 2002, 2004). These were imported into GRASS Geographical Information System (GRASS Development Team, 2006) as raster maps. SST along the north-eastern Atlantic coast, was derived from *in situ* raw data obtained from the International Comprehensive Ocean – Atmosphere Data Set (ICOADS, http://icoads.noaa.gov/, Woodruff *et al*., 1988). To avoid bias due to different daytime measurements, only data from 12 : 00 GMT were used. Point data were imported into GRASS GIS and transformed into monthly raster maps with a spatial resolution of 4 km using a surface interpolation method. Each cell was derived from the 12 nearest data points using the distance squared weighting algorithm (Jarvis & Stuart, 2001). The variables NSAT, CC, PP and SST are thought to be a surrogate not only for the air and sea temperature to which limpets are exposed, but also for other climatic factors that potentially affect the heat budget of intertidal organisms during low tide, like the amount of solar irradiance and the evaporation rate (see Denny & Harley, 2006). Sea salinity is another environmental factor potentially important for the physiology and fitness of limpets (Fischer-Piétte, 1948). Monthly averaged SSS data, with an average resolution of 0.5° latitude $\times$ 0.5° longitude cells, were extracted from SODA-POP v1.2 model data set, available at the National Virtual Ocean Data System live server webpage (http://ferret.pmel.noaa.gov/NVODS/servlets/dataset) and imported into GRASS GIS as raster maps. Because data were only available from January 1958 onwards, the computed average for the decade of 1950s was based solely on 2 years.

The resulting set of 240 (20 years $\times$ 12 months) SST, NSAT, CC, PP and 164 SSS maps was stored in GRASS GIS as monthly layers and sampled along the studied coastline in the locations visited by D. J. Crisp, E. Fisher-Piétte and J. Gaillard during the 1950s (Fischer-Piétte, 1955, 1958, 1963; Crisp & Southward, 1958; Crisp & Fischer-Piétte, 1959; Fischer-Piétte & Gaillard, 1959). Sampled data for each parameter were combined to build a table with each row denoting a geographical location and each column a month/year combination. For SST and NSAT, the general 10-year mean and the average value of winter, summer and reproductive seasons were computed, as well as the mean value of the coldest and hottest month during those seasons. Reproductive season was assumed to be from September to November (according to Ibanez *et al*., 1986, P. A. Ribeiro, unpublished data). For CC and PP, the 10-year average and the values of the percentiles 25, 50 and 75 were calculated. For SSS, only the long-term average value during each studied period was considered. An overview of the climatic variables can be found in Table 1.

On the Atlantic coasts of Europe and North Africa the presence of *P. rustica* is generally associated with steep surfaces found in the upper eulittoral of exposed rocky shores (Fischer-Piétte & Gaillard, 1959; Lima *et al*., 2006). Thus, a quantitative measure of sea exposure was included in the model. Average wave power was used as surrogate for the exposure of sampled locations to the effects of wave action (WA). The EXposure estimates for fragmented Archipelagos (EXA) procedure as described by Ekebom *et al*. (2003) was generally followed, apart from the need to obtain wave climatology parameters from prevailing winds because they were directly incorporated in the exposure model. Hence, that computational step was skipped. Wave climate data were downloaded from the European Environmental Agency database (http://www.eea.europa.eu). Data were only available for regularly spaced offshore locations 50–100 km away from the coastline (Fig. 1). Therefore, in every coastal location the wave parameters of the nearest offshore point were used. The fetch length was measured for each direction

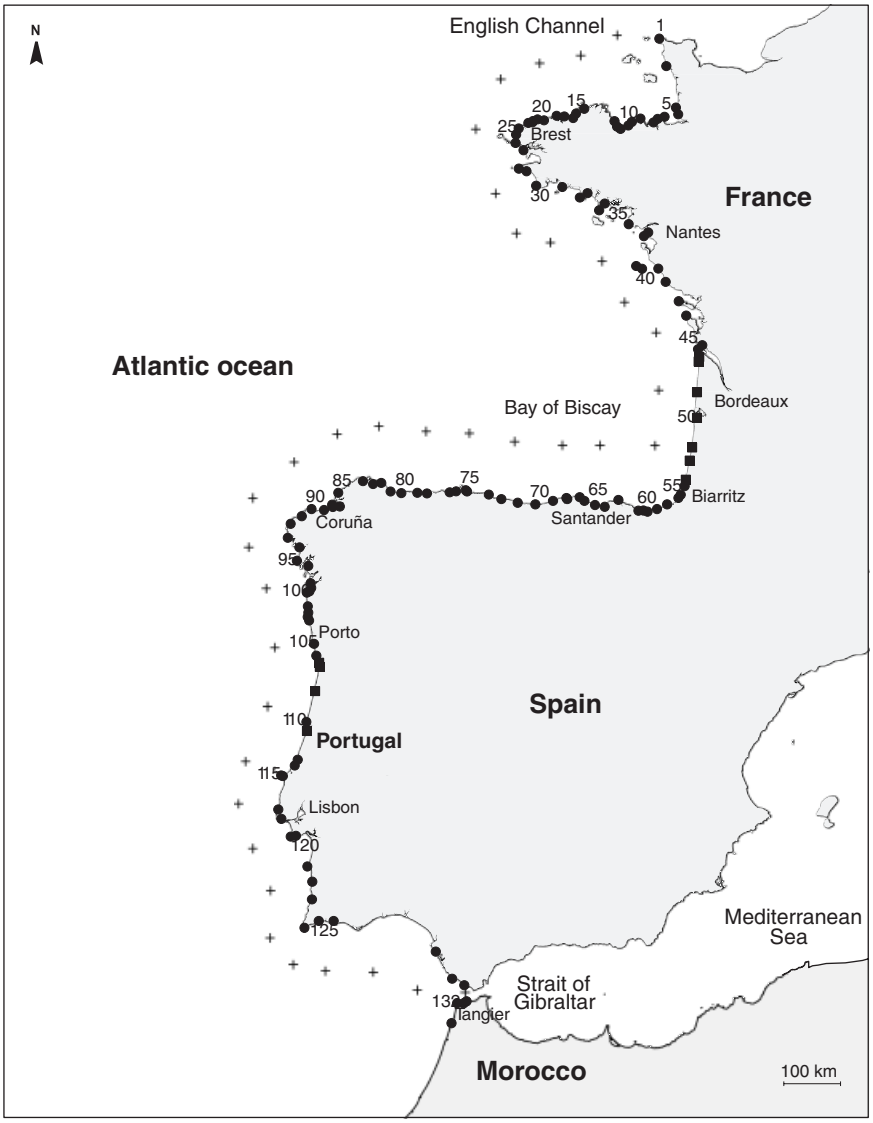

Fig. 1 Study area. Circles represent rocky shore locations and squares represent seawall constructions. Offshore points represent the sites for which wave climatology data were available. 1 – Cap de la Hague, 2 – Cap de Carteret, 3 – Granville, 4 – Carolles, 5 – Pte. Grouin, 6 – Rotheneuf, 7 – Dinard, 8 – Cap Fréhel, 9 – Cap d'Erquy, 10 – Pte. Pléneuf, 11 – Pte. Roselier, 12 – Pte. Pordic, 13 – St. Quay – Portri, 14 – Trestrignel, 15 – Pte. Bihit, 16 – Pte. Locquirec, 17 – Pte. Primel, 18 – Pte. Bloscon, 19 – Greve Poulfoén, 20 – Pontusval, 21 – Guissény, 22 – Greve Vougot, 23 – Greve Lilia, 24 – Trémazan, 25 – Melon, 26 – Le Conquet, 27 – Camaret, 28 – Pte. Raz, 29 – Pte. Lervily, 30 – St. Guénolé, 31 – Trévignon, 32 – Pte. Pen Men, 33 – Port-Louis, 34 – Quiberon, 35 – Pte. Kerbihan, 36 – Pte. Croisie, 37 – St. Michel Chef Chef, 38 – Pte. St. Gildas, 39 – Yeu, Pte. But, 40 – Yeu, Pte. Tranche, 41 – Croix de Vie, 42 – Les Sables d'Olone, 43 – Phare Balenes, 44 – Phare Chassiron, 45 – Royan, 46 – Courdouan, 47 – L'Amelie, 48 – Montalivet, 49 – Lancanau, 50 – Arcachon, 51 – Mimizan, 52 – Contis, 53 – Vieux-Boucau, 54 – Capbreton, 55 – Boucau, 56 – Biarritz, 57 – Cabo Higuer, 58 – San Sebastian, 59 – Zarauz, 60 – Zumaya, 61 – Deba, 62 – Bermeo, 63 – Barrica, 64 – Urdiales, 65 – Santoña, 66 – Noja, 67 – Santander, 68 – Cabo Menor, 69 – Ubiarco, 70 – San Vicente de la Barquera, 71 – Llanes, 72 – Ribadesella, 73 – Luces, 74 – Perlora, 75 – Candas, 76 – Salinas, 77 – San Juan, 78 – Luarca, 79 – Navia, 80 – Cangas de Foz, 81 – Ribadeo, 82 – San Ciprian, 83 – Vivero, 84 – Espasante, 85 – Cobas, 86 – Carnoedo, 87 – La Coruña, 88 – Sta. Cristina, 89 – Arteijo, 90 – Malpica, 91 – Lage, 92 – Mugia, 93 – Finisterre, 94 – Muro, 95 – Corrubedo, 96 – Grove, 97 – Punta Corbeiro dos Castros, 98 – San Miguel de Oia, 99 – Bayona, 100 – Cabo Sillero, 101 – La Guardia, 102 – VP Âncora, 103 – Montedor, 104 – Viana Castelo, 105 – Vila do Conde, 106 – Leixões, 107 – Aguda, 108 – Espinho, 109 – Aveiro, 110 – Cabo Mondego, 111 – Buarcos, 112 – Leirosa, 113 – Nazaré, 114 – S. Martinho Porto, 115 – Cabo Carvoeiro, 116 – Peniche, 117 – Azenhas do Mar, 118 – Cascais, 119 – Cabo Espichel, 120 – Sesimbra, 121 – Sines, 122 – VN Milfontes, 123 – Odeceixe, 124 – Sagres, 125 – Lagos, 126 – Armação de Pêra, 127 – Rota, 128 – Trafalgar, 129 – Punta Paloma, 130 – Punta Malabata, 131 – Tanger, 132 – Cap Spartel, 133 – Arcila.

Table 1 Summary of the independent variables used in the CART modelling of *Patella rustica* distribution

Environmental variables	Acronym	Unit of measurement	Considered periods	Statistics	Temporal resolution	Spatial resolution (raw data)
Sea surface temperature	SST	°C	1950–1959 1991–2000	Mean, minimum and maximum during winter, summer, and reproduction	Monthly	4 km
Near surface air temperature	NSAT	°C	1950–1959 1991–2000	Mean, minimum and maximum during winter, summer, and reproduction	Monthly	10 min
Cloud cover	CC	%	1950–1959 1991–2000	Percentile 25, 50, 75	Monthly	10 min
Precipitation	PP	%	1950–1959 1991–2000	Percentile 25, 50, 75	Monthly	10 min
Sea surface salinity	SSS	‰	1958–1959 1991–2000	Percentile 25, 50, 75	Monthly	10 min
Wave power	WP	kW	1985–2001	Mean	–	Approximately 100 km

by a group of lines radiating from each coastal point with a 7.5° bearing step between them. Lines were trimmed when reaching the coastline. For each one of the remaining lines, wave parameters from the corresponding directional sector were selected and used to estimate the wave power observed in coastal location over that specific direction. Wave power (P) in kW was defined as $P = nE1.56T$ where $n = 0.5$, T = mean significant wave period (in seconds) and E (energy in Joules) = $1/16\,\rho\,g\,H$ where ρ = seawater density ($1020\,\mathrm{kg\,m^{-3}}$) g = acceleration due to gravity ($9.8\,\mathrm{m\,s^{-1}}$) and H = mean significant wave height in metres. For each coastal location, total wave power was computed as the sum of all converging partial estimates from the different directions, weighted by the percentage of time waves were observed in each direction. The online wave data were only available for the period between 1985 and 2001 and records were summarized in statistics relative to that 16-year period without the possibility to extract specific years or months, which forced the use of the whole data set. As a consequence, the parameter WA was considered static (i.e. not varying from the 1950s to 1990s), which may not be entirely true (see Davies & Johnson, 2006).

Modelling approach

Classification and Regression Trees (CART) (Breiman *et al*., 1984) are one of the currently available climate envelope approaches. Traditionally applied to fields such as medical diagnosis, meteorology, plant physiology, soil sciences and wildlife management, they have recently been used to successfully model terrestrial (e.g. Vayssières *et al*., 2000; Edwards *et al*., 2006; Fronzek *et al*., 2006) and marine species distributions (De'ath & Fabricius, 2000; DeVantier *et al*., 2006; Friedlaender *et al*., 2006).

For the purposes of the current study CART models were selected primarily because they are relatively immune to multicollinearity (Breiman *et al*., 1984; Jackson & Bartolome, 2002; Karels *et al*., 2004), and the data set incorporated 20 potentially correlated predictor variables. Other advantages included the possibility of output response variables with more than two levels, the robustness regarding possible outliers and nonnormal distributions of variables (Breiman *et al*., 1984) and the capacity to determine complex interactions among explanatory variables without the need to specify them *a priori* (Vayssières *et al*., 2000; Rouget *et al*., 2001). Additionally, these analyses are easy to interpret (De'ath & Fabricius, 2000) because they provide a hierarchical view of the relationships between species and environmental variables, allowing the identification of those which are the most correlated with the presence of species (Vayssières *et al*., 2000).

Homogeneity of groups was assessed by the Gini purity index (Breiman *et al*., 1984). Because the maximal tree is usually overfitted to training data, a computational step to constrain the tree to its best size is required to avoid the problem of overfitting (Araújo & Guisan, 2006). Overfitted models tend to perform exceptionally well on the training data because they fit

the idiosyncrasies and noise in the data set used to build the model, in addition to the generic relationships between independent and response variables. Thus, a balanced model including sufficient complexity to uncover subtle effects and interactions but not compromising predictive power should be chosen. A common approach in tree-based techniques is to freely allow the maximum growing process and then prune the overbranched tips of the tree (Breiman *et al*., 1984; De'ath & Fabricius, 2000; Questier *et al*., 2005). The initial tree was allowed to attain the maximum size and then pruned using the mode of 50 repeated 10-fold cross-validations (see Breiman *et al*., 1984; De'ath & Fabricius, 2000 for details). For the cross-validation, the data set was divided in 10 parts and each subset was removed in turn, being used as a test sample against predictions based on the remaining 90% of data. This technique is similar but more robust than jack-knifing, as it gives a better reflection of its performance on new data (Fielding & Bell, 1997). The average error rate was plotted in relation to the tree size and the best size for the tree was chosen as the smallest having an error rate within one SE of the minimum (De'ath & Fabricius, 2000).

The predictive power of the model was evaluated using the area under curve (AUC) obtained from the receiver operator characteristic (ROC) plot. AUC is a reliable measure of model performance because it is largely unaffected by the data prevalence, (i.e. the proportion of study sites naturally occupied by the species (Manel *et al*., 2001; McPherson *et al*., 2004; Vaughan & Ormerod, 2005). Because the output of CART is categorical, ROC curves were derived by recursive partitioning (Cook & Goldman, 1984; Raubertas *et al*., 1994). Even though it has been traditionally used to measure the degree to which a classifier can discriminate between two classes, its extension to a multiclass classification may be done by computing a series of binary AUCs for each class vs. all the others (called one- vs. -all approach or OVA). Thus, each one of these binary comparisons has its own AUC, measuring how well each class is identified by the model (Patel & Markey, 2005).

The model was evaluated by feeding it with climatic and oceanographic data from the 1990s, when the distributional gap was bridged, to hindcast the contemporary distribution of *P. rustica* along the NE Atlantic coast. The output was subsequently compared with the real distribution and abundance of *P. rustica* assessed in the 2002–2003 and 2006 surveys.

Results

A total of 122 locations for which data were available from the 1950s were revisited during the 2002–2003 survey, enabling abundance and distribution to be mapped (Fig. 2a and c). In the 40-year period between the two surveys, the most remarkable changes were: (i) the bridging of the distributional gap in northern Portugal (Lima *et al*., 2006); (ii) a global increase in abundance in NW Iberia (Galicia) and on some southern Portuguese shores; and (iii) no extension of the northern boundary of the range at Capbreton, although an increase in abundance was also observed in this region.

The final model, selected and pruned after cross-validation, could be represented as a five leaf tree explaining 94% of data variance for the distribution during the 1950s (Fig. 3). Scores of the AUC obtained from ROC plots for test data were 0.93 for category class 0 (absent), 0.99 for category 1 (rare) and 0.92 for category 2 (abundant), which according to Swets (1988) and Zweig & Campbell (1993) indicates excellent classification accuracy. From the variety of climate data fed into the model, SST and NSAT were selected as determinant factors of the distribution of *P. rustica*. The first data partition, based solely in the observed value of the average NSAT of the coldest month during the reproductive period, explained nearly 38% of data variation and was responsible for identifying the break, which corresponds to the northern boundary of the distribution. The remaining cases were generally explained by the average SST of the coldest winter month, average NSAT and average SST. Nearly half of misclassifications occurred within the distributional gap in Northern Portugal. In this area, the model erroneously predicted the presence of *P. rustica* at two locations where the species had not been observed (Cabo Mondego and Buarcos). It also predicted the existence of limpets at high densities at Vila do Conde, a location where Fischer-Piétte & Gaillard (1959) reported only a single individual during their 1950s survey. In addition, the model overestimated the abundance in three locations in the southern part of the studied distribution (Sesimbra, Rota and Tangier). In one locality in northern Spain (Santander) the predicted abundance was misclassified as rare, while in reality it was abundant (Fischer-Piétte & Gaillard, 1959). Nevertheless, globally the model performed quite well, because the main distributional features such as the northern range limit, the existence of a distributional gap in northern Portugal and the region of low abundance in north-western Iberia were correctly modelled (Fig. 2b).

When the model was fed with environmental data from the 1991–2000 period, scores of the AUC were 0.78 for category class 0 (absent), 0.45 for category 1 (rare) and 0.95 for category 2 (abundant). In particular, the model correctly hindcasted the bridging of the historical gap in Northern Portugal and also the observed increase in densities at Galicia (NW Iberia) and at the

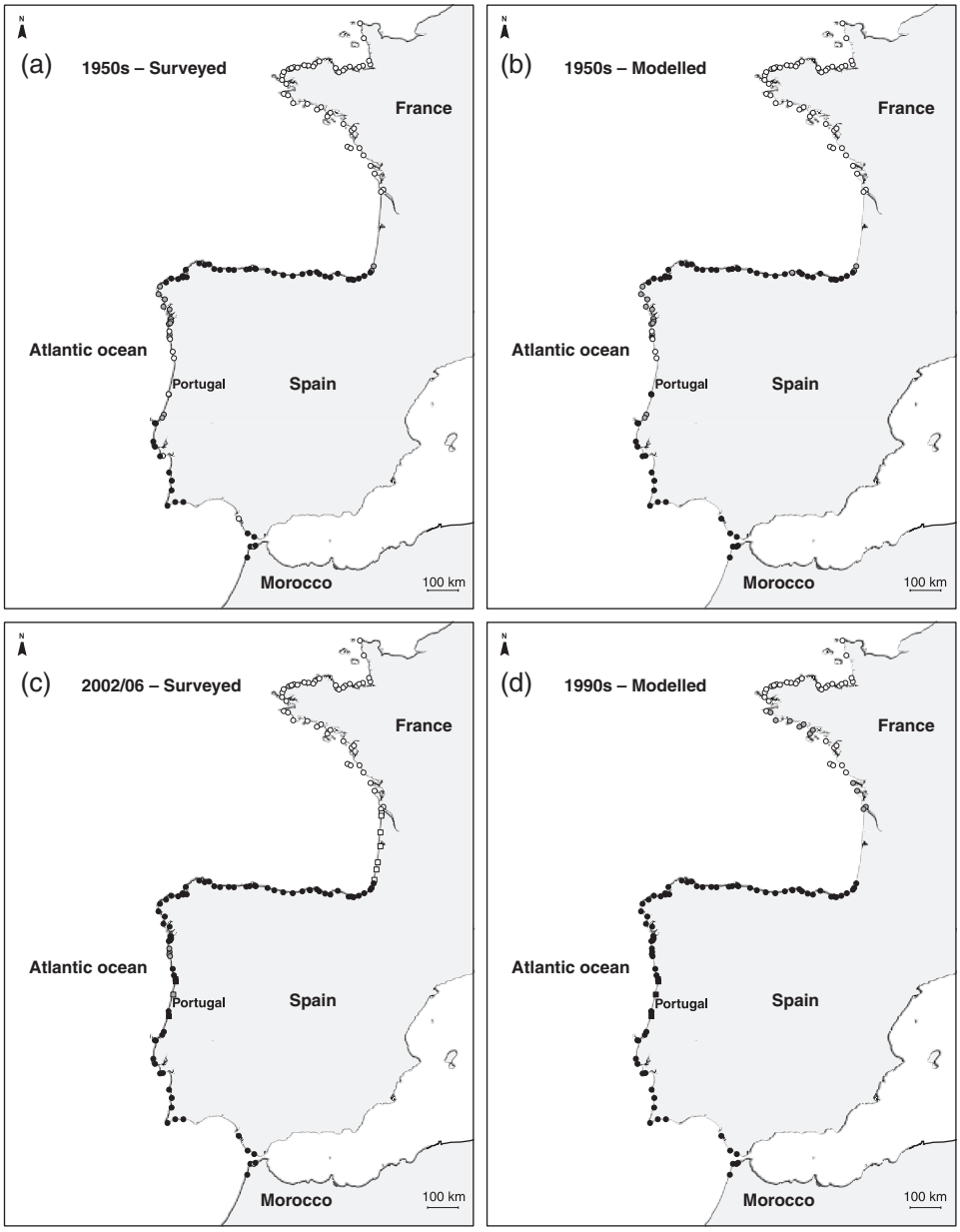

Fig. 2 Abundance of *Patella rustica* along the studied area. Circles represent rocky shore locations and squares represent seawall constructions. White stands for absent, grey represents rare and black means abundant. (a) Historical abundance. (b) Abundance modelled for the 1950s. (c) Contemporary abundance. (d) Model output for 1990s.

current northern limit of the species range (Capbreton). However, it overestimated the densities inside the former distributional gap and more importantly, failed in identifying the actual northern limit of the species range, because it predicted the species presence (although at low densities) in all locations between Capbreton and Ille d'Óléron and further north, between Pointe de Kerbihan and Pointe de Lervily. A new survey made in 2006, from Capbreton towards the Cotentin Peninsula (in the English Channel), revealed that the predicted northern expansion of the species range had not occurred.

Discussion

The CART model suggested that the distribution of *P. rustica* during the 1950s was highly correlated with temperature. According to the model output, the distribution of this species along the European Atlantic coast could be accurately explained by air and sea

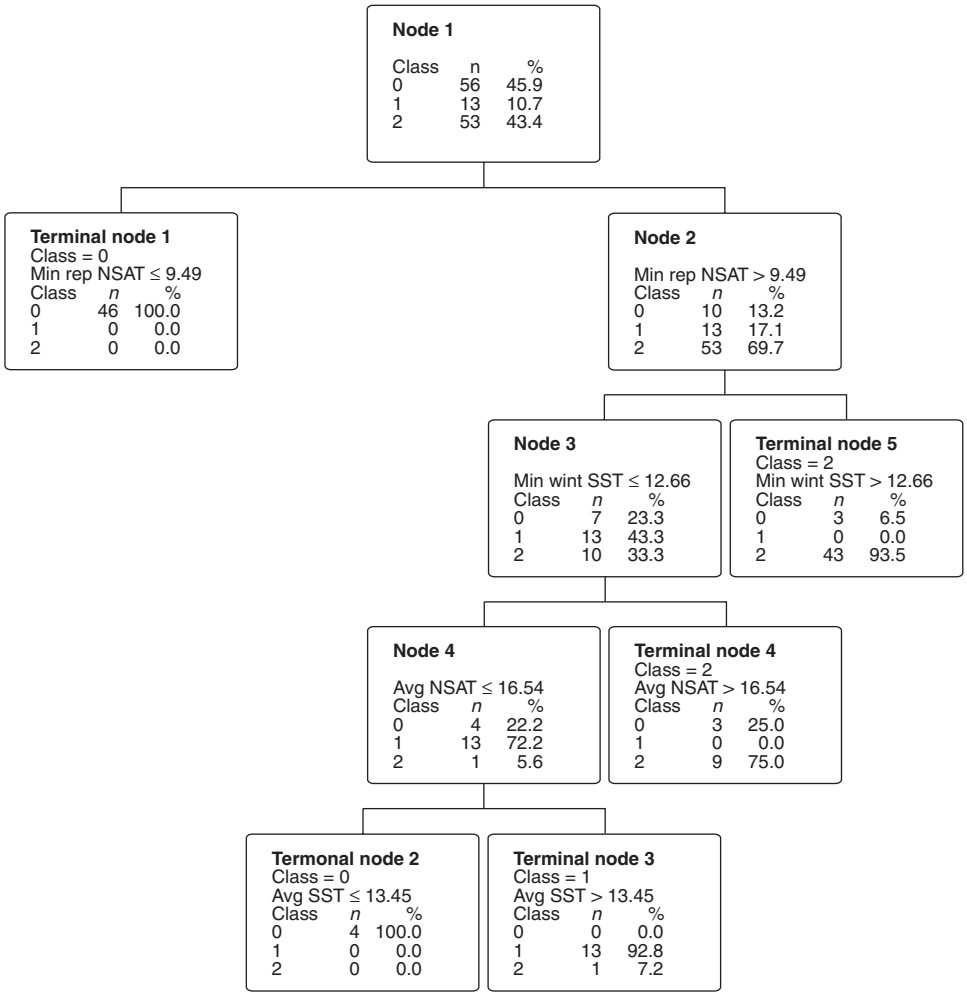

Fig. 3 Decision tree of the CART model built using historical (1950s) survey data. At each partition, the value of the splitting variable is shown. Information featured in the nodes includes the node identification, the level of the predicted response and the value of the split variable associated with that node. Finally, the number and percentage of cases for each level of the predicted response is also displayed. 0 stands for absent, 1 for rare and 2 for abundant. Climatic variables refer to the period of 1950–1959. Min rep NSAT, mean NSAT value of the coldest month during reproductive season; min wint SST – mean SST value of the coldest month during winter; avg NSAT – mean NSAT value during the 10-year period; avg SST – mean SST value during the 10-year period.

temperature patterns, especially during the reproductive (autumn) and initial growth (winter) periods. The model correctly excluded *P. rustica* from locations where temperature was too low, such as most of the French Atlantic coast and in the historical distributional gap in Northern Portugal, where upwelling has a dominant cooling effect on the sea temperature, at least during some periods of the year (Peliz *et al*., 2002, 2005). On the other hand, regions characterized by a warmer climate such as the Southern Portuguese coast and the Bay of Biscay were correctly modelled as having high densities of *P. rustica*.

The use of the climate envelope with contemporary environmental conditions correctly predicted the bridging of the gap and the increase in densities in Northern Spain. This suggests that some of the factors used in the model do determine, to some extent, the distribution of *P. rustica*, at least in this section of the range. Both events are probably connected, constituting an ongoing expansionary episode whose onset was already noticed by Fischer-Piétte & Gaillard (1959). Yearly abundance reassessments made from 2003 to 2006 clearly showed that the number of individuals from the new populations in this area is still increasing (F. P. Lima, unpublished data). The recent expansion of *P. rustica* observed in Northern Portugal, which led to the collapse of a long-term distributional gap in the late 1990s, was coincident with sea surface anomalies recorded over the last decade off the Portuguese coast (Lima *et al*., 2006). The current patterns observed off the Portuguese coast

in the autumn/winter are part of a wider and well-known system called 'Navidad' which affects the Atlantic European continental slope (Pingree & Le Cann, 1992). During winter, a warm water stream flows northward along the east coast of Portugal and Spain. It then splits into two streams, one flowing directly to northern France and the British Isles, and the other moving eastward, following the Spanish coastline, and turning north along the French coast. This stream is tightly correlated with the North Atlantic Oscillation Index (NAO). An extremely negative winter NAO Index was observed during 1996 and 1998, enhancing poleward flow and an anomalous winter warming along the coast of the Bay of Biscay (Pingree & Le Cann, 1992; Garcia-Soto, 2004). Therefore, because the observed changes in the distribution of *P. rustica* are likely to be related with increases in temperature (either from air or sea), a similar expansion was anticipated in Southern France, where an analogous warming trend was observed (Koutsikopoulos *et al*., 1998). Hence, it was interesting to notice the absence of such a phenomenon. The only evidence of a tendency for expansion in the northern portion of the range was the huge increase in densities observed in the two northernmost locations (Boucau and Capbreton) when compared with the data from Fischer-Piétte & Gaillard (1959). However, this evidence is fairly weak, because in these locations the building of coastal defences largely expanded the area of suitable habitat, thus confounding the effect of any other factor, such as environmental changes due to sea or air temperature increase.

The model failed to predict the correct northern range boundary on the Western French coast (Capbreton), extending the 'suitable habitat' for *P. rustica* as far north as Pointe de Lervily. This probably indicates that although both air and sea temperatures could be nowadays favourable to the presence of the species, some other factor, or even a combination of factors are hampering the expansion. It is possible that *P. rustica* is already present in locations north of Capbreton, but at densities which hindered its detection even by experienced observers. This is very unlikely considering the effort of the fieldwork, with more than 50 locations visited between Capbreton and Royan. In addition to the few rocky shores, all artificial coastal defences were also surveyed. It is also possible that certain variables not accounted in the model have a strong limiting influence on survival and/or expansion of this species. For example, recent work suggests that marine populations may be less open than originally thought (Bohonak, 1999; Cowen *et al*., 2000; Levin, 2006) and that sea currents can potentially constitute insurmountable barriers to species with a dispersive larval stage, constraining their ranges even when there is suitable habitat beyond those barriers (Gaylord & Gaines, 2000). The situation becomes even more complicated when effects of dispersal barriers are confounded with effects of physiological limitations imposed by steep clines in climatic factors such as sea temperature, because they usually co-occur in space (Zacherl *et al*., 2003). The ocean circulation in the Bay of Biscay has been studied by numerous authors (Koutsikopoulos *et al*., 1998; Bardey *et al*., 1999; Puillat *et al*., 2004; Planque *et al*., 2006), who pointed out several hydrographical fronts, upwellings, eddies and low salinity plumes from the rivers Loire and Gironde as being responsible for strong discontinuities in oceanic and coastal waters. Thus, these factors may represent considerable dispersal barriers for *P. rustica*.

Another hypothesis could be related with the limited larval dispersal potential of *P. rustica*. Even assuming that oceanographic barriers are not obstructing larval transport, a limited dispersal ability coupled with the lack of suitable habitat may pose a serious problem to expansion, as reported previously by Crisp (1958) for the barnacle *Elminius modestus*. The 200 km of coastline between Capbreton and Royan are mainly sandy and, therefore, do not allow larval settlement. The ability to traverse such a gap depends on the existence of a sufficiently long larval stage, able to survive during the transport towards the north, and even so, favourable currents must exist during larval drift. In the Bay of Biscay, the current flow over the continental slope was determined as having a characteristic northward velocity of $5\,cm\,s^{-1}$ during the winter, and probably reversing during the summer (Aken, 2002). Spawning is thought to happen during the autumn (Ibanez *et al*., 1986) but unfortunately very little is known regarding ocean circulation in this region during that season. However, and considering an average travelling speed of $5\,cm\,s^{-1}$, larvae would need more that 45 days to traverse the sandy coastline, a prohibitive period for a species whose planktonic stage lasts approximately 6 days (P. A. Ribeiro, unpublished data). If this is the main factor limiting the species expansion, why has it not prevented the recent colonisation of several locations in Northern Portugal, leading to the collapse of the historical 280 km gap? Coastal currents off the Portuguese coast display a stronger northward component during the reproductive period of *P. rustica*, with mean velocities of $15.1 \pm 4.4\,cm\,s^{-1}$ (Martins *et al*., 2002). In 6 days, this current may transport larvae for distances of up to 100 km, which is enough to cover the largest sandy stretch of coastline.

Additionally, the construction of a high number of artificial sea defences to protect both the sandy shores and the main city harbours from wave action has most likely increased population connectivity of rocky shore

species on the Portuguese coast. In fact, most sea defences are massive structures hosting typical rocky shore communities (F. P. Lima, unpublished data), where for example it is possible to find intertidal algae (*Porphyra* spp., *Fucus spiralis*), polychaetes (*Sabellaria alveolata*) crustaceans (*Pachygrapsus marmoratus, Pollicipes pollicipes*, *Chtamalus montagui* and *Chtamalus stellatus*) and molluscs (*Mytilus galloprovincialis*, *Littorina neritoides*, *Littorina saxatilis*, *Patella depressa*, *Patella vulgata* and *P. rustica*). These artificial reefs have shortened the largest sandy stretches to approximately 50 km, allowing a much easier expansion. On the contrary, along the 200 km rocky hiatus of the French coast, the construction of sea defences was done in a much more subtle way. With the exception of the two seawalls at Boucau and Capbreton, all constructions are small in height and length, unable to accommodate more than a few ephemeral green algae (genus *Ulva* and *Enteromorpha*), a few mussels (*Mytilus* sp.), oysters, and in some rare occasions, a handful of *P. depressa*. These structures clearly lack the typical habitat of exposed vertical walls required by *P. rustica*. Similarly, Gilman (2006) found that the most likely explanation for the determination of the northern range limit of the intertidal limpet *Collisella scabra* in California was an increase in the distance between populations at the range margin, reducing the chances of dispersing larvae to find suitable habitat for settlement, rather than any climatic constraint. Thus, it is possible that the limits of many marine species can remain unchanged even when peripheral habitat conditions become favourable (Crisp & Southward, 1958; Fields *et al*., 1993).

It has been noted that range expansions, even from those species which eventually become successfully established, are frequently preceded by several failures (Sax & Brown, 2000). In addition, it was shown that species with a similar larval duration to *P. rustica* may take several years to become completely established over an area similar to the one for which the present expansion was anticipated (see Shanks *et al*., 2003; Siegel *et al*., 2003, for a review). Therefore, even with present favourable climatic conditions and assuming that some extraordinary events such as storm-strengthened anomalous currents allowed the species to overpass the sandy barrier, the elapsed time for such a large expansion to occur may still be insufficient. In this perspective, the hindcasted northern range expansion is not completely wrong (see Araújo *et al*., 2005), and might become visible in future years, as long as the sea and air warming phase of the last decades is maintained.

In the light of current results, the hypothesis proposed by Lima *et al*. (2006) that the changes in the geographical distribution of *P. rustica* observed in NW Iberia were largely related to a joint effect of increasing temperature and alteration in oceanic circulation patterns is reinforced. Therefore, the conceptual model here proposed has the ability to simultaneously explain several spatially independent phenomena, giving it a higher degree of confidence. Nonetheless, because other valid explanations could be advanced, future investigations are still needed in this area. Several recent studies indicate that some organisms have the ability to adapt to different conditions at diverse parts of their range (Sagarin *et al*., 2006), and also that environmental variables might not be physiologically limiting at all range edges (Helmuth *et al*., 2006b). Hence, it is even possible that the factors which were until recently limiting the expansion in northern Portugal could be distinct from those currently governing the northern boundary. Nonetheless, the existence of barriers to dispersal, resulting in limited or no connectivity remains the most parsimonious and, thus the most probable scenario. This hypothesis can be tested using a bioclimatic approach coupled with a dispersal simulation model, encompassing information about oceanic currents and habitat availability. This approach would help to definitely solve the question of the relative importance of temperature or transport in establishing limits in the distribution of *P. rustica*. The use of autocorrelation and/or contagion indexes could also be a way to gain some insights on the strength and extension of larval dispersal.

This study also reinforces the idea that intertidal organisms are clearly influenced by both air and water temperature. Although it has already been shown that various aspects of both terrestrial and aquatic climate drive the physiological performance of these species (Helmuth *et al*., 2006a), the use of a nonlinear modelling technique showed that these factors frequently alternate with one another and with nonclimate-related factors, in determining distributional limits (Helmuth *et al*., 2006b).

Although the present results partially support previous suggestions that BEMs may be inadequate for making projections of future events, they also suggest that this approach can be of great utility in gaining further insights into the nature of the relationship between the distribution of the species and the environment (Araújo *et al*., 2005). Therefore, although sometimes bioclimatic envelopes may appear too limited or deterministic, they certainly still have an important role in ecology by helping to effectively work on some explanatory hypothesis, which can subsequently be tested.

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References

Aken HMv (2002) Surface currents in the Bay of Biscay as observed with drifters between 1995 and 1999. *Deep Sea Research Part I. Oceanographic Research Papers*, **49**, 1071–1086.

Araújo MB, Guisan A (2006) Five (or so) challenges for species distribution modelling. *Journal of Biogeography*, **33**, 1677–1688.

Araújo MB, Pearson RG, Thuiller W, Erhard M (2005) Validation of species-climate impact models under climate change. *Global Change Biology*, **11**, 1504–1513.

Bardey P, Garnesson P, Moussu G, Wald L (1999) Joint analysis of temperature and ocean colour satellite images for mesoscale activities in the Gulf of Biscay. *International Journal of Remote Sensing*, **20**, 1329–1341.

Bertness MD, Gaines SD, Hay M (eds) (2001) *Marine Community Ecology*. Sinauer Associates, Sunderland, MA.

Bohonak AJ (1999) Dispersal, gene flow, and population structure. *The Quarterly Review of Biology*, **74**, 21–25.

Breiman L, Friedman JH, Olshen RA, Stone CJ (1984) *Classification and Regression Trees*. Wadsworth, Belmont.

Christiaens J (1973) Révision du genre *Patella* (Mollusca, Gastropoda). *Bulletin du Museum National d'Histoire Naturelle, Paris*, **182**, 1305–1392.

Clark RA, Fox CJ, Viner D, Livermore M (2003) North Sea cod and climate change – modelling the effects of temperature on population dynamics. *Global Change Biology*, **9**, 1669–1680.

Cook EF, Goldman L (1984) Empiric comparison of multivariate analytic techniques: advantages and disadvantages of recursive partitioning analysis. *Journal of Chronic Diseases*, **37**, 721–731.

Cowen RK, Lwiza KMM, Sponaugle S, Paris CB, Olson DB (2000) Connectivity of marine population: open or closed? *Science*, **287**, 857–859.

Crisp DJ (1958) The spread of *Elminius modestus* Darwin in North West Europe. *Journal of the Marine Biological Association of the UK*, **37**, 483–520.

Crisp DJ, Fischer-Piétte E (1959) Repartition des principales espèces intercotidales de la côte atlantique Française en 1954–1955. *Annales de l' Institut Océanographique, Paris*, **36**, 275–388.

Crisp DJ, Southward AJ (1958) The distribution of intertidal organisms along the coasts of the English Channel. *Journal of the Marine Biological Association of the UK*, **37**, 157–208.

Crumpacker DW, Box EO, Hardin ED (2001) Implications of climatic warming for conservation of native trees and shrubs in Florida. *Conservation Biology*, **15**, 1008–1020.

Davies AJ, Jenkinson LS, Lawton JH, Shorrocks B, Wood S (1998) Making mistakes when predicting shifts in species range in response to global warming. *Nature*, **391**, 783–786.

Davies AJ, Johnson MP (2006) Coastline configuration disrupts the effects of large-scale climatic forcing, leading to divergent temporal trends in wave exposure. *Estuarine, Coastal and Shelf Science*, **69**, 643–648.

De'ath G, Fabricius KE (2000) Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology*, **81**, 3178–3192.

Denny MW, Harley CDG (2006) Hot limpets: predicting body temperature in a conductance-mediated thermal system. *The Journal of Experimental Biology*, **209**, 2409–2419.

DeVantier LM, De'ath G, Turak E, Done TJ, Fabricius KE (2006) Species richness and community structure of reef-building corals on the nearshore Great Barrier Reef. *Coral Reefs*, **25**, 329–340.

Dong BW, Sutton RT, Jewson SP, O'Neill A, Slingo JM (2000) Predictable winter climate in the North Atlantic Sector during 1997–1999 ENSO cycle. *Geophysical Research Letters*, **27**, 985–988.

Edwards JTC, Cutler DR, Zimmermann NE, Geiser L, Moisen GG (2006) Effects of sample survey design on the accuracy of classification tree models in species distribution models. *Ecological Modelling*, **199**, 132–141.

Ekebom J, Laihonen P, Suominen T (2003) A GIS-based step-wise procedure for assessing physical exposure in fragmented archipelagos. *Estuarine, Coastal and Shelf Science*, **57**, 887–898.

Fielding AH, Bell JF (1997) A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environmental Conservation*, **24**, 38–49.

Fields PA, Graham JN, Rosenblatt RH, Somero GN (1993) Effects of expected global climate change on marine faunas. *Trends in Ecology and Evolution*, **8**, 361–367.

Fischer-Piétte E (1948) Sur les éléments de prospérité des Patelles et sur leur spécificité. *Journal de Conchyliologie*, **88**, 45–96.

Fischer-Piétte E (1953) Répartition de quelques mollusques intercotidaux communs le long des côtes septentrionales de l'Espagne. *Journal of Conchology*, **93**, 39–73.

Fischer-Piétte E (1955) Repartition le long des cotes septentrionales de l'Espagne, des principales éspèces peuplant les rochers intercotidaux. *Annales de l'Institut Océanographique Paris*, **31**, 37–124.

Fischer-Piétte E (1958) Sur l'écologie intercotidal Ouest – ibérique. *Comptes Rendus de l'Académie des Sciences (Paris)*, **246**, 1301–1303.

Fischer-Piétte E (1963) La distribution des principaux organisms nord – ibériques en 1954–55. *Annales de l'Institut Océanographique Paris*, **40**, 165–311.

Fischer-Piétte E, Gaillard J-M (1959) Les patelles au long des côtes atlantiques Ibériques et Nord – Marocaines. *Journal of Conchology*, **99**, 135–200.

Friedlaender AS, Halpin PN, Qian SS, Lawson GL, Wiebe PH, Thiele D, Read AJ (2006) Whale distribution in relation to prey abundance and oceanographic processes in shelf waters of the Western Antarctic Peninsula. *Marine Ecology-Progress Series*, **317**, 297–310.

Fronzek S, Luoto M, Carter TR (2006) Potential effect of climate change on the distribution of palsa mires in subarctic Fennoscandia. *Climate Research*, **32**, 1–12.

Garcia-Soto C (2004) Prestige oil spill and Navidad flow. *Journal of the Marine Biological Association of the UK*, **84**, 297–300.

Gaylord B, Gaines SD (2000) Temperature or transport? Range limits in marine species mediated solely by flow. *The American Naturalist*, **155**, 769–789.

Gilman SE (2006) The northern geographic range limit of the intertidal limpet Collisella scabra: a test of performance, recruitment, and temperature hypotheses. *Ecography*, **29**, 709–720.

Grass Development Team (2006) Geographic Resources Analysis Support System (GRASS) Software. ITC-irst, Trento, Italy. http://grass.itc.it

Guisan A, Thuiller W (2005) Predicting species distribution: offering more than simple habitat models. *Ecology Letters*, **8**, 993–1009.

Guisan A, Zimmermann NE (2000) Predictive habitat distribution models in ecology. *Ecological Modelling*, **135**, 147–186.

Hampe A (2004) Bioclimate models: what they detect and what they hide. *Global Ecology and Biogeography*, **13**, 469–476.

Heikkinen RK, Luoto M, Araujo MB, Virkkala R, Thuiller W, Sykes MT (2006) Methods and uncertainties in bioclimatic envelope modelling under climate change. *Progress in Physical Geography*, **30**, 751–777.

Helmuth B, Broitman BR, Blanchette CA *et al*. (2006a) Mosaic patterns of thermal stress in the rocky intertidal zone: implications for climate change. *Ecological Monographs*, **76**, 461–479.

Helmuth B, Mieszkowska N, Moore P, Hawkins SJ (2006b) Living on the Edge of Two Changing Worlds: forecasting the responses of rocky intertidal ecosystems to climate change. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 373–404.

Herbert RJH, Hawkins SJ, Sheader M, Southward AJ (2003) Range extension and reproduction of the barnacle *Balanus perforatus* in the eastern English Channel. *Journal of the Marine Biological Association of the UK*, **83**, 73–82.

Hijmans RJ, Graham CH (2006) The ability of climate envelope models to predict the effect of climate change on species distributions. *Global Change Biology*, **12**, 1–10.

Hodkinson ID (1999) Species response to global environmental change or why ecophysiological models are important: a reply to Davis et al. *Journal of Animal Ecology*, **68**, 1259–1262.

Huntley B, Green RE, Collingham YC *et al*. (2004) The performance of models relating species geographical distributions to climate is independent of trophic level. *Ecology Letters*, **7**, 417–426.

Ibanez M, Pena J, Feliu J (1986) Reproduction of *Patella* spp. on the Basque coast of Spain. *Hydrobiologia*, **142**, 327.

Jackson RD, Bartolome JW (2002) A state-transition approach to understanding nonequilibrium plant community dynamics in Californian grasslands. *Plant Ecology*, **162**, 49–65.

Jarvis CH, Stuart N (2001) A Comparison among Strategies for Interpolating Maximum and Minimum Daily Air Temperatures. Part II: the interaction between number of guiding variables and the type of interpolation method. *Journal of Applied Meteorology*, **40**, 1075–1082.

Karels TJ, Bryant AA, Hik DS (2004) Comparison of discriminant function and classification tree analyses for age classification of marmots. *Oikos*, **105**, 575–587.

Kaschner K, Watson R, Trites AW, Pauly D (2006) Mapping world-wide distributions of marine mammal species using a relative environmental suitability (RES) model. *Marine Ecology Progress Series*, **316**, 285–310.

Koutsikopoulos C, Beillois P, Leroy C, Taillefer F (1998) Temporal trends and spatial structures of the sea surface temperature in the Bay of Biscay. *Oceanologica acta*, **21**, 335–344.

Lemos RT, Pires HO (2004) The upwelling regime off the west portuguese coast, 1941–2000. *International Journal of Climatology*, **24**, 511–524.

Lemos RT, Sansó B (2006) Spatio-temporal variability of ocean temperature in the Portugal Current System. *Journal of Geophysical Research*, **111**, C04010, doi: 04010.01029/02005JC003051.

Levin LA (2006) Recent progress in understanding larval dispersal: new directions and digressions. *Integrative and Comparative Biology*, **46**, 282–297.

Levitus S, Antonov JI, Boyer TP, Stephens C (2000) Warming of the World Ocean. *Science*, **287**, 2225–2229.

Lima FP, Queiroz N, Ribeiro PA, Hawkins SJ, Santos AM (2006) Recent changes in the distribution of a marine gastropod, *Patella rustica* Linnaeus, 1758, and their relationship to unusual climatic events. *Journal of Biogeography*, **33**, 812–822.

Manel S, Williams HC, Ormerod SJ (2001) Evaluating presence – absence models in ecology: the need to account for prevalence. *Journal of Applied Ecology*, **38**, 921–931.

Martins CS, Hamann M, Fiúza AFG (2002) Surface circulation in the eastern North Atlantic, from drifters and altimetry. *Journal of Geophysical Research*, **107**, 3217.

McPherson JM, Jetz W, Rogers DJ (2004) The effects of species' range sizes on the accuracy of distribution model: ecological phenomenon or statistical artefact? *Journal of Applied Ecology*, **41**, 811–823.

Mitchell TD, Carter TR, Jones PD, Hulme M, New M (2004) A comprehensive set of high-resolution grids of monthly climate for Europe and the globe: the observed record (1901–2000) and 16 scenarios (2001–2100). *Tyndall Working Paper*, **55**, 1–30.

Mitchell TD, Hulme M, New M (2002) Climate data for political areas. *Area*, **34**, 109–112.

Patel AC, Markey MK (2005) Comparison of three-class classification performance metrics: a case study in breast cancer CAD. In: *Medical Imaging 2005: Image Perception, Observer Performance, and Technology Assessment*, Vol. 5749 (eds Eckstein MP, Jiang Y), pp. 581–589. SPIE, San Diego, CA.

Pearson RG, Dawson TP (2003) Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography*, **12**, 361–371.

Pearson RG, Dawson TP, Berry PM, Harrison PA (2002) SPECIES: a spatial evaluation of climate impact on the envelope of species. *Ecological Modelling*, **154**, 289–300.

Peliz A, Dubert J, Santos AMP, Oliveira PB, Le Cann B (2005) Winter upper ocean circulation in the Western Iberian Basin – Fronts, Eddies and Poleward flows: an overview. Deep Sea Research Part I. *Oceanographic Research Papers*, **52**, 621–646.

Peliz A, Rosa TL, Santos AMP, Pissarra JL (2002) Fronts, jets, and counter-flows in the Western Iberian upwelling system. *Journal of Marine Systems*, **35**, 61–77.

Pingree RD, Le Cann B (1992) Anticyclonic eddy X91 in the southern bay of Biscay, May 1991 to February 1992. *Journal of Geophysical Research*, **97**, 14353–14367.

Planque B, Lazure P, Jegou A (2006) Typology of hydrological structures modelled and observed over the Bay of Biscay shelf. *Scientia Marina*, **70S1**, 43–50.

Puillat I, Lazure P, Jégou AM, Lampert L, Miller PI (2004) Hydrographical variability on the French continental shelf in the Bay of Biscay, during the 1990s. *Continental Shelf Research*, **24**, 1143–1163.

Questier F, Put R, Coomans D, Walczak B, Heyden YV (2005) The use of CART and multivariate regression trees for supervised and unsupervised feature selection. *Chemometrics and Intelligent Laboratory Systems*, **76**, 45–54.

Raffaelli D, Hawkins SJ (1996) *Intertidal Ecology*. Chapman & Hall, London.

Raubertas RF, Rodewald LE, Humiston SG, Szilagyi PG (1994) ROC curves for classification trees. *Medical Decision Making*, **14**, 169–174.

Ridgway SA, Reid DG, Taylor JD, Branch GM, Hodgson AN (1998) A cladistic phylogeny of the family Patellidae (Mollusca: Gastropoda). *Philosophical Transactions of the Royal Society of London B*, **353**, 1645–1671.

Rouget M, Richardson DM, Lavorel S, Vayreda J, Gracia C, Milton SJ (2001) Determinants of distribution of six *Pinus* species in Catalonia, Spain. *Journal of Vegetation Science*, **12**, 491–502.

Sagarin RD, Gaines SD (2002) Geographical abundance distributions of coastal invertebrates: using one-dimensional ranges to test biogeographic hypotheses. *Journal of Biogeography*, **29**, 985–997.

Sagarin RD, Gaines SD, Gaylord B (2006) Moving beyond assumptions to understand abundance distributions across the ranges of species. *Trends in Ecology & Evolution*, **21**, 524–530.

Santos AMP, Peliz A, Dubert J, Oliveira PB, Angelico MM, Re P (2004) Impact of a winter upwelling event on the distribution and transport of sardine (*Sardina pilchardus*) eggs and larvae off western Iberia: a retention mechanism. *Continental Shelf Research*, **24**, 149–165.

Sax DF, Brown JH (2000) The paradox of invasion. *Global Ecology & Biogeography*, **9**, 363–371.

Shanks AL, Grantham BA, Carr MH (2003) Propagule dispersal distance and the size and spacing of marine reserves. *Ecological Applications*, **13**, S159–S169.

Siegel DA, Kinlan BP, Gaylord B, Gaines SD (2003) Lagrangian descriptions of marine larval dispersion. *Marine Ecology Progress Series*, **260**, 83–96.

Simkanin C, Power A, Myers A *et al*. (2005) Using historical data to detect temporal changes in the abundances of intertidal species on Irish shores. *Journal of the Marine Biological Association of the UK*, **85**, 1329–1340.

Southward AJ, Hawkins SJ, Burrows MT (1995) Seventy years' observations of changes in distributions and abundance of zooplankton and intertidal organisms in the Western English Channel in relation to rising sea temperature. *Journal of Thermal Biology*, **20**, 127–155.

Swets JA (1988) Measuring the accuracy of diagnostic systems. *Science*, **240**, 1286–1293.

Tomanek L, Helmuth B (2002) Physiological ecology of rocky intertidal organisms: a synergy of concepts. *Integrative and Comparative Biology*, **42**, 771–775.

Vaughan IP, Ormerod SJ (2005) The continuing challenges of testing species distribution models. *Journal of Applied Ecology*, **42**, 720–730.

Vayssières MP, Plant RE, Allen-Diaz BH (2000) Classification trees: an alternative non-parametric approach for predicting species distributions. *Journal of Vegetation Science*, **11**, 679–694.

Wolcott FG (1973) Physiological ecology and intertidal zonation in limpets: a critical look at 'limiting factors'. *Biological Bulletin*, **145**, 389–422.

Woodruff SD, Diaz HF, Elms JD, Worley SJ (1988) COADS release 2 data and metadata enhancements for improvements of marine surface flux fields. *Physics and Chemistry of the Earth*, **23**, 517–526.

Zacherl D, Gaines SD, Lonhart SI (2003) The limits to biogeographical distributions: insights from the northward range extension of the marine snail, *Kelletia kelletii* (Forbes, 1852). *Journal of Biogeography*, **30**, 913–924.

Zweig MH, Campbell G (1993) Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clinical Chemistry*, **39**, 561–577.

