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The role of habitat gaps and oceanography on the biogeography and population genetics of rocky intertidal gastropods in the Bay of Biscay

by

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A thesis submitted in partial fulfilment for the degree of Doctor of Philosophy

August 2018

Abstract

In biogeography, patterns of species abundance, distribution, size, population genetics and morphology often do not follow simple latitudinal gradients – which are classically assumed in such "rules" as the abundant centre hypothesis and Bergmann's rule. Rather, the biogeography of a species or groups of species are more likely to be associated with abiotic and biotic variables such as availability of suitable habitat, temperature, dispersal potential, and habitat specificity. However, previous biogeographic studies rarely go beyond simple pattern description, particularly for marine species. In this thesis, I find that regional patterns of abundance, distribution, size, morphology, and population genetics are often associated with regional and local differences in environmental and biotic variables, such as upwelling intensity, habitat availability, and intrinsic attributes of species ecology, such as their level of habitat specificity. For example, a lower availability of suitable habitat can result in a fragmented distribution and subsequently lower genetic connectivity for species dependent upon that habitat. Differing abundances and recruitment among localities may also affect intraspecific competition, so density-dependent interactions occur, resulting in smaller/larger individuals. This would not necessarily follow the classically assumed temperature-size rule of smaller individuals usually being found at higher temperatures. Within a species, differing growth rates and adaptations to environmental variables can result in local morphological variation.

These biogeographic concepts are explored in depth using rocky intertidal species of the Bay of Biscay as a model system, which contains a 230 km habitat gap and regional variation in temperature unrelated to a latitudinal gradient. In Chapter 2, the almost complete mitochondrial genome of *Steromphala umbilicalis* is described in relation to other Vetigastropoda. By removing different genes, and using amino acid or nucleotide sequences, different phylogenies are generated. From this, it is proposed that selection mechanisms may be occurring. In Chapter 3, the population genetics of the congeners *S. umbilicalis* (habitat generalist) and *S. pennanti* (habitat specialist), are described over the sympatric portion of their range, using both a newly developed mitochondrial marker and microsatellites. By combining this information with abundance estimates, *S. umbilicalis* is shown to have a more continuous distribution and is more abundant, and therefore shows greater genetic connectivity than *S. pennanti* which has a more fragmented distribution and lower abundance. In Chapter 4, the abundances, size structures and distributions of *S. umbilicalis*, *S. pennanti, Phorcus lineatus*, *Patella vulgata* and *Patella depressa* are explored using multiple linear regression for environmental variables including sea surface temperature, air temperature, wave exposure, distance to the habitat gap, and adjacent rocky substrate. Sea temperature showed a positive relationship with *P. depressa* abundance and the opposite for *P. vulgata*. Subsequent density-dependent interactions in *P. depressa* resulted in smaller individuals being associated with higher abundance between localities, and between quadrats within localities for both *Patella* species. The habitat gap amplified the differences in abundance between the cool water region in northern France dominated by *P. vulgata* and the warm water Basque region dominated by *P. depressa*. In Chapter 5, the aspect ratio of *S. umbilicalis* is explored at the macroscale in relation to multiple environmental variables. Sea temperature shows the best relationship with aspect ratio, both with initial European data and an expanded dataset including museum specimens from the British Isles. Whilst the underlying mechanism is unclear, it may be linked to a decreased growth rate at higher temperature, resulting in a more pointed shell.

This work throws new light on biogeographic patterns and processes, particularly regarding temperature around range centres. It has implications when considering the impacts of global warming, particularly in relation to rocky intertidal biogeography.

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Appendix 7.7 (https://doi.org/10.5258/SOTON/D0618) on the Pure database system contains quadrat information (Quadrats.xlsx) with abundance of species and *Patella* measurements within each quadrat. It also contains *Steromphala pennanti* measurements (G_pen.csv) and *Steromphala umbilicalis* measurements (S_umb_measurements.xlsx). Referencing details for two papers, one of which is published (Wort *et al.*, 2017), one of which is in press (Wort *et al.*, in press) based on chapters from this thesis are included in the reference section.

Academic Thesis: Declaration Of Authorship

I, Edward Wort, declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

The role of habitat gaps and oceanography on the biogeography and population genetics of rocky intertidal gastropods in the Bay of Biscay

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
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   Wort, E.J., Fenberg, P.B. & Williams, S.T. (2017) Testing the contribution of individual genes in mitochondrial genomes for assessing phylogenetic relationships in Vetigastropoda. *Journal of Molluscan Studies*, **83**, 123-128

Signed: .....

Date: .....

Acknowledgements

In writing this thesis, I have not so much stood on the shoulders of giants as crowd-surfed a vast host of people of differing stature and rarely seen much further than them. I am, of course, extremely grateful to my main supervisor Dr Phil Fenberg for his constant support at every stage of my project and his bottomless patience. From my remaining host of supervisors (Dr Ellinor Michel, Dr Marc Rius and Professor Steve Hawkins) I particularly thank Dr Suzanne Williams, whose enthusiasm and watchful eye were at the heart of both genetics chapters in this thesis. I also thank my panel chair Dr Cathy Lucas, who helped provide a realistic structure and goals throughout the PhD.

In the lab, Lisa Smith at the NHM deserves a special mention for the many late nights and showing me the delicate process of DNA extraction, which I passed on to Lucy Henshall, Alex Loveridge and Katherine Ellis during their time in the lab with me. I also thank John Gittins for his quiet supervision in Southampton.

In the field, I must thank Gary Fisher and Moira for their initial help in organisation. I am grateful to Matthew Cobain for volunteering for every bit of fieldwork, including storms and pasta porridge. I am also indebted to Elena Bollati, Katie St. John Glew, Sterre Witte, Claudia Fry, Georgios Margaritis and Rebecca Wilson.

There are many who have kept me on an even keel throughout the PhD. As usual, I cannot help but mention the karate club, particularly Dan, Gavin, Shippy, Edd, Ollie, Steve, Tony, and my karate wife Gabriella. Consider this final proof I don't study snail penises. I also thank Sev, Matt and Harriet for their good cheer and moral support over many lunches, and the Anthonys for providing musical and scientific enrichment. I'm very grateful to Steve Bourne and Matthew Cobain for living with me while we all went through the trials of the last year of a PhD. As a tripod, we could all lean on each other against the storm! Thanks is also due to my office mates, particularly Elwyn, Stuart and Lina.

I thank my loving girlfriend Helen for the silliness and for caring for me when I needed it.

Finally I thank my parents and brother, who have supported me in every way possible before and during this PhD, and my family in general, even if I struggled to explain the "why" to their satisfaction!

Chapter 1: Introduction

1.1 Project outline

Species respond to changing climate over both large spatial and long temporal scales (Harley *et al.*, 2006; Hellberg *et al.*, 2001; Lenoir & Svenning, 2013; Mieszkowska *et al.*, 2006; Neiva *et al.*, 2010; Simkanin *et al.*, 2005; Southward, 1991; Southward & Crisp, 1954; Southward *et al.*, 1995). In a rapidly changing world, understanding the past, present, and forecast future broadscale geographic patterns is essential for putting more local changes into the global context. Rocky intertidal species in the Bay of Biscay have been used in this study as a model system to explore these themes.

1.2 Marine Biogeography

The field of biogeography seeks to identify the biotic and abiotic factors affecting patterns within and between species across large spatial scales (Briggs, 1974). These patterns include geographic distribution (Lima *et al.*, 2007b), abundance (Mieszkowska *et al.*, 2007), morphology (Irie, 2005), population genetics (Fenberg *et al.*, 2010), phylogeography (Donald *et al.*, 2012) and biodiversity of assemblages (Rivadeneira *et al.*, 2015). These trends in biogeography are frequently associated with temperature gradients, namely air temperature (Lenoir & Svenning, 2013) and water temperature (Southward *et al.*, 1995) for terrestrial and aquatic species respectively. Intertidal species, by definition, spend time immersed in water and emersed in air and are therefore affected by both air and water temperatures (Firth *et al.*, 2011; Fischer-Piette & Crisp, 1959; Harley *et al.*, 2006; Helmuth *et al.*, 2006; Seabra *et al.*, 2016). Furthermore, availability or suitability of habitat, principally whether the substrate is hard or sediment for the intertidal zone, can influence species distributions and demographics (Fenberg *et al.*, 2014; Shanks *et al.*, 2014).

1.3 Biogeography of Rocky Shores

There are several advantages to using rocky intertidal invertebrates as model species for biogeographic studies. For example, many rocky intertidal invertebrates are sessile or slow moving, making them easy to sample (Sagarin & Gaines, 2002). Intertidal habitat can also be identified and mapped using satellite imagery with GIS software (Fenberg *et al.*, 2014). Maps can then be used in

combination with high resolution, geo-referenced environmental data (e.g. Tyberghein *et al.* (2012)) to test biogeographical hypotheses and "rules". One such example is the abundant centre hypothesis, where it is assumed that there will be greater abundance towards the geographic range centre of a species and a decrease in abundance towards range edges (Brown *et al.*, 1995; Hengeveld & Haeck, 1982). This is due to the conditions (principally temperature over a latitudinal gradient) being optimal for that species towards the range centre and recruitment from adjacent populations higher than at range edges. However, this would occur only under certain conditions, namely an environmental gradient that co-varies with latitude, consistent local factors and an even distribution of suitable habitat. Whilst a useful starting point, the reality for intertidal biogeography is frequently more complex as regional variation in temperature, such as upwelling of cold water, results in deviation from a simple latitudinal gradient (Sagarin & Gaines, 2002). Moreover, an increasing proportion of unsuitable substrate can decrease recruitment between localities, resulting not only in highly variable abundances, but also potentially genetic and morphological differentiation (Fenberg *et al.*, 2014). Where the principal form of dispersal between localities is transport of larvae, the direction and velocity of the dominant current regimes can also determine connectivity between them, as well as the duration of the larval phase (Pannacciulli *et al.*, 1997; Treml *et al.*, 2008). Local variations in abiotic variables such as wave action can also determine both the abundance and vertical zonation of intertidal species (Ballantine, 1961).

The effect that these environmental factors have on the biogeography of species can be assessed using high spatial and temporal resolution surveying, or targeted surveying of particular species. High spatial resolution options include semi-quantitative and quantitative surveys over long timescales, such as the long running 'MarClim' datasets and their precursors (Bowman & Lewis, 1977; Crisp & Southward, 1958; Hawkins *et al.*, 2013; Kendall & Lewis, 1986; Kendall, 1987; Kendall *et al.*, 2004; Lewis, 1964; Lewis & Bowman, 1975; Lewis, 1986; Lewis *et al.*, 1982; Southward & Crisp, 1954; Williamson & Kendall, 1981). This has contributed to a body of literature targeting rocky intertidal species abundance and distribution throughout the British Isles, focussing on the role of temperature over time (Crisp & Southward, 1958; Herbert *et al.*, 2003; Mieszkowska *et al.*, 2006; Simkanin *et al.*, 2005; Southward, 1991; Southward & Crisp, 1954; Southward *et al.*, 1995). By contrast, other regions, such as the rocky Atlantic coasts of France and Spain, are surveyed more sporadically, both spatially and temporally. In this region, there was a peak in surveys led primarily by Fischer-

Piette in the 1930s to the 1950s (Evans, 1957; Fischer-Piette, 1935, 1936, 1955; Fischer-Piette & Crisp, 1959; Fischer-Piette *et al.*, 1962), a synthesis of regional research over the past half century (Southward *et al.*, 1995) and a recent thesis (Alcock, 2003 PhD Thesis). Other research and surveys covering the region at a similar scale to the MarClim surveys usually targeted the abundance and distribution of a few select species (Lima *et al.*, 2006; Wethey & Woodin, 2008; Wethey *et al.*, 2011), also focussing on the role of temperature. Multi-species biogeographic studies in this region are particularly rare, especially for rocky shore gastropods, here the focus of this thesis.

The role of temperature in controlling abundance can be complex and act in several different pathways. For example, it has been noted that temperature can influence timing, frequency and duration of spawning events in intertidal species such as *Patella depressa* Pennant, 1777 (Moore et al., 2011) and *Perforatus perforatus* (Bruguière, 1789) (Herbert et al., 2003). Increased spawning can have several negative impacts on the sizes of individuals by diverting energy from growth and increasing the number of recruits, thereby increasing intraspecific competition (Blackmore, 1969; Workman, 1983). This intraspecific competition can then reduce growth, preventing the occurrence of large individuals (Boaventura *et al.*, 2002; Boaventura *et al.*, 2003; Moore *et al.*, 2007b; Ribeiro *et al.*, 2009). Furthermore, a second ecological "rule" is that (at least interspecifically) larger organisms are found at the cooler temperatures found at higher latitudes (Bergmann, 1848). As with the abundant-centre hypothesis, Bergmann's rule is subject to caveats, as at the intraspecific level whilst aquatic arthropods show a decrease in body mass with increasing temperature (Forster *et al.*, 2012), terrestrial arthropods often show an increase in mass with higher temperatures (Fenberg *et al.*, 2016; Horne *et al.*, 2015).

Size structures in populations at range edges can also vary depending on which factors determine the geographic limit (Sagarin & Gaines, 2002). For example, a pole-ward range edge with periodic successful recruitment and subsequent low intraspecific competition is normally characterised by large, older individuals and periodic local extinctions (Fenberg & Rivadeneira, 2011; Frank, 1975; Lewis *et al.*, 1982; Mieszkowska *et al.*, 2013). This leading edge is also frequently associated with individuals with lower genetic diversity compared to more central populations (Hellberg *et al.*, 2001; Ribeiro *et al.*, 2010). By contrast, if a geographic limit is determined principally by an absence of suitable habitat beyond, it is still possible for regular recruitment to occur at that locality,

resulting in a large population composed of smaller individuals (Sagarin & Gaines, 2002) and similar genetic diversity compared to more central populations (Wort, personal observation). For some species, a range limit can be defined by a combination of environmental variables, such as habitat gaps coinciding with temperature changes (Lima *et al.*, 2007b; Reid, 2002).

Decreased recruitment between populations across habitat gaps can result in their genetic isolation and subsequent differentiation (Knox *et al.*, 2011; Williams *et al.*, 2011). There are several different types of genetic markers that can be used to identify differentiation between populations. These include mitochondrial DNA commonly used across most taxa such as cytochrome c oxidase (Haupt *et al.*, 2013; Muñoz-Colmenero *et al.*, 2012) and microsatellites (Fenberg *et al.*, 2010; Lourenço *et al.*, 2016) which frequently only amplify within a given genus or species (McInerney *et al.*, 2011). Where sequence data are used, regions with greater rates of nucleotide substitution can be used to evaluate recent genetic transfer between populations at a higher temporal resolution. However, as some regions may be non-neutral markers they can be subject to selection mechanisms (Chrismas *et al.*, 2014; James *et al.*, 2016). In these circumstances, it can be useful both to identify the optimum regions for marker development in population genetics (Neiva *et al.*, 2014) and whether there is positive selection for that particular genetic attribute (Mishmar *et al.*, 2003). If using a mitochondrial DNA marker for population genetics, it is therefore useful to identify genes that are subject to positive or negative selection, as well as showing a high substitution rate.

1.4 Intertidal biogeography of the Bay of Biscay

Recent research has reported a northwards shift in species' ranges on the North-east Atlantic intertidal zone, which has typically been attributed to increasing sea temperatures (Hawkins *et al.*, 2009; Hawkins *et al.*, 2008; Lima *et al.*, 2007b; Mieszkowska *et al.*, 2007; Mieszkowska *et al.*, 2013; Mieszkowska *et al.*, 2006; Simon-Bouhet *et al.*, 2006; Southward *et al.*, 1995; Wethey & Woodin, 2008). On the north coast of Spain, a west to east sea temperature gradient exists from cool upwelling water on the north-west coast of Spain, to warm water around the Basque country (Fischer-Piette, 1955; Fischer-Piette & Crisp, 1959; Southward *et al.*, 1995). A second, more usual, gradient of decreasing temperature with increasing latitude occurs along French Atlantic coast which is aligned approximately from south to north (Fischer-Piette & Crisp, 1959; Koutsikopoulos *et al.*, 1998; Seabra *et al.*,

2011). These temperature gradients have been associated with abundance patterns of many intertidal species (Berke *et al.*, 2010; Duarte *et al.*, 2013; Fischer-Piette, 1955; Hawkins *et al.*, 2009; Neiva *et al.*, 2014; Southward *et al.*, 1995; Wethey & Woodin, 2008; Wethey *et al.*, 2011). Several fucoid and gastropod species, such as *Fucus serratus* (Linnaeus 1753) (Duarte et al., 2013) and *Patella vulgata* Linnaeus, 1758 (Fischer-Piette, 1955), which prefer cold water conditions have been identified as absent from the eastern portion of the north coast of Spain and reappear on the north-west coast. Conversely, increased sea temperature functions well as a predictor of the changing distributions warm water species such as *Patella rustica* Linnaeus, 1758, particularly bridging habitat gaps in northern Portugal (Lima *et al.*, 2006). However, this species has not extended north of a habitat gap of sandy beach some 230 km in length in the south-east of Bay of Biscay, in spite of a local increase in abundance at rocky intertidal localities on its southern edge (Lima *et al.*, 2006). The distance that larvae can disperse may also be affected by the size of the habitat gap itself, as well as north to south longshore currents and low salinity plumes from the rivers Loire and Gironde. For example, the larvae of *Stramonita haemastoma* (Linnaeus, 1767) are capable of long distance dispersal (Claremont *et al.*, 2011). It was first recorded just south of the habitat gap in St Jean de Luz and Biarritz in 1897 (Dautzenberg, 1897) under the name of *Purpura haemastoma* and has not moved north since, suggesting that the habitat gap is restricts to the northern range edge. These examples (*P. rustica and S. haemastoma*) suggest that the habitat gap may act as a connectivity barrier for some rocky shore gastropod species.

Whilst there is no natural rocky habitat across this habitat gap, there is a patchy distribution of artificial habitat, mostly military bunkers with few invertebrate species (Lima *et al.*, 2007a). To evaluate the importance of the habitat gap in Aquitaine, it is necessary to compare it to the wider distribution of rocky intertidal habitat (Warmoes *et al.*, 1992). Generating a habitat map of the coast of the Bay of Biscay will help to resolve questions regarding the distribution of suitable habitat. Indeed, it has been suggested that *Littorina arcana* Hannaford-Ellis, 1978 and *Littorina compressa* Jeffreys, 1865 both have their distribution limited by a lack of suitable habitat to lay eggs (Mill & Grahame, 1990; Warmoes *et al.*, 1992).

There have been numerous population genetics studies with sampling sites in and around the Bay of Biscay, targeting a wide variety of intertidal taxa over differing scales and regions with differing markers (Assis *et al.*, 2014; Campo *et al.*, 2010; Doellman *et al.*, 2011; Muñoz-Colmenero *et al.*, 2015; Neiva *et al.*, 2014; Neiva *et al.*, 2015). Although it has been suggested that genetic

differentiation may under-represent ecological isolation (Hawkins *et al.*, 2016), there are several common results in these studies alluding to differing glacial refugia and subsequent colonisation pathways. For example, there is a genetic break between the west and north coast of the Iberian Peninsula for *Fucus vesiculosus* Linnaeus, 1753 (Assis et al., 2014), *Fucus ceranoides* Linnaeus, 1753 (Neiva et al., 2010, 2012), which is viewed as indicative of different glacial refugia. Whilst the northern edge of both of the species expanded during glacial retreat and subsequently the leading edge population show little genetic differentiation, the populations closer to the rear edge do not mix as much (Assis *et al.*, 2014). *Pollicipes pollicipes* (Gmelin, 1790) in particular shows genetic differentiation between localities in northern France and others, with the suggestion of a northern glacial refugium which has yet to mix with southern populations on the Iberian Peninsula (Campo *et al.*, 2010). The habitat gap coincides with the break between the different populations from glacial refugia, suggesting it may play a role in maintaining genetic isolation.

Bifurcaria bifurcata has largely well mixed populations in the Bay of Biscay with the notable exception of the eastern portion of the northern Iberian Peninsula (Neiva *et al.*, 2015), suggesting that the temperature gradient may also result in genetic isolation. Indeed, *Pelvetia canaliculata* shows several genetically differentiated regions associated with the change in sea temperature along the north Iberian coast (Neiva *et al.*, 2014). It has also been suggested that different genetic populations in the same species are associated different Atlantic glacial refugia (Neiva *et al.*, 2014), as was described for *P. pollicipes* (Campo et al., 2010).

1.5 Thesis aims and objectives

Temperature and availability of suitable habitat can be associated with biogeographic patterns of species distribution, abundance and size. This can frequently result in these biological response variables deviating from broader ecological ‘rules’. In addition, species morphology and population genetics may be associated with the same environmental variables at the macroscale. The wider aim of this study is to explore these biological response variables in a group of species across the same biogeographic region. To achieve this, the biogeography of rocky intertidal gastropod species principally from the Atlantic coasts of France and Spain in and around the Bay of Biscay is investigated. Differing species life history, ecology, and thermal tolerances are also duly considered in any

explanations. All of the aforementioned biological response variables are considered for *Steromphala umbilicalis* (da Costa 1778), as well as its phylogeny in relation to other Vetigastropoda.

Chapter 2 examines the relative importance of different genes on the mitochondrial genome in constructing phylogenies, particularly with regard to selection. To do this, the nearly complete mitochondrial genome of *Steromphala umbilicalis* is assembled and multiple phylogenies for Vetigastropoda are generated. The hypotheses tested are that selection occurs on Vetigastropoda mitochondrial genes and that differing phylogenies are generated for Vetigastropoda depending on which mitochondrial genes are used. This was published in the Journal of Molluscan Studies (Wort *et al.*, 2017) prior to the genus being reassigned from *Gibbula* to *Steromphala* (Affenzeller *et al.*, 2017).

Chapter 3 aims to describe the relationship between habitat specificity, abundance, distribution and population genetics. The hypotheses tested are: *S. umbilicalis* (a habitat generalist) is more abundant than *Steromphala pennanti* (Philippi, 1846) (a habitat specialist); *S. umbilicalis* shows less genetic differentiation than *S. pennanti* between the same localities. In this context, regional variation in abundance, distribution of both species and biological habitat in relation to sea temperature, and associated differences in population genetics of *S. umbilicalis* and *S. pennanti* are explored. This has been accepted as a research paper by the Journal of Biogeography (Wort *et al.*, in press). In addition, Chapter 3 includes population genetics based on the mitochondrial DNA of *S. umbilicalis* (using results from Chapter 2).

Chapter 4 aims to identify the drivers of intertidal species abundance and distribution towards their range centre. The biogeographic patterns of abundance and body size of rocky intertidal gastropods around the Bay of Biscay are explained, namely for the species *Patella vulgata, Patella depressa, Phorcus lineatus* (da Costa, 1778)*, Steromphala umbilicalis*, and *Steromphala pennanti*. The relative importance of different environmental variables, such as temperature, wave exposure and availability of suitable substrate, in predicting abundance and body size are focussed on particularly. This chapter therefore tests multiple hypotheses within the Bay of Biscay, namely that: *Patella vulgata* abundance decreases with increasing sea temperature; *Patella depressa* abundance increases with increasing sea temperature; *Steromphala pennanti* is abundance increases with increasing fucoid abundance; in patellids and *Steromphala* size decreases with increasing abundance; the best descriptive

variables for *S. umbilicalis* and *Phorcus lineatus* are exposure and sea temperature.

Chapter 5 examines whether shell morphology in gastropods is associated with environmental variables at the macroscale. To do this, the biogeography of the aspect ratio of *S. umbilicalis* is explored. This principally examines the role of temperature using both field and museum specimens throughout the range of this species. The hypothesis tested is that shell morphology is associated with sea temperature.

In the concluding remarks the wider implications of the study as whole are discussed, as well as predictions of the likely impacts of increasing sea temperatures in the region. The limitations and areas for future research, both in the Bay of Biscay and in the wider field of biogeography, are described.

Chapter 2: Testing the contribution of individual genes in mitochondrial genomes for assessing phylogenetic relationships in Vetigastropoda

2.1 Introduction

Phylogenetic analyses for molluscs frequently make use of the mitochondrial (mt) markers COX1, 12S and 16S, in part because of the availability of universal primers (Aktipis & Giribet, 2011; Plazzi & Passamonti, 2010; Plazzi *et al.*, 2011; Wakabayashi *et al.*, 2012; Williams *et al.*, 2010). Recent phylogenetic studies have expanded on gene sampling, sequenced entire mt genomes and undertaken phylogenetic analyses using all protein-coding and rRNA genes for determining relationships within Mollusca, generally resulting in well-resolved trees with highly supported nodes (Uribe *et al.*, 2015; White *et al.*, 2011; Williams *et al.*, 2014). However, several papers have pointed out that trees based purely on mt DNA can produce erroneous deep relationships between different molluscan taxa (Bernt *et al.*, 2013b; Stöger & Schrödl, 2013). This is perhaps unsurprising, given that mt DNA generally evolves more quickly than the nuclear genome (Burton & Barreto, 2012), and deeper relationships may therefore be beyond the saturation point of mt DNA. However, with the recent development of next generation sequencing, entire mt genomes can be sequenced rapidly and relatively cheaply. It is therefore important to determine the utility and reliability of the mt genome as a marker for phylogenetic analysis.

Mt DNA has some of the features of an ideal genetic marker, such as being small and highly conserved in size for animals (Burton & Barreto, 2012), strict orthology of protein coding genes (PCG) and rRNAs (Gissi *et al.*, 2008), and possessing no introns and very short intergenic regions (Gissi *et al.*, 2008). However, the neutrality of the mt genome has been questioned recently, particularly due to interactions with the nuclear genome (Burton & Barreto, 2012). Selection in the mt genome has been identified across multiple taxa (James *et al.*, 2016), including mammals such as humans (Kivisild *et al.*, 2005; Ruiz- Pesini & Wallace, 2006), shrews (Fontanillas *et al.*, 2005), killer whales (Foote *et al.*, 2011), as well as other vertebrates such as fish (Jacobsen *et al.*, 2016). Within molluscs,

there exists a very strong correlation between egg size divergence and COX1 gene amino acid divergence in geminate species of the gastropod genus *Nucella*, sampled either side of the Isthmus of Panama (Marko & Moran, 2002), suggesting that selective pressure may also play an important role in the evolution of some mt genes in molluscan taxa. Differing results of phylogenetic analyses based on nuclear or mitochondrial gene markers, or performance of COX and CytB markers relative to ND genes within molluscs has been noted (Havird & Santos, 2014), lending greater weight to this hypothesis.

In this study the robustness of using whole or nearly complete mt genomes based on both nucleotide and amino acid variation to create phylogenies in vetigastropods is assessed, as has been done in other taxa (Kuo *et al.*, 2008; Seixas *et al.*, 2016; Uribe *et al.*, 2015). We ask how the removal of one or more mt genes affects tree structure, and how the resulting phylogenies compare with those based on both nuclear markers and mt DNA. Using a nearly complete mt genome newly obtained for *Steromphala umbilicalis* (Mollusca: Vetigastropoda) as well as 13 published sequences from other vetigastropods, it is shown that different tree structures are supported when different genes are removed from analyses.

The relationships within Vetigastropoda have been subject to several different interpretations based on different genetic and morphological markers (molecular markers: Kano *et al.,* 2009; Aktipis & Giribet, 2010, 2011; Kano *et al.*, 2013; Uribe *et al.*, 2015; Nakajima *et al.*, 2016; morphological markers: Haszprunar, 1988; Hedegaard, 1997; Ponder & Lindberg, 1997; Sasaki, 1998). The new mt genome for *Steromphala umbilicalis* will contribute to a more robust phylogenetic framework for Vetigastropoda, as it is the first mt genome for the highly speciose family Trochidae *sensu* Williams (2012).

2.3 Methods and results

DNA was extracted from 25 mg of ethanol-preserved mantle tissue of *Steromphala umbilicalis* collected from Peveril Point, Dorset, UK using a 'DNeasy Blood and tissue sample' kit following the manufacturer's instructions (Qiagen), including the optional extra elution with 200 µl of buffer 'AE'. Yield and integrity of double-stranded DNA was determined using a 'Qubit' fluorometer 2.0 (Invitrogen, Waltham, MA, USA) and a Tapestation (Agilent, Santa Clara, CA, USA). DNA was fragmented to approximately 550 bp lengths using a 'Covaris' ultrasonicator (Covaris, Woburn, MA, USA). An indexed library was constructed

with a 'TruSeq Nano' DNA sample preparation kit by following the manufacturer's recommendations (Illumina Inc., San Diego, CA, USA), apart from using a six-cycle PCR enrichment rather than the recommended eight cycles. Following verification of library concentration by qPCR, each library was sequenced on 1/5th of a flowcell on an 'Illumina MiSeq' platform (V.3 chemistry, 2x300 bp).

Reads were trimmed with an error probability limit of 0.05 and no ambiguities allowed, then paired with an expected distance of 750bp using GENEIOUS v.8.1.5 (Kearse *et al.*, 2012). Resulting contigs were subsequently assembled to a previously reported *Steromphala umbilicalis* COX1 sequence (GENBANK accession no. GQ232367; Williams *et al.*, 2010). Overlapping reads were mapped and reassembled iteratively, until generating a nearly complete mt genome 16,277 bp (GUMSWAN3; GENBANK Acc. No.KX646541) in length. At this point, the genome could not be extended further without adding reads of uncertain sequence at the 3' end (unknown number of AT repeats).

All 22 putative tRNAs, 13 PCGs and both rRNA genes were identified using MITOS (Bernt *et al.*, 2013a) and checked with ARWEN (Laslett & Canback, 2007). Gene annotation was performed after alignment of the new *Steromphala umbilicalis* sequences with genes from 13 reference mt genomes of other vetigastropods, later used in the phylogenetic analysis.

Nucleotide sequences for the 15 mt genes (13 PCG and two rRNA genes) were each aligned separately using the translational alignment tool in GENEIOUS (Kearse *et al.*, 2012) for protein-coding genes and MUSCLE (Edgar, 2004) for rRNA genes. Phylogenetic analyses were also carried out using amino acid sequences from PCGs, which in other invertebrate phylogenetic trees have outperformed nucleotide trees (Seixas *et al.*, 2016). GBLOCKS was used to remove poorly aligned positions or divergent regions, with DNA in PCG treated as 'codons' and in rRNA genes treated as 'nucleotides' and gap positions in all alignments allowed within the final blocks (Catresana, 2000; Talavera & Catresana, 2007).

Table 2.1: Posterior probability (PP) support values for nodes highlighted with coloured dots in six trees. Support values in brackets are given in order for red/orange/blue/yellow/green dots. Trees based on amino acids in bold.

Tree	Removed genes
A	None (0.95/1/1/1/1), ATP8 (0.97/1/1/1/1), CytB (0.79/0.75/0.75/0.75/1), ND1 (0.99/0.96/0.96/0.96/1), ND2 (0.82/1/1/1/1), ND3 (0.71/1/1/1/1), ND4 (0.69/1/1/1/1), ND4L (0.97/1/1/1/1), ND5 (1/1/1/1/1), ND6 (0.98/1/1/1/1), **ND5 (1/1/0.84/1/1), ND6 (1/1/0.58/0.63/1)**
B	COX1 (0.96), COX2 (0.86), COX3 (0.90)
C	16S (1/1/1/0.98), 12S (1/1/1/1), ATP6 (0.92/0.92/0.92/1)
D	**None (0.95/0.89), ATP6 (0.68/0.55), ATP8 (0.91/0.78), COX1 (0.59/0.84), COX2 (0.96/0.93), CYTB (0.87/0.93), ND1 (1/0.92), ND2 (0.62/0.70), ND3 (0.99/0.92), ND4 (0.88/0.93), ND4L (1/0.99)**
E	**COX3**
F	12S and 16S

The resulting alignments were verified by eye, concatenated, and subjected to phylogenetic analysis using MRBAYES (Ronquist & Huelsenbeck, 2003) on CIPRES (Miller *et al.*, 2010), employing the substitution models recommended by the corrected AIC score (Hurvich & Tsai, 1989) in JMODELTEST (Darriba *et al.*, 2012). In the case of nucleotide variation, this was GTR+I+G for all genes excepting ATP6 and ATP8, where the GTR+G model was selected. Protein substitution model selection was carried out in MEGA 6 (Tamura *et al.*, 2013), with the corrected AIC score (Hurvich & Tsai, 1989) used to determine the suitable model, namely: LG+G+I+F for COX1 and COX2; JTT+G for ATP8; LG+G+F for ATP6, ND1, ND4, ND4L, ND5; mtREV24+G+I+F for COX3 and CYTB; mt REV24+G+F for ND3 and ND6; JTT+G+F for ND2. *Nerita fulgurans* Gmelin, 1791 was used as the outgroup in all analyses. Phylogenetic analyses were also re-run with one gene excluded at a time. These analyses resulted in six well supported, but incongruent trees (Fig. 1) depending on which dataset was used (nucleotide or amino acid) and which genes were removed (Table 2.1). In each tree, most nodes received full support (PP = 1), but in most cases there was at least one node with PP <1. The support for these nodes depended on which genes were excluded.

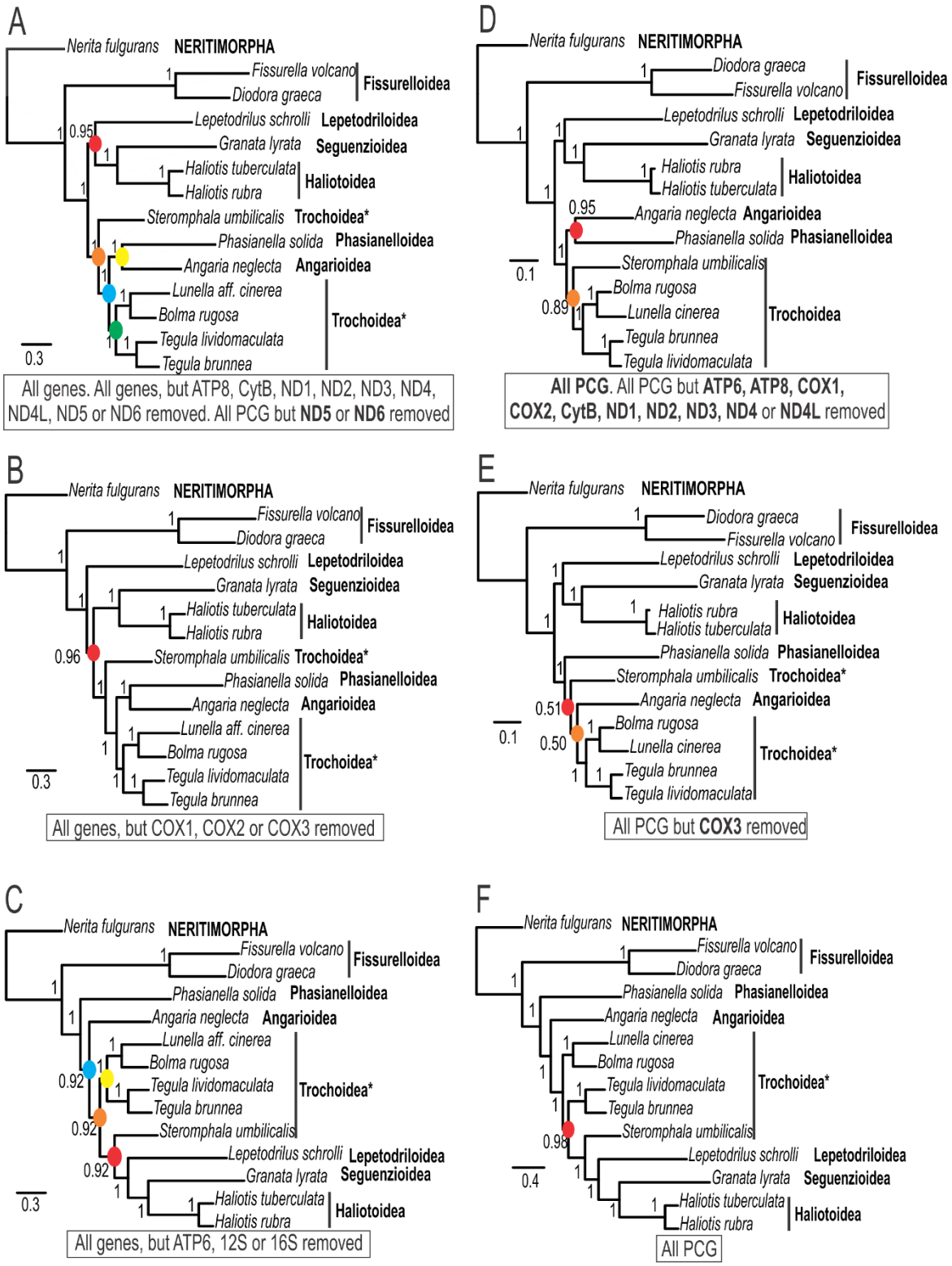


Figure 2.1: Six different tree topologies for Vetigastropoda based on Bayesian analysis. **A.** Tree A. Bayesian inference (BI) tree based on analysis of nucleotide variation for all 15 concatenated genes. The same topology was recovered for analyses based on 14 genes with ATP8, CytB, ND1, ND2, ND3, ND4, ND4L, ND5 or ND6 removed, as well as ND5 and ND6 in amino acid (aa) analyses. **B.** Tree B. BI tree based on analysis of nucleotide variation for 14 genes with COX1 removed. The same topology was recovered for analyses based on 14 genes with COX2 or COX3 removed. **C.** Tree C. BI tree based on analysis of nucleotide variation for 14 genes with ATP6 removed. The same topology was recovered for analyses based on 14 genes with 12S or 16S removed. **D.** Tree D. BI tree based on aa analysis of all 13 protein-coding genes (PCG). The same topology was recovered for analyses based on 12 genes with ATP6, ATP8, COX1, COX2, CytB, ND1, ND2, ND3, ND4, or ND4L removed. **E.** Tree E. BI tree based on aa analysis of 12 PCG with COX3 removed.

F. Tree F. BI tree based on nucleotide analysis of 13 PCG (to compare with Tree D). Text beneath trees show datasets that resulted in the same tree topology; amino acid datasets are in bold font. GenBank accession numbers for each species: *Steromphala* (formerly *Gibbula*) *umbilicalis* KX646541; *Nerita fulgurans* KF_728888, *Fissurella volcano* NC_016953, *Diodora graeca* JN_790612, *Lepetodrilus schrolli* KR_297250, *Granata lyrata* NC_028708, *Haliotis tuberculata* NC_013708, *Haliotis rubra* NC_005940, *Phasianella solida* NC_028709, *Angaria neglecta* KR_297248, *Lunella aff. cinerea* KF_70096, *Bolma rugosa* NC_029366, *Tegula lividomaculata* NC_029367, *Tegula brunnea* NC_016954. *Nerita fulgurans* is used as the outgroup in all trees. Superfamily classification is indicated on the right; those marked with an asterisk are not monophyletic. The definition of Trochoidea is based on Williams and Ozawa (2006).

To investigate the possibility that selection may be acting on mt genes, Tajima's D (Tajima, 1989) was calculated for the codon alignment of each PCG in MEGA 6 (Tamura *et al.*, 2013) (Table 2.2). In addition, a FUBAR analysis (Murrell *et al.*, 2013) was conducted with HYPHY (Pond & Muse, 2005) using individual genes and their gene trees with default values, as well as using the entire PCG alignment and tree based nucleotide sequence from all PCG (Tree F; Table 2.2). Both analyses showed multiple genes undergo significant positive selection ($p < 0.001$; D value > 2.798), with the FUBAR analysis identifying single codons in ATP6, ND4, ND4L and ND6 as undergoing positive selection. These methods are conservative estimates of selection, which frequently fail to detect adaptation when it has occurred (Crandall *et al.*, 1999; Sharp, 1997), moreover, the analyses here do not test for selection in ribosomal genes, so the results may underestimate the importance of selection in the mt genome.

Table 2.2: Evidence for selection at mitochondrial protein-coding genes. Tajima's D values showing significant ($p < 0.001$) positive selection in bold. FUBAR test for positive selection conducted by gene show codon position in the gene, posterior probability values of positive selection > 0.9 [dN/dS > 1], and first nucleotide position. FUBAR analyses by alignment show codon position and first nucleotide position in alignment.

Gene	Tajima's D	FUBAR by gene			FUBAR by alignment		
		Codon	dN/dS>1	Nuc.	Codon	dN/dS>1	Nuc.
ATP6	**2.82755**	32	0.92788	94	-	-	-
ATP8	2.53779	-	-	-	-	-	-
COX1	**2.83303**	-	-	-	-	-	-
COX2	2.74291	-	-	-	-	-	-
COX3	**2.84012**	-	-	-	-	-	-
CytB	2.59975	-	-	-	-	-	-
ND1	2.77333	-	-	-	-	-	-
ND2	**3.42609**	-	-	-	-	-	-
ND3	**3.03519**	-	-	-	-	-	-
ND4	**2.86668**	47	0.92958	139	-	-	-
ND4L	**2.86284**	79	0.90024	235	-	-	-
ND5	**3.06231**	-	-	-	-	-	-
ND6	**3.26115**	36	0.95149	106	3468	0.91145	10402

2.3 Discussion

Phylogenetic trees for gastropods are frequently based on sequence including COX1, 12S and 16S (Aktipis & Giribet, 2011; Colgan *et al.*, 2003; Geiger & Thacker, 2005; Williams *et al.*, 2010), but here it is shown that the removal of these genes individually from phylogenetic analysis results in different trees. This may be linked to the positive selection found in other genes, in which case the mt genome is no longer acting as a neutral marker (Burton & Barreto, 2012; Fontanillas *et al.*, 2005; Kivisild *et al.*, 2005; Mishmar *et al.*, 2003; Ruiz- Pesini & Wallace, 2006), although there was no evidence of selection acting on COX1 (12S and 16S were not tested).

All the trees obtained here differ significantly from previous molecular studies including nuclear and mt markers (Aktipis & Giribet, 2010, 2011; Geiger & Thacker, 2005; Kano *et al.*, 2009; McArthur & Harasewych, 2003; Williams & Ozawa, 2006; Williams *et al.*, 2008; Yoon & Kim, 2005) except where indicated otherwise below. The positions of *Phasianella solida* (Born, 1778), *Angaria neglecta* and the only trochid, *Steromphala umbilicalis*, are particularly problematic.

Tree A and Tree D are both consistent with the topology found by Uribe *et al.* (2015) and (Williams *et al.*, 2014), both of which used mt genomes, albeit with different taxon sampling, models of substitution and using either amino acids or nucleotides. As all the ND genes with the exception of ND1 showed significant positive selection, it is possible that the genes ND5 and ND6 may not be representative of the entire mt genome. In contrast, the node values for nucleotide trees without CytB and without ND1 are lower in tree D, two genes which don't show positive selection either based on Tajima's D or FUBAR analysis.

Tree B differs from Tree A in that the Lepetodriloidea is a sister to the Haliotoidea, Seguenzioidea and Trochoidea *sensu* Hickman and McLean (1990), whereas in Tree A Lepetodriloidea is sister to only the Seguenzioidea and the Haliotoidea. In this respect, Tree B is consistent with the tree recovered using 18S by Yoon & Kim (2005). Tree C differs from Tree A in that *Phasianella solida* and *Angaria neglecta* are sister taxa to the non-monophyletic Trochoidea *sensu* Williams and Ozawa (2006), Lepetodriloidea, Seguenzioidea and Haliotoidea, whilst *Steromphala umbilicalis* is sister to the Lepetodriloidea, Haliotoidea and Seguenzioidea. This is consistent with the phylogeny by Geiger and Thacker (2005) based on COX1, H3 and 18S markers. The Trochoidea are polyphyletic in

Tree C, regardless of whether the definitions of Williams and Ozawa (2006) or Hickman and McLean (1990) are used.

Tree D differs from Tree A in that it is the only tree that recovers the Trochoidea *sensu* Williams and Ozawa (2006) as monophyletic. Tree E recovers *Steromphala umbilicalis* as sister taxa to a clade with the Angarioidea and the remaining Trochoidea *sensu* (Williams & Ozawa, 2006). However, the support values for theses nodes are very low (0.51 and 0.50), which suggests that COX3 is of high relative importance for determining relationships within Trochoidea *sensu* Hickman and McLean (1990).

A notable difference between mt genome trees (Uribe *et al.*, 2015; Williams *et al.*, 2014) and trees obtained using both nuclear and mt markers is that Trochoidea *sensu* Hickman and McLean (1990) is recovered in all mt genome trees except Tree C and Tree F, whereas Trochoidea *sensu* Hickman and McLean (1990) is polyphyletic in trees using both nuclear and mt markers (Aktipis & Giribet, 2011; Geiger *et al.*, 2008; Nakajima *et al.*, 2016; Williams & Ozawa, 2006; Williams, 2012; Williams *et al.*, 2008). If results based on mt genomes are confirmed, these results would resurrect the use of the Angariinae and the Phasianellinae as subfamilies within the superfamily Trochoidea *sensu* Hickman and McLean (1990). Trochoidea *sensu* (Williams & Ozawa, 2006) is obtained only in Tree D, suggesting that the use of amino acid variation is preferable to the use of nucleotide variation for vetigastropod level phylogenies, as might be expected given the age of this clade. However, these conflicting trees show that further work is needed to confidently resolve relationships within Vetigastropoda. A cautious use of mt genomes in phylogenetic analyses is required, as both the methods used for phylogenetic analyses and positive selection may affect the results.

Chapter 3: Contrasting genetic structure of two sympatric congeneric gastropods: do differences in habitat preference, abundance, and distribution matter?

3.1 Introduction

A central question of biogeography asks how the population genetic structure of species is affected by environmental, ecological and life history variables and whether there are any associations with patterns of abundance and distribution (Hellberg *et al.*, 2002; Riginos *et al.*, 2016; Selkoe *et al.*, 2016). It has been classically assumed that early life history traits (e.g. duration of a dispersive phase) are good predictors of population genetic structure (Almeida *et al.*, 2017; McInerney *et al.*, 2012; Rognstad *et al.*, 2014). However, recent meta-analyses are equivocal and approach generalisations with caution because other factors, such as adult body size and microhabitat preferences, can also influence genetic structure (Kelly & Palumbi, 2010; Riginos *et al.*, 2011; Selkoe & Toonen, 2011). Likewise, the distribution of physical habitat (e.g. geological substrate) and environmental conditions appear to influence genetic structure for some species, but not others (Almeida *et al.*, 2017; Haye *et al.*, 2014; McInerney *et al.*, 2012). Recent biogeographic studies have shifted focus to ask whether demographic and ecological traits, such as abundance or habitat specificity, are related to differences in genetic structure (Engler *et al.*, 2014; Kierepka *et al.*, 2016; Selkoe *et al.*, 2016).

The relationship between habitat specificity and abundance with genetic population structure has been explored in terrestrial systems, where habitat generalist species exhibited more genetic connectivity than sympatric congeners with more restrictive habitat preferences and therefore a more fragmented geographic distribution (Engler *et al.*, 2014; Kierepka *et al.*, 2016). There are fewer examples in marine systems, but a recent study showed a positive relationship between estimates of local abundance and genetic structure, although this varied between pairs of synchronously diverging co-distributed species (Dawson *et al.*, 2014). In a review of reef community population genetics, all species with “chaotic” genetic structure (highly variable levels of genetic structuring with no obvious spatial patterning) were habitat generalists, whereas

habitat specialists showed genetic differentiation between regions (Selkoe *et al.*, 2014). Differing habitat preferences and subsequent variation in current and historic distribution in intertidal gastropod congeners have also been associated with population genetic structure (Marko, 2004).

The trochids *Steromphala umbilicalis* and *Steromphala pennanti* were chosen here to explore these patterns for phenotypically similar and sympatrically distributed species along the rocky intertidal shores of the North-east Atlantic. The recorded range of *S. umbilicalis* extends from north-west Scotland (Mieszkowska *et al.*, 2013) to Morocco (Southward *et al.*, 1995). It is found within the mid to low shore across a range of habitats, such as rockpools, cracks, above and under boulders/rock ledges/platforms, and the fucoid zone (Mieszkowska *et al.*, 2013; Muñoz-Colmenero *et al.*, 2015). *Steromphala pennanti* is distributed from the Cherbourg peninsula in northern France to Morocco (Southward *et al.*, 1995) on the low shore, and is more closely associated with fucoid habitat, such as *Fucus serratus*, than *S. umbilicalis* (Bordeyne *et al.*, 2017). *Steromphala pennanti* is therefore a habitat specialist and largely restricted to the fragmented distribution of fucoids along the Bay of Biscay (Wort, personal observation). Regarding dispersal potential, *Steromphala* species are widely held to produce lecithotrophic larvae and are estimated to have approximately the same maximum larval duration of seven to nine days (Keith *et al.*, 2011; Underwood, 1972a, b).

Within the ranges of both species lies 230 km of almost uninterrupted sandy intertidal habitat in south-west France (Castelle *et al.*, 2006). This habitat gap (Figure 3.1) results in the absence of both species along that shoreline and coincides with the northern range limit of the rocky intertidal gastropods *Patella rustica* (Lima *et al.*, 2007b) and *Stramonita haemastoma* (Wort, personal observation). However, no studies to date assess whether the habitat gap affects population connectivity of rocky shore species that are found on both sides of the gap.

Here, differences in the abundance, distribution, and genetic population structure are investigated between habitat generalist (*Steromphala umbilicalis*) and more specialist (*Steromphala pennanti*) congeneric rocky shore gastropods within the sympatric portion of their ranges. In order to evaluate their genetic structure, microsatellite loci of both species were isolated and genotyped. The hypotheses tested are: *S. umbilicalis* (a habitat generalist) is more abundant than *Steromphala pennanti* (Philippi, 1846) (a habitat specialist); *S. umbilicalis* shows

less genetic differentiation than *S. pennanti* where *S. pennanti* has a patchy distribution; in regions where *S. pennanti* and *S. umbilicalis* are both abundant and continuously distributed, there is low genetic differentiation in both species; the habitat gap in the southwest of France is not associated with genetic differentiation.

3.2 Materials and Methods

3.2.1 Sampling

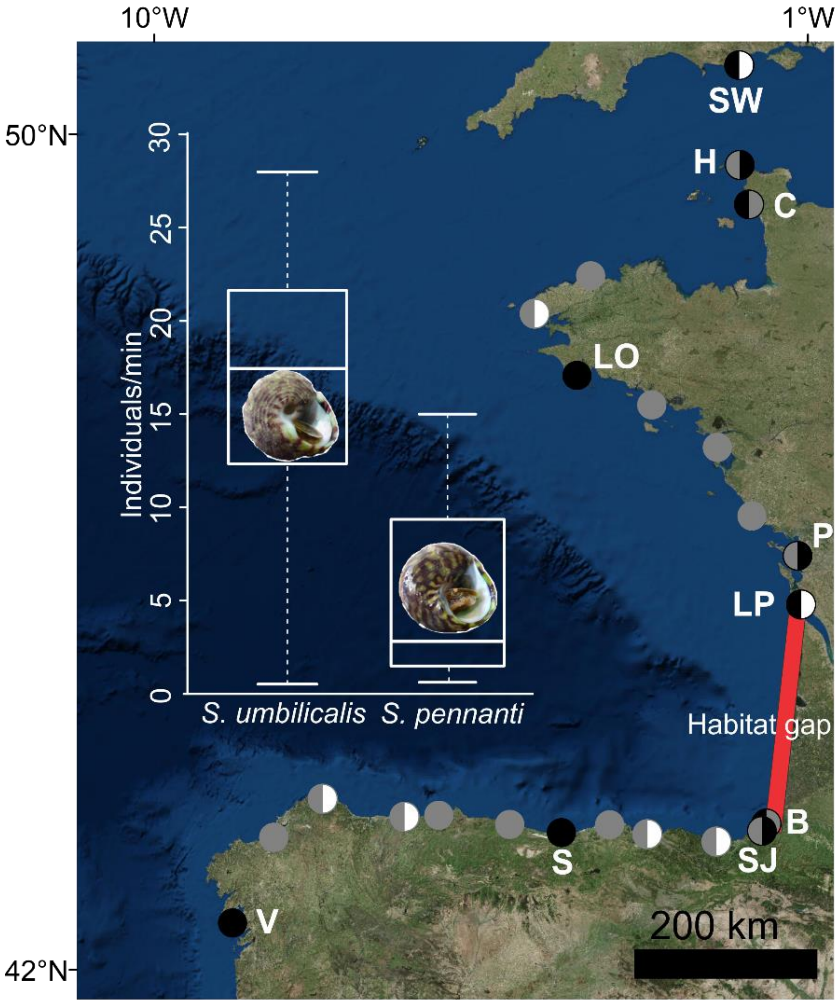


Figure 3.1: Map using World_Aitoff projection in ArcGIS displaying sampling localities as circles, and abundance box plot for localities where both species were present (n=16). Left semi-circles represent *S. umbilicalis*, right semi-circles represent *S. pennanti*: white = absent; grey = present; black = present and used as DNA sample locality. Locality codes correspond with Table 3.1.

Abundance estimates were made at 23 localities (Figure 3.1) at low tide in November 2014, August 2015, April 2016 and August 2016, with multiple visits to several localities. These localities were roughly evenly spaced and equally sampled north and south of the habitat gap (11 north and 12 south). At each

locality, four people searched the mid to low shore in varied microhabitats where both species occur for two to four minutes. To prevent sampling overlap, samplers were spaced at least 10 m apart. Timed searches were part of a wider sampling effort to quantify the presence/absence of rocky shore gastropods at each locality (~3 hours per locality). This additional sampling effort was used to increase confidence of any locality-specific *S. pennanti* absences. For both species, the mean number of individuals found per minute was calculated at each locality where both species were present (n = 16). Normality of abundance distributions was tested using a Shapiro-Wilk test, which showed a non-normal abundance distribution in *S. pennanti*, suggesting a patchier distribution indicative of under-dispersal. Therefore, a Wilcoxon-Mann-Whitney test was used to compare abundances between species over their sympatric range. Abundance measurements were also compared between localities to the north of the habitat gap in south-west France against the localities to the south of the habitat gap for both species.

An average of 27 (SE = 1.04) individuals of *S. umbilicalis* and 26 (SE = 2.30) individuals of *S. pennanti* were collected from three localities to the south of the habitat gap and three to the north (Figure 3.1 and Table 3.1) and stored in 95% ethanol. Where it was not possible to collect enough specimens of each species from the same locality, the nearest locality was used instead for the under-sampled species (usually *S. pennanti* due to its fragmented distribution and often low abundances). *Steromphala umbilicalis* was also collected from Swanage (UK) to investigate whether the English Channel was associated with a genetic break.

Table 3.1: Descriptive statistics from seven *S. umbilicalis* and eight *S. pennanti* microsatellite loci: locality, number of samples (N), the expected (H_e) and observed heterozygosities (H_o) and the number of alleles (Â) found per locus at each locality from north to south, with SW France habitat gap shown as thicker line.

S. umbilicalis					
Code	Locality	N	H_e	H_o	Â ± SD
SW	Swanage	22	0.703	0.234	6.86±1.57
C	Cap de Carteret	27	0.626	0.481	6.43±1.99
LO	Loctudy	29	0.651	0.437	7.29±2.06
LP	Les Pierrieres	29	0.598	0.452	6.86±1.86
B	Biarritz	29	0.589	0.433	7.14±3.48
S	San Vicente de la Barquera	24	0.582	0.379	6.43 ±3.21
V	Vilagarcía de Arousa	27	0.614	0.381	6.71±2.56
S. pennanti					
Code	Locality	N	H_e	H_o	Â ± SD
H	Cap de la Hague	33	0.566	0.245	6.75±4.92
LO	Loctudy	30	0.558	0.259	6.38±4.57
P	Point du Chay	20	0.547	0.270	4.88±2.17
SJ	St Jean de Luz	19	0.649	0.270	5.50±1.69
S	San Vicente de la Barquera	26	0.501	0.296	5.75±4.06
V	Vilagarcía de Arousa	29	0.535	0.291	6.00±3.66

3.2.2 Mitochondrial DNA locus selection and primer design

Mitochondrial (mt) DNA reads were obtained from whole genome shotgun sequence data from a *Steromphala umbilicalis* collected from Ria Formosa, Algarve, Portugal (37.01˚ N, 8.00˚ W) (NHM voucher: NHMUK 20030328) using methods described in Wort *et al.* (2017). Mitochondrial sequence reads were mapped in GENEIOUS v. 8.1.5 (Kearse *et al.*, 2012) onto the nearly complete mt genome of another *S. umbilicalis* collected from a geographically distant location (Peveril Point, Dorset, UK; 50.61˚ N, 1.94˚ W) (Wort *et al.*, 2017) (GenBank acc. No. KX646541). The consensus sequence of the new DNA fragments was then used to generate a second incomplete mt genome (GenBank Acc. No. KX646542). A sliding window analysis of the two mt genomes was used to identify by eye a region of high intraspecific variability, flanked by highly conserved regions suitable for developing PCR primers. This identified a region of DNA 915 base pairs (bp) long which could be amplified using primers ND4L_FWD_BLAST (ACGCCCCCATAGTAATCAG) and CYTB_REV_BLAST (AGGGTCAAATAATCCTTTGGG). The primers spanned a region including the 3' end of ND4L, two tRNAs and the 5' end of cytB.

3.2.3 Obtaining *S. umbilicalis* transcriptome

Approximately 100 mg of foot tissue was dissected from an individual *S. umbilicalis* collected at Southsea, Hampshire, UK and stored in 'RNAlater' (Qiagen, UK). The tissue was then ground with liquid nitrogen and RNA extracted using a Qiagen RNeasy Plant Mini kit (Qiagen, UK), utilising an on-column DNase step as per the manufacturer's recommendation. RNA quantity was approximated using a NanoDrop to ensure sufficient yields. The RNA sample was prepared for sequencing using the 'KAPA stranded mRNA' kit and eight cycles of library amplification via PCR. Following further library quantification, the sample was sequenced on ¼ of a lane of Miseq at the University of Southampton Environmental Sequencing Facility for 300 cycles.

Fastq formatted reads from the Miseq (3.95M pairs) were trimmed of adapters and low quality sequences (phred quality < 5), and short sequences (< 36 nucleotides) removed using TRIMMOMATIC v. 0.32 (Bolger *et al.*, 2014) with settings 'LEADING' (cuts bases off the start of a read, if below a threshold quality) = 5, 'TRAILING' (cuts bases off at the end of a read if below a threshold quality)= 5, 'SLIDINGWINDOW' (performs a sliding window trimming approach starting at 5' end and clipping read once the average quality within the window falls below a

threshold) = 4:15, MINLEN (drop the read if it below a threshold length) = 36. Unpaired reads were excluded and the paired trimmed reads (3.77M reads) were normalised with the script insilico_read_normalization.pl (removing reads with kmer coverage > 30) from within the TRINITY RNAseq analysis package v. 2.0.6 (Grabherr *et al.*, 2011). This reduced the number of reads to assemble to 1.23M pairs. Assembly was carried out with TRINITY with additional settings --min_kmer_cov = 2 (min count for kmers to be assembled by Inchworm), --max_internal_gap_same_path = 15 (max number of internal consecutive gap characters allowed for paths to be merged into single paths), and --max_diffs_same_path = 4 (max allowed differences encountered between path sequences to combine them). These settings were chosen to ignore single copy (potentially error-containing) kmers, and to allow two reads differing by up to a 15 base indel and/or up to four SNPs (single nucleotide polymorphisms) to be assembled, primarily because the assembled individual was likely to be heterozygous at a majority of loci. TRINITY assembles the data into 'components' (loosely akin to genes) and 'transcripts', where each component is made up of one or more transcripts (Grabherr *et al.*, 2011). Primer design proceeded using only one transcript per component. The *S. umbilicalis* transcriptome assembly contained 54,335 transcripts from 44,341 components with a GC content of 42.71% and a total transcriptome length of 45.3MB. Transcript N50, median and mean length were 1236, 548 and 835 bp, respectively.

3.2.4 DNA extraction

DNA was extracted for both species using a 'CTAB' buffer method similar to Williams *et al.* (2003). Approximately 25 mg of ethanol-preserved tissue was blotted dry between a sterile scalpel blade and a sterile Kimwipe before being placed in a microcentrifuge tube containing 500 µl of CTAB extraction buffer (100 mM Tris, 1.4 M NaCl, 23 mM EDTA, 2 % w/v CTAB, 2 % w/v PVP dissolved in sterile distilled water on a hot plate), 0.2 % w/v of β-mercaptoethanol and 10 µl proteinase K (~20 mg/mL) prior to incubation at 60˚C for 3 hours. The tissue extract was then extracted twice with 500 µl of 24:1 chloroform:isopropanol mix before precipitation with 350 µl of isopropanol and then washed with 200 µl of 70 % ethanol. The DNA pellet was dried using a vacuum drier at room temperature for approximately 30 minutes before resuspension in sterile distilled water. DNA concentration was measured using the NanoDrop and resulted between 50 and 500 ng/µl.

3.2.5 Microsatellite identification and primer development

The assembled transcriptome of *S. umbilicalis* was mined for microsatellites using MISA (http://pgrc.ipk-gatersleben.de/misa/) with the minimum number of repeats being: dinucleotide ≥8x, trinucleotide ≥6x and tetranucleotide ≥4x. Loci without >20bp of sequence flanking the microsatellite were removed and longer microsatellites were preferred for primer design. 1951 microsatellites were identified in the *S. umbilicalis* transcriptome, of which ten were tested for PCR amplification. Microsatellite primers were designed using different methods for each species. For *S. umbilicalis*, the selected sequences of transcripts containing simple sequence repeats (SSRs) were entered into PRIMER3 v. 4.0.0 (Koressaar & Remm, 2007; Untergasser *et al.*, 2012) one by one with the SSRs highlighted using default settings. For each locus where primers could be designed, the top primer pair was retained. Any primers were removed if they had: 1) non-zero values for self-complementarity score (any_th), 2) the 3' self-complementarity (3'_th), 3) the value of the melting temperature of the most stable hairpin structure of the primer (hairpin) or 4) low (<60°C) annealing temperatures. Remaining loci were 'BLAST' searched against the mt genome of *S. umbilicalis* in GENEIOUS (Kearse *et al.*, 2012), and any loci potentially belonging to the mt genome were excluded (Wort *et al.*, 2017). Any primers that contained a microsatellite-like repeat were excluded, or in some cases a new primer pair designed with the above conditions.

For *S. pennanti*, DNA from ten specimens from San Vicente de la Barquera and Vilagarcía de Arousa (referred to for the remainder of the text as Vilagarcía) were diluted to approximately equal concentrations as measured by a NanoDrop, mixed to achieve ≥1 µg of DNA and sent to Genoscreen (Lille, France) to develop microsatellite primers. Generation of multiplex-enriched libraries and sequencing was carried out as reported in (Malausa *et al.*, 2011). Briefly, genomic DNA was mechanically fragmented, enriched in microsatellite loci with 8 probes: (TG, TC, AAC, AGG, ACG, AAG, ACAT and ACTC) before amplification by PCR with a High Fidelity Taq. PCR products were purified, quantified and GsFLX libraries were then carried out following manufacturer protocols and sequenced on 1/32 of a 454 GS-FLX PTP. The bioinformatics program QDD (Meglécz *et al.*, 2009) was used to analyse *S. pennanti* DNA sequences to obtain PCR primers. Among 5,945 sequences containing a microsatellite motif, 189 primer sets bioinformatically validated were designed which had "perfect" characteristics. After excluding primer pairs that failed to amplify in the majority of samples and those that were

monomorphic, seven remained that amplified all *S. umbilicalis* individuals, and eight from an initial nine trialled in *S. pennanti* (Table 3.2).

Table 3.2: Microsatellite loci, primer and PCR information. Locus = locus associated primer names, marked "U" for *S. umbilicalis*, or "PEN" for *S. pennanti*; Forward = forward primer sequence; Reverse = reverse primer sequence; SSR = simple sequence repeat; Mix (label) = multiplex mixes (fluorescent label); T = annealing temperature of PCR reaction (°C); A = number of alleles recorded for each locus; R = range length for variation observed among alleles.

Locus	Forward	Reverse	SSR	Mix	T	A	R
U26231	GCAGGGCTGGTTTGAAGATC	GCATGGATTCAGCGCGTTAT	$(ATA)_6$	D_{FAM}	55.7	22	209-332
U23195	GCAGGCAGGTAGAGCTAGAG	TTCGGGCATAAACAGGTCCT	$(GCAG)_4$	A_{FAM}	60	11	156-208
U34184	GCACAGGCCCTCAGATCATT	AACCCTCTCATGTCCACAGC	$(AT)_8$	A_{HEX}	60	12	199-233
U36148	GGCCACCCTGAAGAGATAGG	AGGGGTGGCTCAACTTTCAT	$(ACAG)_5$	A_{NED}	60	11	174-214
U15541	GTATTGGCTTGCTGTCCGTC	TGCCTCCATGATAAGCTTACCA	$(AATG)_4$	B_{FAM}	58.1	14	217-257
U34428	ATAGTATTGGGCAGCGTCCG	TGAGATATCCCGCTGACAAGG	$(TACG)_4$	C_{FAM}	58.1	9	213-249
U62438	TTGAACCCCAAACTCAACGC	TTGTCAAAACTGGTGCTGGC	$(ACA)_8$	C_{NED}	58.1	10	136-178
PEN79	CACGTTTGAGTCCTGTCGAA	CGTTGCATCAGTTTGACGAT	$(AG)_6$	1_{FAM}	55.7	3	115-119
PEN86	TTTTGAGCGATGCTTTATGC	TGCATTTGACTGTTCAATTCAT	$(ATGT)_7$	1_{HEX}	55.7	18	122-214
PEN146	TCTCTTGTAGCCCTTTTGCG	AAATCCCATGTTTCCGTTCA	$(TC)_5$	1_{NED}	55.7	23	172-230
PEN65	CCAGTTCTGCAAGTGAACCA	CAGGATCACCTCTCGCTTCT	$(CAG)_7$	2_{FAM}	58.1	5	92-104
PEN138	ATCATTGCACTTCCTCTCGG	CAGGCAGATAGGGTAGCAGC	$(AG)_5$	2_{HEX}	58.1	5	165-173
PEN104	TCGTCTCTGCTCATTGTTACC	AGAGATGACGCTCCCTCGTA	$(CT)_5$	2_{NED}	58.1	6	133-143
PEN168	CCCAATGTAAGTCCGCTGTT	TTCGATGTGCAGAAGGAATG	$(TTTG)_6$	3_{FAM}	58.1	7	207-231
PEN145	GCCATTAGCAGAGTGACCTTG	ATAGAGAAGGCCGAGCAGC	$(GA)_5$	3_{NED}	58.1	10	169-193

3.2.6 PCR, sequencing and peak scoring

For mt DNA, PCRs using ND4L_FWD_BLAST and CYTB_REV_BLAST were performed in 25 µl containing 1.25 µl of each forward and reverse primer (10 µM), 12.5 µl of 'GoTaq G2 Green Master Mix' (Promega, Madison, WI), 2 µl DNA and 8 µl of distilled sterile water. Thermal cycling was performed with an initial denaturation for 3 min at 95°C, followed by 40 cycles of 45 s at 95°C, 45 s at 58°C, 90 s at 72°C, and a final extension step of 10 minutes at 72°C. PCR amplicons were sequenced in both directions using a 3730xl DNA analyser (Applied Biosystems) at the NHM sequencing facility. Reads were paired and aligned against the mt genome before editing in SEQUENCHER v. 5.3 (GeneCodes Corporation, Ann Arbor, MI). The resulting consensus sequences for each paired read were then aligned in GENEIOUS v. 8.1.5 (Kearse *et al.*, 2012) using the Geneious Alignment with free end gaps and 65 % similarity cost matrix.

For microsatellites, PCR was performed in 25 µl reactions containing 1 µl of forward and reverse primers at 10 µM concentration, 12.5 µl of 'GoTaq G2 Green Master Mix' (Promega, Madison, WI), 2 µl of DNA solution between 50 and 500 ng/µl concentration measured using a Nanodrop, and sterile distilled water.

Thermal cycling was performed with initial denaturation for 5 minutes at 95°C, 40 cycles of 60 s at 95°C, 60 s at the given annealing temperature (Table 3.1), 90 s at 72°C and a final elongation step of 15 minutes at 72°C. PCR product was then diluted to 1:50 volume with sterile distilled water and sent to DBS Genomics (Durham University, UK) for fragment length analysis (FLA) using a 3730 DNA Analyser (Applied Biosystems) with the filter set DS-30, and ROX500 as the size standard. Primers were initially trialled with two samples from three localities by using a temperature gradient PCR for annealing temperatures from 52°C to 62°C, and subsequent FLA. Where amplification of polymorphic loci was unsuccessful regardless of temperature or samples, the primers were excluded.

3.2.7 Microsatellite peak scoring and analysis

Peak scoring was carried out using PEAK SCANNER v. 2.0 (Applied Biosystems). Heterozygote deficiency both by locus and by locality were also tested in GENEPOP ON THE WEB v. 4.2 (Rousset, 1995; Rousset, 2008) with 10,000 dememorization steps, 1000 batches and 10,000 permutations per batch (Fenberg *et al.*, 2010) (Table 7.1). The observed (H_o) and expected (H_e) heterozygosities, and the number of alleles (Â) per locus and locality were calculated with GENALEX v. 6 (Peakall & Smouse, 2006) (Table 3.2, 7.2 and 7.3). GENEPOP ON THE WEB v. 4.2 (Rousset, 1995; Rousset, 2008) was used to obtain pairwise and global F_{ST} (Weir & Cockerham, 1984) and subsequent p-values with default settings and significance ($\alpha = 0.05$) determined following the sequential Bonferroni correction. Nei's pairwise F_{ST} (Nei, 1973) was calculated using the 'hierfstat' package (Goudet, 2005) in 'adegenet' v. 2.0.1 (Jombart, 2008) in RSTUDIO v. 1.0.136 and used to generate a discriminant analysis of principal components (DAPC) using the package 'adegenet' (Jombart, 2008; Jombart *et al.*, 2010) in RSTUDIO.

STRUCTURE v. 2.3.4 (Pritchard *et al.*, 2000) was used with an admixture model on microsatellite data with and without prior populations input to the model. Ten runs were carried out for each of the potential number of populations (k) (1-6 for *S. pennanti*, 1-7 for *S. umbilicalis*) with a burn-in of 30,000 steps followed by 100,000 Markov Chain Monte Carlo (MCMC) iterations to estimate mean and variance of posterior probabilities and log likelihoods of the number of assumed populations. The best estimate of k was determined in STRUCTURE HARVESTER (Earl, 2012) using the peak in the delta k value. Subsequent visualisation of the data as bar graphs was carried out in CLUMPAK (Kopelman *et al.*, 2015) for the best assumed k value. The population structure was also assessed with the package GENELAND 4.0 (Guillot *et al.*, 2005) in RSTUDIO using the

spatial model and uncorrelated frequencies. One million iterations, 100 iterations of thinning and a final burn-in of 10% of the saved iterations were used to test for the best k between one to the maximum number of samples in each species.

AMOVAs (Excoffier *et al.*, 1992) were carried out in RSTUDIO v.1.0.136 using the 'poppr' package v. 2.5.0 (Kamvar *et al.*, 2014) on each species between two groups for *S. pennanti* (north and south of the habitat gap), and for *S. umbilicalis* among three groups (Swanage, north and south of the habitat gap), between sites within those groups, and all individuals. Significance of results was tested using MC tests with 10,000 repeats. The number of migrants per generation was estimated in GENEPOP ON THE WEB using the private alleles method (Barton & Slatkin, 1986) between localities north and south of the habitat gap. Genetic effective sizes (N_E) were calculated with the Linkage Disequilibrium Method implemented in NeEstimator 2.01 (Do *et al.*, 2014).

A Mantel test (Mantel, 1967) was carried out on each species to test for isolation by distance (IBD) using GENEPOP ON THE WEB v. 4.2 (Rousset, 1995; Rousset, 2008) using 10,000 permutations with distances measured as the shortest possible distance over sea between sites in ARCGIS DESKTOP v. 10.5 (Esri, Redlands, California, USA) as in Rousset (1997) and a genetic distance based on Weir and Cockerham (1984) F_{ST} index. For *S. umbilicalis*, this was repeated excluding Swanage, as initial results showed Swanage population to be strongly divergent.

Presence of null alleles and scoring errors following identification of heterozygote deficient loci was tested with MICROCHECKER (Van Oosterhout *et al.*, 2004). As multiple null alleles were detected, the software FREENA (http://www1.montpellier.inra.fr/CBGP/software/FreeNA/) was used to generate corrected F_{ST} values. In most cases, the corrections only affected the third decimal place in the F_{ST} values and they did not affect their significance. Consequently, the effect of presence of null alleles was not considered in further analyses (Chapuis *et al.*, 2008; Pérez- Portela *et al.*, 2017). Multiple null alleles were obtained for both species (Table 7.1). However, this is a common occurrence for microsatellites in gastropod and other molluscan taxa (Hedgecock *et al.*, 2004; Kemppainen *et al.*, 2009) and does not necessarily make the marker invalid (Rico *et al.*, 2017), as found following correction in FREENA (http://www1.montpellier.inra.fr/CBGP/software/FreeNA/).

3.2.8 Mitochondrial DNA analyses

Sequence alignment was converted to a Phylip format in BIOEDIT v. 7.2.5 (Hall, 1999), and the different populations defined in DNASP v. 5.10.01 (Librado & Rozas, 2009). TCS v. 1.21 (Clement *et al.*, 2000) was used to generate the haplotype network. ARLEQUIN v. 3.5.2.2 (Excoffier & Lischer, 2010) was used to generate F_{ST} and corresponding p-values for pairwise comparisons among populations.

3.3 Results

3.3.1 Mitochondrial DNA results

An initial low sample size of *S. umbilicalis* (Table 3.3) showed significant differences between populations at San Vicente de la Barquera and Brest (north of Loctudy) and Les Pierrieres (Table 3.4). However, the haplotype network showed no clear regional structure, possibly suggesting a panmictic genetic structure (Figure 3.2). Following this initial exploration of population genetics, we focussed on using microsatellite data with the expectation that they would show clearer structuring among populations.

Table 3.3: Statistics of *S. umbilicalis* mitochondrial DNA haplotypes, showing number of individuals (N), number of haplotypes (N_h), haplotype diversity (H_d) and average number of character differences (K).

Location	Code	N	N_h	H_d	Average number of differences (K)
Vilagarcía	V	16	15	1	6.81905
San Vicente de la Barquera	S	13	13	1	4.66667
Les Pierrières	LP	18	17	1	7.52941
Cap de Carteret	C	11	10	1	8.15556
Brest	BRE	8	8	1	8.42857
Biarritz	B	17	15	1	7.31429

Table 3.4: Nei's pairwise F_{ST} between populations (above diagonal), p-values below diagonal for mtDNA of *Steromphala umbilicalis*. Locality codes correspond to Table 3.3. No values were significant after Bonferroni correction.

	LP	C	BRE	V	S	B
LP		-0.01401	-0.03626	-0.02505	0.05471	-0.00950
C	0.6536 ±0.0100		-0.01776	-0.00568	-0.00528	-0.02954
BRE	0.9339 ±0.0041	0.6430 ±0.0091		-0.04408	0.06986	-0.00452
V	0.9547 ±0.0038	0.4979 ±0.0082	0.9210 ±0.0060		0.03633	-0.00016
S	**0.0265 ±0.0030**	0.5273 ±0.0083	**0.0440 ±0.0042**	0.0757 ±0.0047		-0.00321
B	0.6268 ±0.0088	0.9481 ±0.0042	0.4585 ±0.0091	0.3980 ±0.0085	0.47537 ±0.0089	

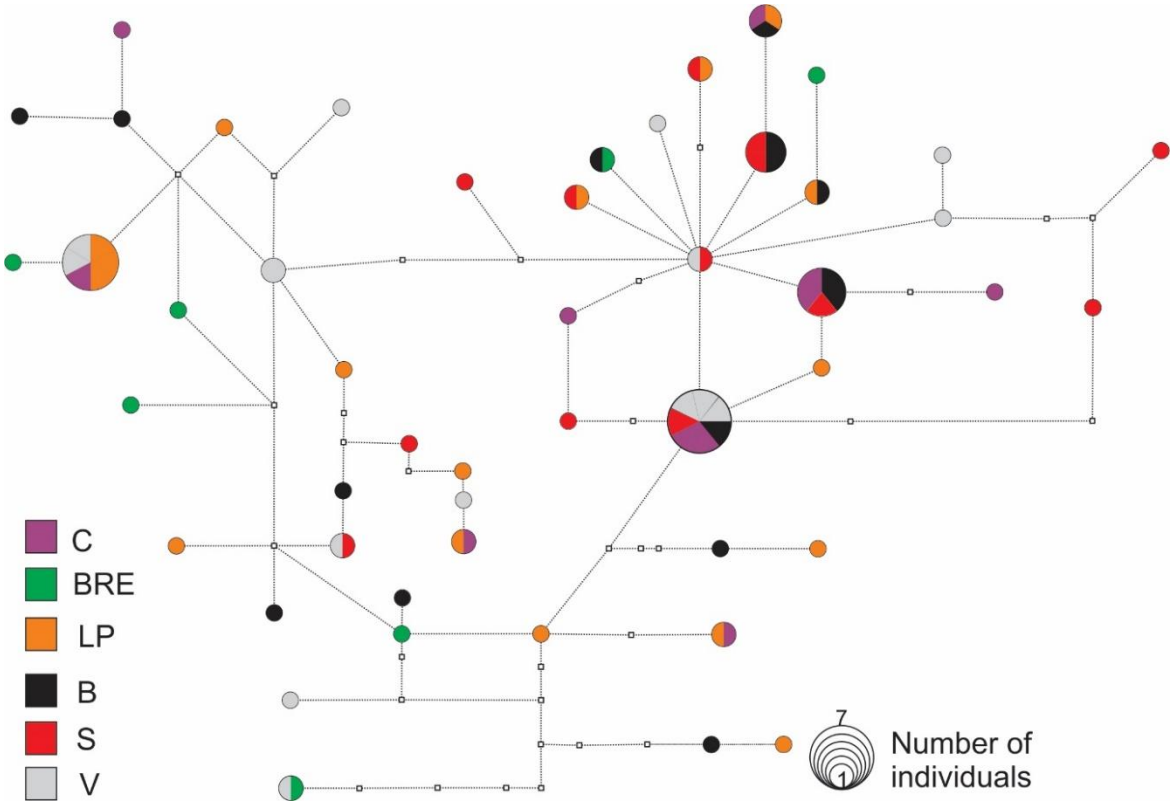


Figure 3.2: Haplotype network of *S. umbilicalis* based on mitochondrial DNA data. Locality codes correspond to Table 3.3.

3.3.2 Abundance distributions

Steromphala umbilicalis was significantly more abundant (Wilcoxon-Mann-Whitney test, W = 219, p = 0.0006) than *S. pennanti* at localities where both species were present (n = 16), with mean abundance of 16.38 (SE = 2.03) individuals per minute search time for *S. umbilicalis* compared to 5.44 (SE = 1.22) for *S. pennanti* (Figure 3.1). *Steromphala pennanti* was absent from six localities within our sampling range, four of which were south of the habitat gap where fucoids were not present, whereas *S. umbilicalis* was present at every locality. Thus, the habitat generalist, *S. umbilicalis*, was more abundant and more continuously distributed than *S. pennanti* within their sympatric range. *Steromphala pennanti* was less abundant south of the habitat gap [3.34 (SE = 1.08)] than north of the habitat gap [7.19 (SE = 1.98)], albeit not significantly (W = 45, p = 0.1949). *Steromphala umbilicalis* showed the opposite pattern, with an abundance of 19.12 (SE = 2.01) south of the habitat gap (n = 13) and 12.17 (SE = 2.55) north of the habitat gap (n = 9) (W = 16.5, p = 0.1149).

3.3.3 Microsatellite population genetics

Microsatellite data were obtained for 187 *S. umbilicalis* and 157 *S. pennanti* individuals (Table 3.1). Results for individual loci are presented in table 7.2 and 7.3. Pairwise F_{ST} results (Table 3.5) showed a larger number of pairwise significant differentiations in *S. pennanti* than *S. umbilicalis* between localities. The only significant pairwise differences for *S. umbilicalis* were found between the British locality (Swanage) and all other populations on the continent. By contrast, *S. pennanti* showed significant differentiation between San Vicente de la Barquera (on the central north coast of the Iberian Peninsula) and all other localities, and Vilagarcía (NW Iberian Peninsula) with Cap de la Hague (Cherbourg Peninsula, France). The habitat gap did not coincide with significant genetic differentiation in either species between the two localities immediately north or south, or when tested using AMOVA (Table 3.6). Analysis of the number of migrants per generation between groups north and south of the habitat gap showed that *S. pennanti* had 6.91 migrants per generation, whereas *S. umbilicalis* had 10.87 migrants per generation (excluding Swanage for *S. umbilicalis*) Using all populations of the dataset (excluding Swanage for *S. umbilicalis*), the number of migrants per generation was 2.71 for *S. pennanti* and 5.43 for *S. umbilicalis*. Because the number of migrants per generation is greater than 1 for both species (and between groups), random genetic drift as a cause of genetic differentiation among samples can be discarded (Wright, 1931).

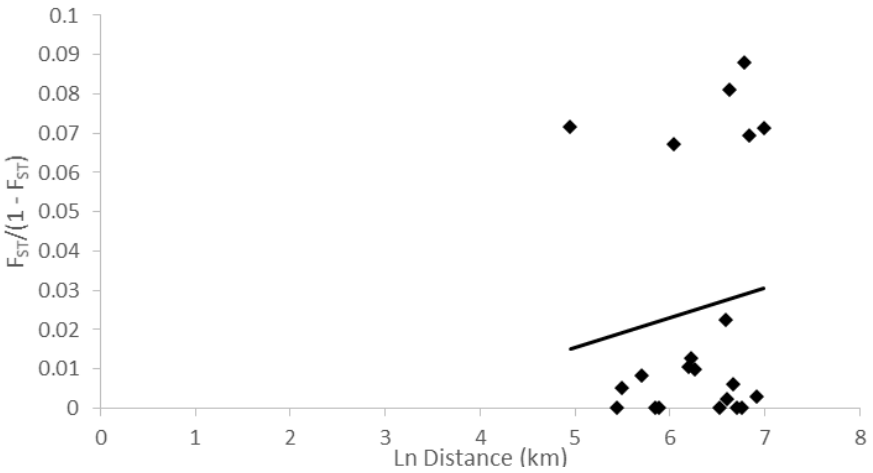


Figure 3.3: Mantel test *S. umbilicalis* showing genetic distance on y-axis and natural log of geographic distance on x axis. N = 21, one-tailed p-value = 0.2737

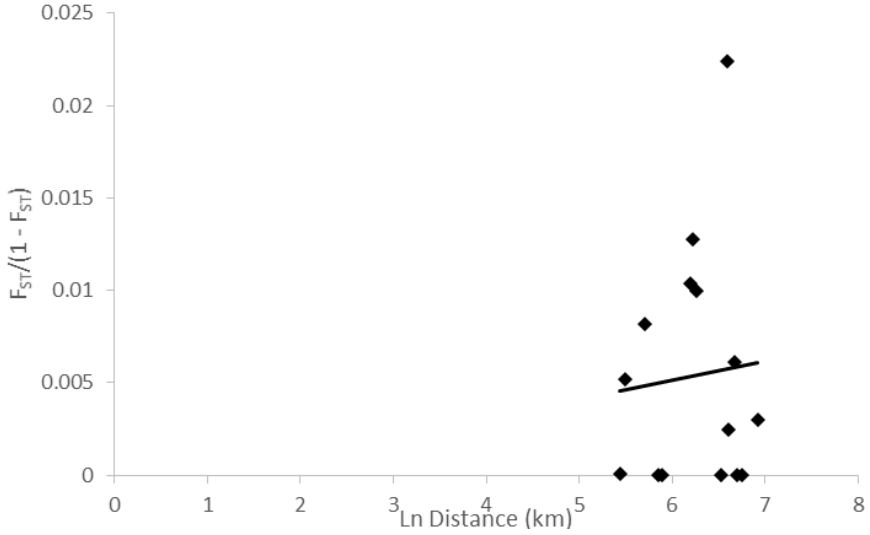


Figure 3.4: Mantel test *S. umbilicalis* without Swanage showing genetic distance on y-axis and natural log of geographic distance on x axis. N = 15, one-tailed p-value = 0.6436.

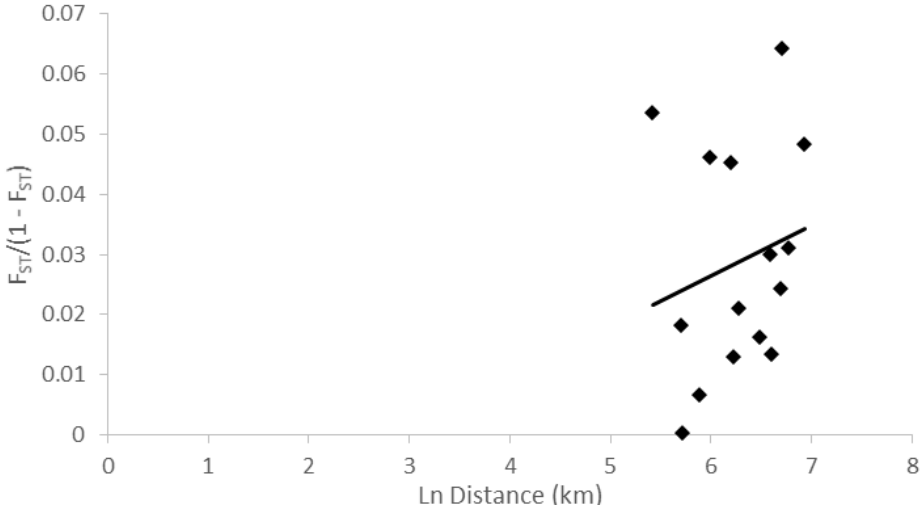


Figure 3.5: Mantel test *S. pennanti* showing genetic distance on y-axis and natural log of geographic distance on x axis. N = 15, one-tailed p-value = 0.1574.

There was a very low coefficient of determination for points around the trend line generated from the Mantel test in either species (Figures 3.3, 3.4 and 3.5). Although the trend line was positive in all cases, the large variation and lack of significant p values shows isolation by distance was too simplistic a model to explain population structure for either *Steromphala* species.

For *S. umbilicalis*, the optimum k was determined to be 3 based on the peak in Δk value, with or without prior population information. For *S. pennanti*, the optimum assumed k = 3 with or without prior population information, determined by the peak in Δk value. However, GENELAND analyses estimated that *S. pennanti* constituted a single genetic unit (k=1) while two different units were found in *S. umbilicalis*: Swanage, and the continental samples. It is possible that

STRUCTURE is less useful (Figure 3.6) due to high gene flow (Waples & Gaggiotti, 2006). The DAPCs for *S. umbilicalis* showed substantial overlap of genetic similarity for most populations (Figure 3.7a), but Swanage (and to a lesser extent, Vilagarcía) is a clear outlier population. By contrast, the DAPC for *S. pennanti* showed less overlap between populations (Figure 3.7b). N_E and their 95% confidence interval were calculated by groups within species. The northern group of *S. pennanti* showed a N_E of 268 (95% CI = 130-3813) while for the southern group was 103 (95% CI = 66-197). Estimates in *S. umbilicalis* were 198 (95% confidence interval = 114-558) in the northern group (Swanage not included) and 835 (95% CI = 198-∞) in the southern group.

Table 3.5: Pairwise comparisons of microsatellite genotypic differentiation (p-value) and F-statistics: localities north of the habitat gap in **bold**, sites ordered from south (top) to north (bottom); significant p-values following sequential Bonferroni correction marked with asterisk (*)**;** F_{ST} WC = F_{ST} calculated according to Weir and Cockerham (1984); F_{ST} WC C = corrected F_{ST} WC values. Where calculations gave negative F_{ST} values, these were reported as 0. Locality codes correspond with Table 3.1. *Steromphala umbilicalis* left, *S. pennanti* right.

Localities	P-value	F_{ST} WC	F_{ST} WC C
V & S	0.074	0.011	0.023
V & B	0.004	0.023	0.023
V & **LP**	0.046	0.007	0.007
V & **LO**	0.126	0.004	0.005
V & **C**	0.072	0.004	0.011
S & B	0.178	0.000	0.010
S & **LP**	0.629	0	0.006
S & **LO**	0.189	0.009	0.017
S & **C**	0.374	0	0.001
B & **LP**	0.079	0.005	0.010
B & **LO**	0.342	0.009	0.011
B & **C**	0.613	0	0.009
LP & **LO**	0.011	0.008	0.009
LP & **C**	0.388	0	0.005
LO & **C**	0.854	0	0.003
SW & V	<0.001*	0.066	0.052
SW & S	<0.001*	0.082	0.104
SW & B	<0.001*	0.066	0.074
SW & **LP**	<0.001*	0.075	0.074
SW & **LO**	<0.001*	0.064	0.061
SW & **C**	<0.001*	0.067	0.082

Localities	P-value	F_{ST} WC	F_{ST} WC C
V & S	0.003*	0.014	0.016
V & SJ	0.005	0.028	0.035
V & **P**	0.124	0.023	0.017
V & **LO**	0.078	0.014	0.017
V & **H**	0.001*	0.047	0.035
S & SJ	<0.001*	0.052	0.071
S & **P**	0.001*	0.044	0.042
S & **LO**	<0.001*	0.043	0.043
S & **H**	<0.001*	0.060	0.057
SJ & **P**	0.474	0.000	0.012
SJ & **LO**	0.021	0.019	0.029
SJ & **H**	0.021	0.029	0.037
P & **LO**	0.051	0.017	0.014
P & **H**	0.189	0.016	0.012
LO & **H**	0.055	0.007	0.008

Table 3.6: Microsatellite data AMOVA results for: (a) *S. umbilicalis* with loci U34184 and U36148 removed from calculations as greater than 5% values missing; (b) *S. pennanti* with loci HEX1, NED1 & FAM3 removed from calculations as greater than 5% values missing.

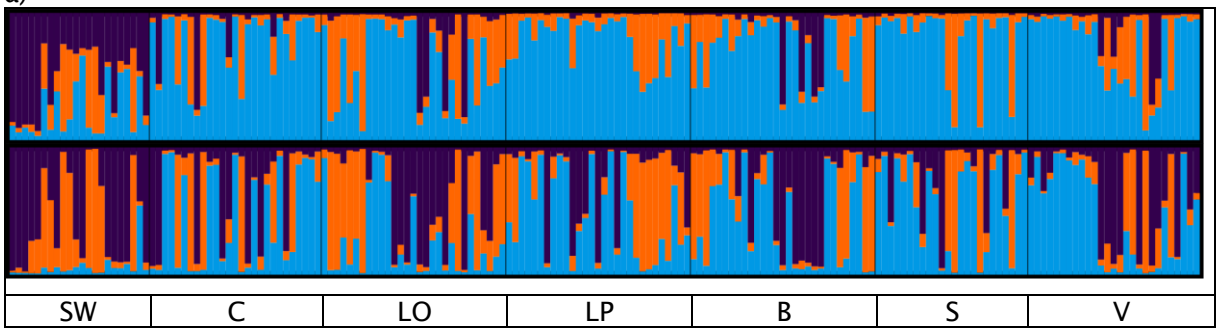
a)

Comparison between:	df	Sum of squares	σ	% variation	ϕ	P-value
Gap groups	2	15.417	0.088	3.251	ϕ_{CT}=0.033	0.058
Localities within gap groups	4	11.313	0.008	0.300	ϕ_{SC}=0.003	0.179
Samples within localities	180	469.048	2.606	96.449	ϕ_{ST}=0.036	0.000

b)

Comparison between:	df	Sum of squares	σ	% variation	ϕ	P-value
Gap groups	1	3.888	-0.008	-0.401	ϕ_{CT}=-0.004	0.395
Localities within gap groups	4	17.610	0.096	4.723	ϕ_{SC}=0.047	0.000
Samples within localities	151	292.955	1.940	95.678	ϕ_{ST}=0.043	0.000

a)



b)

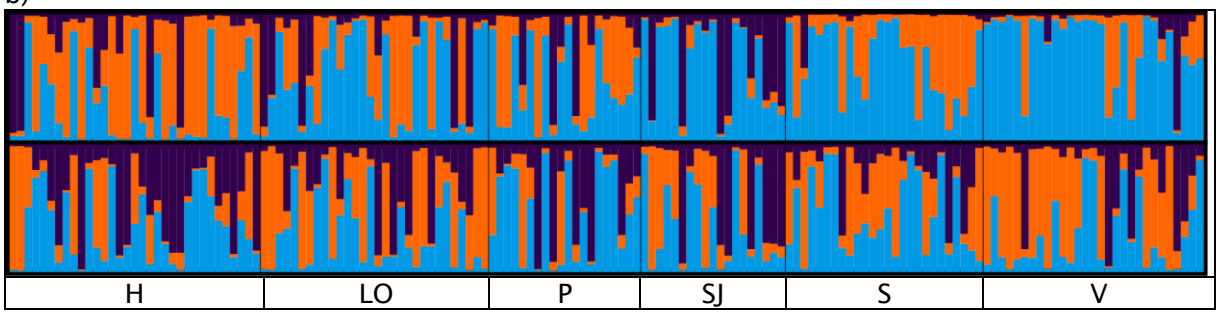
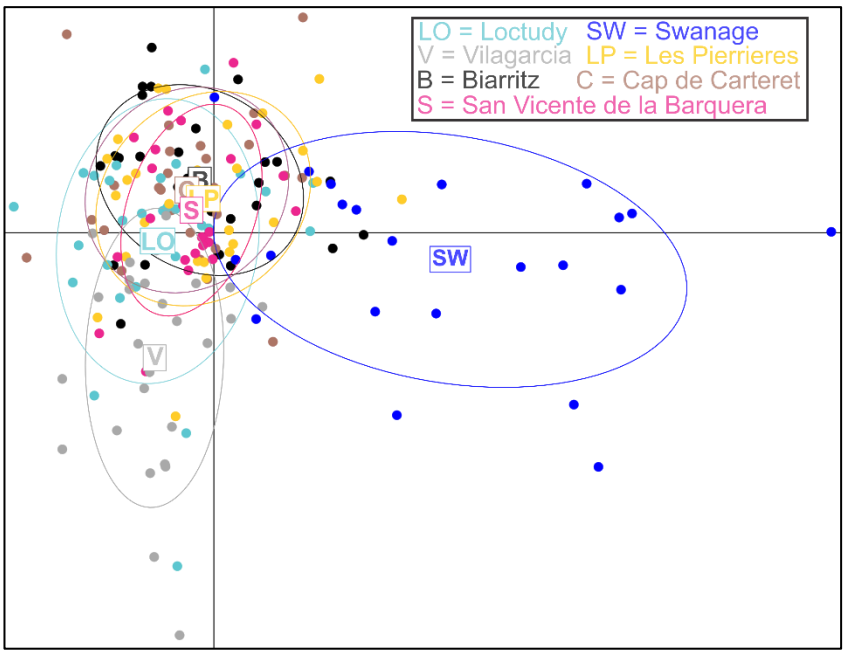


Figure 3.6: STRUCTURE bar plots of the proportion of membership (q) for individuals from sampled localities for: (a) *S. umbilicalis* with prior populations (k=3) (top); without prior populations assumed (k=3) (bottom); (b) *S. pennanti* with prior populations (k=3) (top); without prior populations assumed (k=3) (bottom). Locality codes correspond with Table 3.1.

a)



b)

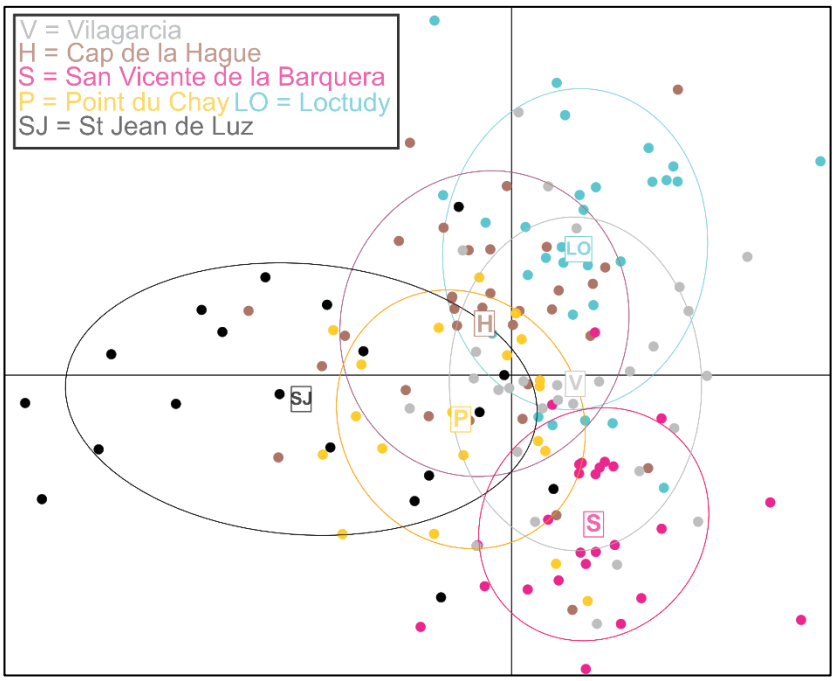


Figure 3.7: Plots of the first two axes obtained by DAPC visualising genetic distance between samples and localities for *S. umbilicalis* (a) and *S. pennanti* (b). Locality codes are indicated at the centre of each group and the ellipses represent 95% confidence interval.

3.4 Discussion

Until recently, studies infrequently accounted for the potential of differing abundance and distribution patterns to help explain contrasting patterns of genetic structure between species. This study joins a small, but growing literature that combines such field data with population genetics for marine species (e.g. Dawson *et al.* (2014)). It attempts to remove some of the potentially confounding factors by examining congeneric species (i.e. they largely share evolutionary histories) along the sympatric portions of their geographic range (i.e. they largely share the same broad-scale environmental conditions). By integrating field data on abundance and distribution and knowledge of their respective ecologies, it is possible to more clearly isolate the ecological and physical mechanisms behind differences in genetic structure of the studied species. The results presented here are in accordance with recent studies of rocky shore invertebrates and fish (Dawson *et al.*, 2014; Selkoe *et al.*, 2014), as well as butterflies and mammals (Engler *et al.*, 2014; Kierepka *et al.*, 2016), showing that specialist species (which are often less abundant with fragmented distributions) exhibit more genetic differentiation compared to generalist species (which are often more abundant with more continuous distributions).

Within the same region, a habitat generalist species with high local abundance and a continuously spread distribution should yield greater reproductive output than a phenotypically similar habitat specialist congener with lower abundance and a more fragmented distribution. Assuming juvenile mobility is similar, then this will result in shorter dispersal pathways among populations and more emigrant offspring for the habitat generalist species, leading to increased gene flow and therefore reduced genetic variation between populations compared to the habitat specialist (Dawson *et al.*, 2014). Other factors such as differences in larval dispersal could also be important (e.g., in congeneric chthamalid barnacles, Pannacciulli *et al.* (1997)), but remain largely unexplored in rocky intertidal invertebrates. The results here show that the widespread and common *S. umbilicalis* exhibits lower genetic differentiation among localities compared to the habitat specialist, *S. pennanti,* which is characterised by low abundances and a more fragmented distribution (Figure 3.1; Table 3.5). Furthermore, *S. umbilicalis* shows a greater number of migrants between the groups north and south of the Biscayan habitat gap than *S. pennanti*. The greatest number of significant pairwise differences for *S. umbilicalis* were found between the ‘islanded’ site at Swanage and the continental localities, whilst the

absence of significant genetic differentiation within the Bay of Biscay suggests a largely panmictic population. Thus, the current regime in the English Channel (Pingree & Maddock, 1977) (which *S. pennanti* does not cross) appears to be a larger barrier to gene flow than the Biscayan habitat gap for *S. umbilicalis*. In *S. pennanti*, the greatest number of significant pairwise differences are found between San Vicente de la Barquera and all other localities. San Vicente de la Barquera (central north Iberian coast) coincides with the most fragmented part of its geographic range sampled (Figure 3.1). In addition, this region (south of the habitat gap) is associated with the lowest abundances and estimates of genetic effective population size in *S. pennanti*. In contrast, this region coincides with highest abundances and estimates of genetic effective population size for *S. umbilicalis*. Below the biological and physical factors that may explain the differences and commonalities of genetic structure between the studied species are discussed.

3.4.1 Differences between species

Steromphala umbilicalis has a continuous distribution within the Bay of Biscay and shows high overall field (census population size) and genetic population sizes (i.e. effective population size). Thus, a panmictic metapopulation fits with predictions for a generalist species such as *S. umbilicalis* (Dawson *et al.*, 2014; Engler *et al.*, 2014; Kierepka *et al.*, 2016). By contrast, *S. pennanti* has a fragmented geographic distribution, lower overall abundance both in field observations and genetic estimations, and is associated with fucoids largely on more sheltered shores (Bordeyne *et al.*, 2017). Given these clear differences between the studied species, locality specific patterns of genetic differentiation can be considered in the light of the abundance and distribution data. For example, significant genetic differentiation is found between San Vicente de la Barquera (central north Iberian coast) and all other localities in *S. pennanti* (Table 3.5). The study surveys show that *S. pennanti* and *Fucus* were largely absent within the intervening section of coastline separating St Jean de Luz (SW France) and San Vicente de la Barquera (225 km). The absence of several fucoid species in this region of the Iberian Peninsula has been attributed to its relatively high sea surface temperature (SST) (Duarte *et al.*, 2013; Southward *et al.*, 1995; Zardi *et al.*, 2015). Fucoids act as a bioengineer once established (Pocklington *et al.*, 2017; Seed & O'Connor, 1981) and are thought to facilitate the recruitment of certain gastropods including *S. pennanti* (Bordeyne *et al.*, 2017). Conversely, there is no such genetic differentiation between any of the mainland sampled *S.*

umbilicalis populations. This may be largely attributable to the presence of *S. umbilicalis* at every sampling locality, reducing isolation among populations. *Steromphala pennanti* is also absent from two of five localities between Vilagarcia (NW Iberian coast) and San Vicente de la Barquera (Figure 3.1). This may explain the significant genetic differentiation between the two localities. Interestingly, whilst *S. umbilicalis* shows no significant genetic difference between these two localities, there is a relatively high F_{ST} value (0.011) and Vilagarcia appears as an outlier population in the DAPC (Figure 3.7a).

Another major difference between species is the presence of *S. umbilicalis* on the British Isles, which is significantly genetically differentiated from all mainland localities (Table 3.5). As *Steromphala* is thought to have originated south of the British Isles (Southward *et al.*, 1995), two possible explanations for extremely rare but successful recruitment of *S. umbilicalis* from mainland to British shores and absence of *S. pennanti* on British shores are: (i) its greater abundance (compared with *S. pennanti*) increases the number of larvae released, thereby increasing the probability of propagules reaching Britain; or (ii) *S. umbilicalis* has a longer larval duration than *S. pennanti*, allowing larvae to disperse further. The significant genetic differentiation between Swanage (Britain) and all other populations (F_{ST} = 0.064 to 0.082, $p \leq 0.001$ for all pairwise comparisons), suggests diminished genetic flow in either direction across the Channel for *S. umbilicalis* which was not attributable to distance (Figure 3.3). This low connectivity in *S. umbilicalis* may be attributable to the Channel acting as a habitat gap, where tidal currents dominate the transport (Pingree & Maddock, 1977). These currents are not driven in a uniform direction, unlike the longshore currents along the sandy habitat gap in south-west France (Castelle *et al.*, 2006; Lazure *et al.*, 2009), although they can be of a comparable order of magnitude (Pingree & Maddock, 1977).

3.4.2 Patterns associated with both species

One of this study's hypotheses is that there should be low genetic differentiation among localities in regions where both species are relatively abundant with continuous distributions. This view is supported by the observation presented here that on the northern mainland coast (Figure 3.1) there is no significant genetic differentiation between localities in either species, as well as a higher genetic effective size for both species. This cooler region has a sustained and well established fucoid habitat (Southward *et al.*, 1995; Zardi *et al.*, 2015), which

is mirrored by the relatively consistent presence and higher abundances of *S. pennanti* in this region relative to south of the habitat gap (Figure 3.1).

Both *Steromphala* species exhibit no significant genetic differentiation between the sampled localities directly north and south of the habitat gap (F_{ST} = 0.005, p = 0.079 for *S. umbilicalis* and F_{ST} = 0.000, p = 0.474 for *S. pennanti*), which has also been found for similar scale sandy habitat gaps for some rocky intertidal species (Ayre *et al.*, 2009). One possible scenario is southward transport of larvae of both species along the surface water layer of this region during late summer (Lazure *et al.*, 2009). Larval transport spanning this gap of ~230 km would require a mean longshore current of approximately 0.4 m/s, which would be possible considering the estimated larval duration of ≤7 days for *S. umbilicalis* (Keith *et al.*, 2011), and a similar assumption could be made for *S. pennanti*. Southward longshore current velocities measured and modelled on this sandy coast mostly range between 0.1 m/s and 0.5 m/s, sometimes reaching 1 m/s (Castelle *et al.*, 2006). These currents may therefore enable southward larval transport over the habitat gap during the spawning season, resulting in genetic connectivity. As such, the results here suggest that currents, larval duration, and spawning season are more important than absolute distances, when considering genetic differentiation across habitat gaps.

The role of coastal currents and oceanographic fronts in influencing population genetics has been assessed for several species in and around our study region, particularly around Brittany in northern France (Almeida *et al.*, 2017; Couceiro *et al.*, 2013). These and other studies highlight the interaction between currents and timing of propagule release and spawning (Dong *et al.*, 2012). Spawning of *S. umbilicalis* peaks from August to November/December and October/November on the north and west coasts of the Iberian Peninsula respectively (both south of the habitat gap) (Bode *et al.*, 1986; Gaudèncio & Guerra, 1986; Lewis, 1986). Assuming a similar spawning season for *S. pennanti*, November and December ocean currents around the western Iberian Peninsula are wind driven from the south-west, which would enable autumn and winter dispersal of larvae north towards the centre of the Bay of Biscay (Puillat *et al.*, 2004; Varela *et al.*, 2005). These currents could drive surface water containing larvae away from the suitable habitat on the north coast of the Iberian Peninsula. The remainder of the year, the western Iberian Peninsula is dominated by a northwest oceanic swell (Zardi *et al.*, 2015), possibly resulting in southwards transport of any larvae (Ribeiro *et al.*, 2010). This would reduce recruitment

between the north and west coast of the Iberian Peninsula, potentially contributing to Vilagarcía (NW Iberian coast) being an outlier for both species.

3.5 Conclusions

The results of the genetic comparison of sympatric study species support the hypothesis that, over their range, a habitat generalist with a continuous distribution and high abundances will exhibit lower genetic differentiation compared to a congener habitat specialist with a fragmented distribution and lower abundances. These genetic differences are likely to be a function of contrasting population sizes and population isolation, which have long been known to influence genetic differentiation in a broad sense (Frankham, 1996; Riginos & Nachman, 2001). However, data presents here a novel addition to parallel studies by providing field data that are consistent with the ecological mechanisms that likely drive the observed differences in population genetics. Compared to the largely panmictic and continuously distributed *S. umbilicalis*, higher genetic differentiation in *S. pennanti* is suggested to be caused by an association between habitat availability (fucoid presence/abundance), which is spatially fragmented on the N. Iberian Peninsula where genetic differentiation is highest in this species. This research broadly supports recent studies that suggest how distribution and abundance data can help the interpretation of comparative population genetic studies (Dawson *et al.*, 2014; Engler *et al.*, 2014; Kierepka *et al.*, 2016). It is practical to incorporate a measure of abundance, distribution, and knowledge of the ecology and life history of species within population genetics studies. This can be combined with information on the spatial distribution of habitat availability and environmental variables to inform subsequent interpretation, particularly through targeted sampling of sympatric congeners.

Chapter 4: Explaining biogeographic patterns of abundance and body size of rocky intertidal gastropods in the Bay of Biscay

4.1 Introduction

4.1.1 Background and hypotheses

Key questions in biogeography are how and why species abundances vary within their geographic ranges (Briggs, 1974; Sagarin *et al.*, 2006; Scrosati & Heaven, 2008). Classically, in the biogeographical literature, it has been assumed that species follow a simple pattern where they are most abundant within the centre of their geographic ranges and decline towards their respective edges (Brown *et al.*, 1995; Hengeveld & Haeck, 1982). Recent studies have been equivocal, however, with species often exhibiting highly variable abundance patterns within their range centres and edges (Fenberg & Rivadeneira, 2011; Hidas *et al.*, 2010; Rivadeneira *et al.*, 2010; Sagarin & Gaines, 2002). It is worth noting that such patterns had been described in considerably older literature on geographic distributions of intertidal species (Crisp & Southward, 1958; Fischer-Piette, 1955; Fischer-Piette & Crisp, 1959; Lewis, 1964; Southward & Crisp, 1954). Therefore, focus should be shifted to identifying the environmental and biological variables that can best predict abundance patterns not only towards range edges, but also within the central portion of species ranges. Such an approach has been enhanced by the recent and widespread availability of high resolution, geo-referenced environmental data [e.g. Tyberghein *et al.* (2012)]. Intertidal species are ideal for these biogeographical investigations as their ranges are effectively one dimensional, with narrow vertical extents of less than tens of metres (Fenberg & Rivadeneira, 2011), unlike on land (Lenoir & Svenning, 2013) or subtidally (Rowe & Menzies, 1969) where altitudinal and depth gradients can extend over hundreds of km. Furthermore, rocky intertidal invertebrates are sessile or slow moving, making them easy to sample (Sagarin & Gaines, 2002).

The environmental variable most commonly thought to influence patterns of distribution and abundance, within and between species, is temperature (Broitman *et al.*, 2008; Hutchins, 1947; Lima *et al.*, 2006; Wethey & Woodin, 2008). However, responses of intertidal species to temperature have been most

commonly investigated at range edges or at local scales, not generally at larger geographic scales covering the central portions of their range. This is likely due, in part, because of concerns about rapid anthropogenic climate change causing species range expansions or contractions (Hawkins *et al.*, 2009; Helmuth *et al.*, 2006; Lima *et al.*, 2007b; Mieszkowska, 2005 PhD Thesis; Rubal *et al.*, 2013; Wethey *et al.*, 2011). Temperature can be considered in different ways when analysing the effects on the ecology of intertidal species. Tidal cycles result in intertidal species alternating between immersion and emersion on the shore. Thus both air and sea temperatures can influence abundances (Seabra *et al.*, 2011). As differing durations of immersion occur at different shore heights, it has been suggested that thermal stress and subsequent survival, particularly when emersed, can differ between the high and low shore populations of the same species and among closely related species (Somero, 2002; Tomanek & Somero, 2000); although this does not always happen (shore height was not significant in relation to limpet and mussel mortality; Harley, 2008). Several intertidal species respire and forage more effectively when submerged (Newell, 1979). For example, in *Patella vulgata* tide out respiration and foraging can occur; but foraging is constrained to damp conditions such as at night or humid days, under dense seaweed canopies or restricted to certain habitats such as vertical faces (Hartnoll & Wright, 1977; Hawkins & Hartnoll, 1982; Hawkins & Hartnoll, 1983; Little, 1989). Metabolism, growth and reproductive output can therefore be considered to be driven primarily by high tide seawater temperatures (Branch *et al.*, 1988). Individual performance will be arrested by temperature stresses particularly during low tides, with subsequent mortality occurring at extreme temperatures (Harley, 2008). Whilst multi-annual mean sea surface temperature (SST) has performed well in explaining some species distributions and abundances in models (Keith *et al.*, 2011; Poloczanska *et al.*, 2008), temperatures fluctuate at daily, seasonal and interannual scales over the life cycle of intertidal biota (Seabra *et al.*, 2011). Therefore, seasonal temperature extremes or occasional extreme events may also affect population responses (Harley, 2008; Mieszkowska *et al.*, 2013; Wethey *et al.*, 2011).

Higher temperature has also been observed to frequently correlate with decreased individual size on geographic scales, particularly in aquatic species (Atkinson, 1994; Forster *et al.*, 2012; Horne *et al.*, 2015), first conceptualised as an interspecific trend in Bergmann's rule (Bergmann, 1848). In addition, size is influenced by other local biological factors such as density-dependent competition (Boaventura *et al.*, 2002; Boaventura *et al.*, 2003; Underwood, 1976),

with density in turn driven by recruitment regimes (Jenkins *et al.*, 2008). This often means that size will correlate negatively with abundance in a population (Boaventura *et al.*, 2003; Kendall & Lewis, 1986) but not temperature in some organisms (Green & Middleton, 2013). The scale and nature of abundance-body size relationships is highly variable (Meager *et al.*, 2011; White *et al.*, 2007); but should be considered when trying to assess why abundance of a species varies across its range. Furthermore, in order to better understand why abundance varies within the ranges of species, size-structure should be compared across populations as they can be a reflection of demographic factors contributing to spatial variation in abundance. For example, the proportion of juveniles is an indication of recruitment success (i.e. high recruitment often leads to high overall abundances) as has been found at some range centres (Fenberg & Rivadeneira, 2011); while the proportion of large individuals can be an indication of density-dependent interactions (i.e. larger individuals take up more space and resources, resulting in fewer individuals occupying a given area, frequently in an allometric relationship (Boaventura *et al.*, 2003; Fenberg, 2013). Therefore, when trying to understand why abundance patterns vary within the ranges of species, at a minimum, spatial patterns of population size-structure should also be taken into account; but they rarely are (Sagarin *et al.*, 2006).

Temperature is also expected to interact with local biotic variables, determining the realised niche and influencing the abundance of a species (Connell, 1961; Guisan & Zimmermann, 2000; Hutchinson, 1957; Moore *et al.*, 2007b; Pearson & Dawson, 2003). For example, congeners and similar species within functional groups with overlapping distributions often have different tolerances of environmental variables (Boaventura *et al.*, 2002; Firth *et al.*, 2009; Hutchinson, 1957; Poloczanska *et al.*, 2008). These can include differing thermal preferences, with one species better suited to warmer temperatures than the other, modulating the outcomes of competition between two or more species (Boaventura *et al.*, 2002; Poloczanska *et al.*, 2008; Somero, 2002; Southward & Crisp, 1954). This balance between congeners can then interact with other biota which may be habitat forming, such as *Patella* Linnaeus 1758 interacting with the engineer species *Fucus vesiculosus* (Hawkins *et al.*, 2008; Moore *et al.*, 2007a; Moore *et al.*, 2007b).

Unrelated to temperature, the abundance of both engineering and other rocky shore species can also be determined by local gradients of wave exposure, with some species such as fucoids more common in sheltered shores, and others such as limpets, mussels and barnacles on more exposed shores (Ballantine,

1961; Lewis, 1964; Raffaelli & Hawkins, 1996). This environmental variable has classically been ranked qualitatively or semi-quantitatively (Boaventura *et al.*, 2001; Hidas *et al.*, 2010; Muñoz-Colmenero *et al.*, 2015) or estimated using map-based methods (Thomas, 1986). There has been a shift to using geographic information system (GIS) data to generate quantitative estimates of exposure based on variables such as wave fetch and prevailing winds, which can be subsequently incorporated into species distribution models (Burrows *et al.*, 2008; Hawkins *et al.*, 2009; Lima *et al.*, 2007b; Mieszkowska *et al.*, 2013).

The regional and local distribution of sediment is also unrelated to temperature, but it can play several key roles in determining rocky intertidal species distribution and abundance. At a regional scale the relative proportion of rocky to sedimentary (sandy and muddy shores) habitats will limit the total area of rocky shores along that section of coast. This might therefore reduce recruitment to rocky shores within the region as adjacent source populations are smaller, fragmented or more distant (Dawson *et al.*, 2014). If there is a large area of sediment shore separating two rocky shores, such a habitat gap can act as a barrier to dispersal depending on the interaction between species life history, ecology and other environmental conditions, such as nearshore currents (Shanks *et al.*, 2014). Habitat gaps can cause isolation of populations, which can be identified by genetic differentiation (Fenberg *et al.*, 2014; Knox *et al.*, 2011; Lourenço *et al.*, 2017), sometimes in association with differences in population size-structure and abundance (Fenberg & Rivadeneira, 2011; Ribeiro *et al.*, 2010). Sediments may affect rocky intertidal biota on local scales by burial in sediment, scour or abrasion, and changes in the physical characteristics of the bottom surface (Airoldi, 2003). For example, if an area of rocky shore is regularly inundated with sand, this can result in mortality of *Patella* species (Airoldi & Hawkins, 2007; Díaz-Tapia *et al.*, 2013; Marshall & McQuaid, 1989).

On the north-east Atlantic coast there lies 230 km of mostly uninterrupted sandy intertidal habitat in south-west France (Castelle *et al.*, 2006). This habitat gap for rocky shore species (Figure 4.1) coincides with the northern range limit of some warm water rocky intertidal gastropods such as *Patella rustica* (Lima *et al.*, 2007b) and *Stramonita haemastoma* (Wort, personal observation). It is also within the overlapping central part of the ranges of several congeners, namely the colder water limpet *Patella vulgata* and warmer water *Patella depressa*, as well as the warm-water trochids *Steromphala pennanti* and *S. umbilicalis* that extend further northwards along with the warm-water *Phorcus lineatus*. However, the

effect of the habitat gap on the abundance of these species either side is largely unknown.

The abundances of these rocky intertidal species may respond differently to the environmental variables along the NE Atlantic coast, including sea surface temperature, air temperature, wave exposure and the local and regional distribution of rocky intertidal habitat. In this study, the abundance and size-structure of rocky intertidal gastropods (*Patella vulgata, Patella depressa, S. umbilicalis, S. pennanti* and *Phorcus lineatus*) were measured along the Atlantic coast of France and the Iberian Peninsula (approximately 1600 km). The resulting data were then tested to show how much of the variation in abundance and size-structure can be explained by sea temperature (expressed as SST), air temperature (AT), wave exposure and habitat availability. It was hypothesized that mean SST would be the best environmental predictor of the abundance of intertidal species within their central region of their ranges, as an ultimate driving factor over-riding more local factors, by modulating density-dependent processes driven by patterns of recruitment. The study also investigated as to whether there is a relationship between temperature and the largest size classes (90th percentile) of individuals across the study region, hypothesizing that warmer temperatures (air or sea temperature) would result in smaller sizes of individuals; which could also affect density-dependent processes, thereby contributing to observed patterns of abundance. Study data were also used to determine if abundance is correlated with body size (measured as shell length/diameter), to test the hypothesis that abundance will be negatively related to body size for each species across the study region. This particular relationship is predicted by both density-dependence and macroecological theory, although for the latter it is usually measured interspecifically (Peters & Wassenberg, 1983; White *et al.*, 2007). The results were also interpreted to take account of the large habitat gap that coincides with the middle of the study region. These analyses will help illustrate how and why abundances of coastal species vary among locations in the central portions of their ranges in relation to heterogeneous environmental variables as well as the distribution of habitat availability.

4.1.2 Study species and focal region

The focal region of study covered a large portion of the central range of all targeted species. The trochids *Steromphala umbilicalis*, *Steromphala pennanti* and *Phorcus lineatus* are phenotypically similar and sympatrically distributed along the north-east Atlantic intertidal zone, occupying slightly different

ecological niches on the rocky intertidal shore (Bordeyne *et al.*, 2017). For *Phorcus lineatus* and *S. umbilicalis*, changes in range, abundance, population structure and genetic responses to climate change, particularly SST and wave exposure, have been both measured and modelled using quantitative and semi-quantitative data in the UK and along a section of the north coast of Spain (Mieszkowska *et al.*, 2006; Muñoz-Colmenero *et al.*, 2015). These studies respectively covered the northern and central portion of the ranges of *S. umbilicalis* and *Phorcus lineatus*. Southern limits for *Phorcus lineatus* were recorded in North Africa (Donald *et al.*, 2012; Lewis, 1986). There has been a recent re-expansion of *Phorcus lineatus* polewards along UK shores associated with the warming climate (Mieszkowska *et al.*, 2007). *Phorcus lineatus* is typically found on the mid-high shore (Crothers, 2001) and has a different diet to the two *Steromphala* species, as shown by its isotopic carbon and nitrogen ratios (Bordeyne *et al.*, 2017). The recorded range of *S. umbilicalis* extends from north-west Scotland (Mieszkowska *et al.*, 2013) to North Africa (Lewis, 1986; Southward *et al.*, 1995), with a habitat preference for the mid to low shore in rockpools, cracks, and around boulders (Mieszkowska *et al.*, 2013; Muñoz-Colmenero *et al.*, 2015), with recent range expansions further along the west coast of Scotland as well as along the English Channel (Mieszkowska *et al.*, 2006). *Steromphala pennanti* is distributed from the Cherbourg peninsula in northern France to Morocco (Southward *et al.*, 1995). The focal study area is therefore closer to its northern range limit than for the other two trochid species, but still covers most of the central range of *S. pennanti*. It is typically found on the low shore and considered more closely associated with fucoids such as *Fucus serratus* and *F. vesiculosus* than *S. umbilicalis* (Bordeyne *et al.*, 2017); thus it is more habitat-specific than the other two trochid species which are found across a wider range of habitats within the rocky intertidal.

The patellid congeners *Patella vulgata* and *Patella depressa* appear to have extremely similar grazing mechanisms and diets (Hawkins *et al.*, 1989; Thompson *et al.*, 2000) and co-occur on the mid-region of moderately exposed shores. Historically *Patella depressa* was distributed from north Wales to Senegal whilst *Patella vulgata* was found from northern Norway to the south of Portugal (Fretter & Graham, 1976; Southward *et al.*, 1995). Recent range shifts include the contraction of the southern limit of the boreal-cold species *Patella vulgata* on the Portuguese coast (Hawkins *et al.*, 2009; Lewis, 1986); whilst *Patella depressa* has increased in abundance near its northern limit (Hawkins *et al.*, 2009; Kendall *et al.*, 2004), and minor range extensions have occurred eastwards on the English

Channel coast (Hawkins, unpublished observations). The focal region is well within the range centres of both of these species. Where the two species overlap, their differing life histories can result in substantial differences in size and abundance. For example, *Patella depressa* has a different reproductive season to *Patella vulgata,* resulting in reduced grazing activity during the spring and summer (Moore *et al.*, 2007a). They show slight differences in microhabitat preferences, with *Patella vulgata* found to preferentially aggregate beneath *Fucus* Linnaeus, 1753 species, unlike *Patella depressa* (Moore *et al.*, 2007b). It has been suggested that the coexistence of these two *Patella* species can be attributed to a stronger effects of intraspecific than interspecific competition at different size classes (Boaventura *et al.*, 2002). In these intraspecific interactions, larger individuals of *Patella depressa* have been found to have a greater negative effect on smaller individuals than vice versa, and may modulate abundance of smaller individuals (Boaventura *et al.*, 2003).

Several fucoid species, such as *F. serratus* and *Ascophyllum nodosum* (Linnaeus) Le Jolis, 1863, are absent east of a boundary that varies at approximately 5˚ W on the north coast of the Iberian Peninsula which has been attributed to higher sea surface temperature (SST) (Duarte *et al.*, 2013; Hawkins *et al.*, 2009; Southward *et al.*, 1995). Fucoids are widely recognised to act as a bioengineers once established (Jenkins *et al.*, 2005; Jenkins *et al.*, 1999; Pocklington *et al.*, 2017; Seed & O'Connor, 1981), therefore their abundance has also been studied as potential habitat for gastropods, especially *S. pennanti*.

4.2 Methods

4.2.1 Surveys

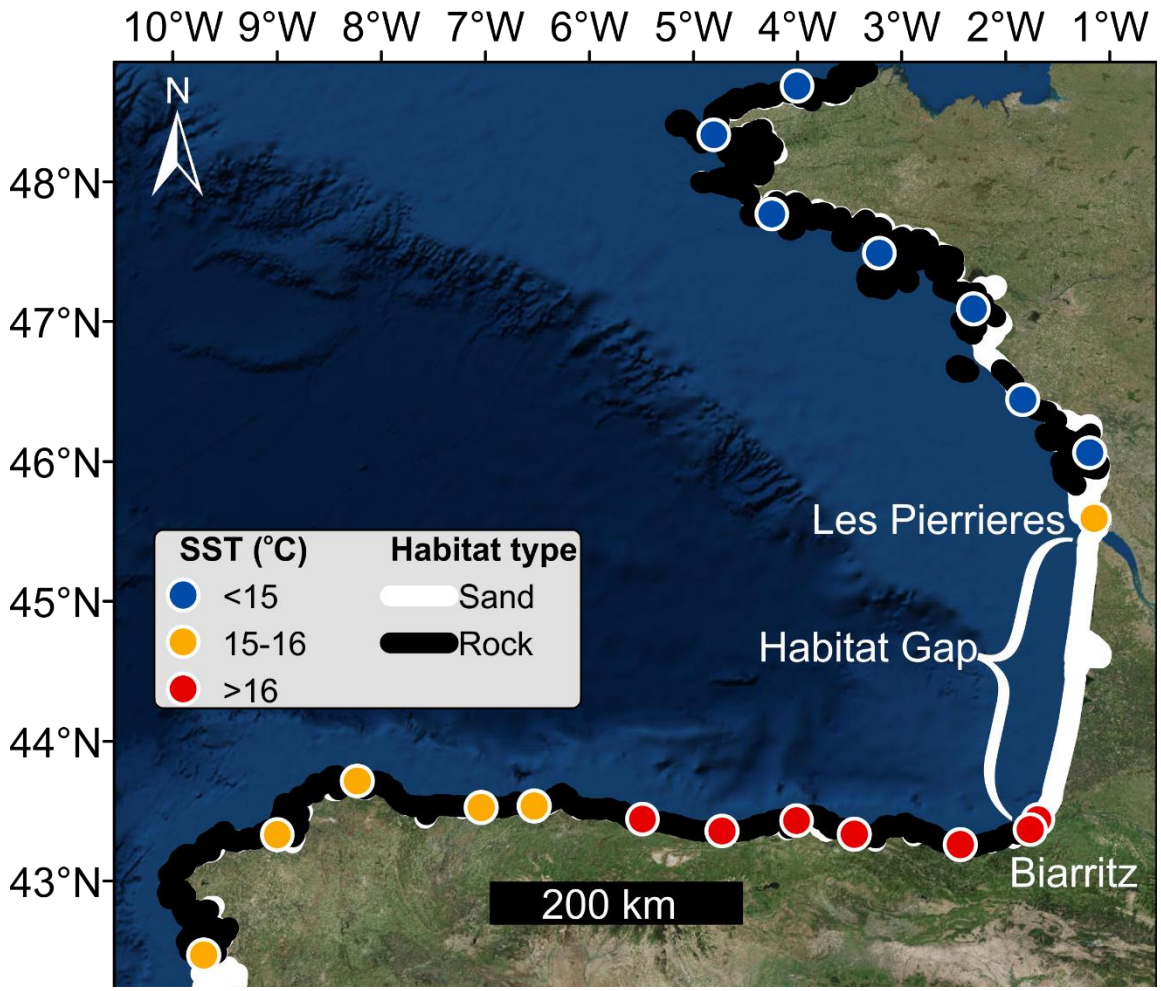


Figure 4.1: Map using World-Aitoff projection in ArcGIS displaying: localities surveyed and their mean annual SST from August 2005 to August 2015; habitat type and the location of the habitat gap.

Sampling was carried out during low tides at 20 localities in August 2015, April and October 2016, with multiple visits to several localities (Figure 4.1 and table 4.1). 0.5 x 0.5 m quadrats were used for haphazard sampling, with ten quadrats on the upper shore and ten on the lower shore. To achieve hierarchical sampling, at each shore height two teams approximately 100-500 m apart sampled a set of five haphazard quadrats within approximately 20 m of each other. The upper shore was defined as being between the splash zone dominated by the *Littorina saxatilis* (Olivi, 1792) species complex or *Melarhaphe neritoides* (Linnaeus, 1758) and the fucoid belt or zone of equivalent macroalgae. The lower shore was defined as from the fucoid belt to the low tide waterline at spring tides. Within each quadrat: *Patella* >5 mm length were identified to species level and shell

length measured to the nearest 0.1 mm using dial callipers; *Steromphala umbilicalis*, *S. pennanti* and *Phorcus lineatus* were identified and counted; and percentage cover of fucoids was estimated by eye. *Ascophyllum nodosum* and *Fucus* species were initially grouped together when describing fucoid cover, as this enabled rapid estimates. *Bifurcaria bifurcata* Ross, 1958 is also a fucoid, although it has a different morphology. Two fucoid cover estimates were therefore produced, one including *B. bifurcata* and one excluding it. Total macroalgae cover for each quadrat was also estimated.

Table 4.1: Localities sampled from north to south (with bold line representing the habitat gap), code, latitude, longitude and mean SST (°C)

Locality	Code	Latitude	Longitude	Mean SST (°C)
Roscoff	ROS	48.72927	-3.98871	13.01993
Brest	BRE	48.37611	-4.76194	13.50411
Loctudy	LOC	47.80944	-4.17556	13.98286
Quiberon	Q	47.53415	-3.14635	13.98030
Pt St Gildas	PSG	47.13736	-2.24669	13.85629
Sables d'Olonne	SOL	46.48729	-1.76996	14.67956
Pt du Chay	PDC	46.10861	-1.14333	14.61894
Les Pierrieres	LP	45.63972	-1.09528	15.10650
Biarritz	BIA	43.48278	-1.56861	16.62547
St Jean de Luz	SJL	43.41078	-1.63763	16.67643
Zumaia	ZUM	43.30122	-2.25981	16.65670
Castro-Urdiales	CU	43.37444	-3.20944	16.48710
Loredo	LOR	43.46974	-3.72677	16.47645
San Vicente de la Barquera	SVB	43.39278	-4.39000	16.41171
Ribadesella	RIB	43.47471	-5.10180	16.26974
San Esteban de Pravia	SEP	43.56539	-6.08149	15.94800
Luarca	LUA	43.55083	-6.55556	15.83608
O Vicedo	VIC	43.7306	-7.68159	15.73493
A Coruna	CO	43.34417	-8.35528	15.16771
Villagarcia	VIL	42.47833	-8.92333	15.34263

At each locality a two-minute timed search (TS) was made by four people for *Steromphala*. This was in the fucoid or equivalent low shore zone where both *S. umbilicalis* and *S. pennanti* would be present. If *Steromphala* were rarer, the search time was doubled to 4 minutes per person. To prevent sampling overlap, samplers were spaced at least 10 m apart and in varied microhabitats to ensure a fair representation of the abundance of both species. The maximum basal diameter of the shell was then measured to the nearest 0.1 mm using dial callipers.

Mean number of individuals/m^2, mean size for both *Patella* species and mean percentage fucoid cover (with and without *Bifurcaria*) were calculated at

each locality, as well as means of high and of low shore data. For *Steromphala*, mean abundance as average number of individuals found per minute for each locality was also calculated. The proportion of quadrats with over 5 % fucoid cover at each locality was calculated to test for a relationship between fucoid abundance and gastropod abundance.

Juvenile recruitment of *Patella* species, which can have an effect on overall abundance, was quantified by dividing the number of individuals <15 mm by the total number of limpets per quadrat using data from August 2015 and October 2016 sampling only and aggregating results by locality. The 90th percentile of shell size was calculated for *Patella* and *Steromphala* species and used to consider the relationship between environmental variables (including temperature) and size using locality data,

4.3.2 Environmental data

Daily mean 0.25 degree resolution SST and 0.5 degree resolution monthly mean air temperature provided by the NOAA/OAR/ESRL PSD, Boulder Colorado, USA was obtained for each locality (https://www.esrl.noaa.gov/psd/data/gridded/ last accessed 12/2/2018) (Fan & Van den Dool, 2008; Reynolds *et al.*, 2007). From these datasets, annual mean SST (SSTM) and air temperature (ATM) in ˚C from August 2005 to 2015, winter mean (December, January and February) from December 2005 to February 2015 (SSTW and ATW) and summer mean (June, July and August) from June 2006 to August 2015 (SSTS and ATS) were extracted for each locality. To avoid bias from using different daytime measurements, only values from 12:00 were used.

A coastline shapefile was obtained from the European Environment Agency (EEA) (https://www.eea.europa.eu/data-and-maps/data/eea-coastline-for-analysis/gis-data/europe-coastline-shapefile last accessed 8/2/2018) and overlaid on the highest available (typically 30 cm) resolution satellite imagery available as a background layer in ArcGIS Desktop v. 10.5 (Esri, Redlands, California, USA). All layers were converted to the ETRS89 coordinate system for use in subsequent operations. The coastline shapefile was divided into sections defined as rock or sediment based on the substrate type on the line or at the nearest land-sea interface. The buffer tool was used to create circles of 1 km and 5 km radius around each locality centroid, and the proportion (as %) of rocky coast to total coastline within each circle calculated. The proportion within 1 km radius (R_1) was used as a proxy for potential sediment inundation or scouring of rocky substrate

of populations. This was on the basis that at this local scale, sediment would be more likely to shift over rock if a higher proportion of the adjacent substrate was sediment. The proportion within 5 km radius (R_5) was used a general estimate of suitable substrate in the immediate area.

Assuming any effects of the habitat gap on species abundance at a given locality would be determined by proximity, the distance using the coastline shapefile between the proximal edge of the habitat gap and each locality was measured in ArcGIS Desktop v. 10.5. As use of logged distance is commonly used in preparation for Mantel tests of genetic differentiation with geographic distance (Mantel, 1967; Rousset, 1997), this was calculated to use as a variable for distance to the habitat gap (GP).

Wave exposure was calculated using the quantitative index developed by Burrows *et al.* (2008). The EEA coastline shapefile covering the extent of the study region was converted to 1 km raster resolution and the wave fetch from angular sectors of 22.5˚ to coastal cells calculated in WaveFetch16v01. Using the nearest weather station to each locality, wind data for Iberian Peninsula, Biarritz and St Jean de Luz was downloaded from AEmet, using monthly mean wind speed and the direction of the maximum wind speed from 2012 (earliest available data) to 2015 (https://opendata.aemet.es/centrodedescargas/productosAEMET last accessed 12/2/2018). Daily mean wind speed and direction from August 2005 to August 2015 for all French localities excepting Biarritz and St Jean de Luz was downloaded from the MeteoFrance website (https://donneespubliques.meteofrance.fr/ last accessed 12/2/2018). The proportion of time records where wind came from the differing 22.5˚ angular sectors was calculated and multiplied by the square of mean wind speed in knots for each sector. This wind energy variable was then multiplied by the fetch to obtain a measure of wave exposure (Burrows *et al.*, 2008).

4.2.3 Statistical analyses

Multiple linear regression analyses were initially performed in RStudio v. 1.0.136 to explore the relationships between each species abundance variable and the aforementioned environmental variables. The dredge function of the R package 'MuMIn' (Barton, 2013) was applied to fit linear models for all possible combinations of explanatory variables. These models were then ranked according to the corrected Akaike Information Criterion (AICc) following Burnham and Anderson (2003). The model-averaged coefficient was then extracted for each

variable present in at least one candidate model, defined as those with $\Delta AIC \leq 7$ (Burnham *et al.*, 2011). Nested models were removed (Richards *et al.*, 2011) using the nested function in 'MuMIn' (Barton, 2013) to reduce selection of overly complex models (Richards *et al.*, 2011). Frequency of each variable in the candidate models was used to determine the importance score, with a score of 1.0 signifying that a variable was present in all candidate models. As well as presenting the most important individual explanatory variable and the model with the lowest AICc score, the individual explanatory variable with the lowest AICc score is also given. R^2 and p values from linear regression models of the most important and lowest AICc scored individual explanatory variables are also presented. This ensured that the explanatory variable with the lowest AICc score was not only the most parsimonious but that any regression was statistically significant.

Local size-density relationship (LSDR) was considered as the relationship between body size of a species and its population density, where all population densities are taken from a single region (White *et al.*, 2007). To do this, the 90th percentile size of each *Patella* and *Steromphala* species was calculated, thereby minimising distortion that would occur if the maximum size were used (Kendall & Lewis, 1986). The same model selection procedure was repeated as described above using the 90th percentile size as the response variable. This procedure was also repeated with % abundance of *P. depressa* and *P. vulgata* juveniles (<15mm shell length). Abundance-body size relationships were further investigated by linear regression analyses between mean size and abundance and log-transformed abundance using both individual quadrat and aggregated locality data for each species.

Linearity, homoscedasticity and normality of data were examined prior to testing for a significant relationship between the different algal cover variables and abundance of gastropod species. To diagnose for linearity and homoscedasticity, residuals were plotted against fitted values. Where the residual plot visibly deviated from a residual values of 0 in a systematic manner, the data violated linearity and homoscedasticity. Normal quantile plots were used to diagnose for normality. Where in the normal quantile plot sampled residuals deviated systematically from a linear relationship with theoretical quantiles, the residuals were skewed or 'heavy tailed'. In either of these cases, data were log-transformed and re-diagnosed. If p values were higher following the transformation, the log-transformed statistics were not included.

To compare the size structure of the different populations, size-frequency histograms were produced for each species at each locality only using data from August 2015 and October 2016. This ensured any comparisons particularly regarding smaller size classes were fair and not due to seasonal variation. Where localities were sampled on both occasions, abundance for size categories was divided by two for data visualisation. Size class intervals of 2 mm were used for *Steromphala* species and 5 mm for *Patella* species. Cumulative frequency size plots were produced for limpets, using groups of approximately equal numbers of localities based on SSTM, namely <15˚C, 15-16˚C and >16˚C (Figure 4.1). This enabled comparisons of the relative proportions of recruits and larger individuals at different sea surface temperatures, which is predicted to be the most important variable.

4.3 Results

4.3.1 Linear model statistics

The outcomes of model selection and linear regression analyses for juveniles and 90th percentile size for *S. umbilicalis*, *S. pennanti*, *Patella vulgata* and *Patella depressa* are summarised in table 4.2 and for abundance of all species and fucoids in table 4.3. Individual genera are then considered in turn.

Table 4.2: Results from model selection and linear regression: Species tested; abundance data analysed, final model with the lowest AICc with value in brackets, highest importance variable, individual variable with the lowest AICc value in brackets, R^2 and p values for individual variable with the lowest AICc value. J = % juveniles; 90 = 90th percentile of size frequency distribution. ATM = mean air temperature; ATS = summer air temperature; ATW = winter air temperature; SSTM = mean sea surface temperature; SSTS = summer sea surface temperature; SSTW = winter sea surface temperature; R_1 = % rock in 1km radius; R_5 = % rock in 5km radius.

Species	Data	Final model	Highest importance	Lowest individual AICc	R^2	p values
P. vulgata	Juvenile	+ATM-R_5-SSTW (173.7)	SSTW (0.54)	-R_5 (178.6)	0.1787	0.0805
P. depressa	Juvenile	-R_1 (180.1)	R_1 (0.47)	-R_1 (180.1)	0.1829	0.05998
P. vulgata	90th	-ATS (108.5)	ATM (0.75)	-ATS (108.5)	0.527	**0.0006474**
P. depressa	90th	-ATW+R_1 (100.2)	ATW (0.8)	-ATW (101.5)	0.4444	**0.001328**
S. umbilicalis	90th	+R_5 (71.3)	R_5 (0.8)	+R_5 (71.3)	0.2439	**0.02687**
S. pennanti	90th	-SSTM+R_5 (42.5)	SSTM (0.76)	-SSTM (43.6)	0.5846	**0.003777**

Table 4.3: Results from model selection and linear regression: species tested, abundance data analysed, final model with lowest AICc with value in brackets, highest importance variable with (score), individual variable with the lowest AICc value in brackets (with negative (-) or positive (+) relationships), R^2 and p values for linear regression analysis for the individual variable with the lowest AICc value. ATS = summer air temperature; ATM = mean air temperature; ATW = winter air temperature; EXP = exposure; GP = Gap proximity; SSTS = summer sea surface temperature; SSTM = mean sea surface temperature; SSTW = winter sea surface temperature; R_1 = % rock in 1 km radius; R_5 = % rock in 5 km radius. Significant p values in **bold.**

Species	Data	Final model	Highest importance	Lowest individual AICc	R^2	p values
Patella vulgata	All	-SSTM (187)	SSTM (0.55)	-SSTM (187.0)	0.5471	**0.000194**
	Low	+EXP-SSTM (169.1)	SSTM (0.78)	-SSTM (170.0)	0.6074	**0.00005115**
	High	+ATS-SSTM (211.6)	SSTM (0.78)	-SSTM (211.9)	0.3514	**0.00588**
Patella depressa	All	-GP-ATM+ATW-R_1 (210.2)	GP (0.9)	+SSTS (216.8)	0.3519	**0.00583**
	Low	-ATS+SSTM (166.6)	SSTM (0.61)	+SSTM (170.9)	0.3287	**0.00822**
	High	-ATM+ATW-GP-R_1 (233.7)	GP (1.00)	+SSTS (247.2)	0.2905	**0.01419**
Steromphala umbilicalis	All	+GP-R_5 (199.8)	GP (0.56)	+GP (201)	0.1867	**0.05707**
	Low	-	-	-	-	**-**
	High	+EXP+GP (209.0)	GP (0.57)	+EXP (210.3)	0.2181	**0.03788**
	Time	+SSTW (139.1)	SSTW (0.52)	+SSTW (139.1)	0.1378	0.1071
Steromphala pennanti	All	-R_5-SSTS (129.5)	R_5 (0.69)	-SSTS (130.5)	0.4343	**0.01975**
	Low	-R_5-SSTS (156.9)	R_5 (0.69)	-SSTS (157.9)	0.4291	**0.02077**
	High	-	-	-	-	**-**
	Time	-SSTS (108.3)	SSTS (0.82)	-SSTS (108.3)	0.5515	**0.005663**
Phorcus lineatus	All	+GP+ATM-ATW-R_5 (171.9)	R5 (0.74)	+EXP (174.1)	0.2306	**0.03213**
	Low	-ATW+SSTW (87.4)	ATW (0.72)	-ATW (90.5)	0.1552	0.08565
	High	+GP+ATM-ATW-R_5 (200.3)	R_5 (0.72)	+EXP (202.1)	0.2318	**0.0316**
Fucoid	All	-SSTM (137.1)	SSTM (0.68)	-SSTM (137.1)	0.4169	**0.002106**
	Low	-SSTM (164.3)	SSTM (0.54)	-SSTM (164.3)	0.2764	**0.01728**
	High	-SSTM-ATS (106.4)	SSTM (0.59)	-SSTM (106.8)	0.5054	**0.0004419**
Fucoid5	All	-SSTM (163.8)	SSTM (0.75)	-SSTM (163.8)	0.6456	**0.00001986**
	Low	-SSTM (189.9)	SSTM (0.77)	-SSTM (189.9)	0.5264	**0.0002939**
	High	-SSTM-ATS (140.1)	ATS (0.69)	-SSTM (141.3)	0.6173	**0.00004041**

4.3.2 Patellids

The environmental variable with the highest importance score for abundance of *Patella vulgata* aggregated by locality or divided between higher and lower shore was SSTM (Table 4.3). In the linear regression with SSTM (R^2 = 0.5471, p = 0.000194) and initial diagnoses, locality-aggregated abundance of *Patella vulgata* showed the locality Les Pierrieres immediately north of the habitat gap as an outlier (Figures 4.2 and 4.3B). High abundances were found in both species adjacent to the habitat gap (Figure 4.3); at the northern boundary in *Patella vulgata*, and the southern boundary for *Patella depressa*.

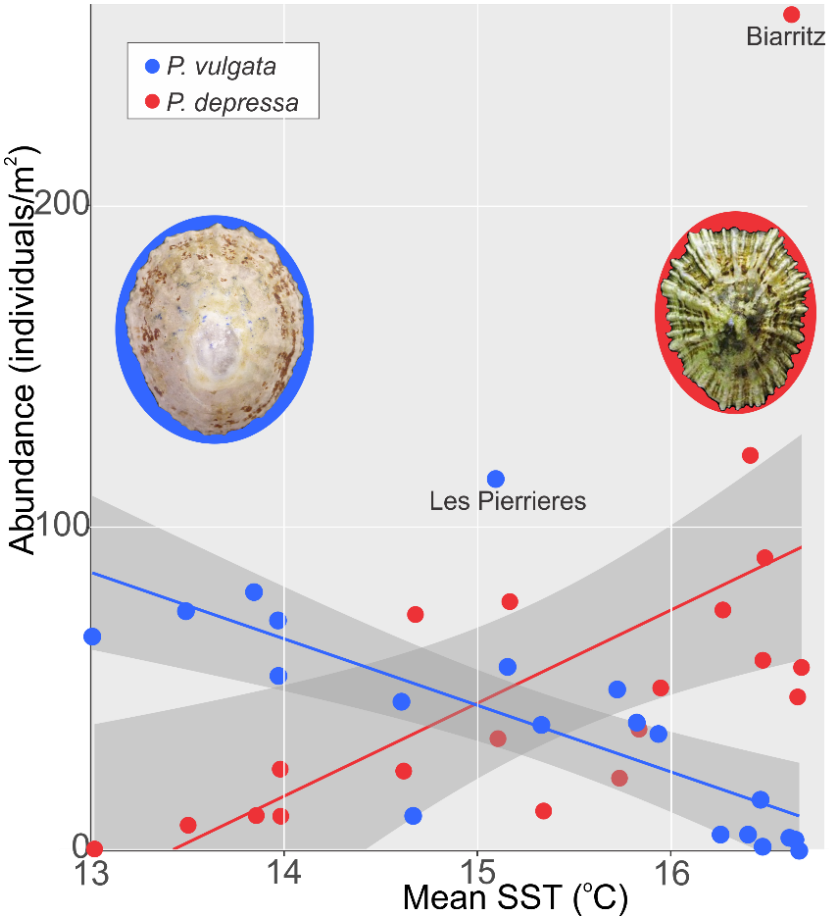


Figure 4.2: Mean annual SST from August 2005-2015 plotted against mean abundance at localities of *Patella vulgata* and *Patella depressa*, with Biarritz and Les Pierrieres outlier localities labelled. Images of *Patella vulgata* (blue background) and *Patella depressa* (red background) are included.

The environmental variable with the lowest AICc was SSTS for *Patella depressa* abundance ($R^2 = 0.3519$, $p = 0.00583$) (Table 4.3), which showed a positive relationship (Figure 4.3A). In addition, Biarritz had a noticeably high abundance of *Patella depressa*, particularly of smaller size classes (Figure 4.3A and 4.4). *Patella depressa* had the highest proportion of recruits at the localities >16˚C SSTM, with populations made up of larger individuals at localities of lower SSTM (Figures 4.3A and 4.4). This trend was revealed by the cumulative frequency plots (Figure 4.5A), whereas R_1 had the lowest AICc score with abundance of juvenile *Patella depressa* ($R^2 = 0.1829$, $p = 0.05998$). This greater proportion of recruits then drives an increase in locality abundance (Figures 4.4).

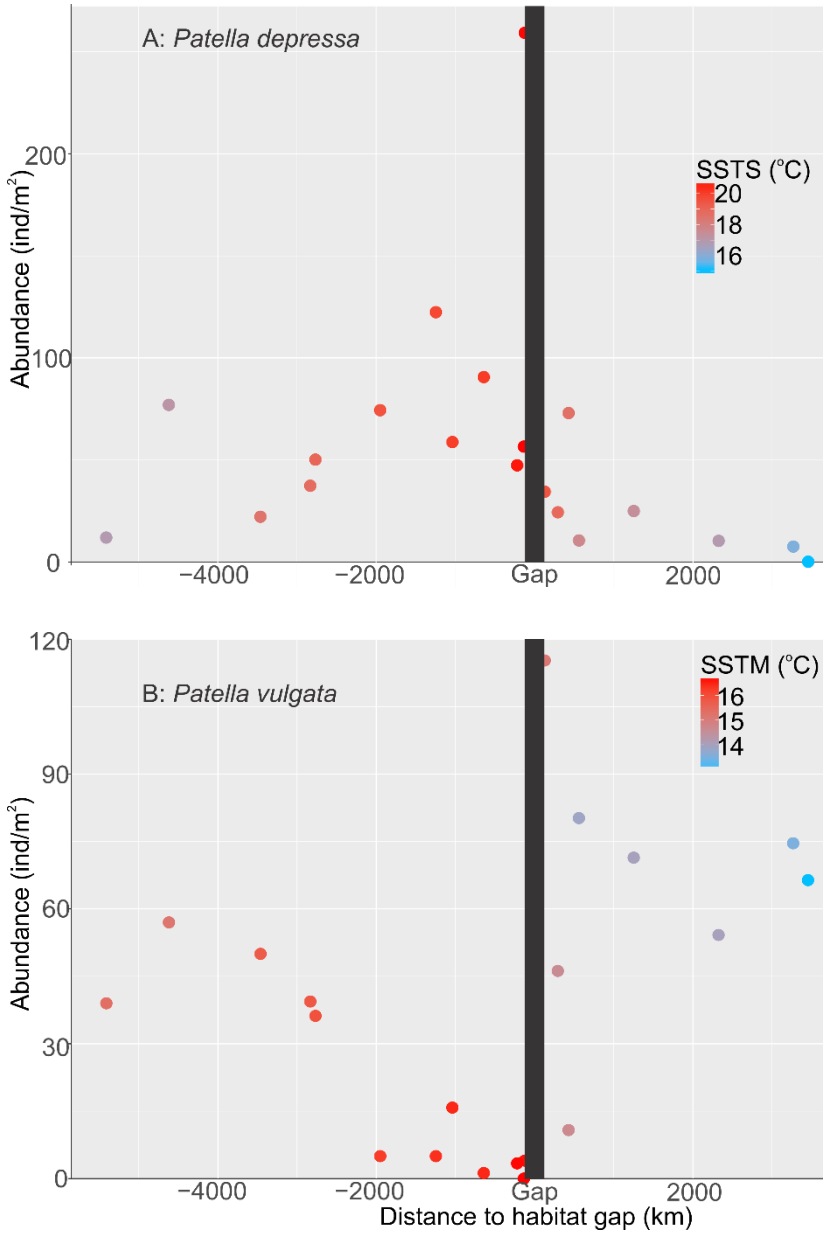


Figure 4.3: Mean abundance of *Patella depressa* (A) and *Patella vulgata* (B) at localities with increasing distance along the habitat gap (shown to scale) with colour indicating summer and mean sea surface temperature for *Patella depressa* and *Patella vulgata* respectively. Negative values indicate localities south of the habitat gap. Note high abundance values in both species adjacent to the habitat gap (north side for *Patella vulgata*, south side for *Patella depressa*).

Temperature had a negative relationship with size for both *Patella* species, namely winter air temperatures for *Patella depressa* 90th percentile of size (R^2 = 0.444, p = 0.001328; Figure 4.6A) and summer air temperature for *Patella vulgata* (R^2 = 0.527, p = 0.0006474; Figure 4.6B). This may be linked to Les Pierrieres having a particularly high abundance of smaller *Patella vulgata* individuals (Figures 4.3B and 4.7). The relationship between *Patella vulgata* recruitment and SSTW is negative, with colder localities in northern France having a higher abundance of recruits than warmer localities (Figures 4.3B and 4.7).

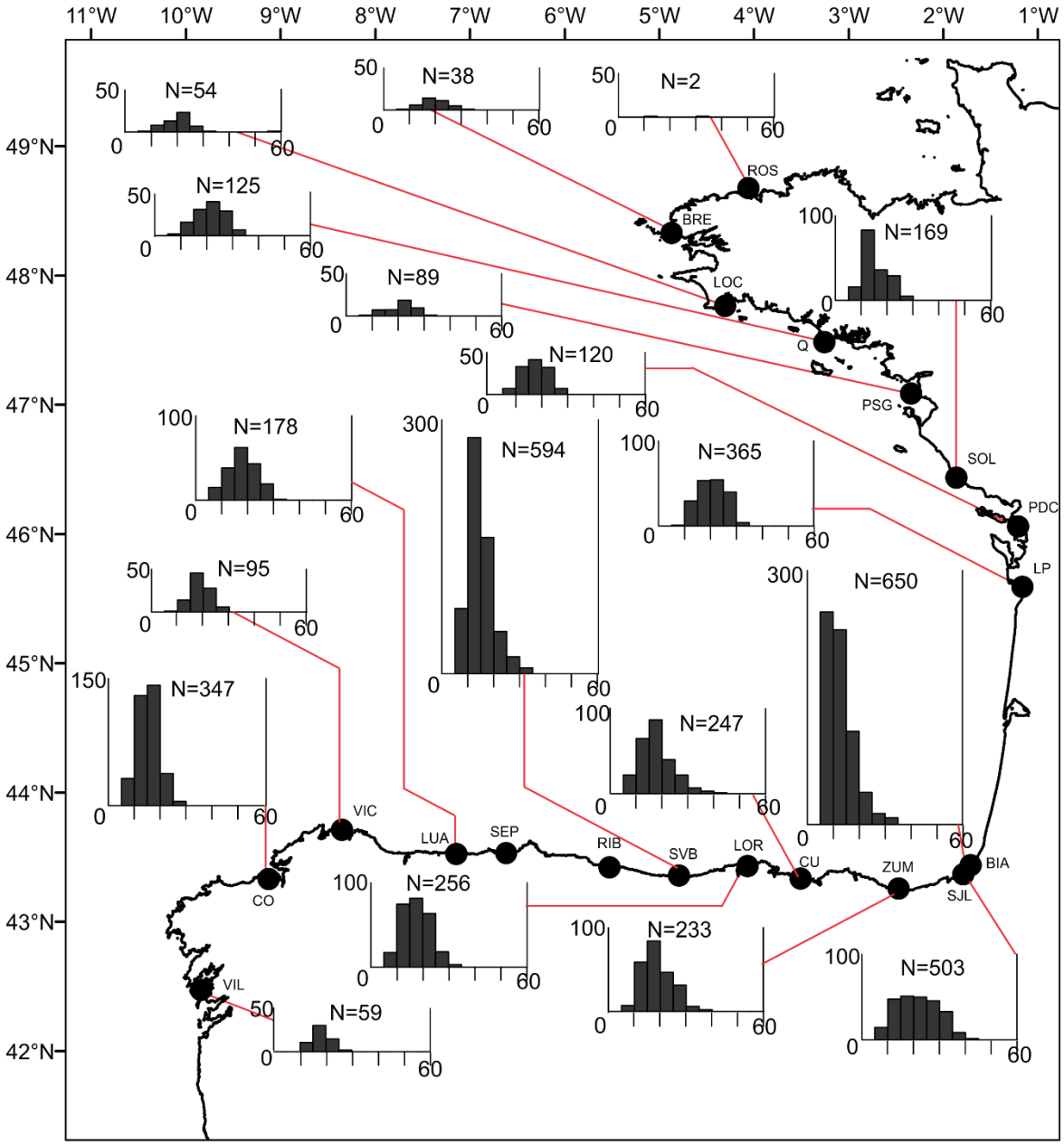


Figure 4.4: Map and locality size-frequency histograms for *Patella depressa* (only representing sampling from August 2015 and November 2016). Shell length (mm) was used as the size variable. Solid circle = species present (including April 2016 sampling). Locality codes correspond with table 4.1.

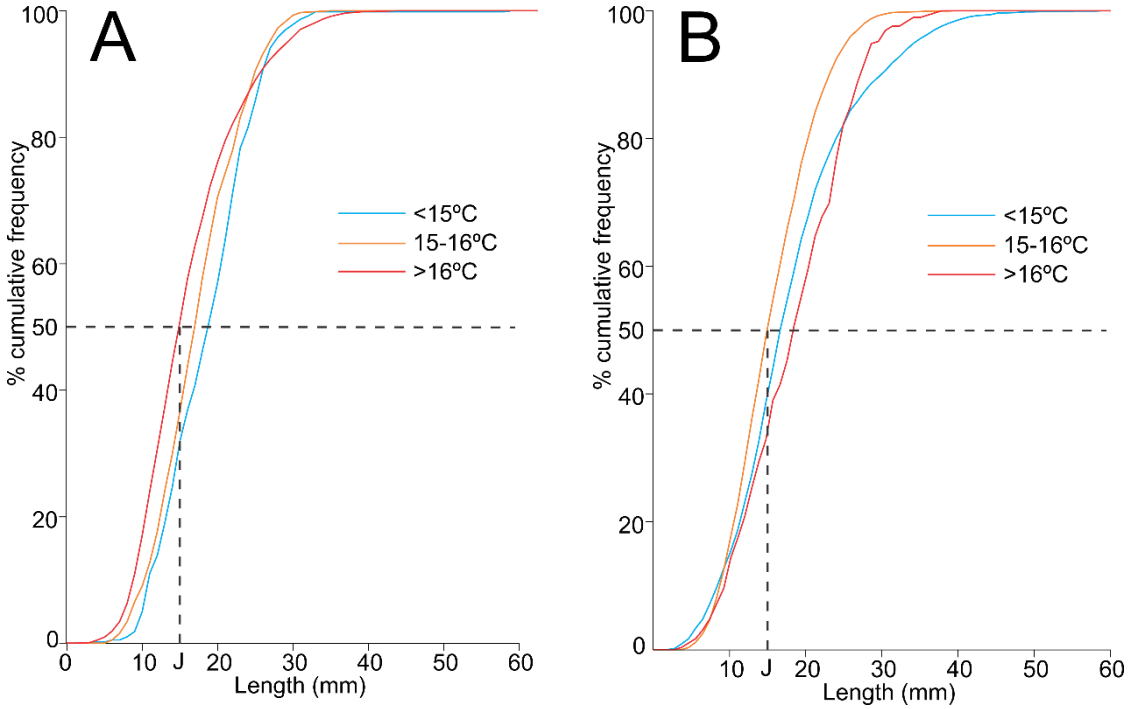


Figure 4.5: Cumulative frequency curves of SSTM grouped *Patella depressa* (A) and *Patella vulgata* (B) using all site data pooled by localities at different temperatures with 50th percentile and juvenile size (15mm) marked.

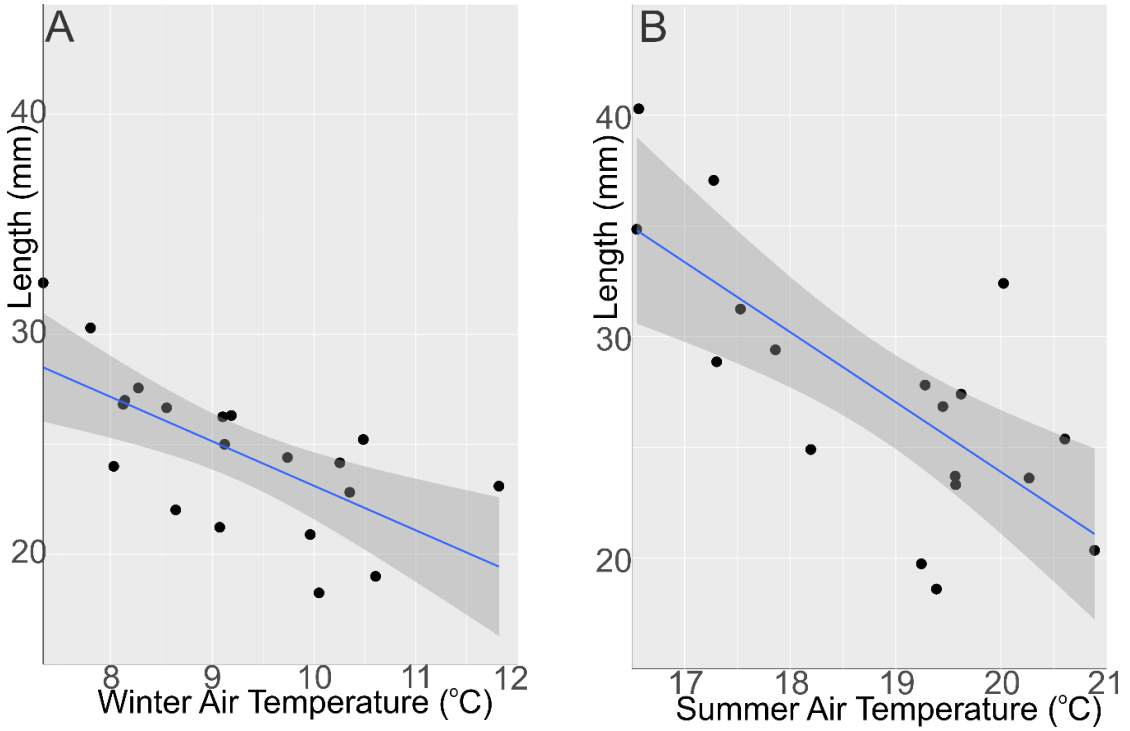


Figure 4.6: *Patella depressa* : 90th percentile shell length plotted against winter air temperature (A) and *P. vulgata* 90th percentile shell length plotted against summer air temperature (B) (both respective final models).

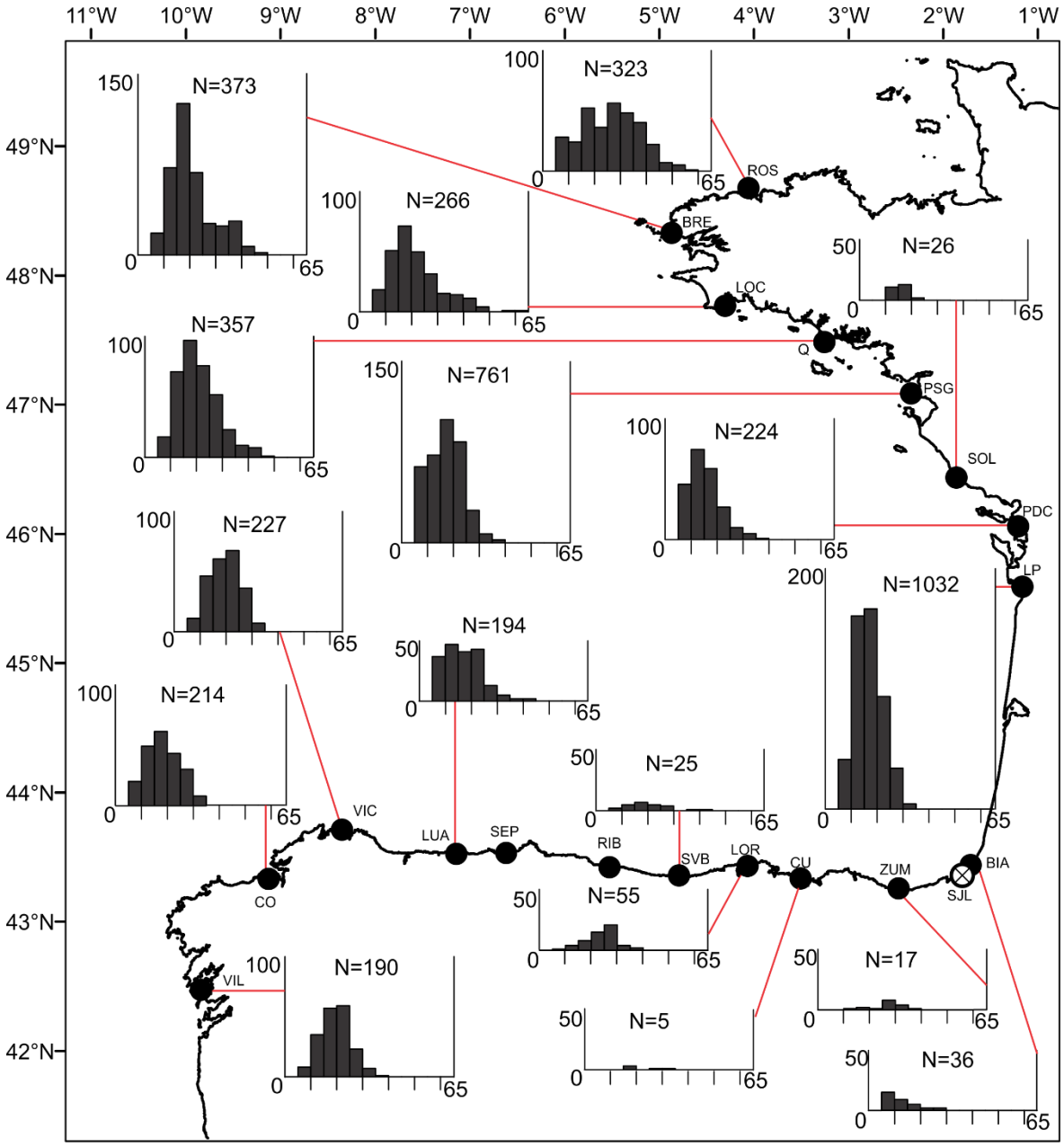


Figure 4.7: Map and locality size-frequency histograms for *Patella vulgata* (only representing sampling from August 2015 and November 2016). Shell length (mm) was used as the size variable. Cross = absence, solid circle = species present (including April 2016 sampling). Locality codes correspond with table 4.1.

For mean size and abundance for individual quadrats, *Patella vulgata* displayed a negative relationship (R^2=0.05733, p=4.708 x 10^{-5}), suggesting that mean size is weakly density-dependent. However, locality mean size and log-transformed abundance (but not non-transformed abundance) showed a significant relationship (R^2=0.3237, p=5.219 x 10^{-3}). *Patella depressa* had stronger significant negative linear relationship between mean size and abundance at the individual quadrat level (R^2=0.1073, p=1.058 x 10^{-8}). When considered between localities, this negative relationship between size and log-transformed abundance is maintained (R^2 = 0.5714, p = 0.0001156; Figure 4.8).

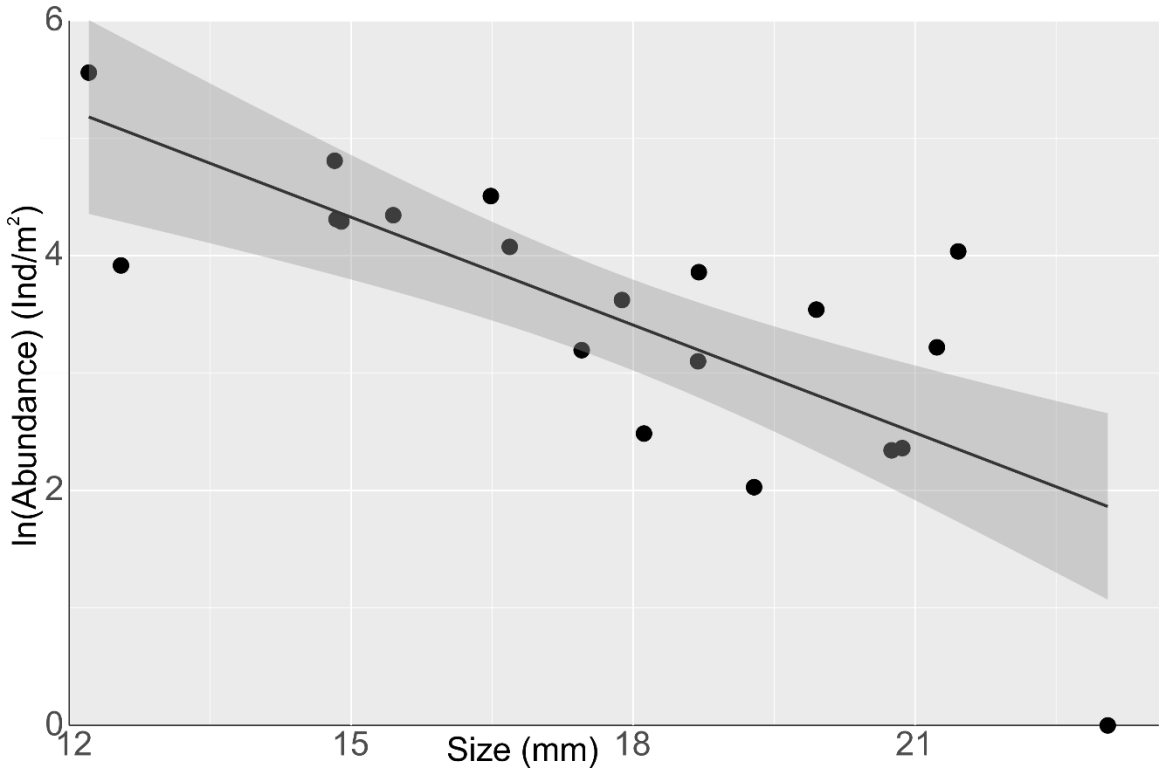


Figure 4.8: Mean size plotted against natural log of mean abundance of *Patella depressa* at localities showing density-dependent relationship.

4.3.3 Trochids

For *S. umbilicalis*, gap proximity (GP) had the highest importance score, and as a single variable had an AICc of 201 compared to the final model lowest AICc of 199.8. This was attributable in part to the low abundances at localities immediately north and south of the habitat gap (Biarritz and Les Pierrieres; Figures 9 and 10), in contrast to *Patella* species and *Phorcus lineatus*, which show a peak in abundance either immediately north or south of the habitat gap. When run as a linear model, the relationship between quadrat-based locality abundance and GP was not quite significant (R^2=0.1867, p=0.05707), nor were any environmental variables when applied to abundance based on timed searches (TS) or low shore mean abundances. The only significant linear relationship between a *S. umbilicalis* abundance measurement and an individual environmental variable was a positive relationship between high shore *S. umbilicalis* abundance and exposure (R^2 = 0.2181, p = 0.03788), with an AICc of 210.3 (final model AICc = 209.0). Using the timed search abundance for *S. umbilicalis*, SSTW had the highest importance score and an AICc = 139.1, although it did not show a significant linear relationship.

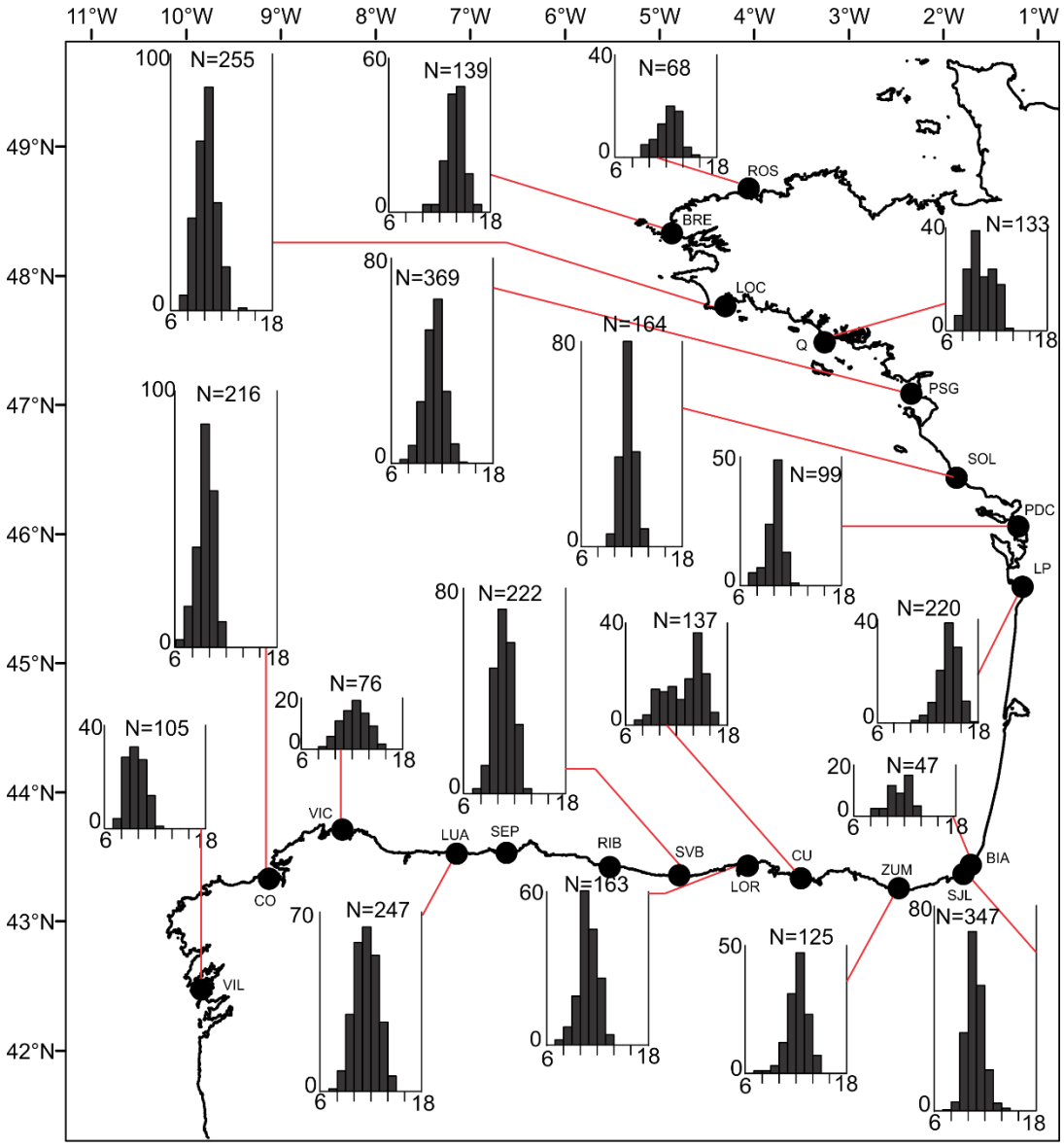


Figure 4.9: Map and locality histograms for *S. umbilicalis* (only representing sampling from August 2015 and November 2016). Shell width (mm) was used as the size variable (derived from timed search data). Cross = absence, solid circle = species present (including April 2016 sampling). Locality codes correspond with table 4.1.

There was a significant negative relationship between mean locality size and aggregated quadrat-based abundance ($R^2 = 0.4993$, $p = 0.000496$) of *S. umbilicalis* (Figure 4.11). This suggests size of *S. umbilicalis* is partly density dependent, with larger individuals found at localities with lower abundance (Figures 4.9 and 4.11). The interaction between the abundance and size around the range centre of this species, and lack of significant environmental variables describing either of these, suggests that recruitment may drive both.

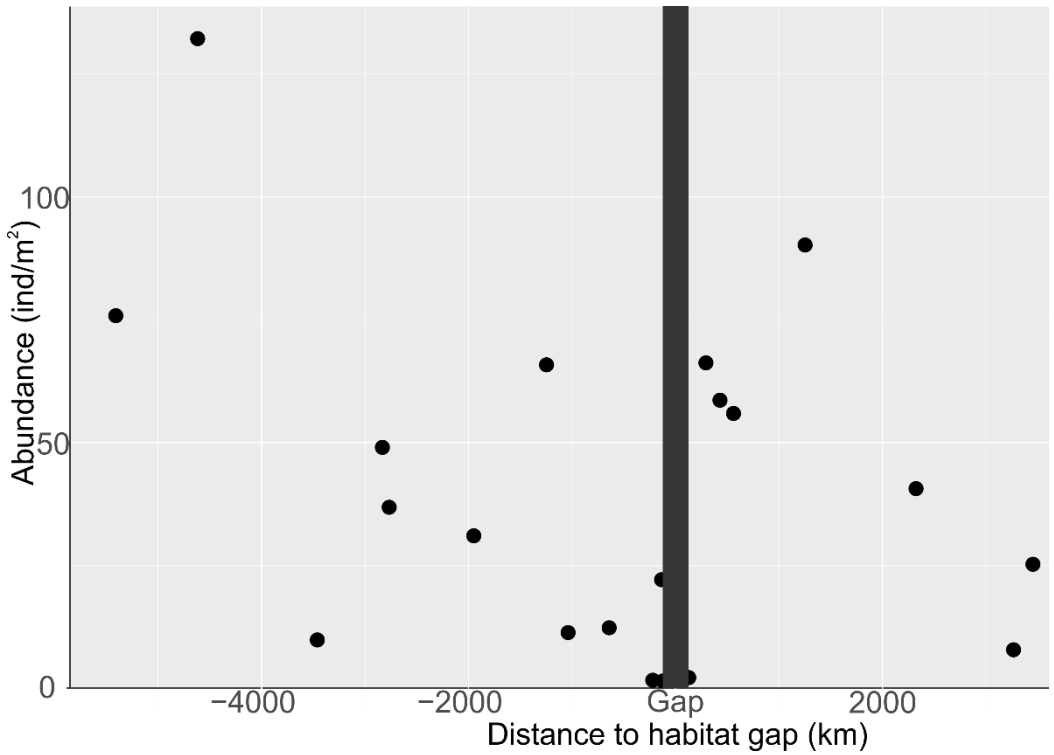


Figure 4.10: Mean abundance of *S. umbilicalis* at localities with increasing distance along the habitat gap (shown to scale). Negative values indicate localities south of the habitat gap.

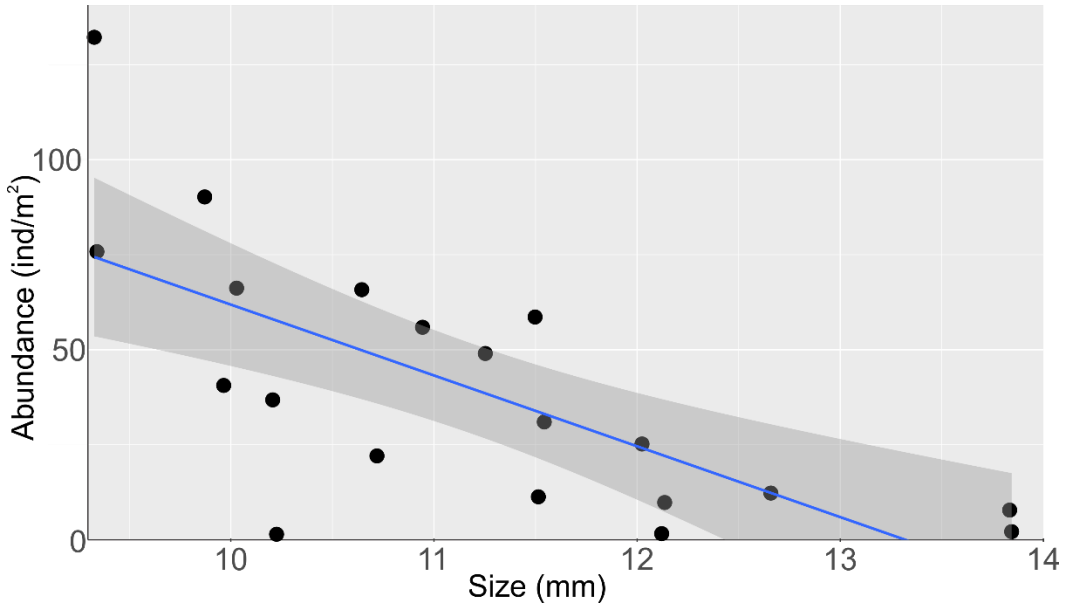


Figure 4.11: Mean size plotted against mean abundance of *S. umbilicalis* at localities showing density-dependent relationship

For *S. pennanti* the individual variable for both quadrat and time search abundance with the lowest AICc was SSTS (130.5 and 108.3 respectively compared to the final model AICc of 129.5), although R_S had the highest importance score (Table 4.3). There was a significant negative linear regression of *S. pennanti* abundance measured in quadrats (Figure 4.13) and by timed searches with SSTS ($R^2 = 0.4343$, $p = 0.01975$ and $R^2 = 0.5515$, $p = 0.005663$ respectively).

Steromphala pennanti showed a significant positive relationship between size and abundance based on timed search data ($R^2 = 0.3573$, $p = 0.003191$), counter to what would be predicted by a density-dependent scenario. This may be attributable to *S. pennanti* being associated with macroalgae; larger individuals are easier to find when searching amongst macroalgae than smaller ones. This means that a locality with larger individuals may appear to have a greater abundance than a locality with the same number of individuals that are smaller.

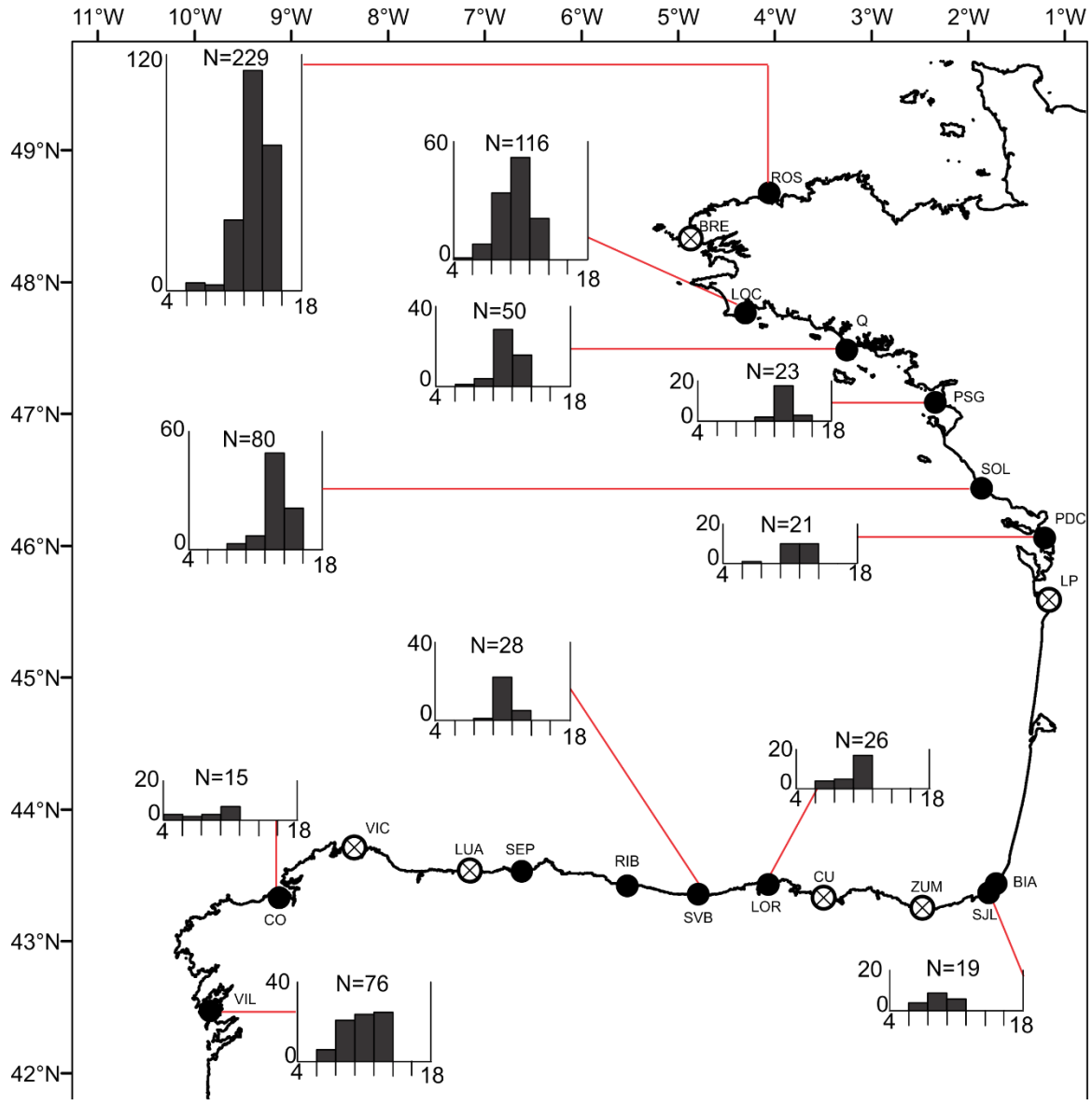


Figure 4.12: Map and locality histograms for *S. pennanti* (only representing sampling from August 2015 and November 2016). Shell width (mm) was used as the size variable (derived from timed search data). Cross = absence, solid circle = species present (including April 2016 sampling). Locality codes correspond with table 4.1.

Steromphala pennanti had a much patchier distribution than *S. umbilicalis* (Figures 4.9 and 4.12) and was completely absent from multiple localities (n = 7) (Figures 4.12 and 4.13). It is rarer around the habitat gap, but as discussed above, that appears to be more attributable to sea temperature being higher at

adjacent localities than the habitat gap itself. The skew towards smaller individuals at these localities, even accounting for any sampling bias, suggests that low or infrequent recruitment may not be the main reason for low abundances.

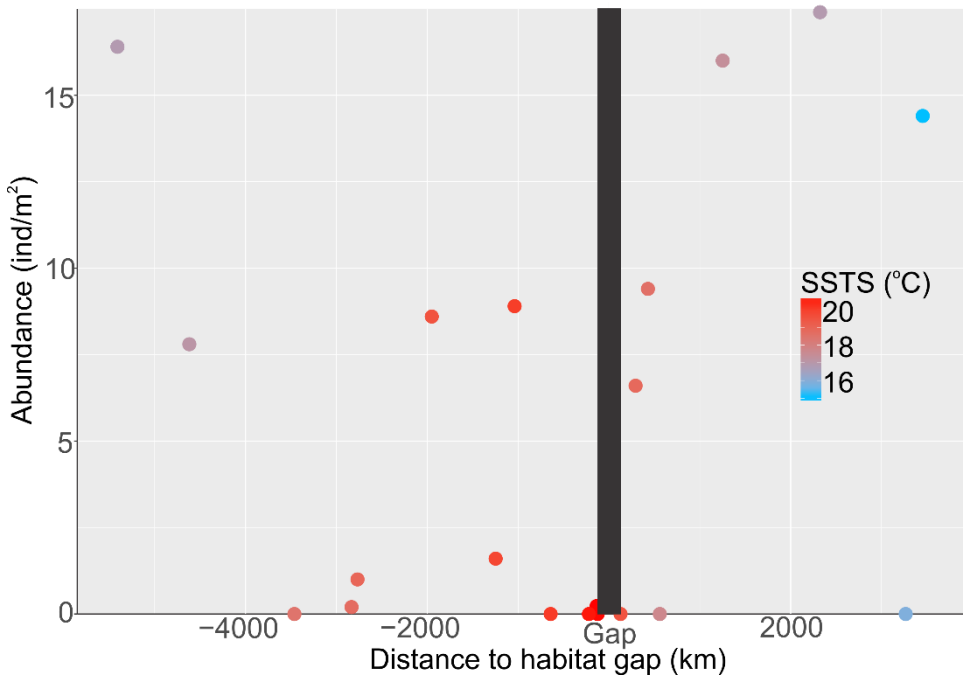


Figure 4.13: Mean abundance of *S. pennanti* at localities with increasing distance along the habitat gap (shown to scale) and colour indicating SSTS. Negative values indicate localities south of the habitat gap.

For *Phorcus lineatus*, exposure is the single variable with the lowest individual AICc of 174.1, producing a significant linear relationship ($R^2 = 0.2306$, $p = 0.03213$). Abundance is greatest at localities immediately north of the habitat gap, with the exception of Les Pierrieres (Figure 4.14). This follows the same abrupt drop in abundance south of the habitat gap as *P. vulgata*.

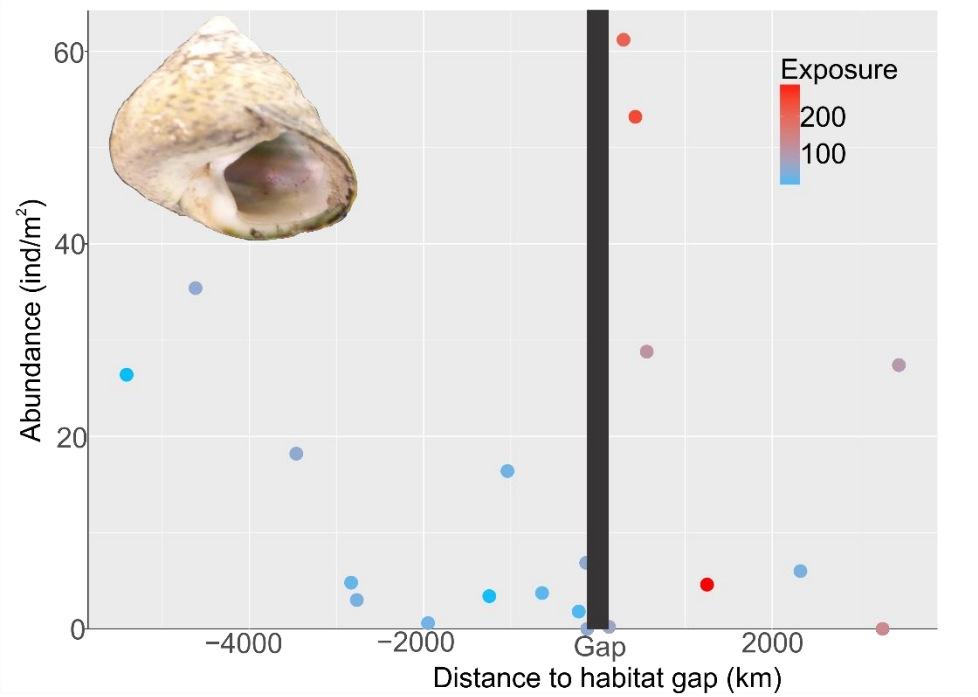


Figure 4.14: Mean abundance of *Phorcus lineatus* at localities with increasing distance along the habitat gap (shown to scale) and colour indicating wave exposure. Negative values indicate localities south of the habitat gap. An image of *Phorcus lineatus* shell is included.

4.3.4 Fucoids

For fucoid cover mean sea surface temperature (SSTM) had the lowest AICc value using any of the fucoid cover variables, with a significant negative linear regression for % fucoid cover ($R^2 = 0.4169$, $p = 0.02106$) (Table 4.3). Any significant regressions with *Patella depressa* and fucoid or macroalgal cover are negative at the individual quadrat level suggesting that *Patella depressa* is less abundant in fucoid covered habitat. Whereas the only positive regression with fucoid cover for *Patella vulgata* was on lower shore quadrats using locality averages (for Fucoid5, Table 4.4). This is possibly indicative of co-linearity between SST and fucoid cover at sites. Indeed, there was also a significant negative regression of *Patella vulgata* with macroalgae by individual quadrats, which may be due to grazing pressure of *Patella vulgata* on macroalgae. There were positive regressions with *S. pennanti* and fucoids with or without *B. bifurcata* and macroalgal cover when individual quadrat data was used, albeit with low R^2 values (Table 4.4).

The relationship between *S. umbilicalis* and macroalgae varies, with timed search abundance estimate of *S. umbilicalis* showing a significantly negative relationship with quadrat-based fucoid cover (Table 4.4). This relationship contrasts with the quadrat results which show weak positive relationships, if any,

between *S. umbilicalis* abundance and fucoid cover either with aggregated or individual quadrats. One possible explanation is that greater macroalgal cover at a locality makes it more difficult to find *S. umbilicalis* within a given time constraint as they are hidden by the canopy, unlike when completely searching in a given quadrat.

Table 4.4: R^2 and p-values for linear regression of species abundance in relation to fucoid cover. Fucoid = % cover *Fucus* and *Ascophyllum* per quadrat (individual quadrats); Fucoid>5 = % of quadrats per site with greater than 5% *Fucus* and *Ascophyllum* (aggregated quadrats); FucoidBB = % cover *Fucus*, *Ascophyllum* and *B. bifurcata* per quadrat; Macroalgae = % cover all macroalgae per quadrat. Significant regression with negative **(-)** or positive **(+)** relationships, logarithmic abundance used (l). Timed search results aggregated by locality.

Species	Quadrats	Fucoid		Fucoid>5		Fucoid BB		Macroalgae	
		R^2	P-value	R^2	P-value	R^2	P-value	R^2	P-value
Patella depressa	All	0.02134	**(-)** 0.0008335	0.2529	**(-)** 0.02383	0.02953	**(-)** 0.0026 (l)	0.07812	**(-)** 8.702e-11
	High	0.01459	0.05126	0.2028	**(-)** 0.04634	0.01537	**(-)** 0.0454	0.0008119	0.6468
	Low	0.02217	**(-)** 0.0165	0.1505	0.09097	0.01877	**(-)** 0.0275	0.04307	**(-)** 0.0007786
Patella vulgata	All	0.002785	0.2296	0.4105	**(+)** 0.002337	0.005305	0.0971	0.1432	**(-)** < 2.2e-16
	High	1.772e-5	0.946	0.2156	**(+)** 0.03915	6.989e-5	0.8931	0.01416	0.0549
	Low	0.001973	0.4766	0.6142	**(+)** 4.348e-5	0.001264	0.569	0.1652	**(-)** 1.01e-11
Steromphala umbilicalis	All	0.0004735	0.6205	0.0006537	0.9148	0.0004098	0.6451	0.001476	0.382
	High	0.02636	**(+)** 0.008591	0.003997	0.7912	0.02002	**(+)** 0.0222	0.04338	**(+)** 0.000709
	Low	0.01087	0.0941	0.01211	0.6442	0.009125	0.125	0.04369	**(-)** 0.000712
	Time	0.3141	**(-)** 0.01016	0.2451	**(-)** 0.02645	0.3489	**(-)** 0.006106	0.01101	0.6598
Steromphala pennanti	All	0.01119	**(+)** 0.01579	0.09729	0.1806	0.01607	**(+)** 0.003783	0.05059	**(+)** 2.18e-07
	High	0.0122	0.07482	0.04654	0.361	0.01469	0.0505	0.01614	**(+)** 0.0403
	Low	0.004125	0.3031	0.09545	0.185	0.005704	0.226	0.009918	0.1098
	Time	0.08757	0.2052	0.1839	0.05923	0.1289	0.1201	0.004778	0.7722
Phorcus lineatus	All	0.006376	0.06886	0.0001013	0.9664	0.009168	**(-)** 0.02902	0.07134	**(-)** 6.059e-10
	High	0.000148	0.8449	1.037e-6	0.9966	2.298e-5	0.9386	0.000595	0.6949
	Low	0.003993	0.3111	0.01883	0.564	0.00494	0.2597	0.08677	**(-)** 1.399e-6

4.4 Discussion

4.4.1 Main findings

Species often exhibit highly variable abundances within the central portions of their geographic range (Lima *et al.*, 2007b; Sagarin & Gaines, 2002). The purpose of this study was: (i) to measure the abundance and size-structure of sympatrically distributed rocky intertidal gastropods within their range centres; (ii) to determine which of the highly heterogeneous environmental variables best predict patterns in these biological response variables; (iii) to examine whether the most useful predictive variables vary by shore height; and (iv) to seek evidence for density-dependent interactions constraining size. The principal observations are that abundance and size-structure of patellid and trochid gastropods are highly variable within their range centres, but that spatial variation in temperature is often the best predictor of these patterns. Temperature is often viewed as an ultimate environmental factor driving rocky intertidal species ecology both at the macroscale as well as when considering microscale processes (Hawkins *et al.*, 2009; Helmuth *et al.*, 2006; Hutchins, 1947; Lima *et al.*, 2006; Seabra *et al.*, 2011; Wethey *et al.*, 2011). Given the association made between temperature and the northern ranges limits of the some of the species studied here (Hawkins *et al.*, 2009; Mieszkowska *et al.*, 2013), the present study postulated that temperature would also be important for predicting how abundance is distributed within their range centres. The results presented above support this view, and it appears to be in part mediated by recruitment and subsequent density-dependent processes (Moore *et al.*, 2007b; Ribeiro *et al.*, 2009). It may be inferred that as the climate and sea temperatures changes, so too will abundances of rocky intertidal species throughout their ranges (Broitman *et al.*, 2008; Hawkins *et al.*, 2009; Helmuth *et al.*, 2006; Lima *et al.*, 2007b; Wethey & Woodin, 2008). The ultimate and proximate factors associated with size and abundance of intertidal species are examined below.

4.4.2 Temperature as an ultimate and proximate factor

Temperature had the greatest role in the abundance of both *Patella* species (Figures 4.2 and 4.3) and *S. pennanti* (Figures 4.12 and 4.13), as well as the size-structures of *Patella*, which are highly variable within the focal region (Figures 4.4-7). These patterns have different signs in the two *Patella* species, which can mostly be explained by their different thermal affinities. As *Patella depressa* is a

warmer water species (Hawkins *et al.*, 2009; Kendall *et al.*, 2004), its abundance increases with sea temperature (Figures 4.2 and 4.3A). The peak in abundance of *Patella depressa* in the warmest region (south of the habitat gap) is because of higher recruitment, evidenced by the higher proportion of juveniles at these greater temperatures (Figures 4.4 and 4.5). However, it should be noted that the northernmost locality Roscoff had a particularly low abundance of *Patella depressa* which may be due to Roscoff being a highly sheltered locality, as well as having the lowest SSTM. *Patella vulgata*, on the other hand, is a cooler water species that has higher abundances and a greater proportion of juveniles at localities with lower temperatures, most of which occur north of the habitat gap (Figure 4.3B and 4.5B). In fact, *Patella vulgata* is extremely rare at localities where mean sea surface temperature (SST) is >16°C, where *Patella depressa* adults and juveniles are most common. This observation supports the finding that sea temperature is the best predictor of the abundances within the range centres of both species, but in opposing directions (Figure 4.2).

The negative response of *S. pennanti* abundance to increasing sea temperature and its occasional absence suggests that it may experience the upper limit of its thermal tolerance in the focal region (Figures 4.12 and 4.13). However, as was predicted, *Fucus* and *Ascophyllum* act as engineer species and hence show positive correlation with *S. pennanti* (Bordeyne et al., 2017). The results agreed with pre-existing literature in identifying sea temperature as the driver of fucoid abundance and distribution. It is a possibility that *S. pennanti* abundance is limited by a combination of sea temperature at the macroscale and fucoid habitat availability at the more local scale (see below). Indeed, *S. pennanti* is abundant at localities west of the Cherbourg peninsula in northern France, including the Channel Islands, with an abrupt north-eastern range limit associated with the English Channel and the frontier between the two Channel tidal gyres (Crisp & Southward, 1958). This suggests that its northern limit is likely a function of abrupt dispersal limitation, possibly related to less rocky habitat being present on the eastern side of the Cherbourg Peninsula and tidal currents unfavourable to northwards dispersions across the Channel (Pingree & Maddock, 1977). Given the high abundances of *S. pennanti* at its northern limit, if more suitable habitat become available (e.g. via added artificial substrate), currents become more favourable for the northwards transport of larvae, the northern range of *S. pennanti* may be predicted to expand in the future. A similar scenario has been suggested to explain northern range expansions of rocky shore gastropods along the coast of southern California (Fenberg *et al.*, 2014).

Intraspecific differences in thermal stress responses are commonly associated with the shore height of individuals, which corresponds to the period of emersion in air and immersion in water experienced (Tomanek & Somero, 2000). The results presented here indicate that regardless of shore height, SST is a better proxy variable for *Patella* abundances than air temperature. However, air temperatures performed better than sea temperature when predicting variation in 90th size percentile of both *Patella* species (Figure 4.6) (Atkinson, 1994; Horne *et al.*, 2015; Wilson-Brodie *et al.*, 2017). There are fewer large *Patella vulgata* individuals at localities where summer air temperatures are high, in agreement with *Patella vulgata* thermal affinities (Figure 4.6B). High mortality rates in other limpet species with northerly distributions [*Lottia scabra* (Gould, 1846)] have been observed to coincide with high summer air temperatures at low tide (Harley, 2008), which may also occur in *Patella vulgata*. Furthermore, larger limpets are found on open areas of flat rock (Bowman & Lewis, 1977; Hartnoll & Hawkins, 1985; Jones, 1948; Kido & Murray, 2003; Rivera-Ingraham *et al.*, 2011) which would be more exposed to solar radiation. This behaviour potentially increases the probability of summer temperature-induced mortality relative to smaller individuals in patchier, more shaded habitat (Harley, 2008; Lima *et al.*, 2016).

In *Patella depressa* (warm-water species), it is winter air temperature, rather than summer air temperature, that shows the best predictive negative relationship with the presence of the largest size-classes. Warmer winter air temperatures in *Patella depressa* may allow for more successful reproduction and recruitment, as their spawning principally occurs in multiple events from September to January in Portugal, (Ribeiro *et al.*, 2009) and in recent years has shown extended duration (March to October) towards the north of its range (Moore *et al.*, 2011). Subsequent intraspecific competition due to a high number of new recruits may then reduce individual sizes (Figure 4.8). The additional energy expenditure of extended spawning duration at higher temperatures may also restrict growth in mature individuals of *Patella depressa* with high reproductive outputs (Blackmore, 1969; Workman, 1983). In contrast, spawning failure years have been shown to occur in *Patella vulgata*, attributed to warmer summers leading to later and less successful spawning in this single brooding species (Moore *et al.*, 2011).

The larger size class of *Steromphala pennanti* also shows a negative relationship between size and temperature, albeit with mean SST as the best proxy. Whereas the two *Patella* species occur at all shore heights, *S. pennanti* exists almost exclusively on the lower shore. Since *S. pennanti* individuals

experience a greater proportion of tidal cycles immersed in sea than most *Patella vulgata* and *Patella depressa*, they may be less influenced by air temperatures. Thus the presence of larger size classes may be determined by general metabolism represented by mean SST rather than the thermal stresses encountered by both *Patella* species (Somero, 2002; Tomanek & Somero, 2000).

The low proportion of *Patella vulgata* recruits and the high abundance of *Patella depressa* recruits at warmer localities supports the hypothesis that sea temperature can be used to predict spatial patterns of recruitment, which in turn drives size-structure and abundance (Moore *et al.*, 2007a; Ribeiro *et al.*, 2009). These results also suggest that adult activity during immersion such as grazing (Branch *et al.*, 1988; Little, 1989), rather than thermally induced mortality during emersion, is a better indicator of long-term species abundance in intertidal gastropods. One major caveat to using mean sea surface temperature as a proxy variable for abundances is that the abundance response to specific extreme temperature events was not examined. As the sampling was not immediately before or after any extreme events (either in temperature or storms), it will not have highlighted their impact on intertidal species, which has found to be considerable in terms of both size-structure and abundance (Harley, 2008; Muñoz-Colmenero *et al.*, 2015; Wethey *et al.*, 2011).

A possible interpretation of the results for *Phorcus lineatus* and *S. umbilicalis* is that in the focal region of the study, their environment is at around their optimal temperature range, and hence their fundamental niche space is weakly defined by other environmental variables (Hutchinson, 1957). If in the focal region these southern species experienced the lower sea temperature that marks the northern limit of their ranges, one might expect a positive relationship with SST (Mieszkowska *et al.*, 2006), as was found with *Patella depressa*. Conversely, it is unknown whether the southern range limits of *Phorcus lineatus* and *S. umbilicalis* are associated with greater sea temperature. Within the central portion of their range in the focal region, they are therefore likely to be within their thermal tolerance. This would result in little relationship with temperature proxy variables and potentially allow individuals to respond behaviourally or physically to other unfavourable variables such as exposure (Walter & Walter, 1953). It follows that in the focal area, *S. umbilicalis* behaved as a habitat generalist.

4.4.3 Habitat gap

The distribution of rocky habitat is unrelated to temperature, but it has the potential to influence patterns of abundance and size structure of populations (Fenberg & Rivadeneira, 2011). For example, the highest overall abundances and greatest proportion of juveniles of *Patella* species occurs just north (high *Patella depressa* abundances at Biarritz) and south (high *Patella vulgata* abundances at Les Pierrieres) of the habitat gap (Figures 4.3, 4.4 and 4.7). There is a similar contrast with high abundance for *Phorcus lineatus* at localities north of the habitat gap (excepting Les Pierrieres for reasons that will be discussed later) and low abundance to the south. The difference in abundance between localities immediately north and south of the habitat gap may be due to its geographic coincidence with other environmental variables, namely temperature for *Patella* (Figures 4.3, 4.4 and 4.7) and exposure for *Phorcus lineatus* (Figure 4.14). This is because SST is much warmer immediately south of the habitat gap than north of the gap (Figure 4.1) and wave exposure is substantially lower (Figure 4.14). However, the habitat gap may accentuate these differences by reducing recruitment between localities north and south. Thus, the geographic coincidence between temperature or exposure change and the habitat gap may have a compounding effect on species abundances in this region.

In the case of Les Pierrieres, the nearest localities are found to the north where *Patella vulgata* is more abundant than *Patella depressa* due to lower temperatures. It follows that recruitment from these localities will be dominated by *Patella vulgata*, resulting in their high abundance at Les Pierrieres. By contrast for Biarritz immediately south of the habitat gap, recruitment will be principally from adjacent warm water localities dominated by *Patella depressa*. A similar but more extreme pattern has been observed for *Patella rustica*. The northern range limit of this warm-water species coincides with the habitat gap, which is believed to prevent recruitment further north in combination with the lower sea temperatures (Fischer-Piette, 1955; Lima *et al.*, 2007b). The dominant flow across the habitat gap is a southward longshore current, sometime reaching 1 m/s (Castelle *et al.*, 2006), which may also hinder southern species from successfully recruiting north of the habitat gap.

4.4.4 Local factors

Whilst temperature and the habitat gap may account for the majority of patterns in recruitment and subsequent abundance and size structure, there are other

potential local drivers of variation in abundance both within and between localities. These local factors include, but are not limited to: proximity to estuaries (Evans, 1957) and local patterns in habitat forming macroalgae (Cefalì *et al.*, 2016; Moore *et al.*, 2007b). Les Pierrieres is located at the mouth of the Gironde, which is the largest estuary within the sampled region. Several environmental conditions for this locality are not included, such as high sediment load, decreased salinity and dissolved oxygen content in the estuary water (Lanoux *et al.*, 2013), which may contribute to the low abundance of *Phorcus lineatus* and *S. umbilicalis* amongst other biological response variables. Indeed, the relationship between % rock in 5 km, and localities' larger size class of *S. umbilicalis* suggests that sediment load can have a negative effect (Fischer-Piette & Crisp, 1959). This may be through sediment burial and mortality events in areas with a lower % rock in 5 km removing the older and larger individuals (Airoldi, 2003; Díaz-Tapia *et al.*, 2013).

Other local factors that may influence the abundance and size structure of gastropods, particularly *Patella*, is substrate type, rugosity, and other microhabitat variables. For example, the lower shore of Les Pierrieres is dominated by oyster beds unsuitable for larger limpets or the fucoids that normally occupy that shore zone. This local lack of fucoid habitat is one probable cause of the absence of *S. pennanti* at this locality, whilst the cold-water species *Patella vulgata* can settle on both upper and lower shore, although low shore *Patella vulgata* would move to the upper shore as they grew (Bowman & Lewis, 1977). This zone is composed of particularly craggy and rugose limestone at Les Pierrieres. It has been noted that individuals of *Lottia gigantea* Gray in G.B. Sowerby I, 1834 (Kido & Murray, 2003) and *Patella ferruginea* Gmelin 1791 (Rivera-Ingraham *et al.*, 2011) are smaller and occur in higher density in areas of rugose rock, as found in as topographically variable or patch habitats. This may account in part for the increased abundance composed of small individuals of *Patella vulgata* at Les Pierrieres (Figures 4.2, 4.3B, 4.7) (Lewis & Bowman, 1975). The same principle can be applied to the high abundance of predominantly smaller individuals of *Patella depressa* found at Biarritz and San Vicente de la Barquera (Figures 4.2, 4.3A and 4.4), which also had highly rugose substrates (Wort, personal observation).

The role of microhabitat and macroalgae in particular can also be considered across localities. It has been shown that fucoids prompt aggregation by *Patella vulgata* at scales of around one metre in the British Isles (Hartnoll & Hawkins, 1985; Hawkins & Hartnoll, 1983; Moore *et al.*, 2007b), but the data did

not support this for individual quadrats. The positive relationship between fucoid cover and *Patella vulgata* abundance may be due to collinearity with fucoid abundance and mean SST, which has been identified here and in similar studies (Duarte *et al.*, 2013; Hawkins *et al.*, 2009; Southward *et al.*, 1995). By contrast, the significant negative relationship between macroalgal cover and both *Patella depressa* and *Patella vulgata* abundances may suggest a top-down control of macroalgae by limpet grazing, which has been experimentally demonstrated in the UK (Hawkins, 1981; Jenkins *et al.*, 2005; Jones, 1948; Jonsson *et al.*, 2006), as well as the coast of northern Spain, although for the warmer Portuguese coast the relationship is unpredictable (Coleman *et al.*, 2006). This is more pronounced in *Patella depressa* for both fucoids and other macroalgae. It could therefore be suggested that these results were a combination of fucoids having a negative effect on *Patella depressa* abundance, whilst higher abundance of limpets prevented establishment of algae (Ballantine, 1961; Ferreira *et al.*, 2015; Hawkins *et al.*, 2017; Moore *et al.*, 2007b). Both of these would have resulted in a negative relationship between *Patella* abundance and macroalgal cover.

Certain environmental variables may have a less significant or different effect on species abundances over the central portion of the species ranges compared to species abundances closer to range limits. For example, there was a positive relationship of exposure with high shore abundance of *S. umbilicalis* and *Phorcus lineatus*. Around the UK, *Phorcus lineatus* probability of presence derived from category abundance data showed a negative relationship with wave fetch at lower mean winter temperatures from 8.3 ˚C to approximately 10.8 ˚C, with some indication of becoming a positive relationship above 10.8 ˚C (Mieszkowska *et al.*, 2013). This change in the relationship between exposure and abundance at the higher temperatures is supported by data presented here for southern French and Iberian Peninsula populations in warmer conditions. By contrast, on the cooler UK shores Mieszkowska *et al.* (2013) showed wave fetch to have a negative relationship with probability of presence of *S. umbilicalis* from mean winter SST of approximately 9.8 ˚C to the maximum of 11.2 ˚C. It is only on the high shore that there is a significant positive regression between *S. umbilicalis* abundance and wave exposure not locality-wide estimates, as used in (Mieszkowska *et al.*, 2013). It is possible that as localities have greater wave exposure, the upper shore is immersed by waves more frequently than less exposed shores. This would lead to less desiccation and therefore a higher abundance of *S. umbilicalis* on the upper shore, but not necessarily for the entire locality.

The relationship between size and abundance was found in both *Patella* species and *S. umbilicalis*, which suggests intraspecific competition as has been described earlier. This may be absent from *S. pennanti* as there is less intraspecific competition, as well as the aforementioned sampling bias towards larger individuals. For *Patella* species, this relationship scaled allometrically, whereas for *S. umbilicalis* the relationship was linear. This suggests that the competition for a limiting resource occurs differently between *Patella* and *Steromphala*.

4.5 Concluding remarks

The principal driver of species abundance and size around range centres appears to be sea temperature, which undergoes regional variation rather a simple gradient with latitude. This may be through controlling recruitment, metabolism, and competitive interactions between congeners. Habitat gaps may also accentuate variation in abundance of species by reducing recruitment to localities with less favourable conditions, such as higher temperatures for *P. vulgata*. Biotic factors that may influence size and abundance, such as fucoid engineer species and density dependent processes, can frequently be understood in the context of temperature gradients. It is therefore unsurprising that range centres are not always associated with higher abundances, as temperature variation at the mesoscale (tens to hundreds km) deviates from a simple latitudinal gradient. As sea and air temperatures continue to increase due to anthropogenic climate change, it is likely that further changes in species size, abundance, and distribution around range centres will be observed.

Chapter 5: Why the point? Trends in shell morphology of *Steromphala*

5.1 Introduction

5.1.1 Background

It is widely recognised that gastropods have high intraspecific shell morphological diversity in response to different environmental conditions and biological processes (Harley *et al.*, 2009; Vermeij, 1973). Intertidal gastropods are particularly useful for testing the abiotic and biotic drivers of shell morphology because they are slow moving and easy to find in the field (Sagarin & Gaines, 2002). In addition, their shells are often well represented in museum collections, giving researchers the potential to use large spatial coverage in which to examine morphological change across the ranges of species (Fenberg *et al.*, 2014; Wilson-Brodie *et al.*, 2017). These intraspecific morphotypes have most often been associated with abiotic variables, such as shore height (Johannesson *et al.*, 1993) and wave exposure (Conde-Padín *et al.*, 2007; Crothers, 1985; Trussell *et al.*, 1993), as well as biological variables such as predation (Cotton *et al.*, 2004; Edgell & Miyashita, 2009; Galindo & Grahame, 2014; Preston & Roberts, 2007) and intraspecific competition with increasing population density (Kemp & Bertness, 1984). It has also been suggested that temperature has an important role in shell morphology (Harley *et al.*, 2009; Miller & Denny, 2011; Queiroga *et al.*, 2011), and hence has been considered when forecasting growth and morphological responses to climate change, sometimes in combination with CO_2 concentration and pH (Melatunan *et al.*, 2013; Rühl *et al.*, 2017). In spite of this growing body of evidence, temperature is not frequently considered as a "main effect" on intraspecific morphology (Urdy *et al.*, 2010). Intraspecific studies, however, are often relatively local in scale (Conde-Padín *et al.*, 2007) or are confined to laboratory experiments (Melatunan *et al.*, 2013; Rühl *et al.*, 2017). Patterns in shell morphology are less frequently investigated across the geographic distribution of a species, where populations experience a wide range of temperatures and other environmental variables (Frank, 1975; Irie, 2005).

Where intraspecific morphological differences have been associated with latitudinal gradients of temperature, it has been suggested that the variation is plastic rather than genetic in origin (Irie, 2005). As temperature can influence

growth rates, both directly by altering metabolism, and indirectly through recruitment influencing density dependent mechanisms, it has been suggested that it may also alter particular morphological traits (Kemp & Bertness, 1984), including overall shell size (Atkinson, 1994; Horne *et al.*, 2015; Wilson-Brodie *et al.*, 2017). The link between growth and morphology can be considered in terms of the rate of growth of the aperture, where exponential growth forms a conical shell shape; whilst a constant rate of growth results in a shell with convex sides [Figure 5.1, Rice (1998)]. With a greater exponential rate of aperture growth, the shell will assume a wider, flatter profile (high aspect ratio). It therefore follows that if aspect ratio of a species shows a relationship with temperature, it could be suggested that the rate of growth does too. This association between the aspect ratio (relationship between shell width and shell length or height) and the growth rate has been noted in *L. littorea*, with globose thin shells indicating rapid growth compared to elongate thicker shells (Kemp & Bertness, 1984). The same trend of older shells being pointier than younger shells was noted in passing for *Tegula funebralis* (Frank, 1975), although aspect ratio and age changed with size.

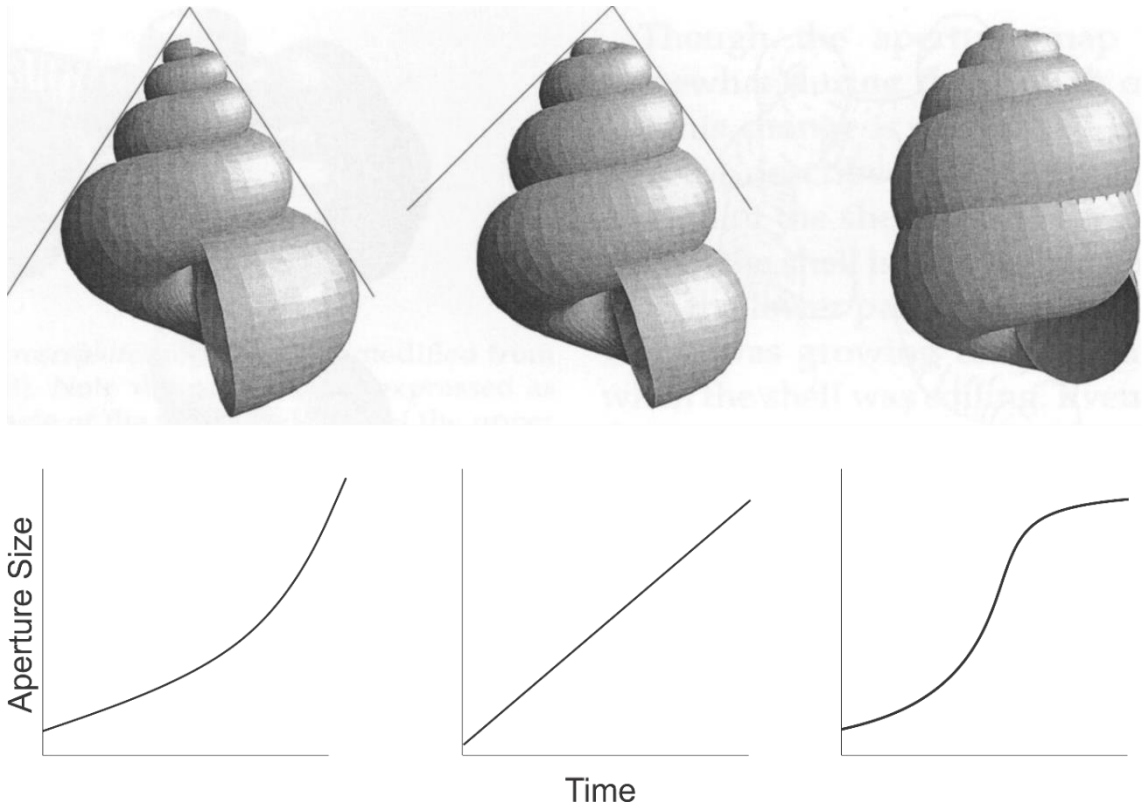


Figure 5.1: Adapted from Rice (1998) showing shells resulting from rate of new shell production proportional to aperture size. Where the aperture (and, by inference, the animal inside) grows exponentially, a conico-spiral shell forms (left). Linear growth (centre) produces a shell with convex sides. A sigmoid growth function builds a shell that expands at first, the coils straight down the axis.

At the interspecific level, the basal dimensions of high intertidal species in cool temperate regions was proportionally lower than warm tropical species (Vermeij,

1973). One possible selection pressure was that species with taller, more narrow shells at lower latitudes have a smaller area exposed to solar radiation, particularly when incoming radiation is considered as perpendicular to the base (Vermeij, 1973). Furthermore, these taller shells would achieve a greater volume without increasing the basal area in contact with the substrate which may also have a high temperature due to solar radiation, therefore reducing risk of overheating (Miller & Denny, 2011). Moreover, at the intraspecific level taller individuals with relatively smaller apertures are less prone to desiccation (Struhsaker, 1968). It follows that aspect ratio could be relevant to both water retention and thermal regulation (Cotton *et al.*, 2004; Melatunan *et al.*, 2013; Vermeij, 1982). These suggestions are based on the periods of emersion experienced by intertidal gastropods where air temperatures are likely to have a greater influence, whilst the association with metabolism and growth may be linked to both air and sea temperatures (Somero, 2002). As recent anthropogenic climate change continues to increase sea and air temperatures, this may cause species morphology (Rühl *et al.*, 2017) as well as species ranges to change (Hawkins *et al.*, 2009). However, as the trend of increasing temperatures at lower latitudes is not constant, but interrupted by regional variation such as coastal upwelling (Lima *et al.*, 2016; Lourenço *et al.*, 2016), latitude may not necessarily be an ideal proxy for temperature.

5.1.2 Study species

Steromphala umbilicalis (da Costa, 1778) has a habitat preference for the mid to low shore in rockpools, cracks, around and on boulders (Mieszkowska *et al.*, 2013; Muñoz-Colmenero *et al.*, 2015). The recorded range of *S. umbilicalis* extends from north-west Scotland (Mieszkowska *et al.*, 2013) to North Africa (Lewis, 1986; Southward *et al.*, 1995). It has undergone a recent range expansion further along the west coast of Scotland as well as along the English Channel which has been associated with increasing sea temperature (Mieszkowska *et al.*, 2013). There are no published differences in colouration (Mieszkowska *et al.*, 2006) or shell sculpture between different microhabitats in *S. umbilicalis*, although juveniles are more common on the lower shore and on the undersides of boulders (Williams, 1964). This makes it an ideal species for examining biogeographical trends, as local “noise” may be relatively limited, unlike highly morphologically variable species such as the *Littorina saxatilis* complex (Galindo & Grahame, 2014; Walker & Grahame, 2011). Furthermore, *S. umbilicalis* have not been commonly harvested historically (Gutierrez & Gonzalez, 2010; Turrero *et al.*,

2014) or at present, so it is highly unlikely that there will be any artificial selection based on traits such as size or shape.

The relationship between aspect ratio, predation and behaviour in four gastropod species including *S. umbilicalis* has been investigated experimentally (Al-Mazrouai, 2008 PhD Thesis; Cotton *et al.*, 2004). *Steromphala umbilicalis* exhibited a plastic response in shell length but not aspect ratio [although the definition of these measurements was slightly different in Al-Mazrouai (2008 PhD Thesis)] and generally less response to predation than several other gastropod species (Al-Mazrouai, 2008 PhD Thesis; Cotton *et al.*, 2004). Since individuals had been collected from the same shore for these experimental investigations, this suggests *S. umbilicalis* is capable of a plastic response in shell morphology.

5.1.3 Hypothesis

It is postulated that temperature will show a relationship with intraspecific gastropod shell morphology across large geographic scales. To test this, comparisons were made of the differences in aspect ratio (measured from field and museum specimens) across most of the range of *Steromphala umbilicalis.* The aspect ratio of this trochid, common to the north-east Atlantic rocky intertidal zone, was compared to multiple environmental factors including air and sea temperature proxies and estimates of wave exposure.

5.2 Methods

5.2.1 Field sampling

Initial sampling was carried out at 20 localities during low spring tides in August 2015, April and October 2016, with multiple visits to several localities (Figure 5.2). At the approximate centre of the initial localities was a 230 km habitat gap in south-west France (Figure 5.2). Locality sampling for *Steromphala* consisted of timed searches for two minutes by four people. This was in the fucoid or equivalent mid to lower shore zone where *S. umbilicalis* would be present. If *Steromphala umbilicalis* was uncommon, the search time was doubled to four minutes per person. To prevent sampling overlap and ensure a fair representation of any differing morphotypes and sizes classes, samplers were spaced at least 10 m apart and in varied microhabitats. Shell width and height were measured to the nearest 0.1 mm using dial callipers (Figure 5.3). Mean abundance as average number of individuals found per minute for each locality was also calculated.

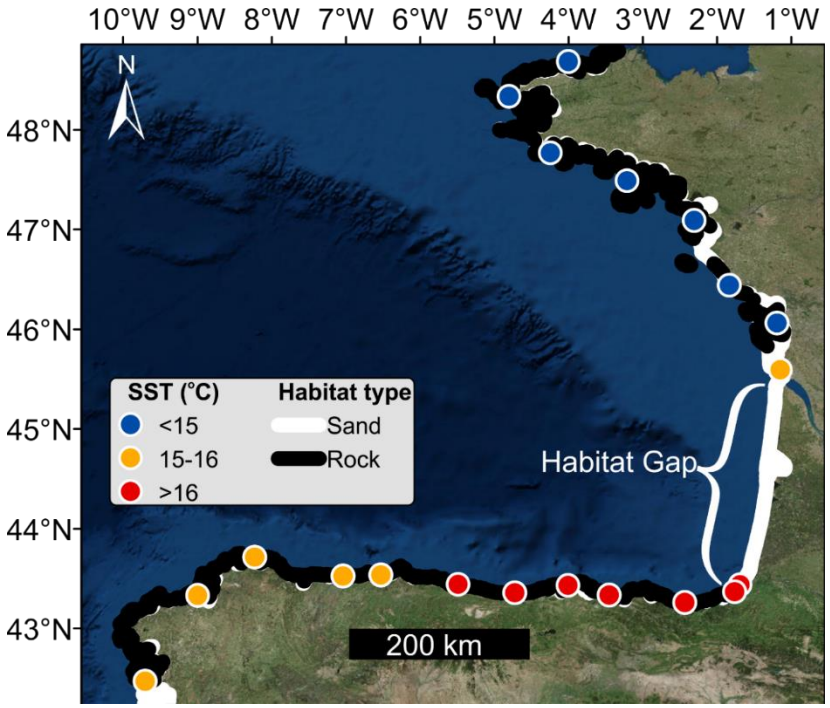


Figure 5.2: Map using World-Aitoff projection in ArcGIS displaying initial sampled localities, mean annual sea surface temperature from August 2005 to August 2015, habitat type and the location of the habitat gap.

Figure 5.3: Shell width (left), shell height (right) used in aspect ratio calculations.

To cover a greater portion of *S. umbilicalis* geographic range, additional samples were collected (using the same methodologies described above) beyond the initial study area (expanded data, Figure 5.4) from localities on the southern UK (Dale, the Channel Islands and Swanage) and northern France (Cherbourg peninsula).

5.2.2 Museum specimens

To expand further upon this dataset temporally and geographically, specimens with time and locality information from the Natural History Museum in London were also measured. These included samples from northern British Isles

(Stromeferry) to south-west France (Biarritz) spanning 1858 to 2009 (museum data, Figure 5.4).

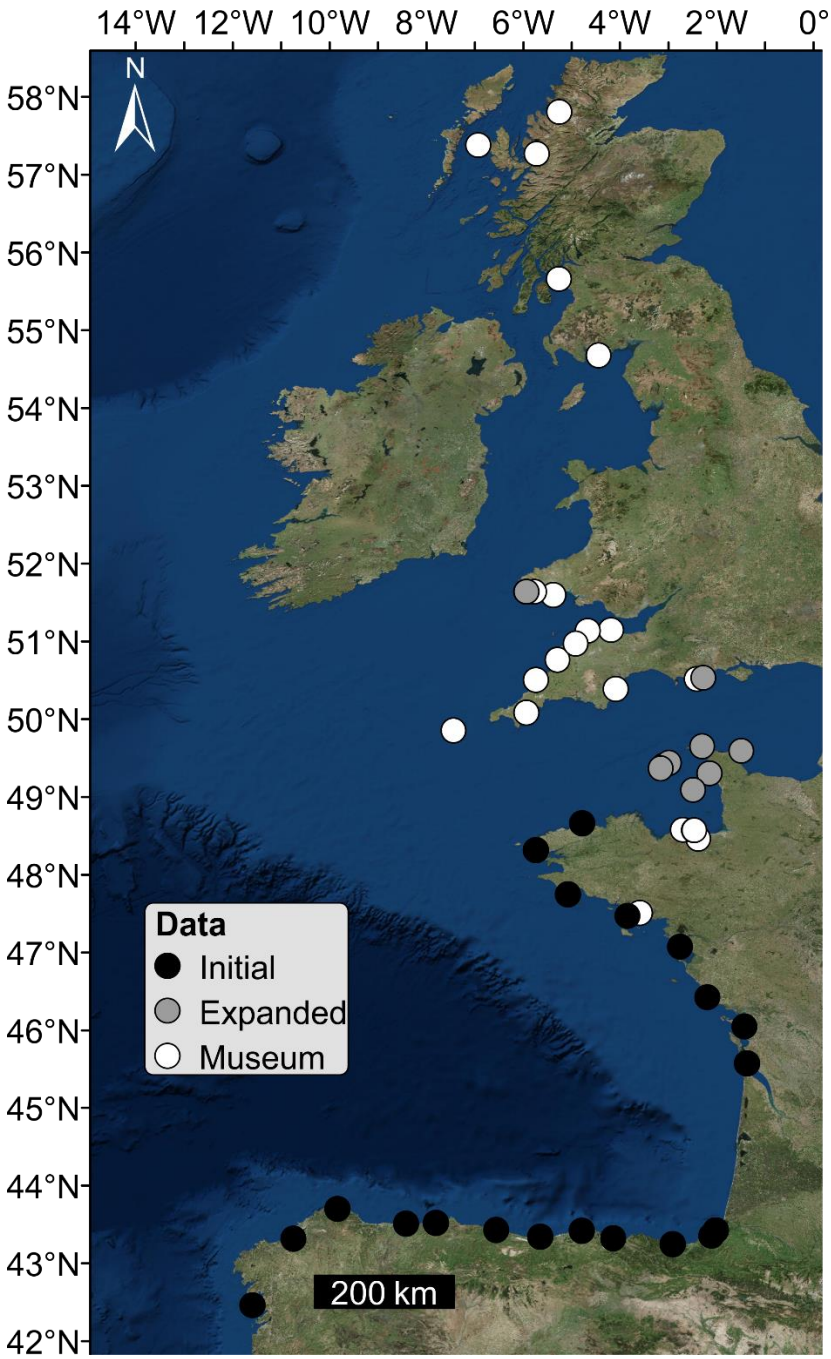


Figure 5.4: Map using World-Aitoff projection in ArcGIS displaying initial sampled localities, expanded sampling localities and museum specimen localities.

5.2.3 Environmental data

Daily mean 0.25 degree resolution sea surface temperature (SST) and 0.5 degree resolution monthly mean air temperature provided by the NOAA/OAR/ESRL PSD, Boulder Colorado, USA was obtained for each locality from the geographically expanded dataset (https://www.esrl.noaa.gov/psd/data/gridded/ last accessed 12/2/2018) (Fan & Van den Dool, 2008; Reynolds *et al.*, 2007). From these

datasets, annual mean SST and air temperature from August 2005 to 2015, winter mean (December, January and February) from December 2005 to February 2015 and summer mean (June, July and August) from June 2006 to August 2015 were extracted for each locality from the geographically expanded dataset. This duration encompassed the temperatures experienced by the oldest individual, as longevity is estimated to be below 5 years for sampled localities (Duchamps, 1992; Lewis *et al.*, 1982; Pelseneer, 1933). To avoid bias from using different daytime measurements, only values from 12:00 were used. To generate SST values for museum specimens pre-dating 1990, the 2 degree resolution monthly mean SST values were used (Huang *et al.*, 2015) and the mean SST calculated for each locality for the decade prior to the collection date. This would cover the life span of all but a few individuals in northern Scotland previously estimated to be 12 years old (Lewis *et al.*, 1982). Where museum specimens could have been collected over a range of years (estimated by collector biographies) rather than an exact collection date (from labelled meta-data), the mean SST for that time range was calculated. If localities were within the same grid cell and had the same decadal mean SST value, data was aggregated before calculating the mean aspect ratio.

For the region covered by the initial dataset, a coastline shapefile was obtained from the European Environment Agency (EEA) (https://www.eea.europa.eu/data-and-maps/data/eea-coastline-for-analysis/gis-data/europe-coastline-shapefile last accessed 8/2/2018) and overlaid on the highest available (typically 30 cm) resolution satellite imagery available as a background layer in ArcGIS Desktop v. 10.5 (Esri, Redlands, California, USA). All layers were converted to the ETRS89 coordinate system for use in subsequent operations. The coastline shapefile was divided into sections defined as rock or sediment based on the substrate type on the line or at the nearest land-sea interface. The buffer tool was used to create circles of 1 km and 5 km radius around each locality centroid, and the proportion (as a percentage) of rocky coast to total coastline within each circle calculated. The proportion within 1 km radius was used as a proxy for potential sediment inundation of populations; the proportion within 5 km radius was used a general estimate of suitable substrate in the immediate area.

A second environmental variable obtained for the initial dataset was wave exposure, which was calculated using the quantitative index developed by Burrows *et al.* (2008). The EEA coastline shapefile covering the extent of the study region was converted to 1 km raster resolution and the wave fetch from angular

sectors of 22.5˚ to coastal cells calculated in WaveFetch16v01. Using the nearest weather station to each locality, wind data for Iberian Peninsula, Biarritz and St Jean de Luz was downloaded from AEmet, using monthly mean wind speed and the direction of the maximum wind speed from 2012 (earliest available data) to 2015 (https://opendata.aemet.es/centrodedescargas/productosAEMET last accessed 12/2/2018). Daily mean wind speed and direction from August 2005 to August 2015 for all French localities excepting Biarritz and St Jean de Luz was downloaded from the MeteoFrance website (https://donneespubliques.meteofrance.fr/ last accessed 12/2/2018). The proportion of time records where wind came from the differing 22.5˚ angular sectors was calculated and multiplied by the square of mean wind speed in knots for each sector. This wind energy variable was then multiplied by the fetch to obtain a measure of wave exposure (Burrows *et al.*, 2008).

Finally, for the initial dataset, assuming any effects of the habitat gap on species abundance at a given locality would be determined by proximity, the distance using the coastline shapefile between the proximal edge of the habitat gap and each locality was measured in ARCGIS DESKTOP v. 10.5. As use of logged distance is commonly used in preparation for Mantel tests of genetic differentiation with geographic distance (Mantel, 1967; Rousset, 1997), this was calculated for distance to the habitat gap variable (gap proximity; GP).

5.2.4 Calculations and statistical analyses

The aspect ratio of the shell of a gastropod species can vary over the life of an individual as the shell can deviate from a conical to a more domed or columnar shape with size/age, resulting in an allometric relationship (Frank, 1975; Rice, 1998). In these situations, aspect ratio can be calculated using the natural log of one or both measurements (Kemp & Bertness, 1984; Parsons, 1997). Another method used to standardise the effects of size is to only use the mean ratio at a specified adult width (Johnson & Black, 2000). To verify if these methods were necessary, height was plotted against width for seven localities with highest *S. umbilicalis* abundance both north and south of the habitat gap (chapter 4). If the ratio was isometric at each locality with increasing size (i.e. a linear relationship between height and width), any skew towards larger individuals being sampled by collectors from museum collections would unlikely bias results. A linear regression was also run on initial sample localities timed search abundances and aspect ratio to see if there was a density dependent relationship.

Once the relationship between height and width was confirmed as isometric, a multiple linear regression analysis was performed to model the relationship between all environmental variables and the mean aspect ratio for the initial sampled localities (Figure 5.2), weighted by the number of samples at each locality. The dredge function of the R package MuMIn (Barton, 2013) was applied to fit linear models for all possible combinations of explanatory variables. These models were then ranked according to the corrected Akaike Information Criterion (AICc) following Burnham and Anderson (2003). The model-averaged coefficient was extracted for each variable present in at least one candidate model, defined as those with $\Delta AIC \leq 7$ (Burnham *et al.*, 2011). Nested models were removed using the nested function in MuMIn (Barton, 2013) to reduce selection of overly complex models (Richards *et al.*, 2011). Frequency of each variable in the candidate models was used to determine the importance score, with a score of 1.0 signifying that a variable was present in all candidate models. The individual explanatory variable with the highest importance score, the final model with the lowest AICc score and the individual explanatory variable with the lowest AICc score were presented. The highest importance scoring and lowest AICc individual explanatory variable were plotted against aspect ratio using a weighted linear model. This ensured that the selected explanatory variable was both the most parsimonious and had a statistically significant regression. Simple linear regression was then repeated using the expanded and museum aspect ratio dataset using the most parsimonious and highest importance scoring explanatory variable. Finally, shell width was plotted against the same explanatory variable as was selected for aspect ratio using a general additive model (GAM) to illustrate that size does not follow the same linear trend.

5.3 Results

A total of 5593 individuals of *S. umbilicalis* were measured, 3836 of which were in the initial dataset. Initial visualisation of data revealed a linear relationship that best described the relationship between shell height and width for each locality (Figures 5.5 and 5.6). This means that there is an isometric relationship between shell height and width, so mean ratio values could be used for subsequent comparisons between locality aspect ratios. Furthermore, abundance showed no significant relationship with aspect ratio (Adjusted $R^2 = -0.05435$, $p = 0.8876$), suggesting that aspect ratio is not a density dependent response variable.

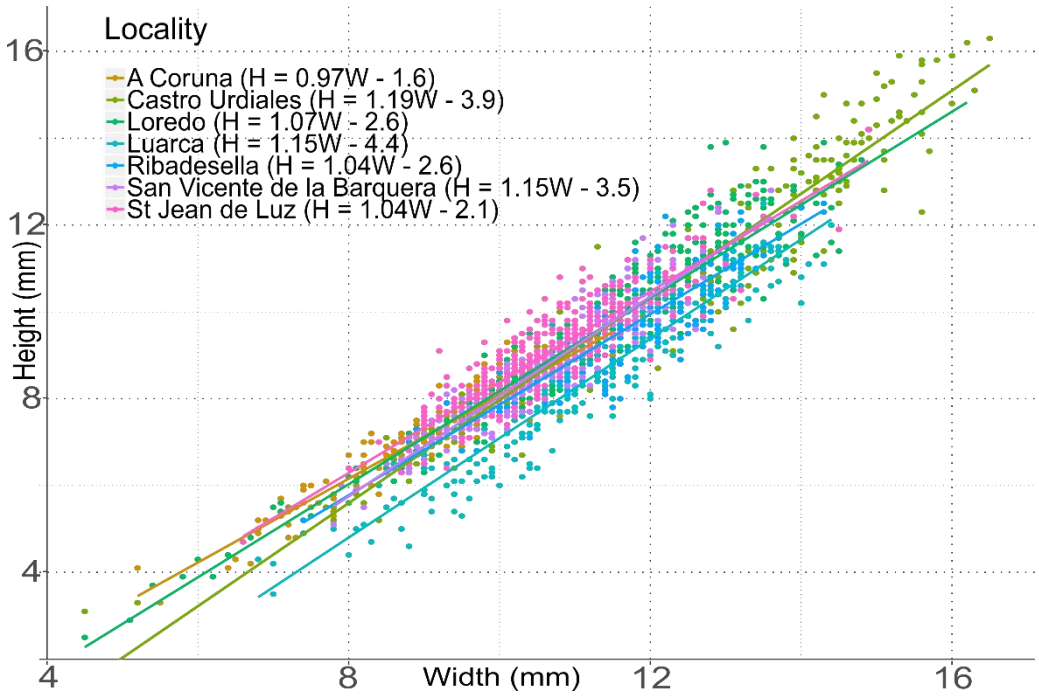


Figure 5.5: Height plotted against width for seven localities with greatest sample size south of the habitat gap, with linear equations for localities given in brackets (H = Height, W = Width). N for: A Coruna = 224, Castro Urdiales = 208, Loredo = 342, Luarca = 253, Ribadesella = 201, San Vicente de la Barquera = 222, St Jean de Luz = 349.

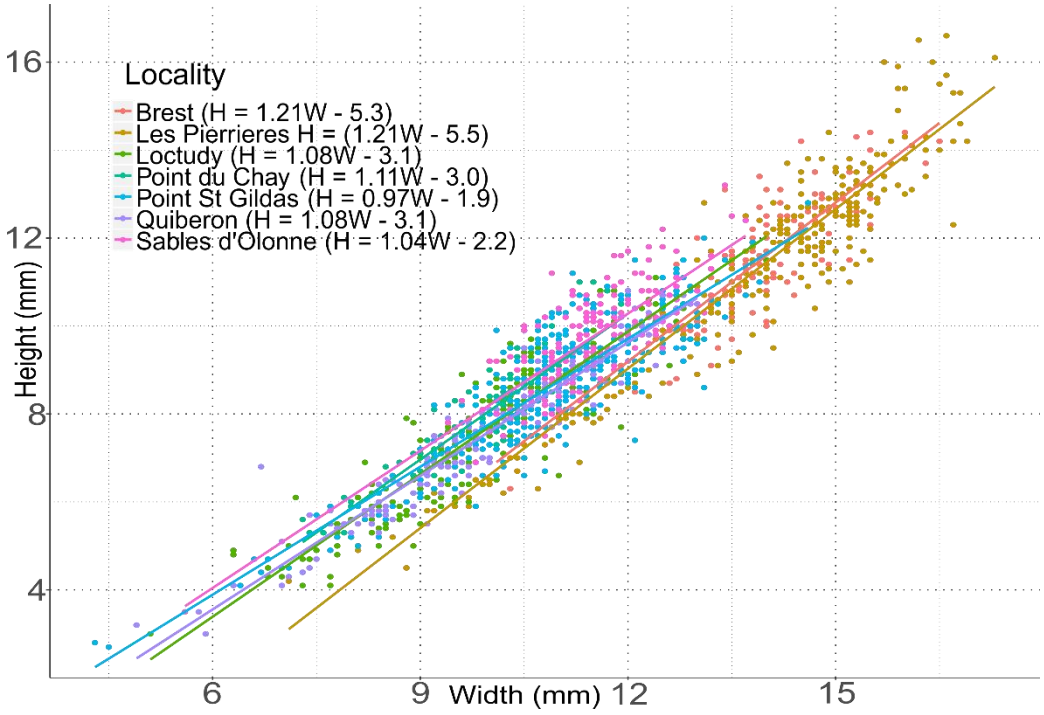


Figure 5.6: Height plotted against width for seven localities with greatest sample size north of the habitat gap, with linear equations for localities given in brackets (H = Height, W = Width). N for: Brest = 139, Les Pierrieres = 303, Loctudy = 270, Point du Chay = 99, Point St Gildas = 378, Quiberon = 143, Sables d'Olonne = 166.

Initial data exploration revealed a general increase in shell aspect ratio with increasing distance from the habitat gap, both to the north along the French

coast and east along the Iberian Peninsula (Figure 5.7). However, the multiple linear regression model with the lowest AICc score for mean aspect ratio using initial locality data was SSTS and summer air temperature (weight = 0.367; AICc = -59.4). SSTS, not the gap proximity variable, was the single variable with lowest AICc score as well as the most important variable (weight = 0.141, AICc = -57.5; importance score = 0.76, Adjusted R^2 = 0.4034, p=0.001564, Figure 5.8). When applied as an explanatory variable to the full dataset, SSTS continued to show a highly significant negative relationship with aspect ratio (Adjusted R^2 = 0.4647, p = 5.803e-8, Figure 5.9). This indicated that shells were flatter at localities with lower SSTS, and more pointed at higher SSTS in the present day around the range centre and at wider geographic and temporal scales.

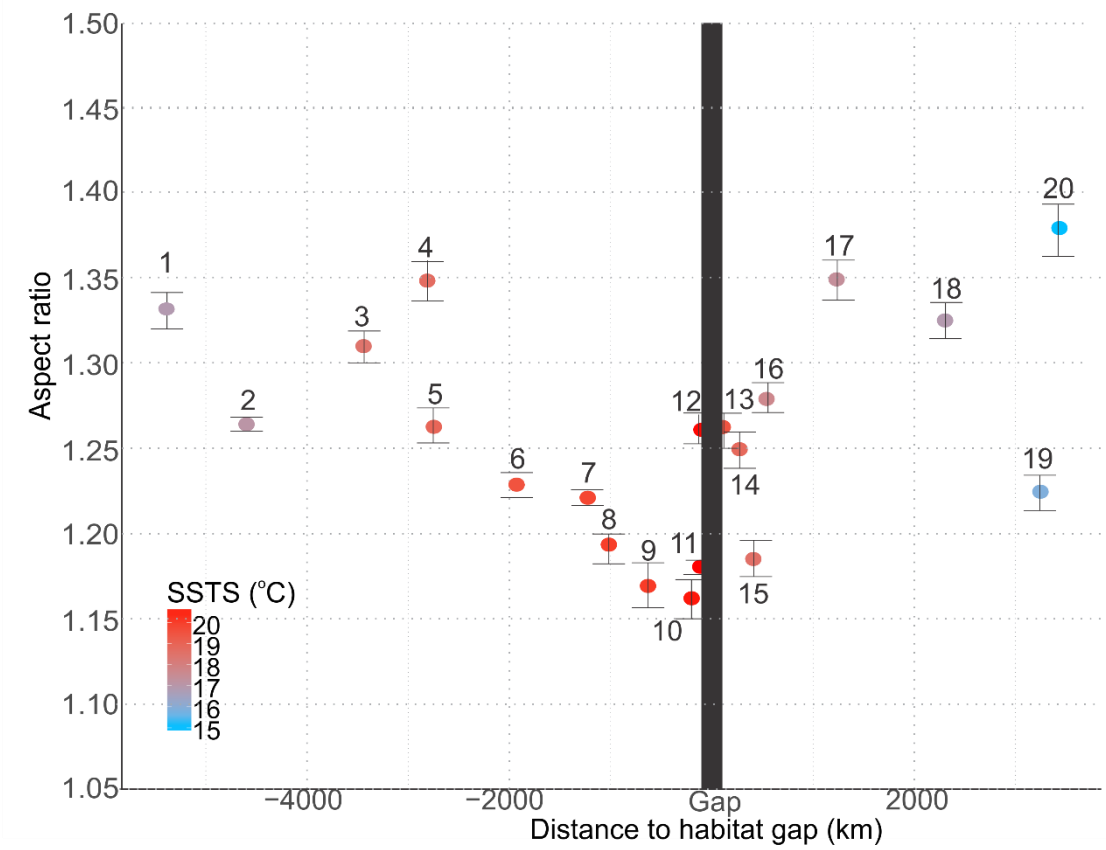


Figure 5.7: Mean aspect ratio with SE bars at localities with increasing distance along the habitat gap (shown to scale) with colour indicating summer sea surface temperature. Locality number written above upper quartile. N for: 1 (Vilagarcia) = 118, 2 (A Coruna) = 224, 3 (O Vicedo) = 76, 4 (Luarca) = 253, 5 (San Esteban de Pravia) = 166, 6 (Ribadesella) = 201, 7 (San Vicente de la Barquera) = 222, 8 (Loredo) = 343, 9 (Castro Urdiales)= 208, 10 (Zumaia) = 128, 11 (St Jean de Luz) = 349, 12 (Biarritz) = 196, 13 (Les Pierrieres) = 303, 14 (Point du Chay) = 99, 15 (Sables d'Olonne) = 166, 16 (Point St Gildas) = 378, 17 (Quiberon) = 143, 18 (Loctudy) = 270, 19 (Brest) = 139, 20 (Roscoff) = 71.

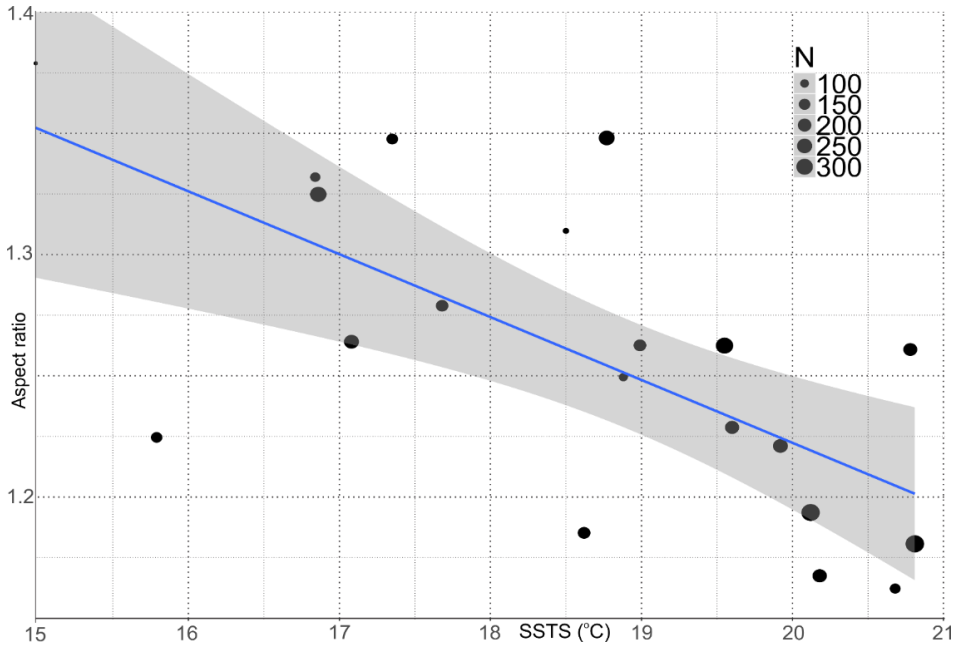


Figure 5.8: Linear regression between summer sea surface temperature (SSTS) and mean locality aspect ratio weighted by number of individuals (N). Larger circles indicate higher N. This is using the initial dataset mostly consisting of localities around the range centre.

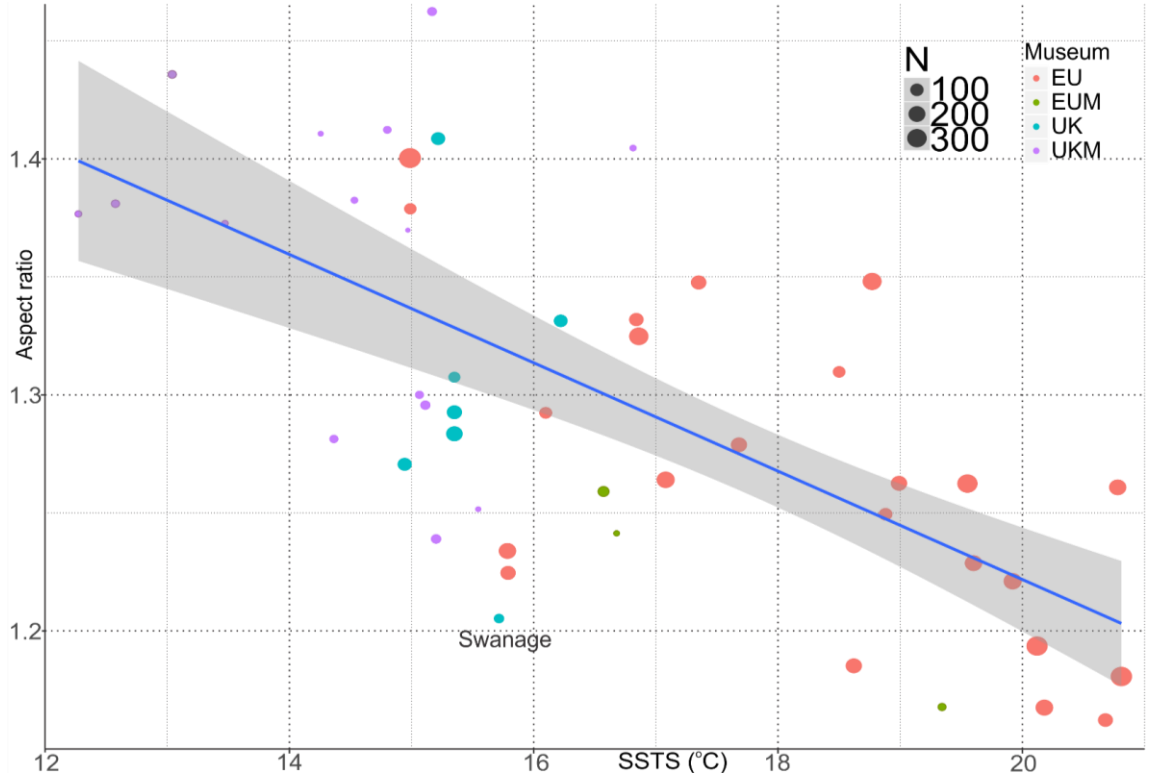


Figure 5.9: Linear regression between summer sea surface temperature (SSTS) and mean locality aspect ratio weighted by number of individuals (N) using the expanded dataset including both museum and wider geographic sampling. Larger circles indicate higher N; EU = initial samples; EUM = museum samples from French localities; UK = Channel Islands and British Isles samples; UKM = museum samples from UK localities. Swanage is marked as an outlier with particularly low aspect ratio.

Whilst mean size generally decreases with increasing sea surface temperature, there is an initial trough in size around 17°C, before increasing again up to 20°C (Figure 5.10).

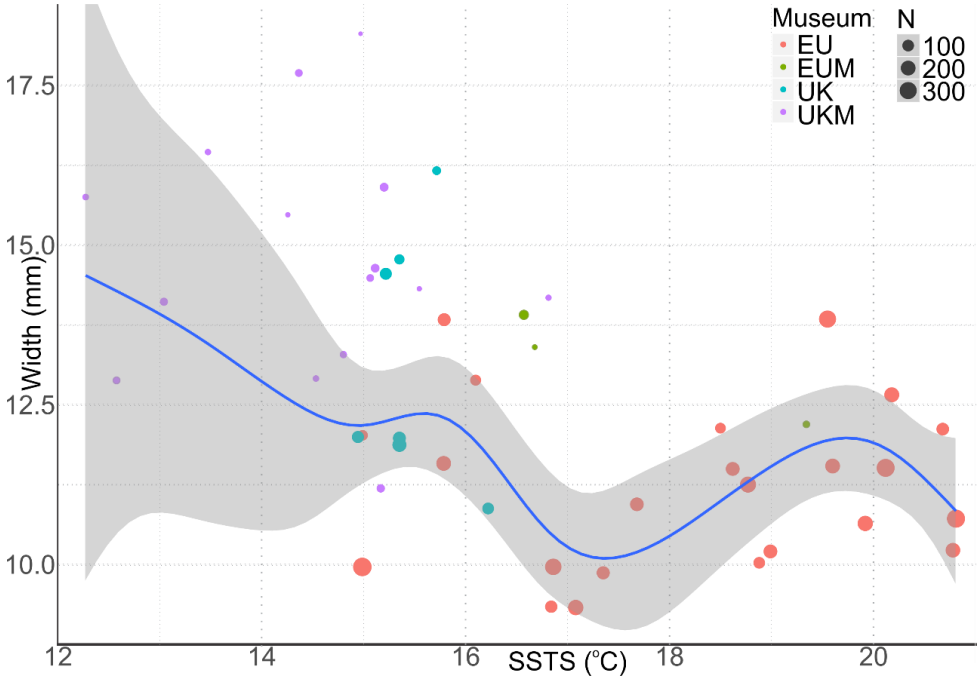


Figure 5.10: General additive model (GAM) between summer sea surface temperature (SSTS) and mean locality shell width (mm) weighted by number of individuals (N). Larger circles indicate higher N; EU = initial samples; EUM = museum samples from French localities; UK = Channel Islands and British Isles samples; UKM = museum samples from British Isles localities.

5.4 Discussion

Shell morphology of intertidal gastropods has been linked with numerous environmental variables at local-scales, but large-scale patterns in shell morphology and their environmental correlates have been considered somewhat infrequently (Dehnel, 1955; Irie, 2005; Vermeij, 1973). Here it can be shown that *Steromphala umbilicalis* aspect ratio has a significant negative relationship with sea temperature, which at the macroscale (i.e. across much of its range) outweighs environmental variables such as wave exposure that have been found to drive local differences in shell morphology in other species (Caley *et al.*, 1995; Conde-Padín *et al.*, 2007; Struhsaker, 1968; Trussell *et al.*, 1993). The lowest aspect ratio values are found south of the habitat gap on the north-east section of the Atlantic coast of Spain, with flatter individuals in the cooler upwelling regions in western Spain. As the gradient of sea temperature approximately corresponds with latitude for northern France and the UK, the more northerly, cooler localities have flattest individuals. This clearly shows that biological variables are driven by

sea temperature, which rarely follows a monotonic spatial or latitudinal gradient along the coast (Figure 5.7).

One initial consideration is whether the variation in aspect ratio is due to phenotypic plasticity or microevolutionary processes (Parsons, 1997). Only one locality (Swanage) showed any genetic difference with other localities, which showed an anomalously low aspect ratio relative to both mainland Europe and British localities. Localities on mainland Europe had different aspect ratios but showed no genetic differentiation earlier in this study. The possibility of differing aspect ratio *S. umbilicalis* being linked to genetics is therefore cautiously eliminated, and plastic morphological responses to temperature are the focus of the remainder of this discussion.

A second factor to consider is density-dependent interactions altering shell growth rate and morphology (Kemp & Bertness, 1984). This need not be through competition for resources such as food, but other density-dependent conspecific interactions such as conspecific chemical signals which stimulate responses, as has been suggested for variation in juvenile *Cypraea annulus* size and morphology (Irie, 2005). A significant relationship between any temperature variables and *S. umbilicalis* abundance was not found for the initial localities earlier in this study. As there was no relationship between abundance and aspect ratio, it is unlikely the relationship between aspect ratio and sea temperature is mediated by density dependant processes. One caveat is that the largest individuals were museum specimens from cooler localities at higher latitudes (north British Isles) with low abundances and higher aspect ratios (Lewis *et al.*, 1982; Mieszkowska *et al*., 2013). This suggests that towards the northern limit of *S. umbilicalis*, decreased intraspecific competition due to lower and more infrequent recruitment may result in increased shell growth and as proposed by Lewis *et al.* (1982), hence the observed decrease in aspect ratio. The same size trend of larger individuals present at higher latitudes has been identified in *T. funebralis*, which occupies a similar latitudinal range (albeit on the west coast of USA) and morphology to *S. umbilicalis* (Frank, 1975). However, these northern larger, older individuals were slow growing and had pointier shells than more southerly specimens (Frank, 1975) – the opposite of the morphological trend identified in *S. umbilicalis*.

Where aspect ratio is greater and shells are flatter, this would suggest more rapid growth of the individual (Rice, 1998). This association also proposed by Kemp and Bertness (1984) for *L. littorea*, would suggest that *S. umbilicalis*

grows more quickly at lower sea temperatures, as was argued by Lewis *et al.* (1982). These cooler conditions are not only associated with latitude, but also regional upwelling, as has been identified on the north-west coast of Spain for some time (Fischer-Piette, 1955), illustrating the role of regional variation within wider biogeographic trends.

In this context, an alternative explanation for *S. umbilicalis* variation in aspect ratio is that increased reproductive output may decrease the body growth rate as a life-history trade-off. Indeed, growth rate in gastropods has been found to decrease at sexual maturity as less energy is available (Duchamps, 1992; Palmer, 1982). It has been noted that on the warm north coast of Spain, spawning occurs throughout the year in *S. umbilicalis*, although it mostly occurs between June and November (Bode *et al.*, 1986). The same is true of the west coast of Portugal, with a marked transition from gonad development to spawning between May and August (Gaudèncio & Guerra, 1986). *Steromphala umbilicalis* in the UK has a later and shorter spawning season that has been associated with lower sea temperature (Lewis, 1986). More energy may therefore be used in reproduction at the localities with high summer sea temperatures, resulting in a lower rate of body growth, subsequently with a lower shell growth rate and pointier shells.

This lower rate of growth at higher temperature initially appears to contradict the seasonal variation found by Williams (1964) where lower winter temperatures were associated with decreased growth rate, possibly due to reduced feeding activity. There are two major differences between this temperature-growth relationship and the one presented here: firstly that seasonal changes in size, growth or aspect ratio were not observed; secondly that differing temperatures based principally on geographic, rather than seasonal differences, were compared. It is therefore possible that at the annual scale individuals grow faster at localities with cooler mean SST, whereas annual growth of individuals in warmer waters is slower due to increased reproductive effort. Indeed, Dehnel (1955) found that whilst rates of growth were generally greater for gastropod larvae kept at higher temperatures, larvae from populations in the cooler region (Alaska) had a considerably greater growth rate than larvae from populations in warmer regions (southern California). By contrast, in the same region, large, slow-growing pointed individuals of the trochid *T. funebralis* occurred in cooler more northerly conditions, whilst smaller, flatter individuals were found at lower latitudes (Frank, 1975). This illustrates that patterns in aspect ratio are variable for different species.

There are several alternative hypotheses based on a plastic response of *S. umbilicalis* to increased temperature. One such hypothesis that has been considered at the interspecific level is the association between thermal radiation and shell shape, where taller shells absorb less solar radiation, hence are less likely to experience thermal stress (Vermeij, 1973). This mechanism would produce the same relationship between higher temperature values and aspect ratio as was found at the intraspecific level, as the shell would be most susceptible to solar radiation during periods of emersion. A more significant relationship with air temperature than sea surface temperature would therefore have been expected if this was the case. Another potential explanation would be a link between the area of contact between the animal and substratum. Flatter individuals with larger apertures would be more susceptible to both heat transfer and desiccation from the substratum during periods of emersion (Vermeij, 1971). However, this too would result in a more significant relationship with air temperature than sea surface temperature. Before discounting these hypotheses, it should be borne in mind that the air temperature grids used were half the resolution of the sea surface temperature grids. The air temperature values used may therefore not be as accurate as the sea surface temperature values, and subsequently show a weaker relationship with aspect ratio.

If there is a morphological trend for intertidal species in relation to air or sea temperature, it follows that in the present day climate change scenario, we are likely to observe corresponding changes in species morphology (Melatunan *et al.*, 2013; Rühl *et al.*, 2017). *Steromphala umbilicalis* on the north-east Atlantic coast will become more pointed, including northerly individuals such as those on the UK coast. As these become more pointed, they may become more vulnerable to predation, for example by crabs such as *Carcinus maenas* (Linnaeus, 1758) (Cotton et al., 2004). However, as these findings contradict other literature regarding latitudinal patterns in trochid morphology (Frank, 1975), there is no obvious biogeographic rule governing trends at the intraspecific level. It follows that morphological responses to climate change may vary from species to species.

Chapter 6: Overview and Synthesis

6.1 Summary of main findings

Spatial variation in abundance, distribution, body size, morphology, and population genetics across different taxa, regions and habitat types are commonly associated with temperature (Horne *et al.*, 2015; James, 1970; Rowe & Menzies, 1969; Southward *et al.*, 1995; Vermeij, 1973) and distribution of suitable habitat (Fenberg *et al.*, 2014; Laurance, 2008; Shanks *et al.*, 2014). What makes this study rare, if not unique, is that it explores all of these biological variables across the same environmentally and physically heterogenous coastal region for species occupying the same general habitat type (rocky shores). These biological responses have all been considered for *Steromphala umbilicalis*, so it was possible to explore whether they are related to the same environmental variables in the same species. Furthermore, this study allows for the relationship, or lack thereof, between these different biological variables to be considered, interspecifically and intraspecifically.

Biogeographic patterns for the different species in this study were commonly associated with the sea temperature. Although latitude is frequently used as a proxy for temperature and distance from the centre of a species range, it is frequently a poor proxy for these two variables and should not be considered as an environmental variable in itself. Sea temperature varies regionally along the complex coastlines in and around the Bay of Biscay, particularly in the well-documented transition from cold upwelling water on the north-west coast of the Iberian Peninsula to warm waters found around the Basque country (Fischer-Piette, 1955). This meant that on the north coast of the Iberian Peninsula where latitude did not vary much between localities, the biological responses of intertidal gastropods were best predicted by sea surface temperature (from satellite and modelled data). As differences in environmental variables and the habitat gap drove the large changes in abundances of target species (Fenberg *et al.*, 2014; Sagarin & Gaines, 2002), the abundant centre hypothesis (Brown *et al.*, 1995; Hengeveld & Haeck, 1982) did not apply to any of the sampled gastropod species, as was predicted.

Where each *Patella* species experienced their preferred temperature conditions (regions of cold water found at upwelling regions associated higher *Patella vulgata* abundance, and warm water associated with higher *Patella*

depressa abundance), their higher abundance was indicative of greater recruitment and survival. The habitat gap in the south-west of France also amplified the differences in the abundance of both *Patella* species between cooler northern French localities and those found around the north-eastern Iberian Peninsula, as well as for *Phorcus lineatus* with a contrast between more exposed localities north of the habitat gap and less exposed localities south. However, *Patella vulgata*, which was rare or absent at localities on the southern edge of the habitat gap, was more common towards the northwest coast of the Iberian Peninsula. It could therefore be suggested that sharp decreases in abundance either side of the gap for (south of the gap for *Patella vulgata* and *Phorcus lineatus*, north of the gap for *Patella depressa*) are mostly associated with differences in environmental conditions rather than limited connectivity. It has also been suggested that it is the coincidence of a decrease in sea temperature, rather than the habitat gap itself, that controls the northern limit of *Patella rustica* in the same region (Lima *et al.*, 2007b). It could be postulated that if mean sea temperatures increase on the French Atlantic coast, species northern limits that are currently at Biarritz, such as *Patella rustica* and *Stramonita haemastoma*, will shift to north of the habitat gap. Furthermore, if the upwelling on north-west Iberian Peninsula coast weakens, the cooler water species in the region, such as *Patella vulgata*, could become less abundant while warmer water species, such as *Patella depressa* and *Patella rustica*, become more abundant.

The habitat gap was not associated with significant genetic differentiation in either *S. umbilicalis* or *Steromphala pennanti*, suggesting that it is not a barrier to connectivity for species with dispersive larval phases. Whilst this is unsurprising given the aforementioned distributions, it does contrast with the genetic differentiation between populations of *Pollicipes pollicipes* or *Pelvetia canaliculata* (Campo et al., 2010; Neiva et al., 2014) around the habitat gap. It could therefore be suggested that whilst habitat gaps may limit connectivity for some species, they are by no means absolute barriers. Instead, it is a combination of both the distribution of habitat and unsuitable environmental conditions that limit dispersal both within and beyond species ranges (Lima *et al.*, 2007b; Reid, 2002), as well as the current regime that may reduce dispersal in a given direction (Ayata *et al.*, 2010). The varied tidal currents in the English Channel may also explain the absence of *S. pennanti* on the British coast and the genetic isolation of Swanage in relation to mainland European samples for *S. umbilicalis*. This also illustrates that a genetic 'isolation by distance' model does not

necessarily apply to species where larvae are not dispersed evenly, but are driven by currents.

Considering the role of biological habitat on connectivity, sea temperature had a negative relationship with fucoid cover, with warmer regions in the southeast Bay of Biscay having less fucoid habitat than cooler regions, to the point that some species are absent (Duarte *et al.*, 2013). As discussed above, relatively lower abundances and a fragmented distribution of the habitat specialist *Steromphala pennanti* was associated this fragmented distribution of fucoids in these regions. This in turn reduced genetic connectivity, possibly leading to the genetic differentiation observed between populations. By contrast, there was little biogeographic variation in abundance of the generalist *S. umbilicalis* and no relationship with temperature within the Bay of Biscay. The lack of genetic differentiation between *S. umbilicalis* mainland Europe populations may be attributable to this continuous distribution. This difference between the two species shows the advantage of combining not only species distribution information (Assis *et al.*, 2013; Neiva *et al.*, 2014), but also more detailed ecological data to inform interpretation of population genetics and biogeography (Dawson *et al.*, 2014; Pannacciulli *et al.*, 1997). To focus on one particular ecological trait, in this case habitat specificity, using congeners has been shown to be a very useful tool (Dawson, 2012; Dawson *et al.*, 2014). From this study, it could be predicted that if sea temperature increases on the French coast, it is likely that fucoids would become more patchily distributed. Over multiple generations of *Steromphala* (the mutation rate of the microsatellites used is unknown), this could result in more isolated populations of *S. pennanti* and decreased genetic connectivity. Similar genetic isolation may occur in other other species reliant on a particular biological habitat whose distribution is influenced by environmental factors (Cefalì *et al.*, 2016; Kierepka *et al.*, 2016), as well as the abiotic habitat (Neiva *et al.*, 2012). However, Vetigastropoda have been shown in this study to undergo positive selection on the mitochondrial genome, so may have the capacity to become adapted genetically to unfavourable conditions.

The greater abundance of a *Patella* species may have led to greater intraspecific competition, so that smaller individuals were observed where the abundance was greater. It can be suggested, as was predicted in the introduction, that as sea temperature can influence abundance and then size through density-dependent processes, temperature can indirectly influence size. This does not necessarily occur as a fixed “temperature-size rule” (Bergmann, 1848; Forster *et*

al., 2012). The same negative relationship between mean size and abundance was found for *S. umbilicalis*, albeit without temperature-dependence. This confirms the hypothesis that size is density-dependent for *Patella depressa*, *Patella vulgata* and *S. umbilicalis*, but not *S. pennanti*. It is possible that as *S. pennanti* was less common than the other species, intraspecific competition for resources was not as much of a limiting factor to growth. It could be postulated that if sea temperature increases for over a decade (approximately enough time for individuals to reach full size), abundance of *Patella vulgata* would decrease and their mean size would increase, whilst the opposite would be true of *Patella depressa*. By contrast, there would be no associated change in the mean size of *S. umbilicalis*, at least within the Bay of Biscay.

Although neither size nor abundance of *S. umbilicalis* was associated with sea temperature in the Bay of Biscay, *S. umbilicalis* aspect ratio had a negative relationship with summer sea temperature. This illustrates that biological responses to temperature at the macroscale can occur throughout the range, even if there is no notable relationship to abundance or size. In this particular case, it was suggested that growth is greater in cooler regions in *S. umbilicalis*, possibly due to a trade-off between growth and reproduction. As with other ecological rules, generally applying to other species or regions without considering the species ecology and life history, as well as other environmental variables, may prove erroneous (Frank, 1975). As increasing sea temperature could alter the morphology of *S. umbilicalis* shells, they may become more vulnerable to other factors, such as predation from crabs (Cotton *et al.*, 2004).

In response to the wider question of the study, it has been illustrated that the same environmental variable, in this case sea temperature, can be used to consider abundance, which in turn can influence size (*Patella depressa* and *Patella vulgata*), and morphology (*Steromphala umbilicalis*). As the same environmental variable can also be associated with the abundance and distribution of habitat-forming species, it can alter the distribution of species (*Steromphala pennanti*) dependent upon a given biological substrate (fucoids). This can sometimes outweigh the importance of the distribution of abiotic substrate (rock or sand) in genetic connectivity (*S. pennanti*). Ecological ‘rules’, such as the abundant centre hypothesis are not applicable unless certain conditions are met, which was not the case in this study. Whilst the size-density rule was more widely applicable (for three of four species tested), it is still highly context-dependent, principally on intraspecific competition.

6.2 Limitations and moving forward

In this study, relatively low (0.25 degree) spatial resolution environmental data revealed the association between temperature and several biological response variables in different species. This does not negate the importance of local variation (~1 m resolution) in temperature, which has been described for rocky intertidal species within the context of macroscale variation (Seabra *et al.*, 2011). A variable for this microhabitat variation in temperature was not included, which may influence all of the biological variables, namely abundances, sizes, genetics (Galindo & Grahame, 2014) and morphologies (Conde-Padín *et al.*, 2007) of the gastropods, examined in this study.

Similarly, this study has exclusively used long term (including seasonal) means and not higher temporal resolution extreme events which have been noted as causing mass mortalities in several species (Wethey *et al.*, 2011). It would be advantageous to explore biological responses to particular events where possible, as well as long term trends. Measuring a biological response to an extreme event is considerably easier if carried out by an adjacent research institute (Muñoz-Colmenero *et al.*, 2015). Likewise, regular observations and experimentation may better reveal interactions such as intraspecific and interspecific competition in patellids around the Bay of Biscay, as has already been established on the coast of Portugal (Boaventura *et al.*, 2002; Boaventura *et al.*, 2003).

Regarding the population genetics in Chapter 3, Swanage was the only British locality for *S. umbilicalis* and a limited number of samples were used. To further explore the role of the English Channel as a habitat gap, as well as the influence of decreasing abundance closer to the northern range limit on population genetics (Fenberg *et al.*, 2014), further genetic data from other British localities would be required (Hoarau *et al.*, 2007). Shell morphology and genetic data for individuals (Irie, 2005; Johnson & Black, 2008), was unfortunately not combined due to the time and equipment available in fieldwork. With both additional genetic samples and associated morphological data, it would be possible to test whether population genetics is associated with differing aspect ratio and other morphological traits (Chrismas *et al.*, 2014).

It would also be interesting to consider the biological role of different genes on the mitochondrial DNA and explore the association with selection. Whilst these differences are more likely to be apparent at the interspecific level (James *et al.*, 2016), they may also be explored at the intraspecific level (Chrismas

et al., 2014), particularly for localities that show both genetic differentiation and different conditions which can be tested.

6.3 Concluding remarks

This study has principally been an exploration of the importance of sea temperature in driving patterns in intertidal species abundances, distribution, size, and morphology. The distribution of rocky substrate and biologically engineered habitat which can be indirectly affected by temperature, have also been shown to play a major role in species distributions, abundances, and genetic connectivity. This study contributes to an understanding of the patterns and processes acting to shape biogeographic trends around range centres and peripheries of coastal species. In the wider context, the results presented here contribute to the growing body of evidence used to consider and test basic biogeographic hypotheses with the broader applied implications for how species may respond in the future to anthropogenic climate change (Harley *et al.*, 2006; Hawkins *et al.*, 2008; Southward *et al.*, 1995).

Appendices

7.1 Appendix: Tests for null alleles

Table 7.1: "H.def" showing *S. umbilicalis* (top) and *S. pennanti* (bottom) Hardy-Weinberg equilibrium heterozygote-deficiency p-values with values below Bonferroni-corrected significance level in **bold**. "MC" showing MICROCHECKER results in determining whether null alleles were present (nulls) or absent (good). Locality codes correspond to Table 3.1.

Locus	U26231		U23195		U34184		U36148		U15541		U34428		U62438	
Code	H.def	MC	H.def	MC	H.def	MC	H.def	MC	H.def	MC	H.def	MC	H.def	MC
SW	**<0.0001**	Nulls	0.0011	-	**<0.0001**	Nulls	**0.0009**	Nulls	**<0.0001**	Nulls	**<0.0001**	Nulls	**<0.0001**	Nulls
C	0.0299	-	0.2711	Good	**<0.0001**	Nulls	0.2366	-	0.0732	Good	0.1836	Good	0.0086	-
LO	**<0.0001**	Nulls	0.1818	Good	**<0.0001**	Nulls	0.1885	Good	0.0030	-	**<0.0001**	Nulls	0.7655	Good
LP	**<0.0001**	Nulls	0.8165	Good	**<0.0001**	Nulls	0.0043	-	0.0155	Good	**<0.0001**	Nulls	1.0000	Good
B	**<0.0001**	Nulls	0.9257	Good	**<0.0001**	Nulls	0.0172	Good	0.0060	-	**<0.0001**	Nulls	0.5656	Good
S	**<0.0001**	Nulls	0.2191	Good	**<0.0001**	Nulls	**0.0608**	-	0.0567	Good	-	Good	0.6693	Good
V	**<0.0001**	Nulls	0.3646	Good	0.0011	-	**<0.0001**	Nulls	**<0.0001**	Nulls	**<0.0001**	Nulls	0.2363	Good

Locus	PEN79 FAM1		PEN86 HEX1		PEN146 NED1		PEN65 FAM2		PEN138 HEX2		PEN104 NED2	
Code	H.def	MC	H.def	MC	H.def	MC	H.def	MC	H.def	MC	H.def	MC
H	0.0635	Good	**<0.0001**	Nulls	**<0.0001**	Nulls	0.0924	Good	**<0.0001**	Nulls	**0.0010**	Nulls
LO	-	Good	**<0.0001**	Nulls	**<0.0001**	Nulls	0.0011	-	**<0.0001**	Nulls	**<0.0001**	Nulls
C	0.0154	Good	0.0023	Nulls	**<0.0001**	Nulls	0.2481	Good	**<0.0001**	Nulls	0.0178	Nulls
SJ	0.0022	-	**<0.0001**	Nulls	**<0.0001**	Nulls	**<0.0001**	Nulls	**<0.0001**	Nulls	**<0.0001**	Nulls
S	1.0000	Good	**<0.0001**	Nulls	**<0.0001**	Nulls	1.0000	Good	0.0061	Nulls	**0.0001**	Nulls
V	0.0297	Good	0.0014	-	**<0.0001**	Nulls	0.1714	Good	**<0.0001**	Nulls	**<0.0001**	Nulls

Locus	PEN168 FAM3		PEN145 NED3	
Code	H.def	MC	H.def	MC
H	0.1042	Good	0.2705	Good
LO	**0.0007**	Nulls	0.0061	Good
C	0.0475	Good	0.1167	Good
SJ	0.0561	Good	0.1718	Good
S	0.0759	Good	0.6306	Good
V	0.0947	Good	0.0498	Good

7.2 Appendix: Information for individual loci

Table 7.2: For individual loci for *S. umbilicalis*: number of samples (N); observed (H_o) and expected heterozygosities (H_e); number of alleles (A) found per locus and locality. Locality codes correspond to Table 3.1.

Locus	U26231				U23195				U34184				U36148				U15541				U34428				U62438			
Code	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A
SW	19	0.755	0.158	8	22	0.451	0.182	5	19	0.752	0.105	8	8	0.833	0.250	5	22	0.746	0.273	7	22	0.751	0.364	6	22	0.632	0.318	9
C	27	0.685	0.481	9	26	0.572	0.654	5	25	0.818	0.44	7	21	0.719	0.571	7	27	0.751	0.593	8	27	0.175	0.148	3	27	0.661	0.481	6
LO	29	0.792	0.310	10	29	0.553	0.517	6	29	0.849	0.345	8	22	0.67	0.591	8	29	0.728	0.552	9	29	0.336	0.172	4	28	0.628	0.571	6
LP	29	0.680	0.448	10	29	0.609	0.655	6	28	0.821	0.429	8	28	0.645	0.393	6	29	0.739	0.655	8	29	0.285	0.103	5	29	0.407	0.483	5
B	29	0.774	0.379	13	29	0.461	0.552	7	29	0.802	0.276	9	20	0.619	0.65	5	29	0.728	0.448	9	29	0.251	0.103	3	29	0.489	0.552	4
S	24	0.689	0.292	12	24	0.592	0.667	6	23	0.843	0.174	7	23	0.730	0.478	6	24	0.739	0.625	7	24	0	0	1	24	0.480	0.417	6
V	27	0.732	0.481	12	27	0.461	0.444	6	22	0.793	0.409	7	21	0.609	0.238	5	27	0.704	0.481	7	27	0.384	0.074	4	26	0.618	0.538	6
Sum	184			74	186			41	175			54	143			42	187			55	187			26	185			42

Table 7.3: For individual loci for *S. pennanti*: number of samples (N); observed (H_o) and expected heterozygosities (H_e); number of alleles (A) found per locus and locality. Locality codes correspond to Table 3.1.

Locus	PEN79				PEN86				PEN146				PEN65				PEN138				PEN104				PEN168				PEN145			
Code	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A
H	33	0.145	0.091	3	32	0.838	0.156	10	31	0.911	0.226	17	33	0.116	0.061	2	33	0.645	0.061	4	33	0.415	0.212	6	29	0.688	0.517	4	30	0.773	0.633	8
LO	30	0	0	1	25	0.86	0.36	11	26	0.911	0.308	15	30	0.188	0.067	3	30	0.681	0.067	4	30	0.354	0.067	5	30	0.701	0.500	6	30	0.772	0.700	7
P	20	0.188	0.1	3	13	0.757	0.385	7	20	0.863	0.300	9	20	0.233	0.150	4	19	0.622	0.105	3	20	0.309	0.150	3	20	0.641	0.45	5	20	0.764	0.600	5
SJ	17	0.269	0.059	3	17	0.840	0.231	8	13	0.825	0.231	7	19	0.613	0.105	4	15	0.687	0.067	4	19	0.546	0.211	6	16	0.647	0.563	6	19	0.761	0.632	6
S	26	0.112	0.115	3	25	0.850	0.280	12	25	0.886	0.200	13	26	0.208	0.231	2	26	0.212	0.077	3	26	0.478	0.231	6	26	0.61	0.423	4	26	0.653	0.808	4
V	28	0.2	0.143	3	20	0.812	0.400	9	25	0.913	0.32	13	29	0.16	0.103	2	28	0.386	0.036	3	29	0.411	0.138	5	28	0.644	0.536	6	29	0.75	0.655	7
Het. Def. P	0				0				0				0				0				0				0				0.0002			
Sum	154			16	132			57	140			74	157			17	151			21	157			31	149			31	154			37

7.3 Appendix: Locality environmental variables

This appendix corresponds to the environmental data used in Chapters 4 and 5.

Table 7.4: Environmental variables for different localities included in table 4.1: SSTM = mean sea surface temperature, AirTM = mean air temperature, Exposure = wave exposure variable; R_1 = % rock in 1 km of locality; R_5 = % rock in 5 km of locality; Dtogap = longshore distance to habitat gap in km, lnDgap = ln(Dtogap), AirTW = winter air temperature, AirTS = summer air temperature, SSTW = winter sea surface temperature, SSTS = summer sea surface temperature.

Locality	SSTM	AirTM	Exposure	R_1	R_5	Dtogap	lnDgap	AirTW	AirTS	SSTW	SSTS
A Coruna	15.16771	14.68	55.55351	49	24	4498.025	8.411394	9.962667	19.27901	13.65	17.08
Biarritz	16.62547	15.8317	53.59008	26	19	3.177	1.155937	10.605	20.885	12.99	20.78
Brest	13.50411	12.8825	133.0948	72	61	3146.546	8.054061	9.188333	16.54567	11.46	15.79
Castro-Urdiales	16.4871	13.7344	19.3109	66	82	521.167	6.256071	9.122333	18.53633	13.31	20.18
Les Pierrieres	15.1065	14.3241	71.78348	65	53	7.9	2.066863	8.141333	20.26434	10.71	19.55
Loctudy	13.98286	13.2294	36.79234	60	37	2204.116	7.698082	9.103667	17.275	11.07	16.86
Loredo	16.47645	13.6481	31.33721	73	34	917.801	6.821981	9.739333	17.86001	13.31	20.12
Luarca	15.83608	15.5568	25.41764	100	76	2712.42	7.905597	10.48433	20.60334	13.4	18.77
O Vicedo	15.73493	14.9031	54.13956	34	54	3341.402	8.114146	10.254	19.447	13.5	18.5
Pt du Chay	14.61894	13.9535	209.1724	53	16	173.803	5.157922	8.033	19.569	10.23	18.88
Pt St Gildas	13.85629	13.1588	111.1515	71	79	440.343	6.087554	8.126	18.195	9.66	17.68
Quiberon	13.9803	12.9193	294.4544	55	44	1132.53	7.032209	8.274667	17.52867	10.41	17.35
Ribadesella	16.26974	15.65542	31.30485	100	61	1829.397	7.511742	11.816	19.56367	13.36	19.6
Roscoff	13.01993	12.1529	97.31354	48	49	3334	8.111928	7.805333	16.56433	11.17	14.99
S Olonne	14.67956	14.1023	243.9056	34	44	309.718	5.735662	8.641667	19.24367	10.74	18.62
San Esteban	15.948	14.7294	36.60914	54	61	2646.974	7.881172	10.049	19.386	13.37	18.99
St Jean de Luz	16.67643	13.007	53.61879	46	44	14.755	2.691582	7.34	18.91133	13.08	20.81
SVDB	16.41171	12.9475	2.035848	66	56	1126.451	7.026827	9.074	17.30334	13.34	19.92
Villagarcia	15.34263	15.0828	1.002126	43	51	5288.623	8.573313	10.351	19.61967	13.76	16.84
Zumaia	16.6567	14.26	14.34292	72	85	99.894	4.60411	8.552667	20.022	13.22	20.68

7.4 Appendix: SACFOR field data

Table 7.5: SACFOR abundances for *Patella* at different localities at different dates including in table 4.1. S = Superabundant, A = Abundant, C = Common, F = Frequent, O = Occasional, R = Rare, NF = Not found.

Locality	Date	Latitude	Longitude	*Patella vulgata*	*Patella depressa*	*Patella ulyssiponensis*	*Patella rustica*
Roscoff	1/8/15	48.72927	-3.98871	A	O	R	NF
Brest	2/8/15	48.37611	-4.76194	A	F	A	NF
Loctudy	3/8/15	47.80944	-4.17556	A	C	C	NF
Quiberon	4/8/15	47.53415	-3.14635	A	C	C	NF
Pt St Gildas	5/8/15	47.13736	-2.24669	S	C	NF	NF
Pt du Chay	6/8/15	46.10861	-1.14333	A	A	NF	NF
Les Pierrieres	7/8/15	45.63972	-1.09528	S	A	NF	NF
Biarritz	10/8/15	43.48278	-1.56861	NF	S	C	A
St Jean de Luz	10/8/15	43.41078	-1.63763	NF	S	P	R
Castro Urdiales	11/8/15	43.37444	-3.20944	R	A	O	NF
San Vicente de la Barquera	12/8/15	43.39278	-4.39	O	A	O	A
Luarca	13/8/15	43.55083	-6.55556	A	A	A	NF
O Vicedo	14/8/15	43.7306	-7.68159	A	C	O	NF
Vilagarcia	15/8/15	42.47833	-8.92333	A	C	C	NF
A Coruna	16/8/15	43.34417	-8.35528	A	A	A	NF
San Esteban de Pravia	5/4/16	43.56539	-6.08149	C	A	F	O
Ribadesella	6/4/16	43.47471	-5.1018	F	A	C	O
Loredo	7/4/16	43.46974	-3.72677	F	A	C	F
Castro-Urdiales	8/4/16	43.38693	-3.22517	R	A	C	O
Biarritz	9/4/16	43.48278	-1.56861	NF	S	A	A
Les Pierrieres	10/4/16	45.63972	-1.09528	A	C	A	NF
Sables d'Olonne	16/4/16	46.48729	-1.76996	F	A	A	NF
St Jean de Luz	18/10/16	43.41078	-1.63763	NF	A	C	NF
Zumaia	19/10/16	43.30122	-2.25981	O	C	C	F

Table 7.6: SACFOR abundances for *Littorina* at different localities at different dates including in table 4.1. S = Superabundant, A = Abundant, C = Common, F = Frequent, O = Occasional, R = Rare, NF = Not found.

Locality	Date	Latitude	Longitude	*Littorina littorea*	*Littorina saxatilis/arcana/compressa*	*Littorina obtusata*	*Littorina fabalis*
Roscoff	1/8/15	48.72927	-3.98871	O	C	O	C
Brest	2/8/15	48.37611	-4.76194	NF	C	NF	R
Loctudy	3/8/15	47.80944	-4.17556	O	A	NF	C
Quiberon	4/8/15	47.53415	-3.14635	O	A	NF	F
Pt St Gildas	5/8/15	47.13736	-2.24669	A	A	NF	C
Pt du Chay	6/8/15	46.10861	-1.14333	S	C	NF	NF
Les Pierrieres	7/8/15	45.63972	-1.09528	C	F	NF	O
Biarritz	10/8/15	43.48278	-1.56861	NF	NF	NF	NF
St Jean de Luz	10/8/15	43.41078	-1.63763	NF	NF	NF	NF
Castro Urdiales	11/8/15	43.37444	-3.20944	NF	NF	NF	NF
San Vicente de la Barquera	12/8/15	43.39278	-4.39	F	NF	NF	NF
Luarca	13/8/15	43.55083	-6.55556	NF	O	NF	NF
O Vicedo	14/8/15	43.7306	-7.68159	F	A	NF	A
Vilagarcia	15/8/15	42.47833	-8.92333	R	A	NF	R
A Coruna	16/8/15	43.34417	-8.35528	NF	C	NF	NF
San Esteban de Pravia	5/4/16	43.56539	-6.08149	NF	C	NF	NF
Ribadesella	6/4/16	43.47471	-5.1018	NF	O	NF	NF
Loredo	7/4/16	43.46974	-3.72677	NF	R	NF	NF
Castro-Urdiales	8/4/16	43.38693	-3.22517	NF	NF	NF	NF
Biarritz	9/4/16	43.48278	-1.56861	NF	NF	NF	NF
Les Pierrieres	10/4/16	45.63972	-1.09528	O	C	NF	NF
Sables d'Olonne	16/4/16	46.48729	-1.76996	O	C	NF	NF
St Jean de Luz	18/10/16	43.41078	-1.63763	NF	NF	NF	NF
Zumaia	19/10/16	43.30122	-2.25981	NF	NF	NF	NF

Table 7.7: SACFOR abundances for trochids at different localities at different dates including in table 4.1. S = Superabundant, A = Abundant, C = Common, F = Frequent, O = Occasional, R = Rare, NF = Not found.

Locality	Date	Latitude	Longitude	*Steromphala umbilicalis*	*Steromphala cineraria*	*Steromphala pennanti*	*Phorcus lineatus*	*Calliostoma zizyphinum*
Roscoff	1/8/15	48.72927	-3.98871	C	C	A	A	O
Brest	2/8/15	48.37611	-4.76194	A	NF	NF	O	R
Loctudy	3/8/15	47.80944	-4.17556	A	F	A	C	R
Quiberon	4/8/15	47.53415	-3.14635	S	C	A	C	NF
Pt St Gildas	5/8/15	47.13736	-2.24669	A	O	C	S	NF
Pt du Chay	6/8/15	46.10861	-1.14333	A	O	C	S	NF
Les Pierrieres	7/8/15	45.63972	-1.09528	C	NF	NF	F	NF
Biarritz	10/8/15	43.48278	-1.56861	F	NF	NF	C	NF
St Jean de Luz	10/8/15	43.41078	-1.63763	S	NF	NF	A	NF
Castro Urdiales	11/8/15	43.37444	-3.20944	A	NF	NF	C	NF
San Vicente de la Barquera	12/8/15	43.39278	-4.39	A	NF	F	C	NF
Luarca	13/8/15	43.55083	-6.55556	S	NF	O	C	NF
O Vicedo	14/8/15	43.7306	-7.68159	A	NF	NF	A	NF
Vilagarcia	15/8/15	42.47833	-8.92333	A	F	C	A	NF
A Coruna	16/8/15	43.34417	-8.35528	S	F	C	A	R
San Esteban de Pravia	5/4/16	43.56539	-6.08149	A	O	R	A	NF
Ribadesella	6/4/16	43.47471	-5.1018	A/C	O	C	C	NF
Loredo	7/4/16	43.46974	-3.72677	A	F	C	A	O
Castro-Urdiales	8/4/16	43.38693	-3.22517	F	R	NF	A	NF
Biarritz	9/4/16	43.48278	-1.56861	Patchy A	O	F	R	NF
Les Pierrieres	10/4/16	45.63972	-1.09528	C	NF	NF	O	NF
Sables d'Olonne	16/4/16	46.48729	-1.76996	A	R	A	A	NF
St Jean de Luz	18/10/16	43.41078	-1.63763	C	R	O	O	NF
Zumaia	19/10/16	43.30122	-2.25981	C	O	R	F	NF

Table 7.8: SACFOR abundances for muricids at different localities at different dates including in table 4.1. S = Superabundant, A = Abundant, C = Common, F = Frequent, O = Occasional, R = Rare, NF = Not found.

Locality	Date	Latitude	Longitude	*Nucella lapillus*	*Ocenebra erinacea*	*Ocenebrina aciculata*	*Stramonita haemastoma*	Other muricids (unidentified)
Roscoff	1/8/15	48.72927	-3.98871	O	R	NF	NF	NF
Brest	2/8/15	48.37611	-4.76194	C	R	NF	NF	NF
Loctudy	3/8/15	47.80944	-4.17556	O	R	NF	NF	NF
Quiberon	4/8/15	47.53415	-3.14635	C	O	NF	NF	NF
Pt St Gildas	5/8/15	47.13736	-2.24669	S	NF	NF	NF	NF
Pt du Chay	6/8/15	46.10861	-1.14333	O	A	NF	NF	NF
Les Pierrieres	7/8/15	45.63972	-1.09528	F	F	NF	NF	NF
Biarritz	10/8/15	43.48278	-1.56861	NF	NF	NF	NF	NF
St Jean de Luz	10/8/15	43.41078	-1.63763	NF	NF	NF	O	NF
Castro Urdiales	11/8/15	43.37444	-3.20944	NF	NF	NF	O	NF
San Vicente de la Barquera	12/8/15	43.39278	-4.39	NF	NF	NF	NF	O
Luarca	13/8/15	43.55083	-6.55556	R	NF	NF	NF	NF
O Vicedo	14/8/15	43.7306	-7.68159	C	R	NF	NF	NF
Vilagarcia	15/8/15	42.47833	-8.92333	C	R	NF	NF	NF
A Coruna	16/8/15	43.34417	-8.35528	R	R	NF	NF	NF
San Esteban de Pravia	5/4/16	43.56539	-6.08149	R	R	NF	O	NF
Ribadesella	6/4/16	43.47471	-5.1018	R	NF	R	F	NF
Loredo	7/4/16	43.46974	-3.72677	A	R	NF	O	NF
Castro-Urdiales	8/4/16	43.38693	-3.22517	NF	R	NF	F	NF
Biarritz	9/4/16	43.48278	-1.56861	NF	Dead shells	NF	O	O
Les Pierrieres	10/4/16	45.63972	-1.09528	C	O	NF	NF	NF
Sables d'Olonne	16/4/16	46.48729	-1.76996	F	R	NF	NF	NF
St Jean de Luz	18/10/16	43.41078	-1.63763	NF	NF	NF	F	NF
Zumaia	19/10/16	43.30122	-2.25981	NF	NF	NF	O	NF

Table 7.9: SACFOR abundances and presence for other gastropods at different localities at different dates including in table 4.1. S = Superabundant, A = Abundant, C = Common, F = Frequent, O = Occasional, R = Rare, NF = Not found, P = Present.

Locality	Date	Latitude	Longitude	*Cingula trifasciata*	*Rissoa spp.*	*Nassarius reticulatus*	*Aplysia punctata*	*Tricolia pullus*	*Melarhaphe neritoides*
Roscoff	1/8/15	48.72927	-3.98871	NF	NF	NF	P	NF	NF
Brest	2/8/15	48.37611	-4.76194	NF	NF	NF	NF	NF	NF
Loctudy	3/8/15	47.80944	-4.17556	NF	NF	NF	NF	NF	NF
Quiberon	4/8/15	47.53415	-3.14635	NF	S	NF	NF	NF	A
Pt St Gildas	5/8/15	47.13736	-2.24669	NF	NF	NF	NF	NF	NF
Pt du Chay	6/8/15	46.10861	-1.14333	NF	NF	NF	NF	NF	A
Les Pierrieres	7/8/15	45.63972	-1.09528	NF	NF	NF	NF	NF	C
Biarritz	10/8/15	43.48278	-1.56861	NF	NF	NF	NF	NF	A
St Jean de Luz	10/8/15	43.41078	-1.63763	NF	NF	NF	NF	NF	A
Castro Urdiales	11/8/15	43.37444	-3.20944	NF	NF	R	NF	NF	S
San Vicente de la Barquera	12/8/15	43.39278	-4.39	O	NF	O	NF	NF	A
Luarca	13/8/15	43.55083	-6.55556	NF	NF	NF	Eggs	NF	C
O Vicedo	14/8/15	43.7306	-7.68159	NF	NF	NF	Eggs	NF	S
Vilagarcia	15/8/15	42.47833	-8.92333	O	NF	NF	NF	NF	O
A Coruna	16/8/15	43.34417	-8.35528	C	NF	NF	NF	NF	C
San Esteban de Pravia	5/4/16	43.56539	-6.08149	NF	NF	C	NF	NF	A
Ribadesella	6/4/16	43.47471	-5.1018	NF	Patchy A	C	P	NF	A
Loredo	7/4/16	43.46974	-3.72677	NF	F	F	NF	NF	S
Castro-Urdiales	8/4/16	43.38693	-3.22517	NF	NF	NF	NF	R	A
Biarritz	9/4/16	43.48278	-1.56861	NF	O	O	NF	NF	A
Les Pierrieres	10/4/16	45.63972	-1.09528	NF	NF	NF	NF	NF	C
Sables d'Olonne	16/4/16	46.48729	-1.76996	NF	P	P	NF	P	A
St Jean de Luz	18/10/16	43.41078	-1.63763	NF	C	P	NF	O	A
Zumaia	19/10/16	43.30122	-2.25981	NF	NF	NF	NF	P	A

Table 7.10: SACFOR abundances and presence for bivalves at different localities at different dates including in table 4.1. S = Superabundant, A = Abundant, C = Common, F = Frequent, O = Occasional, R = Rare, NF = Not found, P = Present.

Locality	Date	Latitude	Longitude	*Lasaea rubra*	Oysters	Mussels
Roscoff	1/8/15	48.72927	-3.98871	P	NF	NF
Brest	2/8/15	48.37611	-4.76194	P	NF	NF
Loctudy	3/8/15	47.80944	-4.17556	NF	NF	NF
Quiberon	4/8/15	47.53415	-3.14635	NF	NF	NF
Pt St Gildas	5/8/15	47.13736	-2.24669	NF	NF	NF
Pt du Chay	6/8/15	46.10861	-1.14333	NF	S	NF
Les Pierrieres	7/8/15	45.63972	-1.09528	NF	S	A
Biarritz	10/8/15	43.48278	-1.56861	NF	NF	NF
St Jean de Luz	10/8/15	43.41078	-1.63763	NF	R	R
Castro Urdiales	11/8/15	43.37444	-3.20944	NF	C	C
San Vicente de la Barquera	12/8/15	43.39278	-4.39	NF	NF	NF
Luarca	13/8/15	43.55083	-6.55556	NF	NF	O
O Vicedo	14/8/15	43.7306	-7.68159	NF	NF	NF
Vilagarcia	15/8/15	42.47833	-8.92333	NF	NF	NF
A Coruna	16/8/15	43.34417	-8.35528	NF	NF	C
San Esteban de Pravia	5/4/16	43.56539	-6.08149	NF	NF	C
Ribadesella	6/4/16	43.47471	-5.1018	NF	NF	NF
Loredo	7/4/16	43.46974	-3.72677	NF	O	O
Castro-Urdiales	8/4/16	43.38693	-3.22517	NF	NF	NF
Biarritz	9/4/16	43.48278	-1.56861	NF	NF	O
Les Pierrieres	10/4/16	45.63972	-1.09528	NF	S	NF
Sables d'Olonne	16/4/16	46.48729	-1.76996	NF	P	P
St Jean de Luz	18/10/16	43.41078	-1.63763	NF	NF	NF
Zumaia	19/10/16	43.30122	-2.25981	NF	NF	NF

Table 7.11: SACFOR abundances and presence for other species at different localities at different dates including in table 4.1. S = Superabundant, A = Abundant, C = Common, F = Frequent, O = Occasional, R = Rare, NF = Not found, P = Present.

Locality	Date	Latitude	Longitude	*Sabellaria alveolata*	*Anemonia viridis*	*Actinia equina*	*Actinia fragacea*
Roscoff	1/8/15	48.72927	-3.98871	NF	NF	NF	NF
Brest	2/8/15	48.37611	-4.76194	NF	NF	NF	NF
Loctudy	3/8/15	47.80944	-4.17556	NF	NF	NF	NF
Quiberon	4/8/15	47.53415	-3.14635	NF	NF	NF	NF
Pt St Gildas	5/8/15	47.13736	-2.24669	NF	NF	NF	NF
Pt du Chay	6/8/15	46.10861	-1.14333	P	NF	NF	NF
Les Pierrieres	7/8/15	45.63972	-1.09528	S	NF	NF	NF
Biarritz	10/8/15	43.48278	-1.56861	NF	NF	NF	NF
St Jean de Luz	10/8/15	43.41078	-1.63763	NF	NF	NF	NF
Castro Urdiales	11/8/15	43.37444	-3.20944	P	NF	NF	NF
San Vicente de la Barquera	12/8/15	43.39278	-4.39	NF	NF	NF	NF
Luarca	13/8/15	43.55083	-6.55556	NF	A	NF	NF
O Vicedo	14/8/15	43.7306	-7.68159	NF	NF	NF	NF
Vilagarcia	15/8/15	42.47833	-8.92333	NF	NF	NF	NF
A Coruna	16/8/15	43.34417	-8.35528	NF	NF	NF	NF
San Esteban de Pravia	5/4/16	43.56539	-6.08149	NF	NF	C	NF
Ribadesella	6/4/16	43.47471	-5.1018	NF	P	C	P
Loredo	7/4/16	43.46974	-3.72677	NF	A	F	NF
Castro-Urdiales	8/4/16	43.38693	-3.22517	NF	NF	C	NF
Biarritz	9/4/16	43.48278	-1.56861	P	NF	C	NF
Les Pierrieres	10/4/16	45.63972	-1.09528	A	NF	NF	NF
Sables d'Olonne	16/4/16	46.48729	-1.76996	S	P	P	P
St Jean de Luz	18/10/16	43.41078	-1.63763	NF	NF	NF	NF
Zumaia	19/10/16	43.30122	-2.25981	NF	NF	NF	NF

7.5 Appendix: Quadrat data and *Steromphala* measurements

Appendix 7.5 (https://doi.org/10.5258/SOTON/D0618) on the Pure database system contains quadrat information (Quadrats.xlsx) with abundance of species and *Patella* measurements within each quadrat. It also contains *Steromphala pennanti* measurements (G_pen.csv) and *Steromphala umbilicalis* measurements (S_umb_measurements.xlsx).

7.6 Appendix: Relationship between different size measurements

This appendix briefly describes the relationship between mean, median and 90th percentile statistics of locality size measurements for *Patella vulgata*, *Patella depressa*, *S. umbilicalis* and *S. pennanti* based on chapter 4. There was a significant correlation between all of these statistics in every species, albeit with some scatter. However, as can been seen from both the scatterplots and normal quantile plots, towards larger sizes mean size does not have a linear relationship with the 90th percentile for *S. pennanti*, *Patella depressa* and, less clearly due to high scatter throughout the data, *Patella vulgata* (Figures 7.1-7.6). Instead, as the 90th percentile size increases, the mean size begins to level off. As both statistics are easy to calculate and their relationship varies, they should continue to be applied separately when considering relationships between size and other factors. For convenience, the normal quantile plots and scatterplots for each species are placed on the same page.

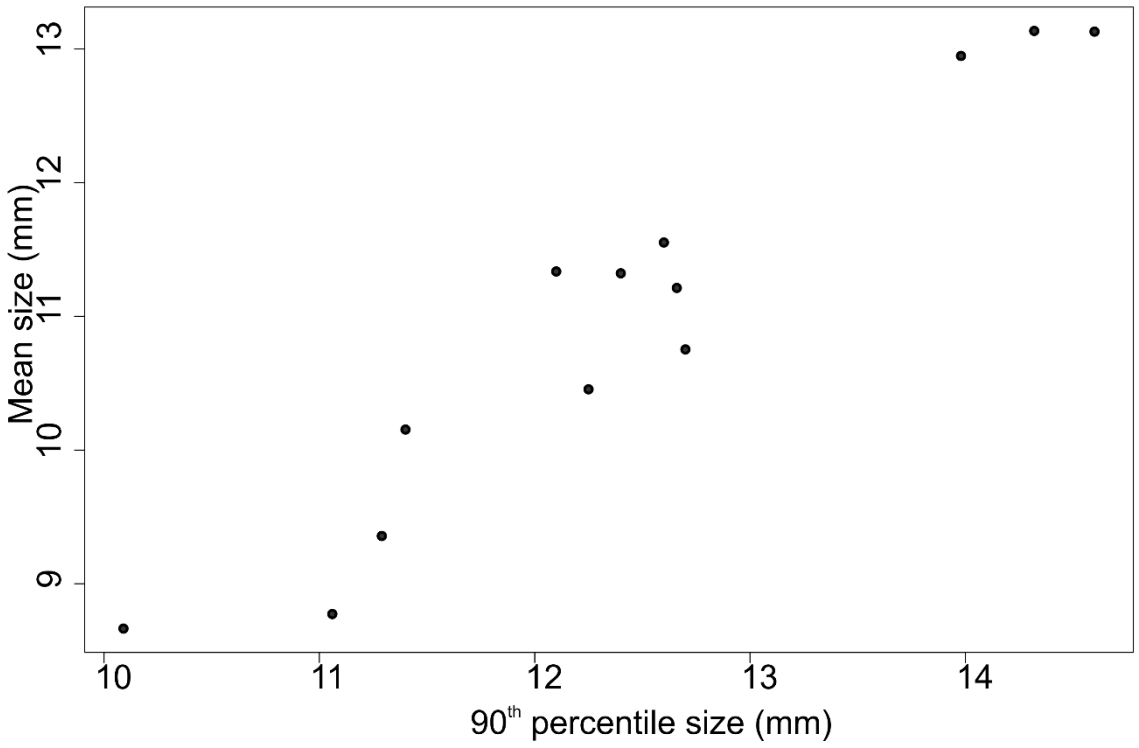


Figure 7.1: Scatterplot showing the relationship between mean and 90th percentile of shell width for *S. pennanti.* As a linear regression, $R^2 = 0.9171$, $p = 1.698 \times 10^{-7}$.

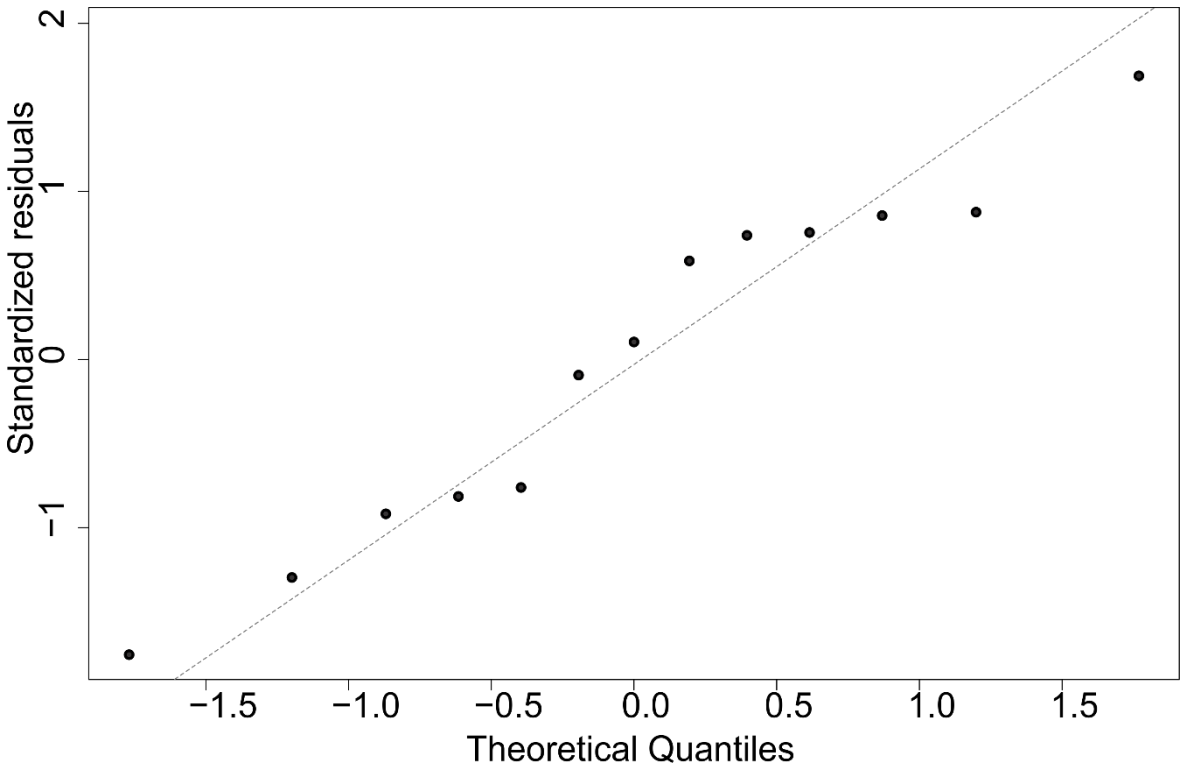


Figure 7.2: Normal quantile plot based on scatterplot showing the relationship between mean and 90th percentile of shell width for *S. pennanti* (Figure 7.1).

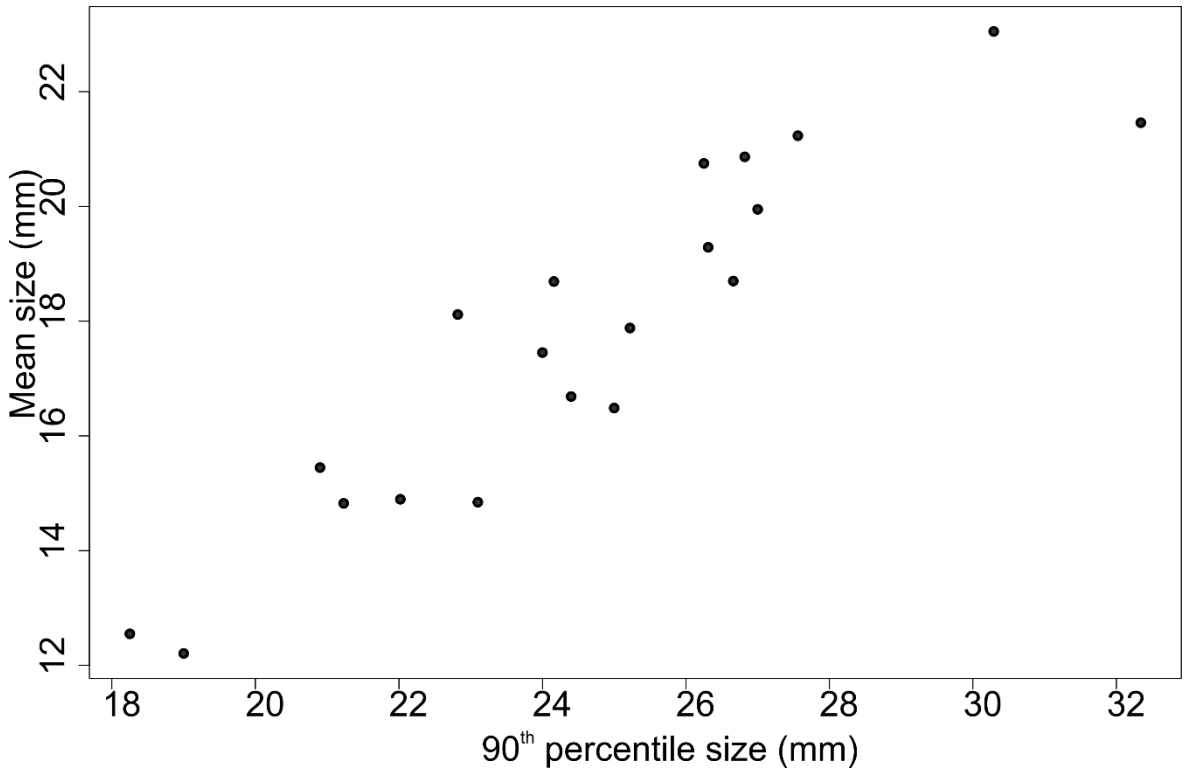


Figure 7.3: Scatterplot showing the relationship between mean and 90th percentile of shell size for *Patella depressa*. As a linear regression, $R^2 = 0.8371$, $p = 9.932 \times 10^{-9}$.

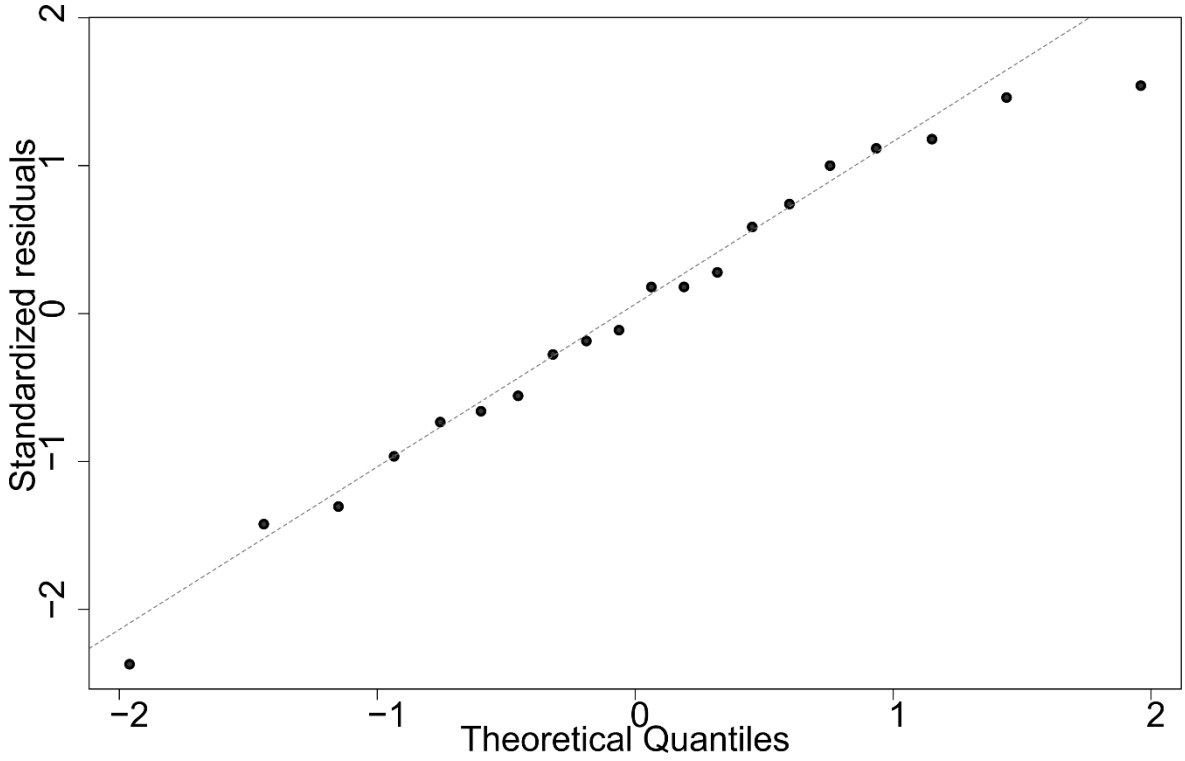


Figure 7.4: Normal quantile plot based on scatterplot showing the relationship between mean and 90th percentile of shell width for *Patella depressa* (Figure 7.3).

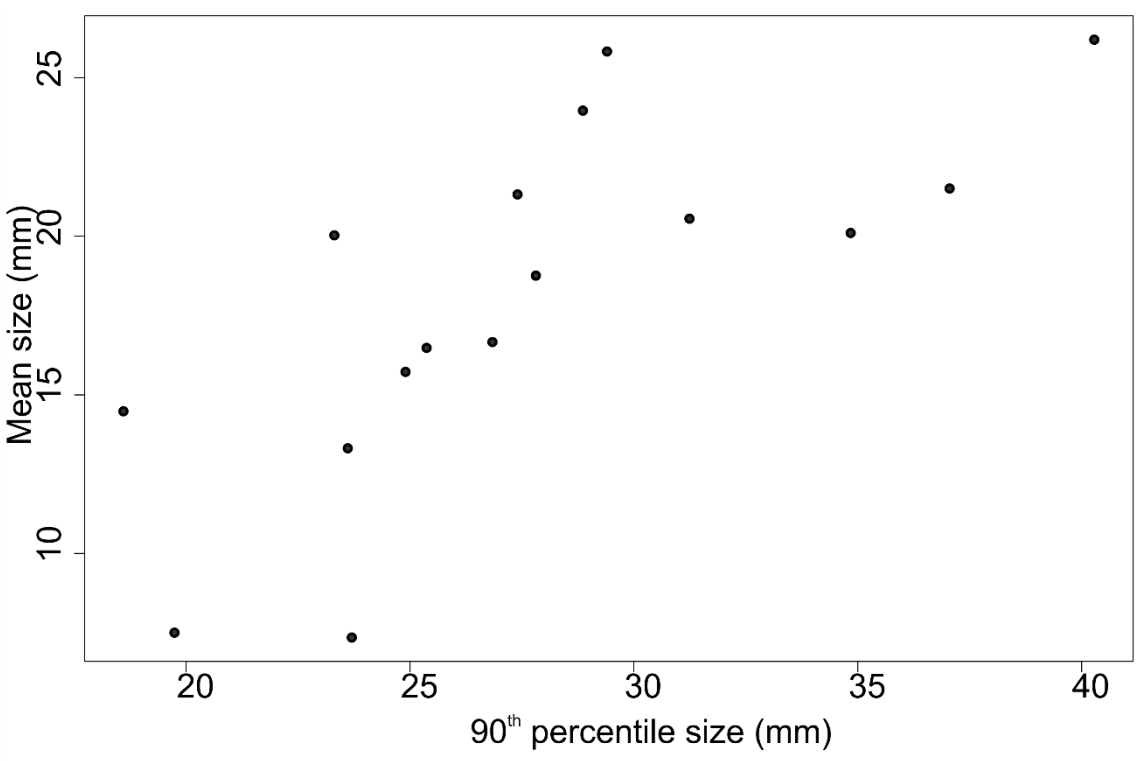


Figure 7.5: Scatterplot showing the relationship between mean and 90th percentile of shell size for *Patella vulgata.* As a linear regression, $R^2 = 0.521$, $p = 0.001594$.

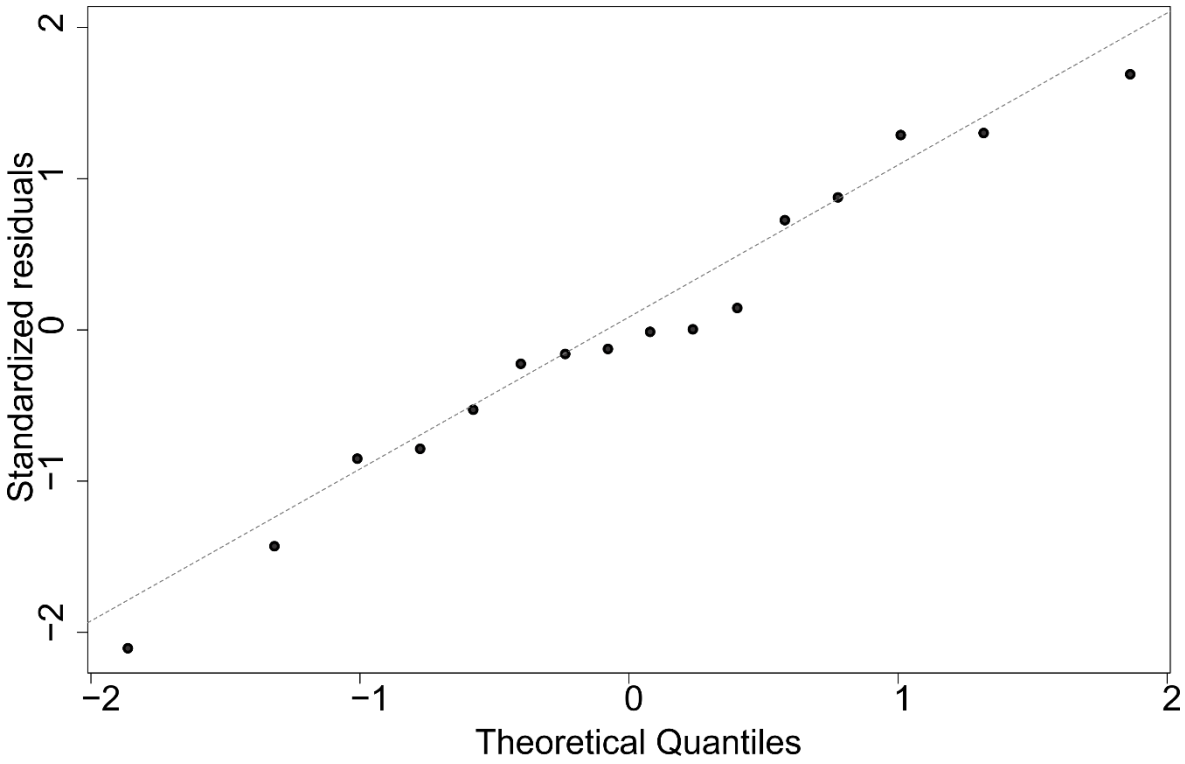


Figure 7.6: Normal quantile plot based on scatterplot showing the relationship between mean and 90th percentile of shell width for *Patella vulgata* (Figure 7.5).

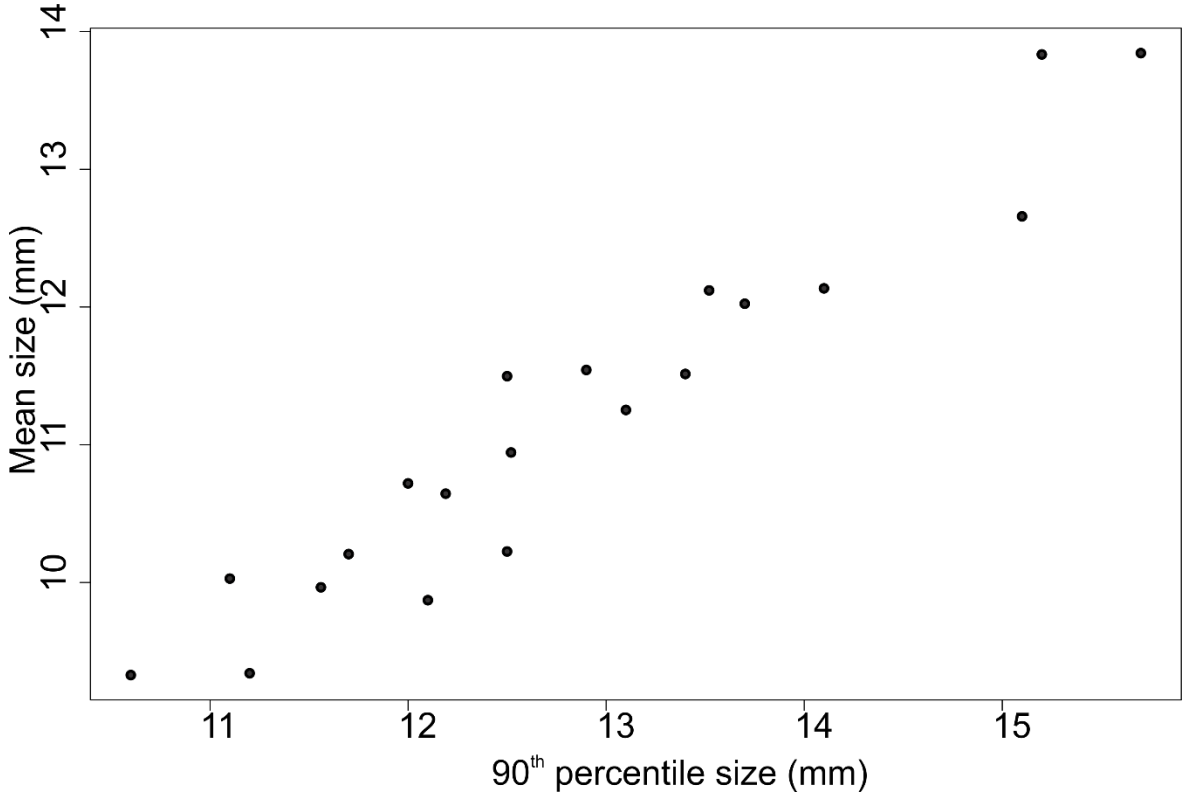


Figure 7.7: Scatterplot showing the relationship between mean and 90th percentile of shell width for *S. umbilicalis*. As a linear regression, $R^2 = 0.9185$, $p = 1.88 \times 10^{-11}$.

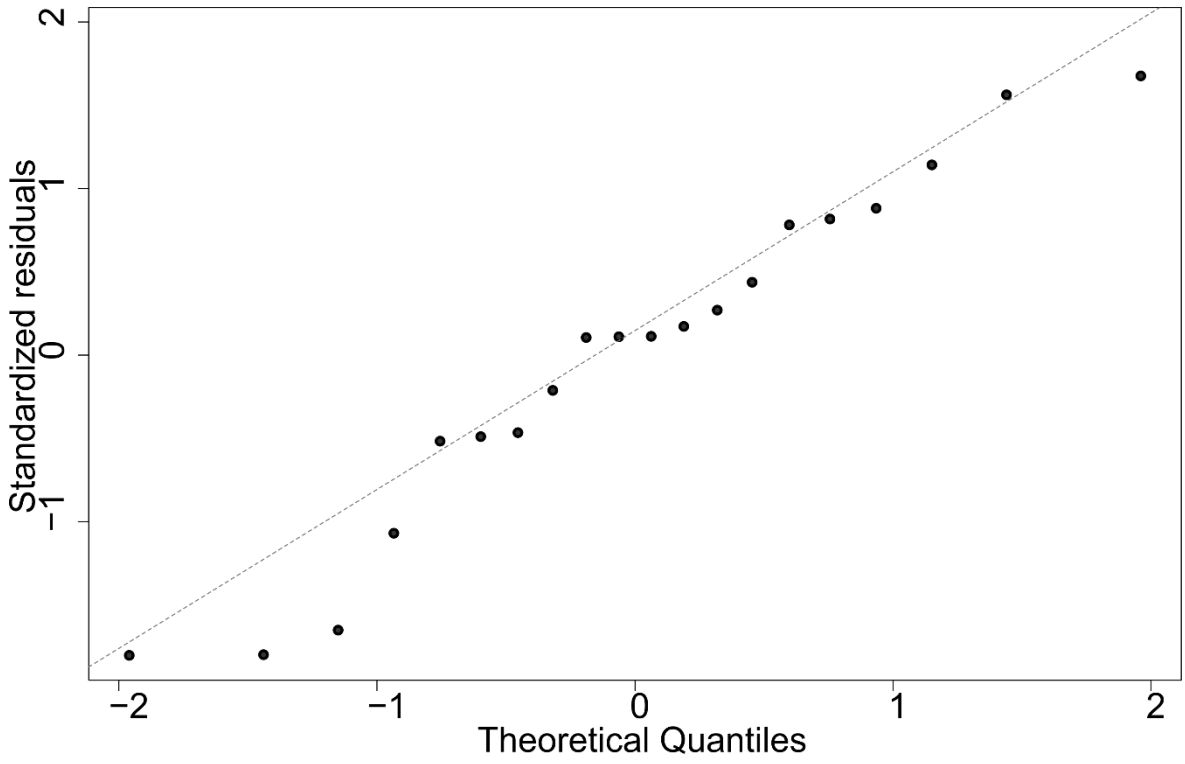


Figure 7.8: Normal quantile plot based on scatterplot showing the relationship between mean and 90th percentile of shell width for *S. umbilicalis* (Figure 7.7).

7.7 Appendix: Quadrat data and *Steromphala* measurements

Appendix 7.7 (https://doi.org/10.5258/SOTON/D0618) on the Pure database system contains quadrat information (Quadrats.xlsx) with abundance of species and *Patella* measurements within each quadrat. It also contains *Steromphala pennanti* measurements (G_pen.csv) and *Steromphala umbilicalis* measurements (S_umb_measurements.xlsx).

Glossary of terms and acronyms

AICc = Corrected Akaike Information Criterion

AMOVA = Analysis of Molecular Variance

Aspect ratio = In this study, shell width divided by shell height

AT = Air Temperature

ATM = Mean Air Temperature

ATS = Summer Air Temperature

ATW = Winter Air Temperature

Bay of Biscay = In this study, the region of the Atlantic on the western coast of France (excluding the English Channel) and northern Iberian Peninsula

Biogeography = A field of study dedicated to identifying the biotic and abiotic factors affecting patterns within and between species across large spatial scales (Briggs, 1974)

Bp = Base pairs

CTAB = Cetyl Trimethylammonium Bromide

Congener = Species of the same genus

DAPC = Discriminant Analysis of Principal Components

EDTA = Ethylenediaminetetraacetic acid

EEA = European Environment Agency

EXP = Wave Exposure

FLA = Fragment Length Analysis

FUBAR = Fast Unconstrained Bayesian Approximation

GP = Distance to the habitat gap

Haplotype = In this study, a particular DNA sequence found at a given locus inherited from a single parent

IBD = Isolation By Distance

Intertidal = In this study, between the spring high tide and low tide water level, including splash zone

Lecithotrophic = Non-feeding larvae (dependent upon egg's yolk reserve)

Locus = In this study, a targeted region of DNA

LSDR = Local Size-Density Relationship

MC = Monte Carlo

MCMC = Markov Chain Monte Carlo

Mt = Mitochondrial

PCG = Protein Coding Genes

PCR = Polymerase Chain Reaction

Phylogenetic = Interspecific genetic lineage

PP = Posterior Probability

Primer = Artificial DNA strand used in PCR to amplify a given locus

PVP = Polyvinylpyrrolidone

rRNA = Ribosomal Ribonucleic Acid

SE = Standard Error

SNP = Single Nucleotide Polymorphism

SSR = Simple Sequence Repeat (synonymous with microsatellite)

SST = Sea Surface Temperature

SSTM = Mean Sea Surface Temperature

SSTS = Summer Sea Surface Temperature

SSTW = Winter Sea Surface Temperature

Tris = Trisaminomethane

tRNA = Transfer Ribonucleic Acid

TS = Timed search

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