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The long-term Recovery of the Bioindicator Species *Nucella lapillus* from Tributyltin Pollution.

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Abstract

In an attempt to control marine biofouling, a co-polymer paint system was developed containing the biocide tributyltin (TBT) in the 1960s. TBT was found to be highly toxic to non-target organisms, particularly to female common dogwhelk (*Nucella lapillus*) in the UK. TBT blocks hormone conversion of testosterone to oestradiol, resulting in the development of a penis and vas deferens (imposex). Females die as male sex organs overgrow the oviduct, leaving them unable to deposit eggs and so suffer internal rupture. The toxicity of TBT led to legislation controlling its use from the mid 1980s and the intention for a total ban by 2008.

Long-term monitoring of *N. lapillus* populations in Plymouth, UK south coast and Isle of Wight has showed recovery from TBT effects since the 1980s, although imposex in some populations has recently increased. Imposex has reduced at sites directly affected by shipping and population recovery has been seen at some sites from where *N. lapillus* had previously been absent. At some sites close to spoil dumping grounds, imposex has risen and may have been adversely affected by TBT associated with dredged harbour sediments. At some south coast sites *N. lapillus* population fluctuations reflected barnacle abundance, highlighting the uncertainty associated with elucidation of cause-effect relationships.

Recovery from TBT impacts through recolonisation and colonisation at some sites has been achieved despite *N. lapillus'* lack of a pelagic stage. Study of man-made groynes at Highcliffe, Dorset, indicated that recruitment may occur due to passive transport from inshore subtidal populations. Reintroduction trials at a formerly occupied site showed that *N. lapillus* were robust enough to survive translocation, but TBT levels prevented re-establishment of a viable population through severe imposex effects. Passive transport may facilitate the use of artificial sites with viable habitat and tolerable TBT levels as stepping-stones for recolonisation of isolated sites that have suffered local extinction.

The broader effects of TBT on UK rocky shore communities through predation by *N. lapillus* are not well understood. On an artificial shore (Highcliffe, Dorset), disturbance was shown to be more influential than *N. lapillus* predation on community structure. This finding may not apply to other, natural, shores in the UK, but demonstrated the importance of stochastic processes in mediating community structure.

The use of *N. lapillus* to measure TBT pollution is reliable, but impacts of *N. lapillus* population changes and predation effects on intertidal community structure are less robust indicators of TBT effects than imposex levels. Moreover, the accurate appraisal of TBT impacts and effectiveness of legislation urgently need to acknowledge the potential of TBT release from dredged spoil to damage further the biota of the UK's rocky shores.

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Chapter 1. INTRODUCTION

1.1 Thesis background

Extensively used worldwide, organotin antifouling paints have proven very effective at inhibiting fouling organisms on ships and stationary structures (Evans, 1970; Champ and Pugh, 1987; Lyons, 1995). Unfortunately these tributyltin oxide (TBTO) based paints have also achieved notoriety as highly toxic compounds adversely affecting non-target organisms (Lee, 1991; Fent, 1996; Watermann, 1999; Kobayashi and Okamura, 2002). Throughout the world, stenoglossan gastropods, in particular, have been proven to be highly vulnerable to organotin pollution (Bryan *et al.,* 1986; Gibbs and Bryan, 1986; Stewart and Demora, 1992; Douglas *et al.,* 1993; Swennen *et al.,* 1997; Reitsema *et al.,* 2002). In the UK the common dogwhelk (*Nucella lapillus*, formerly known as *Thais lapillus*) is the most sensitive non-target organism to tributyltin oxide and females suffer morphological changes and eventual death following chronic exposure to this substance (Bryan *et al.,* 1987; Gibbs *et al.,* 1987). Particularly in areas of commercial shipping and yachting, this impact resulted in the decline and in some cases local extinction of *N. lapillus* populations (Gibbs and Bryan, 1986; Spence *et al.,* 1990; Douglas *et al.,* 1993). The south coast of the UK is particularly severely impacted by TBTO paints and many *N. lapillus* populations have declined (Bryan *et al.,* 1986; Spence *et al.,* 1990).

The overall aim of this research was to examine the long-term recovery, recolonisation and community influence of *N. lapillus,* focusing on UK south coast sites. Using *N. lapillus* as a bioindicator of tributyltin (TBT) pollution, this work builds on previous studies by Spence (1989) and Proud (1994) who investigated TBT-impacted populations in Plymouth Sound. To complement this, the long-term health of populations on the south coast from Osmington Mills (Dorset) to Selsey (West Sussex), and on the Isle of Wight have also been considered. *N. lapillus* have no known pelagic life stage, but have shown an ability to recover at sites at which they became extinct and to colonise new habitats (e.g. Moore, 1940; Herbert *et al.,* 2000). This research also investigates the ability of *N. lapillus* to recolonise and recover from tributyltin impacts and, in addition, methods of reintroducing *N. lapillus* to a formerly populated site are tested. Finally, several authors (e.g. Connell, 1961; Menge, 1976, 1978a) suggest that predatory gastropods, including *N. lapillus*, have a role in structuring rocky shore communities. Work on the influence of *N. lapillus* on community structure was investigated using a series of limestone block groynes at Highcliffe, Christchurch Bay, Dorset as tractable experimental systems.

The rest of this introduction outlines the history of the development of organotin paints and reviews their impacts on non-target organisms with particular reference to *N. lapillus*. A brief overview of the general biology and ecology of *N. lapillus* is also given as much of this is central to the experimental work which follows. Following identification of worldwide legislative actions against organotin paints, work on the recovery of non-target gastropoda is reviewed. Finally the rationale, aims and objectives of the study are presented.

1.2 Biofouling and its prevention

Numerous marine organisms such as sessile animals and algae can grow and survive on hard surfaces at the exposure extremes of rocky intertidal and subtidal habitats. When they attach to man-made structures this is known as fouling and biofouling (Clare, 1995; Callow and Callow, 2000; Yan and Yan, 2003; Konstantinou and Albanis, 2004). The rough surface that biofouling creates leads to increased drag and thus increased fuel consumption and larger bills for shipping and heightened corrosion of stationary structures (Evans, 1981; Edyvean 1985; Ten Hallers-Tjabbes, 1997); these effects are compounded by increased maintenance costs (Ludgate, 1987; Champ, 2001a, 2003). It has been shown that an estimated 50% of the operating costs of commercial vessels are from fuel consumption. A 10 μm increase in hull roughness is predicted to result in a 0.3-1% increase in fuel consumption and a hull with 5% fouling cover will result in a fuel consumption increase of 5-10% (Champ and Lowenstein, 1987); a vessel with 33% fouling cover will lead to 50% increased fuel costs (Ludgate, 1987). This, coupled with regular dry-docking to remove fouling organisms, leads to increased shipping costs, potentially greater environmental issues and decreased profit margins (Champ, 2001a; Champ, 2003).

The International Maritime Organisation (IMO) is at the forefront of the promotion of "..*efficiency of navigation and prevention and control of marine pollution from ships*" (IMO, 2000) and recognises that the major impact of fouling on shipping is one of economic importance (Table 1.1). This has led to methods to improve the efficiency of travel at sea and to reduce fouling of stationary structures such as oil rigs, fish cages (Konstantinou and Albanis, 2004) by the development of antifouling paints which minimise biofouling and thus reduce costs. Despite this, fouling is still acknowledged to have a major impact on the profitability of commercial fleets and research into efficient antifouling coatings remains active (e.g. Alzieu, 1995; Milne, 1996; Watermann, 1999; Champ, 2001b; Phillippi *et al.,* 2001; Konstantinou and Albanis, 2004; Yebra *et al.,* 2004). It is now widely acknowledged, however, that antifouling compounds can have significant detrimental impacts upon non-target marine organisms (Pereira *et al.,* 1999; Konstantinou and Albanis, 2004)

TABLE 1.1 BIOFOULING ISSUES: THE SHIPPING INDUSTRY PERSPECTIVE.
(Modified from IMO, 1999 based on MEPC,1996).

Fouling explained	
What is fouling?	Fouling is an unwanted growth of biological material - such as barnacles and algae - on a surface immersed in water.
How much fouling does an unprotected structures get?	Vessel bottoms not protected by antifouling systems may gather 150 kg of fouling per square metre in less than six months of being at sea. On a very large crude oil carrier with 40,000 square metre underwater areas, this would add up to 6,000 tonnes of fouling.
Why do ships and static structures need antifouling systems?	Small amounts of fouling can lead to an increase of vessel fuel consumption, as the resistance to movement will be increased. A clean ship can sail faster and with less energy. Stationary structures suffer increased corrosion and therefore maintenance costs increase.
How do antifouling systems save a ship owner money?	An effective antifouling system can save a ship owner money in a number of ways: • Direct fuel savings by keeping the hull free of fouling organisms; • Extended dry-docking interval, when the antifouling system provides several years of use; • Increased vessel availability - as it does not have to spend so much time in dry dock.

Over 2000 marine species cause biofouling (Evans, 1970), and of these, barnacles are the most significant (Christie and Dalley, 1987). There are records of attempts to control barnacles and other fouling organisms from as early as 300 BC, when lead sheets were used to cover hulls (Stebbing, 1985; Proud, 1994). From the reign of Henry VIII, when the English Navy was substantially increased (Herman, 2004), there are records of antifouling coating trials of lime or oil laced with sulphur, arsenic and gunpowder (Clare, 1995; Ten Hallers-Tjabbes, 1997; IMO, 1999). Copper sheeting was later used as a relatively effective coating and this led to the development of the first antifouling paints at the turn of the 19th century (Stebbing, 1985).

Most modern antifouling paints became based on organometal compounds which inhibit biofouling effectively (Evans, 1970). The organometal elements are toxic biocides and when applied to a hull or structure cause the death of fouling organisms at the settlement stage (Stebbing, 1985; Christie and Dalley, 1987). Original versions of organometal antifouling paints were based on copper (II) oxide and later arsenic and mercury (Stebbing, 1985; Christie and Dalley, 1987; IMO, 1999). In the 1960s, however, early versions of organotin paints were found to be a more cost-effective method of fouling prevention (Evans, 1970; IMO, 1999). These compounds had originally been developed in the 1920s as a moth inhibitor (Santillo *et al.,* 2002), and used in the 1940s as wood preserver and fungicide (Evans, 1970). For use as an antifoulants, the most effective organic compound of tin was found to be tributyltin oxide (TBT). Although TBT was available in the United States by the late 1960s it was not widely distributed in the UK and other countries until the early 1970s (Stebbing, 1985; Herbert, 1988; Santillo *et al.,* 2002).

TBT was a major breakthrough in antifouling paints, being initially developed as a ' free association' paint which used 'contact leaching' to release the biocide (Champ and Pugh, 1987). Contact-leaching paints include hard epoxy, modified epoxy, oil, and alkyd compounds; the TBT within them leaches out when it is exposed to water (Stebbing, 1985; Nicholson, 2002). The biocide release rate in free association paints was subsequently found to be too high, so in the 1970s self-polishing co-polymer paints were developed (Champ and Pugh, 1987). With co-polymer products the organotin compounds are chemically bonded to a polymer base (Evans, 1970; Champ and Pugh, 1987; IMO, 1999). The biocide discharge rate of these paints is regulated through the reaction of seawater with the surface of the compound and the biocide is slowly released through wave action or due to forward motion wearing the co-polymer paint away. Once the surface covering is worn off biocide release begins again with the next layer (Fig. 1.1). In this way the leaching rate is consistent throughout the life of the paint. This extended the useful life of antifoulants and it became possible for ships to continue commercial operations for up to 60 months without repainting (Callow, 1990; IMO, 1999).

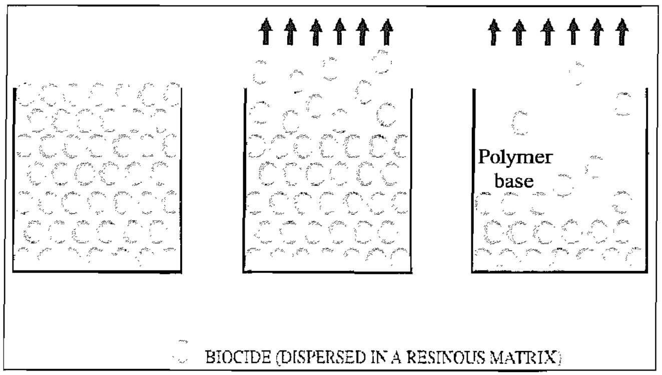

FIGURE 1.1 SCHEMATIC REPRESENTATION OF THE SELF-POLISHING ACTION OF TBT PAINTS.
(Modified from IMO, 1999).

Because of the self-polishing assets of the TBT biocide system, not only were fouling organisms inhibited at an early stage, but also they were unable to attach for prolonged periods due to the unstable substrate. The result was the considerable reduction of draginflated fuel bills for shipping and a lessening of the corrosion of stationary structures (Ludgate, 1987; Clare, 1995).

1.3 TBT effects on non-target species

Although the effectiveness of TBT based paints delivered an efficient solution to the problem of biofouling, concerns were raised regarding the indiscriminate release of the biocide into the marine environment (Thomas, 1969; Alzieu *et al.,* 1986). For example in Canada in the late 1960s, a recommendation was made that TBT use near shellfish farms should be avoided (Thomas, 1969). In the 1970s-1980s links between the toxicity of TBT and its potential effects on non-target organisms were increasingly found (Smith, 1981a,b,c; Feral and Le Gall, 1983; Fridberg, 1985; Alzieu *et al.,* 1986; Wade *et al.,* 1988). By the time that TBT antifouling paints were readily available in the UK it was apparent that there were problems with these compounds. Despite this, their efficiency and wide availability in the 1970s resulted in their burgeoning use in the USA, Canada, Europe and the UK (Evans, 1970; Champ and Pugh, 1987; Santillo *et al.,* 2002).

From the early to mid 1980s to the present day, studies have shown that at low levels organotin compounds can impact non-target species (e.g. Waldock and Thain, 1983; Alzieu *et al.,* 1986; Thain and Waldock, 1986; Bryan *et al.,* 1986; Gibbs *et al.,* 1987; Langston *et al.,* 1990; Waite, *et al.,* 1991; Minchin and Minchin, 1997; Miller *et al.,* 1999; Yan and Yan, 2003; Terlizzi *et al.,* 2004; Roepke *et al.,* 2005). Microscopic components of the marine community such as phyto and zooplankton have been adversely affected. For example, Beaumont and Newman (1986) found that at levels as low as 0.001-1.092 $\mu g/l^{-1}$ TBT restricted the growth of micro algae and U'ren (1983) found that the substance was highly toxic to the copepod *Acartia tonsa.* Dahl and Blanck (1996) also reported that with TBT at levels of 1 ngl^{-1} artificial periphyton communities suffered reduced biomass and photosynthesis, and showed a shift towards tolerant species.

High levels of TBT are generally associated with commercial shipping (e.g. Minchin *et al.,* 1997; Morgan *et al.,* 1998), and sensitive species in areas such as harbours, marinas and oil terminals can be exposed to greater concentrations than reported by Beaumont and Newman (1986). Langston *et al.,* (1994) reported that waterborne TBT at Lymington near Southampton, used largely by pleasure craft, was in the region of 7 ngl^{-1}, although this was a marked decrease from a high in 1988 of 800 ngl^{-1}. In comparison, at the commercial port of Southampton levels in June 1994 were still recorded at 43 ngl^{-1}. TBT at these levels affects the physiology, growth and behaviour of organisms and has caused widespread death in commercial and non-commercial species (e.g. U'ren, 1983, Alzieu *et al.,* 1986; Bryan *et al.,* 1986; Almedia *et al.,* 1999; Gooding *et al.,* 1999; Alzieu, 2000; Jha *et al.,* 2000; Zhou *et al.,* 2001; Birchenough *et al.,* 2002a; Kobayashi and Okamura, 2002).

Although TBT is poisonous to a range of organisms from plankton (U'ren, 1983; Peterson and Gustavson, 2000) to higher level organisms (e.g. Pearce, 1996, 1999; Kannan *et al.,* 1997; Tanabe *et al.,* 1998; Grinwis *et al.,* 1998) and potentially humans (Heidrich *et al.,* 2001; Nielsen and Rasmussen, 2004), worldwide TBT is most toxic to stenoglossan (central toothed radula with 1-3 marginal teeth) gastropods with at least 72 susceptible species (Morgan *et al.,* 1998; see also Ellis and Pattisina, 1990; Fioroni *et al.,* 1991). Although the widespread toxicity of TBT-based paints was apparent in the late 1970s and 1980s, moves to limit their use did not occur until commercial shellfish were affected (Alzieu *et al.,* 1986; Waldock, 1986; Champ, 2000).

Antifouling paints were developed to particularly inhibit barnacles, but paradoxically they are the least affected by TBT (Goldberg, 1986). In contrast the commercial oyster (*Crassostrea gigas*) industry in Arcachon Bay, France, declined at the same time as growers of *C. gigas* along the east coast of England reported abnormal shell forms (Alzieu *et al.,* 1986; Alzieu, 1991; Alzieu, 1995; Champ, 2000). A link to TBT was made by researchers in the UK (Waldock and Thain, 1983; Thain and Waldock, 1986) and France (Alzieu *et al.,* 1986) who suggested that organotins were affecting the oysters. In adult *C. gigas* exposure to levels of TBT as low as 20 ng l^{-1} causes shell deformities (excessive shell calcification resulting in reduced valve volume), and from 1975 this and no spatfall were recorded in the French South Atlantic oyster culturing areas (Alzieu *et al.,* 1986). *In situ* experimentation showed that embryogenesis and larval development of oysters had been inhibited by TBT (Alzieu, 1995; Alzieu, 2000). Subsequent work on *C. gigas* in the River Crouch, Essex, revealed that shell deformities were also occurring within this population (Waldock and Thain, 1983; Thain and Waldock, 1986; Waldock, 1986) and TBT levels of 400 ngl^{-1} were found (Waldock, 1996). The financial loss to the oyster industry from 1975-1982 totalled some $150 million (Langston, 1995; Pullen, 1995). In January 1982 the French Government banned the use of TBT based paints on all vessels under 25m length overall (LOA). This was followed by similar legislation in the UK and USA (Champ, 2000). The effect of the ban became apparent with tin/tissue levels in oysters sampled from Arcachon Bay, falling from 3.40 µg g^{-1} dry weight in August 1982, to 0.50 µg g^{-1} by August 1984 (Alzieu *et al.*, 1986) (see page 16 for further details).

Although legislation was put in place (§ 1.5), waterborne TBT levels improved slowly and it was found that much of the organotin had become bound up in sediments (Langston *et al.*, 1990). TBT is scavenged from the dissolved phase by sediment and this creates a readily bio-available reservoir of the compound (Langston *et al.*, 1990; Langston and Burt, 1991; St. Jean *et al.*, 1999). TBT partitions between sediment and water at ratios of several thousands to one (Langston and Burt, 1991; Proud, 1994; Pullen, 1995; Ruiz *et al.*, 1996) and in water has a suggested half-life of three years (Langston *et al.*, 1994). In contrast, in sediments TBT is slowly leached out and may persist for up to 10 years (Dowson *et al.*, 1996) or as suggested by Macguire (2000) "*there may be a legacy problem* [of TBT] *in sediments in some locations in Canada for perhaps 20 to 30 years after a total ban*" (see also Bryan and Langston, 1992). Although this estimate relates to Canadian harbours, Biselli *et al.*, (2000) showed that TBT concentrations in sediment near to leisure craft marinas in the German Baltic were between 570 to 17000 ngl^{-1}.

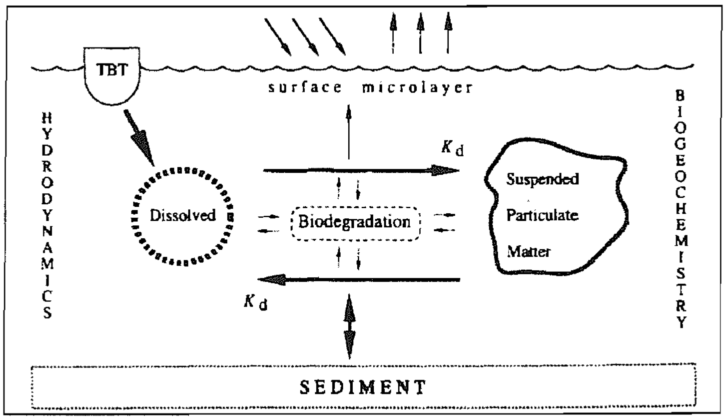

FIGURE 1.2 THE FATE OF TRIBUTYLTIN DUE TO DEPOSITION, HYDRODYNAMICS AND PHOTOLYSIS IN THE COASTAL MARINE ENVIRONMENT.
(where kd is the dissolved/particulate coefficient influenced by pH, dissolved organic matter and SPM (load, size and organic content)) (Ruiz *et al.*, 1996).

TBT persists in the water column and sediments due to hydrodynamic and biogeochemical factors (Ruiz *et al.*, 1996). Hydrodynamic factors such as localised circulation patterns and gyres can determine the time that pollutants remain in the water column. TBT is hydrophobic and therefore accumulates at the air-water interface where TBT breakdown and loss to the atmosphere can be stimulated through photolysis (Macguire *et al.,* 1985; Ruiz *et al.,* 1996). However, TBT has a tendency to bond to suspended particulate matter (SPM) and is deposited on the benthic sediment layer (see Fig. 1.2 for a summary of the process). The long-term residence of TBT in sediment reservoirs and subsequent leaching into the water column may result in sensitive non-target organisms being affected for some years.

1.4 Biology and ecology of *Nucella lapillus*: direct and indirect effects of TBT

1.4.1 Introduction

Numerous workers worldwide have confirmed the adverse effects of TBT on individuals and populations of stenoglossan gastropods (e.g. Smith, 1981a,b,c; Bryan *et al.,* 1986; Stroben *et al.,* 1995; Ruiz *et al.,* 1996; Smith, 1996; Bech, 2002). TBT sensitive species include: *Thais haemastoma* (Spence *et al.,* 1990), *Lynsassa obsoleta* (Say), *Hinia reticulata* (L.) *Ocenebra erinacea* (L.) (Hawkins and Hutchinson, 1990; Gibbs *et al.,* 1991), *Lepsiaella scobina* (Smith, 1996), *Thais orbita* (Rees *et al.,* 2001) *Nassarius reticulatus* (Ruiz *et al.,* 2005) (see review by Ellis and Pattisina, 1990). These species all exhibit 'imposex' (Smith, 1971), in which females of the affected species have male sex organs superimposed upon them (§ 1.4.3 below).

Blaber (1970) first described imposex in *Nucella lapillus* (common dogwhelk) from Plymouth Sound following survey work carried out in 1969, but did not attribute this to TBT pollution. Bryan *et al.,* (1986) showed that Plymouth Sound populations were suffering from TBT-induced imposex, causing their severe decline over the previous fifteen years (Gibbs and Bryan, 1986; Bryan *et al.,* 1987; Gibbs *et al.,* 1987). Due to the females' sensitivity to very low TBT levels a method was developed which allows their masculinisation to be utilised as a bioindicator of local TBT levels (Bryan *et al.,* 1986, 1987; Gibbs and Bryan, 1986) and this has been effectively used to asses the efficacy of legislation limiting the use of TBT (e.g. Spence, 1989; Spence *et al.,* 1990; Evans *et al.,* 1994; Proud, 1994; Minchin *et al.,* 1997; Bray and Herbert, 1998; Morgan *et al.,* 1998; Gibbs *et al.,* 1999; Miller *et al.,* 1999; Herbert *et al.,* 2000).

1.4.2 Reproduction dispersal and morphology of *Nucella lapillus*

N. lapillus are gonochoristic and the, often larger, females are fertilised internally (Crothers, 1985). Dogwhelks reach sexual maturity at around two years old (Feare, 1970; Crothers, 1985), but before this individuals are generally not gregarious (Crothers, 1985). Upon reaching maturity, however, during spawning periods and when sheltering in the winter, *N. lapillus* aggregate in rock crevices in the lower half of the intertidal zone (Feare, 1971; Crothers, 1985). Spawning in the UK normally occurs during April and May (Feare, 1970; Crothers, 1985), but in the south west of England has been seen in the autumn (Spence, 1989) and the early spring (pers. obs.; Hawkins, pers. obs.).

Upon insemination the females coat the fertilised vase-shaped eggs with albumen. Eggs are laid singly after being passed along the oviduct then along a groove to the foot of the sole. The capsules are then held perpendicular to the substrate and attached using secretions (Crothers, 1985). Each egg capsule contains around 600-700 eggs of which 94% are utilised by the remaining 6% as food (Crothers 1985).

Dogwhelk embryos are direct developing and do not have a pelagic larval stage (Kitching *et al.,* 1966; Crothers, 1985). The juveniles take 4-7 months to hatch depending on external temperature (Crothers, 1985; Spence, 1989). Upon emergence from capsules the fully formed juveniles (crawlaways), can migrate down shore where feeding has been observed on the annelid worm *Spirorbis borealis* (Moore, 1936) or small barnacles (Crothers, 1985). Juvenile *N. lapillus* have also been observed at the same tidal level as egg cases sheltering in empty barnacle carapaces (Feare, 1970; Spence, 1989).

Due to the lack of a pelagic larval stage *N. lapillus* populations have become isolated and genetically differentially selected which results in phenotypic differences in shell morphology (Staiger, 1957; Kitching *et al.,* 1966; Cambridge and Kitching, 1982; Kirby, 1994a) often correlating well with environmental pressures such as wave exposure, predation and temperature (Kirby 1994a). Individuals that exist on more sheltered shores tend to have thicker shells due to predation pressures from the shore crab (*Carcinus maenus*) (Feare, 1967; Hughes and Elner, 1979; Currey and Hughes, 1982; Gibbs, 1993; Trussell and Etter, 2001) and in some cases birds (Feare, 1967). In contrast *N. lapillus* from exposed shores have thinner shells as predation risk is less; this allows the growth of a larger foot, reducing the risk of displacement during rough weather (Kitching *et al.,* 1966; Etter, 1988a; Gibbs, 1993; Hughes and Taylor, 1997). Translocation experiments have been made where exposed shore *N. lapillus* morphs were moved to sheltered locations (Gibbs, 1993). The progeny of these animals differed from their parents as they developed in the form of sheltered shore morphs; this may have been a factor which resulted in them surviving 6 years or more. It was concluded that the shell form of *N. lapillus* showed a plastic response as the sheltered morph could be produced from a genetic background of exposed shore animals (Gibbs, 1993).

1.4.3 Direct effects of TBT on *Nucella lapillus*

Having noticed a penis like outgrowth behind the right tentacle of female *N. lapillus*, Blaber (1970) suggested the external polymorphism (e.g. Kitching *et al.*, 1966) between *N. lapillus* populations may extend to a level of morphological hermaphroditism. It was suggested that female penis growth occurred at the end of the breeding cycle (Blaber, 1970). This has been seen in other gastropoda (e.g. apple snail *Ampullaria polita*, Bouvier, 1888 *op cit* Blaber, 1970, and the cone shell *Conus mediterraneus*, Kuschakewitsch, 1913 *op cit* Blaber, 1970). This can lead to difficulties in differentiating between males and females of gonochoristic gastropods. Preserved *N. lapillus* specimens collected from Plymouth Sound by Moore (1938a) were assessed by Bryan *et al.*, (1987) and it was found that none of the females bore a penis like structure. Therefore it now seems likely that Blaber (1970) was recording the first impacts of TBT on *N. lapillus* in Plymouth Sound.

A positive relationship between the tissue burden of TBT in *N. lapillus* females and imposex was made and this was linked to boating activity through transfer experiments (e.g. Bryan *et al.*, 1986). Individuals of *N. lapillus* were translocated from St Agnes in North Cornwall, where boating is not common, to Plymouth Sound. The occurrence of imposex in the transferred animals increased from 0.6% to 60% over a one-year period (Bryan *et al.*, 1986). As a result of individual females being affected, with continuing severe TBT contamination dogwhelk populations declined throughout the UK (Spence *et al.*, 1990). Losses were seen in areas of the UK as far apart as Sullom Voe in the Shetland Isles (Davies and Bailey, 1991; Harding *et al.*, 1997) and the south coast of England (Gibbs *et al.*, 1987; Spence *et al.*, 1990; Langston *et al.*, 1994; Bray and Herbert, 1998; Hawkins *et al.*, 2002).

Langston *et al.*, (1990) found that female *N. lapillus* exposed to TBT showed a significant increase in the production of testosterone, although for some time the physiological reasons for this were not clear. It was subsequently demonstrated that in the presence of TBT, a metabolising protein known as P-450 cytochrome (aromatase), is inhibited (Spooner *et al.*, 1991). Aromatase is responsible for converting androgens (which have male hormone properties), to oestrogens (Morcillo and Porte, 1998). When bound in sediments TBT becomes readily bioavailable and although primary producers can uptake TBT direct from solution, consumption of TBT-tainted food is the most direct route for invertebrates such as gastropoda (Ruiz, *et al.,* 1996). Bryan *et al.,* (1989), however, carried out research to establish the pathway of TBT uptake by *N. lapillus*. Using ^{14}C labelled TBT in water and with *N.* lapillus feeding on mussels, they found that the diet accounted for approximately 50% of the body burden of the compound. TBT is lipophilic and thus many organisms can readily metabolise it to reduce toxicity. Molluscs dependent on the P450 molecule, however, cannot metabolise TBT at a rate sufficient to detoxify the compound due to the slow reaction process (Lee, 1991) so TBT accumulates in tissues.

N. lapillus does not have sex chromosomes; thus androgens and oestrogens are responsible for the expression of sex (Le Gall and Streiff, 1975). However, the entire mechanism behind sexual expression in prosobranch (with gills and operculum) molluscs is not fully established (Smith, 1971). Oestrogen activity is suppressed due to the inhibition of the P450 molecule by TBT which leads to an increase in testosterone and thus the growth of male sex organs in the female (Morcillo and Porte, 1998). Once initiated in female dogwhelks, the growth of the male sex organs does not stop. In addition, if affected females are transferred to areas where TBT levels are low, there is no reversal in imposex (Bryan *et al.,* 1987).

Masculinisation of female *N. lapillus* follows two forms (Gibbs and Bryan, 1986). Initially a vas deferens begins to be formed by infolding of mantle epithelium tissue which eventually fuses to form a tube. This structure grows in an anterior direction away from the vulva thus forming the proximal section of the vas deferens; at this stage a small penis, is also noticeable. The distal section of the vas deferens develops from the base of the penis, eventually fusing with the proximal section; this concludes the early stages of imposex. Following the vas deferens formation, penis growth continues, and where TBT levels are high, this may reach a size close to the male penis. The final stage of imposex is that the vas deferens eventually overgrows and blocks the vulva leaving the female sterile. The female continues to produce egg cases, but due to a blocked oviduct they suffer internal rupture and subsequent death. TBT concentrations in water as low as 1-2 ng l^{-1} can cause females to become sterilised and the penis to reach a size comparable to that of the males (Gibbs *et al.,* 1991). At levels above 4 ng l^{-1} most females in a population are left unable to reproduce (Gibbs *et al.,* 1988). Subsequently methods have been developed to assess the level of imposex as an indicator of individual female and population health, and as an assessment of

TBT levels in water (Bryan *et al.,* 1986, 1987; Gibbs and Bryan, 1986; Gibbs *et al.,* 1987) (§ 2.2).

Cooke (1915) suggested that the British Isles were the *"metropolis"* of the species, but after surveys in the 1980s dogwhelk abundances were compared with previous records (e.g. Crothers, 1975) and it was found that populations of *N. lapillus* were in serious decline (Bryan *et al.,* 1986 Gibbs and Bryan, 1986; Herbert, 1988; Spence, 1989). *N. lapillus* was absent in many areas and with the lack of a pelagic stage recovery has been slow. TBT pollution was most apparent on the south coast of England where all of the dogwhelk populations had been affected to some extent (Spence *et al.,* 1990; Hawkins *et al.,* 1994).

Many dogwhelk populations close to marinas and harbours contained low numbers of mainly older females with high levels of imposex (Bryan *et al.,* 1986; Gibbs and Bryan 1986; Spence *et al.,* 1990). It was suggested that the proportion of females to males in affected *N. lapillus* populations tended to decrease from the norm (e.g. Moore, 1938a; Minchin and Davies, 1999) with increasing imposex, and a shortage of females was apparent in many of the populations then impacted by TBT (Bryan *et al.,* 1986). With females unable to lay eggs, juvenile recruitment was severely reduced or eliminated at the most severely affected sites along the UK south coast, and many *N. lapillus* populations became extinct (Herbert, 1988; Spence *et al.,* 1990).

1.4.4 The role of *Nucella lapillus* in community structure

Due to predation on sessile organisms, it has been suggested that dogwhelks may be influential in the structuring of rocky shore communities (e.g. Crothers, 1976) and that the reduction of their populations due to TBT pollution presents an opportunity to study this (Hughes and Burrows, 1993).

N. lapillus mainly feeds on barnacles (usually *Semibalanus balanoides* or *Chthalamus* species) and mussels (*Mytilus edulis*) (Kitching *et al.,* 1966; Crothers, 1985; Hughes and Taylor, 1997), but when these are scarce dogwhelks will feed upon other intertidal species (see Crothers, 1985). A preference between barnacles and mussels is not clear, as when forced, dogwhelks will switch between them (Fischer-Piette, 1935 *op cit* Hughes and Burrows, 1993; Burrows and Hughes, 1991); Crothers (1985) has suggested that they have a preference for *Semibalanus balanoides*. This selectivity may be because experienced animals can easily prise apart the platelets of larger *S. balanoides* (Crothers, 1985) thereby not needing to expend energy drilling or opening mussels, thus reducing food handling time and yielding greater returns for energy expended (Dunkin and Hughes, 1984).

At small scales the population structure of barnacles may be dictated by *N. lapillus* as larger older individuals are preferentially taken, so reducing competition for space (Connell, 1961; Spence, 1989; Johnson *et al.,* 1998). Without predation, intraspecific (e.g. Bertness *et al.,* 1998) and interspecific (e.g. Connell, 1961) spatial competition can occur in barnacle populations resulting in crushing, overcrowding and undercutting (Spence, 1989; Bertness *et al.,* 1998). These effects have mainly been recorded where crevice habitats are available from which *N. lapillus* range creating a halo effect in barnacle density. Johnson *et al.,* (1998) found a relationship between barnacles' sizes and the abundance of refuges for dogwhelks; median barnacle size increased with distance from crevices. Fairweather (1988) noted a similar pattern and identified that in Australia on the New South Wales coast predatory gastropods resident in crevices had a role in structuring the community immediate to refuges. Over 2 years the removal of whelks at several sites resulted in the disappearance of abundance gradients which had previously existed with distance from the crevices.

It is possible that *N. lapillus* has a role in structuring mussel populations, for example on north east Pacific coasts Dayton (1975) showed that without predation by *Thaid* gastropods (subfamily Thaiidinae), mussels could dominate shore communities. Petraitis (1998) reported, however, that the influence of *N. lapillus* predation on mussels in the Gulf of Maine, USA, was very site specific and that the mortality rates of translocated mussel populations were highly variable between "*similar sites and among years*". The variable mortality rate could not be directly related to the abundance of dogwhelks at each location, therefore no clear role for predation by *N. lapillus* could be clearly identified. Hunt and Scheibling (2001) found that predation by *N. lapillus* was not as important as physical effects in structuring communities and patch dynamics of the mussels *Mytilus trossulus* and *M. edulis*. They concluded that wave shock disturbance (see also Menge, 1976, 1978a,b) removing and redistributing mussels, was more influential than biotic factors, but that the site chosen differed from many others with regard to the relative importance of wave dislodging and predation.

Studies of a community role for *N. lapillus* have shown that dogwhelks can have an impact on barnacle-mussel-algae matrices on north west Atlantic shores (Lubchenco and Menge, 1978). It was found that where *N. lapillus* was naturally restricted due to severe wave action, opportunistic mussel growth excluded *S. balanoides* and stands of the algae *Chondrus crispus*, which were both found where *N. lapillus* was present. It was concluded that predation by *N. lapillus* and starfish had a defining role in setting the zonation patterns on moderately exposed areas of the shore. The study by Lubcheco and Menge (1978) suggests that where wave action is severe predation by *N. lapillus* is confined to foraging in and around the most sheltered crevices available (see also Burrows and Hughes, 1989).

Conversely, on north east Pacific shores in Washington State, North America, Wootton (2002) found that that *Nucella emerginata* had a facilitating role in causing community succession from one mussel species to another. *N. emerginata* preferentially preyed upon *Mytilus trossulus* over *M. californianus*; the former was found to competitively exclude *M. californianus*. With stochastic events such as physical disturbance and the effects of preferential predation, it was found that this dominance could be switched to the less favoured species. *M. californianu*s were able to colonise gaps created by physical disturbance and predation, so shifting the community dominance. Wootton (2002) concluded that differential predation was an important factor in assisting a shift in community structure.

In the UK these effects have been found at small scales for barnacles (Connell, 1961; Johnson *et al.,* 1998; Proud, 1994) and no unequivocal role has so far been identified for *N. lapillus* predation in structuring UK mussel populations, although further work has been suggested (Proud, 1994). From Fairweather (1988) Proud (1994) and Johnson *et al.,* (1998) it may be concluded that predatory gastropods have an important, if localised, role in structuring barnacle size class abundances and potentially reducing overcrowding and hummocking (Bertness, 1988). Thus, due to TBT pollution, the potential effects of the reduction of *N. lapillus* populations propagating to UK rocky shore species and communities have so far not been clearly demonstrated at scales larger than crevices and small habitats.

Southward and Southward (1978) and Hartnoll and Hawkins (1985) also suggested that dogwhelks are not highly influential in the structure of sheltered shores as these communities are inherently stable. On moderately exposed shores, however, *N. lapillus* may be important in influencing changes from barnacle to algal dominated areas with a decline in dogwhelk abundance leading to a dense barnacle mat of mixed ages (Hawkins and Hartnoll, 1983; Hartnoll and Hawkins, 1985; Spence, 1989; Spence *et al.,* 1990; Proud, 1994). A reduction in their predation allows more settling and growth of barnacles which results in increased surface roughness so reducing the effectiveness of grazing species, especially limpets. This leads to areas on which fucoid propagules can settle (Lubchenco, 1983; Hartnoll and Hawkins, 1985).

The communities of rocky shores in Europe can be strongly structured by limpet grazing which prevents more exposed shores from becoming dominated by algae (Southward, 1964; Southward and Southward, 1978; Hawkins, 1981a,b; Hawkins and Hartnoll, 1983; Hawkins and Southward, 1992; Jenkins *et al.,* 2005). Limpets cannot graze upon fucoid propagules where barnacles are dense and 'fucoid escapes' can occur. Th is can lead to patches of mature fucus under which limpets and *N. lapillus* aggregate (Hartnoll and Hawkins, 1985). Fucoid sweeping inhibits barnacle settlement and limpet aggregations further prevent their recruitment (Hawkins, 1983). *N. lapillus* sheltering under the fucoids feed upon the larger barnacles (Proud, 1994) thus creating a halo effect (Johnson *et al.,* 1997) around the clump. Over time the fucoid canopy thins and the limpets and dogwhelks underneath disperse (Hawkins and Hartnoll, 1983). The dispersal of grazers and predators from the denuded fucoid area leaves a clear patch for barnacles to settle upon. The clear role of *N. lapillus* in this cycle has not been fully established and as these interactions asre complex, to design a suitable experiment to explain them would be challenging. It may be expected, however, that in its absence, due to TBT pollution, the cycle of fucoid to barnacle dominance can be interrupted. Johnson *et al.,* (1997), however, warn that this model may be very site-specific.

It would be expected that the demise in *N. lapillus* populations due to TBT impacts on shores where it had been continuously present would have had a marked effect on the intertidal community. Both Spence (1989) and Proud (1994), however, could not clearly establish what effects, if any, had occurred. Although ports usually have the highest levels of TBT contamination, they are normally in sheltered areas where dogwhelks are less common due to general lack of preferred prey. Therefore in such locations *N. lapillus* abundance is naturally low and TBT is affecting these populations at one end of their ecological range (Spence *et al.,* 1990). It has consequently proven difficult to determine the indirect effects of *N. lapillus* reduction on intertidal communities in areas where the species would not necessarily be present, or has a lesser ecological effect (Spence, 1989; Spence *et al.,* 1990; Proud, 1994).

1.5 Legislation

Although the potential for the adverse effects of TBT on non-target marine species had been first suggested in the late 1960s (Thomas, 1969) warnings were not immediately acted upon. The collapse of the oyster industry in Arcachon Bay, France, in the early 1980s, however, resulted in the French Government placing restrictions upon the use of TBT based paints (Alzieu, 1986, 1991). On the 19th of January 1982, the Ministry of the Environment for France put in place a 2 year ban on paints containing more than 3% by weight of TBT (Champ, 1999, 2000). Initially the ban was for vessels less than 25 tonnes in weight and only applied to Atlantic coasts and the English Channel. In September of the same year the ban was expanded to cover all the coastal areas of France and all TBT paints. The ban was also extended so that TBT paints could only be applied to vessels over 25 m length over all (LOA). The exception was aluminium hulls, as copper based paints, the main alternatives to TBT, caused their corrosion (Lewis, 1999).

Although UK researchers were among the first to investigate and suggest the link between TBT and non-target organisms (Bryan *et al.,* 1986; Gibbs and Bryan, 1986) UK legislation was slower to be introduced. In July 1985 the Environment Minister put in place new actions for the control of TBT. These comprised: development of methods to control the sale of the most damaging TBT paints (i.e. free association) and the limiting of the tin content in co-polymer paints; a notification scheme for all new antifouling sales agents; guidelines for removal of old and painting on new antifouling coatings; development of a provisional Environmental Quality Target (EQT) for TBT in water at 20 ngl^{-1}; the co-ordination of research and monitoring schemes to enable the UK Government to assess the efficacy of legislation put in place (Spence, 1989; Morgan, 1998; Champ, 2000; Santillo *et al.,* 2002). Following these proposals, on the 13th January 1986, the UK regulations were enforced under the Food and Environment Protection Act 1985, Part III. Consequently TBT content was restricted to 7.5% in copolymer paints and 2.5% in free association paints. Further to the above measures, on the 1st July 1987 UK legislation banned the use of TBT on vessels less than 25 m LOA (Spence, 1989; Champ, 2000). In addition the use of TBT was banned on aquaculture cages (Balls, 1987; Champ, 2000). Finally in 1987 the EQT for water borne TBT was lowered to 2 ngl^{-1} and replaced by an Environmental Quality Standard (EQS). No equivalent target was set for sediment bound TBT, however, and the levels were not statutory (Langston *et al.,* 1994). The last UK legislation was to ban the retail sale of TBT in May

1987 (Champ, 2000) and currently (2005) UK TBT legislation remains unchanged from that put in place in 1987.

More stringent legislation than in place in Britain and France began to be enforced world-wide. In the United States initial reluctance to legislate against TBT was apparent. The US Navy considered that the problems occurring in Europe were because of the excessive use of free association paints on small vessels. They were reluctant to see legislation against TBT as they estimated that the benefit of its use was around $100-130 million *per annum* (Champ, 2000). By mid 1987, however, coastal USA states were considering the enforcement of TBT restrictions with Virginia the first to apply measures. TBT restrictions initially were at State rather than Federal level with allowable waterborne limits ranging from 10 ngl^{-1} for Virginia (which shortly after changed to 1 ngl^{-1}), to 6 ngl^{-1} in California (Champ, 2000). Following differing state versions of TBT legislation, the Federal Government enacted the Organotin Antifouling Paint Control Act of 1988. Similarly to the UK TBT was banned on vessels less than 25 m LOA and release rates were set (Ten Hallers-Tjabbes, 1997; Champ, 2000).

Similar controls to the USA were introduced in Canada, Australia and New Zealand during 1989 (Ten Hallers-Tjabbes, 1997). Monitoring in Japan found ‘biologically significant’ levels of organotin in the local marine environment (Champ, 2000). Subsequently strict controls were put in place for the use of TBT paints on non-aluminium vessels. The legislation amounted to a ban of TBT on new hulls, and subsequently on its manufacture, use and import (IMO, 1999; Champ, 2000). Worldwide legislation against TBT was enacted, including some countries with no direct link to the sea. Austria and Switzerland banned TBT in their lakes and rivers thus minimising indirect impacts on marine habitats and direct effects on freshwater communities (e.g. Becker Van-Slooten. and Tarradellas, 1994; Stab *et al.*, 1996; Day *et al.*, 1998). Legislation against TBT was increasingly put in place and by 1992 numerous nations had restrictions on the use of the paint (Table 1.2).

The International Maritime Organisation provides an international focal point for the monitoring and introduction of policy aims regarding TBT use on commercial vessels. Within the IMO the formation of policies for the protection of the marine environment is considered within the Marine Environmental Protection Committee (MEPC) (Ten Haller-Tjabes, 1997). The MEPC consists of signatory states to the International Convention for the Prevention of Pollution from Ships (MARPOL 73/78, 1973).

TABLE 1.2 REGULATIONS CONTROLLING THE USE OF TBT PAINTS WORLDWIDE BY COUNTRY UP TO 1992. (Modified from Ortepa, 1999).

Europe	**Year**	**Regulations**
Austria	NA	Banned the use of TBT antifouling paint in fresh water lakes.
Europe (EC states)	1991	Prohibited the use of TBT-based paints on vessels less than 25 m LOA. TBT antifoulants available only in 20 L containers.
Europe (non-EC)	Vary	Prohibited use of TBT-based paints on vessels less than 25 m (most states).
Finland	1991	Prohibited the use of TBT-based paints on boats less than 25 m LOA.
France	1982	Prohibited the use of TBT-based paints on vessels less than 25 m LOA, except for aluminium-hulled vessels.
Germany	1990	Prohibited the use of TBT-based paints on vessels less than 25 m LOA. Ban on retail sale. Ban on its use on structures for mariculture. Regulation for the safe disposal of antifouling paints after removal.
The Netherlands	1990	Prohibited the use of TBT-based paints on vessels less than 25 m LOA Washing/blasting slurry used to prepare TBT antifoulants may be treated as hazardous waste TBT antifoulants available only in 20 L containers All antifoulants must be registered
Norway	1989	Prohibited the use of TBT-based paints on vessels less than 25 m LOA.
Sweden	1989 1992	Prohibited the use of TBT-based paints on vessels less than 25 m LOA. Maximum leaching rate of 4µg/cm2/day for vessels greater than 25 m LOA. All antifoulants must be registered.
Switzerland	1987	The use of TBT-based antifouling paints is banned in fresh water lakes. All antifoulants must be registered
United Kingdom	1985 1987	Sale of TBT-based, restricted, effective bar on TBTO free-association paints. Prohibited the use of TBT-based paints on vessels less than 25 m LOA and on fish-farming equipment. TBT antifoulants available only in 20 L containers. All antifoulants to be registered as pesticides; Advisory Pesticides Committee must approve sale and use. Washing/blasting slurry treated as hazardous.
N. America	**Year**	**Regulations**
Canada	1989	Prohibited the use of TBT-based paints on vessels less than 25 m LOA, except for aluminium-hulled vessels. Maximum leaching rate ofµg/cm2/day for vessels greater than 25 m in length All antifoulants must be registered
United States	1988 1990	Prohibited the use of TBT-based paints on vessels less than 25 m LOA, except for aluminium-hulled vessels. Maximum leaching rate of 4 $\mu g/cm^2$/day for vessels greater than 25 m in length. All antifoulants must be registered. TBT-based antifouling paints can only be applied by certified applicators.
S. h/sphere	**Year**	**Regulations**
Australia	1989	Prohibited the use of TBT-based paints on vessels less than 25 m) LOA. Maximum leaching rate of 5 micrograms per square centimetre per day (µg/cm2/day) for vessels greater than 25m LOA. All dry-docks must be registered with the Environmental Protection Agency because of discharges. All antifoulants must be registered.
New Zealand	1989 1993	The application of TBT copolymer antifouling paint is banned with three exceptions: hulls of aluminium vessels, the aluminium out-drive or any vessel greater than 25 m LOA. The application of TBTO free-association paints is banned Maximum leaching rate of 5µg/cm2/day for vessels greater than 25 m LOA. All antifoulants must be registered. Use of any organotin containing antifouling paint prohibited.
South Africa	1991	Prohibited the use of TBT-based paints on vessels less than 25 m LOA. TBT antifoulants available only in 20 L containers All antifoulants to be registered.
Hong Kong	NA	All TBT antifoulants must have a valid permit for import/supply. All antifoulants must be registered.
Japan	**Year**	**Regulations**
Japan	1990 1992	TBT banned for all new vessels. TBT banned for all vessels.
IMO measures	**Year**	**Regulations**
Worldwide ban proposal	2003-2008	Proposed ban by 1st Jan 2003. All TBT to be gone by 2008. ban slow to be ratified. By November 2004, 9.06% of world shipping tonnage signed up to ban, but for worldwide ratification requires 25% or 25 of the worlds shipping nations.

In 1990 the MEPC agreed resolution MEPC 29 which recommended governments to promote methods controlling and minimising the adverse affects of TBT. Measures were put in place ensuring that users and producers of TBT were registered and controlled. This included that part of the profits from TBT sales be put towards research into alternatives. In addition an 'environmental degradation fund' was created for research into TBT in the marine environment. The resolution agreed the international banning of TBT on vessels less than 25 m LOA and a vessel release rate for TBT/water of 4 $\mu g\ cm^{-2}$ per day (Ten Hallers-Tjabes, 1997; Champ, 1999, 2000). The legislation was taken up internationally with governments carrying out national and local regulations. Research followed legislation using bioindicator species as a guide to improvement of TBT/water levels. For example Batley *et al.,* (1992) showed improvements in the commercial oyster (*Saccostrea commercialis*) population in Sydney Harbour, Australia, following the banning of TBT within the area. After the recommendations of MEPC29, the Japanese called for the worldwide ban of TBT at the 30th MEPC meeting in 1990. Research had found biologically significant levels of TBT in the marine environment and it was suggested that the high incidence of ocean going vessels passing through Japanese waters was to blame (Champ, 1999).

In November 1998 at MEPC 42 several other nations (Belgium, Denmark, France, Norway, the Netherlands, Sweden and the UK) called for a worldwide ban on TBT paints (MEPC, 1998; Champ, 2000). At the MEPC 43rd session (June to July 1999) it was recommended that legislation be passed to regulate antifouling paints with specific reference to phasing out organotin compounds (Champ, 2000). The MEPC subsequently suggested a 10-year period to implement the planned ban. The last allowable date for the application of organotin paints on vessels being set as the 1st January 2003 and a total phase out of organotin antifouling coatings by 1st January 2008 (Champ, 2000).

The decision to ban TBT applications on the 1st January 2003 was contingent on the support of 25 states representing 25% of the world's merchant shipping tonnage (IMO, 1999). The 159 member states of the IMO accept the proposed ban in October 2001 (IMO, 2001; ENS, 2001; ENDS, 2002a). Unfortunately by July 2002 the treaty had not been validated by any of the initial signatory nations. Thus it was identified that the regulations could not be enforced until twelve months after the 25 state signatory figure had been reached (ENDS, 2002a).

Whilst waiting for the ban, two nations attempted to put in place their own national legislation. Belgium in 2000 and Germany in 2001, with the backing of the Danish Ecological Council (Ege and Boye, 2001), attempted to adopt the proposed ban conditions unilaterally. Both attempts were rejected. Belgium was told that it could not introduce the planned law because it had agreed to allow restricted use of TBT under a European Union directive in 1999. The European Commission ruled out new evidence from the Belgian authorities, that high TBT levels in flatfish increased the risk to human consumers, warranting tougher measures (ENDS, 2002a). The German application was rejected on similar grounds. Both nations were advised to wait for worldwide legislation being developed by the IMO.

In November 2002 the European Union's transport committee agreed that the IMO proposal to ban organotins from 2003 should be supported. The Commission noted that the draft regulation, agreed in 2001, was unlikely to take effect from the 1st January 2003 due to procedural delays but that it would be implemented by the summer (ENDS, 2002b). IMO members, however, were reluctant to take up the ban and the deadline of 1st January 2003 passed. The second half of the IMO ban scheduled for 1st January 2008 is still in place. This states that "ships shall not bear such [TBT] compounds on their hulls or external parts or surfaces" (IMO, 2001). However, as member states have been reluctant to start the process, it remains to be seen whether the total removal of TBT will be achieved by 2008.

Some researchers have argued that the decision to ban TBT is unnecessary (e.g. Evans, 1999; Lewis, 1999). Evans (1999) argued that the proposal was premature and unrealistic until suitable alternatives to TBT were investigated and available and that the legislation then in place was sufficiently effective as to render a full ban unnecessary. In addition several authors (Champ, 1999, 2000, 2001b, Abbott *et al.,* 2000; Abel 2000) stated that TBT concentrations in water and sediment had declined to acceptable levels. In addition some workers have questioned the validity of research into the impacts of TBT and suggested that many results were based on generalities (Champ, 2000). Evans *et al.,* (2000a) reported that even though *N. lapillus* was highly sensitive to TBT, predicted population extinctions had not occurred and that dogwhelks were found on all of the UK sites surveyed. Champ (2000) questioned the ability of TBT to bioaccumulate through the food chain and research by Evans *et al.,* (2000b) identified other causes of imposex in *N. lapillus* (e.g. the endocrine disruptor nonylphenol and parasitism by trematode worms) thus questioning its status as a bioindicator for TBT in the marine environment.

At the time of the controversy over TBT impacts the MEPC were continuing to promote research into TBT alternatives (Champ, 2001b; Santos *et al.,* 2002) and some were reported as adequate (Ten Hallers-Tjabbes, 1997). Abel (2000) highlighted, however that there were disincentives towards developing viable TBT alternatives since much of the research was funded by shipping operators. It was felt that if TBT was successfully banned without adequate alternatives the costs would be to shipping operators with the benefits going to paint manufacturers. Therefore shipping companies may have been reluctant to promote less effective and more costly alternatives (Abel, 2000). It was felt that the proposed ban on TBT had been poorly considered and that the adverse effects of a total ban were potentially worse.

As a result ships would travel more slowly, use more fossil fuels and a switch to less environmentally friendly modes of transport such as air and land may occur (Abel, 2000; Strandenes, 2000).

Batt (1999) summarised the issues concerning organotin paint users and producers that had led to what was considered to be a premature ban on TBT. These included that no proven alternatives were available. Furthermore no specific criteria had been set to evaluate them and that those that were already available did not meet US VOC (volatile organic compounds) emissions restrictions. In addition the concerns regarding economic consequences to the shipping industry were seen as important (Ludgate, 1987; Champ, 2001a). Similarly to Champ (2000) it was also felt that the decisions to ban TBT were based on flawed research (Batt, 1999).

Apart from concerns about increased fossil fuel use and a growth in the use of even less environmentally friendly modes of transport, there are also potential problems with the disposal of TBT residue after it has been removed from hulls. Abbott *et al.,* (2000) commented that there is no provision in place for the safe disposal of TBT polluted residue after 2008, hence the residue may be dumped into sensitive areas in unregulated countries in the developing world (e.g. Greenpeace, 2000). Furthermore some advocates of TBT stated that alternatives were ill developed, less effective and had as much potential for insidious pollution (Batt, 1999; Champ, 1999, 2000, 2001a; Abbot *et al.,* 2000).

1.6 Alternatives to TBT

TBT has been proven the most effective antifouling paint commercially available to date (Santillo *et al.,* 2002). It has dominated 70-80% of the antifouling market, effectively hindering the need for research into viable alternatives (Champ, 2001b). Due to the problems associated with organotin compounds, however, research into alternatives has been continuing for some years (Clare, 1995; Cameron, 2000; Champ, 2001b; Santillo *et al.,* 2002). Since 1986 a series of international symposia on antifouling paints have been held (Champ, 2001b). There are several groups of alternatives to TBT and new ideas are being proposed and researched; future antifouling options can be broadly placed into five categories (Table 1.3).

TBT works through being an unstable coating with a biocide component. It is the diffuse pollution caused by the indiscriminate release of the TBT biocide into the water column and sediment which leads to its impact on non-target species. With this in mind much research into alternatives has been in the field of biocide free paints. Cameron (2000) reported that 23 biocide free alternatives had been tested. These included non-toxic non-stick finishes that prevented settlement, self polishing coatings (the same idea as TBT) but without biocides, fibre coverings which prevent firm adhesion, and non-abrasive coatings combined with special cleaning. Several of the biocide free compounds had a promising performance and although fouling still occurred to some degree, the levels were similar to those seen on TBT treated hulls (Cameron, 2000).

TABLE 1.3 CATEGORIES OF ALTERNATIVE ANTIFOULING COATINGS TO TRIBUTYLTIN CO-POLYMER BASED PAINTS. (Modified from SA EPA, 1999)

Alternative type
Electrical Current Systems. These are based upon the generation of hyperchlorite from seawater on the surface of the structure and can sterilise the surface for up to four years. Another similar system uses alternating current and a conductive hull coating.
Ceramic-Epoxy Coatings. They can provide an effective barrier, and can be used on fibreglass and metal hulls.
Foulant-Release ('non-stick') Coatings. Silicon elastomers are effective non-stick antifoulants, with similar service life to TBT copolymer, but are costly and best suited to fast craft.
Epoxy-Copper Flake Paints. A bonded-copper system which may protect boats for up to 10 years but cannot be used on aluminium hulls due to corrosion reactions.
Biological compounds. Marine algae and invertebrates do not become fouled themselves because they secrete chemicals, many with anti-bacterial, anti-fouling, and anti-algal properties. Some of these are currently being tested around the world. At least 50 natural substances have been identified as potentially useful antifoulants. Australia is prominent in this research

Conversely research into biocide-containing compounds has also continued but Evans *et al.*, (2000c) report that booster-biocides researched so far can be equally as toxic as TBT. They suggested that the widespread use of TBT replacement biocides should only be permitted following more dedicated research. One example of an alternative that was thought to be less toxic than TBT is the compound triazine which was sold as Irgarol 1501 (Evans *et al.*, 2000c). Like most organic booster-biocides Irgarol has other applications and can be used as a herbicide. The mode of action of Irgarol is to inhibit photosynthetic activity in algal chloroplasts, thereby preventing growth of unwelcome marine algae.

As Irgarol is leached from vessel hulls in the same way as TBT it was felt that it had the same potential to impact non target organisms. It was also concluded that Irgarol was likely to adversely affect communities associated with algae (Dahl and Blanck, 1996). Thus it was suggested that the fate and effects of Irgarol required further evaluation (Dahl and Blanck, 1996). Subsequently the compound was found to accumulate in sediments and coastal waters (Basheer *et al.,* 2002; Bowman *et al.,* 2003; Karlsson and Eklund, 2004) and to adversely affect non-target organisms (e.g. Scarlett *et al.,* 1999; Kobayashi and Okamura, 2002; Owen *et al.,* 2002). As with TBT, Irgarol was shown to have a long residence time in sediments and can become concentrated at levels above those identified as noxious to non target algae (Biselli *et al.,* 2000; Basheer *et al.,* 2002). Subsequent to research on the effects of Irgarol on algae and seagrass communities (Dahl and Blanck, 1996; Scarlett *et al.,* 1999; Chesworth *et al.,* 2004) Irgarol and another booster biocide Diuron, were banned on recreational craft (< 25 m LOA) in the UK from November 2002 (Chesworth *et al.,* 2004).

Following the reduction of the regular use of TBT, and the ban on recreational vessels, a return to copper based paints occurred, which were common before the development of TBT (Clare, 1995; Santillo *et al.,* 2002). To be as effective as TBT, however, copper systems also require booster biocides and these work with the same (self polishing) action as TBT leading to release to the marine environment and potential effects on non-target organisms. Problems have been identified with copper-based replacements for TBT (Santillo *et al.,* 2002). For example, the copper content of oysters in Arcachon Bay, France, increased following the ban on TBT (Claisse and Alzieu, 1993). In addition Kobayashi and Okamura (2002) found that a copper-biocide paint used as a TBT alternative was considerably more toxic to sea urchins than TBT. Currently other copper based antifouling paints are the main TBT substitute available for small craft, and with no effective alternatives coming forward, problems associated with elevated copper levels in the marine environment may increase (Claisse and Alzieu, 1993). However, silicone coatings have achieved some success in preventing fouling (Ten Hallers-Tjabbes, 1997; Thomas *et al.,* 2004). They provide a non-stick surface that is a ‘physical barrier’ to settlement (Santillo *et al.,* 2002). These coatings are effective on fast vessels, however on slower craft fouling organisms become attached, but hulls are easier to clean (Clare, 1995; Santillo *et al.,* 2002). Silicone coatings have been available for a number of years and are used in significant amounts on leisure craft, having the potential to be a viable alternative to toxic biocide based paints (Burnell *et al.,* 1997; Santillo *et al.,* 2002).

Natural antifouling chemicals have been investigated for some years (for review see Meseguer *et al.,* 2004) as some organisms, despite being sessile, can remain unfouled (e.g. Armstrong *et al.,* 2000; Cowling *et al.,* 2000; Burgess *et al.,* 2003; Murugan and Ramasamy, 2003). Many of these organisms were found to possess natural alternatives to chemically synthesised compounds and included several species of algae, sea squirts and corals (Clare, 1995,1998). The sea grass *Zostera marina* also has the ability to remain relatively unfouled and research has identified a substance now known as zosteric acid. This substance does not repulse all fouling species but has been shown to have promising attributes (Clare, 1995, 1998). Although the research is promising Santillo *et al.,* (2002) commented that commercial application of natural antifouling substances may be some time away.

Research has also focused on the epidermis of larger cetaceans where it was identified that their skin remained relatively unfouled despite them being slow moving and submerged throughout their life cycle. This ability was attributed to 'nanoskin roughness', which prevented a clear laminar flow boundary layer developing, therefore reducing the ability of organisms and particles to remain attached (Baum *et al.,* 2002). This research may prove beneficial for future TBT alternatives. It is not yet clear, however, how it may be applied to inhibit biofouling of man-made structures. One similar development using roughness and instability is micro-fibre coatings. This system is based on the theory of surface texture providing insufficient grip for fouling organisms (Philippi *et al.,* 2001). The process by which the coating is achieved is known as 'flocking' in which electrostatically charged polymer based micro fibres are attracted to a hull that has an adhesive layer. Following trials, Philippi *et al.,* (2001) found that the covering inhibited brown and green algae, but not reds. In addition encrustation of barnacle and mussel species can be inhibited with microfibres, but they were less effective at retarding tube building polychaetes, ascidians and algae (Watermann *et al.,* 2000). In addition, preliminary results from trials in the Mediterranean showed that the coating worked relatively well on slower vessels, but that on high speed craft the micro-fibres created turbulence at the hull which resulted in decreased fuel efficiency (Burt, pers. com. 2000).

1.7 Recovery of affected *Nucella lapillus* populations

Sediment bound TBT can remain in aquatic environments for considerably longer than TBT in the water column (Langston *et al.,* 1994). Following the legislation affecting small vessels, Langston *et al.,* (1994) predicted that TBT levels in the Hamble River near Southampton, one of the busiest recreational moorings in the UK, would fall from a 1987 figure of 500 ngl^{-1} to the EQT of 2 ngl^{-1} by 1995. Mean concentrations of TBT in the Hamble in 1995, however, proved to be ten-fold higher than the EQT (20 ngl^{-1}) and by 1998 further reduction appeared to be slowing (CONSSO, 1998; Langston *et al.,* 1998). It was suggested that sediment bound TBT and the continuing use of organotins on larger vessels may have been responsible for the slow improvement rate.

Since the UK legislation against TBT paints in 1987, populations of *N. lapillus* in certain areas have been recovering (e.g. Bryan and Gibbs, 1991; Evans *et al.,* 1996; Harding *et al.,* 1997; Miller *et al.,* 1999; Hawkins *et al.,* 2002). In many recreational boating areas the problem of imposex in *N. lapillus* populations has diminished, however where commercial ships are prevalent recovery has been slow (Miller *et al.,* 1999). Areas where TBT rich sediment (e.g. Thomas, 2000, 2001) provides a reservoir of the compound show slow recovery or continuing impacts (e.g. Santos *et al.,* 2004). This may result from the constant replenishment of water borne TBT via sediment supply (Bray and Herbert, 1998; Herbert *et al.,* 2000). Alternatively, dredging activities also have potential for impact following the disturbance and dumping of TBT contaminated sediments (Svavarsson *et al.,* 2001; Santos *et al.,* 2004).

Following legislation, imposex indices have been regularly employed to highlight the effectiveness of the measures introduced (e.g. DETR, 2000). Studies in several areas have reported marked imposex declines in *N. lapillus* populations (e.g. Evans, 1999; Evans, *et al.,* 2000b; Birchenough *et al.,* 2002b). Minchin *et al.,* (1997) reported that a 1993 reinvestigation of a 1987 survey showed that there was a significant recovery in areas of fish farming and recreational boats. It was also found, however, that in some areas where commercial vessels were dominant imposex indices increased (Minchin *et al.,* 1997; Quintela *et al.,* 2000; Santos *et al.,* 2002).

To monitor the effectiveness of legislation worldwide, other species of stenoglossan gastropod, and bivalves (e.g. Ruiz *et al.,* 2005) are employed as bioindicators of TBT pollution. For example Smith (1996) reported that imposex in *Lepsiella scobina* had declined around recreational harbours in New Zealand, but where they were found close to commercial ports imposex was present in 90-100% of females. In 2000, Reitsema *et al.,* (2002) compared imposex in the late 1980s (Saavedra Alvarez and Ellis, 1990) and early to mid 1990s (Tester *et al.,* 1996) around Vancouver Island, Canada. In the 1980s there was no clear recovery in the local gastropod populations (*N. canaliculata, N. lamellosa, N. emarginata* and *Searlesia dira*). Following the later (2000) survey work, however, the authors found imposex levels were declining and population abundances improving (Reitsema *et al.,* 2002).

In some areas the locality of populations in relation to their level of recovery can be highly critical. Harding *et al.,* (1997) found that *N. lapillus* populations near Sullom Voe oil terminal in the Shetlands were still impacted by TBT. When compared with 1991, results from 1995 showed a clear improvement in imposex for populations just outside Sullom Voe. Populations closer to the oil terminal, however, had low juvenile recruitment and imposex indices were higher. Overall, current UK legislation has reduced the levels of imposex in *N. lapillus* populations (Evans *et al.,* 1998; Morgan *et al.,* 1998; Hawkins *et al.,* 2002). It is apparent, however, that dogwhelk, and other sensitive gastropod populations will continue to be affected until TBT is phased out globally and levels in sediment have diminished (Morgan *et al.,* 1998). In the Pacific, Rees *et al.,* (2001) reported on the recovery of *Thais orbita* in Port Philip Bay on the New South Wales coast of Australia. It was found that on a regional scale *T. orbita* had largely recovered from TBT and that populations and imposex indices were improving. There were sites, however, where both recreational and commercial boats were used and imposex levels had not improved. Thus recovery is not always clear, and worldwide the continued monitoring of imposex in gastropod bioindicators provides a useful tool to indicate the effectiveness of legislation.

As TBT levels have declined, in many UK *N. lapillus* populations' imposex levels have reduced and they have recolonised former habitats (Bray and Herbert, 1998; Herbert *et al.,* 2000; Colson and Hughes, 2004), but with no pelagic stage how dogwhelks re-establish themselves is not well recognised. Several methods have been suggested as to how non-pelagic species can colonise favourable habitats (e.g. Highsmith, 1985; Bryan *et al.,* 1986; Martel and Chia, 1991a,b), however, as Johannesson (1988) discussed, the method by which this is achieved is not generally appreciated.

1.8 Rationale and aims

The preceding review has highlighted the value of long-term and continuing ecotoxicological studies which, following the methods developed at the Marine Biological Association of the United Kingdom at Plymouth (Bryan *et al.,* 1986; 1987; Gibbs and Bryan, 1986; Gibbs *et al.,* 1987), utilise *N. lapillus* as a bioindicator for marine environmental concentrations of TBT.

Following the work carried out by Spence (1989) and Proud (1994), this work highlights the changes in *N. lapillus* populations following the UK legislation of 1987 with particular reference to variations since 1997.

TBT affected *N. lapillus* populations are skewed towards fewer older animals (Bryan *et al.*, 1987; Gibbs *et al.*, 1987; Herbert, 1988; Spence *et al.*, 1990; Harding *et al.*, 1997, 1999; Hawkins *et al.*, 1999, 2002) as juvenile recruitment reduces and adult mortality increases. After legislation, a change in the structure of *N. lapillus* populations should be observed. An increase in juveniles and enhanced longevity amongst *N. lapillus* adults would be expected, leading to populations with a broad spectrum of age classes. The description of long-term trends in levels of imposex, population distribution and rate of recovery in *N. lapillus* will provide information on the value of current legislation and give an insight into localised impacts and their causes.

Some authors have stated that *Nucella* species do not move more than a few metres yearly (e.g. Palmer, 1984; Crothers, 1985), however, some previously extinct *N. lapillus* populations have re-established at sites polluted by TBT such as Sullom Voe in the Shetlands (Harding *et al.*, 1997) and Watermouth Cove, North Devon (Crothers, 1998). Recovery is less apparent on the UK south coast (Spence *et al.*, 1990; Hawkins *et al.*, 2002). Although the reasons for the lack of recovery in some areas are not fully understood, newly colonised coastal defence habitats provide opportunities to investigate causal or preventative factors. In addition, formerly populated sites offer the chance to investigate the worth of *N. lapillus* reintroduction as proposed by Hawkins *et al.*, (1999). The broader impacts of *N. lapillus* population variation on rocky shore communities due to TBT are not fully understood. Before TBT pollution the community influence of *N. lapillus* (Connell, 1961) and other predatory *Thaid* gastropod species (Connell, 1970; Menge, 1976, 1978a; Lubchenco and Menge, 1978) have been demonstrated on barnacle and mussel population densities and distributions. Their overall role in community structure, however, is less well defined (but see work by: Hawkins and Hartnoll, 1983; Hartnoll and Hawkins, 1985; Proud, 1994; Hunt and Scheibling, 1998, 2001; Johnson *et al.*, 1998; Bertness *et al.*, 1999a; Hamilton and Nudds; 2003; Trussell *et al.*, 2003). In northern Europe *N. lapillus* may have a minor influence on relatively stable shore communities (sheltered and exposed), but have limited control on the communities of moderately exposed coasts (Spence *et al.*, 1990). Manipulation of an unaffected *N. lapillus* population on an exposed to moderately exposed shore should shed some light on what the effects of removal of dogwhelks by TBT might be.

The overall aims of this research were to build on previous work (Spence, 1989; Proud, 1994) by establishing the extent of the long-term recovery and status of *N. lapillus* populations at monitoring sites in Plymouth Sound. To complement Plymouth Sound locations, key sites of TBT impact on *N. lapillus* along the south coast of the UK have also been investigated. The objective of long-term monitoring at all sites is to identify the health of *N. lapillus* populations and the effectiveness of current UK legislation against TBT. Where *N. lapillus* has been extinct at some locations and where new habitats have been provided, *N. lapillus* has shown an ability to establish new populations. As *N. lapillus* lacks a pelagic stage the mechanisms behind this are not well understood. This research aims to clarify further the ability of *N. lapillus* to colonise new habitat. Although recolonisation has taken place at some locations, several sites have not seen *N. lapillus* re-establish. This may be due to high levels of TBT or the lack of a local source population of *N. lapillus*. Reintroducing *N. lapillus* has previously been suggested (Langston *et al.,* 1990; Hawkins *et al.,* 1999). The effectiveness of methods used for a reintroduction trial and the survival and movements of individuals reintroduced to a site within Southampton Water are investigated here. Where *N. lapillus* has become extinct, it may be that communities undergo structural change due to a reduction in predation. The final aim of this research is to establish the importance of *N. lapillus* in structuring shore communities at a manipulated site on the UK south coast.

Chapter 2 provides detail on general methods used throughout this study. This avoids repetition of information on surveying approaches and associated analyses in subsequent sections.

It has been highlighted that TBT has detrimental impacts on *N. lapillus* at the individual and population levels. Chapter 3 continues investigations into the recovery of populations monitored in Plymouth Sound since 1987 and further considers impacts on a regional (south coast) basis, including the Solent hot spot (Herbert, 1988; Langston *et al.,* 1990, 1994, 1997, 1998; CONSSO, 1998; Bray and Herbert, 1998; Herbert *et al.,* 2000). Long-term (13 years for Plymouth Sound populations) and broad scale monitoring will undertake to show whether imposex levels in *N. lapillus* populations are falling significantly (Spence, 1989; Proud,

1994) in response to declining south coast TBT levels (e.g. Langston *et al.,* 1990, 1994; Langston and Burt, 1991).

N. lapillus has no recognised pelagic stage (Crothers, 1985). In spite of this *N. lapillus* has recolonised sites from where they were eradicated by TBT (e.g. Crothers, 1998). Chapter 4 presents information on a population of *N. lapillus* at Highcliffe in Dorset which has colonised rock groynes on a formerly bare sand and shingle shore. The methods by which colonisation is achieved have not yet been identified by previous research and are, as yet, not well understood. The population at Highcliffe provided an opportunity to investigate patterns of colonisation.

Langston *et al.,* (1990) and Hawkins *et al.,* (1999) discussed the need and merits for the reintroduction of *N. lapillus* populations at sites formerly impacted, or still impacted at a low level, by TBT. Chapter 5 investigates the results of a reintroduction trial undertaken at a site influenced by the commercial port of Southampton and considers the feasibility of reintroduction at other UK sites impacted by TBT.

The previous review has highlighted the uncertain or highly location specific role that *N. lapillus* has in structuring rocky shore communities through predation pressure. Chapter 6 presents the results of removal experiments undertaken to establish the role of *N. lapillus* in structuring barnacle and mussel communities on man-made groynes at Highcliffe in Dorset.

Chapter 7 synthesises the information gathered from investigating *N. lapillus* at the individual, population and community levels in the preceding chapters. In addition, the efficacy of current legislation and the potential for continuing impacts are further considered. Finally suggestions for further research are made and conclusions resulting from this research are drawn.

Chapter 2. General methods

2.1 Introduction

During this study a number of generic survey and analytical methods were employed which are common to all chapters. Where different techniques are used these are identified in the appropriate section. All of the experimental work included assessments of *N. lapillus* abundance and population structure and the local shore community. In addition for Chapters 3 and 5 assessments of imposex within *N. lapillus* populations were carried out.

2.2 Assessment of imposex

2.2.1 Introduction

N. lapillus is the most sensitive species to organotin pollution (Huet *et al.,* 1995) showing the effect of the superimposition of male sexual characteristics on the female (imposex) (Smith, 1971). This occurs at waterborne TBT levels down to 1 ngl^{-1} (Bryan *et al.,* 1986 Gibbs *et al.,* 1988; Langston *et al.,* 1994) and propagates from impacting females to populations; the growth of male sex organs on the female results in death due to a blocked oviduct. Populations decline due to *N. lapillus* lacking a pelagic stage thus limiting recruitment from external sources and internal breeding is prevented as no fertile females are available.

Methods to assess imposex developed by Bryan *et al.,* (1986) and Gibbs *et al.,* (1987) have been used in this study. These techniques can indicate the long-term recovery of *N. lapillus* from TBT-related imposex and the efficacy of UK legislation intended to mitigate these impacts. Indicators are female penis size in relation to male (relative penis size index (RPS) and the growth of the male sperm duct, (vas deferens), the development of which has been separated into six stages known as the vas deferens sequence (VDS) (Gibbs *et al.,* 1987).

Suggested refinements and alternative approaches to imposexing methods have been proposed (e.g. Fioroni *et al.,* 1991; Stroben *et al.,* 1992, 1995; Oehlmann *et al.,* 1992, 1993; Huet *et al.,* 1995). The alternative methods have, however been proven too complex as several sub stages are used. Consistent results (e.g. Minchin and Davies, 1999) can be achieved using the methods developed by Bryan *et al.,* (1986) and Gibbs *et al.,* (1987) and these remain the most reliable and widely used.

2.2.2 *Nucella lapillus* collection and storage

Individuals were held in nylon net bags and carried from the shore in a portable tank, with battery air pump, before transfer to the Southampton Oceanography Centre holding tank. Plastic labels were placed in the bags with the name of the source shore on them. Where transport back to Southampton Oceanography Centre was not necessary, animals were assessed at a location close to field sampling sites.

2.2.3 Narcotising and preparation of *Nucella lapillus* specimens

N. lapillus were stored at the aquarium in made up seawater until processed, which was not later than four days after collection. After removal from the aquarium, animals were placed in 8% magnesium chloride solution (MgCl). This solution is isotonic with seawater and does not cause osmoregulation problems for animals, but acts as a narcotising agent (Moore, 1989). Animals were left in the solution for 30 minutes to ensure full anaesthesia enabling specimens to be dissected whilst in a semi relaxed state. Huet *et al.,* (1995) state that narcotisation can lead to an overestimation of penis length when carrying out imposexing. It was found that if animals were processed after 30 minutes immersed in MgCl they were sufficiently relaxed to enable ease of handling, but that the penis length was not overestimated. When fully narcotised, they were cracked open using a ‘G’ clamp and the muscular attachments to the inside of the shell were cut.

2.2.4 Identifying sex

Before imposex impacts in the 1970s and 1980’s, male and female *N. lapillus* could be sexed, on occasion with some difficulty (Feare, 1970; Gibbs *et al.,* 1987), by the presence or absence of a penis; no external characteristics are available to provide this information (Feare, 1970).

Once TBT induced imposex became prevalent, the presence or absence of a penis was no longer a reliable method of identifying the sex of *N. lapillus* (Bryan *et al.,* 1986). A characteristic that has proven reliable in discerning between male and female *N. lapillus* is the sperm ingesting gland which is a reddish brown structure present posterior to the capsule gland (Fig.2.1). This is only present in females including the sexually immature (Gibbs *et al.,* 1987) and does not vary in colour with exposure to TBT thus this characteristic was used for sex identification throughout this study.

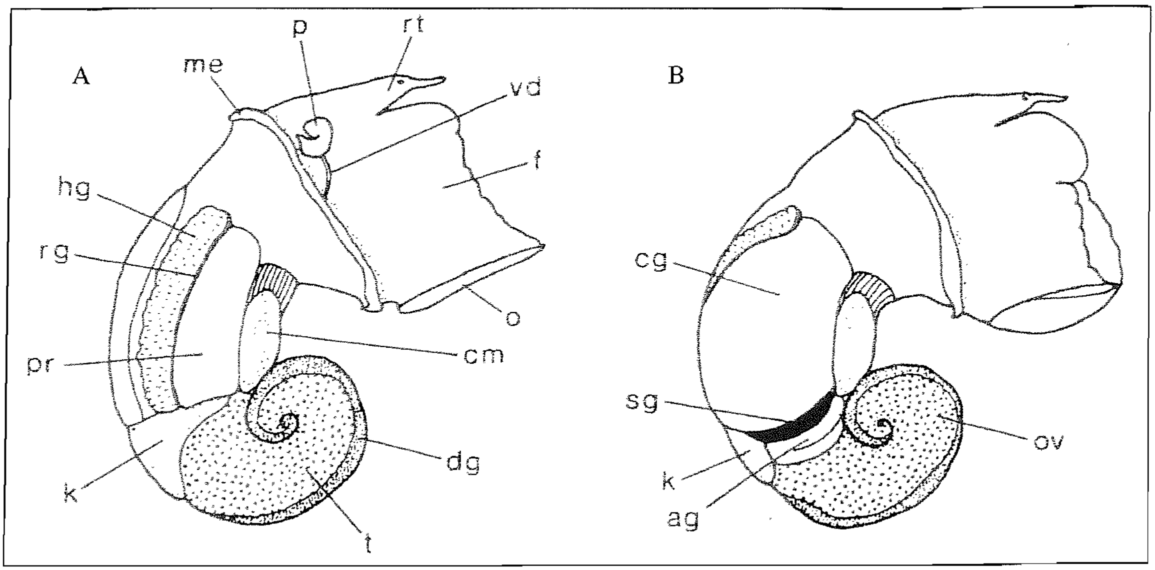

FIGURE 2.1 THE PHYSICAL STRUCTURE OF MALE (A) AND FEMALE (B) *NUCELLA LAPILLUS* FROM THE R/H SIDE AFTER SHELL REMOVAL. Abbreviations: ag, albumen gland; cg, capsule gland; cm, columella muscle; dg, digestive gland; f, foot; hg, hypobranchial gland; k, kidney; me, mantle edge; o, operculum; ov, ovary; p, penis; pr, prostate; rg, rectal gland; rt, right tentacle; sg, sperm ingesting gland; t, testis; vd, vas deferens
(From Gibbs *et al.,* 1987).

2.2.5 Relative penis size index (RPS)

Gibbs *et al.,* (1987) showed that the minimum number of *N. lapillus* necessary to give reliable RPS values was around 30 animals in total and that workers can achieve relatively consistent RPS values from this number with variations in the order of 1-2%. Both Spence (1989) and Proud (1994) found that the sample size suggested was adequate for reliable results; for the present study 35 animals were taken from each population investigated.

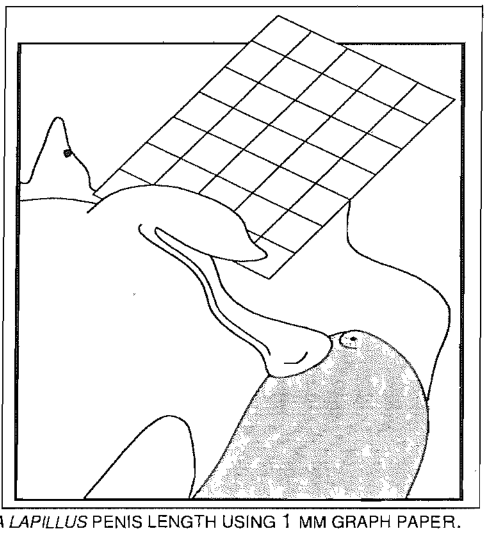

FIGURE 2.2 MEASUREMENT OF *NUCELLA LAPILLUS* PENIS LENGTH USING 1 MM GRAPH PAPER.
(From Proud, 1994).

After being narcotised in the 8% MgCl solution and then detached from their shells, each specimen was sexed and pinned, dorsal side up, in a wax based dish of MgCl solution and examined using a binocular microscope (Kyowa optical SDZPL) under a cold light source (Fort EF1 JOS) to prevent tissue shrinkage. The penis was measured by placing a piece of 1mm graph paper under it (Fig.2.2) and the length was recorded to the nearest 0.1mm. As recommended by Gibbs *et al.,* (1987), no attempt was made to straighten the penis of specimens. The RPS was calculated by comparing the average penis lengths from a sample of both males and affected females from each site using Equation 2.1 below:

(Equation 2.1)

$$\mathbf{RPS} = \frac{\textbf{mean length of female penis}^3}{\textbf{mean length of male penis}^3} \times 100$$

(Bryan *et al.*, 1986).

In this way the bulk of the female penis in relation to the male is given as a % value (Gibbs *et al.,* 1987). In extreme cases values of 100% have been recorded and this indicates that the females of a population have penis sizes equal to the average of the males. Such results indicate a high level of TBT contamination. RPS is a sensitive indicator of TBT levels in an area, but it gives no indication of the reproductive capability of female *N. lapillus*.

2.2.6 The Vas Deferens Sequence

The vas deferens sequence (VDS) is a more useful indicator of imposex because not only does vas deferens development precede the penis, but also its continued growth, formed by infolding of pallial epithelium (Gibbs *et al.,* 1987), causes the pallial oviduct to be occluded. Despite this females continue to produce egg capsules that cannot be released from the capsule gland (Fig.2.1) resulting in rupture and death.

The vas deferens sequence is divided into six stages, (Table 2.1, Fig. 2.3), which relate to the level of imposex and the ability of the female to reproduce. Females are capable of reproduction up to and including stage four of the VDS, but any further growth of the vas deferens results in sterilisation (Gibbs and Bryan, 1986).

Following the assessment of penis length for calculation of the RPS, the mantle skirt of females was cut between the hypobranchial and capsule glands (Fig.2.1) and peeled back to reveal the medial surface. Each prepared specimen was then viewed under a dissecting microscope. An assessment of each females VDS stage was then made following the features detailed (Fig. 2.3).

TABLE 2.1 THE STAGES OF THE VAS DEFERENS SEQUENCE IN FEMALES OF *NUCELLA LAPILLUS*. (Gibbs *et al.*, 1987).

Stage Number.	Characteristics of stage.
Stage 1	Development of the proximal of the vas deferens (vd) commencing with infolding of the mantle cavity epithelium in the region ventral to the genital papilla (gp) with vulva (v).
Stage 2	Development of the penis (p) initiated with the formation of a ridge.
Stage 3	Small penis develops, distal section of vas deferens formed from its base.
Stage 4	Proximal and distal sections of the vas deferens are fused and the penis is enlarging to a size approaching that of the male. Reproduction possible.
Stage 5	Vulva is overgrown by continued development of vas deferens forming a blister (b) and continued growth forms a nodule (n). Reproduction impaired.
Stage 6	Inside of capsule gland (cg) contains material of aborted capsules (ac).

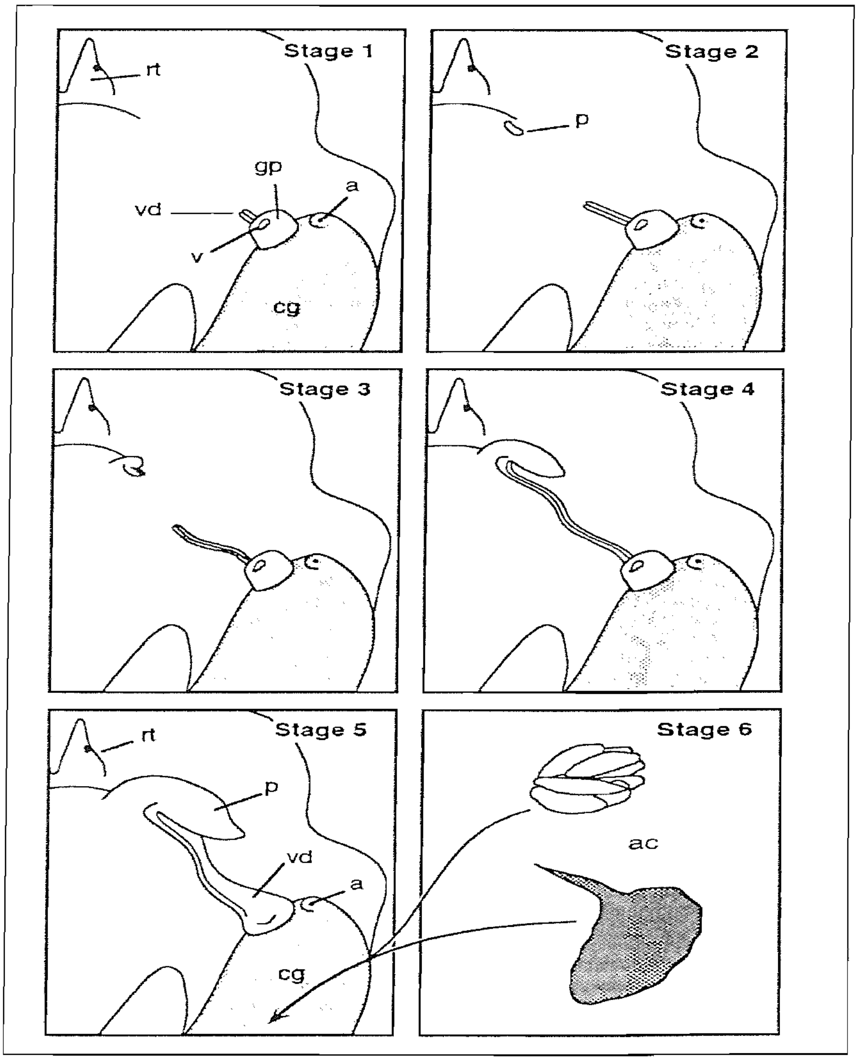

FIGURE 2.3 THE STAGES IN THE DEVELOPMENT OF THE VAS DEFERENS SEQUENCE IN FEMALE *NUCELLA LAPILLUS*. Abbreviations: a, anus; ac, aborted capsules; b, blister; cg, capsule gland; gp, genital papilla, n, nodule; p, penis; rt: right tentacle; v, vulva; vd, vas deferens. (From Gibbs *et al.*, 1987).

Many researchers present VDS data as the female population mean value (e.g. Evans *et al.*, 1998, 2000b; Santos *et al.*, 2002; Minchin, 2003). This is not entirely correct as the six VDS stages defined by Gibbs *et al.*, (1987) are ranks rather than a continuous scale. As the main focus of imposexing work in this study is to build upon historical data from work by Spence

(1989) and Proud (1994) the method follows that devised by them. The median VDS value is presented as a more accurate representation of a population's central tendency VDS. In addition, the modal VDS value is also given, where possible, as this provides an indication of future longevity of population females. Finally the percentage of sterile females (VDS stages 5 or 6) and the percent of females in each population were calculated.

2.3 *Nucella lapillus* population structure

2.3.1 Timed searches

Timed searches were used to asses the numbers and ages of *N. lapillus* populations at study sites. Although quadrats were employed to assess the distribution and availability of *N. lapillus*' prey this method has been found to underestimate dogwhelk abundance due to their scattered distribution, preference for crevices and marked differences in foraging behaviour in relation to weather (Burrows and Hughes, 1990). Timed searches allow accurate assessment of *N. lapillus* abundance through a constant effort of search and recovery over an appropriate period. The searches were carried out over a fixed period where all animals encountered were collected and shell length, aperture and shell lip thickness were measured to the nearest 0.1 mm using metric vernier callipers (Fig. 2.4). For the various treatments that have taken place throughout this study, timed searches have been adapted to appropriate scales for the relevant experiment; these modifications are detailed in the applicable sections.

The most common area to locate *N. lapillus* is between mid-tide level and mean low water neaps (MLWN) (Crothers, 1985). *N. lapillus* is rare below MLWN, particularly on sheltered shores. Sub tidal populations, however, have been reported (Crothers, 1985; Mallinson, pers. com., 2002). In this study the zone between mid tide level and MLWN was used for survey work with the exception of Highcliffe (Chapters 4 and 6).

N. lapillus size class data collected through the time searches can be used to indicate if adults dominate a population or whether juveniles are present suggesting breeding females (Bryan *et al.,* 1987; Gibbs and Bryan, 1987; Herbert, 1988; Spence *et al.,* 1990). Population structure gives a useful temporal comparison within sites, but should be treated with caution between locations. This is due to natural differential growth rates of *N. lapillus* between sites (Etter, 1989) and their phenotypic plasticity in relation to environmental factors and differences in food and refuge availability (Kitching *et al.,* 1966; Hughes and Elner, 1979;

Castle and Emery, 1981; Kirby *et al.,* 1994a,b; Day *et al.,* 1994; Etter, 1996; Son and Hughes, 2000; Trussell and Etter, 2001).

2.3.2 Age classification

Although the structure of dogwhelk populations is a useful indicator of health, there are problems associated with establishing the age of *N. lapillus*. External features such as shell height and whorl numbers do not indicate age and individual growth rates can be related to exposure and prey type (Leung *et al.,* 2001). Whorls stop being produced when maturity is reached and shell height can vary over all ages except for very young juveniles (Crothers, 1985). *N. lapillus* produces dentiform tubercules or 'teeth' (Crothers, 1985) (Fig.2.4), which can be used to identify adults (e.g. Feare, 1969). Unfortunately teeth are not always grown at maturity, but may develop after prolonged starvation (Cowell and Crothers, 1970), or due to being parasitised by trematode worms thus leading to a hormonal imbalance in males (Crothers, 1985; Evans *et al.,* 2000b). This can make age identification and imposex assessment unreliable (Minchin and Davies, 1999; Minchin, 2003). Therefore parasitised individuals were not used for this work.

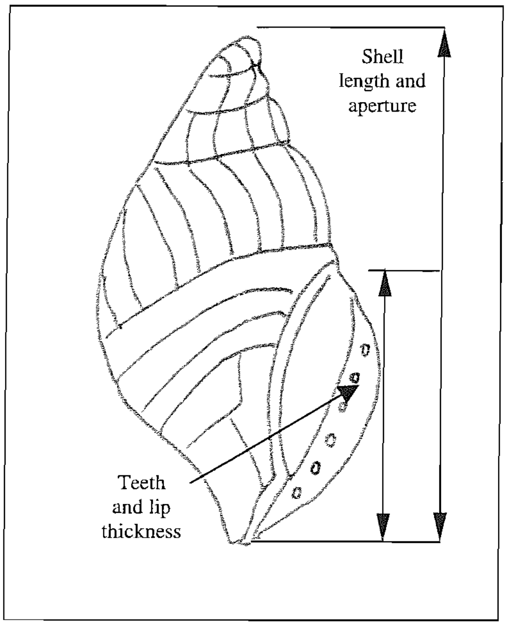

FIGURE 2.4 MEASUREMENT OF *NUCELLA LAPILLUS* SHELL CHARACTERISTICS FOR THE ASSESSMENT OF AGE CLASS. (based on Feare, 1969)

Of the methods for age assessment (Berry and Crothers, 1968; Feare, 1969; Lawrence, 1972 *op cit* Spence 1989) Spence (1989) used the system developed by Feare (1969) in which three age classes are assigned from shell height and lip thickness. Sharp lips are classed as juveniles, thickening as second years and thick, with or without teeth on the inside of the shell aperture, as adults. For continuity this method was employed by Proud (1994) and has been used here. Spence (1989) confirmed the accuracy of this system by assessing individual gonad development from juveniles to adults. In addition to age classification each shell was examined for wear to provide further clarification and following this individuals not used for imposexing were replaced on the shore.

2.4 Community assessment

2.4.1 Role of community assessment in this study

N. lapillus may have an impact on barnacle and mussel populations through predation. Many researchers have worked on the potential role of *N. lapillus* in structuring rocky shore communities (e.g. Connell, 1961; Lubchenco and Menge, 1978; Southward and Southward, 1978; Hartnoll and Hawkins, 1985; Spence, 1989; Proud, 1994). Assessment of the communities at sites used in this study was designed to increase information on this role.

2.4.2 Identification of intertidal zones

Throughout this work Lewis' (1964) modification of Stephenson and Stephensons' (1949) universal zonation scheme was used as a classificatory framework for shore communities (Fig.2.5). Shore exposure was assessed using Ballantines (1961) scale (Fig.2.6).

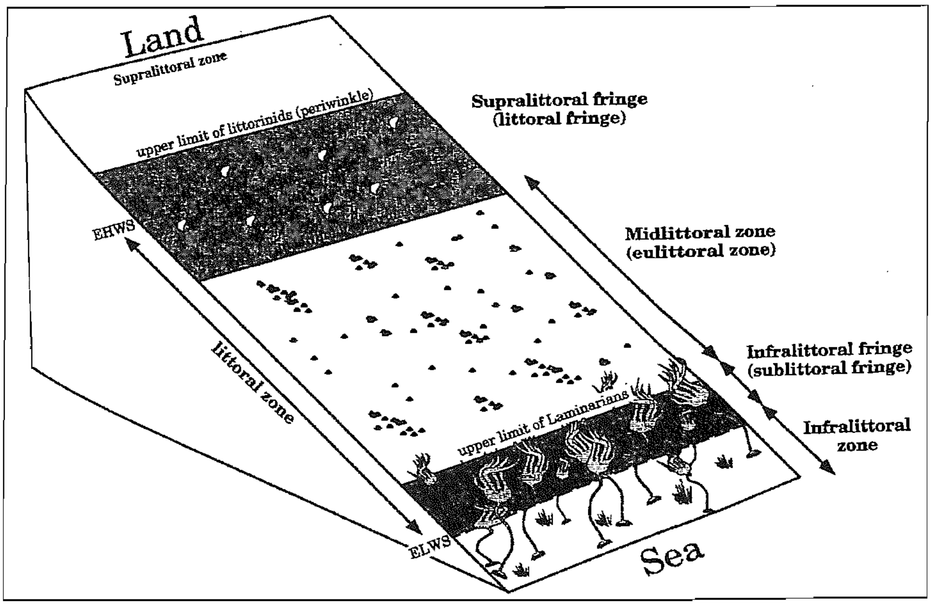

FIGURE 2.5 THE UNIVERSAL ZONATION SCHEME FOR THE DISTRIBUTION OF INTERTIDAL COMMUNITIES.
EHWS (Extreme high water spring). ELWS (Extreme low water spring)
Modified from Stephenson and Stephenson (1972), Lewis' (1964) alternative terminology in brackets.
(From Raffaelli and Hawkins, 1996).

N. lapillus is generally associated with the mid to lower eulittoral intertidal, being more common on moderately exposed shores, but ranging from relatively sheltered to exposed. For this study, the exposure scale and the zone system were used to identify habitats in which *N. lapillus* would normally be present (e.g. *M. edulis* beds and barnacle communities in the mid eulittoral). Sampling within these constraints ensured that a stratified random method could be used for all community quadrat surveys and timed searches. This allows for differences in marine habitat ensuring unrepresentative areas are not sampled (Golet *et al.*, 2002).

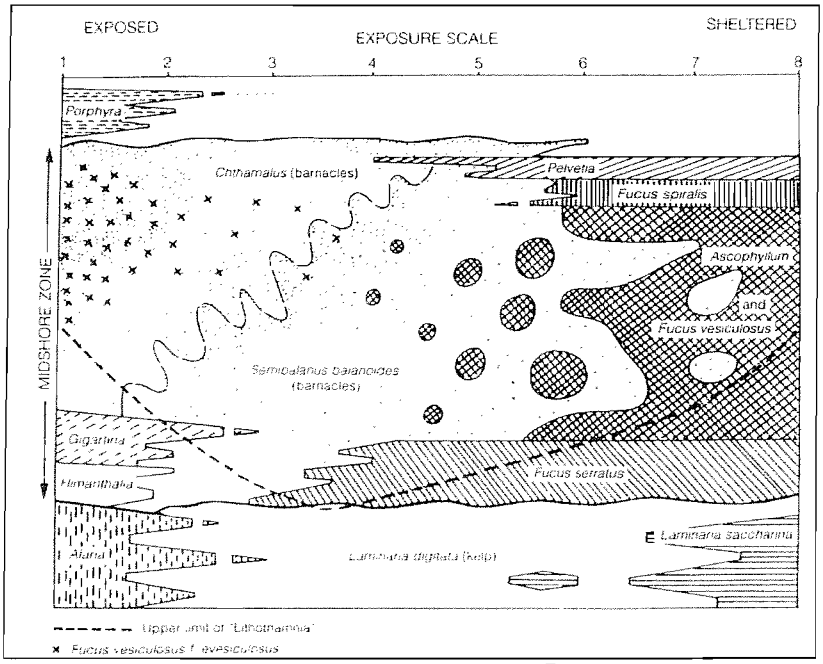

FIGURE 2.6 THE BIOLOGICAL EXPOSURE SCALE FOR ROCKY SHORES BASED ON THE PEMBROKE AREA. (Based on Ballantine, 1961, from Raffaelli and Hawkins, 1996).

2.4.3 Percentage cover

Algae, mussel and barnacle cover were assessed using a 0.5 m x 0.5 m quadrats (Chapter 6, 0.25 m x 0.25 m quadrats also). The quadrat was subdivided into 25 10 x 10 cm squares, each giving a value of 4% of the quadrat area. This approach allows a more accurate assessment of cover than an unstrung quadrat, which can be difficult to assess with a continuous effort (Barbour, 1987). Quadrats were randomly thrown using the stratified method having identified "*biologically defined strata*" (Raffaelli & Hawkins, 1996). Following this rationale all community data were collected from within the eulittoral zone (Fig.2.5) where *N. lapillus* usually occurs. For some aspects of the study, standard quadrat sizes were revised to ensure accurate coverage of the habitat under observation. Where this approach has been used, it is described in the appropriate section.

2.4.4 Optimal quadrats

For continuity of data collection, the number of quadrats used by previous workers dictated the methodology used in some of the investigations for this study and are described where appropriate. Where no protocol was prescribed, a method was utilised to ensure that the optimum number of quadrats were used thus providing an assessment of communities as accurately as possible.

Baseline studies were carried out at experimental sites where stratified random quadrat results were recorded for prey and cover species. From these an estimate of the optimum required quadrats to provide useful data was determined. This was achieved by plotting the standard error of the running mean for percentage cover of taxa versus the quadrat number. The optimal number of quadrats corresponds to the point where the curve bottoms out (Barbour *et al.*, 1987).

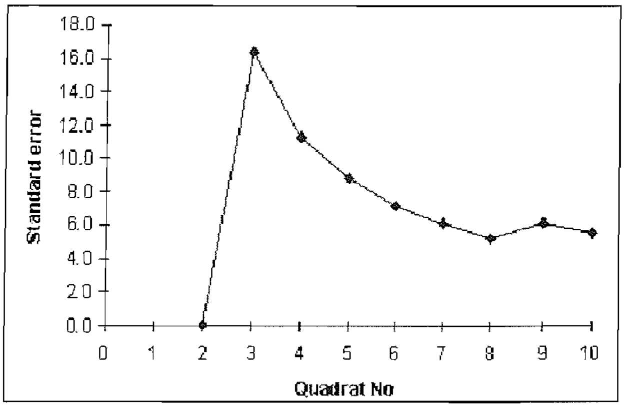

FIGURE 2.7 NUMBERS OF QUADRATS AGAINST SAMPLING ERROR USED TO IDENTIFY OPTIMAL SAMPLING EFFORT.
This example Highcliffe (Dorset) percentage barnacle cover in May 2001 on treatment groyne H8 (see Chapter 6).

Figure 2.7 shows an example of the process where the standard error has been calculated for percentage of barnacle cover. It can be seen that after seven quadrats there is limited value in continuing to sample (standard error quadrats 7-10 = 0.45) when considering tidal restrictions.

2.4.5 Species identification

When unsure of in field identification of species, a field guide was used, e.g. Collins Guide to Rocky Shores (Barrett and Yonge, 1977; Hawkins and Jones, 1992). Where species proved difficult to identify in the field, an example was removed outside of the experimental area, and returned to the laboratory for later identification.

2.4.6 Location of field sites

In cases where locations used by previous workers were re surveyed, the general area and description of these sites were referred to. Where sites have been developed for experimental studies, their locations within a shoreline were marked and referenced to recognisable features on the shore. In addition, site location was recorded in latitude and longitude using a hand held GPS unit (Magellan 2000XL). These units are not entirely reliable unless they are the differential type which was not available, therefore the GPS was used as a general guide.

Chapter 3. Temporal and spatial trends of recovery from TBT pollution by *Nucella lapillus* on the UK south coast

3.1 Introduction

Diffuse TBT pollution has adversely impacted stenoglosssan gastropod populations worldwide (e.g. Smith, 1981a,b,c; Bryan *et al.,* 1986; Gibbs and Bryan, 1986; Ellis and Pattisina, 1990; Hawkins and Hutchinson, 1990; Saaverdra Alvarez and Ellis, 1990; Spence *et al.,* 1990; Smith, 1996; Swennen *et al.,* 1997; Rilov *et al.,* 2000; Strand and Jakobsen, 2002; Gibson and Wilson, 2003, see Bryan and Langston, 1992; Matthiessen and Gibbs, 1998; Macguire, 2000; Birchenough *et al.,* 2002a for reviews). Recent studies continue to show that imposex as a result of TBT pollution is still causing extinction or decline of muricid gastropod populations (e.g. Rilov *et al.,* 2000), although long-term monitoring in some areas has indicated a gradual improvement (e.g. Huet *et al.,* 2004). This chapter describes the extent of *Nucella lapillus* recovery on the south coast of England following legislation in 1987, integrating new survey data with previous work.

Bryan *et al.,* (1986) and Gibbs and Bryan (1986), were first to establish the cause and effect relationship of TBT and imposex affecting UK populations of *N. lapillus* first observed by Blaber (1970) (also see: Miller and Pondick, 1984; Gibbs *et al.,* 1987). Their work around Plymouth Sound was subsequently continued by their students (e.g. Spence, 1989; Proud, 1994) who assessed the effects of the partial ban on TBT use in the UK in 1985 and the subsequent tightening of these regulations in 1987. Spence *et al.,* (1990) showed that other locations on the south coast of England had, in the late 1980s, the highest levels of imposex recorded around the UK; many sites had only male *N. lapillus* present. At numerous locations, such as Cowes on the Isle of Wight, dogwhelks were reported as completely absent (e.g. Herbert, 1988; Spence *et al.,* 1990; Harding *et al.,* 1992, 1999; Langston *et al.,* 1997,1998; Bray and Herbert, 1998; CONSSO, 1998; Herbert *et al.,* 2000).

The south coast of England is the largest area in the UK that has been continually affected by high TBT levels (e.g. Spence *et al.,* 1990; Langston *et al.,* 1998; Herbert *et al.,* 2000; Birchenough *et al.,* 2002b; Hawkins *et al.,* 2002). The region has numerous sheltered coastal waterways, which are used for military, commercial and recreational harbours. The south coast has the highest activity in recreational boating in the UK, being particularly intense around the Isle of Wight and Solent (Royal Yachting Association pers. com., 2003). Langston *et al* (1994) predicted that TBT levels in the Hamble River near Southampton would fall from 500 ngl^{-1} in 1987 to the UK Environmental Quality Target (EQT) of 2 ngl^{-1} by 1995. In 1995, however, mean concentrations of TBT in the Hamble were ten-fold higher than the EQT (Langston, 1995) and by 2000 levels near Southampton docks were still above the EQT at 22-33 ngl^{-1} (Thomas *et al.,* 2000, 2001). Although Langston and Pope, (1995) showed a reduction in TBT, the rate of decrease slowed. In 1997/1998 *N. lapillus* was still absent from many locations directly influenced by the Solent (Langston *et al.,* 1997; CONSSO, 1998). *N. lapillus* has recovered and recolonised in some locations on the south coast and Isle of Wight (Herbert *et al.,* 2000; Birchenough *et al.,* 2002b), but the retarded recovery around the Solent may reflect high commercial shipping levels (Langston *et al.,* 1994; Bray and Herbert, 1998) as well as material stored in the sediment (Thomas *et al.,* 2000, 2001).

Following early surveys of the incidence of imposex in Plymouth Sound, Gibbs *et al.,* (1987) reported that "*around south-west England at least, imposex has advanced to the stage where almost all females of this species are penis bearing*". Spence (1989) and Proud (1994) continued monitoring Plymouth *N. lapillus* to investigate projected imposex changes after legislation had been introduced. Predictions were that imposex would decline and populations recover by the late 1990s. Continued long-term monitoring of *N. lapillus* in the region provided an opportunity to show the effectiveness of UK TBT legislation. A brief preliminary summary of long-term recovery has been provided by Hawkins *et al.,* (2002).

Some sites with continuing high levels of TBT in the UK and world-wide have become known as TBT 'hot-spots' (Evans *et al.,* 1995, 1998; Rilov *et al.,* 2000; Birchenough *et al.,* 2002a,b; Gibson and Wilson, 2003). In the UK these are normally close to industrial ports (e.g. Harding *et al.,* 1997; Morgan *et al.,* 1998; Miller *et al.,* 1999), but elsewhere have been related to dumped industrial port dredge spoil (Santos *et al.,* 2004). Rilov *et al.,* (2000) investigated imposex near two marinas in Israel, which has no current TBT legislation. They found that high organotin levels from the marinas caused imposex in two species of gastropod (*Stramonita haemastoma* and *Hexaplex trunculus*). Imposex significantly decreased away from the marinas. Therefore they were described as TBT 'hot-spots' (Rilov *et al.,* 2000). A monitoring programme officially started in 2001 investigating TBT and other pollutants in port sediments showed that concentrations were in the region of 100ì g/kg

(Barak Herut *et al.,* 2003). In the UK *N. lapillus* populations on the Isle of Wight have continued to be affected (Langston *et al.,* 1997; Bray and Herbert, 1998; CONSSO, 1998; Herbert *et al.,* 2000) with some severely impacted populations at sites on the south coast which are unlikely to have direct shipping influence (Bryan, 1987; Bray, 1996; Bray and Herbert, 1998; Herbert *et al.,* 2000). Study of the impact of TBT on *N. lapillus* at these sites will show whether imposex levels have declined and population abundance increased, thus identifying potential continuing impacts if TBT usage continues.

The overall aim of this chapter is to monitor the recovery of *N. lapillus* populations following legislation introduced in the UK in 1987. This chapter reports on the continued incidence of imposex in *N. lapillus* populations in Plymouth Sound. This work commenced in 1997, 10 years after the UK ban of TBT on vessels less than 25m LOA (Length Over All). To complement these new data, original, often unpublished, results from other workers on the south coast of England and Isle of Wight have been presented to provide a synthesis. Sites where TBT contamination continues at high levels can be identified.

The study areas and sites selected reflect local levels of TBT and long-term data availability. Plymouth Sound is heavily used by Royal Naval, commercial and recreational vessels (Table 3.1) and is the site of some of the earliest observations of imposex (Blaber, 1970, Bryan *et al.,* 1986; Gibbs and Bryan. 1986). In contrast, the sites chosen for the regional south coast study are more impacted by recreational craft, but may be influenced by commercial vessels from minor ports such as Weymouth and Poole. Finally, the severely contaminated Isle of Wight and Solent region (Langston *et al.,* 1994; Herbert *et al.,* 2000) has been used to demonstrate the influence of both commercial and recreational vessels within a relatively enclosed waterway. This chapter contributes to an evaluation of the effectiveness of UK legislation. Predictions of rates of recovery by previous authors on this subject can also be assessed (e.g. Spence *et al.,* 1990; Langston *et al.,* 1990, 1994). The specific objectives are:

1. To investigate *N. lapillus* recovery from imposex at local and regional scales;
2. To quantify the recovery of populations in the Plymouth area following the implementation of legislation in 1987 by means of a long-term data set;
3. To examine the continuing influence of TBT on Isle of Wight populations at potential 'hot-spots' of contamination;
4. To consider other factors affecting *N. lapillus* populations such as availability of prey which could influence abundance and the potential for recovery.

3.2 Methods

3.2.1 Site selection

Sites selected were where TBT concentration gradients were likely to exist or high imposex levels continued. Locations along the south coast were chosen to reflect different levels of contamination from recreational and commercial ports. For long-term monitoring of Plymouth Sound, sites were those used by previous workers (Bryan *et al.,* 1986; Gibbs and Bryan, 1986; Gibbs *et al.,* 1987; Bryan *et al.,* 1987; Spence, 1989; Proud, 1994). Sites on the north shores of the Isle of Wight reflected contamination from the Solent, whereas work on the southern shores continued previous research (e.g. Bryan, 1987).

3.2.2 South coast

Despite being the region where imposex and its relationship with TBT was first identified in the UK, the south coast of England has received less attention compared to east and northern UK ports (e.g. Douglas *et al.,* 1993; Evans *et al.,* 1991, 1994, 1996, 2000a; Morgan *et al.,* 1998; Birchenough *et al.,* 2002a). Following initial recognition of the TBT issue in the south west of England (Blaber, 1970; Bryan *et al.,* 1986; Gibbs and Bryan, 1986) the long-term recovery of *N. lapillus* has not been widely reported for this region (but see Hawkins *et al.,* 2002 for a preliminary report). Some work has been carried out on population recovery, (Spence *et al.,* 1990; Bray and Herbert, 1998; Herbert *et al.,* 2000; Birchenough *et al.,* 2002b) and the persistence of TBT using translocated *N. lapillus* as indicators (Harding *et al.,* 1999).

As the south coast provides many sheltered waterways where vessel use is high, no ideal control sites free from shipping could be identified. For the purposes of this study, changes in imposex levels were related to the long-term recovery and condition of *N. lapillus* at the 'clean' site of St. Agnes Cornwall (Bryan *et al.,* 1986), used by Spence (1989) and Proud (1994) (§ 3.1) as a control site. Sites were based on work by Crothers (1975) where the presence of *N. lapillus* populations was reported during shell morphology survey work. Rocky shores become less common on the south coast eastwards from Swanage. The mainland shore east of Old Harry Rocks (Fig. 3.1, SZ 054 824) consists mainly of Barton sands and gravels; few habitats are suitable for *N. lapillus* though there have been reports of isolated populations (Herbert *et al.,* 2000). Spence *et al.,* (1990) surveyed along the south coast, but did not work on this section. Langston *et al.,* (1990) suggested that a population may have been present slightly to the north west of Browndown Point (Fig. 3.1, SZ 567 991), but at the time were absent. In addition, Langston *et al.,* (1994) reported that a population had been present at Netley foreshore (Fig. 3.1, SU 465 075), but that none could be found in 1993. Numerous *N. lapillus* shells can be seen in the death assemblage at Netley foreshore (pers. obs.). *N. lapillus* is generally absent from points east of Old Harry Rocks until Selsey Bill.

Portland Bill (SY 677 684) (A: Fig. 3.1) consists of limestone with chert. The site is very exposed with high tidal races and is subject to extreme wave action. This has resulted in wave cut platforms to the east of the most southerly point of the Bill. These platforms provide some shelter from wave action and the community on the site is indicative of that associated with 2-3 on Ballantine's (1961) exposure scale (Fig. 2.6).

Osmington Mills (SY 735 817) (B: Fig. 3.1) is a limestone reef with chert deposits. Down to the lower eulittoral zone there are boulders and wave formed reefs running parallel to the beach. Osmington is moderately exposed shore with abundant *G. umbilicalis* and littorinids.

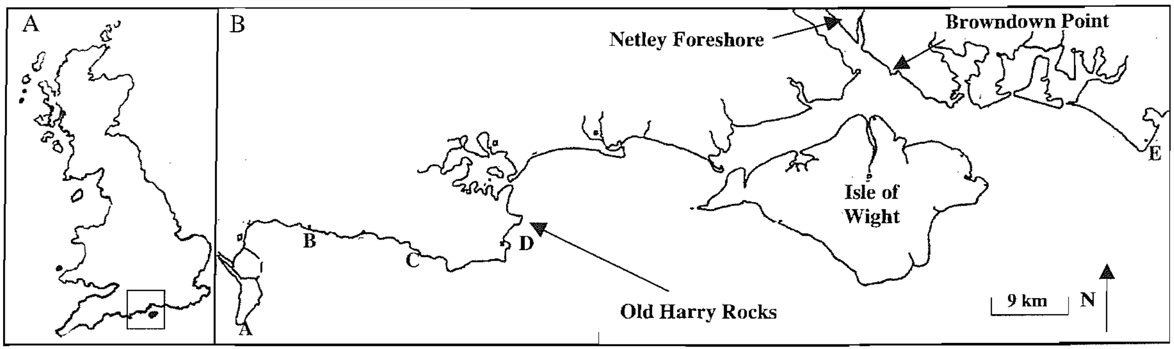

FIGURE 3.1 A: THE CENTRAL SOUTH COAST REGION OF THE UK, B: SITES ON THE SOUTH COAST SURVEYED TO ESTABLISH IMPOSEX LEVELS AND POPULATION STRUCTURES OF *NUCELLA LAPILLUS* IN THE REGION.
Sites: A: Portland Bill, B: Osmington Mills, C: Lulworth Cove, D: Peveril Point, E Selsey Bill.

Lulworth Cove (SY 827 797) (C: Fig. 3.1) is an embayment within the Dorset Heritage Coast. *N. lapillus* are generally associated with a small moderately exposed Purbeck limestone reef on the eastern side of the cove which is perpendicular to the shore.

Peveril Point (SZ 041 787) (D: Fig. 3.1) is located at Swanage in Dorset. The limestone with chert deposit reef is perpendicular to the shore. The site is exposed with sparse barnacle cover, but with a considerable population of limpets and abundant intertidal algae.

Selsey Bill (, SZ 875 945) (E: Fig. 3.1) was chosen as the most easterly survey point for UK south coast work. The site consists of Bracklesham gravel beds overlain with a storm beach of large gravels and cobbles. Although this substrate is relatively mobile, wooden groynes and a sea wall have resulted in some stability. The intertidal community is sparsely distributed although littorinid grazers inhabit large cobbles and concrete debris on the largely flat shore profile.

3.2.3 Plymouth Sound

Plymouth Sound (Fig. 3.2) is a drowned river valley (ria) supporting a variety of estuarine and intertidal habitats (MBA, 1957; TECF, 1998). The intertidal zone is varied, with rock, cobble and fine sediment. In 1996 Plymouth Sound was chosen as a candidate Special Area for Conservation (cSAC) (Langston *et al.,* 2003) and has now achieved this designation.

TABLE 3.1 MAIN VESSEL GROUPS USING PLYMOUTH SOUND AND THEIR TOTAL TONNAGE FOR 1993, 1997 AND 2003. (Modified from Fletcher, 2003).

Indicators of Current Levels of Activity				**Comments**
Annual Traffic for:	**1993**	**1997**	**2001**	
Ministry of Defence port use tonnes				While there has only been a relatively small increase in activity overall, the size of the ships has increased, and the level of MOD activity in the Port is at about capacity.
Warship/RFA	2,882	5,099	3,442	
RMAS /Other Military	6,064	13,478	9,938	
Commercial port use tonnes				While the overall level of activity has marginally decreased, there is still a significant volume of traffic and overall level of Port activity is considered to be quite healthy. Within these broad figures, the amount of oil shipped has remained fairly static, stone has increased, animal feed has increased significantly, clay has increased, fertiliser has remained static, general cargo has decreased, and the fish market is now well served by larger boats.
Commercial		6,172	4,954	
Ferries	1,928	1,014	805	
Fishermen	8,049	6,219	2,566	
Recreational marina use				
Moored craft Nos.	1,564	2,816	3,907	These figures do not reflect the actual numbers of craft using the Sound and its estuaries when the number of trailed and visiting craft is taken into account

RFA: Royal Fleet Auxiliary. RMAS: Royal Maritime Auxiliary Service.

There are considerable and increasing pressures on Plymouth Sound ranging from commercial and military vessels to fishing and recreational craft (Fletcher pers. com., 2003). Data on shipping for Plymouth are not readily available due to sensitivity concerns regarding Royal Naval information. Where available, shipping tonnage figures for the three main classes of vessel use with Plymouth Sound are given in Table 3.1.

Populations of *N. lapillus* within Plymouth Sound declined in the 1980s due to high TBT levels (Bryan *et al.,* 1986; Gibbs and Bryan, 1986) and the sites chosen for study (Fig. 3.2) were previously selected by Spence (1989) to continue the early work. These exposed or moderately exposed locations (Spence, 1989) were believed to be along a contamination gradient of TBT from proximity to Devenport Dockyard within Plymouth Sound. There is also the potential impact from TBT released from dumped dredge spoil at the Rame Head disposal site on *N. lapillus* populations at Tregantle and Renney Rocks which was not realised at the time of site establishment (Fig. 3.2).

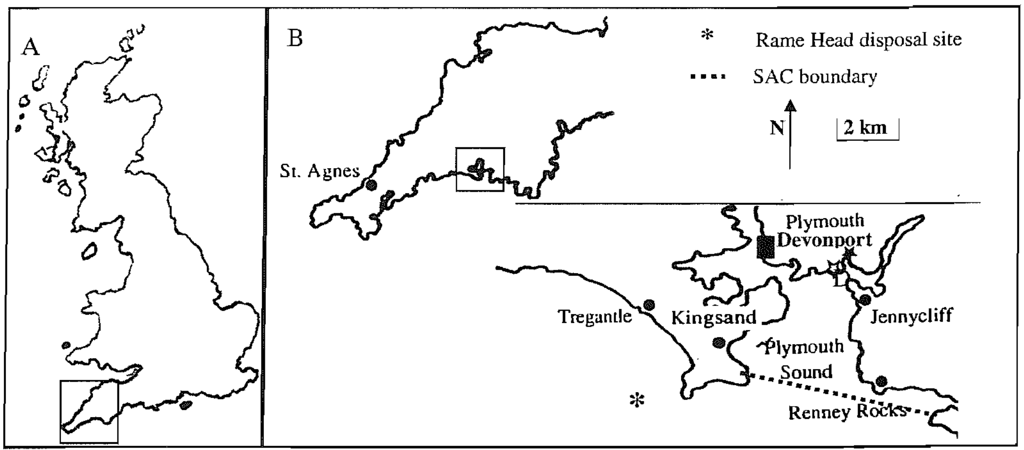

Figure 3.2 A: Cornwall and Devon south west UK, B: study sites in Devon and Cornwall around Plymouth Sound and the reference site at St. Agnes.
Rame Head disposal site and the Special Area of Conservation (SAC)are shown. Modified from Spence (1989) and CEFAS (2003).

Tregantle (SX 387 525) (Fig. 3.2) is moderately exposed to exposed and is located on Whitsand Bay outside and west of Plymouth Sound. The site consists of rock stacks and outcrops vertical to the shore that have become eroded with numerous cracks providing microhabitats and refuge for *N. lapillus*. Limpets are abundant on the rocky outcrops. Mussels dominate the area with barnacles occurring more on eastward faces. The lower section of each rock stack is generally bare, possibly due to scouring.

Kingsand (SX 438 514) (Fig. 3.2) is on the western shore of Plymouth Sound. The site has barnacle-covered reefs perpendicular to the shore, which are interspersed with rocks and boulders. A barnacle covered redundant sewage pipeline crosses the shore. The pipeline and the shore are grazed by abundant littorinids, patellids and trochids.

Renney Rocks (SX 492 487) (Fig. 3.2) are situated on the south east entrance point to Plymouth Sound. This is a moderately exposed reef with numerous blocks and remnant rock stacks covered by barnacles with abundant littorinids, patellids and trochids.

Jennycliff (SX 491 521) (Fig. 3.2) on the eastern shore of Plymouth Sound is nearest to the influence of Devonport Dockyard and the growing commercial activities in the Cattewater and marinas. This shore is moderately exposed and the substrate is shale and large boulders on a rock bed. The eulittoral was dominated by dense barnacle cover with fucoid patches and numerous grazers such as littorinids, trochids and patellids.

Ideally a control site which had communities similar to those found at Jennycliff, Renney Rocks and Kingsand would have been set up during the initial work. At the time of site selection in 1987, there was no sheltered location without mussels available due to the widespread contamination by TBT in the south west (Spence, 1989).

St Agnes, (SW 723 518) (Fig. 3.2) on the north Cornwall coast, was chosen by Spence (1989) as a control site subject to minimal TBT influence. *N. lapillus* from St. Agnes were previously used by Gibbs *et al.,* (1987) as a source population for transplant because of low TBT pollution levels. It is similar to Tregantle with rock stacks and outcrops, but with less sand infill. As at Tregantle, the shore community consisted of mussel beds, barnacle and fucoid patches with associated patellid grazers.

3.2.4 Isle of Wight

Some of the early work establishing the relationship between TBT and imposex was undertaken on the Isle of Wight (e.g. Bryan, 1987). The island was one of the targets of a 1988 Marine Conservation Society survey to establish the national status of *N. lapillus* after the effects of TBT became apparent (Herbert, 1988). Since that time several workers have carried out further studies (e.g. Spence *et al.,* 1990; Langston *et al.,* 1990, 1994; Bray, 1996; Bray and Herbert, 1998; Turner, 1999; Herbert *et al.,* 2000), although this work has not previously been integrated to give a time series.

Although the Isle of Wight does not have a major port on its coastline, shipping entering or leaving Southampton Water or Portsmouth Harbour passes through the Solent (Fig. 3.3). In addition to ship-borne TBT sources, contamination originating from the various docks and terminals (Southampton Docks, Fawley and BP oil terminals, Hamble River and Portsmouth Dockyard) also reaches the Solent. Recreational vessels are present in large numbers on the northern shores of the Isle of Wight with large marinas concentrated in Cowes (Fig. 3.3). Northern sites on the Isle of Wight reflect the long-term use of this area by recreational vessels and the influence of the Solent itself.

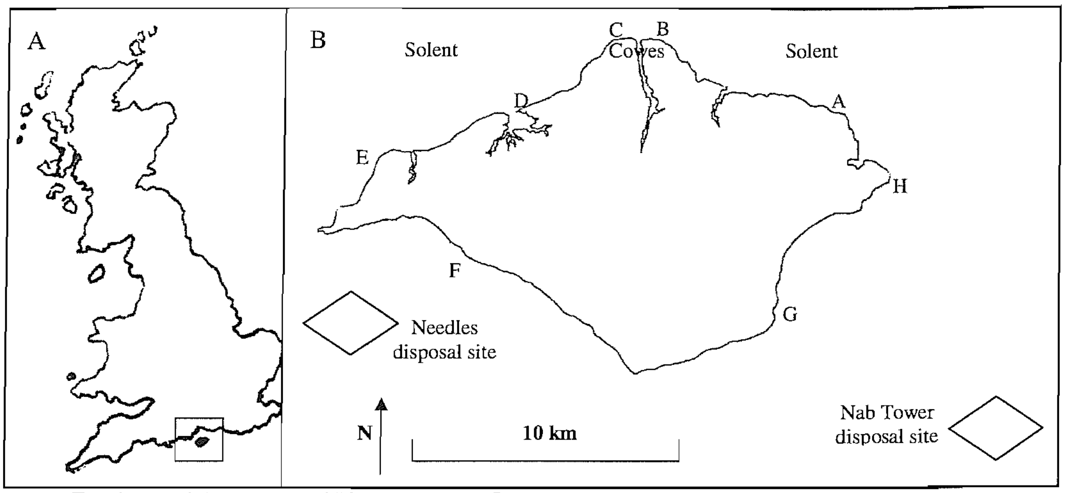

FIGURE 3.3 A: THE ISLE OF WIGHT ON THE UK SOUTH COAST B: THE SITES SURVEYED TO ESTABLISH IMPOSEX LEVELS AND POPULATION STRUCTURES OF *NUCELLA LAPILLUS*.
A: Seaview, B: East Cowes, C: West Cowes, D: Newtown, E: Warden Ledge, F: Hanover Point, G: Shanklin Horse Ledge, H: Bembridge Ledges. Needles and Nab Tower dredge spoil disposal sites also shown (CEFAS, 2001).

On the southern shores of the Isle of Wight, recreational moorings are not readily available. Heavy commercial shipping passes both the east and west of the island out to the English Channel and this may be the major source of TBT affecting southern Isle of Wight *N. lapillus* populations. In addition the source may be from sediment dispersing from the Solent (Bray and Herbert, 1998) or from TBT bound to dredge spoil dumped at the Needles and Nab Tower sites (Fig. 3.3b). Southern Isle of Wight populations have never been reported as fully extinct although some severe imposex levels have been recorded (Bryan, 1987; Langston *et al.,* 1990, 1994). It is possible that these populations provided sufficient numbers of individuals to allow the eventual spread of *N. lapillus* back to formerly severely polluted sites on the north coast. As *N. lapillus* had been widely reported to be absent from shores on the north of the island for some years, only minimal survey and destructive sampling to measure imposex has been undertaken to protect those individuals present and to ensure recovery (Herbert pers. com., 1995, 2002).

The moderately exposed North shores of the Isle of Wight provide suitable habitats for *N. lapillus* with sandstone outcrops and platforms at Seaview (SZ 629 917) (A: Fig. 3.3) and Bembridge (SZ 661 875) (H: Fig. 3.3). At site East Cowes (B: Fig. 3.3) dogwhelks inhabited a concrete breakwater whereas at West Cowes (C: Fig. 3.3) they were found on a rip-rap coastal defence system. Both sites B and C are influenced by pollution from recreational craft. At Newtown (D: Fig. 3.3) *N. lapillus* were previously found on a gravel spit (Bray and Herbert, 1998) and this population was reinvestigated. At Warden Ledge (E: Fig. 3.3) *N.*

lapillus were found on occasional rock and man-made outcrops. At Newtown recreational vessels were common, but at Warden Ledge they were less frequent due to limited shelter.

On the southern shore of the Isle of Wight, the coast is more exposed. Hanover Point (site F, Fig. 3.3) is on the boundary of Freshwater Bay. *N. lapillus* populations occur here on an outcrop of a fossilised forest reef. At Horse Ledge and Bembridge Ledges (sites G and H, Fig. 3.3), *N. lapillus* inhabit wave cut sandstone platforms. The influence of recreational vessels in these areas was minimal as there are few safe anchorages and commercial craft do not pass close to these coasts. As with other south coast sites, no appropriate control with which to compare Isle of Wight populations was available. St. Agnes in north Cornwall was used as a reference site.

3.2.5 Specific methods and materials

General methods used for fieldwork have been described in Chapter 2. For the long-term and broad-scale imposex surveys, the methods used are described in greater detail here. Population structure and abundance were assessed by methods used by previous workers thereby providing internal consistency. Imposex was measured using relative penis size index (RPS) and vas deferens sequence (VDS) (§ 2.2.5 and 2.2.6) (Bryan *et al.,* 1986; Gibbs and Bryan, 1986).

Timed collections were used to measure *N. lapillus* population abundance. These followed the method described in section 2.3.2. Spence (1989) and Proud (1994) carried out ten-minute timed searches in Plymouth Sound. In 1997 this method was further developed to encompass replicates. Three ten minute searches were undertaken at each site for each survey year within the mid to lower eulittoral zone (§ 2.4.2) (Shute, 1997). This method was used for all subsequent surveys at Plymouth. Where small numbers of juveniles were found, a further 5-minute search was carried out. This additional time ensured effective searching in less accessible areas as juveniles can hide in empty barnacle cases, mussel clumps and narrow rock crevices (Crothers, 1985). This protocol again follows that used by Spence (1989). All counts were expressed as numbers per time search.

Isle of Wight and south coast data were collected using a variety of strategies. Bryan (1987) Bray (1996) and Bray and Herbert (1998) used single searches whereas Turner (1999) and Bray (this work) used three ten minute replicates.

For the assessment of imposex *N. lapillus* individuals were collected and processed in the laboratory (§ 2.2). Using the vas deferens sequence (§ 2.2.6, Bryan *et al.*, 1986; Gibbs and Bryan, 1986) the proportion of females at VDS stage 5 or above was estimated. This was used as an index of sterility in populations as females at stage 5 are unable to breed and will eventually die due to rupture of the capsule gland (Fig. 2.1) (Oehlmann *et al.*, 1996). The percentage of females in *N. lapillus* populations was also estimated.

Fewer females than expected may indicate the impact of TBT on *N. lapillus* populations. Where this occurs populations may be suffering from low recruitment with older females at VDS stage 4 (approaching sterility) or above. Various estimates of the 'normal' percent of females in *N. lapillus* populations have been made. In populations unaffected by TBT female proportions have been reported from 41% (Minchin and Davies, 1999) to 58% (Moore, 1938a). The value from Moore (1938a), for populations in Plymouth Sound and Cornwall, has been used as a benchmark for these studies (see also Spence, 1989 and Proud, 1994). This value takes into account an increasing proportion of females to males when considering age and shell height.

The relative penis size index (RPS) (§ 2.2.5) is a relatively precise indicator of TBT levels affecting *N. lapillus* populations (Bryan *et al.*, 1987). In addition to RPS values, raw penis length data were compared using ANOVA to establish whether mean female penis lengths changed over time and in comparison to males within the populations. Care must be exercised when making statistical comparisons of penis length between populations. This is because *N. lapillus* is polymorphic and penis lengths can be related to body mass (Gibbs *et al.*, 1987) with animals from sites where large morphs are present having a correspondingly higher penis length and *vice versa*. In addition, penis length can change seasonally in male *N. lapillus,* which can affect the results of RPS calculations (Spence, 1989). Therefore shell length was correlated against penis length for both sexes to identify if any relationship existed and surveys were carried out within the same season to minimise confounding of results due to male penis growth or decline. At Plymouth sites sampling was carried out in late autumn to early winter, as this is when TBT concentrations in *N. lapillus* are highest (Skarphéðinsdóttir, 1996). Isle of Wight and south coast surveys were slightly more opportunistic but were always between summer and autumn.

N. lapillus size class abundances can be used to indicate levels of recruitment with populations from badly contaminated sites dominated by older, larger individuals. Therefore, to compare population structures, Kolmogorov-Smirnov Z (KSz) tests (Siegel, 1956) were carried out on population size cumulative frequency curves. Where sequential multiple population comparisons of yearly results were necessary, this had the potential to cause a propagating error resulting in acceptance of significant differences when there are none (type 1 error). Normally Bonferroni corrections (Bonferroni, 1936) can be used to correct for the error, but can be too conservative resulting in an unobtainable P value (Prescott pers. com., 2003). Therefore for multiple comparisons significant differences were only accepted at $P<0.01$ or less (Pinn pers. com., 2003; Prescott pers. com., 2003). For other examples of this approach see Connell and Gladsby (1999) and Connell (2000, 2001).

Juvenile abundances were assessed to indicate if TBT was adversely affecting the ability of females to breed thereby reducing recruitment. Before 1997 replicated timed searches were not made. Therefore changes in the relative abundance of juveniles were assessed using a two-way unreplicated ANOVA for the factors site and year. For results collected after 1997 repeated time searches were available. Therefore comparisons were made using a replicated two-way ANOVA for the factors sites and years.

The abundance of the primary prey (percentage barnacle and mussel cover) of *N. lapillus* was also recorded in all Plymouth surveys from 1997 onwards. These data were collected using thirty random 0.5 x 0.5 m quadrats. Where results have been synthesised from this work and previous workers' data, prey abundance is presented when available.

3.3 Results

3.3.1 South coast populations

3.3.1.1 Incidence of imposex

South coast sites (Fig. 3.1) were surveyed in 1995 (Bray, 1996), 1999 (Turner, 1999: Osmington and Lulworth; Bray: Selsey) and 2003 (Bray, this work). Neither Portland nor Peveril point had enough *N. lapillus* (less than 30) to calculate imposex. In addition, there were insufficient numbers at Selsey in 1999 and 2003, and at Lulworth Cove for 2003.

TABLE 3.2 RELATIVE PENIS SIZE (RPS) INDEX RESULTS FOR *NUCELLA LAPILLUS* POPULATIONS IN THE SOUTH COAST REGION. 1995 (Bray, 1996), 1999 Bray and Turner (Turner, 1999), 2003 (Bray, this work). NA: Not available.

Year	**Osmington Mills**	**Lulworth Cove**	**Selsey**
1995	2.54	4.80	4.83
1999	1.39	1.58	NA
2003	0.22	NA	NA

Low RPS values of less than 5 (values of 100 have been recorded, Spence *et al.* 1990) were found at all the south coast sites investigated in 1995 (Table 3.2). These continued to decline at Osmington Mills and Lulworth Cove thus indicating low TBT contamination.

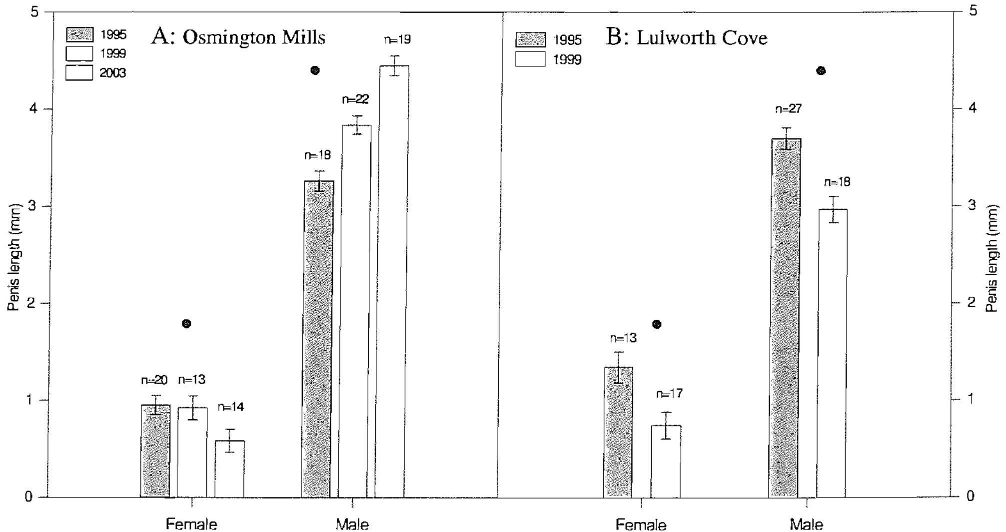

FIGURE 3.4 COMPARISON OF MEAN (+/- 1SE) PENIS LENGTHS FOR FEMALE AND MALE *NUCELLA LAPILLUS* FOR OSMINGTON MILLS AND LULWORTH COVE.

Figure 3.4 shows changes in both male and female penis size at Osmington Mills and Lulworth Cove. The improvement in RPS value at Osmington Mills was due to an increase in male penis length coupled with a decrease in that of the females (Table 3.3A, significant differences for year, sex and year x sex interaction). In contrast at Lulworth Cove both male and female penis lengths decreased over time at a similar rate (Table 3.3B, significant differences for year and sex, but no significant interaction). Comparing Osmington Mills and Lulworth Cove female penis lengths revealed no significant differences (see Fig. 3.4), although changes occurred between years and their magnitude varied between the sites (Table 3.3C, significant for years and year x site interaction). *Post hoc* Tukey's tests (Table 1A, Appendix 1) confirm the importance of larger male penis lengths at Osmington Mills in contributing to the lower RPS values.

Table 3.3 Two-way ANOVA comparison results for penis lengths at Osmington Mills (1995, 1999, 2003), Lulworth Cove (1995, 1999) and Osmington Mills and Lulworth Cove females 1995,1999).

Osmington Mills					
Source	**DF**	**SS**	**MS**	**F**	**P**
Year	2	3.1	1.6	7.9	<0.001
Sex	1	234.5	234.5	1195.9	<0.001
Year x Sex	2	10.5	5.3	26.9	<0.001
Residual	100	19.6	0.2		
Total	105	273.0	2.6		
Lulworth Cove					
Source	**DF**	**SS**	**MS**	**F**	**P**
Year	1	7.7	7.8	23.2	<0.001
Sex	1	91.9	92.0	275.3	<0.001
Year x Sex	1	0.1	0.1	0.2	NS
Residual	71	23.7	0.3		
Total	74	136.2	1.8		
Osmington Mills vs Lulworth Cove (between females 1995 and 1999)					
Source	**DF**	**SS**	**MS**	**F**	**P**
Year	1	1.5	1.5	6.7	<0.05
Site	1	0.2	0.2	0.8	NS
Year x Site	1	1.2	1.2	5.6	<0.05
Residual	59	13.1	0.2		
Total	62	15.8	0.3		

N.B. Insufficient *N. lapillus* found at Lulworth Cove in 2003 for imposex assessment.

Post hoc comparisons between female penis lengths at Osmington and Lulworth showed that in 1995 the penis length of Lulworth females was greater than those at Osmington (P<0.05, Table 1C, Appendix 1). By 1999 mean female penis length at Lulworth had reduced (Fig. 3.4b) and no significant difference between sites was found (Table 1C, Appendix 1).

VDS (§ 2.2.6) results for Portland, Peveril Point, Lulworth and Selsey Bill were limited due to insufficient numbers (or absence) of *N. lapillus*. With all south coast sites no sterile (stages 5 and 6) *N. lapillus* were found between 1995 and 2003. VDS values at Osmington Mills declined over this period (Table 3.4) indicating that TBT pollution had reduced by 2003. The mode in 1995 was stage 4, one below the point at which *N. lapillus* females become sterile. This suggested that populations may have declined further due to lack of recruitment, but by 1999 recovery from TBT impacts was seen with less imposex development. The proportion of females in the population, however, also dropped over this period which may have led to reduced recruitment (Table 3.4).

At Lulworth Cove the ban on use of TBT on small craft may have resulted in the lower VDS values by 1999 (Table 3.4), but in both 1995 and 1999 no sterile females were found. Unlike Osmington, at Lulworth between 1995 and 1999 the percentage of females increased (Table 3.4), but by 2003 the abundance of *N. lapillus* was too low to allow estimates of imposex.

TABLE 3.4 VAS DEFERENS SEQUENCE RESULTS (1995, 1999, 2003) FOR FEMALE *NUCELLA LAPILLUS* COLLECTED IN THE SOUTH COAST REGION FROM OSMINGTON MILLS AND LULWORTH COVE.
Median, mode, range (stage 0-6) and percent sterile (stages 5 and 6). NA: Not available.

Osmington Mills					
Year	**Median**	**Mode**	**Range**	**% sterile**	**% females**
1995	3	4	0-4	0	57
1999	2	2	2-3	0	37
2003	1	0	0-2	0	40
Lulworth Cove					
Year	**Median**	**Mode**	**Range**	**% sterile**	**% females**
1995	3	3	2-4	0	37
1999	2	2	1-3	0	48
2003	NA	NA	NA	NA	NA
Selsey Bill					
Year	**Median**	**Mode**	**Range**	**% sterile**	**% females**
1995	2	2	1-4	0	57
1999	NA	NA	NA	NA	NA
2003	NA	NA	NA	NA	NA

N. lapillus at Selsey Bill were only directly affected by inshore fishing boats. VDS in 1995 was low 8 years after the small craft TBT ban. No females were sterile and the VDS range showed that whilst some females were at stage 4, stage 2 was more common, which does not impede breeding. The number of females in the population was normal (Table 3.4, § 3.2.5). In 1999 and 2003 the numbers of *N. lapillus* found were not sufficient to determine imposex.

3.3.1.2 Comparisons of south coast Nucella lapillus *populations*

Abundances of the populations at Osmington Mills and Lulworth Cove showed similar declines after 1999 (Fig. 3.5) whereas the reported population at Portland Bill (Crothers, 1975), first resurveyed in 1995 (Fig. 3.6A), was absent in 1999. The numbers found at Portland in 1995 were insufficient to imposex therefore only abundance and the population size structure were measured. The population was not dominated by older adults, a sign of severe TBT pollution, and breeding was evident as 45% of the thirty three found were second years and 12% juveniles. No *N. lapillus* were found in 1999 and one adult in 2003 and therefore statistical comparisons were not possible. Crothers (1975) also reported a small *N. lapillus* population at Peveril Point (Fig. 3.1) with large morphs found subtidally, but in 1995 one second year was found despite an extended search and none were seen in 1999 or 2003.

Total abundance at Osmington Mills between 1995 and 1999 remained relatively constant, but by 1999 the *N. lapillus* population was dominated by larger older animals though juveniles had slightly increased. (Fig.3.5A). The 1995 cumulative shell size frequency at Osmington Mills showed that the *N. lapillus* population was skewed towards second years (57%) and adults (47%) (Figs. 3.5A and 3.6B); juveniles were rare (Fig. 3.5A). By 2003 *N. lapillus* at Osmington Mills had severely declined to 14% of those recorded in 1999. Seven juveniles were found in 1999 and 2003 which made up 1.5% and 10% of the population respectively. This suggested that recruitment may have resumed following a general population decline.

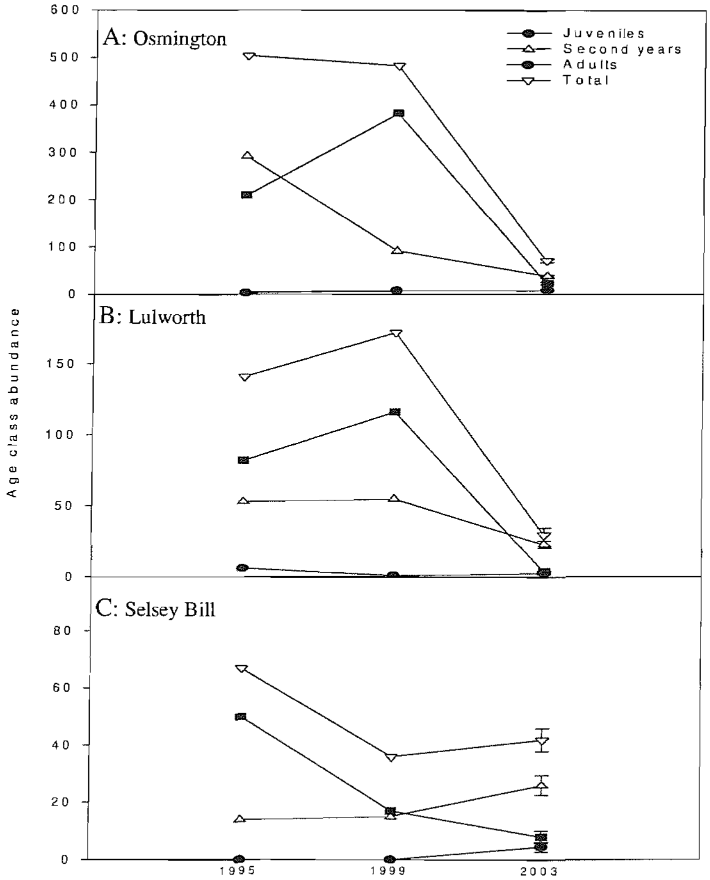

FIGURE 3.5 TOTAL ABUNDANCE OF *NUCELLA LAPILLUS* AND ABUNDANCES OF JUVENILES, SECOND YEARS AND ADULTS FROM SITES IN THE SOUTH COAST REGION FOR 1995, 1999 AND 2003.
1995 and 1999 total counts. Mean +/-1SE per 10 minute timed search 2003 only. Note independent Y axes.

Although lower numbers were always recorded at Lulworth Cove than at Osmington Mills (12.6 km west, Fig. 3.1), the temporal change in population structure at Lulworth (Fig. 3.6C) was similar to Osmington apart from the abundance of juveniles in 1995 (Fig. 3.5B). The total abundances in 1995 and 1999 were similar although in 1999 the population was more skewed towards adults and juveniles had declined (Figs. 3.5B and 3.6C). By 2003 the population at Lulworth Cove had declined to less than one fifth (17%) of its 1999 level, although as at Osmington juveniles abundance increased slightly (Fig. 3.5B).

In 1995 the population of *N. lapillus* at Selsey was dominated by larger animals (Figs. 3.5C and 3.6D); 75% of those found having adult shell morphology. By 1999 total abundance had declined by almost 50%, although juveniles had increased slightly and second years were more common (Fig. 3.5C). By 2003, adults in the population had reduced to 19% of the total found, although total abundance had slightly increased (Fig. 3.6D); the proportion of juveniles was also greater (Fig. 3.5C). Thin shell morphologies were common, indicating juveniles and second years or adults that had grown rapidly. In 2003 the Selsey population had become spatially isolated with several present on wood and iron groynes feeding on barnacles and the remainder on large cobbles feeding upon *L. littorea*.

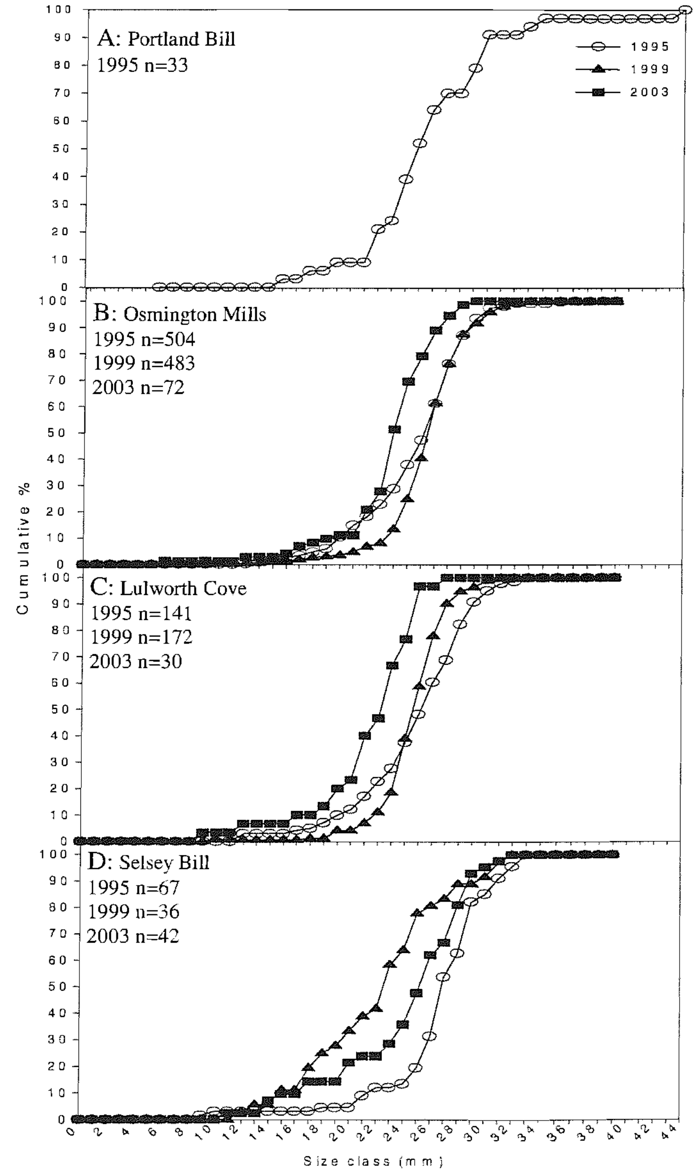

FIGURE 3.6 CUMULATIVE PERCENTAGE SIZE FREQUENCY CURVES FOR SOUTH COAST REGION *NUCELLA LAPILLUS* FOR YEARS WHERE SIGNIFICANT POPULATION STRUCTURE CHANGE OCCURRED.

Kolmogorov-Smirnov two sample tests showed significant differences between the cumulative frequency curves for all years of Osmington Mills and Lulworth Cove results (Table 3.5, Fig. 3.6B,C). At Selsey between 1995 and 1999 the total decline in abundance and recruitment of second years and juveniles was significant (Table 3.5, Fig. 3.6D), but the comparison between 1999 and 2003 was not significant despite the increase in juveniles (Table 3.5, Fig. 3.5C).

TABLE 3.5 KOLMOGOROV–SMIRNOV Z TEST RESULTS FOR CHANGES IN POPULATION STRUCTURE FROM SHELL SIZE CLASS FOR SOUTH COAST SITES.
NS: Not significant.

Year	Osmington Mills	Lulworth Cove	Selsey
1995 vs. 1999	$P<0.01$	$P<0.01$	$P<0.01$
1999 vs. 2003	$P<0.01$	$P<0.01$	NS

3.3.1.3 Nucella lapillus *prey abundance*

During surveys percentage barnacle abundance was recorded (Fig. 3.7A). At Portland Bill and Peveril Point the abundance of barnacles was low throughout and at Selsey they were not recorded where *N. lapillus* were found. Barnacle cover declined at Osmington Mills and Lulworth Cove. At Selsey Bill, where *N. lapillus* were found in two distinct groups, barnacle cover on the cobble substrate was always zero and dogwhelks were found feeding upon *L. littorea*. There were small patches of barnacles and *M. edulis* on the wooden groynes where the other *N. lapillus* were found, but they were not seen feeding on these when emersed.

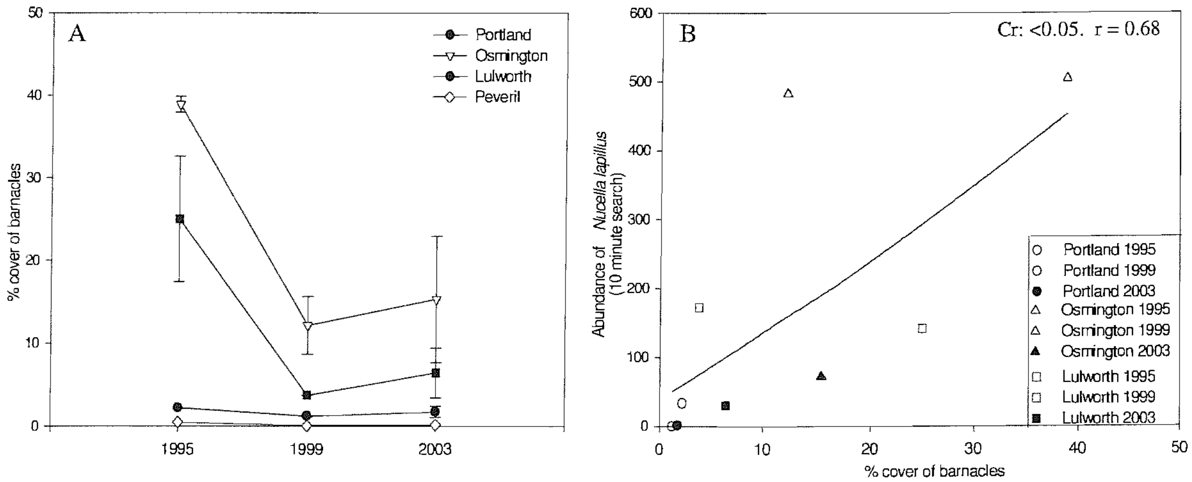

FIGURE 3.7 A: MEAN PERCENTAGE COVER (+/- 1SE) OF BARNACLES FOR SOUTH COAST OF ENGLAND SITES 1995, 1999, 2003 (EXCLUDING SELSEY BILL), B: THE RELATIONSHIP BETWEEN THE PERCENT COVER OF BARNACLES AND THE TOTAL ABUNDANCE OF *NUCELLA LAPILLUS* FOR SOUTH COAST OF ENGLAND SITES (EXCLUDING PEVERIL POINT AND SELSEY BILL).

There was a significant positive correlation between cover of barnacles and the abundance of dogwhelks when data from all sites (except Selsey and Peveril Point) were pooled ($r = 0.68$, $n = 9$, $df = 7$, $P<0.05$). A trend line was fitted ($R^2 = 0.47$, $y = 10.592x + 34.212$, Fig. 3.7B).

3.3.2 Plymouth Sound populations

3.3.2.1 Imposex indices in Plymouth Sound Nucella lapillus *populations*

Even at the height of TBT impacts in the 1980s the populations at St. Agnes were largely unaffected by imposex. The RPS fluctuated little from 1986 to 1994 and was zero from 1997 to 2003 (Fig. 3.8A), with no sterile females ever being found (Fig. 3.8B) and females all within the population parameters suggested by Moore (1938a) before the introduction of TBT.

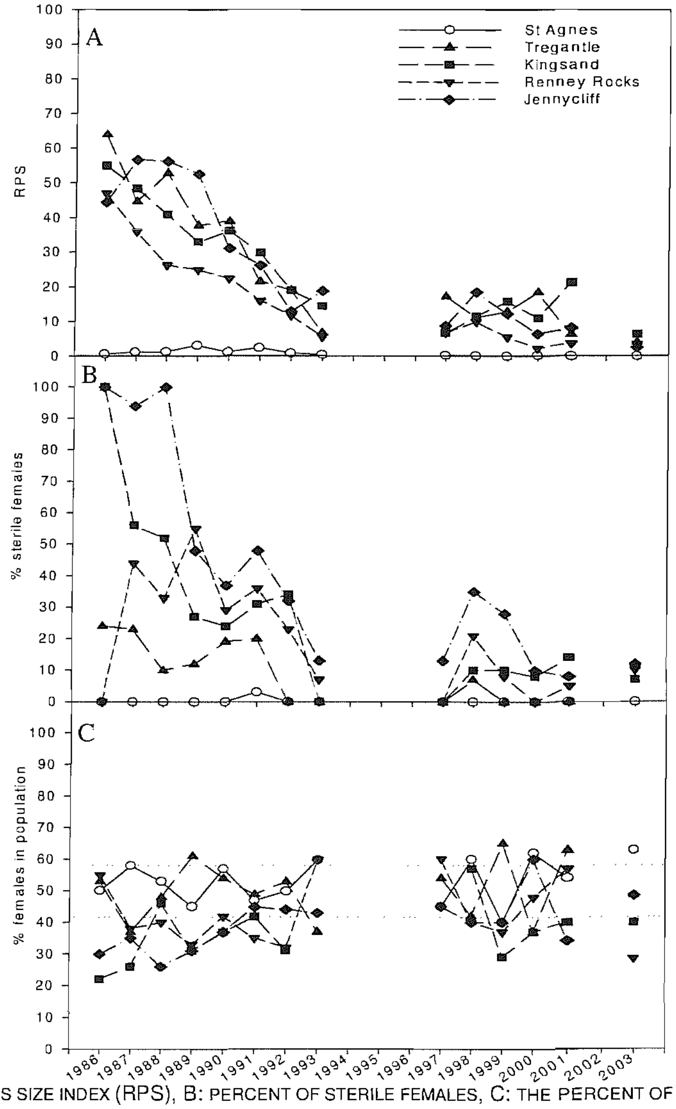

Figure 3.8 A: relative penis size index (RPS), B: percent of sterile females, C: the percent of females in Plymouth Sound and at St. Agnes *Nucella lapillus* populations from 1986-2003.
Dotted lines on Fig. 3.8C represent suggested normal percent of females in population by Moore (1938a) 58% and Minchin and Davies (1999) 41%. No survey 1994-1996 and 2002. Data sources: 1986-1989 Spence (1989), 1990-1994 Proud, (1994), 1997 Shute and Bray (Shute, 1997), 1998-2001/2003 Bray (this work)

Sites in and around Plymouth Sound had high RPS values from the mid 1980s (Fig. 3.8A). After the 1987 TBT ban the RPS declined except at Jennycliff where, from 1986 to 1987, RPS values increased, only falling clearly in the 1990 survey (Proud, 1994). In relation to the suggested concentration gradient, Renney Rocks had RPS values lower than sites inside Plymouth Sound. In contrast *N. lapillus* from Tregantle, also outside Plymouth Sound, had consistently high RPS from 1986 to 1993. From 1997 to 2003 RPS values fluctuated, but did not decline as rapidly as in the early phase of recovery (Fig. 3.8A). At Renney Rocks and Jennycliff values decreased further, but at Tregantle and Kingsand an increase was seen by 2001. By 2003 RPS values at all sites had dropped to their lowest levels over the whole period except at Kingsand where, although still decreasing, RPS was still the highest (6.34) (Fig.3.8A).

Within site two-way ANOVAs were carried out for differences between penis lengths of male and female *N. lapillus*. Raw data were not available for 1986 to 1989, therefore the tests were carried out for 1990 to 2003 results. All data failed both normality and equal variance and could not be transformed. Subsequently data were analysed using the conservative Scheirer-Ray-Hare test (Scheirer *et al.,* 1976; Quigley and Hall, 1999; Hitchmough, 2003) as described by Sokhall and Rolf (1995) and Dytham (1999) (Table 3.6, Fig. 3.9).

Significant differences were found for sex and year comparisons and interactions (Fig. 3.9, Table 3.6), with the exception of a year*sex interaction at Kingsand (Fig. 3.9C). RPS results from 1990 to 2003 showed that female *N. lapillus* penis bulk did not attain that of males, although at Plymouth imposex was severe (Fig. 3.8A). No differences were found at St. Agnes between years, but interactions between sex and year were significant ($P<0.05$). Tukey's test showed that these were due to continuous decline in female penis length from 1991 to 1997 (Table 2, Appendix 1). In males an increase in average penis size was seen, with mean lengths from 1997 onwards significantly greater than 1990-1993. At Tregantle (Fig. 3.9B) pooled penis lengths were significantly different ($P<0.01$, Table 3.6), due to a consecutive increase in average penis length from 1997 onwards (Table 2, Appendix 1). This was partly attributable to changes in the penis lengths of females in 1997, 1999 and 2000, but the effect was clearer in males. Within Plymouth Sound at Kingsand yearly comparisons were significantly different ($P<0.01$, Table 3.6), but interactions were not. Female penis lengths fluctuated with a maximum in 2001, whereas that of males remained relatively constant from 1993 (Fig. 3.9C).

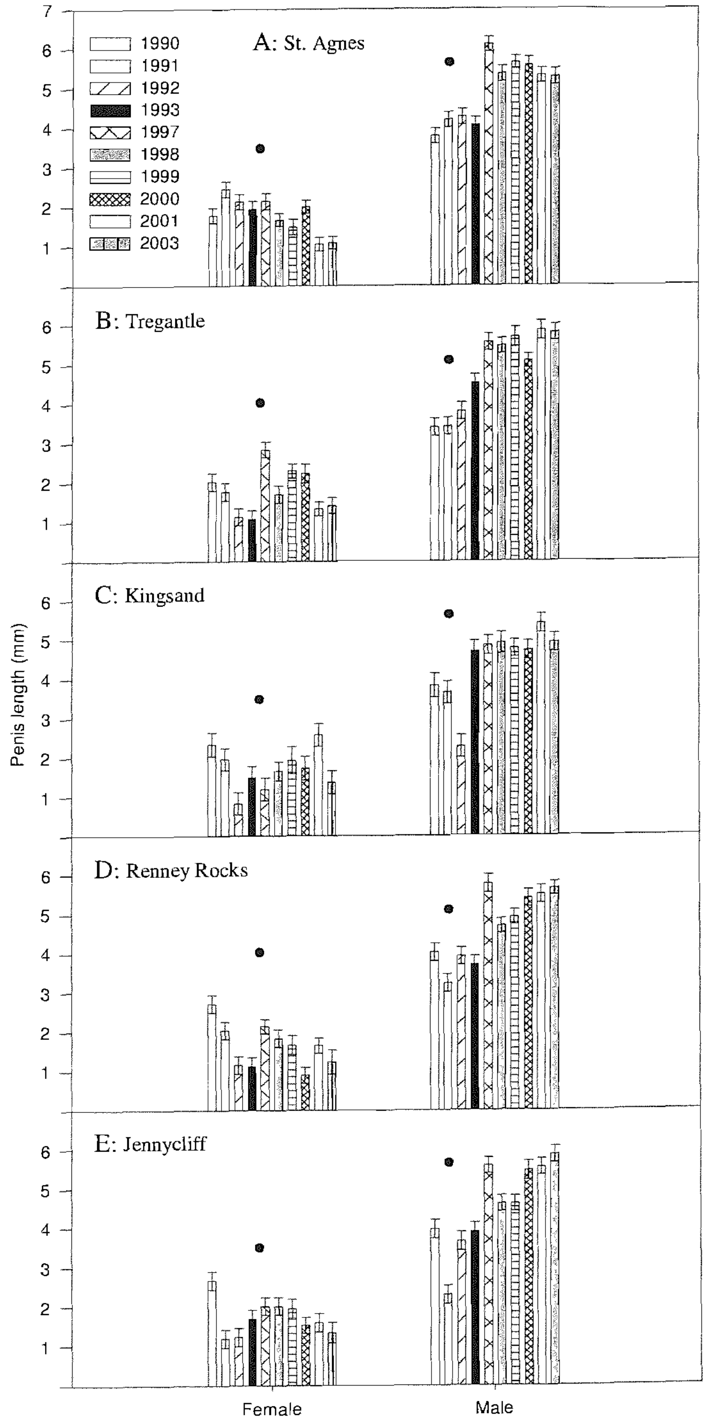

Figure 3.9 Comparison of mean (+/- 1SE) penis lengths for female and male *Nucella lapillus* for sites in Plymouth Sound and at St. Agnes (1990-2003).

At Renney Rocks penis lengths were significantly different between years (P<0.05, Table 3.6, Fig. 3.9D). *Post hoc* tests (Table 2, Appendix 1) showed that pooled lengths in 1997 were significantly greater than all years except 1990, 1999, 2001 and 2003. The 1990 result was due to the largest female penis length when TBT pollution was still high. After this, however, female penis length declined, but male penis lengths increased significantly from

1997 (Fig. 3.9D). Jennycliff penis length comparisons were significantly different between years (Table 3.6) with 1997 pooled values significantly greater (Fig. 3.9E, Table 2, Appendix 1). Within females, however, penis length was only significantly different for 1990, which was greater than 1991, 1992, 2000, 2001 and 2003. Overall female penis length did not clearly decline at Jennycliff, but male values fluctuated from 1990 to 1999 then increased from 2000 to 2003 (Fig. 3.9E).

TABLE 3.6 SCHEIRER-RAY-HARE RANKED ANOVA COMPARISON RESULTS FOR PENIS LENGTHS WITHIN SITES IN PLYMOUTH SOUND AND AT ST. AGNES (1990-2003).

St Agnes

Source	DF	SS	MS	F	$SS_{(factor)}\backslash Ms_{(total)}$	Chi^2	P (1-chi^2)
Year	9	106009.1	11778.8	8.6	11.9	0.001	NS
Sex	1	2002658.5	2002658.5	1449.4	2.3	1.0	<0.001
Year x Sex	9	191601.3	21289.0	15.4	21.7	0.99	<0.05
Residual	306	422794.2	1381.7				
Total	325	2874975.5	8846.1				

Tregantle

Source	DF	SS	MS	F	$SS_{(factor)}\backslash Ms_{(total))}$	Chi^2	P (1-chi^2)
Year	9	271322.0	30147.0	16.7	30.0	0.995	<0.01
Sex	1	1890466.8	1890466.8	1046.3	209.3	1.0	<0.001
Year x Sex	9	191558.6	21284.3	11.8	21.2	0.98	<0.05
Residual	309	558329.9	1807.0				
Total	328	2962164.0	9030.988				

Kingsand

Source	DF	SS	MS	F	$SS_{(factor)}\backslash Ms_{(total)}$	Chi^2	P (1-chi^2)
Year	9	261678.9	29075.4	9.8	29.3	0.995	<0.01
Sex	1	1416812.3	1416812.4	476.5	158.8	1.0	<0.001
Year x Sex	9	121056.6	13450.7	4.5	13.6	0.86	NS
Residual	307	912913.7	2973.7				
Total	326	2907646.5	8919.2				

Renney Rocks

Source	DF	SS	MS	F	$SS_{(factor)}\backslash Ms_{(total)}$	Chi^2	P (1-chi^2)
Year	9	170573.1	18952.6	10.0	19.0	0.97	<0.05
Sex	1	1794291.6	1794291.6	950.3	199.8	1	<0.001
Year x Sex	9	223644.1	24849.3	13.2	24.9	0.995	<0.01
Residual	308	581529.8	1888.1				
Total	327	2936451.0	8980.0				

Jennycliff

Source	DF	SS	MS	F	$SS_{(factor)}\backslash Ms_{(total)}$	Chi^2	P (1-chi^2)
Year	9	278685.5	30965.1	15.1	31.6	0.999	<0.001
Sex	1	1596681.0	1596681.0	780.3	181.1	1	<0.001
Year x Sex	9	227239.2	25248.8	12.3	25.8	0.997	<0.01
Residual	305	624110.2	2046.3				
Total	324	2856884.5	8817.6				

Within each population there were no correlations between shell and penis lengths thus a comparison between female penis lengths from the five sites could be made. These data failed both normality and equal variance and could not be transformed, thus the Scheirer-Ray-Hare test (Scheirer *et al.,* 1976) was also used for analysis. Between site comparisons were significant (Table 3.7) and *post hoc* tests (Table 3, Appendix 1) on pooled data showed that females from Tregantle had significantly longer penis lengths than all other sites (P<0.001, Fig. 3.9). Female penis lengths at Kingsand and Jennycliff (within Plymouth

Sound, Fig. 3.2) were not significantly different, but were greater than Renney Rocks (P<0.001) which was in turn larger than the control population at St. Agnes (P<0.001).

TABLE 3.7 SCHEIRER-RAY-HARE RANKED ANOVA COMPARISON BETWEEN SITE RESULTS FOR FEMALE PENIS LENGTHS 1990-2003 FOR: ST. AGNES, TREGANTLE, KINGSAND, RENNEY ROCKS, JENNYCLIFF.

Source	DF	SS	MS	F	$SS_{(factor)} \backslash MS_{(total)}$	Chi^2	P (1-chi^2)
Site	4	18817592.9	4704398.2	204.4	360.4	1.0	<0.001
Year	9	2119980.8	235553.4	10.2	40.6	0.999	<0.001
Site x Year	36	2257858.3	62718.3	2.7	43.2	0.809	NS
Residual	742	17080174.3	23019.1				
Total	791	41304358.5	52217.9				

Between year comparisons for female penis lengths were also significant (Table 3.7), but interactions between year and site were not. *Post hoc* Tukey's test on year (Table 3, Appendix 1) showed that mean female penis lengths from 1990 were significantly greater than all years except 1997, when longer penis lengths were found at Tregantle and Renney Rocks (Fig. 3.9B,D); the two sites outside Plymouth Sound (Fig. 3.1). Female penis lengths from 1997 to 1999 were significantly greater than in previous years and also greater than those recorded in the 2003 survey.

For Plymouth Sound and adjacent populations at Tregantle and Renney Rocks the VDS varied around a level of 3-4 from 1997 onwards though stage 4 was more common (Table 3.8). Before this a drop in the sterile females (VDS 5-6) was seen four years after the ban, particularly at Jennycliff and Kingsand (Fig. 3.8B, Table 3.8). The incidence of sterile females at Renney Rocks and Tregantle was always low in comparison with sites closer to TBT sources in Plymouth Sound, although their VDS values did not clearly decline until 1993. After 1998 recovery accelerated as seen at the other sites with the proportion of sterile females continuing to decline (Fig. 3.8B).

Until 1993 median VDS values were high (>4) for all impacted sites (Table 3.8). There was some evidence of vas deferens growth at St. Agnes until 1993, but this was always lower than the other sites and from 1997 it had clearly declined. For all of the sites in and adjacent to Plymouth Sound, modal and median VDS values remained high up to 2003 (Table 3.8). The VDS range increased at Jennycliff and Kingsand as TBT pollution decreased although some sterile females were always found. At Kingsand the recovery was less clear between 2000-2001 as older, sterile females remained in the population. In 2003 sterile females were also recorded at Renney Rocks (Fig. 3.9B, Table 3.8).

TABLE 3.8 VAS DEFERENS SEQUENCE RESULTS FOR *NUCELLA LAPILLUS* FOR SITES IN PLYMOUTH SOUND AND AT ST. AGNES. Median, mode and range (stages 0-6 where 1-4 able to breed, 5-6 sterile) NA: Not available. 1986-1989 Spence (1990), 1990-1993 Proud (1994), 1997 Bray and Shute (Shute, 1997), 1998-2003 Bray (this work).

<table>
<tr><th colspan="15">St Agnes VDS measurements 86-03</th></tr>
<tr><th>Year</th><th>86</th><th>87</th><th>88</th><th>89</th><th>90</th><th>91</th><th>92</th><th>93</th><th>97</th><th>98</th><th>99</th><th>00</th><th>01</th><th>03</th></tr>
<tr><td>Median</td><td>3</td><td>3</td><td>3</td><td>3</td><td>3</td><td>3</td><td>3</td><td>3</td><td>1</td><td>0</td><td>1</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>Mode</td><td>NA</td><td>NA</td><td>NA</td><td>NA</td><td>3</td><td>3</td><td>2</td><td>3</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Range</td><td>2-4</td><td>1-4</td><td>2-4</td><td>0-4</td><td>2-4</td><td>3-5</td><td>2-4</td><td>2-4</td><td>0-4</td><td>0-4</td><td>0-3</td><td>0-4</td><td>0-2</td><td>0-2</td></tr>
<tr><th colspan="15">Tregantle VDS measurements 86-03</th></tr>
<tr><th>Year</th><th>86</th><th>87</th><th>88</th><th>89</th><th>90</th><th>91</th><th>92</th><th>93</th><th>97</th><th>98</th><th>99</th><th>00</th><th>01</th><th>03</th></tr>
<tr><td>Median</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td></tr>
<tr><td>Mode</td><td>NA</td><td>NA</td><td>NA</td><td>NA</td><td>3</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td></tr>
<tr><td>Range</td><td>4-6</td><td>4-6</td><td>4-6</td><td>4-6</td><td>3-6</td><td>4-6</td><td>3-4</td><td>2-4</td><td>3-4</td><td>3-5</td><td>2-4</td><td>3-4</td><td>0-5</td><td>0-5</td></tr>
<tr><th colspan="15">Kingsand VDS measurements 86-03</th></tr>
<tr><th>Year</th><th>86</th><th>87</th><th>88</th><th>89</th><th>90</th><th>91</th><th>92</th><th>93</th><th>97</th><th>98</th><th>99</th><th>00</th><th>01</th><th>03</th></tr>
<tr><td>Median</td><td>5</td><td>5</td><td>5</td><td>5</td><td>4</td><td>4</td><td>5</td><td>4</td><td>4</td><td>3.5</td><td>4</td><td>4</td><td>4</td><td>4</td></tr>
<tr><td>Mode</td><td>NA</td><td>NA</td><td>NA</td><td>NA</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td></tr>
<tr><td>Range</td><td>5-6</td><td>4-5</td><td>4-5</td><td>4-6</td><td>4-6</td><td>0-6</td><td>2-5</td><td>2-4</td><td>0-4</td><td>0-5</td><td>3-5</td><td>0-5</td><td>3-5</td><td>2-5</td></tr>
<tr><th colspan="15">Renney Rocks VDS measurements 86-03</th></tr>
<tr><th>Year</th><th>86</th><th>87</th><th>88</th><th>89</th><th>90</th><th>91</th><th>92</th><th>93</th><th>97</th><th>98</th><th>99</th><th>00</th><th>01</th><th>03</th></tr>
<tr><td>Median</td><td>4</td><td>4</td><td>4</td><td>5</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>3.5</td><td>4</td><td>3</td><td>4</td><td>4</td></tr>
<tr><td>Mode</td><td>NA</td><td>NA</td><td>NA</td><td>NA</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>3</td><td>4</td><td>4</td><td>4</td><td>4</td></tr>
<tr><td>Range</td><td>4</td><td>4-6</td><td>4-5</td><td>4-6</td><td>3-6</td><td>3-6</td><td>3-6</td><td>3-5</td><td>2-5</td><td>0-5</td><td>0-4</td><td>2-4</td><td>0-5</td><td>3-5</td></tr>
<tr><th colspan="15">Jennycliff VDS measurements 86-03</th></tr>
<tr><th>Year</th><th>86</th><th>87</th><th>88</th><th>89</th><th>90</th><th>91</th><th>92</th><th>93</th><th>97</th><th>98</th><th>99</th><th>00</th><th>01</th><th>03</th></tr>
<tr><td>Median</td><td>6</td><td>6</td><td>5</td><td>5</td><td>4</td><td>4.5</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td></tr>
<tr><td>Mode</td><td>NA</td><td>NA</td><td>NA</td><td>NA</td><td>4</td><td>4</td><td>3</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td></tr>
<tr><td>Range</td><td>5-6</td><td>6</td><td>5-6</td><td>4-6</td><td>4-6</td><td>4-6</td><td>3-6</td><td>3-6</td><td>3-5</td><td>0-6</td><td>3-5</td><td>2-5</td><td>2-6</td><td>0-6</td></tr>
</table>

Despite sterility being common at both Kingsand and Jennycliff until 1993, the percentage of females in the population increased at both sites (Fig. 3.8C). This increasing trend was seen at the other sites (Tregantle and Renney Rocks). At St. Agnes females remained within the band expected for natural populations. From 1997, female numbers at Kingsand reflected the levels of sterility and imposex recorded (Figs. 3.8A,B). In 2003, Kingsand had the highest RPS and the lowest number of females with the exception of Renney Rocks (Fig. 3.8C).

3.3.2.2 Plymouth Sound and St. Agnes Nucella lapillus *abundance and imposex indices*

For Plymouth Sound the relationship between imposex (as RPS and the percent sterile) and abundance over the period of the observations is shown in figure 3.10. St. Agnes, with permanently low imposex, showed no relationship and has not been plotted.

At the most polluted sites (Jennycliff and Kingsand) abundance was strongly related to the incidence of imposex (Figs. 3.10B,D and 3.10F,H), with RPS being the better predictor at both sites with higher r^2 values. The slopes for both RPS and the percent sterile at each site were very similar. T tests comparing the regression lines showed that there were no significant differences between regression slopes for the RPS (t = 0.0006, 24 df, P = NS) or the percent of sterile females (t = 0.0005, 24 df, P = NS) at Jennycliff and Kingsand. This relationship broke down at the less polluted sites where the percentage sterile were primarily under 50% and RPS values were also generally low by comparison. Clearly at these sites other, predominantly ecological, factors overrode the effects of TBT.

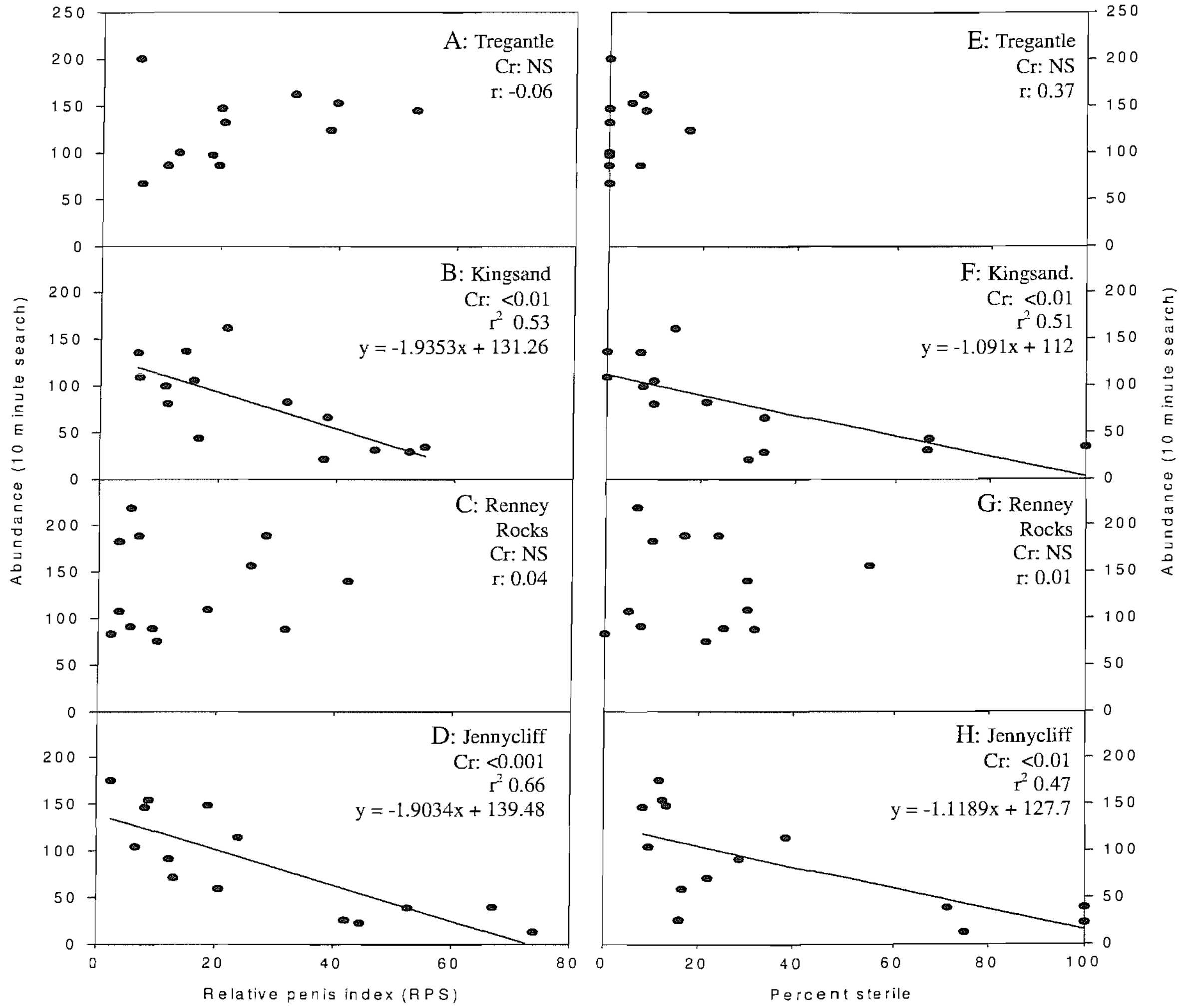

FIGURE 3.10 A-D RELATIONSHIP BETWEEN THE RELATIVE PENIS SIZE INDEX (RPS), E-H PERCENTAGE OF STERILE FEMALES AND THE TOTAL ABUNDANCE OF *NUCELLA LAPILLUS* AT SITES WITHIN AND ADJACENT TO PLYMOUTH SOUND
Cr: Correlation result, r: correlation coefficient, r^2 coefficient of determination (Kingsand and Jennycliff).

3.3.2.3 Comparisons of Plymouth Sound and St Agnes Nucella lapillus *populations*

Although the level of imposex was low (Table 3.8) the abundance of *N. lapillus* at St. Agnes showed a general decline from 1987 to 1993, but was relatively stable in the 1990s (Fig. 3.11). Abundances at Tregantle and Renney Rocks showed similar patterns, with declines in adults in 1990 (Figs. 3.11B,D). Recruitment had occurred at both sites by 1997 and was seen again in juveniles at Renney Rocks in 2000 and Tregantle in 2001.

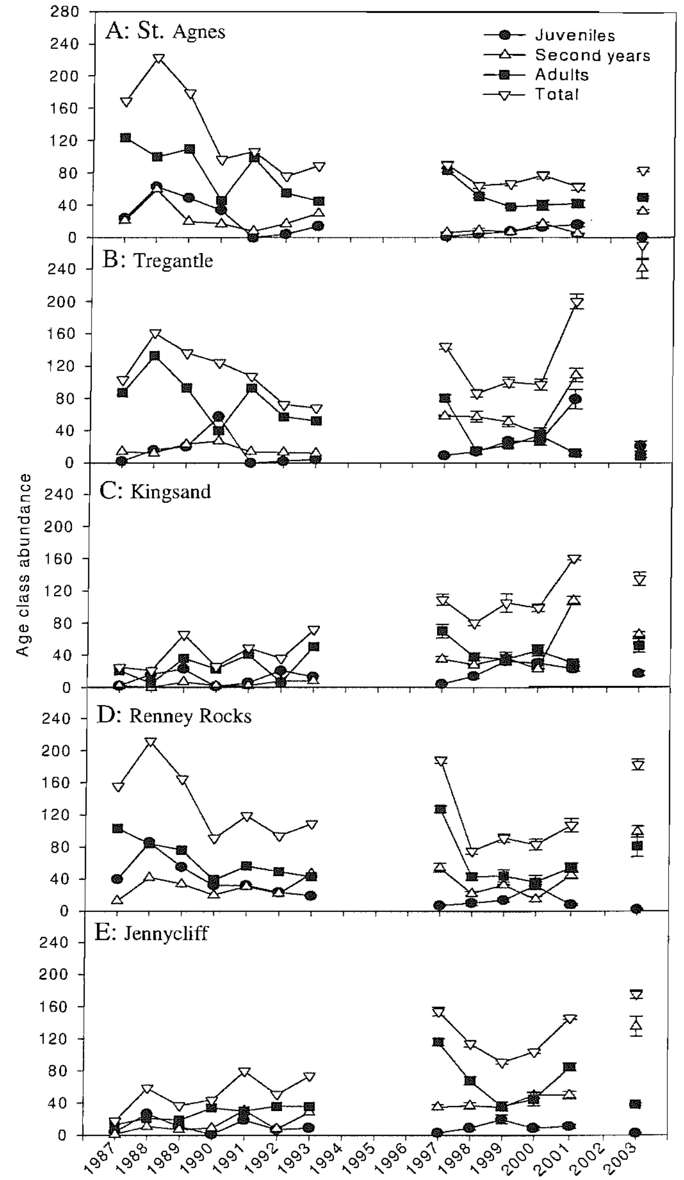

Figure 3.11 Total abundance of *Nucella lapillus* and abundances of juveniles, second years and adults from sites in Plymouth Sound and at St. Agnes (1987-1993, 1997-2001, 2003).
1987-1993 total abundance. 1997-2003, Mean +/- 1SE.

Populations at the two severely impacted sites, Jennycliff and Kingsand, were affected by low recruitment between 1987 and 1991 due to the high level of sterile females (Figs. 3.8C and 3.10F), but in 1992 recruitment increased at Kingsand in particular (Fig. 3.11B,E) as sterility reduced slightly (Table 3.8). From 1997 to 2000 both populations showed similar recruitment and decline but abundances increased and remained relatively stable between 2001 and 2003.

Cumulative frequency plots and statistical comparisons show years at each site where population structure changes were significant (Fig. 3.11, Table 3.9). At St. Agnes significant differences were seen due to natural fluctuations such as the increased recruitment of juveniles in 2001 (Fig. 3.11A and 3.12A).

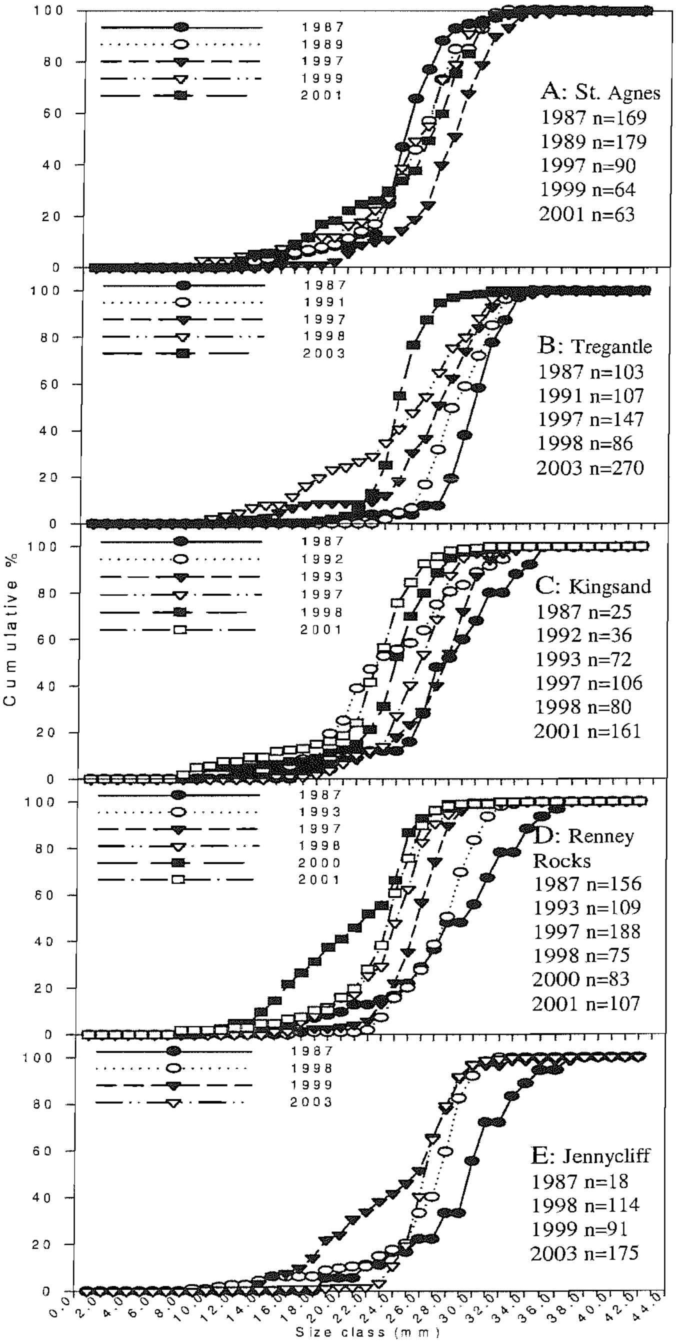

FIGURE 3.12 CUMULATIVE PERCENTAGE SIZE FREQUENCY CURVES FOR PLYMOUTH SOUND AND ST. AGNES *NUCELLA LAPILLUS* FOR YEARS WHERE SIGNIFICANT POPULATION STRUCTURE CHANGE OCCURRED.

At Tregantle, a population dominated by larger older individuals in 1987 saw a significant change in 1991 when recruitment resulted in more small individuals (Fig. 3.11B and 3.12B, Table 3.9). Further significant changes were seen in 1997 and 1998 when recruitment occurred, but adults declined, so by 1998 overall abundance had dropped (Figs. 3.11B and

3.12B). The final significant change was seen in 2003 when a large abundance increase in second years and smaller adults was found, possibly due to the large numbers of juveniles found in the late 1990s having grown to adult size (Fig. 3.11B and 3.12B, Table 3.9).

TABLE 3.9 KOLMOGOROV–SMIRNOV Z TEST RESULTS FOR CHANGES IN POPULATION SIZE STRUCTURE FOR SITES IN PLYMOUTH SOUND AND AT ST. AGNES.
Bold text: years where significant change occurred at P<0.01. NS: Not significant.

St Agnes		Tregantle		Kingsand		Renney Rocks		Jennycliff	
Year	**P**	**Year**	**P**	**Year**	**P**	**Year**	**P**	**Year**	**P**
1987-1988	NS	1987-1988	NS	1987-1988	NS	1987-1988	NS	1987-1988	NS
1987-1989	**<0.01**	1987-1989	NS	1987-1989	NS	1987-1989	NS	1987-1989	NS
1989-1990	NS	1987-1990	NS	1987-1990	NS	1987-1990	NS	1987-1990	NS
1989-1991	NS	**1987-1991**	**<0.01**	1987-1991	NS	1987-1991	NS	1987-1991	NS
1989-1992	NS	1991-1992	NS	**1987-1992**	**<0.01**	1987-1992	NS	1987-1992	NS
1989-1993	NS	1991-1993	NS	**1992-1993**	**<0.01**	**1987-1993**	**<0.01**	1987-1993	NS
1989-1997	**<0.01**	**1991-1997**	**<0.01**	**1993-1997**	**<0.01**	**1993-1997**	**<0.01**	1987-1997	NS
1997-1998	NS	**1997-1998**	**<0.01**	**1997-1998**	**<0.01**	**1997-1998**	**<0.01**	**1987-1998**	**<0.01**
1997-1999	**<0.01**	1998-1999	NS	1998-1999	NS	1998-1999	NS	**1998-1999**	**<0.01**
1999-2000	NS	1998-2000	NS	1998-2000	NS	**1998-2000**	**<0.01**	1999-2000	NS
1999-2001	**<0.01**	1998-2001	NS	**1998-2001**	**<0.01**	**2000-2001**	**<0.01**	1999-2001	NS
2001-2003	NS	**1998-2003**	**<0.01**	2001-2003	NS	2001-2003	NS	**1999-2003**	**<0.01**

At Kingsand the population was dominated by older animals in 1987 (Fig. 3.12C). In 1992, however, the juvenile recruitment caused a significant change in population structure (Table 3.9). Abundance increased by 1993, but the population switched between adult and juvenile dominance. During the period 1998-2001 the numbers at Kingsand had multiplied by a factor of seven over 1987 and in 2001 high recruitment of juveniles was occurring (Figs. 3.11C and 3.12C).

Larger older animals dominated Renney Rocks in 1987. By 1993 recruitment over the intervening years had resulted in a significant change in size structure (Table 3.9) although the population had declined (Fig. 3.12D). Imposex indices continued to decline (Table 3.8, Fig.3.8A) as did population abundance until 1997. Further recruitment was evident resulting in a significant change in 1998, although overall abundance declined. In 2000 the juvenile recruitment caused significant change (Fig. 3.12D), but by 2001 the influence of this size class had diminished (Table 3.9, Fig. 3.12D).

Jennycliff, (closest to Devonport Dockyard and the Cattewater), had fully sterile females (VDS 5,6) up to and including 2003 (Table 3.8). Sterility resulted in low recruitment and thus few significant changes in population structure were observed (Table 3.9, Fig. 3.12E) until 1998 when an improvement began. By 1999 juvenile recruitment was clearly evident at Jennycliff (Fig. 3.12E). The last significant change at Jennycliff was seen in 2003 when the numbers of juveniles had declined from that seen in 1999, although population abundance was three times that recorded in 1988.

As an indication of the impact of female sterility on recruitment rates, an unreplicated general linear model ANOVA on pre 1997 juvenile abundance data was carried out. This showed significant differences between sites ($F_{(4,34)}$, 4.99, $P<0.01$) and years ($F_{(6,34)}$, 3.11, $P<0.05$). *Post hoc* tests showed that juvenile recruitment at Renney Rocks was greater and more stable than at the other sites (Table 4A, Appendix 1). Tukey's tests could not resolve which years were significantly different, but 1987 was indicated ($P = 0.058$).

A two-way ANOVA on the replicated juvenile abundance data collected from 1997 to 2003 was carried out. Data failed normality tests and were square root transformed before the test was performed. Results showed that levels of juvenile recruitment between years was significantly different ($F_{(5,89)}$, 4.185, $P<0.01$). Tukey's tests identified that this was due to juvenile recruitment in 2000 and 2001 being significantly greater than that in 2003 (Table 4B, Appendix 1). Differences between sites were significant ($F_{(4,89)}$, 2.672, $P<0.05$), but the *post hoc* tests could not identify which sites had greater recruitment, although Tregantle compared to St. Agnes was indicated ($P = 0.09$) (Table 4C, Appendix 1).

3.3.2.4 Nucella lapillus *prey abundance*

N. lapillus primary prey abundance was recorded at Plymouth sites from 1997 onwards (§3.2.5, Fig. 3.13). The shores at St. Agnes and Tregantle were characterised by patchy *M. edulis* and barnacles whereas at the other sites only barnacles were found.

At St. Agnes the abundance of the dominant barnacles and *M. edulis* varied little from 1997 to 2000. In 2001 a switch from barnacle domination to *M. edulis* was observed, but by 2003 this was less evident (Fig. 3.13A). Conversely at Tregantle switching between barnacle and *M. edulis* dominance was a common occurrence and particularly apparent in 2001. By 2003 the sessile community had reverted from *M. edulis* to being marginally dominated by barnacle cover (Fig. 3.13B).

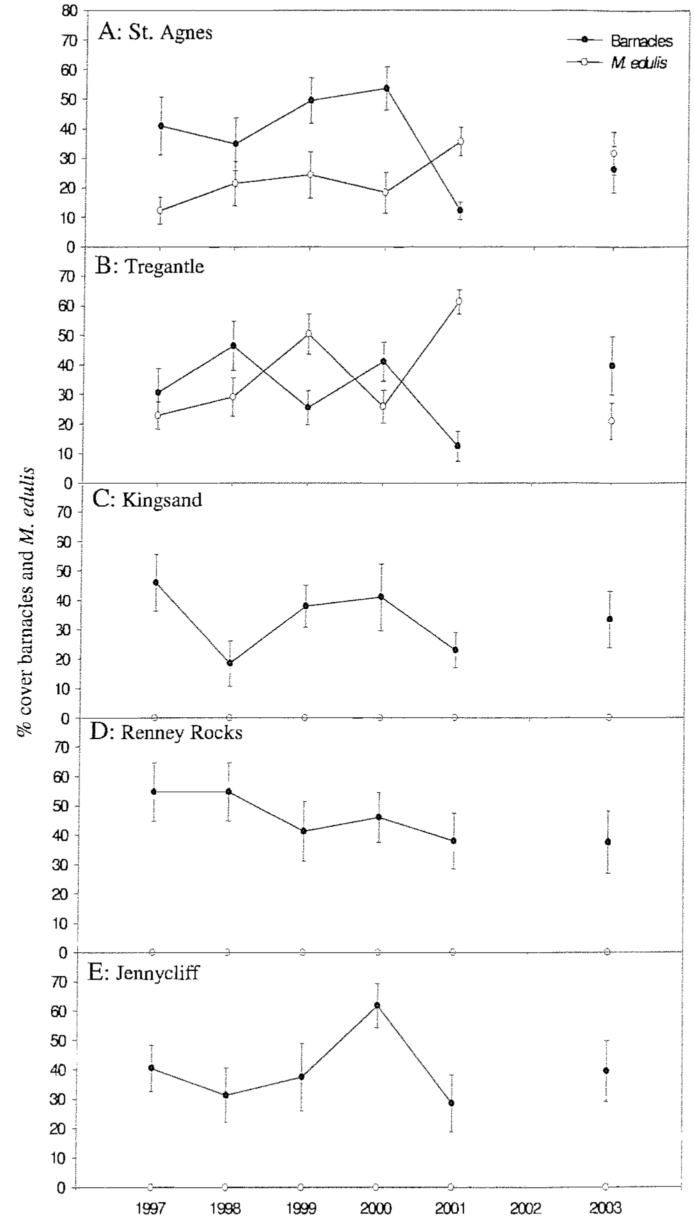

FIGURE 3.13 MEAN PERCENTAGE COVER (+/- 1SE) OF BARNACLES AND *MYTILUS EDULIS* FOR SITES IN PLYMOUTH SOUND AND AT ST. AGNES 1997-2003.

At Kingsand, Renney Rocks and Jennycliff, barnacles were the only readily available prey for *N. lapillus*. At Kingsand (Fig. 3.13C) barnacle abundance fluctuated, with clear declines in 1998 and 2001. At Renney Rocks (Fig. 3.13D) a gradual decline in barnacle cover from 1997 to 2001 was found, although between 2001 and 2003 further losses were not seen. At Jennycliff a barnacle cover decline in 1998 was reversed in 1999 and further recruitment was observed in 2000. Similarly to all other sites at Plymouth, the percentage of barnacle cover between 2000 and 2001 decreased, but recovered slightly by 2003 (Fig. 3.13E)

As the impact of TBT reduced further (Table 3.8) *N. lapillus* populations showed broadly similar patterns of decline and growth from 1997 to 2001 (Fig.3.11). This was with the exception of St. Agnes where a drop in abundance continued, but this did not correlate to the percentage of barnacle and *M. edulis* cover. Further correlations between *N. lapillus* age class abundances, the total population abundance and the percentage of prey cover at the individual sites within and adjacent to Plymouth Sound found no significant relationships. As a broad scale indication of the effect of food availability on dogwhelk populations at Plymouth, barnacle abundance data from all sites within and adjacent to Plymouth Sound were pooled and correlated against pooled *N. lapillus* abundance; a significant relationship was not found.

3.3.3 Isle of Wight populations

3.3.3.1 Imposex indices in Isle of Wight Nucella lapillus *populations*

Survey locations on the Isle of Wight (Fig. 3.3) were representative of suitable habitats on north and south shores of the island for which some earlier data were available. Destructive sampling to enable imposex assessment was restricted where *N. lapillus* numbers were low. RPS results for the northern half of the Isle of Wight were not obtained before 2003 because accurate calculation of the RPS requires a minimum sample of 30 animals (Gibbs *et al.*, 1987), but populations were not large enough to withstand their removal. Table 3.10 shows the RPS values from each Isle of Wight site used to create the aggregated imposex values for northern sites. The individual site values are provided only as an indication of the ambient TBT pollution.

TABLE 3.10 RELATIVE PENIS SIZE (RPS) RESULTS FOR JULY 2003 AT SITES ON THE NORTH COAST OF THE ISLE OF WIGHT.

Site	Number sampled	RPS	RPS for north Isle of Wight.
Seaview	0	None present.	
East Cowes	0	Insufficient No's	
West Cowes	17	14.8	
Newtown	5	25.5	
Warden Ledge	8	5.6	11.9

Of the north shore sites, no *N. lapillus* were found at Seaview, although they had been previously reported there (Herbert, 1988; Kent *et al.*, 2003). At East Cowes *N. lapillus* were not found in sufficient numbers to sample for imposex, but at West Cowes, where dogwhelks were absent in 1985 (Bryan 1987), a population had established on defence blocks fronting a sea wall and 17 individuals were removed for imposexing which showed that TBT levels were still relatively high. At Newtown the RPS result was greater than at West Cowes (Table 3.10) although limited animals were sampled; in comparison, at Warden Ledge the RPS was low. The total RPS for the north Isle of Wight (Table 3.10) showed that TBT still influenced populations, but RPS levels were comparable to those at Plymouth Sound (Fig. 3.8) where dogwhelks were breeding.

RPS, the percentage sterile and the percentage of females in populations for southern coast sites were collated from previous worker's raw data, this work, and values for which raw data were not available (Fig. 3.14). Early results (Fig. 3.14A) showed that the RPS value was increasing to a maximum in 1987 for Bembridge and 1991 for Horse Ledge. After these years a decline was seen at both sites, with some fluctuation at Bembridge, though more variation may have occurred, as data were not available for some years. At Hanover Point the RPS was not generally as high and with fewer observations trends were less clear. Between 1992 and 1997 Hanover RPS did not decline, although by 1999 the index had dropped to a similar value as at Bembridge. Of the three sites on the south coast of the Isle of Wight, Horse Ledge RPS, though declining steadily was still the highest at 9% in 2003 (Fig. 3.14A).

Results for the percentage of sterile females (Fig. 3.14B) generally followed RPS patterns. Numbers of sterile females at Bembridge and Hanover Point declined sharply between 1987-1990 and 1990-1992 respectively. At Horse Ledge, however, the percent sterile in 1992 was higher than those in 1989. Imposex at both Bembridge and Hanover Point continued to decline, but in 2003 at Hanover Point the proportion of sterile females increased to 18 %. In contrast, at Horse Ledge in 2003 the proportion of sterile females had changed little from that in 1997 (Fig. 3.14B).

At Hanover Point the percentage of females in the population rose from a low in 1987 to a peak in 1997, but in 1999 and 2003 the number dropped slightly (Fig. 3.14C). The highest number found at Bembridge was in 1992 and after this female abundance dipped, but by 2003 a slight increase had occurred. At Horse Ledge the percent of females was variable reaching a maximum of 70 % in 1990 at a time when the RPS was at its highest, but the percent of sterile females was low (Fig. 3.14A,B,C). Female numbers then declined to a low in 1992 followed by an increase approaching the suggested norm used for this work (Moore, 1938a), but above that suggested by Minchin and Davies (1999) (Fig. 3.14C; § 3.3).

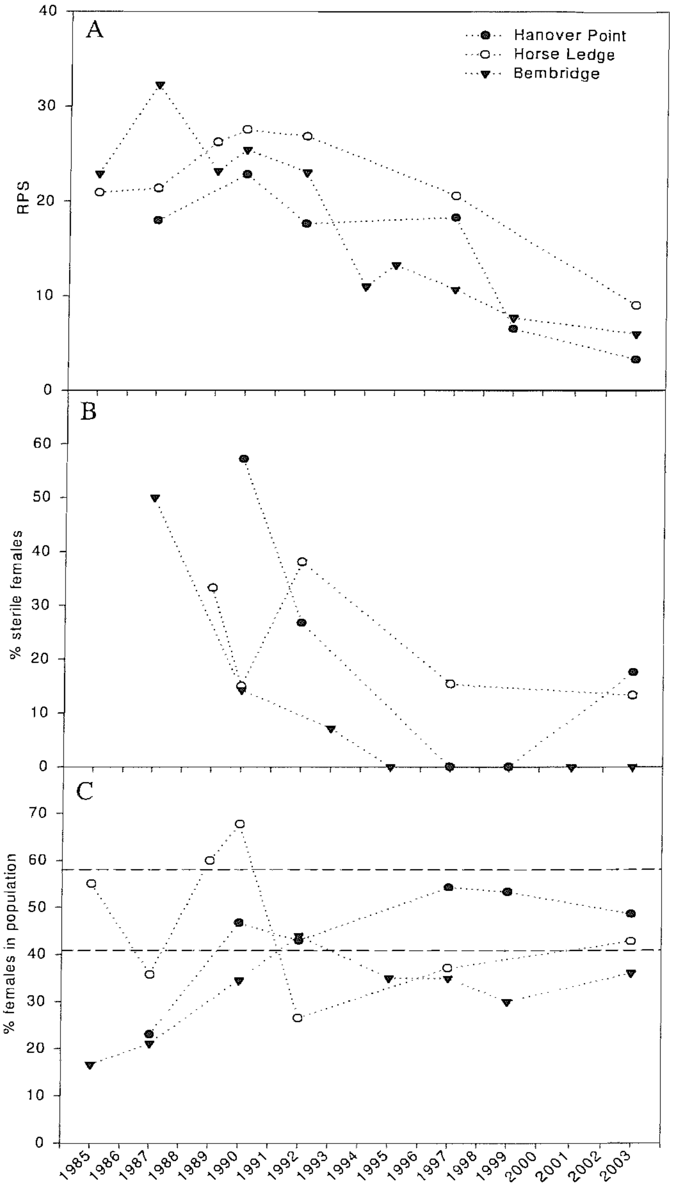

FIGURE 3.14 A: RELATIVE PENIS SIZE INDEX (RPS), B: PERCENTAGE OF STERILE FEMALES, C: PERCENTAGE OF FEMALES IN ISLE OF WIGHT SOUTH COAST POPULATIONS 1985-2003.
Data sources: 1985-1987 Bryan (1987), 1989-1992 Langston *et al.*, (1994), 1994 Herbert (1994), 1995 Bray (1996), 1997 Bray and Herbert (1998), 1999 Turner and Bray (Turner, 1999), 2003 Bray (this work). Dotted lines indicate data not a continuum. Dashed lines on Fig. 3.14C represent suggested normal percent of females in population by Moore (1938a) 58% and Minchin and Davies (1999) 41%.

Two-way ANOVA comparisons for the factors sex and year on penis length confirmed that on the Isle of Wight south coast mean female penis length never attained that of males (Fig. 3.15, Table 3.11). Main effects between years were significantly different at Hanover Point and Bembridge, but not Horse Ledge.

Interactions between year and sex were significant for Bembridge and Hanover Point and multiple comparisons for Hanover Point showed that penis lengths in 1987 were the main contributor to the significant differences between females and males (Table 5, Appendix 1,

Fig. 3.15A). Further to this female penis lengths in 1997 and males in 1997 and 2003 were significantly greater than the 1999 results (Fig. 3.15A).

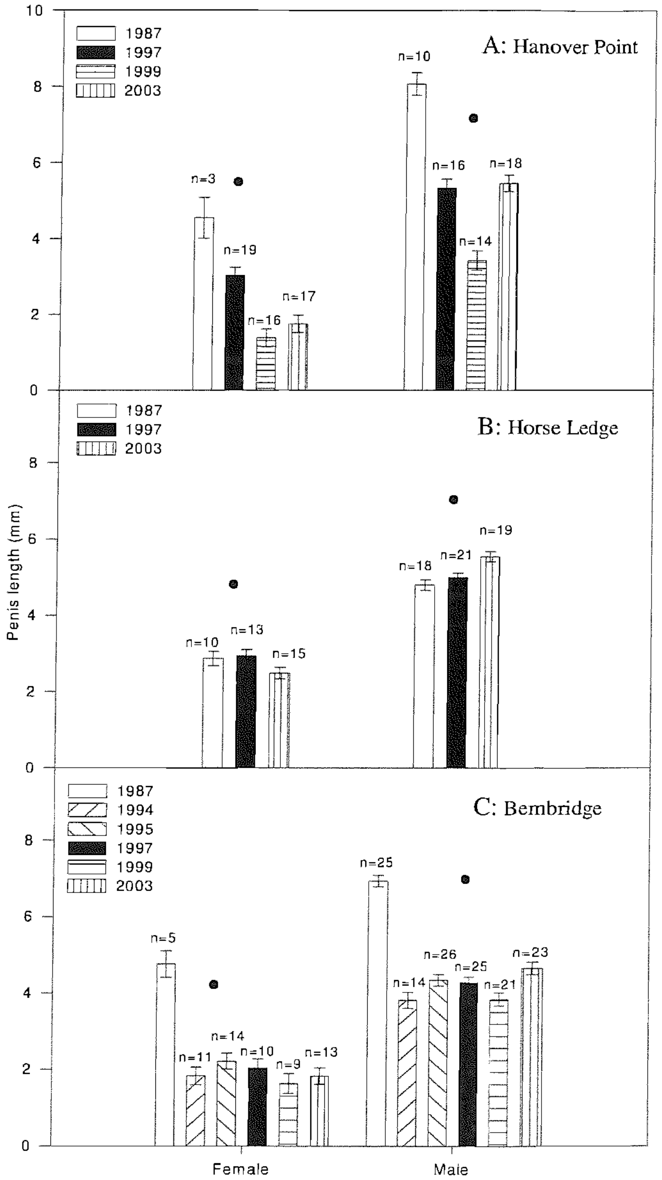

FIGURE 3.15 COMPARISON OF MEAN (+/- 1SE) PENIS LENGTHS FOR FEMALE AND MALE *NUCELLA LAPILLUS* FROM ISLE OF WIGHT SOUTH COAST SITES

At Horse Ledge no significant change was recorded in mean female penis lengths and in males only 2003 was significantly larger (Fig. 3.15B, Table 5, Appendix 1). At Bembridge the pooled 1987 penis lengths results were significantly greater than all the other years (Fig. 3.15C, Table 5, Appendix 1). In males the 1987 raw penis length was significantly greater than results from all the other years ($P<0.001$), in addition male penis lengths in 2003 were significantly greater than those in 1994 and 1999 ($P<0.05$, Table 5, Appendix 1). Whilst the RPS declined at all three sites (Fig. 3.14A) the only real reduction was at Hanover Point, as at

Horse Ledge female penis lengths did not decrease, but the mean of males was larger. At Bembridge there was no significant effect for a reduction in female penis lengths after 1987. Thus the RPS reduction at both these sites was a function of the growth in male penis lengths.

TABLE 3.11 TWO-WAY ANOVA COMPARISON RESULTS FOR MALE AND FEMALE PENIS LENGTHS AT SITES ON THE SOUTH COAST OF THE ISLE OF WIGHT.

Hanover Point					
Source	**DF**	**SS**	**MS**	**F**	**P**
Year	3	121.5	40.5	47.0	<0.001
Sex	1	168.5	168.5	195.4	<0.001
Year x Sex	3	14.7	4.9	5.7	<0.01
Residual	105	90.5	0.9		
Total	112	539.8	4.8		
Horse ledge					
Source	**DF**	**SS**	**MS**	**F**	**P**
Year	2	0.5	0.2	0.7	NS
Sex	1	125.5	125.5	360.2	<0.001
Year x Sex	2	6.1	3.1	8.8	<0.001
Residual	92	32.1	0.3		
Total	97	170.7	1.8		
Bembridge					
Source	**DF**	**SS**	**MS**	**F**	**P**
Year	5	122.0	24.4	40.9	<0.001
Sex	1	197.2	197.2	330.9	<0.001
Year x Sex	5	3.3	0.7	1.1	NS
Residual	183	109.0	0.6		
Total	194	589.3			

Mean female penis lengths were analysed over time to indicate changes in the levels of TBT pollution affecting the Isle of Wight south coast sites. To create a balanced design the years compared were 1987, 1997 and 2003 as these data were common to all sites. No indication of differential effects of TBT pollution were found as female penis lengths from the three sites (Hanover Point, Horse Ledge and Bembridge) were not significantly different ($F_{(2,104)}$, 1.207, P = 0.3).

For south coast Isle of Wight data VDS results were not available for all data where penis lengths were recorded (e.g. Bryan, 1987). Although the north coast Isle of Wight sites were in the Solent (Fig. 3.3) thus impacted by numerous recreational craft movements particularly at Cowes, no sterile females were found in 2003 (Table 3.12). At Newtown (site D, Fig. 3.3), however, all the females were at stage 4, i.e. one stage away from sterility (Table 3.12A).

Compared to 1989-1992 the advanced stages of the VDS (5 and 6) were less common in 1999 and 2003 at all of the south coast Isle of Wight sites. At Hanover Point (site F, Fig. 3.3) the average VDS value for 1990 and 1992 recorded by Langston *et al.,* (1994) (Table

3.12B), suggested a heavily impacted population, which between 1997 and 1999 was improving. In 2003, however, sterile females were found (Fig. 3.14) and the mode VDS was 4 (Table 3.12). As with female penis lengths at Horse Ledge (Fig. 3.15B), between 1997 and 2003 no reduction was found in the VDS of the Horse Ledge population (Table 3.12C). At Bembridge between 1989-1992 sterility was common (Langston *et al.*, 1994, Table 3.12D), but by 1997 a clear improvement was seen (stage 3). In 2003, however, as with Hanover point, evidence was found of a rise in imposex at Bembridge with the mode and median VDS increasing, but some unaffected individuals (stage 0) were also present. In contrast to Hanover Point, no sterile females were found at Bembridge.

TABLE 3.12 VAS DEFERENS SEQUENCE RESULTS FOR *NUCELLA LAPILLUS* FOR SITES ON THE SOUTH COAST OF THE ISLE OF WIGHT. Median, mode and range (stages 0-6 where 1-4 able to breed, 5-6 sterile) NA: Not available. Bembridge and Horse Ledge 1989-1992, Hanover 1990-1992 Langston *et al.*, (1994); Bembridge 1995, Bray (1996); Hanover, Horse Ledge and Bembridge 1997, Bray and Herbert (1998); Hanover and Bembridge 1999, Turner (1999); Hanover, Horse Ledge and Bembridge 2003, Bray (This work).

A:	**North Isle of Wight coast VDS measurements 2003 only**						
Year	**W. Cowes**	**Newtown**	**Warden Ledge**	**All N. Coast**			
Median	4	4	3	3			
Mode	4	4	3	3			
Range	2-4	4	1-3	2-4			
% sterile	0	0	0	0			
% females	41.1	40	60	46.6			
B:	**South coast Isle of Wight VDS measurements**						
	Hanover 1990-2003						
Year	**1989**	**1990**	**1992**	**1995**	**1997**	**1999**	**2003**
Median	NA	NA	NA	NA	3	2	4
Mode	NA	NA	NA	NA	4	2	4
Range	NA	NA	NA	NA	2-4	2-3	1-5
Average	NA	4.6	4.3	NA	NA	NA	NA
C:	**Horse Ledge 1989-2003**						
Year	**1989**	**1990**	**1992**	**1995**	**1997**	**1999**	**2003**
Median	NA	NA	NA	NA	4	NA	4
Mode	NA	NA	NA	NA	4	NA	4
Range	NA	NA	NA	NA	3-5	NA	3-5
Average	4.4	4.3	4.4	NA	NA	NA	NA
D:	**Bembridge 1989-2003**						
Year	**1989**	**1990**	**1992**	**1995**	**1997**	**1999**	**2003**
Median	NA	NA	NA	4	3	3	4
Mode	NA	NA	NA	4	3	3	4
Range	NA	NA	NA	3-5	1-4	1-4	0-4
Average	4.8	5.8	4.3	NA	NA	NA	NA

3.3.3.2 Comparisons of Isle of Wight Nucella lapillus *populations*

The first recolonising *N. lapillus* were recorded on the East Cowes breakwater in 1995 (Fig. 3.16A). Five individuals were found, all with thin shell lips, but up to 30 mm long. This suggests that growth may have been rapid due to minimal competition.

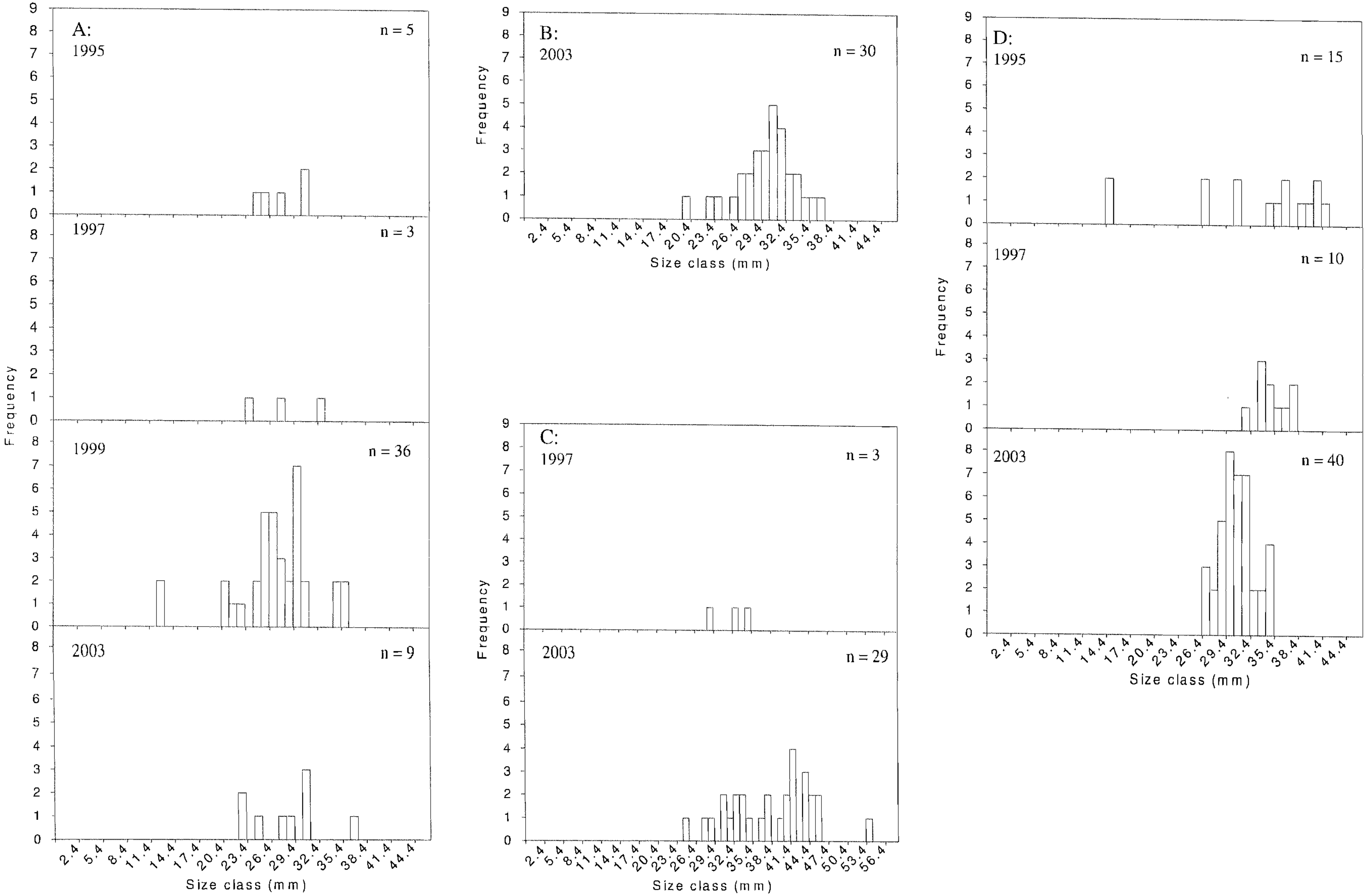

FIGURE 3.16 *NUCELLA LAPILLUS* POPULATION DISTRIBUTION FROM SHELL LENGTH SIZE CLASS: A: EAST COWES, B: WEST COWES, C: NEWTOWN, D: WARDEN LEDGE.

By 1997 3 similar individuals were found at East Cowes but in 1999 a significant level of colonisation had occurred with juveniles, second years and adults present (Fig. 3.16A). By 2003, however, the 36 found in 1999 had reduced to 9 consisting of 3 adults and the rest with thin shell morphology. The population found at West Cowes in 2003 (Fig. 3.16B), comprising 30 second year and adult individuals, was the first record of *N. lapillus* at this site since the mid 1980s.

Newtown and Warden Ledge had similar population characteristics. At Newtown (9 km west of Cowes, Site E, Fig. 3.3) in 1997 3 individuals were found on a shingle spit north of the estuary mouth (Fig. 3.16C). Each had a long shell, up to 35 mm, but the lip thickness of each was 0.3 mm. In 2003 the population increased to 29 which had similar shell morphologies to those observed in 1997. The range was 25-53 mm with the largest having a lip thickness of 0.3 mm. Imposex in this population was high (Tables 3.10 and 3.12), though none were sterile and those found in 2003 were clumped and breeding.

At Warden Ledge (Fig. 3.16D) individuals were generally large (up to 41 mm) with thin shell lips. By 1997 the population had declined slightly and 10 *N. lapillus* were recovered of which, although large, only 40% were greater than 35 mm compared to 55% in 1995. By 2003 a population increase at Warden Ledge occurred with 2% greater than 35 mm. The average shell size in 1995 and 1997 was 35 mm, but by 2003 had reduced to 30.2 mm possibly reflecting greater competition.

On the south coast of the Isle of Wight only data collected in 2003 consisted of repeat time searches for population abundance (Fig. 3.17). Therefore a two-way ANOVA without replication was carried out on juvenile abundances within and between Hanover Point, Horse Ledge and Bembridge. For these comparisons Bembridge results from 1995 were excluded, as data were not collected at the other sites in this year. In addition there were no 1999 results from Horse Ledge, therefore the ANOVA was carried out on results from 1987, 1997 and 2003 for all sites. Although in 1997 peaks of juvenile recruitment were seen at Hanover (Fig. 3.18A) and Bembridge (Fig. 3.18C) and Horse Ledge juveniles also increased slightly (Fig. 3.18B), there were no significant differences ($F_{(2,8)}$, 0.7 $P = 0.5$) and years ($F_{(2,8)}$, 4.0, $P = 0.1$). A further unreplicated ANOVA, including data from 1999, was carried out on Bembridge and Hanover Point results. Main effects were not significant for both factors although the yearly recruitment comparisons suggested an effect ($F_{(3,7)}$, 6.3, $P=0.08$)

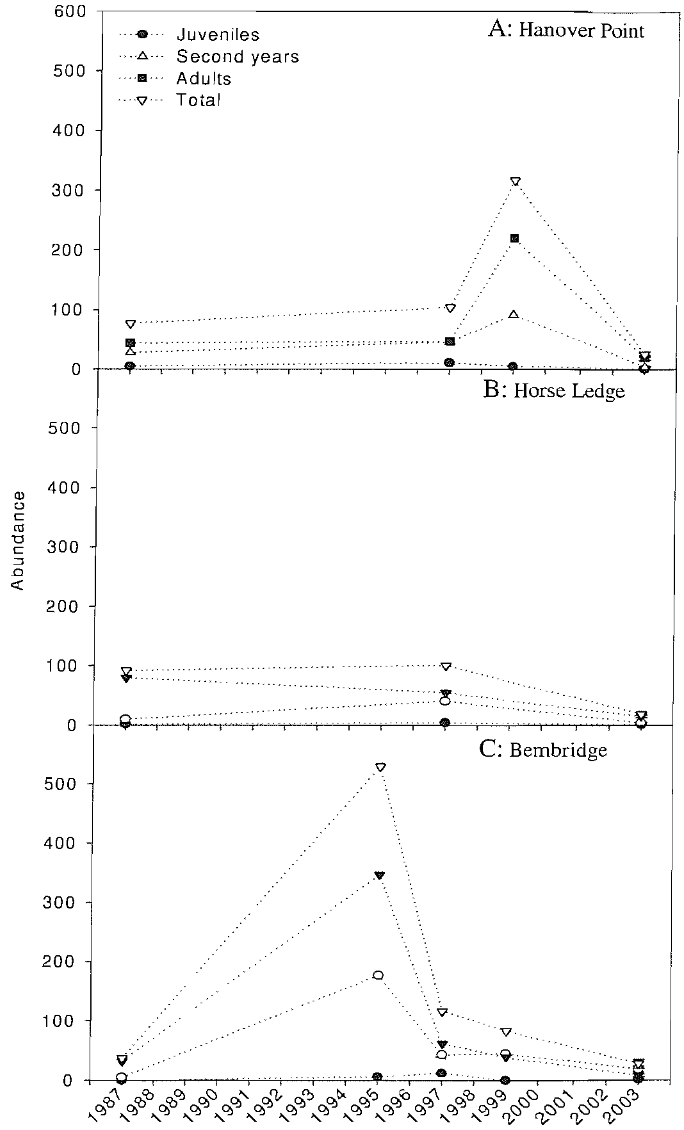

FIGURE 3.17 TOTAL ABUNDANCE OF *NUCELLA LAPILLUS* AND ABUNDANCES OF JUVENILES, SECOND YEARS AND ADULTS FROM SITES FOR ISLE OF WIGHT SOUTH COAST POPULATIONS.
1987-1993 total abundance. 2003 mean +/- 1SE Dotted lines indicate data not a continuum

In contrast to Warden Ledge, Hanover Point (Fig. 3.17A and 3.18A) had low numbers in 1987, but by 1999 abundance was almost three times greater. In 1997 a large cohort of juveniles and second years were recorded and these may have recruited to the adult population by 1999. By 2003 however, abundance had dropped and older larger individuals dominated the remaining population. No individuals had shell lip thickness below 2 mm, which indicated older animals and little recruitment to the population. In 2003 imposex impacts increased at Hanover Point with 18% of females sterile (Fig. 3.14B).

Although no data were available for 1999, broadly comparable population size class patterns to Hanover Point were seen at Horse Ledge (Fig. 3.18B). In 1987 *N. lapillus* were relatively common at Horse Ledge (Fig. 3.17B) although only 2% of the population were juveniles. As at Hanover Point, this population showed a slight tendency toward bimodal distribution particularly in 1997 (Fig. 3.18B). In 1997 total abundance had not greatly increased from 1987 (Fig. 3.17B and 3.18B) and between 1997 and 2003 numbers declined although, unlike Hanover Point, three juveniles were found. The proportion of sterile females found at Horse Ledge in 2003 (15%) was comparable to that seen at Hanover Point (Fig. 3.14B).

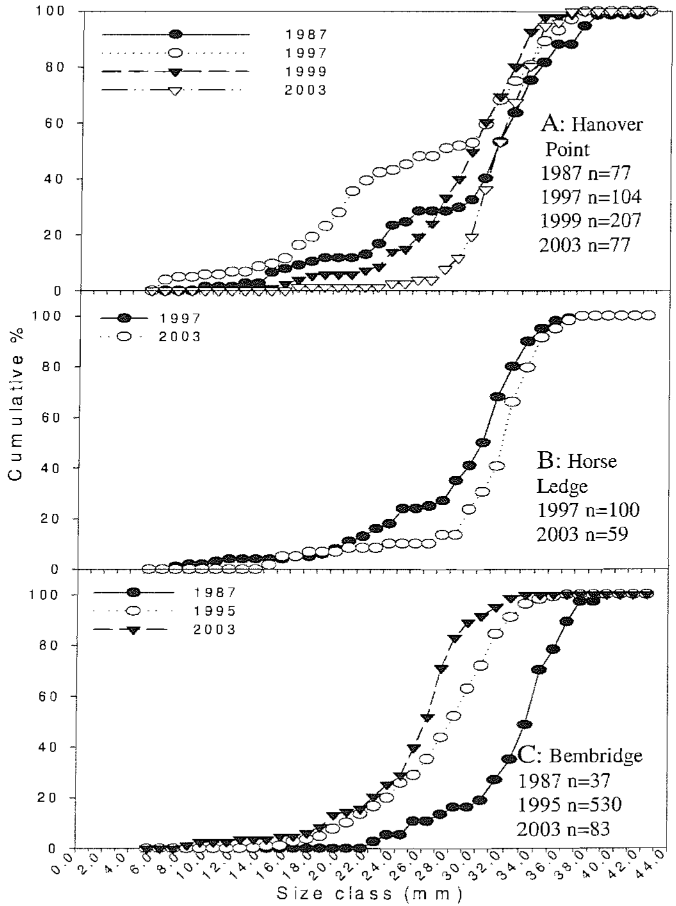

FIGURE 3.18 CUMULATIVE PERCENTAGE SIZE FREQUENCY CURVES FOR ISLE OF WIGHT SOUTH COAST *NUCELLA LAPILLUS* FOR YEARS WHERE SIGNIFICANT POPULATION STRUCTURE CHANGE OCCURRED.

Populations at Bembridge have been the focus of attention on the Isle of Wight since the impacts of TBT were identified. *N. lapillus* at Bembridge showed similar patterns of abundance changes to Hanover Point and Horse Ledge, although the decline was more apparent between 1995 and 1997 (Fig. 3.17C). In 1987 the RPS was 32%, and 50% of the females were sterile (Fig. 3.14A,B); the population being clearly impacted by TBT. By 1995, although VDS stage 5 was still recorded (Table 3.12D), numbers had increased (Fig. 3.17C and 3.18C) and the shell morphology of the population at Bembridge showed that it consisted of juvenile, second year and adult cohorts. Although abundance declined, relatively large numbers of juveniles were found in 1997 (10% of total), which were recruited to second years (50% of total) in 1999 (Fig. 3.17C). In 2003 juveniles and second years comprised 72% of the total population.

Kolmogorov-Smirnov Z (KSz) tests could not be applied to populations from sites on the north coast of the Isle of Wight as abundances were not sufficient for reliable comparison; the changes in population density were evident (Fig. 3.16). In 1997 at Hanover Point (Fig. 3.18A) the population was significantly different from that in 1987 with a bimodal distribution, due to an increase in the numbers of juveniles and second years (Table 3.13). The loss of smaller size classes in 1999 followed by abundance decline in 2003 (Fig. 3.18A) led to further significant differences (Table 3.13).

Table 3.13 Kolmogorov–Smirnov Z test results for changes in shell size class population structure for Isle of Wight south coast sites.
Bold text: years where significant change occurred at P<0.01, NS: Not significant.

Hanover Point		Horse Ledge		Bembridge	
Year	P	Year	P	Year	P
1987vs1997	**<0.01**	1987vs1997	NS	**1987vs1995**	**<0.01**
1997vs1999	**<0.01**	1987vs2003	NS	1995vs1997	NS
1999vs2003	**<0.01**	**1997vs2003**	**<0.01**	1995vs1999	NS
				1995vs2003	**<0.01**

At Horse Ledge (Fig. 3.18B) comparisons between the 1987 result and those from subsequent years were not significant as the population distribution curve pattern was midway between the latter two years. A comparison carried out on 1997 and 2003 cumulative frequency data showed that the lack of juveniles and second years coupled with a decline in the number of adults gave a significant result (Table 3.13). At Bembridge in 1987 the small population was dominated by adults (Fig. 3.18C), but by 1995 recruitment led to a higher representation of *N. lapillus* in all the size classes and a significant difference between cumulative curves (Table 3.13). The comparison between 1995 and 2003 was also significantly different due to the population being skewed toward younger individuals.

3.3.3.3 Nucella lapillus *prey abundance*

Prey abundance at Isle of Wight sites was not recorded for all sites and years as the focus of most researchers was to consider imposex only. Percentage barnacle cover was recorded by Bray (1995, 2003), Turner (1999), and Herbert (2001, 2002) who produced a continuous data set from 1995 to 2002 for Hanover Point and Bembridge. The 2003 data were collected during imposex and population surveys. These data, presented as number of barnacles per cm^2 have been used to provide a continuum of abundance results (Fig. 3.19).

Barnacle density at Hanover Point increased from 1994 to a high in 1998. Density declined sharply in 2000 then stabilised from 2001 to 2003 (Fig. 3.19). Bembridge barnacle density was lower than at Hanover Point and they tended to cluster at a point on the shore where a drop between an upper and lower ledge exists; dogwhelks were seen predating upon these patches. Elsewhere at Bembridge barnacles may not have been able to colonise due to the sweeping action of abundant algae fronds (Southward and Southward, 1978; Hawkins, 1983).

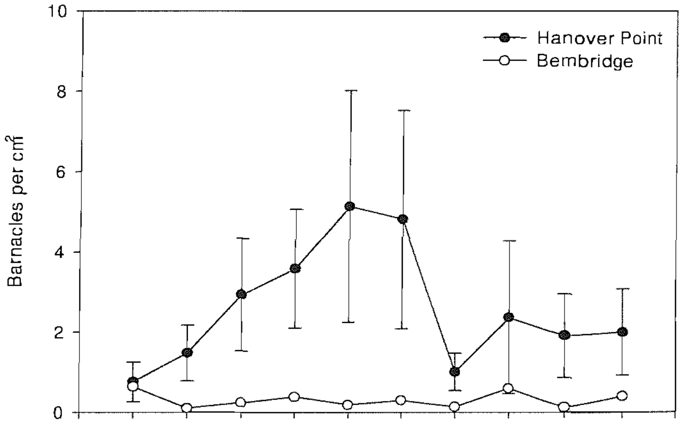

FIGURE 3.19 MEAN (+/- 1SE) DENSITY OF BARNACLES PER CM^2 AT SOUTH COAST ISLE OF WIGHT SITES (HANOVER POINT AND BEMBRIDGE) 1994-2003.
(Data from Herbert, 2003).

Growth and decline in *N. lapillus* abundance, particularly at Hanover Point suggested that prey may have been a limiting factor. *N. lapillus* abundance at Hanover Point increased to its highest in 1999 (Figs. 3.17A and 3.18A) in the same period as the maximum barnacle density (Fig. 3.19). A possible relationship between *N. lapillus* abundance in relation to prey at Bembridge was less clear as numbers reached a high in 1995 (Figs. 3.17C and 3.18C) whereas barnacle density remained relatively constant. Correlations between total *N. lapillus* abundance and barnacle density at both sites produced no significant relationships, though this may have been due to the lack of abundance data for *N. lapillus*. Pooling of barnacle density from both sites also did not show a significant correlation to *N. lapillus* abundance.

3.4 Discussion

3.4.1 Procedural problems

The main focus of this work was the long-term recovery of *N. lapillus* populations from TBT pollution in Plymouth Sound and other sites on the south coast. Despite gaps in these data for Plymouth Sound (1994-1996, 2002), trends were clear. On the Isle of Wight, although data were available for several years, there were also gaps (e.g. 1987-1997) due to several workers collecting the data and the need to limit destructive surveys to reduce their impact. Other south coast sites were only surveyed in the years 1995, 1999, and 2003. The collection of data by several workers, in particular at the Isle of Wight, and to a lesser extent, at Plymouth, may have led to some inconsistencies, but efforts were made to minimise this. In addition at Plymouth, populations were originally assessed by Spence (1989) and Proud (1994) who were able to visit the sites on several occasions each year. This was not possible in this work, although data were always collected and compared from the same season.

The impact of TBT on females results in continued penis and vas deferens growth, but does not affect males (Bryan *et al.*, 1987). In some cases mean male penis lengths were longer than previously recorded (e.g. Osmington Mills Fig.3.4A, Tregantle and Renney Rocks Fig. 3.9B,D), leading to decreases in the RPS. The reasons for the male penis increase are unclear (possibly due to increasing adult age, e.g. Osmington Mills), although the length increases when peak sexual activity is approached (Bryan *et al.*, 1987) and fluctuations can be very pronounced with the onset of winter (Gibbs *et al.*, 1987).

The reliable ageing of *N. lapillus* can be difficult (Gibbs and Bryan, 1986), although the widely used method developed by Feare (1969) was used (§ 2.3.2). *N. lapillus* feeding on *M. edulis*, however, often have thin shells (Crothers, 1975, 1985) and shore exposure can affect shell thickness. At Tregantle, where the main prey were mussels and the site was moderately exposed, thin shells were common leading to problems estimating age. Thin shells have also been found in recolonisers after TBT pollution (Crothers, 1998, 2003). Thus when assessing populations variable shell morphology may have led to uncertainties in assigning age groups.

Anecdotal information was available regarding disturbance and dredging in Weymouth Harbour in 1995-1996 and the disposal of material close to Osmington (Stable pers. com. 2003). The TBT content, amount of sediment and the duration of the dredging project could not be established thus the potential for impact from these activities was not clear.

3.4.2 Recovery of *Nucella lapillus* populations and the continuing impact of TBT

3.4.2.1 Imposex and population assessment

After the 1987 partial ban on TBT, imposex in many UK *N. lapillus* populations improved (e.g. Douglas *et al.,* 1993), but where commercial vessels are common, less recovery was seen (e.g. Morgan *et al.,* 1998). The three geographical scales at which the TBT impact on *N. lapillus* have been considered here confirm that it is reduced, but still present in the water column and impacting dogwhelks even on open coasts (e.g. Tregantle and Horse Ledge).

At sites on the south coast the reasons for population decline were not clear. *N. lapillus* surveyed in the region did not have high levels of imposex, although some evidence of TBT impacts were seen at Osmington in 1995 when dredging at Weymouth and spoil disposal near to Osmington were taking place (Stable pers. com. 2003). Imposex persisted there in 2003, but indices were low with minimal impact on the females so could not be reliably attributed towards the observed population decline. The TBT ban was effective at Lulworth, as imposex there was never high between 1995-2003. Before imposex impacts became common Crothers (1975) collected only sixty dogwhelks from Lulworth for shell shape assessment, indicating a small population before common TBT use. The abundance increase seen at Lulworth in 1995, but decline by 2003, suggests factors other than TBT pollution were affecting the population size; breeding did not appear to be impaired as many were juveniles and second years. At Selsey only adult males were found in 1990 (Spence *et al.,* 1990). In 1992 imposex at Selsey was high and juvenile recruitment was poor (Harding *et al.,* 1992) leading to a subsequent population decline and although imposex decreased by 1995, older animals remained dominant. By 1999 juveniles and second years were becoming more common indicating that recovery of the Selsey population was occurring. Thus it is possible that the older *N. lapillus* were remnants from when Selsey dogwhelks were severely impacted (Harding *et al.,* 1992) and that a healthy breeding population was starting to re-establish.

The fluctuating populations, with periods of older adult dominance followed by juvenile recruitment seen at Osmington and Lulworth, have also been suggested to occur at St. Agnes and Renney Rocks (Spence, 1989). Population decline at St. Agnes between 1989 and 1997 (Spence, 1989; Proud, 1994; Shute, 1997) and low recruitment there and at Renney Rocks was offset by abundant second years and juveniles being found at Renney Rocks in 2000 and at St. Agnes in 2001. These variations appear to have been due to other effects, rather than TBT pollution, particularly at St. Agnes which had low contamination and no sterile females.

As the ability of female *N. lapillus* to reproduce is impaired a change in population age class structure, by a shift towards dominance of older animals and low abundance, can reflect the impact of TBT (Harding *et al.*, 1998). Work on dogwhelk populations from Plymouth Sound (Spence, 1989; Proud, 1994, Bray, this work) and observations on the Isle of Wight and the south coast have shown these effects. Although older *N. lapillus* dominated some populations (e.g. Jennycliff and Kingsand and Hanover Point on the Isle of Wight) TBT may not be the only cause of changes in population structure. Whilst RPS and VDS were good indicators of TBT pollution, Spence (1989) felt that population abundance and structure were not reliable indicators as natural constraints (prey, exposure, predation) masked the effects of TBT. Changes in population structure as observed at St. Agnes and Renney Rocks should be considered in conjunction with imposex indices and natural factors (Harding *et al.*, 1998).

Although few *N. lapillus* were found at Portland and none at Peveril Point, the physical habitats were suitable even if prey densities were low. At Portland *N. lapillus* were previously common (Crothers, 1975), but badly affected by imposex in the early 1990s (Harding *et al.*, 1992). Portland is very exposed and it may be that the high imposex coupled with the loss of nearby *N. lapillus* populations due to TBT pollution or natural factors, resulted in further isolation and decline in this population, as by 2003 only one dogwhelk was found. A small population of dogwhelks was reported at Peveril Point (Crothers, 1975), but the subsequent loss of *N. lapillus* at the site could not be attributed to TBT as none were imposexed. Peveril Point is also relatively isolated, but subtidal dogwhelks were suggested as the source of the population (Crothers, 1975). It seems possible that the limited availability of food was a factor in their absence. This may have been due to the uneven substrate being unsuitable for barnacles (e.g. Berntsson *et al.*, 2004) as where barnacle clusters did exist they were found on chert deposits within the limestone.

The high imposex levels found in the late 1980s to early 1990s in *N. lapillus* populations from Plymouth Sound had clearly reduced (Bryan *et al.*, 1987; Gibbs *et al.*, 1987; Spence, 1989, 1990; Proud, 1994). Spence (1989) found that imposex in these populations had stabilised by the late 1980s and reported that even at the most polluted sites (Kingsand and Jennycliff) breeding females were present. Spence (1989) predicted that the recovery of Plymouth populations would take five to ten years, but would depend on the continued reduction of TBT. Subsequent work at Plymouth (Proud, 1994) showed that waterborne TBT had reduced and a linear relationship between the reduction of female *N. lapillus* penis lengths and time led to the prediction of zero RPS between 1994 and 1995. This was thought to be unlikely, however, due to continued low TBT levels in the water from its continued use on large vessels and dockyard activities. Thus it was concluded that imposex recovery would not remain linear, but decline over a longer period than initially predicted (Proud, 1994).

Observations at Plymouth from 1997 onwards showed that, as predicted, the imposex decline had slowed and was fluctuating around lower levels, although Jennycliff females were still badly impacted due to their proximity to Devonport and the Cattewater. In 1997 female penis length increases at Jennycliff, Renney Rocks and Tregantle may have been a relatively rapid reaction to a short-term rise in TBT, as in 1998 female sterility also rose. This lag may have been due to vas deferens growth from stage 4 to sterility being slow (Davies, 2000), although initially this structure develops more quickly than the penis (Gibbs *et al.*1987). Similarly to the 1997-1998 imposex increases at Plymouth, Huet *et al.,* (2004) reported that *N. lapillus* in the Bay of Brest (Brittany) showed a marked RPS rise in 1998. The only documented event possibly related to this was the launch of a new aircraft carrier that had been coated with TBT-based paint. This highlights the sensitivity of *N. lapillus* to short-term TBT inputs and may help explain the 1997-1998 imposex increase seen at Plymouth sites as in this period naval and support craft movements increased (Fletcher pers. com. 2003).

RPS generally increases with proximity to major TBT sources (Bryan, *et al.,* 1986; Gibbs and Bryan, 1986; Spence *et al.,* 1990; e.g. Santos *et al.,* 2000; Svavarsson, 2000), but on the UK south coast this effect was less clear due to extensive recreational boating (Spence *et al.,* 1990). At Tregantle, which is more exposed and distant from the dockyard, unexpectedly high imposex incidence was often seen (e.g. 1997, 1999, 2000), but RPS and percentage sterile were similar to Jennycliff and Kingsand levels. This blurring of concentration gradients, found in populations distant from large ports, has also been seen on the Portuguese coast (Santos *et al.,* 2002) due to continued TBT use on small boats and marina construction.

N. lapillus at West and East Cowes were relatively abundant before TBT impacts on the Isle of Wight, (Crothers, 1975). By the 1980s and early 1990s these and other north coast populations, including Seaview, Newtown and Warden Ledge were extinct and south coast populations were severely impacted (Bryan, 1987; Herbert, 1988; Langston *et al.,* 1994). *N. lapillus* populations there were primarily affected by TBT painted on small craft, thus stood to benefit from the 1987 legislation. After TBT levels declined in the Solent, particularly at areas of small boat use (Langston *et al.,* 1994) *N. lapillus* were found recolonising at West Cowes and Warden Ledge (Bray, 1996). During a period when abundances at St. Agnes declined despite very low imposex, further recolonisation and abundance increases were seen on the north of the Isle of Wight (Bray and Herbert, 1998; Turner; 1999; Herbert *et al.,* 2000). For the first time in ten years, in 1997 *N. lapillus* were found at Newtown although imposex in the females was high. This demonstrates that they could re-establish at a site where TBT levels in fine sediment may still have been high. By 2003 abundance had declined at East Cowes, but this may have been due to limited prey as at West Cowes imposex was not sufficient to cause extinction; the VDS range showed females were fertile.

Whilst dogwhelk populations on the northern Isle of Wight coast were severely impacted by imposex and declined to extinction in the 1980s (Herbert, 1988), those on southern shores did not die out. These may have been the source of north coast recolonisers; Bryan *et al.,* (1986) suggested that a population at Penzance was maintained at the height of TBT impacts due to external recruitment with juveniles floating in on material. Morphologically the north coast *N. lapillus* did not resemble those on the south coast. *N. lapillus* at Hanover Point and Horse Ledge had robust shells with thick lips whereas those at Bembridge were slightly smaller with thinner shells; crab or bird predation can lead to the phenotypic expression of the stronger shell type (Moore, 1938b; Crothers, 1985; Trussell and Etter 2001; Nagarajan *et al.,* 2002). On the north coast at Warden Ledge in 1995 and 1997 and at Newtown in 2003 several of the recolonising *N. lapillus* were very large with thin shells. Dogwhelks with a similar morphology have recolonised elsewhere (Crothers, 1998, 2003) and have been reported in subtidal habitats (Moore, 1936; Crothers, 1975) including Bramble Bank in the Solent (Mallinson pers. com. 2002). The distance of south coast sites from northern Isle of Wight shores suggests that new populations may have been established from large benthic morphs already present rather than the arrival of south coast *N. lapillus*, although TBT would probably have also affected subtidal habitats. This does not explain the recolonisation of Cowes by dogwhelks which were not large (Bray, 1996). The larger morphs did not persist at Warden Ledge, possibly due to an increase in competition as more individuals arrived.

N. lapillus on the southern Isle of Wight showed severe TBT impacts. Hanover Point and Horse Ledge are not directly affected by a TBT source (e.g. shipping) so the reasons for the high imposex indices were not clear. Bembridge is directly influenced by tidal flushing of the Solent (Fig.3.20A) and there is a gyre that occurs under favourable winds south east of the Isle of Wight which may trap sediment and pollutants (Statham *et al.,* 1999). Despite the impacts of tidal flows and sediment from the Solent, imposex incidence at Bembridge declined and the percentage of females returned to normal, however the population declined between 1999 and 2003. Similarly to Bembridge, complex tidal flows also affect Hanover Point (Fig. 3.20B). These may trap pollutants and thus be partly responsible for the slow decline in imposex at Hanover Point in particular, although overall the reduction there was greater than at Horse Ledge where tidal influences are less. In 2003 sterile females and few juveniles were found at Hanover Point and Horse Ledge and at Hanover Point the VDS had increased from 1999. These populations may have been suffering the impact of TBT from diffuse sources such as sediment (Herbert *et al.,* 2000).

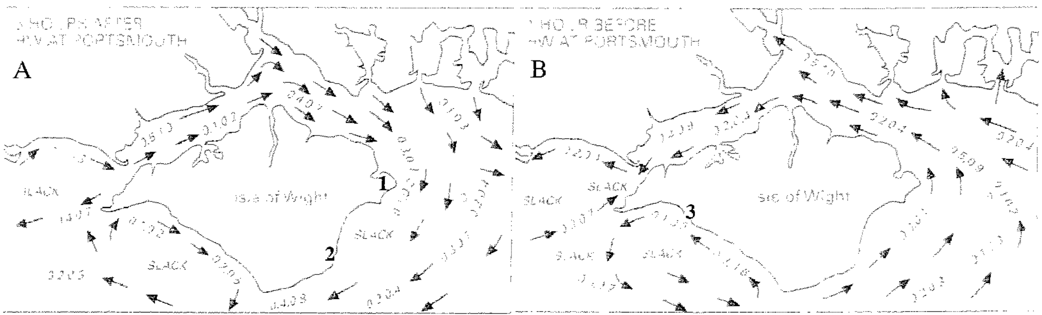

FIGURE 3.20 A: TIDAL FLUSHING OF THE SOLENT AND SOUTHAMPTON WATER AFFECTING BEMBRIDGE (1) AND HORSE LEDGE (2) AND B: THE DAILY TIDAL GYRE RECYCLING WATER OFF OF HANOVER POINT (3).
(Modified from Admiralty Tidal Charts SC2035, Stanfords, 2000).

3.4.2.2 Continued sources of TBT contamination

For the south coast region no TBT data were available, but for Plymouth Sound they are recorded by the Environment Agency and were obtained for 1993-2003 (Fig. 3.21). Samples were taken yearly, close to Plymouth Dockyard, but no fixed numbers or locations were used; thus concentrations were averaged from autumn to autumn of each year as this was when imposexing and population assessments were carried out.

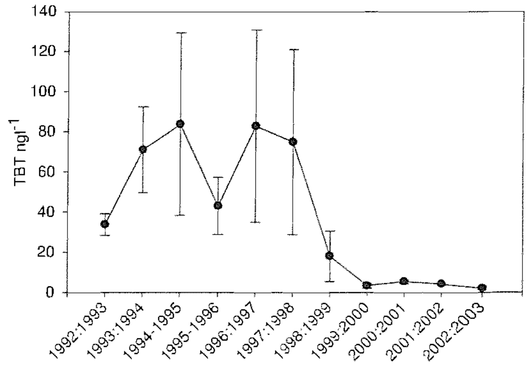

FIGURE 3.21 MEAN (+/- 1SE) TBT LEVELS IN WATER NEAR DEVONPORT DOCKYARD, 1992-2003.
*Period taken from Autumn to Autumn. (Data from Environment Agency 2003).

TBT Levels declined from 1998 with acute pollution events influencing results prior to this. At Jennycliff and Kingsand, within Plymouth Sound, there was a slow reduction in the RPS and the percentage sterile, although after 1998 recovery improved. These indices were correlated with TBT levels with a 1-year time lag to reflect the delayed effect (Fig. 3.22). Correlations between Kingsand imposex indices and TBT levels were not significant. At Jennycliff there was a significant relationship between TBT levels and the RPS ($r = 0.87$, $n = 7$, $df = 6$, $P<0.05$) to which a trend line was fitted ($r^2 = 0.71$, $y = 0.0653x + 3.210$). The comparison between TBT and the percent of sterile females was also significant ($r = 0.91$, $n = 7$, $df = 6$, $P<0.01$) to which a trend line was also fitted ($r^2 = 0.79$, $y = 0.233x + 3.342$). This demonstrated a clear relationship between imposex and TBT concentrations at Jennycliff as imposex levels dropped after 1998, in the same period as TBT levels declined (Fig. 3.21).

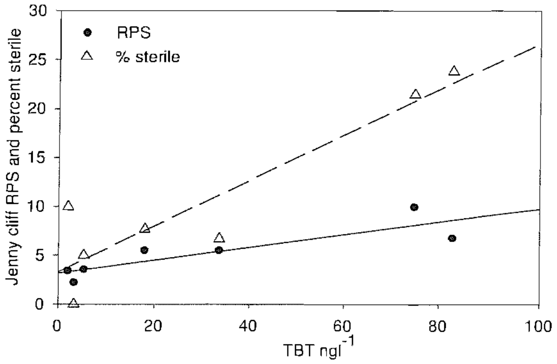

FIGURE 3.22 RELATIONSHIP BETWEEN THE PERCENT OF STERILE FEMALES AND THE RELATIVE PENIS SIZE INDEX (RPS) AT JENNYCLIFF AND TBT LEVELS IN PLYMOUTH SOUND.
Compared with 1-year lag. (Data from Environment Agency, 2003).

Imposex levels at Tregantle and Renney Rocks (Plymouth) and at Horse Ledge and Hanover Point on the Isle of Wight were higher than expected. Dredged material is dumped near to these sites (Figs. 3.2B and 3.23) and at Plymouth re-suspended sediment from the dumpsite has been modelled as impacting Renney Rocks (CEFAS, 2003).

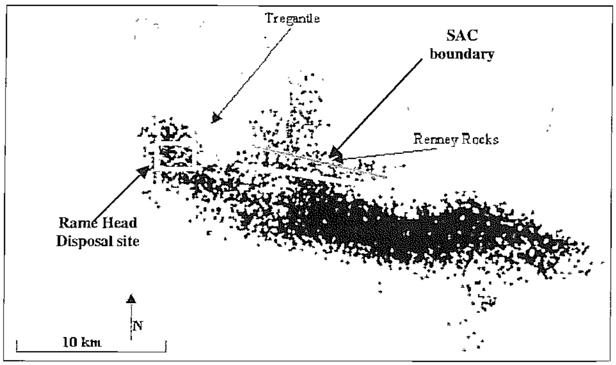

FIG.3.23 THE PATH OF DREDGE SPOIL DUMPED AT THE RAME HEAD DISPOSAL SITE IN RELATION TO TREGANTLE AND RENNEY ROCKS AND THE PLYMOUTH SOUND SAC
(Modified from CEFAS, 2003).

To show if a relationship existed between dredge spoil and imposex indices at Tregantle and Renney Rocks, data for the tonnage of sediment dumped at Rame Head between 1986-2001 (Fig. 3.24) were correlated against the percentage of sterile females and the RPS. The RPS and percentage sterile at Tregantle and Renney Rocks declined similarly to reductions in dredge spoil (Fig. 3.24), apart from the clear difference in 2001. Initially the correlations were not significant, but when results from 2001 onwards were removed significant relationships were found. The RPS was significant for both sites comparing same year results (Tregantle: correlation, $r = 0.76$, $n = 12$, $df = 11$, $P<0.01$, regression, $y = 0.0000493x + 17.645$; Renney Rocks: correlation, $r = 0.59$, $n = 12$, $df = 11$, $P<0.05$, regression, $y = 0.0000329x + 12.360$). 2002 imposex results were not available to establish if imposex increased in relation to tonnage dumped in 2001, but by 2003 the VDS at both sites had risen.

With the time lag, thus allowing for the VDS to develop further, a relationship between sterile females and dredge spoil was only significant for Tregantle ($r = 0.79$, $n = 12$, $df = 11$, $P<0.01$, $y = 0.0000224x + 0.859$). Tregantle is relatively close to the dumpsite, although not modelled as directly impacted (Fig. 3.23). For Renney Rocks the result was not significant, possibly due to the distance over which the spoil would have dispersed (Fig. 3.23), but a relationship was indicated ($r = 0.57$, $n = 12$, $df = 11$, $P = 0.05$). These results should be treated with caution as this cannot be established as a cause-effect relationship and would require further research, particularly when considering the sediment dumped in 2001.

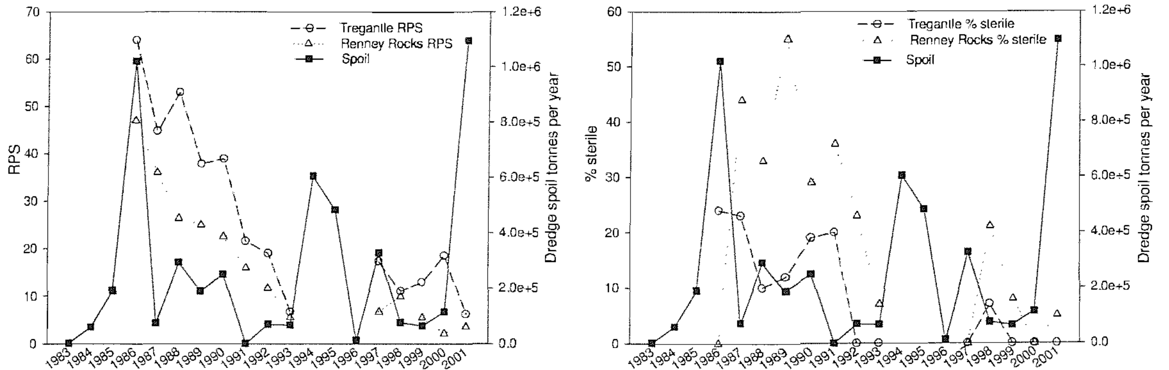

FIGURE 3.24 A: TONNAGE OF DREDGED MATERIAL IN RELATION TO THE RELATIVE PENIS INDEX AT TREGANTLE AND B: THE PERCENTAGE OF STERILE FEMALES AT TREGANTLE, C: DREDGED MATERIAL IN RELATION TO THE RELATIVE PENIS INDEX AT RENNEY ROCKS AND D: THE PERCENTAGE OF STERILE FEMALES AT RENNEY ROCKS.

The tonnages of dredged material dumped at the Needles and Nab Tower (Fig. 3.3) were obtained for 1985-2000 (CEFAS 2001). A positive correlation was found between sterile females at Hanover Point and dumped material at the Needles ($r = 0.98$, $n = 5$, $df = 4$, P<0.01) (Fig. 3.25B). Regression analysis was also significant ($r^2 = 0.97$, $y = 0.001x - 3.239$). These relationships should be treated with caution as they were mainly based on one coincident fall in both values between 1989-1992, also imposex data were not continuous and only five observations were available. No correlation was found between material dumped at the Needles and the RPS at Hanover or any other site. The tonnage dumped at Nab remained high (Fig. 3.25C,D), although the RPS and the percent sterile declined at Bembridge, Horse Ledge and Hanover Point, thus suggesting that no direct relationship was present. Data from this dumpsite did not correlate with RPS or sterility at any south coast Isle of Wight location.

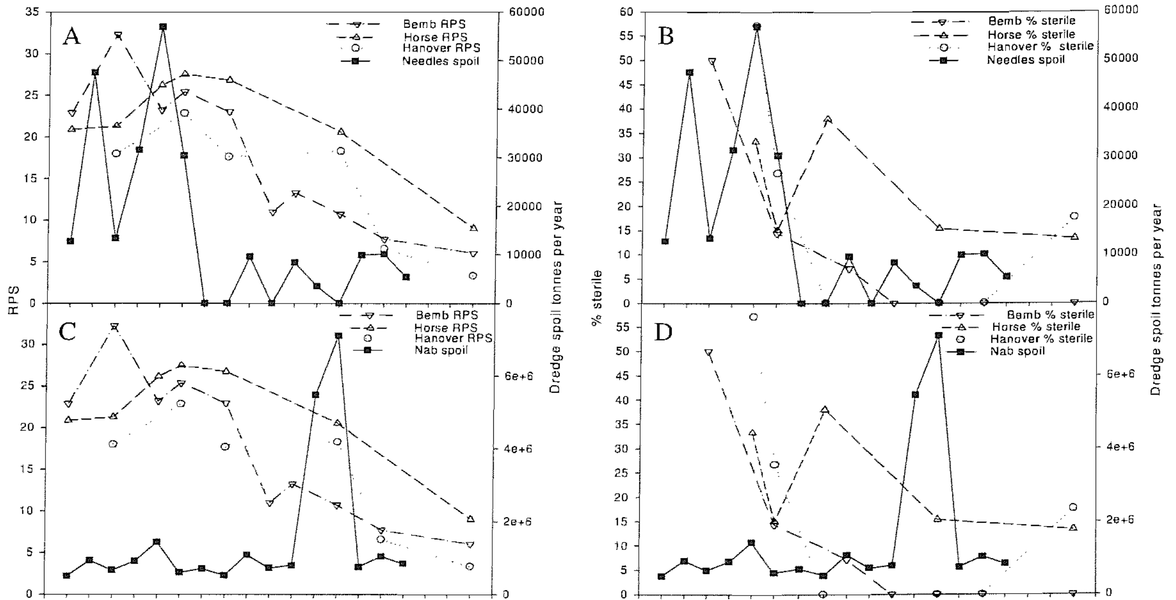

FIGURE 3.25 A: TONNAGE OF DREDGED MATERIAL DUMPED AT THE NEEDLES IN RELATION TO THE RELATIVE PENIS INDEX AT SOUTH ISLE OF WIGHT SITES AND B: THE PERCENTAGE OF STERILE FEMALES AT SOUTH ISLE OF WIGHT SITES AND C: DREDGED MATERIAL DUMPED AT NAB TOWER IN RELATION TO THE RELATIVE PENIS INDEX AT SOUTH ISLE OF WIGHT SITES AND D: DREDGED MATERIAL DUMPED AT NAB TOWER IN RELATION TO THE PERCENTAGE OF STERILE FEMALES AT SOUTH ISLE OF WIGHT SITES.

TBT from dredge spoil has been shown to influence imposex and benthic communities (Quigley *et al.,* 1999; Svavarsson *et al.,* 2001; Santos *et al.,* 2004) and has been implicated in causing imposex in *N. lapillus* (Santos *et al.,* 2004). The potential for the influence of dredged spoil on sensitive marine areas in the UK has also been considered (CEFAS, 2001, 2003). The results found here suggest that TBT from spoil at Plymouth may adversely affect the *N. lapillus* populations at Renney Rocks and Tregantle. At Hanover Point, where the percentage sterile increased in 2003, the same may be occurring. Whilst populations at Bembridge may be affected by water flushed from the Solent, the high incidence of imposex at Horse Ledge may require further consideration, as these observations may have far reaching implications for the management of sensitive marine habitats.

3.4.3 Other factors

N. lapillus predominantly feeds upon barnacles and mussels and, of barnacles, has been suggested to prefer *Semibalanus balanoides* (Crothers, 1985), but Burrows (1988) found that preferences may be more size than species related. Herbert *et al.,* (2003) found that a barnacle species associated with warmer waters (*Balanus perforatus*) had recovered from the severe winters of 1962-1963 and extended its range on the southern UK coast past the Isle of Wight. As an indicator of temperature in the English Channel this suggests that the sea in the region has warmed thus allowing a wider range for the species. The settlement of barnacles, in particular *Semibalanus balanoides,* can be very variable both spatially and temporally (e.g. Hawkins and Hartnoll, 1982; Hansson *et al.,* 2000; Jenkins *et al.,* 2001) and can be driven by fluctuating thermal conditions (Southward, 1991; Southward *et al.,* 1995; Herbert, 2001). *N. lapillus* abundance is related to food availability (Spence *et al.,* 1990) and at south coast sites a correlation was found between pooled barnacle cover for Portland, Osmington and Lulworth and the decline of *N. lapillus.* This suggests that though TBT was present, the major factor in *N. lapillus* decline at these sites was a drop in prey abundance.

Genner *et al.,* (2004) reported that recent years have been the warmest for the past century. Change in sea temperature during this period may have caused barnacle populations to fluctuate and leave total cover low whilst they adjusted to new conditions; barnacle abundance can lag two years behind temperature changes (Southward, 1991; Southward *et al.,* 1995). With *N. lapillus* having a size preference for barnacles and sometimes taking time to learn to handle new prey (Fischer-Piette, 1935 *op cit* Hughes and Burrows, 1993), the populations in the south coast regions appear to have been affected by the barnacle decline. *N. lapillus* at Selsey fed on alternative species to barnacles and mussels (for list of alternative prey see Crothers, 1985) and this may also occur at sites such as Lulworth and Osmington (e.g. Hughes *et al.,* 1992) whilst barnacle density recovers, thus allowing dogwhelks to persist until their preferred prey density increases.

Isle of Wight south coast populations did not correlate with prey abundance and the percentage sterile from Hanover Point only correlated weakly with dredge spoil. Imposex levels were not high enough to cause total extinction on the south coast of the Isle of Wight, but did lead to sterile females, thus reducing recruitment. TBT impacts combined with the decline in prey species seen after 2001 on the south coast of the Isle of Wight may have acted synergistically to cause the population reductions seen at Hanover Point and Horse Ledge.

At St. Agnes significant changes in *N. lapillus* population density were observed (Spence, 1989; this work). These natural fluctuations, particularly in 2000-2001, may have been due to scour of prey communities by sediment at the base of the rock stacks on which the dogwhelk populations were based. *N. lapillus* has also been shown to be sensitive to high temperatures; darker morphs are often associated with exposed shores where thermal stress is lessened and *vice versa* (Crothers, 1985; Etter, 1988b). High summer temperatures have been suggested as possibly influential in increased *N. lapillus* mortality (Gibbs *et al.,* 1999). Correlations were carried out between dogwhelk age class and total abundances at all of the sites surveyed to show if any relationship existed with seasonal high and low temperatures between 1986 and 2003. No significant relationships were found for any of the populations; therefore the short-term effects of changing air temperature did not appear to adversely influence the long-term survival of the *N. lapillus* populations.

3.4.4 Management issues and legislation

In the UK TBT was banned on vessels less than 25 m long in 1987. The ban has been partially effective in reducing imposex, particularly at sites such as Selsey and Lulworth Cove which are remote from large shipping, but where small vessels are common. In the longer term TBT is to be banned on larger vessels worldwide. This is to take place when an international agreement put in place by the International Maritime Organisation is ratified by either 25% of the worlds shipping nations, or when 25% of the worlds shipping tonnage is encompassed by signatories. Take up of the agreement has been slow. It was originally planned to become effective from the 1st January 2003 and all ships to have TBT based paints removed by 2008 (Champ, 2000). By November 2004 only 9.06% of the world's shipping tonnage was covered by the agreement (IMO pers. com. 2004).

Short-term TBT increases (Huet *et al.,* 2004) and organotins bound to sediment (Santos *et al.,* 2004) can affect imposex levels. TBT has been shown to bind to fine sediments leaching out over long periods (Dowson, 1996; Macguire, 2000). With the ban being taken up slowly and the gradual release of TBT from sediments, the implications for the management of impacts from dredging and ships are important. This is particularly the case in sensitive marine areas such as Plymouth Sound and South Wight Maritime SACs. TBT has been shown to impact Marine Protected Areas causing imposex in gastropods, thus highlighting the ability of pollutants to transcend boundaries in the marine environment (Terlizzi *et al.,* 2004). A cause-effect relationship cannot be established from this work, but TBT from sediments may be affecting *N. lapillus* and possibly other sensitive species at the marine habitats on the south of the Isle of Wight and at Plymouth Sound.

Evans (1999) suggests that the total ban on TBT may be premature and argues that the detrimental effects of the compound are outweighed by commercial benefits. In addition Evans (2000) showed that populations declared sterile during the height of imposex have recovered. It was not explained, however, whether this was due to the recolonisation of sites, or whether the local population had recovered. In some areas both recovery and recolonisation have not taken place (Bray and Herbert, 1998; Herbert *et al.,* 2000) therefore such statements must be considered with caution.

3.5 Summary

Over the past fifteen years *N. lapillus* populations have recovered from the severe impacts of TBT seen in the mid to late 1980s with recolonisation taking place at some sites at which extinctions had been recorded. TBT is still causing imposex in some areas and in Plymouth Sound this appears to be directly related to its use on large ships. At sites where small craft are prevalent imposex has declined. Imposex can still be found in dogwhelk populations which may be indirectly impacted by the disposal of contaminated dredge spoil (e.g. Tregantle and Hanover Point).

Changes in population structure may not indicate TBT pollution, but may also be affected by prey availability and physical forcing such as catastrophic losses through storms. Population fluctuations should therefore be considered in conjunction with imposex indices when TBT impacts are being evaluated. In this case the availability of prey at locations on the south coast has apparently driven the decline of populations where imposex was minimal. At other locations, particularly on the Isle of Wight, the impact of diffuse TBT sources coupled with a decline in prey may have acted together to reduce the populations, although the impact of TBT at some sites was significant.

The management of dredged material may require further consideration as this appears to impact sensitive marine habitats. Further research would be required to ascertain whether contaminated spoil was responsible for the imposex levels observed. Contaminated spoil will reduce, though over a long period, if the world's shipping nations agree to take up the legislation proposed by the IMO thus leading to eventual imposex decline.

Chapter 4. Colonisation of new substrate by *Nucella lapillus*

4.1 Introduction

Following UK legislation restricting the use of organotin paints in 1987, imposex declined in many *N. lapillus* populations leading to recolonisation and recovery along much of the coastline of Britain (e.g. Evans *et al.,* 1995; Bray and Herbert, 1998; Crothers, 1998, 2003; Miller *et al.,* 1999; Hawkins *et al.,* 2002), particularly where recreational vessels were common (Crothers, 1998, 2003; Birchenough *et al.,* 2002b). Population recovery from imposex was prevalent along eastern and northern UK coasts (e.g. Douglas *et al.,* 1993; Evans *et al.,* 1994; Birchenough *et al.,* 2002a), although at some sites has been incomplete due to continued TBT use on commercial ships (Harding *et al.,* 1997; Hawkins *et al.,* 2002).

N. lapillus is a direct developing species (Vance, 1973; Crothers, 1985) with no recognised pelagic stage and is generally acknowledged as moving small distances throughout its life (Connell, 1961; Hughes, 1972; Castle and Emery, 1981; Crothers, 1985; Etter, 1996; Cook and McMath, 2001). Therefore how *N. lapillus* achieves dispersal and recolonisation or colonisation of formerly occupied or newly available sites is not well understood. Thus the aim of this chapter is to investigate how *N. lapillus* can colonise new habitats and recolonise areas where local extinctions have occurred due to TBT pollution.

Newly created intertidal habitats present opportunities for rocky shore species to colonise where no suitable substrate formerly existed (Moore, 1939, 1940; Southward and Orton, 1954; Dobbs and Vozaerik, 1983; Chapman and Bulleri, 2003). In addition to recovery at formerly polluted sites, *N. lapillus* has proven able to colonise coastal protection structures (Moore, 1940; Bray and Herbert; 1998; Herbert *et al.,* 2000). Rafting on detritus or seaweed clumps may be how directly developing species can colonise available habitats (Highsmith, 1985; Bryan *et al.*1986; Johannesson, 1988; Martel and Chia, 1991a,b; Ingólfson, 1995; Johnson *et al.,* 2001), but rafting is not well understood (Johannesson, 1988). Ingólfson (1995) recorded infauna of seaweed clumps around Iceland and suggested that rafting may enable dispersal of many intertidal species over distances of hundreds of kilometres or more. For some species of gastropod this may be the only method of colonisation (Ingólfson, 1998). In addition to rafting, other suggested dispersal methods for species such as *N. lapillus* include a hitherto unknown pelagic stage achieved due to a small amount of air trapped within the juvenile shell, although no evidence for this has been presented (Adachi and Wada, 1999). In addition, Scheltema (1989) suggested that adult non-planktonic species such as *N. lapillus* could colonise by crawling from source locations.

A novel method of gastropod dispersal was described by Martel and Chia (1991a,b) who found that directly developing *Lacuna* species dispersed by raising the majority of their foot and accumulating mucus at the unattached area. Water currents then carried the mucus thread and gastropod away. The thread allowed re-attachment when a suitable substrate was encountered. No observations of these methods have been seen in *N. lapillus*. Whether dispersal occurs via largely unpredictable physical processes, such as storms (e.g. Dobbs and Vozarik, 1983), or is restricted to specific age groups such as new hatchlings or juveniles is currently unknown.

On the south coast of the UK improvement in imposex levels has been less apparent in *N. lapillus* populations than elsewhere (Bray and Herbert, 1998; Herbert *et al.,* 2000; Birchenough *et al.,* 2002b). As recently as 1998 reports stated that *N. lapillus* was absent from several formerly occupied south coast habitats (Langston *et al.,* 1998; CONNSSO, 1998). Conversely, the colonisation of man-made habitats (e.g. Moore, 1939, 1940; Bray and Herbert, 1998; Herbert *et al.,* 2000) and recolonisation of sites formerly polluted by TBT (Bray and Herbert, 1998, Crothers, 1998, 2003; Herbert *et al.,* 2000, Birchenough *et al.,* 2002b) has occurred on some south coast shores. Considering the recovery of populations recorded in the UK and Europe (Spence *et al.,* 1990; Harding *et al.,* 1997; Følsvik *et al.,* 1999; Miller *et al,* 1999; Santos *et al.,* 2000, 2002; Hawkins *et al.,* 2002) the ability of *N. lapillus* to disperse and move between sites and habitats may be greater than suggested (e.g. Crothers, 1985).

Man-made sites provide an opportunity to investigate *N. lapillus* dispersal abilities. They may also be important stepping-stones aiding colonisation from less contaminated areas to sites formerly heavily impacted by TBT. A set of rock groynes at Highcliffe in Christchurch Bay, Dorset was constructed in the early 1990s consisting of alternate long and short structures. By 1998 each long groyne had been colonised by populations of *N. lapillus*. As no dogwhelks were reported in the area prior to the groyne construction (Pegoraro, pers. com. 2002), this was an opportunity to manipulate the discrete populations at Highcliffe.

The main aim of this chapter was to investigate recolonisation by *N. lapillus* on manipulated rock groynes at Highcliffe in Dorset. The alternate long groynes were cleared of *N. lapillus* thus enabling investigation of movement and recolonisation patterns on to and between the structures. The specific aims of this study were:

1. To identify age and size groups recolonising suitable habitats,
2. To indicate which areas on the groynes were the main site of arrival,
3. To establish whether colonising cohorts remained at the arrival point or redistributed within and between the groynes,
4. To determine whether colonisation or movement patterns have any relation to weather or hydrodynamic forcing,
5. To propose recolonisation mechanisms of contaminated sites from the patterns observed.

Particular age or size groups dominating the colonisation process can thus be identified and the related physical factors and processes explored.

4.2 Methods

4.2.1 Site description

A site of limestone block groynes (Fig. 4.1, grid ref SZ 213 930), at Highcliffe Beach in Dorset was chosen for both recolonisation (this chapter) and community studies (Chapter 6, see Appendix 2). The geology of the cliffs at Highcliffe consists of clays and gravels (Burton, 1925) and suffers from severe wave erosion. Prior to the construction of rock groynes Christchurch Bay including Highcliffe, consisted largely of fine sand and gravel with remnant wooden groynes. The high erosion rate led to Christchurch Council commissioning gravel injection and the construction of the current groynes at Highcliffe in the early 1990s (Tyehurst, 1994). Long groynes (H0, H2, H4, H6, H8, H10, Fig. 4.1D) were approximately 160 m apart and 60-70 m in length. In between the longer groynes there were shorter (25 m) structures (H1, H3, H5, H7, H9, Fig. 4.1D) which were not used in this research.

The natural sand and gravel habitat at Highcliffe was previously unsuitable for sessile taxa. The gravel injection provided a more varied substrate, but was still too mobile for sessile or slow moving organisms. From 1992 the groyne construction provided a habitat suitable for rocky shore communities. Following a pilot site visit in 1998 it was found that each longer groyne supported a lower eulittoral to supralittoral exposed to moderately exposed low diversity rocky shore community including *N. lapillus* populations of varying abundance.

Further investigations showed that the shorter groynes were too far up the beach to support a typical eulittoral community and only green algae and small numbers of limpets were present. The pilot survey also showed that many of the Highcliffe *N. lapillus* were large morphs with thin shells (see also Crothers, 1998, 2003).

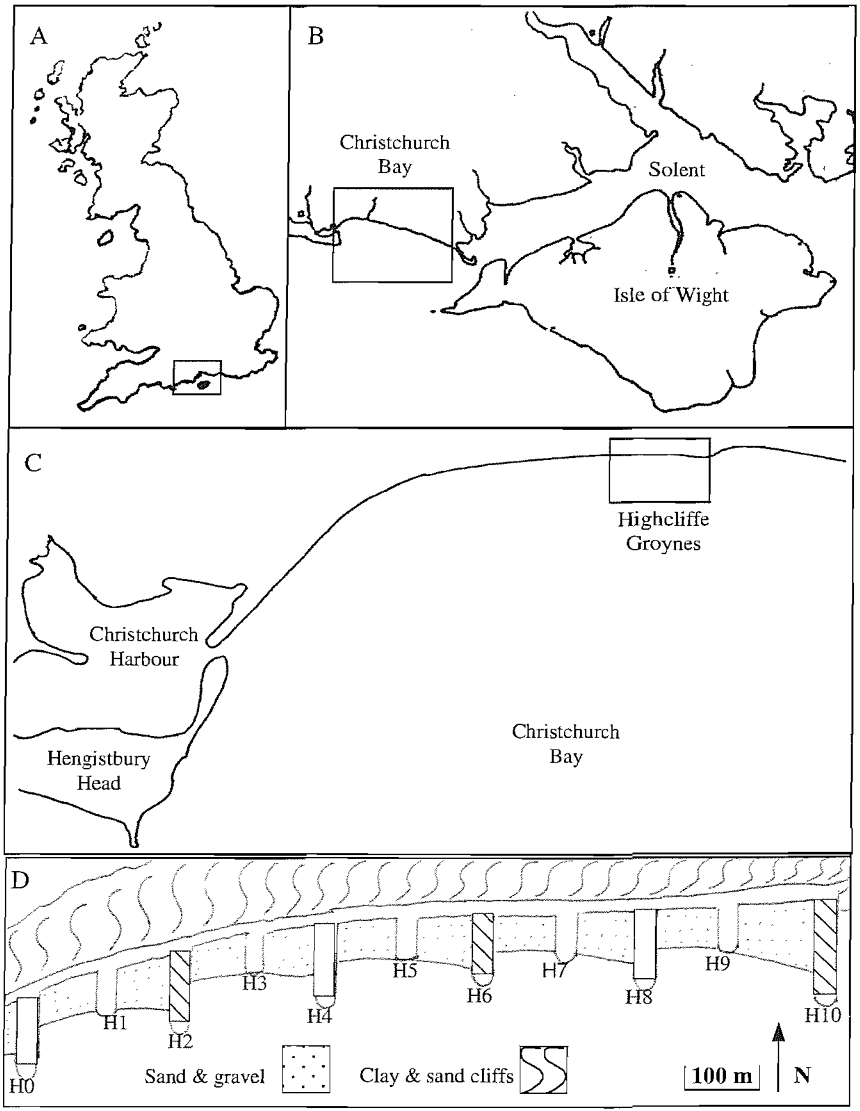

FIGURE 4.1 HIGHCLIFFE STUDY SITE A:UK, B: SOLENT AND ISLE OF WIGHT, C: CHRISTCHURCH BAY, D: HIGHCLIFFE GROYNES. ☐ H0, H4 and H8 *Nucella lapillus* populations removed. ▧ H2, H6 and H10 *Nucella lapillus* populations left in place. Odd numbered groynes were not used in experiments due to their short length and absence of an intertidal community.

4.2.2 Materials and experimental design

The recolonisation experiment at Highcliffe was undertaken concurrently with work on the role of *N. lapillus* structuring rocky shore communities (Chapter 6). A baseline survey and pilot study of the groynes was carried out in May 2001 to establish abundance of *N. lapillus* and the overall composition of community. Groynes were marked into three zones 10 m long on the east (A-C) and west (D-F) groyne faces (Fig. 4.2A). Laterally, these zones were kept spatially separate using 2 m spaces (Fig. 4.2A,B). Zones were marked with Hammerite (^{TM}, ICI PLC) paint, which was renewed monthly. All marked areas and points were recorded with digital photographs to provide a reference. The zone of each groyne was subdivided vertically into three 1 m levels with a 0.5 m exclusion area between them to ensure discrete zones (Fig. 4.2B). The ends of all groynes except H0 (Fig. 4.1D) were not used, as generally the small tidal range at Highcliffe precludes access. Groyne H0 is 50 m long and to ensure spatial comparability the design required the use of its whole length. The end of H0 is accessible at all spring tides regardless of magnitude. Where survey zones A and D (see Fig. 4.2A) abutted on H0, a 1 m exclusion area was left to ensure independence.

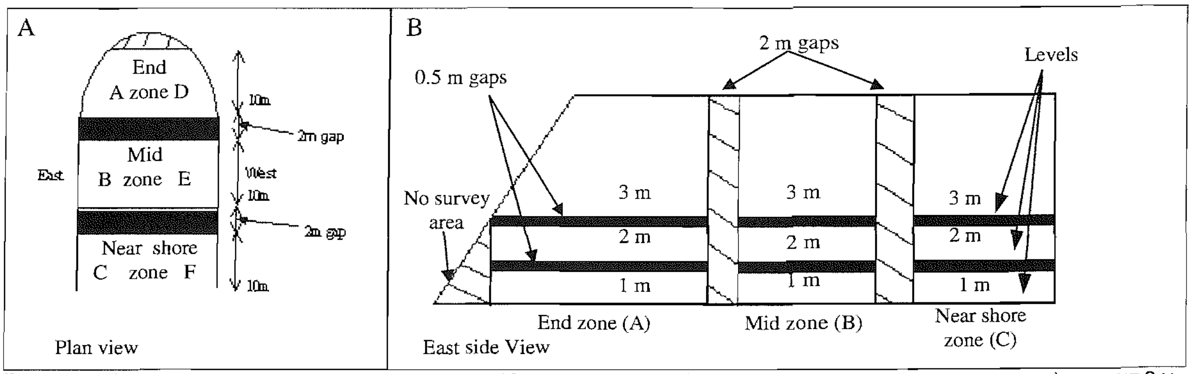

FIGURE 4.2 A: PLAN VIEW OF A LONG GROYNE SHOWING 10 M SURVEY ZONES (END ZONE, MID ZONE, NEAR SHORE ZONE) AND THE 2 M GAPS LEFT BETWEEN THEM TO ENSURE SPATIAL INDEPENDENCE. 4.2 B: SIDE VIEW OF A LONG GROYNE SHOWING THE 1 M LEVELS THAT ZONES WERE DIVIDED INTO AND THE 0.5 M GAPS BETWEEN THEM.
A-F on the plan view show east (A-C) and west (D-F) zones. Numbers 1-3 within each zone on the side view show the levels within zones 0-1 m. 1-2 m and 2-3 m above the sand for both groyne faces. (Not to scale).

The groynes were surveyed for dimensions and topography (length of intertidal on each face, base and crest widths, height and the angle of the east and west groyne faces). The *N. lapillus* population at Highcliffe had abundant large morphs. Therefore individuals were assigned to size classes based on total length of: 0-15 mm (juvenile), 16-25 mm (second year), 26-35 mm (small adults) and over 35 mm (large adults). Size was estimated visually using a 1 mm gauge as disturbance from physical measurement may have led to dislodgement by the incoming tide thus interfering with recolonisation patterns.

After the baseline survey for the recolonisation and community (Chapter 6) experiments, groynes H0, H4 and H8 were designated as treatments (Fig. 4.1D). These groynes, including the spaces between zones and levels, were cleared of *N. lapillus* and their egg masses; eggs were removed throughout the experiment. Subsequent clearances were also carried out in June, July and twice in August, until on the final visit (20/08/01) no individuals were visible. The abundance of each age class and total numbers removed from each groyne were recorded. The groynes had some inaccessible areas thus making it unlikely that all *N. lapillus* were removed. All cleared animals were transplanted to sea defences at Barton on Sea (1.5 km east) in the direction of prevailing longshore drift. Groynes H2, H6 and H10 were designated as controls (Fig. 4.1D) and surveyed using the same methods as treatments throughout the experiment. Dogwhelk numbers were noted at these sites to allow later comparison with treatments. After the clearance work dogwhelks were allowed to recolonise the treatment (H0, H4, H8) groynes naturally although they were cleared from blocks within zones being used for the community experiment (Chapter 6) and replaced within the groyne.

Following the clearance work, the surveying of recolonisation patterns began in September 2001. Observations were made of recolonisation rates monthly, weather permitting, until October 2002. Following a large storm an additional survey was made in September 2001. To record the abundance of recolonising *N. lapillus* in the four size classes, three replicate 1-minute timed searches were made at each level in all of the zones (Fig. 4.2A,B). Weather conditions were noted and measurements of wave energy during the sampling period obtained (source: New Forest District Council, 2003). Spring tides and storms prevented sampling in November 2001, and February and May 2002.

The concurrent experiment on the influence of predation by *N. lapillus* on community structure (Chapter 6) required that 4 blocks within each area on the east side of treatment groynes (zones 1, 2 and 3 on groynes H0, H4 and H8, Fig. 4.2A,B and Fig. 4.1D) were kept clear of recolonising dogwhelks. Where *N. lapillus* individuals were found on these blocks during timed searches to study recolonisation they were removed, but immediately replaced on a block that was not part of the community experiment.

Baseline (May 2001) and recolonisation data (from September 1st 2001) were analysed using a three-way ANOVA in SigmaStat for the factors groyne, zone, and level. Age groups and months were analysed separately as they were not independent. Where pre ANOVA heterogeneity of variance and normality tests were failed, data were square root transformed (Thacker and Bromley, 2001). When transformations were unsuccessful raw data were analysed. It has been suggested (Box, 1953; Underwood, 1981) that ANOVA may be more valid than many tests assuming homogeneity of variance and that the test can still be run if there are sufficient data with a balanced design. Thus where the pre ANOVA tests failed after transformation significance was only accepted at $P<0.01$ (Prescott, pers. com. 2004).

4.3 Results

4.3.1 Baseline conditions and the effectiveness of recolonisation

Before manipulation of the treatment sites, the abundance of *N. lapillus* was assessed on treatment and control groynes in May 2001. The mean abundance of *N. lapillus* on the treatment groynes (H0, H4, H8) was three times higher than on the controls (H2, H6, H10) (Fig. 4.3). In comparison by October 2002 mean *N. lapillus* abundance on the treatments had been reduced to 10% of the original population whereas control sites still had 47% of those found in May 2001 (Fig.4.3).

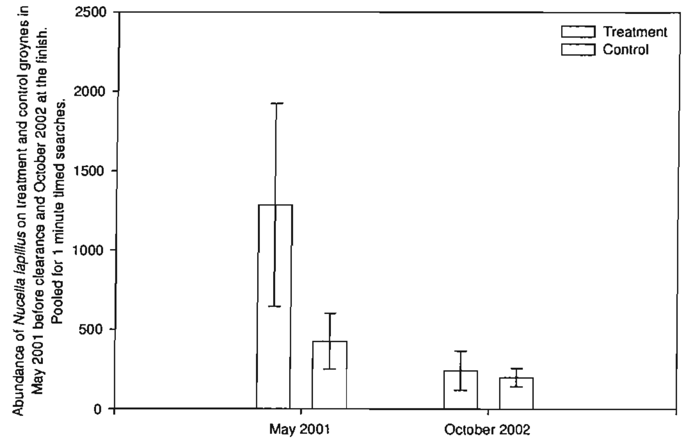

FIGURE 4.3 POOLED MEAN ABUNDANCE (+/- 1SE) OF *NUCELLA LAPILLUS* ON TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H10) GROYNES IN MAY 2001 BEFORE CLEARANCE AND IN OCTOBER 2002 AT THE FINISH OF SURVEYS.

Of the treatment groynes initially H4 and H8 had the highest dogwhelk abundance (Fig. 4.4). By October 2002 H4 and H8 had similar abundances, but on H0 none were found. On the control groynes H2, and H6, *N. lapillus* declined naturally, although on H10 abundance increased by 66% from 104 in May 2001 to 173 in October 2002 (Fig. 4.4).

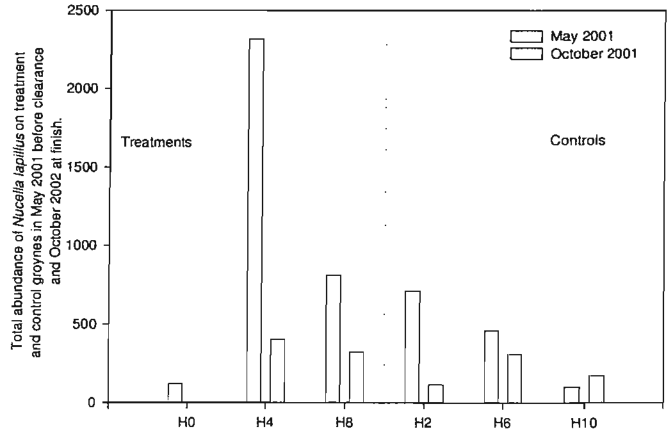

FIGURE 4.4. TOTAL ABUNDANCE OF *NUCELLA LAPILLUS* ON EACH TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H10) GROYNES IN MAY 2001 BEFORE CLEARANCE WORK AND IN OCTOBER 2002 AT THE FINISH OF SURVEYS.

From the results of May 2001 surveys the distributions of the dogwhelk size classes (§ 4.2.2) were assessed in relation to preferred zone and level (Fig. 4.2). A three-way ANOVA (§ 4.2.2) for the factors groyne, zone and level was carried out. Significance for the test was set (P<0.01) as all data failed normality in raw form and when transformed (§ 4.2.2). Tests showed that juveniles and second years were similarly distributed on the groynes (Fig. 4.5A,B). Between groyne comparisons were significantly different (juveniles: $F_{(5,323)}$, 37.9 P<0.001; second years: $F_{(5,323)}$, 73.1 P<0.001). *Post hoc* Tukey's tests showed that the abundance of both these age classes was greater on treatment groyne H4, due to their numbers on the east face groyne end zone at 1 m being greater than all the other sites (P<0.001, Fig. 4.5A,B). Comparisons within groyne H4 showed that in addition to their high abundance in the east face end zone, second years were significantly more abundant on the west face at 1m in the end zone than the mid and near shore zones (P<0.01, Fig. 4.5B).

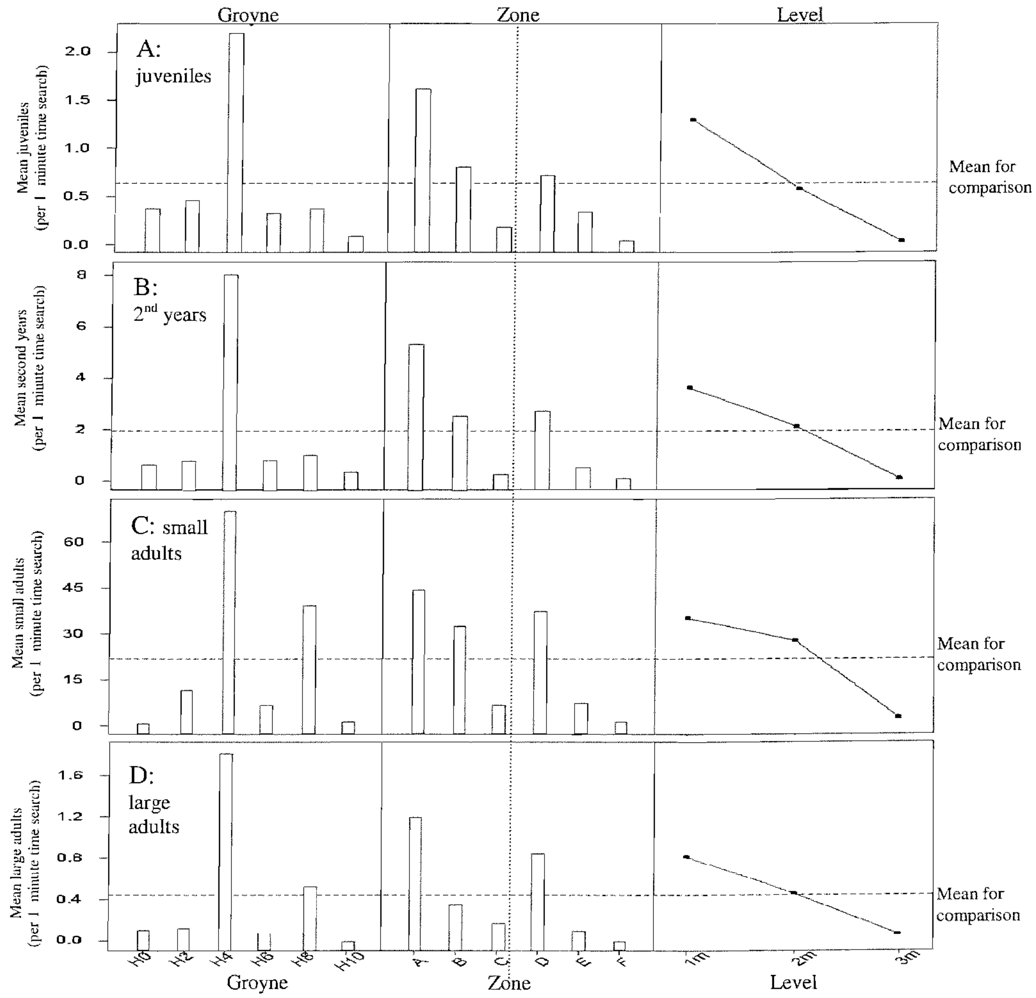

FIGURE 4.5 COMPARISONS OF *NUCELLA LAPILLUS* ABUNDANCES PER 1 MINUTE TIMED SEARCH (3 REPLICATES) IN GROYNES, ZONES AND LEVELS IN MAY 2001
Groynes: H0, H4, H8 treatment, H2, H6, H8 control. Zones abundances pooled: A end zone east face, B mid zone east face, C near shore zone east face, D end zone west face. E mid zone west face, F near shore zone west face. Level abundances pooled at 1 m, 2 m, 3 m within each zone. Note different y scales.

Tests showed that the abundances of small (26-35 mm) and large (>35 mm) adults were significantly different between groynes (small adults: $F_{(5,\ 323)}$, 68.1 P<0.001; large adults: $F_{(5,323)}$, 38.5, P<0.001). On H4 small adults were significantly more abundant than on all the other groynes (P<0.001). Groyne H8 had the second highest abundance which, excluding H4 was greater than all the other treatment (H0) and control (H2, H6, H10) groynes (P<0.01, Fig. 4.5C). *Post hoc* tests on large adult abundances showed that only the population on H4 was significantly greater (P<0<001, Fig. 4.5D). Within H4 both adult classes were more abundant at 1 m on the east face end and mid zones (P<0.01) whereas on H8 their abundances were greatest at 2 m on the east and west groyne end zones (P<0.01).

Clearance work (Fig. 4.6) highlighted that small adults were the dominant age class (Table 4.1) which was also found on the controls in May 2001. The small numbers of *N. lapillus* on groyne H0 (Fig. 4.4) were effectively cleared (Fig. 4.6A). Total *N. lapillus* numbers on H8 declined steadily in response to repeated removals (Fig. 4.6C). On H4 large numbers of *N. lapillus* were cleared in May, with a further 500 being removed in June (Fig. 4.6B). By July, however, numbers had increased, possibly due to animals moving from refuges. After the two visits in August 2001 the population on H4 was clearly reduced to no visible dogwhelks at the start of the experiment (Fig. 4.6B).

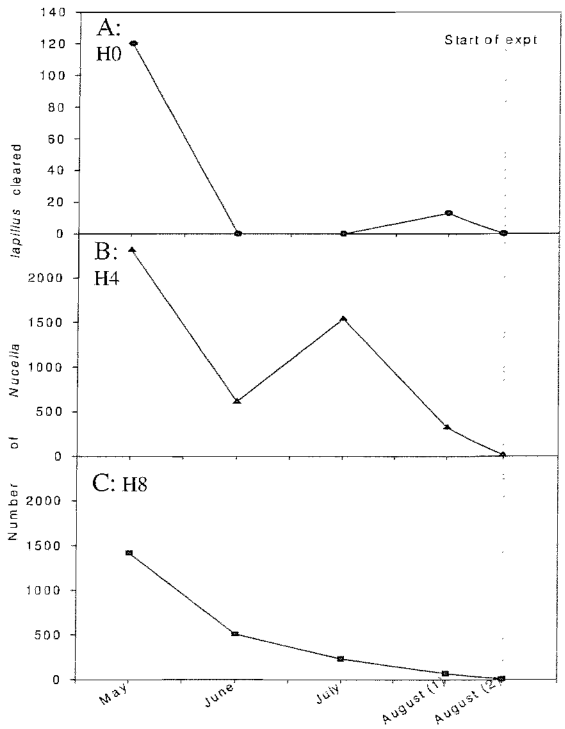

FIGURE 4.6 TOTAL *NUCELLA LAPILLUS* CLEARED FROM TREATMENT GROYNES ON EACH VISIT IN MAY, JUNE, JULY AND TWICE IN AUGUST 2001.
Note independent y axes.

TABLE 4.1 TOTAL SIZE CLASS ABUNDANCES CLEARED FROM ON THE TREATMENT GROYNES (H0, H4, H8) BETWEEN MAY AND AUGUST 2001 AND THEIR CORRESPONDING ABUNDANCES ON CONTROL GROYNES (H2, H6, H10) GROYNES IN MAY 2001.

Age class/Groyne	H0	H4	H8	H2	H6	H10
Juveniles	20	118	22	25	17	5
Second years	35	439	57	33	47	23
Small adults	71	3816	2125	646	392	76
Large adults	7	99	28	7	4	0

The first experimental survey on the 1st September 2001 (Fig. 4.7) showed that the removal of *N. lapillus* had initially been successful with abundance remaining low on all treatment groynes. Natural fluctuations, however, resulted in decline of dogwhelks on control sites. An opportunistic survey was carried out on the 17th of September (Fig. 4.7) following an equinoctial gale, which showed a pulse of increased *N. lapillus* abundance. *N. lapillus* were found on all of the treatment groynes either due to recruitment or exchange between treatment and control groynes, or movement within treatment groynes of individuals missed during clearance work.

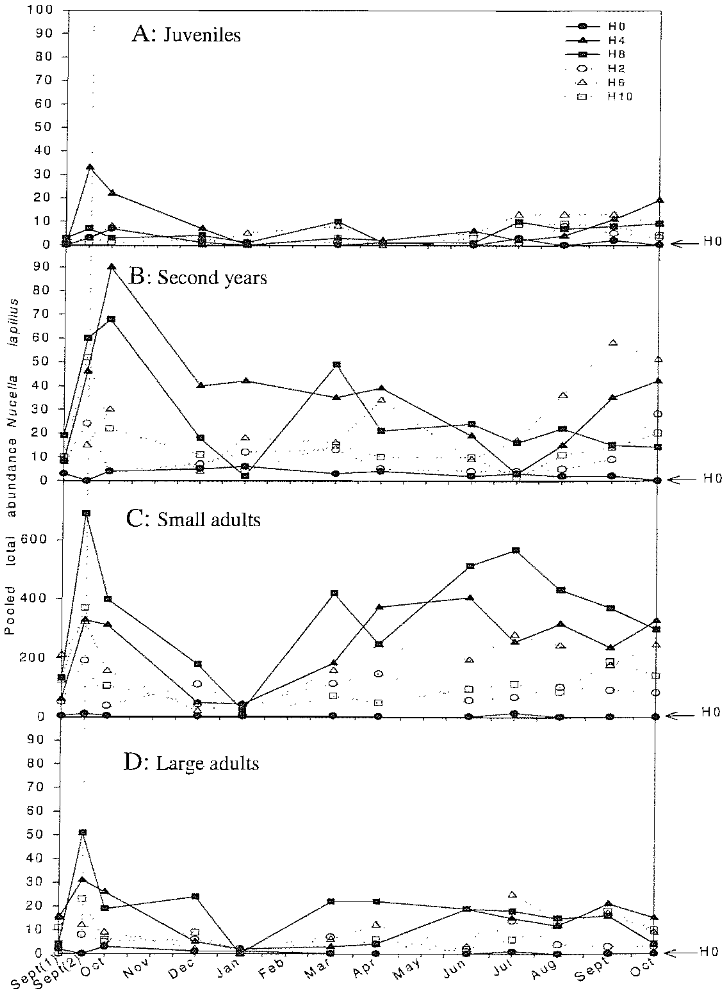

FIGURE 4.7 TOTAL ABUNDANCE OF *NUCELLA LAPILLUS* FOR EACH AGE CLASS ON TREATMENT (H0, H4, H8) AND CONTROL GROYNES (H2, H6, H10) POOLING DATA FROM EAST AND WEST GROYNE FACES, ZONES AND LEVELS FROM SEPTEMBER 2001-OCTOBER 2002. Solid symbols: treatment, open symbols: control. Note different y-axis for small adults (C). Note 2 surveys in September 2001, Sept (1) 1st Sept (1). Sept (2) 17th Sept after storms.

Juvenile abundance recorded on the 1st September 2001 was low (Fig. 4.7A), but after the storm there was a clear increase on treatment groyne H4 which declined slightly after a second gale in October 2001. By December 2001 juvenile abundance on groyne H4 was similar to other groynes. Juveniles increased on H8 in March 2002 and, similarly to H4, numbers rose in the mid to late summer of 2002. On treatment groyne H0 there were occasional increases in juveniles, particularly after the September and October storms in 2001. On control groynes (H2, H6, H10) the only noticeable change in juvenile abundance was on groyne H6 where increases coincided with those on H8 (Fig. 4.7A).

Second year dogwhelks were relatively common but, like juveniles, declined in winter 2001. On groyne H0 they were absent on September 17th, but were found again in October 2001. They remained scarce, but present until October 2002. On treatment groynes H4 and H8 increases in second years occurred after storms in September and October 2001 (Fig. 4.7B). Subsequently they declined on groyne H4, partly due to reconstruction work in December 2001, although dogwhelks were most common in the undisturbed end zone on this groyne. On H8 second years were not apparent initially, possibly sheltering over the winter, then after a brief increase in April 2002 they declined to a relatively constant level. Similar patterns were seen on control groynes, but to a lesser extent. As with juveniles, recruitment or movement of second years from sheltered areas on H6 was seen, with increases in April and late summer 2002. On control groyne H10 second years were abundant on September 17th, but by December had reduced to 20% of previous numbers, remaining uncommon until recruitment (also seen on control groyne H2) in October 2002 (Fig. 4.7B).

The relative proportions of both adult size classes showed similar patterns of habitat preference on the groynes (Fig. 4.7C,D), but large adults were considerably less abundant than small adults, e.g. on H8 the large adult total was 7% of the small adults. As with second years, both small and large adults were rare, but never completely absent on treatment groynes. After the storm in mid September 2001 the abundance of small adults rose on treatment groyne H8, and to a lesser extent on groyne H4 and all of the control groynes (H2, H6, H10). On treatment groyne H0 both adult classes were rare, although slight increases were seen on September 17th 2001 and in July 2002, coincident with the increasing juveniles (Fig. 4.7A). On all of the groynes, with the exception of H4, small and large adults sheltered through the winter resulting in reduced numbers. After winter, abundances fluctuated more on treatment sites H4 and H8 and to a lesser extent control groyne H6 (Fig. 4.7C,D).

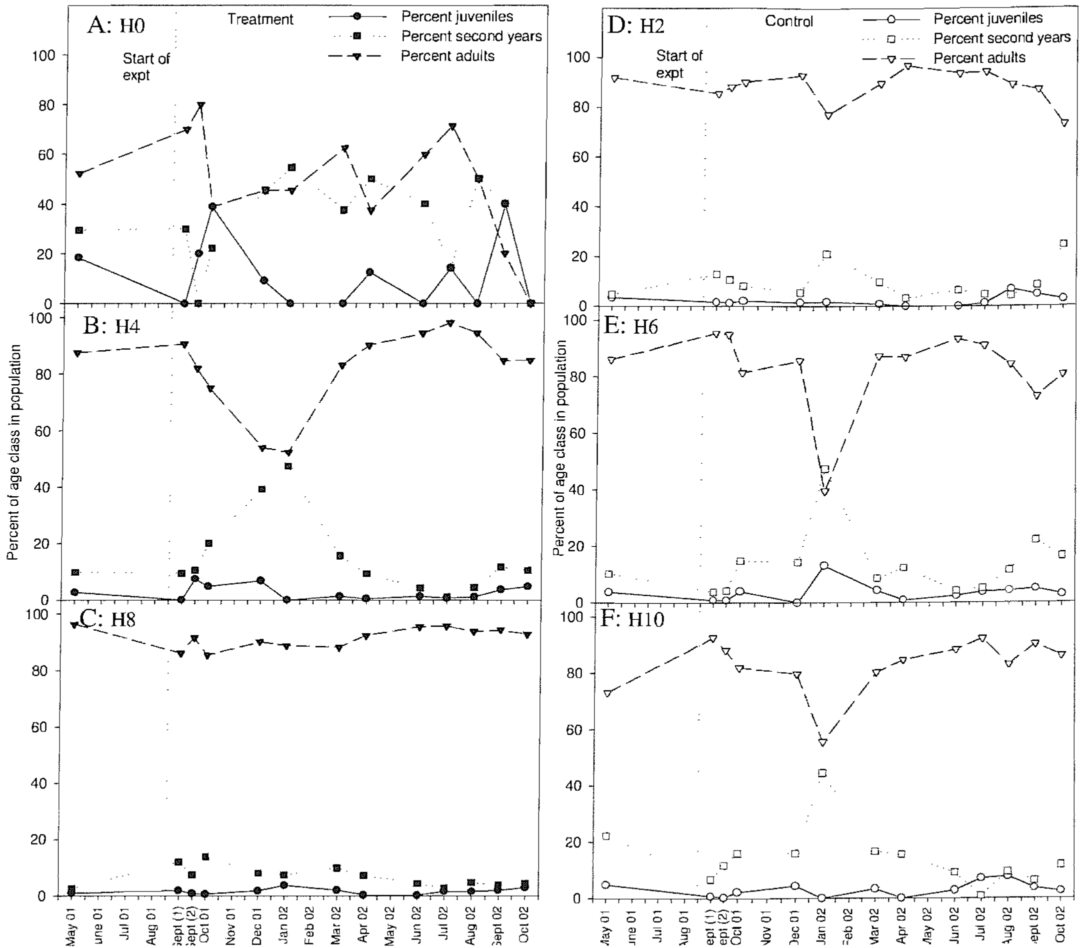

FIGURE 4.8 PERCENTAGE ABUNDANCE OF *NUCELLA LAPILLUS* ADULTS, SECOND YEARS AND JUVENILES IN MAY 2001 (BEFORE CLEARANCE) TO OCTOBER 2002 ON TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H10) GROYNES.
Solid symbols: treatment, open symbols: control.

The proportions of juveniles, second years and total adults (small and large morphs) are shown in figure 4.8. On treatment groyne H0 the proportions of the three age classes fluctuated markedly after clearance (Fig. 4.8A). Juveniles did not follow the pattern of variation seen on the control sites (Fig. 4.8D,E,F). The abundance of second years and adults showed no clear pattern. On treatment groyne H4, although cleared of all *N. lapillus*, the proportions of the three age groups behaved similarly to the controls (Fig. 4.8B). The percentage of adults declined from September 2001 as numbers of second years increased. A small pulse of juveniles was also observed. As smaller age classes declined, adults increased again by spring 2002 and to a lesser extent in September 2002. Adults on treatment groyne H8 resurged by September 17th (Fig. 4.7C,D) and the population did not show the same recruitment patterns seen on groyne H4 and the control sites (Fig. 4.8C). This may have reflected the level of disturbance associated with the experimental clearances.

4.3.2 Patterns of movement and dispersal on treatment and control groynes

Abundance was low on the east face of treatment groyne H0 from September 17th onwards (Fig. 4.9A, which is arranged by groyne location rather than treatment and controls) with all size classes being found generally in the end zones at 1 m (Table 4.2, Fig. 1A, Appendix 2). Dogwhelks were also occasionally found at 2 m in the mid zone (Figs. 1G and 3A, Appendix 2). Those found on the east face may have been uncleared remnants of the original population, but were absent by September 2002. *N. lapillus* were present on the west face of H0 from the experiment start (1/09/01), although their abundance fluctuated (Fig. 4.9B) often with isolated second years and juveniles found on blocks in the end zone (Table 4.2, Fig. 2, Appendix 2). On H0 small adults were not the most abundant age class after clearance.

On the other groynes, irrespective of being treatments or controls, patterns of dogwhelk abundance on the east and west groyne faces up to and including H8 (treatment, Fig. 4.1), did not clearly differ. The removals reduced *N. lapillus* populations on H4 and H8 to a similar level as that on H6 (control), whereas abundance on H2 (control) remained consistently low on the east face and declined slightly on the west (Fig. 4.9C,D, Table 4.2, Fig. 2H, Appendix 2). There was not a noticeable recruitment from the populations on the east faces of H2 or H6 on to west faces of H4 or H8 due to longshore drift. Winter fluctuations did occur on these groynes, particularly on H6 and H8 (Fig. 4.9G-J), which were slightly more pronounced on west faces.

Control groyne H10 was the last groyne in the system, populations on which were highly variable. Although proportions showed broadly similar patterns to the other undisturbed control sites, east face abundances declined with losses in winter 2001-2002 (Fig. 4.9K). On the west face, however, although abundances were consistently low towards the end of sampling, small numbers of all the age classes were found due to recruitment in isolated areas with limited prey and long emersion times (e.g. Fig. 6F, Appendix 2) though these may have been dispersed from within the groyne (e.g. Figs. 4F and 6D, Appendix 2).

Abundance changes within populations appeared to be driven by seasonality and breeding patterns. In addition, numbers on the treatment groynes remained relatively high, thus possibly masking fine scale patterns of movement or dispersal between or onto them. Three-way ANOVA (§ 4.2.2) analyses of the results were carried out to discern any dogwhelk age class related patterns in movement on to or between the groynes (Table 4.3A,B).

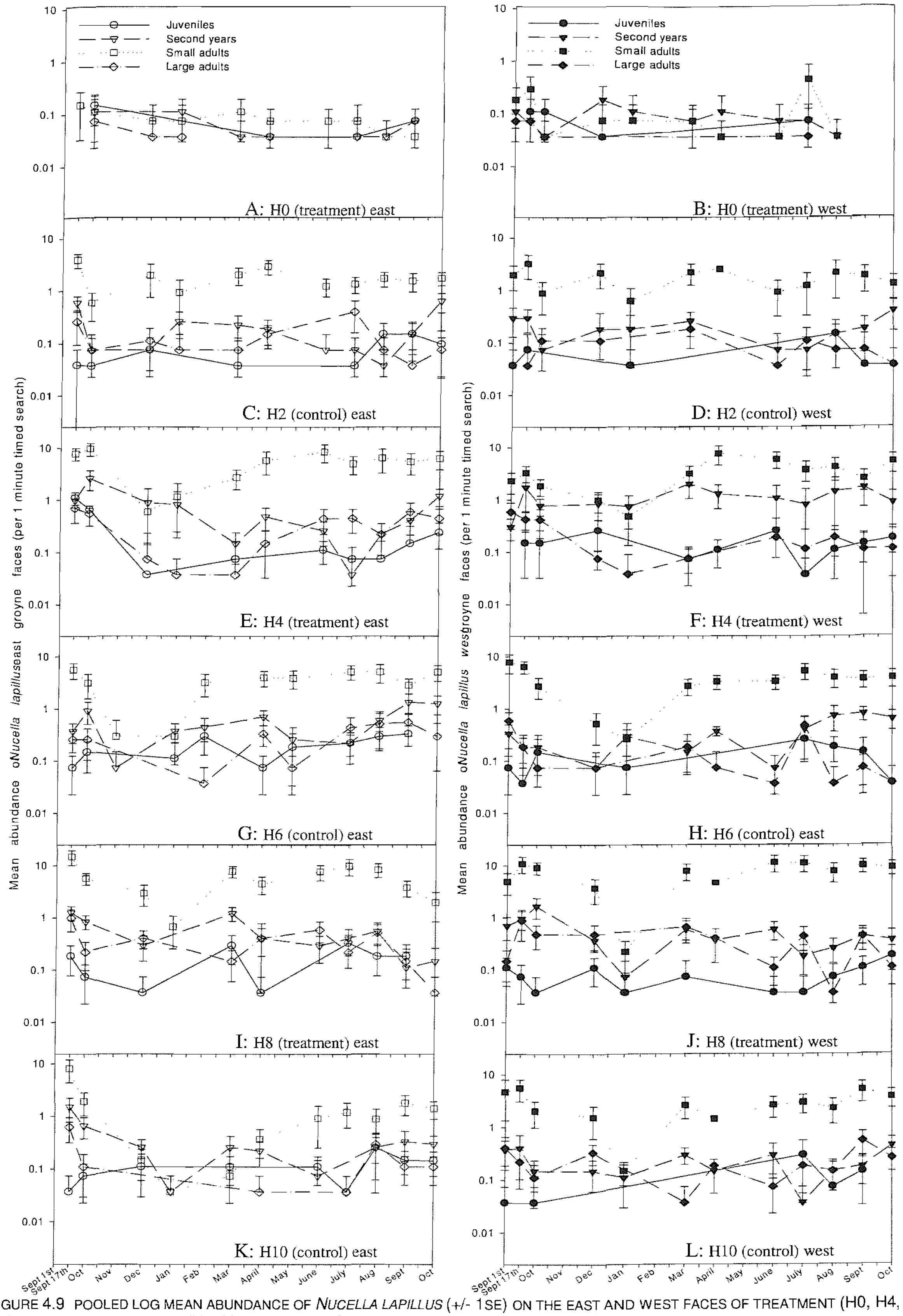

FIGURE 4.9 POOLED LOG MEAN ABUNDANCE OF *NUCELLA LAPILLUS* (+/- 1SE) ON THE EAST AND WEST FACES OF TREATMENT (H0, H4, H8) AND CONTROL GROYNES (H2, H6, H10) FROM SEPTEMBER 2001-OCTOBER 2002.
Figure indicates the interspersed design with groyne results presented in the order of their location from west to east on Highcliffe beach. H0 furthest west then alternate treatment and control groynes. Note two surveys in Sept 2001.

TABLE 4.2 SUMMARIES OF THE KEY MOVEMENTS AND ABUNDANCE OF *NUCELLA LAPILLUS* ON THE EAST AND WEST FACES OF TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H10) GROYNES IN THE END, MID SHORE AND NEAR SHORE ZONES AT 1, 2 AND 3 M LEVELS.

See Figure 4.2 for key to zones and levels.

H0 (treatment)	**H0 whole groyne**	**H0 east face**	**H0 west face**
Summary	Low population in May 2001. Erratic recolonisation of space many arrivals of 2^{nd} years and juveniles.	All age classes rare and only to mid zone mainly at 1m. Rare at 2 m. None in near shore or at 3 m	All age classes rare. But occasional 2^{nd} years and juveniles on bare blocks possibly passive transport. Rarely at 2 m. Never in mid or near shore zones or 3 m.
H2 (control)	**H2 whole groyne**	**H2 east face**	**H2 west face**
Summary	Natural fluctuation in summer 2001. Population abundance remained low throughout.	Low abundance maintained on east face at 1m. Arrival of adults at 2m April 2002. Constant low abundance maintained. One individual at 3 m after Sept 2001 gales. None mid or near shore at 3 m	Slow decline west face at 1m more pronounced at 2 m. None on west face near shore. None at 3 m mid or near shore.
H4 (treatment)	**H4 whole groyne**	**H4 east face**	**H4 west face**
Summary	Recovery after clearance following storm; probably internal. Reconstruction in December 2001 caused loss of community with low reestablishment of *N. lapillus* on east face, more returned to west.	None found in first survey on east face after clearance but abundant after storm, 1 at 3 m end zone. Slow recolonisation of east face after reconstruction in mid zone but few present at 1m March 2002 onwards. None in near shore zone as no prey from December 2001.	Dogwhelks present on west face in first survey at 2 m then small adults increased rapidly on west face at 1m. More rapid reestablishment in mid zone than on east face. None in near shore after engineering.
H6 (control)	**H6 whole groyne**	**H6 east face**	**H6 west face**
Summary	Similarly to H2 with natural loss in summer 2001. Variable recruitment but no apparent increase after construction work disturbance on H4 in winter 2001.	Populations relatively constant though naturally declined in summer 2001. One small adult at 3 m end zone after September 2001 storm. Slight decline at 1m but may have moved up to 2 m from April 2002. Low small adult and 2^{nd} year abundance at 1 m on east face. None at 3m mid or near shore.	West face variable population changes but no signs of loss to others sites. One small adult found at 3 m end zone after Sept 2001 storm. 2^{nd} years and juveniles increased July 2002 onwards. Mid zone populations constant. Occasional recruitment patterns similar to H8. None at 3 m mid and near shore.
H8 (treatment)	**H8 whole groyne**	**H8 east face**	**H8 west face**
Summary	Though cleared, large resurgence after September storms. Often more common at 2 m due to scour on west face, smothering on east.	East face recolonised after Sept gale at all levels, but mainly at 2 m. East face abundance variable and resurged in March 2002. Disturbance in August-September 2002 caused large-scale losses in mid and near shore. None at 3 m mid or near shore.	On west face present from September 1^{st} 2001. One individual found at 3 m after September storm. West face abundance higher at 2m possibly due to scour. Recruitment patterns suggest 2^{nd} years dominant.
H10 (control)	**H10 whole groyne**	**H10 east face**	**H10 west face**
Summary	Population increased sharply after September 2001 gales, then declined again. Some recruitment of 2^{nd} years and adults by late 2002. H10 seems to have arrivals on west face in areas where no food was available. Occasional at 3m in end zone.	Abundant *N. lapillus* found at 1 and 2m east side after September 2001 gale, but declined by October. All gone from east face 1m by August 2002 possible migration up groyne face to 2 m where small adults were found from July 2002. East face 1m small population resident on *M. edulis* patch. Occasional at 2 m. Occasional small adults at 3 m end zone, none in mid or near shore.	Lower numbers persisted mainly at 2 m. At 2m juveniles first seen August 2002. Occasional small adults end zone. Mid zone at 1 m small populations declined and absent by July 2002, but may have moved up the groyne where abundance increased. Near shore zone small adults present at 1 m, but gone by September 2002 then found at 2 m in September 2002 possibly migration up shore, but total abundance increased and no prey at this site; possible passive transport.

The apparent success of clearance work was seen in the September 1^{st} 2001 survey as small adult *N. lapillus* abundance on H6 (control) was significantly greater than that on treatment groyne H0 (Table 4.3; Fig. 4.7C). Between zone and between level comparisons, however, showed that treatment sites (H4, H8) still had significantly higher small adult and second year abundances at 2 m. At this stage movement on to or colonisation of open spaces after clearance work was not seen.

Table 4.3a Summary of ANOVA and *post hoc* tests of significant differences in numbers of juvenile, second year, small and large adult *Nucella lapillus* in the zones and levels on treatment (H0, H4, H8) and control (H2, H6, H10) groynes between September 1st 2001 and April 24th 2002.
EZ: end zone, MZ: mid zone, NSZ: near shore zone NS: Not significant. *: P<0.01. **: P<0.001. Juv: juvenile, 2nd yr: second years, Sm Ad: small adults, L Ad: large adults (T): Treatment, (C): control.

Survey date	Age class	Significant groyne effect	Zone and level Tukey's tests on abundance increases					
			H0 (T)	H4 (T)	H8 (T)	H2 (C)	H6 (C)	H10 (C)
1/09/01	Juv	NS	NS	NS	**West MZ 2m (*)**	**East EZ 1m (*)**	**East EZ 1m (*)**	**East EZ 1m (*)**
	2nd Yr	NS	NS	NS	**West MZ 2m (**)**	NS	**West MZ 2m (**)**	NS
	Sm Ad	**(F$_{5,323}$), 3.2, P<0.01) H6>H0 (*)**	NS	**West EZ 2m (*)**	NS	NS	**West MZ 2m (*)**	NS
	L Ad	NS	NS	**West EZ 2m (**1)**	NS	NS	**West MZ 2m (*)**	NS
17/09/01 Table 4.3 for main effects and interactions	Juv	**H4>all (**)**	NS	**East EZ, MZ, NSZ 1m (**)**	NS	NS	NS	NS
	2nd Yr	**H8>H0, H6 (**); H4 & H10> H0 (*)**	NS	**East MZ 1 m (*)**	**East EZ 1m (*) West MZ 1m (*)**	NS	NS	**East EZ 2m (*)**
	Sm Ad	**H8>all (**) H4, H6, H10>H0 (*)**	NS	**East MZ 2m (*)**	**East & west EZ 2m (**) West MZ 1m (**)**	NS	**East & west EZ2m (*) East & west MZ1m (*)**	**East & west EZ 2m(*) East & west MZ 1m(*)**
	L Ad	**H8>H0, H2, H6 (*)**	NS	**East MZ 2m (*)**	**East EZ 2m (*) West EZ 2m (**)**	NS	NS	**East end zone 2m (*)**
14/10/2001	Juv	NS	NS	NS	NS	NS	NS	NS
	2nd Yr	**(F$_{5,323}$), 7.8, P<0.01) H4>H0, H2, H10 (*) H8>H2, H0 (*)**	NS	**East NSZ 1m (*)**	**West EZ 1m (*)**	NS	**East EZ 1m (*)**	
	Sm Ad	NS	NS	NS	NS	NS	NS	NS
	L Ad	NS	NS	NS	NS	NS	NS	NS
26/03/2002 Table 4.4 for main effects and interactions	Juv	NS	NS	NS	NS	NS	NS	NS
	2nd Yr	**H8>H0, H2,H6 H10 (*)**	NS	NS	**West EZ 1m (**) West MZ 2m (**)**	NS	NS	NS
	Sm Ad	**H8>H0, H4, H2,H6 H10 (**) H4>H0(*)**	NS	**East EZ 1m (*) West EZ 1m (*)**	**East EZ 1m & 2m (*) & MZ 2m (*) West EZ 1m (**)**	**West EZ 2m (*)**	**East EZ 1m (**) West EZ zone 1m (*)**	**West EZ 1m (*)**
	L Ad	**H8> H0, H4, H2,H6 H10 (*)**	NS	NS	**West EZ 1m (**)**	**West EZ 1m (*)**	NS	NS
24/04/2002	Juv	NS	NS	NS	NS	NS	NS	NS
	2nd Yr	**(F$_{5,323}$), 4.2, P<0.01) H4>H0, H2, H10**	**2m>1m(*)**	**East EZ 1m (*) West EZ 1m (**)**	NS	NS	NS	NS
	Sm Ad	**(F$_{5,323}$), 7.7, P<0.01) H4>H0, H2,H10 (**) H8>H0 (*)**	**2m>1m(*)**	**East & west EZ 1m (*)**	NS	NS	NS	NS
	L Ad	NS	NS	NS	NS	NS	NS	NS

Table 4.3b Summary of ANOVA and *post hoc* tests of significant differences in numbers of juvenile, second year, small and large adult *Nucella lapillus* in the zones and levels on treatment (H0, H4, H8) and control (H2, H6, H10) groynes between June 20th 2001 and October 7th 2002
EZ: end zone, MZ: mid zone, NSZ: near shore zone NS: Not significant. *: P<0.01. **: P<0.001. Juv: juvenile, 2nd yr: second years, Sm Ad: small adults, L Ad: large adults(T): Treatment, (C): control.

Survey date	Age class	Significant groyne effect	Zone and level Tukey's tests on abundance increases					
			H0 (T)	**H4 (T)**	**H8 (T)**	**H2 (C)**	**H6 (C)**	**H10 (C)**
20/06/2002	Juv	NS	NS	NS	NS	NS	NS	NS
	2nd Yr	NS	NS	NS	NS	NS	NS	NS
	Sm Ad	**($F_{5,323}$), 38.1, P<0.01) H8=H4>H0, H2, H6, H10 (**) H6>H0 (*)**	NS	**East EZ 1m **) West EZ 1m (**)**	**East EZ 1m (**) West EZ 1m (*) M 2m (*)**	NS	**East MZ 1m (*) West EZ 1m (*)**	**West EZ 1m (*)**
	L Ad	**($F_{5,323}$), 7.1, P<0.01) H4=H8>H0, H2,H10 (*)**	NS	**East EZ 2m (*) West EZ 2m (**)**	**East E & MZ 2m (*)**	NS	NS	NS
27/07/2002	Juv	NS	NS	NS	NS	NS	NS	NS
	2nd Yr	NS	NS	NS	NS	NS	NS	NS
	Sm Ad	**($F_{5,323}$), 17.1, P<0.01) H8>H0,H4,H2,H6,H10 (**) H6>H0 (*)**	NS	NS	**East E & MZ 2m (**) West E & MZ 2m (**)**	NS	**East EZ 2m (*)**	NS
	L Ad	NS	NS	NS	NS	NS	NS	NS
14/08/2002	Juv	NS	NS	NS	NS	NS	NS	NS
	2nd Yr	**($F_{5,323}$), 7.22, P<0.01) H6>H0,H2,H10 (*)**	NS	**East EZ 2m (*)**	**East MZ (*)**	NS	**West EZ 2m (**)**	**East EZ 2m(*)**
	Sm Ad	NS	NS	NS	NS	NS	NS	NS
	L Ad	NS	NS	NS	NS	NS	NS	NS
10/09/2002	Juv	NS	NS	NS	NS	NS	NS	NS
	2nd Yr	**($F_{5,323}$), 7.22, P<0.01) H6>H0,H2,H8,H10 (*)**	NS	**West EZ 2m (*)**	NS	NS	**East EZ 1m (*)**	NS
	Sm Ad	**($F_{5,323}$), 11.42, P<0.01) H8>H0,H2,H6 (*)**	NS	**East EZ 1m (*)**	**West EZ 2m (**) West MZ 1m (*)**	NS	**West EZ 1m (**)**	**West EZ 2m(*)**
	L Ad	NS	NS	NS	NS	NS	NS	NS
12/10/2002	Juv	**($F_{5,323}$), 3.5, P<0.01) H4>H0 (*)**	NS	**East EZ 1 m (*)**	NS	NS	NS	NS
	2nd Yr	**($F_{5,323}$), 6.8, P<0.01) H6>H0,H8 (*) H4>H0 (**)**	NS	**East EZ 1m (**)**	NS	NS	**East E & MZ 1m (**)**	NS
	Sm Ad	**($F_{5,323}$), 13.7, P<0.01) H4>H0,H2,H10 (**) H8>H0,H2 (**) H6>H0 (**)**	NS	**East EZ 1m (**) & 2m (*) West EZ 1m (**) & 2m (*)**	NS	NS	**East EZ 2m (**) West EZ 2m (*)**	NS
	L Ad	NS	NS	NS	NS	NS	NS	NS

On September 17th 2001 significant abundance increases were seen after the storm (Fig. 4.7, Table 4.4). *Post hoc* tests showed this occurred on treatment (H4, H8) and control groynes (H6, H10). Abundance increases were seen in all the age classes, mainly on treatment groynes H4 (east zones, Fig. 4.9E) and H8 (east and west zones, Fig. 4.9I,J) (Table 4.3A). The October 2001 survey showed that only second year abundance continued to increase significantly on groynes H4 (east and west, Fig. 4.9E,F) and H8 (west, Fig. 4.9J, Table 4.3A).

TABLE 4.4 THREE-WAY ANOVA COMPARISONS OF *NUCELLA LAPILLUS* ABUNDANCES AT HIGHCLIFFE ON SEPTEMBER 17TH 2001 FOR A: JUVENILES, B: SECOND YEARS, C: SMALL ADULTS AND D: LARGE ADULTS.
See Figure 4.2 for full key to zones and levels.

A	**Juveniles Sept 17th 2001**				
Source	**DF**	**SS**	**MS**	**F**	**P**
Groyne (H0, H2, H4, H6, H10)	5	3.9	2.8	8.6	<0.001
Zone (end, mid, near shore)	5	4.7	0.9	2.9	NS
Level (1m, 2m, 3m).	2	9.2	4.6	14.2	<0.001
Groyne x Zone	25	16.8	0.7	2.1	<0.01
Groyne x Level	10	25.5	2.6	7.9	<0.001
Zone x Level	10	6.2	0.6	1.9	NS
Groyne x Zone x Level	50	43.8	0.9	2.7	<0.001
Residual	216	70.0	0.3		
Total	323	190.3	0.6		
B	**Second years Sept 17th 2001**				
Source	**DF**	**SS**	**MS**	**F**	**P**
Groyne (H0, H2, H4, H6, H10)	5	50.9	10.2	9.1	<0.001
Zone (end, mid, near shore)	5	44.9	8.9	8.0	<0.001
Level (1m, 2m, 3m).	2	51.5	25.7	22.9	<0.001
Groyne x Zone	25	80.4	3.2	2.8	<0.001
Groyne x Level	10	81.4	8.1	7.3	<0.001
Zone x Level	10	128.2	12.9	11.4	<0.001
Groyne x Zone x Level	50	221.9	4.4	3.9	<0.001
Residual	216	242.0	1.1		
Total	323	901.2	2.8		
C	**Small adults Sept 17th 2001**				
Source	**DF**	**SS**	**MS**	**F**	**P**
Groyne (H0, H2, H4, H6, H10)	5	4643.2	928.6	22.1	<0.001
Zone (end, mid, near shore)	5	6057.7	1211.5	28.9	<0.001
Level (1m, 2m, 3m).	2	4996.8	2498.4	59.6	<0.001
Groyne x Zone	25	3847.6	153.9	3.6	<0.001
Groyne x Level	10	6087.9	608.7	14.5	<0.001
Zone x Level	10	10418.3	1041.8	24.8	<0.001
Groyne x Zone x Level	50	11385.1	227.7	5.4	<0.001
Residual	216	9042.7	41.8		
Total	323	56479.6	174.9		
D	**Large adults Sept 17th 2001**				
Source	**DF**	**SS**	**MS**	**F**	**P**
Groyne (H0, H2, H4, H6, H10)	5	31.3	6.2	5.8	<0.001
Zone (end, mid, near shore)	5	31.5	6.3	5.8	<0.001
Level (1m, 2m, 3m).	2	26.8	13.4	12.4	<0.001
Groyne x Zone	25	46.0	1.8	1.7	NS
Groyne x Level	10	70.1	7.0	6.4	<0.001
Zone x Level	10	57.7	5.7	5.3	<0.001
Groyne x Zone x Level	50	99.7	1.9	1.8	<0.01
Residual	216	233.3	1.0		
Total	323	596.7	1.8		

From December 2001 to February 2002 the abundance of all age classes declined and dispersal patterns were not significant. The re-engineering and disturbance of H4 did not appear to influence populations on the adjacent groynes (H2 and H6) and no significant differences were found between groyne abundances.

Apart from juveniles, significant differences between groyne populations were found after abundances increased in March 2002 (Table 4.5). For second years, recruitment on groyne H8 was significantly different among the zones and levels (Table 4.3A). By April second year abundance on groyne H8 had declined, but risen on the adjacent control groyne H6 (Fig. 4.7B) though this was not significant. For small adults *post hoc* tests showed the abundance increase on groyne H8 was significant (Table 4.3A).

TABLE 4.5 THREE-WAY ANOVA COMPARISONS OF *NUCELLA LAPILLUS* ABUNDANCES AT HIGHCLIFFE ON MARCH 2002 FOR A: SECOND YEARS, B: SMALL ADULTS AND C: LARGE ADULTS.
See Figure 4.2 for full key to zones and levels.

A	**Second years March 2002**				
Source	**DF**	**SS**	**MS**	**F**	**P**
Groyne (H0, H2, H4, H6, H10)	5	26.386	5.277	7.338	<0.001
Zone (end, mid, near shore)	5	25.127	5.025	6.988	<0.001
Level (1m, 2m, 3m).	2	29.154	14.577	20.270	<0.001
Groyne x Zone	25	42.522	1.701	2.365	<0.001
Groyne x Level	10	30.994	3.099	4.310	<0.001
Zone x Level	10	12.920	1.292	1.797	NS
Groyne x Zone x Level	50	73.599	1.472	2.047	<0.001
Residual	216				
Total	323				
B	**Small adults March 2002**				
Source	**DF**	**SS**	**MS**	**F**	**P**
Groyne (H0, H2, H4, H6, H10)	5	1889.0	377.8	32.3	<0.001
Zone (end, mid, near shore)	5	2559.7	511.9	43.8	<0.001
Level (1m, 2m, 3m).	2	1752.5	876.2	75.0	<0.001
Groyne x Zone	25	1639.3	65.5	5.6	<0.001
Groyne x Level	10	1157.6	115.7	9.9	<0.001
Zone x Level	10	2234.3	223.4	19.1	<0.001
Groyne x Zone x Level	50	2632.7	52.6	4.5	<0.001
Residual	216	2521.3	11.6		
Total	323	16386.7	50.7		
C	**Large adults March 2002**				
Source	**DF**	**SS**	**MS**	**F**	**P**
Groyne (H0, H2, H4, H6, H10)	5	6.0	1.2	6.9	<0.001
Zone (end, mid, near shore)	5	11.5	2.3	13.4	<0.001
Level (1m, 2m, 3m).	2	3.1	1.5	9.0	<0.001
Groyne x Zone	25	14.4	0.5	3.3	<0.001
Groyne x Level	10	4.8	0.4	2.8	<0.01
Zone x Level	10	9.8	0.9	5.7	<0.001
Groyne x Zone x Level	50	17.0	0.3	1.9	<0.001
Residual	216	37.3	0.1		
Total	323	104.3	0.3		

In July 2002 the juvenile increase on treatment H8 and control H6, H10 (Fig. 4.7A) was not significant and no further significant results for juvenile population patterns were found until October 2002 (Table 4.3B, Fig. 4.7A). Juvenile recolonisation or recruitment was found on treatment groyne H4 (Fig. 4.7A) and was significant (P<0.001) occurring at 1 m in the east face end and mid groyne zones (Figs. 1C and 3C, Appendix 2).

Second years declined in June and July 2002, but by August significant main effects were found as their abundance increased on control groyne H6 (Table 4.3B). By September 2002 further significant increases on H4 (end zone west face) and H6 (east and west mid zones) were due to rises in abundance at 1 m (Table 4.3B). In October 2002 main effects and interactions were significant (Table 4.3B). Although abundance on control groyne H6 was still the highest, the population had declined whereas on groyne H4 (treatment) the increases were significant.

Significant differences were found within and between small adult populations from June to October 2002 mainly due to fluctuation on groynes H4 and H8 (treatment) (Table 4.3B). This was with the exception of recruitment on the west face of control groyne H10 in September 2002 (Fig. 4.9L, Table 4.3B). Small adults were found in west face zones (groyne end to near shore), which had very low prey abundance, but by October *N. lapillus* abundance had decreased leading to no significant difference within H10.

In June on groyne H4, large adult differences were significant due to increases on both groyne faces (Fig. 4.9E,F); recruitment on the west face was dominant (Table 4.3B). The only other month in which changes were apparent was July 2002 (Fig. 4.7D), but the increasing numbers on control groynes (H2, H6, H10), in particular H6 (Fig. 4.9G,H), did not result in a significant main effect. A difference was suggested between H6 and H0 ($P<0.02$), but this was not accepted as data were not normally distributed in raw or transformed formats. Raw data showed that the change on H6 was due to a slight abundance increase at 1 m on the west groyne end (Fig. 2D, Appendix 2).

4.3.3 Prey abundance and physical factors

Whilst *N. lapillus* abundance was generally higher in end zones, some distribution patterns did not correspond with an optimum habitat for both prey and emersion time (e.g. treatment sites H0 Fig. 2G and H8 Fig. 2O, Appendix 2; control site H10 Fig. 6F, Appendix 2). Therefore correlation tests were carried out on *N. lapillus* abundance and the percentage cover of *M. edulis* and barnacles which were recorded for community analysis (Chapter 6). No relationships were found between the variables thus suggesting that *N. lapillus* distribution could be influenced by factors other than prey availability alone.

Of initial abundances and significant changes, groynes H4 and H8 were notable (Figs. 4.4 and 4.7). Abundance changes on controls H6 and, to a lesser extent, H10 were also often significant (Table 4.3). These changes may have been related to the availability of habitats as groynes H4 and H8 in particular appeared to be sinks for dogwhelks. Therefore, as a measure of physical variability between groynes, a cluster analysis using Bray-Curtis similarity (Bray and Curtis, 1957, Fig. 4.10) and a principal component analysis (PCA, Fig. 4.11) were carried out to determine if the physical habitats on groynes were similar.

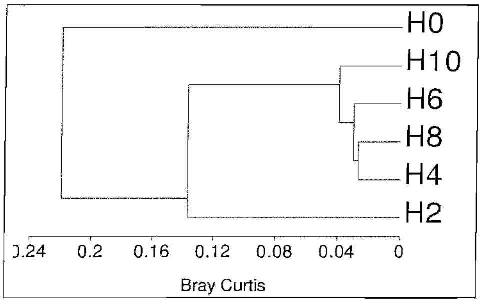

FIGURE 4.10 DENDROGRAM PLOT OF BRAY-CURTIS SIMILARITY MEASURES FOR THE PHYSICAL ATTRIBUTES OF TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H10) GROYNES AT HIGHCLIFFE BEACH.
Attributes: east groyne face mean angle, west groyne face mean angle, top width of groyne, base width of groyne, groyne height, east base length of groyne available for colonisation, west base length of groyne available for colonisation.

Cluster analysis showed that the physical characteristics of H4 and H8 (treatment) and H6 and H10 (control) were closely related (Fig. 4.10). The PCA showed that groynes H4 and H8 were particularly related to the length of colonisable area on the east and west groyne faces (Fig. 4.16). H6 and H10 were also influenced by the groyne height and base width. Although closely clustered to other sites, H10 did not have a large *N. lapillus* population in May 2001 and dogwhelk residence times appeared short (e.g. Fig. 3L, Appendix 2), although they may have been sheltering within the groyne.

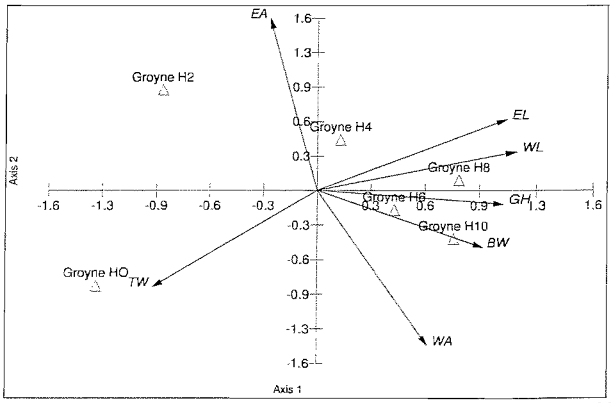

FIGURE 4.11 PRINCIPLE COMPONENT ANALYSIS FOR THE PHYSICAL ATTRIBUTES OF TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H8) GROYNES AT HIGHCLIFFE BEACH.
EA: east groyne face mean angle, WA: west groyne face mean angle, TW: top width of groyne, BW: base width of groyne, GH: groyne height, EL: east base length of groyne available for colonisation, WL: west base length of groyne available for colonisation.

4.4 Discussion

4.4.1 Procedural problems

At the start of experimental work on the groynes H0, H4 and H8 at Highcliffe, including the gaps left between zones and levels, were cleared of *N. lapillus*. Although initial clearance work appeared to be successful, the population on groyne H4 in particular resurged in July 2001. The rapid appearance of numerous *N. lapillus* on open rock faces of H4 and H8 after the storm suggested that, although a large number of *N. lapillus* had been removed, these individuals had been present on inaccessible areas within the groyne. They may have moved to open faces on the blocks as conditions for foraging ameliorated (Burrows and Hughes, 1989). However, by making repeated clearances visible populations were reduced to zero by the start of the experiment.

Dogwhelk population structure appeared to be affected by extremes of hydrodynamic forcing (e.g. September and October 2001). High tides and severe weather were the main conditions when movements may have occurred, but made the groynes inaccessible (e.g. November 2001, May 2002). This prevented the data from being a continuum thus important fluctuations in abundance may have been missed.

The survey design was set up to monitor abundance changes at differing spatial scales and some increases were found in areas where *N. lapillus* were unlikely to remain due to emersion and lack of prey (e.g. H10 near shore zone). Natural fluctuations, particularly on control groynes, however, may have masked some of the changes seen on treatment groynes. The populations on control groynes (H2, H6, H10) declined in summer 2001, although this may have been 'active refuging' in response to high temperatures (Burrows and Hughes, 1989). Fluctuations such as this may have made the identification of recolonisation patterns difficult.

Ideally, recolonisation would also have been monitored using offshore sampling methods such as benthic sampling. Previous workers have attempted to investigate dispersal methods in gastropods and bivalves using off bottom collectors, buckets and plastic bags, to catch drifting direct development species (e.g. Highsmith, 1985; Martel and Chia, 1991). These methods may have been useful at Highcliffe, as individuals were seen rolling in the surf suggesting that passive transport took place.

4.4.2 Colonisation and recolonisation of the rock groynes at Highcliffe

Bryan *et al.,* (1986) suggested that a population of *N. lapillus* affected by TBT pollution had been maintained by unaffected juveniles rafting in. In addition, Moore (1940) showed that dogwhelks could colonise new man-made habitats, although this was at a slower rate than achieved by other early colonisers with larval dispersal phases (e.g. *Semibalanus balanoides, Patella vulgata*) (Moore, 1939). The early 1990s construction of the rock groynes at Highcliffe provided a suitable habitat for *N. lapillus*, but the source of the initial colonisers and subsequent arrivals remains unclear, particularly in the context of the local extinctions in the Solent region. By observing dogwhelk recovery at Watermouth Cove in North Devon it has been reported that it took *N. lapillus* thirty years to cover thirty metres (Crothers, 1998, 2003). Several authors have suggested that *N. lapillus* moves very little throughout its lifetime (e.g. Crothers, 1985; Etter, 1996; Cook and McMath, 2001). This suggests that at Watermouth successive cohorts achieved a slow recolonisation rather than individuals travelling, being passively transported or rafting over large distances and also that after TBT pollution diminishes, recolonisation is a gradual process as conditions improve. Crothers (1998, 2003) also noted that several of the *N. lapillus* were large (> 50 mm) and suggested that they may have arrived on fishing pots from benthic populations, as large morphs have been recorded subtidally (Cooke, 1895, *op cit* Crothers, 1975; Mallinson, pers. com. 2002).

Rafting may take place by attachment to clumps of algae (e.g. Ingólfson, 1995, 1998), but at Highcliffe onshore stranding of seaweed was rare, even after storms, thus suggesting that subtidal algae in Christchurch Bay were uncommon. An alternative is that the population at Highcliffe, which included large adults, may have arrived via passive hydrodynamically forced transport along the seabed. Passive transport of gastropods as a method of population recovery and establishment has been suggested (Colson and Hughes, 2004) and recorded (Berry and Crothers, 1968; Martel and Chia, 1991; Day *et al.,* 1994; Swearer *et al.,* 2002). This may be achieved through processes similar to saltation (rolling along the sea floor), which has been seen in shells of *Littorina littorea* (Amos *et al.,* 2000).

Subtidal populations of *N. lapillus*, including the large morphs, have been observed by scientific divers in the Solent region and west of Christchurch Bay on mussel beds near the Isle of Purbeck (Mallinson, pers. com. 2002). The nearest subtidal feature to Highcliffe which may support *N. Lapillus* is Christchurch Ledge (Fig. 4.12). South-west of the groynes the ledge has mussel cover (Mallinson, pers. com. 2002), but there are no direct observations of *N. lapillus* and grab samples did not produce any (CEFAS, pers. com. 2002). Whether *N. lapillus* had been present on the ledge or otherwise, it cannot be assumed that the dogwhelks at Highcliffe arrived from Christchurch Ledge.

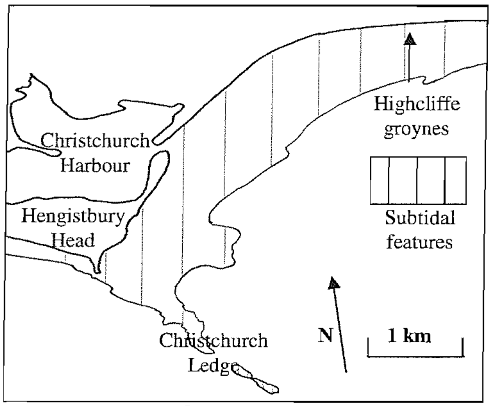

FIGURE 4.12 THE LOCATION OF SUBTIDAL FEATURES WITHIN CHRISTCHURCH BAY AND THE POSITION OF HENGISTBURY HEAD AND CHRISTCHURCH LEDGE IN RELATION TO THE STUDY SITE AT HIGHCLIFFE GROYNES.

Following the clearance work on the Highcliffe treatment groynes, it was planned to determine if one age class dominated recolonisation patterns without its significant decline on the adjacent control sites. To demonstrate reliably that dogwhelks had arrived from an offshore or adjacent site to the treatment groynes, it would have been necessary to show that sufficient numbers of an existing population drifted away and arrived at another groyne to form a new population (Highsmith, 1985). This could not be reliably demonstrated in this study; although clearance work had reduced populations to zero in early September 2001, the pulsed abundance increase on H4 and H8 (treatments) after the mid September 2001 storm was very high and seen in all of the age classes. These may have been dogwhelks that had refuged in inaccessible areas within the treatment groynes or possibly were arrivals from adjacent control sites as *N. lapillus* emerged when foraging conditions improved (Burrows and Hughes, 1989), and were passively transported to treatment groynes. Control populations did not recover to their pre summer levels, therefore these animals may have been 'exchanged' between groynes. This pattern was indicative of the passive transport of dogwhelks within and possibly between the groynes, to blocks formerly occupied by the cleared animals. Many of the cleared blocks were relatively isolated and active movement on to them would have been difficult. There is no groyne west of H0 therefore passive transport on to it is likely to be a much less predictable outcome. This may also explain the very random colonisation of treatment groyne H0 where dogwhelk abundance was never high and did not recover to pre clearance abundances.

4.4.3 Physical and biological interactions

Whilst it could not be established whether the pulse of recolonisation after storms came from offshore arrivals or within or between groyne dispersal, the abundance increases appeared to be weather influenced. The gales in autumn 2001 and winter 2001-2002 appeared to affect populations on both treatment and control groynes. Increases were seen in all of the size classes although juveniles appeared to be less susceptible to storms than the larger animals, as their abundances did not significantly increase with the exception of groyne H4.

As an indication of the influence of severe weather on changes in *N. lapillus* populations, total abundances on treatment and control sites were correlated against wave energy records for the sampling period. Several workers (e.g. Dobbs and Vozarik, 1983; Trussell, 1997) have suggested that storms can significantly redistribute fauna and that this may be related to shell shape and size in gastropods (Kitching *et al.,* 1966; Hughes and Taylor, 1997; Trussell, 1997). Thus in addition to total dogwhelk numbers, abundance results for each age class were also correlated to assess whether shell size was an important factor in dispersal at Highcliffe. Whilst wave energy increased with the onset of winter 2001 (Fig. 4.13) and *N. lapillus* abundances declined over this period, possibly due to sheltering from cold air (Largen 1967), a significant relationship was only found for changes in second year abundance on treatment groyne H8 ($r = 0.62$, $n = 12$, $df = 11$, $P<0.05$).

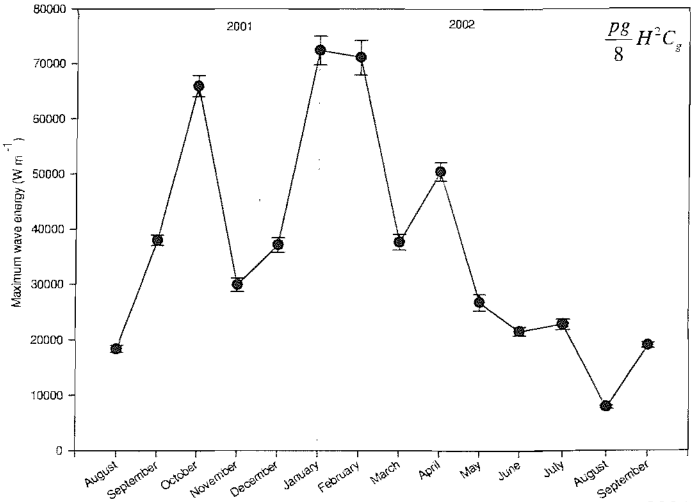

FIGURE 4.13 MAXIMUM WAVE ENERGY VALUES RECORDED IN CHRISTCHURCH BAY, DORSET AUGUST 2001-OCTOBER 2002. (Data from New Forest District Council, 2003). Wave energy: The rate at which wave energy is transmitted across a plain of unit width perpendicular to the direction of wave advance, where p = seawater density (1025kg m^3), g = acceleration due to gravity (9.8m/s^2). H = wave height, C_g = wave group velocity (US Army, 1984).

N. lapillus are polymorphic, displaying different shell morphologies in relation to wave exposure and predation pressures (Kitching *et al.,* 1966; Crothers, 1975; Gibbs, 1993a,b;

Kirby *et al.,* 1994). A large pedal area and thinner shell structure allow relatively tenacious attachment to rock and and have been shown to be beneficial to dogwhelks in exposed habitats (Kitching *et al.,* 1966; Etter, 1988a; Hughes and Taylor, 1997). In addition dogwhelks have been shown to grow more rapidly on a diet of mussels, thus developing thin shells (Moore, 1936). *N. lapillus* at Highcliffe were all thin shelled and large morphs were relatively common, although this morphology was not always associated with *M. edulis* patches. On groyne H8, barnacles were the dominant diet for *N. lapillus* suggesting that wave exposure, rather than diet, was the overriding factor or that the populations were of subtidal origin (see Crothers, 1975).

Once washed away from egg capsules (Moore, 1938b), juvenile *N. lapillus* usually shelter on the lower shore in barnacle cases or deep crevices (Crothers, 1985) or among *Spirorbis borealis* tubes (Moore, 1938b), whereas second years and adults live on the open rock face, but seek shelter in extreme weather (Moore, 1936; Hawkins and Hartnoll, 1983; Crothers, 1985; Burrows and Hughes, 1989). At Highcliffe juveniles were rare, although they were found at both 1 and 2 metre levels in the groyne end zones. A true estimation of their relative abundance in dogwhelk populations, however, is difficult and may require extended time searches (Spence, 1989) which were not possible due to the small tidal range. Sheltering on the extreme lower shore would have been demanding for juveniles as sediments appeared to scour and smother lower shore communities, particularly after storms. As juveniles did not show the same patterns of abundance as the larger size classes, conclusions about fluctuations in their numbers are unreliable and cannot be used to infer colonisation at Highcliffe.

Although a relationship between wave energy and shell size could not be reliably established, the relative proportions of size classes did alter after extreme weather, and in the winter. Not only does the large pedal area, seen in Highcliffe dogwhelks, lessen the possibility of dislodgment, but also allows longer foraging (Hughes and Taylor, 1997), although Denny *et al.,* (1985) found that the stationary adhesive strength was quite low in predatory gastropod species and that foraging is limited to times when it is mechanically safe. Trussell (1997) found that storm effects were counterintuitive; after storms at an exposed site, mean shell aperture and height in a population of *Littorina littorea* decreased. It was concluded that reduced shell height and aperture gave advantages when sheltering from storms. In addition a shorter spire in exposed *N. lapillus* may also be advantageous (Hughes and Taylor, 1997), as it has been found that larger individuals may be more likely to be dislodged (Etter, 1989).

At Highcliffe the redistribution of animals was not easy to quantify. Crevices for shelter were limited due to the nature of the groynes where waves are allowed to pass partially through, thus dissipating energy. It is possible that the adults, particularly the large morphs, were unable to shelter fully and were therefore dislodged by wave action, allowing second years to move into favoured habitats, possibly due to reduced competition. Alternatively second years, possibly out competed for favoured habitats, may have been dislodged due to their smaller aperture and pedal size and then redistributed within and between groynes, thus leading to the observed increases after storms. Birchenough *et al.,* (2002b) found recolonisation at many formerly TBT impacted sites and suggested that this was due to individuals being washed back passively. At Highcliffe this may be the mode of colonisation, but the results did not show any consistent trends so did not allow an unequivocal identification of a dominant shell size or age class in this process.

4.4.4 Habitat disturbance

Crowe (1996) found that observations on the dispersion of *Bembicium auratum*, an intertidal gastropod, were not predictable and concluded that models to forecast the distribution of adults and juveniles on rocky shores will not always be effective. In addition Gladsby and Connell (2001) stressed the need for caution in the use and interpretation of studies using artificial habitats when testing hypotheses about naturally occurring assemblages. Identifying the interactions that affect dogwhelk distribution and dispersal at Highcliffe has proved difficult, and this may be due to the unusual habitat coupled with the level and types of disturbance. Highcliffe is a man-made analogue of a rocky shore and such structures often have low diversity due to frequent disturbance (Bacchiocchi and Airoldi, 2003). However, coastal defences benefit local diversity as they allow the settlement of species that usually live on rock reefs (Bombace *et al.,* 1995), thus Highcliffe may act as a ‘stepping stone’ (Bacchiocchi and Airoldi, 2003) by which *N. lapillus* could recolonise isolated sites from where it has previously been eradicated due to TBT (e.g. Southampton Water, see Chapter 5).

The clearance of dogwhelks on treatment groynes simulated catastrophic losses of *N. lapillus.* Between September 2001 and October 2002 the groynes at Highcliffe were not sufficiently recolonised to enable a return to their former population densities. It did appear, however, that disturbances might have resulted in the random distribution of individual dogwhelks, sometimes resulting in their presence in unfavourable habitats.

Hughes *et al.,* (1992) found that juvenile *N. lapillus* were more abundant on the exposed side of an intertidal reef and that adults were roughly equally distributed on the exposed and sheltered sides. At Highcliffe all of the age classes were more abundant on the more sheltered east groyne face, although this was less pronounced in adults. This possibly reflects the level of scour and abrasion disturbance from the mobile gravels common at the west face of groynes, where dogwhelks were more abundant at 2 m (i.e. more distant from the effects of scour). A trade off between risk and habitat on the east face was also evident, as during the summer months fine sands often smothered end zone blocks, accreting by up to 0.5 m between surveys. Thus whilst natural factors such as desiccation and prey abundance would dictate preferred habitats, the impacts of wave action and sediment mobilisation could have had largely random effects on dogwhelk distribution within the groyne system.

Flow rates can dictate community structure (Leonard *et al.,* 1998) and wave shock (Menge, 1976, 1978a,b) acting on the groyne populations of *N. lapillus* has the potential to be amplified as it passes through voids within the groynes, thus unpredictably redistributing dogwhelks. In addition the currents within Christchurch Bay do not behave in the manner predicted by Admiralty Charts (Riley, 1995). There is a double high tide which is split with two distinct bodies of water meeting in the region of the Highcliffe groynes. It is possible that Christchurch Ledge affects this as one current passes over it and another to the east of the ledge leading to a convergence in Christchurch Bay (Riley, 1995). If the ledge proved to be the source of passively transported recolonisers the path through which these travelled would be less predictable, thus leading to increased randomness of dispersal at the groynes.

Overall the patterns of recolonisation appear to have been relatively random with no clear patterns of age group or location clearly evident in the process, although passive transport between and within the groynes was indicated. Natural disturbances of dogwhelk populations (due to wave shock and scour) may have been augmented by maintenance of the groynes. The rebuilding of groyne H4 led to the total loss of the mid and upper intertidal communities with subsequent recolonisation by diatoms and green algae. However, towards the end of the sampling period, barnacles and dogwhelks were found in the mid zone. Disturbance by maintenance engineering was acute and may have redistributed *N. lapillus* randomly throughout the groynes (e.g. to H10). As several groynes were characterised by limited prey availability (e.g. H0 and H10) the communities may have also been undergoing recovery from previous maintenance, but this had not been documented.

4.4.5 Overview and summary

Natural variability of dogwhelk populations at Highcliffe appeared to have an equal magnitude to the abrupt simulated event in this work. This demonstrates that at Highcliffe the signals of colonisation or dispersal were superimposed on large natural changes and therefore were too small to be resolved by this work. The repopulation of cleared areas appeared initially to be 'pulsed' and may be related to environmental factors such as tidal range and wind and wave energy, suggesting that it was a relatively passive process of redistribution within and between the groynes. Conversely colonisation may be active and related to abiotic factors (active release) or due to biotic pressures (e.g. competition) at the source location (decisions to move on, Burrows and Hughes, 1991a,b; Vadas *et al.,* 1994). Exchanges between groynes of both *N. lapillus* and its prey species may have occurred, but the experiment design was not sensitive enough to demonstrate this.

It was not possible to unequivocally identify a size class, preferred habitat or arrival point for the dogwhelks at Highcliffe due to the 'noise' in the system; population variations appeared to be more associated with stochastic events (e.g. storms) than with deterministic processes of redistribution within and between groynes. The low abundance on groyne H0 and high numbers on H4 and H8, however, indicated that the middle of the groyne system, where erosion was highest, may receive more passively transported dogwhelks, therefore there remain aspects of *N. lapillus* movement to be studied. Highcliffe is not a natural shore and has abiotic factors that may not normally be encountered by rocky shore communities, but the low diversity discrete groynes made this site suitable for testing the recolonisation capabilities of *N. lapillus*. Further study involving marking of dogwhelks and offshore samplers, may provide better insight into the passive or active movements of *N. lapillus*.

To reliably investigate *N. lapillus* population dynamics at Highcliffe would require the identification of source populations, in this case possibly benthic, and genetic analysis of the colonisers at Highcliffe. Sympatric genetically different morphs of *N. lapillus* can exist on the same shore (Rolan *et al.,* 2004) thus it may be possible to identify if the *N. lapillus* at Highcliffe interact as a meta-population (Levin, 1969) with one source group, or if colonisers have arrived from several locations. Potentially this would lead to insights into the methods by which *N. lapillus* can migrate to formerly polluted habitats using man-made sites as stepping-stones.

Chapter 5. Translocation of *Nucella lapillus* and testing feasibility of reintroduction

5.1 Introduction

Introduced in the late 1980s, UK legislation banning tributyltin-based (TBT) antifouling paints on ships less than 25 m length over all, has led to the partial or total recovery of *N. lapillus* populations. Compared to the late 1980s (Bryan *et al.,* 1986, 1987; Gibbs and Bryan, 1986; Gibbs *et al.,* 1987, 1988) the legislation has lessened the incidence of imposex in female dogwhelks on many UK shores (e.g. Evans, *et al.,* 1994, 1996, Harding *et al.,* 1997, 1999, Hawkins *et al.,* 2002; Huet *et al.,* 2004). Imposex levels have diminished in many *N. lapillus* populations adjacent to recreational boating harbours (e.g. Bryan and Gibbs, 1987; Gibbs *et al.,* 1988; Spence *et al.,* 1990; cf Harding *et al.,* 1999; Svavarsson, 2000; Birchenough *et al.,* 2002a,b; Minchin, 2003). There are, however, some UK sites where *N. lapillus* populations are still absent, particularly in areas of high commercial shipping activity, e.g. Southampton Water, Hampshire, UK (Fig. 5.1) (Langston *et al.,* 1994, 1997, 1998; CONSSO, 1998, Harding *et al.,* 1999). In addition, there are some small boat harbours throughout Europe where a reduction in Imposex has been slow (Miller *et al.,* 1999; Barroso and Moreira, 2002; Santos *et al.,* 2002), particularly where the ban appears to have been disregarded (e.g. Huet *et al.,* 1996; Santos *et al.,* 2002).

In some areas sediment provides a reservoir of TBT (Langston *et al.,* 1990; Langston and Pope, 1995; Ruiz *et al.,* 1996), allowing it to be readily bioavailable (Bryan and Langston, 1992; Macguire, 2000). Constant replenishment of water borne TBT from sediment remobilisation can result in TBT concentrations above legislative targets (e.g. UK, Langston and Burt, 1991; France, Ruiz *et al.,* 1996; UK, Thomas *et al.,* 2000, 2001; Egypt, Bakarat *et al.,* 2001; Portugal, Santillo *et al.,* 2002). In the UK *N. lapillus* has demonstrated colonisation and recolonisation abilities inconsistent with its lack of a pelagic stage (Moore, 1939,1940; Crothers, 1998, 2003; Herbert *et al.,* 2000; Birchenough *et al.,* 2002b; Colson and Hughes, 2004), although at some locations recovery has not been seen. In addition to the continuing influence of TBT on the reproductive capability of *N. lapillus*, other reasons for the failure of populations to re-establish may be the lack of a nearby source site or unfavourable hydrodynamic processes limiting passive transport.

Transplants of *N. lapillus* from exposed, unpolluted shores to sheltered areas near industrial ports have previously been undertaken to assess ambient TBT levels (e.g. Bryan *et al.,* 1987; Harding *et al.,* 1999; Davies, 2000; Quintela *et al.,* 2000). There have also been transplants testing differences in adult behaviour and juvenile development between different habitats (Burrows and Hughes, 1989; Gibbs, 1993b; Etter, 1996) and for differing genotype reactions to changing physiological stress (Day *et al.,* 1994; Kirby *et al.,* 1994a,b). To date protocols have not yet been established to reintroduce *N. lapillus* to shores on which TBT-related extinction has occurred.

Langston *et al.,* (1990) suggested that the recovery of *N. lapillus* at some sites would require the immigration of juveniles or the reintroduction of a fertile population. Reintroduction of *N. lapillus* has subsequently been proposed by Hawkins *et al.,* (1999). They suggested that the transplant and monitoring of *N. lapillus* to a trial site would assist method development if wider reintroduction efforts were made. In the UK the survival of *N. lapillus* would, however, be unlikely in areas where TBT levels are still above the Environmental Quality Target (EQT) of 2 ngl^{-1} (Langston *et al.,* 1990). Southampton Water (Fig. 5.1), an estuary busy with vessel movements, is still polluted by TBT from commercial vessels and contaminated sediments (Thomas *et al.,* 2000, 2001). It has previously supported *N. lapillus* populations (Langston *et al.,* 1994), the remnants of which can be seen in death assemblages (pers. obs.). Fluctuating TBT levels have been recorded with a modal waterborne level over 6 years of 1 ngl^{-1} (source Environment Agency, Waterlooville, Hampshire). Therefore TBT concentrations may be low enough to allow *N. lapillus* populations to survive in this area.

Apart from the absence of a pelagic larval stage, the reasons for lack of recolonisation by *N. lapillus* at Southampton may be that there are no local source populations, or that TBT pollution is still too high in the area to allow re-establishment. The nearest known source populations (Bray and Herbert, 1998; Herbert *et al.,* 2000) are located at Cowes on the Isle of Wight (9.6 km SSE, Fig. 5.1) and no current natural recolonisation has been seen. The reintroduction and monitoring of a population at a trial site in Southampton Water should provide information on the future viability of schemes to translocate *N. lapillus* to areas where local extinction has occurred.

The overall aim of this chapter was to investigate the prospects for *N. lapillus* reintroduction to Southampton Water. The transplant would establish whether this site was sufficiently uncontaminated to allow the survival and breeding of *N. lapillus*. Animals from a healthy population were translocated using different methods and different habitats. Survival, dispersion and the rate of imposex development were measured between the various treatments and compared with control (translocated to source site) populations.

The specific objectives were:

1. To establish levels of imposex pre-transfer and after 6 months at the transfer site and to compare these with a source (control) site;
2. To reintroduce *N. lapillus* to a former habitat and to compare survival rates between methods of reintroduction (caged and uncaged) onto artificial and natural habitats;
3. To assess the dispersal of treatment groups between sites as an indication of habitat and prey preference;
4. To consider other external factors influencing rates of survival such as predation on *N. lapillus* and food availability.

5.2 Methods and experimental design

5.2.1 Southampton Water: study site

Southampton Water is located on the mid south coast of the UK (Fig. 5.1). It is a partially mixed estuary with generally weak tidal circulation (Sharples, 2000). Shorelines within Southampton Water consist of Eocene gravels and clay with some areas of intertidal salt marsh and mudflat, particularly on the western side (Velegrakis, 2000).

The estuary is heavily used by commercial shipping, from Southampton Docks, Fawley oil refinery and the BP oil terminal (Fig. 5.1C). Numerous recreational boating centres can be found in the River Hamble, Hythe Marina and at marinas close to Southampton Docks. TBT levels in Southampton Water (Fig. 5.1C) are recorded by the Environment Agency. Sources may be from diffuse shipping release, sediments or wastewater spills from dockyard repair work which lead to a mean value of TBT in the water column from 1996-2001 of 4.8 ngl^{-1} (Fig. 5.2).

The Eastern Shore of Southampton Water consists of intermediate soft sediments and exposed intertidal cobbles and gravels (Velegrakis, 2000). The translocation release site was at Netley Abbey, Royal Victoria Country Park foreshore (Fig. 5.1d). Here clay and fine silt underlie exposed Eocene gravels, cobbles and concrete blocks remaining from a 4 m high seawall construction which was built in 1951-1952 (Hider, pers. com. 2004). The cobble shore supports a mixed community of common littorinids, sparse *M. edulis* and *E. modestus* and in some areas common *C. maenus*. On the vertical surface of the wall a barnacle and littorinid dominated community of species representative of a sheltered to moderately exposed middle and upper intertidal habitat occurs (Ballantine, 1961).

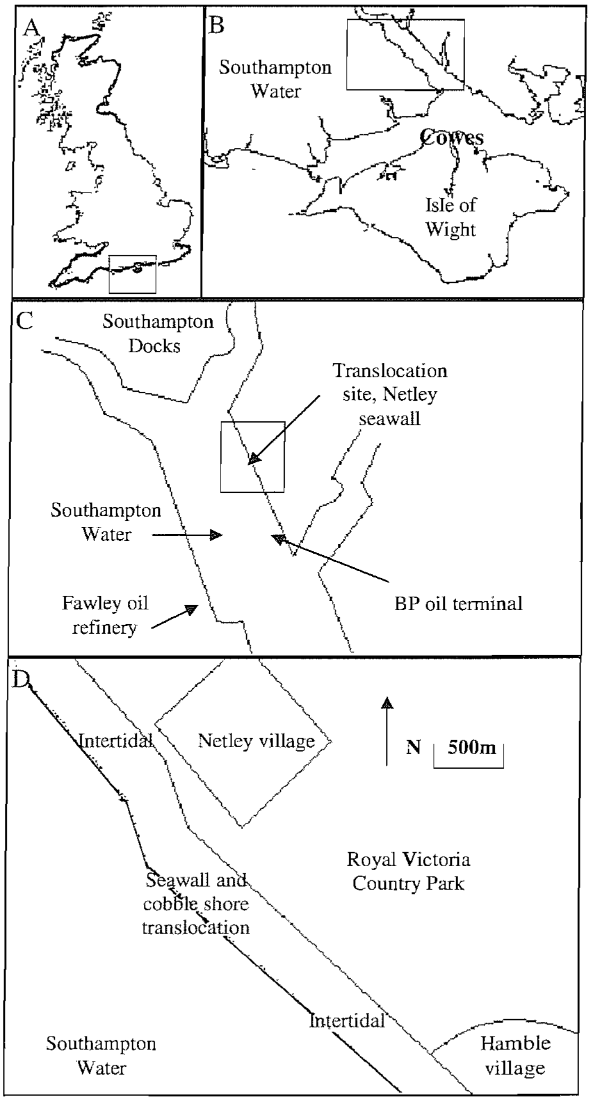

FIGURE 5.1 A:UK AND CENTRAL SOUTH COAST REGION, B: SOUTHAMPTON WATER AND ISLE OF WIGHT, C: SOUTHAMPTON WATER AND NETLEY TRANSLOCATION SITE, D: ROYAL VICTORIA COUNTRY PARK FORESHORE AND TRANSLOCATION SITE.

The foreshore at Netley has been designated as part of the Lee-on-the-Solent to Itchen Estuary SSSI and is included for its geological characteristics and rich wading bird feeding grounds (English Nature, 2003).

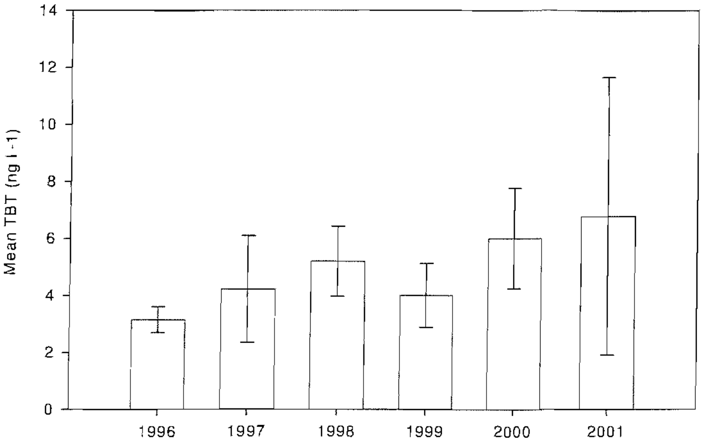

FIGURE 5.2 MEAN (+/- 1SE) WATER BORNE TBT CONCENTRATIONS RECORDED AT SOUTHAMPTON DOCKS (DOCK HEAD, SOUTHAMPTON WATER) 1996-2001.
(Data source, Environment Agency, 2003).

5.2.2 Polzeath: *Nucella lapillus* source site

Both Gibbs and Bryan (1986) and Spence *et al.,* (1990) recorded low levels of imposex at North Cornish shores during periods of higher TBT pollution elsewhere in the UK. North Cornwall was thus chosen as the source of a *N. lapillus* translocation population. Following several surveys of *N. lapillus* abundance, the site used as a translocation population source was an intertidal reef at Hayle Bay, New Polzeath in North Cornwall (Fig. 5.3, SW 931 797).

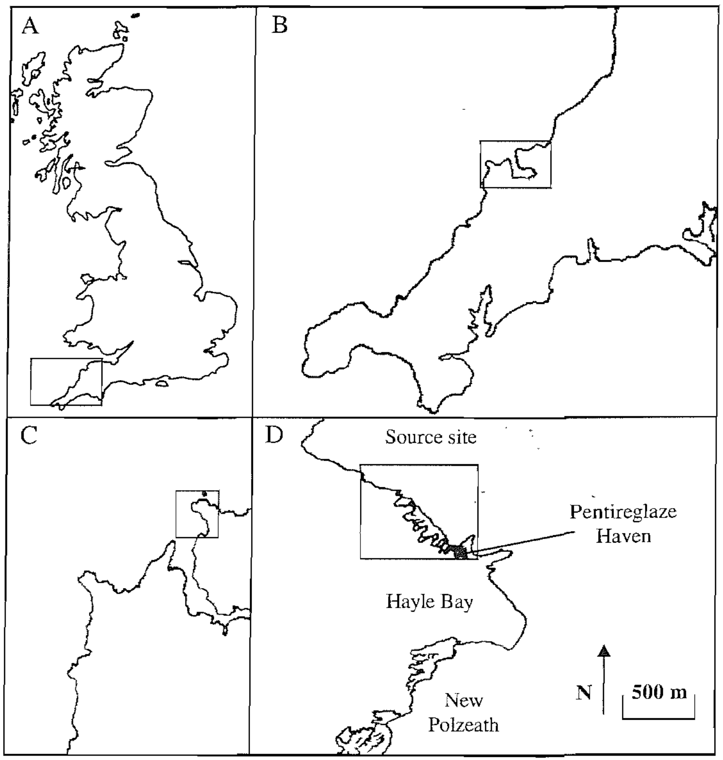

FIGURE 5.3 SOURCE SITE FOR TRANSLOCATED *NUCELLA LAPILLUS* AT POLZEATH IN NORTH CORNWALL A: UK AND SOUTH WEST ENGLAND, B: CORNWALL, C: HAYLE BAY NORTH OF CAMEL ESTUARY, D: SOURCE REEF NORTH HAYLE BAY (PENTIREGLAZE HAVEN).

Polzeath is remote from commercial shipping and recreational boating is uncommon in the region. Characterised by a relatively exposed rocky shore community representative of 2-3 on Ballantine's (1961) exposure scale (Fig. 2.6), *M. edulis* and barnacles dominate open areas of rock with occasional patches of *Fucus vesiculosis evesiculosis* and *Porphyra umbilicalis*. *N. lapillus* are abundant and shelter in crevices from which they generally forage on the mussel beds. They are also common in wave cut inlets in the seaward face of the reef.

5.2.3 Translocation methods: Royal Victoria foreshore

This study compared release methods and subsequent dogwhelk movements in two habitats: the seawall and the exposed cobbles at the Royal Victoria Country Park foreshore (Fig. 5.4). The experiment was designed with sufficient replicates to ensure that survival rates, methods of reintroduction and movement measurements were not confounded by recording from insufficient treatments (Underwood, 1991). Following a preliminary survey, four replicate release sites were surveyed on the cobbles (sites 1,3,5,7, Fig. 5.4). Four locations were also set up on the vertical wall face (sites 2,4,6,8, Fig. 5.4). All sites were photographed, referenced to two fixed points and positions recorded using a Magellan 2000 GPS unit.

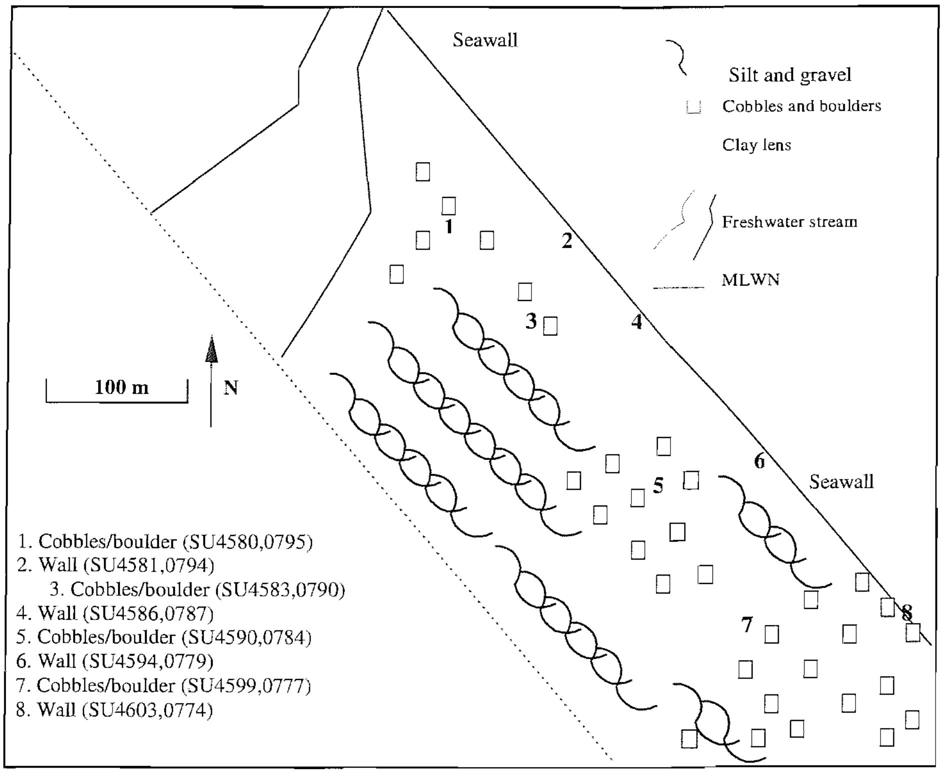

Figure 5.4 Translocated *Nucella lapillus* release site locations on the cobble shore and the seawall at the Royal Victoria Country Park, Netley foreshore, Southampton Water.
Sites 1, 3, 5, 7 cobble translocation sites for 125 caged and 125 uncaged *N. lapillus* on each site.
Sites 2, 4, 6, 8 seawall translocation sites for 125 caged and 125 uncaged *N. lapillus* on each site.

On the 12th February 2002 a baseline survey was made of the transfer habitats to ensure similar physical and biological factors at locations within treatments. For the cobble shore, twenty random 50 x 50 cm quadrats were sampled at each site. The percentage cover of barnacles and algae was recorded. Littorinids were common, but other intertidal species such as patellids, *M. edulis* and *Elminius modestus* were sparsely distributed on the cobbles. Therefore, to give an accurate abundance assessment three 3-minute timed searches were carried out. In addition, although highly mobile, the numbers of *Carcinus maenus* (which is a major predator of *N. lapillus*, (Hughes and Elner, 1979) were also noted. The assemblages on the wall were also surveyed using twenty 50 cm quadrats and three 3-minute timed searches were used to assess mussel, littorinid and limpet numbers.

5.2.4 Translocation methods: Polzeath

N. lapillus was highly abundant at Polzeath with over 700 individuals found during a 10 minute time search in February 2002. Subsequently 2000 adult *N. lapillus* were collected for translocation to the Royal Victoria Country Park foreshore. 50 extra individuals were collected to allow for mortality during transport and another 35 to provide a baseline imposex value (§ 2.2). The *N. lapillus* were transported to Southampton Oceanography Centre aquarium (§ 2.2.2) where they were held in "instant ocean" seawater tanks to avoid contamination, until marked and assigned to a site and reintroduction method.

Translocation of intertidal species requires controls to ensure that any observed effects after relocation are not artefacts of the movement and disturbance process (Chapman, 1986, 1999). Controls for this work were based on methods suggested by Crowe and Underwood (1998). As each treatment group released at the Netley translocation site comprised 125 individuals, groups to control for confounding factors were based on this number. To control for the stress of movement from Polzeath to Southampton, 125 *N. lapillus* were collected along with the 2000 for translocation. These were also taken to the Southampton Oceanography Centre aquarium. The medial shell surface of each specimen was marked with white stripes of Humbrol (TM) enamel paint, which is non-toxic to *N. lapillus* (Bryan *et al.,* 1987; Proud, 1994). These animals were then held in the aquarium until being returned to Polzeath on the 10th March 2002 (Fig. 5.5).

When the *N. lapillus* used to control for the effects of transport were returned to Polzeath, disturbance effects were also considered (Chapman, 1986, 1999; Crowe and Underwood, 1998; Underwood *et al.,* 2004). To control for this 125 *N. lapillus* were removed from the shore at Polzeath, cleaned and dried then marked on the medial shell surface with a blue stripe of enamel paint (Fig.5.5). These individuals were immediately replaced upon the shore in the area in which they were found. Ideally a control would have been put in place to consider the impact of painting itself without handling *N. lapillus* individuals, however this is impractical as cleaning the shell is first required resulting in disturbance itself. Using timed searches, survival of the control population was surveyed in May and August of 2001.

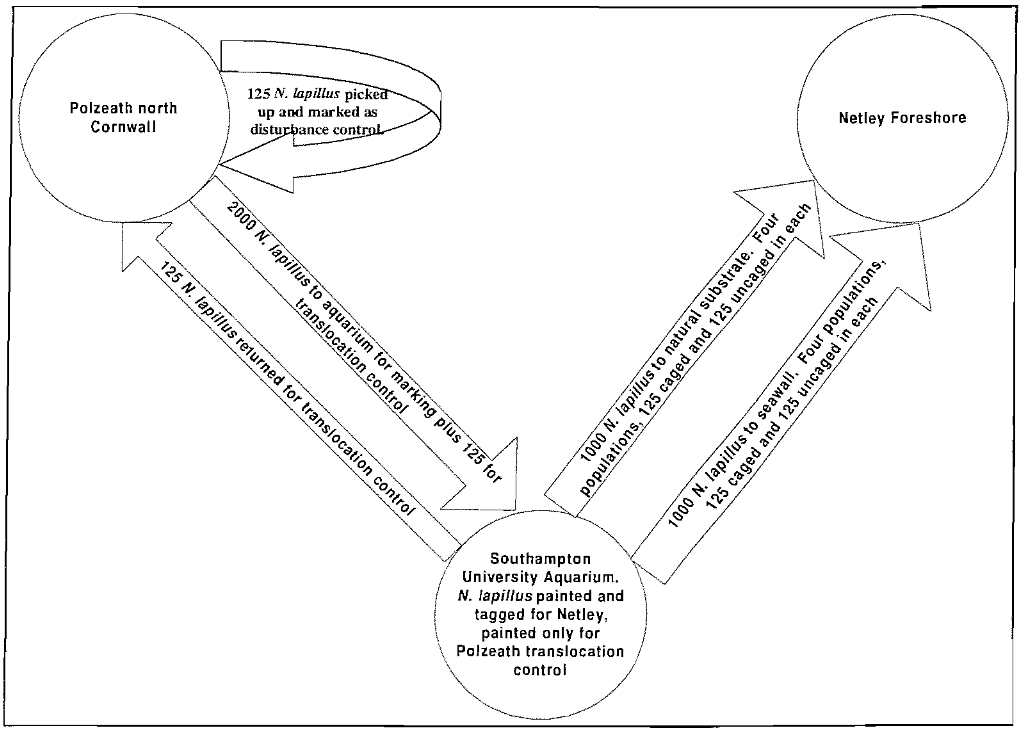

FIGURE 5.5 SUMMARY OF TREATMENT AND CONTROL POPULATION CONTROL PROTOCOLS FOR THE TRANSLOCATION OF *NUCELLA LAPILLUS* FROM POLZEATH, NORTH CORNWALL TO NETLEY FORESHORE AND SEAWALL.

5.2.5 Experimental set up

The experimental design comprised a total of eight treatment populations. Four populations were released onto the cobble shore at sites 1, 3 5 and 7 and four populations onto the seawall at sites 2, 4, 6 and 8 (Fig. 5.4). Each population comprised 250 *N. lapillus* and to further test between methods of reintroduction, these were subdivided into 125 to be caged for the first week of release and 125 uncaged (Fig. 5.6). Cages have been used to place treatment organisms at transfer locations for ease of relocation and to provide an initial level of protection allowing acclimatisation (Davies, 2000; Quintela *et al.,* 2000). Kolmogorov-Smirnov two sample tests (Siegel, 1956) were carried out between the shell size-frequency distributions of each population to check for similarity. Populations 3 and 5 were significantly different due to the presence of one smaller and one larger individual respectively. It was considered that this minor variation would not significantly bias survival results due to population age or size related differences.

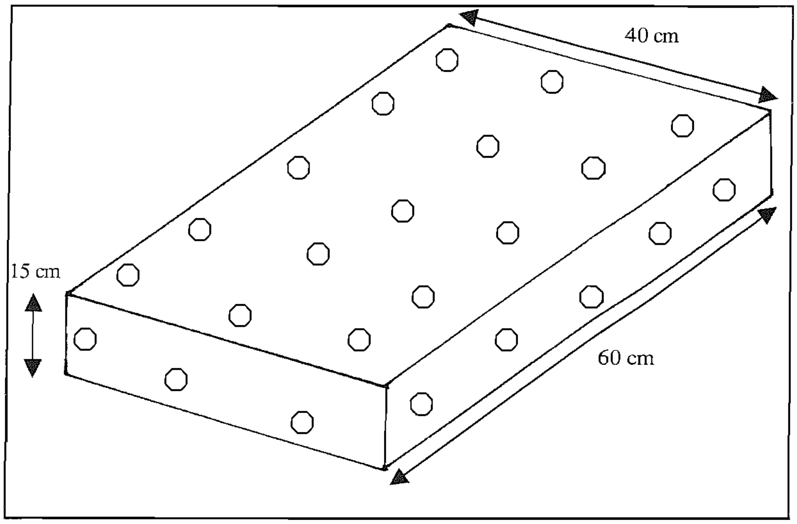

FIGURE 5.6 THE DIMENSIONS OF THE PLASTIC CAGES USED TO PROTECT 125 *NUCELLA LAPILLUS* AT EACH SITE ON THE COBBLE SHORE (SITES 1,3,5,7) AND THE SEAWALL (SITES 2,4,6,8).
(Circles represent 0.5 mm holes to allow free flow of seawater).

Each population was marked with an identifying colour. In addition, using a modification of methods developed by Crowe *et al.,* (2001), a 0.3 g piece of coloured aluminium sheet was attached to each individual using cyanoacrylate glue. This allowed for post-release searching for *N. lapillus* using a waterproof metal detector (White Electronics, Beachcomber 4).

On the 12th March 2002 the 8 caged and uncaged populations were distributed at their allocated sites on the Netley seawall and cobble shore (Fig. 5.4). The cages were modified plastic seed trays with 31 0.5 cm holes drilled in them (Fig. 5.6). These were fixed to the wall and blocks on the shore using 6 stainless steel screws around the cage lip.

Chapman (1986) recommended that post-translocation assessment should begin within the first week of the experiment. Accordingly, after release on the 12th of March, the first measurements of survival and distribution of uncaged individuals were made two days later on the 14th of March and monthly thereafter. The caged populations at each site were released after a further five days and first surveyed on the 20th of March three days later.

Survival rates on the seawall and the monthly average distance moved by treatment groups (caged/uncaged) within each replicate population were recorded east and west and up the wall and down shore of release sites using a 1 m^2 grid system. At each cobble site (Fig. 5.4) a stake with 1 m intervals marked on an attached rope was driven into the centre of each release point. Movement and survival rates were recorded by walking clockwise around the stake at each of the intervals. This resulted in a circular search pattern outward from 1m until the wall was reached. The seawall and cobble communities were resurveyed in May and August 2002 to assess any impact of *N. lapillus* predation on barnacles and *M. edulis*.

At Polzeath, survival of control *N. lapillus* groups was checked the day after replacement on the shore (11/3/2002), 65 days later (15/5/2002), and finally 151 days after the initial return to Polzeath (9/08/2002). In addition, on the last survey, final imposex levels for Polzeath and wall and shore sites at Netley were assessed (§ 2.2).

Univariate analysis was carried out using a standard statistical package (Sigma Stat). Multivariate analysis was carried out using PRIMER (Clarke and Gorley, 2001). This was used to show community variability within and between treatments using non-metric multi dimensional scaling (nMDS) calculated from square root transformed data (Thacker and Bromley, 2001). Stress values on each plot indicate the degree of difficulty of representing the data in two dimensions and its worth for interpretation (Clarke and Gorley, 2001) where <0.05 is excellent, <0.1 is good, <0.2 is useful and >0.3 is largely random placement (Clarke and Warwick, 1994). Finally Species Contribution to Similarity (SIMPER, PRIMER, Clarke and Gorley, 2001) analysis was run to show taxa contribution to Bray-Curtis dissimilarly within communities (Bray and Curtis, 1957).

5.3 Results

5.3.1 Changes in imposex

Baseline imposex results for the Polzeath *N. lapillus* calculated at the time of collection of donor animals showed that the morphological change due to chronic TBT pollution was minimal. The relative penis size index (RPS) result was 1.81 showing that the more sensitive indicator to TBT was low and with a median and modal vas deferens sequence (VDS) at 0 females were largely unaffected and hence able to breed. Thus the long-term survival of transfer populations was unlikely to be affected due to pre-existing TBT-induced morphological change.

Imposex in Polzeath and Netley seawall and cobble shore populations was reassessed in August 2002, 6 months after translocation. In Polzeath *N. lapillus* the VDS result was a mode and median of 0; the highest individual VDS score of 2 occurred three times. August VDS results for animals translocated to Southampton Water seawall and cobble shore populations were a mode and median of 4. A Kolmogorov-Smirnov Z test was carried out on the cumulative percent of females at each VDS stage (1-6, §2.2.6, Fig. 5.7). This showed that Polzeath March and August VDS results were not significantly different and neither were comparisons between the August 2002 results for cobble shore and seawall populations at Netley. Comparisons between both Netley populations and the August result for Polzeath *N. lapillus* showed that the frequency of higher VDS values in translocated animals was significantly different from those at Polzeath ($P<0.01$, Fig. 5.7).

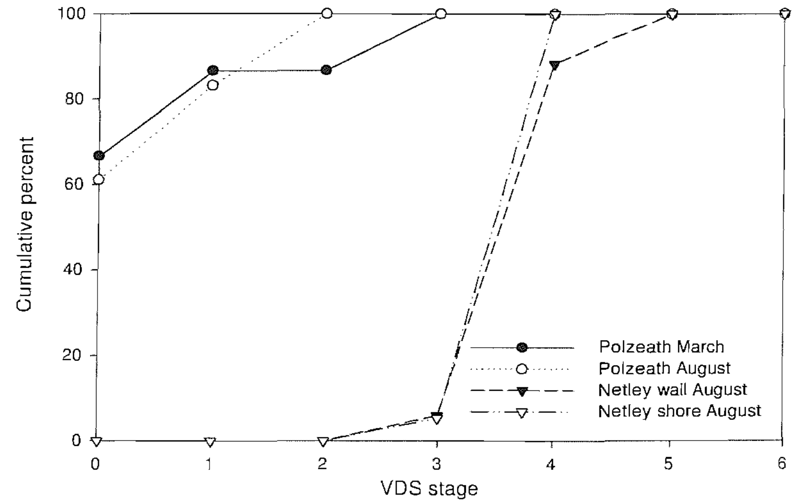

FIGURE 5.7 CUMULATIVE PERCENTAGE OF VAS DEFERENS STAGE FREQUENCIES IN POLZEATH CONTROL *NUCELLA LAPILLUS* (MARCH AND AUGUST 2002) AND NETLEY SEAWALL AND COBBLE SHORE TRANSLOCATED ANIMALS (AUGUST 2002).

Polzeath females had small penises (mean 1.18 mm) one third of the length developed during the short exposure time (6 months) in Southampton female *N. lapillus* (3.29 mm seawall, 3.53 mm, cobble shore). These did not reach the same size as males (Fig. 5.8), but still increased significantly. A two-way ANOVA comparing penis lengths for the factors site and sex was significant for main effects and interactions (Table 5.1). *Post hoc* Tukeys multiple comparisons showed that there was no distinction between female penis lengths from Southampton Water populations, but that a significant difference ($P<0.001$) existed between females from these populations and the controls at Polzeath (Table 1, Appendix 3).

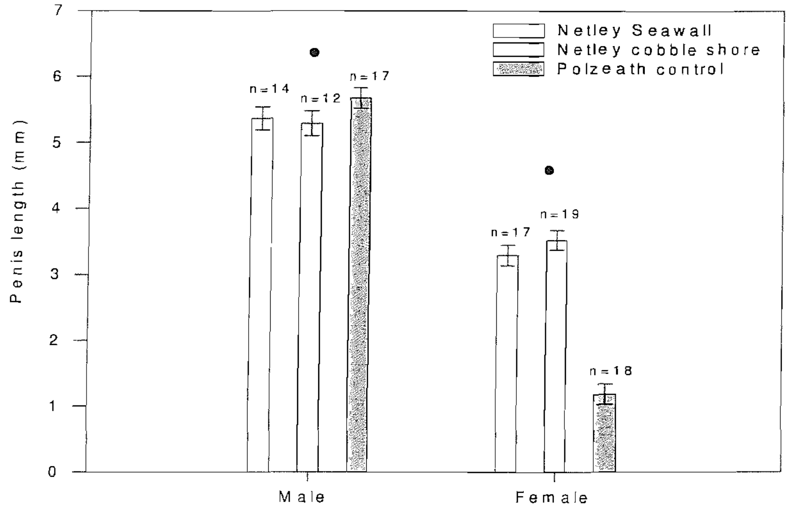

FIGURE 5.8 COMPARISON OF MEAN (+/- 1SE) PENIS LENGTHS FOR NETLEY TRANSLOCATION (SEAWALL AND COBBLE SHORE) AND POLZEATH (CONTROL) *NUCELLA LAPILLUS* POPULATIONS

TABLE 5.1 TWO-WAY ANOVA RESULTS OF PENIS LENGTH COMPARISONS FOR THE FACTORS SITE AND SEX FOR THE TRANSLOCATION POPULATIONS AT NETLEY SEAWALL AND COBBLE SHORE AND THE CONTROL POPULATION AT POLZEATH, NORTH CORNWALL.

August 2002	**DF**	**SS**	**MS**	**F**	**P**
Site Netley Wall/Shore/Polzeath	2	19.5	9.8	23.2	<0.001
Sex (M/F)	1	183.1	183.1	435.1	<0.001
Site x Sex	2	36.7	18.3	43.6	<0.001
Residual	91	38.3	0.4		
Total	96	287.4	2.9		

5.3.2 Survival rates

Following the translocation of *N. lapillus* to both habitats in March 2002, replicate population survival was monitored for six-months (Fig. 5.9). Rapid losses occurred in some initially caged populations on the seawall, although this was variable (Fig. 5.9A). Initially, uncaged individuals on the seawall declined at a similar rate as the caged animals, but quickly found shelter and the loss rate was negligible until May 2002 (Fig. 5.9B). Loss or mortality in caged cobble beach populations was low, but these had clearly declined by April (Fig.5.9C). Whilst considering that animals may have been present, but not found during searches, recovery of translocated populations declined throughout the experiment although uncaged replicates on the seawall had the slowest loss rate with the only major drop in August 2001 (Fig. 5.9B). Animals from uncaged populations on the cobbles declined steadily after translocation and by August 2002 these groups had the lowest abundance (Fig. 5.9D).

On some occasions (e.g. caged cobble shore populations in May 2001, Fig. 5.9C), the numbers of *N. lapillus* found increased. Where the total individuals found in each replicate was greater than in the previous month's survey, the additional individuals were recorded as alive over the intervening period. In addition in August 2002 settlement of *E. modestus* on *N.*

lapillus shells had obscured paint markings, making it difficult to identify from which population *N. lapillus* individuals originated. Therefore the adjusted data up to, and including, July results was formally analysed using a two-way ANOVA for the factors cobble shore, seawall and caged or uncaged release method.

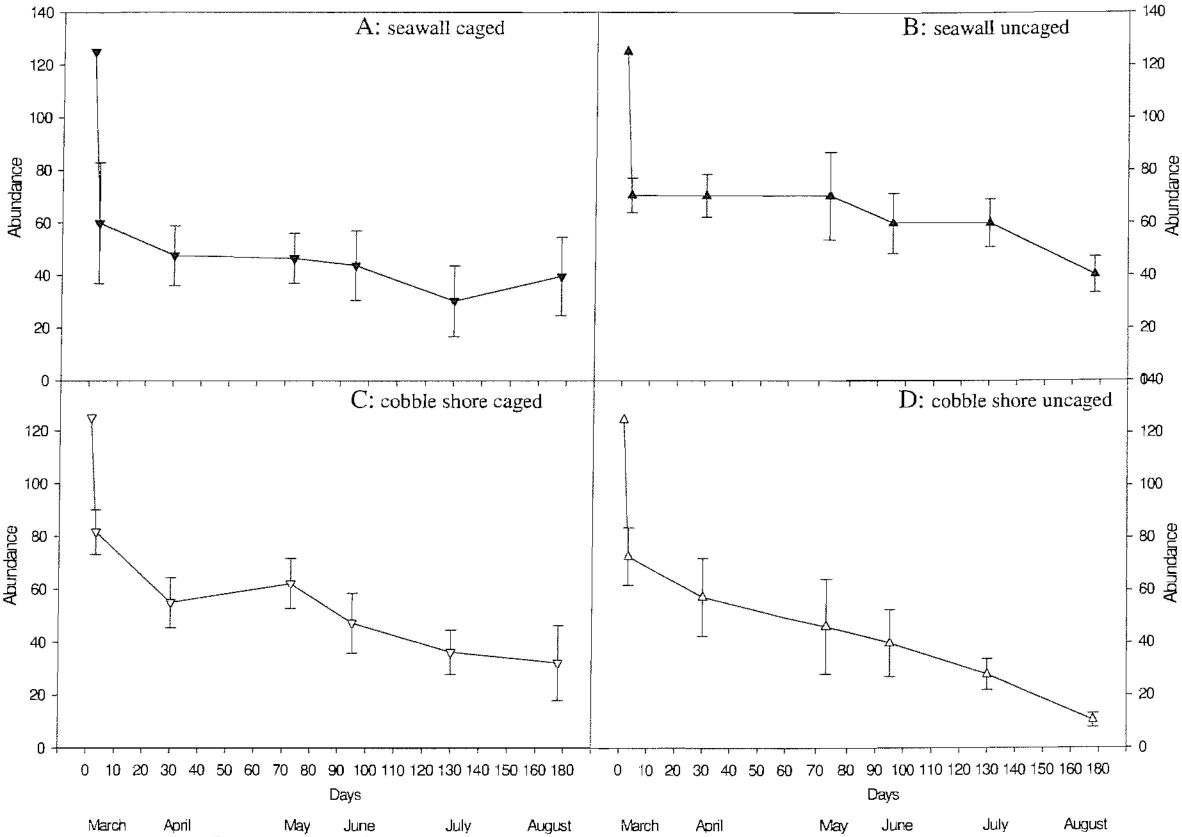

FIGURE 5.9 MEAN (+/- 1SE) SURVIVING *NUCELLA LAPILLUS* IN TRANSLOCATION POPULATIONS OF CAGED OR UNCAGED AND SEAWALL OR COBBLE SHORE GROUPS

The daily mortality rate data failed normality and were transformed to enable the two-way ANOVA. Comparisons between reintroduction site (seawall/cobbles) were not significant ($F_{(1,14)}$ 1.4, P = 0.2) and neither was the reintroduction method (caged/uncaged) ($F_{(1,14)}$ 1.0, P = 0.3). Interactions between reintroduction method and habitat were not significant at 5% ($F_{(1,14)}$ 3.5, P = 0.08), but indicated possible differences in survival.

Results of the Polzeath control *N. lapillus* surveys, carried out one day after return to Polzeath and in May and August 2002 (§ 5.2.4) showed abundance decline in the disturbed and transported control groups, but not the undisturbed (Fig. 5.10A,B). Recovery of control animals was low with 36% of the disturbance and 46% of transported control individuals lost within 1 day of placement back at Polzeath (Fig. 5.10B). By August 2002 few marked animals were found though the undisturbed population was relatively constant (Fig. 5.10A).

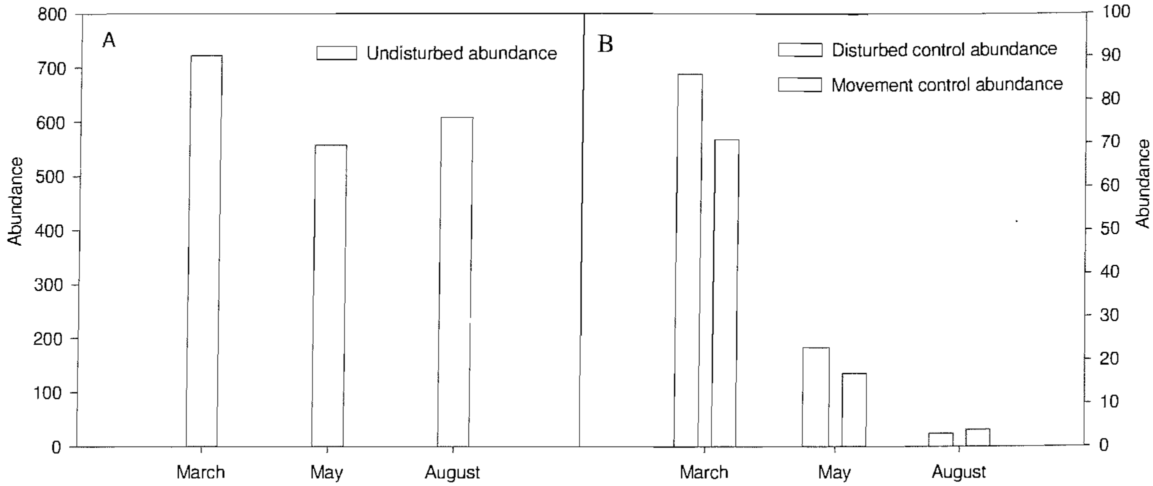

FIGURE 5.10 TOTAL ABUNDANCE OF *NUCELLA LAPILLUS* AT POLZEATH FOR A: UNDISTURBED INDIVIDUALS AND B: THOSE DISTURBED BY PICKING UP AND MARKING AND THOSE TRANSPORTED FROM POLZEATH TO SOUTHAMPTON AND RETURNED.

5.3.3 Movement of translocated *Nucella lapillus*

From March to August 2002, the monthly average movements of *N. lapillus* translocated to Netley foreshore were recorded, although the August results were not used in the final analysis. Results from the seawall populations showed that for site 2 (Fig. 5.11A), at the western end of the seawall (Fig. 5.4) movement patterns indicated a slight bias to the east of the wall, more noticeably in uncaged animals. For sites 4 and 6 the dominant direction was west, which mirrored the prevailing longshore drift (Fig. 5.11B,C). Site 8 (Fig. 5.11D) was the only location where individual *N. lapillus* moved both along and down shore to concrete blocks located 2 m west and 1-3 m down shore from the introduction point (Fig. 5.4). At site 8 animals did not show a directional preference between east and west (Fig. 5.11D)

The difference between initially caged and uncaged groups of *N. lapillus* was due to better early survival of uncaged wall populations (Fig. 5.9B), which allowed a greater dispersal of remaining individuals. In addition, following the first survey, it was found that at site 2 (Fig. 5.11A) some initially caged and uncaged *N. lapillus* moved 6 m and 11 m west respectively. This measurement may not have been due to natural processes, but rather interference by the public as site 2 was the nearest to a shore access point. The animals were left in place and allowed to disperse or to be lost from the site.

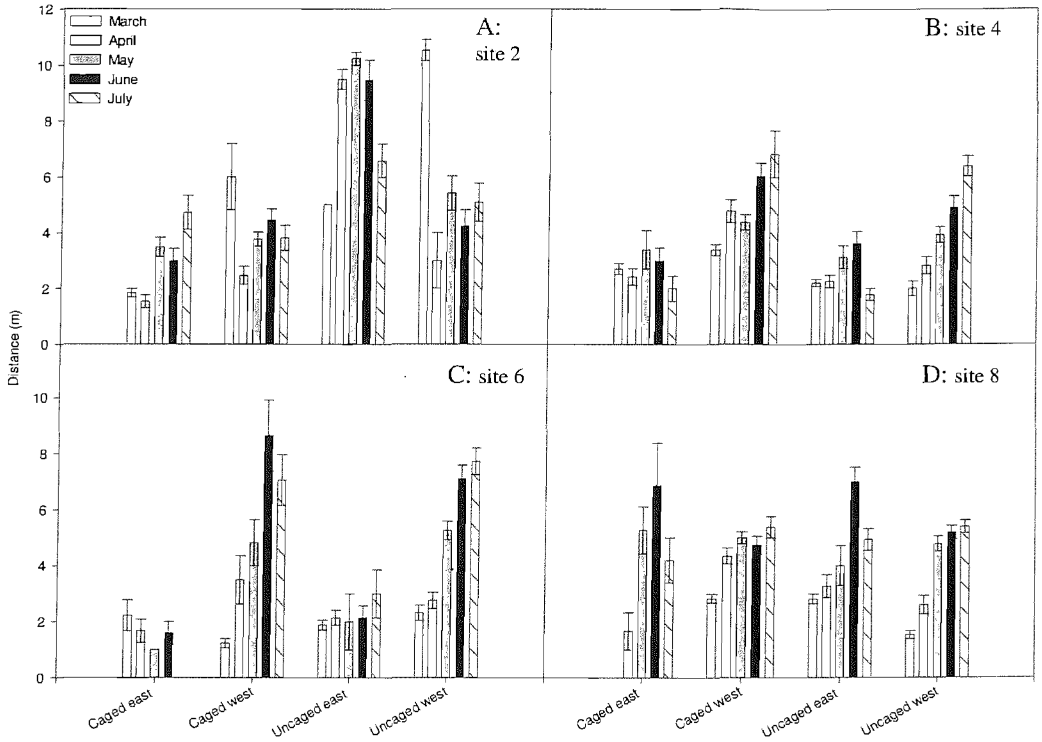

FIGURE 5.11 POOLED MEAN (+/- 1SE) DISTANCE MOVED IN AN EAST OR WEST DIRECTION BY *NUCELLA LAPILLUS* INDIVIDUALS TRANSLOCATED TO SEAWALL CAGED AND UNCAGED GROUPS FROM MARCH TO JULY 2002.

The movement of caged and uncaged *N. lapillus* from the cobble shore populations (Fig. 5.12) was broadly unidirectional (NW) toward the seawall though it is not clear if this was passive movement or active migration. Mortality or loss was high in the cobble shore groups, although individuals were still present when surveys ceased. In July, the last month when groups could be distinguished with certainty, *N. lapillus* from sites 1 (Fig. 5.12A) and 5 (Fig. 5.12C) showed large increases in distances moved (up to 22 m), suggesting that the sediment was unstable or that tidal currents were important vectors.

Many *N. lapillus*, although still on the cobble shore, were found up to 9 m away from their translocation sites having moved or been passively transported towards the wall. The cobble shore sites were located an average distance of 15 m away from the wall and after the 9 m point a band of sandy substrate, influenced by freshwater close to sites 1 and 3 (Fig. 5.4), ran in front of the seawall. Despite these physical barriers *N. lapillus* individuals from shore site 1, 3 and 5 caged and uncaged populations crossed the sand and reached the wall.

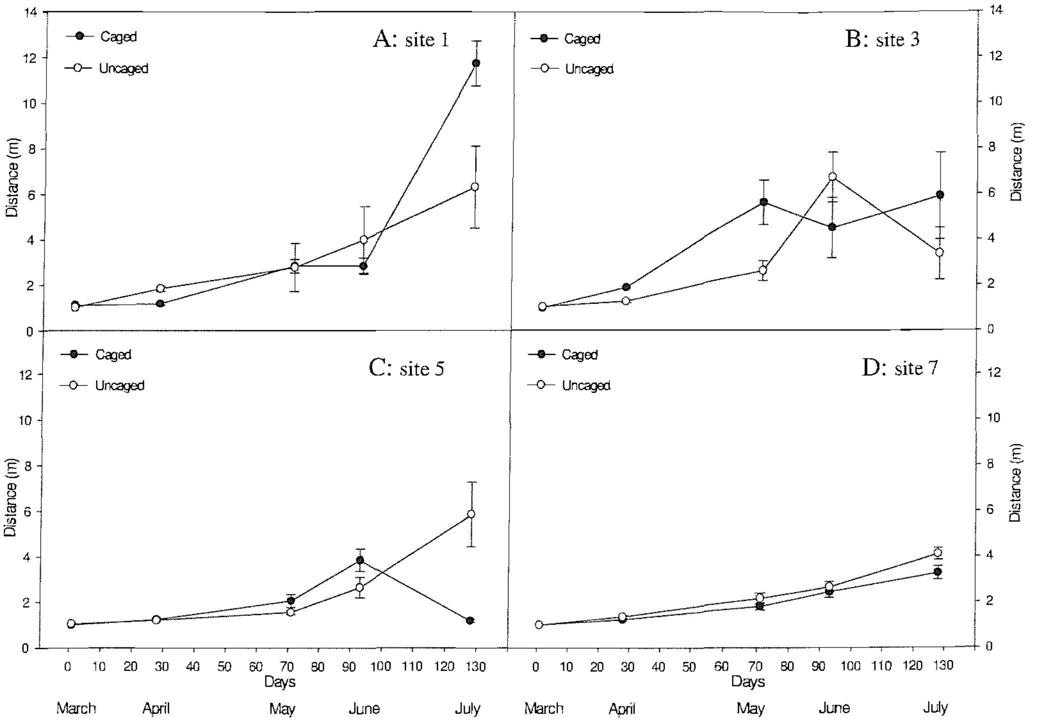

FIGURE 5.12 POOLED MEAN (+/- 1SE) DISTANCE MOVED BY *NUCELLA LAPILLUS* INDIVIDUALS TRANSLOCATED TO COBBLE SHORE CAGED AND UNCAGED GROUPS FROM MARCH TO JULY 2002.

No *N. lapillus* from site 7 (the farthest east, Fig. 5.4), achieved this. Mortality was high at this site with several empty shells and shell fragments recovered. This may have been due to high predation by *Carcinus maenas*, numbers of which increased sharply at this site between May and August 2002 (Fig. 5.13).

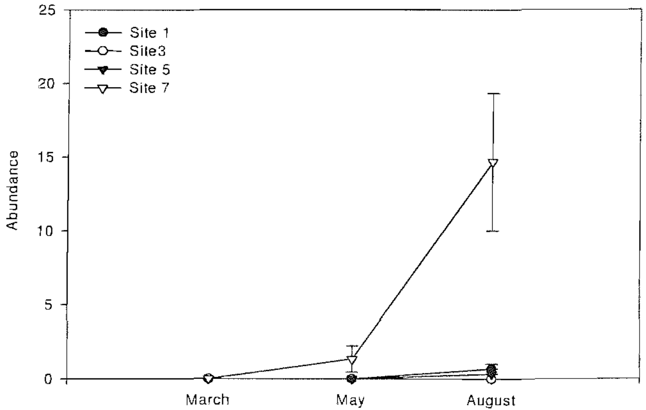

FIGURE 5.13 MEAN (+/- 1 SE) ABUNDANCE OF *CARCINUS MAENAS* AT COBBLE SHORE SITES (1,3,5,7) FROM MARCH TO AUGUST 2002.

Dispersal distances were related to the available *N. lapillus* within each group. As *N. lapillus* abundance declined, a smaller pool of animals was available to move away from their release points. Dispersal from the seawall sites (2,4,6,8) was broadly east or west along the wall. Conversely, those individuals from the cobble shore actively or passively moved towards the wall (sites 1,3,5,7). Due to the randomness of individuals found, distance measurement results were unbalanced and failed homogeneity of variance and normality. Transformations were not successful and sub sampling to create a balanced set was not possible due to the low numbers at site 7 (cobbles) in June and July. Therefore a Kruskall Wallis ranked ANOVA with Dunns multiple comparisons (Table 2, Appendix 3) was carried out. This test did not allow for interactions between site, introduction method and month.

Distances moved by individuals in March (Fig.5.13A) were significantly different (K.W. N = 16 $H_{(15)}$ = 650, P<0.001). Caged and uncaged groups on the seawall initially dispersed significantly greater distances than cobble shore populations. This was with the exception of initially caged *N. lapillus* from seawall site 6 which did not widely disperse due to high losses after the cage was removed (Fig. 5.14A). Uncaged *N. lapillus* from site 2 on the seawall moved a significantly greater distance (median 10 m; Fig. 5.14A) although the public may have moved these. Both caged and uncaged cobble shore groups remained clumped around their release points showing similar, but limited dispersal.

Similar significant differences were seen in April (Fig. 5.14B) (K.W. N = 16 $H_{(15)}$ 401, P<0.001). Some minor differences from the March results were seen with individuals from cobble sites, (1-uncaged and 3-caged), ranging further (Table 2, Appendix 3). Caged *N. lapillus* at site 3 (cobbles) moved significantly greater distances (P<0.05) than all others except uncaged individuals from cobble shore sites 1 and 7. This pattern was largely repeated for May (K.W. N = 16 $H_{(15)}$ 297, P<0.001), with some individual *N. lapillus* from site 3 (Fig.5.14C) travelling up to 15 m from the cobbles to the seawall.

In June there were still significantly greater differences in movements of seawall populations compared to shore groups (K.W. N = 16 $H_{(15)}$ 174, P<0.001) (Table 2, Appendix 3). Several individuals from cobble shore sites (in particular site 3) had arrived at the seawall and remained clustered together. *N. lapillus* from both caged and uncaged translocation groups at seawall site 6 had moved up to 15 m from source. The early high losses from site 6 and subsequent long distance movement towards refuges on the wall suggests a strong influence of water movement. The initial limited movement of *N. lapillus* from some cobble shore sites had increased (sites 1 and 3, Fig. 5.14D), with many reaching the wall thus obscuring differences between treatments (Table 2, Appendix 3). The exceptions to this were sites 5 and 7 (Figure 5.14D) where losses were high and estimates of movement limited.

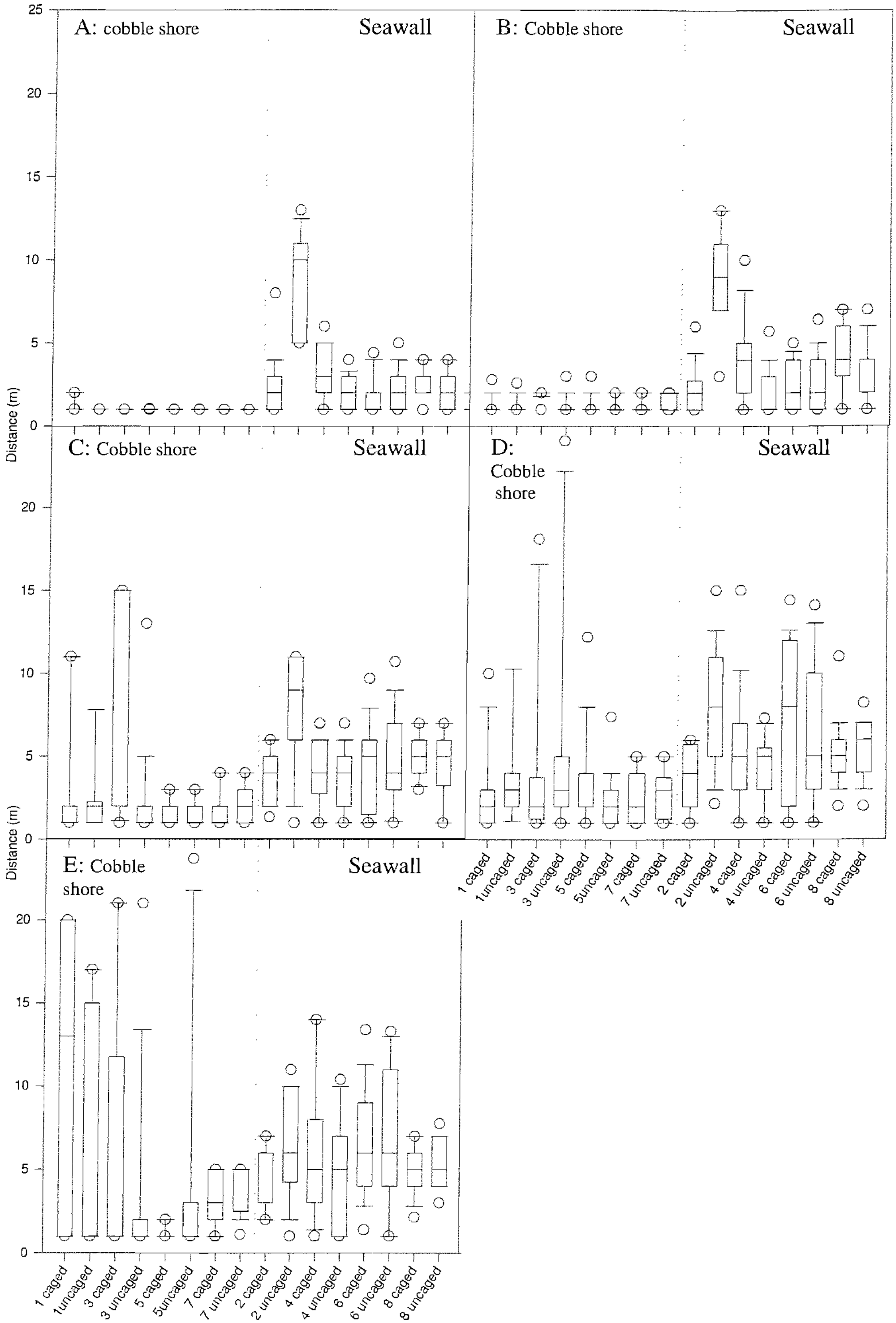

FIGURE 5.14 DISTANCES MOVED BY CAGED (FOR FIRST WEEK) AND UNCAGED *NUCELLA LAPILLUS* AT COBBLE SHORE SITES (1,3,5,7) AND SEAWALL SITES (2,4,6,8).
A: March, B: April, C: May, D: June, E: July, see Fig. 5.2 for map of Netley foreshore and seawall. Median, 25th, 75th (box), 5th and 95th (whiskers) percentiles and outliers

In July (Fig. 5.14E) increasing *N. lapillus* from both caged and uncaged cobble shore populations had arrived on the wall and the significant differences between treatments (K.W. N = 16 $H_{(15)}$ 133, P<0.001) were due to this. The exceptions were site 5-caged, 3-uncaged and both populations from site 7. These were the only groups from which dispersal was limited, possibly due to predation reducing numbers (Fig. 5.13). At seawall site 6, caged and uncaged *N. lapillus* had still moved a significantly greater distance than those from cobble sites 3 and 5 (Table 2, Appendix 3). Dunns comparisons (Table 2, Appendix 3) showed that the major change from previous results was that for the first time individuals from the cobbles (sites 1 and 3) had dispersed a significantly greater distance than the other cobble and seawall groups. This process may have continued with *N. lapillus* from other cobble shore sites arriving on the seawall. Unfortunately by August barnacle settlement on the shells made distinguishing between treatment groups difficult and data collection was discontinued.

5.3.4 Community changes

The survey results in February 2002 of the cobble shore and seawall communities suggested that the assemblages at both locations were broadly similar, but that relative abundances were different. Patterns of variation between locations in February and subsequently in May and August 2002 were assessed using non-metric multi dimensional scaling (nMDS) (Fig. 5.15). A formal comparison was made using a Bray-Curtis (Bray and Curtis, 1957) similarity matrix on square root transformed data (Thacker and Bromley, 2001) using the SIMPER routine in Primer 5 (Clarke and Gorley, 2001) (Table 5.2).

In February 2002 the seawall assemblages (2,4,6,8) were closely clustered indicating a very similar community structure. Cobble assemblages (1,3,5,7) were more widely scattered reflecting the effects of greater physical variety on the shore on community composition (Fig. 5.15A). For example, sites 5 and 7, shown on the lower half of figure 5.15A, were further to the east on the shore in a low-lying region. The outlier replicate at site 5 had limited *E. modestus* cover and the other site 5 and site 7 cluster was due to lower *Littorina littorea* numbers which were dominant at other release points.

SIMPER analysis of February 2002 results (Table 5.2A) showed that the absence of limpets on the shore and the greater abundance of *L. littorea* on the seawall were the main factors responsible for dissimilarity. More significantly prey species of *N. lapillus* such as *E. modestus* and *Mytilus edulis* were more abundant at wall sites and when combined contributed to 30% of the dissimilarities between wall and shore locations.

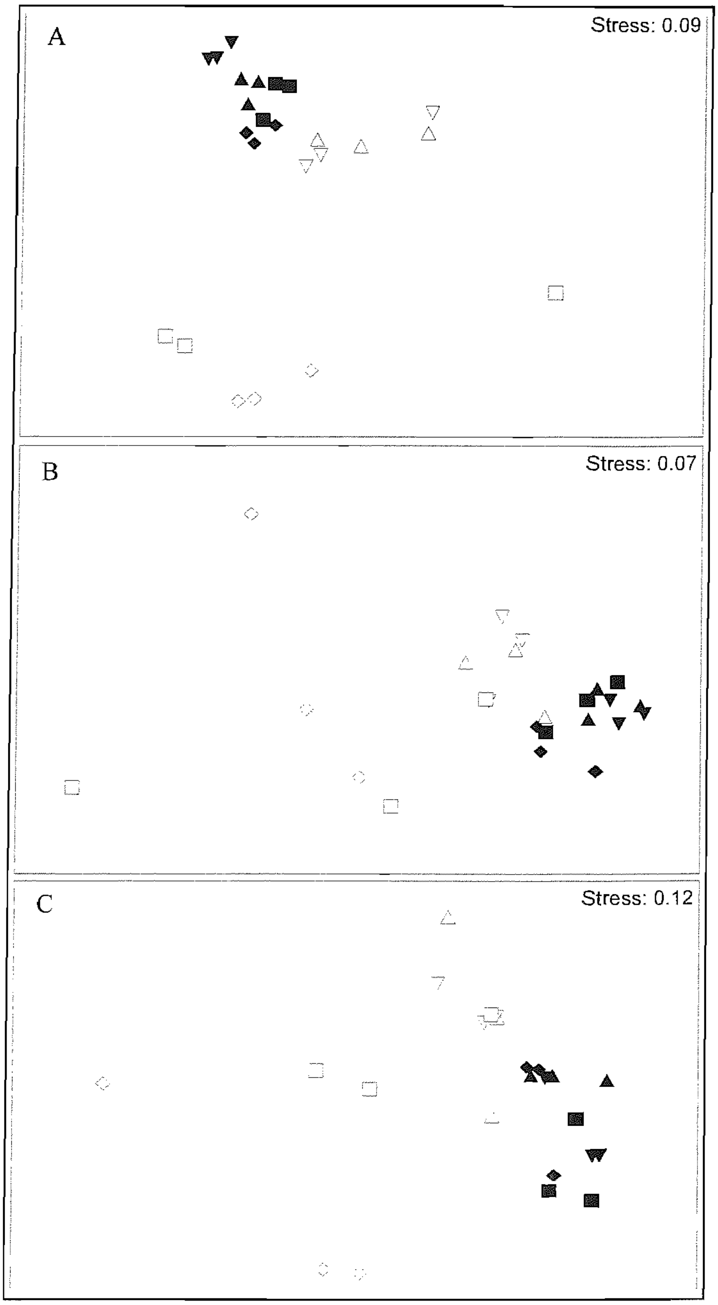

FIGURE 5.15 NON METRIC MULTI DIMENSIONAL SCALING (NMDS) PLOTS OF SEAWALL AND COBBLE SHORE COMMUNITY ASSEMBLAGE RESULTS FOR: A: FEBRUARY 2002, B: MAY 2002, C: AUGUST 2002
Three replicates per site. Wall sites 2▲, 4▼, 6■, 8◆. Cobble/boulder sites 1△, 3▽, 5□, and 7◇.

NMDS plots (Fig. 5.15B,C) and SIMPER comparisons for both May and August 2002 (Table 5.2B,C) showed that wall and shore sites were similarly clustered to March results (Fig. 5.15A). Both May and August 2002 Bray-Curtis dissimilarities remained largely unchanged from March, although clustering was less distinct due to *L. littorea* and *M. edulis* decline on the seawall. For all of the community assemblage surveys the main effect on dissimilarity was limpet abundance, which was greater on the seawall, and this effect did not differ apart from the influence of patches of *Chondrus crispus* in August 2002 (Table 5.2C).

TABLE 5.2 SIMPER (SIMILARITY PERCENTAGE BREAKDOWNS) FOR COMMUNITY ASSEMBLAGE RESULTS FROM THE NETLEY SEAWALL AND COBBLE SHORE TRANSLOCATION RELEASE SITES FOR FEBRUARY, MAY AND AUGUST 2002.
Av.Abund: the average abundance of each species in each group of samples (in this case treatments), Av,Diss: The average dissimilarity among groups of samples, Diss/SD: measure of variation of species contribution to group dissimilarity, Contrib%: the percentage contribution that species gives to between group dissimilarity.

A: February 2002	**Treatment Groups: Seawall and Cobble substrate Average dissimilarity = 37.5**				
Species	**Av.Abund Seawall**	**Av.Abund Substrate**	**Av.Diss**	**Diss/SD**	**Contrib%**
P. vulgata	64.8	0.0	12.6	3.9	33.7
L. littorea	312.9	187.8	11.8	1.2	31.4
E. modestus	84.5	46.8	6.3	1.1	16.7
M. edulis	26.8	3.5	4.9	1.5	13.2
B: May 2002	**Treatment Groups: Seawall and Cobble substrate Average dissimilarity = 34.3**				
Species	**Av.Abund Seawall**	**Av.Abund Substrate**	**Av.Diss**	**Diss/SD**	**Contrib%**
P. vulgata	56.4	1.7	12.05	2.9	35.2
L. littorea	268.3	182.6	10.7	1.2	31.1
E. modestus	75.8	47.3	4.7	0.9	13.7
M. edulis	16.3	2.8	4.2	1.5	12.3
C: August 2002	**Treatment Groups: Seawall and Cobble substrate Average dissimilarity = 37.1**				
Species	**Av.Abund Seawall**	**Av.Abund Substrate**	**Av.Diss**	**Diss/SD**	**Contrib%**
P. vulgata	55.9	1.08	12.1	2.4	32.7
L. littorea	220.8	198.8	10.4	1.3	28.0
E. modestus	72.8	43.0	6.0	1.1	16.2
C. crispus	3.7	4.2	3.2	1.8	8.6
M. edulis	9.4	2.9	3.0	1.5	8.1

Translocated *N. lapillus* were not observed feeding on the abundant *L. littorea* on the seawall and the reason for the decline in this species is not clear although *L. littorea* have been shown to migrate up and down the shore (Newell, 1958; Warner, 2001). *N. lapillus* on the seawall were, however, found feeding primarily on the relatively uncommon *M. edulis* immediately following translocation from the mussel-dominated Polzeath site. They continued this preference until the competition for *M. edulis* appeared to force a switch to barnacles.

Whilst not a primary aim of this work, *M. edulis* abundance on the seawall in particular, appeared to decline following the translocation of *N. lapillus* to Netley. A one-way ANOVA on *M. edulis* abundance results showed that a significant decline in mussels occurred on the seawall ($F_{(2,35)}$ 3.55, $P<0.05$) (Fig. 5.16a). Abundance change results for the cobble shore *M. edulis* populations were not significant ($F_{(2,35)}$ 0.42, $P = 0.6$). Though *N. lapillus* did switch from *M. edulis* to barnacles there were no significant decreases (at 5%) in barnacle cover on the seawall ($F_{(2,119)}$ 2.51, $P = 0.08$) although the ANOVA result suggested some effects (Fig. 5.16B). No significant decline was observed on the cobble shore sites ($F_{(2,119)}$ 0.76, $P = 0.5$).

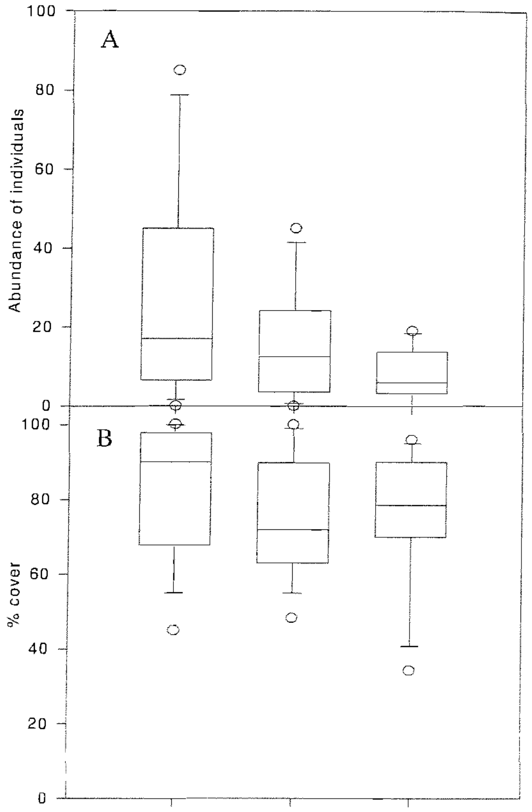

FIGURE 5.16 ABUNDANCE IN FEBRUARY, MAY AND AUGUST 2002 OF A: *MYTILUS EDULIS* (COUNTS OF INDIVIDUALS) AND B: PERCENTAGE BARNACLE COVER AT THE NETLEY SEAWALL SITES (2,4,6,8) FOLLOWING THE TRANSLOCATION OF *NUCELLA LAPILLUS* FROM POLZEATH TO NETLEY.
Median (5th, 25th, 75th and 95th percentiles and outliers).

5.4 Discussion

5.4.1 Procedural problems

There was poor recovery of individuals from all populations and, although not significant, losses were slightly higher for the cobble shore groups. Although not quantifiable, predation by the shore crab *Carcinus maenas* (Moore, 1939; Hughes and Elner, 1979) had the potential to be an important factor for the cobble shore populations, particularly at site 7. Due to selective pressure from predation, wave action and the lack of a pelagic stage, *N. lapillus* populations have developed in relative isolation. Populations show genotypic selection, which is phenotypically expressed as polymorphism in shell shape (Kitching *et al.,* 1966; Hughes and Elner, 1979; Gibbs, 1993b; Pascoe and Dixon, 1994; Etter, 1996; Trussell and Etter, 2001). Relatively unpolluted exposed shore *N. lapillus* which have larger shell apertures enabling an expanded foot and greater resistance to dislodgement are particularly common in the south of the UK. Conversely, sheltered shore morphs with thicker and smaller aperture shells which give protection against desiccation and predation by *C. maenas* (Gibbs, 1993b; Hughes and Elner, 1979; Hughes and Taylor, 1997; Trussell and Etter, 2001), are still rare in this region due to poor recovery from TBT pollution. Ideally sheltered shore morphs would have been used to mitigate the impacts of predation on shore populations, but there are few source sites available in southern Britain. Of the 2000 transplanted to Netley in March 2002, however, 551 animals were still present in August 2002.

The value of control populations in this work has been shown by the high losses of *N. lapillus* at Polzeath in both groups. Removing and replacing *N. lapillus* at the high energy Polzeath control site may have induced wave shock stress resulting in high early losses not seen at Netley where physical conditions were relatively sheltered. The poor control animal recovery at Polzeath was also probably made worse due to the complex physical structure of the habitat with many available refuges and very high *N. lapillus* abundance. Whilst controls were necessary, the rapid decline in these populations did highlight some difficulties with translocation designs.

Initially it was envisaged that six months of reliable observations would be achieved. By July, individuals from shore populations were increasingly arriving at wall sites and this trend would have proven useful to explore further. During July-August 2002, however, the shell markings were obscured by dense settlement of *E. modestus* in Southampton Water, thus further identification of population groups was not reliable.

5.4.2 Imposex in Southampton Water

High concentrations of TBT found in sediment in Southampton Water (e.g. Hythe Marina 5.6 $\mu g/g^{-1}$) have been recorded in sediment and the water column (Thomas *et al.,* 2000, 2001). *N. lapillus* can bioaccumulate TBT from these sources and its prey (Bryan *et al.,* 1989; Ruiz *et al.,* 1996). There are no direct measurements of TBT at Netley although these records from other sites in Southampton Water indicate that concentrations are likely to be high.

It has been suggested that the long term survival of TBT affected *N. lapillus* populations would be unlikely at TBT/water levels above $2ngl^{-1}$ (Langston *et al.,* 1990; Harding *et al.,* 1999). In August 2002, imposex in *N. lapillus* from the seawall and cobbles was assessed. Statistical comparison between the wall, shore and control populations from Polzeath showed that in six months Netley transfer groups had developed significantly greater imposex levels than Polzeath control animals, which remained at the March 2002 level. Animals directly in contact with the cobbles did not develop greater VDS levels than the seawall populations, but mean penis length for shore based females was greater, although not significantly.

Morphological change, with the full development of a vas deferens in females at Netley, had occurred between March and August 2002 with a modal VDS of stage 4 and some individuals at stage 5. The development of imposex through stages 1-3 can be rapid: (half lives of stages 1-2 approximately 1-8 weeks, stage 3 around twice as long (Davies, 2000) though there may be a considerable lag between the onset of stage VDS 4 and progression to sterility at stage 5 (Davies, 2000). Some females at Netley were still able to lay eggs by the end of the regular monitoring period, as several egg clusters on both wall and shore sites were found between May and August 2002. During additional visits in November 2002 and January 2003 more egg clusters were found on the seawall. This demonstrates that VDS stage 4 (or less) was maintained for 6 months by some females although by January the numbers of remaining *N. lapillus* were greatly reduced, possibly due to TBT related mortality. Throughout sampling no juveniles were found despite large egg clusters being seen. Very small juveniles seek suitable shelter, for example in empty barnacle cases, so are difficult to record (Crothers, 1985). In addition imposex develops more rapidly in *N. lapillus* recruits (Spence, 1989; Harding *et al.,* 1997; Davies, 2000), which may have been a factor in high juvenile mortality at the Netley sites.

An interesting point is that reports of the previous populations of *N. lapillus* in Southampton Water were confined to the Netley foreshore (Langston *et al.,* 1994). The seawall was built in the 1950's and since then wave reflection has stripped away the fine sediment creating a moderately exposed cobble and gravel shore with left over blocks from the wall construction. *N. lapillus* appears to be having difficulty recolonising some areas, including locations previously seriously polluted by TBT (e.g. Plymouth Sound). Conversely there have been several examples of recovery such as a formerly polluted inlet in Cornwall (Crothers, 1998, 2003) and at North Sea sites on mainland Europe and the east coast of the UK (Birchenough *et al.,* 2002a,b). At Southampton, however this has not occurred naturally, perhaps due to locally persisting TBT levels impacting recruits, or the loss of nearby source populations due to long-term TBT pollution in the Solent system.

5.4.3 Survival of *Nucella lapillus* at Netley

The impacts of predation and the availability of prey may have been factors in the decline of translocated *N. lapillus* populations. Particularly once released on the seawall, uncaged *N. lapillus* rapidly took up shelter whereas the caged populations already had this advantage. *C. maenas* are common predators upon *N. lapillus* (Hughes and Elner 1979; Hughes and Taylor, 1997; Trussell and Etter, 2001). Birds such as oystercatchers, which are common in Southampton water, also feed on *N. lapillus* (Moore, 1938b; Feare, 1967; Nagarajan *et al.,* 2002) and were seen at Netley during fieldwork. However, predation may not explain the majority of losses from the seawall populations as birds foraged lower on the shore and it is not clear if *C. maenas* could reach the levels at which *N. lapillus* were clustered. *C. maenas* attacks all *N. lapillus* when they are encountered (Hughes and Elner, 1979). As caged populations remained clumped in groups after the cages were removed they may have been easily detected by foraging *C. maenas* resulting in high predation impacts. Although *C. maenas* would not have encountered *N. lapillus* on the Netley shore for several years they can transfer prey-handling skills between similarly shaped species (Hughes and O'brien, 2000). Recovered shells crushed by crabs (Hughes and Elner, 1979) were, however, rare and did not account for the numbers of *N. lapillus* lost. This suggests that *N. lapillus* individuals or shell debris may have been lost due to water movement or that bird, rather than crab predation, was common.

At Polzeath *M. edulis* was the dominant prey of *N.* lapillus. Mussels support less growth than barnacles (Burrows and Hughes, 1990), although this is related to environmental factors such as higher exposure reducing foraging time (Burrows and Hughes, 1989; Etter, 1996). On the cobble shore sites at Netley *M. edulis* was relatively rare whereas isolated clusters were found on the wall. It was found that once *N. lapillus* had dispersed along the wall several individuals sought shelter and prey among *M. edulis* patches. Despite highly abundant *E. modestus* these dogwhelks initially showed a preference for their original prey, a trait which has previously been identified (Fischer-Piette, 1935 *op cit* Hughes and Burrows, 1993; Burrows and Hughes, 1991). *M. edulis* patches on the seawall declined significantly over the experimental period (Fig. 5.16). This may have been due to *N. lapillus* feeding, though ideally this experiment should have been controlled through the monitoring of *M. edulis* patches on the seawall without *N. lapillus* predation, as losses may have been due to other factors such as thermal stress or wave action and sediment abrasion (Harley *et al.,* 2003).

Hughes and Burrows (1993) noted that the long-term persistence of TBT, and its impact on *N. lapillus* populations, may have helped to identify a community structuring role for dogwhelks after predation by them was reduced. In Southampton Water the high levels of TBT pollution resulted in the death of the population at Netley (Langston *et al.,* 1994). Without the impact of TBT on *N. lapillus* at Netley, cobble shore and seawall communities may have developed in a more patchy nature due to predation by dogwhelks, though their influence is thought to be localised (e.g. Connell, 1961).

When competition for *M. edulis* is high, more than one *N. lapillus* will predate on an individual mussel (Moore, 1939). This was seen on the Netley seawall, though over time competition for the reducing *M. edulis* patches became so intense that many *N. lapillus* individuals were forced to switch to barnacles. Desired prey become scarce close to refuges which limits choice, Menge, 1978a,b; Hughes and Burrows, 1993). Switching between preferred organisms has been observed when prey abundance is reduced (Fischer-Piette, 1935 *op cit* Hughes and Burrows, 1993; Largen, 1967; Burrows and Hughes, 1991) and the long-term persistence of *N. lapillus* on the seawall may further alter the age and size structure of *M. edulis* patches. It was also noted that *L. littorea* declined on the wall sites though this may have been due to normal migration between shore zones (Newell, 1958; Warner, 2001). *N. lapillus* were not seen feeding on *L. littorea*, but they were found sheltering with them. It may be that *N. lapillus* fed upon *L. littorea* which they will take when other prey are limited (Moore, 1938b; Largen, 1967; Crothers, 1985). Littorinids will avoid predators and can detect damaged conspecifics (Jacobsen and Stabell, 1999; Keppel and Scrosati, 2004).

5.4.4 Movement of *Nucella lapillus* at Netley

Underwood and Chapman (1989) found a littorinid that had random movement on rough terrain, but was more directional over less complex surfaces. Although *N. lapillus* are reported to move little in their lifetime (Connell, 1961; Hughes, 1972; Castle and Emery, 1981; Crothers, 1985; Etter, 1996; Cook and McMath, 2001) individuals from the cobble shore (sites 1 and 3) were found at increasing distances from their release sites having moved towards the wall. By July some were found up to 22 m away from their release sites, crossing a gap of up to 7 m of sand either actively or passively. *N. lapillus* cannot voluntarily cross sand (Etter, 1996), but have been seen rolling in surf on sandy beaches (Pers. obs.) and whether passive or active this may be how *N. lapillus* moves to new areas. In addition *N. lapillus* cannot tolerate low salinity (Wilson *et al.,* 1983; Crothers, 1985; Stickle *et al.,* 1985) and the influence of the freshwater stream at the western end of the shore (Fig. 5.4) may have prompted some *N. lapillus* (from cobble shore sites 1 and 3 and seawall site 2) to move away. The stream, however, was not large and any influence on these sites was probably minimal.

Lower prey density on the shore may also have caused *N. lapillus* to move further as, when hungry, dogwhelks can make decisions based on the need to forage balanced against shelter and the risk of predation (Burrows and Hughes, 1991a,b; Vadas *et al.,* 1994). Motivated by hunger *N. lapillus* will move in straight lines (Hughes and Dunkin, 1984) and during the summer have been shown to move up shore (Hawkins and Hartnoll, 1983). This suggests that as well as passive transport and seasonal up-shore migration, increased prey density on the seawall may have motivated individuals to move in this direction. In addition to foraging, muricid gastropods seek shelter when conditions are unfavourable (Moran, 1985; Burrows and Hughes, 1989). The open area between shore sites and the seawall was largely barren of both prey species and shelter particularly at sites 5 and 7.

Dispersal of seawall based *N. lapillus* was variable. *N. lapillus* usually forages only when covered by the tide (Palmer, 1984; Burrows and Hughes, 1989) and for seawall sites 4 and 6, dispersal from release points was generally west in the direction of prevailing longshore drift. At seawall sites 2 and 8 dispersal patterns were less well defined, but indicated a choice in response to emersion as both sites were at either end of the wall (Fig. 5.4) and emersed for longer than those towards the middle. Both caged and uncaged individuals from site 2 showed a minor easterly trend whereas those from site 8 moved slightly west, but more often down shore. This pattern suggests that dogwhelks on the seawall moved towards areas submerged for longer thus increasing foraging time and minimising desiccation; *N. lapillus* from exposed shores such as Polzeath are less able to tolerate prolonged exposure to air (Hughes and Taylor, 1997). From March to June, individuals on the seawall dispersed further east and west than the cobble shore groups and sheltered in cracks in the seawall. The cobble shore groups generally remained at their release sites for longer, perhaps inhibited by the rougher terrain. By July, however, they had begun to arrive at the seawall in increasing numbers possibly assisted by wave action.

Survival rates in both caged and uncaged populations were not significantly different though it appeared that clustering in caged groups on the wall and shore may have resulted in immediate losses after cages were removed, particularly on the wall. Conversely the cages provided some initial shelter, particularly for shore groups where uncaged losses were high, but predation later in the experiment may have accounted for losses in caged and uncaged cobble shore groups. Being uncaged on the wall gave the best chance of survival with the robustness of *N. lapillus* allowing them to seek refuge and forage immediately after release. This suggests that the harder, though artificial, substrate with it shelter and abundant prey, was preferred and the abiotic and biotic conditions on the wall were more favourable.

5.4.5 Translocation practicalities

Netley foreshore is an isolated site from which the local *N. lapillus* population was eradicated due to TBT pollution (Langston *et al.,* 1994). Reintroduction of *N. lapillus* at Netley was tested as a pilot for other isolated and sheltered sites where no local recoloniser source of *N. lapillus* is available. This option may not always be appropriate as at some sites *N. lapillus* is recolonising without assistance (Crothers, 1998, 2003; Birchenough, *et al.,* 2002b).

In previous work carried out for the purpose of monitoring TBT levels, permanently caged *N. lapillus* were used (e.g. Harding *et al.,* 1999; Quintela *et al.,* 2000). In the present study cages were utilised to provide greater initial protection from predation (5 days) than was available to free individuals (Quintela *et al.,* 2000) and the possibility to acclimatise to prevailing abiotic conditions (Kirby *et al.,* 1994a). It was found, however that caged populations on the wall declined quickly, therefore caging was not valuable in this case and it was concluded that the translocation of sufficient numbers was more useful.

The early endurance of translocated *N. lapillus* on the Netley cobble foreshore was similar for all populations and these survived longer than the disturbance and transport controls. It is possible that stress from disturbance and movement then return to the exposed conditions at Polzeath resulted in high mortality or loss of control animals. Conversely the relatively benign conditions at Netley would not have been stressful to acclimatise to in terms of exposure, but the risk of predation impacts due to the exposed shore shell morphology of Polzeath *N. lapillus* may have been greater (Kitching *et al.,* 1966; Crothers, 1975).

Results from the reintroduction of exposed shore *N. lapillus* from Polzeath to Netley foreshore suggest that even though the exposed shore, thin shelled morphs are not ideally suited to the local conditions, they appeared robust enough to survive the process. Without the influence of long-term TBT pollution, populations would probably have successfully established. TBT concentrations in Southampton Water would currently prevent long-term survival of *N. lapillus* at Netley, but in the longer term once the total ban against TBT is in force and its levels in sediment decline, reintroduction may be worth a further trial.

The reintroduction of *N. lapillus* also requires consideration of genetics as a controlling factor on the long-term survival of translocated individuals. Gastropods produce some of the best examples of spatial genetic variation due to environmental and predation pressures (Kirby *et al.,* 1994a). Populations of *N. lapillus* may be genetically divergent with exposed shore morphs having 2n:26 and hybrid variation up to 2n:36 diploid pairs of chromosomes (Day *et al.,* 1994; Pascoe *et al.,* 1994). Chromosomal differences are phenotypically expressed with exposed and sheltered shore morphs varying in size, shape and colour.

Often darker morphs are from exposed shores (Etter, 1988b) and are more likely to be thermally stressed on sheltered shores. In addition those with 26 pairs of chromosomes have a smaller mantle cavity which leaves them less able to withstand hyperosmotic stress than sheltered shore morphs (Day *et al.,* 1994). Those from Polzeath were exposed shore morphs, and will have been under greater stress from thermal factors, but were seen dispersing to preferable habitats. Conversely, although the translocation process would have been stressful, exposed shore animals have a larger pedal size (Etter, 1988a; Gibbs, 1993b) and this may have enabled a sufficient number to remain attached to the substrate compared to the apparent loss of control animals at Polzeath where energy was higher. Therefore it is possible that the exposed morphs were suitable to translocate in this case as they easily withstood the wave pressure and sheltered from thermal stress and predation.

The reproductive effort of exposed shore dogwhelks differs from sheltered morphs as they can produce twice as many eggs with twice as many juveniles emerging (Etter, 1989). When raised in uniform laboratory conditions, exposed and sheltered shore juveniles grew similarly (Etter, 1989), but when exposed shore juveniles were placed in sheltered conditions they developed the associated morphology (Gibbs, 1993b). It was predicted that sheltered shore morphs would reappear soon after recolonisation if the plasticity seen in exposed shore morphs (from Bude, north Cornwall) was constant (Gibbs, 1993b).

Direct developing *N. lapillus* has recolonised former habitats and passive transport, although over a relatively short distance, has been suggested by this work. Many workers report that

N. lapillus moves little in its lifetime (e.g. Crothers, 1985; Etter, 1996; Cook and McMath, 2001), but genetic research suggests that passive transport may be over greater distances than seen here. Genetic identity between *N. lapillus* populations in North America and Europe varied little and Day *et al.*, (1994) suggested that passive transport of juveniles significantly contributed to gene flow and that "*long distance dispersal may not be negligible in direct developers*". Sites are being recolonised more rapidly than expected and genetic studies show that this is achieved by individuals from several locations (Colson and Hughes, 2004).

Two years after the reintroduction, no translocated *N. lapillus* were found at Netley, presumably due to the long-term impacts of TBT. Although *N. lapillus* had not arrived at Netley naturally, it is possible that though the site is remote, recolonisation will occur without assistance. Should this not occur following TBT decline in Southampton Water, results here suggest that a large number of exposed shore morphs placed directly on to the seawall without initial protection may have the best chance of long term population survival.

5.5 Summary

Experiments testing *N. lapillus* reintroduction at Netley suggest that the process is viable provided that a suitable design is adapted and long-term pollution levels are favourable. Translocated *N. lapillus* were sufficiently robust to survive the process and it is possible that exposed shore morphs have some advantages. Active dispersal from unfavourable conditions with subsequent passive transport to the seawall was indicated with many of the arrivals sheltering with members of their own cohort.

TBT levels in Southampton Water are apparently still high despite current UK legislation and although the foreshore at Netley Royal Victoria Country Park is not directly affected by the major shipping lanes, TBT influence was clear. Imposex results for both wall and shore groups were high 6 months after reintroduction though breeding was still found 1 year later. This suggests that reintroduction could be considered on a site-by-site basis and that where up to date TBT level data are not available, chemical testing may be appropriate prior to the assisted arrival of *N. lapillus* populations. Overall *N. lapillus* appears robust enough to survive translocation to sheltered, previously TBT impacted locations. The ability of *N. lapillus* to avoid predation, maximise food resource and find suitable habitats, coupled with passive transport would enhance the long-term survival of populations.

Chapter 6. The role of *Nucella lapillus* in structuring shore communities: tests using groynes at Highcliffe, Dorset.

6.1 Introduction

Rocky shore habitats are important resources for the formation and testing of hypotheses relating to ecological interactions (Raffaelli and Hawkins, 1996; Bertness *et al.,* 2002). Predatory gastropods can have a key role in influencing rocky shore communities, although this may be specific to location and habitat (e.g. Menge, 1976, 1978b; Menge *et al.,* 1994; Proud, 1994). In the UK, the role of *Nucella lapillus* in structuring communities is poorly understood. The impact of TBT pollution on *N. lapillus* which reduces their predation influence, has been suggested as an opportunity for further work (Hughes and Burrows, 1993).

Suggestions that dogwhelks mediate a switch from barnacle to mussel-dominated shores were made by Moore (1938b). In addition, Hartnoll and Hawkins (1985) proposed that *N. lapillus* predation may facilitate the cycling of fucoid patches that can occur on moderately exposed shores. Connell (1961), Johnson *et al.,* (1998) and Proud (1994) showed the localised effects of *N. lapillus* predation on barnacle populations, but Proud (1994) suggested that more work was needed on mussel-dominated UK shores to establish whether dogwhelks mediate patch dynamics in mussel and barnacle communities. In the UK the impact of TBT on *N. lapillus* populations could have provided the opportunity to show the importance of dogwhelk predation on rocky shore communities, but these interactions proved difficult to identify (Spence, 1989; Proud, 1994).

In North America (e.g. Menge, 1976, 1978a,b; Moran, 1985; Berlow, 1997; Hunt and Scheibling, 1998; Raimondi *et al.,* 2000) and elsewhere (e.g. Connell, 1970; Fairweather *et al.,* 1984; Navarrete and Menge, 1996; Noda, 1999) predatory gastropods can directly and indirectly affect the structure of intertidal communities. Conversely, in the UK the significance of dogwhelk predation on exposed or sheltered shore communities is thought to be minimal as they are naturally stable, and *N. lapillus* are at the extremes of their range (Little and Kitching, 1996). Following TBT impacts Spence (1989) investigated the decline of *N. lapillus* populations and the consequences of reduced predation on moderately exposed shores. These habitats are unstable due to biotic pressures (Lewis, 1976; Hawkins and

Hartnoll, 1983; Lindegarth *et al.,* 2001) and it was concluded that demonstrating the effects of a reduction in *N. lapillus* on the associated communities was difficult, as natural changes may have masked the effects of predation (Spence, 1989).

Connell (1961) studied the effect of *N. lapillus* predation on *Semibalanus balanoides* on the Isle of Cumbrae, Scotland. It was shown that *N. lapillus* selectively fed on larger *S. balanoides*, thus accounting for different age structures in the barnacle populations on the high and low shore. In addition dogwhelk predation during the summer could result in 100% mortality for those barnacles older than 6 months. Predation by *N. lapillus* also had a facilitating role that benefited the barnacle *Chthamalus*. Following the decline of *S. balanoides*, competition for space was reduced thus allowing *Chthamalus* to colonise and survive lower in the eulittoral than was possible without *N. lapillus* predation.

On the Isle of Man *N. lapillus* predation reduced barnacle density near crevices on vertical faces and amongst algal clumps (Proud, 1994), but this observation may be confounded as barnacle density can also be affected by limpet bulldozing and algal sweeping (Connell, 1961; Hawkins, 1983; Miller and Carefoot, 1989; Johnson *et al.,* 1997). With *N. lapillus* experimentally removed, overcrowding was observed in *S. balanoides* populations, generating a rougher shore surface. Due to this, limpet mobility was restricted, leading to algal escapes. The size and age of fucoid clumps thus increased as *N. lapillus*' presence or absence moderated limpet grazing (Proud, 1994). The restriction of limpets thus had a stronger effect on community structure than removal of *N. lapillus*. These effects, however, were dependent on the abundance of *N. lapillus* which was controlled by environmental conditions, particularly the availability of crevices.

In the UK much less attention has been paid to the influence of dogwhelk predation on *M. edulis* than has been directed towards barnacle populations. Similarly to New England (e.g. Dayton, 1971; Menge, 1976, 2000; Lubchenco and Menge, 1978; McCook and Chapman, 1991) *N. lapillus* may have a function in mediating competition between mussels and barnacles on UK shores, thereby affecting the activity of grazers and algae abundance (e.g. Turner and Todd, 1991). In addition Wilson *et al* (1983) showed that *N. lapillus* predation may be responsible for restricting *M. edulis* to a freshwater stream running across the shore at Porlock, Somerset. Predation restricted *M. edulis* to a realised niche in which *N. lapillus* could not forage, but which *M. edulis* utilised by remaining closed until the returning tide for protection from osmotic stress. This is a relatively rare example, although *M. edulis* being confined to a freshwater stream has also been observed on the Isle of Wight (Herbert, pers. com. 1997) and on the Isle of Man (Hawkins, pers. com. 2005).

This chapter aims to quantitatively study the direct influence of *N. lapillus* predation on rocky shore communities dominated by *M. edulis* and barnacles (*S. balanoides* and *Chthalamus* species). In addition, the indirect role of *N. lapillus* in moderating the ability of limpet species to graze and the consequences for algal assemblages was also examined. The groynes at Highcliffe in Dorset were used (also used for recolonisation studies, Chapter 4) as a test system, as the sandy beach in the space between each groyne allowed the manipulation of spatially independent *N. lapillus* treatment populations with adjacent interspersed controls. This experiment also simulated the extinction of *N. lapillus* on similar UK shores due to the influence of TBT pollution. The specific objectives of this study were:

1. To determine the direct influence of *N. lapillus* predation on *M. edulis* and barnacles;
2. To assess whether *N. lapillus* mediates competition between mussels and barnacles, thereby altering community composition:
3. To establish whether the reduction of *N. lapillus* results in reduced limpet grazing efficiency in areas where barnacle cover increases;
4. To identify if indirect effects on grazers results in an increase in total algal cover.

6.2 Methods

6.2.1 Site description

Experimental studies were carried out on the Highcliffe groynes. The limestone block sea defences are in Christchurch Bay, Dorset (Fig. 6.1, grid ref SZ 213 930, § 4.2.1 for a site description). The beach area formerly consisted of sands and gravels (Tyehurst, 1994) but, following groyne construction in the early 1990s, an artificial rocky intertidal zone was created. The groynes provided a suitable habitat for the development of lower to upper eulittoral exposed to moderately exposed communities (see Fig. 2.5) (2-3 Ballantine exposure scale, 1961, Fig. 2.6), although diversity at the sites was low. Initial surveys showed that of the two groyne lengths, the shorter structures (H1, H3, H5, H7 and H9; Fig. 6.1D) were unsuitable for experimental work, being too high on the shore for colonisation. Thus these were not used in this study and attention was restricted to the larger groynes (H0, H2, H4, H6, H8 and H10; Fig. 6.1D). Before the groyne construction there were no records of intertidal *N. lapillus* and associated communities in the area due to a lack of habitat (e.g.

Crisp and Southward, 1958). Each long groyne (Fig. 6.1D) was, however, found to support dogwhelk populations of varying abundance.

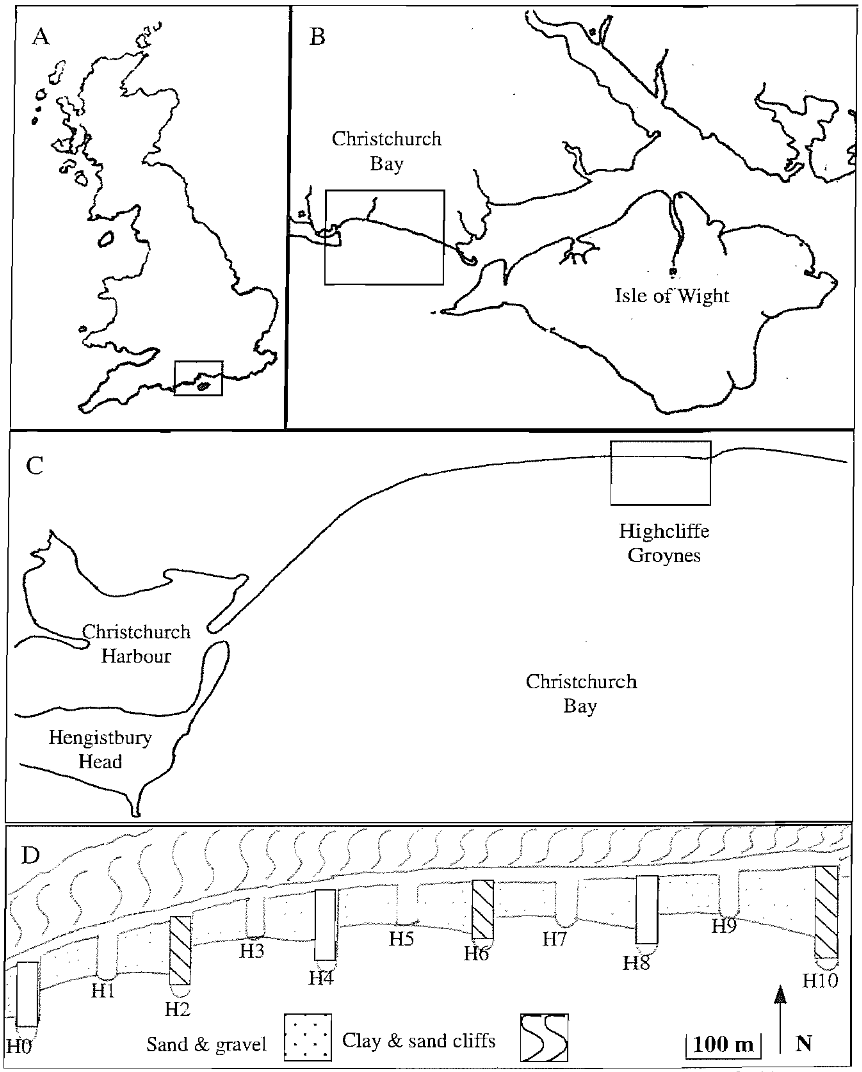

FIGURE 6.1 HIGHCLIFFE STUDY SITE A: UK, B: SOLENT AND ISLE OF WIGHT, C: CHRISTCHURCH BAY, D: HIGHCLIFFE GROYNES. ☐ H0, H4 and H8: *Nucella lapillus* populations removed. ▨ H2, H6 and H10: *Nucella lapillus* populations left in place. Odd numbered groynes were not used in experiments due to their short length and absence of an intertidal community.

6.2.2 Methods and experimental design

Dogwhelk abundances were recorded on each of the long groynes (H0, H2, H4, H6, H10, Fig. 6.1D). It was found that *N. lapillus* were more abundant on the east faces, away from the dominant longshore drift direction. Using paint (Hammerite^{TM}, ICI, PLC), the east faces of the groynes were divided into three 10 m longitudinal zones: A, the groyne end , B, mid groyne and C, the near shore zones (Fig. 6.2). To ensure that the zones were spatially independent, 2 m gaps were left between each. Within these zones, at the base of each groyne, four randomly selected blocks of similar size and aspect were chosen for use in the study and then marked with the paint which was replenished monthly. This resulted in three zones with four marked blocks in each, on the east face of the interspersed treatment (H0, H4, H8) and control (H2, H6, H10) groynes.

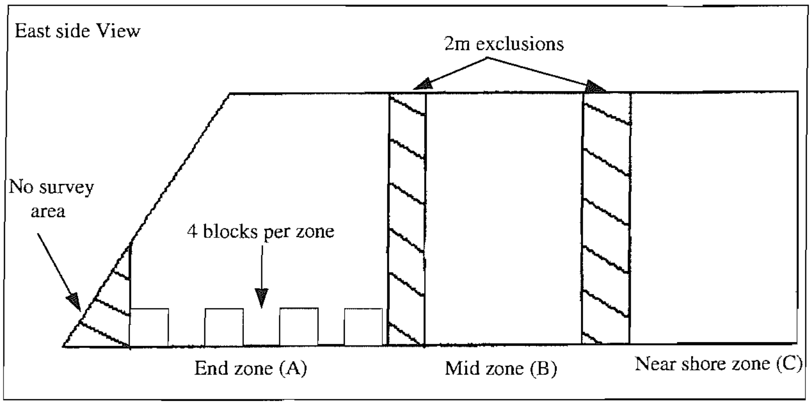

FIGURE 6.2 EAST FACE VIEW OF TREATMENT GROYNE (H0, H4, H8) SHOWING THE THREE ZONES EACH GROYNE WAS DIVIDED INTO AND THE FOUR BLOCKS USED FOR FINE SCALE COMMUNITY ASSESSMENT WITHIN EACH ZONE.
2 m exclusions left between zones to ensure spatial independence. No surveys carried out at end of groynes except for treatment groyne H0 due to shorter length.

The community present before the manipulation of dogwhelk populations was recorded using the optimal number of quadrats (§ 2.4.4) to assess the percentage cover of barnacles, mussels, total algae, bare rock and counts of limpet abundance. The impact of *N. lapillus* predation on the Highcliffe groyne communities was assessed at two scales of resolution. To determine the fine scale influence of dogwhelk predation, five 0.25 m x 0.25 m quadrats were used to assess the community on blocks within zones (Fig. 6.2). For larger scale observations seven 0.50 m x 0.50 m quadrats were used in each groyne zone (Fig. 6.2). Results from the initial community surveys at both scales were used as reference points for subsequent experimental manipulation. In conjunction with recolonisation work (Chapter 4), the treatment groynes (H0, H4, H8) were cleared of all accessible *N. lapillus* between May and August 2001 (§ 4.2.2). The abundance of dogwhelks on the control groynes (H2, H6, H10) was recorded and these populations were not disturbed. The experiment began in August 2001 and was completed in September 2002. Surveys at both scales of resolution were carried out every three months or at the next nearest spring tide cycle when storms restricted shore access.

The groynes were also used for the collection of recolonisation data (Chapter 4). Thus where recolonising *N. lapillus* were found on treatment blocks previously cleared for community assessment, they were removed throughout the study period, but replaced within the same zone. This continued removal ensured minimisation of dogwhelk predation on the community of each cleared block. When surveying at the larger scale (0.50 m x 0.50 m) *N. lapillus* found recolonising in the zones were not disturbed as these observations, including those removed from the blocks and placed within the zone, were part of the recolonisation experiment (Chapter 4). Thus at the larger scale *N. lapillus* were reduced to zero at the experiment start, but their influence as they returned resulted in a marked reduction in predation rather than removal.

A four-way ANOVA analysis of 0.25 m x 0.25 m quadrat data was carried out using GMAV (Underwood *et al.,* 1988) for the factors blocks (Bl), zones (Zn), groynes (Gr) and treatment (Tr). Tests were carried out on pooled results for 0.25 m x 0.25 m quadrat data for the taxa: *M. edulis*, barnacles, limpets, total algae, and also bare rock, to establish whether pre-existing differences were significant and for the analysis of subsequent survey results. For data collected in zones with 0.50 m x 0.50 m quadrats, a three-way ANOVA was used for the factors zone (Zn), groyne (Gr) and treatment (Tr) using the same taxa. In GMAV data are examined for heterogeneity of variance by Cochran's test (Cochran, 1950) and when significantly different ($P<0.05$) $\ln(X+1)$ transformations were applied. Although equal variance is desirable, if data were still not homogenous they were left untransformed. Box (1953) and Underwood (1981) suggest that ANOVA is more valid than many tests assuming homogeneity of variance and that it can still be run if there are sufficient balanced data. Thus where Cochran's test failed after transformation, significance for the ANOVA was set at $P<0.01$ (Prescott, pers. com. 2004). In addition, percentage data in the range 0-100 can lead to clustering at low or high values, therefore these data were arcsine transformed (Zar, 1996).

Unfortunately, due to storms in September-October 2001, groyne H4 (treatment) was damaged, necessitating reconstruction in December 2001. Due to this all biota from the mid and near shore zones (Fig. 6.2) were cleared, therefore data analysis from December 2001 onwards was redesigned. For univariate (ANOVA) and multivariate (NPMANOVA) analysis data from all of the treatment (H0, H4, H8) and control (H2, H6, H10) end zones were analysed. For the mid and near shore zones a separate ANOVA was carried out with the data from control groyne H2 randomly selected for exclusion, thus balancing the design.

Multivariate analysis of data at both scales was carried out using nMDS and SIMPER from PRIMER (Clarke and Gorley, 2001; § 5.2.5). In addition a nonparametric multivariate analysis of variance (NPMANOVA) (Anderson, 2001; McArdle and Anderson, 2001) test was carried out on each set of taxa data; bare rock was not included in community analysis. To homogenise variance, data were square root transformed (Thacker and Bromley, 2001).

6.3 Results

6.3.1 Univariate analysis of changes on cleared blocks (0.25 m x 0.25 m quadrats)

At the start of the experiment in May 2001, before manipulation, ANOVAs showed that there were no significant differences in abundance of *M. edulis*, barnacles, total algae and bare rock between pooled treatment (*N. lapillus* removed, H0, H4, H8) and control (*N. lapillus* undisturbed, H2, H6, H10) groyne results (Fig. 6.3, Table 6.1). A significant difference between pooled totals of limpets was indicated (Fig. 6.3C, Table 6.1C), however all data failed the Cochran's test, therefore the result was not accepted at the P<0.01 level.

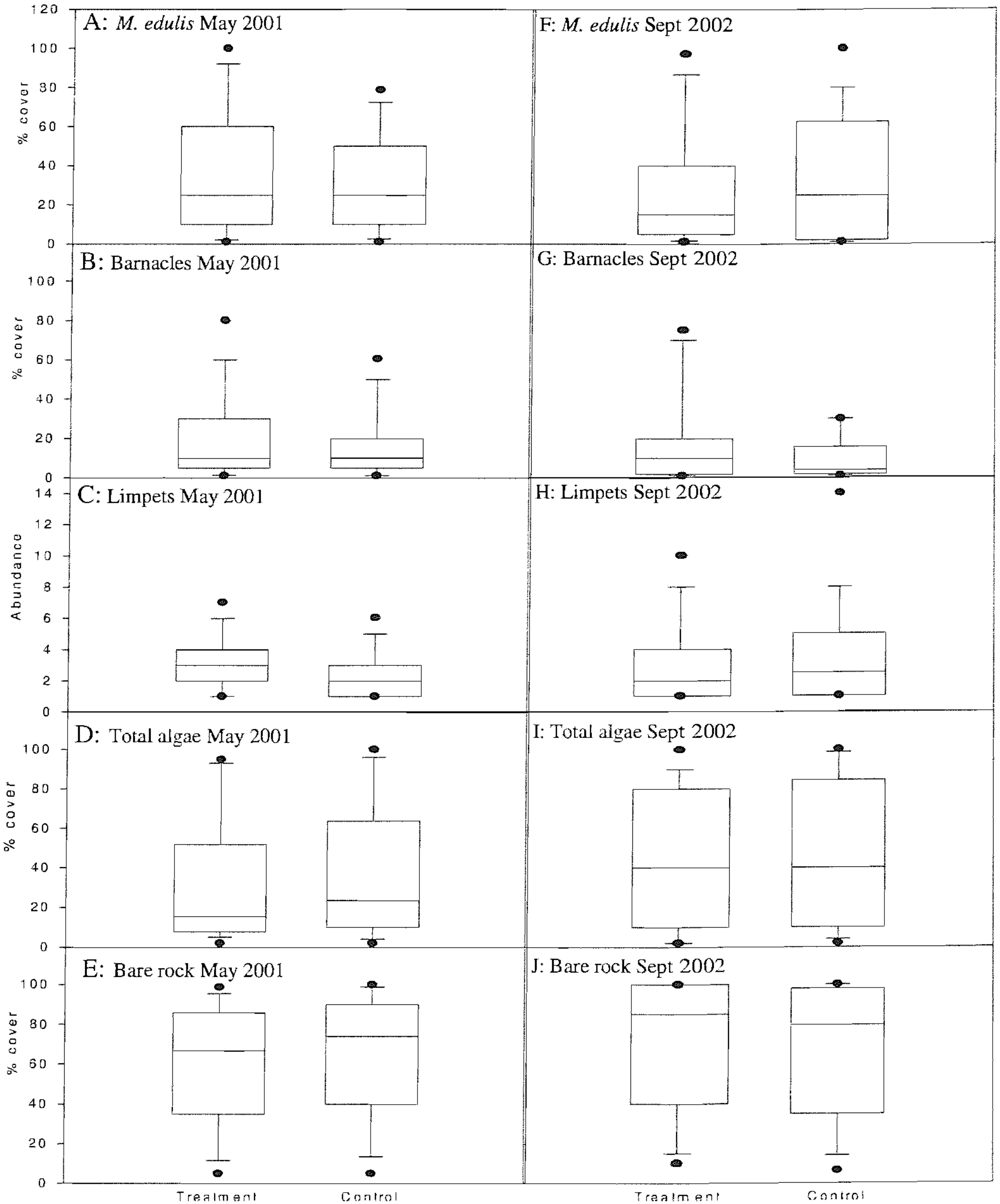

FIGURE 6.3 POOLED TREATMENT AND CONTROL GROYNE ABUNDANCES OF *MYTILUS EDULIS*, BARNACLES, LIMPETS, TOTAL ALGAE AND BARE ROCK ON TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H10) GROYNES IN MAY 2001 BEFORE MANIPULATION (A-E) AND AT THE END OF EXPERIMENTAL WORK IN SEPTEMBER 2002 (F-J) (0.25 M X 0.25 M QUADRATS ON BLOCKS).
Median, 25th, 75th (box), 5th and 95th (whiskers) percentiles and outliers

Table 6.1 Four-way ANOVA Comparisons between and within treatment (H0, H4, H8) and control groyne (H2, H6, H10) of abundances in May 2001 before manipulation. .(0.25 m x 0.25 m quadrats).
NS: Not significant. !: Cochrans test failed. Tr: treatment, Ctrl: control, Gr: groyne, Zn: zone, Bl: block. Data collected with 0.25 m x 0.25 m quadrats on blocks.

A:	***M. edulis***			
Source	**DF**	**MS**	**F**	**P**
Tr vs Ctrl	1	24.8	0.3	NS
Gr(Tr)	4	75.9	22.3	<0.001
Zn	2	12.7	1.3	NS
Bl(TrxGrxZn)	54	3.4	2.7	<0.001
TrxZn	2	1.8	0.2	NS
ZnxGr(Tr)	8	9.5	2.8	NS!
RES	288	1.2		
TOT	359			

B:	**Barnacles**			
Source	**DF**	**MS**	**F**	**P**
Tr vs Ctrl	1	12.3	0.4	NS
Gr(Tr)	4	31.1	9.8	<0.001
Zn	2	1.5	0.4	NS
Bl(TrxGrxZn)	54	3.2	3.2	<0.001
TrxZn	2	4.9	1.1	NS
ZnxGr(Tr)	8	3.7	1.2	NS
RES	288	1.0		
TOT	359			

C:	**Limpets**			
Source	**DF**	**MS**	**F**	**P**
Tr vs Ctrl	1	9.8	11.5	NS!
Gr(Tr)	4	0.9	1.6	NS
Zn	2	1.9	1.8	NS
Bl(TrxGrxZn)	54	0.6	1.5	NS!
TrxZn	2	0.3	0.3	NS
ZnxGr(Tr)	8	1.1	2.0	NS
RES	288	0.4		
TOT	359			

D:	**Total algae**			
Source	**DF**	**MS**	**F**	**P**
Tr vs Ctrl	1	3373.3	0.2	NS
Gr(Tr)	4	14709.3	7.9	<0.001
Zn	2	16147.3	19.9	<0.001
Bl(TrxGrxZn)	54	1843.5	3.6	<0.001
TrxZn	2	6060.6	7.5	<0.05
ZnxGr(Tr)	8	812.1	0.4	NS
RES	288	515.3		
TOT	359			

E:	**Bare rock**			
Source	**DF**	**MS**	**F**	**P**
Tr vs Ctrl	1	3416.3	0.5	NS
Gr(Tr)	4	6467.3	3.2	<0.05
Zn	2	6613.9	3.9	NS
Bl(TrxGrxZn)	54	2044.9	2.6	<0.001
TrxZn	2	1711.9	1.0	NS
ZnxGr(Tr)	8	1666.2	0.8	NS
RES	288	793.8		
TOT	359			

By September 2002 pooled abundances of *M. edulis* and limpets on treatment groynes (H0, H4, H8) had decreased whereas barnacles, total algae and bare rock had increased (Fig.6.3F-J). Barnacle cover on control groynes (H2, H6, H10) declined whereas all other categories showed a slight increase over abundances in May 2001. Overall by September 2002 the removal of *N. lapillus* from treatment groynes did not appear to have directly affected abundance and cover on the treatment groynes (H0, H4, H8) (Fig. 6.3F-J).

Within treatment and control comparisons for May 2001 showed that, except for limpets (Table 6.1C, Fig. 6.4C), there were significant differences between the abundance of taxa and bare rock on all groynes (Fig. 6.4A-E). *M. edulis* distribution was variable and they were found on all of the groynes except H8 (Fig. 6.4A). *Post hoc* Student-Newman-Keuls (SNK) tests showed that within treatment groynes *M. edulis* cover was significantly greater on H4 ($P<0.01$); controls groynes H2 and H6 had greater mussel cover than H10 ($P<0.01$). For barnacle cover no significant differences were found between the control groynes (H2, H6, H10) whereas within treatments (H0, H4, H8) groyne H8 was dominated by barnacles and bare rock (Fig. 6.4B,E) and had significantly higher barnacle cover ($P<0.01$).

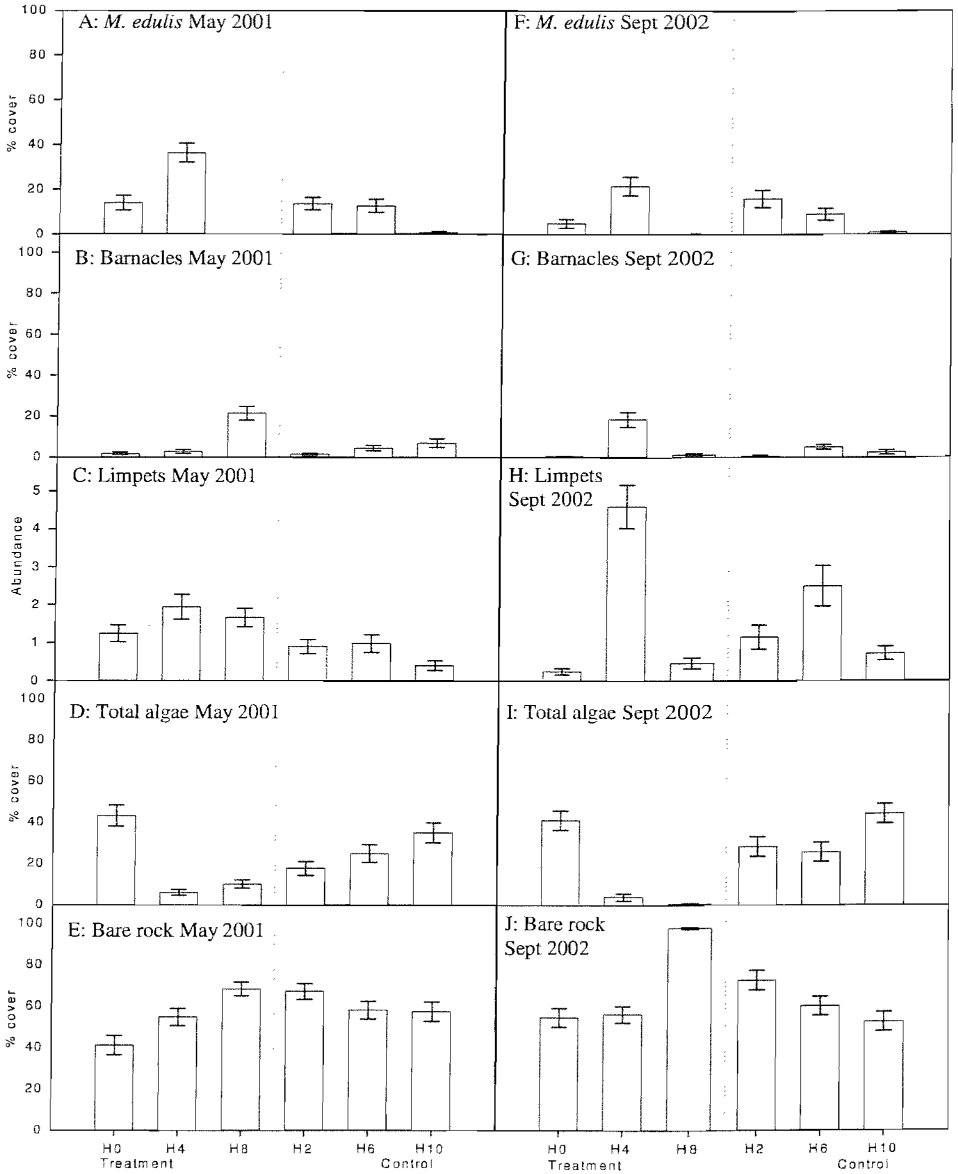

Figure 6.4 Mean (+/- 1 SE) groyne abundance s of *Mytilus edulis*, barnacles, limpets, total algae and bare rock on treatment (H0, H4, H8) and control (H2, H6, H10) groynes in May 2001 before manipulation (A-E) and at the end of experimental work in September 2002 (F-J).
Data collected with 0.25 m x 0.25 m quadrats on blocks.

Within treatment and control groynes no significant differences were found for limpet abundances (Table 6.1, Fig. 6.4C) although they were denser on treatment groynes (Fig. 6.3C). Total algae (ephemeral species and occasional fucoids) cover on groynes appeared to reflect limpet abundance except for treatment H0 (Fig. 6.4C,D). Within treatment and SNK tests showed that abundance of ephemeral algae on H0 was significantly greater than on the other treatment groynes ($P<0.01$); no significant differences were found between control sites (H2, H6, H10). A significant difference ($P<0.05$) between treatment groynes showed that there was more bare rock on groynes H4 and H8 than H0 (Fig. 6.4E, Table 6.1).

Comparisons to show differences between zones in May 2001 were carried out (Fig. 6.5), but statistical assessments between individual blocks (B1 Table 6.1) were not considered further as these communities were highly variable. Only total algal cover was significantly different between zones ($P<0.05$) (Table 6.1D, Fig.6.5C4) due to higher percentage cover in the near shore zones of treatments (H0, H4, H8) and controls (H2, H6, H10). Within treatment groynes, the near shore zone of H0 had significantly higher algal cover ($P<0.01$). The treatment and zone interaction found for total algae (Table 6.1) was due to the near shore zones of control sites having significantly greater algal cover than treatments ($P<0.01$).

By October 2001 the decline of *M. edulis* on treatment groynes H0 and H4 (Fig. 6.5A-C1) reduced the difference between treatments and controls which were no longer significant ($F_{(1, 288)}$ 0.15, P = NS). From August to October barnacle density fluctuated (Fig. 6.5A-C2), but there was no significant main effect between treatment and controls ($F_{(1, 288)}$ 0.46, P = NS) and no significant lower order interactions were found. *M. edulis* and barnacles were the main prey species available, but direct effects of the reduction in *N. lapillus* predation had not significantly impacted their abundances on treatment groynes (H0, H4, H8) by October 2001.

Comparisons between treatment and controls for the indirect influence of *N. lapillus* removal on limpet populations, total algae and bare rock showed no significant main effects. Limpet densities varied within both treatments (H0, H4, H8) and controls (H2, H6, H10) between August and October 2001, particularly the end zones (Fig. 6.5A-C3). The changes were significant by October 2001 ($F_{(4, 288)}$ 1.25, $P < 0.001$) and SNK tests identified that limpet decline on H0 and increases on H4 and H8 (treatments) were significant (H4=H8>H0, $P<0.01$). Limpet density increased on groyne H2 (Fig. 6.5A-C3) thus there were significant differences between controls (H2=H6>H10, $P<0.05$).

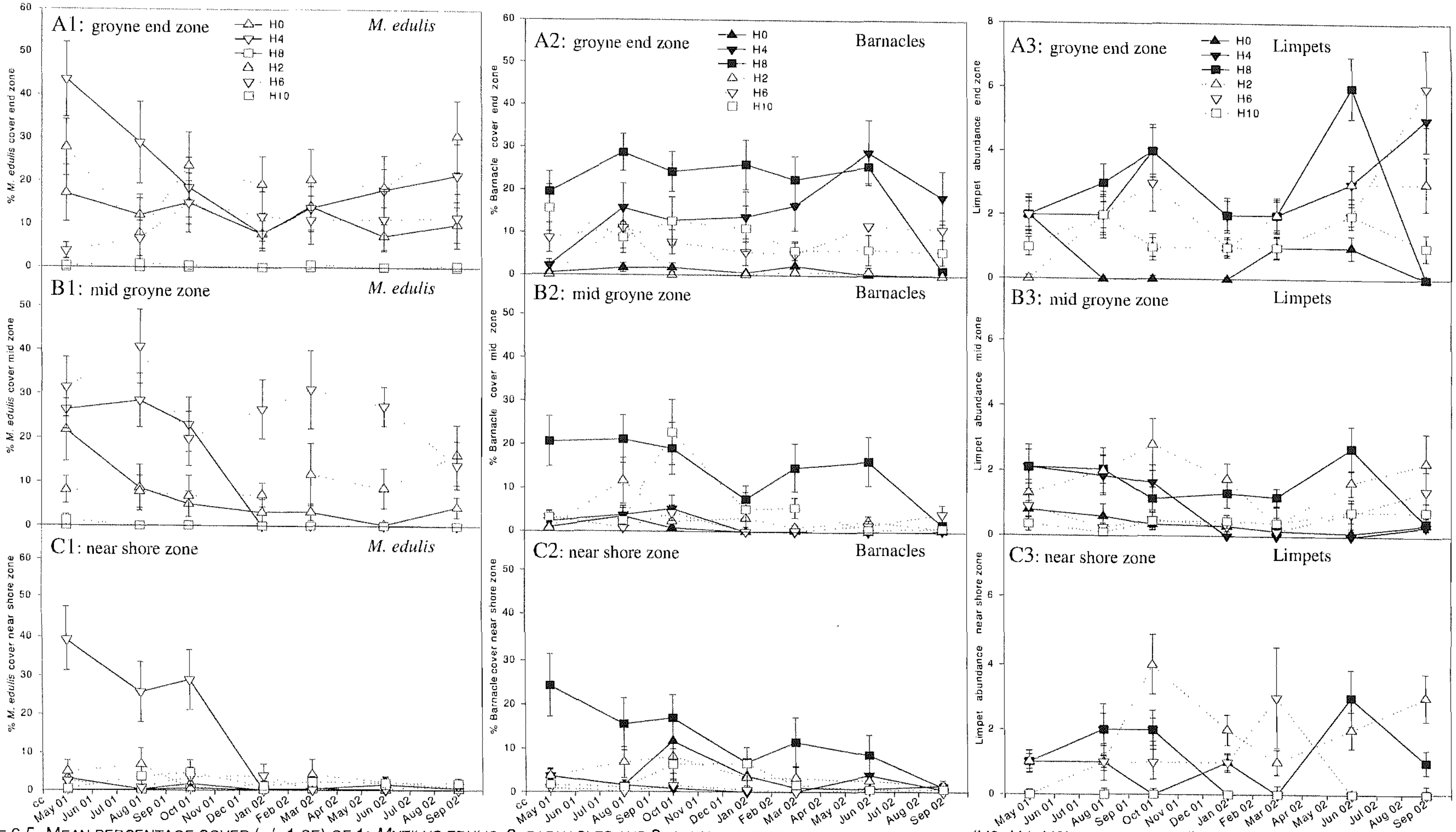

FIGURE 6.5 MEAN PERCENTAGE COVER (+/- 1 SE) OF 1: *MYTILUS EDULIS*, 2: BARNACLES AND 3: ABUNDANCE OF LIMPETS ON TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H10) GROYNES IN THE END, MID AND NEAR SHORE GROYNE ZONES BETWEEN MAY 2001 AND SEPTEMBER 2002. (0.25 M X 0.25 M QUADRATS).
4 blocks per zone, 5 quadrats each block. Mid and upper eulittoral data for H4 and H2 unused after October 2001.

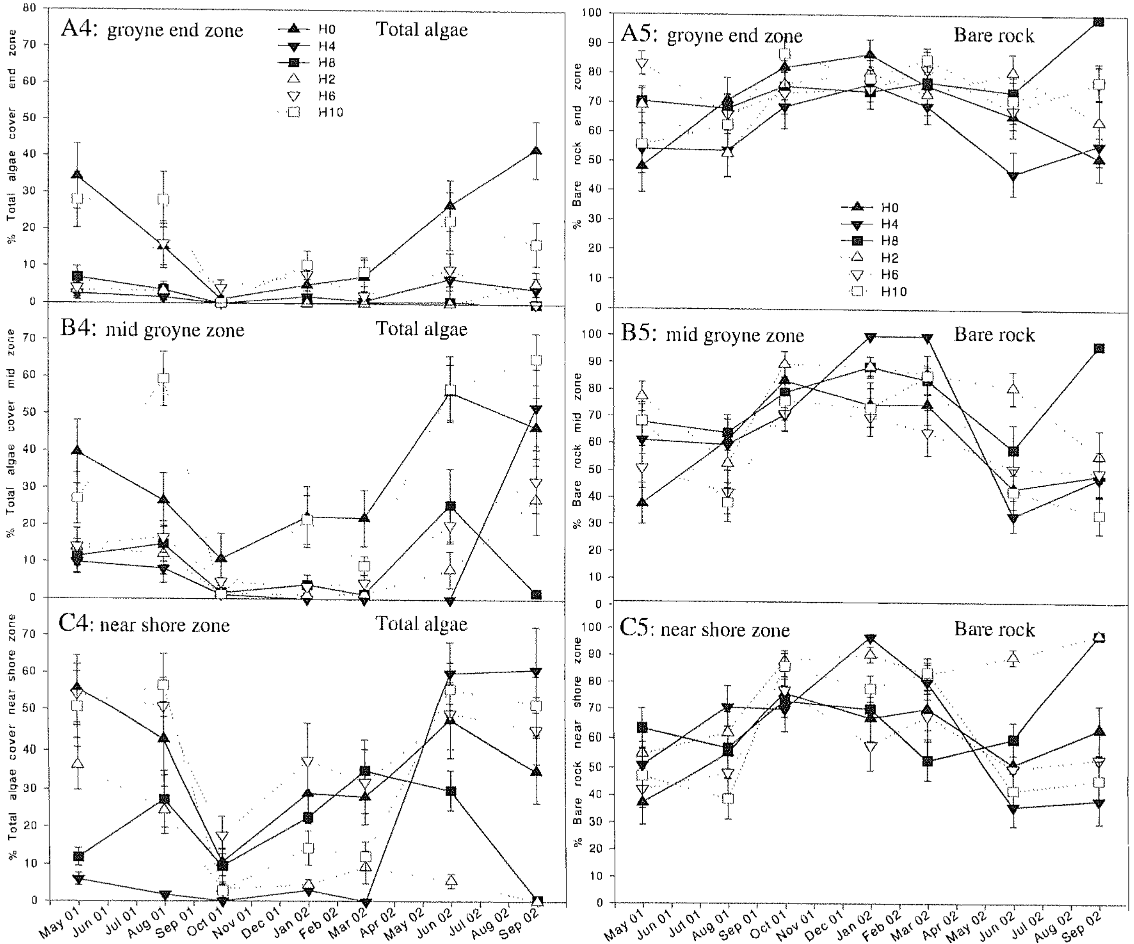

FIGURE 6.5 (CONTD) MEAN PERCENTAGE COVER (+/- 1 SE) OF 4: TOTAL ALGAE AND 5: BARE ROCK ON TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H10) GROYNES IN THE END, MID AND NEAR SHORE GROYNE ZONES BETWEEN MAY 2001 AND SEPTEMBER 2002. (0.25 M X 0.25 M QUADRATS).
4 blocks per zone, 5 quadrats each block. Mid and upper eulittoral data for H4 and H2 unused after October 2001.

The annual cycle of cover in ephemeral algae was clear (Figs. 6.5A-C4). Total algal cover declined from May to October 2001, whereas bare rock increased (Fig. 6.5A-C5), although treatment and control comparisons for both categories were still not significant (algae: $F_{(1, 288)}$ 0.21, P = NS; bare rock: $F_{(1, 288)}$ 1.25, P = NS). Algal cover on control groynes increased from May to August, whereas on treatment groynes this was less evident. This was largely due to an increase in ephemeral algae on H10 mid and near shore zones (Fig. 6.5B,C4) but, as with all of the sites, algal cover had declined sharply by October 2001.

In December 2001 Christchurch Council rebuilt treatment groyne H4 due to erosion which necessitated a redesign of analysis techniques (§ 6.2.2), therefore mid and near shore results from H4 and H2 were still presented (Fig. 6.5) to show trends, but were excluded from formal analysis.

TABLE 6.2 THREE-WAY ANOVA COMPARISONS BETWEEN AND WITHIN TREATMENT (H0, H4, H8) AND CONTROL GROYNES (H2, H6, H10) OF END ZONE ABUNDANCES FOR MARCH AND SEPTEMBER 2002. (0.25 M X 0.25 M QUADRATS).
NS: Not significant, Source key: TR: treatment, Ctrl: control, GR: groyne, Bl: block.

A: *M. edulis* end zone March 2002					**F: *M. edulis* end zone Sept 2002**				
Source	**DF**	**MS**	**F**	**P**	**Source**	**DF**	**MS**	**F**	**P**
Tr vs Ctrl	1	0.3	0.03	NS	Tr vs Ctrl	1	2.6	0.2	NS
Gr(Tr)	4	11.0	2.5	NS	Gr(Tr)	4	17.1	3.4	<0.05
Bl(Tr×Gr)	18	4.3	2.7	<0.01	Bl(Tr×Gr)	18	4.9	3.3	<0.001
RES	96	1.6			RES	96	1.5		
TOT	119				TOT	119			
B: Barnacles end zone March 2002					**G: Barnacles end zone Sept 2002**				
Source	**DF**	**MS**	**F**	**P**	**Source**	**DF**	**MS**	**F**	**P**
Tr vs Ctrl	1	15.5	1.1	NS	Tr vs Ctrl	1	1.6	0.1	NS
Gr(Tr)	4	14.5	4.9	<0.01	Gr(Tr)	4	16.2	5.0	<0.01
Bl(Tr×Gr)	18	2.9	2.2	<0.01	Bl(Tr×Gr)	18	3.2	3.9	<0.001
RES	96	1.4			RES	96	0.8		
TOT	119				TOT	119			
C: Limpets end zone March 2002					**H: Limpets end zone Sept 2002**				
Source	**DF**	**MS**	**F**	**P**	**Source**	**DF**	**MS**	**F**	**P**
Tr vs Ctrl	1	0.3	0.0	NS	Tr vs Ctrl	1	8.8	0.9	NS
Gr(Tr)	4	1.7	2.2	NS	Gr(Tr)	4	8.9	6.6	<0.01
Bl(Tr×Gr)	18	0.8	2.1	NS	Bl(Tr×Gr)	18	1.4	4.7	<0.001
RES	96	0.4			RES	96	0.3		
TOT	119				TOT	119			
D: Total algae end zone March 2002					**I: Total algae end zone Sept 2002**				
Source	**DF**	**MS**	**F**	**P**	**Source**	**DF**	**MS**	**F**	**P**
Tr vs Ctrl	1	1.9	0.4	NS	Tr vs Ctrl	1	2.0	0.1	NS
Gr(Tr)	4	4.8	2.8	NS	Gr(Tr)	4	34.6	8.5	<0.001
Bl(Tr×Gr)	18	1.7	2.2	<0.01	Bl(Tr×Gr)	18	4.1	4.7	<0.001
RES	96	0.8			RES	96	0.9		
TOT	119				TOT	119			
E: Bare rock end zone March 2002					**J: Bare rock end zone Sept 2002**				
Source	**DF**	**MS**	**F**	**P**	**Source**	**DF**	**MS**	**F**	**P**
Tr vs Ctrl	1	1725.2	5.9	NS	Tr vs Ctrl	1	537.6	0.1	NS
Gr(Tr)	4	292.4	0.4	NS	Gr(Tr)	4	7408.8	3.7	<0.05
Bl(Tr×Gr)	18	826.9	1.2	NS	Bl(Tr×Gr)	18	2011.4	3.6	<0.001
RES	96	679.8			RES	96	561.1		
TOT	119				TOT	119			

Comparisons of percentage mussel cover between treatment and control groynes in the end zones for March and September 2002 did not identify any significant main effects (Table

6.2A,F see Fig. 6.3F for September 2002 comparison). Variability within treatments and controls was still seen with *M. edulis* increasing in the end zones of treatment groynes H0 and H4 and control groyne H2 (Fig. 6.5A1); these differences were significant between groynes in September 2002 (Table 6.2F). SNK tests could not differentiate between results, but a significant difference between control groynes was indicated due to the higher recruitment of *M. edulis* in the end zone of H2.

Compared to *M. edulis* the percentage of barnacle cover in end zones was slightly more variable between January and September 2002 (Fig. 6.5A2). There were significant differences within treatment and control groynes for March and September 2002 (Table 6.2,B,G). Whilst abundance on the control groynes remained relatively static, barnacle density increased on the end zone of treatment site H4 (Fig.6.5A2), possibly due to decline of *M. edulis* in the winter (Fig. 6.5A1). SNK tests confirmed significant increases in March on H4 (H4=H8>H0, $P<0.05$), possibly due to recruitment, but by September 2002 a sharp decline on treatment site H8 (Fig. 6.5A2) led to H4 having significantly greater barnacle cover within treatments ($P<0.05$).

Limpet abundances in the end zone declined on treatment groynes H4 and H8 and control groynes H2 and H6 between October 2001 and January 2002 (Fig. 6.5A3), possibly due to smothering or abrasion in winter storms. By March 2002 limpet abundances had stabilised on both treatment and control groynes and no significant main effects were found (Table 6.2C). Recruitment in treatment and control limpet populations occurred after March 2002, and by September abundances had increased particularly on control groynes, although disturbance of H8 in August 2002 impacted the population (Fig. 6.5A3). In September 2002 there were significant differences within treatment and control groynes, although not between them (Table 6.2H). SNK test results could not fully resolve the differences (Table 1 Appendix 4), although indications were that losses on treatment groyne H8 and high recruitment on control H6 were responsible.

In March and September 2002 algal cover and bare rock in the end zone were less common than in the mid and near shore zones (Fig. 6.5A4, and 6.5A5). For algal cover no significant differences were found between treatment and control groynes (Table 6.2D,I). Significance differences were found within groynes for September 2002 (Table 6.2I) due to algal increase on H0 (Fig. 6.5A4, Table 1 Appendix 4). Throughout the experiment, apart from in September

2002, the percentage of end zone bare rock was not significantly different both within and between control sites. In September 2002 the loss of communities on H8 resulted in a significant difference within treatment groynes (Table 6.2J and Table 1 Appendix 4).

TABLE 6.3 FOUR-WAY ANOVA COMPARISONS BETWEEN AND WITHIN TREATMENT (H0, H4, H8) AND CONTROL GROYNES (H2, H6, H10) OF MID AND NEAR SHORE ZONE ABUNDANCES FOR MARCH AND SEPTEMBER 2002. (0.25 M X 0.25 M QUADRATS). NS: Not significant. !: Cochrans failed. Tr: treatment, Ctrl: control, Gr: groyne, Zn: zone, Bl: block. 25x25 quadrats.

A: *M. edulis* mid and near shore zones March 2002					**F: *M. edulis* mid and near shore zones Sept 2002**				
Source	**DF**	**MS**	**F**	**P**	**Source**	**DF**	**MS**	**F**	**P**
Tr vs Ctrl	1	6.8	0.9	NS	Tr vs Ctrl	1	2.1	0.5	NS
Gr(Tr)	2	7.2	5.9	<0.01	Gr(Tr)	2	4.1	2.4	NS
Zn	1	7.9	0.6	NS	Zn	1	2.9	0.6	NS
Bl(Tr×Gr×Zn)	24	1.2	1.6	NS	Bl(Tr×Gr×Zn)	24	1.7	2.9	<0.001
Tr×Zn	1	3.9	0.3	NS	Tr×Zn	1	0.1	0.03	NS
Zn×Gr(Tr)	2	13.9	11.2	<0.001	Zn×Gr(Tr)	2	5.4	3.2	NS
RES	128	0.8			RES	128	0.6		
TOT	159				TOT	159			
B: Barnacles mid and near shore zones March 2002					**G: Barnacles mid and near shore zones Sept 2002**				
Source	**DF**	**MS**	**F**	**P**	**Source**	**DF**	**MS**	**F**	**P**
Tr vs Ctrl	1	4.4	0.2	NS	Tr vs Ctrl	1	0.2	1.8	NS
Gr(Tr)	2	18.6	12.3	<0.001	Gr(Tr)	2	0.1	0.1	NS
Zn	1	2.7	2.1	NS	Zn	1	1.8	1.2	NS
Bl(Tr×Gr×Zn)	24	1.5	1.6	NS	Bl(Tr×Gr×Zn)	24	0.9	1.9	<0.01
Tr×Zn	1	0.03	0.03	NS	Tr×Zn	1	1.8	1.2	NS
Zn×Gr(Tr)	2	1.3	0.8	NS	Zn×Gr(Tr)	2	1.5	1.7	NS
RES	128	0.9			RES	128	0.5		
TOT	159				TOT	159			
C: Limpets mid and near shore zones March 2002					**H: Limpets mid and near shore zones Sept 2002**				
Source	**DF**	**MS**	**F**	**P**	**Source**	**DF**	**MS**	**F**	**P**
Tr vs Ctrl	1	0.04	0.1	NS	Tr vs Ctrl	1	0.2	0.5	NS
Gr(Tr)	2	0.6	1.1	NS	Gr(Tr)	2	0.4	0.7	NS
Zn	1	0.4	0.2	NS	Zn	1	0.4	1.7	NS
Bl(Tr×Gr×Zn)	24	0.6	3.1	<0.001	Bl(Tr×Gr×Zn)	24	0.5	2.1	<0.01
Tr×Zn	1	0.9	0.4	NS	Tr×Zn	1	0.9	3.8	NS
Zn×Gr(Tr)	2	2.4	4.2	NS!	Zn×Gr(Tr)	2	0.2	0.5	NS
RES	128	0.2			RES	128	0.2		
TOT	159				TOT	159			
D: Total algae mid and near shore zones March 2002					**I: Total algae mid and near shore zones Sept 2002**				
Source	**DF**	**MS**	**F**	**P**	**Source**	**DF**	**MS**	**F**	**P**
Tr vs Ctrl	1	3.1	1.6	NS	Tr vs Ctrl	1	116.7	1.6	NS
Gr(Tr)	2	1.9	0.3	NS	Gr(Tr)	2	71.1	11.3	<0.001
Zn	1	47.2	2.5	NS	Zn	1	4.1	1.1	NS
Bl(Tr×Gr×Zn)	24	6.9	3.9	<0.001	Bl(Tr×Gr×Zn)	24	6.3	7.9	<0.001
Tr×Zn	1	9.5	0.5	NS	Tr×Zn	1	9.3	2.4	NS
Zn×Gr(Tr)	2	18.6	2.7	NS	Zn×Gr(Tr)	2	3.8	0.6	NS
RES	128	1.8			RES	128	0.8		
TOT	159				TOT	159			
E: Bare rock mid and near shore zones March 2002					**J: Bare rock mid and near shore zones Sept 2002**				
Source	**DF**	**MS**	**F**	**P**	**Source**	**DF**	**MS**	**F**	**P**
Tr vs Ctrl	1	945.8	0.3	NS	Tr vs Ctrl	1	38223.3	2.1	NS
Gr(Tr)	2	3603.9	2.0	NS	Gr(Tr)	2	18340.6	6.0	<0.01
Zn	1	2780.6	1.5	NS	Zn	1	2520.2	4.1	NS
Bl(Tr×Gr×Zn)	24	1785.6	2.5	<0.001	Bl(Tr×Gr×Zn)	24	3056.5	5.5	NS
Tr×Zn	1	3468.9	1.9	NS	Tr×Zn	1	0.3	0.0	NS
Zn×Gr(Tr)	2	1827.7	1.0	NS	Zn×Gr(Tr)	2	612.1	0.2	NS
RES	128	725.5			RES	128	557.5		
TOT	159				TOT	159			

The abundances of mid and near shore taxa were more stable than in the end zones and few significant differences were found (Table 6.3). *N. lapillus* were generally less common in these zones, thus their potential for community influence was reduced, although prey abundance was also lower.

Mid and near shore differences of *M. edulis* abundance between treatment and control groynes were not significant for March and September 2002 (Fig. 6.5B,C1, Table 6.3A,F). March results were significantly different within groynes (Table 6.3A) due to recruitment in the mid zone of control site H6 (H6>H2>H10, P<0.05). By September 2002 mussels had declined on H6, thus there were no further significant effects. As with mussels, the reduction of dogwhelk predation had little effect on barnacle populations in the mid and near shore zones. Barnacles continued a general decline on treatment groyne H8 and did not clearly increase on the other treatment groynes; control population cover remained relatively constant. Within treatment and control abundance comparisons were significant in March 2002, but not in September (Table 6.3B,G). This was due to recruitment in the mid and near shore zones of treatment groyne H8 (H8>H4=H0, P<0.05) (Fig. 6.5B,C2).

Density fluctuations of limpets in the end zone were also seen in the near shore zone; in contrast mid zones were more stable. This was with the exception of control groyne H2 (not analysed) which had high levels of recruitment and decline in the end and near shore zones (Fig. 6.5B,C3). On all the control groynes (H2, H6, H10) limpet recruitment from June 2002 seemed greater than on treatment sites, although this was not significant (Table 6.3C,H). As with the end zone, limpet recruitment on both treatment and control groynes suggested that the removal of *N. lapillus* did not cause a differential response in limpet density.

Mid and near shore fluctuations in algae and bare rock followed those seen in the end zone although at greater amplitudes (Fig. 6.5B,C4 and Fig. 6.5B,C5). Algal cover in the mid zone was greatest on H0 (treatment) and H10 (control) which were at either end of the groyne system (Fig. 6.1D). In the near shore zone algal cover was similar on all groynes although there was a decline on H8 associated with disturbance in August 2002 (Fig. 6.5B,C4), leading to a reciprocal increase in bare rock (Fig. 6.5B,C5). No significant differences between treatment and control groynes were found for March and September 2002 (Table 6.3), the significant difference within groynes in September was probably due to the disturbance on H8 (SNK results within treatments for algae H0>H8 P<0.05; bare rock H8>H0 P<0.01).

6.3.2 Univariate analysis of changes within zones (0.50 m x 0.50 m quadrats)

Surveys within zones at the larger quadrat scale (0.50 m x 0.50 m) were conducted concurrently with the work on the blocks; *N. lapillus* were not continually cleared from zones. Data were analysed using the same methods as for smaller scale results (§6.2.2). As patterns were broadly similar to those obtained from manipulations within blocks (0.25 m x 0.25 m quadrats) an overview of the results from the larger scale survey is presented.

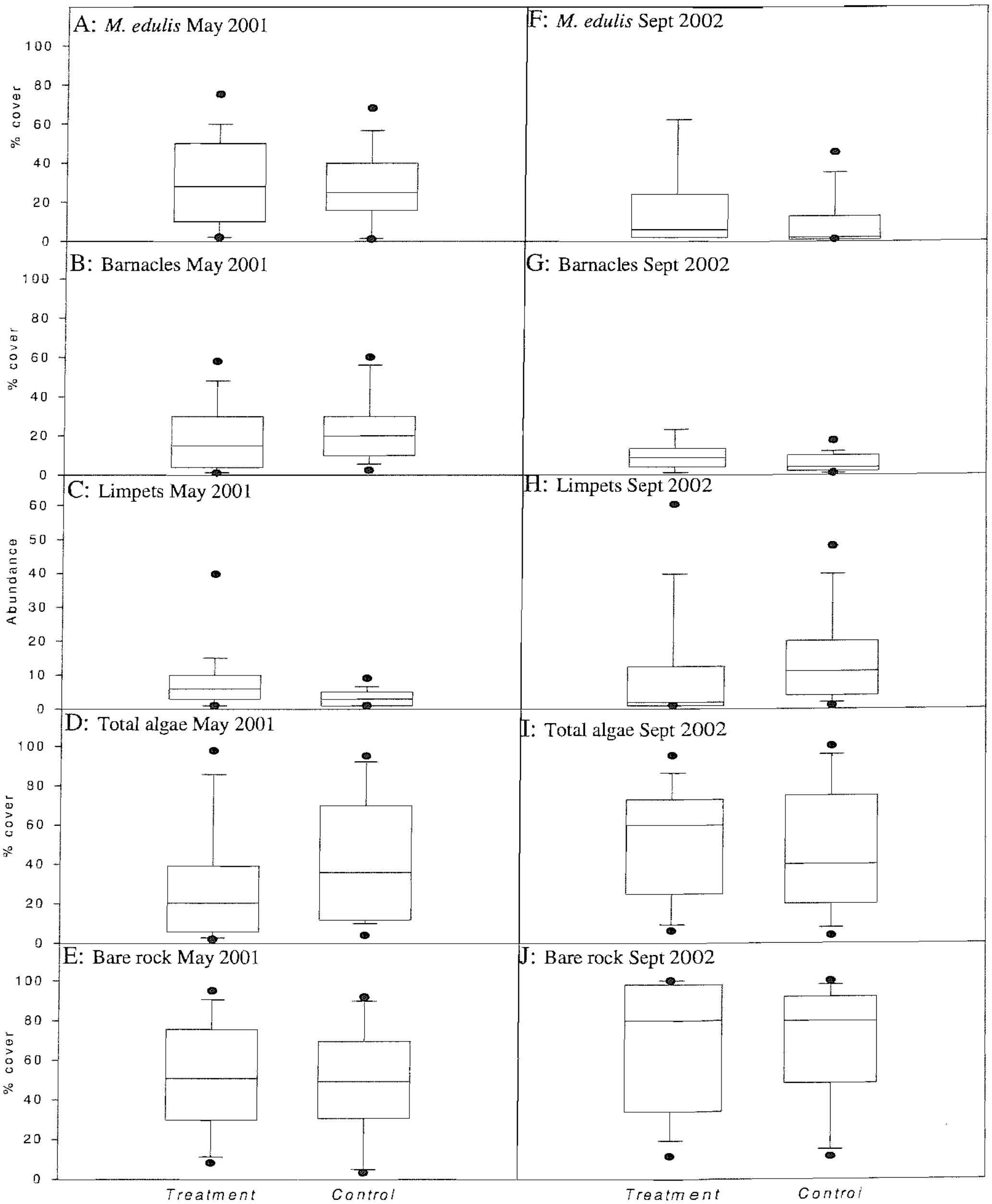

FIGURE 6.6 POOLED TREATMENT AND CONTROL GROYNE ABUNDANCES OF *MYTILUS EDULIS*, BARNACLES, LIMPETS, TOTAL ALGAE AND BARE ROCK ON TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H10) GROYNES IN MAY 2001 BEFORE MANIPULATION (A-E) AND AT THE END OF EXPERIMENTAL WORK IN SEPTEMBER 2002 (F-J) (0.50 M X 0.50 M QUADRATS FROM ZONES).
Median, 25th, 75th (box), 5th and 95th (whiskers) percentiles and outliers.

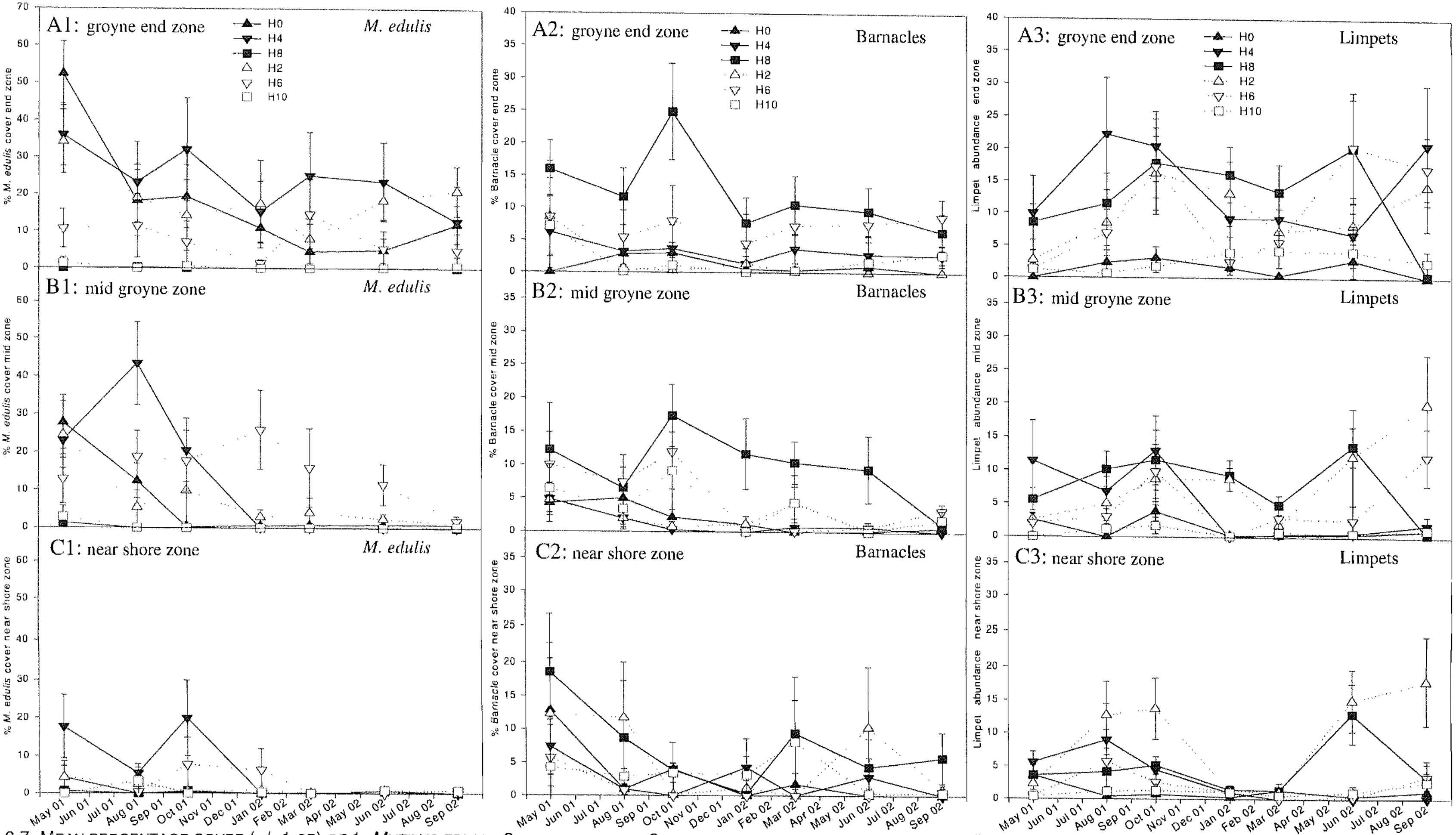

FIGURE 6.7 MEAN PERCENTAGE COVER (+/- 1 SE) OF 1: *MYTILUS EDULIS*, 2: BARNACLES AND 3: ABUNDANCE OF LIMPETS ON TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H10) GROYNES IN THE END, MID AND NEAR SHORE GROYNE ZONES BETWEEN MAY 2001 AND SEPTEMBER 2002. (0.50 M X 0.50 M QUADRATS).
7 quadrats each zone. Mid and upper eulittoral data for H4 and H2 unused after October 2001.

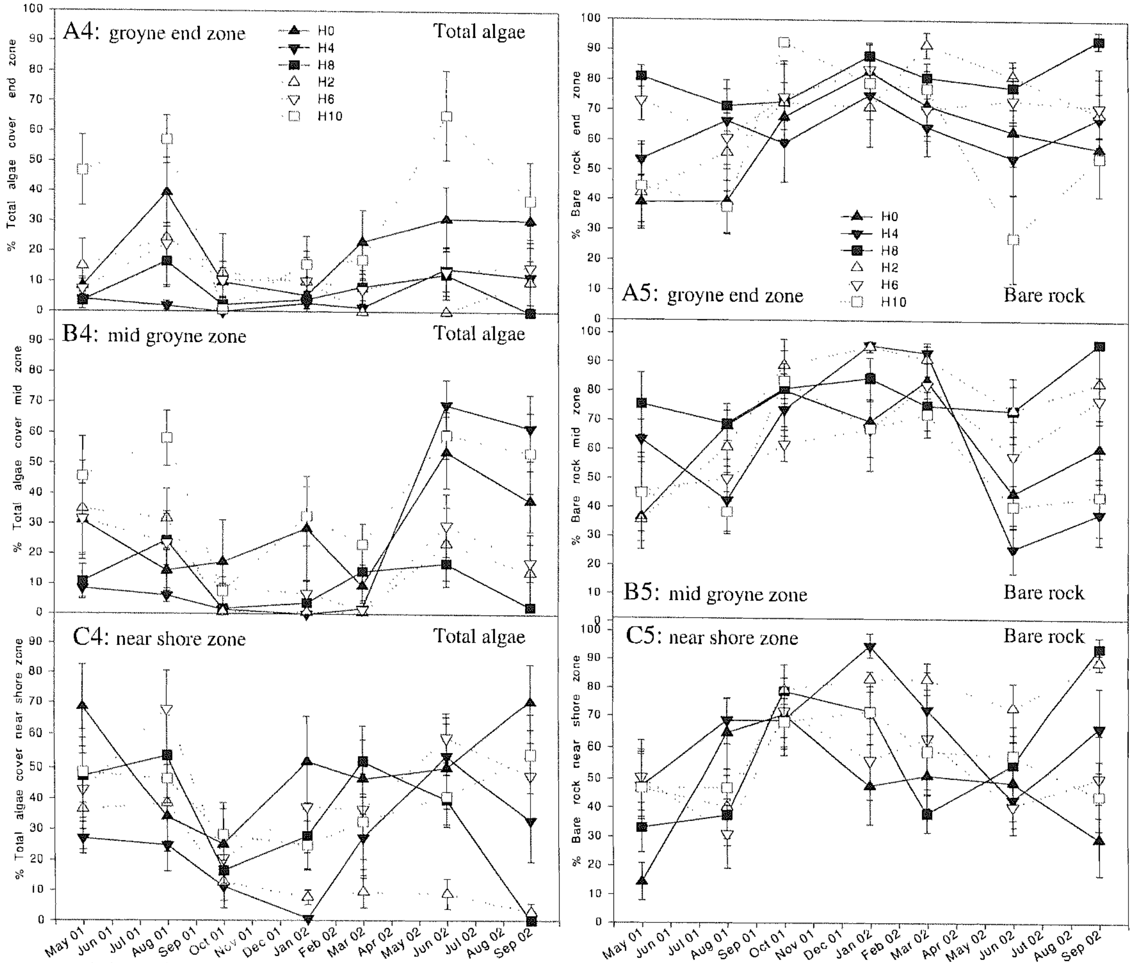

FIGURE 6.7 (CONTD) MEAN PERCENTAGE COVER (+/- 1 SE) OF 4: TOTAL ALGAE AND 5: BARE ROCK ON TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H10) GROYNES IN THE END, MID AND NEAR SHORE GROYNE ZONES BETWEEN MAY 2001 AND SEPTEMBER 2002. (0.50 M X 0.50 M QUADRATS).
7 quadrats each zone. Mid and upper eulittoral data for H4 and H2 unused after October 2001.

There were no significant differences between treatment and control abundances of taxa and bare rock in May 2001, before *N. lapillus* were removed (Fig. 6.6) (Table 3 Appendix 4). By September 2002 declines in *M. edulis*, barnacles and limpets and an increase in algae were seen, particularly on treatment groynes (Fig. 6.6). Within site comparisons were broadly similar to those found at the smaller scale (Table 6.1), although larger scale observations showed overall trends within zones more clearly (Fig.6.7).

M. edulis showed a general decline on both treatment and control groynes throughout the experiment, although this was more evident on treatments H0 and H4 (Fig. 6.7A-C1); losses on H4 in the mid and near shore zones were due to the groyne reconstruction. Differences identified by SNK tests in May 2001 (treatments: H0=H4>H8, $P<0.01$; controls: H2>H6>H10 $P<0.05$) were largely maintained throughout the experiment, although the overall decline led to slight reductions in significance within both treatments and controls. No significant treatment effects were found for end zone comparisons in March 2002 or September 2002 (Tables 4 and 5A,F, Appendix 4), thus a percentage cover increase in *M. edulis* cover on treatments following *N. lapillus* removal was not detected at the larger scale.

Barnacle cover remained relatively constant in the end zone although some decline occurred on treatment site H8 (Fig. 6.7A2) (also seen at the smaller scale, Fig. 6.5A2). Losses were also seen in both the mid and near shore zones on treatments and controls, although the near shore zone populations fluctuated more. No significant end, mid or near shore zone differences were found both between and within treatments and controls for March 2002 (Tables 4 and 5B, Appendix 1). In September the one significant effect (Table 4G, Appendix 1) was due to recruitment on the end zone of control groyne H6 (Fig. 6.7A,2).

At the larger scale, indirect effects of the reduction in *N. lapillus* populations were not detected in limpet abundances or algae and bare rock cover. No treatment and control effects were seen in March or September 2002 for limpets, although differences between end zones within treatment groynes (H0, H4, H8) increased in significance due to the possible disturbance on H8 (Fig. 6.7A3, Tables 4C,H, Appendix 4). Total algal cover and bare rock did not appear to respond to a change in limpet populations on treatment groynes. There were no significant main effects between treatment and control groynes for March and September 2002 (Tables 4 and 5D-J, Appendix 4).

6.3.3 Multivariate analysis of community responses to removal of *Nucella lapillus*

Data used in univariate analyses of taxa and bare rock following the reduction of *N. lapillus* predation were further analysed to show responses in the intertidal communities on treatment groynes (H0, H4, H8) (§ 6.2.2). Results collected at both scales (0.25 m x 0.25 m quadrats on blocks and 0.50 m x 0.50 m quadrats in zones, Fig. 6.2) in the end, mid and near shore zones were pooled. Bare rock was not included in community analysis, mussels, barnacles and limpets were recorded as single taxonomic groups and algae were recorded as species.

In May 2001, before *N. lapillus* were removed from the treatment groynes, communities appeared broadly similar at both scales of resolution (Fig. 6.8). Results from surveys on the blocks (0.25 m x 0.25 m quadrats, Fig. 6.8A) showed that there was little clear differentiation between the communities on treatment (H0, H4, H8) and control (H2, H6, H10) groynes. Stress levels at achieving the plots (§ 5.2.5) showed that the results were a useful representation of the data. Some clustering was seen for data from treatment groynes H4 and H8 (due to *M. edulis* and barnacle dominance respectively, Fig. 6.4A,B) and for control groyne H10 due to the dominance of algal cover and bare rock (Fig.6.4D,E). These patterns were repeated for data collected within zones (0.50 m x 0.50 m quadrats) with H4 in particular showing clustering (Fig. 6.8B).

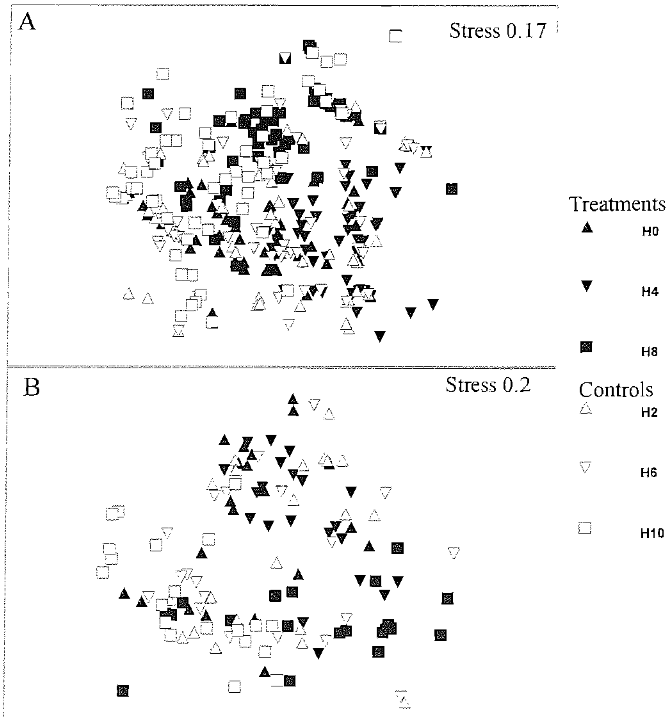

FIGURE 6.8 NON METRIC MULTI DIMENSIONAL SCALING (NMDS) COMPARISONS FOR MAY 2001 OF COMMUNITIES ON TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H10) GROYNES FOR A: 0.25 M X 0.25 M QUADRAT DATA COLLECTED FROM BLOCKS AND B: 0.50 M X 0.50 M QUADRAT DATA COLLECTED WITHIN ZONES.

Following nMDS analysis a nonparametric analysis of variance (NPMANOVA) (§ 6.2.2) was used to show if any pre-existing significant differences existed within and between treatment and control groyne communities. Comparisons for zones (end, mid and near shore) on each groyne at both scales (0.25 m and 0.50 m) showed no significant differences between treatment and control groynes (Table 6.4). As with univariate data, however, significant effects were seen at both scales for comparisons within treatment (H0, H4, H8) and control (H2, H6, H10) groynes (Table 6, Appendix 4). Differences were more significant (P<0.001) within treatment zones for 0.25 m x 0.25 m data, particularly for groynes H4 and H8. Within controls, 0.25 m x 0.25 m data comparisons were less distinct. H2 and H6 end and near shore zones were significantly different (Table 6A,F, Appendix 4), but less so than H10 which was dominated by ephemeral algae. Comparisons for data collected within the zones (0.50 m x 0.50 m) were more similar, particularly for control groynes (Table 6, Appendix 4) although differences were still found particularly between end zones (Table 6J-L, Appendix 4).

TABLE 6.4 NPMANOVA ANALYSIS OF COMMUNITY DATA IN END, MID AND NEAR SHORE ZONES AT THE START OF THE EXPERIMENT (MAY 2001) (0.25 M X 0.25 M AND 0.50 M X 0.50 M QUADRATS).
NS: Not significant. Source key: TR: treatment, Ctrl: control, GR: Groyne.

A: End zone 0.25 m x 0.25 m results treatment and control

Source	df	MS	F	P
Tr vs Ctrl	1	5676.9	0.2	NS
Gr(Tr)	4	26475.5	10.3	<0.001
RES	114	2560.3		
TOT	119			

B: Mid zone 0.25 m x 0.25 m results treatment and control

Source	df	MS	F	P
Tr vs Ctrl	1	2916.8	0.5	NS
Gr(Tr)	4	17892.0	6.9	<0.001
RES	114	2610.5		
TOT	119			

C: Near shore zone 0.25 m x 0.25 m results treatment and control

Source	df	MS	F	P
Tr vs Ctrl	1	22763.1	1.4	NS
Gr(Tr)	4	16508.8	7.5	<0.001
RES	114	2199.9		
TOT	119			

D: End zone 0.50 m x 0.50 m results treatment and control

Source	df	MS	F	P
Tr vs Ctrl	1	4880.8	0.4	NS
Gr(Tr)	4	12084.5	7.3	<0.01
RES	36	1663.53		
TOT	41			

E: Mid zone 0.50 m x 0.50 m results treatment and control

Source	df	MS	F	P
Tr vs Ctrl	1	5630.8	1.2	NS
Gr(Tr)	4	4529.7	2.2	<0.05
RES	36	2034.6		
TOT	41			

F: Near shore zone 0.50 m x 0.50 m results treatment and control

Source	df	MS	F	P
Tr vs Ctrl	1	3706.9	1.2	NS
Gr(Tr)	4	3055.3	1.8	<0.05
RES	36	1684.1		
TOT	41			

A SIMPER (§ 6.2.2) analysis was carried out (Table 6.5) to show which taxa constituted the main influence on patterns at the start of the experiment in May 2001. Results were similar at both scales with the exception of *Fucus* having a minor influence on 0.50 m x 0.50 m results mainly within treatment groynes which was not evident in 0.25 m x 0.25 m quadrat data (Table 6.5B). At both scales the influence of taxa was similar on treatment groynes with *M. edulis* the major factor, but on control sites the cover of ephemeral algae (*Ulva lactuca, Enteromorpha*) had a greater influence on 0.50 m x 0.50 m results (Table 6.5B).

TABLE 6.5 SIMPER COMPARISONS OF TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H10) 0.25 M X 0.25 M AND 0.50 M X 0.50 M QUADRAT RESULTS AT THE START OF THE EXPERIMENT IN MAY 2001.
Av.Abund: the average abundance of each taxa in sample groups, Av, Diss: the average dissimilarity among groups of samples, Diss/SD: measure of the variation of species contribution to group dissimilarity, Contrib%: the percentage contribution that species gives to between group dissimilarity.

A: 0.25 m x 0.25 m. Groups: Treatment (H0, H4, H8) and Control (H2, H6, H10) Average dissimilarity = 75.51

Species / taxa	Av.Abund treatment	Av.Abund control	Av.Diss	Diss/SD	Contrib%
Mytilus edulis	17.2	9.5	18.2	0.9	24.2
Ulva lactuca	10.4	13.1	16.5	0.9	21.8
Enteromorpha	7.8	10.1	13.7	0.9	18.2
Barnacles	8.8	4.5	13.0	0.8	17.2
Limpets	1.6	0.8	6.4	0.9	8.5

B: 0.50 m x 0.50 m. Groups: Treatment (H0, H4, H8) and Control (H2, H6, H10) Average dissimilarity = 67.27

Species / taxa	Av.Abund treatment	Av.Abund control	Av.Diss	Diss/SD	Contrib%
Mytilus edulis	18.4	10.3	14.9	1.0	22.2
Ulva lactuca	11.3	15.9	13.4	1.1	20.0
Enteromorpha	9.6	16.1	13.0	1.1	19.3
Barnacles	9.3	7.6	10.8	0.9	16.1
Limpets	5.7	1.4	7.6	1.0	11.3
Fucus	4.5	1.0	1.6	3.0	0.5

Post hoc SIMPER tests showed the relative influence of taxa contributing to community patterns within and between treatment and control groynes (Tables 7 and 8, Appendix 4). Within treatment groynes 0.25 m x 0.25 m quadrat results from block surveys showed that on H4 *M. edulis* and H8 barnacles had the main dissimilarity effects (Table 7A, Appendix 4). Ephemeral algae dictated patterns in 0.25 m x 0.25 m data from treatment groyne H0, as with control groynes H6 and H10. On H2 (control) the main community dissimilarity was due to *M. edulis* cover (Table 7A, Appendix 4). For 0.50 m x 0.50 m quadrats (Table 7B Appendix 4) Bray-Curtis dissimilarity results were similar to 0.25 m x 0.25 m data, but at the larger scale *M. edulis* was dominant on H0.

SIMPER comparisons between groynes showed that for 0.25 m x 0.25 m results (Table 8A, Appendix 4) the dissimilarity influences of *M. edulis*, barnacles and ephemeral algae were highlighted; results for data collected within zones (0.50 m x 0.50 m) (Table 8B, Appendix 4) were similar. At the larger scale a switch from barnacle and ephemeral algae dominance to *M. edulis*, barnacles and ephemeral algae was seen between treatment groynes H0 and H8; a greater influence from barnacle cover between treatment groyne H4 and control H2, and a decrease in barnacle importance between treatment groyne H8 and control H10. In addition whereas with 0.25 m x 0.25 m quadrat data (Table 8A, Appendix 4) patterns of dissimilarity tended to be dominated by one taxon, with the larger quadrats the effects of the taxa tended to be more evenly distributed (Table 8B Appendix 4).

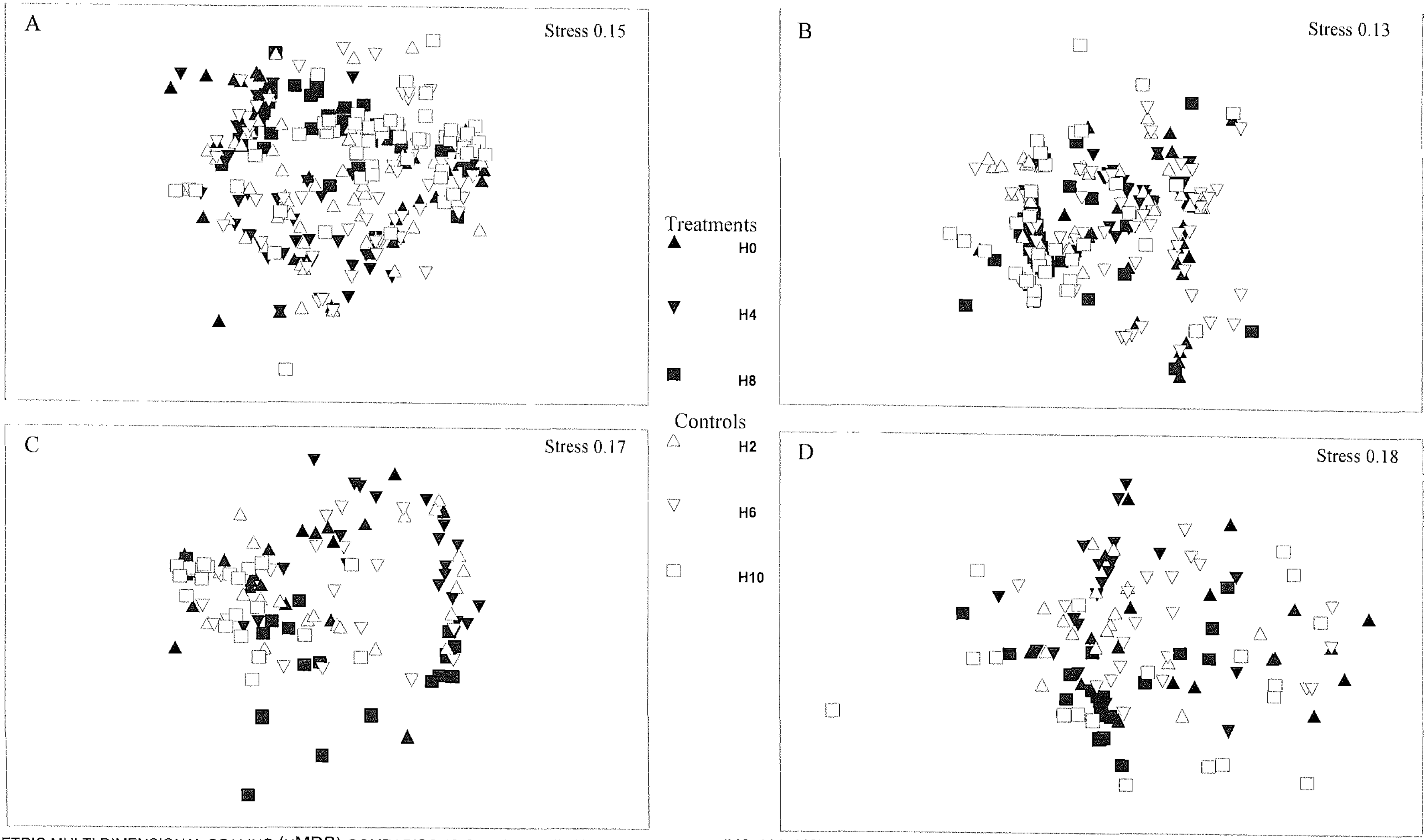

Figure 6.9 Non metric multi dimensional scaling (nMDS) comparisons of communities on treatment (H0, H4, H8) and control (H2, H6, H10) groynes.
A: 0.25 m x 0.25 m quadrat results August 2001, B: 0.25 m x 0.25 m quadrat results October 2001, C: 0.50 m x 0.50 m quadrat results August 2001, D: 0.50 m x 0.50 m quadrat results October 2001.

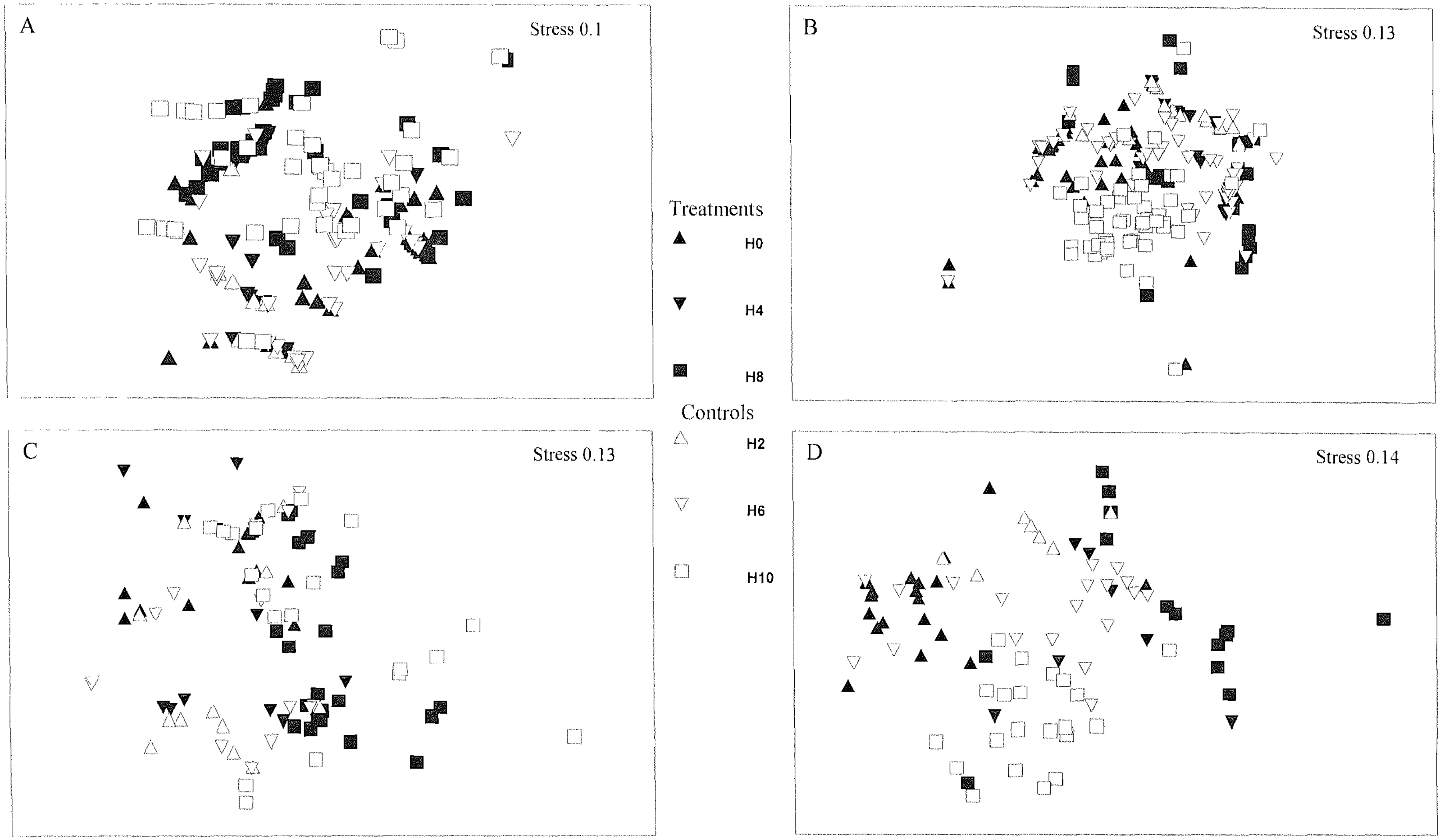

FIGURE 6.10 NON METRIC MULTI DIMENSIONAL SCALING (NMDS) COMPARISONS OF COMMUNITIES ON TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H10) GROYNES
A: 0.25 m x 0.25 m quadrat results March 2002, B: 0.25 m x 0.25 m quadrat results September 2002, C: 0.50 m x 0.50 m quadrat results March 2002, D: 0.50 m x 0.50 m quadrat results September 2002

Change following the removal of *N. lapillus* from treatment groynes (H0, H4, H8) was first assessed in August. These results were included to highlight natural fluctuations as the dogwhelk populations were only fully cleared at the beginning of the month. NMDS plots at both scales (0.25 m x 0.25 m, Fig. 6.9A,B and 0.50 m x 0.50 m Fig. 6.9C,D) did not show clear differences between data from the different groynes; stress levels for the plots were similar to experiment start values (Figs. 6.8A,B). By October no significant main effects between treatments and controls were found for NPMANOVA results at both spatial scales (Table 9, Appendix 4); *post hoc* tests within treatment and control results did not indicate any significant change. SIMPER tests showed that the influence of ephemeral algae on Bray-Curtis dissimilarity declined (Table 10, Appendix 4), which resulted in a slightly increased effect compared to barnacles and *M. edulis*. *M. edulis* decreased slightly whereas barnacle cover remained more stable; both trends were more apparent on treatment groynes. Dissimilarity results at both spatial scales were comparable (Table 10, Appendix 4).

Community surveys were also affected by the reconstruction of treatment groyne H4, thus NPMANOVA analyses followed the experimental redesign (§ 6.2.2). In March and September 2002 nMDS plots for results from blocks (0.25 m x 0.25 m, Fig. 6.10A,B) and zones (0.50 m x 0.50 m, Fig. 6.10C,D) showed a similar lack of clusters to May 2001 (Fig. 6.8A,B) (before *N. lapillus* were removed). Some clustering of results from blocks (0.25 m x 0.25 m) on treatment groyne H8 was seen for March 2002 (Fig. 6.10A). Raw data showed this was due to barnacle density in end, mid and near shore zones indicating that on blocks at the base of the groyne barnacle cover was similar (Fig. 6.5A-C2). For 0.50 m x 0.50 m quadrats no treatment and control effects were seen for March (Fig. 6.10C) or September 2002 results at both scales (Fig. 6.10B,D) although at 0.50 m x 0.50 m some clustering was seen for H0 (treatment) and H10 (control) (Fig. 6.10D) due to increasing ephemeral algae cover (Fig. 6.7A-C4). Due to disturbance, community loss on H8 was seen at both scales (Fig. 6.10B,D) with clustering on both plots due to the remaining limpets and barnacle cover.

As with univariate analysis trends of change were not apparent, therefore only data from surveys in March and September 2002 are presented in more detail as these represent the mid point and end of experimental work. NPMANOVA comparisons between treatment and control results for March and September 2002 were not significant at both spatial scales (Table 6.6). As with results from May 2001, within treatment and control comparisons showed significant differences apart from the mid zone 0.50 m x 0.50 m result for September

2002 (Table 6.6K). *Post hoc* tests (Table 11, Appendix 4) showed that whilst there were still significant differences for results at both spatial scales (0.25 m x 0.25 m, Table 11A-F, Appendix 4 and 0.50 m x 0.50 m, Table 6.11G-L, Appendix 4), dissimilarities within treatment and control groynes in March 2002 had reduced when compared to from May 2001. By September 2002 some increases in significance were seen at both spatial scales in the end and near shore zones (Table 11A,B,E,F,G,H,K,L, Appendix 4). In the mid zones (Table 11C,D,I,J, Appendix 4) groyne comparisons were less significant or unchanged.

TABLE 6.6 NPMANOVA COMPARISONS OF TREATMENT AND CONTROL 0.25 M X 0.25 M AND 0.50 M X 0.50 M QUADRAT RESULTS IN THE END, MID AND NEAR SHORE ZONES IN MARCH AND SEPTEMBER 2002.
NS: Not significant. Source key: TR: treatment, Ctrl: control, GR: Groyne.

A: End zone 0.25 m x 0.25 m data treatment and control March 2002

Source	df	MS	F	P
Tr vs Ctrl	1	4544.4	0.3	NS
Gr(Tr)	4	14594.7	4.7	<0.001
RES	114	3099.2		
TOT	119			

B: Mid zone 0.25 m x 0.25 m data treatment and control March 2002

Source	df	MS	F	P
Tr vs Ctrl	1	5941.5	0.2	NS
Gr(Tr)	4	22196.9	6.7	<0.001
RES	114	3306.8		
TOT	119			

C: Near shore zone 0.25 m x 0.25 m data treatment and control March 2002

Source	df	MS	F	P
Tr vs Ctrl	1	11155.3	1.5	NS
Gr(Tr)	4	7136.5	2.1	<0.001
RES	114	3311.2		
TOT	119			

D: End zone 0.25 m x 0.25 m data treatment and control Sept 2002

Source	df	MS	F	P
Tr vs Ctrl	1	20019.4	0.6	NS
Gr(Tr)	4	31544.1	12.8	<0.001
RES	114	2456.0		
TOT	119			

E: Mid zone 0.25 m x 0.25 m data treatment and control Sept 2002

Source	df	MS	F	P
Tr vs Ctrl	1	39563.6	1.3	NS
Gr(Tr)	4	28446.0	12.3	<0.001
RES	114	2310.7		
TOT	119			

F: Near shore zone 0.25 m x 0.25 m data treatment and control Sept 2002

Source	df	MS	F	P
Tr vs Ctrl	1	30440.3	1.1	NS
Gr(Tr)	4	28098.1	9.6	<0.001
RES	114	2923.6		
TOT	119			

G: End zone 0.50 m x 0.50 m data treatment and control March 2002

Source	df	MS	F	P
Tr vs Ctrl	1	918.1	0.1	NS
Gr(Tr)	4	7116.1	2.5	<0.01
RES	36	2805.6		
TOT	41			

H: Mid zone 0.50 m x 0.50 m data treatment and control March 2002

Source	df	MS	F	P
Tr vs Ctrl	1	2072.8	0.1	NS
Gr(Tr)	4	10895.4	3.6	<0.001
RES	36	2947.1		
TOT	41			

I: Near shore zone 0.50 m x 0.50 m data treatment and control March 2002

Source	df	MS	F	P
Tr vs Ctrl	1	5739.5	1.0	NS
Gr(Tr)	4	5501.0	2.1	<0.05
RES	36	2568.4		
TOT	41			

J: End zone 0.50 m x 0.50 m data treatment and control Sept 2002

Source	df	MS	F	P
Tr vs Ctrl	1	4571.6	0.3	NS
Gr(Tr)	4	12918.0	6.0	<0.001
RES	36	2149.4		
TOT	41			

K: Mid zone 0.50 m x 0.50 m data treatment and control Sept 2002

Source	df	MS	F	P
Tr vs Ctrl	1	1669.0	0.9	NS
Gr(Tr)	4	1738.2	0.5	NS
RES	36	3036.1		
TOT	41			

L: Near shore zone 0.50 m x 0.50 m data treatment and control Sept 2002

Source	df	MS	F	P
Tr vs Ctrl	1	8500.13	0.6	NS
Gr(Tr)	4	13677.4	5.3	<0.001
RES	36	2563.5		
TOT	41			

At both spatial scales SIMPER comparisons for March 2002 (Table 6.7A,B) showed the decreasing influence of *M. edulis* from May 2001 (Table 6.5) indicating that they had not increased on treatment groynes after predation declined. For 0.25 m x 0.25 m results mussels had already declined by August 2001, being initially replaced by *Enteromorpha* (Table 10A, Appendix 4) and by barnacles in October (Table 10B, Appendix 4). In March 2002 *Enteromorpha* and barnacles were dominant at both spatial scales (Table 6.7A,B) and the effect of barnacles in treatments was greater than May 2001 0.25 m x 0.25 m results (Table 6.5). In addition, limpet abundance in 0.50 m x 0.50 m quadrats was a significant factor in community composition (Table 6.7B), suggesting that they were patchily distributed and more effectively quantified with larger quadrats. However, recruitment or decline of limpets from May 2001 values was not found on treatment groynes at either scale (Table 6.7A,B).

Table 6.7 SIMPER comparisons of treatment and control 0.25 m x 0.25 m and 0.50 m x 0.50 m quadrat results in March and September 2002.
Av.Abund: the average abundance of each taxa in sample groups, Av, Diss: the average dissimilarity among groups of samples, Diss/SD: measure of the variation of species contribution to group dissimilarity, Contrib%: the percentage contribution that species gives to between group dissimilarity.

A: 0.25 m x 0.25 m groups: Treatment (H0, H4, H8) and Control (H2, H6, H10)
Average dissimilarity = 78.01 March 2002

Species	Av.Abund treatment	Av.Abund control	Av.Diss	Diss/SD	Contrib%
Enteromorpha	14.7	11.1	23.0	0.9	29.5
Barnacles	12.1	3.6	19.8	0.9	25.4
Mytilus edulis	5.6	12.4	17.7	0.7	22.7
Limpets	1.1	1.41	8.5	0.9	10.9
Ulva lactuca	NA	NA	NA	NA	NA

B: 0.50 m x 0.50 m groups: Treatment (H0, H4, H8) and Control (H2, H6, H10)
Average dissimilarity = 49.06 March 2002

Species	Av.Abund treatment	Av.Abund control	Av.Diss	Diss/SD	Contrib%
Enteromorpha	19.7	15.6	21.4	1.1	30.1
Barnacles	4.9	3.6	11.5	0.8	16.2
Limpets	4.2	3.5	12.7	1.0	17.9
Mytilus edulis	4.1	6.2	10.0	0.6	14.1
Fucus	0.9	2.3	4.3	0.4	6.0
Pelvetia	1.3	0.1	3.5	0.4	4.9
Ulva lactuca	1.6	0.3	3.0	0.3	4.2

C: 0.25 m x 0.25 m groups: Treatment (H0, H4, H8) and Control (H2, H6, H10)
Average dissimilarity = 81.33 September 2002

Species	Av.Abund treatment	Av.Abund control	Av.Diss	Diss/SD	Contrib%
Enteromorpha	20.6	11.3	22.8	0.9	28.1
Ulva lactuca	2.5	19.4	19.0	0.8	23.3
Mytilus edulis	6.8	8.8	13.5	0.6	16.6
Barnacles	4.6	3.3	11.3	0.7	13.9
Limpets	1.2	1.9	9.1	0.8	11.2

D: 0.50 m x 0.50 m groups: Treatment (H0, H4, H8) and Control (H2, H6, H10)
Average dissimilarity = 79.01 September 2002

Species	Av.Abund treatment	Av.Abund control	Av.Diss	Diss/SD	Contrib%
Enteromorpha	21.3	11.0	19.5	0.9	24.7
Ulva lactuca	2.8	18.4	16.5	0.9	20.9
Limpets	4.4	7.7	13.3	1.0	16.9
Mytilus edulis	4.2	4.4	9.6	0.7	12.2
Barnacles	2.74	2.5	8.8	0.8	11.1
Fucus	1.2	2.7	4.0	0.4	5.1

SIMPER comparisons in September 2002 showed that *Enteromorpha* and *U. lactuca* had the most affect on dissimilarity measures (Table 6.7C,D) reflecting the high abundance of algae at both scales in all of the zones (Figs. 6.5A-C4 and 6.7A-C4). *U. lactuca* had clearly increased over cover recorded in March whereas in *Enteromorpha* this was less pronounced. In September 2002 at both scales (0.25 m x 0.25 m and 0.50 m x 0.50 m) *Enteromorpha* cover was dominant on treatment groynes whereas *U. lactuca* was more common on control groynes (Table 6.7C,D). In September 2002 the influence of *N. lapillus* prey species had not demonstrably increased and limpet abundance remained largely similar although slightly increased in control groyne 0.50 m x 0.50 m quadrat results. Overall, the influence of ephemeral algae appeared to be the dominant factor identified by SIMPER analysis with barnacle and mussel decline on treatment and control sites reducing their influence in community dissimilarly by September 2002. Indirect effects of the reduction of *N. lapillus* predation on treatment communities leading to a decline in limpets were not established.

SIMPER *post hoc* within groyne comparisons for March and September 2002 highlighted taxa changes at both scales (Tables 12, Appendix 4). On treatment groynes the influence of *N. lapillus* removal was not seen in the communities. Prey taxa declined, particularly *M. edulis*, (Figs. 6.5A-C1, 6.5A-C2 and 6.7A-C1, 6.7A-C2) resulting in a greater influence from limpets (e.g. treatment groyne H4 Table 12D Appendix 4). At both scales the main effect on community changes in March and September 2002 was the variable cover of ephemeral algae, fluctuating from low (winter) to high (late summer to early autumn) throughout the year (Figs. 6.5A-C4 and 6.7A-C4) particularly on groynes H0 (treatment) and H10 (control). This was particularly seen on H0 where mussel cover declined from May 2001 (Table 7, Appendix 4) to March 2002 (Table 12A,B, Appendix 4) at both scales (Figs. 6.5A-C4 and 6.7A-C4). Comparisons between groynes (Table 13, Appendix 4) further showed the importance of ephemeral algae. The influence of barnacle and *M. edulis* abundance both within treatment and between treatment and control groynes declined as cover was lost. In addition the influence of limpets increased over May 2001 results (Table 8, Appendix 1) as their abundance rose within zones, particularly by September 2002 (Table 12D, Appendix 4).

Community changes within and between treatment and control groynes appeared to be influenced primarily by factors other than predation by *N. lapillus*. The removal of dogwhelk predation did not appear to affect community composition either through increases in barnacle or mussel density, or the reduction of limpets. The large influence of ephemeral algae was indicative of early succession stages which reflected disturbance.

6.3.4 Trajectories of taxa and bare rock

Abundances of taxa and bare rock fluctuated on treatment and control groynes on both blocks (0.25 m x 0.25 m quadrats, Fig.6.5) and zones (0.50 m x 0.50 m quadrats, Fig. 6.7). To clarify changes, within treatment and control groyne comparisons were made between the abundance or cover of taxa and bare rock at the start of the experiment (May 2001) and their fluctuations in the end zones of the groynes at both scales until September 2002 (Fig. 6.11).

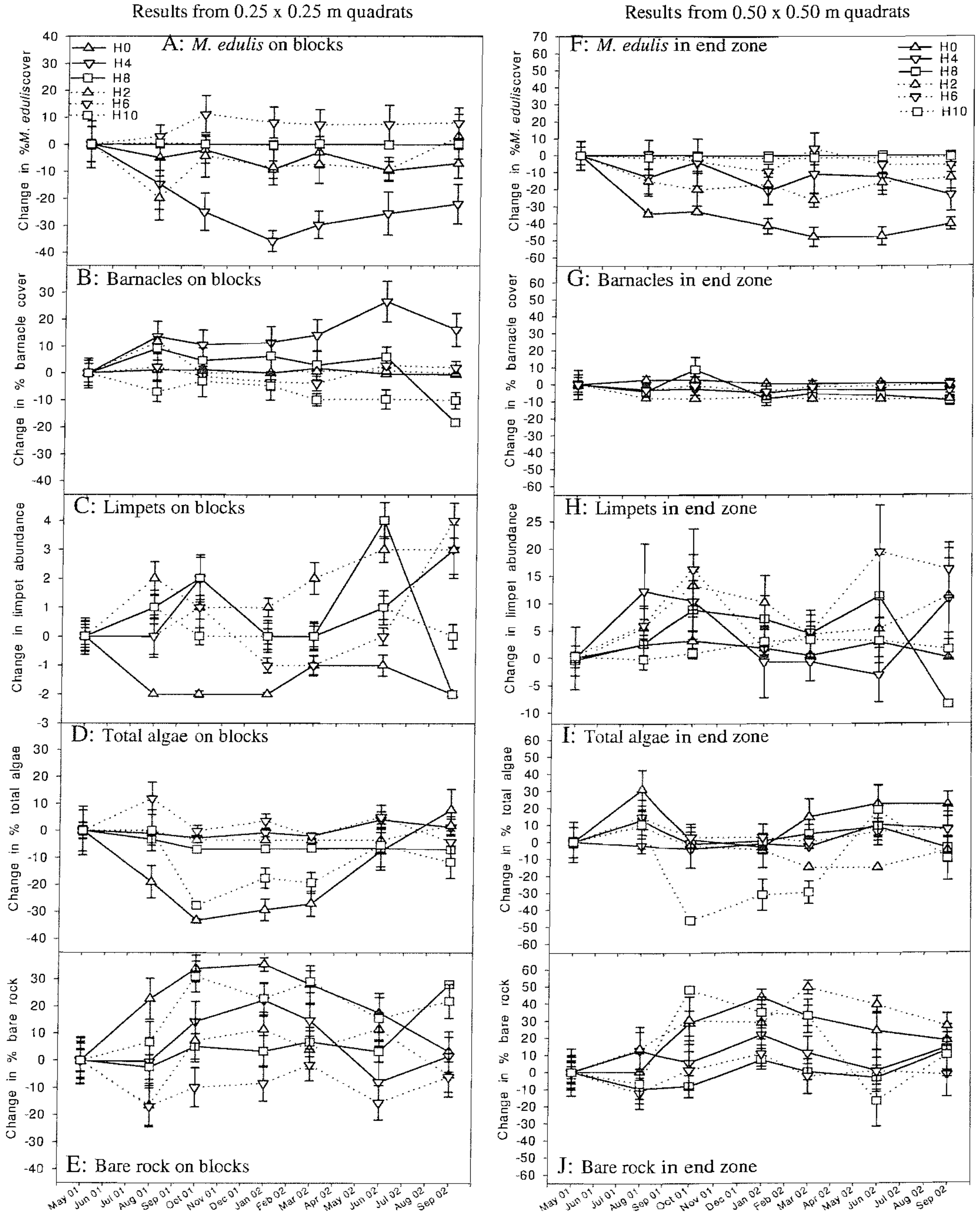

FIGURE 6.11 CHANGES OF MAJOR TAXA ABUNDANCE AND BARE ROCK IN TREATMENT (H0,H4,H8) AND CONTROL (H2,H6,H10) END ZONES FROM MAY 2001 TO SEPTEMBER 2002 FOR A-E 0.25 M X 0.25 M QUADRATS ON BLOCKS AND F-J 0.50 M X 0.50 M IN ZONES. +/- 1SE from mean of each original data set. Abundance in May 2001 set as zero and changes after this recorded as plus or minus from the experiment start results. Data from end zone only due to destruction of H4 mid and near shore zones in December 2001.

One sample T tests were carried out on patterns of temporal change in the groyne end zones (Table 6.8). As data were partially dependent confidence intervals were set at 99% and significance at P<0.01 (Jones, pers. com. 2005). Results showed that declines in *M. edulis* on the blocks (0.25 m x 0.25 m quadrats) and zones (0.50 m x 0.50 m quadrats) were significant on treatments H0 and H4 (Table 6.8, Fig. 6.11A,F). On control groyne H2 mussels declined significantly in the zone, but not the blocks, although an effect was suggested (Table 6.8). Conversely on H6 (control) *M. edulis* cover increased on the blocks, but not in the end zone. A significant decline was also seen on control groyne H10 within the end zone (Table 6.8), although mussel cover there was patchy and may have affected the result.

TABLE 6.8 ONE SAMPLE T-TEST RESULTS FOR TEMPORAL CHANGE FROM THE EXPERIMENT START (MAY 2001) TO FINISH (SEPTEMBER 2002) TAXA AND BARE ROCK ON BLOCKS AND WITHIN ZONE FOR TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H10) GROYNES. Treat: treatment, Ctrl: control. D: decline, I: increase, *: significance indicated (value given), but not accepted. NS: not significant, NA: None present. NS* and bold text show where an effect was indicated, but not significant (P>0.01), figures in bold italics indicate a significant effect P<0.01.

	M. edulis		**Barnacles**		**Limpets**		**Total algae**		**Bare rock**	
Groyne	0.25m x 0.25m quadrats blocks	0.50m x 0.50m quadrats zones	0.25m x 0.25m quadrats blocks	0.50m x 0.50m quadrats zones	0.25m x 0.25m quadrats blocks	0.50m x 0.50m quadrats zones	0.25 m x 0.25m quadrats blocks	0.50m x 0.50m quadrat zones	0.25m x 0.25m quadrats blocks	0.50m x 0.50m quadrats zones
H0 (treat)	***<0.01 D***	***<0.01 D***	NS	**NS* I (P=0.03)**	***<0.01 D***	**NS* I (P=0.02)**	NS	**NS* I (P=0.05)**	***<0.01 I***	***<0.01 I***
H4 (treat)	*<0.01 D*	*<0.01 D*	*<0.01 I*	*<0.01 D*	NS	NS	NS	NS	NS	***<0.01 I***
H8 (treat)	NA	NS	NS	NS	NS	NS	***<0.01 D***	NS	NS	NS
H2 (ctrl)	**NS* D (P=0.05)**	***<0.01 D***	NS	***<0.01 D***	***<0.01 I***	***<0.01 I***	NS	NS	NS	***<0.01 I***
H6 (ctrl)	***<0.01 I***	NS	NS	**NS * D (P=0.03)**	NS	**NS* I (P=0.02)**	NS	**NS* I (P=0.01)**	***<0.01 D***	NS
H10 (ctrl)	NS	***<0.01 D***	***<0.01 D***	***<0.01 D***	NS	**NS* I (P=0.02)**	**NS* D (P=0.01)**	NS	***<0.01 I***	NS

Whilst mussels declined significantly on treatment groyne H0, an effect from an increase in the percentage of barnacle cover end zone (0.50 x 0.50 m quadrats) was indicated although this was not significant (Table 6.8). Conversely on H4 (treatment) blocks barnacle cover significantly increased as *M. edulis* declined, but overall barnacles declined in the end zone (Fig. 6.11B,G). On treatment groyne H8, which was dominated by barnacles (Fig. 6.4B), no effects were seen. On control groynes H2 (zones) and H10 (blocks and zones) the percentage of barnacle cover declined; on H6 barnacle cover also reduced, but not significantly.

As previously highlighted (Figs. 6.5A-C3 and 6.7A-C3), limpet abundances were highly variable at both scales on all of the groynes, possibly reflecting sampling techniques (Figs. 6.1C,H). Abundances of limpets were higher when recorded in zones with the 0.50 m x 0.50 m quadrats. Limpet abundance changes on the treatment groynes were not significant for treatment groynes H4 and H8. On H0 they declined significantly on the blocks but increased overall in the end zone, although this was not significant (Table 6.8). Although declines in algae were only significant on blocks within treatment groyne H8 (Table 6.8, Fig. 6.11D,I) effects were indicated on other groynes (H0 zone, H6, zone, H10 blocks), but results were not significantly different. Bare rock (Fig. 6.11E,J) appeared to mirror the changes in algal cover, particularly on the blocks (Fig.6.11D, I), however the significant responses in bare rock change did not entirely reflect this. Whilst bare rock increased on both blocks and the end zone of H0 (treatment), algae did not respond inversely and this effect may have been due to the significant mussel losses on this groyne (Table 6.8). Similarly on H4 bare rock increased in the end zone whereas mussel cover declined, but on H8 no effect was seen for bare rock, indicating that the barnacle dominated community in the end zone was relatively stable. On control groynes bare rock increases were significant on both H2 and H10 in the end zone and on the blocks respectively (Table 6.8). These changes may have been due to a loss of mussels and barnacles on H2 and of barnacles and algae on H10. Finally H6 (control) was the only groyne where bare rock decreased on the blocks, possibly indicating the effect of the significant increase in the cover of *M. edulis* (Fig. 6.11A,E) .

The temporal changes seen in the prey (*M. edulis* and barnacles) of *N. lapillus* did not reflect the reduction of predation on treatment groynes. In addition, taxa which may have been expected to show indirect effects appeared to respond independently, with algal cover and limpet abundance increasing on both treatment and control groynes. Multiple correlations at both scales of resolution were carried out on the temporal changes in taxa (and bare rock) and *N. lapillus* abundance (recorded for Chapter 4 results) (Table 6.9). As with the t tests on temporal change in taxa and bare rock (Table 6.8), significance for the tests was set at 1% as results were partially dependent, thus avoiding the effects of multiplicity. Due to the low numbers of dogwhelks on treatment groynes after clearance, data from *N. lapillus* abundances recorded in each zone using timed searches were pooled into two age classes; second years were pooled with juveniles and small adults were combined with large adults.

Correlations between temporal changes in *N. lapillus* juvenile and adult abundance and taxa and bare rock did not show any significant relationships. On treatment groyne H4 positive relationships between adult *N. lapillus* and total algal cover were suggested at both scales, but were not statistically significant (Table 6.9). A similar effect was seen for both juveniles and adults on the blocks of groyne H6 (control). Positive relationships were also indicated between juvenile *N. lapillus* and barnacle cover in the end zone of control groyne H6, and a relationship between juveniles and adults and barnacle cover was indicated for control groyne H10 (0.50 m x 0.50 m quadrats). In addition a positive relationship between the abundance of juveniles and adults and percentage of mussel cover was also indicated for the end zone of control groyne H10 (0.50 m x 0.50 m quadrats). On groyne H10 dogwhelk prey were uncommon and very patchily distributed (Fig. 6.7A-C1 and 6.7 A-C2).

TABLE 6.9 CORRELATIONS BETWEEN MAY 2001 AND SEPTEMBER 2002, OF JUVENILE AND ADULT *NUCELLA LAPILLUS*, PERCENTAGE *MYTILUS EDULIS* AND BARNACLE COVER, LIMPET ABUNDANCE AND THE PERCENTAGE OF TOTAL ALGAE AND BARE ROCK ON TREATMENT (H0, H4, H8) AND CONTROL (H2, H6, H10) GROYNES, ON BLOCKS (0.25 M X 0.25 M) AND IN ZONES (0.50 M X 0.50 M).
Treat: treatment, ctrl: control. Juv: juvenile *N. lapillus*, Adu: adults *N. lapillus*, Muss: mussels, Limp: limpets, B.roc: bare rock, Alga: total algal cover, Barn, barnacles. NS: not significant, NA: not analysed as none present. * and bold text show where an effect was indicated, but not significant (P>0.01). Threshold for acceptance was 1% (P<0.01). 25cm: data collected with 0.25 m x 0.25 m quadrats for changes on blocks, 50cm data collected with 0.50 m x 0.50 m quadrats for changes within the end zone.
·N = 7.

	H0 treat		**H4 treat**		**H8 treat**		**H2 ctrl**		**H6 ctrl**		**H10 ctrl**	
	25cm	**50cm**	**25cm**	**50cm**	**25cm**	**50cm**	**25cm**	**50cm**	**25cm**	**50cm**	**25cm**	**50cm**
Juv/ Muss	+0.24 NS	+0.36 NS	-0.67 NS	0.00 NS	NA	-0.63 NS	+0.51 NS	-0.28 NS	+0.75 NS	-0.36 NS	+0.09 NS	*+**0.85** **P=0.03**
Juv/ Barn	-0.23 NS	-0.08 NS	-0.31 NS	-0.54 NS	-0.35 NS	+0.17 NS	-0.65 NS	+0.16 NS	-0.14 NS	*+**0.82** **P=0.04**	-0.17 NS	*+**0.85** **P=0.03**
Juv/ Alga	+0.30 NS	-0.48 NS	+0.01 NS	+0.06 NS	-0.53 NS	-0.27 NS	+0.11 NS	-0.50 NS	*+**0.83** **P=0.04**	-0.20 NS	-0.50 NS	-0.32 NS
Adu/ Muss	+0.54 NS	+0.02 NS	-0.14 NS	-0.02 NS	NA	-0.35 NS	+0.48 NS	-0.51 NS	+0.61 NS	+0.23 NS	-0.10 NS	*+**0.91** **P=0.01**
Adu/ Barn	+0.37 NS	+0.03 NS	+0.78 NS	+0.00 NS	-0.18 NS	+0.07 NS	-0.65 NS	-0.28 NS	-0.26 NS	+0.73 NS	+0.05 NS	*+**0.86** **P=0.02**
Adu/ Limp	+0.00 NS	+0.07 NS	+0.58 NS	-0.30 NS	+0.33 NS	+0.17 NS	+0.06 NS	+0.06 NS	+0.26 NS	+0.37 NS	-0.152 NS	-0.12 NS
Adu/ Alga	-0.44 NS	-0.66 NS	*+ **0.81** **P=0.05**	*+**0.83** **P=0.03**	-0.60 NS	-0.01 NS	+0.01 NS	-0.70 NS	***-0.84** **P=0.03**	-0.66 NS	-0.34 NS	-0.08 NS
Adu/ B. roc	+0.52 NS	+0.72 NS	-0.53 NS	-0.77 NS	+0.61 NS	-0.06 NS	+0.25 NS	+0.72 NS	+0.72 NS	-0.09 NS	+0.33 NS	+0.06 NS
Muss/ Barn	*+**0.83** **P=0.04**	+0.72 NS	+0.10 NS	+0.73 NS	NA	-0.20 NS	-0.53 NS	+0.08 NS	-0.45 NS	-0.21 NS	-0.08 NS	+0.74 NS
Muss/ Alga	-0.34 NS	-0.14 NS	+0.05 NS	-0.51 NS	NA	+0.71 NS	+0.74 NS	+0.34 NS	-0.54 NS	-0.42 NS	+0.43 NS	-0.35 NS
Barn/ Limp	+0.07 NS	*+**0.84** **P=0.03**	+0.11 NS	+0.32 NS	+0.64 NS	+0.36 NS	+0.09 NS	+0.14 NS	+0.59 NS	+0.78 NS	-0.29 NS	+0.03 NS
Barn/ Alga	-0.69 NS	+0.99 NS	*+**0.89** **P=0.05**	-0.35 NS	+0.35 NS	-0.35 NS	+0.14 NS	+0.30 NS	+0.25 NS	-0.00 NS	+0.26 NS	+0.02 NS
Limp/ Alga	-0.20 NS	-0.17 NS	+0.36 NS	-0.19 NS	+0.10 NS	+0.48 NS	+0.53 NS	-0.06 NS	-0.54 NS	+0.34 NS	*+**0.83** **P=0.03**	-0.23 NS
Limp/ B.roc	+0.14 NS	-0.20 NS	-0.04 NS	+0.36 NS	-0.34 NS	-0.57 NS	-0.37 NS	+0.126 NS	-0.08 NS	-0.00 NS	-0.85 NS	+0.21 NS

Interactions were also suggested between taxa and bare rock (e.g. *M. edulis* and barnacles on H0 blocks and limpets and algae on H10 blocks, Table 6.9), but the test results did not meet the accepted significance levels (P<0.01). Overall the only effects accepted at 1% were negative correlations between the percentage of algal cover and bare rock and these were only seen at both spatial scales on H10 (control) (not shown); two groynes (H4 treatment, H6 control) showed no effect for algae and bare rock. Although significant temporal changes in cover of *N. lapillus* prey were found (Table 6.8), these did not appear to relate to the abundance of *N. lapillus*.

6.4 Discussion

6.4.1 Procedural problems

A major difficulty with using Highcliffe as an experimental site for the study of interactions in rocky shore communities was that a generality of patterns could not be made due to the differing communities on each groyne. The general ecology of man made intertidal structures does not necessarily reflect that seen on nearby natural shores (Glasby and Connell, 2001; Bacchiocchi and Airoldi, 2003; Bulleri, 2005). In addition, communities within sites may differ between structures (Connell and Glasby, 1999). Colonisation of a southern UK sea defence structure was shown to be a slow process and problematic for some intertidal organisms to maintain populations (Moore, 1939, 1940). The communities at Highcliffe have developed in relative isolation from each other and, as observed for other work on man-made sea defences (e.g. Bacchiocchi and Airoldi, 2003), have relatively low species richness. When considering community interactions at Highcliffe, care must be made in interpreting these in relation to natural shores. For example Bulleri *et al.,* (2000) demonstrated differing limpet-grazing interactions on man-made and natural shores within the same locality. Conversely Chapman and Bulleri (2003) found that seawalls and natural shores in Sydney Harbour supported similar communities though species abundance differed. Observations at Highcliffe showed that communities differed markedly between groynes with varying gradients of algae and *M. edulis* or barnacle domination. This may be due to each groyne existing as a separate system with little interaction between them.

The set of groynes at Highcliffe initially appeared suitable for work of this nature, being replicated experimental areas (groynes) surrounded by a barrier of sand enabling manipulation of dogwhelk population densities without the problems of cage artefacts (Mac-Nally, 1997; Jenkins *et al.,* 1999). This system proved less tractable than initially thought due to a combination of human interference (re-building of one test groyne), high levels of between-groyne spatial variability and highly variable groyne-specific temporal dynamics. However, exclusions of dogwhelks from blocks on treatment groynes were very effective with low percentages of the initial abundances (recorded in May 2001) removed between September 2001 and September 2002 (Table 6.10). The blocks (surveyed with 0.25 m x 0.25 m quadrats) represented 30-40% of the available habitat within zones (surveyed with 0.50 m x 0.50 m quadrats). Therefore the percentage ranges were factored to indicate the percentage range of *N. lapillus* present within each zone (between September 2001 and September 2002) in comparison to the population in May 2001(Table 6.10). This indicated that the maximum percentage of dogwhelks within zones in comparison to the original population was 21% (H4 mid zone, Table 6.10) suggesting that the reduction of *N. lapillus* populations within the zones was between 79-100% of the initial population.

Table 6.10 Range of percentages of *Nucella lapillus* cleared from blocks on treatment groynes (H0, H4, H8) throughout the experiment (September 2001 to September 2002) and scaled up to indicate the total within zones as a percentage of the original population in the east face zones only.
EZ, end zone, MZ, mid zone, NSZ, near shore zone.

	Total *N. lapillus* abundance In zones May 2001	**Percentage range of *N. lapillus* removed from blocks between Sept 2001-Sept 2002 as a proportion of total numbers in May 2001(before clearance)**	**Percentage range of *N. lapillus* factored to represent total within zones between Sept 2001-Sept 2002 as a proportion of total in numbers in May 2001 (before clearance)**
H0 EZ	60	1.6 – 3.0	4.1- 8.3
H0 MZ	9	0	0
H0 NSZ	3	0	0
H4 EZ	476	0.4 - 8.4	1.0 – 21.0
H4 MZ	964	0.41 - 0.62	1.0 - 1.55
H4 NSZ	250	0.4 - 3.2	1.0 – 8.0
H8 EZ	242	0.4 - 2.8	1.0 - 7.2
H8 MZ	73	5.4 - 8.2	13.6 – 20.5
H8 NSZ	43	2.3 - 4.6	5.8 - 11.6

Results from Chapter 4 indicated some movement of *N. lapillus* between groynes. Between May and September 2001, however, repeated removal of dogwhelks led to a marked reduction in the treatment groyne populations with, initially, none being found during the first experimental survey in September 2001.

The community interactions and patterns investigated in this work did not indicate a specific role for *N. lapillus* predation in structuring the communities on the Highcliffe groynes. Previous work in the UK has also found difficulty in clearly identifying such a role (e.g. Spence, 1989; Proud, 1994), although the importance of *N. lapillus* in affecting barnacle abundance and age structure close to crevices has been shown (Connell, 1961; Johnson *et al.*, 1997). Therefore it may have been appropriate to conduct investigations upon crevice habitats (e.g. Connell, 1961; Johnson *et al.*, 1997; Schmidt *et al.*, 1999; Schmidt and Rand, 1999). The emphasis of this study, however, was on broader scale patterns and processes and due to the nature of the blocks and the spaces between them, crevice habitats were rare. Moreover as Highcliffe has a small tidal range, which only allowed a short working time, surveys at spatial scales less than the 0.25 x 0.25 m and 0.50 x 0.50 m observations taken, were not practicable. Although the sampling effort needed to effectively reduce the associated error was established for each taxa before the May 2001 baseline survey, investigations of community variability between groynes may have benefited from a greater effort at one spatial scale. An alternative to the randomly located 0.25 m x 0.25 m quadrats used to record community changes on the blocks would have been to use the randomly chosen blocks, but to record changes with fixed quadrats, thus ensuring spatial independence. This may then have enabled more effective comparisons of temporal fluctuations, although fixed quadrats may not be representative of the whole shore (Lewis, 1976).

Rocky shore communities are not normally affected by mobile fine and gravel sediment, although this was seen at St Agnes and Tregantle (Chapter 3), but at Highcliffe the dominant sediment is fine sand with injected gravels. The mobility and thus potential for scour of these sediments was seen, as groyne H4 was undermined and required rebuilding. It is probable that sediment mobilisation constituted a major disturbance of these communities, particularly in the lower (1 m) levels in the groyne zones. Therefore a method of evaluating sediment scour or deposition may have been useful in establishing factors causing changes in community patterns and species abundances.

6.4.2 Effects of *Nucella lapillus* in different spatial scales and assemblages

Physical influences coupled with plant and animal interactions dictate community structure in the intertidal zone (Raffaelli and Hawkins, 1996; Bertness *et al.,* 1999a,b). Sheltered shores are inherently stable (Southward and Southward, 1978) and predation has been shown as influential on community structure (Menge, 1976, 1978a,b; Menge and Sutherland, 1987). Conversely exposed shores are characterised by fluctuations in mussel density (e.g. Lewis, 1976) due to disturbance (e.g. wave shock, Menge, 1976, 1978a,b) and competition for limited space, particularly within and between mussels and barnacles (Menge, 1976). In the UK, a community scale impact from the chronic or catastrophic removal of *N. lapillus* on either sheltered or exposed shore communities has so far not been demonstrated.

The study of rocky shores in New England, USA, has led to conclusions that predation is the dominant biological effect on the structure of intertidal communities (Menge, 1976). Several authors have suggested that predatory gastropods have a direct effect on prey abundance (Connell, 1961, 1970; Dayton, 1971; Vadas, 1992, Petraitis, 1998). For example, predation by gastropods has been suggested to mediate intra and interspecific space competition in barnacle and *M. edulis* populations, thus influencing their limits of distribution (Dayton, 1971; Menge, 1976; Bertness, 1989; Bertness *et al.,* 1998). Furthermore indirect effects resulting in community cycling and facilitation due to a reduction of competition for space have also been considered (Lubchenco and Menge, 1978; Vadas, 1992; Hunt and Scheibling, 2001). Many examples of direct and indirect effects are from the north west Atlantic and north east Pacific shores including *N. lapillus* (Menge, 1978a,b) *N. emaginata* and *N. canaliculata* (Noda, 1999) and *N. lima* (Carroll and Highsmith, 1996).

On Pacific shores a predation reduction of two whelks increased mussel and barnacle density (Navarrete, 1996). Following manipulation of *N. emarginata* and *N. canaliculata* numbers, it was demonstrated that the predator-resistant mussel (*Mytilus californianus*) invaded gaps following clearance of the preferred prey species *M. trossulus*. It was thus clearly shown that gastropods had a mediating role in community succession (Wootton, 2002).

Research on northwest Atlantic and UK shores identified that predation by *N. lapillus* affected barnacle density around microhabitats (Connell, 1961, 1970; Menge, 1978a; Hughes and Burrows, 1993; Johnson *et al.,* 1997) and, to a lesser degree, mussels on wave exposed shores in North America (Hunt and Scheibling, 1998). On rocky shores in Maine it was found that predation by *N. lapillus* and bulldozing by *Littorina littorea* were important in clearing patches in populations of the barnacle *Semibalanus balanoides*. This resulted in annual cycling of algae to barnacle patches in the mid and low intertidal (Vadas, 1992). In addition, Petraitis (1990) concluded that predation by *N. lapillus* on barnacles had a facilitating role in providing a rough surface of dead barnacles for juvenile *M. edulis* to settle upon, although no direct effect on mussel density was observed. Following manipulations, however, Menge (1991) concluded that at wave sheltered sites *N. lapillus* reduced mussel density independently of barnacle impacts, but at highly exposed sites no overall effect was found due to dogwhelks being confined to crevices.

Communities on the groynes at Highcliffe broadly represented those associated with exposed to moderately exposed sites (2-3, Ballantine, 1961), therefore study of them followed the suggestion made by Spence (1989) that the impacts of *N. lapillus* may be more apparent on this type of shore. Although relatively close to each other (separated by 160 m), communities on the treatment and control groynes were often asynchronous (e.g. H8 dominated by barnacles and limpets, H4 *M. edulis* and H0 and H10 ephemeral algae). The effect of the removal of *N. lapillus* at unstable locations has the potential to be masked (Spence, 1989) and these variable communities may have confounded clear results.

There was a general decline of *M. edulis* within two of the treatment sites and recruitment was only seen on one control groyne. The major loss of *M. edulis* cover occurred on groyne H4 before the disturbance of the mid and near shore zones due to reconstruction,. This may have been due to the density of *M. edulis* on this groyne, as competition for space can lead to the development of unstable vertical mussel growth or 'hummocks' which can be removed by wave action (Harger and Ladenberger, 1971, Davenport *et al.,* 1996, 1998; Bertness *et al.,* 1998). On exposed and moderately exposed shores in New England, wave action removing and redistributing *M. edulis* has been shown to be of greater importance than *N. lapillus* predation in creating patchy community structure (Menge, 1976, 1978a,b; Lubchenco and Menge, 1978; Hunt and Scheibling, 1998, 2001). Wave shock (Lubchenco and Menge, 1978) removes mussel clumps, thus creating bare areas, and may allow inter and intraspecific competition for space within or between *M. edulis* and *Semibalanus balanoides* (Menge, 1976); barnacles did increase on groyne H4 after *M. edulis* declined. Lubchenco and Menge (1978) suggested, however, that on exposed sites mussels out-compete other space users resulting in a monopoly of the available habitat.

Control groynes H2 and H6 had similar densities of both *M. edulis* and barnacles and small increases in barnacle density were seen on treatment sites H4 and H8. The removal of *N. lapillus* did not have a significant effect on barnacle cover on any groyne. However the barnacle recruitment in the end zones of treatment groynes H4 and H8 may have been due to the reduction in *N. lapillus* numbers. *N. lapillus* were only seen feeding upon *M. edulis*, but this behaviour may have changed to barnacles when submerged as *N. lapillus* can exhibit prey switching, utilising optimal patch choice (Murdoch, 1969; Chase *et al.,* 2002). Barnacle cyprids can settle gregariously (Knight-Jones, 1955; DeWolf, 1973; Crisp, 1974; Thompson *et al.,* 1998; Kent *et al.,* 2003) and are believed to be able to detect mucus from predatory gastropods thereby avoiding the 'ghost of predation future' (Johnson and Strathmann, 1989; Proud, 1994), thus the removal of dogwhelks may have induced this. In addition to predation reduction and avoidance, patterns of barnacle variation may have been due to self-regulation through space competition enhanced in the absence of *N. lapillus* (Connell, 1961; Proud, 1994). Mortality in post settlement barnacles can be due to density dependent factors (Kent *et al.,* 2003) with crowding leading to a dense cover of mature animals and hummock formation, which are vulnerable to wave shock removal (Bertness, 1989, Bertness *et al.,* 1998). Through the selection of larger barnacles by *N. lapillus* (Connell, 1961) space competition may be reduced (Proud, 1994). Though the effects of *N. lapillus* removal trials by Proud (1994) were variable, the inference was that through predation bare patches arise which allow cyprids to settle.

In the UK an indirect role for *N. lapillus* in community interactions was proposed via a 'patchy mosaic model' for moderately exposed shores on the Isle of Man (Hawkins and Hartnoll, 1983; Hartnoll and Hawkins, 1985). Through barnacle predation, *N. lapillus*, was proposed to be involved in a cycle of macro algae-limpet-barnacle dominated shores, although stochastic events, particularly escapes (Burrows and Hawkins, 1998) of algae from limpet grazing, are also acknowledged as being influential in both initiating this process and making it unpredictable (Raffaelli and Hawkins, 1996). Patchy communities were not common at Highcliffe, but were seen on control groynes H2 and H6 comprising barnacles, limpets and *M. edulis*. Limpets were found in the barnacle matrices and were relatively common in all the zones on treatment groyne H8. The keystone role for limpets (Paine, 1966) in preventing fucus-dominated shores in the UK (Southward and Southward, 1978; Hawkins and Hartnoll, 1983; Hartnoll and Hawkins, 1985; Hawkins and Southward, 1992; Lindegarth, 2001; Jenkins *et al.,* 2005) did not appear to be a factor at Highcliffe, although this can vary regionally and in relation to algae propagule supply (Jenkins *et al.,* 2005). Although barnacle patches were present at Highcliffe, *Fucus* species were a relative rarity, being present only on control groynes H2 and H10 and largely in the near shore zones. Lower and mid shore communities had developed in a manner which indicated greater exposure on both the windward and leeward groyne faces, but no gradient of exposure tolerant fucoid species was present and no escapes were seen. In the absence of *Fucus* patches, limpets appeared to graze ephemeral algae species and occasional growths of *Porphyra umbilicalis* and *Mastocarpus stellatus* which were usually associated with *M. edulis*. Though barnacles did increase in the end zones of treatment groynes H4 and H8, limpets did also, which suggests an opportunistic utilisation of available space following *M. edulis* decline. These variations in limpet numbers, particularly in the groyne end and near shore zones, may have been due to sampling variance as quadrat sampling at both scales may have under-represented their abundance and timed searches may have been more appropriate (e.g. Kaustuv *et al.,* 2003).

Whilst barnacle regulation and subsequent limpet facilitation or inhibition have not been clearly established in this study, patchy growth of ephemeral algae was seen on all the groynes possibly indicating the varying degrees of disturbance (Sousa, 1979) on each groyne. Significant differences in algal abundance could not be reliably related to the removal of *N. lapillus* and cycles of algal cover were assumed to be controlled by light and, to a lesser degree, nutrient supply (Pihl *et al.*, 1996; Martins and Marques, 2002). Before disturbance and reconstruction groynes H8 and H4 always had less algae than other sites. Groyne H4 in particular was dominated by *M. edulis*, therefore limpet and algae cycles were not major features. Although H8 was barnacle-dominated, algae were uncommon in all zones, possibly due to grazing by the relatively abundant limpets. The major feature to arise from algal surveys was the communities present on treatment groyne H0 and control groyne H10 were largely different from the other groynes as ephemeral algae were abundant, but all other taxa were poorly represented. This was with the exception of *M. edulis* on groyne H0, which were largely confined to patches underneath blocks. As groynes H0 and H10 are at either end of the group, they do not benefit from protection by adjacent structures, and physical abrasion and disturbance may result in opportunistic colonisation by ephemeral algae (Sousa, 1979; Littler and Arnold, 1982).

At the spatial and temporal scales investigated, the results of this study suggest that direct and indirect influences of *N. lapillus* predation were not evident and that the variations within groyne communities were influenced by other factors, particularly disturbance. Predation by *N. lapillus*, shown in New England to be more important on sheltered shores (Petraitis, 1987, Menge, 1991), may not be a major factor determining community structure at Highcliffe. As the effect of removing *N. lapillus* predation appeared to be negligible, these variations appear to have been more related to stochastic events, natural growth cycles or abiotic influences.

6.4.3 Interactions between ecological and physical processes at Highcliffe

Physical forces at Highcliffe appear to be the dominant factors organising community structure, but from the data obtained it is not possible to make direct inferences regarding the importance of physical stresses in structuring Highcliffe groyne communities. In the UK it has been concluded that on some barnacle-dominated shores physical factors were of greater importance than predation by *N. lapillus* (Proud, 1994), and the same was suggested for Canadian *M. edulis* dominated communities (Hunt and Scheibling, 1998, 2001). Wave shock, smothering by the mobile sandy sediment and disturbance of the groynes due to reconstruction may be the prevalent processes influencing the system at Highcliffe, resulting in a cycling of groyne communities which were driven by these physical influences.

The fetch toward Highcliffe shore is interrupted by Hengistbury Head and Christchurch Ledge (Fig. 6.12), leading to Christchurch Bay having complex tidal circulations which create cycles of macroscale eddies and tidal splits (Riley, 1995). These cycles, coupled with storm-induced wave shock (such as those seen in autumn-winter 2001) may account for the differing communities established on each groyne at Highcliffe. In addition, wave shock had the potential to be enhanced within groynes (e.g. Bulleri, 2005) as spaces were intentionally left between the blocks to allow wave energy to dissipate. This may have resulted in concentrated water flows being driven through interstitial spaces, thus limiting refuges and increasing wave and sediment scour effects. High flow rates can be associated with bottom-up systems (Leonard *et al.*, 1998*)* whereby community structure was driven by wave forces which can also affect larval supply (Fischer-Piette, 1935 *op cit* Hughes and Burrows, 1993; Bulleri, 2005), whereas at low flow sites a top-down structure due to predation pressure was indicated (Leonard *et al.,* 1998). Thus, whilst top-down control of intertidal species abundance through predation has been identified, including predation by *N. lapillus* (e.g. Connell, 1961; Menge, 1976), bottom-up factors such as larval supply, flow rates and algal cover can also influence intertidal community structure (Leonard *et al.*, 1998; Menge, 2000; Bulleri, 2005) and may impact *N. lapillus* abundance (Thompson *et al.,* 2000).

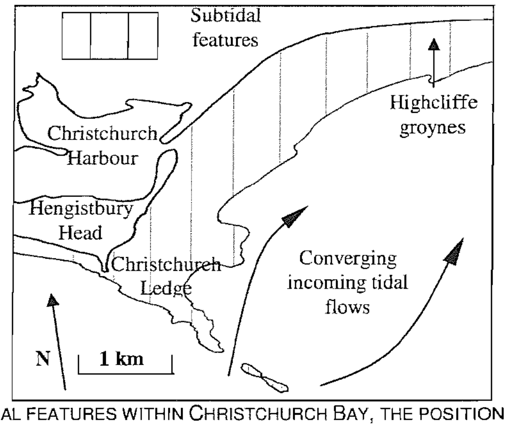

FIGURE 6.12 THE LOCATION OF SUBTIDAL FEATURES WITHIN CHRISTCHURCH BAY, THE POSITION OF HENGISTBURY HEAD AND CHRISTCHURCH LEDGE AND THE CONVERGING TIDAL STREAMS IN RELATION TO THE STUDY SITE AT HIGHCLIFFE GROYNES (Modified from Riley, 1995).

Groyne H4, on which large areas of mussels were lost, was subject to the greatest deposition and removal of sediment through tidal scour (Pers. obs.; Cross, pers. com, 2002). In addition thermal stress can impact *M. edulis* populations (Stephens and Bertness, 1991; Harley and

Helmuth, 2003). At Highcliffe, high temperatures and intraspecific competition, which led to hummocks (Davenport *et al.,* 1996, 1998), may have accounted for the early decline of *M. edulis* which increased with the onset of the autumn and winter storms in 2001. A switch toward a barnacle-dominated community, indicative of greater hydrodynamics in the form of higher flow rates, (Leonard *et al.,* 1998), appeared to be occurring after *M. edulis* losses on groyne H4, although this change was not clearly established.

Short-term changes driven by physical forces appeared to be the major factors dictating community patterns on the Highcliffe groynes. Predation by *N. lapillus*, shown in New England to be more important on sheltered shores (Petraitis, 1987, Menge, 1991), was not found to be important in this study. It may not be appropriate, however, to dismiss the role of predation by *N. lapillus* at Highcliffe entirely, but to consider it in conjunction with physical forces over longer periods. Although predation has been demonstrated as less important than physical effects on moderately exposed shores (Lubchenco and Menge, 1978), *N. lapillus* may have a long-term facilitating role which has not been clearly identified in the timescale over which interactions were investigated in this study. Predation can be concentrated on one organism where it is abundant and this may result in regulation of the prey species (Connell, 1961; Petraitis, 1990). Where *N. lapillus* is abundant it is potentially capable of depleting all available prey, particularly around crevices and where it has aggregated (Hughes and Burrows, 1993).

N. lapillus were initially highly abundant on treatment groynes H4 (4700 removed) and H8 (2500 removed) where they were seen to feed solely on *M. edulis* and barnacles respectively. Over the period of this work their removal did not appear to influence these groyne communities. However, after nine years of monitoring (1925-1933) at Décollé Point, Brittany, France, it was shown that *N. lapillus* initially specialised in barnacles, preferentially taking larger individuals but ignoring *M. edulis* patches (Fischer-Piette, 1935 *op cit* Hughes and Burrows, 1993). Petraitis (1990) has shown that *N. lapillus* barnacle predation can leave empty barnacle carapaces that provide an ideal rough surface for mussels to settle on. In 1930 local hydrographic variation at Décollé Point resulted in high mussel recruitment settling on former barnacle mats finally resulting in a mussel-dominated shore which prevented barnacle settlement (Fischer-Piette, 1935 *op cit* Hughes and Burrows, 1993). *N. lapillus* can relatively readily change diet (Connell, 1961) and at Décollé Point *N. lapillus* had switched to *M. edulis* over a 3 year period. Due to the predation switch, by 1933 mussels had severely declined and the shore had returned to a barnacle-dominated community (Fischer-Piette, 1935 *op cit* Hughes and Burrows, 1993). *N. lapillus* were frequently found sheltering under the mussel hummocks on groyne H4 and may have had a role in their loss. The hummock base layers of *M. edulis* were often eaten by *N. lapillus* which may have lead to instability and subsequent loss due to wave action, thus allowing barnacles to settle. Therefore it is possible that given sufficient larval supply of barnacles and the removal of *M. edulis* domination due to wave action (as seen on H4), if the groynes were left undisturbed the community may have ultimately switched from mussel to barnacle domination (as seen on H8). This in turn would lead to lower abundance of *N. lapillus* as shelter is reduced and they adapt from feeding on mussels to barnacles. Overall it seems unlikely that these alternative states (e.g. Bertness *et al.,* 2002; Paine and Trimble, 2004) existed, or were mediated due to predation by *N. lapillus* at Highcliffe. What was observed was possibly a reflection of the levels of disturbance and larval supply (Petraitis *et al.* 2003; Bulleri, 2005).

Differing levels of disturbance can lead to alternative communities. Dial and Roughgarden (1998) found that at high disturbance in marine communities a dominant (at Highcliffe *M. edulis*) would be driven extinct first. At Highcliffe *M. edulis* were lost in the storms allowing barnacles to settle, and after groyne H4 was rebuilt with the loss of large mussel beds, barnacles were the first sessile taxon to recolonise (e.g. Povey and Keough, 1991). At lower levels of disturbance (due to wave shock and scour etc) coexistence may have occurred; possibly exhibited by groynes H2 and H6 which were never rebuilt. Conversely H4 (mussel dominated) and H8 (barnacle dominated) were the two groynes most affected by erosion and consequent rebuilding, therefore physical factors and differing larval supply (e.g. Bulleri, 2005) may have resulted in the bi-polar communities. Bacchiocchi and Airoldi (2003) found that species richness was low on sea defence structures at the Emillia Romagna coast, Italy and that the communities were similar on each structure. Conversely at Highcliffe communities were remarkably different and previous levels of disturbance may have induced alternative states, thus masking any influence of predation by *N. lapillus*.

6.5 Summary

N. lapillus were removed from three limestone groynes at Highcliffe in Christchurch Bay, Dorset, and the subsequent changes between these and three control groynes were monitored for 13 months. The study investigated the direct influence of *N. lapillus* predation on interactions between prey species and the subsequent indirect effects of facilitation of algal growth due to restricted limpet mobility. All of the groyne communities fluctuated, but at both spatial scales examined changes potentially directly and indirectly attributable to a mediation of interactions by *N. lapillus* were not found.

For both mussels and barnacles the removal of predation by *N. lapillus* did not show a detectable effect at the spatial and temporal scales considered. As shown by Fischer-Piette (1935), however, predation influences may take several years to become apparent. Predation appears to have a minimal impact on the groyne communities when factors such as differential larval supply, complex tidal and exposure patterns, storms, scour and thermal stress are considered. Overall, however, human disturbance of the groyne communities may have been the major driver of change leading to the differing communities. Groynes H4 and H8 had entirely different communities present and both of these sites were subject to very high levels of erosion necessitating their re-engineering. This major disturbance may have 'switched' the intertidal communities to differing states particularly on groyne H4 where barnacles colonised spaces, possibly due to a reduction in *N. lapillus* mucus levels, or opportunistic cyprid settlement following the removal of *M. edulis* hummocks. These appeared to occur following overcrowding and were subsequently lost due to wave shock disturbance, possibly mediated by *N. lapillus* feeding on the basal mussel layer. The indirect impact of restricted limpet mobility due to growth of barnacle mats, leading to a loss of limpets and an increase in algal growth were not seen. This effect, however, may be expected on less exposed shores, and limpet grazing influences can differ regionally.

The Highcliffe groyne system may not be a true representation of what could occur on a natural shore following the removal of *N. lapillus*. Natural sites develop over numbers of decades rather than the fifteen years that those on the Highcliffe groynes have had and are not subject to regular and catastrophic physical changes that parallel engineering work. Previous monitoring on a natural shore has shown that dogwhelk predation over a longer time scale may affect communities on a scale greater than microhabitats and that *N. lapillus* may cause a whole shore community to shift from one prey taxa to another. Although this work simulated both the reduction and extinction of *N. lapillus* due to TBT pollution, on these types of shores it appeared that predation by dogwhelks was not important. Changes in community structure, attributable to reduction and removal of *N. lapillus*, have not been seen in this work, and therefore community changes in this context are not reliable indicators of TBT impacts.

Chapter 7. General discussion and overview

7.1 Overview of thesis

This study has focussed upon the long-term effects of the ubiquitous marine pollutant tributyltin (Strand and Asmund, 2003) upon individuals and populations of the bioindicator species *Nucella lapillus* in the southern UK. The efficacy of legislation to control TBT was previously investigated using dogwhelk populations in Plymouth Sound (Spence, 1989, Proud, 1994) and at other sites on the south coast (e.g. Bryan, 1987). This work has continued and updated these observations (Chapter 3). Population declines due to TBT impacts resulted in sites where *N. lapillus* were locally extinct (Spence *et al.,* 1990; Minchin *et al.,* 1997; Herbert *et al.,* 2000). Even though they travel short distances and have no pelagic larval stage (Connell, 1961; Castle and Emery, 1981) dogwhelks can colonise both former habitats (Herbert *et al.,* 2000) and new ones (Moore, 1939, 1940). How this is achieved is not well understood, although rafting by juveniles has been suggested (Bryan *et al.,* 1986), therefore investigations were carried out to identify if there were patterns within the recolonisation process (Chapter 4). In addition, although TBT levels have declined, at some locations *N. lapillus* have not re-established. Therefore further work in this study examined suitable methods to reintroduce dogwhelks, and investigated whether translocated individuals would find appropriate habitats, breed, and feed at the translocation site (Chapter 5). The impact of TBT reducing dogwhelk populations was previously suggested as an opportunity to clarify the role *N. lapillus* plays in structuring UK rocky shore communities (Hughes and Burrows, 1993). Their role has been demonstrated in the UK at small scales (Crothers, 1961; Proud, 1994; Johnson *et al.,* 1998), but following suggestions for further work on dogwhelks (Proud, 1994) their impact at larger scales, particularly on mussel communities, was investigated using a simulated TBT induced reduction in abundance (Chapter 6).

In the rest of this discussion the following themes are considered: the continued use of *N. lapillus* as a bioindicator of TBT levels; the effects of *N. lapillus* predation on rocky shore communities; the role of legislation against TBT in relation to conservation issues and the long-term legacy of organotin pollution.

7.2 The bioindication of TBT levels

After the partial ban on TBT for small boats early signs of recovery in some *N. lapillus* populations were found (e.g. Morgan *et al.,* 1998). It was reported that RPS values were lower and that recruitment of juveniles and the proportion of females had improved in Northumbrian coast populations (Evans, 1991). Further recovery was recorded throughout the 1990s (e.g. Proud, 1994; Harding *et al.,* 1997; Miller *et al.,* 1999), which led to suggestions that plans for a total ban on TBT were premature and that the economic benefits outweighed the environmental disadvantages (Evans, 1999; Abbot *et al.,* 2000). Recovery, or partial recovery, of dogwhelks from TBT impacts has now been widely demonstrated (e.g. Harding *et al.,* 1997; Morgan *et al.,* 1998), although it has also been suggested that natural effects such as parasitism (Mouritsen and Poulin, 2002) and other chemicals (e.g. nonylpehnol) can induce imposex in *N. lapillus* (Evans *et al.,* 2000b; Leung *et al.,* 2001) and other stenoglossan gastropods (e.g. Nias *et al.,* 1993). As a result of these observations it was suggested that imposex cannot always be attributed to TBT unless there is a known direct source (Evans *et al.,* 2000b). Conversely Matthiessen and Gibbs (1997) stated that imposex is unmistakably linked to a specific pollutant (TBT) and that it is the clearest example of chemical interference with endocrines (Spooner *et al.,* 1991). Based on the clear link between TBT and imposex (Bryan *et al.,* 1986) the recovery of *N. lapillus* populations appears to be a true reflection of current TBT controls. The dogwhelk populations at Plymouth, where the relationship was first shown, are some of the best indicators of long-term TBT levels (see Hawkins *et al.,* 2002).

The continued decrease of imposex at Plymouth Sound (Spence, 1989; Proud, 1994; Hawkins *et al.,* 2002; this work) appears to be related to lowered TBT levels as environmental management and dockyard practices improved (Champ, 2000). There is, however, increasing concern for the legacy from the release of TBT from sediments through dredging or disturbance (Brack, 2001). Difficulties in managing this impact have been highlighted (e.g. Svavarrson, 2001; Santos *et al.,* 2004). In this study imposex surveys from the *N. lapillus* populations immediately outside Plymouth Sound and those on the south coast of the Isle of Wight, together with tidal predictions, indicated that TBT from dredge spoil may be causing the high levels found.

Maritime ports are increasingly having to expand the throughput of containerised transport vessels to meet competition. Throughout Europe there have been plans to increase shipping levels by turning ports into regional hubs (Marcadon, 1999). Such increases in shipping tonnage at sites near to sensitive marine habitats, (e.g. Plymouth and proposals for Southampton Docks, Adams Hendry, 2000), may lead to further recharging of sediments with TBT if the reluctance to accept the proposed total TBT ban continues (IMO, 2001). In addition, as deeper channels will have to be maintained to greater depths to allow larger vessels (Side and Jowitt, 2002), the impact from dumped spoil may yet increase at sites not yet widely affected by TBT (Svavarrson, 2001). There is a variety of estimates of the long-term persistence of TBT in sediments (e.g. 10 years, Dowson *et al.,* 1996 and 20-30 years, Macguire, 2000). If the continued incidences of imposex found in this work and elsewhere (e.g. Fent, 2004; Santos *et al.,* 2004) are reliable indications of the future legacy of TBT from sediments, greater management of potential impacts is an important consideration. The areas at the south of the Isle of Wight and within Plymouth Sound are Special Areas of Conservation, but such designations do little to defend protected areas from transported pollutants (Boersma and Parrish, 1999; Terlizzi *et al.,* 2004). More research is necessary to further establish a cause-effect relationship between dredged spoil and imposex and the potential impact on marine ecosystems. Without this, 'knee jerk' reactions can lead to poor decisions (Champ, 2003) and if regulations on the disposal of dredging spoil were tightened this may lead to increased costs which would impact an already defensive shipping and port industry.

The absence of *N. lapillus* from formerly occupied sites can be viewed as an indication of continuing TBT pollution (Harding *et al.,* 1997; Bray and Herbert, 1998) and recolonisation as a guide to an improvement in TBT levels. *N. lapillus* has demonstrated abilities to recolonise sites (Herbert *et al.,* 2000; Colson and Hughes, 2004) which suggest that this is achieved by rafting (Bryan *et al.,* 1986) or movement from remnant populations (Colson and Hughes, 2004). In the Solent this has been achieved at sites on the Isle of Wight which were formerly highly polluted, but not at other locations which appear suitable for dogwhelks to inhabit (such as Seaview on the north coast). A wider understanding of this process may be necessary, as it would be easy to assume that dogwhelks were absent from Seaview due to continuing organotin pollution whereas this can be influenced by natural factors (Spence, 1989; Harding *et al.,* 1998). This leads to the need for reintroduction trials, as piloted in this work. Such attempts to repopulate sites or augment natural populations can be controversial

(Platenberg and Griffiths, 1999) because of the risks of loss of genetic identity and behavioural deficiencies in the reintroduced populations (McPhee, 2004).

It has been shown that whilst *N. lapillus* appears suitable for translocation and reintroduction, the long-term survival of dogwhelks appears unlikely even when waterborne TBT levels have substantially declined, as was seen at Southampton Water. The groynes at Highcliffe, (examined in chapters 4 and 5), may have provided a "stepping-stone" (Thomas and Jones, 1993; Bacchiocchi and Airoldi, 2003 Gårdmark *et al.,* 2003) to other former habitats. Thus rather than reintroducing *N. lapillus,* or other TBT impacted gastropod species directly to formerly impacted, but potentially still polluted sites it may be better to utilise structures such as those at Highcliffe. In this way the colonisation or reintroduction of TBT affected species into 'strategic patches' may be an alternative to direct reintroduction until the target species can maintain a steady population, rather than extinction outstripping the supply of colonisers (Gårdmark *et al.,* 2003).

Throughout this work the abundance of some *N. lapillus* populations has fluctuated independently of TBT impacts or, in the case of St. Agnes, where little or no TBT effects were seen. Thus natural drivers of population change should be considered before erroneous conclusions about TBT levels are made. This further indicates that population abundance and size structure alone are not reliable tools for detecting the impacts of TBT (Spence, 1989). Declines in abundance have, however, been used to suggest TBT impacts in *Hydrobia ulvae* (Nehring, 2000). Observations have shown that physical factors such as disturbance though wave action and scouring (Etter, 1989; 1996), can dictate the abundance and size classes of *N. lapillus*. However, physical effects can also influence the availability of prey which at south coast and Isle of Wight sites were predominantly barnacles (*Chthalamus* and *Semibalanus balanoides*). Colonisation by *S. balanoides* is highly variable (Hawkins and Hartnoll, 1982; Kendall *et al.,* 1985; Jenkins *et al.,* 2001) largely due to larval supply, physical conditions (O'Riordan *et al.,* 2004) and localised variations (Hawkins and Hartnoll, 1982; Connell, 1985) and the balance between this species and *Chthamalus* has changed over the last 50 years (Southward, 1991). Therefore this variability may explain the abundance changes seen in *N. lapillus* populations particularly at Lulworth and Osmington Mills. Herbert (2001) also showed that these barnacle species were present in low numbers between Portland and Selsey Bill. Barnacles had declined at Lulworth and Osmington Mills and the local dogwhelk population trends indicated a bottom-up response. Unlike at Selsey *N.*

lapillus were not seen switching to alternative prey, although they do forage more at night (Hughes and Burrows, 1993). Therefore it seems likely that abundance in many of the dogwhelk populations investigated is dictated more by prey availability rather than TBT pollution. So whilst TBT impacts are widely recognised, as legislation and management techniques increase in efficacy, care should be taken toward attributing the decline of sensitive species to TBT. This is particularly so when pollution pathways become indistinct, such as with dredge spoil, thus requiring careful research and subsequent regulation.

7.3 Rocky shore communities mediated by TBT effects on *Nucella lapillus*

In the relatively short-term, cellular or molecular biomarkers can be used as indicators of the long-term adverse effects of pollution on populations or communities (Cajaraville *et al.*, 2000). At the molecular level the response of female *N. lapillus* to TBT is the blocking of P450 cytochrome activity (Spooner *et al.*, 1991). As *N. lapillus* use hormones to dictate gonad development (Le Gall and Streiff, 1975) the inhibition of the P450 molecule leads to masculisation of females (Morcillo and Porte, 1998), reproduction failure and eventually population decline (Bryan *et al.*, 1986; Alzieu, 2000). *N. lapillus* is a predatory gastropod and following a reduction in this often dominant biological effect (Menge, 1976) it would be expected that rocky shore communities would show composition changes. In the UK this expectation has only been demonstrated to a minimal effect (Proud, 1994) and in the east Atlantic has only been seen over a long period (Fischer-Piette, 1935 *op cit* Hughes and Burrows, 1993).

Acute pollution events such as oil spills can have both short and long-term consequences at the community level (e.g. Elmgren *et al.*, 1983; Hawkins and Southward, 1992). This may be seen as the loss of a community or a 'keystone' species (Paine, 1966). For example the loss of the keystone limpet *Patella vulgata* following the use of dispersants on the Torrey Canyon oil spill resulted in a 'bloom' of ephemeral algae as grazing was reduced (Southward and Southward, 1978). The acute response to this impact propagated to a long-term community recovery which took 10-15 years (Hawkins *et al.*, 1983; Hawkins and Southward, 1992).

The consequences of longer-term low-level chronic pollution are subtler than acute events and the 'signal' may be confused by natural spatial and temporal variability (Hawkins and

Hartnoll, 1983; Crowe *et al.,* 2000). Thus community responses will only be found if several species are affected (Hawkins *et al.,* 1994) leading to an identifiable change from a known state before the influence of pollution. Krassulya (2001), however, found that analysis of community structure using multivariate methods on a taxonomic class level or a selected group was an effective method of pollution monitoring. Starfish (*Pisaster ochracues*) were the basis for the original keystone species concept (Paine, 1966). Shim *et al.,* (2005), recognising the importance of starfish in marine ecosystems, found that another starfish species (*Asteria pectinifera*) was a significant accumulator of TBT and suggested it may be used for long-term monitoring. Thus TBT has the potential to impact keystone species which, in this case, may lead to a community response.

Although *N. lapillus* are a common rocky shore predator in the UK and can occur in high densities, the effects of TBT on this species has so far not been shown to affect communities, thus indicating that dogwhelks, unlike limpets on UK rocky shores, do not have a pivotal role in community structure. The apparently subtle effects of dogwhelk predation were masked by natural abiotic (scour and wave action) and human (engineering) disturbance in the communities investigated at Highcliffe. This highlights that the value of *N. lapillus* as a biomonitor of TBT pollution is at the individual and population levels.

In common with other man-made shore defence structures (Bacchiocchi and Airoldi, 2003) the groynes at Highcliffe had very low community diversity. Therefore these systems may be ideal for making observations about the impacts of differing levels of perturbation as higher diversity can diminish the effects of disturbance (Allison, 2004). A possible effect in the small scale is the wave dislodgment (Hunt and Scheibling, 1998) of mussel hummocks (Davenport *et al.,* 1996,1998). *N. lapillus* were often found sheltering under these hummocks at Highcliffe and the base layer of mussels was always detached from the rock surface; possibly having been eaten by dogwhelks. This left hummocks unstable and liable to dislodgement and although not seen at the scales investigated here, this may have reduced in the absence of *N. lapillus* and in the longer term lead to community change.

Proud (1994) suggested that the influence of dogwhelks on the community structure of mussel dominated shores required further investigation. In this work it has been found that in both mussel and barnacle dominated habitats, and on sites where a patchy matrix of the two existed, no effect of dogwhelk predation was found. It is possible that dogwhelk abundance is subject to fluctuations in these species as suggested by the results found between barnacle cover and *N. lapillus* abundance in the south coast sites. This indicates bottom-up regulation of dogwhelks rather than their top-down predation effects (Leonard *et al.,* 1998; Menge 2000).

7.4 Legislation and conservation

Legislation against TBT use was first introduced by France as long ago as 1985, following the decline of commercial shellfish stocks (Alzieu, 1986) and measures regulating its use increased worldwide (see Champ, 2000 for review up to 2000). Since the general agreement to the proposals and of the Anti Fouling System Convention (AFS) in 2001, in which the International Maritime Organisation (IMO) set out the terms for the phasing out and eventual ban of TBT by 2008 (Champ, 2001b), there has been a general reluctance to ratify the terms of the ban by the majority of shipping nations (IMO, pers com. 2004).

The main argument against the total ban of TBT is based on economics as the loss of TBT as an option for shipping companies will result in the use of less effective alternatives, which will lead to more frequent dry docking, slower ships and increased fuel bills (Strandenes, 2000). It is recognised that increased costs to industry are frequently passed on to consumers (Schwartz and Gattuso, 2002). Whilst economic arguments appear less altruistic, opponents of the ban also cite greater environmental problems than those associated with TBT if the legislation succeeds (Abbott *et al*, 2000; Champ, 2000). Objections include the suggestion that global warming will be amplified due to increased CO_2 releases as heavily fouled ships (in the absence of TBT) burn more fuel (Jacobson and Willingham 2000; Strandenes, 2000). Furthermore the transport of invasive exotic species could increase as hull attachment becomes easier for unwelcome organisms (Champ, 2000; Terlizzi *et al.,* 2004).

Whilst there are opponents to the ban, and its implementation is being taken up slowly by shipping nations, some organisations are pressing ahead with the legislation. In the European Union (EU) regulation number (EC) 782/2003 required all EU flagged ships to have TBT sealed off at the first dry-docking after the 1st January 2003. This was modified after objections, the IMO legislation does not come into full force until 2008, and now states that TBT need not be sealed off or removed until 1st July 2008 (European Union, 2003). The regulation also requires that non-EU nation craft be restricted from EU waters if TBT is applied to their hulls, thus forcing non-EU nations into further consideration of the full ban. Whilst this appears to be an effective option in the EU it may lead to questions about global competition laws as vessels from those nations which still permit the use of TBT will not be able to trade in the EU. Unfortunately this may be in breach of free trade legislation and highlights the problems with international environmental agreements (Mulkey and Chanon, 2003). Nations unable to compete in the EU may feel compelled to take legal action, and may have a strong case under current international free-trade laws.

Drivers behind the ban on TBT are conservation-based due to the widely known effects of TBT on bio-indicator species (e.g. Gibbs and Bryan, 1986). Links to human health have also been suggested (Nielsen and Rasmussen, 2004). International conservation targets are becoming increasingly important and the drive for better management of marine habitats and environment is increasing (e.g. DEFRA, 2002; English Nature, 2002). Care should be taken, however, that single-species tests (e.g. the effects of TBT on *N. lapillus*), such as acute and chronic laboratory trials cannot take into account whole ecosystem and community functions (Solomon and Sibley, 2002), therefore conservation objectives should not be based wholly on the response to a pollutant of one sensitive organism.

As the realisation that the legacy of TBT in sediments worldwide (e.g. Thomas *et al.,* 2000. 2001; Barakat *et al.,* 2001) will continue for several years after a ban is finally ratified by shipping nations, the management of contaminated sediments and disposal of TBT polluted waste (dockyard spoil) are paramount. It has been shown that TBT from dredged sediments can have influence across conservation designation boundaries (Terlizzi *et al.,* 2004) thereby affecting sensitive species (Santos *et al.,* 2004). So the best long-term approach is to control TBT and other potentially toxic alternatives (Champ, 2001) at the source (ships and dockyards) before they reach the wider marine ecosystem. TBT effects may remain for several decades, particularly given the reluctance to accept a wider ban and the predicted increases in shipping, thus whilst the situation is improving close to ports and regionally, continued management and biomonitoring will remain necessary for the foreseeable future.

7.5 Suggestions for further research

Drives to create larger hub ports in the UK and in Europe (Marcadon, 1999) may result in greater associated environmental impacts including those from TBT. Sensitive gastropod species are ideal biomarkers for the compound, and at some areas in this study have indicated that TBT levels are increasing, therefore long-term trends should continue to be monitored.

Reintroduction methods trialled in this work indicate that the process is not worthwhile at sites where large vessels are still common. On man-made structures, however, colonisation by *N. lapillus* has been achieved and this may lead to these sites being stepping-stones to former habitats. How a species with no pelagic stage achieves this is still not widely understood, although this work indicated that hydrodynamic forcing was responsible for the passive transport of dogwhelks for distances up to 1000 m. The utilisation of spatially distinct man-made structures with offshore off bottom collectors and marking methods may help identify patterns in this process, and lead to an insight on the distances that can be covered. This may indicate whether *N. lapillus* has the capability of reaching former habitats, such as Netley on Southampton water, and could predict natural recolonisation if TBT levels are ultimately reduced.

Study of the community role of *N. lapillus* at two scales did not identify any significant effect following their removal. At the crevice scale effects have been seen on barnacle density and size classes (Connell, 1961; Johnson *et al.,* 1998) with some modification due to the effects of limpet grazing (Proud, 1994). It was suggested that further work was needed to show the effect of *N. lapillus* on UK mussel populations (Proud, 1994) and this may also be indicated here. Whilst the spatial scales did not highlight a change in mussel hummock numbers, it appeared that their loss due to wave action may have allowed barnacles to establish in the resulting patches. Whether feeding by *N. lapillus* on the basal layer of these hummocks cause their eventual detachment is not known, but a synergistic interaction, of predation effects and wave disturbance, may be occurring. Further study of this system may highlight a role for *N. lapillus* in mussel communities which is greater than seen at crevices but smaller than the scales investigated here.

7.6 Conclusions

Whilst legislation partially banning TBT in the UK was introduced in 1987, the results here indicate that along the south coast of England organotin pollution is still common and that further declines in its levels appear to have slowed. The dogwhelk *Nucella lapillus* is a valuable biomonitoring tool with which to establish ongoing and changing levels of TBT, but declines in dogwhelk populations need to be considered in conjunction with imposex indices to ensure that natural factors are not causative of any abundance changes seen. In addition the increases in imposex seen at sensitive areas along the south coast may be associated with the dumping of dredged sediments from which TBT is then released. A cause and effect relationship could not be established in this work, but the continued monitoring of *N. lapillus* populations, or other benthic bioindicators such as *Buccinum undatum* (Mensink *et al.*, 1996), to identify the impacts of TBT contaminated sediments is recommended.

Although predation has been shown as a significant factor in structuring some rocky shore communities, in the UK *N. lapillus* does not appear to have a pivotal role in these processes at the scales investigated here, although levels of disturbance may have masked a subtle role. It is possible that dogwhelks have an influence on mussel hummocks and the availability of space following their loss through wave action, but this requires greater research.

The eventual ratification of the TBT ban seems inevitable despite the reluctance to take up the proposal by some shipping nations. There are concerns about other environmental issues, such as global warming, becoming heightened in the face of the TBT ban and that increased costs would be transferred on to consumers. In contrast to Evans (1999), however, the environmental costs of continued TBT use may outweigh the economic benefits

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Appendix 1 Results for Chapter 3

Table 1 Significant changes in raw penis length from two-way ANOVA with replication *post hoc* Tukeys' multiple comparisons, for the south coast sites Osmington mills and Lulworth Cove.

A Osmington Mills All Pairwise Multiple Comparison Procedures				
Comparison (year)	**Diff of Means**	**p**	**Q**	**P**
2003 vs. 1995	0.4	3	5.5	<0.001
2003 vs. 1999	0.1	3	1.8	0.430
1999 vs. 1995	0.3	3	3.7	0.029
Comparison (sex)	**Diff of Means**	**p**	**Q**	**P**
Male vs. Female	3.0	2	48.9	<0.001
Comparison (sex in 1995)	**Diff of Means**	**p**	**Q**	**P**
Male vs. Female	2.3	2	22.7	<0.001
Comparison (sex in 1999)	**Diff of Means**	**P**	**Q**	**P**
Male vs. Female	2.9	2	26.6	<0.001
Comparison (sex in 2003)	**Diff of Means**	**P**	**Q**	**P**
Male vs. Female	3.9	2	35.0	<0.001
Comparison (year female)	**Diff of Means**	**P**	**Q**	**P**
1995 vs. 2003	0.34	3	3.3	0.052
1995 vs. 1999	0.03	3	0.2	0.984
1999vs. 2003	0.3	3	2.8	0.123
Comparison (year males)	**Diff of Means**	**p**	**Q**	**P**
1995 vs. 2003	1.2	3	11.5	<0.001
1995 vs. 1999	0.6	3	5.8	<0.001
1999vs. 2003	0.6	3	6.2	<0.001
B Lulworth Cove All Pairwise Multiple Comparison Procedures				
Comparison (year)	**Diff of Means**	**p**	**Q**	**P**
1995 vs 1999	0.7	2	6.8	<0.001
Comparison (sex)	**Diff of Means**	**p**	**Q**	**P**
Male vs. Female	2.3	2	23.5	<0.001
Comparison (sex in 1995)	**Diff of Means**	**p**	**Q**	**P**
Male vs. Female	2.4	2	17.1	<0.001
Comparison (sex in 1999)	**Diff of Means**	**p**	**Q**	**P**
M vs. F	2.2	2	16.1	<0.001
Comparison (year female)	**Diff of Means**	**p**	**Q**	**P**
1995 vs. 1999	0.6	2	4.0	0.006
Comparison (year males)	**Diff of Means**	**p**	**Q**	**P**
1995 vs. 1999	0.7	2	5.9	<0.001
C Osmington vs Lulworth females All Pairwise Multiple Comparison Procedures				
Comparisons for factor: Year (1995 vs 1999)				
Comparison (year)	**Diff of Means**	**p**	**Q**	**P**
1995 vs 1999	0.3	2	3.6	<0.05
Comparisons for factors site within 1995				
Comparison (site 1995)	**Diff of Means**	**p**	**Q**	**P**
Lulworth vs Osmington	0.4	2	3.3	<0.05
Comparisons for factors site within 1999				
Comparison (site 1999)	**Diff of Means**	**p**	**Q**	**P**
Lulworth vs Osmington	0.176	2	1.4	0.3

Table 2 Significant male and female *Nucella lapillus* penis length comparisons from *post hoc* Tukeys' tests for sites at Plymouth Sound and St Agnes 1990-2003.

Years: 1:1990, 2:1991, 3:1992, 4: 1993, 5:1997, 6:1998, 7:1999, 8:2000, 9:2001, 10:2003.

St Agnes	
Comparison between years pooled	
Result	**Significance**
NS	
Comparison between sex all years	
M>F	<0.001
Comparison between years female	
2>9,10	<0.001
3>9,10	<0.01
4>9	<0.05
5>9,10	<0.01
8>9,10	<0.01
Comparison between years male	
5>1,2,3,4	<0.001
6>1,4 (6> 2,3)	<0.001(<0.01)
7>1,2,3,4	<0.001
8>1,2,3,4	<0.001
9>1,4 (9>2,3)	<0.001 (<0.01)
10>1,4 (10>2) ((10>3))	<0.001 (<0.01) (<0.05))

Tregantle	
Comparison between years pooled	
Result	**Significance**
5>1,2,3,4	<0.001
6>2,3 (6>1,4)	<0.001 (<0.01)
7>1,2,3,4	<0.001
8>1,2,3 (8>4)	<0.001 (<0.01)
9>2,3 (9>1,4)	<0.001 (<0.01)
10>2,3 (10>1,6)	<0.001 (<0.01)
Comparison between sex all years	
M>F	<0.001
Comparison between years female	
5>3,4,9,10 (5>6) ((5>2))	<0.001 (<0.01) ((<0.05))
7>4 (7>3,9) ((7>10))	<0.001 (<0.01) ((<0.05))
8>3,4	<0.05
Comparison between years male	
5>1,2,3 (5>4)	<0.001 (<0.01)
6>1,2,3 (6>4)	<0.001 (<0.01)
7>1,2,3 (7>4)	<0.001 (<0.05)
8>1,2,3	<0.001
9>1,2,3,4	<0.001
10>1,2,3,4	<0.001

Kingsand	
Comparison between years pooled	
Result	**Significance**
1>3	<0.001
2>3	<0.001
4>3	<0.001
5>3	<0.001
6>3	<0.001
7>3	<0.001
8>3	<0.001
9>2,3 (9>1,5,10)	<0.001 (<0.05)
10>3	<0.001
Comparison between sex all years	
M>F	<0.001
Comparison between years female	
NS	
Comparison between years male	

Renney Rocks	
Comparison between years pooled	
Result	**Significance**
1>4 (1>3) ((1>2))	<0.001 (<0.01) ((<0.05))
5>2,3,4 (5>8) ((5>6))	<0.001 (<0.01) ((<0.05))
6>4 (6>3)	<0.01 (<0.05)
7>4 (7>3)	<0.01 (<0.05)
9>2,3,4	<0.001
10>4 (10>3) ((10>2))	<0.001 (<0.01) ((<0.05))
Comparison between sex all years	
M>F	<0.001
Comparison between years female	
1>3,4,8 (1>10) ((1>7,9))	<0.001 (<0.01) ((<0.05))
2>8	<0.01
5>8 (5>3,4)	<0.001 (<0.05)
Comparison between years male	
5> 1,2,3,4	<0.001
6>2 (6>4)	<0.001 (<0.05)
7>2 (7>4) ((7>3))	<0.001 (<0.01) ((<0.05))
8>1,2,3,4,	<0.001
9>1,2,3,4	<0.001
10>1,2,3,4 (10>6)	<0.001 (<0.01)

Jennycliff	
Comparison between years pooled	
Result	**Significance**
5>2,3,4	<0.001
8>2,3	<0.001
9>2,3 (9>4)	<0.001 (<0.05)
10>2,3 (10>4)	<0.001 (<0.05)
Comparison between sex all years	
M>F	<0.001
Comparison between years female	
1>2,3 (1>8,10) ((1>9))	<0.001 (<0.01) ((<0.05))
Comparison between years male	
1,3,4,6,7 >2	<0.001
5>1,2,3,4 (5>6,7)	<0.001 (<0.05)
8>1,2,3,4	<0.001
9>1,2,3,4 (9>6,7)	<0.001 (<0.05)
10>1,2,3,4,6,7	<0.001

Table 3 Significant female penis length comparisons from *post hoc* Tukeys' tests for sites at Plymouth Sound and St Agnes between years and sites 1990-2003.

Comparisons for factor: Site				
Comparison	**Diff of Means**	**p**	**q**	**P**
Tregantle vs. St Agnes	446.255	5	37.794	<0.001
Tregantle vs. Renney Rocks	198.774	5	16.256	<0.001
Tregantle vs. Jennycliff	104.292	5	8.614	<0.001
Tregantle vs. Kingsand	103.208	5	8.346	<0.001
Kingsand vs. St Agnes	343.047	5	27.844	<0.001
Kingsand vs. Renney Rocks	95.566	5	7.511	<0.001
Kingsand vs. Jennycliff	1.085	5	0.0860	1.0
Jennycliff vs. St Agnes	341.963	5	28.354	<0.001
Jennycliff vs. Renney Rocks	94.481	5	7.575	<0.001
Renney Rocks vs. St Agnes	247.482	5	20.317	<0.001
Comparisons for factor: Year				
Comparison	**Diff of Means**	**p**	**q**	**P**
1990 vs. 2003	190.965	10	10.434	<0.001
1990 vs. 1992	177.776	10	10.070	<0.001
1990 vs. 1993	151.743	10	8.596	<0.001
1990 vs. 2000	116.760	10	6.758	<0.001
1990 vs. 2001	111.161	10	6.528	<0.001
1990 vs. 1998	91.116	10	5.320	<0.01
1990 vs. 1991	83.030	10	4.703	<0.01
1990 vs. 1999	80.824	10	4.480	<0.05
1997 vs. 2003	131.307	10	7.369	<0.001
1997 vs. 1992	118.118	10	6.887	<0.001
1997 vs. 1993	92.085	10	5.369	<0.01
1999 vs. 2003	110.141	10	5.938	<0.01
1999 vs. 1992	96.952	10	5.414	<0.01
1991 vs. 2003	107.935	10	5.939	<0.01
1991 vs. 1992	94.747	10	5.408	<0.01
1998 vs. 2003	99.849	10	5.654	<0.01
1998 vs. 1992	86.661	10	5.102	<0.05
2001 vs. 2003	79.804	10	4.543	<0.05

Table 4 Significant levels of recruitment from Tukeys multiple comparisons for sites at Plymouth Sound Tukeys and St Agnes.

A: Comparisons for factor: Site replicated				
Comparison	**Diff of Means**	**p**	**q**	**P**
Renney Rocks	26.5	5		<0.05
B: Comparisons for factor: Year replicated				
Comparison	**Diff of Means**	**p**	**Q**	**P**
2001 vs. 2003	1.7	6	4.891	<0.05
2000 vs. 2003	1.6	6	4.584	<0.05
C: Comparisons for factor: Site replicated				
Comparison	**Diff of Means**	**p**	**q**	**P**
Tregantle vs. St Agnes	1.12	5	3.575	NS

Table 5 Significnt penis length comparsuons from *post hoc* Tukeys' test for south Isle of Wight sites Hanover Point, Horse Ledge, Bembridge.
M = male, F = female. NS Not significant.

Hanover Point All Pairwise Multiple Comparison Procedures (Tukey Test)				
Comparisons for factor: Year (1987, 1997, 1999, 2003)				
Comparison	**Diff of Means**	**p**	**Q**	**P**
1987 vs.1999	3.9	4	15.8	<0.001
1987 vs.2003	2.7	4	11.1	<0.001
1987 vs.1997	1.7	4	8.7	<0.001
1997 vs.1999	0.5	4	10.9	<0.001
1997 vs.2003	1.2	4	3.7	NS
Comparisons for factor: sex				
Comparison	**Diff of Means**	**p**	**Q**	**P**
M vs. F	2.896	2	19.771	<0.001
Comparisons for factor: sex within 1987, 197, 1999, 2003				
Comparison	**Diff of Means**	**p**	**Q**	**P**
M vs. F	3.5, 2.3, 2.0, 3.7	2	8.140, 10.3, 8.5, 16.7	<0.001
Comparisons for factor: year within females				
Comparison	**Diff of Means**	**P**	**Q**	**P**
1987 vs. 1999	3.2	4	7.7	<0.001
1987 vs. 2003	2.8	4	6.8	<0.001
1987 vs. 1997	1.5	4	3.7	<0.05
1997 vs. 1999	1.7	4	7.4	<0.001
1997 vs. 2003	1.3	4	5.8	<0.001
2003 vs. 1999	0.4	4	1.6	NS
Comparisons for factor: year within males				
Comparison	**Diff of Means**	**P**	**Q**	**P**
1987 vs. 1999	4.6	4	17.1	<0.001
1987 vs. 1997	2.7	4	10.2	<0.001
1987 vs. 2003	2.6	4	10.1	<0.001
2003 vs. 1999	2.0	4	8.7	<0.001
2003 vs. 1997	0.1	4	0.5	0.983
1997 vs. 1999	1.9	4	8.0	<0.001

Table 5 contd. M = male, F = female. NS Not significant.

Horse Ledge All Pairwise Multiple Comparison Procedures (Tukey Test)				
Comparisons for factor: Year (1987, 1997, 2003)				
Comparison	**Diff of Means**	**p**	**Q**	**P**
NS				
Comparisons for factor: sex				
Comparison	**Diff of Means**	**p**	**Q**	**P**
M vs. F	2.4	2	26.8	<0.001
Comparisons for factor: sex within 1987, 1997, 2003				
Comparison	**Diff of Means**	**p**	**Q**	**P**
M vs. F	1.9, 2.0, 3.0	2	11.7, 14.0, 21.4	<0.001
Comparisons for factor: year within females				
Comparison	**Diff of Means**	**P**	**Q**	**P**
1997 vs. 2003	0.5	3	2.9	NS
1997 vs. 1987	0.1	3	0.4	NS
1987 vs. 2003	0.4	3	2.3	NS
Comparisons for factor: year within males				
Comparison	**Diff of Means**	**P**	**Q**	**P**
2003 vs. 1987	0.7	3	5.5	<0.001
2003 vs. 1997	0.6	3	4.3	<0.01
1997 vs. 1987	0.2	3	1.4	NS
Bembridge All Pairwise Multiple Comparison Procedures (Tukey Test)				
Comparisons for factor: Year (1987, 1994, 1995, 1997, 1999, 2003)				
Comparison	**Diff of Means**	**P**	**Q**	**P**
1987 vs. 1999	3.1	6	18.0	<0.001
1987 vs. 1994	3.0	6	17.5	<0.001
1987 vs. 1997	2.7	6	16.0	<0.001
1987 vs. 2003	2.6	6	15.5	<0.001
1987 vs. 1995	2.6	6	15.9	<0.001
1995 vs. 1999	0.5	6	3.8	NS
1995 vs. 1994	0.5	6	3.2	NS
1995 vs. 1997	0.1	6	0.9	NS
1995 vs. 2003	0.04	6	0.3	NS
2003 vs. 1999	0.5	6	3.4	NS
2003 vs. 1994	0.4	6	2.9	NS
2003 vs. 1997	0.08	6	0.6	NS
1997 vs. 1999	0.4	6	2.8	NS
1997 vs. 1994	0.3	6	2.2	NS
1994 vs. 1999	0.08	6	0.5	NS
Comparisons for factor: sex				
Comparison	**Diff of Means**	**P**	**Q**	**P**
M vs. F	2.262	2	25.728	<0.001
Comparisons for factor: sex within 1987, 1994, 1995, 1997, 1999, 2003				
Comparison	**Diff of Means**	**P**	**Q**	**P**
M vs. F	2.1, 1.9, 2.1, 2.2, 2.2, 2.8	2	8.1, 9.0, 11.7, 10.9, 10.0, 14.9	<0.001
Comparisons for factor: year within females				
Comparison	**Diff of Means**	**P**	**Q**	**P**
1987 vs. 1999	3.1	6	10.3	<0.001
1987 vs. 2003	2.9	6	10.2	<0.001
1987 vs. 1994	2.9	6	9.9	<0.001
1987 vs. 1997	2.7	6	9.1	<0.001
1987 vs. 1995	2.5	6	8.9	<0.001
1995 vs. 1999	0.5	6	2.5	NS
1995 vs. 2003	0.3	6	1.9	NS
1995 vs. 1994	0.3	6	1.8	NS
1995 vs. 1997	0.2	6	0.8	NS
1997 vs. 1999	0.3	6	1.6	NS
1997 vs. 2003	0.2	6	0.9	NS
1997 vs. 1994	0.2	6	0.9	NS
1994 vs. 1999	0.2	6	0.8	NS
1994 vs. 2003	0.003	6	0.02	NS
2003 vs. 1999	0.2	6	0.8	NS
Comparisons for factor: year within males				
Comparison	**Diff of Means**	**P**	**Q**	**P**
1987 vs. 1994	3.1	6	17.1	<0.001
1987 vs. 1999	3.1	6	18.9	<0.001
1987 vs. 1997	2.7	6	17.3	<0.001
1987 vs. 1995	2.6	6	16.9	<0.001
1987 vs. 2003	2.3	6	14.5	<0.001
2003 vs. 1994	0.8	6	4.5	<0.05
2003 vs. 1999	0.8	6	4.8	<0.05
2003 vs. 1997	0.4	6	2.4	NS
2003 vs. 1995	0.3	6	1.9	NS
1995 vs. 1994	0.5	6	2.9	NS
1995 vs. 1999	0.5	6	3.1	NS
1995 vs. 1997	0.07	6	0.4	NS
1997 vs. 1994	0.5	6	2.5	NS
1997 vs. 1999	0.4	6	2.7	NS
1999 vs. 1994	0.03	6	0.1	NS

Appendix 2 Results for Chapter 4

Note that the community experiment run to establish the effects of manipulation of predation by *N. lapillus* on barnacle and mussel populations at Highcliffe was run concurrentrly to the recolonisation experiments detailed in Chapter 4. No effects were found.

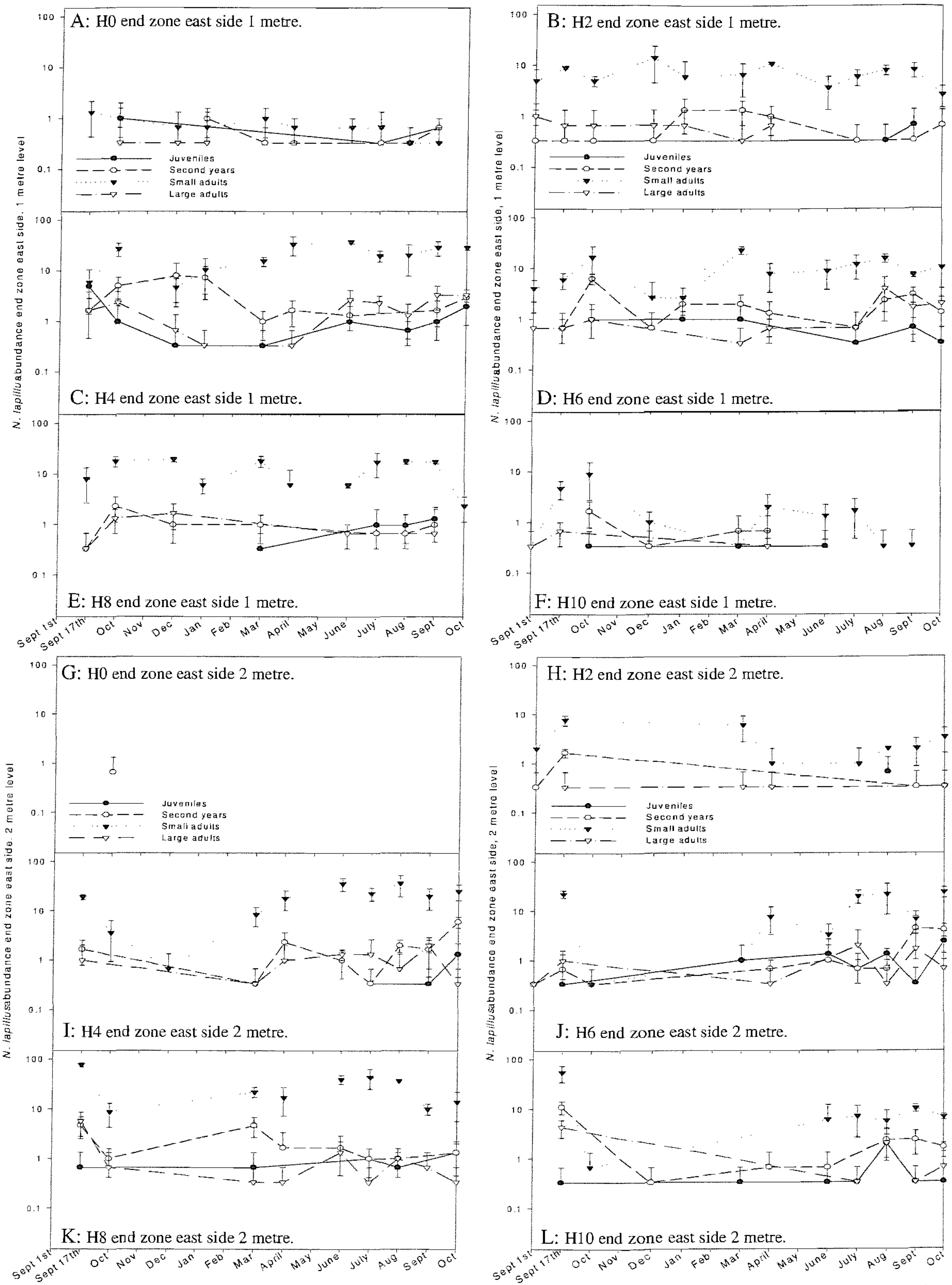

Figure 1 A-F: *Nucella lapillus* log mean abundance (+/- 1SE) at 1 metre and G-L: two metres on treatment (H0, H4, H8) and control groynes (H2, H6, H10) for the east side end zone from September 2001-October 2002.

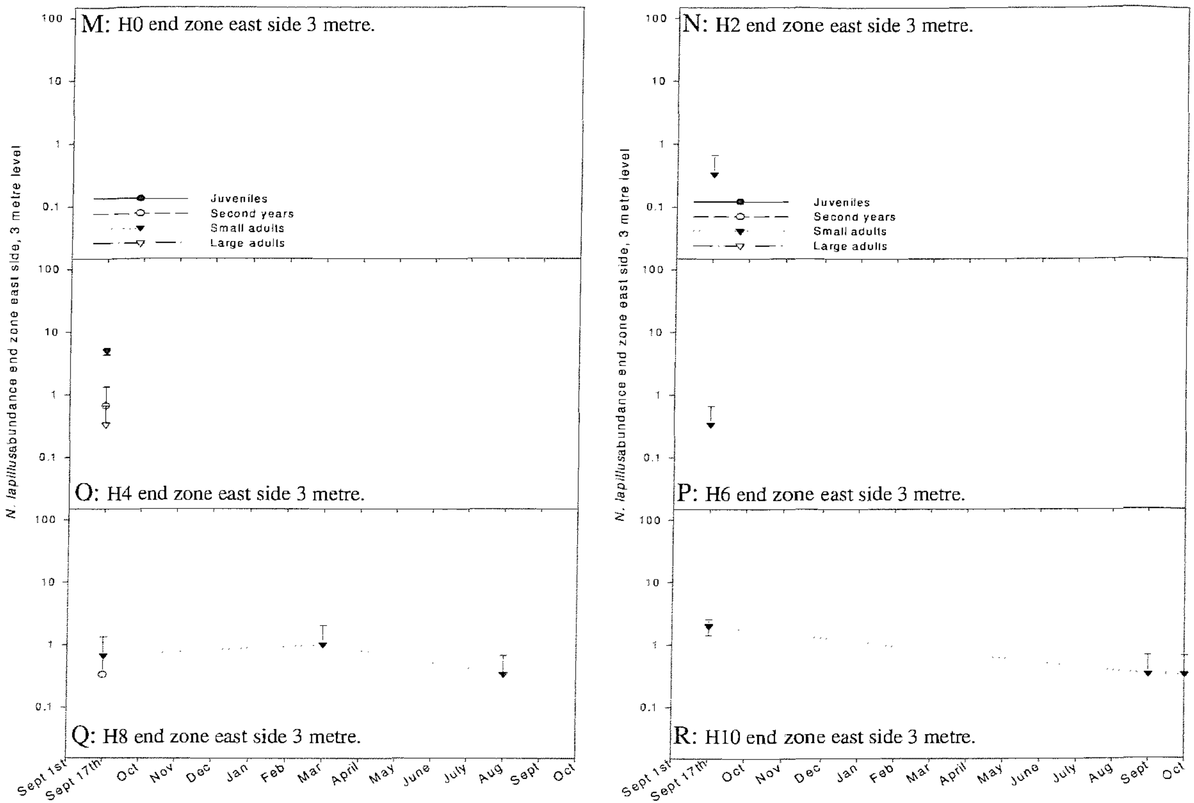

Figure 1 M-R: *Nucella lapillus* log mean abundance (+/- 1SE) at 3 metres on treatment (H0, H4, H8) and control groynes (H2, H6, H10), for the east side end zone, September 2001-October 2002.

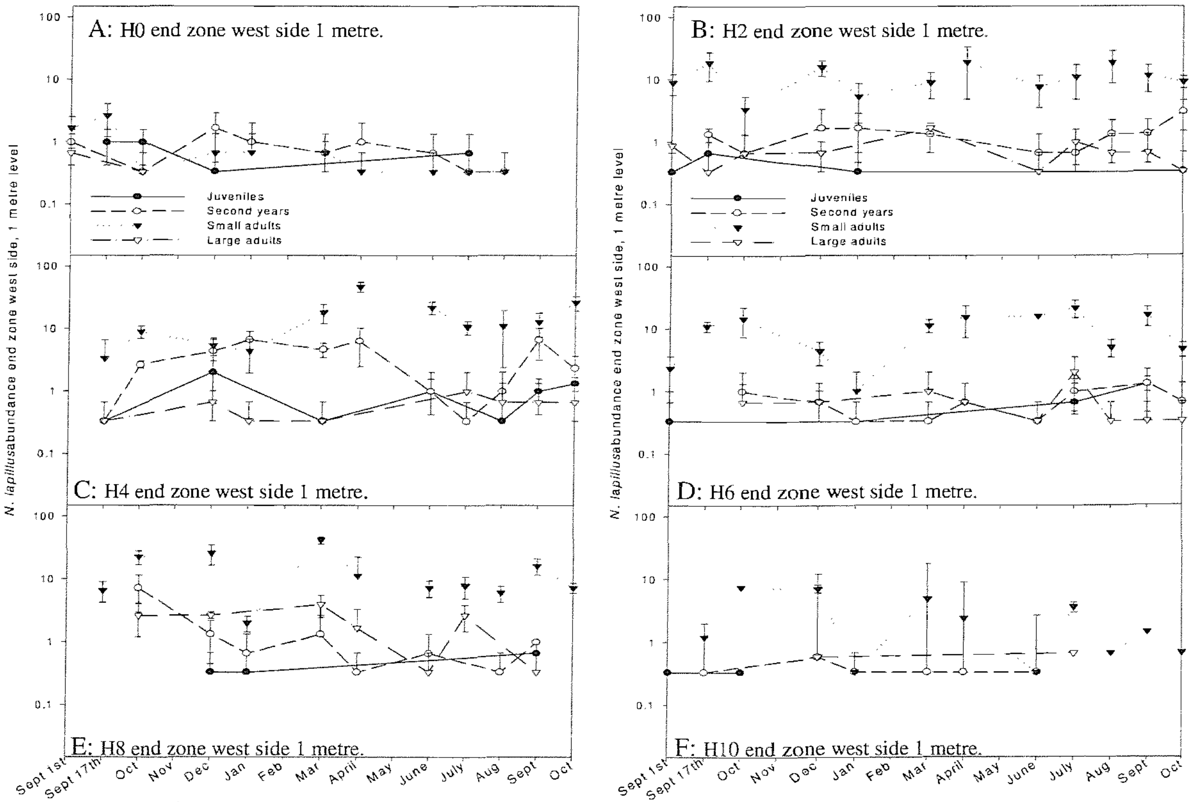

Figure 2 A-F: *Nucella lapillus* log mean abundance (+/- 1SE) at 1 metre on treatment (H0, H4, H8) and control groynes (H2, H6, H10) for the west side end zone, September 2001-October 2002.

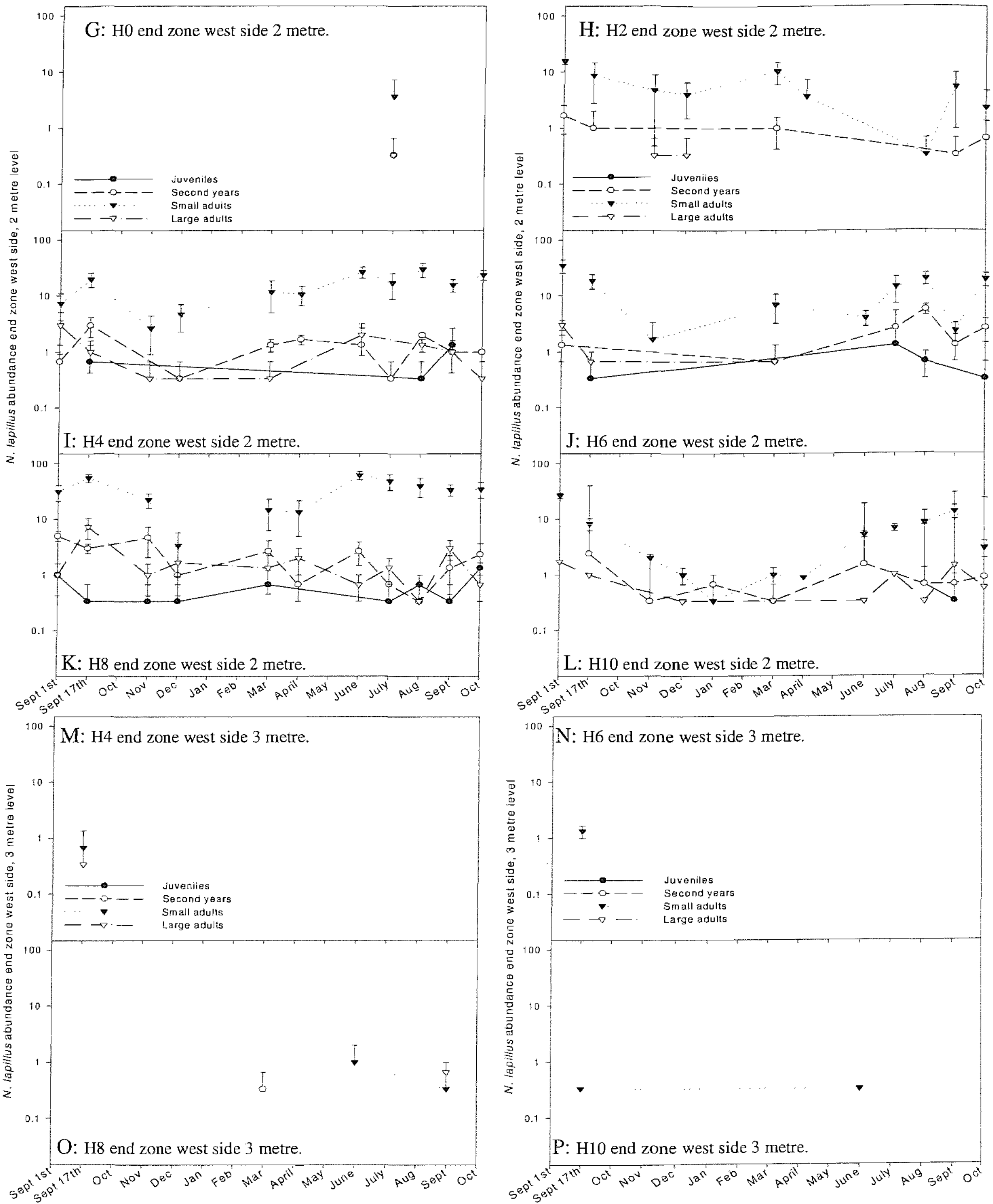

Figure 2 G-L: *Nucella lapillus* log mean abundance (+/- 1SE) at 2 metres on treatment (H0, H4, H8) and control groynes (H2, H6, H10) and M-P 3 metres on treatment (H4, H8) and control (H6, H10) for the west side end zone September 2001-October 2002.

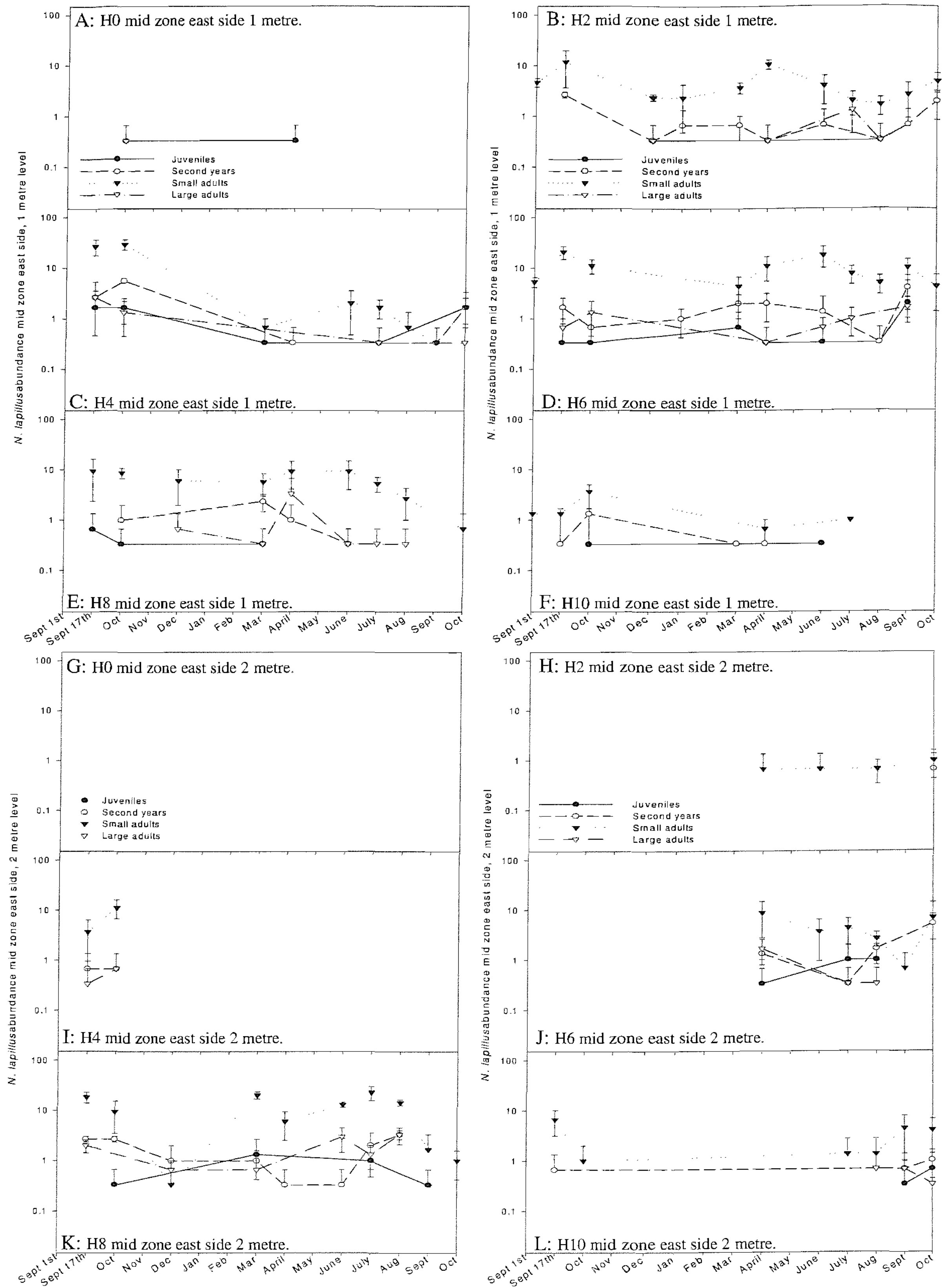

Figure 3 A-F: *Nucella lapillus* log mean abundance (+/- 1SE) at one metre and G-L: two metres on treatment (H0, H4, H8) and control groynes (H2, H6, H10) for the east side mid zone, September 2001-October 2002. Mean +/- 1SE.

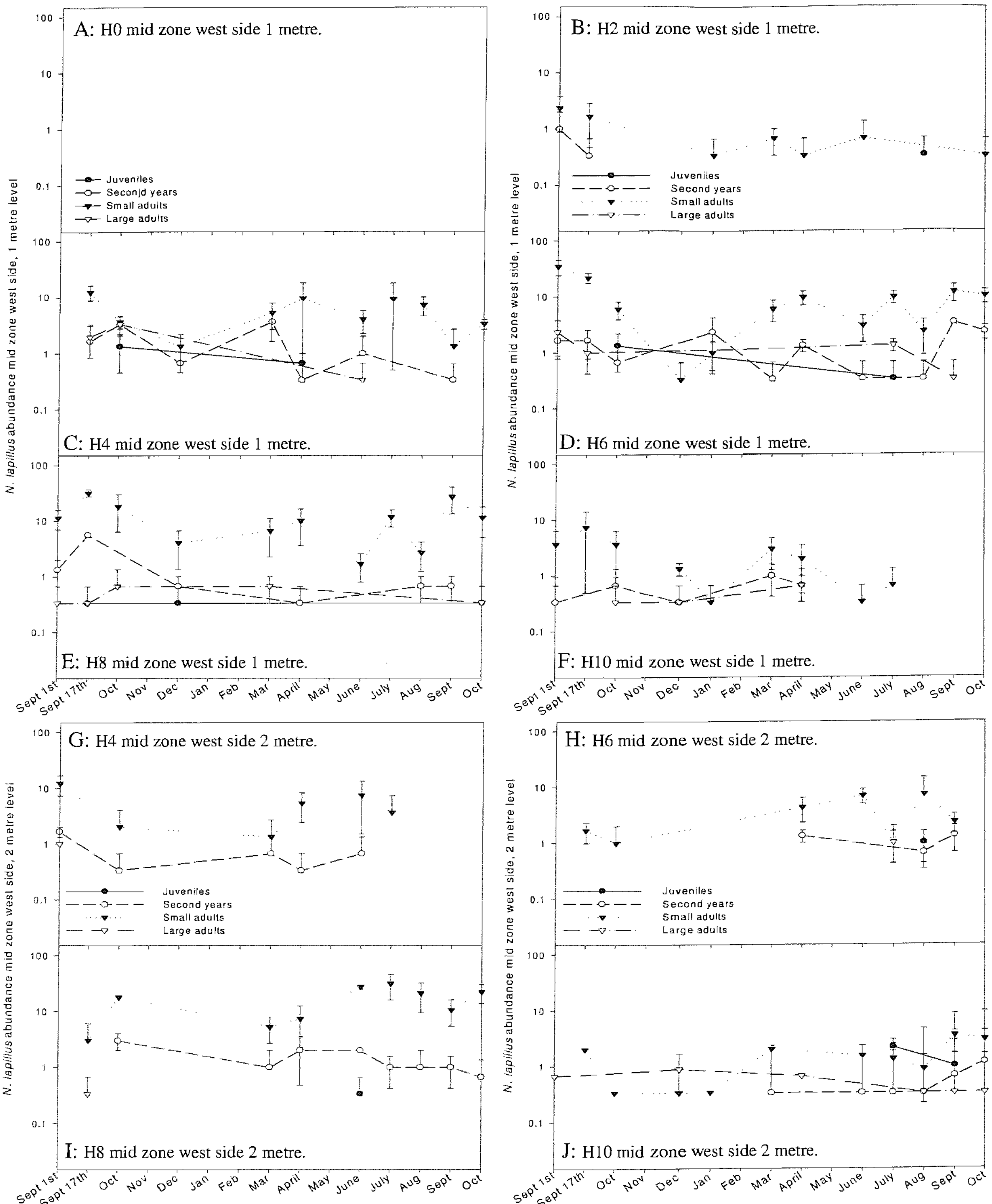

Figure 4 A-F: *Nucella lapillus* log mean abundance (+/- 1SE) at 1 metre on treatment (H0, H4, H8) and control groynes (H2, H6, H10) and G-J: two metres on treatment (H4, H8) and control groynes (H6, H10) for the west side mid groyne zone September 2001-October 2002.

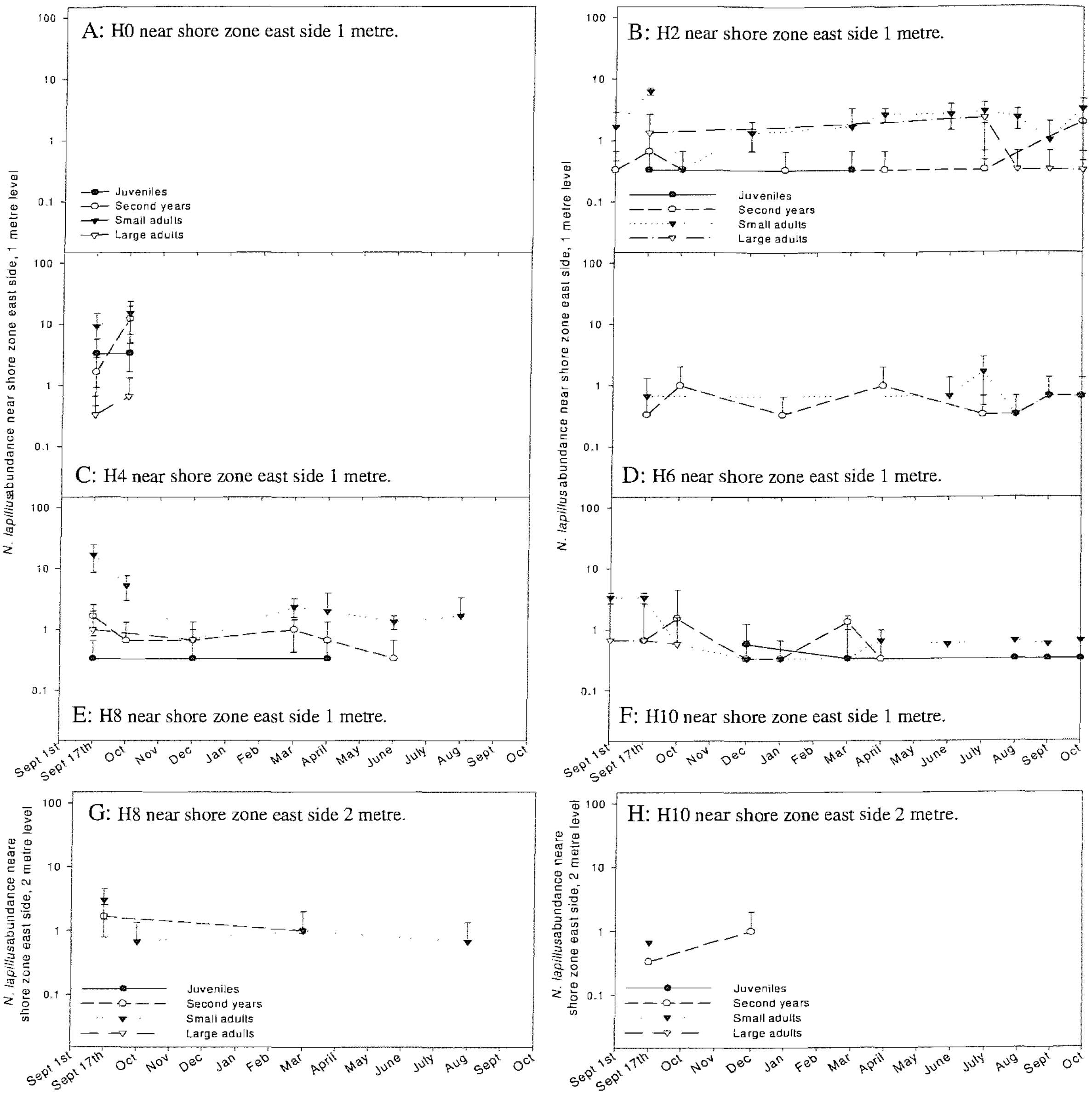

Figure 5 A--F: *Nucella lapillus* log mean abundance (+/- 1SE) at 1 metre on treatment (H0, H4, H8) and control groynes (H2, H6, H10) and G-H at two metres on treatment (H8) and control groynes (H10) for the east side near shore groyne zone September 2001-October 2002.

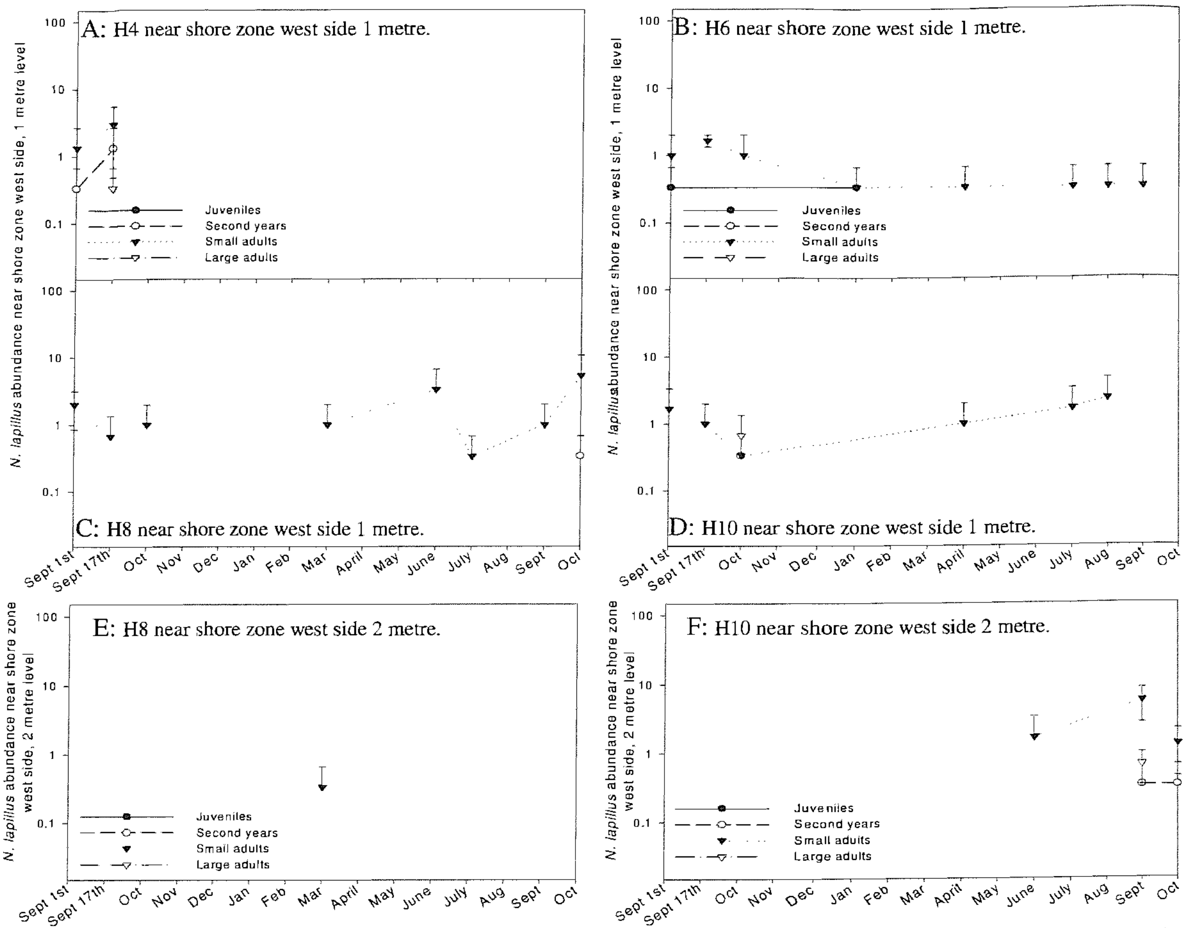

Figure 6 A-D: *Nucella lapillus* log mean abundance (+/- 1SE) at 1 metre on treatment (H4, H8) and control groynes (H6, H10) and E-F: two metres on treatment (H8) and control groynes (H10) for the west side near shore zone September 2001-October 2002. Mean +/- 1SE.

Appendix 3 Results for Chapter 5

Table 1 Significant male and female *Nucella lapillus* penis length comparisons from *post hoc Tukeys'* tests for Polzeath and Netley seawall and shore populations.
Site1: Netley seawall, Site 2: Netley shore, Site 3: Polzeath. Sex 1: males, Sex 2: females.

Comparisons for factor: Site	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
2.000 vs. 3.000	0.976	3	8.510	<0.001	Yes
2.000 vs. 1.000	0.0765	3	0.647	0.891	No
1.000 vs. 3.000	0.900	3	7.934	<0.001	Yes
Comparisons for factor: Sex	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
1.000 vs. 2.000	2.782	2	29.501	<0.001	Yes
Comparisons for factor: Sex within 1	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
1.000 vs. 2.000	2.083	2	12.586	<0.001	Yes
Comparisons for factor: Sex within 2	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
1.000 vs. 2.000	1.771	2	10.470	<0.001	Yes
Comparisons for factor: Sex within 3	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
1.000 vs. 2.000	4.493	2	28.968	<0.001	Yes
Comparisons for factor: Site within 1	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
3.000 vs. 2.000	0.385	3	2.225	0.262	No
3.000 vs. 1.000	0.305	3	1.843	0.397	No test
1.000 vs. 2.000	0.0798	3	0.442	0.948	No test
Comparisons for factor: Site within 2	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
2.000 vs. 3.000	2.338	3	15.497	<0.001	Yes
2.000 vs. 1.000	0.233	3	1.521	0.532	No
1.000 vs. 3.000	2.105	3	13.570	<0.001	Yes

Table 2. Dunns multiple comparisons for distances (m) dispersed by *Nucella lapillus* from Netley seawall (sites 2,4,6,8) and shore (sites 1,3,5,7) populations.
C: caged, U: uncaged, NA: test already completed, NT: no test, NS: not significant.

March 2002 Kruskall-Wallis Dunns multiple comparisons p=<.05.

	1c	1u	3c	3u	5c	5u	7c	7u		2c	2u	4c	4u	6c	6u	8c	8u
1c		NA	NA	NT	NA	NT	NA	NA		2>1	2>1	4>1	4>1	NT	6>1	8>1	8>1
1u	NT		NA	NT	NA	NT	NA	NA		2>1	2>1	4>1	4>1	NT	6>1	8>1	8>1
3c	NT	NT		NT	NT	NT	NT	NA		2>3	2>3	4>3	4>3	NT	6>3	8>3	8>3
3u	NT	NA	NA		NA	NT	NA	NA		2>3	2>3	4>3	4>3	NT	6>3	8>3	8>3
5c	NT	NT	NA	NT		NT	NA	NA		2>5	2>5	4>5	4>5	NT	6>5	8>5	8>5
5u	NT	NA	NA	NT	NA		NA	NA		2>5	2>5	4>5	4>5	NT	6>5	8>5	8>5
7c	NT	NT	NA	NT	NT	NT		NA		2>7	2>7	4>7	4>7	NT	6>7	8>7	8>7
7u	NT	NT	NT	NT	NT	NT	NT			2>7	2>7	4>7	4>7	NS	6>7	8>7	8>7
2c	NA	NA	NA	NA	NA	NA	NA	NA			2u>2c	NT	NT	NT	NA	NT	NT
2u	NA	NA	NA	NA	NA	NA	NA	NA		NA		NA	NA	NA	NA	NS	NA
4c	NA	NA	NA	NA	NA	NA	NA	NA		NA	2>4		NA	NA	NA	NT	NA
4u	NA	NA	NA	NA	NA	NA	NA	NA		NA	2>4	NT		NA	NA	NT	NT
6c	NA	NA	NA	NA	NA	NA	NA	NA		NT	2>6	4>6	NT		NT	8>6	NS
6u	NA	NA	NA	NA	NA	NA	NA	NA		NT	2>6	NT	NT	NA		NS	NT
8c	NA	NA	NA	NA	NA	NA	NA	NA		NA	NS	NA	NA	NA	NA		NA
8u	NA	NA	NA	NA	NA	NA	NA	NA		NA	2>8	NT	NA	NA	NA	NT	

April 2002 Kruskall-Wallis Dunns multiple comparisons p=<.05.

	1c	1u	3c	3u	5c	5u	7c	7u		2c	2u	4c	4u	6c	6u	8c	8u
1c		NS	3>1	NT	NT	NT	NT	NT		NT	2>1	4>1	4>1	NT	6>1	8>1	8>1
1u	NA		NT	NT	NA	NA	NA	NT		NT	2>1	NT	NT	NT	NT	NT	NT
3c	NA	NT		NA	NA	NA	NA	NA		NT	2>3	NT	NT	NT	6>3	NT	NT
3u	NA	NT	3c>3u		NA	NA	NA	NT		NT	2>3	4>3	4>3	NT	6>3	8>3	NT
5c	NA	NT	3>5	NT		NA	NT	NT		NT	2>5	4>5	4>5	NT	6>5	8>5	8>5
5u	NA	NT	3>5	NT	NT		NT	NT		NT	2>5	4>5	4>5	NT	6>5	8>5	8>5
7c	NA	NT	3>7	NT	NA	NA		NT		NT	2>7	4>7	4>7	NT	6>7	8>7	8>7
7u	NA	NT	NS	NT	NA	NA	NA			NT	2>7	4>7	4>7	NT	NT	8>7	8>7
2c	NA	NA	NA	NA	NA	NA	NA	NA			2u>2c	NS	NT	NT	NT	8>2	NT
2u	NA	NA	NA	NA	NA	NA	NA	NA		NA		NA	NA	NA	NA	NT	NA
4c	NA	NA	NA	NA	NA	NA	NA	NA		NA	2>4		NA	NA	NA	NT	NA
4u	NA	NA	NA	NA	NA	NA	NA	NA		NA	2>4	NT		NA	NA	NT	NT
6c	NA	NA	NA	NA	NA	NA	NA	NA		NT	2>6	NT	NT		NT	NS	NT
6u	NA	NA	NA	NA	NA	NA	NA	NA		NT	2>6	NT	NT	NA		NT	NT
8c	NA	NA	NA	NA	NA	NA	NA	NA		NA	NS	NA	NA	NA	NA		NA
8u	NA	NA	NA	NA	NA	NA	NA	NA		NA	2>8	NT	NA	NA	NA	NT	

Table 2 contd. C: caged, U: uncaged, NA: test already completed, NT: no test, NS: not significant.

May 2002 Kruskall-Wallis Dunns multiple comparisons p=<.05.

	1c	1u	3c	3u	5c	5u	7c	7u		2c	2u	4c	4u	6c	6u	8c	8u
1c		NT	NT	NA	NA	NA	NA	NA		NT	2>1	4>1	NT	NT	6>1	8>1	8>1
1u	NA		NT	NA	NA	NA	NA	NA		NT	2>1	NT	NT	NT	NT	NA	NT
3c	NA	NA		NA	NA	NA	NA	NA		NT	2>3	NT	NT	NT	NT	NT	NT
3u	NT	NT	NT		NA	NA	NA	NT		NT	2>3	4>3	NT	NT	6>3	8>3	8>3
5c	NT	NT	NT	NT		NA	NA	NT		NT	2>5	4>5	NT	NT	6>5	8>5	8>5
5u	NT	NS	3>5	NT	NT		NT	NT		2>5	2>5	4>5	4>5	6>5	6>5	8>5	8>5
7c	NT	NT	NT	NA	NT	NA		NT		NT	2>7	4>7	NT	NS	6>7	8>7	8>7
7u	NT	NT	NT	NT	NA	NA	NA			NT	2>7	4>7	NT	NT	6>7	8>7	8>7
2c	NA	NA	NA	NA	NA	NA	NA	NA			2u>2c	NT	NA	NT	NT	NT	NT
2u	NA	NA	NA	NA	NA	NA	NA	NA		NA		NA	NA	NA	NA	NA	NA
4c	NA	NA	NA	NA	NA	NA	NA	NA		NA	NT		NA	NA	NT	NT	NT
4u	NA	NA	NA	NA	NA	NA	NA	NA		NT	2>4	NT		NT	NA	NT	NT
6c	NA	NA	NA	NA	NA	NA	NA	NA		*NA*	NS	NT	NA		NT	NT	NT
6u	NA	NA	NA	NA	NA	NA	NA	NA		NA	NT	NA	NA	NA		NT	NA
8c	NA	NA	NA	NA	NA	NA	NA	NA		NA	NT	NA	NA	NA	NA		NA
8u	NA	NA	NA	NA	NA	NA	NA	NA		NA	NT	NA	NA	NA	NT	NT	

June 2002 Kruskall-Wallis Dunns multiple comparisons p=<.05.

	1c	1u	3c	3u	5c	5u	7c	7u		2c	2u	4c	4u	6c	6u	8c	8u
1c		NT	NT	NT	NT	NA	NA	NA		NT	2>1	4>1	4>1	6>1	6>1	8>1	8>1
1u	NA		NA	NT	NA	NA	NA	NA		NT	NS	NT	NT	NT	NT	NT	NT
3c	NA	NT		NT	NT	NA	NA	NA		NT	2>3	NT	NT	NS	NT	NT	8>3
3u	NA	NA	NA		NA	NA	NA	NA		NT	NT	NT	NT	NT	NT	NT	NT
5c	NA	NT	NA	NT		NA	NA	NA		NT	2>5	NT	NT	NT	NT	NT	8>5
5u	NT	NT	NT	3>5	NT		NT	NT		NS	2>5	4>5	4>5	6>5	6>5	8>5	8>5
7c	NT	NA	NT	NS	NT	NA		NT		NT	2>7	4>7	4>7	6>7	6>7	8>7	8>7
7u	NA	NT	NA	NT	NT	NA	NA			NT	2>7	4>7	NS	6>7	6>7	8>7	8>1
2c	NA	NA	NA	NA	NA	NA	NA	NA			NT	NT	NT	NT	NT	NT	NT
2u	NA	NA	NA	NA	NA	NA	NA	NA		NA		NA	NA	NA	NA	NA	NA
4c	NA	NA	NA	NA	NA	NA	NA	NA		NA	NT		NA	NT	NT	NT	NT
4u	NA	NA	NA	NA	NA	NA	NA	NA		NA	NT	NT		NT	NT	NT	NA
6c	NA	NA	NA	NA	NA	NA	NA	NA		*NA*	NT	NA	NA		NA	NA	NT
6u	NA	NA	NA	NA	NA	NA	NA	NA		NA	NT	NA	NA	NT		NT	NT
8c	NA	NA	NA	NA	NA	NA	NA	NA		NA	NT	NA	NT	NT	NA		NT
8u	NA	NA	NA	NA	NA	NA	NA	NA		NA	NT	NA	NA	NA	NA	NA	

July 2002 Kruskall-Wallis Dunns multiple comparisons p=<.05.

	1c	1u	3c	3u	5c	5u	7c	7u		2c	2u	4c	4u	6c	6u	8c	8u
1c		NA	NA	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	NA	NA	NA
1u	NS		NA	NA	NA	NA	NA	NT		NT	NT	NT	NT	NT	NT	NT	NT
3c	1>3	NT		NA	NA	NA	NT	NT		NT	NT	NT	NT	NT	6>3	NT	NT
3u	1>3	NT	NT		NA	NT	NT	NT		NT	2>3	4>3	NT	6>3	6>3	NS	8>3
5c	1>5	NS	NT	NT		NT	NT	7>5		2>5	2>5	4>5	4>5	6>5	6>5	8>5	8>5
5u	1>5	NT	NT	NA	NA		NT	NT		NT	NT	NT	NT	NS	6>5	NS	NT
7c	1>7	NT	NA	NA	NA	NA		NT		NT	NT	NT	NT	NT	6>7	NT	NT
7u	NT	NA	NA	NA	NA	NA	NA			NT	NT	NT	NT	NT	NT	NT	NT
2c	NT	NA	NA	NA	NA	NA	NA	NA			NT	NT	NT	NT	NT	NT	NT
2u	NT	NA	NA	NA	NA	NA	NA	NA		NA		NA	NA	NT	NT	NA	NA
4c	NT	NA	NA	NA	NA	NA	NA	NA		NA	NT		NA	NT	NT	NA	NT
4u	NT	NA	NA	NA	NA	NA	NA	NA		NA	NT	NT		NT	NT	NT	NT
6c	NT	NA	NA	NA	NA	NA	NA	NA		*NA*	NA	NA	NA		NT	NA	NA
6u	NT	NA	NA	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA		NA	NA
8c	NT	NA	NA	NA	NA	NA	NA	NA		NA	NT	NT	NA	NT	NT		NT
8u	NT	NA	NA	NA	NA	NA	NA	NA		NT	NA	NA	NA	NT	NT	NA	

Appendix 4 Results for Chapter 6

Table 1 Significant changes in limpet numbers and total algal cover as shown by SNK tests for the groyne end zones between January and September 2002.

Limpets Sept 2002 Area 1 only (all groynes)		
Comparison model	**Result**	**Summary**
Gr(Tr) end zone treatment	H4>H8=H0 (p<.01)	H8 end zone decline.
Gr(Tr) end zone control	H6>=H2=H10(p<.05)	H6 recruitment, not fully resolved.
Total algae Sept 2002 Area 1 only (all groynes)		
Comparison model	**Result**	**Summary**
Gr(Tr) end zone treatment	H0>H4=H8 (p<.001)	H0 end zone incfrease
Bare rock Sept 2002 Area 1 only (all groynes)		
Comparison model	**Result**	**Summary**
Gr(Tr) end zone treatment	H8>H0=H4 (p<.05)	H8 increase due to disturbance

Table 2 Significant changes in limpet numbers and total algal cover as shown by SNK tests for the near shore zones groyne end zone between January and September 2002

Total algae Sept 02 end zone 1 only (all groynes)		
Comparison model	**Result**	**Summary**
Gr(Tr) treatment	H0>H8 (p<.01)	Deacrease both zones H8 (disturbance)
Gr(Tr×Zn) treatment mid zone	H0>H8 (p<.01)	Disturnbance
Gr(Tr×Zn) treatment near shore zone	H0>H8 (p<.05)	Disturbance
Total algae Sept 02 end zone only (all groynes)		
Comparison model	**Result**	**Summary**
Gr(Tr) treatment	H8>H0 (p<.01)	Increase both zones H8 (disturbance)
Gr(Tr×Zn) treatment mid zone	H8>H0 (p<.01)	Disturnbance
Gr(Tr×Zn) treatment near shore zone	H8>H0 (p<.01	Disturbance

Table 3 ANOVA for Comparisons between and within treatment (H0, H4, H8) and control (H2, H6, H10) abundances for A: *Mytilus edulis*, B: barnacles, C: limpets, D: total algae and E: bare rock in May 2001 before manipulation. NS: Not significant,. Source key: TR: treatment, GR: Groyne, Zn : Zone. 0.50 m x 0.50 m quadrats.

A:	***M. edulis***			
Source	**DF**	**MS**	**F**	**P**
Tr	1	16.8	0.6	NS
Gr(Tr)	4	27.4	19.2	<0.001
Zn	2	22.1	5.9	<0.05
Tr×Zn	2	0.01	0.00	NS
Zn×Gr(Tr)	8	3.7	2.6	<0.05
RES	108	1.4		
TOT	125			

B:	**Barnacles**			
Source	**DF**	**MS**	**F**	**P**
Tr	1	2.4	0.5	NS
Gr(Tr)	4	4.7	2.5	NS
Zn	2	1.1	0.9	NS
Tr×Zn	2	0.7	0.6	NS
Zn×Gr(Tr)	8	1.3	0.6	NS
RES	108	2.2		
TOT	125			

C:	**Limpets**			
Source	**DF**	**MS**	**F**	**P**
Tr	1	16.3	4.1	NS
Gr(Tr)	4	3.9	4.9	<0.01
Zn	2	0.1	0.1	NS
Tr×Zn	2	0.3	0.3	NS
Zn×Gr(Tr)	8	1.1	1.4	NS
RES	108	0.8		
TOT	125			

D:	**Total algae**			
Source	**DF**	**MS**	**F**	**P**
Tr	1	12.1	2.2	NS
Gr(Tr)	4	5.6	3.0	<0.05
Zn	2	27.6	18.2	<0.01
Tr×Zn	2	3.9	2.6	NS
Zn×Gr(Tr)	8	1.5	0.8	NS
RES	108	1.8		
TOT	125			

E:	**Bare rock**			
Source	**DF**	**MS**	**F**	**P**
Tr	1	77.8	0.1	NS
Gr(Tr)	4	5166.2	7.7	<0.001
Zn	2	2675.0	3.9	<0.05
Tr×Zn	2	2907.1	4.3	<0.05
Zn×Gr(Tr)	8	1070.7	1.6	NS
RES	108	672.5		
TOT	125	834.5		

Table 4 ANOVA End zone comparisons between and within treatment (H0, H4, H8) and control (H2, H6, H10) abundances for A: *Mytilus edulis*, B: barnacles, C: limpets, D: total algae and E: bare rock March and September 2002.
NS: Not significant, Source key: TR: treatment, GR: Groyne, Bl: Block. 0.50 x 0.50 m quadrats.

A: *M. edulis* end zone March 2002

Source	DF	MS	F	P
Tr	1	0.0	0.00	NS
Gr(Tr)	4	6.4	3.67	<0.05
RES	36	1.7		
TOT	41			

B: Barnacles end zone March 2002

Source	DF	MS	F	P
Tr	1	1.9	0.5	NS
Gr(Tr)	4	3.8	3.8	<0.01
RES	36	0.9		
TOT	41			

C: Limpets end zone March 2002

Source	DF	MS	F	P
Tr	1	0.2	0.04	NS
Gr(Tr)	4	4.1	3.2	<0.05
RES	36	1.3		
TOT	41			

D: Total algae end zone March 2002

Source	DF	MS	F	P
Tr	1	0.03	0.0	NS
Gr(Tr)	4	6.8	3.5	NS
RES	36	1.9		
TOT	41			

E: Bare rock end zone March 2002

Source	DF	MS	F	P
Tr	1	572.0	1.3	NS
Gr(Tr)	4	803.2	1.9	NS
RES	36	15503.4	430.7	
TOT	41	18678.1	455.6	

F: *M. edulis* end zone Sept 2002

Source	DF	MS	F	P
Tr	1	0.1	0.01	NS
Gr(Tr)	4	8.2	5.9	<0.001
RES	36	1.4		
TOT	41			

G: Barnacles end zone Sept 2002

Source	DF	MS	F	P
Tr	1	0.6	0.1	NS
Gr(Tr)	4	4.7	5.4	<0.01
RES	36	0.9		
TOT	41			

H: Limpets end zone Sept 2002

Source	DF	MS	F	P
Tr	1	10.3	1.4	NS
Gr(Tr)	4	7.4	5.3	<0.01
RES	36	1.4		
TOT	41			

I: Total algae end zone Sept 2002

Source	DF	MS	F	P
Tr	1	1.1	0.2	NS
Gr(Tr)	4	13.5	6.1	<0.01
RES	36	2.2		
TOT	41			

J: Bare rock end zone Sept 2002

Source	DF	MS	F	P
Tr	1	672.0	0.9	NS
Gr(Tr)	4	2598.0	0.6	<0.05
RES	36	688.95	3.8	
TOT	41	767.9		

Table 5 ANOVA Mid and near shore zone comparisons between and within treatments (H0, H4, H8) and control (H2, H6, H10) abundances for A: *Mytilus edulis*, B: barnacles, C: limpets, D: total algae and E: bare rock March and September 2002. NS: Not significant. !: Cochrans failed. Tr: treatment, Gr: Groyne, Zn: Zone, Bl: Block. 0.50 m x 0.50 m quadrats.

A: *M. edulis* mid and near shore zones March 2002

Source	DF	MS	F	P
Tr	1	0.5	0.4	NS
Gr(Tr)	2	1.2	2.2	NS
Zn	1	1.7	1.3	NS
TrxZn	1	0.7	0.6	NS
ZnxGr(Tr)	2	1.3	2.4	NS
RES	48	0.5		
TOT	55			

B: Barnacles mid and near shore zones March 2002

Source	DF	MS	F	P
Tr	1	2.4	0.9	NS
Gr(Tr)	2	2.6	4.6	<0.05
Zn	1	1.5	0.9	NS
TrxZn	1	5.5	3.1	NS
ZnxGr(Tr)	2	1.8	3.2	<0.05
RES	48	0.6		
TOT	55			

C: Limpets mid and near shore zones March 2002

Source	DF	MS	F	P
Tr	1	1.7	0.8	NS
Gr(Tr)	2	2.3	4.6	<0.05
Zn	1	1.8	1.1	NS
TrxZn	1	0.01	0.01	NS
ZnxGr(Tr)	2	1.7	3.5	<0.05
RES	48	0.5		
TOT	55			

D: Total algae mid and near shore zones March 2002

Source	DF	MS	F	P
Tr	1	9.4	2.7	NS
Gr(Tr)	2	3.6	1.4	NS
Zn	1	22.1	3.4	NS
TrxZn	1	7.7	1.1	NS
ZnxGr(Tr)	2	6.4	2.6	NS
RES	48	2.5		
TOT	55			

E: Bare rock mid and near shore zones March 2002

Source	DF	MS	F	P
Tr	1	707.2	0.9	NS
Gr(Tr)	2	1143.0	1.4	NS
Zn	1	9078.0	11.5	<0.001
TrxZn	1	1216.4	1.5	NS
ZnxGr(Tr)	2	1.446	0.002	NS
RES	48	790.5		
TOT	55	913.5		

F: *M. edulis* mid and near shore zones Sept 2002

Source	DF	MS	F	P
Tr	1	1.4	3.9	NS
Gr(Tr)	2	0.4	1.3	NS
Zn	1	0.1	0.5	NS
TrxZn	1	0.04	0.2	NS
ZnxGr(Tr)	2	0.2	0.8	NS
RES	48	0.3		
TOT	55			

G: Barnacles mid and near shore zones Sept 2002

Source	DF	MS	F	P
Tr	1	0.6	0.5	NS
Gr(Tr)	2	1.3	2.1	NS
Zn	1	1.2	1.4	NS
TrxZn	1	2.2	2.7	NS
ZnxGr(Tr)	2	0.8	1.3	NS
RES	48	0.6		
TOT	55			

H: Limpets mid and near shore zones e Sept 2002

Source	DF	MS	F	P
Tr	1	3.7	2.1	NS
Gr(Tr)	2	1.8	2.1	NS
Zn	1	0.2	0.1	NS
TrxZn	1	3.1	1.4	NS
ZnxGr(Tr)	2	2.3	2.7	NS
RES	48	0.8		
TOT	55			

I: Total algae mid and near shore zones Sept2002

Source	DF	MS	F	P
Tr	1	24.9	0.7	NS
Gr(Tr)	2	38.5	22.3	<0.001
Zn	1	0.4	0.2	NS
TrxZn	1	0.4	0.2	NS
ZnxGr(Tr)	2	1.7	1.0	NS
RES	48	1.7		
TOT	55			

J: Bare rock mid and near shore zones Sept 2002

Source	DF	MS	F	P
Tr	1	3600.0	4.8	NS!
Gr(Tr)	2	3255.8	4.3	NS!
Zn	1	3135.0	4.1	NS!
TrxZn	1	46.4	0.06	NS
ZnxGr(Tr)	2	2730.0	3.6	NS
RES	48	748.2		
TOT	55	1198.9		

Table 6 Experiment start (May 2001) NPMANOVA *post hoc* within treatment and control comparisons for significant differences in community structure for 0.25m x 0.25 m quadrats (within blocks) and 0.50 x 0.50 m quadrats (within zones).

A: Groyne end zone treatment (H0, H4, H8) 25 cm	**P**	**G: Groyne end zone treatment (H0, H4, H8) 50 cm**	**P**
H0 vs H4	<0.001	H0 vs H4	<0.01
H0 vs H8	<0.001	H0 vs H8	<0.01
H4 vs H8	<0.001	H4 vs H8	<0.01
B: Groyne end zone control (H2, H6, H10) 25 cm	**P**	**H: Groyne end zone control (H2, H6, H10) 50 cm**	**P**
H2 vs H6	<0.001	H2 vs H6	NS
H2 vs H10	<0.001	H2 vs H10	<0.01
H6 vs H10	<0.01	H6 vs H10	<0.05
C: Groyne mid zone treatment (H0, H4, H8) 25 cm	**P**	**I: Groyne mid zone treatment (H0, H4, H8) 50 cm**	**P**
H0 vs H4	<0.01	H0 vs H4	NS
H0 vs H8	<0.001	H0 vs H8	<0.05
H4 vs H8	<0.001	H4 vs H8	<0.01
D: Groyne mid zone control (H2, H6, H10) 25 cm	**P**	**J: Groyne mid zone control (H2, H6, H10) 50 cm**	**P**
H2 vs H6	NS	H2 vs H6	NS
H2 vs H10	<0.01	H2 vs H10	<0.05
H6 vs H10	<0.001	H6 vs H10	NS
E: Groyne near shore zone treatment (H0,H4,H8) 25 cm	**P**	**K: Groyne near shore zone treatment (H0, H4, H8) 50 cm**	**P**
H0 vs H4	<0.001	H0 vs H4	NS
H0 vs H8	<0.01	H0 vs H8	NS
H4 vs H8	<0.001	H4 vs H8	<0.05
F: Groyne near shore zone control (H2, H6, H10) 25 cm	**P**	**L: Groyne near shore zone control (H2, H6, H10) 50 cm**	**P**
H2 vs H6	<0.05	H2 vs H6	NS
H2 vs H10	<0.05	H2 vs H10	<0.05
H6 vs H10	<0.01	H6 vs H10	NS

Table 7 Experiment start (May 2001) SIMPER within groyne comparisons, for % Bray–Curtis dissimilarity and taxa relative abundance for A: 0.25 m x 0.25 m quadrats and, B: 0.50 x 0.50 m quadrats.
Ab: Abundance, %: Bray-Curtis % dissimilarity value.

A : 25 cm (within)	**M. edulis**		**Barnacles**		**Limpets**		**Ulva**		**Entero**	
	Ab	**%**	**Ab**	**%**	**Ab**	**%**	**Ab**	**%**	**Ab**	**%**
H0 treatment	14.3	18.6	NA	NA	1.3	10.5	25.9	42.1	26.6	26.5
H4 treatment	37.1	78.7	NA	NA	2.0	11.7	NA	NA	NA	NA
H8 treatment	NA	NA	22.1	57.7	1.7	18.2	3.3	12.9	4.6	10.3
H2 control	14.8	43.7	NA	NA	1.0	15.3	10	14.3	7.4	23.1
H6 control	12.8	23.1	4.5	9.9	1.0	9.7	10.4	35.2	11.3	21.0
H10 control	NA	NA	7.6	19.1	NA	NA	19.2	51.4	11.5	22.1
B : 50 cm (within)	**M. edulis**		**Barnacles**		**Limpets**		**Ulva**		**Entero**	
	Ab	**%**	**Ab**	**%**	**Ab**	**%**	**Ab**	**%**	**Ab**	**%**
H0 treatment	28.2	51.1	NA	NA	NA	NA	19.1	12.5	16.6	29.2
H4 treatment	25.6	49.1	6.2	8.8	9.0	20.6	5.7	16.7	NA	NA
H8 treatment	NA	NA	16.4	40.3	6.2	33.8	9.2	8.8	9.6	12.8
H2 control	21.0	41.3	8.5	9.1	2.5	15.7	15.3	12.3	10.7	19.6
H6 control	8.1	13.4	8.1	9.3	NA	NA	13.7	48.9	12.1	21.7
H10 control	NA	NA	6.3	5.5	NA	NA	19.1	52.8	26.0	35.2

Table 8 Experiment start (May 2001) SIMPER between groyne comparisons, for % Bray–Curtis dissimilarity and taxa relative abundance for A: 0.25 m x 0.25 m quadrats and, B: 0.50 x 0.50 m quadrats.

A: 25 cm (between)	M. edulis	Barnacles	Limpets	Ulva	Enteromorpha
H0 vs H4 (treatment)	33.4	8.67	8.2	24.5	18.5
H0 vs H8 (treatment)	15.7	25.2	7.7	24.8	19.3
H4 vs H8 (treatment)	37.7	24.7	8.4	10.0	10.0
H2 vs H6 (control)	27.7	11.7	8.5	20.5	23.0
H2 vs H10 (control)	20.2	14.5	6.5	25.9	19.4
H6 vs H10 (control)	16.6	16.8	16.6	24.8	20.8
H0 vs H6 (treatment/control)	23.5	10.9	7.8	27.9	23.1
H4 vs H6 (treatment/control)	36.9	16.8	15.1	12.4	9.33
H8 vs H6 (treatment/control)	16.4	28.4	9.1	18.1	17.9
H0 vs H2 (treatment/control)	26.1	7.35	7.9	29.7	22.8
H4 vs H2 (treatment/control)	39.7	10.0	10.1	15.6	15.0
H8 vs H2 (treatment/control)	20.3	28.5	8.7	17.1	16.1
H4 vs H10 (treatment/control)	34.4	13.1	7.5	19.3	13.5
H0 vs H10 (treatment/control)	16.5	13.7	6.7	28.9	22.4
H8 vs H10 (treatment/control)	NS	29.4	9.1	24.2	19.0

B: 50 cm(between)	M. edulis	Barnacles	Limpets	Ulva	Enteromorpha
H0 vs H4 (treatment)	22.2	13.3	14.1	20.0	20.1
H0 vs H8 (treatment)	27.6	18.7	11.1	18.0	17.9
H4 vs H8 (treatment)	28.3	18.0	12.1	14.1	12.5
H2 vs H6 (control)	24.7	17.0	8.8	22.6	19.1
H2 vs H10 (control)	21.6	13.3	7.4	22.3	21.5
H6 vs H10 (control)	13.8	15.7	6.4	21.1	27.4
H0 vs H6 (treatment/control)	29.4	14.0	7.5	23.8	21.4
H4 vs H6 (treatment/control)	25.6	15.4	14.8	15.5	15.2
H8 vs H6 (treatment/control)	12.9	23.1	14.1	20.8	18.9
H0 vs H2 (treatment/control)	26.6	14.9	8.9	23.5	20.6
H4 vs H2 (treatment/control)	25.6	15.4	13.1	18.5	15.4
H8 vs H2 (treatment/control)	23.5	20.8	10.9	17.8	16.9
H4 vs H10 (treatment/control)	25.1	11.3	12.6	15.0	20.5
H0 vs H10 (treatment/control)	27.9	10.8	5.9	23.2	21.5
H8 vs H10 (treatment/control)	4.6	19.5	13.3	22.7	23.4

Table 9 NPMANOVA comparisons for October 2001 of treatment and control groyne community compositions for 0.25 m x 0.25 m and 0.50 m x 0.50 m quadrats for the end, mid and near shore zones.
NS: Not significant. Source key: TR: treatment, Ctr: control, GR: Groyne.

A: End zone 25 cm data treatment and control Oct 2001

Source	df	MS	F	P
Tr vs Ctr	1	38.2	0.3	NS
Gr(Tr)	4	103.7	7.1	<0.001
RES	114	14.5		
TOT	119			

D: End zone 50 cm data treatment and control Oct 2001

Source	df	MS	F	P
Tr vs Ctr	1	4439.5	0.6	NS
Gr(Tr)	4	6430.4	2.9	<0.001
RES	36	2162.8		
TOT	41			

B: Mid zone 25 cm data treatment and control Oct 2001

Source	df	MS	F	P
Tr vs Ctr	1	1355.1	0.08	NS
Gr(Tr)	4	16656.1	5.1	<0.001
RES	114	3209.9		
TOT	119			

E: Mid zone 50 cm data treatment and control Oct 2001

Source	df	MS	F	P
Tr vs Ctr	1	3050.9	0.4	NS
Gr(Tr)	4	7447.7	3.1	<0.001
RES	36	2387.0		
TOT	41			

C: Near shore zone 25 cm data treatment and control Oct 2001

Source	df	MS	F	P
Tr vs Ctr	1	10249.6	0.4	NS
Gr(Tr)	4	21078.1	6.6	<0.001
RES	114	3146.1		
TOT	119			

F: Near shore zone 50 cm data treatment and control Oct 2001

Source	df	MS	F	P
Tr vs Ctr	1	3486.8	0.4	NS
Gr(Tr)	4	8062.5	3.3	NS
RES	36	2422.7		
TOT	41			

Table 10 August and October SIMPER treatment and control comparisons for % Bray–Curtis dissimilarity and taxa relative abundance for A and B 0.25 m x 0.25 m quadrats, C and D 0.50 x 0.50 m quadrats. Av.Abund: the average abundance of each species in each group of samples, Av,diss: The average dissimilarity among groups of samples, Diss/SD: measure of variation of species contribution to group dissimilarity, Contrib%: the percentage contribution that species gives to between group dissimilarity.

A: 25 cm. Groups: Treatment (H0, H4, H8) and Control (H2, H6, H10)
Average dissimilarity = 75.09 August

Species	Av.Abund treatment	Av.Abund control	Av.Diss	Diss/SD	Contrib%
Enteromorpha	14.6	23.4	22.9	1.0	30.6
Mytilus edulis	12.2	10.4	15.5	0.7	20.6
Barnacles	11.0	5.0	15.3	0.9	20.4
Limpets	1.7	1.2	7.0	0.9	9.43
Ulva lactuca	0.7	4.1	6.6	0.6	8.8

B: 25 cm. Groups: Treatment (H0, H4, H8) and Control (H2, H6, H10)
Average dissimilarity = 75.92 October

Species	Av.Abund treatment	Av.Abund control	Av.Diss	Diss/SD	Contrib%
Barnacles	11.6	9.1	23.2	1.0	30.5
Mytilus edulis	11.6	10.3	22.0	0.9	29.0
Enteromorpha	3.2	2.1	8.9	0.5	11.7
Limpets	1.8	1.9	11.9	0.9	14.8
Ulva lactuca	0.4	0.9	4.1	0.4	5.39

C: 50 cm. Groups: Treatment (H0, H4, H8) and Control (H2, H6, H10)
Average dissimilarity = 65.64 August

Species	Av.Abund treatment	Av.Abund control	Av.Diss	Diss/SD	Contrib%
Enteromorpha	16.6	33.0	19.1	1.3	29.1
Mytilus edulis	11.4	6.7	11.4	0.9	17.4
Limpets	7.4	5.0	9.8	1.0	14.9
Barnacles	4.6	3.8	7.9	1.0	12.0
Ulva lactuca	2.2	5.1	7.2	0.8	10.9
Porphyra	1.1	0.5	3.0	0.7	4.5

D: 50 cm. Groups: Treatment (H0, H4, H8) and Control (H2, H6, H10)
Average dissimilarity = 71.14 October

Species	Av.Abund treatment	Av.Abund control	Av.Diss	Diss/SD	Contrib%
Mytilus edulis	10.5	7.1	14.3	0.8	20.1
Limpets	9.0	9.0	14.6	1.2	20.6
Barnacles	6.6	4.2	11.9	0.9	16.8
Enteromorpha	6.3	6.0	12.0	0.7	16.9
Fucus	1.5	4.4	7.4	0.6	10.4
Ulva lactuca	0.8	1.4	4.0	0.5	5.7

Table 11 NPMANOVA post hoc comparisons for March and September 2002 of treatment and control groyne community compositions for 0.25 m x 0.25 m and 0.50 m x 0.50 m quadrats for the end, mid and near shore zones.

A: Groyne comparisons end zone treatment (H0, H4, H8) 25 cm	**P**	**G: Groyne comparisons end zone treatment (H0, H4, H8) 50 cm**	**P**
March H0 vs H4	NS	March H0 vs H4	NS
March H0 vs H8	<0.01	March H0 vs H8	<0.01
March H4 vs H8	<0.01	March H4 vs H8	NS
September H0 vs H4	<0.01	September H0 vs H4	<0.01
September H0 vs H8	<0.01	September H0 vs H8	<0.01
September H4 vs H8	<0.01	September H4 vs H8	<0.05
B: Groyne comparisons end zone control (H2, H6, H10) 25 cm	**P**	**H: Groyne comparisons end zone control (H2, H6, H10) 50 cm**	**P**
March H2 vs H6	NS	March H2 vs H6	NS
March H2 vs H10	<0.01	March H2 vs H10	<0.05
March H6 vs H10	NS	March H6 vs H10	NS
September H2 vs H6	<0.01	September H2 vs H6	<0.05
September H2 vs H10	<0.01	September H2 vs H10	<0.01
September H6 vs H10	<0.01	September H6 vs H10	<0.01
C: Groyne comparisons mid zone treatment (H0, H2, H8 25 cm	**P**	**I: Groyne comparisons mid zone treatment (H0, H2, H8 50 cm**	**P**
March H0 vs H8	<0.01	March H0 vs H8	<0.01
September H0 vs H8	<0.01	September H0 vs H8	NS
D: Groyne comparisons mid zone control (H2, H6, H10) 25 cm	**P**	**J: Groyne comparisons mid zone control (H2, H6, H10) 50 cm**	**P**
March H6 vs H10	<0.01	March H6 vs H10	NS
September H6 vs H10	<0.01	September H6 vs H10	NS
E: Groyne comparisons near shore zone treatment (H0, H2, H8 25 cm	**P**	**K: Groyne comparisons near shore zone treatment (H0, H2, H8 50 cm**	**P**
March H0 vs H8	<0.05	March H0 vs H8	NS
September H0 vs H8	<0.05	September H0 vs H8	<0.01
F: Groyne comparisons near shore zone control (H2, H6, H10) 25 cm	**P**	**L: Groyne comparisons near shore zone control (H2, H6, H10) 50 cm**	**P**
March H6 vs H10	NS	March H6 vs H10	NS
September H6 vs H10	<0.01	September H6 vs H10	<0.01

Table 12 March and September 2002 SIMPER within groyne comparisons, for % Bray–Curtis dissimilarity and taxa relative abundance for A: March 0.25 m x 0.25 m quadrats B: March 0.50 x 0.50 m quadrats, D: September 0.25 m x 0.25 m quadrats and D: September 0.50 x 0.50 m quadrats.

A: 25 cm (within) March	**Mussels**		**Barnacles**		**Limpets**		**Ulva**		**Entero**	
	Ab	**%**	**Ab**	**%**	**Ab**	**%**	**Ab**	**%**	**Ab**	**%**
H0 treatment	8.4	14.9	NA	NA	NA	NA	NA	NA	26.0	77.7
H4 treatment	15.2	31.4	18.2	29.2	2.2	39.0	NA	NA	NA	NA
H8 treatment	NA	NA	18.4	70.1	1.2	20.1	NA	NA	NA	NA
H2 treatment	27.1	61.8	NA	NA	2.3	36.8	NA	NA	NA	NA
H6 treatment	18.3	34.6	NA	NA	1.8	24.2	NA	NA	15.4	36.1
H10 treatment	NA	NA	5.9	39.7	0.6	10.5	NA	NA	10.4	43.9

B: 50 cm (within) March	**Mussels**		**Barnacles**		**Limpets**		**Ulva**		**Entero**	
	Ab	**%**	**Ab**	**%**	**Ab**	**%**	**Ab**	**%**	**Ab**	**%**
H0 treatment	NA	NA	NA	NA	NA	NA	NA	NA	32.3	94.6
H4 treatment	12.5	13.5	NA	NA	4.8	22.5	NA	NA	12.6	55.4
H8 treatment	NA	NA	10.1	33.0	6.5	34.5	NA	NA	14.1	17.3
H2 treatment	7.0	28.8	NA	NA	5.9	55.8	NA	NA	4.9	11.6
H6 treatment	13.4	17.7	NA	NA	3.7	29.5	NA	NA	15.8	44.1
H10 treatment	NA	NA	4.5	17.2	1.9	12.6	NA	NA	22.0	68.1

C: 25 cm (within) September	**Mussels**		**Barnacles**		**Limpets**		**Ulva**		**Entero**	
	Ab	**%**	**Ab**	**%**	**Ab**	**%**	**Ab**	**%**	**Ab**	**%**
H0 treatment	NA	NA	NA	NA	NA	NA	3.4	3.5	40.0	89.7
H4 treatment	21.5	30.0	18.5	25.7	4.6	43.5	NA	NA	NA	NA
H8 treatment	NA	NA	2.5	50.7	0.8	40.8	NA	NA	NA	NA
H2 treatment	30.6	58.0	NA	NA	3.1	37.0	NA	NA	NA	NA
H6 treatment	9.61	9.6	5.4	24.3	2.6	27.0	NA	NA	22.8	35.9
H10 treatment	NA	NA	NA	NA	0.7	4.8	84.9	41.4	2.4	4.3

D: 50 cm (within) September	**Mussels**		**Barnacles**		**Limpets**		**Ulva**		**Entero**	
	Ab	**%**	**Ab**	**%**	**Ab**	**%**	**Ab**	**%**	**Ab**	**%**
H0 treatment	NA	NA	NA	NA	NA	NA	NA	NA	44.5	92.5
H4 treatment	12.8	40.7	2.1	9.3	20.7	44.7	NA	NA	NA	NA
H8 treatment	NA	NA	6.3	66.0	1.8	31.0	NA	NA	NA	NA
H2 treatment	21.0	56.6	NA	NA	14.2	39.9	NA	NA	NA	NA
H6 treatment	2.6	6.9	4.4	21.5	11.4	44.2	NA	NA	20.2	21.3
H10 treatment	NA	NA	1.1	4.2	2.1	3.9	39.0	76.6	NA	NA

Table 13 March and September 2002 SIMPER between groyne comparisons, for % Bray–Curtis dissimilarity and taxa relative abundance for A: March 0.25 m x 0.25 m quadrats, B: March 0.50 x 0.50 m quadrats, D: September 0.25 m x 0.25 m quadrats and D: September 0.50 x 0.50 m quadrats

A: 25 cm (between) March	**Mussels**	**Barnacles**	**Limpets**	**Ulva**	**Enteromorpha**
H0 vs H4 (treatment)	27.1	23.6	11.8	NA	31.3
H0 vs H8 (treatment)	13.9	30.7	9.4	NA	30.7
H4 vs H8 (treatment)	25.7	37.1	11.1	NA	14.3
H2 vs H6 (control)	45.0	11.0	15.2	NA	24.3
H2 vs H10 (control)	36.3	18.8	14.1	NA	20.6
H6 vs H10 (control)	24.8	20.3	10.7	NA	24.8
H0 vs H6 (treatment/control)	30.9	10.1	10.7	NA	41.1
H4 vs H6 (treatment/control)	33.9	26.6	12.0	NA	21.5
H8 vs H6 (treatment/control)	21.0	31.4	10.3	NA	26.2
H0 vs H2 (treatment/control)	37.4	8.3	14.0	NA	35.8
H4 vs H2 (treatment/control)	46.7	32.0	14.0	NA	NA
H8 vs H2 (treatment/control)	34.2	31.9	12.0	NA	12.6
H4 vs H10 (treatment/control)	26.1	29.4	12.4	NA	20.0
H0 vs H10 (treatment/control)	18.5	20.1	8.4	NA	39.2
H8 vs H10 (treatment/control)	4.6	35.8	11.4	5.8	29.2
H8 vs H10 (treatment/control)	3.2	24.3	12.0	40.7	11.2
B: 50 cm (between) March	**Mussels**	**Barnacles**	**Limpets**	**Ulva**	**Enteromorpha**
H0 vs H4 (treatment)	20.7	10.1	16.6	NA	39.1
H0 vs H8 (treatment)	6.1	20.1	17.7	6.1	27.9
H4 vs H8 (treatment)	11.0	18.9	16.9	5.9	21.0
H2 vs H6 (control)	26.2	13.1	22.0	NA	27.4
H2 vs H10 (control)	18.7	15.3	21.1	NA	29.1
H6 vs H10 (control)	16.9	17.2	18.4	NA	31.0
H0 vs H6 (treatment/control)	22.1	11.9	19.0	NA	38.2
H4 vs H6 (treatment/control)	23.1	13.3	19.6	NA	28.1
H8 vs H6 (treatment/control)	12.8	19.2	16.4	5.9	21.7
H0 vs H2 (treatment/control)	21.5	7.1	23.1	NA	40.6
H4 vs H2 (treatment/control)	24.8	11.2	22.7	NA	25.9
H8 vs H2 (treatment/control)	14.5	20.6	15.8	6.1	19.1
H4 vs H10 (treatment/control)	13.8	16.4	16.6	4.7	32.4
H0 vs H10 (treatment/control)	9.8	17.5	13.6	5.7	43.4
H8 vs H10 (treatment/control)	NA	20.9	18.2	8.7	25.3
C: 25 (between) September	**Mussels**	**Barnacles**	**Limpets**	**Ulva**	**Enteromorpha**
H0 vs H4 (treatment)	21.7	18.6	13.0	8.3	34.4
H0 vs H8 (treatment)	10.0	13.3	9.0	8.3	52.3
H4 vs H8 (treatment)	33.2	32.6	20.9	8.3	NA
H2 vs H6 (control)	35.0	14.2	14.0	NA	27.9
H2 vs H10 (control)	27.8	4.8	11.4	38.6	11.3
H6 vs H10 (control)	10.4	12.9	9.5	36.8	21.7
H0 vs H6 (treatment/control)	15.4	14.2	11.2	9.75	41.5
H4 vs H6 (treatment/control)	24.1	24.9	13.2	9.0	22.4
H8 vs H6 (treatment/control)	15.2	21.9	16.8	7.8	32.9
H0 vs H2 (treatment/control)	32.4	NA	13.7	5.9	40.5
H4 vs H2 (treatment/control)	42.2	26.4	14.6	NA	10.4
H8 vs H2 (treatment/control)	45.6	13.1	21.8	NA	16.9
H4 vs H10 (treatment/control)	21.0	19.7	12.2	35.9	5.4
H0 vs H10 (treatment/control)	7.4	6.3	5.0	36.5	35.2
H8 vs H10 (treatment/control)	4.3	13.8	9.5	52.9	10.2
D: 50 cm (between) September	**Mussels**	**Barnacles**	**Limpets**	**Ulva**	**Enteromorpha**
H0 vs H4 (treatment)	16.6	6.8	20.1	10.67	40.2
H0 vs H8 (treatment)	9.5	15.2	8.8	5.7	53.4
H4 vs H8 (treatment)	24.1	21.1	33.5	16.7	NA
H2 vs H6 (control)	27.5	12.9	19.6	5.8	24.5
H2 vs H10 (control)	22.5	4.5	15.4	32.3	10.6
H6 vs H10 (control)	5.7	9.5	16.4	29.9	17.8
H0 vs H6 (treatment/control)	11.1	11.9	20.0	7.2	36.5
H4 vs H6 (treatment/control)	16.6	12.5	25.0	14.1	21.3
H8 vs H6 (treatment/control)	8.1	18.1	26.1	8.8	26.4
H0 vs H2 (treatment/control)	28.1	NA	20.5	NA	41.8
H4 vs H2 (treatment/control)	31.5	9.6	31.5	13.2	12.4
H8 vs H2 (treatment/control)	38.9	17.5	25.4	NA	13.0
H4 vs H10 (treatment/control)	14.9	8.3	20.9	32.8	5.4
H0 vs H10 (treatment/control)	7.4	4.9	5.6	31.0	33.6
H8 vs H10 (treatment/control)	NA	15.0	9.7	43.5	7.1