Tributyltin pollution and the bioindicator *Nucella lapillus*: population recovery and community level responses

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by

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Abstract

The detrimental effects of tributyltin (TBT) have been recorded on many marine organisms. As a result the UK Government imposed a partial ban on the use of organotin antifouling paints on boats less than 25 m in length, in 1987. In 1988 the Isle of Man Government followed suit introducing a licensing procedure restricting all uses of organotins.

At concentrations less than 0.5 ng Sn/l female *Nucella lapillus* develop imposex - the superimposition of male sexual characteristics. To date there have been few studies measuring the recovery of *Nucella* populations after the introduction of restrictions. This study produces evidence of the extent of recovery in *Nucella* populations from sites in the south-west of England and on the Isle of Man. The recovery observed was measured by decreasing values of relative penis size, vas deferens sequence and the percentage of sterile adult females in the population. Following the 1987 ban the recovery of *Nucella* populations in the south-west has shown a linear response allowing predictions to be made for the time scale of complete recovery. In addition concentrations of TBT in the water and tissues of selected indicator organisms also showed decreases. Around the Isle of Man the illegal use of TBT paints was identified and later discouraged by the Marine Administration which was followed by a reduction in TBT concentrations in the water at sites around the Isle of Man. Levels of imposex in dogwhelk populations around the Isle of Man have decreased.

Although effects of TBT on *Nucella* have been well documented at the cellular and individual level, the knock on effects on the community have not been investigated. Manipulative field experiments were used to demonstrate the role of *Nucella lapillus* in structuring shore communities to allow predictions of the effect of TBT to be made. Rather than using the traditional approach of fences and cages, dogwhelks were removed by hand on regular visits to experimental sites creating treatments with reduced abundances of dogwhelks akin to shores affected by TBT. The role of *Nucella* was examined at different stages of a cycle existing on moderately exposed Manx shores where *Fucus vesiculosus* and *Semibalanus balanoides* fluctuate in abundance. The removal of dogwhelks increased the abundance of *Semibalanus balanoides* on the shore and as a result likelihood of algal escapes from grazing by *Patella vulgata* also increased. In addition the removal of *Nucella* increased the size and longevity of newly established *Fucus vesiculosus* clumps. In a factorial experiment the role of *Patella vulgata* and *Nucella lapillus* were examined simultaneously. *Nucella* was found to have an significant effect but less than that of *Patella*. The presence of *Nucella* did, however, mediate the effect of *Patella*. In addition *Nucella* was found to have a direct effect on the level of *Semibalanus balanoides* settlement in the field with the number of barnacles settling in cleared areas being reduced on areas which had been previously occupied by *Nucella*.

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CHAPTER 1

General Introduction

1.1 Biofouling and antifouling

A variety of marine organisms cause the loss of efficiency of immersed structures such as ships, pleasure boats, buoys and off-shore platforms by fouling their surfaces. Barnacles constitute the major problem (Southward & Crisp, 1963; Christie & Dalley, 1987) although the known number of fouling species totals over 2000 (Evans, 1970) including algae, molluscs (mainly mussels and oysters), tube worms, hydroids and sponges (Evans & Smith, 1975; Simmonds, 1986). These fouling organisms produce a roughness that increases the turbulent flow, acoustic noise and drag of a vessel (Champ, 1986) which in turn degrades its performance and increases its fuel consumption (Schatzberg, 1987). A mere 10 micron increase in the average hull roughness can result in a 0.3-1.0% increase in fuel consumption (Champ & Lowenstein, 1987). For large vessels fuel costs may amount to half of the total operational costs (Champ & Pugh, 1987). Figures quoted for the QE II, (Champ & Lowenstein, 1987) for example, show that the fuel bill for 1985-86 was in the region of $17 million, consequently a 1% increase in fuel consumption due to biofouling would amount to a $170,000 increase in costs. Other costs of fouling include the financing of dry docking, long periods out of service, the physical removal of the organisms and then the repainting (Evans & Smith, 1975; Karpel, 1988). There have been various estimates as to the total cost of biofouling to shipping world-wide, in 1987 this figure was calculated to be around two hundred million pounds per annum (Christie & Dalley, 1987).

Measures to counteract biofouling were reported from as early as 300 BC when lead sheets were used to cover the wooden hulled ships to inhibit the action of boring and fouling organisms. Later in the 17th and 18th centuries copper sheeting was used to safe-guard against the action of the ship worm, *Teredo* (Stebbing, 1985). The first antifouling paints were introduced at the turn of the century (Champ & Pugh, 1987) and represented a major change in the control of biofouling. The paint consists of a film-forming material and a pigment, either of which could contain a powerful biocide (Champ, 1986). Small amounts of the biocide are released at the paint surface to kill the settling stages of the fouling organisms.

Many biocides have been tried in antifouling paints. Salts of copper, mainly copper (II) oxide, were amongst the first to be used (Christie & Dalley, 1987). Later organo-mercury and stereoarsenicals were added to enhance the effectiveness of copper salts (Stebbing, 1985). In the 1970's their use was stopped because of their high toxicity and levels of environmental contamination (Champ & Pugh, 1987). At one time DDT was extensively used because of its marked specificity against barnacle settlement. General outcry against persistent chlorinated hydrocarbons later led to its use being banned (Christie & Dalley, 1987).

The most effective biocides available in recent years have been the organo-metallic compounds of arsenic, lead, mercury and tin (Christie & Dalley, 1987). The organotins were toxicologically and environmentally the most acceptable of these. They had previously been used as fungicides and as preservatives for wood, textiles and paper (Champ, 1986; Goldberg, 1986; Champ & Pugh, 1987). Early in the 1960's tributyltin (TBT) compounds were introduced to antifouling paints (Stebbing, 1985) although they were not used in significant quantities in Britain until ten years later. By 1987 between 250,000 and 300,000 lbs of TBT were being used annually in antifouling paints in the US (Champ & Pugh, 1987).

Antifouling paints can be classified into two groups depending upon their method of biocide release (Cardwell & Sheldon, 1986). In the first group the biocide is held in free association with the paint matrix and released exponentially with time (figure 1.1). These are principally based on copper compounds. Sea-water penetration of the paint matrix releases the biocide (figure 1.1), either by contact leaching or dissolving the matrix producing paints which are effective for around two years (Champ, 1986).

The second group of paints, self-polishing co-polymers, originated from the success of tributyltin as a biocide. Here the TBT is chemically attached to a polymer backbone in a bond which is hydrostatically unstable. The biocide is released by chemical hydrolysis of the TBT itself, in a reaction which occurs at a constantly renewed surface layer (figure 1.1) (Champ, 1986; Christie & Dalley, 1987). Apart from the high biocide release during the initial conditioning period there is a constant leaching of the biocide over time (figure 1.1). This coating remains effective for 5-7 years (Champ & Lowenstein, 1987), and because of the self-polishing surface the ships hull remains as smooth as when it was first painted.

Tributyltin based antifouling paints have undoubtedly proved to be amongst the most effective formulations developed so far. The effective life of the TBT self-polishing co-polymers is more than double that of other types of antifoulant representing a substantial financial saving. The US Navy, for example, estimated that the use of TBT antifouling paints would result in a 15% saving in fuel consumption, which would amount to over $150 million annually if the entire fleet was treated with these paints (Goldberg, 1986). Other practical advantages of these paints are in the nature of the constantly renewed surface. This produces a smooth surface to which new layers of co-polymer paints can be added without having to remove the previous co-polymer layers by sand blasting, thus reducing

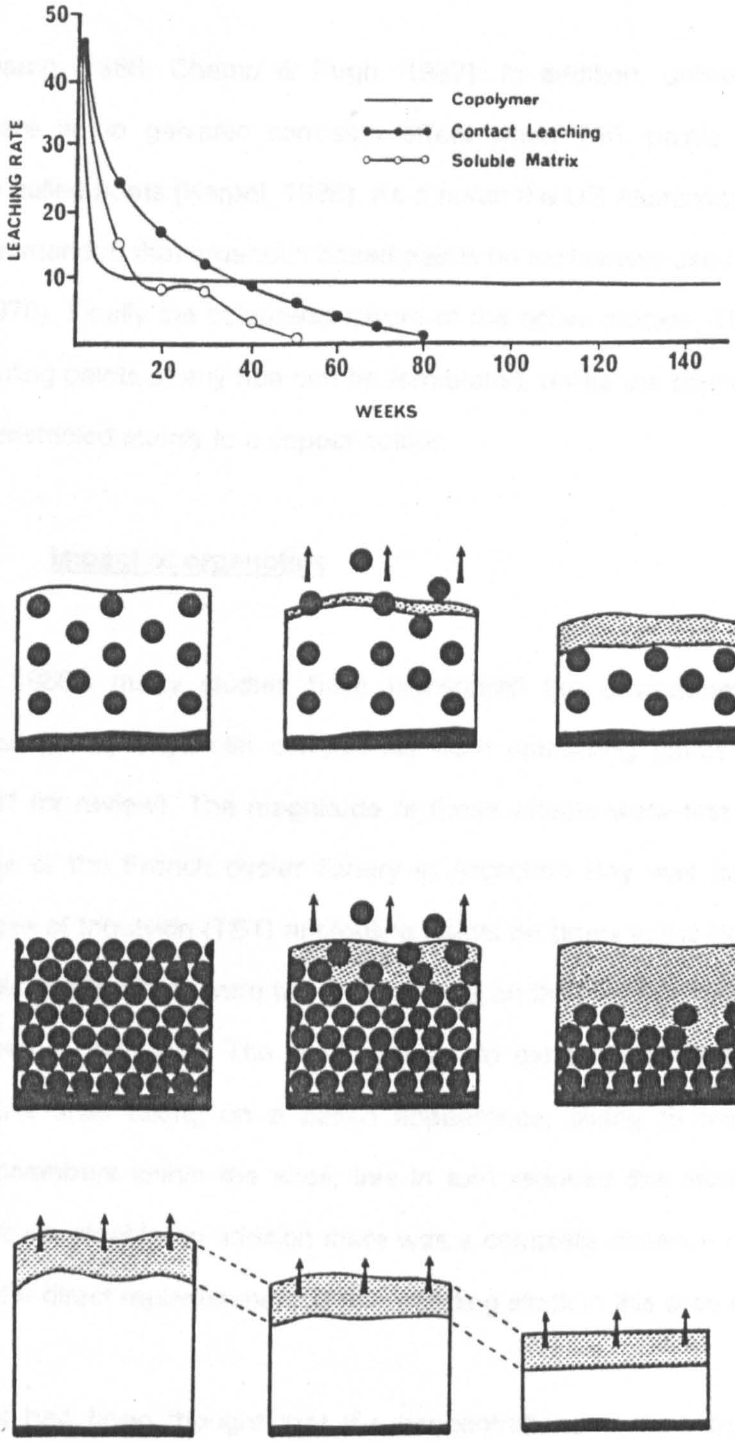


Figure 1.1 Leaching from antifouling paints. Relative leaching rates (A) given in arbitrary units and the diagrammatical representation of leaching from soluble matrix (B), contact leaching (C) and self polishing co-polymer (D) antifoulants (Champ, 1986; Christie & Dalley, 1987).

costs (Champ, 1986; Champ & Pugh, 1987). In addition, unlike copper based paints, there is no galvanic corrosion effect when TBT paints are applied to aluminium hulled boats (Karpel, 1988). As a result the US Aluminium Association in 1967 recommended that organotin based paints be exclusively used on these boats (Evans, 1970). Finally the colourless nature of the active biocide, TBT, also means that antifouling paints of any hue can be formulated, unlike the copper based paints which are restricted mainly to a copper colour.

1.2 Impact of organotins

Since the 1980's many studies have highlighted the environmental impact of pollution caused by organotin compounds from antifouling paints (see Bryan & Gibbs, 1991 for review). The magnitude of these effects were first realised when the collapse of the French oyster fishery in Arcachon Bay was attributed to the increased use of tributyltin (TBT) antifouling paints on boats in the harbours nearby (Alzieu *et al.*, 1986). There were two main effects on the Pacific oyster, *Crassostrea gigas*, farmed in this area. The adults began to exhibit anomalies in the calcic growth of the shell taking on a balled appearance, owing to the formation of numerous chambers within the shell; this in turn reduced the meat yield. These oysters were unsaleable. In addition there was a complete absence of any spatfall, preventing the direct replenishment of the breeding stock in this area (Alzieu, 1991).

Previously it had been thought that the concentrations of TBT from antifouling paints posed no direct threats to marine organisms in view of the large dilution factor and consequently the low concentrations at which it was present (Bellinger & Benham, 1978). When boats are present in a high enough density, however, especially in sheltered conditions, TBT levels may be high enough to kill commercial and non-commercial species plus affect the growth and reproduction of others.

In water, TBT concentrations have been reported ranging from <1 ng/l to >1000 ng/l (see Bryan & Gibbs, 1991 for review). These however are extremes with concentrations generally ranging between 10-100 ng/l in coastal waters in the UK (Cleary & Stebbing, 1985; Cleary & Stebbing, 1987a; Dowson *et al.*, 1992; Dowson *et al.*, 1993). Comparable concentrations have been reported in other countries including the United States (Hall, 1988), Netherlands (Ritsema *et al.*, 1991), France (Alzieu *et al.*, 1989) and the Mediterranean (Gabrielides *et al.*, 1990; Alzieu *et al.*, 1991). Concentrations may, however, vary with season (Hall, 1988), tidal cycle (Clavell *et al.*, 1986; Cleary, 1991) and depth (Cleary & Stebbing, 1987a; Cleary & Stebbing, 1987b). The restricted water exchange in harbours and marinas and the ionic nature of TBT means that high concentrations tend to remain localised (Goldberg, 1986). Enhancement of concentrations in the surface microlayer, up to 27 times greater than that of the subsurface waters (Cleary & Stebbing, 1987a; Cleary & Stebbing, 1987b) are due to the lipophilic nature of TBT and the concentrations of rich organics and lipids found in the top 280-300 µm layer of the water column (Cleary & Stebbing, 1987a). Even 200 km off-shore in the North Sea levels of TBT of up to 1.2 ng/l have been recorded in the microlayer (Coghlan, 1990). This poses a risk to the neuston, including many temporary members of this community such as eggs and larval stages of fish and invertebrates (Cleary & Stebbing, 1987b; Hardy *et al.*, 1987).

The partitioning and removal of TBT to particulates results in concentrations several orders of magnitude greater than those in the water column (Langston & Burt, 1991). In estuaries in south-west England levels in the sediment have been recorded in excess of 0.1 µg/g (Langston *et al.*, 1990; Langston & Burt, 1991). Although the impact of sediment bound tributyltin has received little attention, these high concentrations pose a risk to benthic organisms, especially deposit feeders such as *Scrobicularia plana* (Langston & Burt, 1991).

There is considerable variability amongst organisms with respect to their susceptibility to tributyltin. Gastropods and bivalves appear to be most sensitive (2-14 ng/l), followed by crustaceans (9-14 ng/l), algae (10-35 ng/l) and fish (>2 ng/l) (Rexrode, 1987). Table 1.1 summarises the toxicity data and effects of low levels of organotins in laboratory and field studies upon a variety of organisms (for other reviews see Cardwell & Sheldon, 1986; Champ, 1986; Rexrode, 1987; Bryan & Gibbs, 1991; Hawkins *et al.*, in press). Earlier studies tended to be based mainly upon the acute toxicity of TBT compounds with studies attaining measures of LC_{50} or LD_{50} at concentrations above those found in even the most contaminated waters (for example Bushong *et al.*, 1988). Later the more realistic long-term sub-lethal effects were investigated in the laboratory and in the field. Mammals have been the subject of only limited studies, although death can be induced in mice and rats after inhalation or ingestion of tributyltin (Schweinfurth & Günzel, 1987). The risk to humans is thought to be minimal (Laughlin & Lindén, 1987); it appears that TBT does not represent a mutagenic, carcinogenic or teratogenic hazard either through direct contact or via fish or shellfish (Schweinfurth & Günzel, 1987). Direct contact with eyes and skin is not recommended, however, as TBT can produce severe irritation of the skin and mucus membranes. It is ironic that of all the organisms so far investigated barnacle nauplii, the very organism targeted by antifouling paints, appear to be the most resistant (Goldberg, 1986).

1.3 Gastropods and imposex

Hermaphroditism is relatively common in the mollusca as a whole (Fretter & Graham, 1962) with around 40% of the 5600 mollusc genera being either simultaneous or sequential hermaphrodites (Heller, 1993). These changes are almost exclusively protandric, changing from male to female (Wright, 1988). There

Table 1.1 Summary of the effects of tributyltin (TBT) in water on various organisms arranged in ascending order of concentration. Concentrations of TBT expressed as ng/l.

		Organism	Effect	Reference
<1	Gastropod	*Nucella lapillus*	Imposex initiated	Gibbs *et al.*, 1988
2	Gastropod	*Ilyanassa obsoleta*	Imposex initiated	Bryan *et al.*, 1989a
2	Bivalve	*Crassostrea gigas*	Gel formation and chambering in the shells of adults (long term study)	Alzieu *et al.*, 1989
2-3	Gastropod	*Ocenebra erinacea*	Imposex initiated	Gibbs *et al.*, 1990
6	Bivalve	*Mytilus edulis*	No observed effect concentration for spat	Lapota *et al.*, 1993
4	Gastropod	*Nucella lapillus*	Sterility in a third of females	Gibbs *et al.*, 1988
<8	Bivalve	*Crassostrea gigas*	Upper limit of shell thickening for saleable oysters	Thain *et al.*, 1987
10	Gastropod	*Nucella lapillus*	All females sterile	Gibbs *et al.*, 1988
10	Bivalve	*Mercenaria mercenaria*	Growth inhibition in larvae	Laughlin *et al.*, 1988
10	Bivalve	*Crassostrea gigas*	Reduced ability for spat to compensate for hypoxia	Lawler & Aldrich, 1987
10	Crustacean	*Acartia tonsa*	Impaired egg production	Johansen & Møhlenburg, 1987
20	Bivalve	*Crassostrea gigas*	Reduced growth in spat	Lawler & Aldrich, 1987
20+	Gastropod	*Nucella lapillus*	Oogenesis in females supplanted by spermatogenesis	Gibbs *et al.*, 1988
20-200	Bivalve	*Crassostrea gigas*	Dose related shell thickening	Thain *et al.*, 1987
25	Bivalve	*Mytilus edulis*	Predicted no effect level for juveniles	Salazar & Salazar, 1991
30	Bivalve	*Saccostrea commercialis*	Shell curl and reduced growth	Batley *et al.*, 1989
50	Bivalve	*Mytilus edulis*	Reduced growth and 50% mortality for spat	Lapota *et al.*, 1993
50	Bivalve	*Mercenaria mercenaria*	Reduced larval growth	Laughlin & Lindén, 1987
50	Bivalve	*Crassostrea gigas*	Reduced oxygen consumption and feeding rate in spat	Lawler & Aldrich, 1987
64	Gastropod	*Nucella lima*	Imposex initiated after 2 month exposure	Stickle *et al.*, 1990
70	Bivalve	*Mytilus edulis*	Reduced juvenile growth	Salazar & Salazar, 1991
100	Bivalve	*Cerastoderma edule*	Death after 17 weeks	Beaumont, 1988
100	Echinoderm	*Ophioderma brevispina*	Inhibition of arm regeneration	Walsh *et al.*, 1986
100	Phytoplankton	*Skeletonema costatum*	Virtually no growth	Beaumont & Newman, 1986
100	Bivalve	*Mercenaria mercenaria*	Metamophosis of veligers to pediveligers inhibited	Laughlin *et al.*, 1988
100	Fish	*Morone saxatilis*	Increased Na+ and K+ ATPase in juveniles	Pinkney *et al.*, 1989
100-200	Bivalve	*Crassostrea gigas*	Severe shell balling	Thain *et al.*, 1987

150	Bivalve	*Crassostrea gigas*	Reduced growth in the spat and pronounced shell thickening in the upper valve	Waldock & Thain, 1983
<200	Gastropod	*Lepsiella scobina*	Penis formation induced	Smith & McVeagh, 1991
200	Bivalve	*Anodonta cygnea*	Shell thickening	Machado *et al.*, 1989
300	Crustacean	*Gammarus oceanicus*	Reduced larval survival over 8 weeks	Laughlin *et al.*, 1984
300	Crustacean	*Acartia tonsa*	Copepods moribund	U'Ren, 1983
350	Bivalve	*Pecten maximus*	Detrimental to growth and survival of juveniles	Paul & Davies, 1986 Davies *et al.*, 1986
500	Crustacean	*Uca pugilator*	Development of deformities during limb regeneration	Weis *et al.*, 1987
500	Crustacean	*Uca pugilator*	Acceleration of righting reflex in females, indicative of hyperactivity	Weis & Perlmutter, 1987
500	Crustacean	*Uca pugilator*	Decreased burrowing activity	Weis & Perlmutter, 1987
914	Gastropod	*Nucella lima*	Decreased feeding rate and significant mortality	Stickle *et al.*, 1990
1000	Bivalve	*Scrobicularia plana*	Death	Beaumont, 1988
1000	Phytoplankton	*Pavlova lutheri*	Reduced growth	Beaumont & Newman, 1986
1000	Phytoplankton	*Dunaliella tertiolecta*	Reduced growth	Beaumont & Newman, 1986

is, however, little or no evidence of hermaphroditism amongst the neogastropods (Smith, 1980; Heller, 1993).

In 1970 a number of female *Nucella lapillus* from populations around Plymouth in south-west England were reported to possess a penis like structure (Blaber, 1970). This phenomenon was later observed in *Nassarius obsoletus* (Say) (Smith, 1971) (= *Ilyanassa obsoleta*) and coined 'imposex' - the superimposition of male sexual characteristics on the female (Smith, 1971). Jenner (1979) suggested this phenomenon to be environmentally controlled and further work by Smith (1981a, 1981b) illustrated that imposex was in fact related to a water borne chemical from harbours and marinas. Screening of eight chemicals used on and around boats (Smith, 1981c) and laboratory based studies (Smith, 1981d) established that imposex was related to tributyltin used in antifouling paints.

Since then strong evidence has been provided by Bryan *et al.* (1986, 1988) of the link between the development of imposex in female *Nucella lapillus* and tributyltin. Populations surrounding areas of high boating activity have been shown to have the highest degree of imposex (Bryan *et al.*, 1986; Spence *et al.*, 1990a) with similar detrimental effects having been found near fish farming cages using TBT (Davies *et al.*, 1987; Bailey & Davies, 1989). High tissue burdens of other contaminants used in the shipping industry showed no clear correlations with imposex levels (Bryan *et al.*, 1986).

The degree of imposex is significantly correlated with tributyltin concentrations (Gibbs *et al.*, 1987) and is initiated at < 1 ng/l (0.5 ng Sn/l) (Gibbs *et al.*, 1988). The biochemical changes responsible for the development of imposex are thought to be a consequence of an increase in the steroid hormone testosterone in the female. Gibbs *et al.* (1991a) has suggested that the testosterone titre in the individual is increased on exposure to TBT because the cytochrome p-450 dependent aromatase responsible for the conversion of testosterone to oestraidiol 17ß is inhibited. In the absence of TBT the direct injection of testosterone into female dogwhelks causes an increased expression of imposex (Spooner *et al.*, 1991).

The extent of the development of TBT induced imposex in *Nucella lapillus* can be measured by two indices: relative penis size (RPS) and vas deferens sequence (VDS) between which there is a significant relationship (Gibbs *et al.*, 1987). In order to measure these indices the whelk is first dissected out of its shell and sexed. Females are identified according to the presence of a red/brown sperm ingesting gland found posterior the capsule gland. This is an obvious structure found in all females to which there is no similar feature in males (Fretter & Graham, 1962). The relative penis size index compares the bulk of the female penis to that of the male within the same population (Bryan *et al.*, 1986; Gibbs *et al.*, 1987) and the vas deferens sequence index categorises the development of the penis and vas deferens in the female in six stages (Gibbs *et al.*, 1987). The initial appearance and growth of the penis and vas deferens can be categorised in ranked stages from 1-4. The continual development of the vas deferens subsequently leads to the occlusion of the genital papilla, stage 5, which in turn prevents the release of the egg capsules which build up in the capsule gland, stage 6. These two later stages render the female effectively sterile. Eventually the build up of aborted capsules ruptures the capsule gland in the female leading to a premature death (Gibbs & Bryan, 1986). In highly contaminated areas juveniles may reach stage 5 before reaching sexual maturity, thus never being able to breed (Gibbs & Bryan, 1987). In these cases the population becomes characterised by having few or absent juveniles and ultimately being dominated by adult males, resulting in its complete demise (Bryan *et al.*, 1986; Gibbs & Bryan, 1986; Spence *et al.*, 1990a; Gibbs *et al.*, 1991b). The survival of the population relies on the rafting in of females capable of breeding (Gibbs *et al.,* 1988) since the adults are relatively immobile and *Nucella* does not have a planktonic dispersal phase in its life cycle (Fretter & Graham, 1962).

Imposex has been shown to occur in populations of *Nucella* throughout the British Isles (Bryan *et al.,* 1986; Bailey & Davies, 1989; Spence *et al.*, 1990a), Europe (Fioroni *et al.*, 1991a; Gibbs *et al.*, 1991c; Ritsema *et al.,* 1991; Stroben *et al.*, 1992a; Oehlmann *et al.*, 1993) and North America (Miller & Pondick, 1984). During the 1980's very few populations were found where females did not exhibit some signs of the development of male sexual characteristics, the only examples being from remote sites in Scotland (Spence *et al.,* 1990a). It has been argued that because of the widespread nature of imposex in *Nucella lapillus* that this observation is indicative of the natural occurrence of this phenomena. Evidence against this hypothesis, however, includes the fact that imposex was not recorded before the late 1960's and that preserved material shows that several populations now exhibiting high imposex values were free from any signs of imposex in the past (Bryan *et al.,* 1986; Bailey & Davies, 1988). It would however be surprising if it was only TBT that could induce this phenomena, but the similarities between data on imposex throughout the geographical range of *Nucella* (Gibbs *et al.*, 1991c) and the strength of laboratory and field evidence suggests that for all practical purposes TBT is the only cause (Bryan & Gibbs, 1991).

The occurrence of imposex is not confined to *Ilyanassa obsoleta* and *Nucella lapillus*. To date imposex has been reported in more than 70 species of neogastropod world-wide (see table 1.2). It is only in a few of these species that the link between imposex development and tributyltin has been identified following rigorous laboratory and field studies (table 1.2). The exact expression of imposex in the development of the penis and vas deferens may differ slightly between species.

Although the use of the vas deferens sequence (VDS) and relative penis size (RPS) indexes were developed for *Nucella lapillus* (Gibbs *et al.,* 1987) they have been adapted by other workers to suit other affected neogastropods (for example see Ellis & Pattisina, 1990; Fioroni *et al.*, 1991b; Stewart *et al.*, 1992; Stroben *et al.*, 1992b; Stroben *et al.*, 1992c). As a consequence neogastropods now provide useful bioindicators to world-wide levels of TBT pollution (Ellis, 1991).

Although *Nucella lapillus* becomes effectively sterile when exposed to relatively low concentrations of TBT (Gibbs & Bryan, 1986) this is not the case in all neogastropods (table 1.2). Effective sterilisation also appears to occur in *Thais haemastoma* (Spence *et al.*, 1990b), *Nucella lamellosa* (Bright & Ellis, 1990), *Nucella lima* (Short *et al.*, 1989) *Ocenebrina aciculata* (Stroben *et al.,* 1992c), *Lepsiella vinosa* (Nias *et al.*, 1993), *Thais orbita*, *Haustrum haustorium* and *Lepsiella scobina* (Stewart *et al.,* 1992) (see table 1.2). Population level effects like those observed for *Nucella lapillus* will be masked by those species which have planktonic larval phases. These populations have a greater chance of survival: *T. haemastoma* for example, has a larval dispersal stage in its life cycle (Spence *et al.*, 1990b) enabling re-colonisation of heavily contaminated sites.

The sensitivity of the response to tributyltin, does however, appear to vary between species with *Nucella lapillus* being one of the most sensitive recorded to date (see table 1.2). For example, imposex is initiated at tributyltin concentrations of <1 ng/l in *Nucella lapillus* (Gibbs *et al.*, 1988), 2 ng/l in *Ilyanassa obsoleta* (Bryan *et al.*, 1989a) and between 2-3 ng/l in *Ocenebra erinacea* (Gibbs *et al.*, 1990).

Table 1.2 The occurrence of imposex in neogastropods world-wide and the concentrations at which effects occurred, where known. The evidence used to establish TBT as the cause of imposex development is also given and is expressed as a code where: *, laboratory studies; 0, field transplants; □, measurements of TBT in water or tissues in the natural environment; †, proximity of boating activity; ‡, examination of historical specimens; §, field exposure to TBT (painted on shells); #, work done before the effects of TBT had been extensively studied; x, no details given. Systematic arrangement according to Poppe & Goto (1991).

Superfamily	Family	Species	Location	Level of effect	Effect on reproduction	Codes	Author
Muricacea	Muricidae	*Calotronphon ostrearum*				#	Jenner, 1979
		Cronia margariticola	South-east Asia	Single specimen, not affected	None observed	†	Ellis & Pattisina, 1990
		Drupella rugosa	South-east Asia	None given	None observed	†	Ellis & Pattisina, 1990
		Eupleura caudata				#	Jenner, 1979
		Eupleura caudata etterae				#	Jenner, 1979
		Eupleura sulcidentata				#	Jenner, 1979
		Haustrum haustorium	New Zealand	None given	Sterility observed	†	Stewart *et al.*, 1992
		Lepsiella scobina	New Zealand	<0.2µg/l TBTO induced penis formation in juveniles	No egg capsules or juveniles at sites of high contamination	† 0 * ‡	Smith & McVeagh, 1991
					Sterility observed	† □	Stewart *et al.*, 1992
		Lepsiella albomarginata	New Zealand	<0.2µg/l TBTO induced penis formation in individuals	No egg capsules or juveniles at sites of high contamination	† 0 *	Smith & McVeagh, 1991
		Lepsiella vinosa	South Australia	1ng/l TBTCL induced penis formation in individuals	Vaginal pore observed blocked, aborted egg capsules in genital duct	† □ *	Nias *et al.*, 1993
		Morula musiva	South-east Asia	None given	None observed	†	Ellis & Pattisina, 1990
		Morula marginalba	Eastern Australia	None given	None observed	†	Wilson *et al.*, 1993
		Murex brandaris				x	Fioroni *et al.*, 1991b
		Murex florifer dilectus				#	Jenner, 1979
		Murex pomum				#	Jenner, 1979
		Murex tribulis	Egypt			†	Pers. comm. A. R. Brand, Port Erin Marine Laboratory
		Murex trunculus				x	Fioroni *et al.*, 1991b
		Naquetia capucina	South-east Asia	None given	None observed	†	Ellis & Pattisina, 1990
		Ocenebra erinacea	South-west England & Brittany	Imposex is initiated at 1ngSn/l	Advanced imposex inhibits breeding activity, no larval stage	† □	Gibbs *et al.*, 1990
		Ocenebra lurida	British Columbia, Canada	None given	No apparent potential for sterility	†	Bright & Ellis, 1990
		Ocenebrina aciculata			Sterilisation observed	x	Stroben *et al.*, 1992c
		Taron dubius	New Zealand	None given	None observed	†	Stewart *et al.*, 1992
		Xymene ambiguus	New Zealand	None given	None observed	†	Stewart *et al.*, 1992
	Thaididae	*Nucella canaliculata*	British Columbia, Canada	None given	None observed	† □	Bright & Ellis, 1990

		Nucella emarginata	British Columbia, Canada	None given	None observed	† ‡ □	Bright & Ellis, 1990
		Nucella lamellosa	British Columbia, Canada	None given	Sterile females observed	† ‡ □	Bright & Ellis, 1990
		Nucella lapillus	UK	Imposex initiated at <0.5ngSn/l		† * O □	Gibbs *et al.*, 1988
					Sterility initiated at 1-2ngSn/l at 6-8ngSn/l all females sterile, no larval stage	† * □ §	Gibbs & Bryan, 1987
		Nucella lima	Auke Bay, Alaska	Within a factor of 2 as sensitive as *Nucella lapillus*	Juveniles and egg capsules scarce at contaminated sites	† □ §	Short *et al.*, 1989
			Admiralty Island, Alaska	Imposex initiated at <0.064µg TBT/l after 2 months	Genital papillae observed blocked, effective sterilisation can occur	*	Stickle *et al.*, 1990
		Thais bronni	Japan	Imposex initiated at 10-20ng TBT/g wet weight of tissue	Sterility observed	† □	Horiguchi *et al.*, 1994
		Thais clavigera	Japan	Imposex initiated at 10-20ng TBT/g wet weight of tissue	Sterility observed	† □	Horiguchi *et al.*, 1994
		Thais haemastoma	Azores, Canaries & Spain	None given	Becomes effectively sterile, planktonic larval stage masks any decline in fecundity	†	Spence *et al.*, 1990b
		Thais haemastoma canaliculata				#	Jenner, 1979
		Thais haemastoma floridana				#	Jenner, 1979
		Thais luteostoma (may be an intergrade with *Thais clavigera*)	South-east Asia	None given	None observed	†	Ellis & Pattisina, 1990
		Thais orbita	New Zealand	None given	Sterility observed	†	Stewart *et al.*, 1992
		Urosalpinx cinerea	Essex, UK	Sensitivity may be similar to *Nucella lapillus*	Effectivity sterile	† □	Gibbs *et al.*, 1991d
		Urosalpinx caudata etterae	Maryland, Virginia			#	Griffith & Castagna, 1962
		Urosalpinx cinerea follyensis	Maryland, Virginia			#	Griffith & Castagna, 1962
		Urosalpinx perrugata				#	Jenner, 1979
		Urosalpinx tampaensis				#	Jenner, 1979
Buccinacea	Buccinidae	*Buccinum undatum*	White sea, USSR	None given	None observed	‡	Kantor, 1984
			Roscoff, France	None given	None observed	† ‡	Fioroni *et al.*, 1991b
		Cominella glandiformis	New Zealand	No imposex found in juveniles at 0.6µg/l TBTO	No effects observed	† *	Smith & McVeagh, 1991
		Cominella virgata	New Zealand	None given	None observed	†	Stewart *et al.*, 1992
		Colus halli	British Columbia, Canada	None given	No apparent potential for sterility	†	Bright & Ellis, 1990
		Colus gracilis					
		Neptunea phoenecia	British Columbia, Canada	None given	None observed but morphologically very similar to *N. lapillus*	†	Bright & Ellis, 1990
		Pisania tinctus				#	Jenner, 1979

		Searlesia dira	British Columbia, Canada	None given	No apparent potential for sterility	†	Bright & Ellis, 1990
	Columbellidae	*Amphissa columbiana*	British Columbia, Canada	No imposex found		†	Bright & Ellis, 1990
		Anachis avara				#	Jenner, 1979
		Mitrella lunata				#	Jenner, 1979
	Nassariidae	*Nassarius (Hinia) reticulatus*	Brittany, Normandy	Less sensitive than *N. lapillus*	No sterilisation observed	† □	Stroben *et al.*, 1992a, c
				Imposex initiated at 2-3ng/l	None observed, larval stage in life cycle	x	Bryan & Gibbs, 1991
		Nassarius (Hinia) incrassata	Roscoff, France	None given	None observed	† □	Fioroni *et al.*, 1991b
		Ilyanassa obsoleta	Connecticut	None given		† O*	Smith, 1981b
		(Nassarius obsolentus)	Chesapeake Bay North Carolina	Imposex initiated at around 2ng/l	No evidence of sterility although the number of females in the sample declined with increased imposex, planktonic larvae	† □	Bryan *et al.*, 1989a
		Nassarius vibex				#	Jenner, 1979
		Nassarius trivittatus				#	Jenner, 1979
		Nassarius vibex				#	Jenner, 1979
	Fasciolariidae	*Fasciolaria lilium hunteria*				#	Jenner, 1979
		Fusinus australis	South Australia	None given	None observed	† □	Foale, 1993
		Pleuroploca gigantea				#	Jenner, 1979
	Galeodidae	*Busycon carica*				#	Jenner, 1979
		Busycon contrarium				#	Jenner, 1979
		Melongena corona				#	Jenner, 1979
Volutacea	Olividae	*Olivella biplicata*				#	Jenner, 1979
	Olividae	*Amalda (Baryspira) australis*	New Zealand	None given	None observed	†	Stewart *et al.*, 1992
	Marginellidae	*Marginella apicina*				#	Jenner, 1979
Conacea	Terebridae	*Terebra dislocata*				#	Jenner, 1979
		Terebra protexta				#	Jenner, 1979
	Turridae	*Lora turricula*	UK			#	Smith, 1967
	Condiae	*Conus anemone*	Western Australia	None given	Reproduction normal	†	Kohn & Almasi, 1993
		Conus coronatus	Western Australia	None given	Reproduction normal	†	Kohn & Almasi, 1993
		Conus dorreensis	Western Australia	None given	Reproduction normal	‡ †	Kohn & Almasi, 1993
		Conus klemae	Western Australia	None given	Reproduction normal	†	Kohn & Almasi, 1993
		Conus lischkeanus	Western Australia	None given	Reproduction normal	‡	Kohn & Almasi, 1993
		Conus lividus	Western Australia	No effect seen	Reproduction normal	‡ †	Kohn & Almasi, 1993
		Conus mediterraneus				#	Jenner, 1979
		Conus sponsalis	Western Australia	None given	Reproduction normal	†	Kohn & Almasi, 1993
		Kurtziella cerina				#	Jenner, 1979

1.4 Legislation on tributyltin antifouling paints

The French were the first to introduce restrictive legislation on the use of triorganotins in antifouling paints, banning their use on boats less than 25 m in length in January 1982 (Alzieu *et al.,* 1986; Alzieu, 1991). Their prompt action followed the evidence that the collapse of the oyster fishery in Arcachon Bay was due to the increased use of TBT paints on boats in the harbours nearby.

The UK Government was slower to react. Action was first prompted in July 1985 with suggestions that the tin content for antifouling paints used on boats of 12 m or less should be limited to 0.4 gm/l (Anon, 1985a; Abel *et al.*, 1987). The Paint Makers Association campaigned against any further regulatory action (Anon, 1985b) and at this stage a ban was not imposed. In order to give the paint industry more time to develop new paints based on different biocides, only voluntary regulations were proposed by the Government in July 1985 (Abel *et al.*, 1987).

The United Kingdom Control of Pollution (Antifouling Paints) Regulations 1985 came into operation on 13 January 1986 under sections 100 and 104 (1) of the Control of Pollution Act 1974. Concentrations of tin in dried co-polymer and non co-polymer paints were limited to 7.5% and 2.5% respectively as maximum quantities by weight (Abel *et al.*, 1986; Side, 1986). It was not until 1 July 1987 that legislation came into force announcing a ban on the sale and supply of TBT antifouling paints and its application to pleasure craft, less than 25 m in length, and the nets and cages used in fish farming (Abel *et al.,* 1987; Duff, 1987). This was too late to be effective for the 1987 season, thus allowing the use of TBT paints for an additional year (Spence, 1989).

Similar bans have now been introduced all over the world (table 1.3). In some cases they are more stringent. The legislation introduced on the Isle of Man, for example, although not passed until 1988 introduced a licensing procedure which was applied to the use of organotin paints on boats and structures of all sizes (Marine Administration, 1988; Orme, 1990). Effectively this put a complete stop to any use of organotin paints since it was unlikely that any licences would be granted (pers. comm. D. Ramsbottom, Marine Administration, Isle of Man).

Tidal flushing of contaminated areas may help disperse and dilute the tributyltin (Waldock, 1986). Environmental concentrations will also be reduced as TBT is removed through absorption to lipids and particulate matter (Langston *et al.*, 1987; Unger *et al.*, 1988) and through assimilation and metabolism by plants and animals (Lee, 1985; Cardwell & Sheldon, 1986; Lee, 1986). Natural degradation does occur, breaking down tributyltin to the less toxic forms of dibutyl, monobutyl and inorganic tin (Blunden & Champman, 1982; Maguire *et al.*, 1983). Estimated half lives for TBT in water range from 6 to 60 days depending on the conditions (Thain *et al.*, 1987). The process may be accelerated, for example, by exposure to sunlight (Maguire *et al.*, 1983) or by increased temperatures (Thain *et al.*, 1987). Degradation of TBT to DBT and MBT in sediments appears to be far slower with half lives expressed in years rather than days, especially in anaerobic conditions (Bryan & Gibbs, 1991).

Despite these restrictions there are still new inputs of TBT into the environment due to a number of factors. In the UK ships over 25 m in length are still permitted to use TBT antifouling paints (Abel *et al.*, 1987; Duff, 1987) as are aluminium hulled boats which have so far been exempt from the TBT bans regardless of their size as the alternative copper based paints cause erosion (Karpel, 1988). These exceptions may be revoked as the UK is set to tighten controls on organotin antifouling paints soon (Anon, 1993a). The illegal use of TBT paints appears to be wide-spread

Table 1.3 A summary of the legislation concerning the use of organotin antifouling paints world-wide, with the dates when the legislation became effective, where known.

Date			Place	Legislation	Reference
19	January	1982	France	The use of paints containing over 3% organotin prohibited on boats with a gross registered tonnage under 25 tons	Alzieu, 1991
14	September	1982	France	Ban on the use of organotin paints on boats less than 25m, except those with aluminium hulls	Alzieu, 1991
1	January	1985	North Carolina, US	'Regulatory action against TBT'	Anon, 1985c
	July	1985	Britain	Voluntary suggestions that tin content for paints <12m should be limited to a maximum of 0.4 gm/l	Anon, 1985b; Abel *et al.*, 1987
13	January	1986	Britain	Concentrations of tin in dried co-polymer and non co-polymer paints were limited to 7.5% and 2.5% respectively as maximum quantities by weight	Abel *et al.*, 1986; Side, 1987
8	April	1987	Eire	Restrictions on selling, supplying and application of TBT antifouling paints for boats <25m and nets and cages in fish farming	Spence & Hawkins, 1988
1	July	1987	Britain	Restrictions on selling, supplying and application of TBT antifouling paints for boats <25m and nets and cages in fish farming	Duff, 1987; Abel, *et al.*, 1987
	December	1987	Alaska	'Use of TBT paints restricted'	Short *et al.*, 1989
1	April	1988	Isle of Man	Licence required for any use of TBT antifouling paints. No licences have been applied for, or are likely to be issued	Marine Administration, 1988; Orme, 1990
	June	1988	United States	Use of organotin formulations prohibited on vessels less than 65ft in length and only those with a release rate <4μg/cm²/day are permitted to be sold for use on larger boats. Law passed at Federal level	Karpel, 1988
		1988	Sweden	Use of TBT on boats <25m prohibited	Karpel, 1988
	March	1989	New South Wales	Use of TBT on boats <25m prohibited. Those boats >25m allowed to use TBT paints with a release rate <5μg TBT/cm²/day	Wilson *et al.*, 1993
	June	1989	Victoria, Australia	The use of TBT (or other organotins) prohibited on boats <25m	Foale, 1993
	Late	1989	New Zealand	Partial ban	Smith & McVeagh, 1991
1	July	1989	New Zealand	Prohibition of use of all high release and non copolymer organotin paints but permit the use of low release copolymer paints on vessels >25m and/or with Al hull	Stewart *et al.*, 1992
		1989	Norway	Use of TBT on boats <25m prohibited	Karpel, 1988
	December	1990	Japan	Use of TBT paints prohibited on any new hulls	Ambrose, 1994
		1990	Japan	Manufacture, import and use of TBTO completely prohibited	Horiguchi *et al.*, 1994
		1990	Netherlands	Ban on the use of paints containing organotin on boats <25m	Ritsema *et al.*, 1991

1	July	1991	Western Australia	Ban on the use of paints containing organotin on boats <25m, with a 6 months grace period given	Kohn & Almasi, 1993
1	July	1991	Mediterranean	Use of organotin compounds not permitted on boats <25m or on structures used in mariculture. Applies to contracting parties to the convention for the protection of the Mediterranean sea against pollution	Alzieu *et al.*, 1991
	April	1992	Japan	Use of TBT paints prohibited on all maintenance	Ambrose, 1994
	-		Washington	'Use of TBT paints restricted'	Short *et al.*, 1989
	-		Oregon	'Use of TBT paints restricted'	Short *et al.*, 1989
	-		California	'Use of TBT paints restricted'	Short *et al.*, 1989
	-		Virginia, Maryland, Hawaii, Wisconsin, Michigan	Laws passed at the State level similar to the United States Federal Bill although in some cases more restrictive	Karpel, 1988
	-		Australia	Use of TBT on boats <25m prohibited, only two paints with release rates <5μg/cm²/day could be sold to be used on larger boats.	Anon, 1990
	-		Japan	Voluntary ban on organotin paints used for nets in fish farming	Karpel, 1988
	-		Denmark	Use of TBT restricted to professional boat yards	Karpel, 1988
	-		Tasmania	Use of all TBT containing products prohibited	Karpel, 1988
	-		West Germany	Use of all TBT paints prohibited in the freshwater environment	Karpel, 1988
	-		Switzerland	Use of all TBT paints prohibited in the freshwater environment	Karpel, 1988

Karpel (1988) reports that at the time of his paper going to press there were no reports of restrictions on the use of triorganotins in Eastern Europe, USSR, China, Hong Kong, South Korea, Singapore or Australia, although more recent papers have now reported on restrictions in Australia (Khon and Almasi, 1993; Anon, 1990).

despite efforts to reduce them (Ambrose, 1994), with boat owners continuing to use them because of what they see as a lack of viable alternatives. Concern is also growing about industrial sources of tributyltin and their threat to the environment although curbs now seem imminent following action by France and Germany (Anon, 1987).

It appears from the evidence from France that the introduction of legislation has been effective in reducing environmental levels of TBT and as a consequence reducing the levels of effects seen in sensitive organisms. In Arcachon Bay water concentrations of TBT decreased by 50% in the first year after restrictions were introduced. By November 1986, some three years later, concentrations had been reduced 5-10 times. Recovery was also seen in the oyster *Crassostrea gigas* with a decrease in the percentage of shell malformations and the return to normal spatfall (Alzieu *et al.,* 1986; Alzieu, 1991). Recent evidence from the UK also suggests that there have been reductions in the level of TBT in the water following the introduction of restrictions (Waite *et al.*, 1991; Bryan *et al.*, 1993a; Dowson *et al.*, 1993). In addition there is also some limited evidence of a recovery of some dogwhelk populations in Northumbria (Evans *et al.*, 1991) and on the Isle of Cumbrae (Evans *et al.*, 1994).

With the restrictions on TBT paints there has generally been a return to the traditional copper based formulas. This has happened in Arcachon Bay, for example, and has in turn resulted in a considerable increase in the copper concentrations found in the tissues of oysters close to harbours (Claisse & Alizeu, 1993). In comparison to TBT from antifouling paints, copper is much less toxic to oysters (His & Robert, 1987). These copper based paints are not as long-lasting as the self-polishing co-polymers containing TBT and as a consequence there has been renewed enthusiasm in the development of other alternatives to counteract biofouling. A Japanese company, for example, has developed a new system called MAGPET (marine growth prevention by electrolysis technology) which passes an electrical current through a conductive coating which results in electrolytic action through the surrounding seawater. This produces acid ions around the ships hull preventing the settlement of marine organisms (Anon, 1993b). Other suggestions include the use of low energy surfaces (Callow *et al.*, 1986; Brady, 1994; Schmidt *et al.*, 1994), microbially produced antibiotics (O'Carroll, 1988) and biological control using the limpet *Patella caerulea* (Safriel & Erez, 1987; Anon, 1993c). Paints to rival those containing TBT are now being introduced by manufacturers as viable alternatives to organotin antifouling paints. It appears that BP is testing 'SeaGuardian' a paint which can remain effective for up to three years and that International Paints are developing the first self-polishing co-polymer without TBT. Although what the active biocides in these paints are is currently undisclosed (Anon, 1991). Hempel Marine Paints too have developed a TBT free self-polishing co-polymer which is currently being tested. This is expected to provide the five year protection offered by TBT paints. The active biocides in this paint are described as a mixture of cuprous oxide and organic biocides although again the nature of which organic biocide was not mentioned (Ambrose, 1994).

1.5 Ecology of *Nucella lapillus*

Nucella lapillus, the common dogwhelk, is a carnivore found intertidally mainly at the mid tide level (MTL) and below (Colman, 1933; Lewis, 1964) although vertical migrations up and down the shore do occur with season and age (Feare, 1970a; Coombs, 1973). It is not abundant on shores with high wave exposure and few crevices or at very sheltered sites nor are they tolerant of low salinity conditions (Moore, 1936).

The sexes are separate in *Nucella* and fertilisation is internal (Fretter & Graham, 1962; Fretter & Graham, 1984). The proportion of females within the population increases with increasing age and shell length (Moore, 1938a; Feare, 1970b). In north-east England the main breeding season occurs in spring and early summer (Feare, 1970b) where the egg capsules are laid mainly in April and May (Feare, 1970a). Moore (1936), however, reported seeing capsules all year around at Plymouth in south-west Britain. These capsules are vase-shaped containers which are attached in clusters to the rock surface (Moore, 1938b; Fretter & Graham, 1962; Fretter & Graham, 1984). When first laid they are straw coloured which fades with age. Inside each capsule there may be up to 1000 yolky eggs (Fretter & Graham, 1984). These hatch during September and October, in north-east England, with each capsule releasing an average of 22 protoconchs (Feare, 1970a). Development of the encapsulated embryos is direct, the juveniles emerging as miniature adults; there is no larval stage. If all the nurse cells within the capsule are used up before hatching cannibalism may occur (Largen, 1967). When hatched, individuals with a shell height of about 1 mm shelter in the empty husks of dead barnacles at the same height on the shore as where the egg capsules were laid (Feare, 1970a). Moore (1936), however, suggested that they are washed down onto the lower shore to feed on *Spirorbis borealis*. The main growing season is from

June to November but intermittent feeding by juveniles over the winter is reflected in slow growth over this period (Feare, 1970a). The shell height attained after one winter is related to temperature, as their feeding first reduces and then stops as the temperature decreases (Largen, 1967). Variations in shell height within each age group may also be explained by differences in the habitat as those individuals living in pools grow faster than those on the open shore (Feare, 1970a).

The shells of immature individuals grow rapidly from increments added to the inner lip which remains thin and sharp. When growth ceases in the third year, as the dogwhelk reaches maturity, the inner lip of the shell becomes thicker reaching a width of around 5 mm. Then a series of teeth develop (Moore, 1936). This is thought to strengthen the shell and narrow the opening, protecting against predator attack particularly on sheltered shores (Crothers, 1971; Crothers, 1975a). Additional rows of teeth may be added to the shell lip if growth continues after maturity (Feare, 1970a) or after periods of starvation (Bryan, 1969). Generally the characteristics of the shell edge and length can be used to classify individuals into first years (juveniles), second years (immatures) and adults (Feare, 1970a).

At all times of the year *Nucella lapillus* can be seen on the shore in aggregations. During the summer, between May and October dogwhelks were observed on the Yorkshire coast in aggregations on the open rock. These groups consisted of 20-500 individuals of all age classes which were feeding. The aggregation was thought to protect individuals not just from predatory attack but against dislodgement due to wave action (Feare, 1970a; Feare, 1971a). Winter and pre-breeding aggregations were also observed, with individuals sheltering in clefts and pools on the shore (Feare, 1970a). All age classes are found in these winter aggregations, although the adults arrive first and disperse last (Feare, 1971a). The aggregations prevent individuals from loosing their foothold on the shore which is reduced at cold temperatures. Although adult *Nucella* within these aggregations do not feed, immatures may leave the aggregations for a brief period during periods of calmer weather to do so. The pre-breeding aggregations observed are a continuation of the winter aggregations with the indication of change occurring when the juveniles move away to commence feeding in the spring. These aggregations bring the sexes together for spawning at sites which provide the optimum conditions for the hatching of the egg capsules (Feare, 1971a). Feare (1971a) observed that the same breeding sites were used each year, but since few individuals returned to the same locality in consecutive years the habitual usage of the sites was proposed to be due to suitability rather than any homing behaviour in *Nucella*.

Suppression of a pelagic larva from the life cycle has considerably limited the dogwhelks power of dispersal, which instead relies on the crawling abilities of the adults or the rafting of juveniles on floating debris. They do not cross natural barriers such as sand, mud or deep water voluntarily (Crothers, 1985). Consequently if there is plentiful food and shelter they tend to remain for long periods in the same general area, moving an average distance of 0.16 m between consecutive low tides when feeding (Hughes & Drewett, 1985). Crothers (1985), for example, recovered marked *Nucella* within 30 cm of the release site after 1 year.

1.5.1 Parasite infection in *Nucella lapillus*

Nucella lapillus are commonly found infested with *Cercaria purpurae* Lebour the larval stage of the trematode parasite *Parorchis acanthus* Nicoll (Rees, 1940). *Nucella* acts as the intermediate host with the mature worms being found in the Herring Gull, *Larus argentatus* (Rees, 1940). The eggs hatch in the rectum of the gull and the liberated miracidia penetrate the tissues of the intermediate host, *Nucella*, to proceed to the next stage of their development. Initially the rediae are found near the surface of the digestive gland (Rees, 1940) but eventually they permeate the whole of the digestive gland and gonad (Rees, 1966). The cercariae emerging from the rediae reach the exterior by passing between the body and the shell and out through the shell aperture (Rees, 1966). Light, salinity and temperature are all important factors in controlling the emergence of the larvae from *Nucella* (Rees, 1948). In the affected molluscs the digestive gland is destroyed to varying degrees with most of the damage caused during the early stages of infection (Rees, 1971). In addition the parasite infection also reduces the size of the male penis and castrates the molluscan host (Lauckner, 1980).

Other effects are on the growth and behaviour of *Nucella* infected by *Parorchis acanthus*. Firstly those dogwhelks which are infected are larger in size, indicating that they either continued to grow after maturity or that they grow faster than unaffected individuals (Feare, 1970a; Crothers, 1985). These individuals are also characterised by having multiple rows of teeth on the inside lip of their shells. Feare (1970a) reported that in infected individuals from Robin Hood Bay, North Yorkshire, 56% had at least three rows of teeth and 13% had four. In addition affected whelks could also frequently be identified by their deformed shells sometimes with an extra fourth whorl (Feare, 1970a). *Parorchis acanthus* may also be responsible for the behavioural abnormalities in *Nucella* with individuals not entering the safety of aggregations leaving them more susceptible to attack from avian predators (Feare, 1970a; Crothers, 1985). Oystercatchers, for example, only attack those dogwhelks occurring singly on the shore. For example, in September 1966 when 67% of dogwhelks had already aggregated only 1% were infected by the fluke but of those attacked by oystercatcher 13% contained the parasite (Feare, 1971b).

Lauckner (1980) suggested that the deviations from the 1:1 sex ratio found by Feare (1970a) could possibly be attributed to infection by the parasite. At the time

Feare (1970a) observed fewer males than expected and suggested that differential mortality caused this deviation in the sex ratio.

1.6 Food and feeding of *Nucella lapillus*

Nucella lapillus has been reported to feed on a wide diversity of species (see Largen, 1967; Crothers, 1985) (table 1.4) although barnacles and mussels constitute the majority of its diet on moderately exposed shores. On sheltered shores where barnacles and mussels are sparse, *Nucella* feeds on *Littorina obtusata* and small *Patella vulgata*.

Nucella usually attacks its prey by drilling through the shell, using a combination of mechanical and chemical activity (Benton, 1986). The initial action is made as the accessory boring organ in the sole of the foot secretes an enzymatic mixture softening the organic matrix of the shell. This is followed by the rasping action of the radula (Chétail & Fournié, 1969; Carriker & Williams, 1978). Alternation between the two actions forms a round hole at a rate of 0.36 mm/day (Hughes & Dunkin, 1984a), which reflects the shape of the accessory boring organ. The proboscis is inserted through the hole into the prey injecting a powerful narcotic from the hyperbranchial gland (Carriker, 1981). Following this digestive enzymes are secreted and the rich 'soup' that is formed is sucked up into the gut (Crothers, 1985).

Observations have been made of barnacles having been eaten without drilling (Moore, 1938b; Largen, 1967). In these cases *Nucella* has been observed to prise open the opercular valves of its prey. Barnett (1979) has suggested that *Nucella* may in fact always try this method first, resorting to drilling only after prising has proved unsuccessful. The energy required to prise open a barnacle is considerably

Table 1.4 Recorded prey items of *Nucella lapillus* (adapted from Hughes & Dunkin, 1984b; Largen, 1967; Crothers, 1985).

ANNELIDS	*Spirorbis borealis*	Moore (1938b)
BARNACLES	*Semibalanus balanoides*	Barnett (1979), Largen (1967), Menge (1976, 1978a), Menge & Sutherland (1976), Morgan (1972a), Moore (1936), Connell (1961a)
	Chthamalus stellatus	Moore (1938b), Connell (1961b)
	Elminius modestus	Barnett (1979)
MOLLUSCS	*Nucella lapillus* (cannibalism)	Largen (1967)
	Patella vulgata	Moore (1938b)
	Patella intermedia	Largen (1967)
	Monodonta lineata	Largen (1967)
	Littorina littorea	Moore (1938b)
	Littorina obtusata	Moore (1938b)
	Littorina saxatilis	Largen (1967)
	Hydrobia ulvae	Largen (1967)
	Mytilus edulis	Bayne & Scullard (1978), Hancock (1960); Largen (1967), Lubchenco & Menge (1978), Menge (1976, 1978a), Menge & Sutherland (1976), Moore (1936), Morgan (1972a)
	Cerastoderma edule	Morgan (1972 a, b)
	Ostrea edulis	Hancock (1960)

less than that required to drill through the shell, as is the time taken to perform the operation. For example, prising open a 2 mm barnacle requires only 53% of the time needed for drilling and 63% of the time for a 6 mm barnacle (Dunkin & Hughes, 1984). This is important when it is considered that somewhere between 3 to 24 hours may be required to drill and ingest one barnacle. Inexperienced dogwhelks, for example those conditioned to feeding on mussels, are less likely to use the prising technique until after 6-8 consecutive prey have been eaten (Dunkin & Hughes, 1984).

When *Nucella* is feeding other individuals are attracted, presumably by the leaking body fluids of the prey (Hughes & Dunkin, 1984a). The encounters that follow result in a contest in which the interloper jostles for position with the occupant. The probability of an occupant being displaced by an interloper increases the greater the time elapsed since the occupant initiated the attack (Hughes & Dunkin, 1984a). Around 12% of *Nucella* feeding upon *Semibalanus balanoides* were displaced by interlopers in the laboratory and around 67% when feeding on *Mytilus* (Dunkin & Hughes, 1984). Hence interlopers in effect devalue the prey item by prolonging the handling time or displacing the original occupant. Either way profitability is reduced since this is related to the energy yield per unit of handling time. In order to compensate for this smaller prey may be taken.

During the handling period *Nucella lapillus* will be exposed to environmental conditions which may cause physiological stress or mortality. The main factors involved included high temperatures, desiccation during aerial exposure and the shear forces and risk of dislodgement caused by wave action during high tides. Other factors may include human disturbance and their susceptibility to predation. In order to minimise the handling time *Nucella* learns to select the thinnest part of the shell of *Mytilus edulis* to drill through. By doing this experienced dogwhelks are able to reduce drilling time by 23% and total handling time by 14% (Hughes & Dunkin, 1984a). Before drilling a detailed inspection is carried out where the whelks crawl over the mussel for around an hour during which time any incomplete bore holes are invariably detected and utilised as points of entry (Hughes & Dunkin, 1984a).

It has been suggested that *Nucella lapillus* has a slight inherent preference for mussels rather than barnacles (Moore, 1936; Connell, 1961a; Largen, 1967; Morgan, 1972a) although the opposite has been suggested by Crothers (1985). The selection of prey by *Nucella* may be expected to be in a frequency dependent manner based upon what is encountered. In reality though few animals feed like this, with prey selection being affected by factors such as; detectability, accessibility, nutrient intake, handling time, conditioning and learning, other predators, genetic characteristics, canopy forming algae and other shelter, the weather, for example desiccation or wind speed and the availability of alternative prey (Murdoch, 1969; Morgan, 1972a; Menge, 1978a; Menge, 1978b; Barnett, 1979; Hughes & Dunkin, 1984b; Fairweather, 1985; West, 1986; Burrows & Hughes, 1989). Instead *Nucella* feeds selectively avoiding smaller prey under favourable conditions, acting as an energy maximiser (Hughes & Burrows, 1991).

Many individuals do exhibit a strong prey preference though. Morgan (1972a) showed that the strong preference *Nucella* had for *Semibalanus balanoides* would switch to an alternative prey, *Cerastoderma edule*, when the barnacle abundance dropped. Once the barnacle populations recovered dogwhelks would return to their favoured prey; this switching behaviour would mean that no prey population was drastically reduced, nor is any allowed to become super-abundant. In effect the preferred species is acting as a buffer (Fairweather, 1987). A similar switching by *Nucella* has been observed to occur between barnacles and mussels (Moore, 1938b). These preferences can eventually be altered after laboratory conditioning but previous dietary experiences have an overwhelming influence on prey selection for at least two months (Hughes & Dunkin, 1984b). Starvation also affects dietary preferences for example *Nucella* favouring *Semibalanus balanoides* above *Elminius modestus* could be altered if *Nucella* was starved for ten months after which time both barnacles were equally favoured.

After feeding *Nucella* enters a post-ingestive quiescent phase the duration of which is dependent upon the size of the dogwhelk, its previous dietary history and the environmental conditions of temperature and salinity (Bayne & Scullard, 1978; Stickle & Bayne, 1987). This phase appears to be necessary for the effective digestion and assimilation of the ingested flesh (Burrows & Hughes, 1989). In the laboratory the time spent between feeding is highly predictable depending upon season, temperature and body size. This is not the case in the field. Instead environmental factors affect the length of the post-feeding phase (Burrows & Hughes, 1989).

The natural cycle of foraging and sheltering in *Nucella* is closely associated with changing weather conditions. In sheltered areas foraging is limited in sunny warm weather and in exposed areas is limited by strong wave action (Burrows & Hughes, 1989). During these periods the dogwhelks shelter in refuges on the shore which may take the form of crevices in the rock, *Fucus* clumps, among mussel beds or aggregations on the open rock (Connell, 1961a; Feare, 1971a; Menge & Sutherland, 1976; Hughes & Burrows, 1993). Unfavourable weather forces the whelks to feed close to the shelter of the crevices where prey becomes depleted leaving only the smaller items as the larger prey items have been taken preferentially (Connell, 1961a). Alternatively *Nucella* may actively make the decision not to feed since dogwhelks are able to survive extended periods of starvation (Stickle & Bayne, 1987).

1.7 The role of *Nucella lapillus* in community structure

Nucella lapillus has been shown to be an important predator on rocky shores, regulating the patterns of community structure (Dayton, 1971; Menge, 1976; Menge & Sutherland, 1976; Menge, 1978a; Menge, 1978b), defined as a collection of interacting organisms of all trophic positions occupying a given habitat (Menge, 1976; Menge & Sutherland, 1976). Knowledge of the levels of the intensity of predation and how and why it varies may be the key to understanding the interactions in the community (Menge, 1978b). Clearly the impact of predation is dependent on both the intrinsic characters of the predator and the physical and biotic characteristics of the environment (Murdoch, 1969; Morgan, 1972a; Morgan, 1972b; Menge, 1978a; West, 1986; Burrows & Hughes, 1989) and not necessarily just by the numbers of predators (Menge, 1978a).

Nucella has a direct effect upon the population structure of barnacles (Connell, 1961a; Dayton, 1971). In the summer nearly all of the mortality of barnacles older than 6 months can be accounted for by predation by *Nucella lapillus* (Connell, 1961a). The age structure of the remaining population is strongly affected by predation as the larger barnacles are taken preferentially and the small ones generally ignored (Connell, 1961a; Spence, 1989).

Predation prevents interspecific competition between barnacles and mussels so preventing either species from monopolising the available space on the shore (Connell, 1961a; Connell, 1961b; Dayton, 1971; Menge, 1976). A reduction in the level of predation decreases the diversity of species on the shore through competitive exclusion (Dayton, 1971; Menge & Sutherland, 1976), the so called 'predation hypothesis' (Paine, 1966; Paine, 1971). The community moves towards simplicity with the competitive dominant occupying all available space (Dayton, 1971; Menge & Sutherland, 1976). This move will be halted as predation pressure increases until a level where it becomes so intense that diversity is again reduced (Menge & Sutherland, 1976).

In the absence of predation, interspecific competition occurs between barnacles within a year (Connell, 1961b) with individuals being removed from the shore by over-growth, crushing and under-cutting by larger or faster growing barnacles (Connell, 1961a; Connell, 1961b; Rainbow, 1984). The absence of predation on *Mytilus*, in the north-east Pacific, may lead to a complete monopolisation of mussels in the mid intertidal zone (Dayton, 1975) as they are at the top of the competitive hierarchy above barnacles which are, in turn, above algae (Dayton, 1971). *Mytilus* initially occupies the available secondary space attached to algae and barnacles but eventually also dominates the primary substratum as the underlying barnacles either starve or are smothered (Dayton, 1971).

The effect of predation on communities varies widely (Menge, 1978a). In order to totally understand the role of predation in structuring communities world-wide studies must be carried out (Underwood, 1985). The communities on west coast of America are more diverse than those on the north-eastern coast (Menge & Sutherland, 1976). Secondary carnivore trophic levels exist along with a much larger herbivore guild which includes limpets, chitons and gastropods (Connell, 1970). The region is structured largely by predation which is responsible for maintaining a high species richness (Menge & Sutherland, 1976). However carnivorous gastropods appear to have little effect on this diverse community, instead predators such as the starfish *Pisaster ochraceus* play a more important role because of the level of disturbance and amount of primary space it creates (Paine, 1966). Since the whelks penetrate only one barnacle at a time they do not have the same effect as the starfish which can strip 20-60 barnacles from the shore simultaneously. Even at high whelk densities the effect is not the same as the empty husks of the dead prey are left on the shore (Paine, 1966). New England shores are much simpler (Menge & Sutherland, 1976). Here *Nucella lapillus* plays an important role in the community structure of the shores where there is low wave exposure, but where there is high wave exposure, however, there is a move away from a predator dominated community to one which is competition dominated (Menge & Sutherland, 1976).

Although the community structure of the shores in New England is reported to be similar to those in Britain (Menge & Sutherland, 1976; Menge, 1978a; Menge, 1978b; Menge & Lubchenco, 1981) there are no large patellid limpets. The importance of limpets has been well demonstrated on British shores, where their removal promotes the growth of fucoid algae (Jones, 1948; Lodge, 1948; Burrows & Lodge, 1950; Southward, 1956; Southward, 1964). Moderately exposed shores support a community consisting of patches of *Fucus*, barnacles (mainly *Semibalanus balanoides*), bare rock and grazing *Patella* and predatory *Nucella*. Good examples of these shores are found on the Isle of Man (described in Southward, 1953). These patches form a mosaic which shows considerable temporal variation in the relative abundance of barnacles, bare rock and *Fucus* cover. The mosaics consist of a series of complex positive and negative interactions which creates a cycle of dominance which is mediated by limpets (Hawkins & Hartnoll, 1983; Hartnoll & Hawkins, 1985).

High barnacle densities reduce the foraging efficiency of *Patella* (Hawkins, 1981a; Hawkins, 1981b; Hawkins & Hartnoll, 1982a) thus increasing the likelihood of an 'escape' from grazing for the vulnerable fucoid germlings (Hawkins & Hartnoll, 1983; Hartnoll & Hawkins, 1985). Once the fucoids have reached 3-4 cm in length limpet grazing has little effect upon them (Burrows & Lodge, 1950; Hawkins, 1979) resulting in an increased algal abundance. *Nucella* and *Patella* aggregate beneath the newly formed *Fucus* clumps. Here the barnacle density is reduced as *Nucella* forages from the shelter of the *Fucus* canopy (Connell, 1961a). In addition the settlement of new barnacles under the *Fucus* canopy is limited due to the sweeping or barrier effect of the fronds (Southward, 1956; Lewis, 1964; Dayton, 1971; Menge, 1976; Hawkins, 1983). There is high mortality amongst those that do settle as although limpets do not damage adult barnacles they cause high mortality to settling cyprids during grazing (Connell, 1961a; Dayton, 1971; Hawkins, 1983). Meanwhile the aggregations of *Patella* under the *Fucus* canopy results in reduced grazing intensity on other nearby areas of the shore, in turn leading to the formation of fucoid escapes elsewhere. With time the existing *Fucus* canopy thins and the limpets and dogwhelks disperse, new barnacles recolonise the area of bare rock that remains (Hawkins & Hartnoll, 1983).

On British shores it is unlikely that *Nucella lapillus* has much of an effect on the shore community at exposed sites where they are restricted to foraging close to crevices on the shore (Connell, 1961a). Similarly they are unlikely to have much of on effect on the communities of sheltered shores which are inherently stable (Hartnoll & Hawkins, 1985; Hawkins *et al.*, 1985). However on moderately exposed shores the presence of *Nucella lapillus* may be expected to shape changes in the cycle of fucoid and barnacle dominance observed.

1.8 Rationale and aims of this thesis

Legislation introduced in 1987 in the UK and 1988 on the Isle of Man restricted the use of tributyltin based antifouling paints. In order to assess the effectiveness of these restrictions is it imperative to measure concentrations of this pollutant in the environment. In a previous study, Spence (1989), changes in environmental concentrations of TBT were assessed over a three year period (1986 to 1989) which spanned the year in which the legislation was introduced in the UK. Since the present study commenced in 1990 it provided a unique opportunity to continue some of the established monitoring programmes established by Spence (1989) in order to provide a long term measures of the changes in the environmental concentrations of tributyltin and in the level of effects on *Nucella lapillus* populations. This was especially important since a study spanning more than three years is normally outsides the bounds of a British PhD.

Although the effects of tributyltin on *Nucella lapillus* are now well documented at the cellular, individual and population level (Hawkins *et al.*, in press) the consequences for rocky shore communities have not been explored. As a result there has been only speculation as to the wider implications of the decline in numbers of *Nucella* on rocky shores at the present time.

The introductory section is continued in chapters 2 and 3, where the study sites and general methods used are described fully in order to provide background information which applies to all the chapters presented.

The changes in the concentrations of tributyltin in the environment are assessed in chapter 4 by measuring levels in the water and in the tissues of four common rocky shore species: dogwhelks, mussels, barnacles and limpets. Monitoring continued the work of Spence (1989) at five sites in south-west England.

The recovery of *Nucella* populations are assessed in chapter 5, continuing the data collected by Spence (1989). Changes in the level of imposex development and in the abundance of dogwhelks at five sites in south-west England are used as measures of recovery.

The recovery of *Nucella lapillus* populations around the Isle of Man is assessed in chapter 6. The level and extent of pollution on the Isle of Man differs from that on the south coast where instead of wide-spread TBT pollution, contamination is close to source providing steep gradients away from harbours and marinas. Here a complete picture of the extent of TBT pollution is confirmed by sediment, water and imposex values. The effectiveness of the current legislation is examined.

The role of *Nucella* in shore communities is addressed in chapter 7 using manipulative field experiments to simulate a reduction in dogwhelk abundance. These experiments were conducted on flat ledges on well studied moderately exposed Manx shores. The role of *Nucella* is examined at different stages in the cycle of barnacle and *Fucus* dominance on these shores (Hawkins & Hartnoll, 1983; Hartnoll & Hawkins, 1985). Firstly the direct effects of *Nucella* on the barnacle population and the consequences for the likelihood of *Fucus* escapes is examined. Secondly their effect on the longitivity of the established *Fucus* clumps is examined and thirdly the long and short term effects on barnacle settlement are studied.

CHAPTER 2

Study sites and meteorological data

2.1 Study sites

Studies were made in two general areas of the British Isles, in south-west England primarily around Plymouth, and the Isle of Man.

2.1.1 South-west England

In terms of the level and widespread nature of tributyltin (TBT) pollution, the south coast of England is known to have been one of the most badly affected areas of the UK (Spence *et al.*, 1990a). Whereas elsewhere in the British Isles TBT pollution is localised, close to source (Bailey & Davies, 1988; Spence *et al.*, 1990a), the intensity of boating activity along the south coast has led to high levels of contamination which are not necessarily confined to harbours. Instead large sections of the adjacent coastline are affected by TBT (Bryan *et al.*, 1986). With the marine laboratories at Plymouth, the south-west has been the focus of a number of studies on levels of TBT contamination in the water (Cleary & Stebbing, 1985; Cleary & Stebbing, 1987a), sediment (Langston *et al.*, 1990; Langston & Burt, 1991) and in the biota (Bryan & Gibbs, 1991) of the area. In addition there have been numerous studies into the extent of TBT induced imposex development in neogastropods in the south-west, including those on *Nucella lapillus* (Bryan *et al.*, 1986), *Ocenebra erinacea* (Gibbs *et al.*, 1990) and *Nassarius reticulatus* (Bryan *et al.*, 1993a).

This study has used five sites in the south-west of England (figure 2.1). These were originally selected along a suspected gradient of tributyltin pollution by Spence

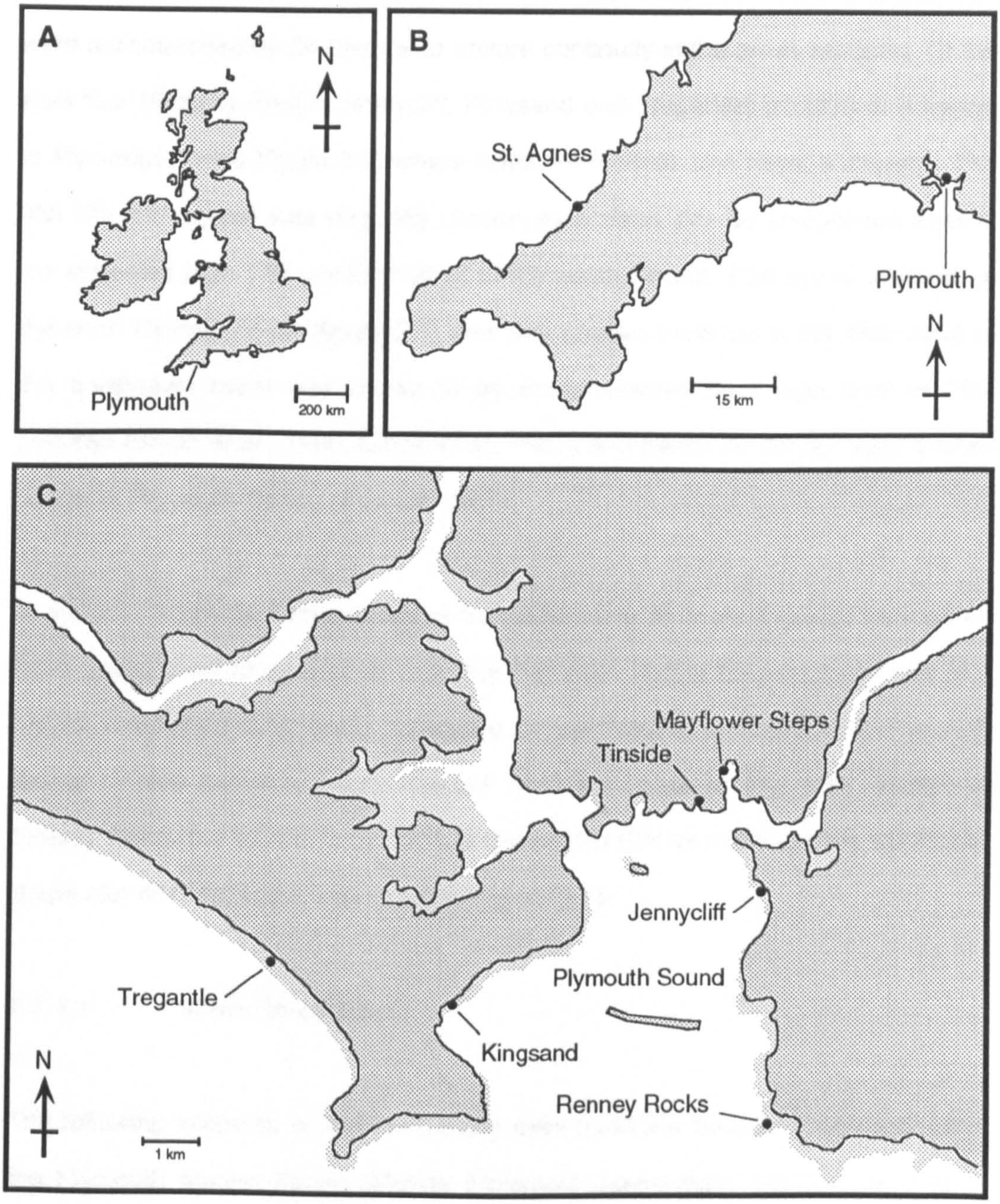


Figure 2.1 Study sites used in the south-west of England, showing the location of Plymouth (A), St. Agnes on the north Cornwall coast (B) and detail of Plymouth Sound (C) marked with all the sites used.

(1989) and used here to continue the time series of data. Initial visits to these sites were accompanied by Dr Spence to ensure continuity in the areas sampled. Of the sites four (Renney Rocks, Jennycliff, Kingsand and Tregantle) occur in or adjacent to Plymouth Sound (figure 2.1) where there are marinas and naval dockyards. The fifth site (St. Agnes) was originally chosen as a clean site for comparison against the expected high TBT contamination of Plymouth Sound. This site is situated on the north Cornish coast (figure 2.1) and was chosen because at the time most of the south-west coast was known to be badly affected by a high level of TBT pollution (Bryan *et al.*, 1986; Gibbs *et al.*, 1987) and hence no control sites existed nearer to Plymouth Sound (Spence, 1989).

In addition to enable comparisons to be made some data are included here which have been collected by workers from the Plymouth Marine Laboratory as part of a regular monitoring programme assessing concentrations of tributyltin in Plymouth Sound. These samples were collected from two sites in Plymouth Sound: at Tinside, below the Marine Laboratory at Citadel Hill (SX 481537) and the Mayflower Steps (SX 484540) in the main harbour (figure 2.1).

2.1.1.1 Shore descriptions

The following accounts of the main study sites used are based on descriptions in the Plymouth Marine Fauna (Marine Biological Association, 1957), Turk (1983), Spence (1989) and personal observations.

In 1990, at the beginning of the present study, dogwhelk populations from all the sites used were affected by tributyltin pollution to some extent. Although dogwhelks were present at all sites, juveniles were rare at Kingsand and Jennycliff. Previous studies at these sites (Gibbs & Bryan, 1987; Gibbs *et al.*, 1987; Spence, 1989;

Spence *et al.,* 1990a) have measured levels of TBT contamination using the two indices of imposex development: relative penis size (RPS) and vas deferens sequence (VDS). Hence these sites can be divided into three categories, according to the level of contamination. St. Agnes is relatively unaffected. Here no sterile females have been recorded and imposex development was low with RPS values of less than 5% and a median VDS stage of 3 for adult *Nucella*. Tregantle, Renney Rocks and Kingsand were and still are, all moderately affected sites. Less than 50% of females sampled were recorded as sterile, RPS values were less than 40% and the median VDS stage was 4. The highest level of contamination has been at Jennycliff where between 50 and 100% of the females sampled were sterile, RPS values were greater than 50% and the median VDS stage was 5 (Spence, 1989).

Spence (1989) recorded *Semibalanus balanoides* as the predominant barnacle species at all of the sites used except at Kingsand, where *Chthamalus montagui* was found to be the most abundant. Three species of limpet were observed at these sites with *Patella vulgata* recorded as the most common, although *P. depressa* was regularly found as were small numbers of *P. aspera*. Of the sites used all are moderately exposed. Exposure values based on Ballantine's biologically defined scale (Ballantine, 1961) were estimated as 3-4 for Jennycliff, Kingsand and St. Agnes, 4-5 for Renney Rocks, and 3 for Tregantle, the most exposed site (Spence, 1989).

St. Agnes

The shore at St. Agnes (SW 722516) consists of areas of Devonian sedimentary rock formed into irregular outcrops, flat rock and boulders. Deep pools separate the reefs which run parallel to the sea. The area was covered by barnacles, with patches of mussels occurring in some areas. Older mussels covered with barnacles blanketed further areas, but these were prone to removal from the shore during rough weather providing bare space for re-colonisation. There was little algal cover. *Nucella* foraged from crevices in the rocks and overhangs on the boulders moving out on to the open shore in the summer. A degree of shelter is afforded from the prevailing south-westerly winds by St. Agnes Head. There is no harbour here, the nearest being at Newquay some 13 km to the north-east.

Tregantle

Expanses of fine sand separate Dartmouth slate outcrops which run at right angles to the shoreline at Tregantle (SX 387525). These were covered in barnacles and mussels. There was little algal cover here although in the spring and early summer there was persistent *Ulva* and *Enteromorpha* on the shore. *Nucella* were observed to feed from the shelter of crevices and from within the *Mytilus* matrix. The shore is subject to wave action from the south-west. There is no harbour at Tregantle. The marinas in Plymouth Sound are around 13 km away.

Renney Rocks

Renney Rocks (SX 492487) is a reef composed of uneven ridges and isolated boulders of Lower Devonian slate. The laminated rock creates many crevices in which *Nucella* were seen to shelter, moving out on to the open rock to feed in the summer. Barnacles dominated the area. The shore is relatively protected on the seaward side by the high tip of the promontory. Renney Rocks borders Plymouth Sound about 6 km from the marina adjacent to the Barbican.

Kingsand

At Kingsand (SX 438514) irregular outcrops of soft Devonian slate are orientated at right angles to the shoreline which form reefs extending into Plymouth Sound. Dense barnacles dominated the area and there was an abundance of littorinid grazers. Algal cover was generally sparse although *Fucus* escapes did occur amongst dense barnacles in some places. *Nucella* were found to occur mainly on and around a sewage pipe crossing the shore to discharge into Plymouth Sound. The shore is subject to wave action from the south-east. Although there is no harbour at Kingsand, occasionally in the summer boats do anchor just off the rocks here. The main marina is some 5 km away.

Jennycliff

The shore at Jennycliff (SX 491521) is composed of Middle Devonian shale rock and large boulders dominated by dense barnacles. The rocks dip seawards forming ridges running parallel to the coast and these are intersected with small gullies. A sewage pipe runs across the shore into Plymouth Sound; most *Nucella* were found along or directly adjacent to this area. The shore is relatively protected from southerly and south-westerly winds by the breakwater in Plymouth Sound. Jennycliff is the closest of the sites studied in Plymouth Sound to the main marina which is only around 2 km away.

2.1.2 Isle of Man

A number of different study sites were used on the Isle of Man. The positions of all of those mentioned in the following chapters are shown in figure 2.2. The monitoring studies, examining levels and effects of tributyltin pollution (chapter 6) were based in harbours around the Island including those of Ramsey, Douglas, Port St. Mary and Port Erin. Niarbyl was used as a control site. Unlike the coast of south-west England, where tributyltin pollution is widespread across the whole coast, around the Island it has remained localised close to source, creating sharp gradients of contamination away from the harbours (Spence *et al.*, 1990a; Spence & Hawkins, 1990). On the open coast dogwhelk populations have been only moderately affected, but close to harbours for example at Port St. Mary, Douglas

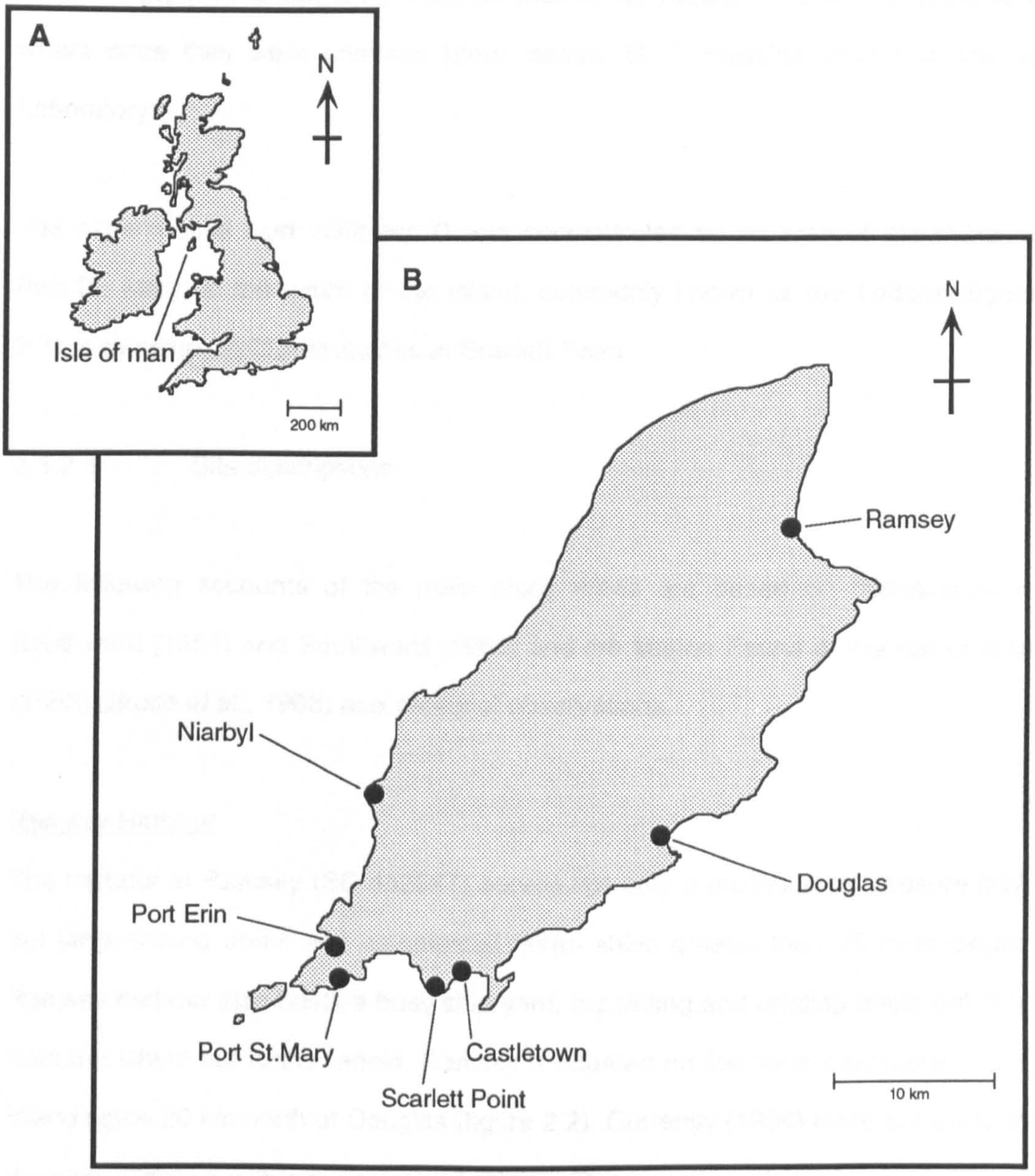


Figure 2.2 Study sites around the Isle of Man, showing the location of the Island (A) and the position of all the sites used around the coast (B).

and Peel (Spence & Hawkins, 1988; Spence *et al.*, 1990a) there are no dogwhelks where once they were common (pers. comm. S. J. Hawkins, Port Erin Marine Laboratory).

The experimental work (chapter 7) was concentrated on an area of the shore at Port St. Mary, in the south of the Island, commonly known as the Ledges (figure 2.3), with some additional studies at Scarlett Point.

2.1.2.1 Site descriptions

The following accounts of the main study areas are based on descriptions in Southward (1951) and Southward (1953) and the Marine Fauna of the Isle of Man (1963) (Bruce *et al.*, 1963) and personal observations.

<u>Ramsey Harbour</u>

The harbour at Ramsey (SC 452947) serves not only a multitude of pleasure craft but large fishing boats and commercial cargo ships greater than 25 m in length. Ramsey harbour also hosts a busy ship yard, repainting and refitting boats not only from the Island but further afield. Ramsey is situated on the north-east coast of the Island some 20 km north of Douglas (figure 2.2). Currently (1994) there are plans to develop a marina within the existing harbour. This would involve impounding water in the part of the harbour which normally dries out at low tide in order to provide a semi-tidal basin achieved by installing a flap gate across the estuary (Allen *et al.*, 1992a).

<u>Douglas Harbour</u>

The largest harbour on the Island is at Douglas (SC 387752) on the east coast (figure 2.2). There is heavy boat traffic which has in addition to pleasure, fishing and cargo craft, two large car ferries and regular visits from tankers, freight and naval vessels. The inner harbour (SC 386750) dries out at low water.

Port St. Mary Harbour

At Port St. Mary about 20 km south of Douglas (figure 2.2) there is a small harbour which is divided in two. The Outer harbour (SC 212674) is used for the local fishing boats and is home to the research vessel R.V. Roagan belonging to Port Erin Marine Laboratory. In the summer months large yachts frequently moor here. The inner harbour (SC 210675) by comparison is used only for small pleasure craft. Larger boats are occasional visitors, usually when they need to be repainted as the inner harbour dries out at low water. In the winter most of the boats are taken out of the water.

Port Erin Harbour

The harbour at Port Erin (SC 193690) in the south-west of the Island (figure 2.2) is very small. It is used mainly in the summer and during the winter months most of the boats are removed from the water. This harbour, which also dries out at low water, is a favourite for small boat owners to use when repainting their boats in the spring. Occasionally flotillas of yachts moor in the Bay.

The Ledges

The carboniferous limestone rock at Port St. Mary (SC 211669) is present as a series of gently sloping, near horizontal platforms descending in steps, commonly referred to as the Ledges (figure 2.3). As a result of its close proximity to the Port Erin Marine Laboratory this area has been extensively studied (for example Jones, 1948; Southward, 1951; Southward, 1953; Hawkins, 1979). The wide gently sloping ledges on this shore make this an ideal site for experimental ecology.

The shore is of moderate exposure sheltered slightly from the prevailing south-west winds. The flat rock in the mid tidal region was dominated by a mosaic of dense barnacles, predominantly *Semibalanus balanoides*, fucoid algae and interspersed with limpets (Burrows & Lodge, 1950; Hawkins, 1979; Hartnoll & Hawkins, 1985). The vertical rocks, forming the steps between the ledges, were again dominated by dense barnacles, but *Fucus* was rarely seen on these areas. Dogwhelks were abundant here, despite the close proximity of the Ledges to the outer harbour at Port St. Mary, only about 0.5 km away. All ages of *Nucella* were commonly observed feeding on the barnacles on the vertical faces or on the flat rock of the ledges, often foraging from the shelter of crevices or fucoids.

Three main areas of the ledges (figure 2.3) were used for monitoring and experimental ecology. The first area (area A, figure 2.3) consists of several vertical rock faces each between 0.5 and 1 m tall and about 20 m long. These run east to west, with the rock surface facing more or less due north. Three of these verticals were used in the experimental investigations of dogwhelk removal (chapter 7). Separating these verticals are flat, gently sloping areas, one of which was about 10 m wide and was dominated by a dense *Fucus* clumps on a barnacle matrix. This area was used in the investigation of the effect of dogwhelk removal on the survival of *Fucus* clumps (chapter 7).

In the experimental area further to the west (area B, figure 2.3) the verticals run north to south with the rock facing due west. These are of approximately the same height and length as those at site A, facing north, and were used for comparison (chapter 7). The flat areas of rock here were bare by comparison with little *Fucus* cover. The barnacle cover was about 80%. This area was used in the barnacle settling experiments (chapter 7).

The third area used is situated further towards the harbour at Port St. Mary (area C, figure 2.3). Here the rock has a uniform slope from the top shore to below low water with no vertical steps. This area was used to monitor the natural changes in the barnacle, bare rock, *Fucus* mosaic (chapter 7) at mid tide level using the monitoring site established by Hawkins (1979) on Southward's transect A (Southward, 1951; Southward, 1953) and some additional areas.

Scarlett Point

Scarlett Point (SC 258662) is also composed mainly of carboniferous limestone. Here the shore on the Castletown side, facing east, is made up of descending ledges similar to those at Port St. Mary but steeper and narrower. The distribution of barnacles, fucoids and dogwhelks is very similar to that at the ledges. Castletown Harbour is about 1 km to the north-east (figure 2.2) (Southward, 1951; Southward, 1953).

Niarbyl

Manx slate makes up this low reef which extends south-west from the coast at Niarbyl (SC 211776). The slate is heavily folded forming irregular masses or steep slopes in contrast to the gentle slopes in the limestone at Port St. Mary or at Scarlett Point. Situated about 10 km south of Peel harbour and the same distance north of Port Erin (figure 2.2) there is no boating activity here.

The slate of this shore makes much of it very uneven. *Nucella* was common moving out from the crevices in the folded slate. Algal cover was scarce and *Semibalanus balanoides* dominated much of the strata. The headland here is exposed to westerly winds (Southward, 1951; Southward, 1953).

2.2 Meteorological data

In order to provide background information and where necessary relate some of the changes observed on the shore with meteorological events, data were collected from Ronaldsway Meteorological Office based at the airport on the Isle of Man. The airport lies only 16 m above sea level and is situated about 8 km north-west of Port St. Mary, consequently it is representative of the conditions at the Ledges and at Scarlett Point.

Information on the changes in the weather at the study sites was considered important since it has long been known that physical factors play an important role in setting the distribution and affecting the recruitment of rocky shore organisms (Lewis, 1964; Lewis, 1977) and in influencing the structure and stability of communities. The natural cycles of foraging and sheltering in the dogwhelk, for example, are associated with changing weather conditions (Burrows & Hughes, 1989). In addition onshore winds around the Isle of Man have been shown to affect the settlement of *Semibalanus balanoides* (Hawkins & Hartnoll, 1982b) and hot weather to directly affect canopy and turf-forming algae (Hawkins & Hartnoll, 1985; Hill, 1993).

Climatic features considered to be important were those of wind, air and water temperature, sunshine and rainfall.

2.2.1 Wind

Onshore winds at Port St. Mary Ledges were taken to be those ranging from 110°-250° (ESE to WSW). Winds from these directions were recorded all year around (figure 2.4) although these were fewest in May and June of each year. Onshore

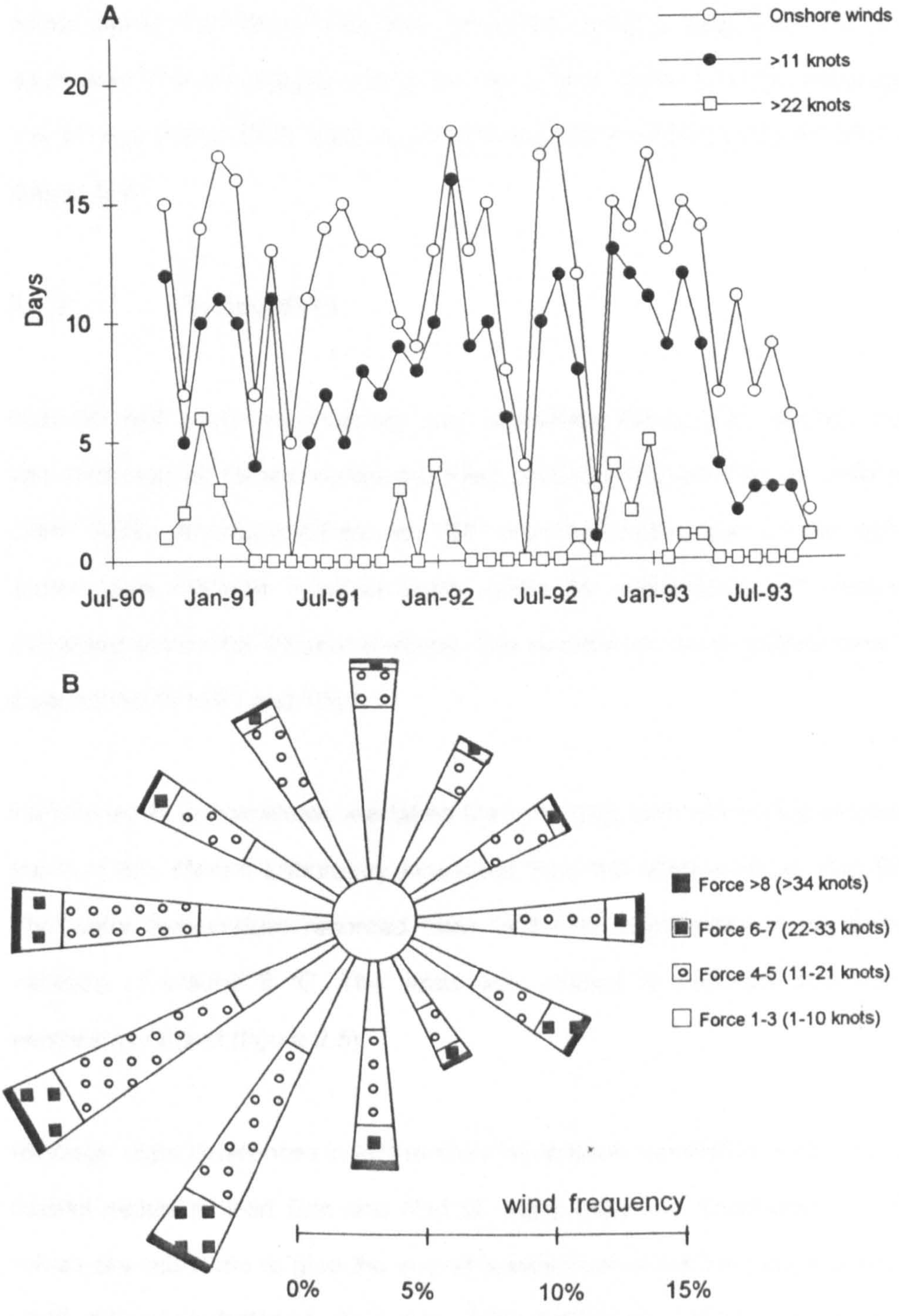


Figure 2.4 Variation in onshore winds (110-250°) at Port St. Mary during period of study (A). Number of days per month that there was an onshore wind, or where the daily mean wind speed onshore exceeded 11 knots (force 4) or 22 Knots (force 6). The wind frequency diagram (B) shows the direction and strength of the prevailing winds on the Isle of Man (1961-1992). Data from Ronaldsway Meterological Office, Isle of Man.

winds greater than force 6 (22 knots) occurred mainly in December and January of each year. The prevailing winds for the Isle of Man, taken from the anemograph for the 31 year period 1961-1992, occur from 200°-220° (SSW) and 230°-250° (WSW) (figure 2.4).

2.2.2 Temperature

Both air and water temperatures vary seasonally (figure 2.5). Monthly maximum and minimum air temperatures recorded generally followed the 40 year average (1947-1986). January and February 1991 were both colder than normal, but the two winters that followed however were milder by comparison with temperatures remaining above the 40 year average. The summer air temperatures were warmer than normal in 1991 and 1992.

Inshore water temperatures are taken from the long-term monitoring programme at the Port Erin Marine Laboratory measured from the Breakwater in Port Erin Bay. The water temperature recorded from Port Erin Breakwater shows a seasonal variation of around 8 °C. The water was coldest in February and March and warmest in August (figure 2.5).

Although slight differences in temperature have been reported to occur between the coastal waters at Port Erin and Port St. Mary (<0.5 °C, Southward, 1953) these values are adequate to give the overall trends throughout the period of study. The small difference between the water temperature on either side of the Island probably has a negligible effect on the organisms (Southward, 1951).

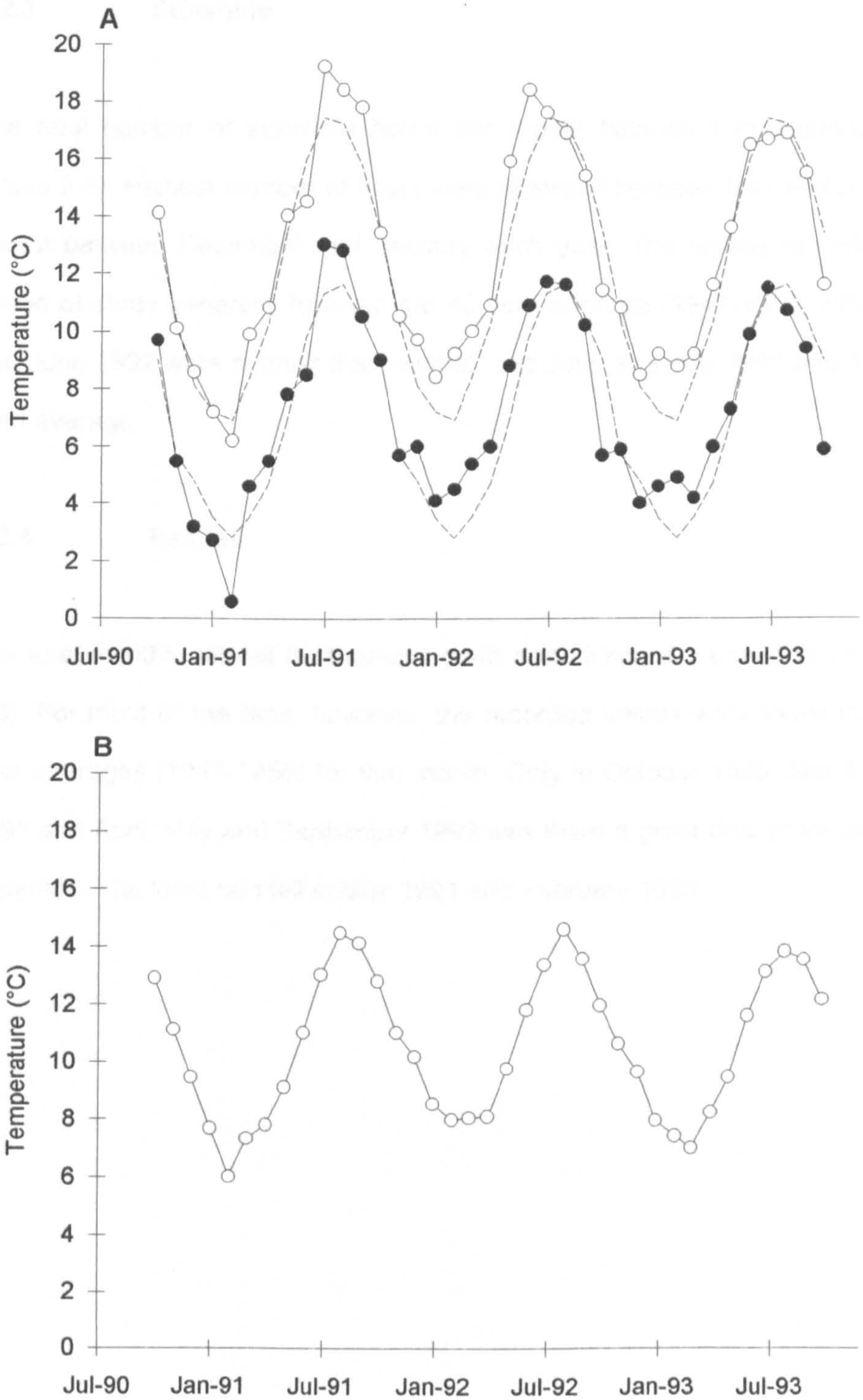


Figure 2.5 Variations in air (A) and inshore water temperatures (B) during the period of study. The monthly mean maximum and minimum air temperatures are shown with 40 year averages (1947-1986) for comparison plotted as dotted lines with no markers. Monthly mean inshore water temperatures were from the breakwater in Port Erin Bay, Isle of Man. Data from Ronaldsway Meterological Office and Port Erin Marine Laboratory, Isle of Man.

2.2.3 Sunshine

The total number of sunshine hours per month fluctuated throughout the year (figure 2.6). Highest number of hours were recorded between May and July and the lowest between December and January each year. The values recorded for the period of study generally followed the 40 year average (1947-1986) although May and June 1992 were sunnier than normal, and June and July 1991 and 1993 duller than average.

2.2.4 Rainfall

The total monthly rainfall fluctuated a great deal during the period of study (figure 2.6). For most of the time, however, the recorded values were lower than the 40 year averages (1947-1986) for that month. Only in October 1990, March and April 1991 and April, May and September 1993 was there a great deal more rainfall than expected. The least rain fell in May 1991 and February 1993.

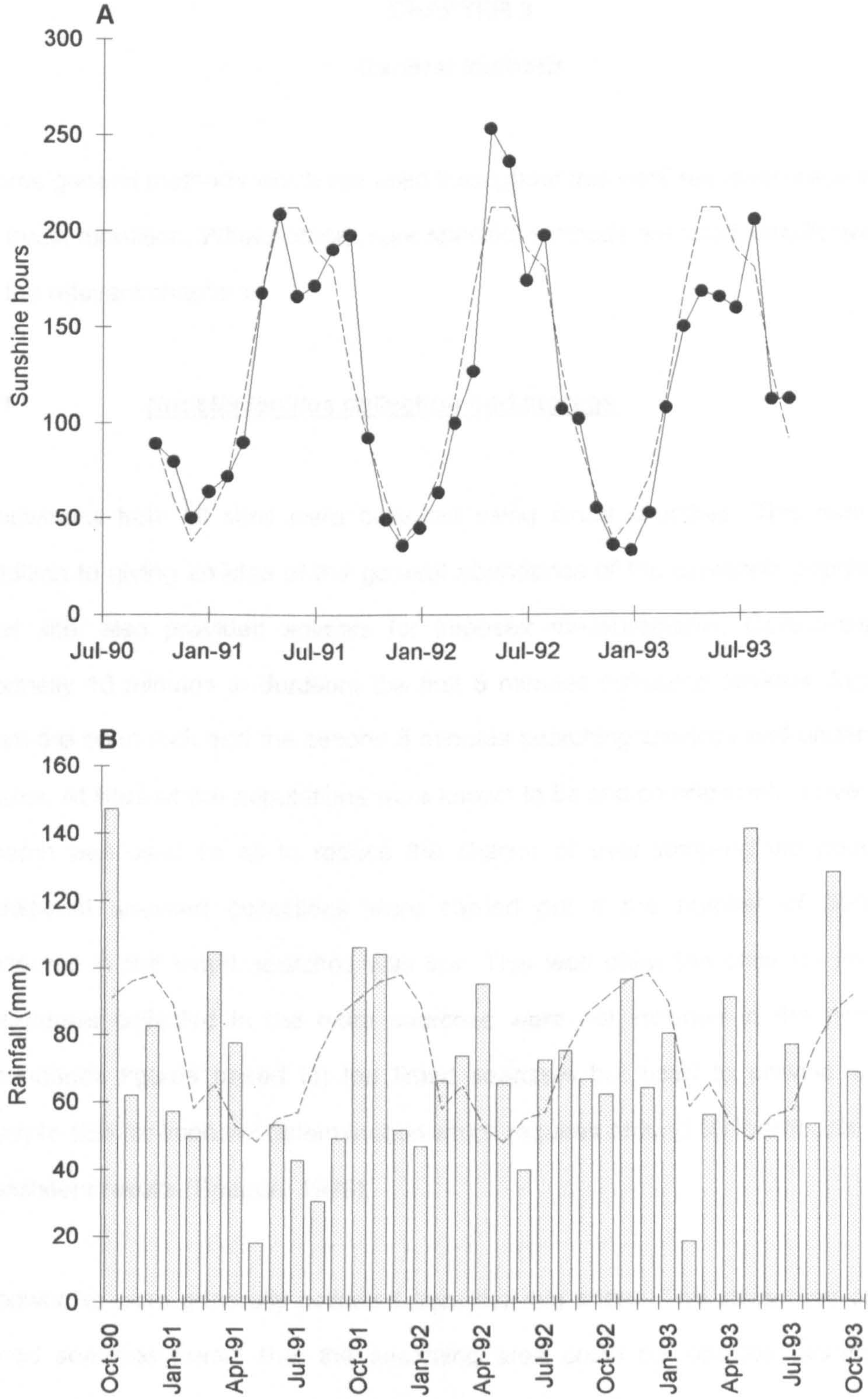


Figure 2.6 Variations in the total number of sunshine hours per month (A) and the total monthly rainfall (B) during the period of study. The 40 year averages (1947-1986) are included for comparison, plotted as dotted lines with no markers. Data from Ronaldsway Meterological Office, Isle of Man.

CHAPTER 3

General Methods

Some general methods which are used throughout this work are given here in order to avoid repetition. Where other, more specific, methods are used, details are given in the relevant chapters.

3.1 *Nucella lapillus* collection and storage

Dogwhelks from all sites were collected using timed searches. This method, in addition to giving an idea of the general abundance of the dogwhelk population at that site, also provided animals for imposex measurements. Collections were normally 10 minutes in duration; the first 5 minutes collecting obvious dogwhelks from the open rock and the second 5 minutes searching crevices and under *Fucus* plants. At sites where populations were known to be scarce often only a five minute search was used so as to reduce the chance of over-sampling the population. Additional un-timed collections were carried out if the number of dogwhelks collected in the timed searches was low. This was often the case for juveniles. Individuals collected in the extra searches were not included in the population abundance figures based on the timed searches but used to provide a larger sample size for imposex determination which requires around 30 individuals to give consistent results (Spence, 1989).

Dogwhelks were generally collected from the mid shore. The advantage of using timed searches meant that the searching area could be adapted, according to seasonal or weather related patterns of dogwhelk distributions, to bands either higher or lower than mid shore. During collection the dogwhelks were placed in net bags with a labelled plant tag for identification. They were transferred to the laboratory with *Fucus* surrounding the mesh bag to keep the animals damp. Once back at the laboratory the mesh bag was placed on the circulation bench with a supply of running sea water. The dogwhelks were usually processed within two weeks, although it was observed that they could survive for a number of months under these conditions.

3.2 Measurement of imposex

Imposex is defined as the superimposition of male sexual characteristics on the female (Smith, 1971). In *Nucella lapillus* it is expressed in terms of the development of a penis and vas deferens. The extent of the development of these two characteristics can be quantified by two indices which are significantly correlated with concentrations of tributyltin in the water (Gibbs *et al.*, 1988): relative penis size (RPS) (Bryan *et al.*, 1986) and vas deferens sequence (VDS) (Gibbs *et al.*, 1987).

3.2.1 Preparation of *Nucella lapillus*

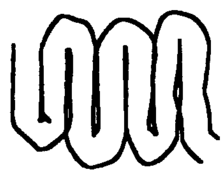
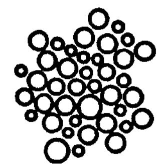
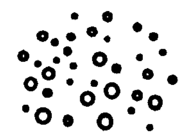
The shell length and aperture of each individual were measured to the nearest 0.05 mm using dial callipers. In addition the general appearance and colour of the shell was noted. Using the thickness of the shell edge as a guide, the age of each individual was assessed using a simplified age system used previously by Feare (1970a) and Spence (1989). Here individuals were placed in one of three age classes: either juveniles with sharp shell edges, second years with thickening edges, or adults with thick or toothed edges to their shells. Often individuals were observed with multiple rows of teeth and were recorded as such. This feature, indicating the continuation of growth after the individual has reached sexual maturity (Feare, 1970a), is often indicative of infection by the trematode parasite *Parorchis acanthus* (Feare, 1970a; Crothers, 1985). All assessments of maturity based on the thickness of the shell edge were later clarified after examination of gonad development (table 3.1).

The shell of the individual was cracked in a small bench vice and the animal removed by separating the columella muscle from its attachment on the inside of the shell using a pair of curved forceps. The animal was placed dorsal side up in a petri dish of sea water and examined under a Wild Heerbrugg M74 binocular microscope, illuminated using a Schott KL1500-T cold light source in order to prevent shrinkage of the penis tissues (Spence, 1989).

3.2.2 Sex determination

Previous to the widespread advent of tributyltin induced effects on dogwhelks the presence or absence of a penis was a clear indicator of the sex of an individual dogwhelk (Feare, 1970b). Clearly this method is now unreliable. Even discriminating between the sexes on the basis of the size of the penis is unrealistic, as even at a moderately affected site the difference between the female and male penis size is often insufficient to distinguish between the two. A variety of other criteria have been used to separate the sexes in the past including the differences in the colour and texture of the gonadal tissue (Hall & Feng, 1976; Miller & Pondick, 1984), the presence of a ventral pedal gland (Fretter & Graham, 1984; Fioroni *et al.*, 1991b), albumen gland (Davies *et al.*, 1987), or of a capsule gland. But criteria based on colour are open to misinterpretation especially with variations caused by seasonal cycles of reproductive activity (Feare, 1970b). The presence of the capsule gland, for example, a creamy white structure, could easy be confused with the yellow of the prostate gland in the male, especially in juvenile individuals.

Table 3.1 Diagrammatical representation of the textural appearance of the seminiferal tubules in the male and of the ovaries in the female used to divide *Nucella lapillus* individuals into three age classes: adults, second years and juveniles.

Age	Male		Female	
Adult	Well developed		Coarse	
Second year	Developed		Medium	
Juvenile	Not developed		Fine	

The definitive structure is the presence of the sperm ingesting gland in the female. This is a distinctive red/brown gland situated immediately posterior to the capsule gland (figure 3.1) to which there is no similar feature in the male (Fretter & Graham, 1962). This feature which is found even in sexually immature individuals is now commonly used in the often difficult distinction between males and females at sites heavily affected by TBT pollution (Gibbs *et al.*, 1987). In addition the sperm ingesting gland does not appear to vary in size or colour with the level of TBT contamination (Gibbs *et al.*, 1988) or reproductive state of the female (Gibbs *et al.*, 1987), unlike the gonad or capsule gland.

Only in juveniles which have shell lengths of less than 15 mm (probably aged less than 6 months) is there any difficulty in distinguishing the sex of the individuals (Gibbs *et al.*, 1987). This is because the sperm ingesting gland is not yet clearly developed. Consequently individuals smaller than 15 mm were not used for imposex assessments.

3.2.3 Relative penis size index

3.2.3.1 Measurement of the penis

The penis, situated behind the right tentacle in both the male and affected females was exposed by drawing back the flap that forms the roof of the mantle cavity. A piece of 1 mm graduated graph paper was placed beneath the penis to measure its length to the nearest 0.1 mm (Bryan *et al.*, 1986; Gibbs *et al.*, 1987) (figure 3.2). The measurement was taken from the tip of the penis to its base at the junction between the body wall and the right tentacle. No attempt was made to straighten out the penis which is a relatively stout and immobile structure. The presence of any abnormalities, for example, in the shape or colour of the penis or in the addition

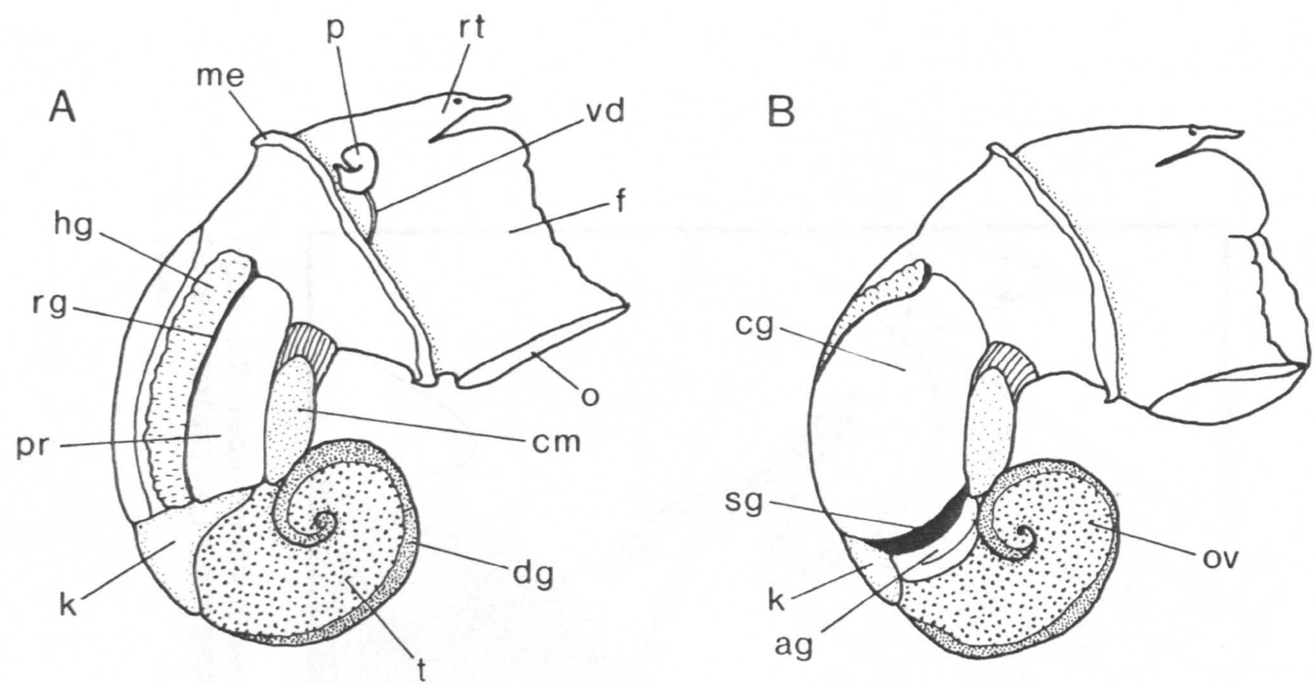


Figure 3.1 External features of mature male (A) and female (B) *Nucella lapillus* after shell removal. Abbreviations: ag, albumen gland; cg, capsule gland; cm, columella muscle; dg, digestive gland; f, foot; hg, hypobranchial gland; k, kidney; me, mantle edge; o, operculum; ov, ovary; p, penis; pr, prostate; rg, rectal gland; rt, right tentacle; sg, sperm-ingesting gland; t, testis; vd, vas deferens (Gibbs *et al.*, 1987).

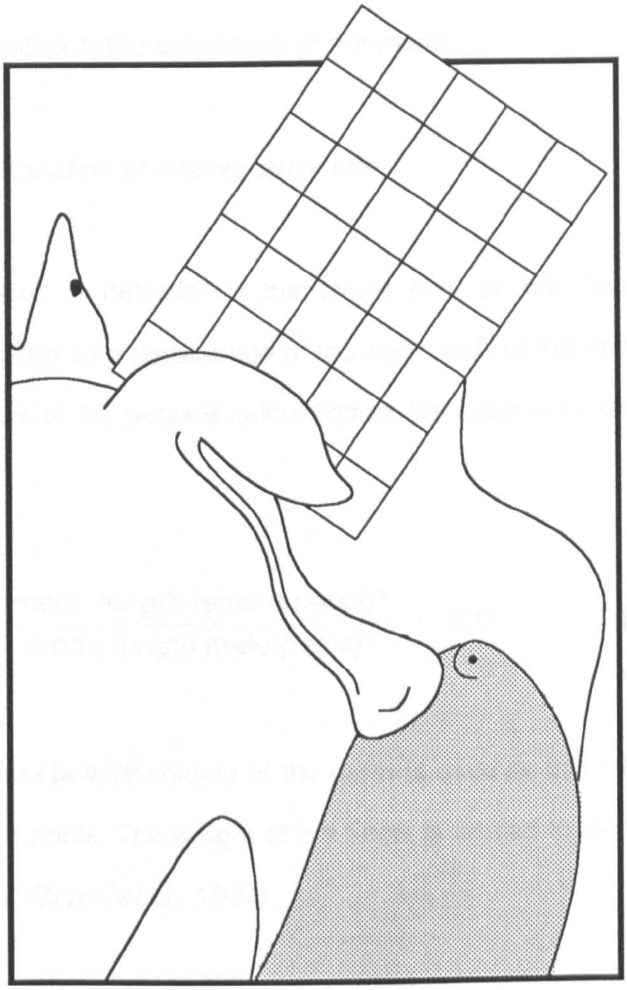


Figure 3.2 Measurement of penis length in *Nucella lapillus* using 1 mm graduated graph paper.

of bumps or nodules were noted. Individuals found with a bi or trifurcate penis were noted and the single longest part measured (Bryan *et al.*, 1986); this feature was relatively common in females but occasionally also found in males. Females found with a ridge or bump but with no measurable penal outgrowth were recorded as zero mm and included in the calculation of the mean.

3.2.3.2 Calculation of relative penis size

Relative penis size is defined as the mean bulk of the female penis in the population expressed as a percentage of the mean bulk of the male penis (equation 3.1) where the bulk of the penis is calculated as the cube of its length (Gibbs *et al.*, 1987).

$$\text{RPS} = \frac{(\text{mean length female penis})^3}{(\text{mean length male penis})^3} \times 100 \qquad \text{Equation 3.1}$$

An estimation of the bulk or volume of the penis is used as this relates more closely to the mass of the penis. The weight of the penis is related to the cube of its length as in equation 3.2 (Bryan *et al.*, 1986).

$$\text{wet weight} = \frac{\text{penis length}^3}{14} \qquad \text{Equation 3.2}$$

Although less accurate than using the penis weight directly the use of penis length makes RPS a quick and accurate index of the level of imposex development within a population (Gibbs & Bryan, 1986).

Since the size of the male penis is known to vary with body size, and body size to vary considerably between sites as a result of environmental factors, the use of RPS allows inter-site comparisons to be made as the average female penis size is related to that of the average male from the same site (Gibbs *et al.,* 1987). Since body size has been removed as a source of variation, the difference between RPS values at different sites is assumed to be due to differing exposure to TBT.

Large sample sizes are not required to give good values of RPS (Gibbs *et al.,* 1987). In adults the variation in the penis size within one population is not large in either males or females. Generally once cessation of growth has stopped variation in penis size in both males and in females is small (Gibbs *et al.,* 1987). Where possible the recommendation of a sample size of at least 30 individuals was followed to calculate RPS values (Spence, 1989).

The nature of the measurement of RPS values means that repeatability between workers is good (Gibbs *et al.,* 1987) allowing good comparisons between data already collected and new work presented here.

3.2.4 Vas deferens sequence index

Although the relative penis size gives an indication of the intensity of imposex, it gives no measure of the reproductive capacity of the females in that population. In addition, since the development of the vas deferens precedes that of the penis, measurement of the vas deferens is especially important in populations exposed to low levels of TBT. The development of the vas deferens was assessed using the vas deferens sequence (VDS) index. This sequence was originally based on a three point scale (Gibbs & Bryan, 1986) which was later extended to six stages

(Gibbs *et al.*, 1987) categorising the development of the vas deferens in the female by examination of the penis, vas deferens, capsule gland and genital papilla.

The VDS stage of each female dogwhelk was assessed by first cutting longitudinally across the mantle skirt of the individual between the hypobranchial gland and the capsule gland. The mantle was then peeled to the left to reveal the medial surface and the state of vas deferens was assessed according to the scale derived by Gibbs *et al.* (1987) (table 3.2, figure 3.3) categorising imposex development from stage 0 (normal) to stage 6 (grossly affected).

Development of the proximal section of the vas deferens close to the genital pallial signals the onset of imposex development (stage 1). There then follows the initial development of the penis (stage 2) and the distal section of the vas deferens (stage 3). The distal and proximal sections of the vas deferens join (stage 4) whilst the penis enlarges to a size comparable to that of the males. Up to stage 4 the female is still capable of releasing egg capsules, but with the proliferation of the vas deferens close to the genital papilla the vulva is eventually occluded (stage 5) rendering the female effectively sterile. With the vulva blocked egg capsules, unable to be released accumulate in the capsule gland forming a tightly bound mass (stage 6) consisting of anything from one egg capsule to over one hundred (Gibbs & Bryan, 1986). In all females observed with blocked or blistered vulva (stage 5) the capsule gland was split with a longitudinal cut and then examined for the presence of aborted egg capsules.

In juvenile *Nucella lapillus* the vulva was often not fully developed and the gonad not differentiated, however, the sperm ingesting gland was still present allowing identification of the sex of the individual. A penis, if present, was obvious and the proximal section of the vas deferens could be observed in the region where the

Table 3.2 Definition of the stages in the development of imposex, based upon the vas deferens sequence (VDS) (Gibbs *et al.*, 1987) illustrated in figure 3.3 (Gibbs & Bryan, 1987) and showing the water concentrations of TBT at which the stages are initiated (Gibbs *et al.*, 1988).

Stage	Vas deferens development	Penis development	TBT in water
0	No male characteristics, 'normal' female.		
1	Development of proximal section of vas deferens, commencing by infolding of the mantle cavity epithelium in the region ventral to the genital papilla.		<0.5 ng Sn/l
2		Penis development initiated with the formation of a ridge behind the right tentacle.	
3	Formation of distal section of vas deferens commences from the base of the penis.	Small penis formed.	
4	Proximal and distal sections of the vas deferens fuse.	Penis enlarging to a size approaching that of the male.	
5	Proliferating vas deferens tissue overgrowing genital papilla cause vulva to be displaced, constricted or no longer visible.		2 ng Sn/l
6	Lumen of the capsule gland contains the material of aborted egg capsules, may comprise single egg or several capsules compressed together to form a translucent or dark brown mass.		

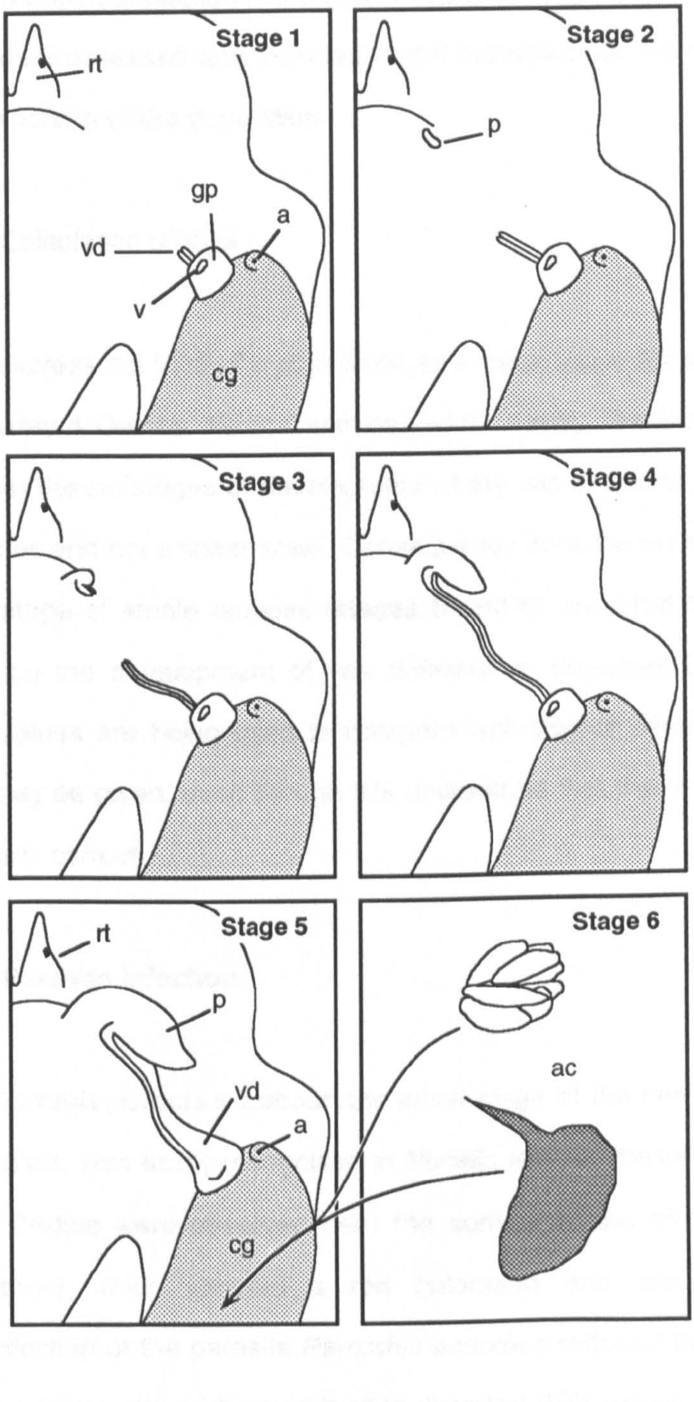


Figure 3.3 Six stages in the development of imposex in *Nucella lapillus* from its initialisation (stage 1) to sterilization (stage 5) and the subsequent accumulation of aborted egg capsules in the capsule gland (stage 6). Abbreviations: a, anus; ac, aborted capsules; cg, capsule gland; gp, genital papillia; p, penis; rt, right tentacle; v, vulva; vd, vas deferens (Gibbs & Bryan, 1987).

vulva would develop. Consequently the extent of the development of imposex in an individual could be assessed and included in the calculation of the imposex value for the juvenile portion of the population.

3.2.4.1 Calculation of VDS

Many authors express the VDS of a population as a mean value (for example Gibbs *et al.,* 1987; Bailey & Davies, 1989; Oehlmann *et al.*, 1991). Statistically this is not strictly correct as the six stages of development of the vas deferens are a series of ranked categories and not a linear scale. Consequently here the median VDS value and the percentage of sterile females (stages 5 and 6) are used to express the effect of TBT on the development of vas deferens in dogwhelk populations. If, however, the values are being used to compare with that of other workers then mean values may be given, even though it is understood that these values are not strictly statistically correct.

3.2.5 Parasite infection

Infestation of *Cercaria purpurae*, Lebour, the larval stage of the trematode parasite *Parorchis acanthus*, was easily recognised in *Nucella lapillus*, the intermediate host (Rees, 1940). Rediae were observed near the surface of the affected digestive gland and gonad which showed a red coloration and generally granular appearance. Infection of the parasite *Parorchis acanthus* reduced the penis size in affected males (chapter 6) which could lead to distorted RPS values. In addition the distinctive red coloration of the gonads of affected individuals often made the sex determination of these whelks difficult as the sperm ingesting gland was often obscured (personal observations). Consequently individuals found infected with parasites were not included in the calculations of RPS and VDS.

3.3 Seawater collection and analysis

Water samples were collected in acid washed glass bottles (McKie, 1987) with wide necks. During collection, the neck of the bottle was submerged halfway so as to include the surface microlayer in the sample due to its role in the food chain. All samples were acidified after collection with the addition of 5 ml of concentrated hydrochloric acid per litre of seawater. Where possible these samples were stored in the dark or diffuse light to reduce photolytic decomposition of tributyltin in the sample (Maguire *et al.*, 1983) and were extracted within 5 days.

Methods of extraction followed those of Bryan *et al.* (1986, 1987). Unfiltered seawater was used in the analysis to include those particles normally available to marine organisms. The samples were extracted in 2 litre glass separating funnels. To achieve standardisation half of each replicate sample was spiked with 25 µl of 1 ppm tributyltin oxide in ethanol. After the addition of 5 ml of hexane these samples were manually shaken for 4 minutes and then left to stand for a couple of minutes. The acidified seawater was drained through the tap on the bottom of the separating funnel and the hexane collected in an acid washed glass vial. Blanks were prepared using distilled water and pre-extracted seawater.

Prior to injection the hexane extract was shaken with 1 ml of 1M sodium hydroxide (NaOH) and allowed to stand for a few minutes allowing the hexane settle on top. This washing process was used to remove dibutyltin (DBT) from the sample thus allowing the tributyltin (TBT) fraction to be measured on its own (Bryan *et al.*, 1986; McKie, 1987). The hexane extract was injected in 50 µl aliquots into a Perkin Elmer 76B graphite furnace attached to a Perkin Elmer 603 atomic absorption spectrophotometer. Information on the conditions and settings used for the determination of tin using the carbon furnace and atomic absorption spectrophotometer are given in Bryan, *et al.* (1986). The detection limit was in the order of 0.5 ng Sn/l.

3.4 Tissue analysis

Analysis of tissues for tributyltin (TBT), dibutyltin (DBT) and total tin (TBT + DBT) was carried out using samples pooled from a number of individuals from a selected site as recommended for pollutant studies (Bryan *et al.*, 1985). This type of analysis smoothes out the effects of small scale individual variations at selected sites which is useful when long term variations in contaminant bio-availability are of interest (Phillips & Segar, 1986).

The methods for the extraction and determination of the levels of contaminants followed the methods of Bryan *et al.* (1986, 1987) which gave detection limits in the order of 0.02 µg/g tin dry weight.

The soft tissues to be analysed had been previously pooled in weighed vials, re-weighed and frozen for future analysis. These samples were allowed to defrost and then homogenised with 2 ml of distilled water. Aliquots of the homogenate, each containing about 0.2 g of dry tissue were placed in each of three, 30 ml stoppered boiling tubes. The remaining homogenate was dried at 60 °C for 24 hours, then used to determine the water content of the sample.

The first of the three tubes was spiked with 20 µl of 10 ppm tributyltin oxide in ethanol, the second with 20 µl of 10 ppm dibutyltin dichloride and the third was left containing just the homogenate. All of these tubes were mechanically mixed and then allowed to stand for at least 1 hour. Concentrated hydrochloric acid, in 5 ml aliquots was added to each tube which was then mixed again, this time being allowed to stand for a minimum of 30 minutes. Each of the tubes was shaken mechanically for 15 minutes after the addition of 5 ml of hexane and then centrifuged at 200 rpm for 5 minutes. After the addition of 5 ml of distilled water they were centrifuged again, using the same speed settings, for 5 minutes. All the samples were left to stand in the fridge overnight prior to analysis the next day. Reagent blanks were put through the same procedure and were consistently found to be below detection limits.

Hexane extracts from each tube were injected into the carbon furnace in 50 µl aliquots (see section 3.3 for information on the carbon furnace). Then, 2 ml of the hexane extract from each of the tubes containing the TBT spiked homogenate was mixed with 1 ml of sodium hydroxide (NaOH) in acid washed glass vials and shaken for 1 minute, in order to remove DBT from the samples. These 'washed' hexane extracts were then injected. The NaOH washing procedure was repeated for the hexane from each of the tubes containing the unspiked homogenate samples. Hexane from the tubes containing homogenate spiked with DBT were not washed, or re-injected. Tin as TBT was determined from the NaOH treated extracts and DBT from the subtraction of the TBT data from the untreated extractions (Bryan *et al.*, 1986). Earlier studies on dogwhelk tissues spiked with organotins showed that this procedure largely separates TBT and DBT from each other and from monobutyltin (MBT) (Bryan *et al.*, 1986). However it has since been stressed that these relatively simple methods do not provide complete speciation of TBT and its metabolites (Bryan *et al.*, 1993b).

Unless otherwise stated concentrations of TBT or DBT fractions throughout this work are expressed as µg/g dry weight or ng/l of tin as this was what was measured, rather than as concentrations of TBT or DBT which would be about 2.5 or 2 times higher respectively (Bryan *et al.*, 1987; pers. comm. P. E. Gibbs, Plymouth Marine Laboratory). Where necessary, for example for comparison with other work, a conversion factor of 2.44 was used for TBT (Gibbs *et al.*, 1991b) and 1.97 for DBT (pers. comm. P. E. Gibbs, Plymouth Marine Laboratory). Concentrations of total tin, TBT and DBT in the tissues of dogwhelks can be converted from a dry weight basis to a wet weight basis by dividing the values by 3.3 for males and 3.0 for females (Bryan *et al.*, 1987).

3.5 Marking of experimental areas

Experimental areas were marked using numbered tags. Holes for the tags were drilled using a two-stroke Ryobi petrol-driven hammer-action drill with a 7 mm drill bit. A 3 cm galvanised screw was placed through a 2 x 2 cm piece of fluorescent plastic 'twinglo' tape and screwed firmly into a rawlplug which had been inserted into the hole. The 'twinglo' tape was of the sort used to mark off areas during road maintenance work and was orange on one side and yellow on the other. This allowed experimental areas to be colour coded, for example orange for areas without dogwhelks and yellow for areas with dogwhelks. For additional identification the tape was marked with a number and letter code using a permanent marker.

The brightness of the tape made areas visible from about 10 m away. This was far enough away to be able to relocate the sites, until they became familiar, but equally far enough away to prevent curious attention from people on the top of the shore. Although the initial brightness of the tape dulled after a month on the shore the tags persisted for over 15 months, the duration of the longest experiments. Only the coded numbers and letters needed regular renewing.

3.6 Measurements of percentage cover

The percentage cover of *Fucus vesiculosus*, *Semibalanus balanoides* and bare rock was measured using a 0.5 x 0.5 m quadrat. This was sub-divided with string attached across the quadrat, creating a 5 cm border around the inside edge and dividing the remaining area into 10 x 10 cm squares. Consequently the quadrat was sectioned into four 5 x 5 cm areas, sixteen 10 x 5 cm and sixteen 10 x 10 cm areas and created 25 intersection points. If the quadrat had been divided up solely by 10 x 10 cm squares only 16 intersection points would have been created, unless the edges of the quadrat were included as intersection points, in which case there would have been 36. Using the sides of the quadrat, however, creates an edge effect especially when sampling adjacent areas, where points on the common side of connecting quadrats will be counted twice (for example see Hawkins & Hartnoll, 1983). The use of a border around the inside of the quadrat, as described, eliminates any edge effects.

The percentage cover of *Fucus* canopy was measured under the quadrat by using the string intersections as sighting points. The number of intersections under which *Fucus* was found, out of a possible 25, was recorded and expressed as a percentage. *Fucus vesiculosus* plants were recorded as canopy once they were around 10 cm tall which was the observed length at which they did not to stand upright any longer but instead laid flat against the substrate. Whilst they were still standing upright (<10 cm) they were classed as under-storey algae (Hawkins & Hartnoll, 1983). Substrate cover of barnacles, bare rock or under-storey algae under the quadrat was estimated subjectively as the total number squares occupied and was measured to the nearest 2%.

3.7 Barnacle clearance areas

When barnacle clearance areas were to be used a 5 x 5 cm quadrat was randomly located on the rock and existing barnacle matrix. The number of living and dead barnacles within this area was counted and recorded. The areas were cleared using a flat bladed paint scraper and the quadrat as a template. Once scraped, shell debris from the area was removed by washing these areas with seawater and a toothbrush.

3.8 Counts of newly settled barnacles

Counts of newly settled barnacles were recorded as numbers of cyprids and metamorphosed spat. The colour of the metamorphosed barnacle appeared pinky-orange in the first instant but became white after about 2 days. Generally, settling cyprids metamorphosed after 2-4 tides (1-2 days) (personal observations).

3.9 Statistical methods

Where possible, all analysis was performed using parametric rather than non-parametric statistical methods as the former are more powerful when used correctly (Zar, 1984). Parametric testing assumes that the data agrees with a number of criteria. The most important of these assumptions are that the data are normally distributed and that they have homogeneous variances. Although analysis of variance is relatively robust to slight deviations from these assumptions (Underwood, 1981; Zar, 1984) all data were tested for normality using the NSCORE command in the statistical software package MINITAB and for homogeneity of variances using the F-max test (Winer *et al.*, 1991; Fowler & Cohen, 1992). Where necessary data was transformed and then re-tested. Transformations used included

$\log_{10}$ and square root methods, or where zero values were found in the data, $\log_{10}(x + 1)$ and square root $(x + 0.5)$. Arc sin transformations were used for percentage data. Where transformed data still showed large deviations from normality and high heterogeneity non-parametric tests were used instead.

Multiple comparisons tests were performed where ANOVA was found to be significant and where more than two treatments were involved. There are a multitude of tests available to test for significant differences between levels of a treatment and much confusion in which are the best to use. Following recommendations by Mortimer (pers comm. M. Mortimer, Liverpool University) and (Maxwell & Delaney, 1989) I have opted to use the Tukey test for unplanned comparisons. The Tukey test is calculated in a identical manner to the Student-Newman-Keuls procedure (SNK test) recommended by Underwood (1981) differing only in the calculation of the critical value of the F statistic making the Tukey test slightly more conservative than its SNK counterpart.

3.10 Scientific names

Scientific nomenclature throughout this thesis follows Bruce *et al.* (1963) except where otherwise stated.

3.11 Grid references

All grid references given in this thesis are taken from Ordnance Survey maps in the 1:50000 Landranger series and are given with letters and a six figure reference referring to the British National Grid, and giving accuracy to the nearest 100 m.

CHAPTER 4

Levels of Tributyltin in the environment after the introduction of legislation in 1987: measured in water and the tissues of the bioindicator *Nucella lapillus*

4.1 Introduction

As employed here the term 'bioindicator' or 'biomonitor' denotes aquatic species which accumulate pollutants in their tissues, and which may therefore be analysed in order to monitor the bio-availability of such pollutants in the environment (Rainbow & Phillips, 1993). The use of biological indicators to determine temporal and geographical variations in the bio-available concentrations of pollutants in coastal and estuarine water is now well established (Bryan *et al.*, 1980; Phillips, 1980; Bryan *et al.*, 1985). Essentially their use provides a direct index of pollutant bio-availability rather than of pollutant concentration (Phillips & Segar, 1986). Furthermore they reflect contamination over a period of time, instead of giving a 'snap shot' figure which occurs when measuring contaminants directly from the water (Phillips, 1977; Phillips & Segar, 1986; Rainbow & Phillips, 1993). This is especially relevant since concentrations of pollutants in the water can show considerable temporal variation (e.g. over a tidal cycle Clavell *et al.*, 1986) and spatial variation (e.g. with the position in the water column Cleary & Stebbing, 1987a; Cleary & Stebbing, 1987b). In addition measurement of the levels of contaminants in the water does not give any idea of the availability of the contaminant to organisms (Phillips, 1980). The ideal bioindicator should meet certain criteria; they should be sedentary, abundant, easy to identify and be suitable for laboratory studies and field transplants (see Phillips, 1977; Bryan *et al.*, 1980; Phillips, 1980; Bryan *et al.*, 1985). The selection of the bioindicator is very important and needs careful consideration.

The accumulated metal concentration within the tissues of the chosen bioindicator will reflect pollutant availability for that particular organism. Thus ultimately the concentration will be influenced by the routes of pollutant uptake, residence time within the tissues and the rate of loss, metabolisation and degradation. The possible routes of pollutant uptake include those from solution, suspended particles, sediment and via food (Rainbow & Phillips, 1993). Consequently the levels of contamination will, therefore, depend upon the feeding mechanisms of the organism concerned. Primary producers obtain their nutrients directly from seawater and hence would be expected to reflect ambient levels of contamination in the water column. Heterotrophs, by comparison, have two possible mechanisms of pollutant uptake: directly from the water and via their food. Similarly, suspension and deposit feeders have in addition to contaminant uptake via the water and food a possible uptake route through contaminated sediments.

Tributyltin compounds were introduced as biocides in antifouling paints in the 1960's (Stebbing, 1985). They are now recognised as probably the most toxic pollutants deliberately introduced into the marine environment (Goldberg, 1986). The detrimental effects of low concentrations on marine life are well established (see chapter 1 and Bryan & Gibbs, 1991 for reviews) with damaging effects at levels far below those yet recorded for other marine pollutants.

In water organotin concentrations have been reported as high as 40 ng/l in inshore waters and up to 880 ng/l in harbours and marinas in the south-west of England (Cleary & Stebbing, 1985). These concentrations will, however, vary with season (Hall, 1988) and tidal cycle (Clavell *et al.*, 1986; Cleary, 1991). High concentrations tend to remain localised because of the ionic nature of TBT (Goldberg, 1986) and restricted water exchange of harbours and marinas. Enhancement of the concentrations occurs in the surface microlayer due to the lipophilic nature of TBT and the concentrations of rich organics and lipids found in this top 300 μm layer of the water (Cleary & Stebbing, 1987a). At some sites, organotin levels in the microlayer have been recorded as being 27 times higher than in the sub-surface water (Cleary & Stebbing, 1987b). This poses an obvious risk to the neuston which includes micro-organisms, protozoa and larvae from most invertebrate taxa (Cleary & Stebbing, 1987b; Hardy *et al.*, 1987). In addition the high organotin concentration in the surface microlayer may also affect organisms resident in the littoral zone, since they will be exposed to the microlayer deposited on the sub-strata as the tide recedes (Cleary & Stebbing, 1987a). The nature of exposure for littoral organisms is therefore different and potentially much greater than for other communities that dwell in sub-surface waters. Since TBT readily absorbs to surfaces, organisms that live and graze on them may be exposed to even higher concentrations than those in the water would suggest (Cleary, 1991).

Bioindicators used to measure tributyltin in the environment have included the tellinid bivalve, *Scrobicularia plana* (Langston & Burt, 1991), Pacific Oyster, *Crassostrea gigas* (Alzieu *et al.*, 1986), Atlantic Salmon, *Salmo salar* (Davies & McKie, 1987) and Atlantic dogwhelk, *Nucella lapillus* (Bryan *et al.*, 1986; Bryan *et al.*, 1987; Gibbs *et al.*, 1987; Gibbs *et al.*, 1988; Spence, 1989). *Nucella lapillus*, especially, has an unique combination of characters that make it ideal as an indicator of TBT contamination (Gibbs *et al.*, 1987). Briefly *Nucella* has the following characteristics: it is widely distributed, easily recognisable, common, has a limited potential for dispersal since development is direct and adults are relatively immobile, is hardy, allowing transplantation and laboratory based studies and is relatively long-lived. Thus satisfying all the criteria for a suitable bioindicator (Phillips, 1977; Bryan *et al.*, 1980; Phillips, 1980; Bryan *et al.*, 1985).

Studies of pathways to evaluate uptake of *organotins* have, in the most part, concentrated on uptake in aquatic organisms via direct exposure to TBT, for example in mussels (Laughlin *et al.*, 1986). This is an approach more relevant to filter feeders or small organisms at low trophic levels which characteristically have a large surface areas to volume relationship facilitating uptake.

Evidence from previous studies, using heavy metals, has highlighted the importance of the diet in the accumulation of contaminants in *Nucella lapillus*. The diet was found to be the major source of Zn, Fe (Young, 1977) and As (Klumpp, 1980) in *Nucella* feeding on *Semibalanus balanoides* and *Littorina littoralis* (probably *Littorina obtusata*) respectively. Bryan *et al.* (1989b) concluded that under favourable laboratory conditions *Nucella* could obtain half or more of its body burden of TBT from a diet of *Mytilus edulis*. Similar laboratory studies using *Semibalanus balanoides* (Spence, 1989) however, gave unclear indications as to the importance of diet for TBT uptake, generally suggesting that the diet was unimportant. In the field the diet may account for less than 50% of the body burden (Bryan *et al.*, 1989b). This is probably because short term factors such as the weather (Burrows & Hughes, 1989) and longer term seasonal variation in feeding behaviour (Feare, 1970a) affect the food uptake in longer term field studies but are controlled in the laboratory based experiments.

The type of prey that *Nucella* is feeding upon is bound to be important in determining the ultimate levels of contamination, especially if the diet is as important a source of TBT as indicated in laboratory studies (Bryan *et al.*, 1989b). Spence (1989) reported a higher body burden of TBT in the tissues of filter feeding mussels and barnacles than in the tissues of grazing limpets from the same sites. Consequently *Nucella* feeding on their more usual prey of barnacle or mussels would be expected to have higher tissue burdens of TBT than those feeding on limpets.

The aims of the work reported in this chapter were to monitor changes in levels of tributyltin in the environment following the introduction of legislation restricting the use of tributyltin antifouling paints in 1987 (Abel *et al.*, 1987; Duff, 1987). To do this the levels of tributyltin were measured in water and in the tissues of *Nucella lapillus,* chosen as the bioindicator. Analyses were made of body burdens of tributyltin (TBT), its breakdown product dibutyltin (DBT) and total tin (TBT + DBT) in *Nucella* and its principal prey *Mytilus edulis* and barnacles (mainly *Semibalanus balanoides*) from five sites in south-west England. In addition concentrations were also recorded in limpets (*Patella vulgata*), a major keystone species on British shores and a recorded prey organism. Thus by looking at concentrations of TBT in the water and food organisms of *Nucella* pathways of uptake can be examined in the field. These can be compared to previous studies by other workers where laboratory based studies have been used.

Changes in the degree of body burdens of TBT in *Nucella* are related to levels in the water and its principal prey organisms, and to known rates of metabolism and loss of TBT from the tissues. Correlations are used to relate the levels of TBT and total tin in the organisms studied to levels of contamination in the water with and without a time lag.

4.2 Materials and Methods

4.2.1 Collection and analysis of samples

Seawater and animals for tissue samples were collected from five sites in the south-west of England: Jennycliff, Kingsand, Renney Rocks, Tregantle and St. Agnes (see chapter 2 for site locations and descriptions). Collections were usually made twice yearly, early in the year between January-March and later, between July-August. These times were chosen to correspond to the previously observed lowest and highest inputs of tributyltin into the environment (Bryan *et al.*, 1987).

Data from the period 1986 to 1989 comes from Spence (1989). Tissue samples collected in the spring and summer of 1990, before this studentship started, were collected by Dr Spence but I analysed them; water samples during the same period were collected and analysed by Dr Spence. All data from October 1990 onwards were collected and analysed by myself.

4.2.1.1 Seawater samples

Water samples were collected from the five sites in 1 litre acid washed glass bottles with wide necks using the methods outlined in chapter 3. All samples were collected at low water (± 30 minutes) on a spring tide, minimising variation in TBT concentrations caused by changes in the tidal cycle (Cleary, 1991). The day time low tide on the springs cycle occurs around 13.00 (Greenwich mean time) at Plymouth (Admiralty tide tables). This also allowed inter-site comparisons to be made and fluctuations in contamination between sampling dates to be put into context. In addition samples taken at high and low water at Tinside (SX 481537) and Mayflower steps (SX 484540) in Plymouth Sound (figure 2.1) are included for reference. These samples form part of a regular monitoring programme by workers at Plymouth Marine Laboratory and represent concentrations of TBT in the environment close to source (pers. comm. G. W. Bryan, Plymouth Marine Laboratory).

Replicate samples were hexane extracted as described in chapter 3 and in Bryan *et al.* (1986). The hexane extracts from these samples were collected in acid washed glass vials and stored in a refrigerator overnight. These were usually analysed the next day after first being washed with sodium hydroxide (chapter 3).

4.2.1.2 Tissue analysis

From each of the five sites adult, second year and juvenile dogwhelks, limpets and barnacles were collected for tissue analysis. In addition mussels were collected from those sites where they were common (Tregantle and St. Agnes). The dogwhelks used in the tissue analysis had been collected for imposex assessment, from each of the sites, using the methods described in chapter 3. Mussels and barnacles were collected within a size range known to be preferred by *Nucella*: mussels 20-25 mm in length (Hughes & Dunkin, 1984a), barnacles 4-5 mm opercular diameter (Dunkin & Hughes, 1984). Analysis was carried out on pooled samples as recommended for pollutant studies (Phillips & Segar, 1986; Bryan *et al.*, 1987). The soft tissues of five individuals in the case of dogwhelks, mussels and limpets and 100 individuals for barnacles were used. Dogwhelks were analysed in five different categories: adult males, adult females, second year males, second year females and juveniles. Any individuals found infected with the parasite *Parorchis acanthus* were not used in the analysis. All of the mussels analysed were *Mytilus edulis* and all the limpets *Patella vulgata*. In November 1990, however, additional samples were taken from the five sites to compare the level of contamination in *P. vulgata* with its commonly co-occurring species *P. depressa*. The barnacles analysed from each site represented the food available; at four of the sites this was predominantly *Semibalanus balanoides* and at the fifth site (Kingsand) this was *Chthamalus montagui* (Spence, 1989). Burrows (1988) reported that it is the size and not the barnacle species which is important in the prey selection of *Nucella lapillus*.

The extraction and determination of tributyltin (TBT), dibutyltin (DBT) and total tin (TBT + DBT) in the tissue samples followed the methods described in chapter 3 and in Bryan *et al.* (1986, 1987), which gave detection limits in the order of 0.02 µg Sn/g dry weight.

4.2.2 Statistical methods

Data were analysed following the methods described in chapter 3. Two-way analysis of variance was used when testing for differences in the degree of contamination in the body burdens of TBT, DBT and total tin and in the water at the five sites over time. All data were log transformed before analysis. Since replicate water samples had not been taken on each sampling date and tissue samples were based on pooled analysis the two-way ANOVA was calculated without replication. This analysis assumes that no interaction occurs between the two factors, which in natural systems is often difficult to predict. However, if a significant result is obtained this may be accepted although there is an increased risk of a type II error, that is that the test may be over conservative.

Differences in the body burdens of TBT, DBT or total tin between different ages and sexes of *Nucella*, barnacles mussels or limpets were tested using a randomised block analysis of variance, since the statistical term 'block' is an extension of the term 'pair' and the resultant test can be more powerful than unpaired ANOVA (Zar, 1984), especially where a gradient is expected. Again there was no replication within the blocks. The null hypothesis of differences between the blocks is generally not of interest in these tests and anyway would require the calculation of an interaction term and thus was not tested for.

Correlations were calculated between concentrations of tributyltin in water and the levels of contamination in the tissues of the organisms sampled with and without a lag phase.

4.3 Results

4.3.1 Tributyltin concentrations in the water

The average level of TBT in Plymouth Sound varied with the tidal cycle and with the season (figure 4.1). Generally, higher concentrations were recorded at low water than at high water and were seasonally higher in the summer, than during the winter months. Concentrations were highest at the Mayflower Steps, the site closest to a large marina (figure 2.1), but a sharp decrease in levels of contamination was shown with lower concentrations at Tinside only half a mile away, and in the open water of Plymouth Sound. The general trend in the level of TBT contamination has been downward at these two sites since the summer of 1987.

The concentrations of TBT in the water at the other sites sampled all showed fluctuations over time (figure 4.2). These fluctuations did not reflect the seasonality observed in the samples from the Mayflower Steps and Tinside. Variation between the replicate samples when taken was low suggesting sampling methodology was adequate. The only sample where standard errors indicate above 10% variation was at Kingsand in May 1987, when the weather was bad and there was considerable sediment in the water (Spence, 1989). Concentrations in the water at St. Agnes remained the lowest of the sites sampled throughout the period of study. By 1993 levels of contamination in the water at Tregantle and Renney Rocks, on the outskirts of Plymouth Sound (figure 2.1), had dropped close to the limits of detection of the methods employed.

The degree of TBT contamination in the water at the five study sites was significantly different (table 4.1). Multiple pairwise comparisons (table 4.1) suggest

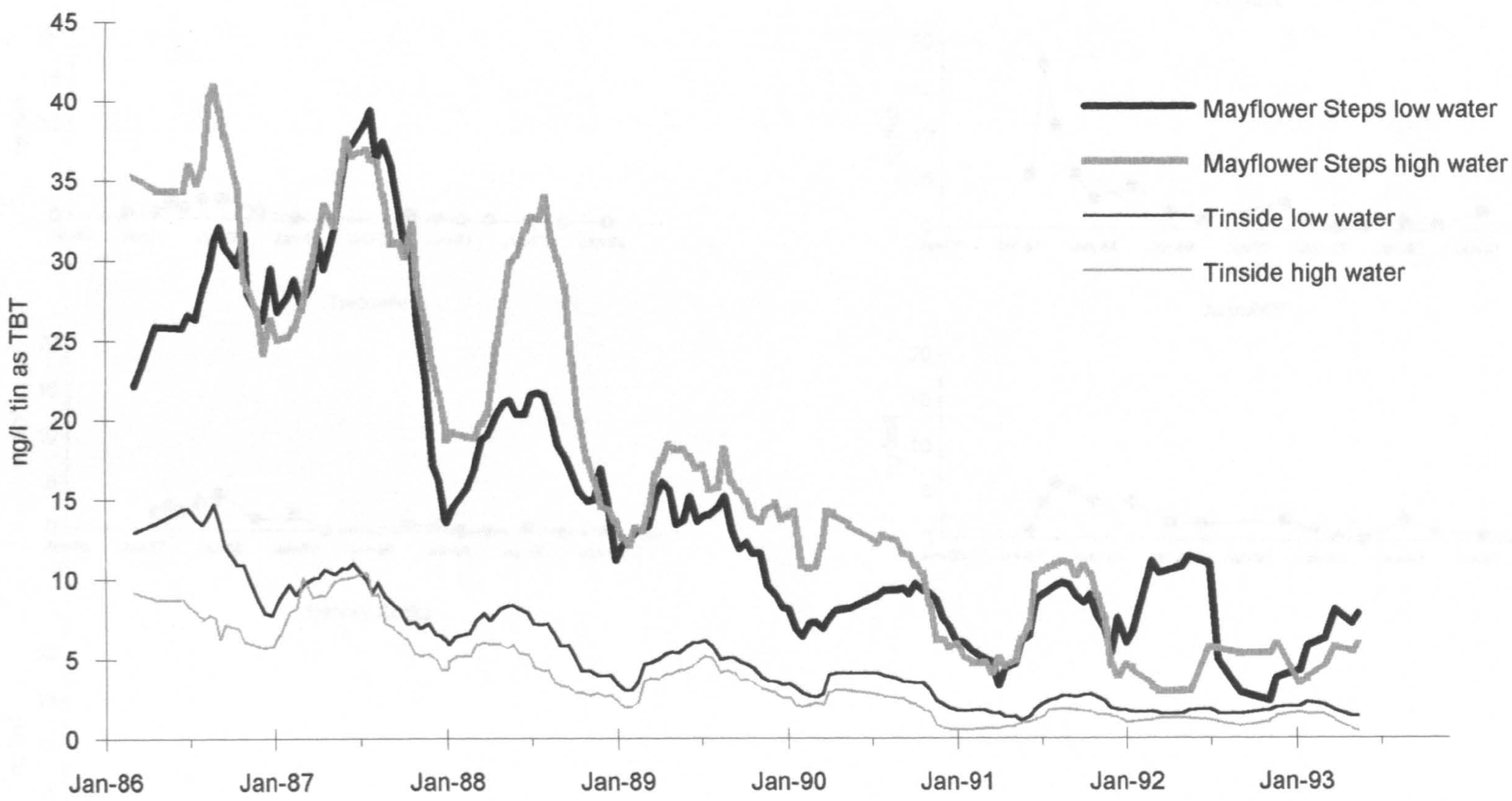


Figure 4.1 Water concentrations of tributyltin (ng Sn/l) in Plymouth Sound, plotted as running means from samples taken twice monthly at high and low water by workers at Plymouth Marine Laboratory.

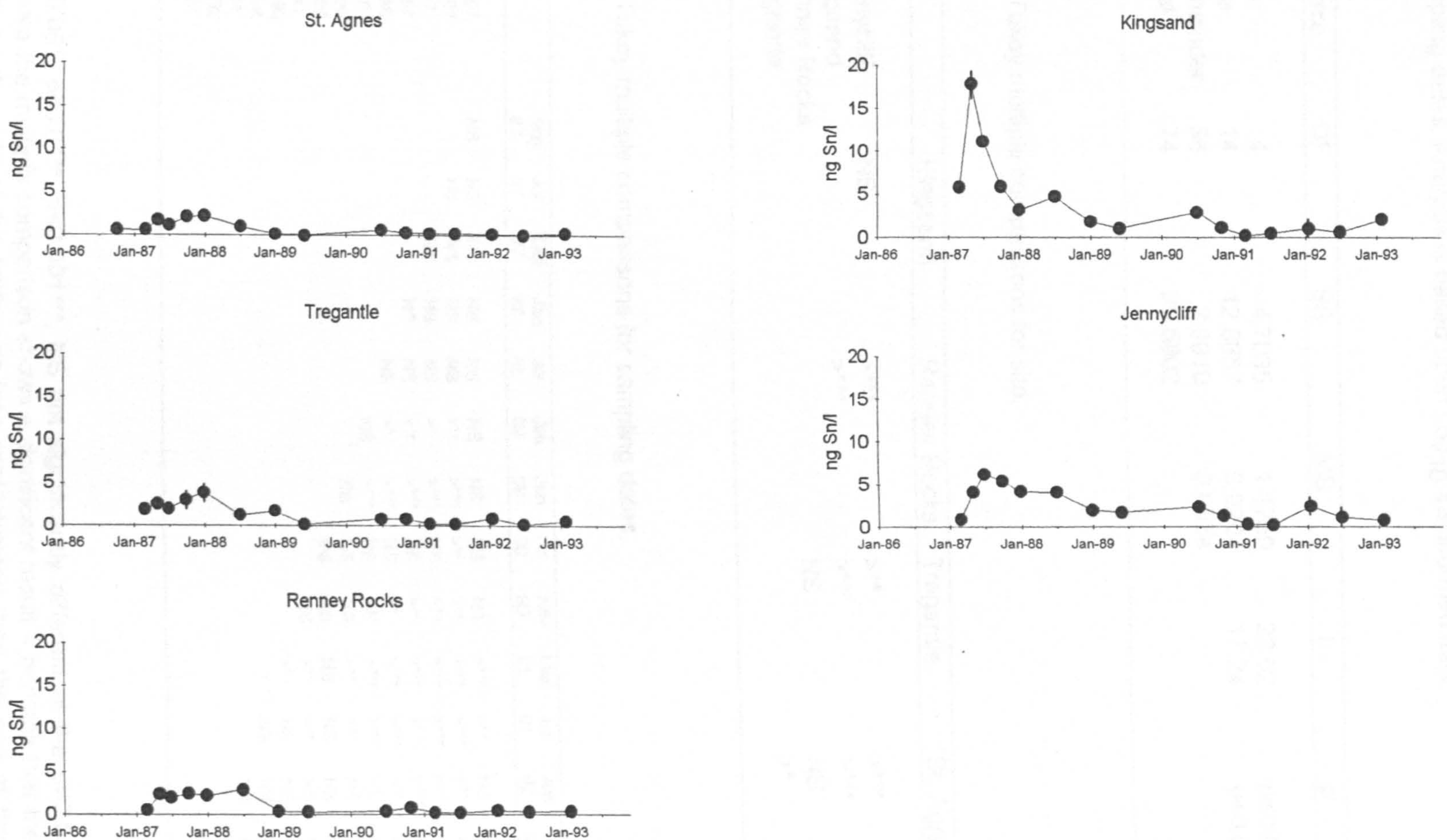


Figure 4.2 Mean water concentrations of tributyltin (ng Sn/l) taken at low water from five sites in the southwest of England. Error bars ± 1 SD. Sites arranged in order of decreasing distance from Tinside (St. Agnes furthest away to Jennycliff closest).

Table 4.1 Two factor analysis of variance without replication of TBT contamination in seawater between the five study sites in south-west England and different sampling dates. Analysis is based upon Log_{10} transformed data.

Source	DF	SS	MS	F	p
Site	4	4.7035	1.1759	22.02	p<0.001 ***
Date	14	12.8857	0.9204	17.24	p<0.001 ***
Remainder	56	2.9910	0.0534		
Total	74	20.5802			

(a) Tukey multiple comparisons for site.

	Kingsand	Renney Rocks	Tregantle	St. Agnes
Jennycliff	NS	>***	>**	>***
Kingsand		>***	>***	>***
Renney Rocks			NS	NS
Tregantle				>*

(b) Tukey multiple comparisons for sampling dates.

	May 87	Jul 87	Oct 87	Jan 88	Jul 88	Jan 89	Jun 89	Jul 90	Nov 90	Mar 91	Jul 91	Jan 92	Jul 92	Feb 93
Mar 87	NS	NS	NS	NS	NS	NS	NS	NS	NS	>***	>**	NS	>*	NS
May 87		NS	NS	NS	NS	>**	>***	>**	>**	>***	>***	>***	>***	>***
Jul 87			NS	NS	NS	>*	>***	NS	>**	>***	>***	>**	>***	>***
Oct 87				NS	NS	>**	>***	NS	>**	>***	>***	>***	>***	>***
Jan 88					NS	>*	>***	NS	>*	>***	>***	>**	>***	>**
Jul 88						NS	>***	NS	NS	>***	>***	>*	>***	>*
Jan 89							NS	NS	NS	>**	>**	NS	NS	NS
Jun 89								NS	NS	NS	NS	NS	NS	NS
Jul 90									NS	>**	>**	NS	NS	NS
Nov 90										>*	NS	NS	NS	NS
Mar 91											NS	NS	NS	NS
Jul 91												NS	NS	NS
Jan 92													NS	NS
Jul 92														NS

p<0.05*, p<0.01**, p<0.001***, NS not significantly different at the p=0.05 level. Where the multiple comparison shows a significant result < denotes that the site or date on the side of the table was less contaminated than the one at the top, > indicates the opposite.

that the sites can be arranged with decreasing contamination in the order Kingsand > Jennycliff > Tregantle > Renney Rocks > St. Agnes.

A general downward trend was seen in the level of TBT contamination during the period of study. This reflected a 5-10 fold reduction at all the sites and was significant over time (table 4.1). After the ban was introduced in 1987, overall levels did not drop significantly until 1989 (table 4.1), two years after the ban had first been imposed. From 1990 there was little difference in TBT concentrations in water although levels did continue to decline. Generally seasonal changes in TBT concentrations in water were not significant, the only exception being changes in concentrations between November 1990 and the higher concentrations recorded in March 1991 (table 4.1).

4.3.2 Tissue burdens

The concentrations of TBT and total tin (TBT + DBT) in *Nucella*, barnacles, mussels and limpets also varied with time and space at the sites sampled (figures 4.3-4.7). In general a downward trend in the body burdens of total tin and TBT was recorded for all organisms at all sites. This was masked only by seasonal variations which were observed in some organisms in the period up to 1989. Analysis showed the decreases in the body burdens of TBT (table 4.2), DBT (table 4.3) and total tin (table 4.4) were all significant with time. Decreases in the concentrations of these contaminants were not significantly less until 1990, three years after the Government ban was introduced. The significant interaction terms (table 4.2, 4.4) show that the body burdens of TBT and total tin responded differently at the different sites with time whilst DBT did not (table 4.3).

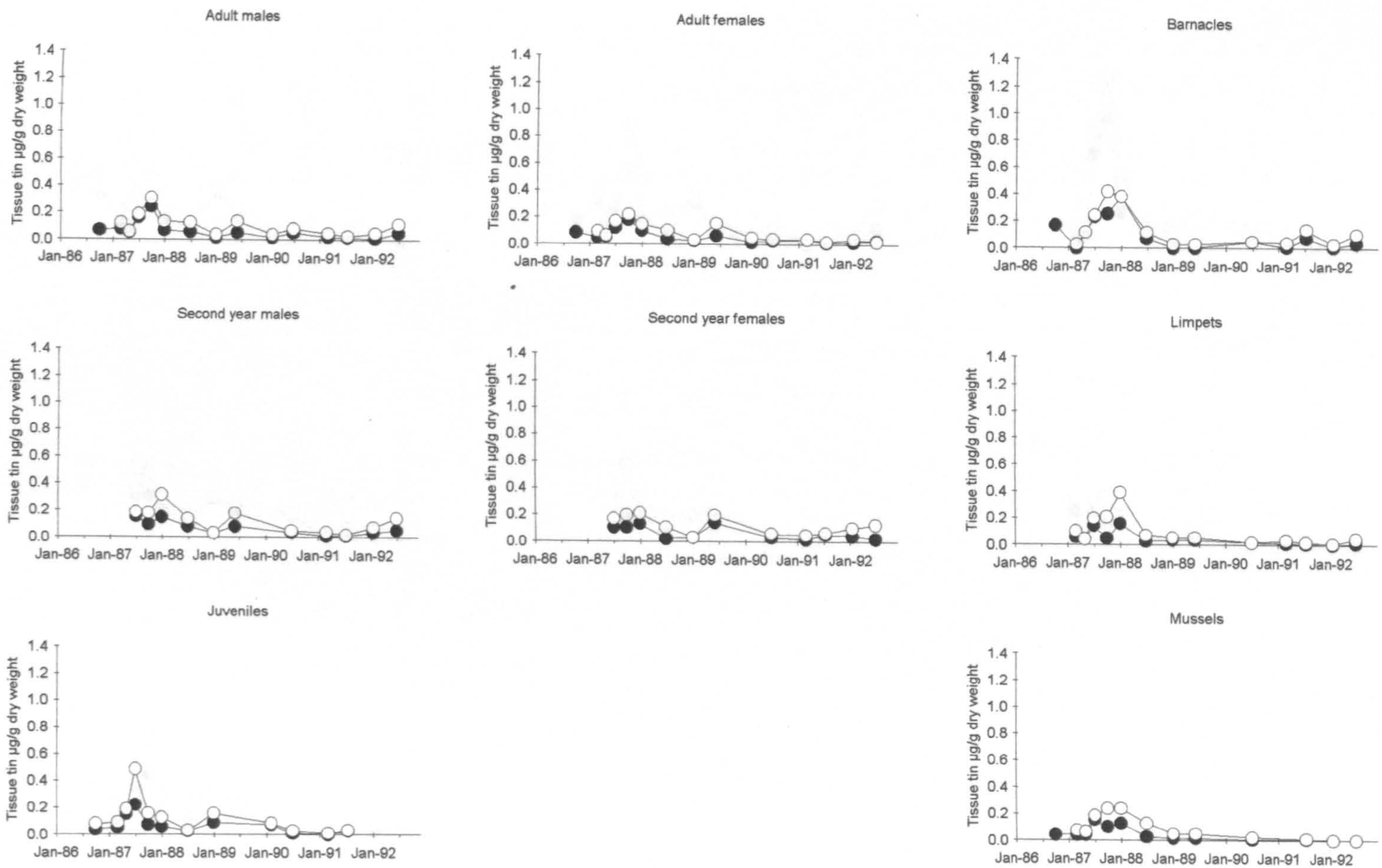


Figure 4.3 Tissue burdens of total tin (TBT + DBT) and TBT in *Nucella* and its principal prey from St. Agnes. Total tin plotted as open symbols, TBT as closed symbols.

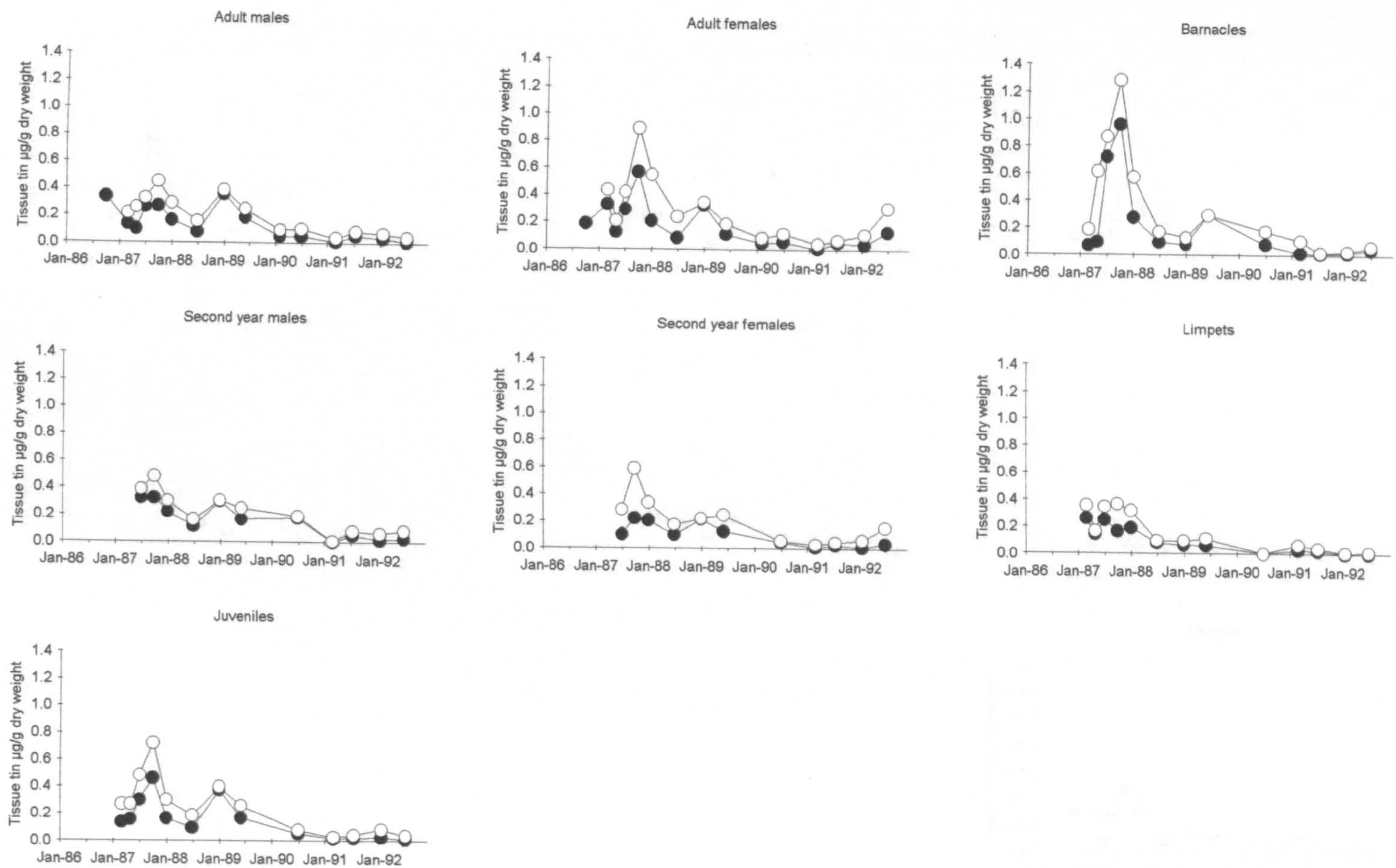


Figure 4.4 Tissue burdens of total tin (TBT + DBT) and TBT in *Nucella* and its principal prey from Renney Rocks. Total tin plotted as closed symbols, TBT as closed symbols.

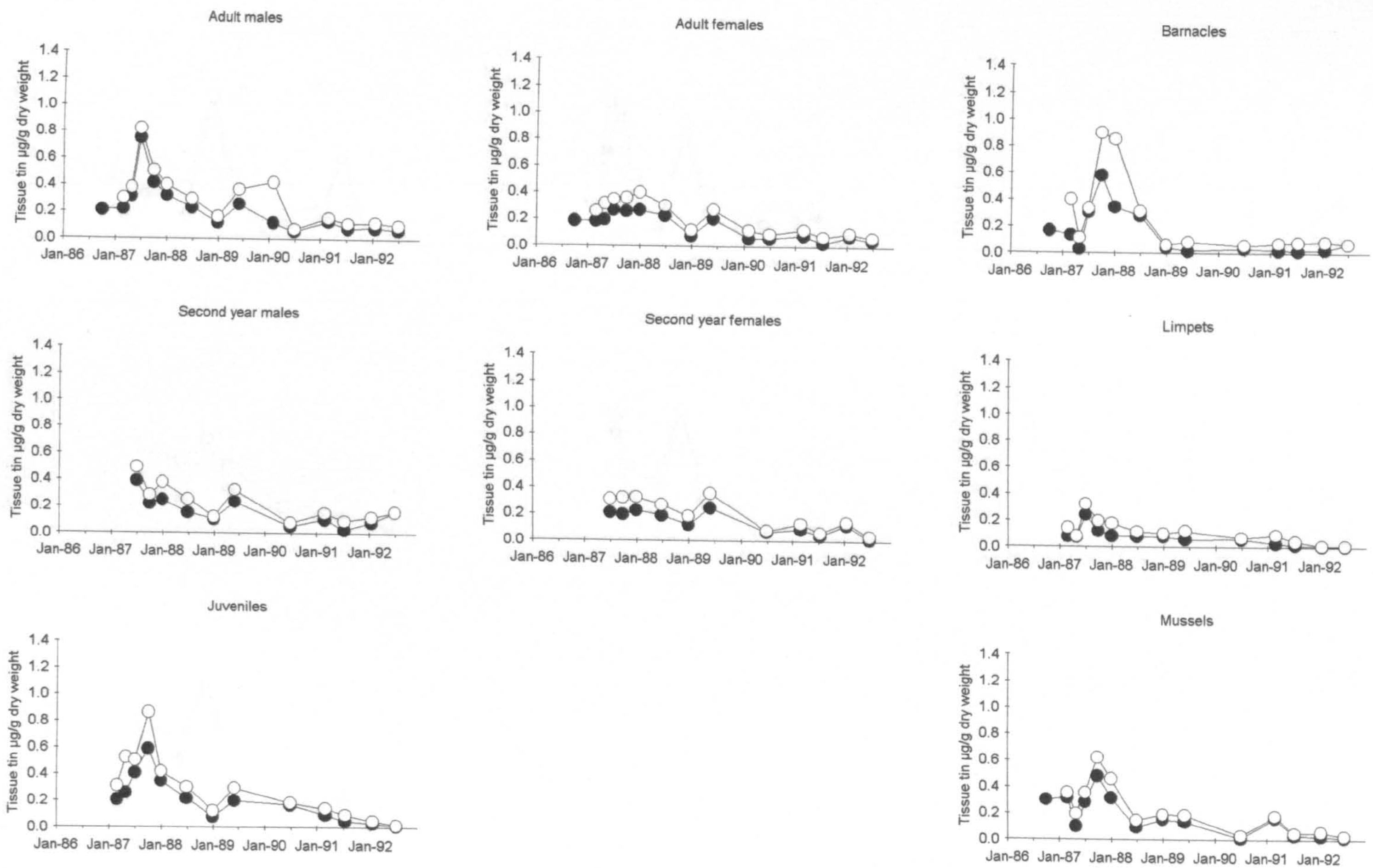


Figure 4.5 Tissue burdens of total tin (TBT + DBT) and TBT in *Nucella* and its principal prey from Tregantle. Total tin plotted as open symbols, TBT as closed symbols.

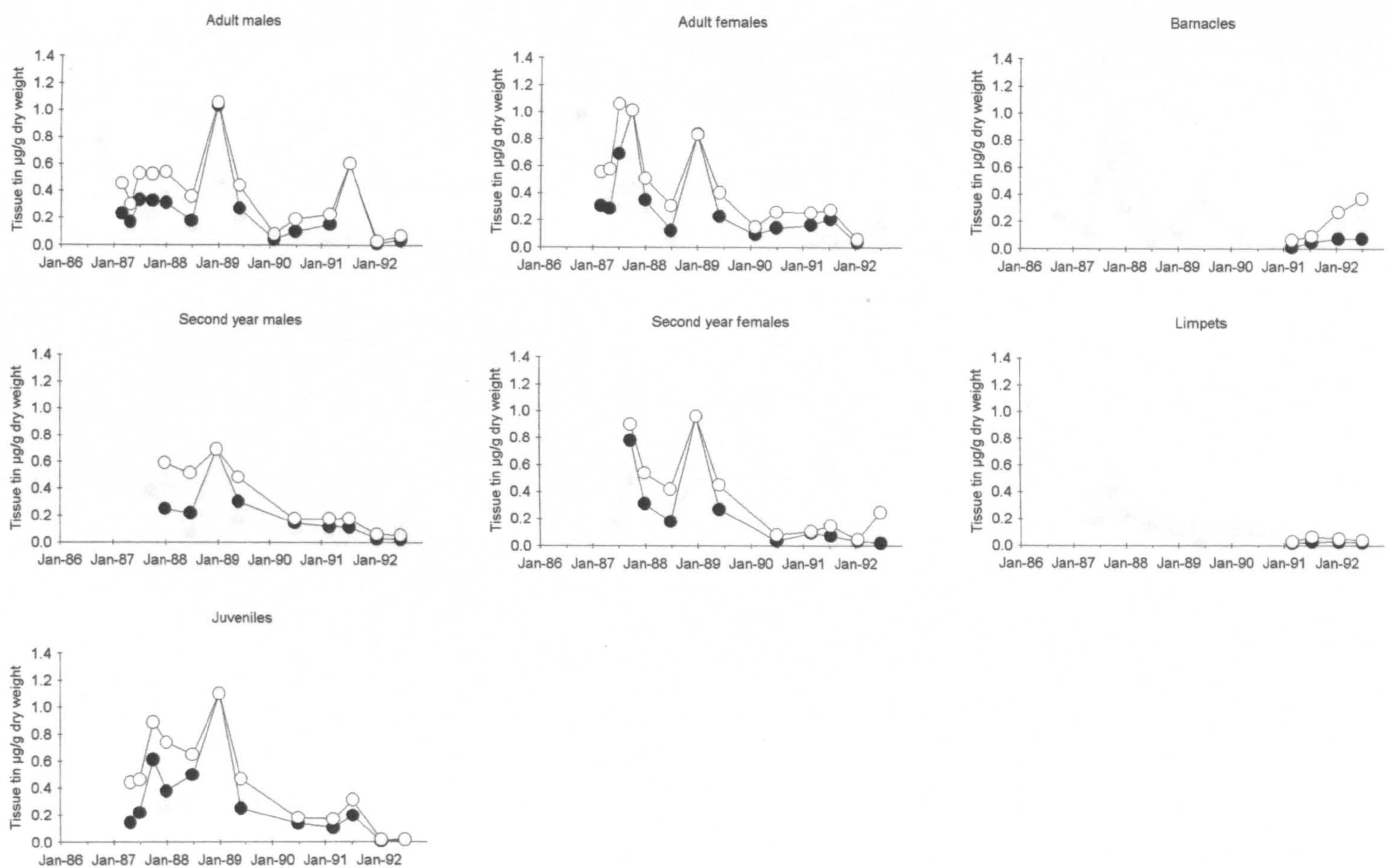


Figure 4.6 Tissue burdens of total tin (TBT + DBT) and TBT in *Nucella* and its principal prey from Jennycliff. Total tin plotted as open symbols, TBT as closed symbols.

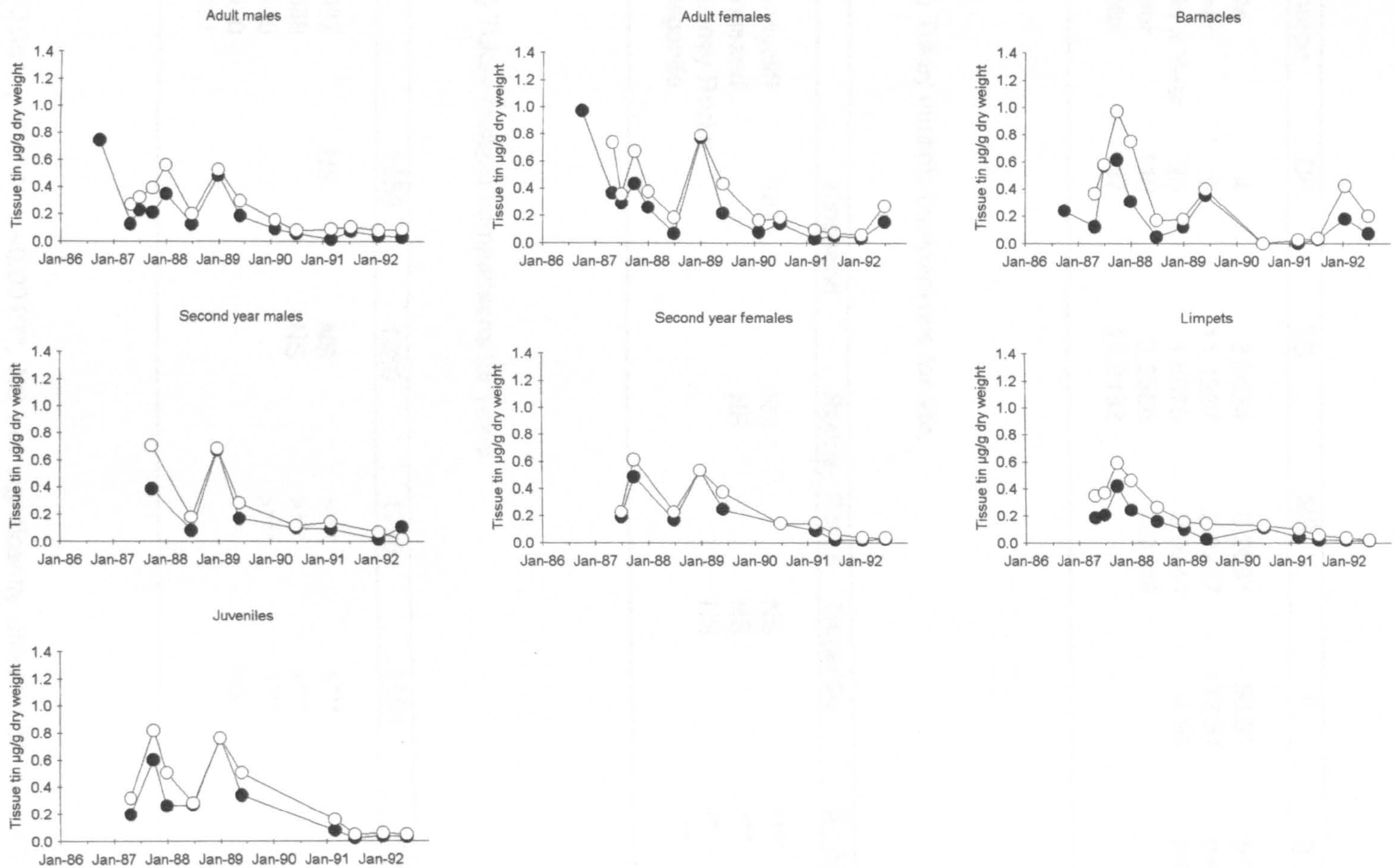


Figure 4.7 Tissue burdens of total tin (TBT + DBT) and TBT in *Nucella* and its principal prey from Kingsand. Total tin plotted as open symbols, TBT as closed symbols.

Table 4.2 Two factor analysis of variance comparing the body burden of total tin (TBT + DBT) in dogwhelks between the five study sites in south-west England and in different years. Analysis is based upon Log_{10} (x + 0.01) transformed data.

Source	DF	SS	MS	F	p
Site	4	3.8524	0.9631	50.81	p<0.001 ***
Year	5	11.1237	2.2247	117.37	p<0.001 ***
Site x Year	20	1.8879	0.0944	4.98	p<0.001 ***
Error	118	2.2366	0.0189		
Total	147	19.0132			

(a) Tukey multiple comparisons for site.

	Kingsand	Renney Rocks	Tregantle	St. Agnes
Jennycliff	NS	NS	NS	>***
Kingsand		NS	NS	>**
Renney Rocks			NS	>*
Tregantle				>**

(b) Tukey multiple comparisons for years.

	1988	1989	1990	1991	1992
1987	NS	NS	>***	>***	>***
1988		NS	>***	>***	>***
1989			>***	>***	>***
1990				NS	NS
1991					NS

p<0.05*, p<0.01**, p<0.001***, NS not significantly different at the p=0.05 level. Where the multiple comparison shows a significant result > denotes that the site or year on the side of the table was more contaminated than that one at the top.

Table 4.3 Results of two factor analysis of variance comparing the body burden of DBT in dogwhelks between the five study sites in south-west England and in different years. Analysis based upon Log_{10} (x + 0.01) transformed data.

Source	DF	SS	MS	F	p
Site	4	1.9195	0.4799	11.38	p<0.001 ***
Year	5	7.2046	1.4409	34.18	p<0.001 ***
Site x Year	20	1.2393	0.0619	1.47	NS
Error	118	4.9744	0.0422		
Total	147	15.2412			

(a) Tukey multiple comparisons for site.

	Kingsand	Renney Rocks	Tregantle	St. Agnes
Jennycliff	none were sensitive enough			
Kingsand				
Renney Rocks				
Tregantle				

(b) Tukey multiple comparisons for years.

	1988	1989	1990	1991	1992
1987	NS	NS	>***	>***	>**
1988		NS	>***	>**	>**
1989			NS	NS	NS
1990				NS	NS
1991					NS

p<0.05*, p<0.01**, p<0.001***, NS not significantly different at the p=0.05 level. Where the multiple comparison shows a significant result > denotes that the site or year on the side of the table was more contaminated than that one at the top.

Table 4.4 Two factor analysis of variance comparing the body burden of TBT in dogwhelks between the five study sites in south-west England and in different years. Analysis is based upon Log_{10} (x + 0.01) transformed data.

Source	DF	SS	MS	F	p
Site	4	4.5418	1.1354	50.04	p<0.001 ***
Year	5	12.6590	2.5318	111.58	p<0.001 ***
Site x Year	20	2.5830	0.1291	5.69	p<0.001 ***
Error	118	2.6775	0.0227		
Total	147	22.3691			

(a) Tukey multiple comparisons for site.

	Kingsand	Renney Rocks	Tregantle	St. Agnes
Jennycliff	NS	NS	NS	>***
Kingsand		NS	NS	>***
Renney Rocks			NS	NS
Tregantle				>**

(b) Tukey multiple comparisons for years.

	1988	1989	1990	1991	1992
1987	NS	NS	>***	>***	>***
1988		NS	>**	>***	>***
1989			>***	>***	>***
1990				NS	NS
1991					NS

p<0.05*, p<0.01**, p<0.001***, NS not significantly different at the p=0.05 level. Where the multiple comparison shows a significant result > denotes that the site or year on the side of the table was more contaminated than that one at the top.

Body burdens of total tin in *Nucella* were lower at the control site, St. Agnes, than at Kingsand, Jennycliff, Renney Rocks and Tregantle (table 4.2). The levels of TBT in the tissues of *Nucella* from St. Agnes were significantly different from those at Kingsand, Jennycliff and Tregantle but not Renney Rocks (table 4.4). A gradient of tissue burdens of TBT or total tin within Plymouth Sound was not seen.

At Jennycliff (figure 4.6) obvious peaks in the body burden of TBT and total tin (TBT + DBT) were recorded in all ages and sexes of dogwhelks in January 1989. Similarly, peaks in contamination were recorded at the other sites within Plymouth Sound (Kingsand (figure 4.7) and Renney Rocks (figure 4.4)) in all dogwhelks sampled in January 1989. Changes in the total tin (TBT + DBT) burden of all the organisms sampled appeared in general to be mirrored by similar changes in TBT suggesting that a relatively constant body burden of DBT occurred. Body burdens measured in limpets generally appeared to be lower than in the tissues of dogwhelks, barnacles and mussels.

Analysis using matched pairs (randomised block) ANOVA showed that there was no significant differences in the body burdens of DBT between any of the organisms tested at St. Agnes, Jennycliff, Tregantle or Renney Rocks (table 4.5). The exception was at Kingsand, but a Tukey pairwise comparison was not sensitive enough to determine between which of the organisms this difference occurred. There was no difference between the body burdens of TBT, DBT or total tin between all of the different ages and sexes of dogwhelks sampled. Similarly no difference was found between the body burdens (TBT, DBT, total tin) found in the three prey items sampled. Of those tests that proved significant, all showed limpets to have a difference in body burdens of contaminants when compared to the different age and sexes of dogwhelks. Limpets had lower body burdens of TBT than adult female, adult male, second year males and juvenile dogwhelks at

Table 4.5 Randomised block analysis of variance to test for differences in the body burdens of TBT, DBT and total tin between all ages and sexes of dogwhelks and barnacles, mussels and limpets, at each of the five sites in south-west England.

Source	DF	MS	F	p	Tukey test
(a) St. Agnes					
TBT	7	0.0645	1.3782	NS	NA
Error	42	0.0468			
DBT	7	0.0390	0.4216	NS	NA
Error	42	0.0925			
Total tin	7	0.0342	0.9421	NS	NA
Error	42	0.0363			
(b) Tregantle					
TBT	7	0.2353	5.2173	p<0.001 ***	Li ≠ AdM, AdF, 2M, Ju
Error	70	0.0451			
DBT	7	0.0338	0.9602	NS	NA
Error	70	0.0352			
Total tin	7	0.1563	5.3711	p<0.001 ***	Li ≠ AdM, AdF, 2M, 2F, Ju
Error	70	0.0291			
(c) Renney Rocks					
TBT	6	0.1634	3.4618	p<0.01 **	Li ≠ AdF
Error	60	0.0472			
DBT	6	0.1303	1.5948	NS	NA
Error	60	0.0817			
Total tin	6	0.2181	4.4419	p<0.001 ***	Li ≠ AdF
Error	60	0.0491			
(d) Kingsand					
TBT	6	0.1187	1.5082	NS	NA
Error	36	0.0787			
DBT	6	0.2174	2.5789	p<0.05 *	Not sensitive enough
Error	36	0.0843			
Total tin	6	0.0727	1.1821	NS	NA
Error	36	0.0615			
(e) Jennycliff					
Dogwhelks					
TBT	4	0.0507	1.8436	NS	NA
Error	28	0.0275			
DBT	4	0.0099	0.1291	NS	NA
Error	28	0.0767			
Total tin	4	0.0103	0.3056	NS	NA
Error	28	0.0337			
Barnacles and limpets					
TBT	1	0.0905	3.4542	NS	NA
Error	3	0.0262			
DBT	1	0.7200	7.0588	NS	NA
Error	3	0.1020			
Total tin	1	0.4871	7.8311	NS	NA
Error	3	0.0622			

Codes used for Tukey test: NA, not applicable (no significant difference after ANOVA); Li, limpet; Dogwhelks - AdM, adult male; AdF, adult female; 2M, second year male; 2F, second year female; Ju, juvenile; ≠ signifies a significant difference ($p<0.05$) between concentrations in these tissues, others not different.

Tregantle and adult females at Renney Rocks. In addition limpets also had lower concentrations of total tin than all ages and sexes of dogwhelks sampled at Tregantle and adult females from Renney Rocks.

At Jennycliff, tested separately, because of the lack of limpet and barnacle data prior to 1990, no differences were found in the levels of TBT, DBT or total tin contamination between different aged and sexed dogwhelks or between barnacles and limpets (table 4.5).

4.3.3 Correlations between tissue and water contamination

Peaks in contamination in the tissue burdens of organisms sampled rarely matched those in the water. In general they appeared in the organisms between 6 to 12 months after the peak levels were first recorded in the water. Significant correlations occurred between the levels in the water and those (TBT or total tin) in the tissues of the organisms sampled at the same time (table 4.6). However introducing a lag phase into the correlation of 6 months produced even better correlations in all animals sampled except barnacles. With a six month lag the concentration in the water accounted for around 70% of the variance in the body burden of TBT in *Nucella*, mussels and limpets and between 63-80% of total tin. Body burdens of TBT and total tin in limpets showed the strongest correlations with TBT in the water after 12 months. Correlations with a lag phase of less than 6 months produced few significant results, nor did those with a lag of between 6-12 months.

Significant positive correlations occurred between the concentrations of total tin and TBT in the tissues of dogwhelks and with body burdens in either barnacles, mussels or limpets sampled at the same time (table 4.7). In general the introduction of a lag

Table 4.6 Correlations between the concentrations of TBT in the water of Plymouth Sound and the body burdens of TBT and total tin (TBT + DBT) in the tissues of dogwhelks, barnacles, mussels and limpets sampled at the same time (0 months) and after a 6 or 12 month time lag.

(a) TBT in water vs TBT in tissues.

lag time	0 months			6 months			12 months		
	r	n	p	r	n	p	r	n	p
Adult male	0.466	65	***	**0.759**	25	***	0.579	21	**
Adult female	0.602	64	***	**0.714**	24	***	0.367	20	NS
Second year male	0.436	50	**	**0.701**	24	***	0.531	20	*
Second year female	0.510	53	***	**0.683**	24	***	0.676	21	**
Juvenile	0.551	60	***	**0.787**	24	***	0.718	20	***
Barnacle	**0.608**	56	***	0.542	22	*	-0.002	19	NS
Limpet	0.636	55	***	0.787	22	***	**0.824**	19	***
Mussel	0.678	26	***	**0.711**	10	***	0.651	9	NS

(b) TBT in water vs total tin (TBT + DBT) in tissues.

lag time	0 months			6 months			12 months		
	r	n	p	r	n	p	r	n	p
Adult male	0.513	64	***	**0.781**	25	***	0.570	21	**
Adult female	0.650	64	***	**0.759**	24	***	0.429	20	NS
Second year male	0.501	50	***	**0.638**	24	***	0.542	20	*
Second year female	0.565	53	***	**0.690**	24	***	0.493	21	*
Juvenile	0.611	60	***	**0.789**	24	***	0.682	20	***
Barnacle	**0.624**	56	***	0.529	22	*	-0.012	19	NS
Limpet	0.652	55	***	0.745	22	***	**0.755**	19	***
Mussel	0.757	25	***	**0.802**	10	**	0.665	9	NS

$p<0.05$ *, $p<0.01$**, $p<0.001$***, NS not significantly different at the $p=0.05$ level, the best correlations are given in bold.

Table 4.7 Correlations between the body burdens of total tin (TBT + DBT) in the tissues of dogwhelks and barnacles, limpets and mussels, sampled at the same time (0 months) and after a 6 and 12 month time lag. Data included from all five south-west sites.

(a) Barnacle total tin (TBT + DBT) correlated with total tin in all ages and sexes of dogwhelks sampled.

lag time	0 months			6 months			12 months		
	r	n	p	r	n	p	r	n	p
Adult male	**0.581**	55	***	0.559	22	**	0.575	17	*
Adult female	**0.597**	54	***	0.536	21	*	0.315	16	NS
Second year male	0.488	47	***	0.312	21	NS	**0.612**	16	*
Second year female	**0.560**	47	***	0.481	21	*	0.513	17	*
Juvenile	0.578	52	***	0.544	21	*	**0.672**	16	**

(b) Limpet total tin (TBT + DBT) correlated with total tin in all ages and sexes of dogwhelks sampled.

lag time	0 months			6 months			12 months		
	r	n	p	r	n	p	r	n	p
Adult male	**0.687**	55	***	0.667	22	***	0.574	17	*
Adult female	**0.666**	55	***	0.568	21	**	0.528	16	*
Second year male	**0.640**	45	***	0.529	21	*	0.486	16	NS
Second year female	**0.714**	47	***	0.545	21	*	0.505	17	*
Juvenile	0.762	52	***	**0.802**	21	***	0.728	16	**

(c) Mussel total tin (TBT + DBT) correlated with total tin in all ages and sexes of dogwhelks sampled.

lag time	0 months			6 months			12 months		
	r	n	p	r	n	p	r	n	p
Adult male	**0.797**	25	***	0.563	10	NS	0.397	8	NS
Adult female	**0.892**	25	***	0.816	10	**	0.693	8	NS
Second year male	**0.710**	21	***	0.415	10	NS	0.243	8	NS
Second year female	**0.696**	21	***	0.385	10	NS	0.411	8	NS
Juvenile	**0.841**	24	***	0.837	9	**	0.686	7	NS

$p<0.05$*, $p<0.01$**, $p<0.001$***, NS not significantly different at the $p=0.05$ level, the best correlations are given in bold.

phase here reduced the amount of variation which could be accounted for by the levels in the prey items.

4.4 Discussion

The frequency of visits to Plymouth to take samples from the monitoring sites was undoubtedly not enough to pick up short-term patterns, such as the seasonal variations in water and tissue contamination. However gradients of contamination, longer-term trends indicating recovery and the usefulness of *Nucella* as a bioindicator can be discussed.

4.4.1 Gradients of contamination

Steep dilution gradients have been reported away from harbours and marinas at sites around the UK, for example on the south coast (Cleary & Stebbing, 1985; Cleary & Stebbing, 1987a; Cleary & Stebbing, 1987b; Langston *et al.,* 1987). Thus confirming that antifouling paints are the major source of TBT in the environment. Overall, levels of contamination were much lower in samples taken in Plymouth Sound, away from the Mayflower Steps and Tinside. The concentrations recorded at Tinside in the open water of Plymouth Sound, 800 m from the large marinas adjacent to the Mayflower steps indicate a rapid fall away in TBT contamination from source. However, the dilution factor decreased as TBT concentrations decreased, so initially when the levels of pollution at the Mayflower steps were higher in the mid 1980's the dilution factor to sites in the Sound were also high. Subsequently as the level of TBT in water at the Mayflower Steps decreased the dilution factor also decreased. Consequently by the end of the survey in February 1993 levels at Jennycliff, for example were only 4 times less than at the Mayflower steps compared to 35 fold in March 1987.

The levels of pollution measured at the four sites studied in Plymouth Sound, however, do not directly reflect the distance away from the marina of each of the sites, as may be expected. Instead Kingsand was the most polluted site and Tregantle was more affected than Renney Rocks, suggesting that there was greater contamination on the west side of Plymouth Sound. There are two possible reasons for this. Firstly the levels of TBT may be affected by currents and tidal flow. In Plymouth Sound the tidal flow is generally anticlockwise (Southward & Boalch, 1993). Secondly the position of the navigational channel used by large boats entering Plymouth Sound is of possible importance. Large boats always seem to enter the harbour in Plymouth on the west side of the breakwater (personal observations) passing close to the shore at Kingsand. Following restrictions on TBT on smaller boats, the main source of contamination is the larger vessels of 25 m plus. Spence (1989) surveying these sites in 1986-1989 ranked them with increasing TBT contamination in the order Tregantle, Renney Rocks, Kingsand and Jennycliff - an order which directly relates to the distance away from the main marina. It is possible that at the time the large numbers of pleasure craft using organotin based antifouling paints masked the effect of the larger ships.

Gradients of contamination in and around Plymouth Sound were not as apparent in the tributyltin burden of the tissues of dogwhelks, barnacles, mussels and limpets. Small scale fluctuations between the sites were not shown, instead all the sites, Jennycliff, Kingsand, Renney Rocks and Tregantle, were found to have similar levels of contamination only differing from the control site, St. Agnes.

4.4.2 Legislation and recovery

Prior to the restrictions on the use of organotin paints in 1987, surveys of the level of tributyltin pollution in the waters around the south coast of Devon and Cornwall in 1984 (Cleary & Stebbing, 1985) and again in 1986 (Cleary & Stebbing, 1987a; Cleary & Stebbing, 1987b) showed that Plymouth was one of the worst affected sites in this area. Even though a number of other sites in the south-west had similar numbers of pleasure craft the high concentrations of TBT recorded in Plymouth Sound were caused by a low degree of tidal mixing and water exchange within the area (Cleary & Stebbing, 1987a; Cleary & Stebbing, 1987b). Concentrations of tributyltin *in the sub-surface waters at the Sutton Harbour Marina in* Plymouth Sound were recorded as high as 350 ng Sn/l and 143 ng Sn/l in 1984 and 1986 respectively (Cleary & Stebbing, 1985; Cleary & Stebbing, 1987a).

The first restrictions on the use of TBT, introduced by the UK Government in January 1986 (Abel *et al.*, 1986; Side, 1986), appeared to have little effect on the levels of TBT in the environment (Waldock *et al.*, 1987). However, following the 1987 Government ban there has been a significant decrease in the levels of tributyltin in the waters of Plymouth Sound. This reduction was not instant, only being apparent in 1989 two years after the ban had been first introduced. This was not surprising, especially since the timing of the legislation was such that it was introduced too late to stop the paints being used over the summer boating season in 1987 (Abel *et al.*, 1987; Duff, 1987). This resulted in higher concentrations of TBT in water in 1987 than 1986 at half of the sites in the south-west of England (Cleary, 1991).

Over the period 1987-1993 a 5-10 fold reduction was recorded in TBT concentrations in Plymouth Sound reflecting the rate of recovery recorded in French waters after legislation was first introduced there in 1982 (Alzieu *et al.*, 1986). Reductions appear to have occurred at a similar rates in the water throughout the south-west. Similar rates were recorded by Bryan *et al.* (1993a) over the same period and Cleary (1991) in concentrations in sub-surface waters in sites in south Devon and Cornwall. TBT concentrations in water along the east coast of England have also declined (Dowson *et al.*, 1993).

Despite these reductions high concentrations of TBT were still being recorded at some sites. At Kingsand and Jennycliff in February 1993 concentrations still exceeded the environmental quality standard (EQS) target set in March 1989 at 2 ng/l (Cleary, 1991) (equivalent to 0.8 ng Sn/l), as did levels at Tinside and the Mayflower steps surveyed by workers at Plymouth Marine Laboratory. Environmental concentrations are likely to remain high because the legislation in the UK, although restricting the amount of organotin in antifouling paints for use on vessels over 25 m, has not banned their use altogether and large boats are still permitted to use them (Abel *et al.,* 1987; Duff, 1987). This usage may account for the differences in recovery rates found between some sites (Bryan *et al.,* 1993a) as detrimental effects have been observed near harbours serving these large vessels (Davies & Bailey, 1991). In spite of the regulations in France abnormal inputs were noted by Alzieu *et al.* (1989) several years after the ban. The continued, illegal use, of TBT has been suggested by a number of workers as reason for levels in the water remaining high. In addition levels in the water will be affected by tidal flushing and degradation.

Degradation in water involves the sequential debutylation of TBT to inorganic tin which occurs through both chemical and biological degradation. Half lives for the degradation of TBT in sea water have been calculated based on laboratory studies. These appear to be very variable ranging from 6-60 days depending on the conditions (Thain *et al.,* 1987). Despite this variation, these values for half lives are much faster than could explain the rates of recovery seen at the sites around Plymouth Sound. Ultimately, the main difference between the situation in Plymouth Sound and controlled studies is the continued input of TBT both from the continued use of TBT and possibility of resuspension of TBT from sediments.

Tributyltin readily absorbs onto particles in the water which settle out into the sediment. TBT is persistent in sediments and as such may act as a sink providing a new source of TBT for the water (Langston & Burt, 1991). It is possible that during rougher weather some of the sediment could be resuspended. There is no doubt that there is some truth in this since the water sample with the greatest variation taken by Spence (1989) in May 1987 at Kingsand had considerable sediment in the sample after a period of bad weather. Resuspension could in part account for some of the fluctuations in the TBT levels observed some years after the ban.

As water concentrations of TBT decrease the seasonal cycles of contamination become less clear reflecting decreases in new inputs. These seasonal cycles were not as clear in the samples collected from the five sampling sites and probably reflect the difference in sampling methodology and the dilution factor away from the source. In contrast to the samples taken at the Mayflower Steps and Tinside the water I collected from Kingsand, Jennycliff, Tregantle, Renney Rocks and St. Agnes included the surface microlayer. Since TBT concentrations in this layer remain relatively constant varying very little with the tidal cycle or with the season (Cleary, 1991) the values are effectively stabilised over the year. In addition the frequency of water collections at these sites is probably not enough to pick up the seasonal variations. As the concentrations decrease variations also reduce as one is less likely to sample hot spots.

4.4.3 Pathways of TBT uptake

Correlations between tissue and water concentrations in *Nucella lapillus* have been examined elsewhere, for example by Bryan *et al.* (1987), in the laboratory. Surprisingly, no one has examined the relationship for a lag phase, instead always relating the tissue burdens to the water concentrations at the same time. Most laboratory studies involving uptake in *Nucella* have been short-term with no consideration of a lag phase. This is despite the fact that a small lag phase of around 16 days was shown in carbon 14 studies in the laboratory in the uptake of TBT in *Nucella,* in the tissues not directly in contact with the water, for example the kidney (Bryan *et al.,* 1993b). Consequently analysis in the whole dogwhelk would expect some lag phase in relation to concentrations in the water.

In the field, few time series in the natural environment have been plotted to show changes in the body burden of TBT in *Nucella* in relation to the water which may illustrate a lag phase in uptake. One of the few exceptions is Bryan *et al.* (1993a) where the changing body burdens of *Nassarius reticulatus* in relation to concentrations of TBT in the water were shown; but since only one sample was taken per year insufficient data points were provided to examine a lag phase.

The data presented here shows that there are significant correlations with the water at time zero confirming previous laboratory studies. However body burdens of TBT and total tin in *Nucella* showed stronger correlations with the TBT in the water after the introduction of a six month lag period. This was also the case for mussels although not for barnacles where the strongest correlations were with water concentrations taken at the same time. Surprisingly body burdens of TBT and total tin in limpets showed the best correlations with water TBT after the introduction of a 12 month lag in uptake. These differences are probably due to differences in feeding mechanisms (e.g. between limpets and barnacles) or body sizes (e.g. between barnacles and mussels).

A closer fit was also seen in the relationship between the TBT in the water and the total tin in the tissues. This is hardly surprising as TBT is the preferential form absorbed and its degradation within the animal is the main source of tissue DBT

(Bryan *et al.,* 1987). There was more DBT in the tissues of dogwhelks 1988 and 1989 than in the later years.

Diet has been reported as important sources of TBT in other organisms, for example in mussels (Laughlin, 1986; Laughlin *et al.*, 1986). Bryan *et al.* (1989b) calculated that under favourable conditions 50% or more of the body burden of TBT in *Nucella* may originate from the diet. These calculations were based upon a laboratory study and as a consequence the true value may be less when taking into consideration the variation in feeding rates dependent upon weather. The uptake from prey may become more important as a source of TBT as the environmental concentration of TBT increases (Spence, 1989). In the field it appears that the water is probably the most important source of TBT in *Nucella lapillus*. Correlations of the concentrations of TBT and total tin in *Nucella lapillus* are stronger with concentrations in the water than concentrations in possible prey items, with or without a time lag. In addition there is no apparent time lag between the concentrations recorded in barnacles, mussels or limpets and those recorded in *Nucella* as perhaps would be expected if the food was the most important source of contamination.

Langston *et al.* (1987) found great variation in the levels of TBT in the tissues of several organisms from the same site. One possible suggestion was that this was connected to differences in the lipid content of these organisms, but a more likely explanation was that it was to do with differences in the feeding mechanisms, or mode of nutrition (Langston *et al.*, 1987). This may explain the differences in body burdens observed between *Nucella lapillus* and *Patella vulgata*. In tune with findings here with *Nucella lapillus*, Bryan *et al.* (1993a) found no difference in the body burdens of male and female *Nassarius reticulatus*. The concentration of TBT in the tissues of dogwhelks did not differ significantly between different age groups.

It can thus be assumed that firstly that *Nucella* is able to metabolise or degrade TBT and secondly that the extent of this ability is the same for all ages of dogwhelk. Supporting evidence is that the major source of DBT in the body is from the breakdown of TBT (Bryan *et al.,* 1987) and there is no difference in the body burdens of DBT between different ages and sexes of *Nucella*. Essentially bioaccumulation was observed in *Nucella* but food chain magnification was not.

In laboratory studies the loss of TBT in *Nucella* transferred to clean water gave a half life of 50-60 days, after exposure to TBT at 107 ng/l for 121 days. The half life of TBT in tissues after exposure to a pulse of contamination at 107 ng/l for 14 days was between 48 to 58 days (Bryan *et al.,* 1987). Another laboratory degradation experiment showed an exponential decrease giving a half life of between 90 and 125 days again from a high concentration, this time 200 ng/l (Bryan *et al.,* 1988). In the field a transplant of *Nucella* from Torcross (south Devon) where RPS values were 44% to Bude, a cleaner site, gave a half life of 100 days. After 9 months the concentrations in the tissues were approaching those of the native animals (Bryan *et al.,* 1987). All these experiments involved a sudden drop in TBT concentrations, which is not the situation in the natural environment.

4.4.4 Bioindicators

The importance of frequent sampling is indicated by fluctuations in the level of TBT in the water collected at high and low tide by the Plymouth Marine Laboratory (figure 4.1). Since the water samples were taken at low water at the five Plymouth sites, they are probably related to the highest levels since the difference between low tide and high tide concentrations could be expected to be two fold (Waldock *et al.,* 1987; Cleary, 1991). Fluctuation of TBT concentrations decreases with increasing distance from source with the lack of episodic events (Spence, 1989).

The fluctuations observed in the water samples reflect the advantage in using the biological indicators which provide a time integrated picture of the level of contamination. This smoothes out the short term fluctuations giving more distinct trends. Body burdens of TBT in the tissues of *Nucella lapillus*, barnacles, mussels and limpets have all shown a recovery over the sampling period. This recovery did not happen as quickly as that measured in the water and is probably due to the rates at which *Nucella* absorbs and metabolises TBT as seen in the lag phase. This was also the case in the tissues of *Nassarius reticulatus* (Bryan *et al.,* 1993a). Alzieu and co-workers (Alzieu *et al.,* 1986) found significant reductions in the tissues of oysters sampled two years after the ban in France and where the decrease in contamination was particularly noticeable in August 1984 (Alzieu *et al.,* 1986). This timescale of recovery was identical to the one recorded for the water at the same site. However, in the present study concentrations of TBT in the tissues of *Nucella lapillus* took longer to recover. The difference in recovery rates between the tissues of the oysters and the dogwhelks could be in a different ability to metabolise TBT. However Lee (1991) found that all molluscs have a low rate at which they metabolise TBT due to low levels of cytochrome P-450 and mixed function oxygenase activity in the digestive gland. Equally the difference could be due to feeding methods.

Not only is *Nucella lapillus* a good indicator because it satisfies all the criteria for a bi-indicator (see Phillips, 1977; Bryan *et al.,* 1980; Phillips, 1980; Bryan *et al.,* 1985) but also because of its development of a dose dependent response to the TBT in its tissues or in the water. This response where the female develops male characteristics, imposex, is directly related to concentrations in the body or water (Gibbs *et al.,* 1988). The recovery of populations of this indicator from TBT pollution are discussed in the next chapter.

4.4.5 Conclusions

Following the ban by the UK Government on organotin antifouling paints in 1987 there has been a significant decrease in the levels of TBT in the waters of Plymouth Sound. This decrease over the period 1987-1993 has been 5-10 fold, reflecting the rate at which levels of TBT decreased in French waters after legislation was introduced there in 1982. Despite the reductions, however, concentrations at two of the sites in Plymouth Sound in February 1993 still exceeded the EQS of 2 ng/l TBT (0.8 ng Sn/l) set in March 1989. These levels are still high enough to induce imposex in *Nucella lapillus* (chapter 5).

In the natural environment it appears that water, rather than diet, is the most important source of TBT for *Nucella*. Body burdens of tributyltin and total tin (TBT + DBT) in *Nucella*, barnacles, mussels and limpets all showed significant correlations with water concentrations taken at the same time. In *Nucella* and *Mytilus*, however, these correlations were stronger when levels of water contamination were correlated with tissue burdens six months later, hereby incorporating a lag phase.

CHAPTER 5

The recovery of *Nucella lapillus* populations in south-west England following the restrictions on the use of organotin based antifouling paints in 1987

5.1 Introduction

The effects of tributyltin leachates from antifouling paints on *Nucella lapillus* are now well documented at the cellular, individual and population level (figure 5.1) (see Bryan & Gibbs, 1991; Hawkins *et al.*, in press for reviews). Exposure of female *Nucella* to tributyltin in seawater (Bryan *et al.*, 1986) or the tissues of prey items (Bryan *et al.*, 1989b) leads to the development of imposex, the superimposition of male characteristics on the female (Smith, 1971).

The biochemical mechanism responsible for the development of imposex is thought to be a consequence of an increase of the steroid hormone testosterone in the female. Gibbs *et al.* (1991a) has suggested that exposure to tributyltin inhibits the cytochrome P-450 dependent aromatase responsible for the conversion of testosterone to oestradiol 17ß in the female. Indeed the direct injection of testosterone into the female in the absence of TBT causes an increased expression of imposex (Spooner *et al.*, 1991).

Nucella lapillus develops the characteristics of imposex in a dose dependent manner which is initiated at water concentrations as low as 0.5 ng Sn/l (Gibbs *et al.*, 1988). The extent of imposex development can be measured by two indices: relative penis size (RPS) and vas deferens sequence (VDS) between which there is a significant relationship (Gibbs *et al.*, 1987). The relative penis size is used as a measure of penis development in the female and compares the bulk of the female penis to that of the male from the same population (Bryan *et al.*, 1986; Gibbs *et al.*,

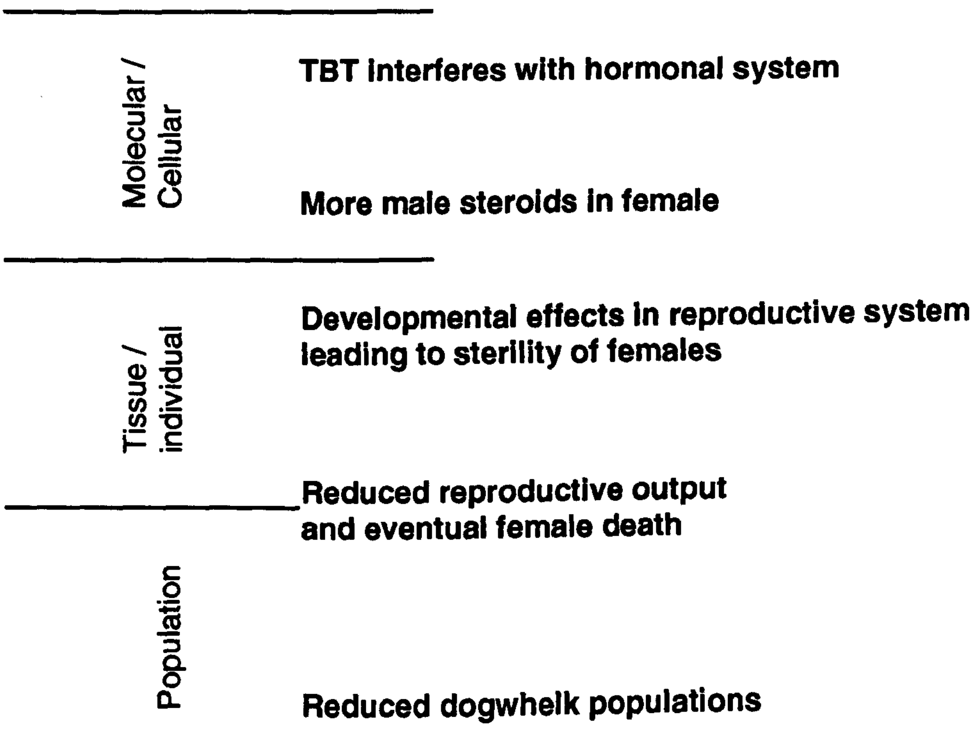


Figure 5.1 Summary of the known effects of exposure to tributyltin on *Nucella lapillus* at different levels of organisation. See text for references, adapted from Hawkins *et al.*, in press.

1987). The vas deferens sequence is a six stage index used to categorise the development of the vas deferens in the female giving a measure of the reproductive capacity of the individual (Gibbs *et al.*, 1987). The initial development and growth of the vas deferens and penis are represented by stages 1-4. The continual development of the vas deferens eventually occludes the vulva, stage 5, and leads to the accumulation of aborted egg capsules in the capsule gland which are unable to be released, stage 6. Stages 5 and 6 render the female effectively sterile (Gibbs *et al.*, 1987). The accumulation of aborted egg capsules appears ultimately to cause the rupture of the capsule gland which can lead to the premature death of the female (Gibbs & Bryan, 1986).

With the lack of a planktonic larval phase in the life history of *Nucella lapillus* and the relative immobility of adults each population is effectively isolated and self sustaining. The effective sterilisation of females affected by tributyltin means that the recruitment of juveniles is eliminated. Populations become characterised by having few females, absent juveniles and then ultimately being dominated by adult males (Bryan *et al.*, 1986; Gibbs & Bryan, 1986). This eventually leads to the complete demise of the population (Spence *et al.*, 1990a). Survival or recovery of a population that is badly affected relies on the rafting in of females capable of breeding (Gibbs *et al.*, 1988). Although juveniles recently emerged from egg capsules at one site may reach another on floating debris, this is likely to be a rare phenomenon (Bryan *et al.*, 1986). If the level of contamination at a site remains high even immature females rafted in may become effectively sterilised before ever being able to breed (Gibbs & Bryan, 1987).

Imposex has been shown to occur in populations of *Nucella lapillus* throughout the British Isles (Bailey & Davies, 1989; Spence *et al.*, 1990a) Europe (Fioroni *et al.*, 1991a; Gibbs *et al.*, 1991c; Ritsema *et al.*, 1991; Stroben *et al.*, 1992a; Oehlmann *et al.*, 1993) and North America (Miller & Pondick, 1984). At many sites across its European range *Nucella* populations have effectively been wiped out (Gibbs *et al.*, 1991c), most noticeably in or adjacent to harbours or marinas where the concentration of TBT in the water has risen above the critical 2 ng Sn/l level. This threshold level of contamination, sufficient to cause sterilisation (Gibbs *et al.*, 1988) was often observed to be exceeded by several orders of magnitude (Cleary & Stebbing, 1985). The situation has been especially severe on the south coast of England due to the level of boating activity throughout the whole area (Spence *et al.*, 1990a; Gibbs *et al.*, 1991b). Only at a few isolated sites have populations been found with no incidence of imposex development (Bryan *et al.*, 1986; Bailey & Davies, 1988).

The French were the first to introduce restrictive legislation on the use of tri-organotins in antifouling paints, banning their use on boats less than 25 m in January 1982 (Alzieu *et al.*, 1986; Alzieu, 1991). Their prompt action followed evidence that the collapse of the oyster fishery in Arcachon Bay was due to the increased use of TBT paints on the boats in the harbours nearby. The monitoring of the effectiveness of the restrictions centred around the commercially important shell fish, *Crassostrea gigas*, which had been so badly affected by the pollutant. These effects had been two fold, causing firstly, a complete absence of spatfall in the area, preventing any direct replenishment of breeding stocks, and secondly anomalies in the calcic growth of shells, with individuals forming numerous extra chambers, taking on a ball shape with very little soft tissue. After the ban the concentrations of TBT in the water column dropped by 50% in the first year and by November 1985 some three years later the concentrations had been reduced by 5-10 times (Alzieu *et al.*, 1986). In addition normal spatfall resumed in 1983, but although the incidence of shell thickening also declined a significant percentage of the oysters (40%) were reported to still have shell malformations in 1985 (Alzieu *et* *al.*, 1986). In 1989, some seven years after the ban, levels of TBT pollution were still sufficient to maintain some incidence of shell malformation in *Crassostrea gigas* (Alzieu, 1991) and a high level of imposex in dogwhelks in these areas (Gibbs *et al.*, 1991c).

The UK government were slower to react. The first controls were introduced in January 1986 restricting the types of antifouling paints which could be used, to those with reduced release rates (Abel *et al.*, 1986; Side, 1986). It was not until July 1987 that an effective ban was imposed on TBT paints (Abel *et al.*, 1987; Duff, 1987) and this was too late to be effective for the 1987 season (Spence, 1989). Since then a reduction in the levels of TBT in seawater has been recorded at a number of sites around the UK (Waite *et al.*, 1991; Bryan *et al.*, 1993a; Dowson *et al.*, 1993; chapter 4) at a rate which reflects the improvement in French waters. Evidence of recovery in oysters (Dyrynda, 1992) and in the netted whelk *Nassarius reticulatus* (Bryan *et al.*, 1993a), both affected by tributyltin, has also been shown at sites around the UK. But neither of these indicators were affected to the same extent as *Nucella lapillus*, or at as low a concentration. The effectiveness of the legislation in promoting recovery in dogwhelk populations is largely unknown, although there is some evidence of a reduction in relative penis size in adult *Nucella* in Northumbria (Evans *et al.*, 1991) and on the Isle of Cumbrae (Evans *et al.*, 1994) accompanied by an increase in abundance.

Following these changes in the legislation the detection of any decrease in the intensity of imposex becomes imperative with respect to monitoring changes in environmental levels. *Nucella lapillus* is an ideal bioindicator. It has a unique combination of characters which make it a prime candidate for use as an indicator of TBT pollution (chapter 4). Most important of these characteristics is its sensitivity to TBT, as manifested by imposex, which is seemingly unrivalled (Gibbs *et al.*, 1987).

The aims of the work reported in this chapter were to monitor recovery of *Nucella lapillus* populations using the two measurements of imposex coupled with assessment of the population abundance and structure. With the effects of imposex being irreversible in *Nucella* (Bryan *et al.*, 1987; Gibbs *et al.*, 1987; Bryan *et al.*, 1988), juveniles were used for short term indication of the level of environmental contamination since they had been exposed for 6-12 months (Gibbs *et al.*, 1987). Adults provide a longer term perspective and the state of the reproductive potential for this population. Abundance assessments employed two methods, fixed areas and timed collections, and the relative merits of each will be discussed. The results are used to examine the effectiveness of the UK legislation and some predictions for the timescale of recovery are given.

5.2 <u>Materials and methods</u>

5.2.1 Collection and analysis for imposex

Dogwhelks were collected for measurements of imposex from five sites in the south-west of England: Jennycliff, Kingsand, Renney Rocks, Tregantle and St. Agnes (see chapter 2 for site locations and descriptions). Collections were usually made twice yearly, early in the year between January-March and later, between July-August. The methods used for the collection, storage and preparation of *Nucella* for imposex analysis are described fully in chapter 3. The level of imposex within each population was assessed using the two indices: vas deferens sequence and relative penis size, details of which are described in chapter 3 and in Gibbs *et al.* (1987).

Data from the period 1986-1989 comes from Spence (1989). Data collected in February and July 1990, before this studentship started, were also collected by Dr Spence. Results from October 1990 onwards were collected and analysed by myself for the present study.

5.2.2 Population abundance assessments

The abundance of dogwhelks at the study sites in the south-west were assessed using two methods, timed searches and fixed monitoring areas.

5.2.2.1 Timed searches

Timed searches were conducted as described in chapter 3, generally searching for 10 minutes over the shore at, or just below the mid tide level. The use of a generalised area of shore such as this meant that searches could be made to take account of variations in the behaviour of the dogwhelks such as aggregation (Feare, 1971a) or migrations (Feare, 1970a) and the search shifted accordingly.

All dogwhelks collected using the timed search methods were used for imposex analysis and their shell heights measured and assessment on ages made in laboratory.

5.2.2.2 Fixed monitoring areas

A second method using fixed monitoring areas was used as a comparison to the timed search methods. These areas, each about 4 m^2, were originally established by Spence (1989) and continued here. Due to the low population abundance of dogwhelks at Jennycliff and Kingsand, the two sites closest to the marinas in Plymouth Sound (figure 2.1), these monitoring areas were only set up at Renney Rocks, Tregantle and St. Agnes. Each area, where possible, was chosen at around mid tide level in an area isolated from the rest of the shore preferably by a natural barrier to the mobility of dogwhelks, such as sand or mud (Crothers, 1985). It was also a requirement that each site be accessible for monitoring, have an adequate prey availability and shelter from predators or adverse weather conditions, for all ages and size classes of *Nucella* (Spence, 1989).

At Renney Rocks the monitoring area was effectively isolated by a boulder field and with a 'skirt' of algae around the bottom of the rock. This large rock was barnacle dominated with a sparse *Fucus vesiculosus* cover on the top. *Nucella* sheltered mainly amongst the crevices in the rock.

The monitoring area at Tregantle consisted of a large rock isolated from others by an expanse of sand. The rock was densely packed with mussels in a band across the top and with a strip of barnacles below. *Nucella* were found amongst the *Mytilus* matrix and in several crevices in the rock.

The nature of the shore at St. Agnes, with large continuous rocky outcrops did not provide an isolated area, instead the monitoring area was a section of such an outcrop facing landward and sheltered from the waves and prevailing winds. The horizontal surface of this area was mussel dominated changing to mainly barnacles on the vertical face. *Nucella* foraged from the crevices and mussel clumps.

During each visit to these monitoring sites all of the dogwhelks within the area were removed. The shell length of each dogwhelk was measured, from the apex to the point of the siphonal canal, and an assessment of its age made according to the thickness of the shell edge (chapter 3). Shell state and colour were also recorded. After measurement all dogwhelks were replaced. The presence of any egg capsules were recorded. Photographs were taken of each of these monitoring areas to gain an overall impression of the nature of the habitat and its changes with time.

5.2.3 Statistical methods

For data in the form of proportions or percentages it is normal to arc sine transform them prior to analysis using parametric statistics, since data expressed in this way are characterised by having heterogeneous variances (Underwood, 1981). This results from the upper and lower constraints that are placed on the variance of data collected at the limits of the scale (proportions must be $0<p<1$ and percentages $0<p<100$). This does not apply to relative penis size values, which are ratios expressed as a percentages. The nature of the penis development in the female means that it is possible to obtain a RPS value of over 100, where the female penis is larger than that of the male from the same population. At the other end of the scale a RPS value of zero for a population is extremely rare. Since TBT concentrations in the water as low as <1 ng/l induce penis development in the female (Gibbs *et al.*, 1988) even populations extremely isolated from any boating or aquaculture activity have been recorded with a RPS value of 5%. However all RPS data was tested for homogeneity of variance normality prior to analysis, using the F-max and NSCORE tests respectively (chapter 3).

Two-way ANOVA was used to compare the changes in RPS values between sites and with time. The RPS values for adults were used to assess long term changes. Since there was only one relative penis value per site, per sampling date, two-way ANOVA without replication was used for the analysis. With only one observation in each cell a test for the interaction of the two factors is impossible since both the interaction and the error variability cannot be calculated (Zar, 1984). Hence analysis using two-way ANOVA without replication must assume that there is no interaction between the two factors (Fowler & Cohen, 1992). This is often difficult to predict in natural systems. However if a significant result is observed this may be accepted although there is a slight increase in the chance of a type II error (Zar, 1984), that is that the test may be on the conservative side.

The problem with using two way ANOVA without replication is that a balanced design must be used (Zar, 1984; Fowler & Cohen, 1992), in this case that there must be a relative penis size value for each site on each sampling occasion. Without this there will be empty cells and computation of the significance of the changes will be impossible. Consequently analysis was performed only on sampling dates when RPS values were obtained at all sites. For adults this meant that comparisons could be made on 15 of the 16 occasions when samples were taken. For second years there were 9 dates when RPS values were obtained for all sites but for juveniles there were only 5 of the possible 16 dates. This low number for the juveniles was due to the absence of juveniles at Jennycliff in 1986 and 1987 and the occasional absence of juveniles at Kingsand, Tregantle and Renney Rocks over the period of monitoring. Consequently two-way ANOVA was not performed on the changes in RPS for juvenile *Nucella* as it was felt that the low number of sampling dates for the analysis would give a false impression of the changes over time.

When significant differences were found between the sites or sampling times multiple comparisons were performed using the Tukey test (chapter 3).

Simple linear regression was used to calculate a line of best fit through the relative penis size results with time. Although values were obtained from 1986 onwards only those results from 1 July 1987 were used as this was when the legislation restricting the use of TBT was introduced (Abel *et al.*, 1987; Duff, 1987). This produced an equation relating changes in time (x variable) with changes in relative penis size (y variable). Data were tested to see if they satisfied the assumptions of the analysis (see Zar, 1984; Fowler & Cohen, 1992) which included homogeneity of variance, even though regression analysis is known to be robust to some violations of its assumptions (Zar, 1984). The significance of the regression was tested using the analysis of variance procedure (Zar, 1984).

The slopes of two or more lines obtained by regression analysis were compared using analysis of covariance (Zar, 1984), testing the homogeneity of the regression coefficients. If no differences were found between the slopes of the lines being compared the procedure was repeated testing instead for differences in the elevations. When significant differences were found between the slopes or between the elevations of three or more lines being compared, multiple comparison tests were performed to find which lines were different from which others. Here the Tukey Honest Significant Difference test was used (Zar, 1984).

The balance of females to males in the adult populations was tested against an expected ratio of 7:5 using the chi-squared test. There appeared to be no clear evidence in the literature of what proportions of males to females constitute a 'normal' population, one unaffected by TBT pollution. Fretter and Graham (1984) said that the sexes are equal in numbers and Evans *et al.* (1991) took a ratio of 1:1 to be the norm for comparison with imposex affected populations. However this ratio can probably be considered as conservative for adult *Nucella*. Moore (1938a) in a study in Plymouth Sound before the advent of tributyltin pollution, found the proportion of females in the population increased with age and shell height giving an overall ratio of females to males of around 7:5. Similarly Feare (1970b) tested a population on the North Yorkshire coast against a sex ratio of 1:1 and concluded that the proportion of females increases with age. The reason given was differential mortality of the sexes. Bryan *et al.* (1986) used a normal of 56.8% females in the population for comparisons of TBT affected adult *Nucella* populations.

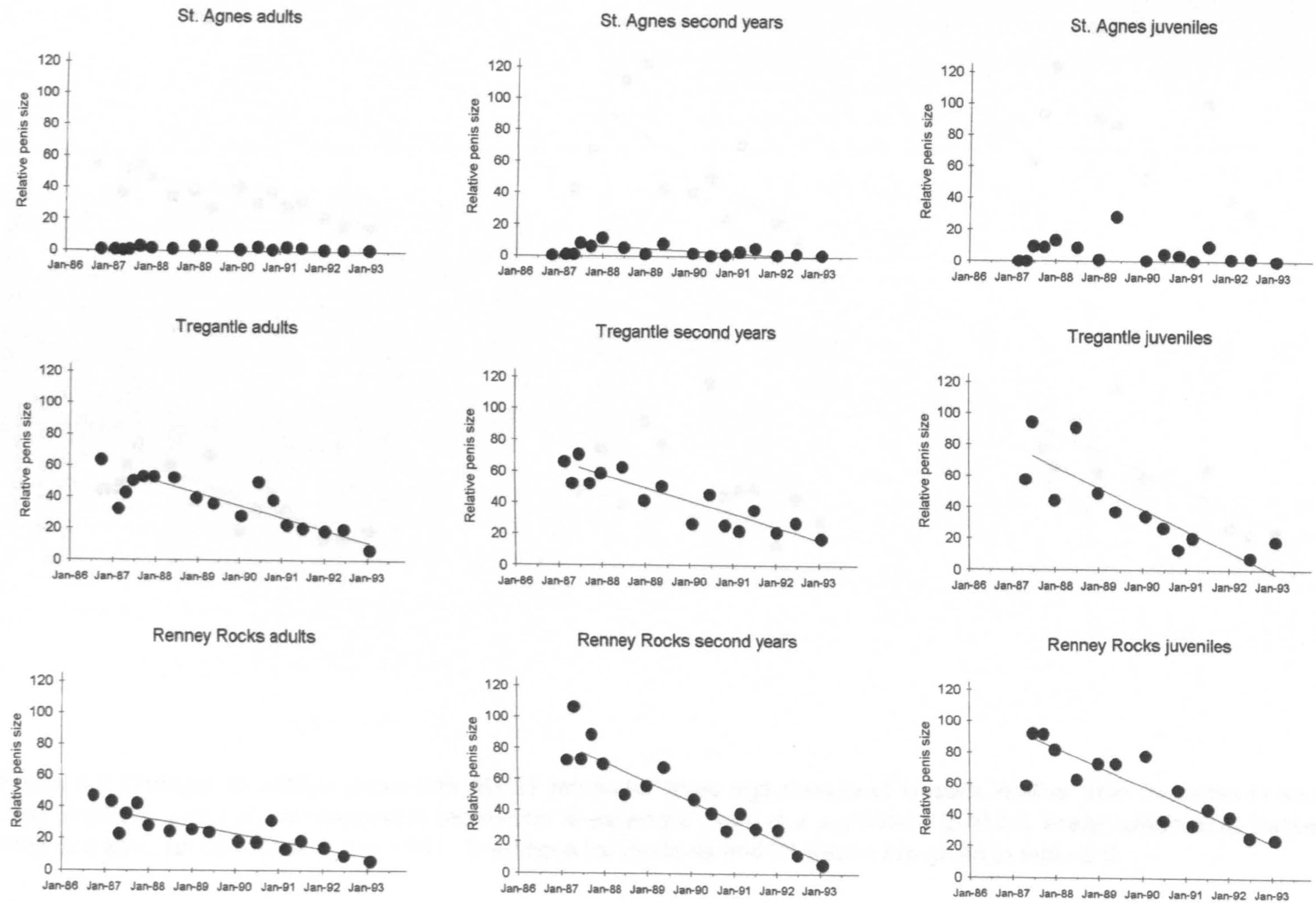
5.3 Results

5.3.1 Relative penis size

There is a downward trend in the relative penis size of dogwhelk populations of all ages from Tregantle, Renney Rocks, Kingsand and Jennycliff (figure 5.2). At the less contaminated site of St. Agnes, by comparison, the RPS values remain relatively unchanged throughout the period of the two studies, with RPS values for adults, second years and juveniles remaining mostly within a range of 0-10.

Two-way analysis of variance showed there to be a significant difference in RPS values between the five sites and over time for both adults (table 5.1) and for second years (table 5.2) (juveniles not tested). St. Agnes had significantly lower RPS values for both adults and for second years than at the other four Plymouth sites (juveniles not tested). Analysis of RPS values over time showed that after the ban in July 1987 adult RPS values did not change significantly until February 1990. However results for February were uncharacteristically low and consequently RPS values in July 1990, which were higher, were not significantly lower than those recorded before the ban. Consistently lower RPS values for adults were recorded in 1992, five years after the ban had first been imposed. The recovery of second years reflected the recovery of adults with significant changes in relative penis size occurring in July 1992 five years after the ban.

The trend of decreasing relative penis size values appears to be due to a decrease in the average length of the female penis rather than to changes in the male penis for adult (figure 5.3) and second year (figure 5.4) *Nucella*. In juvenile *Nucella*, however, the decrease in the female penis size appears to be accompanied by a slight decrease in the male penis size at Tregantle, Renney Rocks, Jennycliff and


St. Agnes adults
St. Agnes second years
St. Agnes juveniles
Tregantle adults
Tregantle second years
Tregantle juveniles
Renney Rocks adults
Renney Rocks second years
Renney Rocks juveniles
Relative penis size
0
20
40
60
80
100
120
Jan-86
Jan-87
Jan-88
Jan-89
Jan-90
Jan-91
Jan-92
Jan-93

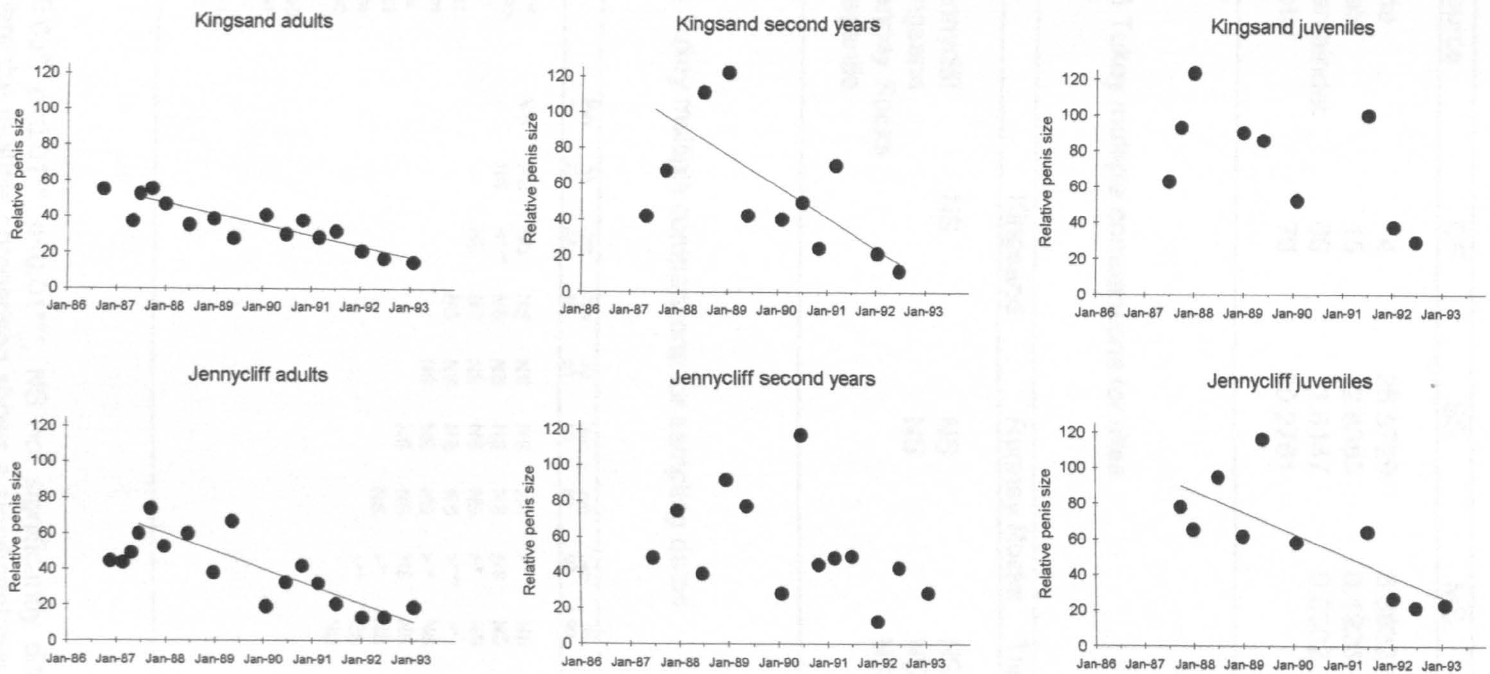


Figure 5.2 Changes in relative penis size (RPS) values for three age classes of *Nucella lapillus* from five sites in south-west England. Lines shown represent regression lines where there is a significant ($p<0.05$) linear relationship between RPS and time, on data after 1 July 1987. Equations for the lines and R^2 values are given in table 5.3.

Table 5.1 Two factor analysis of variance without replication on adult relative penis size values ($\log_{10}$ transformed) at the five study sites on each sampling date.

Source	DF	SS	MS	F	p
Site	4	25.5729	6.3932	211.69	p<0.001 ***
Date	15	2.8885	0.1926	6.37	p<0.001 ***
Remainder	60	1.8147	0.0302		
Total	79	30.2761			

(a) Tukey multiple comparisons for sites

	Kingsand	Renney Rocks	Tregantle	St. Agnes
Jennycliff	NS	NS	NS	>***
Kingsand		NS	NS	>***
Renney Rocks			NS	>***
Tregantle				>***

(b) Tukey multiple comparisons for sampling dates

	May 87	Jul 87	Oct 87	Jan 88	Jul 88	Jan 89	Jun 89	Feb 90	Jul 90	Nov 90	Mar 91	Jul 91	Jan 92	Jul 92	Feb 93
Oct 86	NS	NS	NS	NS	NS	NS	NS	>*	NS	NS	NS	NS	>***	>***	>***
May 87		NS	<***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	>**	>***
Jul 87			NS	NS	NS	NS	NS	>*	NS	NS	NS	NS	>***	>***	>***
Oct 87				NS	NS	NS	NS	>***	>*	>*	>***	>***	>***	>***	>***
Jan 88					NS	NS	NS	>**	NS	NS	NS	NS	>***	>***	>***
Jul 88						NS	NS	NS	NS	NS	NS	NS	>***	>***	>***
Jan 89							NS	>*	NS	NS	NS	NS	>***	>***	>***
Jun 89								>**	NS	NS	NS	NS	>***	>***	>***
Feb 90									NS	NS	NS	NS	NS	NS	>***
Jul 90										NS	NS	NS	>**	>***	>***
Nov 90											NS	NS	>**	>***	>***
Mar 91												NS	NS	>**	>***
Jul 91													NS	>*	>***
Jan 92														NS	>*
Jul 92															NS

p<0.05*, p<0.01**, p<0.01***, NS not significantly different at the p=0.05 level. Where the multiple comparison shows a significant result > denotes that the site or year on the side of the table had a greater RPS value than the site or date on the top, < indicates the opposite.

Table 5.2 Two factor analysis of variance without replication on second year relative penis size values ($\log_{10}$ transformed) at the five study sites on each sampling date.

Source	DF	SS	MS	F	p
Site	4	15.1241	3.7810	50.96	p<0.001 ***
Date	8	1.8958	0.2370	3.19	p<0.05 *
Remainder	32	2.3738	0.0742		
Total	44	19.3936			

(a) Tukey multiple comparisons for sites

	Kingsand	Renney Rocks	Tregantle	St. Agnes
Jennycliff	NS	NS	NS	>***
Kingsand		NS	NS	>***
Renney Rocks			NS	>***
Tregantle				>***

(b) Tukey multiple comparisons for sampling dates

	Jan 89	Jun 89	Jul 90	Nov 90	Mar 91	Jan 92	Jul 92	Feb 93
Jul 88	NS	NS	NS	NS	NS	NS	>**	>*
Jan 89		NS	NS	NS	NS	NS	>**	>*
Jun 89			NS	NS	>**	NS	>**	>**
Jul 90				NS	NS	NS	NS	NS
Nov 90					NS	NS	NS	NS
Mar 91						NS	NS	NS
Jan 92							NS	NS
Jul 92								NS

p<0.05*, p<0.01**, p<0.01***, NS not significantly different at the p=0.05 level. Where the multiple comparison shows a significant result > denotes that the site or date on the side of the table had a greater RPS value than the site or year on the top.

Kingsand (figure 5.5). This gives a distorted view of changes in the RPS value, effectively slowing the rate at which it decreases even though the female penis is reducing in size (figure 5.2). Similarly in some cases the changes in the RPS value at any one sampling date may have been exaggerated by small fluctuations in the length of the male penis rather than those occurring in females. For example, the rise in RPS value seen in the adult population at Tregantle in November 1990 is not due to an increase in the size of the female penis but instead due to a decrease in that of the males (see figure 5.2 and 5.3). Similarly, a reduction in the RPS value does not necessarily correspond to smaller penis sizes in the females but may indicate an increased male penis as seen for example, in November 1990 at Kingsand when the RPS value for *Nucella* in their second year dropped (figure 5.2). This was due to an increase in the average male penis length, not to changes in the female penis (figure 5.4). In some cases fluctuations recorded in both the male and female penis occurred when sample sizes were low, as was often the case in the numbers of juveniles collected.

The penis size of the male increases from juvenile to second years and reaches an average size of about 4.5 mm in adult dogwhelks (figure 5.3). The average length of the adult female penis was always observed to be smaller than that of the male, with the greatest difference occurring between the sexes at St. Agnes. In *Nucella* in their second year the average female penis length approached that of the male at Renney Rocks, Kingsand and Jennycliff (figure 5.4). The average female penis length for juveniles at Kingsand and at Jennycliff was of a greater size than that of the males from the same site on a number of sampling occasions, giving rise to RPS values greater than 100 (figure 5.2).

Regression lines fitted to the relative penis size values after 1 July 1987 show significant linear relationships between time and changes in RPS for most ages of

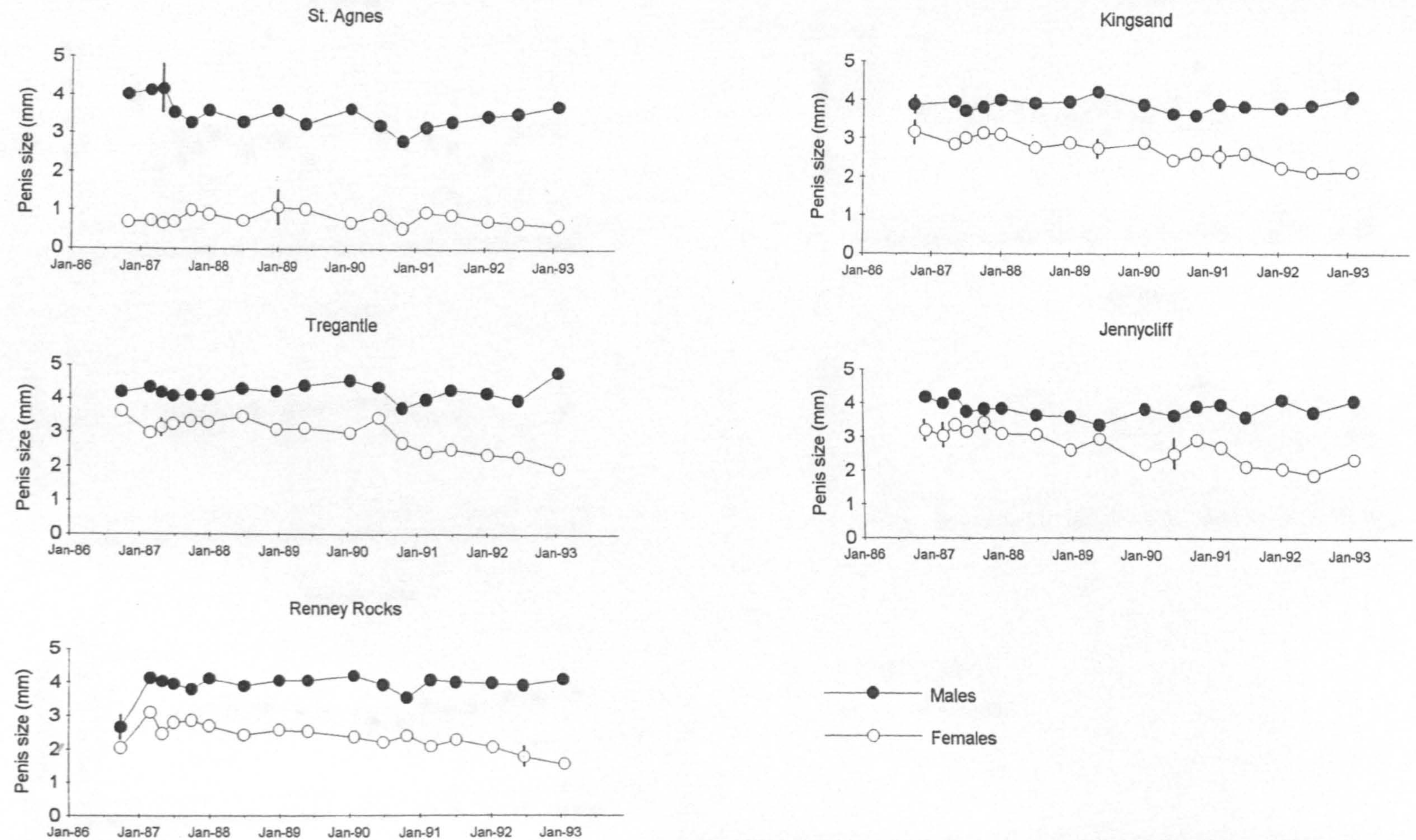


Figure 5.3 Changes in penis size in adult male and female *Nucella lapillus* from five sites in south-west England. Error bars ± 1 SE.

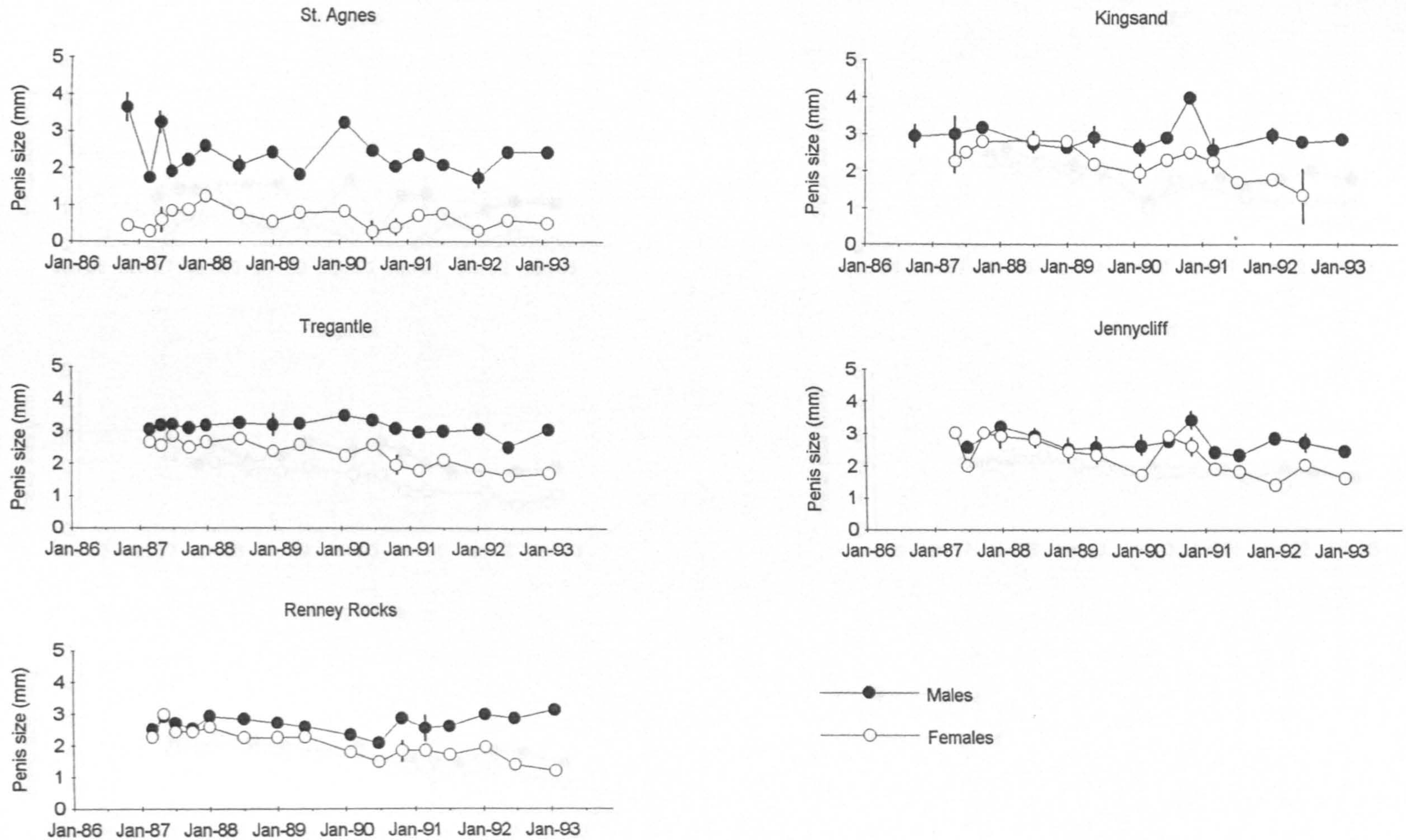


Figure 5.4 Changes in penis size in second year male and female *Nucella lapillus* from five sites in south-west England ± 1 SE.

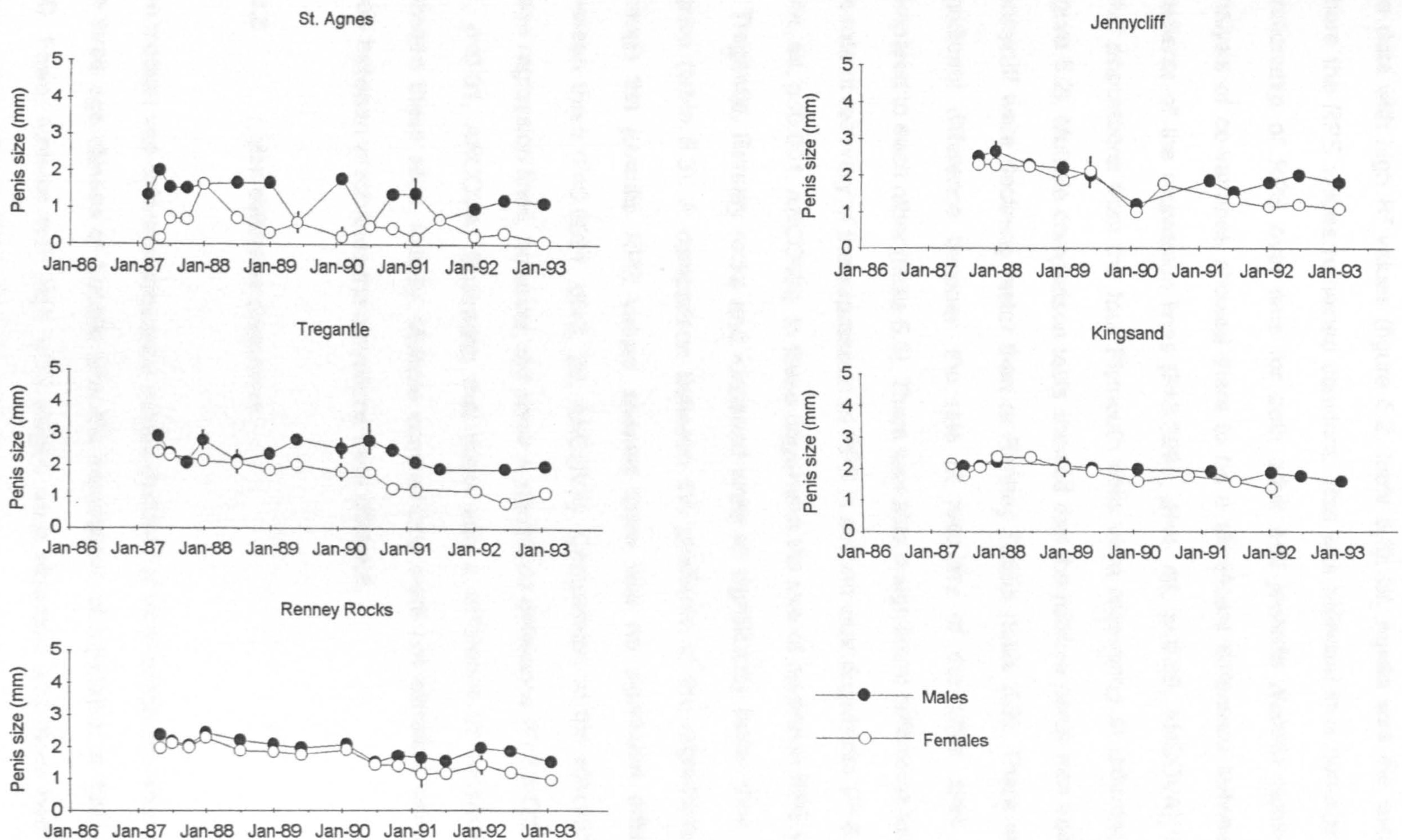


Figure 5.5 Changes in penis size in juvenile male and female *Nucella lapillus* from five sites in south-west England ± 1 SE.

dogwhelks from all sites (table 5.3). Most of the regression lines show good fits to the data with high R^2 values (figure 5.2, table 5.3). St. Agnes was the exception where the RPS values remained constant. This was reflected in a non-significant relationship of RPS over time for both adult and juvenile *Nucella* (table 5.3). Analysis of co-variance showed there to be a significant difference between the gradients of the regression lines ($F=3.3995$, $df=4, 48$, $p<0.05$, ANCOVA), hence adult populations from the four Plymouth sites were recovering at different rates (figure 5.2). Multiple comparison tests showed that the relative penis size values at Jennycliff were declining faster than at Renney Rocks (table 5.3). There was no significant difference between the rate of recovery of the other sites when compared to each other (table 5.3). There was also a significant difference between the rate of recovery of the expression of RPS in second year dogwhelks ($F=8.3189$, $df=4, 44$, $p<0.001$, ANCOVA). In these dogwhelks the rate of decline in RPS values at Tregantle, Renney rocks and Kingsand were all significantly faster than at St. Agnes (table 5.3). A comparison between the gradients of the regression lines through the juvenile RPS values showed there was no significant difference between them ($F=0.0891$, $df=3, 29$, ANCOVA). Comparison of the elevations of these regression lines, however, did show a significant difference ($F=6.6423$, $df=2, 31$, $p<0.01$, ANCOVA) illustrating that there was a difference in contamination between these sites, initially. Multiple comparisons were not sensitive enough to show between which lines the elevations were different.

5.3.2 Vas deferens sequence

The median vas deferens sequence values declined at most of the five sites and in the three age classes of *Nucella* after the introduction of legislation in 1987 (table 5.4). Fewer females with high VDS stages were recorded and more had lower stages (2 and 3) by 1993. Adult *Nucella* expressing vas deferens sequence stages

Table 5.3 Regression analysis results for changes in relative penis size in three age classes of *Nucella lapillus*, over time, after the introduction of legislation on 1 July 1987 (see figure 5.2). In the equations Y = Relative penis size (RPS) and X = time in Julian days, for example 1 July 1987 = 31959. NS = not significant.

(a) St. Agnes

	Equation	Source	DF	MS	F	R^2	P
Adults	Y = 18.3 - 0.0005 X	Regression	1	1.5	1.54	4.0	p=0.238 NS
		Residual	12	0.9			
Second years	Y = 121 - 0.0036 X	Regression	1	73.8	10.88	43.2	p<0.01 **
		Residual	12	6.8			
Juveniles	Y = 185 - 0.0054 X	Regression	1	170.0	3.43	15.7	p=0.089 NS
		Residual	12	49.6			

(b) Tregantle

	Equation	Source	DF	MS	F	R^2	P
Adults	Y = 732 - 0.0212 X	Regression	1	2586.2	50.13	79.1	p<0.001 ***
		Residual	12	51.6			
Second years	Y = 791 - 0.0228 X	Regression	1	3012.1	43.28	76.5	p<0.001 ***
		Residual	12	69.6			
Juveniles	Y = 1252 - 0.0369 X	Regression	1	5783.8	19.28	64.6	p<0.01 **
		Residual	9	300.1			

(c) Renney Rocks

	Equation	Source	DF	MS	F	R^2	P
Adults	Y = 457 - 0.0132 X	Regression	1	1007.8	34.64	72.1	p<0.001 ***
		Residual	12	29.1			
Second years	Y = 1167 - 0.0341 X	Regression	1	6716.4	93.86	87.7	p<0.001 ***
		Residual	12	71.6			
Juveniles	Y = 1150 - 0.0332 X	Regression	1	6358.6	63.23	82.7	p<0.001 ***
		Residual	12	100.6			

(d) Kingsand

	Equation	Source	DF	MS	F	R^2	P
Adults	Y = 578 - 0.0165 X	Regression	1	1574.9	45.67	77.5	p<0.001 ***
		Residual	12	34.5			
Second years	Y = 1607 - 0.0471 X	Regression	1	6246.6	8.09	44.1	p<0.05 *
		Residual	8	771.8			
Juveniles	Y = 951 - 0.0267 X	Regression	1	2755.7	3.87	26.4	p=0.090 NS
		Residual	7	711.7			

(e) Jennycliff

	Equation	Source	DF	MS	F	R^2	P
Adults	Y = 925 - 0.0269 X	Regression	1	4184.8	33.32	71.3	p<0.001 ***
		Residual	12	125.6			
Second years	Y = 637 - 0.0177 X	Regression	1	1550.7	2.03	7.9	p=0.182 NS
		Residual	11	765.3			
Juveniles	Y = 1135 - 0.0326 X	Regression	1	4913.7	10.41	51.1	p<0.05 *
		Residual	8	472.1			

(f) Tukey multiple comparison tests between the slopes of regression lines through relative penis size data in adults.

	Renney Rocks	Kingsand	Jennycliff
Tregantle	NS	NS	NS
Renney Rocks		NS	<*
Kingsand			NS

(g) Tukey multiple comparison tests between the slopes of regression lines through relative penis size data in second years.

	Tregantle	Renney Rocks	Kingsand
St. Agnes	<***	<***	<**
Tregantle		NS	NS
Renney Rocks			NS

Table 5.4 Vas deferens sequence median stage, range and percent sterile females (stage 5-6) from three age groups of *Nucella lapillus*, where, ns, not sampled, nf, no dogwhelks found and n, number of females.

(a) St. Agnes

	Adults				Second years				Juveniles			
Date	n	Median	Range	% Sterile	n	Median	Range	% Sterile	n	Median	Range	% Sterile
Nov 86	10	3	2-4	0	2	3	3	0	ns	ns	ns	ns
Mar 87	21	3	1-4	0	11	2	1-3	0	1	1	1	0
May 87	18	3	1-4	0	3	3	2-3	0	13	2	0-3	0
Jul 87	16	4	1-4	0	16	3	1-4	0	14	3	3-4	0
Oct 87	15	3	3-4	0	12	3	2-3	0	6	3	2-3	0
Jan 88	17	3	2-4	0	2	3	3	0	10	3	3	0
Jul 88	15	3	2-4	0	10	3	2-3	0	21	3	2-3	0
Jan 89	14	3	1-4	0	10	3	1-3	0	15	2	0-3	0
Jun 89	13	3	2-4	0	10	3	3	0	16	3	1-3	0
Feb 90	16	3	2-4	0	4	3	3	0	2	2	2-3	0
Jul 90	18	3	3-4	0	2	2.5	2-3	0	10	3	2-3	0
Nov 90	30	3	2-4	0	6	3	2-3	0	16	3	2-4	0
Mar 91	19	4	3-5	5.0	5	4	3-4	0	8	2.5	1-3	0
Jul 91	50	3	3-4	0	12	3	3	0	6	3	3	0
Jan 92	53	3	2-4	0	5	3	2-3	0	4	2.5	2-3	0
Jul 92	21	3	2-4	0	3	3	3-4	0	1	3	3	0
Feb 93	23	3	2-4	0	15	3	2-4	0	4	2	2-3	0

(b) Tregantle

	Adults				Second years				Juveniles			
Date	n	Median	Range	% Sterile	n	Median	Range	% Sterile	n	Median	Range	% Sterile
Oct 86	16	4	4-6	24.2	ns	ns	ns	ns	ns	ns	ns	ns
Mar 87	12	4	4-6	7.2	14	4	3-5	14.3	ns	ns	ns	ns
May 87	8	4	4-5	25.0	4	4	4	0	9	4	4	0
Jul 87	9	4	4-6	33.3	16	4	3-5	6.2	3	4	4	0
Oct 87	15	4	4-5	26.7	5	4	4	0	nf	nf	nf	nf
Jan 88	13	4	4-5	7.7	5	4	4	0	5	3	3	0
Jul 88	16	4	4-6	12.5	8	4	4	0	4	4	4	0
Jan 89	21	4	4-5	4.8	5	4	4	0	10	4	4	0
Jun 89	10	4	4-6	20.0	9	4	4	0	5	4	4	0
Feb 90	17	4	4-6	11.8	16	4	4	0	4	3	3	0
Jul 90	14	4	4-6	28.6	9	4	4	0	1	3	3	0
Nov 90	24	4	4-6	16.7	12	4	3-4	0	19	3	3-4	0
Mar 91	20	4	4-6	40.0	19	3	2-4	0	11	3	3-4	0
Jul 91	39	4	3-4	0	7	4	3-4	0	nf	nf	nf	nf
Jan 92	38	4	3-4	0	8	4	4	0	6	2.5	2-3	0
Jul 92	27	4	4	0	3	4	3-4	0	1	3	3	0
Feb 93	19	4	2-4	0	5	3	3-4	0	3	3	3-4	0

(c) Renney Rocks

Date		Adults				Second years				Juveniles			
		n	Median	Range	% Sterile	n	Median	Range	% Sterile	n	Median	Range	% Sterile
Oct	86	5	4	4	0	ns	ns	ns	ns	ns	ns	ns	ns
Mar	87	11	4	4-6	36.4	16	4	3-5	12.5	ns	ns	ns	ns
May	87	11	4	4-6	45.5	4	4.5	4-5	50.0	14	4	4	0
Jul	87	8	5	4-6	62.5	9	4	4-5	44.4	7	4	4	0
Oct	87	16	4	4-6	30.0	6	4	4-5	16.7	9	4	4	0
Jan	88	18	4	4-5	16.7	12	4	4-5	25.0	23	4	4-5	4.3
Jul	88	6	4.5	4-5	50.0	20	4	4-5	30.0	13	4	4	0
Jan	89	11	5	4-6	54.5	11	4	4	0	22	4	4	0
Jun	89	9	5	4-6	55.6	7	4	4	0	8	4	4	0
Feb	90	12	5	4-6	33.0	7	4	2-4	0	3	3	3-4	0
Jul	90	14	4	4-6	21.4	6	4	3-4	0	12	4	4	0
Nov	90	19	4	4-6	31.6	6	4	4	0	9	4	3-4	0
Mar	91	7	4	4-6	42.8	5	3	3-4	0	2	3	3	0
Jul	91	20	4	4-6	30.0	14	4	3-4	0	17	4	3-4	0
Jan	92	15	4	4-6	20.0	8	4	4	0	3	4	4	0
Jul	92	8	4	4-6	25.0	11	4	3-4	0	5	4	4	0
Feb	93	15	4	4-5	6.7	15	4	3-4	0	7	3	3-4	0

(d) Kingsand

Date		Adults				Second years				Juveniles			
		n	Median	Range	% Sterile	n	Median	Range	% Sterile	n	Median	Range	% Sterile
Oct	86	6	5	5-6	100	nf	nf	nf	nf	nf	nf	nf	nf
May	87	5	5	4-6	60.0	2	4	4	0	3	4	4	0
Jul	87	6	4	4-5	33.3	2	4.5	4-5	50.0	1	4	4	0
Oct	87	4	5	4-5	75.0	4	4.5	4-5	50.0	2	4.5	4-5	50.0
Jan	88	3	5	4-5	66.7	nf	nf	nf	nf	4	4	2-5	20.0
Jul	88	8	4	4-5	37.5	3	4	4	0	3	4	4	0
Jan	89	15	4	4-6	33.3	2	4	4	0	6	4	4	0
Jun	89	10	5	4-6	20.0	4	4	4	0	4	4	4	0
Feb	90	7	4	4-6	28.6	5	4	4	0	2	4	4	0
Jul	90	5	4	4-6	12.5	2	4	4	0	nf	nf	nf	0
Nov	90	10	4	4-6	30.0	2	4	4	0	1	4	4	0
Mar	91	5	4	3-5	40.0	2	4	4	0	nf	nf	nf	0
Jul	91	19	4	4-6	21.1	1	4	4	0	1	4	4	0
Jan	92	5	4	4	0	7	4	3-4	0	4	4	3-4	0
Jul	92	3	5	4-5	67.0	2	3.5	3-4	0	10	3	2-4	0
Feb	93	17	4	2-4	0	3	3	2-4	0	4	4	2-4	0

(e) Jennycliff

Date	Adults n	Median	Range	% Sterile	Second years n	Median	Range	% Sterile	Juveniles n	Median	Range	% Sterile
Dec 86	7	6	5-6	100	nf	nf	nf	nf	nf	nf	nf	nf
Mar 87	5	6	6	100	nf	nf	nf	nf	nf	nf	nf	nf
May 87	2	6	6	100	1	4	4	0	nf	nf	nf	nf
Jul 87	2	6	6	100	1	4	4	0	nf	nf	nf	nf
Oct 87	4	5	4-6	75.0	1	5	5	100	1	4	4	0
Jan 88	2	5	5	100	2	5	5	100	1	4	4	0
Jul 88	7	5	5-6	100	7	4	4-5	28.6	7	4	4-5	28.6
Jan 89	8	4	4-6	25.0	3	4	4	0	3	4	4	0
Jun 89	7	5	4-6	71.4	5	4	4	0	2	4	4	0
Feb 90	11	4	4-6	27.3	3	4	4	0	2	4	4	0
Jul 90	3	5	4-6	66.7	1	4	4	0	2	3	3	0
Nov 90	12	4	4-6	16.0	8	4	4	0	nf	nf	nf	nf
Mar 91	5	5	4-6	80.0	1	4	4	0	nf	nf	nf	nf
Jul 91	12	4	4-6	16.7	12	4	4	0	6	4	4	0
Jan 92	25	4	4-6	36.0	6	4	4	0	6	4	4	0
Jul 92	11	4	4-5	22.0	3	4	4	0	5	4	3-4	0
Feb 93	15	4	3-6	13.3	12	4	3-4	0	2	4	4	0

below 2 were only recorded at St. Agnes. Within each of the samples collected the vas deferens stage expressed varied very little, usually spanning only two of the six stages.

The percentage of sterile females within the population (the proportion with VDS stages 5 and 6) clearly showed the recovery in the reproductive capability of the females within these populations (table 5.4, figure 5.6). At St. Agnes there were no records of sterile second years or juveniles and on only one occasion was a sterile adult found, a single stage 5 specimen in March 1991. Adults from the populations sampled in Plymouth Sound, on the other hand, have been extremely badly affected, especially at Kingsand and Jennycliff (figure 5.6). However, since 1987 there has been some reduction in the percentage of stage 5 and 6 females. This has now fallen from 100% in 1986 to 13.3% at Jennycliff and to 0% at Kingsand in 1993. Samples taken in February 1993, some 6 years after TBT was effectively banned, show around 10% of adult females at Renney Rocks and Jennycliff are still effectively sterile (table 5.4, figure 5.6). Only occasionally have the juveniles from populations in Plymouth Sound been recorded as being sterile, mostly in the late 1980's (table 5.4), although no juveniles were recorded at Jennycliff between December 1986 and October 1987. There was, however, sometimes difficulty in deciding whether a juvenile was sterile because the genital papilla is not fully developed.

5.3.3 Changes in *Nucella lapillus* populations

5.3.3.1 Sex ratios

There was no deviation from a female to male sex ratio of 7:5 in the adults sampled from St. Agnes throughout the period of study (table 5.5, figure 5.7). At Jennycliff

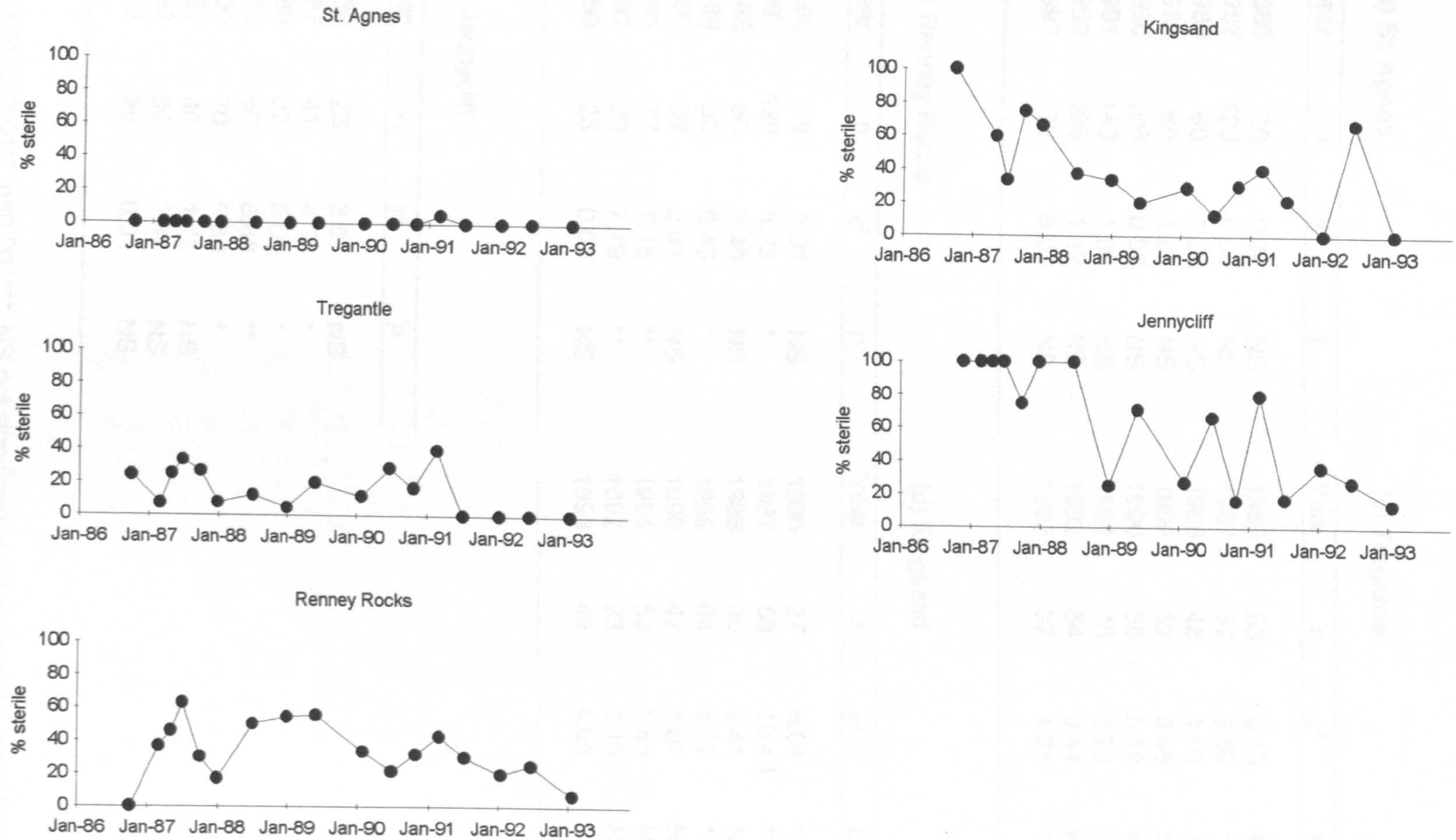


Figure 5.6 The percentage of sterile adult female *Nucella lapillus* (vas deferens sequence stages 5 and 6) at five sites in south-west England.

Table 5.5 Deviations from a female to male ratio of 7:5 in adult *Nucella lapillus* populations at five sites in south-west England over time, data pooled for years. Analysis using the chi-square test.

(a) St. Agnes

Year	n	χ^2	p
1986	20	0.24	NS
1987	120	0.00	NS
1988	60	0.26	NS
1989	60	1.83	NS
1990	106	0.08	NS
1991	140	1.96	NS
1992	196	1.12	NS
1993	38	0.03	NS

(b) Tregantle

Year	n	χ^2	p
1986	53	0.13	NS
1987	37	9.66	**
1988	48	1.03	NS
1989	52	0.46	NS
1990	55	0.19	NS
1991	46	3.29	NS
1992	54	0.44	NS
1993	37	4.23	*

(c) Renney Rocks

Year	n	χ^2	p
1986	9	0.01	NS
1987	120	8.23	*
1988	60	3.46	NS
1989	60	6.43	*
1990	99	2.81	NS
1991	77	7.15	**
1992	70	7.79	**
1993	25	0.01	NS

(d) Kingsand

Year	n	χ^2	p
1986	27	6.04	*
1987	58	10.48	**
1988	30	2.41	NS
1989	64	4.07	*
1990	47	1.07	NS
1991	53	1.55	NS
1992	23	2.19	NS
1993	49	4.69	*

(e) Jennycliff

Year	n	χ^2	p
1986	23	3.07	NS
1987	41	4.11	*
1988	32	5.01	*
1989	50	6.88	**
1990	70	5.39	*
1991	40	1.72	NS
1992	80	2.44	NS
1993	35	0.01	NS

$p<0.05$*, $p<0.01$**, $p<0.001$***, NS not significantly different at the p=0.05 level.

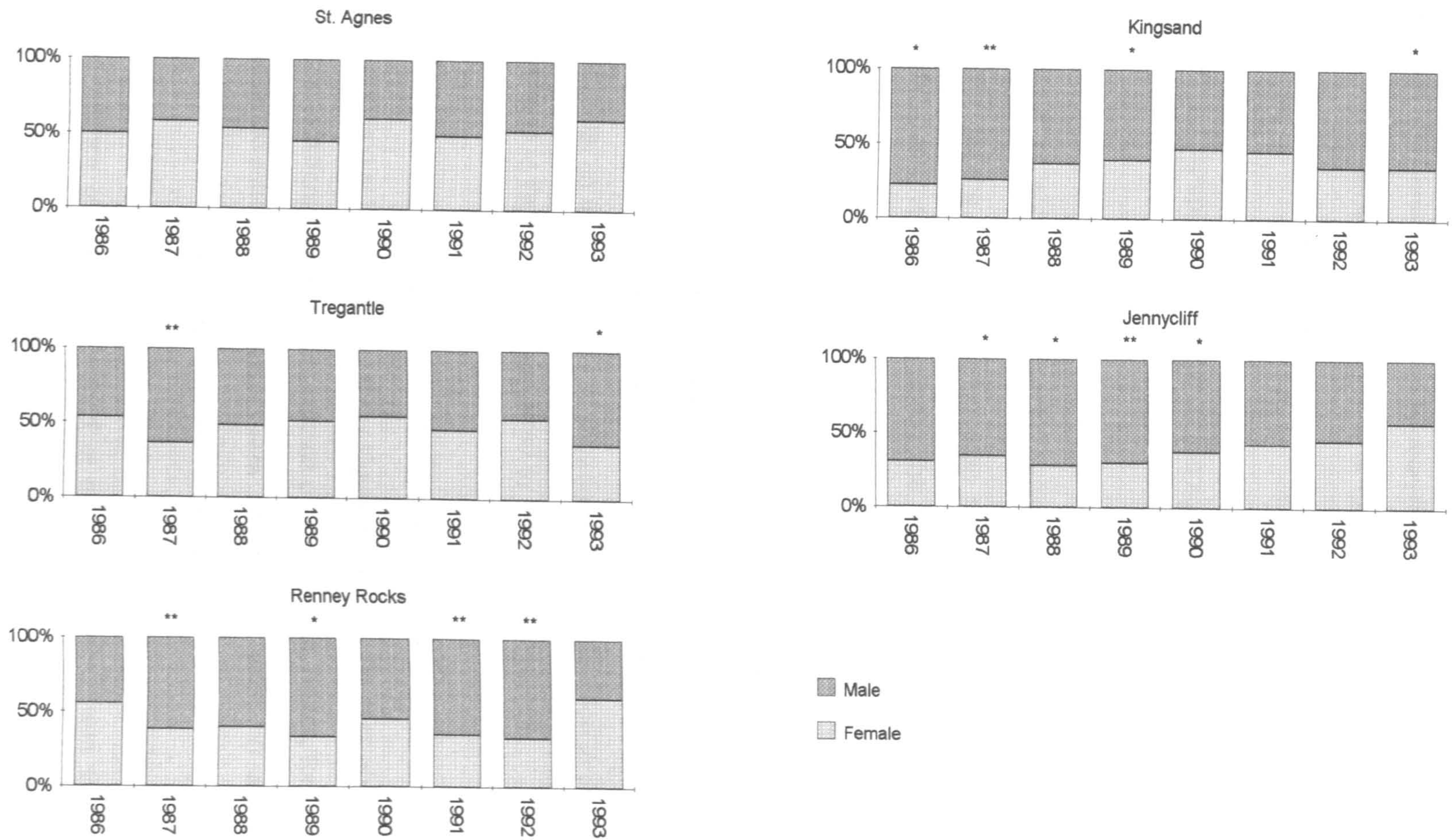


Figure 5.7 The percentage of males and females in adult *Nucella lapillus* populations, pooled for each year. Significant deviation from female to male ratio of 7:5 (Chi-square test) indicated by * $p<0.05$, ** $p<0.01$ or *** $p<0.001$. For full results of the Chi-square test see table 5.5.

the ratio of females to males differed significantly from the expected numbers between 1987 and 1990 but then appeared normal. At the other sites in Plymouth Sound the balance in the proportions of each sex did deviate from the ratio, but there appeared no clear trends on the occurrence of these deviations.

5.3.3.2 Population abundance and age structure

Changes in the total abundance of dogwhelk populations sampled tended to vary depending on the method used to measure abundance (figure 5.8). Only at Tregantle did the fixed monitoring area results appear to mirror the changes observed from the timed collections. The data from the monitoring area at St. Agnes gave the impression that dogwhelk abundance was much lower at this site than at Tregantle and Renney Rocks. However, the timed collections showed that dogwhelk abundance at St. Agnes was as high, if not higher than at Tregantle or Renney Rocks. At Kingsand and Jennycliff dogwhelk abundance increased although it was initially much lower than at the other south-west sites. St. Agnes and Tregantle on the other hand all showed a gradual reduction in the abundance of dogwhelks at these sites as measured by the timed collections.

The differences in total dogwhelk abundance as measured by timed collections (figure 5.9) and at the monitoring areas (figure 5.10) at all the sites appeared mainly to be due to differences in the abundance of adults. At St. Agnes, for example, 50-100 adult dogwhelks on average were collected in each 10 minute search compared to 0-50 at Jennycliff. In general irrespective of the sampling method used or the site collected from, adults made up over 50% of the population (figure 5.11). The abundance of dogwhelks varied at each of the sites, with lower numbers collected in timed searches from Jennycliff than from St. Agnes, for example (figure 5.9). Juveniles made up the smallest percentage of the dogwhelk populations at all

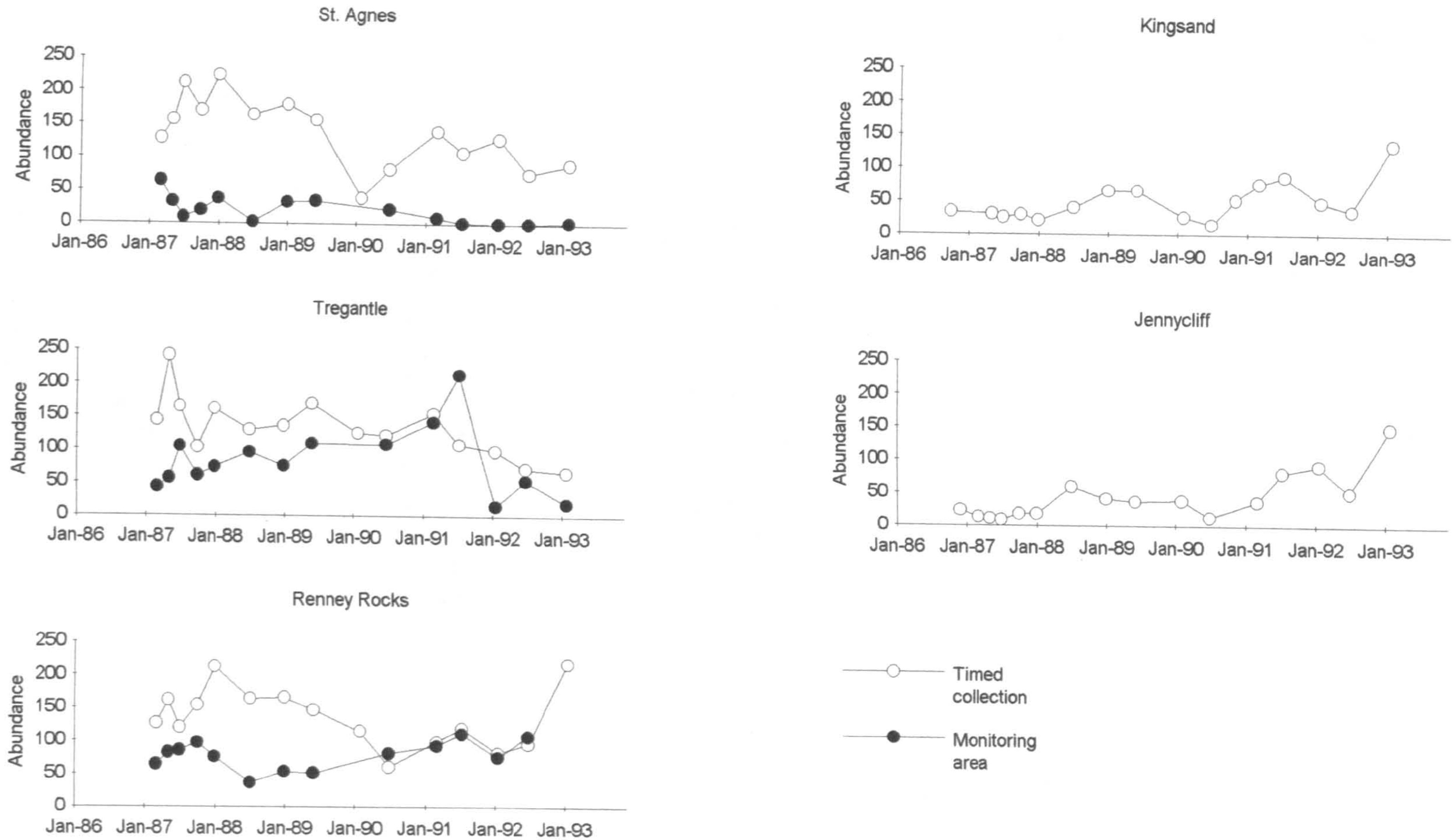


Figure 5.8 Total abundance of *Nucella lapillus* measured at five sites in south-west England. Abundance on the Y-axis represents for the fixed monitoring areas the number of dogwhelks at the monitoring site (4 m²) and for timed collections the number of dogwhelks collected in a 10 minute search.

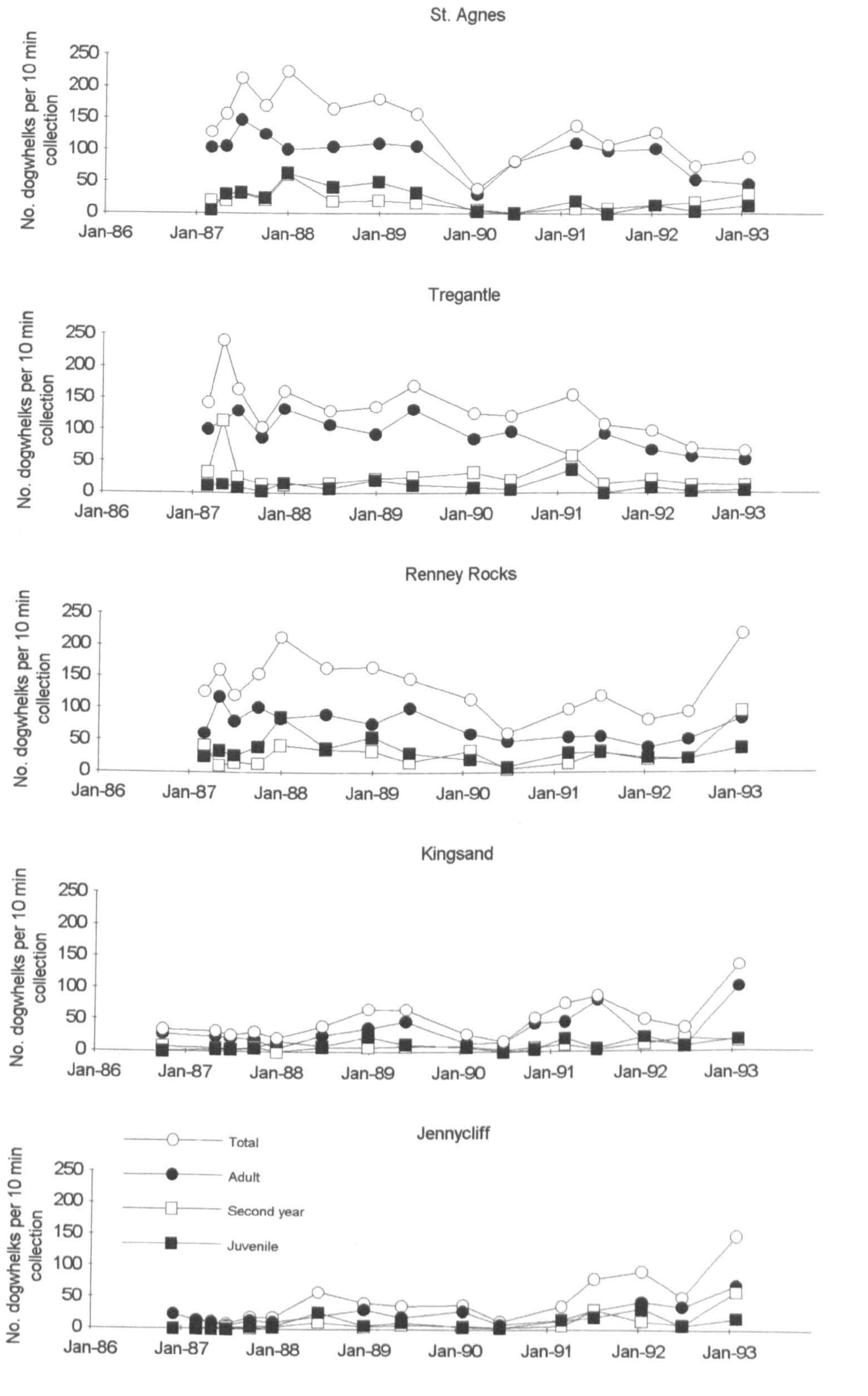


Figure 5.9 Abundance of *Nucella lapillus* collected from five sites in south-west England, measured as the number of dogwhelks collected per 10 minute search.

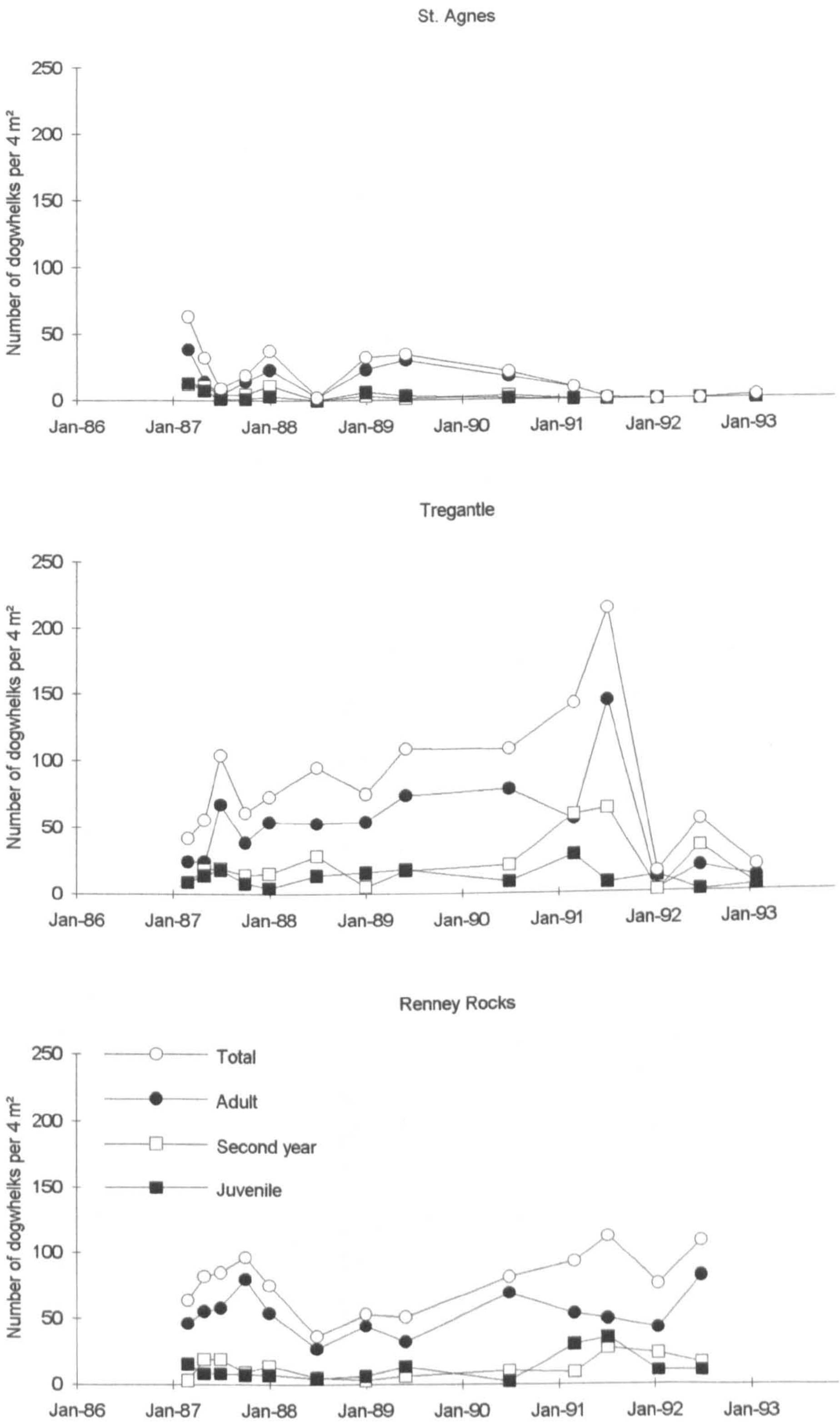


Figure 5.10 Abundance of *Nucella lapillus* within monitoring areas (4 m²) at St. Agnes, Tregantle and Renney Rocks in south-west England.

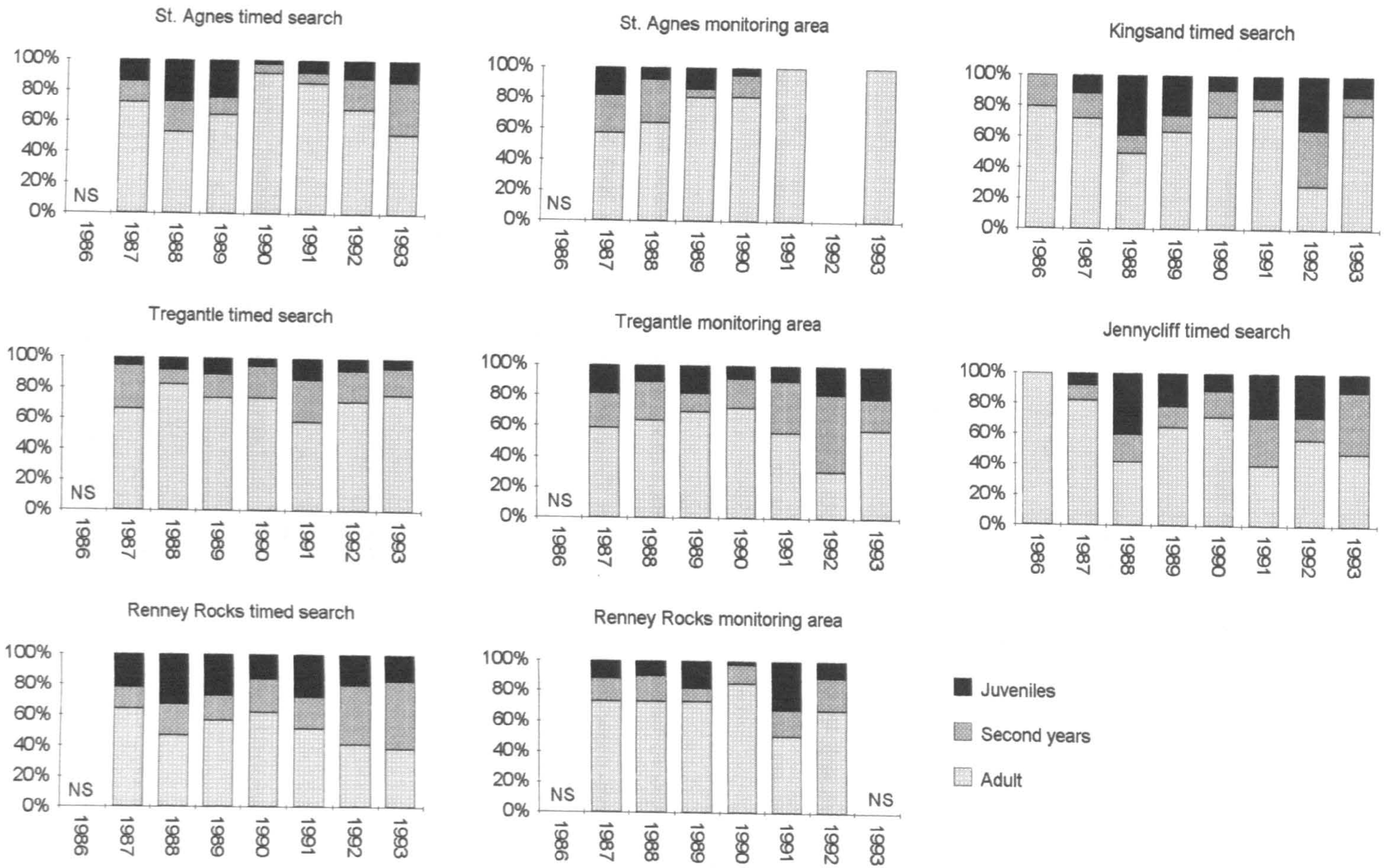


Figure 5.11 The percentage of adult, second year and juvenile *Nucella lapillus* in samples collected from monitoring areas and by timed collections at five sites in south-west England. Years where no samples were taken are represented by NS. Where no bars appear no dogwhelks were found.

sites and most sampling dates. In some cases no juveniles were recorded, most noticeably from Kingsand and Jennycliff in 1986, and at the monitoring area at St. Agnes in 1991 and 1993.

5.3.4 Changes in the community of the monitoring areas

Generally there was little change in the community of any of the monitoring areas during the period of study. At Renney Rocks the dense barnacle cover remained relatively constant whilst the cover of *Fucus* declined from a dense canopy in November 1990 to a few sparse plants by February 1993.

At St. Agnes the most significant change in the community of the monitoring area occurred in January 1990 (pers. comm. S. K. Spence, Plymouth Marine Laboratory) when winter storms ripped the dense mussel covering from the horizontal surface of the area. This area by November 1990 had been recolonised in part by barnacle settlement of that year. By January 1992 juvenile mussels were recolonising the horizontal area, forming small patches near crevices in the rock. These patches increased in size until February 1993 where winter storms removed the larger individuals from within the patches. Throughout the study period the barnacle cover on the vertical surface remained relatively constant.

The greatest habitat changes occurred at Tregantle where changes in the sand level effectively halved the area of the monitoring rock. In November 1990 the monitored rock rose 120 cm above the sand level and by March 1991 the dropping of the sand level by 30 cm created a band of bare rock below the barnacles. The sand level then remained constant until February 1992 where on return to the monitoring area only 70 cm remained above the sand, leaving only the mussel zone above the sand.

During this time the mussel zone also altered, the dense older barnacle covered individuals slowly declined in density until by August 1992 the area was a patchy distribution of barnacles and younger mussels each occupying around 50% of the available space.

5.3.5 Relationship between dogwhelk abundance and imposex

There is a general trend of increasing dogwhelk abundance with decreasing relative penis size and the percentage of sterile females (figure 5.12). When the relationship is assessed using data for all the years when samples were collected and at all the sites used the correlation is only a weak to moderate one (table 5.6, figure 5.12). Correlations of abundance and relative penis size or the percentage of sterile females are stronger in some cases when each of the years or sites are considered separately. This is especially the case in the late 1980's (table 5.6, figure 5.13) and at the more polluted sites (Kingsand, Jennycliff and Tregantle) (table 5.6, figure 5.14). Generally the intensity of the correlation is greater between abundance and the percentage of sterile females than with RPS (table 5.6).

5.3.6 Relationship between concentrations of TBT in the water and imposex and abundance

There is a significant positive correlation between concentrations of tributyltin in the water (chapter 4) and the relative penis size and percentage of sterile adult females, and a significant negative correlation with dogwhelk abundance (table 5.7, figure 5.15). These correlations are stronger after the introduction of a 6 month lag phase (table 5.7, figure 5.16). When the relationships are considered at each site separately the strongest correlations generally occur after a six month time lag.

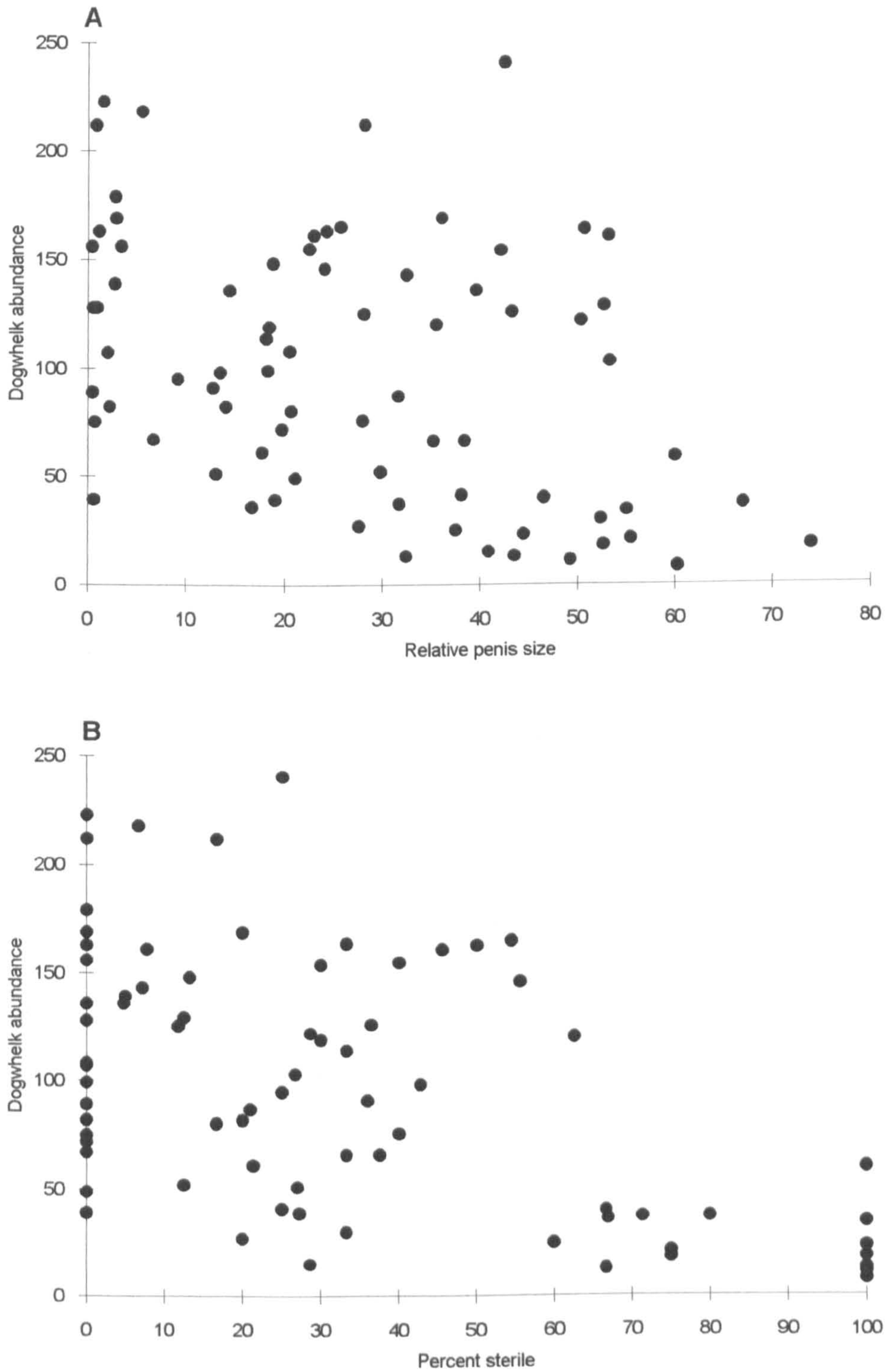


Figure 5.12 The relationship between the relative penis size of adults (A) or the percentage of sterile adult females (vas deferens stages 5 and 6) (B) and dogwhelk abundance, measured as the number of dogwhelks (all ages) collected in a 10 minute search. Data are included from all of the five sampling sites in south-west England, on all the dates sampled.

Table 5.6 Correlations between total dogwhelk abundance, the relative penis size of adults and the percentage of sterile females (arc sine transformed data). Correlations tested either within years (using all sites) or within sites (using all data from 1986-1993), see figures 5.12-5.14.

(a) Abundance vs relative penis size

Year or site	n	r	p
All sites and all years	80	-0.390	p<0.001***
1987	20	-0.603	p<0.01**
1988	10	-0.665	p<0.05*
1989	10	-0.615	p<0.05*
1990	10	0.082	NS
1991	10	-0.551	NS
1992	10	-0.534	NS
St. Agnes	16	0.270	NS
Tregantle	16	0.493	p<0.05*
Renney Rocks	16	0.147	NS
Kingsand	16	-0.557	p<0.05*
Jennycliff	16	-0.572	p<0.05*

(b) Abundance vs percentage of sterile females

Year or site	n	r	p
All sites and all years	80	-0.519	p<0.001***
1987	20	-0.681	p<0.001***
1988	10	-0.783	p<0.01**
1989	10	-0.314	NS
1990	10	-0.307	NS
1991	10	-0.552	NS
1992	10	-0.471	NS
St. Agnes	16	0.014	NS
Tregantle	16	0.534	p<0.05*
Renney Rocks	16	-0.063	NS
Kingsand	16	-0.373	NS
Jennycliff	16	-0.635	p<0.01**

NS, not significant different at the p=0.05 level.

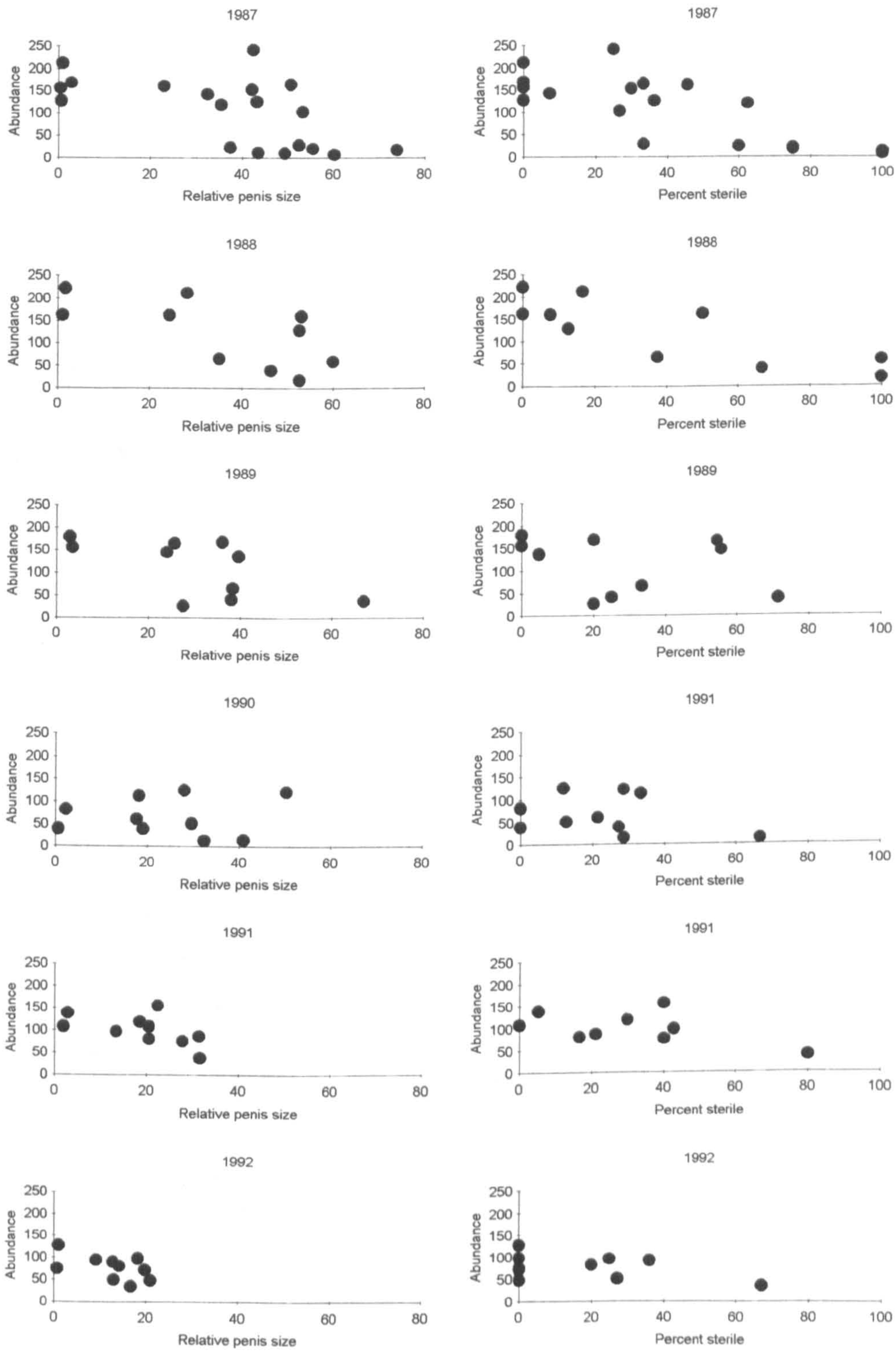


Figure 5.13 The relationship between the relative penis size of adults (left) or the percentage of sterile adult females (right) (vas deferens stages 5 and 6) and dogwhelk abundance, measured as the number of dogwhelks (all ages) collected in a 10 minute search, sampled between 1987-1992. Data are included from all of the five sampling sites in south-west England.

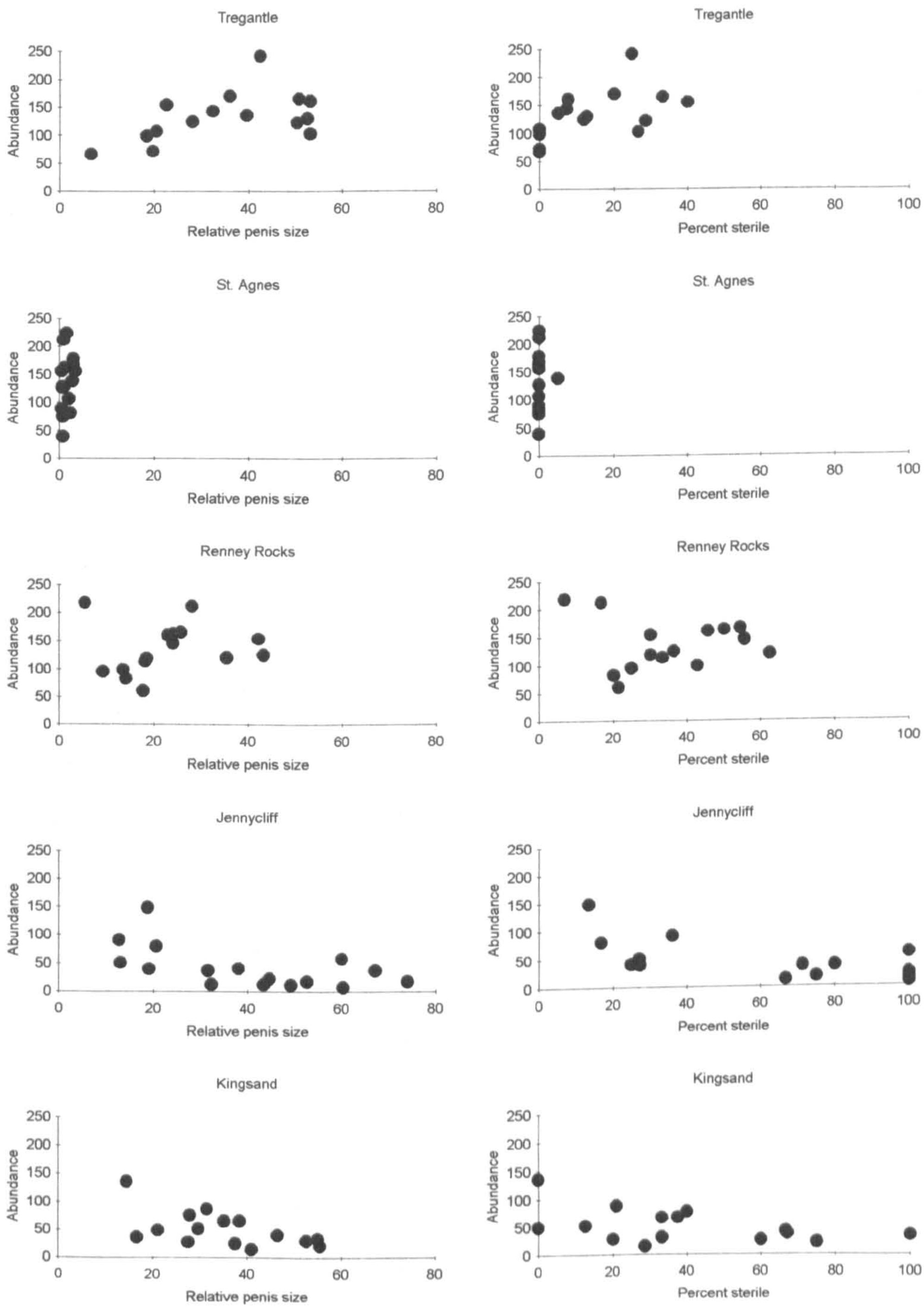


Figure 5.14 The relationship between the relative penis size of adults (left) or the percentage of sterile adult females (right) (vas deferens sequence stage 5 and 6) and dogwhelk abundance, measured as the number of dogwhelks (all ages) collected in a 10 minute search, sampled between 1986-1993. Data is represented at each of the sites in south-west England for all sampling times.

Table 5.7 Correlations between levels of tributyltin in seawater, the relative penis size of adult dogwhelks, the percentage of sterile adult females and the total abundance of *Nucella,* as measured by the total number collected in a 10 minute search (all ages). Correlations tested at each site on $\log_{10}$ transformed data (percentage of sterile females was arc sine transformed first) from 1986-1993. Data correlated with water concentrations with and without a 6 month time lag.

Lag time	0 months			6 months		
	r	n	p	r	n	p
(a) St. Agnes						
Relative penis size	-0.152	16	NS	**-0.441**	13	NS
Percent sterile	**-0.184**	16	NS	-0.167	12	NS
Abundance	**0.440**	15	NS	0.422	12	NS
(b) Tregantle						
Relative penis size	0.613	15	NS	**0.615**	13	NS
Percent sterile	0.352	15	p<0.01	**0.410**	13	p<0.05
Abundance	0.432	15	NS	**0.490**	12	NS
(c) Renney Rocks						
Relative penis size	0.518	15	NS	**0.678**	13	p<0.01
Percent sterile	0.170	15	p<0.05	**-0.396**	13	NS
Abundance	0.372	15	NS	**0.544**	12	p<0.05
(d) Kingsand						
Relative penis size	0.017	15	NS	**0.531**	13	p<0.05
Percent sterile	0.157	15	NS	**0.645**	13	p<0.01
Abundance	**-0.481**	15	NS	-0.449	13	NS
(d) Jennycliff						
Relative penis size	**0.562**	15	p<0.05	0.549	13	p<0.05
Percent sterile	**0.539**	15	p<0.05	0.469	13	NS
Abundance	**-0.479**	15	NS	-0.425	13	NS
(e) All sites, all dates						
Relative penis size	0.398	76	p<0.001	**0.450**	65	p<0.001
Percent sterile	0.386	76	p<0.001	**0.472**	65	p<0.001
Abundance	-0.422	75	p<0.001	**-0.440**	62	p<0.001

NS, not significantly different at the p=0.05 level, the best correlations are given in bold.

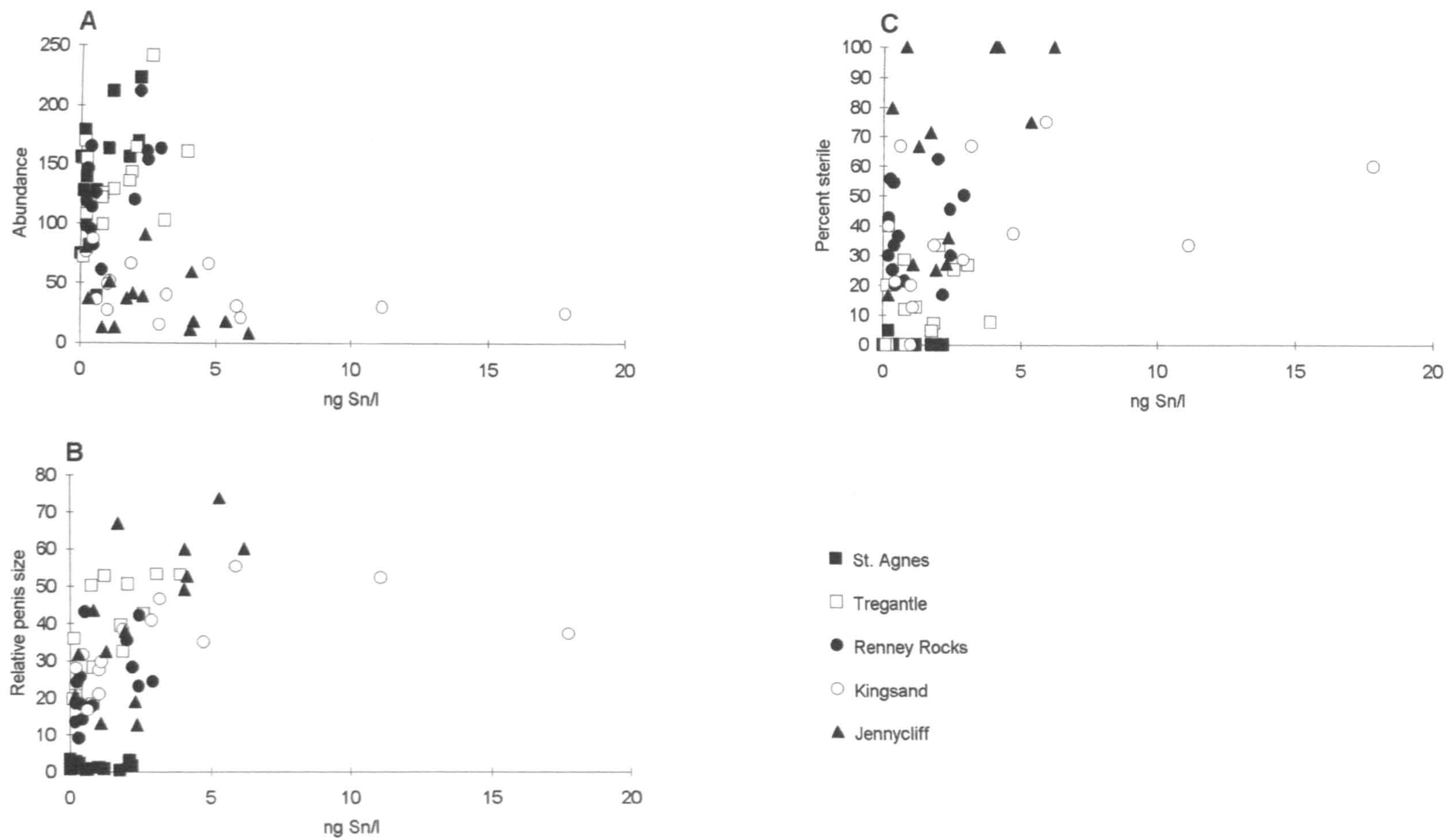


Figure 5.15 The relationship between concentrations of tributyltin (ng Sn/l) in sea water and the abundance of dogwhelks (all ages) measured as the number collected in a 10 minute search (A), the relative penis size value of adults (B) and the percentage of sterile adult females (C) with no time lag.

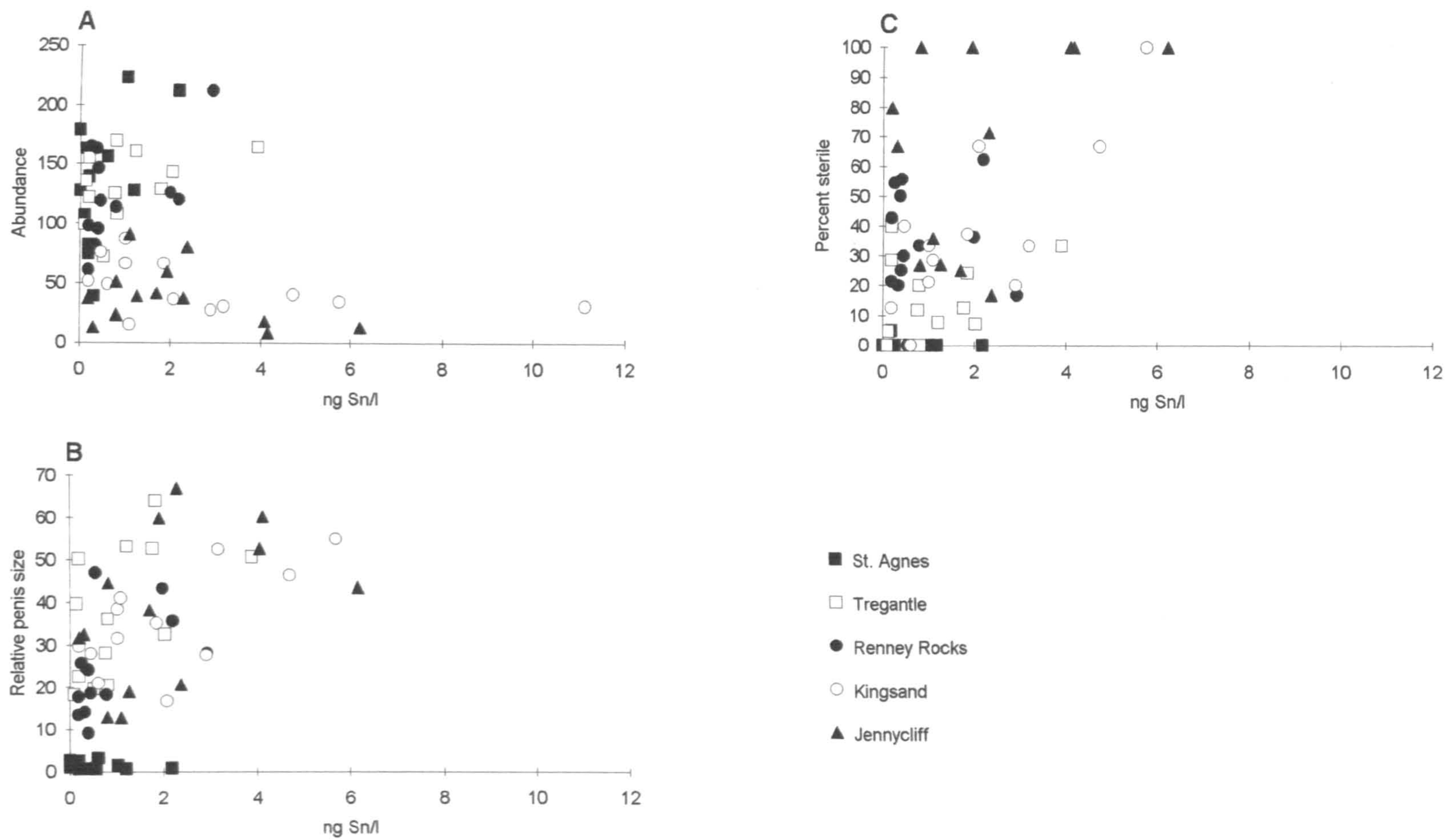


Figure 5.16 The relationship between concentrations of tributyltin (ng Sn/l) in sea water and the abundance of dogwhelks (all ages) measured as the number collected in a 10 minute search (A), the relative penis size value of adults (B) and the percentage of sterile adult females (C) with a 6 month time lag.

Relative penis size shows the strongest correlations with TBT in the water and the total dogwhelk abundance shows the weakest.

5.4 Discussion

5.4.1 Levels of effect

Levels of imposex in *Nucella lapillus* throughout this study are comparable to those recorded by other workers on the south coast (Spence *et al.*, 1990a; Gibbs *et al.*, 1991b). Unlike the steep gradients of imposex values observed away from harbours on the Isle of Man (chapter 6) or elsewhere in the UK (Bailey & Davies, 1988; Spence *et al.*, 1990a), *Nucella* populations at all the sites in and around Plymouth Sound showed similar expressions of relative penis size and vas deferens sequence. Levels of imposex development in *Nucella* were less at St. Agnes, by comparison, confirming it as a relatively clean site.

Of the indices used to measure the extent of the effect of TBT on *Nucella lapillus* relative penis size appeared to be the most sensitive to changes over time. In addition changes in the RPS value measured gave the best correlation with changes in the levels of TBT in the water, confirming the relationship observed in previous studies (Gibbs *et al.*, 1987). The introduction of a six month lag phase produced stronger correlations. This lag phase was incorporated since body burdens of TBT in *Nucella* were shown to have the best relationships with water contamination after 6 months (chapter 4). Surprisingly, as was the case for the relationship between TBT in water and in tissues, it appears that no one has correlated imposex expression and water concentrations using a lag phase. However Bryan *et al.* (1988) did report that after an exposure period of 14 days to TBT in water the penis length of female *Nucella* continued to increase for 2 months.

Changes in relative penis size values do not, however, give an indication of the changes in the reproductive capacity of *Nucella*. Instead the percentage of sterile females (stages 5 and 6) was the best measure of changes in reproductive capacity over time. This was better than using the median vas deferens sequence which appeared to be very stable over the period of study. The stability in the median VDS stages measured is thought to be due to the fact that the level of imposex development probably reflects TBT concentrations prevailing during the first year of the life of an individual. Consequently there is a tendency for one or two stages to be prevalent in any age class (Gibbs *et al.*, 1988).

A decline in the abundance of *Nucella lapillus* at sites of high contamination has been observed at a number of sites on the south coast of England (Bryan *et al.*, 1986). Surprisingly, although levels of imposex have been correlated with TBT concentrations in water and tissues (Bryan *et al.*, 1986; Gibbs *et al.*, 1987), and with the percentage of females in the population (Bryan *et al.*, 1986), the relationship of environmental concentrations with abundance has not been examined. Here a moderate correlation was shown when data from all sites were included. *Nucella* abundance showed a weak, but significant, relationship with increasing relative penis size values, but correlated best with changes in the percentage of sterile females in the population. A significant negative correlation was recorded between *Nucella* abundance and environmental levels of TBT. The amount of scatter observed in these correlations is not surprising given the amount of variation in the apparent day to day abundance of *Nucella* which is affected by factors such as the weather (Burrows & Hughes, 1989).

5.4.2 Recovery

It is clear that recovery has occurred following the introduction of legislation restricting the use of tributyltin antifouling paints in 1987. In addition to decreasing concentrations in the water (chapter 4) there has been a recovery in the level of effect on *Nucella lapillus*. This is characterised by reductions in the percentage of sterile females, reduction in the median vas deferens stage expressed and in the relative penis size. These are all factors that were used initially to measure the impact on *Nucella lapillus* individuals and populations.

Following the introduction of legislation in 1987 the recovery of dogwhelks would be expected to be first shown in juveniles (Gibbs *et al.,* 1987). These individuals characterise short term contamination since they have only been exposed to levels in the environment for at most 12-18 months (Gibbs *et al.,* 1987). This time span covers dogwhelks classified here as juveniles and as second years. It is surprising then, that the rate of recovery in this study, as measured in changes in relative penis size, appears to have been mirrored amongst all ages of dogwhelks. A factor that other workers may not have taken into account are changes in the juvenile male penis size with decreasing TBT contamination as observed here. The average male penis size was observed to decrease in response to declining TBT levels in the south-west mirroring changes observed in the female penis length. Consequently it is possible that these changes are connected with decreases in environmental concentrations of TBT. Since TBT affects the balance of male and female hormones (Gibbs *et al.*, 1991a; Spooner *et al.*, 1991) it can be speculated that the TBT causes an increase in the male hormone, testosterone, in the juveniles males, promoting penis development. This would affect juveniles still developing but not adults which are fully developed. The consequence of this is that it will of course lead to distorted RPS values, where the decline in both male and female penis sizes would lead to relative penis size values remaining constant or declining more slowly than would be expected. This could explain the slower than expected rate of recovery seen in juveniles and why the rates are not different between adults, juveniles and second years.

The rate of change in adults and second years indicates that a significant reduction in the levels of imposex had taken place by the time five years had passed after the ban was first introduced. Since imposex is irreversible (Bryan *et al.*, 1987; Gibbs *et al.*, 1987; Bryan *et al.*, 1988) this reduction is measured through replacement of the worst affected dogwhelks by the younger, less affected ones, coming into the population. In Plymouth Sound, Gibbs *et al.* (1988) predicted that the marginal sites, for example Renney Rocks would recovery fastest. However, in some respects those populations where TBT had reduced the proportion of females could be expected to recover faster since the worse affected adult females would have died. This has been observed in the faster recovery rate of adult *Nucella* at Jennycliff in comparison to Tregantle, Renney Rocks and Kingsand. By comparison the survival of adult females at other sites will reduce the apparent rate of recovery since they will continue to be included in assessments of the level of imposex for adults in that population for a number of years. This is probably the case at some sites where the populations have been moderately affected (e.g. Renney Rocks and Tregantle).

To date results of the recovery of *Nucella lapillus* populations have only been reported from the Isle of Cumbrae (Evans *et al.*, 1994) and on the Northumbria coast (Evans *et al.*, 1991) where significant reductions in the level of imposex were reported to have occurred in 1988 and 1989 respectively. These results indicate these populations are recovering at a faster rate than that found in the present study, in Plymouth Sound. The populations studied by Evans and co-workers, however, were not as badly affected as those in Plymouth Sound, with much lower RPS values initially.

There is little background information for abundances of *Nucella* prior to the introduction of TBT. This is unfortunate since it means that it is difficult to assess the extent of any decline in *Nucella* abundance or make predictions about recovery.

In comparison changes in imposex values can be easily used to assess the point when a population is back to normal. Bryan *et al.* (1986) had to use data collected from a survey in Plymouth Sound by Moore in 1936 (Moore, 1936) and by Crothers in 1975 (Crothers, 1975b) to show that populations had declined in the 1970's and early 1980's in this area.

The abundance of *Nucella* populations is difficult to assess anyway. The measurement of mobile organisms is not easy and in *Nucella* this is especially the case with daily (Burrows & Hughes, 1989) and seasonal (Feare, 1970a) variations in the distribution and aggregative behaviour (Dayton, 1971; Feare, 1971a) of individuals. Comparison of two methods used to assess abundance showed the timed searches to be the more reliable. Fixed monitoring areas gave a false impression of the overall abundance of *Nucella* at some sites: particularly at St. Agnes where dogwhelks were generally observed to be very abundant and imposex values were comparatively low, but the fixed monitoring sites showed there to be few dogwhelks at the site. This was solely due the changes in the habitat at the monitoring area which became very bare after 1990 which meant that there was little food or shelter available. Dogwhelks were abundant less than 10 m away from the fixed monitoring site. The use of replicated areas would have perhaps solved this bias, but for consistency with previous work the methods of Spence (1989) were used. General trends in the population abundance of *Nucella* after the 1987 ban can still be used to show recovery, however. The most obvious changes in abundance occurred at Kingsand and Jennycliff, the two worst affected sites studied, where the most sterile females were initially recorded.

Care should be taken when drawing conclusions about the levels of effect of TBT on *Nucella* populations when it is based on the abundance and presence of juvenile dogwhelks (e.g. surveys by the Marine Conservation Society (Loretto, 1991; Loretto *et al.*, 1994)). The juveniles are very cryptic and shelter amongst crevices and *Fucus* clumps (chapter 7) consequently the overall abundance estimated may often not reflect the true situation. In *Nucella* populations in Plymouth Sound before the widespread effects of TBT occurred, Moore (1936) found dogwhelks occurred in a ratio of 3 adults to every one immature. Results here show that by comparison populations badly affected by TBT may actually have a bigger proportion of juveniles than expected. This is similar to findings by Spence (1989) who suggested the reason for increased juvenile abundance was due to increased reproductive output by unaffected females triggered by increased food availability. Alternatively it could just be due to there being fewer adults in the population.

Changes in the ratio of males to females appears to be masked by large fluctuations recorded in consecutive samples which was probably caused by the small sample sizes obtained in some instances. Since sterile females were only recorded on one occasion at St. Agnes, this population can be deemed normal. The balance of males and females here were never significantly different from an expected ratio of males to females of 7:5 (Moore, 1938a). Surprisingly even at what is considered badly affected populations (e.g. Kingsand and Jennycliff) the balance of males to females was not shifted to any great extent consistently. The patterns observed here do not reflect to such a great extent the male bias observed in *Nucella* populations by other workers (Bryan *et al.*, 1986; Evans *et al.*, 1991).

5.4.3 Predictions for the future

The linear relationship expressed between changes in relative penis size and time enable predictions for the future to be made (figure 5.17). This would suggest that at the present rate of decline that imposex as measured by the relative penis size in adult, second year and juveniles would reach zero at all sites between 1994 and

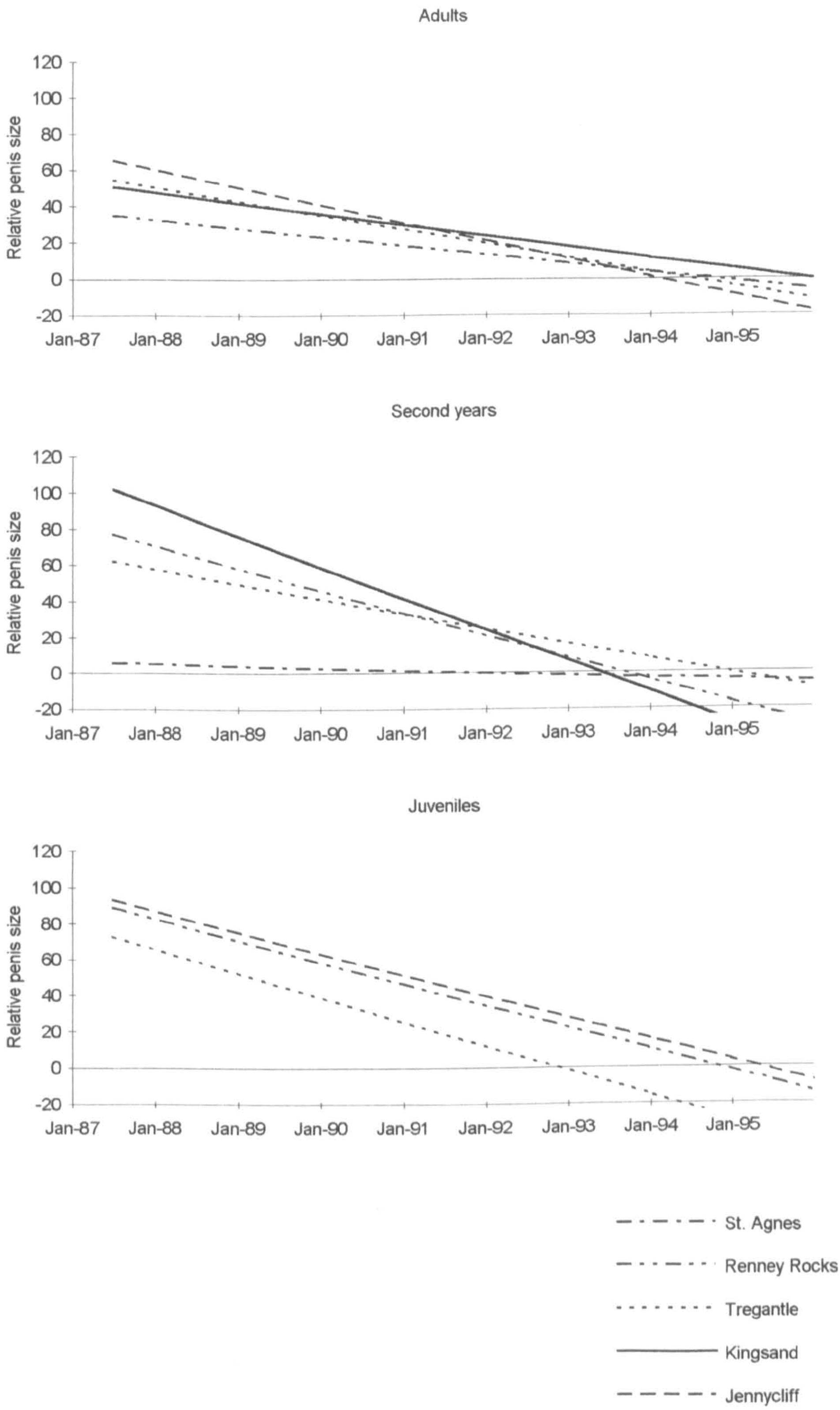


Figure 5.17 Summary of regression lines for adult, second year and juvenile *Nucella lapillus* from five sites in south-west England and the predictions for recovery.

1995. In reality it is unlikely that this will be the case. This is for a number of reasons. The first of these is that it is unlikely that the relationship will remain linear. Since the 1980's few populations of dogwhelks have been found without some level of imposex development (Gibbs *et al.,* 1987). Since TBT is still legally allowed to be used on boats greater than 25 m in length there are currently still some new inputs into the water. Consequently as dogwhelks are extremely sensitive to TBT, whilst there is any TBT in the environment imposex will be expressed to some extent. It would be expected then that the slope will eventually level out as the concentration of TBT in water approaches zero then the recovery will slow. This is seen in part in the rate of recovery at St. Agnes as compared to the other sites. Instead of the recovery being as fast as in the sites from Plymouth Sound the changes are such that the rate of recovery was slower at St. Agnes.

The observation that female juvenile *Nucella* from all sites surveyed are still developing male characteristics means that they must be exposed to tributyltin in the environment. Although water concentrations have dropped there is still measurable TBT concentrations in the water in Plymouth Sound (chapter 4). In addition TBT concentrations in the tissues of barnacles are still high at some sites (chapter 4). These concentrations are unlikely to decline completely whilst TBT is still being used on boats >25 m although the UK Government now seem ready to tighten restrictions further (Anon, 1993a). Despite recording considerable declines in the concentrations of TBT in French waters after the legislation was introduced, seven years after the ban levels in the water were still enough to cause a significant level of imposex in *Nucella lapillus* (Gibbs *et al.*, 1991c).

Nucella in addition to developing imposex on continued exposure to low levels of TBT in the environment also is sensitive to episodic inputs which have a similar detrimental effect (Bryan *et al.,* 1987). Consequently boats visiting Plymouth from countries where legislation is still not in place may prolong the length of time needed for *Nucella* populations to recover.

5.4.4 Conclusions

Since the introduction of legislation restricting the use of tributyltin based antifouling paints in 1987 a recovery has been shown in *Nucella lapillus* populations in the south-west of England. This recovery was measured by decreasing values of relative penis size, median vas deferens sequence and the percentage of sterile adult females in the populations. The rate of recovery appeared to occur at similar rates in all ages of dogwhelks and did not occur in juveniles first as had been expected. This may be due to decreases observed in the penis size of both juvenile male and female *Nucella*. The rate of recovery as measured in changes in relative penis size was faster at the more polluted sites in south-west England.

There is a linear relationship between changes in relative penis size in all ages of dogwhelks and time. Based on this, predictions for the future would suggest levels of imposex would approach zero by 1995. In reality zero imposex is unlikely, however, whilst TBT continues to be used on boats >25 m and concentrations in the water are still relatively high (chapter 4).

Levels of imposex development, as measured by changes in relative penis size, correlated well with concentrations of TBT in the water. This correlation was stronger with the introduction of a six month lag phase. Relative penis size also provided the best measure of changes in the level of imposex in *Nucella* over time as the median VDS remained relatively constant throughout the period of study. The best measure of changes in the reproductive capacity of females came from the percentage sterile females in the population.

CHAPTER 6

Tributyltin pollution around the Isle of Man: contamination in the water and the recovery of *Nucella lapillus* populations

6.1 Introduction

The phenomenon of imposex, the superimposition of male sexual characteristics on the female (Smith, 1971), has been observed in response to tributyltin (TBT) pollution in populations of *Nucella lapillus* throughout the British Isles (Bryan *et al.*, 1986; Herbert, 1988; Bailey & Davies, 1989; Langston *et al.*, 1990; Spence *et al.*, 1990a; Gibbs *et al.*, 1991b), Europe (Fioroni *et al.*, 1991a; Ritsema *et al.*, 1991; Stroben *et al.*, 1992a; Oehlmann *et al.*, 1993) and North America (Miller & Pondick, 1984). The level of sensitivity and development of this response appears to be uniform throughout the distributional range of *Nucella* (Gibbs *et al.*, 1991c).

Much of the work has concentrated on the south coast of England where imposex in *Nucella lapillus* was first reported (Blaber, 1970) and where later its development was first attributed to tributyltin pollution (Bryan *et al.*, 1986; Bryan *et al.*, 1988; Gibbs *et al.*, 1988). The degree of TBT contamination throughout the south coast has been severe, being much worse than elsewhere in the country (Spence *et al.*, 1990a; Bryan & Gibbs, 1991). In some areas concentrations in excess of 1000 ng Sn/l have been recorded in the water column (Cleary & Stebbing, 1987a). This is due to the high level of boating activity in the area and the sheer numbers of harbours and marinas distributed along the coast. Since imposex is initiated at water concentrations of less than 0.5 ng Sn/l, and levels above 2 ng Sn/l cause effective sterility in females (Gibbs *et al.*, 1988), all dogwhelk populations in the area have been badly affected. In addition since *Nucella* does not have a planktonic larval phase in its life cycle, and adults are relatively immobile, sterility in females induced by TBT pollution means that at many sites dogwhelks are now absent where once they were common (Bryan *et al.*, 1986; Spence *et al.*, 1990a; Bryan & Gibbs, 1991; Gibbs *et al.*, 1991b).

The levels and distribution of tributyltin contamination along the south coast, however, have not been typical of the British Isles as a whole. In contrast to the widespread contamination of the south coast, TBT pollution is localised close to source, producing sharp gradients of contamination away from harbours and marinas to relatively unaffected sites on open coasts (Spence & Hawkins, 1988; Spence & Hawkins, 1990). The gradients of contamination recorded around the Isle of Man reflect the situation in much of the British Isles (Spence *et al.*, 1990a). Consequently these gradients have created two levels of effect on dogwhelk populations (Spence *et al.*, 1990a). Firstly, at harbour sites where dogwhelks have been badly affected, populations where present are characterised by having a high percentage of sterile females. This is comparable to the level of effect which has been observed on the south coast of England (Bryan *et al.*, 1986; Bryan & Gibbs, 1991; Gibbs *et al.*, 1991b). Secondly, a short distance away from the harbour are moderately affected sites where females exhibit only the early stages of imposex development and there is no sterility (Spence *et al.*, 1990a).

On the Isle of Man legislation to restrict the use of tributyltin based antifouling paints was introduced in April 1988 (Marine Administration, 1988; Orme, 1990), a year later than the UK mainland (Abel *et al.*, 1987; Duff, 1987). Its format differed slightly, instead of restrictions on the sale of the paints the Manx Government introduced a bill which affected only the use of the paints. It became a legal requirement to have a licence from the Government in order to use paints containing organotins. This legislation, therefore, applies not only to the small yachts and structures, but to larger boats, for example greater than 25 m in length which are presently exempted from the UK Government ban. In this way the Isle of Man legislation was more stringent than that of the UK Government with their current restrictions. In addition, since any licences applied for would not be granted (pers. comm. D. Ramsbottom, Marine Administration, Isle of Man) in theory no TBT paints have been used on the Isle of Man since 1988. It is possible, however, that boats greater than 25 m in length will have visited the Island from the UK, possibility painted with TBT paints, which they are still legally allowed to use (Abel *et al.*, 1987; Duff, 1987).

The rate of recovery of dogwhelk populations around the Isle of Man is likely to be faster than that observed on the south coast (see chapter 5). Firstly the moderately affected dogwhelk populations where breeding has not been affected, would be expected to recover fairly quickly. Although imposex is irreversible (Bryan *et al.*, 1987; Gibbs *et al.*, 1987; Bryan *et al.*, 1988) the level of imposex development in the population will be lowered as less affected juveniles come into the adult population. Secondly the chances of recolonisation of the severely affected sites is much better than is the case on the south coast. Here since the dogwhelk populations close to harbour areas have not declined as has been seen elsewhere it is possible females capable of breeding will raft in from the nearby sites (Gibbs *et al.*, 1988). Thirdly, in theory since the legislation was introduced in 1988 there have been no new inputs of tributyltin to the environment from Manx boats. The only legal possibility of new sources of TBT comes from visiting UK boats >25 m.

On the Isle of Man there is no monitoring of the effectiveness of the legislation concerning the use of TBT paints nor checks that boat owners are conforming to the licensing procedure by Government bodies. The Marine Administration were hopeful that such paints would become more difficult to obtain in the future

(Ramsbottom, 1990), but without thorough checks on boat owners illegal use is likely.

The aims of the work reported in this chapter were to monitor the effectiveness of the current legislation restricting TBT paints on the Isle of Man and assess the recovery of dogwhelk populations. To do this a water sampling programme was established at key sites around the Island and the extent of imposex development was measured in adult and juvenile dogwhelks as long and short term indicators of pollution respectively (Gibbs *et al.*, 1987). Imposex development was measured using two indices: relative penis size (RPS) and the vas deferens sequence (VDS) (Bryan *et al.*, 1986; Gibbs *et al.*, 1987) and compared to values obtained in previous studies by other workers (Spence & Hawkins, 1988; Bell, 1990; Bell *et al.*, 1990; Spence *et al.*, 1990a). Gradients of contamination were identified using the known correlations between RPS, VDS and tributyltin in the water (Bryan *et al.*, 1986; Gibbs *et al.*, 1987; chapter 5). Since dogwhelks are now absent from some of the previously badly affected areas a transplant study was used to determine the chances of survival for any individuals rafting into the affected areas.

During the examination of dogwhelks to assess levels of imposex it was observed that the larvae of the trematode parasite *Parorchis acanthus* were common amongst populations from some sites. Since the infection of *Nucella lapillus* is known to cause castration (Lauckner, 1980) the occurrence of *Parorchis acanthus* amongst dogwhelk populations around the Isle of Man was investigated. The only study made on the occurrence of this parasite in *Nucella* since Rees (1940), was that of Feare (1970a) when growth and behavioural differences of individuals infected with *Parorchis acanthus* were examined.

6.2 Materials and methods

6.2.1 Harbour Survey

In order to relate the levels of contamination of tributyltin in the water and imposex values in dogwhelk populations from sites a broad scale survey was conducted at nine of the harbours on the Island. All sites were visited on the same day (19 July 1993) to allow comparisons to be made around the Island. Weather conditions were relatively rough for the time of year (Wind WNW, average 10 knots, maximum gust 25 knots - force 6-7) therefore boats were more likely to be in the harbour areas. Similarly, the survey was done on a weekday, rather than on a weekend, so as not to under estimate the number of pleasure craft.

Boats at each harbour were scored into three size categories (<10 m, 10-25 m and >25 m) and two classes of use (commercial or pleasure). The size of each vessel was estimated after pacing alongside the boat on the quayside. The category for commercial boats was used to include anything from excursion boats and small open lobster potters to fishing trawlers and cargo vessels. Those classed as pleasure craft included small day motor boats with a cabin or yachts.

6.2.2 Water samples

Water samples were collected from selected sites around the Isle of Man (figure 6.1, appendix A). Port St. Mary Ledges was used as a comparison against the harbours at Ramsey, Douglas, Port St. Mary and Port Erin. Samples were first collected in November 1991, taken monthly until May 1992 and thereafter once every 6-8 weeks. The last collection was made in June 1993. Since the initial site chosen in Douglas Harbour, at the ferry berth, proved to be difficult to sample in

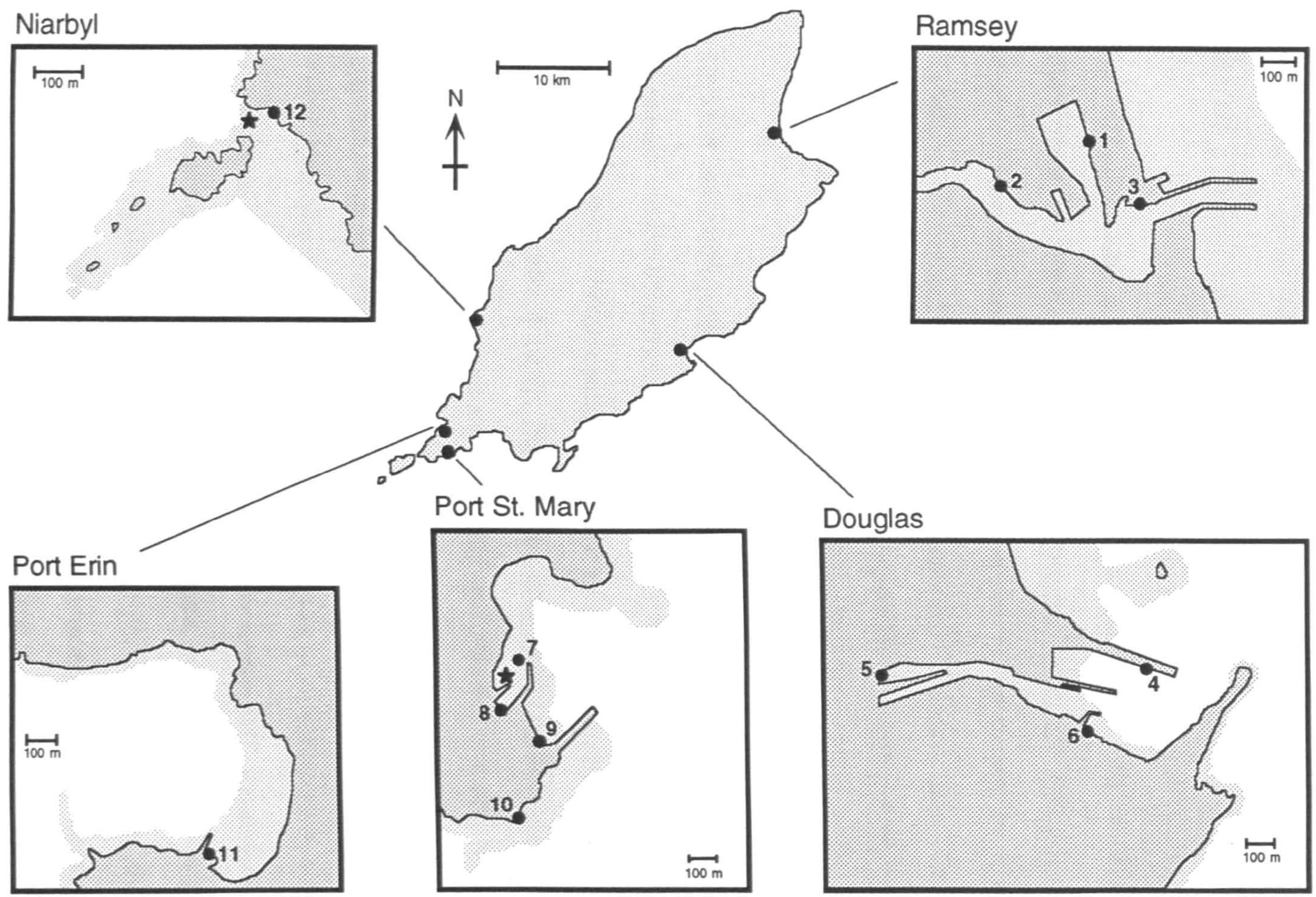


Figure 6.1 Main study sites around the Isle of Man marked with the position for water sample collections (● 1-12) and dogwhelk transplant experiments (★ at Niarbyl and Port St. Mary).

rough weather, samples were taken from an additional more sheltered site in the harbour after July 1992. Additional samples were taken at Ramsey (27 July 1992), as part of an environmental impact assessment study for a proposed marina (Allen *et al.*, 1992a). These and extra samples taken in Douglas Harbour (16 July 1992) were used to examine variations in TBT contamination in water on a local scale.

Three replicate water samples were collected from each site, in 2.5 litre acid washed glass Winchester bottles with wide necks, using the methods outlined in chapter 3. Samples were collected at high water (± 1.5 hours) on a spring tide minimising variations in TBT contamination caused by the tidal cycle (Cleary, 1991). The day time high tide on the springs cycle occurs around midday (Greenwich mean time) on the Isle of Man (Admiralty tide tables).

To coincide with surveying the dogwhelk transplant study (section 6.2.5) additional water samples were collected from Niarbyl and Port St. Mary inner harbour at low tide (± 30 minutes). Unlike collections at the other sites these samples were not collected on as regular a basis, but instead only when collecting dogwhelks for imposex analysis in March, April and July 1992 and January and July 1993.

All samples were extracted with hexane as described in chapter 3 and in Bryan *et al.* (1986). The hexane extracts from these samples were collected in acid washed glass vials and frozen at -15 °C in order to minimise TBT degradation. These samples were transported to Plymouth for analysis, surrounded by ice packs in a cold box. On arrival at Plymouth after an average journey of 10 hours, the samples were stored in a refrigerator overnight and usually analysed the following day, after first being washed with sodium hydroxide (chapter 3).

6.2.3 Sediment analysis

Replicate sediment samples were collected from the harbour at Port St. Mary (SC 210676) and at Port Erin (SC 193690). For comparison samples were also collected from an area of sand at Niarbyl (SC 211776). At mid tide level at each site three replicate samples, each of about 10 g of sediment were collected from the top 5 cm of substrate in acid washed glass vials. No attempt was made to determine the properties of the sediments at each site. It was realised, however, that these may differ between localities, and it was known that properties such as organic content or particle size could influence the level of contaminants in the sediment (Phillips, 1977; Langston & Spence, 1994). Instead the interest was purely in determining the level of contamination at a polluted site in comparison to a clean one.

These samples were taken in November 1992, at the end of the summer boating season on the Isle of Man levels of contamination were expected to be at their highest. The samples were frozen at -15 °C and transported to Plymouth for analysis surrounded by ice packs in a cold box along with the water samples (section 6.2.2). Assessment of the levels of tributyltin, dibutyltin and total tin followed the methods of extraction for tissue samples given in chapter 3 and those of Langston *et al.* (1987).

6.2.4 Assessment of imposex development and the level of parasite infection

Dogwhelks were collected from 24 sites around the Isle of Man over a six year period: in 1987 by Spence (see Spence & Hawkins, 1988; Spence *et al.*, 1990a), in 1989 by Bell (see Bell, 1990; Bell *et al.*, 1990) and in 1991 and in 1993 for the present study (appendix B). Rocky shores are distributed along most of the coast of the Isle of Man except in the north between Peel and Ramsey. At some sites a number of samples were taken to examine any gradient effects away from harbours, for example at Port St. Mary and at Castletown. The methods for the collection, storage and preparation of *Nucella* are described fully in chapter 3. The level of imposex development within each population was assessed using the two indices: relative penis size (RPS) and the vas deferens sequence (VDS), following the methods of Bryan *et al.* (1986) and Gibbs *et al.* (1987) and chapter 3.

Although imposex development was assessed in *Nucella lapillus* populations around the Isle of Man data on abundance are not presented here. This is because unlike the samples collected in south-west England (chapters 4 and 5), around the Isle of Man samples were not collected at consistent times of the year nor were the methods of collection standardised between each of the workers who contributed to the surveys over the six year period.

In addition the digestive gland and gonad of each *Nucella* was examined for the presence of the larval stage of the trematode parasite *Parorchis acanthus* (chapter 3). Any dogwhelks found to be infected were not included in the calculation of relative penis size or vas deferens sequence for the population but used to assess the level of parasite infection within the population.

6.2.5 Transplant study

In March 1992, a sample of 640 adult dogwhelks was collected from Niarbyl (SC 211777) on the west coast of the Isle of Man. These were of an average shell length of 28.47 ± 2.14 mm (n=200) with thick or toothed shell edges. Imposex development was assessed in a sample of 40 of the animals collected in order to gain an idea of the extent of the development of male characteristics in the females before the transplant began.

The dorsal surface of the shell of each dogwhelk was marked with a dot of red Humbrol enamel paint. Enamel paint was used since in field experiments previously carried out it had been shown not to induce imposex in *Nucella* (Bryan *et al.*, 1987). These marked animals were kept overnight on the laboratory circulation bench and then 300 were taken and released at the original site of capture at Niarbyl, whilst the remaining 300 were introduced to a rocky outcrop in Port St. Mary inner harbour (SC 210677). The outcrop chosen had a plentiful supply of barnacles and there were suitable crevices providing shelter for the released individuals. Dogwhelks were reportedly once present here (pers. comm. S. J. Hawkins, Port Erin Marine Laboratory) but were now absent. Previous repeat searches of the area had found a total of 14 dogwhelks, all adults marked with either yellow paint or red dynamo tape from transplant studies carried out in 1989 (Bell, 1990; Guirguis, unpublished). These were collected and the extent of imposex development measured.

At intervals over the next 16 months samples of 40 dogwhelks were collected from the Port St. Mary site. Only those with clear paint marks were taken to ensure that no dogwhelks from any previous transplant experiments by other Port Erin Marine Laboratory students were sampled. Concurrent samples were collected from Niarbyl. Here in addition to the collection of 40 marked individuals, 40 unmarked dogwhelks were gathered from an area at the same tidal height but about 50 m to the south (SC 210776). Comparison of imposex development between the two samples collected at Niarbyl were used to check that the paint marking the dogwhelks did not have any effect on the level of imposex development. Relative penis size and vas deferens sequence measurements were calculated for each group of individuals collected.

Each time samples of dogwhelks were collected from the two sites replicate water samples were also taken for a comparison in the level of tributyltin contamination (section 6.2.2).

6.2.6 Statistical methods

Comparisons of the level of tributyltin contamination in sediments taken at sites around the Isle of Man were tested using one-way analysis of variation, after first testing the data for normality and homogeneity of variance (chapter 3). When a significant difference was found Tukey multiple comparisons were used to determine between which sites the differences occurred (chapter 3).

The recovery of dogwhelk populations around the Isle of Man was tested using Wilcoxon matched pairs tests (Fowler & Cohen, 1992) for all sites where data were collected before and after the introduction of legislation restricting the use of TBT paints.

6.3 Results

6.3.1 Harbour survey

The survey conducted in July 1993 provided a general picture of the size of the harbours around the Isle of Man and the types of boats moored in them (figure 6.2). Although the survey was conducted on only one day the numbers and types of boats recorded were representative of observations made on other occasions during the summer months when taking water samples or collecting dogwhelks in the areas. Approximately half of the number of boats were observed in the water over the winter at these sites.

The largest harbours on the Isle of Man were found to be at Douglas, Ramsey and Peel. These were the only sites where ships greater than 25 m in length were observed in the harbour. The boats in this category were container vessels or cargo ships as seen at Peel ('Mannin' and 'Ben Vane'), Ramsey ('Silver River'), and at Douglas ('Peveril') where in addition one of the passenger ferries owned by the Isle of Man Steam Packet Company ('The Lady of Mann') was moored alongside the pier. The hulls of all of these ships were observed to be the characteristic colour of copper antifouling paint.

At Port Erin and Derbyhaven all the boats in the harbours were less than 10 m long. These were mainly small yachts or day boats, some of which were obviously used for lobster and crab fishing. The provision of two harbours at Port St. Mary meant that there is a segregation in their use: the Inner Harbour had mostly small pleasure craft; whereas the outer harbour had boats of which about half are commercial vessels. Those in the Outer harbour which were between 10 and 25 m long are

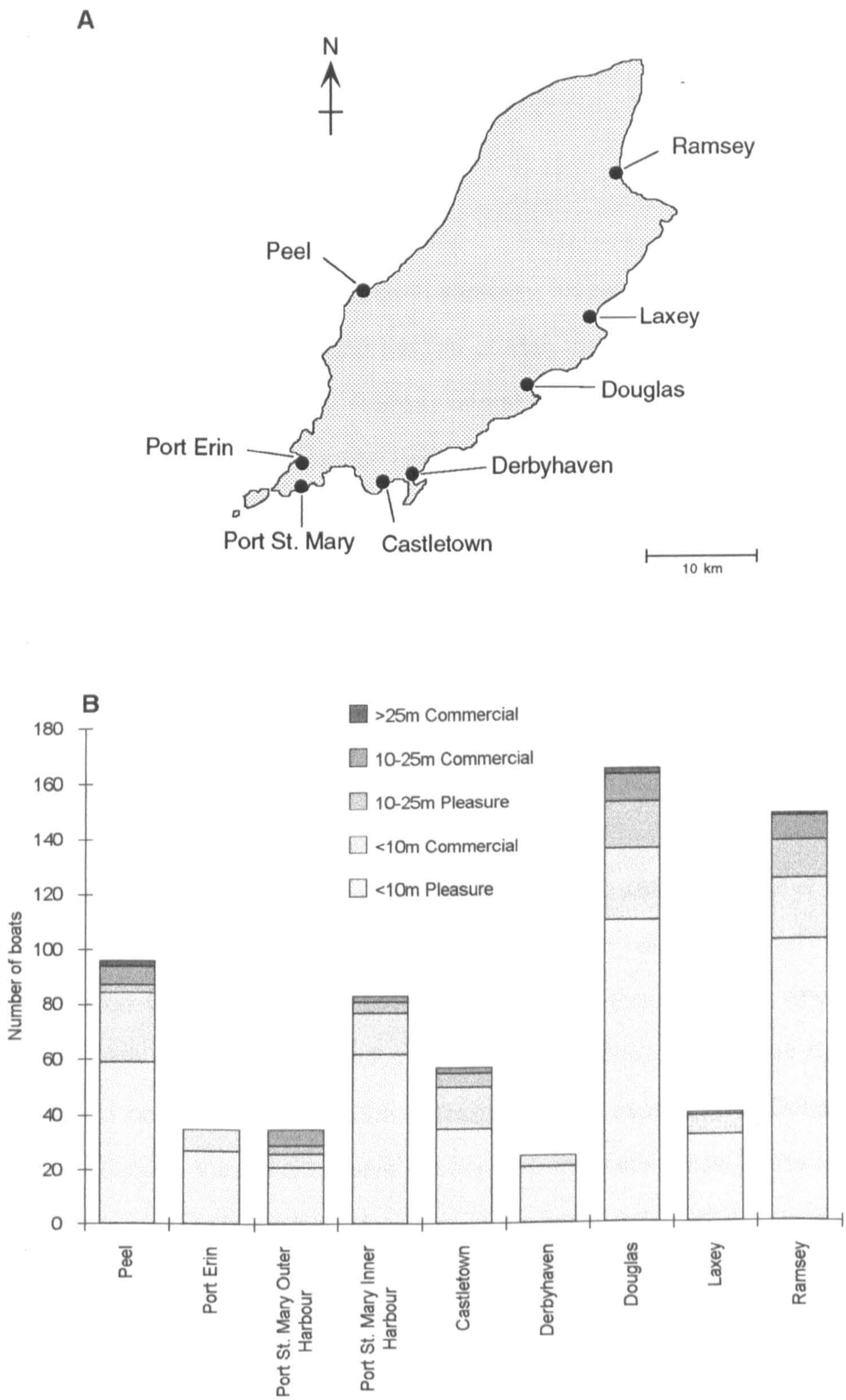


Figure 6.2 Boat survey on 19 July 1993 with the location of the sites used around the Isle of Man (A) and the numbers and types of boats recorded in the harbours (B).

mainly used for scallop dredging. Port St. Mary, Ramsey and Peel are generally recognised as the yachting centres for the Island.

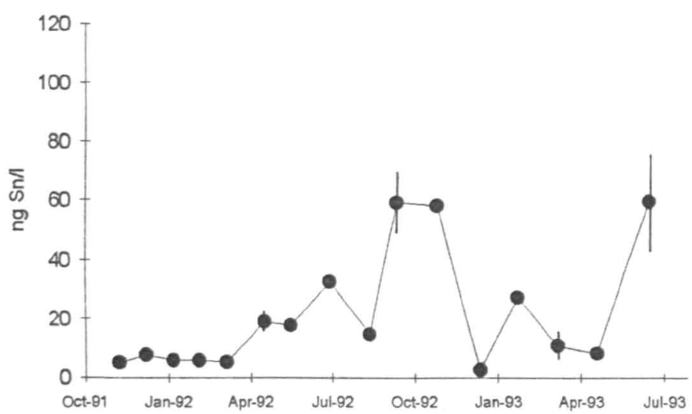
6.3.2 Water contamination

The levels of tributyltin contamination in seawater showed great variation between the different sites sampled around the Isle of Man (figure 6.3). The highest levels were measured at Port St. Mary, Ramsey and at Douglas where a maximum of 300 ng Sn/l was recorded in December 1992. The levels of contamination within each site showed considerable variation during the period of study. At Port Erin, Ramsey and Port St. Mary a peak in the level of TBT in the water was recorded in July 1992, and later in the year another smaller peak was observed at Ramsey and at Port St. Mary Inner Harbour, at the end of October. Levels at the control site at Port St. Mary Ledges remained below 2 ng Sn/l throughout the period of study, similarly levels at the Ferry Berth in Douglas were also low, remaining below 5 ng Sn/l.

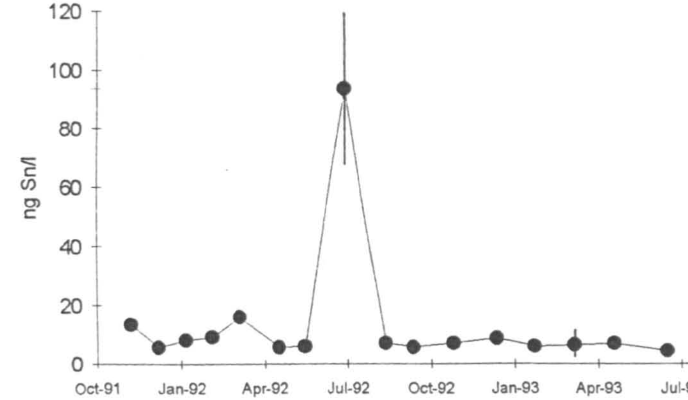
Variation between the replicates taken was generally low within each set of samples taken. Only when contamination levels were high did the level of TBT contamination vary to any great extent. On some of the occasions when these unusually high and variable samples were recorded small paint flakes had been observed to be in the water at the collections sites, especially at Douglas and Ramsey. Although these flakes were observed in the water close to the substrate on the bottom and hence were not present in the water collected, it is possible that they could go some way to explaining the large variations in TBT concentrations seen at these sites.

Samples taken on the same date around the harbour at Ramsey and Douglas showed the extent to which tributyltin contamination varied on a local scale (figure

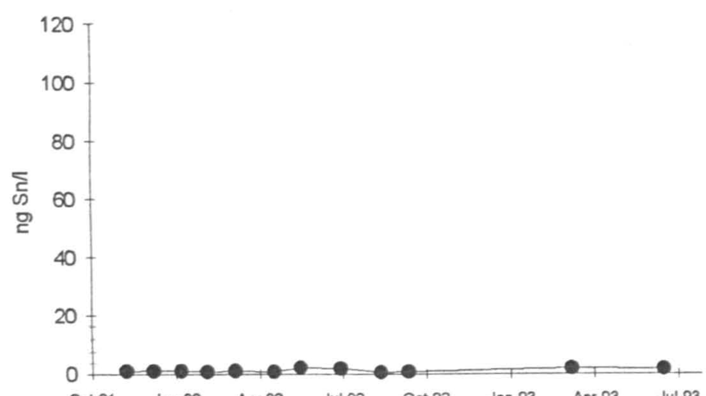
Port St. Mary Inner Harbour



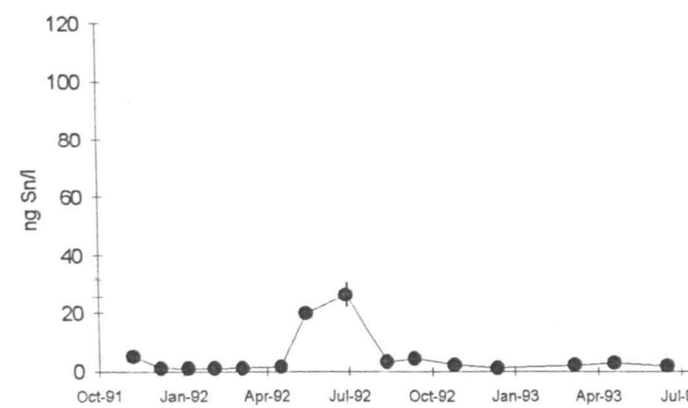
Port St. Mary Outer Harbour



Port St. Mary Ledges



Port Erin Harbour



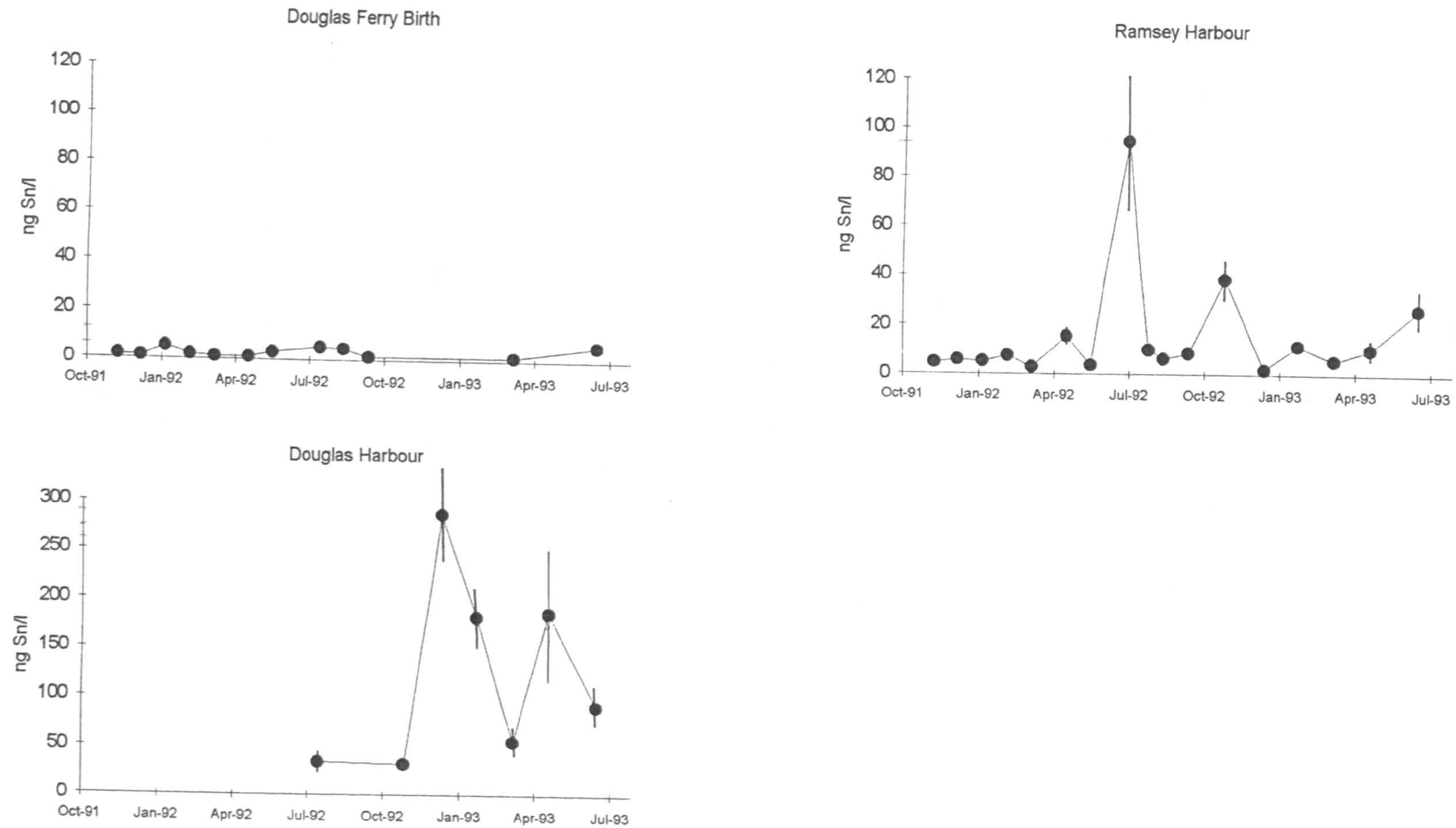


Figure 6.3 Mean water concentration of tributyltin (ng Sn/l) in samples collected from sites around the Isle of Man. Error bars are ± 1 SE. Note the use of a different scale for Douglas Harbour.

6.4). In the main harbour area at Ramsey the levels of contamination were some three times higher than recorded upstream of both the main harbour and the ship yard. The level of contamination in the water increased slightly downstream towards the narrow harbour entrance to the sea. Tributyltin levels in the harbour at Douglas by comparison were six times lower at the entrance to the harbour, as measured at the Ferry Berth, than recorded at the blind ending harbour, upstream.

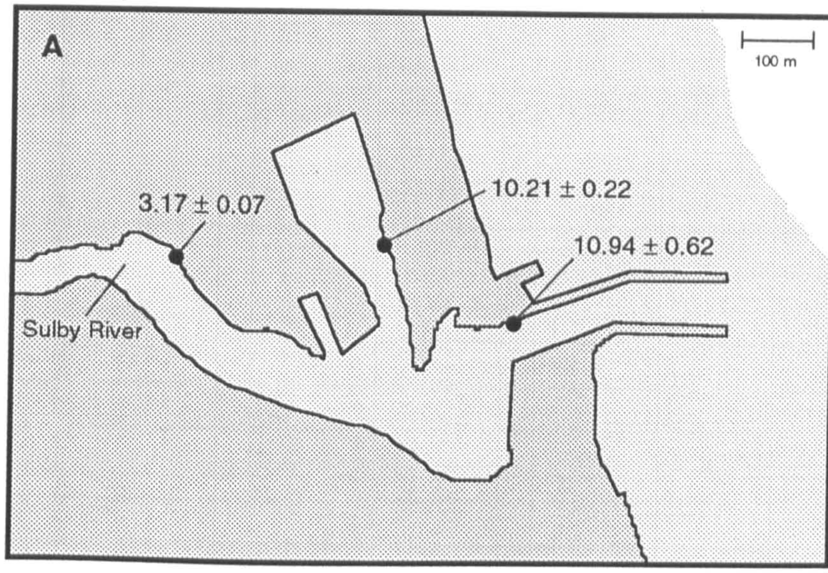
6.3.3 Sediment analysis

The levels of tributyltin (TBT), dibutyltin (DBT) and total tin (TBT + DBT) in the sediment collected from Port St. Mary in November 1992 were around 20 times higher than those recorded in the samples taken from either Niarbyl or Port Erin (table 6.1). There was no difference in the levels of TBT in the sediment between Port Erin and Niarbyl, but both were significantly less contaminated than sediment from Port St. Mary (table 6.1). Little variation was found between the concentrations in replicate sediment samples at each site although the variation was increased when contamination levels were high at Port St. Mary. The percentages of tributyltin and dibutyltin making up the concentrations of total tin in the sediment varied between sites. The sediment at Port St. Mary had the highest percentage of DBT and the lowest percentage of TBT as a measure of total tin, this situation was reversed at Niarbyl.

6.3.4 Imposex survey

In the first survey (Spence & Hawkins, 1988; Spence *et al.*, 1990a) the highest values for VDS, RPS and the percentage of females sterile occurred at the sites nearest to the harbours (figures 6.5-6.9). In the 1993 survey a third of the sites where sterile females had previously been recorded now had no dogwhelks (figure

Ramsey Harbour



Douglas Harbour

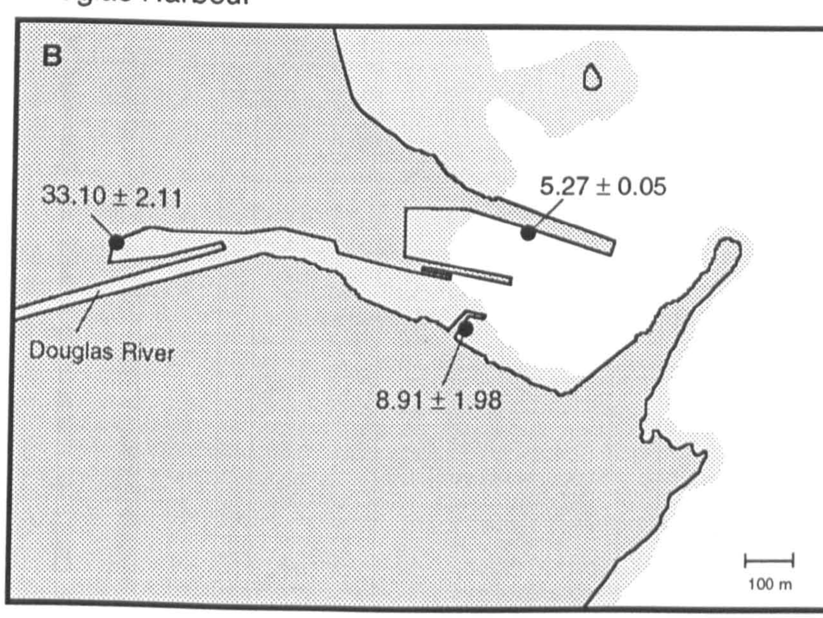


Figure 6.4 Concentrations of tributyltin (ng Sn/l) in water samples taken at sites in (A) Ramsey Harbour (27.7.92) and in (B) Douglas Harbour (16.7.92), expressed as mean (n=3) ± standard error.

Table 6.1 Mean concentrations of tributyltin (TBT), dibutyltin (DBT) and total tin (TBT + DBT) in sediment samples taken at Port St. Mary, Port Erin and Niarbyl in November 1992 (a). Concentrations are expressed as mean µg Sn/g as TBT or DBT per gram of dry sediment and the percentage of TBT in total tin (TBT + DBT), ± 1 standard deviation. One-way analysis of variance, on $\log_{10}$ transformed data, comparing concentrations of TBT in the sediment at the different sites (b) and Tukey multiple comparisons between sites (c).

(a)

Site	n	TBT	DBT	Total tin	% TBT
Port St. Mary Inner Harbour	3	0.026 ± 0.010	0.019 ± 0.008	0.046 ± 0.017	58.200 ± 5.923
Port Erin Harbour	3	0.001 ± 0.001	0.000 ± 0.001	0.002 ± 0.001	68.533 ± 10.174
Niarbyl	3	0.001 ± 0.001	0.000 ± 0.001	0.001 ± 0.000	84.400 ± 30.717

(b)

Source	DF	SS	MS	F	p
Site	2	3.3835	1.6917	60.07	p<0.001***
Error	6	0.1690	0.0282		
Total	8	3.5525			

(c)

	Niarbyl	Port Erin
Port St. Mary	>***	>***
Niarbyl		NS

p<0.05*, p<0.01**, p<0.001***, NS not significant at the p=0.05 level. Where a multiple comparison shows a significant result > denotes that the sediment from the site on the left of the table was more contaminated that from the site on the top.

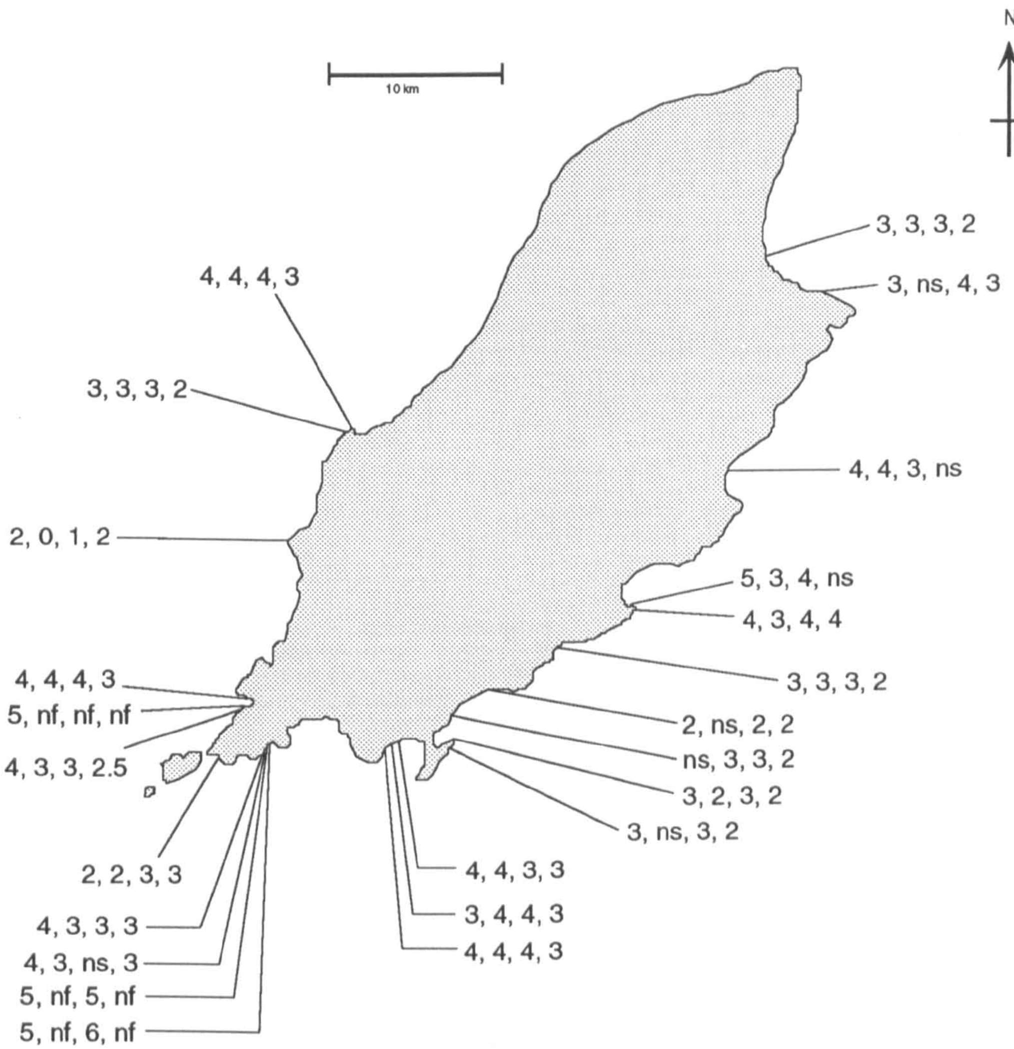


Figure 6.5 Median vas deferens sequence (VDS) values for adult *Nucella lapillus* around the Isle of Man from 1987 to 1993. Values given as 1987, 1989, 1991, 1993 (left to right), ns = not sampled, nf = none found. Data for 1987 from Spence & Hawkins (1988) and for 1989 from Bell (1990).

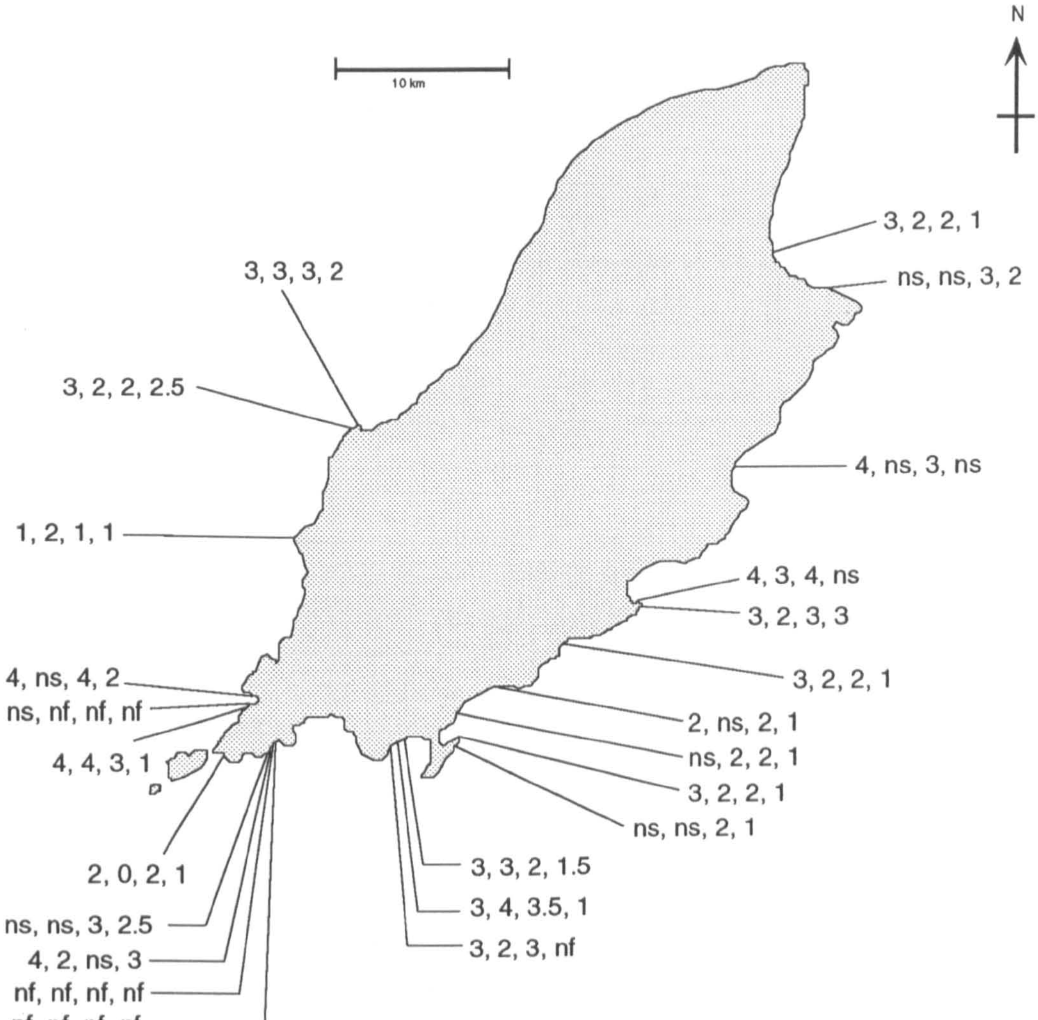


Figure 6.6 Median vas deferens sequence (VDS) values for juvenile *Nucella lapillus* around the Isle of Man from 1987 to 1993. Values given as 1987, 1989, 1991, 1993 (left to right), ns = not sampled, nf = none found. Data for 1987 from Spence & Hawkins (1988) and for 1989 from Bell (1990).

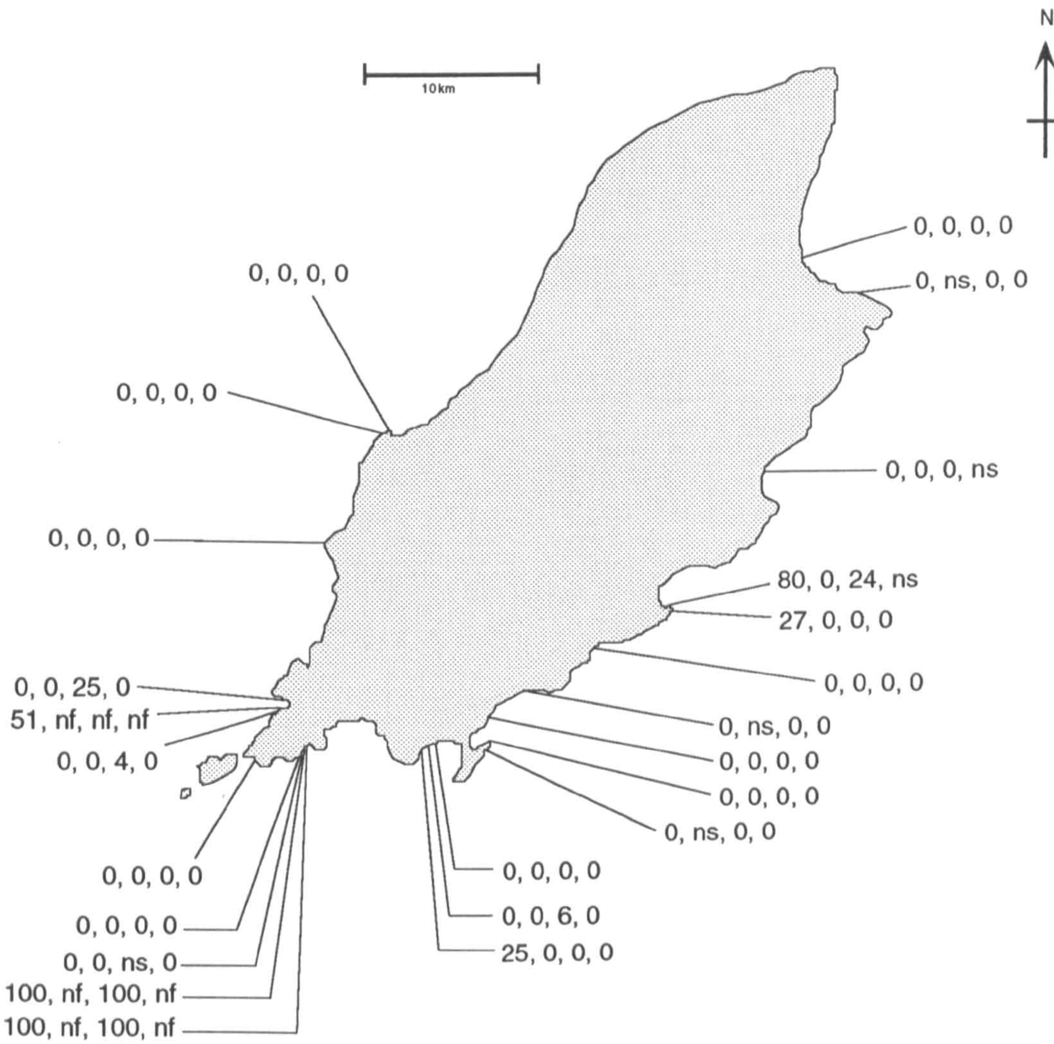


Figure 6.7 Percentage of sterile adult females in dogwhelk populations from around the Isle of Man from 1987 to 1993. Values given as 1987, 1989, 1991, 1993 (left to right), ns = not sampled, nf = none found. Data for 1987 from Spence & Hawkins (1988) and for 1989 from Bell (1990).

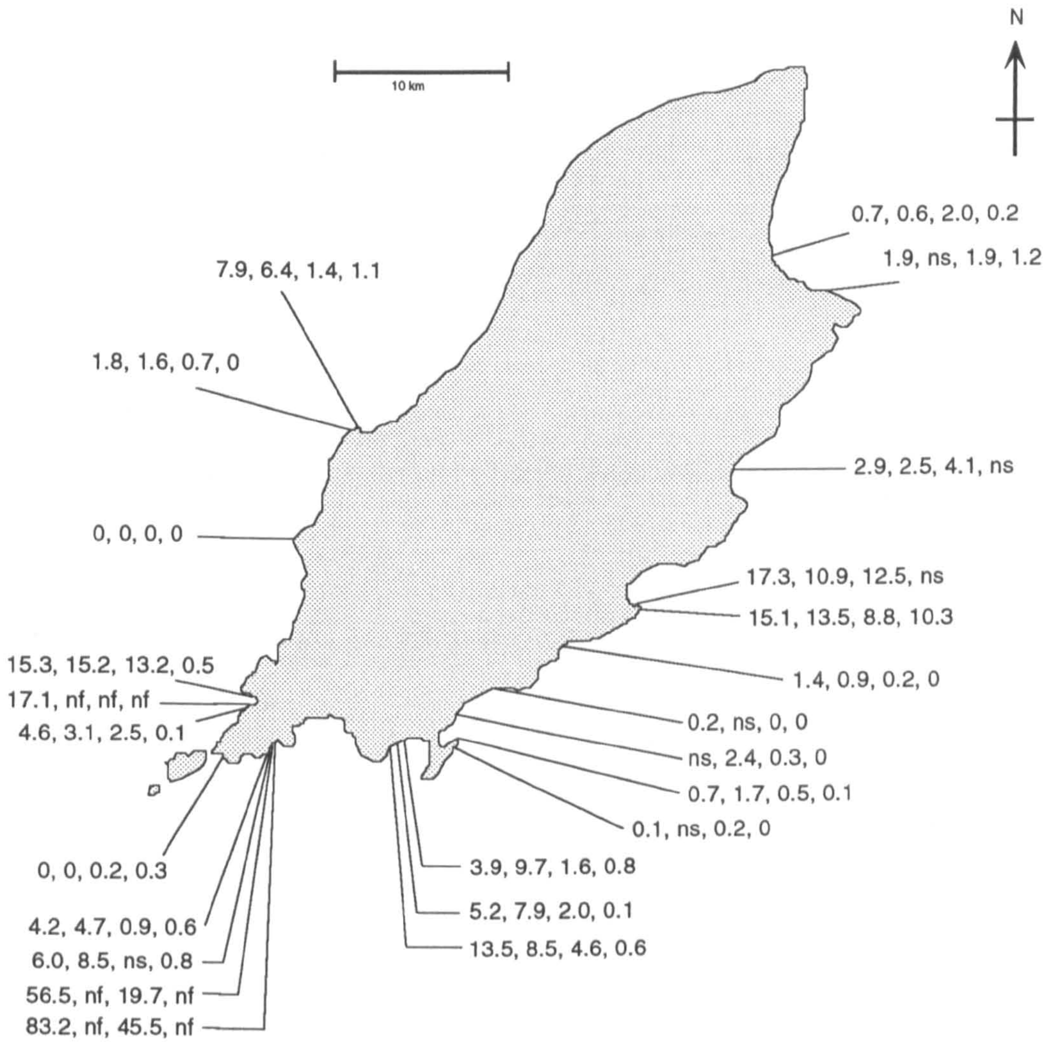


Figure 6.8 Relative penis size values for adult *Nucella lapillus* around the Isle of Man from 1987 to 1993. Values given as 1987, 1989, 1991, 1993 (left to right), ns = not sampled, nf = not found. Data for 1987 from Spence & Hawkins (1988) and for 1989 from Bell (1990).

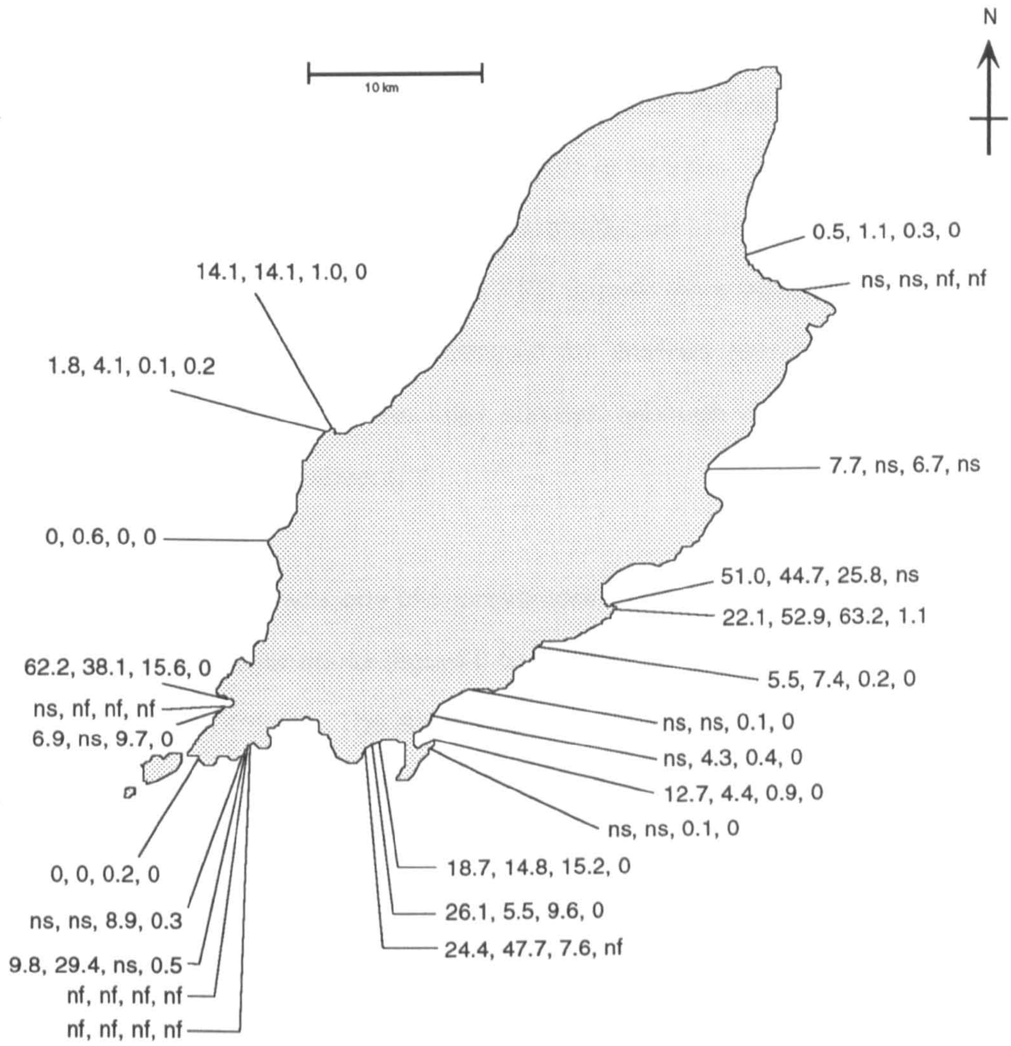


Figure 6.9 Relative penis size values for juvenile *Nucella lapillus* around the Isle of Man from 1987 to 1993. Values given as 1987, 1989, 1991, 1993 (left to right), ns = not sampled, nf = none found. Data for 1987 from Spence & Hawkins (1988) and for 1989 from Bell (1990).

6.7). These sites were close to the harbours at Port St. Mary and Port Erin. These sites also had the highest relative penis size values (figure 6.8-6.9, appendices C-E).

Measurements of RPS, VDS and percentage sterile females showed clear gradients of contamination away from the harbours at Douglas, Castletown, Port St. Mary and Port Erin (figures 6.5-6.9). These were reflected in all ages of dogwhelks sampled although the changes in Juvenile RPS appear more variable (figure 6.9), reflecting the smaller samples sizes collected for analysis (appendix E). These gradients were still apparent in the later surveys although not as clear where dogwhelks were recorded as absent.

During this study the trend was one of improvement at sites around the Isle of Man. The median VDS values for adults (figure 6.5) and for juveniles (figure 6.6) showed a reduction at most locations. The exceptions were at the Sound and at Niarbyl, where although adult VDS values were low they increased slightly during the period of study. There was a highly significant reduction in RPS values between 1987 and 1993 for the adults (Wilcoxon matched pairs test, $T=0.08$, $N=13$, $p<0.001$) and juveniles (Wilcoxon matched pairs test, $T=0$, $N=12$, $p<0.001$) where the median VDS values had mostly fallen to 3 or below by 1993. The pattern of recovery was also reflected in the percentage of sterile females (figure 6.7) where prior to the introduction of legislation concerning TBT by the Manx Government the incidence of sterility in adult female dogwhelks was high. Of the 24 sites sampled, 9 had females with a median VDS stage of 5 or 6. By comparison in the 1993 survey no sterile females were found at any of the sites (figure 6.7) although in the harbours at Port St. Mary and Port Erin no dogwhelks were present. Relative penis size values calculated for adults (figure 6.8) and juveniles (figure 6.9) fell significantly between 1987 and 1993 (Wilcoxon matched pairs test, adults $T=3$, $N=17$, $p<0.001$;

juveniles T=0, N=11, $p<0.001$). In adult dogwhelks RPS values were recorded as high as 83 in 1987. In 1993 most of the populations sampled had RPS values of less than 1, although no dogwhelks were found at the sites where the RPS values had been highest in 1987 (figure 6.8). Similar reductions in RPS values were observed in juveniles between 1987 and 1993 at most sites (figure 6.9). At sites where both adult and juvenile *Nucella* were found the RPS values recorded for juveniles were generally higher than those for the adults.

6.3.5 Transplant experiment

The levels of tributyltin measured in the water at low tide at Niarbyl were always below 1 ng Sn/l (figure 6.10). At Port St. Mary the mean low water TBT concentrations were around seven times higher, rising from 5 ng Sn/l in March 1992 to 23 ng Sn/l in January 1993. This pattern of increasing contamination reflected the levels recorded in Port St. Mary inner harbour at high tide (figure 6.3).

Whereas the relative penis size of the Niarbyl natives and marked individuals remained close to zero there was an rapid increase in the RPS value of the dogwhelks transferred to Port St. Mary (figure 6.10), reaching 7% after 16 months. This increase was due to an increase in the mean size of the female penis and not a decrease in the size of the males penis (figure 6.10) which remained constant throughout the duration of the experiment. The level of variation in the penis length for the females transplanted at Port St. Mary remained relatively constant indicating a uniform response throughout the population exposed to higher environmental TBT concentrations. The penis size did not differ between the Niarbyl marked and unmarked dogwhelks for either the males (two-sample t-test, $t=0.0863$, $df=25$) or females (two-sample t-test, $t=0.5346$, $df=35$) at the end of the experiment (July 1993).

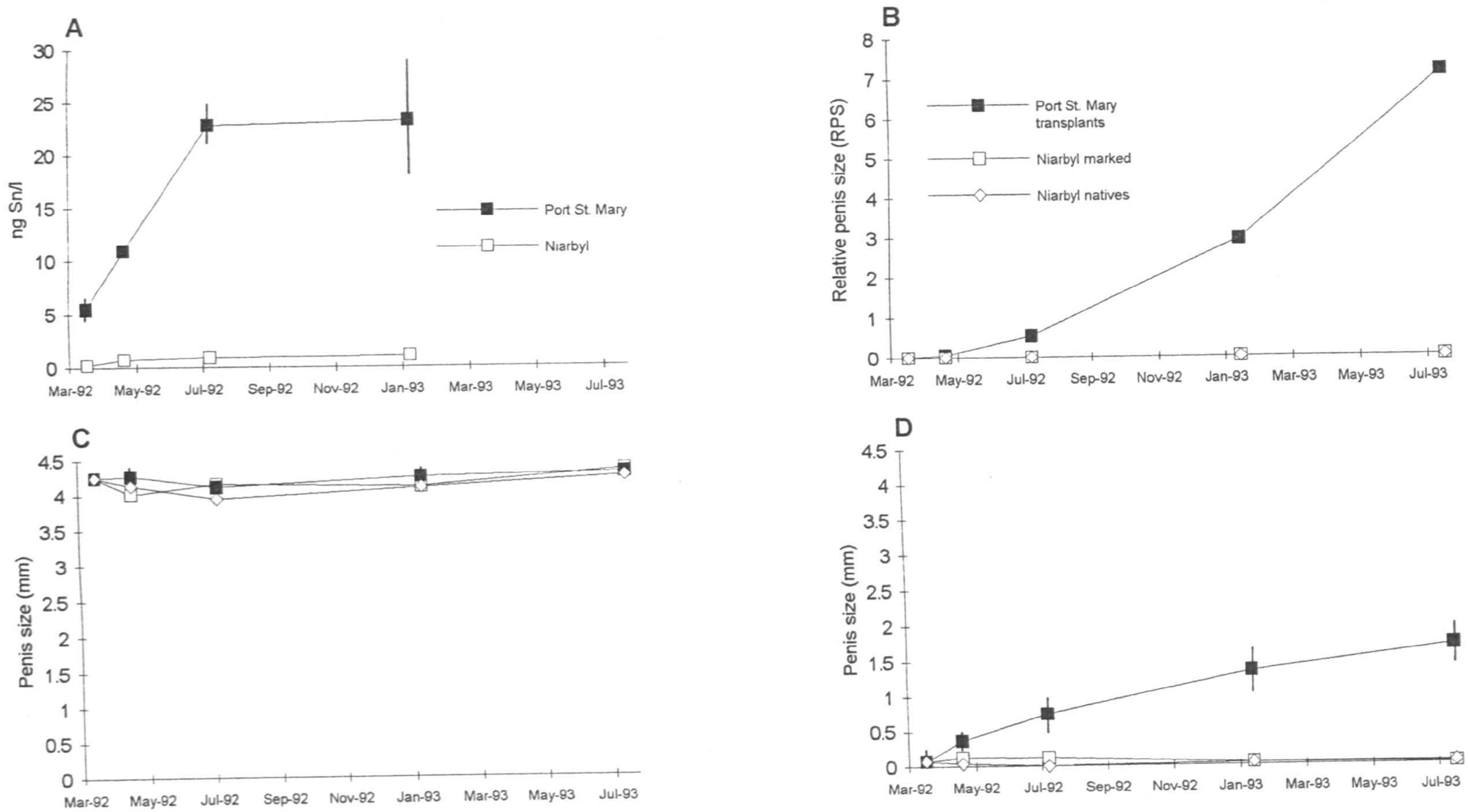


Figure 6.10 Mean water concentration of tributyltin (ng Sn/l) taken at low tide at Niarbyl and at Port St. Mary Inner Harbour (A) and the changes in relative penis size (RPS) (B), male (C) and female (D) mean penis length for the transplant experiment. Error bars ± 1 SE.

There were small fluctuations in the median vas deferens sequence (table 6.2) stage of the females from Niarbyl with both the natives and the marked individuals around stages 1 and 2. By comparison those transplanted to Port St. Mary showed a clear increase in the median VDS stage expressed and a shift in the range of stages expressed from 0-3 to 2-4.

A total of 20 dogwhelks were collected in repeat searches of the Port St. Mary transplant site on three occasions prior to the start of the transplant experiment. The shells of all of these dogwhelks showed remnants of previous markers from other transplant experiments by Port Erin Marine Laboratory students. Examination showed that 5 of the individuals collected were females, three of which were sterile. The median VDS stage expressed was 5 and RPS value was calculated as 28%. These dogwhelks had probably been at the site for about 3 years.

6.3.6 Parasite infection

The majority of the dogwhelk populations infected by the parasite *Parorchis acanthus* were in the south of the Isle of Man (figure 6.11). At most of these sites less than 10% the individuals sampled were infected. The three sites sampled at Castletown and one site at Port St. Mary were the exceptions. Here between 12% and 27% of the dogwhelks examined in 1991 or 1993 contained parasites.

The parasites were found mainly in the digestive gland of the infected individuals, three quarters of which were male. In some badly affected individuals the dark orange coloration of the tissues and the high numbers of parasites packed into the digestive gland often made sexing the individual difficult as the sperm ingesting gland could be obscured. Most of the affected dogwhelks appeared to be the older

Table 6.2 Vas deferens sequence (VDS) median stage and range for adult *Nucella lapillus* used in the transplant experiment from Niarbyl to Port St. Mary Inner Harbour. *n*, number of females.

Date	Dogwhelks	n	Median VDS	VDS range
March-92	Niarbyl natives	16	2	0-3
April-92	Niarbyl natives (unmarked)	12	2	1-3
	Niarbyl marked (controls)	14	2	0-3
	Port St. Mary transplants	22	2	1-3
July-92	Niarbyl natives (unmarked)	13	1	0-3
	Niarbyl marked (controls)	14	1	0-2
	Port St. Mary transplants	14	2	1-3
January-93	Niarbyl natives (unmarked)	19	2	0-3
	Niarbyl marked (controls)	22	1	0-3
	Port St. Mary transplants	20	3	2-4
July-93	Niarbyl natives (unmarked)	8	2	0-3
	Niarbyl marked (controls)	19	2	0-3
	Port St. Mary transplants	21	4	2-4

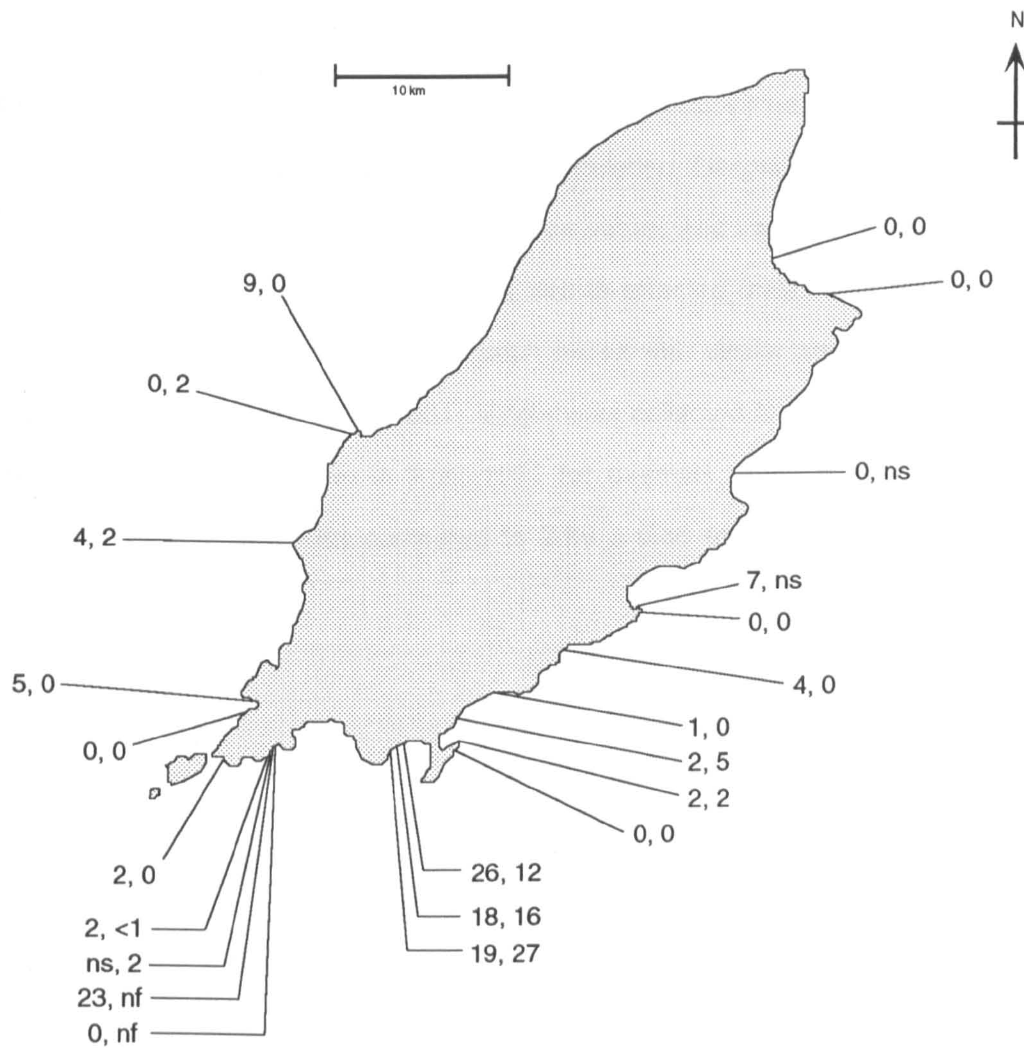


Figure 6.11 Parasite infection in *Nucella lapillus* around the Isle of Man in 1991 and 1993. Values expressed as a percentage of the number of animals examined, both sexes and all ages, and given as 1991, 1993 (left to right), ns = not sampled, nf = none found.

and larger individuals from the population with multiple rows of teeth on the inside lip of the shell. None of the juveniles examined were found to contain visually detectable parasites.

The expression of imposex in the parasitised females appeared to be comparable to that in unaffected adults from the same population. Despite this they were not included in the calculation of relative penis size values and vas deferens sequence calculations for the population. The penis size in affected males was significantly smaller when compared to unaffected adult males with similar shell lengths (two-sample t-test, $t=4.86$, $df=41$, $p<0.001$). Dogwhelks collected from the site near the harbour mouth in Castletown in April 1991 for example, had an average penis length of 4.07 ± 0.27 ($n=26$) compared to 2.99 ± 0.88 ($n=17$) for the individuals infected by parasites.

6.4 Discussion

The number and frequency of water and sediment samples taken around the Isle of Man were limited due to the lack of available equipment to measure tributyltin on the Isle of Man. The sheer volume of water to be processed and transported to Plymouth meant that in many cases it was impossible to record TBT concentrations at as many sites nor as frequently as would have been desired. Consequently small scale temporal and spatial variations may have been missed. Despite these limitations it is still possible to consider the effectiveness of legislation, recovery and gradients of contamination around the Isle of Man.

6.4.1 Effectiveness of the legislation

Legislation restricting the use of tributyltin based antifouling paints has been shown to be effective in reducing environmental levels of TBT in water. In France, for example, three years after a ban was introduced concentrations of tributyltin in the water were between 5-10 times lower than those before (Alzieu *et al.,* 1986; Alzieu, 1991). Strict enforcement of the legislation was carried out by the French Government and was probably what saved the French oyster fishery (Alzieu, 1991).

In the UK regional bodies such as the National Rivers Authority are responsible for the enforcement of legislation concerning the marine and freshwater environment. Since the legislation concerning the use of tributyltin antifouling paints was introduced in 1987 (Abel *et al.,* 1987; Duff, 1987), a number of actions have been taken against boat owners which have resulted in fines. Like the situation in France, the legislation in the UK has been effective in reducing the concentrations of TBT in the water (Waite *et al.*, 1991; Bryan *et al.*, 1993a; Dowson *et al.*, 1993), with significant reductions occurring after two years (chapter 4).

If the legislation introduced on the Isle of Man had been as effective as seen in France and the UK, then significant reductions in the concentrations of TBT in the water would be expected after two years. Since the legislation was introduced in 1988 then these significant decreases would be expected to have occurred by 1990. Unfortunately the extent to which water concentrations have dropped at sites around the Isle of Man is difficult to assess since no samples were taken prior to this study to allow a comparison to be made. Despite this it would still be reasonable to expect, based on the work in south-west England (chapter 4), that the concentration of TBT in the first set of water samples taken in November 1991 should be relatively low, since if the legislation was effective a 5 fold reduction in the levels of TBT in the water could be expected three years after the ban. However since concentrations taken in November 1991 were recorded as 14 ng Sn/l at Port St. Mary Outer Harbour, it can therefore be assumed that either water concentrations before 1988 were very high or the legislation introduced has not been effective.

In the period immediately after the Isle of Man Government introduced the legislation requiring that a licence be applied for in order to use organotin paints, there was no monitoring of the levels of TBT in the environment nor enforcement of the legislation. Since no licences were applied for (pers. comm. D. Ramsbottom, Marine Administration) there should have been no use of organotin paints. After 1991 some of the results obtained in this survey were used as part of an long-term environmental monitoring survey of contamination around the Isle of Man (Allen *et al.*, 1992b; Allen *et al.*, 1993) commissioned by the Isle of Man Government. In response to the findings that elevated levels of TBT occurred in the first summer of sampling (1992) a reminder of the current legislative restrictions on TBT was sent to all yacht clubs and harbours on the Island. The last samples taken under the present study in June 1993 seem to suggest that the reminders have worked since the elevated levels recorded in Port St. Mary Outer Harbour and at Port Erin the previous year were not repeated. Unfortunately this was not the case at all sites as elevated levels were again recorded in Port St. Mary Inner Harbour and Ramsey.

There seems little doubt that the fluctuations and elevated levels of TBT recorded in the water at sites around the Isle of Man indicate the continued illegal use of organotin paints. Variations observed by other workers for example in the tidal cycle (Clavell *et al.*, 1986; Cleary, 1991) or depth of sampling (Cleary & Stebbing, 1987a; Cleary & Stebbing, 1987b) were purposely controlled for. In addition the inclusion of the surface microlayer in the sample should have stabilised the replicate samples since concentrations of TBT in the surface microlayer have been shown to vary very little with season or the state of the tide (Cleary, 1991). This is illustrated in the small differences shown between samples taken at high and low tide in Port St. Mary Inner harbour. These samples although not taken on the same day showed little variation, and certainly not the 2-20 fold variations recorded at some harbours elsewhere (Clavell *et al.*, 1986; Cleary, 1991) again probably because of the inclusion of the microlayer.

The absorption of TBT from water to sediments is reversible and hence it is possible that contaminated sediments may act as sources of dissolved TBT (Unger *et al.*, 1988). It seems unlikely, however, that resuspension of TBT from the sediment would cause the elevation of concentrations to those observed in the samples taken from the harbours around the Isle of Man. Especially since any resuspension of the TBT laden sediment would probably be weather dependent, requiring prolonged periods of onshore winds. Most of the occasions when elevated levels were recorded, for example at harbours like Port St. Mary, were in the summer when the weather was usually calm. Samples collected at depth, in other harbours have not shown elevated concentrations even when taken close to sediments (Cleary & Stebbing, 1987a). Langston *et al.* (1987) concluded that in Poole Harbour, which is very sheltered, wind from any direction would not be enough to resuspend the tributyltin from the sediment. Consequently, although the concentration of tributyltin in the sediment was higher in samples collected from Port St. Mary than those from Niarbyl and Port Erin it is unlikely that this would account for the highly elevated levels recorded in the water. On the other hand, small-scale fluctuations in the concentrations of TBT in the water during the winter months may be due to increased sediment bound TBT in the collected samples, especially since the water was not filtered before analysis.

In the first few months of sampling concentrations of TBT in the water were higher in Port St. Mary outer harbour than in the inner harbour. Since this harbour is used mainly by mid size fishing vessels it was originally suspected that if the illegal use of organotin paints was taking place it was in fact these boats that were using them. This now seems unfounded since in most cases these boats display the characteristic red hulls of the copper antifoulants. Instead it is likely that the culprits are the small pleasure boats removed from the water for the winter and repainted in the spring before being put back in the water for the summer. This theory ties in with the patterns of elevated concentrations observed through the year and also with the harbours in which these peaks occur (Port St. Mary Inner harbour and Port Erin) since these are the sites occupied primarily by small pleasure craft. The smallness of these boats means that they require little paint each year and consequently they are probably being painted with oddments left from previous years. The current accessibility of TBT paints on the Island is unknown, but it is highly likely that old cans are still being used up.

Proving that boat owners are still using TBT paints is not easy. Short of taking paint scrapes from all the boats there is little chance of identifying the culprits. The problem is that elevated levels recorded at one harbour may be caused by a single boat being painted with TBT paint.

Fluctuations at Ramsey Harbour may originate from tributyltin input from the ship yard. Boats from the UK were regularly observed there. Although no boats over 25 m were seen on the slipway, if present they would legally be allowed to use organotin paints.

6.4.2 Recovery

Water concentrations of tributyltin around the Isle of Man were in most cases higher than those currently recorded on the south coast of England (chapter 4). Levels on the open coast, for example at Port St. Mary Ledges and Niarbyl were however comparable to the clean sites, for example at St. Agnes. Levels recorded in Douglas Harbour were very high and were characteristic of those concentrations recorded in the harbours on the south coast in the late 1980's prior to the introduction of regulations (Cleary & Stebbing, 1985; Cleary & Stebbing, 1987a; Cleary & Stebbing, 1987b). The reason for these high concentrations can only be speculated on. The illegal use of TBT is one possibility since the sampling took place close to moored boats one of which could still be using TBT, see previous section. Another factor could be the paint flakes that were observed in the water and sediment at the times of collection. These flakes are probably the result of previous repainting operations at the site. Since the harbour dries out at low tide it is a favoured site for the repainting of small boats in the Spring. Although these flakes were not collected within the sample they were regularly observed amongst the sediment. It has been expected that the leaching of TBT from old paint flakes would slow the decline in seawater concentrations (Bryan & Gibbs, 1991).

Concentrations of TBT in sediments vary seasonally, reaching a maximum in summer (Langston *et al.*, 1987), consequently the levels recorded at Port St. Mary, Port Erin and Niarbyl were probably not the maximum for the year. The differences in concentrations recorded at the three sites are probably not connected due to different sediment characteristics since these sediments are made up of particles of similar size (Southward, 1951). Instead they were likely to be due to differences in the level of boating activity at the sites. Despite the concentrations in the sediment at Port St. Mary being higher than at the other sites they were low compared to concentrations recorded at most other sites in England and Wales (Langston *et al.*, 1987; Langston & Burt, 1991; Dowson *et al.*, 1992; Dowson *et al.*, 1993). They are also below the levels known to affect sediment dwelling organisms like *Scrobicularia plana* (Langston & Burt, 1991) and *Arenicola marina* (Matthiessen & Thain, 1989). Tributyltin is persistent in sediments and as such it may act as a sink providing a potential source of TBT (Langston & Burt, 1991). Since concentrations of TBT in the sediments from sites examined around the Island were low their potential as a major source of contamination is unlikely.

Despite the obvious illegal use of tributyltin paints around the Island, in general concentrations have obviously decreased to some extent: relative penis size, vas deferens sequence values and the percentage of sterile females have all declined. The correlation of these indices and levels of TBT in water are well established (Gibbs *et al.*, 1987; Gibbs *et al.*, 1988; chapter 5).

Imposex is irreversible in *Nucella lapillus* (Bryan *et al.*, 1987; Gibbs *et al.*, 1987; Bryan *et al.*, 1988) and consequently the recovery of adults as measured by relative penis size and the vas deferens sequence relies on less affected individuals coming into the population. Hence the changes in imposex values in adults from populations around the Isle of Man correspond to long term changes in tributyltin contamination in the environment. The recovery observed in juveniles by comparison relates to levels of contamination in the short term (Gibbs *et al.*, 1988) since juveniles have been exposed to TBT in the water for around 6-12 months. They are considered to be more sensitive than adults to TBT (Gibbs *et al.*, 1988) and consequently usually express higher levels of imposex. This was the case in values of RPS in juveniles were in most cases higher than in the adults in most years. In 1993 though these values are lower than for the adults and hence they probably reflect a decrease in water concentrations in the last year of study.

The lack of suitable habitat for dogwhelks along some areas of the coast around the Isle of Man means that the levels of contamination and recovery are difficult to assess. This is one of the reasons that the sediment dwelling *Nassarius reticulatus* has been used as an indicator in some areas (Bryan *et al.*, 1993a). As dogwhelks are absent at a number of sites, most noticeably at Port St. Mary, the recovery of these areas will depend on the rafting in and survival of females capable of breeding. Since dogwhelks on the open coast are only moderately affected there is a greater chance of this on the Isle of Man than is perhaps the case on the south coast of England. The transplant experiment illustrated that adult dogwhelks would be able to survive close to the harbour in Port St. Mary, where they had occurred previously, if they were introduced there. Despite levels in the water being high and the relative penis size and vas deferens sequence values increasing the transplanted dogwhelks survived 18 months with no incidence of sterility. Although no egg capsules were observed at the transplant site in the Spring of 1993 in theory there is no reason why the dogwhelks transplanted there cannot breed. Not all the females collected from the previous transplant studies were sterile after being close to the harbour for about three years. It should be noted, however, that the transplant study used adults which are less sensitive to TBT than juveniles (Gibbs *et al.*, 1988). Whilst the concentration of TBT in the water remains high at Port St.

Mary the progeny of the transplanted adults may well be effectively sterilised before being able to breed themselves. Consequently the population may be able to survive in the short term but the possibly of a long term future for the transplanted *Nucella* seems less assured.

Port St. Mary is one of the worst affected sites around the Island. Since adult dogwhelks were able to survive here females rafting into other sites would also be able to reproduce. Viable populations could be created at these sites with decreasing concentrations of TBT in the water as observed at some of the harbours, thus allowing the long-term survival of dogwhelks population introduced to the area. The rafting in process is likely to be slow however as adults are relatively immobile. Instead it will probably rely on the transport of juveniles on drifting algae (Gibbs *et al.*, 1988). It could be speeded up by the artificial transplants of dogwhelks into these areas. This however carries a number of problems including the fact that in some of the affected areas there is no background information on the abundance of dogwhelks prior to the effects of organotin paints. The introduction of too many *Nucella* could have a worse effect on the community than the reduction in the number of *Nucella* has had (chapter 7).

The dogwhelks which are most likely to raft in are those from the open coast. These dogwhelks are characterised by having small squat shells with large apertures (Crothers, 1985). By comparison the native sheltered shore *Nucella* have elongated shells and narrow openings. Sheltered shore *Nucella* survive longer when crabs are present (Kitching & Ebling, 1967) and usually there are more crabs on sheltered shores (Crothers, 1975a). Consequently the successful recolonisation of shores where dogwhelk populations are now absent not only relies on the levels of TBT in the water declining and females capable of breeding rafting in but also on the exposed shore variety of *Nucella* surviving predation. The physical transplanting of dogwhelks from shores of similar wave exposure would not be too difficult on the Isle of Man since shores of similar exposure where dogwhelks not affected exist. This would be difficult on the south coast, for example, where virtually all sheltered shores have been affected.

6.4.3 Gradients of contamination

Although the water samples are higher than those recorded on the south-coast (chapter 4) the gradients are steep. This is seen over a short distance in the harbours at Ramsey and Douglas and over a slightly longer distance at Port St. Mary. Imposex values confirm the steepness of these gradients away from the harbour areas. These are steeper than the gradients observed on the south coast where characteristically median VDS values are all around stage 4. With time the steepness of these gradients away from the harbours decreased as imposex values from *Nucella* populations close to harbours reduced faster than those on the open coast.

The steepness of the gradient away from the harbour did not relate solely to the size of the harbour, as measured by the number of boats (cf. Port Erin to Port St. Mary) but also to the amount of water exchange in the area. Comparison of the levels of contamination around the harbours at Douglas and at Ramsey, both similar in size and which are two of the Islands largest harbours, illustrates this. At Douglas the wide harbour entrance facilitates a great deal of water exchange and consequently the levels near the harbour mouth are much lower than those recorded in the main harbour. At Ramsey although the narrow harbour entrance is likely to restrict water exchange and levels are higher at the harbour mouth than in the main marina. This problem could be increased in the future if the proposals for a marina are developed (Allen *et al.*, 1992a). The building of a swing gate here will further reduce the water exchange of this harbour. The consequences for *Nucella* populations are important to consider here. Since *Nucella* often occupies rocks at harbour entrances the level of water exchange within the harbour will be directly important. Imposex values near Castletown, for example, which has a restricted harbour entrance are higher than those at Peel which is a larger harbour but has wide harbour mouth facilitating the dilution of TBT.

6.4.4 Parasite infection

Trematode infection has been reported to affect male penis size in the neogastropods *Buccinum undatum* (Køie, 1969) and *Nassarius pygmaeus* (Køie, 1975). Infection of *Nucella lapillus* with the parasite *Parorchis acanthus* appears to have had a similar effect with the penis size of affected males much reduced. Consequently the use of individuals infected by parasites in imposex assessment is not advisable as they would give a distorted view of the relative penis size for a population. The occurrence of the parasite in *Nucella* populations appears to be related to the proximity of harbours probably because this is a favoured location of the herring gull, the final host in the life cycle of the parasite. As a result of the distribution of the parasite occurrence it is possible that infection by *Parorchis acanthus* could effect the rate of decline of *Nucella* populations near some harbour sites.

6.4.5 Conclusions

The data presented here give an overall view of the level of tributyltin contamination away from the severe effects seen on such a large scale on the south coast. Elevated levels of TBT in the water at harbours around the Isle of Man, especially in the summer, are unlikely to be caused by factors such as sediment in the water, changes due to the tidal cycle or weather effects. Instead the illegal use of organotin paints is the suggested cause. This illustrates the importance of not only legislation concerning TBT but also the enforcement and monitoring of the effectiveness of the legislation. After a reminder about the current procedures concerning the Isle of Man legislation in late 1992 water concentrations did decrease. It is imperative that the monitoring of water concentrations now continues to some extent.

Dogwhelks are now absent from sites where once they were common, most noticeably at Port St. Mary. Despite the elevated concentrations of TBT in some harbours, at other sites *Nucella* populations around the Isle of Man are recovering. The levels of imposex at Niarbyl makes this dogwhelk population one of the least effected, not just on the Isle of Man but in the UK. The continuation of monitoring of the transplanted dogwhelk population in Port St. Mary harbour will allow predictions of the long term survival of populations rafted into affected areas, especially the survival of juveniles.

CHAPTER 7

The role of *Nucella lapillus* in structuring shore communities

7.1 Introduction

The effects of tributyltin pollution on *Nucella lapillus* individuals and populations are well documented (see Bryan & Gibbs, 1991; Hawkins *et al.*, in press and chapter 1 for reviews). Exposure of females to water concentrations as low as 0.5 ng Sn/l (Gibbs *et al.*, 1988) leads to the development of male sexual characteristics in the female. This phenomenon, termed imposex (Smith, 1971), develops in a dose dependent manner and leads to the expression of a penis and vas deferens in females. At concentrations above 2 ng Sn/l females become effectively sterile as the vas deferens development occludes the vulva preventing the release of egg capsules (Gibbs *et al.*, 1988). Since there is no planktonic larval phase in the life cycle of *Nucella*, and adults are relatively immobile, each population is effectively isolated and self sustaining. When TBT concentrations are high enough to cause sterility dogwhelk populations become characterised by having few or absent juveniles and then ultimately being dominated by adult males (Bryan *et al.*, 1986; Gibbs & Bryan, 1986). This eventually leads to the complete demise of the population (Bryan *et al.*, 1986; Spence *et al.*, 1990a).

Nucella lapillus has now been exterminated from many areas throughout its European range (Norway to Portugal) (Gibbs *et al.*, 1991c). Dogwhelks are most noticeably absent on shores adjacent to harbours or marinas where the TBT concentrations have extended above the critical 2 ng Sn/l, often by several orders of magnitude (Cleary & Stebbing, 1985; Cleary & Stebbing, 1987a). In many other places their density has been reduced (Bryan *et al.*, 1986; Spence *et al.*, 1990a). The significance of TBT pollution and its effects on *Nucella lapillus* have now been extensively examined at all levels, from cellular to population (Hawkins *et al.*, in press). One of the remaining unanswered questions is what are the ecological consequences that the reduction in numbers of *Nucella lapillus* has had on shore communities around the UK ?

The difficulty in understanding what knock-on effects the reduction in numbers of *Nucella lapillus* has had on rocky shore communities is two-fold. Firstly there is a lack of data concerning both changes in dogwhelk abundance and changes in shore communities over long periods of time both before and after TBT usage first occurred. Secondly there is still a lack of understanding of the complex interactions between *Nucella lapillus* and other major species on British rocky shores.

There appear to be no long term data sets which could show the changes in dogwhelk abundance and other key species on rocky shores at clean and polluted sites. Bryan *et al.* (1986) used abundance data collected in 1936 and 1975 (Moore, 1936; Crothers, 1975b) to show that *Nucella* populations had declined in Plymouth Sound in the 1980's. Unfortunately these were not in combination with measurements of abundance of other organisms such as barnacles, limpets and *Fucus*. In natural systems there is a great deal of fluctuation and often it is difficult to attribute changes to one factor alone. As a consequence only speculation (e.g. Spence *et al.*, 1990a; Hughes & Burrows, 1993) currently exists concerning what effect the reduction in the numbers of dogwhelks, as a result of TBT pollution, has had on the rocky shore communities in the UK.

It is known already that *Nucella lapillus* is an important predator on rocky shores, feeding on the major space-occupying organisms, barnacles and mussels. To date much of the work on the role of predation by thaid whelks has concentrated on the east and west coasts of America where *Nucella* and other species have been shown to have some effect on regulating patterns of community structure (Dayton, 1971; Menge, 1976; Menge & Sutherland, 1976; Menge, 1978a; Menge, 1978b). The effect of predation on communities varies widely (Menge, 1978a) so, in order to understand the role of predation in structuring communities, world-wide studies must be carried out (Underwood, 1985). The communities on the east and west coasts of America, however, are very different from British shores (Menge & Sutherland, 1976). On the west coast the diverse community is structured largely by predation (Paine, 1966; Menge & Sutherland, 1976) with a secondary carnivore trophic level and a much larger herbivore guild (Connell, 1970). Shores on the east coast are much simpler than those on the west coast of America (Menge & Sutherland, 1976). They have been reported to be similar to British rocky shores (Menge & Sutherland, 1976; Menge, 1978a; Menge, 1978b; Menge & Lubchenco, 1981), although a major difference is that there are no large patellid limpets which play an important part in structuring north-east Atlantic shores (Jones, 1948; Lodge, 1948; Burrows & Lodge, 1950; Southward, 1956; Southward, 1964; Southward & Southward, 1978; Hawkins, 1981a; Hawkins, 1981b; Hawkins & Hartnoll, 1983; Hartnoll & Hawkins, 1985; Hawkins *et al.*, 1992).

On British shores previous studies have shown that *Nucella lapillus* has a direct effect on the population structure of barnacles in the field, as the larger individuals are taken preferentially and the smaller ones generally ignored (Connell, 1961a; Spence, 1989; Hughes & Burrows, 1990). In the summer nearly all the mortality of barnacles over six months can be accounted for by predation by *Nucella lapillus* (Connell, 1961a).

In order to examine changes in rocky shore communities, for example those caused by pollution incidents, there is first a need to understand community dynamics and interactions. Moderately exposed shores on the Isle of Man have been well studied and are relatively well understood (Hawkins *et al.,* 1992). Experimental work has examined the interactions within barnacle (*Semibalanus balanoides*) and *Fucus vesiculosus* patches occurring at the mid tide level. These patches form mosaics which show considerable temporal variation in the relative abundance of barnacles, bare rock and *Fucus* cover (Hartnoll & Hawkins, 1985). Examinations of the dynamics of these interactions have concentrated around the limpet *Patella vulgata* which has the greatest level of effect. Consequently, although *Nucella lapillus* is present within these areas, little attention has been given to the role of *Nucella* within the ecosystem.

A natural cycle has been proposed for these shores which is mediated by limpets (Hartnoll & Hawkins, 1985, figure 7.1). This shows the mechanisms generating the mosaics observed on these rocky shores. This cycle of dominance is generated in part by stochastic recruitment fluctuations but also by deterministic cyclic events following escapes of fucoids from grazing limpets.

High barnacle densities reduce the foraging efficiency of *Patella vulgata* (Hawkins, 1981*a*; Hawkins, 1981*b*; Hawkins & Hartnoll, 1982a) allowing vulnerable algal germlings to escape grazing (Hawkins & Hartnoll, 1983; Hartnoll & Hawkins, 1985). This increases the likelihood of an algal escape from grazing so the *Fucus* forms a clump on the barnacle matrix. Once the *Fucus* reaches 3-4 cm in length limpet grazing has little impact upon them (Burrows & Lodge, 1950; Hawkins, 1979) resulting in an overall increased algal abundance. These algal clumps form a valuable resource for food and shelter for many intertidal organisms. *Nucella* and *Patella* congregate underneath these *Fucus* clumps. Beneath the *Fucus* plants the barnacle density is reduced as *Nucella* forages from the shelter of the *Fucus* canopy (Connell, 1961a). New barnacle settlement is limited as the *Fucus* canopy creates a sweeping and a barrier effect (Lewis, 1964; Southward, 1956; Dayton,

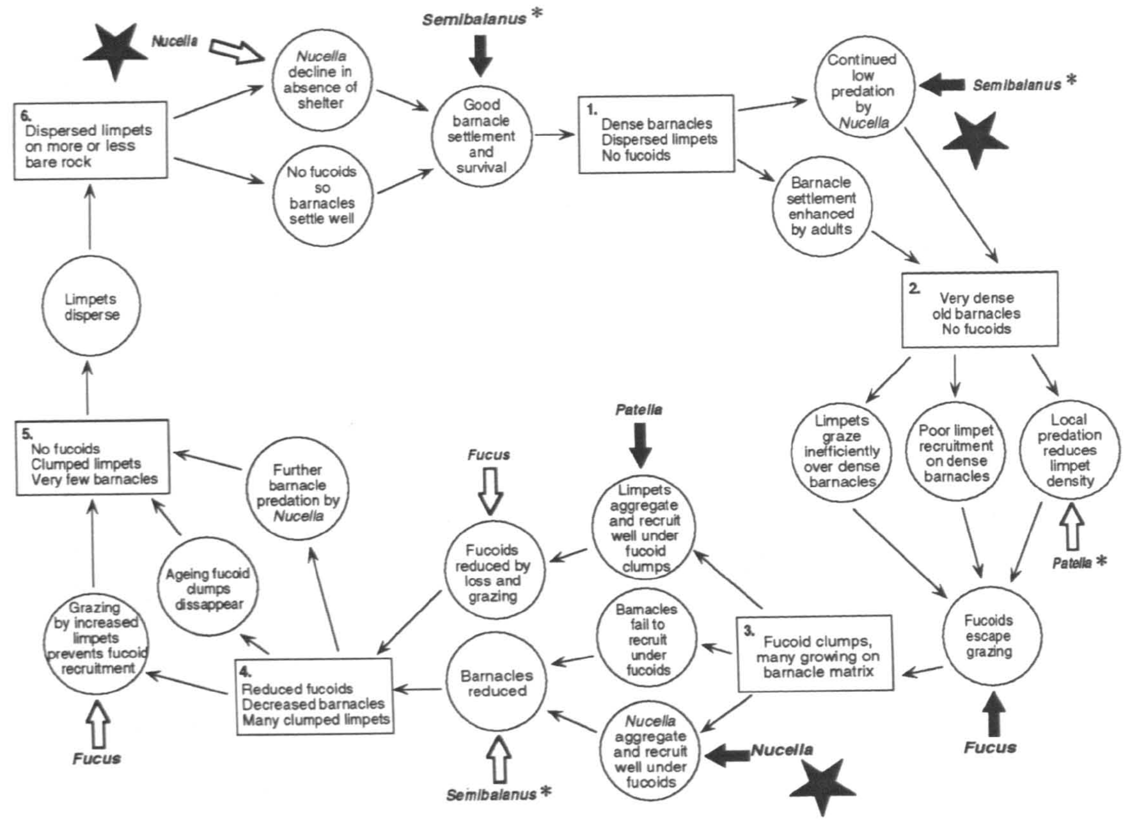


Figure 7.1 A simplified flow chart to represent the sequence of events at the mid-tide level of a moderately exposed shore on the Isle of Man, over several years. The numbered rectangles are changes in the cycle. The circles are intrinsic biological processes generating and maintaining the cycle. The heavy arrows are where very good recruitment of the named species either promotes (solid arrows) or inhibits (open arrows) the progress of the cycle. The asterisks indicate where settlement is from a highly variable planktonic phase. The large stars indicate where *Nucella lapillus* has been examined experimentally in this study. Figure modified from Hartnoll and Hawkins (1985) and Hawkins and Jones (1992).

1971; Menge, 1976; Hawkins, 1983) reducing the numbers of settling cyprids. There is high mortality of those that do settle since although limpets do not damage adult barnacles, they cause some mortality to settling cyprids and newly metamorphosed barnacles during grazing (Connell, 1961a; Dayton, 1971; Hawkins, 1983). It has been proposed that the aggregations of *Patella* under the *Fucus* clump reduces grazing pressure elsewhere creating likely conditions for algal escapes elsewhere (Hartnoll & Hawkins, 1985). With time the *Fucus* canopy thins and the limpets and dogwhelks disperse to other *Fucus* clumps. This leaves areas of rock with sparse barnacles which are colonised by newly settling cyprids (Hawkins & Hartnoll, 1983).

To demonstrate the biological interactions generating these changes the role of *Patella* within this cycle has been determined experimentally (Hawkins, 1979; Hawkins, 1981a; Hawkins, 1981b; Hawkins, 1983; Hartnoll & Hawkins, 1985). There has been little or no attention paid to the role of *Nucella*. Consequently in order to gain an understanding of the role of *Nucella* in structuring shore communities experiments were carried out to examine the effects at each stage within this cycle where *Nucella* is known to occur.

The aim of the work reported in this chapter was to gain some understanding of the role of *Nucella* in structuring shore communities in order that some predictions could be made on the effect of TBT pollution on rocky shores. The initial section provides background information and a description of the general approach adopted in this chapter. The main experiments are organised into three more specific sections examining the influence of *Nucella* at different stages in the natural cycle of barnacle and *Fucus* domination on moderately exposed Manx rocky shores (figure 7.1) using manipulative field experiments. Both the methods and results are given in each section to aid comprehension.

Firstly, the role of *Nucella lapillus* was investigated in influencing the rate of establishment of new *Fucus* clumps as a direct result of its predation on the barnacle population; this was extended to study the relative importance of *Nucella* in comparison to limpets in structuring the community. Secondly the effect *Nucella* has on the dynamics of *Fucus vesiculosus* clumps once established was determined, and thirdly the effect of *Nucella* on the re-colonisation of areas of bare rock by affecting the settlement of barnacles in both the long and short-term was studied.

7.2 The general approach

Rather than using the common experimental approach of mesh fences or cages to exclude *Nucella* (Connell, 1961a; Dayton, 1971; Menge, 1976; Menge & Sutherland, 1976), dogwhelks were removed, by hand, on regular visits to the experimental sites. This approach avoided the need for extra controls to examine cage or fence effects. The frequent removal by hand created controls with dogwhelks present and treatment areas with reduced numbers of dogwhelks, akin to shores with reduced or absent *Nucella* populations resulting from TBT contamination.

The main experimental site used was the Ledges at Port St. Mary (see chapter 2). Here the shore consisted of a number of accessible large flat ledges and abundant dogwhelks suitable for these experiments. In addition, the site was chosen as it is relatively close to the laboratory facilitating the large number of visits needed for the effective removal of dogwhelks.

7.2.1 Natural fluctuations

The natural fluctuations in the abundance of *Semibalanus balanoides*, *Fucus vesiculosus*, *Patella vulgata*, *Actinia equina* and *Nucella lapillus* on the shore at Port St. Mary are shown here to provide background information. The data are from observations made in a 2 x 1 m area at the mid tide level (2.5 m above chart datum) at site C (figure 2.3). This area was originally set up in January 1977 (Hawkins, 1979; see also Hawkins & Hartnoll, 1983; Hartnoll & Hawkins, 1985) to investigate the dynamics of rocky shore communities and has been monitored every 6-8 weeks since. Between October 1990 and May 1992 I was responsible for the continuation of this time series. Changes in this area illustrate the natural cycle of changes in the barnacle and *Fucus* cover over several years and also shows the natural changes in abundance of dogwhelks feeding on the open rock at this site. The changes in the percentage cover of barnacles, bare rock and *Fucus* for ten years from 1983 onwards are shown in figure 7.2. For data collected before 1983 see Hartnoll and Hawkins (1985) and Hawkins and Jones (1992).

Considerable variations are evident in the abundance of all the species sampled in the monitoring area over the ten year period shown (figure 7.2) - *Fucus vesiculosus* from 0-70%, barnacle cover from 10-60%, adult *Patella vulgata* from 17-35 m^{-2}, *Nucella* from 0-8 m^{-2} and *Actinia* from 0-8 m^{-2}. Some of the variation is a result of seasonal cycles of growth and behaviour (Hartnoll & Hawkins, 1980; Hawkins & Hartnoll, 1983) but the main changes are of longer periodicity (Hartnoll & Hawkins, 1985). The most obvious changes occurring between 1983 and 1993 were in the cover of fucoids which were at their highest between 1987-89. This peak in *Fucus* cover was accompanied by a reciprocal change in barnacle cover and an increase in the abundance of *Nucella*, *Actinia* and *Patella*. The period from 1991 onwards shows the monitoring area in a bare phase with *Fucus* cover <5% and the absence of *Nucella* and *Actinia*.

7.2.2 Preliminary experiments

Experimental work was started initially on the horizontal ledges at site B (figure 2.3) in order to explore the role of *Nucella lapillus* in communities where the focus of work had previously been on the limpet/fucoid and barnacle interactions (Hawkins, 1981a; Hawkins, 1981b; Hawkins, 1983; Hartnoll & Hawkins, 1985). Preliminary experiments to investigate the role of *Nucella* involved the removal or addition of individuals from areas of the shore and monitoring the changes in barnacle and fucoid abundance.

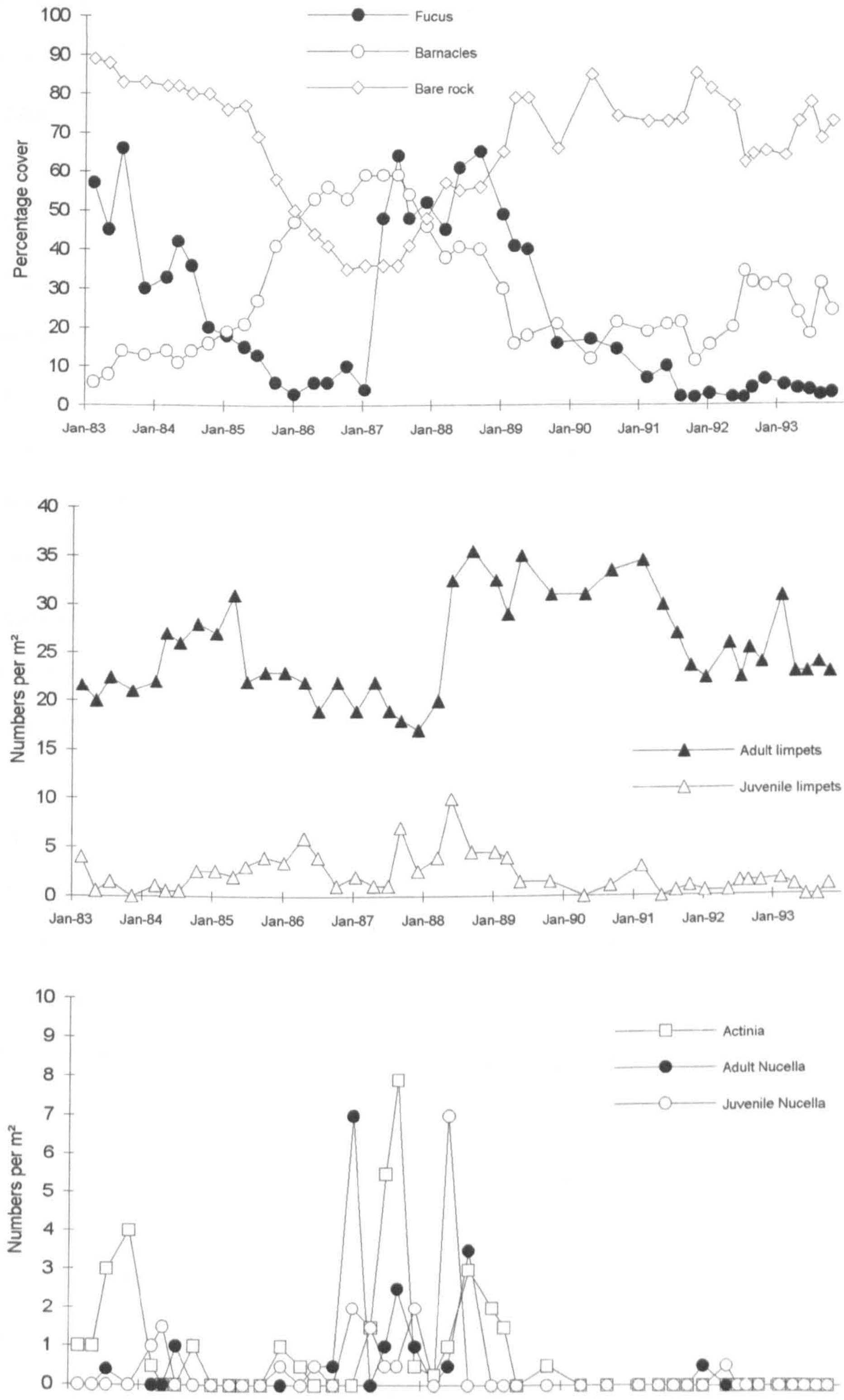


Figure 7.2 Average abundance of barnacles, bare rock and *Fucus* (A), adult and juvenile limpets (B) and adult and juvenile ***Nucella lapillus*** and ***Actinia*** (C) in a 2 x 1 m area at Port St. Mary (Hartnoll & Hawkins, 1985; Hawkins & Jones, 1992; pers. comm. S. J. Hawkins, Port Erin Marine Laboratory).

In the first preliminary experiment twenty 1.0 x 0.5 m areas were randomly selected on a flat ledge at site B (figure 2.3) and marked at the corners with numbered tags (chapter 3). These areas were a minimum of 2 m apart. The percentage cover of *Semibalanus balanoides*, bare rock and *Fucus vesiculosus* was measured using a 0.5 x 0.5 m quadrat (see chapter 3). The average barnacle cover was high in these areas at the start of the experiment in August 1991 (80.8% ± 8.7) and *Fucus vesiculosus* was found in each of the areas (29.8% ± 18.9). Initially *Nucella* was relatively abundant over the whole area (around 10 per m^2) and the 20 areas were randomly assigned to be either treatment (dogwhelk removal) or control (dogwhelks present) areas. Dogwhelks were removed from the treatment areas every week. However, dogwhelk abundance decreased and it soon became apparent that dogwhelks were not generally feeding on the open rock here as *Fucus* cover declined and there were few crevices to provide alternative refuges. The experiment was abandoned in June 1992.

In a second preliminary experiment in July 1992, 800 dogwhelks were collected from a site on the Ledges at Port St. Mary, away from other experimental areas. These were transplanted to just outside Port St. Mary harbour, an area badly affected by tributyltin pollution (chapter 6). Four large areas of rock, each about 6 m^2 and with a 45° slope, were chosen for the experiment, these were effectively isolated from each other by boulder fields. These areas were barnacle dominated with a percentage cover of 75.2% ± 1.6. There was no *Fucus* cover, but a number of crevices ran across the areas. No dogwhelks were found in the experimental areas although they had previously been present here (pers. comm. S. J. Hawkins, Port Erin Marine Laboratory). On 24 July 1992 400 dogwhelks were transferred into two of the four areas. On the first return visit after one week none of the transplanted dogwhelks could be found. Later observations showed that these rocks all faced directly into the sun. It became apparent that the dogwhelks had all moved around to the other side of the rocks which faced northwards and were in the shade.

The lack of success in the preliminary experiments prompted an alternative approach. Instead of adding dogwhelks to areas where they were absent or manipulating naturally occurring low densities experiments were conducted where *Nucella* abundances where high. In addition instead of using large areas of shore and measuring general changes in the community, the role of *Nucella* was examined at specific places in the natural cycle of dominance on these shores (figure 7.1) (Hartnoll & Hawkins, 1985).

Observations at Port St. Mary Ledges showed that the greatest abundance of *Nucella* was either on vertical rock faces or under *Fucus* clumps on horizontal areas. Consequently the role of *Nucella* in influencing the rate of establishment of *Fucus* clumps was investigated by the removal of dogwhelks from areas of vertical rock rather than horizontal areas. Experiments investigating the role of *Nucella* in *Fucus vesiculosus* clumps and on barnacle settlement were done on horizontal ledges. The experimental designs used in all of these experiments are summarised in table 7.1.

7.2.3 Repeated measures designs

The design of some of the manipulative experiments necessitated repeat sampling. This was because the size of the experimental plots and the number of times that sampling was planned to take place meant that it would have been logistically impossible to set up and maintain enough areas on the shore so that each area would be sampled only once. As well as the practical considerations of increased effort for such a large experiment there may be more subtle problems of site

Table 7.1 Summary of the experimental designs for manipulative field experiments used in chapter 7.

		Design and manipulation	Number of replicates	Parameters measured on a regular basis. The number of sub-samples measured in each replicate experimental area is given in brackets where applicable
Section 7.3 The effects of *Nucella lapillus* on barnacle populations and the consequences for the formation of *Fucus* clumps				
Vertical 1	Control	*Nucella lapillus* present	6	Percentage cover of bare rock (9), living (9) and dead (9) barnacles and cover of ephemeral algae (9) and *Fucus vesiculosus*, numbers of *Nucella lapillus* present or removed
	Treatment	*Nucella lapillus* removed	6	As above
Vertical 2	Control	*Nucella lapillus* present	3	As above
	Treatment	*Nucella lapillus* removed	3	As above
Vertical 3	Control	*Nucella lapillus* present	3	As above
	Treatment	*Nucella lapillus* removed	3	As above
Vertical 4	Control	*Nucella lapillus* and *Patella vulgata* present	4	Percentage cover of bare rock (4), living (4) and dead (4) barnacles and cover of ephemeral algae (4) and *Fucus vesiculosus*, numbers of *Nucella lapillus* and *Patella vulgata* present or removed
	Treatment 1	*Nucella lapillus* present, *Patella vulgata* removed	4	As above
	Treatment 2	*Nucella lapillus* removed, *Patella vulgata* present	4	As above
	Treatment 3	*Nucella lapillus* removed, *Patella vulgata* removed	4	As above
Section 7.4 The effects of *Nucella lapillus* on *Fucus vesiculosus* clumps				
Fucus vesiculosus clumps	Control	*Nucella lapillus* present	12	Clump size, plant density (10), plant length (10) abundance of *Patella vulgata* and *Actinia equina*, numbers of *Nucella lapillus* present or removed
	Treatment	*Nucella lapillus* removed	12	As above

Section 7.5 The effects of *Nucella lapillus* on barnacle settlement

Barnacle settlement in fixed areas on vertical 2	Control	*Nucella lapillus* present	3	Numbers of cyprids and metamorphosed barnacles (3)
	Treatment	*Nucella lapillus* removed	3	As above
Barnacle settlement in fixed areas on vertical 3	Control	*Nucella lapillus* present	3	As above
	Treatment	*Nucella lapillus* removed	3	As above
Barnacle settlement in random quadrats on vertical 2	Control	*Nucella lapillus* present	3	Numbers of cyprids and metamorphosed barnacles (10)
	Treatment	*Nucella lapillus* removed	3	As above
Barnacle settlement in random quadrats on vertical 3	Control	*Nucella lapillus* present	3	As above
	Treatment	*Nucella lapillus* removed	3	As above
First mucus effect experiment, on stones	Control	Nothing added	10	Numbers of cyprids and metamorphosed barnacles
	Treatment 1	*Littorina littorea* added initially	10	As above
	Treatment 2	*Nucella lapillus* added initially	10	As above
Second mucus effect experiment, in cleared areas	Control	Nothing added	5	As above
	Treatment 1	*Littorina littorea* added initially	5	As above
	Treatment 2	*Nucella lapillus* added initially	5	As above
Third mucus effect experiment, in cleared areas	Control	Nothing added	8	As above
	Treatment 1	*Littorina littorea* added initially	8	As above
	Treatment 2	*Littorina littorea* added continually	8	As above
	Treatment 3	*Nucella lapillus* added initially	8	As above
	Treatment 4	*Nucella lapillus* added continually	8	As above

specific variation if the experiment was distributed over a large area. This would naturally undermine any statistical advantage of none repeated sampling.

The repeat sampling of replicate areas obviously creates problems with independence. Samples collected from the same replicate area on more than one occasion are not independent with time and so are obviously going to be correlated with each other (Hurlbet, 1984; Green, 1993; Underwood & Anderson, 1994). However, individual results considered at one point in time are not repeat measures. Consequently analysis was performed at each or selected (e.g. at the start and end of an experiment) sampling times between treatments and consequently time was not included as a factor. This followed methods of statistical analysis used by other workers (e.g. see Dungan, 1987; Chapman, 1990; Creed, 1993; Hill, 1993).

7.3 <u>The effects of *Nucella lapillus* on barnacle populations and the consequences for the formation of *Fucus* clumps</u>

7.3.1 Materials and methods

7.3.1.1 Experimental aims and design

Nucella lapillus may be expected to have an indirect effect on the formation of *Fucus vesiculosus* escapes since it has a direct effect on the population structure and abundance of barnacles (Connell, 1961a; Spence, 1989) and dense barnacles reduce the foraging efficiency of limpets (Hawkins, 1981a; Hawkins, 1981b; Hawkins & Hartnoll, 1982a). Hence, the direct effect of *Nucella* on the density of barnacles and as a consequence on the likelihood of algal escapes from limpet grazing was examined. In addition the aim was also to compare the relative importance of *Nucella* and *Patella* in structuring communities. These aims were examined firstly by reducing the densities of *Nucella* on barnacle dominated vertical areas and comparing the changes that occurred with those on areas where dogwhelk densities were not manipulated. Secondly the relative effects of limpets and dogwhelks were examined by removal of both species, or each species singly, in a factorial experiment with an un-manipulated control, again on barnacle dominated verticals.

The first experiment, in which only dogwhelks were removed, used three vertical rock faces. The largest vertical face (vertical 1) was divided into 12 experimental areas and the other two (verticals 2 and 3) had six areas on each. These were randomly assigned to be either control or treatment areas, creating six replicates of each on vertical 1 and three of each on verticals 2 and 3. The control areas on the verticals had dogwhelks at densities which were un-manipulated. In the treatment areas dogwhelks were regularly removed from the experimental plots providing a reduced dogwhelk density.

In the second experiment a fourth vertical (vertical 4) was used where both limpets and dogwhelks were removed in a factorial experiment. This was divided into 16 experimental plots which were randomly assigned to be either control areas or one of the three treatments, creating four replicates of each of the treatments and the control. Control areas had dogwhelk and limpet densities un-manipulated on the vertical faces. The first treatment consisted of un-manipulated dogwhelk densities and a reduced density of limpets where they were regularly removed from the vertical rock. In treatment two dogwhelks were regularly removed from the vertical faces, providing a reduced density, and limpets un-manipulated. In the third treatment dogwhelks and limpets were both regularly removed to provide reduced densities of each (table 7.1).

7.3.1.2 Orientation of vertical rock faces and dogwhelk abundance

A survey was carried out to investigate the abundance of *Nucella lapillus* on areas of vertical rock at Port St. Mary Ledges with different orientations in order to explain some of the natural variations in dogwhelk abundance observed. This also provides background information for comparison with the experimental manipulations described later.

Surveys were carried out on two separate occasions. Firstly on 8 July 1993 when the weather was overcast and heavy rain was recorded (maximum air temperature 15.0 °C, minimum 13.2 °C, 3.5 mm rain, 0 sunshine hours, SW wind force 3) and when low tide occurred at 09:20 (Liverpool). The second occasion was on 12 August 1993 on a dry and sunny day (maximum air temperature 15.0 °C, minimum

9.4 °C, 0.9 mm rain, 6.1 sunshine hours, WNW wind force 2) when low tide occurred at 13:01 (BST, Liverpool). The number of *Nucella lapillus*, adult (>15 mm) and juvenile (<15 mm) limpets were recorded on the open rock in 0.5 x 0.5 m quadrats placed randomly at intervals along the length of selected vertical faces. In addition only areas of the vertical faces which were greater than 0.6 m high were measured, to allow a 0.1 m zone at the bottom where the habitat was observed to be different. Only areas without crevices or *Fucus* cover were surveyed. The aspect of the vertical face was measured using a compass. This was repeated for as many verticals as could be found spanning the mid tidal zone in the time available.

As part of the survey of dogwhelk abundance on vertical rocks faces with different orientations relative humidity and temperature measurements were taken over the period of a low tide to compare the physical environment with different aspects. Measurements were made using a Jenway temperature and relative humidity meter (model 5075) by hanging the probe down the vertical, against the rock, at the half way point. The survey was done on 16 August 1993 when low tide occurred at 17:33 (BST, Liverpool). Air temperatures on this day reached a maximum of 17.3 °C and a minimum of 10.7 °C, there was a gentle NW breeze (force 3) and 13.5 sunshine hours (the highest for that month). Verticals facing 045°, 150°, 215° and 320° (approximately NE, SE, SW and NW) were used and readings were taken every 30 minutes from 15:00 to 19:00.

7.3.1.3 Dogwhelk removal from vertical faces

Three barnacle dominated vertical rock faces at Port St. Mary were chosen for this experiment. At the start of the experiment there was no *Fucus* cover on any of the areas.

Vertical 1 was the longest continuous vertical face selected. It faced northwards, towards land and consequently was in the lee of the wave action. Along the length of the vertical rock 12 experimental plots with similar areas were selected (1.3 m^2 ± 0.5), each plot being separated by at least 0.5 m. These areas were randomly assigned to be either control (dogwhelk present) or treatment (dogwhelks removed) areas (table 7.1).

Vertical 2 ran parallel to vertical 1 but was located slightly to the south (site A, figure 2.3). Six experimental plots with similar areas (1.5 m^2 ± 0.4) were selected at intervals along its length. These were randomly assigned to be three control (dogwhelk present) and three treatment (dogwhelks removed) areas (table 7.1).

Vertical 3 located at site B (figure 2.3) faced eastwards in direction and consequently was more exposed than verticals 1 or 2 because of the seaward direction it faced. Six areas were selected along its length and randomly assigned to be one of three control (dogwhelks present) and three treatment (dogwhelks removed) areas (table 7.1). These were all of a similar size to each other (2.7 m^2 ± 0.4), although larger than those selected on verticals 1 and 2.

Within each of the experimental plots on the verticals 1, 2 and 3 the percentage cover of bare rock, living and dead barnacles and number of limpets was measured. The percentage cover was assessed using a 10 x 10 cm perspex square with 25 holes each 2 mm in diameter drilled through it at regular intervals to form a grid. Regularly spaced holes were used rather than random ones because they were easier to sample. It is unlikely that there is a difference in precision because of this (Foster *et al.*, 1991). The perspex square was randomly positioned against the rock and each of the 25 holes scored as either bare rock, living or dead barnacles. Dead barnacles were identified by the absence of their opercular plates.

quadrat appeared to have landed across part of a dead barnacle and part of a live barnacle the result was scored to the barnacle whose opercular plate was closest to the centre of the hole. If in the random selection of the location for the perspex plate it was placed over a limpet the perspex plate was moved to the immediate right.

Sampling of experimental areas on the vertical faces was stratified. The experimental plots were divided into three: a 30 cm band on the horizontal area at top of the vertical, the section from the top of the vertical to half way down the vertical area, and from the middle of the vertical to the bottom. Within each of the three areas of each experimental plot three sub-samples were measured. Thus in all a total of nine 25 x 25 cm quadrats were counted in each of the experimental plots at each sampling time (table 7.1). The experimental areas on all of these verticals were marked with a coloured tag (see chapter 3) placed on the top of the vertical to the left of the experimental area.

The experiment commenced on 12 June 1992 when dogwhelks were first removed from the areas randomly assigned to be for the treatment. Dogwhelks were removed from within the experimental areas and in a 20 cm boundary around the plot. During the initial removal all those dogwhelks in the treatment areas were removed and placed into buckets and released elsewhere on the Ledges at Port St. Mary, away from other experimental areas. Subsequently when these areas were checked on regular visits, dogwhelks were picked off the areas and placed at the bottom of the vertical face either to the left or right of the experimental area depending on which side it appeared they had encroached from. The frequency of these visits varied from every tide (half a day) when the dogwhelks were very active to once a week during the winter months. In the spring of 1993 onwards the frequency of the removals from the treatment areas increased as more dogwhelks were encroaching the experimental plots. Occasionally (about once every two months) the numbers of dogwhelks arriving in these areas made the placing of the dogwhelks to the left or right impossible. Then the dogwhelks were collected into buckets and released elsewhere. The numbers of dogwhelks removed from each of the areas was recorded. On most, although not all, of these visits the number of dogwhelks within the control areas were also counted.

At regular intervals, about every two months, the percentage of dead and living barnacles and the cover of bare rock was assessed using the 10 x 10 cm perspex plate. The percentage cover of ephemeral greens was also assessed. These measurements were not taken in a 10 cm boundary area at the edge of each of the experimental plots. *Fucus vesiculosus* cover was measured on the vertical areas using a 0.5 x 0.5 m quadrat (chapter 3).

7.3.1.4 Dogwhelk and limpet removal in a factorial experiment

The vertical chosen for this factorial design experiment (vertical 4) was located at site A on Port St. Mary Ledges (figure 2.3). It ran parallel to verticals 1 and 2, facing in a northerly direction. Along its length 16 experimental plots were selected with similar areas ($0.8\ m^2 \pm 0.3$) and with a minimum of 0.5 m between them. The initial survey measured the percentage cover of bare rock, living and dead barnacles and ephemeral green algae using the perspex plate described earlier. Sampling was not stratified as for areas on verticals 1-3, but on each sampling occasion four random quadrats were examined within each replicate area which were then averaged for each experimental plot (table 7.1). Experimental plots were randomly assigned to either control or treatment plots. The dogwhelks and limpets were removed from their respective treatment areas on 25 August 1992. The dogwhelks removed were collected in a bucket and released on another area of the shore away from other experimental areas and limpets were removed with a knife and their shell lengths measured. The numbers of dogwhelks removed were recorded, and the numbers of dogwhelks and limpets present in the other experimental areas, in which they were allowed, were also recorded.

The removal of dogwhelks and limpets was checked regularly, on average every 4 days. Any dogwhelks found in areas assigned to treatments 2 and 3 (table 7.1) were removed, placing them to the left or right of the experimental plot depending on the apparent direction they had come from. Limpets were removed from the rock with a sharp knife from areas assigned to treatments 1 and 3 (table 7.1). Dogwhelks and limpets found in a 20 cm boundary zone around the experimental plots were also removed. The percentage cover of barnacles (living or dead), bare rock or ephemeral algae was recorded approximately every two months using the 10 x 10 cm perspex plate. *Fucus vesiculosus* cover was measured using a 0.5 x 0.5 m quadrat (chapter 3).

7.3.1.5 Statistical methods

Data were analysed following the methods described in chapter 3, with all percentage cover data arc sine transformed before analysis. Differences in the percentage cover of bare rock, living and dead barnacles and *Fucus* and *Enteromorpha* cover between treatment and control areas on verticals 1, 2 and 3 were tested at each sampling time using one-way analysis of variance (see discussion of repeated measures designs, section 7.2.3).

Two-way analysis of variance at each sampling time was used for testing the limpets and dogwhelk effects in the factorial experiment on vertical 4. Following the recommendations of Zar (1984) if the interaction between the limpet and dogwhelk effect was insignificant the results were not re-analysed. Pooling of the interaction and within cells sums of squares (SS) and degrees of freedom (DF) has been suggested by some workers if the interaction is not significant, from which a pooled mean square (MS) can be calculated (see Zar, 1984). In not calculating a pooled MS there may be an increased probability of a type II error, that is that the test may be on the conservative side, but the probability of a type I error is at the stated α level (Zar, 1984). Where the interaction effect was found to be significant multiple comparisons were performed using Tukey tests (chapter 3).

7.3.2 Results

7.3.2.1 Orientation survey

A total of 164 quadrats were sampled on 8 July 1992 and 150 on 12 August 1993. These quadrats were sampled on vertical rock faces of all directions, although verticals facing between 090-119° were rare on the Ledges, consequently only one sample was recorded, from within this range of bearings, on 8 July and none on the 12 August.

Overall less dogwhelks were recorded on the vertical faces on the sunny dry day (12 August) than on the rainy and overcast day (8 July) (table 7.2). On both sampling occasions verticals facing in a north to east direction (landward) with bearings between 330-089° had the most dogwhelks present on them (figure 7.3). These vertical rocks had the orientation as experimental verticals 1, 2 and 4. On the sunny day the fewer *Nucella* were recorded as being on the south and east facing vertical faces (seaward) with bearings in the direction 120-329° (figure 7.3). These vertical faces had the same orientation as vertical 3. Although the abundance of dogwhelks on the southerly facing verticals was also low on the damp day the average numbers of dogwhelks on the verticals with this orientation was higher than recorded on the sunny day (figure 7.3).

The abundance of adult *Patella vulgata* varied very little between the two sampling days (table 7.2, figure 7.4). On both occasions, however, slightly more *Patella* were present on the northerly facing vertical faces (300-089°). The number of juvenile *Patella vulgata* recorded was higher on the damp day than on the dry day.

Table 7.2 The abundance of *Nucella lapillus* and adult and juvenile *Patella vulgata* on vertical rock faces with different orientations. Data collected on an overcast day with heavy rain (8 July 1993) and a sunny dry day (12 August 1993). n is the number of quadrats sampled and mean values are given as the number of dogwhelks or limpets per $m^2 \pm 1$ SD (summarised in figure 7.3 and 7.4).

(a) 8 July 1993

Bearing	n	*Nucella lapillus*	Adult *Patella vulgata*	Juvenile *Patella vulgata*
000 - 029°	20	36.8 ± 26.4	35.0 ± 13.8	3.4 ± 5.5
030 - 059°	31	31.1 ± 25.8	36.3 ± 14.4	3.7 ± 4.1
060 - 089°	8	32.0 ± 45.7	45.0 ± 20.0	7.0 ± 9.0
090 - 119°	1	0.0	12.0	0.0
120 - 149°	6	11.3 ± 11.9	26.7 ± 11.2	0.0
150 - 179°	19	1.5 ± 4.0	23.8 ± 17.6	0.6 ± 2.0
180 - 209°	5	8.0 ± 6.3	27.2 ± 9.1	2.4 ± 3.6
210 - 239°	35	3.6 ± 5.2	22.2 ± 9.7	0.4 ± 1.3
240 - 269°	10	3.6 ± 5.5	26.4 ± 8.0	2.4 ± 3.4
270 - 299°	11	11.6 ± 9.5	28.4 ± 9.0	3.3 ± 4.3
300 - 329°	5	4.0 ± 4.0	32.0 ± 12.6	0.8 ± 1.8
330 - 359°	13	17.2 ± 28.3	41.0 ± 9.4	1.8 ± 3.1

(b) 12 August 1993

Bearing	n	*Nucella lapillus*	Adult *Patella vulgata*	Juvenile *Patella vulgata*
000 - 029°	9	12.8 ± 10.3	41.7 ± 17.2	0.4 ± 1.3
030 - 059°	24	16.0 ± 15.9	33.0 ± 14.7	1.3 ± 2.8
060 - 089°	9	14.7 ± 12.9	33.7 ± 19.6	0.0
090 - 119°	0			
120 - 149°	1	0.0	24.0	0.0
150 - 179°	7	0.0	22.8 ± 11.7	1.1 ± 3.0
180 - 209°	10	2.0 ± 5.1	22.4 ± 12.9	2.8 ± 4.2
210 - 239°	47	1.8 ± 4.3	20.8 ± 10.2	1.0 ±2.2
240 - 269°	16	2.0 ± 4.4	22.0 ± 12.4	0.5 ± 1.4
270 - 299°	20	5.6 ± 7.5	22.2 ± 12.9	1.6 ± 2.4
300 - 329°	5	1.6 ± 2.2	30.4 ± 7.2	1.6 ± 2.2
330 - 359°	11	15.3 ± 15.9	27.6 ± 13.3	1.4 ± 3.2

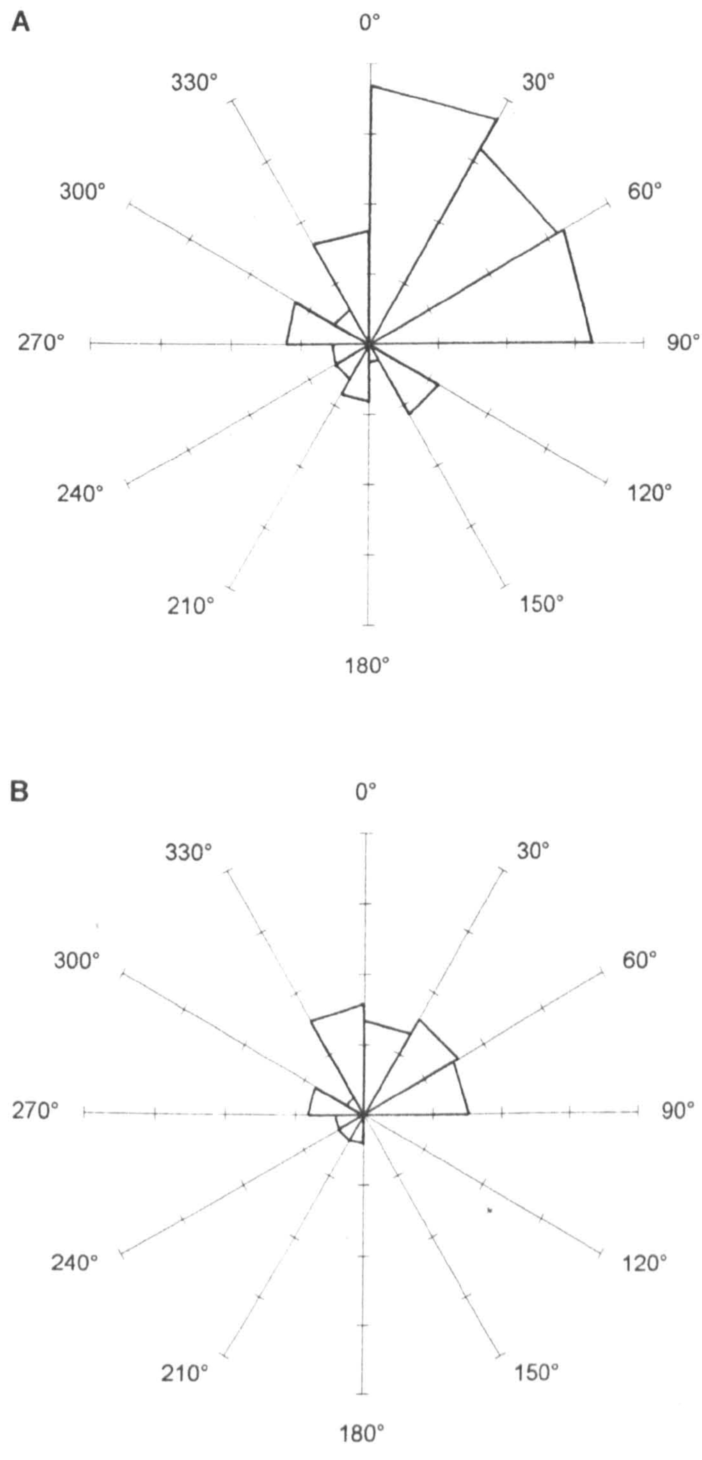


Figure 7.3 The abundance of *Nucella lapillus* on areas of vertical rock with different orientations, on an overcast day with heavy rain, 8 July 1993 (A) and a dry day with bright sunshine, 12 August 1993 (B). Values given as average numbers of dogwhelks per m² (see table 7.1 for SD and n values). Tick marks on axes represent multiples of 10 dogwhelks per m².

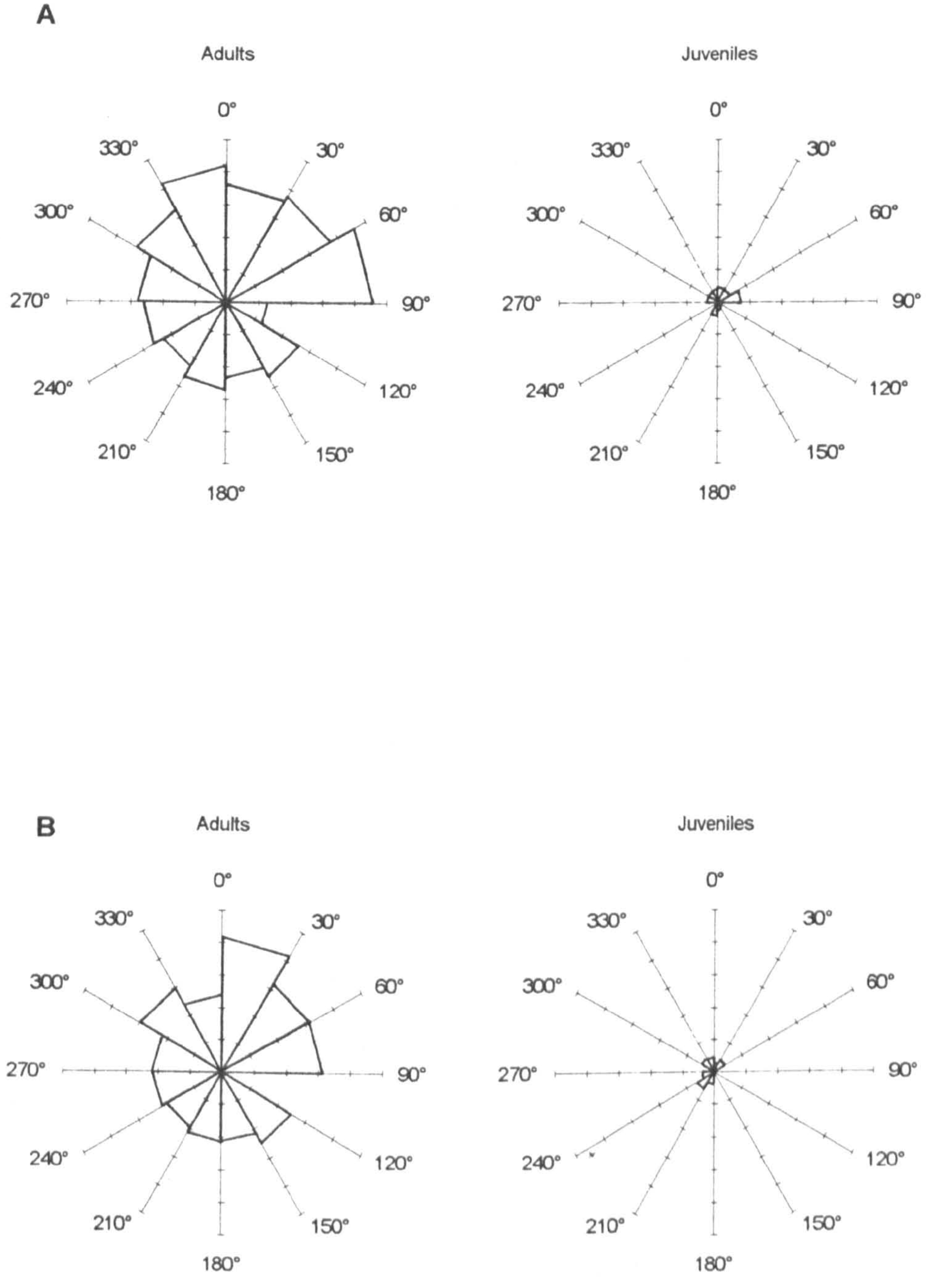


Figure 7.4 The abundance of *Patella vulgata* adults (>15 mm) and juveniles (<15 mm) on verticals with different orientations an a overcast day with heavy rain, 8 July 1993 (A) and on a dry day with bright sunshine, 12 August 1993 (B). Values given as average numbers of limpets per m² (see table 7.1 for SD and n values). Tick marks on axes represent multiples of 10 limpets per m².

Relative humidity increased on verticals of all orientations over the low tide period. Relative humidity was generally recorded as highest on vertical rock with orientations of 320° or 045° (NW, NE) and lowest on verticals of 215° and 150° (SW, SE) (table 7.3). The temperature recorded on the surface of the vertical rock decreased for all four orientations in readings taken between 3 and 7 pm. Generally the highest temperatures were recorded on the vertical rock which faced approximately north-west (320°) (table 7.3).

7.3.2.2 Dogwhelk removal from vertical faces

Dogwhelks were initially removed from the treatment areas on verticals 1, 2 and 3 on 12 June 1992. Removals every week thereafter during the first 8 months of the experiment were sufficient to keep to keep the numbers of dogwhelks in the treatment areas in most cases to a density below 10 dogwhelks per m^2 (figure 7.5). From February 1993 onwards the frequency of the removals was increased to compensate for a higher influx rate. Visits between every tide or a maximum interval of two days were needed to keep the density below 10 dogwhelks per m^2. On all three vertical rock faces used the frequent dogwhelk removal was sufficient to keep the general abundance of *Nucella* lower on the treatment areas than on the control plots on the vertical rock faces (figure 7.5).

The average dogwhelk abundance was greater on vertical 1 than on vertical 2 which in turn was greater than on vertical 3 (figure 7.5). Consequently the removal visits for vertical three could be made less frequently. The average dogwhelk abundance in the control areas fluctuated over the duration of the experiment (figure 7.5). On vertical 1 the highest densities were recorded in June 1992 and again in May and June 1993 when densities of over 50 dogwhelks per m^2 were

Table 7.3 Changes in temperature and relative humidity on vertical rock faces with different orientations over the period of a low tide on 16 August 1993. Values given as average temperature (°C) or relative humidity ± 1 SD. Values are the average of 4 replicate measurements.

(a) Temperature

Bearing	3:00	3:30	4:00	4:30	5:00	5:30	6:00	6:30	7:00
320°	23.7 ± 0.7	21.7 ± 0.1	22.7 ± 0.5	24.1 ± 0.7	23.1 ± 0.6	20.2 ± 1.1	18.8 ± 1.1	18.8 ± 0.5	17.7 ± 0.4
045°	22.6 ± 0.5	20.7 ± 0.7	21.9 ± 0.6	21.8 ± 1.5	19.9 ± 1.4	19.9 ± 0.3	19.2 ± 0.6	19.1 ± 0.4	18.3 ± 0.8
150°	21.0 ± 0.7	22.9 ± 0.2	21.4 ± 0.6	20.8 ± 0.3	20.4 ± 0.4	20.7 ± 0.5	18.2 ± 0.1	19.1 ± 0.2	17.4 ± 0.1
215°	22.3 ± 0.4	20.5 ± 0.8	23.0 ± 0.5	22.5 ± 1.0	21.6 ± 0.6	22.0 ± 1.4	19.2 ± 0.8	18.7 ± 0.5	17.6 ± 0.4

(b) Relative humidity

Bearing	3:00	3:30	4:00	4:30	5:00	5:30	6:00	6:30	7:00
320°	74.1 ± 2.1	71.6 ± 1.8	79.3 ± 1.7	64.5 ± 0.5	72.2 ± 4.6	73.8 ± 5.6	75.4 ± 5.1	75.5 ± 4.2	79.0 ± 3.4
045°	69.8 ± 2.1	73.1 ± 1.9	78.9 ± 3.3	68.0 ± 4.0	73.2 ± 3.9	72.3 ± 3.3	74.6 ± 4.8	73.1 ± 2.4	75.7 ± 4.2
150°	76.9 ± 1.8	64.8 ± 2.1	77.9 ± 1.2	70.0 ± 2.7	68.8 ± 0.9	69.0 ± 1.4	73.2 ± 1.3	71.0 ± 0.7	77.6 ± 0.5
215°	73.1 ± 1.6	67.5 ± 3.1	71.8 ± 2.4	63.5 ± 3.3	60.8 ± 1.0	64.6 ± 2.7	67.7 ± 2.9	72.6 ± 2.1	75.6 ± 1.6

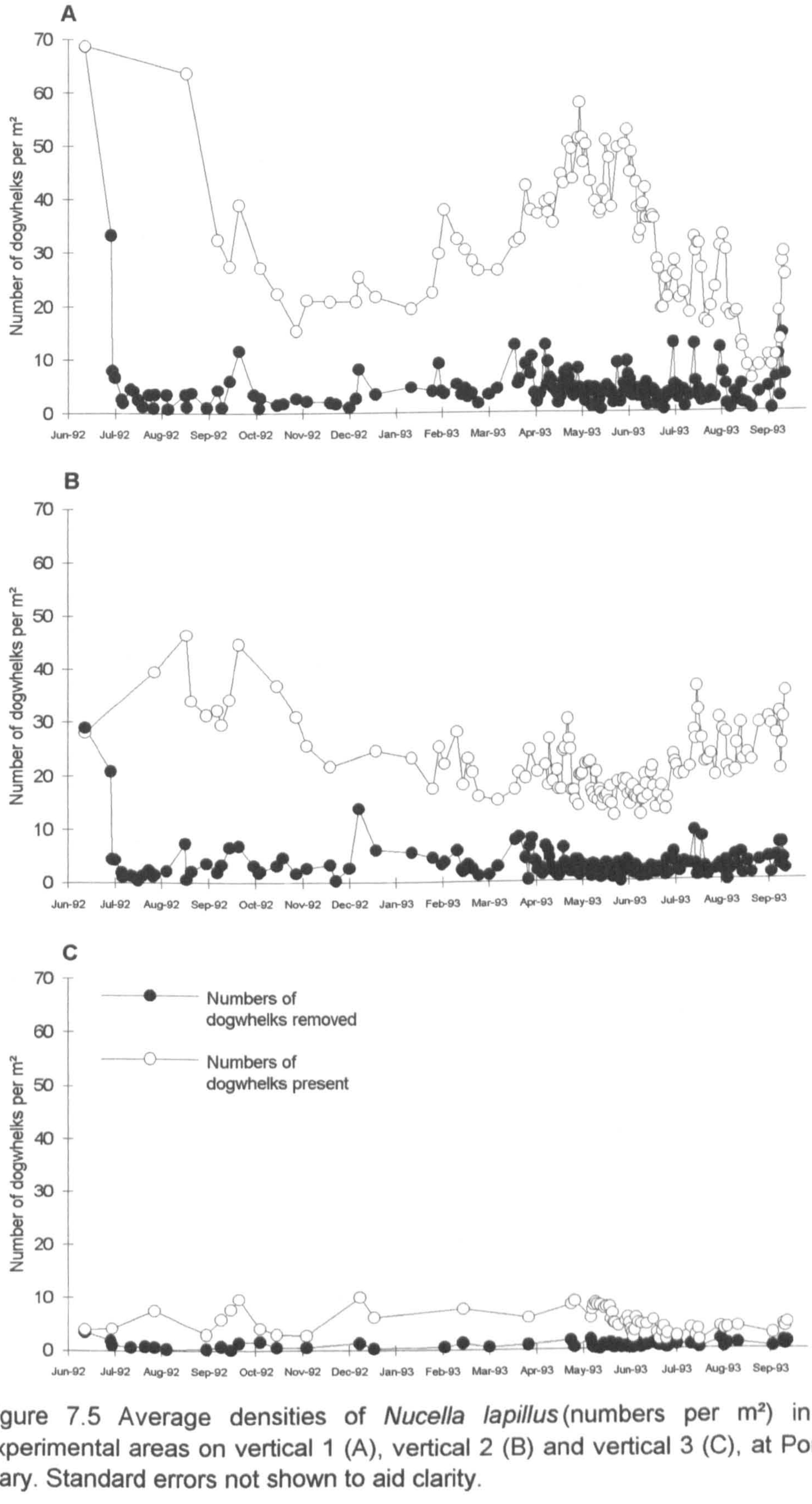


Figure 7.5 Average densities of *Nucella lapillus* (numbers per m²) in the experimental areas on vertical 1 (A), vertical 2 (B) and vertical 3 (C), at Port St. Mary. Standard errors not shown to aid clarity.

recorded. The lowest densities of *Nucella* on vertical 1 were recorded over the period October 1992 to January 1993.

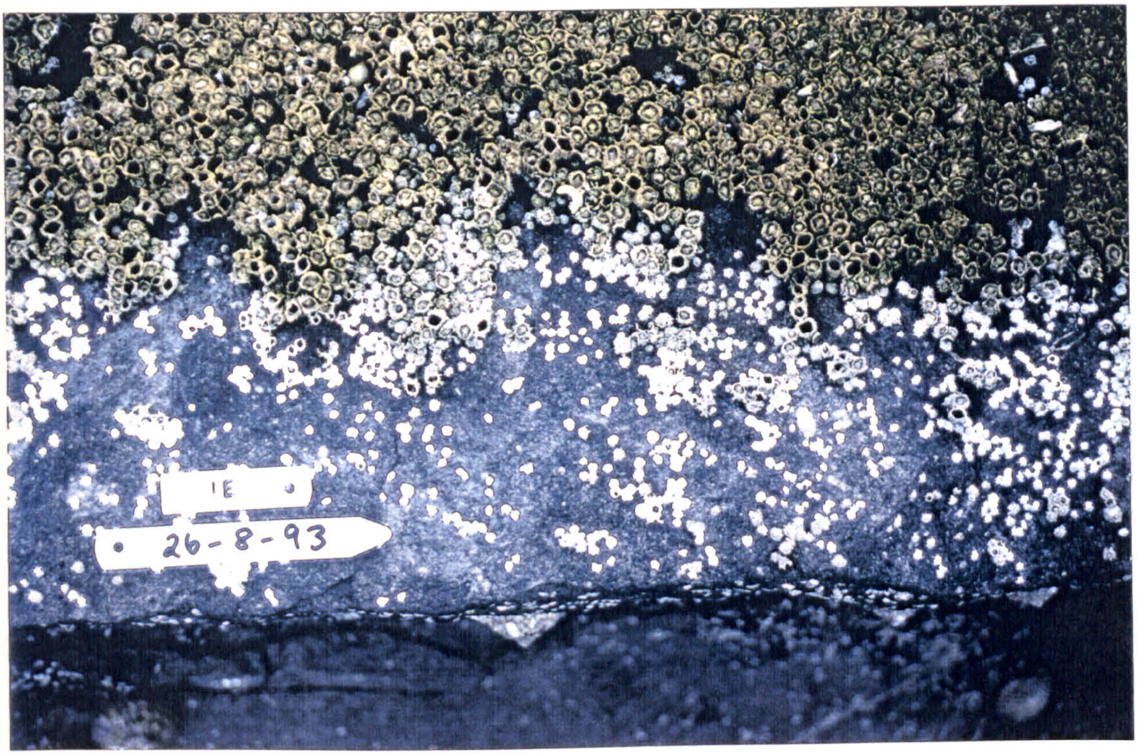
There were fewer fluctuations in the seasonal abundance of *Nucella* on the other two verticals (verticals 2 and 3). Daily fluctuations in abundance were especially apparent on verticals 1 and 2 towards the end of the experiment when there appeared to be some rhythmicity in the abundance of dogwhelks recorded in the control areas (figure 7.5).

Dogwhelks were observed feeding on the vertical faces of all the control areas. In periods of strong onshore winds or very cold weather the adults tended to aggregate into groups of 10-50 individuals on the vertical face or at the base of the vertical rock where there was sometimes a horizontal crevice. These aggregations were especially apparent in October and November 1992. In April 1993 fewer aggregations were observed and the dogwhelks were distributed across the area of the experimental plots. Individuals on verticals 1 and 2 were observed to move up the vertical in a advancing line eventually feeding on the barnacles on the horizontal area at the top of the vertical face. This eventually created a zone where barnacles were absent on the top of the vertical rock in the control areas (plate 1A, 1B). Juveniles were observed amongst the barnacles at the top of the vertical faces in the spring. Often they were wedged in between the densely packed barnacles which were found here. Instead of feeding on these barnacles by drilling or prising open the opercular plates, drill holes were observed in the sides of these barnacles which had grown tall and elongated. These dogwhelks created bare haloes of space in the barnacle matrix around them. As barnacle densities decreased adult *Nucella* were observed feeding on o-group barnacles in July 1993.

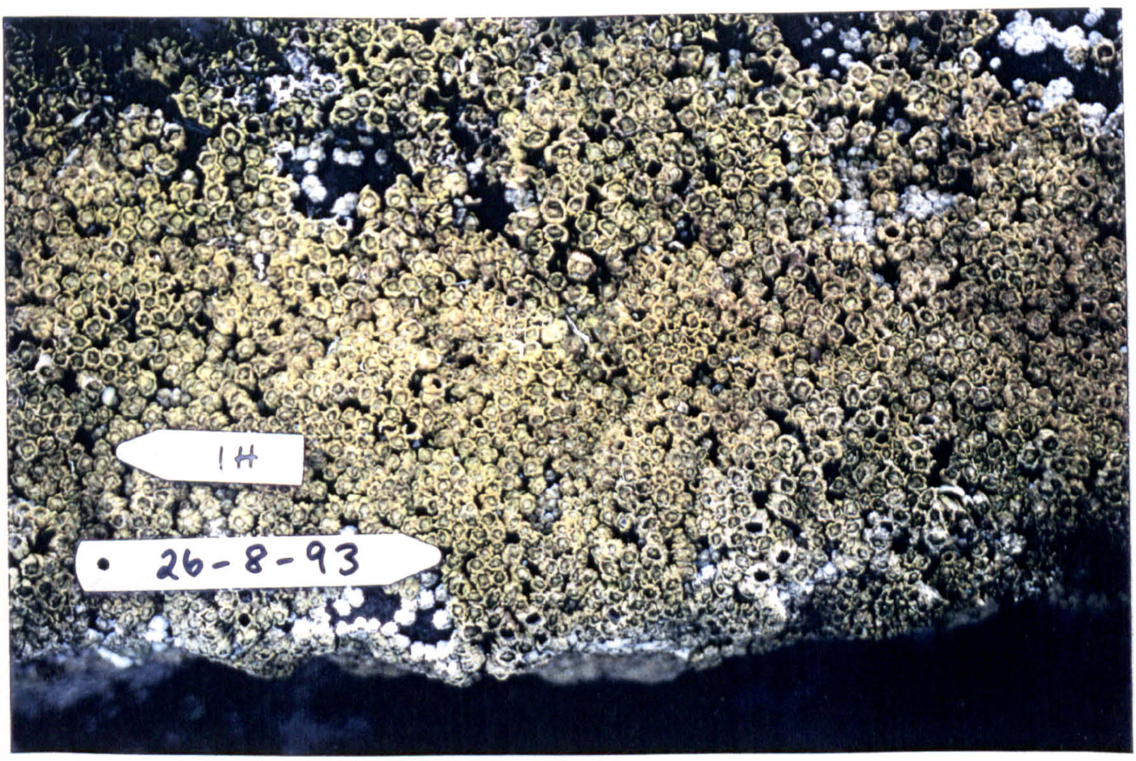
Plate 1

The top of experimental areas on vertical 1 in September 1993 after 16 months where *Nucella lapillus* has either been left un-manipulated (A) or continuously removed (B).

A



B



After the last dogwhelk removal was made on 17 September 1993 the areas were left alone for a number of weeks. A month after the removals had stopped observations showed there to be more dogwhelks in the treatment areas than on the control areas on verticals 1, 2 and 3. Counts were made on verticals 1 and 2 on 15 November 1993, 2 months after the last removal had taken place. On vertical 1 and average density of 1.6 ± 0.7 dogwhelks per m^2 was recorded in the control areas and 33.6 ± 13.7 dogwhelks per m^2 in the treatment areas, where dogwhelks had been previously removed. The situation on vertical 2 was very similar with an average of 7.2 ± 4.2 dogwhelks per m^2 in the control areas and 25.3 ± 14.6 dogwhelks per m^2 in the treatment areas. Return visits to the experimental verticals in March and July 1994 showed that densities of dogwhelks on the experimental verticals had declined in both treatment and control areas to <5 per m^2.

The percentage cover of bare rock, living and dead barnacles varied over the duration of the experiment in response to seasonal cycles on verticals 1, 2 and 3 (figure 7.6-7.8). Out of the three parameters measured, the percentage cover of dead barnacles remained the most constant on all of the experimental verticals. The percentage cover of living barnacles increased in both the treatment and control areas between April and June in 1993 with the settlement of new barnacles and the growth of the previous years settlement. Changes in the percentage cover of living barnacles was accompanied by a reciprocal change in the amount of bare rock on the vertical areas in both the control and treatments. At the end of the experiment the differences in the amount of bare rock and the percentage cover of barnacles between the treatment and control areas was visually apparent (plate 2A, 2B, 3A).

On verticals 1 and 2 as the living barnacle cover increased in the treatment areas, the barnacles at the top of the vertical face were observed to have grown elongated and formed dense hummocks (plate 2B, 3B). These hummocked barnacles were

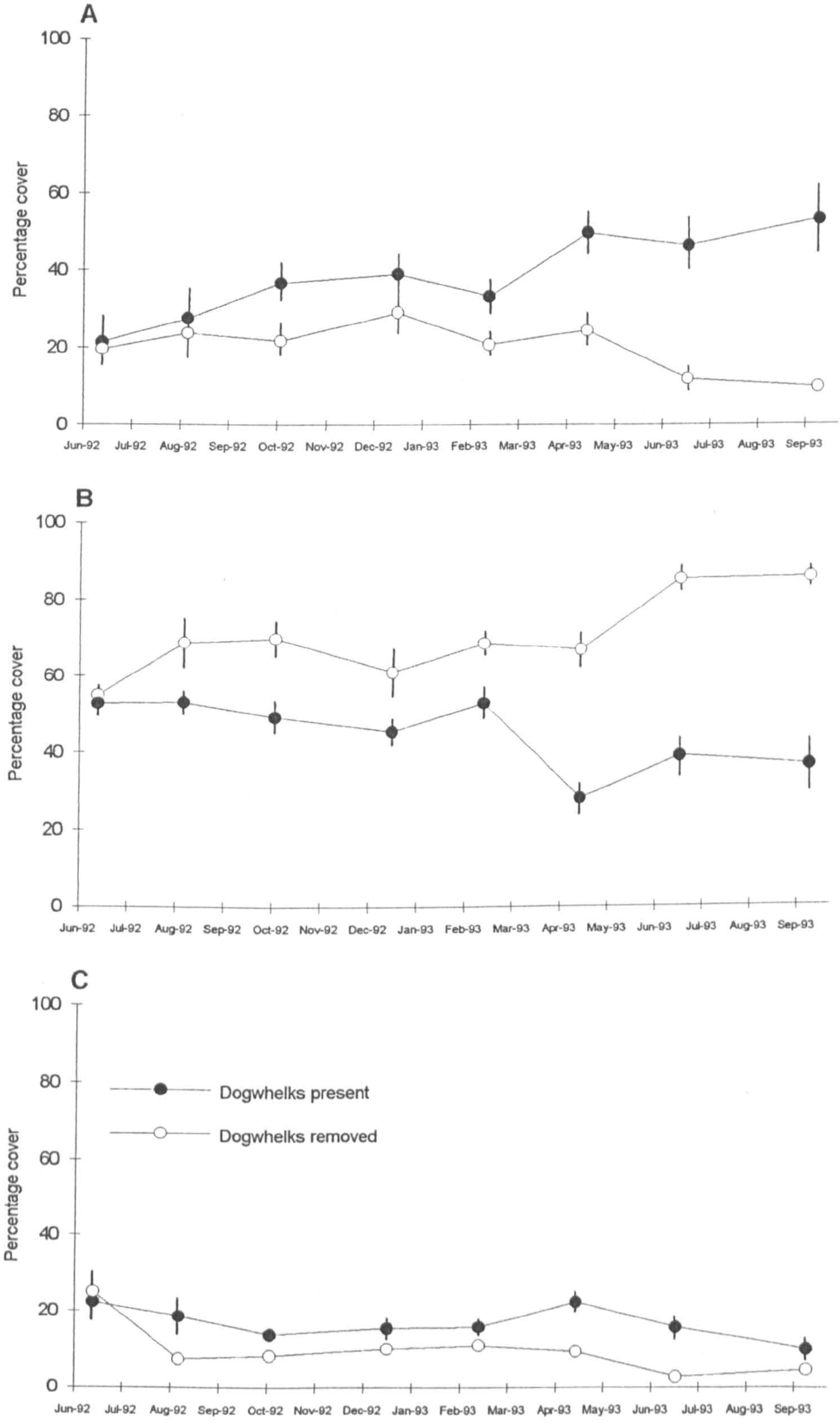


Figure 7.6 Percentage cover of bare rock (A), living (B) and dead (C) barnacles on areas on vertical 1 where dogwhelks have been either removed or remained un-manipulated. Average values ± 1 SE.

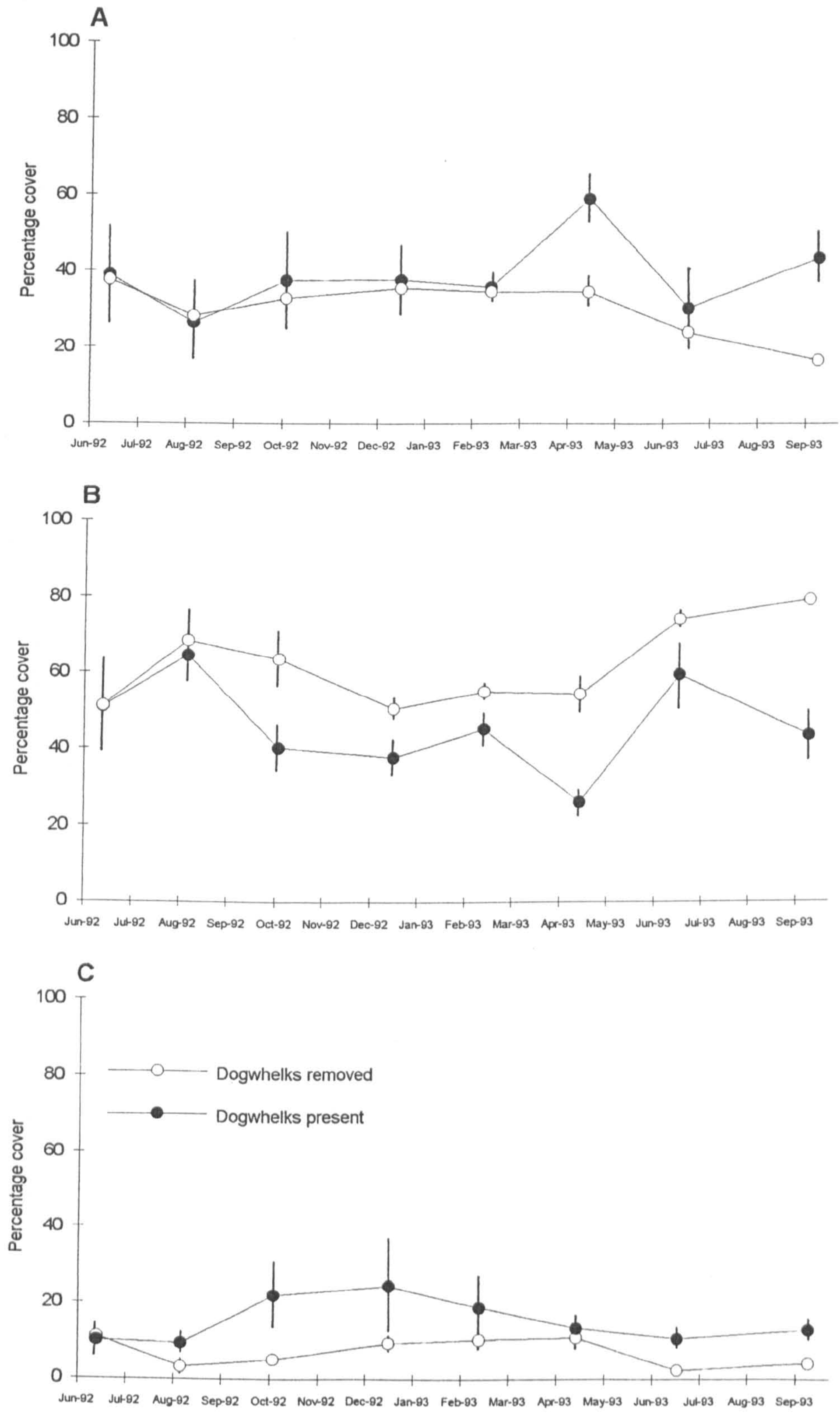


Figure 7.7 Percentage cover of bare rock (A), living (B) and dead (C) barnacles on areas on vertical 2 where dogwhelks have been either removed or remained un-manipulated. Average values ± 1 SE.

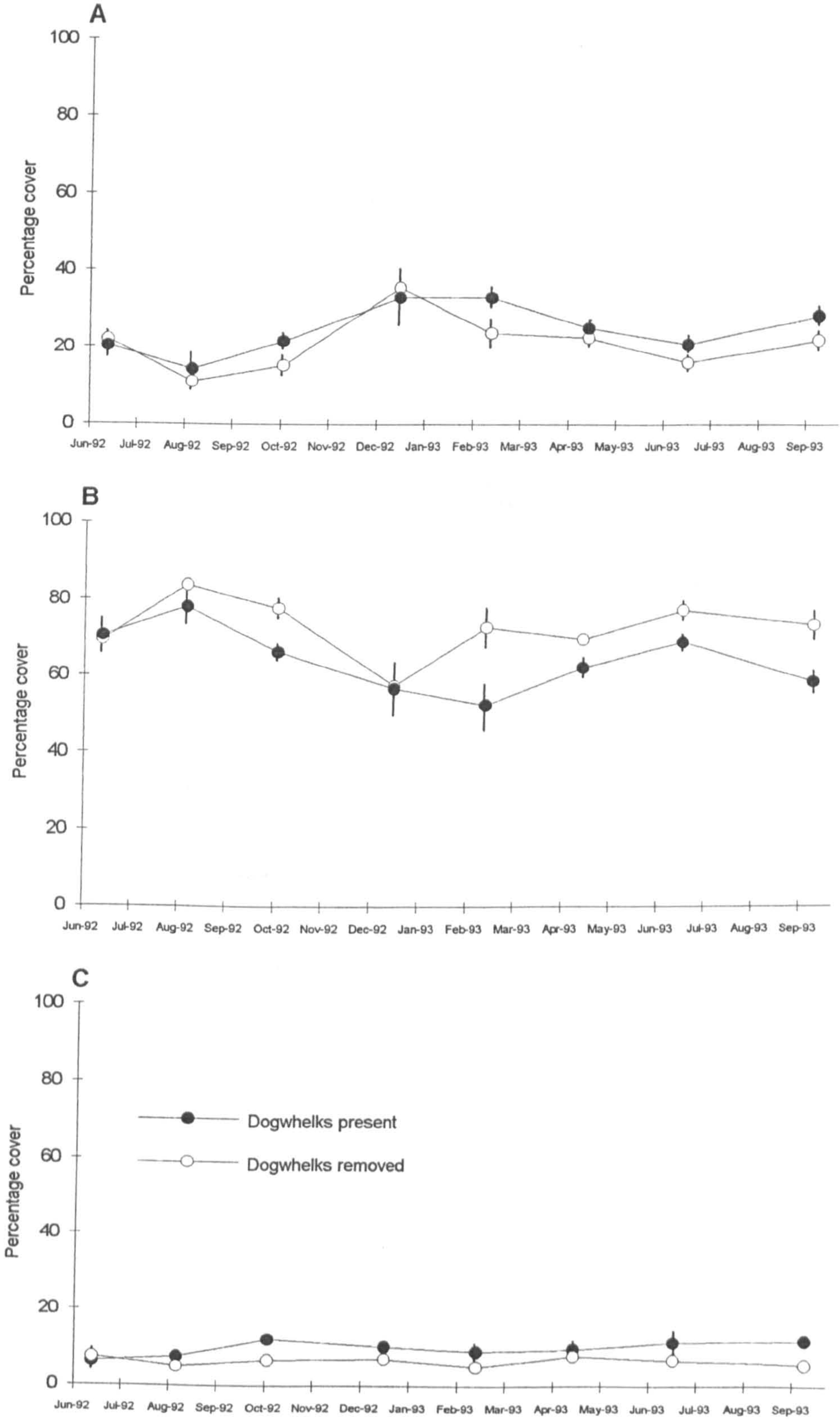


Figure 7.8 The percentage cover of bare rock (A), living (B) and dead (C) barnacles on experimental areas on vertical 3 where dogwhelks have been either removed or remained un-manipulated. Average values ± 1 SE.

Plate 2

Experimental areas on vertical 2 in September 1993 after 16 months where *Nucella lapillus* has either been left un-manipulated (A) or continuously removed (B). Note the dense barnacles in hummocks at the top of the treatment area (B).

A

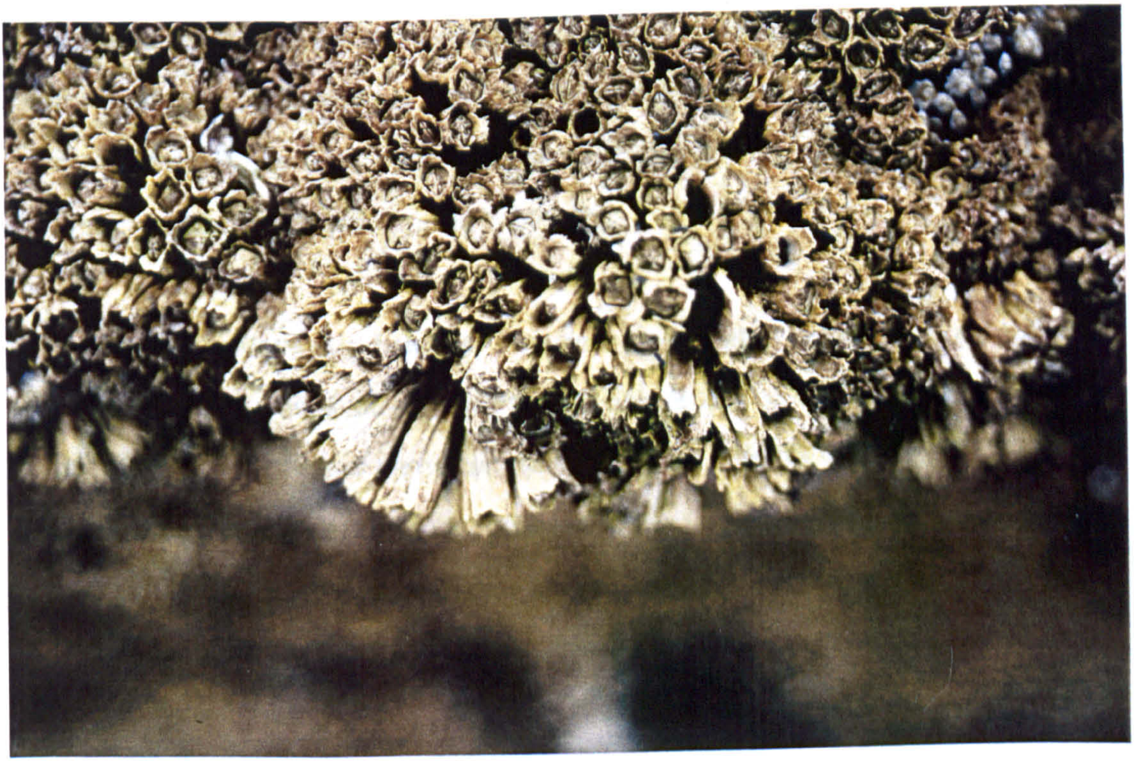
B

Plate 3

Experimental areas on vertical 1 in September 1993 showing an area where *Nucella lapillus* has been continuously removed (on the right) and an area where *Nucella lapillus* has been present (on the left) for the previous 16 months (A). The top of an experimental area on vertical 1 where in the absence of dogwhelks *Semibalanus balanoides* has grown to form dense hummocks (B).

A

B



not a feature of the rock topography and were not observed on vertical 3 nor in the control areas on vertical 1 and 2.

The shells of dead barnacles were observed to stay attached to the vertical surface of the rock for a number of months after being eaten. In the spring of 1993 during some rough weather areas of the dead barnacle matrix were removed from the shore. The rough weather at this time also removed some of the dense hummocked barnacles on verticals 1 and 2 creating small (1-4 cm^2) areas of bare rock amongst the dense barnacle matrix (plate 2B). On other occasions part of the barnacle hummocks were observed to have been removed from the top of the vertical face in the treatment areas on verticals 1 and 2. When this happened part of the barnacle shells were left attached to the rock (plate 3B) as though the barnacles had been snapped off. This feature could be a result of wrasse predation (Stubbings, 1975).

On all three of the vertical faces the percentage cover of bare rock, living and dead barnacles in the treatment and control areas were not significantly different from each other at the start of the experiment (tables 7.4-7.12). However by the end of the experiment, in September 1994, the percentage cover of bare rock, living and dead barnacles was on every vertical face significantly different between the treatment and control areas (tables 7.4-7.12).

On vertical 1 differences between treatment and control areas were statistically significant from 8 weeks after the start of the experiment onwards in the case of the percentage cover of dead barnacles (table 7.4) and after 16 weeks in the case of the percentage cover of bare rock and living barnacles (table 7.5, 7.6). On vertical 2 differences between the percentage cover of bare rock and living barnacles in the treatment and control areas were not significant until after 44 weeks (table 7.7, 7.8), although these differences became insignificant again after the barnacle settlement

Table 7.4 One-way analysis of variance at each sampling date between the percentage cover of bare rock on vertical 1 where *Nucella lapillus* has either been present or continuously removed. Data arc sine transformed.

Date	Weeks		df	MS	F	p
13 June 1992	0	Treatment	1	4.0	0.07	0.796
		Error	10	56.2		
6 August 1992	8	Treatment	1	15.0	0.14	0.715
		Error	10	107.0		
4 October 1992	16	Treatment	1	247.6	5.67	0.039 *
		Error	10	43.7		
7 December 1992	26	Treatment	1	112.8	2.04	0.184
		Error	10	55.3		
17 February 1993	35	Treatment	1	165.6	6.94	0.025 *
		Error	10	23.8		
20 April 1993	44	Treatment	1	748.7	13.65	0.004 **
		Error	10	54.8		
29 June 1993	53	Treatment	1	1356.1	24.89	<0.001 ***
		Error	10	54.5		
17 September 1993	65	Treatment	1	2310.0	21.74	<0.001 ***
		Error	10	106.0		

$p<0.05$ *, $p<0.01$ **, $p<0.001$ ***

Table 7.5 One-way analysis of variance at each sampling date between the percentage cover of living barnacles on vertical 1 where *Nucella lapillus* has either been present or continuously removed. Data arc sine transformed.

Date	Weeks		df	MS	F	p
13 June 1992	0	Treatment	1	5.9	0.51	0.493
		Error	10	11.6		
6 August 1992	8	Treatment	1	482.0	4.54	0.059
		Error	10	106.0		
4 October 1992	16	Treatment	1	702.7	10.70	0.008 **
		Error	10	65.7		
7 December 1992	26	Treatment	1	373.5	4.54	0.059
		Error	10	82.3		
17 February 1993	35	Treatment	1	389.5	9.49	0.012 *
		Error	10	41.0		
20 April 1993	44	Treatment	1	2001.3	41.05	<0.001 ***
		Error	10	48.7		
29 June 1993	53	Treatment	1	3896.4	80.79	<0.001 ***
		Error	10	48.2		
17 September 1993	65	Treatment	1	4272.2	65.89	<0.001 ***
		Error	10	64.8		

$p<0.05$ *, $p<0.01$ **, $p<0.001$ ***

Table 7.6 One-way analysis of variance at each sampling date between the percentage cover of dead barnacles on vertical 1 where *Nucella lapillus* has either been present or continuously removed. Data arc sine transformed.

Date	Weeks		df	MS	F	p
13 June 1992	0	Treatment	1	7.7	0.14	0.713
		Error	10	53.9		
6 August 1992	8	Treatment	1	127.1	5.19	0.046 *
		Error	10	24.5		
4 October 1992	16	Treatment	1	31.5	15.23	0.003 **
		Error	10	2.1		
7 December 1992	26	Treatment	1	29.6	4.57	0.058
		Error	10	6.5		
17 February 1993	35	Treatment	1	24.5	6.83	0.026 *
		Error	10	3.6		
20 April 1993	44	Treatment	1	170.7	46.39	<0.001 ***
		Error	10	3.7		
29 June 1993	53	Treatment	1	162.9	28.68	<0.001 ***
		Error	10	5.7		
17 September 1993	65	Treatment	1	28.9	5.10	0.047 *
		Error	10	5.7		

$p<0.05$ *, $p<0.01$ **, $p<0.001$ ***

Table 7.7 One-way analysis of variance at each sampling date between the percentage cover of bare rock on vertical 2 where *Nucella lapillus* has either been present or continuously removed. Data arc sine transformed.

Date	Weeks		df	MS	F	p
13 June 1992	0	Treatment	1	1.0	0.01	0.923
		Error	4	135.0		
7 August 1992	8	Treatment	1	1.7	0.02	0.896
		Error	4	88.0		
4 October 1992	16	Treatment	1	16.0	0.15	0.718
		Error	4	106.0		
9 December 1992	26	Treatment	1	3.1	0.07	0.810
		Error	4	46.7		
16 February 1993	35	Treatment	1	1.1	0.19	0.682
		Error	4	5.8		
20 April 1993	44	Treatment	1	385.4	12.10	0.025 *
		Error	4	31.8		
28 June 1993	53	Treatment	1	25.4	0.41	0.555
		Error	4	61.4		
17 September 1993	65	Treatment	1	406.6	16.85	0.015 *
		Error	4	24.1		

$p<0.05$ *, $p<0.01$ **, $p<0.001$ ***

Table 7.8 One-way analysis of variance at each sampling date between the percentage cover of living barnacles on vertical 2 where *Nucella lapillus* has either been present or continuously removed. Data arc sine transformed.

Date	Weeks		df	MS	F	p
13 June 1992	0	Treatment	1	0.0	0.00	0.972
		Error	4	131.0		
7 August 1992	8	Treatment	1	13.9	0.16	0.712
		Error	4	88.6		
4 October 1992	16	Treatment	1	380.3	6.35	0.065
		Error	4	59.9		
9 December 1992	26	Treatment	1	97.8	6.49	0.063
		Error	4	15.1		
16 February 1993	35	Treatment	1	62.4	4.39	0.104
		Error	4	14.2		
20 April 1993	44	Treatment	1	477.8	27.82	0.006 **
		Error	4	17.2		
28 June 1993	53	Treatment	1	183.9	3.31	0.143
		Error	4	55.6		
17 September 1993	65	Treatment	1	1063.0	46.41	0.002 **
		Error	4	22.9		

$p<0.05$ *, $p<0.01$ **, $p<0.001$ ***

Table 7.9 One-way analysis of variance at each sampling date between the percentage cover of dead barnacles on vertical 2 where *Nucella lapillus* has either been present or continuously removed. Data arc sine transformed.

Date	Weeks		df	MS	F	p
13 June 1992	0	Treatment	1	0.6	0.06	0.813
		Error	4	9.6		
7 August 1992	8	Treatment	1	18.1	7.23	0.055
		Error	4	2.5		
4 October 1992	16	Treatment	1	146.6	4.13	0.112
		Error	4	35.5		
9 December 1992	26	Treatment	1	122.7	1.50	0.288
		Error	4	81.7		
16 February 1993	35	Treatment	1	37.3	1.25	0.327
		Error	4	29.9		
20 April 1993	44	Treatment	1	3.3	0.52	0.512
		Error	4	6.4		
28 June 1993	53	Treatment	1	34.2	13.82	0.021 *
		Error	4	2.4		
17 September 1993	65	Treatment	1	37.3	27.82	0.006 **
		Error	4	1.3		

$p<0.05$ *, $p<0.01$ **, $p<0.001$ ***

in June. Differences between the percentage cover of dead barnacles on vertical 2 were significant after 53 weeks (table 7.9). Differences on vertical 3 were less marked. Only on the last sampling occasion was a significant difference recorded between control and treatment areas in all three variables (table 7.10, 7.11, 7.12). Before then occasional differences were recorded in living and dead barnacles (table 7.11, 7.12). It is worth noting, however, that the average cover of living barnacles was consistently higher where dogwhelks were absent on all occasions after the first. Furthermore the average percentage cover of dead barnacles was consistently lower in the dogwhelk removal area. Bare rock was also lower where dogwhelks were present on all but one sampling occasion. These observations on vertical 3 suggest a slight but real and consistent difference between control and treatment areas.

No ephemeral green algae were observed on any of the treatment areas on any of the three verticals during the experiment. Towards the end of the experiment *Fucus vesiculosus* was recorded, firstly in 1 of the treatment areas on vertical 1 and later in treatment areas on verticals 2 and 3 (figure 7.9). No *Fucus vesiculosus* was observed on any of the control areas (figure 7.9). The percentage of *Fucus* cover on the three verticals was not tested because of the large numbers of zero results.

In March 1994 observations made on verticals 1 and 2 showed that the control areas remained relatively bare with a low percentage cover of living barnacles. The treatment areas by contrast still had very little bare rock and were covered by a dense matrix of barnacles but instead of these being alive around 90% were now dead. In July 1994 percentage cover of barnacles on the control areas had increased slightly, after the settlement of new individuals in May and June, although the areas still looked very bare. The treatment areas showed a marked contrast to previous observations and were dominated by bare rock with few barnacles after

Table 7.10 One-way analysis of variance at each sampling date between the percentage cover of bare rock on vertical 3 where *Nucella lapillus* has either been present or continuously removed. Data arc sine transformed.

Date	Weeks		df	MS	F	p
12 June 1992	0	Treatment	1	1.4	0.36	0.580
		Error	4	3.9		
7 August 1992	8	Treatment	1	5.3	0.79	0.425
		Error	4	6.8		
5 October 1992	16	Treatment	1	19.7	4.99	0.089
		Error	4	3.9		
19 December 1992	27	Treatment	1	3.1	0.09	0.780
		Error	4	34.4		
16 February 1993	35	Treatment	1	46.1	4.99	0.089
		Error	4	9.2		
20 April 1993	44	Treatment	1	3.7	1.45	0.295
		Error	4	2.6		
24 June 1993	53	Treatment	1	11.8	4.09	0.113
		Error	4	2.9		
17 September 1993	65	Treatment	1	21.8	9.26	0.038 *
		Error	4	2.4		

$p<0.05$ *, $p<0.01$ **, $p<0.001$ ***

Table 7.11 One-way analysis of variance at each sampling date between the percentage cover of living barnacles on vertical 3 where *Nucella lapillus* has either been present or continuously removed. Data arc sine transformed.

Date	Weeks		df	MS	F	p
12 June 1992	0	Treatment	1	1.7	0.10	0.769
		Error	4	17.3		
7 August 1992	8	Treatment	1	42.4	2.20	0.212
		Error	4	19.3		
5 October 1992	16	Treatment	1	140.9	17.31	0.014 *
		Error	4	8.1		
19 December 1992	27	Treatment	1	0.1	0.00	0.959
		Error	4	40.3		
16 February 1993	35	Treatment	1	353.5	7.19	0.055
		Error	4	49.1		
20 April 1993	44	Treatment	1	48.1	9.83	0.035 *
		Error	4	4.9		
24 June 1993	53	Treatment	1	73.3	10.55	0.031 *
		Error	4	6.9		
17 September 1993	65	Treatment	1	199.5	12.01	0.026 *
		Error	4	16.6		

$p<0.05$ *, $p<0.01$ **, $p<0.001$ ***

Table 7.12 One-way analysis of variance at each sampling date between the percentage cover of dead barnacles on vertical 3 where *Nucella lapillus* has either been present or continuously removed. Data arc sine transformed.

Date	Weeks		df	MS	F	p
12 June 1992	0	Treatment	1	0.6	0.65	0.466
		Error	4	0.9		
7 August 1992	8	Treatment	1	3.5	16.87	0.015 *
		Error	4	0.2		
5 October 1992	16	Treatment	1	14.3	588.13	<0.001 ***
		Error	4	0.0		
19 December 1992	27	Treatment	1	4.9	11.99	0.026 *
		Error	4	0.4		
16 February 1993	35	Treatment	1	7.7	7.43	0.053
		Error	4	1.0		
20 April 1993	44	Treatment	1	1.4	0.75	0.435
		Error	4	1.9		
24 June 1993	53	Treatment	1	10.8	3.82	0.122
		Error	4	2.8		
17 September 1993	65	Treatment	1	18.9	19.69	0.011 *
		Error	4	1.0		

$p<0.05$ *, $p<0.01$ **, $p<0.001$ ***

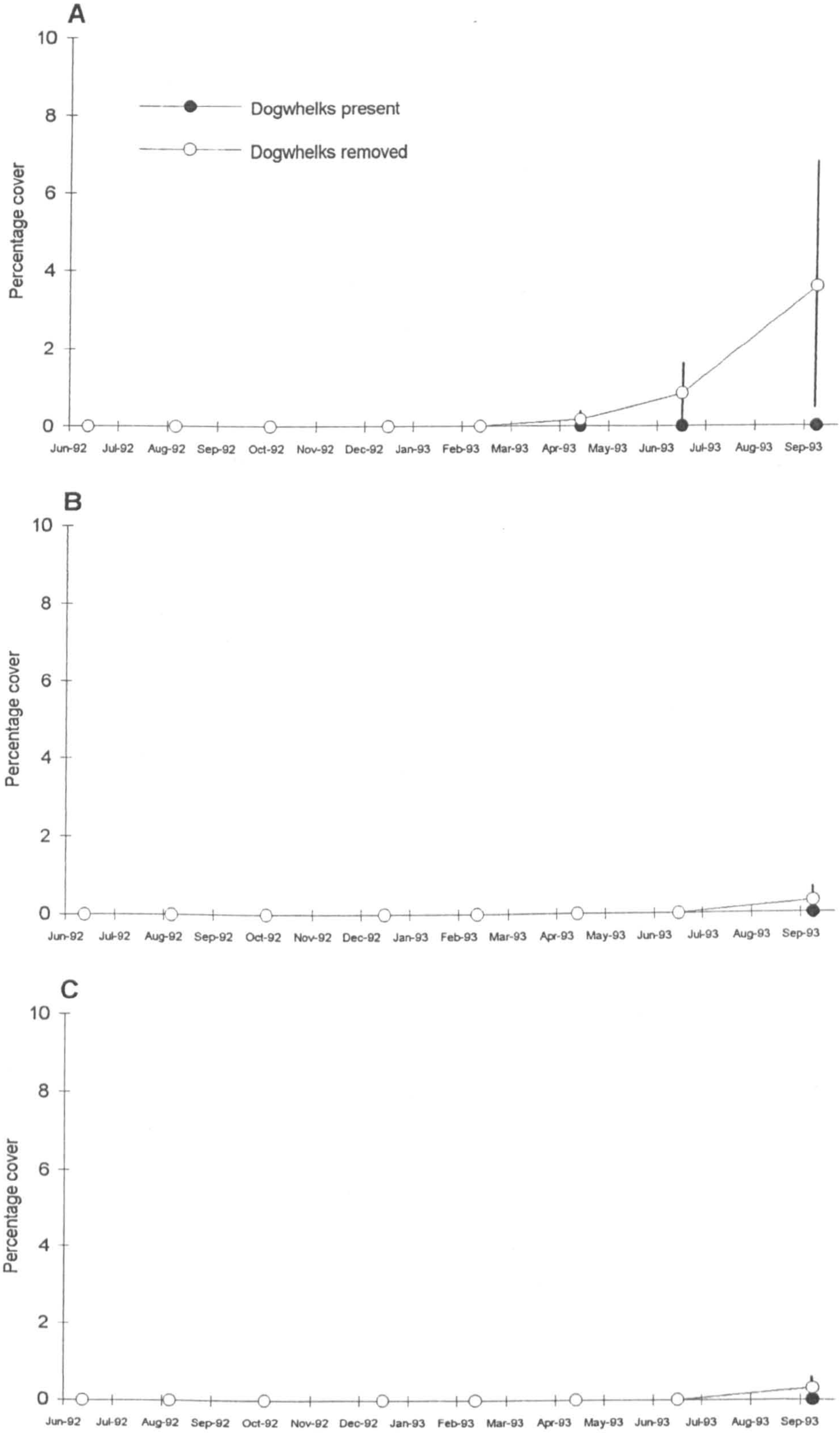


Figure 7.9 Percentage cover of *Fucus vesiculosus* on vertical 1 (A), vertical 2 (B) and vertical 3 (C) in areas where dogwhelks have either been removed or remained un-manipulated. Average values ± 1 SE.

the matrix of dead barnacles had prevented high settlement on the areas in May and June. The matrix of dead barnacle shells had been removed from the areas by July.

7.3.2.3 Dogwhelk and limpet removal in a factorial experiment

Counts of limpets and dogwhelks on control and treatment areas on vertical 4 were not significantly different from each other at the start of the experiment (limpets - one-way ANOVA, $F=0.16$, $df=3, 15$, $p=0.921$; dogwhelks - one-way ANOVA, $F=0.13$, $df=3, 15$, $p=0.940$). The density of *Nucella lapillus* in the experimental areas varied on a daily basis (figure 7.10) but more were recorded in the control and treatment areas, where they were un-manipulated, than in the areas where they were continually removed. Similar numbers were removed from the two dogwhelk removal treatments (with and without limpets) and similar numbers were present in the control and dogwhelks present treatment. Limpet abundance remained relatively unchanged in the areas where limpets were present with a density of between 40-50 per m^2 (figure 7.10). Very few limpets moved into the areas where they had been previously been cleared from even though areas were only checked once every 1-2 weeks. Those that did move in and were removed were usually juveniles, less than 15 mm in shell length.

The percentage of bare rock was not significantly different between the control and any of the treatment areas at the start of the experiment in August 1992 (table 7.13). In the control and all treatments there was initially an increase in the amount of bare rock between August and February and then a decrease reaching a minimum in June 1993 (figure 7.11). After February the amount of bare rock in the areas where no dogwhelks and no limpets were present continued to decrease. In contrast in the other areas the amount of bare rock in the areas increased.

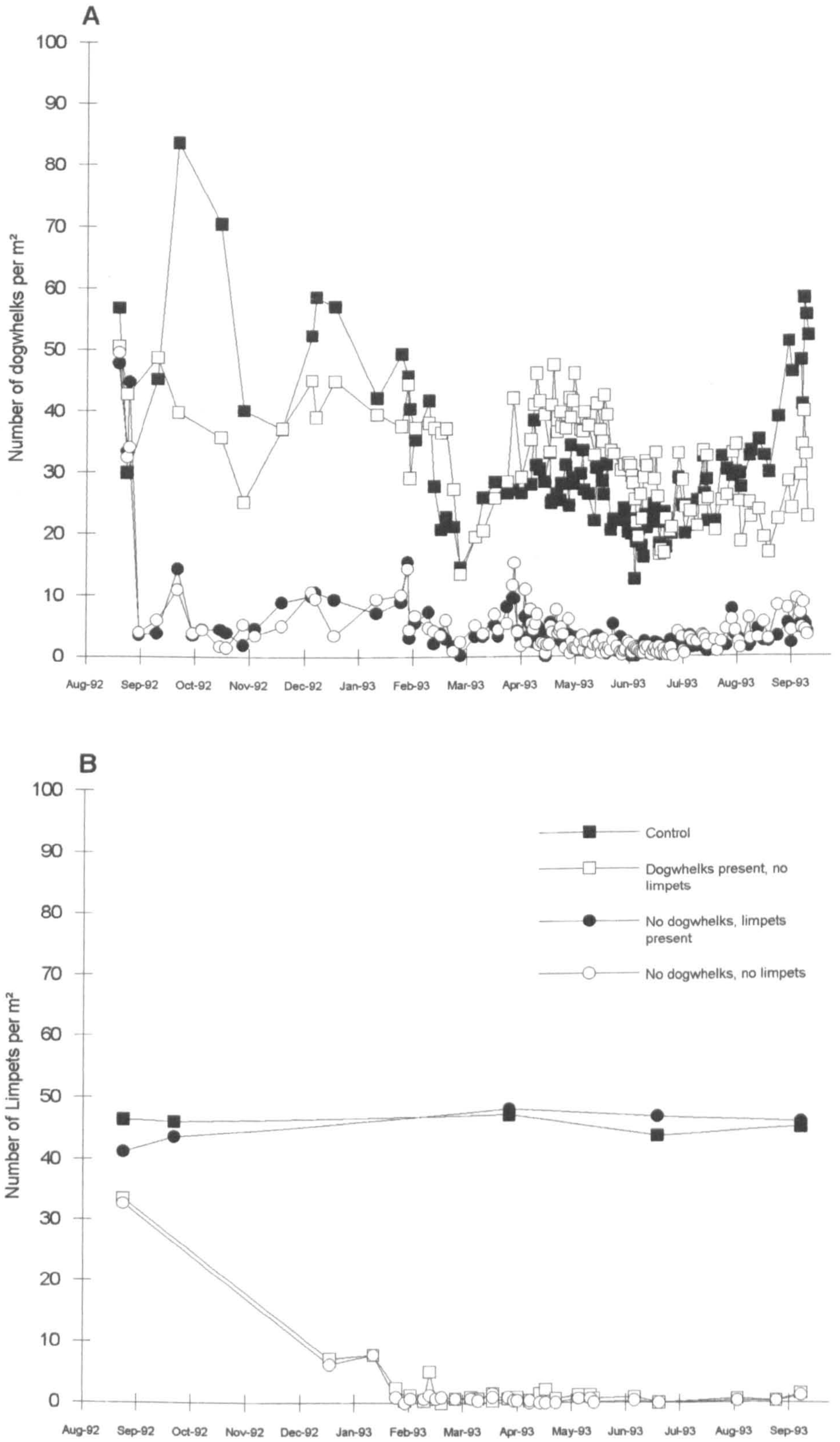


Figure 7.10 Average numbers of *Nucella lapillus* (A) and *Patella vulgata* (B) removed or present in the experimental areas on vertical 4. Standard errors not shown to aid clarity.

Table 7.13 Two-way analysis of variance at each sampling date on the percentage cover of bare rock on vertical 4 in a factorial experiment where *Nucella lapillus* and *Patella vulgata* have either been present or have been continuously removed. Data arc sine transformed.

Date	Weeks		df	SS	MS	F	p
24 August 1992	0	Dogwhelk	1	2.6	2.6	0.58	0.461 NS
		Limpet	1	2.7	2.7	0.60	0.454 NS
		Dogwhelk x limpet	1	0.1	0.1	0.00	0.949 NS
		Error	12	53.6	4.5		
		Total	15	58.9			
1 February 1993	23	Dogwhelk	1	485.3	485.3	7.07	0.021 *
		Limpet	1	0.1	0.1	0.00	0.966 NS
		Dogwhelk x limpet	1	0.1	0.1	0.00	0.973 NS
		Error	12	824.2	68.7		
		Total	15	1309.7			
14 April 1993	33	Dogwhelk	1	246.6	246.6	24.92	<0.001 ***
		Limpet	1	23.2	23.2	2.34	0.152 NS
		Dogwhelk x limpet	1	9.6	9.6	0.98	0.343 NS
		Error	12	118.7	9.9		
		Total	15	398.2			
28 June 1993	44	Dogwhelk	1	55.6	55.6	4.66	0.052 NS
		Limpet	1	152.1	152.0	12.75	0.004 **
		Dogwhelk x limpet	1	0.0	0.0	0.00	0.965 NS
		Error	12	143.1	11.9		
		Total	15	350.8			
16 September 1993	55	Dogwhelk	1	1482.2	1482.2	41.49	<0.001 ***
		Limpet	1	112.1	112.1	3.14	0.102 NS
		Dogwhelk x limpet	1	299.6	299.6	8.39	0.013 *
		Error	12	428.7	35.7		
		Total	15	2322.6			

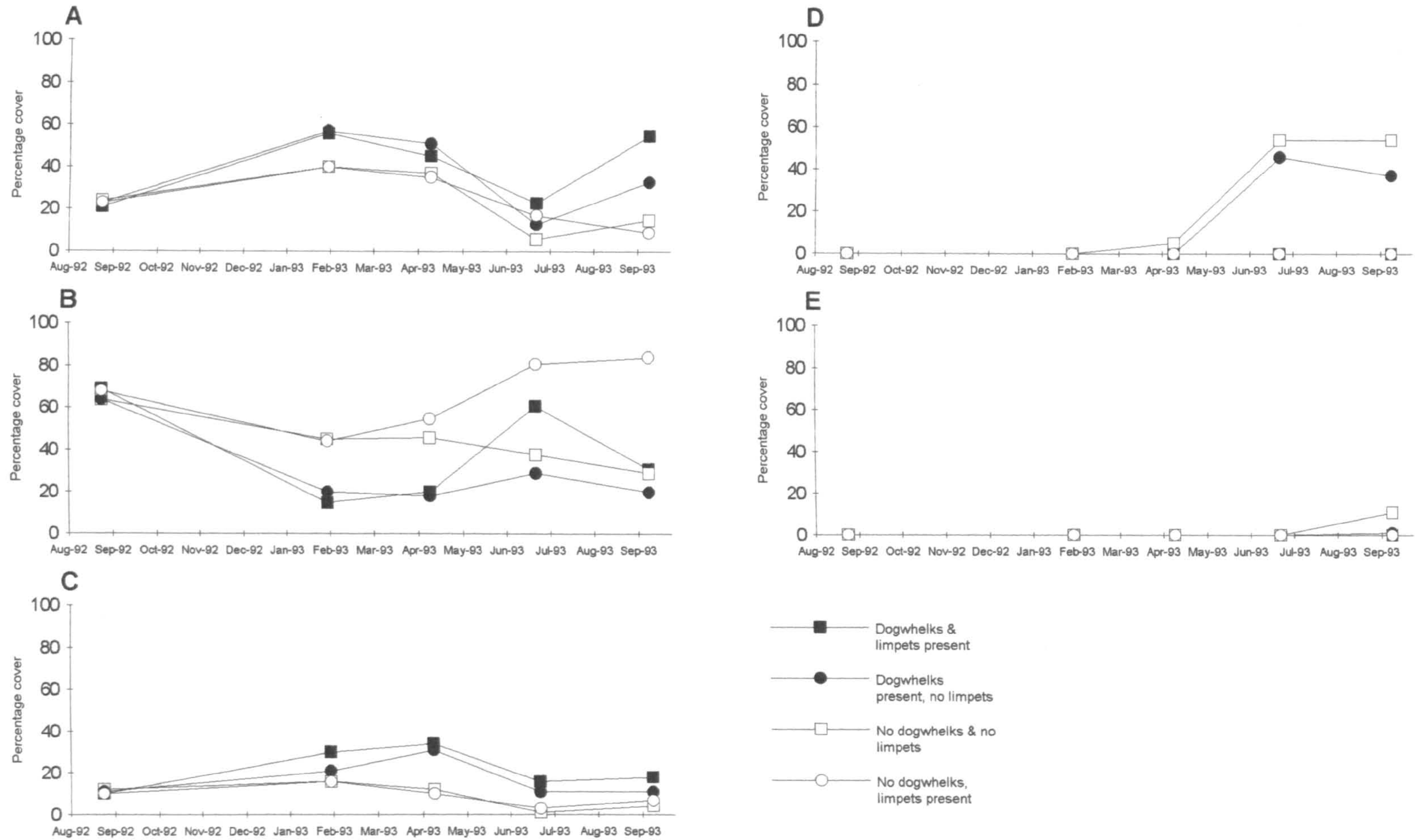


Figure 7.11 Percentage cover of bare rock (A), living (B) and dead (C) barnacles, ephemeral greens (D) and *Fucus* canopy (E) on vertical 4. Standard errors not shown for clarity.

Dogwhelks had a significant effect on the amount of bare rock on the experimental areas. Areas where dogwhelks were present had a significantly higher cover of bare rock than areas where *Nucella* was absent when the experiment was sampled after 23, 33 and 55 weeks (table 7.13). There was no significant effect on the amount of bare rock with the presence or absence of *Nucella* at 44 weeks after the barnacle settlement season although there was more bare rock in areas where *Patella* was present (table 7.13, figure 7.11). At the end of the experiment, in addition to there being a significant effect between the presence or absence of *Nucella*, there was a significant interaction between dogwhelks and limpets (table 7.13). Here there was significantly higher cover of bare rock in the control areas (dogwhelks and limpets present) than in any of the treatment areas (Tukey tests, $p<0.05$) and the treatment with dogwhelks present and limpets removed had more bare rock than those areas where there had been no dogwhelks but limpets had been present (Tukey tests, $p<0.05$).

The percentage cover of living barnacles was the same in the control and all the treatments at the start of the experiment (table 7.14). A mirror image of the changes observed in the percentage of bare rock was observed in the first 6 months of the experiment, as the percentage cover of living barnacles decreased between August 1992 and February 1993. In February and in April there was a significant dogwhelk effect where there was a higher percentage cover of living barnacles where dogwhelks had been removed. After the new barnacle settlement in April, there was no significant dogwhelk effect but barnacle cover was highest on the experimental areas where dogwhelks had been removed but limpets were still present creating a significant limpet effect (figure 7.11, table 7.14). At the end of the experiment the percentage cover of barnacles was significantly affected by both dogwhelks and limpets and there was a significant interaction effect between them (table 7.14). This resulted in a higher percentage cover of living barnacles in the treatment in

Table 7.14 Two-way analysis of variance at each sampling date on the percentage cover of living barnacles on vertical 4 in a factorial experiment where *Nucella lapillus* and *Patella vulgata* have either been present or have been continuously removed. Data arc sine transformed.

Date	Weeks		df	SS	MS	F	p
24 August 1992	0	Dogwhelk	1	1.8	1.8	0.14	0.712 NS
		Limpet	1	38.9	38.9	3.07	0.105 NS
		Dogwhelk x limpet	1	0.3	0.3	0.02	0.880 NS
		Error	12	152.3	12.7		
		Total	15	193.4			
1 February 1993	23	Dogwhelk	1	1044.8	1044.8	39.13	<0.001 ***
		Limpet	1	15.0	15.0	0.56	0.468 NS
		Dogwhelk x limpet	1	8.3	8.3	0.31	0.587 NS
		Error	12	320.4	26.7		
		Total	15	1388.6			
14 April 1993	33	Dogwhelk	1	1478.1	1478.1	57.05	<0.001 ***
		Limpet	1	51.0	51.0	1.97	0.186 NS
		Dogwhelk x limpet	1	19.3	19.3	0.75	0.405 NS
		Error	12	310.9	25.9		
		Total	15	1859.4			
28 June 1993	44	Dogwhelk	1	495.9	495.9	4.53	0.055 NS
		Limpet	1	2625.8	2625.8	23.97	<0.001 ***
		Dogwhelk x limpet	1	97.9	97.9	0.89	0.363 NS
		Error	12	1314.4	109.5		
		Total	15	4534.0			
16 September 1993	55	Dogwhelk	1	2031.6	2031.6	66.25	<0.001 ***
		Limpet	1	2213.0	2213.0	72.17	<0.001 ***
		Dogwhelk x limpet	1	1188.3	1188.3	38.75	<0.001 ***
		Error	12	368.0	30.7		
		Total	15	5800.8			

which dogwhelks had been removed but limpets were present compared to the control and other treatment areas (Tukey tests, $p<0.05$).

The percentage cover of dead barnacles was highest in the control areas when sampled on all occasions after August 1992 (figure 7.11). This difference was significantly higher than in areas where dogwhelks had been removed on all sampling occasions after 14 April (table 7.15). On the last sampling limpets had a significant effect on the percentage cover of dead barnacles (table 7.15).

At the start of the experiment there was no ephemeral green algae on any of the areas of vertical rock. After April ephemeral green algae were recorded on experimental areas where limpets had been removed but not on areas where they were present (figure 7.11). Although there was a significant effect of limpet removal on the percentage cover of ephemeral algae this was not the case for dogwhelks (table 7.16). *Enteromorpha* was first noticed, in February 1993, in the areas where both dogwhelks and limpets had been removed. It was observed growing on the barnacles in these areas and not on the bare rock. A dense covering smothered the barnacles in May and June 1993 and this prevented the settlement of new barnacles into the experimental areas. By the beginning of April green algae was also present on the experimental areas where dogwhelks were present but limpets had been removed. Later *Fucus vesiculosus* was recorded on the limpet removal areas, with a greater percentage cover on the areas where dogwhelks had also been removed (plate 4A, 4B), although this was not significant (table 7.16).

Table 7.15 Two-way analysis of variance at each sampling date on the percentage cover of dead barnacles on vertical 4 in a factorial experiment where *Nucella lapillus* and *Patella vulgata* have either been present or have been continuously removed. Data arc sine transformed.

Date	Weeks		df	SS	MS	F	p
24 August 1992	0	Dogwhelk	1	0.2	0.2	0.06	0.807 NS
		Limpet	1	3.5	3.5	1.19	0.297 NS
		Dogwhelk x limpet	1	0.2	0.2	0.06	0.808 NS
		Error	12	35.5	2.9		
		Total	15	39.4			
1 February 1993	23	Dogwhelk	1	133.2	133.2	2.96	0.111 NS
		Limpet	1	36.1	36.1	0.80	0.388 NS
		Dogwhelk x limpet	1	32.7	32.7	0.73	0.410 NS
		Error	12	539.6	44.9		
		Total	15	741.7			
14 April 1993	33	Dogwhelk	1	641.8	641.8	21.13	<0.001 ***
		Limpet	1	0.9	0.9	0.03	0.861 NS
		Dogwhelk x limpet	1	13.6	13.6	0.45	0.516 NS
		Error	12	364.5	30.4		
		Total	15	1020.8			
28 June 1993	44	Dogwhelk	1	182.7	182.7	12.68	0.004 **
		Limpet	1	11.3	11.3	0.79	0.393 NS
		Dogwhelk x limpet	1	3.1	3.1	0.22	0.651 NS
		Error	12	172.8	14.4		
		Total	15	370.0			
16 September 1993	55	Dogwhelk	1	104.9	104.9	20.08	<0.001 ***
		Limpet	1	31.8	31.8	6.09	0.030 *
		Dogwhelk x limpet	1	6.1	6.1	1.18	0.299 NS
		Error	12	62.7	5.2		
		Total	15	205.6			

Table 7.16 Two-way analysis of variance at each sampling date on the percentage cover of algae on vertical 4 in a factorial experiment where *Nucella lapillus* and *Patella vulgata* have either been present or have been continuously removed. Data arc sine transformed.

Date	Weeks		df	SS	MS	F	p
(a) Ephemeral algae cover							
14 April 1993	33	Dogwhelk	1	8.2	8.2	2.78	0.122 NS
		Limpet	1	8.2	8.2	2.78	0.122 NS
		Dogwhelk x limpet	1	8.2	8.2	2.78	0.122 NS
		Error	12	35.6	2.9		
		Total	15	60.3			
28 June 1993	44	Dogwhelk	1	38.6	38.6	0.22	0.647 NS
		Limpet	1	4028.7	4028.7	23.00	<0.001 ***
		Dogwhelk x limpet	1	38.6	38.6	0.22	0.647 NS
		Error	12	2101.9	175.2		
		Total	15	6207.8			
16 September 1993	55	Dogwhelk	1	127.7	127.7	3.19	0.099 NS
		Limpet	1	2951.8	2951.8	72.78	<0.001 ***
		Dogwhelk x limpet	1	127.7	127.7	3.19	0.099 NS
		Error	12	480.1	40.0		
		Total	15	3687.3			
(b) *Fucus vesiculosus* cover							
16 September 1993	55	Dogwhelk	1	30.4	30.4	1.90	0.193 NS
		Limpet	1	44.3	44.3	2.77	0.122 NS
		Dogwhelk x limpet	1	27.3	27.3	1.71	0.216 NS
		Error	12	192.0	16.0		
		Total	15	294.1			

Plate 4

Experimental areas on vertical 4 in September 1993 after 13 months where *Nucella lapillus* was present and *Patella vulgata* removed (A) or where both *Nucella lapillus* and *Patella vulgata* have been continuously removed (B).

A

B

7.4 The effects of *Nucella lapillus* on *Fucus vesiculosus* clumps

7.4.1 Materials and methods

7.4.1.1 Experimental aims and design

Nucella lapillus would be expected to affect the size and longevity of *Fucus vesiculosus* clumps. This is because individuals have been observed to use the clumps as a refuge from which to forage (Connell, 1961a) and they prey upon the barnacles to which the clump is anchored. Consequently the direct effects of *Nucella lapillus* on the size and quality of *Fucus* clumps was examined. Indirect consequences of any effect of *Nucella* on other organisms using the clump for shelter or for food were also investigated. The approach adopted was that of reducing the densities of *Nucella* within treatment *Fucus* clumps and comparing the growth and survival of these clumps with controls where *Nucella* densities were not manipulated.

The experiment was designed to use newly established *Fucus* clumps which were selected to be of similar size and plant density. These were randomly assigned to be either control or treatment clumps. The control *Fucus* clumps contained dogwhelks at un-manipulated densities within the *Fucus* clump. *Nucella lapillus* was regularly removed from the treatment *Fucus* clumps providing a reduced dogwhelk density.

7.4.1.2 Measurement of physical parameters

Towards the end of the experiment, in August 1993, when a number of different sized and density *Fucus vesiculosus* clumps were present, the temperature and humidity were measured both inside and outside *Fucus* clumps over the period of a low tide. Measurements were taken using a Jenway temperature and relative humidity meter (model 5075) by placing the probe in the centre of the *Fucus* clump or on the rock close by. The survey was done on 15 August 1993 when low tide occurred at 16:41 (BST, Liverpool). Air temperatures on this day reached a maximum of 17.3°C and a minimum of 11.0°C. There was a gentle WNW breeze (force 3) and a total of 10.3 sunshine hours (above the monthly average). The *Fucus* clumps were first uncovered by the tide at 13:45 and covered again at 18:15. Readings were taken in 10 clumps of similar size (0.19 m^2 ± 0.09) and plant density (2.85 plants per 25 cm^2 ± 1.00) and at three different locations on the rocks close by. These readings were taken every 30 minutes throughout the period of low tide. In addition temperature and humidity readings were taken once an hour in all of the 24 clumps, at 14:30, 15:30, 16:30 and 17:30. This was done in order to assess any differences in the temperature or relative humidity between *Fucus vesiculosus* clumps of different sizes or densities.

7.4.1.3 Experimental methods

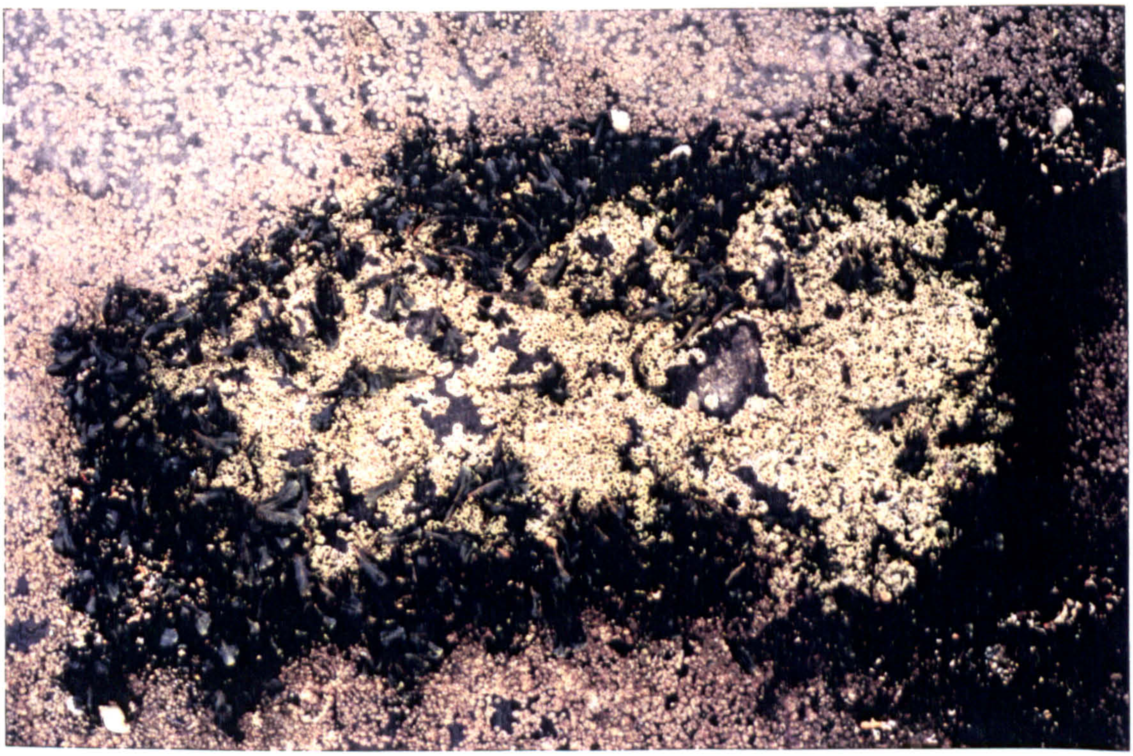
The experiment was set up at site A on Port St. Mary Ledges (figure 2.3). Here *Nucella* was abundant sheltering under clumps of *Fucus vesiculosus* which had established in previous years. The experiment was designed to run over the spring and summer when the dogwhelks would be most active. *Fucus vesiculosus* escapes which had formed in the previous autumn (1992) were used (plate 5A). These escapes were distributed over a ledge which covered an area of approximately 450 m^2. Within this area thirty of the new *Fucus* escapes were selected in November 1992 and each marked with a numbered tag in the rock 15-20 cm away from the clump (see chapter 3). The distribution of the clumps, and the gentle slope of the ledge meant that there was a maximum of 25 minutes between

<u>Plate 5</u>

A new *Fucus vesiculosus* clump with *Nucella lapillus* feeding on *Semibalanus balanoides* close to the clump edge (A). A casualty of the winter storms, where the centre of a *Fucus vesiculosus* clump has been completely removed revealing a scoured patch of dead *Semibalanus balanoides* (B).

A

B



the first and last clump being exposed by the ebbing tide. The area of rock which the *Fucus* clump covered (henceforth referred to as clump size) was calculated by placing a 0.5 m x 0.5 m quadrat over the *Fucus vesiculosus* and counting the total number of 10 x 10 squares occupied (chapter 3).

Soon after, following a period of force 4-5 onshore winds, some of these clumps were recorded as having accumulated a large amount of shell debris amongst the dense plants. Later, during late December and early January, a series of winter storms dramatically thinned all of the clumps. In some cases the centre of the clump was completely removed leaving only a thin circle of plants around what was the edge of the clump. In the centre of the clumps the barnacle matrix remained but it was a brilliant white colour, with most of the barnacles being dead (plate 5B). As a result of this the clump size measurements were repeated in January. This time the clump size was measured using plastic sheeting. A sheet of polythene was laid over the *Fucus vesiculosus* clump and the area the plants covered was traced onto it using a permanent marker pen, this procedure was repeated for each clump. On return to the laboratory the tracing from each clump was laid over a grid of 5 x 5 cm squares and its area calculated to the nearest 25 cm^2. The area of each tracing was calculated blind twice and the average of the two measurements taken. This method appeared to give consistent results and generally the areas of the tracings measured twice agreed within 1-2 squares (25-50 cm^2).

Plant length and density were measured within each of the *Fucus* clumps using ten randomly placed 5 x 5 cm quadrats. The length of all the plants within the quadrat were measured from the base of the stipe to the tip of the frond until 10 plants had been measured in each clump. Plant density was measured using the same 5 x 5 cm quadrat and counting the number of plants which came within it. Ten sub-samples were taken within each *Fucus* clump (table 7.1).

The numbers of dogwhelks under each *Fucus* clump were also recorded. The size of each dogwhelk was estimated and placed in one of three size categories based on shell length: <10 mm, 10-20 mm, >20 mm. The dogwhelk shell lengths were estimated, rather than measured exactly which would have involved removing them from the substrate, in order to reduce the disturbance created. In addition the number of juvenile (<15 mm) and adult (>15 mm) limpets sheltering beneath the clump was recorded. This size differentiation was used because 15 mm is approximately the size at which *Patella vulgata* takes up a home scar; until that point they are still relatively mobile (Jones, 1948; Hawkins, 1979). Other species observed under the *Fucus vesiculosus* plants were also recorded.

Six of the thirty *Fucus* clumps originally marked in November 1992 had been more or less destroyed by the December storms, consequently the remaining 24 were randomly assigned to be either control or treatment clumps, creating 12 replicates of each. Dogwhelks were first removed from the clumps, to which that treatment had been assigned, on 26 January 1993. A pair of plastic forceps was used to systematically search through the *Fucus* plants to remove all the dogwhelks present whilst creating a minimum disturbance. Dogwhelks in a 10 cm boundary zone around the clump were also removed. The same systematic search procedure was used to record the number of dogwhelks present in the control clumps during the same or next low tide. Thus the control clumps were subjected to a similar handling regime. These dogwhelks were recorded as numbers seen in each of the three size classes (<10 mm, 10-20 mm, >20 mm).

Thereafter the 12 treatment clumps were checked approximately weekly. Any dogwhelks found on these return visits were removed and recorded using three size categories. This interval between visits was chosen in order to balance the need for regular dogwhelk removal, but also create the minimum disturbance to the *Fucus* clumps.

Approximately once every 4-6 weeks measurements were taken of the plant density, plant lengths and the size of the *Fucus* clump, which was measured using the plastic sheeting. At the same time the numbers of limpets, anemones and other organisms were also recorded (table 7.1).

The experiment was terminated on 14 September 1993, 8 months after the dogwhelks had first been removed. At the end of the experiment, after the final plant length, plant density and clump size measurements had been taken, the plants from each of the *Fucus* clumps were removed using a pair of scissors. The stipe of each plant was cut as close to the point of attachment of the plant to the substrate as possible. As these plants were being removed the substrate to which each plant was attached was tallied as either rock, barnacle or limpet. Once all the plants had been removed the cover of barnacles was measured beneath each of the clumps.

The plants from each of the clumps were put separately into bags and weight of each clump measured. Many of the plants were observed to be covered in epiphytes, mainly *Enteromorpha*. Since there appeared to be a difference in the epiphyte cover between different clumps the percentage of plants within each clump covered in epiphytes was subjectively estimated. Each clump was recorded as having either 0-25%, 26-50%, 51-75% or 76-100% of its total *Fucus* plants covered in epiphytes. Finally, 16 of the 24 clumps (8 control, 8 treatment) were randomly selected and the lengths of all the plants within the clump measured in order to construct frequency histograms.

For spatial comparison with the experimental *Fucus* clumps used additional measurements of *Fucus vesiculosus* clump size and density and the abundance of *Nucella* were taken in February 1993. These measurements were taken at Scarlett Point (SC 258662, see chapter 2) and on another area of the Ledges at Port St. Mary (SC 209669, see chapter 2). The same methods for assessing clump size and density were used, as in the experimental clumps, and in each case 20 replicate *Fucus vesiculosus* clumps were measured.

7.4.1.4 Force required to remove *Fucus vesiculosus* plants

The force required to remove individual *Fucus vesiculosus* plants attached to either bare rock, living or dead barnacles was assessed on 13 July 1993. Plants were selected at random, attached to one these different substrates. When a plant had been selected a bulldog clip was attached about half way up the stipe and the hook of a 'Little Samson' 4 lb spring balance was looped through the holes in the clip. A force applied at a slow consistent rate at 90° to the substrate. The force at which the *Fucus* plant became unattached or that the stipe broke was recorded in ounces and converted to Kg (1 oz = 28.35 g). In a second experiment the force required to remove plants of similar lengths from each of the three substrate types was measured. The same methods were used and a total of 60 plants with lengths of around 17 cm were removed.

7.4.1.5 Statistical methods

Data were analysed following the methods described in chapter 3. Differences in the size, density and plant lengths of the *Fucus* clumps were tested at each sampling time using one-way analysis of variance (see repeated measures designs, section 7.2.3). All data were tested for normality and homogeneity of variance before analysis, see chapter 3, and transformed and re-tested. Percentage data were arc sine transformed before analysis.

Differences in the relative humidity and temperature inside and outside of *Fucus* clumps were tested with two-sample t-tests (Fowler & Cohen, 1992). Two-sample t-tests were also used for comparisons of plant abundance and weight and the percentage cover of barnacles and bare rock under the *Fucus* canopy at the end of the experiment. Other statistical tests used at the end of the experiment were for differences in the frequency of plant attachment to different substrates, using a chi-square test (Fowler & Cohen, 1992), and between the cover of *Enteromorpha* on the *Fucus* plants from clumps with and without dogwhelks, using a Kolmogorov Smirnov (K-S) two-sample test (Meddis, 1975).

The level of relationships or associations between variables were tested using correlations. Data were first tested to ensure they satisfied the underlying assumptions of the analysis (normality and homogeneity of variance) and the product moment correlation coefficient (r) calculated (Fowler & Cohen, 1992).

7.4.2 Results

7.4.2.1 Measurement of physical parameters

The *Fucus* clumps used for the relative humidity and temperature readings sampled on 15 August 1993 were uncovered for a total of 4.5 hours, from 13:45 till 18:15. During this time the average relative humidity inside the *Fucus* clumps remained relatively constant (figure 7.12) ranging between 87.9 and 91.1. By comparison the measurements taken on the open rock varied a great deal (figure 7.12) fluctuating between 71.0 and 84.4. The highest relative humidity values at each sampling time were always recorded in the *Fucus vesiculosus* clumps in comparison to that on the rock surface (figure 7.12). These differences were tested at 2 hour intervals over the period of low tide and were found to be statistically different in the first reading taken after the clumps were first uncovered by the ebbing tide (two-sample t-test, $t=3.64$, $df=11$, $p<0.05$) and at 16:00 half way through the sampling time (two-sample t-test, $t=4.09$, $df=11$, $p<0.01$). In the last measurement taken prior to the *Fucus* clumps being covered by the flood tide there was no difference in the relative humidity values recorded inside and outside of the *Fucus* (two-sample t-test, $t=1.66$, $df=11$, $p=0.12$).

There is a moderate positive correlation between clump size and density and relative humidity (table 7.17, figure 7.13). The relationship between relative humidity and *Fucus vesiculosus* clump size is stronger than with clump density (table 7.17). None of the correlations were significant at 14:30, in the first measurements taken after the tide had receded. The strongest correlation occurred between relative humidity and clump size at 16:30 (table 7.17).

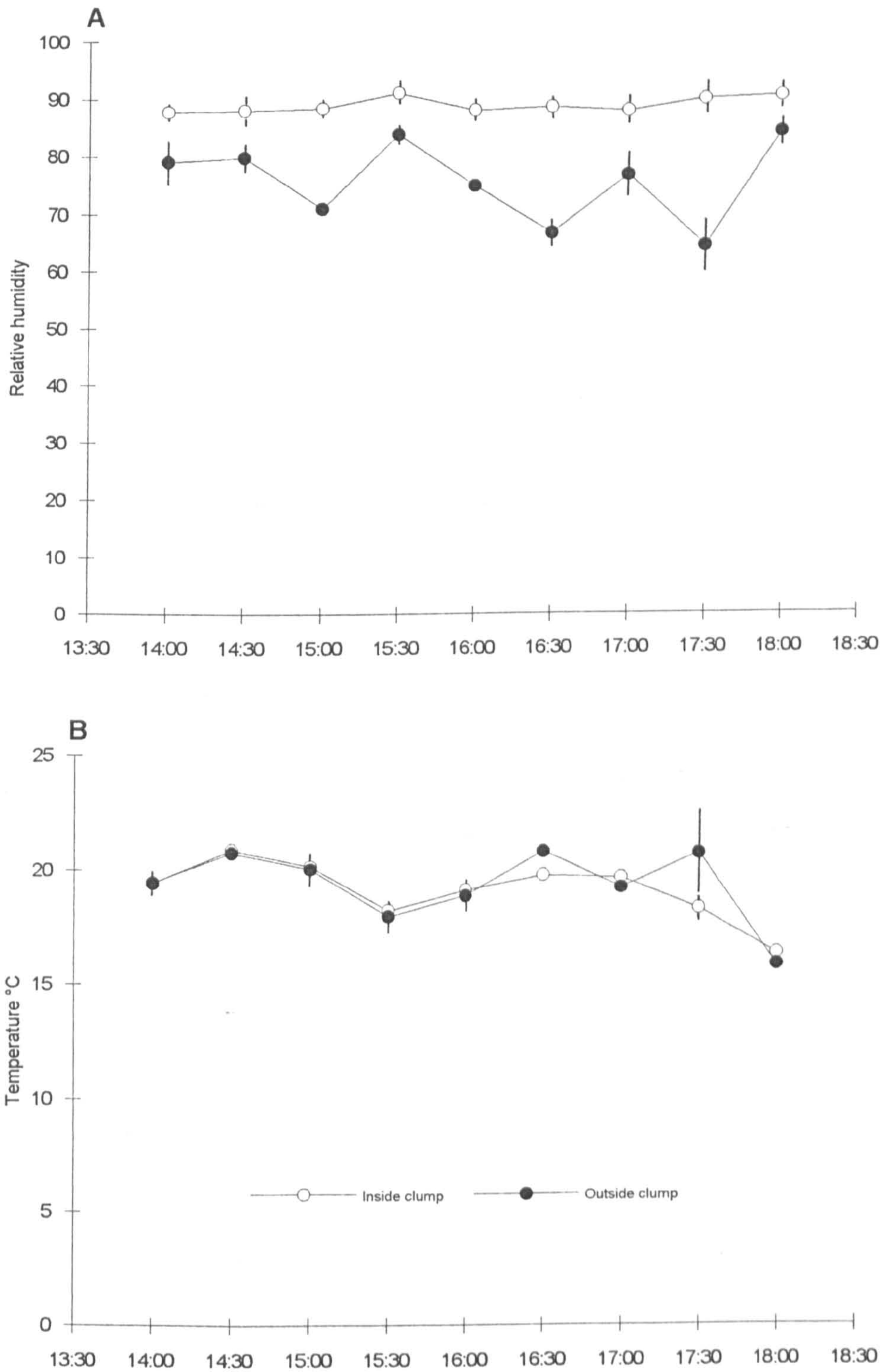


Figure 7.12 Relative humidity (A) and temperature (B) recorded inside *Fucus vesiculosus* clumps and on the rock outside. Readings taken on 15 August 1993 when low tide was at 16:41 (Liverpool, BST). Maximum air temperature 17.3 °C, minimum 11.0 °C, no rainfall, 10.3 hours sunshine, wind WNW, average 8 knots,

Table 7.17 Correlations between relative humidity and temperature and *Fucus vesiculosus* clump size and plant density at different times. Values of temperature and relative humidity taken on 15 August 1992 and correlated with *Fucus vesiculosus* clump size and density measurements taken on 12 and 16 August 1992.

(a) Relative humidity

		14:30		15:30		16:30		17:30	
	n	r	p	r	p	r	p	r	p
Clump size	27	0.054	NS	0.540	**	0.599	***	0.581	**
Clump density	27	0.091	NS	0.459	*	0.309	NS	0.448	*

(b) Temperature

		14:30		15:30		16:30		17:30	
	n	r	p	r	p	r	p	r	p
Clump size	27	0.375	NS	-0.219	NS	-0.185	NS	0.297	NS
Clump density	27	0.365	NS	-0.484	*	0.093	NS	0.430	*

$p<0.05$*, $p<0.01$**, $p<0.001$***, NS not significantly different at the $p=0.05$ level.

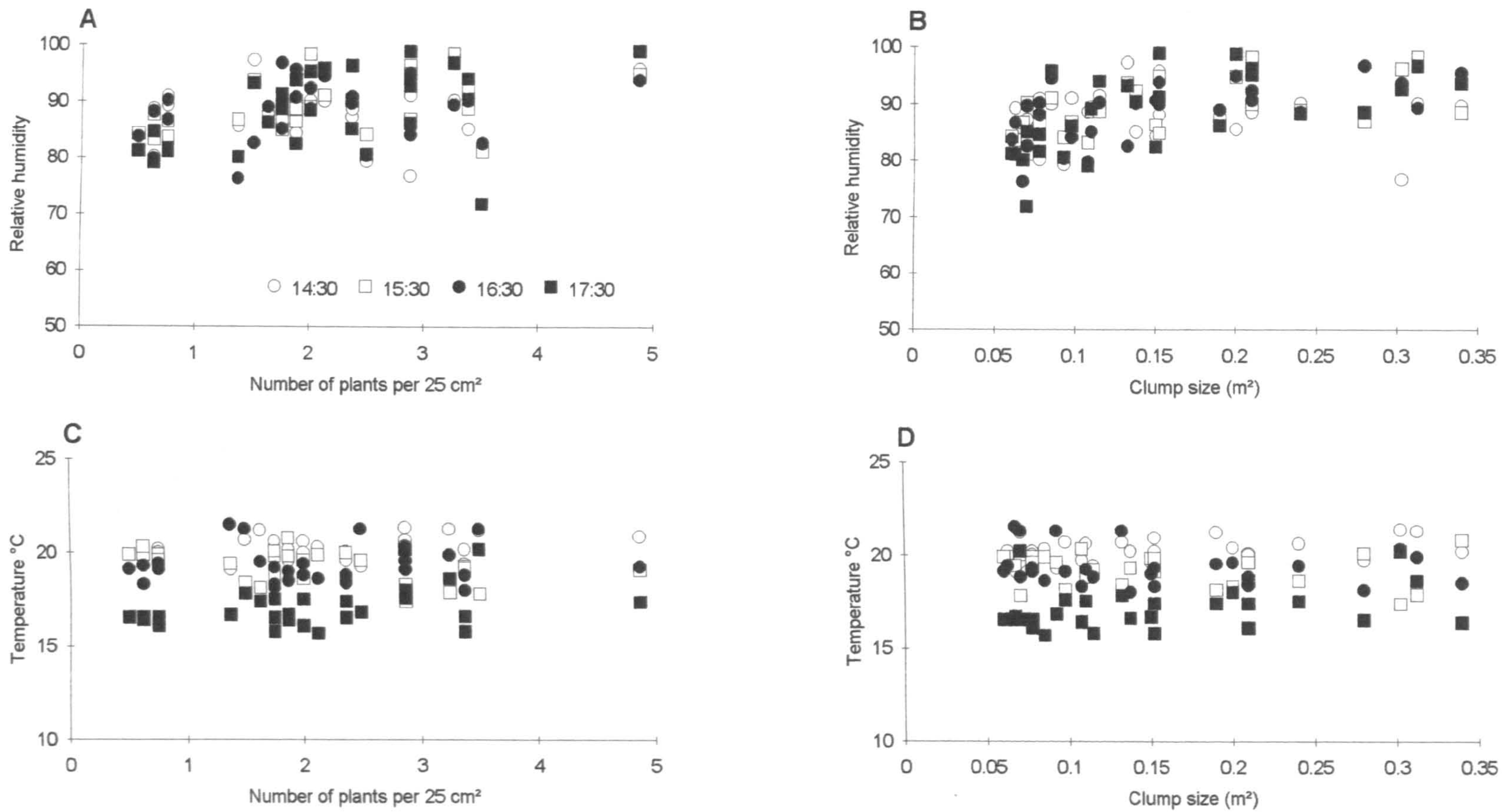


Figure 7.13 The relationship between relative humidity and the density of plants (A) and the size of *Fucus vesiculosus* clumps (B) and between temperature and the density of plants (C) and the size of the *Fucus vesiculosus* clumps (D). For comparison the relative humidity outside of the *Fucus* clumps was 77.3 ± 0.8 at 14:30, 78.0 ± 3.1 at 15:30, 75.4 ± 2.7 at 16:30 and 77.7 ± 1.4 at 17:30 and temperatures were 19.2 ± 0.8 °C at 14:30, 19.2 ± 0.6 °C at 15:30, 19.7 ± 0.3 °C at 16:30 and 16.8 ± 0.3 °C at 17:30. Correlations are given in table 7.17.

The temperature recorded in both the *Fucus* clumps and on the rock fluctuated over the period of low tide (figure 7.12). Generally the average temperature was slightly lower on the open rock than inside the *Fucus vesiculosus* clump. These differences were small however and when tested at 2 hourly intervals over the period of low tide no significant differences were found in either the first readings taken after the clumps were uncovered (two-sample t-test, $t=-0.02$, $df=11$, $p=0.98$) or half way through the sampling period at 16:00 (two-sample t-test, $t=0.55$, $df=11$, $p=0.59$). In the last reading taken before the clumps were again covered by the incoming tide, however, the temperature within the *Fucus* clumps was significantly higher than on the open rock (two-sample t-test, $t=2.47$, $df=11$, $p<0.05$).

Temperature showed little relationship with *Fucus* clump size (table 7.17, figure 7.13). Both negative (at 15:30) and positive (at 17:30) significant relationships were found between the density of the plants in the clumps and temperature (table 7.17).

7.4.2.2 *Nucella lapillus* amongst the *Fucus* clumps

Dogwhelks were found in the *Fucus vesiculosus* clumps throughout the experimental period. They were found either singly or more commonly in groups of 5-20, especially those with shell lengths greater than 20 mm. Occasionally these aggregations under the *Fucus* plants centred around an *Actinia equina* which was squashed between the dogwhelks. Juvenile dogwhelks were usually found singularly sheltering either wedged between barnacles or in the empty shells of dead barnacles. The smallest dogwhelk recorded under any *Fucus vesiculosus* clump had a shell length of only 4 mm. Dogwhelk egg capsules were first observed under one of the control *Fucus* clumps in May. They appeared, in total, in a third of the control clumps throughout the early summer, occurring in batches ranging in quantity from 3-50 egg capsules.

The number of dogwhelks in the control clumps peaked in April when an average of 21.9 dogwhelks per clump was recorded (figure 7.14). This declined throughout the summer months. The lowest density was recorded as an average of 1.7 dogwhelks per clump in August. From May onwards dogwhelks were regularly observed feeding on the open rock away from the shelter of *Fucus* plants, especially on early morning low tides or after periods of calm weather. On some occasions oystercatchers, *Haematopus ostralegus*, were observed in the experimental areas whilst walking down to the site. When reaching the experimental areas it was observed that some of the dogwhelks on the open rock were lying with their opercula facing upwards, suggesting dislodgement by birds.

The total number of *Nucella lapillus* present in the control clumps was always higher than the numbers removed from the treatment clumps (figure 7.14). The numbers of dogwhelks which needed to be removed on the regular sampling visits declined over the period of the experiment. On the first removal on 26 January 1993 an average of 10.4 dogwhelks per clump were removed from the treatment clumps, after this the average number removed per clump ranged from a maximum of 8.0 in April to a minimum of 0.7 in August.

Juvenile dogwhelks with shell lengths of less than 10 mm constituted the majority of dogwhelks both present in the control clumps or removed from the treatment clumps, from January to April. After April dogwhelks with shell lengths greater than 20 mm, mainly adults, were the main component and those with shell lengths <10 mm were seldom recorded.

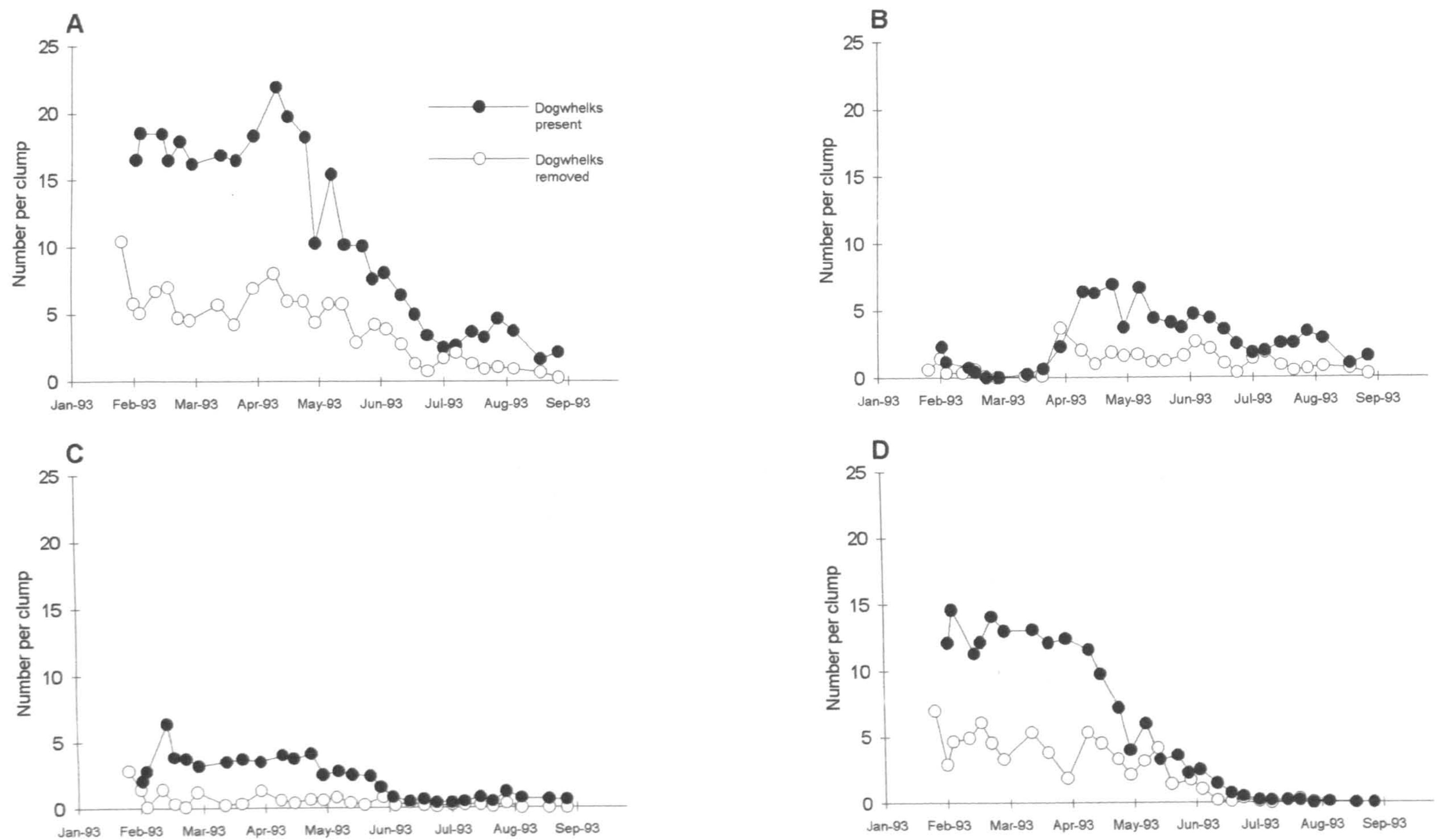


Figure 7.14 The number of *Nucella lapillus* which were either removed from the experimental *Fucus* clumps or recorded as present, expressed as total dogwhelks (A), those >20 mm (B), 10-20 mm (C), or <10 mm (D) in shell length. Each value is the average from 12 *Fucus* clumps, standard errors not shown to aid clarity.

7.4.2.3 Changes in the *Fucus vesiculosus* clumps

Fucus vesiculosus clumps arose as escapes from grazing in the late summer around August to September in 1992. These formed small clumps of very dense plants each about 2-3 cm in length. The plants were attached to the barnacle matrix, the holdfast being fixed to the sides of the barnacles shells (figure 7.15). As the plants grew bigger the holdfast overgrew other barnacles or additionally became attached to bare rock, adjacent to the barnacles. These new clumps appeared to attract a lot of shell debris especially during rough weather. The shell debris remained amongst the dense plants between October and December.

The average area that the *Fucus* clump covered decreased over the period from December to January, before the experiment started, by about 5%. Those clumps which had previously been recorded as containing shell debris showed the biggest decreases, with six of the 30 clumps being destroyed completely. From January onwards the average areas covered by the remaining 24 *Fucus* clumps increased (figure 7.16). These average increases were the case in both the treatment and control clumps, although the increase in the treatment clumps was greater with a doubling in their average size after the experiment had run for 34 weeks. There was no difference in the clump sizes at the start of the experiment (table 7.18) nor at 6, 12 or 17 weeks after the dogwhelks were initially removed from the treatment clumps (table 7.18). However, 23, 30 and finally 34 weeks after the dogwhelks were first removed there was a significant difference between the size of the *Fucus* clumps in the dogwhelk removal treatments compared to the controls (table 7.18) (plate 6A, 6B).

The density of plants within the *Fucus vesiculosus* clumps generally decreased from January onwards (figure 7.16). The sharpest decreases occurred in the first

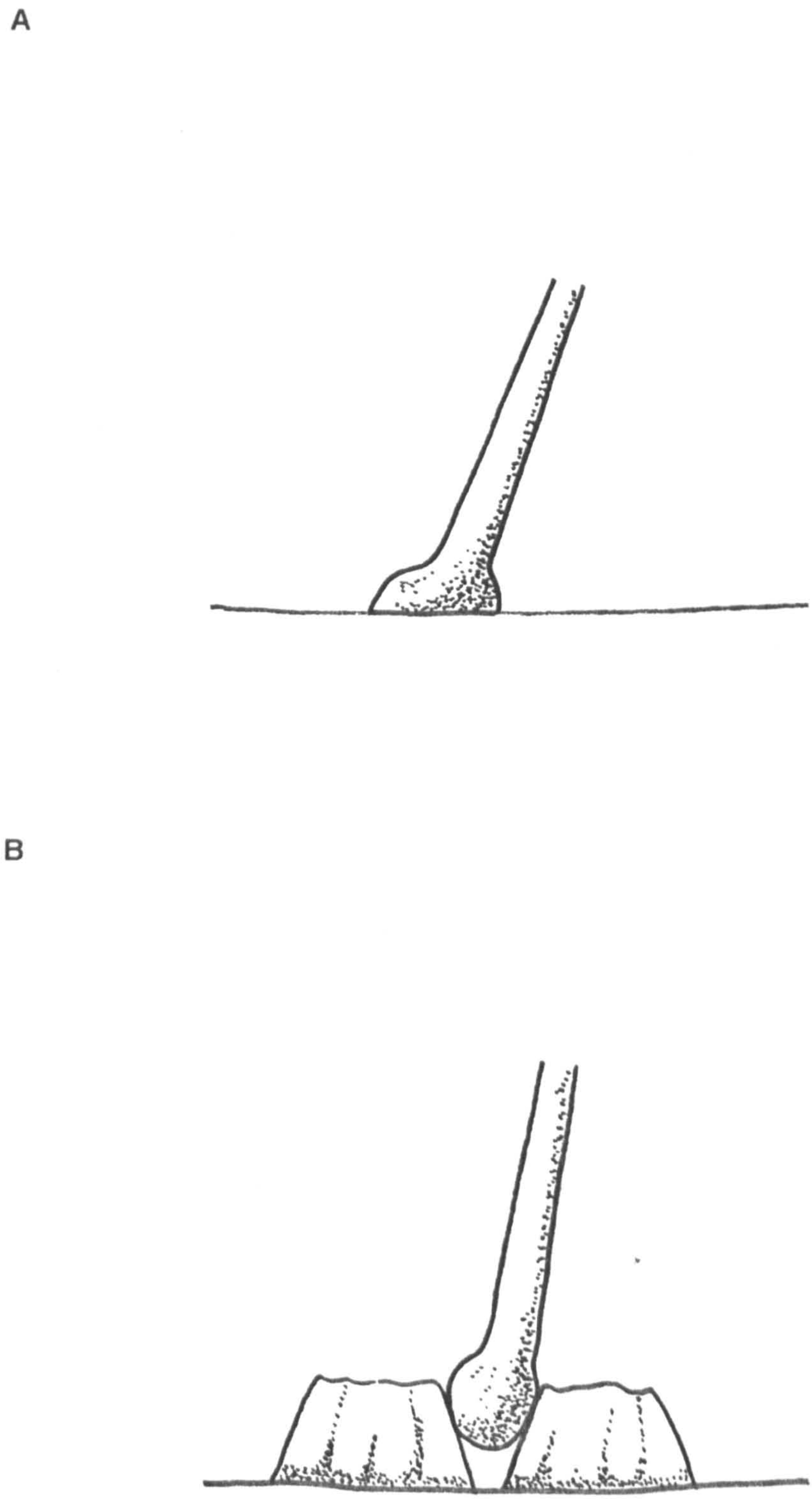


Figure 7.15 Attachment of *Fucus vesiculosus* to bare rock (A) and *Semibalanus balanoides* (B).

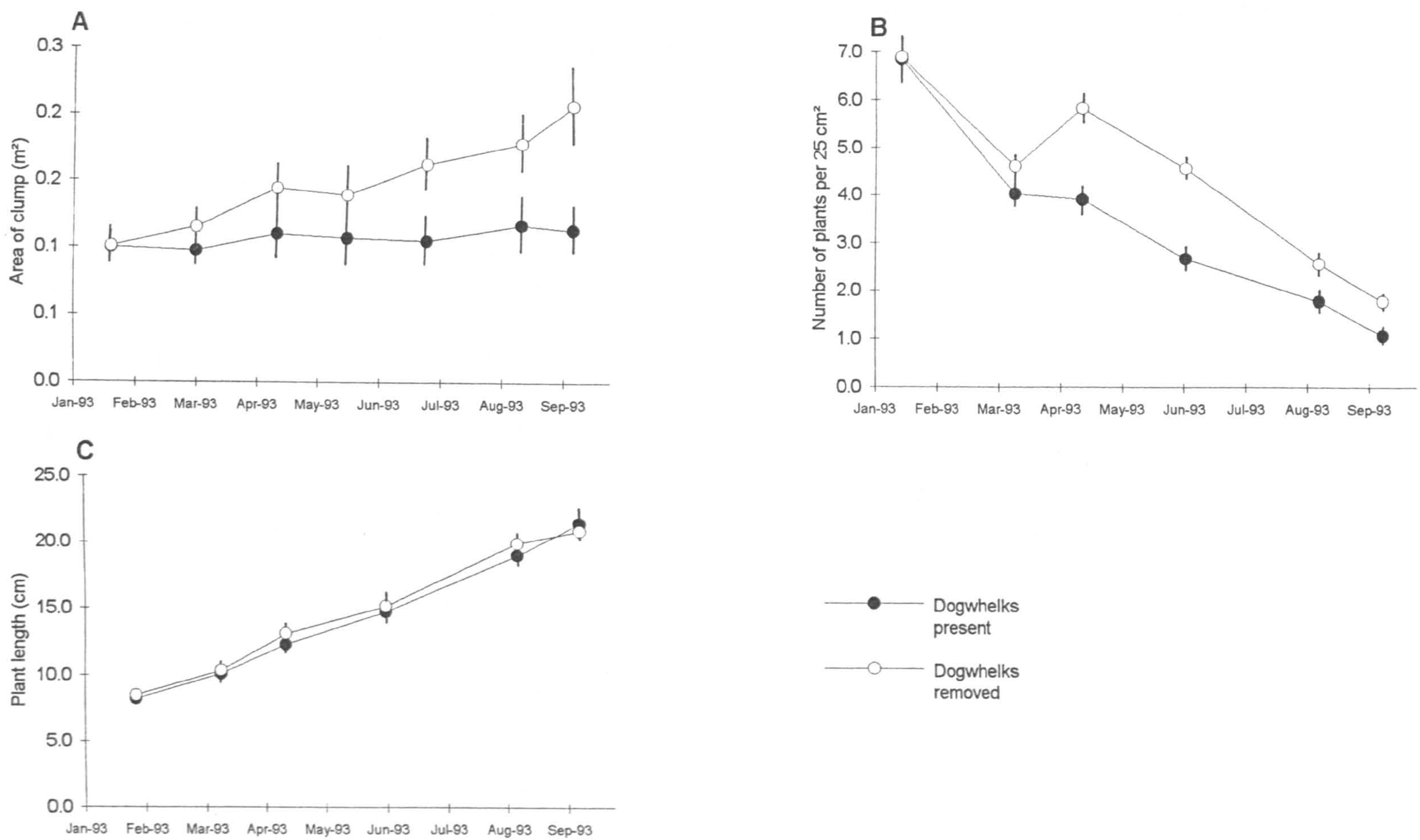


Figure 7.16 Changes in the size (A) density (B) of *Fucus vesiculosus* clumps and the average length of plants (C) in clumps where *Nucella lapillus* was either present or regularly removed.

Table 7.18 One-way analysis of variance at each sampling time between *Fucus vesiculosus* clump sizes where densities of *Nucella lapillus* have either been manipulated or left. Data $\log_{10}$ transformed.

Date	Weeks		df	MS	F	p
20 January 1993	0	Treatment	1	0.0001	0.00	0.950
		Error	22	0.0346		
4 March 1993	6	Treatment	1	0.0267	0.89	0.357
		Error	22	0.0302		
14 April 1993	12	Treatment	1	0.1043	2.16	0.155
		Error	22	0.0482		
20 May 1993	17	Treatment	1	0.1154	1.69	0.208
		Error	22	0.0684		
29 June 1993	23	Treatment	1	0.2777	5.42	0.029 *
		Error	22	0.0512		
16 August 1993	30	Treatment	1	0.2377	5.32	0.031 *
		Error	22	0.0477		
11 September 1993	34	Treatment	1	0.4556	8.55	0.008 **
		Error	22	0.0533		

$p<0.05$*, $p<0.01$**, $p<0.001$***.

Plate 6

Fucus vesiculosus clumps in September 1993 after 8 months where *Nucella lapillus* has been either left un-manipulated (A) or continually removed (B).

A

B

two months of the experiment. There was no difference in the plant densities at the start of the experiment in January (table 7.19) when the average plant density was around 7 plants per 25 cm^2. The density in the control clumps decreased by 85% and after 34 weeks the average plant density was only 1 plant per 25 cm^2. The density of plants in the clumps where dogwhelks had been continually removed also showed a decrease over the experimental period, although there was a slight increase in plant density between March and April (figure 7.16). Overall the density of plants was reduced by 74% in the treatment clumps resulting in an average plant density of nearly 2 plants per 25 cm^2. The density of *Fucus* plants in the dogwhelk removal clumps was significantly higher than in the control clumps during the last six months of the experiment (table 7.19).

At various intervals new germlings were observed amongst the older *Fucus* plants. This seemed to be the case, especially at the end of March and in August. On the latter occasion new germlings were observed on the barnacle matrix on horizontal ledges at Port St. Mary close to the experimental sites.

At the end of the experiment the removal of all the plants from the substrate showed that the clumps consisted of between 10 and 148 individual *Fucus vesiculosus* plants. However, there was a great deal of variation amongst the 24 *Fucus* clumps. The control clumps had on average 46.0 ± 36.1 plants per clump and the treatment clumps had an average of 54.9 ± 32.3 per clump; this was not significantly different (two-sample t-test, $t=0.64$, $df=22$, $p=0.53$). These plants were weighed after removal and had a wet weight of between 0.4 and 2.7 Kg per clump. These was no difference between the average weight of the control (0.9 Kg ± 0.6) and treatment (1.0 Kg ± 0.2) clumps (two-sample t-test, $t=0.52$, $df=22$, $p=0.61$).

Table 7.19 One-way analysis of variance at each sampling time between the density of *Fucus vesiculosus* plants in clumps where numbers of *Nucella lapillus* have either been manipulated or left.

Date	Weeks		df	MS	F	p
14 January 1993	0	Treatment	1	0.00	0.00	0.982
		Error	22	7.51		
12 March 1993	7	Treatment	1	2.08	2.38	0.137
		Error	22	0.87		
12 April 1993	12	Treatment	1	21.95	15.03	0.001 ***
		Error	22	1.46		
6 June 1993	19	Treatment	1	21.62	19.23	0.001 ***
		Error	22	1.12		
12 August 1993	29	Treatment	1	3.77	3.67	0.068
		Error	22	1.03		
13 September 1993	34	Treatment	1	3.02	9.77	0.005 **
		Error	22	0.31		

$p<0.05^{*}$, $p<0.01^{**}$, $p<0.001^{***}$.

The plants within each of the clumps were attached either to bare rock, barnacles or to limpets. Most of the plants were attached to barnacles, in both the treatment and control clumps (figure 7.17). The percentage of plants attached to bare rock was greater in the *Fucus* clumps where dogwhelk densities had not been manipulated. The difference in the frequency of attachment to the different substrates in the control and treatment clumps was highly significant (χ^2=77.72, df=2, p<0.001). The percentage cover of barnacles under the treatment *Fucus* clumps (29.1% ± 9.33) was significantly higher than under the control *Fucus* clumps (17.1% ± 8.55) at the end of the experiment (two-sample t-test, arc sine transformed data, t=3.27, df=22, p<0.01).

The average plant length measured from January to September (figure 7.16) increased 2.5 times in both the treatment and control clumps. There was no significant differences between the average plant lengths in the treatment and control clumps on any of the sampling occasions (table 7.20). Length frequency graphs produced for 16 of the 24 *Fucus vesiculosus* clumps appeared to show no difference in the overall frequency of plant lengths in the treatment and control clumps (figure 7.18).

In June *Porphyra umbilicalis* was observed to be attached to the *Fucus vesiculosus* plants in some of the clumps. Later *Enteromorpha* became abundant. A survey of the amount of plants within any *Fucus vesiculosus* clump which was covered with epiphytes was done blind at the end of the experiment. The clumps ranged in the percentage of plants which had epiphytes on them from 0-100%. Each of the clumps were assigned to one of the four categories depending on the percentage of plants with epiphytes (figure 7.17). The modal class for the control clumps, where dogwhelks were present, was 26-50% of plants with epiphytes attached. The modal class for the treatment clumps was <25% covered. None of the treatment clumps

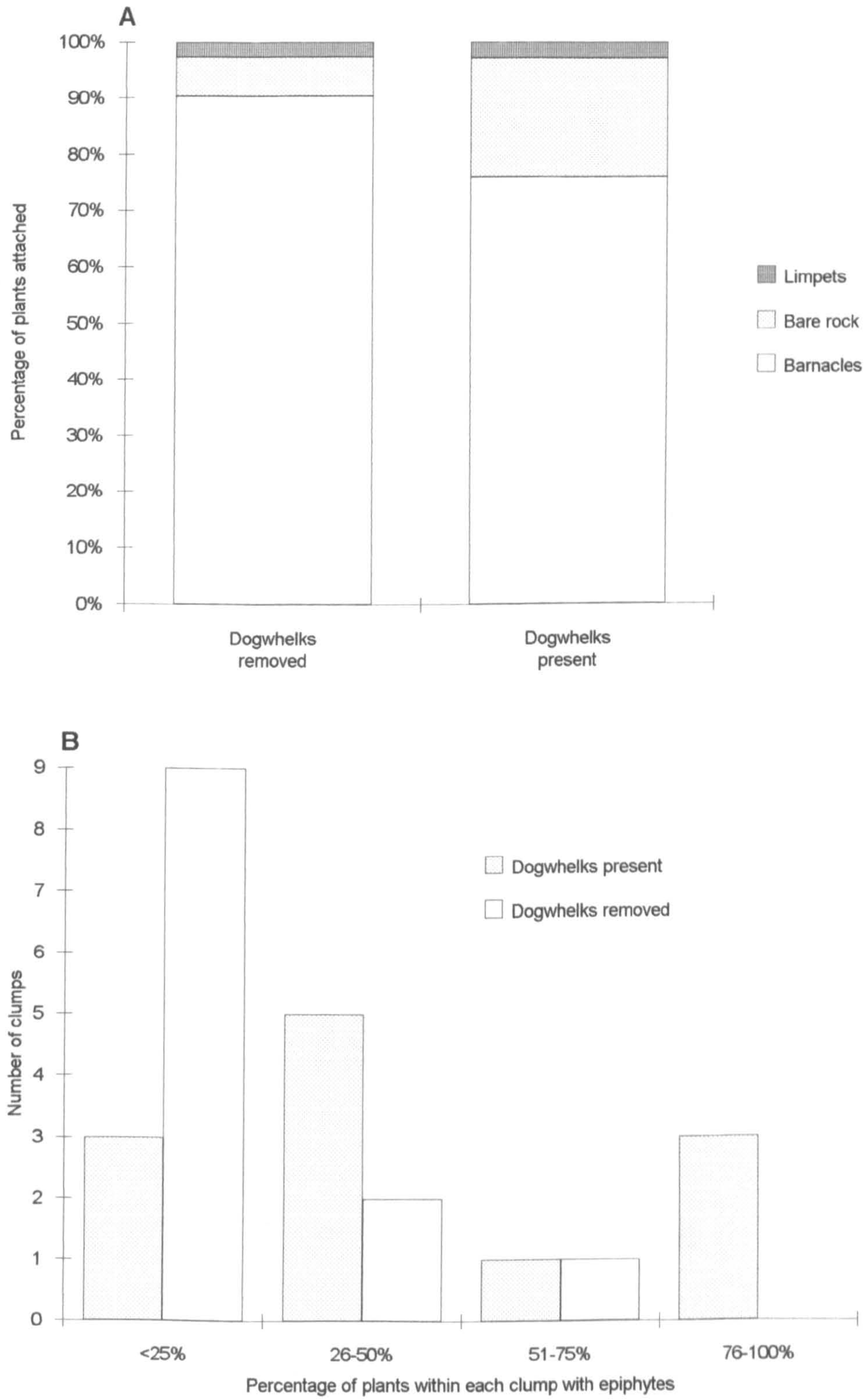


Figure 7.17 Results at the end of the experiment (14 September 1993). The percentage of plants attached to either barnacles, bare rock or the shells of limpets (A), plotted as the average number of plants from 12 clumps with or without dogwhelks. The percentage of plants within each of the 24 clumps which had epiphtes on them (B), plotted as the number of clumps which have that percentage of epiphytes.

Table 7.20 One-way analysis of variance at each sampling time between *Fucus vesiculosus* plant lengths in clumps where densities of *Nucella lapillus* have either been manipulated or left.

Date	Weeks		df	MS	F	p
27 January 1993	0	Treatment	1	0.51	0.71	0.409
		Error	22	0.72		
12 March 1993	7	Treatment	1	0.45	0.16	0.691
		Error	22	2.75		
15 April 1993	12	Treatment	1	3.94	1.59	0.221
		Error	22	2.48		
5 June 1993	19	Treatment	1	0.98	0.17	0.687
		Error	22	5.84		
12 August 1993	29	Treatment	1	5.38	1.62	0.217
		Error	22	3.33		
13 September 1993	34	Treatment	1	1.96	0.22	0.647
		Error	22	9.06		

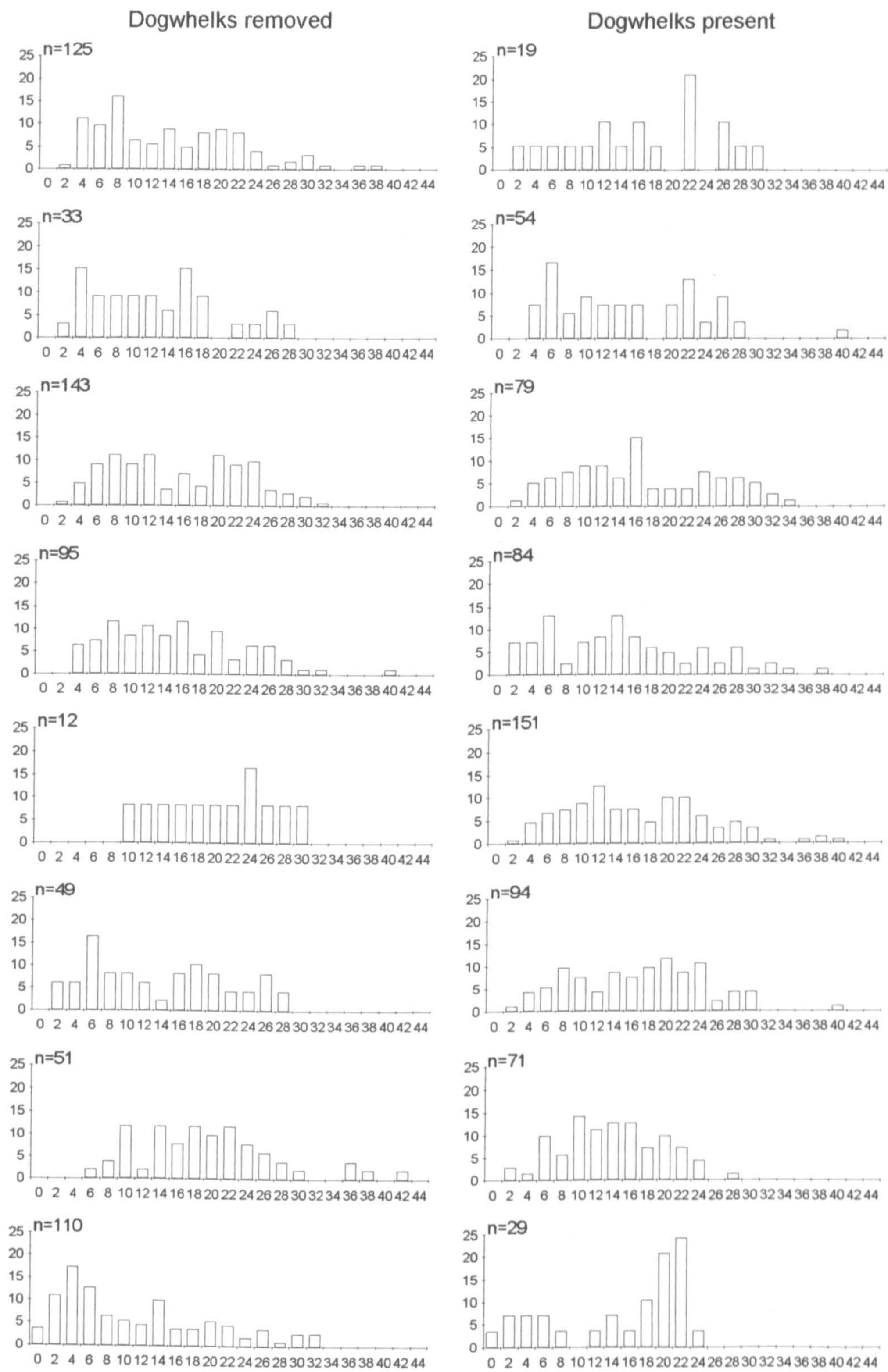


Figure 7.18 Percentage frequency diagrams for the length of all *Fucus vesiculosus* plants in 8 clumps where dogwhelks have been removed and 8 clumps where dogwhelks have been present. Measurements taken on 14 September 1993. n is the total number of plants in that clump.

were classified as having over 75% of their plants from any one clump covered with *Enteromorpha* on them but 3 of the 12 control clumps were (figure 7.17). Thus overall there was a significant difference in the percentage cover of epiphytes in the clumps from which dogwhelks had been removed, compared to the clumps where dogwhelks had been present (K-S two-sample test, $K=1.2247$, $p<0.05$).

The average number of *Actinia equina* found in the control and treatment clumps ranged between 1 and 3 throughout the experiment (figure 7.19). These were always of the red or brown morphs. Green *Actinia* although common elsewhere on the ledges at Port St. Mary (personal observations) were never observed under the *Fucus* canopy. On one occasion an individual *Actinia* was observed on consecutive tides on the bare rock moving away from a *Fucus* clump which had been drastically thinned after a bad storm. The number of juvenile *Patella vulgata*, those with shell lengths of less than 15 mm was usually around 1-2 per *Fucus* clump (figure 7.19) and remained similar in numbers inside both the control and the treatment clumps. The number of adult limpets per clump, however, increased from March onwards (figure 7.19). They started at about 1 per clump and reached an average of around 7 per *Fucus* clump for both the treatment and control clumps. The average number recorded in the clumps where dogwhelks were being removed was always higher than in the control clumps, although there was a great deal of variation.

Other species apart from *Nucella lapillus*, *Patella vulgata* and *Actinia equina* were found amongst the *Fucus* plants. Most of these other species of organisms observed in the *Fucus* clumps were common to both the control and treatment clumps. Although the abundance of these species was not recorded observations on the presence or absence of anything found in the clumps was noted. These species included *Gibbula cineraria*, *G. umbilicalis*, *Calliostoma zizyphinum*, *Helcion pellucidum* and *Lepidochitona cinerea*. Many *Idotea* were also found. In addition to

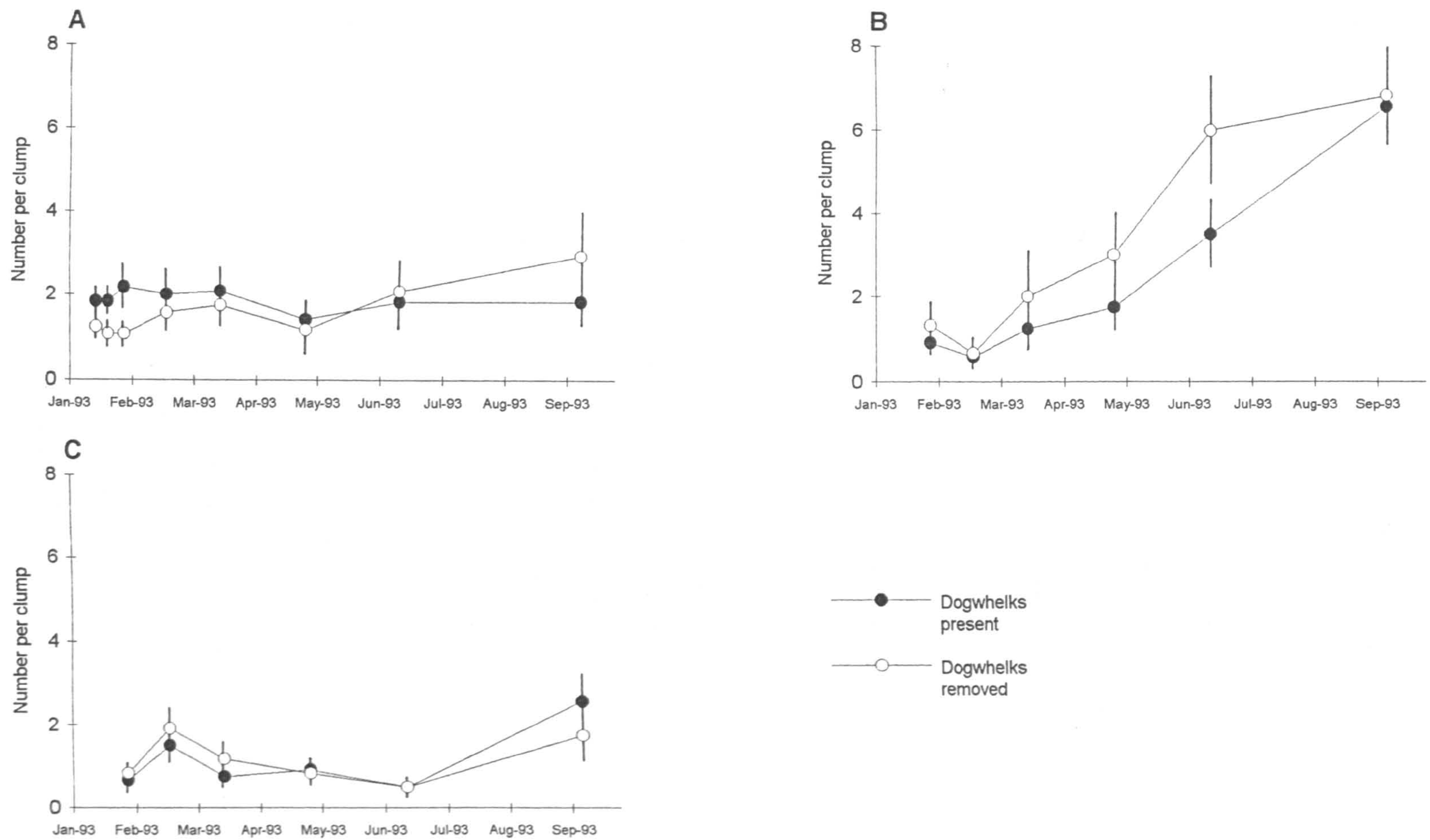


Figure 7.19 The abundance of *Actinia equina* (A) and adult (B) and Juvenile (C) *Patella vulgata* in *Fucus vesiculosus* clumps where *Nucella lapillus* was either present or regularly removed.

Actinia equina, *Bunodactis verrucosa* was observed on occasion in a number of *Fucus vesiculosus* clumps. In March to April nemertines were observed amongst the barnacles and *Fucus* stipes. These were mainly the small yellow *Tetrastemma* spp. but occasional *Lineus ruber* were also observed. Juvenile *Carcinus maenas* ranging in carapace width from 2-5 cm were commonly recorded, especially from June onwards. *Littorina obtusata* was common in both the control and treatment clumps from January to April, but after this time appeared to occur regularly only in the treatment clumps. Small *Mytilus edulis* (0.5-1.0 cm in shell length) were found under both the control and treatment clumps surveyed in January but not in the control clumps after March.

The *Fucus vesiculosus* clumps measured at Scarlett Point and at the extra site at Port St. Mary were around 20% smaller than those used in the experimental manipulation, however, they all showed similar plant densities. The main difference between the three locations was in the dogwhelk abundance recorded in the *Fucus* clumps at each site. The average dogwhelk abundance was around 18 dogwhelks per clump at the experimental site, 4 per clump at the other Port St. Mary site and 2 per clump at Scarlett Point. Densities of *Patella* and *Actinia* were about the same.

7.4.2.4 Force required to remove *Fucus vesiculosus* plants

The force required to remove *Fucus vesiculosus* plants from different substrates increased with increasing plant length (figure 7.20). Correlations between force and plant length were weak but significant when values recorded for removal from all substrate types were included ($r=0.270$, $n=82$, $p<0.05$). When the substrates were considered separately correlations were not significant between plant length and the force needed for removal from either living barnacles ($r=0.101$, $n=33$, NS) or dead barnacles ($r=0.069$, $n=25$, NS). The correlation between force and plant size

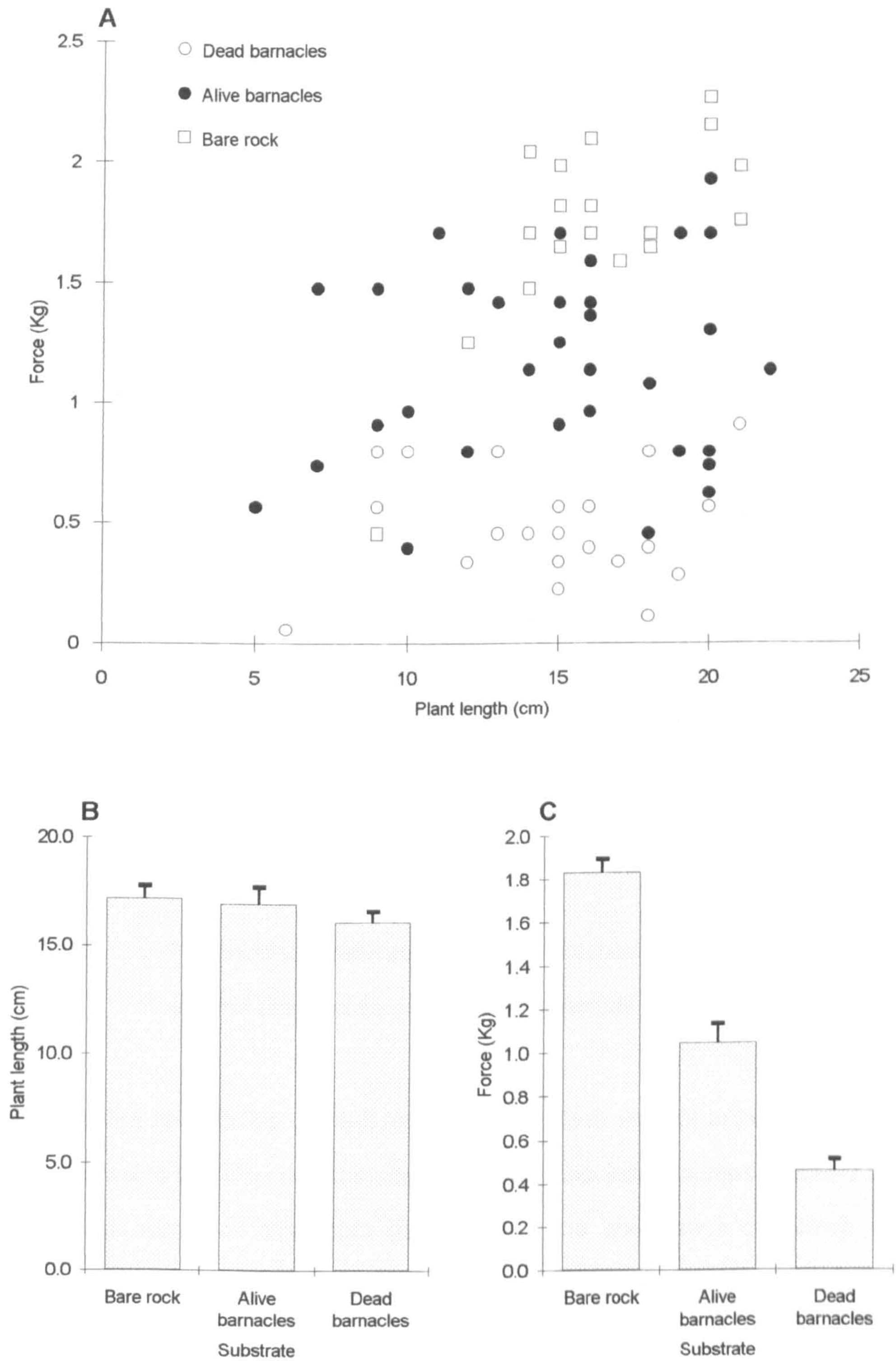


Figure 7.20 The relationship between the length of *Fucus vesiculosus* plants and the force required to remove them from different substrates (A). The size of plants (B) used to assess the force required to remove individual plants from either bare rock, living or dead barnacles (C), error bars ± 1 SE.

attached to bare rock, however, showed a significant and strong correlation ($r=0.711$, $n=25$, $p<0.001$). In the removal of *Fucus* from bare rock the stipe of the plant frequently broke. Out of 20 plants removed from bare rock the stipe broke before the plant came away at the holdfast in 15 cases. The stipe did not break where the bulldog clip was attached, but about 2 cm from holdfast. The stipe never snapped when plants were being pulled from their attachment to either living or dead barnacles.

The force required to remove plants of a similar size (around 17 cm in length) from either bare rock, barnacles or dead barnacles varied. This force was significantly different between the three substrates (one-way ANOVA, $F=104.06$, $df=2, 57$, $p<0.001$). Tukey tests showed these all to be significantly different from each other ($p<0.001$). The strength of the force required was greater in bare rock than on living barnacles which in turn was greater than dead barnacles. There was no difference between the plant lengths of those removed from the different substrates (one-way ANOVA, $F=0.83$, $df=2, 57$, $p=0.442$).

7.4.2.5 Relationships between *Fucus vesiculosus* clump size, plant density and the abundance of *Patella* and *Actinia*

Although the abundance of adult and juvenile *Patella vulgata* appeared to increase with increasing the size and density of *Fucus vesiculosus* clumps (figure 7.21) the correlations were not significant (table 7.21). The abundance of *Actinia equina*, however, showed a significant positive correlation with increasing clump density and clump size (table 7.21).

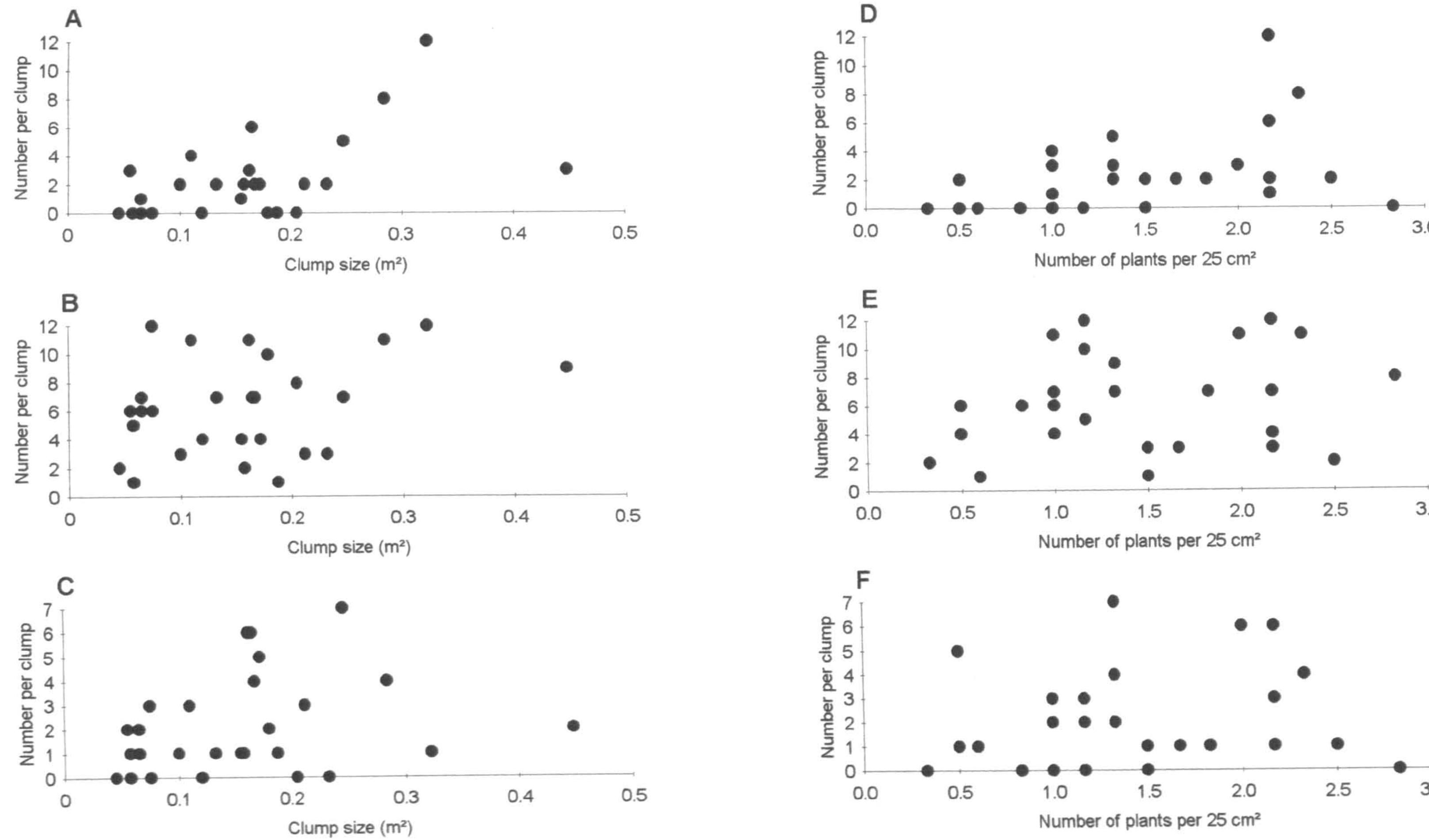


Figure 7.21 The relationship between *Fucus vesiculosus* clump size and the number of *Actinia equina* (A), adult *Patella vulgata* (B) and juvenile *Patella vulgata* (C) and plant density and *Actinia* (D), adult limpets (E) and juvenile limpets (F). Comparisons made on the date the experiment was last sampled, 14 September 1993.

Table 7.21 Correlations of *Fucus vesiculosus* clump size and density with the abundance of *Actinia equina*, adult and juvenile *Patella vulgata* in September 1993.

(a) *Fucus vesiculosus* clump size

	r	n	p
Actinia equina	0.566	27	**
Adult *Patella vulgata*	0.324	27	NS
Juvenile *Patella vulgata*	0.249	27	NS

(b) *Fucus vesiculosus* clump density

	r	n	p
Actinia equina	0.419	27	*
Adult *Patella vulgata*	0.206	27	NS
Juvenile *Patella vulgata*	0.109	27	NS

$p<0.05$*, $p<0.01$**, NS not significantly different at the $p=0.05$ level.

7.5 The effects of *Nucella lapillus* on barnacle settlement

7.5.1 Materials and methods

7.5.1.1 Experimental aims and design

Nucella lapillus is an important predator of barnacles (Connell, 1961a). Since barnacles are known to use environmental cues to assess potential settlement sites, it was hypothesised that settling cyprids would be able to detect the presence of a potential predator and settle away from areas where *Nucella* densities were highest. Consequently the aims of these experiments were to determine whether the presence of *Nucella* affected the density of the settlement of *Semibalanus balanoides* both in the long-term (over a period of weeks or months) and in the short-term (over the period of two or three tides). Long-term effects were examined by measuring the barnacle settlement on areas of vertical rock on which dogwhelks had either previously been excluded or allowed to feed for 10 months. Effects in the short-term were assessed using of the presence or absence of mucus left by *Nucella* on barnacle settlement areas.

Long-term effects of the presence or absence of *Nucella lapillus* were assessed on areas of barnacle dominated vertical rock at Port St. Mary Ledges used in the dogwhelk removal experiments (verticals 2 and 3, section 7.3). The settlement of barnacles was measured in two experiments. The first experiment measured settlement within previously cleared areas in the barnacle matrix. In the second experiment repeat random samples were taken within each of the experimental areas; numbers of adult barnacles were counted and the amount of bare rock assessed to relate the numbers of settling barnacles to the numbers of adults present and available space for settlement.

Short-term effects of the presence or absence of *Nucella lapillus* were examined on the settlement of barnacles on areas of horizontal Ledges at Port St. Mary (site B, figure 2.3). In three experiments the settlement of barnacles was compared in the presence of seawater controls or mucus from the feet of individual *Nucella lapillus* (predator) or *Littorina littorea* (grazer). In the first experiment mucus was applied to stones which were then cemented down amongst the existing barnacle matrix on the shore. In the second and third experiments mucus was applied to cleared areas in the barnacle matrix (table 7.1).

7.5.1.2 Methods used to determine long-term effects

Fixed areas

For the first experiment three 5 x 5 cm areas were cleared in the barnacle matrix on each of the six experimental areas on vertical 2 and 3 (section 7.3). These verticals each had three areas where dogwhelk densities were un-manipulated and three areas where dogwhelk densities had been reduced for the previous 10 months. Areas were cleared on 31 March 1993 using the methods described in chapter 3. The areas chosen to be cleared were not random, but selected within each of the experimental plots to contain between 70-100 living barnacles. This was to try and eliminate the influence of differences in settlement due to differences in the number of conspecifics providing gregarious settlement cues. Areas were checked regularly and once barnacles had started to settle counts were made of numbers of settling cyprids and metamorphosed barnacles on average every three days, until 22 June 1993 (vertical 3) and 2 July 1993 (vertical 2).

Random quadrats

In the second experiment barnacle settlement was monitored on the same two verticals (2 and 3, section 7.3) this time using barnacle counts in ten random 5 x 5 cm quadrats in each of the 6 experimental plots on each vertical. Within each quadrat, counts were made of the number of living and dead adult barnacles and the number of cyprids and newly metamorphosed barnacles. The first counts were made on 16 May 1993.

7.5.1.3 Methods used to determine short-term effects

Stones

In the first experiment 30 pieces of Manx slate were used. These were collected from Gansey Point (SC 216680) as part of an experiment for another research student. As a by-product a series of small (about 70 cm^2) flat slates with virgin surfaces were produced after the rocks had been split. These were utilised to examine the effect of the presence of *Nucella lapillus* on barnacle settlement. These stones were measured and numbered. The position of the number was used as locator for the 5 x 5 cm quadrat which was placed in the stone to record the number of cyprids and metamorphosed barnacles once the rocks were transplanted to the shore. The stones were divided into three sets and placed face upwards in the bottom of three enamel trays. *Nucella lapillus* and *Littorina littorea* were collected from Port Erin (SC 194696) on 21 May 1993 and 150 individuals of each added to trays 1 and 2 respectively. Each of the trays were placed in separate mesh bags (used for the collection of scallop spat) which were tied to prevent escape of the dogwhelks or littorinids. These were placed on the circulation bench with a supply of running seawater on 21 May 1993. The third tray, containing neither dogwhelks nor littorinids, was also placed in a mesh bag and kept on the circulation bench. On 23 May 1993 the dogwhelks and littorinids were removed from the trays and the rocks taken to Port St. Mary Ledges. They were cemented to a large flat area of rock dominated by barnacles (site B, figure 2.3) using Blue Hawk Ltd quick dry cement mixed with seawater in a 3:1 ratio. The percentage cover of barnacles on this ledge was 52.0% ± 12.2 and the *Fucus* cover was low, 1.1% ± 2.3. The stones were grouped in threes, one from each of the treatments and a control, randomly across this ledge. Close to one of the groups of stones three areas of the barnacle matrix, each 5 x 5 cm, were scraped to reveal bare rock (see chapter 3). These were surveyed on the following six consecutive tides and then approximately every week. They were last sampled on 21 May 1993.

Cleared areas

In the second mucus effect experiment 15 areas, each 5 x 5 cm, were cleared in the barnacle matrix on an area of flat rock close to where the stones were cemented at site B (figure 2.3). The percentage cover of barnacles on this ledge was 52.0% ± 12.2 and the *Fucus* cover was low, 1.1% ± 2.3. The areas to be cleared were randomly chosen over this ledge but were a minimum of 1.5 m apart. The barnacles were cleared on 25 May 1993 using methods described in chapter 3. These 15 areas were randomly assigned to control or treatment areas. Ten littorinids were placed on each of the five areas randomly selected to be for the first treatment and ten dogwhelks were placed on each of the five cleared areas randomly selected to be for the second treatment. The five control areas were left. The dogwhelks and littorinids were placed on the cleared areas facing inwards so that they had to turn around before being able to move away, hereby ensuring that a trail was left. The number of cyprids and metamorphosed barnacles in the areas were recorded on the next five consecutive tides. The areas were monitored every 4-5 days after that and last recorded on 28 June 1993. This experiment had been specifically set up on spring tides. On the Isle of Man low tide on springs occurs early in the morning and in the early evening. Consequently these squares could be monitored on every low tide over the period of several days with each tide occurring in daylight. In addition the experiment was set up as the tide went out enabling time for the dogwhelks and littorinids to move away from the treatment areas before the tide came in. The early evening tide on which the experiment was set up facilitated this as it was cooler and damper and consequently the dogwhelks were likely to be more mobile (personal observations). The numbers of cyprids and metamorphosed barnacles in these areas were counted last on 21 June 1993.

The methodology of the second mucus effect experiment was repeated for the third, which was conducted on a ledge close by (site B, figure 2.3). The percentage cover of barnacles on this ledge was 46.9% ± 11.6 and the *Fucus* cover was low, 2.5% ± 3.6. This experiment used 40 cleared areas each of 5 x 5 cm in the barnacle matrix. These areas were randomly selected and cleared on 4 June 1993, with the numbers of living and dead barnacles removed from within the areas recorded. These 40 areas were randomly assigned to be either control or treatment areas. As in the second mucus effect experiment the first treatment was the addition of littorinids to the cleared areas, the second treatment was the addition of dogwhelks and the control areas were left alone. In addition, however, two other treatment were added to the experimental design. These were the continual addition of littorinids or the continual addition of dogwhelks. In these two treatments 10 individuals were placed on the areas assigned to these treatments on the next 11 consecutive tides. There were 8 replicates of each of the treatments and control (table 7.1). The number of cyprids and metamorphosed barnacles in the areas were recorded on the following 11 consecutive tides and then after every 4-5 days until 28 June 1993.

7.5.1.4 Statistical methods

Data were analysed following the methods described in chapter 3. All data were tested for normality and homogeneity of variance using the methods described in chapter 3, and transformed and re-tested where necessary. Differences in the numbers of metamorphosed barnacles settling in the fixed areas on verticals 2 and 3 were tested at the end of the barnacle settling season using one-way analysis of variance. Counts of the numbers of barnacles present in the areas were $\log_{10}$ transformed before analysis.

One-way analysis of variance was also utilised to test for differences in the numbers of cyprids and metamorphosed barnacles in the short term mucus effect experiments. Instead of testing both numbers of cyprids and metamorphosed barnacles at all sampling times, analysis was performed at selected intervals. For the counts of the numbers of cyprids this was done on the first four tides after the experiment was set up. The numbers of metamorphosed barnacles were tested at the end of the barnacle settling season. When analysis of variance was found to be significant multiple comparisons were performed using the Tukey test (chapter 3). All barnacle count collected for the short-term experiments was $\log_{10}(x + 1)$ transformed before analysis.

7.5.2 Results

General searches amongst the barnacle matrix were made every couple of days throughout March 1993 to search for the first settling barnacle cyprids. The first cyprids were seen on 21 March 1993 on a horizontal ledge close to vertical 2. Over the next week a few cyprids were seen settling amongst the barnacle matrix on both vertical and horizontal areas of rock. It was not until 12 May, however, that they were apparent settling in any numbers. The barnacle settling season appeared to last throughout the rest of May and early June. By 20 June 1993 few cyprids were observed settling amongst the barnacle matrix.

7.5.2.1 Long-term effects

<u>Fixed areas</u>

In the 5 x 5 cm clearance squares an average of 90.1 ± 21.4 living barnacles were removed from the control areas on vertical 2 and 108.2 ± 6.6 from the treatment areas. On vertical 3 an average of 96.1 ± 19.3 living barnacles were removed from the nine clearance squares on the control areas and 109.9 ± 10.3 from the treatment areas. There was no significant difference in the numbers of living barnacles removed from the treatment or control areas on either vertical 2 (two-sample t-test, data $\log_{10}$ transformed, t=-1.41, df=4, p=0.23) or vertical 3 (two-sample t-test, data $\log_{10}$ transformed, t=-1.10, df=4, p=0.33).

Although barnacle cyprids were first observed amongst the barnacle matrix on the Ledges at the end of March none settled in the clearance squares until 11 May 1993 when 1 cyprid was recorded in a square on an area where dogwhelks had been removed. This was six weeks after the areas had been scraped clear of adults.

The numbers of barnacles settling in the fixed areas varied on both vertical 2 and 3 on a daily basis (figure 7.22, 7.23), with the peak in settlement occurring on both verticals on the 20 May. On vertical 2 the number of cyprids settling into the cleared areas where dogwhelks had been removed was consistently higher (figure 7.22). Consequently this produced a cumulative effect where the numbers of metamorphosed barnacles in the squares at the end of the experiment were higher. This difference, however, was not significant (table 7.22). The numbers of settling cyprids and metamorphosed barnacles in the fixed areas on vertical 3 were very similar in the control and treatment areas over the settling season (figure 7.23). Consequently at the end of the experiment there was no difference in the numbers of barnacles in the treatment and control areas (table 7.22).

Random quadrats

Random quadrat measurements were taken on verticals 2 and 3 four times over the settlement season, the last occasion being on 22 June (vertical 2) and 24 June (vertical 3). Higher numbers of both adult and metamorphosed barnacles were recorded on vertical 3 than on vertical 2 at the end of June (figure 7.24). On vertical 3 there were significant negative correlations between the numbers of living adult barnacles and the number of newly metamorphosed barnacles in both the control (log10 (x + 1) transformed data, $r=-0.644$, $n=30$, $p<0.001$) and treatment (log10 (x + 1) transformed data, $r=0.731$, $n=30$, $p<0.001$) areas. On vertical 2, however, the final results were different. The correlation between the number of adult barnacles and the number of metamorphosed barnacles in the control areas was positive (log10 (x + 1) transformed data, $r=0.771$, $n=30$, $p<0.001$). The correlation for the dogwhelk removal areas on vertical 2 was not significant (log10 (x + 1) transformed data, $r=0.144$, $n=30$, NS).

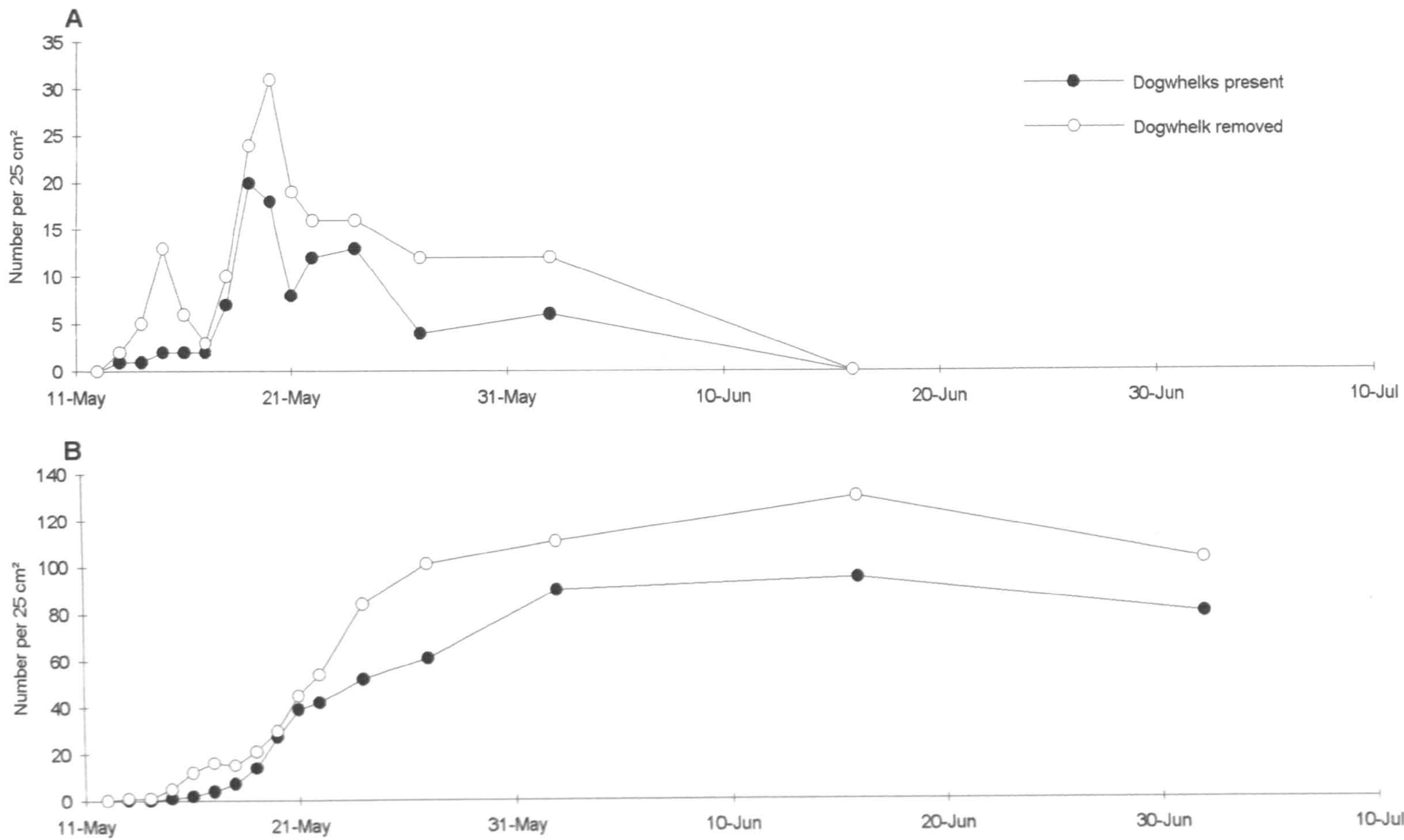


Figure 7.22 Barnacle settlement on vertical 2 in the presence or absence of *Nucella lapillus*, expressed as average numbers of cyprids (A) and metamorphosed barnacles (B) in fixed quadrats cleared of adult barnacles on 31 March 1993.

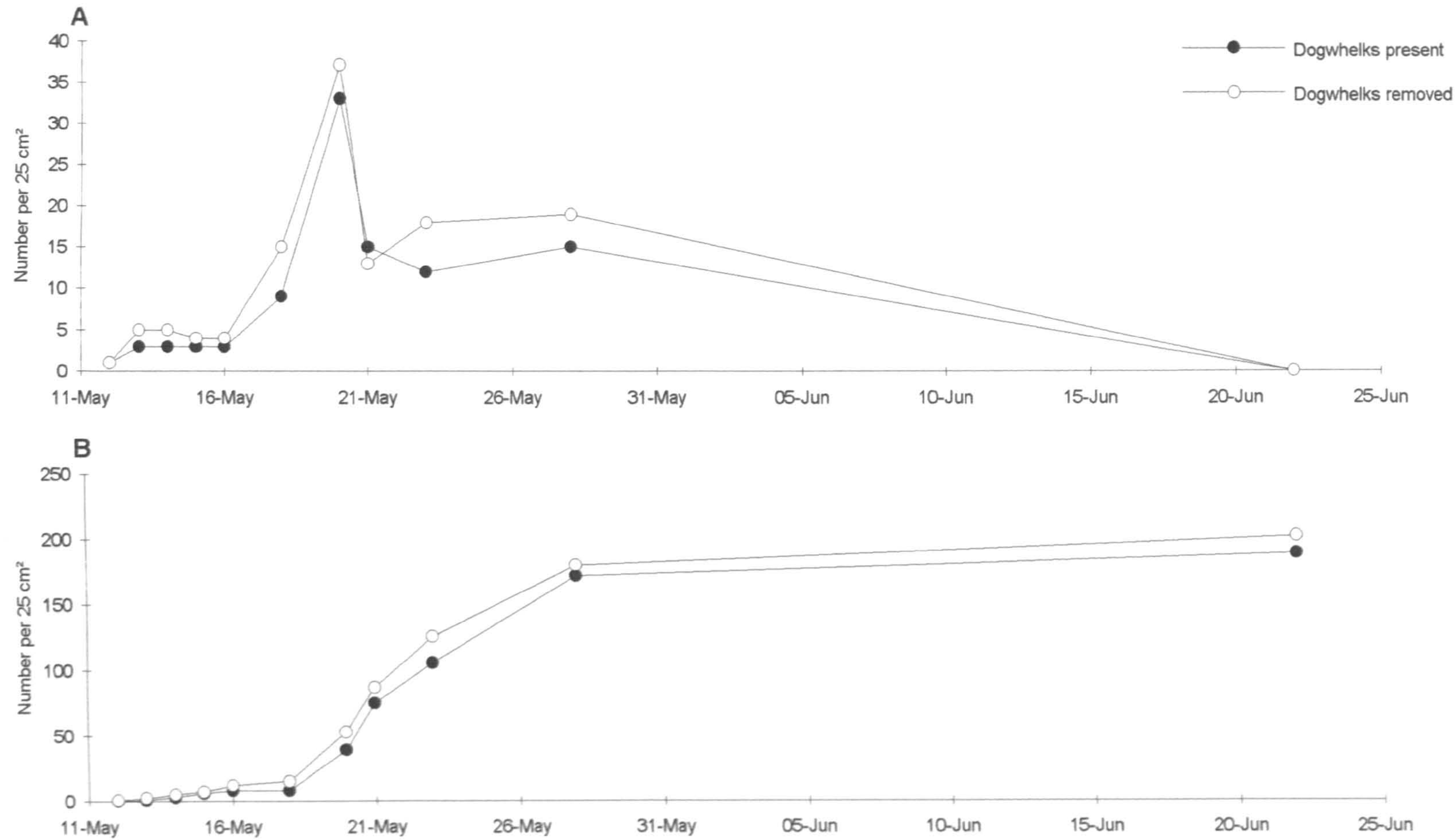


Figure 7.23 Barnacle settlement on vertical 3 in the presence or absence of *Nucella lapillus*, expressed as average numbers of cyprids (A) and metamorphosed barnacles (B) in fixed quadrats cleared of adult barnacles on 31 March 1993.

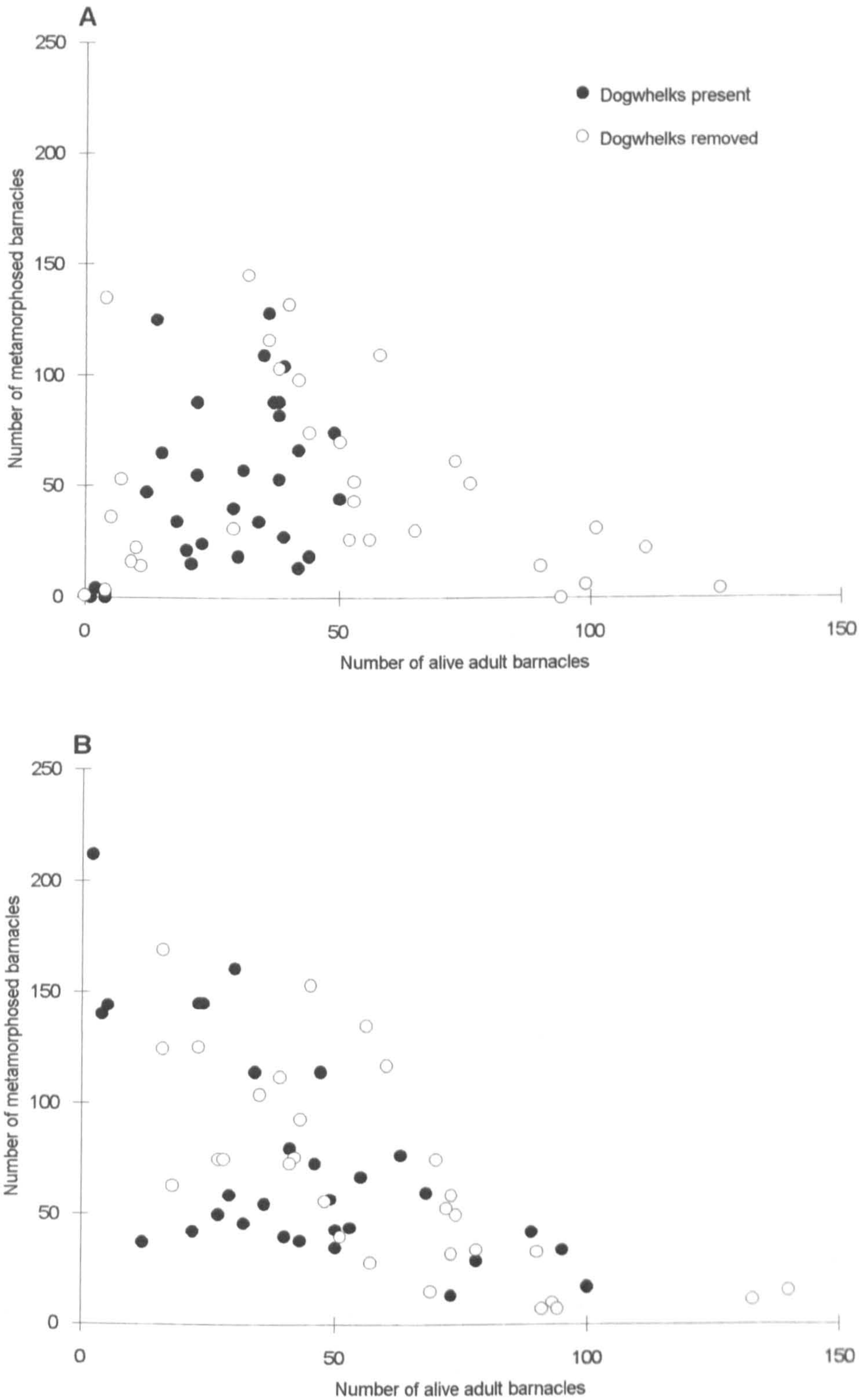


Figure 7.24 Barnacle settlement on vertical 2 (A) and vertical 3 (B) in the presence or absence of *Nucella lapillus,* expressed as the number of metamorphosed barnacles in relation to the number of alive adult barnacles in random 5 x 5 cm quadrats measured on 22 June 1993 (vertical 2) and 24 June 1993 (vertical 3).

The metamorphosed barnacles observed on the vertical rock at the end of the settling season appeared to differ in size and shape between those on the control areas and those on the treatment areas. On the treatment areas those barnacles settling amongst the existing dense barnacle matrix were relatively large and oval in shape. By comparison those on the control areas where there was fewer adult barnacles were relatively small and star-like in shape.

7.5.2.2 Short-term effects

Stones

The stones were examined on the next low tide after they had been cemented on the shore (low tide Liverpool 19:49, BST, Admiralty tide tables). One stone had been lost and no cyprids were found on any of the remaining 29 stones. On the three scraped areas nearby, however, an average of 10.0 ± 4.5 cyprids were recorded. The barnacle settlement on the stones was checked over the following six consecutive tides over which period the number of newly settled barnacles increased on the cleared horizontal areas nearby. By the 3 June the 3 cleared squares contained an average of 68.7 ± 14.3 metamorphosed barnacles in each and between 5-10 cyprids were counted in each of the areas on each tide. However, by 3 June only 0-2 metamorphosed barnacles were present in the 5 x 5 cm quadrats on the stones. Outside of the quadrats, where the surface of the stone was in some cases quite rough, up to 9 metamorphosed barnacles were recorded on any one rock where the average was 0.5 on dogwhelk stones, 0.4 on control stones and 3.2 on littorinid stones. There were no changes in the number of metamorphosed barnacles on the stones when they were sampled again on 9 June although there was now an average of 83.0 ± 5.5 metamorphosed barnacles in each of the cleared areas close by. After another week the stones started to become covered in green ephemeral algae which eventually overgrew any barnacles which had settled on them.

Cleared areas

In the second mucus effect experiment the areas were scraped clear and the dogwhelks added as the tide went out on the evening tide on 25 May 1993 (low tide Liverpool 21:08, BST, Admiralty tide tables). On return to the areas after about 90 minutes around half of the littorinids and a third of the dogwhelks had already moved off the cleared areas. On return to the site on the next low tide (low tide Liverpool 09:43, BST, Admiralty tide tables) there were no dogwhelks nor littorinids in any of the cleared areas. The littorinids had moved either to crevices close by or into intertidal rock pools on the same ledge. Most of the dogwhelks had also moved into the crevices although some were feeding on the barnacles close by. Cyprids were found in the clearance squares on the first tide after the barnacles had been cleared with an average number of 2.2 ± 0.4 in the littorinid areas, 1.8 ± 1.7 in the dogwhelk areas and 2.6 ± 0.5 in the control areas, although these were not significantly different from each other (table 7.22). The numbers of cyprids recorded in the areas reached a maximum on the 27 May (figure 7.25). On this date the highest number of cyprids were in the control areas (21.6 ± 2.9), fewer were recorded in the littorinid areas (15.0 ± 4.2) and the lowest number were found in the dogwhelk areas (12.4 ± 2.9). The numbers of cyprids recorded in the control areas were significantly higher than in either the littorinid or dogwhelk areas (table 7.22). The numbers of metamorphosed barnacles increased between the start of the experiment and 13 June at which point the highest number of metamorphosed barnacles were recorded in the areas where littorinids had been previously and the lowest were where the dogwhelks had been present. Although the numbers of metamorphosed barnacles were consistently highest in the littorinid areas and

Table 7.22 One-way analysis of variance at various sampling times on the effect of the presence of *Nucella lapillus* on the numbers of settling barnacles in long and short-term experiments.

(a) Long-term effects. Numbers of metamorphosed barnacles in fixed areas at the end of the barnacle settling season. Data $\log_{10}$ transformed.

	Date		df	MS	F	p
Vertical 2	2 July 1993	Treatment	1	0.0314	0.99	0.376 NS
		Error	4	0.0317		
Vertical 3	24 June 1993	Treatment	1	0.0154	1.89	0.241 NS
		Error	4	0.0081		

(b) Short-term effects. Numbers of cyprids and metamorphosed barnacles in cleared areas on the first 4 low tides after the start of the experiment (cyprids) and at the end of the barnacle settlement season (metamorphosed). Data $\log_{10}(x + 1)$ transformed.

Date		df	MS	F	p	Tukey test
(i) Second mucus effect experiment						
Cyprids						
26 May 1993 am	Treatment	2	0.0572	1.42	0.280	NA
	Error	12	0.0403			
26 May 1993 pm	Treatment	2	0.0752	3.50	0.063	NA
	Error	12	0.0215			
27 May 1993 am	Treatment	2	0.0704	9.40	0.004 **	Ct ≠ Dw, Lt
	Error	12	0.0075			
27 May 1993 pm	Treatment	2	0.0042	0.65	0.541	NA
	Error	12	0.0065			
Metamorphosed						
22 June 1993	Treatment	2	0.0300	1.10	0.365	NA
	Error	12	0.0274			
(ii) Third mucus effect experiment						
Cyprids						
5 June 1993 am	Treatment	4	0.1717	2.73	0.045 *	Ct ≠ Dwi
	Error	35	0.0630			
5 June 1993 pm	Treatment	4	0.2433	3.26	0.023 **	Lti ≠ Dwi
	Error	35	0.0747			
6 June 1993 am	Treatment	4	0.0247	5.32	0.002 **	Lti ≠ Dwi, Dwc
	Error	35	0.0463			
6 June 1993 pm	Treatment	4	0.1066	2.24	0.084	NA
	Error	35	0.0475			
Metamorphosed						
21 June 1993	Treatment	4	0.2422	6.19	0.001 ***	Lti ≠ Dwi, Dwc
	Error	29	0.0391			Ct ≠ Dwi, Dwc

Codes used for Tukey test: NA, not applicable (no difference); Ct, control; Dw, dogwhelk; Lt, littorinid; i, initial addition; c, continuos addition; ≠ signifies a significant difference ($p<0.05$) between numbers of barnacles in the different areas, others not different; NS, not significantly different at the $p=0.05$ level.

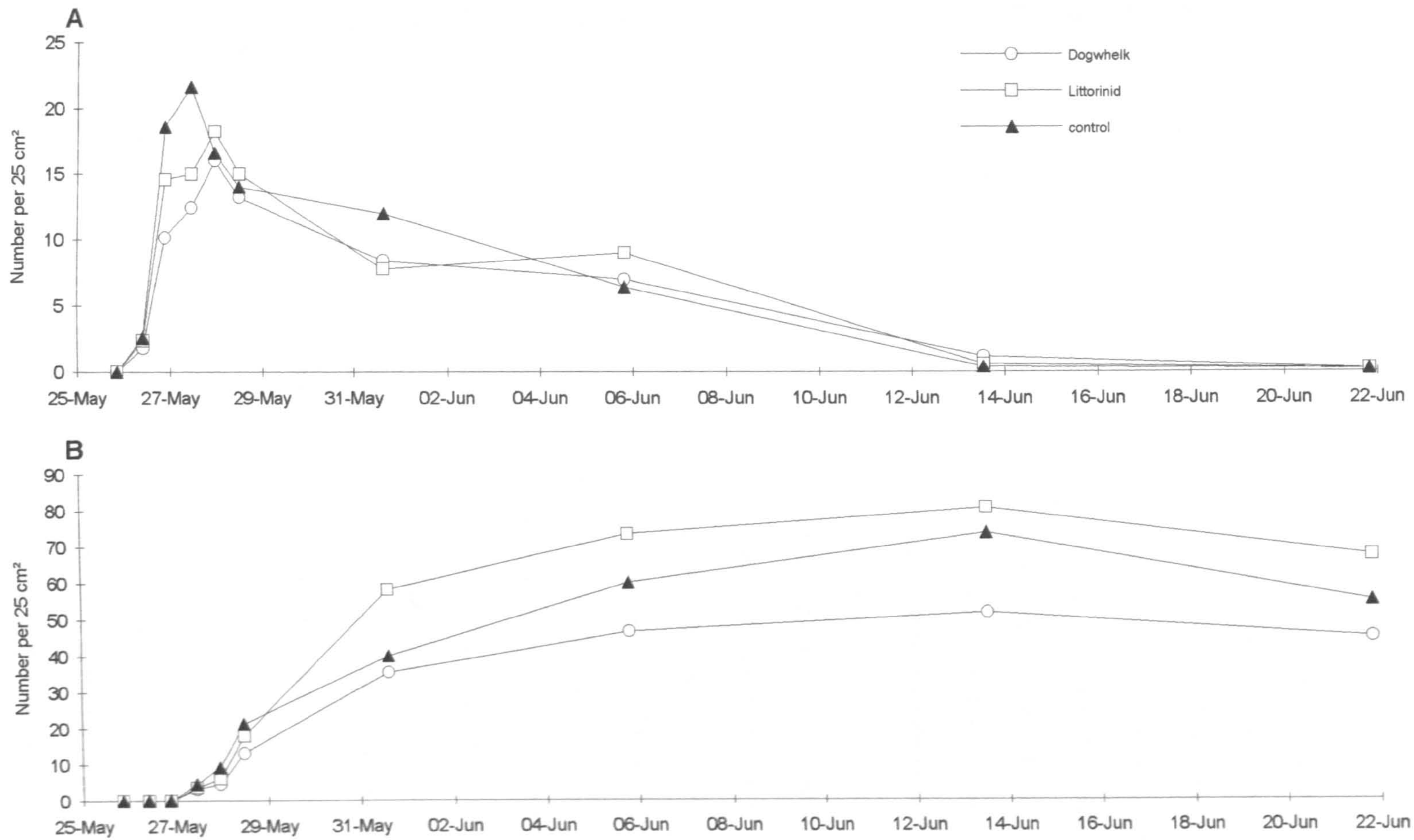


Figure 7.25 Barnacle settlement in the second experiment examining the effects of the presence of *Nucella lapillus* and *Littorina littorea* mucus on the average numbers of cyprids (A) and metamorphosed barnacles (B) in cleared areas, SE not shown to aid clarity.

lowest in the dogwhelk areas from 1 June onwards at the end of the settling season these differences were not significant (table 7.22).

In the third mucus effect experiment, as in the second experiment, dogwhelks and littorinids were added to the squares as early in the low tide period as possible to allow time for them to move away before the tide came in. This was important as there was a danger of creating a barrier effect if the dogwhelks or littorinids remained on the areas beyond the period of low tide. This was especially the case in this experiment since dogwhelks and littorinids were added to some areas on consecutive tides. However, the littorinids moved away from the cleared areas very fast with around half of them moving away before the low tide period was over. Dogwhelks moved away more slowly. On occasion some of the *Nucella* did not even attach their foot to the substrate and as the tide came in they would be rolled away from the areas. On only one occasion was a dogwhelk found in any of the removal areas on the following low tide. On the same occasion three littorinids were found in treatment squares as well. To monitor the dispersal rate of dogwhelks and littorinids from these areas they were examined at intervals after the tide had covered the squares using SCUBA equipment on 25 June 1993. On the first dive over the areas, two hours after the tide covered the removal squares, there were no dogwhelks in any of the areas. However, in one square 5 of the 10 littorinids placed there remained. On a repeat dive after another hour and a half 4 littorinids remained in one square. When the tide uncovered these squares again 3 littorinids were still present in one of the areas.

The numbers of cyprids settling on the removal areas in the third mucus experiment varied on a daily basis with the maximum settlement occurring on the 6 and 9 June. On both these occasions settlement was highest in the areas which had had littorinids on them initially (figure 7.26). The average numbers of metamorphosed

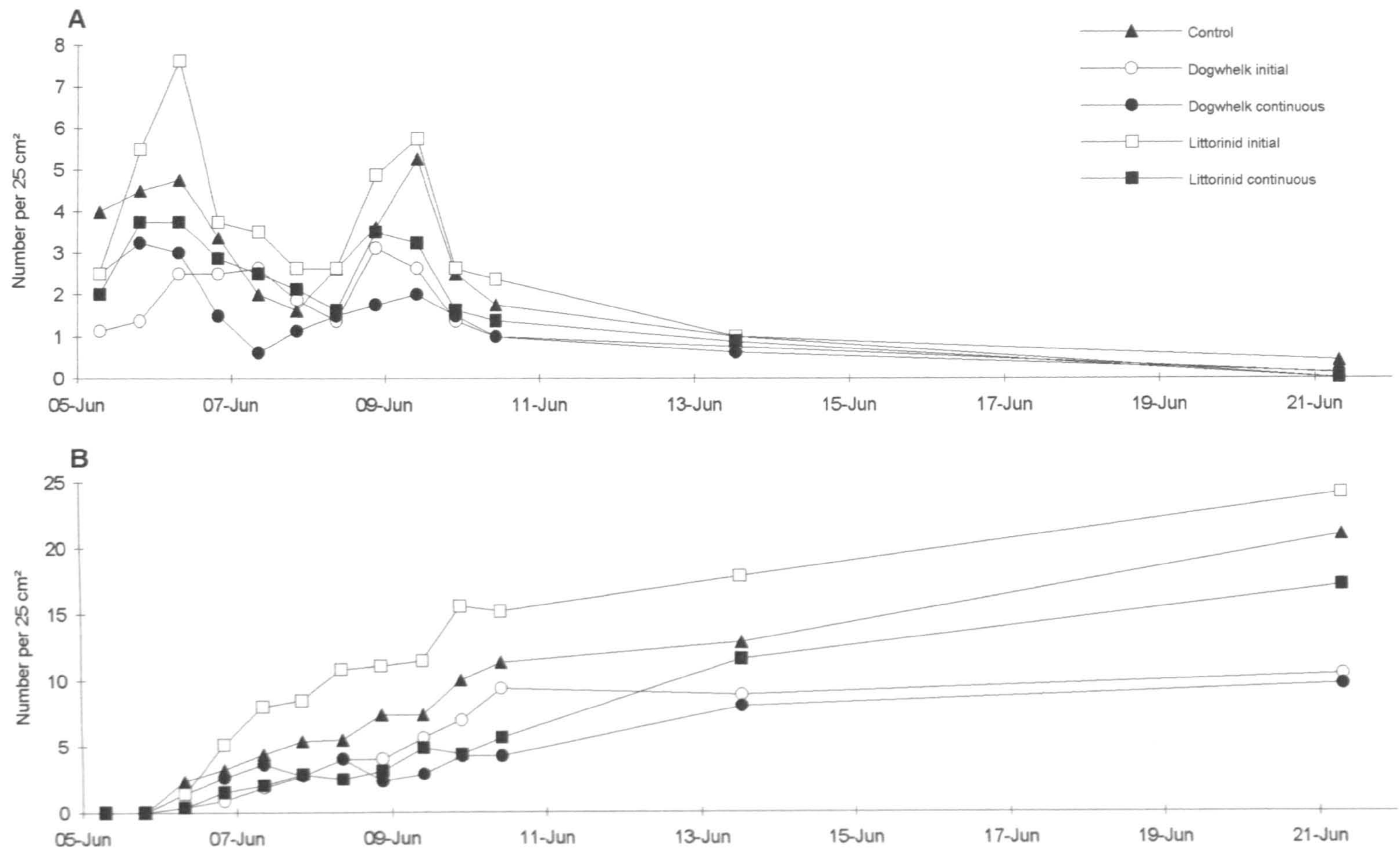


Figure 7.26 Barnacle settlement in the third experiment examining the effects of the presence of *Nucella lapillus* and *Littorina littorea* mucus on the average numbers of cyprids (A) and metamorphosed barnacles (B) in cleared areas, SE not shown to aid clarity. In the littorinid and dogwhelk continuous treatments additions were made every tide until 11 June 1993.

barnacles on the areas increased over the 16 days in which the experiment was sampled. After the first tide significantly higher numbers of cyprids were recorded in the control areas than those where dogwhelks had been added (table 7.22). On the following 2 tides the numbers of cyprids were significantly higher in the littorinid initial treatment than in the areas where dogwhelks had been added. After the 6 June the numbers of metamorphosed barnacles in the littorinid initial treatment areas was consistently higher than the control and other treatments (figure 7.26). The lowest numbers were recorded in the areas where dogwhelks had been added initially and continuously. These differences were significant with the both the control and the areas where littorinids had been added initially begin significantly higher than in either of the dogwhelk treatments (table 7.22).

7.6 Discussion

The ultimate goal in ecology is to discover general truths about how communities and ecosystems are regulated (Menge, 1991). The results from the manipulative field experiments presented in this chapter showed a great deal of variation and in some cases, especially where only a few replicates were used, results were not found to be significant even though gross changes were observed. Despite this, the effects of the removal or addition of *Nucella lapillus* from specific areas of the shore can be discussed; direct and indirect effects of predation by *Nucella lapillus* are considered and these effects compared to those of limpets. Finally the role of *Nucella* in structuring shore communities is discussed.

7.6.1 Direct effects

The fact that *Nucella lapillus* has a direct affect on barnacle populations has been well reported in laboratory and field studies (e.g. Connell, 1961a; Dayton, 1971; Dunkin & Hughes, 1984). Under favourable circumstances *Nucella* feeds selectively, avoiding the smallest barnacles and acting as an energy maximiser (Hughes & Burrows, 1991). *Nucella* thereby alters the population structure of the remaining barnacles as the large ones are taken preferentially and the small ones generally ignored (Connell, 1961a; Spence, 1989). In the absence of predation by *Nucella* Connell (1961a) observed that *Semibalanus balanoides* grew densely in caged areas (<50 cm^2) forming hummocked structures (Connell, 1961a). Reducing the density of *Nucella lapillus* from areas of vertical rock in this study produced comparable results where the percentage cover of adult barnacles was observed to increase and individuals formed dense hummocks. This hummock formation has been reported elsewhere, either where barnacle settlement was high (Barnes &

Powell, 1950) or on exposed shores (Southward, 1951; Southward, 1953), where *Nucella* foraging is likely to be restricted to crevices.

Environmental constraints clearly affect the behaviour of *Nucella* (Hughes & Burrows, 1993). The variation in the numbers of dogwhelks observed on a day to day basis, as recorded for the experimental verticals, is likely to be a result of changes in the weather (Connell, 1961a; Burrows & Hughes, 1989). The spatial distribution and abundance of *Nucella* also depended on environmental considerations, for example the aspect of the vertical rocks; more dogwhelks were found on the northerly facing vertical rock faces which were in the lee of the waves and faced away from the sun. This observed spatial distribution of *Nucella* helps to explain the differences in the significance of effects recorded when *Nucella* was removed from the vertical rocks used in the removal experiment. Dogwhelk densities were lowest on vertical 3 which was east facing; here the effects of dogwhelks were not significant until 10 months after they had first been removed. Dogwhelk abundance was higher on vertical 2 and higher still on vertical 1. This is reflected in the timescale of significant effects observed on the verticals, with significant effects of dogwhelk removal being observed first on vertical 1 then vertical 2.

On a shore where both barnacles and mussels are present it may be expected that *Nucella* would show a switching behaviour (Murdoch, 1969), feeding on an alternative prey to prevent the total eradication of one species. With the absence of mussels on the areas of vertical rock used in the manipulative experiments this was impossible. On some of the control areas *Nucella* effectively eliminated the adult barnacles. In April 1993 a front of foraging dogwhelks were observed to move up the vertical faces and onto the horizontal areas, probably as a direct response to reductions in the number of adult barnacles on the vertical faces in the control areas. Similar fronts of foraging dogwhelks have been observed as *Nucella* emerges from over-wintering sites (Feare, 1970b; Hughes & Burrows, 1993) and as prey becomes depleted close to refuges (Menge, 1978a; Menge, 1978b; Hughes & Burrows, 1993). Equally Connell (1961a) reported a shift in the vertical distribution of *Nucella lapillus* when food availability was reduced at one tidal level. The movement of *Nucella* onto the top of the vertical face represents a calculated risk as to obtain larger prey items they risk encountering less shelter and lower relative humidity. This is also the case when foraging away from the refuge of a *Fucus* clump or crevice. The fact that this move towards feeding on the top of the vertical face was only observed in treatment areas on verticals 1 and 2 reflects the scarcity of prey in these areas in relation to those on vertical 3.

In control areas where dogwhelks had eaten virtually all the adult barnacles, *Nucella* were observed feeding on newly metamorphosed barnacles which had settled six weeks earlier. This has been observed elsewhere when adult barnacles are not available (Connell, 1961a). When prey became scarce under the control *Fucus* clumps it is possible that *Nucella* fed on *Littorina obtusata* as they do on sheltered shores in the absence of barnacles or mussels. This may explain the lack of *Littorina obtusata* under control *Fucus* clumps and the differential cover of ephemeral green algae on the *Fucus* plants in treatment and control clumps in the experiment.

In most cases it is unlikely that the significant effects of *Nucella* on barnacle populations would be long-lived. On verticals 2 and 4, for example, the significant effects of *Nucella* in control areas on the percentage cover of living barnacles were effectively eliminated after the settlement of barnacles in 1993. The differences in percentage cover of living barnacles on the control and treatment areas on vertical 1, however, was much greater and was observed for longer. Even after the settlement of barnacles in 1993 there was a significantly lower percentage cover of barnacles in the control areas.

Apart from predation, other direct effects of *Nucella* on barnacles are seen during settlement. Barnacles use environmental cues to assess potential settlement sites. These cues may be associated with choosing favourable sites (Connell, 1961a; Crisp, 1985; Raimondi, 1988) or avoiding particular habitats (Moyse & Hui, 1981). Johnson and Strathmann (1989) showed that the settlement of the barnacle *Balanus glandula* in the field was reduced on slate tiles that the predatory whelk *Nucella lamellosa* had previously occupied.

Prior occupation of *Nucella lapillus* in cleared area in the barnacle matrix was shown to have a significant effect on the level of settlement of *Semibalanus balanoides*. However, these results were very variable. As a consequence the first of the two experiments examining the effects of *Nucella* on settlement in the short-term, where there were only five replicate areas of each control and treatment, only showed a significant effect on the tide when the maximum numbers of cyprids settled. The use of eight replicates in the second experiment gave a greater level of statistical power.

The prior occupation of the cleared areas by *Nucella lapillus* may have changed the substratum in three ways to make the site less attractive to settling barnacles. The presence of *Nucella* may have firstly removed something from the substrate; secondly may have added something; or thirdly have disrupted or changed the area in some way. The most likely hypothesis is that *Nucella* left behind some substance which affected the settlement of barnacles into the cleared areas. Whatever the change is it is likely to originate from the mucus secreted as individuals moved away rather than be an alteration of the surface by the mechanical action of their movement across the areas or the physical presence of the mucus. This is likely because, although the presence of *Nucella* was observed to be inhibitory, the presence of *Littorina* by comparison was found to be attractive.

It can be hypothesised that the reasons for the inhibitory effect of *Nucella* is that barnacles are taking into account the long-term future risks of settling in an area where there are potential predators ('the ghost of predation future' - Johnson & Strathmann, 1989). The reason why the presence of *Littorina* makes a substrate more attractive to settling cyprids is less obvious, although it is possible that the presence of a grazer suggests that barnacles are less likely to be smothered by algae. Johnson and Strathmann (1989) also showed that the presence of other species could be attractive to barnacle settlement. For example the presence of the nudibranch *Archidoris montereyensis* increased the settlement of *Balanus glandula*. However, they suggested the reason for this was a change in the experimental protocol used rather than attractiveness on the part of the nudibranch.

The continuous addition of *Nucella* and *Littorina* did not have a significantly greater affect than the single addition of either at the start of the experiment. There was little evidence to suggest that settlement had been influenced by a barrier effect created when dogwhelks and littorinids were added to the areas continuously. Higher numbers of settling cyprids were recorded when littorinids were added continuously than when *Nucella* was added initially. It is possible that the presence of newly metamorphosed barnacles (Wethey, 1984) which had settled on the cleared areas provided a stronger attraction to settling cyprids than the continuous presence of dogwhelks constituted a deterrent.

The lack of settlement in the first experiment using stones cemented to the shore is unlikely to be a result of the presence of *Littorina* or *Nucella* on the surfaces. It is more likely to have been due to the flat smooth surface topography of the stones used (Wethey, 1986). These surfaces were fresh and had not been conditioned first consequently no biofilm would have been present on the surface. The presence of lipids and polysaccharides which make up this biofilm layer are known to be attractive to settling cyprids. Settlement in the cleared areas amongst the barnacle matrix close by was high by comparison, but these surfaces had the added benefit of the attraction of adult barnacles which had been cleared from the areas on the previous tide. The fact that the stones stood proud of the natural surface may have affected the surface drying of these areas and consequently also reduced their attractiveness for settlement.

The removal of barnacles to create fixed areas on verticals 2 and 3 purposely chose areas of the barnacle matrix where there were similar numbers of adults. This was designed to eliminate the differences in attractiveness of different levels of cues to the cyprids in order to observe whether presence of *Nucella* had an effect on settlement over a longer timescale. Although slightly more barnacles were removed from the treatment than control areas these differences were not significant. In addition the removal of the adults six weeks before the first cyprids started to settle should have eliminated any effects of slight differences because chemical cues are short lived (Wethey, 1984). Settlement in the fixed areas on verticals 2 and 3 was higher in the treatment (dogwhelk removal) areas, although these differences were not significant. Unlike the experiments examining effects of the prior occupation of the substrate by *Nucella* where individuals were placed into clearance squares it is possible that no dogwhelks entered these cleared areas on the vertical rock, instead they may have been close by. This suggests that the inhibitory effect of the presence of *Nucella* was a direct consequence of contact with mucus left by *Nucella* and the general presence of *Nucella* in the area has little effect.

The random survey on vertical 3 showed there to be a negative correlation between the numbers of newly metamorphosed barnacles and the number of adult barnacles. This suggests that there was a positive relationship between settlement and the amount of bare rock present. The fact that there appeared to be little difference in the level of association of metamorphosed and adult barnacles in the treatment and control areas suggests that the presence of dogwhelks on this experimental vertical face had little effect. On vertical 2 the relationship was different. Numbers of settled metamorphosed barnacles showed a positive relationship with the numbers of adult barnacles on the control areas. An effect likely to be a direct result of the large amount of bare rock on the vertical areas. Instead of showing correlations with the percentage of bare rock the relationship was strongest with the numbers of conspecifics. On areas on vertical 2 where dogwhelks had been removed there was, surprisingly, no significant correlation between the numbers of metamorphosed barnacles and the number of adult barnacles. The reasons for this are unknown.

7.6.2 Indirect effects

Most theoretical studies on the effects of predators emphasise direct effects (Sih *et al.*, 1985). In this study direct effects of *Nucella lapillus* on *Semibalanus balanoides* have been shown but some indirect effects were also apparent.

The level of effect of *Nucella* on barnacles has a knock-on effect which is indirectly related to predation pressure on the barnacles. A reduction in the predation rate has a direct effect on the barnacles which was demonstrated in the formation of densely packed barnacles seen on the shore. Such high barnacle densities reduce the foraging efficiency of *Patella* (Hawkins, 1981a; Hawkins, 1981b; Hawkins & Hartnoll, 1982a) allowing vulnerable algal germlings to escape grazing (Hawkins &

Hartnoll, 1983; Hartnoll & Hawkins, 1985). Fucoid algae have a tendency to become established more readily among barnacles than on bare rock (Menge, 1976; Hawkins & Hartnoll, 1983). *Fucus vesiculosus* was observed on the treatment areas on vertical 1. This vertical showed the significant effects of dogwhelk removal before verticals 2 and 3. *Fucus* formed from amongst the barnacle matrix on verticals where dogwhelk had been removed. *Enteromorpha* did not develop on the areas where dogwhelks had been removed.

Amongst the *Fucus* clumps which formed the experimentally reduced predation on the barnacles underneath the clump allowed the clump to grow to a larger size and higher density. Elsewhere the presence of an algal canopy was shown to increase the feeding of predatory snails (Menge, 1978a). Connell (1961a) suggested that similar mechanisms might contribute to the reduction of barnacle abundance under fucoid canopies in the British Isles.

By feeding on the barnacles that the *Fucus* clump is attached to *Nucella* effectively undermines the roots of the clump which is consequently more likely to be removed from the shore. The force required to remove plants attached to dead barnacles was shown to be much lower than that required to remove similar sized plants from either bare rock or living barnacles. Similar results were found by Barnes and Topinka (1969) in the force required to remove *Fucus vesiculosus* from barnacles and bare rock. They reported that there was a less effective adhesion to the shells of living barnacles than to bare rock. When they measured the force required to remove *Fucus* plants they were measuring the separation of the holdfast from the barnacle shells. In this study however the force required to remove the *Fucus* plants from the attachment of dead barnacles was not the force separating the barnacle and the algae, but the force removing the empty barnacle husk from the shore. Although the loss of a *Fucus* plant from the shore is not dependent on the barnacle being killed (Barnes & Topinka, 1969) the lower force required to remove *Fucus* from dead barnacles as seen here suggests that the plants are more readily lost from areas where *Nucella* has been feeding.

The changes in the size and density of the *Fucus* clump has a knock-on effect for other species: the abundance of *Actinia equina*, for example, showed a direct relationship with the clump size. In *Fucus* clumps where plants had thinned dramatically *Actinia* were observed moving away from clumps. There is a direct relationship between relative humidity and increasing plant size and density in the *Fucus* clump. Relative humidity was higher in the clumps than on the rock. This is of importance for organisms which rely on the shelter the *Fucus* clump provides.

Another indirect effect of *Nucella* predation is the creation of microhabitats (Hughes & Burrows, 1993). The method of attack that *Nucella* uses leaves prey firmly attached to the rock. This provides a habitat for an assemblage of crevice dwellers. Some, such as *Littorina neglecta*, have an almost obligate relationship with empty barnacle shells on certain shores (Raffaelli, 1978). Although empty shells may reflect different sources of mortality a large proportion result from predation by *Nucella lapillus* (Connell, 1961a).

7.6.3 The relative importance of *Nucella*

Both *Nucella* and *Patella* exert effects on the barnacle population. The relative importance of *Nucella* in relation to effects of *Patella* were examined in the factorial removal experiment on vertical 4. The effect of *Nucella* was observed to be significant although less so that of *Patella*. The removal of limpets either experimentally (Jones, 1948; Lodge, 1948), after pollution effects (Southward & Southward, 1978; Hawkins & Southward, 1992) or red tides (Southgate *et al.*, 1984), produces a dense *Fucus* canopy. *Nucella* moderates the level of the limpets effect. Removal of both dogwhelks and limpets produced greater algal cover than removal of limpets alone where dogwhelks were still present. The interaction occurring between *Nucella* and *Patella* is an example of indirect mutualism proposed by Dungan (1987) as one of the consequences of non-trophic effects on rocky intertidal communities. Basically the presence of *Nucella* removes barnacles which is beneficial to *Patella* (Hawkins & Hartnoll, 1982a) and the presence of *Patella* in turn means there is less algae which in turn means there are more barnacles for *Nucella* to feed on. Algal grazing especially by limpets appears to increase the abundance of barnacles by freeing space on the rock surface and thereby enhancing settlement by preventing algae from over growing barnacles or by eliminating frondose algae which sweep the surface and impede recruitment (Jones, 1948; Hawkins, 1983; Hawkins & Hartnoll, 1983).

Although *Nucella* was shown to have a significant effect on the size and density of *Fucus* clumps, the winter storms in December 1992 and January 1993, before the experimental manipulations began in earnest, undoubtedly had a greater effect on the *Fucus* clumps than that of *Nucella* over the following 8 months. Those clumps which had accumulated a large amount of shell debris were destroyed to the greatest extent by these storms. The barnacles underneath had been smothered and scoured which caused virtually the whole *Fucus* clump to be removed as the barnacles underneath were killed. Ultimately any catastrophic effect either biological or physical, which kills barnacles in multiples is likely to have a greater effect that of *Nucella* which kills of barnacles individually. This situation has been shown on the west coast of America where carnivorous gastropods appear to have little effect on this diverse community instead predators such as the starfish *Pisaster ochraceus* play a more important role because of the level of disturbance and the amount of primary space they create as they can remove 20-60 barnacles from the shore simultaneously (Paine, 1966).

7.6.4 The role of *Nucella lapillus* in structuring communities

Nucella has an important effect on the population dynamics of barnacles. On the Isle of Man this effect is localised, being restricted to crevices or areas with suitable refuges (e.g. vertical rocks with a specific orientation or *Fucus vesiculosus* clumps). Even though a significant effect can be shown on barnacle populations this does not illustrate that *Nucella* has a critical influence on the community as a whole. In the absence of *Nucella* the dense hummocks of barnacles that form will eventually become unstable and be washed off the rocks (Connell, 1961a and personal observations). This is an intrinsic property of the barnacle population itself (Barnes & Powell, 1950) together with stochastic elements which could generate a patchwork of bare rock and barnacles, as observed on treatment areas on verticals 1 and 2. In the absence of *Nucella* this would be kept in equilibrium by the annual recruitment of new barnacles. The presence of *Nucella* spreads the cycle of replacement by cropping the larger barnacles creating a more homogenous barnacle cover over the shore. On broken and fissured shores the direct effects of *Nucella* may be less obvious since instead of being restricted close to crevices the dogwhelks are likely to be evenly distributed over the whole shore preventing the localised extinction of prey as seen on the moderately exposed shores on the Isle of Man. As a consequence when the effect of *Nucella* is taken into account over the whole shore the reduction in the level of predation pressure exerted by *Nucella* may be equivalent only to a particularly high settlement of barnacles.

On moderately exposed shores on the Isle of Man *Nucella* has some affect on three main stages in the cycle of dominance occurring between *Fucus* and barnacles; the formation of *Fucus* clumps, the dispersal of *Fucus* clumps and the settlement of barnacles. The absence of *Nucella* effectively speeds up some sections of the cycle and slows down others, creating a shift towards the *Fucus* part of the cycle when denser barnacle assemblages are formed as a result of reduced predation.

The effects of predation on communities vary widely (Menge, 1978a). In order to totally understand the role of predation in structuring shore communities world-wide studies must be carried out (Underwood, 1985). These experiments help explain some of the interactions of *Nucella* in the spatial and temporal fluctuations that occur on the shore at Port St. Mary on the Isle of Man. Care should be taken in extrapolating these findings to moderately exposed shores around the UK however since these the results obtained are likely to have been highly influenced by the differences in the abundance of *Nucella* which is in turn affected by a number of environmental factors.

Communities on the west coast of America are more diverse than those on the north-eastern coast (Menge & Sutherland, 1976). The region is structured largely by predation which is responsible for maintaining a high species richness (Menge & Sutherland, 1976). However, since the whelks penetrate only one barnacle at a time they do not have as dramatic effect as the starfish which can remove 20-60 barnacles from the shore simultaneously. Even at high whelk densities the effect is not as dramatic because the empty husks of the barnacle are left on the shore (Paine, 1966).

7.6.5 Conclusions

The removal of *Nucella lapillus* from different areas of a moderately exposed shore on the Isle of Man produced significant results. In the absence of *Nucella*, on areas of vertical rock, dense overcrowding of *Semibalanus balanoides* was observed with hummocks of barnacles being formed. This in turn promoted the formation of *Fucus* escapes. The removal of dogwhelks from under *Fucus* clumps increased the size and longevity of newly established *Fucus* clumps. The observed effects of *Nucella* were not as significant as those observed following the removal of limpets, but it was found that the presence of *Nucella* had a moderating effect on the effects of *Patella* and that the two organisms exhibited indirect mutualism. The level of any effects seen were dramatically influenced by the density of *Nucella* which was in turn affected by factors such as the weather, the aspect of the rock surface and the frequency of crevices for shelter.

The presence of *Nucella* had a significant effect on the level of barnacle settlement. Settlement of *Semibalanus balanoides* was reduced in cleared areas which had been previously occupied by *Nucella lapillus*. This was a short term effect. In contrast the level of settlement in areas where *Nucella* had been present or removed from the general area was not different suggesting that the inhibitory effect observed in the other experiments works only in when cyprids come into direct contact with mucus produced by *Nucella*.

CHAPTER 8

General Discussion

This chapter considers three main themes: bioindicators on rocky shores and different levels of organisation, recovery from pollution events and the reasons why no community level responses were observed in response to tributyltin pollution. In addition suggestions for further work are included, which have emerged from the work presented in chapters 4-7.

8.1 Bioindicators and levels of organisation on rocky shores

Many rocky shore organisms have been used as bioindicators (Bryan, 1984; Bryan *et al.*, 1985). In addition they have been used as sources of bioindicator molecules such as metallothioneins (e.g. *Littorina littorea*: Langston & Zhou, 1986), as providers of cellular indices (e.g. *Littorina littorea*: Moore *et al.*, 1982), or as indicators at the individual level (e.g. scope for growth in *Mytilus edulis*: Widdows *et al.*, 1980). Clearly bioindicators at lower levels of organisation correlate more directly with environmental levels of a known stressor than those at higher levels. Difficulties abound at the population or community level. This is especially so in species with recruitment through planktonic larvae, such as *Mytilus*, *Patella vulgata* or *Littorina littorea*, which can colonise contaminated sites from unaffected populations (see comments in Underwood, 1989; Underwood & Fairweather, 1989). Numbers settling from the plankton may render negligible the reduction in abundance or reproduction in a population at a polluted site (Underwood & Peterson, 1988). As a result of the large scale dispersal of many marine species the continued chronic pollution of a relatively small geographic locality may have negligible consequences for most local populations, however long the pollutants continue to enter the system. This will only occur if the pollutants do not kill the newly recruited larvae as they arrive in the area (Underwood & Peterson, 1988). That such a marked population level effect of tributyltin has been seen in *Nucella* is due to the nature of the individual level effect which interferes with the reproductive system and the inability of populations to recruit from elsewhere as a result of the direct developmental mode exhibited.

Ultimately the utilisation of measures of pollution at different levels of organisation is an important strategy since such measures serve different purposes (Underwood, 1989). Cellular and individual levels of monitoring provide early warnings of future deterioration and may be the most sensitive. Population and community measures are, in contrast, better indicators of the consequences of pollution to the processes of economic and social values vested in a marine ecosystem (Underwood, 1989).

There are, however, problems with using bioindicators and one bioindicator is not necessarily suitable for all occasions. Certain organisms (e.g. dogwhelks) can be very sensitive indicators of particular contaminants (e.g. TBT) but not others (e.g. arsenic which they regulate: Klumpp, 1980). Broad-scale larval dispersers (e.g. mussels, *Littorina littorea, Patella vulgata*) are often excellent indicators at the sub-individual and individual level but the unpredictability of recruitment makes population level monitoring difficult. In communities where these species occur recruitment variation makes it very difficult to separate natural fluctuations from any pollution effects unless the impacts are gross, such as in massive oil spills. Ironically, barnacles showed themselves to be good indicators of global change but have shown little discernible response to *Nucella* decline along the south coast (Southward, 1991). One of the major problems with measuring the level of pollution at levels of organisation lower than the population is that they are usually assessed on only one species (Underwood & Peterson, 1988) and as a consequence are difficult to relate to community effects.

Despite the above considerations it is clear that studies at different levels of organisation are very important since understanding the level of effect at, for example, a cellular level promotes a greater understanding of consequent effects at the individual level. A considerable amount of effort has gone into developing ever more sensitive sub-individual indicators of contamination (e.g. George & Langston, in press; Pulsford *et al.*, in press). These are superb early warning systems and can also give an idea of the furthest extent of sub-lethal effects of a point source or a generalised effect within a contaminated area. These sensitive indices are often criticised as being over specific (the 'so what' syndrome). For a lay person it is easier to accept an ecological impact if there are fewer of a species (population level) or if species composition changes (community level).

At the population level the best documented example of bioindicators on rocky shores is the response of *Nucella lapillus* to TBT, a species with direct development (Fretter & Graham, 1962; Fretter & Graham, 1984). No similar effects have been found in other marine systems which give a response so specific as to affect the reproduction of one species over such a wide geographical area. In soft benthos organisms are surrounded by sediments which act as sinks to pollutants. The amount of organic enrichment of the sediment will affect deposit feeding infauna and in extreme cases render the sediments and the surface layer of the water anoxic, so effects on organisms tend to be direct. In freshwater streams most pollution comes from point sources making it is easy to have an upstream control and look at dilution effects downstream of the source. The species used as indicators have a unidirectional medium flowing over them or live amongst sediments and are exposed in a more predictable manner. They are also generally short lived, and many are larval stages of insects, thus making very good community level bioassay organisms (Hynes, 1960; Wright *et al.*, in press). Rocky shore communities in contrast typically have multi-directional water movement with three dimensional mixing and dispersal. Large fluctuations in recruitment and a community structured by intense interactions between organisms results in a variable community, often making pollution effects difficult to predict (e.g. Lewis, 1976; Underwood, 1989; Hawkins & Hartnoll, 1983).

In order to understand the effects of pollution on communities experimental manipulations are needed (Underwood, 1989). What is also required, however, is a change in the emphasis in experimental studies on stress and pollution. Currently the trend is to design experiments so that the existing effects can be explained (Underwood, 1989). According to Underwood (1989) consequences of stress on natural populations will only be understood when the natural history of organisms and their interactions are understood. The latter requires experimental investigation (Underwood, 1989) as reported here (chapter 7). Ecological studies need to move away from descriptive studies towards manipulative experiments in order to unravel the complex interactions within these communities (Underwood, 1989), a move that is now evident in the literature (Sih *et al.*, 1985). The knowledge of what is happening ecologically is the ultimate aim of all monitoring and is needed to predict 'safe levels' of pollutants (Lewis, 1976).

8.2 Recovery

A prime use of bioindicators should be in the monitoring of recovery, a fact that is often ignored. Studies of recovery are needed, for example, after banning of sale and use of organotin paints (chapters 5 and 6), but equally after catastrophic environmental disasters, for example, oil spills. Such studies are often neglected or are not funded for long enough. They are best carried out at as high a level of organisation as possible, at the ecosystem or community levels, to create a meaningful interpretation of the level or scale of effect. As an effect becomes more subtle, population or individual level responses can then be monitored using selected bioindicators.

Recovery would be expected to occur first at the sub-cellular level which would then proceed to individuals and populations (Underwood & Peterson, 1988). Consequently true recovery can only be measured at the community level. The difficulty lies in establishing what is a normal state within a community since there is considerable variation over time and space in many natural populations (Underwood, 1989). In an ideal world, measurements of the level of impact of stress within a community would use a BACI (before/after and control/impact) sampling design (Underwood, 1992). The community structure of an affected area could then be compared with measurements taken prior to pollution effects, which would allow an assessment of when the community had recovered completely. Obviously this idealised impact assessment only works when the pollution or stress incidents have been planned (e.g. a new marina development or siting a new effluent outflow).

Nucella lapillus is an excellent bioindicator with which to measure recovery. Although the effects of tributyltin pollution were obviously not planned, a BACI sampling design can be used to measure recovery since current populations of *Nucella* can easily be compared with preserved specimens from the same site and data on abundance compared with surveys carried out before the 1980's (see chapters 5 and 6).

Identification of world-wide bioindicators of imposex in neogastropods is potentially important for monitoring world-wide recovery in TBT contamination. Currently females of over 70 different species (table 1.2) have been reported as developing male sexual characteristics, although in many of these studies concentrations of TBT have not been measured in the environment (e.g. Ellis & Pattisina, 1990;

Spence *et al.*, 1990b; Kohn & Almasi, 1993). Imposex development in these individuals has still, however, been attributed to TBT, in most cases on the basis of the proximity to marinas and harbours. Based on evidence from other studies (Smith, 1981a; Smith, 1981b; Smith, 1981c; Smith, 1981d; Bryan *et al.*, 1986; Bryan *et al.*, 1988) it is highly likely that TBT is the cause. However, without studies directly relating environmental levels of TBT to the extent of imposex development, the use of these species will provide only a gross bioassay of recovery. Despite this the world-wide level of scientific and public interest in this phenomena has ensured the prompt action of Governments around the world in banning the use of these paints (chapter 1).

8.3 Why were no community effects detected in response to TBT ?

As a direct result of tributyltin pollution *Nucella lapillus* has been exterminated from many areas throughout its European range (Gibbs *et al.*, 1991c). The effects of TBT have been widely reported at levels of organisation from cellular to population (see Hawkins *et al.*, in press, chapter 1) but currently no community effects have been observed. Instead the effects of TBT on the community have only been speculated (Spence *et al.*, 1990a; Hughes & Burrows, 1993).

The most badly affected dogwhelk populations have been reported on sheltered shores (chapter 1) where dogwhelks may have a lesser ecological effect. Dogwhelk abundance changes naturally along an exposure gradient, becoming lower at sheltered sites (Moyse & Nelson-Smith, 1963; Lewis, 1964). Consequently where the TBT contamination has generally been greatest (sheltered sites and harbours) pollution effects have been acting at the edge of the dogwhelks natural range (Spence *et al.*, 1990a). Barnacles are often rare here with the few dogwhelks present seeming to feed on *Littorina obtusata*, *Littorina mariae* and juvenile *Patella* *vulgata* where barnacles are not present. Sheltered shores are characterised by being inherently stable (Hartnoll & Hawkins, 1985; Hawkins *et al.*, 1985). Only on moderately exposed shores are dogwhelks likely to have much of an effect on the community, particularly if the shore is broken or fissured (Hughes & Burrows, 1993).

It is on the south coast of England, where dogwhelks have been reduced to the greatest extent (Spence *et al.*, 1990a; Bryan & Gibbs, 1991; Gibbs *et al.*, 1991b) that we would have expected the widespread effects of TBT on the community to be seen. That these effects have not been detected (e.g. Southward, 1991) can be partly attributed to the lack of long term observations of dogwhelks in the context of the whole community on such shores. An explanation also lies in the nature of the communities themselves. On the Isle of Man, experimental removal of dogwhelks showed that effects were significant on a localised scale but less so than those of limpets (chapter 7). Subtle effects were shown such as the fact that the removal of dogwhelks enhanced the effects of limpet removal. This occurred within a reasonably simple interaction web with a species of barnacle capable of heavy settlement producing a dense population in one year (e.g. *Semibalanus balanoides*). With the more diverse communities of the south coast, the effects of removal of any one species is likely to have lesser effects, especially as space is rarely saturated by the relatively slow growing *Chthamalus* species (Burrows, 1988; Southward, 1991). Similarly, on mussel dominated shores complex interactions are likely. Unfortunately, to date, no experimental work in Europe has teased out the interactions on the types of community observed on the south coast to enable predictions, unlike those observed on the moderately exposed shores on the Isle of Man.

The lack of apparent responses does not necessarily imply that the population or community has not been affected (Underwood, 1989). A gradual reduction in the numbers of *Nucella* on some shores is unlikely to have had as dramatic a community effect as, for example, an oil spill which creates an instant effect (Southward & Southward, 1978; Hawkins & Southward, 1992). Obviously there have been some community effects even if only that there are fewer dogwhelk shells available for hermit crabs (Southward, 1991) or fewer refuges for small grazing littorinids (Hughes & Burrows, 1993). These effects may have been subtle, equally they may be still happening now.

It is important not to assume that TBT has not influenced communities purely because the results are not immediately obvious. There is a danger that, unless a community level response can be demonstrated, TBT in paints will not be banned completely in the UK. Even without an obvious community level response there is no doubt that TBT is one of the most dangerous chemicals to have been deliberately added to the environment (Goldberg, 1986) as the cellular, individual and population level effects have shown.

8.4 Suggestions for further work

The effects of tributyltin on *Nucella lapillus* are now well documented (see Bryan & Gibbs, 1991 and chapter 1). The main area in which future work should now be concentrated is the monitoring of the recovery of affected *Nucella* populations. Predictions made in chapter 5 suggested that at the present rate of recovery imposex levels in populations of *Nucella* from Plymouth Sound should reach zero by 1995. This was based on a projection of the rate of decline of imposex, measured by relative penis size, which has been observed so far. With the continued use of TBT antifouling paints on boats >25 m in length, however, it is unlikely that *Nucella* populations will be recorded as having no imposex development in areas like Plymouth Sound. Elsewhere in the UK at sites away from harbours serving large boats, it is possible that females in the population may show no development of male characteristics; this was seen in some individuals at Niarbyl on the Isle of Man (chapter 6). It is imperative that monitoring continues in order that the true timescale of recovery can be assessed, at sites which have been subjected to different levels of effect and possibly that world wide legislation be introduced to ban their use.

The ultimate measure of recovery will be the natural re-colonisation of areas such as Port St. Mary Inner Harbour (chapter 6) and the return of dogwhelk abundances to pre-tributyltin levels. In other cases where *Nucella* has been effectively eliminated, for example after an oil spill (Southward & Southward, 1978) or a red tide (Southgate *et al.*, 1984), dogwhelks have re-colonised the shore after a year. In the case of TBT, however, re-colonisation of affected shores requires not only the influx of dogwhelks from nearby sites but also a sufficient decline in environmental concentrations of TBT to levels where juveniles can survive.

The recovery of one *Nucella lapillus* population will be of special interest. One dogwhelk enclave at Dumpton Gap, Kent, has survived despite those from sites close by being wiped out (Gibbs, 1993). This population appears to have a genetic disorder which leads to the incomplete development of the male genital system - the 'Dumpton syndrome' (Gibbs, 1993). It is estimated that around 10% of males in this population are affected, showing non-development or under-development of the penis. This defect is also reflected in the development of imposex in the female and as a result the effect of imposex is lessened, allowing breeding to continue in some females (Gibbs, 1993). As environmental concentrations of TBT decrease (chapter 4) the breeding capability of this population will be reduced in comparison to populations unaffected by the Dumpton syndrome.

At the community level the work carried out on barnacle dominated moderately exposed shores on the Isle of Man showed the role of *Nucella* to be significant although less important than that of *Patella vulgata*. On other similar shores the subtle effects of reduced numbers of *Nucella* may also be expected. These subtle effects, however, are likely to have been masked by fluctuations in the larval supply to these shores. Other rocky shores may have been affected to differing extents and the role of *Nucella* may be more important. For example research on the patch dynamics of mussel shores in the UK is urgently needed (Hawkins *et al.*, in press) as they differ markedly from other mussel dominated shores investigated elsewhere (e.g. Dayton, 1971; Menge, 1976); limpets are likely to play a more important role in the north-east Atlantic. Even if community level effects are not observed in response to reduced abundances of *Nucella lapillus* effects may be seen within other shore communities world-wide. Currently sterility has been reported in 9 of the 70 or so neogastropod species in which imposex has been observed (chapter 1). Population level effects like those observed in *Nucella lapillus* will be masked in those species which have planktonic dispersal phases. In others, however, community level effects may be seen.

The avoidance behaviour of *Semibalanus balanoides* of a substrata previously occupied by a mobile predator has not been explored to its full potential. Currently only the work of Johnson and Strathmann (1989) on *Balanus glandula* and *Nucella lamellosa* have shown any similar effects.

8.5 Conclusions

Population level bioindicators are attractive because tangible effects can be demonstrated but they are rare in coastal ecosystems, especially on rocky shores. Dogwhelks are a particularly good example, although only as bioindicators for tributyltin pollution. At the community level rocky shores are highly variable and hence often unsuitable for monitoring subtle effects. They do have distinct advantages when monitoring recovery from catastrophic events because they are easy to sample non-destructively and the interactions within some of the communities are reasonably well understood. In the UK there are still some major gaps in knowledge, however, particularly in communities dominated by *Mytilus edulis* on wave-beaten rocky shores. Offshore or intertidal soft benthos provides better communities for demonstrating community level effects probably because the impacts, whether they be organic enrichment or toxic compounds, end up in the sediments which act like a sink, hence the closely associated infauna are directly affected. On rocky shores the lack of infauna and the mixing of the water column result in impacts being less direct. Any impact tends to be masked by fluctuations in larval supply from remote sources.

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Appendices

A The location of water sampling sites around the Isle of Man with grid references and site numbers corresponding to those in figure 6.1.

B The sites used in the Isle of Man imposex survey, with grid references and the dates sampled by Spence and Hawkins (1988), Bell (1990) and for the present survey.

C Imposex survey results for adult *Nucella lapillus* sampled around the Isle of Man in 1991 and 1993.

D Imposex survey results for second year *Nucella lapillus* sampled around the Isle of Man in 1991 and 1993.

E Imposex survey results for juvenile *Nucella lapillus* sampled around the Isle of Man in 1991 and 1993.

Appendix A

The location of water sampling sites around the Isle of Man with grid references and site numbers corresponding to those in figure 6.1.

Site number	Site name	Grid reference
1	Ramsey main Harbour	SC 452947
2	Ramsey below road bridge	SC 450946
3	Ramsey Harbour mouth	SC 454946
4	Douglas ferry birth	SC 387752
5	Douglas Harbour	SC 378752
6	Douglas slipway	SC 386751
7	Port St. Mary inner Harbour (low tide)	SC 211669
8	Port St. Mary inner Harbour	SC 210675
9	Port St. Mary outer Harbour	SC 212674
10	Port St. Mary Ledges	SC 209670
11	Port Erin	SC 193690
12	Niarbyl (low tide)	SC 211776

Appendix B

The sites used in the Isle of Man imposex survey, with grid references and the dates sampled by Spence and Hawkins (1988) (survey 1), Bell (1990) (survey 2) and for the present study (surveys 3-4).

Site	Grid reference	Survey 1	Survey 2	Survey 3	Survey 4
Peel below harbour wall	SC 242857	Jan-87	Feb-90	Jan-91	Jul-93
Peel south of castle	SC 241855	Jan-87	Feb-90	Jan-91	Jul-93
Niarbyl	SC 210776	Jan-87	Nov-89	Jan-91	Jul-93
Port Erin swimming pool	SC 195694	Jan-87	Mar-90	Nov-90	Aug-93
Port Erin Raglan	SC 191691	Jan-87	Mar-90	Nov-90	Aug-93
Port Erin Breakwater	SC 189691	Jan-87	Mar-90	Dec-90	Aug-93
Calf Sound	SC 172667	Jan-87	Mar-90	Oct-90	Apr-93
Port St. Mary Ledges 1	SC 209669	Apr-87	Nov-89	Nov-90	Apr-93
Port St. Mary Ledges 2	SC 212674	Jan-87	Nov-89	-	Jul-93
Port St. Mary Outer harbour	SC 213674	Jan-87	Nov-89	Nov-90	Jul-93
Port St. Mary Inner harbour	SC 210676	Jan-87	Nov-89	Nov-90	Jul-93
Castletown harbour mouth	SC 267674	Apr-87	Jan-90	Apr-91	Aug-93
Castletown +100m	SC 268675	Apr-87	Jan-90	Apr-91	Aug-93
Castletown +200m	SC 269676	Apr-87	Jan-90	Apr-91	Aug-93
St. Michaels Isle cove	SC 296673	Apr-87	-	Apr-91	Jul-93
St. Michaels Isle jetty	SC 193674	Apr-87	Nov-89	Apr-91	Jul-93
Derbyhaven	SC 293681	May-87	Feb-90	Apr-91	May-93
Port Grenaugh	SC 316704	Apr-87	-	Apr-91	Aug-93
Port Soderick	SC 348725	Apr-87	Jan-90	Apr-91	Aug-93
Douglas below lighthouse	SC 389748	Apr-87	Jan-90	Apr-91	Aug-93
Douglas outside harbour	SC 386754	Apr-87	Jan-90	Apr-91	-
Laxey	SC 446837	Jan-87	Feb-90	Jan-91	-
Port Lewaigue	SC 469931	Apr-87	-	Apr-91	Aug-93
Ballure	SC 458936	Apr-87	Mar-90	Apr-91	Aug-93

Appendix C

Imposex survey results for adult *Nucella lapillus* sampled around the Isle of Man in 1991 (i) and 1993 (ii). Penis size given in mm; ns, not sampled; nf, none found.

Site		n	% female	Male penis size	Female penis size	RPS	Median VDS	VDS range	% sterile
Peel below harbour wall	i	44	56.8	4.0 ± 0.2	1.0 ± 0.4	1.4	4	3-4	0
	ii	22	68.2	4.0 ± 0.2	0.9 ± 0.5	1.1	3	2-4	0
Peel south of castle	i	37	48.6	4.0 ± 0.2	0.8 ± 0.3	0.7	4	2-4	0
	ii	12	58.3	4.1 ± 0.8	0.3 ± 0.4	0.1	2	1-3	0
Niarbyl	i	59	51.0	4.0 ± 0.1	0.1 ± 0.2	0.0	1	0-3	0
	ii	20	40.0	4.2 ± 0.3	0.0 ± 0.1	0.0	2	0-3	0
Port Erin swimming pool	i	48	41.7	3.6 ± 0.4	1.8 ± 0.4	13.2	4	4-5	25
	ii	11	58.3	4.2 ± 0.3	0.7 ± 0.5	0.5	3	2-3	0
Port Erin Breakwater	i	48	54.2	3.7 ± 0.5	1.1 ± 0.3	2.5	4	3-6	3.8
	ii	10	80.0	4.0 ± 0.1	0.4 ± 0.5	0.1	2.5	2-4	0
Calf Sound	i	35	48.6	4.4 ± 0.4	0.5 ± 0.5	0.2	3	1-3	0
	ii	34	67.6	4.4 ± 0.4	0.7 ± 0.3	0.3	3	2-4	0
Port St. Mary Ledges 1	i	128	52.3	3.8 ± 0.3	0.8 ± 0.3	0.9	4	2-4	0
	ii	57	36.8	3.9 ± 0.2	0.7 ± 0.3	0.6	3	2-4	0
Port St. Mary Ledges 2	i	ns	ns	ns	ns	ns	ns	ns	ns
	ii	23	60.9	3.9 ± 0.2	0.8 ± 0.4	0.8	3	1-4	0
Port St. Mary Outer harbour	i	13	8.3	3.9 ± 0.4	2.3	19.7	5	5	100
	ii	nf	nf	nf	nf	nf	nf	nf	nf
Port St. Mary Inner harbour	i	8	12.5	4.0 ± 0.4	3.1	45.5	6	6	100
	ii	nf	nf	nf	nf	nf	nf	nf	nf
Castletown harbour mouth	i	21	38.1	4.1 ± 0.3	1.5 ± 0.4	4.6	4	3-4	0
	ii	13	61.5	4.2 ± 0.3	0.8 ± 0.3	0.6	3	3-4	0
Castletown +100m	i	39	43.6	4.0 ± 0.2	1.0 ± 0.4	2.0	4	3-5	5.8
	ii	12	58.3	3.9 ± 0.2	0.4 ± 0.4	0.1	3	2-3	0
Castletown +200m	i	42	35.7	4.0 ± 0.3	1.0 ± 0.1	1.6	4	3-4	0
	ii	21	57.1	4.0 ± 0.2	0.8 ± 0.3	0.7	3	2-4	0
St. Michaels Isle cove	i	23	43.5	3.9 ± 0.3	0.5 ± 0.3	0.1	3	2-3	0
	ii	19	47.4	3.9 ± 0.1	0.3 ± 0.3	0.0	3	1-3	0
St. Michaels Isle jetty	i	30	40.0	3.7 ± 0.5	0.6 ± 0.3	0.4	4	2-4	0
	ii	14	35.7	3.9 ± 0.3	0.3 ± 0.5	0.1	2	1-3	0
Derbyhaven	i	61	54.1	3.6 ± 0.3	0.5 ± 0.3	0.3	3	2-4	0
	ii	12	66.7	4.0 ± 0.7	0.2 ± 0.3	0.0	2	1-3	0
Port Grenaugh	i	41	41.5	4.0 ± 0.2	0.3 ± 0.3	0.0	3	1-3	0
	ii	9	55.5	4.4 ± 0.2	0.0	0.0	2	1-2	0
Port Soderick	i	21	42.8	3.7 ± 0.3	0.5 ± 0.2	0.2	3	3	0
	ii	9	77.8	3.8	1.2 ± 0.2	0.0	2	1-3	0
Douglas below lighthouse	i	39	58.9	3.8 ± 0.3	1.7 ± 0.3	8.7	4	4	0
	ii	9	55.5	3.9 ± 0.1	1.8 ± 0.2	10.3	4	3-4	0
Douglas outside harbour	i	46	45.6	4.0 ± 0.2	2.0 ± 0.4	12.5	4	4-6	23.8
	ii	ns	ns	ns	ns	ns	ns	ns	ns
Laxey	i	14	57.1	3.8 ± 0.2	1.3 ± 0.3	4.1	4	3-4	0
	ii	ns	ns	ns	ns	ns	ns	ns	ns
Port Lewaigue	i	25	60.0	4.0 ± 0.6	1.0 ± 0.3	1.9	4	3-4	0
	ii	15	66.7	4.0 ± 0.3	0.9 ± 0.4	1.2	3	2-4	0
Ballure	i	18	55.5	4.2 ± 0.2	1.2 ± 0.5	2.0	3	3-4	0
	ii	11	54.5	4.0 ± 0.1	0.2 ± 0.5	0.0	2	2-3	0

Appendix D

Imposex survey results for second year *Nucella lapillus* sampled around the Isle of Man in 1991 (i) and 1993 (ii). Penis size in mm; ns, not sampled; nf, none found.

Site		n	% female	Male penis size	Female penis size	RPS	Median VDS	VDS range	% sterile
Peel below harbour wall	i	41	34.1	3.0 ± 0.3	0.7 ± 0.4	1.6	3	2-4	0
	ii	16	18.7	2.9 ± 0.5	0.3 ± 0.2	0.1	3	2-3	0
Peel south of castle	i	26	42.3	3.1 ± 0.3	0.5 ± 0.3	0.5	3	2-4	0
	ii	28	58.3	2.3 ± 0.3	0.2 ± 0.3	0.1	2	1-3	0
Niarbyl	i	20	70.0	2.8 ± 0.5	0.1 ± 0.1	0.0	2	1-3	0
	ii	17	40.0	2.8 ± 0.5	0.0	0.0	1	1-2	0
Port Erin swimming pool	i	10	70.0	2.0 ± 0.1	1.4 ± 0.4	10.2	4	3-4	0
	ii	7	71.4	2.7 ± 0.2	0.7 ± 0.4	1.7	3	2-3	0
Port Erin Breakwater	i	14	42.8	2.3 ± 0.6	0.6 ± 0.3	1.5	3	3-4	0
	ii	10	63.6	3.0 ± 0.1	0.0	0.0	2	1-2	0
Calf Sound	i	10	40.0	3.2 ± 0.1	0.6 ± 0.4	0.6	3	1-3	0
	ii	7	75.0	2.8 ± 0.3	0.1 ± 0.2	0.0	2	2-3	0
Port St. Mary Ledges 1	i	21	66.7	2.9 ± 0.2	0.7 ± 0.2	1.3	3	3-4	0
	ii	21	52.4	2.6 ± 0.3	0.4 ± 0.3	0.5	3	2-3	0
Port St. Mary Ledges 2	i	ns	ns	ns	ns	ns	ns	ns	ns
	ii	15	53.3	2.7 ± 0.3	0.5 ± 0.3	0.6	3	2-3	0
Port St. Mary Outer harbour	i	nf	nf	nf	nf	nf	nf	nf	nf
	ii	nf	nf	nf	nf	nf	nf	nf	nf
Port St. Mary Inner harbour	i	1	100	nf	2.9	nf	6	6	100
	ii	nf	nf	nf	nf	nf	nf	nf	nf
Castletown harbour mouth	i	1	0	2.1	nf	nf	nf	nf	nf
	ii	7	28.6	2.8 ± 0.5	0.5 ± 0.1	0.7	3	3	0
Castletown +100m	i	10	80.0	3.0	1.1 ± 0.3	5.3	3	3-4	0
	ii	8	50.0	2.0 ± 0.2	0.2 ± 0.2	0.1	2.5	1-3	0
Castletown +200m	i	7	71.4	3.0 ± 0.1	0.9 ± 0.3	3.1	3	3-4	0
	ii	20	80.0	2.5 ± 0.3	0.2 ± 0.2	0.1	2	1-3	0
St. Michaels Isle cove	i	8	50.0	2.3 ± 0.6	0.0	0.0	2	2	0
	ii	17	52.9	2.3 ± 0.4	0.2 ± 0.2	0.1	3	1-3	0
St. Michaels Isle jetty	i	33	42.4	1.6 ± 0.5	0.3 ± 0.3	0.4	2	2-3	0
	ii	18	44.4	2.3 ± 0.4	0.1 ± 0.2	0.0	1	0-3	0
Derbyhaven	i	42	40.0	2.8 ± 0.4	0.2 ± 0.2	0.0	2	2-3	0
	ii	9	55.5	2.8 ± 0.3	0.0	0.0	2	1-3	0
Port Grenaugh	i	10	40.0	2.5 ± 0.5	0.1 ± 0.3	0.0	2	1-3	0
	ii	12	44.4	3.0	0.0	0.0	1	0-2	0
Port Soderick	i	18	56.3	2.1 ± 0.6	0.4 ± 0.3	0.6	3	2-3	0
	ii	11	45.5	2.6 ± 0.4	0.1 ± 0.2	0.0	1	1-3	0
Douglas below lighthouse	i	3	0	2.5	nf	nf	nf	nf	nf
	ii	3	66.7	2.5	0.9 ± 0.1	4.7	3	3	0
Douglas outside harbour	i	9	33.3	2.9 ± 0.2	2.0 ± 0.11	36.9	4	4	0
	ii	ns	ns	ns	ns	ns	ns	ns	ns
Laxey	i	7	42.8	3.0 ± 0.6	0.7 ± 0.3	1.1	3	3-4	0
	ii	ns	ns	ns	ns	ns	ns	ns	ns
Port Lewaigue	i	2	50.0	3.0	0.6	0.8	3	3	0
	ii	9	88.8	3.0	0.6 ± 0.4	0.7	3	2-3	0
Ballure	i	6	66.7	3.1	0.5 ± 0.5	0.4	3	2-3	0
	ii	7	42.8	2.8 ± 0.5	0.0	0.0	1	1-2	0

Appendix E

Imposex survey results for juvenile *Nucella lapillus* sampled around the Isle of Man in 1991 (i) and 1993 (ii). Penis size in mm; ns, not sampled; nf, none found.

Site		n	% female	Male penis size	Female penis size	RPS	Median VDS	VDS range	% sterile
Peel below harbour wall	i	38	55.5	1.6 ± 0.4	0.3 ± 0.2	1.0	3	1-3	0
	ii	12	41.7	1.6 ± 0.2	0.1 ± 0.1	0.0	2	2-3	0
Peel south of castle	i	57	47.37	1.6 ± 0.5	0.2 ± 0.2	0.1	2	1-3	0
	ii	10	40.0	1.6 ± 0.2	0.2 ± 0.2	0.2	2.5	1-3	0
Niarbyl	i	31	58.1	1.3 ± 0.4	0.1 ± 0.1	0.0	1	0-3	0
	ii	12	58.3	1.3 ± 0.4	0.0	0.0	1	0-3	0
Port Erin swimming pool	i	19	36.8	1.6 ± 0.3	0.8 ± 0.2	15.6	4	3-4	0
	ii	7	71.4	1.5	0.0 ± 0.1	0.0	2	0-3	0
Port Erin Breakwater	i	28	57.1	1.1 ± 0.2	0.5 ± 0.3	9.7	3	2-4	0
	ii	5	60.0	1.2	0.0	0.0	1	1-2	0
Calf Sound	i	6	66.7	1.0 ± 0.7	0.1 ± 0.2	0.2	2	1-3	0
	ii	5	60.0	1.6 ± 0.2	0.0	0.0	1	1-2	0
Port St. Mary Ledges 1	i	26	46.2	1.2 ± 0.5	0.5 ± 0.2	8.9	3	2-4	0
	ii	14	57.1	1.5 ± 0.3	0.2 ± 0.2	0.3	2.5	2-3	0
Port St. Mary Ledges 2	i	ns	ns	ns	ns	ns	ns	ns	ns
	ii	10	10.0	1.2 ± 0.3	0.2	0.5	3	3	0
Port St. Mary Outer harbour	i	nf	nf	nf	nf	nf	nf	nf	nf
	ii	nf	nf	nf	nf	nf	nf	nf	nf
Port St. Mary Inner harbour	i	nf	nf	nf	nf	nf	nf	nf	nf
	ii	nf	nf	nf	nf	nf	nf	nf	nf
Castletown harbour mouth	i	8	37.5	1.7 ± 0.5	0.7 ± 0.2	7.6	3	3	0
	ii	2	0.0	1.5 ± 0.5	nf	nf	nf	nf	nf
Castletown +100m	i	7	28.6	1.4 ± 0.4	0.6 ± 0.2	9.6	3.5	3-4	0
	ii	3	33.3	1.2 ± 0.3	0.0	0.0	1	1	0
Castletown +200m	i	8	37.5	1.5 ± 0.5	0.8 ± 0.3	15.2	2	1-3	0
	ii	4	50.0	1.4 ± 0.2	0.0	0.0	1.5	1-2	0
St. Michaels Isle cove	i	41	31.7	0.8 ± 0.2	0.1 ± 0.1	0.1	2	0-3	0
	ii	14	50.0	1.2 ± 0.4	0.1 ± 0.2	0.0	1	0-3	0
St. Michaels Isle jetty	i	37	38.3	0.6 ± 0.2	0.1 ± 0.2	0.9	2	1-3	0
	ii	17	52.9	1.1 ± 0.3	0.0	0.0	1	0-2	0
Derbyhaven	i	23	52.2	1.1 ± 0.2	0.2 ± 0.2	0.4	2	1-3	0
	ii	17	64.7	1.2 ± 0.4	0.0	0.0	1	0-2	0
Port Grenaugh	i	18	44.4	1.0 ± 0.2	0.1 ± 0.1	0.1	2	1-3	0
	ii	4	50.0	1.5 ± 0.5	0.0	0.0	1	0-2	0
Port Soderick	i	29	58.6	1.0 ± 0.2	0.1 ± 0.2	0.2	2	1-3	0
	ii	5	80.0	1.3	0.0	0.0	1	0-2	0
Douglas below lighthouse	i	67	56.7	0.8 ± 0.2	0.3 ± 0.3	63.2	3	2-4	0
	ii	2	50	1.8	0.4	1.1	3	3	0
Douglas outside harbour	i	9	44.4	2.0 ± 0.9	1.3 ± 0.2	25.8	4	2-4	0
	ii	ns	ns	ns	ns	ns	ns	ns	ns
Laxey	i	10	60.0	1.4 ± 0.5	0.6 ± 0.3	6.7	3	2-4	0
	ii	ns	ns	ns	ns	ns	ns	ns	ns
Port Lewaigue	i	1	100	nf	0.5	nf	3	3	0
	ii	1	100	nf	0.0	nf	2	2	0
Ballure	i	3	33.3	1.0	0.1 ± 0.2	0.3	2	1-3	0
	ii	7	85.7	1.5	0.0	0.0	1	1-2	0