PHYSIOLOGICAL ADAPTATION AND GENETIC COMPOSITION IN THE LITTORAL DOG-WHELK, *NUCELLA LAPILLUS (L.)*

Deborah Morgans

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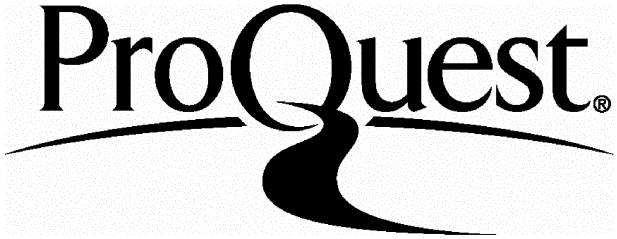

ABSTRACT

Between Peartree Point and Prawle Point, a distance of ca. 5km along the south Devon coast, *Nucella lapillus* exhibits coincident variation in genetic composition and phenotype, which has been proposed to covary with an environmental gradient. In this study, laboratory and shore experiments have investigated the physiological consequences of genetic and phenotypic variation. Sample sites were at Peartree Point and Prawle Point and allelic variation at six polymorphic loci confirmed differences in allele frequencies at two clinal loci, *Lap-2* and *Mdh-1*.

Shell shape, life history characteristics and growth rates varied between sites. Juveniles reared from Prawle Point had lower growth rates than at Peartree Point and variation in tissue growth rate exhibited a direct association with *Lap-2* or *Pep-1* genotypes in the laboratory. Shore juveniles also demonstrated variation in shell growth, feeding rates and growth efficiency among *Lap-2*, *Pep-1* and *Mdh-1* genotypes. The hierarchy of growth differences among genotypes was in agreement to the observed 'population' differences and site-specific allele frequencies; genotypes common in Prawle Point samples had lower growth rates, which coincided with reduced feeding rates and growth efficiency.

Measured temperature, humidity and desiccation rate varied across the cline and may therefore be important environmental pressures influencing prey abundance and foraging behaviour. Wave intensity was similar between sites. Food availability and temperature have been varied in the laboratory. Juveniles reared from Peartree Point (low temperature, high humidity and potentially higher food availability site) showed a greater reduction in growth with decreasing food rations and tended to exhibit a more costly physiology when starved. Growth differences between sites were reduced at higher temperatures, but indicated that feeding rates were higher for Peartree Point juveniles. Similar differences in foraging behaviour were also suggested under a simulated tidal regime. Interpretation of the results have been discussed in view of the concordance of genetic, phenotypic and environmental variation, and the establishment of a site-specific physiology along this region of coast.

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CHAPTER ONE:

General Introduction

Evolutionary biology integrates complementary disciplines such as the synthesis of genetics and ecology (Berry and Bradshaw, 1992) to help understand how and why variation is maintained. Since Darwin's (1859) 'Origin of species', numerous studies have attributed the distribution of variation to the influence of natural selection. If phenotypic variation results in differences in fitness and has a heritable genetic component, selection can result in the differential contribution of individuals to future generations and the evolution of an adaptive phenotype. The 'fittest' phenotype in a particular environment is one which maximises its genetic input to the next generation. Consequently it is the whole phenotype, and not just one aspect of an organism's physiology, ecology or morphology that is subject to the rigours of selection

Classic examples of change in relative abundance of different morphs as a consequence of differences in selective regime include studies of industrial melanism in the Peppered Moth, *Biston betularia* (reviewed in Kettlewell, 1973) and shell banding patterns and colouration in the land snail, *Cepaea nemoralis* (reviewed in Cain and Provine, 1992). In these examples, predation has been shown to be a selective agent responsible for the patterns seen in different locales, but the effect of temperature, notably upon the *Ldh* locus in *Fundulus* (Crawford and Powers, 1989 and refs. therein) and *Adh* variants of *Colias* (Watt, 1983; Watt *et al.*, 1983), has also provided studies of selection. However, although natural selection is widely recognised as a powerful force in moulding populations (Endler, 1986), the advent of gel electrophoresis renewed the neutralist-selectionist debate and questioned the adaptive significance of genetic variation. The observed level of variation in electrophoretic loci (Lewontin and Hubby, 1966; Harris, 1966) was considered too high to be maintained by natural selection alone (King and Jukes, 1969; Kimura and Ohta, 1971). It was suggested that variation at the majority of loci was neutral and did not affect the phenotype or its fitness (Kimura, 1968, 1979). Considerably more genetic variation has since been uncovered with DNA sequence data, and relatively long sequences are highly conserved among species, which have lent support to neutral theory (Kimura, 1993). Reality is probably a compromise whereby some gene loci are under the influence of some form of natural selection, whilst variation at others is neutral in its effect (Avise, 1994).

Selection is more rapid and precise in stressful environments (Berry *et al.*, 1979) where stress is described as 'any stimulus which acts to adversely affect normal metabolic activity' (Koehn and Bayne, 1989). Problems may arise in identifying the major environmental variables affecting phenotypic diversity in terrestrial studies and cryptic variation in habitat has been suggested when variation in some species exhibits an apparently random and patchy distribution in a seemingly 'uniform' environment (Cain and Currey, 1963). Conversely, the predominant stresses encountered by animals in the intertidal zone are well known (Newell, 1979) and so present an ideal opportunity to study the association between genotype, phenotype and habitat. Intertidal species are primarily of marine origin and emersion confers some degree of stress in addition to the effects of temperature, desiccation and salinity (Crisp, 1964; Sandison, 1966). Shores are frequently categorised with respect to these environmental variables and the degree of wave action; sites are typically described as 'exposed' when high wave forces are encountered and 'sheltered' when the predominant environmental pressures are temperature and desiccation (Lewis, 1964).

Intertidal molluscs are popular subjects in studies of adaptation to shore life, but relatively few species, namely *Mytilus* spp. (Hilbish and Koehn, 1985; Hilbish *et al.*, 1994 and refs. therein) and littorinids (eg. Boulding and Van Alstyne, 1993; Tatarenkov and Johanesson, 1994 and refs. therein), have been studied for variation in a number of genetic and phenotypic traits. Recent work undertaken on the common dog-whelk, *Nucella lapillus*, suggests that this species could present itself as an ideal model organism for such an integrated approach (Kirby, 1992; Kirby *et al.*, 1994 a, b). *N. lapillus* is a carnivorous gastropod found on rocky shores around the British Isles, extending also along the coast of Western Europe and the East Coast of America (Crothers, 1985). Dog-whelks are gonochoristic and breeding can occur over a few months each year to an apparently continuous event at different sites (Moore, 1938; pers. obs.). Breeding adults form aggregations and females deposit clusters of egg capsules, with between 2 and 50 (pers. obs.) juveniles emerging per capsule at hatching (Feare, 1970a). A planktonic larval stage often present in the life history of a number of marine organisms (Hedgecock, 1986;

Strathmann, 1990; Koehl and Powell, 1994) is therefore omitted in this species. Direct development coupled with the limited movement of adults often within a few metres (Feare, 1970a; Hughes, 1972), limits gene flow and can lead to marked population structuring over relatively short distances (Grant and Utter, 1988; Goudet *et al.*, 1994).

Shell shape and coloration varies greatly in this species and often correlates with shore type (reviewed by Crothers, 1985; Etter, 1988b). Shells tend to be white and elongate at sheltered sites, whereas on exposed shores shells are rounded with more banded and coloured morphs (Berry and Crothers, 1974). The white form is considered to reduce temperature stress associated with more sheltered locales, through its greater albedo (Etter, 1988b). The elongate shells retain a greater volume of water during emersion than more rounded ones in relation to dry tissue weight (Kirby *et al.*, 1994a), which reduces osmotic changes and the effects of elevated air temperatures through evaporative cooling (Coombs, 1973a; Osborne, 1977).

Genetic composition of both karyotype and electrophoretic loci varies in *N. lapillus*. A number of electrophoretic loci are polymorphic (Day and Bayne, 1988), and alleles are easily resolved. The frequencies for some alleles are coincident with mean chromosome number and wave exposure (Day, 1990; Day *et al.*, 1994; Kirby *et al.*, 1994a b). Chromosomes exhibit Robertsonian translocations (Staiger, 1957; Bantock and Cockayne, 1975); the diploid number ranging from 26, the most common form to 36 (Staiger, 1957; Hoxmark, 1970). The 2n=36 form appears along the South Coast of Britain to be restricted to sheltered sites (Bantock and Cockayne, 1975). Eight chromosome pairs are common to both karyotypes with an additional variable number of metacentrics or acrocentrics to give the 2n=26 and 36 forms respectively (Staiger, 1957; Bantock and Page, 1976). However, these two forms are at least partially fertile and intermediate karyotypic forms are frequently encountered in the field (Staiger, 1957). It is unlikely that a complete speciation event has occurred although sub-species status could be argued and the nature of the polymorphism must restrict gene exchange through reduced recombination between different karyotypes.

Previous work has identified a region in the South West of England where *N. lapillus* exhibits a number of these genetic and phenotypic variations; between Peartree Point (SX820366) and Prawle Point (SX776353), a distance of ca. 5km, *N. lapillus* exhibits a steep cline in allozyme frequencies and shell shape (fig.1) (Day, 1990; Kirby, 1992; Kirby *et al.*, 1994a,b). *N. lapillus* either side of the cline are also differentiated with respect to karyotype (Bantock and Cockayne, 1975; Kirby *et al.*, 1994a), growth rates (Kirby *et al.*, 1994a) and their physiological response to hyperosmotic stress (Kirby *et al.*, 1994b). It has been suggested that this variation might covary with an environmental gradient in temperature and shore exposure along this region of coast (Kirby *et al.*, 1994a, b). It is important to show whether phenotypic variation is adaptive, either through selection or physiological experiments, otherwise the correlation between phenotypic variation and environment may be merely coincidental (Clarke, 1978).

Clinal variation can arise either (I) when differentiated populations come into secondary contact either through chance or by one or more of the distinct populations extending its adaptive range: or (II) in continuous populations where spatial differentiation results by chance such as genetic drift, or an adaptive significance to the variation in response to an environmental gradient (Endler, 1977). The correlation between allozyme frequencies and karyotype is maintained outside of the region between Start Point and Prawle Point (Day *et al.*, 1993), and so it is unlikely that random genetic drift could result in the mosaic distribution of the genetic forms along the South Coast of England. Similarly, variation due to drift is only likely to persist if gene flow between populations is less than one individual per generation; gene flow would perhaps be expected to exceed this level considering the longevity of this species (6 years+ (Feare, 1970a)) and the close proximity of sites differing in genetic composition.

The cline between Peartree Point and Prawle Point is therefore likely to be either a result of primary or secondary contact between populations that adaptively radiated in response to localised environmental pressures. Mitochondrial DNA is inherited through the maternal lineage, independent of the nuclear genome and without recombination, therefore mtDNA variation can be used to analyse the history of populations and species (Avise, 1994).

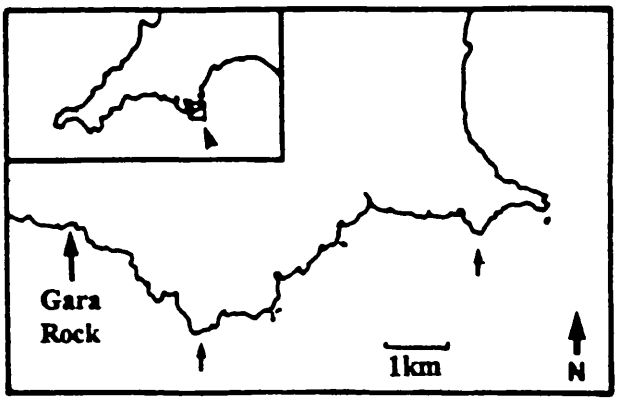
Fig. 1 Location and patterns of clinal variation in genetic composition and phenotype for *Nucella lapillus*.

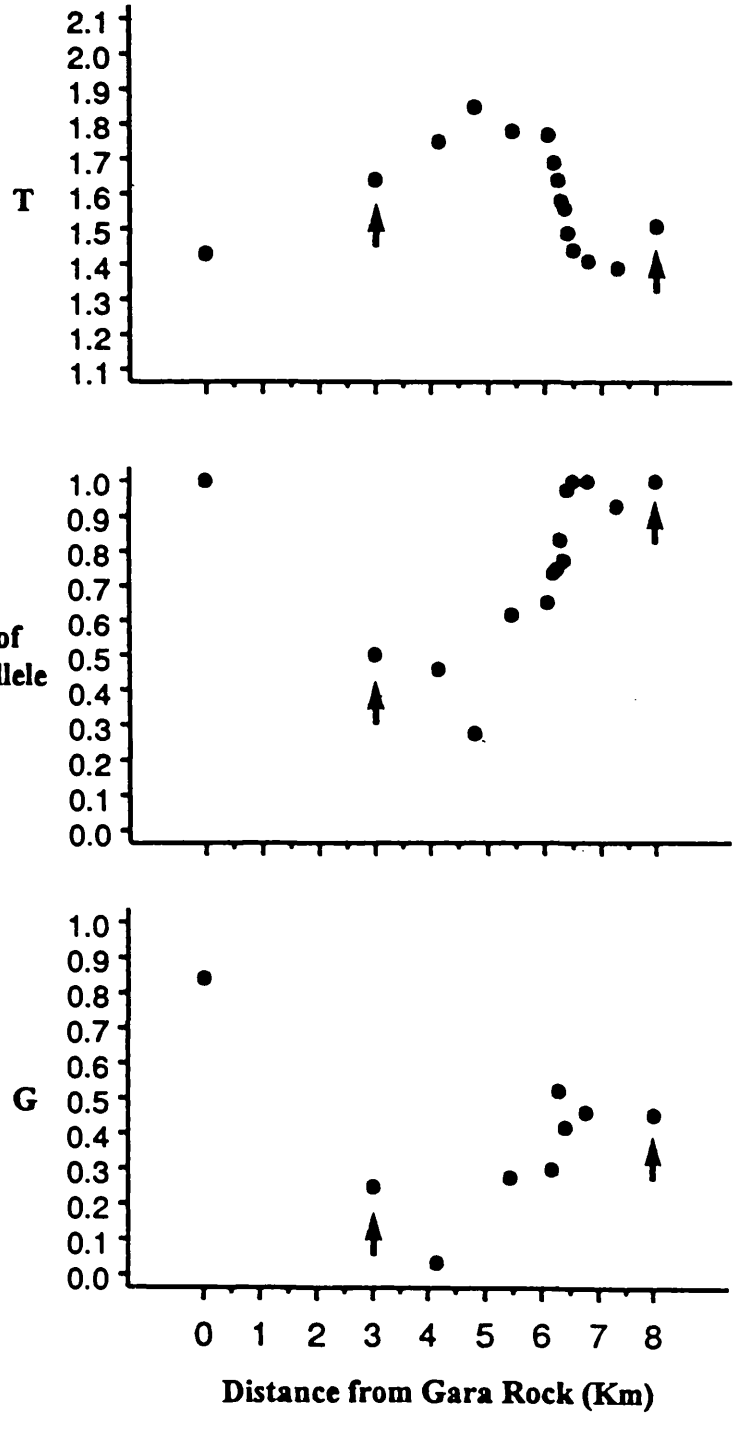
a. Location of clinal variation along the South Devon coast, South West England. Prawle Point (2n=36) and Peartree Point (2n=26) were the sites used in this study and are situated at 3 and 5km respectively from Gara Rock.

b. Shell Shape variation (from Kirby, *et al.* 1994). Shells change from a more elongate shape at Prawle Point to the rounded form found at Peartree Point. The translation rate, T, is an index of shell shape (see Raup, 1966).

c. Allozyme variation. Four loci (*Lap-2*, *Mdh-1*, *Est-3* and *Pep-2*) exhibit similar clinal variation (Day, 1990), but only *Lap-2* is presented here for clarity (from Kirby, *et al.*, 1994). The frequency of the '10' allele decreases towards Prawle Point, and corresponds to the increased frequency of the '9' allele.

d. Mitochondrial DNA variation (taken from Kirby and Berry, in prep.). The frequency of the G-haplotype, a single 'silent' base transition, increases in frequency towards Peartree Point. Prawle Point is characterised by a relatively higher frequency of the A-haplotype.


Gara
Rock
1km
N


T
Frequency of
Lap-2 '10' allele
G
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0 1 2 3 4 5 6 7 8
Distance from Gara Rock (Km)

Studies on mtDNA in *N. lapillus* in this region (Kirby and Berry, in prep.) (fig. 1) suggest that secondary contact is the likely scenario for genetic divergence, which lends support to theories of 'northern' and 'southern' races as based upon karyotype (2n=26 and 36 respectively (Hoxmark, 1970) and morphology (Crothers, 1985). The small differences in mtDNA sequences in sites either side of the cline also suggests a recent divergence, and there appears to be introgression of the Prawle Point haplotype across the cline towards Peartree Point (Kirby and Berry, in prep.). It is still not certain if secondary contact has resulted from chance founder events or extension of the adaptive range of disjunct populations, or whether this cline is stable or transient as dog-whelks in this region have only been studied since 1990.

The aim of the present study was to investigate the physiological consequences of the previously observed variation in genetic composition and phenotype. An expressed phenotype has a genetic and environmental component to the variance, therefore by standardising the environmental component through laboratory studies, the observed phenotype can be mainly attributed to the genetic variability. Such 'common environment' experiments are best after one or more generations in the laboratory to reduce maternal effects upon the phenotype (eg. Reznick *et al.*, 1990). Attempts to obtain sufficient juvenile numbers from laboratory crosses have had limited success (pers. obs.) as a low proportion of crosses produced capsules after approximately 12-18 months in the laboratory. It has however been argued that acclimatisation in the laboratory could give rise to phenotypes that have 'evolved' to an "inestimable" degree in response to the novel laboratory environment (Bernado, 1994). This is probably more important in studies where phenotypic variation is correlated with genetic variation, rather than the direct result of variation at one or more loci. This study has used laboratory juveniles that had been reared from egg capsules collected from the field in an attempt to balance the advantages and disadvantages of using either first generation or acclimatised laboratory populations.

To increase the likelihood of significant differences in genetic variation, study sites were at Peartree Point and Prawle Point. (fig. 1), which were chosen from opposite ends of the

cline. Laboratory experiments were designed, based upon measurements of environmental variables recorded at each site, to identify possible site-specific differences in physiological response to environment. It was hoped that a greater knowledge of the physiology and habitat of *N. lapillus* would further the understanding of "why is what where?" (Berry, 1989) for this species.

CHAPTER TWO:

Laboratory studies of *Nucella lapillus*

2.1 INTRODUCTION

The environment can be viewed as posing a number of "problems" to which the organisms inhabiting them must find a "solution" (Lewontin, 1978). The average phenotype observed in nature tends to be the result of numerous trade-offs among many traits and is rarely the optimal phenotypic solution to the current environmental problem, if it was it would imply that organisms possess a prior knowledge of its future environment (Lewontin, 1978). It has been suggested that phenotypic variation in a population is constantly 'chasing' the optimal phenotype in an ever changing environment (Van Valen, cited in Lewontin, 1978). However, the observed mean phenotype, which aims to optimise fitness, is generally the 'best' solution given the plasticity of the response and phylogenetic constraints imposed on evolutionary change.

The fitness of an organism is optimised through those traits that serve to increase the ability to survive to maturity, reproduce and maximise the number of progeny in the next generation (See Sibly and Calow, 1986). Any physiological, behavioural or morphological characteristic that serves to increase fitness in a particular environment will tend to be preferentially selected and represented in the next generation through the influence of natural selection. This is based upon the assumption that there is a genetic basis to such phenotypic variation and considering the trade-off among traits. The concept of trade-offs is particularly important, for example offspring tend to be smaller when a larger number of offspring are produced on any one occasion. A smaller size can confer a higher risk of mortality, and so maximising a trait (eg. number of offspring) may adversely affect another (eg. survival) (reviewed in Sibly and Calow, 1986). Considerations of trade-offs are important in the advocation of the study of the 'integrated whole' (Gould and Lewontin, 1979), as opposed to reductionist and minimalist approaches (Slobodkin, 1986).

The stresses associated with life on the shore are recognised to include wave force, temperature, desiccation and salinity (reviewed in Newell, 1979). Adaptation, the result of natural selection, has lead to the formation of specialised morphologies, physiologies and behaviours in response to these intertidal stresses (Lewontin, 1978). It is recognised that life

history strategies as the result of the trade-off among these traits (Stearns, 1976, 1989; Partridge and Sibly, 1991; Sibly and Antonovics, 1992) and variation in a number of life history traits have been linked in theories of r-, K- and adversity selection (Southwood, 1977; Greenslade, 1983). Those populations that are subject to variable and fragile environments are likely to exhibit the r-strategy where the intrinsic rate of increase in population numbers is high; this confers an advantage in colonising situations. Individual r-strategists are typically characterised by high fecundities and density-dependent mortalities. K-strategists exhibit the converse characteristics; low fecundities and density-independent mortalities, and these populations tend to be maintained at their carrying capacity and inhabit relatively stable habitats. As with many generalised rules in biology, there are examples which deviate from this 'accepted scheme', however, reference to r- and K- strategy still remains a useful unifying concept recognised in ecology and evolutionary biology, and also as a comparative tool in population studies.

It is perhaps not surprising that many studies on the evolution of life history strategies have looked to the marine intertidal for examples of intraspecific variation (eg. Willows, 1987; Bosman and Hockey, 1988) and include *N. lapillus* (Feare, 1970b, 1971; Etter, 1989). Shores are known to exhibit gradients in environmental variables and are ideal for the study of demographic variation in response to localised habitat. In this study, the number of juveniles per capsule at hatching and subsequent mortality has been measured for both Peartree Point ('exposed') and Prawle Point ('sheltered') to determine site-specific strategies in survivorship and to estimate female reproductive effort, which is comparatively easy to determine for molluscs exhibiting direct development.

Morphological variation is also common in littoral organisms with numerous examples demonstrating the plasticity of characters such as shape and coloration. Hydrodynamic, predatory or physiological explanations have often been postulated to explain why a certain morphology has arisen (Seed, 1978; Hughes and Elner, 1979; Vermeij, 1982; Palmer, 1985b). To determine whether localised variation in morphology is adaptive and not merely influenced by the environment or a random "area effect" (Cain and Currey, 1963), it is necessary to

identify a heritable genetic component to this variation and demonstrate that different morphs are 'better adapted' to a particular environment. Shape is to a certain extent an environmentally determined trait (eg., Palumbi, 1986), but a genetic basis has been identified for *Littorina* spp. (Boulding and Van Alstyne, 1993), and more recently in *Nucella lapillus* reared in the laboratory from sites sampled in the same geographic region as this study (Kirby, 1992; Kirby *et al.*, 1994a).

In the example of *N. lapillus*, shell shape and colouration have been shown to covary with environmental variation and demonstrated to have an adaptive significance (Etter, 1988b; Kirby *et al.*, 1994a, b). At sheltered sites, shells are predominantly white and elongate, which respectively increase the level of reflected insolation and the volume of extra-corporeal water on the basis of tissue weight when emersed, therefore tending to reduce temperature stress and osmotic changes (Etter, 1988b; Kirby *et al.*, 1994a). The narrower aperture of elongate shells has also been suggested to reduce the success of crab predation, which is thought to be generally greater on sheltered shores (Kitching *et al.*, 1966; Seed, 1978; Hughes and Elner, 1979; Lawton and Hughes, 1985). On exposed shores, shells are rounded, and the increase in exposure appears to enable the occurence of banded and coloured morphs (Berry and Crothers, 1974). The rounded shell morph has been shown to possess a relatively larger foot that is thought to help attachment in high wave energy environments (Kitching *et al.*, 1966; Etter, 1988a). Pedal surface area does not differ between juveniles hatched in the laboratory from sheltered and exposed sites suggesting that the surface area of the foot is a plastic response requiring an environmental cue for the site-specific differences to manifest (Etter, 1988a). Shell shape differences are no doubt adaptive, but the actual reasons for the variation cannot be identified with certainty; a combination of environmental and intrinsic factors could underlie this variation. Shell shape variation between sample sites has been investigated in this study to identify if the association between morphology and genetic composition in laboratory reared juveniles is maintained at sites not included in Kirby's (1992) study.

Variation in growth rate is frequently used as an index of fitness; rapid growth is generally thought to be advantageous if a fitness deficit is not incurred (reviewed in Sibly and Calow,

1986) and is often associated with a higher maintenance metabolism (Bayne and Newell, 1983). Under certain conditions when energy acquisition is expected to be lower than maintenance levels, for example during low food abundance or adverse environmental conditions (Burrows and Hughes, 1989), those individuals with high intrinsic maintenance requirements would be expected to be more adversely affected than those with more conservative metabolic demands. Higher growth rates could result in higher mortality rates; those individuals requiring high food intakes may be open to a greater risk of predation and physiological stress by leaving refuges more frequently in search for food (Burrows and Hughes, 1991; Hughes *et al.*, 1992). Fast growth rates are therefore not always 'fitter'. It is necessary to understand the environmental factors affecting growth and distinguish between variation due to these environmental variables and that determined by the genotype before the adaptive significance can be inferred and tested.

Dog-whelks are carnivorous and feed mainly upon mussels and barnacles (Fretter and Graham, 1962) in the mid-tidal range although other prey species have also been identified in their diet (Moore, 1938; Largen, 1967a; *pers. obs*). A small hole is bored through the prey's shell or operculum by a combination of enzymatic and mechanical means (Webb and Saleuddin, 1977) and the tissue ingested. Energy obtained from the diet can be partitioned in energy budgets, where the remaining energy after the maintenance requirements have been met is generally channelled into growth and/or reproduction and is referred to as the scope for growth (SFG) (Warren and Davies, 1967):

$$SFG = C - F - R - U$$

where C is the energetic value of ingested food and F, R and U are the energetic quantities lost as faeces (unabsorbed fraction of ingested food), metabolic heat losses and excretion respectively. Variation in feeding rates is the major factor affecting growth rates as expected. Actual somatic growth has often been shown to closely follow the predicted SFG (Dame, 1972; Bayne and Worrall, 1980), but diverges at the onset of gametogenesis (see Bayne and Newell, 1983 and refs. therein) where a greater proportion of energy is directed to reproduction. Therefore the underlying variation in metabolism and the efficiency in which ingested food is

allocated to growth affects an organism's fitness and warrants attention in studies of physiological adaptation.

Population differences in SFG could result from environmental heterogeneity affecting either energy acquisition or allocation, and could therefore reflect physiological plasticity rather than adaptation *per se.* If population differences are shown to have a genetic basis either through breeding experiments or under common environment, a more convincing adaptationist explanation can be postulated. Selection is more rapid and precise under conditions of stress (Berry *et al.*, 1979), and so it would be expected that different populations living in habitats differing in the degree and nature of stress would become differentiated, in time, with respect to a number of traits. The marine intertidal influences energy acquisition either directly, through environmental influences and the tidal cycle (Burrows and Hughes, 1989), or indirectly, through the effects of 'exposure' upon species assemblages and the relative abundance of prey, predators and competitors (Menge, 1978).

The growth, feeding and respiration rates of samples of *N. lapillus*, previously hatched and reared in the laboratory, were determined for each sample site in the present study. Kirby (1992) has suggested growth variation among populations characterised by different frequencies of four clinal loci (fig. 1); a direct association with the *Lap-2* locus was identified. Five months after hatching, he recorded size measurements for all juveniles and calculated the mean size for each capsule, which was taken to reflect growth rate, and found that 'exposed' shore juveniles were larger than 'sheltered' shore individuals. However, size variation at a set age after hatching could reflect differences in size at hatching, differential survival of genotypes in the two populations or different effects of food abundance upon growth. All of these would affect the mean juvenile size per capsule without necessarily resulting from differences in post-hatching growth rate. In the present study, Kirby's (1992) methodology has been used for Peartree Point and Prawle Point samples reared in the laboratory, but actual growth rates have also been determined. Feeding rates and respiration rates were also measured to identify whether growth variation can be attributed to differences in energy gains or losses.

Energy acquisition has been manipulated in the present study to determine if there are population differences in growth under reduced food availabilities. Feeding rates are easily recorded for the dog-whelk (discrete prey items are attacked with little tissue left after ingestion), but diet manipulation is more difficult to achieve and standardise compared to filter feeding molluscs for which the concentration of algal cells and particulates can be altered. Previously, *N. lapillus* has been rationed by reducing the number of days food is available (Bayne and Scullard, 1978b; Bloomer, 1987). Disturbance can have a profound affect upon feeding activity (pers. obs.) and this method of rationing would be expected to have a marked affect upon the dog-whelk behaviour; physical removal of mussels from a feeding chamber at regular intervals can affect normal feeding activity. Standardising food intake will also be difficult using this approach; some individuals are likely to have a greater or less ability to exhibit 'catch-up feeding' after a period of starvation. Rapid feeding induced by a period of starvation has been found to result in similar growth rates between 'rationed' and *ad libitum* fed individuals using this methodology (Bloomer, 1987). Here dog-whelks have been maintained at one of three ration levels, *ad libitum*, low and starved levels. The 'low' ration was achieved by providing each whelk with one mussel per week and so minimising the effects of disturbance. Growth efficiencies of these juveniles were hoped identify if site specific variation in growth under reduced food availability had a genetic basis or was purely a eco-phenotypic response.

2.2 MATERIALS AND METHODS

2.2.1 General methods

2.2.1.1 Laboratory seawater facilities

All animals were maintained in experimental aquaria on a closed ~4500l recirculating seawater system. Seawater temperature and salinity were held at 15±1°C and 35±2ppt respectively throughout the rearing of *N. lapillus* juveniles and subsequent experiments. Total seawater changes every six to eight weeks helped maintain the water quality.

2.2.1.2 Establishment and rearing of laboratory populations

More than forty egg capsules were collected from both Peartree Point (O.S. Ref SX819366) and Prawle Point (O.S. Ref SX775352) in February, 1992. One capsule per cluster was collected unless the number within a cluster was over ca.100, in which instance two were removed from opposing sides. This sampling strategy coupled with the large area covered during sampling was to ensure a representative sample for the site and minimised the likelihood of capsules being laid by the same female.

In the laboratory, capsules were individually maintained non-tidally in 'Toby Teaboys' (Aldridge Plastics Ltd., U.K.). These containers are rigid polypropylene cages to which 224µm mesh had been securely attached (plate 2.2.1), and have previously been used to successfully rear *N. lapillus* hatchlings (Kirby, 1992). The teaboys were inspected daily and the date of hatching (approx. two months after collection) recorded for each egg capsule. Hatchlings were fed every two weeks with 30g of mussel spat (*Mytilus edulis* <8mm) per teaboy. There were abundant live mussels remaining every time the spat was replenished, and the exterior sides of the teaboys were cleaned at this time to ensure adequate water exchange across the mesh. Mussels were collected at Whitsand Bay (O.S.Ref SX390523) due to plentiful spat at this site and also its accessibility to the laboratory. The mussel spat was rinsed thoroughly in tap water in the laboratory, carefully inspected for juvenile whelks and fed once with an algal diet of *Isochrysis galbana* before being introduced into the teaboys.

Plate 2.2.1: Toby Teaboys used for the rearing of *N. lapillus* hatchlings in the laboratory. Each teaboy consists of two halves; the upper and lower views show the exterior and interior containing mussel spat and juvenile dog-whelks.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Whelks were reared for four months after hatching when size measurements (section 2.2.1.3.1) and the numbers of juveniles surviving from each capsule were recorded. Juveniles were considered too susceptible to damage during handling before this age. At this time, individuals were labelled on the apical whorl with 'beetags' (Christian Graze Inc., West Germany) attached with cyanoacrylate glue ('superglue'). They were then transferred to population stock tanks and subjected to physiological experiments (section 2.2.2).

2.2.1.3 Measurements

2.2.1.3.1 Size measurements

Four size measurements were recorded; length, aperture, tissue weight and shell weight. Shell lengths and apertures (plate 2.2.2) were measured with vernier calipers to the nearest 0.05mm. The relationship between length and aperture measurements provided an indication of shell shape. Tissue and shell mass were distinguished using the method devised by Palmer (1982), which required sacrificing initial samples of dog-whelks ('destructive sample') from the laboratory 'populations' to obtain actual shell and tissue weights. The calculated regression coefficients could then be used to estimate these measures for live experimental animals.

Destructive sample

A wide size range of dog-whelks, previously fed *ad libitum* in population stock tanks, was used as the destructive sample (n>30 per population). All weight measurements (± 0.5 mg) were made with a Mettler (type H16) mechanical balance.

Whelks were individually weighed on platforms suspended in filtered seawater. The specific gravity of tissue approximates to zero in seawater, which results in a linear relationship between this 'immersed' weight and actual shell mass (Palmer, 1982). After the immersed weight had been determined, extra-visceral water was removed from the mantle cavity by gently pressing filter paper strips against the side of the operculum until no water was visible on the wicks. The whelks tended to withdraw into their shells when touched, which helped to expel water from the mantle cavity, and were then left to air-dry for approximately

Plate 2.2.2: Length and aperture measurements of *N. lapillus*.


Length
Aperture

20 minutes during which time shell length and aperture measurements were taken. The total wet weight was measured and the animal dissected into tissue and shell fractions. The wet weight of body tissues was immediately recorded, and then the shell and tissue fractions were freeze dried to determine their respective dry weights. Regression coefficients were calculated and used to estimate separate shell and tissue weights for live whelks, which were used in subsequent experiments:

Peartree Point:

Shell Dry Weight = (1.4119 (± 0.0252) * Immersed Weight) + 0.0006 (± 0.0052)

Prawle Point:

Shell Dry Weight = (1.4119 (± 0.0252) * Immersed Weight) + 0.0088 (± 0.0041)

Dry tissue weights required for standardising physiological rates to a standard animal tissue weight, were estimated for each population using the following equations;

Peartree Point:

$\log_{10}$ Dry Tissue Weight = (3.3180 (±0.2744) * Log_{10} Length) - 5.0906 (±0.3386)

Prawle Point:

$\log_{10}$ Dry Tissue Weight = (3.3180 (±0.2744) * Log_{10} Length) - 5.2324 (±0.3258)

Experimental animals

Immersed weight, length, aperture and total wet weight were measured directly; dry shell weight was estimated using the appropriate population equation above and wet tissue weight was estimated by subtracting the calculated shell dry weight from the measured total wet weight.

2.2.1.3.2 Feeding rates

Mussels (*Mytilus edulis*) were collected from Whitsand Bay (O.S.Ref SX390523) every two weeks, and maintained in the laboratory for at least two weeks without being fed (except low levels of algae present in the seawater system) before being introduced to the juvenile dog-whelks.

The size of mussel provided as prey varied on the basis of the equation derived by Bayne and Scullard (1978b) relating preferred mussel length to whelk length:

$M_{lt} = N_{lt} / (0.46 (\pm 0.08) + (0.36 (\pm 0.05) * N_{lt}))$

where M_{lt} and N_{lt} is the mussel and whelk length (cm) respectively. The size of mussel was varied throughout the feeding rate experiments to account for growth, whenever length measurements were re-measured for the dog-whelks.

The mussel density (number of mussels per whelk) varied in different experiments and depended on how frequently the number of mussels eaten was recorded, although for *ad libitum* fed juveniles it was never less than three per whelk for more than 24 hours.

The lengths of eaten mussels were measured and the dry weight of consumed mussel flesh derived from the relationship:

$\log_{10} DT = (2.5142 (\pm 0.2194) * \log_{10} ML) - 4.8864 (\pm 0.2604)$

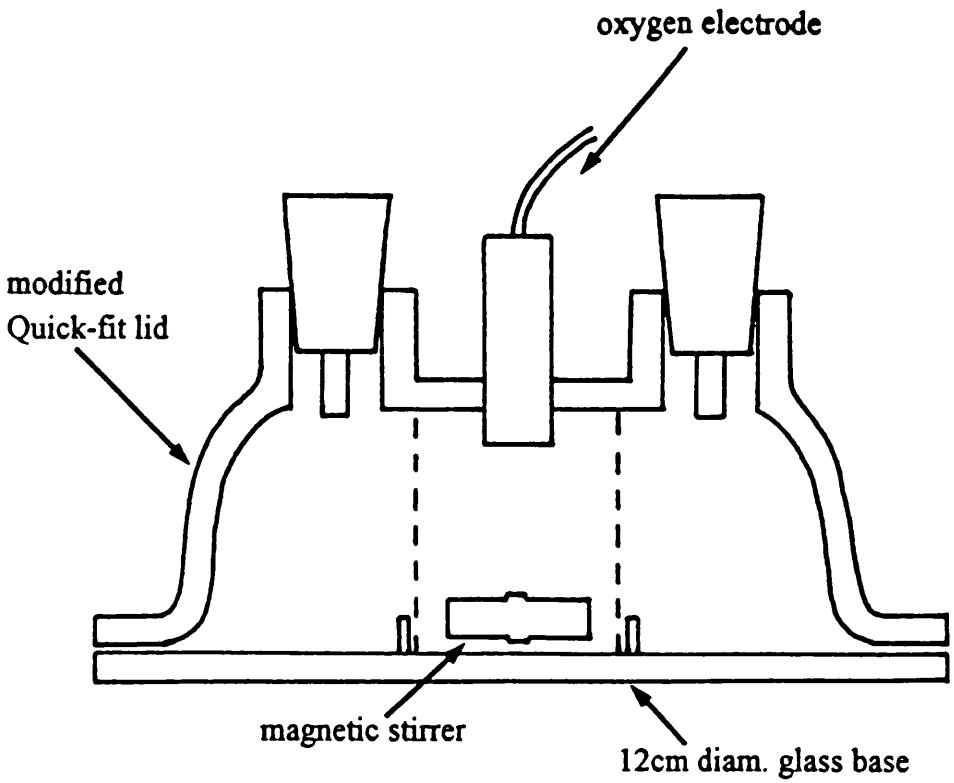
where DT is dry tissue weight (g) and ML is mussel length (mm). The total weight of flesh consumed was summed for each individual over the duration of the experiment and converted to Joules assuming a calorific value of 5.2 $cal.mg^{-1}$ mussel tissue (Bayne and Scullard, 1978b) and 4.184 $J.cal^{-1}$.

Population differences in feeding rates were tested by an analysis of covariance of the relationship between initial whelk tissue weight (either wet or dry weight) and the energetic value of the total mussel flesh consumed by each individual over the duration of the experiment.

2.2.1.3.3 Respiration rates

The methods used to determine respiration rates for *Nucella lapillus* were modified from Kirby (1992). Individual respiration rates were measured in chambers sealed with a modified quickfit lid (Fig. 2.2.1). The seal was improved by using high vacuum silicone grease (Dow Corning) and was impervious to oxygen exchange (pers. obs.). A radiometer oxygen electrode (model E5046) was centred in the top of the quickfit lid and was sensitive to the partial pressure of

Figure 2.2.1 Chamber used to measure individual respiration rates for *N. lapillus* juveniles


oxygen electrode
modified
Quick-fit lid
magnetic stirrer
12cm diam. glass base

oxygen (PO_2). Respiration rates were determined indirectly by measuring the decline of PO_2 as opposed to directly monitoring the evolution of CO_2. The electrode was coupled to a Strathkelvin amplifier (model 781b) connected to a 100mV Rikadenki chart recorder.

An individual whelk was placed within the pot and oxygen saturated filtered seawater (15°C, 35ppt) carefully poured into the chamber via the spout to avoid air bubbles. A high partial pressure of oxygen and constant temperature were respectively achieved by gently aerating a reservoir of seawater and maintaining all glassware within a constant temperature water bath before and during the measurement period. Oxygen consumption rates were determined after at least twenty minutes had elapsed since the introduction of the whelks to the chambers and then monitored over a period of approximately one hour or until 80% PO_2 was reached. Glass rods were attached to the stoppers to prevent animals crawling into the spouts of the quickfit lids, which could result in inaccurate readings as mixing within the spouts is probably reduced.

Difficulties arise in assessing at what stage in their feeding cycle individuals have reached without disturbing the normal feeding pattern (pers. obs.), and so respiration rates were not measured on direct transfer from food. Measurements were undertaken either two (intermediate between standard and routine rates) or seven days (standard rate) after all available food had been removed. Respiration rates decline to standard rates five to ten days post-feeding between 15 and 20°C (Bayne and Scullard, 1978a). All rates were weight corrected to a standard dry tissue weight of 120mg according to the equation:

$$\text{Rate}_{std} = (W_{std} / W_{obs})^b \times \text{Rate}_{obs}$$

where Rate_{std} and Rate_{obs} are the weight corrected and uncorrected respiration rates ($mlO_2/h/g$) and. W_{std} and W_{obs} are the standard and individual dry tissue weights (g) respectively. The b-exponent is equal to the gradient of the linear relationship between $\log_{10}$ respiration rates and $\log_{10}$ dry tissue weight. Dry tissue weights were either obtained by freeze-drying individuals after determining the respiration rates or estimated according to the length-dry weight regressions outlined in section 2.2.1.3. Analysis of variance was used to test for population differences in weight standardised respiration rates.

2.2.1.4 Electrophoresis

All tissues and homogenates were stored at -70°C; homogenates were prepared from foot tissue. The electrophoretic mobilities of six soluble polymorphic enzyme loci, using starch gel electrophoresis, were determined using the methods outlined in Day and Bayne (1988) with modifications (Day, 1990; Kirby, 1992). The following enzymes were scored: malate dehydrogenase (*Mdh-1* E.C.1.1.40), leucine aminopeptidase (*Lap-2* E.C.3.4.11), peptidase (*Pep-1* E.C.3.4.11), mannose phosphate isomerase (*Mpi* E.C.5.3.1.8) and two phosphoglucomutase loci (*Pgm-1* and *Pgm-2* E.C.2.7.5.1). *Lap-2* and *Mdh-1* have previously been shown to exhibit clinal variation in allele frequencies between the two sites used in the present study (fig. 1; Day, 1990; Kirby *et al.*, 1994a). Allele nomenclature followed that used by Day (1990) and Kirby *et al.* (1994a).

2.2.1.5 Statistical analyses

All statistical tests were performed using the Statistical Analysis System (SAS) package (SAS Institute Inc., Cary, NC, USA). The General Linear Model procedure (proc GLM) for regression analyses and ANOVA tests were used to analyse the majority of the collected data unless indicated otherwise; 'non-significant' effects in the model statement were systematically removed and the model retested for 'significant' effects at the 5% level. Type III sums of squares, which allow for unbalanced sample sizes between treatments, were used.

2.2.2 Size at four months of age

A re-examination was undertaken of previous work which described a correlation between population, growth and genotype (Kirby, 1992) within and between sites along this cline.

All individuals surviving to four months after hatching were measured for length, aperture, shell and tissue weights (section 2.2.1.3). An analysis of covariance was used to test for significant effects of family size upon size measurements.

Juveniles were used in subsequent experiments (described in sections 2.2.4 and 2.2.5) at the end of which whelks were scored for the six polymorphic loci described in section 2.2.1.4. Following Kirby (1992) both populations were divided into two size groups; one containing the three largest and another containing the three smallest juveniles from each egg capsule. Size

was based on the pooled ranks of the four size measures: length, aperture, shell weight and tissue weight. Genotype differences between the two size groups were tested with both populations pooled and again for each population separately using Chi-square analysis.

2.2.3 Number of individuals per capsule at hatching and subsequent mortality

Samples of capsules were collected in 1994 to determine the number of hatchlings per capsule at hatching, and mortality over one month. Details of the sampling strategy used for collecting egg capsules are described in chapter four. Prior to hatching, each capsule was maintained in a separate teaboy without food and inspected twice daily for hatching. Counts were made one day after hatching had begun and individuals from each egg capsule kept in a teaboy to which mussel spat had been added; any hatchlings emerging at a later date were added to the teaboy containing their sibs. The total number of hatchlings was summed for each capsule when the egg capsule was empty, which was checked using a binocular microscope. One month after hatching, all live juveniles were counted after carefully sorting through the mussel spat within each teaboy. These juveniles were then transferred to tidal tanks, keeping the two samples separate, for experiments described in chapter four. An attempt to quantify the post-hatching mortality rate was made by counting the number of empty shells each time the mussel spat was replenished. However, problems arose during the early post-hatch stages due to the small size of dead individuals and their delicate shells, which could be easily damaged rendering direct counts difficult to determine. Mortality was therefore measured indirectly by comparing the number alive per capsule at hatching and one month later.

Mortality measurements are binomially distributed with a set upper limit, ie. the number dying cannot exceed the number hatching, therefore analyses requiring normally distributed measures cannot be used. Maximum likelihood estimates were used in this instance to test for significant population differences in mortality using the LOGISTIC procedure in SAS (SAS Institute Inc., Cary, NC, USA).

2.2.4 Population growth rates

Size at four months gives an indication of possible differences in growth (section 2.2.2), but population differences in size at this age could merely reflect differences in size at hatching

and not growth rates *per se*; larger hatchlings are more likely to attain a larger size at a set age than smaller hatchlings. It is also possible that the number of juveniles per capsule (ie. number of juveniles per teaboy) and 'crowding' effects could influence growth to a different degree for the two laboratory populations (Kirby, 1992). In this experiment, the growth rate of juveniles maintained in separate containers was monitored over a four week duration, in addition to periodic measurements of feeding and respiration rates.

Forty whelk juveniles from a wide size range were removed from each population stock tank. Immersed and total weights, length and aperture measurements (section 2.2.1.3) were determined for each individual at 0, 14 and 28 days after the start of the experiment. Individual shell and dry tissue weights were estimated using equations provided in section 2.2.1.3. Respiration rates were measured twice (two and four weeks after the start of the experiment) for each individual and were weight-standardised according to estimated dry tissue weight and the methods in section 2.2.1.3.3. These measurements were made one day after size measurements had been recorded and mussels removed, and therefore correspond to one-day post-feeding rates.

Over the four week experimental period, each juvenile was maintained in a separate feeding pot with five mussels whose sizes were within 1mm of the preferred mussel length (section 2.2.1.3.2). Feeding rates were determined for each individual using the general methods outlined in section 2.2.1.3.2. Eaten mussels were measured daily and replenished as required to maintain a predator:prey ratio of 1:5. The dry weight of any tissue remaining in the valves after feeding was measured and then subtracted from the estimated total tissue weight of the mussel as based on length measurements (section 2.2.1.3.2). Increases in tissue and shell weight per individual over the entire experimental period (0-28 days) were related to total joules of mussel flesh ingested per whelk as an indication of gross growth efficiency. At the end of four weeks, juveniles were frozen at -70^{o}C for later electrophoresis (section 2.2.1.4).

Statistical analysis

Growth rates over three time durations (0-14 days, 14-28 days and 0-28 days) were analysed using modified Ford-Walford plots (Walford, 1946), which plot the relationship between initial

size and either final size or size increase over a given time period; variation in either the slope or intercept can indicate differences in growth rate between two samples. Analysis of covariance was used to test for time and population effects upon increase in size. If growth rates were similar between 0-14 days and 14-28 days, growth over the whole experiment (0 to 28 days) was used in the analysis of covariance for differences among genotypes; each population and locus combination was tested separately to reduce the probability of obtaining a significant result by chance.

The total weight of mussel tissue eaten over the four-week experimental period was summed for each individual and converted to total joules eaten (section 2.2.1.3.2). Analyses of covariance were used to test for significant population effects upon the the relationship between initial size and total joules eaten (feeding rate; section 2.2.1.3.2) and also gross growth efficiency relating total growth to total Joules consumed.

The significance levels of population and time effects upon the b-exponent relating $\log_{10}$ respiration rate to $\log_{10}$ dry tissue weight were determined using an analysis of covariance. Calculated b-exponents were then used to weight standardise respiration rates (section 2.2.1.3.3) based upon length measurements and their estimated dry tissue weights (section 2.2.1.3.1). Analysis of variance was also used to test for significant population, time and genotypic effects upon standardised respiration rates.

2.2.5 Feeding ration experiments

Three ration levels were used to investigate the influence of food availability upon growth and provide some indication of whether growth efficiencies under different feeding rations could explain the suggested differences in growth between these populations.

Five juveniles of at least four months old were selected at random from each of thirty families per population and maintained for six weeks at one of the following ration levels; *ad libitum* (1), low ration (1) or starved (3). Numbers in parentheses indicate the number of juveniles per family at each ration; at the end of the experiment, the total sample sizes for both populations combined were n=59 for each of the *ad lib* and low ration levels, and n=177 for the starved

ration. Size measurements (section 2.2.1.3) were taken at the start of the experiment and then every two weeks over the six week period.

Juveniles maintained on a diet of mussels (both the *ad libitum* and low rations) were kept in separate numbered teaboys for the six week duration with a set density of mussels. Juveniles fed *ad libitum* were each provided with ten mussels within 1mm of the preferred prey size (section 2.2.1.3.2). Eaten mussels were measured every week and replaced to maintain a density of ten mussels per whelk. Low ration individuals were each given one mussel per week whose size was also determined by the equation outlined in section 2.2.1.3.2. Again, eaten mussels were measured and replaced each week. For both the *ad lib* and low rations, the mussel size was recalculated every two weeks to adjust for increases in whelk size and a new stock of mussels used. Population differences in feeding rates were tested using the analysis of covariance described in section 2.2.1.3.2.

The starved juveniles were fed at *ad libitum* ration levels for two weeks in population stock tanks prior to four weeks starvation. These stock tanks held a wide size range of mussels and when removed during the starvation phase of the experiment, juveniles were checked twice daily to ensure that cannibalism did not occur.

At the end of the experiment and after the final size measurements were recorded, all animal tissues from each ration level were stored at -70°C before genetic analysis for electrophoretic variation (section 2.2.1.4). Juveniles previously held at the low and starved ration were also dissected to calculate the actual final shell weight. The effects of ration level upon the Palmer relationship between immersed shell weight and actual shell weight were tested using an analysis of covariance. The results from this test determined how the growth data were analysed and what statistical test should be used to test for population differences. If there were no ration effects upon the Palmer regression, a repeated analysis of variance could be used as intermediate shell weights (and therefore also wet tissue weights, ie. the difference between shell and total weight) could be estimated using the equations in section 2.2.1.3. If ration had a significant effect upon the Palmer regression, then modified Ford-Walford plots relating final to initial size (section 2.2.4) and corresponding analysis of covariance were used instead. Intermediate estimates of shell weight could not be accurately estimated in this case due to

unknown interactions between immersed weight, population and ration over time. Genotype effects upon growth were tested separately for each locus and population combination to reduce the likelihood of a chance significant result.

2.3 RESULTS

2.3.1 Number at hatching

A significantly higher number of hatchlings per capsule emerge from capsules collected from Peartree Point than from Prawle Point (fig. 2.3.1) ($F_{1, 34}$=12.13, p=0.0014). Differences among females in number per capsule at hatching were significant even though a sample size of only two capsules per female ($F_{40, 34}$=5.61, p=0.0001) as the sampling strategy (chapter four) had assumed that adjacent capsules were laid by the same female. The female effect was further investigated (fig. 2.3.2). The correlation between the number hatching from one capsule and that from its pair was significant ($F_{1,32}$=29.82, p<0.001, r^2=0.482) with no population differences in this relationship ($F_{1,32}$=0.23, p=0.63).

2.3.2 Number of juveniles at four months of age

At four months after hatching, a higher mean number of juveniles per capsule survived for Peartree Point capsules (fig. 2.3.3a), than for capsules collected from Prawle Point (fig. 2.3.3b) ($F_{1, 55}$=12.80. p<0.001). The maximum number did not exceed 26 juveniles per capsule for either population, which is considerably less than the maximum number of 48 per capsule at hatching (fig. 2.3.1) and suggests that mortality could be as high as 50% during the first four months following hatching.

2.3.3 Post-hatching mortality

Mortality was recorded one month after hatching for the 1994 cohort (fig. 2.3.4). Maximum likelihood estimates indicated significant population differences in the proportion of hatchlings dead one month post-hatching (fig. 2.3.5) (Wald Chi-sq=67.962, p<0.001); Prawle Point has a significantly higher proportion of juveniles dying during the first month after hatching than Peartree Point. This method of analysis assumes that the proportion dead per capsule is independent of the number per capsule at hatching and remains constant, however the residuals increased in magnitude and sign. For both populations, the greater the number of hatchlings, the greater the proportion dying (Peartree Point d= 1.636, p<0.01; Prawle Point d= 1.653, p<0.01). Even though the mean number of hatchlings per capsule is less for Prawle Point (section 2.3.1), they have a higher mortality over the first month of hatching.

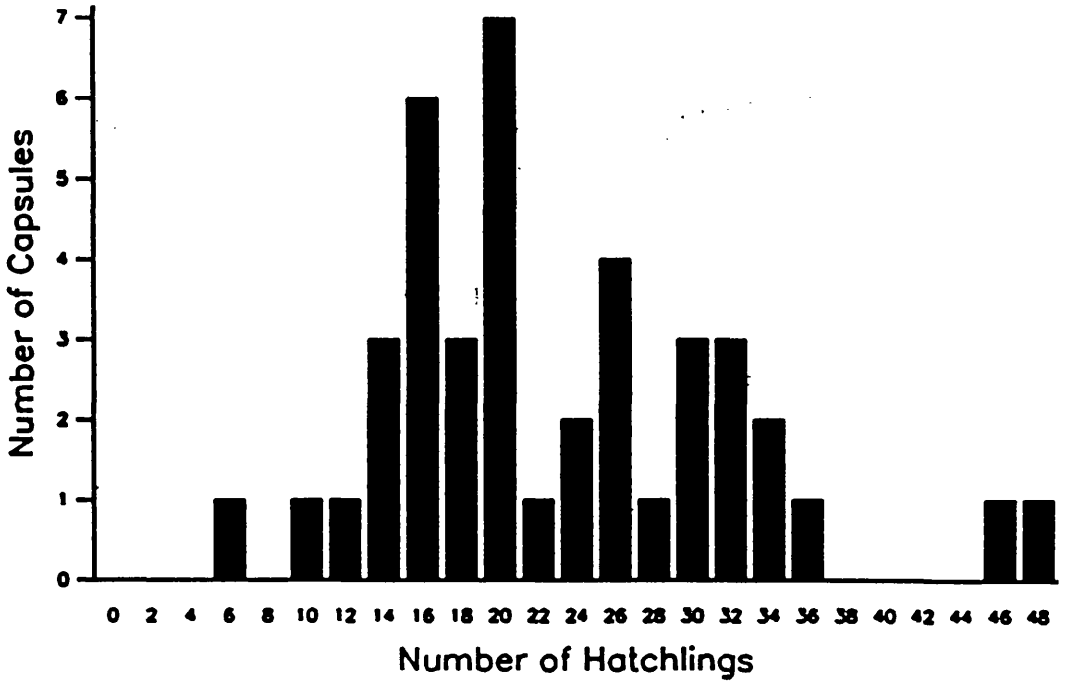
2.3.1 Frequency distribution of the number of hatchlings per capsule at hatching

a. Peartree Point

Mean $\pm$ 2SE = 22.45 $\pm$ 1.7898 hatchlings per capsule

b. Prawle Point

Mean $\pm$ 2SE = 17.72 $\pm$ 2.0432 hatchlings per capsule


Number of Capsules
0
1
2
3
4
5
6
7
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48
Number of Hatchlings

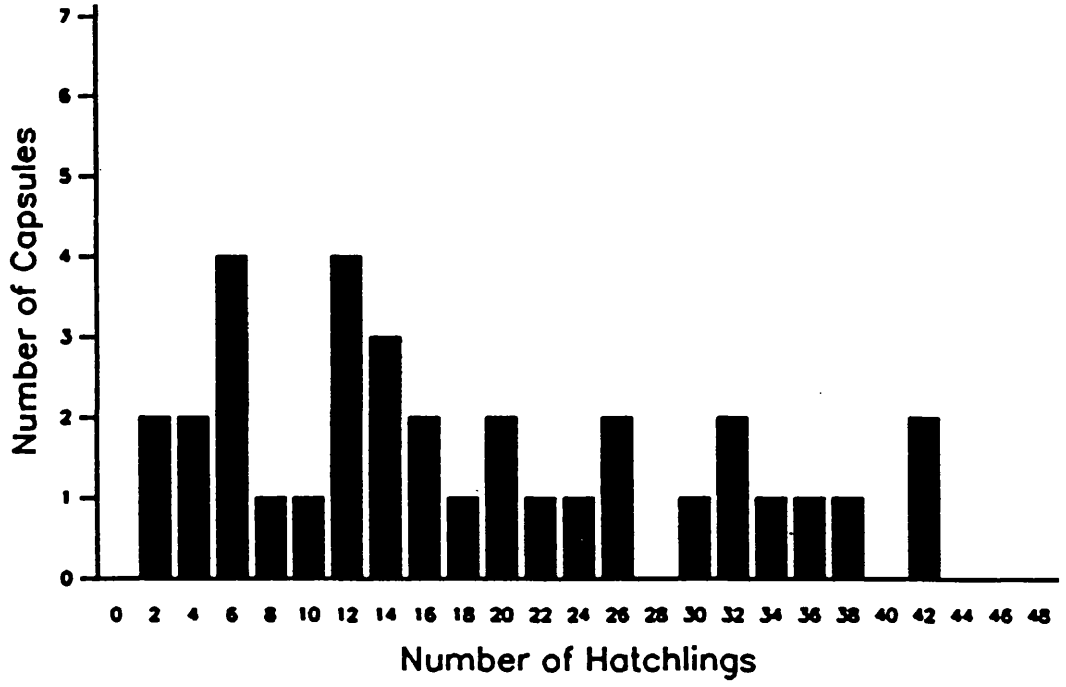

Number of Capsules
0
1
2
3
4
5
6
7
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48
Number of Hatchlings

Fig 2.3.2 Correlation between the number of hatchlings emerging from one capsule and that recorded for the adjacent egg capsule. A capsule within a pair was randomly assigned 'capsule one' and its pair 'capsule 2' for analysis purposes. Peartree Point = closed squares, Prawle Point = open circles.

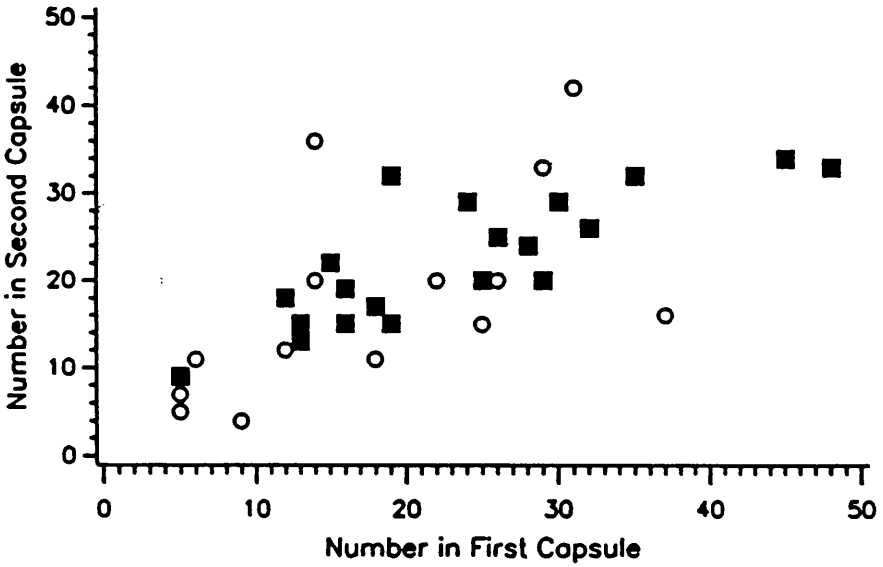

Number in Second Capsule
50
40
30
20
10
0
0
10
20
30
40
50
Number in First Capsule

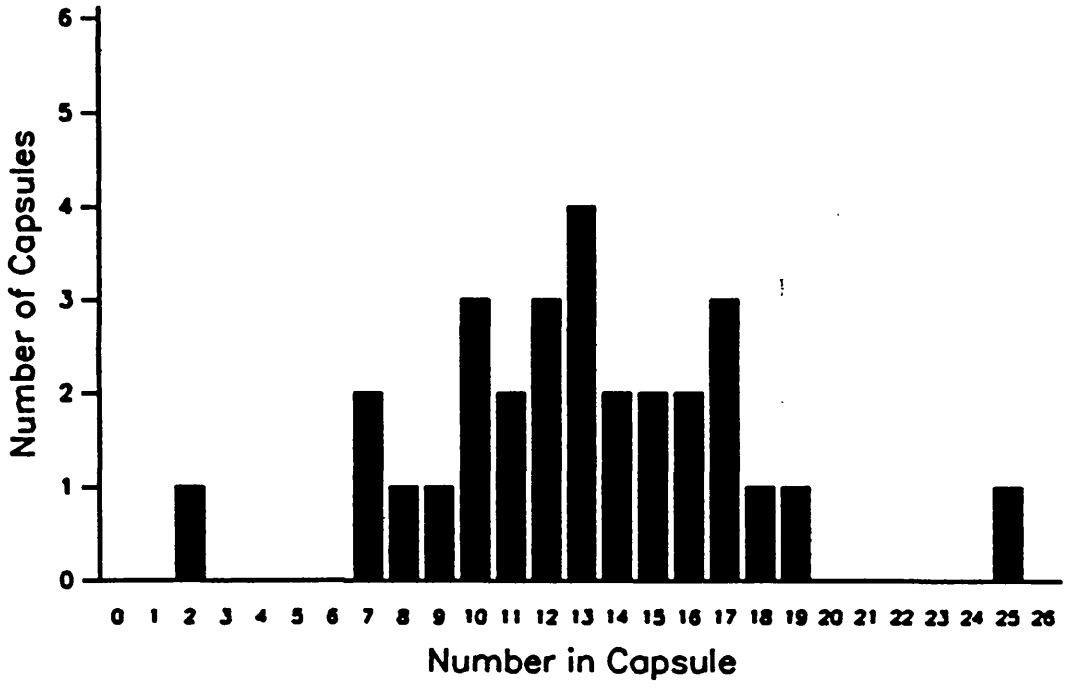
Fig. 2.3.3 Frequency distribution of the number of juveniles per capsule surviving to four months after hatching.

a. Peartree Point

Mean $\pm$ 2SE = 12.966 $\pm$ 1.7233 juveniles per capsule

b. Prawle Point

Mean $\pm$ 2SE = 8.6428 $\pm$ 2.4160 juveniles per capsule


Number of Capsules
0 1 2 3 4 5 6
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
Number in Capsule

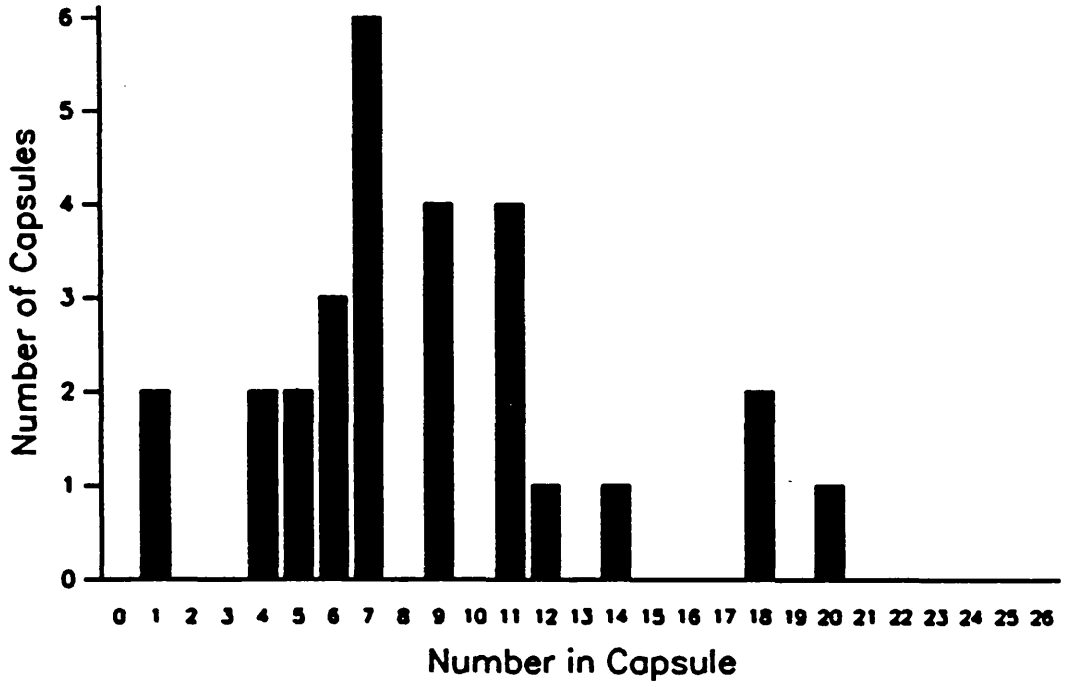
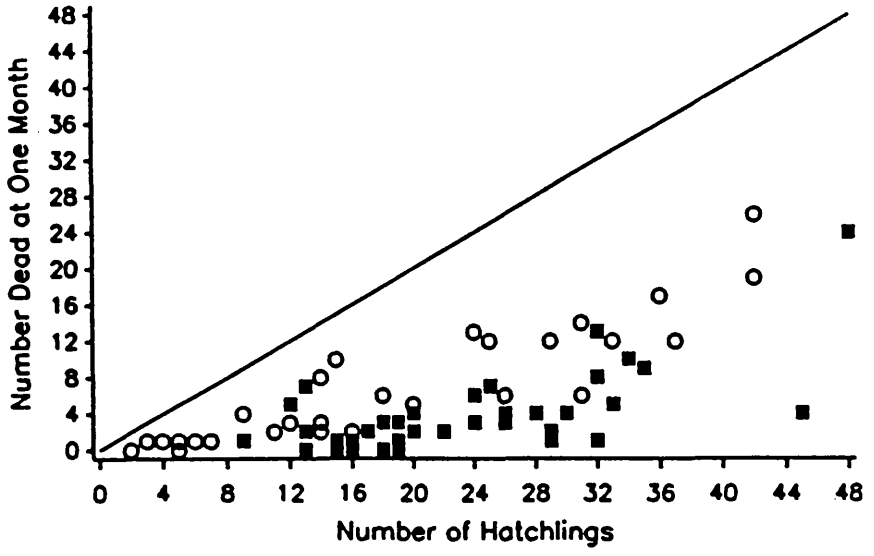
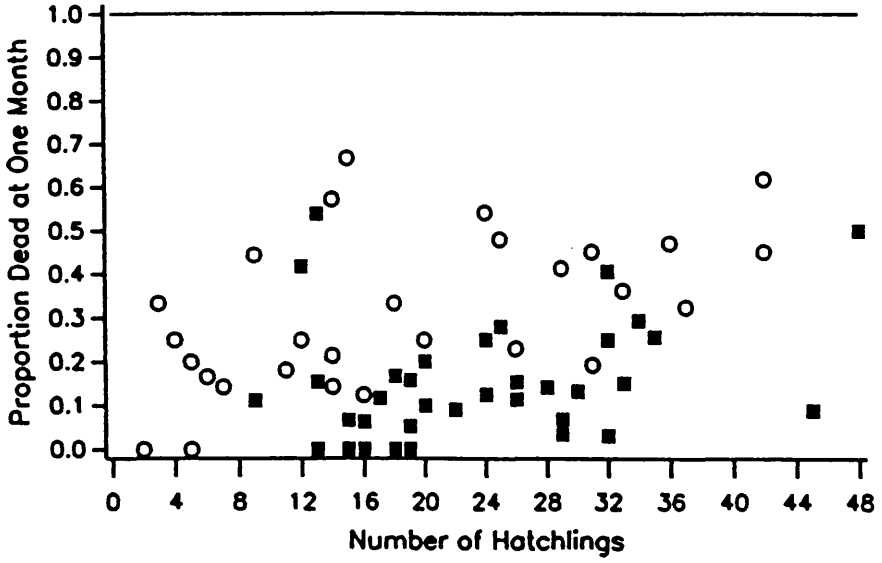

Number of Capsules
0 1 2 3 4 5 6
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
Number in Capsule

Fig. 2.3.4 Relationship between the number of dead juveniles at one month of age and the number of hatchlings at hatching. Peartree Point = closed squares, Prawle Point = open circles, solid line = 100% mortality.

Fig. 2.3.5 Proportion dead at one month of age as a function of the number of hatchlings at hatching. Peartree Point = closed squares, Prawle Point = open circles, solid line = 100% mortality.


Number Dead at One Month
0
4
8
12
16
20
24
28
32
36
40
44
48
Number of Hatchlings


Proportion Dead at One Month
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
4
8
12
16
20
24
28
32
36
40
44
48
Number of Hatchlings

The sampling strategy assumed that adjacent capsules were laid by the same female, which could result in maternal effects upon post-hatching mortality. The proportion dead in one capsule was compared with that of its pair within a population and was not found to be significant at the 5% level (fig. 2.3.6) ($F_{1,27}$=1.67, p=0.2076).

2.3.4 Shell shape and size measurements

Juveniles used to determine the regression coefficients for the relationship between immersed and actual shell weight (section 2.2.1.3.1), were used to test for population differences in shell shape; analysis of covariance indicated significant population effects upon the relationship between length and aperture ($F_{1,76}$=12.84, p=0.0006). For a given length, Prawle Point juveniles possess a longer aperture than juveniles reared from Peartree Point, which would result in a smaller length/aperture ratio for Prawle Point juveniles.

Significant population differences were also seen in length - dry tissue weight relationships ($F_{1,76}$=58.07, p=0.0001, population regression equations are presented in section 2.2.1.3.1); for a given shell length, Peartree Point juveniles have a greater body mass. Results also suggested that Prawle Point juveniles had a greater shell weight for a given length, but were not significant at the 5% significance level ($F_{1,76}$=2.97, p=0.0886). Analysis of covariance indicated that the regression between immersed weight and actual shell weight was significantly different between population samples ($F_{1,76}$=19.58, p=0.0001, population regression equations are presented in section 2.2.1.3.1). For a given immersed shell weight, the actual measured shell weight was greater for Prawle Point juveniles than for Peartree Point individuals.

2.3.5 Size at four months of age

Frequency distributions of length (Fig.2.3.7) and mean total weight (Fig.2.3.8) suggest that Peartree Point juveniles attain a greater mean size than those sampled from Prawle Point. However, family effects on size measurements are significant (Figs.2.3.9-2.3.13; Kirby, 1992), where the number of juveniles per capsule has a significant effect upon the family mean length ($F_{1,54}$=13.02, p<0.001), mean total weight ($F_{1,54}$ = 15.96, p<0.001), mean shell weight ($F_{1,54}$ = 17.95, p<0.001), mean tissue weight ($F_{1,54}$=19.86, p<0.001) attained and the total family weight

Fig. 2.3.6 Correlation between the proportions dying in one capsule and its pair at one month of age. A capsule within a pair was randomly assigned 'capsule one' and its pair 'capsule 2' for analysis purposes. Peartree Point = closed squares, Prawle Point = open circles.

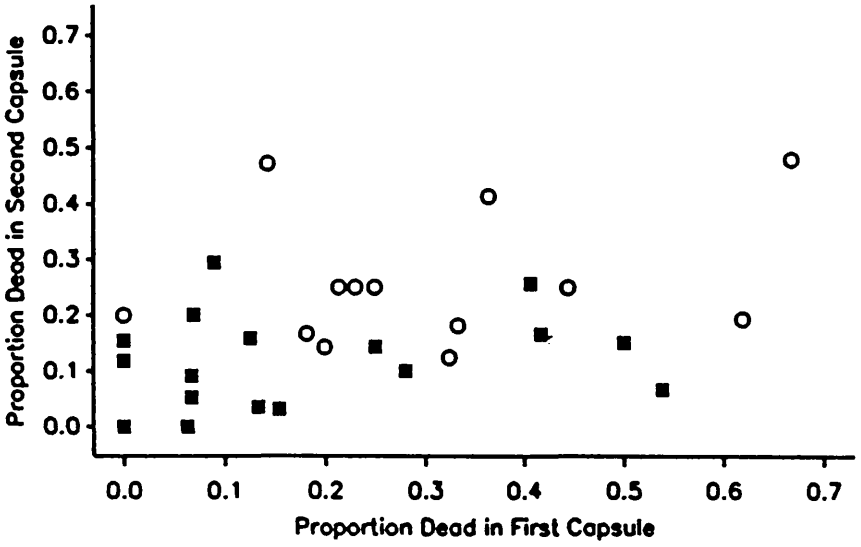
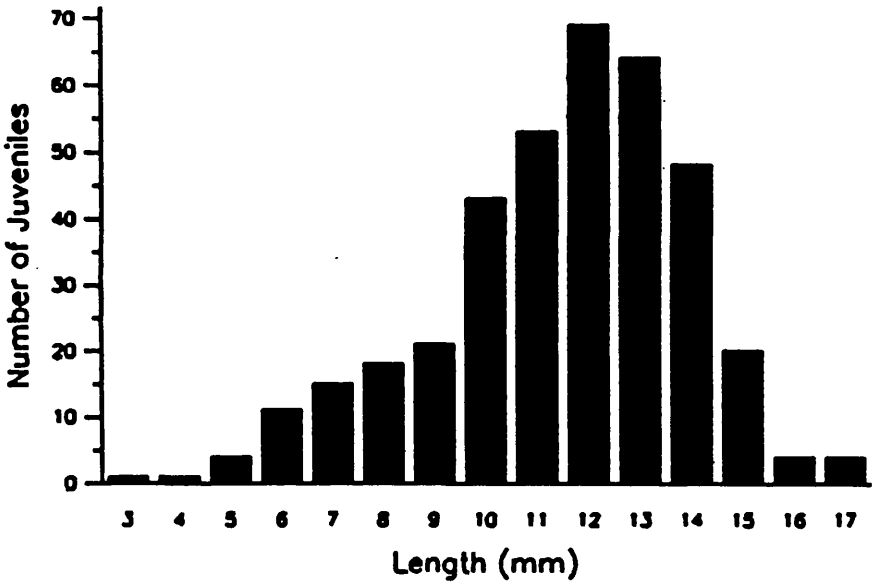

Proportion Dead in Second Capsule
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Proportion Dead in First Capsule

Fig. 2.3.7 Shell length frequency distribution of juveniles at four months after hatching.

a. Peartree Point

b. Prawle Point


Number of Juveniles
0
10
20
30
40
50
60
70
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Length (mm)

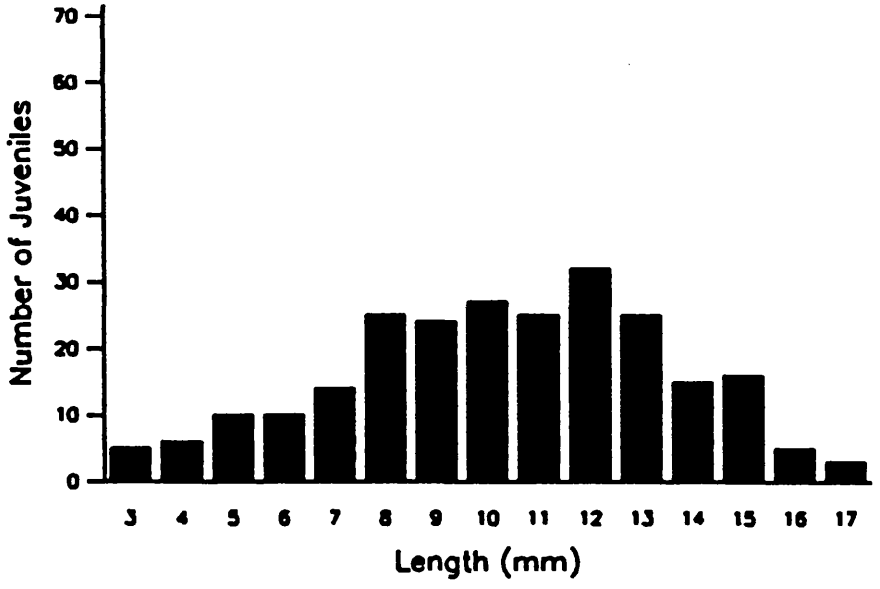
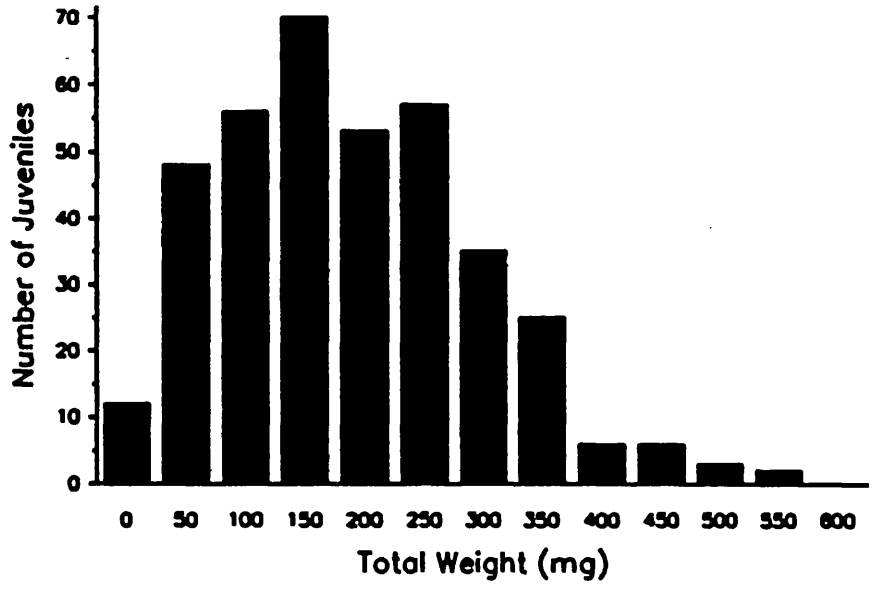

Number of Juveniles
0
10
20
30
40
50
60
70
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Length (mm)

Fig. 2.3.8 Frequency distribution of individual total wet weight for juveniles at four months after hatching.

a. Peartree Point

b. Prawle Point


Number of Juveniles
70
60
50
40
30
20
10
0
0 50 100 150 200 250 300 350 400 450 500 550 600
Total Weight (mg)

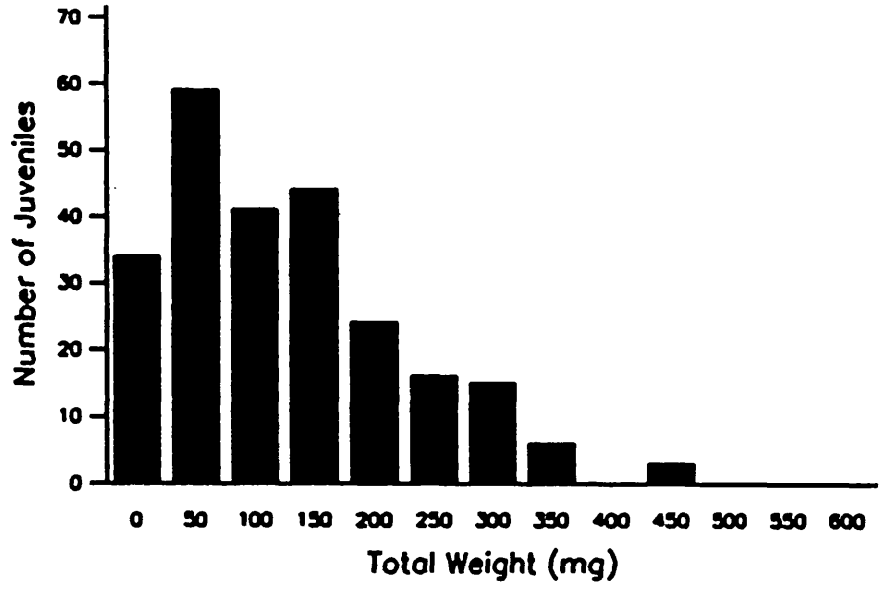
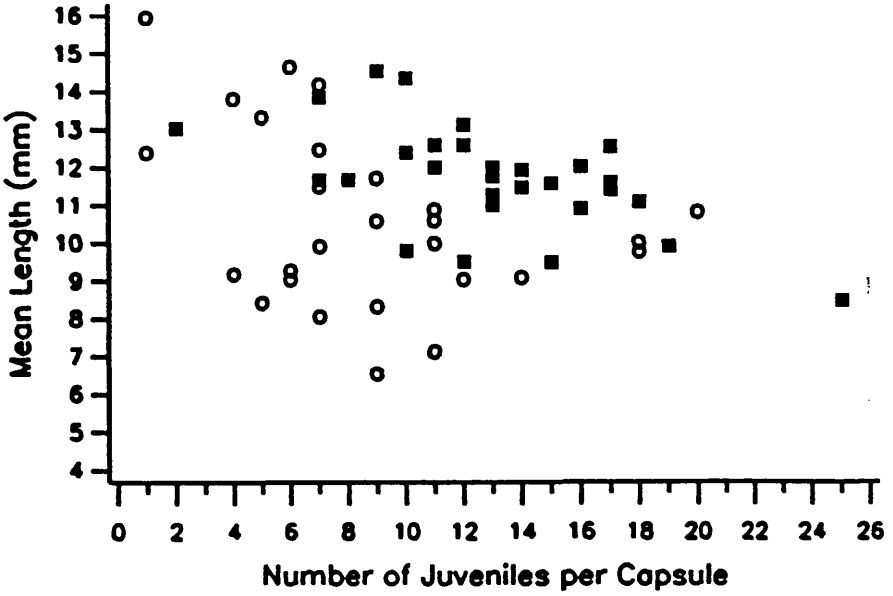

Number of Juveniles
70
60
50
40
30
20
10
0
0 50 100 150 200 250 300 350 400 450 500 550 600
Total Weight (mg)

Fig. 2.3.9 The effects of the number of juveniles per capsule at four months of age upon family mean length. Peartree Point = closed squares, Prawle Point = open circles.

Fig. 2.3.10 The effects of the number of juveniles per capsule at four months of age upon family mean dry tissue weight. Peartree Point = closed squares, Prawle Point = open circles.


Mean Length (mm)
16
15
14
13
12
11
10
9
8
7
6
5
4
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Number of Juveniles per Capsule

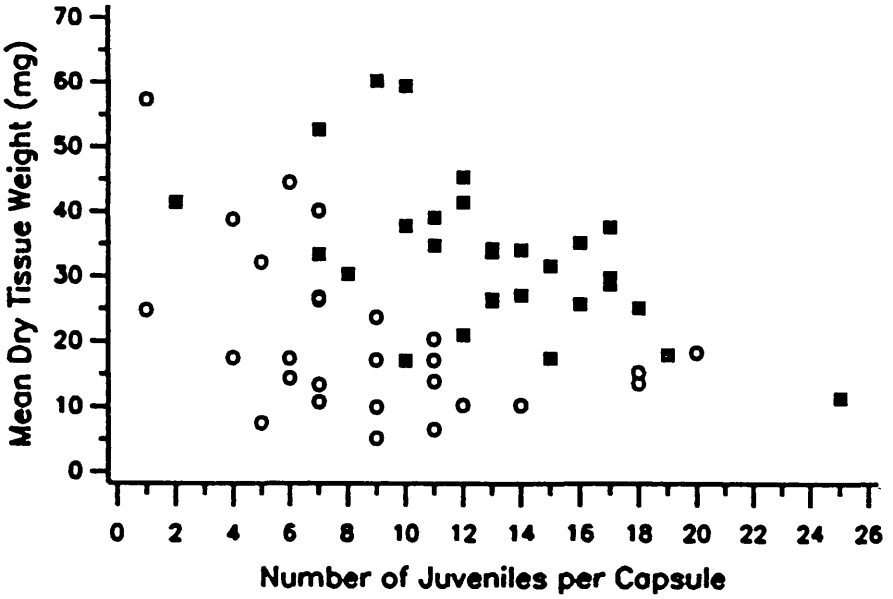
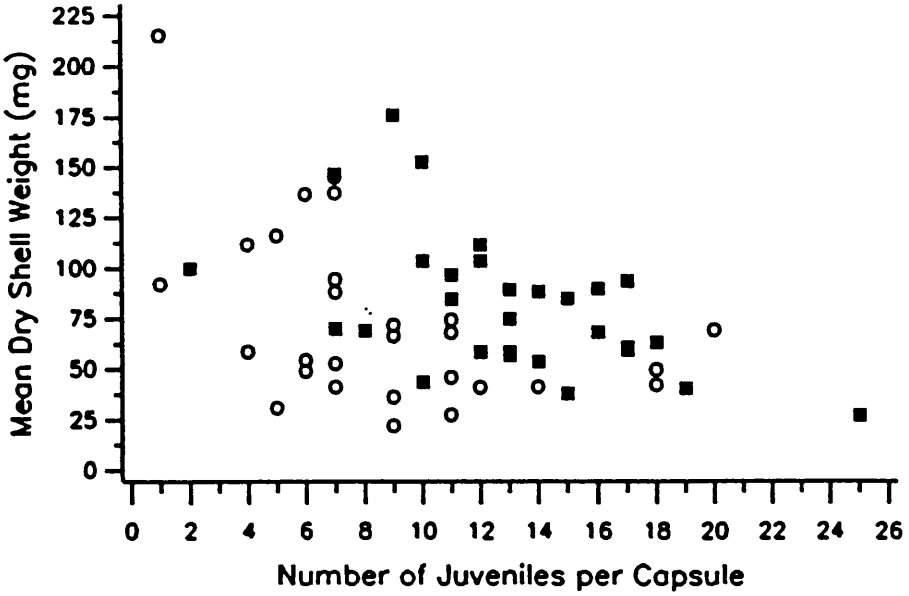

Mean Dry Tissue Weight (mg)
70
60
50
40
30
20
10
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Number of Juveniles per Capsule

Fig. 2.3.11 The effects of the number of juveniles per capsule at four months of age upon family mean dry shell weight. Peartree Point = closed squares, Prawle Point = open circles.

Fig. 2.3.12 The effects of the number of juveniles per capsule at four months of age upon family mean total weight. Peartree Point = closed squares, Prawle Point = open circles.


Mean Dry Shell Weight (mg)
225
200
175
150
125
100
75
50
25
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Number of Juveniles per Capsule

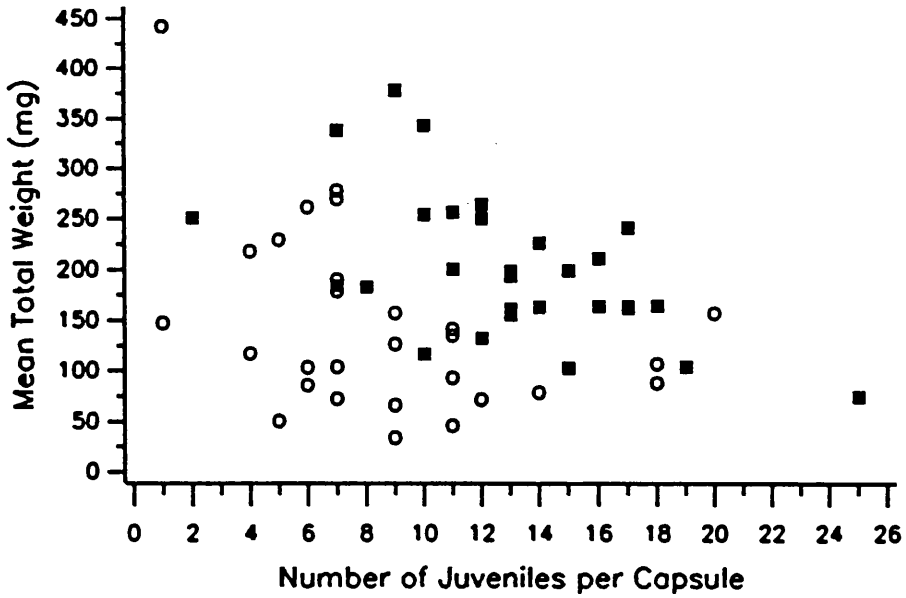
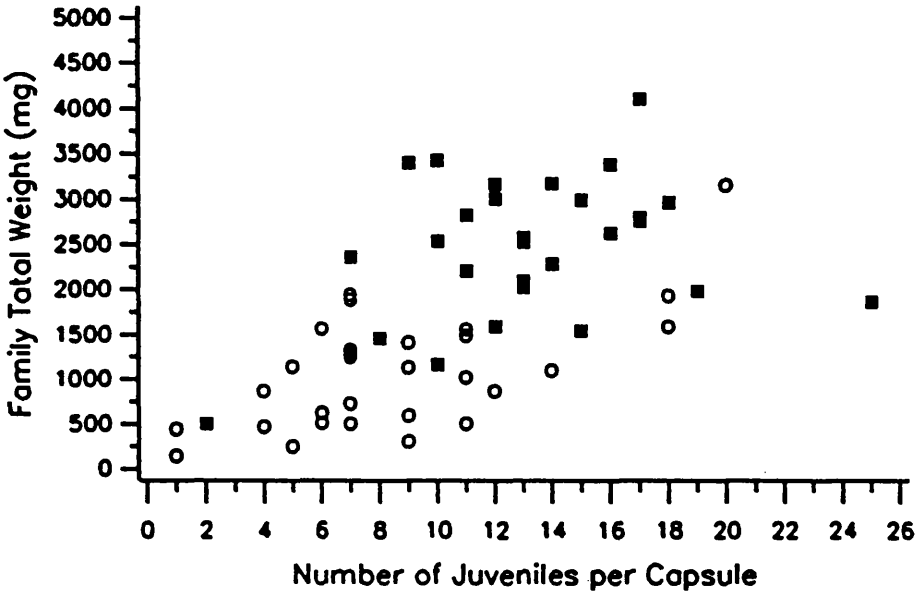

Mean Total Weight (mg)
450
400
350
300
250
200
150
100
50
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Number of Juveniles per Capsule

Fig. 2.3.13 The effects of the number of juveniles per capsule at four months of age upon family total weight. Peartree Point = closed squares, Prawle Point = open circles.


Family Total Weight (mg)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
Number of Juveniles per Capsule

($F_{1,54}$ = 16.24, p<0.001). For a given number of hatchlings per capsule, Peartree Point juveniles are longer ($F_{1,54}$=11.74, p=0.0012), with a greater total weight ($F_{1,54}$ = 19.47, p<0.001), shell weight ($F_{1,54}$=6.49, p=0.014), and tissue weight ($F_{1,54}$ = 32.47, p<0.001) than Prawle Point juveniles. When the total weight of each juvenile is summed for every capsule, Peartree Point samples have a greater total weight per capsule than for Prawle Point for a given number of individuals per capsule ($F_{1,54}$=27.70, p<0.001).

2.3.6 Size at four months of age and genotype

All juveniles that had been measured for size at four months were used in later experiments, and so mortality prevented the scoring of electrophoretic loci for some individuals. Following a similar method of analysis to that used by Kirby (1992), the genotypic frequencies for each of the six loci scored in this study were compared between the large and small size groups for each population; the three largest and three smallest juveniles from each capsule were added to the large and small groups respectively and so allow for the significant effects of family number upon size (section 2.3.5). The genotypic differences between the 'large' and 'small' groups were compared within each population separately, the frequencies of *Pep-1* genotypes differed between the two size groups within the Peartree Point sample (table.2.3.1). The percentage of individuals possessing the 11.11 genotype was higher in the 'large' group (large 7.32%; small 1.30%), whereas a greater percentage of the individuals possessing the 11 allele in the small group were heterozygous (large 26.83%; small 44.16%) (table.2.3.2a). To overcome the differences in number of juveniles between each group size, the percentage of juveniles within each genotypic class was calculated separately for each size category. Variation at the *Pep-1* locus was not associated with the 'large' and 'small' size groups from Prawle Point (table 2.3.1); *Lap-2* was the only locus to approach the 5% significance level (table 2.3.2b).

2.3.7 Population growth rates

2.3.7.1 Genotypic frequencies

The genotypic frequencies at the *Lap-2* and *Mdh-1* loci were significantly different between population samples at the 5% probability level (table.2.3.3).

Table 2.3.1 Chi-square analysis for differences in genotype frequencies within two size categories; 'Large' and 'Small' (see text) for each population * Fisher's Exact Test (two-tailed) was used when any of the cell frequencies were less than five.

Locus	Peartree Point			Prawle Point		
	d.f.	χ^2	Pr>χ^2	d.f	χ^2	Pr>χ^2
Mpi	2	3.073	0.215	2	0.856	0.652
Mdh-1			1.000*	2	0.698	0.705
Pgm-1			0.374*			0.476*
Pgm-2	1	0.131	0.718			0.580*
Lap-2			1.000*	2	5.594	0.061
Pep-1			0.028*			1.000*

Table 2.3.2 Chi-square analysis for differences in genotype frequencies between 'large' and 'small' size categories (see text). * Fisher's Exact Test (two-tailed) was used when any of the cell frequencies were less than five.

a) Peartree Point (Fisher's Exact Test (2-tailed), p=0.028)

Pep-1 genotype	Large		Small	
	n	freq	n	freq
10.10	54	65.85	42	54.55
10.11	22	26.83	34	44.16
11.11	6	7.32	1	1.30

b) Prawle Point (χ-sq = 5.594, 2df, p=0.061)

Lap-2 genotype	Large		Small	
	n	freq	n	freq
10.10	24	41.38	23	44.23
9.10	32	55.17	21	40.38
9.9	2	3.45	8	15.38

Table 2.3.3 Allele frequencies and test for population differences in genotype frequencies in the samples used to determine population growth rates over four weeks. * Fisher's Exact (2-tailed) test was used if any cell count was less than five * * All cell counts were greater than five and so a χ-squared test was used.

Locus	Allele	Frequency at Peartree Point	Frequency at Prawle Point	*Test for population differences in genotype frequencies
Lap-2	10	1.0	0.588	
	9	0	0.412	p<0.0001
Mdh-1	10	0.960	0.462	
	11	0.040	0	
	9	0	0.538	p<0.0001
Pep-1	10	0.803	0.888	
	11	0.184	0.112	
	9	0.013	0	p=0.259
Pgm-1	10	0.947	0.95	
	9	0.053	0.05	p=1.0
Pgm-2	10	0.934	0.825	
	9	0.066	0.175	p=0.076
Mpi	10	0.474	0.488	
	9	0.526	0.512	**χ^2=0.211 p=0.9

2.3.7.2 Growth rates

Figures 2.3.14 to 2.3.17 show the total size increment in relation to initial size for the entire experimental period (28 days). Peartree Point juveniles had higher growth rates than juveniles reared from Prawle Point for three measures of growth (shell length, dry tissue weight and dry shell weight, figs.2.3.14 to 2.3.16) during the two 14-day growth periods (summary of all analysis of covariance analyses in table 2.3.4). The exception was length and shell growth during the second 14-day growth period; growth rates for these two measures were indistinguishable between the two laboratory populations. Variation in total weight growth (fig.2.3.17) over the total 28-day duration was also similar between populations (table 2.3.4d). Identical results were obtained if the increase in length, shell weight and total weight were instead related to dry tissue weight recorded at the beginning of the period over which growth was determined. Population effects upon the relationship between tissue growth and shell growth were statistically significant (table 2.3.5). For a given increase in tissue weight, Prawle Point juveniles deposit more shell than Peartree Point individuals.

2.3.7.3 Genotypic differences in growth rates

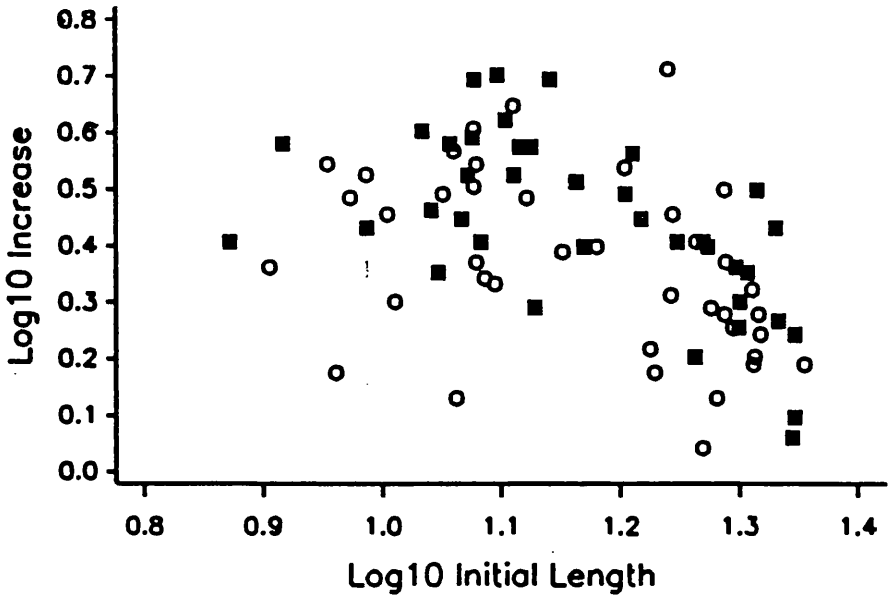
Total growth in dry tissue weight over the four-week experimental period was compared for each population and locus combination to reduce the likelihood of spurious significant results arising by chance. For Prawle Point, variation at the *Pep-1* locus could explain a significant proportion of the variation in tissue growth ($p<0.01$, table 2.3.6). Genotypic variation at the *Lap-2* locus was also associated with differences in growth for Prawle Point juveniles. None of the scored loci could explain the variation in growth rates measured for Peartree Point juveniles.

2.3.7.4 Feeding rates

For each individual, the dry weight of mussel tissue consumed was summed for the first and second 14 day periods. The total dry weights of mussel flesh consumed were converted to Joules using the conversion provided in section 2.2.1.3.2 (p.39). Within each population, there were no differences in feeding rate between the first and second 14 day periods and so feeding rates were summed over the total 28 day duration (fig.2.3.18). Both populations had similar feeding rates (table 2.3.7).

Fig. 2.3.14 Population growth rates over four weeks - length measurements. Peartree Point = closed squares, Prawle Point = open circles.

Fig. 2.3.15 Population growth rates over four weeks - dry tissue weight measurements. Peartree Point = closed squares, Prawle Point = open circles.


Log10 Increase
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.8
0.9
1.0
1.1
1.2
1.3
1.4
Log10 Initial Length

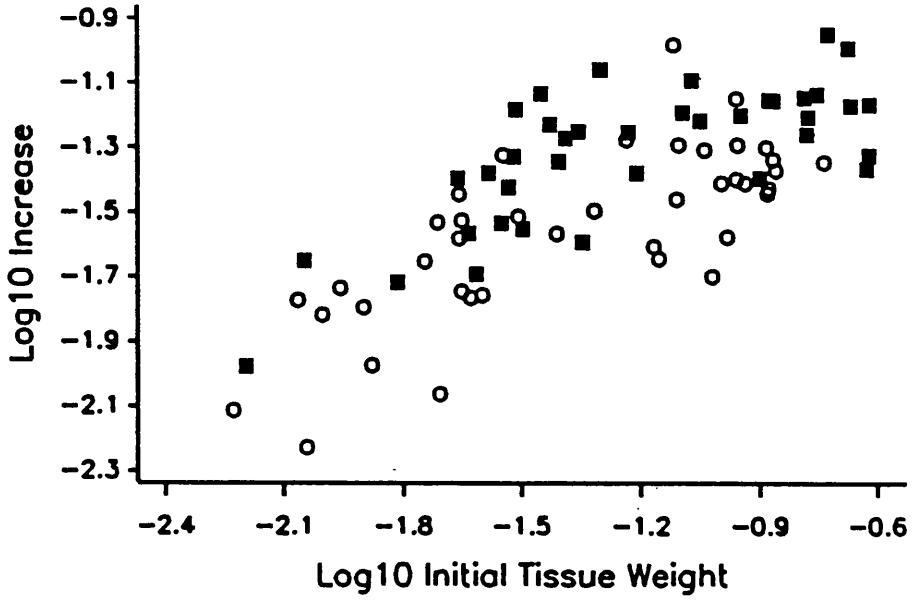
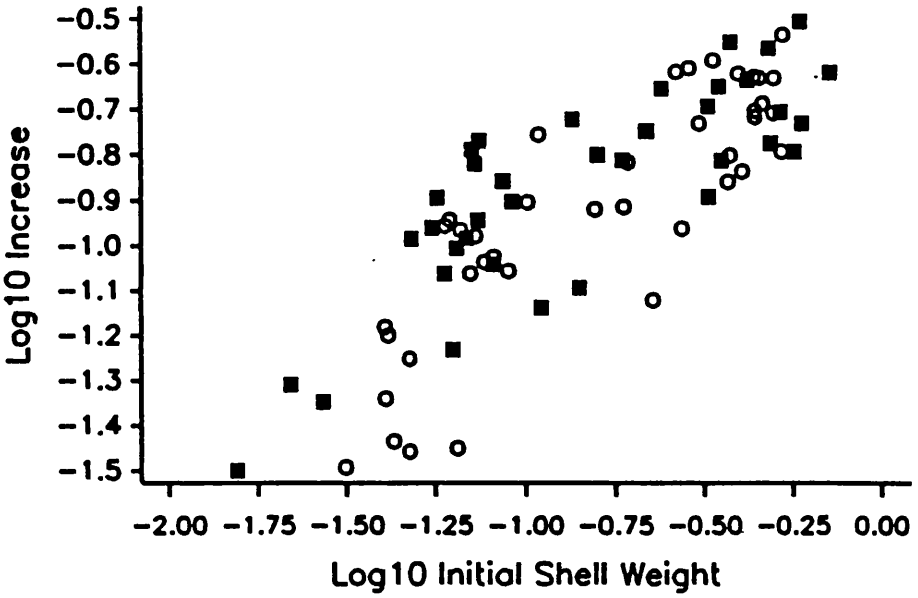

Log10 Increase
−0.9
−1.1
−1.3
−1.5
−1.7
−1.9
−2.1
−2.3
−2.4
−2.1
−1.8
−1.5
−1.2
−0.9
−0.6
Log10 Initial Tissue Weight

Fig. 2.3.16 Population growth rates over four weeks - dry shell weight measurements. Peartree Point = closed squares, Prawle Point = open circles.

Fig. 2.3.17 Population Growth rates over four weeks - total wet weight measurements. Peartree Point = closed squares, Prawle Point = open circles.


Log10 Increase
−0.5
−0.6
−0.7
−0.8
−0.9
−1.0
−1.1
−1.2
−1.3
−1.4
−1.5
−2.00 −1.75 −1.50 −1.25 −1.00 −0.75 −0.50 −0.25 0.00
Log10 Initial Shell Weight

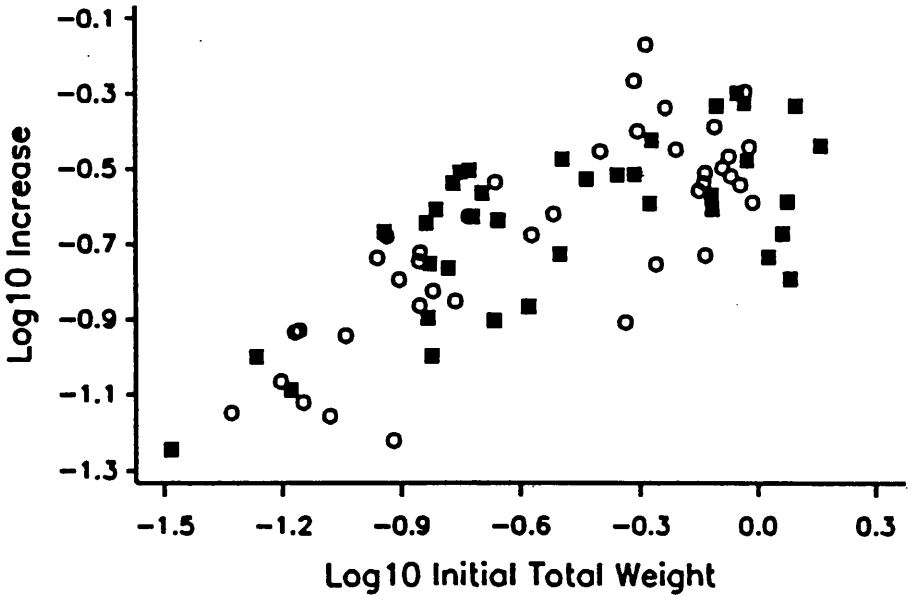

Log10 Increase
−0.1
−0.3
−0.5
−0.7
−0.9
−1.1
−1.3
−1.5 −1.2 −0.9 −0.6 −0.3 0.0 0.3
Log10 Initial Total Weight

Table 2.3.4 Analysis of covariance for population effects upon growth. The relationship between the total increase in measurement (dependent variable) and that recorded at the start of the particular growth period (independent variable) is tested for population differences.

a) Length

Growth Period	Dependent Variable	Source of Variation	df	F-value	Pr>F
days 0-14	Log_{10}Increase 1	Log_{10} Length 1	1,75	13.09	0.0005
		Population	1,75	6.58	0.0123
days 14-28	Log_{10} Increase 2	Log_{10} Length 2	1,74	4.64	0.0344
		Population	1,74	2.78	0.0998
days 0-28	Log_{10}Total Increase	Log_{10} Length 1	1,75	22.89	0.0001
		Population	1,75	5.55	0.0211

b) Dry Tissue

Growth Period	Dependent Variable	Source of Variation	df	F-value	Pr>F
days 0-14	Log_{10} Increase 1	Log_{10} Tissue Wt 1	1,75	101.93	0.0001
		Population	1,75	18.56	0.0001
days 14-28	Log_{10} Increase 2	Log_{10} Tissue Wt 2	1,74	34.19	0.0001
		Population	1,74	7.90	0.0063
days 0-28	Log_{10}Total Increase	Log_{10} Tissue Wt 1	1,75	89.93	0.0001
		Population	1,75	20.51	0.0001

Table 2.3.4 (continued)

c) Dry Shell

Growth Period	Dependent Variable	Source of Variation	df	F-value	Pr>F
days 0-14	Log_{10} Increase 1	Log_{10} Shell Wt 1	1,75	241.37	0.0001
		Population	1,75	6.36	0.0138
days 14-28	Log_{10} Increase 2	Log_{10} Shell Wt 2	1,75	87.16	0.0001
		Population	1,75	1.75	0.1905
days 0-28	Log_{10}Total Increase	Log_{10} Shell Wt 1	1,75	171.47	0.0001
		Population	1,75	5.31	0.0240

d) Total weight

Growth Period	Dependent Variable	Source of Variation	df	F-value	Pr>F
days 0-14	Log_{10} Increase 1	Log_{10} Total Wt 1	1,73	48.58	0.0001
		Population	1,73	0.74	0.3914
days 14-28	Log_{10} Increase 2	Log_{10} Total Wt 2	1,74	55.88	0.0001
		Population	1,74	0.35	0.5566
days 0-28	Log_{10}Total Increase	Log_{10} Total Wt 1	1,74	88.13	0.0001
		Population	1,74	0.04	0.8426

Table 2.3.5 Population effects upon the relationship between tissue growth and shell growth of juveniles used for the determination of population growth rates. Results of the analysis of covariance are presented for the entire growth period (0-28 days) only.

Dependent Variable	Source of Variation	df	F	Pr>F	r^2
Log_{10} increase in shell weight	Log_{10}increase in tissue weight	1,75	402.65	0.0001	0.845
	Population	1,75	45.76	0.0001	

Table 2.3.6 Summary of tests of association between variation in tissue growth rate and genotype for Peartree Point (a) and Prawle Point (b). The *Lap-2* locus was monomorphic for the 10.10 genotype in the Peartree Point sample, and so was excluded from this analysis.

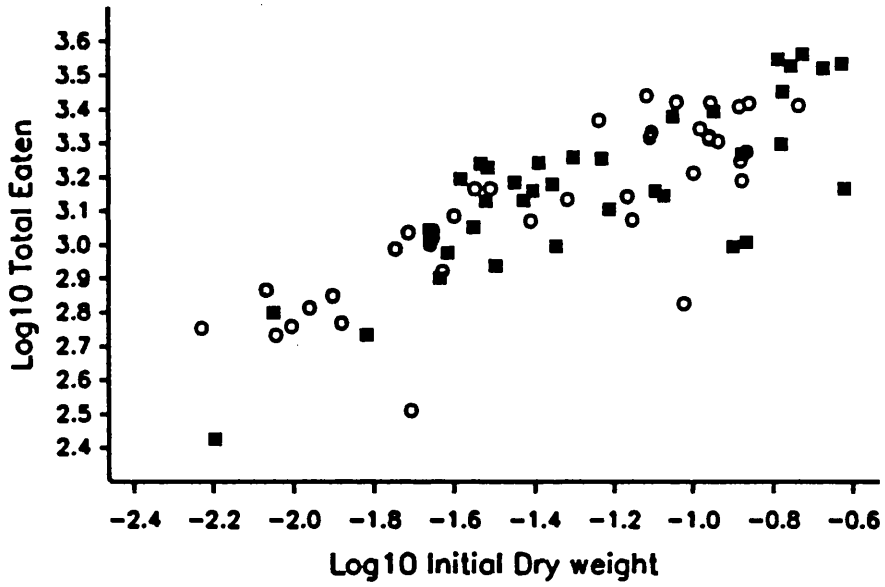
(a) Peartree Point

Locus	F	df	p>F
Pep-1	1.01	2, 34	0.376
Lap-2	.	.	.
Mdh-1	0.07	1, 35	0.786
Mpi	0.41	2, 34	0.666
Pgm-1	0.02	1, 35	0.878
Pgm-2	0.13	1, 35	0.719

(b) Prawle Point

Locus	F	df	p>F
Pep-1	7.54	1, 36	0.009
Lap-2	2.63	2, 36	0.086
Mdh-1	0.85	2, 36	0.434
Mpi	2.10	2, 36	0.137
Pgm-1	0	1, 37	0.970
Pgm-2	1.12	2, 36	0.337

Fig. 2.3.18 Feeding rates of juveniles fed *ad libitum* in the determination of population growth rates. Peartree Point = closed squares, Prawle Point = open circles.


Log10 Total Eaten
3.6
3.5
3.4
3.3
3.2
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.4
-2.4
-2.2
-2.0
-1.8
-1.6
-1.4
-1.2
-1.0
-0.8
-0.6
Log10 Initial Dry weight

2.3.7.5 Gross growth efficiency

An indication of gross growth efficiency was obtained by comparing the total tissue growth for each individual with the total calorific value of mussel tissue ingested (fig. 2.3.19). For a given amount of mussel tissue ingested (Joules), Peartree Point juveniles have a higher rate of tissue growth than Prawle Point individuals, but shell growth is similar for both populations (table 2.3.8 and fig. 2.3.20).

2.3.7.6 Respiration rates

Separate b-exponents used to standardise respiration rates for weight (section 2.2.1.3.3), were calculated for each population and growth period. Within each population, the b-exponents calculated at 14 and 28 days after the start of the experiment were alike (Peartree Point $F_{1,74}$=0.97, p=0.328; Prawle Point $F_{1,77}$=0.04, p=0.833). Respiration rates obtained from the two sampling occasions were therefore pooled separately for each population before population effects upon b were tested and found not to be significant at the 5% level ($F_{1,154}$=3.16, p=0.0774). The b-exponent was calculated to be 0.575 $\pm$ 0.072 ($\pm$ 2SE).

Standard respiration rates (120mg dry tissue weight) (fig.2.3.21) did not differ between time points for both populations (Peartree Point $F_{1,75}$=0.92, p=0.339; Prawle Point $F_{1,78}$=0.06, p=0.808), although mean values indicate slightly lower rates on the second measurement. Repeated analysis of variance indicated that respiration rates were not significantly different between populations at the 5% probability level ($F_{1,75}$=2.09, p=0.153).

2.3.7.7 Genotypic differences in respiration rates

To reduce the probability of obtaining a chance significant result, separate analyses were performed for all population and locus combinations each time respiration rates were determined. Results of the analysis of covariance for the effects of genotype upon standardised respiration rates measured on the first sampling occasion (14 days after the start of the experiment) are presented in table 2.3.9. Similar results were obtained for respiration rates measured at the end of the experiment (28 days). Allozymal variation in the scored loci was not associated with variation in respiration rates.

Table 2.3.7 Analysis of covariance to test for population differences in feeding rates. Juveniles were provided with an *ad libitum* supply of mussels over the four week experimental period.

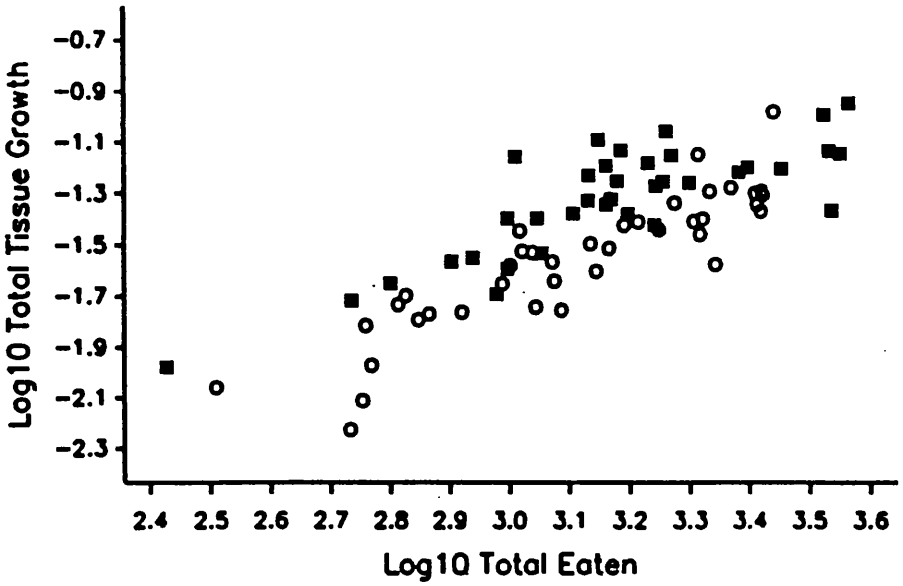
Growth Period	Dependent Variable	Source of Variation	df	F-value	Pr>F
days 0-14	Log_{10} total eaten 1	Log_{10} tissue weight 1	1,74	72.33	0.0001
		Population	1,74	0.01	0.9088
days 14-28	Log_{10} total eaten 2	Log_{10} tissue weight 2	1,74	67.57	0.0001
		Population	1,74	0.23	0.6320
days 0-28	Log_{10}total eaten 1& 2	Log_{10} tissue weight 1	1,75	107.73	0.0001
		Population	1,75	0.17	0.6809

Table 2.3.8 Analysis of Covariance for the effects of population upon the gross growth efficiency over the whole four week experimental period. * - denotes an interaction between the dependent variable, log total eaten, and population, which indicates that each population has a separate gradient when relating tissue growth to the total amount of food eaten.

	Dependent Variable	Source of variation	df	F	Pr>F
Tissue Growth	Log_{10}increase in tissue weight	Log_{10} total eaten	1, 74	186.16	0.0001
		Population	1, 74	6.24	0.0147
		Log_{10} total eaten * population	1, 74	4.05	0.0477
Shell Growth	Log_{10} increase in shell weight	Log_{10} total eaten	1, 75	260.52	0.0001
		Population	1, 75	0.04	0.8338

Fig. 2.3.19 Gross growth efficiency of juveniles fed *ad libitum* in the determination of population growth rates. The relationship between the total increase in dry tissue weight and the total joules of mussel flesh eaten over four weeks is presented. Peartree Point = closed squares, Prawle Point = open circles.

Fig. 2.3.20 Gross growth efficiency of juveniles fed *ad libitum* in the determination of population growth rates. The relationship between the total increase in dry shell weight and the total joules of mussel flesh eaten over four weeks is presented. Peartree Point = closed squares, Prawle Point = open circles.


Log10 Total Tissue Growth
-0.7
-0.9
-1.1
-1.3
-1.5
-1.7
-1.9
-2.1
-2.3
2.4 2.5 2.6 2.7 2.8 2.9 3.0 3.1 3.2 3.3 3.4 3.5 3.6
Log10 Total Eaten

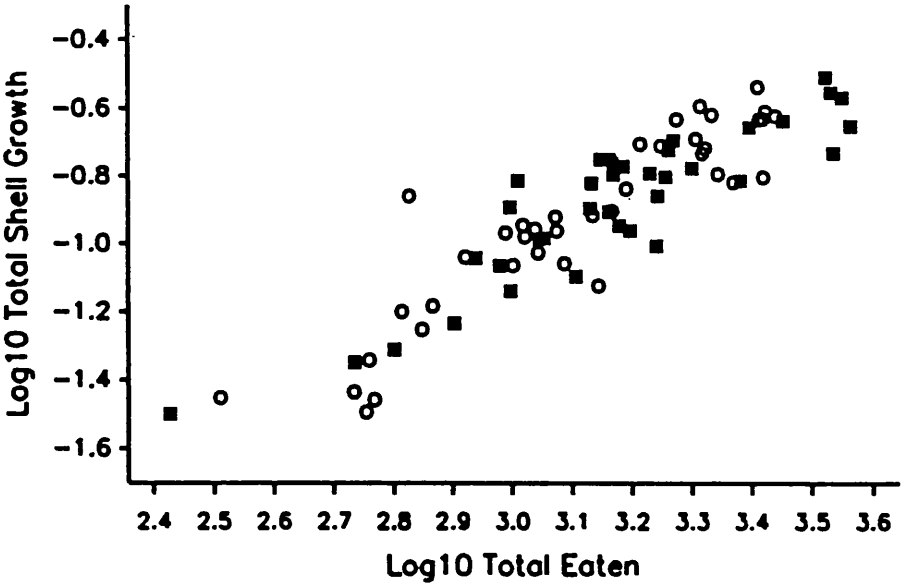

Log10 Total Shell Growth
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
2.4 2.5 2.6 2.7 2.8 2.9 3.0 3.1 3.2 3.3 3.4 3.5 3.6
Log10 Total Eaten

Fig. 2.3.21 Weight standardised respiration rates. A common b-exponent of 0.575 was used to standardise respiration rates to a standard animal size of 120mg dry tissue weight. Means ± 2SE are presented for each measurement taken 14 and 28 days after the start of the experiment. Peartree Point - squares Prawle Point - circles.

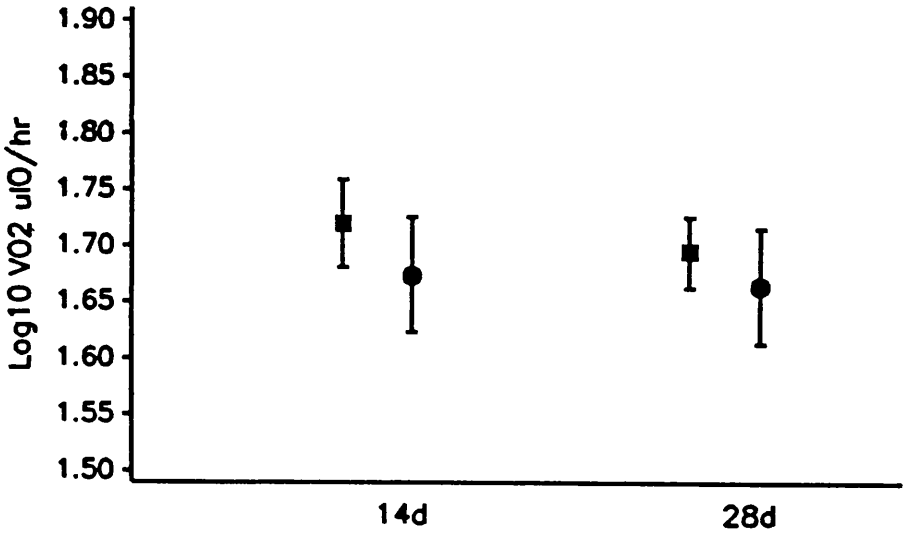

1.90
1.85
1.80
1.75
1.70
1.65
1.60
1.55
1.50
Log10 VO2 ul0/hr
14d
28d

Table 2.3.9 Summary of tests of association between variation in respiration rate measured 14 days after the start of the experiment and genotype for Peartree Point (a) and Prawle Point (b). The *Lap-2* locus was monomorphic for the 10.10 homozygote in the Peartree Point sample and was excluded from this analysis.

(a) Peartree Point

Locus	F	df	p>F
Pep-1	1.39	2, 35	0.2627
Lap-2	-	-	-
Mdh-1	0.31	1, 36	0.5839
Mpi	0.29	2, 35	0.7512
Pgm-1	1.12	1, 36	0.2962
Pgm-2	0.68	1, 37	0.4151

(b) Prawle Point

Locus	F	df	p>F
Pep-1	0.64	1, 38	0.4279
Lap-2	0.52	2, 37	0.5993
Mdh-1	0.59	2, 37	0.5585
Mpi	0.95	2, 37	0.3946
Pgm-1	0.04	1, 38	0.8408
Pgm-2	0.39	2, 37	0.6783

2.3.8 Feeding ration experiments

2.3.8.1 Genetic analysis of population samples

The genotypic frequencies at the *Lap-2* and *Mdh-1* loci were significantly different between population samples of laboratory reared juveniles maintained at each feeding ration ($p<0.0001$, tables 2.3.10-2.3.12). The *Pep-1* genotype frequencies were also significantly different between Peartree Point and Prawle Point in the starved samples ($p<0.0001$, table 2.3.12); the *Pep-1* '9' allele was present and the '11' allele present in the heterozygous condition for Peartree Point juveniles, whereas the majority (75%) of Prawle Point juveniles were homozygous for the '10' allele.

2.3.8.2 Size regressions

Shell weights recorded at the end of the experiment were used to re-calculate the regression equations relating immersed weight to actual shell weight; feeding ration was seen to have a significant effect upon this relationship (table 2.3.13). For a given immersed weight, the actual shell weight measured in ascending order of magnitude was low ration, *ad libitum* and starved samples. Population differences were only found within the *ad libitum* fed samples. The 'new' Palmer regressions were:

Ad libitum

Peartree Point

Dry Shell weight (g) = immersed weight (g) *1.412($\pm$0.025) + 0.0006 ($\pm$0.005)

Prawle Point

Dry Shell weight (g) = immersed weight (g) *1.412 ($\pm$0.025) + 0.0088 ($\pm$0.004)

Low ration

Dry Shell weight (g) = immersed weight (g) *1.515 ($\pm$0.104) - 0.009($\pm$0.019)

Starved

Dry Shell weight (g) = immersed weight (g) *1.398 ($\pm$0.073) + 0.024 ($\pm$0.014)

Table 2.3.10 Allele frequencies and test for population differences between Peartree Point (n=25) and Prawle Point (n=30) genotype frequencies in samples of laboratory reared juveniles fed *ad libitum*. *Fisher's Exact (2-tailed) test was used. * * All cell counts were greater than five and so a χ-squared test was used.

Locus	Allele	Frequency at Peartree Point	Frequency at Prawle Point	* Test for population differences in genotype frequencies
Lap-2	10	1.0	0.633	
	9	0	0.350	
	8	0	0.017	p<0.0001
Mdh-1	10	0.940	0.467	
	11	0.060	0	
	9	0	0.533	p<0.0001
Pep-1	10	0.720	0.900	
	11	0.240	0.100	
	9	0.040	0	p=0.095
Pgm-1	10	0.880	0.983	
	9	0.120	0.017	p=0.102
Pgm-2	10	0.840	0.833	
	9	0.160	0.167	p=0.876
Mpi	10	0.480	0.517	
	9	0.520	0.483	$^{**}\chi^2$=0.166 p=0.920

Table 2.3.11 Allele frequencies and test for population differences between Peartree Point (n=29) and Prawle Point (n=28) genotype frequencies in the samples of laboratory reared juveniles fed on the low ration *Fisher's Exact (2-tailed) test was used. * * All cell counts were greater than five and so a χ-squared test was used.

Locus	Allele	Frequency at Peartree Point	Frequency at Prawle Point	* Test for population differences in genotype frequencies
Lap-2	10	1.00	0.732	
	9	0	0.250	
	8	0	0.018	p<0.0001
Mdh-1	10	0.948	0.500	
	11	0.017	0	
	9	0.035	0.500	p<0.0001
Pep-1	10	0.759	0.821	
	11	0.207	0.179	
	9	0.034	0	p=1.00
Pgm-1	10	0.914	1.00	
	9	0.086	0	p=0.052
Pgm-2	10	0.914	0.857	
	9	0.086	0.143	p=0.358
Mpi	10	0.483	0.536	
	9	0.517	0.464	**χ^2=1.179 p=0.555

Table 2.3.12 Allele frequencies and test for population differences between Peartree Point (n=71) and Prawle Point (n=71) genotype frequencies in the samples of starved laboratory reared juveniles. *Fisher's Exact (2-tailed) test was used. * * All cell counts were greater than five and so a χ-squared test was used.

Locus	Allele	Frequency at Peartree Point	Frequency at Prawle Point	*Test for population differences in genotype frequencies
Lap-2	10	1.00	0.697	
	9	0	0.296	
	8	0	0.007	p<0.0001
Mdh-1	10	0.958	0.438	
	11	0.035	0	
	9	0.007	0.562	p<0.0001
Pep-1	10	0.680	0.833	
	11	0.292	0.167	
	9	0.028	0	p<0.0001
Pgm-1	10	0.944	0.979	
	9	0.056	0.021	p=0.203
Pgm-2	10	0.868	0.882	
	9	0.132	0.118	p=0.920
Mpi	10	0.514	0.521	
	11	0.007	0	
	9	0.479	0.497	p=0.558

Table 2.3.13 Effects of feeding ration and population upon the regression between immersed weight and actual shell weight. Only significant sources of variation at the 5% probability level are presented.

	Dependent Variable	Source of Variation	df	F-value	Pr>F
ad lib v. low ration	Dry Shell Weight	Immersed weight	1, 131	4045.84	0.0001
		Experiment	1, 131	4.05	0.0461
		Imm*Expt	1, 131	7.08	0.0088
ad lib v. starved	Dry Shell Weight	Immersed weight	1, 219	2924.15	0.0001
		Experiment	1, 219	13.81	0.0003
		Population	1, 219	5.62	0.0186
low ration v. starved	Dry Shell Weight	Immersed weight	1, 197	2217.02	0.0001
		Experiment	1, 197	4.79	0.0298

2.3.8.3 Growth rates

Although size was measured every two weeks, growth rate was taken as the total change in shell and tissue weight from the start of the ration period to the end of the experiment. At these times, accurate Palmer regressions were available; intermediate measurements could not be calculated due to the uncertain interactions with immersed weight and population/ration over time (section 2.3.8.2).

Juveniles from both populations maintained on the *ad libitum* ration were alike for either wet tissue weight growth ($F_{1,53}$=0.03, p=0.8739) or shell growth ($F_{1,53}$=2.37, p=0.1299). Population differences in growth were seen for the low and starved rations (table 2.3.14 and figs. 2.3.22 & 2.3.23).

When fed on the low ration (table 2.3.14a and fig. 2.3.22), Prawle Point juveniles had higher tissue and shell growth rates than those measured for Peartree Point juveniles.

Prawle Point juveniles starved for four weeks lost less tissue weight (fig. 2.3.23a and table 2.3.14b) and had lower rates of shell growth for a given animal size (fig.2.3.23b and table 2.3.14b) than Peartree Point individuals.

2.3.8.4 Feeding rates

Mussel dry tissue weight was calculated for each mussel eaten using the calculated length-dry weight regression in section 2.2.1.3.2. The total weight of mussel tissue eaten over the six week experimental period was summed for each individual and converted to Joules assuming 5.2 $cal.mg^{-1}$ (Bayne and Scullard, 1978b) and 4.184 $J.cal^{-1}$. For the *ad libitum* samples, feeding rate was not significantly different between Peartree Point and Prawle Point juveniles ($F_{1,55}$=0.15, p=0.6971) (fig.2.3.24). However, Prawle whelks had a significantly higher calorific intake than that of Peartree Point individuals maintained on the low feeding level ($F_{1,53}$=5.40, p=0.0240) (fig.2.3.25).

2.3.8.5 Growth rate and genotype

The effects of genotypic variation upon growth over the entire four week experimental period were only tested for the starved samples (table 2.3.15); *ad libitum* fed samples did not show

Table 2.3.14 Analysis of covariance for population effects upon growth in wet tissue and shell weight. Tissue weights are estimated from the difference between the total weight and the shell weight (from the regressions in section 2.3.8.2). Interaction terms that failed to reach the 5% significance level are not presented.

a) Low ration

	Dependent Variable	Source	df	F-value	Pr>F
Wet Tissue Growth	$\log_{10}$final tissue weight	$\log_{10}$ initial tissue weight	1, 52	471.28	0.0001
		Population	1, 52	1.81	0.1846
		Interaction	1, 52	5.83	0.0193
Shell Growth	$\log_{10}$ final shell weight	$\log_{10}$ initial shell weight	1, 53	889.65	0.0001
		Population	1, 53	4.17	0.0461

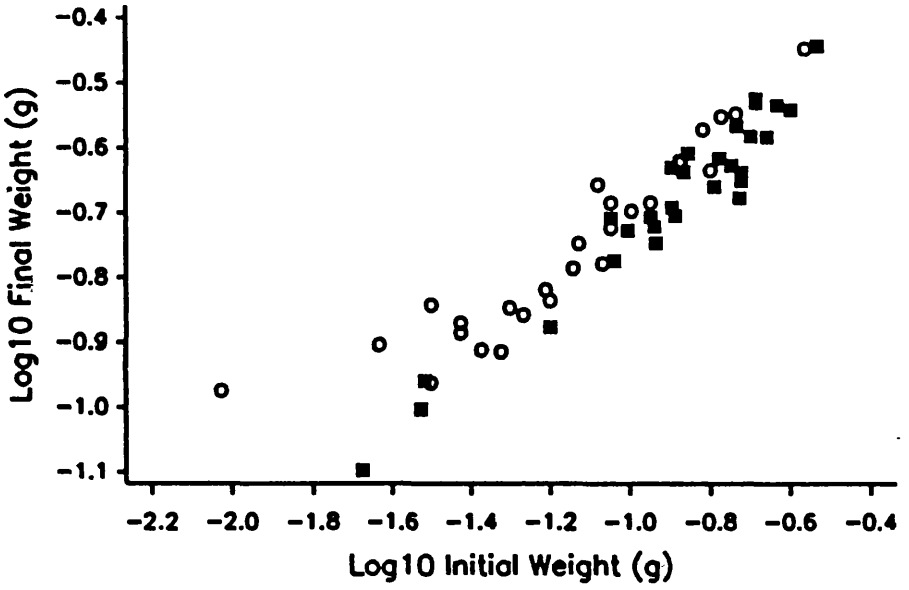
b) Starved ration

	Dependent Variable	Source	df	F-value	Pr>F
Wet Tissue Growth	$\log_{10}$final tissue weight	$\log_{10}$ initial tissue weight	1,159	4527.99	0.0001
		Population	1,159	23.49	0.0001
		Interaction	1,159	6.39	0.0125
Shell Growth	$\log_{10}$ final shell weight	$\log_{10}$ initial shell weight	1,159	12817.2	0.0001
		Population	1,159	2.16	0.1432
		Interaction	1,159	28.71	0.0001

Fig. 2.3.22 Growth of juveniles maintained on a low ration (see text) for six weeks. Peartree Point = closed squares, Prawle Point = open circles.

a. Wet tissue growth

b. Dry shell growth


-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1.0
-1.1
Log10 Final Weight (g)
-2.2
-2.0
-1.8
-1.6
-1.4
-1.2
-1.0
-0.8
-0.6
-0.4
Log10 Initial Weight (g)

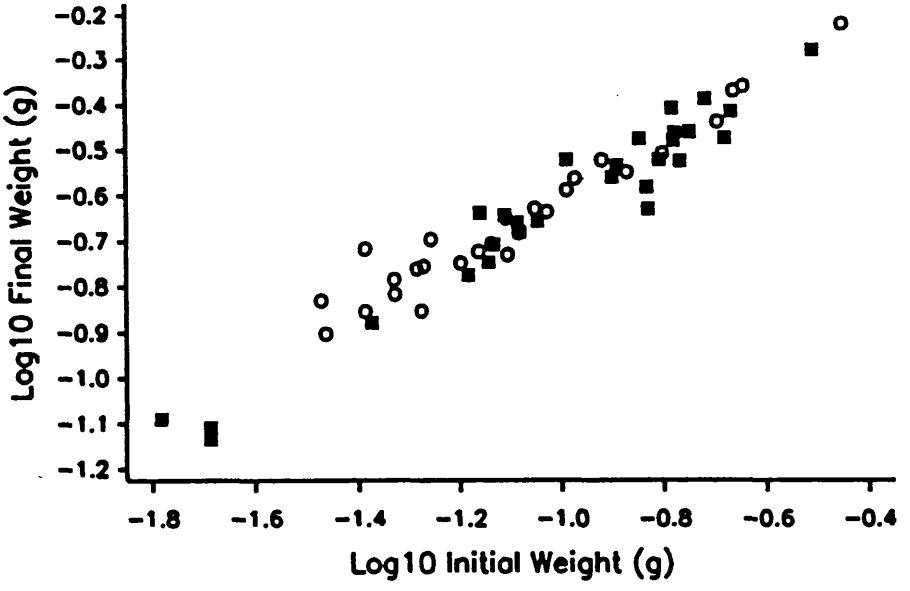
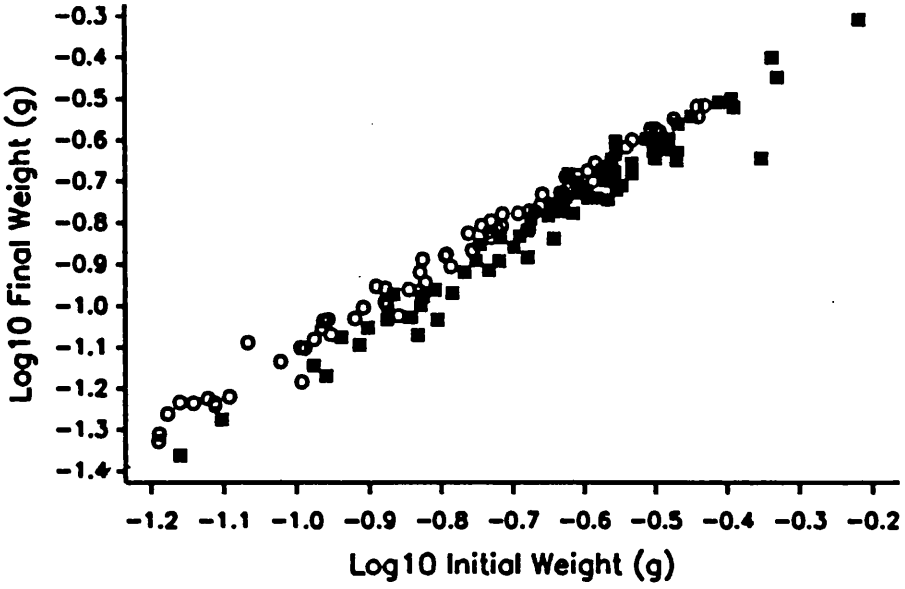

-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1.0
-1.1
-1.2
Log10 Final Weight (g)
-1.8
-1.6
-1.4
-1.2
-1.0
-0.8
-0.6
-0.4
Log10 Initial Weight (g)

Fig. 2.3.23 Growth of juveniles starved for four weeks. Peartree Point = closed squares, Prawle Point = open circles.

a. Wet tissue growth

b. Dry shell growth


Log10 Final Weight (g)
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1.0
-1.1
-1.2
-1.3
-1.4
-1.2 -1.1 -1.0 -0.9 -0.8 -0.7 -0.6 -0.5 -0.4 -0.3 -0.2
Log10 Initial Weight (g)

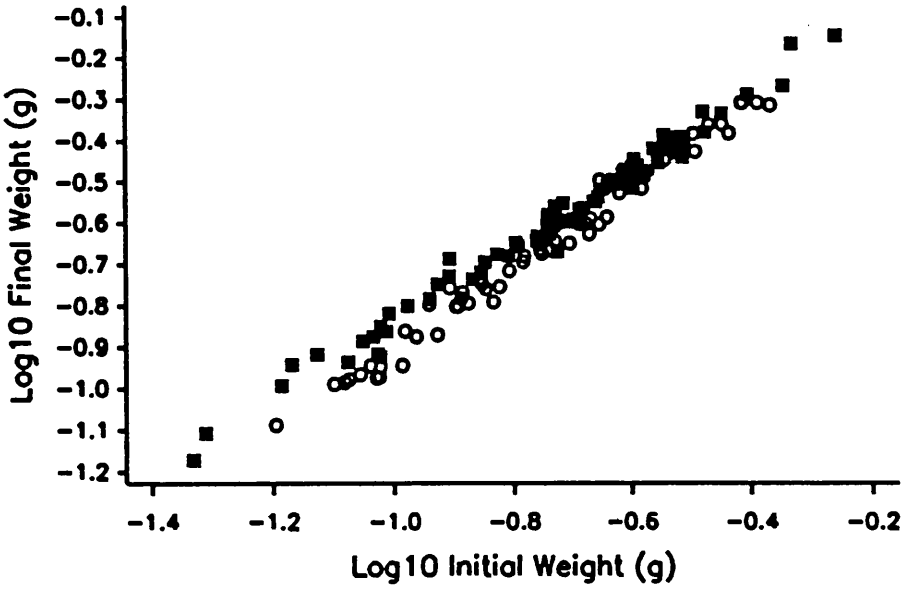
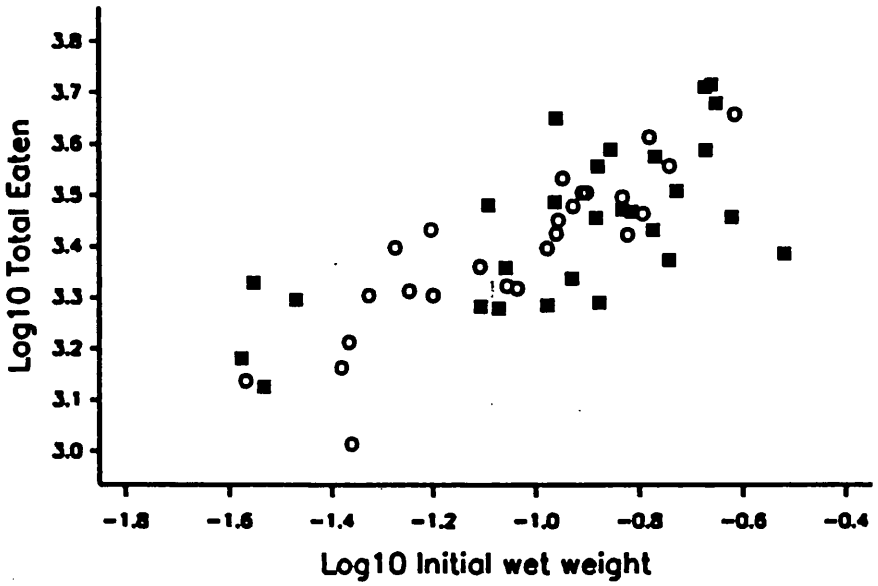
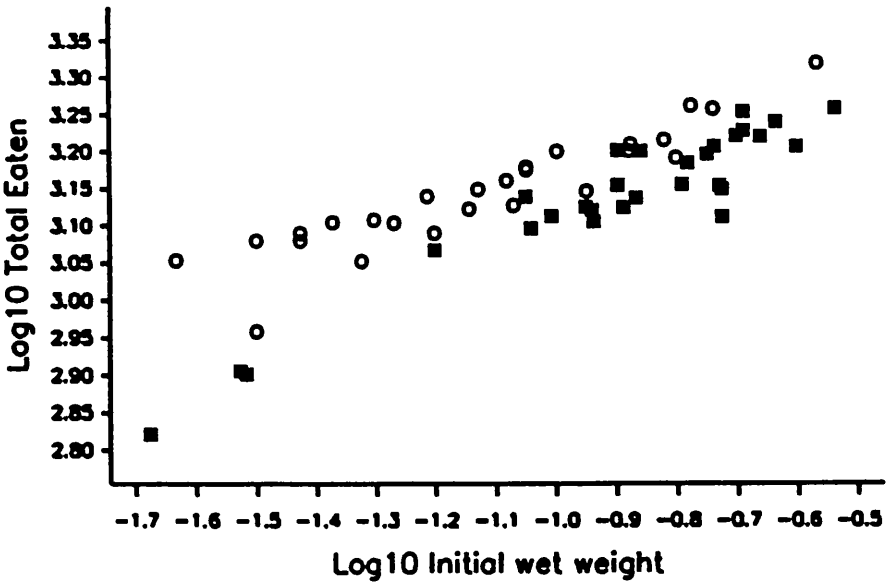

Log10 Final Weight (g)
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1.0
-1.1
-1.2
-1.4 -1.2 -1.0 -0.8 -0.6 -0.4 -0.2
Log10 Initial Weight (g)

Fig. 2.3.24 Feeding rates for juveniles fed *ad libitum* in the feeding ration experiments. Peartree Point = closed squares, Prawle Point = open circles.

Fig. 2.3.25 Feeding rates for juveniles maintained on the low ration (see text) for the feeding ration experiments. Peartree Point = closed squares, Prawle Point = open circles.


Log10 Total Eaten
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3.0
-1.8
-1.6
-1.4
-1.2
-1.0
-0.8
-0.6
-0.4
Log10 Initial wet weight


Log10 Total Eaten
3.35
3.30
3.25
3.20
3.15
3.10
3.05
3.00
2.95
2.90
2.85
2.80
-1.7
-1.6
-1.5
-1.4
-1.3
-1.2
-1.1
-1.0
-0.9
-0.8
-0.7
-0.6
-0.5
Log10 Initial wet weight

population differences in growth rate or feeding rate, and so genotypic differences were considered unlikely coupled with the low sample size scored for the electrophoretic loci (table 2.3.10). The difference in feeding rate between population samples maintained on the low ration precluded further analysis of growth rate in these juveniles.

For the starved samples, each locus was analysed separately for differences in tissue and shell growth rates. Genotype effects were tested for after population effects (section 2.3.8.3) had been removed from the model statement relating total weight increase with the weight measured at the start of the experiment. The relationship between shell growth rate and *Lap-2* genotype was significant (table 2.3.15), the highest rate of shell growth was seen in juveniles with the 10.10 genotype.

Table 2.3.15 Summary of tests of association between variation in growth and genotype for individuals reared from Peartree Point and Prawle Point that had been starved for four weeks.

(a) Tissue Growth

Locus	F	df	p>F
Pep-1	0.47	4, 135	0.7590
Lap-2	1.39	3, 134	0.2494
Mdh-1	1.51	3, 136	0.2154
Mpi	0.54	3, 136	0.6555
Pgm-1	0.44	1, 138	0.5067
Pgm-2	1.10	2, 137	0.3366

(b) Shell Growth

Locus	F	df	p>F
Pep-1	0.41	4, 135	0.8004
Lap-2	5.56	3, 134	0.0013
Mdh-1	0.52	3, 136	0.6659
Mpi	1.86	3, 136	0.1395
Pgm-1	0.12	1, 138	0.7330
Pgm-2	0.15	2, 137	0.8640

2.4 DISCUSSION

2.4.1 Genetic composition of laboratory populations

Sample sites were chosen to maximise the genetic differences in karyotype and allozyme frequencies that have previously been identified for *N. lapillus* along this region of coastline (Day, 1990; Kirby, *et al.*, 1994a). Only *Lap-2* and *Mdh-1* of the four known clinal loci were scored in this study and confirmed genetic divergence between Peartree Point and Prawle Point; the *Lap-2* and *Mdh-1* '9' alleles were present in the Prawle Point sample whereas individuals reared from Peartree Point were fixed for the $Lap\text{-}2^{10.10}$ genotype and had either the *Mdh-1* '10' or '11' alleles. These results are consistent with those of Day (1990) and Kirby *et al.*, (1994a). Allele frequencies of the four non-clinal loci scored (*Pep-1*, *Mpi*, *Pgm-1* and *Pgm-2*) were invariant between laboratory populations and served as controls in the association between phenotype and genotype; statistically significant correlations due to chance would be expected in an approximately equal proportion of the tests undertaken for each locus (clinal and non-clinal) and population.

2.4.2 Life history strategy

Total female reproductive effort (as defined in Stearns, 1992 or Roff, 1992) has not been measured in this study; the total number of capsules and the size of hatchlings produced per female during a breeding season and lifetime, and the relative proportion of resources allocated to reproduction were not recorded. However, the number of juveniles emerging per capsule at hatching was higher for Peartree Point than for Prawle Point suggesting a site-specific difference in reproductive strategy. Egg capsules were collected from the shore sites and so it is not possible to determine from the present results whether differences in reproductive strategy between shores were due to inherent genetic differences, were the direct consequence of environmental variation, or were the result of the integrated effects of both. The parental genotypes were not scored in this study and paternity is also difficult to determine as females often mate with several males (Kirby, 1992) and are also capable of storing sperm for prolonged periods (Fretter and Graham, 1962). Personal attempts to breed dog-whelks in the laboratory have met with limited success for these sites, but was probably due to using

laboratory-reared juveniles as shore collected juveniles from other sample sites have been successfully bred on maturation (pers. obs.). The between and within population crosses established for Peartree Point and Prawle Point were primarily aimed to determine the inheritance of the clinal loci and karyotypes, but would also have helped determine if reproductive strategy had a site-specific genetic basis or was merely a response to local environment.

Females which produce a higher number of juveniles per capsule at hatching have previously been found to deposit more capsules and have higher overall reproductive efforts, even when balanced against the decrease in hatchling size with increases in number per capsule (Etter, 1986, 1989). Higher reproductive efforts are generally found in dog-whelks inhabiting wave-exposed sites (Bloomer, 1987; Etter, 1989; Geller, 1990; Kirby, 1992). It would be reasonable to expect that at Peartree Point, where dog-whelks exhibit the phenotypic characteristics (including shell morphology) normally associated with 'exposed' sites, each female would also deposit a higher number of capsules each of which would also hatch a larger number of small juveniles.

Higher reproductive efforts on exposed shores have been suggested to reflect reduced habitat stability, as a consequence of increased wave action and the risk of dislodgement, and so r and K selection (defined in section 2.1) have been proposed to help explain differences in life history. However, information on density-dependent or independent causes of mortality is needed to infer the type of selection. Direct measurements of environmental variation suggest that maximum wave forces were similar between Peartree Point and Prawle Point (chapter three). Alternative theories on the evolution of life history have looked to the relative juvenile and adult mortality rates (reviewed in Partridge and Harvey, 1988). In order to explain the lower reproductive effort at Prawle Point, the mortality risks for juveniles should be relatively higher than that of adults at this site. Dislodgement, physiological stress during emersion and predation are the major causes of death for dog-whelks on the shore; the latter two causes are thought to predominate on 'sheltered' shores (Etter, 1988b; Burrows and Hughes, 1989) and in the juvenile stages (Feare, 1970; reviewed in Crothers, 1985). The capsules collected from Prawle Point hatched a lower number of juveniles per capsule, which is likely to result in larger

sized hatchlings. Large hatchlings have a better ability to withstand the adverse effects of elevated temperatures and potential desiccation rates (Spight, 1976) such as those measured at Prawle Point (chapter 3). However, more data is required on the relative survival of adults and juveniles, and the causes of mortality on these shores.

In the laboratory, the post-hatching mortality of juveniles increased with the number of juveniles hatching from each capsule, and was higher for juveniles reared from Prawle Point than from Peartree Point when compared for a given number of juveniles per capsule (fig. 2.3.4/5, p 56). Although both populations were reared in the laboratory from hatching, it is again difficult to determine if these mortality differences were genetically based or were the remnant of maternal effects and environmental differences prior to collection from the shore. The previous microhabitats experienced on the shore were likely to be similar as egg capsules are normally deposited in crevices or under an algal canopy, where they are generally protected from the influences of climatic and hydrodynamic effects (pers. obs.). The aggregation of egg capsules must also help to buffer changes in external environment and so confer some degree of protection. Prior environmental variation is more likely to arise from maternal effects such as female reproductive investment. For example, offspring survival is generally increased by increase in investment per offspring (Charnov and Downhower, 1995) and size (Spight, 1976).

Juvenile mortality can be due to dislodgement by waves, physiological stress, predation, maternal condition determining the size at hatching, or genetic composition/abnormalities. The latter two causes were likely to be the main reasons for mortality in the laboratory, but are difficult to differentiate without knowledge of the dietary history, resource allocation to reproduction and the genotype of the parents. Genetic abnormalities are included as a number of deformed hatchlings, such as individuals with an abnormally shaped foot or lacking a shell, were observed at hatching and none survived to one month after hatching in this study (pers. obs.). The number of deformed hatchlings appeared to be associated with particular mothers rather than show differences between sites. Death may also result from cannibalism, which is demonstrated in *Nucella lapillus* and other predatory gastropods particularly in the early juvenile stages (pers. obs.; Morton, 1987 and references therein).

2.4.3 Morphological variation between laboratory populations

Dog-whelks were expected to have elongated shells when reared from Prawle Point and more rounded shells if reared from Peartree Point, assuming a genetic basis to such variation and in view of previous results for shore-collected dog-whelks in this region (Day, 1990; Kirby, 1992; Kirby *et al.*, 1994a). Shell shape differences should be indicated by differences in the relationship between shell length and aperture measurements (L/Ap ratios; see Crothers, 1985). However, Prawle Point juveniles had longer apertures than Peartree Point for a given shell length suggesting the converse relationship. The association between shell shape and shore type has also departed from expectation in other studies (eg. Crothers, 1974, 1975a; Cambridge and Kitching, 1982). Kirby (1992) has attributed these findings to the use of indirect measures of shell shape, eg. L/Ap ratios, and the failure to identify the major environmental correlate underlying the shape polymorphism; wave force, temperature and predation intensity often covary with variation in shell morphology (reviewed in Crothers, 1985; section 3.4.4, this study and references therein). In the present study, the environmental component to shell shape variation was standardised in the laboratory, and the shell shape differences due to genetic composition may have been lower than has been recorded previously (Kirby, 1992; Kirby *et al.*, 1994a). It is therefore possible that the one-dimensional relationship between shell length and aperture may be too imprecise to reveal variation in the overall three dimensional shell shape of laboratory-reared juveniles.

The size of animal enclosed within the shell would be expected to vary depending upon the shape of the shell; Peartree Point juveniles had a heavier body mass for a given shell length, which would be expected for whelks with a more rounded shell shape due to geometry and the internal shell volume. The relationship between length and dry tissue weight therefore contradicts the L/Ap results and agrees with previous estimates of shell shape in adults and juveniles sampled from these shores (Day, 1990; Kirby, 1992; Kirby *et al.*, 1994a; chapter 3, this study). Consequently, these two indirect estimates of shell shape were measured for all dog-whelk samples in this study (chapters 3 and 4).

2.4.4 Population differences in growth rate

Four months after hatching, juveniles reared from Peartree Point were larger in size than those reared from Prawle Point. Kirby (1992) found a comparable result when the size at five months after hatching was measured for juveniles which had been reared from samples with similar genetic differences as the sites sampled in the present study (section 2.4.1). He suggested that size differences could be due to post-hatching growth rate associated with the genetic composition of the population. However, other plausible explanations are possible; population differences in initial hatching size, differential mortality, feeding rates and the possible effects of rearing within teaboys can influence subsequent growth rates or the size attained at a given age.

Direct measurements of growth rate in this study confirmed that Peartree Point juveniles had faster rates of tissue and shell growth. However, Prawle Point juveniles deposited more shell for a given increase in tissue which may be explained by the relationship between the shape and total internal volume of the shell; a dog-whelk with an elongated shell would need to grow a longer shell in order to accommodate a given increase in body size. Alternatively, the differences in growth strategy could reflect variation in the 'costs' associated with shells, which Palmer (1981) described (in relation to shell thickness) to include the 1. cost of shell deposition, 2. the cost of transporting the shell, and 3. the maximum rate of tissue growth may be set by the maximum rate of shell deposition, and not necessarily through the energetic cost of the shell. Elongate shells may be more costly to produce and transport (whelks with the elongate shape have a heavier shell for a given tissue weight), tending to reduce the resources available for tissue growth, assuming an energetic trade-off between shell and tissue growth. The shape and total internal volume of the shell may incur the structural constraints described in 3; an elongate shell shape with its reduced shell volume may limit the rate of tissue growth.

The variation in dry tissue and shell growth rates between populations were not due to differences in feeding rate and resulted in site-specific variation in gross growth efficiencies; Peartree Point juveniles grew more tissue than those reared from Prawle Point for a given feeding rate. The shell growth efficiency was similar for both populations, and reinforces the previous result where Prawle Point juveniles had a higher ratio of shell to tissue mass than

juveniles from Peartree Point. Differences in growth efficiency could result from differences in absorption efficiencies, or variation in the partitioning of absorbed energy to excretion, respiration and production (growth and reproduction combined). Absorption efficiencies and excretion rates (chapter four) were not measured in this experiment and are discussed in section 4.4. Respiration rates were similar between Peartree Point and Prawle Point, although rates were slightly lower in Prawle Point juveniles. The gradient in the relationship between dry weight and respiration rate, i.e. the b- exponent used to standardise respiration rates for weight, were also similar for the two laboratory populations indicating that there was no functional heterogeneity among size classes for either population. Previous work has shown that the stage reached in the feeding cycle has a marked effect upon the rate of oxygen consumption (Bayne and Scullard, 1978a). In the present experiment, it was intended that one day after the removal of mussels would be sufficient to ensure that juveniles were at similar stages in their feeding cycle. However, if the interindividual variation was great, any between population difference may have been obscured. Population differences in respiration rate have been found by Kirby (1992), but only in response to hyperosmotic stress.

Phenotypic variation in growth rate was associated with the *Pep-1* locus and to a lesser degree the *Lap-2* locus in the Prawle Point sample. Similarly these two loci were also associated with size attained at four months for the Peartree Point (*Pep-1*) and Prawle Point *(Lap-2)* samples. The results suggest that other loci also underlie the population differences in growth rates, otherwise equivalent *Lap-2* genotypes from either sample site would exhibit similar growth rates. It is much more likely that loci linked with this allelic variation are responsible particularly with a trait as complex as growth, the rate of which can be affected by variation in a number of metabolic processes and pathways.

The lack of population differences in total weight (combined shell and tissue) growth can be explained by shell morphology; the volume of water retained within the mantle cavity is dependent upon the shell shape (Osborne, 1977; Kirby *et al.*, 1994a) and relative size of animal (Coombs, 1973a). An attempt to remove this extra-corporeal water was made prior to measuring total weight with the aim to reduce the measurement error. However, not all this water can be removed without disturbing the animal to such an extent that several days may

elapse before feeding resumes (pers.obs.). Therefore, any residual water remaining within the shell whilst measuring total weight (section 4.3.1.2) would lead to inaccuracies in these measurement and add to the within-site variation in growth rate. Marine molluscs are often polymorphic for shell shape, and so the present results suggests that growth studies relying solely upon the measurement of total weight or tissue wet weight (without minimising the influence of variation in mantle cavity fluid) should be regarded with caution.

2.4.5 Size measurements and ration

The relationship between immersed and shell weights changed during the course of the experiment depending upon the previous feeding ration. The method of distinguishing shell and tissue weights on live animals is dependent upon the specific gravity of the body tissue approximating to zero during measurements of the immersed weight (Palmer, 1982). It is perhaps not surprising that ration affects this relationship because reduced food availability will inevitably alter the biochemical composition of tissue and its relative density in seawater. Palmer (1982) noted that reproductive status affected the relationship between immersed and actual shell weight, which was presumably due to the change in the relative lipid content. Care must therefore be exercised whenever this method is used as feeding, growth or reproductive status can vary due to experimental manipulation in the laboratory or environmental influences on the shore.

2.4.6 Growth and reduced food availability

The effects of ration upon the relationship between shell and immersed weight (section 2.4.5) meant that repeated measures analysis of growth rate could not be used, and that growth over the entire experimental period had to be calculated as the total change in wet weight over the six weeks. Unlike the previous experiment (see section 2.4.4), the two laboratory-reared populations had similar shell and tissue growth rates when fed *ad libitum.* Any population differences will possibly be undetected in this second experiment due to the use of wet weight measurements. *Ad libitum* fed animals were not sacrificed at the end of the experiment and it is possible that the shell-immersed weight regression for these juveniles changed over the six-week experimental period leading to inaccuracies in estimating the final shell and wet tissue

weights (section 2.4.5). Wet weight measurements were calculated as the difference between total weight and estimated shell weight, and as such were subject to the problems discussed in section 2.4.4. Greater accuracy and reliability are therefore likely to be attained with the use of dry weight measurements (section 2.4.4).

Feeding rates for the *ad libitum* fed juveniles were similar for both populations, but Prawle Point had higher feeding rates in the low ration. This is likely to be a consequence of using length measurements to standardise ration level (Bayne and Scullard, 1978b); shell shape variation (section 2.4.3) and the allometry of shell and tissue growth (section 2.4.4) results in Prawle Point juveniles having a relatively smaller body size for a given shell length. Providing food on the basis of whelk shell length (see equation, section 2.2.1.3.2) resulted in Prawle Point juveniles receiving a relatively greater amount of food for a given body weight. Therefore the growth rates of these juveniles cannot be used to identify any population specific responses to low ration levels. This experiment demonstrated that errors can arise when using an inapproprate size measurement to standardise a particular physiological rate. A similar view was expressed by Kirby (1992) in discussing the general use of length measurements in comparative growth studies when shape differences could be of importance (see section 3.4.6).

Juveniles that had been starved for four weeks were not subject to the problems of standardising feeding rations between the populations. Prawle Point juveniles lost less tissue weight and produced less shell than Peartree Point juveniles. The larger number of juveniles used in this experiment (n=180) and the close fit of the model in the analysis of covariance (r=0.99) suggested that wet tissue weight was a reasonable measure in calculating growth rate on this occasion. The possible errors in measuring wet weight have been discussed previously (section 2.2.4) and would tend to obscur population differences in growth due to increased individual measurement error; errors would arise from using shell-immersed weight regressions at the start of the experiment, and total weight measurements. At the end of the experiment, a greater effort to remove the confounding effects of mantle cavity fluid and increase the measurement accuracy was made by inserting filter paper wicks deep into the shells. Shell growth rates were not complicated by shell morphology; accurate shell-immersed regressions were available for each population sample at the start and shells were weighed at the end of the

starvation experiment. Starved Peartree Point juveniles deposited more shell than those reared from Prawle Point, and so continue to deposit shell under periods of food limitation when it would be considered costly to do so in terms of the energy budget (Palmer, 1981).

The differences in shell growth rate amongst *Lap-2* genotypes in the starved Prawle Point sample indicated that in descending order of growth rate 10.10>9.10>9.9. It is unlikely that variation at one locus can be solely responsible for variation in growth (discussed in section 2.4.4). However, it is possible that *Lap-2* and linked loci, or *Lap-2* merely acting as a marker locus for variation at linked loci, which influence growth rate. Variation at the *Lap-2* and *Pep-1* loci, which has been linked to variation in growth rate (section 2.4.4) was not associated with variation in tissue growth rate in the starved ration.

CHAPTER THREE:

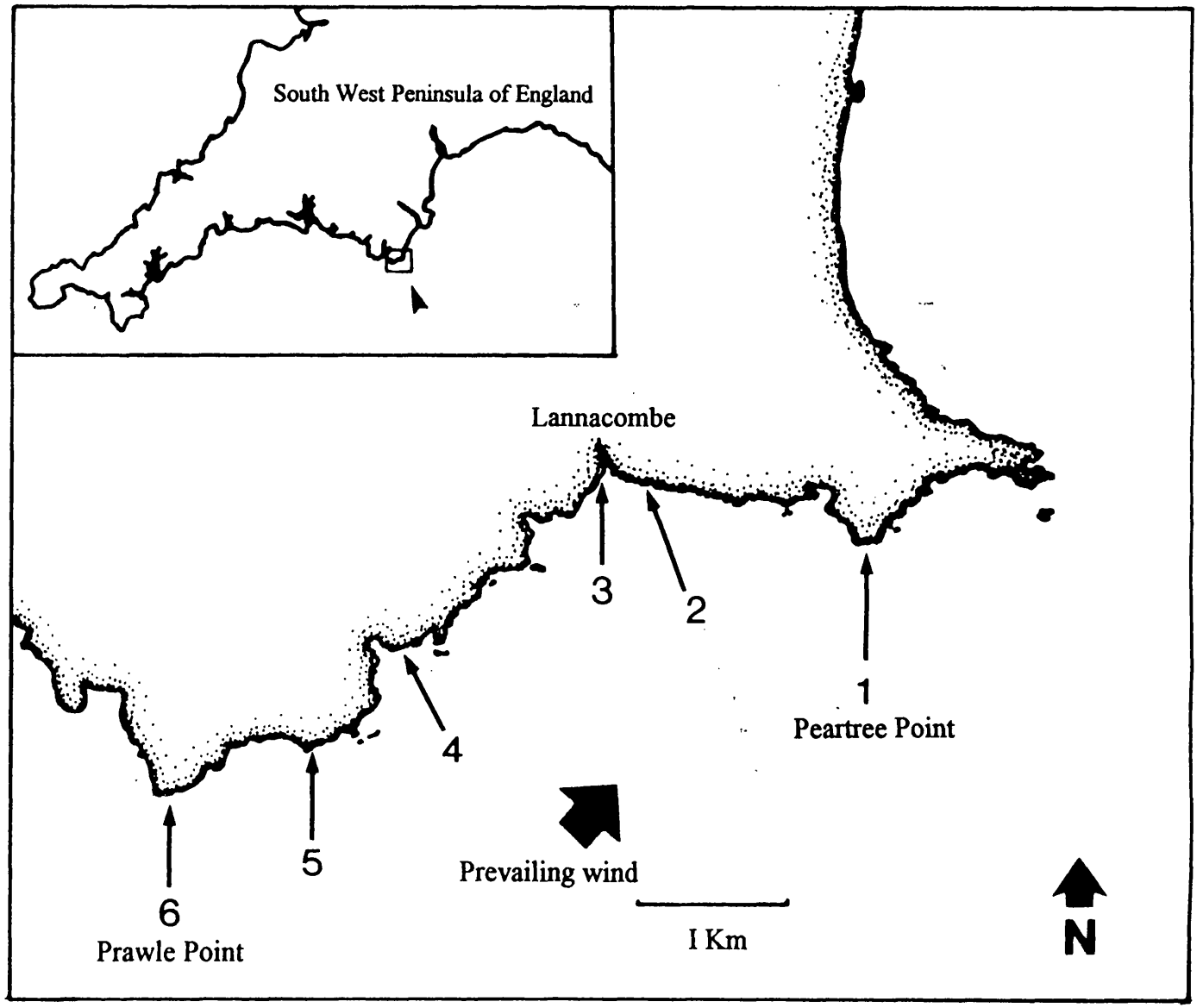
Studies of shore *Nucella lapillus* and environmental variables

3.1 INTRODUCTION

The marine intertidal exhibits well-studied gradients in 'exposure' and so it is perhaps not surprising that clinal variation is evident in a number of traits for species inhabiting these environments (eg. Phillips *et al.*, 1973; Wolcott, 1973; Duineveld and Jenness, 1984; Grahame and Mill, 1992; Mill and Grahame, 1992). Because marked differences in 'exposure' are so apparent, it provides an ideal habitat to study adaptation, but care is needed to avoid improper speculation. The common use of the term 'exposure' is associated with the amount of wave action, but during emersion intertidal organisms are 'exposed' to other environmental factors such as temperature; at more 'sheltered' sites, generally considered to have lower wave forces, individuals are likely to experience higher air temperatures than at 'exposed' sites (reviewed in Newell, 1979). The present chapter primarily deals with the measurement of the intertidal variables, wave force, air temperature and desiccation rates, along the cline of *N. lapillus* in South Devon and the phenotype of whelks inhabiting the region of the cline at Peartree Point and Prawle Point (fig. 3.1).

Estimates of exposure have historically been based upon meteorological data and/or the aspect of the shore in relation to prevailing wind direction (reviewed by Ballantine, 1961 and Newell, 1979). Other estimates have been based upon the number of degrees open to the sea as measured on a compass (Baardseth, 1970; Russell, 1977). It has long been recognised that exposure, especially wave action, influences the presence and relative abundance of certain key species on the shore (eg, Stephenson and Stephenson, 1949). In response to the need for improved methods of distinguishing shores, biological scales were devised, which were more responsive to environmental variation. These scales provided ways of 'quantifying' exposure indirectly through repeated quadrat counts of these species, which included representatives from most of the major phyla. The two main scales used were Ballantine's (1961) exposure scale, which distinguished eight types of shore (scale 1, extremely exposed, to scale 8, extremely sheltered), and that of Lewis (1964), which was more conservative in that five shore types were classified. On the basis of Ballantine's scale, Peartree Point has been classified as exposed (scale 2; McCarter and Thomas, 1980) and Prawle Point as sheltered (scale 4-6;

Figure 3.1: Sample sites used to determine the environmental variables over the allozyme cline in *Nucella lapillus*


South West Peninsula of England
Lannacombe
3
2
1
Peartree Point
4
5
6
Prawle Point
Prevailing wind
I Km
N

McCarter and Thomas, 1980; Day, 1990). Although these scales have been invaluable in a number of comparative studies among shores, their biological nature renders them susceptible to factors other than wave exposure and they are also unable to differentiate between the major 'exposure' factor/s. Of the environmental variables associated with shore exposure, wave force is the most difficult to measure. Devices based upon their erosional properties, such as plaster of paris and cement blocks have been used (Doty, 1971; Craik, 1980; Koehl and Alberte, 1988), but are vulnerable to the interference effects of water currents and possibly rain. Jones and Demeteropolis (1968) devised a cheap mechanical method of measuring intertidal wave force for use in ecological studies and this has formed the basis of many studies since (eg. Palumbi, 1984; Bell and Denny, 1994). Other techniques have also been used, but these are often complex or subject to design complications (Denny, 1983; Galley, 1991).

In this study, wave force dynamometers (Bell and Denny, 1994) were used to quantify wave force. These devices do not record the force of individual waves for the calculation of mean wave force, but measure the maximum force experienced between sampling occasions. Although the potential environmental stress experienced by an organism is rarely temporally constant, particularly in the intertidal, it is the maximum wave force that is crucial to those organisms at risk of dislodgement (Denny, 1993). Similarly, it only takes one catastrophic event such an exceptionally hot summer's day or stormy sea, within the life-time of an individual to 'test' its fitness. Although catastrophies are widely recognised, they are sometimes thought either too rare an event to be of importance or more commonly ignored as outside the time limits of most ecological studies (Weatherhead, 1986). This study aimed to provide some indication of the major differences in wave force between sites, if present, and measurements were undertaken during the spring equinoctial storms at times when it was considered that any site differences would be maximised.

Obtaining measurements of other potential stressing factors, such as temperature, humidity and desiccation rates, during emersion has been less problematical. Desiccation rates have been measured on animals by recording the total weight loss during emersion (eg. Davies, 1969; Etter, 1988b), but these measurements are subject to errors in the intertidal; littoral gastropods retain seawater in their mantle cavity during low tide (Segal and Dehnel, 1962; Coombs,

1973a; Hughes, 1986) and retreat into their shell when disturbed which artificially increases the volume of water lost (pers. obs.). Measurement error would tend to distort or obscure any interpopulation variation in weight loss as a consequence. Desiccation rates of snails have also been indirectly measured by noting the change of osmolarity of the mantle cavity fluid (Boyle, *et al.*, 1979; Kirby *et al.*, 1994b). Both these measurements of water loss from the animal are complicated by intra- and interspecific variation in shell shape and mantle cavity volume. If the weight loss of dog-whelks at Peartree Point and Prawle Point (fig. 3.1) were measured on the shore, assigning how much water loss was due to possible site variation in habitat, shell shape and behaviour would be difficult. In this study, desiccation pots have been designed to contain water-saturated florist oases (see section 3.2.1.3 for description), which are easy to use and are not subject to the same complications as measurements undertaken on animals sampled from the shore.

These environmental variables (temperature humidity and wave force) act to modify the abundance and biology of those organisms inhabiting the intertidal zone, and also the ecological interactions between them. Knowledge of how sites along the cline vary in 'exposure' may help understand why samples of dog-whelks from this region vary in their laboratory physiology (Kirby, 1992; Kirby, *et al.*, 1994a, b; chapter two, this study) and can form the basis of more stringent testing for the occurrence of selection. The genetic and phenotypic cline could merely reflect the outcome of historical processes; chance founder events and the limited amount of movement and dispersal of whelks could explain the observed pattern (reviewed in Endler, 1977). However, if different morphs differ in their physiological response to the measured environmental variable and in a way that can be predicted by their geographical distribution, the cline suggests that some form of differential selection has occurred. The environmental measurements recorded here formed the basis of the experiments outlined in chapter four, which aimed to identify how laboratory-reared populations varied in their physiological response to manipulated laboratory conditions.

In the present chapter, juvenile dog-whelks have been measured for shore growth rates *in situ* and their physiology (growth, feeding and respiration rates) under standardised laboratory conditions to help understand the relative importance of the environmental influence upon

phenotypic variation. *N. lapillus* is an ideal subject for field observations due to the limited movement of animals on the shore; marked individuals can be retrieved with a relatively high success rate (Hughes and Burrows, 1994). Field work was conducted during the summer, when growth rates tend to be high (Phillips and Campbell, 1968; Hughes, 1972; Etter, 1989); dog-whelks tend to be inactive, retreating to crevices and ceasing to feed during the winter (Feare, 1971). Low growth rates in winter will tend to result in a relatively greater measurement error between successive measurements and so obscure possible growth differences between sites. Any physiological variation seen for the field samples maintained in the laboratory not previously observed for laboratory-reared juveniles (chapter two) would suggest the requirement of an environmental cue for the expression of a particular phenotype.

3.2 MATERIALS AND METHODS

3.2.1 Environmental Variables

3.2.1.1 Maximum wave force

Wave force dynamometers (plate 3.2.1) were calibrated using the methods described in Bell and Denny (1994). Known masses were successively hung from each spring and the resulting spring extensions measured (± 0.05mm). The linear regression between force (= mass (Kg) multiplied by 9.81) and spring extension yielded values of k (slope) and c (intercept) for each spring and were used in the calculation of wave velocity, u:

$$u = [(kx + c) / a]^{1/b}$$

where a and b were were taken to equal 0.575 and 1.93 respectively, and were coefficients of drag for a drogue of this type and size (Bell and Denny, 1994). x was the measured spring extension (mm).

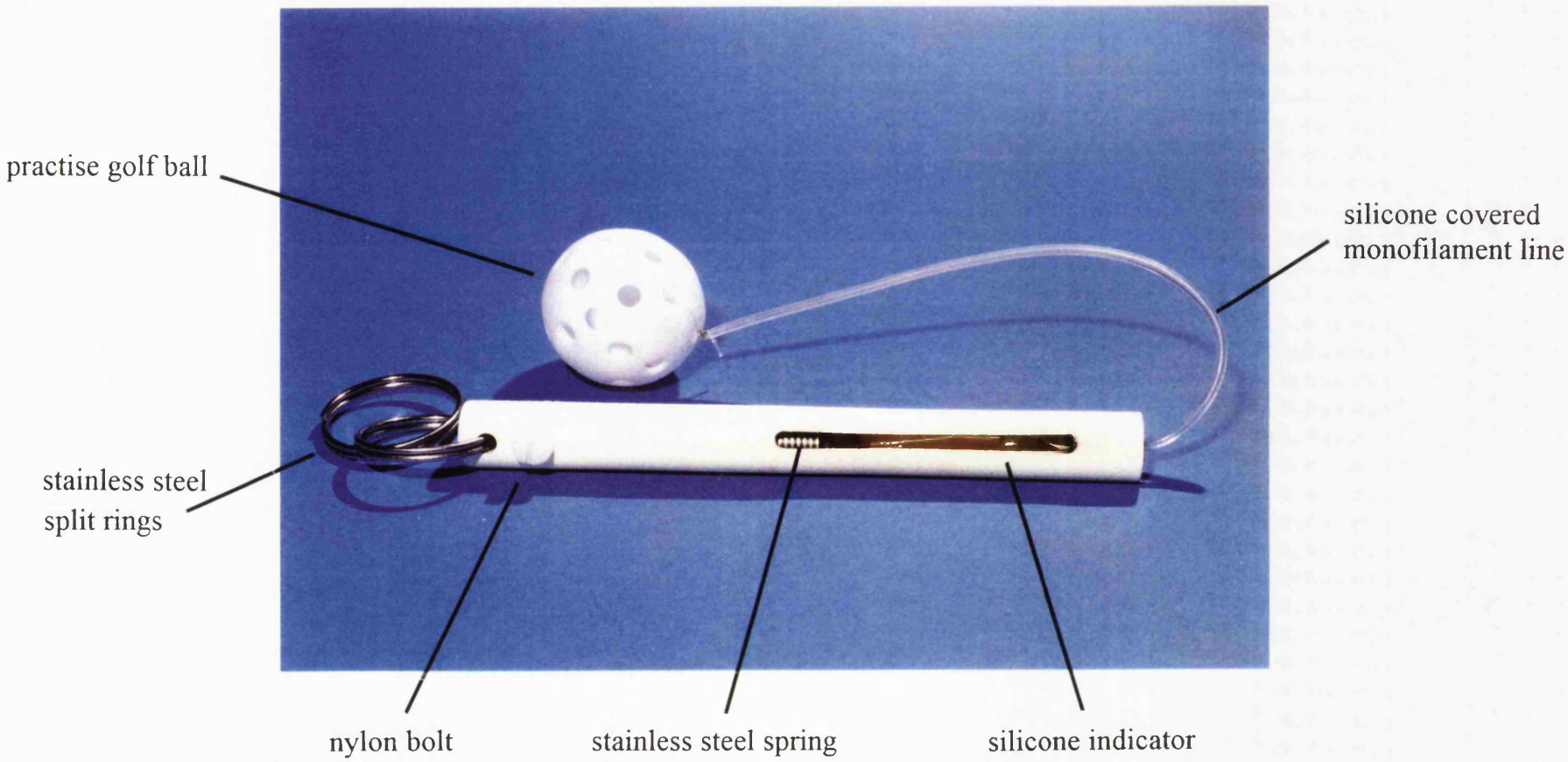
Eight calibrated dynamometers were deployed at Peartree Point and Prawle Point and readings were recorded on eight occasions during the period from 1st February to 19th March 1995. Each reading represented the maximum wave force experienced over the preceeding 24 hour period, which covered two tidal cycles and coincided with spring equinoctial tides. Information on wind speed and direction for this period was obtained from the nearest meteorological office based at Berry Head (SX947566), approximately 24 km east from Peartree Point. This provided an indication of the likely sea conditions at the time wave force measurements were taken.

At each site, four dynamometers were attached to angle iron stakes positioned at least 1m apart over a $15m^2$ area at ~1m above extreme low water.

3.2.1.2 Temperature, humidity and desiccation rates

Temperature and humidity were recorded close to the rock surface using a pocket thermohygrometer (Analog and Numeric Devices Ltd., Leicestershire, U.K.). Desiccation rates were measured using two types of 'desiccation pots' (plate 3.2.2) designed to estimate the desiccation potential on the shore as indicated by the rate of water evaporation from a cube of

Plate 3.2.1: Dynamometers used to quantify maximum wave force. This design is based upon that of Bell and Denny, 1994.


practise golf ball
silicone covered
monofilament line
stainless steel
split rings
nylon bolt
stainless steel spring
silicone indicator

florist's oasis*. Both types of desiccation pot were constructed using a 250ml wide-necked polypropylene bottle with a screw-top lid. One type of pot had an ~27cm^3 block of oasis glued to the inside of the lid (plate 3.2.2), while the other had the oasis glued inside the base of the bottle. The initial sealed total dry weight of each assembled pot was determined, and then water was added to the oases to saturation point with any excess water removed using absorbant paper. The pots were then resealed, reweighed and transferred to the shore in styrofoam boxes to reduce temperature fluctuations in transit.

Twenty desiccation pots (ten of each type) were each deployed at Peartree Point and Prawle Point and opened between 12.45 and 14.45 BST on the 10th July; temperature and humidity readings were recorded at fifteen intervals during the same two hour period. The desiccation pots were opened and placed on the rock surface at least 1m apart with the inside lid and neck of the bottle facing upwards. After two hours, the pots were resealed and weighed when returned to the laboratory. The total water loss was used as an indication of the desiccation rate at each shore. The two pot types were designed to differentiate between the effects of temperature, humidity and wind combined (oasis positioned on the lid) and the desiccation rate mainly attributed to temperature (oasis positioned inside the bottle).

This experiment was repeated on the 12th July, but the number of sample sites was increased to six to include intermediate sites across the cline (fig.3.1). On this occasion only the desiccation pots with the oasis glued to the inside of the lid were used. The standard error calculated in the previous experiment, indicated that the number of pots deployed at each site could be reduced; five pots were synchronously deployed at each of the six sites between 14.00 and 16.00 BST.

3.2.2 Laboratory Physiology of Field Juveniles

Juveniles were collected from Peartree Point (n=40) and Prawle Point (n=42) (fig.3.1) in May 1992 and had shell lengths ranging between 10-13mm. Duplicate samples (n=30 from each shore, shell length 8-19mm) were taken on the same day for the 'destructive' sample in the calculation of the population regression equations using the methods of Palmer (1982) (section

* Oasis is the name given to the rigid sponge-like blocks, which are normally green in colour and used for flower arrangements.

Plate 3.2.2: Desiccation Pot designed to measure rates of water loss on the shore; pots were opened as shown in this plate whilst measurements were taken. Another type of pot (not illustrated) was constructed where the oasis (see text) was positioned inside on the base of the bottle rather than on the lid as shown here.

florist oasis

2.2.1.3.1). Juveniles were sampled from a large area on the shore to reduce the likelihood of relatedness among individuals and ensure a representative sample from each site. Only animals with uninterrupted and active shell growth, as indicated by a 'smooth' shell with a thin margin, were included in these analyses. A 'smooth' shell in this instance is one with no unusually shaped ridges which are commonly the result of a period of starvation or a disturbance event such as crab attack or dislodgement. *Nucella* spp. often have minor ridges in their shells as a result of seasonal growth patterns.

All experimental juveniles were immediately measured (section 2.2.1.3.1) following collection from the shore and again three weeks later. Shell weight was estimated from the measured immersed weight, and tissue dry weight estimated from shell length measurements taken at the start of the experiment (section 3.3.2.1). Over the first three weeks, each juvenile was maintained separately (section 2.2.1.3.2) with a constant whelk:mussel ratio of 1:10 and a wide range of mussel sizes was provided as food during this time. As no differences in preferred prey size were observed between populations (section 3.3.2.4), over the following four weeks diets were standardised only for individual whelk size; each whelk was provided with five mussels of a size calculated from the equation derived by Bayne and Scullard (1978b; section 2.2.1.3.2). The sizes of mussels offered as prey were within 2mm of this calculated value and the whelk:mussel ratio (1:5) was restored every day when eaten mussels were measured and replaced. The dry weight of mussel flesh eaten was calculated from the mussel length:

$$\log_{10} DT = (2.567 (\pm 0.194) * \log_{10} ML) - 4.798 (\pm 0.230)$$

where DT is dry tissue weight (g) and ML is mussel length (mm). Coefficients are mean values $\pm$2S.E. The total amount of mussel tissue eaten over the duration of the seven week experiment was summed for each individual and population differences in feeding rate were tested using the methods outlined in section 2.2.1.3.2.

Rates of oxygen consumption (section 2.2.1.3.3) were determined two and seven days after the removal of food at the end of the seven-week experiment. Respiration rates exponentially decline from routine to standard rates between 5-10 days after the removal of food (Bayne and Scullard, 1978a), and so measurements recorded in this study represent intermediate and

standard rates respectively. After the final measurement of respiration rate, juveniles were dissected into shell and tissue, which were then freeze-dried to obtain final shell and tissue dry weights used in the calculation of growth and respiration rates. Freeze-dried tissue was stored at -70°C for later analysis for electrophoretic loci (section 2.2.1.4).

Genetic and population differentiation in growth, feeding and respiration rates were tested using the analysis of covariance (see statistical analysis in section 2.2.4). Physiological measurements were analysed separately for each locus and population to identify which loci had significant physiological variation among genotypes at the 5% probability level.

3.2.3 Field Growth Rates

Two methods of marking dog-whelks in the field were employed. Bright enamel paint (Humbrol Ltd., U.K.) was painted on the shell apex; the colour permitted quick recognition in the field and distinguished samples obtained on different occasions. A numbered 'beetag' (Christian Graze Inc., W. Germany) was also attached to the apical whorl using cyanoacrylate glue. Both marking methods had previously been tested at Whitsand Bay (O.S.ref. SX390523) and found to persist for at least six months in the field with less than 5% losing both shell marks (pers. obs.). The Whitsand Bay site has a high density of *N. lapillus* and is a 3km wave-swept shore, which is predominantly sandy with isolated rock masses reducing the movement of whelks over large areas (Harris, 1988). Dog-whelks were therefore easily recovered from the field and the abrasive action of sand and debris was considered an extreme test of the permanency of the marking techniques. No deaths occurred in a sample of labelled animals maintained in the laboratory for one week, but the long-term effects upon survival and mortality in the field are unknown. However, several whelks were feeding within a few days of being labelled suggesting that disturbance had been minimal for these individuals.

At Peartree Point and Prawle Point (fig. 3.1), all whelks visible within an area of approx. $20m^2$ in the mid-tidal zone were labelled and measured for shell length and aperture (total per site >280). The two sites were sampled between 25th to 27th May, two weeks later (11th-13th June) and then every subsequent month (see section 3.3.3.2 for exact dates). Sampling occurred during low water spring tides over the summer period between May and September, during

which time additional whelks were measured and labelled to ensure an adequate number of smaller sized juveniles as the season progressed (Sainsbury, 1980). Length and aperture measurements taken on each occasion provided six monthly growth rates per site and length-dry weight relationships (see below and section 3.3.3.1) were used to distinguish between shell and tissue weight for experimental animals on the shore.

A size range of whelks (n=30) was collected from each sample site in both May and September. These animals were used to determine length-dry weight relationships for shell and tissue weights, which may differ between sites due to site-specific shape variation (section 3.3.2.1) and possible differences in nutritional and reproductive status in the field. The sample obtained in September was used to determine whether these relationships had altered over the summer months for both within- and between-site comparisons. In previous experiments shell and tissue weights were measured using the methods devised by Palmer (1982, section 2.2.1.3.1), but it was not considered appropriate in this experiment since the large number of individuals that would have to be measured would require animals to be removed from the field and maintained in the laboratory for approximately two days. This in itself would cause a disturbance effect upon feeding and behaviour (pers. obs.), in addition to those incurred by using the Palmer (1982) methodology as previously noted in the laboratory (chapter two).

An increasing proportion of apparently non-growing whelks are found if the growth of a subset of a population is monitored over a period of time. This presumably results from the onset of reproduction and approach to the asymptotic size for an increasing number of individuals. Previous attempts to overcome these problems have included the omission of whelks with a growth rate below a certain limit or whose size is over the mean asymptotic size determined by one of a number of growth models (eg. Etter, 1989). In this study, a dry tissue weight of 160mg was chosen as the upper size limit of whelks to be included in the growth rate analyses, rather than using a standard shell length due to differences in shell shape; it corresponds to a shell length of ca. 25mm for Peartree Point and ca.29mm for Prawle Point.

3.3 RESULTS

3.3.1 Environmental variables

3.3.1.1 Maximum wave force

No site-specific differences in maximum wave force were found. Both Peartree Point and Prawle Point had similar variation in the calculated 24 hour maximum wave velocities (fig.3.3.1).

3.3.1.2 Temperature and humidity

The temperatures recorded at Prawle Point were consistently and significantly higher than those recorded at Peartree Point (fig.3.3.2a) (Wilcoxon signed- rank test; $d = -2.666$, $p<0.01$). Prawle Point also experienced lower relative humidities than Peartree Point (fig. 3.3.2b) (Wilcoxon signed-rank test, $d = -2.52$, $p<0.01$). Humidities at both sites reached their highest values at approximately 13.00 BST.

3.3.1.3 Desiccation rates

The sites did not differ significantly in the rates of desiccation from oases attached inside the pot (fig. 3.3.3) ($t = -0.91$ 18 d.f. $p=0.377$). However, the oases attached to the lid had a significantly higher rate of evaporation at Prawle Point than those at Peartree Point ($t=-6.107$ 18 d.f. $p=0.00$). These differences also occurred two days later (fig. 3.3.4) when additional sites were sampled; rates of water loss at all the intermediate sites were higher than those measured at Peartree Point. The highest desiccation rate recorded was at site 3; sites 2, 4 and 5 were the same, and the desiccation rates at Prawle Point were lower than at both sites 2 and 4.

3.3.2 Laboratory physiology of field juveniles

3.3.2.1 Size and shape analysis

The following analyses were undertaken on whelks collected from Peartree Point and Prawle Point for the destructive sample (unless indicated otherwise). The regressions relating immersed to actual shell weight, and also between shell length and dry weight were significantly different between populations ($p<0.0005$, section 2.2.1.3.1). The coefficients given are mean values $\pm$ 2SE:

Figure 3.3.1 Maximum wave force recorded at Peartree Point (closed squares) and Prawle Point (open circles) over eight 24 hour periods. Mean values ± SE are presented (n=4 per mean).

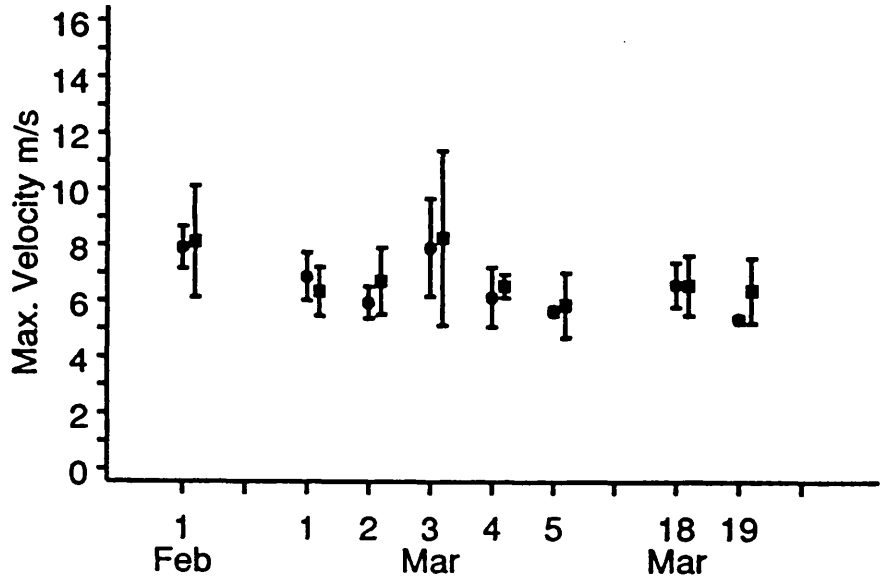
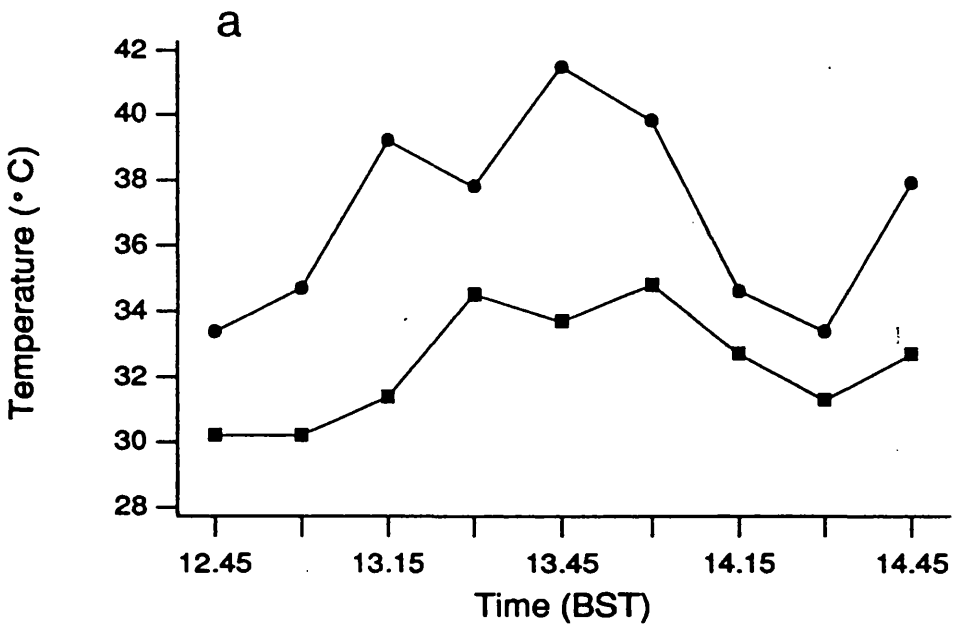

Max. Velocity m/s
16
14
12
10
8
6
4
2
0
1
Feb
1
2
3
4
5
18
19
Mar
Mar

Figure 3.3.2 Temperature (a) and humidity (b) readings recorded over two hours during low water at Peartree Point (closed squares) and Prawle Point (circles). Measurements were taken on a hot summers day in July, 1994. BST - British Summer Time


a
Temperature (° C)
42
40
38
36
34
32
30
28
12.45
13.15
13.45
14.15
14.45
Time (BST)

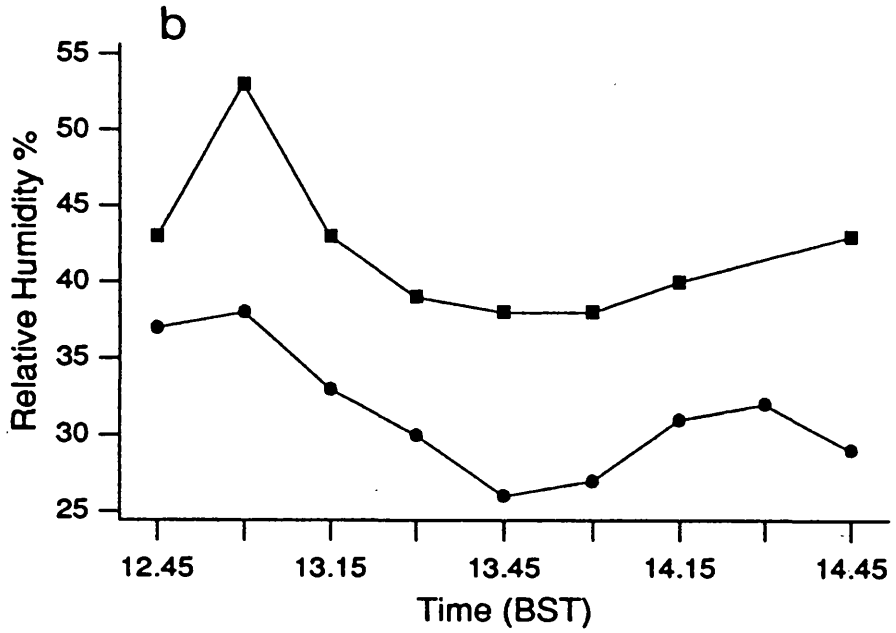
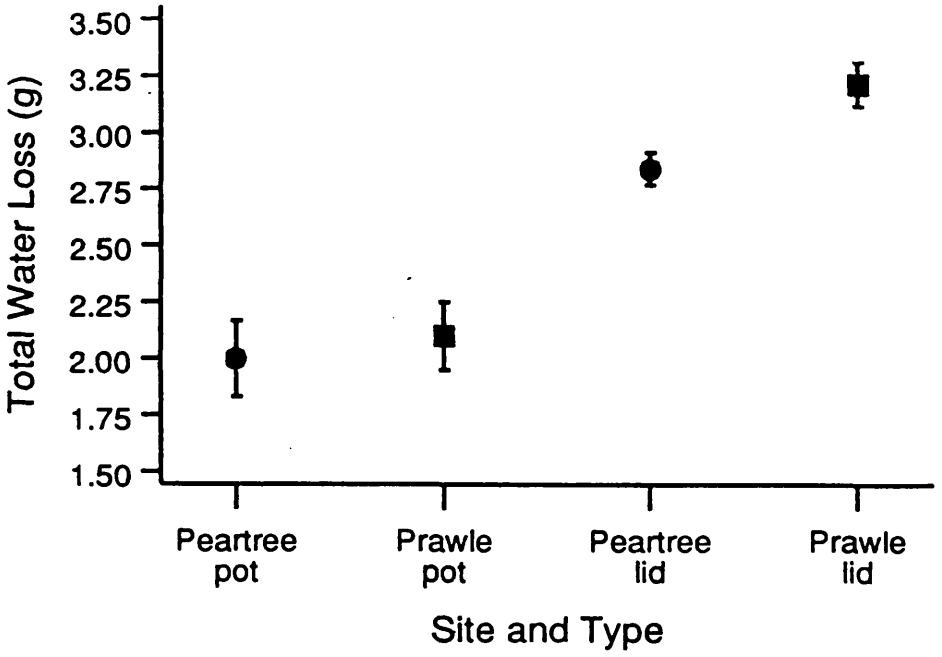
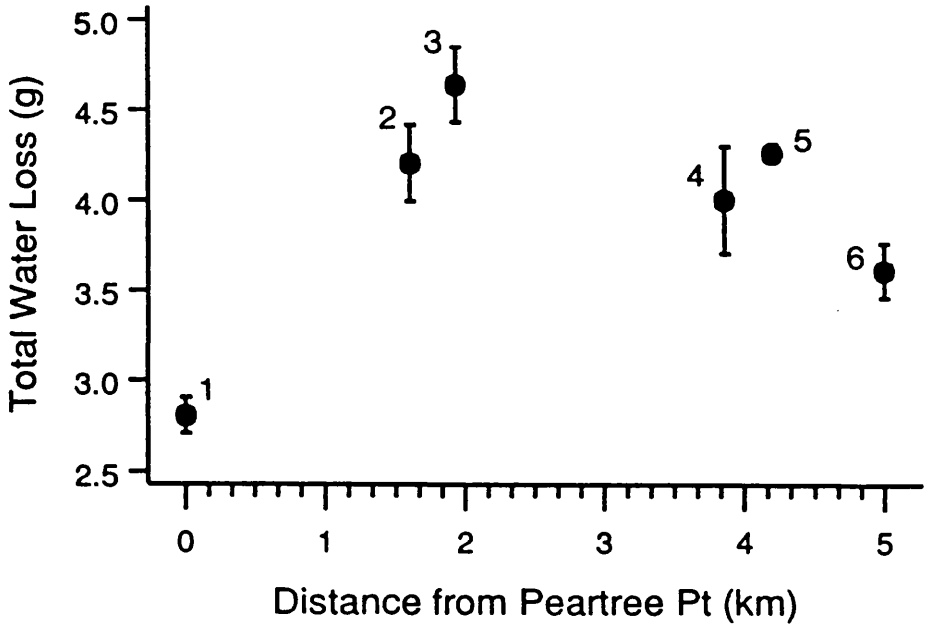

b
Relative Humidity %
55
50
45
40
35
30
25
12.45
13.15
13.45
14.15
14.45
Time (BST)

Figure 3.3.3 Desiccation rates of pots deployed at Peartree Point (circles) and Prawle Point (squares) over a two hour period during low tide. The mean (±2SE) weights of water lost from the two types of desiccation pots (see text) are presented. Each data point is the mean of ten readings.

Figure 3.3.4 Desiccation rates of pots deployed at sites covering the region of the allozyme cline in *N. lapillus*. The mean (±2SE) weights of water lost over a two hour period are presented. Site numbers are indicated (see figure 3.1 for location).


Total Water Loss (g)
3.50
3.25
3.00
2.75
2.50
2.25
2.00
1.75
1.50
Peartree pot
Prawle pot
Peartree lid
Prawle lid
Site and Type


Total Water Loss (g)
5.0
4.5
4.0
3.5
3.0
2.5
1
2
3
4
5
6
0
1
2
3
4
5
Distance from Peartree Pt (km)

Immersed-shell weight relationship:

For a given immersed weight, Peartree Point juveniles have a heavier dry shell weight ($F_{1,62}$ = 14.95, p=0.0003, r^2 = 0.999).

Peartree Point: Shell Weight (g) = (1.602 $\pm$0.0094 * immersed weight (g)) - 0.0016 $\pm$0.0042

Prawle Point: Shell Weight (g) = (1.602 $\pm$0.0094 * immersed weight (g)) - 0.0096 $\pm$0.0041

Length-dry weight relationship:

For a given length, Peartree Point juveniles have a greater dry tissue weight ($F_{1,61}$ = 25.98, p=0.0001, r^2 = 0.844).

Peartree Point: Log_{10} tissue weight (g) = (2.505$\pm$0.276 * $\log_{10}$ length (mm)) - 4.462 $\pm$ 0.312

Prawle Point: Log_{10} tissue weight (g) = (2.505$\pm$0.276 * $\log_{10}$ length (mm)) - 4.611 $\pm$ 0.324

Population differences in shell shape were indicated in the analysis of covariance of the relationship between shell length and aperture measurements. For a given shell length, Peartree Point juveniles collected in the field have a longer aperture (table 3.3.1). The ratio of shell length to aperture length remains constant after seven weeks in the laboratory for experimental animals sampled from either Peartree Point ($F_{1, 77}$ = 2.42, p=0.124) or Prawle Point ($F_{1, 80}$ = 0.04, p=0.836).

3.3.2.2 Genetic analysis of experimental samples

Population differences at the *Lap-2* and *Mdh-1* loci were seen between experimental animals sampled from Peartree Point and Prawle Point (table 3.3.2).

3.3.2.3 Growth rates

Regressions coefficients were calculated (section 3.3.2.1) at the time when experimental animals were collected from the field. Other experiments have indicated that the level of feeding and growth rate can have an effect upon these relationships (chapters 2 and 4), and so it seemed likely that the relationship between immersed and actual shell weight would change with time in the laboratory. A destructive sample was unfortunately not taken after three weeks

Table 3.3.1 Population differences in the physiology of juveniles collected from the shore and maintained in the laboratory. The direction of population differences are indicated; Peartree Point (a) and Prawle Point (b). Bold-type p-values are significant at the 5% significance level. See text for further details.

Analysis of Covariance		d.f.	F	p
Shell shape		1, 62	19.95	**0.0001**, a>b
Growth rate	Tissue	1, 77	8.39	**0.005**, a>b
	Shell	1, 77	3.60	0.062, a>b
Preferred mussel length		2, 236	1.19	0.276, a=b
Feeding rate		1, 78	2.55	0.1998, a=b
Respiration rate	2d	1, 77	5.07	**0.027**, a>b
	7d	1, 77	0.17	0.679, a=b

Table 3.3.2 Numbers of juveniles within each genotypic class for field collected individuals from Peartree Point and Prawle Point (May, 1992).

Locus	Genotype	Peartree Point	Prawle Point
Lap-2	10.10	39	21
	9.10	1	17
	9.9	-	2
Mdh-1	10.11	3	-
	10.10	36	19
	9.10	1	12
	9.9	-	9
Pep-1	11.11	2	1
	10.11	15	11
	10.10	23	28
Mpi	10.10	9	13
	9.10	20	16
	9.9	11	10
Pgm-1	10.10	33	38
	9.10	6	2
	9.9	1	-
Pgm-2	10.10	27	32
	9.10	12	8
	9.9	1	-

in the laboratory and so growth rates were determined over the entire seven week experimental period, rather than over four weeks following three weeks acclimation to laboratory conditions.

Peartree Point juveniles had higher rates of tissue growth than juveniles sampled from Prawle Point (fig. 3.3.5a; table 3.3.1). There was an indication that Peartree Point juveniles also have higher shell growth rates (fig. 3.3.5b, table 3.3.1), but if a 5% significance level was adopted, these population differences were not significant. Both populations also exhibited similar increases in shell growth for a given increase in tissue (fig. 3.3.6) ($F_{1,76}$ = 1.26, p=0.266), indicating there was no apparent variation in the differential allocation of resources to shell or tissue growth between the population samples. The relationship between tissue and shell weight at the start of the experiment showed that Prawle Point juveniles have a heavier shell for a given dry tissue weight of animal in the field ($F_{1,79}$ = 103.49, p=0.0001), but after seven weeks in the laboratory, both populations have a similar ratio of shell to tissue weight ($F_{1,77}$ = 3.45, p=0.067).

3.3.2.3 Growth variation among genotypes

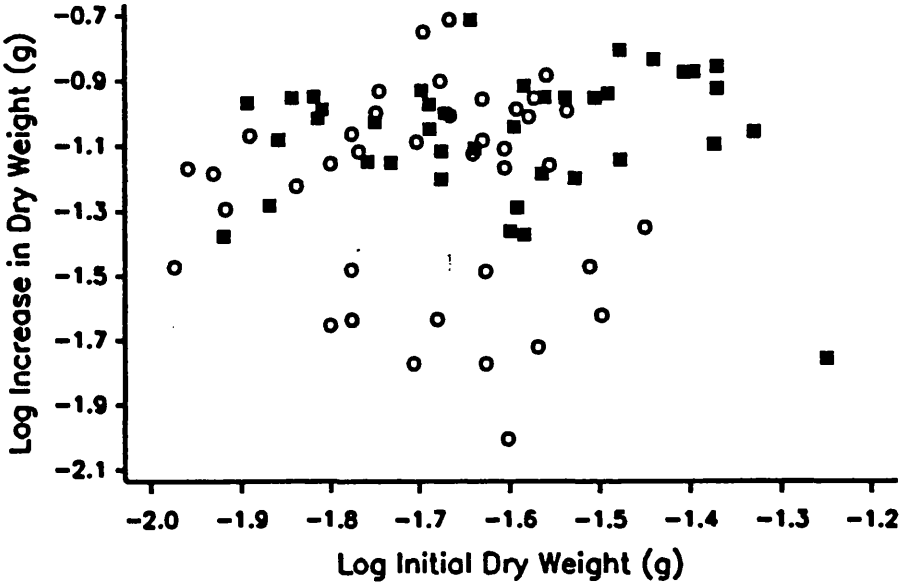
Differences in growth among genotypes were tested separately for each population, and only one locus was considered per analysis of covariance (table 3.3.3). None of the genotypic variation seen in the Peartree Point sample correlated with variation in tissue or shell growth rate (table 3.3.3a).

Genotypic differences at *Lap-2*, *Mdh-1* and *Pep-1* loci coincided with tissue and shell growth differences in juveniles collected from Prawle Point (table 3.3.3b). Prawle Point juveniles with either the *Lap-2* or *Mdh-1* 10.10 genotype, tend to have higher rates of growth than those individuals either homozygous or heterozygous for the 9-alleles (figs. 3.3.7 and 3.3.8). The 10.11 *Pep-1* genotype is present in those Prawle Point juveniles exhibiting higher growth rates (Fig. 3.3.9). Although figures 3.3.7-3.3.9 show tissue growth variation among genotypes, similar plots were obtained for shell growth variation.

Figure 3.3.5 Growth of field juveniles sampled from Peartree Point (closed squares) and Prawle Point (open circles) over seven weeks in the laboratory. Log_{10} transformed data are presented.

a) Tissue growth

b) Shell growth


Log Increase in Dry Weight (g)
-0.7
-0.9
-1.1
-1.3
-1.5
-1.7
-1.9
-2.1
-2.0 -1.9 -1.8 -1.7 -1.6 -1.5 -1.4 -1.3 -1.2
Log Initial Dry Weight (g)

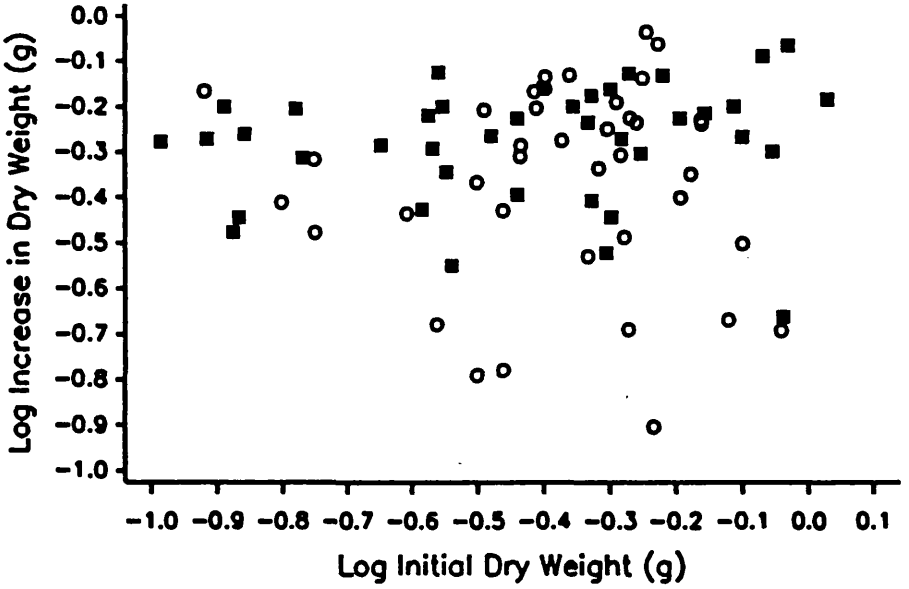

Log Increase in Dry Weight (g)
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1.0
-1.0 -0.9 -0.8 -0.7 -0.6 -0.5 -0.4 -0.3 -0.2 -0.1 0.0 0.1
Log Initial Dry Weight (g)

Figure 3.3.6 Relationship between shell and tissue growth for field juveniles maintained in the laboratory for seven weeks. Log_{10} transformed data are presented. Peartree Point = closed squares. Prawle Point = open circles.

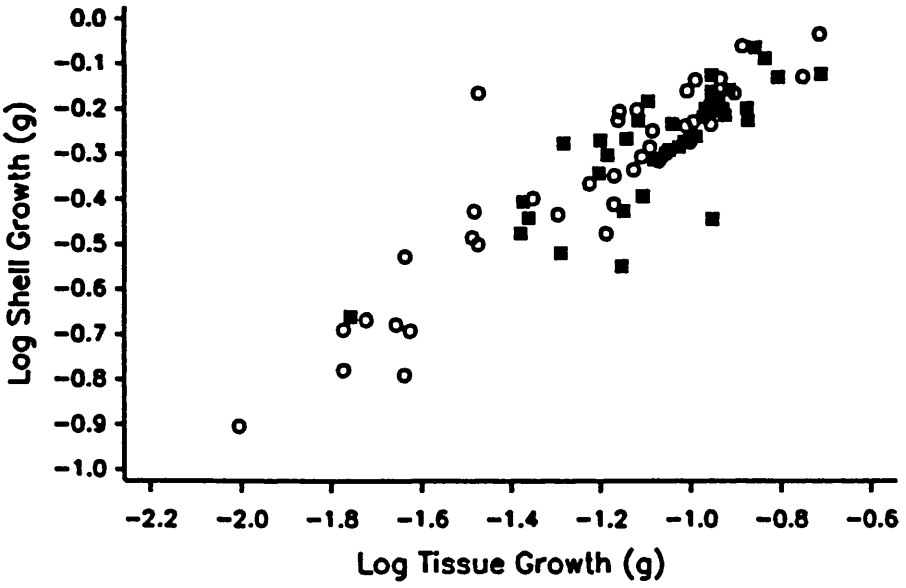

0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1.0
Log Shell Growth (g)
-2.2
-2.0
-1.8
-1.6
-1.4
-1.2
-1.0
-0.8
-0.6
Log Tissue Growth (g)

Table 3.3.3: Genotypic variation in physiology for field collected juveniles maintained in the laboratory for seven weeks. Bold type p-values are significant at the 5% probability level.

(a) Peartree Point

	Tissue Growth			Shell Growth			Feeding Rate		
Locus	F	df	p>F	F	df	p>F	F	df	p>F
Pep-1	1.21	2, 36	0.310	0.22	2, 36	0.806	1.75	2, 36	0.188
Lap-2	0.01	1, 37	0.925	0.10	1, 37	0.755	0.04	1, 37	0.838
Mdh-1	0.33	2, 36	0.718	0.31	2, 36	0.739	0.11	2, 36	0.900
Mpi	1.67	2, 36	0.203	3.19	2, 36	0.053	2.21	2, 36	0.124
Pgm-1	1.95	2, 36	0.158	2.65	2, 36	0.084	2.15	2, 36	0.131
Pgm-2	1.98	2, 35	0.154	1.83	2, 35	0.176	1.83	2, 35	0.175

(b) Prawle Point

	Tissue Growth			Shell Growth			Feeding Rate		
Locus	F	df	p>F	F	df	p>F	F	df	p>F
Pep-1	5.37	2, 36	**0.026**	4.72	1, 36	**0.037**	6.13	2, 36	**0.018**
Lap-2	19.70	2, 35	**0.0001**	7.68	2, 33	**0.002**	15.81	2, 35	**0.0001**
Mdh-1	10.17	2, 35	**0.0003**	5.98	2, 35	**0.006**	9.88	2, 35	**0.0004**
Mpi	0.41	2, 34	0.664	0.73	2, 34	0.488	0.36	2, 34	0.698
Pgm-1	1.10	1, 36	0.301	0.81	1, 36	0.373	1.38	1, 36	0.248
Pgm-2	2.91	1, 36	0.097	3.50	1, 36	0.070	3.53	1, 36	0.068

Table 3.3.3 (continued)

a) Peartree Point

	Tissue Growth Efficiency			Shell Growth Efficiency			Respiration Rates 2 Days Starvation			Respiration Rates 7 Days Starvation		
Locus	F	df	p>F	F	df	p>F	F	df	p>F	F	df	p>F
Pep-1	0.03	2, 36	0.966	0.46	2, 36	0.633	0.74	2, 31	0.487	1.92	2, 34	0.163
Lap-2	0.26	1, 37	0.613	0.45	1, 37	0.506	0.09	1, 32	0.764	0.11	1, 35	0.745
Mdh-1	0.43	2, 36	0.656	0.43	2, 36	0.653	0.90	2, 31	0.417	0.10	2, 34	0.907
Mpi	0.38	2, 36	0.684	2.03	2, 36	0.146	2.48	2, 31	0.100	0.11	2, 34	0.897
Pgm-1	0.02	2, 36	0.977	1.02	2, 36	0.370	5.91	2, 30	**0.02**	0.39	2, 34	0.679
Pgm-2	0.33	2, 35	0.718	0.29	2, 35	0.747	0.03	2, 31	0.970	0.01	2, 34	0.991

b) Prawle Point

	Tissue Growth Efficiency			Shell Growth Efficiency			Respiration Rates 2 Days Starvation			Respiration Rates 7 Days Starvation		
Locus	F	df	p>F	F	df	p>F	F	df	p>F	F	df	p>F
Pep-1	6.71	1, 35	**0.014**	0.11	1, 36	0.745	1.51	1, 37	0.228	1.32	1, 38	0.258
Lap-2	4.15	2, 33	**0.025**	9.58	2, 33	**0.0005**	3.16	2, 36	0.054	0.76	2, 37	0.473
Mdh-1	5.51	2, 33	**0.009**	3.87	2, 33	**0.031**	0.53	2, 36	0.595	0.76	2, 37	0.476
Mpi	3.41	2, 34	**0.045**	1.57	2, 34	0.222	0.28	2, 35	0.761	0.32	2, 36	0.727
Pgm-1	0.01	1, 36	0.939	0.00	1, 36	0.957	0.67	1, 37	0.418	7.32	1, 38	**0.010**
Pgm-2	0.06	1, 36	0.815	0.51	1, 36	0.480	4.84	1, 36	**0.03**	1.35	1, 38	0.253

3.3.2.4 Feeding rates

Over the first three weeks of the experiment, there were no significant ($p>0.05$) population differences in preferred mussel length (table 3.3.1).

The total joules eaten per whelk over the seven weeks were not dependent upon animal size estimated at the start of the experiment (fig. 3.3.10; $F_{1, 78} = 1.67$, $p = 0.1998$) and there were also no population differences in feeding rate (table 3.3.1).

3.3.2.5 Feeding rates and genotype

Each locus and site was tested separately for feeding rate variation among genotypes. There were no correlations between genotypic and feeding rate variation for the Peartree Point sample (table 3.3.3a). Feeding rates varied among *Lap-2*, *Mdh-1* or *Pep-1* genotypes in the Prawle Point sample ($p<0.02$; table 3.3.3). The 10.10 genotype for both the *Lap-2* and *Mdh-1* loci had higher feeding rates than those whelks either homozygous or heterozygous for the 9-alleles. Individuals with the 10.11 *Pep-1* genotype also show higher rates of feeding.

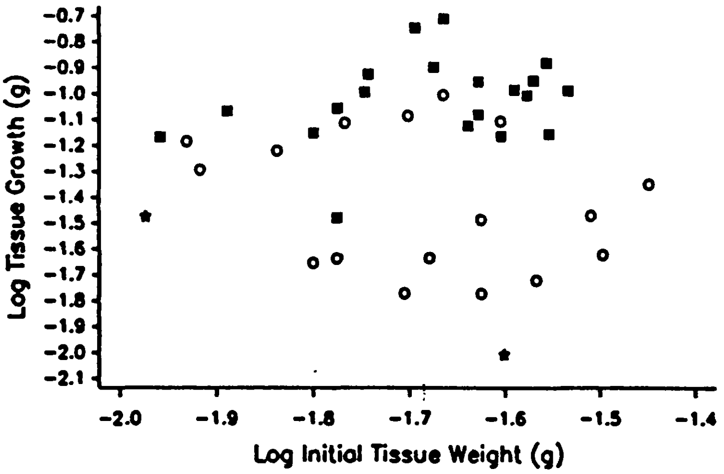
3.3.2.6 Gross growth efficiency

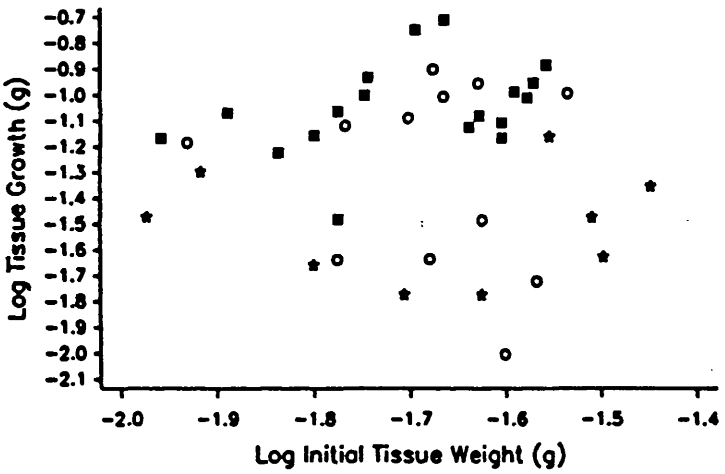
Heterogeneous gradients were found between sites for the relationship between the total amount eaten and the total growth in both tissue (fig. 3.3.11a; $F_{1, 75}= 11.73$, $p= 0.001$) and shell weight (fig. 3.3.11b; $F_{1, 75}= 8.12$, $p= 0.006$). The Johnson-Neyman technique (Huitema, 1980) was used to identify the critical range of feeding rates for which the two population samples did not differ in either their total tissue or shell growth. These regions of 'non-significance' at the 5% probability level were found to be 3849-6158J and 3016-4850J for tissue and shell growth efficiency respectively. The gradients calculated for the two sites suggests that Peartree Point animals have higher growth efficiencies below the lower critical value, whereas Prawle Point juveniles had higher growth efficiencies when feeding rates exceeded the upper limit. However, these results should be viewed with caution due to the paucity of data within these outlying regions.

Figure 3.3.7 Variation in tissue growth among the *Lap-2* genotypes sampled from Prawle Point only. Log_{10} transformed data are presented. 10.10 (closed squares), 9.10 (open circles) and 9.9 (closed stars) genotypes are presented.

Figure 3.3.8 Variation in tissue growth among the *Mdh-1* genotypes sampled from Prawle Point only. Log_{10} transformed data are presented. 10.10 (closed squares), 9.10 (open circles) and 9.9 (closed stars) genotypes are presented.

Figure 3.3.9 Variation in tissue growth among the *Pep-2* genotypes sampled from Prawle Point only. Log_{10} transformed data are presented. 10.10 (closed squares) and 10.11 (open circles) genotypes are presented.


Log Tissue Growth (g)
Log Initial Tissue Weight (g)


Log Tissue Growth (g)
Log Initial Tissue Weight (g)

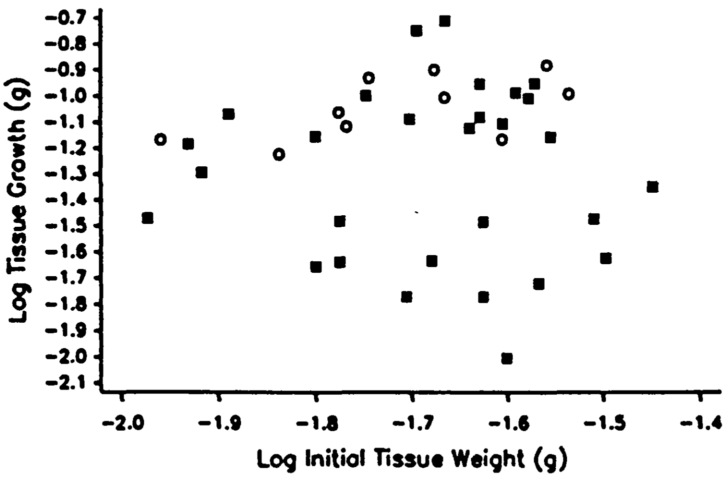

Log Tissue Growth (g)
Log Initial Tissue Weight (g)

Figure 3.3.10 Feeding rates of field juveniles collected from Peartree Point (closed squares) and Prawle Point (open circles), and maintained in the laboratory for seven weeks. Log_{10} transformed data are presented.

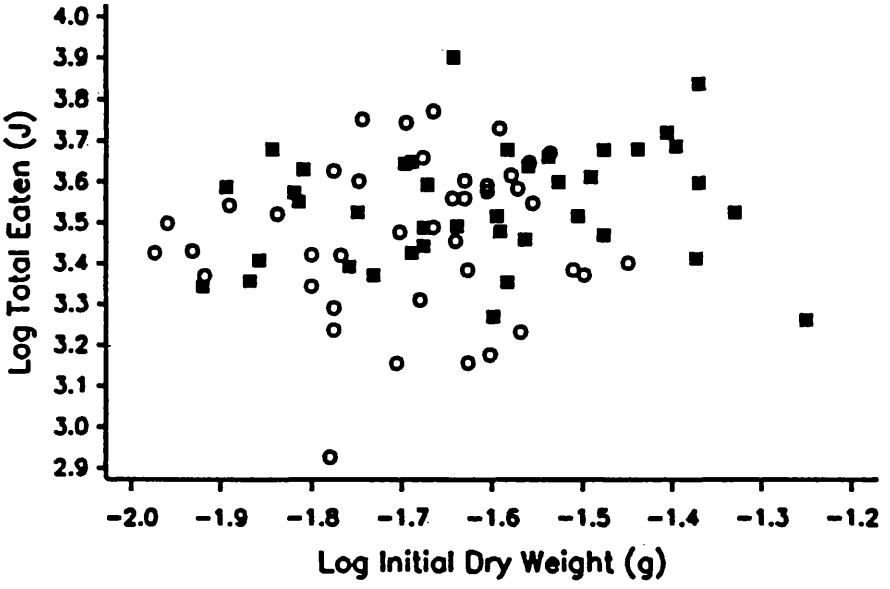
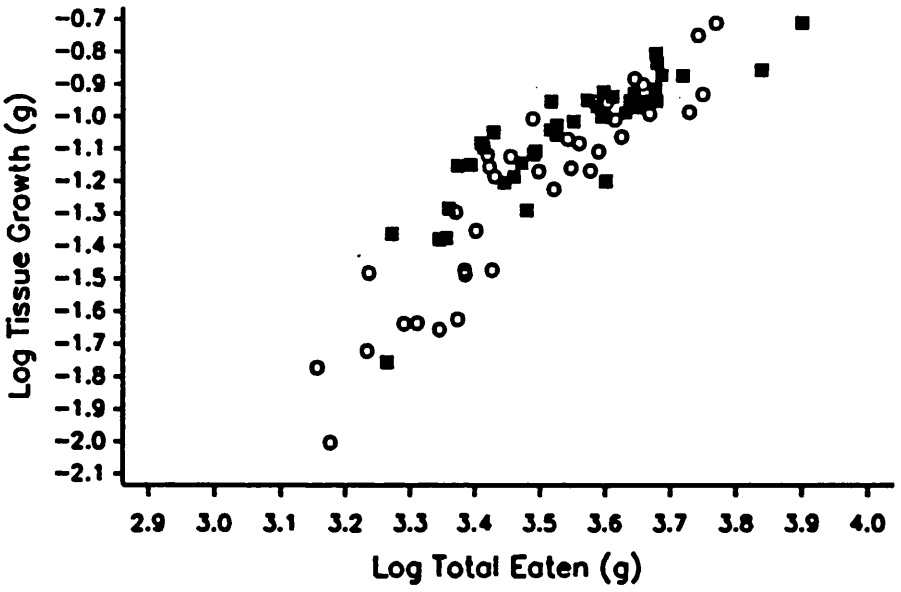
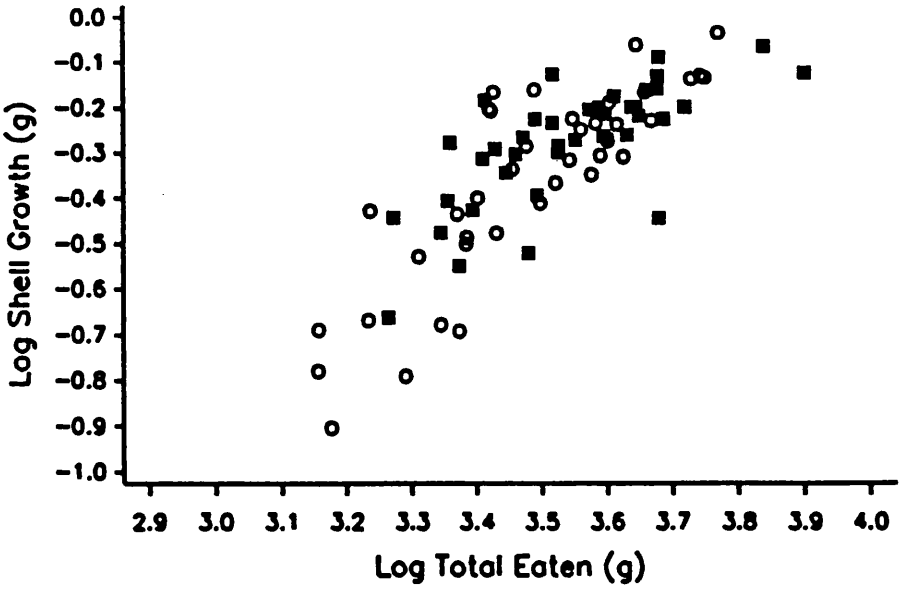

Log Total Eaten (J)
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3.0
2.9
−2.0
−1.9
−1.8
−1.7
−1.6
−1.5
−1.4
−1.3
−1.2
Log Initial Dry Weight (g)

Figure 3.3.11 Growth efficiency of field juveniles maintained in the laboratory for seven weeks. Log_{10} transformed data are presented. The upper and lower critical values for the range of feeding rates for which the two sites did not vary ($p>0.05$) are indicated (see text for derivation and values). The upper critical feeding rate was outside the axis range for tissue growth efficiency and so is not indicated here. Peartree Point = closed squares, Prawle Point = open circles.

a) Tissue

b) Shell


Log Tissue Growth (g)
-0.7
-0.8
-0.9
-1.0
-1.1
-1.2
-1.3
-1.4
-1.5
-1.6
-1.7
-1.8
-1.9
-2.0
-2.1
2.9 3.0 3.1 3.2 3.3 3.4 3.5 3.6 3.7 3.8 3.9 4.0
Log Total Eaten (g)


Log Shell Growth (g)
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1.0
2.9 3.0 3.1 3.2 3.3 3.4 3.5 3.6 3.7 3.8 3.9 4.0
Log Total Eaten (g)

3.3.2.7 Gross growth efficiency and genotype

In the Prawle Point sample, variation at the *Mdh-1* and *Lap-2* loci correlated with the growth efficiency of both tissue and shell, and variation at the *Pep-1* locus was associated with tissue growth efficiency (table 3.3.3b). Juveniles homozygous for either the *Mdh-1* and *Lap-2* 10 alleles grew more shell and tissue for a given amount of food eaten than individuals with either the 9.10 or 9.9 genotype. The *Pep-1* 10.11 and *Mpi* 10.10 genotypes were present in those juveniles which have the higher growth efficiency rates for tissue. The association between genotype and the efficiency of tissue growth for Prawle Point juveniles are presented in figures 3.3.12 - 3.3.14. No other loci showed significant difference at the 5% probability level in either population (table 3.3.3).

3.3.2.8 Respiration rates

For both populations, respiration rates were higher after two days than seven days starvation (Peartree Point; $F_{1,76}$=14.57, p=0.0003; Prawle Point; $F_{1,78}$=8.98, p=0.0037), therefore population differences were tested at each time point. After two days starvation, Peartree Point individuals have higher rates of oxygen consumption than Prawle Point juveniles, but rates were similar between sites after seven days starvation (table 3.3.1).

3.3.2.9 Respiration rates and genotype

The results of the analysis of covariance for variation in respiration rates among each locus and population are given in table 3.3.2. For the 2-day starvation respiration rates, *Pgm-1* in the Peartree Point sample and *Pgm-2* for the sample from Prawle Point were associated with variation in respiration rate (table 3.3.3). Similarly after seven days without food, variation in respiration rate was related to differences at the *Pgm-1* locus in the Prawle Point sample (table 3.3.3b).

3.3.3 Field growth rates

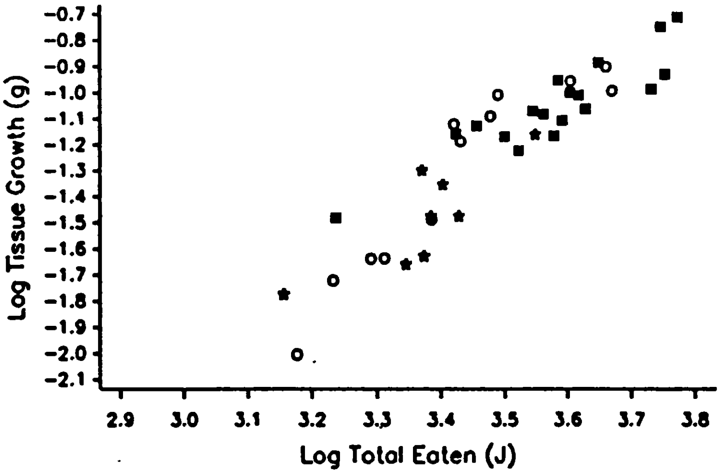
3.3.3.1 Shell shape and weight regressions

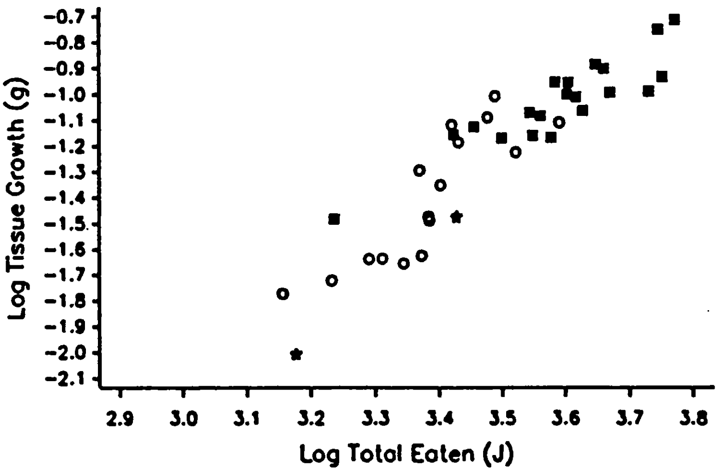
Samples of dog-whelks were taken from each site at the start of the field experiment (May) and again at the end (September). These samples were used to test for population and seasonal

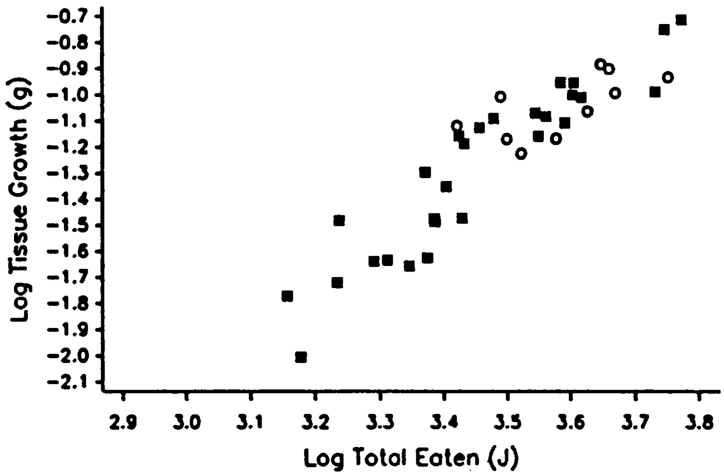
Figure 3.3.12 Association of genotypic variation at the *Lap-2* locus and the efficiency of tissue growth for juveniles collected from Prawle Point and maintained in the laboratory for seven weeks. Log_{10} transformed data are presented. 10.10 = closed squares, 9.10 = open circles and 9.9 = closed stars.

Figure 3.3.13 Association of genotypic variation at the *Mdh-1* locus and the efficiency of tissue growth for juveniles collected from Prawle Point and maintained in the laboratory for seven weeks. Log_{10} transformed data are presented. 10.10 = closed squares, 9.10 = open circles and 9.9 = closed stars.

Figure 3.3.14 Association of genotypic variation at the *Pep-1* locus and the efficiency of tissue growth for juveniles collected from Prawle Point and maintained in the laboratory for seven weeks. Log_{10} transformed data are presented. 10.10 = closed squares and 10.11 = open circles.


Log Tissue Growth (g)
Log Total Eaten (J)


Log Tissue Growth (g)
Log Total Eaten (J)


Log Tissue Growth (g)
Log Total Eaten (J)

variation in shell shape and length-dry weight regressions. All coefficients given in this section are mean values $\pm$2S.E.

The relationship between length and aperture, as an indication of shell shape, was similar for May and September samples for either sample site (Peartree Point, $F_{1,57}$= 1.20, p= 0.28; Prawle Point, $F_{1,56}$= 0.33, p= 0.57), but length-aperture ratios were significantly different between populations ($F_{1,115}$= 9.65, p= 0.002). For a given shell length, Peartree Point whelks had a longer aperture:

Peartree Point: Log_{10} aperture (mm) = (0.9404$\pm$ 0.0316* $\log_{10}$ length (mm))- 0.0454$\pm$0.0412

Prawle Point: Log_{10} aperture (mm) = (1.0026$\pm$ 0.0355* $\log_{10}$ length (mm))- 0.1424$\pm$0.0470

The regression between length and dry tissue weight differed between May and September at Peartree Point ($F_{1,57}$= 21.86, p= 0.0001), whereas samples from Prawle Point did not vary between sampling occasions ($F_{1,56}$= 1.04, p= 0.31). In both months, population differed in the length-dry tissue weight regression (May, $F_{1,87}$= 132.58, p= 0.0001; September $F_{1,85}$= 43.51, p= 0.0001); Peartree Point whelks had a larger tissue weight for a given shell length on both occasions.

Peartree Point:

May: Log_{10} dry tissue weight (g) = (3.1762$\pm$ 0.1704* $\log_{10}$ length (mm))- 5.2103$\pm$0.2230

Sept: Log_{10} dry tissue weight (g) = (3.1762$\pm$ 0.1704* $\log_{10}$ length (mm))- 5.3252$\pm$0.2230

Prawle Point:

Log_{10} dry tissue weight (g) = (3.492$\pm$ 0.2432* $\log_{10}$ length (mm))- 5.9114$\pm$0.3212

The length-shell weight regressions were also significantly different between samples collected in May and September from the Peartree Point site ($F_{1,56}$= 9.39, p= 0.0034), whereas whelks from Prawle Point had similar length-shell weight regressions for the two sampling occasions ($F_{1,56}$= 0.53, p= 0.47). Significant population effects upon the relationship between length and shell weight were seen in the May ($F_{1,86}$= 13.57, p= 0.0004) and September ($F_{1,85}$= 23.37, p= 0.0001) samples. Peartree Point whelks had a heavier shell for all size classes in September

whereas the differences between sites in May were more complicated due to heterogenous slopes ($F_{1,86}$= 3.57, p= 0.0001) intersecting at a shell length of 14.7mm. A Johnson-Neyman test (Huitema, 1980) was used to identify the range of shell lengths for which whelks from the two sites did not differ in shell weight. The region of 'non-significance' at the 5% probability level was ca. 7-39 mm. The range of shell lengths in these samples was well within this critical region, and so it can be concluded that the relationship between shell length and weight was similar for sites in May.

Peartree Point:

May: Log_{10} dry shell weight (g) = (3.531$\pm$ 0.1653* $\log_{10}$ length (mm))- 4.400$\pm$0.2151

Sept: Log_{10} dry shell weight (g) = (3.167$\pm$ 0.1761* $\log_{10}$ length (mm))- 3.918$\pm$0.2289

Prawle Point:

Log_{10} dry shell weight (g) = (3.119$\pm$ 0.1137* $\log_{10}$ length (mm))- 3.919$\pm$0.1502

It was intended that the relationship between dry tissue and shell weight would give an indication of the relative allocation of resources to shell and tissue (as shown in section 3.3.2.3) and was significantly different between samples obtained from the Peartree Point site in May and September ($F_{1,57}$= 18.88, p= 0.0001). For a given dry tissue weight, shells were heavier in the September sample than those sampled in May. At Prawle Point, the dry tissue and shell weight relationship was similar for both months ($F_{1,56}$= 0.17, p= 0.68) and was significantly different from that of Peartree Point in May ($F_{1,86}$= 19.19, p= 0.0001) and September ($F_{1,84}$= 5.92, p= 0.0171). These latter population differences were identified in the interaction term between tissue weight and population indicating separate population gradients. The gradient relating shell to tissue weight was greater in the Peartree Point sample indicating that for a given tissue weight, Peartree Point whelks have a heavier shell:

Peartree Point:

May: $\log_{10}$dry shell weight (g) = (1.0208$\pm$0.0627 * $\log_{10}$dry tissue weight (g))+ 1.2921$\pm$0.0802

Sept: $\log_{10}$dry shell weight (g) = (1.0208$\pm$0.0627 * $\log_{10}$dry tissue weight (g))+ 1.4205$\pm$0.0867

Prawle Point:

$\log_{10}$ dry shell weight (g) = (0.8459 $\pm$ 0.0560 * $\log_{10}$ dry tissue weight (g)) + 1.2921 $\pm$ 0.0782

The coefficients calculated in the length-dry weight regressions for Peartree Point whelks varied over the summer period as described above and so were interpolated to provide more accurate estimates of separate shell and tissue weights for the experimental animals measured over the summer months (table 3.3.4).

3.3.3.2 Growth

Tissue and shell dry weights were estimated from the appropriate length-dry weight regression (section 3.3.3.1 and table 3.3.4). Samples obtained in September were excluded from these analyses due to severe weather conditions and the low number of labelled whelks collected at this time ($n<30$ at Peartree Point). For each sample site, growth rates over a month were compared among samples and did not vary significantly at the 5% probability level for either Peartree Point (tissue growth, $F_{4,102}= 2.10$, $p=0.087$; shell growth, $F_{4,102}= 2.05$, $p=0.093$) or Prawle Point (tissue growth, $F_{4,220}= 1.39$, $p=0.237$; shell growth, $F_{4,220}= 1.46$, $p=0.215$). The five estimates of growth rate per month for each site were therefore pooled over the entire summer period before population variation was investigated. Peartree Point whelks had higher growth rates on the shore than those measured at Prawle Point for the size range of whelks measured in this study (fig. 3. 3. 15; table 3.3.5). Daily growth rates (calculated as the total growth between successive measurements divided by the number of days elapsed) were used in these analyses to accommodate for slight differences in the time intervals between measurements.

Table 3.3.4 Estimated coefficients for separate length-weight regressions for shell and tissue weights of Peartree Point whelks only. Values are interpolated from mean coefficients calculated in May and September; standard errors were inestimable for intermediate samples.

a) Tissue Weight

$$Log_{10}\text{Dry Tissue Weight} = (3.1762 * Log_{10}\text{Length}) - c$$

Sample Date	Intercept (c)
26/27 May	-5.2013
12/13 June	-5.2190
24/25 June	-5.2332
13/14 July	-5.2556
24/25 July	-5.2686
12/13 Aug	-5.2910
22 Aug	-5.3028

b) Shell Weight

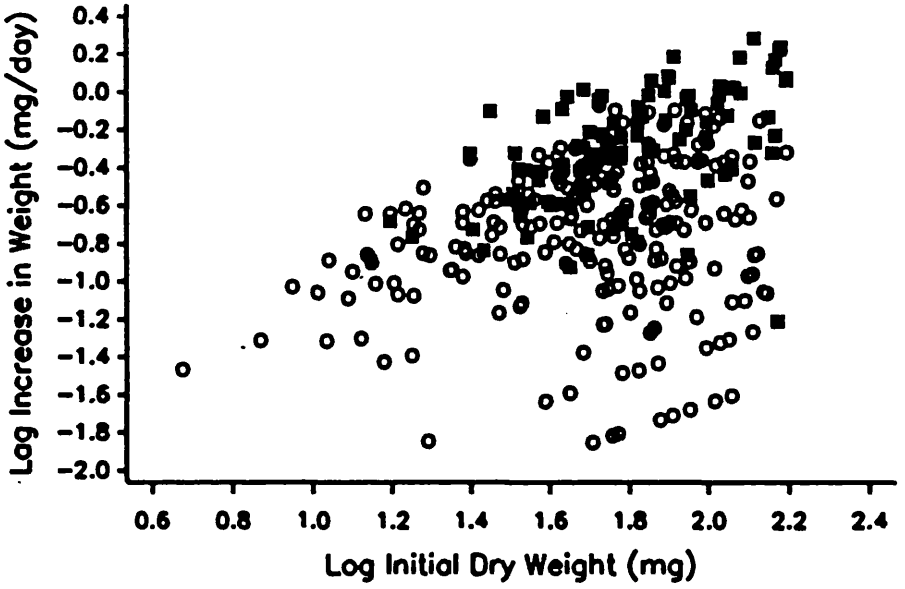
$$Log_{10}\text{Dry Shell Weight} = (b * Log_{10}\text{Length}) - c$$

Sample Date	Gradient (b)	Intercept (c)
26/27 May	3.531	-4.40
12/13 June	3.479	-4.331
24/25 June	3.437	-4.276
13/14 July	3.372	-4.189
24/25 July	3.333	-4.139
12/13 Aug	3.268	-4.052
22 Aug	3.233	-4.006

Figure 3.3.15 Daily growth rates of whelks at Peartree Point (closed squares) and Prawle Point (open circles). Size measurements were recorded each month and the daily growth rate calculated (see text). Log_{10} transformed data are presented.

a) Tissue growth

b) Shell growth


Log Increase in Weight (mg/day)
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
-2.0
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
Log Initial Dry Weight (mg)

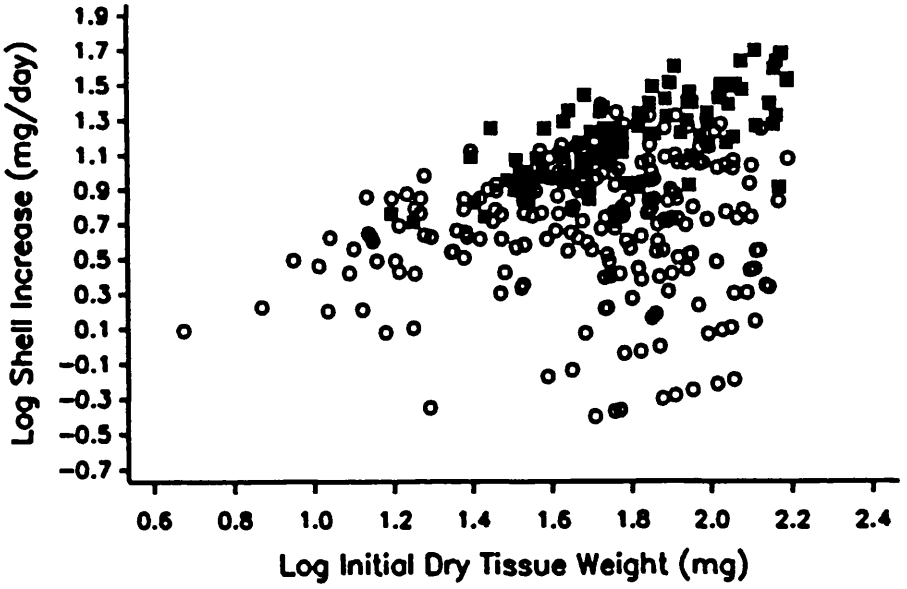

Log Shell Increase (mg/day)
1.9
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
-0.3
-0.5
-0.7
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
Log Initial Dry Tissue Weight (mg)

Table 3.3.5 Summary of the analysis of covariance for differences in growth rate between Peartree Point and Prawle Point measured on the shore during the summer, 1994.

	Dependent Variable	Source of Variation	df	F-value	Pr>F
Tissue Growth	$\log_{10}$ tissue growth	$\log_{10}$ initial tissue weight	1, 330	29.58	0.0001
		Population	1, 330	1.16	0.2816
		Interaction	1, 330	6.34	0.0123
Shell Growth	$\log_{10}$ shell growth	$\log_{10}$ initial shell weight	1, 330	28.27	0.0001
		Population	1, 330	4.32	0.0384
		Interaction	1, 330	10.41	0.0014
Length Growth	$\log_{10}$ length growth	$\log_{10}$ initial shell length	1, 330	5.96	0.0152
		Population	1, 330	8.88	0.0031
		Interaction	1, 330	13.51	0.0003

3.4 DISCUSSION

3.4.1 Maximum wave force

Maximum wave force measurements were similar between sites, contrary to previous estimates of exposure on the basis of Ballantine's (1961) biological exposure scale; Peartree Point has been classified as 'exposed' (scale 2; McCarter and Thomas, 1980) and Prawle Point as 'sheltered' (scale 4-6: McCarter and Thomas, 1980; Day, 1990). The similarity in measurements taken in the present study may be a true reflection of the relative wave forces experiences on these shores, but it should be noted that readings were recorded over a limited period of six weeks and may not necessarily reflect long-term variation in wave force. The spatial distribution of indicator species used in the biological scales could reflect variation in other aspects of wave action such as water turbulence, which would not be apparent from maximum wave force measurements and would also be influenced by the topography of the shore. Topographical complexity will also tend to increase the maximum wave force and the measurement variance, and perhaps explain why maximum wave force measurements sometimes differ from that anticipated from the indicator species present and other *a priori* considerations (Bell and Denny, 1994).

Measurements of maximum wave force were recorded during the spring equinoctial storms, which were considered to maximise any site differences in wave force. Information received from the local meteorological office (ca. 15km east of the sample sites) indicated that rough weather* prevailed during this period, although more severe sea conditions can be encountered in this area (Brixham Coastguard, pers.comm.). It is possible that site differences between Peartree Point and Prawle Point could manifest under worse weather conditions; the fetch, previous sea state and the length of time the wind acts in a particular direction would all influence wave height, turbulence and force reaching the shore. However, when the aspect of this coastline (fig. 3.1) was considered, the similarity in maximum wave forces at these sites was perhaps expected; both Peartree Point and Prawle Point are 'exposed' to the prevailing south west winds. Unfortunately, maximum wave forces were not sampled for sites between

* Mean wind speed ~10-25 knots, mean gust ~25-43 knots, maximum gust ~25-55 knots. These values were calculated for onshore winds only (90-250° true).

Prawle Point and Lannacombe beach (fig. 3.1) as this stretch of coast has a more south-easterly aspect and may experience lower wave forces than those measured at either Prawle Point and Peartree Point. On the basis of the present results obtained on maximum wave forces at opposite ends on the cline, wave force therefore cannot be eliminated as a possible environmental covariate to the clinal variation in dog-whelks from this region.

3.4.2 Temperature and relative humidity

The maximum air temperature recorded was 41°C at Prawle Point, which is comparable to maximum temperatures recorded in other studies undertaken on British shores (Evans, 1948). This temperature may appear exceptionally high, but these measurements were taken in direct sun and above the rock surface as they were intended to reflect the likely temperatures experienced by whelks if foraging for prey. The temperatures recorded at Prawle Point were consistently higher than those measured at Peartree Point where the maximum temperature recorded was 34°C. The relative humidities were inversely related to temperature; higher temperatures corresponded to lower humidities and vice versa over the two hour period at either site. Similarly at Prawle Point, where higher temperature readings prevailed, lower relative humidities were recorded than those at the Peartree Point site. The minimum relative humidity recorded was 25% at the Prawle site.

The higher abundance of mussels at Peartree Point (McCarter and Thomas, 1980) would offer greater protection from the effects of temperature (Lintas and Seed, 1994) and coupled with the higher abundance of algae, would act to increase the humidity and reduce temperatures at this site through the cooling effects of water evaporation. Measurements were recorded on calm days during the summer when the influence of wave action was considered to be reduced and similar for the two sites; sea spray would locally increase the humidity and reduce temperatures. The lower temperatures and higher humidities at Peartree Point are likely to be more amenable to foraging activity than those measured at Prawle Point, where the maximum recorded temperature exceeds previous estimates of the lethal air temperature of 36°C for *N. lapillus* (Sandison, 1967). Considering 36°C as the lethal temperature, dog-whelks should be dead at Prawle Point! However, estimates of lethal temperature are dependent upon the methods used to determine the critical limit (discussed in section 4.1) including the exposure

time; the temperature at Prawle Point was greater than 36°C for one hour. The internal tissue temperatures of whelks in direct sunlight has been suggested to be as much as 10°C higher than air temperatures (Etter, 1988b), but these ambient air temperatures were obtained from a shaded thermocouple. White shells, which tend to reflect incident sun, and evaporative cooling of the mantle cavity fluid would both help reduce the internal temperatures.

3.4.3 Desiccation rates

The higher temperatures and lower humidities, as a consequence of the low abundance of algae and mussels, would explain why water loss from the desiccation pots (section 3.2.1.2) were higher at Prawle Point. If water loss from the mantle cavity was also higher leading to higher salinities (Boyle *et al.*, 1979; Kirby *et al.*, 1994b) at this site relative to Peartree Point, the more conservative response of Prawle Point whelks when in hyperosmotic seawater and the shell shape differences could be explained (Kirby, *et al.*, 1994b; chapter four, this study).

3.4.4 Morphological variation between shore sites

Peartree Point juveniles collected from the shore had a longer aperture for a given shell length than whelks collected from Prawle Point for all shore samples. This agrees with studies that found that dog-whelks predominantly had the round shell shape and lower length/aperture ratios at Peartree Point, whereas the elongate shell shape was found at Prawle Point (Day, 1990; Kirby, 1992; Kirby, *et al.*, 1994a). These morphological differences, which would be expected to affect the internal shell volume and the size of animal (Palmer, 1981; section 2.4.3, this study), can explain why sample sites also differed in the relationship between shell length and dry tissue weight; Prawle Point whelks (elongate shell shape) generally had a lower tissue weight than Peartree Point whelks (rounded shell shape) for an equivalent shell length.

Variation in shell shape has frequently been attributed to shore exposure and the predominant selection pressures encountered (eg. *Nucella*, Crothers, 1973, 1975a b, 1985 (review); Kitching, 1976, 1977; Seed, 1978; Etter, 1988a: *Gibbula cinerea*, Frid and Fordham, 1994: littorinids, Newkirk and Doyle, 1975; Johannesson, 1986). Measurements from the present study, failed to identify differences in maximum wave force, but may be a consequence of this type of measurement for reasons described previously (section 3.4.1). The round shell shape, which dominates the Peartree Point samples, is thought to be less susceptible to dislodgement,

and whelks possessing this shell shape tend to have a relatively larger foot for a given body size which increases tenacity. The elongate shell shape, as found in Prawle Point samples, and its narrow aperture is considered to reduce the success of crab predation, thought to be generally greater on 'sheltered' shores (Kitching *et al.*, 1966; Hughes and Elner,1979; Lawton and Hughes, 1985). The tenacity of dog-whelks and the relative intensity of crab predation on the shore has not been assessed in this study. Air temperature and humidity measurements on the shore differed between sites. It is possible that morphological variation can be explained in terms of these environmental variables, temperature and desiccation rate (Kitching 1986; Kirby, 1992), which are also known to vary among shores differing in 'exposure' (section 3.1 and refs. therein). The elongate shell shape is thought to reduce temperature stress through retaining a larger volume of seawater in the mantle cavity for a given tissue weight, and so maintaining lower salinities and temperatures during emersion (chapter one and refs. therein). Such a shell shape would be beneficial at the Prawle site.

No within-population differences in mean shell shape were seen over the duration of the experiment although whelks were maintained in the laboratory under constant conditions for seven weeks (section 3.2.2), and individuals were presumably in the active stages of growth as initial shells ranged in length from 10 to 13 mm and possessed thin shell lips. Kirby (1992) found a slight change in shell shape where both shore samples approached the length/aperture ratios obtained for laboratory-reared populations, but this change in shape was not statistically significant as adult whelks with slower growth rates had been used. Spight (1973) found that over 6-12 months shell shape changed as the juveniles of a related species *Nucella lamellosa* grew. A longer period in the laboratory may be necessary before any changes in shape, due to altered environment, would be observed. However, it may be possible that shell shape is predetermined in the early life stages and that later changes in environment would not necessarily influence the shell shape of an individual; the shape of the preceding whorl is important to the formation of successive whorls and shell shape (Hutchinson, 1989), which would tend to limit any changes in morphology. It should be remembered that whelks collected from the shore are samples of populations that have already undergone modification and some degree of selection on the shore, and so the present results may be limited to the size and age range used here (~one year old, Coombs, 1973b; pers obs.)

3.4.5 Size measurements and seasonal variation

The following section discusses the results obtained for the destructive shore samples taken in the spring and early autumn of 1994 to enable growth rates to be determined on the shore. Regression coefficients did not vary between May and September samples collected from Prawle Point. For Peartree Point whelks, a lower tissue and shell weight was found in September when compared to the May sample for a given shell length. Shell production continues at a reduced rate and the tissue weight decreases during starvation (Palmer, 1981; section 2.3.8.3), which would be expected over winter when dog-whelks tend to become inactive and cease feeding (Feare, 1971). The regressions of shell length and tissue or shell weight would be expected to indicate that dog-whelks have a heavier shell and lighter body tissue weight (for a given shell length) in the spring (after low winter feeding rates) than if measured after a period of intensive feeding. Growth rates are higher over the summer months, in part due to the increased temperature (Bayne and Scullard, 1978b) and feeding rates, resulting in a lighter shell, and possibly a heavier tissue weight, for a given shell length in September compared to May samples. In the present study, the relative changes in shell weight conform to this expectation, shells are heavier in May than in September for a given shell length, whereas changes in tissue weight requires further explanation. Whelks at Peartree Point produce egg capsules throughout the summer months (pers. obs.), and so the decrease in the relative tissue weight seen from May to September may be due to reproductive expenditure (Stickle, 1975), and may reflect loss through the production and release of capsules and sperm (Chow, 1987). Prawle Point whelks appear to have a restricted breeding season in late winter/spring (pers. obs.), and so relative weight changes associated with reproduction would not be expected during summer. More information about the reproductive cycle of dog-whelks at these sites is required to confirm this interpretation.

3.4.6 Growth of shore whelks

Growth rates on the shore did not vary over the summer; other studies have also found invariant growth rates within the same season (Etter, 1989). Hughes (1972) assumed that growth was constant during the 'growth season' in his calculations of shore growth rates, but this assumption was necessary because of the unpredictable and erratic growth patterns of

individual whelks. Such individual variability would add to the total variance within each site sample, which may explain why growth rates were similar among monthly samples and would tend to conceal any possible differences between sites. Peartree Point whelks however had higher tissue and shell growth rates than individuals sampled from Prawle Point both on the shore and in the laboratory, although the higher shell growth rates of Peartree Point whelks in the laboratory were not significant at the 5% significance level ($p=0.062$). However, whelks from both sites indicated that the relative allocation to shell and tissue was similar, which also suggests that shell growth rates were indeed faster for Peartree Point whelks in the laboratory.

Etter (1989) found that whelks sampled at sheltered sites had higher growth rates, but these differences in growth were based on shell length measurements and may be due to shell shape differences between sites of varying exposure (section 3.4.4); the shell length of elongate shells will appear to grow faster relative to rounded forms due to geometry. The discrepancy between the results of Brown and Quinn (1988) and the present study may result from the same reason. Higher growth rates on 'exposed' shores, relative to 'sheltered' shores, have been attributed to size and higher consumption rates at these sites (Burrows and Hughes, 1990). When absolute growth rates were calculated to account for differences in body size and the amount of food eaten at the two sites, Burrows and Hughes (1990) found that the sheltered site whelks in fact have higher growth rates. It is not possible to attribute the differences between the results of Burrows and Hughes (1990) and those of the present investigation to the complicating influence of shape variation and the use of shell length measurements, as shell and tissue weights were distinguished in both studies when calculating growth rates.

The present study was undertaken over the summer during which time the temperature and relative humidity at Prawle Point reached 41°C and 25% respectively (section 3.4.2). The extreme conditions experienced during low tide at this site could have been sufficient to reduce feeding rate and consequently the growth rate of Prawle Point whelks. Feeding rates have not been measured for Peartree Point and Prawle Point whelks on the shore, but measurements of environmental variables (this study), and the presence and relative abundance of prey types in this coastal region are known to vary between sites (McCarter and Thomas,1980); whelks would be expected to have lower feeding rates at the Prawle Point site.

The differences in laboratory growth rate between site samples were not explained by population variation in feeding rate in the laboratory as both populations had similar feeding rates. Whelks were to be maintained in the laboratory for three weeks prior to the measurement of growth rates over the following four weeks. This was not possible due to the problems of changes in the immersed-shell weight regressions (through laboratory and feeding rate effects: section 2.3.8.2) unknown at the time of this experiment. Growth rates were therefore taken for the entire period that whelks were maintained in the laboratory. Again, possible variation in feeding rates prior to collection from the shore could explain the growth variation in the laboratory. Differences in previous dietary history could reflect either variation in the main prey species attacked at each site (Hughes and Dunkin, 1984b; Moran *et al.*, 1984; West, 1986), or through localised environmental differences among sites influencing the foraging behaviour. Climatic variation and the risks associated with adverse environmental conditions affect the rate of energy acquisition for this species (Burrows and Hughes, 1989). My samples (to determine laboratory physiology) were collected in May before the high summer temperatures and desiccation rates could affect the foraging behaviour, but it is possible that other environmental variables, including the risk of crab predation, could be involved. In some instances, it can be several days before whelks resume feeding after being transferred from the shore (pers. obs.), and any previous differences in body reserves would result in growth differences at these times regardless of measured feeding rates in the laboratory.

Considering the similarity of feeding rates among population samples in the laboratory, it could be argued that the growth and growth efficiency variation for these animals are due to differences in metabolic expenditure (reviewed in Bayne and Newell, 1983). Respiration rates differed between laboratory samples; Peartree Point juveniles had higher rates of oxygen consumption than Prawle Point whelks after two days starvation, but rates are similar between population samples if measured after seven days starvation. Peartree Point juveniles would perhaps be expected to exhibit the more conservative respiration rates considering the growth rates and growth efficiency results, but higher rates after two days starvation may reflect differences in post-feeding behaviour and physiology. Respiration rates are generally lower in the absence of food (Bayne and Newell, 1983), but can be increased by elevated foraging

activity in the search for food (Calow, 1974; Bayne and Scullard, 1978b; Hughes and Dunkin, 1984a). The constant or increased respiration rates seen for *N. lamellosa*, a related whelk species (Stickle and Duerr, 1970), could also be explained in terms of activity levels during starvation. The similarity in respiration rates between sites seven days after the removal of food suggests that basal metabolism was similar between sites.

3.4.7 Genotype and laboratory physiology

It is not surprising that the genotypes at a low number of gene loci had statistically significant variation in physiology; statistical reasons can explain why approximately 5% of the tests undertaken would be 'significant' at the 5% probability level. Caution is therefore needed when interpreting these results. For example, genotypic variation at the *Mpi* and *Pgm-1* loci were only associated with variation in shell growth rates in the Peartree Point sample. However, a consistent relationship was found in the Prawle Point sample where genotypic variation at the *Lap-2*, *Mdh-1* and *Pep-1* loci coincided with both tissue and shell growth rates, and feeding rates. It is this consistent relationship that suggests that the results for these loci are not statistical anomalies and that growth rates do vary among the scored genotypes. Juveniles collected from Prawle Point that were homozygous for the 10-allele at either the *Lap-2* and *Mdh-1* loci or possessed the 10.11 genotype for the *Pep-1* locus exhibited

1. higher tissue and shell growth rates

2. faster feeding rates and

3. higher growth efficiencies (as a product of feeding and growth rates).

Only one juvenile from Peartree Point was heterozygous for the 9-allele for either the *Lap-2* or *Mdh-1* locus, and so the association between these loci and growth or feeding rate could not be determined for this site. Genotypic variation at the *Pep-1* locus for Peartree Point juveniles was sufficient to identify differences among genotypes, but the lack of correlation for these animals suggests that variation at the *Pep-1* locus either acts as a marker locus or is only partially involved in the observed physiological variation. Kirby (1992) on two separate occasions also identified variation in feeding and growth rates (also active respiration rates) among *Pep-1* genotypes and suggested overdominance at this locus. The results of the current study however

indicate that genotypic variation at this locus is insufficient to explain the associated growth and feeding rate variation seen for Prawle Point whelks.

CHAPTER FOUR:

The effects of temperature and desiccation stress upon the physiology of laboratory-reared dog-whelks

4.1 INTRODUCTION

Variation in air temperature between sites has been suggested as a possible reason why *Nucella lapillus* varies in its genetic composition (karyotype, allelic variation and mitochondrial haplotype) and phenotype (shell shape and physiology) along a region of coastline in South Devon (fig. 1) (Kirby, *et al.*,1994 a, b; this study and references therein). Measurements of environmental variables taken in this study (chapter three) indicate that maximum temperatures vary between sites, but the correlation between genotype, phenotype and habitat may be merely coincidental (Clarke, 1978). It is necessary to demonstrate that different genotypes have different phenotypic responses to the environmental variability among sites before an adaptationalist explanation can be postulated to explain the observed phenotypic and genetic distribution. It may be that this variation is only manifested under periods of physiological stress (Rodhouse and Gaffney, 1984; Gentilli and Beaumont, 1988; Koehn and Bayne, 1989).

Variation in temperature and desiccation rates can influence the abundance and distribution of prey species (Dayton, 1971), and so indirectly affect the rate of energy acquisition (Palmer, 1983, 1984). The relative abundance of mussels and barnacles, the preferred prey of dog-whelks, differs between Peartree Point and Prawle Point (McCarter and Thomas, 1980; pers. obs.) and must modify the feeding ecology of dog-whelks at the two sites; very few mussels can be found at Prawle. Temperature also directly affects the behaviour and physiology of whelks (Largen, 1967b; Manzi, 1970; Bayne and Scullard, 1978a, b; Garton and Stickle, 1980). Gradual temperature increases within a critical range (5-20°C) often results in increased feeding behaviour (Bayne and Scullard, 1978b). The effects of temperature upon physiology of marine invertebrates have been well studied (e.g. reviewed by Kinne, 1970 and Newell, 1979), but intraspecific variation in response to temperature is generally investigated in reference to tidal height on the shore (Dayton, 1971; Coombs, 1973a; Underwood, 1979), or seasonal variation (Bayne and Scullard, 1978a, b). Wave action and the risk of predation have generally received more attention in studies on site differences in *Nucella* spp. (see review by Crothers, 1985). Comparatively few studies have attempted to investigate the effects of temperature and desiccation during aerial exposure at different shore sites.

The total time required to search for the preferred type and size of prey, and for the drilling and ingestion stages of the feeding cycle often results in whelks remaining on exposed rock

surfaces during low tide (Menge, 1976; Hughes and Drewett, 1985; Hughes *et al.*, 1992) when the influence of temperature and humidity predominate. High temperature and low humidity are experienced during the summer months when dog-whelks tend to be more active, in part due to the warmer temperatures (Bayne and Scullard, 1978b.). Whelks are generally inactive or have limited movement when emersed (Hull and Evans, 1986). At these times, whelks need to able to tolerate the combined effects of temperature and desiccation, particularly in direct sun. Between feeding bouts, whelks tend to seek the relative shelter afforded by crevices or an algal canopy and so alleviate the effects of severe environmental temperatures (Burrows and Hughes, 1991).

Temperature tolerance has been suggested to account for the geographical distribution of this species, which occurs between the -1 and +20°C isotherms (oceanic seawater temperatures: Moore, 1936). Dog-whelks cease to feed at water temperatures exceeding 20°C (Bayne and Scullard, 1978b) and a further increase in temperature results in a progressive reduction of movement with ultimate heat coma at 28°C and death at seawater temperatures of 33°C (Sandison, 1967) or even 40°C (Evans, 1948). The generally higher lethal temperature in air (36°C, Sandison, 1967) is likely to be due to the ameliorating effect of evaporative cooling of the mantle cavity fluid retained during emersion (Boyle *et al.*, 1979). The discrepancy in these critical temperatures may be due to differences in the criteria used to determine the point of death, whether temperatures were constant or gradually increased (Fraenkel, 1960) or the exposure duration. The upper critical temperatures, and those measured in chapter three, were taken into consideration in designing the experiments presented in the following chapter.

Reciprocal transplant experiments have been successful in studies investigating site-specific differences in physiology (e.g. Burrows and Hughes, 1990; Kautsky *et al.*, 1990). However, there are difficulties in interpreting results from this type of study; if differences in physiology exist between samples, are these differences directly due to the effects of transplantation? Whelks are often quiescent for some time after disturbance particularly if feeding has been interrupted. Transplanted whelks also tend to remain in the crevices to which they were introduced (pers. obs.: introduction to crevices increases the likelihood that whelks will attach to the substratum

before the tide rises). Several days may therefore elapse before feeding resumes. Site-differences in physiology could also be due to prior environmental differences including previous feeding rates and prey-type availability, eg. dog-whelks tend to continue feeding upon a particular prey type (Hughes and Dunkin, 1984b). Transplant experiments often involve transferring individuals from exposed to sheltered sites and vice versa, which are known to vary in their relative abundance of mussels and barnacles (section 3.1 and references therein.). A delay in feeding (and therefore growth) would perhaps be expected for transplanted whelks until they 'learn' the new prey type (Dunkin and Hughes, 1984); an acclimation period in the novel environment should be permitted before recording measurements. However, for similar reasons to those suggested by Bernado (1994) for laboratory studies, selection in the 'field' may eliminate those extreme forms which contribute to the population differentiation.

Reciprocal transplant experiments are preferable in that the complex interactions among environmental variables influence the observed phenotype, however it is this complexity which causes difficulty in assigning which environmental variables are important. For the above reasons this approach was therefore not considered ideal in the present study. It is also possible that introduced individuals may have survived to maturity, reproduced and so altered the native genetic composition of the sample sites, affecting future work in this region. Caged experiments can prevent loss of individuals, but are difficult to erect in wave-swept environments and can influence the results obtained (Edwards *et al.*, 1982) particularly if the effects of temperature and wave action are to be investigated; the cage may act as a partial barrier to incident sun and waves.

In the present chapter, samples of dog-whelks have been studied to identify whether juveniles reared from Peartree Point and Prawle Point (fig. 3.1), differ in their physiological response to temperature, humidity and food availability. Phenotypic variation among genotypes can be revealed in the laboratory by standardising the environmental component to phenotypic variation. The physiological response of laboratory-reared juveniles to controlled manipulation of laboratory conditions can provide an insight into 'why is what where?' (Berry, 1989) for *N. lapillus*. This 'horizontal' approach, the study of geographically separated populations, is not as satisfactory as selection experiments (Berry, 1977) identifying which allele frequencies and phenotypes alter over several generations under certain environmental conditions. However, *N.*

lapillus has relatively long generation times of ca. two-three years (Feare, 1970b; Hughes, 1972) rendering this latter approach outside the time constraint imposed on this study. The first series of experiments aimed to distinguish population differences in physiology at different temperatures and food availability. Juveniles were continually immersed throughout these experiments, and so avoided the complicating effects of desiccation and salinity stress; the upper temperature limit was set at 20°C. Although this exposure temperature has been shown to result in maximal feeding rates (Bayne and Scullard, 1978b), heat stress has also been reported at this temperature (Stickle *et al.*, 1985b).

The integrated effects of tidal emersion and temperature, humidity and desiccation stress were studied in the second experiment presented here. Tidal simulation experiments on laboratory-reared animals have been used as an alternative approach to reciprocal transplant experiments. Rearing juveniles in the laboratory can provide a large number of small juveniles of approximately equal age with a similar rearing history. Obtaining sufficient numbers of the important critical early life-history stages can be difficult in shore studies (Etter, 1989; chapter 3, this study). The influence of environmental variation in aerial exposure can also be observed without the complicating effects of other shore variables (e.g. prey density, whelk density, wave action, predation, etc.). Air temperature, humidity, and potential desiccation rates, were manipulated in the laboratory. Because of the likely adverse effects of long-term environmental stress upon feeding rates and metabolism, high temperatures (33-34°C) and low humidities (25-33%) were only maintained during the morning low tide. No refuges were provided in these tidal tanks to ensure that all individuals experienced identical conditions. Possible behavioural variation within and between populations may have affected the results. Only juveniles with light coloured shells from each population were used in the following experiments as shell colouration is known to influence the internal temperature (Heath, 1975; Cook & Freeman, 1986; Etter, 1989) and behaviour of snails (Bantock, 1980; Cook, 1986a, b).

4.2 MATERIALS AND METHODS

4.2.1 Temperature experiments

4.2.1.1 Collection of egg capsules and rearing of juveniles

A total of ninety capsules were collected from Peartree Point and Prawle Point (fig. 3.1). From each shore three adjoining capsules, assumed to have been laid by the same female, were collected from 15 separate clusters, which were located over a wide area. Capsules laid by the same female tend to remain attached by a continuous basal disc and so adjoining capsules were recognised to provide 'replicate' capsules from an individual female. The problems of egg capsule inviability, low numbers of hatchlings per capsule and obtaining a large number of capsules, meant that collecting only one capsule per female could have lead to an insufficient number of suitably sized juveniles. These problems were considered more acute at the Prawle site where egg capsules are difficult to find, the number of capsules per cluster is often less than ten and post-hatching mortality is high (section 2.3.3). The sampling strategy outlined above was intended to overcome these problems as it was the aim, *a priori*, that all three capsules for at least ten females would hatch to provide a sufficient number of hatchlings per female at the start of the experiments.

Each capsule was placed in a separate numbered 'teaboy' (plate 2.2.1; Aldridge Plastics Ltd., U.K.) on return to the laboratory and capsules hatched over a three week period approximately two months later. Each capsule was individually reared (section 2.2.1.2) at a constant temperature ($15\pm1^{o}C$) and salinity (35 ± 2ppt). Every two weeks, when the mussel spat was replenished, one half of each 'teaboy' was exchanged for a clean replacement to ensure algae and other possible foulants did not hinder the exchange of water across the mesh. Approximately four months after hatching, all whelk juveniles were labelled with 'beetags', measured for shell length (section 2.2.1.3.1) and transferred to separate population stock tanks. Populations were labelled on alternate days over a four day period and individuals were maintained in these stock tanks for two weeks, which was hoped to overcome the possible family and crowding effects upon growth and feeding rates resulting from rearing in 'teaboys' (Kirby, 1992; chapter two, this study).

4.2.1.2 Experimental design

The experiment started over a two week period to ensure that respiration and excretion rates could be measured for all individuals after five weeks at a particular temperature and feeding ration level. Twelve juveniles from each of ten families per laboratory population were equally divided at random between the six experimental treatments (table 4.2.1). After initial size measurements were obtained (immersed and total weights, shell length and aperture; section 2.2.1.3.1), experimental juveniles were maintained for 24 hours without food in a tank held at an intermediate temperature (17.5 or 13.5°C) until transfer to the final temperature and ration level.

Table 4.2.1: Experimental design to identify population differences in physiology under different temperatures and feeding levels (see main text for details). Whelks were maintained in each treatment for five weeks and numbers provided in this table are the total sample sizes for both populations combined.

	Temperature	
Ration	**12°C**	**20°C**
Ad libitum	n=40	n=40
low	n=40	n=40
starved	n=40	n=40

Animals were maintained at constant temperature (12 or 20±1°C) in flow through tanks; animals kept in 'teaboys' were maintained in one tank per temperature (further details in section 4.2.1.4.3). All tanks were held in one insulated water bath per temperature and seawater inflows were passed through a series of glass coils immersed in the water bath to attain the required temperature. Water was circulated in these water baths to prevent the establishment of any thermal gradients. Daily temperatures were measured with an immersed mercury thermometer. Temperature regulation had previously been monitored in each tank using temperature probes, connected to a calibrated 100mV Rikadenki chart recorder. Any daily and

long term fluctuations were continuously recorded and did not deviate by more than one degree from the required temperature. Salinity was held at 35±2ppt throughout the experiment.

4.2.1.3 Size measurements and growth rates

Shell weights were determined using the Palmer (1982) method, and dry tissue weights estimated from shell length-dry weight regressions (sections 2.2.1.3.1 and 4.3.1.2). Two destructive samples were removed per population, one at the start and the other after two weeks, to determine if the regression coefficients changed during the two weeks over which the experiment started. Measurements of shell and tissue weights for all experimental animals were then estimated at the start of the experiment using the appropriate population equation (section 4.3.1.2).

At the end of five weeks and immediately after respiration and excretion rates had been determined (section 4.2.1.5), juveniles were frozen at -70°C to be dissected at a later date. Shell, digestive gland complex and foot tissues were freeze-dried separately and the foot tissue homogenised for electrophoresis (section 2.2.1.4). The dry weights of body tissue (digestive gland and foot weights combined) and shell attained at this point and those estimated at the start of the experiment were used to calculate growth rates over the five week experiment. Population growth rates using modified Ford-Walford plots (section 2.2.4) were compared within and between temperatures and ration levels. In these analyses, the relationship between shell or tissue weight increase and initial tissue weight was compared among treatments and populations; statistically significant variation in the intercept value indicates growth difference among the classes tested. The relative weight of the digestive gland complex to total dry tissue weight was contrasted between populations to provide an indication of any likely differences in allocation of resources to storage/reproduction. For some individuals, namely those in the *ad libitum* feeding rations, the total mass of the digestive gland complex will include a reproductive tissue component.

4.2.1.4 Feeding rates

Small mussels (*Mytilus edulis*; 6-30mm) were collected from Whitsand Bay (O.S. Ref SX390523) and then starved for two weeks in the laboratory. The shell lengths of mussels (13-

18mm) were measured with vernier calipers (±0.05mm) and divided into 1mm size classes which were kept separate in mesh bags. After two weeks in the laboratory, mussels were directly transferred to either one of the experimental temperatures. This was repeated two weeks later for a second collection of mussels. For the first mussel collection, a size range of mussels (6-30mm) was removed every 0, 3, 7 and 14 days after transfer to either temperature to determine if the relative dry weight and organic content varied over this period due to the possible combined influences of temperature and starvation. Only one group of mussels was removed from the second mussel sample, two weeks after collection from Whitsand Bay, for comparison with the previous mussel sample. All mussel samples were stored at -70°C before dissection. The dry tissue weight was determined by freeze-drying and the organic weight taken to be the difference in dry weight before and after placing the mussel tissue in a muffle furnace at 450°C for 4 hours. Any deviations in the relationships between mussel length and either dry tissue or organic weights were accounted for when calculating the juveniles' feeding rates, otherwise any variation in growth rates among whelk samples could possibly be due to variation in the quality of the mussel flesh. Stock and uneaten mussels were replaced after four weeks in the laboratory with mussels from the second collection; the maintenance of mussels without an algal diet increased the correlation coefficient for the length-dry weight relationships, but would be expected to reduce the flesh quality as storage tissue becomes depleted and the shell thickness increases rendering the mussel more difficult to bore (pers.obs.).

4.2.1.4.1 Prey size

Shell shape differences have been associated with Prawle and Peartree Point populations, which would account for the previously observed differences in tissue weight for a given shell length (see chapters two & three). Therefore the size of mussels offered to each individual whelk was determined on the basis of tissue weight, rather than shell length as in previous experiments (see section 2.2.1.3.2). Initial dry weights were estimated for the experimental juveniles using the appropriate population length-dry weight regression (section 4.3.1.2) and then reconverted into length units using the dog-whelk length-dry weight equation of Bayne and Scullard (1978b). This 'new' length was then used to estimate the preferred prey size

(section 2.2.1.3.2; mussel length range 13-18mm); the mussel size offered to the whelks was within 1mm of this estimated mussel length. Two weeks after the start of the experiment, whelk juveniles were remeasured for shell length, which was then used to adjust the mussel size offered as prey.

4.2.1.4.2 Feeding rations

The following feeding regimes were upheld over the five week experiment;

Starved juveniles were held without food in 'teaboys', which had been divided in two by positioning ca. 0.25mm^2 net between the two halves of the 'teaboy' to avoid cannibalism and halve the space required to maintain this number of individuals.

Low ration juveniles were provided with one mussel (size determined in section 4.2.1.4.1) per week and housed in 'teaboy's divided in an identical manner to that described above for starved individuals and for similar reasons. Eaten mussels were measured every week.

Ad libitum juveniles were maintained at a predator:prey density of 1:3 within separate feeding pots (section 2.2.1.3.2). Mussel size was determined using the methods outlined in section 4.2.1.4.1. Eaten mussels were measured daily with vernier calipers (±0.05mm) and replaced at this time to restore the constant predator:prey ratio. Uneaten mussels were completely replaced every two weeks with a new stock of animals to avoid mussels being kept longer than two weeks without food at either of the experimental temperatures (section 4.2.1.4.1).

4.2.1.5 Respiration and excretion rates

At the end of five weeks, respiration and excretion rates were determined consecutively.

4.2.1.5.1 Respiration rates

A different method of determining respiration rates to that outlined in section 2.2.1.3.3 was employed for this series of experiments to enable a greater number of animals to be measured simultaneously.

Measurements were carried out in 100ml glass syringes (Weber Scientific International Ltd., U.K.), which can easily be sealed by inserting the attached syringe needles into silicone bungs. Whelks can be housed in swimfeeders (commonly used by freshwater anglers to contain live bait) to prevent their attachment to the syringe barrel or over the syringe tip, both of which would make sampling difficult and affect the readings obtained. This apparatus had previously been tested and shown to be impervious to the diffusion of oxygen as the partial pressure of oxygen (PO_2) declined within the syringe; a possible route for oxygen diffusion was along the barrel or at the junction of the syringe tip and needle. Seawater salinity was held constant (35±2ppt) for all measurements of respiration rate. The PO_2 of fully oxygen saturated seawater was calculated each day according to the daily air pressure and experimental temperature:

$$\text{mmHg } O_2 = [\text{air pressure (mmHg)} - (\text{S } (0.03 \times \text{temperature}^2))] \times 0.2095$$

where 0.03 is the approximate ionic concentration of 35ppt filtered seawater (FSW), which does not vary with temperature. The solubility of oxygen, S, at 35ppt, was taken to be 6.05mlO_2/l at 12°C and 5.17 mlO_2/l at 20°C. The proportion of oxygen present in air was 0.2095. The oxygen electrodes and meters were calibrated each day and previous checks confirmed that temporal fluctuations in the PO_2 for fully oxygen saturated FSW were not significant over nine hours during which respiration rates were measured.

Ten animals were measured simultaneously. Controls were not considered necessary as previous recordings of syringes set up in an identical manner minus experimental animals, had shown that the PO_2 did not change over a time period in excess of that needed to determine respiration rates. At least 20 minutes elapsed after adding the juveniles to the syringes before readings commenced in order to reduce the effects of disturbance, which would be expected to temporarily increase the respiration rates. Syringes were mixed by gently inverting the syringe twice before replicate 2ml samples (sufficient volume to completely flush the cell) were delivered into a thermostatted cell (model DS66014, Radiometer Ltd., UK) containing an oxygen electrode (model E5046, Radiometer Ltd., UK). Readings, recorded from a Strathkelvin (model 781) meter connected to the electrode, were recorded three minutes after injecting a sample when readings had stabilised. The total sample volume delivered at each time point was

4ml. Syringes were carefully resealed to avoid introducing air bubbles after obtaining the two replicate readings. Two syringes were sampled at one time as two thermostatted cells were available but consecutive readings for each syringe were recorded from the same electrode.

At least three readings per individual were obtained and the total time over which these readings were taken was reduced for those individuals with high respiration rates to ensure the PO_2 did not fall below 110 mmHg ie.~70% oxygen saturation. For starved juveniles, five readings were taken due to their low respiration rates and to further increase the accuracy of these readings, the total initial volume within the syringe was 80ml, which therefore elevated the rate of oxygen depletion. Immediately after measuring respiration rates, excretion rates were measured for all individuals (section 4.2.1.5.2).

Respiration rates ($\mu lO_2/g/min$) were calculated (equation in section 2.2.1.3.3) and an average rate determined for each individual. Animal volumes were estimated according to juvenile length using data collected in chapter two:

$\log_{10}$ whelk volume (ml) = (3.763 * $\log_{10}$ whelk shell length (mm)) -5.203

Volume was measured as the volume of seawater displaced by placing a whelk in a 50ml measuring cylinder, not the internal volume of the mantle cavity. Whelk volumes were subtracted from the syringe volume, which was corrected after taking each sample (2x2ml). The initial volume of the syringe was determined by weighing each syringe when empty, and then reweighing after filling with distilled water; the difference in weight was used to estimate volume assuming 1ml distilled water approximates 1g in mass. Respiration rates were standardised to a dry tissue weight of 100mg using a b-exponent of 0.575 (section 2.2.1.3.3)

4.2.1.5.2 Excretion rates

Juveniles were kept within the swimfeeders used during respiration rate determinations (section 4.2.1.5.1) and were transferred directly from the respirometry syringes to separate 180ml capacity beakers. During transfer, absorbant tissue was used to remove most of the excess water retained in the swimfeeder to reduce seawater carryover; any residual water still remaining was not considered to significantly (<5%) affect the volume of filtered seawater

(FSW) within each beaker. The swimfeeders ensured that each juvenile did not crawl above the seawater level and affect the results. Each beaker contained either 150, 100 or 60ml FSW depending on whether the juveniles had previously been fed on either the *ad libitum*, low or starved rations respectively. Seawater was thoroughly mixed before removing two replicate samples (2x5ml) immediately after the juveniles had been introduced to the beakers and again after 120 minutes for the determination of ammonia concentrations. Primary amine excretion was not determined. Two control beakers without animals were also sampled each time a group of juveniles were measured for excretion rate.

Procedures of ammonia analysis followed the Solorzano's (1969) method using ammonium chloride as the standard. All sample tubes were placed in a muffle furnace at 450°C for at least four hours and all other glassware was acid washed with 10% hydrochloric acid to remove organic residues before use. Rates of ammonia excretion were standardised (see equation in section 2.2.1.3.3 for respiration rates) to a dry tissue weight of 100mg using a b-exponent (the gradient in the correlation between animal size and excretion rate) of 0.6 (Stickle and Bayne, 1982).

4.2.2 Tidal experiments

4.2.2.1 Establishment and rearing of laboratory populations

In March 1994, three adjoining egg capsules per cluster were collected from in excess of thirty clusters per site following the methods described in section 4.2.1.1. Egg capsule clusters were sampled from a large area per site to ensure representative population samples and were maintained non-tidally on return to the laboratory in separate 'teaboys' (plate 2.2.1). All egg capsules were kept in the laboratory until non-viable capsules could be distinguished. At this time, two viable capsules were chosen at random from each female and any excess capsules discarded. The date of hatching was recorded for the remaining capsules and hatched capsule cases kept until all the hatchlings had emerged (section 2.2.3). Mussel spat was introduced to a 'teaboy' immediately after the capsule had hatched (section 2.2.1.2) and juveniles had been counted; this was the only time food was added to the 'teaboys' during the first month following hatching.

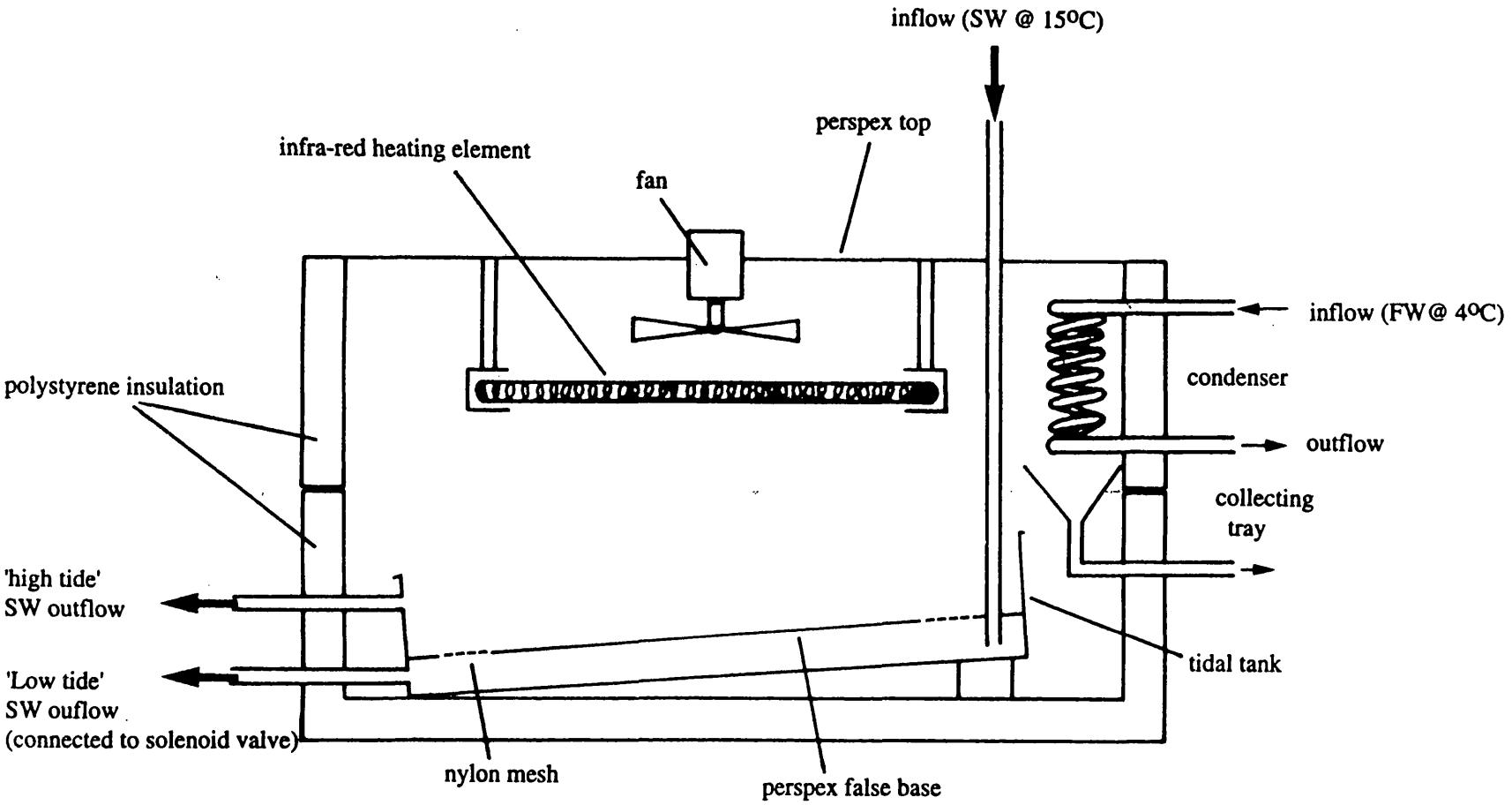
One month after hatching, all hatchlings were transferred from the 'teaboys' into one of two tidal tanks (one for each population) containing an abundant supply of mussel spat; low water was scheduled between 06.00-10.00 and 18.00-22.00 BST. At this time, no control over ambient temperature or humidity was attempted during low tide, but populations were maintained under identical conditions. Juveniles surviving from either 41 (Peartree Point) or 35 (Prawle Point) capsules, which had each been laid by twenty females per site, were transferred to the tidal tanks as none or only one capsule was viable for some females. The two populations were reared separately with periodic additions of mussel spat to the tidal tanks until 3-4 months after hatching when the experiment commenced; juveniles were considered to have attained a large enough size at this age to avoid damage whilst handling.

4.2.2.2 Experimental equipment

Two tidal systems were designed to expose juvenile whelks to either high temperature and low humidity ($33\pm1^{o}C$; $28\pm4\%$) conditions or low temperature and high humidity ($17\pm1^{o}C$; $85\pm8\%$) during the morning 'low tide' (fig. 4.2.1). In the evening, both tidal tanks were at similar ambient air temperatures and humidities during low tide; high temperatures and low humidities were not maintained at these times due to likely adverse effects of extended periods of stress. The timing of the diurnal tidal cycle was identical to the rearing period (section 4.2.2.1) and the seawater temperature and salinity during 'high tide' was held constant at $15\pm1^{o}C$ and 35 ± 2ppt respectively. The times of low tide were to reduce the likely influences of ambient air temperatures on temperature control when low temperatures were required.

Temperature variation over the high temperature emersion period was continuously monitored by a probe positioned centrally within the tidal tank. The temperature probe was calibrated and coupled to a 100mV Rikadenki chart recorder and a relay switch, which achieved temperature regulation by switching the electricity supply to the infra-red heating elements on or off as required (fig. 4.2.1). Air temperatures within a low tide were within $1^{o}C$ of the mean, which varied between 32 and $34^{o}C$ over the course of the experiment. A calibrated humidity probe also

Figure 4.2.1 Tidal system used to regulate high temperature (32-34±1°C) and low humidity (28±4%) during the morning low tide; the low temperature (15-17±1°C) and high humidity (85±8%) tidal set-up was similar, but without the fan, heating elements or 'de-humidifier'. SW - seawater, FW - freshwater


inflow (SW @ 15°C)
perspex top
infra-red heating element
fan
inflow (FW@ 4°C)
condenser
outflow
collecting
tray
polystyrene insulation
'high tide'
SW outflow
'Low tide'
SW ouflow
(connected to solenoid valve)
tidal tank
nylon mesh
perspex false base

connected to the Rikadenki chart recorder, continuously recorded the relative humidity and air temperature. Maximum temperature was achieved after ca.15 minutes, whereas the relative humidity declined exponentially to attain minimum values ca. 2-3 hours after the heating elements had been switched on. The humidity would have increased when high air temperatures were maintained due to evaporation of seawater from the tanks and animals, therefore condensers (glass coils circulating water at approximately 4°C) were positioned inside the polystyrene box to enable a relative humidity as low as 25% to be achieved, which was similar to shore measurements taken at the Prawle Point sample site (chapter three). Water condensing on these coils was allowed to drain out of the tidal apparatus to avoid re-evaporation and high humidity. The electric fan helped prevent the establishment of any temperature or humidity gradients.

The cool temperature tidal system was similar in basic design to the high temperature set-up (fig. 4.2.1). The required temperature was often below ambient, and so a fan was not included that would tend to recirculate warmer air and reduce the relative humidity. Heating elements and condenser unit were also not required in this design. Measurements taken at the end of the low tide with a thermohygrometer (Analog and Numeric Devices Ltd., Leicestershire, UK.) indicated that the air temperature in this system did not exceed 18°C during the morning low tide.

4.2.2.3 Response to elevated temperature and reduced humidity during tidal emersion

4.2.2.3.1 Experimental samples of juveniles

Twenty five juveniles (shell lengths 6-18mm) from each population stock tank were sacrificed to obtain length-dry weight regressions for shell and tissue weight for each population at the start of the experiment. These relationships were then used to estimate initial shell and tissue weights from shell length measurements for experimental animals.

Two samples of thirty juveniles were selected from each population on the basis of shell length to provide a size range of 10-16 mm shell length. At the time shell measurements were recorded, juveniles were marked with a plastic beetag and enamel paint (Humbrol Paint, UK.),

the colour of which was population specific. The paint was used to replicate the number of the whelk as large as possible without interfering with the shell growth; the large number helped identify the whelk without disturbing the animal which would interfere with the feeding pattern. One sample per population was placed in each of the hot or cool tidal system (section 4.2.2.2).

4.2.2.3.2 Feeding rates

Mussel (*Mytilus edulis*) spat was collected from Whitsand Bay (O.S.Ref. SX390523) and maintained in the laboratory without an algal diet for two weeks before being divided between two tidal holding tanks maintained at the same temperatures as the experimental whelks (fig. 4.2.1). After four weeks in the laboratory, all mussels were replaced with mussels collected two weeks after the first collection of mussels; these later mussels were also maintained for two weeks in the laboratory before being used as the replacement food source for the experimental whelks.

On the basis of length-dry weight relationships, the sizes of mussels fed to the whelks were calculated in a similar manner to the method described previously (section 4.2.1.4). In this way, prey size was determined on a dry weight rather than on shell length basis to account for differences in shell shape and relative shell length v. dry weight regressions between laboratory populations of whelk juveniles (chapters 2 & 3, this study). Three mussels of each preferred size (±1mm; total n=180/tank; mussel length 12-16mm) were positioned in the tidal tanks on clear perspex strips which were perpendicular to the base. Each strip was marked with a separate letter and 15 positions were marked along the upper edge of the perspex. Mussels were measured with Vernier calipers (±0.05mm) and recorded before being attached to the perspex using cyanoacrylate glue ('superglue') taking care not to glue the two valves closed. Mussels did not appear to be adversely affected by this method of attachment and very few became detached during the experiment. This 'grid' enabled the size of the mussel to be determined without interfering with the feeding behaviour of the whelk juveniles. During each low tide at 09.00 and 18.00 BST, the location of each feeding whelk was recorded and any eaten mussels

replaced with one of a similar size from the holding tank. Any uneaten mussels were removed the day before the end of the experiment.

Samples of thirty mussels (shell length 11-17mm) from the first collection of mussels were sacrificied at 0, 7 and 14 days after being in each tidal system. Shell length-dry weight relationships were calculated and the corresponding organic content, which was determined as the difference in weight before and after placing mussel tissue in a muffle furnace at 450°C for 4 hours. Total mussel tissue weight consumed per juvenile over the four weeks was calculated, taking into consideration any variation in dry tissue/organic weight variation with tidal treatment and time.

4.3 RESULTS

4.3.1 Temperature experiments

4.3.1.1 Genetic variation

Fisher's exact method was used to test for variation in genotype frequencies between and within population samples. Significant population variation ($p<0.01$) in genotype frequencies at the *Lap-2* and *Mdh-1* loci was found between samples for each temperature and ration combination (table 4.3.1); for both loci, the 9-allele was more common in the Prawle Point samples than those sampled from the Peartree Point population (see tables 4.3.2 and 4.3.3). Allelic variation at the *Pep-1* locus was statistically different ($p<0.05$) between population samples held at 20°C and in the low ration at 12°C (table 4.3.1). The frequency of *Pgm-1* genotypes also varied between populations in the *ad libitum* ration at 20°C and the starved ration at 12°C.

Preliminary comparisons within populations had shown that for each population, samples subjected to the different temperatures and food rations had similar genotype frequencies ($p>0.3$; table 4.3.4).

4.3.1.2 Size analysis

The coefficients in the regressions relating shell dry weight to immersed weight did not differ between samples taken at the start and end of the two week period over which the experiment commenced for either Peartree Point ($F_{1,53} = 2.83$, $p=0.0984$) or Prawle Point ($F_{1,54} = 0.36$, $p=0.5521$) whelks. Similarly, the relationship between length and dry tissue weight was also invariant between the two samples (Peartree Point, $F_{1,53} = 0.01$, $p=0.9434$; Prawle Point, $F_{1,55} = 0.02$, $p=0.8917$). Data from the two time samples were therefore pooled for each population separately before the following analysis of covariance for variation between the two populations was undertaken. The coefficients given below are mean values $\pm$ 2SE:

Immersed-shell weight regression: Laboratory populations did not vary in the relationship between immersed and shell weight ($F_{1,110} = 1.75$, $p=0.1891$):

Dry Shell Weight (g) = (1.3992 $\pm$ 0.0296 * Immersed Weight (g)) + (0.0192 $\pm$ 0.0061)

Table 4.3.1 Fisher's exact tests for population differences in genotype frequencies among each ration and temperature combination. Probability values in large bold type are significant at the 5% significance level.

		12°C	20°C
Ad lib.	*Lap-2*	**$3.35e^{-9}$**	**$3.34e^{-6}$**
	Mdh-1	**$3.05e^{-10}$**	**$5.80e^{-10}$**
	Pep-1	0.23	**$5.72e^{-3}$**
	Pgm-1	0.154	**$8.32e^{-3}$**
	Pgm-2	0.329	0.067
	Mpi	0.050	0.374
Low	*Lap-2*	**$5.82e^{-8}$**	**$3.05e^{-10}$**
	Mdh-1	**$3.05e^{-10}$**	**$5.82e^{-9}$**
	Pep-1	**0.023**	**$8.32e^{-3}$**
	Pgm-1	0.127	0.091
	Pgm-2	0.523	0.868
	Mpi	0.563	0.528
Starved	*Lap-2*	**$7.71e^{-7}$**	**$3.35e^{-9}$**
	Mdh-1	**$1.45e^{-11}$**	**$3.35e^{-9}$**
	Pep-1	1.000	0.057
	Pgm-1	**$1.23e^{-3}$**	0.273
	Pgm-2	0.096	0.258
	Mpi	0.458	1.000

Table 4.3.2 Peartree Point genotype frequencies in each ration and temperature sample

		12°C					20°C				
		9.9	9.10	10.10	10.11	11.11	9.9	9.10	10.10	10.11	11.11
Ad lib.	***Lap-2***	0	0	1.0	-	-	0	0	1.0	-	-
	Mdh-1	0	0	1.0	0	-	0	0	0.9	0.10	-
	Pep-1	-	-	0.60	0.30	0.10	-	-	0.55	0.15	0.30
	Pgm-1	0.10	0.25	0.65	-	-	0.05	0.30	0.65	-	-
	Pgm-2	0.05	0.40	0.45	0.10	-	0	0.50	0.45	0.05	-
	Mpi	0.35	0.50	0.15	-	-	0.35	0.45	0.20	-	-
Low	***Lap-2***	0	0.05	0.95	-	-	0	0	1.0	-	-
	Mdh-1	0	0.05	0.90	0.05	-	0	0.05	0.90	0.05	-
	Pep-1	-	-	0.60	0.30	0.10	0.20	0.15	0.65	0.15	0.20
	Pgm-1	0	0.35	0.65	-	-	0.05	0.25	0.70	-	-
	Pgm-2	0	0.5	0.5	-	-	0.05	0.35	0.55	0.05	-
	Mpi	0.35	0.40	0.25	-	-	0.40	0.40	0.20	-	-
Starved	***Lap-2***	0	0	1.0	-	-	0	0	1.0	-	-
	Mdh-1	0	0	0.95	0.05	-	0	0	0.95	0.05	-
	Pep-1	-	-	0.75	0.20	0.05	-	-	0.65	0.10	0.25
	Pgm-1	0	0.45	0.55	-	-	0.05	0.30	0.65	-	-
	Pgm-2	0	0.50	0.50	0	-	0	0.65	0.30	0.05	-
	Mpi	0.35	0.45	0.20	-	-	0.20	0.50	0.30	-	-

Table 4.3.3 Prawle Point genotype frequencies in each ration and temperature sample

		12°C					20°C				
		9.9	9.10	10.10	10.11	11.11	9.9	9.10	10.10		10.11
Ad lib.	*Lap-2*	0.25	0.65	0.10	-	-	0.30	0.40	0.30	-	-
	Mdh-1	0.50	0.45	0.05	-	-	0.60	0.35	0.05	-	-
	Pep-1	-	-	0.95	0.05	0	-	-	0.95	0.05	0
	Pgm-1	0	0.10	0.90	-	-	0	0	1.0	-	-
	Pgm-2	0.05	0.25	0.70	-	-	0.05	0.20	0.75	-	-
	Mpi	0.10	0.45	0.45	-	-	0.20	0.40	0.40	-	-
Low	*Lap-2*	0.15	0.75	0.10	-	-	0.15	0.80	0.05	-	-
	Mdh-1	0.40	0.60	0	-	-	0.50	0.45	0.05	-	-
	Pep-1	-	-	0.95	0.05	0	-	-	1.0	0	0
	Pgm-1	0	0.10	0.90	-	-	0	0.05	0.95	-	-
	Pgm-2	0	0.35	0.65	-	-	0.05	0.45	0.50	-	-
	Mpi	0.25	0.30	0.45	-	-	0.25	0.40	0.35	-	-
Starved	*Lap-2*	0.15	0.60	0.25	-	-	0.15	0.75	0.10	-	-
	Mdh-1	0.50	0.50	0	-	-	0.50	0.40	0.10	-	-
	Pep-1	-	-	0.80	0.02	0	-	-	0.90	0.10	0
	Pgm-1	0	0	1.0	-	-	0	0.15	0.85	-	-
	Pgm-2	0.05	0.20	0.75	-	-	0.05	0.45	0.50	-	-
	Mpi	0.15	0.55	0.30	-	-	0.15	0.45	0.35	-	-

Table 4.3.4 Genotypic variation among treatments for Peartree Point and Prawle Point samples. Fisher's exact method was used as some cell counts were less than five. No significant differences ($p>0.3$).

	Probability level	
Locus	Peartree Pt.	Prawle Pt.
Lap-2	1.000	0.319
Mdh-1	0.926	0.874
Pep-1	0.481	0.324
Pgm-1	0.900	0.404
Pgm-2	0.736	0.570
Mpi	0.980	0.930

Length-dry weight regression:

Population differences in the relationship between length and dry weight were identified by difference in the gradient as indicated by the interaction term in the analysis of covariance ($F_{1,111}$ = 226.36, p=0.0001). For a given shell length, Peartree Point juveniles have a heavier tissue mass:

Peartree Point

Log_{10} Dry Tissue Weight (g) = (3.4294 $\pm$ 0.1253 * Log_{10} Length (mm)) - (5.1773 $\pm$ 0.1463)

Prawle Point

Log_{10} Dry Tissue Weight (g) = (3.2630 $\pm$ 0.1208 * Log_{10} Length (mm)) - (5.1773 $\pm$ 0.1463)

Relative tissue weight: It was interesting to note that the relationship between the estimated wet tissue weight (total whelk weight minus shell weight) and the actual dry tissue weight was statistically different between populations ($F_{1,111}$ = 4.10, p=0.0452); for a given dry tissue weight, the wet tissue weight is greater for Prawle Point juveniles than that measured for individuals reared from Peartree Point.

4.3.1.3 Shell shape analysis

The ratio between shell length and aperture, which was used as an indication of shell shape, was compared among populations and temperatures for the *ad libitum* ration at the start and end of the experiment. There were no differences between populations ($F_{1,76}$ = 3.19, p=0.0782) or temperatures ($F_{1,76}$ = 0.14, p=0.7127) at the start of the experiment. After the five week experiment, temperature effects upon the relationship were not statistically significant at the 5% level ($F_{1,76}$ = 1.90, p=0.1720), but population differences were evident ($F_{1,76}$ = 6.25, p=0.0146); for a given shell length, Peartree Point juveniles had a longer shell aperture than juveniles reared from Prawle Point. The population differences at the end of the experiment suggested differences in the allometry of growth; Peartree Point whelks had a relatively greater increase in shell aperture length for a given increase in total shell length than Prawle Point whelks ($F_{1,76}$ = 15.98, p=0.0001).

4.3.1.4 Mussel length-dry weight relationships

The following analyses were intended to identify any possible differences in the relationship between shell length and either dry tissue weight or organic weight among mussel samples maintained for up to two weeks at either 12 or 20°C; any differences may be attributed to temperature effects upon maintenance metabolism and tissue weight loss during starvation. Analysis of covariance indicated that within each temperature regime, the shell length-dry tissue or organic weight regressions were similar among mussel samples over the first seven days. The length-dry weight relationships for the pooled 0-7 day samples were invariant to the day 14 sample at 12°C ($F_{1,99}$ = 2.965, p=0.1066); mussel samples maintained at 12°C were therefore pooled before calculating the regression coefficients (table 4.3.5a). However, at 20°C pooled 0-7 day samples were statistically different to the regression line obtained on day 14 ($F_{1,91}$ = 10.38, p=0.0018); coefficients used for the 20°C samples for the period 24-29 August were calculated by interpolating coefficients obtained for the preceding 0-7 day period (16-23 August) with those obtained for the day 14 sample on the 30 August (table 4.3.5).

The two collections of mussels from the shore (section 4.2.1.4) had significant differences in intercepts ($F_{1,46}$ = 7.75, p=0.008) and gradients ($F_{1,46}$ = 6.70, p=0.013) calculated after each sample had been kept in the laboratory for two weeks. As a time course for the effects of temperature was taken only once for each temperature using only one of the shore samples of mussels, the percentage variation in the coefficients for the two initial samples was used to estimate the coefficients for mussel samples maintained during the second two week period at either 12 or 20°C (table 4.3.5).

4.3.1.5 Digestive gland complex : foot tissue

The relationship between the digestive gland dry weight and the total dry tissue weight was compared among treatments. Population differences were only found for the low ration held at 20°C (table 4.3.6); Prawle Point juveniles had a relatively heavier digestive gland complex for a given total dry tissue weight than samples reared from Peartree Point.

Table 4.3.5 Coefficients used to estimate mussel dry tissue weight from measured shell length. Standard errors were inestimable for those coefficients estimated by interpolated values and are therefore omitted.

$$\log_{10} \text{dry tissue weight} = (b * \log_{10} \text{shell length (mm)}) + c$$

a) 12°C

Date	Gradient (b)	Intercept (c)
23/8/93-6/9/9	2.4066	-4.5714
7/9/93-20/9/93	2.7292	-4.9803
21/9/93-	2.7292	-4.9803

a) 20°C

Date	Gradient (b)	Intercept (c)
16/8/93-23/8/93	2.5415	-4.7353
30/8/93	2.5415	-4.7970
31/8/93-6/9/93	2.8821	-5.1567
13/9/93	2.8821	-5.2239
14/9/93-0/9/93	2.8821	-5.1567

Table 4.3.6 Analysis of covariance for population differences in the relationship between total dry tissue weight and the weight of the digestive gland complex. Population differences were deemed significant if $p<0.05$ and are indicated by bold type.

	Temperature	
Ration Level	**20 °C**	**12 °C**
ad libitum	$F_{1, 37} = 2.36$, p=0.1331	$F_{1, 37} = 0.0$, p=0.9695
low	**$F_{1, 37} = 7.18$, p=0.0109**	$F_{1, 37} = 1.09$, p=0.3024
starved	$F_{1, 37} = 0.0$, p=0.9813	$F_{1, 36} = 0.78$, p=0.3837

4.3.1.6 Growth rates

All individuals increased in size during the experiment, except those in the starved ration. Juveniles in this ration lost weight and so negative tissue growth rates, which were also taken as the difference between initial and final tissue weights, were changed to positive values for analysis purposes; the logarithmic (base 10) transformation is not possible for negative values. 'Higher' tissue growth rates in the starved ration were therefore indicative of a greater tissue weight loss.

a. Population differentiation in growth rate

Each temperature and ration combination were analysed separately for population differences in tissue (figs. 4.3.1 and 4.3.2) and shell growth rate (figs. 4.3.3 and 4.3.4; table 4.3.7). Statistically significant differences ($p<0.05$; see table 4.3.7) in growth rates are given below; within each of the remaining temperature and ration combinations, shell or tissue growth rates were similar for Peartree Point and Prawle Point juveniles (table 4.3.7).

Tissue Growth: Peartree Point juveniles had higher tissue growth rates in the *ad libitum* and low rations at 12°C, and although also higher in the *ad libitum* ration at 20°C, population differences were only marginally significant at the 5% probability level ($p=0.047$) (table 4.3.7).

Shell Growth: In the 12°C *ad libitum* and 20°C low feeding ration, Prawle Point juveniles deposited more shell than Peartree Point juveniles (table 4.3.7). The population differences in shell growth in the starved ration at 12°C, were inconclusive; analysis of covariance suggested that Peartree Point whelks had higher rates of shell deposition, but the region of non-significance at the 5% probability level was not estimable using the Johnson-Neyman technique. This method was used due to the heterogeneity of regression gradients, i.e. the statistically significant interaction term in the analysis of covariance.

b. Temperature effects upon growth rate

Within each population, seawater temperature affected the shell and tissue growth in all three rations; animals held at 20°C generally had higher shell and tissue growth rates than those maintained at the lower temperature and when starved, juveniles lost more tissue weight at

Figure 4.3.1 Tissue growth at 12°C for Peartree point and Prawle Point juveniles maintained for five weeks at one of three feeding levels. Log_{10} transformed data are presented.

ad libitum: closed black squares= Peartree Point; closed blue circles= Prawle Point
low ration: open green squares= Peartree Point; open red circles= Prawle Point
starved: closed green squares= Peartree Point; closed red circles= Prawle Point

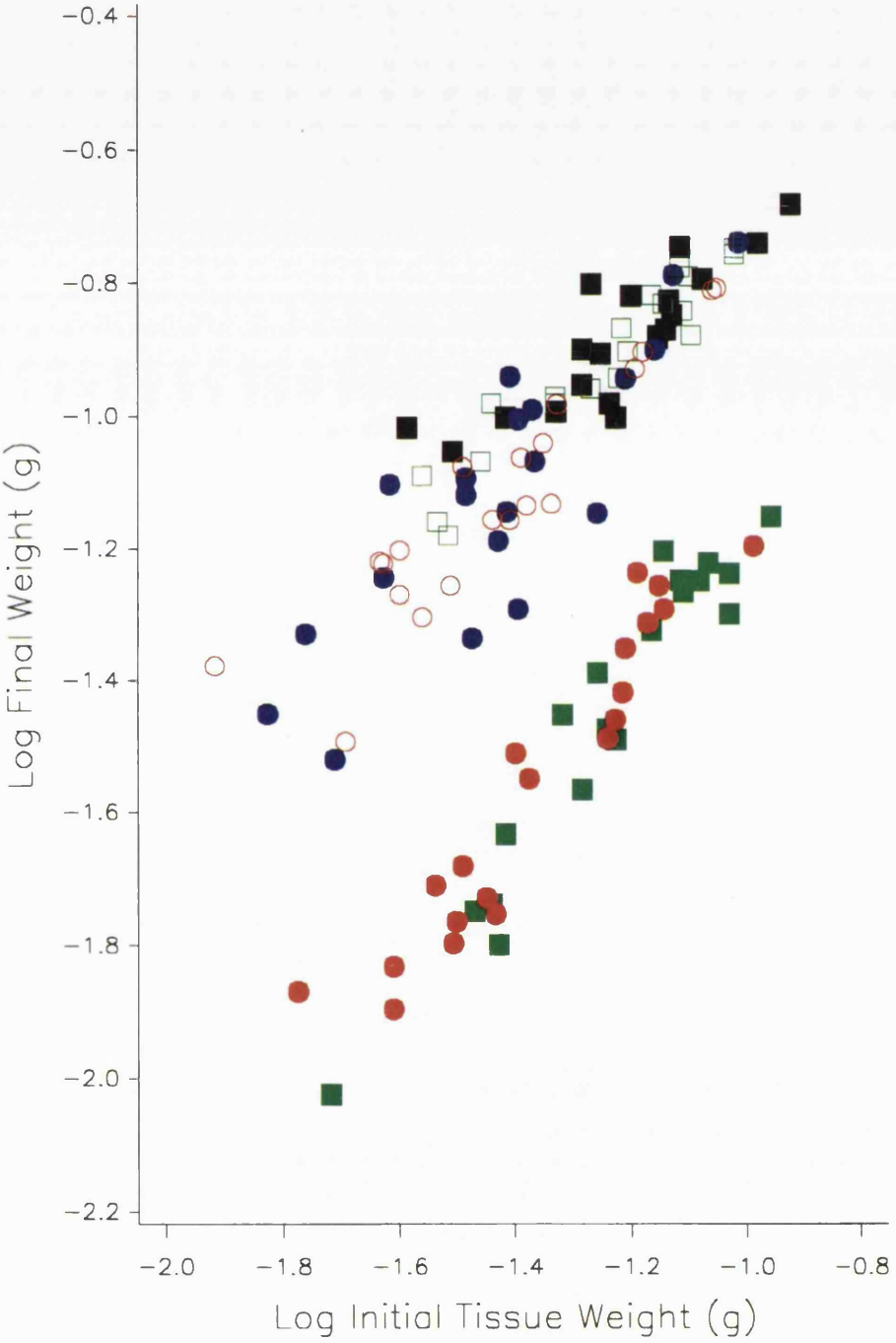

−0.4
−0.6
−0.8
−1.0
−1.2
−1.4
−1.6
−1.8
−2.0
−2.2
Log Final Weight (g)
−2.0
−1.8
−1.6
−1.4
−1.2
−1.0
−0.8
Log Initial Tissue Weight (g)

Figure 4.3.2 Tissue growth at 20°C for Peartree point and Prawle Point juveniles maintained for five weeks at one of three feeding levels. Log_{10} transformed data are presented.

ad libitum: closed black squares= Peartree Point; closed blue circles= Prawle Point
low ration: open green squares= Peartree Point; open red circles= Prawle Point
starved: closed green squares= Peartree Point; closed red circles= Prawle Point

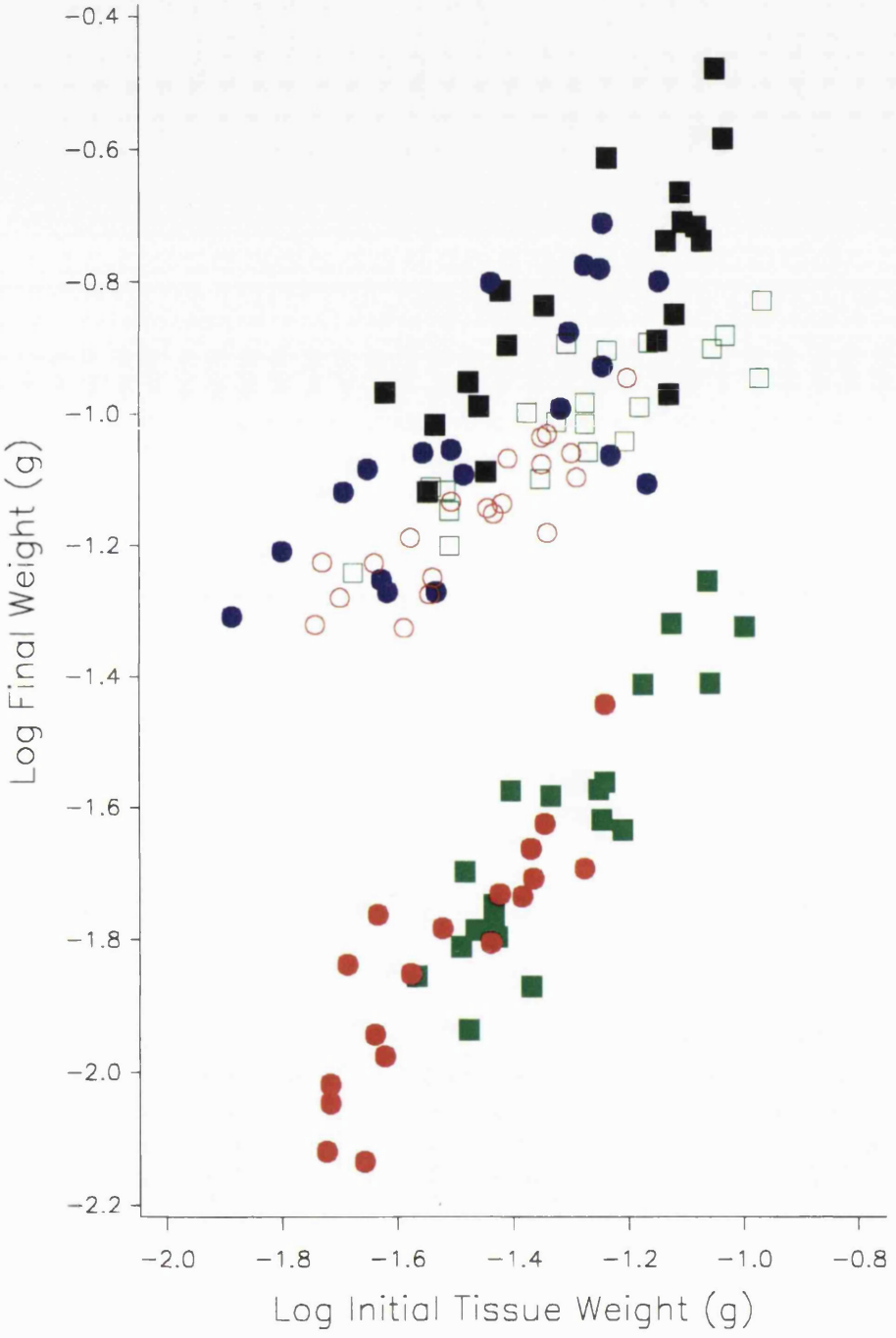

−0.4
−0.6
−0.8
−1.0
−1.2
−1.4
−1.6
−1.8
−2.0
−2.2
Log Final Weight (g)
−2.0
−1.8
−1.6
−1.4
−1.2
−1.0
−0.8
Log Initial Tissue Weight (g)

Figure 4.3.3 Shell growth at 12°C for Peartree point and Prawle Point juveniles maintained for five weeks at one of three feeding levels. Log_{10} transformed data are presented.

ad libitum: closed black squares= Peartree Point; closed blue circles= Prawle Point
low ration: open green squares= Peartree Point; open red circles= Prawle Point
starved: closed green squares= Peartree Point; closed red circles= Prawle Point

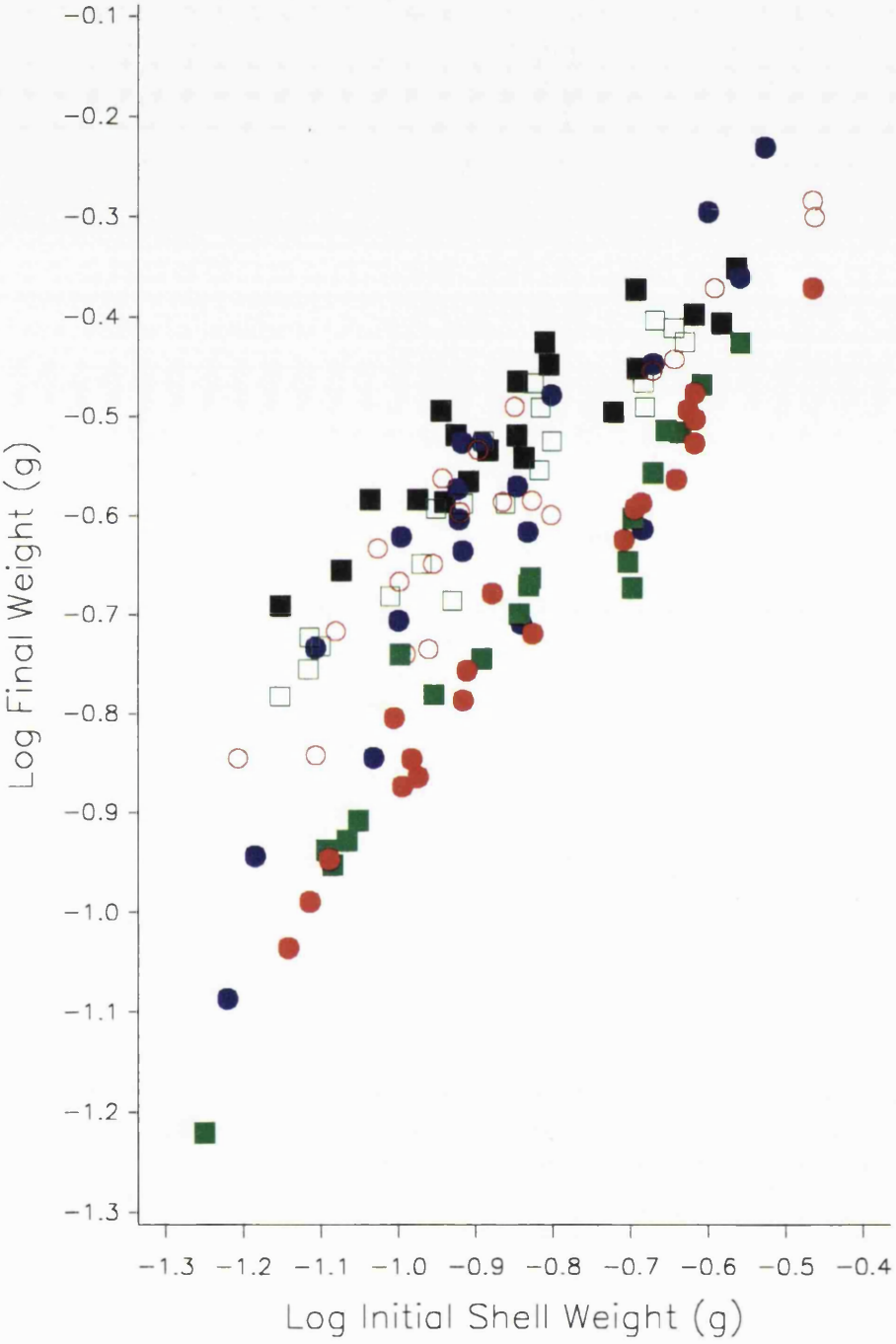

−0.1
−0.2
−0.3
−0.4
−0.5
−0.6
−0.7
−0.8
−0.9
−1.0
−1.1
−1.2
−1.3
Log Final Weight (g)
−1.3 −1.2 −1.1 −1.0 −0.9 −0.8 −0.7 −0.6 −0.5 −0.4
Log Initial Shell Weight (g)

Figure 4.3.4 Shell growth at 20°C for Peartree point and Prawle Point juveniles maintained for five weeks at one of three feeding levels. Log_{10} transformed data are presented.

ad libtium: closed black squares= Peartree Point; closed blue circles= Prawle Point
low ration: open green squares= Peartree Point; open red circles= Prawle Point
starved: closed green squares= Peartree Point; closed red circles= Prawle Point

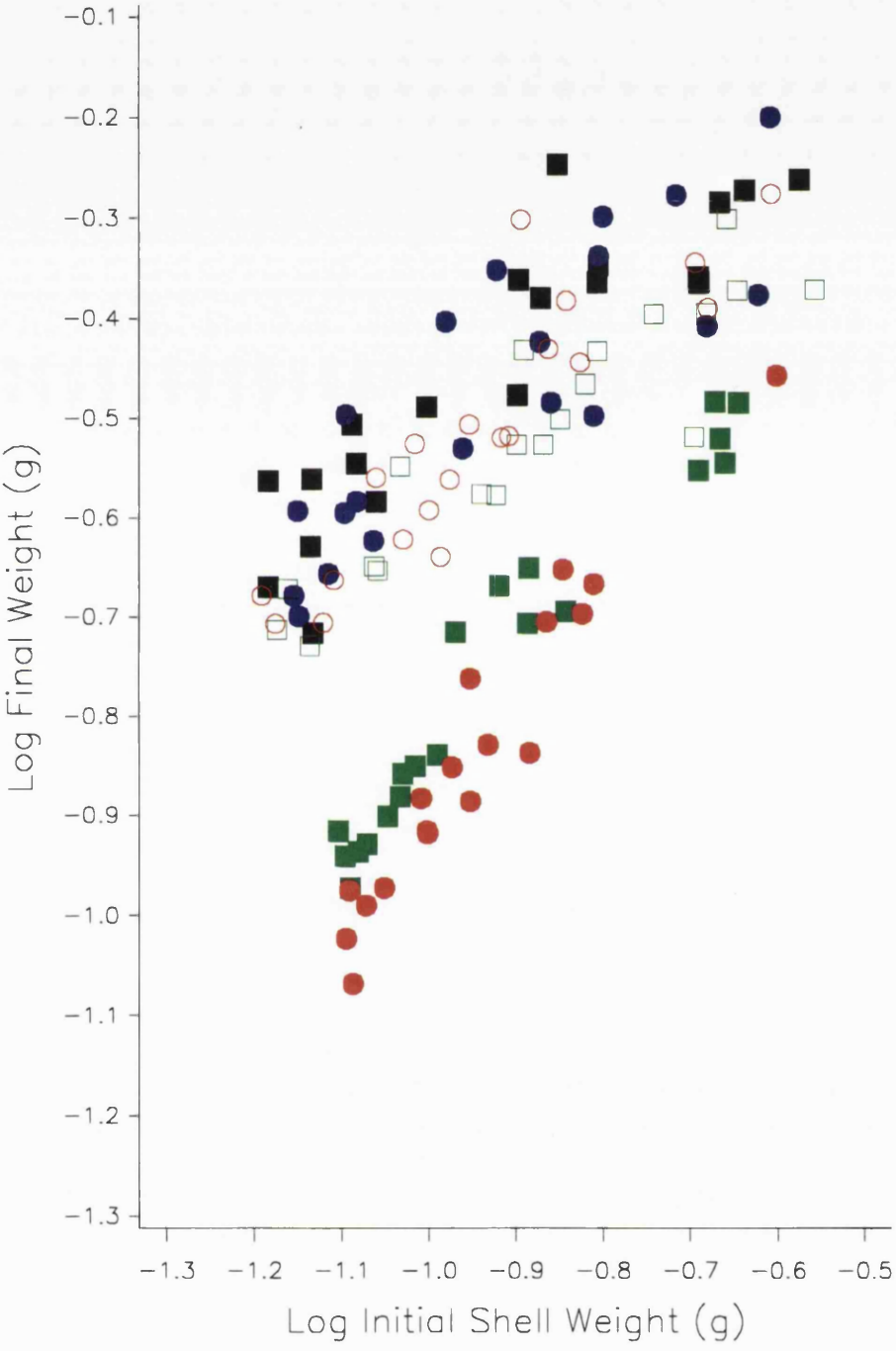

Log Final Weight (g)
−0.1
−0.2
−0.3
−0.4
−0.5
−0.6
−0.7
−0.8
−0.9
−1.0
−1.1
−1.2
−1.3
−1.3
−1.2
−1.1
−1.0
−0.9
−0.8
−0.7
−0.6
−0.5
Log Initial Shell Weight (g)

Table 4.3.7 Summary of the analysis of covariance for population differences in physiology for each ration level (see main text for details) maintained at 12 and 20°C. Statistically significant interaction terms (initial dry tissue weight*population) at the 5% probability level are indicated for shell growth rates. Analysis of variance was used to test for population differences in respiration and ammonia excretion rates. Probability values in bold type are significant at the 5% significance level and the direction of differences indicated. a - Peartree Point, b - Prawle Point. R.G.E.- relative growth efficiency; the analysis of covariance between the total growth in tissue or shell dry weight divided by the initial tissue or shell dry weight respectively, and the total amount eaten.

a) 12°C

	Tissue Growth	**Shell Growth**	**Feeding Rate**
Ad libitum	**$F_{1,37}$=6.03, p=0.019 a>b**	***[1]$F_{1,36}$=5.40, p=0.026 b>a**	$F_{1,37}$=0.00, p=0.978 a=b
Low	**$F_{1,37}$=12.46, p=0.001 a>b**	$F_{1,37}$=1.46, p=0.234 a=b	$F_{1,36}$=0.05, p=0.819 a=b
Starved	$F_{1,36}$=1.51, p=0.227 a=b	***[2]$F_{1,36}$=8.70, p=0.006 a>b**	-

continued...

	Tissue R.G.E.	**Shell R.G.E.**	**Respiration Rate**	**Excretion Rate**
Ad libitum	$F_{1,37}$=0.86, p=0.3591 a=b	$F_{1,37}$=0.23, p=0.6354 a=b	$F_{1,38}$=2.83, p=0.1008 a=b	**$F_{1,36}$=15.81, p=0.0003 a>b**
Low	$F_{1,36}$=1.57, p=0.2184 a=b	**$F_{1,36}$=8.77, p=0.0054 b>a**	**$F_{1,38}$=4.39, p=0.0429 a>b**	$F_{1,38}$=0.41, p=0.5238 a=b
Starved	-	-	$F_{1,37}$=0.11, p=0.7372 a=b	$F_{1,37}$=0.23, p=0.6346 a=b

*[1] the interaction term, $F_{1,36}$=6.35, p=0.0163, b>a

*[2] only the interaction term only is presented here, population as a main effect was not significant (p>0.05). Johnson-Neyman analysis was unable to identify the region of non-significance.

Table 4.3.7 continued ...

b) 20°C

	Tissue Growth	Shell Growth	Feeding Rate
Ad libitum	**$F_{1,37}$=4.22, p=0.047 a>b**	$F_{1,37}$=1.12,=0.295 a=b	$F_{1,37}$=4.06, p=0.0512 a=b
Low	$F_{1,37}$=2.54, p=0.120 a=b	**$F_{1,36}$=8.14, p=0.007 b>a**	$F_{1,37}$=0.44, p=0.5109 a=b
Starved	$F_{1,35}$=0.10, p=0.752 a=b	$F_{1,35}$=1.10, p=0.758 a=b	-

continued ...

	Tissue R.G.E.	Shell R.G.E.	Respiration Rate	Excretion Rate
Ad libitum	$F_{1,37}$=0.14, p=0.7133 a=b	**$F_{1,37}$=4.86, p=0.0338 b>a**	$F_{1,37}$=2.00, p=0.1652 a=b	$F_{1,38}$=0.16, p=0.6919 a=b
Low	$F_{1,37}$=0.0 p=0.9595 a=b	**$F_{1,37}$=14.91, p=0.0004 b>a**	$F_{1,38}$=1.76, p=0.1927 a=b	$F_{1,38}$=0.05, p=0.8257 a=b
Starved	-	-	**$F_{1,38}$=8.76, p=0.0053 a>b**	**$F_{1,36}$=5.38, p=0.0262 a>b**

Table 4.3.8 Summary of the analysis of covariance tests for temperature differences in physiology for Peartree Point (a) and Prawle Point (b) juveniles maintained at each ration level (see main text for details). Statistically significant interaction terms (initial dry tissue weight*temperature) at the 5% probability level are indicated for tissue growth rates. Analysis of variance was used to test for temperature differences in respiration and ammonia excretion rates. Probability values in bold type are significant at the 5% significance level and the direction of differences indicated.

a) Peartree Point

	Tissue Growth	**Shell Growth**
Ad libitum	**$F_{1,37}$=7.71, p=0.009 20>12**	**$F_{1,36}$=11.13, p=0.018 20>12**
Low	***$F_{1,36}$=11.89, p=0.002 12>20**	**$F_{1,37}$=18.35, p=0.0001 20>12**
Starved	**$F_{1,36}$=10.52, p=0.003 20>12**	**$F_{1,36}$=11.20, p=0.002 20>12**

continued ...

	Feeding Rate	**Respiration Rate**	**Excretion Rate**
Ad libitum	**$F_{1,37}$=626.1, p=0.0001 20>12**	$F_{1,38}$=2.27, p=0.1404 12=20	$F_{1,38}$=3.25, p=0.0793 12=20
Low	**$F_{1,37}$=210.2, p=0.0001 20>12**	**$F_{1,38}$=7.46, p=0.0096 20>12**	**$F_{1,38}$=27.15, p=0.0001 20>12**
Starved	-	$F_{1,37}$=0.00, p=0.9481 12=20	**$F_{1,36}$=11.92, p=0.0015 20>12**

* interaction term, $F_{1,36}$=8.52, p=0.006, 12>20

Table 4.3.8 continued ...

b) Prawle Point

	Tissue Growth	Shell Growth
Ad libitum	**$F_{1,37}$=4.50, p=0.041 20>12**	**$F_{1,37}$=28.33, p=0.0001 20>12**
Low	$F_{1,37}$=0.09, p=0.771 12=20	**$F_{1,37}$=48.73, p=0.0001 20>12**
Starved	**$F_{1,37}$=9.25, p=0.004 20>12**	**$F_{1,36}$=9.71, p=0.004 20>12**

continued ...

	Feeding Rate	Respiration Rate	Excretion Rate
Ad libitum	***$F_{1,37}$=674.96, p=0.0001 20>12**	$F_{1,37}$=1.64, p=0.2082 12=20	**$F_{1,36}$=27.42, p=0.0001 20>12**
Low	**$F_{1,36}$=136.98, p=0.0001 20>12**	**$F_{1,38}$=8.76, p=0.0053 20>12**	**$F_{1,38}$=16.89, p=0.0002 20>12**
Starved	-	**$F_{1,38}$=9.89, p=0.0032 12>20**	**$F_{1,38}$=6.02, p=0.0188 20>12**

* the interaction term (initial dry tissue weight*temperature) only is presented here, temperature as a main effect was not significant at the 5% probability level. For the size range used, feeding rates were higher at 20°C.

20°C than at 12°C (table 4.3.8). The exceptions were the tissue growth rates of juveniles in the low ration; Peartree Point juveniles maintained at 12°C had higher growth rates than at 20°C, whereas Prawle Point juveniles did not exhibit significant ($p>0.05$) variation in tissue growth rate between temperatures (table 4.3.8).

c.Effect of ration level upon growth rate

Starved juveniles had the lowest shell and tissue growth rates for each temperature and population combination (figs. 4.3.1-4.3.4).

Tissue growth: At 20°C, *Ad libitum* fed juveniles had higher tissue growth rates than low rationed juveniles sampled from either population (Peartree Point, $F_{1,36} = 10.26$, $p=0.0028$; Prawle Point, $F_{1,37} = 7.43$, $p=0.0097$), but tissue growth rates were similar between these rations at 12°C (Peartree Point, $F_{1,36} = 0.50$, $p=0.482$; Prawle Point, $F_{1,37} = 0.08$, $p=0.778$).

Shell growth: Peartree Point whelks had higher shell growth rates if fed *ad libitum* than on the low diet at either temperature (12°C, $F_{1,37} = 14.52$, $p=0.0005$; 20°C, $F_{1,37} = 21.40$, $p=0.0001$). *Ad libitum* and low fed juveniles reared from Prawle Point had equivalent rates of shell growth at both temperatures (12°C, $F_{1,37} = 1.22$, $p=0.276$; 20°C, $F_{1,37} = 1.75$, $p=0.1942$).

4.3.1.7 Feeding rates

The total dry weight of mussel tissue eaten during the experiment was summed for each individual; analysis of covariance was used to test for within and between population and temperature variation in the relationship between initial dry tissue weight and total amount of mussel flesh ingested. In the *ad libitum* ration at 20°C, Peartree Point juveniles had marginally higher feeding rates ($p=0.0512$) than those of Prawle Point individuals, but both populations had similar feeding rates at 12°C and in the low rations (table 4.3.7). Feeding rates were higher at 20°C than at 12°C for either populations (table 4.3.8).

At 12°C, both populations had higher feeding rates in the low ration than if fed *ad libitum* (Peartree Point, $F_{1,37} = 6.42$, $p=0.0157$; Prawle Point, $F_{1,36} = 11.85$, $p=0.0015$), but *ad libitum* feeding rates were higher than the low diet at 20°C (Peartree Point, $F_{1,37} = 72.80$, $p=0.0001$; Prawle Point, $F_{1,37} = 58.72$, $p=0.0001$).

4.3.1.8 Gross growth efficiency

Gross growth efficiency has previously been compared between and within populations by the analysis of covariance between the total dry mussel tissue eaten and the tissue or shell growth over a certain time period (sections 2.3.7.5 & 3.3.2.6). However, due to the suggestion of feeding rate variation among juveniles of equivalent size (section 4.3.1.7), whelks that have similar feeding rates are likely to have different growth rates due to variation in initial dry tissue weights. Here, the analysis of covariance between the total amount eaten and the relative growth rate (RGR) has been undertaken to overcome these problems in the analysis of gross growth efficiency associated with initial size variation. The RGR was taken as the total growth in tissue or shell dry weight divided by the initial tissue or shell size respectively. Only population variation in gross growth efficiency is presented here as the differences in feeding rates between temperatures or among ration levels for each separate population was considered too large for valid comparisons among treatments.

Prawle Point juveniles had higher shell growth efficiencies than Peartree Point individuals if maintained on the *ad libitum* or low diet at 20°C or the low ration at 12°C (table 4.3.7). Tissue and shell growth efficiencies for juveniles in the remaining treatments were similar between populations ($p>0.05$; table 4.3.7).

4.3.1.9 Respiration rates

Respiration rates were weight standardised to a common dry tissue weight of 100mg using a b-exponent of 0.575 (section 2.3.7.4). Multivariate analysis of covariance indicated significant temperature ($F_{1,231} = 3.78$, $p=0.0531$), ration ($F_{2,231} = 218.70$, $p=0.0001$), interaction effects between temperature and ration ($F_{2,231} = 7.96$, $p=0.0005$), and population differences ($F_{1,231} = 14.49$, $p=0.0002$). Separate univariate analyses were used to identify where these differences occurred. Respiration rates varied between population samples of juveniles maintained on the low ration at 12°C and if starved at 20°C (table 4.3.7); Peartree Point individuals had higher respiration rates than Prawle Point juveniles in both instances (fig. 4.3.5). Population samples

had similar respiration rates within each of the remaining temperature and ration combinations ($p>0.05$, table 4.3.7).

Respiration rates were similar between temperatures in the *ad libitum* ration, but were higher at 20°C for either Peartree Point or Prawle Point juveniles maintained on the low ration level (table 4.3.8). When starved, Peartree Point juveniles did not show any difference in respiration rate between the two temperatures, but rates were higher at 12°C than at 20°C for starved Prawle Point juveniles (table 4.3.8).

The analysis of variance was used to test for significant variation among the different ration levels for each temperature and population separately, however this analysis does not identify which classes are significantly different when more than two classification levels are used, in this example, there were three ration levels; Scheffe's multiple comparison test was used to test which pairs of data were significantly different from one another (SAS Institute Inc., 1989). For each temperature and population combination, the respiration rates between the *ad libitum* and low ration levels were invariant, whereas the starved respiration rates were significantly lower ($p<0.05$) than either of the higher ration levels (fig. 4.3.5; table 4.3.9).

4.3.1.10 Excretion rates

Excretion rates (fig. 4.3.6) were corrected for any changes in the ammonia concentration in the control beakers and standardised to an animal size of 100mg dry tissue weight using a b-exponent of 0.6 (Stickle and Bayne, 1982). Multivariate analysis of covariance identified significant temperature ($F_{1,223} = 75.43$, $p=0.0001$), ration ($F_{2,223} = 3.50$, $p=0.0317$) and population effects ($F_{1,223} = 11.64$, $p=0.0008$), and also the interaction term between all three effects (temperature*ration*population; $F_{7,223} = 3.07$, $p=0.0042$). Separate univariate analyses were used to identify which comparisons among treatments were different (tables 4.3.7 & 4.3.8). Peartree Point had higher excretion rates in the *ad libitum* ration at 12°C and also in the starved ration at 20°C (table 4.3.7), but rates were similar between population samples in the remaining temperature and ration combinations ($p>0.05$; table 4.3.7).

Figure 4.3.5 Mean (± 2 S.E.) respiration rates for juveniles maintained for five weeks on one of three feeding ration levels at either 12°C or 20°C (see text for details). Individual rates were standardised to a dry tissue weight of 100mg and pooled standard errors presented. Green squares - Peartree Point; Red circles - Prawle Point.

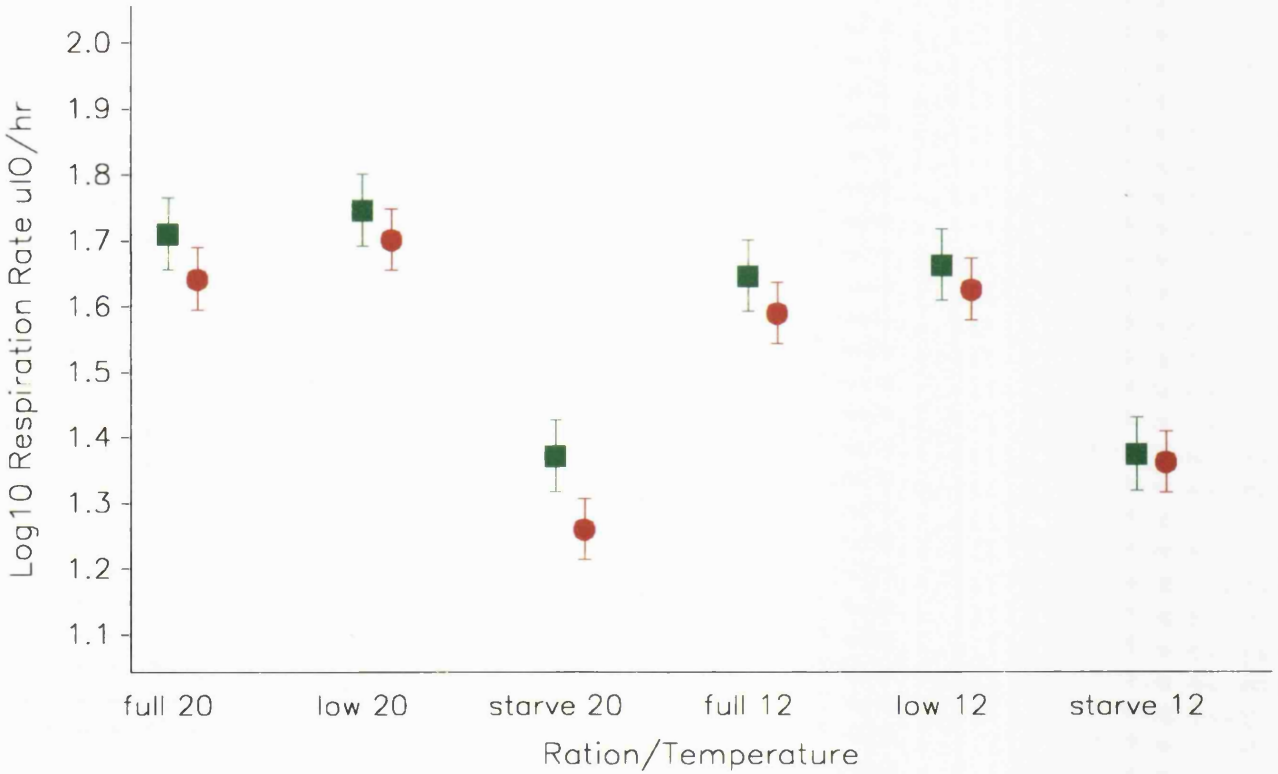

Log10 Respiration Rate ul0/hr
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
full 20
low 20
starve 20
full 12
low 12
starve 12
Ration/Temperature

Table 4.3.9 Variation in mean log respiration (a) and excretion (b) rates among ration levels for each temperature and population sample. Respiration and excretion rates were standardised to a dry tissue weight of 100mg. Means with the same letter are not significantly different at the 5% probability level (α=0.05) using Scheffe's multiple comparison tests.

a. Respiration rates

Peartree Point		Prawle Point	
12°C:-			
Ad libitum	A	*Ad libitum*	A
Low	A	Low	A
Starved	B	Starved	B
20°C:-			
Ad libitum	A	*Ad libitum*	A
Low	A	Low	A
Starved	B	Starved	B

b. Excretion rates

12°C:-			
Ad libitum	A	*Ad libitum*	A
Low	A	Low	B
Starved	A	Starved	B
20°C:-			
Ad libitum	A	*Ad libitum*	A B
Low	A	Low	A
Starved	A	Starved	B

Figure 4.3.6 Mean (± 2 S.E.) ammonia excretion rates for juveniles maintained for five weeks on one of three feeding ration levels at either 12°C or 20°C (see text for details). Individual rates were standardised to a dry tissue weight of 100mg and pooled standard errors presented. Green squares - Peartree Point; Red circles - Prawle Point.

Log10 Excretion Rate uM NH /hr
−0.1
−0.3
−0.5
−0.7
−0.9
−1.1
−1.3
−1.5
−1.7
full 20
low 20
starve 20
full 12
low 12
starve 12
Ration/Temperature

For both populations, excretion rates were higher at 20°C than at 12°C in both the low and starved rations (table 4.3.8). In the *ad libitum* ration, Prawle Point excretion rates were also higher at 20°C, but Peartree Point samples had similar rates between the two temperatures although a slightly higher rate at 20°C is suggested (p=0.08; table 4.3.8a).

At both 12°C and 20°C, Peartree Point juveniles do not show any variation in excretion rates among rations (12°C, $F_{2,56}$ = 1.52, p=0.2270; 20°C, $F_{2,55}$ = 1.52, p=0.2288; table 4.3.9). Conversely, ration level does have a significant (p<0.05) influence upon the excretion rates of Prawle Point juveniles at 12°C ($F_{2,55}$ = 7.83, p=0.0010) and 20°C ($F_{2,57}$ = 3.46, p=0.0381). The Scheffe method for multiple comparisons indicated that at 12°C, a*d libitum* rationed juveniles had the lowest excretion rates, the low and starved rations had similar excretion rates at this temperature, whereas they were significantly different at 20°C (table 4.3.9). At the higher temperature, *ad libitum* rates were similar to either the low or starved ration (table 4.3.9).

4.3.1.11 Temperature sensitivity, Q_{10}

Q_{10} values for respiration and excretion rates were calculated separately for each population and ration (table 4.3.10). Higher Q_{10} values suggest increased sensitivity to temperature.

Respiration rates: Peartree Point juveniles was more sensitive to temperature changes than those of Prawle Point juveniles, irrespective of feeding ration. Q_{10} was lower in the starved ration for both populations.

Excretion rates: With decreased food availability, Peartree Point juveniles were more sensitive to temperature whereas Q_{10} values for Prawle Point suggested decreases in sensitivity.

4.3.1.12 Genotype and physiology

Each temperature, ration, population and gene locus combination was tested separately for genotypic variation in shell and tissue growth and growth efficiency, and feeding, respiration and excretion rates. The results are not presented here due to 1. the large number of tests undertaken (72 per physiological rate), 2. the number of 'significant' tests were not different to the number that would be expected by chance alone using a significance level of 5% (Huitema, 1980), 3. the inconsistent association of genotypic variation with population, temperature and ration, and 4. the low number of juveniles and genotypes (section 4.2.1.2 and 4.3.1.1). These

Table 4.3.10 Q_{10} values for respiration and ammonia excretion rates of dog-whelks maintained at three feeding levels. Experimental temperatures were at 12 and 20°C.

	Respiration rates		Excretion rates	
	Peartree Pt	Prawle Pt	Peartree Pt	Prawle Pt
ad libitum	1.32	1.16	1.42	3.29
low	1.27	1.24	1.77	2.03
starved	0.99	0.74	2.95	1.40

experiments hoped to identify population differentiation in physiology under different feeding levels and temperatures, and as such the samples sizes were established to enable one person to conduct the experiment. Testing for genotypic variation in physiology would have required prohibitively large sample sizes for the experimental design used here and maintaining the higher number of animals would be difficult due to space limitations (section 2.2.1.1) incurred during this series of experiments.

4.3.2 Tidal experiments

4.3.2.1 Size and shape analysis

The relationships between shell length and either dry tissue or shell weights were significantly different between laboratory populations reared from Peartree Point and Prawle Point (dry tissue weight, $F_{1,47}$ = 94.05, p=0.0001; dry shell weight, $F_{1,47}$ = 7.84, p=0.0074). The coefficients given below are means $\pm$2SE:

Peartree Point:

Log_{10} Dry Tissue Weight (g) = (3.1165 $\pm$ 0.1295 * Log_{10} Length (mm)) - (4.8934 $\pm$ 0.1379)

Log_{10} Dry Shell Weight (g) = (3.3057 $\pm$ 0.0730 * Log_{10} Length (mm)) - (4.6253 $\pm$ 0.0778)

Prawle Point:

Log_{10} Dry Tissue Weight (g) = (3.1165 $\pm$ 0.1295 * Log_{10} Length (mm)) - (5.0674 $\pm$ 0.1423)

Log_{10} Dry Shell Weight (g) = (3.3057 $\pm$ 0.0730 * Log_{10} Length (mm)) - (4.6536 $\pm$ 0.0803)

The relationship between shell and tissue weight indicated that Prawle Point juveniles had a heavier shell for a given tissue weight than juveniles reared from Peartree Point ($F_{1,47}$ = 79.89, p=0.0001). The analysis of covariance between shell length and aperture differed for the laboratory-reared populations; both the intercept ($F_{1,46}$ = 6.21, p=0.0164) and slope ($F_{1,46}$ = 6.67 p=0.0130) varied between laboratory samples. Further analysis using the Johnson-Neyman technique indicated that the region of non-significance (p>0.05) ranged between shell lengths of ca. 7-20mm. This region encompassed the total size range used except for three data points (of a total of 50) and so for the size range used here, populations did not exhibit statistically significant differences in shell shape using these shell measurements.

4.3.2.2 Mortality

During the course of the experiment, two (1 Peartree Point, 1 Prawle Point) and eight (3 Peartree Point, 5 Prawle Point) juveniles died in the cool and hot tidal system respectively. Mortality appeared to be size related rather than indicating population specific variation, as dead whelks tended to be at the small end of the size range. One Prawle Point juvenile 'escaped' from the cool tidal tank as no attempt to retain the whelk juveniles was made, which would have caused design difficulties in regulating the temperature and humidity.

4.3.2.3 Growth rate

Some juveniles lost weight over the four week experiment if maintained in the tidal system with high air temperatures and low humidities during the morning low tide. Therefore a dry tissue weight of 0.027g was added to all tissue growth rates to ensure all rates were positive values in the analyses; the logarithmic (base 10) transformation cannot be performed on negative values. Absolute values are therefore not presented in the following results, however it is the relative population and tidal treatment differences which are of interest.

Population differences within each tidal treatment:

Tissue growth rates (fig. 4.3.7) were similar for both populations maintained in the cool tidal system, but Peartree Point juveniles had higher growth rates than Prawle Point juveniles in the hot tidal treatment (table 4.3.11). Peartree Point juveniles also had higher rates of shell growth in both the cool and hot tidal tanks (fig.4.3.8; table 4.3.11). When the relative shell and tissue growth rates were compared (fig.4.3.9; table 4.3.11), Peartree Point juveniles had higher rates of shell growth than Prawle Point whelks for a given tissue growth rate.

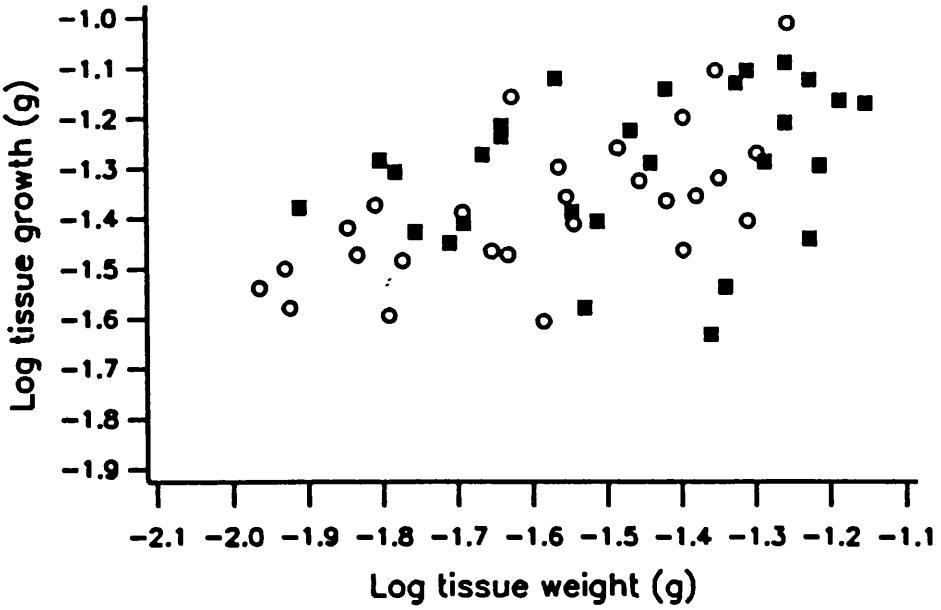
Temperature effects within each population:

Shell and tissue growth rates were higher in the cool tidal tanks for both Peartree Point and Prawle Point juveniles (table 4.3.12). Tissue and shell growth was reduced by 32 and 41% for Peartree Point whelks and 41 and 63% for Prawle Point juveniles in the hot tidal tank; these percentages were calculated from the antilog transformation of adjusted means estimated from the analysis of covariance. The relative growth of shell and tissue weight did not vary between

Figure 4.3.7 Tissue growth rates of juveniles maintained tidally for four weeks. See main text for description of tidal systems. Log_{10} transformed data are presented. Closed squares- Peartree Point, open circles - Prawle Point.

a. Cool tidal

b. Hot tidal


Log tissue growth (g)
-1.0
-1.1
-1.2
-1.3
-1.4
-1.5
-1.6
-1.7
-1.8
-1.9
-2.1 -2.0 -1.9 -1.8 -1.7 -1.6 -1.5 -1.4 -1.3 -1.2 -1.1
Log tissue weight (g)

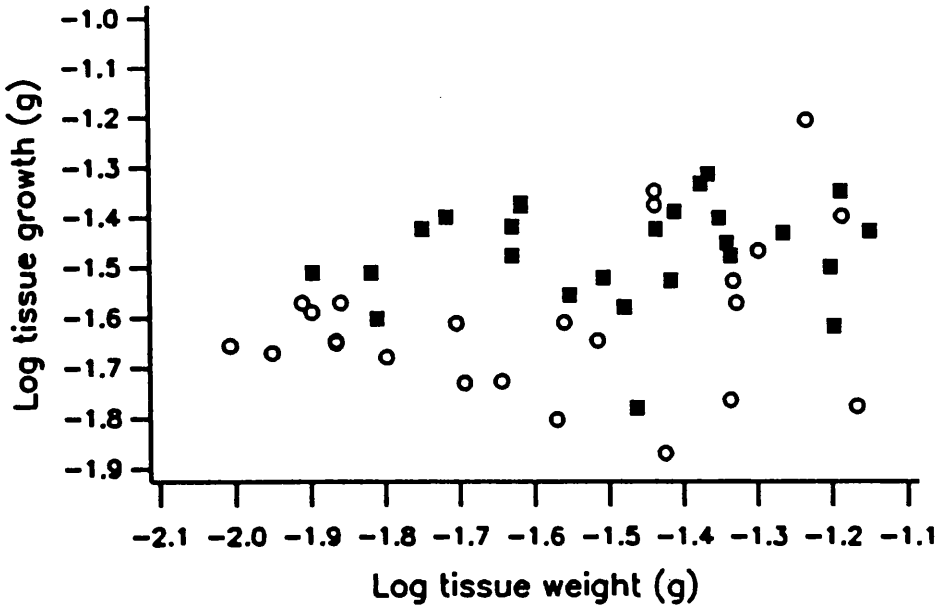

Log tissue growth (g)
-1.0
-1.1
-1.2
-1.3
-1.4
-1.5
-1.6
-1.7
-1.8
-1.9
-2.1 -2.0 -1.9 -1.8 -1.7 -1.6 -1.5 -1.4 -1.3 -1.2 -1.1
Log tissue weight (g)

Table 4. 3. 11 Summary of the analysis of covariance for population differences in size and physiological measurements under two tidal regimes experiencing either high ('hot' tidal, 33±1°C) or ambient temperatures ('cool' tidal, 17±1°C) during the morning low tide respectively. Statistically significant results at the 5% level are presented in bold type and the direction of population differences indicated; a - Peartree Point, b - Prawle Point.

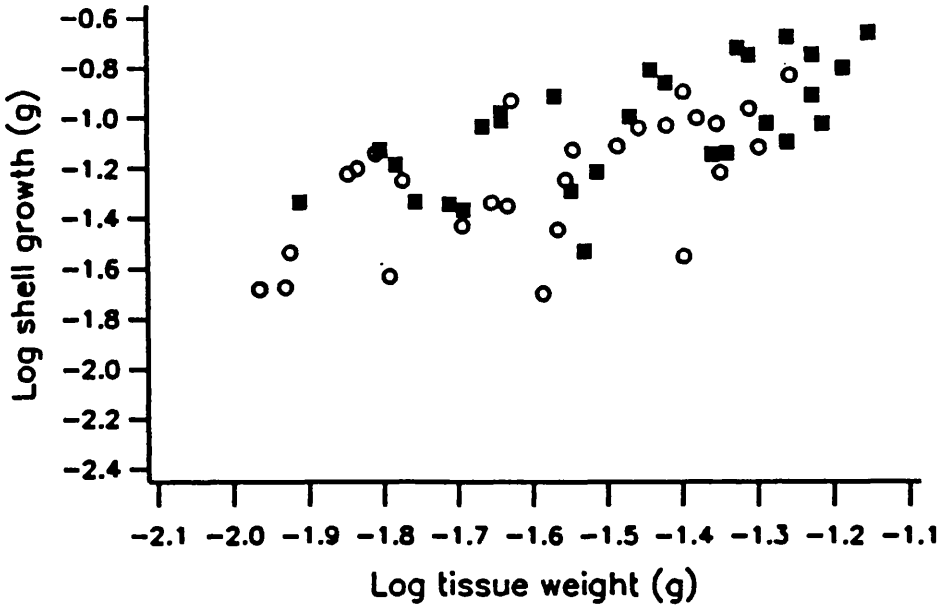
	cool tidal				**hot tidal**			
	F	**df**	**p**	**r^2**	**F**	**df**	**p**	**r^2**
Tissue growth rate	1.44	1,52	0.235 a=b	0.28	**10.58**	**1,48**	**0.002 a>b**	0.26
Shell growth rate	**5.83**	**1,52**	**0.019 a>b**	0.51	**24.93**	**1,48**	**$1x10^{-3}$ a>b**	0.63
Relative growth rate	**5.79**	**1,52**	**0.020 a>b**	0.64	**7.99**	**1,48**	**0.007 a>b**	0.07
Feeding rate	0.00	1,52	0.97 a=b	0.22	**7.12**	**1,48**	**0.01 a>b**	0.28
Tissue growth efficiency	0.16	1,52	0.694 a=b	0.004	***6.72**	**1,47**	**0.013 a>b**	0.16
Shell growth efficiency	1.05	1,52	0.311 a=b	0.18	**11.47**	**1,48**	**0.001 a>b**	0.31

* interaction term $F_{1,47}$ =7.41, p=0.009

Figure 4.3.8 Shell growth rates of juveniles maintained tidally for four weeks. Log_{10} transformed data are presented. See main text for description of tidal systems. Closed squares - Peartree Point, open circles - Prawle Point.

a. Cool tidal

b. Hot tidal


Log shell growth (g)
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
-2.0
-2.2
-2.4
-2.1 -2.0 -1.9 -1.8 -1.7 -1.6 -1.5 -1.4 -1.3 -1.2 -1.1
Log tissue weight (g)

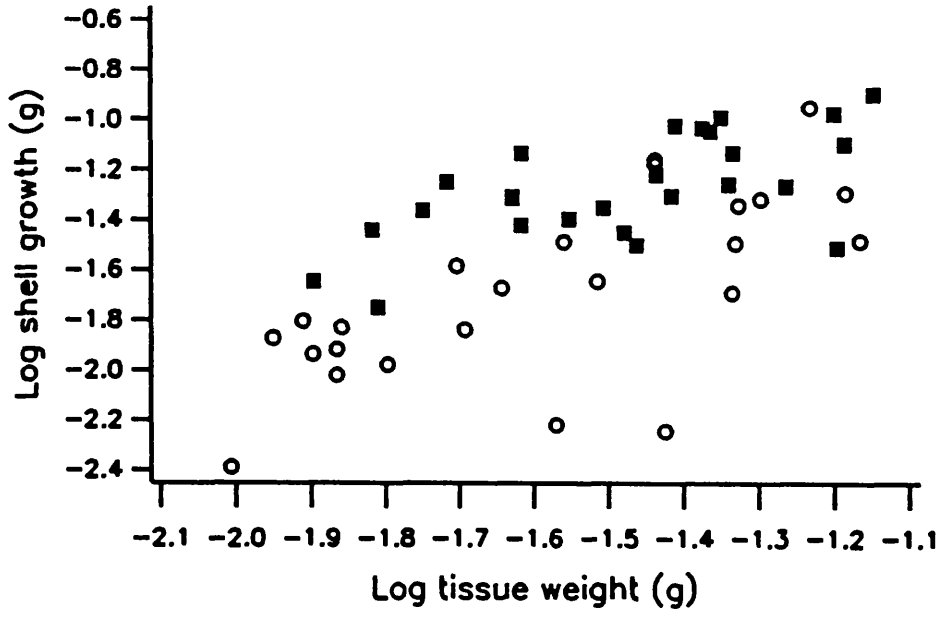
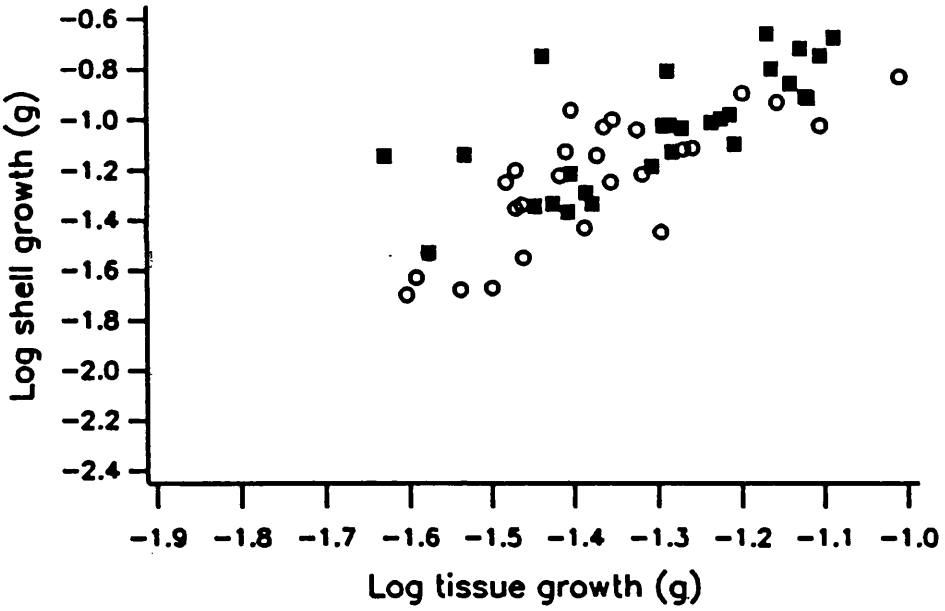

Log shell growth (g)
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
-2.0
-2.2
-2.4
-2.1 -2.0 -1.9 -1.8 -1.7 -1.6 -1.5 -1.4 -1.3 -1.2 -1.1
Log tissue weight (g)

Figure 4.3.9 Relative shell and tissue growth rates of juveniles maintained tidally for four weeks. Log_{10} transformed data are presented. See main text for description of tidal systems. Closed squares - Peartree Point, open circles - Prawle Point.

a. Cool tidal

b. Hot tidal


Log shell growth (g)
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
-2.0
-2.2
-2.4
-1.9 -1.8 -1.7 -1.6 -1.5 -1.4 -1.3 -1.2 -1.1 -1.0
Log tissue growth (g)

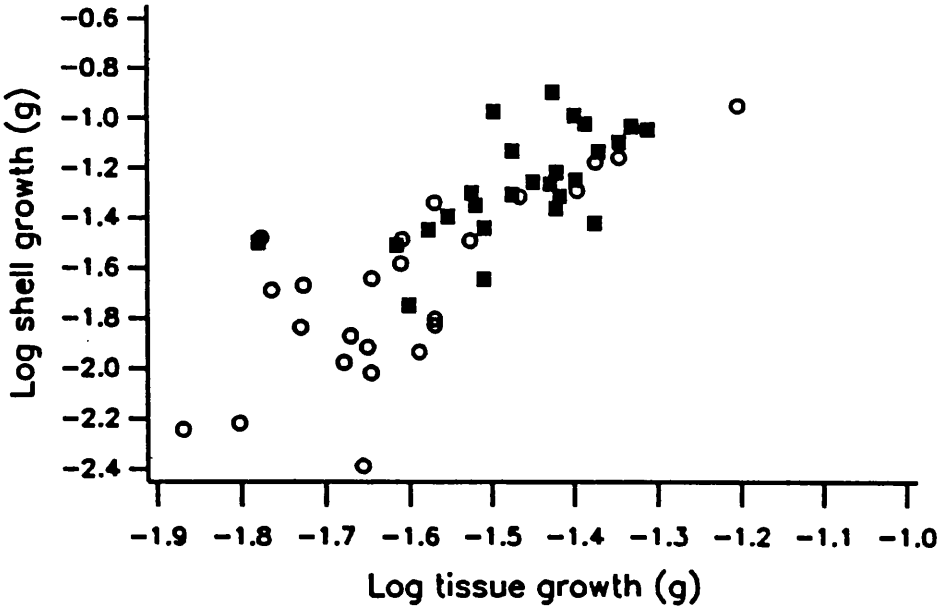

Log shell growth (g)
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
-2.0
-2.2
-2.4
-1.9 -1.8 -1.7 -1.6 -1.5 -1.4 -1.3 -1.2 -1.1 -1.0
Log tissue growth (g)

Table 4.3.12 Summary of the analysis of covariance for differences in physiological measurements under two tidal treatments for Peartree Point and Prawle Point juveniles. Statistically significant results at the 5% level are presented in bold type and the direction of differences between tidal treatments indicated; h- 'hot' tidal, c- 'cool' tidal (see section 4.2.2.2 for details of tidal regime).

	Peartree Pt.				**Prawle Pt.**			
	F	**df**	**p**	**red.**	**F**	**df**	**p**	**red.**
Tissue growth rate	**24.07**	**1,51**	**0.0001 c>h**	32%	**35.48**	**1,48**	**0.0001 c>h**	41%
Shell growth rate	**23.94**	**1,51**	**0.0001 c>h**	41%	**42.63**	**1,48**	**0.0001 c>h**	63%
Relative growth rate	0.28	1,51	0.6004 c=h	-	0.97	1,48	0.3322 c=h	-
Feeding rate	**129.99**	**1,51**	**0.0001 c>h**	86%	**244.11**	**1,48**	**0.0001 c>h**	92%

tidal tanks for Peartree Point and Prawle Point juveniles; for a given tissue growth rate, shell growth was equivalent for the hot and cool tidal tanks (table 4.3.12).

4.3.2.4 Mussel length v. dry weight regressions and relative organic weight

There were no differences in the relationships between $\log_{10}$ length and either $\log_{10}$ dry tissue weight ($F_{1,52} = 0.87$, $p=0.36$) or organic weight ($F_{1,52} = 2.54$, $p=0.12$) between the two field samples of mussels taken two weeks apart and after each had been in the laboratory for two weeks. Data from both field samples were therefore combined before comparisons with samples held at either temperature for seven or 14 days were undertaken (summarised in table 4.3.13).

Mussel samples maintained in the cool tidal system did not vary in the relationships between length and dry tissue weight, or the relative weight of organic and total tissue weight among samples taken at 0, 7 and 14 days after being transferred to the cool tidal tank (table 4.3.13). Samples were therefore pooled before determining regression coefficients.

In the hot tidal system, mussel samples did vary (table 4.3.13) and so two separate regression coefficients were calculated for 0-7 days, and 7-14 day periods; length v. organic weight regressions were calculated due to changes in the relative organic and dry tissue weight among samples maintained in the hot tidal system. This relationship did not vary for samples in the hot tidal system between days 0-7 ($F_{1,80} = 2.63$, $p=0.1085$) or 7-14 ($F_{1,53} = 0.58$, $p=0.4485$), and so coefficients did not need to be interpolated for intermediate days (see table 4.3.13).

4.3.2.5 Feeding rate

The organic weight of mussel tissue ingested was used in these analyses assuming it is the organic fraction of the diet that is absorbed and assimilated (Conover, 1966), and considering the effects of experimental regime upon mussel dry tissue weight in this study (section 4.3.2.4). Some juveniles in the hot tidal system did not eat any mussels over the course of the experiment and so a value of 0.002g was added to each individual feeding rate to overcome the problems of zero values in the analyses and enable relative feeding rates to be compared within and between population samples. After initial size measurements were obtained at the start of

Table 4.3.13 Variation among mussel samples transferred to either experimental tidal system for 0, 7 and 14 days. All data were transformed using the $\log_{10}$ transformation. Coefficients provided in the regression expressions are mean values $\pm$2SE. Probability values in bold type are significant at the 5% significance level.

Temp	comparison	length v. dry weight	organic weight v. dry weight
12°C	0 v. 7 days	$F_{1,80} = 2.26$, p=0.1366	$F_{1,80} = 1.14$, p=0.2889
	0 v. 14 days	$F_{1,80} = 1.98$, p=0.1634	$F_{1,80} = 1.16$, p=0.2839
20°C	0 v. 7 days	**$F_{1,80} = 7.54$, p=0.0074**	**$F_{1,80} = 12.69$, p=0.0006**
	0 v. 14 days	$F_{1,80} = 1.82$, p=0.1808	$F_{1,80} = 3.42$, p=0.0681

Cool tidal (model $F_{1,109} = 489.71$, p=0.0001, r^2=0.818)

$\log_{10}$ organic weight (g) = [$\log_{10}$ length (mm) * 3.1571 $\pm$ 0.2853] - 6.3005 $\pm$ 0.3308

Hot tidal

Days 0-7 (model $F_{1,81} = 4349.38$, p=0.0001, r^2=0812):

$\log_{10}$ organic weight (g) = [$\log_{10}$ length (mm) * 3.2581 $\pm$ 0.3486] - 6.4187 $\pm$ 0.4044

Days 7-14 (model $F_{1,54} = 316.94$, p=0.0001, r^2=0.854):

$\log_{10}$ organic weight (g) = [$\log_{10}$ length (mm) * 3.1599 $\pm$ 0.3550]- 6.2899 $\pm$ 0.4117

the experiment, juveniles were not seen to feed before days two and ten in the cool and hot tidal tanks respectively. The analysis of covariance between initial juvenile dry tissue weight and the total organic weight of mussel tissue consumed over the four weeks revealed that Peartree Point juveniles have higher feeding rates than Prawle Point juveniles in the hot tidal system, whereas no population differences were apparent in the cool tidal system (table 4.3.11). Within population comparisons showed that feeding rates were higher in the cool tidal system than those measured for juveniles maintained in the high temperature tidal tanks for both Peartree Point and Prawle Point samples (table 4.3.12). Feeding rates were estimated to be reduced by 86 and 95% for Peartree Point and Prawle Point juveniles respectively in the hot tidal tanks as calculated from the adjusted means (antilog values) estimated from the analysis of covariance.

4.3.2.6 Gross growth efficiency

For similar reasons to those outlined in section 4.3.1.8, the relative growth rates, ie. the ratio of total growth to initial dry weight for either shell or tissue, rather than the absolute growth rate has been used here. The analysis of covariance between the relative growth rate and the total organic weight of mussel tissue eaten was used for between and within population comparisons of growth efficiency. In the cool tidal tank, both populations had equivalent shell and tissue growth efficiencies, but in the hot tidal system, Peartree Point had higher growth efficiencies than Prawle Point (table 4.3.11).

4.4 DISCUSSION

4.4.1 Genetic analysis of laboratory population samples

Genetic differences between laboratory-reared samples were similar to previous results for Peartree Point and Prawle Point sites (Day, 1990; Kirby *et al.*, 1994a; chapters 2 & 3, this study). Peartree Point whelks were almost fixed for the *Lap-2*$^{10.10}$ or *Mdh-1*$^{10.10}$ genotypes. In contrast, higher frequencies of the '9' alleles at *Lap-2* and *Mdh-1* loci, 0.45-0.58 and 0.7-0.78 respectively, were found in Prawle Point samples. Statistical analysis failed to identify any variation in genotype frequencies among treatments within each laboratory population, which suggests that the selection procedure used to obtain experimental samples was appropriate. The similarity between these and previous estimates of allele frequencies for these sites (chapters 2 & 3, this study and references therein) indicates that animals maintained in the tidal simulation experiment were also likely to be differentiated between population samples with respect to allozyme frequencies.

4.4.2 Palmer regressions and shell morphology

Similar results were obtained to those previously found for laboratory reared populations of whelks from these sites; Peartree Point whelks had a heavier tissue and shell weight for a given shell length which was probably due to the shape differences between these two sites (discussed in sections 2.4.3 and 3.4.4). The results also indicated that although the Palmer (1982) regressions enabled good approximations for wet tissue weight (the difference between shell and total animal weight), Prawle Point juveniles had a heavier wet tissue weight for any given dry tissue weight. It is unlikely that the weight-specific water content of body tissues varied between populations as this relationship tends to vary with feeding level, temperature or salinity (e.g. Ansell and Sivadas, 1973; Pierce and Amende, 1981; Stickle *et al.*, 1985a). However, the morphological differences between populations could be responsible; whelks from Prawle Point tend to have a more slender, elongate shell shape (chapters 2 & 3, this study and references therein). Elongate shells retain a more water within their mantle cavity than the spherical shell typical of Peartree Point whelks for a given tissue weight (Kirby *et al.*, 1994a). The elongate shell shape would be beneficial in high temperature environments such as Prawle

Point, due to a greater capacity for evaporative loss reducing temperature and salinity changes within the mantle cavity (Boyle *et al.*, 1979; Kirby *et al.*, 1994a, b).

4.4.3 Temperature effects upon physiology

Results are summarised in table 4.4.1. Except for tissue growth in the low ration, both populations had higher growth rates at 20°C than at 12°C in the fed rations. When starved at 20°C, juveniles lost more tissue weight and deposited more shell (whether either absolute or relative shell growth was considered) than if maintained at the lower temperature. These experiments established that physiological differences between temperatures were dependent upon the sample site from which juveniles were reared and also the food availability:

Ad libitum ration: Higher growth rates at 20°C in the *ad libitum* diet can be attributed to higher feeding rates in the absence of increased metabolic losses (respiration and excretion rates) at this temperature (table 4.4.1). Higher feeding rates at 20°C were expected (Largen, 1967b; Bayne and Scullard, 1978b) and since food was abundant in this ration, it can be assumed that differences in feeding rates between the temperatures were due to variation in behaviour and/or physiology (Largen, 1967b; Bayne and Scullard, 1978a, b; Hughes, 1986); temperature increases reduce the time spent drilling and ingesting prey, and also the time between feeding bouts (Bayne and Scullard, 1978b). Higher respiration and excretion rates were also expected at 20°C (Bayne and Scullard, 1978a; Stickle and Bayne, 1982; 1987), however rates were similar between the temperatures in this study with the exception of higher excretion rates at 20°C for the Prawle Point whelks. It is possible that the five week duration of the experiment was sufficient for temperature acclimation to have occurred (reviewed by Bayne and Newell, 1983), and explain why respiration and excretion rates were similar between temperatures for both populations. The apparent discrepancy between previous work and the results of this study may be due to the previous history of the whelks used; only young juveniles (<14mm) which had been reared non-tidally, with abundant food and under constant temperature in the laboratory were used in this study. The results from previous studies may be complicated due to the effects of season including the reproductive and feeding status of dog-whelks prior to collection from the shore and temperature manipulation (Connell, 1961; Bayne and Scullard,

Table 4.4.1: Summary of physiological differences at three feeding ration levels. Statistically significant results at the 5% probability levels are indicated in bold type. R.G.E. - relative growth efficiency; the total increase in size is adjusted for initial size variation at the start of the experiment, and then related to the total weight of prey consumed (full details in section 4.3.1.8).

a) Population differences: a-Peartree Point, b-Prawle Point.

	Tissue Growth	Shell Growth	Feeding Rate	Tissue R.G.E.	Shell R.G.E.	VO_4	VNH_4
12°C							
Ad libitum	**a>b**	**b>a**	a=b	a=b	a=b	a=b	**a>b**
Low	**a>b**	a=b	a=b	a=b	**b>a**	**a>b**	a=b
Starved	a=b	**a>b**	-	-	-	a=b	a=b
20°C							
Ad libitum	**a>b**	a=b	a=b	a=b	**b>a**	a=b	a=b
Low	a=b	**b>a**	a=b	a=b	**b>a**	a=b	a=b
Starved	a=b	a=b	-	-	-	**a>b**	**a>b**

b) Temperature differences: 12 and 20 are the experimental temperatures used in degree centigrade.

	Tissue Growth	Shell Growth	Feeding Rate	VO_4	VNH_4
Peartree Point					
Ad libitum	**20>12**	**20>12**	**20>12**	12=20	12=20
Low	**12>20**	**20>12**	**20>12**	**20>12**	**20>12**
Starved	**20>12**	**20>12**	-	12=20	**20>12**
Prawle Point					
Ad libitum	**20>12**	**20>12**	**20>12**	12=20	**20>12**
Low	12=20	**20>12**	**20>12**	**20>12**	**20>12**
Starved	**20>12**	**20>12**	-	**12>20**	**20>12**

1978b; Ansell, 1981). The acclimation time, which aimed to standardise initial feeding rates in these other studies, may not have been sufficient to reduce a possible energy deficit incurred by reduced food availability on the shore. It may also be several days before dog-whelks commence feeding in the laboratory after retrieval from the shore (pers. obs.), which would be expected to influence subsequent feeding and metabolic rates.

Low ration: Feeding rates were lower at 12°C although diets were standardised for whelk size in this ration and should have been equivalent between temperatures. However, not every juvenile ate one mussel per week at 12°C and resulted in temperature differences in whelk size measured two weeks after the start of the experiment. Size measurements taken at this time were used to adjust the preferred mussel size (Bayne and Scullard, 1978b) and because whelks were larger at 20°C, these juveniles were subsequently provided with larger sized mussels as food during the remaining three weeks of the experiment. All juveniles maintained on this ration increased in tissue and shell weight. Although shell growth and feeding rates were higher at 20°C for both populations, differences in tissue growth rates between temperatures varied according to population; tissue growth rates were lower at 20°C for Peartree Point whelks, but temperature-independent for Prawle Point juveniles. Respiration and excretion rates were higher at 20°C unlike the rates measured for the *ad libitum* ration and may reflect the increased feeding rates at this temperature. When food is in limited supply, higher respiration rates can result from the increased activity in the search for food (Calow, 1974; Stickle and Bayne, 1982). Juveniles maintained on the low ration were comparatively more energy stressed at 20°C than at 12°C, which was reflected in the relative feeding rates between the two rations; *ad libitum* feeding rates were higher than those of the low ration at 20°C, but similar between these rations at 12°C for either population. Higher excretion rates at 20°C can be explained by the relatively greater importance of protein catabolism during periods of limited food availability, which is discussed below for starved juveniles. These results suggested that both populations showed a reduction in net energy balance at 20°C; the total energy expenditure at 20°C formed a sufficiently high proportion of the total ingested ration to reduce tissue growth rates below or equal to that measured at 12°C for Peartree Point or Prawle Point juveniles respectively (table 4.4.1b). Energy loss at 12°C was a lower proportion

of the total energy consumed such that the scope for growth (see section 2.1 for description) was higher even though feeding rates were lower than at 20°C.

Starved ration: Higher tissue weight losses in juveniles starved at 20°C were accompanied by higher excretion rates when compared to juveniles starved at 12°C. Similar results have been obtained for other marine species where increases in nitrogenous excretion have been suggested to be due to the greater relative importance of protein as a respiratory substrate during starvation (e.g. Ansell and Sivadas, 1973; Mayzaud, 1973; Stickle and Bayne, 1982). In contrast, respiration rates for starved juveniles appeared to be temperature independent for Peartree Point whelks, but were actually lower at 20°C than at 12°C for Prawle Point juveniles. On direct transfer to higher temperatures, dog-whelks have higher respiration rates (Bayne and Scullard, 1978a), but during starvation it would be advantageous to adopt a more conservative physiology to reduce energy expenditure. The present results suggest that juveniles from both populations reduce their respiration rates at the higher temperature; temperature acclimation (reviewed by Bayne and Newell, 1983) can explain the similarity between temperatures in the Peartree Point samples and the apparent reduction in basal respiration rates at higher temperatures for Prawle Point juveniles. Lower respiration rates at 20°C for Prawle Point animals may also reflect reduced activity at this temperature (see low ration section above). The higher weight losses incurred at 20°C were possibly caused by the likely higher energetic losses during the initial stages of the experiment as energy reserves are catabolised to meet the energetic requirements for standard metabolism during starvation (Stickle and Bayne, 1982). The combination of the high temperature and lack of food may have resulted in a greater reduction in energy loss in the later stages of the experiment.

4.4.4 Population differentiation in physiological response to temperature

Results are summarised in table 4.4.1.

Ad libitum and low rations: Peartree Point juveniles had higher tissue growth rates, but lower rates of relative shell growth than those measured for Prawle Point juveniles when maintained on either of the fed rations at 12°C (table 4.4.1). Similar results have been obtained for laboratory-reared and shore-collected juveniles fed *ad libitum* at 15°C (chapters two and three

respectively). Although Prawle Point juveniles continued to exhibit a relatively higher shell growth rates at 20°C, it is the similarity between populations in tissue growth at this temperature that is of particular interest. Previously high relative shell growth rates for Prawle Point juveniles has been linked to reduced tissue growth at 12°C (this chapter) and 15°C (section 2.3.7.2). The higher temperature in this current study has not revealed a similar relationship here at 20°C; population variation in tissue growth only just attained statistical significance ($p=0.047$) in the *ad libitum* ration and could be due to marginally higher feeding rates for Peartree Point juveniles ($p=0.051$). Tissue growth rates were also similar between populations for the low ration at this temperature.

Variation in feeding rate is the main component of the energy budget underlying growth rate variation, but both populations had similar feeding rates in the *ad libitum* and low rations. Peartree Point juveniles tended to have higher respiration rates, but were only statistically significant in the low ration at 20°C. Similarly, variation in excretion rates cannot explain the growth differences between populations. It is possible that some other component of the energy budget underlies the growth variation seen here. It should also be noted that although respiration rates were measured here, these are only indirect estimates of total metabolic losses (reviewed in Bayne and Newell, 1983). Absorption efficiency and primary amine excretion were not measured in this study. Obtaining accurate absorption efficiencies would have been difficult in this study as the measurement error associated with the method commonly used (Conover, 1966) can be high (see Kirby, 1992). This is partly due to the small amount of faeces produced by dog-whelks, particularly in the small juvenile stages and when feeding rates are low (per. obs.). Therefore, it was unlikely that any differences in absorption efficiencies between populations would be found; population differences (if present) were considered to be low for dog-whelks reared under identical conditions in the laboratory.

Starved ration: Tissue weight loss was similar for Peartree Point and Prawle Point juveniles at either temperature. Shell growth rates were also similar between populations at 20°C. At 12°C, there was a suggestion from the heterogeneity of regression gradients that Peartree Point juveniles grew more shell at this temperature, but the size range of non-significance between

populations was not estimable using the Johnson-Neyman method (Huitema, 1980). Previous experiments have found that starved Peartree Point whelks lost more tissue and gained more shell (section 2.4.6), but this experiment was conducted at 15°C with a greater sample size (90 juveniles per population) and without an acclimation period as animals had been reared at this temperature. At 20°C, starved Peartree Point juveniles had higher respiration and excretion rates than Prawle Point juveniles. These differences in energy losses at the higher temperature and starvation diet may have lead to population differences in weight loss or shell growth if the experiment was extended, but this is merely speculative.

4.4.5 Response to the integrated effects of temperature, humidity and desiccation stress

Eight dog-whelks died in the 'hot' tidal tank (32-34°C; 25-33%) and two died in the tank maintained at ambient temperatures ('cool' tidal; 15-18°C; 77-92%) suggesting that the temperature and humidity experienced during aerial exposure in the hot tidal tank were stressful. Seawater temperatures were held at 15°C for both tidal systems. There were no population differences in mortality within either tidal treatment. These experiments were not designed to identify such differences, however it would be interesting to repeat the experiment with larger sample numbers and perhaps over a longer duration considering the population differences in feeding strategy discussed below. A more sensitive indication of stress in these animals was provided by reduced feeding and growth rates when high temperatures were used (results are summarised in table 4.2.2). No mussels were eaten in these tanks until at least 10 days after the start of the experiment (three days under the 'cool' tidal regime) and some individuals did not feed and lost weight over the course of the four-week experiment in this treatment.

Tissue growth rates were similar between populations in the cool tidal tank, whereas Peartree Point juveniles had higher growth rates in the hot tidal tank. The cool tidal tank, also used to rear the dog-whelk juveniles, was provided as the control for the hot tidal tank in this experiment. It is interesting to note that the population differences seen previously for laboratory reared juveniles under continuous immersion at 15°C (section 2.3.7.2) were not evident under the tidal regime with a seawater temperature of 15°C and ambient air

Table 4.4.2: Summary of differences in physiology between populations and aerial exposure regimes. c- cool tidal, h- hot tidal, a- Peartree Point, b- Prawle Point. See section 4.2.2 for experimental protocol. Relative growth rate - shell growth in relation to tissue growth rate.

	Population		**Air temperature**	
	Peartree	**Prawle**	**cool**	**hot**
Tissue growth rate	**c>h**	**c>h**	a=b	**a>b**
Shell growth rate	**c>h**	**c>h**	**a>b**	**a>b**
Relative growth rate	c=h	c=h	**a>b**	**a>b**
Feeding rate	**c>h**	**c>h**	a=b	**a>b**
Tissue growth efficiency	-	-	a=b	**a>b**
Shell growth efficiency	-	-	a=b	**a>b**

temperatures (<18°C) during emersion. Growth rates have previously been found to be alike for the two populations when held at elevated seawater temperatures of 20°C (section 4.3.1.6), and so the similarity in growth rates in this 'cool' tidal regime may be due to the higher temperatures experienced during emersion. A tidal tank maintained at lower ambient temperatures was not established, which may have confirmed the relative influence of tidal emersion and air temperature upon the comparative growth rates between populations. A literature search of work undertaken on *Nucella* spp. failed to find a study where a manipulated tidal simulation had been attempted to investigate the effects of aerial exposure on aspects of the physiology of dog-whelks. Air temperatures are known to change rapidly in the intertidal and may consequently form a worthwhile subject for further work on the ecophysiology of this species.

In the hot tidal tank, Peartree Point whelks exhibited greater tissue growth rates. This result may appear contrary to expectations; Prawle Point whelks possess a shell shape thought to be adaptive in high temperature environments (see section 4.4.2). These juveniles would perhaps be expected to have higher growth rates as internal temperatures should be theoretically lower at a given ambient temperature and so reduce the short-term influence of temperature upon physiology. Lower internal temperatures would be expected to reduce energetic losses particularly when food stressed, which was incurred in this tidal tank due to the severe air temperatures. Although both populations had very low feeding rates in this tidal tank, Prawle Point juveniles had reduced their feeding rates to a lower rate than Peartree Point individuals. Similar differences in feeding have previously been suggested at 20°C (section 4.3.1.7). Animals may reduce feeding excursions in an attempt to reduce the risk of physiological stress and the difference between populations may represent variation in feeding behaviour in response to stress (section 4.1 and references therein.). Providing some type of refuge from the high temperature and low humidity may have confirmed behavioural differences between the two populations, but were not included in the present experiment to ensure that all individuals were similarly stressed.

In both tidal tanks, Peartree Point whelks had higher shell growth rates than those calculated for Prawle Point (table 4.4.2). This difference between populations was repeated when shell

growth rates were related to increases in tissue weight. The majority of experiments conducted previously on samples from these populations have shown that Prawle Point juveniles exhibited higher shell growth rates for a given increase in tissue weight. It is possible that the shift in relative shell growth rates is related to the higher temperatures experienced during tidal emersion or the reduced time available for feeding, incurred from simulated low tides. Prawle Point juveniles reduced the rate of shell deposition to a greater degree than Peartree Point juveniles in the hot tidal system, a reduction of 63 and 41% respectively. Prawle Point juveniles therefore had lower energetic costs associated with shell growth at times when such a strategy would be beneficial.

CHAPTER FIVE:

General Discussion

The results presented in this thesis (summarised in table 5) have been discussed at the end of each respective chapter: genetic and shell shape variation (chapters 2, 3 and 4), life history (chapter 2), physiology (chapters 2 and 3) and the physiological response to food, temperature and aerial exposure stresses (chapter 4). The present chapter brings together the reasons why this variation may have arisen along this region of coast.

Environmental variation along the cline

It has been suggested that the genetic and phenotypic variation in dog-whelks between Peartree Point and Prawle Point may reflect environmental heterogeneity in wave exposure (Day, 1990; Kirby *et al.*, 1994a, b) and temperature (Kirby *et al.*, 1994a, b; Kirby and Berry, in prep.). Previous workers have classified Peartree Point as wave exposed (Ballantine scale 2: McCarter and Thomas, 1980) and Prawle Point as sheltered (Ballantine scale 4-6: McCarter and Thomas, 1980; Day, 1991). Environmental variables measured in this study (chapter 3), indicate that temperature, relative humidity and desiccation are the main climatic factors to vary between sites, and the differences observed were in agreement with those expected between 'exposed' and 'sheltered' shores. The lower maximum temperatures and higher relative humidities measured at Peartree Point could result from the high abundance of algae, mussels and barnacles at this site, leading to a greater scope for evaporation. In contrast, these epifaunal species are much less common at Prawle Point (McCarter and Thomas, 1980) and therefore the higher temperatures, lower humidities and higher desiccation rates at this site were not unexpected.

Maximum wave forces were similar for Peartree Point and Prawle Point when measured using wave dynamometers (Bell and Denny, 1994). This would be expected from the aspect of the shore; both sites were open to the prevailing south-westerly wind and waves. In contrast the region of the coast between Peartree Point and Lannacombe beach (fig.3.1) was likely to be 'sheltered' due to a more south-easterly aspect. This is supported by the 'sheltered' scoring on Ballantine's (1961) scale (Day, 1990). Future work should consider measuring wave forces at intermediate points, and over a longer period. In the absence of wave force information across the region of the cline (the present results are restricted to opposing ends of the cline),

Table 5: Summary of site differences in genetic and phenotypic variation, and environmental variables established in the present study. Further details are provided in the text. GR - growth rate, * Relative shell growth rate - the relationship between shell and tissue growth rates.

	PRAWLE POINT	PEARTREE POINT
GENETICS: (Frequency of the '10' allele)		
Lap-2	0.42 - 0.74	0.98 - 1.0
Mdh-1	0.22 - 0.62	0.94 - 0.98
ENVIRONMENT:		
	High temperatures	Low temperatures
	High desiccation rates	Low desiccation rates
	Low humidity	High humidity
	Wave forces similar	
PHENOTYPE:		
Shell shape:	Elongate shell	Round shell
Life history:	Low number per egg capsule	High number per egg capsule
	High hatchling mortality	Low hatchling mortality
Physiology:		
• Tissue GR:	Prawle Point<Peartree Point	
• Relative Shell GR*:	Prawle Point>Peartree Point	
• Feeding rates:	Prawle Point=Peartree Point	
• Respiration rates:	Prawle Point≤Peartree Point	
• Differences in growth, feeding rates and growth efficiency among *Lap-2, Mdh-1* and *Pep-1* genotypes		
STRESS RESPONSE:		
Effects of reduced food availability:		
• Reduction in tissue GR:	Prawle Point≤Peartree Point	
• Reduction in shell GR:	Prawle Point>Peartree Point	
• Energetic 'cost' when starved:	Prawle Point≤Peartree Point	
Effects of temperature (*ad libitum* ration):		
• Growth rates: 20°C	Prawle Point≤Peartree Point	
12/15°C	Prawle Point<Peartree Point	
• Feeding rates: 20°C	Prawle Point≤Peartree Point	
12/15°C	Prawle Point=Peartree Point	
Effects of aerial exposure:		
Low temperature/High RH:		
• Feeding rates:	Prawle Point=Peartree Point	
• Tissue GR:	Prawle Point=Peartree Point	
• Relative Shell GR*:	Prawle Point<Peartree Point	
High temperature/Low RH:		
• Feeding rates:	Prawle Point<Peartree Point	
• Tissue GR:	Prawle Point<Peartree Point	
• Relative Shell GR*:	Prawle Point<Peartree Point	

wave force cannot be eliminated as a possible environmental covariate. Dog-whelks at Prawle Point may reflect the distributional limit of the 'sheltered' genetic and phenotypic morph along this stretch of coast. Dog-whelks have not been found along the coast to the immediate west of Prawle Point and samples at the first site where dog-whelks are encountered (ca. 3km west of Prawle Point) exhibit the genetic characteristics of whelks to the east of the cline at Peartree Point (Kirby *et al.*, 1994a).

Local variation in shell shape

The shell shape differences previously identified for whelks from Peartree Point and Prawle Point (Day, 1990; Kirby *et al.*, 1994a) were confirmed in this study. Two out of seven samples did not conform to this result, but were considered to be a consequence of using indirect measures of shell shape (Length/Aperture ratios (L/Ap); discussed in section 2.4.3) as it is possible for two whelks to have the same shell and aperture lengths, yet have differences in overall shell shape. It is also possible that shell shape variation due to genetic composition is lower than had been reported previously for laboratory-reared dog-whelks (Kirby *et al.*, 1994a). More sophisticated methods of shell shape analysis (e.g. Raup, 1966; Kitching, 1976, 1977, Humphries *et al.*, 1981; Ekaratne and Crisp, 1983) are preferred when samples reared under similar environments are to be compared; the genetic basis of shell shape variation may be too small to affect L/Ap estimates in the absence of a suitable environmental cue.

The relationship between shell length and tissue weight were perhaps the best estimates of shape in this study. Round shells would be expected to enclose a comparatively larger animal and so have a higher tissue weight for a given shell length; Peartree Point whelks had a consistently higher body mass for any given shell length. The consequences of shell shape differences are intuitive. Dog-whelks tend to retain water within their mantle cavity during their emersion at low tide, the volume of which varies according to the shell shape (Kirby *et al.*, 1994a) and size of the animal (Coombs, 1973a). Prawle Point dog-whelks have a more elongate shell, which retains a greater volume of seawater for a given animal tissue weight (Kirby *et al.*, 1994a). This provides a greater scope for evaporative water loss thus helping to ameliorate the higher temperatures (Coombs, 1973a) and buffer salinity changes (Boyle *et al.*,

1979; Kirby *et al.*, 1994b) under the conditions at this site. Shell shape differences between sites and the putative "adaptedness" (Endler, 1986) of this variation, complement the environmental changes in temperature and desiccation rate (chapter three; figs. 3.3.2-4). Dog-whelks at Peartree Point possess the rounded shell shape where temperature and desiccation stresses are reduced.

The present discussion has focused upon the temperature and osmotic consequences of shell shape in view of the environmental variation identified between sites in this region (this study) and the site-specific physiological response of whelks to temperature and desiccation/salinity stress (chapter four, this study; Kirby *et al.*, 1994b). Alternative theories for the significance of shell morphological variation in *N. lapillus* have been discussed in chapter 3, and include predatory defence and dislodgement due to wave action. The extent of predation and tenacity of whelks on the shore have not been quantified in this study.

Life history variation

Supporting previous conclusions about the reproductive strategy of *N. lapillus* on 'exposed' and 'sheltered' shores (e.g. Feare, 1970b, 1971; Bloomer, 1987; Etter, 1989; Geller, 1990), dog-whelks from Peartree Point ('exposed' site) appear to have a higher reproductive investment than individuals at the Prawle Point site ('sheltered' site). This was indicated by a higher number of juveniles per capsule at hatching for Peartree Point samples (chapter 2) and the relative tissue weights of shore collected whelks (chapter 3). The latter could be explained by the breeding season being extended, individual reproductive effort being higher or proportionately more whelks were reproducing at Peartree Point. A higher number of juveniles per capsule at exposed sites has been associated with a higher number of capsules laid per female and smaller juvenile size at hatching, resulting in a greater overall reproductive effort (Etter, 1989).

Reproductive investment is frequently used as an index of fitness; within a particular environment, individuals should aim to maximise the number of viable offspring surviving to maturity. However, the 'costs' of reproduction tend to constrain reproductive rates due to trade-offs with survival and future fecundity, which include parental condition, behaviour, offspring

size/number and growth (reviewed in Partridge and Harvey, 1988; Partridge and Sibly, 1991; Roff, 1992; Stearns, 1992; McNamara and Houston, 1996). Reasons for the differences in reproductive effort between exposed and sheltered shores, have included r-K selection and the relative mortalities of adults and juveniles (Osborne, 1977, cited in Crothers, 1985; Etter, 1989). These are referred to as deterministic and stochastic models for life history evolution respectively (Stearns, 1976). The habitat stability of wave exposed and sheltered shores tends to favour either an r- or K- type strategy; information on whether mortality is acting in a density-dependent or independent manner is necessary to infer the type of selection. With the stochastic model, estimates of adult mortality are generally higher on 'exposed' shores, presumably as the result of the greater risk of dislodgement for large whelks in wave-swept environments (Etter, 1989). Juvenile mortality is more difficult to assess due to the problems inherent in locating small individuals, but mortality rates are considered to be higher in 'sheltered' locales where they have been attempted; the causes of juvenile mortality include predation and physiological stress (Etter, 1989), both thought to be important on 'sheltered' shores (Etter, 1988b; Burrows and Hughes, 1989, and references therein). When juvenile mortality was measured under laboratory conditions in this study, Prawle Point hatchlings incurred higher mortalities than in Peartree Point samples. The differences in reproductive strategy between sites could therefore reflect the relative importance of adult or juvenile mortality at Peartree Point and Prawle Point respectively. Confirmatory evidence from age- and size-specific mortality and reproduction rates at these sites is required.

Local shore conditions also have a major effect upon reproductive effort. Egg capsules are laid by mature dog-whelks, which may have been subjected to potentially different site-specific environmental influences described previously to include prey abundance and climate (chapter 3). The nutritional status of the parents influences the total amount of resources available for reproduction even before physiological 'decisions' on how the resource is allocated to growth, maintenance and reproduction. It is therefore difficult to be certain how much of the variation in the number of hatchlings and post-hatching mortality rate observed in this study was due to genetics, environment or both. Feeding rates are higher on 'exposed' shores due to the higher abundance of mussels and reduced physiological stress during emersion (Burrows and Hughes,

1989, 1990) suggesting that whelks at these sites are more likely to have a greater investment in reproduction due to higher energy intakes. The emerging explanation for differences in reproductive strategy can therefore be summarised to include the relative survival of age/size classes and food availability on the shore.

Such site-specific differences in reproductive strategy suggest that *Nucella lapillus* would be an ideal subject for a more detailed study to distinguish the genetic and environmental components to this variation. Life history analysis and breeding for laboratory-reared cohorts experiments (personal attempts have met with limited success, section 2.4.2) would also provide valuable fecundity and mortality information for the different sites, genotypes and karyotypes, and assist in interpreting the differences in juvenile growth rates among sites.

Variation in growth rate

Dog-whelks reared from two sites in the region of the South Devon cline and possessing similar differences in genetic variation as in this study, exhibit differences in growth rate associated with genetic composition (Kirby, 1992; Kirby *et al.*, 1994a; discussed in section 2.4.4). Similar growth differences were found in this study; whelks reared from a site characterised by high frequencies of the *Lap-2* and *Mdh-1* '10' alleles and the low 2n=26 chromosome number (Peartree Point) exhibit higher tissue growth rates. In contrast, individuals reared from a site with low frequencies for these alleles and high 2n=36 chromosome numbers (Prawle Point) had lower growth rates. Peartree Point whelks also have higher respiration rates likely to reflect the higher metabolic rates associated with elevated growth rates (reviewed in Bayne and Newell, 1983). These differences in respiration rates were only statistically significant at the 5% level for shore juveniles maintained in the laboratory, but the direction of site differences was consistent for all experiments.

Relative shell growth rates, the amount of shell deposited in relation to the tissue growth rate, provide important information on relative growth strategies. For example, even when absolute shell growth rates were lower than those of Peartree Point whelks, Prawle Point whelks tended to deposit more shell for a given tissue growth rate. This could reflect the differences in the energetic cost associated with growing a particular shell shape, assuming there is a trade-off

between shell and tissue growth; elongate shells may be more expensive to grow. The rate of tissue growth could also be limited by the maximum rate at which shell can be deposited (Palmer, 1981). Prawle Point whelks could therefore have lower rates of tissue growth as a consequence of both the rate of shell growth and the reduced internal shell volume associated with their elongate shape.

Differences in relative shell growth could reflect variation in shell thickness reported for 'sheltered' and 'exposed' dog-whelks (Kitching *et al.*, 1966; Hoxmark, 1971; reviewed in Crothers, 1985). However, this is unlikely as shell thickness among laboratory-reared juveniles does not differ between sites when measured accurately on regions of the shell away from the shell lip (Kirby, 1992). The growing margins of shells tend to be thinner in faster growing animals and become thickened during starvation (chapter 2; pers. obs.), perhaps explaining the sometimes imprecise correlation of shell thickness and 'exposure' among shore animals (e.g. Moore, 1938; Hoxmark, 1971). Continued growth during periods of food stress suggests that either shell deposition is relatively inexpensive in energetic terms (Palmer, 1981) or that its regulation is reduced.

Growth rates were measured for juveniles maintained under standardised laboratory conditions, so differences in growth between samples from different sites were not necessarily the outcome of differences in habitat or allocation to reproduction. They also demonstrated a heritable component in growth rate variation. Higher growth rates can lead to a shorter juvenile stage or larger body size at maturity, depending on whether maturity is size- or age dependent, and so influence the subsequent fecundity and mortality schedules of the parents and offspring. For example, larger females tend to be more fecund (Roff, 1992; Stearns, 1992). For reasons previously discussed (p. 253/54), growth rates may therefore reflect the evolutionary outcome of differences in age or size-specific mortality rates and fecundity.

Phenotypic variation among genotypes

Differences in growth and feeding behaviour between genotypes were identified in this study. The hierarchy of genotypic differences in growth rate (sections 2.3.6 and 3.3.2) agreed with that predicted by site allele frequencies and the overall 'population' differences in growth rate.

Higher tissue growth rates were recorded for Prawle Point whelks possessing the *Lap-2*$^{10.10}$, *Mdh-1*$^{10.10}$ or the *Pep-1*$^{10.11}$ genotypes, which predominated in the Peartree Point samples. Whelks with the 9-alleles for *Lap-2* and *Mdh-1*, or the *Pep-1*$^{10.10}$ genotype grew more slowly. This hierarchy of genotypic differences was repeated for shell growth, feeding rate and growth efficiency in the laboratory for whelks collected from Prawle (section 3.3.2); high growth rates coincided with higher feeding rates, and higher tissue and shell growth efficiencies.

Variation between genotypes mainly occurred in Prawle Point samples; variation among *Lap-2* and *Mdh-1* genotypes was not identified for whelks from Peartree Point, perhaps reflecting the low incidence of the 9-alleles at this site. The concordance of genotype and phenotype was more apparent for shore collected samples suggesting that the phenotypic response among genotypes was enhanced under shore conditions. Alternatively, these loci may have been either markers to loci affecting growth or possibly only partially responsible for the physiological variability. The number of successive physiological measurements undertaken for each whelk in some experiments reduced sample numbers to the extent that the observed lack of association could be a statistical artefact. Sample numbers, however, were sufficient to identify overall 'population' differences, suggesting again that the clinal loci are markers linked to the physiological variation. Biopsy methods to enable the preselection of specific genotypes would be preferable to the reliance upon sufficient numbers within each genotypic class for *a posteriori* comparisons. The development of a suitable technique would be worthwhile; juveniles differing in allelic variation at the clinal loci could be compared within families (juveniles hatching from one or connected capsules, see section 4.2) whose juveniles are likely to have a greater similarity in genetic background than that of random samples of juveniles.

CONCLUSIONS

Dog-whelks from Peartree Point grow faster than Prawle Point juveniles, and the variation between samples varies according to genetic composition. These differences are likely to reflect variation in local selection pressures along this coast, as growth rate directly affects the fitness of an individual particularly under periods of stress. Temperature, desiccation and food availability are potential stresses that vary between sites in this study. Dog-whelks have a low

food availability at Prawle Point as there are comparatively few barnacles and mussels (McCarter and Thomas, 1980, pers. obs.). Whelks are frequently observed eating non-preferred prey such as limpets and top-shells at Prawle Point (Kirby et al., 1994; pers. obs.); this behaviour occurs when preferred prey are rare or absent (Crothers, 1985) and suggests some degree of food stress at this site. The high maximum air temperatures and desiccation rates (chapter 3) are also likely to affect dog-whelk behaviour and reduce feeding rates (Largen, 1967b; Bayne and Scullard, 1978b; Burrows and Hughes, 1989; chapter 4, this study).

Individuals with faster growth rates under *ad libitum* rations tend to be more adversely affected when food stressed than individuals with lower growth rates. Moreover, such animals often need to venture away from protected refuges more frequently to meet increased energy demands, and so incur a higher risk of mortality through predation, dislodgement or physiological stress (Burrows and Hughes, 1989; Hughes and Burrows, 1994). Consistent with this interpretation, Peartree Point juveniles have a more energetically expensive physiology when starved: juveniles tended to deposit more shell, and in one sample lost more tissue weight than juveniles reared from Prawle. When ambient seawater temperatures were increased to 20°C, starved Peartree Point whelks exhibited higher energetic losses (respiration and excretion rates), although growth rates were similar to those from Prawle Point. When the sample size was large, genotypic differences among Prawle Point juveniles indicated that shell growth during starvation was higher for juveniles with the *Lap-2* 10-allele (common at Peartree Point) than the 9-allele (common at Prawle Point). The reduction in feeding ration generally reduced the growth differences between sites, which were found when juveniles were fed *ad libitum*: Peartree Point juveniles had a greater reduction in tissue growth, whereas Prawle Point juveniles showed a greater reduction in relative shell growth rates (table 4.3.7).

The site-differences in tissue growth rate (chapters 2, 3 and 4) were not obvious for juveniles fed *ad libitum* at 20°C; marginally higher growth rates for Peartree Point juveniles are explained by higher feeding rates at this temperature. Tissue growth rates were also similar for Peartree Point and Prawle Point juveniles when maintained on the standardised low ration at this temperature. The site differences in tissue growth rate were therefore reduced at the higher temperature, even though Prawle Point whelks continued to exhibit higher relative shell growth

rates. Variation in feeding rate between Peartree Point and Prawle Point juveniles was also found when juveniles were subjected to a simulated tidal cycle with high temperatures and low humidities during aerial exposure; Prawle Point juveniles ate less during periods of thermal stress. Although the long-term effects of such a behaviour and its significance can only be guessed, reduced feeding bouts could confer a higher short-term fitness when the risk of physiological stress is high. Shell shape variation and its assumed adaptive significance (discussed above p251/2) may be important in influencing the behaviour of dog-whelks subjected to the thermal stresses encountered at Prawle Point.

The association of genetic and phenotypic variation with environment in *N. lapillus*

The association between phenotype (shell shape and physiology), genetic composition and environmental variation presented in this thesis is suggestive of differential selection along this coastline, and concurs with the results of previous studies in this region (Day, 1990; Kirby *et al.*, 1994a, b; Kirby and Berry, in prep., reviewed in chapter one). The concordance of genetic and environmental variation over wider geographical scales along the South Coast of England also suggests that this association was unlikely to have arisen solely due to chance effects (although a series of founder events along this coastline cannot be dismissed). If genetic variation is not merely an outcome of random effects and reduced gene flow, which may be acting to some degree considering the reduced dispersal capacity of this species, then it may reflect the selection of specific phenotypes at points along this region of coast (Endler, 1977). Temperature and food availability (either due to local prey abundance or prevailing temperature regime influencing behaviour) were identified to be important environmental pressures in this study (chapter 3 and 4). The phenotypic differences between sites could therefore represent contrasting strategies in accordance to these local differences in environment.

The differences among laboratory-reared juveniles indicate a genetic component to the phenotypic variation as the experiments were conducted under standardised laboratory conditions. Although a direct association between growth and genotype was found, variation at the clinal loci was not sufficient to explain the overall 'population' differences. This suggests

that genetic differences linked to the observed clinal loci are likely to be involved. Chromosomal variation (reviewed in White, 1978), and possible interaction effects among loci (Clarke, 1966), could also act to accentuate differentiation along this region of coast. Observations of hybrids in natural populations of *N. lapillus* suggest at least partial fertility (Bantock and Cockayne, 1975). The karyotypic differences observed between the two sites would therefore tend to favour the establishment of coadapted gene complexes particularly if located on chromosomes involved in the numerical polymorphism. Breeding experiments are needed, not only to determine whether life history variation covaries with genotype, but also to provide information on the viability and fecundity of hybrid karyotypes, and the inheritance of clinal loci and associated phenotypic traits.

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