INTRA-SPECIFIC VARIATIONS IN THE LIFE-HISTORY TRAITS OF TWO LACUNIDS (GASTROPODA : PROSOBRANCHIA)

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A Thesis Submitted for the Degree of PhD
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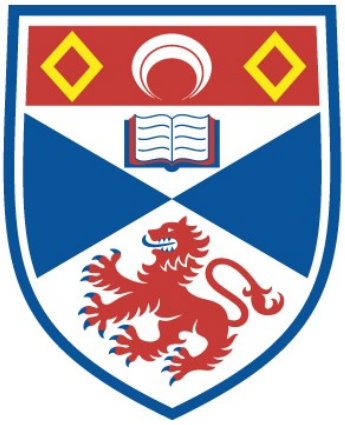
University of St Andrews



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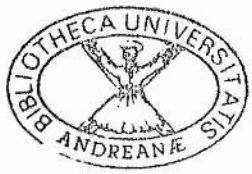

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ABSTRACT

Within life-history trait variations for two herbivorous intertidal lacunids, *Lacuna pallidula*, a direct developer and *Lacuna vincta* a planktotroph, were compared and related to their ecology and to marine invertebrate life-history theory. Aspects of life-history theory covered included; reproductive investment, the relationship between egg size and egg fecundity, the Egg-Juvenile-Period (EJP), the implications of egg size for offspring status and maternal effects.

Similar patterns of growth and reproductive investment were observed for adult females of both species, although absolute rates of growth and reproduction were differently affected by macroalgal diet. Further, differences in response to the favourability of macroalgal diet were observed for the two species, notably in the manner in which eggs were packaged.

Variations in both egg size and egg numbers in spawn masses were observed for the two species, among populations within both species and within *Lacuna pallidula* populations. For *Lacuna pallidula*, these variations were shown to be mediated by both maternal macroalgal diet and population source. Maternal diet directly affected the size of hatching offspring but not the size of eggs produced. Consequently, egg size was not a good indicator of hatching size for either species.

The EJP was determined for both species for a range of temperatures. Greater variations in the EJP and juvenile size were observed in *Lacuna vincta*. This was attributed to the ability of the larvae of this species to delay metamorphosis and to display positive growth during the delay phase. Both temperature and microalgal diet were shown to affect patterns of growth and development in *Lacuna vincta* larvae.

Sources of naturally occurring cues for inducing settlement and metamorphosis in *Lacuna vincta* larvae were investigated to understand further the distribution patterns of this species on macroalgal types. Extension of the work investigated the suitability of various artificial cues for inducing metamorphosis and the effects of larval age and nutritional status of larvae upon latency of response to established inducing cues.

Overall, *L. pallidula* displayed greater variation in traits and was more sensitive to environmental change than *L. vincta*. This finding is discussed in light of the two species larval strategy.

CHAPTER 1

GENERAL INTRODUCTION

1.1. The Lacunidae

The family Lacunidae belongs to a Superfamily of prosobranch molluscs, the Littorinacea, which are regarded as being close to the base of the mesogastropod stem (Barnes, 1987). The definitive morphological characteristics of the Lacunidae are their small size (5-10mm adult shell length), thin transparent periostracum, large aperture and a hollow umbilicus, the latter feature being that from which the Lacunidae derive their name (Fretter and Graham, 1980). The Lacunidae are typically marine, short-lived and herbivorous.

To date, four species of lacunid have been documented in the British Isles, *Lacuna vincta* (Montague, 1803), *Lacuna pallidula* (da Costa, 1778), *Lacuna parva* (da Costa, 1778) and *Lacuna crassior* (Montague, 1803) (Fretter and Graham, 1980). *L. parva* and *L. crassior* have received little attention (Ockleman and Nielson, 1981) because they are both rare. However, *L. vincta* and *L. pallidula* are seasonally abundant and have been studied extensively (e.g. Smith, 1973). Previous investigations have concerned observations of the spawn mass (Goodwin, 1979) and sexual dimorphism of *L. pallidula* (Gallien and Laramburgue, 1938) and of the veliger larvae and population ecology of *L. vincta* (Fretter, 1972; Waddell, 1973; Fralick *et al.*, 1974; Russell-Hunter and McMahon, 1975; Fretter and Manly, 1977; Thomas and Page, 1983; Maney and Ebersole, 1990; Martel and Chia, 1991a). Other studies have directly and explicitly compared the population ecology and reproduction energetics of *L. pallidula* and *L. vincta* (Smith, 1973; Grahame, 1977, 1982, 1985, 1994; Southgate, 1982).

Interest in comparing the biology of *Lacuna vincta* and *Lacuna pallidula* arises partly from the observed similarities in their distribution and habitat. Both species occur on sheltered to moderately exposed rocky shores, extending from LWST into the sublittoral, and are associated with various macroalgal species upon which they graze and spawn (e.g. Smith, 1973; Southgate, 1982; Grahame,

1985). Further, both species attain similar adult size and display similar seasonal and annual life-cycles (see Tables 1.1. and 1.2.). However, despite these similarities, *L. vincta* and *L. pallidula* display very different larval strategies. *L. vincta* produces many more and smaller eggs which hatch as planktotrophic veliger larvae, whereas *L. pallidula* produces fewer and larger lecithotrophic eggs which hatch as benthic juveniles. These two species therefore have been deemed suitable for comparative studies, testing hypotheses for relationships between larval type and other life-history traits (e.g. Grahame, 1977, 1982, 1994). The overall aim of the present work was to compare the phentotypic variation in traits which have been previously investigated for these two species and to examine some which have not been addressed specifically for these two species before.

1.2. Selection for larval type

Marine invertebrates display many different larval types which are categorised on the basis of whether they are pelagic or non-pelagic in their development and on the basis of their nutritional status; i.e. feeding or non-feeding (e.g. Thorson, 1946, 1950; see Levin and Bridges, 1995 for review). The majority of species can be classified as either having pelagic feeding larvae or non-pelagic, non-feeding larvae, as is the situation in the present study, although many variations of this simple classification are known (e.g. Eckert, 1995).

The evolution of larval types has received close attention over the last fifty years. To date, evidence does not unambiguously point towards any one larval type as being the ancestral form (see Salvin-Plawen, 1985; Strathmann, 1990, 1993). However, notwithstanding these difficulties, the general consensus is that the transitions between feeding and non-feeding and pelagic and non-pelagic development have been unidirectional and that non-feeding and non-pelagic development are derived conditions (see Strathmann, 1978). In view of this, much study has been dedicated to understanding the selective processes which may have been involved in these transitions and why some species have conserved the ancestral larval form (e.g. Strathmann, 1978; 1985, 1986; Hadfield and Iaea, 1989; Kempf and Todd, 1989). Consequently, the advantages and disadvantages which are conveyed by these different larval types have been speculated upon (see Grahame and Branch, 1985; Strathmann,

Table 1.1. Data for the life-cycle of *Lacuna vincta* obtained from previous studies.

Parameter		Location	Reference
Egg diameter (μm)	108 - 128	Plymouth, S. England	Lebour, 1937
	160 - 180	Cork, S. Ireland	Southgate, 1982
	110	Laboratory, N.E. England	Grahame, 1977
	94 - 125	Whitburn, N. E. England	Smith, 1973
Egg / spawn mass	1209	Whitburn, N. E. England	Smith, 1973
	1087	Laboratory, N.E. England	Grahame, 1977
	1050	Cork, S. Ireland	Southgate, 1982
Spawning period	Jan - June	Whitburn, N. E. England	Smith, 1973
	Jan - July	Robin Hood's Bay, N.E. England	Grahame, 1986
	Jan - March	Plymouth, S. England	Fretter and Manly, 1977
	Jan - May	Plymouth, S. England	Lebour, 1937
	Jan - Mar	Cork, S. Ireland	Southgate, 1982
	Mar - June	Woods Hole, USA	Russell-Hunter and McMahon, 1975
	Jan - Oct	Isefjord, Denmark	Ramussen, 1973
	June - August	New Brunswick, Canada	Thomas and Page, 1983
	Continuous	Maine, USA	Maney and Ebersole, 1990
	Continuous	Vancouver, British Columbia	Martel and Chia, 1991
	Continuous	California, USA	Langan, 1984
	March - July	White Sea	Milekovsky, 1970
	March - August	The Sound	Thorsen, 1946
Settlement period	June - Oct	Whitburn, N. E. England	Smith, 1973
	July - Sept	Robin Hood's Bay, N.E. England	Grahame, 1986
	May - July	Plymouth, S. England	Fretter and Manly, 1977
	May - July	Woods Hole, USA	Russell-Hunter and McHanon, 1975
	May - July	Cork, S. Ireland	Southgate, 1982
	All year round	Subtidal, Maine, USA	Maney and Ebersole, 1990
	April - September	Vancouver, British Columbia	Martel and Chia, 1991
Egg to hatching	26 Days	Heligoland, Germany	Hertling, 1927
	15 Days	Plymouth, S. England	Lebour, 1937
	2.5 - 3.5 weeks	Vancouver, Canada	Martel and Chia, 1991
Larval period	7 - 9 weeks	Vancouver, Canada	Martel and Chia, 1991
	2 - 3 months	Whitburn, N. E. England	Smith, 1973
	1 - 2 months	Philidephia USA	Waddell, 1973
	2 months	New Brunswick, Canada	Thomas and Page, 1983
	38 - 55 days	California, USA	Langan 1984
Juvenile size (μm)	550	Plymouth, S. England	Fretter and Manly, 1977
	450	Cork, S. Ireland	Southgate, 1982
	450 - 800	Intertidal, S. England	Fretter and shale, 1973
	450	Plymouth, S. England	Lebour, 1937

Table 1.2. Data for the life-cycle of *Lacuna pallidula* obtained from previous studies.

Parameter		Location	Reference
Egg diameter (μm)	270 - 300	The Sound	Thorsen, 1946
	270 - 280	Isefjord, Denmark	Ramussen, 1973
	250 - 297	Whitburn, N. E. England	Smith, 1973
	303 - 324	Aberystwyth, Wales	Goodwin, 1979
	271 - 279	Aberystwyth, Wales	Goodwin, 1979
	254 - 256	Heligoland, Germany	Hertling and Ankel, 1927
	263 - 269	Robin Hood's Bay, N.E. England	Grahame, 1977
	254 - 276	Wimereux, France	Pelseneer, 1910
Egg/spawn mass	49 (13 - 75)	The Sound	Thorsen, 1946
mean (range)	62 (15 - 113)	Whitburn, N. E. England	Smith, 1973
	91 (52 - 105)	Robin Hood's Bay, N.E. England	Grahame, 1977
	139 (69 - 247)	Aberystwyth, Wales	Goodwin, 1979
	133 (72 - 263)	Aberystwyth, Wales	Goodwin, 1979
	80 (43 - 133)	Isle of Cumbrae, Scotland	Goodwin, 1979
	110 - 125	Wimereux, France	Pelseneer, 1910
	123	Intertidal, S. Ireland	Southgate, 1982
	(9 - 60)	Isefjord, Denmark	Ramussen, 1973
	(60 - 150)	Heligoland, Germany	Hertling and Ankel, 1927
Spawning period	Jan - May	Whitburn, N. E. England	Smith, 1973
	Jan - May	Robin Hood's Bay, N.E. England	Grahame, 1986
	Jan - April	Intertidal, S. Ireland	Southgate, 1982
	Jan - Mar		Goodwin, 1979
	Continuous	White Sea	Kuznetsov, 1963
Egg to hatching	8.5 - 14 weeks	Whitburn, N. E. England	Smith, 1973
Settlement period	April - July	Whitburn, N. E. England	Smith, 1973
	April - July	Intertidal, S. Ireland	Southgate, 1982
	May - July	Aberystwyth, Wales	Goodwin, 1979
Hatching size (μm)	700 - 750	The Sound	Thorsen, 1946
	700	Whitburn, N. E. England	Smith, 1973

1990, 1993; Havenhand, 1995). Theoretical considerations of the respective advantages of these larval types have stressed the importance of dispersal, gene-flow, energetics, predation, adult body size, phylogeny and trophic relationships (see Day and McEdward, 1984; Grahame and Branch, 1985; Strathmann, 1986 for reviews).

1.3. Trade-offs between life-history traits

Another important consideration has been the relationships which might exist between larval type and other life-history traits that organisms display (e.g. Roughgarden, 1989; Havenhand, 1993). An unusually marked clustering of life-history traits is observed in marine species (see Grahame and Branch, 1985; Havenhand, 1995 for reviews). This has been attributed to the trade-offs which exist between the traits an organism displays. For example, increasing the somatic growth rate will reduce reproductive output, and producing larger offspring will result in the production of fewer offspring (Stearns 1989, 1992; Strathmann, 1990). Hence, selection is thought not to act upon individual traits *per se* but upon combinations of these traits, resulting in the steady fixation of selectively advantageous suites of traits (Stearns, 1976, 1977, 1989, 1992; Ebenman, 1992). The associations between larval strategy and other life-history traits in marine species have received particularly close attention because larval type has been shown to have many implications both for the demographic patterns of later stages and for the magnitude of gene flow between geographically seperated populations.

1.4. Reproductive investment and larval type

The amount of energy that an organism invests into reproducing offspring, or the relative amount thereof, has been considered to be an important component of life-history strategy (see Grahame and Branch, 1985). In view of the variation in the energy invested in eggs producing different larval types, marine ecologists have investigated relationships between reproductive investment and larval type as an explanation for the evolution of larval type and, alternatively, as a constraint for the evolution of larval type (Chia, 1974; Menge, 1975; Grahame, 1977, 1982, 1994; Todd, 1979; Havenhand and Todd, 1988a, b; Strathmann, 1990). An important facet in this area of work has

been parental size, which is considered to affect directly the absolute amount of resources available for reproduction (e.g. Chia, 1974; Todd, 1979; Havenhand and Todd, 1988b). A consistent pattern has not been shown (Todd, 1985; Strathmann, 1986) but it has been suggested that this may be partly attributable to variations in methods for measuring absolute and relative reproductive investment (Havenhand and Todd, 1989).

1.5. Duration of life-cycle and larval type

More recently the relationship between larval type and duration of the Egg-Juvenile-Period (EJP) has been considered (e.g. Roughgarden, 1989; Havenhand, 1993). This is because the duration of the EJP has been shown to vary for different larval types and to greatly affect the demographics of later stages in the life-cycle of some molluscs (e.g. Miller and Hadfield, 1990; Miller, 1993). The EJP can potentially vary in different larval types, notably because of the delay capabilities of some pelagic larvae (e.g. Kempf, 1981) and because the importance of environmental conditions during the larval phase can vary for species displaying different larval types (Sinervo, 1990).

1.6. Egg size and larval type

Another much studied aspect in larval strategy theory has arisen from the commonly observed relationship between egg size and larval type. In an early model, Vance (1973a, b) investigated the consequences of egg size for larval type and for the survival of offspring hatching from variously sized eggs. Since then there has been much discussion regarding Vance's model and variations in egg size and their consequences for offspring status have been studied within species to test hypotheses regarding the importance of egg size for the selection of larval strategy (e.g. Sinervo, 1990; McEdward and Chia, 1991; Clarke and Gore, 1992). More recently, the importance of environmental conditions, such as food availability, upon the expression of egg size and egg production have been addressed (e.g. George *et al.*, 1991; George, 1994).

1.7. Intraspecific variation in traits

Close attention has been given to the causes of intraspecific variation in quantitative traits of marine species. Important questions in this field include the extent to which phenotypic variation in life-history traits is heritable, the extent to which covarying traits are genetically correlated and, most relevant to the present work, the interactions of these covariances with the environment (Levin and Huggett, 1990; Levin *et al.*, 1991).

In some cases these variations have been attributed to the selection of traits for populations in differing environmental conditions and the heritability of these traits has been demonstrated on several occasions (e.g. Mashiko, 1990; Levin *et al.*, 1991). However, in other studies it has been shown that such variations can be attributed to differences in environmental conditions acting to prevent the full expression of the genotype. For example, several studies have shown that changes in parental diet can greatly affect the phenotypic expression of traits (e.g. George *et al.*, 1991; George, 1994). It is assumed here that such variation is attributed to processes operating upon ecological rather than evolutionary timescales (Parker and Begon, 1986).

Consequently, Willows (1990), among others, has suggested that selection for traits will also be influenced by variations in environmental conditions which are acting on ecological timescales. For example, Todd (1979) suggested that the unpredictability of *Adalaria proxima* individuals obtaining resources for reproduction may have been important for the selection of lecithotrophy in this species. Perhaps more importantly it therefore might be expected that the impact of environmental conditions and their implications for the phenotypic expression of traits may differ for species which display different reproductive and larval strategies. One of the aims of the present work was to assess the importance of environmental conditions upon the expression of traits in *Lacuna vincta* and *Lacuna pallidula*.

1.8. Previous studies on the growth and reproduction in *Lacuna*

Several studies have already compared the growth, reproduction and respiration for *Lacuna pallidula* and *Lacuna vincta* to determine relationships between reproductive effort (RE) (see Chapter II) and larval type (Grahame, 1977, 1982, 1994). Grahame (1977) compared the reproductive output (RO) for these two species in terms of (1) the ratio of spawn weight to body weight and (2) the time for cumulative spawn weight to exceed body weight. Experimental animals were obtained from a single population in Robin Hood's Bay (NE England) and were maintained in the laboratory on a diet of *Fucus serratus*. For both measures, RO for *L. vincta* was significantly greater than for *L. pallidula*. However, significant differences in the mean number of eggs per spawn mass and rates of spawn production (weight) were reported for both species, with *L. pallidula* displaying the greater intraspecific variability.

Todd and Havenhand (1983) analysed the data from Grahame's earlier experiment and found that whilst a positive allometric relationship existed between maximum body size and total reproductive output for the larger species, *L. pallidula*, no allometric relationship existed for *L. vincta*. This suggested that parental size was more important for increasing reproductive output for *L. pallidula* than for *L. vincta*. Another important result from Grahame's earlier work was that females of both species continued to grow during the spawning period, albeit at a reduced rate. This is in marked contrast to some nudibranchs which generally degrow following first reproduction (Havenhand and Todd, 1988a, but see Hall and Todd, 1986).

In a further analysis, Grahame constructed partial energy budgets during the spawning period for both species (Grahame, 1982). Animals were maintained in the laboratory at constant temperature (10 °C). Grahame reported that the reproductive effort in the two species, using this method, was much closer than previously estimated. This was attributed to the higher energy turnover per unit mass of soma in *L. vincta*. Grahame (1982) also calculated the absolute amount of energy invested in reproduction and concluded that, energetically, both larval strategies were an option for both

species. He concluded, therefore, that the larval strategies displayed by *L. pallidula* and *L. vincta* were governed by demographic rather than energetic constraints.

Grahame (1994) published energy budgets for both species which extended back to the pre-spawning period. Animals were maintained in the laboratory at either 10 or 5 $^{\circ}$C, depending upon the time of their collection, and were fed *Fucus serratus*. He reported that both species displayed depressed respiration rates at the onset of spawning but that the two species were differently affected by temperature. Grahame also manipulated the availability of sperm to spawning females and found that the energy budget changes in females denied access to males were different in the two species. When denied access to mates, *Lacuna pallidula* females reduced their growth rates at spawning and began to produce infertile spawn, whereas *Lacuna vincta* females showed a smaller reduction in growth rates but did not produce any spawn.

1.9. Aims

The aims of the present work were to:

- To compare patterns of growth and reproduction between *Lacuna pallidula* and *Lacuna vincta* fed on a range of macroalgae species.
- To compare the variation in the EJP between *L. pallidula* and *L. vincta*.
- To compare the variation in egg size, egg allocation in spawn masses and juvenile size between *L. pallidula* and *L. vincta*, both among and within populations, and to compare relationships for these three life-history traits between these two species.
- To compare the effects of maternal diet upon the phenotypic expression of egg size, egg allocation and juvenile size between the two species and between *L. pallidula* populations.
- To determine naturally occurring sources for inducing settlement of *L. vincta* larvae to understand the distribution of this species during the early juvenile stages of this species life-cycle.

CHAPTER 2

The effects of macroalgal diet upon female adult growth and reproductive investment.

2.1. INTRODUCTION

2.1.1. Energetic considerations

The energy that is assimilated by an organism through feeding is finite and must be divided amongst maintenance demands (ATP or respiratory demand) and production (somatic growth and reproduction). The manner in which an organism allocates assimilated energy to each of these life processes is conveniently studied by constructing simple energy budgets.

$$\text{i.e. } \mathbf{C = P + R + U + F,}$$

where C is energy consumption, P is production (both somatic and reproductive), R is respiration, U is excretory waste, and F is faeces (Crisp, 1971). These budget components may be expressed joules, or in quantities of specific elements such as carbon or nitrogen (see Aldridge, 1982).

Clarke (1987) has suggested a modification to the respiration term in the above formula which takes account of the ontogenetic changes in maintenance demand (e.g. Zuethen, 1953; Grahame, 1994), and of adaptations to temperature. The respiration term in the formula is separated into four components which represent the amount of energy required to fuel (1) basal metabolism, Rb, (2) mechanical work, Ra, and the conversion of assimilated energy into (3) somatic tissue, Rs, and (4) reproductive tissue, Rr. U and F remain as above.

$$\text{i.e. } \mathbf{C = \underline{Ps + Rs} + \underline{Pr + Rr} + \underline{Rb} + \underline{Ra} + U + F.}$$

Each underlined section represents a separate physiological sink for which energy allocation should be measured.

The relative amount of assimilated energy invested in reproduction affects the amount of energy available for other life-history traits such as growth (Fisher, 1930; Stearns, 1992). Such trade-offs are deemed to place constraints upon selection for the maximisation of each individual life-history trait and it is the trade-offs themselves which are considered to be under natural selection (e.g. MacArthur and Wilson, 1967; Murphy, 1968; Vance, 1973a, b; Pianka, 1970, 1974; Stearns 1976, 1977, 1992). Hence, individual life-history traits are assumed not to evolve independently but to covary, resulting in the steady fixation of selectively advantageous combinations (Ebenman, 1992).

Much interest has focused on correlating reproductive investment to investment in other life-history traits especially for species which display contrasting suites of life-history traits (e.g. Menge, 1975; Grahame, 1977, 1982, 1994; Todd, 1979; Yoshioka, 1986). Areas of particular interest include reproductive pattern (semelparity or iteroparity, within annual, biennial or perennial life-cycles), the onset of maturation and larval strategy (planktotrophy, lecithotrophy and brooding).

2.1.2. Measuring reproductive investment

The relative amount of assimilated energy that an organism apportions to reproduction (Reproductive Effort, RE) has been measured by several methods. The disparity in methodology and terminology in the literature results from the difficulties in measuring true RE and to disagreements over the validity of using more accessible alternatives.

The most theoretically accepted method of measuring RE is to determine:

$$RE = \frac{(Pr + Rr + Rab)}{A}$$

Here, Rab is the energy allocated to any behavioural activity associated with reproduction and A is the assimilated energy (Tinkle, 1969; Hirschfield and Tinkle, 1975; Tinkle and Hadley, 1975). Several workers (e.g. Thompson, 1982; George, 1994) have simplified this formula to give a more practical working model;

$$RE = Pr / (Pr + Pg + R).$$

This simplification is particularly appropriate for those sessile species which do not brood their young and for those species which do not show elaborate courting behaviour (Parry, 1982). However, because of the technical and practical difficulties in assessing RE in terms of energy budgets few studies have been undertaken and presented (e.g. Tinkle and Hadley, 1975; Calow and Woolhead, 1977; Calow, 1978b, 1979; Hart and Begon, 1982; Thompson, 1982; Grahame, 1982, 1994; Woolhead, 1983; Todd and Havenhand, 1988). The majority of workers have opted for more accessible methods of questionable validity. The most popular and easier alternative is to determine the Reproductive Output (RO) (Pianka, 1970, 1976; Browne and Russell-Hunter, 1978; Hughes and Roberts, 1980; Russell-Hunter and Romana, 1981 for suggested modifications of the RO formula). RO can be calculated by:

$$RO = Pr / B,$$

whereby B is the energy equivalent of the mean biomass of the individual, or by:

$$RO = Pr / (Pr + Pg).$$

There are several ways in which parameters for the determination of RO have been measured (e.g. Grahame, 1973a, b, 1977; Menge, 1974, 1975; Todd, 1979; Hughes and Roberts, 1980; Perron, 1982). Pr has been presented as gonad weight and offspring weight. B has been presented variously as body weight at first spawning, final body weight and the geometric mean body weight (GM), depending upon the growth pattern of the organism under study. These parameters have been quantified in units of mass (wet weight, dry weight and ash free dry weight) and as joule equivalents.

The appropriateness and validity of methods which estimate the true RE have been examined by Todd and Havenhand (1987). A particular problem is that the parental body weight of many species

changes during the reproductive period. In some cases individuals gain weight or grow (e.g. Grahame, 1977), while in other species, especially in nudibranchs, individuals lose weight or 'degrow' (e.g. Todd, 1979). Todd and Havenhand (1988) therefore proposed a new method of estimating RE, specifically designed to estimate RE for two nudibranch species which degrow following the onset of spawning and which they termed the Reproductive Index (RI). This is given by,

$$RI = \frac{Pr + Ps}{W}$$

Here, W is the energy equivalent of body weight immediately after each spawning event. They suggested that RI measurements for each spawning event could be plotted against time to give a dynamic analysis of RE which can range from negative to positive. They further suggested that summing RI values throughout the spawning period will give an overall performance value (ΣRI)for any given individual.

Whilst the determination of RO is widely accepted and implemented, there are a number of features which must be considered when relating this measure to true RE (e.g. see Todd and Havenhand, 1983; Grahame 1982; Clarke, 1987) (1) RO assumes that there is an isometric relationship between body size (B) and reproductive investment (Pr) (Calow, 1979, 1983). Different relationships have been found and in some cases there has been no relationship at all (e.g. Spight and Emlen, 1976; Todd 1979; Todd and Havenhand, 1983; Grahame, 1994) (2) In cases in which RO is determined by mass, it is assumed that the energy value per unit weight of reproductive material is the same as somatic tissue. Measurements by different weight parameters have sometimes given contradictory results (e.g. Picken, 1980) (3) Unlike RE, RO is not affected by changes in respiratory rate (Calow, 1983, 1985), which in turn is affected by other factors such as temperature (Clarke, 1987). Clarke (1987) demonstrated that RO is not a valid comparative measure of RE particularly when

considering organisms from differing latitude and hence contrasting environmental temperature regimes. However, he suggested that when comparative studies are undertaken for species from similar thermal regimes, and whose size and ecology are similar, differences between respiratory rates may be negligible and hence RO may well be a valid indicator of true RE. However, some studies have demonstrated significant variations in respiratory rates in comparable species and also have found differences in their respiratory response to variations in temperature regime (e.g. Hirschfield and Tinkle, 1975; Grahame, 1982, 1994).

2.1.3. The implications of reproductive investment for life-history theory of marine invertebrates

Somatic growth and reproduction can be regarded as sinks competing for energy which is surplus to maintenance requirements (Gadgil and Bossert, 1970; Calow, 1985; Sibly and Calow, 1986). The manner in which this energy surplus is allocated to growth and reproduction is related to the Darwinian fitness of an organism and has therefore been a central concept in the development of life-history theory (Tinkle, 1969; Pianka, 1970; Stearns, 1976, 1977; Todd, 1985; Havenhand, 1995). For example, whilst a larger allocation of energy resources to a single reproduction event may support the production of a larger number of offspring, concomitant reductions in somatic growth and parental survival probability will reduce the chances of producing offspring in the future (Fisher, 1930). If, on the other hand, a larger proportion of energy is allocated to further growth, fewer offspring might be produced but there will be a greater probability of parental survivorship and future reproduction.

These two scenarios describe semelparity and iteroparity, respectively which are important components of marine invertebrate life-history theory. They emphasise that there are trade-offs between a large commitment in reproduction and future reproductive potential. Only a restricted number of species have been shown to display a life history in which such a trade-off does not apparently exist (e.g. high reproductive effort, early reproduction and long life; see Calow, 1979).

Various models have been constructed to describe the selection for iteroparity and semelparity in marine invertebrates. The best known of these is r-K selection theory. The r-K selection model considers trade-offs selected for when present in an expanding population (density independent regulation) or in a population which has reached its full carrying capacity (density dependent regulation) and where competition for available resources is intense (MacArthur and Wilson, 1967; Pianka, 1970, 1974, 1976; Stearns, 1976, 1977, 1992). Selection for r and K is mutually antagonistic and involves trade-offs between increasing numbers (r) and efficiency of resource utilisation (K). For individuals in an expanding population, greater representation in subsequent generations is achieved by a relatively high reproductive effort (semelparity) early in life because a greater number of offspring can take advantage of a resource for which there is less competition. On the other hand, the offspring of individuals in a population at full carrying capacity will be subject to greater competition and will therefore have less chance of gaining adequate resources. The optimal strategy for individuals in a population at full carrying capacity may be to compromise their reproductive effort and have greater residual reproductive fitness (iteroparity). This model has been widely used to describe the selection for iteroparity and semelparity but is not without criticism, primarily because it does not consider demographic patterns of mortality and it does not consider mortality caused, for example, by harsh environmental conditions (Stearns, 1992). It is therefore perhaps not surprising to find that the r-K selection model does not conform to results obtained in a large proportion of studies (see Stearns, 1992, for review).

Bet-hedging theory (Murphy, 1968; Stearns, 1976) proposes that the relative mortality rates of juveniles and adults in a population comprise an important selection pressure upon lifetime RE. For example, in a population for which there is a relatively higher adult mortality rate, selection for a high reproductive output early in the life-cycle (semelparity) would be advantageous. In a population in which there is a relatively higher juvenile mortality rate, the optimal strategy would be to exert a lower reproductive effort and have greater chances of surviving to produce more offspring in the future (iteroparity).

2.1.4. The onset of sexual maturity

Body size at reproduction also has been considered in reproductive strategy theory. Most organisms have to grow and develop before they can begin to channel energy into reproduction. The optimum body size at which an organism should begin to channel energy into reproduction has been considered using a simple energetic argument. It is thought that the amount of energy available for production increases (though not infinitely) with increasing body size because although both energy assimilation and maintenance costs increase with increasing body size, maintenance costs increase at a decreasing rate with increasing body size. The body size at which there is the greatest difference between the amount of energy assimilated and the amount of energy required for maintenance has been suggested to be the optimal body size (W_{opt}) at which an organism should begin to channel surplus energy into reproduction (Calow, 1979). There are, however, additional constraints which have been considered. These are;

(1) As noted above, life-history models, such as r-K selection theory, include iteroparity and semelparity in their formulations. In the r-K selection model, r-selected populations are assumed to begin to reproduce early in their life-cycle and K-selected populations are assumed to reproduce relatively later in their life-cycle. Hence body size at reproduction in r-selected populations may be smaller than the optimal body size predicted in energetic models. In contrast, in K-selected populations, the onset of maturity may exceed the predicted optimal body size if the act of reproduction at an earlier age puts the parent at risk.

(2) The model assumes that mortality is independent of body size. As in bet-hedging theory, whenever an individual has low probability of surviving to W_{opt} the switch to reproduction at a relatively smaller body size would be selected for.

(3) The body size required to accommodate the gonad may exceed W_{opt}.

(4) The efficiency with which energy is converted into reproductive tissues is often higher than the efficiency with which it is converted into somatic tissue (Calow, 1979). In this situation, a relatively smaller body size would be selected for.

(5) In some species, the switch from growth to reproduction is marked whereas in others the change is gradual; some species continue to grow (e.g. Grahame, 1977) while others begin to degrow (Todd, 1979).

In conclusion, while energetic models are useful for emphasising that trade-offs exist in allocating energy into life-traits such as growth and reproduction, they cannot alone explain the selection of reproductive strategy (Grahame and Branch, 1985; Grant, 1989). Demographic factors also have important implications for the selection of reproductive investment. Nevertheless, close attention has been given to applying these energetic models to species which display different larval types.

2.1.5. The implications of energetic constraints upon selection for invertebrate larval strategy

The aspect of life-history theory most relevant to the present work is the potential relationship between reproductive investment and larval strategy. It is generally accepted that the planktotrophic larval form is ancestral and that the lecithotrophic larval form is an evolutionary derivative (Strathmann, 1978, 1985). Hence much interest has focused upon forces that might govern selection towards lecithotrophy in some species whilst conserving the ancestral larval form in others. It has been suggested that some larval types may be more costly to produce than others and that this may place an important constraint upon the evolution of larval strategy. This suggestion has been addressed in several review papers (e.g. Strathmann, 1985; Grahame and Branch, 1985; Todd, 1985; Havenhand, 1995) and in a number of empirical studies (e.g. Menge, 1974, 1975; Grahame, 1977, 1982, 1994; Clarke, 1979; Todd, 1979; Hughes and Roberts, 1980; Perron, 1982; Parry, 1982; Havenhand and Todd, 1988a, b; Todd and Havenhand, 1988) which have revealed no consistent pattern (see Christiansen and Fenchel, 1979; Todd and Havenhand, 1983; Todd, 1985; Strathmann,

1986 for reviews). For example, Vance (1973a, b) postulated that the energy costs of reproduction would be greatest in species which produced lecithotrophic eggs because the cost of producing at least one lecithotrophic egg which would successfully recruit to the next generation would greatly exceed the energy required for a species producing planktotrophic larvae to undertake the same task. In contrast, Crisp (1974) and Chia (1974) proposed that the energy costs of reproduction for species producing planktotrophic larvae would be greater because very large numbers of offspring would have to be produced to offset pelagic mortality. Vance's (1973) model was supported in a study by Menge (1974) (see also Perron, 1982) who reported that the reproductive cost in *Leptasterias hexactis*, which broods its offspring, was greater than for a larger sized species, *Pisaster ochraceus*, which produces planktotrophic larvae. However, Grahame (1977, 1982) demonstrated that the reproductive effort in two species of *Lacuna*, which display contrasting larval strategies, was approximately the same.

Todd (1979) compared the reproductive effort of two ecologically similar and comparable species of nudibranchs which had contrasting larval strategies. *Adalaria proxima* attains a greater adult size and produces lecithotrophic larvae. *Onchidoris muricata* is smaller and produces many, less well nourished eggs which hatch as planktotrophic larvae. Todd (1979) found that whilst RE was greater for individual *O. muricata*, a greater absolute amount of energy was invested by *A. proxima* individuals. Todd (1979) also reported that there was a direct relationship between parental size and reproductive output in *O. muricata* but that this relationship was lacking for *A. proxima*. He suggested that, energetically, *A. proxima* had both larval strategies open to it but had been selected for lecithotrophy to offset the individuals' unpredictability of allocating sufficient resources to produce a viable number of planktotrophic eggs.

Parental size has been one of the major considerations in this theory, partly because of the commonly observed prevalence of brooding in species with small parental size (e.g. Chia, 1974; Strathmann and Strathmann, 1982; Hess, 1993). The amount of energy available for reproduction is deemed to

vary in individuals which attain different parental body sizes. Generally, larger individuals are deemed to have greater energy resources available for reproduction than smaller individuals. Chia (1974) therefore suggested that the absolute amount of energy that an individual can invest in reproduction may be an important determinant of larval strategy because the production of different sized eggs would have variable energetic costs (Todd, 1979; Underwood, 1979). However, again, the relationship between parental size and larval mode has not proved to be consistent, even in studies which have involved very closely related and sympatric species (see Grahame and Branch, 1985; Todd, 1985; Strathmann, 1986 for reviews).

For example, Grahame (1977, 1982) calculated the absolute energy values apportioned to reproduction in *Lacuna vincta*, a planktotroph, and *Lacuna pallidula,* a lecithotroph, and showed that, despite *L. pallidula* being the larger of the two, the absolute amount of energy apportioned into reproduction was very similar. He also reported that *Lacuna parva,* which is much smaller than either of the above *Lacuna* species produced lecithotrophic eggs.

Various explanations have been proposed for these inconsistencies. Underwood (1979) has suggested that there may be two size thresholds operating on the selection for larval type in prosobranchs and that individuals of different body sizes have possibly different options of larval strategy available to them. Grahame and Branch (1985) suggested that studies on the larval strategies of meiofaunal species may be useful for testing Underwood's hypothesis. However they concluded that observed inconsistencies in this relationship were because parental size is a relative measurement among species and proposed that demographic constraints may be more important than energetic constraints in governing the selection for larval strategy in some species.

Strathmann and Strathmann (1982) also came to similar conclusions when considering the prevalence of brooding in species with small adult size (Chia, 1974; Chaffee and Lindberg, 1986; Roughgarden, 1989; Hess, 1993). They proposed that brooding of offspring would become less

favourable with increasing parental size because while total reproductive output is proportional to length^3, brood area is proportional to length^2. Thus the reproductive output of large individuals would be constrained by the available brood area: larger individuals would not be able to brood all the offspring they could produce. Evidence for this allometric-constraint hypothesis comes from various studies, especially those concerned with brooding echinoderm species (e.g. Strathmann *et al.*, 1984).

Hess (1993) pointed out that the outcome of results in these studies is dependent upon the application of appropriate regression techniques. She noted that previous studies had used Model I regressions to determine allometric-constraint relationships which assume that there is no error in the x-variate and that the x and y variates are independent of each other. Hess (1993) using Model II regressions, which are more appropriate, re-analysed data from previous studies and found no evidence for allometric constraint of brood size. Hess (1993) therefore proposed an alternative explanation for the limitation of brood size in small species. She noted that larger broods may be more difficult to ventilate and that this frequently results in extended development times of offspring or smaller juveniles. Hess (1993) suggested that this placed a constraint upon the number of broods a larger species could produce if it sequentially spawned throughout the spawning season.

2.1.6. Latitudinal and trophic constraints for reproductive investment

Variations in parental nutrition and environmental conditions can also be a potential source for observed inconsistencies in theoretically derived relationships and indeed for variations in reproductive output and reproductive effort within a species.

The amount of energy available for production is surplus to the amount of energy required for maintenance and is dependent upon the amount of energy assimilated from feeding. Maintenance demands vary (e.g. with temperature) and the amount of energy assimilated is dependent upon resource availability and quality. Thus, individuals from geographically separate populations of the same species can display different amounts of energy available for production. As such, reproductive

effort can also vary. Intraspecific variations in RE caused by variations in temperature regime and parental nutrition may outweigh any perceived interspecific variations (e.g. Parry, 1982).

Todd (1979, 1987) suggested that selection therefore may operate upon fixed responses to conditions of variable resource availability. For example, for individuals which continue to grow while reproducing, thresholds of rationing are necessary to maintain both growth and reproduction. However, during periods of low resource availability, further growth may be sacrificed to maintain threshold levels required for reproduction, thereby increasing RE (Emlet *et al.*, 1987; Xu and Barker, 1990; George *et al.*, 1991). On the other hand, an increase in resource availability may result in a greater absolute energy input in both growth and reproduction but may, or may not, result in greater RE (e.g. Thompson, 1982; Hall and Todd, 1986). These assertions are supported by various studies for species of fish (Reznik, 1983), lizards (Ballinger, 1977; Siegel and Ford, 1992), nematodes (Scheimer *et. al.*, 1980), echinoderms (Thompson, 1982) and molluscs (Hall and Todd, 1986; Todd, 1987).

2.1.7. Rationale

Willows (1990) proposed that individuals which are able to maximise their life-time reproductive investment under various potential environmental conditions would be selected for. Havenhand (1995) therefore suggested that if larval type were at all influenced by reproductive allocation, it would most likely respond to variability in that allocation rather than to fixed differences in levels of reproductive investment.

Whilst a considerable amount of data concerning the reproductive energetics of *Lacuna pallidula* and *Lacuna vincta* have been collected (Grahame, 1977, 1982; 1994), the effects of parental nutrition upon growth and reproduction have not yet been investigated. This perhaps is very relevant to obtaining realistic estimations of reproductive investment in these two species because they graze upon various macroalgal species. Adult *L. pallidula* are generally confined to *Fucus serratus*, even

when this macroalga is relatively scarce (Smith, 1973, Grahame, 1977, 1985), but have also been collected from laminarians and various red algal turfs (Smith, 1973; Goodwin, 1979; Southgate, 1982; pers. obs.). *L. vincta* is associated primarily with laminarians but can also be collected from fucoids and red algal turfs in substantial numbers (Smith, 1973; Grahame, 1977, 1985; Southgate, 1982; pers. obs.).

The aim of the work presented in this chapter was to quantify the variability in growth and reproductive investment for populations of *Lacuna vincta* and *Lacuna pallidula* fed on a range of macroalgal diets and to relate these measurements to observed macroalgal preferences. Variability in patterns of spawn production with respect to diet were also compared for both species.

2.2. MATERIALS AND METHODS

2.2.1. General

Lacuna vincta and *Lacuna pallidula* were collected from *Fucus serratus* (L.) and *Laminaria digitata* (Hudson) at low water of spring tides from Kingsbarns, Fife, Scotland, on 1 December 1993, before the spawning period had commenced. Individuals were sexed and allocated to a mating partner at random. Mating pairs were placed in mesh cages (Toby 'Teaboys', Aldridge Plastics Ltd.), which were maintained in two flow-through seawater tanks, at ambient seawater temperature (4.5°C - 7.0°C from 1 December 1993 to 4 April 1994). Cages were held in plastic frames and were submerged throughout the experiment. Ambient photoperiod was set throughout the entire experiment. Males that died during the experiment were replaced to ensure that the females would have a continual supply of sperm to fertilise their eggs. The experiment was terminated on 4 April 1994.

2.2.2. Experimental design

One of four macroalgal species, *Laminaria digitata*, *Fucus serratus*, *Fucus vesiculosus* (L.) or *Mastocarpus stellata* (Stackhouse), was provided *ad libitum* to mated pairs throughout the experimental period. Each diet treatment had six replicate pairs of both species. Replicate treatments were randomly distributed in the two experimental tanks and had no set position throughout the experiment.

2.2.3. Measurement of growth

Growth of female snails was estimated throughout the experiment by using two separate methods, based on measurements of shell length (see Grahame, 1977), and on weight under water (see Havenhand and Todd, 1988a, b). Shell lengths were measured to the nearest 0.01 mm using a Wild M8 binocular microscope. For *Lacuna pallidula*, the measurement was taken from the anterior margin of the shell lip to the posterior margin of the body whorl. For *Lacuna vincta*, the measurement was taken from the anterior margin of the shell lip to the apex of the spire (see Calow,

1975a; Grahame, 1977). Weights under water were measured on a Mettler ME22 analytical microbalance to the nearest 0.01mg. Shell lengths and body weights under water were taken weekly prior to spawning, at least fortnightly thereafter and immediately after each spawning. Estimations of ash free dry body weight (AFDW) were obtained from calibrations relating AFDW to shell lengths and weights under water. For calibrations, female *Lacuna pallidula* (40) and female *Lacuna vincta* (44) were collected from Kingsbarns, Fife, Scotland, measured, rinsed in 0.9% (w/v) isotonic ammonium formate (NH_4HCO_2) to remove excess salts and deep frozen (-20°C). Samples were dried in a vacuum freeze dryer (Chemlab) for 16 h, weighed for dry weight, and then placed in a muffle furnace at 520 °C for 5 h. Estimations of AFDW were then calculated by subtracting the ashed dry weight from the dry weight of each sample. Model I regression equations were obtained for both species and the size range of snails used in the calibrations ensured that all estimations of AFDW could be made without extrapolation. Regression equations involving shell length were determined for log transformed data. The data were then re-analysed to obtain Model II regression equations since both variables were prone to natural variation and measurement error (see Ricker, 1973; Havenhand and Todd, 1988b). The new gradient (v2) was calculated by dividing the slope from the model I regession equation (v1) by its correlation coefficient (r). The intercept (u) was then calculated by

$$\mathbf{u = mean\ y - mean\ x\ (v2)}$$

2.2.4. Analysis of growth data

To observe patterns of growth, data were analysed by calculating individual weight-specific growth rates (WSGR) (mg (growth).mg (GM body weight) $^{-1}$.day^{-1}) (GM = geometric mean) for each interval between measurements. WSGR was determined by

$$\frac{t_2 - t_1}{\text{antilog } 0.5\ (\log t_2 + \log t_2)} \text{ /Days}$$

Where t_1 is body weight at first measurement, t_2 is body weight at second measurement, 'Days' is the number of days between measurements.

2.2.5. Measurement of reproductive investment

Reproductive output was assessed in terms of the number of spawn masses produced, the total AFDW of spawn masses (mg), the total number of eggs produced and the mean daily rates of production thereof. Cages were examined daily and any newly laid spawn masses were removed. The AFDW and the number of eggs for each spawn mass were estimated from calibrations relating to non-destructive measurement parameters (Grahame, 1977). For *Lacuna pallidula* spawn masses, estimations of spawn mass weight were made from direct counting of the number of eggs. For *Lacuna vincta* spawn masses, estimations of AFDW and egg number were determined from the 'mean' spawn mass diameter (see Grahame, 1977). Measurements were taken within 24 h of the spawn mass being deposited to minimise over-estimating values due to swelling of the spawn masses with water. Spawn masses for calibrations were obtained from females (Kingbarns) which were maintained on a mixed macroalgal diet in the laboratory. 41 *L. pallidula* spawn masses and 35 *L. vincta* spawn masses were used and the size range of spawn masses for calibrations ensured that all estimations for experimental spawn masses were possible without extrapolation. 39 spawn masses produced by experimental *L. pallidula* females were sacrificed for determination of a seperate calibration for comparison.

2.2.6. Analysis of reproductive investment data

One objective of the data analysis was to take variations in maternal size into account (see Grahame, 1977; Havenhand and Todd, 1983). Differences between diets were analysed by ANCOVA when a significant allometric relationship between production and maternal body weight was obtained. In these cases, weight specific differences between females on different diets could be determined. When no common relationship existed, data were checked for normality and then analysed by ANOVA and Tukey's post-hoc test. The post-spawning geometric mean body weight (GM) was used in ANCOVAs for mean daily rates of production since snails continued to grow throughout the spawning period. GM was determined by the antilog of 0.5 ($\log t_2 + \log t_2$), where t_1 is maternal body weight at first spawning and t_2 is the final maternal body weight.

2.3. RESULTS

2.3.1. Measurement of growth

Model I and model II regression equations relating data for ash free dry body weight (AFDW) (mg) to data for shell length (mm) and weight under water (mg) are presented for both species (Tables 2.1. and 2.2.). All calibrations showed highly significant regression coefficients. The gradients of these relationships were within the range of those derived previously in both theoretical and empirical studies (Calow, 1975a; Grahame, 1977). AFDW's were estimated for each sample female using regression equations derived from both procedures. A Wilcoxon pairwise comparison test revealed no significant difference between the two sets of data in either species (*Lacuna pallidula*; T+=508, T-=312, $T_{2,40}$=264 at the 5% level; *Lacuna vincta* T+=506, T-=444, $T_{2,44}$=327 at the 5% level).

Estimations of AFDW derived from shell length measurements either increased or remained the same over time possibly because reductions in body weight can not result in 'degrowth' of the shell. However, estimations of AFDW derived from weight under water measurements increased, decreased or remained the same over time, reflecting changes both in body weight gain and loss and in egg production prior to spawning. Weight under water measurements therefore are perhaps more meaningful measures for quantifying relative allocations to reproduction output. On the other hand, shell length measurements are perhaps more appropriate when assessing true growth. Another source of variation in estimations of AFDW derived from shell length measurements may be attributable to intraspecific variation in shell geometry. In view of this, estimations for growth were derived from shell length measurements, while estimations for reproductive output were derived from weight under water measurements. Examples of growth patterns of experimental females in the various diet treatments, using the two methods, are presented for both species (Figures 2.1a-c. and 2.2a-c.). There were large variations in patterns of growth. In addition, estimations derived from the two methods differed somewhat at the onset of spawning. This was most noticeable in *Lacuna pallidula* females in the *Fucus serratus* treatment.

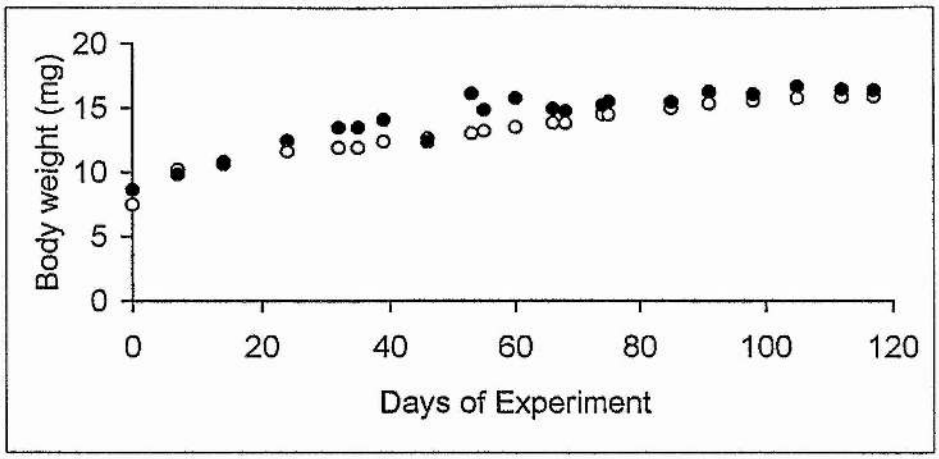
Table 2.1. ***Lacuna pallidula.*** Model I and II regressions for estimations of body weight (n = 40) showing values for the y-intercept, the fitted slope and the correlation coefficient (r) (AFDW = ash free dry weight and ADW =ashed dry weight)

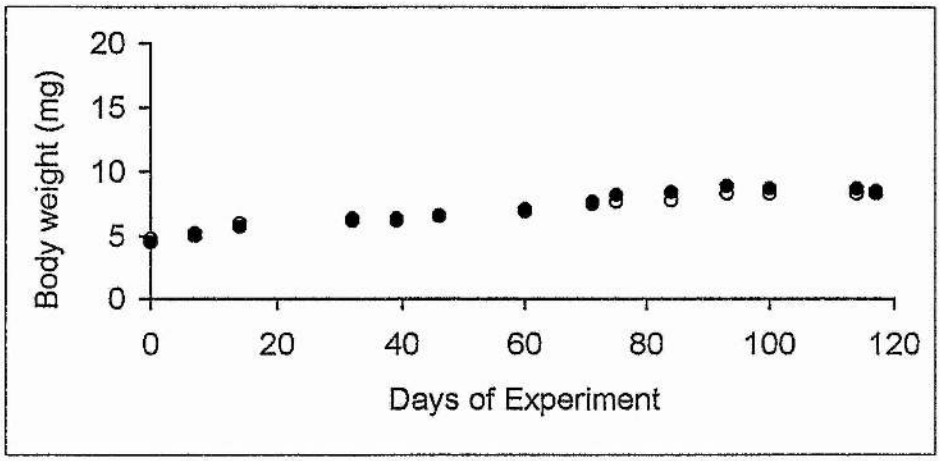
Relationship	Model I	r	Model II
Dry weight/weight under water	0.69 + 1.98	0.99	0.38 + 2.00
log dry weight/log shell length	(-) 0.91+ 2.82	0.99	(-) 0.92 + 2.86
AFDW/weight under water	2.19 + 0.52	0.93	1.56 + 0.56
Log AFDW/log shell length	(-) 0.74 + 2.06	0.92	(-) 0.88 + 2.23
Log AFDW/log shell length	(-) 0.74 + 2.06	0.92	(-) 0.88 + 2.23
ADW/weight underwater	0.14 + 1.41	0.98	(-) 0.76 + 1.45
Log ADW/log shell length	(-) 1.07 + 2.82	0.98	(-) 1.12 + 2.88

Table 2.2. ***Lacuna vincta.*** Model I and II regressions for estimations of body weight (n = 44) showing values for the y-intercept, the fitted slope and the correlation coefficient (r) (AFDW weight and ADW =ashed dry weight)

Relationship	Model I	r	Model II
Dry weight/weight under water	0.37 + 2.19	0.99	0.22 + 2.20
log dry weight/log shell length	(-) 0.72 + 2.46	0.98	(-) 0.75 + 2.51
AFDW/weight under water	0.35 + 0.86	0.98	0.20 + 0.88
Log AFDW/log shell length	(-) 0.10 + 2.31	0.97	(-) 1.05 + 2.39
ADW/weight underwater	0.30 + 1.32	0.99	(-) 0.24 + 1.33
Log ADW/log shell length	(-) 1.02 + 2.56	0.98	(-) 1.07 + 2.62

Figures 2. 1a - c. ***Lacuna pallidula.*** Examples of individual growth patterns of females in the various diet treatments (a, top, *Fucus serratus*; b, middle, *Laminaria digitata*; c, bottom, *Mastocarpus stellata*). Organic body weights were estimated from measurements of weights under water (closed circles) and from measurements of shell length (open circles).


Body weight (mg)
20
15
10
5
0
0
20
40
60
80
100
120
Days of Experiment


Body weight (mg)
20
15
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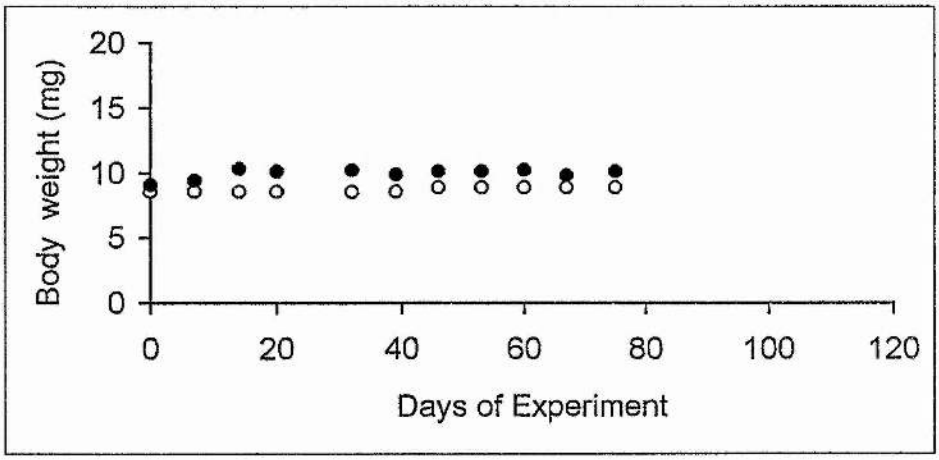
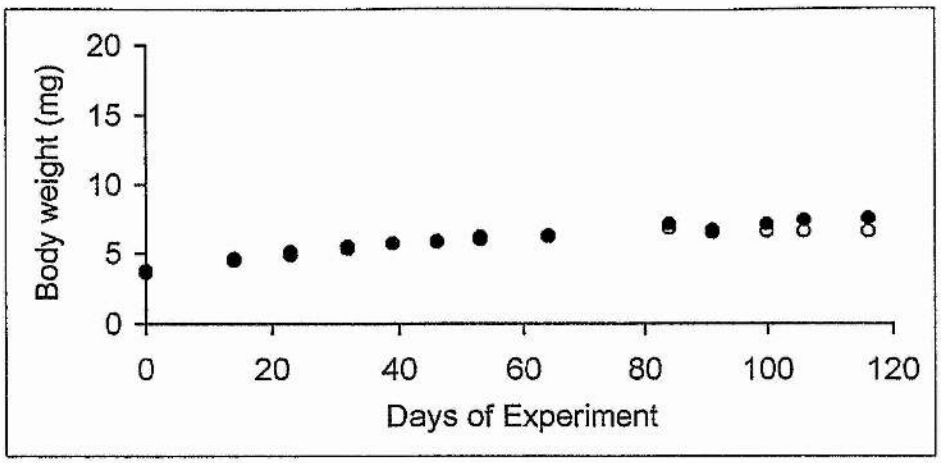
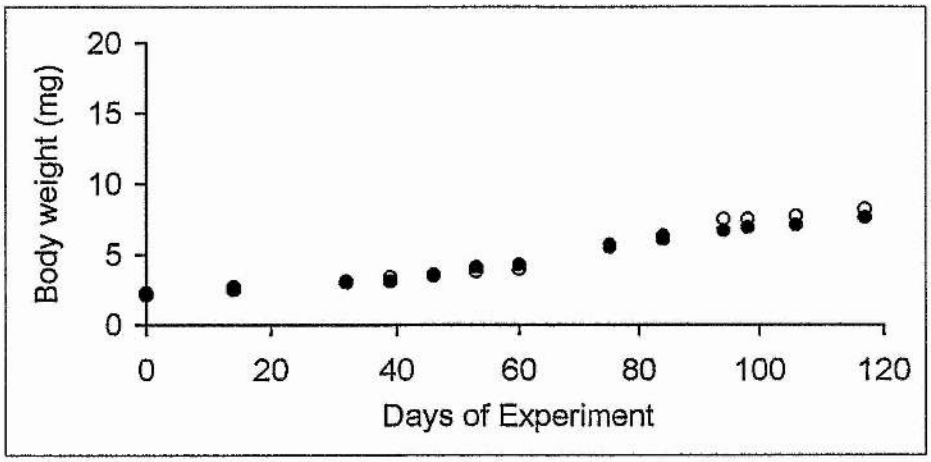
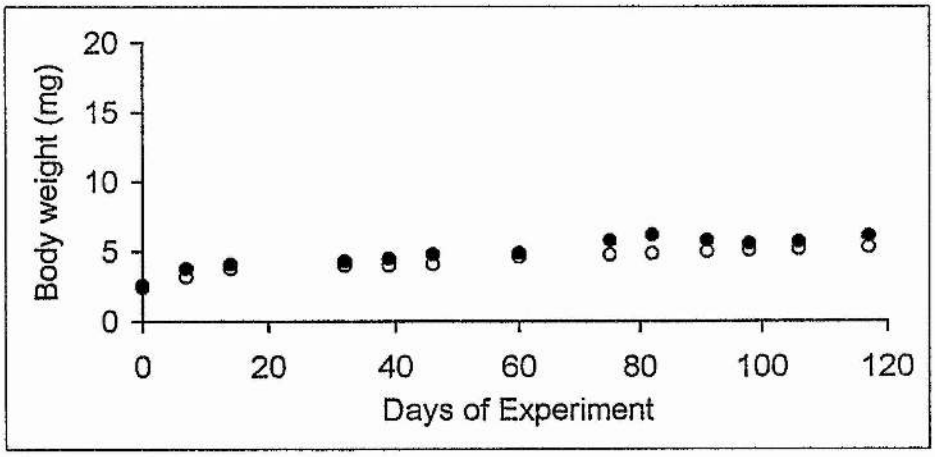
80
100
120
Days of Experiment


Body weight (mg)
20
15
10
5
0
0
20
40
60
80
100
120
Days of Experiment

Figures 2. 2a - c. ***Lacuna vincta.*** Examples of individual growth patterns of females in the various diet treatments (a, top, *Fucus serratus*; b, middle, *Laminaria digitata*; c, bottom, *Mastocarpus stellata*). Organic body weights were estimated from measurements of weights under water (closed circles) and from measurements of shell length (open circles).


Body weight (mg)
20
15
10
5
0
0
20
40
60
80
100
120
Days of Experiment


Body weight (mg)
20
15
10
5
0
0
20
40
60
80
100
120
Days of Experiment


Body weight (mg)
20
15
10
5
0
0
20
40
60
80
100
120
Days of Experiment

2.3.2. Survival and spawning

2.3.2.1. *Lacuna pallidula*

Data for survival and spawning of *Lacuna pallidula* females are presented in Table 2.3. All *Lacuna pallidula* females in the *Fucus serratus* and *Fucus vesiculosus* treatments survived and spawned during the experiment. All *L. pallidula* females in the *Laminaria digitata* treatment spawned but one female died after spawning twice. Only one *Lacuna pallidula* female in the *Mastocarpus stellata* treatment survived the experiment and none of the females in this treatment spawned.

2.3.2.2. *Lacuna vincta*

Data for survival and spawning of *Lacuna vincta* females are presented in Table 2.4. Although all females in the *Fucus serratus* and *Laminaria digitata* treatments spawned, two females from both treatments died before the end of the experiment. Three *Lacuna vincta* females in the *Fucus vesiculosus* died, all but one female spawned. Three *L. vincta* females in the *Mastocarpus stellata* treatment died and only four females spawned during the experiment.

Females that died during the experiment were included, where appropriate, in determinations of pre-spawning growth, body weight at first spawning, time to first spawning and mean weight of spawn mass produced.

2.3.3. Initial body weight, growth rates and WSGR's

The AFDW body weights of *Lacuna pallidula* females on 1 December ranged from 2.47 mg to 9.38 mg (mean = 4.6 mg). *Lacuna vincta* females were slightly smaller; body weights ranged from 1.34 mg to 5.92 mg (mean = 3.4 mg). One-way analyses of variance (ANOVA) did not show any significant differences in body weight among diet treatments at the beginning of the experiment for either species ($F_{3,20}$=0.18, P>0.05, $F_{3,20}$ = 0.63, P>0.05, respectively). Henceforth initial body weight refers to body weight at the time of collection.

Table 2.3. ***Lacuna pallidula.*** Data for percent survival, percent spawning, mean body weight and mean growth rates of females in the various diet treatments.

Diet	*Fucus serratus*	*Laminaria digitata*	*Fucus vesiculosus*	*Mastocarpus stellata*
% survival (n = 6)	100%	83%	100%	17%
% spawning (n =6)	100%	100%	100%	0%
Mean pre-spawning growth rate ($mg.day^{-1}$)	0.085 +/- 0.09	0.054 +/- 0.014	0.049 +/- 0.016	0
Mean body weight (mg) at first spawning	8.95 +/- 1.93	9.17 +/- 1.62	8.3 +/- 0.70	*
Mean post-spawning growth rate ($mg.day^{-1}$)	0.031 +/- 0.009	0.018 +/- 0.006	0.019 +/- 0.004	0
Final body weight (mg)	10.96 +/- 2.51	10.1 +/- 2.28	9.38 +/- 0.95	*
Days spawning	59.83 +/- 5.37	45.5 +/- 3.52	52.83 +/- 6.31	*

Table 2.4. ***Lacuna vincta.*** Data for percent survival, percent spawning, mean body weight and mean growth rates of females in the various diet treatments.

Diet	*Fucus serratus*	*Laminaria digitata*	*Fucus vesiculosus*	*Mastocarpus stellata*
% survival (n = 6)	66%	66%	50%	50%
% spawning (n =6)	100%	100%	84%	66%
Mean pre-spawning growth rate ($mg.day^{-1}$)	0.041 +/- 0.014	0.103 +/- 0.031	0.070 +/- 0.031	0.042 +/- 0.021
Mean body weight (mg) at first spawning	5.21 +/- 1.03	7.45 +/- 2.56	6.10 +/- 1.15	6.28 +/- 1.52
Mean post-spawning growth rate ($mg.day^{-1}$)	0.020 +/- 0.013	0.043 +/- 0.031	0.026 +/- 0.015	0.004 +/- 0.001
Final body weight (mg)	6.06 +/- 1.13	8.61 +/- 2.55	6.87 +/- 1.56	8.67 +/- 2.02
Days spawning	35.75 +/- 14.60	55.25 +/- 2.6	37.5 +/- 13.60	40.25 +/- 11.78

Mean pre-spawning and post-spawning growth rates ($mg.day^{-1}$) were determined for *Lacuna pallidula* and *Lacuna vincta* females in the various diet treatments (Tables 2.3. and 2.4.). Mean pre-spawning growth rates for females of both species in all diet treatments greatly exceeded mean post-spawning growth rates. Although growth rates were comparable for the two species there were clear differences between the two species with respect to response to diet treatment during the pre-spawning period. Whilst ANOVA revealed that the pre-spawning growth rates of *Lacuna pallidula* females in the *Fucus serratus* treatment were significantly greater than those in the other diet treatments, the pre-spawning growth rates of *Lacuna vincta* females in the *Laminaria digitata* treatment were significantly greater than those in the other diet treatments (see Tables 2.5. and 2.6.). Females of both species in the *Mastocarpus stellata* treatment displayed little (*L. vincta*) or no (*L. pallidula*) positive growth.

Mean WSGR's (mg (growth).mg $^{-1}$ (GM body weight).day $^{-1}$) were calculated for females of both species in the various diet treatments (Figures 2.3a - c and 2.4a - d.). As indicated by the data for mean growth rates, gradual declines in the mean WSGR's over time were observed in females of both species in all diet treatments. However, again, differences between species with respect to diet treatment were observed during the pre-spawning period. ANOVA revealed that the mean WSGRs of *Lacuna pallidula* females in the *Fucus serratus* diet treatment during the pre-spawning period were significantly greater than females in the other diet treatments ($F_{2,16} = 4.89$, $P<0.05$,). The mean WSGRs of *Lacuna vincta* females in the *Laminaria digitata* diet treatment during the pre-spawning period were also significantly greater than females in the other diet treatments ($F_{2,21} = 5.69$, $P<0.05$).

Large variations in the WSGRs were observed among females of both species within diet treatments (see Figures 2.3. and 2.4.). This may be due to variations in female body size (where the relationship between growth and body size is not isometric) or in stages of development. Mean pre-spawning and mean post-spawning growth rates were therefore plotted against the pre- or post-spawning geometric mean body weights of females (Figures 2.5., 2.6., 2.7. and 2.8.) and the data were analysed by ANCOVA (results for ANCOVA are given in Tables 2.5. and 2.6.).

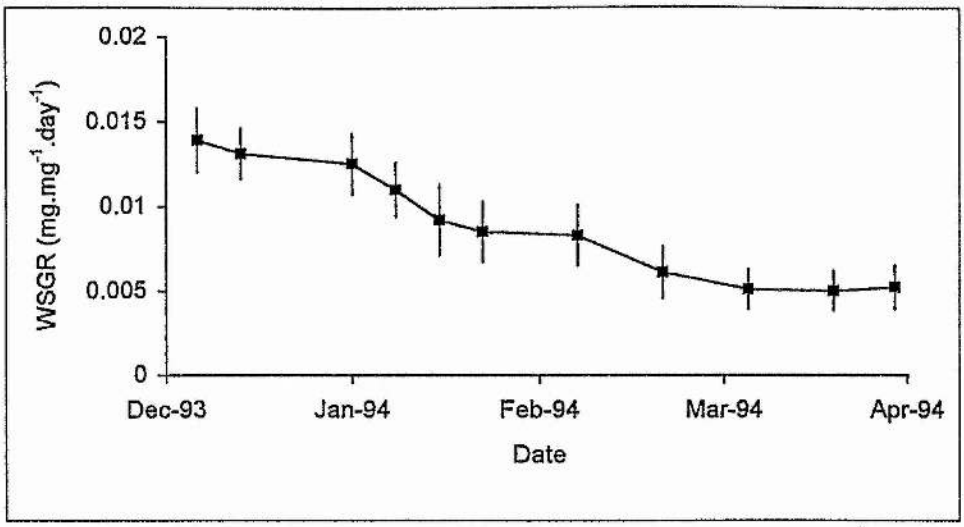
Table 2.5. ***Lacuna pallidula.*** Analysis of variance and covariance of growth data for females in the various diet treatments.

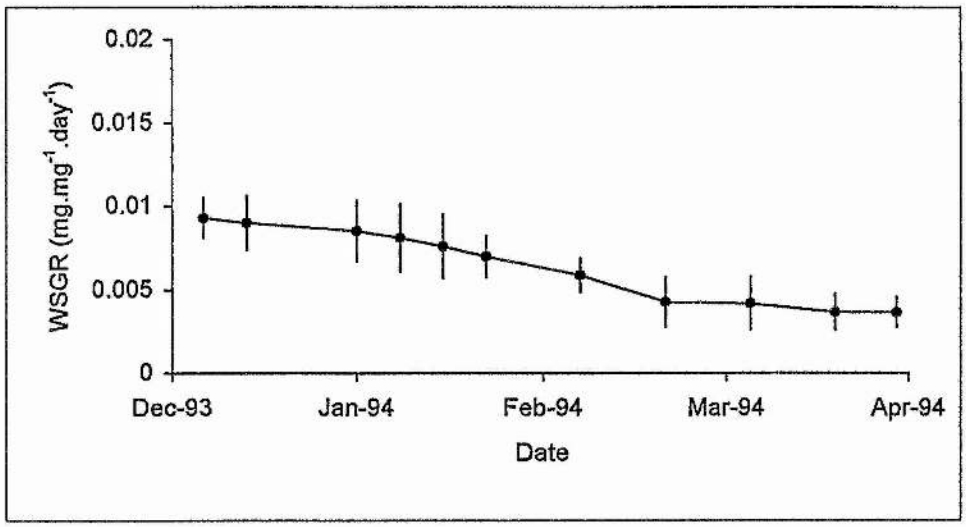
Relationship	ANCOVA		ANOVA	Tukeys HSD test
	Common slope	Elevation in slope		
Spawning body weight and initial body weight (mg)	$F_{1,16}$ = 112.93, P<0.05	$F_{2,15}$ = 0.50, P>0.05		
Days to first spawning and initial body weight (mg)	$F_{1,16}$ = 16.60, P<0.05	$F_{2,15}$ = 4.71, P<0.05		
Mean pre-spawing growth rates (mg.day^{-1}) and pre-spawning GM body weight (mg)	NS		$F_{2,16}$ = 4.57, P<0.05	Fs Fv Ld
Mean post-spawing growth rates (mg.day^{-1}) and post-spawning GM body weight (mg)	$F_{1,15}$ = 8.53, P<0.05	$F_{2,14}$ = 3.31, P>0.05		

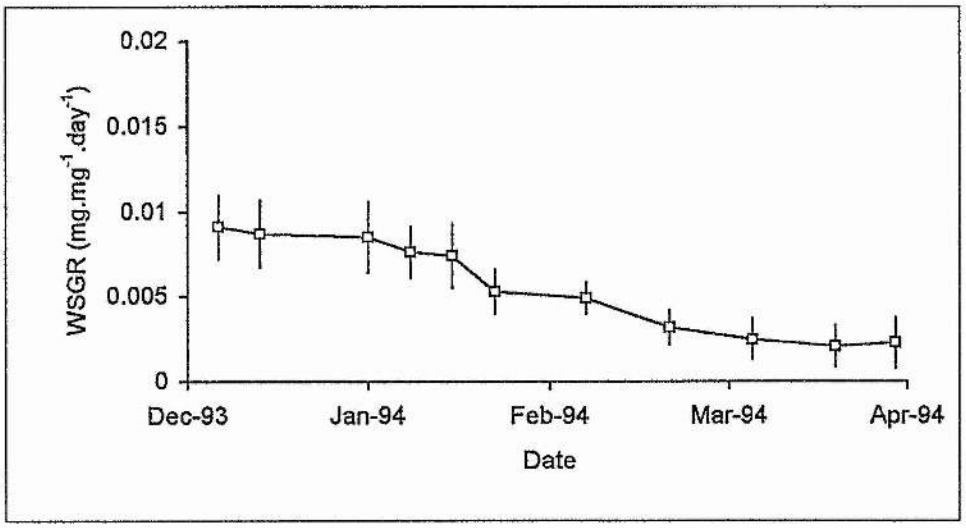
Table 2.6. ***Lacuna vincta.*** Analysis of variance and covariance of growth data for females in the various diet treatments.

Relationship	ANCOVA		ANOVA	Tukeys HSD test
	Common slope	Elevation in slope		
Spawning body weight and initial body weight (mg)	$F_{1,21}$ = 21.76, P<0.05	$F_{3,19}$ = 1.48, P>0.05		
Days to first spawning and initial body weight (mg)	$F_{1,21}$ = 4.68, P<0.05	$F_{3,19}$ = 0.40, P>0.05		
Mean pre-spawing growth rates (mg.day^{-1}) and pre-spawning GM body weight (mg)	$F_{2,21}$ = 5.99 P<0.05	$F_{3,21}$ = 5.48, P<0.05		
Mean post-spawing growth rates (mg.day^{-1}) and post-spawning GM body weight (mg)	$F_{2,12}$ = 6.35, P<0.05	$F_{3,10}$ = 2.21, P>0.05		

Figures 2. 3a - c. *Lacuna pallidula.* Mean weight-specific growth rates (WSGR) ($mg.mg^{-1}.day^{-1}$) (+/- S.E) (n=6) of females in the various diet treatments (a, top, *Fucus serratus*; b, middle, *Laminaria digitata*; c, bottom, *Fucus vesiculosus*).


0.02
0.015
0.01
0.005
0
WSGR (mg.mg-1.day-1)
Dec-93
Jan-94
Feb-94
Mar-94
Apr-94
Date


0.02
0.015
0.01
0.005
0
WSGR (mg.mg-1.day-1)
Dec-93
Jan-94
Feb-94
Mar-94
Apr-94
Date


0.02
0.015
0.01
0.005
0
WSGR (mg.mg-1.day-1)
Dec-93
Jan-94
Feb-94
Mar-94
Apr-94
Date

Figures 2. 4a - d. *Lacuna vincta.* Mean weight-specific growth rates (WSGR) (mg. mg $^{-1}$ .day^{-1}) (+/- S.E) (n=6) of females in the various diet treatments (a, top, *Fucus serratus*; b, second from top, *Laminaria digitata*; c, second from bottom, *Mastocarpus stellata*; d, bottom, *Fucus vesiculosus*).

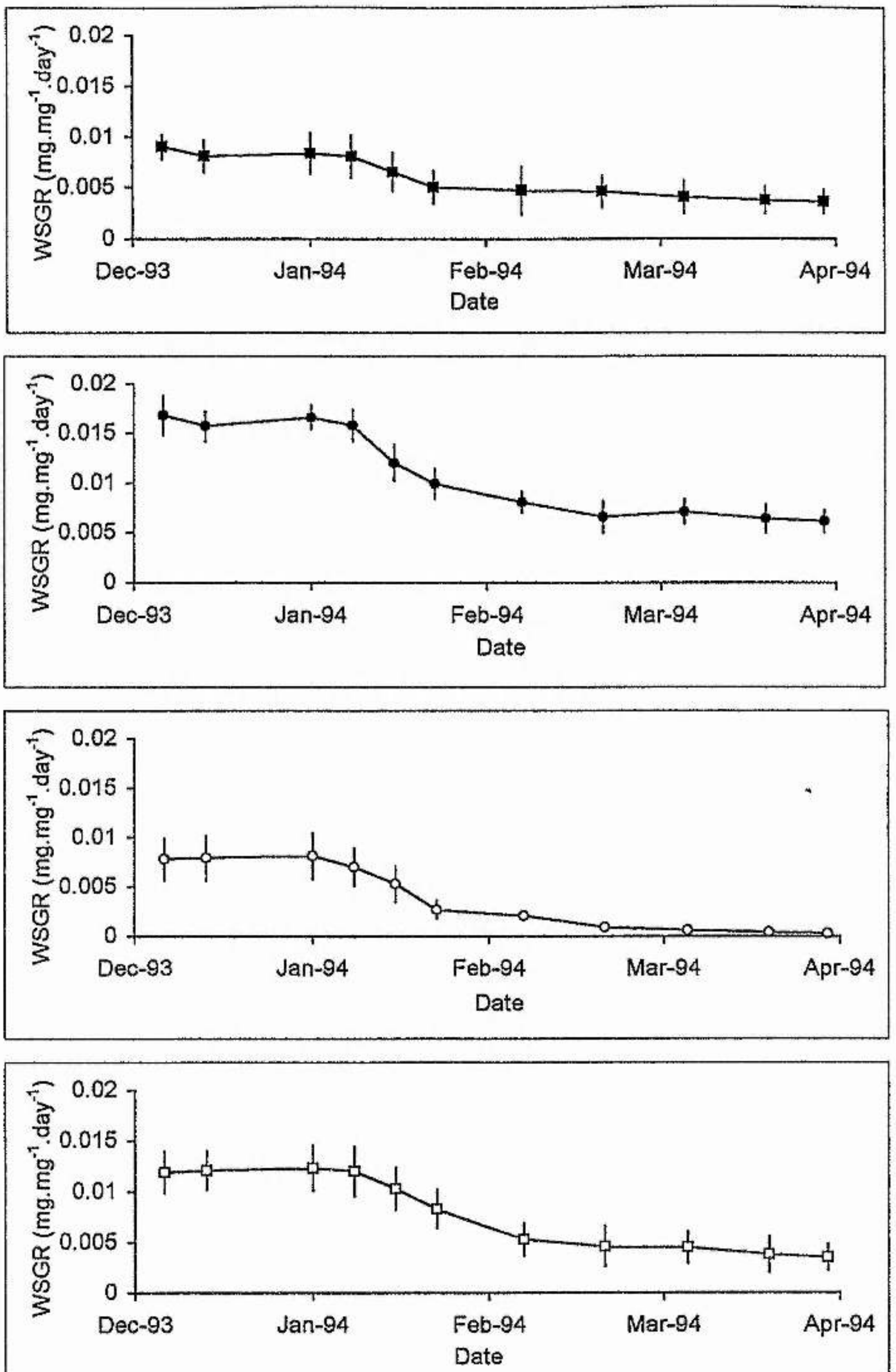
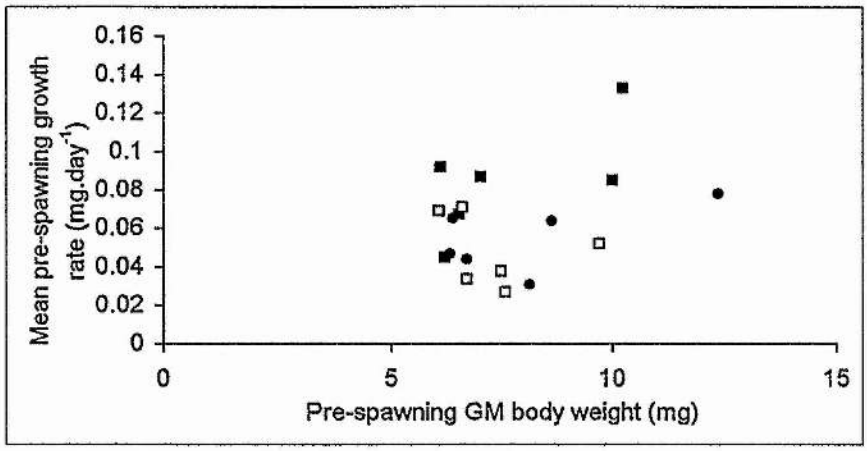

WSGR (mg.mg-1.day-1)
0.02
0.015
0.01
0.005
0
Dec-93
Jan-94
Feb-94
Mar-94
Apr-94
Date

Figure 2. 5. ***Lacuna pallidula.*** (top) Mean pre-spawning growth rates ($mg.day^{-1}$) of females in the various diet treatments as a function of the pre-spawning GM body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles and *Fucus vesiculosus,* open squares).

Figure 2. 6. ***Lacuna pallidula.*** (bottom) Mean post-spawning growth rates ($mg.day^{-1}$) of females in the various diet treatments as a function of the post-spawning GM body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles and *Fucus vesiculosus,* open squares).


Mean pre-spawning growth rate (mg.day-1)
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
5
10
15
Pre-spawning GM body weight (mg)

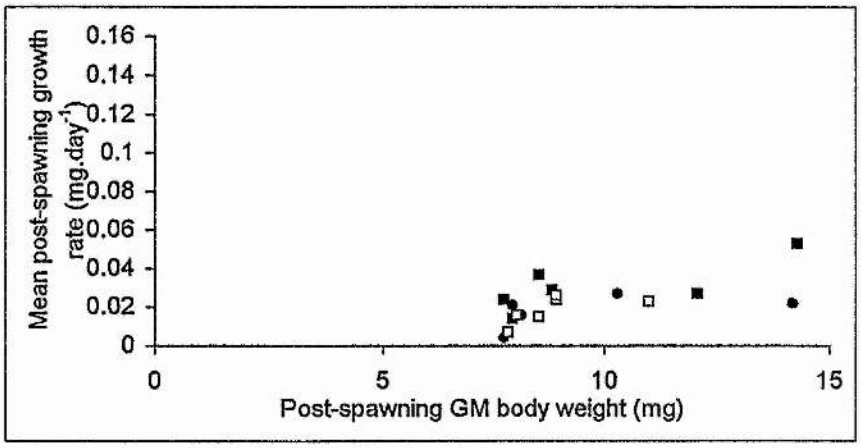
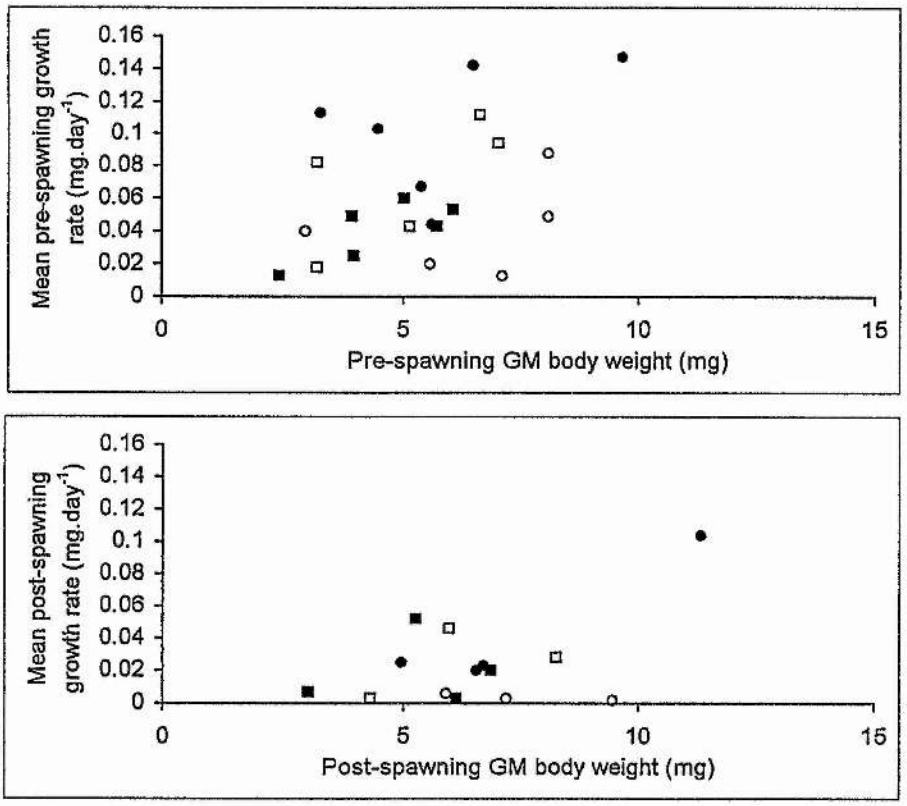

Mean post-spawning growth rate (mg.day-1)
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
5
10
15
Post-spawning GM body weight (mg)

Figure 2. 7. ***Lacuna vincta.*** (top) Mean pre-spawning growth rates ($mg.day^{-1}$) of females in the various diet treatments as a function of the pre-spawning GM body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles, *Fucus vesiculosus,* open squares and *Mastocarpus stellata,* open circles).

Figure 2. 8. ***Lacuna vincta.*** (bottom) Mean post-spawning growth rates ($mg.day^{-1}$) of females in the various diet treatments as a function of the post-spawning GM body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles, *Fucus vesiculosus,* open squares and *Mastocarpus stellata,* open circles).


Mean pre-spawning growth rate (mg.day-1)
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
5
10
15
Pre-spawning GM body weight (mg)
Mean post-spawning growth rate (mg.day-1)
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
5
10
15
Post-spawning GM body weight (mg)

ANCOVA did not reveal a significant common slope for the mean pre-spawning growth rates and the pre-spawning GM body weight for *Lacuna pallidula* females. However, a significant common positive relationship (coefficient = 0.003) between mean post-spawning growth rates and post-spawning GM body weights was shown. Further analysis revealed no significant difference in elevation of the slope with respect to diet treatment.

Unlike for *Lacuna pallidula*, ANCOVA showed a significant common positive slope during both periods for *Lacuna vincta* (coefficient = 0.004 and 0.003 respectively). Significantly different elevations with respect to diet were revealed for mean pre-spawning growth rates, but not for mean post-spawning growth rates. *Lacuna vincta* females in the *Laminaria digitata* diet treatment displayed significantly greater weight-specific pre-spawning growth rates than females in the other diet treatments.

To understand further the processes affecting the onset of spawning (i.e. is it influenced by body size, by diet or by season), body weights at first spawning and times (days after collection) to first spawning were plotted against initial body weights for both species (Figures 2.9. and 2.10.) and the data were analysed by ANCOVA (Tables 2.5. and 2.6.).

For both species, ANCOVA showed a significant common positive slope (coefficient was 0.9 for *Lacuna pallidula* and 1.1 for *Lacuna vincta*) for spawning body weight and initial body weight, but there were no significant variations in elevation of the slope with respect to diet treatment for either species. ANCOVA showed a significant common negative slope for the number of days to spawning and initial body weight for both species. However, whilst *L. pallidula* females in the *Laminaria digitata* treatment spawned significantly later in the season than females in the other diet treatments (Figure 2.9) there was no significant variation in elevation of the slope with respect to diet treatment for *L. vincta* (Figure 2.10.). Therefore, whilst diet affected the onset of spawning in *L. pallidula*, diet had no discernible affect upon *L. vincta*. Both body size at first spawning and onset of spawning were influenced by initial body weight for both species.

Figure 2. 9. ***Lacuna pallidula.*** Number of days to first spawning of females in the various diet treatments as a function of initial body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles and *Fucus vesiculosus,* open squares).

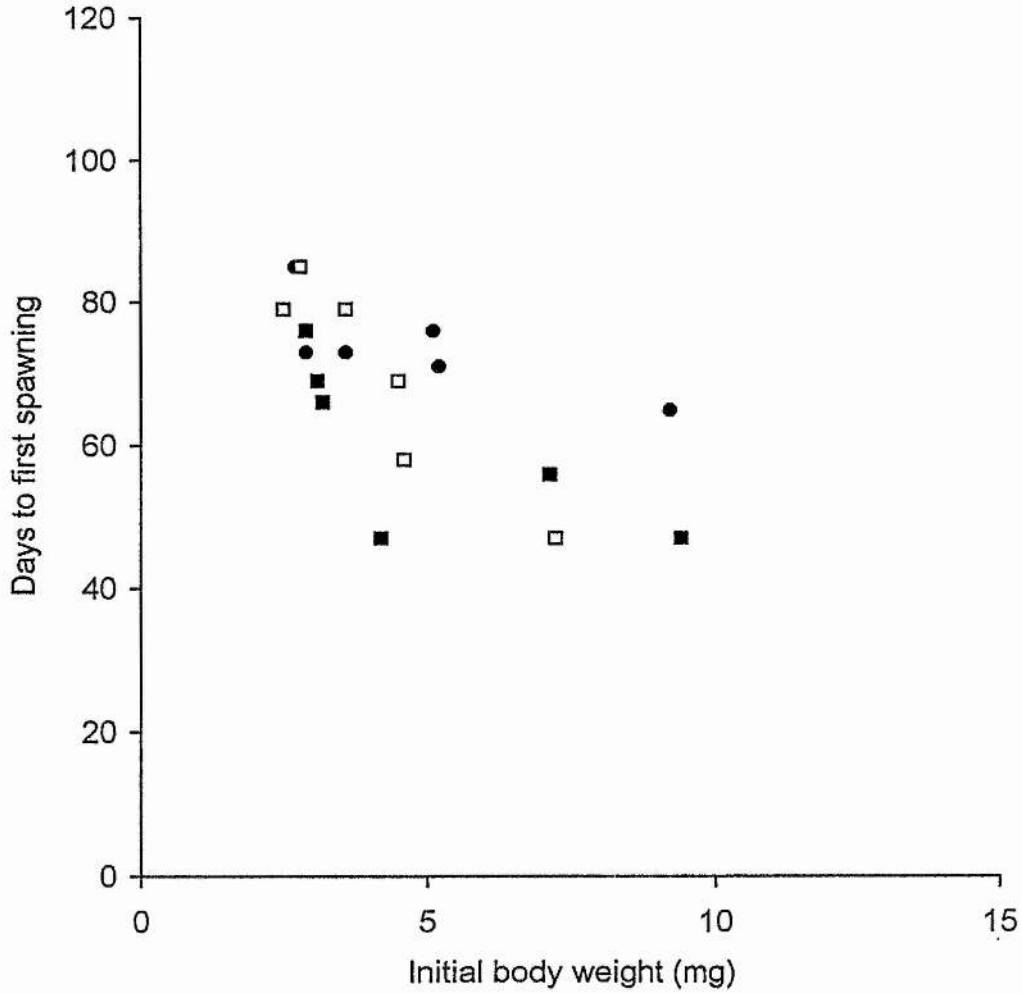
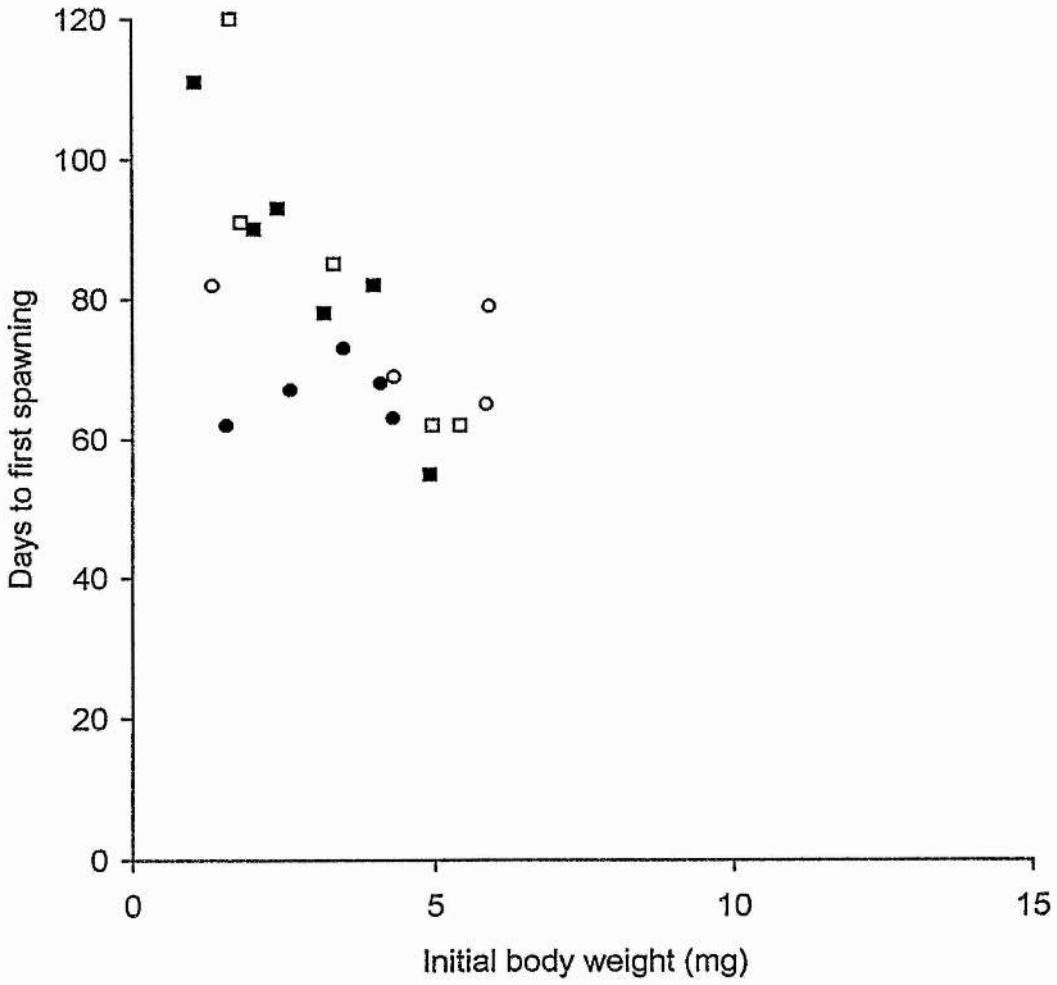

120
100
80
60
40
20
0
Days to first spawning
0
5
10
15
Initial body weight (mg)

Figure 2. 10. ***Lacuna vincta.*** Number of days to first spawning of females in the various diet treatments as a function of initial body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles, *Fucus vesiculosus,* open squares and *Mastocarpus stellata,* open circles).


120
100
80
60
40
20
0
Days to first spawning
0
5
10
15
Initial body weight (mg)

2.3.4. Reproductive investment

2.3.4.1. *Lacuna pallidula*

A significant simple linear relationship between the AFDW (mg) of a spawn mass and the number of eggs in a spawn mass was determined for *Lacuna pallidula* spawn masses;

$$\mathbf{Y = 0.154 + 0.0393\ x\ (n=41,\ r^2=0.74,\ P<<0.05).}$$

Spawn masses produced by experimental *Lacuna pallidula* females were sacrificed for comparison among females in the various diet treatments. Spawn mass weights (mg) were plotted against the numbers of eggs in spawn masses (Figure 2.11.). ANCOVA showed a significant common slope, but no significant difference in elevation of the slope with respect to diet treatment (Table 2.7.).

The total number of spawn masses produced, the total number of eggs produced and total spawn mass production (mg) were plotted against initial body weights (Figures 2.12., 2.13. and 2.14.). In all cases, ANCOVA confirmed that production increased with increasing body size but that there were significant differences in elevation of slope with respect to diet treatment (Table 2.7.). The weight-specific total reproductive outputs (total number of spawn masses, total egg numbers and total AFDW of spawn masses) of females in the *Laminaria digitata* treatment were significantly less than those in the *Fucus serratus* and *Fucus vesiculosus* treatments.

Because the spawning period varied among females and females continued to grow throughout the spawning period, mean days between spawning, mean daily rates of egg production and mean daily rates of spawn mass production (mg) were plotted against post-spawning GM body weights (Figures 2.15, 2.16 and 2.17.). In the first case, ANCOVA did not show a common slope but an ANOVA found significant differences with respect to diet (Table 2.7.). Females in the *Laminaria digitata* treatment produced significantly fewer spawn masses. ANCOVA revealed significant common positive slopes both for rates of egg production and rates of spawn mass production (Table 2.7.). Significant differences

Table 2.7. ***Lacuna pallidula.*** Analysis of variance and covariance for reproductive output data of females in the various diet treatments.

Relationship	ANCOVA		ANOVA	Tukey's HSD test
	Common slope	Elevation in slope		
Total number of spawn masses and initial body weight (mg)	$F_{1,15} = 27.31$, $P<0.05$	$F_{2,14} = 9.96$, $P<0.05$		
Total number of eggs and initial body weight (mg)	$F_{1,15} = 24.99$, $P<0.05$	$F_{2,14} = 8.14$, $P<0.05$		
Total spawn mass weight (mg) and initial body weight (mg)	$F_{1,15} = 26.68$, $P<0.05$	$F_{2,14} = 4.52$, $P<0.05$		
Mean days/spawning event and post-spawning GM body weight (mg)	NS		$F_{2,14} = 5.62$, $P<0.05$	Fs Fv Ld
Mean rate of egg production (eggs.day^{-1}) and post-spawning GM body weight (mg)	$F_{1,15} = 19.36$, $P<0.05$	$F_{2,14} = 6.8$, $P<0.05$		
Mean rate of production (mg.day^{-1}) and post-spawning GM body weight (mg)	$F_{1,15} = 20.98$, $P<0.05$	$F_{2,14} = 6.45$, $P<0.05$		
Mean number of eggs in a spawn mass and body weight (mg) at first spawning	$F_{1,15} = 16.15$, $P<0.05$	$F_{2,14} = 4.26$. $P<0.05$		
Weight of spawn mass and the number of eggs in a spawn mass	$F_{1,38} = 123.82$, $P<0.05$	$F_{2,36} = 2.36$, $P>0.05$		

Figure 2. 11. ***Lacuna pallidula.*** Weight (mg) of spawn masses produced by females in the various diet treatments as a function of the number of eggs in a spawn mass (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles and *Fucus vesiculosus,* open squares).

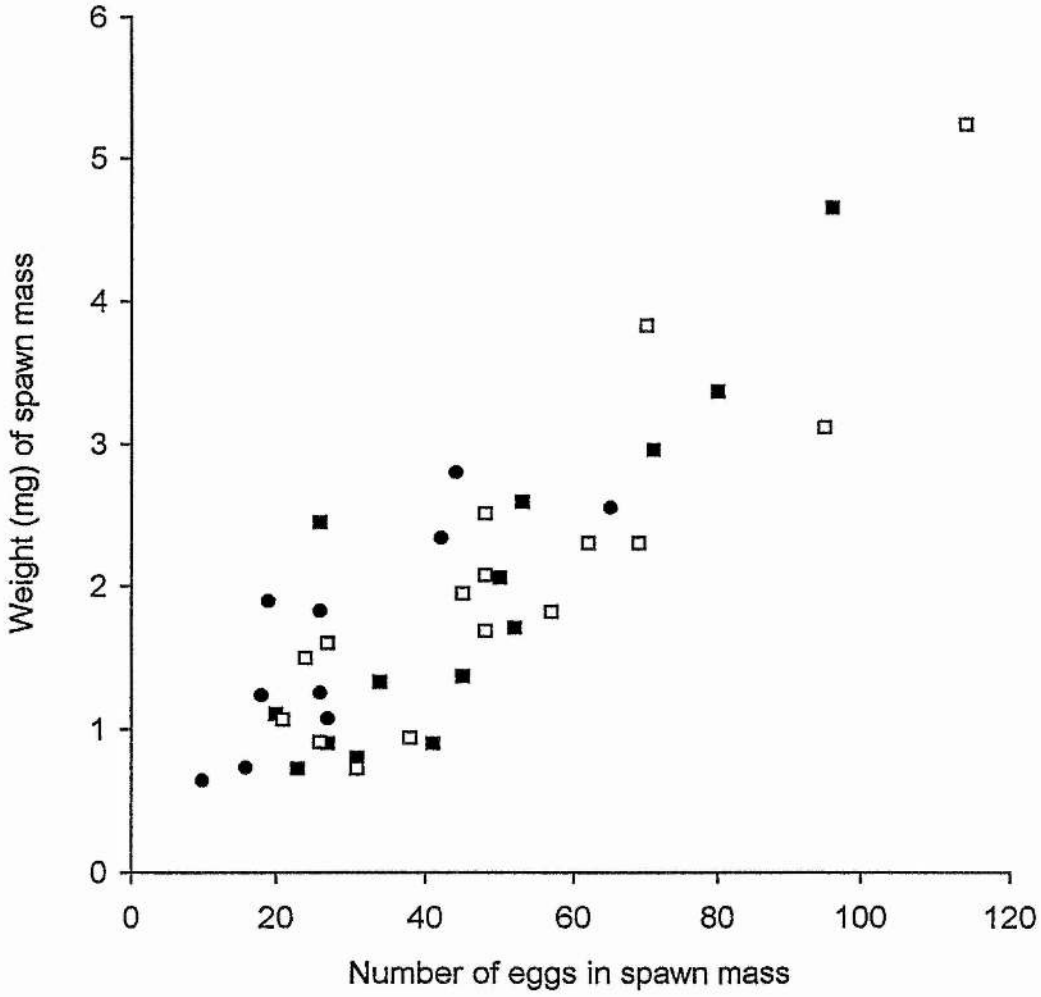

Weight (mg) of spawn mass
Number of eggs in spawn mass
0
1
2
3
4
5
6
0
20
40
60
80
100
120

Figure 2. 12. ***Lacuna pallidula.*** Number of spawn masses produced by females in the various diet treatments as a function of initial body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles and *Fucus vesiculosus,* open squares).

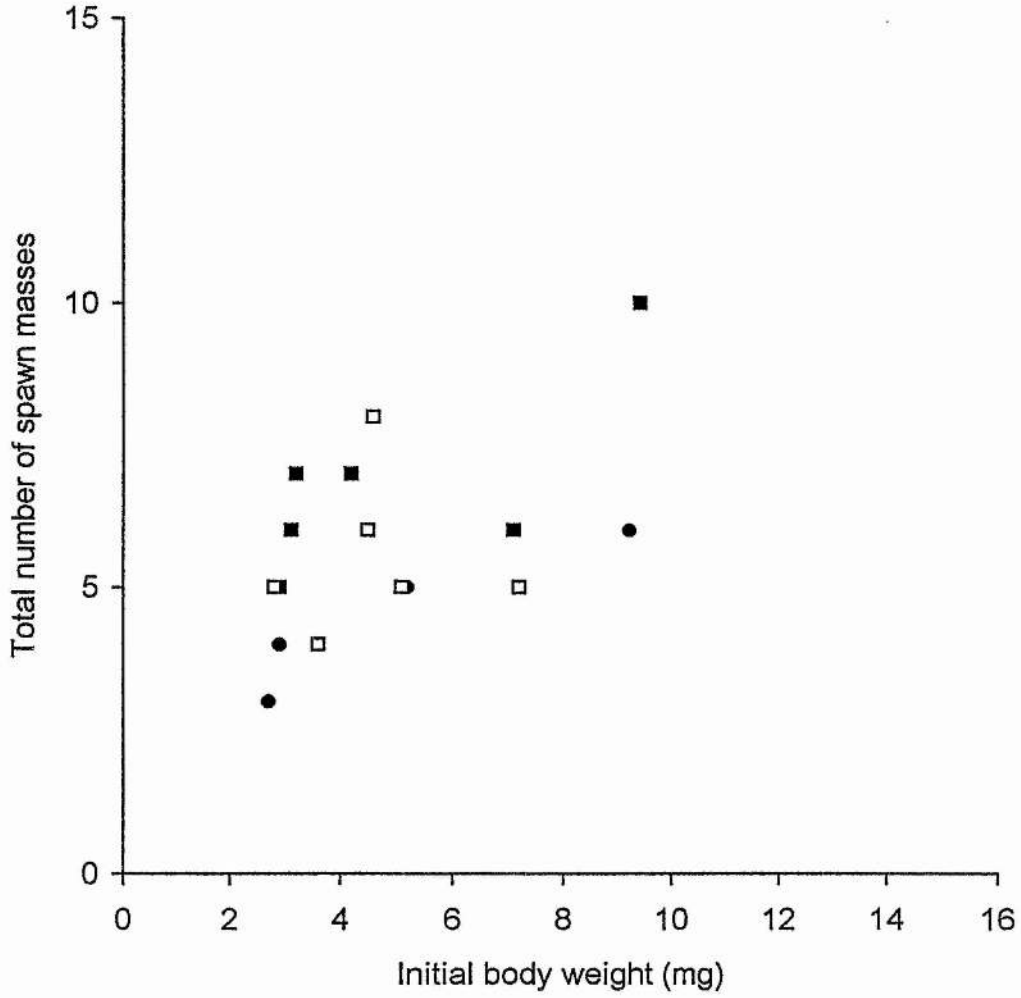

Total number of spawn masses
15
10
5
0
0
2
4
6
8
10
12
14
16
Initial body weight (mg)

Figure 2. 13. ***Lacuna pallidula***. Total number of eggs produced by females in the various diet treatments as a function of initial body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles and *Fucus vesiculosus,* open squares).

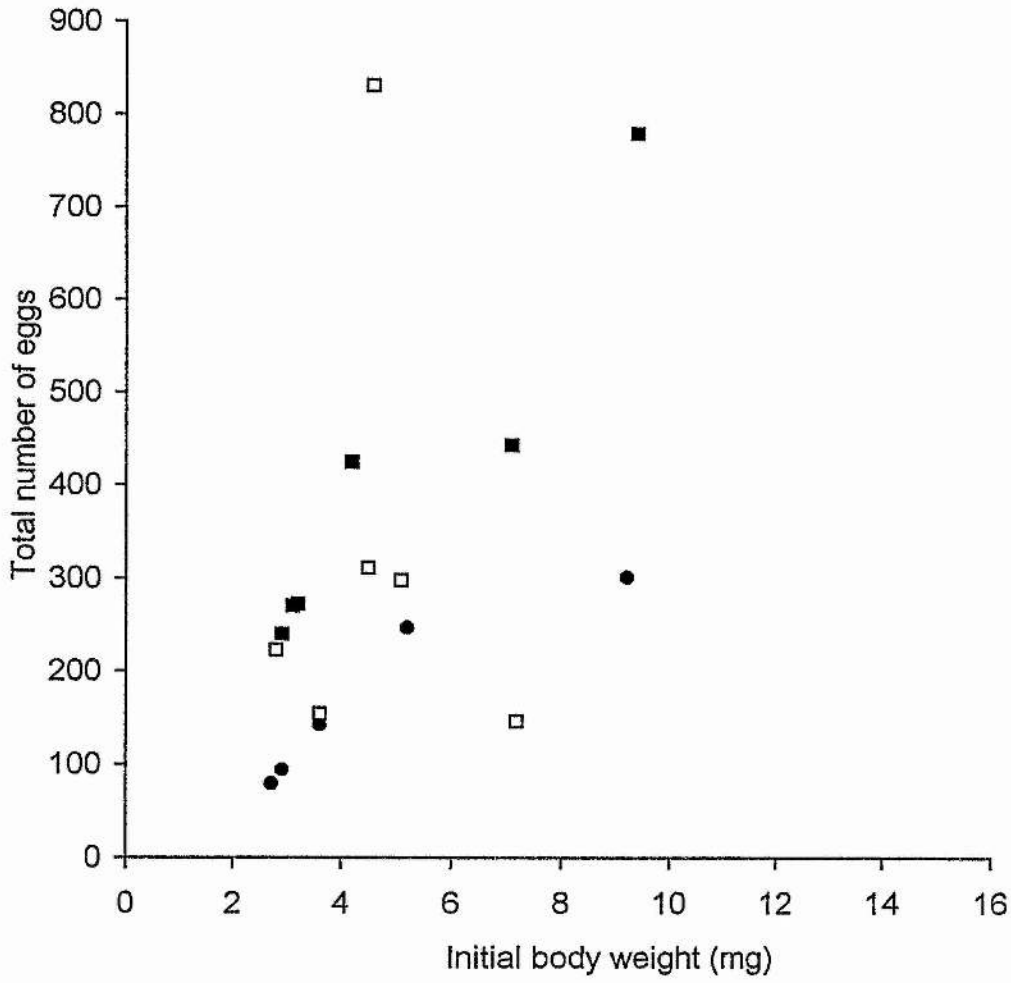

900
800
700
600
500
400
300
200
100
0
Total number of eggs
0
2
4
6
8
10
12
14
16
Initial body weight (mg)

Figure 2. 14. ***Lacuna pallidula.*** Total spawn mass production (mg) of females in the various diet treatments as a function of initial body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles and *Fucus vesiculosus,* open squares).

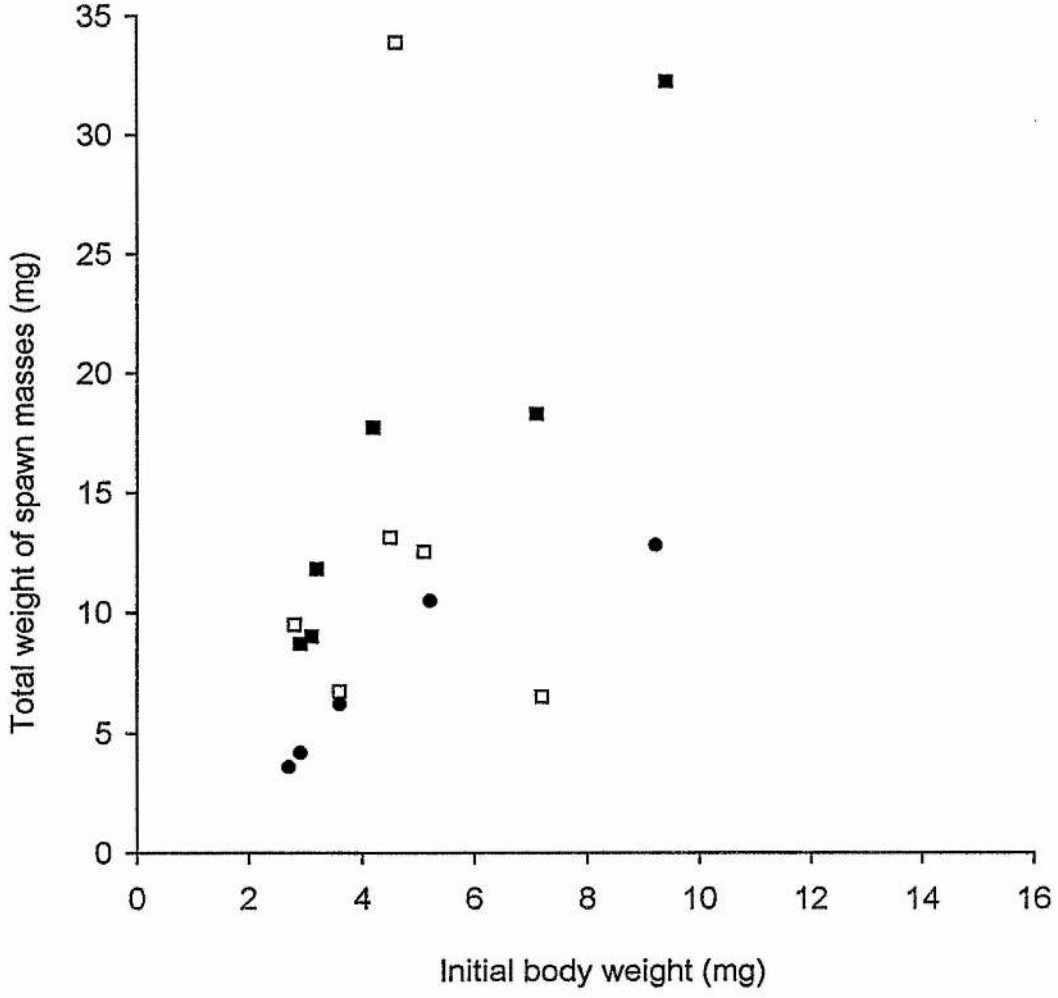

Total weight of spawn masses (mg)
35
30
25
20
15
10
5
0
0
2
4
6
8
10
12
14
16
Initial body weight (mg)

Figure 2. 15. ***Lacuna pallidula.*** Mean days between spawning of females in the various diet treatments as a function of the post-spawning GM body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles and *Fucus vesiculosus,* open squares).

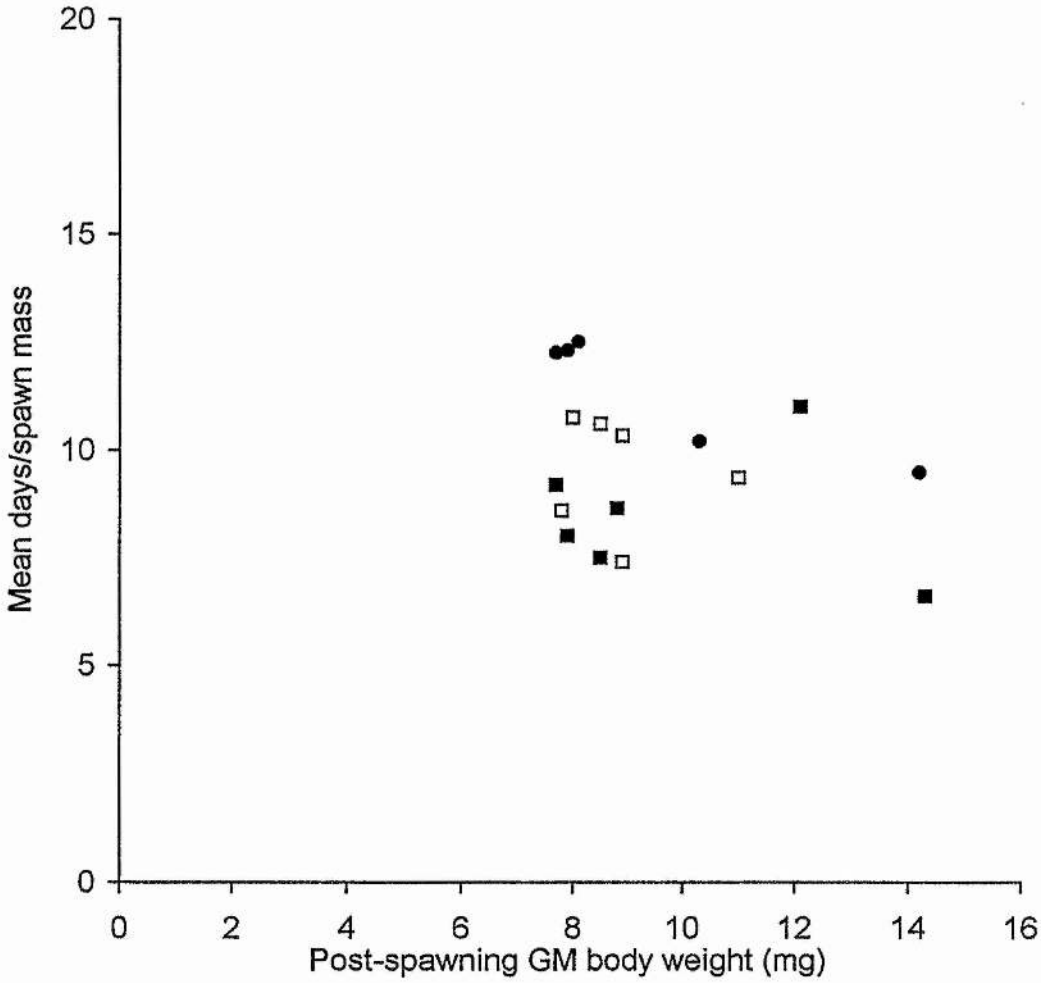

20
15
10
5
0
Mean days/spawn mass
0
2
4
6
8
10
12
14
16
Post-spawning GM body weight (mg)

Figure 2. 16. ***Lacuna pallidula.*** Mean rates of egg production (eggs.day^{-1}) of females in the various diet treatments as a function of the post-spawning GM body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles and *Fucus vesiculosus,* open squares).

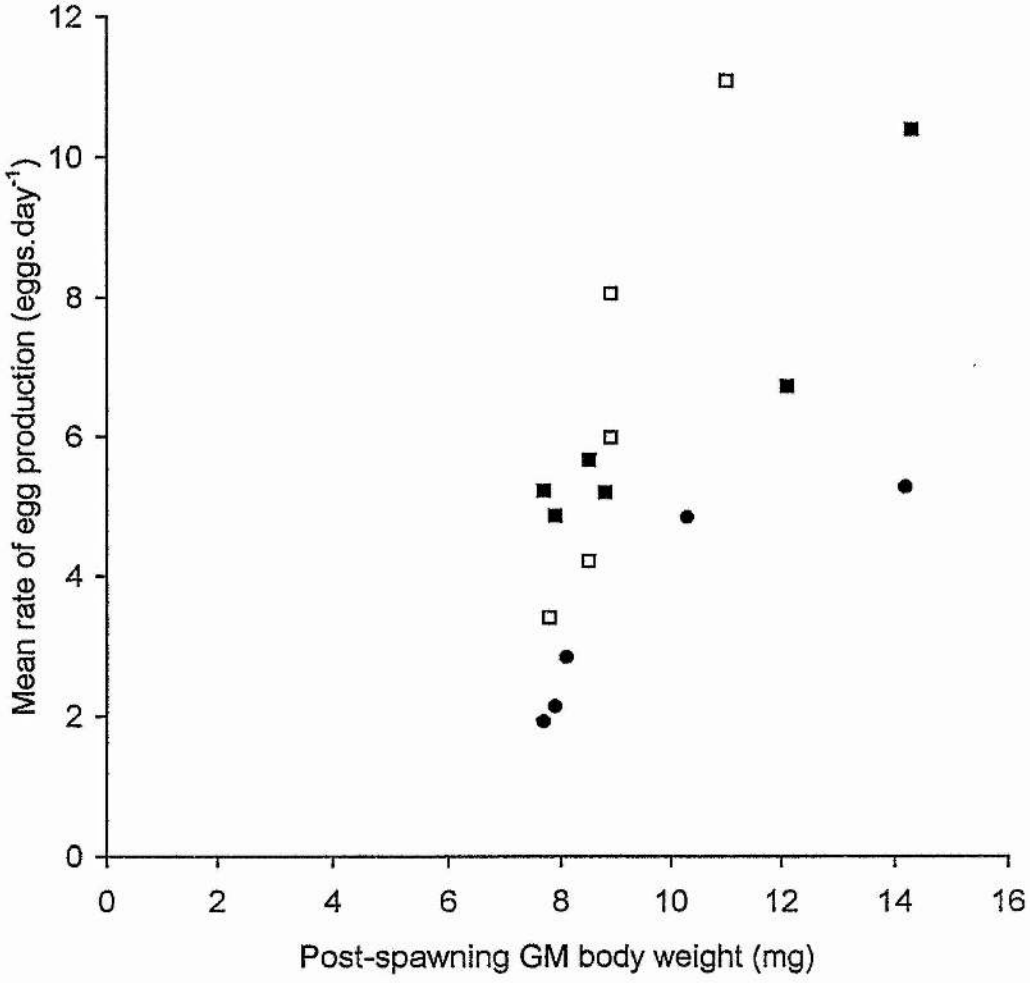
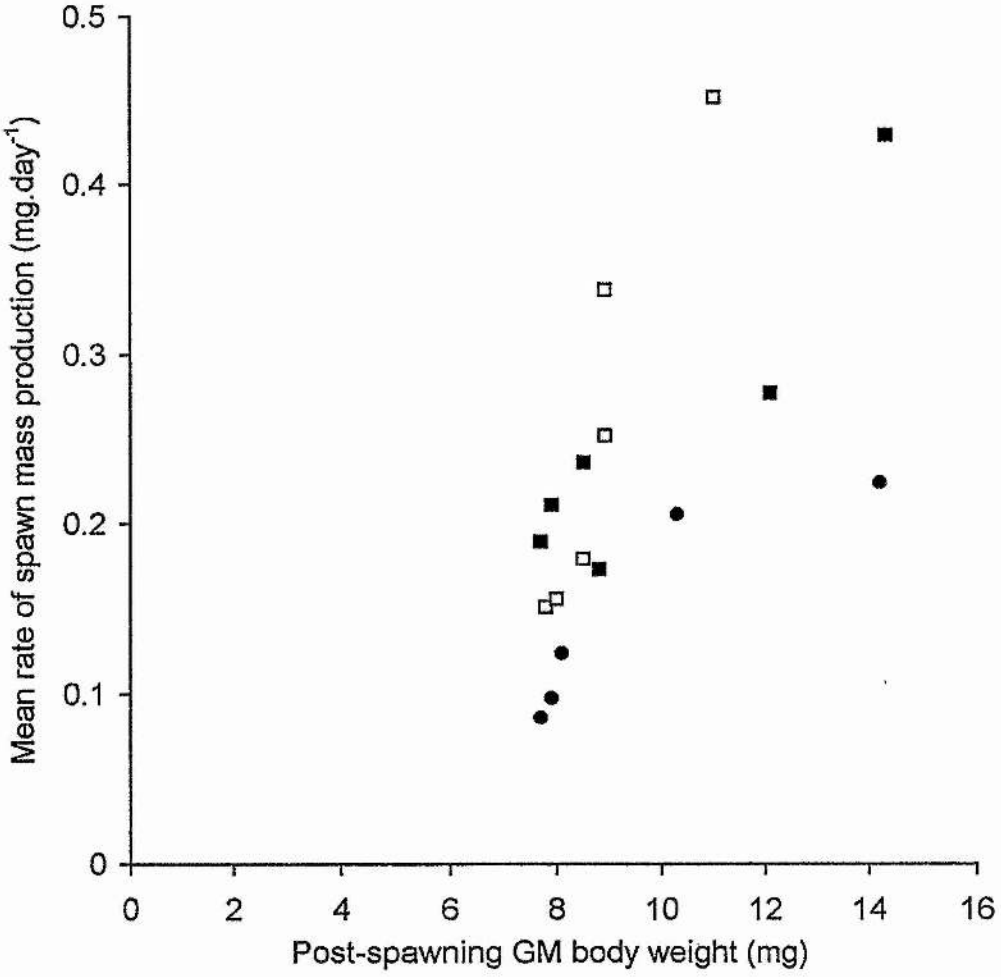

Mean rate of egg production (eggs.day-1)
Post-spawning GM body weight (mg)
0
2
4
6
8
10
12
14
16

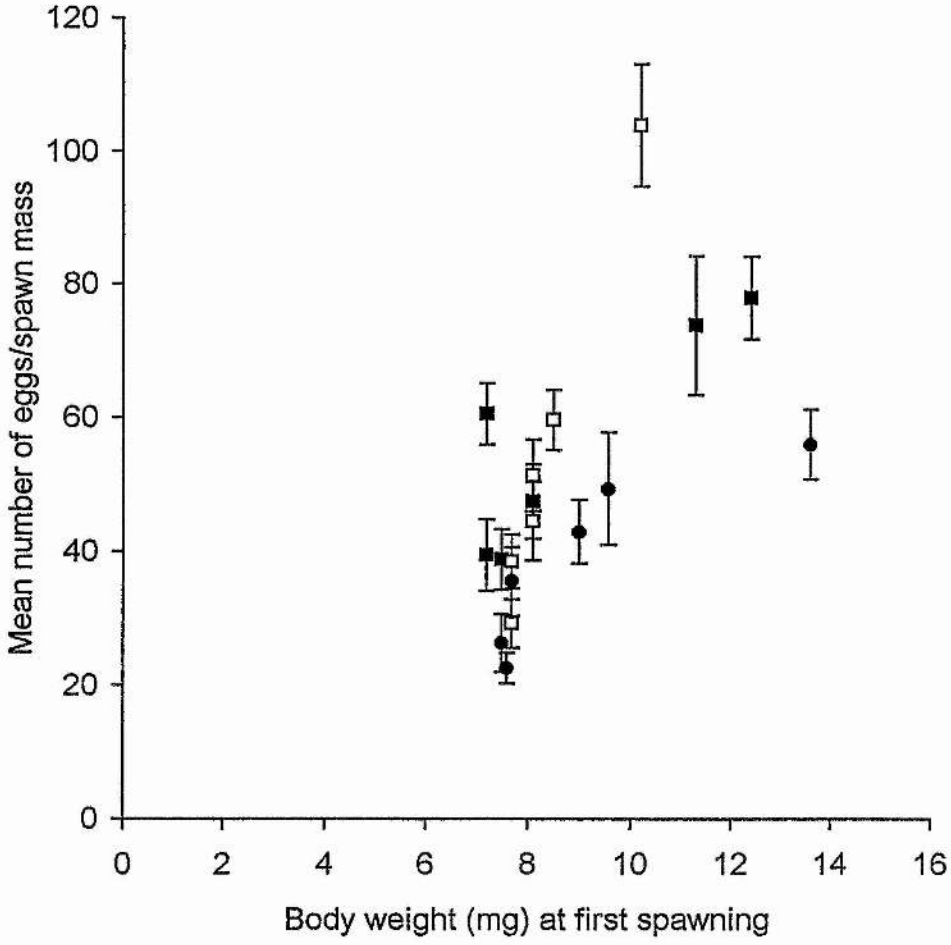
Figure 2. 17. ***Lacuna pallidula.*** Mean rates of spawn mass production ($mg.day^{-1}$) of females in the various diet treatments as a function of the post-spawning GM body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles and *Fucus vesiculosus,* open squares).


Mean rate of spawn mass production (mg.day-1)
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
16
Post-spawning GM body weight (mg)

in elevation of slope with respect to diet were also shown. Weight-specific rates of production for *Lacuna pallidula* females in the *L. digitata* treatments were notably lower.

The mean numbers of eggs in spawn masses produced by *Lacuna pallidula* females were plotted against body weights at first spawning (Figure 2.18.). ANCOVA (Table 2.7.) showed both a significant common positive slope and a significant difference in elevation of slope with respect to diet treatment. *L. pallidula* females in the *Laminaria digitata* treatment produced relatively fewer eggs in spawn masses than females of comparable size in the *Fucus serratus* and *Fucus vesiculosus* treatments.

Figure 2. 18. ***Lacuna pallidula.*** Mean number of eggs/spawn mass produced by females in the various diet treatments as a function of body weight (mg) at first spawning (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles and *Fucus vesiculosus*, open squares).


120
100
80
60
40
20
0
Mean number of eggs/spawn mass
0
2
4
6
8
10
12
14
16
Body weight (mg) at first spawning

2.3.4.2. *Lacuna vincta*

Significant simple linear regression equations, relating the log AFDW weight (mg) of a spawn mass to the log 'mean' spawn mass diameter (mm) and the log number of eggs in a spawn mass to the log 'mean' spawn mass diameter (mm) were determined for *Lacuna vincta* spawn masses,

log AFDW spawn (mg) = -1.08 + 2.52 log diameter of spawn mass(mm) (n=20, R^2=0.79, P<<0.05)

log number of eggs = 2.11 + 2.43 log diameter of spawn mass(mm) (n=15, R^2=0.76, P<0.05).

As for *Lacuna pallidula*, the total number of spawn masses produced, the total number of eggs produced and total spawn mass production were plotted against initial body weights at the time of collection for each *Lacuna vincta* female (Figures 2.19., 2.20. and 2.21.). In the first case, an ANCOVA did not reveal a common slope but ANOVA showed significant differences among females with respect to diet treatment (Table 2.8.). Unlike *L. pallidula*, *L. vincta* females in the *Laminaria digitata* treatment produced significantly more spawn masses than those in the other diet treatments. In the latter two cases, ANCOVA revealed significant common positive slopes and significant differences in elevation of slope with respect to diet treatment. Again, weight-specific reproductive outputs were significantly greater for females in the *L. digitata* treatment.

As for *Lacuna pallidula*, the spawning period of *Lacuna vincta* females varied, and females continued to grow throughout the spawning period. Data for mean days between spawning, mean daily rates of egg production and mean daily rates of spawn mass production were therefore plotted against the post-spawning GM body weight (Figures 2.22, 2.23. and 2.24.). In the first case, an ANCOVA did not show a common slope but ANOVA showed significant differences with respect to diet (Table 2.8.). In the latter two cases, ANCOVA revealed common slopes and significant differences in elevation of slopes with respect to diet treatment. In contrast to *L. pallidula*, *L. vincta* females in the *Laminaria digitata* treatment displayed significantly greater weight-specific rates of production than those in the other three diet treatments

Table 2.8. ***Lacuna vincta.*** Analysis of variance and covariance for reproductive output data of females in the various diet treatments.

Relationship	ANCOVA		ANOVA	Tukey's HSD test
	Common slope	Elevation in slope		
Total number of spawn masses and initial body weight (mg)	NS		$F_{3,10} = 7.55$, $P<0.05$	Ld Fs Fv Ms
Total number of eggs and initial body weight (mg)	$F_{1,12} = 17.45$, $P<0.05$	$F_{3,10} = 14.18$, $P<0.05$		
Total spawn mass weight (mg) and initial body weight (mg)	$F_{1,12} = 16.09$, $P<0.05$	$F_{3,10} = 22.04$, $P<0.05$		
Mean days/spawning event and post-spawning GM body weight (mg)	NS		$F_{3,10} = 8.97$, $P<0.05$	Ld Fs Fv Ms
Mean rate of egg production (eggs.day^{-1}) and post-spawning GM body weight (mg)	$F_{1,12} = 5.31$, $P<0.05$	$F_{3,10} = 10.25$, $P<0.05$		
Mean rate of production (mg.day^{-1}) and post-spawning GM body weight (mg)	$F_{1,12} = 9.25$, $P<0.05$	$F_{3,10} = 4.04$, $P<0.05$		
Mean number of eggs in a spawn mass and body weight (mg) at first spawning	$F_{1,12} = 5.31$, $P<0.05$	$F_{3,10} = 0.03$, $P>0.05$		

Figure 2. 19. ***Lacuna vincta.*** Number of spawn masses produced by females in the various diet treatments as a function of initial body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles, *Fucus vesiculosus,* open squares and *Mastocarpus stellata,* open circles).

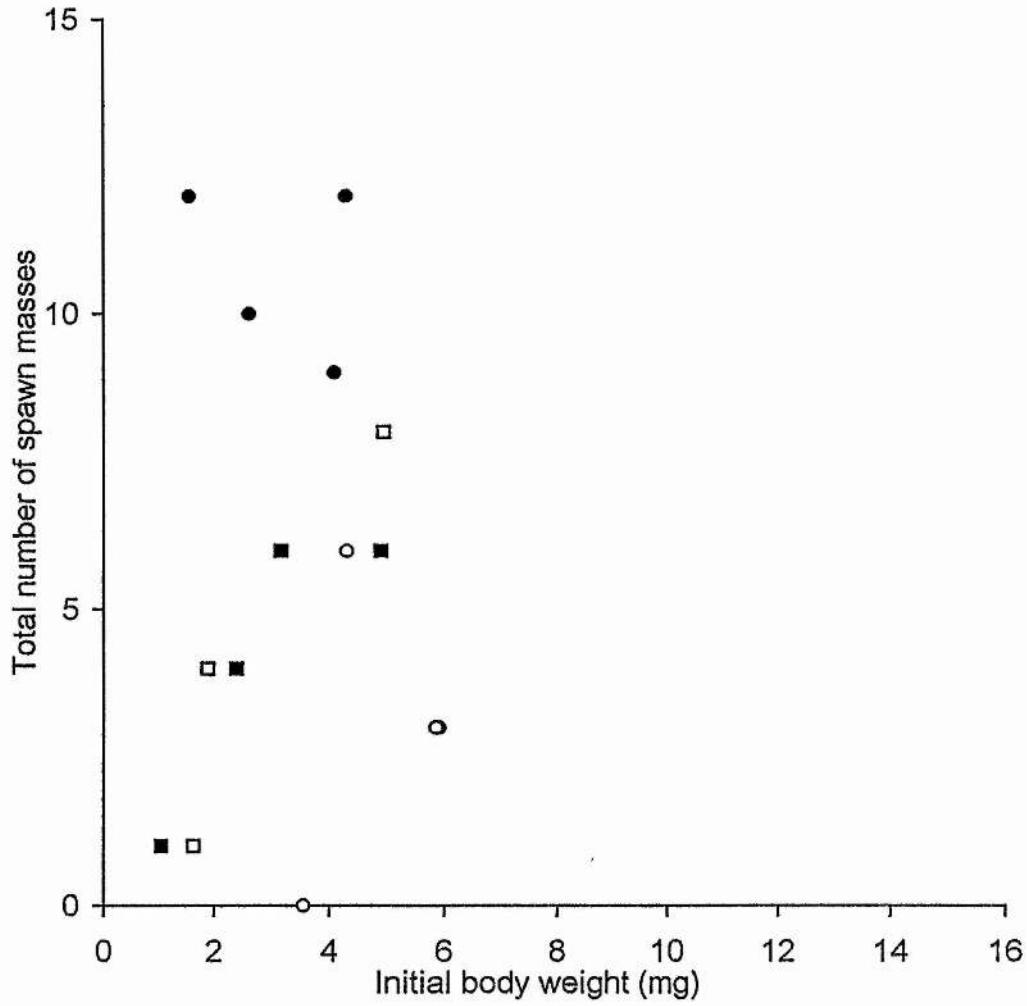

15
10
5
0
Total number of spawn masses
0
2
4
6
8
10
12
14
16
Initial body weight (mg)

Figure 2. 20. ***Lacuna vincta.*** Total number of eggs produced by females in the various diet treatments as a function of initial body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles, *Fucus vesiculosus,* open squares and *Mastocarpus stellata,* open circles).

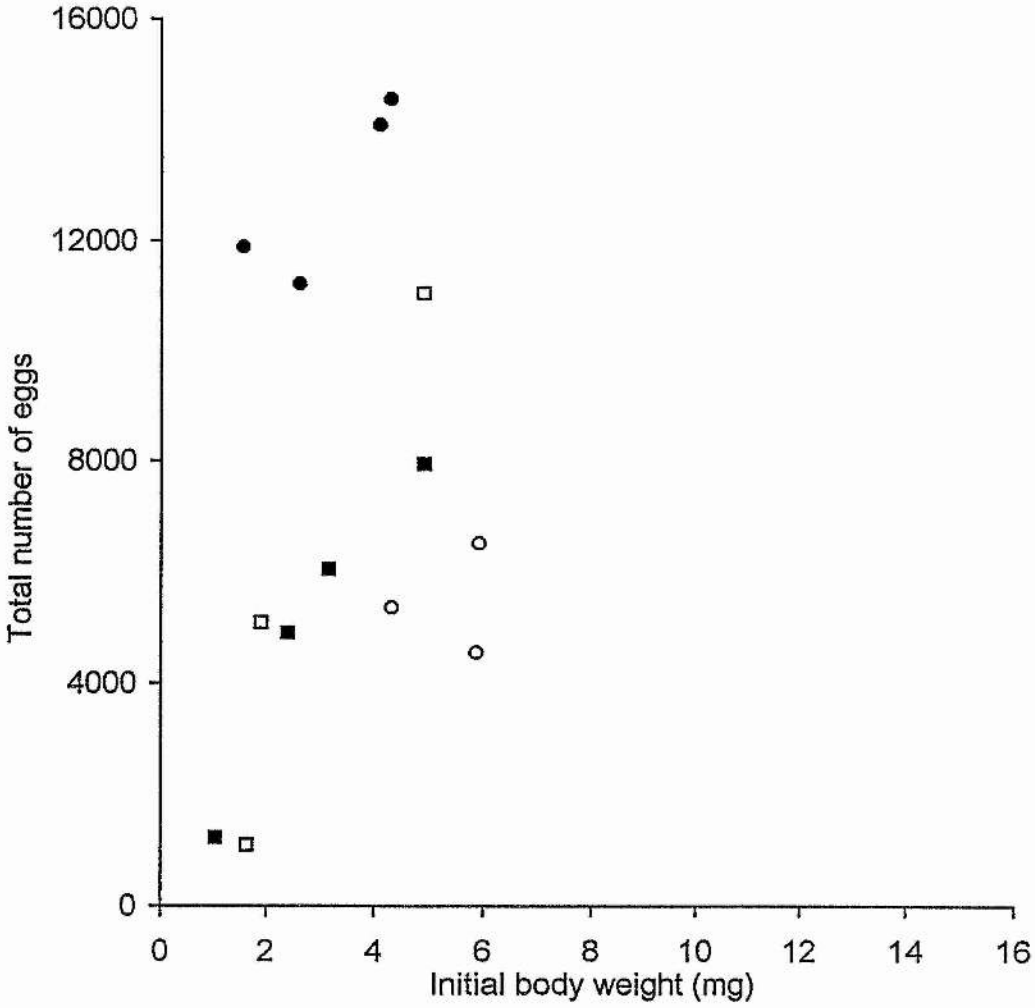

16000
12000
8000
4000
0
Total number of eggs
0
2
4
6
8
10
12
14
16
Initial body weight (mg)

Figure 2. 21. ***Lacuna vincta.*** Total spawn mass production (mg) of females in the various diet treatments as a function of initial body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles, *Fucus vesiculosus,* open squares and *Mastocarpus stellata,* open circles).

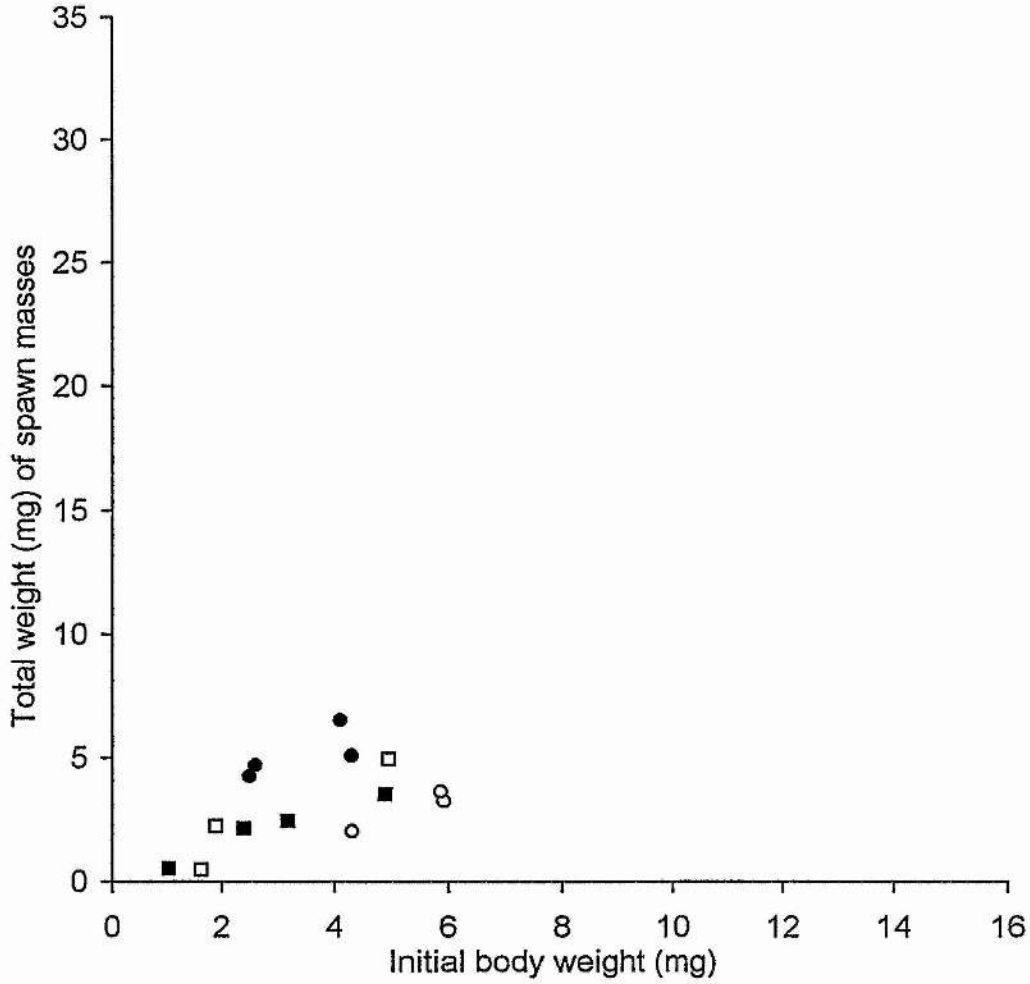

Total weight (mg) of spawn masses
Initial body weight (mg)
0
5
10
15
20
25
30
35
0
2
4
6
8
10
12
14
16

Figure 2. 22. ***Lacuna vincta.*** Mean days between spawning for females in the various diet treatments as a function of the post-spawning GM body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles, *Fucus vesiculosus,* open squares and *Mastocarpus stellata,* open circles).

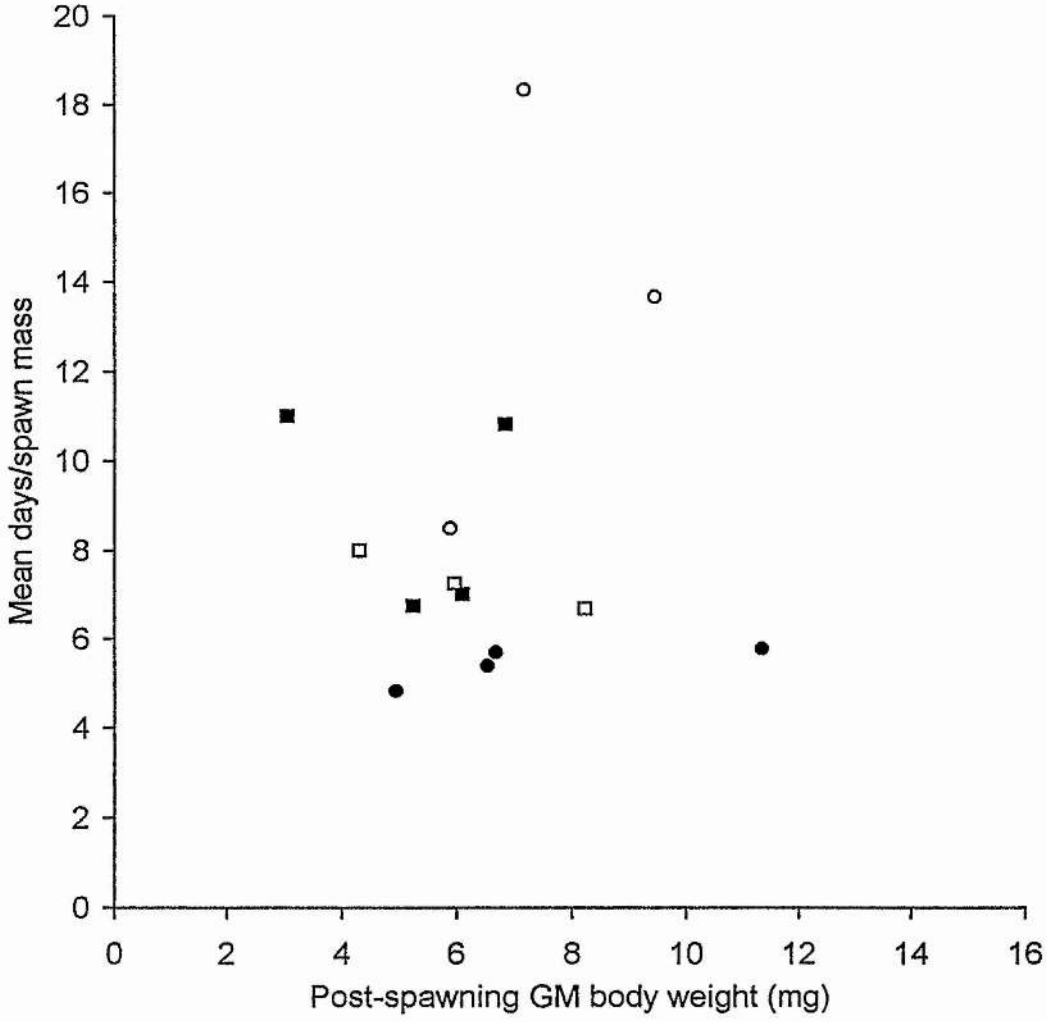

Mean days/spawn mass
Post-spawning GM body weight (mg)
0
2
4
6
8
10
12
14
16
18
20

Figure 2. 23. ***Lacuna vincta.*** Mean rates of egg production (eggs.day^{-1}) of females in the various diet treatments as a function of the post-spawning GM body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles, *Fucus vesiculosus,* open squares and *Mastocarpus stellata,* open circles).

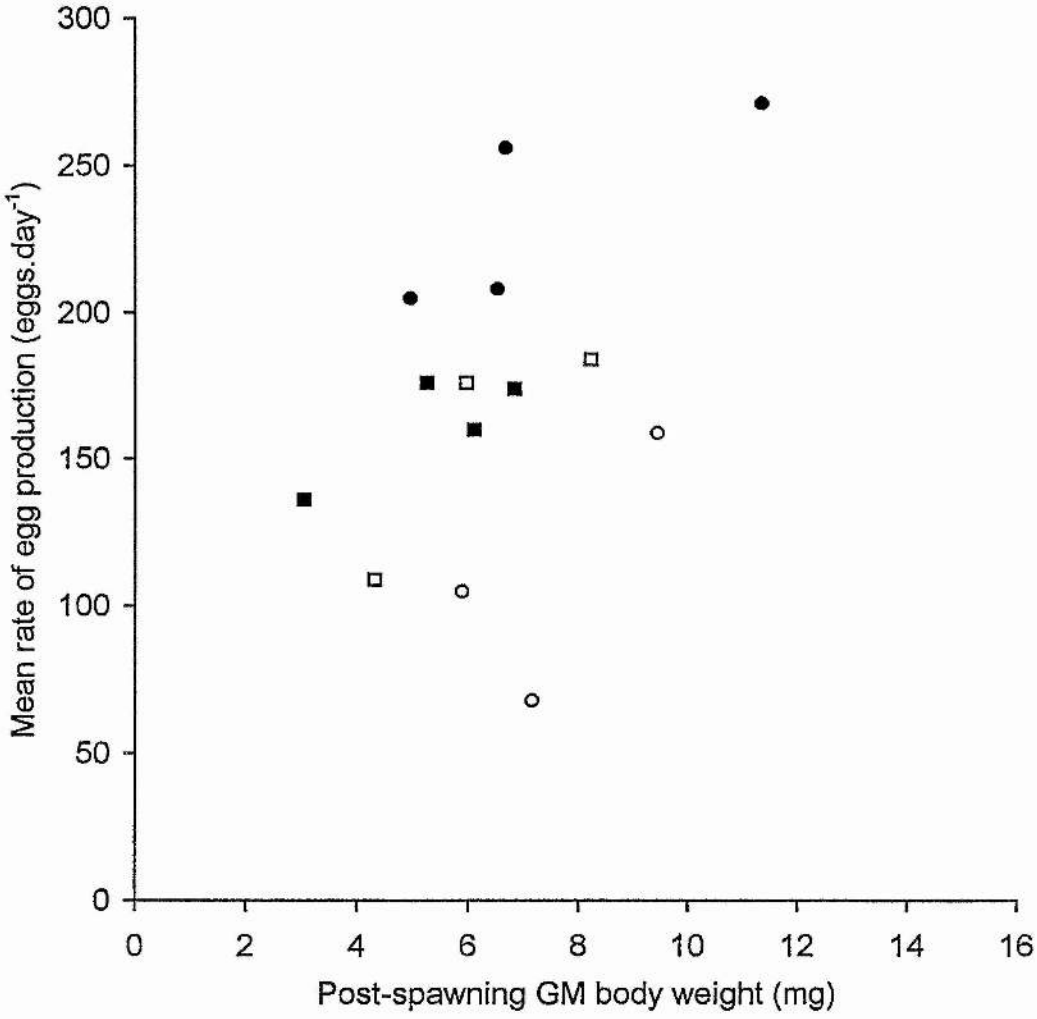
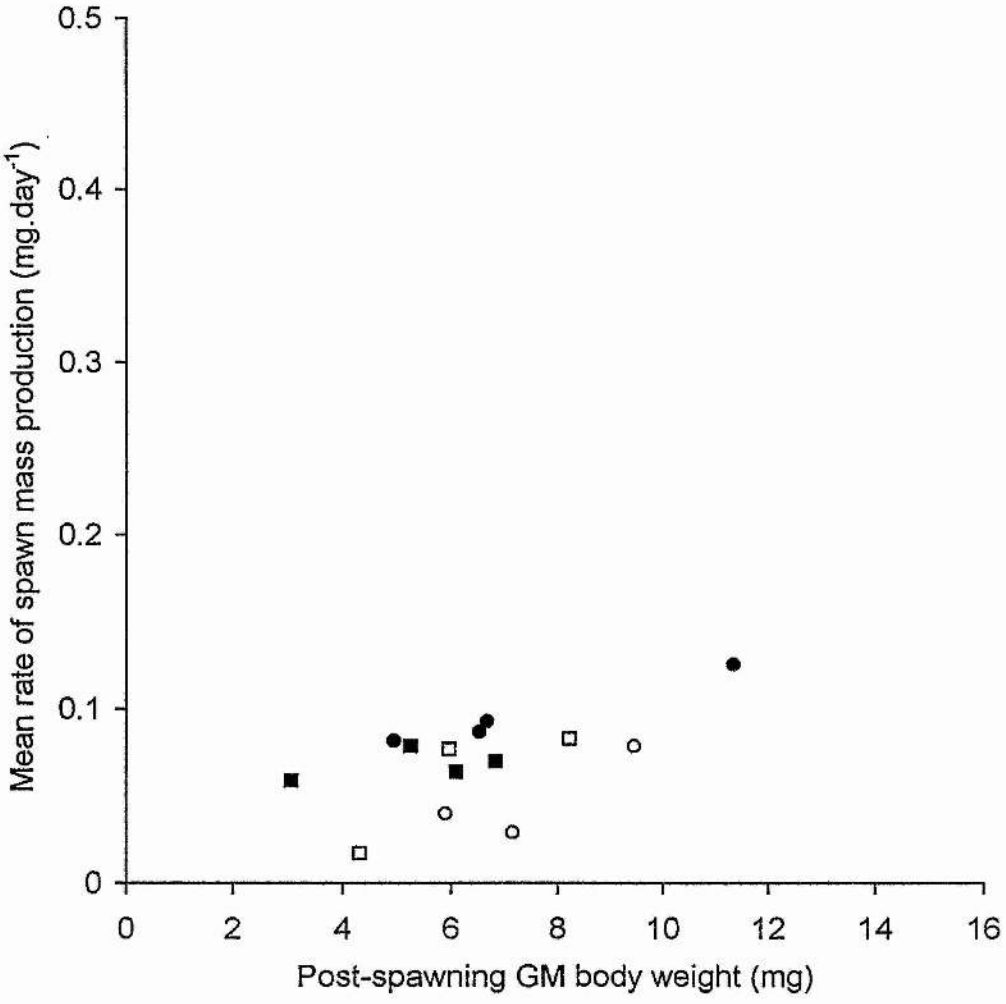
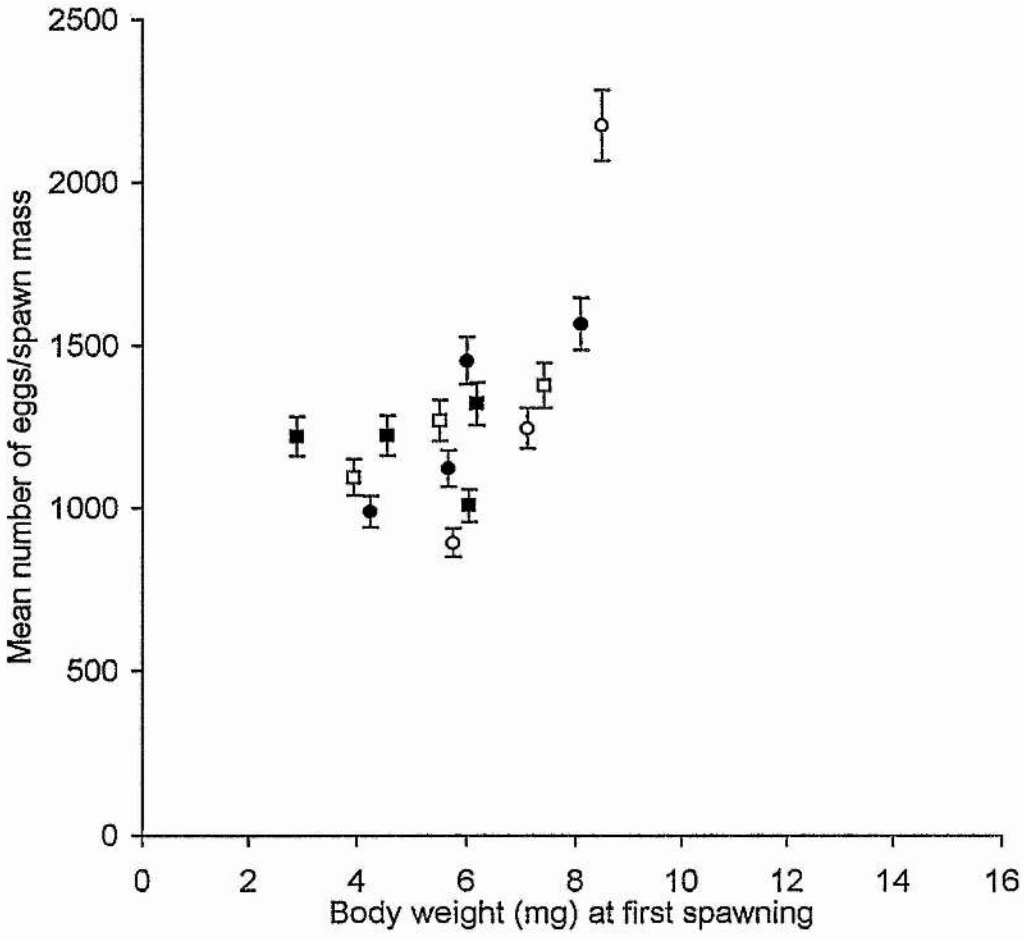

Mean rate of egg production (eggs.day-1)
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
Post-spawning GM body weight (mg)

Figure 2. 24. ***Lacuna vincta.*** Mean rates of spawn mass production ($mg.day^{-1}$) of females in the various diet treatments as a function of the post-spawning GM body weight (mg) (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles, *Fucus vesiculosus,* open squares and *Mastocarpus stellata,* open circles).


Mean rate of spawn mass production (mg.day-1)
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
16
Post-spawning GM body weight (mg)

The mean numbers of eggs in spawn masses produced by *Lacuna vincta* females were plotted against body weights at first spawning (Figure 2.25.). Unlike for *Lacuna pallidula*, ANCOVA revealed a significant common slope but no significant variation in the elevation of slopes with respect to diet treatment (Table 2.8.).

Figure 2. 25. ***Lacuna vincta.*** Mean number of eggs/spawn mass produced by females in the various diet treatments as a function of body weight (mg) at first (*Fucus serratus,* closed squares, *Laminaria digitata,* closed circles, *Fucus vesiculosus,* open squares and *Mastocarpus stellata,* open circles).


2500
2000
1500
1000
500
0
Mean number of eggs/spawn mass
0
2
4
6
8
10
12
14
16
Body weight (mg) at first spawning

2.4. DISCUSSION

2.4.1. General

The intention of the present study was to examine variations in the growth and reproduction for *Lacuna pallidula* and *Lacuna vincta*, given a range of ecologically relevant macroalgal substrata (Smith, 1973; Southgate, 1982; Grahame, 1985). The results indicate that whilst there are several similarities between the two species, there is much potential variation in both growth and reproduction in the field, for both species, when faced with a variable resource supply.

There are, however, a number of factors concerning the protocol which should be addressed. These are:

(1) For practical reasons, the number of replicates in each treatment was small (n=6), dwindling down to one in some cases. However survival was clearly influenced by macroalgal diet. While the total number of each species used (n=24) was comparable to the numbers used in other studies (Grahame, 1977, 1982, 1994; Hall and Todd, 1986; Havenhand and Todd, 1988a, b), a greater number of replicates in each diet treatment would have allowed for greater power of statistical discrimination for significant differences between diet treatments (Zar, 1984; Searcy-Bernal, 1994).

(2) The use of newly hatched siblings would have reduced the variability in past history of females in the experiment. Dietary experiences during early stages may affect the subsequent performance of animals on various diets in later life (Hall *et al.*, 1982, 1984, ; Rossiter, 1987; Mousseau and Dingle, 1991). Preliminary observations of survival and growth of juveniles during the present study suggested that, unlike the adults, juveniles of both species can achieve good growth and survival on a diet of *Mastocarpus stellata* (pers. obs). There therefore are possible ontogenetic changes in the generality of dietary suitability. This is supported by reports of juvenile *Lacuna pallidula* and *Lacuna vincta* grazing on epiphyton on red algal turfs in the field (pers. obs., Fretter and Graham, 1980; Southgate, 1982, also see Williams and Seed, 1992 for similar observations for littorinids). On the other hand, these observations may reflect a preference for habitat, in that juveniles are

provided with better shelter (Fretter and Manly, 1977), or that there are no dietary preferences in the early stages at all. Observations in the field during the present study suggested that juveniles of both species are more widely distributed on various macroalgal species in the lower intertidal than adults (pers. obs).

(3) Ideally such experiments should be conducted in the field, but for practical reasons detailed observations of growth and reproduction could only be conducted in the laboratory. In doing this it had to be assumed that results obtained in the laboratory would reflect the realised situation in the field. Attempts were made to provide realistic conditions by allowing the water temperature and light regime to vary according to local ambient seawater temperatures and photoperiod. Tidal regime was not considered, however it is likely not to be of great importance because both species are rarely aerially exposed in the field. Attempts were also made to provide similar conditions for all experimental animals. The two tanks used in the experiment were adjacent to each other and recieved the same air and water supply. Further, to avoid block effects, animals were not allocated to a specific position in the tanks or to a specific tank during the experiment.

The main problem relates to the quality of macroalgal substrata and to the lack of diversity of dietary choice in the laboratory. While experimental snails were only provided with the vegetative tissue of a selected macroalgal species, snails would have the opportunity to prey upon a range of tissues and on a range of macroalgal species in the field (Watson and Norton, 1987). In addition, experimental snails were provided with 'damaged' fronds which are known to release secondary metabolites, such as phenols, from their cut margins (Hay *et. al.*, 1988). This was a particular problem for snails fed on *Laminaria digitata* which secreted copious amounts of mucilage. Cages holding *L. digitata* had to be frequently cleaned and the algae had to be replenished on a daily basis. Some comparisons of egg production for females maintained in the laboratory and for those in the field are presented in the next chapter.

(4) During the experiment, differences in offspring quality were observed. Hence an increase in reproductive output may not simply relate to increased fitness if the offspring being produced are of 'poor quality'. This also is addressed in the next chapter.

(5) As noted in the Introduction, AFDW measurements may not reliably reflect energy content. The amount of energy per unit weight of somatic and reproductive tissue may be different for females of the same species in the various diet treatments and for species. However, Grahame (1977) reported that the energy content per unit weight of somatic tissue and of reproductive tissues are comparable for the two species.

(6) The effect of quantity of resources upon growth and reproduction was not considered. This is an obvious direction for further study.

2.4.2. Comparisons between the two species

Previous studies have shown that *Lacuna pallidula* and *Lacuna vincta* display similar patterns of growth and reproduction (Grahame, 1977, 1982, 1994). Grahame (1994) has shown that both species display reduced growth and respiration rates following the onset of spawning. The present study observed similar patterns of growth and reproduction and also found that patterns of energy allocation in conditions of variable resource availability also were comparable.

Diet significantly affected the rates of pre-spawning growth and reproductive output for both species. Following the onset of spawning, the growth rates and WSGRs for females of both species were much reduced and there were no significant differences between females of either species in the various diet treatments. This was because most of the assimilated energy was now being channelled into reproduction.

For both species, the weight-specific post-spawning growth rates of females displaying the greatest rates of reproductive output were not significantly greater than those females which displayed lower

rates of reproductive output. It has been shown in other species that reproductive output may be increased at the cost of growth in conditions of poor food supply (Thompson, 1982; Brody *et al.*, 1983; Emlet *et al.*, 1987; Todd, 1987; Xu and Barker, 1990; George *et al.*, 1991). For example, George *et al.* (1991) reported that female *Luidia clathrata* fed low food rations increased their levels of reproductive output at the cost of reducing their growth rates. The potential for the two species in the present study to do this may differ. While the rate of reproductive output for *Lacuna pallidula* was an order of ten magnitude greater (0.1 mg - 0.5 mg. day $^{-1}$) than its rate of growth during the spawning period (0. 01 mg. day $^{-1}$), the reproductive output of *Lacuna vincta* was of the same magnitude as that for its growth rate (0. 01 mg. day $^{-1}$). Hence, any sacrifices in growth for *L. vincta* potentially would have greater consequences for its reproductive output than for *L. pallidula*.

It is not known why these two *Lacuna* species continue to grow during the spawning period. Hall and Todd (1986) have suggested that the advantage of continued growth in *Aeolidia papillosa* may be to enhance success of predation on anemone prey. For *Lacuna*, the advantages in continued growth may lie in less susceptibility to predation. Chia (1974) has suggested that no further gonad growth may occur during the spawning phase and that therefore an increase in resources available during the spawning period will inevitably be assimilated in storage in the body. In the present study, the rate of reproductive output was not observed to increase over time in individuals despite their continuing to grow (also see Grahame, 1977).

Both species began to spawn in the first two months of the year but *Lacuna vincta* tended to initiate spawning rather later. The reasons for this are unclear but the results indicated that the onset of spawning was influenced by both season and the existence of a minimum size threshold in both species. Diet affected the onset of spawning in *Lacuna pallidula* but not for *L. vincta*. Diet may therefore be more influential in gonad growth and development for *L. pallidula*. This is perhaps very important because similar sized female *L. pallidula* in favourable food conditions are likely to produce more offspring than those in unfavourable conditions by spawning earlier in the season. Maternal body size was shown to greatly influence both growth and reproductive output in both

species. Because spawning is seasonal this will have important implications for the production of offspring earlier in the season which in turn will have a longer period of time to grow before spawning the following year.

Lacuna pallidula females generally displayed greater absolute levels of energy available for reproduction, although there was significant variation in the energy invested in reproduction for both species in the various diet treatments. Further, for both species, the number of eggs in spawn masses increased with maternal body size. Grahame (1982), working with *Lacuna* populations from NE England, reported that *L. pallidula* invested only marginally greater amounts of energy in reproduction. In view of the large variations in total reproduction investment and in weight-specific reproduction investment shown in this study, and in view of the findings from previous studies, it may be concluded that, in this case, there is no consistent pattern between reproductive investment and larval strategy. Grahame's (1982) conclusion that the selection for larval strategy in *Lacuna* spp. cannot be explained by energetic arguments alone is supported.

However the present study observed variations in the manner in which the two species responded with respect to the favourability of the different diets, notably the manner in which the two species packaged their offspring. For *Lacuna pallidula*, increased weight-specific rates of reproduction were achieved by earlier onset of spawning, increased rates of spawning and by producing relatively larger spawn masses at each spawning event. However, for *Lacuna vincta*, increased weight-specific rates of reproduction were achieved only by increased spawning frequency. This has also been observed in comparative studies on polychaete species (Tenore, 1977; Tenore and Chesney; 1985; Levin and Creed, 1986). Eckelbarger (1980) has suggested that individuals producing lecithotrophic offspring are more likely to increase their egg production in conditions of favourable food supply because lecithotrophic eggs contain proportionally greater quantities of heterosynthetic yolk than planktotrophic eggs (also see Levin and Creed, 1986). Another contributory factor may be that, unlike *L. vincta*, the costs of manufacturing the protective matrix in which *L. pallidula* eggs are deposited in places constraints upon the number of eggs that can be produced in spawn masses.

2. 5. SUMMARY

- Growth and reproduction were compared for *Lacuna pallidula* and *Lacuna vincta* females, from a single population, fed a range of ecologically relevant macroalgal diets.
- Similar patterns of energy allocation to growth and reproduction were observed for the two species and for females of the same species in the various diet treatments.
- Macroalgal diet significantly affected the survival, the pre-spawning growth rates and the rates of reproductive output for females of both species, although the responses of the two species to the various diet treatments were markedly different.
- Maternal body size significantly affected the onset of spawning, body size at first spawning and rates of growth and reproduction for both species.
- *Lacuna pallidula* females were generally larger than *Lacuna vincta* females and displayed greater rates of reproductive output. However, large variations in these traits were mediated by both macroalgal diet and maternal size for both species.
- Differences in the responses of females to the 'favourability' of ' macroalgal diet were observed for the two species. Whilst diet significantly affected both the onset of spawning and the size of spawn masses produced by similar sized *Lacuna pallidula* females, diet had no apparent affect on these traits for *Lacuna vincta*.

CHAPTER 3

Inter- and intra-population variations in Egg-Juvenile-Period and offspring size.

3.1. INTRODUCTION

3.1.1. The importance of egg size in marine invertebrate larval strategy theory

Variations in egg size and their consequences for offspring have been greatly considered in marine invertebrate larval strategy theory (e.g. Vance 1973 a, b; Pechenik, 1979; Grahame and Branch, 1985; Sinervo and McEdward, 1988; Roughgarden, 1989; Levitan, 1993; Havenhand, 1993, 1995). Traditionally, emphasis has been placed upon the importance of egg size variations because of the commonly observed association between egg size and larval nutritional mode (Thorson, 1946, 1950). This relationship has been explained in terms of the contrasting energetic contents of different sized eggs and the implications thereof to the energy status of the offspring (Vance, 1973 a, b). In the earliest model, Vance (1973 a, b, 1974) postulated that offspring hatching from relatively small eggs are less likely to have sufficient intrinsic energy reserves to complete their pre-juvenile development and therefore require additional energy to successfully complete development. This usually is obtained by entering the water column and feeding upon the plankton, necessitating the selection for specialised feeding and swimming apparatus (planktotrophy) (Strathmann, 1985, but see Manahan, 1990). In contrast, offspring derived from larger eggs are more likely to be provided with sufficient energy reserves to complete their larval development and therefore are without the pre-condition of entering the plankton to obtain additional energy for successful development (pelagic and non-pelagic lecithotrophy). In this latter situation a specific feeding apparatus is redundant (Strathmann, 1985).

A strong inverse relationship between egg size and fecundity is also commonly observed in marine invertebrate species. While production of small eggs equates to greater fecundity, the energy reserves contained within eggs, and hence fitness of offspring, decreases with increasing fecundity. Egg size variation therefore implies a number of consequences for offspring in terms of larval nutritional mode, larval development, larval habitat (non-pelagic/pelagic), duration of larval development, fecundity and larval size. A substantial amount of empirical data, broadly comparing

interspecific variations in reproductive and larval traits, has demonstrated these relationships (Thorson, 1946, 1950; Hadfield and Switzer-Dunlap, 1984; Emlet *et al.*, 1987; Mauchline, 1988).

Thorson (1946) was the first to emphasise the potential adaptive significance of egg size variation when he identified the three most common larval strategies of marine invertebrates (1) Pelagic planktotrophy - whereby many larvae hatch from small eggs and are required to feed in the plankton once the apparatus for feeding is functional (2) Pelagic lecithotrophy - whereby fewer larvae hatch from larger eggs into the plankton but do not feed since they have sufficient intrinsic energy reserves to complete their development and (3) Non-pelagic or benthic lecithotrophy - whereby fewer offspring, also derived from large eggs, do not enter the plankton. Thorson noted that the relative frequency of these three larval types varied with latitude, such that the relative number of species displaying pelagic planktotrophy decreased towards the poles. Since this early classification many more types of larval developmental mode have been reported (e.g. Eckert, 1995; see Levin and Bridges, 1995 for review); however, much larval strategy theory has been based upon Thorsons simplified classification.

3.1.2. Vance's Model

Thorson's (1946) classification of larval types was used in an early theoretical model which has become the basis of larval strategy theory. Vance (1973 a, b) presented a simple mathematical model which attempted to calculate the relative reproductive efficiencies (i.e. the number of metamorphosing offspring per unit energy devoted to reproduction) of adults producing eggs of variable energetic content. Egg energy content was considered in relative terms whereby a value of 1 was complete lecithotrophy and values towards zero approached planktotrophy. He assumed that the relative amount of energy invested into an egg would determine the fecundity, the nutritional status (feeding period or no feeding period required) and hence the relative benthic and pelagic periods of the developing offspring. Egg energy contents approaching 1 would have a longer benthic existence since sufficient energy resources were available to discount the need to feed in the plankton. Egg energy contents approaching zero would have a relatively shorter benthic period and a longer feeding period in the plankton since energy resources would be utilised earlier in

development. Vance also incorporated the additional energy required to retain eggs away from the plankton (whereby eggs are deposited in a spawn mass or are brooded by the adult). The cost of this was set as a function of the number of eggs produced. Mortalities during benthic and pelagic periods were determined by natural logarithmic rates of predation. When reproductive efficiency is plotted against egg energy content (s; 0-1) an upwardly concave curve is produced. The model therefore predicted that only the extremes of egg size, and hence the extremes of larval strategy (pelagic planktotrophy or non-pelagic lecithotrophy), would be evolutionarily stable. In addition, as a result of the asymmetry of the curve, planktotrophy was predicted to be more efficient than lecithotrophy if planktonic food was abundant (thereby decreasing the developmental time) and/or if planktonic predation was low. Clarke (1987) later added that increases in temperature would also reduce the developmental period in the plankton and thus would also favour planktotrophy.

Vance's model is still considered to be an important theoretical basis underlying some of the principal selective pressures operating upon egg size and the implications thereof for offspring fecundity, larval nutritional mode, larval development period and larval habitat. However, some of the predictions of the model are neither consistent with other models of larval evolution nor with some empirical observations. Probably the two most important problems which have been addressed are the existence of intermediate egg sizes and mixed larval strategies in marine invertebrates and the complete lack of support for the theory and evidence that suggests that the non-feeding larva is an evolutionary derivative of the feeding larva (Strathmann, 1985, 1993; Kempf and Todd, 1989).

There has been much debate about the problems of Vance's model and other aspects have been bought to light. The failings of Vance's model have been attributed in part to the omission of other selective forces, both energetic and demographic, operating upon egg size and which might outweigh the principal selective forces advocated in Vance's model (e.g. dispersal potential and hence gene flow, Jablonski, 1986; Scheltema, 1989; Vermeij *et al.*, 1990; implications of duration of larval development period, Todd and Doyle, 1981; Roughgarden, 1989; Todd, 1991; Miller and Hadfield, 1993; Havenhand 1993; fertilisation success, Levitan, 1993). Another important area of research has arisen from the proposed invalidity of some of the assumptions which are implicit to Vance's model

(e.g. Christiansen and Fenchel, 1979; Pechenik, 1979; Caswell, 1981, Strathmann, 1985; Clarke, 1987). An example of the latter is the simple assumption that selection for egg size for all species in a given environment will be the same and of equal magnitude. The co-existence of species producing differently sized eggs but displaying similar larval strategies provides evidence that larval growth and mortality schedules vary widely among species (Strathmann, 1987). Further, while some of the predicted relationships between egg size and larval status are upheld generally there is less agreement when these relationships are considered within given phyla (Underwood, 1974; Hadfield and Switzer-Dunlap, 1984; Hadfield and Miller, 1987 for reviews on molluscs).

However, Vance (1974) argued that interspecific comparisons are of limited value for testing the predictions of his model because the evolution of reproductive and larval traits is driven by natural selection acting upon variations within a species (Vance, 1974). The best evidence bearing upon the selection for egg size and its effect upon larval status is therefore obtained from studies involving single species. Such studies enable the testing of correlations between egg size and larval traits without the problem of differences in genetic expression and of demonstrating the relationships that produce such correlations (Sinervo and McEdward, 1988).

3.1.3. Testing for relationships between egg size and larval traits.

Because shifts between developmental modes within a species are rare, adaptive changes have been mostly observed within a given developmental mode (but see Hadfield, 1972; Eyster, 1979; Levin, 1984, Levin and Creed, 1986; Levin *et al.*, 1991). The energy reserves of an egg are utilised in the production of cells and tissues of variable complexity and in the maintenance of cells once they are produced. Energy surplus at hatching serves as an additional energy store. Hence intraspecific variability in egg size could give rise to variations in larval survival, hatching size, developmental complexity and growth rates. For example, Mashiko (1985) demonstrated that an increase in egg size in the freshwater prawn *Palaemon paucidens* increased early survival, early developmental rates and starvation tolerance in hatched larvae (also see Krauter *et al.*, 1982; Luxmoore, 1982; Hadfield and Miller, 1987; Lima and Lutz, 1990; George *et al.*, 1990; Monteleone and Houde, 1990).

Echinoderms have been used for investigating the relationships between egg size and larval form because of the regulative nature in the formation and elaboration of larval feeding structures (McEdward, 1986; Sinervo and McEdward, 1988; Emlet *et al.*, 1987; George *et al*, 1990). Sinervo and McEdward (1988) were able to demonstrate that manipulation of the energy content of *Strongylocentrotus droebachiensis* eggs could affect the hatching size, body form (in terms of the number of larval arms of the feeding apparatus) and the rate of development through the early feeding stages. They reported that a reduction in size of *S. droebachiensis* eggs produced a simpler larval form, similar to another species, *S. purpuratus*, which produces smaller eggs. They concluded that evolutionary changes in egg size can influence the morphogenesis of larval form in echinoderms, and likewise, that natural selection acting upon the functional consequences of larval form can result in correlated changes in egg size.

However, while egg size has been shown to be influential in the early pre-juvenile developmental stages of individual offspring, there appears to be less influence upon later stages of pre-juvenile development, particularly in species with planktotrophic/pelagic larvae (Krauter *et al.*, 1982; Sinervo and McEdward, 1988; George *et al.*, 1990, but see Emlet *et al.*, 1987; Paulet *et al.*, 1988). This perhaps is because the fate of offspring of species displaying this larval strategy is affected by additional factors such as food availability and predation in the plankton. Sinervo (1990) suggested that planktotrophic offspring derived from variously sized eggs may ultimately have equivalent fitness and therefore that selection for egg size would be relatively less in planktotrophic eggs.

3.1.4. Selection for intraspecific variation in egg size

Population differences in egg size have been noted in species of crustacean (Mashiko, 1980, 1982; 1990; Skadsheim, 1984; Clarke *et al.*, 1985; 1991; Belk *et al.*, 1990; Duggan *et al.*, 1991; Clarke and Gore, 1992; Clarke, 1993), echinoderm (Emlet *et al.*, 1987; George, 1990, 1994; George *et al.*, 1990, 1991) mollusc (Goodwin, 1979) and fish (Montelone and Houde, 1990; Scott and Barbour, 1992). Variations in latitude or temperature (Clarke, 1979; Luxmore, 1982; Wagele, 1987; Dugan *et al.*, 1991; Clarke and Gore, 1992), food supply (George, 1990), hydrogeographic regime (Mashiko,

1982) and salinity (Mashiko, 1992) have all been shown to be factors which will bear upon the survival of offspring. This has led to the suggestion that variations in egg size among populations of the same species are the result of natural selection for an optimal egg size in variable environments. For populations which invest similar quantities of energy in reproduction, an increase in egg size will inevitably result in a decrease in fecundity. The trade-off between egg size and fecundity is therefore implicit in the natural selection of the optimal egg size ('Principle of Allocation', Cody, 1966). Several models which incorporate this concept predict the production of many small eggs in environments favourable for offspring and the production of fewer, larger eggs in unfavourable ones (Smith and Fretwell, 1974; Morris, 1987; McGinley *et. al*, 1987; Sibly *et. al*, 1987). Some empirical data have supported these predictions (eg. Mauchline, 1988; Mashiko, 1990, 1992). However egg size and fecundity can also vary independently of each other, with some populations producing many large eggs or fewer smaller eggs (Willows, 1987; Montelone and Houde, 1990; Clarke *et* al., 1991; George, 1994). Other models which place greater emphasis upon the effects of parental food availability, parental size and parental care upon the selection for egg size predict that the production of more and larger eggs is advantageous in favourable environments (Parker and Begon, 1986; Sargent, 1987).

If egg size is indeed under strict genetic control then it must be demonstrated that egg size is heritable, is not affected in the short term by environmental change and that any increase or decrease in reproductive investment will result in an increase or decrease in fecundity. For example, Mashiko (1990, 1992) studied the variation in egg and clutch size among populations of the prawn *Macrobrachium*, along the Sagami river, Japan. He reported that populations in the upper enclosed freshwater basin produced relatively few large eggs, populations in brackish waters produced a moderate number of intermediate sized eggs and populations at the river mouth produced many small eggs. Mashiko (1990, 1992) bred reciprocal crosses for populations of this species from the freshwater basin with those from the river mouth and hybrids from these crossings were ongrown. Egg and clutch size in hybrids were compared to controls. Mashiko (1990, 1992) reported that hybrids produced a moderate number of eggs of intermediate size, implying that egg size was a polygenic trait. Further, while variation in clutch size was observed for wild populations there was

no difference between experimental animals from different populations (also see Thompson, 1982; Mashiko, 1980, 1982; Xu and Barker, 1990; George *et al.*, 1991; George, 1994, for other studies demonstrating the selection for egg size)

3.1.5. Phenotypic expression of egg size - environmental influences

Variations in egg size have also been observed within single populations and individual parents (Skadsheim, 1984; Emlet *et al.*, 1987; Ferrand *et al.*, 1988; George *et al.*, 1991). There is some evidence to suggest that such variations are caused by variations in food supply. For example, the production of larger eggs by food-limited females has been reported in a terrestrial isopod (Brody and Lawlor, 1984) an echinoderm (George *et al.*, 1991; George 1994) and for some fishes (Bagenal, 1969; Townsend and Wooton, 1984). Clearly in such cases egg size is not under strict genetic control.

This led Sinervo and McEdward (1988) to propose that the influence of environmental factors, such as food supply, upon the phenotypic expression of egg size and clutch size could be of great importance because these epigenetic effects could impose constraints upon the evolution of larval strategy. Likewise selection may operate upon the responses of parents to variable environmental conditions (e.g. McKillup and Butler, 1979). Consequently some work has been focused upon the effects of maternal conditions such as food supply upon offsring production and quality (George, 1990; George *et al.*, 1991; Rossiter, 1991a, b) or timing of spawning (Skadsheim, 1984; Scott and Barbour, 1992). As for the results in Chapter II, it perhaps is reasonable to expect that species displaying different larval strategies may respond differently to changes in nutrient conditions and accordingly that their offspring also will be differently affected by changes in parental food supply.

3.1.6. Rationale

The aim of this section of the work was to compare the intraspecific variation in offspring size and development in *Lacuna pallidula* (a direct developer) and *Lacuna vincta* (a planktotrophic larvae) and to compare the influences of maternal food supply upon the phenotypic expression of these traits

3.2. MATERIALS AND METHODS

3.2.1. Duration of the Egg-Juvenile-Period

3.2.1.1. Collection and maintenance of adults and spawn masses

Twenty adult *Lacuna pallidula* and seventeen adult *Lacuna vincta* females were collected from St Andrews Bay, Fife, in January 1993 and were maintained on a mixed macroalgal diet (*Fucus serratus* and *Laminaria digitata*) in continuous flow seawater tanks at approximately 1°C above prevailing ambient seawater temperature (5.2 - 7.3 ^{0}C). Spawn masses were collected over a three week period during January. Newly deposited spawn masses were removed from the substratum with a scalpel and were transferred to hot-air sterilised petridishes containing 30 ml of 0.22 μm twice filtered seawater (TFSW) which was replaced daily (Todd and Havenhand, 1984). Twelve spawn masses of both species were incubated at each temperature treatment, 6 °C, 10 ^{0}C or 15 °C. Spawn masses in the 15 °C temperature treatment were brought up to a temperature of 15 °C over a two day period to minimise the effects of temperature shock. Stages of development were observed and the EJP (i.e. time to first hatching) was recorded in days (d).

3.2.1.2. Larval culture methods for *Lacuna vincta* larvae

Lacuna vincta larvae hatching from incubated spawn masses were cultured through the larval phase to metamorphosis at three temperatures (6°C = 3 cultures, 10°C = 6 cultures, 15°C = 10 cultures). Larvae were maintained initially at 2. ml^{-1}, and were fed the flagellate *Rhodomonas* at an initial concentration of 10^{4}. cells ml $^{-1}$ (Walne, 1963). TFSW and microalgal food (not bacteria free) were replenished every 5 days according to the methods described by Todd and Havenhand (1984) and Todd (1991) for the culturing of nudibranch larvae. Culture changes were made by concentrating larvae in a mesh bottomed plastic cup, which were then pipetted into fresh cultures (Scheltema, 1962). As the larvae grew, meshes of larger pore size (40, 80, 125 μm mesh size) were used during culture changes to allow ciliates and debris to fall through the mesh while retaining the larvae. Once larvae had attained shell lengths greater than 500 μm, changes were made by pipetting larvae

individually into clean cultures (Pilkington and Fretter, 1970) since the larvae were too large to concentrate on the mesh bottom used in the usual culture change procedure. Antibiotics were administered occasionally, consisting of 50μg. ml^{-1} of Streptomycin sulphate and 60μg. ml^{-1} Penicillin G (Todd, 1991). Larval cultures were maintained in continuous light to maintain the quality of microalgal food and to minimise bacterial growth in the larval cultures (Pilkington and Fretter, 1970). Cultures were not aerated because preliminary studies had shown adverse affects of aeration upon larval growth and survival.

Stages of development were noted and the shell lengths of 20 larvae from each culture were measured every five days. The onset of competence to metamorphose was determined by placing samples of 10 larvae in petri dishes with pieces of *Laminaria digitata* frond, a cue previously reported to induce metamorphosis (Fretter, 1972; Fretter and Manly, 1977). Metamorphosis was recorded according to the criteria reported by Fretter (1972), namely by loss of the velar lobes.

Microalgae were batch cultured in 'F/2' medium (Walne, 1963) at room temperature (20-25°C) and in continuous light. Once algal cultures displayed a colour indicating the nearing of the exponential phase of growth, 250 ml aliquots were twice centrifuged at 2500 rpm for 10 minutes to remove the nutrient medium. The resulting pellet was resuspended in TFSW and diluted to give the appropriate concentration of algae in larval cultures (Todd and Havenhand, 1984; Todd, 1991).

In addition, three *Lacuna vincta* spawn masses containing hatching larvae were each divided into four (4 x 250 larvae) and were cultured at 12.5 °C as described above on one of four microalgal diets. *Rhodomonas*, Tahitian strain of *Isochrysis*, *Pavlova lutheri* and an equal numerical mixture of all three. Larvae were kept at a concentration of one larvae. ml^{-1} and microalgal food was provided at a concentration of 10^{4} cells. ml^{-1}. Shell growth, larval survival and time to first competence were recorded during the first twenty days post-hatching.

3.2.2. Variations in egg diameters, numbers of eggs in spawn masses and juvenile size

3.2.2.1. Experiment one- Effects of maternal diet upon offspring size

Spawn masses produced by experimental animals of both species reported in Chapter II (Kingsbarns population) were used to assess the influence of variations in maternal diet upon egg size and hatching size of offspring. Spawn masses were obtained from *Lacuna vincta* females feeding upon *Laminaria digitata, Fucus serratus, Fucus vesiculosus* and *Mastocarpus stellata.* Spawn masses were obtained from *Lacuna pallidula* females feeding upon *L. digitata, F. serratus* and *F. vesiculosus.* The experiment was conducted between January and April 1994. The diameter of ten eggs in *L. pallidula* spawn masses and of twenty eggs in *L. vincta* spawn masses were measured to the nearest 0.001 mm. Spawn masses were then incubated at 10°C as described in section 3.2.1.1. The shell lengths of twenty offspring hatching from spawn masses were measured to the nearest 0.001 mm. *L. vincta* larvae were not cultured since survival of larvae in spawn masses produced by females in the *F. serratus, F. vesiculosus* and *M. stellata* diet treatments was poor.

Statistical analysis of the data varied for the two species because of the different numbers of spawn masses collected for both individual females and for females maintained on the different diets. For *Lacuna pallidula*, data for egg diameters were analysed by nested analysis of variance using mean measurements for spawn masses, with 'Diet' as a factor and with 'Females' as a nested factor within diets. Some females therefore had to be eliminated from the analysis to give a balanced design. Insufficient data were obtained to examine to include analysis of variation among spawn masses within individual females. The relationships between egg diameters and numbers of eggs, and hatching size and numbers of eggs, for females in the different diet treatments were analysed by ANCOVA.

For *Lacuna vincta*, egg diameters were analysed by one-way analysis of variance (with diet as a factor) because insufficient data were collected for each female to have 'females' as a nested factor.

However, sufficient data for hatching size were obtained for individual females which were analysed by nested analysis of variance with 'Diet' as a factor and with 'Females' as a nested factor. The relationship between hatching size and egg diameter in the various diet treatments was analysed by ANCOVA.

3.2.2.2. Experiment two - Inter-population variation in offspring size

Following observations of differences in maternal size, egg spawn mass size (i.e. egg numbers in spawn masses) and egg size in two geographically separate populations of *Lacuna pallidula* namely Kingsbarns (Fife) and Clachan Seil (Argyll), 30 *L. pallidula* egg spawn masses and 30 *Lacuna pallidula* females were collected from various macroalgal plants at both these sites in March 1994 and were maintained in the laboratory as described in section 3.2.1.1. Spawn masses and females also were collected from a third site, St Andrews Bay, which is 6km north of the Kingsbarns site.

Egg numbers in spawn masses collected from the field were compared to egg numbers in spawn masses deposited by females in the laboratory which were obtained over a fourteen day period (for both n=30). Egg diameters (n=10) were measured for the spawn masses deposited by *Lacuna pallidula* females in the laboratory (Clachan Seil n=10, Kingsbarns n=17). The shell lengths (n=20) of juveniles hatching from these spawn masses also were recorded. Egg number data were analysed by one-way analysis of variance. Egg diameter and juvenile size data were analysed by nested analysis of variance with 'Population' as a factor and with 'Spawn masses' as a nested factor. As such, some spawn masses had to be randomly eliminated from the analysis to give a balanced design. In view of the number of females used, and the short time in which the spawn masses were collected, it was assumed unlikely that any individual female had contributed more than one spawn mass to the data set.

Lacuna vincta spawn masses (for Clachan Seil n=13 and for Kingsbarns n=13) and *Lacuna vincta* females (For Clachan Seil n=14 and for Kingsbarns, n=22) also were collected from both sites in

March 1994. Egg numbers in spawn masses collected from the field and in the laboratory were compared (n=13 for both field collected spawn masses and for spawn masses obtained from captive individuals). Egg diameters were measured in spawn masses which had been deposited in the laboratory. These spawn masses then were incubated at 10 °C and the shell lengths (n=20) of hatching larvae were compared for the two populations. As for *Lacuna pallidula*, egg number data were analysed by one-way analysis of variance, while egg diameter and larval size data were analysed by nested analysis of variance.

3.2.2.3. Experiment three - Effects of maternal diet upon offspring size for three *Lacuna pallidula* populations

Previous work (Experiments one and two) had shown significant variation in egg numbers in spawn masses, egg diameters and juvenile shell lengths among *Lacuna pallidula* females from the same population, among different populations of *Lacuna pallidula* and among groups of females from the same population under different dietary regime. To investigate further these variations, 12 *L. pallidula* females were collected from each of three sites in Scotland, Kingsbarns (Fife), St Andrews Bay (Fife) and Clachan Seil (Argyll) in September 1994. The first two sites were 6 km apart on the east coast of Scotland, the third site was on the west coast of Scotland. The shell lengths of females from each population were recorded and six females from each population were fed either *Fucus serratus* or *Laminaria digitata ad libitum.* It was ensured that the body sizes of females in the diet treatments were comparable. Animals were maintained as described in Chapter II. Spawn masses were collected in January, February and March 1995. Egg numbers in spawn masses, egg diameters and shell lengths of hatching offspring were recorded as described above. Egg number data were analysed by two-way analysis of variance (with 'Population' and 'Diet' as factors), with 'Females' as a nested factor within each of the six treatments (3 populations and 2 diets). Egg diameter data and juvenile size data were analysed by two-way analysis of variance (factors being 'Population' and 'Diet') with spawn masses from different females nested within each treatment. Again, in both cases

some females were randomly eliminated to give a balanced design. Insufficient data were obtained to assess the variation among spawn masses within individual females.

Statistical tests were carried out with Minitab software using the methods described by Zar (1984) and Sokal and Rohlf (1981).

3.3. RESULTS

3.3.1. Egg-Juvenile-Period (EJP) of *Lacuna vincta* and *Lacuna pallidula* offspring

The ranges of EJP (d) of *Lacuna pallidula* and *Lacuna vincta* offspring incubated at the various temperatures (6, 10 and 15 °C) are summarised in Tables 3.1, 3.2 and 3.3. EJPs for *L. pallidula* were determined from the egg stage to first hatching of juveniles (Table 3.1). EJPs for *L. vincta* were determined from the uncleaved egg stage to first metamorphosis (Tables 3.1 and 3.2). Variations within temperature regimes were observed for both species. The EJPs of *L. vincta* were less than those of *L. pallidula* at all experimental temperatures (Table 3.3), but *L. vincta* larvae were able to delay metamorphosis for at least two months (see Figures 3.1 and 3.2) and hence *L. vincta* offspring displayed the greatest variation in EJP. Q_{10} values for the EJP were calculated by determining the coefficient of the regression for a conventional Arrhenius plot over the temperatures used (Todd, 1991) (Table 3.3). Q_{10} estimations were highly comparable.

The shell lengths of *Lacuna vincta* larvae at hatching ranged from 198-220 μm. Larvae attained competence to metamorphose at a shell length of 450-500 μm (Table 3.4). *Lacuna pallidula* offspring hatched at 602-705 μm in shell length (Table 3.4). However, *L. vincta* larvae continued to grow during the competent phase, attaining juvenile shell lengths of 450 to 1000 μm (Figures 3.1 and 3.2). It was interesting to note that the pattern of shell length growth in *L. vincta* larvae in the various temperature treatments followed different trajectories (Figures 3.1 and 3.2). Shell growth of larvae incubated at 15°C was rapid during the pre-competent phase and for a short time thereafter before levelling off. However, shell growth of larvae incubated at 10 °C and 6 °C levelled off almost immediately after attaining competence. Juvenile size therefore was more variable in *L. vincta* offspring, since *L. vincta* larvae were able to grow during the competent phase.

Table 3.1. Embryonic development time from first cleavage to hatching (* no data).

	Lacuna vincta			*Lacuna pallidula*		
Temperature (°C)	6	10	15	6	10	15
Number of spawn masses	12	12	12	12	12	12
Days to gastrula	*	*	*	16	9	4
Appearance of eyes	*	*	*	71	34	20
Days to hatch	22-25	14-17	13-15	115-126	58-67	35-40

Table 3.2. Larval development times from hatching to first attainment of competence in *Lacuna vincta.*

Temperature (°C)	6	10	15
Number of spawn masses	3	6	10
Days to competence	65	25	15

Table 3.3. Total larval period from first cleavage to juvenile.

	Lacuna vincta			*Lacuna pallidula*		
Temperature (°C)	6	10	15	6	10	15
Larval period (days)	87-90	39-42	27-28	115-126	58-67	35-40
Q_{10} (6 °C - 10 °C)	4.4			4.3		

Table 3.4. Egg size, hatching size and juvenile size (* data not relevant).

Species	*L. vincta*	*L. pallidula*
Egg diameter range (µm)	90-144	230-290
Hatching shell length (µm)	198-220	425-705
Shell length at first competence (µm)	450	*
Juvenile shell length (µm)	>450	602-705

Figure 3. 1. ***Lacuna vincta*** - Mean (+/- SE) shell lengths (μm) of larvae cultured at 15 °C (closed circles) (n = 10 cultures), 10 °C (open circles) (n = 6 cultures) and 6 °C (closed squares) (n = 3 cultures).

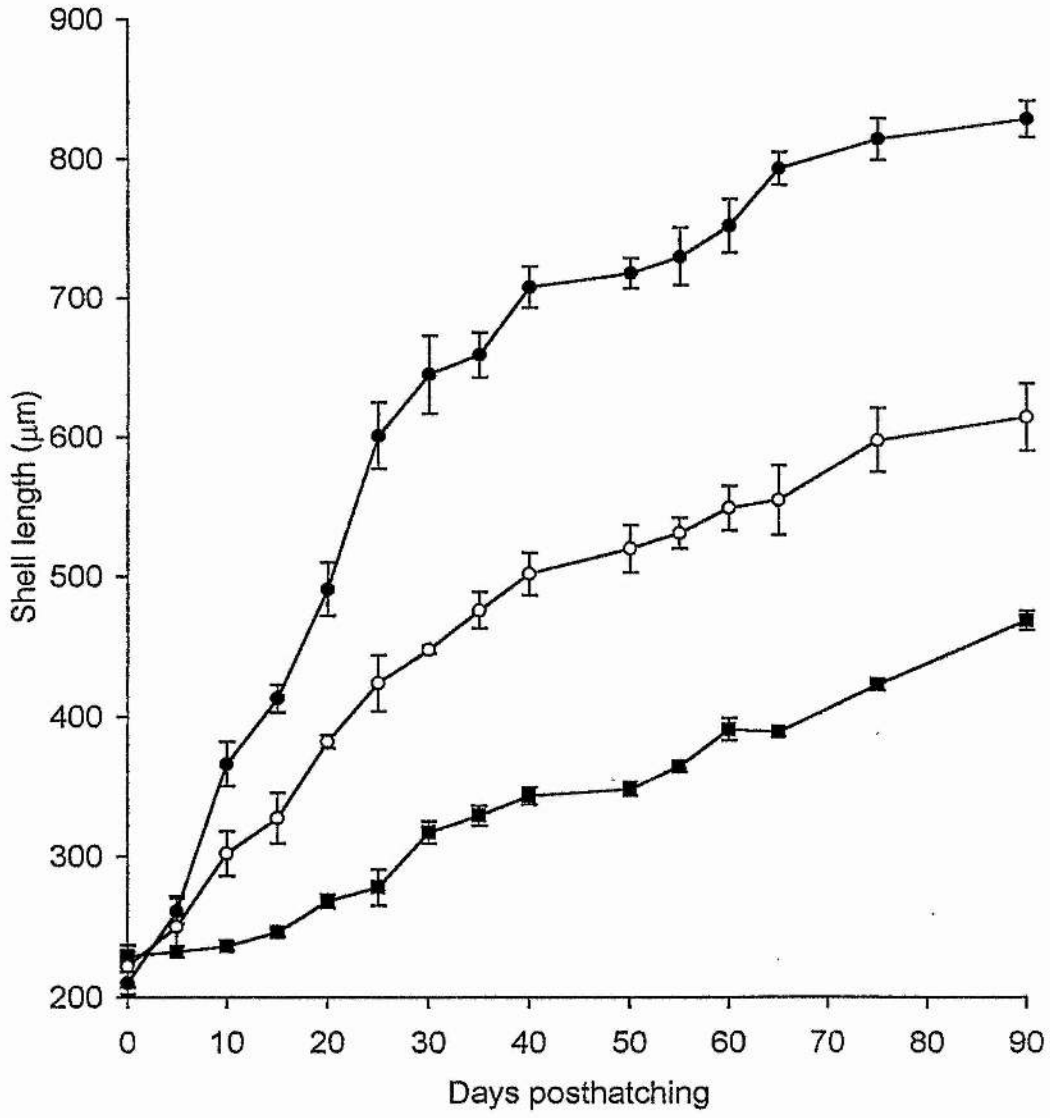
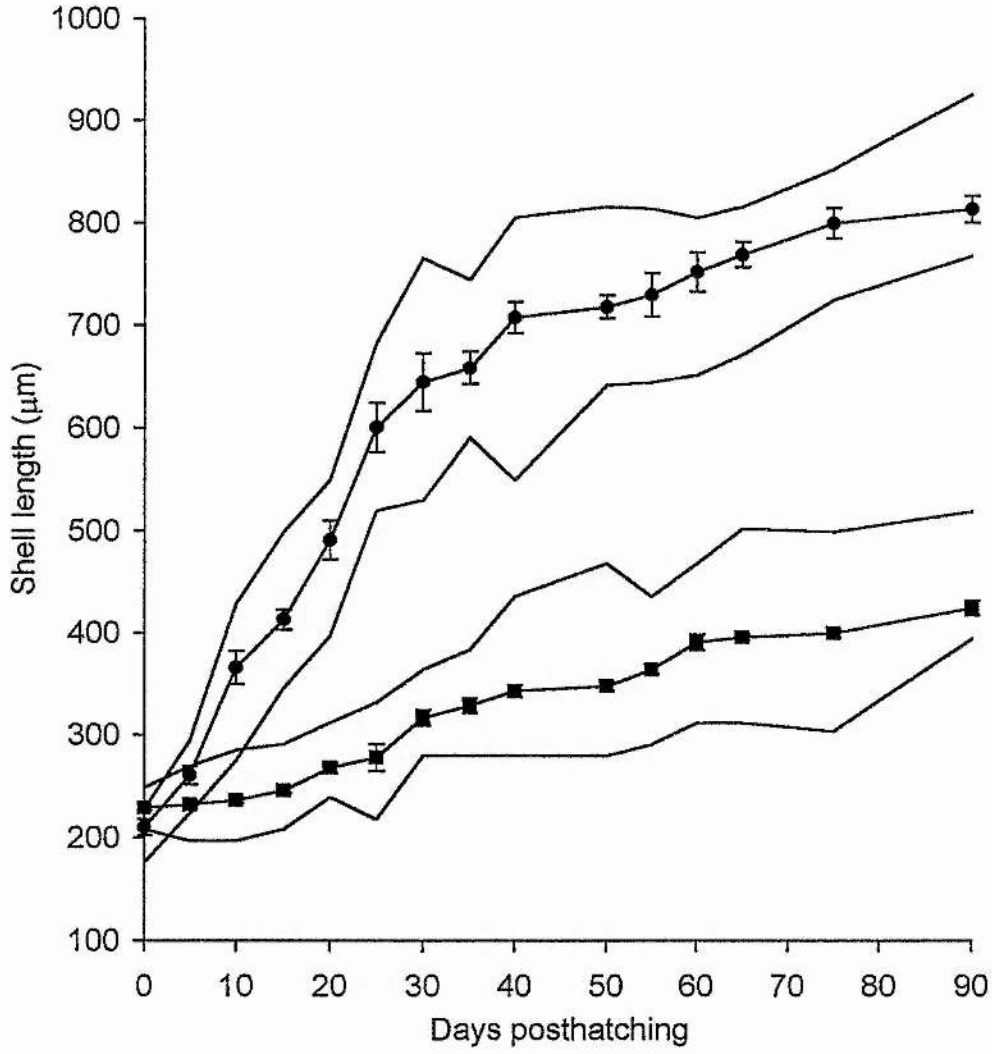
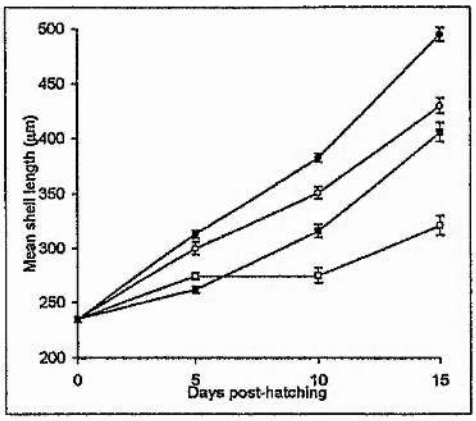

900
800
700
600
500
400
300
200
Shell length (µm)
0
10
20
30
40
50
60
70
80
90
Days posthatching

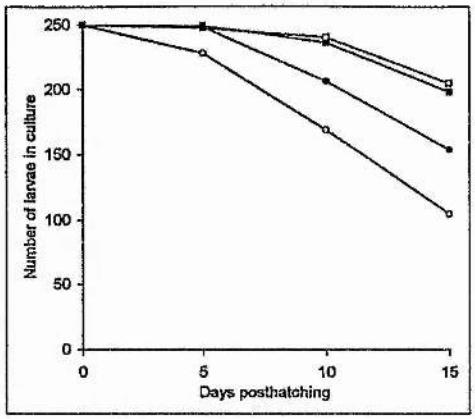
Figure 3. 2. ***Lacuna vincta*** - Mean (+/- SE), mimimum and maximum shell lengths (μm) of larvae cultured at 15 °C (closed circles) (n = 10 cultures) and 6 °C (closed squares) (n = 3 cultures).

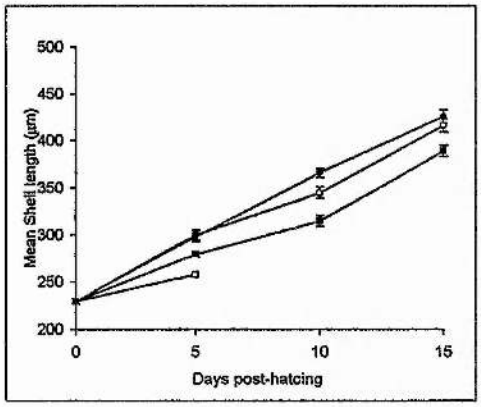

1000
900
800
700
600
500
400
300
200
100
Shell length (µm)
0
10
20
30
40
50
60
70
80
90
Days posthatching

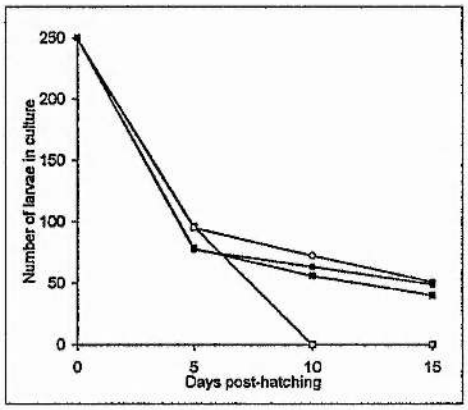
Variations both in shell growth and survival of *Lacuna vincta* larvae in the various microalgal diet treatments were observed (Figure 3.3). Larvae maintained on a diet of *Pavlova lutheri* displayed poor growth and survival. Larvae maintained on Tahitian strain *Isochrysis galbana* and on a mixed diet consisting of equal numerical proportions of all three microlagal species generally displayed greater growth rates and better survival. Variations in growth and survival were also observed between larvae from different spawn masses. Larvae from spawn mass (a) were larger at hatching and displayed greater rates of shell growth than larvae from the other two spawn masses. Time to first metamorphosis was 15 days in Tahitian strain *Isochrysis* and mixed diet treatments, and 20 days in *Pavlova lutheri* and *Rhodomonas* treatments.

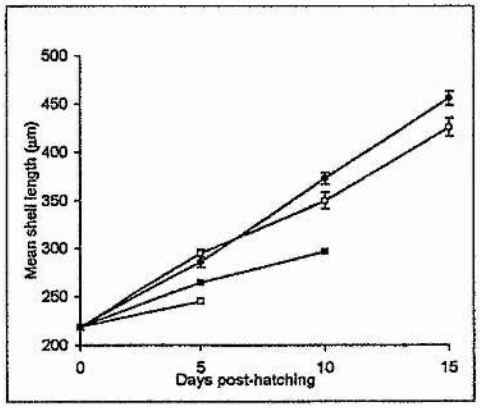
Figure 3. 3. ***Lacuna vincta*** - Mean shell lengths (μm) (+/-SE) (first column) (n=20 larvae) and survival (second column) of larvae from three different spawn masses (a = top row, b = middle row and c = bottom row) in the various microalgal diet treatments (*Pavlova lutheri* = open squares, *Rhodomonas* = closed squares, Tahitian strain *Isochrysis galbana* = open circles and mixed diet = closed circles).

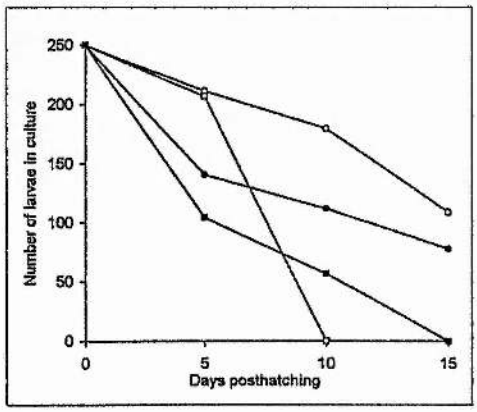

Mean shell length (µm)
500
450
400
350
300
250
200
0
5
10
15
Days post-hatching


Number of larvae in culture
250
200
150
100
50
0
0
5
10
15
Days posthatching


Mean Shell length (µm)
500
450
400
350
300
250
200
0
5
10
15
Days post-hatcing


Number of larvae in culture
250
200
150
100
50
0
0
5
10
15
Days post-hatching


Mean shell length (µm)
500
450
400
350
300
250
200
0
5
10
15
Days post-hatching


Number of larvae in culture
250
200
150
100
50
0
0
5
10
15
Days posthatching

3.3.2. Variations in offspring size

The relationship between investment in individual eggs and the number of eggs in a spawn mass in *Lacuna pallidula* and *Lacuna vincta* was initially addressed by calculating the predicted mean organic weight of eggs using the regression equations derived in Chapter 2 (Havenhand and Todd, 1988b). Different relationships were observed for the two species (Figures 3.4 and 3.5.). The predicted mean weight of *Lacuna pallidula* eggs (and attendant gel) declined with increasing number of eggs in a spawn mass, levelling off at 40μg for 60 or more eggs in a spawn mass. The predicted weight of *Lacuna vincta* eggs remained constant at 7×10^{-4} μg within the range of the number of eggs in spawn masses observed in this experiment.

3.3.2.1 Experiment one - Effects of maternal diet upon offspring size

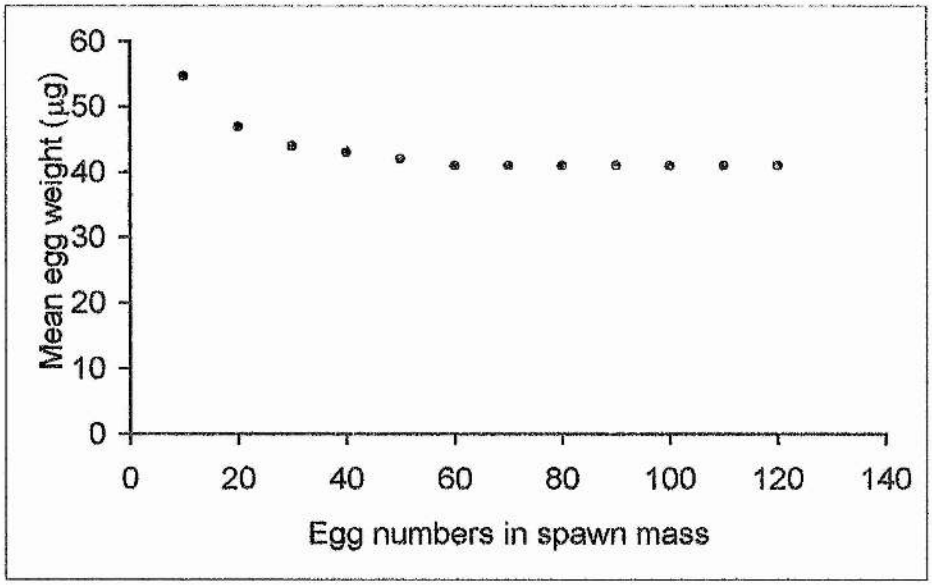
Lacuna pallidula

The mean diameters (n=10) of eggs from 42 *Lacuna pallidula* spawn masses produced by females in the various macroalgal diet treatments (*Fucus serratus*, *Fucus vesiculosus* and *Laminaria digitata*) are presented in Figure 3.6. Nested analysis of variance, using data for mean egg diameters in spawn masses, was used to examine variations in egg size among females in different diet treatments and among females within diet treatments ('diet' was a factor and 'females' was a nested factor). Results of the analysis revealed significant variation among females only (results are presented in Figure 3.6).

In view of the predictions from the regression equations derived in chapter II, it was suggested that this may be because females were producing different numbers of eggs and hence eggs of different sizes (see Figure 3.4). Mean egg diameters therefore were plotted against egg numbers in spawn masses produced by females in the various diet treatments (Figure 3. 7). As predicted from the regression equation, analysis of covariance (see Figure 3.7) revealed that egg diameters significantly decreased with increasing egg numbers in spawn masses. This suggested therefore that *Lacuna pallidula* females producing larger spawn masses were also producing smaller eggs. However, it was

Figure 3. 4. ***Lacuna pallidula*** - Predicted organic weights (μg) of eggs as a function of egg numbers in spawn masses derived from the regression equation obtained in section 2.3.

Figure 3. 5. ***Lacuna vincta*** - Predicted organic weights (μg x 10^4) of eggs as a function of egg numbers in spawn masses derived from regression equations obtained in section 2.3.


Mean egg weight (µg)
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
Egg numbers in spawn mass

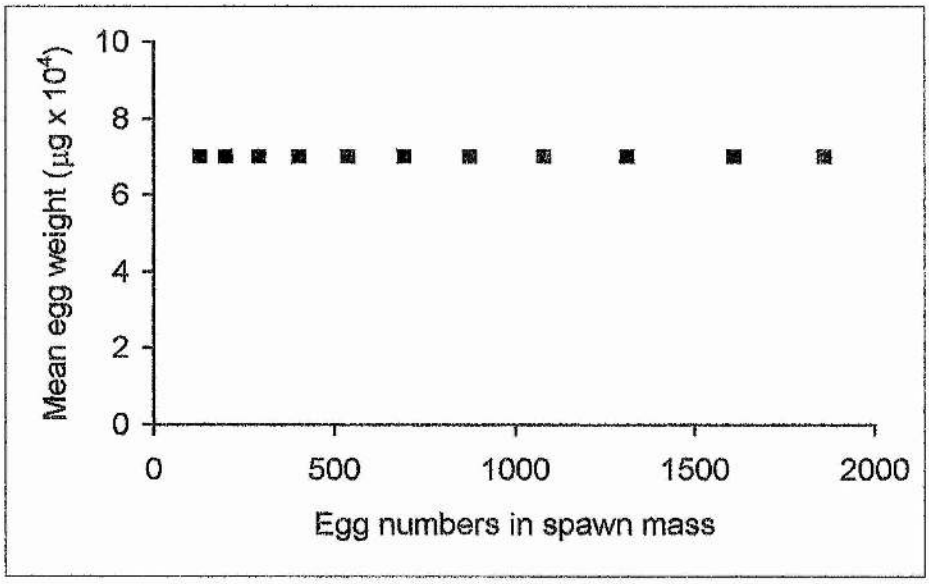
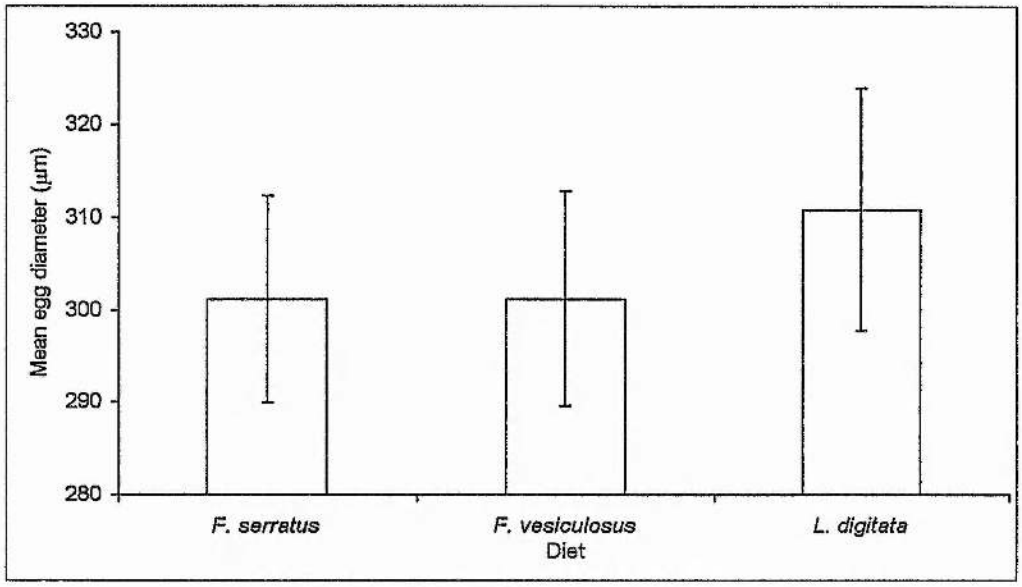
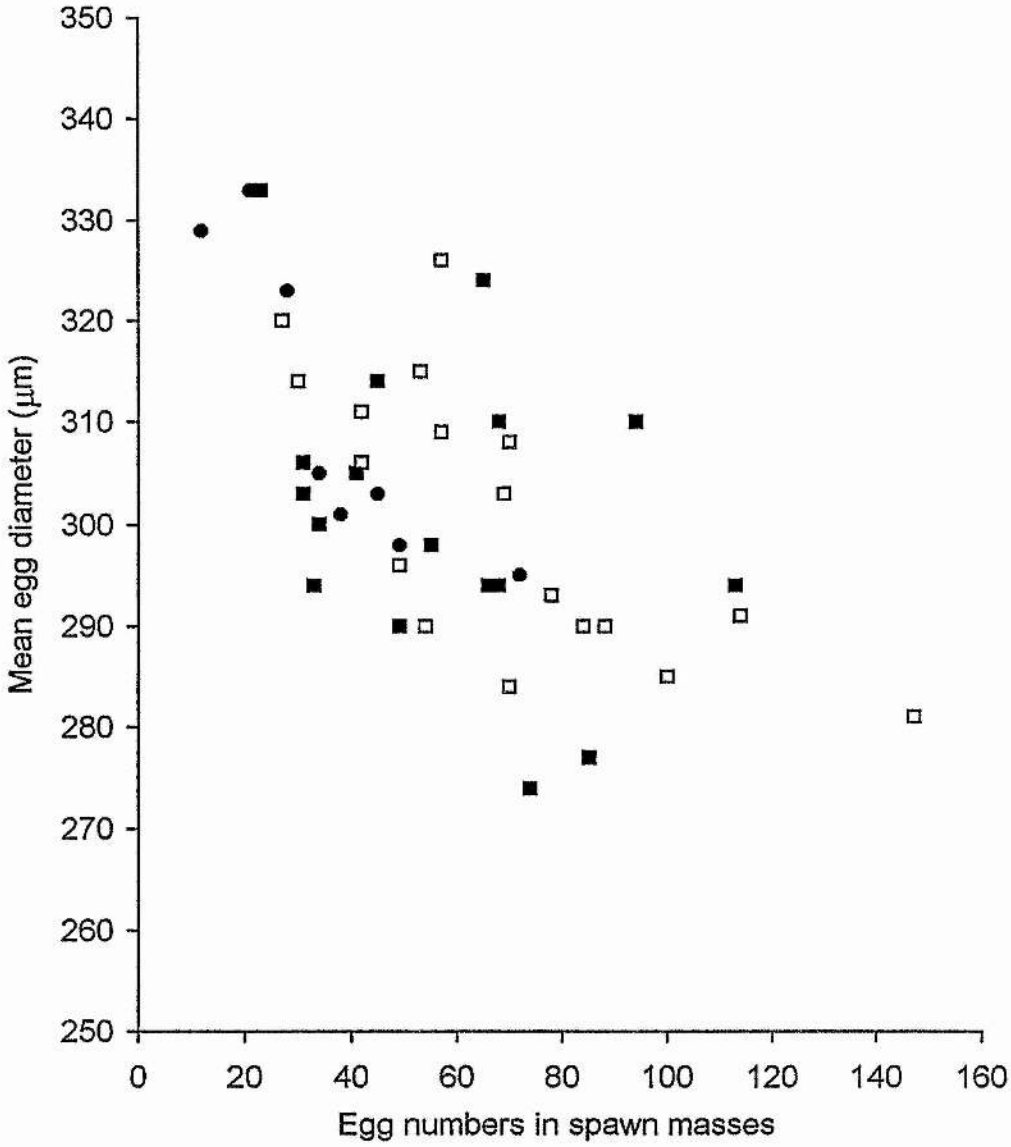

Mean egg weight (µg x 10⁴)
10
8
6
4
2
0
0
500
1000
1500
2000
Egg numbers in spawn mass

Figure 3. 6. ***Lacuna pallidula*** - Mean (+/- SE) diameters (μm) of eggs in spawn masses produced by females in the various diet treatments (*Laminaria digitata* n=8, *Fucus serratus* n=17, *Fucus vesiculosus* n=18). Nested analysis of variance showed no significant variation among females in the various diet treatments but significant variation among females (see table below).



Source	DF	Seq SS	Adj SS	Adj MS	F	P
Diet	2	770.8	576.7	288.3	2.28	0.122
Female (Diet)	9	4085.7	4085.7	454	3.58	0.005
Error	27	3419.2	3419.2	126.6		
Total	38	8275.7				

Figure 3. 7. ***Lacuna pallidula*** - Mean (n=10) diameters (μm) of eggs in spawn masses produced by females in the *Fucus serratus* (open squares), *Fucus vesiculosus* (closed squares) and *Laminaria digitata* treatments (closed circles) as a function of the egg numbers in spawn masses. Analysis of covariance showed a significant common negative relationship ($F_{1,41} = 20.95$, $P < 0.05$) but no significant variation in elevation of this relationship for the various diet treatments ($F_{2,40} = 0.31$, $P > 0.05$).


Mean egg diameter (µm)
350
340
330
320
310
300
290
280
270
260
250
0
20
40
60
80
100
120
140
160
Egg numbers in spawn masses

thought that probably some bias may have been introduced in this result because the data set was derived from females which did not have equal weighting for the number of spawn masses they contributed to the analysis. Further, because individual females were producing variously sized spawn masses, it also was deemed important to determine whether this relationship was upheld for spawn masses produced by individual females.

Mean egg diameters, therefore, were plotted against egg numbers in spawn masses for each female within each diet treatment (Figures 3.8, 3.9 and 3.10.). Within all three diet treatments there was a general decline in egg diameter with egg numbers in a spawn mass, although there was one notable exception with one female in the *Fucus serratus* diet treatment producing relatively large numbers of large eggs (see Figure 3.8, open circle). Analysis of covariance along with the plots revealed that there was no consistent pattern in individual females (see Figures 3.8, 3.9 and 3.10). For example, while some females produced eggs of similar diameter over a range of egg numbers in spawn mass (e.g. Figure 3. 9, closed diamond), in others, the mean egg diameter decreased with increasing egg numbers in a spawn mass (e.g. females in the *F. serratus* treatment, Figure 3.8).

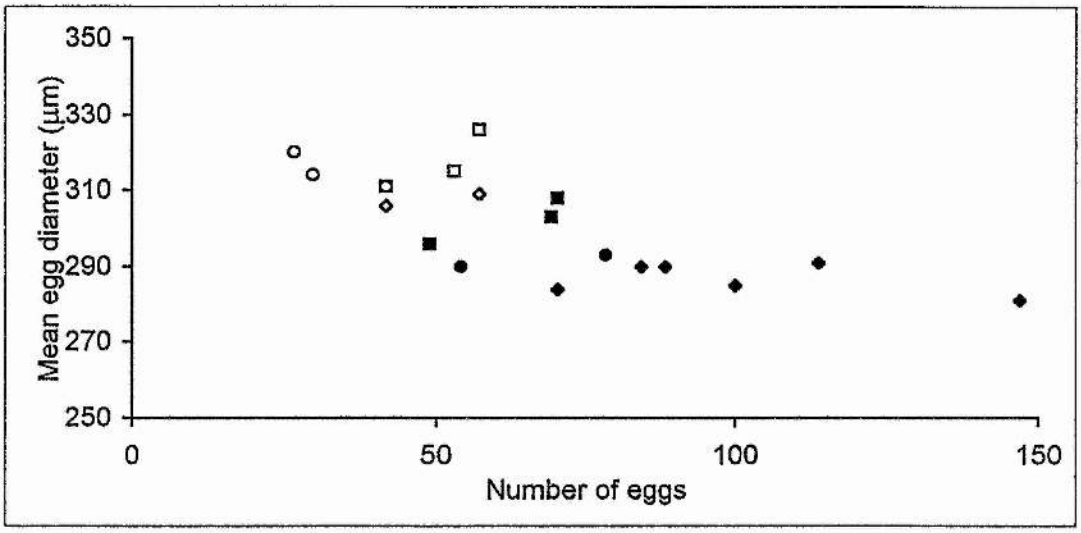
Twenty eight *Lacuna pallidula* spawn masses were successfully incubated at 10°C to hatching. Mean shell lengths of hatching offspring produced by females in the various macroalgal diet treatments were plotted against egg numbers in spawn masses (Figure 3.11.). Analysis of covariance (see Figure 3. 11) showed a significant common negative slope, but significant variation in the elevation of slope with respect to diet treatment. Offspring hatching from spawn masses produced by females in the *Laminaria digitata* treatment were relatively smaller than offspring hatching from similar sized eggs which had been produced by females in the *Fucus serratus* and *Fucus vesiculosus* treatments. Hence, while maternal diet did not significantly directly affect the size of eggs produced it did directly affect the size of hatching offspring derived from similarilar sized eggs. Numbers of eggs in spawn masses influenced both egg size and the size of hatching offspring.

Figure 3. 8. (top) ***Lacuna pallidula*** - Mean (n=10) diameters (μm) of eggs in spawn masses produced by females in the *Fucus serratus* treatment as a function of egg numbers in spawn masses. Analysis of covariance showed a significant common negative relationship for all females ($F_{1,16} = 8.69$, $P < 0.05$) but significant variation in elevation of slopes ($F_{5,12} = 5.89$, $P < 0.05$).

Figure 3. 9. (middle) ***Lacuna pallidula*** - Mean (n=10) diameters (μm) of eggs in spawn masses produced by females in the *Fucus vesiculosus* treatment as a function of egg numbers in spawn masses. Analysis of covariance showed no common relationship for females ($F_{1,17} = 0.3$, $P > 0.05$) but significant variation among females ($F_{5,13} = 8.41$, $P < 0.05$).

Figure 3. 10. (bottom) ***Lacuna pallidula*** - Mean (n=10) diameters (μm) of eggs in spawn masses produced by females in the *Laminaria digitata* treatment as a function of egg numbers in spawn masses. Analysis of covariance showed no common relationship for females($F_{1,7} = 0.4$, $P > 0.05$) but significant variation among females ($F_{3,5} = 2.2$, $P < 0.05$).


Mean egg diameter (µm)
350
330
310
290
270
250
0
50
100
150
Number of eggs


Mean egg diameter (µm)
350
330
310
290
270
250
0
50
100
150
Number of eggs


Mean egg diameter (µm)
350
330
310
290
270
250
0
50
100
150
Number of eggs

Lacuna vincta

The mean diameters of eggs produced by *Lacuna vincta* females in the various diet treatments *(Laminaria digitata, Fucus serratus, Fucus vesiculosus* and *Mastocarpus stellata*) are presented in Figure 3.12. As for *Lacuna pallidula*, one-way analysis of variance showed no significant variation in the diameter of eggs with respect to diet treatment (see Figure 3.12). Insufficient egg size data were obtained to determine whether there were any significant variations in diameters of eggs produced by different females.

The mean shell lengths of *Lacuna vincta* offspring hatching from spawn masses produced by females in the various diet treatments are presented in Figure 3.13. Nested analysis of variance ('diet' was a factor, 'spawn masses' was a nested factor) showed significant variation with respect to diet but no significant variation among spawn masses within diet treatments (see Figure 3.13). Offspring hatching from spawn masses produced by females in the *Laminaria digitata* diet treatment were significantly larger. Mean shell lengths of hatching offspring were then plotted against the mean diameters of eggs produced by females in the four diet treatments (Figure 3.14). Analysis of covariance did not show a significant relationship between hatching shell length and egg size, but the shell lengths of larvae hatching from spawn masses produced by females in the *L. digitata* diet treatment were significantly larger (see Figure 3.14). Hence, as for *Lacuna pallidula*, diet affected the size of offspring derived from similarily sized eggs.

Figure 3. 11. ***Lacuna pallidula*** - Mean (n=20) shell lengths (μm) of juveniles hatching from spawn masses produced by females in the *Fucus serratus* (open squares), *Fucus vesiculosus* (closed squares) and *Laminaria digitata* treatments (closed circles) as a function of numbers of eggs in spawn masses. Analysis of covariance showed a significant decline in juvenile size with increasing egg numbers ($F_{1,27} = 8.6$, $P < 0.05$) and significant variation in this relationship among diet treatments ($F_{2,26} = 8.06$, $P < 0.05$).

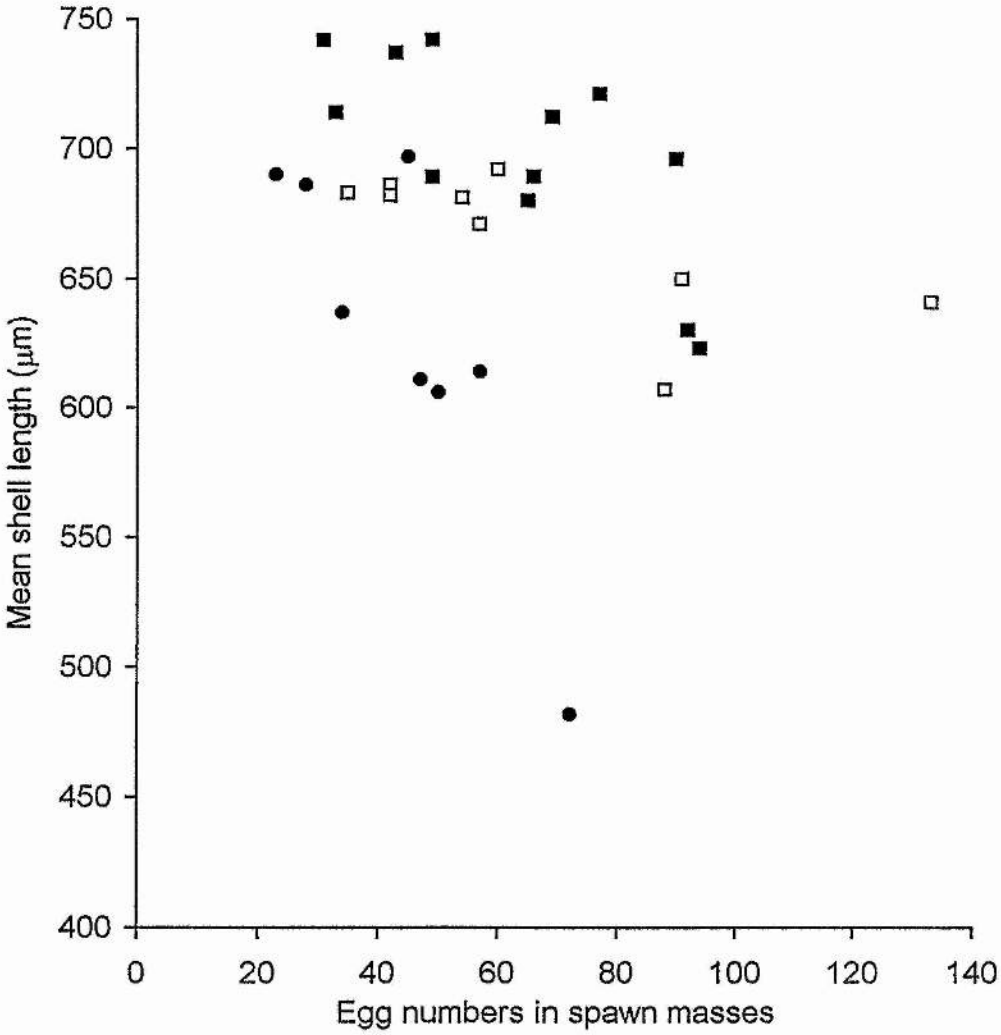

750
700
650
600
550
500
450
400
Mean shell length (μm)
0
20
40
60
80
100
120
140
Egg numbers in spawn masses

Figure 3. 12. ***Lacuna vincta*** - Mean (+/- SE) diameters (μm) of eggs in spawn masses produced by females in the various diet treatments (*Laminaria digitata* n=12, *Fucus serratus* n=5, *Fucus vesiculosus* n=7 and *Mastocarpus stellata*, n=8). One-way analysis of variance showed no significant variation in egg size among diet treatments ($F_{3,636} = 1.33$, $P > 0.05$).

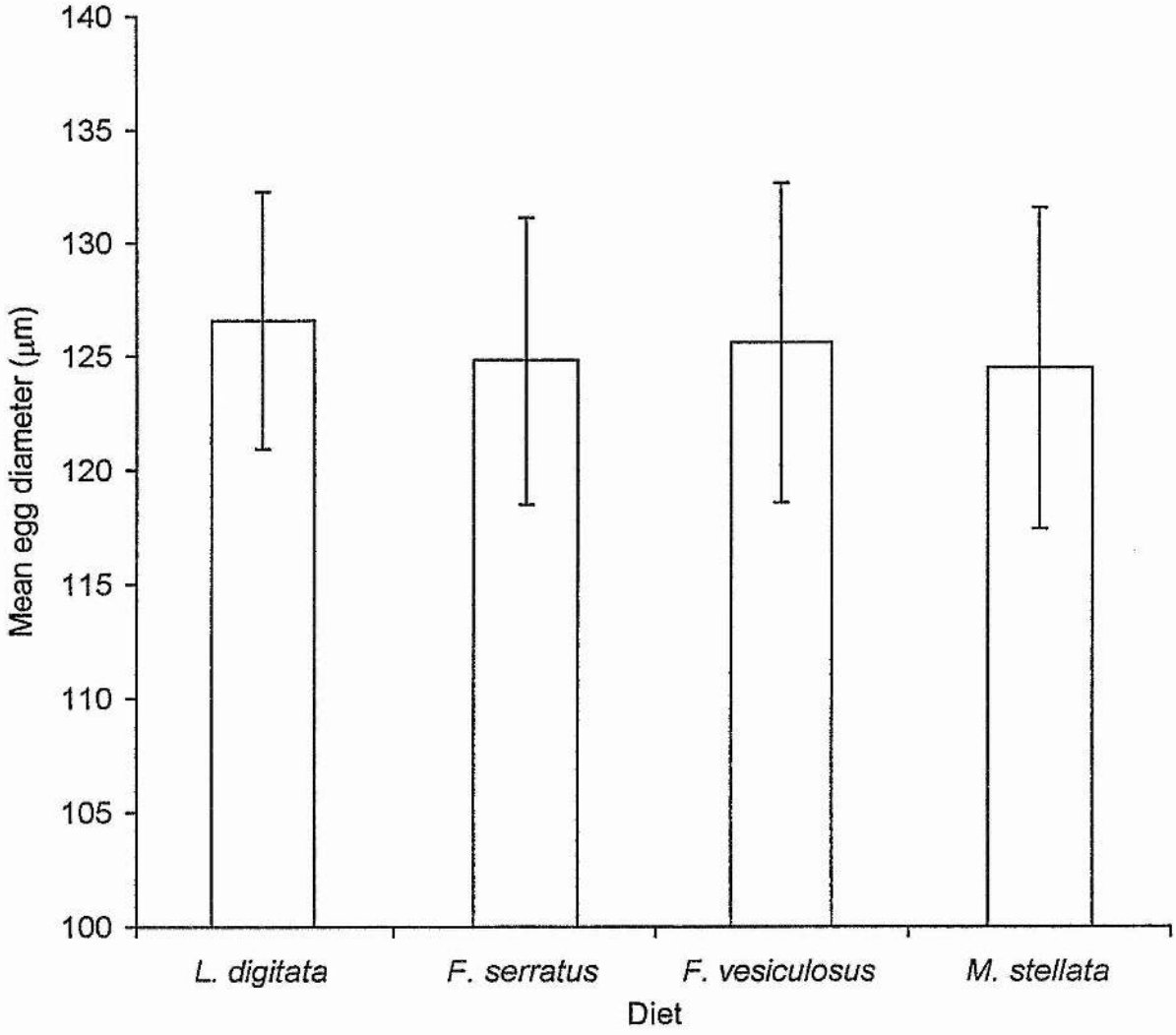
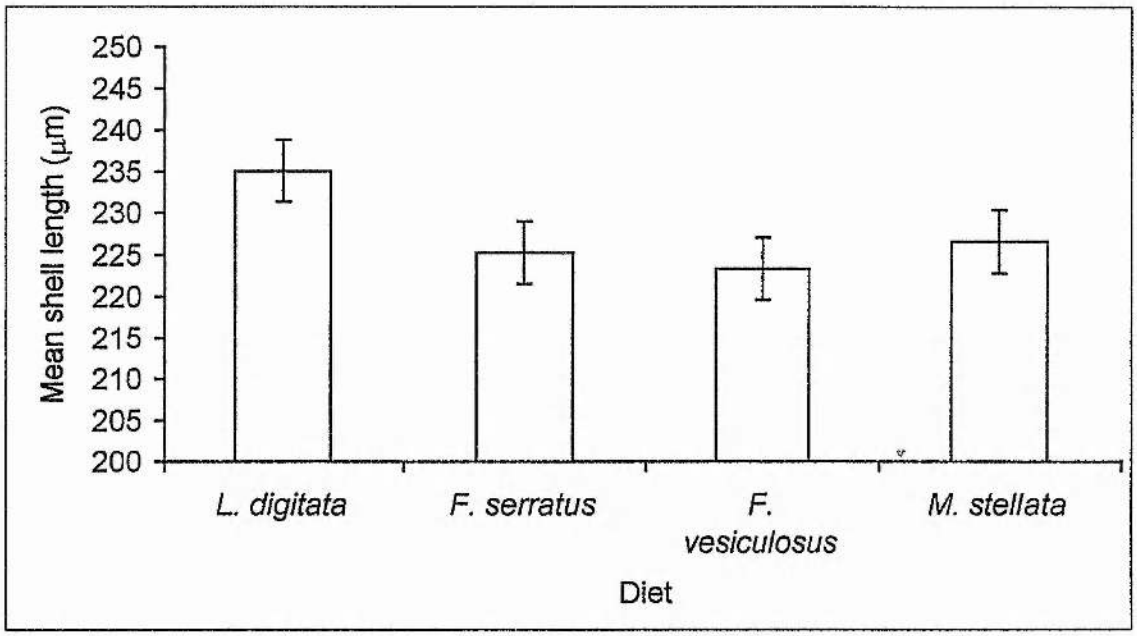
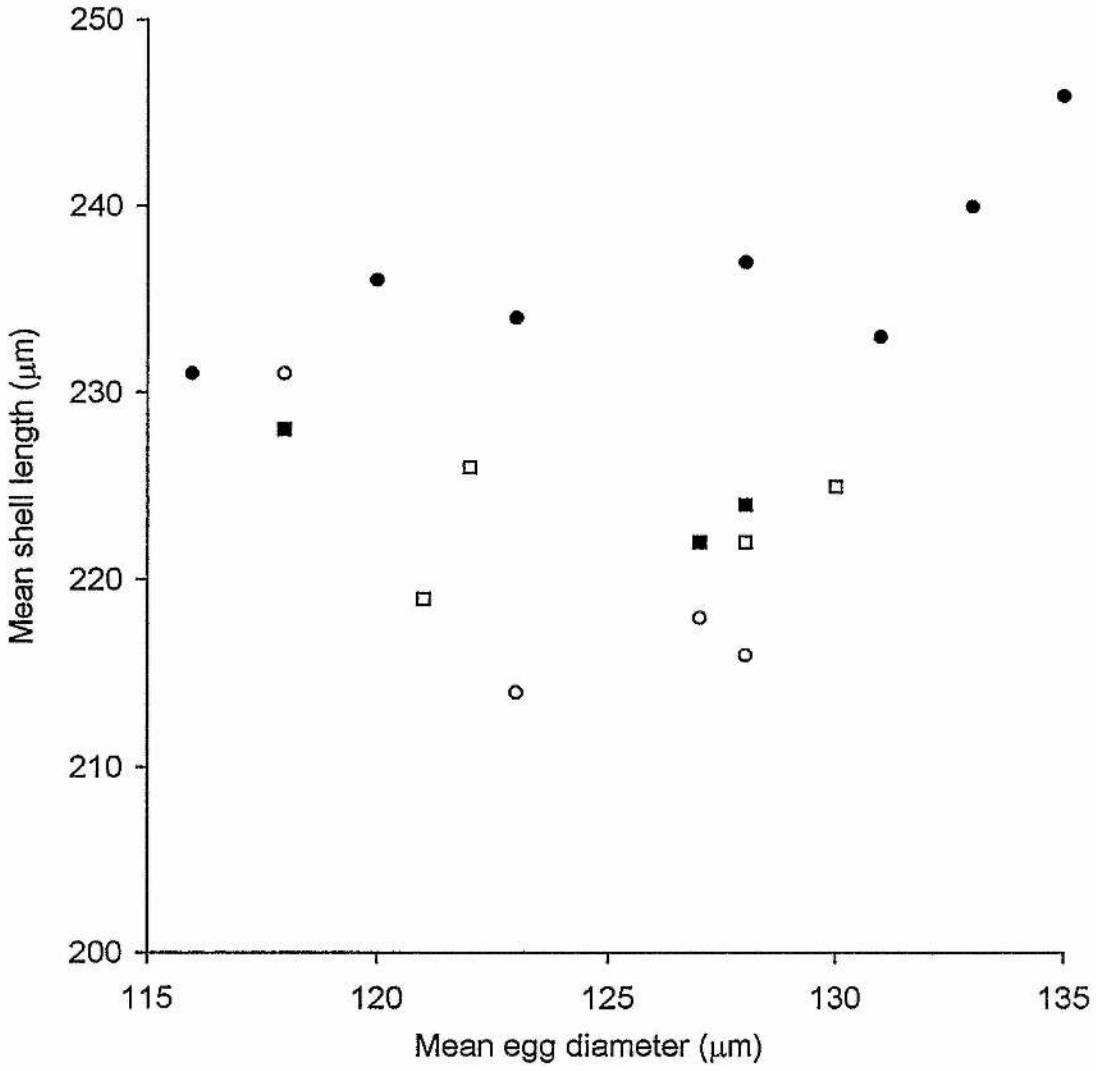

140
135
130
125
120
115
110
105
100
Mean egg diameter (µm)
L. digitata
F. serratus
F. vesiculosus
M. stellata
Diet

Figure 3. 13. ***Lacuna vincta*** - Mean (+/- SE) shell lengths (μm) of larvae hatching from spawn masses produced by females in the various diet treatments (*Laminaria digitata* n=11, *Fucus serratus* n=7, *Fucus vesiculosus* n=8 and *Mastocarpus stellata*, n=8). Nested analysis of variance showed significant variation among diets, but no significant variation among spawn masses within diet treatments (see table below).



Source	DF	Seq SS	Adj SS	Adj MS	F	P
Diet	3	10221.4	11063	3687.7	19.21	<0.001
Females (diet)	8	2259.9	2259.9	282.5	1.47	0.164
Error	597	114608.7	114608.7	192		
Total	608	127090				

Figure 3. 14. ***Lacuna vincta*** - Mean (n=20) shell lengths (μm) of hatching juveniles in spawn masses produced by females in the *Fucus serratus* (open squares), *Fucus vesiculosus* (closed squares), *Laminaria digitata* (closed circles) and *Mastocarpus stellata* diet treatments (open circles) as a function of the mean egg diameter (μm). Analysis of covariance revealed no significant common relationship ($F_{1,16} = 0.029$, $P > 0.05$) but showed significant variation with respect to diet ($F_{3,14} = 11.28$, $P < 0.05$).


250
240
230
220
210
200
Mean shell length (μm)
115
120
125
130
135
Mean egg diameter (μm)

3.3.2.2. Experiment two - Inter-population variation in offspring size

Lacuna pallidula

Thirty *Lacuna pallidula* spawn masses were each collected from two sites, Clachan Seil and Kingsbarns, and, in addition, from another site, St Andrews Bay, which is 6km to the north of Kingsbarns. Females from all three populations also were collected and maintained in the laboratory.

Mean numbers of eggs allocated to spawn masses for both field-collected and laboratory-collected spawn masses are presented in Figure 3.15. Egg allocations for spawn masses collected from field sites were similar to those produced by females originating from the same site but which were maintained in the laboratory. Hence, it therefore was assumed that egg numbers in spawn masses produced by females maintained in the laboratory would be a true reflection of those which were produced at field sites. One-way analyses of variance showed significant differences in egg allocation among populations for both field and laboratory collected spawn masses (see Figure 3.15.). *Lacuna pallidula* females from the Clachan Seil population deposited significantly more eggs in spawn masses than females from the Kingsbarns and St Andrews Bay populations (see Figure 3.15.).

The mean diameters of eggs produced by captive *Lacuna pallidula* females from Clachan Seil and Kingsbarns are presented in Figure 3.16. The mean diameter of eggs produced by females from Kingsbarns were significantly larger than those produced by females from Clachan Seil. However, significant variation was also shown among spawn masses (see Figure 3.16.). As before, it was suggested that this variation may be due in part to differences in egg numbers in spawn masses. Analysis of covariance therefore was performed upon data for mean egg diameters and egg numbers in spawn masses (see Figure 3.17). In contrast to the results obtained in Experiment one, egg diameters increased with increasing numbers of eggs in spawn masses for both populations. Hence, while egg diameters were observed to decrease with increasing numbers of eggs for different populations, patterns among spawn masses within populations were varied. Perhaps it is important

Figure 3. 15. ***Lacuna pallidula*** - Mean numbers of eggs in spawn masses produced by females from Clachan Seil, Kingsbarns and St Andrews Bay populations in the field (white) (n=30) and in the laboratory (grey) (n=30). One-way analysis of variance showed significant variation among populations in both cases (field $F_{2,87} = 36.14$, $P<0.05$, laboratory $F_{2,87} = 21.93$, $P<0.05$).

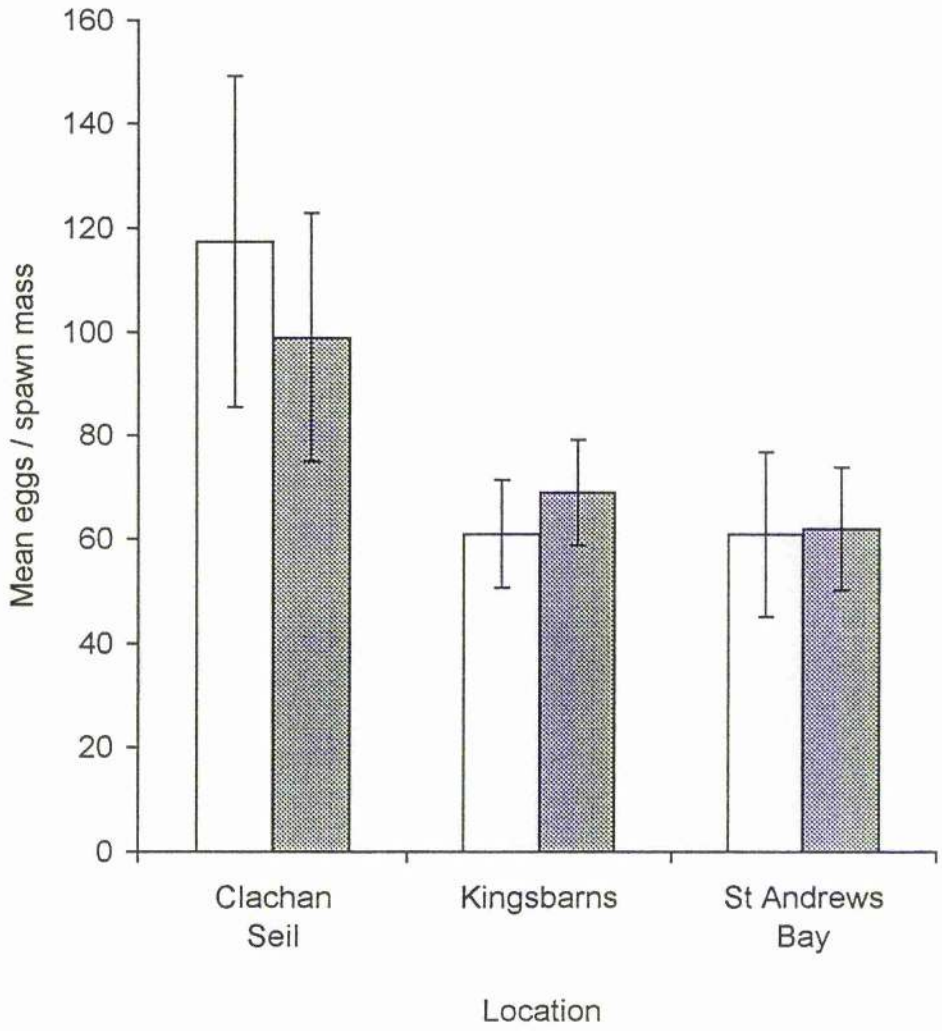
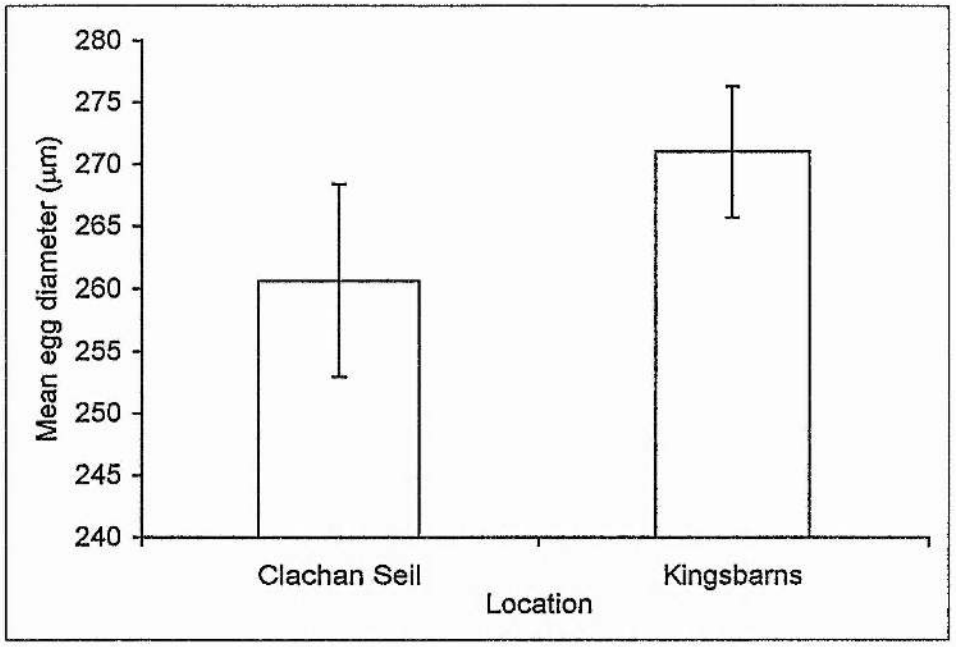
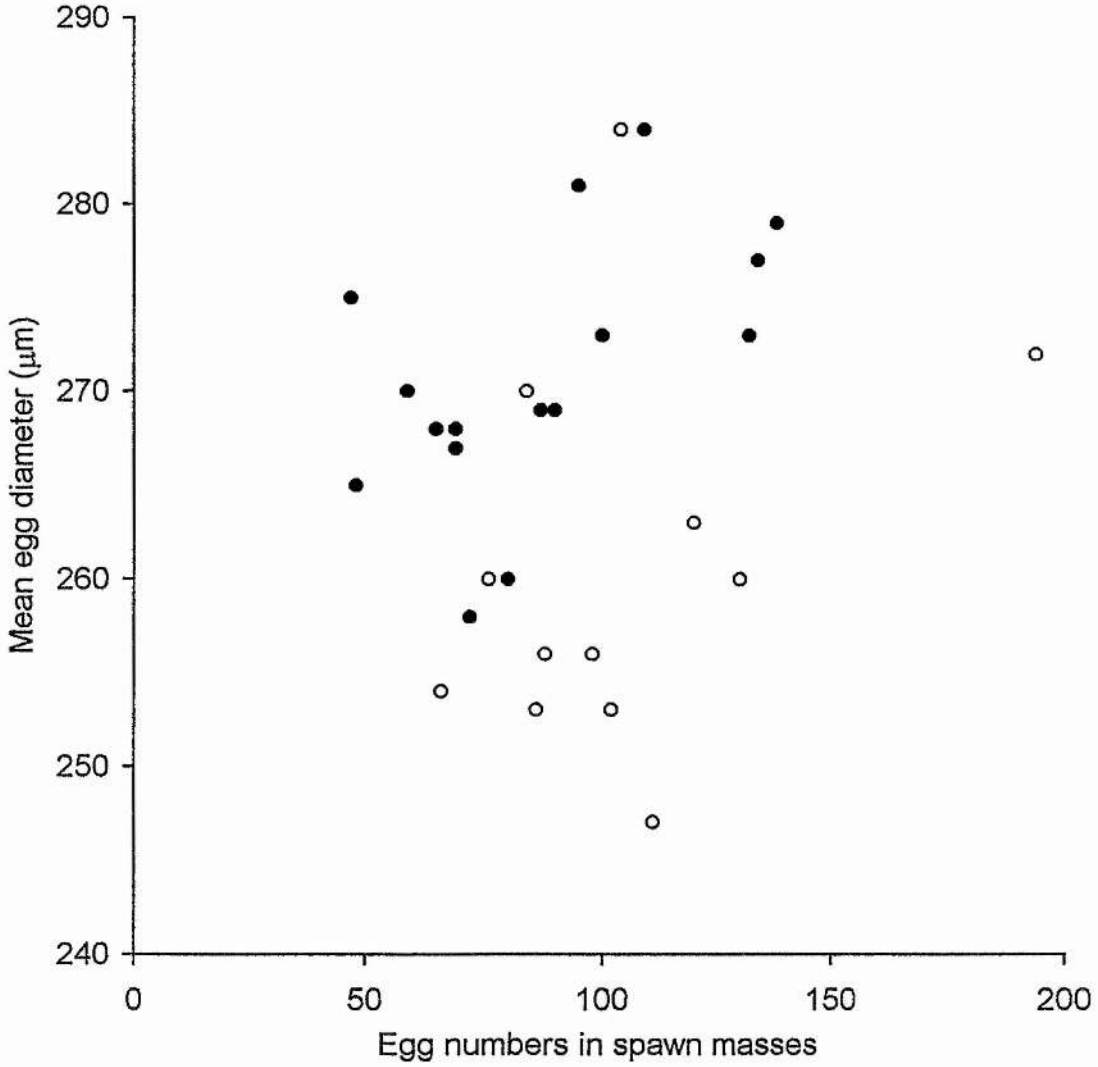

160
140
120
100
80
60
40
20
0
Mean eggs / spawn mass
Clachan Seil
Kingsbarns
St Andrews Bay
Location

Figure 3. 16. ***Lacuna pallidula*** - Mean diameter (μm) of eggs produced by females from both Clachan Seil (n=12) and Kingsbarns (n=17) populations. Nested analysis of variance showed significant variation between populations and between spawn masses produced by females within a population (see table below).



Source	DF	SS	MS	F	P
Population	1	4794	4794	33.36	<0.001
Spawn masses (population)	18	17827	990	6.89	<0.001
Error	180	25869	144		
Total	199	48491			

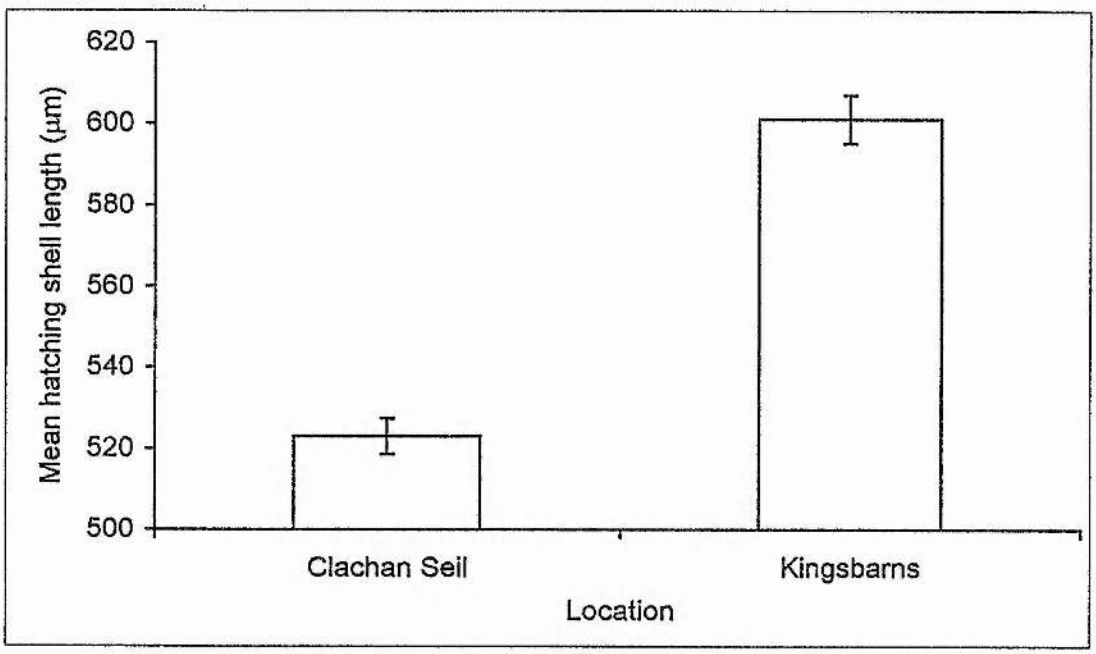
Figure 3. 17. ***Lacuna pallidula*** - Mean (n=10) egg diameters (μm) of eggs as a function of numbers of eggs in spawn masses produced by females from both Clachan Seil (open circles) and Kingsbarns (closed circles) populations. Analysis of covariance showed a significant common positive relationship ($F_{1,27} = 5.83$, $P < 0.05$), but significant variation in elevation of slope ($F_{1,27} = 16.87$, $P < 0.05$).


290
280
270
260
250
240
Mean egg diameter (μm)
0
50
100
150
200
Egg numbers in spawn masses

to note here, however, that while the female source was known for spawn masses from experiment one, the origins of spawn masses in this experiment were not known and that the spawn masses were obtained from many more females.

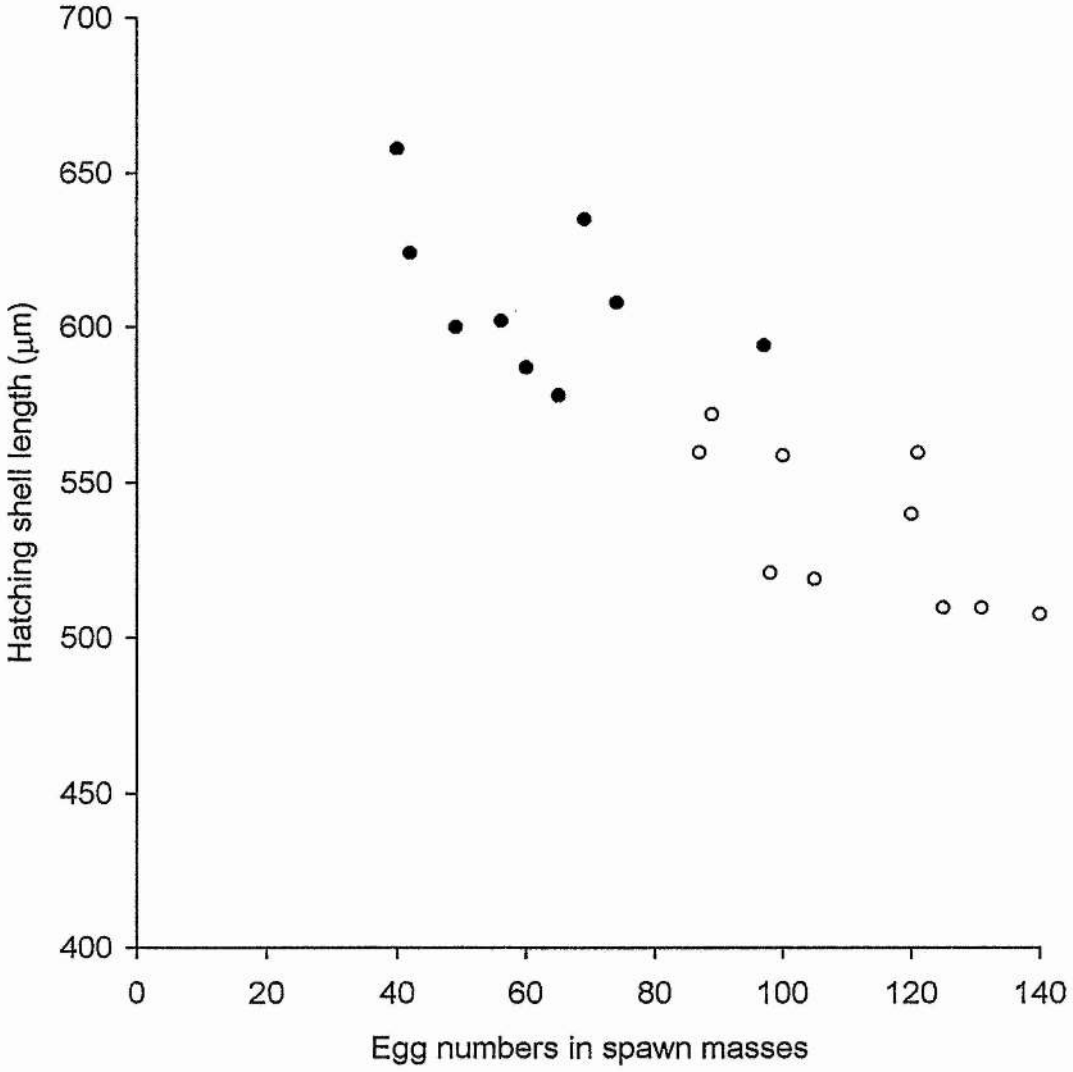
Spawn masses collected from Clachan Seil and Kingsbarns were incubated and the shell lengths of hatching offspring were compared for the two populations (Figure 3.18). Nested analysis of variance showed that the juveniles from the Kingsbarns population were significantly larger than those from the Clachan Seil population, but, in addition, that there was significant variation among spawn masses within each population (Figure 3.18). To further investigate this variation among spawn masses, data were plotted against numbers of eggs in spawn masses for both populations (Figure 3.19). Analysis of covariance showed a significant common negative slope but no significant difference in elevation of slope (see Figure 3.19). Hence females from Clachan Seil were producing more but smaller eggs and juveniles than females from the Kingsbarns population.

Figure 3. 18. ***Lacuna pallidula*** - Mean (n=10) shell lengths (μm) of juveniles hatching from spawn masses produced by females from Clachan Seil (n=10) and Kingsbarns (n=10) populations. Nested analysis of variance showed significant variation between populations and between spawn masses produced by females within a population (see table below).



Source	DF	SS	MS	F	P
Population	1	298335	298335	206.82	<0.001
Spawn masses (population)	18	276977	15388	10.67	<0.001
Error	180	259642	1442		
Total	199	834955			

Figure 3. 19. ***Lacuna pallidula*** - Mean (n=10) shell lengths (μm) of juveniles hatching from spawn masses produced by females from both Clachan Seil (open circle) and Kingsbarns (closed circle) populations as a function of egg numbers in spawn masses. Analysis of covariance showed a significant common negative slope ($F_{1,17} = 8.04$, $P < 0.05$) and no significant difference in elevation of slope ($F_{1,17} = 3.49$, $P>0.05$).


700
650
600
550
500
450
400
Hatching shell length (µm)
0
20
40
60
80
100
120
140
Egg numbers in spawn masses

Lacuna vincta

As for *Lacuna pallidula*, egg numbers in *Lacuna vincta* spawn masses collected from field sites were compared to those obtained from captive females (Figure 3.20). One-way analysis of variance did not show any significant differences between egg numbers in spawn masses and hence it was assumed that spawn masses obtained from females during short durations of captivity were no different to those collected from the field. Unlike *L. pallidula*, one-way analyses of variance did not show any significant variation in egg allocation for the two populations (see Figure 3.20).

However, nested analysis of variance showed significant variations in egg diameters both among populations and among spawn masses within a population (see Figure 3.21). Females from Clachan Seil produced significantly larger eggs. Nested analysis of variance revealed that larvae hatching from spawn masses produced by females from Clachan Seil also were significantly larger (see Figure 3.22.).

Figure 3. 20. ***Lacuna vincta*** - Mean (+/- SE) number of eggs (μm) in spawn masses produced by females from Clachan Seil (n=13) and Kingsbarns (n=13) populations in both the field (white) and in the laboratory (grey). One-way analysis of variance did not show significant variation between populations in either the field or in the laboratory (field, $F_{1,25} = 0.19$, $P > 0.05$, laboratory $F_{1,25} = 0.01$, $P > 0.05$).

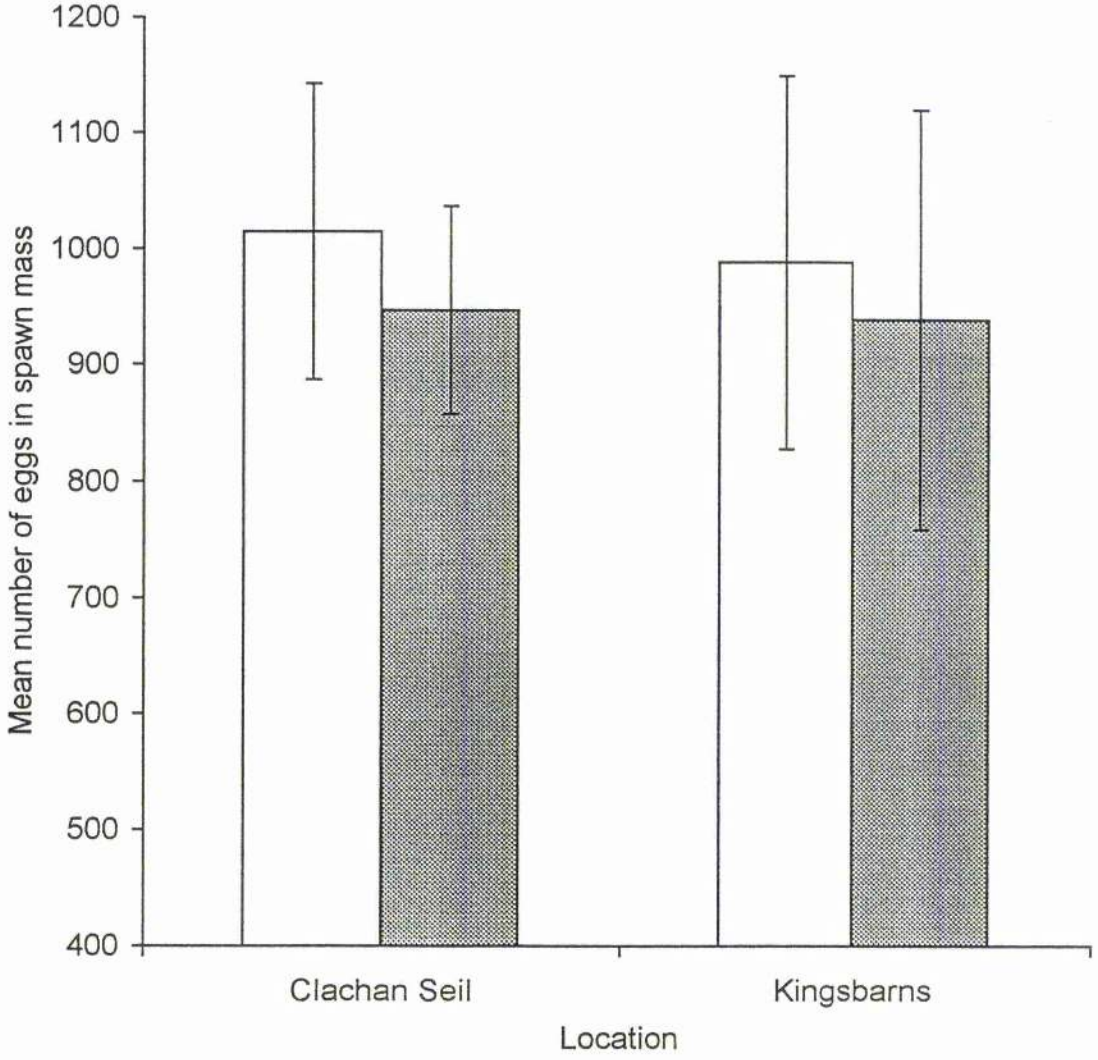
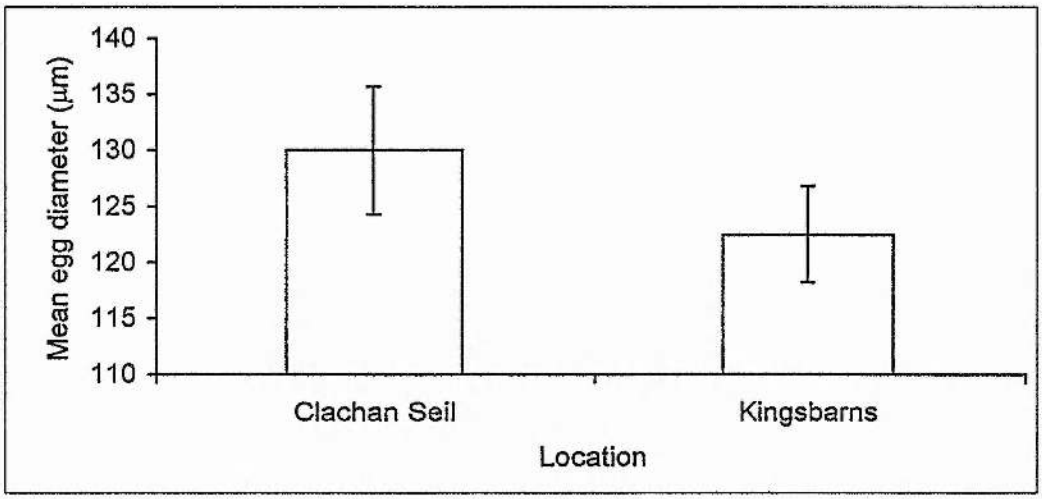
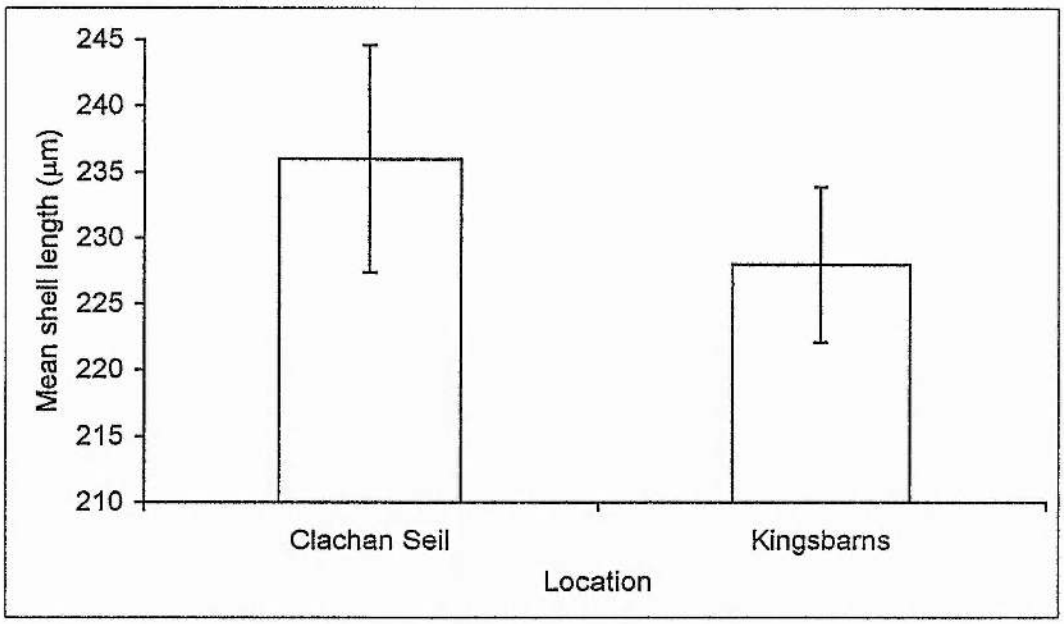

1200
1100
1000
900
800
700
600
500
400
Mean number of eggs in spawn mass
Clachan Seil
Kingsbarns
Location

Figure 3. 21. ***Lacuna vincta*** - Mean egg diameters (μm) of eggs in spawn masses produced by females from Clachan Seil (n=7) and Kingsbarns (n=7) populations in the laboratory. Results for nested analysis of variance (with population as a factor, and with spawn masses as a nested factor) are shown in the table below.



Source	DF	Seq SS	Adj SS	MS	F	P
Population	1	1646	2083	2083	14.49	<0.001
Spawn masses (population)	12	25025	25025	2085	14.5	<0.001
Error	214	30767	30767	143		
Total	227	57438				

Figure 3. 22. ***Lacuna vincta*** - Mean shell lengths (μm) (n=20) of larvae hatching from spawn masses collected from Clachan Seil (n=7) and Kingbarns (n=7). Results for nested analysis of variance (population is a factor and spawn masses is a nested factor) are shown in the table below.



Source	DF	SS	Adj SS	MS	F	P
Population	1	2110	2696	2696	19.4	<0.001
Spawn masses (population)	12	31112	31112	2592	18.8	<0.001
Error	214	29535	29535	128		
Total	227	62758				

3.3.2.3. Experiment three - Effects of maternal diet upon offspring size for three *Lacuna pallidula* populations

The mean shell lengths of *Lacuna pallidula* females collected from Clachan Seil, Kingsbarns and St Andrews Bay in September 1994 are shown in Figure 3.23. Large variations were observed within populations as indicated by the large standard errors. However, females from Clachan Seil were notably larger.

Figure 3.24 shows the egg numbers in spawn masses produced by *Lacuna pallidula* from the three populations maintained either on a diet of *Fucus serratus* or *Laminaria digitata*. The data were statistically analysed by a two-way analysis of variance ('population' and 'diet' were factors) with females as a nested factor within each treatment (see Figure 3.24). Significant variation was only shown with respect to population; as before, females from the Clachan Seil population produced significantly greater numbers of eggs in spawn masses. In addition, however, the analysis also revealed a significant interaction effect between population and diet (see Figure 3.24.). Whereas females from Kingsbarns and St Andrews Bay produced relatively smaller spawn masses when on a diet of *L. digitata*, as opposed to *F. serratus*, females from Clachan Seil produced relatively larger spawn masses when on a diet of *L. digitata*. As before, significant variation was also shown among females.

Data for egg diameter measurements are presented in Figure 3.25. Two-way and nested analysis of variance (with population and diet set as factors and spawn masses from different females nested within treatments) did not show any significant variation with respect to either population or diet (see Figure 3.25). However, there was a significant interaction effect. The mean diameter of eggs in spawn masses produced by females from Clachan Seil in the *Fucus serratus* diet treatment was larger than those which were fed *Laminaria digitata*. Likewise, females from Kingsbarns and St Andrews Bay populations in the *F. serratus* diet treatment produced smaller eggs than females in the *L. digitata* diet treatment. Hence an inverse relationship was observed between egg numbers and egg

Figure 3. 23. ***Lacuna pallidula*** - Mean (n = 12) (+/- SE) shell lengths (μm)of females from Clachan Seil, Kingsbarns and St Andrews Bay populations on the 1st December 1994.

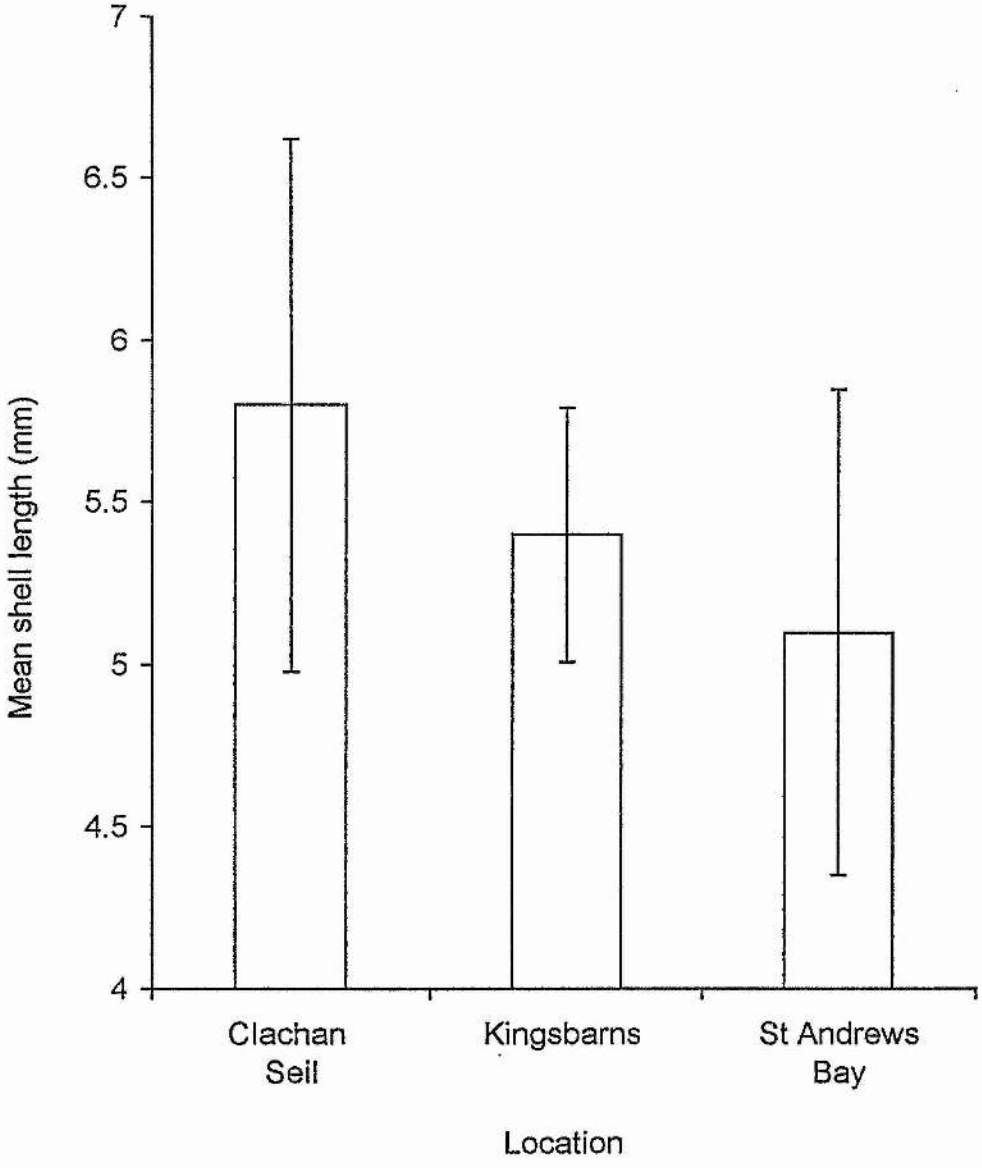
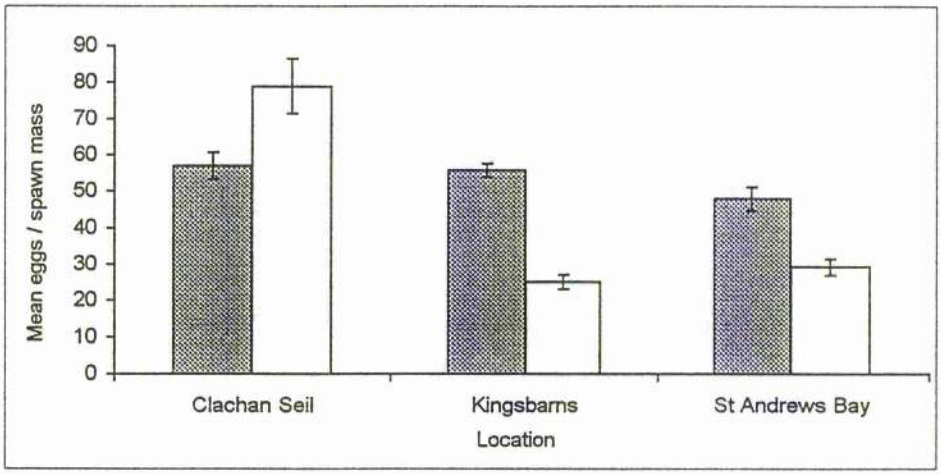
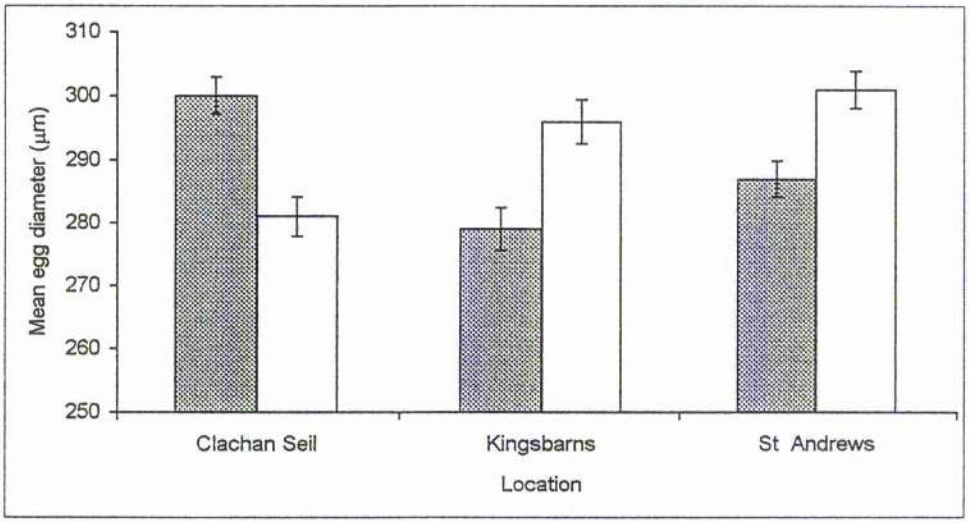

7
6.5
6
5.5
5
4.5
4
Mean shell length (mm)
Clachan Seil
Kingsbarns
St Andrews Bay
Location

Figure 3. 24. ***Lacuna pallidula*** - Mean number of eggs in spawn masses produced by females, which were maintained on either *Fucus serratus* (grey) or *Laminaria digitata* (white), from Clachan Seil (n=41), Kingsbarns (n=72) and St Andrews Bay (n=35) populations. Results of two-way analysis of variance (population and diet are factors) with females as a nested factor are shown in the table below.



Source	DF	SS	Adj SS	Adj MS	F	P
Population	2	16679	18048	9024	65.56	<0.001
Diet	1	6478	2580	2580	18.74	<0.001
Population x diet	2	16296	15539	7770	56.44	<0.001
Females	30	20434	20434	681	4.95	<0.001
Error	116	15968	15968	137		
Total	151	75857				

Figure 3. 25. ***Lacuna pallidula*** - Mean (n=10) diameter of eggs (μm) in spawn masses produced by females from Clachan Seil, Kingsbarns and St Andrews Bay populations on a diet of *Fucus serratus* (grey) or *Laminaria digitata* (white). Results of two-way analysis of variance (with population and diet as factors) with spawn masses from different females as a nested factor are shown in the table below.

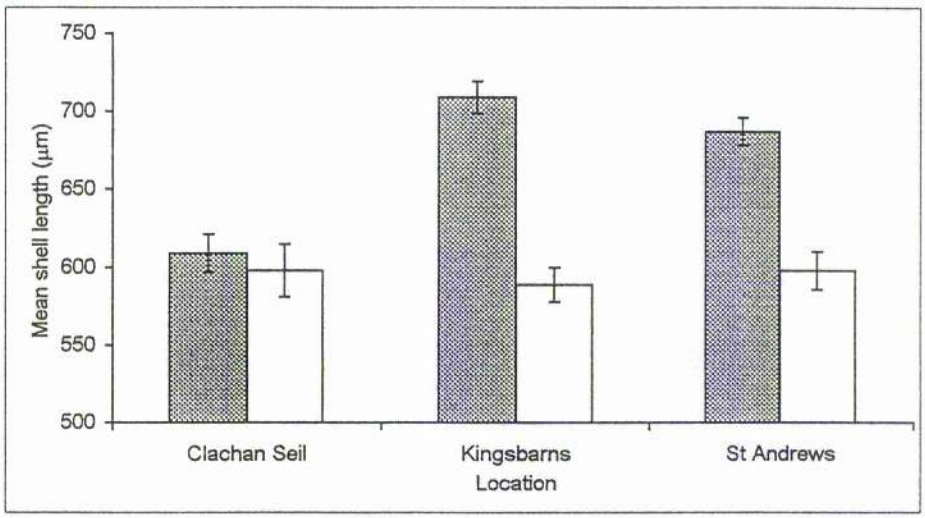


Source	DF	SS	Adj SS	Adj MS	F	P
Population	2	1606	1606	803	2.91	0.057
Diet	1	70	70	70	0.25	0.615
Population x diet	2	7127	7127	3563	12.93	<0.001
Spawn masses	12	24831	24831	2067	7.5	<0.001
Error	162	44638	44638	276		
Total	179	78254				

size within each population, but the diets which mediated these relationships differed for populations.

Mean shell length measurements of hatching juveniles were not significantly different among the three populations (see Figure 3.26). However, significant variation was observed both among diet treatments and among females. Perhaps most importantly, a significant interaction effect between maternal diet and population was shown. While the shell lengths of juveniles from Clachan Seil were comparable in the two diet treatments, juveniles from Kingsbarns and St Andrews Bay were significantly larger in spawn masses produced by females in the *Fucus serratus* treatment.

Figure 3. 26. ***Lacuna pallidula*** - Mean (n=10) shell lengths (μm) of juveniles hatching from spawn masses produced by females from Clachan Seil, Kingsbarns and St Andrews Bay populations on a diet of either *Fucus serratus* (grey) or *Laminaria digitata* (white). Results for two-way analysis of variance (with population and diet as factors) with spawn masses from different females as a nested factor are shown in the table below.



Source	DF	SS	Adj SS	Adj MS	F	P
Population	2	256	256	256	1.05	0.652
Diet	1	7458	7458	7458	22.1	<0.001
Population x diet	2	9654	9654	2798	62.5	<0.001
Spawn masses	12	45423	45423	850	8.2	<0.001
Error	162	18569	18569	115		
Total	179	81360				

3.4. DISCUSSION

3.4.1. The Egg-Juvenile-Period (EJP)

One of the aims of this work was to compare the variability and time taken for *Lacuna pallidula* and *Lacuna vincta* offspring to develop from the egg to the juvenile stage (i.e. the Egg-Juvenile-Period, EJP, see Todd, 1991; Havenhand, 1993). The duration of the EJP is considered to be under natural selection since it has been shown in both theoretical and empirical studies to have important implications for the demographics of post-settlement stages in the life-cycle (Todd and Doyle, 1981; Emlet, 1986; Levin *et al.*, 1987; Todd, 1991; Miller, 1993) and for the duration of the entire life-cycle (e.g. Ayal and Safriel, 1982; Werner, 1986; Roughgarden, 1989; Miller and Hadfield, 1990; Havenhand 1993). Interest in comparing the EJP in species which display different larval strategies arises from observations, notably in opisthobranchs, that larval type and EJP are correlated; pelagic lecithotrophy and non-pelagic lecithotrophy generally yield a shorter EJP than does planktotrophic development (Todd and Doyle, 1981; Calow, 1983; Hadfield and Switzer-Dunlap, 1984; Thompson and Jarman, 1985; Emlet *et. al.*, 1987; Strathmann, 1987; Rumrill, 1990; Havenhand, 1993). Todd and Doyle (1981) were the first to emphasise the potential importance of the EJP in the selection for larval type in their Settlement-Timing Hypothesis in which they proposed that the gap between the time of egg production and availability of juvenile food may select for that particular larval type having an appropriate EJP (also see Todd, 1991). Havenhand (1993) presented a conceptual model to show how a reduction in the EJP could result in increased fitness (i.e. intrinsic rate of population growth (r)) by either increasing the size at reproduction in species with fixed duration cycles, or by decreasing the overall generation times in species with a fixed age or size at reproduction. Havenhand (1993) suggested that the strong correlation between shorter EJP's and lecithotrophic development potentially reflected a selective pressure for the evolution of lecithotrophic development. In turn, Havenhand's model suggests that the EJP can have various consequences for the duration of the life-cycle. The EJP of *Lacuna pallidula* and *Lacuna vincta* were assessed and considered in relation to their life-cycles.

In the present study it was found that the EJPs of *Lacuna vincta* offspring were shorter than those for *Lacuna pallidula* offspring at 6, 10 and 15°C (see Table 3.3.). However, *Lacuna vincta* larvae were able to delay their settlement for substantial periods of time in the absence of an appropriate settlement inducing cue (at least two months, see Figure 3.1). The delay capabilities of *Lacuna vincta* larvae have also been noted in the field (e.g. Fretter and Shale, 1973; Waddell, 1973; Martel and Chia, 1991a). Hence in the realised situation it might be expected that the EJP of *Lacuna vincta* offspring could exceed that of *Lacuna pallidula* offspring because *Lacuna vincta* are delaying their settlement until a suitable substratum for settlement is located. Smith (1973) reported that the EJP of *L. pallidula* was 60-91 days, depending on how late spawn masses were laid in the season. Developmental times from the egg stage to hatching for *Lacuna vincta* in the field have been estimated to be 15 - 25 days (Lebour, 1937; Martel and Chia, 1991a). The larval period of *L. vincta* in the field has been variously estimated to be between 30 and 90 days (Smith, 1973; Waddell, 1973; Thomas and Page, 1983; Langan, 1984; Martel and Chia, 1991a). Data from this study fall well within the range of estimations derived from field studies (See table 3.4).

Variations in growth and development of *Lacuna vincta* larvae were mediated by microalgal diet. While growth and survival of larvae in mixed and Tahitian *Isochrysis* diet treatments were high, growth and survival of larvae in the *Pavlova lutheri* microalgal diet treatment was poor. While different microalgal species are known for their varying antibacterial effects (Skjermo *et al.*, 1993) and for reducing levels of ammonia in cultures (Alderson and Howell, 1973), it is not thought that this was the major cause of the observed variation in growth and survival of larvae in this study. Instead it is suggested that variation in growth and survival of *Lacuna vincta* larvae in the various microalgal diet treatments was caused by the varying lipid composition in the different microalgal species. The proper development of motile, nervous and photoreceptive tissues in developing feeding larvae is dependent upon the acquisition of long chain polyunsaturated fatty acids (PUFA's), in particular Docosahexaenoic acid (DHA, 22:6 n-3), in the diet (Neuringer *et al.*, 1988; Sargent, 1995). While Tahitian-*Isochrysis,* which yielded good growth and survival in larvae, has a high proportion of its fatty acids as DHA (16.7%), *Pavlova lutheri*, which yielded poor survival and

growth of larvae has a much lower proportion of its fatty acids as DHA (8.7%) (Thompson *et al.*, 1990). Another possible cause for this variation is the varying amino acid composition in different microalgal species which are important for osmotic regulation in marine invertebrates (see Fhyn and Serigstad, 1987, for review).

In this study, *Lacuna vincta* larvae were competent to settle once they had grown to a shell length of 450 μm (see also Fretter and Shale, 1973, Fretter and Manly, 1977; Southgate, 1982; Martel and Chia, 1991a). However, *Lacuna vincta* larvae continued to grow, albeit at a reduced rate (but see Calow, 1979) while delaying settlement. There is evidence that *Lacuna vincta* larvae continue to grow during the delay phase in the field. For example, Martel and Chia (1991a) followed a cohort of *Lacuna vincta* larvae in the plankton which attained competence to settle at a shell length of 500 μm but continued to grow and attained shell lengths of 775-875μm before settling (also see Fretter and Shale, 1973). Several species with feeding larvae also continue to grow while delaying settlement (e.g. see Domanski, 1984; Pechenik and Lima, 1984; Emlet, 1986) although other larvae do not grow during this phase (e.g. Highsmith and Emlet, 1986; Kempf and Todd, 1989). Species with non-feeding larvae generally display degrowth while delaying settlement and hence unlike species which continue to grow during the delay phase, juvenile size can decreases with increasing time in the plankton (Emlet, 1986; Highsmith and Emlet, 1986).

Werner (1986) suggested that the optimum EJP, or the optimal time to settle is determined by a trade-off between the ratios of mortality to growth rates in the pre-settlement and post-settlement habitats. He predicted that if the mortality / growth ratio is higher in the pre-metamorphic habitat then selection will favour early metamorphosis. This has been shown in species whose larvae do not continue to grow during the delay phase (e.g. Emlet, 1986; Miller, 1993). However, Hadfield and Miller (1990) suggested that the detrimental effects of larvae delaying metamorphosis in *Phestilla sibogae* was due to a temporary hiatus of development and not due to reduced growth (also see Miller, 1993). It is not known to what extent the adverse effects of delaying metamorphosis are

compromised when larvae continue to grow during the delay phase, as for *Lacuna vincta*. It was interesting to note that the growth trajectories of *Lacuna vincta* larvae greatly differed in the various temperature treatments. *Lacuna vincta* larvae cultured at 15 °C continued to grow rapidly for a short time after delaying settlement (see Figures 3.1 and 3.2). The cause of this may be due to a higher rate of metabolism, resulting in additional energy available for growth (Pechenik, 1980) or it may be due to the better quality of algae at higher temperatures. In view of Werner's model, this possibly could have important implications for the fitness of offspring which are delaying settlement in later stages of the life-cycle. However Roughgarden (1989) suggests that high mortalities of offspring remaining in the plankton are likely to offset any advantages which might occur from continued larval growth during the delay phase.

In conclusion, *Lacuna vincta* offspring display more varied EJPs and juvenile size than *Lacuna pallidula* offspring (see Tables 3.3. and 3.4.). This is caused by the various influences of temperature and diet during the larval phase of *L. vincta* and the delay capabilities and continued growth of this species' larvae during the competent phase. It is therefore suggested that *L. vincta* will display greater variations in its life-cycle than will *L. pallidula*. Field studies on intertidal populations of *L. pallidula* and *L. vincta* have indicated that the spawning and recruitment periods of these two species are protracted but seasonal, the spawning period occurring from January to April, and the recruitment period occurring from May to September. These observations suggest that the EJP of both species is similar and fairly consistent from year to year. However, more recent studies on subtidal populations of *L. vincta* have shown that the life-cycle of individuals in this species is more variable (Maney and Ebersole, 1991). While spawning peaks during the winter and spring months, spawn mass production is continuous all year round. Recruitment of juveniles occurs episodically throughout the year. These findings are supported by Fretter and Shale (1973) and Fretter and Manly (1977) who reported that *L. vincta* larvae could be found in the plankton all year round. Further study comparing the life cycles of intertidal and subtidal populations of *L. vincta* would help in clarifying contrasting reports on the life-cycle of *L. vincta*.

3.4.2. Variations in offspring size

The manner in which energy is packaged in the production of offspring is an important concept in marine invertebrate life history strategy theory (Vance, 1973a, b). More recently, closer attention has been given to variations in the manner in which offspring are packaged within single species and the consequences that this may have for offspring (e.g. Clarke *et al.*, 1991; Clarke and Gore, 1992; George, 1994). The adaptive significance of such variations and the extent to which these are mediated by environmental conditions have been considered in several models (e.g. Parker and Begon, 1986; Rossiter, 1991a, b).

One of the aims of this work was to examine the intraspecific variability in egg size, egg numbers in spawn masses and juvenile or hatching size in *Lacuna pallidula* and *Lacuna vincta* and to determine any relationships between these three life-history traits. In addition, the effects of maternal diet upon these life-history traits were compared for these two species. Intraspecific variations in these traits were observed for individuals on various diet treatments (Experiment one) and for populations (Experiment two). The interaction effects of maternal diet and population source upon these traits were examined only in *L. pallidula* (Experiment three).

Mean weights of eggs (plus attendant gel) were plotted against egg numbers for Kingsbarns spawn masses using the equations derived in Chapter II to determine whether any relationship between investment per egg and the number of eggs in a spawn mass may be expected in either species. Very different relationships were found. While the mean egg investment decreased exponentially with egg numbers in *Lacuna pallidula* spawn masses, mean investment per egg remained constant in *Lacuna vincta*. The mean investment per *L. pallidula* egg (organic weight) was 40μg. Grahame (1977) estimated the mean dry weight of eggs (plus attendant gel) produced by *L. pallidula* females from a population in the north-east of England to be approximately 30 μg ; however females from that population produced smaller eggs (mean diameter =266 μm) than did females from the Kingsbarns population in the present study. For spawn masses containing sixty eggs or more, the mean

investment per *L. pallidula* egg remained relatively constant. It is therefore interesting to note that the mean numbers of eggs in spawn masses produced by *Lacuna pallidula* females from both the Kingsbarns and St Andrews Bay population also was approximately sixty (see Figure 3.24.). It has been suggested that selection for numbers of eggs in spawn masses may be mediated by the advantages of eggs being 'cheaper' to produce when greater numbers of eggs are deposited in a spawn mass (Christiansen and Fenchel, 1979). This is thought to especially be the case when the eggs are encapsulated in structurally complex protective spawn masses, as is the situation for many prosobranch species, which like *L. pallidula* (see Goodwin, 1979), produce non-pelagic lecithotrophic offspring. The assumption here is that the protective covering per egg decreases with increasing egg numbers because the ratio of the volume of a spawn mass to the area of a spawn mass will increase with increasing egg numbers in spawn masses (Christiansen and Fenchel, 1979). Weighing the spawn mass matrix and the protective covers of spawn masses which contained different numbers of eggs would determine whether this applies to the spawn masses of *L. pallidula*.

An alternative argument to explain this relationship in *Lacuna pallidula* spawn masses produced by females from a single population is that the investment per egg decreases with increasing numbers in a spawn mass. In Experiment one the diameters of eggs in spawn masses produced by females in all diet treatments were observed to significantly decrease with increasing numbers of eggs. In addition, the diameters of eggs also decreased in spawn masses produced by individual females in the *Fucus serratus* treatment.

However, upon closer inspection of all females, no consistent pattern was observed. Further, the diameters of eggs in spawn masses produced by females collected from the field in Experiment two increased with increasing egg numbers. Parker and Begon (1986) have proposed that such variability within a population is inevitable because females are of different sizes (and hence will have differently sized gonads) and will have varying amounts of energy available to them during the spawning period. Hence, while some females may produce fewer smaller eggs, others may produce many more larger eggs. Diet was not found to directly affect this relationship in *Lacuna pallidula*

spawn masses, but it is perhaps important to note that very different types of relationships were observed in females within each diet treatment.

In contrast, for *Lacuna vincta* spawn masses, the energy costs per egg were predicted to remain constant despite changes in egg numbers in spawn masses. Unfortunately, data were unavailable to explicitly examine this relationship. However, given that the number of eggs produced is dependent upon the amount of energy available in both species, it is suggested here that variations in the amounts of energy available for reproduction may have very different implications for the production and packaging of offspring in these two species.

In the present study, significant variations in egg diameters, egg numbers in spawn masses and offspring hatching shell length were found among three populations of *Lacuna pallidula*. Females from the Clachan Seil population on the west coast of Scotland were larger and they produced significantly more and smaller eggs than did females from the Kingsbarns and St Andrews Bay populations on the east coast of Scotland. Consequently, offspring hatching from spawn masses produced by females from the Clachan Seil population were significantly smaller.

An inverse relationship between egg diameters and the number of eggs in spawn masses therefore was observed among populations of *Lacuna pallidula*. Other studies also have found an inverse relationship between egg size and the numbers of eggs produced among populations (e.g. Mashiko, 1982; Qian and Chia, 1991). If it is assumed that these variations reflect selection for egg size and egg numbers then this study supports models which incorporate an optimal egg size for the survivorship of offspring in various physical environments (e.g. Smith and Fretwell, 1974; Roff, 1992). These models propose that females in favourable environments for the survival of offspring should produce smaller eggs since less investment per egg is required for offspring to survive. Consequently, energy is available for the production of more eggs. Likewise females in environments which are less favourable for the survival of offspring should produce larger and hence fewer eggs to optimise representation in the next generation (Sibly *et al.*, 1987).

However for *Lacuna vincta*, females from Clachan Seil produced similar numbers of eggs but significantly larger eggs and hence larger larvae at hatching than did females from Kingsbarns. Life-history models which incorporate the nutritional state of the females and the size of females predict this relationship (e.g. Parker and Begon, 1986; McGinley, 1989; Venable; 1992; but see Clarke, 1987). These models predict that females from populations which have large amounts of energy available for reproduction should produce large amounts of large eggs since offspring hatching from larger eggs are better able to compete for resources (Bevren and Chadra, 1988; Sinervo, 1990) and have faster growth rates (Sargent *et. al*, 1987). The importance of larval hatching size in species which produce planktotrophic larvae may not be great since larvae will experience various conditions during the pelagic phase (Sinervo, 1990). However, larvae hatching from large eggs produced by the seastar, *Arbacia lixula* (planktotrophic) displayed higher survival rates and faster growth and development than smaller larvae hatching from smaller eggs (George *et al.*, 1990). Although no quantitative data are presented, larger *Lacuna vincta* larvae hatching from spawn masses generally did display better survival and greater growth rates than smaller larvae hatching from spawn masses (see Figure 3.3.).

George (1994) has suggested that the production of large numbers of large eggs will be selected for in populations where competition between individuals is high. If this is indeed the case then it may be predicted that competition between *Lacuna pallidula* offspring in the Clachan Seil population is not so intense as to place a selection pressure upon the production of larger eggs and hence larger offspring. Experiments which compare the density of juveniles per unit surface area or weight of algae for the two sites may address this hypothesis. Likewise, for *Lacuna vincta*, experiments which investigated the effect of larval hatching size upon subsequent growth and survival for larvae at the two sites would address the possible advantages of producing larger eggs.

Variations in the diameters of eggs and in the number of eggs produced were observed among *Lacuna* populations. A clear difference was observed between the two *Lacuna pallidula* populations on the east coast of Scotland (Kingsbarns and St Andrews) with the *L. pallidula* population on the

west coast (Clachan Seil). Further, females from the Clachan Seil population were affected differently by maternal diet. It is suggested here that this is because of variations in food availability at the three sites. While the site at Clachan Seil is sheltered and has a greater abundance of kelp, the two sites on the east coast are more exposed and hence have a more sparse algal cover which predominantly consists of fucoid species. While *L. pallidula* females from the Clachan Seil populations produced better quality offspring when fed *Laminaria digitata*, females from the other two populations produced better quality offspring when fed *Fucus serratus*. Hence, it is suggested that these populations have adapted to local conditions and that the production of offspring is dependent both upon the selection processes operating at different sites and as an adaptation to utilising the local food supply. It is only until quite recently that maternal diet has been shown to have important implications for the phenotypic expression of egg size and numbers. Poor food supply has been shown to adversely affect egg size, fecundity and larval viability for sea urchins and seastars (George, 1990; George *et. al*, 1990, 1991).

In life-history theory it has been assumed that egg size and the energy content of eggs are correlated. This relationship has not been shown for some species, especially for those that produce small eggs (Strathmann and Vedder, 1977; Krauter *et al.*, 1982; McEdward, 1986; McEdward and Chia, 1991). McEdward and Coulter (1987) suggested that the failure of egg size to predict offspring quality may result from the swelling of eggs. While egg size variations among populations of both species was here a good predictor for variations in hatching size, egg size variations among females from the same population, notably, on different diet treatments, did not account for variations in hatching size. Several studies have addressed the effects of the quantity of food available to females and the quality of food available for females for offspring quality (Tenore, 1977; Thompson, 1982; George *et al.*, 1991; Qian and Chia, 1991; Rossiter, 1991a, b). These studies have shown that while maternal diet has little effect upon egg size, offspring survival, egg fertilisability and size at hatching are greatly affected. Both lipid composition and protein content have been shown to be important in the development of offspring (Bayne, 1972; Helm *et al.*, 1973; Clarke *et al.*, 1985; Marsh *et al.*, 1990; George *et al.*, 1990; George, 1994). It is interesting to note that offspring quality varied differently

in the various diet treatments in *Lacuna pallidula* and *Lacuna vincta* and among populations of *Lacuna pallidula*. It therefore is suggested that the quality of offspring is not correlated with the nutritional content of various macroalgal foods available to females but is correlated with the ability of females to ingest and process energy acquired from various macroalgae species.

3.5. SUMMARY

- Variations in the EJP were compared for *Lacuna pallidula* and *Lacuna vincta* offspring over a range of ecologically relevant temperatures.

- The EJP of *L. vincta* was more variable. This was attributed to the delay capabilities of *L. vincta* veligers during the competent phase and to the influences of microalgal diet during the pelagic feeding phase.

- Both microalgal diet and temperature affected the pattern of growth of *Lacuna vincta* veligers during the pelagic feeding period.

- Juvenile size was also more variable for *Lacuna vincta*. This was attributed to the positive growth displayed by larvae during the delay phase.

- Variations in offspring size and egg numbers in spawn masss were examined in *Lacuna pallidula* and *Lacuna vincta*. Variations were observed to be mediated by both diet and by population source.

- The relationship between egg numbers in spawn masses and egg diameter differed markedly in the two species. While an increase in fecundity generally resulted in smaller eggs being produced by *Lacuna pallidula*, this was not observed for *Lacuna vincta*. However, variations in this relationship were observed for females from the same population and among *L. paliidula* populations.

- Maternal diet directly affected the size of off-spring hatching from similar sized eggs. Hence, in the present study, egg diameter was not a good indicator of hatching size for either speices.

CHAPTER 4

Induction of settlement and metamorphosis in *Lacuna vincta* larvae.

4.1. INTRODUCTION

4.1.1. General Introduction

Temporal and spatial larval settlement patterns greatly influence the recruitment patterns of marine invertebrate species with pelagic larvae and play an integral part in structuring marine benthic communities (e.g. Keough and Downes 1982; Underwood and Fairweather, 1989; Grosberg and Levitan, 1992). Consequently, the processes involved in the mediation of larval settlement have received much attention. Various studies have found that settlement patterns are the culmination of a complex series of events experienced by larvae and are dependent upon physical, biological and chemical processes operating at various scales of magnitude (e.g. Crisp, 1974; Eckman, 1987; Tamburri *et al.*, 1992; Rodriguez *et al.*, 1993; Tamburri and Zimmer-Faust, 1996).

The area in which larvae settle is potentially crucial to their chances of surviving, especially for species with sessile adult forms or with specialised or restricted adult prey diets. It is therefore not surprising to find that larval settlement is not an entirely random event, as was once thought (Peterson, 1913; Colman, 1933; Yonge, 1937), and that many larvae can display active choices. The ability of larvae to delay settlement when in unfavourable areas or to actively settle when in areas favourable for survival is deemed to be under natural selection (e.g. Strathmann and Branscomb, 1979; Woodin, 1986, 1991). Close attention has focused on the nature of the cues which species can evolve to respond to.

On many occasions it has been demonstrated that chemical cues released into the environment by biologically active sources can both deter and promote larval settlement and metamorphic responses

(see Pawlik, 1992 for review). Further, while some cues have been found to induce the entire settlement process (Morse, 1990) others induce only stages (i.e. settlement or metamorphosis) of the process (e.g. Coon *et al.*, 1988; Bonar *et al.*, 1990; Fitt *et al.*, 1990; Tamburri *et al.*, 1992; Zimmer-Faust and Tamburri, 1994). Interest in studying these chemical cues has gained momentum over recent years with the acknowledged importance of recruitment in structuring benthic marine communities (e.g. Grosberg and Levitan, 1992) with continued interest in aquaculture (e.g. Kingzette *et al.*, 1990; Ambrose *et al.*, 1992; Parsons *et al.*, 1993: Pascual and Zampatti, 1995) and with efforts to develop less toxic antifoulant products (e.g. Price *et al.*, 1995).

4.1.2. Naturally occurring sources of chemical inducing cues

Naturally occurring sources of chemical cues which induce larval settlement and metamorphic responses divide broadly into conspecifics, associated species (such as prey or host species) and microbial films. Examples of settlement and metamorphic responses of larvae to cues associated specifically with conspecifics and with prey or host species can be found across a wide range of invertebrate phyla and are listed in reviews by Hadfield (1984), Pawlik (1992) and Rodriguez *et al.* (1993). Marine microbial films and associated bacteria also have been shown to greatly affect larval settlement and metamorphic responses across a wide range of invertebrate phyla (e.g. Zobell and Allen, 1935; Scheltema, 1974; Ryland, 1974; Cameron and Hinegardner, 1974; Mihm *et al.*, 1981; Brancato and Woollacott, 1982; Weiner *et al.*, 1985; Chen and Run, 1989; Roberts *et al.*, 1991; Maki *et al.*, 1992; Pawlik, 1992; Holmstrom *et al.*, 1992; Parsons *et al.*, 1993; Todd and Keough, 1994; Keough and Raimondi, 1995) and there is evidence that the inducing capacity of microbial films can vary with age (e.g. Maki, 1988, 1989; Pierce and Scheibling, 1991; Becker, 1993; Keough and Raimondi, 1995). However, the capacity for biofilms to induce larval settlement and metamorphic responses greatly varies among species (e.g. Mihm *et al.*, 1981; Keough and Chernoff, 1987; Todd and Keough, 1994; Keough and Raimondi, 1995).

To date only a few naturally occurring chemical cues have been isolated and unambiguously characterised (e.g. Kato *et al.*, 1975; Cuomo, 1985; Pawlik, 1986). However, it is evident in the literature that various organic components (e.g. polysaccharides, proteins, glycoproteins and fatty acids) can be potential inducers of larval settlement and metamorphosis (e.g. Morse *et al.*, 1979; Kirchman *et al.*, 1982; Pawlik, 1986; Pawlik and Faulkner, 1988; Morse, 1991, see Hirata and Hadfield, 1986; Burke, 1986; Pawlik, 1992; Rodriguez *et al.*, 1993 for reviews) and that the responses of larvae to these cues is normally dose dependent (e.g. Burke, 1984; Jensen and Morse, 1984; Pawlik, 1986; Pawlik and Faulkner, 1988; Pennington and Hadfield 1989).

Many chemical cues are bound to the surface of the natural source (e.g. Kirchman *et al.*, 1982; Morse and Morse, 1984; Cuoma, 1985; Pawlik, 1986; Maki *et al.*, 1988, 1989; Rowley, 1989; Pearce and Schiebling, 1991; see Morse, 1990 for review), although some cues may be released into the environment and do not therefore operate specifically at the surface of the natural source (e.g. Highsmith, 1982; Burke, 1984; Clare *et al.*, 1994). More recently, interest has been focused upon the importance of small polar waterborne cues which enter the water column (e.g. Coon *et al.*, 1988; Pennington and Hadfield, 1989; Bonar *et al.*, 1990; Hadfield and Pennington, 1990; Tamburri *et al.*, 1992; Zimmer-Faust and Tamburri, 1994; Lambert and Todd, 1994). Some of these studies provide evidence that larvae of some species respond to both waterborne cues and cues bound to the surface but that the function of these cues are different (e.g. Tamburri *et al.*, 1992; Zimmer-Faust and Tamburri 1994; Lambert and Todd, 1994). For example, Bonar *et al.* (1990) report that larvae of the bivalve, *Crassostrea virginica* will metamorphose with direct contact of extracellular polysaccharides bound on bacterial cell walls but are previously induced to search the substratum by a waterborne inducer, ammonia.

4.1.3. Artificial chemical inducing cues

Various artificial chemicals have also been shown to induce settlement and metamorphic responses in invertebrate larvae (e.g. Coon *et al.*, 1985; Pawlik, 1986, 1990: Pechenik, 1990: Rogriguez *et al.*,

1993). Cues from artificial sources have been used frequently to induce metamorphic responses in larvae since they are a convenient source and can be used in known quantities for studying the transduction mechanisms involved in metamorphosis (see Pechenik, 1990). The convenience of using artificial cues is demonstrated by the many studies which have investigated the potential application of artificial cues in aquaculture (e.g. Pascual and Zampatti, 1995). Artificial chemical cues divide broadly into (1) elevated concentrations of monovalent and divalent ions (2) choline derivatives and (3) amino acid derivatives such as L-DOPA, the catecholamines and γamma amino-butyric acid (GABA).

Increasing external concentrations of monovalent and divalent ions in excess of seawater concentrations has been shown to induce metamorphic responses in larvae. This is most clearly illustrated by studies involving potassium (K^+), which appears to have the widest application for inducing metamorphic responses in a wide range of phyla (e.g Baloun and Morse, 1984; Yool *et al.*, 1986; Pechenik and Heyman, 1987; Todd *et al.*, 1991). The metamorphic responses of larvae to excess potassium concentrations is dose dependent and is comparable among species, ranging between 10mM and 20 mM excess. At higher concentrations metamorphosis can be inhibited (e.g. Yool *et al.*, 1986; Todd *et al.*, 1991). It is perhaps important to note however that potassium, despite having a wide application, is by no means universal in its capacity to induce metamorphic responses (e.g. Rittschof *et al.*, 1986). Other cations which can effect metamorphosis in larvae in a dose dependent fashion include caesium, rubidium, lithium and calcium (e.g. Pechenik, 1984).

Choline is a pre-cursor of the neurotransmitter, acetylcholine, which acts at neuronal-neuronal and neuronal-effector synapses (Knuffler *et al.*, 1984). Choline, like several of its derivatives, has been shown to be an effective inducer of metamorphosis in *Phestilla sibogae* and *Adalaria proxima* (Bonar, 1976; Hadfield, 1978, 1984; Todd *et al.*, 1991). The optimal inducing concentration is similar in the two species, ranging between 10^{-2} and 10^{-3} M. Again, however , choline is not universal in its application for inducing complete metamorphic responses and can have toxic or

lethal effects (e.g Morse *et. al.*, 1979; Akashigi *et al.*, 1981; Levantine and Bonar, 1986; Pawlik, 1986).

Exposing larvae to specific concentrations of L-beta-3,4-dihydroxyphenylalanine (L-DOPA) and other tyrosine derivatives, such as dopamine, epinephrine and nonepinephrine (catecholamines), for specific periods of time provides another tool for inducing metamorphosis in certain marine invertebrate larvae. Again, however, the effects are variable (e.g. Morse *et al.*, 1979; Akashigi *et al.*, 1981; Hadfield, 1984; Jensen and Morse, 1984; Coon *et al.*, 1985; Levantine and Bonar, 1986, Pawlik, 1990).

Morse *et al.* (1979, 1980) were the first to report that another amino acid derivative, GABA, which is the decaboxylation product of glutamic acid, could induce metamorphosis in the abalone, *Haliotis rufescens* at 10^{-6} M. GABA also induces metamorphosis in *S. droebachiensis* but at higher concentrations (10^{-4} to 10^{-3} M) than those reported for *Haliotis rufescens* (Pearce and Scheibling, 1991). However, GABA has no inductive effect upon *Alcyonium siderium* (Sebens, 1983), *Phestilla sibogae* (Hadfield, 1984), *Mytilis edulis* (Cooper, 1982) or *Crepidula fornicata* (Pechenik and Heyman, 1987).

4.1.4. Rationale

Lacuna vincta is common in the lower intertidal and subtidal of rocky shores and is associated with a wide range of macroalgal species. *Lacuna vincta* larvae can delay settlement, may settle in high densities and may therefore have a major grazing impact upon the macroalgal canopy (Fralick *et al.*, 1974). Hence *Lacuna vincta* is a potentially important species when considering factors affecting rocky shore macroalgal communities (Underwood and Fairweather, 1989). Despite this, the natural sources of the chemical inducing cues (s) have not been directly and explicitly examined in this species (Fretter, 1972).

The aim of this investigation was to determine naturally occurring sources of settlement and metamorphic inducing chemical cues, to screen a variety of known artificial cues for their effectiveness in inducing metamorphosis, to determine whether metamorphic responses to natural and artificial cues were comparable and, finally, to determine the effect of larval age and nutritional status (starving larvae for defined periods during the facultative period) upon metamorphic responses to established inducing chemical cues.

4.2. MATERIALS AND METHODS

4.2.1. Production of *Lacuna vincta* larvae

General methods for collecting adult spawning stocks and for culturing larvae are described in section 3.2. For these experiments, adult *Lacuna vincta* were collected from Kingsbarns (Fife, Scotland) during the spawning season (January through to June) in both 1994 and 1995 and were maintained at 10 °C in large glass tanks. Newly deposited spawn masses were removed from the tanks and were separately incubated at 10 °C in hot-air sterilised petridishes. Twice filtered seawater (TFSW) (10 μm and then 0.22 μm) was replenished daily and antibiotics were used only occasionally. This protocol consistently produced healthy larvae which hatched after 14-17 d.

Larvae were cultured in hot-air sterilised 1l glass beakers at 10 °C in continuous light (densities were 1-2 larvae. ml^{-1}). TFSW and microalgal food (10^4 cells. ml^{-1} *Rhodomonas* spp., not bacteria free) were replenished every 5 d, for the first 20 d, and then more frequently (2-3 d), to reduce the amount of bacterial filming on the surface of culture beakers. Larvae generally attained competence within 25 d posthatching.

4.2.2. Assays

Larvae were pipetted from cultures into hot-air sterilised glasses petridishes (50mm in diameter and 18mm in height), containing 30ml of TFSW, and were incubated at 10 °C. Water was not replenished in treatment petridishes, and food was not provided during the course of the assay so as to minimise disturbance to larvae and to preclude the possibility of inducing larvae to metamorphose by components in microalgal food which were not bacteria free (Rodriguez *et al.*, 1993). Where possible, both positive (15 mM elevated external concentrations of potassium chloride or $2cm^2$ *Laminaria digitata* fronds) and negative (TFSW) controls were included alongside experimental treatments. Metamorphs and dead larvae were observed at timed intervals (at 12 or 24 h intervals for up to 5 d) using a Wild M8 stereomicroscope and both of these categories of individuals were

immediately removed. Metamorphs were kept one day thereafter to ensure that they had successfully completed metamorphosis.

4.2.3. Experimental design and data handling

Treatments dishes in each experiment were kept on a single tray and were randomly positioned therein. Treatments were replicated three times, and each replicate contained 10 larvae. Sibling larvae were used within each experimental assay. Metamorphosis in each replicate was recorded as the percentage of larvae metamorphosing and data were arc-sine transformed. The means and standard errors presented for each treatment are for back-transformed arc-sine data. One-way or two-way analysis of variance tests were performed upon arc-sine transformed data. Where appropriate, Tukey multiple pairwise comparison tests were performed upon arc-sine transformed data to determine which treatment means were significantly different from others (Minitab). Data used in statistical analyses were generally those collected at the end of the trial; however, if metamorphosis in any one treatment was 100 % before the end of the experiment, data collected at that point in time were used instead.

4.2.4. Assays for artificial chemical inducing cues

A range of artificial chemicals (as described in section 4.1) wasssayed for their capacity to induce metamorphosis in *Lacuna vincta* larvae, and hence, their suitability for use as a positive control when assaying possible natural cues.

Experiment one: Elevated potassium ion concentrations

The concentration of potassium in normal seawater is approximately 9mM. The inductive capacity of a range of elevations in potassium concentrations was investigated (Yool *et al.*, 1986). Concentrations referred to here are elevated levels above that in normal seawater (i.e. 10mM 'excess' = 19mM in seawater). In the first experiment the treatments were 5, 10, 15, 20, 25 and 30 mM excess potassium chloride (KCl) added to twice filtered natural seawater. The experiment was

repeated four times using four different sibling larval cultures. Percentage metamorphosis of larvae was recorded after 24, 48 and 72 h.

Experiment two: External concentrations of choline

The treatments were 10^{-2}, 10^{-3} and 10^{-4} M external concentrations of choline chloride. Percentage metamorphosis of larvae was recorded after 24, 48, 72, 96 and 120 h.

Experiment three: External concentrations of Gamma-amino-butyric acid (GABA)

The treatments were 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M external concentrations of GABA. Percentage metamorphosis of larvae was recorded after 24, 48, 72, 96 and 120 h.

Experiment four: External concentrations of L-DOPA

The treatments were 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} M external concentrations of L-DOPA. Percentage metamorphosis of larvae was recorded after 24, 48, 72, 96 and 120 h.

4.2.5. Assays for naturally occurring chemical inducing cues

Experiment five: Macroalgal species associated with *Lacuna vincta*

Lacuna vincta may variously be associated with the macroalgae *Laminaria digitata*, *Fucus serratus* and *Mastocarpus stellata*. Accordingly, 2 cm^2 sections were cut from the thalli of these three species and were presented to *Lacuna vincta* larvae. In addition, *Fucus spiralis*, which is found in the upper intertidal, but is not associated with *Lacuna vincta*, was also assayed to determine whether metamorphosis was specific to cues released by macroalgae species associated with *Lacuna vincta*. Percentage metamorphosis of larvae was recorded in each treatment dish after 24, 48 and 72 h.

Experiment six: Other naturally occurring sources

Other potential sources of the metamorphic inducer of larvae were also tested. *Lacuna vincta* adult females and spawn masses were tested to determine whether larvae would metamorphose in the

presence of conspecifics. A biofilmed substratum, formed by placing petridishes upside down in continuous flow seawater tanks for one week, was tested for its inducing capacity to determine whether larvae would respond to a fouled surface. *Laminaria digitata* fronds, which were either heavily grazed or ungrazed were also tested. Another treatment consisted of *Laminaria digitata* fronds which had been boiled for 5 minutes in TFSW. This latter treatment was included to determine whether the inducing cue found in *Laminaria digitata* was heat labile. Finally, unfiltered seawater was directly added to sterile petridishes to determine whether larvae would metamorphose in response to the organic particulates in seawater. Percentage metamorphosis of larvae was recorded after 24 h.

Experiment seven: Filtered natural seawater

This experiment was conducted to determine whether bacteria and diatoms on the surface of macroalgae, or whether particulates emanating from macroalgae could induce metamorphosis of *Lacuna vincta* larvae. Two hundred grams (wet weight) of freshly collected *Laminaria digitata* fronds was placed in a 5l glass jar and 5l of TFSW was added. The mixture was continuously agitated using a magnetic stirrer. After one day the seawater was treated with a series of filters; 200μm, 40μm, 8μm, and 2μm, and the filtrate from each stage was placed in petridishes. Unfiltered seawater was also used as a treatment for comparison. Percentage metamorphosis of larvae was recorded after 24, 48 and 72 h.

Experiment eight: Cultured bacteria

Three strains of gram negative non-toxic marine bacteria, *Psychrobacter immobilis* (NCIMB 1 308), *Photobacterium phosphoreum* (NCIMB 1 844) and *Pseudomonas* 3-1-1 were raised at 20 °C to log phase growth in marine broth ("Difco" 2216). Bacterial cultures then were twice centrifuged at 1,900g for 10 mins and washed in NaCl buffer (3.2%) to remove the broth. Bacteria were resuspended in artificial seawater (ASW) (Sigma chemicals) and standardised in concentration to an absorbency of 0.5 at 570 nm indicating a concentration of approximately 5×10^7 cells. ml^{-1}.

Bacterial strains then were mixed in equal proportions and serially diluted to give three concentrations; 10^3, 10^5 and 10^7 cells. ml^{-1}. ASW was run as a negative control. Percentage metamorphosis of larvae was recorded after 12, 24, 36 and 48 h.

Experiment nine: Laminarin (polysaccharide)

Laminarin is a polysaccharide which is released from the cut margins of kelp and is routinely used to induce immune responses in marine species (V. Smith, pers. comm.). It therefore was deemed appropriate to determine whether laminarin could induce metamorphic responses in larvae. Laminarin was dissolved in ASW at five concentrations, 0.01, 0.05, 0.1, 0.5 and 1.0 $mg.ml^{-1}$ and was presented to larvae. The experiment was repeated three times. Percentage metamorphosis of larvae was recorded after 24, 48 and 72 h.

4.2.6. Comparison of metamorphic responses to artificial and naturally occurring chemical cues

Experiment ten: Inhibition by 30 mM excess potassium

Previously published experiments including a range of excess potassium concentrations indicated that 30mM excess potassium was either inhibitory or non-inductive (e.g. Todd *et al.*, 1991). It was therefore deemed appropriate to assess the effect of 30mM excess potassium in combination with *Laminaria digitata* which was found to be the most effective naturally occurring inducing cue. Four separate sibling larval cultures were simultaneously presented with 30mM excess potassium and *L. digitata*. Additionally, other treatments consisted of larvae presented with either 15mM excess potassium or *L. digitata*. Percentage metamorphosis of larvae was recorded after 24, 48 and 72 h.

Experiment eleven: 15 mM excess potassium and *L. digitata*

Data for metamorphic reponses of larvae in natural and artificial cue treatments indicated that the time taken for larvae to metamorphose differed with respect to the inducing cue. To confirm these observations, six separate sibling larval cultures were presented with either 15 mM excess potassium or *Laminaria digitata*. Percentage metamorphosis of larvae was recorded after 24, 48 and 72 h.

4. 2.7. The effects of age and condition of larvae

Experiment twelve: Effect of larval age upon metamorphosis

Lacuna vincta larvae were able to delay settlement in the absence of appropriate inducing cues, providing an ideal opportunity to investigate the effects of delay period upon metamorphosis in response to artificial and natural cues. Two separate sibling larval cultures were sampled after 25 (first attainment of competence), 35, 45 and 55 days posthatching and were presented with either 15 mM excess potassium or *Laminaria digitata*. Percentage metamorphosis of larvae was recorded after 24, 48 and 72 h.

Experiments thirteen and fourteen: Effect of starvation period upon metamorphosis

The effect of starving larvae upon metamorphic responses to artificial and naturally occurring cues was investigated. In experiment thirteen, larvae from two separate sibling larval cultures (45 days posthatching) were starved for 5 days before being presented with either 15 mM excess potassium or *Laminaria digitata*. In experiment fourteen, older larvae from one of the larval cultures used in the above experiment (now 10 days older and hence 55 days posthatching) were starved for either 5 or 8 days prior to presentation of either 5 mM excess potassium, 15 mM excess potassium, or *Laminaria digitata*. Sibling larvae, of the same age, that had previously not been deprived of food were set up as controls in both experiments. Because sibling larvae were used in these experiments, the results obtained allowed for assessment of whether there was any interactive effects of larval age and nutritional status of larvae upon metamorphosis in response to the various cues and in negative controls. Percentage metamorphosis of larvae was recorded after 24, 48 and 72 h, in experiment thirteen, and after 12, 24, 36 and 48 h in experiment fourteen.

4.3. RESULTS

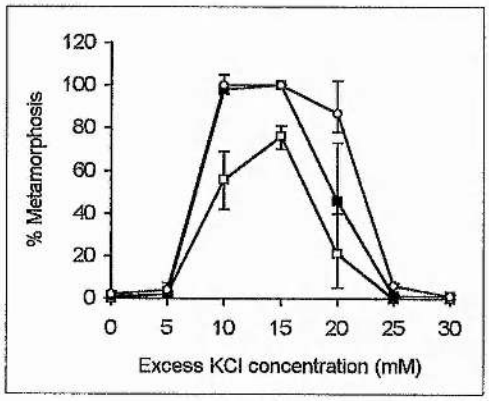
4.3.1. Artificial chemical cues

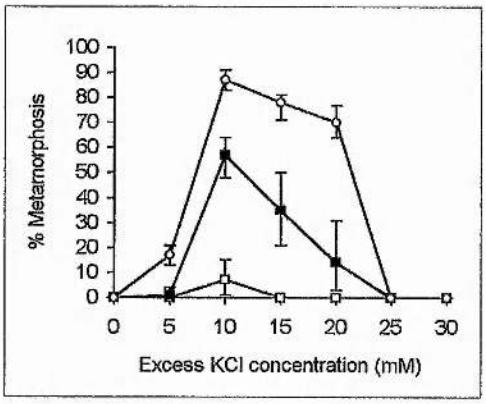
Experiment one: Excess potassium concentrations

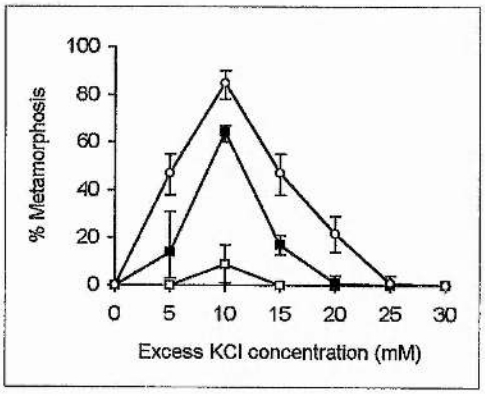
Excess concentrations of potassium consistently induced complete larval metamorphosis with no discernible lethal effects or mortality of larvae. The mean percentage response after 72 h continuous exposure was concentration dependent in all four larval cultures used in assays (Figures 4.1a-d.). Notwithstanding the variation in maximal levels of percent metamorphosis and in the general shape of the dose response plots, comparable qualitative results were obtained for all sibling larval groups. The optimal inducing concentration was between 10 mM and 20 mM in excess of seawater concentrations (9 mM) and was thereby set at 15mM 'excess' for use as a positive control in other experiments. Concentrations outside the optimal range were considered sub- or supra-optimal. Larvae did not metamorphose in either the negative control (0.22 μm TFSW) or in the 30 mM excess potassium treatment.

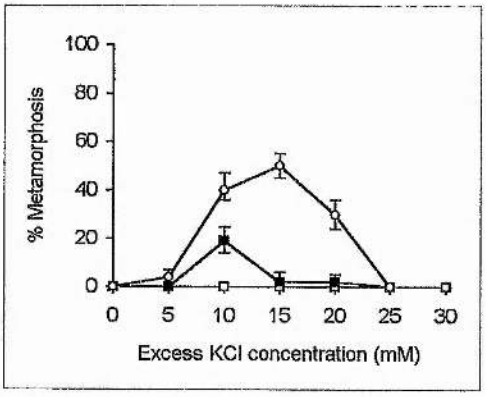
The behavioural response of larvae varied with potassium concentration. Larvae in the negative control (TFSW) continuously swam and were rarely observed on the bottom of the petri dish. In optimal inducing concentration treatments (10-20 mM), however, larvae remained stationary, lying on their dorsal side on the bottom of the petri dish. The larvae in these treatments rapidly contracted their velar lobes in a regular fashion. The behavioural response in 30mM excess potassium treatments was indicative of a paralytic effect.

Figures 4. 1a-d. Experiment one: Mean metamorphic responses of *Lacuna vincta* larvae from four separate sibling groups to a range of excess potassium concentrations. Data are back-transformed arc-sine mean percentage responses after 24 h (open square), 48 h (closed square) and 72 h (open circle) continuous exposure.


% Metamorphosis
120
100
80
60
40
20
0
0 5 10 15 20 25 30
Excess KCl concentration (mM)


% Metamorphosis
100
90
80
70
60
50
40
30
20
10
0
0 5 10 15 20 25 30
Excess KCl concentration (mM)


% Metamorphosis
100
80
60
40
20
0
0 5 10 15 20 25 30
Excess KCl concentration (mM)


% Metamorphosis
100
80
60
40
20
0
0 5 10 15 20 25 30
Excess KCl concentration (mM)

Experiment two: External concentrations of choline

External concentrations of choline chloride induced metamorphosis but also had a lethal toxic effect. At 10^{-5} and 10^{-4} M larvae continuously swam but did not metamorphically respond. At 10^{-3} M and 10^{-2} M larvae initiated metamorphosis, but here the results were compromised, more so in the higher concentration treatment, by high mortality and clogging of the mantle cavity by a secreted white solid. This was in marked contrast to the 100 % final response in the positive control (15 mM excess potassium).

Experiment three: External concentrations of GABA

Whilst the final mean percentage response in the positive control (15 mM excess potassium) was 100%, larvae in GABA concentration treatments did not metamorphose.

Experiment four: External concentrations of L-DOPA

As for the previous experiment larvae did not metamorphose in response to L-DOPA. After 24 h the L-DOPA had oxidised in treatments causing 100% mortality of larvae.

4.3.2. Naturally occurring chemical cues

Experiment 5: Metamorphic responses of *Lacuna vincta* larvae to macroalgal species

Figure 4.2 presents the metamorphic responses of *Lacuna vincta* larvae following 24, 48 and 72 h continuous exposure to four intertidal macroalgal species. 15 mM excess potassium was included here as a positive control, having being deduced as the optimal dose in Experiment one. Responses were observed in all macroalgal treatments within the first 24 h. However, it was clear that the magnitude of response varied with macroalgal species and that the pattern across species was retained over the time course of the experiment.

One-way analysis of variance for the 72 h data showed significant differences between treatment means ($F_{3,12} = 9.25$, $P < 0.05$) and Tukey's test separated the treatment means into two groups (see Figure 3.5.). *Laminaria digitata* was the most effective inducer of metamorphosis, with a 100% response after 72 h, followed by *Mastocarpus stellata* (61%) and *Fucus serratus* (52%). The mean percentage response in *Fucus spiralis* treatments was relatively low (15%) but this was compromised by high mortality which had reached 69% after 72 h. The negative (TFSW) and positive (15 mM KCl) controls showed 10 % and 100 % metamorphosis respectively. Whilst larvae in the positive control responded within 24 h, the latency of response in the negative control was between 48 and 72 h.

Experiment six: Other naturally occurring sources

The metamorphic responses of larvae in *Laminaria digitata* (grazed, ungrazed and boiled), adult female conspecifics, spawn mass, biofilm and unfiltered seawater treatments after 24 h continuous exposure are presented in Figure 4.3. Metamorphic responses were observed in all treatments, except in those in which *L. digitata* fronds had been previously boiled. In this treatment mortality had reached 100 % after 24 h. One-way analysis of variance showed significant differences between treatments ($F_{6,21} = 1.39$, $P <$

Figure 4. 2. Experiment five: Mean metamorphic responses of *Lacuna vincta* larvae to a range of intertidal macroalgal species, *Laminaria digitata* (black), *Mastocarpus stellata* (grey vertical stripe), *Fucus serratus* (grey dot), and *Fucus spiralis* (dark grey diagonal stripe), to 15 mM excess potassium (positive control, white) and in 0.22 μm TFSW natural seawater (negative control, horizontal black stripe). Data are back-transformed arc-sine mean percentage responses after 24 h, 48 h and 72 h continuous exposure. One-way analysis of variance on data collected after 72 h showed significant variation in metamorphic responses among the macroalgae treatments ($F_{3,12} = 9.25$, $P < 0.05$). Groupings for Tukey multiple pairwise comparison test are shown above data collected after 72 h.

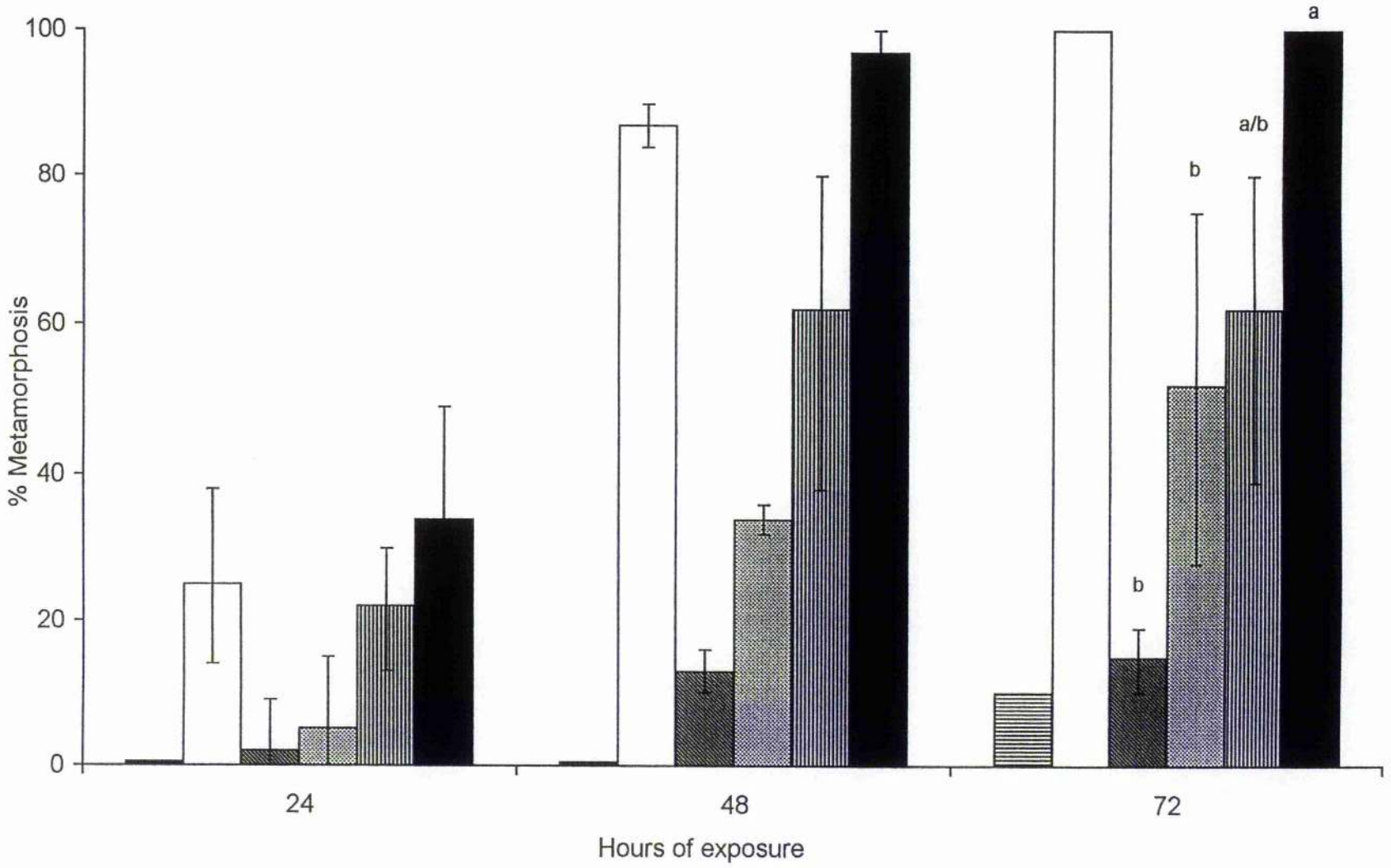
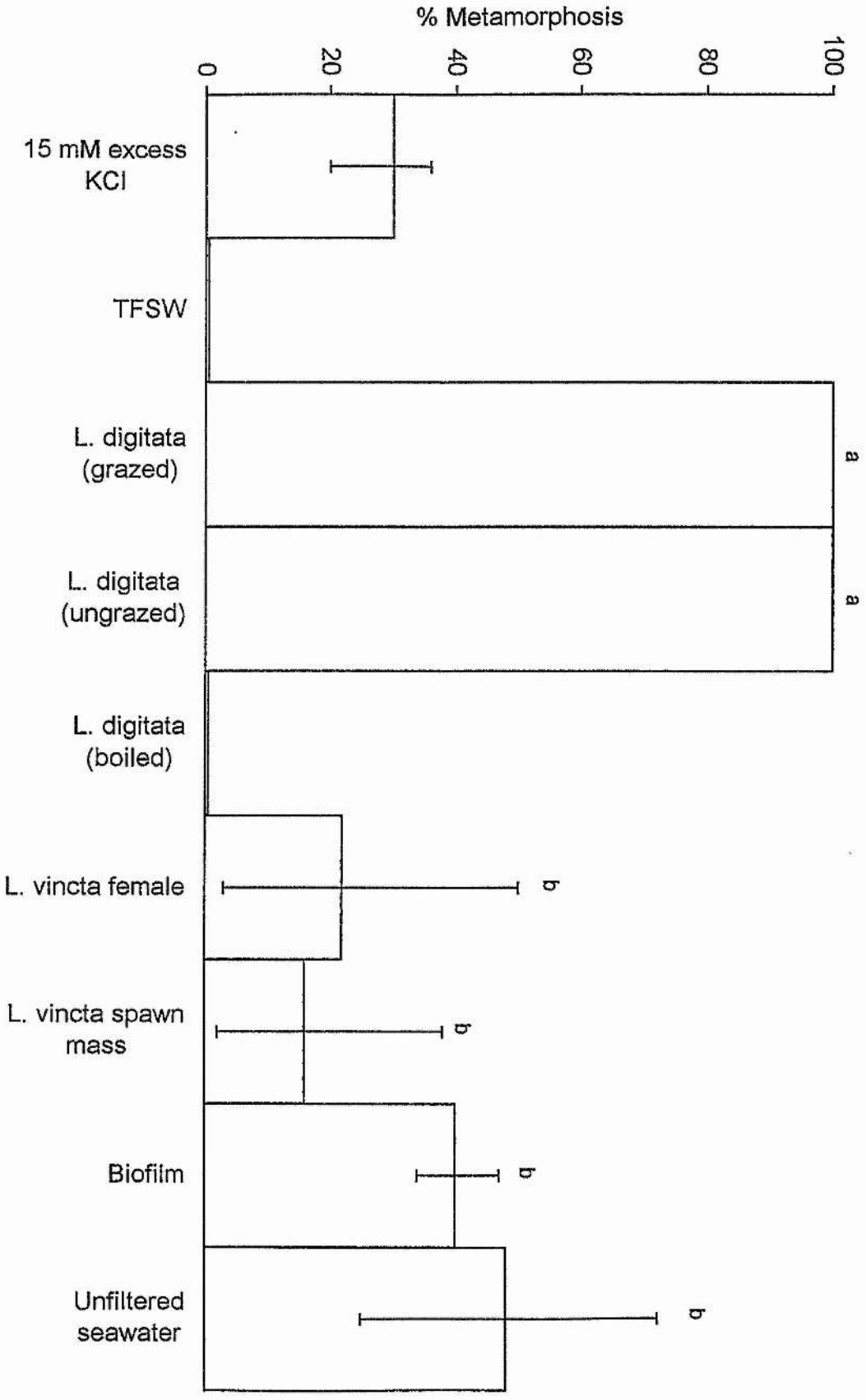

100
80
60
40
20
0
% Metamorphosis
24
48
72
Hours of exposure
a
a/b
b
b

Figure 4. 3. Experiment 6: Mean metamorphic responses of *Lacuna vincta* larvae to a range of putative naturally occurring metamorphic cues, including manipulated *Laminaria digitata*, the presence of adult female *Lacuna vincta*, *Lacuna vincta* spawn masses, particulates in unfiltered natural seawater and a biofilm developed on a glass petridish after one week. Data are back-transformed arc-sine mean percentage responses after 24 h. One-way analysis of variance showed significant variation in metamorphic responses among the cue treatments ($F_{6,21}$ =1.39, $P < 0.05$). Groupings for Tukey multiple pairwise comparison test are shown above columns of data.


% Metamorphosis
0
20
40
60
80
100
15 mM excess KCl
TFSW
L. digitata (grazed)
L. digitata (ungrazed)
L. digitata (boiled)
L. vincta female
L. vincta spawn mass
Biofilm
Unfiltered seawater
a
a
b
b
b
b

0.05). Tukey's test separated the grazed and ungrazed *L. digitata* treatments (both 100%) from unfiltered seawater (48%), biofilm (40%), adult female (22%) and spawn mass (16%) treatments (see Figure 4.3.). The responses in the negative (TFSW) and positive controls (15 mM KCl) were 0% and 30 % respectively.

Experiment seven: Filtered natural seawater

Metamorphic responses of larvae in the various filtered seawater treatments are shown in Figure 4.4. Responses were observed in all treatments within 24 h. However, one-way analysis of variance did not show significant variation in metamorphic responses among the treatments ($F_{4,15} = 1.43$, $P > 0.05$). The greatest metamorphic response was observed in the positive control (*Laminaria digitata*), reaching 100 % after 48 h.

Experiment eight: Cultured bacteria

Figure 4. 5. shows the metamorphic responses of larvae in the various bacterial concentration treatments. While some larvae in the positive control (*Laminaria digitata*) had responded within 12 h, the latency of response in bacterial concentration treatments was between 12 and 48 h. After 48 h, there was a trend of decreasing mean response with increasing bacterial concentration (ranging from 100% in 10^3. ml^{-1}, down to 11% in 10^7. ml^{-1}). However, one-way analysis of variance on data after 48 h did not show any significant differences among the treatments ($F_{2,6} = 7.58$, $P > 0.05$).

Experiment nine: Laminarin (polysaccharide)

The metamorphic responses of larvae from three separate sibling cultures to various concentrations of laminarin are shown in Figures 4.6 a-c. While larvae in the positive control (*Laminaria digitata*) responded within 24 h, the first response of larvae in the laminarin treatment was observed after 24 and 96 h. Two-way analysis of variance showed no significant variation in mean responses with respect to

Figure 4. 4. Experiment 7: Mean metamorphic responses of *Lacuna vincta* larvae to a range of natural seawater filtrates, including non-filtered (white) 200 μm filtrate (black horizontal stripe), 40 μm filtrate (light grey dot), 8 μm filtrate (black vertical stripe) and 2 μm filtrate (black). Mean metamorphic responses of larvae in positive the control (*Laminaria digitata*, dark grey dot) and in the negative control (0.22 μm TFSW, black diagonal stripe) are also shown. Data are back-transformed arc-sine mean percentage responses after 24 h, 48 h and 72 h continuous exposure. One-way analysis of variance using data collected after 72 h did not show significant variation in metamorphic responses among the filtrate treatments ($F_{4,15} = 1.43$, $P > 0.05$).

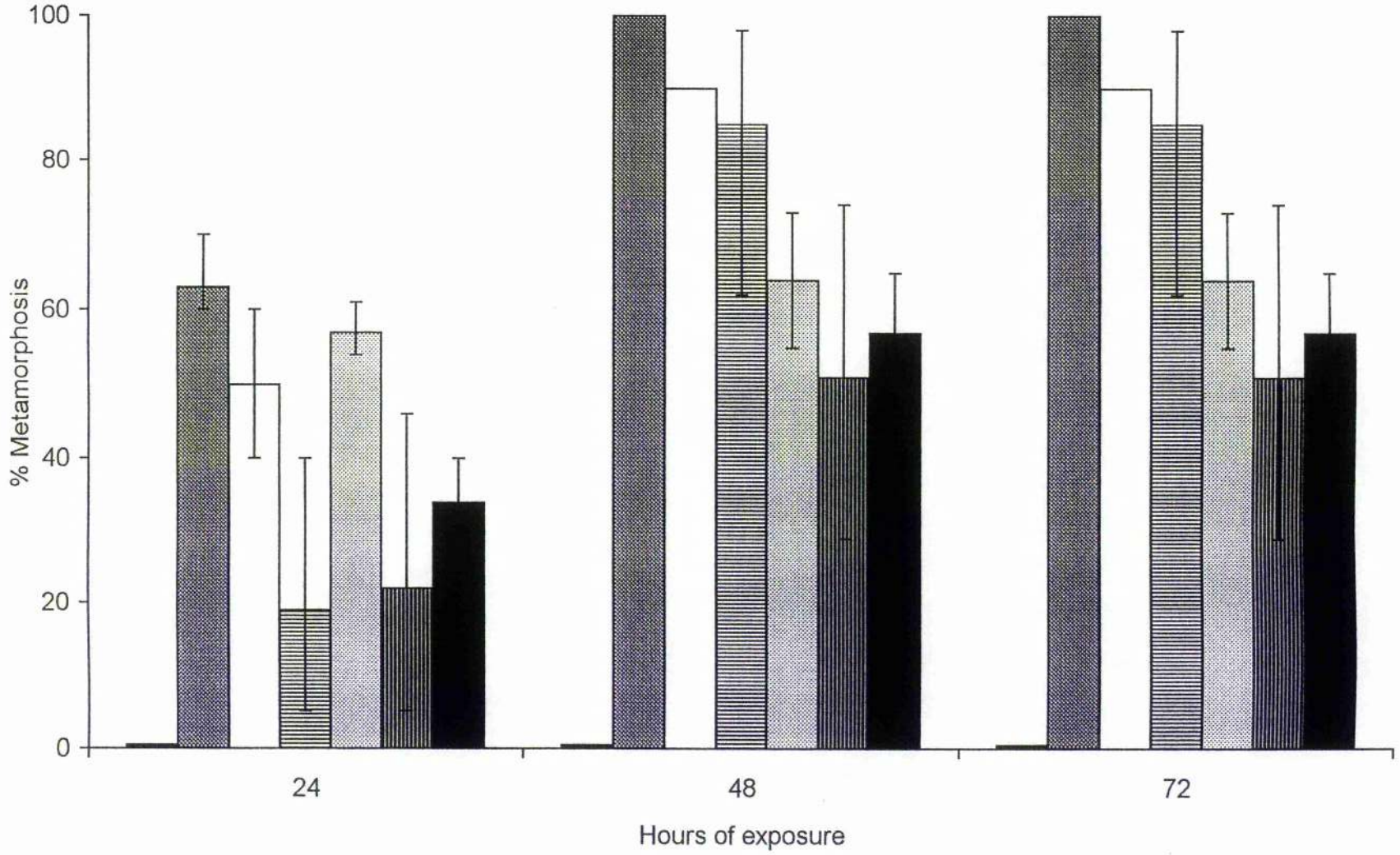
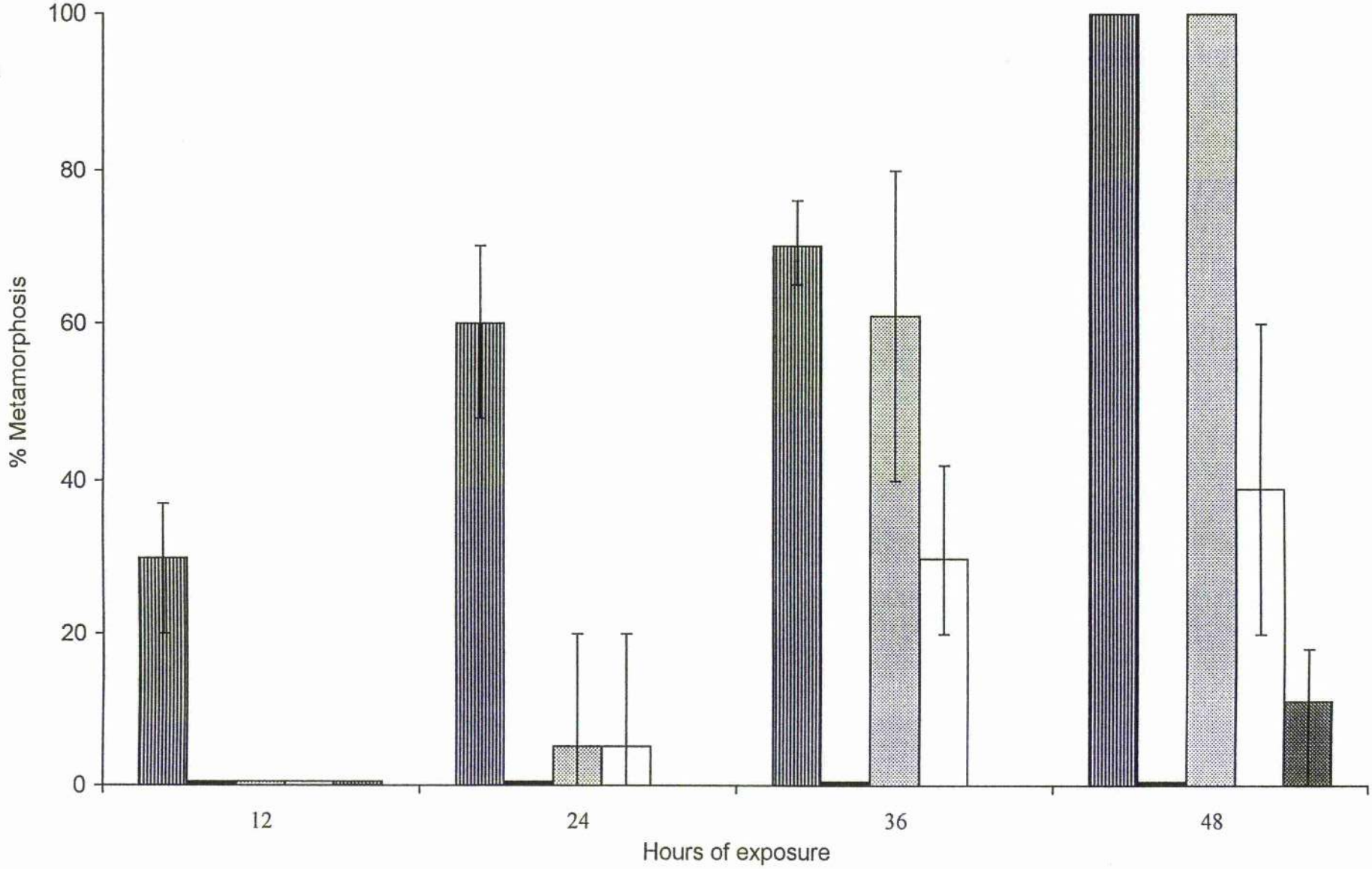
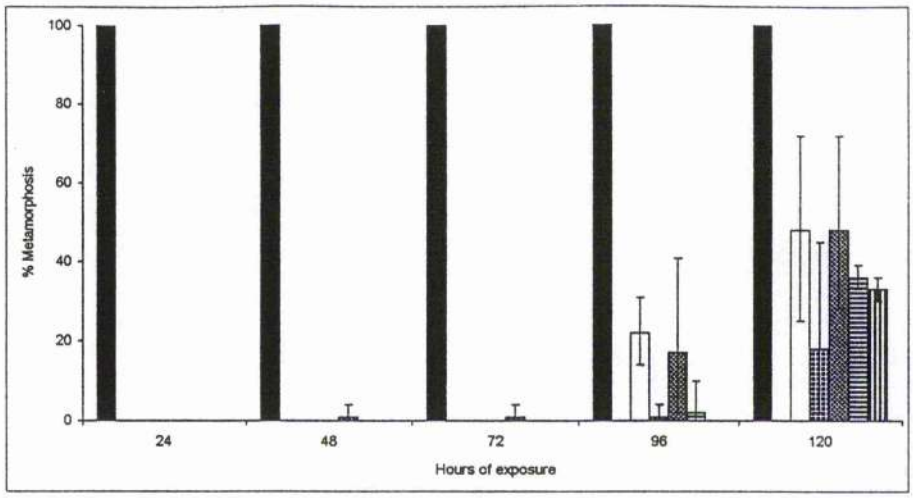
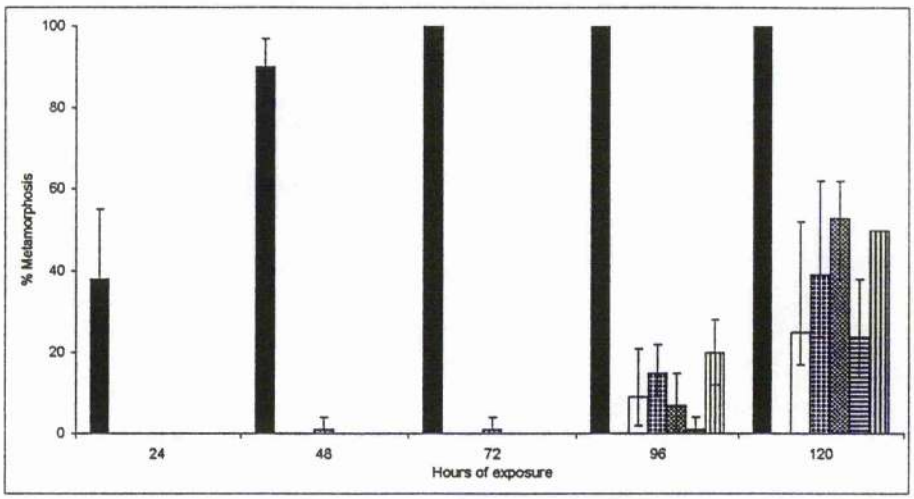

100
80
60
40
20
0
% Metamorphosis
24
48
72
Hours of exposure

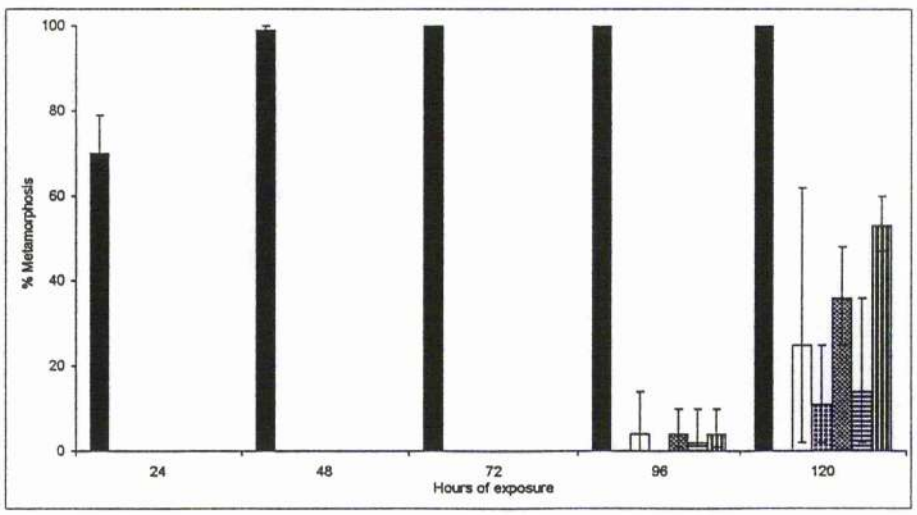
Figure 4. 5. Experiment 8: Mean metamorphic responses of *Lacuna vincta* larvae to 10^3 cells. ml $^{-1}$ (light grey dot), 10^5 cells. ml $^{-1}$ (white) and 10^7 cells. ml $^{-1}$ (black check) cultured marine bacteria in artificial seawater (equal numerical mixtures of *Psychrobacter immobilis*, *Photobacterium phosphoreum* and *Pseudomonas* 3-1-1) and in positive (*Laminaria digitata*, black vertical stripe) and negative (artificial seawater, black diagonal stripe) controls. Data are back-transformed arc-sine mean (n = 2) percentage responses after 12 h, 24 h, 36 h and 48 h continuous exposure. One-way analysis of variance using data collected after 48 h did not show significant variation in metamorphic responses among the bacterial concentration treatments ($F_{2,6}$ = 7.58, $P > 0.05$).


100
80
60
40
20
0
% Metamorphosis
12
24
36
48
Hours of exposure

Figures 4. 6a-c. Experiment 9: Mean metamorphic responses of *Lacuna vincta* larvae from three separate sibling larval cultures in 0.01 mg. l^{-1} (white), 0.05 mg. l^{-1} (black check), 0.10 mg. l^{-1} (black criss-cross), 0.50 mg. l^{-1} (black horizontal stripe) and 1.00 mg. l^{-1} (black vertical stripe) concentrations of laminarin in artificial seawater and in positive control (*Laminaria digitata* in artificial seawater black) and negative control (artificial seawater, black diagonal stripe) treatments. Data are back-transformed arc-sine mean percentage responses after 24 h, 48 h, 72 h, 96 h and 120 h continuous exposure.


100
80
60
40
20
0
% Metamorphosis
24
48
72
96
120
Hours of exposure


100
80
60
40
20
0
% Metamorphosis
24
48
72
96
120
Hours of exposure


100
80
60
40
20
0
% Metamorphosis
24
48
72
96
120
Hours of exposure

either laminarin concentration or culture source ($F_{4,15} = 1.11$, $F_{4,15} = 0.61$ respectively, for both, $P > 0.05$).

4.3.3. Comparison of cues

Experiment ten: Inhibition of metamorphosis by 30 mM excess potassium

Results from experiment one suggested that 30mM excess potassium was either inhibitory or non-inductive. To investigate this further larvae were presented with 30 mM excess potassium with *Laminaria digitata*, the most effective inducing natural cue. Table 4.1 shows the metamorphic responses of larvae in treatments which contained both 30 mM excess potassium and *Laminaria digitata*, 30mM excess potassium, 15mM excess potassium or *Laminaria digitata*. 30 mM excess potassium was inhibitory both in isolation and when included with *Laminaria digitata*.

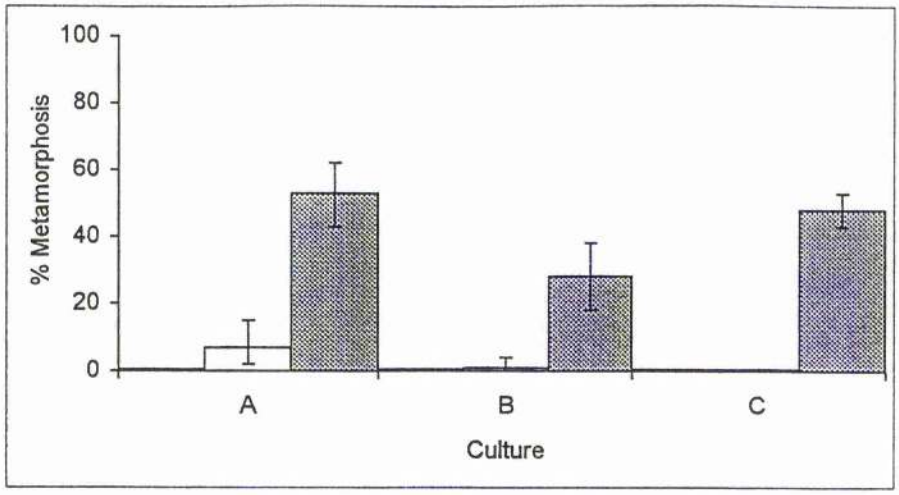
Experiment eleven: 15 mM excess potassium and *Laminaria digitata*

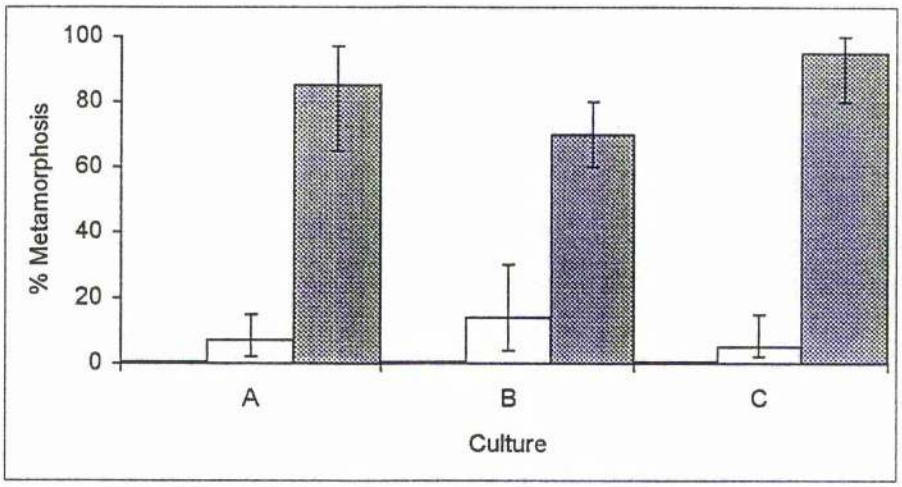
The metamorphic responses of larvae to the optimal inducing concentration of excess potassium and to *Laminaria digitata* were compared using larvae from six separate sibling larval cultures (see Figures 4.7a. and 4.7b.). The three larval cultures from 1994 (A-C) were 35 days posthatching and the three cultures from 1995 (D-F) were 54 days posthatching. Of necessity, therefore, these data were treated as two separate experiments in the analysis. Results for two-way analysis of variance (factors were cue source and larval culture) on data after 24 h are shown for both experiments in Tables 4.2 and 4.3. In both cases the metamorphic responses of larvae in the *Laminaria digitata* treatment was significantly greater than those obtained in the 15 mM excess potassium treatment.

Table 4. 1. Experiment ten: Mean metamorphic responses of *Lacuna vincta* larvae in TFSW (negative control) 15mM excess potassium, *Laminaria digitata* (positive controls), 30mM excess potassium and 30mM excess potassium with *Laminaria digitata* treatments after 24 h continuous exposure. Data are back-transformed means and standard errors of arc-sine data.

Larval culture	TFSW	*L. digitata*	15 mM excess potassium	30 mM excess potassium	*L. digitata* and 30mM excess potassium
1	0	100	83 (81, 86)	0	0
2	0	100	85 (81, 90)	0	0
3	0	100	40 (36, 47)	0	0

Figure 4. 7a. Experiment eleven: Mean metamorphic responses of *Lacuna vincta* larvae from three separate sibling larval cultures (A-C, 1994) in the 15mM excess potassium treatment (white), in the *Laminaria digitata* treatment (grey) and in the negative control (0.22 μm TFSW, black). Data are back-transformed arc-sine mean percentage responses after 24 h (top), 48 h (middle) and 72 h (bottom) continuous exposure. Results of two-way analysis of variance (factors are cue source and larval culture) are shown in Table 4.2.


100
80
60
40
20
0
% Metamorphosis
A
B
C
Culture


100
80
60
40
20
0
% Metamorphosis
A
B
C
Culture

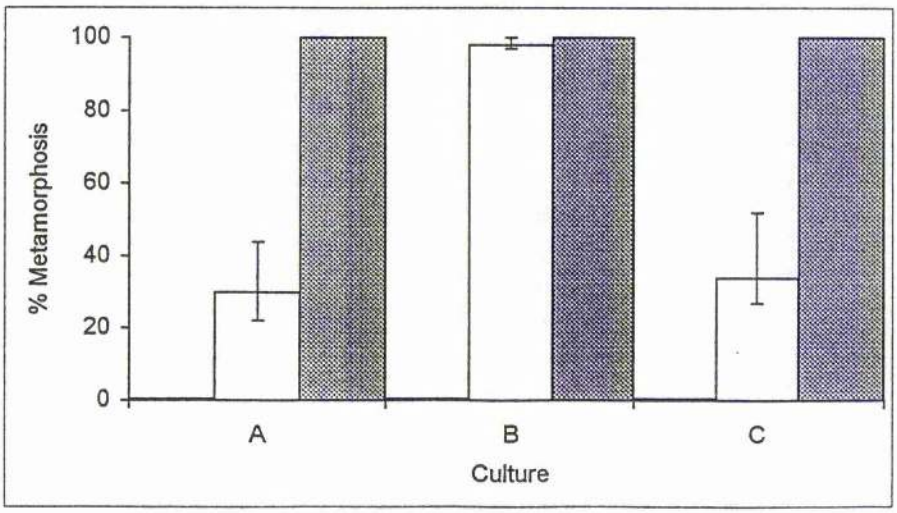
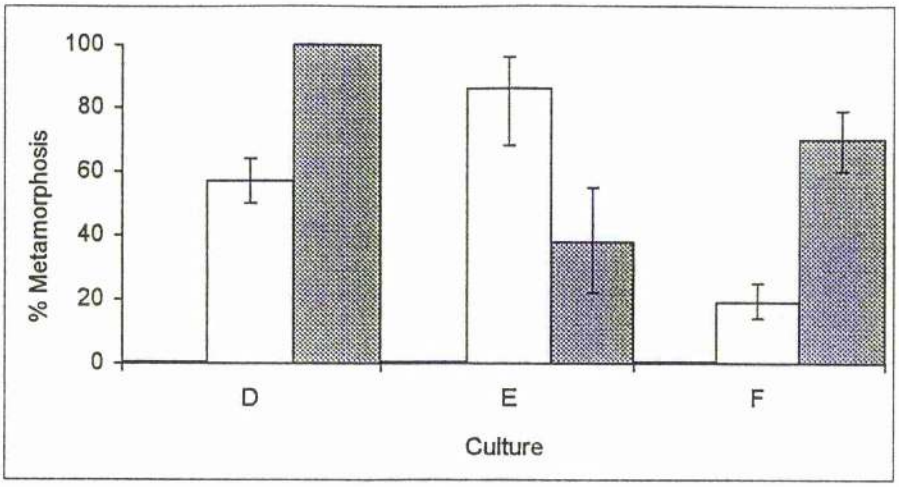
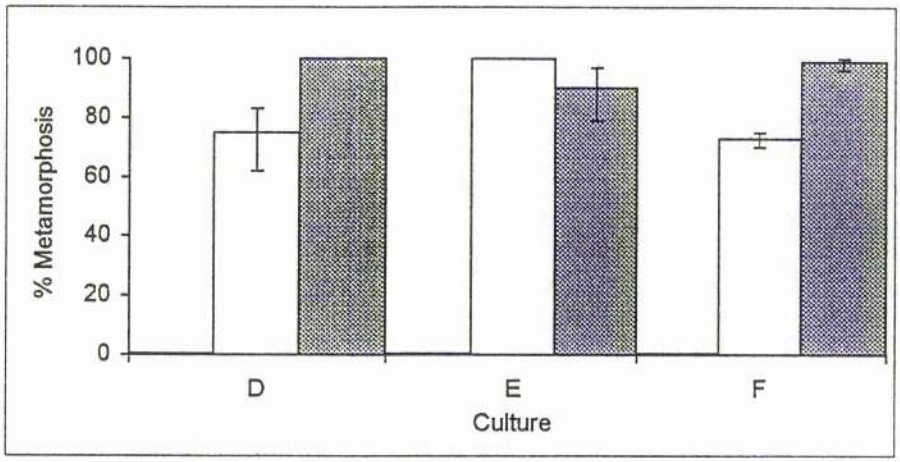
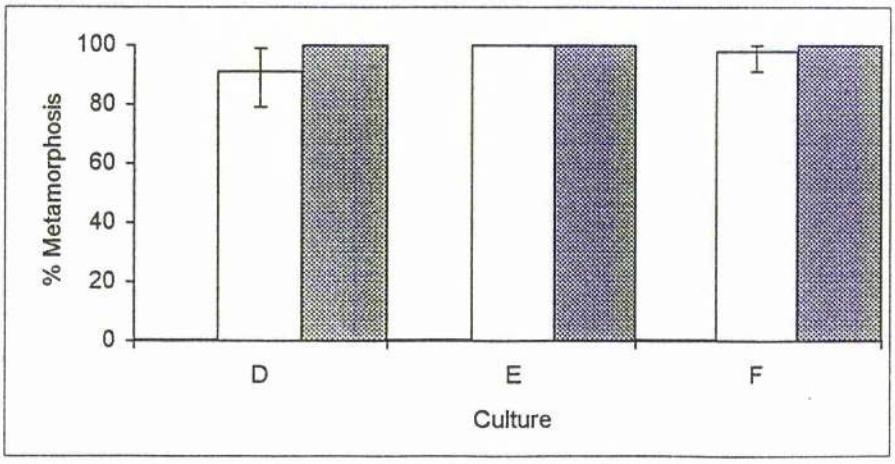

100
80
60
40
20
0
% Metamorphosis
A
B
C
Culture

Figure 4. 7b. Experiment eleven: Mean metamorphic responses of *Lacuna vincta* larvae from three separate sibling larval cultures (D-F, 1995) in 15mM excess potassium (white) and *Laminaria digitata* (grey) treatments and in the negative control (0.22 μm TFSW, black). Data are back-transformed arc-sine mean percentage responses after 24 h (top), 48 h (middle) and 72 h (bottom) continuous exposure. Results of two-way analysis of variance (factors are source and larval culture) are shown in Table 4.3.


100
80
60
40
20
0
% Metamorphosis
D
E
F
Culture


100
80
60
40
20
0
% Metamorphosis
D
E
F
Culture


100
80
60
40
20
0
% Metamorphosis
D
E
F
Culture

Tables 4. 2. (top) **and 4. 3.** (bottom) **Experiment eleven**: Two-way analysis of variance (factors are cue source and larval culture) on data for metamorphic responses of *Lacuna vincta* larvae to *Laminaria digitata* and 15 mM excess potassium in 1994 (top) and 1995 (bottom).

Source	DF	SS	MS	F	P
Cue treatment	1	4608	4608	51.49	<0.001
Larval culture	2	327	163.5	1.83	0.203
Interaction	2	363	181.5	2.03	0.174
Error	12	1074	89.5		
Total	17	6372			

Source	DF	SS	MS	F	P
Cue treatment	1	854.2	854.2	5.64	<0.05
Larval culture	2	2430.1	1215.1	8.02	<0.01
Interaction	2	4397.4	2198.7	14.51	<0.001
Error	12	1818.7	151.6		
Total	17	9500.4			

4.3.4. Age and condition of larvae

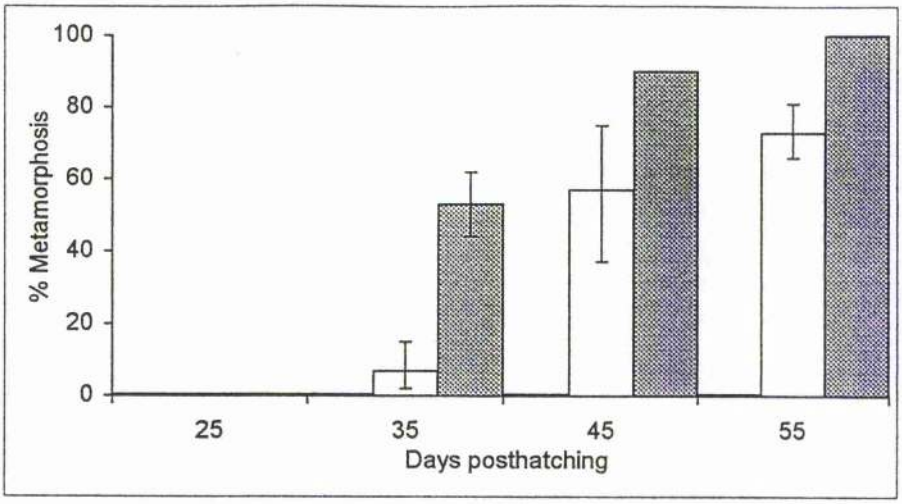
Experiment twelve: Effect of larval age upon metamorphosis

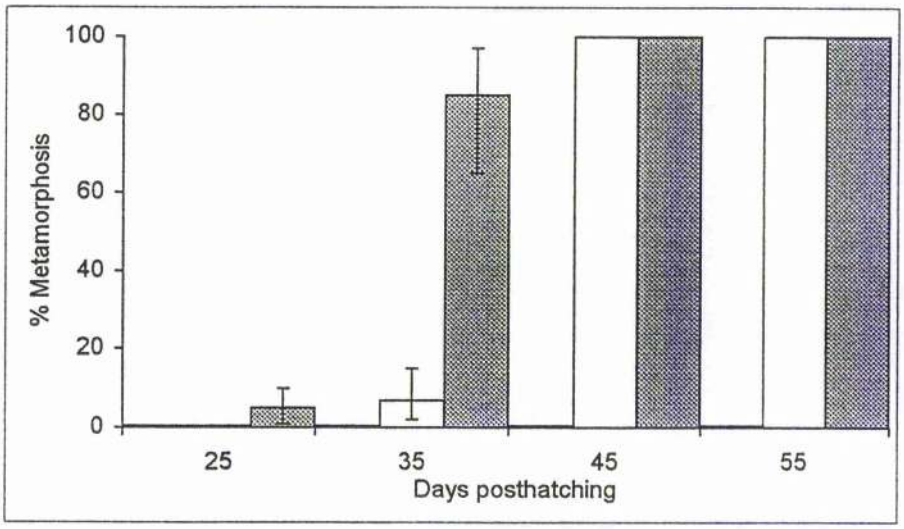
Figures 4.8a. and 4.8b. show the metamorphic responses of variously aged larvae from two separate sibling larval cultures in *Laminaria digitata* and 15 mM excess potassium treatments. Results for two-way analysis of variance (factors were cue source and larval age) on data after 24 h showed that the metamorphic response of larvae significantly increased with increasing larval age and was significantly greater in the *Laminaria digitata* treatment (see Tables 4.4. and 4.5.).

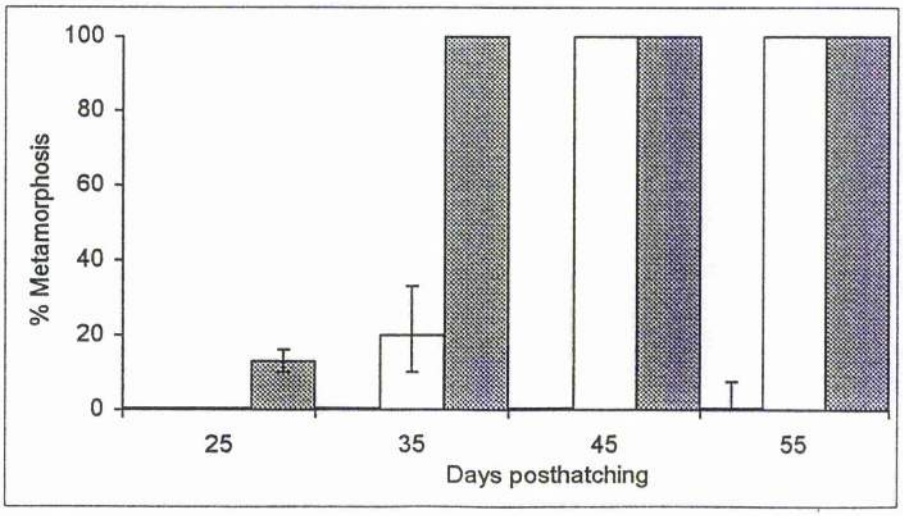
Experiments thirteen and fourteen: Effect of starvation period upon metamorphosis

The metamorphic responses of variously starved groups of larvae from two separate sibling larval cultures in 15mM excess potassium and *Laminaria digitata* treatments are presented in Figure 4.9. One-way analysis of variance showed significant variation among starved groups in the 15 mM excess potassium treatment only. Here, metamorphic responses increased with increasing starvation period. The same result also was obtained when the experiment was repeated again with older larvae (Experiment fourteen, Figure 4.10.). Additionally, one-way analysis of variance showed an increased metamorphic response with increasing starvation period in the negative control (see Figure 4.10.). To investigate the interactive effects of larval age and starvation period upon metamorphosis in the various cue treatments and in the negative control, two-way analyses of variance (factor levels were larval age and starvation period) were used on data from the two experiments which are shown in Tables 4.6, 4.7. and 4.8. Significant variations among variously aged groups were shown in the 15mM excess potassium, the *Laminaria digitata* treatment and in the negative control (see Tables 4.9., 4.10. and 4.11.). Significant variation among starved groups was shown in the 15mM excess potassium treatment and in the negative control but not in the *Laminaria digitata* treatment. Consequently, significant interaction effects were shown only in the 15mM excess potassium treatment and in the negative control.

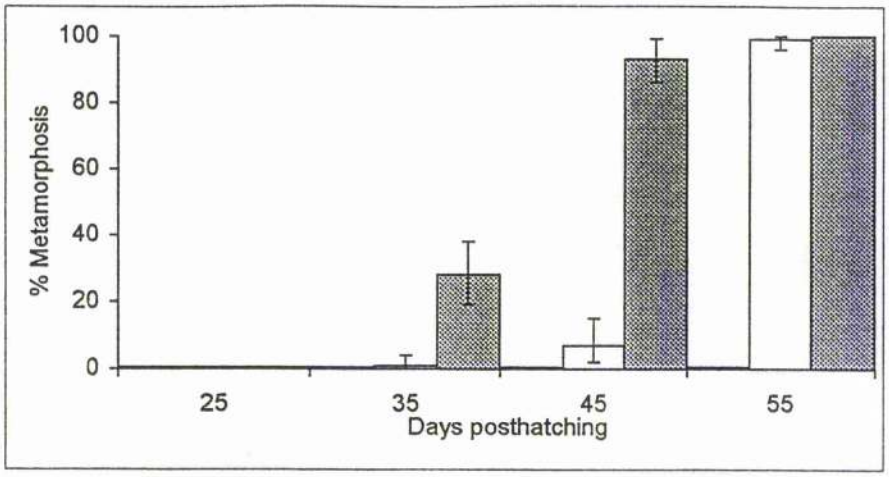
Figures 4. 8a. Experiment twelve: Back-transformed arc-sine mean percentage responses of differently aged larvae (25, 35, 45 and 55 days posthatching) from two separate sibling groups in the negative control (0.22 μm TFSW, black) and in 15 mM excess potassium (white) and *Laminaria digitata* (grey) treatments after 24 h (top), 48 h (middle) and 72 h (bottom) continuous exposure. Results of two-way analysis of variance (factors are cue source and larval culture) are shown in Table 4.4.

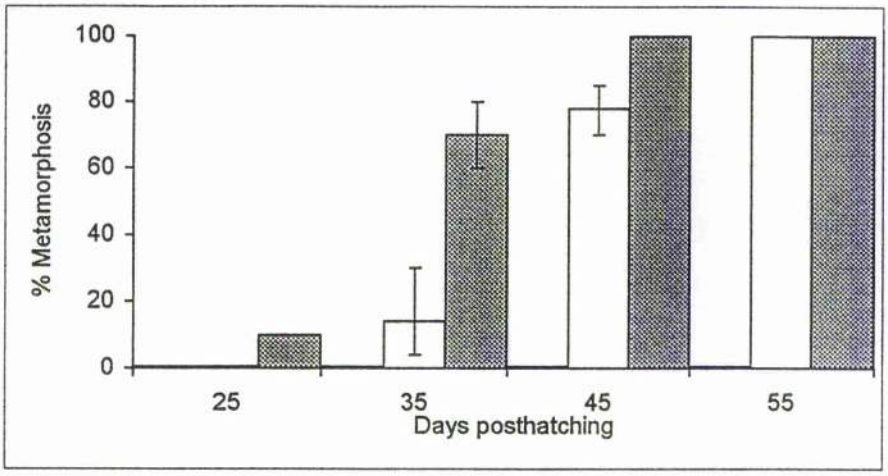

100
80
60
40
20
0
% Metamorphosis
25
35
45
55
Days posthatching

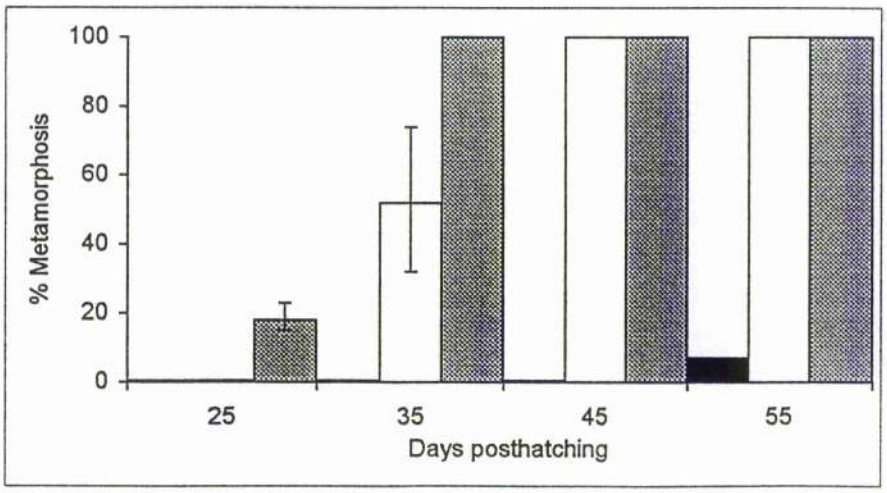

100
80
60
40
20
0
% Metamorphosis
25
35
45
55
Days posthatching


100
80
60
40
20
0
% Metamorphosis
25
35
45
55
Days posthatching

Figures 4. 8b. Experiment twelve: Back-transformed arc-sine mean percentage responses of differently aged larvae (25, 35, 45 and 55 days posthatching) from two separate sibling groups in negative control (0.22 μm TFSW, black), 15 mM excess potassium (white) and *Laminaria digitata* (grey) treatments after 24 h (top), 48 h (middle) and 72 h (bottom) continuous exposure. Results of two-way analysis of variance (factors are cue source and larval culture) are shown in Table 4.5.


% Metamorphosis
100
80
60
40
20
0
25
35
45
55
Days posthatching


% Metamorphosis
100
80
60
40
20
0
25
35
45
55
Days posthatching


% Metamorphosis
100
80
60
40
20
0
25
35
45
55
Days posthatching

Tables 4. 4. (top) **and 4. 5.** (bottom) **Experiment twelve:** Two-way analysis of variance (factors are cue source and larval age) on data for metamorphic responses of variously aged *Lacuna vincta* larvae from two separate sibling cultures to *Laminaria digitata* and 15 mM excess potassium.

Source	DF	SS	MS	F	P
Larval age	3	21358.1	7119.4	139.6	<0.001
Cue Treatment	1	2223.1	2223.4	43.6	<0.001
Interaction	3	838.1	279.4	5.48	<0.01
Error	16	816	51		
Total	23	25235.6			

Source	DF	SS	MS	F	P
Larval age	3	20731.5	6910.5	82.64	<0.001
Cue Treatment	1	4537.5	4537.5	54.26	<0.001
Interaction	3	2749.5	916.5	10.96	<0.001
Error	16	1338	83.6		
Total	23	29356.5			

Figure 4. 9. Experiment thirteen: Back-transformed arc-sine mean percentage metamorphic responses in differently starved (0 or 5 days pre-exposure to cue) larval groups from two sibling larval cultures (a-first column, b-second column) in the negative control (0.22 μm TFSW, black) and in15 mM excess potassium (white) and *Laminaria digitata* (grey) treatments after 24 h (top), 48 h (middle) and 72 h (bottom) continuous exposure. One-way analysis of variance showed significant variation among starved groups in 15mM excess potassium treatments (in a $F_{1,6}$ = 31.69, $P < 0.05$, in b $F_{1,6} = 8.56$, $P < 0.05$) but no significant variation in *Laminaria digitata* treatments (in a - $F_{1,6} = 1.00$, $P > 0.05$, in b - $F_{1,6} = 0.15$, $P > 0.05$).

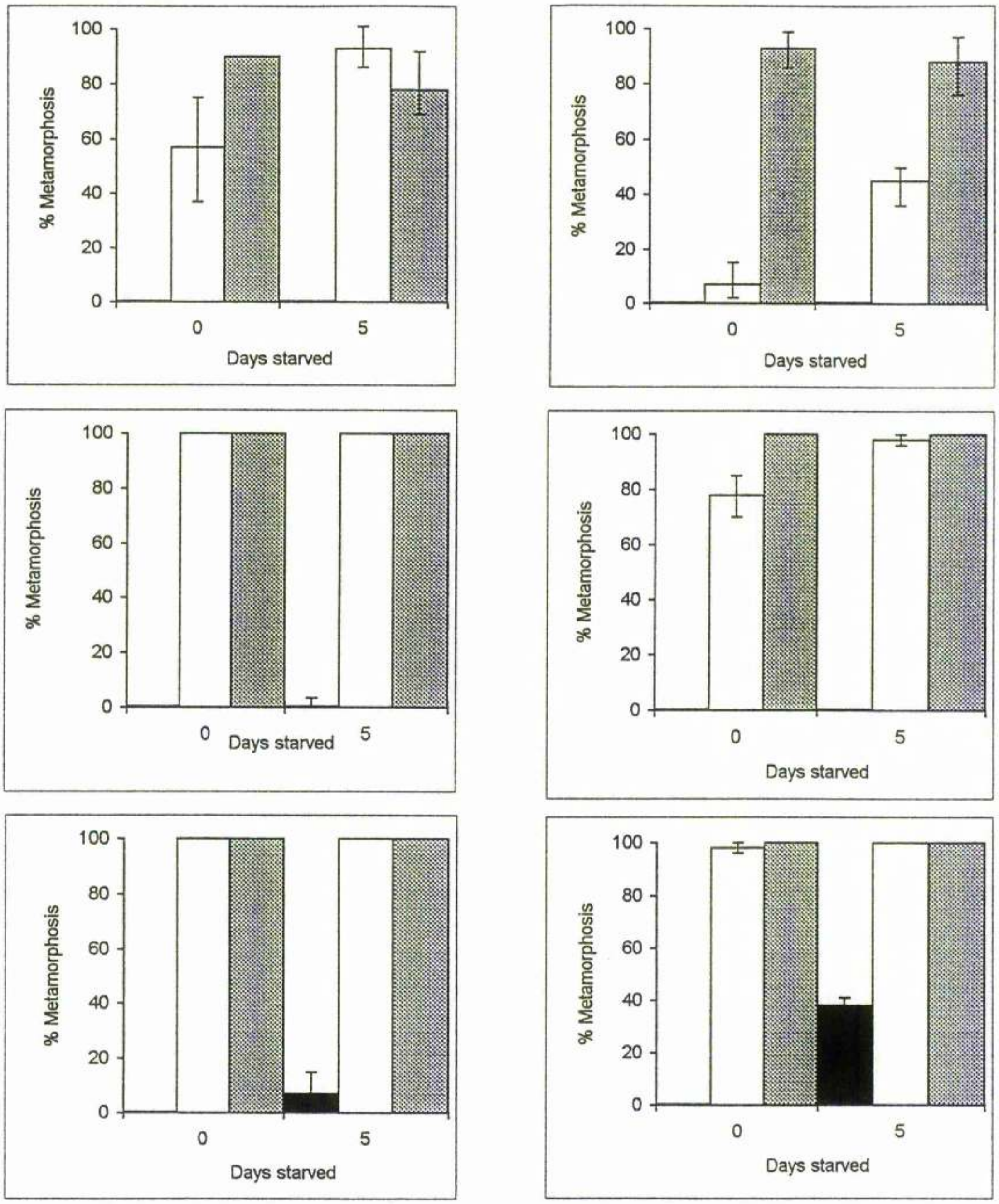
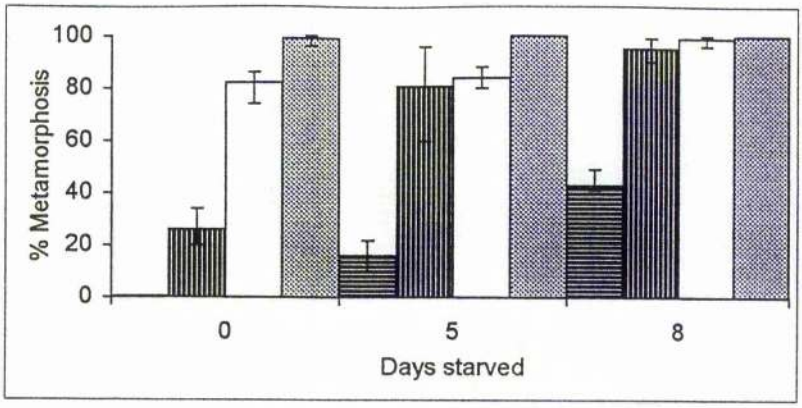
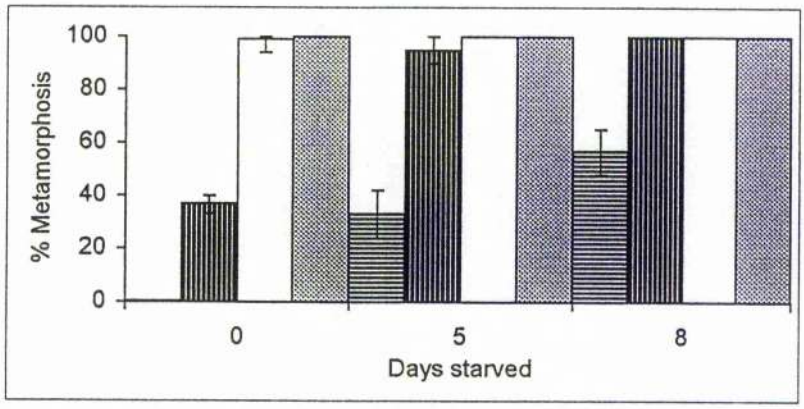
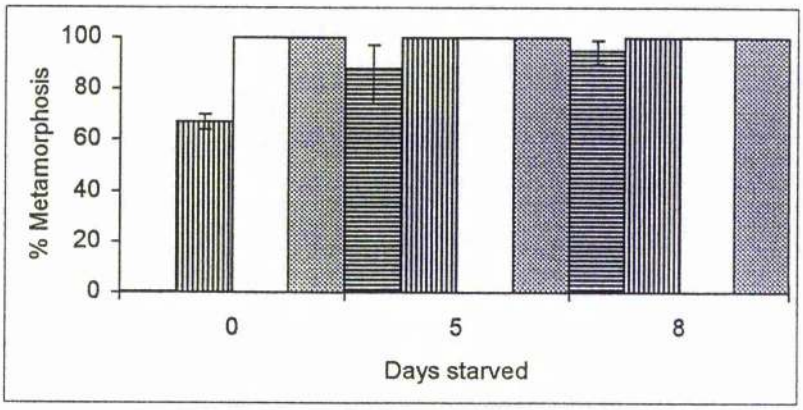
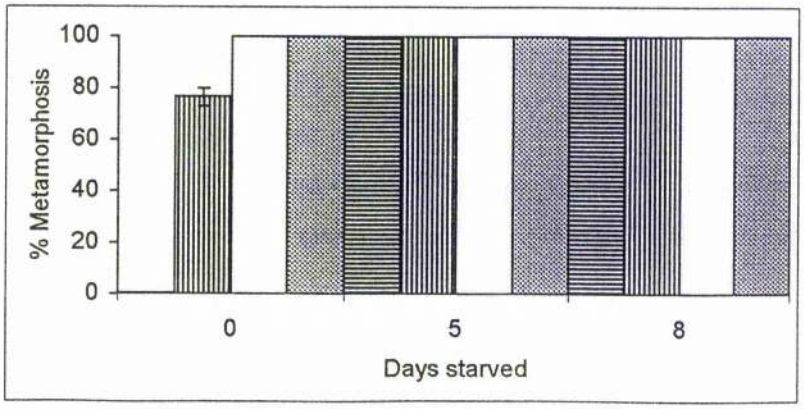

100
80
60
40
20
0
% Metamorphosis
0
5
Days starved

Figure 4. 10. Experiment fourteen: Back-transformed arc-sine mean percentage metamorphic responses in differently starved (0, 5 or 8 days pre-exposure to cue) sibling larval groups in the negative control (0.22 μm TFSW, black horizontal stripe), and in 5 mM excess potassium (black vertical stripe), 15 mM excess potassium (white) and *Laminaria digitata* (grey) treatments after 12 h (top), 24 h (second from top), 36 h (third from top) and 48 h (bottom) continuous exposure. One-way analysis of variance showed significant variation among differently starved groups in the negative control ($F_{2,9} = 43.69$, $P < 0.05$), and in the 5mM excess potassium ($F_{2,9} = 7.5$, $P < 0.05$) and the 15 mM excess potassium ($F_{2,9} = 5.69$, $P < 0.05$) treatments, but no significant variation in the *Laminaria digitata* treatment ($F_{2,9} = 1.00$, $P > 0.05$).


% Metamorphosis
100
80
60
40
20
0
0
5
8
Days starved


% Metamorphosis
100
80
60
40
20
0
0
5
8
Days starved


% Metamorphosis
100
80
60
40
20
0
0
5
8
Days starved


% Metamorphosis
100
80
60
40
20
0
0
5
8
Days starved

Tables 4. 6. (top), **4. 7.** (middle) and **4. 8.** (bottom) **Experiments 13 and 14:** Mean metamorphic reponses of variously aged and starved groups of sibling *Lacuna vincta* larvae in 0.22 μm TFSW (top), 15mM excess potassium (middle) and *Laminaria digitata* (bottom) treatments. Data are back-transformed means and standard errors of arc-sine transformed data. Results of two-way analysis of variance are shown in Tables 4.9., 4.10. and 4.11.

Hours of exposure	0	24	48	72
45 days, not starved	0	0	0	0
45 days, starved 5 days	0	0	0	38 (35, 41)
55 days, not starved	0	0	0	0
55 days, starved 5 days	0	35 (24, 42)	100	100

Hours of exposure	0	24	48	72
45 days, not starved	0	7 (2, 15)	78 (73, 79)	98 (96, 99)
45 days, starved 5 days	0	45 (38, 50)	98 (96, 99)	100
55 days, not starved	0	99 (98, 100)	100	100
55 days, starved 5 days	0	100	100	100

Hours of exposure	0	24	48	72
45 days, not starved	0	93 (91, 95)	100	100
45 days, starved 5 days	0	88 (86, 90)	100	100
55 days, not starved	0	100	100	100
55 days, starved 5 days	0	100	100	100

Tables 4. 9. (top), **4. 10.** (middle) and **4. 11.** (bottom) **Experiments 13 and 14:** Two-way analysis of variance (factors are larval age and starvation period) on mean metamorphic responses of variously aged and starved groups of sibling larvae in 0.22 μm TFSW (top), 15mM excess potassium (middle) and *Laminaria digitata* (bottom) treatments.

Source	DF	SS	MS	F	P
Larval age	1	918.75	918.75	43.75	<0.001
Starvation level	1	918.75	918.75	43.75	<0.001
Interaction	1	918.75	918.75	43.75	<0.001
Error	8	168	21		
Total	11	2924.25			

Source	DF	SS	MS	F	P
Larval age	1	3780.8	3780.8	60.73	<0.01
Starvation level	1	4680.7	4680.7	75.19	<0.01
Interaction	1	546.7	546.7	8.78	<0.05
Error	8	498	62.3		
Total	11	9506.3			

Source	DF	SS	MS	F	P
Larval age	1	918.8	918.8	7.38	<0.05
Starvation level	1	18.7	18.7	0.15	0.708
Interaction	1	18.7	18.7	0.15	0.708
Error	8	996	124.5		
Total	11	1952.3			

4.4. DISCUSSION

4.4.1. Naturally occurring chemical cues

The capacity of various naturally occurring sources to induce larval settlement and metamorphic responses in *Lacuna vincta* was investigated assuming that responses would be induced by chemicals originating from the presented source. Four intertidal macroalgal species were chosen, *Laminaria digitata*, *Fucus serratus*, *Mastocarpus stellata* and *Fucus spiralis*. These species were chosen because they are of ecological relevance to *L. vincta*. While *Laminaria digitata*, *Fucus serratus* and *Mastocarpus stellata* are found below MLWN, and are species with which *L. vincta* may be associated, *Fucus spiralis* is found higher up on the shore and is not exploited by *L. vincta*.

Larval metamorphic responses were observed in all macroalgal treatments. However, there was significant variation in the magnitude and latency of response among macroalgal treatments. *Laminaria digitata* induced the greatest metamorphic response, followed by *Mastocarpus stellata*, *Fucus serratus* and *Fucus spiralis*. However, data for the *F. spiralis* treatment were confounded by high larval mortality, possibly due to the release of toxic compounds (e.g. phenols) from the cut margins of this alga (Hawkins and Hartnoll, 1983; Watson and Norton, 1987).

There are two possible explanations for the significantly greater responses of larvae in the *Laminaria digitata* treatment. First, it could be an adaptive response since *Laminaria digitata* has been shown to yield high growth and survival in *Lacuna vincta* during post-settlement stages (see Chapter II) and would provide an appropriate cue for larvae to settle low down on the shore where they are found (e.g. Raimondi, 1988). However, responses also were observed in the other macroalgal treatments and in treatments which consisted only of biofilms and filtered seawater, suggesting instead that the inducing chemical (s) is (are) broadly distributed in the environment. In a similar vein, Pawlik (1989) reported

that while larval *Aplysia juliana* metamorphose in response to its major food source, the red alga *Laurencia*, responses also are induced by several other macroalgal species, including some which are not associated with any stage of its life-cycle. He suggested that *A. juliana* larvae metamorphically respond to a wide range of macroalgal species and after settlement are able to migrate to their preferred food source. This also may be the case for *Lacuna vincta* since juveniles can migrate between areas by drifting in the water column (Martel and Chia, 1991b,c). Observations during field studies in this work indicate that juvenile *L. vincta* are more broadly distributed on macroalgal species in the intertidal than are the adults, although no quantitative data are available to support these observations (but see Smith, 1973; Fretter and Manly, 1977). A field experiment in which settlement of *Lacuna vincta* is quantified on various macroalgal species which have been transplanted to various heights on the shore may address the importance of *Laminaria digitata* for settlement in *Lacuna vincta*. However, problems may arise from transplanting macroalgal species to zones in which they are unable to survive or in which their physiology is modified (Hawkins and Hartnoll, 1983).

Second, the greater responses of *Lacuna vincta* larvae in the *Laminaria digitata* treatment may have been attributable to the large quantities of organic particulates released from the cut margins of this alga which in turn increased the rate of bacterial proliferation in the water. Paradoxically, metamorphic responses also were observed in the laminarin treatment which was made up with artificial seawater and therefore was deemed to be bacteria free. However, it is likely that some bacteria were introduced into the laminarin treatment when larvae were pipetted from culturing vessels to treatment petri dishes. The greater latency in response of larvae in the laminarin treatment as opposed to the responses of larvae in the positive control, *L. digitata*, may support this since bacterial concentrations in the laminarin treatment would have been initially very much lower. In addition, the negative response in the negative control (ASW) which also would have been contaminated with some bacteria lends further support to the

suggestion that it was the proliferation of bacteria in nutrient rich water which greatly induced metamorphic responses in *L. vincta*.

The inductive capacity of the cue in *Laminaria digitata* was abolished upon boiling (see Rowley, 1989; Pierce and Scheibling 1991 for examples of similar results). However, this result was confounded by high larval mortalities, possibly due to the lethal effects of breakdown products.

In conclusion, results here suggest that the settlement patterns of *Lacuna vincta* larvae in the field would be relatively non-specific since larvae metamorphically respond to chemical cues which are broadly distributed in the environment. However, other factors also will influence settlement patterns and there is much evidence to suggest that flow regime at the surface of the settling substratum is important for *L. vincta* (Fretter and Manly, 1977; Martel and Chia, 1991a). Finally, while *L. vincta* larvae will delay metamorphosis if an appropriate chemical cue is not present, they nonetheless will metamorphically respond to many naturally occurring sources, including unfiltered seawater. Perhaps it is important to consider here that some larvae can habituate, or down regulate, to low concentrations of the cue during the pre-competent phase and become more sensitive to cue concentrations during the competent phase (e.g. *Phestilla sibogae*, Hadfield, 1984). A comparison of the metamorphic responses of *L. vincta* larvae cultured in filtered and unfiltered seawater during the pre-competent phase may address this problem.

4.4.2. Comparison of metamorphic responses in cue treatments

From the range of artificial cues assayed, excess potassium concentrations were shown to be the most effective inducers of metamorphosis in *Lacuna vincta* larvae. The metamorphic response of larvae to excess potassium was dose dependent, the optimum, 15 mM excess, falling well within the reported range in other species (e.g. Todd *et al.*, 1991). *Laminaria digitata* was found to be the most effective natural cue. Observations during earlier experiments had indicated that the latency of responses for

larvae varied in these two cue treatments. A comparison of the metamorphic responses of larvae to *L. digitata* and 15 mM excess potassium therefore was conducted using variously aged and starved larvae.

Latency of response for larvae decreased with increasing age of larvae in both cue treatments. This is a common phenomenon and has been reported for larvae of several other species (see Pechenik, 1990; Pechenik *et al.*, 1995 for reviews). It is thought that as larvae age they may become either responsive to lower threshold concentrations of cues, or they may respond more quickly to cues (e.g. Degnan and Morse, 1994). Degnan and Morse (1994) have suggested that greater responsiveness to cues with increasing age may be mediated by increasing the production of transcriptional or regulatory factors important for metamorphosis. Because known quantities of cues were presented in treatments it is concluded here that *Lacuna vincta* larvae responded more quickly to cues as they aged.

Latency of response of larvae also varied among cue treatments. Larvae in the *Laminaria digitata* treatments displayed significantly shorter latencies of response than those in the 15 mM excess potassium treatment. Other studies of similar design to the present also have found that the latency of response of larvae to naturally occurring cues is shorter than those to artificial ones (e.g. Hadfield, 1984; Hirata and Hadfield, 1986; Pawlik; 1986; Bonar *et al.*, 1990; Coon *et al.*, 1990; Todd *et al.*, 1991; Pechenik *et al.*, 1995). For example, Pechenik *et al.*, (1995) found that the latency of response to potassium for larvae of the nudibranch *Phestilla sibogae* greatly exceeded the latency of response to natural cues. Further, it has also been shown in some species that whilst larvae need to be continuously exposed to artificial cues to induce metamorphosis, larvae need only be exposed to natural cues for relatively short periods of time to metamorphically respond (Coon *et al.*, 1985; Pechenik and Heyman, 1987; Todd *et al.*, 1991).

These studies have suggested that while naturally occurring cues act at surface receptors, artificial cues such as potassium act endogenously and depolarise nerve cells which effect metamorphic responses (Coon *et al.*, 1985; Pechenik *et al.*, 1995, but see Morse *et al.*, 1979; Morse, 1990; Ilan *et al.*, 1993). Alternately, Todd *et al.*, (1991) have proposed that potassium may act possibly directly at sites on target tissues by causing the release of cAMP. Barlow (1988) further suggested that some artificial cues may act internally to arrest larval swimming, and that in a stressed state metamorphosis will indirectly follow.

Clearly then, if the endogenous action of potassium is assumed, then the latency of response of larvae in artificial cue treatments will be dependent upon the rate at which the artificial chemical cue is incorporated into the larvae (see Hadfield and Pennington, 1990). There are two lines of evidence in the present work which lend some support to these suggestions. First, metamorphic responses of larvae in the *Laminaria digitata* treatment were precluded by 30 mM excess. A second line of evidence is that the latency of the metamorphic responses of larvae significantly decreased with increasing starvation level in both 5 mM and 15 mM excess potassium treatments, implicating the importance of the energetic status of the larvae for preventing the influx of potassium into the body and effecting metamorphosis.

No significant variations in the responses of variously starved larvae were observed in the *Laminaria digitata* treatment. However, the latency of response was short and may have masked any effects of starving larvae in this treatment. Metamorphic responses also were observed for starved larvae in the negative control, although the latency of response was significantly greater than the responses of larvae in the cue treatments. It is not known whether the metamorphic responses of the larvae in the negative control were spontaneous or whether the larvae were responding to very low concentrations of bacteria in the water. Pechenik (1980, 1990), among others, has emphasised the importance of energetics of larvae for delaying metamorphosis and has suggested that larvae may spontaneously metamorphose when they

4.5. SUMMARY

- *Lacuna vincta* larvae metamorphosed in response to a range of putative naturally occuring cues, although the responses of larvae to *Laminaria digitata* were significantly greater than those for other cue treatments.

- *Lacuna vincta* larvae responded to elevated external concntrations of potassium in seawater in a dose dependent manner. The optimal concentration was between 10 mM and 15 mM. 30 mM excess potassium was inhibitory.

- The latency of response of larvae to potassium and *Laminaria digitata* significantly decreased with increasing larval age.

- Starvation period was shown to affect the responses of larvae in potassium treatments and in filtered seawater controls but had no apparent affect for larvae in the *Laminaria digitata* treatment.

can no longer sustain energy requirements for delaying settlement any longer. Preliminary results here lend some support to this hypothesis.

CHAPTER 5

GENERAL DISCUSSION

5.1. General

The overall aim of the present work was to (1) compare the importance of macroalgal diet for the expression of some traits in *Lacuna pallidula*, a lecithotroph and in *Lacuna vincta*, a planktotroph, and (2) to compare the manner in which maternal diet mediated any variation. Previous studies have compared the life-history traits of these two species, notably for examining relationships between reproductive effort and larval strategy (e.g. Grahame, 1977, 1982, 1994), but have not considered the variations within these traits which can, for example, be bought about by variable food supply and maternal body size (e.g. Todd and Havenhand, 1983; George *et al.*, 1991). The effects of macroalgal diet upon the expression of these traits in these two species is perhaps important for obtaining realistic estimates from the field because individuals, from both within and among populations, can be found on several macroalgal species (Smith, 1973; Grahame, 1986). An additional aim of the present work was to examine the EJP of the two species and to relate the results to their larval ecology. Settlement cues for *L. vincta* were also investigated since these were considered to play important roles in both the EJP and dietary status for individuals of this species. The results in the present work indicated that qualitative variations in maternal diet greatly affected survival, adult pre-spawning growth, reproductive output and offspring size for both species. However, some differences were observed for the two species in their responses to the various macroalgal diets and also in the manner in which they responded to favourable and unfavourable diets.

5.2. Variations in response of the two species to the different macroalgal diets

It is perhaps interesting to note that no single macroalgal species was shown to be the most favourable diet for either the two species or for populations of *Lacuna pallidula*. For example, results in Chapters 2

showed that whilst the most favourable diet for a population of *L. pallidula* individuals was *Fucus serratus*, the most favourable diet for *Lacuna vincta* individuals from the same site was *Laminaria digitata*. Further, results in Chapter 3 indicated that the suitability of different diets for maximising fitness in *L. pallidula* varied among populations.

In Chapter 2 *Lacuna pallidula* females in the *Mastocarpus stellata* diet treatment did not display any positive growth or produce any spawn. In a field study, Southgate (1982) found that while juvenile *L. pallidula* were abundant on *M. stellata*, adults would 'migrate' to *Fucus serratus* to spawn. He suggested that the large lamellar foot of *L. pallidula* was unable to obtain a secure attachment to the narrow fronds of *M. stellata* and that they would be swept off. However in this study *L. pallidula* females were observed crawling along the fronds of this algae. It is therefore suggested here that *L. pallidula* is unable to complete its life-cycle on *M. stellata* and as such may avoid this species of algae in the later stages of its life. *Lacuna parva*, which is smaller than *Lacuna pallidula,* is associated specifically with red macroalgal turfs (Ocklemann and Nielson, 1981). This species produces similar spawn masses to *L. pallidula*, except that the spawn masses contain far fewer eggs (2-16 eggs per spawn mass). It is suggested here that these two species may be an example of niche partitioning.

In Chapter 2, the weight-specific mean rates of pre-spawning growth and reproductive output of *Lacuna pallidula* females in the *Fucus serratus* and *Fucus vesiculosus* diet treatments were significantly greater than those in the *Laminaria digitata* and *Mastocarpus stellata* diet treatments. At the site of collection, *L. pallidula* were collected mostly from *F. serratus*. *F. vesiculosus* was found above the usual tidal range of *L. pallidula*. Smith (1973) reported that *L. pallidula* could be found on *F. vesiculosus* when it was growing below its usual tidal range. However, *L. pallidula* was also found on *L. digitata*. These individuals may have strayed from their preferred dietary source but this observation supports the expectation of considerable variability in RO in the field.

Unlike *Lacuna pallidula*, *Lacuna vincta* individuals were able to survive, grow and reproduce on all four macroalgal diet treatments, perhaps reflecting its broader ecological distribution and dietary preference in the field (see also Smith, 1973; Southgate, 1982; Grahame, 1986). It was interesting to note, however, that the spawn masses produced by females in the *Mastocarpus stellata* treatment were all green as opposed to the usual yellow colour. Green spawn masses were observed at the site of collection and have been reported in other studies, although it has not been attributed to diet before (Lebour, 1937; Waddell, 1973). Waddell (1973) reported that the spawn masses produced by a *L. vincta* population in red algal turfs were green. Lebour (1937) reported green spawn masses that were morphologically similar to *L. vincta* spawn masses and attributed these to another species. The green hue is likely to be due to the breakdown products of the pigment in red algae and demonstrates that converted dietary constituents of this algae are being channelled into reproduction.

5.3. Dietary preferences

The results showed that there is much potential variation for both the growth and reproduction in both species, when faced with a variable resource supply, and that performance correlates with observed distribution patterns on different macroalgal types. The preferences of herbivores for various macroalgal species have been extensively studied in the littorinids (Watson and Norton, 1985, 1987; Norton *et al.*, 1990) because they are deemed to be important for influencing the structure of macroalgal communities (Hawkins and Hartnoll, 1983) and for further understanding plant-herbivore interactions (see Williams and Seed, 1992 for review).

Investigations have found that the 'edibility' (i.e. the speed at which dietary material is ingested and will satisfy the physiological need of the grazer), the 'attractiveness' (for providing shelter and a substratum for spawning) and the release of anti-grazing compounds are important influential factors for dietary and substratum preferences (Nicotri, 1980; Hay *et. al.*, 1988; Manley, 1989; Imrie *et al.*, 1989).

Hay and Fenical (1988) suggested that small herbivore species, like *Lacuna,* would select macroalgal types primarily on the basis of their value as 'safe living' sites and secondarily on the basis of the value of their food because of predation (see also Stoner, 1980). However, in this study the two species displayed greater survival, better growth rates, greater RO and produced relatively larger offspring when feeding on their preferred macrolagal species. Food was given *ad libitum* and therefore it was presumed that it was mostly the qualitative differences in the macroalgal species which mediated this result. Studies on other species have also shown the enhanced performance of individuals when feeding on their preferred diet (e.g. Hall and Todd, 1986; Shepherd and Steinberg, 1992). Quite clearly the two species have adapted to grazing on different species of macroalgae. Such adaptations may take the form of small differences in the structure and functioning of the feeding apparatus which have been shown to impose dietary restrictions and promote the partitioning of food resources in other littorinids (Steneck and Watling, 1982).

5.4. The importance of nutrient requirements and digestibility of diet

Differences in the responses of the two species to the various macroalgal diets presented may be attributable to differences in their nutrient requirements for essential amino acids and lipids and/or to their digestive or metabolic physiology (Mai *et al.*, 1995). The requirements for these two species may be determined by evaluating the proteins and lipids in various body tissues and in eggs for (Mai *et al.*, 1994). Aswell as being important for energy acquisition these nutrients also are important for other processes in the body. For example, free amino acids (FAA) present in tissues are important for energy metabolism processes in molluscs (Nie *et al.*, 1986; Tjeerdema *et al.*, 1991). Further, gastropods have high concentrations of FAA in tissues such as taurine alanine, glycine and arginine which have been shown to be important for osmoregulation (Campbell and Bishop, 1970; Bishop *et al.*, 1983). Essential fatty acids are important for maintaining cell functions, as components of biomembrane phospholipids and in animal reproduction as pre-cursers of prostaglandins and other eicosanoids (Sargent *et al.*, 1989).

Lipids, particularly those rich in PUFAs (polyunsaturated fatty acids) have been shown to accumulate to very high levels in the gonads of molluscs, as they are maturing (Robinson, 1992), and consequently are considered to be important for reproductive success and offspring survival (Soudant *et al.*, 1996).

Different macroalgal species will contain variable quantities of these nutrients and therefore may contain limiting quantities of essential amino acid or fatty acids. For example Mercer *et al.* (1993) have shown that the nutrient requirements for two abalone species, *Haliotis discus hannai* (Ino) and *Haliotis tuberculata*, can be met only by providing a mixed macroalgal diet consisting of species which contain high levels of either arginine or methionine which are essential for growth.

Alternatively, it is well documented that certain amino acids when offered in excessive amounts or in disproportion in the diet of the animal, can result in adverse reactions such as depressed appetite, retarded growth, pathological lesions and death (Harper *et al.*, 1970). This can be bought about by reducing digestibility of the food or by physiological inhibition of biosynthesis processes. Physiological inhibition can also be bought about by the release of toxic substances or secondary metabolites such as phenols from damaged plants (Hay *et al.*, 1988; Borowsky and Borowsky, 1990). Perhaps the two species have different tolerance levels towards toxic substances released from different species of macroalgae. Survival of animals in Chapter II was greatly influenced by diet for both species.

However a satisfactory level and balance of nutrients in the diet cannot guarantee that ingestion of the diet will satisfy the nutrient requirements of an animal (Wilson, 1989). Alternatively differences in responses may be attributable to differences in the digestibility of the macroalgae in the two species. The protective cellular structures and/or tannins and phenols in some plants result in poor digestibility (Mai *et al.*, 1994). An experiment in which *Lacuna* were presented with a mixed macroalgal diet or with various parts of plants would be useful for determining whether *Lacuna* graze on a variety of macroalgae

species or whether they have adapted to grazing on various parts of a particular macroalgal species (see Johnson and Mann, 1986).

5.5. Dietary breadth and the effects of macroalgal diet on the responses of the two species

Maternal effects contributed to the phenotypic expression of life-history traits in the present study. If maternally-induced modifications of phenotype influence the fitness of off-spring then maternal effects have the potential to alter population growth by changing parameter values for life-history traits which are critical to growth and mortality (Rossiter, 1991a, b).

However, while diet effected the onset of spawning and the size of spawn masses produced by *Lacuna pallidula,* this was not apparent in *Lacuna vincta.* The results indicate that *Lacuna vincta* has a broader dietary breadth than *Lacuna pallidula.* While *L. vincta* was able to survive, grow and reproduce on all four macroalgal diet treatments, *L. pallidula* was not. This is also supported by observations indicating that *L. vincta* larvae metamorphically respond to a range of macroalgal treatments. Further, differences also were observed among populations of *L. pallidula* which could be related to the availability of the various macroalgal species at the different locations.

5.6. The importance of the dispersal capacity and ecology of the offspring

The dispersal potential of larvae has frequently been inferred from their different larval type. Pelagic larvae are usually assumed to have relatively widespread dispersal whereas non-pelagic larvae are presumed to have a more restricted dispersal (Jablonski, 1986; Scheltema, 1989; Vermeij *et al.*, 1990, but see Cohen, 1990). The dispersive potential of larvae away from parental populations is assumed to play an important and integral role in gene flow between geographically separated populations. Not withstanding that there are other mechanisms for increasing gene flow between populations, (e.g. Martel

and Chia, 1991b, c; Ryland and Bishop, 1993), pelagic larvae are assumed to confer a degree of panmixis, which leads to the prevalence of generalist traits, decreased interpopulation variation and lower speciation and extinction rates. Conversely, non-pelagic larvae are deemed to confer reduced gene flow, resulting in decreased intrapopulation variation, increased interpopulation variation and increased rates of speciation and extinction rates (Jablonski and Lutz, 1983; Jablonski, 1986, Scheltema, 1989; but see also Hedgecock, 1986; Johannesson, 1988). Morphological, physiological and electrophoretic data have been collected for species which display different larval types to test the hypothesis that larvae with pelagic larvae have more generalist traits than species with non-pelagic larvae (e.g. Yamada, 1989).

Clark and Goetzfried (1978) suggested that the loss of the pelagic larval stage in marine species may be highly advantageous if this should increase survival of offspring and ensure that offspring would hatch into the adult habitat where resources are presumably plentiful. However the loss of a pelagic larval stage also may result in a reduction in dispersive capacity which in turn will effect the long-term durability of a species by rendering it more susceptible to local extinction (Jablonski and Lutz, 1983; Endler, 1986). Results here suggest that *Lacuna pallidula* populations have adapted to utilising the food types available at the different sites. As suggested by Hansen (1978, 1980), while the reproductive strategy of *L. pallidula* implies the selection of utilisation of predictable local resources and hence greater sensitivity to changes in maternal diet, the reproductive strategy of *L. vincta* implies the utilisation of resources on a broader scale and hence less sensitivity to changes in maternal diet.

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