Early life history traits of *Placopecten magellanicus* (Gmelin): behaviours, lipid condition and vertical distribution of veligers at micro- and meso-scales

by

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a mi recordada Nanita

a mis padres, Guilfido y Luisa

a mi familia, Yanko, Sebastian y Carolina

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Abstract

The distribution of pelagic marine invertebrate larvae has been considered mainly to be driven by physical characteristics of the ecosystem, but there is evidence that behaviour influences vertical distribution. I examined the role of active swimming behaviours on the vertical distribution of sea scallop *Placopecten magellanicus* larvae by combining a series of experimental observations in microcosms and mesocosms.

Scallop larvae showed consistent behaviours that may be responsible for their retention in areas of patchy distribution of phytoplankton, and could result in aggregations in areas of high productivity. Larval swimming throughout ontogeny consisted basically of two modes: vertically oriented helical patterns and vertical straight lines, either ascending or descending. In the helical mode, larval vertical displacement rates during ascent increased with larval age from 0.09 to 1.05 *mm* s^{-1} (VVU), however, during descent displacement rates remained relatively constant with age between 0.20 and 0.37 *mm* s^{-1} (VVD) which may result from increased drag due to velum growth. When swimming in straight lines, larvae ascend and descend (sinking) only by rotating around the vertical axis, and display much faster swimming speeds than during helical swimming. The range of swimming speeds increased with larval age and varied during ascent between 0.72 and 1.27 *mm* s^{-1} (LVU) and during descent between 0.94 to 1.73 *mm* s^{-1} (LVD).

I concluded that helical motion favours feeding while restricting vertical distribution of larvae. Therefore, this behaviour can enhance retention of larvae in particular areas. Fast sinking and rising can affect vertical positioning in response to immediate changes in the environment. Young larvae swim slowly, are more buoyant, and have higher lipid levels than older larvae. As larvae grow and density increases due to shell deposition, swimming becomes more energetically costly. At intermediate stages lipids are lowest, increasing again before metamorphosis. I showed that the proportion of triacylglycerides (TG) to total lipids was greatly reduced from eggs (13%) to the formation of Prodissoconch I (29%) and during the Prodissoconch II at 13 days (8%), increasing again in late Prodissoconch II at 22 days (20%).

At a larger scale, I monitored vertical distributions of early veligers in a series of mesocosm simulations that included diel light cycle, food availability and salinity stratification. Diel vertical migrations occurred in well mixed food conditions in a 10 *m* water column, corresponding to the classical nocturnal ascent of most microzooplankton. Larval mean depth was found to vary between a minimum of 2.8 *m* at night and a maximum of 6.3 *m* during the day. However, migration of larvae appear to be suppressed in the absence of food and with stratified food in salinity gradients. The observed diel migrations in the mesocosm simulations were comparable to those found in mixed and stratified areas of the Bay of Fundy and Baie de Chaleurs.

Behavioural traits and larval condition influenced the vertical distribution of larvae on the scale of my observations, and responses of larvae to food availability and diel light cycles resembled those in nature. My studies are a first step to establish a behavioural baseline for the early life history of this unique and valuable pectinid. My results provide new techniques and insights to address questions of larval ecology of marine invertebrates at intermediate scales comparable to the natural environment.

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CHAPTER 1

General Introduction

1.1 Background

Natural populations of the benthic sea scallop *Placopecten magellanicus* are found only in coastal and shelf areas of the Northwest Atlantic ocean. Their geographic distribution ranges from the Northern tip of Newfoundland (Squires, 1962) to the southern extreme of Cape Hatteras (Posgay, 1957). The sea scallop fishery has been one of the most valuable fisheries in Atlantic Canada, behind cod and lobster and representing over C$ 100 millions in revenue. The major offshore concentrations of sea scallop adults are found on Georges Bank and Brown's Bank, and in inshore areas in the Bay of Fundy, Gulf of St. Lawrence and Port au Port Bay in Newfoundland (Young-Lai and Aitken, 1986). Although Georges Bank is the largest known offshore aggregation and has sustained a fishery for over a century, there are also reports of scallop exploitation in inshore areas since the 1600's (Bourne, 1964).

Management of sea scallop fisheries began in 1918 (Anonymous, 1920 in Black *et al.*, 1993) and has evolved until the present to include numerous regulations such as meat counts, enterprise allocation regimes, seasonal fishing restrictions and gear regulations, particularly for offshore regions (Black *et al.*, 1993). The natural sustainability of scallop populations depends on the contribution to recruitment by different main spawning periods in the different regions; however, the role of early life history stages of sea scallops upon recruitment success is not well understood.

The temporal and spatial variability in the abundance and distribution of planktonic organisms has been studied for many species of zooplankton and for meroplanktonic larvae of benthic invertebrates (Cushing, 1951; Scheltema, 1964; Pearre, 1973; Dagg, 1977; Davis, 1984; Geller, 1986; Scheltema, 1986; Jonsson, 1989), but the controlling mechanisms that drive the observed distributions still remain poorly known

(Pearre, 1979; Strathmann, 1982; Price *et al.*, 1988). There are two main approaches that have been proposed to explain the distribution of planktonic organisms. The first approach is based on the strength of large scale of physical processes that affect marine systems and therefore may drive the distribution of planktonic organisms at a macroscale (*e.g.*: Andrews, 1983; Davis, 1984). The second recognizes that the biological interactions within systems also influence the distribution of plankters (Pearre, 1973; Geller, 1986; Forward, 1988; Forward and Hettler, 1992). These two approaches are interactive since the main difference between the two is the temporo-spatial scale at which they influence the abundance and distribution of planktonic organisms.

Thorson (1950) suggested that as a general rule, marine invertebrates need to be replaced with at least one reproductive individual to maintain a population at steady state. However, the fate of a larval cohort in the field and its progression into recruitment to adult populations is the result of complex interactions among physical and biological mechanisms, which are difficult to assess from larval sampling alone. Our inability to make direct observations on deep sea populations such as sea scallops, has hampered our understanding of the role of larval behaviours as adaptive traits to respond to environmental variability.

One of the most puzzling problems concerning sea scallops and other commercially important invertebrates and fish involves the sources of the apparently high recruitment variability these populations withstand (Sinclair *et al.*, 1985). Based only on scallop landings, it appears that water temperature (Dickie, 1955; Caddy, 1979) and long-period tidal cycles of about 20 years affect recruitment of sea scallops in the Bay of Fundy and Georges Bank (Black *et al.*, 1993).

The pelagic phase of marine bivalves with complex life histories, that is, life cycles with metamorphosis (Ebenman, 1992); are usually planktotrophic larvae that are important components of the microzooplankton living in a nutritionally diluted environment (Conover, 1968), prior to settling in benthic areas of adequate substrate. Sea

scallop larvae reared in the laboratory have a planktonic period of about 35-40 days at 14 °C, which it is thought to be similar in natural conditions (Fig.1.1) The horizontal distributions of planktonic larvae have been attributed mainly to passive interaction with the physical environment and, while the role larval behaviour plays in vertical distribution is recognized, its influence on larger scale distributions and benthic population dynamics (Cameron, 1986) has seldom been considered.

Timing of adult spawning behaviour of sea scallops appears to coincide with spring and fall phytoplankton blooms and with oceanographic conditions that may enhance survival and retention of larvae near the parental grounds (Starr *et al.*, 1990). Although the fate of planktonic larvae will affect population density, little is known about the active role, if any, planktotrophic larvae play in their dispersal, or on the structure and hence, sustainability of established populations.

Nonetheless, it is well known that early developmental stages of many benthic organisms, such as, crabs, lobsters, echinoderms, clams, mussels, oysters and scallops, are characteristically planktonic and capable of independent locomotion (Chia *et al.*, 1984). It is this feature that characterizes organisms with complex life histories, which switch niches through their lives and "use different modes of swimming at different stages of the life cycle: a ciliated larvae and a muscular adult form" (Sleigh and Blake, 1977). Adult individuals inhabit benthic grounds of various characteristics and, either release larvae (*e.g.*: decapod crustaceans) or gametes (*e.g.*: bivalves, annelids, echinoderms). Eggs are fertilized externally developing into a planktonic larval stage in a few hours. Many planktonic larval stages are planktotrophic prior to developing a competent stage that settles in the benthos to develop into a juvenile stage, and later into an adult, as in the case of sea scallops (Fig. 1.1). The question of whether competent larvae settle near or on the adult grounds has not been resolved for most species. However, there is a general consensus that since they are vulnerable to predation they

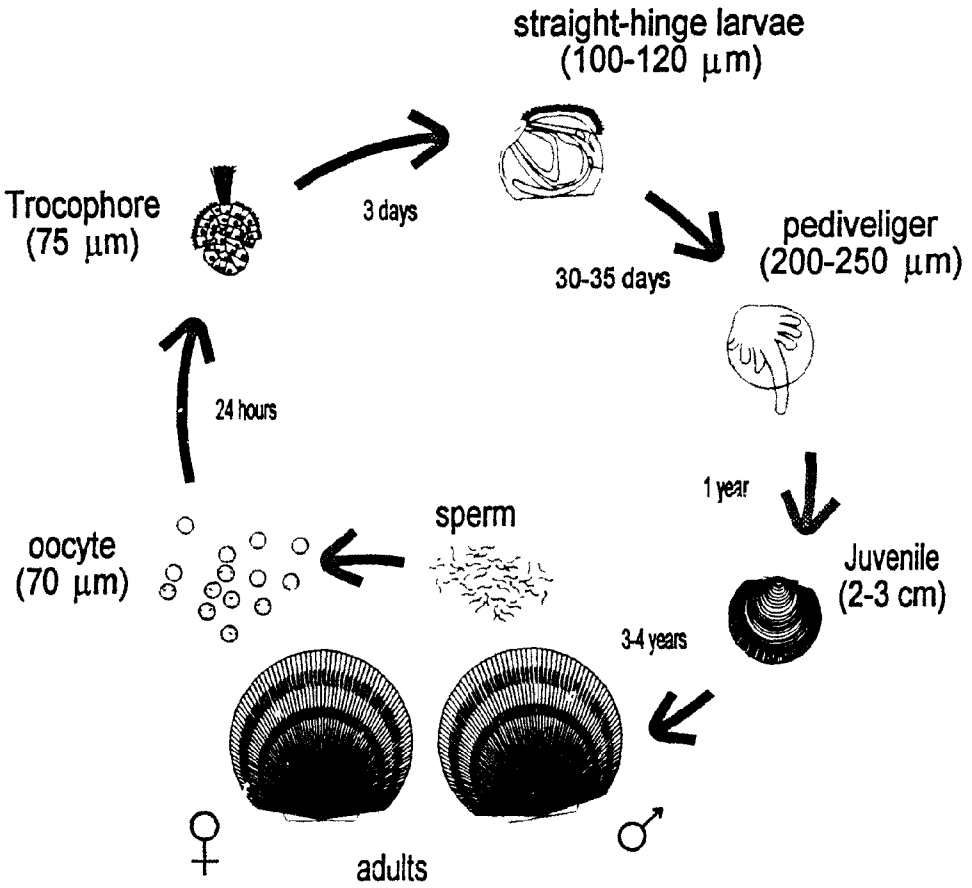

Figure 1.1 Schematic representation of the life history of sea scallop *Placopecten magellanicus*. (Trocophore and straight-hinge larvae adapted after Drew, 1906; temporal scale from Drew, 1906, Culliney, 1974 and Couturier, 1986).

likely occupy particular areas which diminish this risk of losses by predation (Young and Chia, 1987).

Until recently, population dynamic studies of sea scallops have centered on adult individuals (*i.e.*: distribution, age structure, reproductive physiology, ecology) (Posgay, 1957; Naidu, 1970; 1975; Thompson, 1977; Caddy, 1979; Chouinard, 1984: MacDonald and Thompson, 1986; DuPaul *et al.*, 1989; Dibacco *et al.*, 1995). This comprises an extremely valuable literature on the adult stages of the life history of scallops; however, the literature on similar aspects of early developmental stages is limited. The question of evaluating the influence of early life history strategies on the sustainability of populations is only beginning to be asked at the time when scallop species are dwindling and the maintenance of a healthy fishery is at stake. It has become clear that knowledge of the complete life history of many invertebrate species is necessary if sustainable management of marine resources is to be accomplished.

Larval stages of marine invertebrates are an important component of the microzooplankton during the spring and/or fall, following population specific spawning periods. However, there is a general lack of monitoring programs to assess distribution and abundance of planktonic larvae in relation to other zooplanktonic components and food availability at appropriate scales. A unique series of studies on larval distribution of sea scallop larvae was undertaken by Tremblay and Sinclair (1988, 1990a,b, 1992), that provides a wealth of information on the abundance and distribution (vertical and horizontal) of larvae. However, the coupling of larval behavioural capabilities to environmental variability needs to be examined at smaller scales to understand the larger scale observations.

Questions about the abundance and distribution of sea scallop larvae and other ephemeral plankters that eventually leave the plankton to return to parental populations (Galtsoff, 1964) include the nature of controlling mechanisms that affect them. Abundance and distribution of planktonic larvae may be the result of biological

interactions and physical forcing, as has been suggested for micro-zooplankters that complete their entire life cycle in the plankton. For benthic invertebrates, a complex life history strategy appears to optimize reproductive investment by maximizing gamete production that may increase the chance of egg fertilization and development of planktonic larvae (Bayne, 1983; Cameron, 1986). Arguably, an important part of this strategy is to return recruits to the population to secure sustainability of its genetic pool (Sastry, 1979; Burton and Feldman, 1982).

Sinclair (1988) reviewed the concept of population regulation and found that essentially two approaches, theoretical and descriptive have been used. In theory, there should be a minimum relationship between population size and recruitment generated. However, descriptive studies (Hjort, 1914) proposed two generalizations: a) that a year class size is determined early in the life history, prior to recruitment to the fishery and b) that year class is not a simple function of egg production. The recruitment hypotheses to be tested were defined as the effect of food availability and the plausible inter-annual variability of local oceanographic circulation as an influence on the geographic distribution of eggs and larvae. Cushing (1975) proposed the match/mismatch theory that added an important advance to Hjort's (1914) first hypothesis of early life-history events. Cushing (1975) included the importance of processes of seasonal stratification and de-stratification in relation to food availability and spawning grounds. Although a detailed account of the theories related to population regulation is beyond the scope of this thesis, some of these aspects need to be assessed to evaluate in experimental conditions the significance of these processes on benthic invertebrates with complex life histories such as sea scallops.

1.2 Behavioural traits of planktonic larvae

Bivalve veligers are usually small, measure less than 300 μm, are negatively buoyant and swim weakly (Mann, 1986a), and during their ephemeral life in the plankton

they must swim and feed aided by the ciliary activity provided by the velum. Veliger locomotion has been characterized for a number of species, and a common feature is the upward movement in vertically oriented helical patterns or straight lines (Bayne, 1963, 1964; Cragg and Gruffydd, 1975; Cragg, 1980; Mann and Wolf, 1983), commonly alternating with periods of either active or passive sinking (Carriker, 1961, Cragg and Gruffydd, 1975, Isham and Tierney, 1953; Lough and Gonor 1971, Mann and Wolf, 1983) This swimming behaviour has been generally observed for specific larval stages but rarely throughout the entire larval ontogeny of different species

Moreover, only upward helical patterns have usually been characterized since it has been assumed that the function of this behaviour combined feeding with a mechanism for retaining larvae in the upper part of the water column My focus on sea scallop larvae from Georges Bank as a case study was mainly based on the possibility that behavioural traits varying through development could be linked to known oceanographic features and was intended to couple vertical and horizontal distributions in nature to recruitment patterns of this extremely valuable fishery

1.3 Condition of planktonic larvae

The sources of energy of a developing bivalve larvae can be divided in two phases The first is an early lecithotrophic stage in which the developing embryo reaches the Prodissoconch I. This first stage is mainly fueled by endogenous reserves The second is a planktotrophic stage that mainly depends on the acquisition of nutrients from the environment (Bayne, 1983) The initial larval stages of sea scallops are less dense (1 02 g cm^{-3}) than the successive stages (1.26 g cm^{-3} for 11 day old larvae) (Jackson, 1992) mainly due to increasing shell deposition. Consequently, energy reserves play a major role in the capability of older larvae to remain swimming

To stay suspended in the water column, planktonic larvae must swim or evolve buoyancy organs (Alexander, 1990) to overcome the effect of gravity Swimming however, is an energetically expensive endeavour that must be fueled consistently

throughout larval ontogeny. Depending on the species, lipids or proteins or both can account for the energetic requirements of larval activity.

My focus on the lipid class composition of scallop larvae was mainly based on the particular role that triacylglycerides may have on providing a source of both energy and buoyancy. The partitioning of lipids into class composition allows the comparison of lipid ratios through larval ontogeny that may provide insights into the larval condition and also into the identification of the role of lipids in providing energy and buoyancy. By relating the observed changes in behaviour and the condition of larvae we might gain insights into understanding of larval adaptations to remain planktonic.

1.4 Vertical distribution of planktonic larvae

Vertical migration of planktonic organisms has been known to occur for more than 150 years, but the precise advantages that it may confer are not well understood (Longhurst, 1976; Roff, 1991). Diel vertical migration of zooplankton has been described as common (Russell, 1927; Cushing, 1951; Pearre, 1973; Lee and Williamson, 1975, Southward and Barrett, 1983; Fogg *et al.*, 1985) and appears to result from either behavioural responses to exogenous factors (light, gravity, temperature, salinity, hydrostatic pressure and food) or to endogenous changes in behaviour and physiology (age, size, condition, biological rhythms), or both (Forward, 1988).

During the earliest phase of their life history, the horizontal distribution of marine invertebrate larvae closely resembles the distribution of the benthic parental class, therefore include areas of suitable settling substrate (Young and Chia, 1987). Distribution and dispersal of larvae depends primarily on the length of larval life, swimming behaviour of larvae, predation, and hydrographic regimes. Furthermore, the controlling mechanisms of distribution are most probably those related to the swimming and sinking responses to environmental stimuli which can restrict larval activity and consequently change the basic patterns of vertical distribution. Horizontal distribution is then governed by major current circulations and flow regimes at various depths and times.

In my study early veliger larvae of sea scallops were selected to gain insights by microscale observations into the responses larvae may be able to display under a series of environmental conditions that can be related to lipid condition

1.5 The specific questions

The main aims of my experimental study were to evaluate some of the physiological and ecological aspects inherent in the development of scallop larvae through ontogeny and the vertical distribution of early veligers in relation to the availability of food and physical stratification. The specific questions asked were

1) Do larval locomotory activity, feeding and behavioural traits vary through ontogeny?
2) Does lipid class composition of larvae vary through ontogeny?
3) Does vertical distribution of veligers vary in response to experimental simulations of mixed and stratified water columns in a 10 *m* mesocosm?

The results of this study are presented in the following three chapters

Chap 2 A micro-scale approach to characterize swimming patterns and behavioural traits through larval ontogeny

Chap 3 Temporal variability of lipid class composition through larval ontogeny

Chap 4 A mesocosm approach to study the vertical distribution of sea scallop larvae in response to environmental variability

The significance of each individual aspect and of all combined has served to demonstrate that sea scallop larvae present an array of adaptations that, in turn, could result in specific behaviours in response to stimuli in particular regimes which may affect their distribution in nature Furthermore, the development of my research has provided new insights into the biological interactions occurring at the scale of individual planktonic larvae, the methods available to study the phenomena and the relations to

environmental variability that should be considered when planning studies on the early stages of other benthic invertebrates.

1.6 Thesis outline

My work is concentrated on the early life history of *P. magellanicus*, and I began by investigating the basic behavioural patterns of larvae through early ontogeny under laboratory conditions (Chapter 2). This is one aspect that had never been studied for this species and could provide useful insights into the responses of larvae to environmental variability, and into their capabilities and limitations. The characterization of swimming behaviours of individual larvae through development established a basis for investigating the interaction of biological mechanisms with large scale distribution of planktonic larvae. Planktonic ciliated larvae swim and feed aided by a ciliated velum; therefore, the response of 6 day old veligers previously fed and starved was evaluated with respect to variability of the helical swimming pattern in a medium containing food and in one without it.

Variability through ontogeny of lipid class content, from unfertilized eggs to late veligers (22 days) was examined and used as an indicator of larval condition (Chapter 3). These findings can help in gaining insights into energy acquisition and energy available for activity and growth.

These two micro-scale studies on individuals identified key behavioural and density changes through larval ontogeny which could affect larval distribution. Chapter 4 focuses on the assessment of diel changes in the vertical distribution of early veligers considering the suite of behaviours in response to an array of simulated environmental variables and provides the basis to test hypotheses about active vertical migration in a series of mesocosm simulation experiments. The observed capabilities and limitations of behavioural traits of scallop larvae were evaluated in these mesocosm simulations in various conditions of food availability and salinity stratification.

Finally, Chapter 5 includes a general discussion of identified factors that may affect the distribution of planktonic larvae in nature. It focuses on the ways in which physiological changes and behavioural flexibility, as revealed in my study, may interact with environmental variability to affect vertical distribution and enhance survival and retention of sea scallop larvae on Georges Bank.

CHAPTER 2

A microscale approach to characterize swimming patterns and behavioural traits through larval ontogeny

2.1 Introduction

The early life-history of most marine and estuarine invertebrates commences with a free planktotrophic larva that lasts from a few hours to several months in the plankton, before leaving the pelagic phase to search for a suitable substrate to settle and to complete metamorphosis. Although, diversity of morphology, size and behaviour of planktonic larvae is great among invertebrate taxa, one shared characteristic is their ability to swim independently (Chia *et al.*, 1984). Nonetheless, little is known about the extent to which free swimming larvae affect larval dispersal and later recruitment to benthic populations of distinct life-history characteristics.

Placopecten magellanicus is a dioecious benthic bivalve characterized by a complex life-history that includes a meroplanktonic larva in its life cycle (Fig. 1.1), and it is found distributed in the coast and shelf waters of the Northwest Atlantic (Posgay, 1957; Squires, 1962). Concentrations of adult sea scallops vary along the latitudinal range, but the world's largest known aggregation of scallops is found in the area of Georges Bank. Smaller aggregations are known to exist in the Bay of Fundy and Browns Bank and along the Northwest Atlantic coast. Natural populations of sea scallops on Georges Bank have sustained a valuable commercial fishery for well over a century. Recruitment, however, appears to fluctuate widely and the factors influencing this variability remain to be addressed (Sinclair *et al.*, 1985) in order to improve our current understanding of the sea scallop's life-history and to develop sustainable management policies for this important marine resource.

An interesting question with profound implications for the larval ecology of benthic invertebrates and for the management of a marine invertebrate fishery is whether

planktonic larvae can control their distribution in the water column in response to environmental variability, and hence ultimately affect their recruitment to particular areas. The problem is complex and a better understanding of the physical forces encountered by meroplanktonic larvae and the mechanisms of adaptation of larvae to successfully survive this period is needed (Boicurt, 1982; Burton and Feldman, 1982).

A review of the literature on the distribution and dispersal of larval stages of bivalves, other molluscs and crustaceans reveals considerable controversy on the roles of passive and active processes on larval dispersal (Andrews, 1979,1983; Sheltema, 1986; Mann, 1986b; Stancyk and Feller, 1986; Mann *et al.*, 1991). In some cases, larval dispersal has been suggested to result from passive mechanisms, which consider planktonic stages are mere drifters at the mercy of major circulation patterns and currents (Korringa, 1941, 1952; Manning and Whaley, 1954; Quayle, 1964, 1969; Andrews, 1979, 1983; Zinsmeister and Emerson, 1979; Boicurt, 1982; Seliger *et al.*, 1982); this view requires that larval behavioural traits be overridden by physical conditions.

In other instances, larval dispersal has been hypothesized to result from the capability of some larvae to regulate their vertical distribution to a certain degree as in the case of some marine bivalves, gastropod and crustacean larvae (Nelson, 1911; Roughley, 1933; Isham and Tierney, 1953; Nelson, 1953, 1955; Turner and George, 1955; Carriker, 1961; Konstantinova, 1966; Lough and Gonor, 1971; Wood and Hargis, 1971; Mileikovsky, 1973; Cragg and Gruffydd, 1975; Gruffydd, 1976; Hidu and Haskin,1978; Cragg, 1980; Mann and Wolf, 1983; Sulkin, 1984).

A compromise explanation is that larval dispersal is the result of both passive and active mechanisms, which allows selective coupling of larval swimming activity and circulation patterns to drive larval distribution (Carriker, 1951,1961; Nelson 1953,1955; Kunkle,1957; Wood and Hargis, 1971; Mann, 1985; Mann *et al.*, 1991). This explanation is not easy to test in the field. In fact, not even the geographical scale of dispersal of planktonic larvae of benthic species is well understood (Le Fevre and Bourget, 1992).

A fair question to ask is, are these differences real or are they artifacts of the diversity of approaches used by different authors (*i.e.*: field versus laboratory)? How can we link the distinctive life-history strategies of the various larval species as adaptive traits to the various environments? There isn't a simple answer. In some cases it is not possible to make comparisons, although, with caution some generalizations can be made from an evolutionary point of view. The identification of behavioural traits of larvae through ontogeny is therefore a fundamental aspect of marine ecology that needs to be evaluated since it can provide relevant information to test the frequency of specific behaviours which could influence larval dispersion under natural or simulated conditions.

Distinct life-history adaptations must represent trade offs that would enhance population sustainability. Their assessment requires a multidisciplinary approach to the distinct and integrative aspects of a species' life-history in relation to the environmental variability to which it is exposed, at appropriate temporal and spatial scales. One way is to elucidate life-history strategies of offshore (non-coastal) marine invertebrates, particularly during the planktonic stages which are not well known. Here, one could examine the different adaptations which enable larvae to feed and move and hence determine their capability to regulate vertical distribution during the planktonic phase.

Early development of *P. magellanicus* has been described by Drew (1906), and by Culliney (1974); however, behavioural studies and analyses of the components of swimming behaviour and patterns of locomotion are lacking for larval stages, making their role on vertical distribution and recruitment difficult to assess.

Bivalve veligers are small, usually less than 300 μm in maximum shell length, negatively buoyant and swim weakly (Mann, 1986b) aided by a ciliated velum with a dual role of capturing particles and swimming (Cragg, 1980). Bivalve larval swimming has been described as upward movement in vertically oriented helices or straight lines (Bayne 1963,1964; Cragg and Gruffydd, 1975; Cragg, 1980; Mann and Wolf, 1983), alternating with periods of either active or passive sinking during which the velum may

be trailed or the valves closed (Isham and Tierney, 1953, Carriker, 1961, Lough and Gonor, 1971, Cragg and Gruffydd, 1975, Mann and Wolf, 1983) Moreover, bivalve larvae have generally been found to swim at speeds in the range of 0 67 to 2 0 *mm* s^{-1} (Mileikovsky, 1973), although a strict comparison of behaviour, swimming components and swimming speed estimates among estuarine and marines species cannot be made due to the diverse life-histories of the various species and the diverse methods employed by the various authors during their studies

Upward and downward swimming in helical paths and straight lines are recognized behaviours of bivalve larvae although only upward helices have been generally studied. Swimming behaviour of larvae is also reported to be influenced by changes in light, gravity, temperature, hydrostatic pressure, and salinity, particularly for estuarine species (Bayne, 1963, 1964, Thorson, 1964, Cragg and Gruffydd 1975, Hidu and Haskin, 1978) adding potential sources of variability The sustainability of any population of benthic invertebrates with a planktonic larva would either depend on recruitment of passively drifting larvae from elsewhere, or on larval capability to control their distribution by appropriate vertical movements within an oceanographic regime The latter capability requires larval traits to match environmental conditions in such a manner that they will insure the return of recruits to adult populations by a combination of active and passive mechanisms Mann (1985) suggested the possibility of larval dispersal of bivalves in a seasonally stratified coastal system, to be the result of both active and passive processes

The possibility that specific larval traits or behaviours can sustain the benthic species continuity from generation to generation has not been adequately examined for bivalve species, and a detailed study on the variability of the components of swimming activity of planktonic larvae and their responses to environmental changes is lacking Furthermore, understanding the role of planktonic larvae in the successful recruitment of adult populations requires knowledge of the physical oceanographic characteristics of

areas adjacent to known benthic populations in relation to the field distributions of larvae. Recent mathematical models have begun to describe larval distribution using some of the swimming characteristics measured in laboratory (Tremblay *et al.*, 1994). However, in order to introduce more realistic information into modeling efforts, we need to understand the ontogenetic changes of larval behaviour during their planktonic journey since morphology, size, swimming capability and behaviour change through development. The temporal and spatial coupling of oceanographic regimes coinciding with the pelagic phase of invertebrate larvae with appropriate behavioural traits could enhance retention or dispersal of planktonic larvae and need to be further investigated.

Here I present the results of an investigation of the basic swimming patterns and swimming components of pre-competent sea scallop larvae of *P. magellanicus* to determine whether active swimming, (determined here as the helical pattern described by larvae during upward and downward movement) and passive sinking, (which denotes swimming in straight vertical lines) remains constant or vary through ontogeny. Constant swimming behaviour is easy to model, but unlikely to be adaptable. On the other hand, behaviours that change through development suggest adaptability and larvae may, as well, have the capability to respond to the immediate environment. Thus, to determine scallop larvae responses to food availability, the swimming components and vertical and helical velocity were assessed on early veligers considering previous feeding condition and food availability.

2.2 Methods

The aim of this study was two fold, first to determine the basic behavioural patterns of sea scallop larvae through ontogeny, referred to as Experiment I throughout the text; and, secondly to determine the effect of larval feeding condition and food availability on the swimming pattern of early veligers, referred to as Experiment II

2.2.1 Spawning procedure

Two methods were used to induce scallop spawning. The first included raising temperature 3 $^{\circ}C$ to 5 $^{\circ}C$ above normal. If this did not stimulate spawning I injected 0.2 *ml* of 2 μM serotonin (a well known neurotransmitter) into the adductor muscle (Couturier, 1986).

Ripe adult scallops (a minimum of 5 females and 3 to 5 males) from Georges Bank were selected after visual examination of gonad coloration and fullness I cleaned their shell surfaces of encrustations by scrubbing and rinsing in freshly filtered seawater All selected individuals were air exposed for about 30 minutes at room temperature prior to placement in filtered seawater. Scallops were kept separated, with each individual scallop placed in a 7 *l* plastic container filled with freshly filtered seawater at the selected temperature. The length of spawning induction varied between 3 and 4 hours, and water was exchanged at the end of every hour for all containers The water exchange permitted elimination of faeces and secretions that could have provided substrate for infection of gametes at the time of release.

Once spawning began, eggs and sperm were collected separately by gently sieving gametes through a double set of Nytex® mesh of appropriate size. Eggs were retained on 33 μm mesh after larger particles were collected on 102 μm mesh Sperm were collected after passing through a double Nytex mesh of 102 μm and 20 μm Gametes were kept separated in 20 *l* containers. After one hour all combined eggs were mixed with some of the sperm collected and left to fertilize undisturbed for 30 *min* to 1 *h*.

After fertilization was observed, embryos were gently washed and retained on 44 μm Nytex® mesh and incubated in 20 *l* plastic containers with 1 μm filtered seawater at an initial density of 30 *eggs* ml^{-1} Temperature was kept at 14 $^{\circ}C$ with gentle aeration in the absence of light (covered with black plastic sheets) during the next 96 hours.

All seawater used for adult spawning and larval rearing originated from the AQUATRON system (Dalhousie University) and was filtered through two propylene filters of 1 *μm* connected in line (Filterite Corp., Timonium, Maryland, USA) prior to or at the same time it was needed.

2.2.2 Larval rearing

Basic methods for rearing of scallop larvae were described by Couturier (1986) and only a general description is included here. Ninety six hours after fertilization, all straight hinge larvae (Prodissoconch I) were retained on 44 *μm* Nytex® mesh after being passed through 152 *μm* Nytex® mesh. All larvae were mixed and three random samples of 5 *ml* each were taken from a 7 *l* volume to estimate growth and survival. Seawater was changed every second or third day throughout the rearing period, at the same time larvae were fed on a diet consisting of an equal proportion on a dry weight basis of three phytoplankton species *Chaetoceros muelleri*, *Chaetoceros calcitrans*, and *Isochrysis galbana* at an initial concentration of 50.000 *cells* ml^{-1}.

The initial larval concentration in cultures at day four was 5 *larvae* ml^{-1} in 1 *μm* filtered seawater without the addition of antibiotics or UV radiation.

2.2.3 Algal culture

Algal species were originally obtained from the Center for Culture of Marine Phytoplankton at the Bigelow Laboratory for Oceans Sciences (West Boothbay Harbour, Maine). The general methods followed for algal culture have been described earlier by Enright (1984), therefore only a general description is included here.

To obtain an approximate 20 *l* volume of each algal species at each scheduled water change, stock cultures were initiated in 125 *ml* flasks containing 50 *ml* of sterile seawater and f/2 medium (Guillard and Ryther, 1962). Cultures were kept under a 12:12 light:dark regime at 22 °*C*, and every four days these cultures were used to inoculate 1 *l* flasks containing 250 *ml* sterile seawater and f/2 medium. Subsequently, every three to

four days these flasks were used to inoculate 20 *l* glass carboys also filled with sterile seawater and f/2 medium. A silicate supplement was added to the diatom cultures two days after inoculation.

All phytoplankton species used to feed scallop larvae were harvested at four days after carboy inoculation, screened through a 44 *μm* mesh and sampled to determine culture condition and cell concentration. All samples were first examined live under a compound microscope at 40x power for visual determination of contamination by ciliates or bacteria. Freshly fixed algae in 1% formalin were counted on a hemotocytometer (Neubauer) to determine the exact volume to feed

2.2.4 Experimental observations

To determine the basic larval behaviour through ontogeny, scallop larvae were collected from larval cultures throughout development (Experiment I) beginning on day 4 with the first shelled D-stage (Prodissoconch I), and through subsequent stages at days 8, 12, 16, 22 and 30 (Prodissoconch II) until they had reached the pediveliger stage. All larvae were collected during the water exchange period. Methods of recording swimming activity are described in the next section.

To determine the effect of a short period of starvation on swimming activity (Experiment II), larvae were collected at 6 days old from a different larval batch originated from adults from the same population and placed in filtered seawater for 3 hours prior to experimental recordings.

Previously fed larvae were used in Experiment I after being left for 30 to 60 *min* in filtered seawater prior to recording of swimming activity. Samples of about 2000 larvae were kept undisturbed in 250 *ml* glass beakers at 14 *C* before placing larvae in experimental chambers. During Experiment I all larvae were previously fed except 4 days old, and all recordings were made in filtered seawater.

Larvae were placed in a 5 *ml* glass cuvette or a home made glass-chamber of 250 *ml* at a density of 10 to 20 *larvae* ml^{-1} and recorded after 30 *min* of acclimatization.

Each recording session lasted for about 30 *min* during Experiment I and 45 *min* during Experiment II.

The second set of experiments (Experiment II) included the following treatments: a) previously fed larvae observed in filtered seawater (**F/S**), and after the addition of food (**F/F**); b) larvae previously starved for 3 h observed in filtered seawater (**S/S**), and after the addition of food (**S/F**); and c) a second group of larvae previously starved for 3 h were observed in filtered seawater (**C/S**) and after the addition of filtered seawater (**C/W**). This last group (C) served as control group to determine if observed changes in behavioural patterns resulted from the level of turbulence caused by the addition of seawater or were generated by larvae in the presence of phytoplankton. Approximately 50,000 *cell ml* $^{-1}$ of *I. galbana* (TISO) were added to the experimental larvae (**F/F** and **S/F**). The control group which consisted of previously starved larvae (**C/S**) received filtered seawater instead of algae (**C/W**).

2.2.5 Recording and analysis of swimming behaviour

All larvae were recorded while swimming at 14 *C* in either 5 *ml* glass cuvettes or a 250 *ml* glass chamber. Larvae aged 4 and 6 days in Experiment I were recorded for shorter duration in 5 ml cuvettes inside a cold room. Older larvae were recorded in the three section glass container which allowed for recording of larvae swimming in the middle chamber (250 ml) at 14 *C* by monitoring the temperature of the outer chambers while the observer remained at room temperature. The recordings for Experiment II used a temperature control system that allowed longer recording periods in 5 ml glass cuvettes.

Two similar experimental apparatuses were used to record larval behaviour during the first and second experimental observations (Fig. 2.1 and Fig. 2.2). In both Experiments I and II only larvae swimming at the centre of the experimental chambers were used in the analysis to minimize wall effects (Winet, 1973).

During Experiment I, larval swimming activity was recorded using a video-microscopy system that included a low light-intensity RCA® videocamera fitted to a

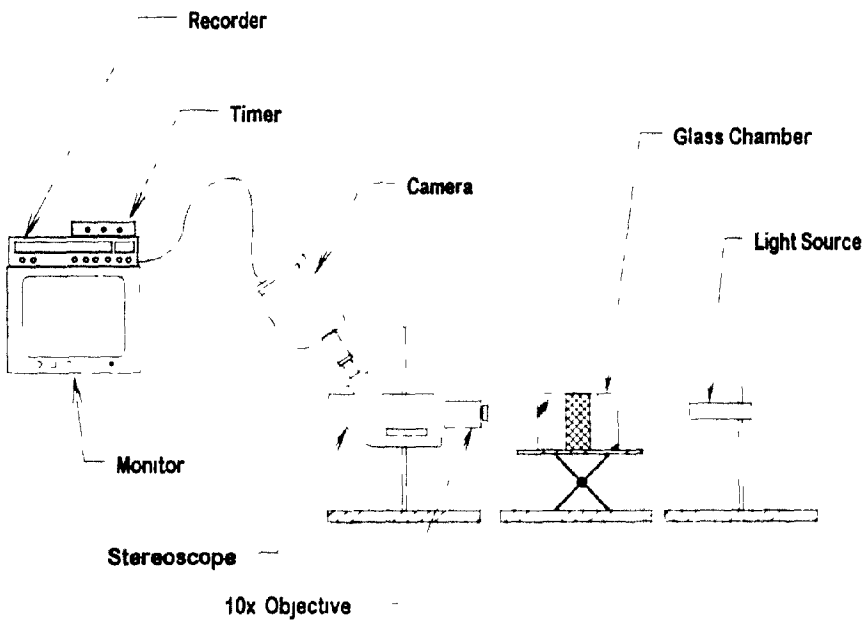

Figure 2.1 Experimental apparatus (not to scale) used to examine and record swimming behaviour of sea scallop larvae through ontogeny. A video camera is shown affixed to a stereoscope with a 10x Objective lens facing a glass chamber containing larvae. The glass chamber was mounted over a vertically movable jack and illuminated from the side with a low intensity lamp. The images were observed on a TV monitor and recorded on a VCR linked to a time code generator for playback analysis.

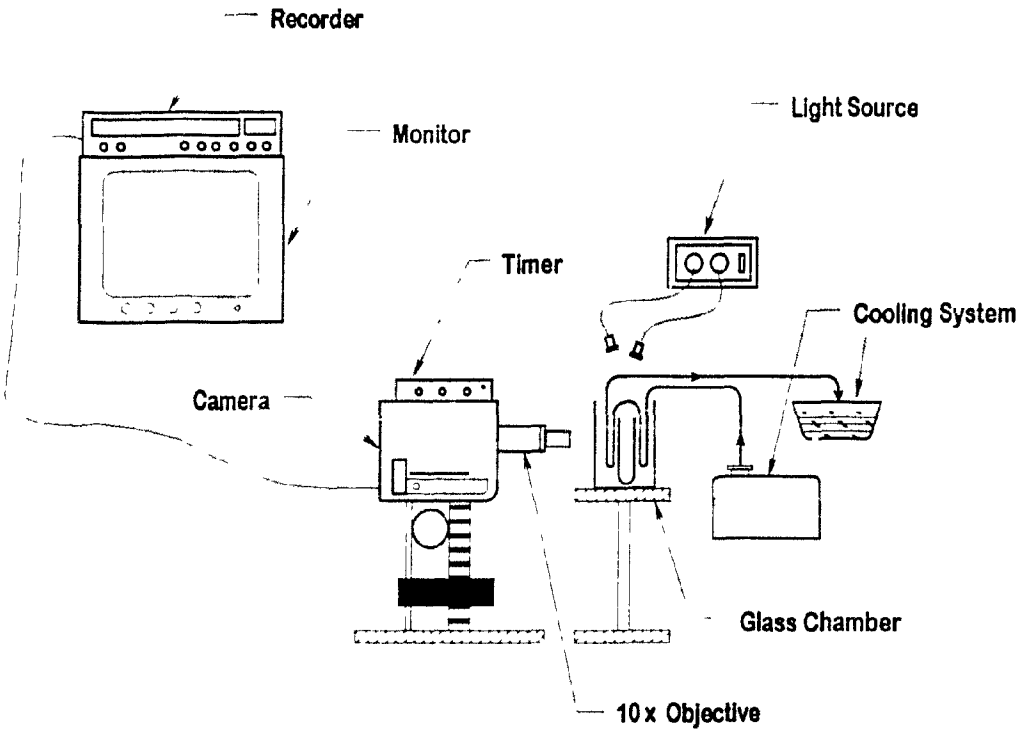

Figure 2.2 Experimental apparatus (not to scale) used to examine and record swimming behaviour of sea scallop larvae during the early veliger response to food availability. A video camera is shown affixed to a 10x Objective lens, mounted over a vertically movable jack, and facing a temperature controlled glass chamber containing larvae. Larvae were illuminated from the side with a cold infrared light source. The images were observed on a TV monitor and recorded on a VCR linked to a time code generator for playback analysis.

horizontally placed compound microscope and connected to a BETA-1[®] cassette recording system, a monitor and a time code-generator. In this design, the recording apparatus was fixed in one position and the glass chamber containing larvae was manually positioned in the vertical axis to follow the swimming activity of larvae (Fig 2.1). All larvae were recorded while illuminated from the side under static conditions and at $14 \pm 1\ ^{\circ}C$.

During Experiment II, all recordings of larval activity were from a video-microscopy system that consisted of a vertically movable Panasonic Digital 5000[®] video camera fitted with a 10x microscope objective that allowed the glass chamber containing larvae to remain in a fixed position (Fig 2.2). A VHS system and a monitor were connected to the Panasonic camera to videotape all experimental treatments. A fibre-optic light-source moved to illuminate the glass cuvette from directly opposite to the video camera. All larvae were filmed while in static conditions and kept at constant temperature of $14 \pm 1\ ^{\circ}C$ by circulating cold water through the outer chamber in which the cuvette containing larvae was installed.

Description of basic swimming patterns (Experiment I) was possible by analyzing the swimming components during playback of video recordings made of larvae in filtered seawater at 4, 6, 12, 16, 22 and 30 days after fertilization. Four swimming modes were identified: a) upward/straight, b) upward/helical, c) downward/straight, and d) downward/helical. Measurements of the swimming path of 5 larvae from each helical mode (upward and downward) and 3-10 larvae from each straight-line mode (upward and downward) were analyzed during Experiment I. During the last developmental stage, pediveligers were not observed to swim in helical patterns, although a few ($n = 3$) did swim in the "straight-line" pattern.

I used the following definitions for estimated swimming velocity following a helical path: Vertical displacement per unit time during upward and downward helical

patterns was defined as the "Vertical Velocity" (**VV**) and "Helical Velocity" (**HV**), which correspond to the (net) vertical velocity and gross or absolute velocity respectively (Cragg, 1980; Mann and Wolf, 1983). Helical Velocity Upwards (**HVU**) and Helical Velocity Downwards (**HVD**), are the distances per unit time travelled by larvae along the helical path; the net vertical velocity during helical swimming was identified as Vertical Velocity Upwards (**VVU**) and Vertical Velocity Downwards (**VVD**), respectively. Upward and downward velocity following a straight-line path were distinguished as Linear Velocity Upwards (**LVU**) and Linear Velocity Downwards (**LVD**).

The second set of experimental observations (II) were made on 6 day old veligers, in which the effect of larval feeding condition and availability of food was investigated. Here only vertical displacement in upward/helices was assessed from the analysis of 4 to 8 larvae per treatment providing data on **VVU** and **HVU**.

All larval trajectories were traced manually over a transparent acetate film covering the monitor screen, during frame by frame analysis of video recordings. In both cases, a grid was filmed prior to the beginning of the recordings and the experimental cuvette was kept in the same position throughout the recording periods.

To determine velocities during the helical swimming path, I measured the diameter of the helix (D), the height of one complete spiral (H), and the time (T) taken to rise or descend a known vertical distance (K) from two-dimensional recordings to calculate **HVU** and **HVD**, using the equations:

$$\text{Helical (absolute) Velocity} = \frac{\sqrt{H^2 + (\pi D)^2}}{T};$$

the pitch of the helix (σ) as,

$$\tan \sigma = \frac{H}{\pi \cdot D}; \text{ and}$$

Vertical Velocity (**VVU** and **VVD**) as,

$$\text{Vertical (net) Velocity} = \frac{K}{T}$$

Although several larval batches were produced and maintained, it was not possible to obtain complete recordings from all of them due to logistic constraints. One complete set of observations from a larval batch is presented for the first trial and another partial set from the spawning batch used in the second trial.

2.2.6 Statistical analysis

The statistical analysis of the data for this chapter and for all others will use the method described by Rodger (1974, 1975). This is an statistical procedure for evaluating contrasts considered as being selected post-hoc. The method is comparable to that of Sheffe (1953) and Tukey (1953) but is less harsh than both of these. Unlike the Newman (1939), Keuls (1952), and Duncan (1952) procedures, the Rodger method is not restricted to comparisons only; but it is somewhat harsher than both the Newman-Keuls and the Duncan methods.

After analysis of variance of J treatment groups, the Rodger method is used to find a set of J-1 contrasts, preferably mutually orthogonal but certainly linearly independent of one another, to constitute a decision set. Some (limited) number of these, will be null contrast rejections (by Rodger criterions) and some may be null acceptances. The Rodger F-criterion was computed to ensure that the rate of true null contrast rejection (*i.e.* type I errors) would be at the pre-chosen level $E\alpha$. Rodger symbolises his criterion by $F[E\alpha];\nu_1,\nu_2$ to distinguish it from the traditional values $F\alpha;\nu_1,\nu_2$ where ν_1 and ν_2 are the degrees of freedom.

A numeric illustration of the detailed calculations is given in Appendix A. The data were assessed statistically by the method of Rodger (1974, 1975) using his $E\alpha = 0.05$ (Appendix B1, B2, B3, B4, B5). This procedure allows for the post-hoc assessment of

contrasts across the group means following analysis of variance From the contrasts assessed, a decision set is chosen that fits the sample data well, makes biological sense and describes how the true population means appear to be related to one another.

2.3 Results

Development of sea scallop larvae through 30 days of growth and differentiation in filtered seawater (1 μm), fed a mixed diet of phytoplankton at 32‰ and at 14 C produced the normal ontogeny of larvae as earlier described by Culliney (1974) and Couturier (1986).

Hatchery reared larvae grew and developed under laboratory conditions at rates comparable to those reported by Culliney (1974) and Couturier (1986). The first shelled larval stage correspond to a D-stage or straight-hinge (104 μm), and growth rates varied from 3.5 to 4.95 $\mu m\ d^{-1}$ until larvae attained the umbonate veliger stage (163 μm), larvae continued to grow at 5.42 $\mu m\ d^{-1}$ until the pediveliger stage (239 μm) (Fig. 2.3)

All swimming larvae followed vertically oriented paths, independent of the direction of illumination, food availability or period of starvation. This is consistent with observations of other bivalve larvae. The normal position of a swimming larva in the water column is one with the hinge lowermost, in which the ciliated velum is uppermost but extended and tilted toward the direction of the helical path (Fig. 2.4). As it moves around the helix it also rotates around an axis perpendicular to the hinge

Helical swimming corresponds to "active swimming" and can be easily identified by the tilted position of the ciliated velum pointing towards the direction of movement. Although a larva may appear to withdraw spontaneously from a helical path to "passive" sinking (Fig. 2.5), it may also be responding to a disturbance such as occurs when other larvae pass nearby, apparently disturbing its own flow generated field. During upward straight-line movement, the larval shell appears slightly open as it ascends in the experimental chamber and the velum is not fully extended.

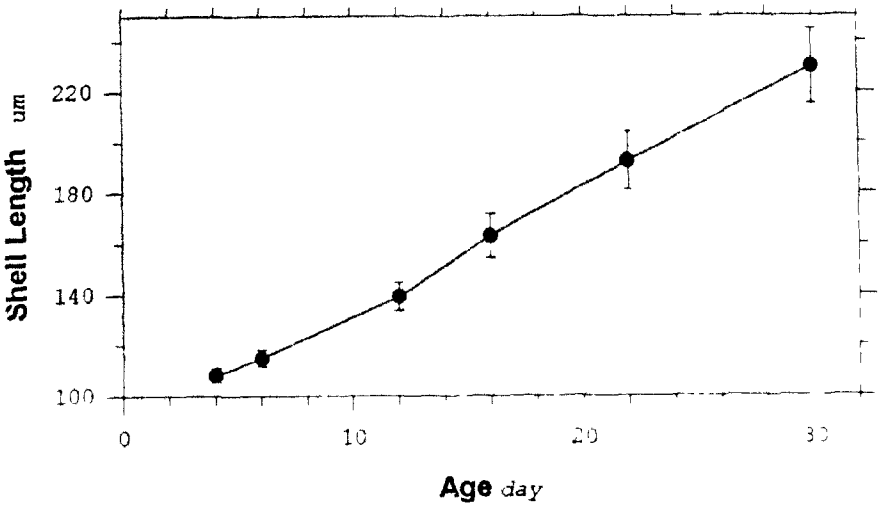

Figure 2.3 *Placopecten magellanicus*. Growth rate of sea scallop larvae fed with a mixed algal ration (50 10^6 *cells ml*$^{-1}$) composed of: *Isochrysis galbana* (TISO), *Chaetoceros calcitrans* and *Chaetoceros gracilis*. Values shown are mean shell length (n=25). Standard deviations are indicated as vertical bars

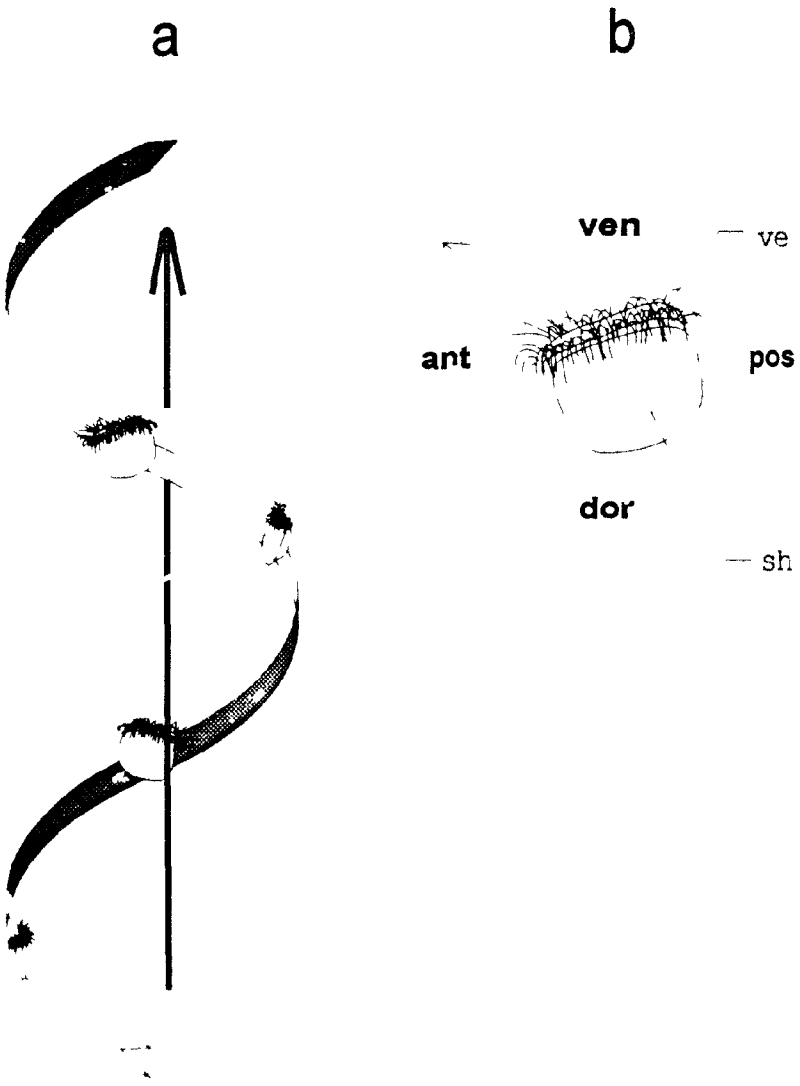

Figure 2.4 Schematic representation of a typical swimming upward helical path (a) showing the larval posture (b). Larval regions are identified as anterior (ant), posterior (pos), dorsal (dor) and ventral (ven). Indicated are the position of the shell (s) and velum (ve). (Adapted from Cragg, 1980).

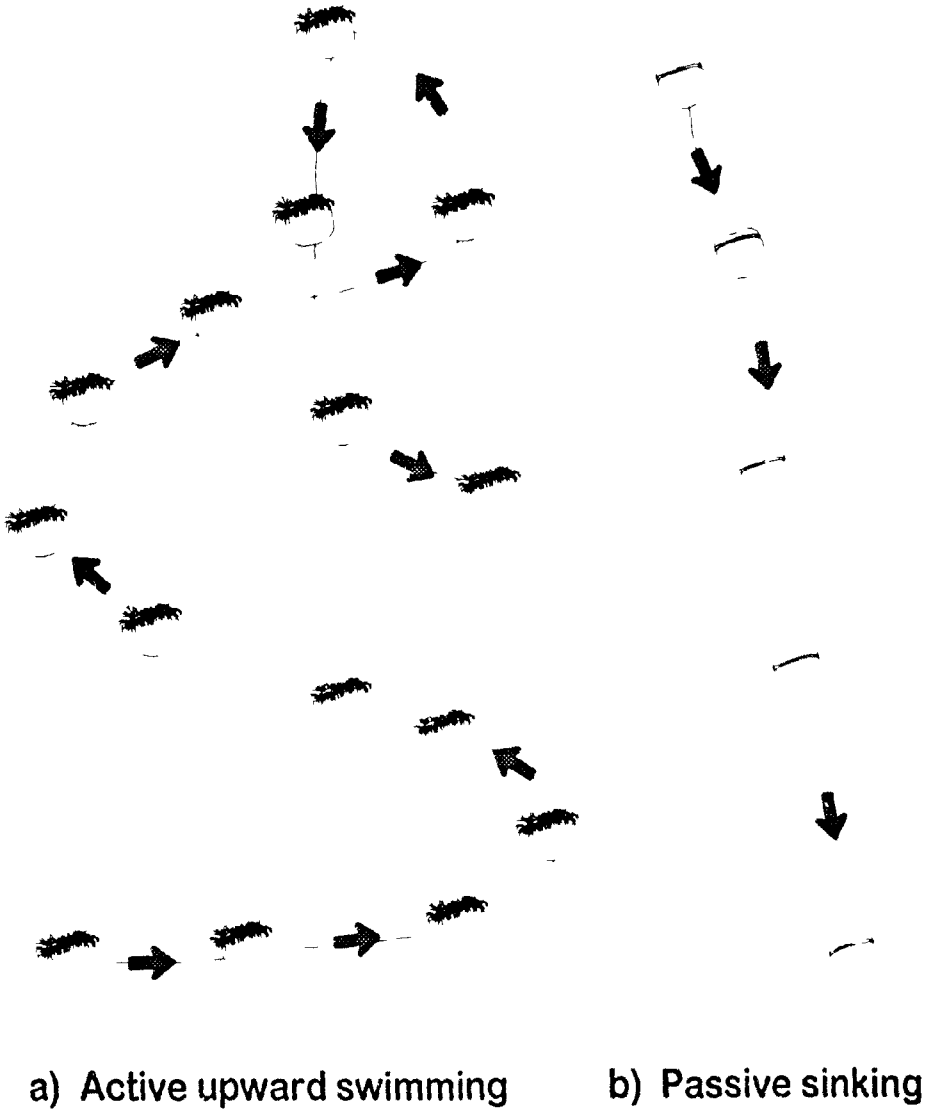

Figure 2.5 *Placopecten magellanicus* Schematic representation of a typical swimming path observed on sea scallop larvae (a) active upward helix and, (b) passive sinking in straight-line pattern

Swimming components, Vertical velocity (**VV**) and Helical velocity (**HV**) of larvae were estimated for larvae at 4, 6, 12, 16, and 22 days after fertilization during Experiment I and did not include observations on pediveligers, at day 30. Linear velocity was calculated for all veligers including pediveligers, for which helical displacement was not observed; larvae appeared to be searching for substrate and only a few exhibited short rises, followed by passive sinking.

The results obtained during the second experimental trial, in which early veligers, 6 days old (110 *μm*), swam in response to phytoplankton availability included only measurements of active swimming in upward helices.

There is, in general, a swimming speed increase through larval development, at least until day 22, although patterns differed between helical and linear swimming, and between upward and downward activity within each mode.

2.3.1 Helical or active swimming

Overall the dimensions of helical swimming components, helix height and helix radius (Fig. 2.6) tended to increase with developmental stage up to day 16 after fertilization although a decrease in helix height occurred at 22 days. The helix radius of descending larvae was usually wider than that of ascending larvae, and tended to increase with development (Fig. 2.6). The increasing width of helix radius described by larvae during downward activity through development has not been reported before for any other molluscan larvae, nor has its significance in feeding, since only upward helices have been associated with feeding.

The height of each helix tended to increase with development until day 16 in which ascending larvae present higher helices than descending, both showing a decrease in height at the last sampled stage (Appendix B1). Older larvae have higher helices during upward motion (2.24 to 1.38 *mm*) than during descent (1.49 to 0.86 *mm*), between 16 and 22 days after fertilization (Fig. 2.6).

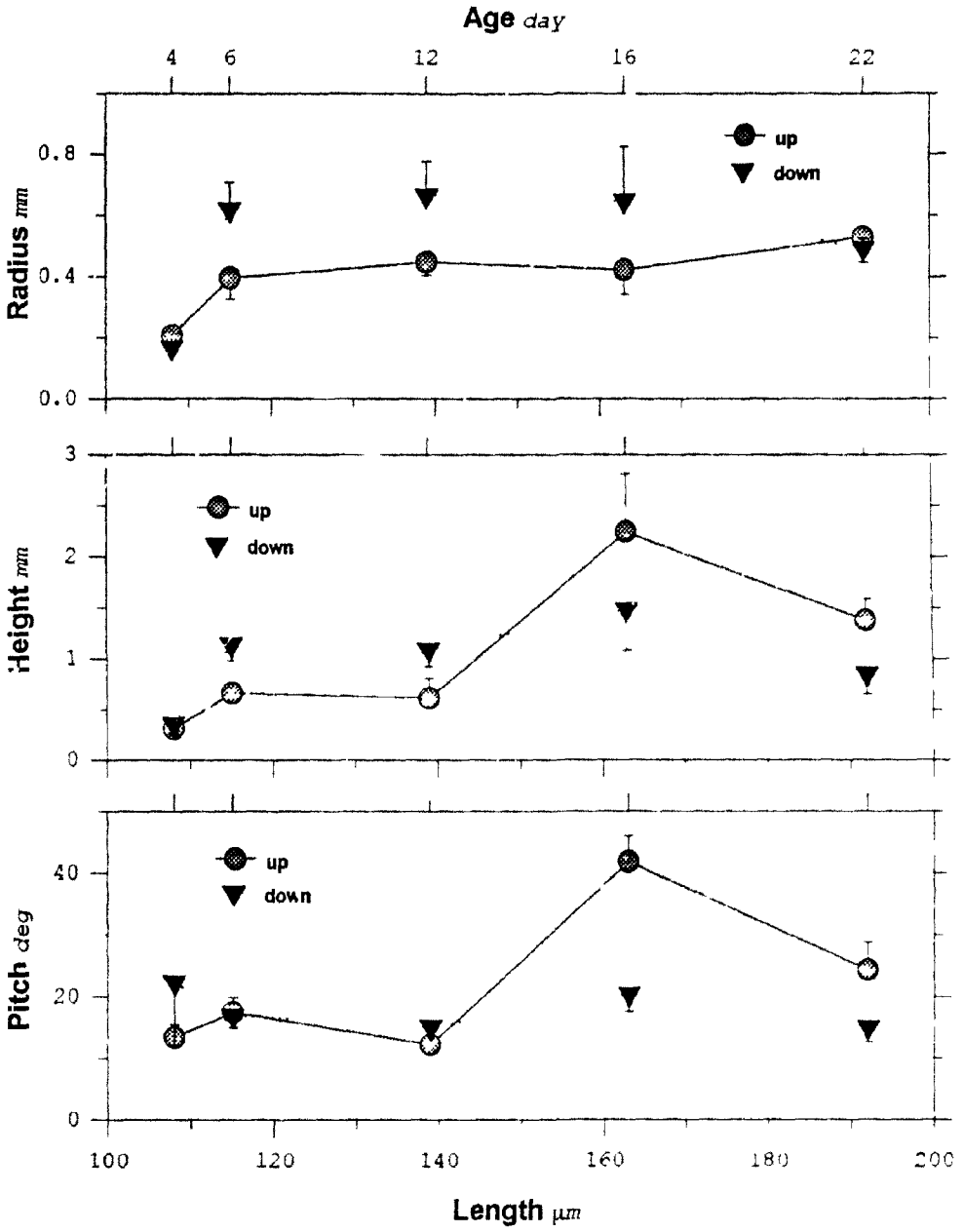

Figure 2.6 *Placopecten magellanicus* swimming components of helical path through larval ontogeny (a) helix radius, (b) helix height and, (c) helix pitch, associated with active ascent and active descent in the water column. Standard error is indicated as vertical bars.

The angle of an ascending larvae in its helical path increased from 13.39° (day 4) to 41.83° (16 days) and then decreased to 24.32° (22 days) (Appendix B1). However, descending larvae displayed a flatter angle varying between 22.34° (day 4) to 20.41° (day 16) which decreased even further to 15° (22 days) in older larvae (Fig. 2.6).

The helical velocity (**HV**) calculated for larvae while ascending and descending in the water column showed a comparable increase through ontogeny (Fig. 2.7), although, ascending (**HVU**) larvae display an almost four fold increase from 0.46 to 1.62 *mm* s^{-1} while descending (**HVD**) larvae shows a lesser two fold increase from 0.56 to 1.10 *mm* s^{-1} between day 4 and 16 after fertilization, respectively. A decrease in both **HVU** and **HVD** was found in 22 day old larvae (Fig. 2.7; Appendix B1), which is consistent with findings on other bivalve larvae (Cragg, 1980; Mann and Wolf, 1983).

A similar trend was observed in vertical velocity (**VV**) calculated for planktonic larvae swimming in helical patterns through development (Fig. 2.7); there was a significant increase during ascending helices (**VVU**) from 0.09 *mm* s^{-1} at 4 days old to 1.05 *mm* s^{-1} at 16 days (163 *μm*), followed by a decrease to 0.42 *mm* s^{-1} for larvae 22 days old. A different pattern occurred for **VVD**, which remained relatively constant varying between 0.20 (4 days) to 0.37 (16 days) and 0.25 *mm* s^{-1} (22 days) (Fig. 2.7).

In general, swimming rates of larvae following helical paths showed two distinct phases, the first for younger larvae up to 12 days old (139 *μm*), in which **HVU** is slower than **HVD**, and the opposite trend for older larvae of 16 and 22 days old, exhibiting a faster **HVU** than **HVD**. Within this second phase, there is a distinct decrease in both at the last sampled stage (22 days).

2.3.2 Straight-line swimming

Upward and downward straight-line displacements occurred throughout development including pediveliger larvae (239 *μm*). During the linear upward motion, it appeared that larvae held their shells slightly open, and more tightly closed during descent, although a number of variations appeared to occur but could not be quantified

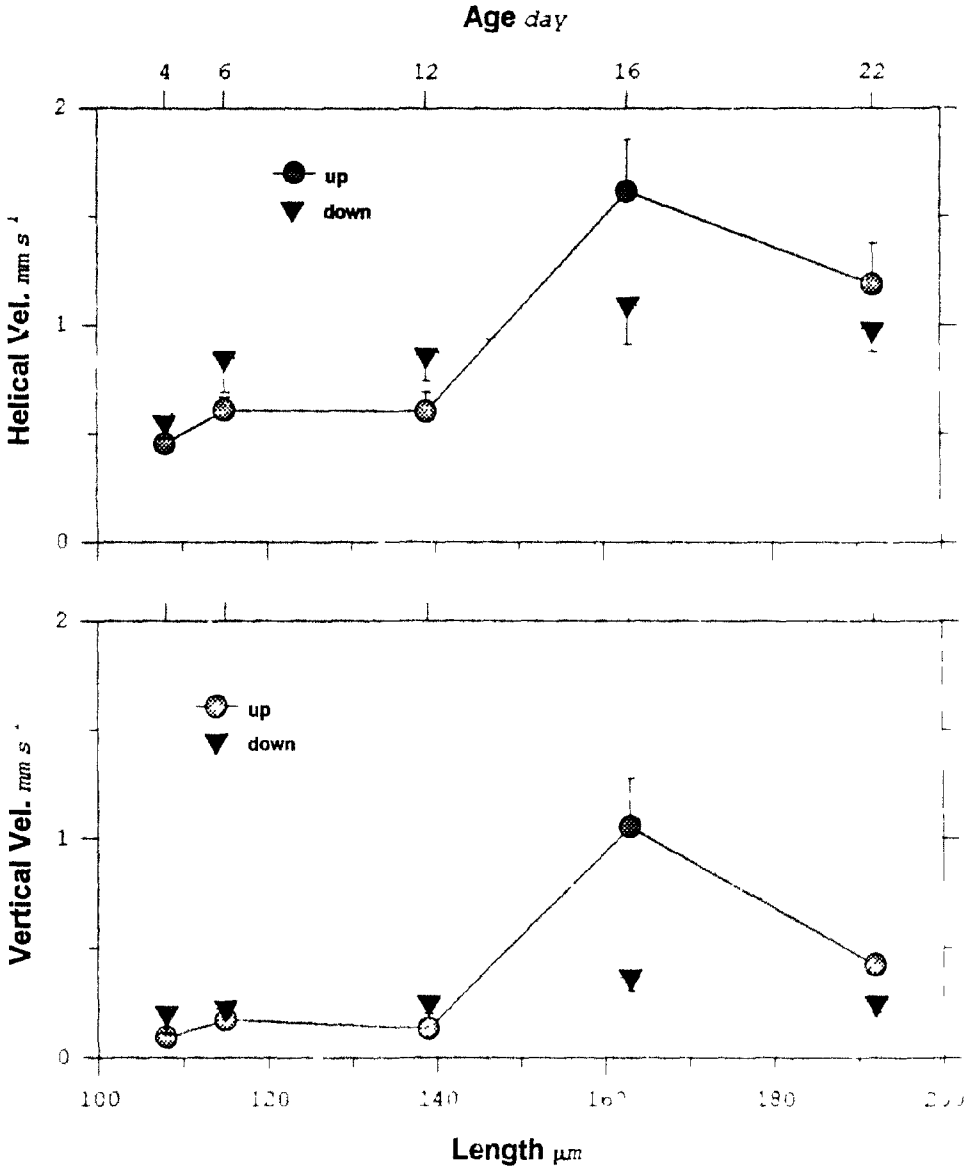

Figure 2.7 *Placopecten magellanicus*. Swimming velocities through larval ontogeny for upward and downward helical patterns. Top panel: Helical Velocity , bottom panel: Vertical Velocity . Standard error is indicated as vertical bars

due to the resolution of the optical system. During linear activity, **LVU** of larvae increased from 0.98 to 1.27 *mm* s^{-1} (4-16 days) with a slight decrease at the later stage to 0.88 *mm* s^{-1} (30 days old) (Fig. 2.8; Appendix B2).

"Passive sinking" followed a similar trend, with **LVD** increasing through development from 0.95 to 1.78 *mm* s^{-1} between days 4 to 22, and then decreases slightly to 1.43 *mm* s^{-1} at 30 days (Fig. 2.8) which represents an average five fold increase over downward helices. The maximum sinking rate occurred in older larvae (22 days) with a seven fold increase in downward movement (Appendix B2).

To allow me to compare relative swimming speeds of scallop larvae with other microzooplankton in the literature, I calculated vertical (**VV**), helical (**HV**), and linear (**LV**) displacement, in terms of body lengths per unit time (Fig. 2.9). Minimum and maximum swimming rates estimated for **VVU** (vertical velocity upwards), were 0.85 and 6.45 *body-length* s^{-1}, while **VVD** remained relatively constant between 1.79 to 2.25 *body-length* s^{-1}. During the last stage, both swimming rates during ascent and descent decreased to 2.2 and 1.3 *body-length* s^{-1}, respectively (Fig. 2.9)

Swimming rates of larvae in helical pattern (**HV**), were at least 4 *body-length* s^{-1} reaching a minimum and maximum **HVU** of 4.22 and 9.91 *body-length* s^{-1} (days 4 to 16), while **HVD** varied between a minimum and maximum value of 5.1 to 7.3 *body-length* s^{-1} (days 4-6) and then decreased to 5.12 *body-length* s^{-1} (day 22) (Fig. 2.9).

During passive sinking (**LVD**) scallop larvae fell in the water column at rates that fluctuated between 10.29 and 5.98 *body-length* s^{-1}. The maximum applies to 16 day larvae and the minimum to pediveligers, although only a few pediveliger larvae were measured. Maximum and minimum swimming rates during **LVU** were 9.04 and 4.16 *body-length* s^{-1} at days 4 and 30 after fertilization (Fig 2.9). The observed variability may reflect changes in buoyancy or drag of larvae through development, although, general values are well within the range described for other microzooplankton (Buskey *et al.*,

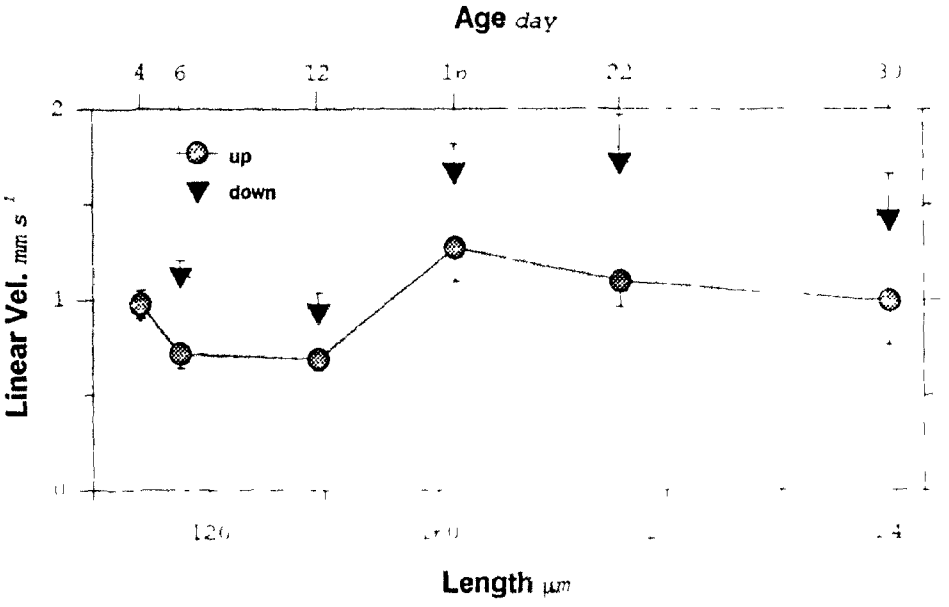

Figure 2.8 *Placopecten magellanicus*. Linear velocity through larval ontogeny, during passive sinking and upward straight-line swimming. Standard error is indicated as vertical bars.

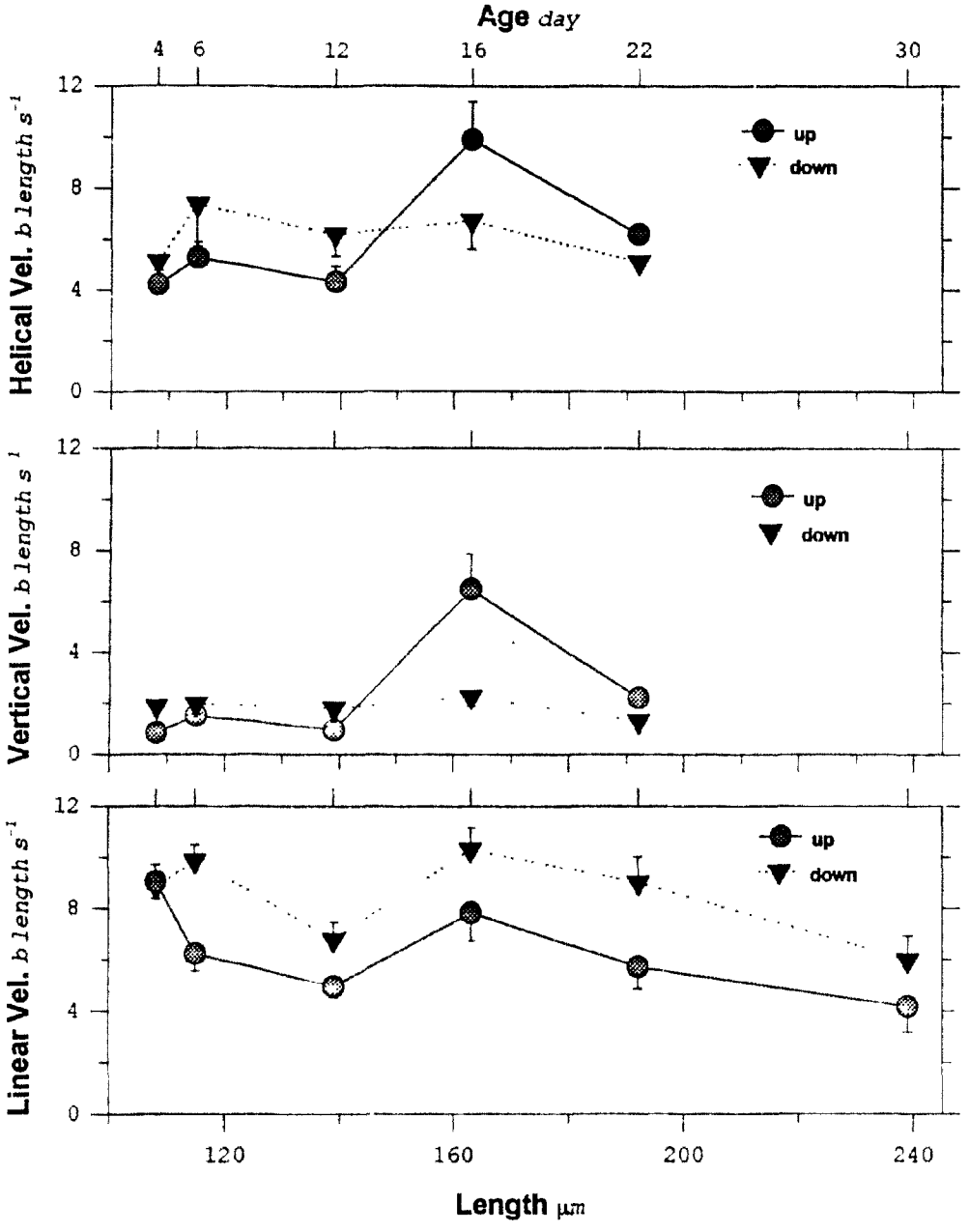

Figure 2.9 *Placopecten magellanicus*. Body-length scaled swimming velocities through larval ontogeny for helical swimming (a;b) and straight-line patterns (c). Top panel: Helical Velocity, middle panel: Vertical Velocity and, bottom panel: Linear Velocity including upward (**U**) and downward (**D**) swimming. Standard error is indicated as vertical bars.

199' and for other bivalve larvae. Comparisons with other larval species are difficult since most authors do not distinguish direction of movement or mode of locomotion.

2.3.3 Response to food availability and feeding condition

Fed and starved veligers responded differently to the availability of food and, although, larvae in both conditions modified the helix radius and helix height, the extent of the changes varied, probably reflecting the larval condition (Fig. 2.10). Fed larvae (**F/S**, **F/F**) had a greater increase in helix height (0.30 to 0.50 *mm*) and helix radius (0.10 to 0.30 *mm*) in comparison with starved larvae (**S/S**, **S/F**) which showed a lesser increase in helix height (0.85 to 0.96 *mm*) and helix radius (0.08 to 0.12 *mm*) (Appendix B3). Both fed (**F/F**) and starved (**S/F**) larvae decreased the pitch of the helix in the presence of phytoplankton from 27° to 16° and from 64° to 45°, respectively, although overall starved larvae had the greatest pitch (Fig. 2.10).

After food was added, both fed and starved larvae showed a decrease in vertical velocity (**VVU**) from 0.17 to 0.13 *mm* s^{-1} (**F/F**) and from 0.43 to 0.22 *mm* s^{-1} (**S/F**); starved larvae (**S/S**) moved faster vertically overall (Fig. 2.11). Helical velocity (**HVU**) of previously fed (**F/S**) larvae showed the reverse trend, exhibiting an increase in **HVU** after the addition of food (Fig. 2.11) (Appendix B3). The two fold increase of helical velocity of starved larvae in the presence of phytoplankton may indicate the increasing scanning of the water column therefore increasing feeding rate.

Larvae that had experienced three hours of starvation and received only filtered seawater instead of phytoplankton were the last group to be filmed and showed a slight decrease of helix radius, helix height and pitch (Fig. 2.10) causing a minimal decrease in **HVU** and a slight increase in **VVU** (Fig. 2.11) which indicates that the addition of filtered seawater failed to elicit a larval response of similar magnitude to that of either fed or starved larvae after the addition of food.

Larval response to the presence of food is evident for both fed and starved larvae as shown by the increase in helix height and radius, and in both cases the larvae reduced

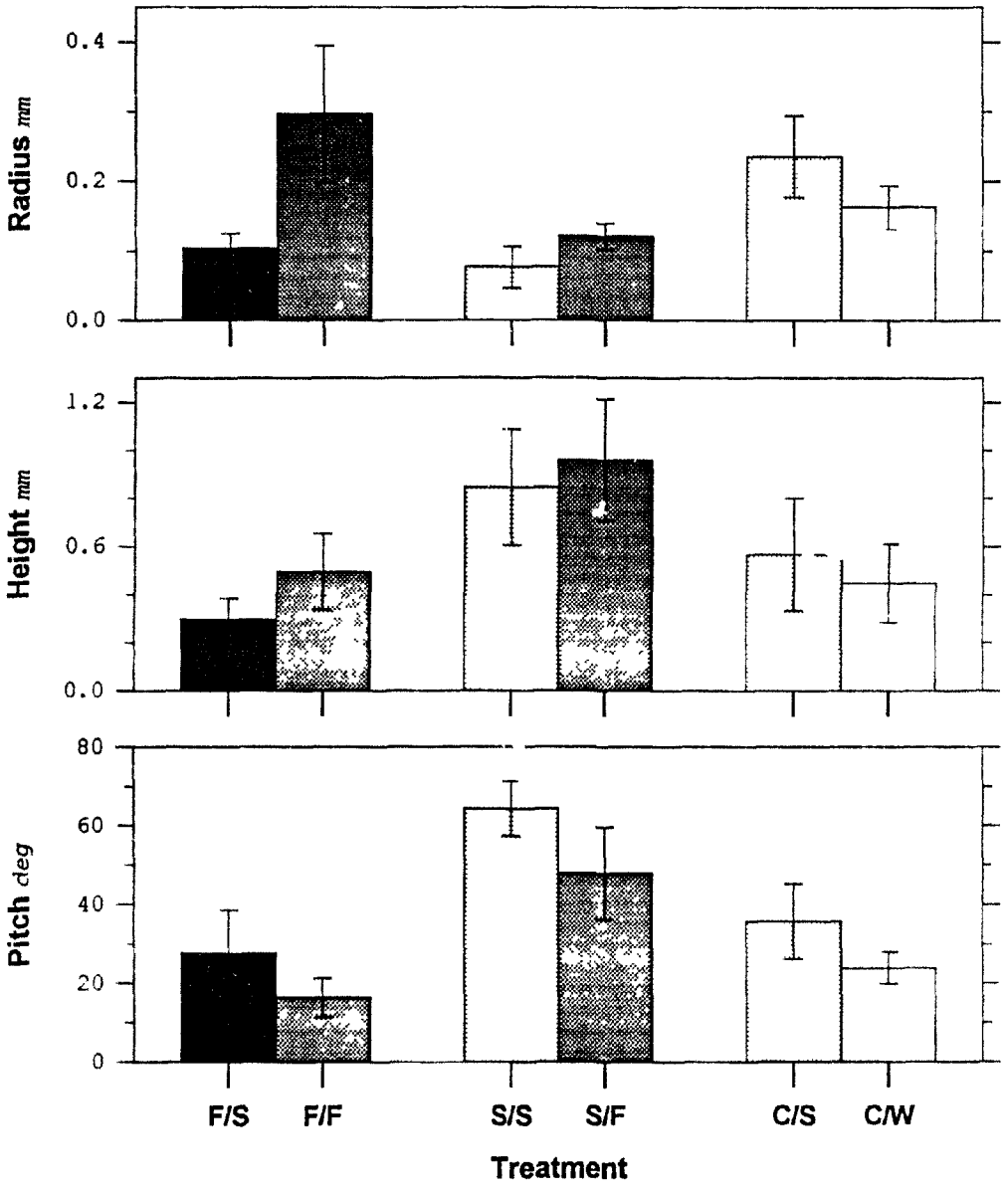

Figure 2.10 *Placopecten magellanicus*. Variability of swimming components of helical patterns, in response to the addition of microalgae (*Isochrysis galbana*, TISO) and filtered seawater in early veligers (110 μm). Conditions are: Fed (**F/S, F/F**) and Starved (**S/S, S/F, C/S, C/W**) larvae. Standard error is indicated as vertical bars.

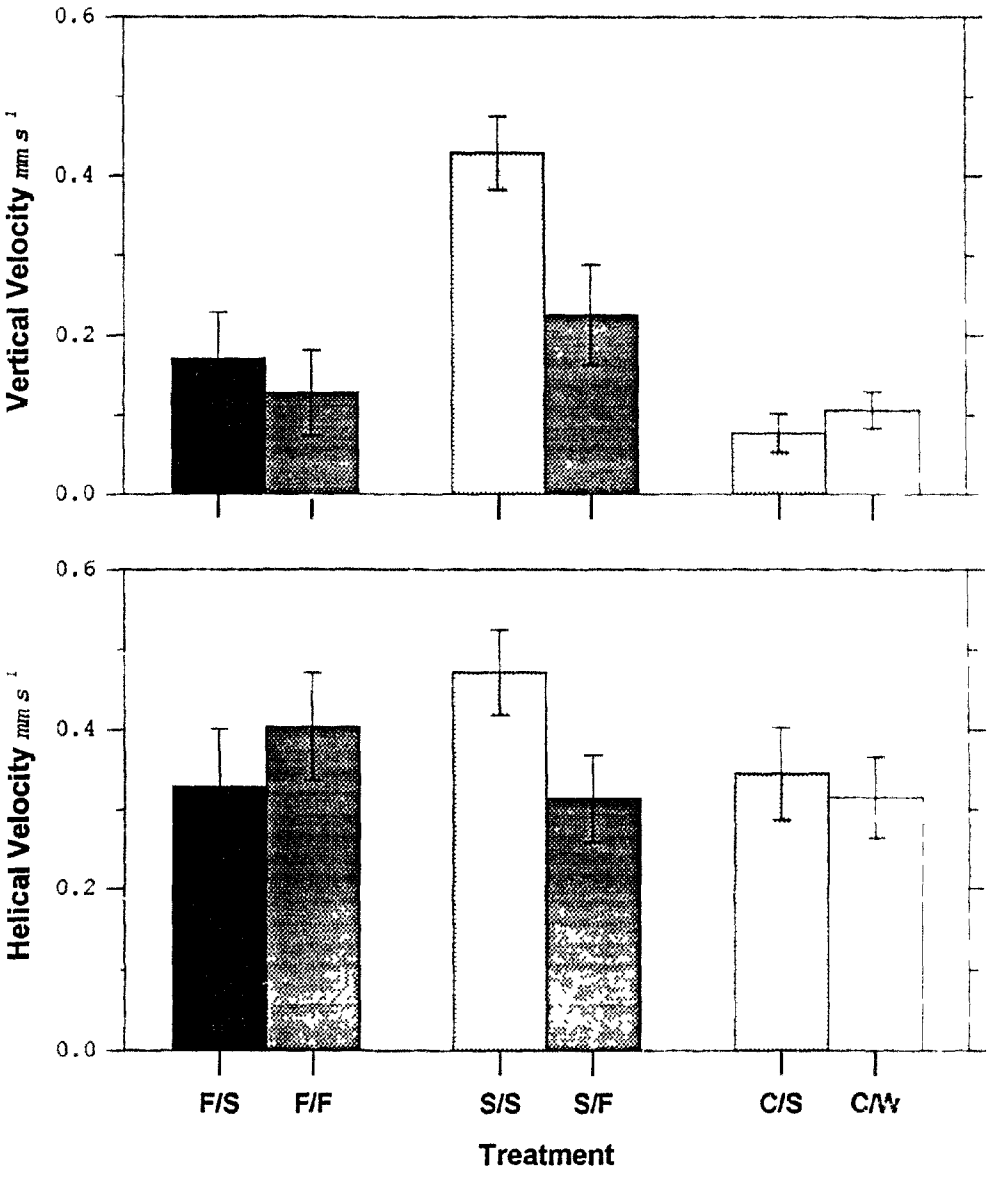

Figure 2.11 *Placopecten magellanicus*. Variability of Helical Velocity (a) and Vertical Velocity (b), in response to the addition of microalgae (*Isochrysis galbana*, TISO) and filtered seawater in early veligers (110 μm). Conditions are: Fed (**F/S, F/F**) and Starved (**S/S, S/F, C/S, C/W**) larvae. Standard error is indicated as vertical bars.

the angle of the described helix relative to swimming in filtered seawater Similar behavioural responses may occur during the planktonic development of larvae These modifications of the geometry of the helical path would provide an advantageous adaptive mechanism for feeding in a diluted environment oy increasing the scanning time and volume of water filtered in the water column and at the same time maintaining the vertical position while hovering in areas of elevated food availability Sea scallop larvae display **VVU** and **HVU** in the range previously described for other molluscan bivalve larvae (Cragg, 1980, Mann and Wolf, 1983, Mann *et al*, 1991) and swimming rates are in the same range of other microzooplankton. Moreover, straight line movements allow larvae to gain some control over depth regulation

2.4 Discussion

Most studies of veliger larvae focus on the pattern of alternating helical ascents and linear descents typically used by "middle-aged" larvae to feed in shallow containers Because the overall context of the present study included mesocosm-scale migrations (Chapter 4), it also monitored helical descents and linear ascents, which are less common but potentially important during feeding and vertical migration. I propose that it is the plasticity of these behavioural traits that allow larvae to take advantage of environmental conditions to enhance their growth by adjusting behaviours to increase feeding and to display active mechanisms to ensure their later return to benthic grounds

The major factors influencing locomotor parameters are the relative sizes of the velum and shell, which affect hydrodynamics, and the relative quantities of the shell mineral and lipid which affect density (Cragg, 1980,1989, Cragg and Crisp, 1991; Jonsson *et al*, 1991) After rapid changes in the locomotor parameters in the first few days, probably associated with development of the velum, the general pattern of locomotor activity stabilizes Absolute (helical) velocities generally increase with size during the first half of the larval phase, but relative to body length, there is little change However, active swimming during ascent (**HVU**, **VVU**) appears to decline as the larvae

approach settlement, perhaps because the effective area of the velum no longer increases as fast as body size once the transition to the pediveliger begins. More surprising is the fact that passive sinking rates (**LVD**) increase by less than 50% with a 2.2 fold increase in larval length. Swimming at low Reynolds numbers is characteristic of planktonic larvae and, if they are considered as falling spheres, Stoke's law predicts that, for spheres of equal density, velocity should increase as the square of the radius ($U_s = 2r^2 g (\rho-\rho_w)/9\mu$) (Vogel, 1981). Thus the observed change is only a tenth of that predicted. This suggests a significant decrease in density (ρ) as lipid reserves accumulate to fuel metamorphosis at settlement. If sinking forces are actually reduced, then the decline in **VVU** represents a fairly dramatic decline in the forces supplied by the velum.

My study described the ontogenetic variability of a pectinid larva, considering all modes of upward and downward swimming, by quantifying the geometry and Vertical, Helical and Linear Velocities of larvae from the straight hinge or D-stage (4 days) until the pediveliger stage (30 days). Helical and linear "straight-line" swimming were observed to occur throughout larval ontogeny, however, pediveligers were not observed describing helices but rather searching for substrate and sporadically displaying short bursts of straight-line swimming. Between 4 and 16 days of age, well-fed larvae during active swimming exhibited an increase in Vertical Velocity Upward (**VVU**) from 0.09 to 1.05 *mm* s^{-1} but **VVD** remained relatively constant between 0.20 to 0.37 *mm* s^{-1}. At the last sampled stage (22 days) which correspond to larvae near the pediveliger stage, there was a drastic decrease of **VVU** to 0.42 *mm* s^{-1}, and a lesser decline in **VVD** to 0.25 *mm* s^{-1}. Earlier studies on locomotion of bivalve larvae have evaluated only certain aspects of swimming behaviour under conditions different from those used here. Nevertheless, a comparison is worthwhile to assess similarities and differences that would allow the recognition of general patterns (Table 2.6). Swimming rates of scallop larvae are comparable to those found by Cragg (1980), Mann and Wolf (1983), Mann *et al.* (1991),

Table 2.6 Swimming rates of bivalve larvae during active swimming (helical swimming upward and downward) and passive swimming in straight lines (sinking and upward movement)

Species	Length *μm*	Vertical velocity *mm s*$^{-1}$				Source
		Active swimming		Passive swimming		
		upward	downward	upward	downward	
P. maximus †	N/A	0.17 – 0 63	N/A	N/A	N/A	Cragg, 1980
A. islandica †	170 – 202	0 20 – 0.52	N/A	N/A	N/A	Mann and Wolf, 1983
S. solidissima ‡	95 6 – 196 1	0 18 – 0 49	0 15 – 0 37	N/A	0.64 – 2.23	Mann *et al.*, 1991
M. lateralis ‡	88.9 – 159 7	0 25 – 0.50	0 17 – 0 52	N/A	0.67 – 1.30	*ib.*
R. cuneata ‡	103 – 168 5	0 18 – 0 53	0.25 – 0 59	N/A	2.31 – 1.74	*ib.*
P. magellanicus §	104 – 239	0 09 – 1 05	0.20 – 0.37	0 72 – 1.27	0.94 – 1 73	present study

† includes response to hydrostatic pressure

‡ includes response to salinity gradient

§ well fed larvae in filtered seawater

for larvae of other species and they may reflect typical values of vertical displacement of ciliated larvae during active and passive motion

A behaviour has been shown that promotes movement toward the surface by planktonic larvae in response to conservative parameters such as hydrostatic pressure (Cragg, 1980; Mann and Wolf, 1983, Sulkin *et al.*, 1983, Guan 1993) Cragg (1980) reported that *Pecten maximus* larvae swam at velocities between 0.172 to 0.464 *mm* s^{-1} at atmospheric pressure and between 0.207 to 0.718 *mm* s^{-1} when the atmospheric pressure increased 2 bar, although he only examined upward helices during the study Similarly Mann and Wolf (1983) found that larger larvae (170-202 *µm*) of *Arctica islandica* swam at velocities of 0.28 to 0.37 *mm* s^{-1} at atmospheric pressure and of 0.23 to 0.49 *mm* s^{-1} when pressure was increased to 4 bar they also did not quantify downward helices or straight-line movement Sea scallop larvae from two populations (shallow and deep) also increased upward swimming rates with increasing hydrostatic pressure at most stages of development (Guan 1993)

A more recent study by Mann *et al* (1991) included the effect of salinity gradients on the swimming behaviour of three mactrid species and included larval ascent and descent in helical paths (**VVU** and **VVD**) and sinking (**LVD**) Although, Mann *et al* (1991) did not measure helical swimming they found consistent increases in mean upward swimming rates (**VVU**) between 0.18 to 0.53 *mm* s^{-1} and mean downward rates (**VVD**) from 0.15 to 0.59 *mm* s^{-1} through development Maximum values were obtained at the umbo stage followed by a decrease at the pediveliger stage These findings are consistent with the ontogenetic changes of larval weight, specific gravity and velum development as reviewed by Chia *et al.* (1984), but only partially with the present study of sea scallop larvae since **VVD** remained relatively constant through ontogeny (Table 2.3) Passive sinking (**LVD**) was found to increase significantly through the ontogeny of two of the three mactrid species studied by Mann *et al* (1991), which is also consistent with the present study of scallop larvae Swimming speeds of *P. magellanicus* larvae

exhibit a marked overall increase through early ontogeny despite the increasing density of the larval shell, and probably coincide with the development of a highly sophisticated ciliary arrangement as found earlier in *P. maximus* (Cragg, 1980,1989; Crag and Crisp, 1991) and *C. hastata* (Hodgson and Burke, 1988).

The similarity of findings in my study with others in the literature is indicative both of common physical limits and the adaptive value of the common traits of larvae which are meroplanktonic for a short period of their life cycle. Early stages sink slowly, probably due to high lipid contents and lower shell density; pediveligers, on the other hand, need to be evaluated differently since at this stage they are seeking a substrate where they can complete metamorphosis. Changes in swimming rates of older larvae likely relate to the experimental conditions, the lack of some environmental stimulus (*e.g.* substrate, flow) and the physiological condition of larvae, since late stages (30 days) are in the process of losing the ciliated velum and beginning to use the ciliated foot to search for substrate. In this study I observed late larvae at the bottom of the experimental chamber extending the foot and using it as an anchor to change position by flipping from one shell to the other and in other cases actually advancing by carrying its shell in steps. Certainly by the latest observed pediveliger stage many larvae were no longer planktonic and the static conditions did not elicit swimming.

My detailed study of swimming behaviour through ontogeny clearly indicates that a helical pattern must enhance feeding activity and that passive sinking can potentially be the mechanism responsible for regulating the vertical position of larvae. Passive sinking would be advantageous for larvae to actively escape from unfavourable situations and thus benefit their persistence in the plankton during the early stages of development. As larval development proceeds and approaches the competent stage, increasing sinking rates would also be beneficial as larvae are preparing to leave the water column in search for appropriate substrate to settle.

Scallop larvae also decreased sinking rates during helical swimming patterns, but this is probably the result of a combination of the increasing drag by increasing the diameter of the velum, a decrease in ciliary frequency (Arkett *et al.*, 1987) and the asymmetric density distribution of the larval body (Jonsson, 1989; Pennington and Strathmann, 1990). This density asymmetry of the larval body is caused by the dense aragonitic crystals of calcium carbonate deposited in the shell, as in the case of sea scallop larvae (Hurley *et al.*, 1987) and the less dense ciliated velum which creates a gravitational torque due to the separation of the larval centre of gravity and the centre of buoyancy as proposed for *Ceratodesma edule* larvae (Jonsson *et al.*, 1991). Reported values of density of the whole organism indicate that larvae are generally denser than seawater and vary between 1.1 to 1.29 $g\ cm^{-3}$ (Gallager, 1985, Eriksson and Jonsson unpublished in Jonsson *et al.*, 1991, Jackson, 1992). *P. magellanicus*, unfertilized eggs, and larvae at 3, 11 and 13 days seem to vary in density from 1.02, 1.29, 1.26 and, 1.24 $g\ cm^{-3}$ respectively, which place sea scallop larvae among the most dense bivalves (Jackson, 1992).

The most efficient way for a bivalve larva to counteract the forces induced by the shell would be to store neutral lipids that could provide cheap and light fuel. In fact, neutral lipid granules at the base of each cilia and in the digestive gland seem to be characteristic of bivalve larvae (Gallager, 1991). This, coupled with increasing velar ciliation as larvae grow (Cragg, 1989; Cragg and Crisp, 1991), must provide enough force to lift the larvae as it swims. Although the role of lipids in buoyancy and as an efficient source of energy in bivalve larvae has been suspected by many researchers, details of the dual role of lipids on the energetic requirements of bivalve larva locomotion are lacking. Some estimations of the cost of swimming of bivalve larvae indicate that almost 70% of the total metabolism is required to satisfy swimming activities (Manning, 1985; Gallager, 1991); therefore, only appropriate timing of planktotrophic stages of benthic bivalves during periods of high food availability would allow larvae to meet the

cost of swimming and growing to maintain themselves in the plankton, providing a selective advantage to the sustainability of benthic populations.

Planktotrophic veligers of benthic bivalves and other molluscan species meet their nutritional requirements by filtering particulate material, mainly phytoplankton, as they swim through the surrounding seawater using the bifunctional ciliated velum, which provides both a feeding and a propulsive mechanism (Strathmann *et al.*, 1972; Strathmann and Leise, 1979). Descriptions of the outline of the perimeter of the velum are similar for the three pectinid species *Placopecten magellanicus*, *Pecten maximus* and, *Chlamys hastata* (Culliney, 1974; Hodgson and Burke, 1988 and, Cragg, 1989, respectively). This reflects one set of adaptive similarities of these species that occupy similar niches during their early developmental stages. The typical pattern of velar ciliation of *P. maximus* consists of an inner pre-oral ring (shorter cilia), long pre-oral cirri, an adoral tract and post oral cilia, fitting the "opposed band" method as earlier described for other invertebrate larvae (Strathmann *et al.*, 1972; Strathmann and Liese, 1979, Nielsen, 1987), in which the pre-oral cirri overtake suspended particles and sweep them toward the adoral cilia. The capture of particles occurs at the recovery stroke of the pre-oral cirri and/or by the action of the post-oral cilia (Cragg, 1989). Although my present study did not focus on the characterization of ciliary activity due to the lack of such fine resolution with the available system, similarities among pectinid larvae are expected.

Feeding rates were not measured in this study, although, the observed increase in helix diameter of early veliger larvae when phytoplankton was present appears to be a good indicator of increasing feeding rates. Feeding condition also influences the response of larvae, as was observed in the decrease in vertical swimming rates and higher **HVU** of starved larvae (**S/F**) which can increase feeding rates of starved larvae. In fact starved larvae of mussels (Sprung, 1984) and clams (Gallager, 1988) have shown higher feeding rates than well fed larvae but these studies did not evaluate swimming activity.

Gallager (1991) estimated that the perceptive volume of a hovering mactrid larvae will be 40-fold greater than a swimming or sinking larvae, increasing the chances of encounter by a predator mechano-sensory mechanism as it feeds. It is likely then, that "hovering" is an adaptive mechanism to enhance feeding efficiency by bursts of feeding when enough food is available such as in conditions of stratification, although larvae may also feed in the nutrient diluted environment of a well-mixed water column. The periodic sinking of larvae will therefore diminish the risk of larvae being fed upon by predators but this needs further investigation.

All planktonic larvae confront somewhat similar constraints in the plankton and therefore have similar basic requirements. However the adaptive mechanisms to overcome the dangers of staying suspended, like finding food, avoiding predators and avoiding currents that would disperse larvae (Chia *et al*., 1984), are most probably dependent upon the life-history characteristics of individual species as determined by the location of adult habitats (Day and McEdward, 1984).

Helical ascent and descent have been typically described for a number of planktotrophic larvae of benthic invertebrates as a mechanism associated with feeding activity. The role of "passive sinking" on larval distribution has been overlooked and may have an important role on determining the extent of larval migratory capability at appropriate scales as it is proposed here. In my study, active swimming in helical patterns is most likely to be associated with efficient feeding mechanisms. Hovering is suggested as a mechanism for confining feeding activity to horizontally distributed food patches, such as those that would occur above pycnoclines as described by Tremblay and Sinclair (1990b). The powered climbing and sinking behaviours observed in my study may be the behaviours related with active depth regulation since it allows for faster changes on the larval position in the water column and these are identified as intrinsic mechanisms associated with active depth regulation.

Although my study encountered a number of limitations, it has provided the basis for further research on ecological and physiological aspects of larvae that have permitted identification of the early life-history of sea scallops as one of the most important components of the sustainability of populations, a fact that is beginning to be recognized by fisheries managers. Current studies using more sophisticated optic equipment and a double camera system to simultaneously evaluate helical patterns, swimming patterns and Net versus Gross displacement are already underway (Jackson *et al.*, 1994) yielding comparable values of swimming components and swimming speeds in our laboratory. The availability of software for tracking particles is a promising addition to the fast growth of microzooplankton research.

Even understanding the gross patterns of larval movements documented in this thesis and seen in the field requires a better discernment of locomotory and feeding mechanisms. If the change in helix angle in the presence of food documented here is purely a mechanical result of particle capture, or if changes in velocities in thermal gradients (Gallager *et al.*, in press) are directly linked to viscosity, the veliger's behaviours become more predictable from knowledge of design differences and physics alone.

CHAPTER 3

Variability of lipid classes through larval ontogeny

3.1 Introduction

Increasing concern for the sustainability of sea scallop populations of *P magellanicus* has prompted numerous studies on ecological and physiological aspects of adult individuals; by contrast, little information is available on the physiology and ecology of early developmental stages. This bias on the focus of research toward the adult component of the population has mainly resulted from difficulties associated with the capture and identification of larva in the field (Day and McEdward, 1984; Mann, 1986b; Tremblay *et al.*, 1987). At present the growing interest in marine invertebrates as potential candidates for aquaculture, and the recognition that a clear understanding of early life history is needed to assess the sustainability of benthic populations, particularly bivalves, have prompted the re-focus earlier life history stages. Many benthic species, particularly bivalves, can be successfully reared under laboratory conditions, and this has provided the opportunity to study larval fitness of various species in such conditions (Bayne, 1983).

Benthic invertebrates with feeding larvae (planktotrophic) usually produce smaller and more numerous eggs than those species with non-feeding larvae (lecithotrophic) resulting in a reduced parental investment per offspring (Strathmann, 1987). A common characteristic of benthic species with complex life histories is the development of planktotrophic larvae adapted to the acquisition of nutrient during their ephemeral journey in the plankton that results from simultaneous swimming and feeding activity.

Early observations of swimming activity of sea scallop larvae in microcosms (Chapter 2) indicated that larvae display helical patterns of swimming as well as almost linear ascent and descent throughout ontogeny. Subsequently I proposed two hypotheses,

the first, that feeding must occur during helical swimming and the second, that regulation of vertical distribution results from linear ascent and descent (Chapter 2). Testing of these hypotheses in nature is not yet completely feasible and would be an expensive venture. A logical next approach is to begin with smaller scale assessments that can be related to natural situations and that can focus on the variability of energy resources available at each developmental stage. These resources must cover the metabolic costs associated with swimming, feeding and differentiation of larvae in the plankton (Bayne, 1983)

Knowledge of several aspects of physiological energetics of bivalve larvae has been derived mainly from studies conducted to increase hatchery production of selected molluscan larvae for aquaculture purposes, primarily for oysters (Bayne, 1983). The constraints on the early life history of bivalves are those imposed by morphological and physiological adaptations in response to environmental variability (Pechenik, 1987). However, little is known about the variability that occurs across taxa, species, and even populations of the same species in their natural habitat (Price and Paffenhofer, 1984).

The role of lipid and protein reserves in providing energy during the initial embryonic development of benthic invertebrates, reserves which are supplied by parentally derived endogenous material in spawned eggs, has been frequently acknowledged in the literature (Millar and Scott, 1967; Holland and Hannant, 1974; Manning, 1985; Mann and Gallager, 1985; Gallager and Mann, 1986a,b; Whyte *et al.*, 1990a,b, 1992) In most studied bivalve larvae, lipids have been shown to be the major energy reserves (Holland, 1978), while carbohydrates and proteins are secondary (Millar and Scott, 1967; Bayne, 1972; Holland and Spencer, 1973; Holland and Hannant, 1974). Carbohydrates do not seem to play a major role on the provision of energy at any stage of development and remain relatively constant throughout the planktonic period of many of the species that have been studied (Holland and Hannant, 1974; Day and McEdward, 1984; Whyte *et al.*, 1987; Whyte *et al.*, 1990a,b, 1992).

For bivalves, the content of eggs provides the energy required for the initial embryogenesis until the formation of the feeding stage (Prodissoconch I or straight hinge larvae). From 50 to 70% of the total energy reserves in eggs, depending on species, has been found necessary for embryogenesis. Gallager *et al.* (1986) found that in two species of bivalves (hard clam, *Mercenaria mercenaria* and eastern oyster, *Crassostrea virginica*) most of the energy was provided by parentally derived lipids, while Whyte *et al.* (1987) found that both protein and lipids were equally important in the Japanese scallop (*Patinopecten yessoensis*). The only study on *P. magellanicus* found that lipids in a Newfoundland population, were also the primary source of energy (between 50 and 69%), but adult conditioning may influence this result (Manning, 1985). During and after the transition from the initial short lecithotrophic stage to full planktotrophy, further catabolism of endogenous reserves continues to occur in the Japanese scallop (*P. yessoensis*) larvae as long as 14 days after initiation of planktotrophy, during which time larvae appear unable to assimilate sufficient energy from algal diets (Whyte *et al.* 1987).

During the subsequent development of bivalve larvae in the plankton, distinct increases in shell deposition and velum diameter occur as well as an increase in organogenesis; at the end of this stage larvae will be prepared to enter the benthic stage (Cragg, 1989). Thus, the role of physiological adaptations of planktotrophic larvae throughout ontogeny in response to environmental variability need to be better understood. The relationship between acquisition and utilization of nutrients and the supply of required energy at different stages can vary depending on the life history stages of individuals and may have consequences on larval development.

Planktonic larvae swim and feed aided by the ciliary activity of the velum, and the energy acquired must be partitioned into the requirements for maintenance, swimming and growth. The likelihood of larval survival has been thought to depend mainly on larval fitness and the risk of predation (Korringa, 1941; Thorson, 1946). The possibility that low levels of larval fitness are related to starvation has been discussed extensively in the

literature, but more recently, the starvation hypothesis has found less support (Bayne, 1965; Holland and Spencer, 1973; Olson *et al.*, 1987; Olson and Olson, 1989) since larvae of many species seem to occur in the plankton during periods of high primary productivity (Himmelman, 1975; Starr *et al.*, 1990). Moreover, some invertebrate larvae can utilize bacteria and dissolved organic matter as alternative sources of food (Manahan and Crisp, 1982; Olson *et al.*, 1987; Manahan, 1990).

One method that can be used to determine larval fitness is to assess the energetic content of larvae as an indicator of the likelihood of survival, since metabolic requirements need to be met. However, the major problem is that of determining quantitative changes of biochemical components in such minute individuals (Holland and Gabbott, 1971). Larval condition is primarily influenced by food availability, but is also dependent on the changing levels of metabolic activity through development which may increase energetic demands of larvae. As earlier indicated, there is strong evidence indicating that either lipids or proteins or both play major roles in providing the energetic requirements of developing planktonic larvae. Many larval stages of fish, crustaceans and bivalves are known to store and use mainly lipids to fuel the energetic requirements (Holland and Hannant, 1974; Sasaki, 1984; Holland, 1978; Gallager and Mann, 1986a,b; Fraser *et al.*, 1987, 1988). Triacylglycerides in particular seem to vary greatly, reflecting the importance of this particular lipid class in the metabolism of early developmental stages (Holland and Spencer, 1973; Gallager and Mann, 1981,1986a; Delaunay *et al.*, 1992; Marty *et al.*, 1992;).

The availability of energy sources in a planktonic larva may determine the adaptive mechanisms used by species with complex life histories which switch niches during their life cycle. Moreover, switches from lipids as a source of energy during planktonic stages to carbohydrates as a main source of energy during adulthood are common (Holland and Hannant, 1974; Holland, 1978; Manning, 1985) reflecting the importance of available food in the plankton and benthos respectively.

Studies on the physiological ecology of Georges Bank scallops have focused on adult reproductive physiology (Dibacco *et al.*, 1995) and variability of lipid composition (Napolitano, 1991). Scallop adults are benthic filter feeders which obtain their energy from available phytoplankton and the lipid contents of female gonads have been found to show marked seasonality associated with reproductive activities (Robinson *et al.*, 1981; Napolitano, 1991). Most recently, a series of studies of larval distribution in the field have been reported (Tremblay, 1991) which also included the assessment of lipid content in some older larvae. However, long term studies in the field are impossible to pursue due to logistic constraints.

My study focused on the variability of lipid classes and lipid ratios of eggs and larvae of the sea scallop *P. magellanicus* from the Georges Bank population and includes the analysis of: unfertilized eggs, fertilized eggs, and 4, 13 and 22 days old larvae. The main objectives were to determine if ontogenetic changes in development are reflected in the lipid composition and to determine a larval condition index based on the triacylglycerol-sterol lipid ratio as proposed by Fraser (1989).

Marine lipids are energetically and biochemically important components in the functioning of marine animals (Joseph, 1982), and are characterized by a number of properties such as fluidity at low temperature, high degree of unsaturation, and a high content of non-saponifiable materials such as fat soluble vitamins (Sargent, 1976). Also, lipids provide a high energy reserve available in a concentrated form that may also confer enhanced buoyancy on planktonic animals in the marine environment. Neutral lipid in particular has a lower density than seawater, thereby conferring buoyancy that may provide a great advantage for life in the oceans (Sargent, 1976).

Current hatchery techniques and the possibility of obtaining live samples of adult scallops from Georges Bank provided the opportunity to examine several aspects of the physiological ecology of scallop larvae, including behavioural studies (Chapter 2),

vertical distribution of larvae in mesocosms (Chapter 4), and this study which includes an analysis of the variability of lipid classes through ontogeny.

3.2. Methods

3.2.1 Larval culture

Adult scallops *P. magellanicus* from Georges Bank were obtained from commercial scallop draggers and from the Department of Fisheries and Oceans research cruises in October, 1987 and 1988. Once in the laboratory, scallops were kept in running sea water at the AQUATRON facilities, Dalhousie University, and after 24 h, were fed ad-libitum on a mixed diet composed of *Isochrysis galbana* (strain TISO), *Chaetoceros calcitrans* and *Chaetoceros muelleri* every second day prior to induction of spawning.

All adults were in spawning condition as appraised by visual examination of gonads. Gonad coloration is evident in mature animals, females usually having a distinct intense orange colour and males having a characteristic ivory colour. Four female and three male individuals were selected based on the colour and degree of fullness of the gonad prior to spawning induction. The selected animals were placed in filtered seawater for two to three hours to allow elimination of faeces and were then cleaned thoroughly by scrubbing and washing the external shell in filtered seawater. All individual were then left exposed to air for 30 *min* before spawning. The general spawning procedure used was the one described by Couturier (1986) and therefore only general aspects are described here.

Adult scallops were separated and placed in individual 7 *l* plastic containers filled with 1 *µm* filtered seawater at temperatures between 3-5 °*C* above ambient seawater (14 °*C*). Spawning induction usually lasted 2-3 *h*, and the whole content of each bucket was replaced hourly to avoid accumulation of faeces and mucus.

Once spawning was initiated, eggs were sieved through a 102 *µm* Nytex® mesh to retain large particles and then collected in a 33 *µm* Nytex® mesh. Sperm were sieved

through a stack of two screens of 102 and 20 *µm*, with only the sperm that passed through both screens used. Gametes were kept in separate containers until artificially fertilized by gently mixing a small amount of sperm into all eggs spawned by the different females and leaving them undisturbed for 1 *h*. Prior to fertilization three samples of 10 *ml* each were taken to visually assess fertilized gametes prior to sampling for lipids and after fertilization to assess stage of development. Fertilized eggs were gently washed with fresh filtered seawater and incubated at a concentration of 30 *egg ml*$^{-1}$ in 120 *l* buckets. Gentle aeration was provided and all culture containers covered with a black plastic sheet during the complete rearing period. At day four, larval density was reduced to 5 *larvae ml*$^{-1}$ and a phytoplankton diet consisting of a mixture of *Isochrysis galbana* (strain TISO), *Chaetoceros muelleri* and *Chaetoceros calcitrans* was added at an initial concentration of 50,000 *cell ml*$^{-1}$. The ration was subsequently fed after each water exchange every second to third day. This mixed diet that contained phytoplankton in the size spectrum between 3-9 *µm*. This particle size range has been found to be common in Georges Bank phytoplankton during the fall (Rao Durvasula, S. Bedford Institute of Oceanography, Darmouth, N.S., pers. comm.).

All larvae were removed from each culture container at day four after fertilization and every second to third day thereafter to allow water exchange and cleanup of containers with a dilute bleach solution to avoid bacterial growth since antibiotics were not used. Samples of unfertilized eggs, fertilized eggs (after 1 *h*) were collected immediately after sampling. Samples of larval stages at day 4, 13 and 22 days after fertilization were collected after 3 *h* in filtered seawater.

3.2.2 Lipid classes analysis

Replicated samples of at least 100,000 unfertilized and fertilized eggs and larvae were obtained from four batches of eggs from different scallops and from two batches of larval cultures at days 4, 13 and 22 (Table 3.1). Collection of larvae after sieving into an

Table 3.1 Summary of developmental stages sampled for lipid contents in October 1987 and 1988 Each lipid sample was analyzed with 2 to 5 replicates.

Developmental Stage	Date	Samples
Non-Fertilized eggs	Oct 87	2
	Oct 88	2
Fertilized eggs	Oct 87	2
	Oct 88	1
4 day-old larvae	Oct 87	1
	Oct 88	1
13 day-old larvae	Oct 87	1
	Oct 88	1
22 day-old larvae	Oct 87	1
	Oct 88	1

appropriate Nytex® mesh was possible by gentle suction using a micropipette to collect eggs and transfer them with minimum amount of water into 20-40 *ml* glass vials.

Lipid extraction was performed in two stages. The first, in which lipids were fixed immediately after collection by adding a mixture of chloroform:methanol (2:1), and flushed with atmospheric nitrogen. All vials were covered with teflon lined caps, mixed and then stored at -17°*C*. The final extraction (Bligh and Dyer, 1959) was performed immediately before lipid analysis.

Lipid classes were qualitatively determined by thin layer chromatography (TLC) and then quantitatively determined by Iatroscan® thin layer chromatography flame ionization detection (TLC-FID) as described by Ackman (1981).

The separation of lipid classes was possible by using Prekote® silica gel plates (200 *μm* particle size) (Applied Science Laboratories, College Park PA, USA) previously cleaned by developing in ethyl acetate and activated by heating at 110° C for 30 *min*. The lipid extracts in chloroform solution were applied using disposable micropipettes (Microcaps®, Drummond Scientific Company, USA). The development of plates in solvent saturated glass tanks containing hexane:diethyl ether:acetic acid (80:20:1 v/v/v) allowed the visual identification under UV light of the lipid classes after the silica plates had been sprayed with 1% 2,7' - dichlorofluorescein solution in ethanol. Standards and standard mixtures were spotted beside the extracts.

The quantification of each lipid class was possible using an Iatroscan® analyzer (Iatron Laboratories, Inc., Tokyo, Japan) with a flame ionization detector (FID) after separation of lipid classes on Chromarods-SII® (Silica gel coating) (Sipos and Ackman, 1978; Ackman, 1981). Before chromarods were spotted with samples, used chromarods were pre-soaked overnight in 30% nitric acid, washed and blank scanned on the FID of the Iatroscan® analyzer. One to 5 *μl* of chloroform solution containing the lipid extract were applied to Chromarods-SII® with Drummond disposable micropipettes. The frame containing the 10 chromarods was then placed into a solvent saturated glass chamber

containing hexane:diethyl ether:formic acid (97:30:1.5 v/v/v). Developed rods were oven dried at 110 °C for 3 *min* to evaporate the solvents and then transferred to the scanning frame of the Iatroscan® analyzer. A mixture of triacylglycerides from fish oil was used as a lipid standard, using a total of 20 μg per rod.

The scanning procedure was performed as follows:

- hydrogen flow rate: 160 $ml\ min^{-1}$,
- air flow rate: 2,000 $ml\ min^{-1}$,
- voltage range of the detector: 8 mV full scale and,
- scanning speed: 0.42 $cm\ s^{-1}$.

Peak areas were recorded and integrated on a Spectra Physics SP 4200® computing integrator.

Data obtained on the lipid composition at each developmental stage represent the average of two batches of eggs and two batches of larvae, each batch having between two and five replicates. All data are presented as % wet weight of the total lipids extracted as obtained from the printout of each analysis and, therefore, must be considered approximate values. Statistical analysis of data using the Rodger's (1974,1975) method of analysis of variance and comparisons were performed and are shown in Appendix B4.

3.3 Results

The lipid composition of eggs and larvae of sea scallops spawned and reared under laboratory conditions exhibited high variability of the three main lipid classes. Triacylglycerides (TG), phospholipids (PL), and sterols (ST) were easily identified. Minor traces of sterol esters, free fatty acids, and hydrocarbons were also identified in some samples and they were pooled as other lipids (OL). Examples of chromatograms

showing the separation of total lipid from sea scallop eggs and larvae are depicted in Fig. 3.1.

Observed lipid composition varied significantly throughout development (Fig 3.2) as shown by the results of one way ANOVA performed for all the lipid classes (Appendix B4). Immediately after fertilization of eggs, Triacylglycerides and Phospholipids remained relatively unchanged with respect to unfertilized eggs while sterols increased slightly from 3% to 4% (Fig. 3.2).

The most distinctive feature was the high relative content of TG of maternal origin in both unfertilized (43%) and fertilized eggs (44%) which was subsequently reduced to 29% of total lipids as found in 4 days old larvae (Appendix B4). This latter stage corresponded to the first shelled stage, and was reached in the absence of added food.

The opposite trend was found to occur for phospholipids which increased from 43% to 59% of the total lipids, which is inversely comparable to the TG losses (Fig. 3.2). The proportion of sterols also increased from 3% to 4% of total lipids with respect to the initial content of unfertilized eggs.

Veliger larvae that had been fed for nine consecutive days after the first feeding showed a further decrease of TG content with respect to unfertilized eggs at day 13 to 8% of total lipids, which was the lowest level detected for this class. PL and ST continue to increase to 71% and 6%, respectively (Appendix B4). These findings indicate that larvae have continued to catabolize TG in spite of being fed, as was also found by Whyte *et al* (1987) in larvae of the japanese scallop *P. vessoensis*.

Larvae sampled at 22 days had been fed continually for 18 days and at this stage TG increased again to 20%. Values for phospoplipids increased to 71% from fertilization to day 13, then decreased slightly to 65%; sterols increased from fertilization through to day 22 (Fig. 3.2).

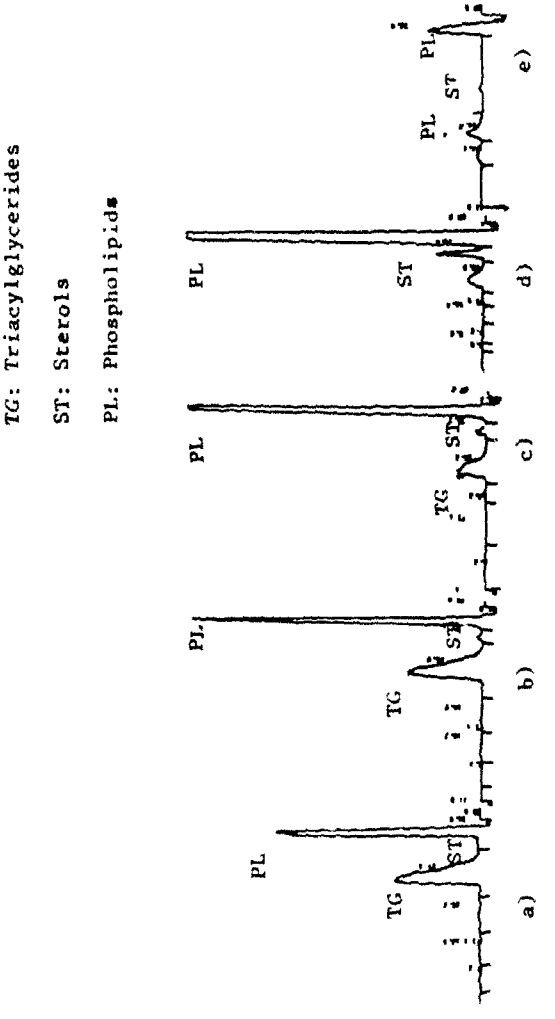

Figure 3.1 TLC chromatograms showing lipids classes in non-fertilized eggs (a), fertilized eggs (b), 4 day old larvae (c), 13 day day old larvae (d), and 22 days old larvae (e). The developing direction is from left to right and all chromatograms were recorded at an attenuation of 8 mV- full scale deflection.

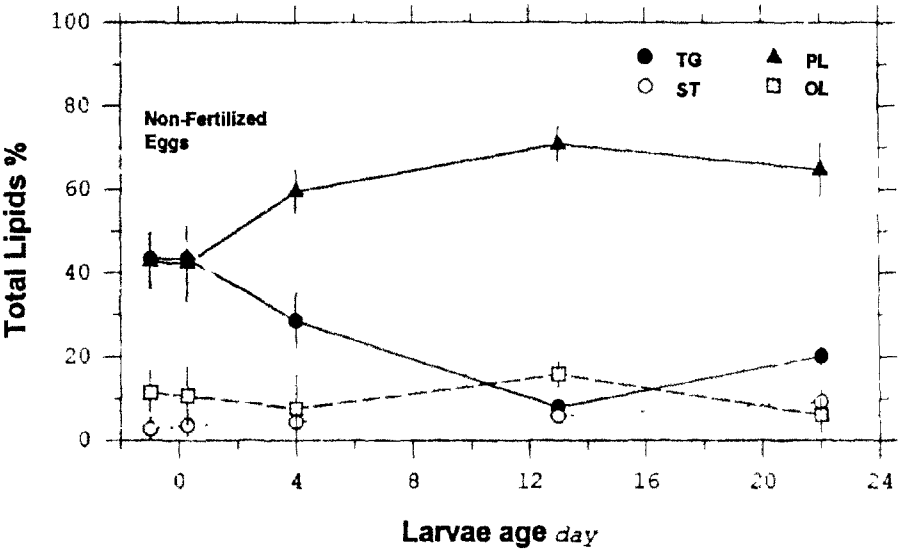

Figure 3.2 *Placopecten magellanicus*. Variation of individual triacylglycerides (**TG**), phospholipids (**PL**), sterols (**ST**) and other lipids (**OL**), expressed as percentage of total lipid found during the development of scallop larvae, from unfertilized eggs to 22 day old larvae.

Thus, scallop larvae had a relatively low proportion of TG though the first half of the larval period with TG increasing toward the end of the larval period The observed variability in lipid composition (Fig 3.2) indicates that neutral lipids (TG) were being metabolized to greater extent during the ontogeny of scallop larvae and therefore TG may indeed play a major role in larval energetics as found earlier in other bivalve larvae (Gallager *et al.*, 1986 a,b), and crustacean larvae (Sasaki, 1984)

Triacylglycerides content is directly affected by exogenous food intake and theyare rapidly catabolized when larvae are nutritionally stressed, however knowledge of TG content is required to assess the viability of larvae. Since it is always difficult to determine the weight of bivalve larvae, it is reasonable to use the method proposed by Fraser (1989) to express the variability of tricaylglycerides based on another lipid class that remains relatively constant as is the TG/ST ratio

The TG/ST ratio decreased through development from 16.7 in unfertilized eggs to 1.4 in 13 day larvae before increasing slightly to 2.2 at 22 days (Fig. 3.3) The TG/PL ratio also decreased through development from unfertilized eggs (1.02) to 13 day (0.11) larvae before increasing slightly at 22 days (0.31) The ratio of PL/ST decreased from 16.4 in unfertilized eggs to 7.0 in 22 day old larvae (Fig 3.3)

3.4 Discussion

The early development of many marine organisms is characterized by a post-hatching period during which the larvae are largely dependent on endogenous energy reserves, mainly in the form of storage of lipids. Triacylglycerol (TG) is the major lipid class stored in animal cells (Lehninger, 1975) The relative content of TG is usually depleted during the early stages of larval development, until the energetic demands of growth and metabolism are met from exogenous sources as has been found in several species of fish larvae (Fraser *et al*., 1987, 1988), lobster larvae (Sasaki, 1984) and bivalve larvae (Gallager *et al.*, 1986 a,b) Fraser (1989) reviewed the tricylglycerol content as a condition index for fish, bivalve and crustacean larvae and found that TG begins to be

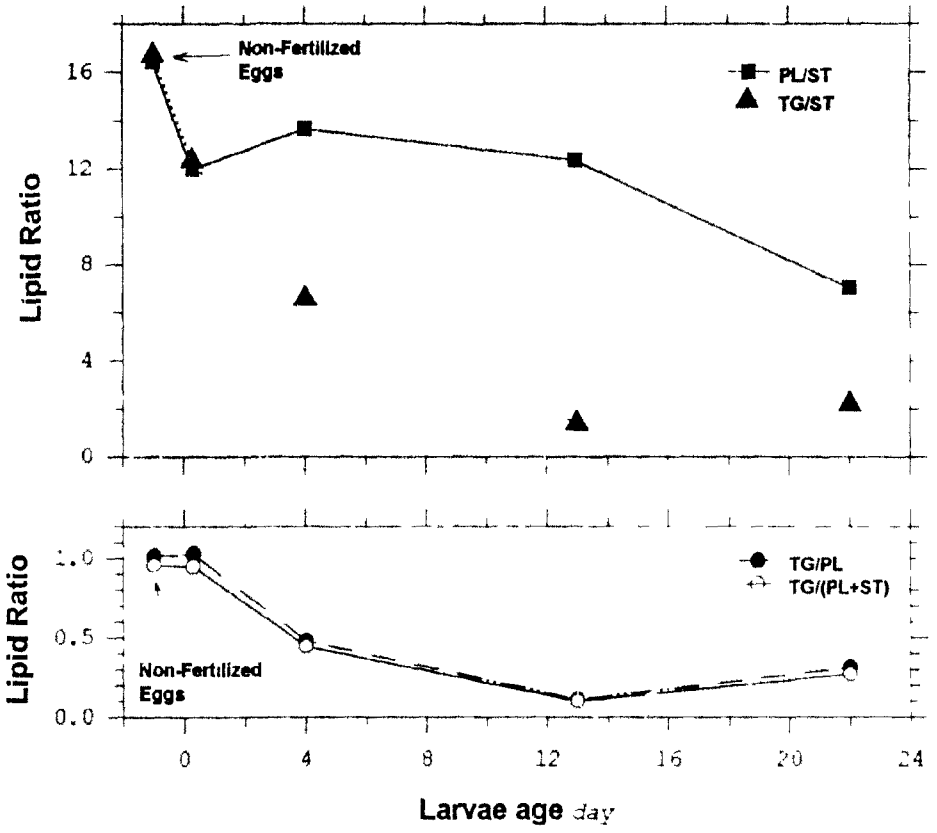

Figure 3.3 *Placopecten magellanicus* Variation of lipid ratios **PL/ST, TG/ST** (top panel); **TG/PL** and **TG/(PL+ST)** expressed as percentage of total lipid during the development of scallop larvae

stored only when exogenously derived energy exceeds the immediate metabolic demands of a larvae as previously found in fish larvae by Ehrlich (1974) and Fraser *et al.*, (1987).

Identifying the composition of lipid classes of developmental stages of scallop larvae and the determination of the relative proportions of TG, ST and PL, has served to recognize the potential importance of individual lipid classes in the basic metabolism of planktonic stages of a benthic invertebrate. My study showed that triacylglycerides were an energy source throughout the entire planktonic period

Initially, eggs contained almost equal proportions of both triacylglycerides and phospholipids; but only TG subsequently showed a significant decrease. The variability of lipids, and in particular the lowest level of TG observed at day 13, is similar to the decrease of lipids found in P yessoensis (Whyte *et al.*, 1987). These authors suggested that the high lipid catabolism may have been the result of lack of feeding due to an incomplete gut system It is also possible, however, that it reflects increasing energy requirements to satisfy swimming activities.

The relatively consistent increase in the concentration of PL through larval ontogeny is a good indicator of healthy larval growth, since there is no indication of polar lipids being catabolized, with the exception of the small decrease at the last sampled stage Similarly, the concentration of ST shows a clear trend to increase through development from 4% to 9% between days 4 and 22. Phospholipids and sterols are structural components of cell membranes and represent a constant proportion of the wet weight of a broad range of eukaryotic organisms (Nes, 1974; Fraser *et al.*, 1987). Their increase reflects cellular proliferation. Furthermore, Fraser (1989) suggested that sterol content will correlate highly with the dry weight of bivalve larvae for a wide range of species. Although phospholipids also correlate highly with dry weight of Atlantic herring larvae, he suggested that sterol content is easier to quantify.

The similarities between egg composition of unfertilized and fertilized eggs are mainly related to the maternal lipid condition. The variability of lipid composition

through development however, is the result of the interaction of several factors: phytoplankton diet, larval growth, and swimming activity. All developmental stages were reared under similar condition and fed equal amounts of the same mixed phytoplankton diet, therefore changes should reflect only the normal development of larvae in hatchery conditions.

The onset of ciliary feeding starts with the formation of the velum and is known to occur in sea scallops between the third and fourth day (Culliney, 1974). Further growth also implies a larger velum and a denser shell (Cragg, 1989). In Chapter 2, I showed that early veligers between the ages of 10 and 16 days increased their helical swimming speeds. It is tempting to link the lowest level of triacylglycerols found at day 13 to the higher energy requirements for these swimming activities. Since all examined larvae originated from a single population (i.e. Georges Bank adults) with similar timing of development, further studies of populations with different timing will could use simultaneous analyses to resolve this feature.

Since larvae generally accumulate fatty acids from the phytoplankton diet (Whyte *et al.*, 1989, 1990a,b), further studies to characterize the larval fatty acid components of lipids will be necessary to assess the fatty acid oxidation that provides the main source of energy. Of particular interest will be a comparison of scallop larvae from different locales and also within and between different spawning seasons. A comparison of laboratory reared larvae with those found in the plankton should be of interest to assess the physiological condition of larvae. The only study on fatty acid composition of the lipid classes of sea scallop eggs found no significant differences between shallow and deep water scallops located in Newfoundland, but this finding only reflected the lipid content of female gonads (Napolitano *et al.*, 1992).

The proportions of both main lipid classes (TG and PL) in eggs of Georges Bank were near 43% while Napolitano *et al.* (1992) found that Newfoundland scallops eggs have a higher proportion of TG (~60%) than PL (~39%). Furthermore, the proportion of

ST determined here for Georges Bank eggs is almost five fold larger (2.59%) than those from Newfoundland (~0.57).

Napolitano (1991) found that the relative proportions of TG, PL and ST in female gonads of GB scallops vary throughout the year, but generally higher concentrations of PL were found in the gonad during winter, spring and summer. The only season in which the proportions of both PL and TG are relatively similar, near 50%, was during the fall which corresponds to the time in which adult individuals readily spawned eggs after thermal stimulation in the laboratory.

In my study, the focus was on the analysis of lipid variability that will reflect the development of sea scallop larvae in laboratory conditions, fed a high quality diet in order to assess the importance of the various lipid classes throughout ontogeny. The hatchery conditions outlined by Couturier (1986) were appropriated for this study and the interference with larval development was kept to a minimum. Conditions of temperature and salinity were kept constant and were comparable to conditions known to occur in Georges Bank at the time that larvae are present in the plankton. Delaunay *et al* (1992) found that larvae of *Pecten maximus*, which usually settle and begin metamorphosis between the 23 to 25 day of culture at 18 °C also varied in lipid composition through development, in some cases with very low TG concentrations toward the end of the planktonic life However, since they focussed on the maximum production of larvae, a variety of diets were used and may not therefore reflect the natural condition of larvae.

There are many factors that could affect the lipid composition of larvae that should also considered in further studies to make comparisons among species and across taxa feasible, including: the origin of adults, diet provided in the laboratory, time of the year, culture conditions, volume of culture containers, and the use of antibiotics that could preclude the availability of bacteria that may cause variation in larval condition.

A further study of lipid composition should include the analysis of fatty acid composition in relation to light cycle and feeding. Until now it has been thought that

planktonic bivalve larvae are continually feeding and swimming but the level of activity has not been determined between day and night. All analyses of larval lipids reported in this study were on larvae collected during the day, as well as observations of behaviour in Chapter 2, but it would be necessary to assess swimming rates and lipid content during the night. Only vertical distribution of larvae was determined during night samples and larvae were found to migrate upwards in the simulated mesocosms (Chapter 4).

Negatively buoyant organisms must swim to remain in the same vertical position until they accumulate sufficient lipid reserves through feeding activity to achieve neutral buoyancy (Sargent, 1976). Therefore, the presence of lipid reserves serves a dual role as a source of both energy and buoyancy. Consequently, in conditions of high availability of food, lipid reserves would be enhanced, probably also increasing buoyancy. Raby *et al.* (1994) found a higher feeding index in bivalve larvae located higher in the water column during the night than during the day in both stratified and mixed water conditions. The same authors also found that the vertical position of larvae overlapped the distribution of food during the night. Buoyancy of larvae was not determined in their study, although it is possible that increased buoyancy may be one of the consequences of nocturnal feeding up in the water column. Furthermore, the same authors found that diurnal descent coincided with a lower feeding index. The pattern of vertical distribution of early veligers in a series of mesocosms simulations (Chapter 4) was similar to that of the Baie de Chaleur study. Although a feeding index was not calculated, the similarities of behaviour are striking. Furthermore, larvae that have food available in the water column of a mesocosm performed small-amplitude vertical migrations while those in the absence of food did not (Chapter 4). It is possible that larval migration would only occur when enough food is available to resupply energy requirements during swimming. Further research in this area will be needed to assess the feeding condition of larvae at different levels of activity. It will also be necessary to evaluate the response of larvae in mesocosm simulations in the presence of predators.

In addition to smoothing out daily fluctuations in energy demand and supply, TG is accumulated to fuel metamorphosis. Catabolism of TG during metamorphosis is known to occur (Holland and Spencer, 1973) which reflects the reliance on endogenous reserves during this period in which larvae do not feed (Bayne, 1965, Hickman and Gruffydd, 1971), although this study did not extend to this late stage.

Several studies on the metabolism of mollusc bivalves have shown that while planktonic larvae have essentially a lipid based metabolism, benthic post-metamorphic stages switch to carbohydrate based metabolism. The adaptive significance of this strategy is, in part, related the fact that lipids yields a greater quantity of metabolic energy per unit weight that does carbohydrate or protein (Sargent, 1976), but there is also the additional advantage of buoyancy regulation. Apparently only lipid will satisfy the energetic requirements of a highly active larva in the plankton but other resources will serve for less active benthic stage

CHAPTER 4

A mesocosm approach to assess vertical distribution of sea scallop veligers in response to environmental variability

4.1 Introduction

Planktonic organisms display numerous adaptations to complete their life cycle and successfully respond to their immediate needs of locating and gathering food, remaining suspended in the water column, dispersing to new areas, navigating, and avoiding predators or unfavourable conditions (Chia *et al.*, 1984). Meroplanktonic stages of benthic invertebrates encounter the same challenges as holoplanktonic organisms during short periods of time before switching to benthic life, nevertheless, little is known about their underlying mechanisms of adaptation.

Forward (1988) reviewed the occurrences of vertical migrations of zooplankton and found that they are commonly described as the result of either behavioural responses to exogenous factors such as light, gravity, temperature, salinity, oxygen and hydrostatic pressure or endogenous changes in behaviour and physiology attributed to age, condition, and/or biological rhythms. Almost all zooplankton species are known to undergo diel vertical migration to some extent (Forward and Hetler, 1992). Three general patterns have been identified: nocturnal, twilight, and reverse migration. Although there seems to be a considerable variation between and within species, the most often described pattern of vertical migration corresponds to nocturnal diel migration characterized by a single daily ascent to a minimum depth reached between sunset and sunrise and a descent to a maximum depth during daylight hours. Another common pattern is twilight migration with ascent just before sunrise and sunset and descent during the night and day (Forward, 1988).

Highly evolved sensory and locomotory capabilities have allowed holoplankton to adapt to a permanent planktonic life. It is therefore expected that ephemeral

meroplanktonic organisms must develop comparable capabilities if they are to survive. This transitional period in the life history of an estimated 70% of benthic invertebrates (Thorson, 1950) must contain significant ecological advantages for the sustainability of benthic populations of adults of reduced locomotion. However, there is limited information on the vertical distribution of bivalve larvae and larval adaptive traits to environmental variability.

Bivalve larvae are generally small (<300 μm), and at certain times of the year they are important components in the planktonic ecosystem (Jorgensen, 1981). Most larvae undertake vertical movements in the water column, this may result from ontogenetic changes in their photoresponse (Thorson, 1964), but little is known about the underlying physiological and behavioural mechanisms used by planktonic larvae to secure a successful switch of niches and ultimately sustain the temporal and spatial continuity of benthic populations. Hence, research on the early life history of benthic invertebrates is needed to assess the capability of larvae to respond to environmental changes, which must focus on studies at appropriate scales to better understand the role of planktonic stages in determining their vertical distribution.

One of the main difficulties in obtaining data on field distribution of bivalve larvae has been the problems associated with larval identification from plankton samples, and the lack of long-term research programs for meroplanktonic larvae of invertebrates. The distribution of identifiable bivalve larvae has been generally thought to result from passive mechanisms driven mainly by local oceanographic circulation (Korringa, 1952; Boicurt, 1982; Seliger *et al.*, 1982). Some studies have proposed a second alternative explanation which considers the capabilities of active depth regulation of veligers in response to environmental stimuli inherent to planktonic development (Nelson, 1911; Nelson, 1953,1955, Carriker, 1951,1961; Kunkle, 1957; Haskin, 1964; Wood and Hargis, 1971; Mann and Wolf, 1983; Mann, 1986a). This last hypothesis has been given less consideration. The conflicting views arise from the different scales taken into account by

field and microscale studies. Moreover, the role of "active" and "passive" mechanisms in the distribution of bivalve larvae or any other planktonic organism cannot be determined easily and continues to be a major challenge in larval ecology (Mann, 1988a,b). Mann (1986a) recognized that both active depth regulation (swimming) and passive mechanisms (*i.e.*: major circulation patterns) contribute to larval distribution of bivalves; however, relative contributions are difficult to assess.

Several studies have found evidence of diel vertical migrations of bivalve larvae, such as in a shallow coastal area of Nova Scotia (Harding *et al.* 1986). Here, a diel pattern of vertical migration occurred, with the median depth of the population lower in the water column by day and close to the surface at night. In another study of vertical distribution of a broad range of planktonic organisms in the Western Irish Sea, Scrope-Howe and Jones (1986) counted bivalve larvae during sampling series in mixed isothermal and stratified water column conditions. Lamellibranch veligers, not identified, in mixed isothermal conditions exhibited various vertical movements or moved toward a particular depth in the water column at night. In stratified waters, larvae had either the same behaviour as during the mixed time series or they moved upward toward the surface at night.

Using laboratory reared larvae, Kaartvedt *et al.* (1987) used 4 *m* deep plastic enclosures (12 *m*) to study the vertical distribution of *Pecten maximus* larvae. Kaartvedt found a pattern of larval migration to shallower depths at night, based on a positive correlation between larval depth and the amount of incident radiation, and a negative correlation between vertical dispersion and radiation.

Vertical distribution data on offshore bivalve larvae are rare, although a unique series of field studies has been reported for larvae of *Placopecten magellanicus* (Tremblay and Sinclair, 1990a, b, 1992; Tremblay, 1991) in the Georges Bank area and in the Bay of Fundy (Northwest Atlantic). Sea scallop larvae were identified (Tremblay *et al.*, 1987) and found to be distributed throughout the water column in well-mixed waters

and aggregated above the pycnocline in stratified areas in Georges Bank (Tremblay and Sinclair, 1990b,1992). However, in an outer area of the Bay of Fundy, there were only small amplitude changes in the vertical distribution where larvae appeared to undertake diel vertical migration (Tremblay and Sinclair, 1990a).

Larval dispersal is thought to depend primarily on endogenous factors inherent to the length of larval life and larval behaviour and on exogenous factors that correspond to the hydrographic regime that larvae encounter (Young and Chia, 1987). Horizontal distribution of larvae seems to be mainly driven by physical forces (Robinson *et al.*, 1991); nevertheless, there has been growing recognition that planktonic organisms may be able to regulate their vertical distribution. Earlier studies have shown that bivalve larvae display both a helical pattern of swimming that may enhance feeding activity but does not significantly alter the larvas' vertical position, as well as passive sinking and ascent in vertical lines that could significantly affect their vertical position (Chapter 2). Veliger larvae swim and feed using the ciliated velum while ascending and sinking in microcosms throughout development prior to metamorphosis (Bayne, 1963, 1964; Cragg and Gruffydd, 1975; Cragg, 1980; Mann and Wolf, 1983). Numerous environmental variables appear to modify this behavioural pattern, such as light (Thorson, 1964); salinity (Haskin, 1964; Hidu and Haskin, 1978); temperature (Bayne, 1964; Verwey, 1966); and pressure (Bayne, 1963; Cragg and Gruffydd, 1975; Guan, 1993). During my short term study of the effect of food availability and larval feeding conditions (Chapter 2), larvae appeared to respond to the presence of food by increasing the diameter of the helical pattern. This response indicated the capability of larvae to modify their behaviour in response to biological changes. Behavioural changes could certainly occur in natural environments and could enhance the retention of larvae in areas of high productivity.

It is not possible to define the large scale processes of the open ocean without *in situ* observations, but small scale laboratory studies have provided a description of species-specific traits that may contribute to the understanding of vertical distribution of

planktonic organisms and thus influence dispersal and recruitment (Sulkin, 1986) The lack of comprehensive understanding of the planktonic distribution of larvae is most probably due to the absence of studies that consider both alternatives, "passive and active processes" in the design of sampling and analysis of vertical distribution of larvae.

In an attempt to understand the coupling of active locomotory and feeding capabilities of larvae in relation to passive environmental constraints imposed by natural processes, I developed this study as a logical next step in the assessing of vertical distribution of scallop larvae in a series of mesocosm simulations. Considering the behavioural traits observed earlier (Chapter 2), the response of larvae to exogenous factors were assessed from observed vertical distributions. The feasibility of rearing larvae of *P. magellanicus* in the laboratory and the availability of one of the largest mesocosms in the world at Dalhousie University provided a unique opportunity to design a series of vertically mixed and vertically stratified water columns to include the effects of a diel light cycle, food availability, and salinity stratification.

These studies initiated a comprehensive research program at the Biology Department of Dalhousie University to assess the capability of larvae from different locales to regulate vertical distribution through the planktonic stage. Here, only larvae originated from sea scallops from Georges Bank were used as a test case to address the following questions:

i. Is there an observable pattern of vertical distribution of sea scallop larvae through a diel light cycle?
ii. Does a diel light cycle coupled with food availability have any effect on the vertical distribution of sea scallop larvae?
iii. Do diel light cycle, salinity stratification and food availability at two haloclines affect the vertical distribution of sea scallop larvae?

Vertical distribution of *P. magellanicus* larvae in these mesocosm simulations can either remain uniform throughout the water column or vary according to the simulated

environmental conditions in each case. If it remains constant it will be possible to hypothesize that larvae are acting as inanimate particles and do not have the capability to alter vertical distribution under the given conditions. On the other hand, if larval vertical distribution changes throughout the simulations and within profiles, it will be possible to substantiate the hypothesis proposed in Chapter 2 that larvae have the capability to actively regulate vertical distribution. The observed vertical distribution of larvae found in each of nine mesocosm simulations will be evaluated and discussed in the context of the overall role of larval swimming capability (active swimming) within the temporal and spatial scale of this study and with those in the literature.

4.2 Methods

4.2.1 Mesocosm facilities

A concrete tower of 11.7 *m* capacity and 10.4 *m* depth by 3.6 *m* internal diameter (Balch *et al.*, 1978) (Fig. 4.1) was used in my study. Seawater is pumped from the Northwest Arm of Halifax Harbour, passing through Graver sand pressure filters that remove particles larger than 50 *μm*. Prior to enter the Aquatron tower the seawater line was further filtered to remove particles larger than 1*μm*, by flowing seawater through a set of 16 filter cartridges of 1 *μm* mesh. Light is provided from six overhead lamps, four phosphor-coated metal halide lamps (1000 *W*) and two mercury lamps (400 *W*) that provided a maximum illumination equivalent of up to 40% of the mid-day summer light at this latitude (44°N) (Balch *et al.*, 1978). Two sets of light timers provide successive decreasing or increasing light conditions, simulating dusk and dawn effects while maintaining a diel cycle of 12.12 *h* corresponding to 7 am: 7 pm normal day time. After the first mesocosm simulation, the air cooling system was turned on to diminish the effect of heat generated by the light system on the surface of the water column.

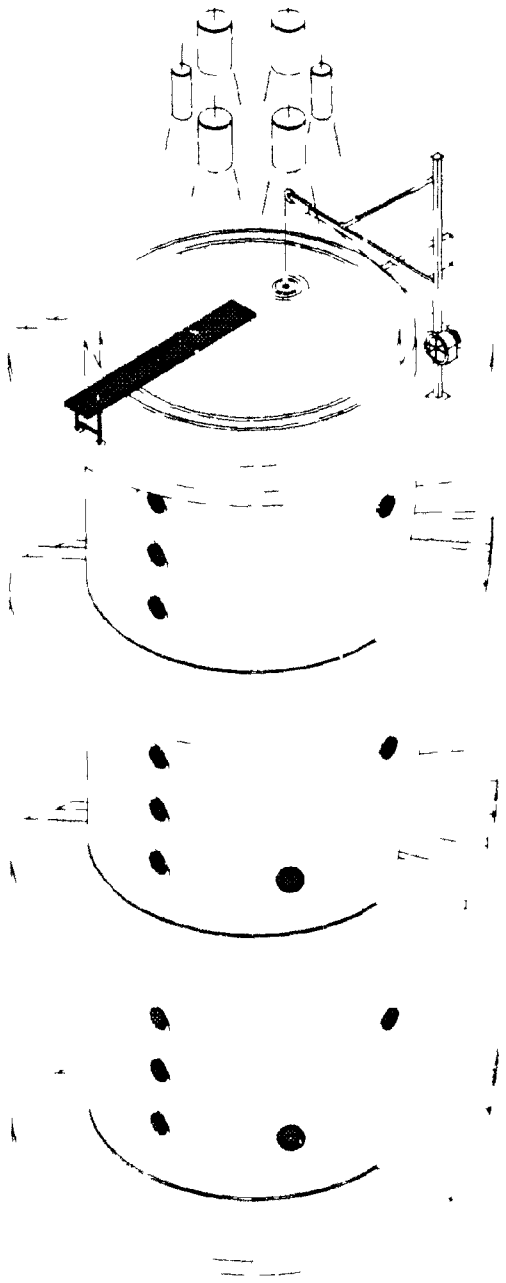

Figure 4.1 The 10 *m* vertical mesocosm at Dalhousie University. Shown are glass viewing ports, overhead lightning system and spiraling staircase used to access sampling ports

4.2.2 Larval culture

All larvae were obtained by inducing spawning of mature scallops from the Georges Bank area, followed by artificial fertilization of gametes and larval rearing in the Aquatron facilities at Dalhousie University, Halifax, N.S., as indicated in Chapter 2. During the rearing phase, all eggs and larvae were grown and maintained in 1000 *l* insulated XACTICS tanks at constant temperature of $14 \pm 1°C$ with gentle aeration. The eneral schedule of water exchange, feeding, and sampling was kept as described in Chapter 2.

A mixture of three species of phytoplankton at an initial concentration of 50,000 *cells ml* $^{-1}$, was added every second to third day from day four after fertilization. All larvae utilized for mesocosms simulations were harvested between 4-10 days after fertilization and were held for 4-6 hours in freshly filtered seawater (1 μm) in the absence of food prior to being placed into the mesocosm.

Four species of phytoplankton (*Chaetoceros muelleri, Isochrysis galbana, Chaetoceros calcitrans* and *Thalassiosira pseudonana* (3H)) were axenically grown (Guillard and Ryther, 1962) and harvested during the exponentially growing phase. During 1988, only the first three species were used. During 1989 *T. pseudonana* was used instead of *C. calcitrans*. The selected diet was added at the beginning of the simulations and every second day thereafter until the experiment ended.

4.2.3 Experimental design

To investigate the vertical distribution of sea scallop veligers and their response to the water column structure, I designed five experimental studies to record the vertical distribution of larvae under the following conditions (Table 4.1): 24 *h* (12:12) diel light cycle in January, 1987 (**S1**), diel light cycle in the absence of added food (**S2**) and patchy food at the surface layer in September, 1988 (**S3**), diel light cycle and surface patchy food (**S4**) and uniformly distributed food in December, 1989 (**S5**); diel light cycle, salinity

Table 4.1 *Placopecten magellanicus* Summary of all experimental mesocosm simulations in 10 *m* water column

Simulation		Date	Larval Concentration x 10^6	Larval age *days*	Profiles	Sampling Period *h*	Sampling Intervals *h*	Sampled Volume *l*
Diel Light Cycle (DCL)	1 No Food	Jan 87	3.27	7	5	24	4 - 6	20-40
DLC	2 No Food 3. Patchy Food	Sep 88	0.82	9	6	72	12 - 24	40
DLC	4. Patchy Food 5. Uniform Food	Dec 89	1.11	10	11	108	4 - 12	20
DLC Salinity Stratification	6. Food at 31‰ 7 Food at 33‰	Aug 89	1.36	4	5	60	12	7-20
DLC Salinity Stratification	8. Food at 31‰ 9. Food at 33‰	Oct 89	2.10	8	7	84	12	7-20

stratification, and food availability at two distinct haloclines at 31‰ (**S6**) and 33‰ (**S7**) in August, 1989 (in this study a dye was added to the haloclines to identify thickness) repeated without dye in October, 1989 (**S8** and **S9**, respectively).

During the first simulation (**S1**) the whole mesocosm was used (117 m^3) as one experimental column (Fig. 4.2). All other experimental mesocosms were run using half of the vertical tank (58.5 m^3) for each experimental situation. A flexible divider (polyethylene sheet) was installed to physically separate two water columns of 58.5 m^3 allowing two simultaneous experimental situations (Fig. 4.2).

The first experimental simulation (**S1**), investigated the effect of diel cycle in a vertically homogeneous water column. The vertical tank was filled with 50 μm sand-filtered seawater, the light cycle was set at 12:12 h (light:dark) and 3.27 x 10^6 larvae were added over the surface layer. A total of 5 profiles were obtained from integrated samples using an "octopus sampler"

The second simulation (**S2, S3**) investigated the effect of light cycle and food added to 1 μm filtered sea water. During September, 1988 one experimental column contained only larvae in 1 μm filtered seawater (**S2**) and the other contained food added by gravity to the upper 2 - 3 m of the water column (**S3**) by gravity 3 - 4 h prior to the first and fourth profiles sampled. During December, 1989, one experimental column contained larvae and patchy food added by gravity in the upper 2 m (**S4**) and the other contained larvae and uniformly distributed food (**S5**). A total of 0.82 x 10^6 *and* 1.11 10^6 larvae were added into each column in September, 1988 and December, 1989, respectively

The last simulation series aimed to investigate the combined effect of diel light cycle, salinity stratification and food availability during August, 1989 (**S6, S7**) and October, 1989 (**S8, S9**). In both cases the salinity stratified water column contained a stepwise gradient of predetermined salinities from 30‰ to 34‰ from top to bottom. The salinity gradient was developed slowly by adding brine to 1 μm filtered 32‰ seawater

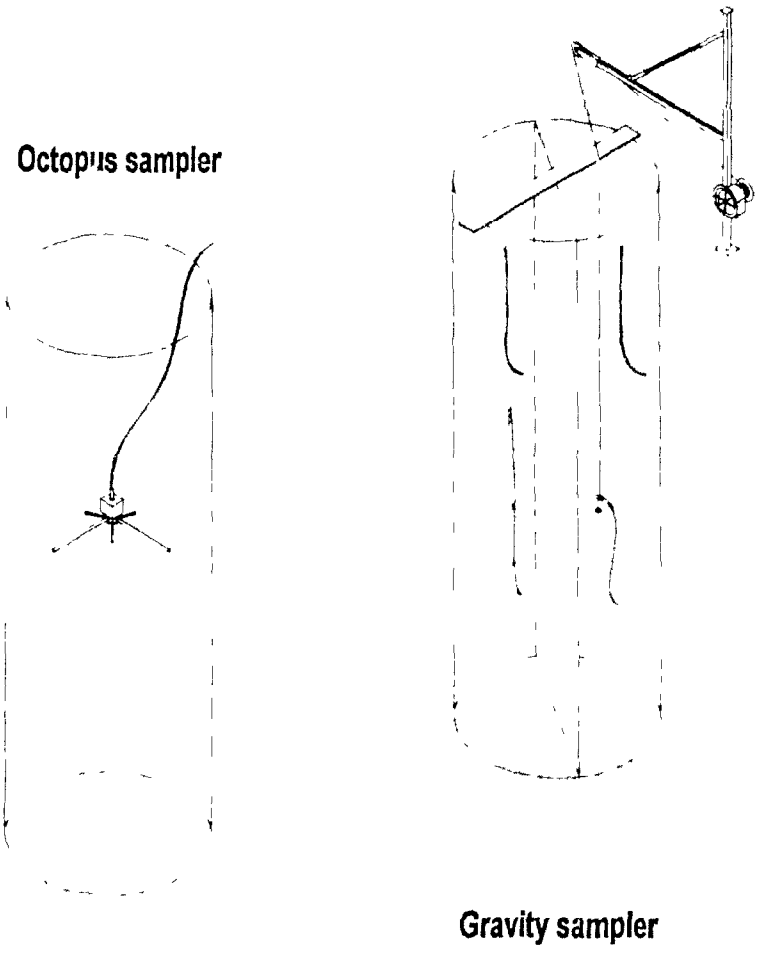

Figure 4.2 Diagrams of the vertical tank with the sampling systems. "Octopus sampler" used during **S1** (left) and the vertically divided tank with a gravity sampler system (right).

until the 34‰ layer (bottom) layer was obtained. The middle layer was slowly layered on with normal 32‰ salinity seawater flow ; onto a sheet of floating plywood to avoid mixing. The top 30‰ layer was obtained by mixing normal salinity water with filtered dechlorinated water. By carefully monitoring salinity and water flow, two columns were simultaneously constructed with a salinity gradient consisting of three well defined uniform horizontal layers (30, 32 and 34‰) Two varying microlayers averaging 31 and 33 ‰ of approximately 0.5 *m* height were formed at haloclines as a result of the slow mixing and could easily be detected by salinity readings. During August 1989, 100 *ml* of diluted Rhodamine dye (2%) was added at haloclines in 31 and 33‰ water to help visualize their height and location but no dye was added during October, 1989. A total of 1.36 x 10^6 (August, 1989) and 2.10 x 10^6 (October, 1989) larvae were siphoned by gravity into the centre (5 *m*) of each middle layer (32‰). A mixture of phytoplankton was made available by siphoning by gravity a similar amount into the 31‰ microlayer in one column (**S6, S8**) and at the 33‰ microlayer on the other column (**S7, S9**).

4.2.4 Sampling procedure

Water and larval samples throughout the mesocosm simulations were obtained using two physical sampler types; an "octopus sampler" activated by an electric surface pump during the diel cycle simulation (**S1**) and a gravity sampler in all others.

The "octopus sampler" used during the first simulation (**S1**) consisted of an array of six sets of 1.5 *cm* (diameter) PVC pipes of different lengths all connected to a central pipe which connected to an extension hose and to an electric pump (Fig. 4.2). The "gravity sampler" used during all other simulations consisted of two sets of 4 *m* long flexible hose (2 *cm* diameter) with one end attached to a valved sample port through the wall of the tank and the other end attached to a weighted "T" joint, secured to a metered rope that allowed an operator at the surface to manoeuvre the hose during sampling at predetermined depths.

Integrated samples were obtained by coordinating activities through walkie-talkie communication between one operator in charge of the vertical and horizontal movements of the intake hose located at the top of the mesocosm and a second individual controlling the valve and collecting the samples at two depths in the tank. Sample volumes of 40 *l* were collected during the first profile of January, 1987 and during September, 1988. All other samples were of 20 *l* for each integrated depth, except in the 0.5 *m* haloclines of the last simulation series which were only 7 *l*.

Integrated samples from 0-3, 3-6 and 6-10 *m* were collected during simulations **S1** to **S5**, in each side of the mesocosm during the sampling period. Integrated samples from 0-3, 3-3.5, 3.5-6.5, 6.5-7 and 7-10 *m* were taken during simulations **S6** to **S9**. A sampling period every 4 hours was included on the last day of sampling to verify the trend of larval distribution at a higher frequency since all other simulations had a 12 *h* frequency.

To avoid contamination of samples, 10 *l* of water were discarded prior to each sampling and between each depth. Larvae were screened through 180 *µm* mesh to remove large particles, retained on 44 *µm* screens and preserved in 4% formalin buffered with sodium borate in labelled flasks for later counts under dissecting microscope.

All larvae obtained in each of the samples were counted under a dissecting microscope and used to calculate the number of larva per liter in each sample by dividing the total found by the sample volume. Subsequently, to estimate the total number of larvae that would have been found in each stratum, the number of larvae per liter was multiplied by the total volume of each stratum. Therefore, the concentration of larvae shown in each layer is the proportion of the total number estimated for each profile assuming uniform distribution of larvae within each stratum.

4.2.5 Water column characteristics

To characterize the structure of the water column, temperature and salinity were measured in situ using a Beckman® salinometer. A LiCor® Li-188AB radiometer was used to obtain a light intensity profile during the first simulation. Determinations of

Chlorophyll-*a* were done during the first five diurnal profiles for **S4** and **S5**.; during the second diurnal profile of **S6** and **S7**, and for all day and night profiles during **S8** and **S9**. In each case, 250 *ml* water samples were collected and filtered through a 22 *um* mesh following larval sampling; immediately after, all samples were further filtered using GF/F Whatman filters which were kept frozen (-20C) for later analysis following the method proposed by Parsons *et al.* (1984).

4.2.6 Data analysis

All data from the 63 larval profiles (295 samples) are presented as percentage of the total larvae found in the water column per integrated depth (*i.e.*: 0-3 *m*, 3.0-3.5 *m*) to facilitate comparisons within profiles for each simulation and between simulations

The data were assessed statistically by the method of Rodger (1974, 1975) using his $E\alpha = 0.05$. That procedure allows for the post-hoc assessment of contrasts across the group means following analysis of variance and are shown in Appendix B5 for each simulation.

Mean depth of sea scallop larvae during each of the 63 sampling profiles was calculated as the median depth or centre of mass (**ZCM**) as proposed by Fortier and Leggett (1982):

$$ZCM = \sum_{i=1}^{N} p_i z_i$$

where, p_i is the proportion of the total number of larvae collected within the i-th depth interval (*i.e.*: 0-3, 3-6, 6-10 *m*; 0-3, 3-3.5, 3.5-6.5, 6.5-7, 7-10 *m*) and z_i is the i-th interval mid-depth (*e.g.*, for the depth interval 3-3.5 *m*, z_i is 3.25 *m*).

4.3 Results

The overall observed larval distribution appears to indicate a consistent median depth of larvae (**ZCM**) deeper in the water column during the day and higher in the water column during the night. Although variations were found depending on the characteristics of each simulation.

4.3.1 Simulation of diel light cycle

During the first simulation (**S1**) the vertical distribution of larvae showed a distinct pattern moving from a high concentration at the surface at night toward the lower portion of the tank during daylight sampling and returning to a higher concentration near the surface during the last nocturnal sampling period (Fig. 4.3). There was a significant difference between day and night **ZCM** as shown I Appendix B5. Larvae were found throughout the vertical column in all samples representing estimates of the total number of larvae between 16 to 100 %. The larval median depth (**ZCM**) depicted in Fig. 4.4 indicates that the mean depth of larvae for the three nocturnal samples is significantly higher than the two daylight samples. Although, this simulation contained 50 *µm* filtered sea water and no added phytoplankton it is likely that there was food was available within the particle size that larvae feed upon but no measurements of chlorophyll-*a* were made.

4.3.2 Simulation of diel light cycle and food availability

During the first experimental column (**S2**) larvae were placed in 1 *µm* filtered seawater (no food) and they were observed to distribute throughout the entire depth of the tank except in the bottom layer of the second profile of **S2**. Larvae appeared mostly concentrated in the middle and top layers throughout the entire sampling period (Fig. 4.5) and there were no significant differences between day and night (Appendix B5).

The second experimental column of this simulation (**S3**) contained a phytoplankton layer added at the surface which appeared to alter the vertical distribution of larvae (Fig. 4.5). During two of the three nocturnal sampling sessions larvae appear

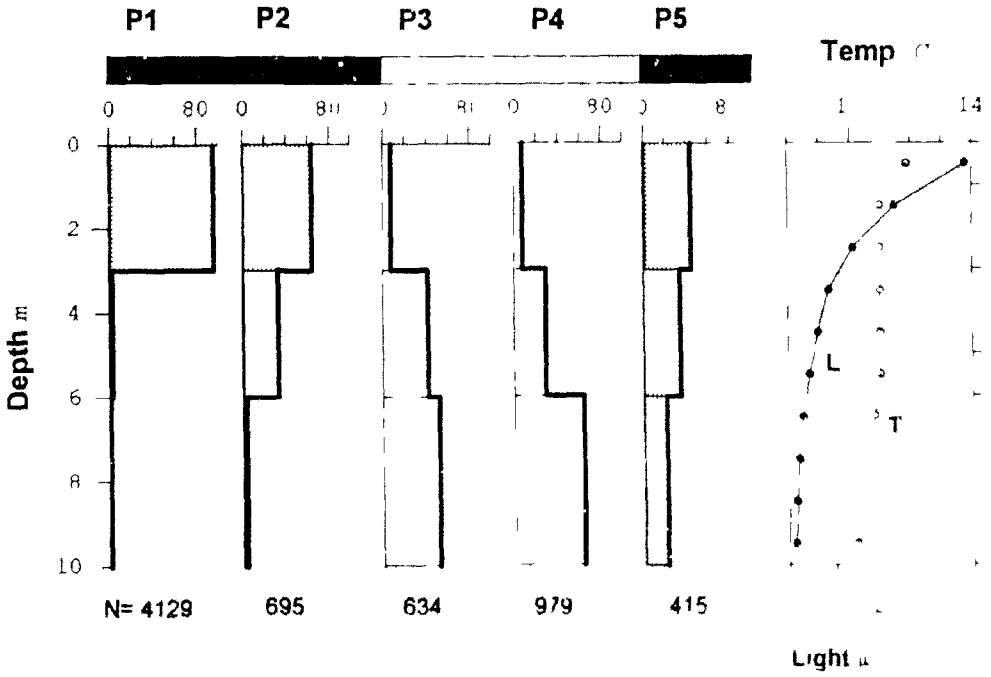

Figure 4.3 *Placopecten magellanicus* Vertical distribution of scallop larvae over a 24 *h* period (**S1**) in a single 10 *m* height mesocosm (117 *m*) under 12 12 light regime The width of each of the three strata represents the percentage of larvae from the total estimated for each profile The height of each stratum corresponds to the sampled depth interval (0-3 3-6 6-10 *m*) **P**: indicates sampling profile, **N** total number of larvae in the water column The horizontal bar represent day (white) and night (black) Right column, temperature and light intensity during daylight prior to sampling

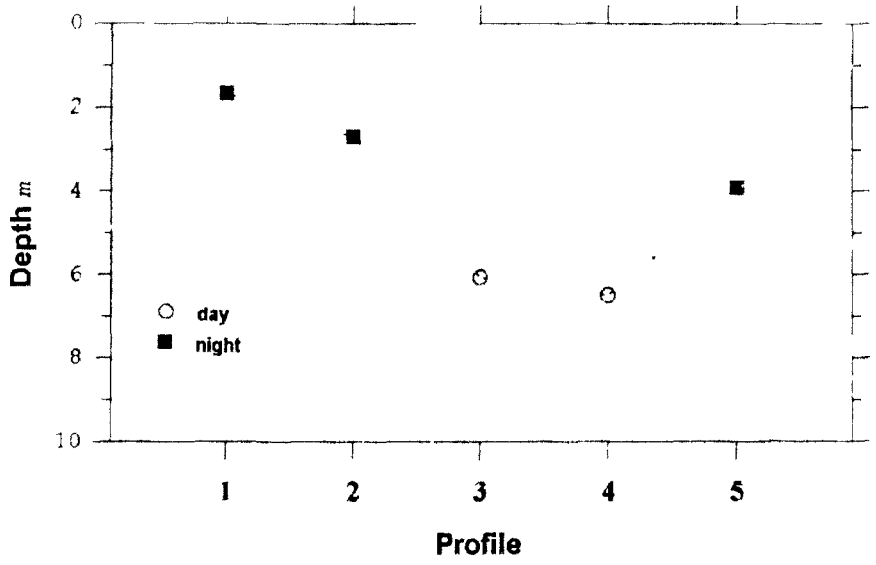

Figure 4.4 *Placopecten magellanicus*. Larval median depth (**ZCM**) for each profile during the 24 *h* sampling period (**S1**).

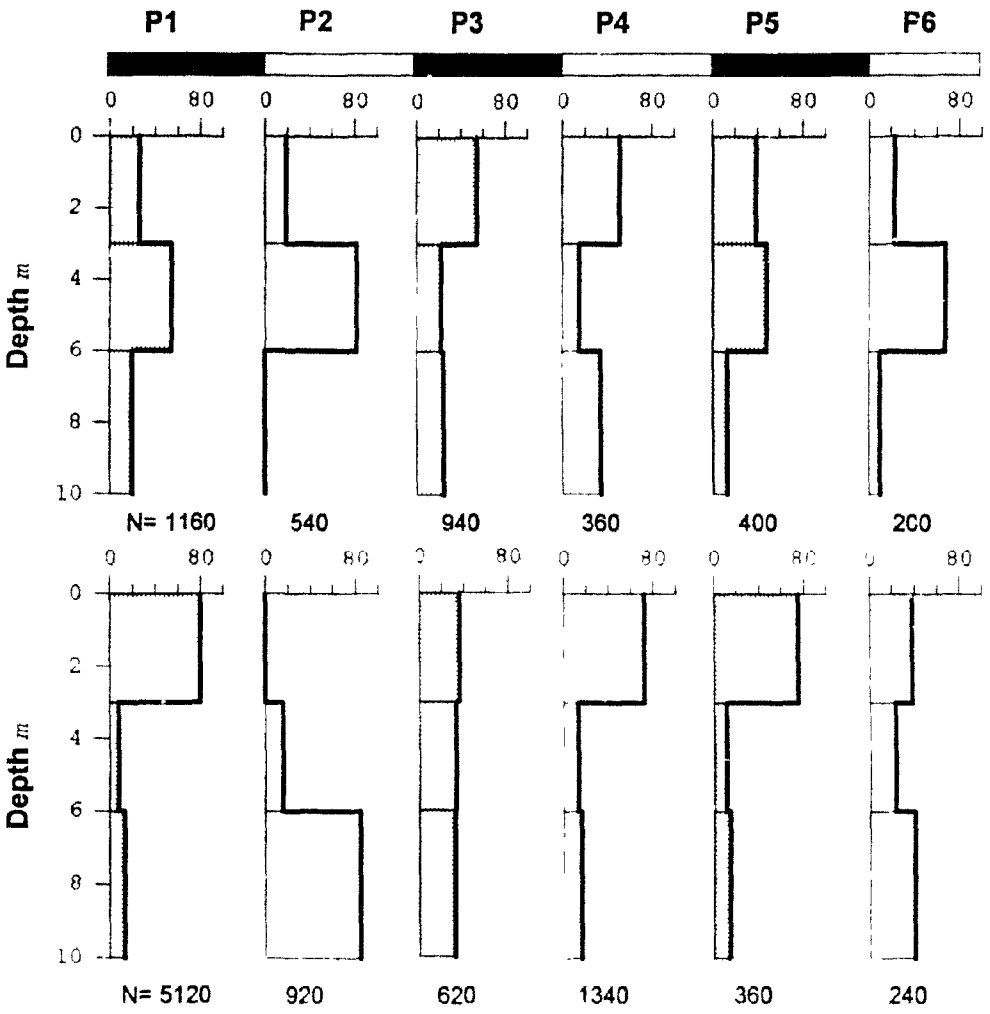

Figure 4.5 *Placopecten magellanicus* Vertical distribution of scallop larvae in two experimental 10 *m* water column, uniform water column without added food (**S2**) (top panel) and patchy distribution of phytoplankton near the surface (**S3**) (bottom panel) over a 72 *h* period. The width of each of the three stratum represent the percentage of larvae from the total estimated for each profile. The height of each stratum correspond to the sampled depth interval (0-3, 3-6, 6-10 m).

significantly concentrated in the upper portion of the tank (Appendix B5), while during diurnal sampling larval distribution was more variable No larvae were found in the top layer of the first diurnal sampling, but they were highly concentrated in the top layer during the second diurnal sampling and more evenly distributed during the last diurnal sampling The fact that the phytoplankton mixture was added to the top layer (2 - 3 *m*) just 3-4 *h* prior to the first nocturnal sampling and second diurnal sampling may have influenced the pattern of distribution in the diurnal sample of the fourth profile of **S3**.

The calculated larval median depth (**ZCM**) shown in Fig. 4.6 indicates a relatively constant mean larval depth throughout simulation **S2** in the absence of food while it is more variable during **S3**. Mean depth of larvae (**ZCM**) during nocturnal sampling (**S3**) was generally higher in the water column than during daylight sampling, though, the diurnal sampling following the addition of food in the water column (profile 4) did not show the trend of diurnal descent seen on the other days, perhaps because the opportunity to graze upon the food layer held larvae in the upper layer.

The last set of experimental mesocosms investigating the effect of food availability was performed in December, 1989 and included a column with a mixture of phytoplankton added to the top layer (**S4**) and the other with the same amount of food evenly distributed throughout its depth (**S5**) (Fig. 4.7, 4.8). The phytoplankton mixture was added prior to the first and fifth sampled profile (day) but, as Fig 4 9 shows, the patch was not stable and rapidly equilibrated. A sampling period every 4 hours was included on the last day of sampling to verify the trend of larval distribution at a higher frequency since all other simulations had a 12 *h* frequency. Higher concentrations of larvae were found in the top layer during all nocturnal sampling during **S4**, while during the day larvae did not show a consistent pattern but rather appeared to be either evenly distributed, somewhat concentrated toward the bottom layer or more rarely concentrated in the upper layers (Fig. 4.7; 4.8). Overall, there were significant differences between day and night samples as shown in Appendix B5.

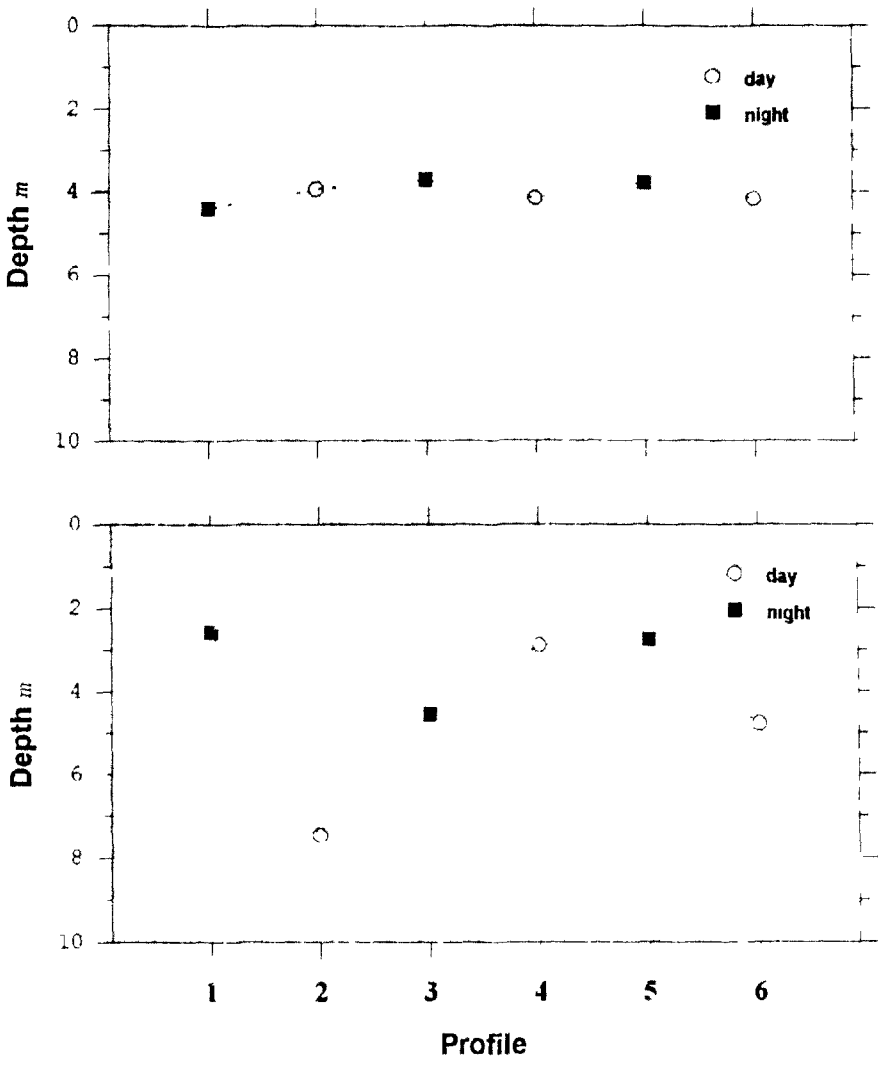

Figure 4.6 *Placopecten magellanicus* . Larval median depth (**ZCM**) for each profile during the 72 *h* sampling period. Water column without added food (**S2**) (top panel), water column with near surface patchy distribution of food (**S3**) (bottom panel).

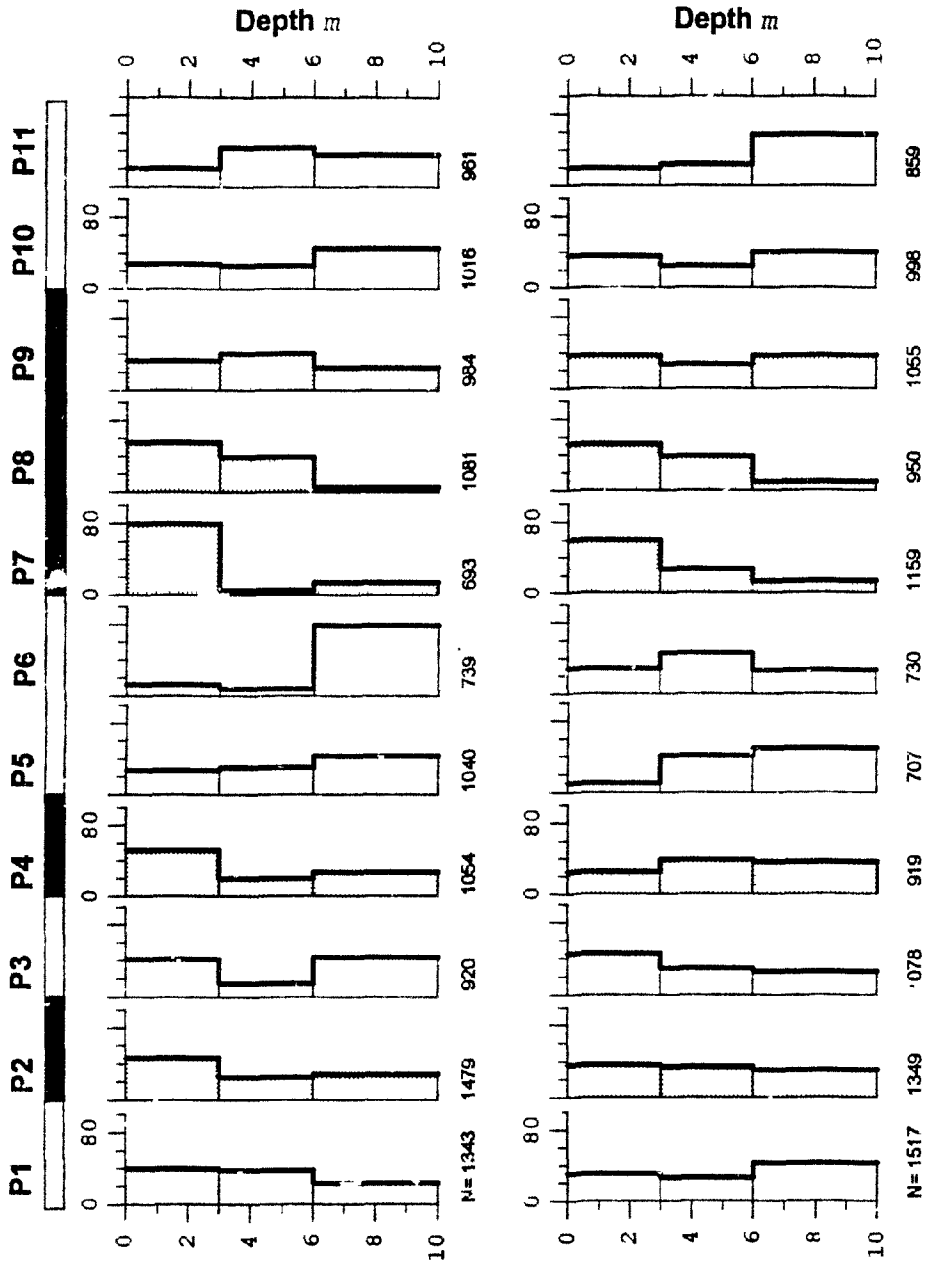

Figure 4.7 *Placopecten magellanicus*. Vertical distribution of scallop larvae in two experimental 10 *m* water column, patchy distribution of phytoplankton near the surface (**S4**) (top panel) and uniformly distributed phytoplankton along the water column (**S5**) (Lottom panel). A total of eleven profiles were sampled in each column over a period of 108 *h*. Samples were collected at midnight and midday and every 4 hours during the last 24 hours of sampling. The width of each of the three strata represent the percentage of larvae from the total estimated for each profile. The height of each stratum correspond to the sampled depth interval (0-3, 3-6, 6-10 *m*).

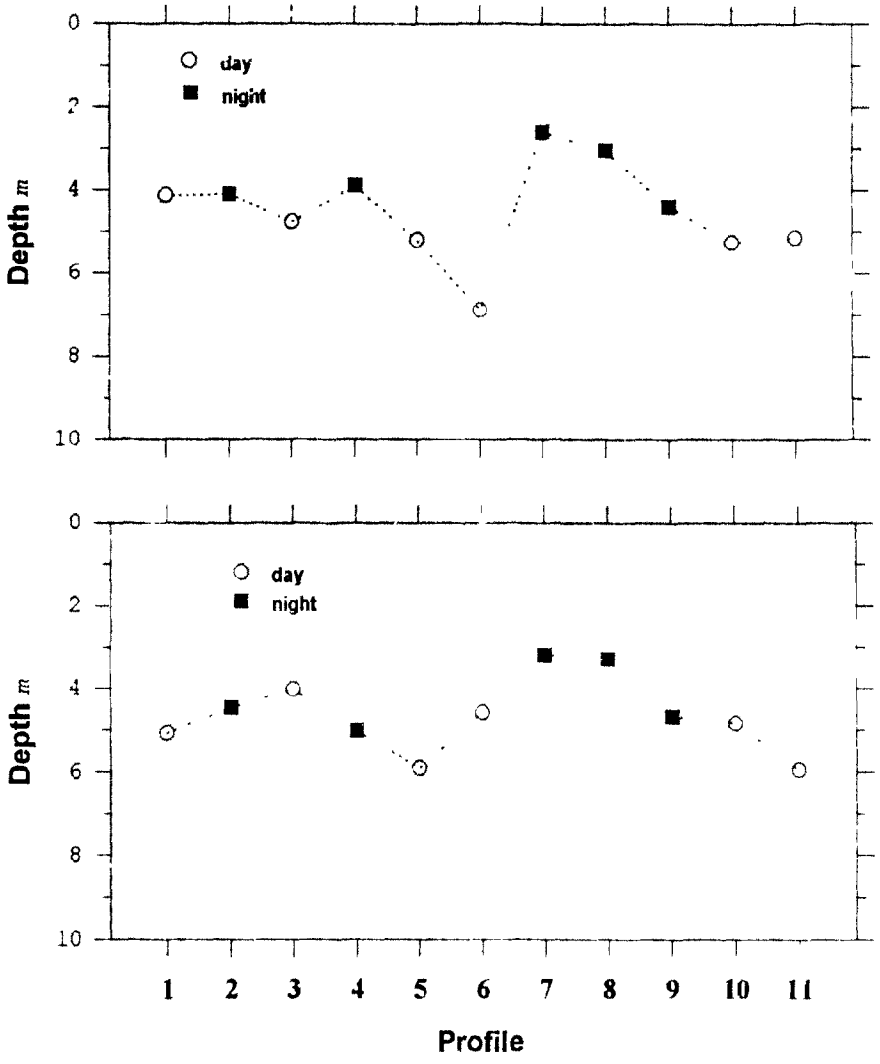

Figure 4.8 *Placopecten magellanicus.* Larval median depth (**ZCM**) for each profile during 108 *h* sampling period. Water column with near surface patchy distribution of food (**S4**) (top panel), water column with food uniformly distributed (**S5**) (bottom panel).

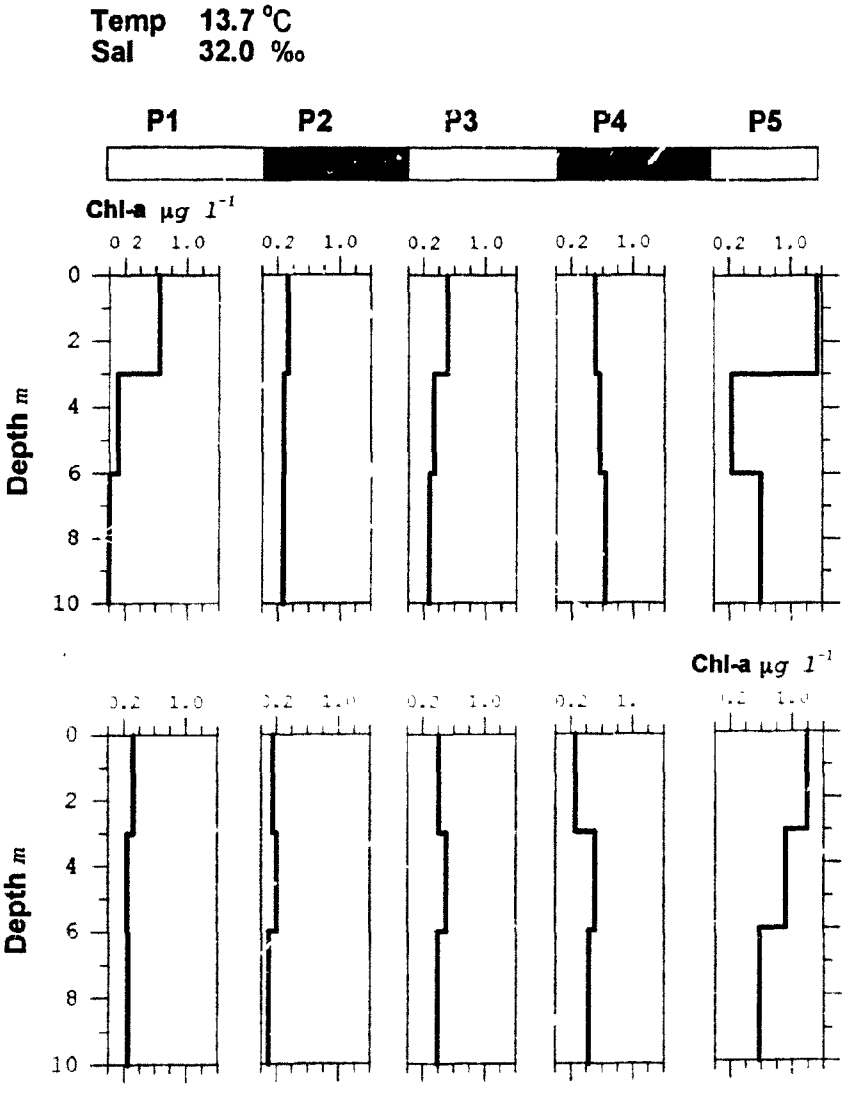

Figure 4.9 Profiles of the concentration of chlorophyll-*a* < 30 *μm* during the first five profiles from Simulation 4, with top patchy distribution of food (**S4**) (top panel) and Simulation 5, with uniform distribution of food (**S5**) (bottom panel).

When food was available in the entire column of **S5**, the nocturnal concentration of larvae near the surface observed earlier was evident only in two profiles, the others show a more even distribution throughout the water column. Daylight distribution on the other hand, showed a reverse trend, a more consistent pattern of higher concentrations toward the middle and bottom layers of the water column (Fig. 4.8) was detected. Larval mean depth (**ZCM**) was consistently higher in the water column during nocturnal sampling (**S4**) than diurnal sampling (Fig. 4.7, 4.8). Overall, there was evidence of migration in both simulations (**S4** and **S5**) (see Appendix B5)

4.3.3 Simulation of diel light cycle, food availability and salinity stratification

Larval distribution per stratum showed very distinct features in the columns in which the haloclines were dyed (**S6** and **S7**) compared with distributions where no dye was used (**S8** and **S9**). In **S6** and **S7** larvae were almost consistently absent or in very low numbers in the top layer and mostly aggregated toward the bottom layer (34%) (Fig. 4.10) which bore no relationship with the level of available chlorophyll-*a* estimated from the last diurnal profile (**P4**) (Fig. 4.10). There were significant differences between the calculated larval median depth (**ZCM**) for day and night samples during these simulations **S6** and **S7** (Appendix B5), eventhough, the larval median depth of one night profile was lower during the night (Fig 4.11). The salinity gradient was relatively stable throughout the complete sampling period, although temperature increased slightly (Fig. 4.12) as shown by the calculated Sigma-t (Fig 4.13).

The last simulations revealed a pattern different from that observed in **S6** and **S7** In columns **S8** and **S9**, larvae aggregated mostly in the middle layer where they were originally placed, subsequently moving to the top layer and bottom layer at different times (Fig. 4.14). Two of the three nocturnal samples in **S8** showed a net larval movement toward the top layer (**P4**, **P6**) as seen in the top panel of Fig. 4.14. Diurnal samples showed larvae to be aggregated in the middle layer and a small proportion always appeared in the top layer, no statistical differences were found in **S8** (Appendix

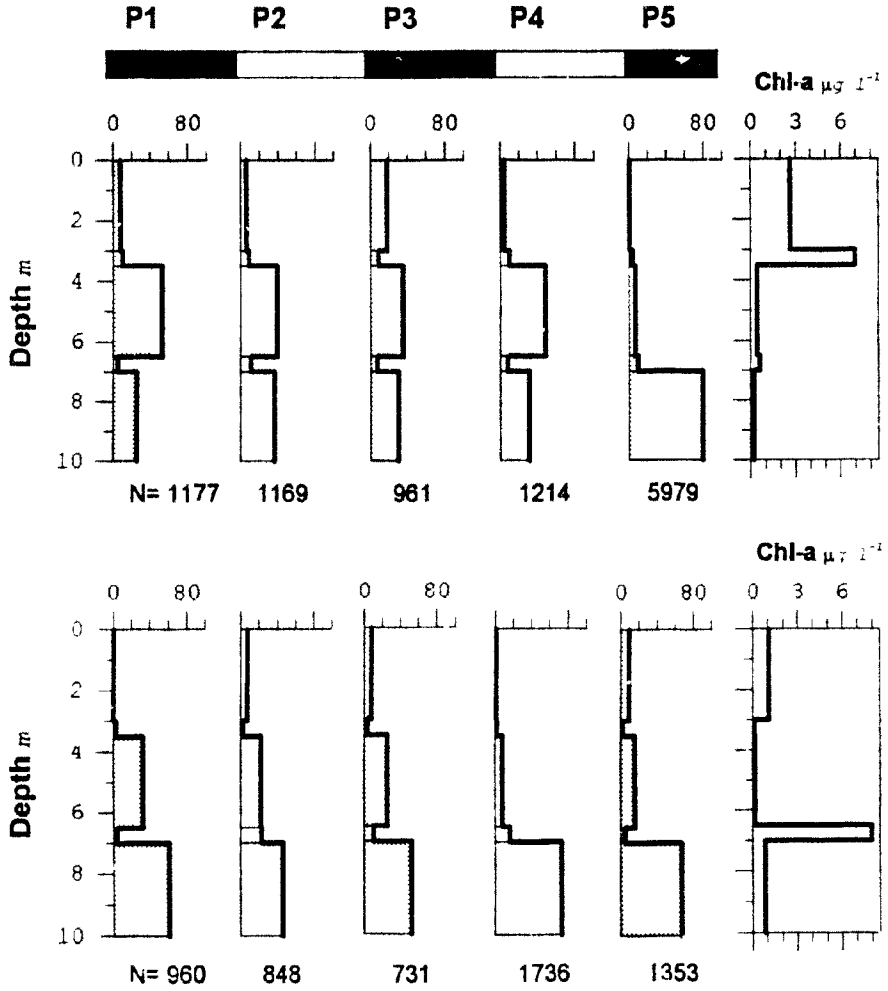

Figure 4.10 *Placopecten magellanicus* Vertical distribution of scallop larvae in two experimentally stratified 10 *m* columns with a salinity gradient from 30 to 34‰ from top to bottom. Food was available in one column at the 31‰ halocline (**S6**) (top panel) and in the other at the 33‰ halocline (**S7**) (bottom panel). A total of five profiles were sampled in each column over a period of 60 hours. Samples were collected every midnight and midday and every four hours during the last 24 hours.(Rhodamine dye was added to each halocline). The width of each of the five strata represent the percentage of larvae from the total estimated for each profile. The height of each stratum correspond to the sampled depth interval (0-3, 3-3.5, 3.5-6.5, 6.5-7, 7-10 *m*). Right column: chlorophyll measurements at each depth interval during the last diurnal profile.

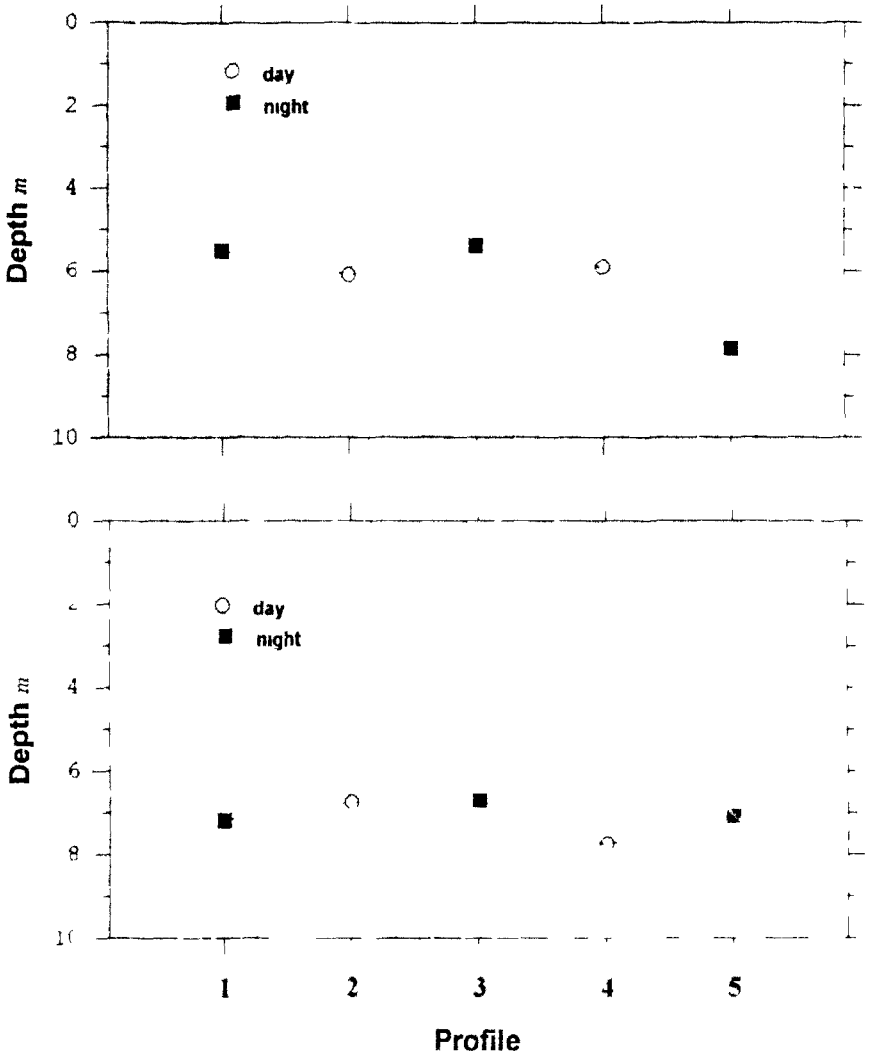

Figure 4.11 *Placopecten magellanicus* Larval median depth for each profile during 60 *h* sampling period in a salinity stratified water column. Food availability at 31‰ halocline (**S6**) (top panel), food availability at 33‰ halocline (**S7**) (bottom panel).

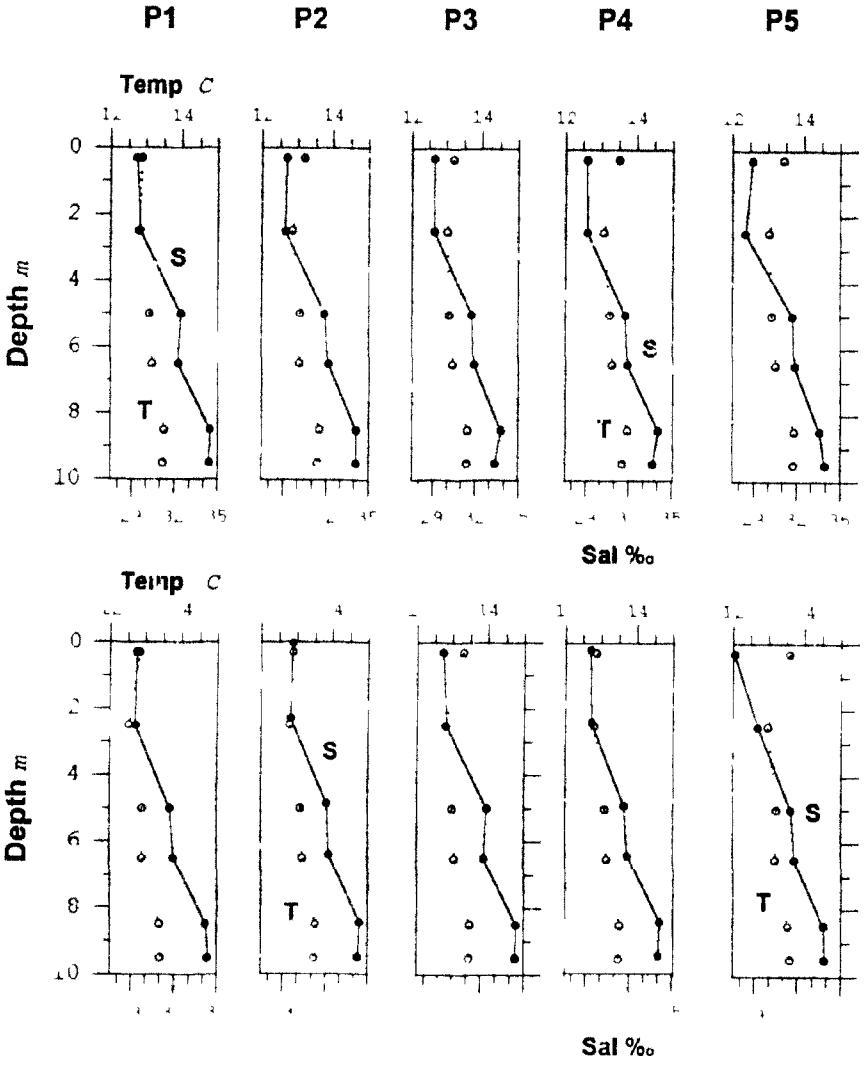

Figure 4.12 Profiles of temperature and salinity during of each sampling period in Simulation 6 (top panel) and Simulation 7 (bottom panel) Solid line is salinity and dotted line is temperature

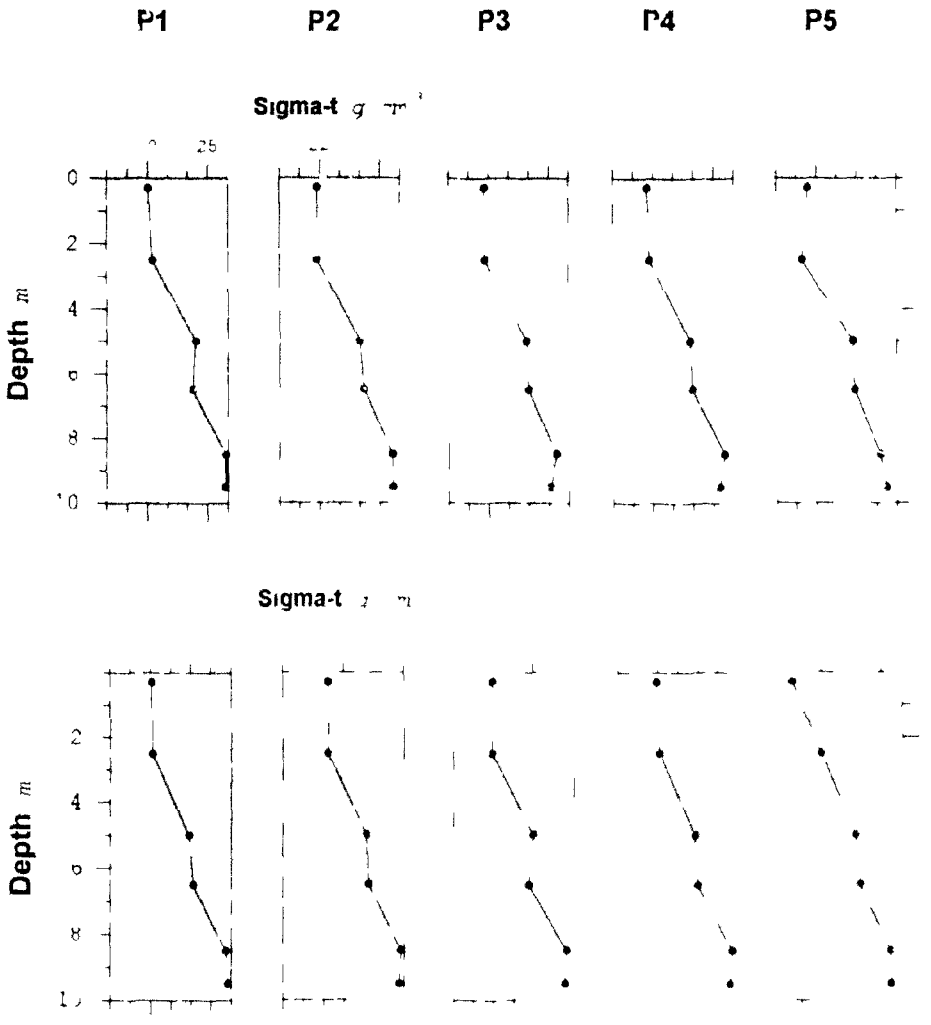

Figure 4.13 Profiles of density during each sampling period in Simulation 6 (top panel) and Simulation 7 (bottom panel)

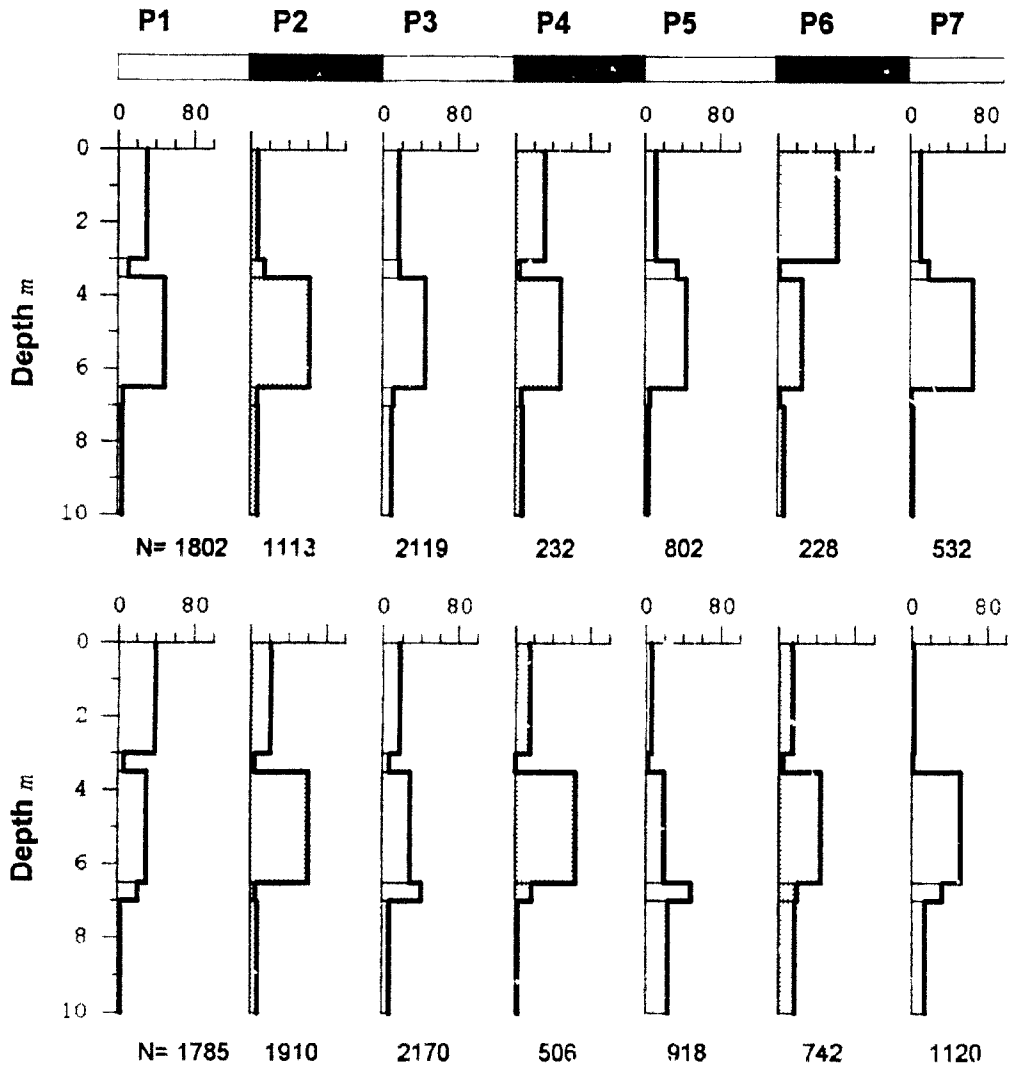

Figure 4.14 *Placopecten magellanicus*. Vertical distribution of scallop larvae in two experimentally stratified 10 m water column with a salinity gradient from 30 to 34‰ from top to bottom. Food was available in one column at the 31‰ halocline (**S8**) (top panel) and in the other at the 33‰ halocline (**S9**) (bottom panel). A total of seven profiles were sampled in each column over a period of 84 *h*. Samples were collected every midday and midnight. The width of each of the five strata represent the percentage of larvae from the total estimated for each profile. The height of each stratum correspond to the sampled depth interval (0-3, 3-3.5, 3.5-6.5, 6.5-7, 7-10 *m*).

B5). Vertical distribution of larvae fed at 33‰ (**S9**) was somewhat different to that of larvae fed at 31‰ (**S9**), in this last simulation larvae were mostly concentrated in the middle layer and showed a trend to aggregate in the bottom layer during the last three sampling periods (Figs. 4.14; 4.15). Diurnal profiles showed a broader distribution of larvae throughout the water columns, although, there were significant differences between day and night samples (Appendix B5).

The mean depth of larvae (**ZCM**) of each profile during both simulated conditions (**S8** and **S9**) was generally higher with food offered at the upper halocline and lower when food was offered at the lower halocline (Fig. 4.15; 4.16). The salinity stratified water column (Fig. 4.17, 4.18) affected the vertical distribution of larvae, with larvae remaining near their original depth and moving only slightly into the closest area of higher concentration of food at the haloclines (Fig. 4.16). The earlier trend toward nocturnal ascent (**S1**, **S3**, **S4**, **S5**) observed during a uniform water column remained in **S9**, but it was damped.

In general, throughout all nine experimental water columns larvae appeared to be located lower in the 10 *m* water column by day and higher at night (Fig. 4.19), in particular during **S1**, **S3**, **S4** and **S5**, larval vertical distribution remained relatively constant during **S2** in the absence of food (Appendix B5). The presence of a salinity gradient clearly limited the extent of larval movement in the water column as seen during the last four simulations.

There were no differences in **ZCM** found in **S2** in the absence of food, but a clear pattern of higher **ZCM** was consistently found in those columns that contained available food (**S1**, **S3**, **S4**, **S5**) (Appendix B5). The **ZCM** of the last four simulations shows that larvae remained mostly within a narrow band near the haloclines. Larval mean **ZCM** was marginally higher, but statistically significant (Appendix B5) in the column during the night and lower in the water column during the day for **S7** and **S9** but no statistical differences were found during **S8** (Appendix B5).

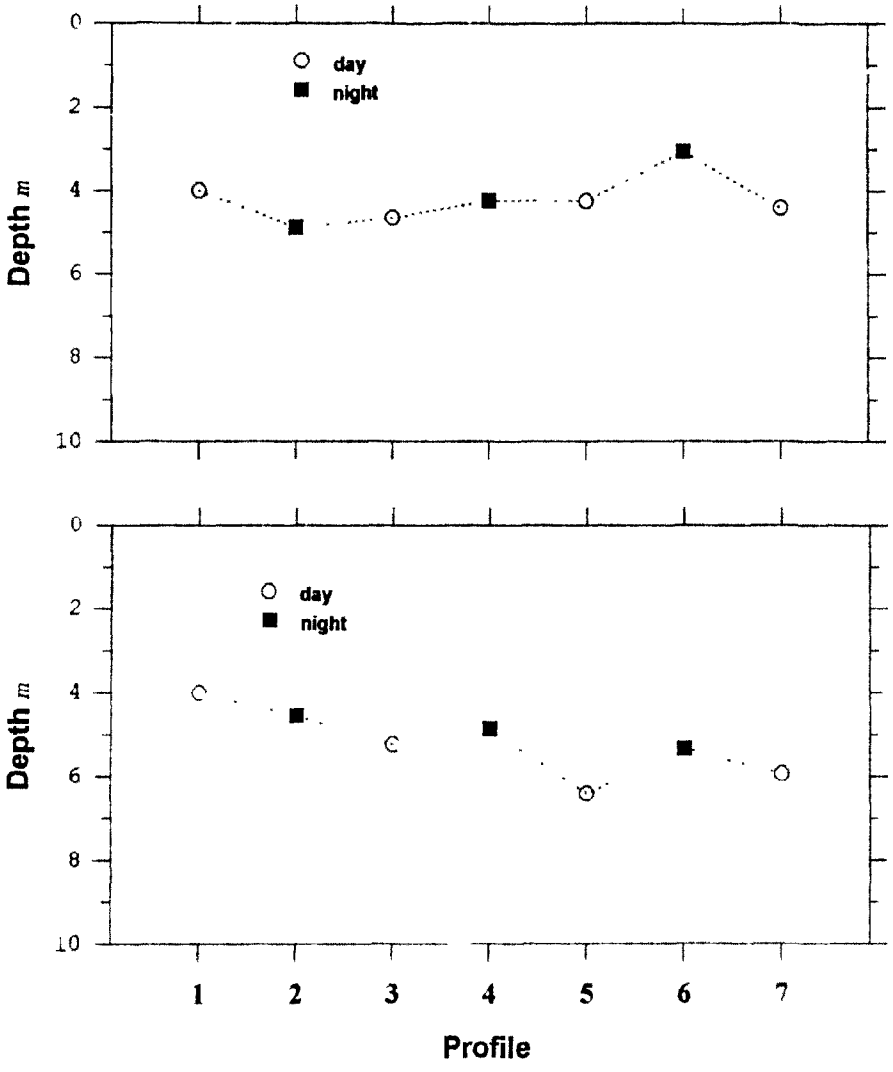

Figure 4.15 *Placopecten magellanicus*. Larval median depth (**ZCM**) for each profile during 84 *h* sampling period in a salinity stratified water column. Food availability at 31‰ halocline (**S8**) (top panel) and at 33‰ halocline (**S9**) (bottom panel).

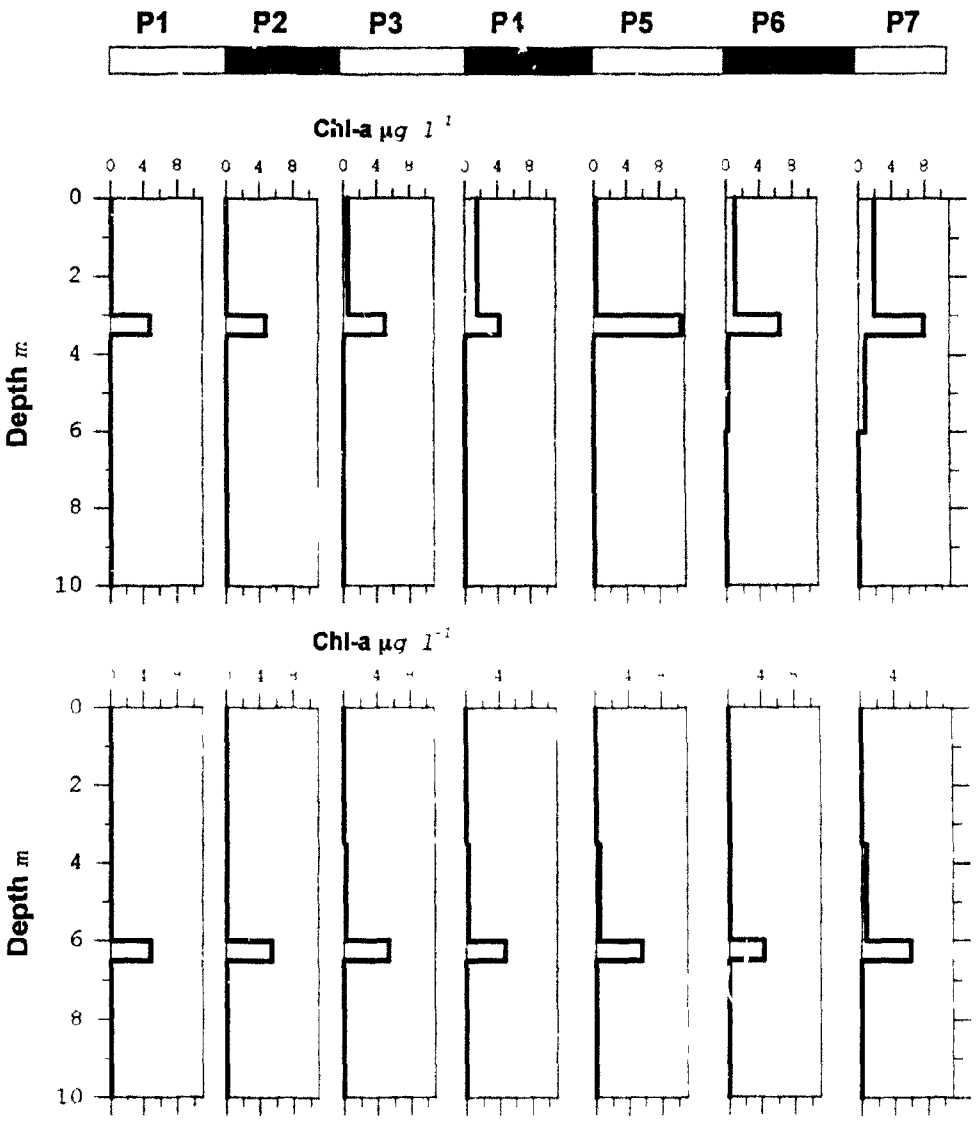

Figure 4.16 Profiles of chlorophyll-*a* < 30 *µm* during each sampling from Simulation 8, with top food at 31‰ (top panel) and Simulation 9, with bottom food at 33‰ (bottom panel).

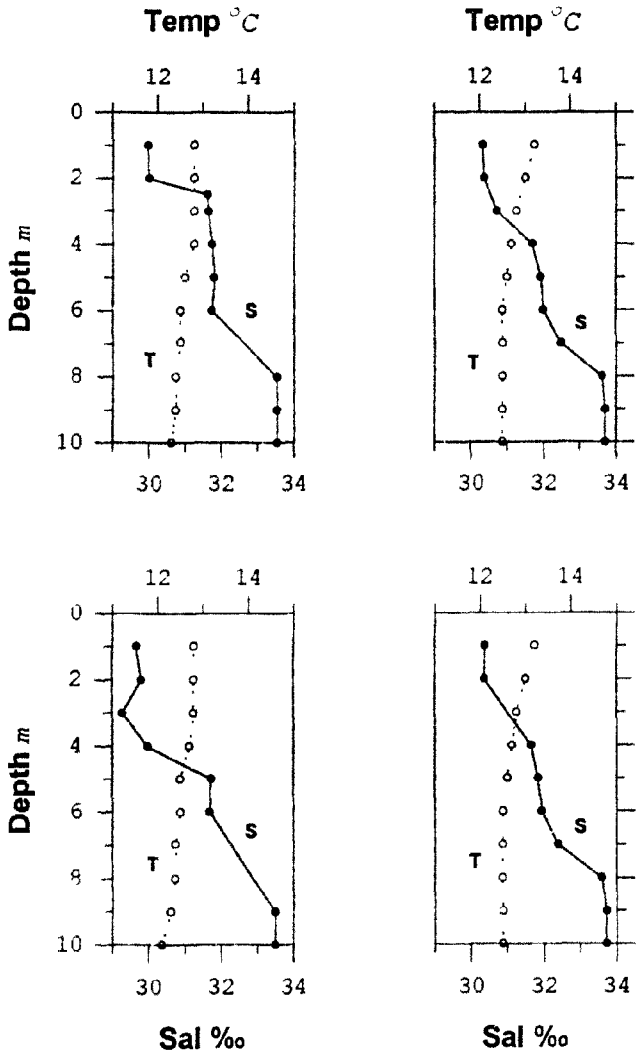

Figure 4.17 Profiles of temperature and salinity during the first sampling period (left panels) and last sampling (right panel) in Simulation 8 (top panels) and Simulation 9 (bottom panels). Solid line is salinity and dotted line is temperature.

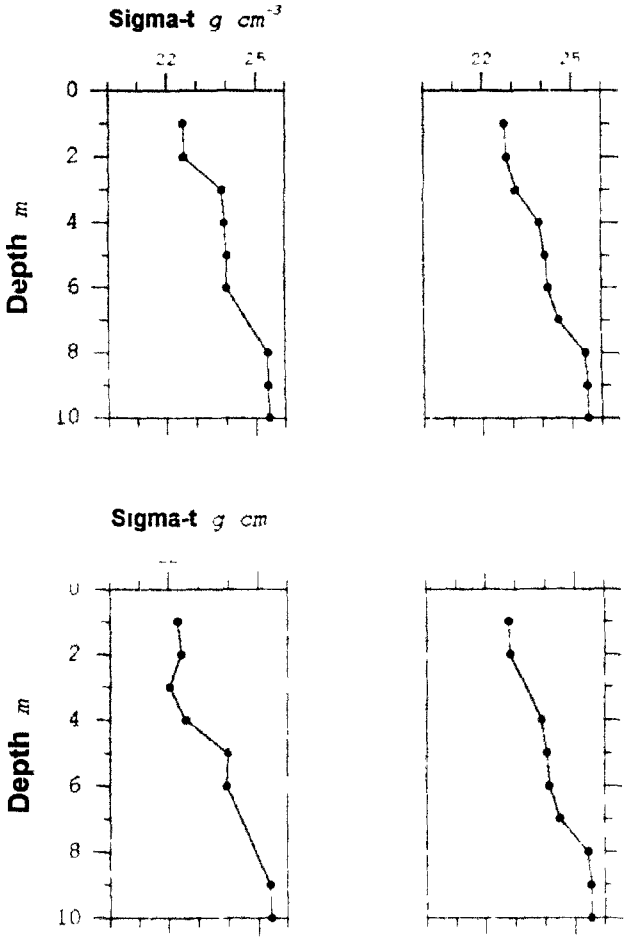

Figure 4.18 Profiles of density corresponding to the first and last sampling period in Simulation 8 (top panel) and Simulation 9 (bottom panel).

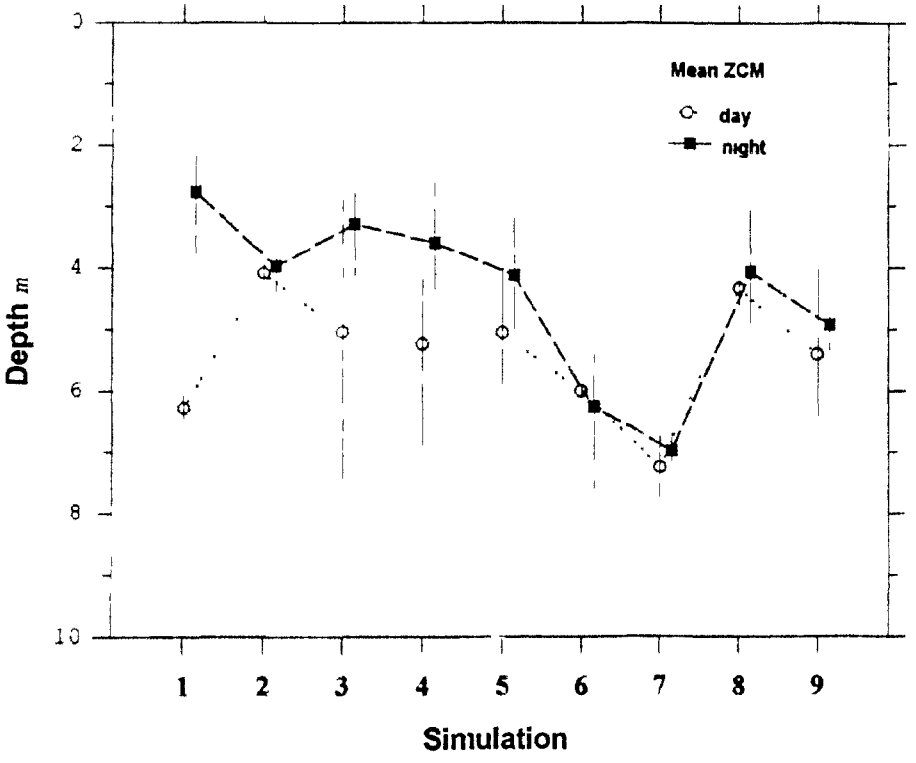

Figure 4.19 *Placopecten magellanicus* Mean larval median depth for all day and night profiles for each of the nine simulations. Vertical bars represent minimum and maximum values.

4.4 Discussion

One of the main objectives of this study was to initiate a series of investigations on the factors determining the vertical distribution of planktonic sea scallop larvae in semi-natural conditions. The feasibility of rearing larvae of sea scallops *P. magellanicus* in the laboratory and the availability of one of the world's mesocosms at the Aquatron (Dalhousie University) made this pilot study on the vertical distribution of veligers under a series of simulated conditions possible.

The 10 *m* mesocosm has been previously used for short term experiments on the distribution of zooplankton and zooplankton feeding (Boher, 1983, Price, 1989; Collins, 1989) providing valuable information on the ecological aspects of planktonic organisms; the present study reports the first series of mesocosm simulations investigating the vertical distribution of a bivalve larvae in an indoor mesocosm.

The spatial distribution (vertical and horizontal) of planktonic larvae has been considered to be mainly driven by passive mechanisms, *i.e.*: the local oceanographic regime (Tremblay and Sinclair, 1988,1990b,1992, Robinson *et al* , 1991), although the possibility that planktonic larvae can regulate their distribution by displaying active mechanisms prior to benthic settlement has been hypothesized for several estuarine and coastal bivalve species (Boicurt, 1982, Burton and Feldman, 1982, Day and McEdward, 1984; Pennington and Emlet, 1986). I investigated links between active mechanisms observed in larvae swimming in microcosms and the vertical distribution in simulated mixed and stratified conditions which had not been investigated for more oceanic species such as sea scallops. Survivors of this planktonic period must eventually leave the water column to settle in adequate substrate and later recruit into pre-established adult populations or colonize new areas (Crisp, 1976). In nature, adult sea scallops live more or less confined to particular benthic areas and although they are well known jet swimmers when escaping from predators, there is little evidence that they migrate from the adult grounds. Adult aggregations in Georges Bank are probably a self-sustaining macro-

population in which larvae from one location may be seeding the next aggregation as a result of the well known clockwise circulation over the bank (McGarvey *et al*., 1992,1993; Tremblay *et al*., 1994)

Does vertical distribution of veligers vary or remain constant through a diel light cycle? This was the question in the first simulation. The observed vertical distribution of larvae clearly varied over the 24 *h* sampling period. Larvae moved from a high concentration at the surface during the first night to a high concentration in the middle and bottom layers during the day, and then to the upper layer during the next day although in lesser concentrations. In this particular mesocosm, the octopus sampler used was deployed using a mechanical crane in the middle of the tank, and therefore collected larvae only in the middle section. The decrease in the total number of larvae collected after the first sampling session may have resulted from sampling at a larval patch during the first profile but not during the others since larvae were originally placed in the top layer. Nevertheless, larvae were found to distribute away from the upper layer, well into the lower part of the tank during the day and to ascend to the upper layer during the second night. Even though food was not added into this simulation, the 50μm filtered seawater likely contained enough food particles in the size range that larvae feed upon, less than 30μm (Rao Durvasula S., Bedford Institute of Oceanography, Darmouth N.S. unpublished data). Therefore, this simulation is a baseline for larval behaviour of a previously fed larva in a moderate food environment. When food was absent, mean larval **ZCM** was 4.1 *m* during the day and 4 *m* during the night (Fig. 4.19).

Three additional simulations to analyze questions about the effect of environmental variability in semi-natural conditions on the vertical distribution of larvae were possible given the available technical controls of the mesocosm.

Is the vertical distribution of larvae in response to a diel light cycle constant or does it vary with food availability? The vertical distributions of larvae in the first five

experimental columns (**S1-S5**) indicate that larvae can be found distributed throughout the water column, although they mostly migrated to the upper layer during the night and descended in the water column during the day when food was available, as shown by the calculated larval median depths. When the water column contained only filtered seawater (**S2**) most larvae remained in the upper portion of the column which suggests that searching for food using upward helices tends to bring larvae to the top, while feeding and/or satiety reduce upward movement. Thus, when food was added just prior to midday sampling in **S3** (profile 4), larvae appeared in the upper layer during the day. I propose that availability of food plays a role on the migrating behaviour of larvae which may indicate a preference for nocturnal feeding in the upper portion of the column as found in **S1**, **S3**, **S4** and **S5**, although feeding may still occur deeper in the column during the day. Earlier observations of larval locomotory capabilities did not find any evidence of long term cessation of activities in early veligers so the effects of feeding likely last at most a few hours (pers. obs). Overall, the mean larval **ZCM**s observed in the presence of phytoplankton vary between 5.1 (**S5**) and 6.3 *m* (**S1**) during the day, and between 4.1 (**S5**) and 2.8 *m* (**S1**) during the night (Fig. 4.19).

Does vertical distribution of larvae change or remain constant in response to diel light cycle, food availability and salinity stratification? The general trend of nocturnal ascent and diurnal descent in the water column was not statistically supported in **S8**, but there were significant differences found in **S6**, **S7** and **S9** (Appendix B5). Most sampled larvae remained concentrated in the middle and bottom layers throughout the sampling period and the pattern of nocturnal ascent and diurnal ascent was reduced considerably. This finding could indicated that larvae were retained in this area of patchy food, as it has been found to occurr in some copepods (Dagg, 1977) and consistent with Chapter 2. The only obvious differences between the August and October simulations was the use of a dye in the haloclines of the former which could have had an effect on light penetration in the column, or some unknown deleterious effect on the algae and larvae. Another factor

that could have had an effect on the observed distribution is the origin of larvae and larval condition, but both series used larvae originated from adults of the same Georges Bank population.

The extent of vertical migration in this last series was limited by the presence of a salinity gradient (Fig. 4.17) but may have been driven by the presence of food at the haloclines. In stratified conditions (**S6-S9**), the mean **ZCM** during the day were shifted between experiments but, overall, averaged 5.2 *m* when food was offered in the upper 31‰ halocline and 6.3 *m* when food was at the lower 33‰ halocline. The average **ZCM** during the night for the upper halocline fed columns showed no change at 5.2 *m*, but lower haloclines fed columns (**S7. S9**) moved upwards slightly to 6 *m*. The consistently shallower **ZCM**'s when food is at the upper halocline suggest that the decreased vertical movement is the result of the horizontal food patches rather than the salinity changes themselves. This pattern could be explained by changes in microscale seen in Chapter 2.

What are the advantages and limitations of simulations in the mesocosm? The main advantage was the possibility of designing simulations that mimic a natural environment to provide an important step in the study of the characteristics of planktonic larvae of particular species. The observed vertical distribution of larvae under the array of simulated conditions raises important questions regarding the adaptive value of behavioural traits in the early life history of sea scallops. It is certainly more economically feasible to conduct simulations in the available mesocosm to answer particular questions of the early life history of benthic invertebrates than to conduct long term surveys in nature where the underlying behavioural mechanisms of larvae can not be assessed.

The salinity gradient created during the last two simulations was maintained throughout the sampling period and varied only slightly. This indicates that the sampling method used did not disrupt the structure nor the temperature of the experimental column. However, there were drawbacks, particularly the fact that no true replication was

possible. This limited the strength of statistical analysis, as did the lack of continuous characterization of the water column because of logistics and concern about mixing.

Although, I report only data obtained from physical samples, I did attempt to develop a non-destructive sampling method. However, techniques for underwater photography and underwater video that I used simultaneously with physical sampling did not produce reliable information. The random integrated sampling used during this study may have not have appropriately sampled larval patches that are known to occur in most microzooplankton, but overall sampling estimated an average of 61% of the total larvae in the water column (within sampling error).

As a direct result of these experimental studies a new system of non-destructive sampling was developed to make possible the study of vertical distribution of larvae over time (Gallager *et al.*, in press). This new method of video sampling allows the direct observation of larval patches at a similar scale of that used during the characterization of larval behaviour observed in microcosms and provides a more detailed account for distribution of larvae in the temporal and spatial scale related to larval size

The question of active versus passive migration can be in part assessed from the observed vertical distributions of larvae in a column of uniform temperature with and without food and from the salinity stratified columns. Under mixed conditions, larvae appeared in the upper layers during the night (75% of cases) and descended to bottom layers during the day (76% of cases). Active migration of larvae occurred when food was available but not in the absence of food (**S2**). In these simulations, temperature and salinity were relatively uniform and therefore there were no physical constraints opposing larval behaviour.

The salinity stratified simulations however, imposed a physical barrier that could affect larval behaviour by limiting the control of behavioural traits. Simulations 6 and 7 showed a consistent aggregation in the bottom layer, and larvae never appeared in the surface layer; however in the last two simulations larvae remained mostly in the middle

layer showing up in the upper layer in most profiles and avoiding the bottom dense layer even though the gradients were as similar as possible. It is not known how salinity gradients affects larval behaviour, although the observed changes in vertical distribution were greatly diminished relative to those observed in mixed water columns. The physical barriers of the haloclines may have reduced larval mobility and retained some initial bias in larval distribution, but both simulations show a clear trend for the larvae to be higher when the food is available higher. To elucidate the effect of salinity gradients on the behaviour of scallop larvae further studies should be conducted.

Although, the interaction of diel light cycle and the presence of phytoplankton may have driven the observed larval distribution, larvae seemed to avoid the upper part of the column during the day, as shown by the fewer larvae found in most of the diurnal samples. Ringelberg (1995) recently reviewed the effect of light intensity on diel vertical migration comparing the marine and fresh water environments and found that relative changes in light intensity were the primary causes of migration, the presence of predators however, enhanced the phototactic response but it was inhibited in low food concentration.

My study therefore showed evidence of what is known as diel vertical migration although there were conditions in which this pattern is suppressed (low food and salinity stratification with patchy food) Pronounced diel vertical migration (**DVM**) has been commonly observed in populations of both calanoid copepods and euphausids in conditions of abundant food, although when food is scarce, they remain in the surface waters (Huntley and Brooks, 1982; Johnsen and Jakobsen 1987; Verheye and Field, 1992). Even though there is little evidence that bivalve larvae can take vertical migrations as extensive as those found in zooplankton. Tremblay and Sinclair (1990a) did find vertical migration of scallop larvae of similar amplitudes in a shallow embayment (24 *m*) of the Bay of Fundy within a similar temperature range. Also several bivalve larvae

sampled in Baie de Chaleurs showed a similar pattern in mixed and stratified conditions (Raby *et al*., 1994).

I therefore hypothesize that larval distribution in nature is the result of the complex interaction of behaviour which may be expressed to different degrees depending on the environmental characteristics (*i.e.* level of stratification, food availability) and recent feeding experience of individual larvae. By simply modifying their behavioural repertoire, larvae can take advantage of appropriate conditions for feeding and/or sinking out of a particular depth after nocturnal feeding in a vertically mixed water column rich in food (Raby *et al*., 1994). In conditions of high stratification, as those simulated in salinity gradients, behavioural traits are less likely to alter vertical distribution and larvae will probably be found aggregated near discontinuities (Sullivan, 1993). Such aggregations probably reflect both physical constraints on the larvae and the tendency for the same constrains to concentrate phytoplankton.

Planktonic larvae are adapted for feeding and swimming while acquiring nutrients (Strathmann *et al*., 1972) and remaining suspended probably to optimizes its short residence in the rich phytoplankton blooms using the very simple behaviours observed earlier (Chapter 2). As a direct result of these experimental studies a new system of non destructive sampling was developed to make possible the study of vertical distribution of larvae over time (Gallager *et al*., in press). This new method of video sampling allows the direct observation of larval patches at a similar scale of that used during the characterization of larval behaviour observed in microcosms and provides a more detailed account for distribution of larvae in the temporal and spatial scale related to larval size.

The overall results of this study have highlighted some of the complex interaction of early life history of invertebrates and the dynamism of the environment that should be considered for a sustainable management and development of natural populations of benthic invertebrates with complex life cycles. Furthermore, the study of interactive

relationships of larval and microzooplankton ecology with the environment can be addressed in mesocosms which are more comparable to natural conditions (Lampert and Loose, 1992; Sullivan, 1993).

CHAPTER 5

General discussion

5.1 Introduction

Natural populations of bivalve molluscs are important components of benthic ecosystems, occupying a great diversity of substrates, and are presumed to be continually replenished with periodic recruitment of new individuals of the same species. However, survival and recruitment of planktotrophic larvae of bivalves appear to be highly variable from year to year (Sinclair *et al*., 1985, Sinclair and Iles, 1985), thus the influence of stochastic and deterministic mechanisms must be reviewed to determine their significance throughout larval ontogeny to understand and protect these marine resources.

Understanding of the mechanistic basis for the process of recruitment of bivalve populations requires a comprehensive review of the role of a planktonic larva in securing its survival and later recruitment of competent stages into appropriate areas. Knowledge of specific adaptations that may control larval survival are therefore crucial since these adaptations could ultimately affect recruitment within a particular environmental regime (Day and McEdward, 1984, Hines, 1986, Sastry, 1986).

As a first step toward such a comprehensive review, I designed a series of experimental studies on sea scallop larvae to resolve three basic questions:

1) Do larval locomotory activity, feeding and behavioural traits vary throughout ontogeny?
2) Does lipid class composition of larvae vary throughout ontogeny?
3) Does vertical distribution of veligers vary in response to mixed and stratified water column conditions?

The positive response to all three questions was the product of a combination of micro-scale laboratory observations and analyses with meso-scale experiments that provide an integrative approach to the study of the larval ecology of bivalve molluscs.

Although each aspect was studied separately to be able to assess individual contributions (larval behaviour, lipid class variability and vertical distribution of sea scallop larvae), the overall vertical distributions of larvae are expected to reflect both behavioural traits and condition of larvae in response to simulated environmental variability.

5.2 Do larval locomotory activity, feeding and behavioural traits vary throughout ontogeny?

I found that sea scallop larvae throughout ontogeny ascended and descended in helical and straight lines. Larvae usually alternated between helical swimming to straight line swimming. This pattern may be the result of cyclic velum-wide ciliary arrests caused by a propagated Ca^{++} dependent action potential as found in gastropod veligers (Arkett *et al.*, 1987), which seems to be a generalized physiological phenomenon occurring across veliger species. It causes the velum to be withdrawn within the larval shell, thereby causing larvae to sink in straight lines. Arkett *et al.* (1987) also presented evidence that showed that the external Ca^{++} concentration may affect this cycle or it may be stopped altogether by using anaesthetics. Although this aspect has not been studied for species other than gastropods, the swimming activity of a bivalve larvae from estuarine and oceanic areas may vary considerably and would undoubtedly make comparisons complex.

Compared to initial characterization of behaviours by well fed larvae in a medium that contained only filtered seawater, the behaviour of younger, well-fed and previously starved larvae showed a small decrease in the vertical component of helical swimming and a considerable increase in helical velocity in starved larvae. Although there was a great deal of variability, this response is consistent with results for *Bankia gouldi* and *Argopecten irradians* (Gallager, 1991) under similar conditions. Unfortunately, I did not assess later stages where the responses might be more evident. In microzooplankton studies, Price and Paffenhofer (1984) found that the copepod (*Eucalanus pileatus*) alters its behaviour depending on the previous feeding condition including changes in the

particle size spectra of cells captured and ingested over time. It is not known if such selective behaviours also occur in meroplanktonic organisms, but certainly this is an essential question to be resolved considering the implications that it may have on the trophic dynamics and energy flow in the aquatic environment (Dickie and Smith , 1989).

The most conspicuous change in the swimming ability of sea scallop larvae occurred in 10-16 days old (140-160 μm) larvae in which there was a noticeable increase in vertical and helical velocity that may have resulted from increasing strength resulting from a larger ciliated velum and perhaps the addition of compound cilia that it is known to occur in other bivalve larvae (Cragg, 1989; Gallager, 1991). The significance of this change could imply a better controlling mechanism to remain suspended in the water column. At the same time, the extended velum could help to maximize drag during descent in helical patterns (Emlet, 1983).

Considering the characteristics of each swimming mode it is reasonable to propose that physiological changes occurring in larvae must act in concert with specific adaptations for increasing feeding efficiency and diminishing the risk of sinking off the water column. Helical swimming seems to provide an efficient mechanism for increasing feeding, and since it works in both directions (upward and downwards), his could enhance particle contact rates and also help larvae to remain suspended (Levandowsky *et al.*, 1988, Rothschild and Osborn, 1988; Gallager, 1991,1993).

Feeding and growing result in the addition and growth of structures including increasing density by shell deposition, therefore, swimming larvae would benefit from mechanisms that enhance buoyancy and hence diminish the energetic cost of swimming which could be provided by the presence of lipids of low density.

5.3 Does lipid class composition of larvae vary throughout ontogeny?

The variability of lipid composition has been used as indicator of larval condition for many larval fish, microzooplankton and holoplanktonic organisms (Fraser, 1989). The main aspect of lipid variability that has often been overlooked, but commonly referred to.

is their role in providing compact energy storage and buoyancy (Sargent, 1976). Triacylglycerides in particular are less dense than other lipid classes but are usually difficult to assess. In this study, only the relative proportion of lipid classes as a function of total lipids was examined. The relative amount of triacylglycerides greatly diminished through development from 43.3 to 7.84% of total lipids, which led to the conclusion that lower density or at least, higher buoyancy is conferred on earlier stages by this lipid class alone. Although, larval densities were not measured in this study, Jackson (1992) estimated the density for unfertilized eggs and larvae and found that early stages were indeed less dense than later stages. Current data on scallop larvae indicates that earlier stages also present scattered globules of neutral lipids throughout the complete larval body, while it become more concentrated in the digestive gland in later stages (Jackson, D. Dalhousie University, pers. comm.).

The triacylglycerol-sterol ratio has been proposed as a good condition index for larval stages of fish, crustaceans and bivalves that can be used without the need of relative weight of total lipids, since sterols have shown to be highly correlated with larval dry weight (Fraser, 1989). In scallop larvae it is evident that this ratio decreases through times; however, the implications of this finding are not clear. Because of the shear required for particle capture there may be a trade off between feeding efficiency and density (Strickler, 1982), such a that some reduction in lipid stores or buoyancy is necessary for optimal feeding.

In theory it is assumed that larval condition will increase as it grows, and it is well known that lipids are important energetic reserves for planktotrophic larvae, particularly during the period of embryogenesis and later during metamorphosis when larvae seem unable to feed (Whyte *et al.*, 1987). However, during the initial planktotrophic stage there seems to be a transitional period in which some larval species continue to catabolize lipids of maternal origin, with an increase in lipid reserves only occurring toward the end of the planktonic period. As in the comparison of swimming behaviour of larval species,

it seems that estuarine species and those with shorter planktotrophic periods enter the water column with greater amounts of lipids, which may not be necessary for a larva such as a sea scallop. However, this question needs to be further addressed by quantification of lipid reserves and lipid classes relative to larval weight and density. A comparison among estuarine and marine species would aid in providing answers to this central question on the role of lipids in different environments.

Larvae of estuarine invertebrates may require higher lipid contents both because of energetic requirements and because of the greater quantity of lipid needed to become neutrally buoyant in comparison with oceanic species. Fully marine larvae of invertebrates live in more stable environments may have fewer problems in maintaining an adequate level of buoyancy, because the ocean is much denser. The combination of behavioural traits provided by effective swimming and lipids, particularly triacylglycerides, would contribute to the success of benthic populations in maintaining a planktotrophic larvae as a response to relatively stable environments and should be further investigated.

5.4 Does vertical distribution of veligers vary in response to experimental simulations of mixed and stratified water columns in a 10 *m* mesocosm?

The broader question of passive versus active regulation of vertical distribution of larvae can only be hinted at since the experimental observations were limited to the response of veliger larvae in mixed and stratified conditions of food availability in the mesocosms, and it was not possible to simulate the current shear often present in normal water bodies. However, small amplitude changes in the larval centre of mass showed a clear trend toward nocturnal aggregation in the upper layers, moving deeper in the water column during the day, which resembles the pattern of diel vertical migration described earlier for most other planktonic organisms. Similar findings were reported by Tremblay and Sinclair (1990a) in a shallow area of the Bay of Fundy and by Raby *et al.* (1994) in a mixed and stratified location in Baie de Chaleurs.

Most recently, Raby *et al* (1994) found the same patterns of larval aggregation at shallow depths during the night associated with maximum chlorophyll-*a* concentration, and deeper in the water column below the maximum chlorophyll-*a* concentration during the day time in stratified waters In well mixed conditions, they found that larvae also migrated shallower at night and deeper during day, despite the presence of evenly distributed chlorophyll-*a* concentrations (Raby *et al.*, 1994).

The present study included one simulation (**S2**) that contained a column of filtered sea water with virtually no food (1 μm filtered seawater), where no net differences were found between the diurnal and nocturnal distribution of larvae. Ringelberg (1995) found found similar results in low food concentrations. The hypothesis of vertical migration in relation to the depth of the maximum chlorophyll-*a* concentration cannot be unequivocally supported in the present study because of the lack of measurements, and it was not supported by Raby *et al.* (1994)

A series of behavioural traits that seem to suit the requirements of planktonic life, such as remaining in the plankton by a combination of ciliary swimming in helical patterns and the accumulation of lipids has been proposed (Gallager, 1991) The triacylglycerides ratio decreased drastically from embryogenesis until day 13, which may reflect the higher metabolic requirements of larvae (Manning, 1985), but showed a slight increase at day 22 prior to metamorphosis. Larval swimming activity requires a large amount of energy which in many bivalve larvae is thought to be supplied mainly by lipids accumulated from the available diet. Lipid condition of larvae in the mesocosms studies were not assessed but this should be considered in future research plans to determine the effect of varying food availability in stratified and mixed conditions

The relationship of bivalve larval feeding index and chlorophyll-*a* was found to vary according to the level of stratification of the water column in Baie de Chaleurs, indicating that in stratified waters the feeding index was higher at the depth of maximum chlorophyll-*a*, while no differences were found in mixed waters (Raby *et al.*, 1994).

Furthermore, the same authors found a higher feeding index during nocturnal samples (14 to 31%) compared to diurnal samples in both levels of stratification which supports the hypotheses that hunger may cause upward migration and satiation causes donward migration, as proposed by Pearre (1979). Further research is needed to estimate the rate at which each of the identified behaviours occurs in nature, the natural diets and the feeding index of larvae in the water column under various environmental conditions.

Micro-scale observations can contribute to this by providing a basic understanding of behavioural traits of scallop larvae through ontogeny; the further examination of vertical distribution of veligers in meso-scale experiments helps to identify the larval response to food availability. Although my study mainly focused on the effect of food availability which may be more relevant for early developmental stages, there is a need to continue to investigate the response of older larvae in similar conditions as the primary drive becomes finding settlement sites. Trying to decouple the various mechanistic bases for the vertical distribution of larvae is only possible in mesocosm simulations and efforts have been made to carry on with this line of research at Dalhousie. Following the completion of this study, a new series of simulations were undertaken with the addition of a system that allows the generation of a temperature stratified column and video sampling which indicates that larvae can also vary their response to temperature gradients through ontogeny.

Active depth regulation is a possible scenario that can be supported from the capability of larvae to sink and ascend in microcosms. Veliger larvae of scallops at 32‰ salinity were found to ascend in helical patterns at a mean vertical velocity varying between 0.09 to 1.05 $mm\ s^{-1}$ and to descend at 0.20 to 0.37 $mm\ s^{-1}$. When larvae display straight line patterns no apparent feeding occurs, and this particular behaviour is proposed to be purely related to changing vertical distribution. If we consider each observed larval behaviour to be sustained for a period of one hour, larvae could produce swimming velocities of 0.7 to 1.3 $m\ hr^{-1}$ during ascending and descending helices,

respectively. Ascent and descent in straight lines could reach 2.6 to 4.8 $m\ hr^{-1}$, respectively. In small containers neither of the observed behaviours were found to last for long periods of time. Instead a continuous shift from helical to straight swimming occurred which is probably the result of velum wide ciliary arrests caused by depolarization of the membrane as observed in gastropod larvae (Arkett *et al.*, 1987). The continuous sinking after upward and helical swimming could be the means by which a larva regains its vertical position at small scale to optimize feeding rates; however, most observations of well fed larvae where done in a medium containing filtered seawater. When fed and starved larvae are compared clear differences in the potential effect that swimming in either helical or straight lines patterns may have on larval vertical distribution are seen.

With the techniques available for these mesocosm studies it was not possible to identify the micro-scale temporal or spatial variability of swimming larvae; however, since most experimental simulations contained adequate food, larvae were expected to spend most of their time feeding, and therefore, maintaining their vertical position in the water column Nonetheless, there were conspicuous changes in the mean depth of larvae between day and night samples that lend support to the hypotheses of diel vertical migration. Based in this study, satiated larvae will sink and regain position in relation to physiological changes caused by feeding, and should be the focus of further studies.

Studies of microzooplankton locomotion and behaviour are more common and have usually found significant differences among instars of crustacean larvae (Sulkin, 1984). Bivalve larvae are only ephemeral inhabitants on the plankton which usually coincide with periods of high primary productivity (spring and fall) (Day and McEdward, 1984); however, there are very few studies that have focused on the adaptive capabilities of larvae to successfully complete planktonic development prior to settlement. The swimming activity of bivalve larvae has been thought to affect vertical distribution in some species but not in others. In this study, it is proposed that passive sinking and ascent

in vertically oriented straight lines has an important role on the vertical distribution of larvae, which may also affect its horizontal distribution.

Moreover, the characteristic helical patterns represent a common adaptation to efficient feeding as found in many other planktonic microzooplankton. This particular behaviour could help larvae to remain in phytoplankton patches which could increase retention of larvae in or near areas of high productivity. Further work is needed to determine threshold response to chlorophyll-*a*, temperature and salinity levels that may elicit changes in behaviour throughout ontogeny and could provide useful insights into the feeding ecology of bivalve larvae.

Changes in the vertical distribution of larvae in nature may also be affected by the presence of predators (Bollens and Frost 1989; Bollens and Stearns, 1992, Ringelberg, 1995) and further investigations are needed to understand the dynamics of predator-prey interactions affecting larvae. The 10 *m* mesocosm provides an ideal intermediate scale to address all the suggested ecological questions which could lead to the formulation and testing of the complex interaction found in the early life history of benthic molluscs

Notes on Statistical Appendices

The statistical analysis of all data obtained during the experimental recording of swimming behaviour, determination of lipid classes and during the assessment of vertical distribution, were assessed by one way analysis of variance followed by a post-hoc assessment of contrasts across the group means as proposed by Rodger (1974,1975).

Appendix A contains an explanation of the assessment of contrasts using the data obtained for Helix Height during the recording of helical swimming throughout larval development and shows that there are no differences between ascent and descent during the first sampled stage (4 days). It also shows that the height of the helix is higher in ascending larvae at 16 days. The other larval stages have intermediate helix height and are therefore situated between the lower and higher helix height.

Appendices B1, B2, B3, B4, and B5 includes the summary tables for each set of data analyzed. In general, younger larvae have lower values of individual swimming components as well as vertical and helical velocity; older larvae have the higher values, and all F[0.05];9,40= 1.080 (Appendix B1). When linear velocity was analyzed, the older larval stages (16,22, 30 days) again have higher sinking velocities but these are matched by the 16 days ascending veligers; all others are considered equal. Analysis of variance is well above the critical F[0.05];11,90= 0.95 (Appendix B2).

Appendix B3 shows the results of the analysis of data of swimming components and helical swimming during ascent and shows that there were significant differences among helix radius, helix pitch and vertical velocity since the F-values were well above F[0.05];5,32= 1.490. The helix radius was particularly large in the fed larvae in a medium containing food and very reduced for starved larvae in filtered seawater, result consistent with retention in vertical patches. The other four groups had variable radii. The helix pitch and vertical velocity were higher in both groups of starved larvae which is likely indicative of an active searching mode in these larvae. Bothe helix height and helical velocity were similar, independent of the treatment and no significant differences were found among the groups.

Appendix B4 shows the results of the analysis of each lipid class present in the total lipid fraction. Triacylglycerides were lower at older stages that during eearlier stages at well above F[0.05];4,43= 1.650. Sterols and phospholipids increased with age and the older the larvae, the larger the Sterols and Phospholpids composition.

Appendix B5 contains a detailed analysis of variance and contrasts within each simulation and confirmed the presence of a distinct pattern between day and night distribution of larvae. In this analysis only, the sample volumes were equated to either 20 or 7 l , the results of the analysis of variance and the night versus day contrasts for each simulation confirmed that there were clear differences except in simulations 2 and 8

Appendix A

The object of the assessment of contrasts *post-hoc* is to discover a set of NG-1 contrasts which resolve and reflect the differences between the NG groups means. Those contrasts must be linearly independent of one another; preferrably mutually orthogonal. The process is illustrated with the Helix height data.

The sample means (each based on N = 5) in order of size were:

	m_1	m_6	m_3	m_2	m_{10}	m_7	m_8	m_5	m_9	m_4	s_2
or	m_{4u}	m_{4d}	m_{12u}	m_{6u}	m_{22d}	m_{8d}	m_{12d}	m_{22u}	m_{16d}	m_{16u}	
=	0.31	0 36	0 62	0.74	0.86	1.00	1.10	1.38	1.49	2 24	0.35

The overall F-ratio for these ten groups is Fm = 4 929. This is much larger than Rodger's (1975) criterion F[.05]; 9,40 = 1.080; so there are contrasts across the means wich will also give F-ratios greater tan 1 080 For example, one of the differences between the largest three means:

$$\sum c_j \mu_j = c_4\mu_4 + c_5\mu_5 + c_9\mu_9 = \mu_4 - 2\mu_5 + \mu_9 = 0$$

by the F procedure

$$F = N\frac{\left(\sum c_j m_j\right)^2}{\nu_1 s^2 \sum c_j^2} = 5 \times \frac{(0\,976)^2}{(9 \times 0.344 \times 6)} = 0\,256$$

wich is clearly less than 1.080, so we would accept the null contrast

The decision set finally selected (with F values in parentheses) was

$$\mu_2 - \mu_3 = 0 \qquad (0.012)$$
$$\mu_{10} - \mu_7 = 0 \qquad (0\,016)$$

$$\mu_{10} + \mu_7 - \mu_5 - \mu_3 = 0 \qquad (0.100)$$
$$4\,\mu_8 - \mu_2 - \mu_3 - \mu_5 - \mu_{10} = 0 \qquad (0.112)$$

which establishes the equality of these five means.

$$\mu_4 - \mu_9 = 0 \qquad (0.464)$$
$$\mu_4 + \mu_9 - 2\,\mu_6 = 0 \qquad (0.256)$$

which establishes the equality of these three means

$$\mu_6 - \mu_1 = 0 \qquad (0.002)$$

which equates those two.

$$2\,\mu_1 + 2\,\mu_{\;} + 2\,\mu_9 - 3\,\mu_{\;} - 3\,\mu_2 > 0 \qquad (3.614)$$

which demonstrates that the top three differ from the bottom pair of means

Finally,

$$\mu_{\;} + \mu_1 + \mu_{\;} + \mu_{\;} + \mu_{\;} - \mu_{\;} - \mu_{\;} - \mu_{\;} - \mu_{\;} - \mu_{\;} = 0 \qquad (0.351)$$

which establishes that the middle group of five means ($\mu_{\;}$, $\mu_{\;}$, $\mu_{\;}$, $\mu_{\;}$, $\mu_{\;}$ and $\mu_{\;}$) sit in the middle "as" at the average of the top three ($\mu_{\;}$, $\mu_{\;}$, $\mu_{\;}$) and the bottom pair (μ_1, $\mu_{\;}$).

We may conclude that, within the limits of what Type I statistical decision error permits

$$\mu_{\;} = \mu_{\;} = \mu_{\;} = \mu_{\;} = \mu_{\;} > \mu_{\;} = \mu_{\;} > \mu_1 = \mu_{\;}$$

or

$$\mu_{\;} = \mu_{\;} = \mu_{\;} = \mu_{\;} = \mu_{\;} > \mu_{\;} = \mu_{\;} > \mu_1 = \mu_{\;}$$

Hereafter the individual decision sets are not reported.

Appendix B1 (Helical movement)

Results of the analysis of variance of helical movement from scallop larvae throughout development are shown below.

Only the final results of analysis, carried out in the fashion shown in Appendix A, are given. The sample means (m_i) and the populations means implied by the analysis are shown. Sample sizes were the same for all variates ($N = 5$) after random removal of observations. All F-ratios are based on 9 and 40 degrees of freedom, for wich the corresponding critical value at 0.05 significance level is: $F_{[.05];\,9,40} = 1.080$.

The group codes are 4, 6, 12, 16 and 22 days, code U represents upward movement and code D downward movement. Height and radius were measured in *mm*, pitch in degrees and vertical and helical velocity in *mm* s^{-1}.

HELIX RADIUS											**MSE**	**F**
m_i	0.17	0.21	0.39	0.42	0.45	0.49	0.53	0.60	0.65	0.67	0.042	3.434
u	4D=	4U<	6U=	16U=	12U=	22D=	22U<	6D=	16D=	12D		

HELIX HEIGHT												
m_i	0.31	0.36	0.62	0.74	0.86	1.00	1.10	1.38	1.49	2.24	0.344	4.929
u	4U=	4D<	12U=	6U=	22D=	6D=	12D<	22U=	16D=	16U		

HELIX PITCH												
m_i	11.98	13.3 2	14.9 0	15.1 5	15.6 7	18.5 0	19.8 6	20.2 1	23.5 3	40.3 1	70.909	4.702
u	12U=	4U=	22D=	12D=	6D=	6U=	4D=	16D=	22U<	16U		

HELICAL VELOCITY												
m_i	0.46	0.55	0.60	0.64	0.86	0.98	0.99	1.10	1.19	1.62	0.105	5.932
u	4U=	4D=	12U=	6U<	12D=	22D=	6D=	16D=	22U<	16U		

VERTICAL VELOCITY												
m_i	0.09	0.13	0.19	0.20	0.25	0.25	0.26	0.37	0.42	1.05	0.037	10.122
u	4U=	12U=	6U=	4D=	12D=	22D=	6D<	16D=	22U<	16U		

Note: analysis for unequal sample sizes led to the same conclusions as those given above.

APPENDIX B2 (Linear movement)

Results of the analysis of variance of linear velocities (straight) from scallop larvae throughout development are shown below.

Only the final results of analysis, carried out in the fashion shown in Appendix A, are given. The sample means (m_i) and the populations means implied by the analysis are shown. The sample sizes (N) are noted in the table below. The F-ratio had 11 and 90 degrees of freedom, for wich the corresponding critical value at 0.05 significance level is: $F[.05], 11,90 = 0.950$.

The group codes are 4, 6, 12, 16 and 22 days, code U represents upward movement and code D downward movement. Linear velocity was measured in *mm* s^{-1}.

LINEAR VELOCITY **MSE: 0.138** **F: 8.10**

N	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
m_i	0.69	1.72	1.81	.64	0.95	1.38	1.1[illegible]	.4	1.[illegible]	1.45	2.[illegible]	1.[illegible]
u	12U =	6U =	30U =	12D=	4D=	0	22D	6D	16D	D	6D	22D

Note: When group sizes are equated to N = 6 for ten of the groups (excluding 30U and 30D) by random removals the above conclusion were confirmed.

APPENDIX B3 (Treatments)

Results of analysis of variance of helical movement in early veligers (6 days) of each treatment: fed, starved and control. The group codes for treatments were **F/S** (fed in seawater); **F/F** (fed in the presence of food); **S/S** (starved in seawater); **S/F** (starved in food); **C/S** (control starved in seawater), and **C/W** (control starved after the addition of seawater).

Only the final results of analysis carried out in the fashion shown in Appendix A, are given. The sample sizes (N_j = 6,5,7,5,7,8) were the same for all variates and are given in the table. The sample means (m_j) and the population means implied by the analysis are shown. All F-ratios are based on 5 and 32 degrees of freedom, for wich the corresponding critical value at 0.05 significance level is $F[.05]; 5\ 32 = 1.490$

HELIX RADIUS							**MSE**	**F**
N_j	7	6	5	8	7	5		
m	0 08	0 11	0.12	0 16	0.24	0 30	0 014	2.985
u	**S/S=**	**F/S=**	**S/F<**	**C/W<**	**C/S=**	**F/F**		

HELIX HEIGHT								
N_j	5	8	6	7	5	7		
m_j	0 30	0.45	0 50	0.57	0 85	0 96	0 258	1 441
u_j	**F/S=**	**C/W=**	**F/F=**	**C/S=**	**S/S=**	**S/F**		

HELIX PITCH								
N_j	6	8	5	7	7	5		
m	16.41	23.93	27 78	35.72	47.87	64.32	423.565	5.215
u_j	**F/F=**	**C/W=**	**C/S=**	**F/S<**	**S/F=**	**S/S**		

HELICAL VELOCITY								
N_j	8	6	7	5	7	5		
m_j	0.31	0.32	0.33	0.35	0.40	0.47	0.022	1.175
u_j	**S/F=**	**C/W=**	**F/S=**	**C/S=**	**F/F=**	**S/S**		

VERTICAL VELOCITY

N_i	7	8	5	6	5	7		
m_i	0.08	0 11	0.13	0 17	0 23	0 43	0 012	9.612
u_i	**C/S=**	**C/W=**	**F/F=**	**F/S<**	**S/F<**	**S/S**		

Note: When group sizes are equated (to N = 5) by random removals, the above conclusions were confirmed. The difference in Pitch between the large **S/F** and **S/S** and the three smaller means (F = 1.480) did not quite reach the criterion $F_{[.05],\,5,32} = 1.490$, therefore all six groups are accepted as equal.

APPENDIX B4 (Lipids classes)

Results of the analysis of variance of lipid classes composition from eggs to 22 days larvae of sea scallop are shown below.

Only the final results of analysis, carried out in the fashion shown in Appendix A, are given. The sample means (m_j) and the populations means implied by the analysis are shown. The sample sizes (N) are noted in the table below and the F-ratio was based on 4 and 43 degrees of freedom, for wich the corresponding critical value at 0.05 significance level is: $F_{[.05],4,43} = 1.650$.

The group codes are unfertilized eggs (NF), fertilized eggs (F), 4 days (4D), 13 days (13D) and 22 days old larvae (22D). The variate measured was percentage of individual lipid classes obtained from the Iatroscan analyzer.

TRIACYLGLYCERIDES						MSE	F
N	5	5	11	11	16		
m	7 84	20 15	28.60	43.32	43 50	33 709	52 524
u_i	13D<	22D<	4D=	NF=	F		

STEROLS							
N	16	11	11	13	22		
m_i	2.59	3.53	4.35	5 71	9 18	8 512	5 340
u	NF=	F=	4D<	13D<	22D		

PHOPHOLIPIDS							
N	16	11	11	5	5		
m	42.26	42 65	59 48	64 62	70 67	44 505	31 409
u_i	F=	NF<	4D<	22D<	13D		

Note: Analysis of equal sample sizes after random removal of observations led to the same genaral conclusions.

APPENDIX B5 (Mean depth)

Results of the analysis of variance of larval mean depth determined for sea scallop larvae in each simulation are shown below.

The unequal sample sizes (N_i), the sample means (m_i), MSE, overall F-ratio (Fm) and the F-ratio for night versus day groups (F n/d) are shown.

Simulation 1 contained 50 *μm* filtered seawater; Simulation 2 contained only 1 *μm* filtered seawater; Simulations 3 and 4 initially contained patchy food at the surface; Simulation 5 contained vertically uniform distribution of food. Simulations 6-9, contained a salinity stratified water column with patchy food at the haloclines.

Because of the very large d.f. for error, the critical value used will be $F[0.05]_{1,\infty} = 1.551$, $F[0.05]_{5,\infty} = 1.372$; and $F[0.05]_{10,\infty} = 0.973$. These Rodger (1975) post-hoc criteria are much higher than the F-equivalent of a t-test ($F[0.05]_{1,\infty} = 3.841$) because they are effectively $4F[0.05]_{4,\infty} = 6.204$, $5F[0.05]_{5,\infty} = 6.860$; and $10F[0.05]_{10,\infty} = 9.730$.

The only simulations showing no night/day difference in mean depths were simulations S2 and S8.

SIMULATION 1

	Night			Day			MSE	Fm	Fn/d
N	2144	471	318	607	935				
m	1.79	3.21	4.35	5.98	6.44		2.648	1720.746	685.889

SIMULATION 2

	Night			Day			MSE	Fm	Fn/d
N_i	1160	940	400	540	360	260			
m	3.45	3.65	4.22	3.80	3.94	4.08	4.646	14.71	0.884

SIMULATION 3

	Night			Day			MSE	Fm	Fn/d
N	5120	620	360	920	1340	240			
m_i	2 40	2 60	4 24	2 68	4 42	7 31	4 348	39 705	118 345

SIMULATION 4

								MSE	Fm	Fn/d
Night	N	1479	1054	673	1081	984				
	m_i	2 39	2 96	3 58	3 80	4 15				
Day	N	1343	920	1040	739	1016	951			
	m_i	3 89	4 37	4 86	4 87	4 90	6 61	6 165	168 275	103 52

SIMULATION 5

								MSE	Fm	Fn/d
Night	N	1349	919	1159	950	1055				
	m_i	3 02	3 17	4 17	4 34	4 72				
Day	N	1517	1078	707	730	998	859			
	m_i	3 75	4 33	4 48	4 71	5 59	5 61	6 175	106 339	32 785

SIMULATION 6

	Night			Day		MSE	Fm	Fn d
N	508	517	3051	449	667			
m_i	5 07	5 16	7 00	5 42	5 64	3 673	264 861	2 690

SIMULATION 7

	Night			Day		MSE	Fm	Fn/d
N	413	376	599	523	766			
m_i	6 35	6 66	6 75	6 56	7 1	3 350	13 06,	3 112

SIMULATION 8

	Night			Day				MSE	Fm	Fn/d
N	660	114	94	963	1380	583	308			
m_i	3 42	4 66	4 66	3 89	3 91	4 15	4 63	2 960	27 163	0 201

SIMULATION 9

	Night			Day				MSE	Fm	Fn/d
N	913	279	456	1136	1682	705	762			
m_i	4 77	5 72	5 73	5 51	5 94	6 37	6 53	2 758	124 89	20 571

REFERENCES

Ackman, R.G., 1981. Flame ionization detection applied to thin layer chromatography on coated quartz rods. In J.M. Lowenstein [ed.], Methods in Enzymology. Vol. 72. Academic Press, London, pp. 205–252.

Alexander, R.M., 1990. Size, speed and buoyancy adaptations in aquatic animals. Amer. Zool. 30: 189–196.

Andrews, J.D., 1979. Pelecypoda: Ostreidae. In A.C. Giese and J.S. Pearse [eds.], Reproduction of Marine Invertebrates, Vol. 5. Academic Press, New York, pp. 239–339.

Andrews, J.D., 1983 Transport of bivalve larvae in James River, Virginia. J. Shellfish Res. 3: 29–40.

Arkett, S.A., G.O. Mackie and C.L. Singla, 1987. Neuronal control of ciliary locomotion in a gastropod veliger (*Calliostoma*) Biol. Bull. 173: 513–526.

Balch, N., C.M. Boyd and M. Mullin, 1978. Large-scale tower tank systems. Rapp P.-v. Reun. Int. Explor. Mer. 173: 13–21.

Bayne, B.L., 1963. Responses of *Mytilus edulis* larvae to increases in hydrostatic pressure. Nature 198. 406–407.

Bayne, B.L., 1964. The responses of the larvae of *Mytilus edulis* (L) to light and gravity Oikos 15: 162–174.

Bayne, B.L., 1965 Growth and delay of metamorphosis of the larvae of *Mytilus edulis* (L). Ophelia 2: 1–47.

Bayne, B.L., 1972. Some effects of stress in the adult on the larval development of *Mytilus edulis*. Nature 237. 459.

Bayne, B.L., 1983. Physiological ecology of marine molluscan larvae. In N.H. Verdonk, J.A.M. Van den Biggelaar and A.S. Tompa [eds.], The Mollusca, Vol. 3 Development. Academic Press, New York, pp. 299–343.

Black, G.A.P., R.K. Mohn, G. Robert and M.J. Tremblay, 1993. Atlas of the biology and distribution of the sea scallop *Placopecten magellanicus* and Iceland scallop *Chlamys islandica* in the Northwest Atlantic Can. Tech. Rep. Fish. Aquat. Sci. No. 1915: 40p.

Bligh, E.G. and D.J. Dyer, 1959. A rapid method for total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911–917.

REFERENCES

Ackman, R.G., 1981. Flame ionization detection applied to thin layer chromatography on coated quartz rods. In J.M. Lowenstein [ed.], Methods in Enzymology. Vol. 72. Academic Press, London, pp. 205–252.

Alexander, R.M., 1990. Size, speed and buoyancy adaptations in aquatic animals. Amer. Zool. 30: 189–196.

Andrews, J.D., 1979. Pelecypoda: Ostreidae. In A.C. Giese and J.S. Pearse [eds.], Reproduction of Marine Invertebrates, Vol. 5. Academic Press, New York, pp. 239–339.

Andrews, J.D., 1983. Transport of bivalve larvae in James River, Virginia. J. Shellfish Res. 3: 29–40.

Arkett, S.A., G.O. Mackie and C.L. Singla, 1987. Neuronal control of ciliary locomotion in a gastropod veliger (*Calliostoma*). Biol. Bull. 173: 513–526.

Balch, N., C.M. Boyd and M. Mullin, 1978. Large-scale tower tank systems. Rapp. P.-v. Reun. Int. Explor. Mer. 173: 13–21.

Bayne, B.L., 1963 Responses of *Mytilus edulis* larvae to increases in hydrostatic pressure. Nature 198: 406–407.

Bayne, B.L., 1964 The responses of the larvae of *Mytilus edulis* (L) to light and gravity Oikos 15: 162–174.

Bayne, B.L., 1965 Growth and delay of metamorphosis of the larvae of *Mytilus edulis* (L). Ophelia 2: 1–47.

Bayne, B.L., 1972 Some effects of stress in the adult on the larval development of *Mytilus edulis*. Nature 237. 459.

Bayne, B.L., 1983 Physiological ecology of marine molluscan larvae In N.H. Verdonk, J.A.M. Van den Biggelaar and A.S. Tompa [eds.], The Mollusca, Vol. 3. Development. Academic Press, New York, pp. 299–343.

Black, G.A.P., R.K. Mohn, G. Robert and M.J. Tremblay, 1993. Atlas of the biology and distribution of the sea scallop *Placopecten magellanicus* and Iceland scallop *Chlamys islandica* in the Northwest Atlantic. Can. Tech. Rep. Fish. Aquat. Sci. No. 1915: 40p.

Bligh, E.G. and D.J. Dyer, 1959 A rapid method for total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911–917.

Bohrer, R.N., 1983. Experimental studies on diel vertical migration. **In** W.C. Kerfoot [ed.,] Evolution and Ecology of Zooplankton communities. Special Symposium. Vol 3. American Society of Limnology and Oceanography, University Press of New England, pp. 11-121.

Boicourt, W.C., 1982. Estuarine larval retention and mechanisms on two scales. **In** V.S. Kennedy [ed.], Estuarine Comparisons. Academic Press, New York, pp. 235–246.

Bollens, S.M. and B.W. Frost, 1989. Zooplanktivorous fish and variable diel vertical migration in the marine planktonic copepod *Calanus pacificus*. Limnol. Oceanogr. 34: 1072–1083.

Bollens, S.M. and D.E. Stearns, 1992 Predator-induced changes in the diel feeding cycle of a planktonic copepod. J. Exp. Mar. Biol. Ecol, 156: 179–186.

Bourne, N.F.F., 1964. Scallops and the offshore fisheries of the Maritimes Bull. Fish. Res. Board. Can. 145: 60pp.

Burton, R.S. and M.W. Feldman, 1982 Populations genetics of coastal and estuarine invertebrates: does larval behaviour influence population structure? **In** V.S. Kennedy [ed.], Estuarine Comparisons. Academic Press, New York, pp. 537–551.

Buskey, E.J., C. Coulter and S. Strom, 1993. Locomotory patterns of microzooplankton: potential effects on food selectivity of larval fish. Bull. Mar. Sci. 53: 29–43.

Caddy, J.F., 1979 Long-term trends and evidence for production cycles in the Bay of Fundy scallop fishery. Int. Council. Explor. Sea. Rapp. P.-V. Reun. 175: 97–108.

Cameron, R.A., 1986. Introduction to the invertebrate larval biology workshop: a brief background. Bull. Mar. Sci. 39: 145–161.

Carriker, M.R., 1951. Ecological observations on the distribution of oyster larvae in New Jersey estuaries. Ecol. Monogr. 21. 19–38.

Carriker, M.R., 1961. Interrelation of functional morphology, behaviour and autoecology in early stages of the marine bivalve *Mercenaria mercenaria* J. Elisha Mitchell Sci. Soc. 49: 85–125.

Chia, F.-S., J. Bucklands-Nicks and C.M. Young, 1984 Locomotion of marine invertebrate larvae: a review. Can. J. Zool. 62: 1205–1222.

Chouinard, G.A., 1984. Growth of the sea scallop (Placopecten magellanicus) on the Tormentine bed, Northumberland strait, Canada ICES, C.M. 1984/k 40, 7p.

Collins, T.M., 1989. A comparative study of the vertical migration of marine zooplankton in the field and laboratory. M.Sc. Thesis. Dalhousie University, Halifax, Nova Scotia, Canada. 184pp.

Conover, R.J., 1968. Zooplankton-life in a nutritionally dilute environment. Am. Zool. 8: 107–118.

Couturier, C.Y., 1986. Aspects of reproduction and larval production in *Placopecten magellanicus* held in a semi-natural environment. M.Sc. Thesis, Dalhousie University, Halifax, Nova Scotia. 108pp.

Cragg, S.M., 1980. Swimming behaviour of the larvae of *Pecten maximus* (L.) (Bivalvia). J. Mar. Biol. Assoc. U.K. 60: 551–564.

Cragg, S.M., 1989. The ciliated rim of the velum of larvae of *Pecten maximus* (Bivalvia: Pectinidae). J. Moll. Stud. 55: 497–508.

Cragg, S.M. and D.J. Crisp, 1991. The biology of sea scallop larvae. **In** S.E. Shumway [ed.], Scallops: Biology, Ecology and Aquaculture. Developments in Aquaculture and Fisheries Science, Vol. 21, Elsevier Science Publishers, The Netherlands. pp. 75–132.

Cragg, S.M. and Ll.D. Gruffydd, 1975. The swimming behaviour and the pressure responses of the veliconcha larvae of *Ostrea edulis* (L.) **In** H. Barnes [ed.], Proceedings of the 9th European Marine Biology Symposium. Oban, Scotland, 1974, Aberdeen University Press, Aberdeen, Scotland. pp. 43–57.

Crisp, D.J., 1976. The role of the pelagic larva. **In** P. Spencer-Davies [ed.], Perspectives in Experimental Biology, Vol. 1, Zoology, Pergamon Press, Oxford. pp. 145–155.

Culliney, J.L., 1974. Larval development of the giant sea scallop *Placopecten magellanicus* (Gmelin). Biol. Bull. 147: 321–332.

Cushing, D.H., 1951. The vertical migration of planktonic crustacea. Biol. Rev. 26: 158–192.

Cushing, D.H., 1975. Marine Ecology and Fisheries. Cambridge University Press, London. 278pp.

Davis, C.S., 1984. Interaction of a copepod population with the mean circulation on Georges Bank. J. Mar. Res. 42: 573-590.

Dagg, M., 1977. Some effects of patchy food environments on copepods. Limnol. Oceanogr. 22: 99–107.

Day, R. and L. McEdward. 1984. Aspects of the physiology and ecology of pelagic larvae of marine invertebrates. In K.A. Steidinger and L.M. Walker [eds.] Marine Plankton Life Cycle Strategies. CRC Press, Boca Raton, Fla., pp. 93–120.

Delaunay, F., Y. Marty, J. Moal and J.-F. Samain. 1992. Growth and lipid class composition of *Pecten maximus* (L.) larvae grown under hatchery conditions. J. Exp. Mar. Biol. Ecol. 163: 209–219.

Dickie, L.M., 1955. Fluctuations in abundance of the giant sea scallop *Placopecten magellanicus* (Gmelin), in the Digby area of the Bay of Fundy. J. Fish. Res. Board. Can. 12: 797–856.

Dickie, L.M. and P.C. Smith. 1989 Fisheries ecology program (FEP): General introduction. Can. J. Fish. Aquat Sci. 46: 538.

Dibacco, C., G. Robert and J. Grant., 1995. Reproductive cycle of the sea scallop, *Placopecten magellanicus* (Gmelin, 1791) on Northeastern Georges Bank J Shellfish Res. 14: 59–69.

Drew, G.A., 1906 The habits, anatomy and embryology of the giant scallop *Pecten tenuicostacus* (Mighels). University of Maine Studies. No. 6. 3–71

Duncan, D.B., 1952. On the properties of the multiple comparisons test Virginia J Sci 3: 50-67.

Ebenman, B., 1992. Evolution of organisms that change their niches during the life cycle. Amer. Nat. 139: 990-1021.

DuPaul, W.D., J.E. Kirkley, and A.C. Schmitzer, 1989. Evidence of a semiannual reproductive cycle for the sea scallop, *Placopecten magellanicus* (Gmelin 1971), in the mid-Atlantic region J Shellfish Res. 8: 173-178.

Ehrlich, K.F., 1974 Chemical changes during growth and starvation of larval *Pleuronectes platessa*. Mar. Biol 24: 39–48.

Emlet, R.B., 1983 Locomotion, drag and the rigid skeleton on larval echinoderms. Biol. Bull. 164: 433–445.

Enright, C.T., 1984. Determination of the relative value of phytoplankton for feeding the juvenile oyster, *Ostrea edulis*. Ph.D. Thesis, Dalhousie University, Halifax, Nova Scotia, Canada, 218pp.

Fogg, G.E., F.R.S., B. Egan, G.D. Floodgate, D.A. Jones, J.Y. Kassab, K. Lochte, E.I.S. Rees, S. Scrope-Howe and C.M. Turley, 1985. Biological studies in the vicinity of a shallow-sea tidal mixing front. VII. The frontal ecosystems. Phil. Trans. R. Soc. Lond. B 310: 555–571.

Fortier, L. and W.C. Leggett, 1982. Fickian transport and the dispersal of fish larvae in estuaries. Can. J. Fish. Aquat. Sci. 39: 1150–1163.

Forward, R.B.Jr., 1988. Diel vertical migration: zooplankton photobiology and behaviour. Oceanogr. Mar. Biol. Annu. Rev. 26. 361–393.

Forward, R.B.Jr. and W.F. Hettler Jr., 1992. Effect of feeding and predator exposure on photoresponses during diel vertical migration of brine shrimp larvae. Limnol. Oceanogr. 37: 1261–1270.

Fraser, A.J., 1989. Triacylglycerol content as a condition index for fish, bivalve, and crustacean larvae. Can. J. Fish. Aquat. Sci. 46: 1868–1873.

Fraser, A.J., J.R. Sargent, J.C. Gamble and P. MacLachlan, 1987 Lipid class and fatty acid composition as indicators of the nutritional condition of larval Atlantic herring. American. Fish. Soc. Symp. 2: 129–143.

Fraser, A.J., J.C. Gamble and J.R. Sargent, 1988. Changes in lipid class composition and fatty acid composition of developing eggs and unfed larvae of cod (*Gadus morhua*). Mar. Biol. 99: 307–313.

Gallager, S.M., 1985. Buoyancy regulation and swimming energetics in larvae of *Bankia gouldi* (Bartsch) (Terenidae, Bivalvia). Abstract of the 77th Annual NSA Meeting, Norfolk, VA. p8.

Gallager, S.M., 1988. Visual observations of particle manipulation during feeding in larvae of a bivalve mollusc. Bull. Mar. Sci. 43: 344–365.

Gallager, S.M., 1991. Locomotion and feeding in marine bivalve larvae Ph.D. thesis, Boston Univ., Mass., 293pp.

Gallager, S.M., 1993 Hydrodynamic disturbances produced by small zooplankton: case study for the veliger larvae of bivalve mollusc. J. Plankton Res. 15: 1277–1296.

Gallager, S.M. and R. Mann, 1981 Use of lipid-specific staining techniques for assaying condition in cultured bivalve larvae J. Shellfish Res. 1: 69–73.

Gallager, S.M. and R. Mann, 1986a Individual variability in lipid content of bivalve larvae quantified histochemically by absorption photometry J. Plankton Res. 8: 927–937.

Gallager, S.M. and R. Mann, 1986b Growth and survival of larvae of *Mercenaria mercenaria* (L.) and *Crassostrea virginica* (Gmelin) relative to broodstock conditioning and lipid contents of eggs. Aquaculture 56: 105–121.

Gallager, S.M., R. Mann and G.C. Sasaki, 1986. Lipid as an index of growth and viability in three species of bivalve larvae. Aquaculture 56. 81–103.

Gallager, S.M., J. Manuel, D.A. Manning and R.K. O'Dor.. 1995. Ontogenetic changes in the vertical distribution of the sea scallop larvae *Placopecten magellanicus* in 9m deep mesocosms as a function of light, food and temperature. Mar Biol. (in press).

Galtsoff, P.S.. 1964. The American oyster *Crassostrea virginica* Gmelin. U S. Fish. Wildl. Serv. Fish. Bull. 64: 480pp.

Geller, W.. 1986. Diurnal vertical migration of zooplankton in a temperate great lake (L Constance): A starvation avoidance mechanism? Arch. Hydrobiol. 74. 1–60.

Gruffydd, Ll.D.. 1976 The development of the larvae of *Chlamys islandica* in the plankton and its salinity tolerance in the laboratory (Lamellibranchia, Pectinidae) Astarte 8: 61–67.

Guillard, R.R.L. and J.H. Ryther. 1962 Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. Can. J. Microbiol. 8 229–239.

Guan, Y.-H., 1993. Genetic differences in larval behaviours among scallop (*Placopecten magellanicus*) populations. M.Sc. Thesis. Dalhousie University. Halifax, Nova Scotia, Canada, 85pp.

Harding, G.C., W.P. Vass, B.T. Hargrave and S. Pearre Jr.. 1986 Diel vertical movements and feeding activity of zooplankton in St Georges Bay, N.S., using net tows and a newly developed passive trap Can. J Fish. Aquat. Sci. 43: 952–967.

Haskin, H.H.. 1964. The distribution of oyster larvae In N Marshall H P Jeffries, T.A. Napora and J M. Sieburth [ed.], Symposium on experimental marine ecology. Graduate School of Oceanography, University of Rhode Island Occ Publ. 2: 76–80.

Hickman, R.W. and Ll. Gruffydd. 1971 The histology of the larvae of *Ostrea edulis* during metamorphosis. In D.J. Crisp [ed.], Fourth European Marine Biology Symposium. Cambridge University Press, Cambridge U K. pp 281–294

Hidu, H. and H.H. Haskin. 1978. Swimming speeds of oyster larvae *Crassostrea virginica* in different salinities and temperatures. Estuaries 1 252–255

Himmelman, J.H., 1975. Phytoplankton as stimulus for spawning in three marine invertebrates. J. Exp. Mar. Biol. Ecol 20: 199–214

Hines, A.H., 1986. Larval problems and perspectives in life histories of marine invertebrates. Bull. Mar. Sci. 39: 506–525

Hjort, J.. 1914. Fluctuations in the great fisheries of northern Europe viewed in light of biological research. Int. Explor. Mer. Rapp. P.-V. Reun. 20: 1–228.

Hodgson, C.A. and R.D. Burke. 1988. Development and larval morphology of the spiny scallop *Chlamys hastata*. Biol. Bull. 174: 303–318.

Holland, D.L., 1978. Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. In P.L. Malins and J.R. Sargent [eds.], Biochemical and biophysical perspectives in marine biology. Academic Press, London and New York. pp. 85–123.

Holland, D.L. and P.A. Gabbott, 1971. A micro-analytical scheme for the determination of proteins, carbohydrate, lipids and RNA levels in marine invertebrate larvae J. Mar. Biol. Ass. U.K. 51: 659–668.

Holland, D.L. and P.J. Hannant, 1974. Biochemical changes during growth of the spat of the oyster *Ostrea edulis* L. J. Mar. Biol. Assoc. U.K. 54: 1007–1016.

Holland, D.L. and B.E. Spencer, 1973. Biochemical changes in fed and starved oysters, *Ostrea edulis* (L.) during larval development, metamorphosis and early spat growth. J. Mar. Biol. Ass. U.K. 53: 287–298.

Huntley, M. and E.R. Brooks. 1982. Effects of age and food availability on diel vertical migration of *Calanus pacificus*. Mar. Biol. 71: 23–31.

Hurley, G.V., M.J. Tremblay and C. Couturier. 1987. Age estimation of sea scallop larvae (*Placopecten magellanicus*) from daily growth lines on shells. J. Northw. Atl. Fish. Sci. 7: 123–129.

Isham, L.B. and J.Q. Tierney. 1953. Some aspects of the larval development and metamorphosis of *Teredo (Lyrodus) pedicellata* de Quatrefages. Bull. Mar. Sci. Gulf Carib. 2: 574–590.

Jablonsky, D. and R.A. Lutz. 1983. Larval ecology of marine invertebrates: paleobiological implications. Biol. Rev. 58: 21–89.

Jackson, D.L., 1992 Physiological ecology of *Placopecten magellanicus*, food availability and larval growth response. M.Sc. Thesis, Dalhousie University, Halifax, Nova Scotia, Canada, 69pp.

Jackson, D.L., R.K. O'Dor and S.M. Gallager. 1994. Scallops in space: microgravity experiments with veliger locomotion. Can. Tech. Rep. Fish. Aquat. Sci. 1994: 45–52.

Johnsen, G.H. and P.J. Jakobsen, 1987. The effect of food limitation on vertical migration in *Daphnia longispina*. Limnol. Oceanogr. 32: 873–880.

Jonsson, P.R., 1989. Vertical distribution of planktonic ciliates - an experimental analysis of swimming behaviour. Mar. Ecol. Prog. Ser. 52: 39–53.

Jonsson, P.R., C. Andre and M. Lindegarth. 1991. Swimming behaviour of marine bivalve larvae in a flume boundary-layer flow: evidence for near bottom confinement. Mar. Ecol. Prog. Ser. 79: 67–76.

Jorgensen, C.B., 1981. Mortality, growth and grazing impact of a cohort of bivalve larvae, *Mytilus edulis* L. Ophelia: 185–192.

Joseph, J.D., 1982 Lipid composition of marine and estuarine invertebrates. Part II: Mollusca. Prog. Lipid Res. 21: 109–153.

Kaartvedt, S., D.L. Aksnes and J.K. Egge. 1987. Effect of light on the vertical distribution of *Pecten maximus* larvae. Mar. Ecol. Prog. Ser. 40: 195–197.

Keuls, M., 1952 The use of studentized range in connection with an analysis of variance. Euphytica 1 112-122

Konstantinova, M.I., 1966 Characteristics of movement of pelagic larvae of marine invertebrates Dokl. Akad. Nauk. SSSR 170. 726–729.

Korringa, P., 1941. Experiments and observations on swarming pelagic life and setting in the European flat oyster *Ostrea edulis* L. Arch. Neerl. Zool. 5. 1–249.

Korringa, P., 1952 Recent advances in oyster biology Q. Rev Biol. 27: 266–308 & 339–365.

Kunkle, D.R., 1957. The vertical distribution of oyster larvae in Delaware Bay Proc Natl. Shellfish. Ass. 48: 90–91.

Lampert, W. and C.J. Loose. 1992 Plankton towers: bridging the gap between laboratory and field experiments. Arch. Hydrobiol. 126. 53–66

Lee, J.W. and D.I. Williamson. 1975 The vertical distribution and diurnal migration of copepods at different stations in the Irish Sea. J. Oceanogr. Soc. Jpn. 31. 199–211

Le Febre, J. and E. Bourget. 1992. Hydrodynamics and behaviour: transport processes in marine invertebrate larvae Trends in Ecology and Evolution 9. 288–289.

Lehninger, A.L., 1975 Biochemistry. The molecular basis of cell structure and function. 2nd edition. Worth Publishers Inc. New York. N.Y., 1104pp.

Levandowsky, M., J. Klafter and B.S. White. 1988. Feeding and swimming behavior in grazing microzooplankton. J. Protozool. 35: 243–246.

Longhurst, A.R., 1976. Vertical migration. In D.H. Cushing and J.J. Walsh [eds.], The ecology of the seas. Blackwell Scientific Publications, Oxford. pp. 116–137.

Lough, R.G. and J.J. Gonor, 1971. Early embryonic stages of *Adula californiensis* (Pelecypoda: Mytilidae) and the effect of temperature and salinity on development rate. Mar. Biol. 23: 11–17.

MacDonald, B.A., 1989. Physiological energetics of Japanese scallop *Patinopecten yessoensis* larvae. J. Exp. Mar. Biol. Ecol. 120: 155–170.

MacDonald, B.A. and R.J. Thompson, 1986. The influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus* III Physiological ecology, the gametogenic cycle and the scope for growth. Mar. Biol. 93: 37-48.

Manahan, D.T., 1990. Adaptations by invertebrate larvae for nutrient acquisition from seawater. Am. Zool. 30: 147–160.

Manahan, D.T. and D.J. Crisp, 1982. The role of dissolved organic matter in the nutrition of pelagic larvae: amino acid uptake by bivalve veligers. Amer. Zool. 22: 635–646.

Mann, R., 1985. Seasonal changes in the depth-distribution of bivalve larvae on the southern New England Shelf. J. Shellfish Res. 5: 57–64.

Mann, R., 1986a. *Artica islandica* (Linneus) larvae: active depth regulators or passive particles?. Amer. Malac. Union Spec. Ed. No.3: 51–57.

Mann, R., 1986b. Sampling of bivalve larvae. Can. Spec. Publ. Fish. Aquat. Sci. 92: 107–116.

Mann, R., 1988a. Distribution of bivalve larvae at a frontal system in the James River, Virginia. Mar. Ecol. Prog. Ser. 50: 29–44.

Mann, R., 1988b. Field studies of bivalve larvae and their recruitment to the benthos. A commentary. J. Shellfish Res. 7: 7–10.

Mann, R., B.M. Campos and M.W. Luckenback, 1991. Swimming rate and responses of three mactrid bivalves to salinity discontinuities. Mar. Ecol. Prog. Ser. 68: 257–269.

Mann, R. and S.M. Gallager, 1985. Physiological and biochemical energetics of larvae of *Teredo navalis* (L) and *Bankia gouldi* (Bartsch) (Bivalvia: Teredinidae). J. Exp. Mar. Biol. Ecol. 85: 211–228.

Mann, R. and C.C. Wolf, 1983. Swimming behaviour of larvae of the ocean quahog *Artica islandica* in response to pressure and temperature. Mar. Ecol. Prog. Ser. 13: 211–218.

Manning, D.A., 1985. Aquaculture studies of the giant scallop *Placopecten magellanicus* (Gmelin). Conditioning of broodstock and energy requirements of the larvae and juveniles. M.Sc. Thesis. Memorial University of Newfoundland, St. John's, Newfoundland, 201pp.

Manning, J.H. and H.H. Whaley, 1954. Distribution of oyster larvae and spat in relation to some environmental factors in a tidal estuary. Proc. Natl. Shellfish. Ass. 45: 56–65.

Marty, Y., F. Delaunay, J. Moal and J.F. Samain, 1992. Changes in the fatty acid composition of *Pecten maximus* (L.) during larval development. J. Exp. Mar. Biol. Ecol. 163: 221–234.

McGarvey, R., F.M. Serchuk and I.A. McLaren, 1992. Statistics of reproduction and early life history survival of the Georges Bank sea scallop (*Placopecten magellanicus*) population. J. Northw. Atl. Fish. Sci. 13: 83–99.

McGarvey, R., F.M. Serchuk and I.A. McLaren, 1993. Spatial and parent-age analysis of stock-recruitment in the Georges Bank sea scallop (*Placopecten magellanicus*) population. Can. J. Fish. Aquat. Sci. 50. 564–574.

Mileikovsky, S.A., 1973 Speed of active movement of pelagic larvae of marine bottom invertebrates and their ability to regulate their vertical position. Mar Biol. 23: 11–17.

Millar, R.H. and J.M. Scott, 1967. The larvae of the oyster *Ostrea edulis* during starvation. J. Mar. Biol. Ass. U K. 47: 475–484.

Naidu, K.S., 1970 Reproduction and breeding cycle of the giant scallop *Placopecten magellanicus* (Gmelin) in Port au Port Bay, Newfoundland. Can J. Zool 48 1003-1012.

Naidu, K.S., 1975. Growth and population structure of a northern shallow-water population of the Giant scallop *Placopecten magellanicus* (Gmelin) ICES C M 1975/k:37.

Napolitano, G.E., 1991 Marine food chains: effect of phytoplankters and depth on the organ lipids of the sea scallop *Placopecten magellanicus* (Gmelin) and of intertidal invertebrates on the fat reserves of shorebirds. Ph.D. Thesis. Dalhousie University, Halifax, Nova Scotia, Canada, 184pp.

Napolitano, G.E., B.A. MacDonald, R.J. Thompson and R.G. Ackman, 1992 Lipids in eggs and adductor muscle of the scallop *Placopecten magellanicus* from different habitats. Mar. Biol. 113: 71–76.

Nelson, J., 1911. Report of the biologist. Rep. New Jers. St. Agric. Exp. Stn. June, 1910: 183–218.

Nelson, T.C., 1953. Some observations on the migrations and setting of oyster larvae Proc. Natl. Shellfish Assoc. 43: 99–104.

Nelson, T.C., 1955. Observations on the behaviour and distribution of oyster larvae. Proc. Natl. Shellfish Assoc. 45: 23–28.

Newman, D., 1939. The distribution of range in samples from a normal population, expressed in terms of an independent estimate of standard deviation. Biometrika 31: 20-30.

Nielsen, C., 1987. Structure and function of metazoan ciliary bands and their phylogenetic significance. Acta Zool. 68: 205–262.

Olson, R.R., I. Bosh and J.S. Pearse, 1987 The hypothesis of Antarctic larval starvation examined for the asteroid *Odontaster validus*. Limnol. Oceanog. 32: 686–690.

Olson, R.R. and M.H. Olson, 1989 Food limitation of planktonic marine invertebrate larvae: does it control recruitment success? Annu. Rev Ecol. Syst. 20: 225–247.

Parsons, T.R., I. Maita and C.M. Lalli, 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford, 173pp.

Pearre, S.Jr., 1973. Vertical migrations and feeding in *Sagitta elegans* Verril. Ecology 54: 300–314.

Pearre, S.Jr., 1979. Problems of detection and interpretation of vertical migration. J. Plankton Res. 1: 29–44.

Pechenik, J.A., 1987 Environmental influences on larval survival and development. In A.C. Giese, J.S. Pearse and V.B. Pearse [eds.], Reproduction of Marine Invertebrates Vol 9 General Aspects: Seeking Unity in Diversity. Blackwell Scientific Publications, Palo Alto, California, pp. 551–608.

Pennington, J.T. and R. Emlet, 1986. Ontogenetic and diel vertical migration of a planktonic echinoid larva, *Dendraster excentricus* (Eschsholtz) occurrence, cause and probable consequences. J. Exp. Mar. Biol. Ecol. 104: 69–95.

Pennington, J.T. and R.R. Strathmann, 1990. Consequences of the calcite skeletons of planktonic echinoderm larvae for orientation, swimming, and shape. Biol. Bull. 179: 121–133.

Posgay, J.A., 1957. The range of the sea scallop. Nautilus 71: 55–57.

Price, H.J., 1988. Feeding mechanisms in marine and freshwater zooplankton Biol. Bull. Mar. Sci. 43: 327–343.

Price, H.J., 1989. Swimming behaviour of krill in response to algal patches: A mesocosm study. Limnol. Oceanogr. 34: 649–659.

Price, H.J. and G.-A. Paffenhofer, 1984. Effects of feeding experience in the copepod *Eucalanus pileatus*: a cinematographic study. Mar. Biol. 84: 35–40.

Price, H.J., G.A. Paffenhofer, C.M. Boyd, T.J. Cowles, P.L. Donaghay, W.M. Hamner, W. Lampert, L.B. Quetin, R.M. Ross, J.R. Strickler and M.J. Youngbluth, 1988. Future studies of zooplankton behaviour: questions and technological developments. Bull. Mar. Sci. 43: 853–872.

Quayle, D.B., 1964. Distribution of introduced mollusca in British Columbia waters. J. Fish. Res. Board. Can. 21: 1155–1181

Quayle, D.B., 1969. Pacific oyster culture in British Columbia Bull. Fish. Res. Board. Can. 169.

Raby, D., Y. Lagadeuc, J.J. Dobson and M. Mingelbier, 1994 Relationship between feeding and vertical distribution of bivalve larvae in stratified and mixed waters. Mar. Ecol. Prog. Ser. 103: 275–284

Ringelberg, J., 1995 Changes in light intensity and diel vertical migration: a comparison of marine and fresh water environments. J. Mar. Biol. Ass. U.K. 75: 15–25.

Robinson, S.M.C., J.D. Martin, R.A. Chandler, G.J. Parsons and C.Y. Couturier. 1991. Larval settlement patterns of the giant scallop (*Placopecten magellanicus*) in Passamaquoddy Bay, New Brunswick Can. Atl. Fish. Sci. Adv. Committee, 25pp.

Robinson, W.E., W.E. Wehling, P. Morse and G.C. McLeod, 1981. Seasonal changes in soft body components indices and energy reserves in the Atlantic deep-sea scallop *Placopecten magellanicus*. Fish. Bull. 79: 449–458.

Rodger, R.S., 1974. Multiple contrasts, factors, error rate and power Br. J math statist. Psychol. 27: 179–198.

Rodger, R.S. 1975. The number of non-zero post-hoc contrasts from ANOVA and error rate I. Br. J. math. statist. Psychol. 28: 71–78.

Roff, D.A., 1991. Life history consequences of bioenergetic and biomechanical constraints on migration. Amer. Zool. 31: 205–215.

Rothschild, B.J. and T.R. Osborn, 1988. Small-scale turbulence and plankton contact rates. J. Plankton Res. 10: 465–474.

Roughley, T.C., 1933. The life history of the Australian oyster *Ostrea commercialis*. Proc. Linnean Soc. of New Wales. 58: 275–333.

Russell, F.S., 1927. The vertical distribution of plankton in the sea. Biol. rev 2. 213-256.

Sargent, J.R., 1976. The structure, metabolism and function of lipids in marine organisms. **In** D. Malins and J.R. Sargent [eds.], Biochemical and Biophysical perspectives in Marine Biology, pp. 159–212.

Sasaki, G.C., 1984. Biochemical changes associated with embryonic and larval development in the American lobster *Homarus americanus* Milne Edwards. Ph.D. Thesis MIT/WHOI. 457pp.

Sastry, A.N., 1986. Pelagic larval Physiology and ecology of benthic marine invertebrates in the context of the Indian Ocean. **In** M.-F. Thompson, R. Sarojini and R. Nagabhushanam [eds.], Biology of Benthic Marine Organisms, pp. 387–401. A..A. Balkema, Rotterdam.

Scheffee, H., 1953. A method for judging all contrasts in the analysis of variance. Biometrika 40. 87-104.

Scheltema, R.S., 1964 Origin and dispersal of invertebrate larvae in the North Atlantic Am. Zool. 4. 299–300.

Scheltema, R.S., 1986. On dispersal and planktonic larvae of benthic invertebrates. An eclectic overview and summary of problems. Bull. Mar. Sci. 39: 290–322.

Scrope-Howe, S. and D.A. Jones, 1986 The vertical distribution of zooplankton in the Western Irish Sea. Est. Coastal Shelf Sci. 22: 785–802.

Seliger, H.H., J.A. Boggs, R.B. Rivkin, W.H. Biggley and K.R. Aspden. 1982 The transport of oyster larvae in an estuary. Mar. Biol. 71: 57–72.

Silva, M.A. and R.K. O'Dor, 1994. Early life history traits of sea scallops, *Placopecten magellanicus*, from the Georges Bank population: vertical distribution of larvae **In** N.F. Bourne, B.L. Bunting and L.D. Townsend [eds.], Vol. 1. Can. Tech. Rep. Fish. Aquat. Sci., pp. 67–75

Sinclair, M., 1988 Marine populations: an essay on population regulation and speciation. University of Washington Press, Seattle and London, 252pp

Sinclair, M.M. and T.D. Iles, 1985 Population regulation and speciation in the oceans J. Cons. Int. Explor. Mer. 45: 165–175.

Sinclair, M., R.K. Mohn, G. Robert and D.L. Roddick, 1985. Considerations for the effective management of Atlantic scallops. Can. Tech. Rep. Fish. Aquat. Sci. No. 1382: vii + 113pp.

Sipos, J.C. and R.G. Ackman, 1978 Automated and rapid quantitative analysis of lipids with chromarods. J. Chrom. Sci. 16: 443–447.

Sleigh, M.A. and J.R. Blake, 1977 Methods of ciliary propulsion and their size limitations. **In** T.J. Pedley [ed.], Scale Effects in Animal Locomotion. Academic Press, London, pp. 243–256.

Southward, A.J. and R.L. Barrett, 1983 Observations on the vertical distribution of zooplankton, including post-larval teleosts, off Plymouth in the presence of a thermocline an a chlorophyll dense layer. J. Plank. Res. 5: 599–618.

Sprung, M., 1984 Physiological energetics of mussel larvae (*Mytilus edulis*) II Food uptake. Mar. Ecol. Prog. Ser. 17: 295–303.

Squires, H.T., 1962 Giant scallops in Newfoundland coastal waters. Bull. Fish. Res. Board. Can. 135. 29pp.

Stancyk, S.E. and R.J. Feller, 1986. Transport of non-decapod invertebrate larvae in estuaries: an overview. Bull. Mar Sci. 39: 257–268.

Starr, M., J.H. Himmelman and J.-C. Therriault, 1990 Direct coupling of marine invertebrate spawning with phytoplankton blooms. Science 247. 1071–1074.

Strathmann, R.R., 1982. Selection for retention or export of larvae in estuaries **In** V S. Kennedy [ed.], Estuarine Comparisons. Academic Press, New York, pp 521–536.

Strathmann, R.R., 1987. Larval Feeding **In** A.C. Giese, J.S. Pearse and V.B. Pearse [eds.], Reproduction of Marine Invertebrates Vol 9 General Aspects Seeking Unity in Diversity. Blackwell Scientific Publications, Palo Aito, California, pp. 465–550.

Strathmann, R.R., T.L. Jahn and J.R.C. Fonseca, 1972 Suspension feeding by marine invertebrate larvae: Clearance of particles by ciliated bands of a rotifer, pluteus and trocophore. Biol. Bull. 142: 505–519.

Strathmann, R.R. and E. Leise, 1979 On feeding mechanisms and clearance rates of molluscan veligers. Biol. Bull. 157: 524–535.

Strickler, J.R., 1982. Calanoid copepods, feeding currents, and the role of gravity. Science 218: 158-160

Sulkin, S.D., 1984. Behavioral bases of depth regulation in the larvae of brachyuran crabs. Mar. Ecol. Prog. Ser. 15: 181–205.

Sulkin, S.D., 1986. Application of laboratory studies of larval behavior to fisheries problems. Can. J. Fish. Aquat. Sci. 43: 2184–2188.

Sulkin, S.D., W.F. Van Heukelem and P. Kelly, 1983. Behavioral bases of depth regulation in hatching and post-larval stages of the mud crab *Eurypanopeus depressus*. Mar. Ecol. Prog. Ser. 11: 157–164.

Sullivan, B.K., 1993. How does water column structure influence copepod populations in coastal marine systems? Bull. Mar. Sci. 53: 240–246.

Thompson, R.J., 1977. Blood chemistry, biochemical composition, and the annual reproductive cycle in the Giant scallop, *Placopecten magellanicus*, from Southeast Newfoundland. J. Fish. res. Bd. Can. 34: 2104-2116.

Thorson, G., 1946. Reproduction and larval development of Danish marine bottom invertebrates. Medd. Komm. Danm. Fisk.-og Havunders ser. Plankton 4: 523pp.

Thorson, G., 1950. Reproductive and larval ecology of marine bottom invertebrates Biol. Rev. 25: 1–45.

Thorson, G., 1964. Light as an ecological factor on the dispersal and settlement of larvae of marine bottom invertebrates. Ophelia 1: 167–208.

Tremblay, M.J., 1991. Sea scallop larvae *Placopecten magellanicus* in the Georges Bank region: distribution, abundance and condition. Ph.D. Thesis, Dalhousie University, Halifax, Nova Scotia, Canada. 201pp.

Tremblay, M.J., J.W. Loder, F.E. Werner, C.E. Naimie, F.H. Page and M.M. Sinclair, 1994. Drift of sea scallop larvae *Placopecten magellanicus* on Georges Bank: a model study of the roles of mean advection, larval behavior and larval origin. Deep-Sea Research II. 41: 7–49.

Tremblay, M.J., L.D. Meade and G.V. Hurley, 1987. Identification of planktonic sea scallop larvae (*Placopecten magellanicus*) (Gmelin). Can. J. Fish. Aquat. Sci. 44: 1361–1366.

Tremblay, M.J. and M. Sinclair, 1988. The vertical and horizontal distribution of sea scallop (*Placopecten magellanicus*) larvae in the Bay of Fundy in 1984 and 1985. J. Northw. Atl. Fish. Sci. 8: 43–53.

Tremblay, M.J. and M. Sinclair, 1990a Diel vertical migration of sea scallop larvae *Placopecten magellanicus* in a shallow embayment. Mar. Ecol. Prog. Ser. 67: 19–25.

Tremblay, M.J. and M. Sinclair, 1990b. Sea scallop larvae *Placopecten magellanicus* on Georges Bank: vertical distribution in relation to water column stratification and food. Mar. Ecol. Prog. Ser. 61: 1–15.

Tremblay, M.J. and M.M. Sinclair, 1992. Planktonic sea scallop larvae (*Placopecten magellanicus*) in the Georges Bank region: broadscale distribution in relation to physical oceanography. Can. J. Fish. Aquat. Sci. 49: 1597–1615.

Tukey, J.W., 1953. The problem of multiple comparisons. Umpublished, Princeton University.

Turner, H.J. and C.J. George. 1955. Some aspects of the behaviour of the quahog, *Venus mercenaria* during the early stages. In Eighth report of investigations of the Shellfisheries of Massachusetts. Department of Natural Resources, Division of Marine Fisheries, Amherst, MA., pp. 5–14.

Verheye, H.M. and J.G. Field, 1992. Vertical distribution and diel vertical migration of *Calanoides carinatus* (Kroyer, 1849) development stages in the southern Benguela upwelling region. J. Exp. Mar. Biol. Ecol. 158: 123–140.

Verwey, J., 1966. The role of external factors in the vertical migration of marine animals. Neth. J. Sea Res 3: 245–266.

Vogel, S., 1981 Life in Moving Fluids: The Physical Biology of Flow. Princeton University Press, 352pp.

Whyte, J.N.C., N. Bourne, N.G. Ginther and C.A. Hodgson, 1992 Compositional changes in the larva to juvenile development of the scallop *Crassadoma gigantea* (Gray). J. Exp. Mar. Biol. Ecol. 163: 13–29.

Whyte, J.N.C., N. Bourne and C.A. Hodgson, 1987. Assessment of biochemical composition and energy reserves in larvae of the scallop *Patinopecten yessoensis* J Exp. Mar. Biol. Ecol. 113: 113–124.

Whyte, J.N.C., N. Bourne and C.A. Hodgson, 1989 Influence of algal diets on biochemical composition and reserves in *Patinopecten yessoensis* (Jay) larvae Aquaculture 78: 333–347

Whyte, J.N.C., N. Bourne and N.G. Ginther, 1990a Biochemical and energy changes during embryogenesis in the rock scallop *Crassadoma gigantea* Mar. Biol 106: 239–244.

Whyte, J.N.C., N. Bourne and C.A. Hodgson, 1990b Nutritional condition of rock scallop *Crassadoma gigantea* (Gray), larvae fed mixed algal diets Aquaculture 86: 25–40.

Winet, H., 1973 Wall drag on free moving ciliated micro-organisms J. Exp Biol. 59: 753–766.

Wood, L. and W.J.Jr. Hargis, 1971. Transport of bivalve larvae in a tidal estuary In D.J. Crisp [ed.], Proc. 4th Eur. Mar. Biol. Symp. Cambridge University Press. Cambridge pp. 29–44.

Young, C.M. and F.-S. Chia, 1987 Abundance and distribution of pelagic larvae as influenced by predation, behavior, and hydrographic factors. In A.C. Giese, J.S. Pearse and V.B. Pearse [eds.], Reproduction of Marine Invertebrates, Vol 9. Blackwell, Palo Alto, California, pp. 385–463.

Young-Lai, W.W. and D.E. Aitken, 1986. Biology and culture of the giant scallop, *Placopecten magellanicus*: a review. Can. Tech. Rep. Fish. Aquat. Sci. 1478. 21pp.

Zinsmeister, W.J. and W.K. Emerson, 1979. The role of passive dispersal in the distribution of hemipelagic invertebrates, with examples from the tropical pacific ocean. Veliger 22: 32–40.