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A Comparison of Natural and Laboratory Diets for the Culture of Marine Invertebrate Larvae American Oyster, Crassostrea virginica, Queen Conch, Strombus gigas, and Milk Conch, Strombus costatus

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A Comparison of Natural and Laboratory Diets for the Culture of Marine Invertebrate Larvae: American Oyster, *Crassostrea virginica*, Queen Conch, *Strombus gigas*, and Milk Conch, *Strombus costatus*.

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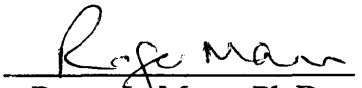

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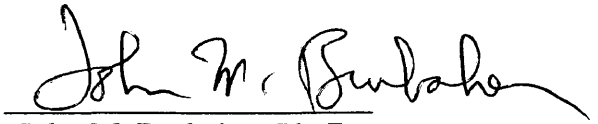

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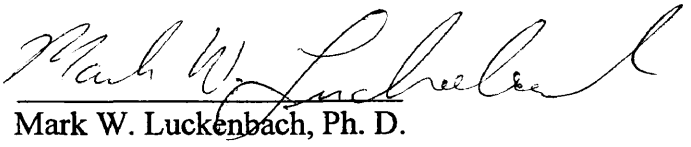

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DEDICATION.

To my Mother and to the memory of my Father, for their love, encouragement and unwavering faith in me.

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Abstract

Invertebrate larvae have been cultured for descriptive and experimental purposes for many years; however, I have been unable to find a critical comparison of growth and development of laboratory cultured larvae with sibling larvae maintained *in situ* in the natural environment. Consequently there remains the question, how far it is legitimate to extrapolate from laboratory studies to the ecology of the organism in the field?

The objective of this investigation was to determine whether growth and development rates of larvae cultured on natural diets are significantly different from those maintained on cultured algal diets. A small scale mesocosm was developed for the study of *in situ* culture of marine invertebrate larvae.

The mesocosm or Submersible Larval Incubation Chamber (SLIC) was powered by a 12 volt battery and controlled by two programmable time-delay relays to enable either constant flow or pulsed flow through the larval incubation chamber. The SLIC was tested in two ecosystems: temperate high nutrient system, using larvae of *Crassostrea virginica* and tropical oligotrophic system using larvae of *Strombus* spp. Sibling larvae of all species were divided into treatments and cultured under different dietary regimes, either in situ in the mesocosm or in the hatchery on mixed algal diets. For the larvae of *S.gigas* and *S. costatus*, cultured algae were not available, therefore the *in situ* data were compared with data from other studies on the same species that had been cultured on algal diets.

For *C. virginica*, a significantly higher growth rate was found on natural river diet than on mixed algal diet (comparison of 95% confidence intervals of the regression curves); larvae also became competent to settle significantly earlier when cultured on natural river diet ($t=-3.30$, $P=0.016$) For *S.gigas*, growth rates obtained on natural diets were similar to those obtained from the literature; however, the larvae began to display competence to settle at 16 days, which is several days earlier than quoted in the literature (21-28 days) for larvae cultured on algae. For *S. costatus*, the growth and development rates observed in this study were higher or equivalent to growth rates found on cultured algal diet. The mesocosm functioned well using the pulsing configuration in the tropical ecosystem; however, it suffered from severe fouling problems in the temperate system.

To conclude, the comparison of *in situ* and laboratory culture of three species of molluscan larvae in two different ecosystems, shows that significantly different growth and development rates can be obtained using natural and cultured algal diets. The optimal development observed in the laboratory is probably not typical of larvae in their natural environment and should be considered a separate phenomenon. The mesocosm concept is tractable, but the SLIC is not optimal for use with small organisms in ecosystems with high organic loading because of prohibitive fouling problems.

A Comparison of Natural and Laboratory Diets for the Culture of Marine Invertebrate Larvae: American Oyster, *Crassostrea virginica*, Queen Conch, *Strombus gigas*, and Milk Conch, *Strombus costatus*.

GENERAL INTRODUCTION:

The larval phase, defined as the period between emergence of the planktonic stage and metamorphosis into the permanently settled benthic organism, is critical in many species of marine invertebrates. Recruitment to the benthic community is determined by the successful metamorphosis from the pelagic larva to the attached feeding juvenile and the subsequent survival of the juvenile organism. Keough and Downes (1982) recognized three phases in the colonization of habitats by marine organisms with planktonic larvae: development (including dispersal as a planktonic form), testing of a habitat for suitability, and settlement. For sessile invertebrates, the latter also includes attachment to the substratum and metamorphosis. Recruitment is defined by Keough and Downes (1982) as those organisms which survive to be detected as part of the post metamorphic population. The settlement phase is merely the passage of larvae into metamorphosis. Clearly larval and post metamorphic processes, which affect recruitment, can influence the abundance and distribution of the adult population.

Availability of suitable food in the natural environment can influence larval growth and development rates (Fenaux, Strathmann and Strathmann; 1994) and possibly the recruitment success of a cohort, as hypothesized by Olson (1985). Food is not always sufficiently limiting to cause mortality; however larval malnutrition can adversely affect the subsequent stages of metamorphosis, growth, development and reproduction (Miller, 1988; Rodriguez, 1990). Holland and Spencer (1973) concluded that lipids are important in the larval development of the bivalve, *O. edulis* and greater lipid reserves at settlement are correlated with higher growth rates of post metamorphic oyster spat (Laing and Millican, 1986).

Interest in nutritional requirements of invertebrate larvae has arisen partly because of

the expansion in culture systems for commercially important organisms (Nellen et al 1980; Fernandez de Puelles, 1985; Utting, 1986). For economic reasons, the bivalve molluscs are among the more intensively studied classes. They are also an important functional group in many benthic systems. Originally, the success of various diets was determined in culture experiments, by trial and error, with different food sources. Laboratory studies on artificial assemblages of cells yield only empirical evidence for larval nutritional requirements. Recently, quantitative methods, such as biochemical analysis of organisms, have provided more direct evidence of nutritional requirements and effects of various deficiencies on energetic reserves and how they relate to the condition of the animals (Holland and Spencer, 1973; Holland, 1978; Rodriguez et al, 1990)

Clearance and Ingestion Rates

There have been numerous expressions used in the literature to describe the feeding parameters of filter feeding organisms. To alleviate ambiguity, the terms used in this text are described as follows:

- Pumping rate is the total quantity of water pumped over the feeding organ per unit time.

  Clearance rate is the volume of water from which all particles (usually of a predetermined size range) are removed per unit time.

Clearance rate is always lower than the pumping rate since organisms are not 100% efficient at removing particles. Filtration and clearance are often used synonymously.

Consumption is dependent on, but not necessarily linearly related to, the clearance

rate; some particles removed from a suspension may not be ingested, but disposed of as pseudofaeces. Removal of particles depends on endogenous (physiologically mediated) and exogenous parameters such as temperature, particle size and food concentration (Sprung, 1984; Gerdes, 1983). Research into filtration, clearance and ingestion of invertebrate larvae has produced a range of data for volumes or numbers of cells ingested, volumes of water cleared and numbers of cells retained (Baldwin, and Newell, 1991; Sprung, 1984; Gerdes, 1983) Since water temperature, larval size, cell concentrations and larval species of the investigations were not standardized their results cannot be compared directly, however, certain trends have been demonstrated.

- Filtering activity was higher on mixed than single culture diets for similar sized larvae of *Crassostrea gigas* (Gerdes, 1983), which contributes to, but does not completely account for, the increased growth rates usually observed when larvae are fed mixed algal species.

  Filtration and clearance rates can adapt to particle concentration. Gallager and Mann (1980) observed clearance rate with various filter feeding planktonic organisms, over a range of cell concentrations. Highest clearance rates were observed at intermediate food concentrations; probably due to decreased filtering activity at low food levels and limitations in handling ability or gut capacity at high particle concentrations.

  If food quality is poor, the ingestion rate is not necessarily a good correlate of growth rate. Assimilation efficiency (consumption - egestion/consumption) may also be high but produce low growth efficiency (growth/consumption) Organisms may attempt to increase the filtration rate to compensate for the low food quality of cells

ingested. Settled juveniles, or spat, of *Ostrea edulis* were fed various algal diets (Laing and Millican, 1986); with lower quality food items, size specific clearance rates and metabolic demand (oxygen consumption) of the spat were higher, but growth rates were lower. The ingestion-growth relationship can also be confounded by temperature.

Size of food particle limits retention capability. Various maximum and minimum particle size retention capacities have been reported from different studies. Oyster larvae have a lower limit of retention, with particles the size of heterotrophic bacteria (1-2μm) (Gerdes, 1983; Sprung, 1984), and an upper limit (constrained by the width of the food groove) reported between 20-30μm (Baldwin and Newell, 1991) Echinoderm larvae can capture larger cells than can veliger larvae, but are relativley inefficicent at capturing small cells in the 2-3μm range (Strathmann, 1987) The differential cell size preference is due to the morphological constraints of the different feeding mechanisms.

Filter feeders can regulate their feeding to optimize available quality and quantity of food. Shumway et al (1985) defined three possible mechanisms by which bivalve filter feeders can select food items: preferential clearance, pre-ingestive selection on the labial palps and differential absorption in the gut. Echinoplutei have been shown to select particles on the basis of flavour (Rassoulzadegan et al., 1984) as distinct from size or shape.

Information on efficiency of utilization of different food particles, at different concentrations, is important for formulation of aquaculture feeding programs. Defining the

limits of particle size and concentration for optimal ingestion, could also advance estimation of food limitation in natural ecosystems.

Biochemical Requirements of Marine Invertebrate Larvae

Marine invertebrate larvae have been analyzed to determine composition of fatty acids, proteins, carbohydrates and various classes of lipids during development. This information has been used to infer the biochemical nutritional requirements of the organisms.

Fatty acids are the fundamental structural components of lipids. The chain length and number of double bonds (degree of saturation) of the fatty acid, determines the physical properties of the lipid. Most marine invertebrate larvae require certain essential fatty acids in their diet. These are long chain polyunsaturated fatty acids, or PUFA's, which cannot be synthesized internally and are derived from marine algae. The more important essential fatty acids are ecosapentanoic acid or EPA (20:5*w*6; which represents a 20-carbon chain with 5 double bonds; the first bond being after the 6th carbon) and decosahexanoic acid or DHA (22:6 *w*6), which play an important role in membrane integrity. There are consistently high levels of PUFA's maintained in the phospholipid component of the total lipids, even during food stress. Triacylglycerols (TAG) function not only as an energy reserve but also serves as a storage depot for PUFA's, which are later transferred to the polar lipid fraction for incorporation into cellular membranes (Napolitano et al, 1988).

The importance of neutral lipids such as triacylglycerols (TAG), wax esters and sterols to invertebrate larval development has been well documented. Larval *Crangon spp.*,

were collected from the field in two consecutive years (Kattner et al., 1994) In the first year, the levels of EPA and DHA were low, as were larval abundance and recruitment. In the second year, higher levels of EPA and DHA corresponded with high recruitment. Larvae of the American lobster, *H. americana* were subjected to periods of starvation to determine their utilisation of fatty acids. Enven under prolonged starvation, EPA, DHA and arachodonic acid were conserved (Sasaki, 1984) Holland and Spencer (1973) found the growth rate of *Ostrea edulis* to correlate closely to the neutral lipid concentration of the developing larvae. The percentage of TAG (as a function of total organic material) increased throughout larval development until metamorphosis. During metamorphosis, TAG concentration rapidly decreased. In contrast, the concentration of phospholipid (polar lipids) proteins and carbohydrates remained approximately constant during larval development and metamorphosis. During periods of starvation, neutral lipid and protein accounted for 75% of the total organic material metabolized.

Carbohydrate concentration increases as development proceeds to metamorphosis and lipid reserves are utilized. Carbohydrates are the primary source of energy in the adult oyster; this respiratory substrate is more suited to the lifestyle of adult, since they can be periodically exposed to hypoxic conditions. Carbohydrates can be metabolized anaerobically, for a limited period, whereas lipids cannot. Oysters utilize proteins as a respiratory substrate primarily during metamorphosis or during starvation when their lipid reserves are depleted.

Particulate Diet of Marine Invertebrate Larvae

Little is known about the composition of natural particulate diets of planktonic

invertebrate larvae. Most of the information to date on the effects of diet on growth, survival and development time, has been from laboratory studies using specific types of cells.

Experiments on larval invertebrate growth have shown that unialgal diets (with very few exceptions) are insufficient to support optimal growth (Tan Tiu et al, 1989). Laing and Millican (1986) studied relative growth efficiency of *O.edulis* spat on combinations of five marine algal species. Except those cultured on *Chaetoceros calcitrans* (which appears to be an almost complete diet for *O. edulis*), they found that growth rates were consistently higher on mixed algal cultures, than with single species diets. A synergistic effect was observed when the larvae were fed a combination of species; by mixing the cultures, the growth rates were higher than the additive effects of feeding single cultures. This may vary with the biochemical content of different cell types. Essential nutrients, absent from the poor food items, may be growth-limiting factors; when these nutrients are supplied by another cell type, the larvae can use the poor food types more efficiently. The quality of an algal culture also depends on the composition of the medium in which it is grown (Jaekle and Manahan, 1983). If the medium is deficient in elements necessary for the synthesis of essential larval nutrients, the value of the algae as a larval food will be reduced.

Phytoplankton is not the sole source of particulate food available to invertebrate larvae. They have been shown to ingest (Baldwin and Newell, 1991; Bell, 1991; Manahan, 1990) and assimilate (Dioullet, 1993) heterotrophic flagellates, picoplankton and cyanobacteria. These cell types can make up a considerable portion of material ingested by larvae besides the conventionally recognized phytoplankton component of the diet. Dissolved organic material (DOM) has also been proposed as a significant dietary supplement

(Manahan, 1983; Laing and Millican, 1986) and will be discussed in greater detail later in the text. By employing such techniques as epifluorescence microscopy, radioactive labeling (Baldwin and Newell, 1991) and flow cytometry (Shumway and Cucci, 1991; Cucci et al, 1985) it is possible to detect the range of particles that larval invertebrates remove from the surrounding water body.

Baldwin (1991) investigated the feeding behavior of prodissoconch II larvae of *C. virginica*. Phototrophs, labeled with C^{14} bicarbonate and heterotrophic bacteria, and phagotrophic protozoans, labeled with H^3-thymidine, were used to determine the clearance rates of various naturally occurring cells in a coastal marine ecosystem. The heterotrophs included free bacteria, attached bacteria, ciliates and flagellates; phototrophs included cyanobacteria and large dinoflagellates. The larvae were found to selectively remove cells between 20 and 30 μm at high clearance rates. This size class was represented primarily by large dinoflagellates. Ciliates, flagellates and heterotrophic bacteria (although clearance rates were low) were also consumed. Previous studies (Riisgard et al, 1980) suggest that larvae do not efficiently remove cells >6um. It is possible that the larvae are actively selecting large cells, despite handling difficulty, for their high food value. This is not unlikely since selectivity has been demonstrated in *Mercenaria mercenaria* larvae (Gallager, 1988). Although there appears to be ample evidence for selection mechanisms in filter feeding organisms, Rassoulzadegan and Fenaux (1979) observed that planktonic filter feeders graze largely in the size ranges where particle concentration is high; therefore removal of a particular size class is a function of density rather than a selection for a particular cell size.

The importance of small planktonic cells is unclear but they may play an important

supplementary role in nutrition, especially in phytoplankton deficient waters. There is considerable variation between species of invertebrates in their plasticity and ability to overcome food limitation. It would be advantageous to be able to exploit a wide range of organic materials, particularly in oligotrophic waters where food is usually less abundant than shallow coastal areas.

Significance of DOM in Invertebrate Larval Nutrition

The ability of larvae to take up DOM directly from sea water, has been well documented (Stephens, 1988; Wright and Manahan, 1989). Manahan (1990) concluded that invertebrate larvae are well adapted to take nutritional advantage of the dissolved organic material in their environment. The larval velum is the site of transport for dissolved nutrients; the developing gill buds take over this role at metamorphosis followed by the gill in the adult (Manahan, 1990); a common feature of these structures is their high surface area to volume ratio. In both adults and larvae, absorption rates of dissolved nutrients follow Michaelis-Menton kinetics (Stephens, 1988), which indicates an ability to actively take up nutrients from the water.

Much of the work to date on DOM utilization, has been done on specific quantifiable components of the DOM pool, such as amino acids, rather than DOM as a whole. Most DOM exists as complex macromolecules that are difficult to characterize. Dissolved carbohydrates, proteins and organic acids make up less than 10% of total dissolved organic carbon in sea water (Manahan, 1983).

There are good demonstrations of the nutritional importance of DOM to invertebrate

larvae. Experiments by Manahan (1990) demonstrated that larval *C. virginica* are capable of increasing their biomass on particle free (<0.2μm filtered) water. As the larvae increase in size, their metabolic demand increases in direct proportion. The increase in transport capacity of DOM, as bivalve larvae develop, has been calculated to be sufficient to support growth (Manahan, 1990); however, the contribution of DOM to nutritional demand may be limited by the substrate concentration in the environment.

Many invertebrate larvae can supplement their particulate diet by active uptake of dissolved organic carbon from their surroundings. Development of the non-feeding larval stage of *Haliotis rufescens* could not be accounted for by endogenous reserves, which implies the uptake of DOC from the environment. (Jaeckle and Manahan, 1990) Echinoderm larvae are capable of developing without a particulate food supply (Strathmann; 1987), although when fed phytoplankton cells, growth and development was faster and larvae were larger. It is possible the unfed animals are utilising DOM as well as endogenous food reserves.

Effects of Food Limitation on Larval Invertebrates

Most experiments to date address the question of whether food is limiting to growth and development, by culturing animals in natural sea water at various particle concentrations and recording growth rates (Lucas, 1982; Paulay et al, 1985; Strathman, 1987; Olson and Olson, 1989; Fenaux, 1994) The results of these, and the few in situ studies documented, are inconclusive.

Lucas (1982) carried out studies on 'crown of thorns' starfish larvae, *Acanthaster planci* in the laboratory with natural seawater. The results indicated that food is a possible

limiting factor in successful recruitment of larvae to the adult population. Initially, Olson (1985) also hypothesized that starvation in *A. planci* controlled natural populations; however, in situ mesocosm studies led to his rejection of the hypothesis for this particular species since larvae developed as rapidly, or more so, in situ, than in the laboratory. White (1976) determined growth rates of larvae of *O. edulis* decreased at concentrations of less than 50-100 cells μl^{-1} of *I. galbana*. Natural concentrations of this nanoplankton species, over oyster beds rarely exceeds 10 cells μl^{-1} (Drinkwaard, 1961); therefore food limitation is indicated in this environment. According to Walne (1963), growth rates in nature and the laboratory are similar, therefore there must be an energy source apart from phytoplankton, that has not been considered. As previously discussed, larval invertebrates have been shown to utilize many non phototrophic cells and DOM and levels of algal cells or chlorophyll may underestimate food availability in natural marine systems.

The importance of food limitation changes with species and environment (Olson and Olson, 1989). Generally, food limitation is more likely to occur in offshore oceanic locations and is less likely to be important in coastal and inland systems that generally experience higher nutrient input. For the same reason, temperate areas are less likely to be nutrient deficient than tropical areas. There are, however, exceptions to these generalizations (see Paffenhoffer, 1988).

The type and concentrations of algal cells required to culture invertebrate larvae have been established for a number of species. The variety of the diet is limited and may have sub optimal levels of essential nutrients. The natural diet of marine larval invertebrates has not been quantified in the field. It is conceivable, given the spatial and temporal variability

of phytoplankton in tidal coastal areas, that larvae are food limited for at least some of their planktonic phase (Paulay et al, 1985). If food limitation occurs in the natural environment, it is possible that cultured organisms supplied with constant abundant food, show better growth and survival than larvae in natural populations. The relative advantage of the aquaculture and natural systems may also change temporally with seasonal fluctuations of nutrient input in the environment. Food limited growth has implications for life-history, ecology and evolution; if growth and development rates are sub optimal, this influences dispersal of the planktonic stage and recruitment to the adult community. The extrapolation of laboratory or hatchery experiments to field nutrition must be made with consideration of potential artifacts.

Variability in Natural Marine Ecosystems

The planktonic environment was originally thought to be homogeneous and at equilibrium over large scales; an alternative theory of a "contemporaneous disequilibrium" was proposed in 1970 (Harris, 1980) which assumed the aquatic environment was not homogeneous, but that temporal and spatial disruption existed.

In vivo fluorescence techniques assisted the elucidation of patchiness in phytoplankton populations and the subsequent introduction of more sophisticated techniques (spectral analysis) has enabled description of spatial variation in phytoplankton populations in lakes and oceans. The degree of spatial and temporal variability depends on degree of mixing and turnover in the water body. Stratification is can be broken by wind stress, internal waves, freestream eddies, to form patches of water of variable quality. A well mixed water body

however, is more homogeneous by definition.

Harris (1980) observed that phytoplankton species diversity increased during summer stratification periods. He explained this by hypothesizing that each cell size can exploit a given patch of favorable water quality, which is maintained for a given spatial and temporal distribution depending on the nature of the patch. He concluded that the degree of species diversity reflects the degree of patchiness in the water body and assigned the bloom phenomenon to periods of very low physical activity and strong stratification.

Litaker et al (1987) studied the short term physical and biological variation in a shallow tidal estuary in the Outer Banks, NC. The estuary was extensively sampled over two years. Hourly measurements of 28 parameters were taken to establish extent of temporal and spatial variability, and effects on plankton communities. Results indicated that high frequency (cell generation time) processes are important in characterizing phytoplankton ecology; wheras phytoplankton biomass was regulated by temporal distribution of dissolved inorganic nitrogen (DIN). Variation was non-random, mostly occurring on time scales of 12 to 96 hrs.

Evaluation of Field Nutrition

Much of the work on larval nutrition has been prompted by requirements to develop diets for high intensity aquaculture operations. This approach sustains no necessity or incentive for field investigations, so laboratory experiments have dominated research on nutrition of larval invertebrates. Investigations into the impact of larval nutrition on the ecology of a species have been few by comparison. Laboratory experiments have the advantage of being quantifiable, because environmental conditions can be controlled and

described. Results of laboratory studies generally have less variation and are more easily explained than field data; however, information from laboratory data cannot necessarily be extrapolated to explain or describe natural ecosystems.

There is a recognized need for investigations into organisms in their natural environment (Olson and Olson, 1989; Thorson, 1946; Mann, 1988); however, field experiments on nutrition are often inconclusive. This may be due to uncontrolled or unquantified variables such as age of larvae, developmental stage, history of nutrition and energy reserves at hatching. In a highly variable system, extensive sampling may be necessary to give the required power to a statistical test, but there are potential logistic constraints on sample size.

Mesocosms

Mesocosm studies have bridged the gap between field evaluation and reductionist laboratory experiments as a means of answering ecological questions. Mesocosms are experimental ecosystems which are deployed *in situ* in the environment. They are small enough to allow manipulation, but large enough to simulate (to a limited extent) a natural ecosystem. The advantage over field investigation is the increased control and quantification of experimental variables; the advantage over a laboratory experiment is the increased realism, which can help validate laboratory data (Fairchild and Little, 1993).

Mesocosms are not perfect solutions to the laboratory vs. field experiment dilemma; many are closed systems, which introduces a significant artifact since natural ecosystems are open and dynamic. Mesocosms can however, offer a tractable compromise between the

laboratory and natural ecosystems. They have been used for investigations into impacts of toxicants, (Gladyshev, 1993) water quality (Fairchild and Little, 1993), nutrition (Olson, 1985), predation rates and many other ecological questions (Grice et al, 1980; Keller, 1987; Fairchild and Little, 1993).

There are a few experiments documented in the literature where larvae have been cultured on a small scale in the field to investigate their growth and development. For various reasons, these experiments generally met with little success. A brief summary is as follows.

- MacKay (1929) reared lobster larvae in cages, but had high mortality due to fouling of the larval carapace with diatoms.

  Thorson (1946) developed cages of mesh cylinders suspended from a line for rearing of larvae in situ. Larvae of various genera were placed in the cages and submerged to 16m. After 3 weeks they were retrieved, but only one larva of one genus was found alive. Thorson was convinced these methods would prove useful if logistical problems could be overcome.

  Preston (1985) used mesh cages to rear penaeid prawn larvae in an estuary, but found them unsatisfactory. This was probably due to lack of water exchange into the cages and impairment of larval feeding or oxygenation.

Few successful attempts at small scale in situ culturing were reported in the literature.

Haynes (1977) reared shrimp larvae *Pandalus goniurus*, in flasks covered with 0.57 mm mesh and submerged to a depth of 20 m. He found lower mortality and a greater synchrony of development in the field raised shrimp compared with those reared in the laboratory.

Olson et al (1985), developed a mechanized system for rearing of *A. planci* that proved repeatedly successful in locations in the Great Barrier Reef, Australia, Antarctica and Japan.

Davis (1994c) cultured larval Queen conch in floating mesocosms (200 liter capacity) in the Exuma cays, Bahamas. The larvae reached metamorphosis is 14 days, one week earlier than previously described from laboratory studies.

This investigation:

The primary objective of this investigation is to determine whether there is any significant difference between growth and development rates of larvae cultured under different dietary regimes. Larvae fed mixed algal assemblages were compared with those cultured in water from their natural environment.

Larvae from two different ecosystems were used as a comparison of dietary influence under different natural food levels. The ecosystems used are described in the objectives. This study is not intended to determine an optimal diet for mass larval culture; it aims to address the validity of drawing conclusions on ecological questions, from laboratory studies.

Larvae were exposed to their ambient environment using a small scale *in situ* mesocosm, similar to that used by Olson, 1985, to assess growth and development of three species of marine larval invertebrates under 'natural' conditions. The initial task was, therefore, to construct the mesocosm. After several attempts, the Submersible Larval Incubation Chamber (SLIC) was developed. The two experimental systems permitted evaluation of the SLIC in different environments.

Objectives

1. Development of a small scale 'mesocosm' to facilitate larval culture under natural environmental conditions and to evaluate the utility of the mesocosm in different ecosystems.
2. To determine whether there is a significant difference between growth and development rates of larvae cultured with mixed algal assemblages compared with those cultured in water from their natural environment.

Experimental systems elected for this investigation.

- Larvae of the American Oyster, *Crassostrea virginica* (Gmelin) (Mollusca, bivalva) in the temperate, high nutrient ecosystem of the Chesapeake Bay, VA

  Larvae of the Queen conch, *Strombus gigas* and Milk Conch, *Strombus costatus* (Mollusca, gastropoda) in the tropical ecosystem of the Exuma Cays, Bahamas.

Statement of Null Hypothesis:

Differences between natural and mixed algal dietary regimes do not significantly affect the growth and development of the elected molluscan larvae.

Alternative Hypothesis: (H1)

Differences between natural and mixed algal dietary regimes significantly affect the growth and development of the elected molluscan larvae.

Chapter 1

Development and Evaluation of the Submersible Larval Incubation Chamber (SLIC)

Objective: Develop and construct a mesocosm to facilitate larval culture under natural environmental conditions and to evaluate the utility of the SLIC in different ecosystems.

Introduction

Development

The original concept was that of a small cage that could be deployed to study the growth and nutritional status of invertebrate larvae cultured in their natural environment. The first design was a simple construction of 3.5" PVC (schedule 40) piping of 1 liter capacity. Nitex screen was held in place over the open ends of the piping with push-fit connectors. The device was weighted using 1kg of lead diving weights, secured with cable ties. A styrofoam float was used to maintain the cage at a depth of 1m in the water column. There was keel of 7mm thick plexiglass attached to the underside in an attempt to maintain orientation of the cage into the current.

The cage was stocked at a density of 3 larvae ml^{-1}, with 4 day old *Crassostrea virginica* veliger larvae, reared at the VIMS oyster hatchery. It was then tied to a rope and pulley system and suspended approximately 5m away from the VIMS Ferry Pier in 3-4 m of water. After two days, the cage was retrieved and opened. No larvae were found alive. Several more attempts were made, each time with the same result.

Faults in the design of the cage caused inhibition of flow through the larval chamber. The screens used were of small mesh size (75-130 μm) and resisted water flow; this was exacerbated by screen blockage from sediment and fouling organisms. The current was not consistently strong enough to maintain flow through the cage.

A simple experiment was carried out using "Lifesavers" hard sugar candy rings, to determine differences in flow rate inside and outside the chamber. A "Lifesaver" was suspended with fine cotton in each of the following positions:

Inside the chamber, between the Nitex screens.

Outside the screens at either end of the pipe.

Suspended 10cm below the cage in the water column.

After 15 minutes the candies were removed. Although the candies were not weighed, there was obviously much less dissolution of the one inside the chamber, than those outside. This was repeated several times in different current speeds, with similar results. It was inferred from this experiment, that passive currents were not strong enough to overcome the resistance of the screens and maintain sufficient flow through the chamber to sustain larval oyster development.

The next step was to use an upwelling system (Fig. 1) York River water was passed into a trough via a 100μm mesh bag filter. The upweller units used were plastic cylinders, of 38 liter capacity. There was a screen of 50, 90 or 130μm mesh (depending on the size of the veligers) covering the base. The outlet was also screened to prevent the larvae being flushed away. The units were suspended in the water by means of two 2.5cm diameter PVC poles, secured with cable ties. The water would flow into the trough, up through the upwelling unit and through the outlet into a drain, thus producing a continuous flow of water through the unit. Although this arrangement was not strictly *in situ*, the water was pumped continuously from the York River and it was considered sufficiently close to natural conditions to be used as a surrogate.

The screen produced enough resistance to the upward flow of water that it

flooded over the standpipe of the enclosing tank, instead of flowing through the unit. The larvae survived quite well, but upwelling would decrease very rapidly as the screen blocked. This could be delayed by reducing the flow into the trough and using a 50 μm bag filter instead of 100 μm; however, the screen did eventually block. The system was modified to standardize food availability and reduce sediment in the unit. The troughs were drained and refilled daily at high tide, with York River water. The stocking density was reduced to 2 ml^{-1} ; every other day the units were rinsed with clean 1 μm filtered river water and a subsample of 10 larvae were removed from each replicate for measurement. This experiment was used as a preliminary for the main investigation.

The final step in the evolution of an *in situ* culture chamber, was the conception of the Submersible Larval Incubation Chamber (fig. 2) This was designed to counteract the problems of low flow and screen blockage by actively pumping water through the larval chamber and periodically reversing direction of flow to flush the screens. The larvae were held in a 1 liter capacity incubation chamber (plastic piping with 50 or 90μm Nitex screen ends). Full operation and control details are given in the Technical Report Appendix I. The internal plumbing was supported by a 1" PVC framework. Small holes were drilled in the frame in a few places to allow water to flood in and make it negatively buoyant. The displacement volume in the watertight box is such that the underwater weight of the box and contents is approximately 1.4kg. This made the box negatively buoyant but not so heavy that the SLIC sank into the substrate.

Configuration used in temperate high nutrient ecosystem

In the (original) configuration, flow was continuously pumped through the cage and was intermittently reversed by timed opening and closing of the battery actuated solenoid valves. The pump and valves were powered by a 12 volt sealed acid battery and controlled electronically with a circuit board. The pump capacity was 360 gph but flow was reduced using a voltage regulator to lower the voltage to the pump motor. The valves were actuated by the battery and were alternately opened and closed at one hour intervals by two programmable time delay relays. The battery, circuit board, voltmeter (to indicate battery power) and wire connections were enclosed in a watertight plexiglass box so the entire system could be submerged if necessary.

Configuration used in tropical oligotrophic system

For this experiment, three replicate units were run from the same circuit board. Since the three pumps together were drawing more amps (2.5 x 3) than the potentiometer was capable of regulating, a small rheostat was substituted to control the pump motor speed. The system was powered by two 6 volt Golf cart batteries (220 amp hours each) in series to produce a 12 volt supply. It was difficult to keep the flow rate constant; with three pumps and the time delay relays drawing power, the voltage of the batteries would decline quickly. Once the voltage decreased below 11.5 volts, the pumps would cut out and the flow would stop. This problem was

remedied by altering the circuit board so that the time delay relays controlled the pump instead of the solenoid valves. This allowed the flow to be 'pulsed' rather than continuous: The time delay relays were left on "recycle" but the timer on TDR1 was set for 58 minutes and TDR2 for 2 minutes. Using this configuration, the pumps would come on for two minutes every hour. Using 12 volts power (and 130 μm screen on the filter) the flow rate was 4 liters^{-1} with the 250 μm screen on the chamber and 2.75 liters min^{-1} with the 130 μm screen. Two minutes of pumping was sufficient to produce a full water exchange inside the incubation chamber whilst greatly reducing the power consumption.

The solenoid valves, when not powered, are closed and had to be replaced with manual valves. Flow reversal was originally incorporated in the SLIC design to flush the chamber screens and keep them clean in a high sediment environment. This was not necessary in the clear tropical waters of the Bahamas, especially with the introduction of the pulsed flow system. When the chambers were removed for cleaning and subsampling, the valves were switched over to reverse the flow around the internal plumbing and flush out any organisms or debris that may have accumulated.

For both of these experiments, the SLIC was operated near shore and the battery box remained on land, with cable connecting it to the mesocosm (fig. 3) This was easier than having to retrieve the box to change the battery. The wires enter through holes in the box lid (which are sealed with 'plumbers goop') and attach to the

appropriate wires on the circuit board via quick release connections. The circuit board was attached to the lid of the box to enable removal of the battery for charging and replacement. The pump was set high on the frame to reduce blockage of the intake by benthic sediment. An in-line cartridge filter was placed between the pump outlet and the valve system to remove larger particles. The original cartridge was stripped and replaced with a 90μm or 130μm Nitex sock.

Possible Artifacts Associated with the Mesocosm

- Stationary retention of organisms in the water column exposes them to nutrients in a way that would not necessarily mimic that of a natural system. As previously mentioned, the distribution of nutrients in the estuarine ecosystem is patchy, therefore the food concentration at any given time and location will vary between estimable limits. Planktonic organisms are susceptible to physical forces such as currents, turbulence and fronts that may expose them to favorable or unfavorable local environments. If the same forces simultaneously affect the source of nutrients for the planktonic organisms, their food supply would be relatively constant. Stationary retention of organisms in the water column uncouples this system and exposes the organisms to patches of nutrients and changes in water characteristics.

  Confining planktonic organisms may disrupt responses to environmental cues. Confining organisms at unnaturally high densities could artificially create food limitation or density dependent growth inhibition in some species.

Fluid flow through the larval containment chamber is generated artificially, therefore will be different from that of the ambient water body; turbulence and boundary layers would be created within the chamber.

The effects of predation on survival are eliminated, thus eliminating any recruitment/survival assessments that can be made.

Each artifact should have a control to assess the direct, additive or interactive effects of treatment and artifact; the ideal control would be the treatment without containment, which is not always possible. An estimation of effects of artifacts can be obtained by changing their intensity and evaluating the consequences.

Evaluation of SLIC Environmental Parameters

Oxygen Levels

Dissolved oxygen requirements of different larval stages of *C. virginica* have been determined in previous studies (Baker, 1994; Widdows et al, 1989). For larvae of stage IV (late veliger) , the O_2 demand was found to be 700 pmoles hr^{-1} $larva^{-1}$. The York River experiences low dissolved oxygen content in the summer, especially below the pycnocline during periods of stratification. Dissolved oxygen measurements were taken for the water used in the upwelling tanks and in the river at 2m below the surface; the lowest DO measured was 5mg l^{-1} (pers. data; summer 1994). A flow rate of approximately 9ml hr^{-1} would provide sufficient oxygen for contained oyster larvae, (at a density of 4 ml^{-1}), even in the lower oxygen concentrations of the river. The York River estuary may become stratified to various

degrees during the summer months causing the bottom waters to become hypoxic. Wind stresses can locally disturb the stratification and bring oxygen depleted water into the water body. The larvae held in the chambers cannot move in response to low oxygen, however these events are not long term and pumping of water into the chambers would aid in oxygenation.

Research on oxygen consumption by *Strombus gigas* (Erickson, 1984) showed that larvae at age 13 days, consumed 0.76 μg O_2 day^{-1}. A stocking density of 10 larvae l^{-1}, creates a total O_2 requirement of 7.6μg O_2 l^{-1} day^{-1}. The dissolved oxygen content of tropical water is approximately 7 mg l^{-1}. In one liter of water, there would be 10^3 times more oxygen than required by 10 larvae in 24 hours; therefore oxygen should not be a limiting factor in the chambers.

Particle Flux

Clearance rates of *C. virginica* larvae reported in the literature (Widdows et al, 1989; Baldwin and Newell, 1991) for different particle sizes, range from 0.0017 ml $larvae^{-1}$ hr^{-1} (veliger larvae on natural plankton assemblages <30μm) to 0.109 ml $larvae^{-1}$ hr^{-1} (pediveligers on cultured ciliates). These numbers are consistent with other marine bivalve molluscs of comparable size (Sprung, 1984; Crisp et al, 1985). Using hatchery density of 4 larvae ml^{-1} (or 4000 $liter^{-1}$) gives an hourly depletion of 400ml of water in a 1 liter chamber. If a flow rate of 400 ml hr^{-1} was maintained through the 1 liter chamber, the larvae would theoretically not be capable of depleting their food supply.

Studies on ingestion and feeding of *Strombus gigas* on algal diets, have produced mixed

results of requirements for optimal growth, ranging from 1000-30000 cells ml^{-1} with a density of 150 larvae l^{-1} (Davis and Hesse; 1983) Without information on natural cell densities or larval clearance rates, this information is of limited assistance in determining flow rates required.

Flow Speeds

Flow effects on settlement are a potential treatment artifact in *in situ* vs hatchery comparisons, since incubation chambers are continuous flow and the hatchery tanks are static. With too high a flow rate, the larvae are in danger of being damaged against the screen; alternatively, they may close up, sink (and therefore stop feeding) in response to stress. Numerical modelling of larval settlement (Gross et al, 1992) indicates that most settlement will occur during periods of slack water whereas comparatively little will occur during stronger flows. Prytherch (1929) states "no larvae could be swimming in the water when the flood current had reached a velocity of 0.6 feet $second^{-1}$" (20 cm $second^{-1}$). Laboratory studies by the same author reported that oyster larvae sank to the bottom at speeds as low as 0.3 ft $second^{-1}$ (10 cm $second^{-1}$). This is more likely to be due to turbulence than flow speed; since larvae are carried along in flow streams it is unlikely that speed of the water parcel alone was causing the larva to sink. The flow rate within the chamber therefore, must be high enough to replenish food and oxygen , but low enough not to cause mortality or disruption of settlement. A flow rate between 400 and 3600 ml $hour^{-1}$ would fall within the appropriate range for oyster larvae. Similar information is not available for *Strombus* larvae.

Analysis of Flow through Larval Chambers

Experiments were carried out in large tanks to observe flow patterns passing through the chambers. This was done by introducing fluorescein or red food dye into the pump intake. The dye was used to indicate any turbulence created within the cages and to show how the water behaves when the flow is reversed. A hose was fitted to one of the chamber exhausts so that water leaving the chamber emptied into a graduated beaker; this enabled measurement of flow through the chamber. A suitable flow rate and time interval was determined from the flow analyses.

At flow rates of 500 ml min^{-1}, there are low levels of turbulence in the chamber. When forced through very quickly, a plume of fast moving fluid extends along the length of the chamber before the main body of water in the chamber becomes colored; the high flow rates created a strong cross chamber gradient and less mixing. When the water is moving slowly, it gently passes through the screen as one mass. A slow moving plume is preferable to the fast flow configuration because the water is mixed more evenly and is less disruptive to normal larval behavior patterns.

Figure 1: Diagram of upwelling units used in pilot project culture of *Crassostrea virginica* larvae in the York River, VA

Upwelling Flow-through system

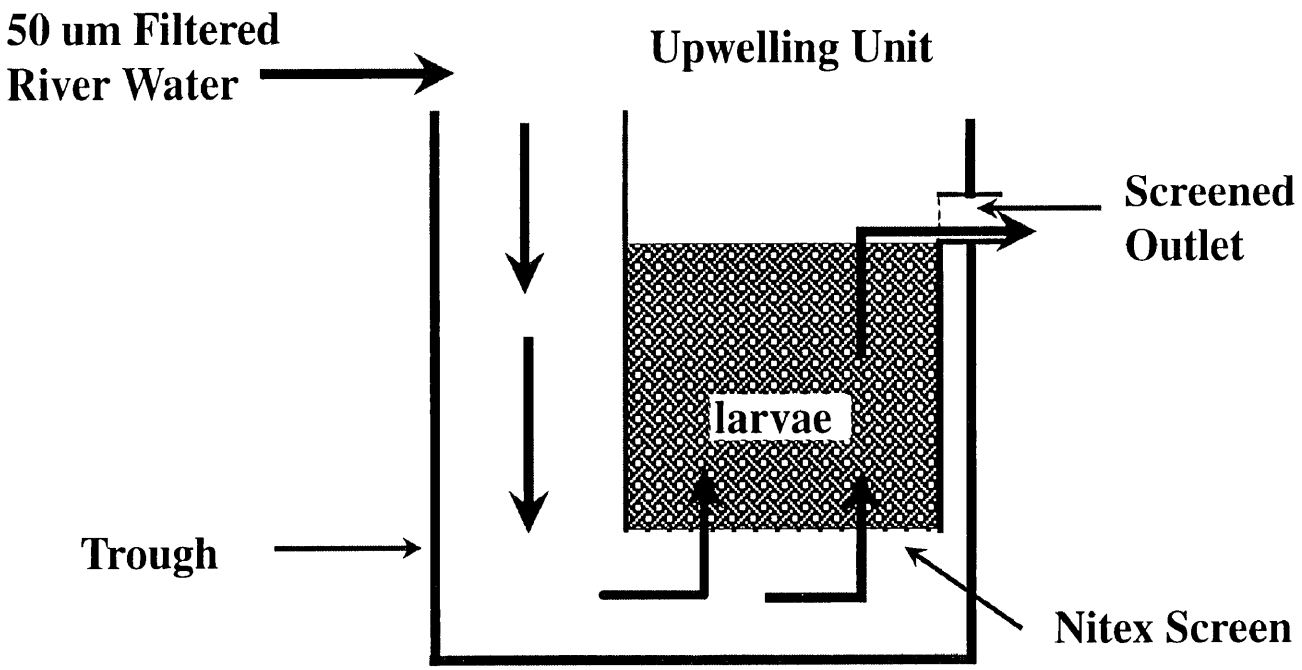


Figure 2: Submersible Larval Incubation Chamber (SLIC). Plan view of functional unit, showing water flow in one direction.

PLAN VIEW OF SLIC SHOWING FLOW IN ONE DIRECTION THROUGH THE UNIT

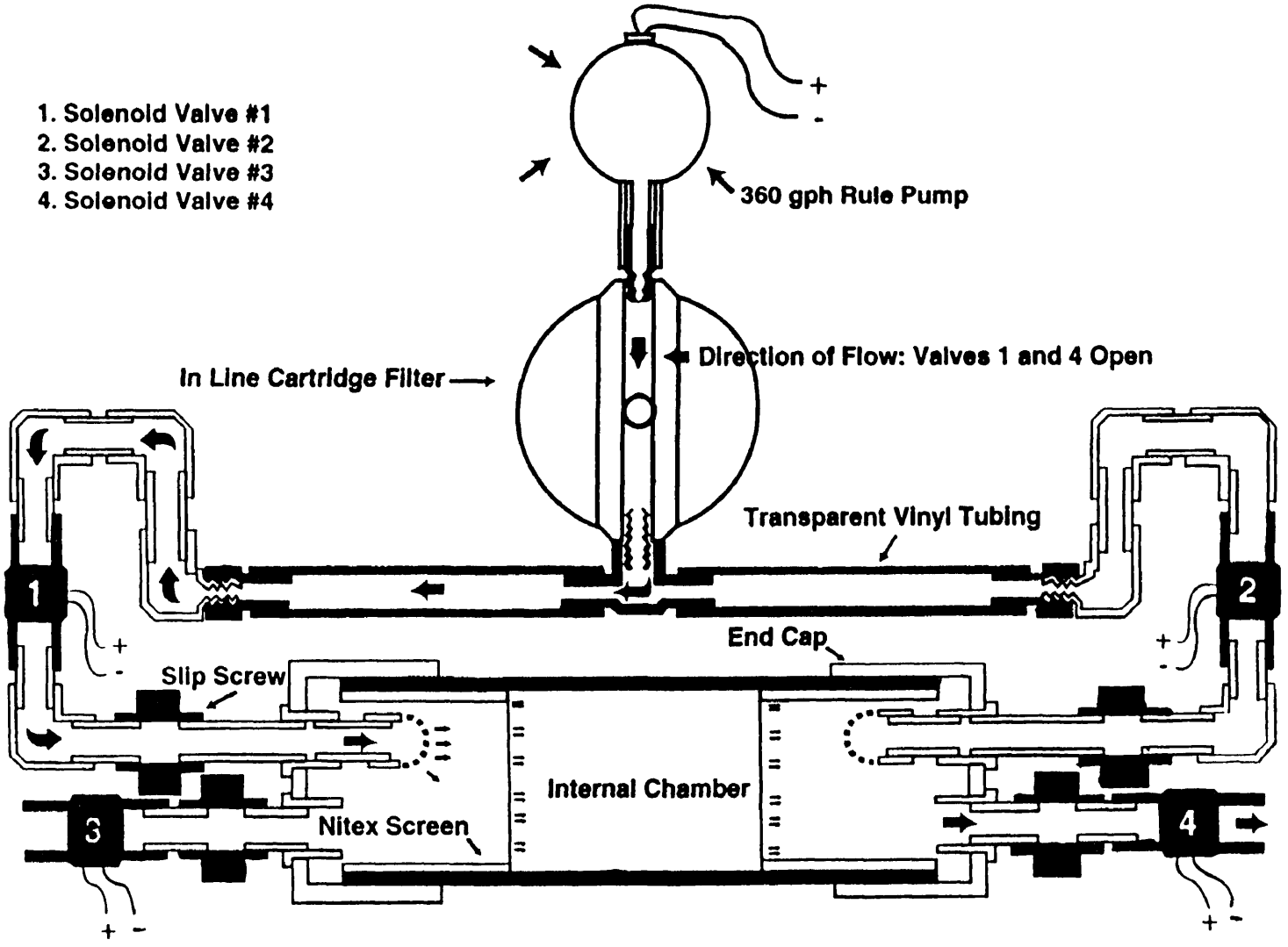
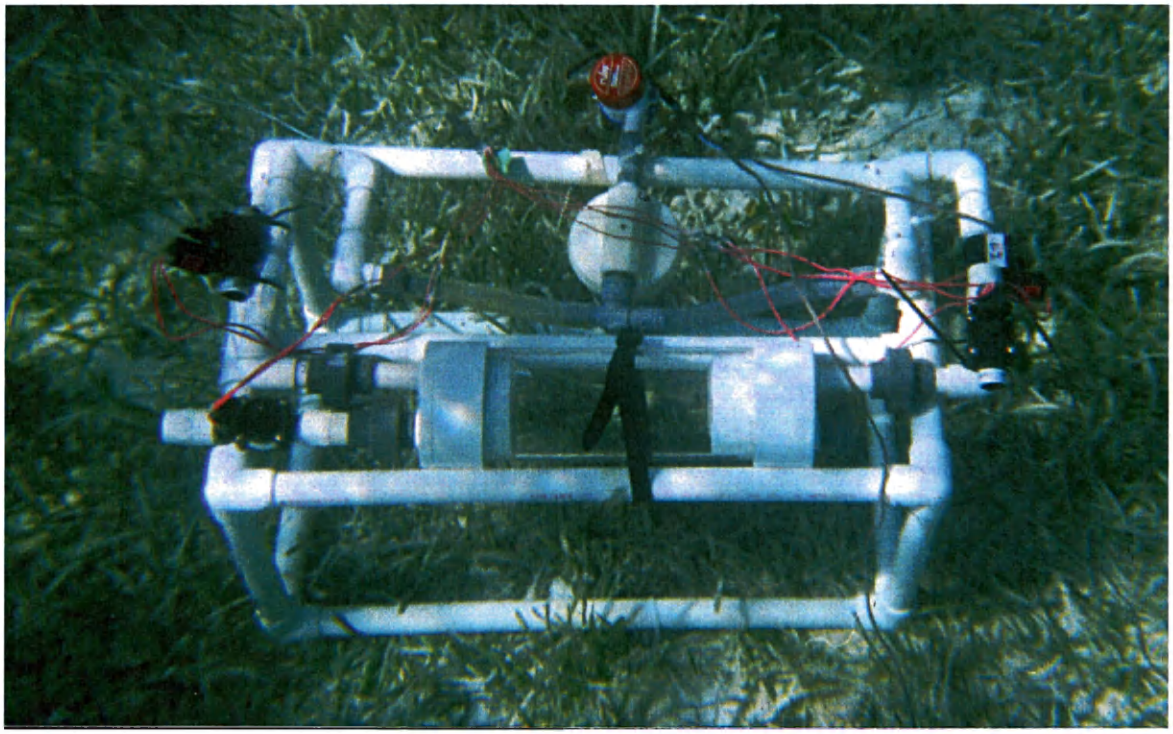
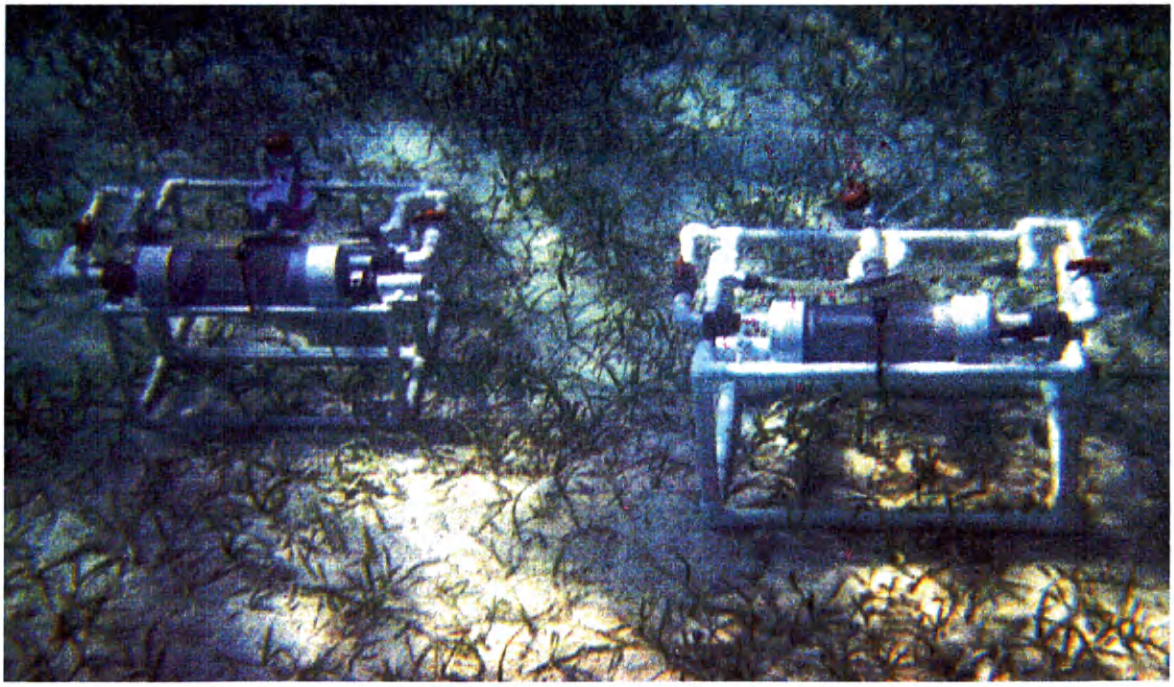


Figure 3: Photographs of SLIC in operation in a shallow (3m) bed of *Thalassia testudinium*. Lee Stocking Island, Bahamas.

a



b



Evaluation of utility of SLIC in different ecosystems

High nutrient temperate ecosystem

There were problems associated with using the mesocosm in the high sediment loading of this ecosystem. The primary problem was fouling, both biotic and abiotic.

Abiotic fouling caused severe and rapid blockage of the Nitex screens of the filter and chamber. Although the filter screen was of a smaller mesh size than the chamber screens, the fine sediments could still pass through. Any reduction in flow energy allows particles to fall out of suspension and settle. Initially, energy of the flow decreases due to friction as it passes through the plumbing. There is further reduction in flow speed as the water enters the chamber and the diameter of the pipe increases from 1.95cm to 11.25cm. Flocculated clay may be forced through the filter screen, but re-aggregate in the lower energy flow in the chamber. Blockage of the screens further reduces the energy of the flow, creating a positive feedback situation. Sediment accumulation creates a treatment artifact; if the flow rate is not constant, neither is the nutrient or oxygen flux. Increasing the pumping to a point where the flow could back flush the screens would have been counter productive; it would have increased the quantity of sediment and possibly damaged the larvae.

Biotic fouling was caused by epiphytes growing on the valves of the larvae, and protozoa and bacteria invading the soft tissues. Indirect biotic problems came from the entry of very high numbers of planktonic organisms, representing various phyla. The most common were copepodites, but also present were other crustaceans,

gastropod larvae, polychaete larvae and small flagellates. These were potential competitors for food and oxygen and their presence in the water could increase the BOD to a point where it became limiting, especially when compounded by blockage of the screens and high summer temperatures. During the late summer, the river water contains a high level of bacteria. The chambers, may have been concentrating bacteria and other such undesirable organisms, creating stress and possibly larval mortality.

An operational problem arose when the larvae were removed from the chamber and the apparatus was cleaned. Removing the larvae from the mesocosm was a lengthy and difficult procedure. The problems were primarily related to physical manipulation of the apparatus. The SLIC was easily removed from the water using a winch and davit. Once it was placed on a stable surface, the chamber unit was detached using the slip screws. If handled very gently, the chamber could be maintained full of water due to the surface tension on the small meshed screen. If the chamber was knocked or jolted, this surface tension would be released and the chamber rapidly empty, potentially stressing the animals. I tried releasing the chamber while it was still partially submerged, but this increased the risk of losing the chamber. Removal of the end caps without pulling off the Nitex screen was another challenge; if the screen was pulled or damaged, the larvae would be lost. Sediment would collect in the junction between the chamber and the end caps, making them very difficult to remove. Re-submerging the chambers while the screens were wet was very difficult, since the water would not flow back into the chamber against surface tension. The chamber had to be replaced with dry mesh and also filled before attaching to the end caps to avoid air in the chamber.

Oyster larvae are convenient to work with in culture experiments because of their relative robustness and ability to survive at high densities. The disadvantage is their small size, which necessitated fine mesh on the chambers and exacerbated the fouling problem. Such small organisms were also time consuming to retrieve from the chamber during cleaning and subsampling. The current experiment was also carried out during a season of very high water temperature. The larvae were often exposed to water temperatures of 32°C, which was not only stressful to the organisms but induced rapid bacterial growth and scope for mortality from pathogenic attack.

Oligotrophic tropical coastal ecosystem

The SLIC was much easier to work with in the oligotrophic environment than the Chesapeake ecosystem; the substrate was heavy sand rather than fine particles; the visibility was 20m rather than 0.5m, and the location was shallow enough to allow chambers to be changed in the water rather than having to retrieve the apparatus for servicing. The level of fouling and non target organisms was lower than in the high nutrient system. Larger mesh sizes were used in this experiment therefore surface tension is not such a problem as in the temperate ecosystem experiment.

There was one observed potential artifact in the oligotrophic environment which was not observed in the high nutrient system; the temperature inside the chamber was occasionally higher (0.5-1°C) than ambient. I think this was due to the high level of irradience on a small enclosed body of water. The chamber was flushed

every hour, but this may not have been sufficient to prevent an increase in internal temperature. This artifact can be overcome by increasing the flushing frequency.

Conclusions

The mesocosm concept is tractable but there are problems inherent in every system; which have to be dealt with and accounted for. The SLIC functioned well in the tropical ecosystem but the design may not be tractable for use in an ecosystem with high organic content because of the high degree of fouling.

The pulsed configuration of the SLIC was superior to the continuous flow since it reduced power consumption and fouling levels . If used in an ecosystem with high insolation, the unit should be shaded to prevent temperature increase inside the chamber.

Chapter 2

Effects of Natural vs Laboratory Culture on Larvae of the American Oyster, *Crassostrea virginica* (Gmelin)

Objectives:

- To investigate the effects of *in situ* vs laboratory culture on growth and development of larvae of the American Oyster, *Crassostrea virginica* (Gmelin).

Section 2.1: Pilot project using upwelling units to expose larvae of *Crassostrea virginica* to natural York River water.

Section 2.2: In situ culture of *Crassostrea virginica* larvae using the SLIC mesocosm on Eastern Shore of Virginia.

Introduction

Life cycle and natural history of *Crassostrea virginica*

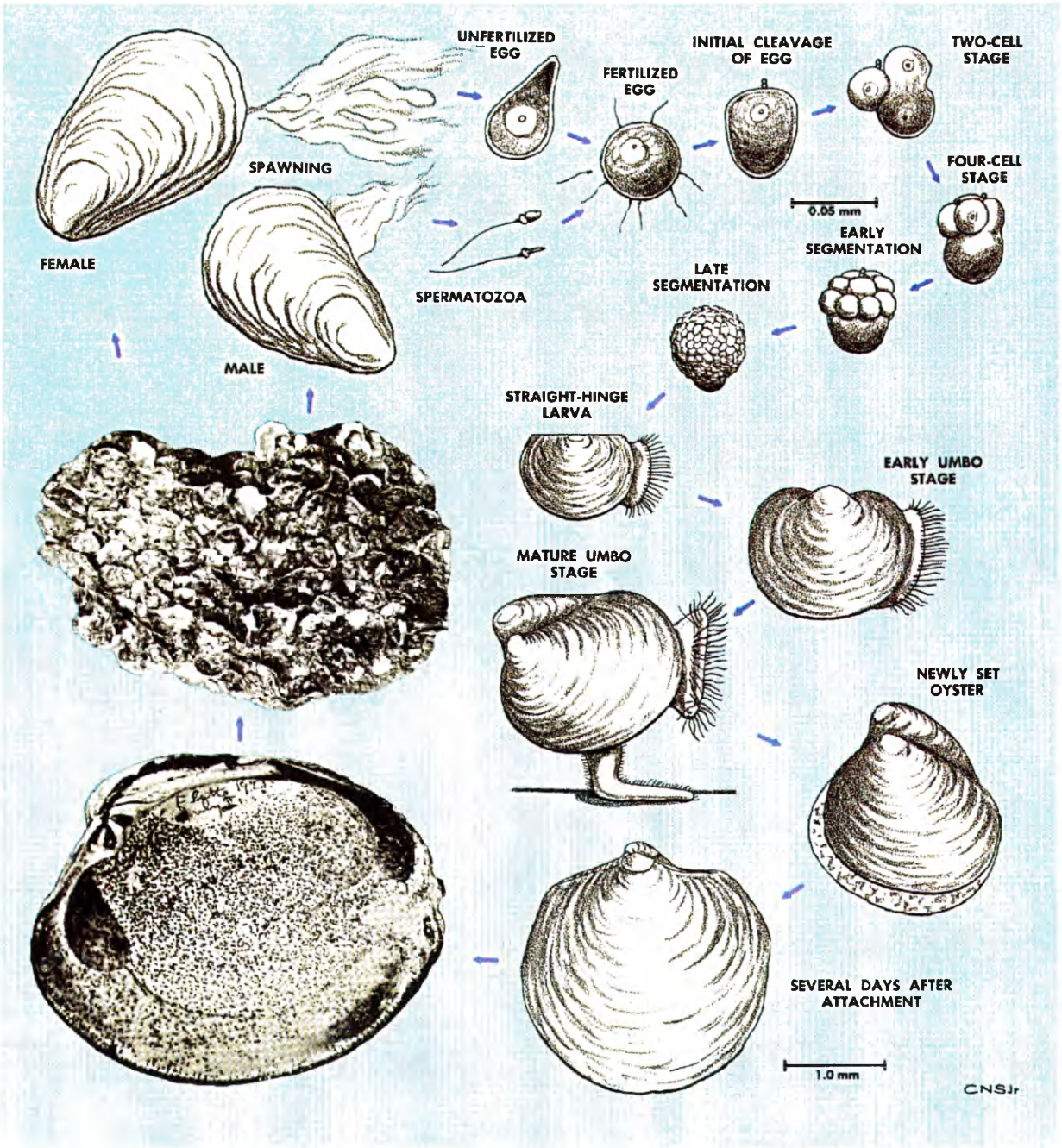
Oysters produce gametes in response to seasonal changes in water temperature. In the Chesapeake Bay a period of "hibernation" occurs generally from mid-December to mid-March. Water temperatures of 19-22°C indicate optimum conditions for gamete production; spawning begins around the end of June and continues intermittently during July , August and September. Peak spawning months occur from June through September. Oysters continue spawning seasonally throughout adult life.

Figure 4 shows *C. virginica* lifecycle. Male oysters release sperm into the water column, which stimulates the females to release their eggs. Eggs and sperm fuse and ~ 24 hours after fertilization, the trochophore larva is formed. This stage lasts only 24-48 hours, then proceeds to the veliger stage. The veliger posesses a velum, composed of two ciliated lobes in *C.virginica* larvae. Large cilia beat to create swimming currents and small ones at the base of the velum carry food particles towards the mouth. The initial veliger stage is the prodissoconch, also referred to as 'D-stage' or 'straight hinge' due to the shape. Major changes take place in the appearance of the larvae as it develops and grows through the "umbo" and "pediveliger" stages to become competent for metamorphosis.

Competence to metamorphose is indicated by the presence of a dark pigmented eye spot, which disappears after metamorphosis. The larvae settle, metamorphose and become attached juveniles or spat. During metamorphosis, the larval organs disappear and there is an anatomical reorganization of the permanent organs (Baker and Mann, 1994). The settlement is irreversible in oysters since one of the valves becomes cemented to the substrate.

Figure 4: Lifecycle of the American Oyster, *Crassostrea virginica* (Gmelin)

Life Cycle of *Crassostrea Viginica*



Feeding Mechanism of Bivalve Mollusc Veligers

Strathmann and Leise (1979) described the direction of the ciliary currents in the bivalve molluscan veliger larvae. The lobed velum of the larvae creates both feeding and locomotory currents. Suspended particles are concentrated between two opposed bands of cilia which line the velar edge. The preoral band (prototroch) consists of long compound cilia which produce the swimming and feeding currents. The post oral band (metatroch) consists of shorter cilia which beat towards the preoral band; the combination of these two bands captures and retains particles. Between these bands is a food groove with very small cilia which transport food towards the mouth.

The cilia on the velar lobe remove excess material as pseudofaeces. Material passes into the stomach via the oesophagus. In the stomach, the particles are rotated by large cilia at the anterior end and by the smaller cilia on the wall of the stomach. Simultaneously, the crystalline style is rotating in the style sac; the direction is either anti clockwise or clockwise, depending on the species or individual. Extracellular digestion begins in the stomach. Partially digested material is passed to the digestive diverticula, where intracellular digestion is completed. Residual perticulate material is returned to the stomach. Particles leave the stomach via the mid gut and continue down the alimentary tract and out of the anus. The feces are rolled into a ball by the cilia in the mantle cavity and are then expelled (Strathmann and Leise, 1979)

Section 2.1: Pilot project using upwelling units to expose larvae of *Crassostrea virginica* to natural York River water.

Before embarking on the main project, a pilot project was carried out to determine the extent to which diet affects growth and develoment of invertebrate larvae. The mesocosm was in development during this season therefore upwelling units were used to expose the larvae to river water in lieu of *in situ* culture in the river

Treatments:

Sibling larvae at 24 hours , were used at a density of 4 ml^{-1} for all treatments.

Treatment 1.

Larvae were cultured at the VIMS Oyster Hatchery in 1μm filtered York River water in a 1500 liter tank with diet of mixed algal cultures (Appendix II). This diet is used for the pilot scale commercial rearing of *C. virginica* from spawn to settlement in the culture system.

Treatment 2

Larvae were cultured in an upwelling unit outside and supplied with 100 μm filtered York River Water. No supplementary food was used for culture.

Treatment 3

An upwelling unit was placed inside a tank and supplied with the same algal diet as in treatment 1. This treatment was included to determine whether there were any effects of screening and containment on the larval growth rates.

An orthogonal experimental design would require a treatment in a 1500 liter tank filled with river water; however, due to logistical contraints, this treatment was not included.

Experimental Design

Dependent variables	Shell height and length (μm)	
Independent variable	Age (days)	
Factor	Enclosure type	Diet type
Levels	E1 = 1500 liter tank	D1 = Hatchery diet
	E2 = Upwelling unit	D2 = River water

	E1	E2
D1	Treatment 1	Treatment 3
D2	No Treatment	Treatment 2

Stocking density:	4 larvae ml^{-1}	Number of replicates:	2
Number of spawns:	4	Larvae per sample:	20

Materials and Methods

Study Site

This work was carried out at the oyster hatchery facilities of the Virginia Institute of Marine Science, located near the mouth of the York River (fig. 5) The river is a tidally flushed, mesohaline system, which experiences wide ranges of temperature and salinity. Haas (1974) recorded surface temperatures ranging from 2 (February) to 26°C (July) and salinities from 15ppt (April) to 22 ppt (November). Salinity increases with depth to a maximum of 27 ppt at 18m in June. Tides are semi diurnal with mean amplitude of 0.7m and mean tidal excursion of 7km. The estuary oscillates between periods of extreme stratification during the

winter months and periods of intense vertical mixing during July and August, especially during the spring tides. The maximum daily chlorophyll levels recorded by Haas (1974) ranged from as low as ~5 μgl^{-1} in February to 25 μgl^{-1} in October. The phytoplankton community was dominated by nanoplankton (<15 μm) which reached a peak in the late summer months and accounted for 65-90% of the total chlorophyll a productivity.

Oyster larvae were supplied by the Virginia Institute of Marine Science hatchery at Gloucester Point. Filtered (1μm) York River water is used in the aquaculture system; ambient salinity and temperature of the hatchery water were taken daily (fig. 6) Larvae were maintained in 1500 liter aerated tanks with water changes every two days and daily food replenishment (Refer to Appendix II for dietary details). When the water was changed, the tanks were drained and larvae collected on Nitex screens of appropriate mesh size. The dead, moribund and abnormally small larvae were discarded and only those actively swimming in the water column were returned to the tanks.

At 24 hours after spawning, a subsample of larvae was removed and allocated into treatments. These larvae were at the prodissoconch stage (70-80 μm shell length) and were just starting to feed.

Treatment 1

These larvae were maintained on cultured algal diet, as described in Appendix 2a.

Treatment 2

York River water was passed into a trough via a 100 μm mesh bag filter. The upwelling units were constructed from 38 liter plastic cylinders with a screen base of 50, 90 or 130 μm mesh, depending on the size of the Veligers. The outlet was also screened to

prevent the larvae being flushed away. The units were suspended in the water by means of two 2.5 cm diameter PVC poles, secured with cable ties. The water flows into the trough, up through the upwelling unit and out through the outlet into a drain, thus producing a continuous flow of water up through the unit. With this continous flow-through configuration, the screen base became blocked with sediment very rapidly. This treatment was therefore changed to a static water system whereby the upwelling trough was refilled daily. Water was taken at high tide when the sediment level is lower and the oxygen content higher than low tide.

Treatment 3

A 180 liter tank was filled with 1μm filtered York River water and supplied with algal diet as in treatment 1. The upwelling unit was stocked at a density of 4 larvae ml^{-1}, suspended in the carboy by means of two 2.5 cm PVC poles and secured with cable ties. Every other day the water was drained and replaced. Food was replenished on a daily basis. Every two days, subsamples of 20 larvae from each treatment were removed for growth rate and lipid content analysis as described below.

1. Growth Rate Measurements

The larvae were drawn into a flattened capillary tube and examined under x10 magnification. Shell height (dorso-ventral) and length (anterior-posterior) of individual larvae were measured using a Zeiss IM 35 microscope with an ocular micrometer, which measures to 1μm resolution. Shell height is distance from hinge to the opposite side of the valve. Length is perpendicular to height and is usually the widest span of the shell.

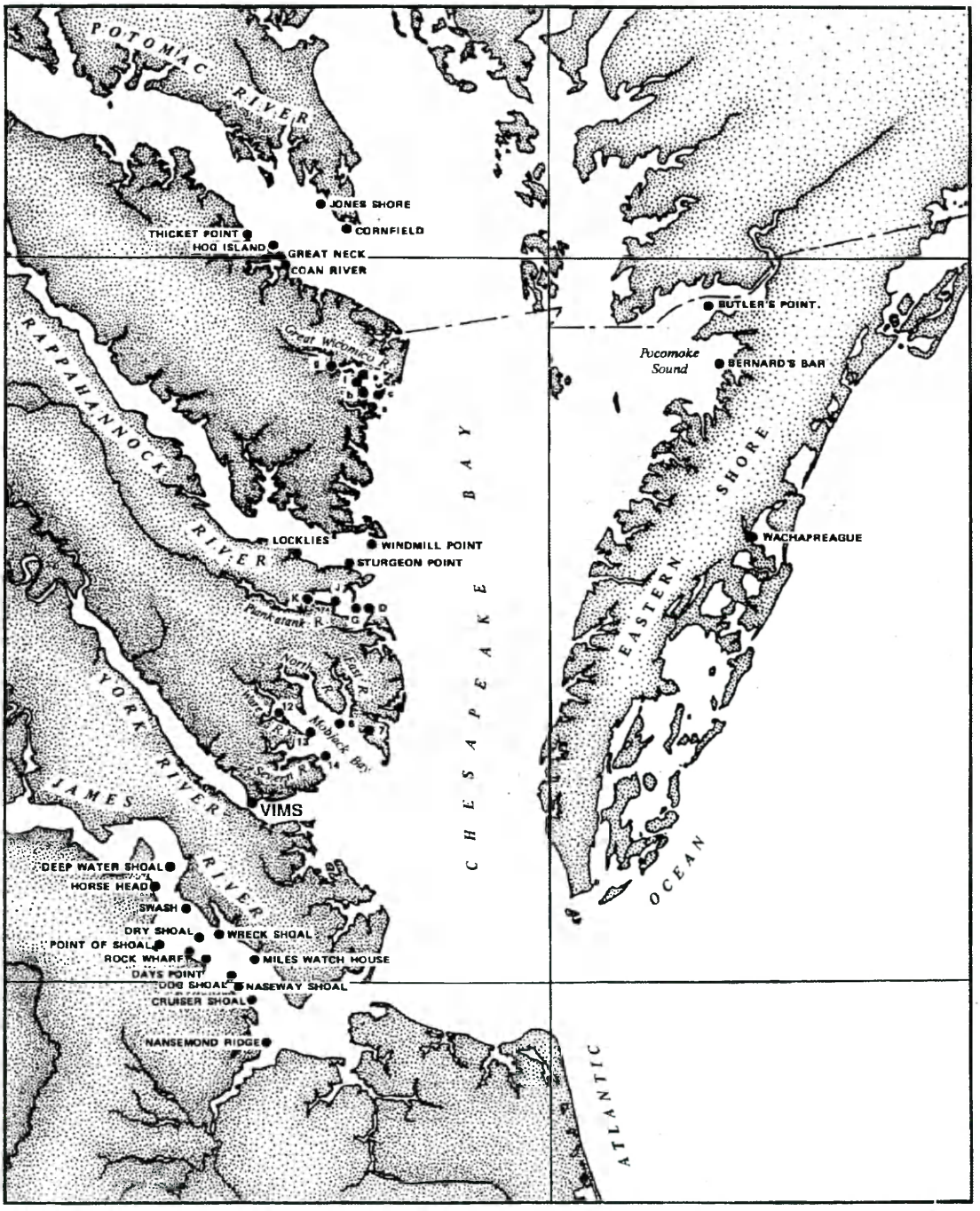
Statistical Analyses:

Levenes test for homogeneity of variance determined all the data sets to be heteroscedastic. Parabolic and $\log_{10}$ transformations were applied to the data, but the variance distributions remained non homogeneous and the outcomes of the statistical analyses were unchanged by either transformation. The statistical analyses performed on the data are robust to non homogeneity of variance, but care must be taken in the interpretation of the results because the chance of committing a type I error will be increased with non homogeneous data. Regression analysis was used to establish a dependence relationship between age and shell dimension , and to determine a growth rate coefficient for each diet. ANCOVA General Linear Model was performed to determine whether the effect of diet produced a significantly different growth response ($\alpha = 0.05$) and to detect any interaction between diet and age. Since an interaction effect was found in all data sets, the 95% confidence intervals of the regression lines were used to determine whether diet had a significant effect on larval growth.

Figure 5: Map of study sites:

- Pilot project at the VIMS hatchery facility on the York River.

  Main study at VIMS Wachapreague facility on the Eastern Shore of VA.


POTOMAC RIVER
JONES SHORE
CORNFIELD
THICKET POINT
HOG ISLAND
GREAT NECK
COAN RIVER
RAPPAHANNOCK RIVER
Great Wicomico R.
BUTLER'S POINT
Pocomoke Sound
BERNARD'S BAR
CHESAPEAKE BAY
EASTERN SHORE
LOCKLIES
WINDMILL POINT
STURGEON POINT
WACHAPREAGUE
Piankatank R.
North R.
East R.
Mobjack Bay
YORK RIVER
Severn R.
VIMS
JAMES RIVER
DEEP WATER SHOAL
HORSE HEAD
SMASH
DRY SHOAL
WRECK SHOAL
POINT OF SHOAL
ROCK WHARF
MILES WATCH HOUSE
DAYS POINT
DOG SHOAL
NASEWAY SHOAL
CRUISER SHOAL
NANSEMOND RIDGE
ATLANTIC OCEAN

Results:

The data for all treatments was plotted together on the same axis for shell height (fig. 6a) and shell length (fig. 6b); there appeared to be no treatment effect on larval growth and regression analyses of shell dimension vs age produced similar trends for all treatments (figs. 7a-c and 8a-c). Tables 1 through 6, show the results of the regression analysis of shell height and length on age for different diets. The Null hypothesis (slope of the line=0) was rejected (α=0.05); the predictor (age), therefore has a significant effect on the response (shell dimension), which is to be expected of a growth curve. The r^2 was >0.8, meaning that >80% of the size was acounted for by age.

Tables 7 and 8 show ANCOVA's of age vs shell height and length for all treatments. A significant interaction between age and shell dimension was found for both shell height (p=0.00) and shell length (p=0.05) The interaction indicates an age dependent response to dietary differences, which can be explained by change in nutritional requirements of the larvae as they develope. This significant interaction between predictor and response invalidates the use of the ANCOVA to establish statistical differences between treatments. When an interaction is found, the upper and lower 95% confidence intervals of each regression curve can be compared instead of analysis of variance. If the confidence intervals overlap, the regression curves are not significantly different. Shell height and length data was compared using the 95% confidence intervals; growth curves of the river and algal diets were not significantly different, but the upwelling algal diet produced a significantly faster growth rate than either of the other treatments.

Higher larval growth rate found in the algal upwelling treatment may have been due

to concentration of algal food in the upwelling unit; however, from observation of the units during the experiment the opposite appeared to be true; the water inside the units appeared to be more food depleted than that outside. The larvae may have been grazing and reducing the food concentration; standing stock does not necessarily reflect food availibility. Alternatively, the submersible pump caused a slight increase in water temperature (1-2 ^{0}C), which may have been sufficient to increase growth rate (in the presence of an adequate food supply), above that of the other two treatments.

Development rates

There was no observable difference between development rates of the treatments. There was however, much within-treatment variation which may have masked any dietary effects. The larvae were maintained for a maximum of 18 days, when the shell length was approaching 300 μm in all treatments.

Water Quality

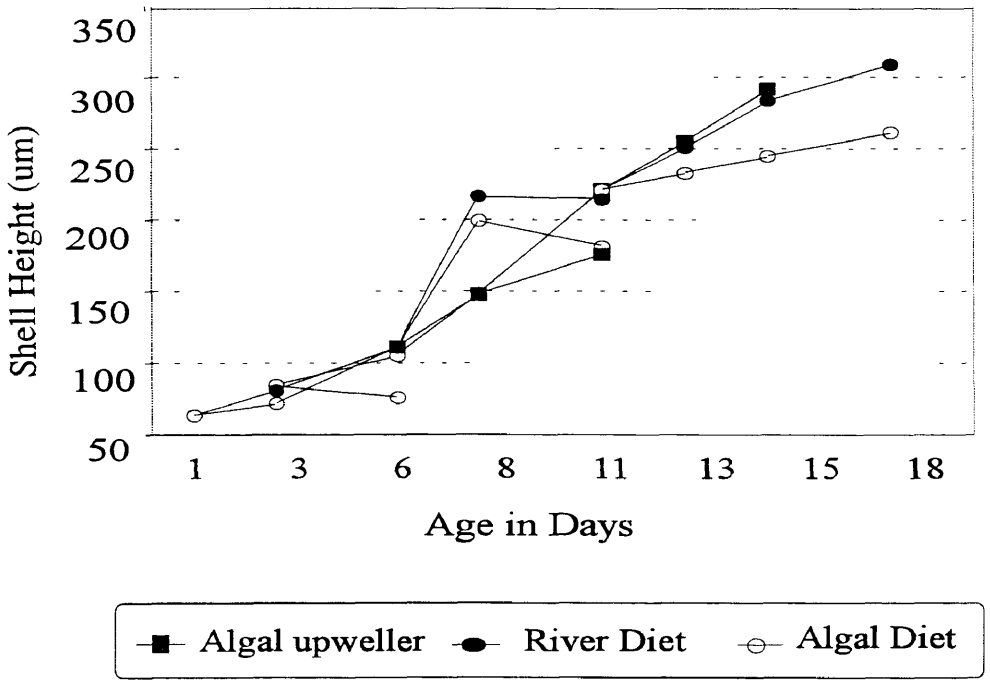
The experiment was conducted during the summer months in the York River. Temperature and salinity data are shown in figure 9.

Figure 6: Growth curves for *Crassostrea virginica* larvae

a. Shell height (μm day^{-1}) against age (days) for different treatments

b. Shell length μm day^{-1}) against age (days) for different treatments

a. Shell height vs age



b. Shell length vs age

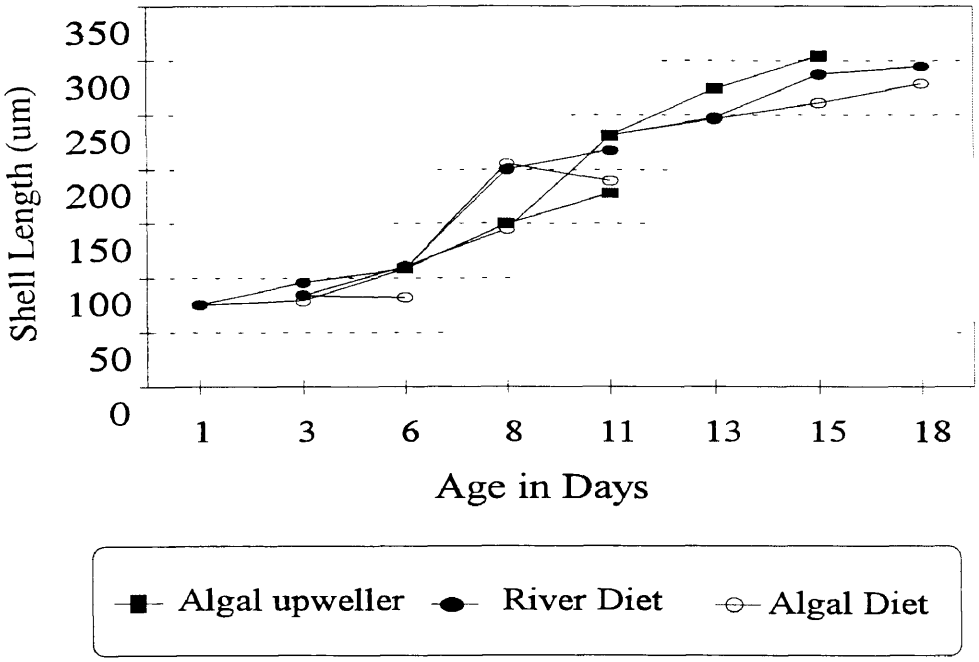


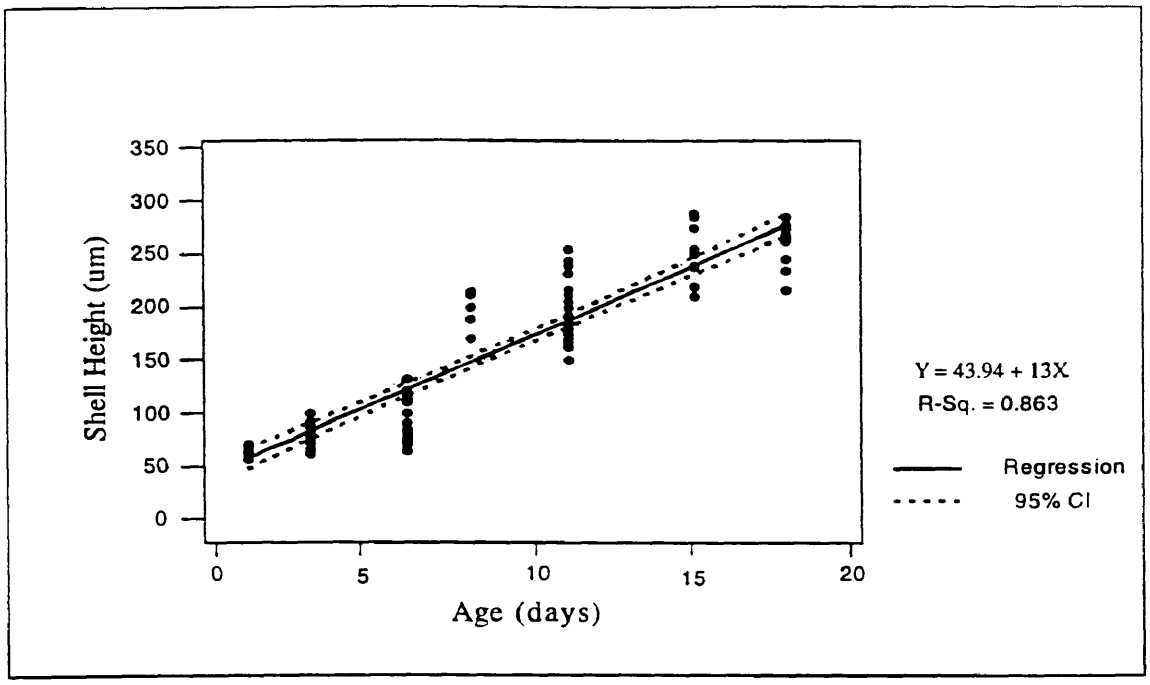
Figure 7: Linear regression fitted line plots for shell height (μm day^{-1}) against age (days) for different treatments. Hatched lines represent 95% confidence intervals.

a. Treatment 1: Hatchery algal diet.

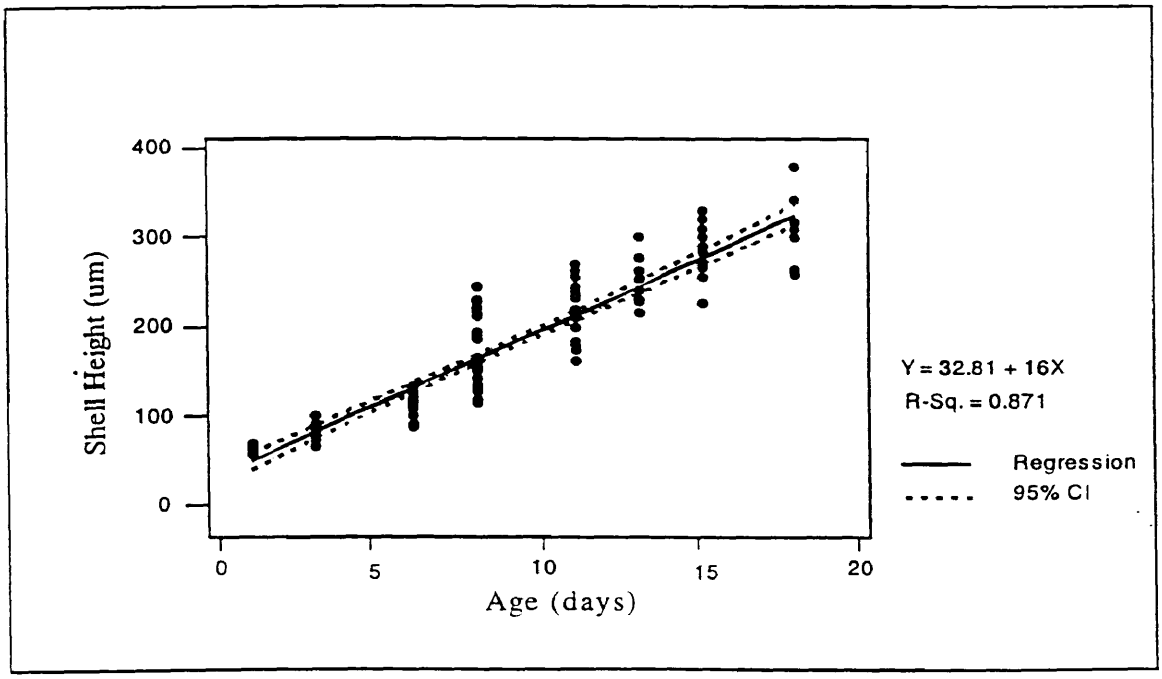
b. Treatment 2: Upwelling unit supplied with York River water.

c. Treatment 3: Upwelling unit supplied with hatchery algal diet.

a.


Shell Height (um)
350
300
250
200
150
100
50
0
0
5
10
15
20
Age (days)
Y = 43.94 + 13X
R-Sq. = 0.863
Regression
95% CI

b.


Shell Height (um)
400
300
200
100
0
0
5
10
15
20
Age (days)
Y = 32.81 + 16X
R-Sq. = 0.871
Regression
95% CI

c.

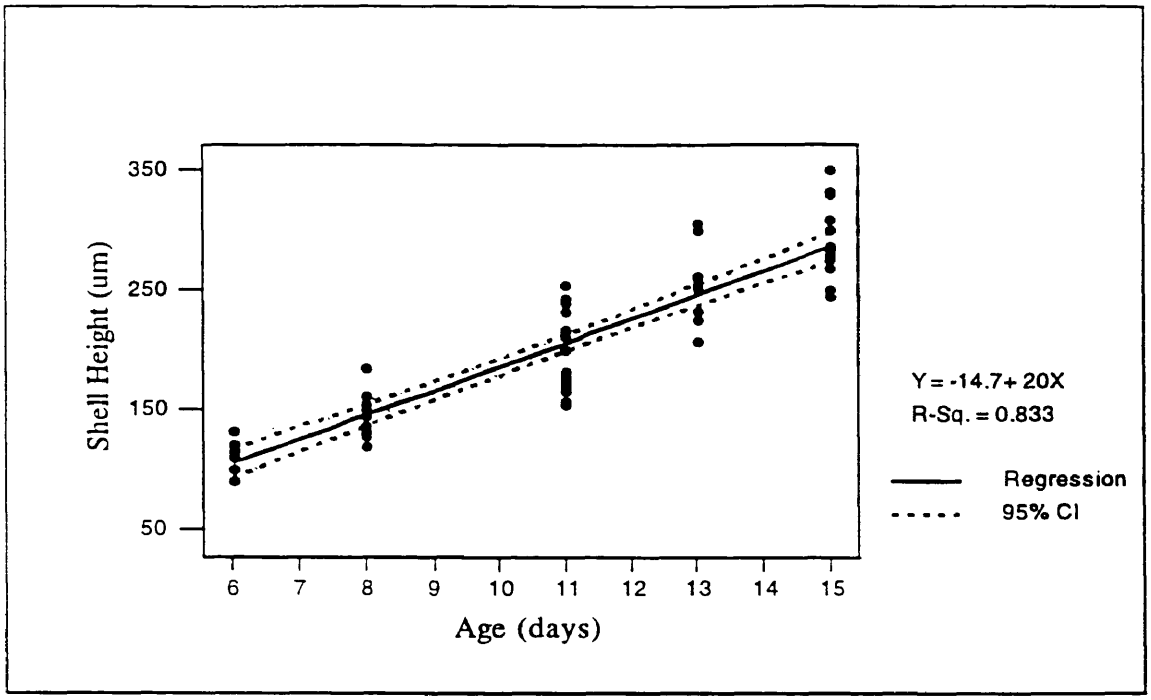

Shell Height (um)
350
250
150
50
6
7
8
9
10
11
12
13
14
15
Age (days)
Y = -14.7+ 20X
R-Sq. = 0.833
Regression
95% CI

Table 1: Linear regression analysis of *C. virginica* shell height on age for algal diet.

Regression equation:

Shell height = 43.9 + 13 age $R^2 = 86.3\%$

	Coefficient	95% lower	95% upper
Intercept	43.94	33.77	54.11
Age	12.97	11.97	13.96

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	5.22×10^5	5.22×10^5	668.39	0.00
Residual	106	8.27×10^4	780		
Total	107	6.04×10^5			

Table 2 : Linear regression analysis of *C. virginica* shell height on age for river diet.

Regression equation:

Shell height = 32.8 + 16 age. $R^2 = 87.1\%$

	Coefficient	95% lower	95% upper
Intercept	32.81	22.50	43.13
Age	16.21	15.13	17.29

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	7.51×10^5	7.51×10^5	884.29	0.00
Residual	131	1.11×10^5	849		
Total	132	8.62×10^5			

Table 3: Linear regression analysis of *C. virginica* shell height on age for algal upwelling diet.

Regression equation:

Shell Height = - 14.8 + 20 age $R^2 = 83.3\%$

	Coefficient	95% lower	95% upper
Intercept	-14.77	-39.16	9.63
Age	20.04	17.82	22.27

	DF	Sum Sq	Mean Sq.	F-value	P-value
Regression	1	2.56×10^5	2.56×10^5	323.58	0.00
Residual	65	5.14×10^4	790		
Total	66	3.07×10^4			

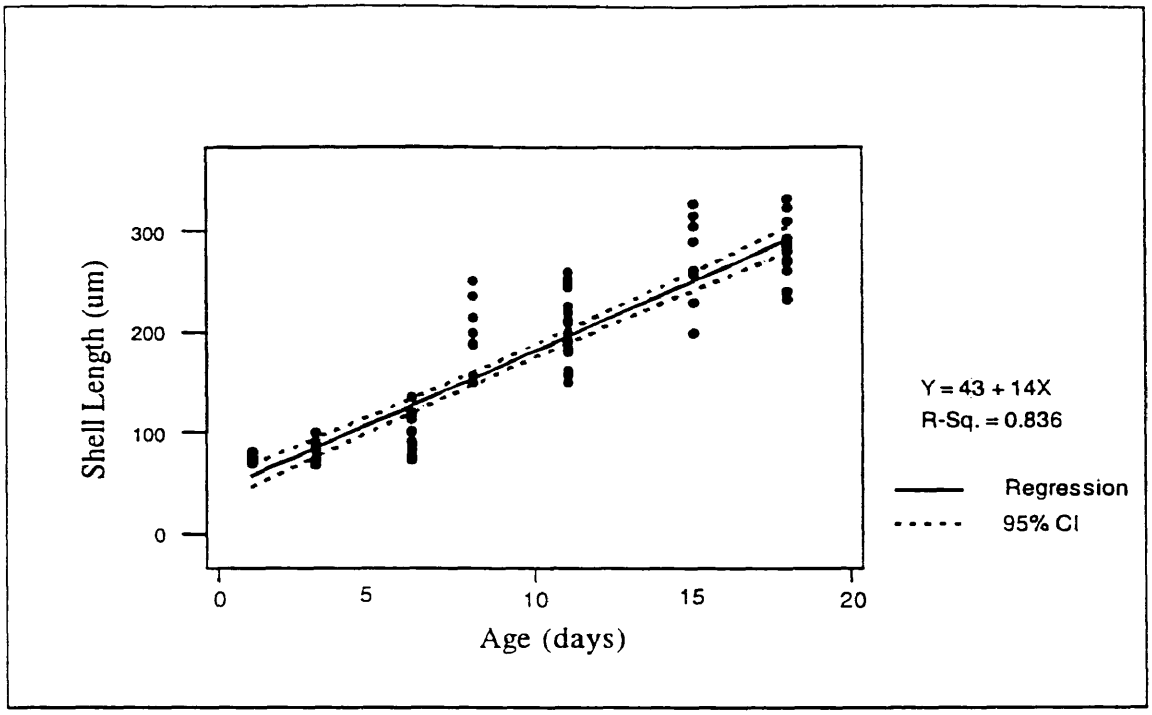
Figure 8: Linear regression fitted line plots for shell length (μm day^{-1}) against age (days) for different treatments. Hatched lines represent 95% confidence intervals.

a. Treatment 1: Hatchery algal diet.

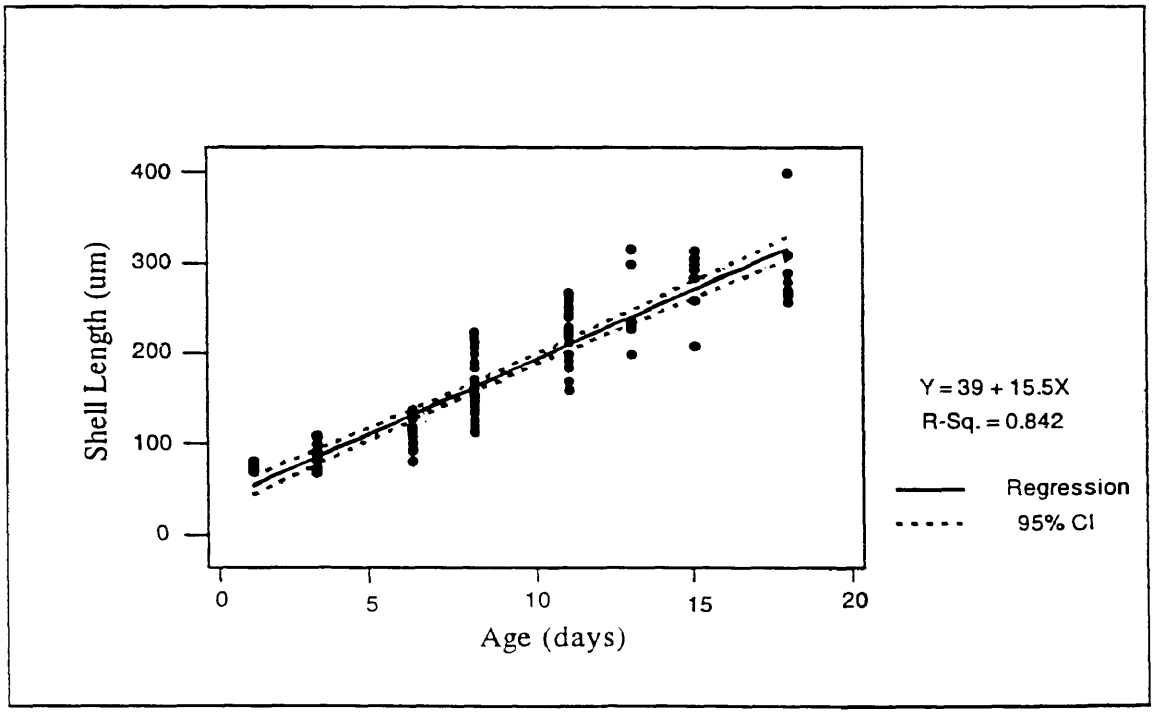
b. Treatment 2: Upwelling unit supplied with York River water.

c. Treatment 3: Upwelling unit supplied with hatchery algal diet.

a.


Shell Length (um)
300
200
100
0
0
5
10
15
20
Age (days)
Y = 43 + 14X
R-Sq. = 0.836
Regression
95% CI

b.


Shell Length (um)
400
300
200
100
0
0
5
10
15
20
Age (days)
Y = 39 + 15.5X
R-Sq. = 0.842
Regression
95% CI

c.

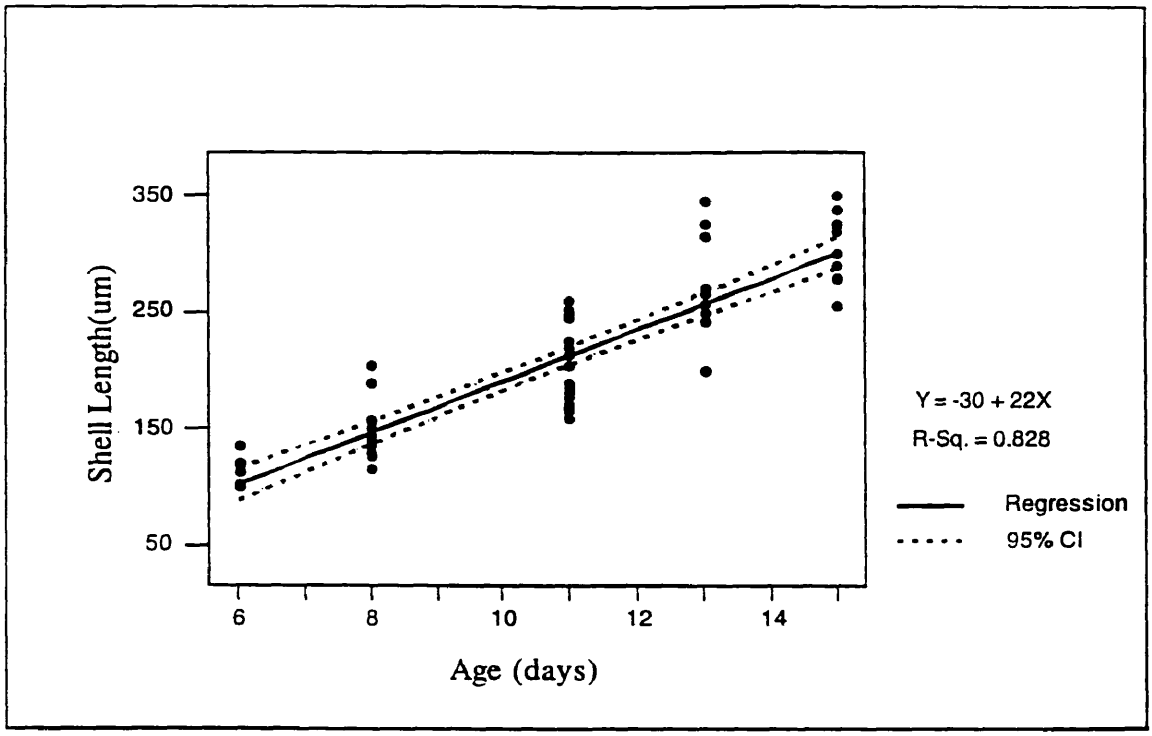

Shell Length(um)
350
250
150
50
6
8
10
12
14
Age (days)
Y = -30 + 22X
R-Sq. = 0.828
Regression
95% CI

Table 4: Linear regression analysis of *C. virginica* shell length on age for algal diet

Regression equation:

Shell length = 43.2 + 14 age $R^2 = 83.6\%$

	Coefficient	95% lower	95% upper
Intercept	43.16	30.95	55.37
Age	13.80	12.63	15.00

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	5.81×10^5	5.81×10^5	545.94	0.000
Residual	107	1.14×10^5	1.06×10^3		
Total	108	6.95×10^4			

Table 5 : Linear regression analysis of *C. virginica* shell length on age for river diet

Regression equation:

Shell length = 39.0 + 15 age R-sq = 84.2%

	Coefficient	95% lower	95% upper
Intercept	38.96	27.68	50.25
Age	15.44	14.26	16.61

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	6.49×10^5	6.49×10^5	676.69	0.000
Residual	127	1.22×10^5	960		
Total	128	7.71×10^5			

Table 6 : Linear regression analysis of *C. virginica* shell length on age for algal upwelling diet

Regression equation:

Shell Length = - 30.05 + 22.0 age $R^2 = 82.8\%$

	Coefficient	95% lower	95% upper
Intercept	-30.05	-57.334	-2.76
Age	22.04	19.55	24.53

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	309334	309334	312.91	0.000
Residual	65	64257	989		
Total	66	373591			

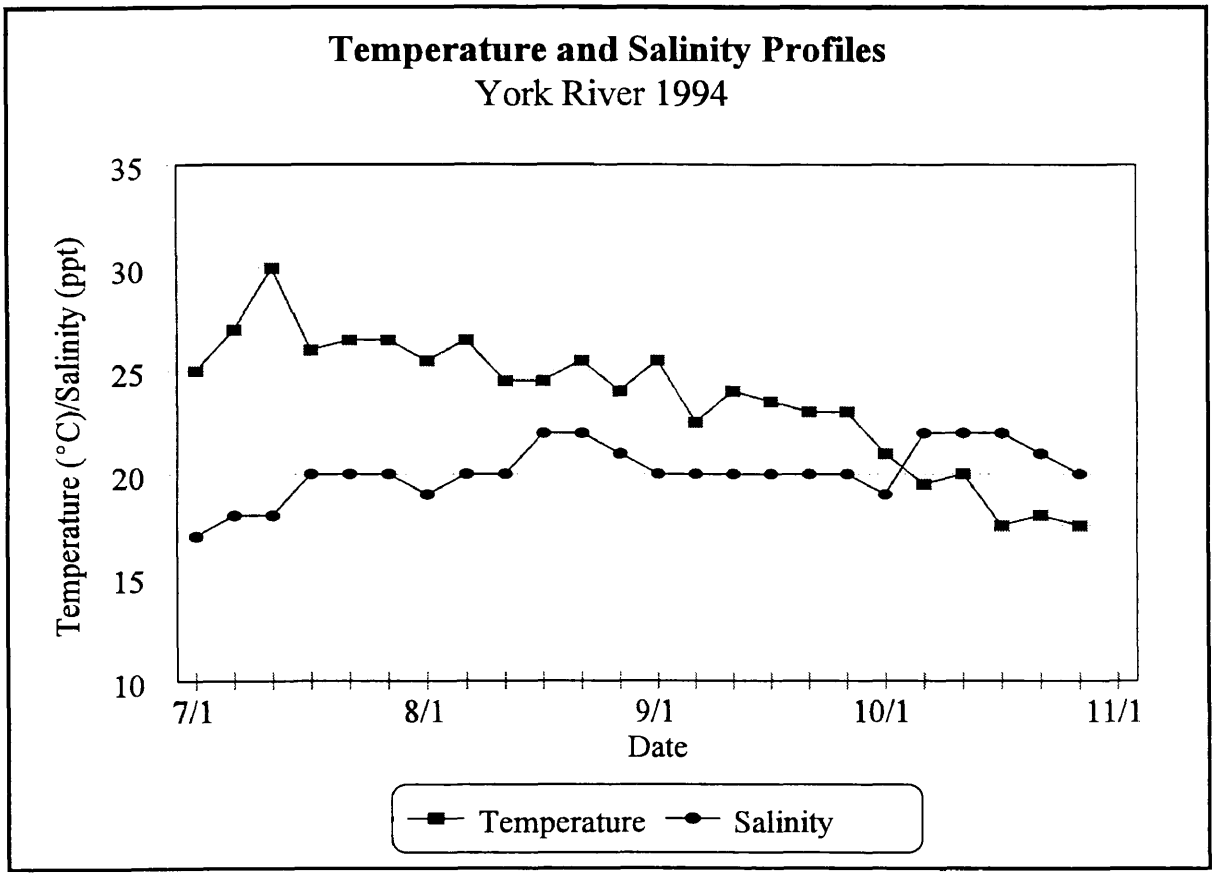
Table 7: ANCOVA of Age vs Shell Height for *C. virginica* for all diets

	Df.	Seq SS	Adj SS	Adj MS	F	P
Age	1	$6.23x10^5$	$6.4x10^5$	$6.4x10^5$	695.23	0.00
Diet	2	$2.65x10^4$	$2.30x10^4$	$1.15x10^4$	12.44	0.00
Diet*age	2	$3.19x10^4$	$3.19x10^4$	$1.60x10^4$	17.21	0.00
Error	202	$1.87x10^5$	$1.87x10^5$	927		
Total	207	$8.69x10^5$				

Table 8: ANCOVA of Age vs Shell Length for *C. virginica* for all diets

	Df.	Seq SS	Adj SS	Adj MS	F	P
Age	1	$7.26x10^5$	$7.57x10^5$	$7.57x10^5$	635.00	0.00
Diet	2	$1.17x10^4$	$2.54x10^4$	$1.27x10^4$	10.67	0.00
Diet*age	2	$3.29x10^4$	$3.29x10^4$	$1.65x10^4$	13.80	0.00
Error	205	$2.44x10^5$	$2.44x10^5$	$1.19x10^3$		
Total	210	$1.01x10^6$				

Figure 9: York River temperature and salinity data; July-November 1994.


Temperature and Salinity Profiles
York River 1994
Temperature (°C)/Salinity (ppt)
35
30
25
20
15
10
7/1
8/1
9/1
10/1
11/1
Date
Temperature
Salinity

Section 2.2: In situ culture of *Crassostrea virginica* larvae, using the SLIC mesocosm, on the Eastern Shore of Virginia.

The following section investigates the effect of natural river and mixed algal diets on growth and development of larvae of *C. virginica*. As well as static culture, a small scale mesocosm (SLIC) was used to investigate effects of nutrition *in situ* in the ambient environment. The primary differences between the mesocosm and the static culture are the flow fields and variation in water quality experienced by the larvae in the SLIC chamber as opposed to a static system, in which the water supply was changed once daily.

Treatments:

Sibling larvae at 7 days post-spawn were obtained from a commercial hatchery on the Eastern Shore of Virginia. A density of 3-4 larvae ml^{-1} were used in all treatments.

Treatment 1.

Mixed algal culture: Larvae were maintained in 1 μm filtered River water in 20 liter buckets and supplied with a mixed algal diet.

Treatment 2

Static river water culture: Larvae were maintained in 25 μm filtered river water in 20 liter buckets.

Treatment 3

Flow through river water culture: Larvae were maintained in the functional unit of the SLIC which was submerged in a 750 liter tank with filtered (25 μm) York River water.

Treatment 4

In situ culture: Larvae were maintained in the SLIC mesocosm, which was deployed in Willis Creek, Wachapreague.

To complete an orthogonal experimental design, the incubation chamber (E2) and the SLIC (E3) would need to be maintained in large tanks and supplied with algal diet. Due to constraints of apparatus and algal availbility, these treatments were not included. It was not essential to have full orthogonal design, since potential treatment artifacts are controlled for by pairwise comparisons of existing treatments.

Comparison of T1 and T2 determines the effect of diet only since all other conditions are the same. The comparison of T2 and T3 determines whether there is a treatment artifact from containing the larvae in the flow through system; the diet is the same, static river water which is changed daily. Comparing T3 and T4 investigates the difference between holding the larvae in a recirculating system with constant ambient conditions, and exposing them to the variation inherent in the open river.

Experimental Design

Dependant variables:	Shell height/length	
	Number of larvae competent to metamorphose.	
Independent variable:	Age in days	
Factor:	Enclosure type	Diet Type
Levels:	E1 = 20 liter bucket	D1 = Hatchery Diet
	E2 = Incubation Chamber	D2 = River Water
	E3 = SLIC	

	E1	E2	E3
D1	Treatment 1	No treatment	No treatment
D2	Treatment 2	Treatment 3	Treatment 4

Number of Spawns: 1 Number of larvae per sample: 20

Number of Replicates: T1, T2, T3:2; T4:1 Number competent to settle: Daily %

Materials and Methods:

Study Site

This experiment was carried out at the VIMS Wachapreague facility on the Eastern Shore of Virginia (fig. 5). The SLIC was deployed in Willis Creek, a tidal creek close to the laboratory. Water depth varied between 3m and 5m depending on tidal stage. The other treatments were housed in a shore based hatchery and water was pumped directly from the creek; high tide water was used since it was less turbid and more oxygenated than at low tide.

Oyster larvae were supplied by a commercial hatchery at Chincoteague, VA. They were obtained at age 7 days and had been cultured on a mixed algal diet.

Treatment 1

Larvae were kept in 1 μm filtered river water in 20 liter buckets and supplied with a diet of mixed algal cultures (Appendix 2b). This diet is used for the commercial rearing of *C. virginica* from spawn to settlement the culture system at the source hatchery in Chincoteague. The water was changed daily, because of the risk of bacterial infection in the high summer temperatures. Larvae were siphoned out of the containers and collected on Nitex screens; they were rinsed with 1μm filtered seawater and replaced in clean water.

Treatment 2

For this treatment, the 20 liter containers were supplied with filtered (25 μm) water which was pumped directly from Willis Creek. The prefiltering was intended to reduce the number of small planktonic competitors and potential predators but does not exclude the range of particles consumed by the oyster larvae. Ambient conditions and water changes were the same as Treatment 1.

Treatment 3

Larvae were placed in a 1 liter incubation chamber within a recirculation system. This is the functional unit of the mesocosm, without the electronic control of flow speed and back flushing. A submersible pump was connected to a rheostat to facilitate control of flow rate. A clear vinyl $^{3}/_{4}$" hose was fitted to one of the exhaust valves and water was pumped through the unit into a graduated beaker. A flow rate of 1 liter min^{-1} was selected (for each screen size used) and the rheostat set accordingly. Flow was periodically reversed manually (using opposing two-way valves), to flush the screens. This was done hourly (approximately) between 8am and 12 midnight, but was left overnight. A cartridge filter, fitted with a 90 μm Nitex screen sock, was placed between the pump and the valves. The recirculation unit was placed in a 750 liter tank and was filled with 25 μm filtered river water. The prefiltering of the water rendered the in-line filter somewhat redundant; however, to facilitate comparison with treatment 4, the configuration of the functional unit was kept as similar to the SLIC as possible. The water was filtered to 25μm to validate comparison with treatment 2 in order to evaluate effects of retaining larvae in the incubation chamber.

The water from the large tank was drained daily, then refilled with water collected at

high tide and recirculated for 24 hours. Every two days, the larval chambers were detached and cleaned. After removing a subsample, the larvae were replaced in the chamber.

Treatment 4

Larvae were placed in the Submersible Larval Incubation Chamber , which allowed larvae to be place *in situ* in Wllis Creek. This treatment was exposed to the ambient environment. Detailed information on the SLIC can be found in the Technical Report, Appendix 1.

A flow rate of 1 liter min^{-1} (for each screen size used) was selected. To calibrate the flow, a 3/4" hose was fitted to one of the exhaust valves, water was pumped through the unit into a graduated beaker and the potentiometer (which controls the pump voltage) was set accordingly. The SLIC has a flow reversal system to back flush the screens of the chamber and reduce fouling. Various time intervals were tested; the shorter intervals were marginally better than the longer ones at keeping the screens clean; too short an interval (<3 minutes) greatly increased turbulence in the chamber. An interval of 1 hour was elected for continuous operation. In a further attempt to reduce fouling, a Nitex screen sock, of 90μm mesh size, was placed on the cartridge of the in-line filter.

The internal plumbing was supported by 1" PVC framework. Small holes were drilled in the frame to allow water to flood in allow it to sink. The SLIC was deployed in 3 meters of water in Willis Creek, Wachapreague. For the duration of the experiment a barge was tethered to the dock in Willis creek; the apparatus was maneuvered in and out of the water from the barge platform with an electric winch and boom. Every two days, the larval chamber was detached, cleaned and, after removing a subsample of 20, the larvae were replaced.

Growth Rate Measurements

Shell height (dorso-ventral) and length (anterior-posterior) were measured using a Zeiss IM 35 inverted microscope fitted with an ocular micrometer, which measures to 1 μm resolution. Shell height is the distance from the hinge to the opposite side of the valve. Length is perpendicular to height and is usually a measure of the widest span of the shell. These larvae were then stained for qualitative examination of lipid content.

Examination of Lipid Content

The larvae were stained with a lipid stain (Oil Red O) observed under a light microscope. The protocol is according to Gallager and Mann (1981)

To prepare the stain, 0.75g Oil Red O was dissolved in in 100 ml Ethylene glycol and heated to ~60°C. The hot solution was filtered through Whatman No. 2 paper and refrigerated. On cooling it was re-filtered one more. To stain the larvae, the sample was placed in a vial and 5 drops of formalin were added. The larvae were alowed to settle and the liquid was removed with a pipette, leaving the larvae in the bottom of the vial. One ml of Oil red O was added and left for a minimum of 1 hour. The stain was then removed with a pipette and 1ml of ethylene glycol was added and left standing for a minimum of 30 minutes to clear the excess stain from the larval tissue. The discoloured liquid was removed and replaced with new ethylene glycol. Clearing was complete when excess stain ceased to color the medium.

The stained larvae were drawn into an optically flattened capillary tube and examined under x10 magnification using a Zeiss IM 35 inverted microscope fitted with an ocular micrometer. The larvae were examined and the degree of staining evaluated qualitatively.

Determination of Time to Competence and Settlement

As oyster larvae progress towards metamorphosis, an 'eye' or pigmented spot develops, which is visible through the shell. In the absence of successful settlement, this pigmentation cue can be used as a surrogate indication of developmental progress. The developmental stage of the larvae in each treatment was noted and the percentage of larvae with dark spots was recorded; this 'eye' begins to show at 180-200μm shell length. This is an easily recognizable physiological indicator, but behavioural changes such as swimming close to the benthos and probing the substrate, confirm the onset of competence to settle. Metamorphosis occurs at approximately 300 μm shell length.

Oyster larvae have been shown to respond to cues from bacterial films found on shells of established oysters (Fitt et al, 1990; Tritar et al, 1992). To increase settlement in the hatchery, the settlement substrate of ground, cleaned, oyster shell is soaked in broodstock tanks (with mature adults) for 24 hours prior to exposure to the competent pediveligers.

A settlement substrate was introduced into the larval chambers in treatments 3 and 4 and into the tanks in treatments 1 and 2. Ground oyster shell would have been impractical to evaluate, therefore porcelain squares of 2.5cm x 2.5cm were used as an optimal substrate. Every two days, the substrate was removed, the number of settled animals counted, then replaced with new substrate. This continued until no more settlement was observed. Replacing the tiles with new substrate avoided the problem of build up of bacteria which may have affected the intensity of settlement cues and recounting of spat. Since time to peak settlement was of interest, rather than actual numbers set, differences in absolute numbers between the treatments did not interfere with the analysis.

Water Quality Measurements:

Temperature and salinity were recorded daily in the river for treatment 4. For treatments 1, 2 and 3, temperature and salinity were recorded before each water change. To ensure there was no more than a 2^0C difference in temperature, the clean collected water would be left to stand in trash cans in the cold room, until the temperature difference was within acceptable limits. The river temperature was usually higher than the hatchery.

As previously discussed (Chapter 2: Section 2.1), Oxygen levels should not be limiting in this experiment. To verify that O_2 did not fall critically low, measurements were taken in each of the treatments just before daily water change. measurements were taken with a YSI model oxygen probe with incorporated temperature and salinity meter.

Results: *Crassostrea virginica* 1995

Growth rates

Growth curves were plotted for all treatments together for both shell height and length (fig. 10a, b); there is a definate difference in growth rate between those larvae cultured on natural river water and those cultured on hatchery diet.

Regression analysis was performed on all the data sets and is summarised in tables 9-12 for shell height and tables 13-16 for shell length. For all treatments, the H_0 (slope of the line=0) was rejected (α=0.05); the predictor (age) therefore has a significant effect on the response (shell dimension), as expected for a growth curve (figs. 11a-d and 12a-d). The mesocosm data is insufficient to determine any growth response to diet; however the data serves to prove that *C. virginica* larvae can survive and grow in mesocosm conditions.

The regression analysis was performed on all the data sets, but the ANCOVA was applied to the river and algal diets only. For the chamber and SLIC treatments, the retrieval of larvae at each subsampling was very low (1-2%), so after the second water change only 1 or 2 larvae were retrieved form the original 4000. The same larvae must be exposed to a treatment for several days to determine the effect of the treatment on growth and development, and there was insufficient data to create a statisitcally valid data set for the Chamber and SLIC treatments.

Tables 17 and 18 summarise the ANCOVA's for shell height and length against age for the river and algal diets. There was a significant difference found between the different treatments, but also a significant interaction effect between age and shell dimension, therefore the ANCOVA, cannot be used to compare the regression curves of different treatments. The

95% confidence intervals for the regression lines did not overlap, and the growth curves for algal and natural river diets were considered significantly different.

Lipid Content

The larvae were examined and photographed to investigate qualitative differences in lipid content between different diets. Those larvae maintained in the static cultures were generally well fed with a dark red gut, but due to the variation within the samples it was not possible to establish whether one diet was preferable to the other. The larvae from the chamber and SLIC treatments also appeared well fed but in some cases, the shells were quite heavily infected with epiphytes and did not appear as healthy as those in the static culture. Figure 13 (reproduced with permission from Gallager and Mann, 1981) shows two sets of larvae, the upper group (13a) are well fed; the lower (13b) are starved; in this situation the difference in colour intensity is apparent, but the data from this experiment did not show such an obvious difference in lipid content.

Determination of Time to Competence

Competence was determined by the presence of an "eyespot". Eyespots were first observed at age 12 days and the number of larvae exhibiting this pigmentation, was counted for each the static algal and river cultures until the experiment was terminated. The results were plotted (fig. 14) and tabulated (table 19) A students t-test (two-tailed) was used to compare the development rates of larvae under different feeding regimes. Results are shown in table 20; a significant difference was found ($t=-3.30$, $p=0.016$) between the two treatments.

The only competence data set recorded for the larvae in the mesocosms, was from treatment 3 at age 14 days. The percentage larvae showing "eyespots" was determined as 87% ($^{34}/_{39}$ x100). The larvae had been in the chamber for three days only. Since so few mesocosm larvae were retrieved at each water change, a time series was not available to establish treatment effects.

Water quality

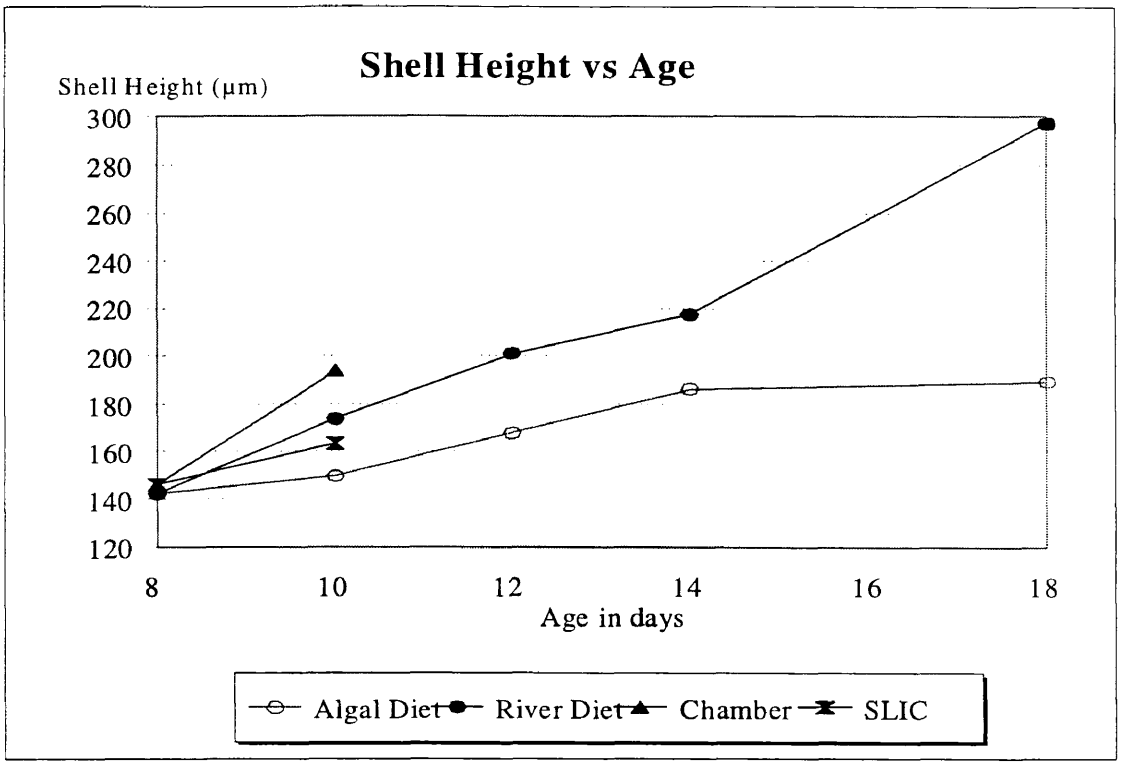
The experiment was conducted during the months of July and August, which are among the warmest of the summer. The river temperature reached as high as 32°C for several days consecutively (fig.15) At such elevated temperatures, the larvae are temperature-stressed and bacterial populations thrive, neither condition is conducive to optimal growth.

Figure 10: Growth curves for *Crassostrea virginica* larvae.

a. Shell height (μm day^{-1}) against age for all treatments

b. Shell length (μm day^{-1}) against age for all treatments

a.


Shell Height vs Age
Shell Height (µm)
300
280
260
240
220
200
180
160
140
120
8
10
12
14
16
18
Age in days
Algal Diet
River Diet
Chamber
SLIC

b.

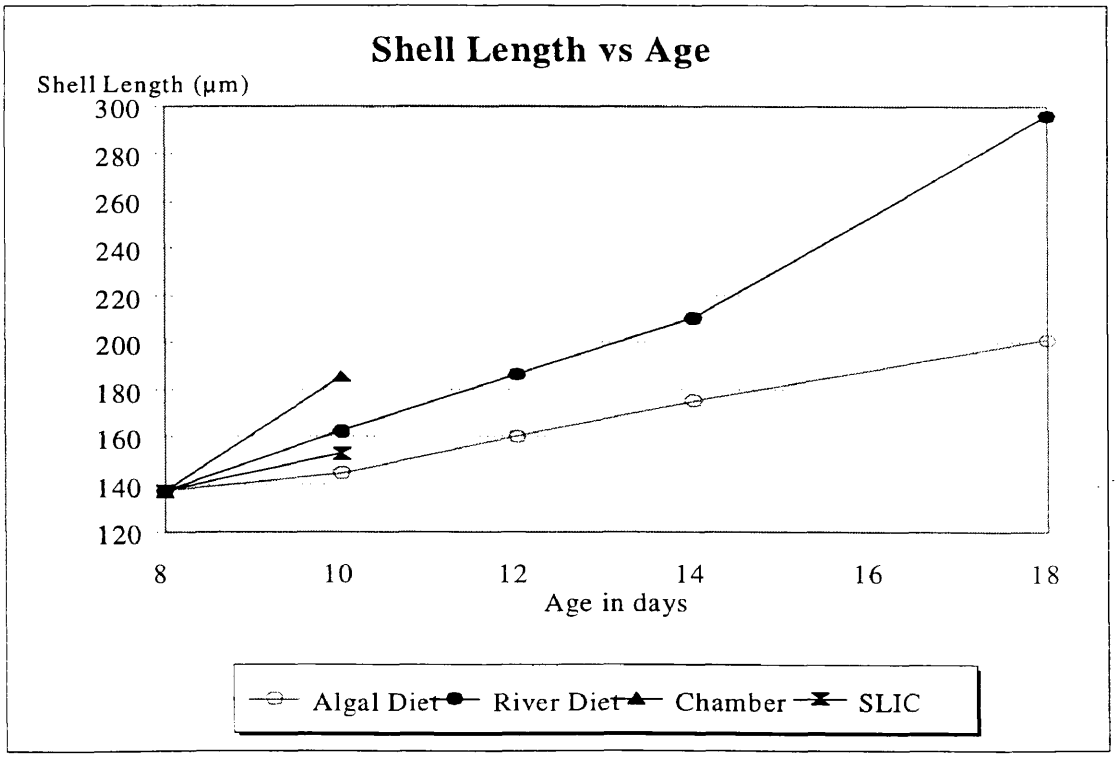

Shell Length vs Age
Shell Length (µm)
300
280
260
240
220
200
180
160
140
120
8
10
12
14
16
18
Age in days
Algal Diet
River Diet
Chamber
SLIC

Figure 11: Linear regression fitted line plots for shell height (μm day^{-1}) against age (days) for different treatments. Hatched lines represent 95% confidence intervals.

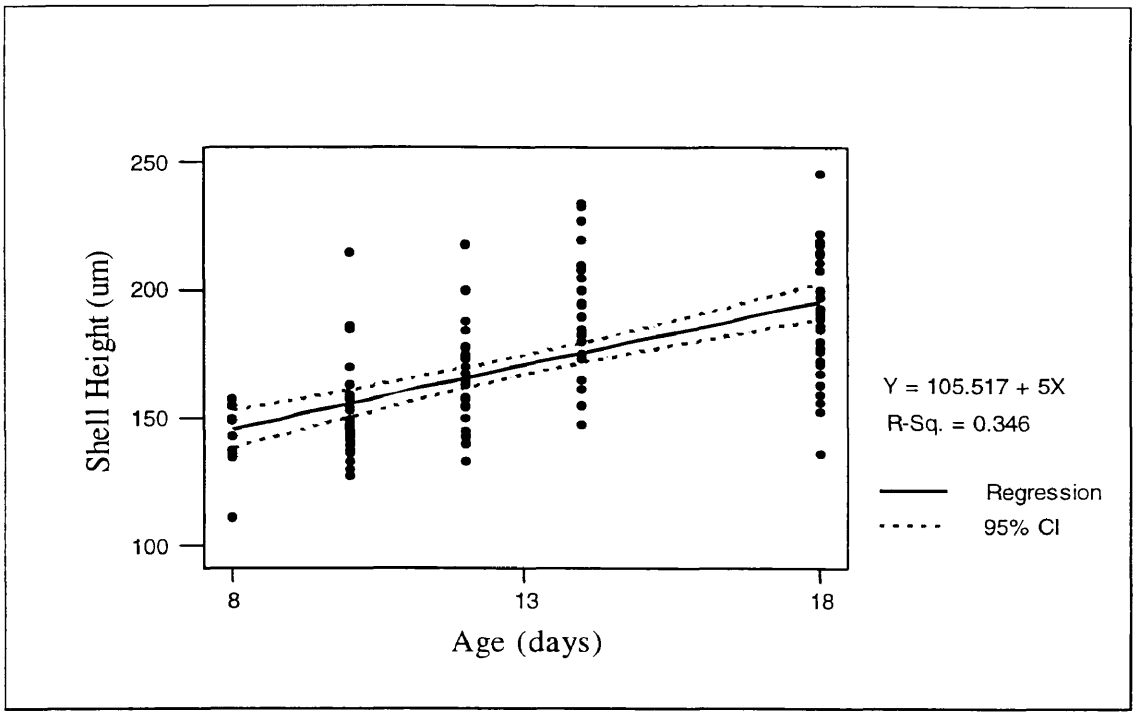
a. Treatment 1: Algal diet in buckets.

b. Treatment 2: River water in buckets.

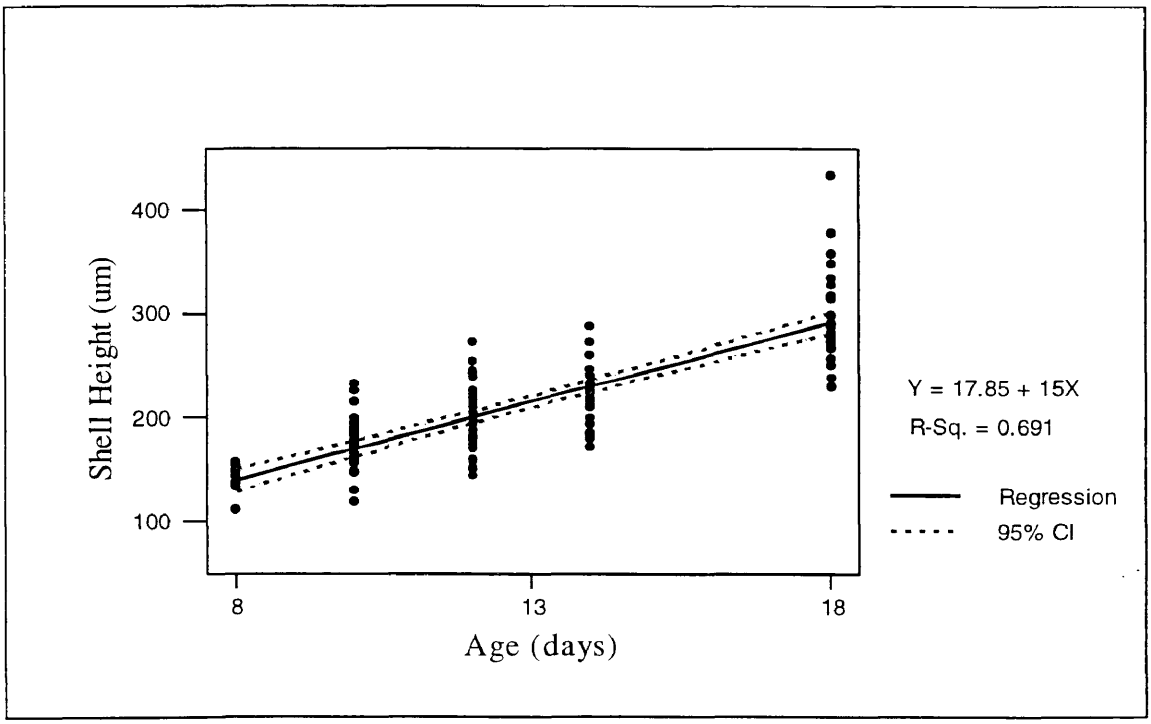
c. Treatment 3: Chamber treatment

d. Treatment 4: SLIC *in situ* in Willis Creek. .

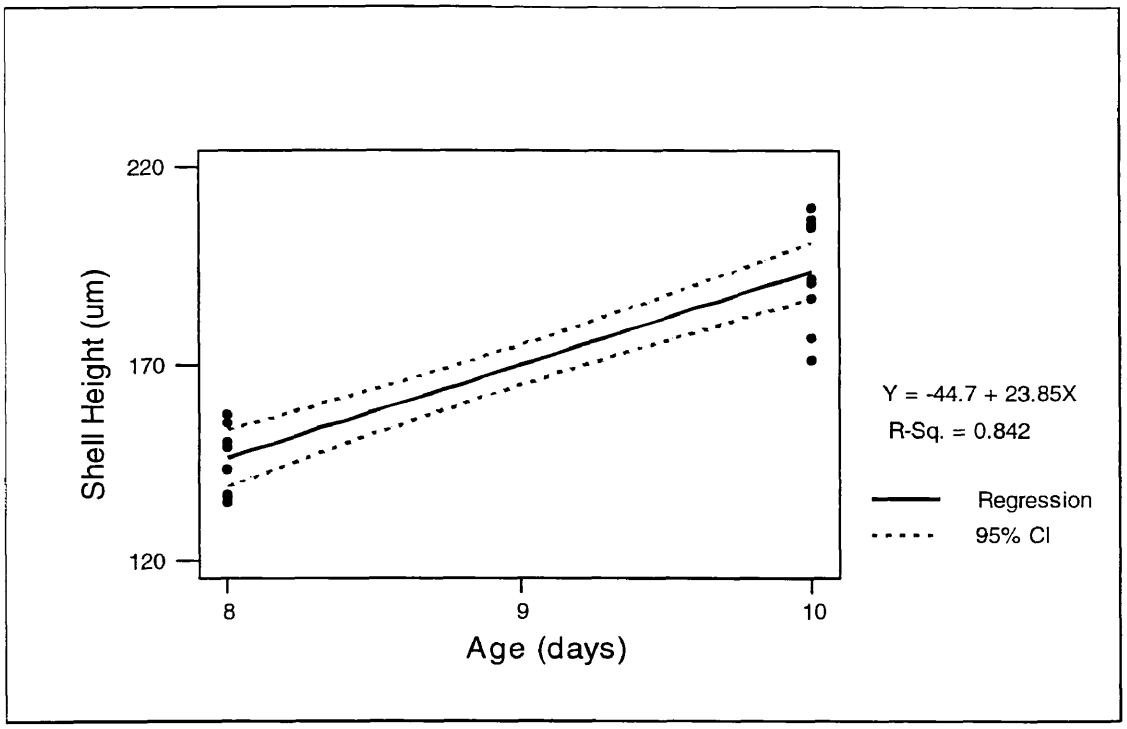
a.


Shell Height (um)
250
200
150
100
8
13
18
Age (days)
Y = 105.517 + 5X
R-Sq. = 0.346
Regression
95% CI

b.


Shell Height (um)
400
300
200
100
8
13
18
Age (days)
Y = 17.85 + 15X
R-Sq. = 0.691
Regression
95% CI

c.


220
170
120
Shell Height (um)
8
9
10
Age (days)
Y = -44.7 + 23.85X
R-Sq. = 0.842
Regression
95% CI

d.

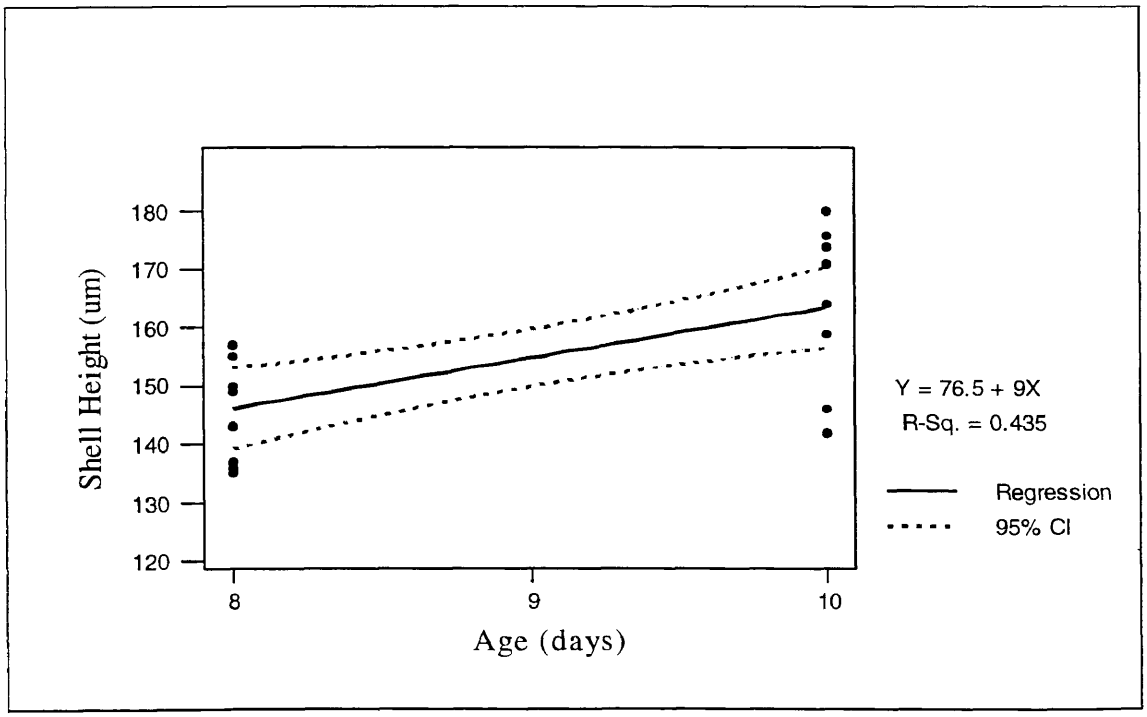

180
170
160
150
140
130
120
Shell Height (um)
8
9
10
Age (days)
Y = 76.5 + 9X
R-Sq. = 0.435
Regression
95% CI

Table 9: Linear regression analysis of *C. virginica* shell height on age for algal diet

Regression equation:

Shell height = 105.5 + 5 age $R^2 = 34.6\%$

	Coefficient	95% lower	95% upper
Intercept	105.52	89.33	121.70
Age	5.00	3.80	6.20

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	3.32×10^4	3.32×10^4	67.70	0
Residual	128	6.28×10^4	491		
Total	129	9.61×10^4			

Table 10: Linear regression analysis of *C. virginica* shell height on age for river diet

Regression equation:

Shell height = 17.9 + 15.2 age $R^2 = 69.1\%$

	Coefficient	95% lower	95% upper
Intercept	17.85	-6.03	41.73
Age	15.18	13.40	16.95

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	3.06×10^5	3.06×10^4	286.58	0
Residual	128	1.37×10^5	1.37×10^5		
Total	129	4.43×10^5			

Table 11: Linear regression analysis of *C. virginica* shell height on age in chamber

Regression equation:

Shell height = - 44.7 + 23.8 age R^2 = 84.2%

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	$1.14x10^4$	$1.14x10^4$	95.85	0
Residual	18	$2.14x10^3$	119		
Total	19	$1.35x10^4$			

Table 12: Linear regression analysis of *C. virginica* shell height on age in SLIC

Regression equation:

Shell height = 76.5 + 8.7 age R^2 = 43.5%

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	$1.5x10^3$	$1.5x10^3$	13.85	0.002
Residual	18	$1.97x10^3$	109.3		
Total	19	$3.48x10^3$			

Figure 12: Linear regression fitted line plots for shell length (μm day^{-1}) against age (days) for different treatments. Hatched lines represent 95% confidence intervals.

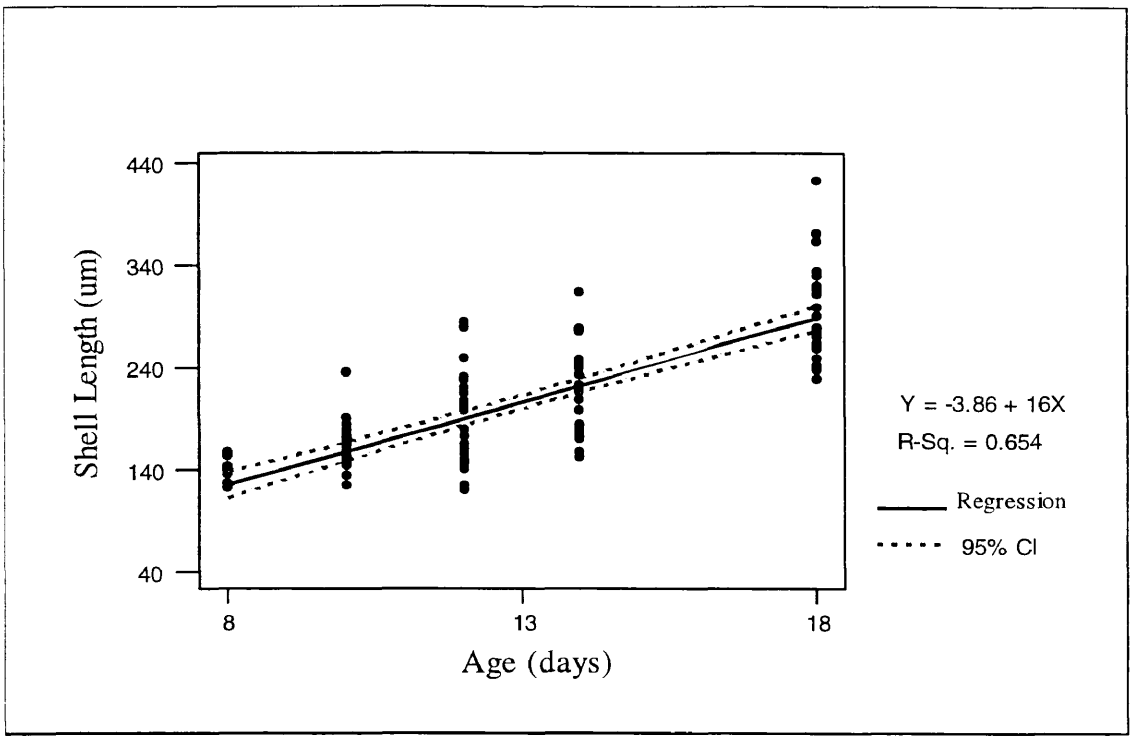
a. Treatment 1: Algal diet in buckets.

b. Treatment 2: River water in buckets.

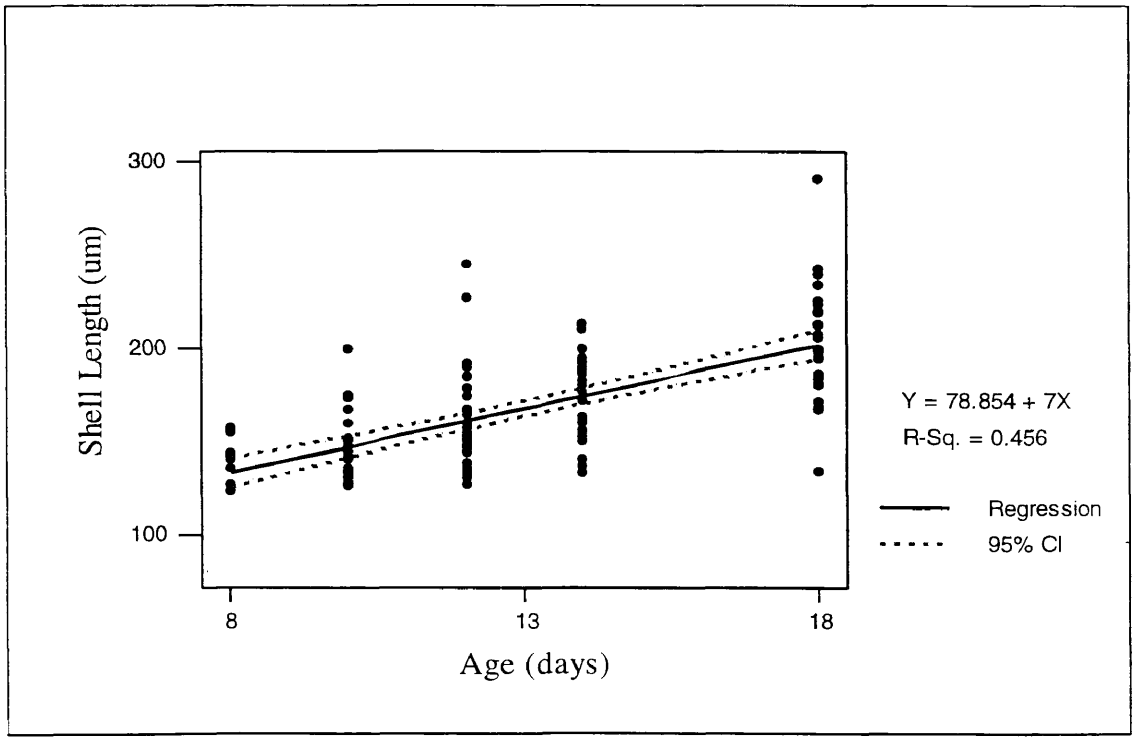
c. Treatment 3: Chamber treatment

d. Treatment 4: SLIC *in situ* in Willis Creek. .

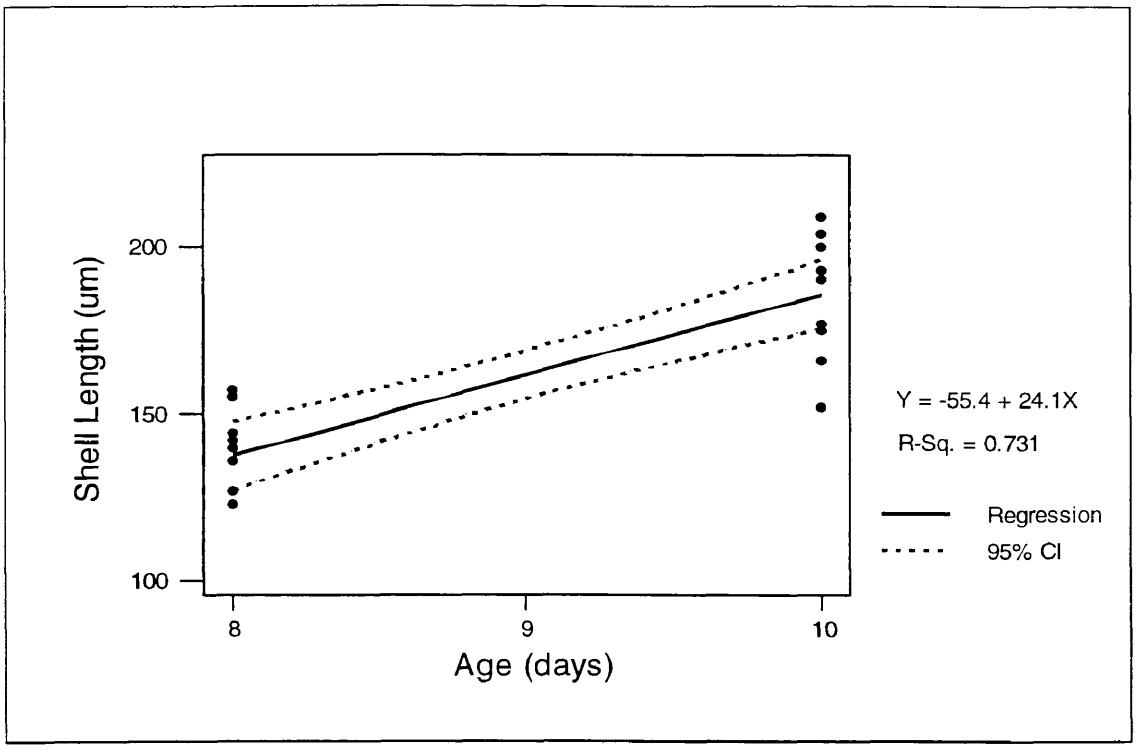
a.


Shell Length (um)
440
340
240
140
40
8
13
18
Age (days)
Y = -3.86 + 16X
R-Sq. = 0.654
Regression
95% CI

b.


Shell Length (um)
300
200
100
8
13
18
Age (days)
Y = 78.854 + 7X
R-Sq. = 0.456
Regression
95% CI

c.


Shell Length (um)
200
150
100
8
9
10
Age (days)
Y = -55.4 + 24.1X
R-Sq. = 0.731
Regression
95% CI

d.

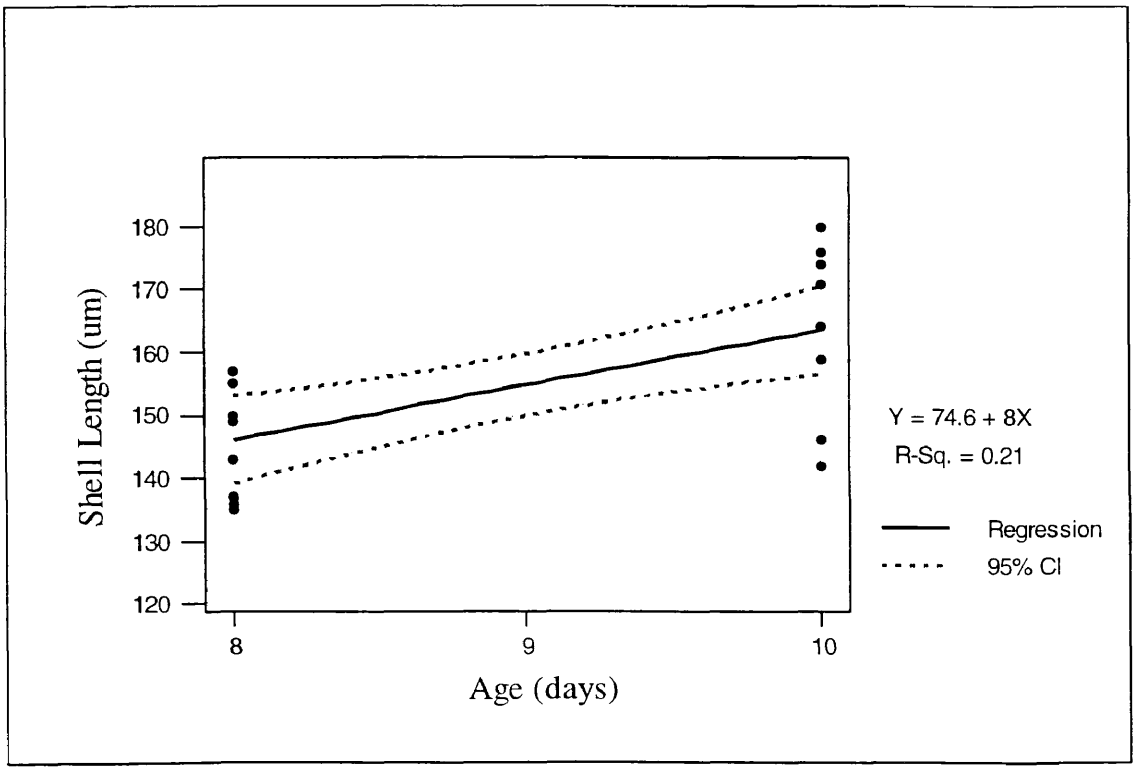

Shell Length (um)
180
170
160
150
140
130
120
8
9
10
Age (days)
Y = 74.6 + 8X
R-Sq. = 0.21
Regression
95% CI

Table 13: Linear regression analysis of *C. virginica* shell length on age for algal diet

Regression equation:

Shell length = 78.9 + 6.8 age R^2 = 45.6%

	Coefficient	95% lower	95% upper
Intercept	78.85	61.35	96.36
Age	6.81	5.51	8.11

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	6.16×10^4	6.16×10^4	107.24	0.00
Residual	128	7.35×10^5	574		
Total	129	1.35×10^5			

Table 14 : Linear regression analysis of *C. virginica* shell length on age for river diet

Regression equation:

Shell length = -3.9 + 16.2 age R^2 = 65.4%

	Coefficient	95% lower	95% upper
Intercept	-3.87	-31.64	23.90
Age	16.20	14.14	18.26

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	3.49×10^5	3.49×10^5	241.48	0.00
Residual	128	1.85×10^5	1.45×10^3		
Total	129	5.34×10^5			

Table 15: Linear regression analysis of *C. virginica* shell length in chamber.

Regression equation:

Shell length = - 55.4 + 24 age $R^2 = 73.1\%$

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	$1.16x10^4$	$1.16x10^4$	48.80	0.00
Residual	18	$4.29x10^3$	238		
Total	19	$1.59x10^4$			

Table 16: Linear regression analysis of *C. virginica* shell length in SLIC.

Regression equation:

Shell length = 74.6 + 8 age R-sq = 20.1%

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	$1.23x10^3$	$1.23x10^3$	4.53	0.047
Residual	18	$4.90x10^3$	272.0		
Total	19	$6.13x10^3$			

Table 17: ANCOVA of Age vs Shell Height for *C virginica* for all Diets

	Df.	Seq SS	Adj SS	Adj MS	F	P
Diet	3	$1.70x10^5$	$3.10x10^4$	$1.03x10^4$	14.80	0.00
Age	1	$2.80x10^5$	$2.74x10^4$	$2.74x10^4$	39.27	0.00
Diet*age	3	$7.24x10^4$	$7.26x10^4$	$2.42x10^4$	34.71	0.00
Error	292	$2.04x10^5$	$2.04x10^5$	697		
Total	299	$7.26x10^5$				

Table 18: ANCOVA of Age vs Shell Length for *C. virginica* for all diets

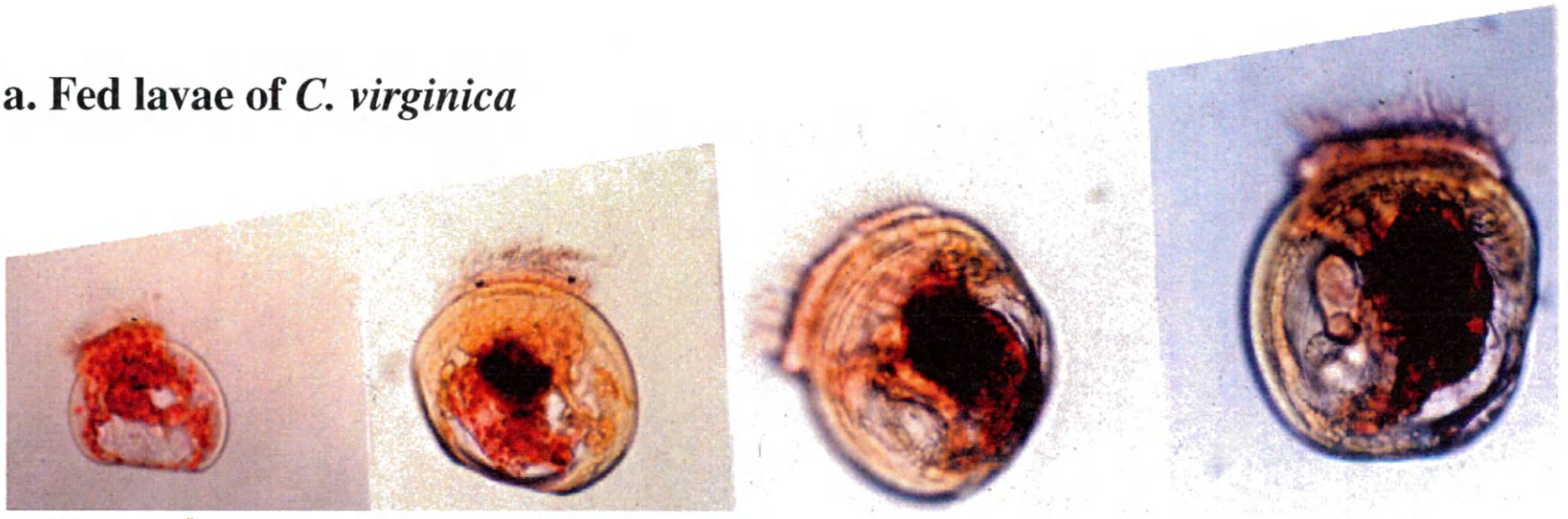
	Df.	Seq SS	Adj SS	Adj MS	F	P
Diet	3	3.49	0.41	0.14	6.05	0.00
Age	1	9.05	0.99	0.99	44.26	0.00
Diet*age	3	0.99	0.99	0.33	14.93	0.00
Error	292	6.52	6.52	0.02		
Total	299	20.05				

Figure 13: Larvae of *Crassostrea virginica* showing the development stages from straight hinge to pediveliger. The red coloration is due to a lipid specific stain (Oil Red O); the degree of color reflects the lipid content of the larvae.

a. Series of well fed, darkly stained larvae.

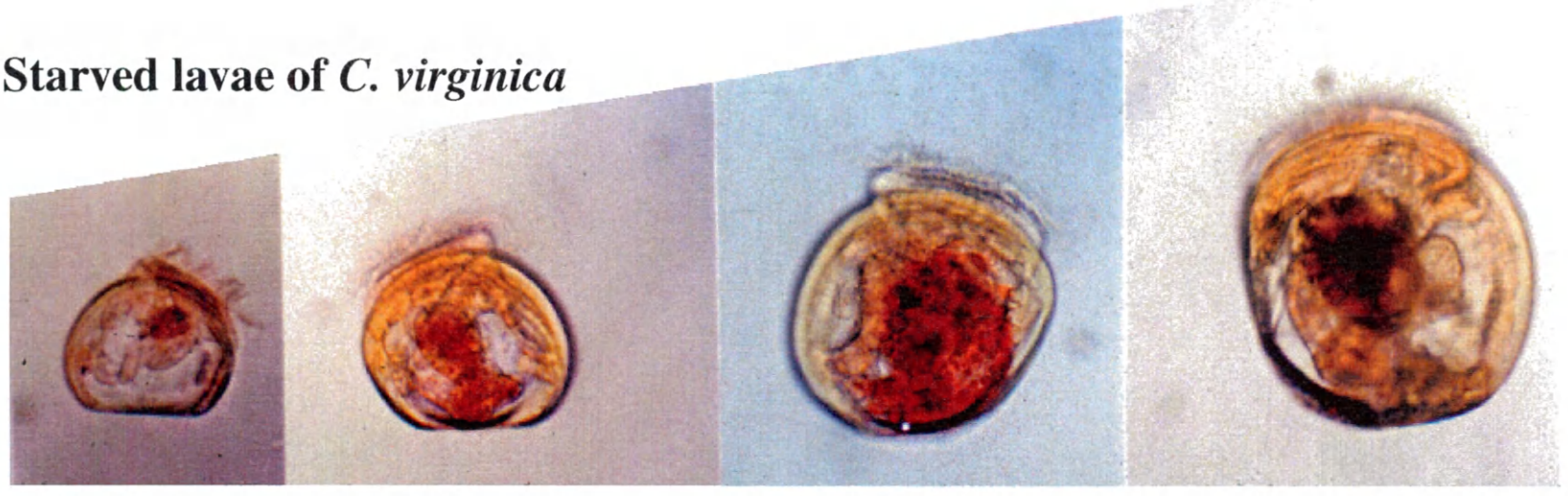
b. Series of starved, lightly stained larvae.

a. Fed lavae of *C. virginica*



Straight hinge | Early umbo | Late umbo | Pediveliger

b. Starved lavae of *C. virginica*



Straight hinge | Early umbo | Late umbo | Pediveliger

Figure 14: Plot of percent larvae competent to settle against age for river and algal diets

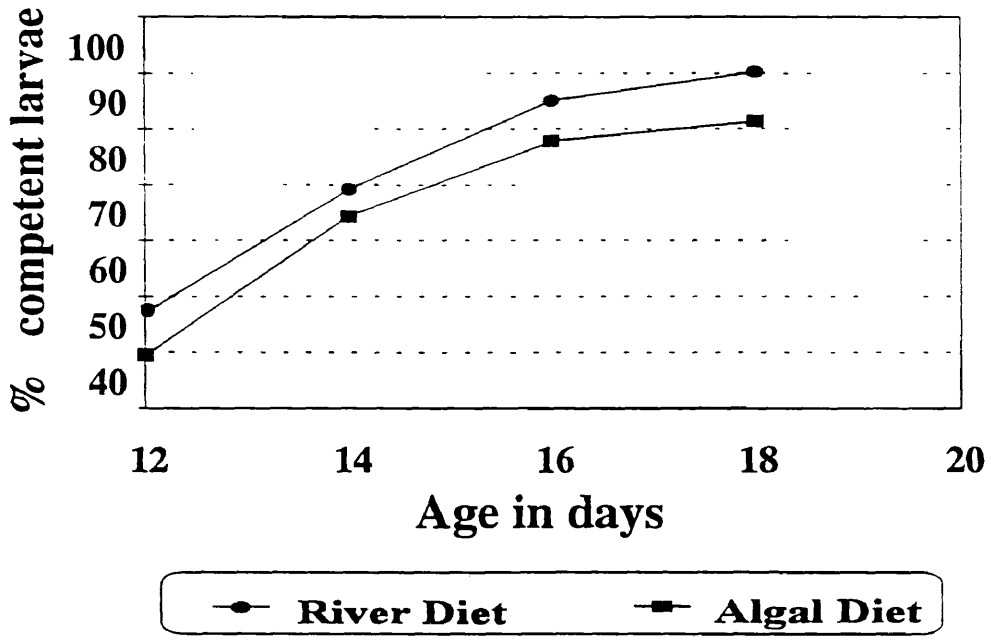

% competent larvae
100
90
80
70
60
50
40
12
14
16
18
20
Age in days
River Diet
Algal Diet

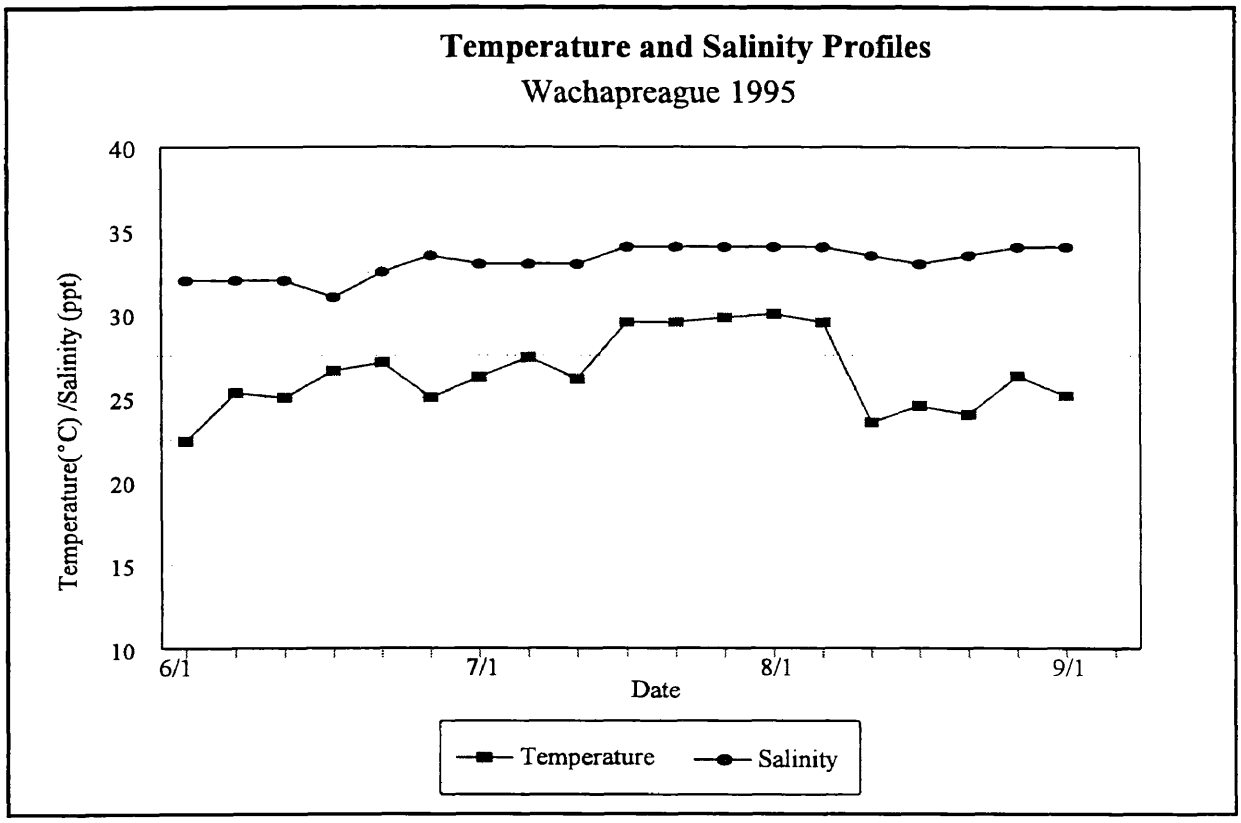
Table 19: *C. virginica*: Percentage of eyed larvae in algal and river culture. Numbers are means for two replicates of n>50 per replicate.

Age in days	Algal Culture	River Culture
12	39.60 (σ = 0.00)	47.06 (σ = 0.00)
14	64.38 (σ = 4.38)	69.22 (σ = 4.19)
16	77.85 (σ = 0.55)	85.15 (σ = 1.25)
18	81.50 (σ = 1.00)	90.38 (σ = 0.63)

Table 20: Two tailed t-test to compare % competent larvae from each treatment

	Algal Culture	**River Culture**
Mean	69.58	76.65
Variance	235.80	259.61
Observations	7	7
t value	-3.30	
t critical (T)	2.45	
P(T≤t)	0.016	

Figure 15: Temperature and salinity profiles for 1995, taken from Willis Creek Wachapreague, VA


Temperature and Salinity Profiles
Wachapreague 1995
Temperature(°C) /Salinity (ppt)
40
35
30
25
20
15
10
6/1
7/1
8/1
9/1
Date
Temperature
Salinity

Discussion

Section 2.1: 1994 Pilot Project

The control treatment for the upwelling unit was discontinued due to malfunction of the system, therefore there is no available information regarding treatment artifact of the upwelling system. If such an artifact had been operating, it would probably have been to the detriment of the larvae in the upwelling system since the screen base of the unit became blocked very quickly and would have excluded some potential food items. The filling of the unit with river water, (despite filtering) also introduced other filter feeding microzooplankton competitors, primarily copepodites.

Although there was no significant difference between the growth rates for the different treatments, the mean size of larvae cultured in river water was higher than the algal treatment; the data therefore indicates that there may be an advantage to culture on natural diet over the mixed algal diet used in the hatchery.

Section 2.2: 1995 Main Study

The data from the static treatments indicate a significant advantage to culturing *C. virginica* larvae on natural river water over culture on a mixed algal diet. As reviewed in the general introduction, marine invertebrate larvae require certain essential components that cannot usually be supplied adequately by a single food type; for example, essential free fatty acids. It is possible that, although the cultured algae can fulfill larval energetic requirements, variability in the natural river diet provided micronutrients required for optimal development. The biochemical composition of the two diets was not analyzed and the reason for the

observed difference in growth and development remains speculative.

Those larvae cultured on algae in this investigation showed growth rates that were lower than described in the literature for mixed algal diets. Larvae of *C. virginica* reached 230µm by age 10 days (Chu and Webb, 1984) when cultured on a mixture of *I. galbana* and *Pavlova (monochrysis) lutheri*. Different aquaculture techniques differ in the suite of cells they use, but many of them include a strain of the small flagellate *Isochrysis spp.* Larval food requirements for optimal growth have been investigated using laboratory culture. There is however, limited information on larval nutritional requirements and the apparently high levels of phytoplankton cells needed for optimal growth, may simply be an artifact of nutritionally sub optimal diets.

In calculations of food available in the field the standing stock is evaluated; however, this gives only a 'snapshot' of the full picture. The are many grazing organisms in the water column that are continuously removing the phytoplankton; microzooplankton (such as invertebrate filter feeding larvae) represent 40% of the total planktonic particulate matter and can remove 70% of the daily organic carbon produced by phytoplankton (Rassoulzadegan and Fenaux, 1979). There may be more algal food items accessible to the larvae than is represented by standing stock chlorophyll counts. Phytoplankton peak production occurs during the day, but many grazers rise to the upper part of the water column at night to feed, producing a diurnal variation in the phytoplankton levels.

The results of the ANCOVA's indicated the presence of an age dependent effect of diet. Previous studies on larval nutrition (His and Mauer, 1988; Chu and Webb, 1984) have determined that the nutritional requirements of larvae change with age and developmental

stage, therefore one diet may be more advantageous than the other at different stages of the larval development. The nutritional mode of larvae also changes with age (His and Mauer, 1988) Initially, the food is derived from endogenous reserves (endotrophy), then by a combination of vitelline reserves and exogenous food items (mixotrophy) and finally, as all the endogenous reserves have been used, to complete reliance on exogenous food (exotrophy) in the water.

In a comparison of effects of natural and experimental algal diets, His and Mauer (1988) followed a single cohort of *C. gigas* larvae in the Bay of Arcachon in France; every two days larvae were subsampled and shell length and dry weight were measured. They compared the data with larvae of the same age cultured on an algal diet in the laboratory. For the first 9 days there was no difference in size or weight; however, as the larvae progressed towards metamorphosis, those cultured in the laboratory grew faster and metamorphosed 8 days earlier than those sampled from the field. The authors explained the observed difference by speculating that the larvae were using endogenous reserves for the first period, after which, the difference in exogenous food supply affected the growth and development rates.

The phenomenon of utilization of different food reserves during different parts of the larval life would explain the significant interaction between age and dietary effect observed in the present study. Echinoderm larvae are able to exploit larger cells with increasing age, but the ability to remove smaller cells (<4 m) diminishes. Veliger larvae cannot adapt in the same way and exploit larger food items (>30 m) because the width of the food groove determines the upper limit on ability to manipulate cells towards the mouth. Larvae may preferentially remove some cell types over others on the basis of size (Baldwin, 1991), taste

(Rassoulzadegan et al, 1984), or other parameters. Alternatively, there is evidence (Rassoulzadegan and Fenaux, 1979) that larvae preferentially remove the type of cell with the highest abundance. When one cell type is exhausted, the larvae clear the cells in the next most abundant size category. in summary, the type and concentration of different cells available in the medium affects clearance by the larvae; therefore quality of diet cannot be assessed using energetic content alone.

The issue of food limitation in natural environments is an important one to ecology of marine invertebrates. Growth, development, survival and dispersal capacity of larvae can affect the population dynamics of the individual species, and those species upon which it has an influence. Calculations by Mann (1988) estimated the number of cells ml^{-1} ($5.4x10^2$ to $6.77x10^4$ cells ml^{-1}) using chlorophyll a levels encountered (April-December) off the coast of New England. Applying these calculations to the range of chlorophyll levels measured by Haas (1974) in the York River (5-20 ml^{-1}), gives a range of $2.7x10^7$ to $4.2x10^8$ cells ml^{-1}. These values for particulate food availability are very approximate, however, they indicate that there is sufficient food available in a temperate coastal systems to prevent nutrition becoming a growth-limiting resource to planktotrophic invertebrate larvae. Strathmann (1987) also states that concentration of food in coastal waters is often high enough to prevent starvation but is rarely sufficient to attain optimum growth. He also points out however, that high estimates for food requirements on algal diets may be an artifact of poor culture techniques.

The results obtained from this experiment are relevant only to the time, place and species studied. In a temperate estuarine ecosystem, there is a seasonal variation in nutrition available for planktotrophic organisms. In the York River in 1975, chlorophyll a levels

ranged from 5-20μg l^{-1} during the spawning period of *C. virginica.* Nanoplankton (<15 μm) dominated the phytoplankton community, accounting for 70-90% of the total chlorophyll a level in the river (Haas, 1975). No such data is available for the Eastern Shore site, but it is probable that community structure of the water column follows a similar trend, although the absolute values may be different. Chlorophyll a levels cannot be interpreted as total food available. Work by Ducklow at the University of Maryland, has shown that in the upper Chesapeake Bay, bacterial biomass may be equal to that of phytoplankton (Mann 1988). Levels of phytoplankton and other nutrients will vary in time and space; the oysters spawn from May through to the end of September and the variation in larval food during the spawning season may be sufficient to create a seasonal difference for growth and development rates.

Conclusions

This study is not intended to determine a preferable diet for mass larval culture; it rather serves to address the validity of drawing conclusions on ecological questions, using results of laboratory studies. Larvae can feed on an extensive array of size and type of particles, as well as bacteria and DOM. In nature the relationship ship between nutrition, larval growth, and development does not depend solely on the level of phytoplankton available in the medium. Providing larvae with fixed food concentrations in the laboratory to discover optimal growth and development rates, and then extrapolating the results to nature, is problematic. The optimal development observed in the laboratory is probably not typical of larvae in their natural environment and should be considered a separate phenomenon.

Regarding the use of the mesocosm; it did not function well in the environmental

conditions of the experiment and a larger organism would also be easier to manipulate. The concept; however, is tractable and has other ecological applications besides nutrition, such as pollutant stress monitoring or effects of red tides.

Chapter 3

In Situ Culture of Larvae of Queen Conch, *Strombus gigas* and Milk Conch, *Strombus costatus*

Objective:

- To investigate the effects of *in situ* vs laboratory culture on growth and development of larval marine invertebrates

Introduction

Strombus species, particularly *Strombus gigas* the Queen conch, is an important fishery throughout the Caribbean region. Conch was exploited at the subsistence level by local communities for many years before it became commercially important throughout the Caribbean region, especially with increased tourist demand. Current status of the fishery ranges from severely depleted in Florida, to well exploited in the more remote areas.

Demand for conch meat and threat of over exploitation has stimulated interest in potential for aquaculture of larval strombids. The Queen conch, *Strombus gigas,* has been the focus of most of the studies on growth and development rates; however, some work has also been done on the milk conch *S. costatus* and hawkwing conch *S. raninus*, two of the other most common, but less commercially important species in the range.

Lifecycle

Figure 16 illustrates the lifecycle of a *Strombus* species. The adults gather together in localized areas on the coastal shelves at about 20m depth between the months of July and September. Fertilization occurs internally and the eggs are laid in strands of approximately 400,000 on clean, low organic sand (Stoner and Sandt, 1992). The female remains over the crescent shaped egg mass until hatching, protecting it with the flared lip of the shell. Egg masses have also been found sitting on bare sand, with no female protection; the masses are, however, covered with sand particles which makes them cryptic.

The larvae require 5-6 days to develop within the egg capsule (fig. 17a) , after which they emerge as free swimming veligers that suspension feed on plankton. On emergence the

shell is approximately 300 μm long and the larvae have two small velar lobes (fig. 17b) equipped with bands of cilia to facilitate swimming and feeding. After 4-6 days, the velum splits to form four lobes (fig 17c), then at 6-9 days the anterior lobe divides (fig. 17d) to form the final 6 lobed velar configuration (fig. 17e). There is considerable variation in the literature on both *S.gigas* and *S. costatus,* regarding growth and development rates of larvae from hatching to metamorphosis. Davis (1994) and Brownwell (1977) observed *S. gigas* larvae ready to metamorphose from between 14 and 28 days old (950-1200 μm shell length); D'Asaro (1965) reported metamorphosis as late as 40 days.

The larvae, after reaching competence to metamorphose, must be stimulated to settle by the presence of a settlement cue (Davis, 1994b). Natural cues include detrital blades of *Thallassia testudinum*, and old branches of the red alga *Laurencia poitei*. The larvae must settle into their nursery area within 6 days of reaching competence or they subsequently lose their ability to metamorphose (Davis 1994b). This limited ability to control competence period has ramifications on larval dispersal ability and population dynamics. It is unusual that the competence period is so short. In general, for planktonic larvae of benthic marine invertebrates, the competence period is at least as long as the pre-competence period (Strathmann, 1987). Sandt and Stoner (1993) suggested that if larvae do not require specific juvenile habitat, and are induced to settle by a range of cues, it is not necessary to have a long competence period.

During metamorphosis, the *Strombus* larvae change their respiratory and vascular system. The adult heart grows during development and takes over in function from the larval heart, which gradually atrophies (D'Asaro 1965; Davis 1994b). The velum atrophies

through metamorphosis and is replaced by the adult true gill. The larval behavior progresses from the planktonic swimming, suspension feeding form, through a swim-crawl stage when the larvae are testing the benthic substrate (this is a behavioral indication of competence to metamorphose), to a crawling stage when the feeding mode changes from suspension feeding to grazing on benthic algal assemblages.

The post metamorphic juveniles hide in the roots of seagrass beds during the day and emerge at night to feed. Little is known about the first nine months of benthic life (Sandt and Stoner 1993). At one year old (80 to 100mm shell length) the juveniles emerge from the sediment and feed epibenthically in seagrass meadows that provide ample algal and detrital food. (Randall 1964, Stoner and Waite 1991). The development of the flared lip of the shell precedes sexual maturity at 3½ years (180-270 mm shell length). At the beginning of the spawning season, the adults migrate to the deeper (15-28m) sandy slopes to mate.

Larval Gastropod feeding mechanism

The feeding mechanism of gastropod larvae is similar to that of other species which utilize ciliated bands to capture particles (Strathmann 1987). As in the oyster larvae, there is a prototroch band of long stiff cilia on the preoral edge of the velar lobe and an oral groove with very small fine cilia which pass the food particles to the mouth. The metatroch is a row of medium length cilia on the post oral edge of the velum; it is hypothesized that their function is to assist the prototroch cilia in particle capture but the actual mechanism by which they do this is not clear. (Strathmann 1987). There is a particle selection function which operates at the mouth and another which is simply the size limitation of the oral groove.

Figure 16: Lifecycle of *Strombus gigas*

Life Cycle of *Strombus gigas*

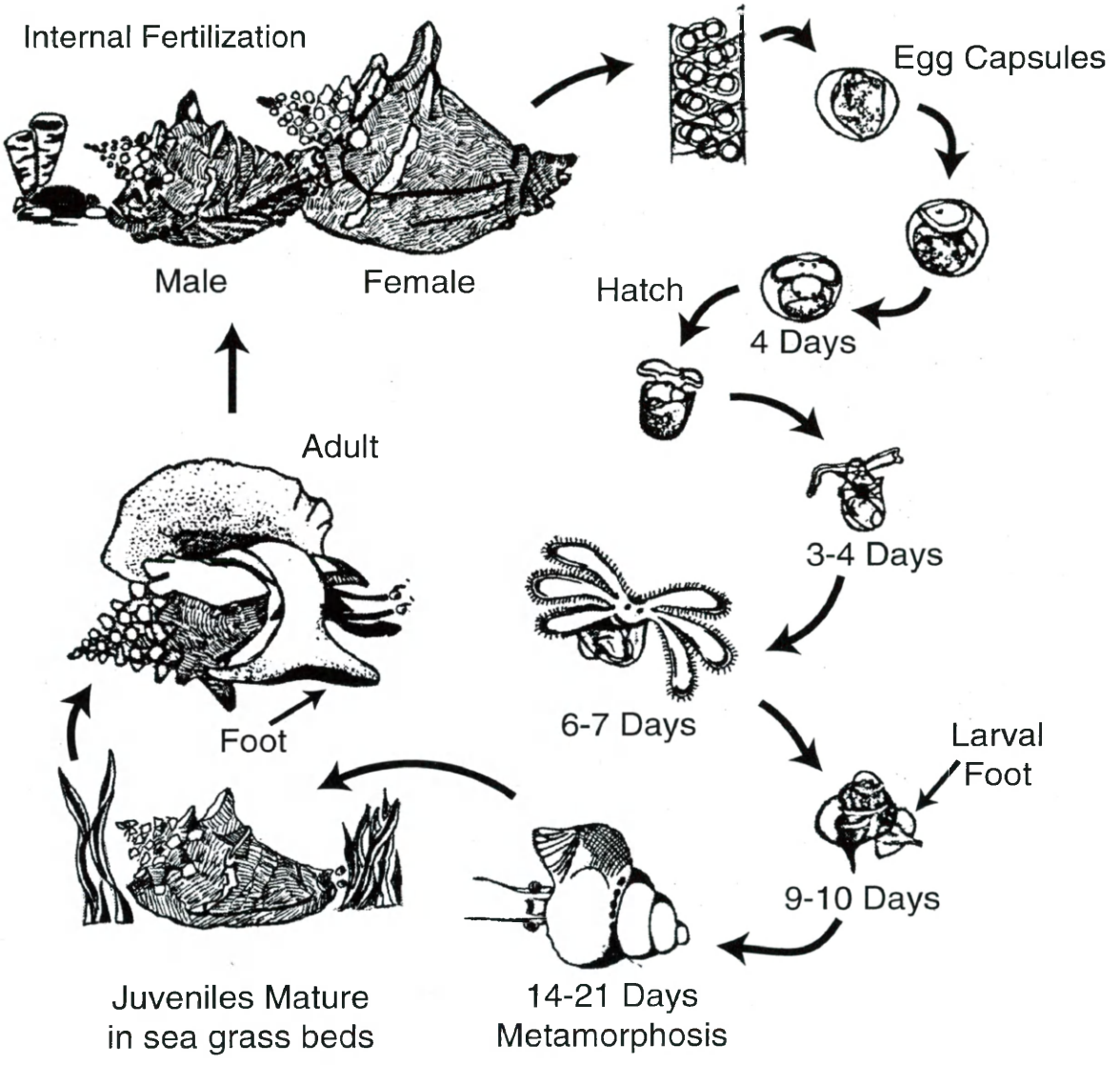


Figure 17: Photographs of *Strombus gigas* larvae, showing stages of development.

a. Egg mass

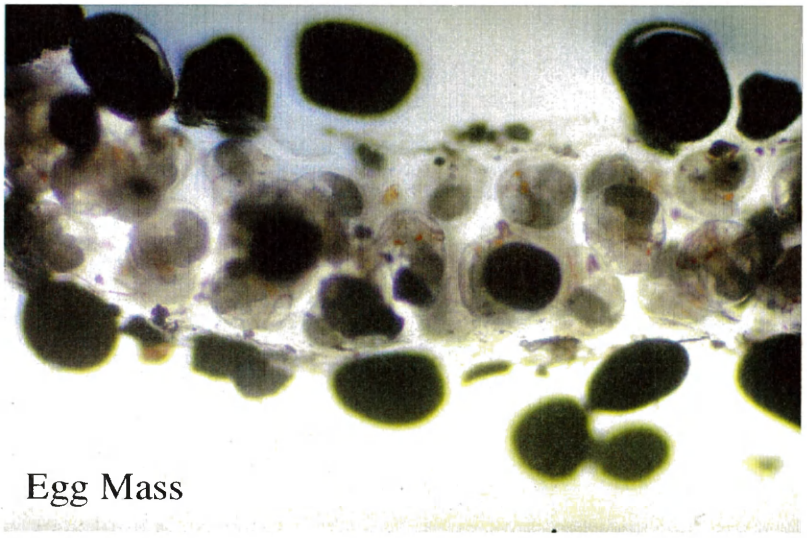
b. Two lobed larva, 24 hours post hatch (200-250 μm).

c. Four lobed larva, 3-4 days of age (350-450 μm).

d. Six lobed larva; 5-6 days of age (450-600 μm).

e. Six elongated lobes; 6 days of age to onset of competence (600-1100 μm).

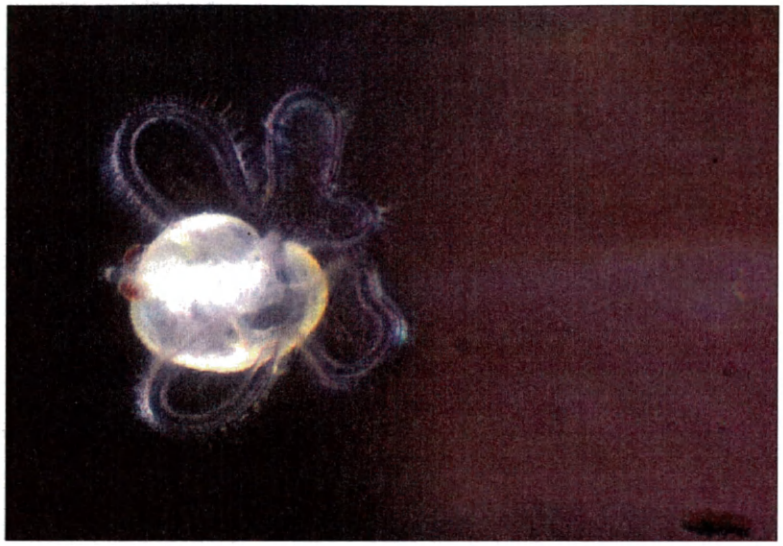
a



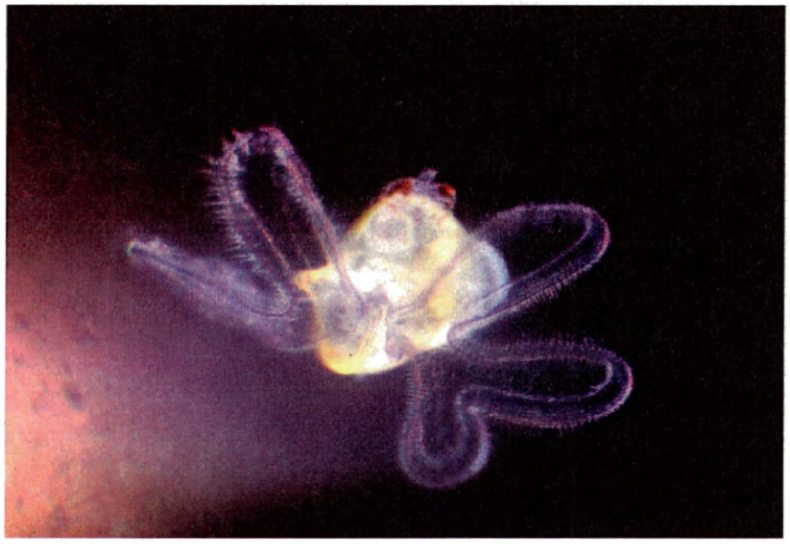
Egg Mass

b

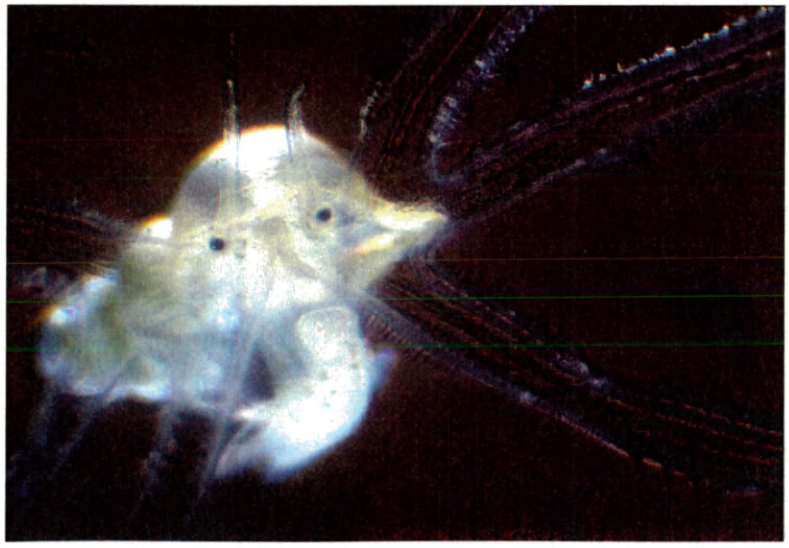
c



d



e



Treatments:

It was not possible to directly compare *in situ* culture with an algal diet, since there were no algal culturing facilities available at the study site on Lee Stocking Island. A literature search was carried out to determine the "typical" growth and development rates found with this species when cultured on algal diet (table 21). The data from the literature was then compared with the observed growth and development rates for this experiment.

Study Site

This part of the investigation was carried out at the CMRC research centre on Lee Stocking Island in the Exuma Cays, Bahamas (fig. 18) The study site (where the mesocosm was deployed), on the south coast of Lee Stocking Island, is influenced by two water masses; the relatively phytoplankton rich (100-150 ng l^{-1}) deep oceanic water of the Grand Bahama Bank and the phytoplankton poor (25-50 ngl^{-1}) water of the shallow Exuma Sound (fig. 19). The larvae in the mesocosm were exposed to both water masses to determine if high and low phytoplankton water had any significant effect on growth and development of the larval strombids. In the event that a significant effect of water mass was observed, it would be useful to know the degree of influence for interpretation of the mesocosm data.

Sibling larvae at 24 hours post hatch (300µm shell length) were used for all treatments. Initial stocking density was 10 per liter. At 10 days post hatch, the density was reduced to 5 $liter^{-1}$. Two replicates were maintained for treatments 1 and 2; three replicates for treatment 3.

Figure 18: Map of the Exuma Sound, Bahamas.

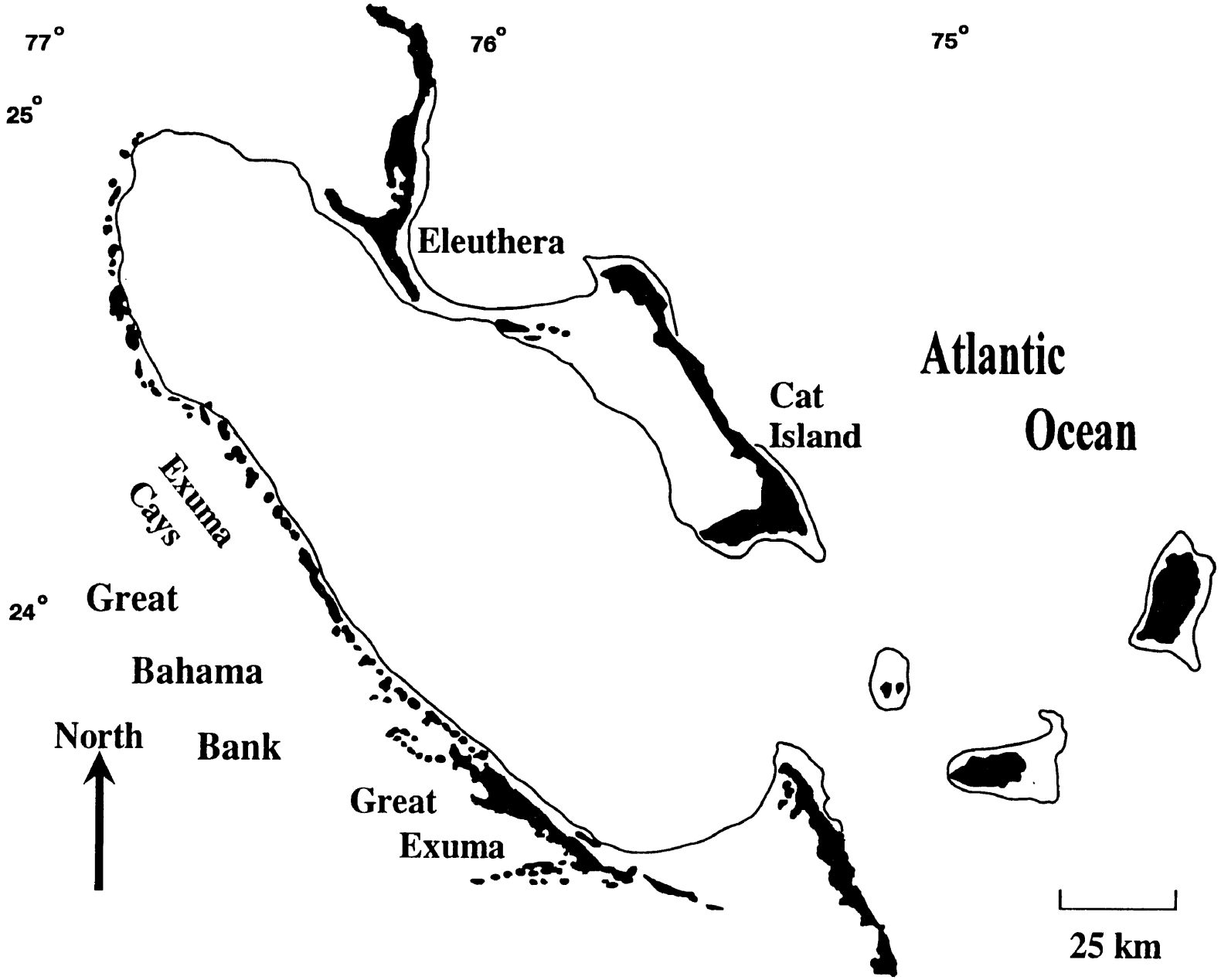
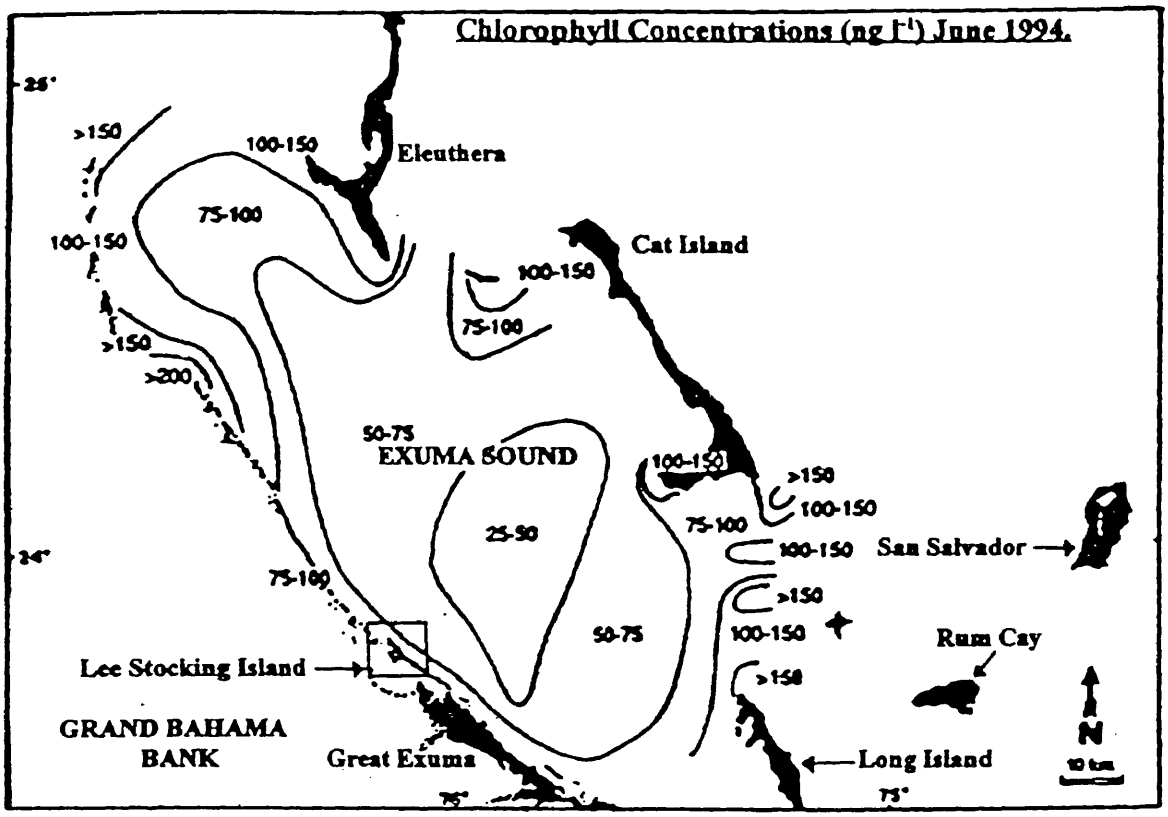

77°
76°
75°
25°
24°
Eleuthera
Cat
Island
Atlantic
Ocean
Exuma
Cays
Great
Bahama
Bank
North
Great
Exuma
25 km

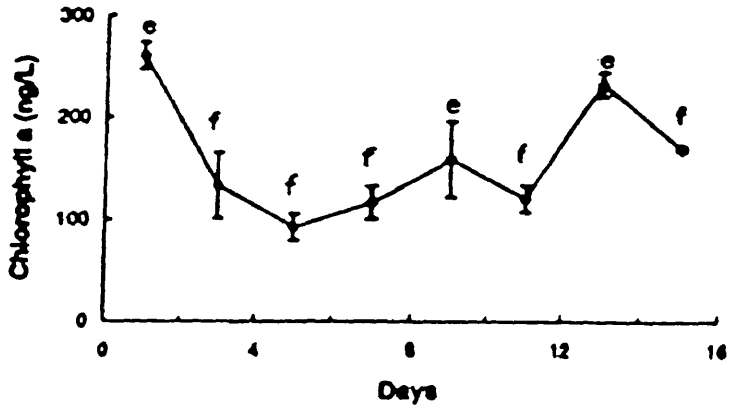
Figure 19: Chlorophyll a distribution in Exuma Cays study area.

Chlorophyll levels at flood (f) and ebb (e) tides are also indicated.

Chlorophyll Map of Exuma Cays, Bahamas



Chlorophyll Concentrations at Ebb and Flood Tides.



Treatment 1

High phytoplankton diet: Larvae were cultured in water collected at 2 hours after high tide, when water floods in from the Great Bahama Bank and the chlorophyll concentration is richest (100-150 ng ml^{-1}) at the water collection station on Lee Stocking Island (Davis personal comm., 1995).

Treatment 2

Low phytoplankton diet: Larvae were cultured using water collected two hours after low tide, when water from the shallow Exuma sound passes by the water collection station and chlorophyll concentration is low (<50 ng ml^{-1})

Treatment 3

In situ diet: The larvae were cultured in the SLIC mesocosm, in a shallow (3-4 meters) bed of *Thalassia testudinum.*

Experimental Design

Dependent variables: Shell Height/Diameter

Number competent/metamorphosed.

Independent variable: Age (days)

Factor:	Enclosure type	Diet type
Levels:	E1 = 7 liter containers	D1 = High phytoplankton
	E2 = SLIC	D2 = Low phytoplankton
		D3 = In situ in SLIC

	E1	E2
D1	Treatment 1	No treatment
D2	Treatment 2	No treatment
D3	No treatment	Treatment 3

Number of spawns: *S. gigas*:1; *S. costatus*: 1 Number of larvae per sample: 10

Number of replicates: T1, T2, T3 :2; T4:3. Number of larvae competent

Materials and Methods:

A *Strombus gigas* egg mass was collected on 7th September 1995, from a sandy area on the South side of the Island, at a depth of 60 ft. A second egg mass was found on the 23rd of September and was subsequently identified as *Strombus costatus*, the Milk Conch. The methods used for both species were identical.

The crescent shaped, sand covered egg mass was gently teased apart to allow good water circulation and placed in a 4 liter plastic container with a mesh base. This was placed in ambient seawater to a depth of approximately 10 cm. The bottom of the container was raised above the surface on small blocks and a constant gentle stream of seawater was directed into the container to create a slow downwelling. The water temperature remained between 30 and 32° C.

The eggs of *Strombus gigas* hatched overnight on the 10th-11th of September and those of *Strombus costatus* on the 24th-25th September. After 24 hours, the larvae were individually counted into 7 liter plexiglass containers at a density of 10 per liter. The culture water was collected 2 hours after high tide (as in treatment 1), when the phytoplankton rich

bank water has entered the Exuma Sound (Davis pers. Comm., 1995). The water was changed daily by transferring the larvae (with a pipette, to avoid the stress of sieving) into a container of clean water. After use, the containers were cleaned with fresh water and rinsed with distilled water and left to air dry. No bleach or disinfectant was used. After 5 days, the larvae were divided amongst the three different treatments. Due to the limitation in the number of larvae, they were not exposed to the potentially stressful treatments 2 and 3 until they had been well fed for 5 days. This was to avoid the risk of high mortality in the fragile early stages. The different feeding regimes were included to cover the extremes of the range of water quality in a tidal cycle. Since the larvae in the laboratory are kept at such low density, those cultured in the phytoplankton rich water may have access to more food items over a 24 hour period than those exposed to the full tidal cycle in the mesocosm.

Treatment 1

Larvae were kept in 7 liter plexiglass containers and maintained in a thermostatically controlled incubator set at 29° C. The diurnal light regime was 12 hours dark, 12 hours light. The larvae were cultured using water collected at 2 hours after high tide, when the chlorophyll concentration is richest. The water was pre-filtered with 105 μm sieve to remove potential predators and competitors. Every day the larvae were pipetted individually into clean containers of fresh seawater. Every second day, a subsample of 10 larvae was taken from each replicate for examination.

Treatment 2

The larvae were incubated under the same conditions as in treatment 1. This treatment was, however, cultured using water collected two hours after low tide when the chlorophyll

concentration is lower. This water was pre-filtered with 105µm sieve. As with treatment 1, larvae were pipetted into clean containers of fresh seawater daily. Every second day, a subsample of 10 larvae was taken from each replicate for examination.

Treatment 3

The larvae were maintained in the SLIC mesocosm; details of the SLIC are described in the Technical Report (Appendix 1). The SLIC was set up a little differently from the configuration described in chapter 1; the pump recieved power for two minutes then was cut off for 58 minutes, creating a periodic pulsed flow through the chamber. This design served to reduce power consumption and fouling of the screens. The larvae in the chamber were also exposed to less continuous turbulence, since the water was static for most of the time. This was advantageous since conch larvae react to turbulence by retreating into their shells, and consequently not feeding.

The solenoid valves, when not powered, are closed and had to be replaced with manual valves. Flow reversal was originally incorporated in the SLIC design to back flush the chamber screens and keep them clean in a high sediment environment. This was not necessary in the clear tropical waters of the Bahamas, especially with the introduction of the pulsed flow system. When the chambers were removed for cleaning and subsampling, the valves were switched over to reverse the flow around the internal plumbing and flush out any organisms or debris that may have accumulated.

Every two days the mesocosm chambers, filters and screens were cleaned. The chambers were detached from the SLIC under water and placed in a submerged bucket. The lid was sealed and the bucket pulled onto the shore. Whilst still upright in the bucket of

water, one end of the chamber screen was removed. The larvae were pipetted out individually. After photographing and measuring the larvae from all replicates, the cleaned chambers were replaced in the SLIC. There was a 1cm diameter hole in the chamber wall, which allowed air to escape and flood the chamber, and was the route by which larvae were replaced, using a pipette. A rubber stopper prevented larvae escaping.

1. Shell length

The shell length was measured using a dissecting microscope fitted with an ocular micrometer, which measures to 1μm accuracy. Length is defined as distance from the tip of the beak to the tip of the last whorl. Diameter is defined as distance across the widest part of the largest whorl. These measurements were used to determine growth rates.

2. Developmental stage

The developmental stage was described for each group, using diagrams and descriptions from Davis (1994a).

3. Lipid Content

Every four days a subsample of 10 larvae from each treatment was fixed using 10% Phosphate buffered paraformaldehyde. The larvae were stained with Nile Red, a lipid specific stain, according to the protocol described in Mann and Gallager (1981). The larvae were photographed and a qualitative assessment of lipid content was made.

4. Determination of time to competence/metamorphosis

As the larvae develop there are some distinct morphological changes that can be used as developmental cues (Davis 1994a) As the larvae become competent to settle, their behavior changes from swimming in the water column to swim/crawl behavior in which the larvae remain longer on the substrate and probe actively with the newly developed foot. Competence in conch veligers is recognized morphologically by outward migration of the eyes, tentacles of equal length, and a change in pigments on the foot from orange to green. The number of larvae competent to settle was recorded for each treatment.

Competent *Strombus* larvae require an environmental cue to induce metamorphosis (Davis 1994b). Detrital material of the seagrass, *Thalassia testudinum* is a natural cue and a commonly used artificial cue is an extract of (old) stalks of the red macroalga, *Laurencia poitei* (Davis 1994b). When the larvae began to display signs of competence, 5 large detrital blades of *T. testudinum* were introduced into the culture container, after rinsing with seawater to remove sand and debris.

5. Water quality assessment

Temperature and salinity measurements were taken at high and low tide for the first week, there after temperature was recorded daily. The temperature in the incubator for treatments 1 and 2 ranged between 27 and 30° C. The seawater temperature ranged between 29 and 32° C. The salinity was constant at 35 ppt. Dissolved oxygen concentration was not considered a limiting factor in well circulated oceanic water.

Table 21: Summary of growth and development rates found in the literature for *S. gigas* and *S. costatus*.

Author	Species	Food Supply	Growth Rate (μd^{-1})	Age at Competence (d)
Davis (1994)	*S. gigas*	Exuna shelf ;107 ngChl al^{-1} Bahama bank;176 ngChla l^{-1} Algae diet; 470 ngChl a l^{-1} Mesocosm	11 29 29 43	- -
Davis (1993)	*S. gigas* *S. costatus*	*Isochrysis* (5,000 cells ml^{-1}) *C. gracilis* (3,000 cells ml^{-1})	39 28	21 32
Aldana Aranda (1989)	*S. costatus*	*I. galbana / Tetraselmis chui* (3,000 cells $larva^{-1} d^{-1}$)	40	26-28
Pillsbury (1985)	*S. gigas*	*Dunaliella tertiolecta* *Prorocentrum minimum*	13 53	 -
Ballantine and Appeldoorn (1983)	*S. gigas* *S. costatus*	*Isochrysis* *T. chui* *Thalssiosira fluviatilis*	50-55 51	12-22 14-18
Brownwell (1977)	*S. gigas* *S. costatus*	Enriched seawater $1x10^5$ cells ml^{-1}	-	18-21 (metamorphosis 27d) 15-18 (metamorphosis 36d)

Results: *Strombus gigas*

Growth rates

Plots of shell dimenesion against age indicate that growth is comprised of an initial rapid rate which becomes almost asymptotic after approximately 18 days, at a shell height of approximately 900µm. For the 'high phytoplankton' diet, the slope of the curve is steeper and the plateau is reached earlier than for the low phytoplankton diet. The growth rates became asymptotic once competence was attained (fig. 20a, b) and larvae remained in a state of competence without growing or undergoing metamorphosis. Regression analysis was performed on the linear part of the growth curve, since the asymptotic section is not representative of growth rates; regression fits (figs. 21 a, b and 22 a, b) were significant for all treatments ($P<0.05$) on both shell dimensions. Tables 22 and 23 summarise the regression analysis for shell height on high and low diets and the regressions for shell diameter are summarised in tables 24 and 25.

The ANCOVA indicated no significant difference between diets, nor was a significant interaction effect found between age and diet for shell height (table 26). The growth response to diet was therefore independent of age in this experiment. Table 27 shows the results of a one-way ANOVA, which indicated no significant effect of diet on shell height ($F=0.92$; $p=0.34$). The age/diet interaction factor was found to be significant for shell diameter, (table 28); therefore the ANCOVA could not be used to compare the regression lines. The 95% confidence intervals of the regression analysis (tables 24 and 25) overlap, indicating no significant difference between the growth rates of the two treatments.

Development rates

The mesocosm was behaving erratically during this experiment, because of inconsistent power regulation and flow rates. Consequently, the data for the SLIC treatment was discarded. The larvae did survive and grow in the mesocosm while it was functioning, therefore the mesocosm environment was not prohibitive to *Strombus* larval survival and growth. After 6 days in the SLIC, the mean shell height of the larvae (912 μm) was very similar to those maintained on the 'high' phytoplankton diet (913 μm). After 11 days of exposure in the mesocosm (age 25 days), one larva was observed crawling; it had undergone metamorphosis. This was not statistically valuable data however, since the mesocosm had malfunctioned (power loss) and the rest of the larvae died.

There are several developmental changes that occur as the larvae approach competence (Davis, 1993). Onset of developmental factors indicating competence were initially observed at 14-15 days in the high diet animals, the frequency increased over a period of a ten days until almost all the larvae sampled from in the high diet indicated competence. None appeared to metamorphose, even after exposure to detrital blades of *Thalassia testudinum.*

Development was slower in the 'low diet' treatment; however, by day 18, the larvae were beginning to show traits of competence. Photographs were taken (fig. 23a-e) under a dissection microscope (x5) and developmental stage was identified using shell morphology, from descriptions by Davis (1993). By the termination of the experiment, most of the larvae in this treatment were competent to metamorphose, but none underwent metamorphosis, despite exposure to detrital blades of *Thalassia testudinum.*

Lipid Content

No difference was observed between the two treatments. The qualitative nature of the lipid assessment was not sufficiently sensitive to detect small differences. Significant differences in nutritional status would have been reflected in the degree of staining, however, most of the larvae in both cultures appeared to have a well stained gut area (photographs in fig 23, were stained with oil Red O and show heavy red gut staining); no difference could therefore be determined between the two treatments.

Water quality

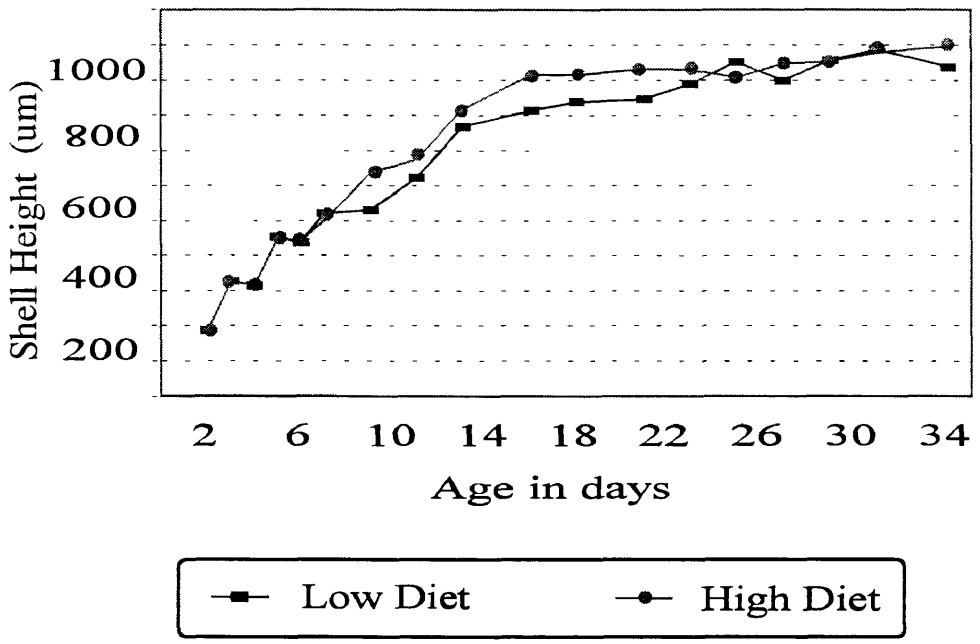
The larvae in this experiment were cultured in 7 liter containers in an incubator. The thermostat was set at 28°C, but varied from 26.5-31°C as determined by maximum-minimum thermometers. The ambient water temperature was taken at each collection of new water (fig. 24) If there was more than 2°C difference in temperature between the new and old culture water, the larvae were left out of the incubator in the same room as the new water to allow the temperatures the equalize, thus avoiding temperature shock on water change. Salinity was 35ppt consistently.

Figure 20: Growth curves for *Strombus gigas* larvae.

a. Shell height (μm day^{-1}) against age for high and low phytoplankton diets.

b. Shell diameter (μm day^{-1}) against age for high and low phytoplankton diets.

a.


Shell Height (um)
1000
800
600
400
200
2
6
10
14
18
22
26
30
34
Age in days
Low Diet
High Diet

b.

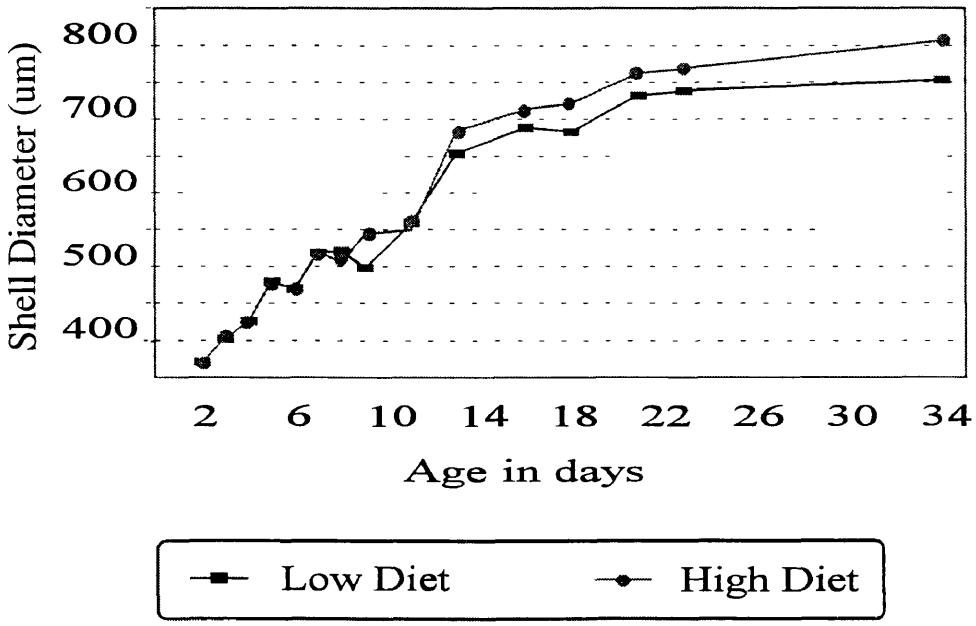
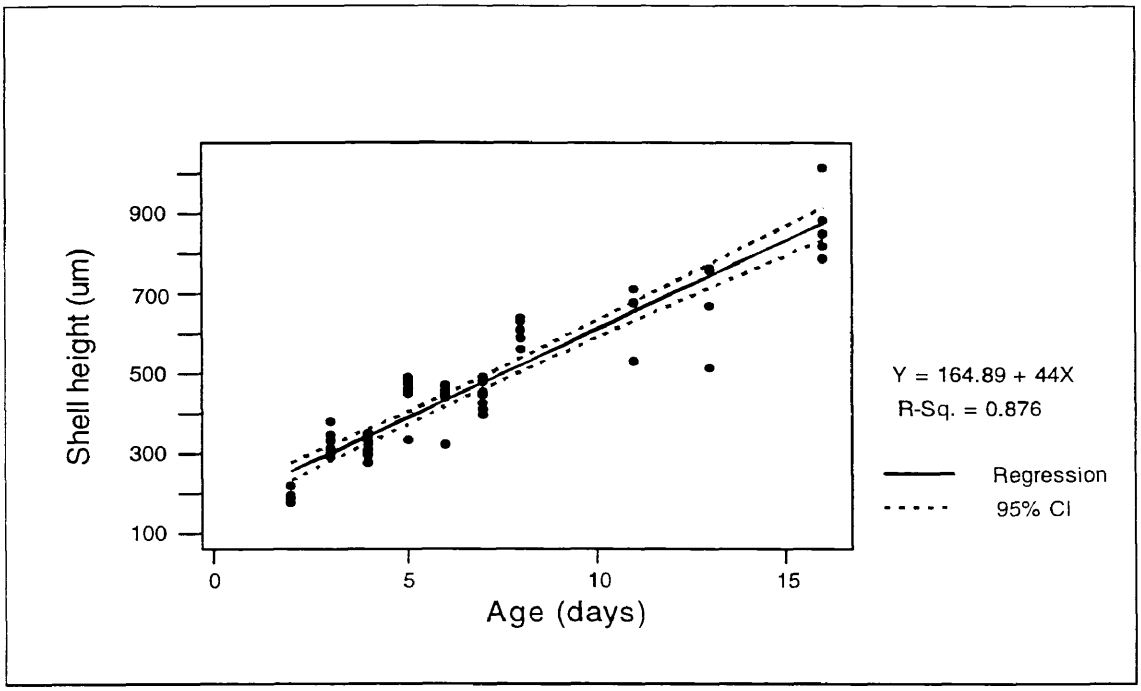

Shell Diameter (um)
800
700
600
500
400
2
6
10
14
18
22
26
30
34
Age in days
Low Diet
High Diet

Figure 21: Linear regression fitted line plots for shell height (μm day^{-1}) against age (days) for different treatments. Hatched lines represent 95% confidence intervals

a. Treatment 1: High phytoplankton diet.

b. Treatment 2: Low phytoplankton diet

a.


Shell height (um)
900
700
500
300
100
0
5
10
15
Age (days)
Y = 164.89 + 44X
R-Sq. = 0.876
Regression
95% CI

b.

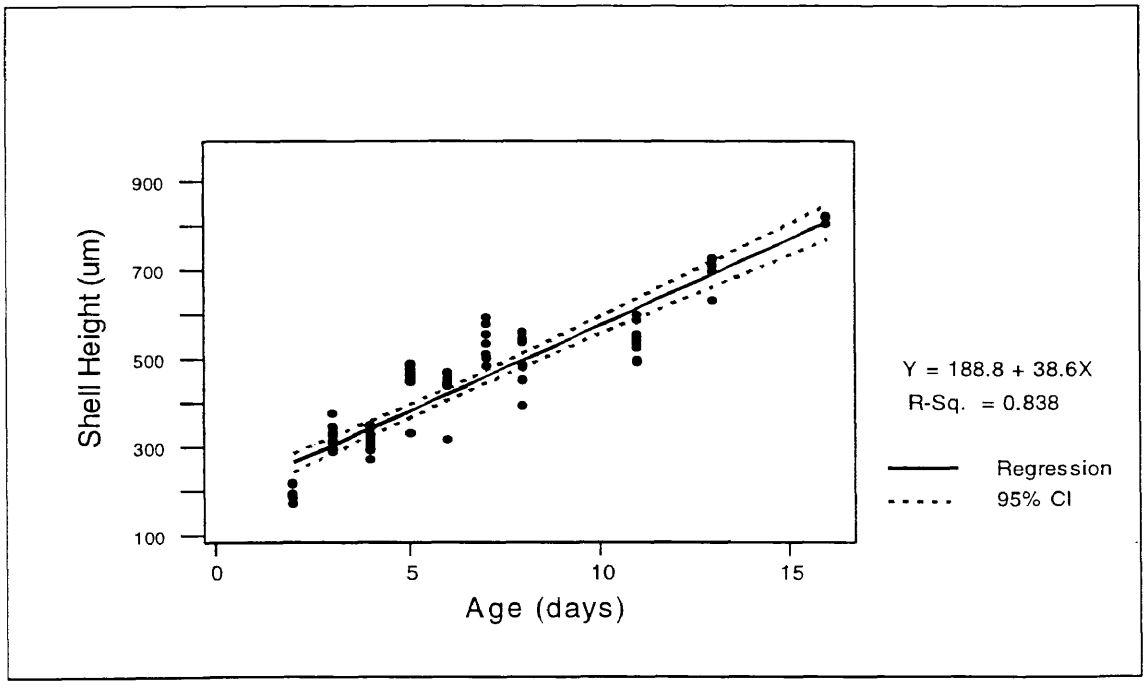

Shell Height (um)
900
700
500
300
100
0
5
10
15
Age (days)
Y = 188.8 + 38.6X
R-Sq. = 0.838
Regression
95% CI

Table 22: Linear regression analysis of *S. gigas* shell height on age for high diet.

Regression equation:

Shell height = 138.7 + 51.5 age $R^2 = 87.6\%$

	Coefficient	95% lower	95% upper
Intercept	138.74	109.29	168.19
Age	51.50	47.30	55.64

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	2.25×10^6	2.25×10^6	517.89	0.00
Residual	73	3.7×10^5	4.34×10^3		
Total	74	2.57×10^5			

Table 23: Linear regression analysis of *S. gigas* shell height on age for low diet

Regression equation:

Shell height = 173 +43 age $R^2 = 83.8\%$

	Coefficient	95% lower	95% upper
Intercept	173.13	142.97	203.29
Age	43.16	39.13	47.20

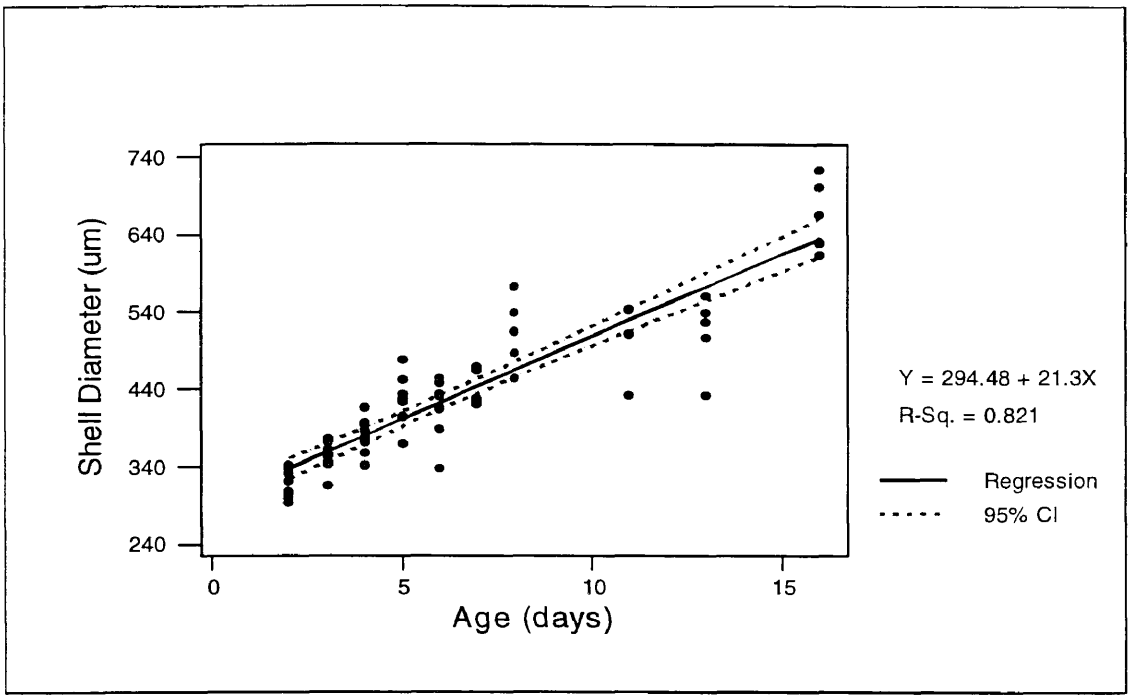
	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	1.63×10^6	1.63×10^6	410.0	0.00
Residual	79	3.15×10^5	3.99×10^3		
Total	80	1.95×10^6			

Figure 22: Linear regression fitted line plots for shell diameter (μm day^{-1}) against age (days) for different treatments. Hatched lines represent 95% confidence intervals

a. Treatment 1: High phytoplankton diet.

b. Treatment 2: Low phytoplankton diet

a.


740
640
540
440
340
240
Shell Diameter (um)
0
5
10
15
Age (days)
Y = 294.48 + 21.3X
R-Sq. = 0.821
Regression
95% CI

b.

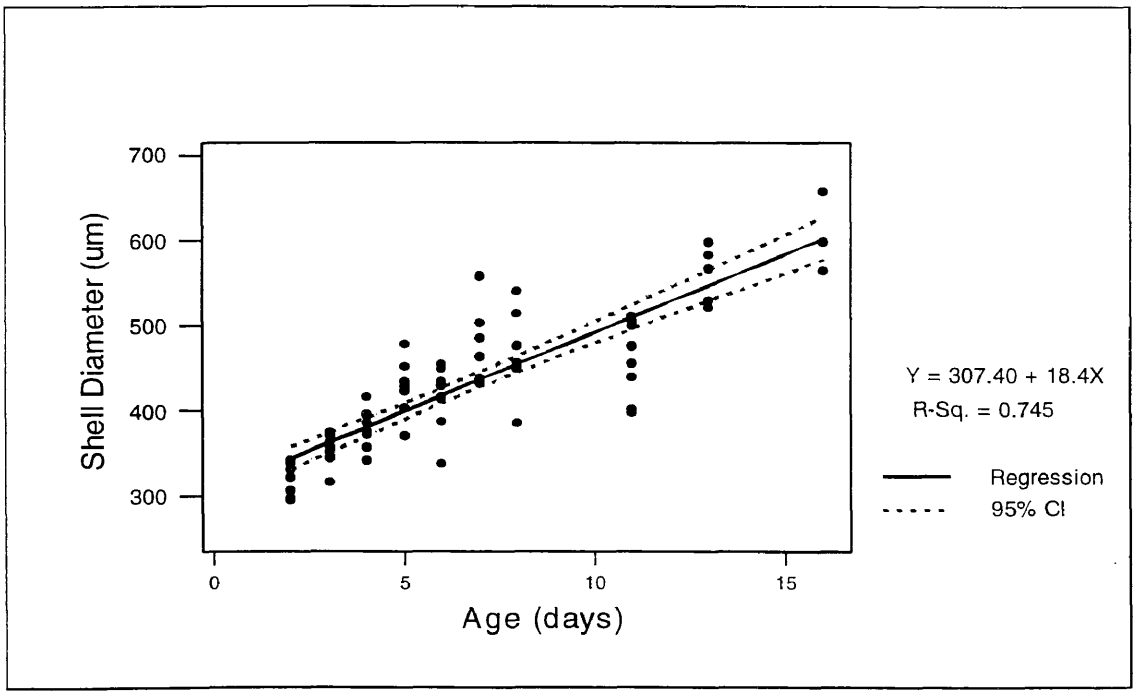

700
600
500
400
300
Shell Diameter (um)
0
5
10
15
Age (days)
Y = 307.40 + 18.4X
R-Sq. = 0.745
Regression
95% CI

Table 24: Linear regression analysis of *S. gigas* shell diameter on age for high diet

Regression equation:

Shell diameter = 282.3+24.7 age $R^2 = 82.1\%$

	Coefficient	95% lower	95% upper
Intercept	282.29	264.59	300.00
Age	24.72	22.21	27.22

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	$5.2x10^5$	$5.2x10^5$	329.14	0.00
Residual	72	$1.14x10^5$	$1.58x10^3$		
Total	73	$6.39x10^5$			

Table 25: Linear regression analysis of *S. gigas* shell diameter on age for low diet

Regression equation:

Shell diameter = 293.3 +21.8 age $R^2 = 74.5\%$

	Coefficient	95% lower	95% upper
Intercept	293.30	275.47	311.12
Age	21.81	19.43	24.20

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	$3.7x10^5$	$3.7x10^5$	230.73	0.00
Residual	79	$1.27x10^5$	$1.6x10^3$		
Total	80	$4.97x10^5$			

Table 26: ANCOVA of age vs shell height for *S.gigas* for high vs low diets

	Df.	Seq SS	Adj SS	Adj MS	F	P
Age	1	$1.80x10^7$	$1.80x10^7$	$1.80x10^7$	$1.64x10^3$	0.00
Diet	1	$8.74x10^3$	615	615	0.06	0.81
Diet*age	1	$1.54x10^3$	$1.54x10^3$	$1.54x10^3$	0.14	0.71
Error	262	$2.87x10^6$	$2.87x10^6$	$1.09x10^4$		
Total	265	$2.09x10^7$				

Table 27: ANOVA of age vs shell height for *S. gigas* for high vs low diets

	DF	Sum Sq	Mean Sq	F	p
Diet	1	$7.28x10^4$	$7.28x10^4$	0.92	0.34
Error	264	$2.08x10^7$	$7.88x10^4$		
Total	265	$2.08x10^7$			

Table 28: ANCOVA of age vs shell diameter for *S. gigas* for high vs low diets

	Df.	Seq SS	Adj SS	Adj MS	F	P
Age	1	$2.03x10^6$	$1.96x10^6$	$1.96x10^6$	672.51	0.00
Diet	1	$3.47x10^3$	$5.37x10^3$	$5.37x10^3$	1.84	0.18
Diet*age	1	$2.09x10^4$	$2.09x10^4$	$2.09x10^4$	7.17	0.01
Error	177	$5.16x10^5$	$5.16x10^5$	$2.92x10^3$		
Total	180	$2.57x10^6$				

Figure 23: Photographs of *Strombus gigas* larval shells, showing stages of development.

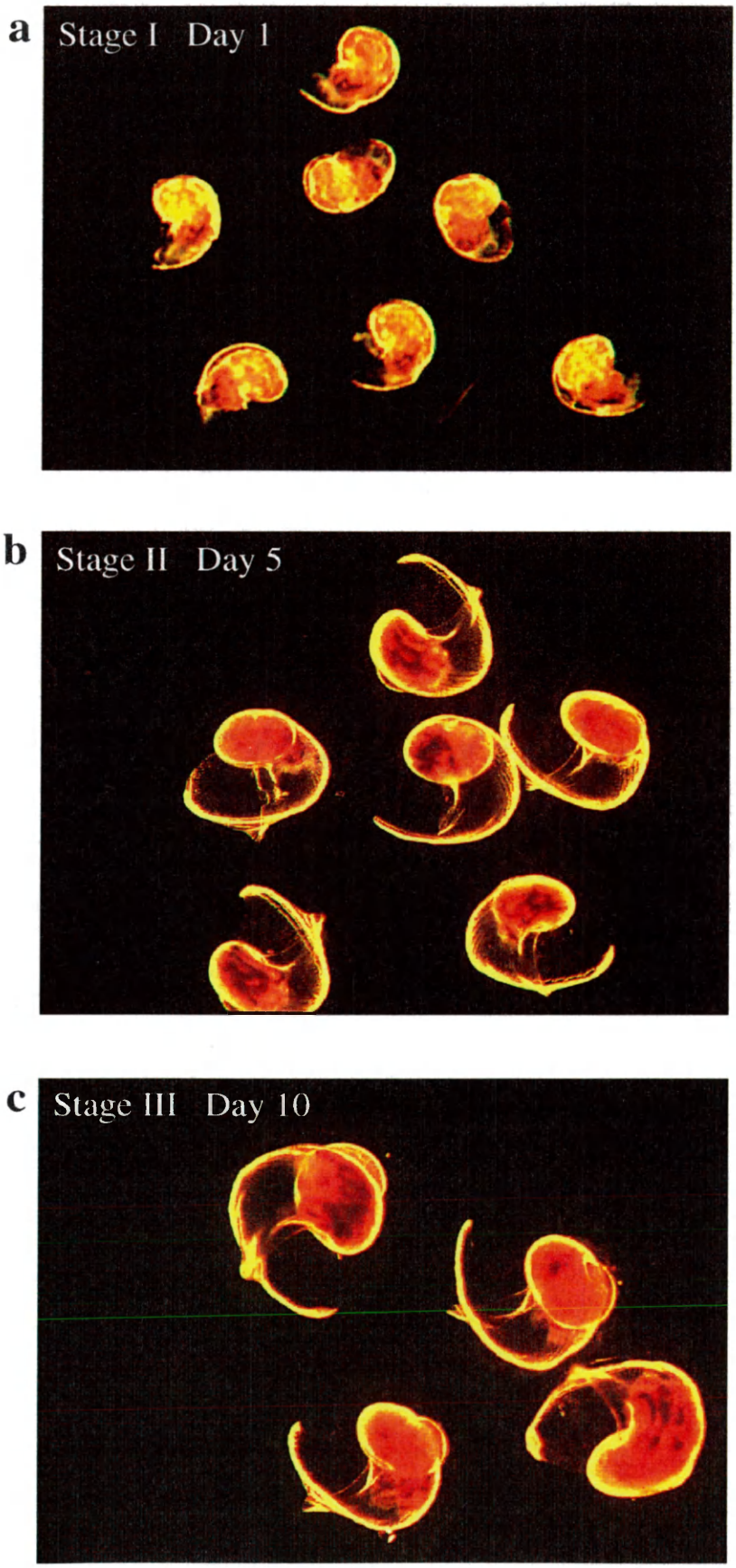
a. Stage I; age 1 day (200-250 μm).

b. Stage II; age 5 days (500-600 μm)

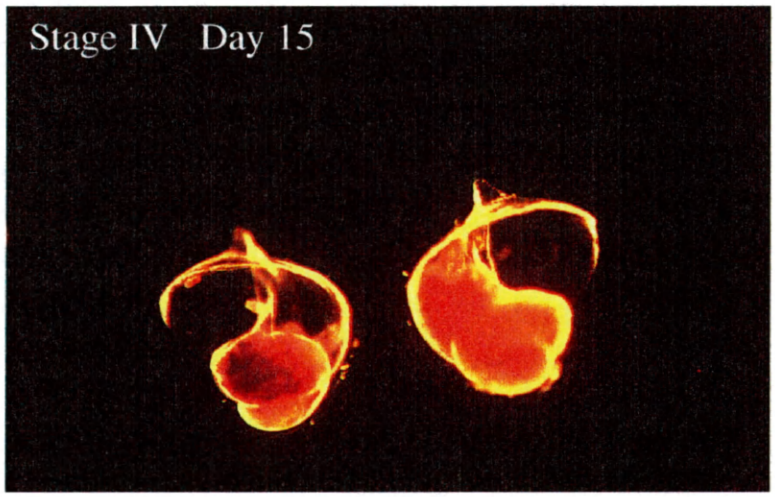
c. Stage III; age 10 days (650-700 μm).

d. Stage IV; age 15 days (850-950 μm).

e. Stage V; age 20 days (950-1050 μm).


a
Stage I Day 1
b
Stage II Day 5
c
Stage III Day 10

d



e

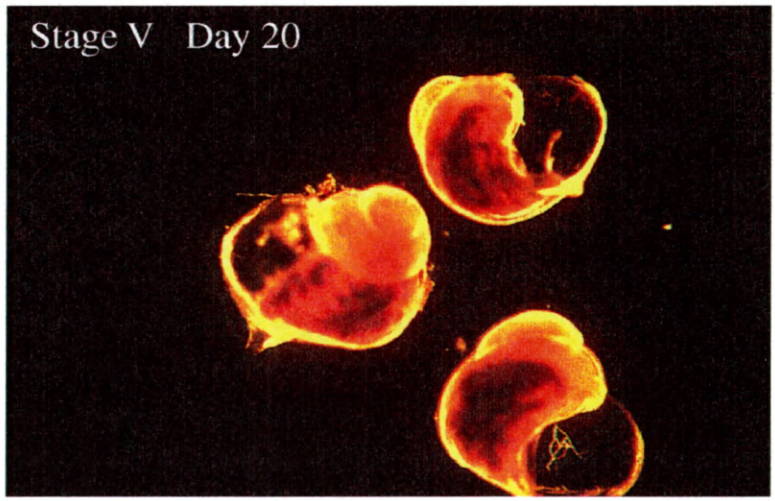
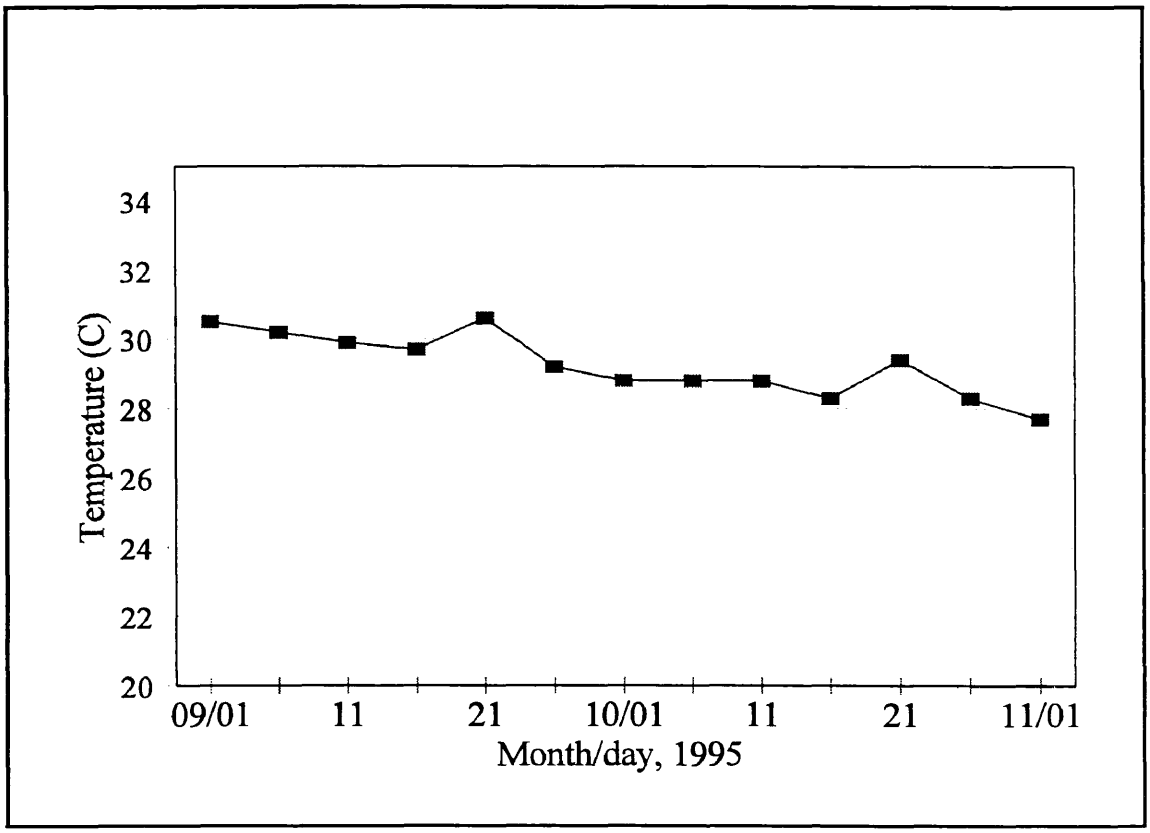


Figure 24: Water temperature (°C) at Lee Stocking Island, September-October 1995.


34
32
30
28
26
24
22
20
Temperature (C)
09/01
11
21
10/01
11
21
11/01
Month/day, 1995

Results: *Strombus costatus*

The larvae survived and grew in the mesocosm, therefore SLIC data was included in the analyses. The statistical analyses performed on the *Strombus costatus* data were the same as for *Strombus gigas.*

Growth rates

Growth curves were created for both shell dimensions for each treatment (figs. 25 a,b) The growth curves do not tend to reach a plateau as did *S. gigas.* Since *S. costatus* takes longer to reach competence, it is possible that the measurements were taken from the linear phase of larval growth. The regression analyses are summarised in tables 29-31 for shell height on each diet and presented graphically in figures 26a-c. Similarly, for shell diameter, the regression analyses are tabulated (tables 31-34) and plotted (figs. 27a-c). The regressions were significant for all treatments ($P<0.05$), as expected of an age-size growth relationship. The data closely fit the linear regressions, producing r^2 values between 87.1% and 92.1%.

The ANCOVA for both shell height (table 35), and shell diameter (table 36) vs age, indicated significant interaction effect between age and shell size. The 95% confidence intervals of regression of the mesocosm larvae vs age, did not overlap with either of the other treatments, indicating a significant difference between the mesocosm animals and those cultured on the high and low diets in the static system. The confidence intervals of the regression curves for the larvae cultured on high and low diets did not overlap, therefore no significant difference in growth response was indicated for the static cultures.

During the course of the experiment, there was on occasions, a 0.5-1.0^{0}C increase above ambient temperature inside the mesocosm which would have introduced a treatment

artifact during the periods of higher temperature. I also noticed that during these time periods, the fouling of the larval shells by epiphytes increased. It did not appear to interfere with the larval behavior, but epiphytic infestations are normally associated with poor culture conditions. Since the ambient water temperature was high, the occasional further increase in temperature inside the chamber could be sufficient to induce low level stress, leading to reduced growth rates. Overheating of the chamber water could be overcome by shading the transparent part of the chamber. With the pulsed configuration, one litre of water is static in the chamber and exposed to constant irradiation from tropical insolation for almost an hour before a water exchange. Alternatively, the pulsing system can be set on smaller time intervals.

Development rates

There was no noticeable difference in growth rates between the three treatments, although the mesocosm animals were smaller than those in static culture, this did not appear to impede their development. Competence was recognized by a change in foot pigmentation from orange to green, eye migration up the tentacles to form eyestalks and onset of swim-crawl behavior, in which veligers use the foot to drag themselves along the substrate. By age 20 days, when the experiment was terminated, approximately 75% of the larvae of all treatments showed signs of competence. This is 12 days earlier and approximately 150 μm greater shell length, than recorded by Davis et al (1993), when larvae were cultured on *Isochrysis galbana* and *Chaetoceros gracilis,* and similar to the development recorded by Brownwell (1977) when culturing *S. costatus* larvae on enriched natural seawater. The

developmental stages were verified using shell morphology as described by Davis (1993), and photographed under dark field dissection microscopy (x5).

Lipid Content

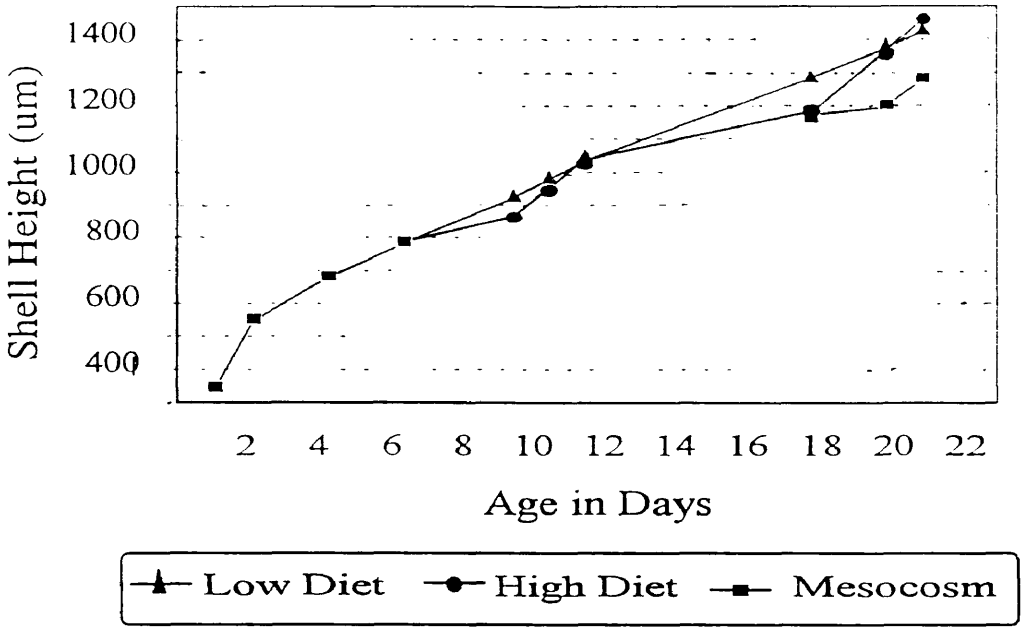
There was no observable variation between the lipid content of the different treatments. The guts of all the larvae observed (except those that were obviously unhealthy) were red and full (fig. 28, b-f). A more quantitative method of lipid analysis would be required to distinguish small differences between treatments.

Figure 25: Growth curves for *Strombus costatus* larvae.

a. Shell height (μm day^{-1}) against age for all treatments

b. Shell diameter (μm day^{-1}) against age for all treatments

a. Shell height vs age



b. Shell diameter vs age

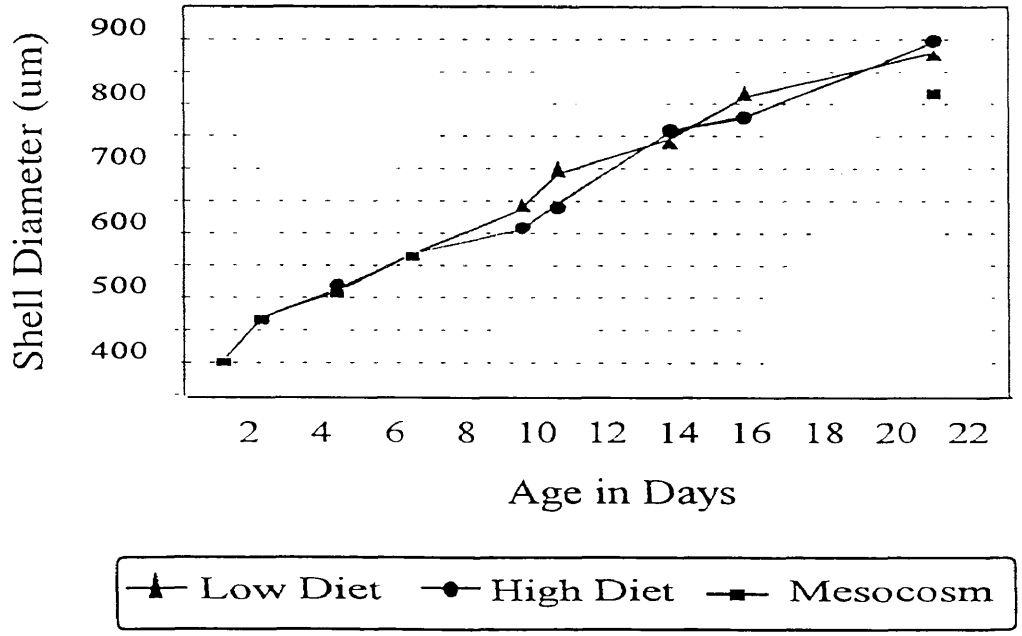


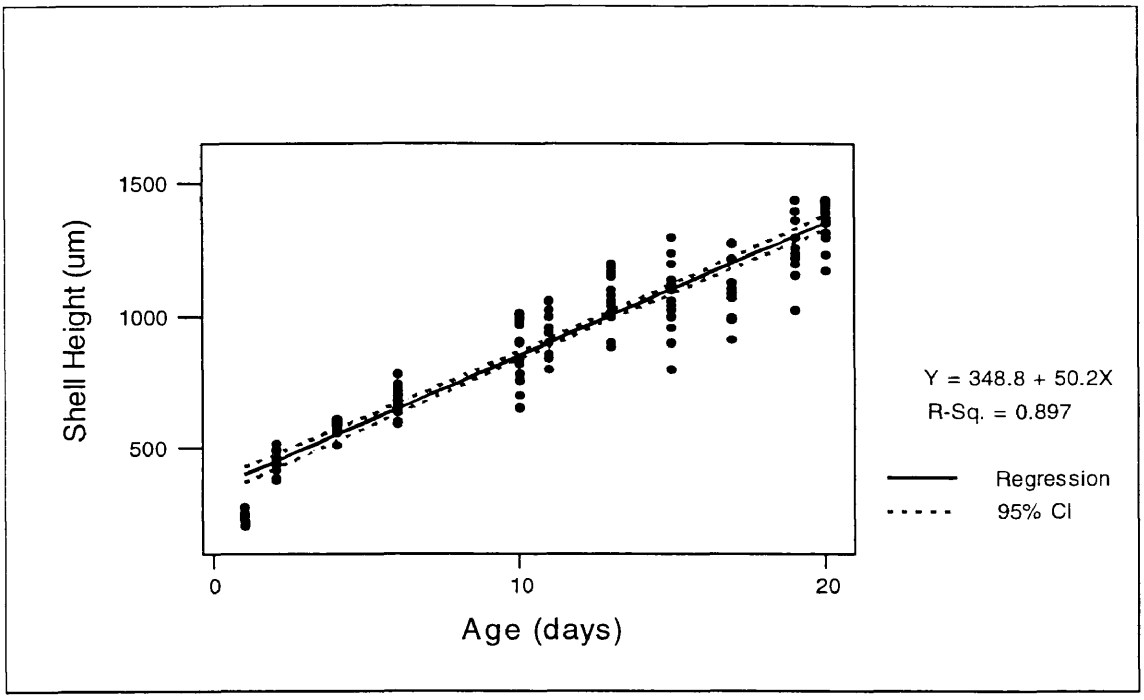
Figure 26: Linear regression fitted line plots for *Strombus costatus;* shell height ($\mu m\ day^{-1}$) against age (days) for different treatments. Hatched lines represent 95% confidence intervals

a. Treatment 1: High phytoplankton diet.

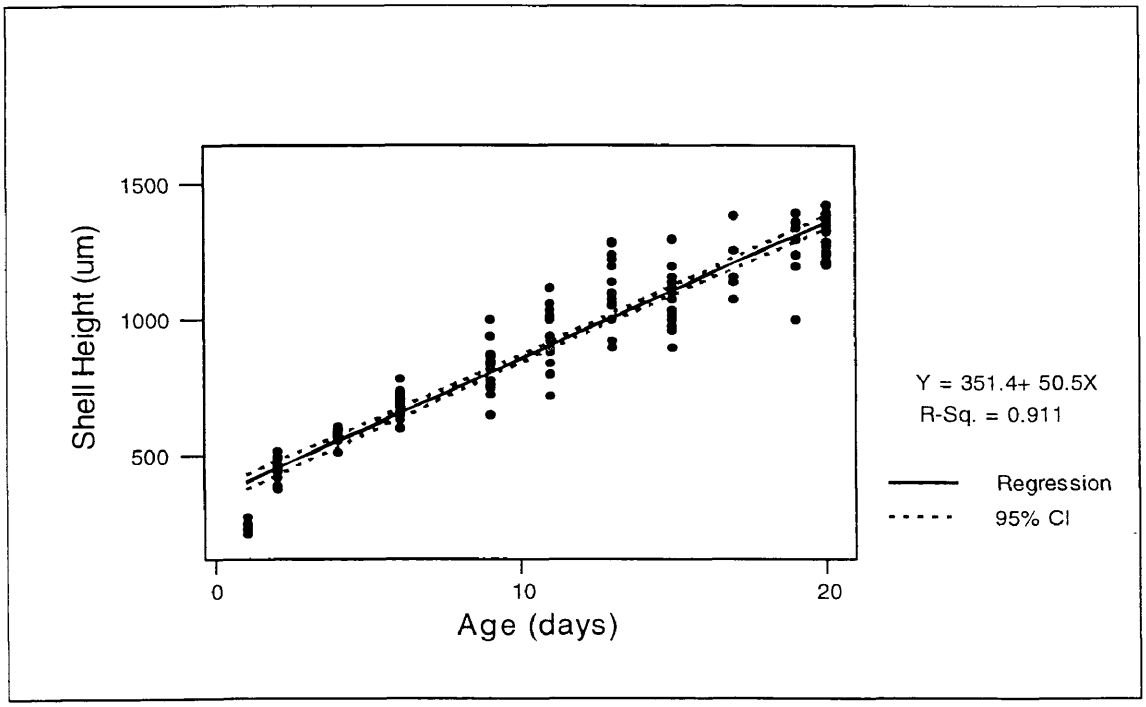
b. Treatment 2: Low phytoplankton diet

c. Treatment 3: larvae cultured in the SLIC.

a.


Shell Height (um)
1500
1000
500
0
10
20
Age (days)
Y = 348.8 + 50.2X
R-Sq. = 0.897
Regression
95% CI

b.


Shell Height (um)
1500
1000
500
0
10
20
Age (days)
Y = 351.4+ 50.5X
R-Sq. = 0.911
Regression
95% CI

c.

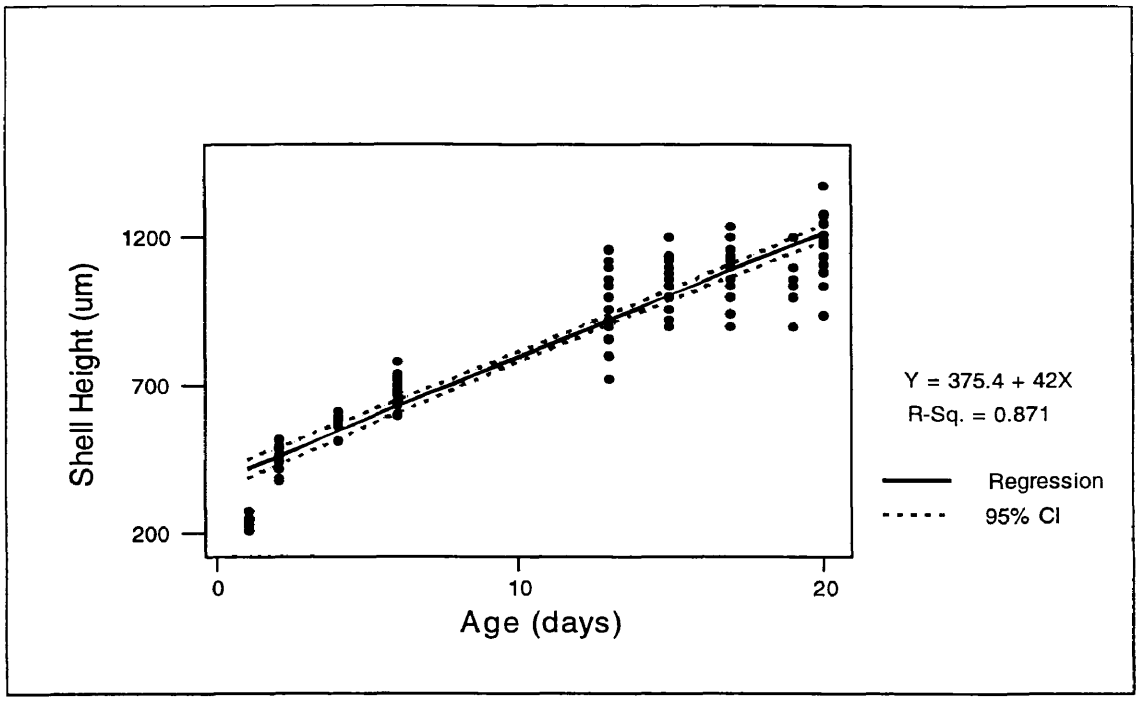

Shell Height (um)
1200
700
200
0
10
20
Age (days)
Y = 375.4 + 42X
R-Sq. = 0.871
Regression
95% CI

Table 29: Linear regression analysis of *S. costatus* shell height on age for high diet

Regression equation:

Shell Height = 348.8 + 50.2 age $R^2 = 89.7\%$

	Coefficient	95% lower	95% upper
Intercept	362.63	317.14	380.39
Age	23.86	47.68	52.65

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	1.67×10^7	1.67×10^7	1588.74	0.00
Residual	183	1.93×10^6	1.05×10^4		
Total	184	1.87×10^7			

Table 30: Linear regression analysis of *S. costatus* shell height on age for low diet

Regression equation:

Shell height = 351.4 + 50.5 age $R^2 = 91.1\%$

	Coefficient	95% lower	95% upper
Intercept	351.4	321.86	380.95
Age	50.50	48.17	52.82

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	1.72×10^7	1.72×10^7	1836.89	0.00
Residual	179	1.67×10^6	9350		
Total	180	11.89×10^7			

Table 31: Linear regression analysis of *S. costatus* shell height on age in SLIC

Regression equation:

Shell height = 375.4 + 42 age R^2 = 87.1%

	Coefficient	95% lower	95% upper
Intercept	375.35	340.99	409.72
Age	41.99	39.43	44.57

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	1.18×10^7	1.18×10^7	1042.5	0.00
Residual	155	1.76×10^6	1.14×10^4		
Total	156	1.36×10^7			

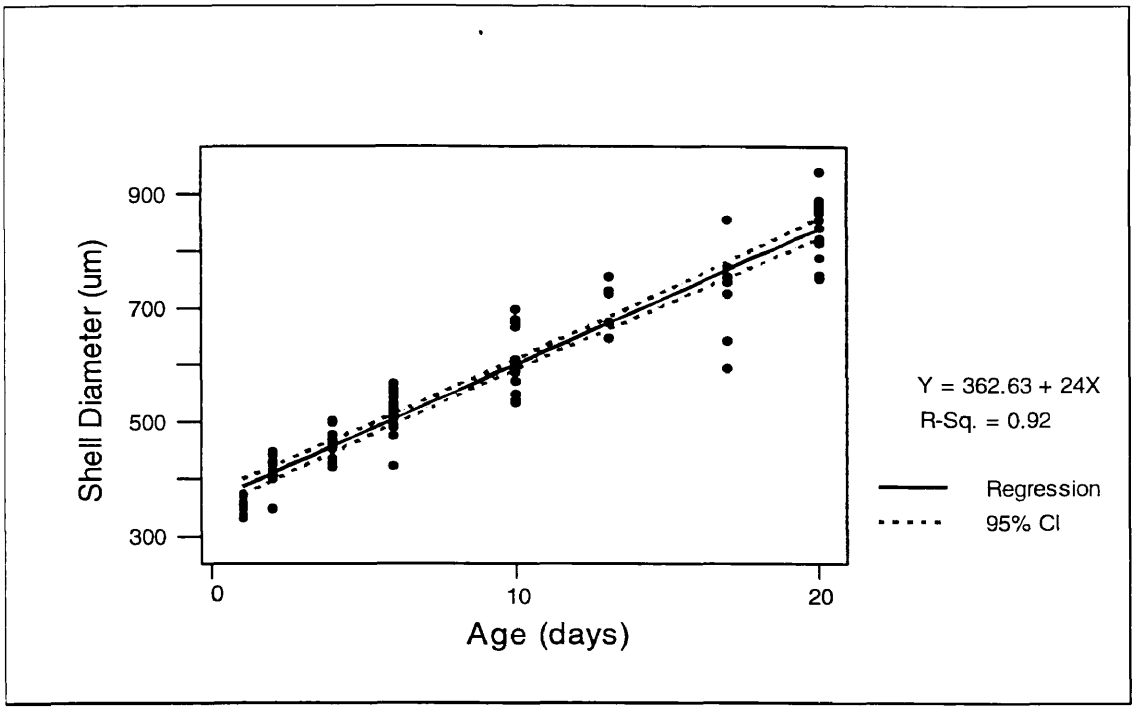
Figure 27: Linear regression fitted line plots for *Strombus costatus;* shell diameter ($\mu m\ day^{-1}$) against age (days) for different treatments. Hatched lines represent 95% confidence intervals

a. Treatment 1: High phytoplankton diet.

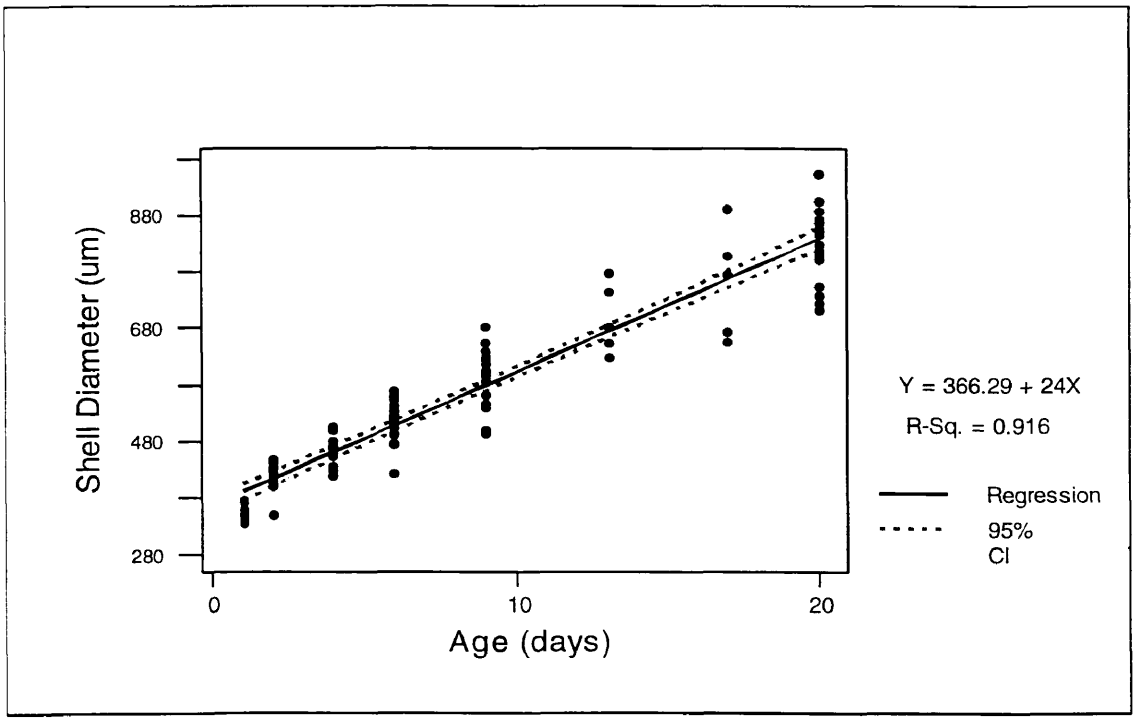
b. Treatment 2: Low phytoplankton diet

c. Treatment 3: larvae cultured in the SLIC.

a.


Shell Diameter (um)
900
700
500
300
0
10
20
Age (days)
Y = 362.63 + 24X
R-Sq. = 0.92
Regression
95% CI

b.


Shell Diameter (um)
880
680
480
280
0
10
20
Age (days)
Y = 366.29 + 24X
R-Sq. = 0.916
Regression
95%
CI

c.

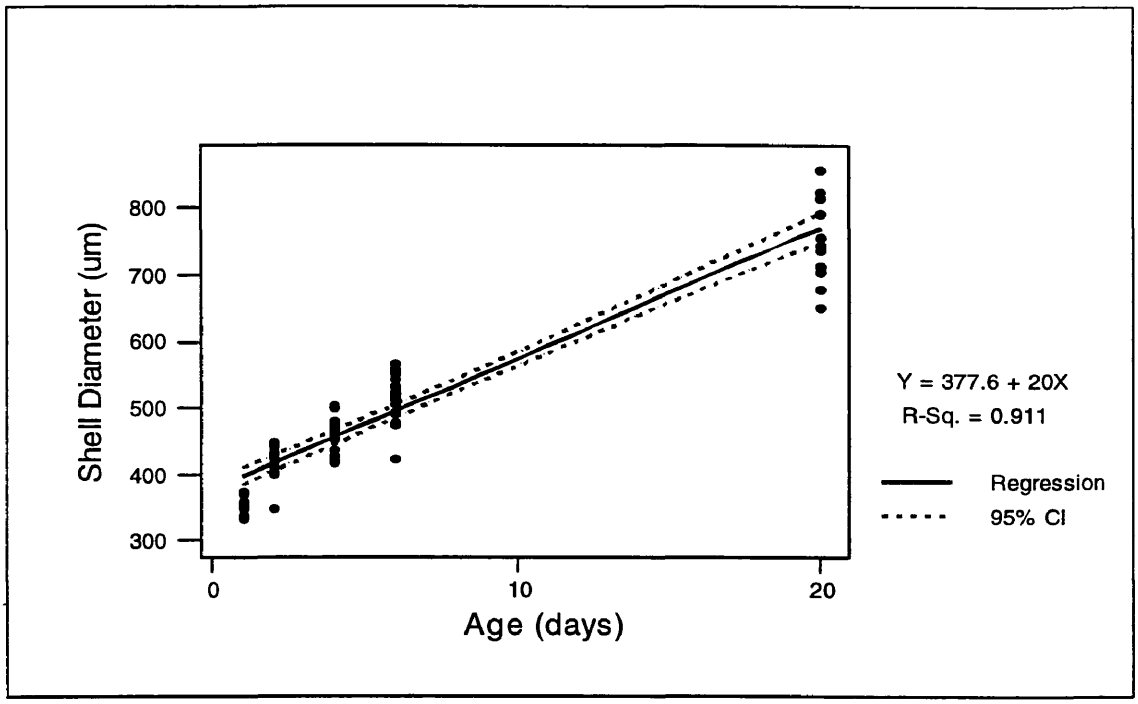

Shell Diameter (um)
800
700
600
500
400
300
0
10
20
Age (days)
Y = 377.6 + 20X
R-Sq. = 0.911
Regression
95% CI

Table 32: Linear regression analysis of *S. costatus* shell diameter on age on high diet

Regression equation:

Shell Diameter = 362.6 + 23.9 age $R^2 = 92.1\%$

	Coefficient	95% lower	95% upper
Intercept	362.63	347.95	377.31
Age	23.86	22.50	25.22

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	2.51×10^6	2.51×10^6	1213.67	0.00
Residual	104	2.15×10^5			
Total	105	2.73×10^6			

Table 33: Linear regression analysis of *S. costatus* shell diameter on age on low diet

Regression equation:

Shell Diameter = 366.3 + 23.7 age $R^2 = 91.6\%$

	Coefficient	95% lower	95% upper
Intercept	366.29	351.00	381.58
Age	23.66	22.25	25.07

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	2.55×10^6	2.55×10^6	1106.76	0.00
Residual	102	2.35×10^5	2307		
Total	103	2.79×10^6			

Table 34: Linear regression analysis of *S. costatus* shell diameter on age in the SLIC

Regression equation:

Shell Diameter = 377.6 + 19.7 age $R^2 = 91.1\%$

	Coefficient	95% lower	95% upper
Intercept	377.61	363.55	391.66
Age	19.69	18.25	21.13

	DF	Sum Sq.	Mean Sq.	F-value	P-value
Regression	1	1.32×10^6	1.32×10^6	740.85	0.00
Residual	72	1.28×10^5	1.78×10^3		
Total	73	1.45×10^5			

Table 35: ANCOVA of age vs shell height for *S. costatus* for high vs low diets

	Df.	Seq SS	Adj SS	Adj MS	F	P
Age	1	$4.51x10^7$	$4.54x10^7$	$4.54x10^7$	$4.38x10^3$	0.00
Diet	2	$5.51x10^5$	$1.66x10^4$	$8.3x10^3$	0.80	0.45
Diet*age	2	$3.11x10^5$	$3.11x10^5$	$1.55x10^5$	15.00	0.00
Error	517	$5.36x10^6$	$5.36x10^6$	$1.04x10^4$		
Total	522	$5.13x10^7$				

Table 36: ANCOVA of age vs shell diameter for *S. costatus* high vs low diets

	Df.	Seq SS	Adj SS	Adj MS	F	P
Age	1	$6.49x10^6$	$6.11x10^6$	$6.11x10^6$	$2.93x10^3$	0.00
Diet	2	$1.62x10^4$	$4.46x10^3$	$2.22x10^3$	1.07	0.34
Diet*age	2	$4.09x10^4$	$4.09x10^4$	$2.05x10^4$	9.82	0.00
Error	278	$5.79x10^5$	$5.79x10^5$	$2.08x10^3$		
Total	283	$7.12x10^6$				

Figure 28: Photographs of *Strombus costatus* larval shells, showing stages of development.

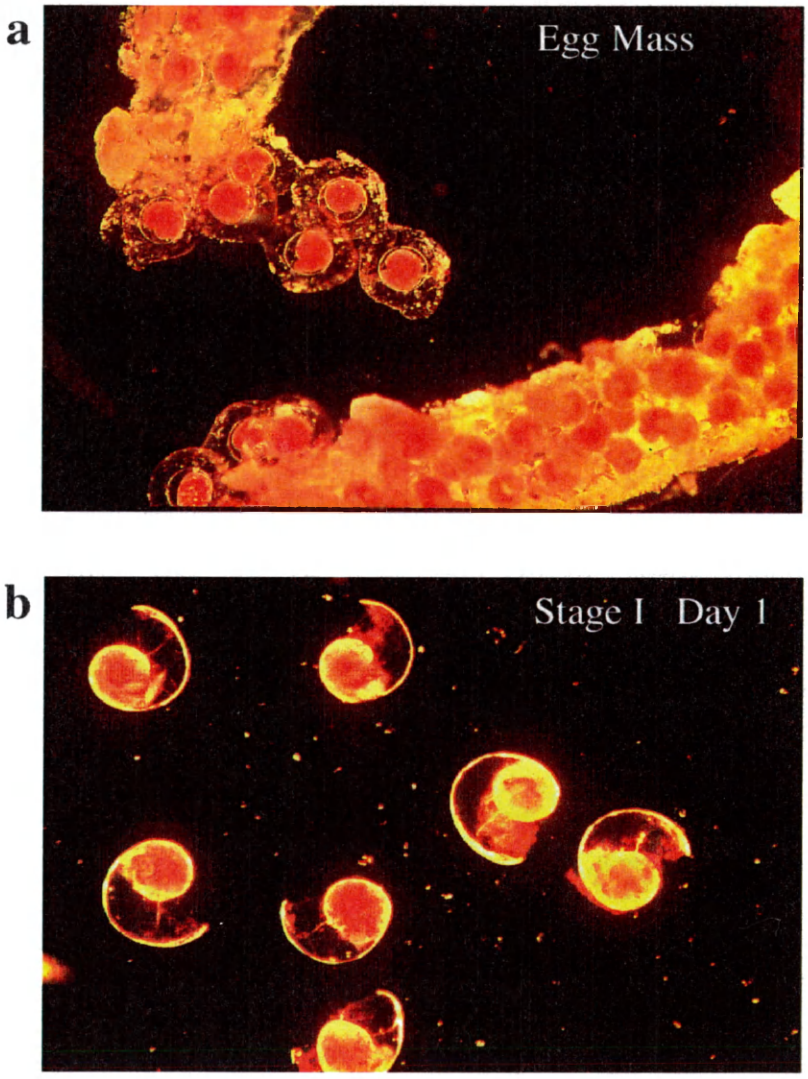
a Egg mass showing larvae within capsules.

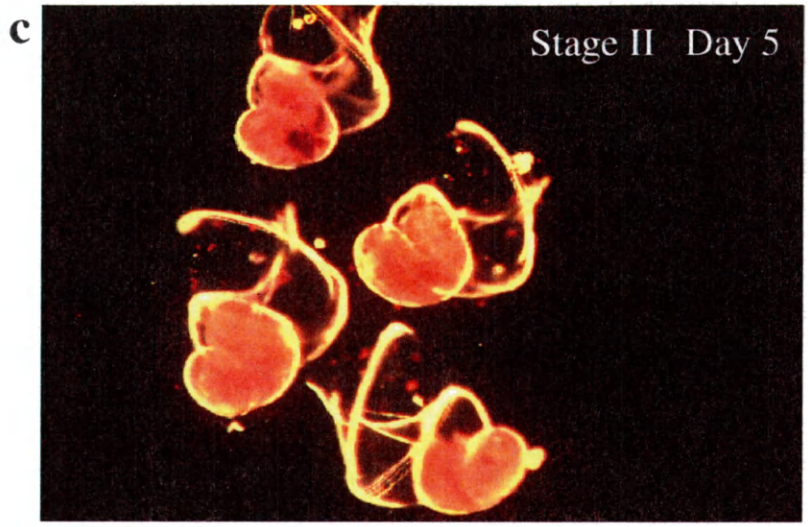
b. Stage I; age 1 day (300-350 μm).

c. Stage II; age 5 days (700-800 μm).

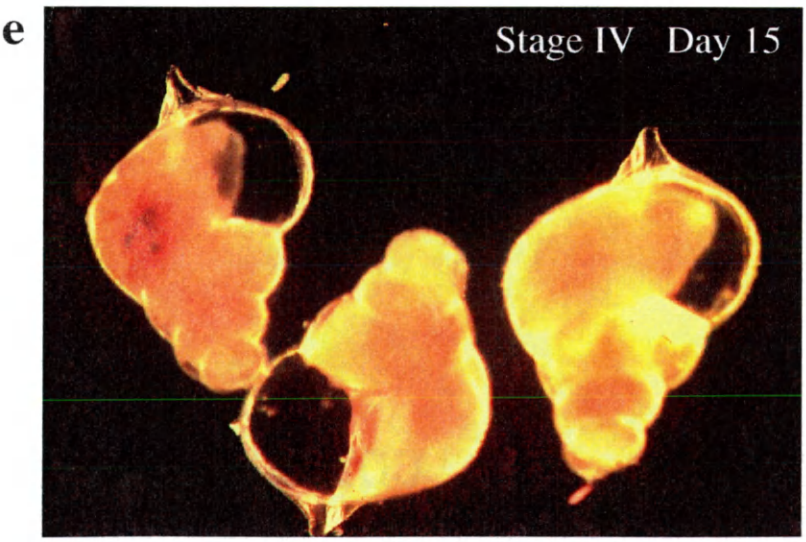
d. Stage III; age 9 days (900-1000 μm).

e. Stage IV; age 15 days (1200-1300 μm).


a
Egg Mass
b
Stage I Day 1


c
Stage II Day 5


d
Stage III Day 9


e
Stage IV Day 15

Discussion

Growth and Development Rates: *S. gigas*

The regression equations for both diets produced growth coefficient of 33 μday^{-1}. Davis (1993) cultured three species of *Strombus* larvae (*S. gigas; S. costatus; S pugilis*) on mixed algal diets. The fastest growing species was *S. gigas* at 39$\mu m\ day^{-1}$. Davis (1994c) cultured *S. gigas* larvae in a large mesocosm (capacity), fed by the phytoplankton rich waters of the Grand Bahama Bank. Observed growth rates (50$\mu m\ day^{-1}$) and time to metamorphosis (14 days) were faster than previously recorded in the literature. Previous studies have cultured larvae on algal diets; Davis was the first investigator that successfully cultured *S. gigas* larvae in the field.

S. costatus: Growth and Development Rates

There was no significant difference in growth and development rates between the treatments. The high and low phytoplankton diets served as an upper and lower limit of the range of phytoplankton that the mesocosm animals were exposed to.

As mentioned in the results section, much faster growth (51 vs 28 $\mu m\ day^{-1}$) rates and much earlier signs of metamorphosis (20 vs 32 days) were observed in *S costatus* on natural diet than on algal diet in a study carried out by Davis et al (1993). When grown on enriched seawater in a study in Venezuela; however, the growth and development times were similar to the results of this study (Brownwell, 1977). Ballantine and Appeldoorn (1982) recorded similar growth rates (51$\mu m\ day^{-1}$) and time to metamorphosis (16 days) on a mixed algal diet of *Isochrysis, Tetraselimis chui* and *Thalssiosira fluviatilis*. This is a finding of

some importance in the evaluation of the ecology of the species. It has been shown here that larval life *can* be much shorter than found previously in laboratory studies; if extrapolating from laboratory data to the ecology of the organism in it's natural environment, this decrease in duration of the larval lifecycle could have significant consequences for the estimation of larval dispersal capacity and growth rates.

If larvae from supposedly nutrient deficient, poor phytoplankton waters were taken and fed high levels of algae, a dramatic increase in growth and development rates might be expected. This is not the case in the present study, in fact the reverse seems to occur. As mentioned in the introduction, there is ample evidence that larval invertebrates can exploit sources other than phytoplankton cells for nutrition, which indicates that measuring phytoplankton does not necessarily give a good indication of food quality. Throughout the course of evolution, animals are selected upon to become adapted to their environments. There is always a degree of flexibility or adaptive behavior, within which the animal can compensate for environmental fluctuations, if it cannot, it dies. This premise will also apply to fluctuations and deficiencies in levels of nutrition. I could find no evidence in the literature however, that the abilty to exploit alternative sources of nutrition is any more prevalent in nutrient poor than nutrient rich environments.

The velar lobes of the Strombid larvae divide twice during development and elongate until the edge area is large. Strathmann (1987) observed that the tissue dry weight increased non linearly with velar length; as the larvae became larger, their growth accelerated (this is not necessarily reflected in shell growth, which does not increase linearly with tissue mass accumulation). The increase in velar length therefore, may be an adaption for life in low

particulate environments, which allows them to exploit more of the food items in the water body. Since there have been no thorough definitive studies on correlation of velar area with environmental nutritional status, this is however, just a hypothesis.

A large velar area of gastropods facilitates swimming with a larger body mass than is possible for a bivalve with their velar configuration. This is significant in terms of size at settlement; Strombid larvae metamorphose between 950-1400 µm, wheras oysters are approximately 300 µm at metamorphosis. A larger size may confer advantage to the jeuvenile in terms of protection from predaton and greater energy reserves.

There are many sources of potential mortality to marine invertebrate larvae, including starvation, offshore transport, disease and predation. Some larvae are capable of extending their competent phase, which allows them to search for suitable substrate or disperse over large distances, the ultimate example of this is teleplanic larvae. The costs involved with traversing such large distances are increased risk of predation and transport to an area with no settlement substrate. The ramifications of remaining for extended periods of time in the plankton are exposure to these potential source of mortality for longer periods of time.

Marine ecosystems are patchy; if a larva encounters a nutrient poor patch of water it may encounter a good patch shortly afterwards. Calculations that determine that environments are food limited are based on growth rates obtained on algal cultures and compared with phytoplankton in natural systems (Lucas 1978).

Optimal growth rates have been determined from laboratory studies using higher phytoplankton levels than the organism under investigation enounters naturally (Davis, 1994; Ballantine and Appeldoorn, 1983). The unaturally high food levels required to acheve

the optimal growth may be a consequence of suboptimal culture diets. Larvae can compensate (within limits) for lack of calories, (development slows, larval period is extended), but if an essential nutrient is limiting in the diet, the larvae cannot compensate and development is affected.

Conclusions

In Situ versus Cultured Algal Diets

Evidence from this investigation and other studies (Ballantine and Appeldoorn, 1983; Davis, 1993; Davis, 1994c) illustrates that there is a difference in growth and development rates between larvae cultured in the ambient environment compared with those cultured on algal diets (Table 21). This investigation illustrates the limitations of extrapolating from data derived from laboratory experiments to presumptive situations on the field. It is important to undertstand growth and development rates of a given species over the range of nutrient levels available to that species in the natural environment. Differences in culture methods may account for the differences between the observed results in experimental studies.

General Conclusions

The species studied in this investigation produced faster or equivalent growth and development rates when cultured *in situ* than when cultured on algal diets. These results; however, are specific to the species, location and season under investigation.

The comparison of *in situ* and laboratory culture of three species of molluscan larvae in two different ecosystems, shows different growth and development rates using natural and cultured algal diets. This illustrates the limitations of drawing conclusions regarding the ecology of an organism from data generated in the laboratory using cultured algal diets. The optimal development observed in the laboratory is probably not typical of larvae in their natural environment and should be considered a separate phenomenon. It is not always logistically possible to culture organisms in situ or use natural water supplies; however, data should be interpreted with the understanding that the conditions of the laboratory experiment may not reflect the situation in the field.

Strombus larvae exhibited similar growth and development rates when grown on two different levels of phytoplankton. Larvae may have been were compensating for low nutrients whilst in the low phytoplankton water, by increasing clearance rates. It is also probable that larvae were deriving nutrition from a source other than phytoplankton (bacteria, DOM).

The SLIC functioned well in the oligotrophic tropical ecosystem, but the design may not be tractable in an ecosystem with high organic content because of the high degree of fouling. The pulsed configuration of the SLIC was superior to the continuous flow since it reduced power consumption and fouling levels. If used in an ecosystem with high insolation, the chamber should be shaded to prevent temperature increase inside the chamber.

Appendix 1. Technical report on the construction and operation of the Submersible Larval Incubation Chamber (SLIC)

1a: Submersible Larval Incubation Chamber - Technical Report

External Framework

This is constructed from Schedule 40 PVC 1" piping. The frame measures 45cm width x 90cm length x 57 cm height. It is a basic cuboid shape with a crossbar across the top to support the chamber. The components are bonded using clear PVC primer and cement (1). Use of pipe cleaner prior to priming is also recommended. A baseboard of 1/2" plexiglass (2) supports the battery box; this has a plexiglass bracket to prevent the box moving on the baseboard and a bracket to hold it securely in place. The baseboard is attached to the frame by nylon nuts and bolts (1). There are holes drilled in the framework to allow water to enter and prevent the frame being positively buoyant.

Internal Plumbing

Standard Schedule 40 PVC plumbing parts (1) are used throughout except in one section. Some flexibility is required to disconnect the chamber from the slip screws; to reduce strain on the joints, transparent flexible 3/4" hose is used between the filter and the piping holding the chamber. A full list of parts used can be found in the appendix. The internal plumbing is held to the framework by cable ties. The chamber is suspended from it's support by a rubber belt with plastic quick release buckle (3).

Pump

This is a RULE 360 gph 12 volt, 2.5 amp bilge pump (4). The intake is at the base of the pump, therefore it needs to be set high up on the frame to avoid excessive sediment intake, especially in soft, muddy benthos. The water outlet is a 3/4" male hose fitting.

These pumps are designed to function in standing water but they are not generally totally submerged for extended periods of time; therefore the wire outlet is sealed with 'Plumbers Goop' (1) for extra leak protection. The pump is attached to the framework by means of a plastic bracket.

Filter

These are Amteke filter cartridges, listed as "cold water housing with a styrene acrylonitrile SAN sump" (5) The dimensions are 12 1/16" H x 5 1/8" diameter. The filter itself is covered with a 90 um Nitex (6) sock and held in place with a cable tie. The function of this is to crudely prefilter the water before it reaches the Nitex screen inside the chamber. Despite being flushed periodically, fouling is still a potential problem.

Valves

These are TORO (7) 24 v AC, remote control valves with 3/4" outlet pipe. They are actuated remotely by the battery. Two valves are placed at each end of the chamber. One controls the inlet flow and the other the outlet. When the former is closed the latter is open and vice versa. The state of the valve is controlled by the electronic circuit inside the battery box. At preset time intervals, the state of each valve is switched, creating a flow reversal through the chamber.

Incubation Chamber.

The body of the chamber is a cylinder of clear acrylic piping (8) 4.5" in diameter and 12" long. Two schedule 40 PVC 4.5"end caps (1) enclose the cylinder. Two holes were drilled in the end caps, to accommodate two 3/4" couplers. These house the inlet and outlet pipes; these are fitted with slip screws (1) to allow easy removal and exchange of the

chamber during sampling. Inside the main chamber are two pieces of 3.5" PVC connectors (8.5cm long) these are used to hold the Nitex screen securely and also serve as manifolds to allow the flow stream to disperse before entering the larval chamber.

Battery Box

This is an Ikelite (9) 1/4" plexiglass box (model 5910) . The lid has a rubber seal and an O-ring; it is held closed by metal clamps. There are ten holes drilled in the top of the lid for the wires (from the valves and the pump) to pass through. These are sealed with 'Plumbers Goop' to make them watertight. Inside the box is a bracket for the battery to prevent movement. From the displacement value of the box volume (14229 cc = 14.32 kg of water) and the weight of the battery (12 kg), the underwater weight of the box and contents is 2.32kg.

Battery

This is a Powersonic rechargeable 12 volt, lead-acid battery ; Model PS-12330 (10). The dimensions are 13.2 cm width x 19.7 cm length x 18.2 cm height. Weight is 12 kg. The nominal capacity is 33 amp hours at 20 hour rate. Two such batteries are required to keep the SLIC running continuously since they will need to be removed for recharging.

Electronic Circuit

The electronic circuit controls the state (open or closed) of the valves on a preset, continuously-cycling time interval and regulates the voltage to the pump to control the flow rate into the chamber.

The circuit was designed on a protoboard. A diagram of the final circuit was photocopied onto toner transfer paper (11) and transferred to the 9 x 4.5" copper plated

circuit board (11) using a household iron. The board was then submerged in an Archers etchant solution (12) for 35 minutes. This has to be changed frequently and agitated to remove the background copper plating, leaving the copper circuit diagram in relief on the board. Once the board had been etched, holes were drilled into the board to attach the components. A 1/16" drill bit was used for the smaller components and a 1/8" bit for the heavier (grade 22) wires. The components were then soldered into the appropriate holes using an Ungar Controlled Soldering System.

Power is supplied to the circuit by the 12 volt battery. The leads are permanently clamped to the terminals of the battery, and the power supply is switched on and off from outside the battery box via a magnetic reed switch (13). The switch is mounted on the inside of the box but is actuated by a magnet on the outside. When the magnet is placed above the switch (note: orientation is important) the connectors inside are pulled together and current passes through. When the magnet is removed the connectors inside the switch spring apart and the switch is off. A 3.5" digit LCD voltmeter (14) (input range +/- 19.99v) is attached to the inside of the box to indicate battery power.

Time delay relays (TDR) model CNT-35-26 (13), control the operation of the solenoid valves. (The TDR needs to be mounted on a surface mount screw terminal socket (13) model S7E123) . They can be programmed for time intervals from 1 to 999 seconds, minutes or hours. There are 10 possible timer functions; the 'Power/Control Recycle' function was used on both TDR's. For this function, the output relay is turned on at the end of a programmed time interval. The relay stays on for a preset time interval then turns off . The cycle continues until 'reset' or power cut switches the relay off. The SLIC circuit board has

two TDR's which function together. TDR 1 runs continuously; it changes modes after preset time interval and simultaneously activates TDR 2. They run together in different modes for an equal time interval; TDR 2 is switched off when TDR1 switches back to the original mode. When the TDR's switch modes, the state (open or closed) of each valve changes, reversing the flow through the chamber.

The pump flow rate is controlled via an adjustable voltage regulator (12), plus heat sink (12) and a cermet (10 k ohms) potentiometer (12). This allows the voltage to the pump and hence the motor speed to be regulated. The flow rate is a function of the motor speed therefore by altering the resistance on the potentiometer, the pump output can be controlled. The voltage regulator also requires a 240 ohm resistor (12) and a 1uF capacitor (12) to function correctly (the wiring diagrams are printed on the back of the component packages). The current passes from the positive terminal, through the reed switch, through the voltage regulator system and through a DPDT (double pull double throw) 5 amp relay (12), before reaching the pump. The DPDT relay also controls current passing from positive to the TDR's and all components are activated simultaneously. A schemeatic representationof the circuit board is shown in figure 29.

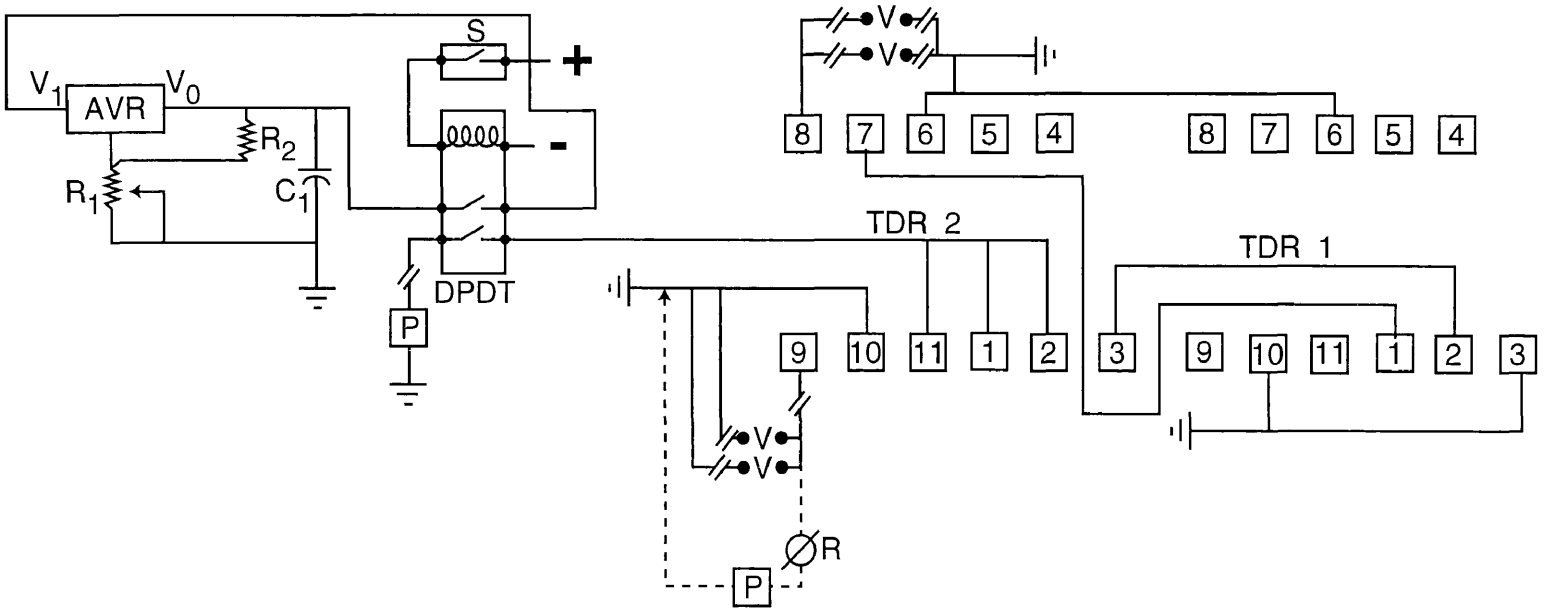
The wires from the circuit board are connected to those of the battery, valves and pump by nylon connectors (1) for ease of coupling/uncoupling. This is necessary since the wires from the external components enter through (and are sealed into) holes in the lid of the box. The lid must be detachable for battery recharging, maintenance of the circuit board etc.

* Suppliers are listed in Appendix 1b, by number in parentheses.

Addresses are listed in Appendix 1c.

Figure 29: Schematic of the SLIC control circuit

Schematic For SLIC Circuit Board



C_1 = Capacitor
R_2 = 270Ω Resistor
R_1 = 10kΩ Potentiometer
AVR = Adjustable Voltage Regulator
DPDT = Double Pull Double Throw
12 VDC PC Relay
------ = Alternative Configuration for Pulsed Flow

P = Pump
V = Solenoid Valve
S = Reed Switch
+/- = Power Terminals
1-11 = Terminals of Time Delay Relay
R = Rheostat

Appendix 1b: Components used in the construction of the SLIC

Component (No. of Units)	Source	Cost per Unit
Circuit Board Plate (1)	Digi-Key Corporation	$ 8.06
Transfer Paper (1)	Digi-Key Corporation	$ 27.95
Time Delay Relay (2)	Newark Electronics	$ 77.62
Surface Mount Screw Terminal (2)	Newark Electronics	$ 7.81
Voltage Regulator (1)	Radio Shack	$ 1.99
Heat Sink (1)	Radio Shack	$ 1.99
Variable Resistor 10K Ohms (1)	Radio Shack	$ 1.49
Resistor (1)	Radio Shack	$ .49
Capacitor (1)	Radio Shack	$ .49
DPDT Switch (1)	Radio Shack	$ 3.99
Digital Display Voltmeter (1)	D1 International Inc.	$ 29.50
Battery Box (1)	Kay Gee Plastics	$ 98.85
Battery (12v) 33 AH Nom. Cap. (1)	Battery Factory Outlet	$ 72.75
In-Line Cartridge Filter (1)	Carters	$ 27.00
360 gph Bilge Pump (1)	Jordan Marine	$ 16.00
Battery Actuated Solenoid Valves (4)	Green Planters Nursery	$ 22.00
Plumbers Goop (1)	Ace Hardware	$ 5.00
3/4" Ell (2)	Ace Hardware	$ 0.59
3/4" Pushfit-Female Coupler (8)	Ace Hardware	$ 0.69
3/4" Slip Screw Unit (4)	Ace Hardware	$ 3.89

3/4" Female-Hose Tee (1)	Ace Hardware	$ 1.98
3/4" Pushfit-Female Ell (2)	Ace Hardware	$ 0.92
3/4" Male-Hose (2)	Ace Hardware	$ 0.79
4" End Cap (2)	Ace Hardware	$ 5.99
3/4" Sched 40 PVC Piping (20ft)	Ace Hardware	$ 2.99
1" Ell (8)	Ace Hardware	$ 0.98
1" Tee (14)	Ace Hardware	$ 1.25
1" Sched. 40 PVC piping (30ft)	Ace Hardware	$ 2.99
Acryllic Piping (1)	Norva Plastics	$ 25.00
Reusable Cable Ties (12)	Carters	$ 0.49
Nitex Screen (90um) (1sq meter)	Tetko Inc	$ 32.20
Plexiglass Plate (50x45 cm)	Norva Plastics	$ 40.00

Appendix 1c: List of Suppliers

1. Ace Hardware,

Gloucester Supply,

Old Rt 17,

Hayes, Va 23072

Tel: (804) 642-5300

2. Rohm and Haas,

Philadelphia, PA 19105

3. Dacor Diving supplies.

c/o Divers Supply,

Virginia Beach,

VA

4. Rule Industries Inc.,

Cape Ann Industrial Park,

Gloucester, MA 01903.

Tel: (617) 272-4084

8. Norva Plastics,

2609 Monticello Ave.,

Norfolk, VA 23517.

Tel: 1-804-622-9281

9. Kay Gee Plastics,

3317 Tait Terrace,

Norfolk, VA 23513

Tel (804) 853-7651

10. Power-Sonic Corp.,

PO Box 5242,

3106 Spring Street,

Redwood City, CA 94063

Fax (415) 366-3662

11. Digi-Key,

701 Brooks Ave. South,

PO Box 677,

Theif River Falls, MN 56701

Tel: 1-800-344-4539

5. Cole Palmer Instrument Co.,

7425 North Oak Park Ave.,

Niles, IL 60714 - 9930.

Tel: 1-800-323-4340

6. Tetko Inc.,

3335 Highland Ave.,

Briarcliff Manor. NY 10510

Tel: (914) 941-7767

7. The TORO Company,

Irrigation Division,

Riv., CA.

12. Radio Shack,

PO Box 338,

Hayes, VA 23072

Tel: (804) 642-6255

13. Newark Electronics,

8100 Three Chopt Rd.,

Richmond. VA 23229-4833

Tel: (804) 282-5671

14. D1 International Inc.,

95 East Main St.,

Huntingdon, NY 11743.

Tel: (516) 673-6893

Appendix 2: Algal feeding regimes for the culture of larval *C. virginica:*

Appendix 2a: VIMS Hatchery Feeding Regime

Days 1 and 2:

T-ISO: 20, 000 cells ml^{-1}

Days 3 and 4:

T-ISO: 20,000 cells ml^{-1}

Cc - Tetra: 10,000 cells ml^{-1}

Days 5 to 13:

T-ISO: 25,000 cells ml^{-1}

Cc - Tetra: 25,000 cells ml^{-1}

(50% of each by volume)

'T-ISO' (*Isochrysis galbana*): Small Flagellate; 7-10 mm

'Cc' (*Chaetoceros calcitrans*): Diatom, with spines; ~17mm.

[During handling most lose the spines to yield; ~7mm]

'Tetra' (*Tetraselmas maculata*): Flagellate; ~20 mm

Appendix 2b: Feeding regime used on the Eastern Shore of Virginia

Day 1		**Day 5**	
T-iso	20, 000 cells ml^{-1}	T-iso	40, 000 cells ml^{-1}
Cc-Tetra	10, 000 cells ml^{-1}	Cc-Tetra	30, 000 cells ml^{-1}
Day 2		**Day 6**	
T-iso	20, 000 cells ml^{-1}	T-iso	40, 000 cells ml^{-1}
Cc-Tetra	20, 000 cells ml^{-1}	Cc-Tetra	40, 000 cells ml^{-1}
Day 3		**Day 7**	
T-iso	30, 000 cells ml^{-1}	T-iso	50, 000 cells ml^{-1}
Cc-Tetra	20, 000 cells ml^{-1}	Cc-Tetra	40, 000 cells ml^{-1}
Day 4		**Day 8**	
T-iso	30, 000 cells ml^{-1}	T-iso	50, 000 cells ml^{-1}
Cc-Tetra	30, 000 cells ml^{-1}	Cc-Tetra	50, 000 cells ml^{-1}

Continue as Day 8 until metamorphosis.

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