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Feeding and growth of prosobranch veligers

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University of Hawaii, 1993
FEEDING AND GROWTH OF PROSOBRANCH VELIGERS

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI'I IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY

MAY 1993

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ACKNOWLEDGEMENTS

I gratefully acknowledge the support, patience and guidance of my chairperson, Dr. Michael Hadfield. He gave direction and focus to this research and has helped to shape my scientific thinking. I thank the other members of my committee: Drs. E. Alison Kay, Robert A. Kinzie III, Stephen R. Palumbi, Andrew D. Taylor and Richard R. Young for their insight and discussion. Thanks are due to Dr. Marilyn Dunlap and my fellow graduate students, Dr. Cynthia Hunter, Dr. Stephen Kempf, Dr. Stephen Miller, and Dr. Amy Ringwood for technical assistance and discussion. Members of the Palumbi laboratory, Paul Armstrong, Dr. Scott Baker, Bailey Kessing, Dr. Andrew Martin, Owen McMillan, Ed Metz and Sandra Romano, shared their computers and humor which eased the completion of this dissertation. I thank Steven Chow for moral support and help with the darkroom work. The staff of Kewalo Marine Laboratory kept the seawater flowing and I thank them for that. Special thanks are due to my parents, Marion and Alexander Bell, who fostered my education at an early age and supported the completion of this dissertation at a late age.

This research was supported by the Hawaiian Malacological Society, Southwestern Malacological Society, Lerner-Gray Fund, Sigma-Xi, ARCS of Hawaii, Charles H. and Margaret B. Edmondson Fund, and the Department of Zoology, University of Hawaii. I am grateful for all financial contributions to this research.
ABSTRACT

For marine invertebrates, larval growth rates in the plankton impact dispersal, recruitment and the timing of metamorphosis. Previous laboratory studies of gastropod larvae have suggested that adequate growth is achieved only when concentrations of algal food are much higher than phytoplankton concentrations in the ocean, especially those in oligotrophic environments. The few studies of larvae feeding on food in natural seawater have suggested two possibilities: larvae are food-limited or larvae are growing at near-maximal rates.

The effects of larval concentration, food diversity, nannoplankton concentration and picoplankton abundance on larval growth and survival were evaluated for the prosobranch gastropods *Crepidula aculeata* and *Crucibulum spinosum*. Larvae were grown on particles in natural seawater or on single or multiple species diets of the laboratory-cultured algae Tahitian *Isochrysis galbana*, *Nannochloropsis oculata*, and *Chaetoceros gracilis*. Larval growth rates increased with the number of algal cells available per larva. Highest growth rates were achieved at the lowest larval density (20 larvae/liter), and larvae in this low concentration grew well, even with algal concentrations as low as those in ambient seawater. This larval density, unusually low for laboratory culture, was designed to simulate ocean conditions. When algal concentration and diversity of algal cell types were compared, larval growth rates were not influenced by diversity; total algal biomass as indicated by dry weight had a significant effect on growth rates.
Two factors in natural seawater, picoplankton and availability of patches, may contribute to its superiority as a food resource. Larvae grown on particles from natural seawater demonstrated higher growth rates and percent survival than larvae grown on cultured-algal diets of the same particle concentration as the seawater. When natural seawater was enhanced with cultured algae, larval growth rates were significantly higher than in natural seawater alone. Thus growth was limited by food available in ambient seawater. Particles in seawater were separated into size fractions by reverse filtration, and ambient phytoplankton particles (> 2 μm) were concentrated (3X) to simulate patches. Larval growth rates in the concentrated fraction were higher than those in ambient seawater. Thus the food-limited growth resulted from insufficient phytoplankton biomass in ambient seawater, not from a nutrient deficiency corrected by enhancement with laboratory-cultured algae. Larvae grew at rates close to maximum when reared with particles from a naturally occurring patch of plankton. Nannoplankton particles in the patch were 30 times more abundant than those in average ambient seawater. Since larvae are food-limited at average ambient phytoplankton concentrations, larval growth and survival are enhanced when phytoplankton patches are available.

Significant larval growth resulted from a diet of only picoplankton particles (< 2 μm). Larvae cultured in the picoplankton fraction enhanced with cultured algae grew significantly faster than larvae in Millipore-filtered seawater enhanced in the same manner. Thus the major contribution to increased growth was picoplankton and not dissolved organic matter (DOM). The lack of significant growth in the 0.22 Millipore-filtered flow-through control also demonstrated little contribution from DOM.
In Hawaiian coastal waters 25-50% of total chlorophyll a was derived from picoplankton particles. In offshore waters, picoplankton accounts for 60-80% of the chlorophyll a. In oligotrophic oceans, larvae may derive a considerable portion of their nutrition from picoplankton.

Most information about larval growth and life-span has come from laboratory studies. However, laboratory conditions (temperature, light, food and antibiotics) probably do not closely simulate conditions encountered in the plankton. Our knowledge of larval life-span would be enhanced if the age of field-collected larvae could be assessed. For gastropods, the statolith, an integral part of the statocyst sensory apparatus, may furnish the age of a larva. Statoliths of *C. spinosum, C. aculeata*, and *Littoraria scabra* were examined with light microscopy and scanning electron microscopy (SEM). In laboratory culture, the number of incremental layers in the statoliths was highly correlated with larval age in days. The statolith diameter also increased in a linear relationship with larval age. Knowledge of the age of field-caught larvae will allow us to answer questions about larval growth, metamorphosis, dispersal and longevity in the plankton.
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GENERAL INTRODUCTION

Most benthic marine invertebrates are sedentary, and many spend a portion of their developmental period as larvae in the plankton. The duration of the larval stage of marine benthic invertebrates affects larval dispersal, recruitment, and gene flow among populations. Estimates of larval duration (or larval life span) are used to predict several important aspects of larval and adult biology, such as dispersal capabilities (Thorson 1961, Scheltema 1971a, 1986, Crisp 1976, Ayal and Safriel 1982, Palmer and Strathman 1981), recruitment success (Obrebski 1979, Jackson and Strathmann 1981), life histories strategies (Menge 1975, Ayal and Safriel 1982), and speciation and extinction rates (Jablonski 1986).

Despite the importance of larval duration, little is known about actual life spans larvae experience in the plankton. Evidence from plankton surveys and species distribution studies show that planktonic larvae disperse to both local and distant populations. However, direct assessment of larval life span, growth, abundance, distribution, and mortality is lacking because of the difficulty in tracking minute organisms in the vast three dimensional space of the ocean. Most information about larval life has come from laboratory experiments, although some inferences concerning larval life-span have been made from the study of distinct cohorts of larvae in the field (Jorgensen 1981, Gaines and Roughgarden 1985, Yoshioka 1986). Since larvae in the plankton may be exposed to environmental conditions (e.g. temperature, light, or food) which differ markedly from those used in laboratory culture, the laboratory model may not simulate conditions in the plankton.
Planktotrophic larvae grow and/or develop until they are competent to metamorphose. Once they are competent they will metamorphose if the suitable chemical or physical inducer to metamorphosis is available. The growth rate of larvae during the precompetent period dictates the minimum duration of the planktonic stage. When growth is rapid, larval life span can be short, thus reducing the potential for dispersal and duration of exposure to planktonic predators (Thorson 1950). Many factors influence larval growth rate (e.g., larval density, temperature, salinity, and composition and concentration of food), and their relative importance has been difficult to resolve. Two principal factors that have been examined in laboratory studies are temperature and nutrition (Scheltema 1986). The relative impact of these factors on larval growth rate may change depending on the larval environment. Tropical oceans, in contrast to temperate ones, have narrower temperature ranges and lower plankton biomass (Raymont 1980). In such an environment, nutrition, rather than temperature, may have a greater influence on the range of larval life span.

The larval period will be relatively short if nutrition and temperature are optimal, and longer if conditions are sub-optimal. A larva's chance of being eaten increases the longer it remains in the plankton and the smaller its size (Thorson 1950). Compared to a slow-growing larva, a fast-growing larva has a reduced risk of mortality by being larger and by shortening the time from hatching to metamorphic size. Thus the influence of temperature and nutrition on larval life-span affects not only the proximity to recruitment sites but also the number of larvae available for recruitment. While the influence of temperature on larval development and growth has been well established
(Costlow et al. 1960, Scheltema 1967, Pechenik 1984), many questions remain about the role of larval nutrition.

Considerable debate has centered on whether larval survival and growth are food-limited in the ocean (Lucas 1982, Paulay et al. 1985, Olson 1987, Olson et al. 1987). A few species have been directly observed in the field, but most larvae are too small to be seen without optical magnification and their life-spans extend beyond practical underwater observation time. Another approach has been to make inferences concerning larval life-span from the study of distinct cohorts of larvae in the field (Jorgensen 1981, Gaines and Roughgarden 1985, Yoshioka 1986). In most cases, generalizations about growth rates of larvae have been based on laboratory experiments where larvae were reared with diets of cultured algae. Usually in such studies, larvae were maintained at high densities in continuous light and fed high concentrations ($10^4-10^5$ cells/ml) of one or two unicellular algal species (Switzer-Dunlap and Hadfield 1977). Clearly, these conditions do not imitate the ocean where larval density and food concentration are lower and food diversity is greater.

To understand what factors influence larval growth and life span in the field, it is necessary to better simulate conditions in the ocean. While a physical factor such as temperature is easily controlled and well studied (Pechenik 1980, 1984, Zimmerman and Pechenik 1991), a biological factor such as nutrition includes many variables.

Few studies have attempted feeding with ambient phytoplankton (Olson 1985, Paulay et al. 1985), presumably because the food levels were considered too low to sustain larvae (Lucas 1982). Paulay et al. (1985) grew larvae in standing cultures of natural seawater and suggested that "natural
food supplies must limit growth and development of larvae." However, larval density in culture is usually more than an order of magnitude higher than it is in the plankton, and Paulay et al. (1985) used 250 molluscan larvae per liter, and 75 sand dollar larvae per liter. However, Olson (1985) used in situ larval growth chambers with pumps to feed starfish larvae ambient phytoplankton. In both the Great Barrier Reef (Olson 1985) and Antarctica (Olson et al. 1987), larvae grew well on the food available in seawater. Scheltema (1986) suggested natural food supplies may not limit growth if larval densities are at their natural levels. To understand larval growth rates, the tradeoffs among larval density and food concentration and quality need to be examined.

In addition to phytoplankton, another possible source of nutrition may be picoplankton. This size group consists of living cells in the range of 0.2-2.0 μm including bacterioplankton and photoautotrophic plankton, (i.e. chroococcoid cyanobacteria, prochlorophytes and eucaryotic microalgae). Stockner and Antia, in their 1986 review of algal picoplankton, suggested that larval marine invertebrates have the potential to retain such particles. This could be a significant larval energy source in Hawaiian waters where photoautotrophic picoplankton may account for at least 60% of the biomass production (Stockner and Antia 1986).

A growing body of data suggests that picoplankton is a significant source of larval nutrition. Antarctic starfish larvae of two species ingested tritium-labeled bacteria but not 14C-labeled phytoplankton (Rivkin et al. 1986). Using the same technique, Bosch and Rivkin (1988) demonstrated uptake of bacteria by starfish larvae in the Sargasso Sea. Preliminary observations by Richard Strathmann (pers. comm.) showed that gastropod veligers can efficiently capture particles as small as 2 μm.
This evidence suggests that at least bacterioplankton, and possibly photoautotrophic picoplankton, may contribute to larval nutrition and growth in oligotrophic environments. It is generally accepted that energy in the microbial loop is transferred from picoplankton, to flagellates, to ciliates, which are ingested by mesozooplankton. Perhaps larvae and small mesozooplankton, such as cladocerans and copepod nauplii, can bypass the loop and graze directly on picoplankton.

The purpose of this dissertation was to examine the duration of larval life in the plankton. In Chapter 1, I described investigations of nutritional factors that promoted larval growth and survival, and assessed the relative importance of these factors in tropical oceans. Effects of larval density, algal food species and algal concentrations on larval growth and survival were tested on the prosobranch gastropods *Crepidula aculeata* (Gmelin, 1791) and *Crucibulum spinosum* (Sowerby, 1824).

The second chapter details investigations of factors in the ocean that promote larval growth and survival, and assessed the extent of variability in larval growth rates under conditions larvae might encounter in the ocean. Effects of larval density, natural planktonic food, phytoplankton patches and picoplankton on larval growth and survival were tested with larvae of the prosobranch gastropods *Crepidula aculeata* and *Crucibulum spinosum*.

In Chapter 3, I describe techniques to determine the age of larvae using statoliths and their application to estimate actual field growth rates. In this study, statoliths from larval gastropods were examined to determine if one increment is added each day. The statoliths of many species were examined to demonstrate a broad usefulness of the technique. Environmental factors were manipulated during larval growth to determine if any could change the
number of increments produced per day. Growth rates of larvae collected in the plankton were estimated from statolith increment counts and compared with growth rates in laboratory cultures. Larvae were obtained from eight mesogastropods (*Crepidula aculeata* [Gmelin 1791], *Crucibulum spinosum* [Sowerby 1824], *Epitonium ulu* [Pilsbry 1921] *Epitonium sp.*, *Littoraria scabra* [Linnaeus 1758], *Philippia oxytropis* A. Adams 1855, *Serpulorbis variabilis* Hadfield and Kay 1972, and *Styliferina goniochila* [A. Adams 1860]), a nudibranch (*Phestilla sibogae* Bergh 1905), and a pulmonate (*Siphonaria normalis* Gould 1846).

*Littoraria scabra* was chosen for the experimental part of this study because its extensive range from South Africa to Hawaii (Reid 1986) suggests a long planktonic duration. *Crucibulum spinosum* and *Sepulorbis variabilis* were used for the plankton studies because they have large larvae which were common in plankton tows.

In the final chapter, I describe investigations of the larval life of the gastropod *Epitonium ulu* (Pilsbry 1921), a symbiont of the solitary coral *Fungia scutaria* Lamarck. In the case of a symbiotic relationship, aspects of larval biology such as larval life span and conditions inducing metamorphosis may be constrained.
CHAPTER 1

GROWTH AND SURVIVAL OF VELIGERS OF CREPIDULA ACULEATA AND CRUCIBULUM SPINOSUM: EFFECTS OF LARVAL DENSITIES, ALGAL FOOD SPECIES AND ALGAL CONCENTRATIONS

INTRODUCTION

The growth rate of planktotropic larvae of marine benthic invertebrates affects the duration of the planktonic stage. When growth is rapid, larval life span can be short, thus reducing the potential for dispersal and duration of exposure to planktonic predators (Thorson 1950). Many factors influence larval growth rate (e.g., larval density, temperature, salinity, and composition and concentration of food), and their relative importance has been difficult to resolve (Pechenik 1980, 1984, Paulay et al. 1985). Two principal factors that have been examined in laboratory studies of larvae are temperature and nutrition (Scheltema 1986). The relative impact of these factors on larval growth rate may change in different areas of the ocean. Tropical oceans, in contrast to temperate ones, have narrower temperature ranges and lower plankton biomass (Raymont 1980). In such an environment, nutrition, rather than temperature, may have a greater influence on the range of larval life span.

Considerable debate has centered on whether larval survival and growth are food-limited in the ocean (Lucas 1982, Paulay et al. 1985, Olson
1987, Olson et al. 1987). A few species with large larvae have been directly observed in the field (Young 1986). However, most larvae are too small to be seen without optical magnification, and their larval life-spans extend beyond practical underwater observation time. Another approach has been to make inferences concerning larval life-span from the study of distinct cohorts of larvae in the field (Jorgensen 1981, Gaines and Roughgarden 1985, Yoshioka 1986). In most cases, however, generalizations about growth rates of larvae have been based on laboratory experiments where larvae were reared with diets of cultured algae. Usually in such studies, larvae are maintained at high densities in continuous light and fed high concentrations \((10^4-10^5 \text{ cells/ml})\) of one or two unicellular algal species (e.g., Switzer-Dunlap and Hadfield 1977). Clearly, these conditions do not imitate the ocean where larval density and food concentration are lower and food diversity is greater.

The factors influencing larval growth and life span in the ocean can be clarified by simulating conditions in the ocean. While a physical factor such as temperature is easily controlled and well studied (Pechenik 1980, 1984, Zimmerman and Pechenik 1991), a biological factor such as nutrition is more difficult to manipulate because it includes many variables. The major variables to consider are larval density, algal concentration and algal diversity. In tropical oceans algal concentrations are lower than in temperate oceans (Raymont 1980) and may seem inadequate for larval growth (Lucas 1982). However, because densities of zooplankton and larvae are low in tropical oceans (Raymont 1983, Finn 1993), low algal concentrations may be sufficient. Phytoplankton in most areas of the ocean is comprised of many species (Raymont 1980) and several may be needed to fill larval nutritional requirements (Fretter and Montgomery 1968, Pilkington and Fretter 1970).
On the other hand, given sufficient biomass, one algal species may fulfill most nutritional requirements, while others may be poor foods for various reasons (Walne 1963). An alga may be difficult for a larvae to capture due to size or shape, difficult to digest, or of poor nutritional quality (Strathmann et al. 1972, Bass et al. 1990).

Most of the growth and/or development of plantotrophic larvae occurs during the pre-competent stage before they are competent to metamorphose. Once they are competent they will metamorphose if the suitable chemical or physical inducer to metamorphosis is available. Larvae of some species can remain in culture as competent larvae for long periods of time (e.g., 288 days for Aplysia juliana [Kempf 1981]). However, larvae of other species metamorphose in culture without a specific inducer a few weeks after becoming competent (Pechenik 1980, 1985). Metamorphosis without a deliberate trigger from a specific inducer can be termed "non-specific" metamorphosis. For larvae with "non-specific" metamorphosis, the length of the pre-competent period may be the major portion of the larval life span. The growth and development rates of larvae during the pre-competent period dictate the duration of this stage. Thus variations in larval growth affect the minimum larval duration in the plankton and the minimum time for potential dispersal.

It is important to understand how the wide variability of phytoplankton diversity and abundance in the ocean (Raymont 1980), affects larval growth and survival. To investigate the effects of differing phytoplankton abundance and diversity, larval growth and survival were investigated in a variety of nutritional conditions. Effects of larval density, algal food species and algal concentrations on larval growth and survival of pre-competent veligers were
tested on the prosobranch gastropods *Crepidula aculeata* (Gmelin 1791) and *Crucibulum spinosum* (Sowerby 1824).
METHODS

*Crepidula aculeata* and *Crucibulum spinsosum* and the rocks on which they lived were collected in Kaneohe Bay, Oahu, Hawaii, and all experiments were conducted at the Kewalo Marine Laboratory, University of Hawaii, Oahu, Hawaii. The animals deposited egg masses on subtidal rocks near shore and brooded them under the mantle near the head. In the laboratory, removal of the animals from the rocks revealed the egg masses. Preliminary observations showed that the veliger larvae of both of these species were ready to hatch when pigment had developed along their intestines and, additionally, on the velum of *C. aculeata*. Larvae, released by tearing open the egg capsules with forceps, were counted and transferred by pipet into appropriate experimental containers.

Unless otherwise stated, cultures were maintained in clean 2- or 5-liter beakers standing in seawater tables to maintain ambient temperatures. Temperatures ranged from 24 to 27°C during the entire study but were consistent during each experiment. Water and food were changed every other day.

Experiments were designed to examine how veliger growth and survival differ in response to 3 variables: (1) larval density, (2) algal concentration, and (3) algal food species. For all cultures larvae were added to 0.45 µm Millipore-filtered seawater (MFSW). Algal species were cultured in 300 ml batches of Provasoli's ES media (Provasoli *et al.* 1957) in constant light and used for feeding when they were in the exponential growth phase. The diets of laboratory-cultured algae (LCA) consisted of one, two or three
different algal species. In preliminary experiments the species were the flagellates *Isochrysis galbana* (I, Tahitian strain) and *Ochromonas* sp. (O, a species isolated from Hawaiian waters), and the diatoms, *Phaeodactylum tricornutum* (P) or *Chaetoceros gracilis* (C). In multiple-species diets, equal cell numbers of each algal species were mixed. Various volumes of each single-species diet and multiple-species diet mixture were added to the larval cultures ($10^3$, $5 \times 10^3$, $10^4$, and $5 \times 10^4$ cells/ml) to achieve final feeding concentrations. In preliminary experiments, the algal culture medium was removed by centrifugation before feeding. Because flagellate species never recovered full motility after centrifugation, and larval growth was not affected by the addition of the algal culture medium with the algae, algae were added to larval cultures with their culture medium. The concentrations $10^3$ and $5 \times 10^3$ cells/ml are common for phytoplankton in oligotrophic areas (e.g., Hawaiian waters) of the ocean (Chapter 2; Raymont 1980). The concentration $10^4$ cells/ml has been used in many studies of larval gastropods (e.g., Pilkington and Fretter 1970, Switzer-Dunlap and Hadfield 1977, Hubbard 1988, Paige 1988). The concentration of $5 \times 10^4$ cells/ml was used as a control for maximum potential larval growth rate. In preliminary experiments, larvae grew no faster when algal concentrations were higher than $5 \times 10^4$ cells/ml. This concentration or higher has also been used in other studies of larval gastropods (Pechenik 1980 1984, Hubbard 1988).

To obtain growth data, ten actively swimming larvae were removed from each culture every two to five days. Maximum shell lengths were measured with the aid of an ocular micrometer on a compound microscope, and the larvae were returned to the culture. Mean shell length for the larvae of each culture was calculated for each sampling day.
Preliminary Experiments: Effects of Larval Density, Algal Concentration and Algal Diet on Growth and Survival

Larvae of *Crepidula aculeata* and *Crucibulum spinosum* are obligatory planktotrophs. When larvae (20/L) were maintained in MFSW without particulate food, they all died within ten days (Chapter 2).

Preliminary experiments were performed to determine the "best" culture conditions, that is, the levels of the three variables which would promote the maximum growth and survival of larvae of *Crepidula aculeata* and *Crucibulum spinosum* (Table 1.1). Three criteria for the relative success of culture conditions were: percent survival, days to competence, and days to non-specific metamorphosis. The objective of the preliminary experiments was to maximize the first criterion (percent survival) and minimize the latter two. Conditions common in many studies of larval gastropods were used in the first preliminary experiment: 1000 larvae/liter and $10^4$ cells/ml of food composed of one or two algal species. In subsequent preliminary experiments, levels of the three variables were modified to meet the objective. Larval densities ranged from 20/L to 1000/L; algal densities ranged from $10^3$ to $5 \times 10^4$ cells/ml; and the number of algal species ranged from one to three.

The levels of the three variables which met the objective for the "best" culture conditions (maximum survival, and minimum time to competence and metamorphosis) were: a larval density of 20/L, an algal concentration of $5 \times 10^4$ cells/ml, and a diet of three algal species. At these high algal concentrations larvae cultured in a three-species diet grew slightly faster than
### TABLE 1.1

**GROWTH AND SURVIVAL OF CREPIDULA ACULEATA AND CRUCIBULUM SPINOSUM WITH VARIOUS LARVAL DENSITIES, ALGAL SPECIES AND ALGAL CONCENTRATIONS (PRELIMINARY EXPERIMENTS)**

<table>
<thead>
<tr>
<th>PRE. EXP. NO.</th>
<th>SPECIES</th>
<th>NO. LARVAE/L</th>
<th>ALGAL CON. CELLS/ML</th>
<th>ALGAL SP.</th>
<th>%SURVIVAL</th>
<th>SURVIVAL TO DAY</th>
<th>DAYS TO COMP SIZE</th>
<th>NS MET</th>
<th>COMPOSSIBLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 a</td>
<td>C. aculeata</td>
<td>1000</td>
<td>1 X 10⁴</td>
<td>I</td>
<td>&lt;10</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
<td></td>
<td>IC</td>
<td>&lt;10</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td></td>
<td></td>
<td></td>
<td>IP</td>
<td>&lt;10</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>&lt;10</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 a</td>
<td>C. aculeata</td>
<td>1000</td>
<td>1 X 10⁴</td>
<td>IP</td>
<td>&lt;25</td>
<td>38</td>
<td>42</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
<td></td>
<td>IC</td>
<td>&lt;25</td>
<td>38</td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 a</td>
<td>C. aculeata</td>
<td>600</td>
<td>1 X 10⁴</td>
<td>I&amp;P</td>
<td>&lt;10</td>
<td>26</td>
<td>29</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>4 a</td>
<td>C. spinosum</td>
<td>600</td>
<td>1 X 10⁴</td>
<td>I&amp;P</td>
<td>&lt;10</td>
<td>33</td>
<td>27</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>5 a</td>
<td>C. spinosum</td>
<td>600</td>
<td>1 X 10⁴</td>
<td>IP</td>
<td>&lt;25</td>
<td>29</td>
<td>38</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
<td></td>
<td>IC</td>
<td>&lt;25</td>
<td>29</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 a</td>
<td>C. spinosum</td>
<td>400</td>
<td>1 X 10⁴</td>
<td>OC</td>
<td>&lt;10</td>
<td>31</td>
<td>32</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
<td></td>
<td>IC</td>
<td>&lt;50</td>
<td>31</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 a</td>
<td>C. spinosum</td>
<td>400</td>
<td>1 X 10⁴</td>
<td>IP&amp;O</td>
<td>&lt;25</td>
<td>25</td>
<td>32</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
<td></td>
<td>IP</td>
<td>&lt;50</td>
<td>25</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 a</td>
<td>C. spinosum</td>
<td>200</td>
<td>1 X 10⁴</td>
<td>IP</td>
<td>&lt;50</td>
<td>50</td>
<td>51</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
<td></td>
<td>IC</td>
<td>&lt;50</td>
<td>50</td>
<td>56</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td></td>
<td></td>
<td></td>
<td>O</td>
<td>&lt;25</td>
<td>50</td>
<td>65</td>
<td>51</td>
<td></td>
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</tbody>
</table>
**TABLE 1.1 (CONTINUED) GROWTH AND SURVIVAL OF *CREPIDULA ACULEATA* AND *CRUCIBULUM SPINOSUM* WITH VARIOUS LARVAL DENSITIES, ALGAL SPECIES AND ALGAL CONCENTRATIONS (PRELIMINARY EXPERIMENTS)**

<table>
<thead>
<tr>
<th>PRE. EXP. NO.</th>
<th>SPECIES</th>
<th>ALGAL CONCENTRATION</th>
<th>ALGAL SPECIES</th>
<th>% SURVIVAL</th>
<th>SURVIVAL TO DAYS</th>
<th>DAYS TO COMP SIZE</th>
<th>DAYS UNTIL NS MET</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 a</td>
<td><em>C. spinosum</em></td>
<td>300 1 X 10⁴</td>
<td>IPO</td>
<td>&lt;75</td>
<td>23</td>
<td>32</td>
<td>29</td>
</tr>
<tr>
<td>10 a</td>
<td><em>C. spinosum</em></td>
<td>100 5 X 10³</td>
<td>IPO</td>
<td>&lt;1</td>
<td>23</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>11 a</td>
<td><em>C. aculeata</em></td>
<td>20 1 X 10⁴</td>
<td>IPO</td>
<td>&lt;100</td>
<td>25</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>12 a</td>
<td><em>C. spinosum</em></td>
<td>20 1 X 10⁴</td>
<td>IPO</td>
<td>&lt;100</td>
<td>15</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>13 a</td>
<td><em>C. aculeata</em></td>
<td>20 5 X 10⁴</td>
<td>IPO</td>
<td>&lt;100</td>
<td>24</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>14 a</td>
<td><em>C. spinosum</em></td>
<td>20 5 X 10⁴</td>
<td>IC</td>
<td>100</td>
<td>13</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>15 a</td>
<td><em>C. aculeata</em></td>
<td>20 5 X 10⁴</td>
<td>IC</td>
<td>100</td>
<td>13</td>
<td>11</td>
<td>13</td>
</tr>
</tbody>
</table>

*a* I (Isochrysis galbana, Tahitian strain), C (Chaetoceros gracilis), P (Phaeodactylum tricornutum), O (Ochromonas sp.); species listed together were part of the diet from day 1; species listed after the & symbol were combined with the other species after day 10.

*b* Larvae were grown until they reached competent size of 700 μm, but not tested for competence with an inducer.

*c* Days until at least 1 larva metamorphosed non-specifically (NS MET) in culture without a specific inducer.
those in a two-species diet. Results of the preliminary experiments (Table 1.1) indicated that one species should be *I. galbana* (Tahitian stain) and the other a diatom. The choice of the third was not clearly defined.

Four experiments were designed to expand upon the preliminary experiments and further test the effects of algal abundance and diversity on larval growth. Experiment 1 was conducted during the preliminary experiments to examine larval ingestion of the algal species being tested. Experiment 2 was designed to test the hypothesis that competent larvae will metamorphose more frequently in waters with high phytoplankton concentrations than in waters with little or no phytoplankton; competent larvae for experiment 2 were derived from preliminary experiment 9a. The "best" culture conditions from the preliminary experiments were used as the baseline in further experiments (3 and 4) designed to test the relative importance of each variable. In Experiments 3 and 4 *Ochromonas* sp. was replaced by the chlorophyte, *Nannochloropsis oculata* (N) due to the poor ingestion of *Ochromonas* sp. in Experiment 1 (see Results). Of the two diatoms used in the preliminary experiments, *C. gracilis* (C) was chosen because it grew more consistently in culture.

*Crepidula aculeata* and *Crucibulum spinosum* displayed similar development times and responses to the three variables. *C. aculeata* was chosen for experiments 3 and 4 because the adults and egg masses were more abundant.

**Experiment 1: Effects of Algal Concentration on Ingestion**

Short term (24 hr) feeding experiments were performed to determine how algal species and concentration affected larval feeding. Larvae of
Crepidula aculeata and Crucibulum spinosum were tested with Isochrysis galbana to examine how ingestion was affected by algal concentration. Crucibulum spinosum was tested with Ochromonas sp. to determine if larvae were ingesting this species. In some preliminary experiments (6, 7 and 8), larval survival was poor when Ochromonas sp. was in the diet.

Different volumes of a stock suspension of laboratory-cultured algae (LCA) were added to MFSW (0.22 μm) to prepare algal feeding suspensions at three initial concentrations (5 x 10^3, 10^4 and 5 x 10^4 cells per ml). For each concentration, there were two or three experimental replicates and one control, each containing 50 ml of the algal feeding suspension. One hundred larvae were placed in each of the experimental replicates, while the controls contained only algae. To measure significant ingestion in 24 hours, larval densities had to be high enough to lower algal concentrations, unlike growth experiments where food supplies remained constant.

All particle suspensions were measured on a Coulter Counter®. MFSW (0.22 μm) was used as the blank, and its counts were subtracted from the experimental and control measurements as the background signal. Initial particle suspensions were measured before beakers were placed in an incubator at 25°C with continuous dim light. After 24 hours, veligers were removed by filtration on a Nitex screen and particle suspensions in all experimental replicates and controls were counted. Ingestion rates (cells ingested per larva per hour) were calculated according to Frost (Frost 1972). Algal growth rate was calculated from the initial and final concentrations of the controls.
Experiment 2: Effects of Starvation on Metamorphosis

Larvae of *C. spinosum* and *C. aculeata* metamorphosed without a specific inducer shortly after attaining competence (Table 1.1). Phytoplankton concentrations are low in offshore waters near Hawaii (Taguchi and Laws 1988), and food concentration may have an effect on the occurrence of non-specific metamorphosis. The second experiment tested whether competent larvae would metamorphose non-specifically in waters without phytoplankton.

Experiment 2 measured the duration of fed and starved competent veligers and tested which would metamorphose sooner. Larvae of *C. spinosum* from preliminary experiment 9a (Table 1.1) were used when they had reached competent size. On day 33 post-hatch, larvae from each replicate of preliminary experiment 9a were divided equally into a starved group and a fed group. The starved groups contained 57 and 92 larvae and the fed groups contained 58 and 91 larvae. Larvae were maintained in the same culture conditions as preliminary experiment 9a except no food was added to the starved groups whereas the fed groups were given the same food as before. Periodically (two to six days) cultures were examined for dead or metamorphosed individuals.

Experiment 3: Effects of Larval Density and Algal Concentration on Veliger Growth and Survival in *Crepidula aculeata*

The third experiment tested the relative importance of larval density and algal concentration on growth and survival of veligers of *Crepidula aculeata*. Larvae were grown at 24°C in nine treatments with four replicates each.
Treatments consisted of a two-way design of three larval densities (20, 100, and 300 per liter) and three algal concentrations (5 X 10^3, 10^4, and 5 X 10^4 cells per ml) (Table 1.2).

Shell lengths were measured on days 1, 5, 10, 15, 19 and 24 post-hatching, and values for days and shell lengths were log-transformed to produce the straightest growth curves. A separate linear regression was done on each replicate. Mean growth rate for a treatment was the mean of the slopes of the four replicates. Analysis of variance (ANOVA) was conducted to test the effects of the independent variables larval density and algal concentration and of their interaction with each other. Growth rate was the dependent variable.

To further analyze the interaction between algal concentration and larval density, the former was divided by the latter to compute food available per larva. These calculated food concentrations (FC:cells/larva) were plotted against the mean growth rate for each treatment, and food concentration was included in a multiple regression along with linear and quadratic terms in larval density, algal concentration and their interaction.

**Experiment 4: Effects of Algal Diversity on Veliger Growth and Survival in *Crepidula aculeata***

The fourth experiment evaluated the effects of one, two, or three algal-species diets on veliger growth and survival of *Crepidula aculeata*. Larvae were grown at 27°C in 21 treatments of two replicates each. Treatments consisted of a two-way design of three algal concentrations (10^3, 5 X 10^3, and 5 X 10^4 cells per ml) and 7 diets (C, I, N, IC, CN, IN and INC).
TABLE 1.2

Effects of algal concentration and larval density on veliger growth of *Crepidula aculeata*. Mean growth rates (see methods for calculation of growth rate) were compared for three larval densities, three algal concentrations and all treatments. The treatments with a † were not significantly different from each other (Tukey's Studentized Range test; P>0.05). All other pairwise comparisons of the nine treatments were significantly different (P<0.05). All three larval densities averaged over all algal concentrations were significantly different from each other (P<0.05; means of columns). All three algal concentrations averaged over all larval densities were significantly different from each other (P<0.05; means of rows). N = 4 for each treatment; treatment numbers are in parentheses.

<table>
<thead>
<tr>
<th>ALGAL CONCENTRATIONS</th>
<th>20/L</th>
<th>100/L</th>
<th>300/L</th>
<th>MEANS OF ROWS</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 X 10^4</td>
<td>0.41 (1)</td>
<td>0.37 (4)</td>
<td>0.29 (7)†</td>
<td>0.35</td>
</tr>
<tr>
<td>1 X 10^4</td>
<td>0.35 (2)</td>
<td>0.24 (5)</td>
<td>0.14 (8)</td>
<td>0.24</td>
</tr>
<tr>
<td>5 X 10^3</td>
<td>0.30 (3)†</td>
<td>0.18 (6)</td>
<td>0.10 (9)</td>
<td>0.19</td>
</tr>
<tr>
<td>MEANS OF COLUMNS</td>
<td>0.35</td>
<td>0.27</td>
<td>0.17</td>
<td></td>
</tr>
</tbody>
</table>
(In preliminary trials of Experiment 4 with the same algal concentrations as Experiment 3, growth rates did not differ significantly among concentrations for certain diets, hence $10^3$ was substituted for $10^4$ to enhance differences) All larval densities were 20/l.

Shell lengths were measured on days 1, 3, 7, 10, 13, and 15 post-hatching, and values for days and shell length were log-transformed to produce the straightest growth curve. A separate linear regression was done on each replicate. Mean growth rate for a treatment was mean slope of the four replicates. Analysis of variance (ANOVA) was conducted to test the effects of the independent variables larval density and algal concentration and of their interaction with each other. Growth rate was the dependent variable. Biochemical compositions of each algal species (Brown 1991) were combined to calculate the dry weight and the protein, lipid and carbohydrate content of each diet. Growth rate of each treatment was plotted against biochemical compositions of the diets.
RESULTS

Experiment 1: Effects of Algal Concentration on Ingestion.

Veligers of *Crepidula aculeata* and *Crucibulum spinosum* ingested increasing numbers of cells of *Isochrysis galbana* as the cell concentrations increased (Figure 1.1). Veligers of *C. spinosum* ingested more cells of *I. galbana* at all algal concentrations than did veligers of *C. aculeata*. Veligers of *C. spinosum* ingested *I. galbana* at a faster rate than they did *Ochromonas* sp. Ingestion of *Ochromonas* sp. at the highest algal concentration (5 x 10⁴ cell/ml) was significantly lower than ingestion at 10⁴ cells/ml.

Experiment 2: Effects of Starvation on Metamorphosis

Starvation did not induce larval metamorphosis (Figure 1.2). A few larvae had metamorphosed non-specifically on day 29, indicating that they were capable of non-specific metamorphosis before the starvation experiment began on day 33. A few starved veligers of *Crucibulum spinosum* (4%) metamorphosed non-specifically within six days, but most (96%) died within ten days. Fed veligers metamorphosed non-specifically throughout the experiment until the last one metamorphosed on day 87; thus, maximum duration of fed competent veligers was 58 days (=87-29). Of the fed veligers 62% metamorphosed and 38% died.
Figure 1.1. Mean ingestion rates (± SE) of Isochrysis galbana (I) and Ochromonas sp. (O) by newly hatched larvae of Crepidula aculeata and Crucibulum spinosum.
Figure 1.2. Effects of starvation on metamorphosis and mortality of competent veligers of *Crucibulum spinosum*. Cumulative mean percent metamorphosed (± SE) of starved and fed cultures. Starvation began on day 33 post-hatching. Termination of starved or fed cultures occurred when all veligers in that culture had metamorphosed or died.
Experiment 3: Effects of Larval Density and Algal Concentration on Veliger Growth and Survival in *Crepidula aculeata*

When effects of larval density are analyzed alone, veligers of *Crepidula aculeata* grew fastest in the lowest larval density and increasingly slower as density increased (Figs. 1.3 and 1.4). Growth rates of larvae reared in the three larval densities were significantly different from one another (Table 1.2; Tukeys Studentized Range test; P<0.05; [Sokal and Rohlf 1981]). When effects of algal concentration are analyzed alone, veligers grew fastest in the highest concentration and increasingly slower as concentration decreased (Figs. 1.3 and 1.4). Growth rates of larvae reared in the three algal concentrations were significantly different from one another (Table 1.2; Tukeys Studentized Range test; P<0.05; [Sokal and Rohlf 1981]).

In ANOVA, the two independent variables, larval density and algal concentration had a significant interaction (F-test; P,<0.01). However, even though the slopes for growth rate plotted by larval density with respect to algal concentration were not parallel, the three algal concentrations maintained their relative positions for all three larval densities (Fig. 1.4). In other words, the larvae reared in the highest algal concentration grew fastest in each larval density; larvae reared in the lowest algal concentration grew slowest in each larval density. When all nine treatments were compared, growth rates of larvae in all treatments were significantly different from one another except in the following pair: 20/L, 5,000 cells/ml LCA and 300/L, 50,000 cells/ml LCA (Treatments 3 and 7, Figs. 1.3 and 1.5a Table 1.2; Tukey’s Studentized Range Test; P<0.05; [Sokal and Rohlf 1981]). At high larval densities of 300/L,
Figure 1.3. Effects of algal concentration and larval density on veliger growth (mean shell length ±SE for each treatment) of *Crepidula aculeata* (Experiment 3). Algal concentrations are given as cells/ml laboratory-cultured algae (mix of equal cell numbers of 3 species: *Isochrysis galbana*, *Chaetoceros gracilis* and *Nannochloropsis oculata*). Larval densities are given as number of larvae/L. Treatment numbers are in parentheses. Log transformed (In) slopes all differ significantly from each other except treatments 3 and 7 (Tukey's Studentized Range test P<0.05).
Figure 1.4. Effect of interaction between algal concentration and larval density on veliger growth of *Crepidula aculeata*. Mean growth rates ±SE were plotted for each treatment. Growth rates are slopes of regressions of ln shell length on ln days. Numbers are treatment numbers as in Table 2.
Figure 1.5a. Effect of food concentrations (cells/larva) on growth rates (mean ± SE) of veligers of _Crepidula aculeata_. Treatment numbers are given next to each symbol. See Table 2 for explanation of treatments. All mean growth rates were significantly different from each other except 3 and 7 (Table 2; Tukey's Studentized Range test P < 0.05).
larvae grew significantly faster with a high algal concentration similar to typical laboratory cultures (50,000 cells/ml, Treatment 7), than with a low algal concentration similar to oceanic levels (Chapter 2; 5,000/cells/ml, Treatment 9, Fig. 1.4). However, when algal concentration was low (5,000 cells/ml), larvae grew significantly faster with the two lower larval densities (20/L, Treatment 3 and 100/L, Treatment 6) than with the highest larval density (300/L, Treatment 9, Fig.1.4). Thus, high food concentrations are needed only to sustain the high larval densities used in typical laboratory cultures, not the lower larval densities in the ocean.

Due to the interaction between algal concentration and larval density, the food concentration (FC) or cells available/larva was the best predictor of larval growth rate (Fig. 1.5a). Larvae grew fastest in the lowest larval density and highest algal concentration (Treatment 1, Fig. 1.5a), and larvae grew slowest in the highest larval density and lowest algal concentration (Treatment 9, Figure 1.5a).

When growth rate and food concentration were plotted as numeric rather than logarithmic values (Fig. 1.5b), the increase in growth rate was approximately linear until it plateaued at approximately 200 μm/day. At food concentrations above 600,000 cells/larva, growth rate increased little (190 μm/day to 210 μm/day) when food concentration increased five-fold.

Variables used in multiple regression (Sokal and Rohlf 1981) to explain variation in larval growth rate were: larval density(LD), larval density squared (LD^2), algal concentration (AC), algal concentration squared (AC^2), the interaction between the first 2 variables (LD X AC), and natural log of food concentration (FC:cells/larva). The variables explaining a significant amount
Figure 1.5b. Effect of food concentrations (cells/larva) on growth rates of veligers of *Crepidula aculeata*. Growth rates are slopes of regressions of shell length on days. Data from Exp. 3 were not log transformed before calculating slopes as they were in Fig. 1.5a.

\[ Y = 38.41 \ln(X) - 332.14 \]

\[ R^2 = 0.97 \]
of variation in larval growth rate were natural log of food concentration, the interaction between algal concentration and larval density, and larval density squared. Ln of food concentration alone (FC) explained most of the variation (Fig. 1.5a). (Independent variables are listed in order of most variation explained: Growth rate [ln shell length/ln days] = 0.5433608FC + 0.00000001LD X AC - 0.0000007L02 - 0.00585095; R2 = 0.98; P < 0.15).

Survival to day 24 was always 100% when algal concentration was 50,000 cells/ml (Fig. 1.6). When algal concentration was reduced to 10,000 cells/ml, 100% survival was maintained in cultures of 20/l but not 100 or 300/l. With an algal density of 5,000 cells/ml, survival was high in cultures of 20 and 100/l, but only 42% in cultures of 300/l. Variance in survival was not homogeneous across treatments, so differences in survival were evaluated with standard errors. At the highest larval density survival, in algal concentrations of $10^4$ and $5\times10^4$ cells/ml (Treatments 8, and 9) did not differ from each other but did differ significantly from survival in all other treatments. All other treatments (1-7) did not differ significantly from each other. When treatments were ranked from highest to lowest by growth rates or survival rates, the rank order was approximately the same for either rate.

Experiment 4: Effects of Algal Diversity on Veliger Growth and Survival in *Crepidula aculeata*

As in the other experiments, when effects of algal concentration were analyzed alone, veligers grew fastest in the highest concentration and slower in the lower concentrations (Fig. 1.7); growth rates of larvae reared in the
Figure 1.6. Effects of algal concentration and larval density on survival to day 24 of veligers of *Crepidula aculeata*. Abbreviations same as Fig. 3. Arc-sine transformed mean survival (±SD) of four replicates in each treatment.
Figure 1.7. Effects of algal concentration and composition of algal diet on growth rates of veligers of *Crepidula aculeata*. All larval densities were 20/L.
three algal concentrations were significantly different from one another (Tukey's Studentized Range test; \( P < 0.05 \); [Sokal and Rohlf 1981]).

When effects of diet were analyzed alone, larvae did not grow fastest in the three-species diet (Fig. 1.7). This result suggests that diversity of algal species was not the most important factor influencing larval growth. The only diet effect consistent across all algal concentrations was that larval growth was always slowest on a diet of *N. oculata*.

There was significant interaction between the effects of diet and algal concentration on growth rates (ANOVA F-test; \( P < 0.01 \) [Sokal and Rohlf 1981]). If diets were ranked by growth rate from highest to lowest, the rank order would not be maintained at different algal concentrations (Fig. 1.7). In other words, the diet with the highest growth rate at the highest algal concentration is not the diet with the highest growth rate at another algal concentration. The rank of diets by percentage of larval survival also changed with each algal concentration (Fig. 1.8). For some diets (*I. galbana*, *C. gracilis* and the three-species diet), survival rates of larvae were the same at the middle (5x10³ cells/ml) and lowest (10³ cells/ml) algal concentrations (Tukey's Studentized Range test \( P < 0.05 \) [Sokal and Rohlf 1981]; Fig. 1.8). This result suggests that for certain algal diets, larval survival remained high (>80%) even at low algal concentrations similar to those in tropical oceans (Chapter 2).

At the lowest algal concentration (5 X 10³ cells/ml), larvae grew fastest in cultures with *I. galbana* or *C. gracilis* and without *N. oculata* (Fig. 1.7). They grew slower in cultures of mixed diets with *N. oculata*, and slowest in cultures where *N. oculata* was the only food.

When larvae were grown on diets with the middle algal concentration (10⁴ cells/ml), diets of *C. gracilis* alone and those involving *I. galbana* and
Figure 1.8 Effects of algal concentration and composition of algal diet on survival of veligers of *Crepidula aculeata*. Survival was determined to day 10 for algal concentrations of 50,000 cells/ml, to day 15 for algal concentrations of 5,000 cells/ml, and to day 13 for algal concentrations of 1,000 cells/ml. Arc-sine transformed mean survival (±SD) of 2 replicates in each treatment.
C. gracilis were the best for larval growth (Fig. 1.7). The next highest growth rates occurred in cultures with a three-species diet of I. galbana, N. oculata and C. gracilis and a two-species diet of C. gracilis and N. oculata. Growth rate was faster with these four diets than with I. galbana alone. Larvae grew slowest with diets which included N. oculata, especially N. oculata alone and I. galbana with N. oculata.

At the highest algal concentration, larval growth was fastest in a two- or three-species diet involving I. galbana and N. oculata (Fig. 1.7). Larval growth was slower in some diets involving Chaetoceros gracilis but survival was severely limited (Fig. 1.8). This effect of C. gracilis on survival was absent at the two lower concentrations and in the three-species diet at the highest concentration where C. gracilis was only a third of the diet. Thus, at the highest concentration, when a diet was composed of 50% or 100% C. gracilis, survival was extremely low (Fig. 1.8).

Growth rates were correlated with the protein, lipid and carbohydrate in each diet at each concentration (Figs. 1.9-1.11). The correlation was weakest at the highest algal concentration. No nutrient correlated more strongly with growth than any other, so it appears that algal dry weight may be a good predictor of growth rate, except at highest algal concentrations when growth rate is near maximal (Fig 1.12).
Figure 1.9. Effect of total protein in the diet on growth rates of veligers of *Crepidula aculeata*. Protein content of algal species from Brown, 1991.
Figure 1.10. Effect of total lipid in the diet on growth rates of veligers of *Crepidula aculeata*. Lipid content of algal species from Brown, 1991.
Figure 1.11. Effect of total carbohydrate in the diet on growth rates of veligers of *Crepidula aculeata*. Carbohydrate content of algal species from Brown, 1991.
Figure 1.12 Effect of total dry weight in the diet on growth rates of veligers of *Crepidula aculeata*. Dry weights of algal species from Brown, 1991.
DISCUSSION

The results described here demonstrate that the growth rate of larvae varies with the density of larvae and, presumably, with the density of other herbivores. If larval densities are low, larvae will grow when reared in algal concentrations as low as those commonly found in tropical oceans (Raymont 1980, Chapter 2). Larvae are plastic in their responses to different algal concentrations: when algal abundance is low, larval growth and development rates drop; when algae are concentrated, development and growth rates are maximized, and larvae are capable of settling much sooner. In the experiments described here, survival rates did not decrease at lower algal concentrations ($10^4$ and $5 \times 10^3$ cells/ml) unless larval density was high (300/ml). This plasticity in sustainable growth and development should allow larvae to survive in a wide variety of situations they may encounter in the sea, and indeed, the capacity of larvae to continue growing at very low phytoplankton concentrations is remarkable.

The diversity of the diet is not as important as the individual algal species which comprise it. Some species are good foods when used alone at all concentrations. For example, in this study larvae grew and survived well with all concentrations of *I. galbana* (Tahitian strain). Walne (1963) also found that a low concentration of a good food like *Isochrysis galbana* produced the same accumulation of carbohydrate and nitrogen:glucose ratio in juvenile clams as did all levels of a relatively poor diet. On the other hand, Pilkington and Fretter (1970) compared two prosobranch gastropods and found the algal species *Exuviaella baltica* promoted rapid larval growth in
**Crepidula fornicata** and not in *Nassarius (Hinia) reticulatus*. In contrast, some foods are always less than adequate, and the benefits of some vary with their concentration. In this study, growth rates for diets of *N. oculata* alone were always lower than growth rates for other diets with the same total dry weight (Fig. 1.12). In addition, high concentrations of *C. gracilis* were detrimental to survival (Fig 1.8), and ingestion decreased at high concentrations of *Ochromonas* sp. (Fig. 1.1). Similarly, Pilkington and Fretter (1970) found several algal species were always "poor" foods, and the growth rate of *Crepidula fornicata* decreased with increased concentration of *Pyramimonas grossii*. Whether an algal species is suitable food for a larva will be highly specific for that combination of algal and larval species. In the ocean, the abundance of "good" foods and "poor" foods for a particular larval species will vary over time and space. A larval diet may consist of a combination of "poor, adequate and superior" foods.

In this study both *Crepidula aculeata* and *Crucibulum spinosum* ingested cells at a rate similar to echinoderm larvae of the same size (Strathmann 1971). However, newly hatched *C. aculeata* (320 μm) and *C. spinosum* (330 μm) ingested fewer cells than newly hatched *Crepidula fornicata* (400 μm) (Pechenik 1980). Methods in this study differed from the ones used by Pechenik (1980). In his study larvae were without food for 10 hours prior to the three-hour ingestion experiment; in this study, larvae were fed before the 24-hour ingestion experiment. Normal larval ingestion is more accurately measured for fed larvae in 24 hours than for hungry larvae in three hours. Also, the algal concentration used by Pechenik (1980) was $2 \times 10^5$ cells/ml, four times higher than the highest ($5 \times 10^4$) used in this study.
In many feeding studies, ingestion increased with algal concentration until it reached a plateau at high algal concentrations (Frost 1972). A plateau for ingestion rate was not discerned, in this study because there were no experiments for algal concentrations between $10^4$ and $5 \times 10^4$ cells/ml or higher than $5 \times 10^4$ cells/ml. Despite the fact that a plateau for ingestion was not discernible, veliger growth rate of *C. aculeata* reached a plateau when the algal concentration was close to $5 \times 10^4$ cells/ml; at this concentration, ingestion and assimilation rates for veligers of *C. aculeata* may be close to maximum.

Evidence from this study suggests that some veligers ingest different algal species at different rates. However, evidence from oysters indicates that their larvae do not select the best food cells in natural seawater. Given natural phytoplankton densities and assemblages, larvae of the oyster, *Crassostrea virginica* select for small size particles (<10 μm), but among small particles feed non-selectively among types or species of cells (Fritz *et al.* 1984). Larvae may change their feeding rate depending on the desirability of an algal species in a mono-culture, but they may be unable to select desirable species from a mixed culture.

Ingestion is only the first step in the process of deriving nutrition from an algal cell. In the second step, a larva must be able to digest the algal species to derive nutrition from it. Bass *et al.* (1990) found that juveniles of the hard clam, *Mercenaria mercenaria* captured *Nannochloropsis* sp. and *Pseudoisochrysis paradoxa* efficiently, but that assimilation was lower with *Nannochloropsis* sp. than for *P. paradoxa*, a chrysophyte known to promote growth of hard clams. Since the clams filtered *Nannochloropsis* sp., it was not the small size of the cells which prevented it from being a good food.
Rather, low absorption efficiencies may be due to the indigestible polymerized carotenoid sporopollenin, found in the walls of some chlorophyte cells (Bass et al. 1990).

The third step in the process of deriving nutrition from an algal cell involves the amount of energy which is provided by a particular algal species once it has been digested. While the amino acid compositions of microalgae are similar, the composition of polysaccharides differs significantly between species and classes (Brown 1991). For example *Phaeodactylum tricornutum*, used in the preliminary experiments, has lower glucose concentrations but higher mannose levels than the three species (*I. galbana*, *N. oculata* and *C. gracilis*) used in experiments three and four. While those three species have similar glucose concentrations, they have different concentrations of rhamnose and arabinose. Variations in sugar composition could contribute to differences in the nutritional value of some species, since animals digest polysaccharides of different composition at different rates (Brown 1991).

All three components of energy storage in algal tissue (proteins, lipids and carbohydrates) were correlated with larval growth rate and each other. No component was more important than the others perhaps because the relative amounts of each component were similar for the three algal species chosen. Thus the dry weight of the diet (Fig. 1.12) and the cells available for each larva (Fig. 1.5a) were correlated with growth rate. Since the diet for Figure 1.5a was the same for all treatments, a plot of dry weight per larva would be identical with that of cells/larva. Dry weight of the diet for the three algal species used seemed to be a good predictor of growth rate if the algal species were digestible. While a small algal species may be a good food
when larvae are small, the total available biomass may become too low as larvae grow.

In the present study, larvae cultured at 20/l grew much faster than those cultured at 100/l with the same concentration of laboratory-cultured algae. This occurred even at high algal concentrations (5 \times 10^4 \text{ cells/ml}) where food could not be depleted. While clear explanations for this phenomenon are lacking, perhaps the larvae interfere with each other and thus feed less efficiently when present at high density (Scheltema 1986). For example, Fritz et al. (1984) found grazing rates of larval *Crassostrea virginica* to be inversely proportional to larval density. Alternatively, the lower growth rate could be due to a metabolite from the larvae which increases at higher larval densities.

When larval densities and algal concentrations are experimentally adjusted to simulate those encountered in the ocean, results demonstrate that average natural food concentrations are generally adequate for growth of the larval gastropods in this study. In particular, mortality due to starvation does not occur unless food is extremely low, lower than in tropical coastal waters (Chapter 2). However, in the field, mortality may be higher for slower-growing larvae because of increased time of exposure to predators (Thorson 1950). On the other hand, the capacity of larvae for rapid growth allows increased growth and development when phytoplankton concentrations are high. In the ocean, circulation and mixing influence growth and aggregation of phytoplankton, creating patches which exist on varying temporal and spatial scales (Seliger et al. 1981, Bienfang et al. 1984, Haury et al. 1986). The capacity for rapid growth allows for an increase in growth rates if larvae encounter a phytoplankton patch when they first come to the surface or when
they drift from offshore to coastal waters. Thus larval gastropods are adapted to a wide variety of conditions, in that they can survive in extremely low phytoplankton concentrations and take advantage of higher concentrations.

The gastropod larvae studied here are flexible enough to grow and survive in a variety of nutritional conditions. The algal-species composition of their diets is not as important as the total biomass or dry weight of the food as long as the algal species are digestible. Larval growth rates varied in response to algal concentration and algal species composition suggesting that larvae can grow in the wide range of phytoplankton species and abundances they may encounter in the ocean.
CHAPTER 2

GROWTH AND SURVIVAL OF VELIGERS OF CREPIDULA ACULEATA AND CRUCIBULUM SPINOSUM: EFFECTS OF NATURAL PLANKTONIC FOOD, PHYTOPLANKTON PATCHES AND PICOPLANKTON

INTRODUCTION

The duration of the larval stage of marine benthic invertebrates affects larval dispersal, recruitment, and gene flow among populations. Evidence from plankton surveys and species-distribution studies shows that planktonic larvae disperse to both local and distant populations (Scheltema 1986, McShane et al. 1988, Sammarco and Andrews 1989). Dispersal distance is affected by larval life-span, which limits the time available for dispersal, and by current speeds which limit the distance dispersed (Scheltema 1986). Together larval life-span and current speeds determine whether larvae will recruit only locally or have the potential to reach distant habitats. Accurate estimates of larval life-span in the plankton can be combined with available current speed data to enhance our knowledge of dispersal. Without an understanding of dispersal and recruitment, the community dynamics of benthic invertebrates must remain speculative (Ebert 1983, Connell 1985).

Larval growth and survival contribute to variation in larval life-span of planktotrophic (feeding) larvae. Temperature and food ration are two major factors affecting growth and development of planktotrophic larvae (Scheltema
The larval period may be relatively short if nutrition is optimal and temperature is within a suitable range; it may be longer if conditions are sub-optimal. A larva's chance of being eaten increases the longer it remains in the plankton and the smaller its size (Thorson 1950, Rumrill et al. 1985, Pennington et al. 1986). Compared to a slow-growing larva, a fast-growing larva has a reduced risk of mortality by being larger sooner and by shortening the time from hatching to the attainment of metamorphic size. Thus the influence of temperature and nutrition on larval life-span affects not only the proximity to recruitment sites but also the number of larvae available for recruitment.

The relative impact of temperature and nutrition on larval life-span may change in different areas of the ocean. Tropical oceans, in contrast to temperate ones, have narrower temperature ranges and lower plankton biomass, (Raymont 1980). In such an environment, nutrition, rather than temperature, may have a greater influence on the range of larval life-span. While the influence of temperature on larval development and growth has been well established (Costlow et al. 1960, Scheltema 1967, 1986, Pechenik 1980, 1984, Zimmerman and Pechenik 1991), many questions remain about the role of larval nutrition.

Considerable debate has centered on whether larval survival and growth rate are food-limited in the ocean (Lucas 1982, Paulay et al. 1985, Olson 1987, Olson et al. 1987). Underlying the debate is the assumption that phytoplankton is the major nutritional source in the ocean since it is a sufficient food in the laboratory (Lucas 1982). However, phytoplankton concentrations in the ocean are often below the concentrations required for larval growth in the laboratory. This is especially true in oligotrophic
environments such as the tropics, the poles and the open ocean (Raymont 1980). A few studies have addressed the question of larval growth with natural phytoplankton concentrations. For example, growth and survival of the tropical seastar *Acanthaster* were low when they were fed phytoplankton concentrations comparable to those in the ocean (based on chlorophyll *a* measurements; (Lucas 1982)), but, *in situ* experiments on these same larvae and those of an Antarctic asteroid demonstrated that the larvae grew well in the field (Olson 1987, Olson et al. 1987). In most cases, however, generalizations about growth rates of larvae have been based on laboratory experiments where larvae were reared with diets of cultured algae. Usually in such studies, larvae are reared at high densities in continuous light and fed high concentrations (10^4-10^5 cells/ml) of one or two unicellular algal species (e.g., Switzer-Dunlap and Hadfield 1977). These conditions do not imitate conditions in the ocean where larval density and food concentration are often lower and food diversity is usually higher.

Several factors may affect the growth of larvae in tropical oceans: densities of competing herbivorous zooplankton and larvae; diversity or quality of food particles; dissolved organic matter (DOM); phytoplankton patchiness; and presence of picoplankton. Because densities of zooplankton and larvae are low in tropical oceans (Raymont 1983, Finn 1993), the low algal concentrations found there may be sufficient for larval growth. The benefits of low larval density in cultures of low food concentrations were supported by laboratory experiments with cultured algae (Chapter 1).

Diversity of algal species does not appear to affect larval growth rate, at least in experiments with laboratory-cultured algae (Chapter 1). However, the algal-species composition in tropical oceans is considerably more diverse than
the three-species diet used in the laboratory (Gilmartin and Revelante 1974, Raymont 1980, Venrick 1989). DOM, ubiquitous in the ocean, could affect larval growth; larvae of gastropods, bivalves and echinoids can take up DOM (Manahan 1983a, 1983b, Manahan et al. 1983, Jaekle and Manahan 1989). Larvae may grow faster in a phytoplankton patch than in average phytoplankton concentrations. Phytoplankton patches occur in the ocean when circulation and mixing either supply limiting nutrients or cause aggregation of passive particulates (Bowman and Iverson 1978, Seliger et al. 1981). Gastropod larvae are essentially passive particles (Hannon 1984) that may encounter a patch at hatching or be included in a patch as it forms. If larval growth is food-limited under typical oceanic conditions, growth could increase by intensified feeding in a patch. In experiments with laboratory-cultured algae, ingestion rate and growth rate increased with higher algal concentrations (Chapter 1).

Picoplankton, consisting of particles in the range of 0.2 to 2.0 μm in diameter, could be an important source of nutrition in the ocean, a potential that has been neglected in laboratory experiments. Picoplankton includes cyanobacteria, heterotrophic bacteria, and some eukaryotic green algae, the first being the most abundant component. Prior to 1986, picoplankton was considered too small for larval food because it was thought that larvae could capture particles only 2-30 μm in diameter, the size range of most laboratory-cultured algae (Thorson 1950, Fretter and Montgomery 1968, Strathmann et al. 1972). However in 1986, Rivkin et al., demonstrated uptake of bacteria by starfish larvae using double isotope labeling. If picoplankton is a larval food, it may have a significant impact on larval growth due to its substantial biomass and widespread geographical distribution (Sieburth et al. 1978, Sieburth 1979,
Waterbury et al. 1979). To be useful as nutrition for larvae, picoplankton particles must not only be ingested, but must contribute to larval growth and development.

This study investigated factors in the ocean that promote larval growth and survival, and assessed the extent of variability in larval growth rates under conditions larvae might naturally encounter. To test the hypothesis that larval growth and survival are food-limited in the ocean, larvae were cultured in coastal ocean seawater with its natural compliment of phytoplankton and in various concentrations of laboratory-cultured algae. To test the hypothesis that picoplankton is an important food resource in oligotrophic waters, larvae were cultured in coastal ocean seawater with all nannoplankton removed and/or replaced with laboratory-cultured algae. To test the hypothesis that larvae will grow faster in the higher phytoplankton concentrations found in a plankton patch, larvae were cultured in coastal ocean seawater with its natural compliment of nannoplankton increased three-fold and in coastal ocean seawater from a dense phytoplankton patch that occurred naturally. Effects of larval density, natural planktonic food, phytoplankton patches and picoplankton on larval growth and survival were explored with larvae of the prosobranch gastropods Crepidula aculeata (Gmelin 1791) and Crucibulum spinosum (Sowerby 1824).
METHODS

Crepidula aculeata and Crucibulum spinosum are circumglobal in distribution (Kay 1979), and larvae of both species are large and easily cultured relative to many other gastropods. Adults and the rocks on which they lived were collected in Kaneohe Bay, Oahu, Hawaii, and transported to the Kewalo Marine Laboratory, University of Hawaii, Oahu, Hawaii, where all experiments were conducted. Collection of egg masses and hatching of veligers are described in Chapter 1.

Unless otherwise stated, cultures were maintained in clean 2- or 5-liter beakers standing in seawater tables to maintain ambient temperatures. Temperatures ranged from 24.5 to 28.5°C during the entire study but were consistent during each experiment.

Initially two sources of planktonic food were used: plankton in laboratory seawater (Laboratory Plankton:LP) drawn from the Kewalo Marine Laboratory seawater system; and the plankton in coastal ocean seawater (Ocean Plankton:OP) collected by bucket directly from the ocean near the Kewalo Marine Laboratory. In eleven preliminary experiments larvae of C. spinosum were cultured in 400 ml baskets which floated in a laboratory seawater table and received a continuous input of dripping laboratory seawater with its compliment of plankton. Most of these larvae grew slowly and died within a week, although one larva in one culture metamorphosed. Thus the first experiment was designed to test the hypothesis that laboratory plankton (LP) lacked a component essential for growth and survival. Kewalo Laboratory seawater is drawn through only a coarse filter (1 cm mesh) and
was used directly as it came from the laboratory spigot. (The Kewalo Laboratory seawater system draws its water from approximately 300 m offshore at a depth of approximately 7 m; inside the seawater pipes are populations of sessile filter-feeding invertebrates.) In the experiments OP was passed through 41 μm Nitex® mesh to remove predators and competitors. Particle counts of both phytoplankton sources were made on a Coulter Counter® or Elzone 180 Particle Counter®.

Experiments were designed to examine how larval growth and survival differ in response to various food sources: (1) all naturally occurring plankton in ocean seawater; (2) various size fractions of plankton in ocean seawater; (3) laboratory-cultured algae (LCA); and (4) all plankton in ocean seawater supplemented with laboratory-cultured algae. All experimental treatments are summarized in Table 2.1. The larval diet of laboratory-cultured algae (LCA) consisted of equal densities of three different algal species. In Experiments 1-3 the species were the flagellates *Isochrysis galbana* (Tahitian strain) and *Ochromonas* sp. (a species isolated from Hawaiian waters), and a diatom, *Phaeodactylum tricornutum*. In Experiments 4 and 5, the second and third algal species were replaced by a chlorophyte, *Nannochloropsis oculata*, and a diatom, *Chaetoceros gracilis*. Equal cell numbers of each algal species were mixed and diluted to final feeding concentrations with 0.45 μm Millipore-filtered seawater (MFSW). (In this Chapter 0.45 μm will be the filter size for MFSW. When 0.22 μm filters were used the filter size will be specified as 0.22 μm MFSW.) Feeding concentrations were $10^3$, $3 \times 10^3$, $10^4$, and $5 \times 10^4$ algal cells/ml. The first ($10^3$ cells/ml) and second ($3 \times 10^3$ cells/ml) are common phytoplankton concentrations in Hawaiian waters and approximate concentrations in average ambient OP and plankton-rich patches of OP.
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<td></td>
<td>MFSW</td>
<td>LCA/1,000/EVERY DAY</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>MFSW</td>
<td>LCA/1,000</td>
<td>20</td>
</tr>
</tbody>
</table>
TABLE 2.1. (CONTINUED) EXPERIMENTAL CONDITIONS

<table>
<thead>
<tr>
<th>EXP. TREAT</th>
<th>GASTROPOD SPECIES</th>
<th>WATER SOURCE</th>
<th>FOOD/DENSITY (cells/ml)</th>
<th>LARVAL DENSITY (#/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXP. 5</td>
<td>C. aculeata</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>MFSW</td>
<td>LCA/50,000</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>OP</td>
<td>NATURAL</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Conc. OP</td>
<td>NATURAL</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Reduc. OP</td>
<td>NATURAL</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>OPC</td>
<td>NATURAL</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>MFSW</td>
<td>LCA/1,000</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>OPC</td>
<td>NAT &amp; LCA/1,000</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>MFSW</td>
<td>LCA/3,000</td>
<td>20</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>OPC</td>
<td>NAT &amp; LCA/3,000</td>
<td>20</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>MFSW (0.22)(^\text{i})</td>
<td>DOM(^\text{k})</td>
<td>20</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>MFSW /cont. drip(0.22)(^\text{i})</td>
<td>DOM</td>
<td>OPEN B(^\text{m})</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>OP PATCH</td>
<td>NATURAL PATCH(^\text{n})</td>
<td>20</td>
</tr>
</tbody>
</table>

\(a\) 0.45 Millipore filtered seawater
\(b\) Laboratory-cultured algae, an equal mix of 3 species described in text
\(c\) Ocean plankton
\(d\) All particles available in that water source (mean = 1,000 particles/ml)
\(e\) Plankton in laboratory seawater
\(f\) 200 larvae in 400 ml basket with new water supplied continuously
\(g\) Concentrated (3-fold) ocean plankton
\(h\) Reduced (one-half) ocean plankton
\(i\) Water and food changed every day; all treatments not specified were changed every other day
\(j\) 0.22 \(\mu\)m Millipore-filtered seawater in static beakers as other treatments
\(k\) All dissolved organic matter available in 0.22 \(\mu\)m Millipore-filtered seawater
\(l\) 0.22 \(\mu\)m Millipore-filtered seawater dripping continuously
\(m\) 20 larvae in 400 ml basket with new water supplied continuously
\(n\) Natural phytoplankton-rich patch in coastal ocean (30,000 particles/ml)
respectively (see Table 2.2; Bienfang et al. 1984). The third concentration, 10^4 cells/ml, has been a common algal-cell concentration employed in many studies of larval gastropods (e.g. (Pilkington and Fretter 1970, Switzer-Dunlap and Hadfield 1977, Hubbard 1988, Paige 1988). The last, 5 X 10^4 cells/ml, was used as a control for maximum potential growth rate (Chapter 1).

To obtain growth data, ten actively swimming larvae were removed from each culture every two to five days. Maximum shell lengths were measured with the aid of an ocular micrometer on a compound microscope, and the larvae were returned to the culture. Mean shell length for the larvae of each culture was calculated for each sampling day.

In larvae of *Crucibulum spinosum* and *Crepidula aculeata*, the shell develops a circular flange (termed a brim by Pechenik [1980] and shown in Thiriot-Quievreux and Scheltema [1982]) at metamorphic competence which transforms a coiled larval shell to a limpet-like adult shell. Larvae in each culture were examined to determine the day brims first appeared.

**Experiment 1: Effects of Phytoplankton Source and Larval Density on Veliger Growth and Survival in *Crucibulum spinosum***

The first experiment tested whether ocean plankton (OP) is a better source of nutrition than laboratory plankton (LP) and which larval density allows the best sustainable growth and survival in OP. Larvae of *C. spinosum* were grown at 28.5°C in 5 treatments of two replicates each: (1) MFSW with 10^4 cells/ml LCA and 100 larvae/liter; (2) OP with 20 larvae/liter; (3) OP with 100 larvae/liter; (4) laboratory seawater with its naturally occurring plankton (LP) dripping into 400 ml baskets (floating in the laboratory seawater tables)
<table>
<thead>
<tr>
<th>Location &amp; Seawater Fraction</th>
<th>Particle Size (μm)</th>
<th>Chlorophyll a (μg/l)</th>
<th>Particle #/ml</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surface Coastal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kewalo Marine Laboratory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OP&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;2</td>
<td>0.53</td>
<td>932</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>0.2 &lt; X &lt; 2</td>
<td>0.16</td>
<td>57,392</td>
<td>''</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.69</td>
<td></td>
<td>''</td>
</tr>
<tr>
<td>Conc OP&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;2</td>
<td>1.72</td>
<td>2,345</td>
<td>''</td>
</tr>
<tr>
<td></td>
<td>0.2 &lt; X &lt; 2</td>
<td>0.22</td>
<td>88,827</td>
<td>''</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1.94</td>
<td></td>
<td>''</td>
</tr>
<tr>
<td>OPC&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&gt;2 (&lt;3 μm)</td>
<td>0.05</td>
<td>270</td>
<td>''</td>
</tr>
<tr>
<td></td>
<td>0.2 &lt; X &lt; 2</td>
<td>0.10</td>
<td>82,835</td>
<td>''</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.15</td>
<td></td>
<td>''</td>
</tr>
<tr>
<td>Kahe Point</td>
<td>Total</td>
<td>0.10</td>
<td></td>
<td>Bienfang &lt;i&gt;et al.&lt;/i&gt; 1984</td>
</tr>
<tr>
<td>Hawaiian Islands: Mean 9 stations</td>
<td>Total</td>
<td>0.09</td>
<td></td>
<td>Gilmartin and Revelante 1974</td>
</tr>
</tbody>
</table>
### TABLE 2.2. (CONTINUED) ANALYSIS OF PARTICLES FROM VARIOUS SEAWATER FRACTIONS IN HAWAIIAN WATERS

<table>
<thead>
<tr>
<th>Location &amp; Seawater Fraction</th>
<th>Particle Size (μm)</th>
<th>Chlorophyll a (μg/l)</th>
<th>Particle #/ml</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neretic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaneohe Bay</td>
<td>0.2 &lt; X &lt; 2</td>
<td>0.35</td>
<td></td>
<td>Taguchi and Laws 1988</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1.4</td>
<td></td>
<td>&quot;</td>
</tr>
<tr>
<td>Kaneohe Bay</td>
<td>Total</td>
<td>0.26</td>
<td></td>
<td>Gilmartin and Revelante 1974</td>
</tr>
<tr>
<td>Offshore</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hawaiian Islands: Mean 5 stations</td>
<td>Total</td>
<td>0.05</td>
<td></td>
<td>Gilmartin and Revelante 1974</td>
</tr>
<tr>
<td>Molokai</td>
<td>0.2 &lt; X &lt; 2</td>
<td>0.06</td>
<td></td>
<td>Taguchi and Laws 1988</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>0.075</td>
<td></td>
<td>&quot;</td>
</tr>
</tbody>
</table>

*a* Ocean Plankton: Particles in whole ambient ocean seawater collected outside Kewalo Marine Laboratory (Not put through reverse filtration).

*b* Concentrated Ocean Plankton: Particles in whole ambient ocean seawater collected outside Kewalo Marine Laboratory concentrated 3-fold by reverse filtration.

*c* Ocean Picoplankton: Particles in whole ambient ocean seawater collected outside Kewalo Marine Laboratory which passed through the 2 μm Nucleopore filter.
containing 200 larvae; and (5) LP in static culture (same as 1-3) with 100 larvae/liter.

**Experiment 2: Food-Limited Growth and Survival of Larval *C. spinosum* and *C. aculeata* in Ocean Plankton.**

The second experiment tested whether larval growth of both gastropod species was limited by the food available in ocean plankton (OP) and which larval density was best for larvae of *Crepidula aculeata*. Laboratory-cultured algae (LCA) were used to enhance OP, and as a separate diet in MFSW to compare with OP. Larvae of *C. spinosum* (A) and *C. aculeata* (B) were each raised at 26°C in three treatments of two replicates each: (1) $10^4$ cells/ml LCA and 20 larvae/l; (2) OP and 20 larvae/l; (3) OP enhanced with $10^4$ cells/ml LCA and a larval density of 20/l. A fourth treatment of two replicates was for *C. aculeata* only, as *C. spinosum* had been tested in Experiment 1: (4) OP and 100 larvae/l.

**Experiment 3: Interaction of Plankton Patches and Picoplankton with Larval Density of *Crepidula aculeata*.**

The third experiment tested the relative benefits of plankton patches and picoplankton as food sources for larvae at different densities. The particles in ambient OP were size-fractionated by reverse filtration through a 2 μm polycarbonate filter (Nucleopore®). A treatment to simulate a plankton-rich patch of OP was produced by concentrating three-fold the nannoplankton-sized particles (> 2μm) which did not pass through the filter. Nannoplankton particles are defined as those 2-20 μm; the diameter of particles in the water collected for this study never exceeded 13 μm. This treatment of
concentrated OP was called Conc. OP. The filtrate, containing only particles under 2 \( \mu m \), was used as the ocean-picoplankton treatment (OPC). The lower limit of sustainable growth was tested by diluting the particle concentration in OP with an equal volume of MFSW. This reduced plankton treatment (Reduc. OP) contained half the particle concentration of OP and was designed to simulate offshore waters where plankton concentrations are lower than in coastal waters. Water samples of ambient OP, the filtrate (OPC), and the concentrated fraction (Conc. OP) were measured for particle numbers and chlorophyll-a concentrations to determine food concentrations and check the effectiveness of the filtration apparatus.

Particle numbers and chlorophyll-a concentrations were determined for the same replicate samples. Chlorophyll-a samples were filtered consecutively through polycarbonate (Nucleopore®) filters with pore sizes of 2.0 \( \mu m \) and 0.2 \( \mu m \). Volumes of 200 ml for the OP and OPC, and 100 ml for the Conc. OP were filtered. To avoid damage to cells during filtration, 47 mm diameter filters were used at a vacuum pressure of <100 mm Hg (Goldman and Dennett 1985). The filters were immediately placed in dark plastic vials containing 100% acetone and stored for at least 24 h at -20°C before further processing. Chlorophyll a and phaeopigments were determined on a Turner fluorometer using a procedure recommended by Holm-Hansen et al. (1965). The polycarbonate (Nucleopore®) filter-induced increase in background fluorescence was subtracted from the readings, which were taken in the linear portion of the fluorometer response curve (between 40 and 80 units on scale), at the maximum sensitivity setting of the fluorometer (Venrick 1987).

Larvae of \textit{C. aculeata} were raised at 24.5°C in eight treatments of two replicates each: (1) \( 5 \times 10^4 \) cells/ml LCA and larval density of 20/l; (2) OP
and larval density of 20/l; (3) OP and larval density of 100/l; (4) Conc. OP and larval density of 20/l; (5) Conc. OP and larval density of 100/l; (6) Reduc. OP and larval density of 20/l; (7) Reduc. OP and larval density of 100/l; and (8) OPC and larval density of 20/l.

Experiment 4: Comparison of Particles in Coastal Ocean Seawater with Laboratory-Cultured Algae as Food for Veliger Larvae of Crepidula aculeata.

The fourth experiment tested the relative benefits of OP and LCA at the same nannoplankton concentrations, as well as the effect of changing water every day to replenish the food supply. A concentration of $10^3$ cells/ml was chosen because it was the average nannoplankton concentration in ambient coastal ocean seawater near the Kewalo Marine Laboratory. Larval density was 20/l for all treatments. Larvae of C. aculeata were raised at 27°C in four treatments: (1) OP changed every day; (2) OP changed every other day; (3) $10^3$ cells/ml LCA changed every day; and (4) $10^3$ cells/ml LCA changed every other day.

Experiment 5: Importance of Plankton Patches and Picoplankton in Larval Diets of Crepidula aculeata.

The fifth experiment evaluated the relative importance of the various nutritional components in ambient coastal ocean seawater. Treatments were designed to evaluate the importance of patches, picoplankton, nannoplankton particle diversity and dissolved organic matter (DOM) to larval nutrition. In addition to treatments used in previous experiments, four treatments used LCA at the same concentration as the ocean plankton treatment ($10^3$ cells/ml
LCA to simulate OP) or as the concentrated ocean plankton treatment (3 X 10^3 cells/ml LCA to simulate Conc. OP); each of these two concentrations of LCA was combined with MFSW and OPC to assess the importance of picoplankton. Larval density was 20/l for all treatments. Water and food were changed every other day. In addition, to assess any nutritional contributions from DOM, 0.22 μm MFSW was dripped continuously into floating baskets.

Larvae of *C. aculeata* were grown at 24.5°C in 11 treatments: (1) 5 X 10^4 cells/ml LCA in 0.22 μm MFSW; (2) OP; (3) Conc. OP; (4) Reduc. OP; (5) OPC; (6) 10^3 cells/ml LCA in 0.22 μm MFSW; (7) 10^3 cells/ml LCA in OPC; (8) 3 X 10^3 cells/ml LCA in 0.22 μm MFSW; (9) 3 X 10^3 cells/ml LCA in OPC; (10) 0.22 μm MFSW; and (11) continuously dripped 0.22 μm MFSW.

Later, larvae of *C. aculeata* were grown at 24.5°C in two treatments: (1) 5 X 10^4 cells/ml LCA in 0.22 MFSW (same as treatment 1 above); and (12) OP collected at a time when natural ocean plankton outside Kewalo Marine Laboratory was denser than usual (Particle concentrations given in Fig. 2.6; total nannoplankton: 30,000 particles/ml.)
RESULTS

Experiment 1: Effects of Phytoplankton Source and Larval Density on Veliger Growth and Survival in *Crucibulum spinosum*.

In Experiment 1 (Fig. 2.1) the highest rate of larval growth occurred in OP with larval densities of 20/liter (Treatment 2). Shell brims appeared on day nine, and earliest non-specific metamorphosis (i.e. metamorphosis without exposure to a specific inducer) occurred on day nine. At the higher larval density, 100/l, larvae grew faster in LCA (Treatment 1) than they did in OP (Treatment 3). In LCA brims appeared on days 11 and 15, and earliest non-specific metamorphosis occurred on day 23 for both replicates; in OP brims did not appear on larval shells during the 25 days of the experiment. Larvae grew slowly and died before the end of the experiment in LP in the drip-through baskets (Treatment 4) and in beakers (Treatment 5). Larval growth rates in all treatments were found to be significantly different from each other by Tukey’s Studentized Range test (P<0.05; [Sokal and Rohlf 1981]). Thus OP is a better source of nutrition than LP, and a larval density of 20/l allows for better growth and survival than 100/l. Particles larger than 2 μm were found to be twice as abundant in OP as in LP, probably because filter-feeding animals in the laboratory seawater pipes depleted available particles in the laboratory seawater.
Figure 2.1. Effects of plankton source and larval density on veliger growth of *Crucibulum spinosum*. For abbreviations see Table 2.1.
Experiment 2: Food Limited Growth and Survival of Larval *C. spinosum* and *C. aculeata* in Ocean Plankton.

In Experiment 2A and 2B (Figs. 2.2 & 2.3), larvae of both species grew fastest in enhanced OP (Treatment 3) and slightly slower in LCA (Treatment 1). The difference was significant for *C. spinosum* (Experiment 2A, Fig. 2.2) but not for *C. aculeata* (Experiment 2B, Fig. 2.3). All other treatment comparisons of larval growth rates within a gastropod species differed significantly (Tukey's test; P<0.05; [Sokal and Rohlf 1981]). Growth of both species was slowest in OP (Treatment 2). Larvae of *C. aculeata* reared at 100/l in OP (Treatment 4) had all died by day 9, while those reared at 20/l (Treatment 2) continued to grow and lived for the duration of the experiment (Fig. 2.3). For *C. spinosum* reared in enhanced OP (Treatment 3; Fig. 2.2), brims appeared on day 7 and the earliest non-specific metamorphosis occurred on days 6 and 11 in the two replicates. In LCA (Treatment 1), brims appeared on day 9 and earliest non-specific metamorphosis on day 11. Thus, larvae of *C. spinosum* grew significantly faster, developed brims, and metamorphosed earlier in the enhanced OP than in LCA. Larvae of *C. aculeata* did not grow significantly faster, but did develop brims sooner, in enhanced OP than in LCA. Larvae of both species grew in OP (Treatment 2), showing that at low larval densities (20/l), ocean plankton is an adequate source of nutrition.
Figure 2.2. *Crucibulum spinosum*. Food-limited growth of veligers in ocean plankton and laboratory-cultured algae (Experiment 2A). For abbreviations see Table 2.1.
Figure 2.3. *Crepidula aculeata*. Food-limited growth of veligers in ocean plankton and laboratory-cultured algae (Experiment 28). For abbreviations see Table 2.1.
Experiment 3: Effects of Plankton Patches, Picoplankton, and Larval Density on Veliger Growth in *Crepidula aculeata*.

The contribution of picoplankton and concentrated patches of nannoplankton to larval growth were examined in Experiment 3. Given the same seawater and food conditions, larvae grew faster in those treatments with larval densities of 20/l than they did in those of 100/l (Fig. 2.4). Larvae reared in $5 \times 10^4$ cells/ml LCA (Treatment 1) grew significantly faster than they did in all other treatments. This treatment was used as a control for maximum growth potential. Preliminary experiments showed that larvae reared in concentrations of LCA above $5 \times 10^4$ cells/ml did not grow any faster. Brims appeared on day 7, and non-specific metamorphosis occurred on days 12 and 17 in the two replicates. Larvae reared at 20/l in Conc. OP (Treatment 4) grew significantly faster than those in OP (Treatment 2). Brims appeared on larvae in Conc. OP (Treatment 4) on day 22 and non-specific metamorphosis started on day 24. No brims appeared on larvae in OP (Treatment 2) before the termination of the experiment. Concentrated OP was used to simulate plankton-rich patches in the ocean, and larvae grew faster in a "plankton patch" than in ambient ocean plankton. The growth rates of larvae reared at 20/l in OP (Treatment 2), Reduced OP (Treatment 6), and OPC (Treatment 8), were not significantly different from each other. This suggests a significant role for picoplankton in larval nutrition since larvae in ambient ocean plankton did not grow faster than those fed only picoplankton. If nannoplankton particles were more important, halving their numbers by diluting with filtered seawater (Reduced OP) should have an effect. Percent survival to day 14 under different food treatments showed trends similar to
Figure 2.4. *Crepidula aculeata*. Effects of plankton patches, picoplankton and larval density on veliger growth. For abbreviations see Table 2.1.
those for growth rates. Survival was highest in LCA (100%), slightly lower in Concentrated OP and similar for the other three treatments (Fig. 2.5).

At the higher larval density, simulated plankton patches did not enhance growth, but reduced plankton density significantly lowered growth rates. Larvae reared at 100/l in Concentrated OP grew at the same rate as those in OP, but larvae in both treatments grew significantly faster than those in Reduced OP. Although effects of food concentration on larval growth differed between the 2 larval densities, effects on larval survival to day 14 were similar. At 100 larvae/l survival was highest in Concentrated OP and lower but similar in OP and Reduced OP (Fig. 2.5). Particle counts and chlorophyll a concentrations are given in Table 2.2 and Fig. 2.6.

Experiment 4: Effects of Particle Source and Depletion on Larval Growth of Crepidula aculeata.

In Experiment 4 there were no significant differences in larval growth rates between cultures in which OP was changed every day and every other day (Fig. 2.7). Similarly, for LCA, growth rates did not differ between cultures changed every day or every other day. This result indicated that even at nannoplankton concentrations of 1,000 cells/ml (OP and LCA), larval grazing did not reduce food concentrations enough to affect growth in two days. However, larvae in the two OP treatments grew significantly faster and had higher survival than those in the two LCA treatments. This suggests that at the same nannoplankton concentration, OP is a better source of nutrition than LCA.
Figure 2.5. *Crepidula aculeata*. Larval survival to day 14 in Experiment 3. Values are means (± SE) of two replicates. For abbreviations see Table 2.1.
Figure 2.6. Densities of all particles in whole seawater (OP) and of particles size-fractionated by reverse filtration. A 2 \( \mu \text{m} \) Nucleopore filter was used to retain nannoplankton-size particles and increase their concentration 3-fold (Conc. OP). The filtrate (OPC) contained mostly picoplankton-size particles.
Figure 2.7. *Crepidula aculeata*. Effects of particle type and depletion on veliger growth. For abbreviations see Table 2.1.
Experiment 5: Importance of Phytoplankton Patches and Picoplankton to Larval Growth in *Crepidula aculeata*.

Experiment 5 evaluated the importance of patches, picoplankton, nannoplankton, particle diversity and DOM to larval nutrition. Larvae grew fastest when reared in $5 \times 10^4$ cells/ml LCA (Treatment 1), the control for maximum potential growth (Fig. 2.8). Larvae in Concentrated OP (Treatment 3) grew significantly faster than those in OP and OPC (Treatments 2 and 5). Thus, concentrating the nannoplankton, as would occur in a natural plankton patch, resulted in a faster larval growth rate. Larval growth rates in OP and OPC (Treatments 2 and 5) were similar, and both were significantly higher than in Reduced OP (Treatment 4). This suggests that most larval nutrition must have been provided by picoplankton because growth rates were no higher in OP which contained nannoplankton than in OPC which contained no nannoplankton. And while growth rates were not affected by a lack of large particles, they were significantly lower when particle density of both nannoplankton and picoplankton was reduced by half (Treatment 4). The growth rate of larvae in OP (Treatment 2) was significantly higher than in $10^3$ cells/ml LCA (Treatment 6). Both treatments had the same nannoplankton density, but OP included natural nannoplankton and picoplankton while LCA had only cultured nannoplankton.

Larval growth rates were similar in the two treatments of OPC combined with $3 \times 10^3$ cells/ml LCA and $1 \times 10^3$ cells/ml LCA (Treatments 9 and 7), even though the former contained more LCA. These treatments simulated Concentrated OP and OP (Treatments 3 and 2). However, when
Figure 2.8. *Crepidula aculeata*. Importance of plankton patches and picoplankton on veliger growth.
LCA was used with MFSW (Treatments 8 and 6) instead of OPC (Treatments 9 and 7), larval growth was significantly higher in the treatment with the higher concentration of LCA (3 \times 10^3 \text{ cells/ml}, Treatment 8). At these two concentrations of LCA, which simulate ambient and concentrated patches of nannoplankton, picoplankton must contribute enough to growth to negate any advantage a higher LCA concentration would have in MFSW. When treatments with identical concentrations of LCA (3 \times 10^3 \text{ cells/ml}, Treatments 9 and 8; 10^3 \text{ cells/ml}, Treatments 7 and 6) were compared, larval growth was significantly faster in cultures with picoplankton than without it. When larvae were cultured in static and continuously dripping 0.22 \mu m MFSW (Treatments 10 and 11), no sustainable growth was evident and larvae died by days 11 and 12, respectively. In addition, since other all treatments would have contained DOM, the differences in growth rates must have been due to the presence of picoplankton.

In a comparison of natural particles vs. laboratory-cultured algae, when picoplankton is equalized, LCA appears to be a better nutrition source. Larval growth rates were higher when larvae were reared in OPC with 3 \times 10^3 \text{ cells/ml LCA} (Treatment 9) than in Concentrated OP (Treatment 3). Similarly, they were higher in OPC with 10^3 \text{ cells/ml LCA} (Treatment 7), than in OP (Treatment 2). Perhaps there are more edible cells in LCA than in OP, or the higher particle diversity in OP is not important.

When larval growth on ambient particles is compared with growth on LCA, picoplankton appears to provide a significant portion of the nutrition. Larvae cultured in OP (Treatment 2) grew significantly faster than those in 10^3 \text{ cells/ml LCA} (Treatment 6). Either picoplankton provided a significant portion of the nutrition or diversity of nannoplankton in OP is important. However,
there was no difference in the growth rates of larvae reared in Concentrated OP (Treatment 3) and $3 \times 10^3$ LCA (Treatment 8). Thus diversity of cell types in ambient seawater does not appear to affect larval growth. While picoplankton provided a significant portion of larval nutrition in ambient plankton, its contribution may have been less important in concentrated plankton where it was a smaller component of the biomass.

The growth rate of larvae in Reduced OP (Treatment 4) was not significantly different than in $10^3$ cells/ml LCA in MFSW (Treatment 6). The former (Reduced OP) contained half the nannoplankton concentration of the latter ($10^3$ cells/ml LCA), but it also contained picoplankton (1/2 the ambient number of particles), and the nannoplankton was natural, while the latter contained LCA and no picoplankton. Here again, picoplankton appears to compensate for a low concentration of nannoplankton.

Larval growth was significantly enhanced in a natural plankton patch (Treatment 12). Larvae grew almost as fast as their maximum potential, and some larvae were competent on day eight. Particle concentration in the natural OP patch was an order of magnitude higher than in the simulated patch (Concentrated OP) produced in the laboratory (Fig. 2.6).

Larval survival to day 19 was approximately 70% in treatments with the slower growth rates (OP, Reduced OP, and OPC) and slightly higher in the Concentrated OP (Fig. 2.9). Larval survival was higher in the natural plankton-rich patch (89%) and highest in the control for maximum potential growth (99%).
Figure 2.9. *Crepidula aculeata*. Larval survival to day 19 in Experiment 5. Values are means (±SE) of two replicates. For abbreviations see Table 2.1.
Larval Life Span

Larval growth rates were used to predict minimum larval life-spans under various conditions. Larvae of *C. aculeata* are competent when shell length is 700 μm (Chapter 1). Larval growth as measured by shell length becomes more linear after the initial growth phase (Chapter 1 and this study). For this reason, larval growth rates were calculated as slopes of growth after day 14 and 10 in Experiments 3 and 5 respectively. These slopes were used to calculate the number of days to reach competent size (Fig. 2.10). In normal coastal waters of Oahu it would take a larva of *C. aculeata* 40-56 days to reach competent size. If it encountered a patch such as the one simulated in the laboratory, that time could be reduced to 25-38 days. However, natural plankton patches can have up to 30,000 nannoplankton-size particles/ml, and in those patches larvae can become competent in eight days. If larvae drifted offshore and encountered water with 1/2 the particles available in coastal seawater, the time to competent size could increase to 73 days. If only picoplankton was available, growth to competence would take 38-67 days.
Figure 2.10. *Crepidula aculeata*. Larval life-spans of veligers reared with different sources of nutrition. Life-spans predicted from growth rates in Experiments 3 and 5. Ranges are given where growth rates varied significantly. For abbreviations see Table 2.1.
DISCUSSION

The experimental results described here demonstrate that gastropod larvae have a tremendous plasticity in their response to various concentrations of particulate plankton (i.e., food). When plankton density is low, growth and developmental rates drop; when plankton is dense, developmental and growth rates increase, and larvae are capable of settling much sooner than when plankton is less concentrated.

Experiments with high concentrations of laboratory-cultured algae, ocean plankton supplemented with cultured algae, and concentrated ocean plankton all demonstrated that growth and development of larvae of *Crepidula aculeata* and *Crucibulum spinosum* can be much faster than in "typical" coastal ocean plankton. Similarly, decreased growth rates in ocean plankton with reduced particle counts and in low concentrations of laboratory-cultured algae demonstrate that larvae can develop more slowly than they do in "typical" coastal ocean plankton and still survive to metamorphic competence.

This plasticity in sustainable growth and development allows larvae to survive in a wide variety of situations they may encounter in the sea. The capacity of larvae to sustain growth at low particle concentrations is remarkable. Dissolved organics alone are not sufficient to sustain the larval species examined here and may not contribute significantly to growth even in combination with particulates. However, because of the ubiquitous nature of picoplankton in the ocean (Sieburth *et al.* 1978, Sieburth 1979), some particulates will always be available as larval food even when nannoplankton concentrations are low. Experiments in this study demonstrated that larvae grew when the ambient coastal seawater particulate concentration was
reduced by one-half and when all nannoplankton was removed. The lowest
level of particulates which will sustain larval growth was not determined here,
but it is definitely less than one half the concentration of ambient particles in
coastal seawater outside Kewalo Marine Laboratory.

On the other hand, the capacity of larvae for rapid growth means that
larvae grow and develop faster when phytoplankton concentrations are high.
In the ocean, circulation and mixing influence growth and aggregation of
plankton, creating patches which exist on varying temporal and spatial scales.
The capacity for rapid growth allows for an increase in growth rates when
larvae encounter a phytoplankton-rich patch or drift from offshore to coastal
waters. Thus larvae are opportunistic in their ability to take advantage of a
wide variety of feeding conditions; they can survive in extremely low plankton
concentrations and grow rapidly if they encounter higher concentrations.

Experiments with and without picoplankton demonstrate that larvae
derive a considerable portion of their nutrition from small particles.
Picoplankton is the principle component of plankton biomass in oligotrophic
water, in contrast to temperate waters where larger phytoplankters
predominate (Raymont 1980, Fogg 1986, Stockner and Antia 1986, Taguchi
and Laws 1988). In the present study picoplankton accounted for 63% of the
biomass in near-shore ocean water if particle volume is estimated from
particle diameter and counts. Picoplankton would be even more important in
offshore waters where the relative ratio of picoplankton to nannoplankton
typically increases (Fogg 1986; Table 2.2). While picoplankton contained 40%
of the total chlorophyll a in Kaneohe Bay on Oahu, Hawaii, it accounted for
80% in surface waters offshore of Maui and Molokai (Table 2.2; Taguchi and
Laws 1988).
Volume estimates from particle counts give a more accurate estimate of picoplankton biomass than chlorophyll $a$, since many picoplankton particles do not contain chlorophyll $a$ (Fogg 1986, Stockner and Antia 1986). In the present study picoplankton accounted for 23% of the available food if measured with chlorophyll $a$ and 63% if biomass is estimated from particle counts (Table 2.2). Probably a combination of chlorophyll-$a$ measurements and particle counts will give a more accurate picture of larval food resources.

Actual plankton content in offshore waters is probably lower than the "reduced ocean plankton" used in this study. With less food, larvae would grow slower and drift even further before becoming competent. The chlorophyll $a$ content of the "reduced ocean plankton" treatment was 0.35 $\mu$g/l, a level 4-5 times higher than that measured in the open ocean surface waters off Molokai and Maui (0.075 $\mu$g/l, Table 2.2; Taguchi and Laws 1988). The chlorophyll-$a$ value for the offshore waters provided by Taguchi and Laws (1988) is close to the value from the present study for picoplankton (0.10 $\mu$g/l), and in fact they found 80% of the biomass in the waters offshore was comprised of picoplankton. In the present study the best estimates of larval growth rates in offshore waters may come from larval growth rates in picoplankton rather than larval growth rates in "reduced ocean plankton".

The present investigation has revealed that, under natural conditions, larvae of the studied species are food-limited in the sense that concentrations of plankton they encounter are typically below those necessary for maximum growth and developmental rates as determined by laboratory studies. However, enough food is available in the "typical" oligotrophic sea for slow sustained growth. Paulay et al. (1985) also found larval growth of the sand dollar *Dendraster excentricus* to be food-limited in natural seawater;
the pre-competent period lasted six days longer than that of larvae grown at maximum growth rates on laboratory-cultured algae. The difference between growth rates in natural seawater and maximum growth rates may be smaller in temperate waters than in the present study because plankton is more abundant in temperate waters. The experiments of Paulay et al. (1985) on larvae of *D. excentricus* were conducted using coastal seawater at Friday Harbor, Washington, where phytoplankton densities were about 2.5 times greater than those found in Hawaii in the current studies (as determined by chlorophyll-\(a\) concentrations: 1.75 \(\mu g/l\) at Friday Harbor [Paulay et al. 1985]; 0.69\(\mu g/l\) in Hawaii [Table 2.2]).

Larval growth rates measured in ambient seawater fall between the maximum and minimum sustainable growth rates determined by laboratory experiments, thereby ruling out starvation as a typical larval condition in the sea. Larvae of *Crepidula aculeata* were still able to grow when ambient particle concentration was reduced by one half (0.35\(\mu g/l\) chlorophyll-\(a\)). Paulay et al. (1985) found larvae of *D. excentricus* could grow in seawater in which particle concentration was reduced to 1/5 of the normal concentration, translating to a chlorophyll-\(a\) concentration of 0.35 \(\mu g/l\), a figure identical to that for the reduced ration in the present study. Larval growth at these chlorophyll-\(a\) concentrations is expected because the chlorophyll-\(a\) concentration in the reduced rations of both studies falls in the middle of the range of chlorophyll \(a\) concentrations for tropical waters (0.1-0.5 \(\mu g/l\); [Lucas 1982, Bienfang et al. 1984, Olson 1987, Taguchi and Laws 1988]).

Larvae of *Crepidula aculeata* (this study) and *Dendraster excentricus* (Paulay et al. 1985) grew faster when the plankton in ambient seawater was enhanced with laboratory-cultured algae. These results also demonstrate that
larvae do not grow at their maximum growth potential in ambient seawater. The increase in larval growth in the LCA-enhanced ocean plankton was probably due to an overall increase in biomass of food. Cultured algae did not supply a nutrient absent or in low concentration in ambient seawater, because in Experiments 4 and 5 larvae grew faster in ocean plankton than in cultured algae at the same particle concentration.

Diversity of plankton species may also contribute to the superiority of ocean plankton over laboratory-cultured algae as a growth medium for invertebrate larvae. With laboratory-cultured algae, larval growth in a three-species diet was not enhanced over a two-species diet. (Chapter 1). However, many more phytoplankton species occur in ocean plankton, and this diversity may contribute to larval nutrition. Evidence from oysters indicates that larvae do not select the best food cells in natural seawater; given natural plankton densities and assemblages, larvae of *Crassostrea virginica* select smaller particles (<10 μm), but among small particles feed non-selectively among types or species of cells (Fritz *et al.* 1984).

Dispersal distances can be predicted by combining larval life-span estimates from this study with available current speed data. Larvae of *Crepidula aculeata* and *Crucibulum spinosum* will take 40-56 days to reach competence if they remain in the coastal waters around an island (Fig. 2.10). However, if they are taken offshore by currents, the precompetent period could extend for as long as 73 days. In the Subtropical Countercurrent, which extends from the Western Pacific to the Hawaiian Islands at 20°N, the mean current speed is 6.6 cm/sec, but a maximum flow of 16.5 cm/sec may be sustained for several months (Grigg 1981). At that current speed, the travel time from Johnston Island to Hawaii would be 50 days; and from Wake Island,
187 days (Grigg 1981). These distances and current speeds are the same or greater than those between other island groups in the Pacific Ocean. Larvae of *Crepidula aculeata* and *Crucibulum spinosum* would reach other island groups in the Pacific near the end of their precompetent period or during their competent period. Larval dispersal may account for their circumglobal distribution, but their common occurrence in bays and harbors suggests that adults may raft on ship bottoms.

In general, food supplies in oligotrophic areas of the ocean are adequate for larval growth. Larvae are very plastic in their response to various concentrations of particulate plankton. Larval growth rates and thus minimum duration in the plankton will vary with the water mass in which they are transported. If they encounter a dense plankton patch, their growth rate will increase and they could be metamorphically competent in a relatively short time. On the other hand, if larvae drift offshore where phytoplankton densities are typically lower, their growth rate will slow, but they can still survive even if picoplankton is the only food. The life-spans of larvae are sufficient for dispersal and maintenance of gene flow given the current speeds and distances between Pacific Islands.
CHAPTER 3

THE USE OF INCREMENTS IN STATOLITHS OF LARVAL GASTROPODS TO ESTIMATE GROWTH RATES IN THE PLANKTON

INTRODUCTION

Most benthic marine invertebrates are sedentary, and many spend a portion of their developmental period as larvae in the plankton. Estimates of the length of time larvae spend in the plankton (larval life-span) are used to predict several important aspects of larval and adult biology, such as dispersal capabilities (Thorson 1961, Scheltema 1971a, 1986, Crisp 1976, Palmer and Strathmann 1981, Ayal and Safriel 1982, Chapter 2), recruitment success (Obrebski 1979, Jackson and Strathmann 1981), life histories strategies (Menge 1975, Ayal and Safriel 1982), and speciation and extinction rates (Jablonski 1986). Despite the importance of larval duration, little is known about the actual life-spans larvae experience in the plankton.

Life-span, growth, abundance, distribution, and mortality of larvae have not been directly measured because of the difficulty in tracking these minute organisms in the vast three-dimensional space of the ocean. Most information about larval life has come from laboratory experiments. Investigations of planktotrophic (feeding) larvae in laboratory culture have shown that larval growth and life-span vary with temperature, nutrition and availability of metamorphic cues and suitable recruitment sites (Pilkington and Fretter 1970,

Some inferences concerning larval life-span have been made from the study of distinct cohorts of larvae in the field (Jorgensen 1981, Gaines and Roughgarden 1985, Yoshioka 1986). Since larvae in the plankton may be exposed to environmental conditions (e.g. temperature, light, or food) which differ markedly from those used in laboratory culture, the laboratory model may not simulate conditions in the plankton. Although knowledge of actual life-spans and their variability is crucial to a better understanding of dispersal, recruitment, genetic variation, and speciation, most predictions of larval growth and life-span based on laboratory studies have not been tested in the ocean.

A method for assessing the age of larvae collected from the plankton would enhance our knowledge of larval life-spans for marine invertebrates as it has for marine fishes. Such a method has long been available for marine fish larvae: studies which assess larval age by daily otolith increments have provided extensive information concerning the larval life of marine fishes (see Jones [1986] and Wellington and Victor [1992] for reviews). Photoperiod and feeding have been postulated to affect increment deposition in fish otoliths (Jones 1986).

Many mollusks have structures similar to fish otoliths, the statoliths of their statocyst balance organs. Like otoliths, the statoliths of squids are composed of aragonitic calcium carbonate and are used to detect rapid changes in acceleration and gravity (Stephens and Young 1978, Radtke 1983). The aragonite crystals in squid statoliths are deposited incrementally in a protein matrix to form layers which are similar to the daily increments in
fish otoliths (Brothers et al. 1976, Spratt 1978). The number of increments is positively correlated with size of the squid, and, in many cases, the increments have been demonstrated to be formed daily (Dawe et al. 1985, Lipinski 1986, Jackson 1989, Nakamura and Sakurai 1990). Other molluscan groups, including gastropod larvae, also have statocysts containing calcified concretions. If gastropod larval statoliths are similar to squid statoliths and grow incrementally, the increments might be enumerated to estimate the age of a larva.

To test the hypothesis that statoliths of larval marine gastropods grow by discrete daily accretion, statoliths from ten species were examined. These observations revealed spherical layering in the statolith. Light-dark cycles and feeding times of larval gastropod cultures were experimentally varied to test whether or not the increments in the statoliths were related to photoperiod or feeding intervals. In addition, statoliths of field-collected larvae were used to assess growth rates in the plankton.

The capability of determining the ages of larvae caught in the plankton would allow comparisons of growth and mortality rates of larvae of a single species caught in different areas.
METHODS

Species and Sites

Statoliths were obtained from larvae of eight mesogastropods (*Crepidula aculeata* [Gmelin 1791], *Crucibulum spinosum* [Sowerby 1824], *Epitonium ulu* [Pilsbry 1921] *Epitonium sp.*, *Littoraria scabra* [Linnaeus 1758], *Philippia oxytropis* A. Adams 1855, *Serpulorbis variabilis* Hadfield and Kay 1972, and *Styliferina goniochila* [A. Adams 1860]), a nudibranch *Phestilla sibogae* (Bergh 1905), and a pulmonate *Siphonaria normalis* (Gould 1846). Species were identified by descriptions in Kay (1979) and Taylor (1975).

Statoliths from all species were examined for evidence of incremental growth.

*Littoraria scabra* was chosen for the experimental part of this study because its extensive range from South Africa to Hawaii (Reid 1986) suggests a long planktonic duration. *Crucibulum spinosum* and *Serpulorbis variabilis* were used to assess growth rates in the plankton because they have large larvae which were common in plankton tows.

Larvae of *Crucibulum spinosum*, *Crepidula aculeata*, *Epitonium spp.*, *Littoraria scabra*, *Philippia oxytropis*, *Serpulorbis variabilis*, *Styliferina goniochila* and *Siphonaria normalis* were obtained from surface plankton tows in Kaneohe Bay, Oahu, Hawaii and on the south side of Oahu near Kewalo Marine Laboratory. In addition, larvae of *Crucibulum spinosum*, *Crepidula aculeata*, *Epitonium ulu*, *Littoraria scabra*, and *Phestilla sibogae* were cultured at Kewalo Marine Laboratory, Oahu, Hawaii.

Adults of *Epitonium ulu* were collected from the underside of *Fungia scutaria* in Kaneohe Bay, Oahu, Hawaii. In seawater tables, *E. ulu* were placed under *F. scutaria* and checked daily for egg capsule production.
Adults of *Phestilla sibogae* were obtained from the coral *Porites compressa*, which was collected from Kaneohe Bay, Oahu, Hawaii and maintained in seawater tables. For *E. ulu* and *P. sibogae*, capsules were removed from the corals and placed in aerated 0.22 μm Millipore-filtered seawater (MFSW) until hatching. Adults of *Crucibulum spinosum* and *Crepidula aculeata* were obtained from rocks near shore in Kaneohe Bay, Oahu, Hawaii. Egg masses were deposited on the rocks and brooded under the females’ shells; they were collected just prior to hatching (Chapter 1). Adults of *Littoraria scabra* were collected from rock walls at Coconut Island, Kaneohe Bay, Oahu, Hawaii. The species is ovoviviparous and larvae were collected in 0.22 μm MFSW after their release from the mantle cavity of a female.

Larvae of *E. ulu* were reared at a density of 1000/l in beakers containing 600 or 800 ml of 0.22 μm MFSW. Water and a diet of laboratory-cultured algae (LCA) were changed every other day according to the methods of Switzer-Dunlap and Hadfield (1977). The diet of laboratory-cultured algae consisted of three different algal species: the flagellates *Isochrysis galbana* Tahitian strain (I) and *Pavlova lutheri* (P), and the diatom *Phaeodactylum tricornutum* (Pt). Equal cell numbers of each algal species were mixed and diluted to the final feeding concentration of approximately 10⁴ cells per ml with 0.22 μm MFSW. Larvae of *P. sibogae* were reared according to the methods of Miller and Hadfield (1986).

Larvae of *Crucibulum spinosum*, *Crepidula aculeata* and *Littoraria scabra* were cultured in 1- or 2-liter glass beakers. Millipore-filtered seawater (0.45 μm) and a diet of laboratory cultured-algae (LCA) were changed every other day according to methods of Switzer-Dunlap and Hadfield (1977). The diets of laboratory-cultured algae (LCA) consisted of equal numbers of two or
three different algal species. In Experiments 1 and 2 the species were the same as for larvae of *E. ulu* (LCA-I,P,Pt). In Experiment 3, *I. galbana* was used with the diatom *Chaetoceros gracilis* (C) in a two-species diet (LCA-I,C). In multiple-species diets, equal cell numbers of each algal species were mixed to achieve feeding concentrations. For all cultures, algae were diluted to final feeding concentrations (10^4 and 5 × 10^4 cells/ml) with 0.45 μm MFSW. The concentration 10^4 cells/ml has been used in many studies of larval gastropods (Switzer-Dunlap and Hadfield 1977, Hubbard 1988, Paige 1988). The higher concentration of 5 × 10^4 cells/ml was used as a control for maximum potential growth rate.

To obtain growth data, ten actively swimming larvae were removed from each culture at 2-5 day intervals. Maximum shell lengths were measured with the aid of an ocular micrometer on a compound microscope, and the larvae were returned to the culture. Mean shell length for the larvae of each culture was calculated for each sampling day.

**Statolith Analyses**

Larvae from cultures and plankton tows were prepared for dissection on glass slides. A drop of distilled water was added to each larva and withdrawn by a drawn-out Pasteur pipette. This distilled water wash was repeated three times to remove all external salt. A glass cover slip or a minuten insect needle, mounted on the end of a wooden stick with epoxy resin, was used to break the shell of each larva. The tissue was teased into very small pieces with two mounted minuten insect needles and spread out to reveal the statoliths. The tissue was allowed to dry in place (5-10 min.), and a line was drawn on the bottom of the glass slide around the area of the
dissected tissue with a waterproof marker. A drop of alcohol (95-100%) was pipetted onto the tissue area to disperse the lipids. When the alcohol evaporated the process was repeated four to five times. After the preparation thoroughly dried following the last drop of alcohol, a cover slip was mounted with a permanent mounting medium (Pro-tex®).

Statoliths were examined by light microscopy at 2000X, and phase contrast or polarized light was used to increase contrast of the image. Polarized light gave the best contrast. I made repeated increment counts of statoliths without knowledge of age or size of the larva. The final value represented the mean of four counts. For statoliths with mean counts less than 12, repeat counts varied by less than three. For statoliths with mean counts greater than 12, repeat counts varied by less than four. Statolith diameters were measured after increments were counted.

An increment consisted of a wide, light (translucent) ring and a narrow dark (refractile) ring. These rings alternated from the inner core to the edge of the statolith. Increments were counted beginning at the edge of the inner core. Statolith diameters from newly-hatched larvae were measured to confirm the size of the inner core prior to hatching and to establish the location to begin counting layers. The layers were not in sharp focus or easily seen. The layers being counted were smaller (L. scabra: 0.11 μm, C. aculeata: 0.43 μm, S. variabilis: 0.17) than the resolution limit of the light microscope. Thus the light-dark layers being counted cannot be actual images of the layers but a distortion of the light field caused by the layers or some other phenomenon such as discontinuities on the surface of the statolith or diffraction rings associated with the size of the statolith. Experiment 2 described below although designed for other purposes tested the hypothesis that the layers
counted are artifacts (e.g. light diffraction rings) which increase in number with the size of the statolith.

**Scanning Electron Microscope Preparation**

Statoliths were examined in the Scanning Electron Microscope (SEM) to verify the presence of the microincrements seen in the light microscope. Of the species cultured in the laboratory, only *Crepidula aculeata* and *Crucibulum spinosum* had statoliths large enough to be prepared for the SEM. After preparing the larvae as described above for the light microscope, the statoliths were dragged away from the tissue with the needle. When a small number of statoliths (four to eight) was assembled on the slide, they were washed with a drop of ethanol (95-100%), allowed to dry and further rinsed with a drop of distilled water. Quick drying epoxy was spread thinly with a needle onto a small piece of broken cover glass, and the statoliths were transferred with the needle to the epoxy. After the surface epoxy dried, the piece of cover glass was transferred to an SEM stub and glued with epoxy. The surface of the preparation was etched with 0.5% HCl for 35-40 seconds, or 0.1% EDTA (disodium ethylene diaminetetraacetate) buffered with NaOH to pH 8.0 for two to ten minutes, then rinsed with distilled water. The HCl preparation revealed increments better than the EDTA preparation. After etching, SEM stubs were dried in an 80°C oven for 1 hour. All samples were coated with gold/palladium in a Hummer II sputter coater before viewing in a Hitachi S-800 field emission SEM. After viewing, some samples were etched again with HCl and viewed again. A few attempts were made to polish the sample surface with 0.05 μm alumina paste to remove the gold/palladium coat.
before the second etching. However, this procedure usually dislodged the statolith.

Experiments

Experiment 1: Growth of shell and statolith of *Littoraria scabra*

The first experiment investigated the relationship between larval shell growth and the growth and number of increments of the larval statolith. Immediately after hatching, 1,000 larvae of *Littoraria scabra* (from many females) were established in each of four one-liter beakers. Throughout the study, MFSW and a diet of laboratory-cultured algae (I,P,Pt) at a density of $10^4$ cells/ml were changed every other day. Two of the beakers (Cultures 2 & 3) were maintained in seawater tables where the temperature was 24-25°C. They were controls to determine growth rate of the larvae and age at onset of metamorphic competence. After the mean shell length had reached 300 μm, five to ten larvae were removed each day and placed in a bowl of MFSW with either a twig from a mangrove or a small piece of concrete which had been kept in the seawater tables for two to three days. The most reliable method for inducing metamorphosis was a mangrove twig. If larvae metamorphosed in 24 hr, they were counted as competent on the day they were removed from the culture. Two of the beakers were maintained in the ambient laboratory where the light:dark (L:D) cycle was approximately 14:10 hr, and the temperatures ranged from 25-28°C. Larvae from one beaker (Culture 1) were used for statolith analyses. Samples of larvae were removed and frozen each day for 26 days. Later samples were defrosted, shell lengths measured, and four to eight larvae of each sample prepared for statolith dissection. Those from the other beaker were used to determine larval survival. All larvae were
counted at three to eight day intervals and all live larvae returned to the culture.

Experiment 2: Effects of Light and Food on Statolith Growth and Formation in Larvae of *Littoraria scabra*

The second experiment examined how the light:dark cycle and the feeding schedule might affect the formation of increments in the statolith. Immediately after hatching 2000-3000 larvae of *L. scabra* (from many females) were established in each of three one-liter beakers containing MFSW with $10^4$ cells/ml LCA-I,P,Pt. Larval density was higher than in other experiments because of the need to sample larvae in darkness. Cultures were maintained in the laboratory where the temperatures ranged from 25-28°C. Treatment 1 (Light) was maintained in constant light from a fluorescent bulb. Treatment 2 (Dark) was maintained in total darkness 24 hr/day; the beaker was kept in a box in a darkroom and all water changes, additions of algal food, and sampling were done in the dark. Treatment 3 (Restricted Food) was maintained in the 14:10 L:D cycle (same as Experiment 1) but laboratory-cultured algae were available only every other day. Laboratory-cultured algae were added to the culture on the first day, but on the second day larvae were transferred to fresh MFSW and no algae were added. On the third day the food cycle was repeated and LCA was added. Samples for larval shell growth and statolith analyses were taken on days 1, 3, 6 and 11, except for statolith samples of the Dark treatment which were taken on day eight instead of six. Samples from Experiment 1 were used as controls for these treatments.
Experiment 3: Growth Rates of Larvae Reared in Ocean Plankton

In the third experiment, growth rates of larvae reared in ocean water with its natural complement of phytoplankton (OP) were compared with those reared in a maximal concentration of laboratory-cultured algae. Growth rates of larvae of *Littoraria scabra* reared in ocean plankton were compared with growth estimates based on statoliths from larvae reared in laboratory-cultured algae from Experiment 1. Growth rates of larvae of *Crucibulum spinosum* reared in ocean plankton were compared with growth estimates based on statoliths from field-caught larvae.

Natural ocean water was collected by bucket directly from the ocean near the Kewalo Marine Laboratory and passed through 41-µm Nitex® mesh to remove predators and competitors. Particle counts in the ocean water were made on a Coulter Counter® or Elzone 180 Particle Counter®. The algal diet was a two-species mixture of *I. galbana* and *C. gracilis* (LCA-I,C).

Larval cultures of *Littoraria scabra* and *Crucibulum spinosum* were maintained in clean five-liter beakers standing in seawater tables where temperature was steady at 26°C. Larval densities were low to simulate densities in oligotrophic oceans (Chapter 2). Larvae of *C. spinosum* were reared at a density of 20/L in three treatments of two replicates each: (1) OP (mean particle concentration $10^3$/ml); (2) $10^4$ cells/ml LCA-I,C; and (3) $5 \times 10^4$ cells/ml LCA-I,C. Larvae of *L. scabra* were reared in a density of 50/L in two treatments of two replicates each: (1) OP (mean particle concentration $10^3$ cells/ml); and (2) $5 \times 10^4$ cells/ml LCA-I,C.
Growth Estimates Based on Statoliths from Field-caught Larvae

Tropical waters contain larvae of many species of gastropods, but only those with distinctive forms or colors can be easily distinguished to species. Larvae of *Crucibulum spinosum*, *Crepidula aculeata*, *Epitonium* spp., *Littoraria scabra*, *Philippia oxytropis*, *Serpulorbis variabilis*, *Siphonaria normalis* and *Styliferina goniochila* were identified, but larvae of only *C. spinosum* and *S. variabilis* were abundant enough to obtain a growth curve for a particular sampling day. Larvae of *C. spinosum* were from samples taken in Kaneohe Bay on April 30, 1985. Larvae of *S. variabilis* were from samples taken on the south side of Oahu near Kewalo Marine Laboratory on August 4, 1983. Shell lengths were measured and statoliths analyzed. Growth rates in the plankton were estimated from the regression of shell lengths on the number of increments on the statoliths, assuming one increment is added each day of free-swimming larval life.
RESULTS

Statoliths from larvae of *Crepidula aculeata*, *Crucibulum spinosum*, *Epitonium ulu*, *Epitonium sp. Littoraria scabra*, *Phestilla sibogae*, *Philippia oxytropis*, *Serpulorbis variabilis* and *Styliferina goniochila* were examined for evidence of incremental growth. All had layers which could be distinguished under light microscopy. Larger larvae had larger statoliths, making them much easier to investigate with the techniques described in this study.

Scanning Electron Microscope Analysis

Fine rings counted on the light microscope were difficult to photograph because they are close together and inside a spherical statolith (Fig. 3.1), and SEM techniques were used to confirm that these rings exist. Because of the difficulty in preparing and etching statoliths for the SEM, only a few statoliths were etched exactly to a plane through the central core. Statoliths were often lost, buried in epoxy, etched too much or not enough (Fig. 3.2).

When viewed with the SEM, the center of the statolith of *Crepidula aculeata* consisted of an inner core region which coincides with the diameter of the statolith at hatching (Fig. 3.3). Surrounding the central core are concentric layers of crystals which are presumably comprised of calcium carbonate (Fig. 3.3). The crystal structure of the statolith looks similar to the crystal structure of squid statoliths when viewed with the SEM (Radtke 1983). Although Fig. 3.3 reveals some increments it does not show the 12 increments expected for the statolith of a 12 day old larva. Enlargement of Figure 3.3 shows the layers in more detail (Fig. 3.4). Two distinct increments are visible: one near the edge of the statolith and one which could be the edge of the
Fig. 3.1. *Littoraria scabra*. Statolith of a 12 day old larva.
Fig. 3.2. *Crepidula aculeata*. Statolith of ten day old larva. Etching with 5% HCl eroded the edges of the statolith but not the central core.
Fig. 3.3. *Crepidula aculeata*. Statolith of 12 day old larva. Growth increments are concentric rings near the outer edges. Arrow points to an increment which could be the edge of the central core present at hatching. At least two increments are visible and enlarged in Fig. 3.4.
Fig. 3.4. *Crepidula aculeata*. Enlargement of Figure 3.3. Arrows point to a distinct increment near the edge of the statolith and one which could be the edge of the central core.
central core. More or less etching may reveal more increments between the
two distinct ones. SEM analysis verified that statoliths contain increments
formed by concentric layers of crystals. These layers may be the increments
viewed in the light microscope but the number of increments expected from a
known age larva was not verified.

The increments were not as clear when etched with 0.1% EDTA (Fig. 3.5) as they were when etched with HCl. However, etching with EDTA often
revealed the central plane and since the process is slower, it is easier to
control. EDTA could be used to etch to the central plane, and then HCl could
be applied to reveal layers in the crystal structure.

**Growth and metamorphosis of *Littoraria scabra*: Experiment 1**

Larvae of *Littoraria scabra*, reared at a larval density of 1000/L and
provided with laboratory-cultured algae at $10^4$ cells/ml, grew from about 120
μm shell length at the time of release from the female to about 420 μm in 45
days. Metamorphosis was first induced on day 35, when mean shell length
was 380 μm (Fig 3.6). In the one culture where survivorship was measured,
93% of larvae survived to become metamorphically competent. Non-specific
metamorphosis was rare, and without an inducing surface such as mangrove
twig, larvae continued to swim until the experiment was terminated after 77
days.
Fig. 3.5. Crepidula aculeata. Statolith etched with 0.1% EDTA.
Figure 3.6. *Littoraria scabra*. Veliger growth and size at metamorphic competence in three separate cultures (Exp. 1). Error bars are 95% confidence intervals. Arrow indicates size at metamorphic competence.
Statolith Growth and Formation: Experiments 1 and 2

The diameter of statoliths of larvae of *Uttoraria scabra* increased in direct relation to the age in days of the larvae (\( Y = 7.66 + 0.345 \times X; r^2 = 0.94 \); Exp. 1; Fig. 3.7). The number of layers in the larval statoliths was directly proportional to the age in days of the larvae (\( Y = 0.156 + 0.882 \times X; r^2 = 0.99 \); Fig. 3.8). The slope of the regression of increment number on larval age was less than one, and the statolith increment on any given day was one to two days less than the larval age. If the first increment is added during day 1 post-hatching, then statoliths removed from two-day-old larvae would have one increment.

Larvae of *Uttoraria scabra* grew as well as those in Experiment 1 when reared in constant light (Light: Treatment 1), slower when reared in constant darkness (Dark: Treatment 2), and slowest when reared with food every other day (Restricted-Food: Treatment 3) (Exp. 2; Fig. 3.9). Experiment 2 was terminated on day 11 because the cleanliness of the culture and the number of larvae caught in the seawater surface tension could not be monitored in the Dark treatment. Statolith layers were impossible to distinguish for larvae reared in the Light or Dark treatments. For larvae reared in the Restricted-Food treatment, statolith layers were visible and increased with the number of days until day six.

Experiment 2 also resolved the hypothesis that rings could be caused by light diffraction. This hypothesis is ruled out because the statolith increased in size in the Light and Dark treatments without forming any visible rings.
Figure 3.7. *Littoraria scabra*. Relationship between the diameter of statoliths and larval age in days (Exp 1). Each symbol is one larva.

\[ Y = 0.345X + 7.66; \quad r^2 = 0.94 \]
Figure 3.8. *Littoraria scabra*. Relationship between the number of increments in statoliths and larval age in days (Exp 1).
Figure 3.9. *Littoraria scabra*. Growth of veligers in constant light, constant darkness, and restricted food (Exp. 2). In the restricted food treatment larvae were fed on alternative days. Error bars are 95% confidence intervals.
Statolith diameter increased in all three treatments except after day 6 for those larvae cultured in the Restricted-Food treatment (Fig. 3.10). Thus, statoliths from larvae reared in constant light or dark grew but did not form layers, as did to statoliths from larvae reared in a natural light:dark cycle (Figs. 3.7 and 3.8). For larvae reared in the Restricted-Food treatment, shells continued to grow after day six (Fig. 3.9), but statolith diameters did not increase after day six (Fig. 3.10) nor did increment numbers.

**Larval Growth of *Crucibulum spinosum* and *Littoraria scabra* in Natural Ocean Plankton: Experiment 3**

At low larval densities (50/L) and high concentrations of laboratory-cultured algae (50,000 cells/ml), larvae of *L. scabra* reached metamorphic size (375 μm) in 18 days (Fig. 3.11). Larvae grew more slowly when they were reared with only the plankton available in natural ocean water as a source of food (Fig. 3.11). A few larvae reared in natural ocean plankton (OP) reached metamorphic size on day 23, the day the experiment was completed. While no larvae achieved competence in natural ocean plankton, if the experiment had been continued, the mean shell length of larvae on day 24 would have been equal to metamorphic size of larvae in other cultures. Larvae fed LCA in experiment 3 grew faster and reached metamorphic size sooner (day 18) than those in experiment 1 where larval density was higher and algal food supply lower.

At larval densities and algal food concentrations promoting fastest growth (Chapter 1), larvae of *Crucibulum spinosum* reached metamorphic size (700 μm) in 10 days (Fig. 3.12). There was no difference in growth rate between larvae cultured in $10^4$ and $5 \times 10^4$ cells/ml as long as the larval
Figure 3.10. *Littoraria scabra*. Growth of statoliths from veligers cultured in constant light, constant darkness and restricted food (Exp. 2). In the restricted food treatment larvae were fed on alternative days. Error bars are 95% confidence intervals.
Figure 3.11. *Littoraria scabra*. Growth of larvae in natural ocean plankton (OP) and in LCA (50,000 cells/ml) (Exp. 3). Larval density was 50/L for all treatments. Symbols are means of two replicate treatments (±SE). Arrow indicates size at metamorphic competence.
Figure 3.12. *Crucibulum spinosum*. Growth of larvae in natural ocean plankton (OP) and high concentrations of LCA (cells/ml) (Exp. 3). Larval density was 20/L for all three treatments. Symbols are means of two replicate treatments (±SE). Arrow indicates size at metamorphic competence.
density was low (20/L). Larvae grew more slowly when they were reared with ocean plankton (OP) as the only source of food (Fig. 3.12), and did not reach metamorphic size before the end of the experiment on day 13. If larvae reared in ocean plankton had continued to grow at the same rate as they had between day 3 and 13, they would have reached metamorphic size on day 28.

Growth Estimates Based on Statoliths from Field-caught Larvae

Not enough larvae of *Littoraria scabra* were collected from plankton tows in any one sampling period to estimate growth curves of larvae in the plankton. Statoliths from twenty-one larvae of *C. spinosum* collected on April 30, 1985 in Kaneohe Bay were analyzed to estimate growth rates in the plankton. There was a correlation between the larval shell length and the number of layers in the statoliths (Fig. 3.13). When a linear regression was applied to the data points in Fig. 3.13, the size predicted for day 0 was 370 urn, which is close to the hatching-shell length (320 urn) for larvae of *C. spinosum*. The estimated growth rate of the shell was 23 urn per day. The same regression predicts that larvae will reach metamorphic size (700 urn) in 14 days, much less than the 28 days predicted by growth rates of larvae reared in the laboratory with natural concentrations of ocean plankton (Experiment 3; Fig. 3.12).

Statolith diameters of the 21 larvae of *C. spinosum* correlated with both larval shell length (Fig. 3.14) and the number of layers in the statolith (Fig. 3.15), demonstrating that as larvae grow, statoliths grow and add layers. A linear regression of the data points in Fig. 3.14 predicted that when larvae were hatching size (320 urn), the statolith diameter was 23.5 urn. This is the statolith diameter measured in newly-hatched larvae, and the inner core of
Figure 3.13. *Crucibulum spinosum*. Relationship between shell length and number of increments in the statoliths of veligers collected from plankton tows.
Figure 3.14. *Crucibulum spinosum*. Relationship between diameters of statoliths and shell length of veligers collected from plankton tows.
Figure 3.15. *Crucibulum spinosum*. Relationship between diameters of statoliths and number of statolith increments in veligers collected from plankton tows.
23.5 μm which developed prior to hatching had no distinct visible layers. Counting of layers in the statolith began at the perimeter of the inner core. A linear regression of the data points in Fig. 3.15 predicted that when statolith diameter was 24 μm the number of layers was zero.

Statoliths from eight larvae of *Serpulorbis variabilis* also were analyzed to estimate growth rates in the plankton. Larval shell length was correlated with the number of layers in the statoliths (Fig. 3.16). A linear regression of the data points in Fig. 3.16 predicted a hatching size on day one of 475 μm, the estimated growth of the shell to be 9.4 μm per day and the time to metamorphic size to be 20 days. Shell length at hatching of larvae of *S. variabilis* is 270 μm (Hadfield *et al.* 1972) and shell length at metamorphosis is 660 (Taylor 1975). Since seven of the eight larvae were close to metamorphic size and might have been in a plateau phase of growth, linear regression may not predict overall larval growth. The data fit a logarithmic growth curve as is common for other species that have reached the plateau phase (Chapter 1); in fact the logarithmic curve for the eight data points passed through 285 μm shell length on Day 1 which is near the known hatching size (Day 1: 270 μm; Fig. 3.16). Perhaps larval shell length increases rapidly from 270 to 550 μm and then reaches a plateau above 550 μm. Statolith diameter of the eight larvae of *S. variabilis* was correlated to both larval shell length (Fig. 3.17) and the number of layers in the statolith (Fig. 3.18).
Figure 3.16. *Serpulorbis variabilis*. Relationship between shell length of veligers collected from plankton tows and number of increments in statoliths. The known size at hatching is 270 μm.
Figure 3.17. *Serpulorbis variabilis*. Relationship between shell length of veligers collected from plankton tows and diameter of their statoliths.

\[ Y = 0.0114X + 14.76; r^2 = 0.45 \]
Figure 3.18. *Serpulorbis variabilis*. Relationship between diameter of statolths of veligers collected from plankton tows and number of statolith increments.

\[ Y = 0.157X + 19.4; \quad r^2 = 0.78 \]
Our understanding of larval life in the plankton would be enhanced if data on growth rates during the precompetent period could be derived from planktonic larvae. Adult growth rates in many invertebrate groups, especially mollusks, have been estimated from quantifiable changes in mineral deposition (Pannella and MacClintock 1968, Nishiwaki et al. 1975, Brothers et al. 1976, Lutz and Rhoads 1977, Spratt 1978, Bretos 1980, Peretz and Adkins 1982, Ekaratne and Crisp 1984, Deith 1985, Jones et al. 1989). While most studies used growth lines in the external shell, a few have used statoliths from adult or paralarval squid (Radtke 1983, Bigelow 1992). Prior to this study, statoliths from larval gastropods had not been examined.

Statoliths were formed by layers, and these layers were present in the larvae of all ten gastropods examined here. Alternating rings of light and dark were counted but were narrow and difficult to see. Since the rings being counted were smaller than the resolution limit of the light microscope, they could not be actual images of the layers. Experiment 2 ruled out the hypothesis that the rings seen could be light diffraction rings associated with the size of the statolith. However, they could be a distortion of the light field caused by the incremental layers or by discontinuities on the surface of the statolith.

SEM techniques were used to confirm that crystal layers were present which could be the basis for the fine rings counted on the light microscope. Because of the difficulty in preparing and etching statoliths for the SEM, verification of increment counts from known age larvae was not accomplished.
Further study with a more elaborate etching protocol may reveal more increments.

Statoliths in larval *Littoraria scabra* grew as the larvae grew. Statolith diameter increased in direct proportion to shell length in laboratory-reared larvae. When larvae were provided with food only on alternative days, larval growth was slow and the statoliths did not increase in diameter after day six. The number of statolith increments increased with larval age in days in a 1:1 ratio indicating daily deposition. Initial increment deposition began within one day of hatching.

Manipulation of photoperiod and feeding times provided insight into increment deposition in larvae of *L. scabra*. In constant light or dark, statoliths grew in size but did not form increments. Since layers were formed in a normal light:dark photoperiod, photoperiodicity is required for the formation of layers. The same is true for otoliths of most fish species (Jones 1986).

Increment deposition in statoliths of *Littoraria scabra* was also affected by the number of feeding periods, when feeding periods were fewer than one per day. Under these conditions increments were deposited on a daily basis until day six. Statoliths did not grow from day six to eleven and no new layers were deposited. The effect of more than one feeding period per day was not examined. Increment deposition may be disrupted if larvae fail to feed. In fish larvae feeding time does not alter increment formation, but starvation disrupts increment deposition in a few species (Jones 1986). Larval gastropods are able to grow and survive on extremely low concentrations of food (Chapters 1 and 2). Whether or not low concentrations will have the same effect as absence of food was not examined but may be critical to interpreting age of
field-collected larvae. Until laboratory studies verify the hypothesis that daily increment formation is a universal phenomenon even under sub-optimal conditions, there will be some doubt about the accuracy of aging field-collected larvae using statolith increments.

Two factors render statoliths of gastropod larvae less useful than otoliths in fish larvae or statoliths in squid paralarvae. First, larval gastropod statoliths are an order of magnitude smaller than larval fish otoliths. The diameters of statoliths in this study ranged from 8-31 μm in diameter. The diameters of otoliths in larval fish and paralarval squid may be as small as 20-30 μm at hatching, but during larval periods of less than 52 days, the fish otoliths grow to 300-500 μm (Radtke 1984, Wellington and Victor 1992) and squid statoliths to 300 μm (Bigelow, 1992). Because of small size and large nucleus, increment widths of larval gastropods are an order of magnitude narrower than those of squid or fish young. Thus the increments in larval fish otoliths are wider and easier to count than those in statoliths from larval gastropods.

The second factor which makes statoliths of gastropod larvae less useful than otoliths in fish larvae is the different growth histories of larval fish and larval gastropods. When fish larvae did not settle after they were competent, they grew at a reduced rate, and increments in their otoliths were significantly narrower than they were prior to competence (Wellington and Victor 1992). The number of narrow increments was used to estimate the length of the competent period before settlement. Growth of larval gastropods in most species stops shortly after they attain metamorphic competence, and it is likely that the statolith stops growing at the same time. Therefore
examination of the statoliths may not reveal the extent of larval duration during the competent period.

Larval growth rates and minimum larval durations predicted from statolith counts of field-collected larvae fell within the expected range and corresponded to growth rates expected from available food concentrations. In general, minimum larval durations are inversely correlated with food concentration (Chapter 2). The minimum larval duration of *C. spinosum* predicted from statolith counts of plankton samples (14 days), was within the range for laboratory-reared larvae (28 days for natural ocean plankton and 10 days for high concentrations of laboratory-cultured algae) and indicates a relatively high growth rate in Kaneohe Bay water. Thus, increment counts can be used to estimate ages in *C. spinosum* but data from *Serpulorbis variabilis* are less clear.

Larval shells of different species differ in shape, and growth measured by shell length may be linear in one species and logarithmic in another or may change within one species in different culture conditions (Chapter 1 and 2). Most of the larvae of *Serpulorbis variabilis* from plankton tows were close to metamorphic size, and if age estimates are accurate, require very rapid growth at smaller sizes to fit the known hatching size. Larvae of *S. variabilis* grow in a barrel shape rather than the typical gastropod spiral shape and the growth curve measured by shell length may differ from that of other species.

The estimation of ages of larval gastropods from their statoliths has the potential to be a useful tool in the study of larval life spans. Increments were present in all species examined. Deposition of increments is probably a daily phenomenon induced by a diel photoperiod. While deposition may not be affected by the number of feeding periods, it may be interrupted at low food
concentrations. Minimum larval durations estimated from field-collected larvae appear to fit within the expected range of known larval durations under known conditions. Statolith techniques will be best applied to species with large larvae and will require verification of increment deposition under sub-optimal conditions. Knowledge of the age of planktonic larvae will allow us to answer questions about minimum planktonic duration and larval growth rates under various conditions larvae encounter in the plankton.
CHAPTER 4

LARVAL GROWTH AND METAMORPHOSIS OF *EPITONIUM ULU*, A GASTROPOD SYMBIONT OF THE SOLITARY CORAL, *FUNGIA SCUTARIA*

INTRODUCTION

The larval stage is the major dispersal phase for relatively sedentary benthic marine invertebrates (Scheltema 1971b, 1986). In many species adults have little or no ability to move long distances and must live either near or exactly where they settled and metamorphosed as larvae. Moreover, larvae of most species do not appear to exert much control over their own dispersal because they are transported as virtually passive particles unable to swim faster than ocean currents (Hannon 1984). However, larvae can respond to various environmental cues and regulate their position in the water column, an activity which may allow them to ride currents and change their horizontal distribution (Cronin and Forward 1986). Larvae of many species metamorphose only in response to a specific inducing substance emanating from the benthic habitat, for example, the adult food, adult substratum or the adult itself (see Hadfield, 1986, for a review).

The ability of larvae to settle on or near a substratum which is suitable for the adult stage is crucial to the survival of the species and has been the focus of much research (Scheltema 1961, Crisp 1974). One might expect this
ability to be strong in species whose adults require a highly specific food or substrate, as in a symbiotic species for which there is no marginal habitat. For such a species the larvae should settle and metamorphose only in response to a specific inducer, or the planktonic period should be relatively short or non-existent, keeping offspring close to the adult habitat.

A few molluscan species have a direct or obligatory relationship with living corals (Hadfield 1976). These species may be more specifically adapted to their biological hosts than the many other molluscs which are generally adapted to a reef ecosystem. Special adoptions can occur between symbionts, and for marine mollusks, the larval or developmental period may be affected by the need to settle near a particular coelenterate. For example, the coral, *Porites compressa* is both the habitat and prey for the coral-eating nudibranch *Phestilla sibogae* (Hadfield 1978). A compound released by the coral induces larvae to metamorphose in proximity to the coral.

In this paper I report on larval life and metamorphosis of the wentletrap *Epitonium ulu* (Pilsbry 1921) (Gastropoda: Epitoniidae), which occurs on the underside of the solitary coral *Fungia scutaria* Lamarck. Wentletraps are common symbionts of certain coelenterates (Robertson 1981). Extensive life-history information on epitoniid snails is lacking except for a few species (McDermott 1981, Robertson 1981, 1983) of which two (*Epitonium millecostatum* and *Epitonium albidum*) live in the tropics. Adults of *Epitonium ulu* have a thin white shell and are usually found with numerous white egg capsules attached to the underside of *F. scutaria*. Bosch (1965) suggested that maturation from settlement to reproduction took only 3 weeks.

In this study I investigated the larval life-span and metamorphic induction of *E. ulu*. If larval life is extremely short, larvae may never drift away
from the coral reef and potential coral hosts. If larval life-span of *E. uiu* is long, larvae may locate a host by metamorphosing only in the presence of an inducing substance given off by *Fungia scutaria*.
METHODS

Adult maintenance and larval growth

Adult *Epitonium ulu* were collected from *Fungia scutaria* on several patch reefs in Kaneohe Bay, Oahu, Hawaii, U.S.A. On two reefs all *F. scutaria* and associated snails were examined and enumerated. The animals were kept in flow-through seawater tables at the Kewalo Marine Laboratory, University of Hawaii, Oahu, Hawaii. In the seawater tables, one *E. ulu* was placed under each of eight *F. scutaria* and checked for egg capsule production during four separate 24 hr periods. Capsules were removed from the coral and placed in filtered aerated seawater (26-27°C) until hatching. Thirty capsules were measured with the aid of an ocular micrometer on a dissecting microscope and the eggs counted from three capsules. Thirty uncleaved eggs from two females were measured with the aid of an ocular micrometer on a compound microscope. Larvae were reared in beakers containing 600 or 800 ml of 0.22 μm Millipore-filtered seawater (MFSW), with and without antibiotics (60 μg/ml Penicillin G and 50 μg/ml Streptomycin sulfate). Water was changed every other day by pouring the culture through a 41 μm Nitex® sieve seated in another beaker. Fresh MFSW was poured into the sieve until it replaced the old MFSW as it overflowed the bottom beaker. Larvae, concentrated in the small volume of water at the bottom of the floating sieve, were pipetted into a beaker of fresh MFSW.

Larval cultures were supplied daily with a diet of laboratory-cultured algae (LCA) consisting of equal numbers of two or three different algal species. Equal cell numbers of each algal species were mixed and diluted to
the final feeding concentration of approximately $10^4$ cells per ml with 0.22 μm MFSW. In three of the larval growth cultures the algal species were the flagellates *Isochrysis galbana* (Tahitian strain) and *Pavlova lutheri*. In the fourth larval growth culture and all the antibiotic experiments, the diatom *Phaeodactylum tricornutum* was added to the diet mixture.

To examine larval growth, ten individual larvae were removed every 2-3 days, and shell lengths (the longest shell dimension) were measured with the aid of an ocular micrometer on a compound microscope. Mean shell lengths and confidence intervals of those ten individuals were calculated for each culture on each sampling day.

To determine shell mass and ash-free tissue mass, larvae were pipetted onto filter paper that absorbed the small volume of seawater, leaving the larval shell free from salts. Larvae were dried at 80°C for 24 hr, weighed to 0.1 μg on a Cahn electrobalance, ashed at 500°C for 3 hr and reweighed (Kempf 1981). Ten aluminum foil pans containing larvae were weighed for each sampling date. Individual larvae from hatching to 18 days old have insufficient mass for significant values on the Cahn electrobalance, and thus two to ten larvae were weighed in each pan. Numbers of larvae per pan on each sampling day are given in Table 4.1. Tissue and shell masses were calculated from pre- and post-ash weighings. Shell mass was considered to be equal to the total ash mass since shell makes up most of the inorganic substance of the larva. Adult tissue of *E. ulu* contained only 10% ash (Bell unpublished data).
TABLE 4.1.
NUMBER OF LARVAE USED PER PAN TO DETERMINE LARVAL MASS

<table>
<thead>
<tr>
<th>Larval Age (days)</th>
<th>No. Larvae/Pan</th>
<th>Larval Age (days)</th>
<th>No. Larvae/Pan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-9</td>
<td>10</td>
<td>1-7</td>
<td>10</td>
</tr>
<tr>
<td>12-14</td>
<td>5</td>
<td>9-11</td>
<td>5</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>12-17</td>
<td>2</td>
</tr>
<tr>
<td>&gt;17</td>
<td>1</td>
<td>&gt;17</td>
<td>1</td>
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</table>
Larval cultures were set up to investigate larval growth and metamorphosis. Cultures were maintained on the laboratory bench where temperatures ranged from 25-27°C. Larvae from two cultures, fed the two- and three-species diets respectively, were used to measure shell growth before and after metamorphosis. Two cultures, derived from the same egg mass and fed the two-species diet, were used for: (1) measuring shell growth; (2) determining onset and maintenance of metamorphic competence; and (3) measuring growth of larval tissue and shell mass. Both linear and log-linear regression analyses were performed to test the relationship between shell length and mass of the larval tissue and shell.

**Antibiotic experiments**

In preliminary trials, larval survival rates were higher when larvae were cultured with antibiotics but larval growth seemed to be slower. Thus, to determine the effect of antibiotics on larval growth, larvae from the same egg mass were reared under identical conditions with and without antibiotics. Two trials of two replicates each were run, at 25°C. Cultures from both trials were sampled for shell lengths, and cultures from the second trial were sampled additionally for larval mass.

**Metamorphosis**

In preliminary experiments larvae metamorphosed in culture without a specific inducer when they were approximately 35-45 days old and 400 μm in shell length. Three methods were compared to determine the best inducer for metamorphosis. Ten larvae of competent size were placed in a finger bowl
with: 1) 200 ml MFSW and an 8 cm diameter *F. scutaria*; 2) 200 ml of MFSW seasoned with *F. scutaria* for 24 hr; and 3) 200 ml of MFSW and 3 adult *E. ulu*. To season MFSW with *F. scutaria*, an 8 cm *F. scutaria* was placed in 1 liter of MFSW for 24 hr. Once an inducer had been established, larvae from larval growth experiments were tested to find the earliest day and minimum size that larvae became competent. Larvae in a culture were considered competent if 30% metamorphosed when induced. Testing of larvae for competence was started when the mean larval shell length for a culture was 320 μm.

Larvae from one culture were maintained in culture conditions after they were competent to determine if they could remain competent when not induced by their symbiotic host. After competence was established, larvae were transferred daily to clean dishes of fresh MFSW. Every two to four days larvae were counted as live, dead, or metamorphosed. Only live larvae were returned to the culture. Metamorphosis which occurred in culture without induction by *F. scutaria* was termed non-specific metamorphosis.

To test how long juveniles could survive if they were not on an *F. scutaria*, newly metamorphosed juveniles were starved for 32 days. Thirty juveniles which metamorphosed on day 34 without a specific inducer were sampled for mass and shell length analysis. They were maintained in MFSW with no food until day 66, when they were sampled again.
RESULTS

Life history observations

*Epitonium ulu* had a clumped distribution in relation to *Fungia scutaria*. On one patch reef 12% of the corals had snails, but that reef held only 28 *F. scutaria*. On a second patch reef, with 289 *F. scutaria*, only 4% of the corals had snails. However, one coral had 29 snails (2-14 mm shell length) imbedded in a mass of egg capsules.

Egg capsules were found in clusters attached to the undersides of *F. scutaria* and often attached to adult *E. ulu*. The capsules were connected to each other and to the coral by an elastic mucous thread which stretched when the capsules were pulled apart. The capsules were elliptical with thick walls and many projections (see Robertson, 1980). Mean capsule size was 1.32 mm wide and 1.76 mm long. The capsules contained 520-578 eggs, with a mean of 558 for three capsules. Mean diameter for uncleaved eggs was 78 μm (range 75-83; n=30; eggs from two females). Adults produced a mean of 32 egg capsules per 24 hr based on observations of eight individuals over four separate 24 hr periods. Using the mean number of capsules laid per day (32) and the number of eggs per capsule (558), the estimated egg production was 18,000 eggs per day per female.

Intracapsular embryonic development lasted six days at 26°C, and embryos left the capsules as planktotrophic veligers with a bilobed velum. Initial hatching size varied among females. Mean shell length at hatching was 133 ± 12 μm S.D. (range 115 to 145; n=5 egg masses).
Larval growth

The coiling of the shell at hatching was nearly planispiral, but slightly orthostrophic. The transparent shell consisted of one whorl. When larvae reached metamorphic competence, the shell consisted of three whorls and the aperatural edge was smooth and rounded. This rounded edge or lip was present in older, larger larvae and may denote a cessation in shell growth and competence for metamorphosis. The surface of the shell was smooth with faint axial lines, and a mark after the first whorl may represent a transition zone formed at hatching.

After hatching, the growth rate, as measured by shell length, was fairly linear until it leveled off between 380 and 420 µm (Figure 4.1). In the fastest-growing culture this non-growth phase began on day 18. The addition of the diatom *Phaeodactylum tricornutum* to the two-species diet may have caused the faster growth rate. The three-species diet was used in subsequent cultures.

Initial hatching size did not affect the final size at competence. The final shell lengths of larvae shown in Fig. 4.1 were the same even though initial hatching sizes in the cultures covered a 30 µm range. Results of these experiments do not clarify whether slower growth of larvae affects shell length at competence. Cultures grew at different rates, but larvae from all cultures had the same final size.

Larval tissue, measured as µg of ash-free dry weight (AFDW), increased until day 24 (Fig. 4.2) and then reached a plateau. Mean tissue mass was not significantly different from day 24 to day 42. A similar plateau was evident in shell mass (Fig. 4.2) and shell length (Fig. 4.1).
Figure 4.1. Shell growth of larvae of *Epitonium ulu*. Symbols are means of ten individuals and error bars are 95% confidence intervals. Vertical arrows indicate the day larvae became competent. Horizontal arrow indicates size of smallest larva induced to metamorphose.
Figure 4.2. Tissue mass (AFDW) and shell mass of larvae and starved juveniles of *Epitonium ulu*. Symbols are means of ten pans containing various numbers of larvae (Table 4.1). Error bars are 95% confidence intervals. Juveniles were starved from day 34 to day 66.
Variability in mass increased with age of the larvae, possibly due to the decrease in the number of larvae weighed in each pan as larvae increased in size and age. Juvenile tissue mass decreased significantly to the same mass as newly-hatched larvae during starvation for 32 days. Tissue mass decreased without causing death of the larvae.

Larval tissue expressed as a percentage of total larval mass remained on average between 25-35% from hatching to competence (Fig. 4.3). Tissue mass of newly-metamorphosed individuals was 2.2 µg AFDW, which did not differ significantly from the tissue mass of larvae from days 24-42.

To examine whether shell length measurements represent overall growth of larvae of *E. ulu*, shell lengths were compared to tissue mass and shell mass:

\[
\text{Log tissue mass} = 2.85(\text{log shell length}) - 16.2 \quad r=0.98.
\]

\[
\text{Log shell mass} = 2.90(\text{log shell length}) - 15.57 \quad r=0.99.
\]

Thus there were no changes in tissue mass or shell mass which were not adequately reflected in shell length. In *E. ulu* shell lengths accurately represent larval growth and are easy to determine.

Larvae reared in MFSW treated with antibiotics grew significantly slower than larvae reared in MFSW which was not treated (Fig. 4.4). All aspects of growth were affected, shell length as well as tissue mass and shell mass (Figs. 4.5 and 4.6). Experiments were continued until day 29 when 60% of the larvae in cultures without anti-biotics were competent to metamorphose; no larvae in cultures with antibiotics were competent on day 29. Antibiotics, commonly used to enhance the survival of cultures, may actually inhibit growth in larvae of *E. ulu*.
Figure 4.3. Percent of total mass contributed by tissue (AFDW) for larvae of *Epitonium ulu*. Arrow indicates time of metamorphic competence.
Figure 4.4. Larval growth of *Epitonium ulu* with and without antibiotics.
Figure 4.5. Larval tissue mass (AFDW) of *Epitonium ulu* reared with and without antibiotics.
Figure 4.6. Larval shell mass of *Epitonium ulu* reared with and without antibiotics.
Metamorphosis

MFSW which had been seasoned with one *F. scutaria* for 24 hr was the best inducer of metamorphosis. When larvae of competent size (400 μm) from the same culture were tested with the three methods described above, those with a live *F. scutaria* died; those with adults of *E. ulu* remained as swimming larvae; those in MFSW seasoned with *F. scutaria* all metamorphosed. Larvae with the live coral were not induced to metamorphose because they were caught in the mucous released by the coral. In another trial with live coral, larvae were eaten by hydrozoans living on the underside of the *F. scutaria*.

In the slowest growing culture, larvae were first competent on day 44 post-hatching. In two cultures from the same egg mass larvae were first competent on day 26 post-hatching. The larvae which were competent were large for their age. Mean shell length on day 26 for the two cultures was 380 μm and 371 μm respectively, and competent larvae were 410-420 μm in shell length, larger than the 95% CI for mean shell length on day 26. By day 29 larvae began to metamorphose in culture without a specific inducer. The metamorphosed individuals had a mean shell length of 400 μm (range 356-428; n=20) on day 34 and were no different in size from larvae which were induced to metamorphose. In a test for competence with another culture, only the largest larva (380 μm) was induced to metamorphose; the other larvae were less than 364 μm.

Experiments were conducted to determine the potential duration of the competent period and to test whether larvae would metamorphose only when induced by water seasoned with *F. scutaria*. Larvae were competent on day 26 and the experiment was started with 463 larvae (Fig. 4.7).
Figure 4.7. Cumulative percent of competent larvae of *Epitonium ulu* which metamorphosed non-specifically in culture (without a specific inducer).
By day 29, larvae had begun to metamorphose non-specifically. Between day 32 and day 34, approximately 40% of the larvae metamorphosed and by day 36, 50% had metamorphosed. Most larvae (78.1%) metamorphosed by the end of the experiment on day 60; 20% died without metamorphosing.
DISCUSSION

The life history and reproduction of *Epitonium ulu* is similar to that of others in the same genus. It is distributed in clumps as is *Epitonium albidum* (Robertson 1981). Adult *E. ulu* lays 32 egg capsules per day (this study); *E. albidum* lays 16 (Robertson 1981) and *E. rupicola*, a temperate species, lays 54 (McDermott 1981). Mean diameter of uncleaved eggs is 78 µm for *E. ulu* (this study), 68 µm for *E. albidum* (Robertson 1983) and 73 µm for *Epitonium millecostatum* (Robertson 1981). The mean number of eggs per capsule is 558 for *E. ulu* and 431 for *E. rupicola* (McDermott 1981).

The larvae of *E. ulu* have a long developmental period in the plankton but not a long competent period. A precompetent period of 26 days is not short compared to other gastropod larvae (Kempf 1981, Pechenik 1984, Pechenik and Lima 1984, Perron 1986, Hubbard 1988, Chapter 1, Chapter 3), and larval life-span of congeners is unknown. Larvae of *E. ulu* did not remain competent for a long period of time; early in the competent stage, larvae began to metamorphose without any specific inducer from their requisite host.

The length of the precompetent larval period varies widely among species of gastropods and is constrained by developmental type and rate. The precompetent period can range from a few hours (Jackson 1985, 1986, Olson 1985) to several months (Phillips *et al.* 1979, Domanski 1984). Results of the experiments presented here suggest that larvae of *E. ulu* have a precompetent period of 26 days, which is in the middle of the range for larvae of prosobranch gastropods (Pechenik 1984, Pechenik and Lima 1984, Perron 1986, Zimmerman and Pechenik 1991). If larvae of *E. ulu* have a similar
precompetent period in the field, they could disperse a long distance. High fecundity and wide dispersal may be a good strategy for locating a host with a clumped distribution such as *F. scutaria*. On the other hand, *E. ulu* larvae could be retained in Kaneohe Bay where *F. scutaria* is abundant.

Gastropod larvae vary in their ability to maintain competence. Species which require a specific adult prey or habitat typically maintain competence longer than those with more general adult requirements. *Epitonium ulu* began to metamorphose without a specific inducer 3 days after becoming competent. *Littoraria scabra*, found on mangroves, remains competent for long periods of time (Chapter 3). *Aplysia juliana*, which feeds on one algal species, became metamorphically competent at 28 days and remained so for 288 days (Kempf 1981). Species with less specific adult requirements, i.e., *Crucibulum spinosum*, (Chapter 1), *Crepidula aculeata* (Chapter 1) and *Crepidula fornicata* (Pechenik 1984), undergo non-specific metamorphosis. A non-specific settlement cue such as a bacterial film, a hard substrate or a chemical from any coral may still enable larvae to settle on a coral reef which might include *F. scutaria*. Results presented here suggest that juveniles can remain alive for 32 days without any food. Perhaps they settle on a reef and then "search" for *F. scutaria*, and juveniles may feed on diatoms, bacteria or benthic algae before switching to their adult prey (Hadfield 1963).

Effects of antibiotics on larval growth have rarely been tested because larval survival in antibiotic-treated cultures is often significantly higher than in cultures without antibiotics (Switzer-Dunlap and Hadfield 1981). Pilkington and Fretter (1970) reared unfed gastropod larvae with and without antibiotics. Those without antibiotics grew faster than those with antibiotics. One interpretation of their results is that bacteria in the untreated seawater
provided some food. Kempf (1981) cultured larvae of *Aplysia juliana* in MFSW without antibiotics but only after rearing larvae with antibiotics during the growth phase (first 18 days). The suppression of growth by antibiotics suggests that they should not be used in studies where maximum growth or length of larval life is important. If larval densities are low, the use of antibiotics may become unnecessary for some species (Chapter 1 and 2).

Larvae of *Epitonium ulu* appear to have little flexibility in choosing a settlement site. A short time after a larva becomes competent it will metamorphose non-specifically if it has not earlier encountered a *Fungia scutaria*. However, in embayments like Kaneohe Bay, Hawaii, larval retention may be such that larvae of *E. ulu* have a high probability of encountering a host within hours of becoming competent.
EPILOGUE

The duration of the larval stage of marine benthic invertebrates affects larval dispersal, recruitment, and gene flow among populations. Despite the importance of larval duration to population dynamics of marine invertebrates, little is known about actual life spans larvae experience in the plankton.

Tropical oceans, in contrast to temperate ones, have narrower temperature ranges and lower plankton biomass. In a tropical oligotrophic environment such as Hawaii, nutrition, rather than temperature, may have a greater influence on the range of larval life span. To test the effects of nutrition on larval life span, laboratory conditions (e.g. temperature, larval density, and food) were designed to simulate conditions in coastal waters of Hawaii. In this study I examined duration of larval life of four prosobranch gastropods: *Crepidula aculeata*, *Crucibulum spinosum*, *Littoraria scabra* and *Epitonium ulu*.

The gastropod larvae studied here are flexible enough to grow and survive in a variety of nutritional conditions. The algal-species composition of their diets is not as important as the total biomass or dry weight of the food, as long as the algal species are ingestible and digestible. Larval growth rates varied in response to both algal concentration and algal species composition suggesting that larvae can grow in the wide range of phytoplankton species and abundances they may encounter in the ocean.

In general, food supplies in tropical oligotrophic areas of the ocean are adequate for larval growth. Despite the low phytoplankton biomass in Hawaiian waters, starvation is probably not a typical condition for gastropod
larvae. They can bypass the microbial loop and graze directly on picoplankton.

The larvae studied here are plastic in their response to various concentrations of particulate plankton. Larval growth rates and thus minimum duration in the plankton will vary with the water mass in which larvae are transported. If they encounter a dense plankton patch, their growth rate will increase and they may become metamorphically competent in a relatively short time. On the other hand, if larvae drift offshore where phytoplankton densities are typically lower, their growth rate will slow, but they can still survive even if picoplankton is the only food. Given the current speeds and distances between Pacific islands, life-spans of larvae are sufficient for dispersal and maintenance of gene flow among the islands.

The estimation of ages of larval gastropods from their statoliths has the potential to be a useful tool in the study of larval life spans. Deposition of increments is probably a daily phenomenon induced by a diel photoperiod. While deposition may not be affected by the number of feeding periods, it may be interrupted in low food concentrations. This phenomenon may be significant in oligotrophic oceans. Minimum larval durations estimated from field-caught larvae appear to fit within the expected range of known larval durations under known conditions. Statolith techniques will be best applied to species with large larvae and will require verification of increment deposition under sub-optimal conditions. Knowledge of the age of planktonic larvae will allow us to answer questions about minimum planktonic duration and larval growth rates under various conditions larvae encounter in the plankton.

Larval growth rate affects the duration of the larval period until the beginning of metamorphic competence. After that the duration of the
competent period may be a species-specific phenomenon. Larvae of some species will metamorphose only in response to a specific inducer. Other species become less specific in their response over time and may settle on any hard surface with or without a bacterial film. *Littoraria scabra* maintains specific requirements for metamorphic induction for at least 6 weeks after metamorphic competence. *Crepidula aculeata, Crucibulum spinosum* and *Epitonium ulu* require specific inducers for a week or less.
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