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Feeding ecology in the early life stages of the crown-of-thorns starfish, *Acanthaster planci* (L.)

Thesis submitted by
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ABSTRACT

Evaluating factors affecting survivorship during early life stages of the crown-of-thorns starfish, *Acanthaster planci* (L.), is essential to understand mechanisms of the population outbreaks. The objective of this study was to determine whether food availability is a crucial factor controlling the growth, development and survival of the larvae and the juveniles of *A. planci*. This was done through field and laboratory studies on their feeding ecology.

Larvae were reared in an *in situ* rearing apparatus to assess the relative importance of different natural foods. This apparatus was designed to create different food environments inside its chambers by progressive filtration: 100 μm mesh filtered seawater (100 μm FSW), 1 and 0.2 μm filtered seawater (1 μm FSW and 0.2 μm FSW), and activated charcoal filtered seawater (ACF). In a preliminary rearing experiment, larvae successfully developed through to metamorphosis in 100 μm FSW, 1 μm FSW and 0.2 μm FSW, but not in ACF. These results suggested that adequate amount of particulate and dissolved foods was available in ambient seawater to support larval development. However, subsequent deployment of the apparatus revealed that chlorophyll *a* concentrations inside the rearing chambers, except for ACF, were significantly higher than in ambient seawater. The initial objective was not achieved, because this apparatus created enriched food environments. The importance of food limitation needed to be assessed by means other than *in situ* larval rearing.

To test food limitation, larvae were reared in freshly collected and coarse filtered seawater, and the same seawater treated by further filtration or food enrichment. Larvae were also reared in nutrient enriched seawater (NES), where the concentration of natural phytoplankton was elevated by adding nutrient solution and incubating for a few days. Larvae reared in 2 μm filtered seawater consistently failed to develop. Larval development and survival were nil or significantly slower in the coarse filtered seawater than in the same seawater enriched with cultured microalgae, dissolved free amino acids (DFAA), or in NES. When larvae were reared in NES having different fixed phytoplankton (chlorophyll *a*) concentrations, a change from modest larval survival to optimal survival through rapid development occurred between 0.5 and 0.8 $\mu g\ L^{-1}$. This range is slightly higher than typical chlorophyll *a* levels found in the Great Barrier Reef (GBR) waters.

These results altogether suggest that, if total chlorophyll a concentration is used as the sole index of food availability, larvae are usually food limited. However, there are also the factors of particle size that the larvae can filter and ingest, and the contribution of dissolved organic matter (DOM) to the larval nutrition.

The feeding rates of larvae on naturally-occurring particles, plastic beads of different sizes or cultured microalgae were measured to determine the dependence of larval feeding on particle size. The clearance rate of larvae feeding on eukaryotes (3.6-4.6 μm, mean equivalent spherical diameter) was 118-358 μL larva^{-1} h^{-1}, while the rate when feeding on cyanobacteria (1-2 μm) was 0.035-0.349 μL larva^{-1} h^{-1}. This was despite ambient conditions where cyanobacteria were magnitudes more abundant, showing that larvae selectively feed on nanoplankton (> 2 μm). Clearance rates of cultured microalgae were significantly higher than those of 6 and 20 μm beads, suggesting that feeding is also dependent on chemical properties of food particles.

The net uptake rates of three DFAA, alanine, glutamic acid and arginine, by larvae were measured to estimate their potential contribution on the larval nutrition. Larvae selectively took up alanine (neutral amino acid) over glutamic acid (acidic) and arginine (basic). The net uptake rates of alanine from the initial concentrations of 148, 150 and 465 nM were 8.9, 15.4 and 36.9 pmol larva^{-1} h^{-1}, respectively. The alanine uptake at these substrate concentrations could account for 13.1, 23.1 and 55.3 % of the basic metabolic demand of a larva. Neutral DFAA are a potentially important food source, however, their ambient concentrations (trace - ca. 200 nM) generally seem too low to make a significant contribution to the larval nutrition.

Post-metamorphic juveniles were reared in the laboratory and deployed in the field to test the effect of different coralline algae on growth rates. Juveniles feeding in the laboratory on the coralline alga, *Lithophyllum insipidium*, grew significantly faster than those feeding on the algae, *Neogoniolithon clavacymosum* and *Lithothamnium pseudosorum*. The estimated growth rate of juveniles in the field was relatively high, compared to rates in the laboratory, and was similar to the previous growth data obtained with animals feeding on multi-specific natural food. Because a wide range of algae is accessible to juveniles, food quality may not influence growth rate and hence survivorship in relatively shallow reef environments.

Phytoplankton and DFAA were identified as potential food sources for larvae. Comparison between potential contributions of these food sources to the larval nutrition,

in terms of ambient concentrations and the ability of larvae to utilise these food sources, indicates that nanoplankton are the major food. Because the availability of phytoplankton (chlorophyll *a* concentration) in the GBR waters is lower or marginal to the critical range, larvae are usually food limited. Food limitation is likely to have a significant impact not only on larval survival by extending the vulnerable planktonic period, but also on juvenile survival by reducing juvenile size at metamorphosis. This study confirms that food is a crucial environment factor controlling the growth, development and survival of *A. planci* larvae in the GBR waters.

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Chapter One

General introduction

Population outbreaks of the large corallivore starfish, *Acanthaster planci* (L.), have caused extensive damage on many coral reefs in the Indo-West Pacific over the past three decades (Moran, 1986; Birkeland and Lucas, 1990). Together with ever increasing demands for developing coral reef resources, outbreaks of *A. planci* have become one of the greatest concerns to both managers and scientists (Potts, 1981; Birkeland and Lucas, 1990). As a result, nearly a thousand scientific papers and articles on *A. planci* have been published (Birkeland and Lucas, 1990). Such a large body of information has tremendously improved our understanding of biology and ecology of *A. planci*, yet the cause or causes of the outbreaks remain unknown (Birkeland and Lucas, 1990).

A. planci is very fecund, producing up to 50 million eggs during one spawning event (Kettle and Lucas, 1987). Survivorship during planktonic larval and juvenile stages is undoubtedly crucial in determining the size of adult populations (e.g. Lucas, 1972; Birkeland, 1982; Moran, 1986; Birkeland and Lucas, 1990). Thus, a number of experimental studies have been conducted to evaluate factors affecting the growth, development and survival during early life stages of *A. planci*. Larvae are influenced by temperature and salinity (Lucas, 1975; Johnson and Babcock, 1994), and quality and quantity of phytoplankton food (Lucas, 1982). The growth and survival of juveniles are influenced by the availability of coral diet (Yamaguchi, 1974; Lucas, 1984), and predation (Keesing and Halford, 1992b). Birkeland and Lucas (1990), in reviewing the literature, identified further research needs in the following four areas: dispersal of larvae; food limitation in the growth, development, and survival of larvae; predation on juveniles; and the timing of juveniles switching their diet from algae to corals.

The present study focuses on food availability as a factor controlling the growth, development and survival of *A. planci* during its early life stages. In this chapter, relevant information on larval and juvenile *A. planci*, and other marine invertebrates with similar life history, is reviewed in order to clarify what knowledge is required in coping with the theme.

1.1 Are *Acanthaster planci* larvae food limited?

1.1.1 Food limitation in planktotrophic larvae of marine invertebrates

Are the growth, development and survival of planktotrophic larvae limited by the supply of food in nature? This question has long been a focal point for debate concerning the early life history processes of marine invertebrates (Thorson, 1950; Strathmann, 1985; Emlet et al., 1987; Pechenik, 1987; Olson and Olson, 1989; Rumrill, 1990). Shortage of food could potentially lead to mortality of planktotrophic larvae both directly, by starving them to death, and, indirectly, by limiting the rates of growth and development, thus exposing them to greater risk of predation (Thorson, 1950). Evaluating the extent of starvation, or food limitation, is a significant matter in understanding patterns of larval dispersal, recruitment, and temporal variation in adult populations (Pechenik, 1987).

Many authors have investigated the possible occurrence and importance of food limitation in larvae of crustaceans (e.g. Checkley, 1980; Huntley and Boyd, 1984; Hansson et al., 1990), molluscs (e.g. Bell, 1991; Baldwin and Newell, 1991; Gallager et al., 1994) and echinoderms (e.g. Paulay et al., 1985; Hart and Scheibling, 1988; Fenaux et al., 1994). The growth, survival and subsequent recruitment of crustacean larvae are often limited by the food supply (e.g. Connel, 1985), but not always (Scheltema and Williams, 1982; Huntley and Boyd, 1984). Despite some evidence for food limited growth and developmental plasticity with different food rations in some mollusc and echinoderm larvae (e.g. Paulay et al., 1985; Fenaux et al., 1988; Fenaux et al., 1994), the extent of food limitation in the field remains equivocal (cf. Olson and Olson, 1989).

The possibility of food limitation was suggested by laboratory studies of temperate echinoid larvae. Paulay et al. (1985) showed that the growth and development of the larvae of the echinoid *Dendraster excentricus* slowed as the supply of phytoplankton was reduced. Fenaux et al. (1994) demonstrated not only that metamorphosis of the larvae of the echinoid *Paracentrotus lividus* reared in natural seawater was delayed compared to those reared in natural seawater enriched with cultured microalgae, but also that naturally-occurring larvae showed "food-limited form". These authors suggested that food limitation commonly occurred in the field, because concentrations of phytoplankton in the natural habitat were lower than the level at which maximum growth and development of larvae were supported. They also implied that an extended larval period might have a significant impact on the survival of larvae. A similar situation was reported for temperate asteroid larvae. The growth, development and survival of the larvae of the

asteroids *Luidia foliorata* and *Asterina miniata* were influenced by variation in quantity of phytoplankton food (George, 1994; Allison, 1994).

Extrapolating laboratory results to the field has been questioned, particularly in the light of evidence demonstrating the significance of food sources other than phytoplankton, such as dissolved organic matter (hereafter referred to as DOM) and bacteria (Manahan et al., 1983; Manahan et al., 1989; Rivkin et al., 1986). Manahan and co-workers (1983, 1989) demonstrated that the larvae of the echinoid *Strongylocentrotus purpuratus* were capable of taking up DOM as alternative food. A series of studies with the larvae of the asteroid *Odontaster validus* suggested that food limitation was unlikely to occur in antarctic waters. These larvae were able to ingest bacteria and to take up DOM (Bosch et al., 1988; Rivkin et al., 1991; Shilling and Manahan, 1991). Concentrations of these potential diets in antarctic waters seem to be sufficient to support maximal rates of growth, development and survival (Welborn and Manahan, 1991; Shilling and Manahan, 1994).

From these studies of echinoderm larvae, the importance of food limitation in larval ecology can not be generalised. It may vary not only with species, but also with quantity and quality of food in the specific body of water, which larvae encounter. In evaluating potential food limitation, it is necessary to identify the major dietary components, and to investigate variation in food concentrations in the habitat.

1.1.2 Food limitation in *A. planci* larvae

Whether *A. planci* larvae are food limited in the field has been a matter of controversy. Birkeland (1982) proposed that phytoplankton blooms, resulting from nutrient rich river runoff, enhanced the survivorship of larvae, leading to increases in adult populations on coral reefs adjacent to land masses (terrestrial runoff hypothesis). Lucas (1982) supported this hypothesis by demonstrating that optimal growth and survival of *A. planci* larvae were achieved only at algal concentrations that were rarely encountered in the Great Barrier Reef waters (hereafter referred to as GBR). Then Lucas suggested that starvation was the major cause of larval mortality (larval starvation hypothesis).

To test the larval starvation hypothesis in the field, Olson and co-workers (1985, 1987, 1988) reared *A. planci* larvae using an *in situ* larval rearing apparatus (see Plate 2-1 in Chapter 2). *A. planci* larvae reared *in situ* developed at near optimal rates and settled successfully, despite phytoplankton concentrations being much lower than the optimal

algal concentration suggested by Lucas (1982). These *in situ* larval rearing experiments produced no evidence of food limitation in *A. planci* larvae. Olson and Olson (1989) explained the difference between the results of laboratory and *in situ* experiments as being due to the ability of *A. planci* larvae to utilise food sources other than phytoplankton, such as bacteria and DOM.

Recent studies on the nutrition of *A. planci* larvae have yielded results contrary to those of Olson and Olson (1989). Examination of the gut contents of *A. planci* larvae revealed their diet consisted of mainly relatively large phytoplankton, but no bacteria (P. Dixon, unpubl., cited in Birkeland and Lucas, 1990). Subsequent feeding experiments also suggested that *A. planci* larvae were unable to utilise bacteria (Ayukai, 1994). Hoegh-Guldberg (1994) suggested that transepidermal uptake of dissolved free amino acids (DFAA) could provide a sizable amount of energy to *A. planci* larvae at high substrate concentrations, but ambient concentrations of DFAA in the GBR waters seemed too low to make a significant contribution to the larval nutrition (Ayukai, 1993).

Food limitation in *A. planci* larvae has received much attention in relation to the management of water quality in the GBR. Outbreaks of *A. planci* may be exacerbated by phytoplankton blooms through the increased nutrient runoff from anthropogenic sources, such as agricultural fertilisers and sewage effluent (Bell, 1992; Brodie, 1992). Although proof of eutrophication has not yet been obtained (Furnas, 1990), there is increasing concern over the possible link between increasing nutrient inputs and occurrence and intensity of *A. planci* outbreaks (Brodie, 1992). Such concern has been highlighted in relation to past records showing that primary outbreaks of *A. planci* occur around latitude 16 °S (Moran et al., 1992), where GBR coral reefs come close to the mainland.

Evaluating the larval starvation hypothesis is not only a scientific challenge, but has significant implications for management. This goal can not be achieved without further understanding potential food sources and their relative availability in the putative habitat of *A. planci* larvae.

1.2 Are post-metamorphic *Acanthaster planci* food limited?

1.2.1 Food limitation in juvenile echinoderms

A number of studies have shown that mortality of echinoderms is very high in the post-metamorphic juvenile stages and dependent on many factors, including habitat type, settlement density and predation (e.g. Birkeland and Chia, 1971; Andrew and Choat, 1982; Rowley, 1990; Sewell and Watson, 1993).

Food availability as a factor controlling mortality of a juvenile asteroid was suggested by Birkeland (1974). Birkeland suggested that the variability in survivorship of juvenile *Hippasteria spinosa* was due to the animal being strongly dependent on the availability of its diet, small sea pens. Many workers have considered food availability as a factor controlling growth of juveniles. Variable growth rates in post-metamorphic juvenile stages with different food supplies have been observed in echinoids (e.g. Birkeland and Chia, 1971; Ito et al., 1987). Raymond and Scheibling (1987) suggested that the growth of the juveniles of the echinoid *Strongylocentrotus droebachiensis* was food limited in the barren zone due to lack of fleshy macroalgae. Food limited growth is usually unlikely to be the major cause of juvenile mortality in the field, but it may affect subsequent growth and ultimate size (Mead, 1901 cited in Lawrence and Lane, 1982; Ebert, 1968; Nauen, 1978).

1.2.2 Food limitation in juvenile *A. planci*

Survivorship in the post-metamorphic, alga-eating stages also has significant consequences for the population dynamics of adult *A. planci* (cf. Birkeland and Lucas, 1990). Early juveniles of *A. planci* are very small (0.5 - 0.8 mm) and vulnerable to various environmental factors, including predation (Keesing and Halford, 1992a). Keesing and Halford (1992b) measured mortality rates of *A. planci* juveniles in manipulated field experiments. The mortality rate of post-metamorphic juveniles (1 month old) was extremely high (6.49 % day^{-1}) in comparison to 4 months old juveniles (1.24 % day^{-1}) and 7 months old ones (0.45 % day^{-1}), indicating size-dependent mortality.

There are three main potential causes of high mortality in juvenile *A. planci*, i.e. predation, disease and food availability (Birkeland and Lucas, 1990). In the experiments carried out by Keesing and Halford (1992b), mortality rates of juveniles in natural benthic

substrates (dead coral rubble) were significantly higher than those in bleached rubble. As starvation over 6 days deployment had little effect on the survivorship of juveniles, Keesing and Halford (1992b) suggested that the major cause of mortality was predation by epibenthic fauna.

Diseases that cause necrotic tissues on the body of juveniles may become an important cause of mortality, but the occurrence of such infection has only been reported for a small number of juveniles in the late stages of disintegration (Zann et al., 1987; Habe et al., 1989; cf. Birkeland and Lucas, 1990). The bacteria or any other pathogens causing the necrosis are not known.

Food availability is not thought to affect the mortality of juvenile A. planci, because larvae settle selectively on coralline algae that are a ubiquitous component of coral reef (Birkeland and Lucas, 1990; cf. Littler, 1973; Klumpp and McKinnon, 1989). However, it is possible that the quality of dietary coralline algae may indirectly influence survivorship. If growth of juvenile A. planci is limited by quality of coralline algae, they would be exposed for a longer period to the high predation pressure on small size (Keesing and Halford, 1992a; Birkeland, 1989). Also, food limited growth may delay the timing of diet transition to coral, possibly resulting in a long period to sexual maturity (cf. Yamaguchi, 1975; Lucas, 1984).

Zann et al. (1987) and Habe et al. (1989) succeeded in locating and measuring the growth of naturally-recruited juvenile A. planci, but their observations on food availability were circumstantial. It is necessary to describe the relationship between quality of natural food and growth of juvenile A. planci.

1.3 Objectives

The overall objective of this study was to determine whether food availability is a factor controlling the growth, development and survival of *A. planci* during its early life stages in the field. This study had five specific aims, which correspond to each of the following chapters. These aims were:

(i) To assess the relative importance of different natural food sources for the growth, development and survival of *A. planci* larvae using the *in situ* larval rearing apparatus of Olson (1987) (Chapter Two).

(ii) To determine the critical range of food concentration for *A. planci* larvae, based on laboratory rearing experiments (Chapter Three).

(iii) To measure the feeding rates on naturally occurring food particles by *A. planci* larvae and to determine the dependence of feeding rates on particle size (Chapter Four).

(iv) To measure the net uptake rates of dissolved free amino acids by *A. planci* larvae and to estimate their potential contribution on the larval nutrition (Chapter Five).

(v) To test the effect of different coralline algae, in the laboratory and in the field, on the growth of juvenile *A. planci* (Chapter Six).

The final chapter evaluates the importance of food limitation on the growth, development and survival of larval and juvenile *A. planci*, based on the results obtained in this study and data on the availability of their food sources in GBR waters (Chapter Seven).

Chapter Two

In situ studies of larval nutrition of *Acanthaster planci*

2.1 Introduction

The central premise of the larval starvation hypothesis (Lucas, 1982) is that *A. planci* larvae frequently starve in the GBR waters because phytoplankton concentrations are below the threshold necessary to sustain their growth and survival. The criticism of this is that variety of natural foods other than phytoplankton may compensate for the shortfall in phytoplankton concentration (Olson, 1985).

To address this question, Olson (1985, 1987) measured the growth and development of *A. planci* larvae using an *in situ* larval rearing apparatus. He demonstrated, through a series of *in situ* rearing experiments conducted in the GBR and in Okinawa, that most *A. planci* larvae reared *in situ* settled in 14 days, similar to the larval period obtained under optimal laboratory conditions (Lucas, 1982). Overall survival rates of ca. 60 % were also similar to those achieved in laboratory experiments (Birkeland and Lucas, 1990), despite ambient chlorophyll *a* concentrations being below the level at which Lucas (1982) found *A. planci* larvae would not survive. Furthermore, Olson (1987) reared *A. planci* larvae in chambers filled with seawater enriched with cultured microalgae. These additional experiments showed little difference in development and survival rate of *A. planci* larvae between ambient seawater and alga-enriched treatments. Olson (1987) concluded that growth, development and survival of *A. planci* larvae were not food limited.

Olson and Olson (1989) concluded that the discrepancy between the laboratory and *in situ* rearing experiments was due to *A. planci* larvae being able to utilise non-phytoplankton food sources, such as bacteria and DOM. Several other studies of echinoderm species have demonstrated or suggested the significance of bacteria and DOM in the nutrition of larvae (e.g. Manahan et al., 1983; Rivkin et al., 1986). To test the hypothesis that *A. planci* larvae utilise bacteria and DOM, Birkeland and Lucas (1990) suggested that the *in situ* larval rearing apparatus be modified to exclude food materials progressively down to DOM with a series of in-line filters.

The aim of this study was to assess the relative importance of potential food sources in different size fractions for the growth and survival of *A. planci* larvae. In-line filters of different pore sizes were added to the flushing system of the *in situ* larval rearing

apparatus of Olson (1987) to create different food environments inside the *in situ* chambers. Preliminary rearing experiments were conducted using the modified apparatus, but the finding of mesh screens being fouled caused concern that the food environment inside the *in situ* rearing chambers might not be the same as the ambient food environment (Okaji, 1993). This was noted by Olson and Olson (1989), but they did not present any data on environmental parameters inside the chambers. Olson and co-workers (1987, 1988) took seawater samples for measurement of chlorophyll *a* concentration from next to the rearing apparatus, not inside. In this study, chlorophyll *a* concentrations inside and outside the *in situ* larval rearing chambers were measured to clarify this potential problem.

2.2 Materials and methods

2.2.1 Modified *in situ* larval rearing apparatus and performance tests

The *in situ* larval rearing apparatus of Olson (1987) was modified with in-line filter cartridges and one-way valves on the outflow windows. With values on the outflow windows, the apparatus retained filtered seawater inside the chambers more securely than the original one. Specifications and performance of two *in situ* larval rearing apparatuses, the "original" and the "modified" models, were compared.

The original apparatus was described in detail by Olson (1987, also see Plate 2-1). The apparatus consisted of three components: four larval rearing chambers, a seawater flushing system and a perspex base plate (hereafter referred to as the culture table). Each chamber consisted of a cylindrical casing and a cone shaped lid. Each chamber had three openings covered with 100 μm mesh screen: one round opening (40 mm in diameter) was placed at the top of the lid for a seawater inlet and the other two rectangular openings (40 x 120 mm) were on the side wall of the cylindrical casing for seawater outlets. The chamber was attached to the culture table upside down, so that the upward casing created an air pocket at its top. The seawater flushing system was equipped with an electric bilge pump powered by a submersible 12 V battery. The timing circuit wired between the pump and the battery was programmed to turn the pump on for 30 seconds every hour. The pump outlet and seawater inlets of each of four chambers were connected with plastic tubing. These items, including four detachable rearing chambers, were mounted on the culture table (50 x 50 cm).

The apparatus was modified to create four different food environments: 100 μm mesh filtered seawater (100 μm FSW), 1 μm FSW, 0.2 μm FSW and activated charcoal filtered seawater (ACF). The major modification was installation of three 25 cm filter cartridges (1 μm, 0.2 μm and activated charcoal, Onga Pump Pty. Ltd.) and their housings in the seawater flushing line. A 15 μm pore sized paper prefilter was also added between the 100 μm FSW chamber and 1 μm filter.

A diagram of seawater flow is shown in Figure 2-1. Seawater was drawn in through the 100 μm mesh screen at the pump intake and sent to the first chamber (100 μm FSW treatment), and to a 15 μm prefilter plus a 1 μm filter cartridge. Filtered seawater from the 1 μm cartridge feeds into the second chamber (1 μm FSW treatment) and a 0.2 μm cartridge. This seawater was then pushed into the third chamber (0.2 μm FSW), and to

an activated charcoal filter cartridge and finally to the fourth chamber (ACF treatment). Equal seawater flow to each chamber was maintained by adjusting control valves in the tubing.

Along with the addition of in-line filters, the larval rearing chambers were also modified to prevent inflow of ambient seawater outside (see Plate 2-2). Each of the two seawater outlets of a chamber was covered with a perspex box. Two rubber one-way valves (regulator exhaust valves, SCUBAPRO Inc.) were fixed onto each of these boxes. Each one-way valve was held by a short perspex tube and plugged with a rubber stopper if required. Other minor modifications included the replacement of a pump and reprogramming of the electric circuit for the enhancement of seawater flushing frequency.

The modified apparatus was tested in a large aquarium and in the field to check the performance of each component. The waterflow was visualised by releasing a fluorescein dye solution at the pump intake. The volume of seawater delivered to each chamber was measured by collecting outflowing filtered seawater at the end of the tubing.

The modified apparatus with two original and two modified chambers was deployed to compare efficiency of seawater flushing. Several mL of a fluorescein dye solution were injected into each of four chambers. The pump was immediately activated for 40 s and initial 50 mL samples were withdrawn from each chamber through a 100 μm mesh screen with a syringe (t=0). Sampling was then made at 30 min (t=30), 60 min (t=60), and 150 min (t=150), following 40 s flushing each time.

Fluorescently-labelled microalgae were injected into the chambers to examine if particle concentrations decreased as predicted from the calculated flushing rates. A culture of *Tetraselmis* sp. was heat-killed and labelled with the fluorescence stain, 5-(4,6-dichlorotriazin-2-yl) aminofluorescein (DTAF), following the procedure described by Sherr et al. (1987). Ten mL of DTAF-labelled *Tetraselmis* suspension (7.10 X 10^6 cells mL^{-1}) were injected into each of four chambers at the beginning of experiments. Sampling intervals were the same as described above.

All seawater samples were kept frozen. Relative changes in concentration of fluorescein dye were measured using a Turner Design Fluorometer. Concentrations of DTAF-labelled cells in each sample were determined by epifluorescence microscopy: DTAF-labelled cells were easily distinguished by their bright yellow-green fluorescence under blue excitation light (450-490 nm) (Sherr et al., 1987).

2.2.2 *In situ* larval rearing experiment using the modified apparatus

A. planci larvae used for the experiment were obtained from the mass-larval rearing facility in the Australian Institute of Marine Science (see Appendix I).

The modified apparatus was deployed at Davies Reef lagoon in January 1992. The apparatus was moored at 2 m depth near the AIMS weather station. The apparatus was assembled, except for the larval rearing chambers, and taken to the experiment site. The larval chambers with their one-way valves being closed by stoppers were filled with 0.5 µm cartridge filtered seawater. Two hundred bipinnaria larvae (4 day old) were introduced into each chamber. The chambers were taken to the experiment sites and mounted on the culture table of the apparatus. The electric pump was switched on immediately after the one-way valves were opened and kept running for the first few minutes to replace the seawater inside the chambers.

The submersible battery and a 100 µm mesh screen at the pump intake were changed every day. The chambers were retrieved and brought back to the laboratory every second day. Surviving larvae in each chamber were gently sieved with a 60 µm mesh screen and transferred to a glass dish with 0.5 µm filtered seawater. Each larva was examined under a dissecting microscope to record their developmental stages according to criteria described by Lucas (1982, see Table 2-1). After examination, larvae were returned to a cleaned chamber filled with 0.5 µm filtered seawater and deployed again. When late brachiolaria larvae first appeared, pieces of coralline algae were placed in the chambers as settlement substrates.

2.2.3 Measurements of chlorophyll *a* concentrations inside the *in situ* larval rearing apparatus

Two trials with the modified apparatus were made at Davies Reef lagoon in March and May, 1992, and one with the original apparatus at Flora Reef in January 1995. Chlorophyll *a* concentration inside the chambers was measured without *A. planci* larvae, but the apparatuses were deployed and tended in the same manner as the rearing experiment.

In the first trial with the modified apparatus, duplicate 100 mL seawater samples were withdrawn from each of four chambers just before retrieval of the chambers. Sampling was conducted underwater using clean syringes. At the same time, 300 mL seawater

samples were collected near the pump intake. Samples for chlorophyll *a* measurements were filtered onto Whatman GF/F glass fibre filters and kept frozen for fluorometric analysis in the laboratory (Strickland and Parsons, 1972).

In the second trial, the modified apparatus was used in two different settings. The electric pump was programmed to flush the chambers either once per hour (1 flush) or twice per hour (2 flush). Seawater samples were taken in the same manner as the March experiment.

In the third trial, with the original apparatus, a minor modification was made on the chambers to avoid damaging the 100 μm mesh screens with a syringe needle. Each chamber had a small hole drilled on the middle part of its side wall to insert a rubber sampling plug (Septum, SUBA-SEAL Co. Ltd.). Duplicate 100 mL seawater samples were collected from the approximate centre of each chamber through the sampling plug using a syringe with a long (89 mm) needle. Seawater samples were also collected directly from the plastic tubing. Before sampling, the end of each of the four tubes was connected to the spigot valve of an empty 3 L collapsible plastic container (Cole-Parmer Co. Ltd.). Then the pump was turned on for 40 seconds. The valves on the four plastic containers were securely closed, and the tubing was removed. Seawater samples were kept frozen for the fluorometric analysis.

2.3 Results

2.3.1 Performance of modified versus original *in situ* larval rearing apparatus

The modified apparatus delivered 1.3 L of seawater to each of the four chambers every 30 minutes. No major leaks were found on the filter system or the tubing. In the aquarium deployment, fluorescein dye tended to be trapped in eddies near the base (i.e. the lid) of chambers during flushing. In the field, however, fluorescein dye was quickly mixed with seawater inside chambers, because the apparatus moved due to wave action. While seawater contained in the modified chambers remained undisturbed between each flushing, concentrations of fluorescein dye in the original chambers were gradually diluted by diffusion out through the side wall screen outlets. Consequently, relative dye concentration in the original chambers decreased much faster than those in the modified ones (Fig. 2-2). Slopes of the fitted lines calculated from exponential transformation were -0.009 for the modified and -0.016 for the original.

Changes in relative concentration of DTAF-labelled phytoplankton in each of two modified and two original chambers are shown in Fig. 2-3. Slopes of fitted lines were -0.006 for the modified and -0.009 for the original. In both types of chambers, relative concentrations of DTAF-labelled cells decreased slightly slower than those of fluorescein dye.

2.3.2 *In situ* larval rearing experiment using the modified apparatus

Changes in proportions of each developmental stage of *A. planci* larvae within each of the four larval rearing chambers are shown in Fig. 2-4. *A. planci* larvae reared in 100 μm started developing to the late brachiolaria stage between 10 and 12 days after fertilisation (12 days after fertilisation is hereafter referred to as Day 12, etc.). Larvae reared in 1 μm FSW and 0.2 μm FSW developed slightly slower, few reached late brachiolaria stage by Day 12. Larvae reared in ACF did not develop beyond the early brachiolaria stage and gradually regressed from Day 8.

It should be noted that the data on proportions of each developmental stage on Day 14 were based on a small sample of larvae, because high mortality occurred in all chambers between Day 12 and Day 14 (Fig. 2-5). The cause of this mortality is unknown, but it is suspected that predators were inadvertently introduced into the chambers when pieces of coralline algae were added.

A. planci larvae inside the rearing chambers tended to remain at the air-seawater interface. When the chambers were being flushed, larvae were pushed onto the mesh screen of each water outlet. Larvae had similar morphology to those reported in the previous *in situ* rearing study (Olson et al., 1988): larval arms were not elongated and their bodies were rounded and relatively small in comparison with laboratory-reared larvae. The stomachs of brachiolaria larvae reared in 100 μm FSW, 1 μm FSW and 0.2 μm FSW were coloured slightly yellow-green.

During the experiments, the 100 μm mesh screen on the pump intake was heavily fouled despite daily replacement. The whole apparatus was covered by sediment during strong wind conditions. Mesh screens of the modified chambers were also fouled with light-yellow filamentous particles. This result was unexpected because the screens were completely separated from ambient seawater by one-way valve boxes. Slight fouling in the chambers was likely to be due to contamination during the replacement of chambers underwater.

2.3.3   Concentrations of chlorophyll *a* inside the *in situ* larval rearing chambers

Mean concentrations of chlorophyll *a* in ambient seawater and in the modified larval rearing chambers are presented in Table 2-2. In the first trial (March 1992), the mean chlorophyll *a* concentrations in 100 μm, 1 μm and 0.2 μm FSW were significantly higher than those in ambient seawater (P < 0.001, ANOVA). In the second trial (May 1992), the mean chlorophyll *a* concentrations in 100 μm FSW for 1 flush per hour setting were more than two fold higher than those in ambient seawater (P < 0.01, ANOVA). Obviously, these elevated concentrations of chlorophyll *a* reflect an increase of algal biomass inside the seawater flushing system and the chambers.

Table 2-3 presents mean chlorophyll *a* concentrations inside the chambers and the tubing of the original larval rearing apparatus and in ambient seawater 2, 4 and 6 days after deployment. The mean chlorophyll *a* concentrations inside the tubing were similar to those in ambient seawater. However, the mean chlorophyll *a* concentrations inside the chambers were significantly higher than those in ambient seawater (P < 0.01, 2 days after deployment, P < 0.001, 4 and 6 days after deployment). The results clearly show that algal biomass had increased inside the chambers during the deployment.

2.4 Discussion

Microcosms, such as the apparatus of Olson (1987), have been used in freshwater and marine studies of microorganism ecosystems. For instance, the complete larval development cycle of a crustacean was described by *in situ* rearing (Haynes, 1978). A number of authors have studied the effects of zooplankton grazing on natural phytoplankton communities using field-deployed enclosures of various sizes (e.g. Turner and Granéli, 1992; Thatcher et al., 1993). However, it is recognised that the environment within enclosures is still different from the environment that animals encounter in their natural habitats (cf. Lafontaine and Leggett, 1987; Ravera, 1992).

The result of *in situ* larval rearing in 100 µm mesh filtered seawater indicated that *A. planci* larvae were not food limited in the field (100 µm FSW treatment in Fig. 2-4), supporting the conclusion of Olson (1987). Larvae were able to develop, at least to later developmental stages, even in 0.2 µm filtered seawater with low concentrations of phytoplankton and bacteria (0.2 µm FSW treatment in Fig. 2-4). It was only in activated charcoal filtered seawater that there was no significant larval development (ACF treatment in Fig. 2-4). These results suggest that DOM was an essential food source for positive growth. However, these results can also be explained by elevated concentrations of chlorophyll *a* inside the chambers. Although no data on chlorophyll *a* concentrations were taken during the course of the rearing experiment, it is probable that *A. planci* larvae had access to phytoplankton grown or accumulated in the chambers. It is noteworthy that the stomachs of larvae reared in the chambers flushed with ultrafiltered seawater (1 µm and 0.2 µm FSW) were coloured (see section 2.3.2). This observation indicates that the larvae had fed on some food material, as the stomachs of larvae reared in uncontaminated, ultrafiltered seawater do not become opaque (personal observation).

Concentrations of chlorophyll *a* in the modified chambers flushed with 100 and 1 µm filtered seawater were significantly higher than those in ambient seawater. Even in the chambers flushed with 0.2 µm and activated charcoal filtered seawater, the chlorophyll *a* concentration was equivalent to or higher than ambient levels in one of the two measurements (Table 2-2). The source of chlorophyll *a* is likely to be due to algal contaminants that intruded and proliferated in the chambers. Intrusion of ambient phytoplankton into the apparatus was unavoidable, because the chambers and the filter system had to be disconnected underwater during the deployment and retrieval procedures. Such confinement of phytoplankton *in situ* is known to result in remarkable changes in biomass and species composition (Venrick et al., 1977).

The environments inside the original *in situ* larval rearing chambers were not the same as the ambient environment (Table 2-3). The experiments with fluorescein dye showed the likelihood of incomplete flushing of seawater inside the original chambers. Fluorescently-labelled phytoplankton remained within the chambers longer than expected from the dye experiments (see Fig. 2-2, 2-3), suggesting particle retention near the base of the chambers. The surface of any material immersed in seawater is almost immediately covered by organic matter, leading to the formation of algal film (ZoBell, 1943 cited in Lee and Fuhrman, 1991). The elevated concentration of chlorophyll *a* can result from both accumulation and proliferation of algal contaminants within the chambers.

The aim of assessing the relative importance of different foods for *A. planci* larvae was not achieved. The modified apparatus did not remove food particles progressively down to DOM as originally planned. Rather, both modified and original apparatuses create enriched food environments due to algal contaminants. The concept of *in situ* larval rearing is very appropriate, but results obtained from this apparatus are very dubious. Thus, the larval starvation hypothesis needs to be assessed by means other than *in situ* larval rearing.

Table 2-1 Criteria of each developmental stage of *Acanthaster planci* larvae (after Lucas, 1982).

Advanced bipinnaria:	with fully developed bipinnaria arms
Early brachiolaria:	with first appearance of the paired brachiolar arms as little stamps
Mid brachiolaria:	with starfish primordium opaque and asymmetrical along posterior border in lateral view; still positively buoyant
Late brachiolaria:	with arms of the incipient starfish evident around the starfish primordium in lateral view; primordium distinctly broader than larval body in lateral view; negatively buoyant and showing searching behaviour*
Juvenile:	settlement and metamorphosis

*Late brachiolaria search the bottom substrate with three elongate brachiolar arms

Table 2-2 Concentrations of chlorophyll a in ambient seawater and inside the modified larval rearing chambers with four different treatments. 100 μm FSW: 100 μm mesh filtered seawater, 1 μm FSW: 1 μm cartridge filtered seawater, 0.2 μm FSW: 0.2 μm cartridge filtered seawater, ACF: activated charcoal filtered seawater. Values are mean ± 1 SD (N=6) with ranges in parentheses.

Treatment	Chlorophyll a concentration ($\mu g \, L^{-1}$)
March 1992	
Ambient seawater	0.64 ± 0.08 (0.60 - 0.76)
100 μm FSW	1.58 ± 0.43 (1.11 - 2.15) [*1]
1 μm FSW	0.99 ± 0.44 (0.49 - 1.55) [*1]
0.2 μm FSW	1.23 ± 0.38 (0.69 - 1.70) [*1]
ACF	0.56 ± 0.25 (0.37 - 0.91)
May 1992	
Ambient seawater	0.40 ± 0.14 (0.22 - 0.55)
100 μm FSW [#]	1.16 ± 0.20 (0.96 - 1.49) [*2]
100 μm FSW [#]	1.13 ± 0.32 (0.88 - 1.63) [*2]
100 μm FSW	0.49 ± 0.16 (0.40 - 0.77)
0.2 μm FSW	0.34 ± 0.05 (0.29 - 0.39)
ACF	0.16 ± 0.06 (0.10 - 0.26) [†]

Higher than ambient, *1: $P < 0.001$, *2: $P < 0.01$
Lower than ambient, †$P < 0.05$
#: 1 flush per hour (2 flush per hour for the rest)

Table 2-3   Chlorophyll *a* concentrations in ambient seawater, flushing tubing and larval rearing chambers of the original *in situ* larval rearing apparatus 2, 4 and 6 days after deployment. Values are mean ± 1 S.D. with ranges in parentheses.

Samples	Days after deployment		
	2	4	6
Ambient seawater	0.51 ± 0.03 (0.49 - 0.53) [†]	0.44 ± 0.08 (0.35 - 0.52) [††]	0.55 ± 0.10 (0.48 - 0.62) [†]
Flushing tubing	0.46 ± 0.06 (0.39 - 0.57)	0.37 ± 0.05 (0.25 - 0.41)	0.45 ± 0.08 (0.36 - 0.60)
Larval chambers	1.01 ± 0.18 (0.70 - 1.23) [*]	0.94 ± 0.18 (0.70 - 1.21) [**]	2.12 ± 0.47 (1.51 - 2.71) [**]

*P < 0.01, **P < 0.001 Higher than ambient seawater
[†]Subsamples N=2, [††]N=4 and N=8 for the rest
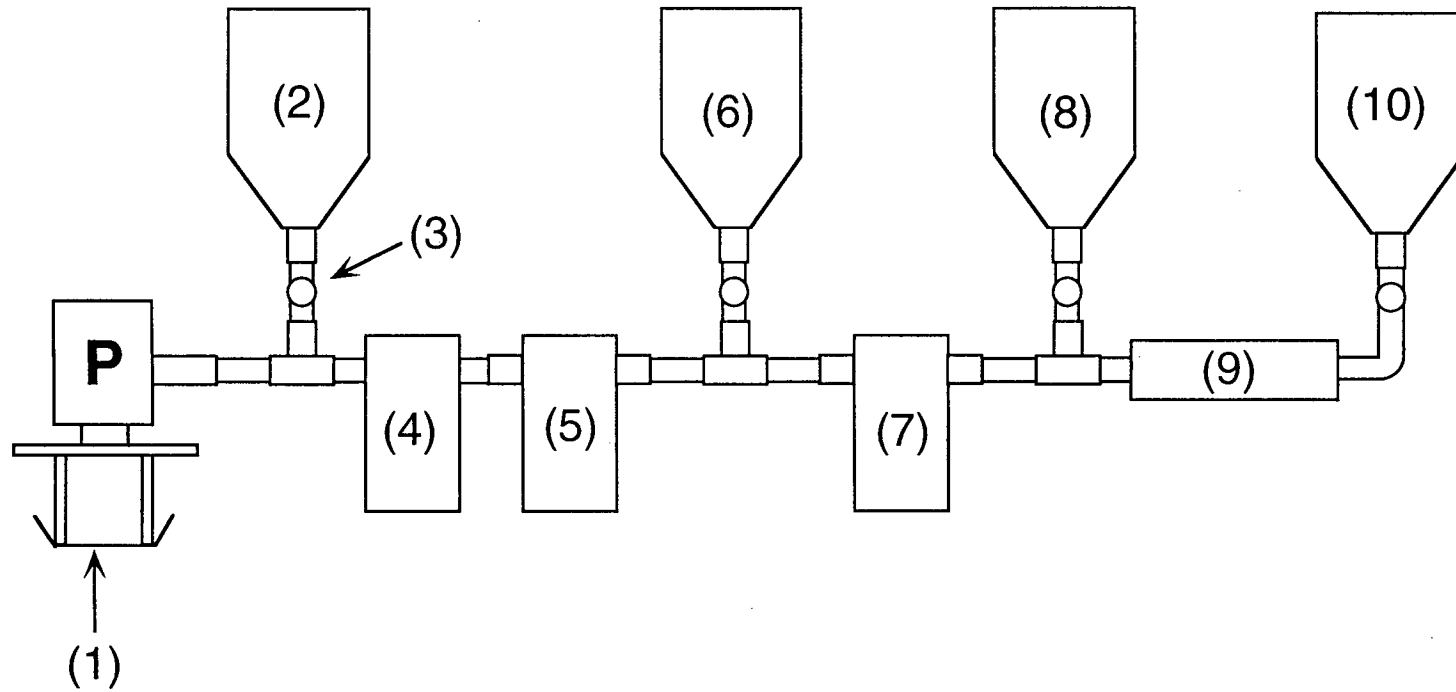
Figure 2-1 Flow diagram of seawater in the modified larval rearing apparatus. Seawater that had passed through a 100 µm mesh screen (1) was supplied to the first chamber (2), 15 µm prefilter (4) and 1 µm paper filter cartridge (5) by an electric bilge pump (P). Then 1 µm filtered seawater was pushed through a 0.2 µm filter cartridge (7) to an activated charcoal filter cartridge (9), thus flushing the third (8) and the fourth chamber (10). The amount of seawater supply to each chamber was equalised with a control valve (3) in each tube.
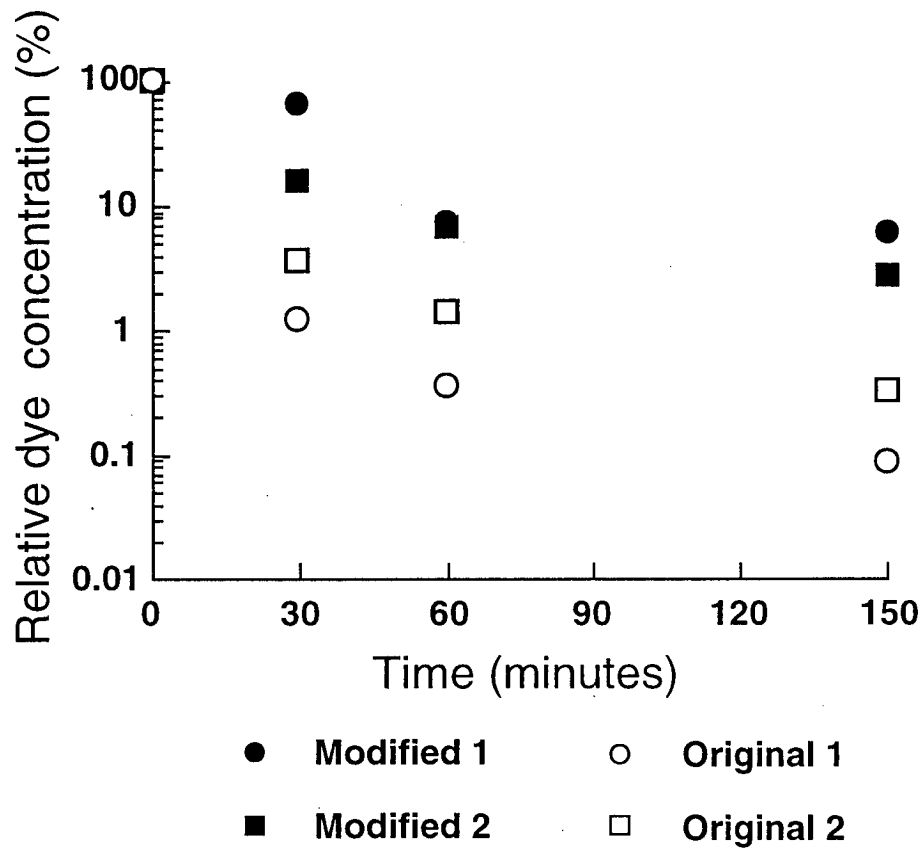
Figure 2-2 Changes in relative concentration of fluorescein dye in each of two modified (■ and ●) and two original (□ and O) *in situ* larval rearing chambers.
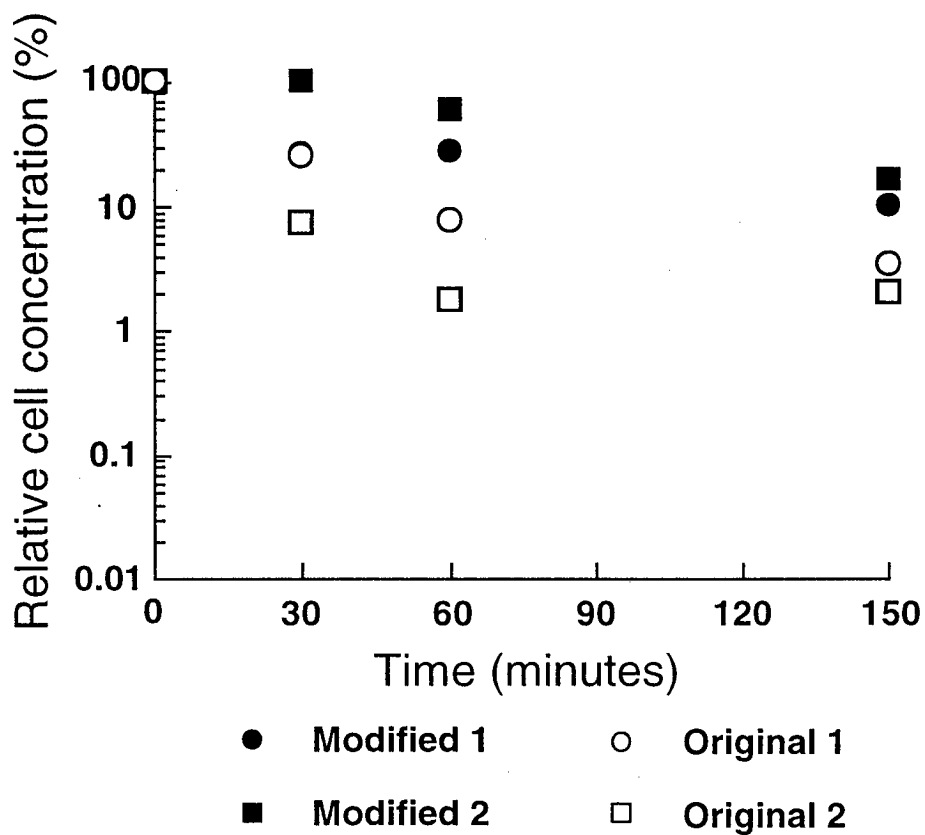
Figure 2-3   Changes in relative concentration of fluorescently-labelled
cultured microalgae (*Tetraselmis* sp.) in each of two
modified (■ and ●) and two original (□ and O) *in situ*
larval rearing chambers.
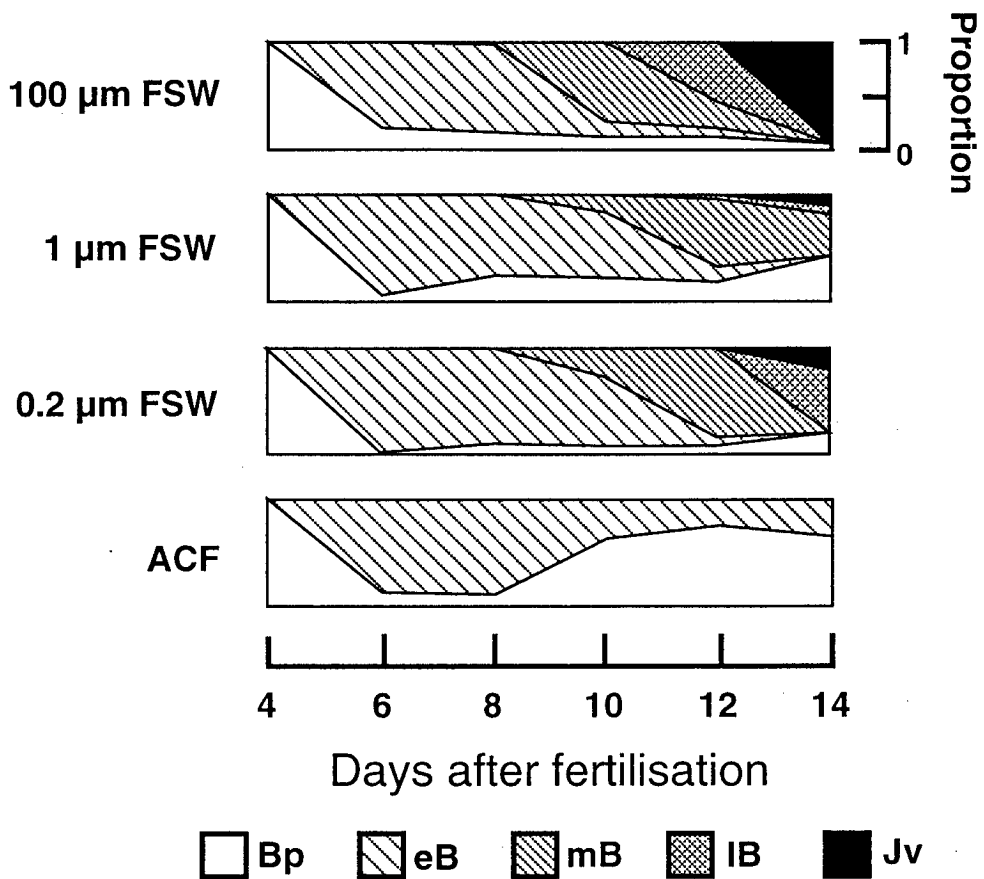
Figure 2-4   Development of *Acanthaster planci* larvae reared in *in situ* chambers of four different seawater treatments. 100 μm FSW: 100 μm mesh filtered seawater, 1 μm FSW: 1 μm cartridge filtered seawater, 0.2 μm FSW: 0.2 μm cartridge filtered seawater, ACF: activated charcoal filtered seawater. Bp: bipinnaria stage, eB: early brachiolaria stage, mB: mid brachiolaria stage, lB: late brachiolaria stage, Jv: metamorphosed juvenile.
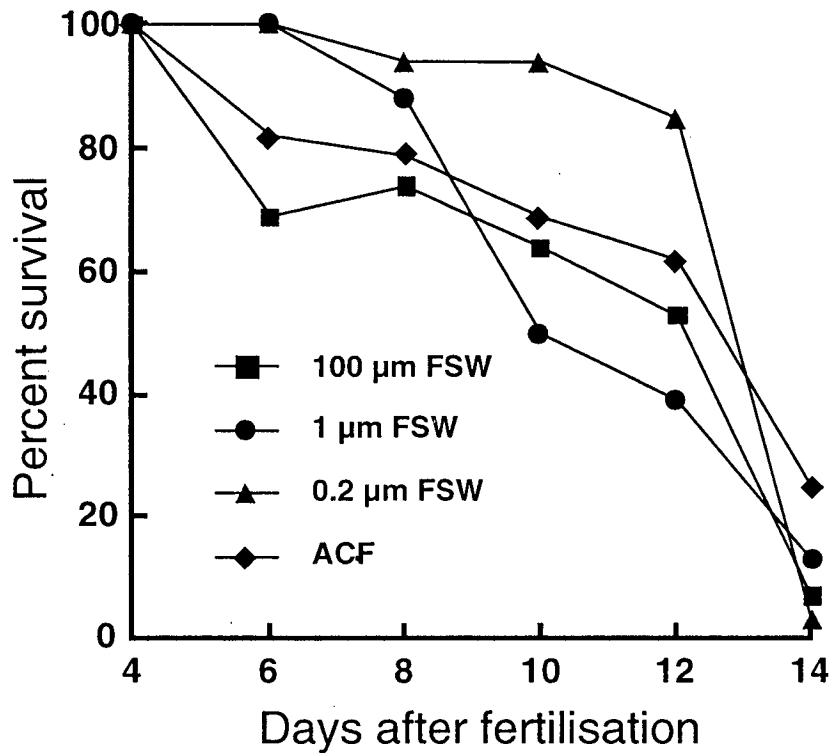
Figure 2-5 Changes in survival rates of *Acanthaster planci* larvae reared in *in situ* chambers of four different seawater treatments. 100 μm FSW (■): 100 μm mesh filtered seawater, 1 μm FSW (●): 1 μm cartridge filtered seawater, 0.2 μm FSW (▲): 0.2 μm cartridge filtered seawater, ACF (◆): activated charcoal filtered seawater.

Plate 2-1   *In situ* larval rearing apparatus: the modified model upon deployment.
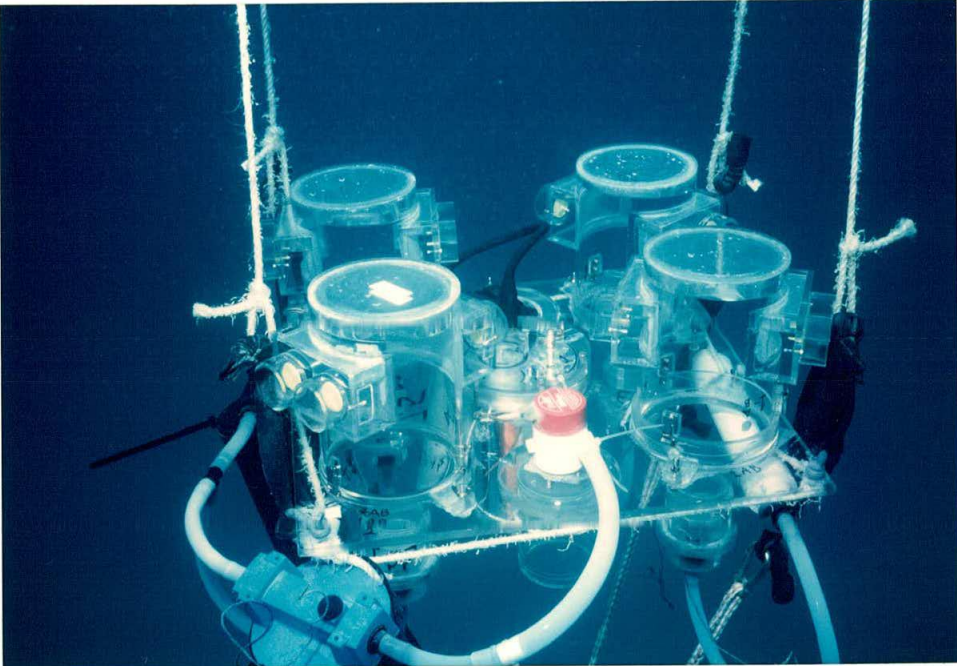
Plate 2-2 *In situ* larval rearing chambers: the original (left) and the modified one (right) fitted with one-way valves.
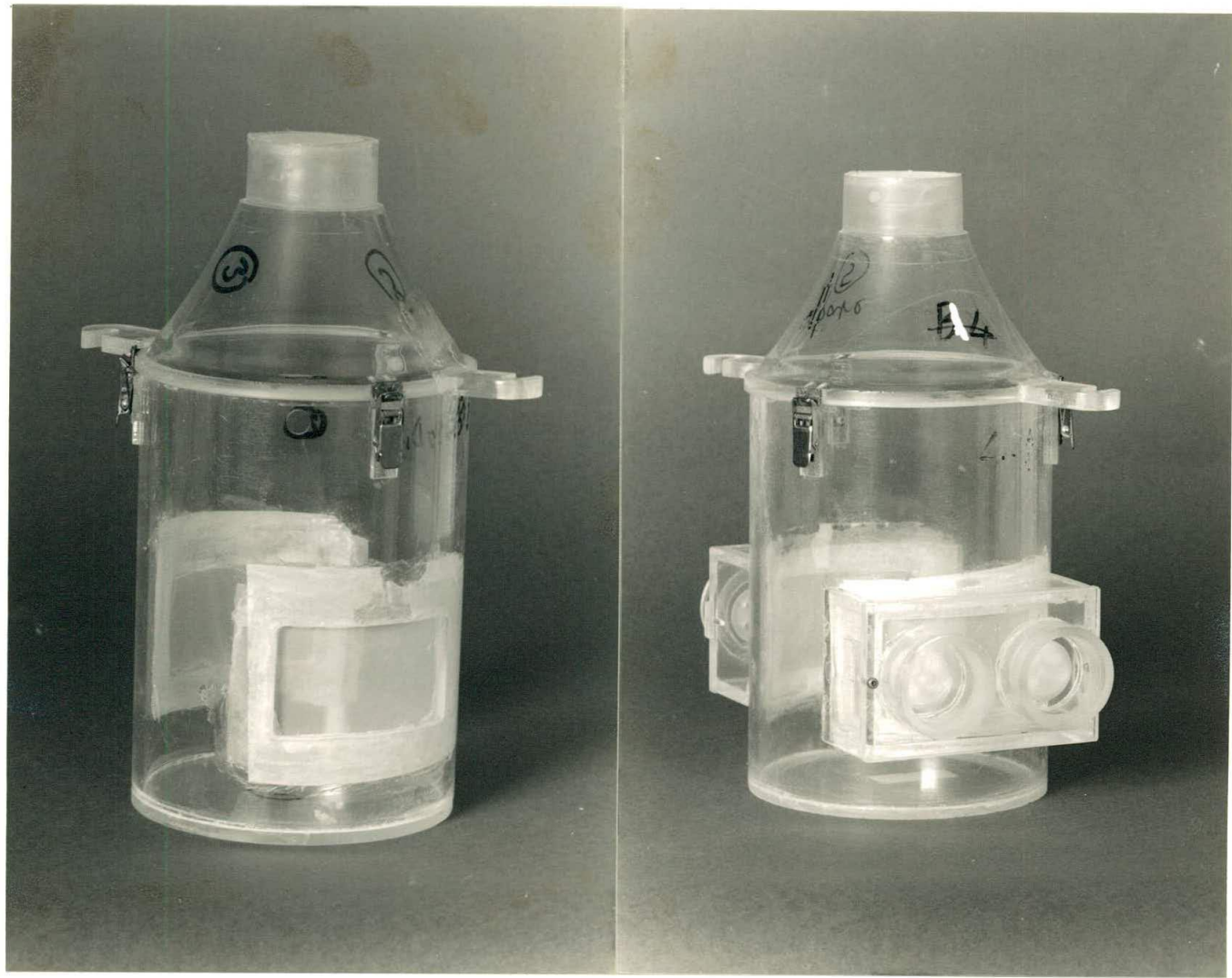
Chapter Three

Laboratory studies of larval nutrition of *Acanthaster planci*

3.1 Introduction

Thorson (1950) categorised levels of food availability to larvae as: the level at which larvae starve to death; the level at which larvae are able to survive and vegetate for some time; and the level at which optimal growth, development and survival of larvae are supported. These categories are distinguished by two threshold food concentrations: the compensating and the critical concentration. It is necessary to determine the critical range between these food concentrations to judge whether larvae are food limited in the field.

Phytoplankton concentrations in GBR waters are usually too low to support the growth, development and survival of *A. planci* larvae, according to Lucas (1982). However, this estimate was a matter of debate, because it was based on data from *A. planci* larvae utilising cultured microalgae. Laboratory experiments with only cultured foods are inadequate to assess the field situation. *In situ* larval rearing was problematic in achieving this objective (Chapter 2), but rearing larvae in natural seawater is still valid. The practicality of such "semi-natural" larval rearing experiments in the laboratory has been proven in studies of food limitation of other echinoderm larvae (Paulay et al., 1985; Fenaux et al., 1994).

The aim of this study was to determine the critical range of food concentration for *A. planci* larvae. Larvae were reared in the laboratory using freshly-collected and coarse filtered (25 μm mesh filter) seawater and the same seawater treated by further filtration (0.45 or 2 μm membrane filter) or food enrichment (cultured microalgae or a mixture of 18 amino acids). Larvae were also reared in nutrient enriched seawater, where the concentrations of natural phytoplankton were elevated by adding nutrient solution to the coarse filtered seawater and incubating for a few days.

3.2 Materials and methods

3.2.1 Larval rearing experiments using membrane filtered seawater, coarse filtered seawater and alga and amino acid enriched seawater

Experiments were conducted at the Lizard Island Research Station, GBR, in November and December, 1992, and at the University of the Ryukyus in Okinawa, Japan, in June and July, 1993.

Batches of *A. planci* larvae were reared at both facilities using a method similar to Birkeland and Lucas (1990). The sex of adult *A. planci* was determined by syringe biopsy. A few lobes of ovary and testis were dissected from a pair of adult starfish. The ovary lobes were placed in a glass dish with 300 mL of 0.45 μm membrane filtered seawater. Several mL of 10^{-3} M 1-methyladenine solution were added to the glass dish to induce maturation of oocytes and contraction of the ovary walls. After 30-45 minutes, the oocytes released from the ovaries were transferred to a 2 L glass pan filled with filtered seawater. Spermatozoa were obtained by spraying a few drops of the 1-methyladenine solution on to testis lobes in a dry glass dish. Two to three drops of the dense spermatozoa suspension were added to the glass pan and stirred to fertilise the oocytes. The fertilised oocytes were gently sieved with 60 μm mesh screen and rinsed twice with filtered seawater. Then they were transferred to a 5 L glass beaker filled with filtered seawater. The number of the oocytes was reduced to form one layer on the bottom of the beaker. Minute air bubbles were kept flowing through a pasteur pipette near the seawater surface. The beaker was covered with a sheet of aluminium foil and immersed in a water bath at 28 °C.

Approximately 20-24 hours after fertilisation (Day 1), aeration was stopped for about an hour to allow gastrulae to swarm near the seawater surface. Abnormal embryos and oocytes crawling or resting on the bottom were siphoned out. Then actively swimming gastrulae with ≈1 L of seawater were transferred to another beaker. Seawater was agitated by gentle aeration from the bottom of the beaker. On Day 2, when most of gastrulae had developed to the early bipinnaria stage with the completed alimentary canal, aeration was stopped and healthy larvae were collected from the top quarter of the beaker for the rearing experiments.

Seawater used for the experiments was collected daily at the beach in front of the Lizard Island Research Station or at the front of Chatan Reef in Okinawa. Seawater was filtered

through a 25 μm mesh screen immediately after collection to remove predators and miscellaneous detritus. This coarse filtered seawater is hereafter referred to as 25 μm FSW. Seawater was further filtered through either a 2 μm polycarbonate filter or a GF/B glassfibre filter (nominal pore size of 0.45 μm). These membrane filtered seawater are referred to as 2 μm FSW and 0.45 μm FSW. For the alga enriched treatment (referred to as ALE), cultured microalgae, *Dunaliella primolecta*, were added to 25 μm FSW at a concentration of 3,000-4,000 cells mL^{-1}, which Lucas (1982) reported as a sufficient concentration for the development and survival of *A. planci* larvae. For amino acid enriched treatment (referred to as AAE), a standard amino acid mixture* was added to 25 μm FSW at the total concentration of 5 μM.

At Lizard Island, two batches (Batch 1 and 2) of *A. planci* larvae were reared with four different food treatments: ALE, 25 μm FSW, 2 μm FSW and 0.45 μm FSW. In Okinawa, two batches (Batch 3 and 4) of larvae were reared in ALE, AAE, 25 μm FSW and 2 μm FSW. Experiments were run in triplicate for each of the four treatments.

Duplicate seawater samples, 100-200 mL for chlorophyll *a* measurements and 5 mL for enumeration of phytoplankton, were withdrawn from each treatment container every day. Each seawater sample for chlorophyll *a* measurement was filtered onto a GF/F glass fibre filter (25 mm, Whatman) and kept frozen for fluorometric analysis (Strickland and Parsons, 1972). Each sample for enumeration of phytoplankton was filtered onto a black stained filter (25 mm, Millipore) and mounted on a slide glass using Zeiss immersion oil. The filter was then examined under an epifluorescence microscope equipped with a blue excitation filter set (450-490 nm) to determine the number of eukaryotic algae and coccoid cyanobacteria in the sample (epifluorescence microscopy, cf. Haas, 1982).

One hundred early bipinnaria larvae (2-day old) were introduced into each container (1 L glass jars at Lizard Is. and 2 L beakers at Okinawa) at the start of experiments. The seawater was gently aerated during the course of experiments. Larvae were sieved with a

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*Equimolar mixtures of 18 amino acids were supplied by SIGMA, WAKO and Ajinomoto. Contents were:

Glycine	Isoleucine	Histidine	Glutamic acid
Alanine	Leucine	Arginine	Proline
Cystine	Tyrosine	Asparagine	Ammonia
Valine	Phenylalanine	Threonine	
Methionine	Lysine	Serine	

60 μm mesh screen and transferred to another set of containers with freshly prepared seawater every day. Seawater temperature was kept in the range between 26.5 and 29 °C.

A. planci larvae were individually examined under a dissecting microscope every second or fourth day. The developmental stages of larvae were recorded according to the criteria set by Lucas (1982) (see Table 2-1 in Chapter 2). Regressed or abnormal larvae were included in bipinnaria stage. The survivorship in each jar was also recorded. On the day when larvae developed to late brachiolaria stage, aeration was reduced and a few small pieces of coralline algae (*Lithothamnium pseudosorum* or *Porolithon* sp.) were placed in each container as a settlement substrate. These pieces of coralline algae were checked daily to count metamorphosing larvae or settled juveniles.

The development of *A. planci* larvae was analysed by comparing proportions of surviving larvae in the most advanced developmental stage at a given day. Percentile data were arcsine transformed and their mean values were compared with One-way ANOVA and Scheffe method (cf. Zar, 1984). As late brachiolaria larvae are competent to settle (Lucas, 1982), the proportion of late brachiolariae and settled juveniles were combined in the analysis.

Overall survival rates were not analysed, because the survivors included abnormal or regressed larvae. Abnormal larvae were subjectively distinguished from healthy ones, but there were no rigorous criteria to judge them. Instead, mean numbers of larvae that had completed development by the final day, were compared using either t-test or One-way ANOVA.

3.2.2 Larval rearing experiments using membrane filtered seawater, coarse filtered seawater and nutrient enriched seawater

Experiments were conducted at the Lizard Island Research Station in October and November, 1993. Bipinnaria larvae of *A. planci* used for these experiments were obtained in the same manner as described in 3.2.1.

Seawater used for the experiments was collected every day approximately 1 km offshore from the research station. Collected seawater was immediately filtered with a 25 μm mesh screen (25 μm FSW) and brought back to the laboratory. Seawater was then filtered with a 2 μm polycarbonate filter (2 μm FSW). Two mL of a nutrient mixture

(Guillard's f/2 solution) were added to 20 L of 25 μm FSW. This nutrient enriched seawater (NES) was prepared daily and incubated in 20 L polycarbonate containers for two days. These containers were immersed in a 500 L outdoor aquarium and illuminated only in the daytime by sunlight.

Two batches (Batches 5 and 6) of *A. planci* larvae were reared with four different food treatments: 2 μm FSW, 25 μm FSW, mixture of 50 % 25 μm FSW and 50 % nutrient enriched seawater (50 % NES), and 100 % nutrient enriched seawater (100 % NES). Experiments were run in triplicate for each of four treatments.

Duplicate seawater samples, 200 mL for chlorophyll *a* and 5 mL for enumeration of phytoplankton, were withdrawn from each treatment every second day. Each seawater sample for chlorophyll *a* measurement was size fractioned, i.e. 100 mL for < 25 μm and 100 mL for < 2 μm fraction. Chlorophyll *a* concentration was measured fluorometrically. The numbers of eukaryotes and cyanobacteria were determined by epifluorescence microscopy.

At the beginning of the experiment, one hundred 2-day old bipinnaria larvae were introduced into 2 L polycarbonate bottles (Cole-Palmer Co.). These bottles were incubated at 27-29 °C with gentle agitation. Larvae were sieved with a 60 μm mesh and transferred to freshly prepared bottles every day. All surviving larvae were individually examined every fourth day to record their developmental stages. Body length of randomly selected larvae (10-20) in each treatment was measured using an ocular micrometer. Measurement was made along the longest axis when the larva relaxed its body. On the day when late brachiolaria larvae appeared, a few small pieces of coralline alga (*Lithothamnium pseudosorum*) were placed in each bottle. The pieces were checked daily to count and remove metamorphosing larvae and settled juveniles. The data on the development and survival of *A. planci* larvae were analysed using the same method described in 3.2.1.

3.2.3 Larval rearing experiments using nutrient enriched seawater: rearing at ten different chlorophyll *a* concentrations

Experiments were conducted at the Australian Institute of Marine Science (AIMS) from October to December, 1994. Bipinnaria larvae of *A. planci* used for these experiments were obtained in the same manner as described in 3.2.1.

Seawater was collected off Cape Bowling Green once a week and stored in 500 L tanks. Nutrient enriched seawater was prepared daily, in the same manner as described in 3.2.2, and incubated for three days. The chlorophyll a concentration in the nutrient enriched seawater was determined fluorometrically before use. Then the nutrient enriched seawater was diluted with 0.45 μm filtered seawater to rear two batches of $A.$ $planci$ larvae at ten different chlorophyll a concentrations: 1.60, 0.80, 0.40, 0.20, 0.10 μg L^{-1} for Batch 7 and 1.00, 0.75, 0.50, 0.25, 0 μg L^{-1} for Batch 8. Experiments were run in duplicate for each chlorophyll concentration.

Approximately one hundred and fifty 2-day old bipinnaria larvae were introduced into 2 L polycarbonate bottles at the beginning of experiments. Randomly withdrawn larvae ($\approx$50) were individually examined every second day. Other procedures were the same as those described in 3.2.2. In addition to the analysis of larval development described in 3.2.1, a mean number of days required to reach 50 % late brachiolariae and settlement was calculated for each treatment.

3.3 Results

3.3.1 Larval rearing experiments using membrane filtered seawater, coarse filtered seawater, and alga and amino acid enriched seawater

In Batch 1 and Batch 2, *A. planci* larvae developed to late brachiolaria stage only in ALE treatment (Fig. 3-1). Larvae of both batches reared in 25 μm FSW treatment did not develop beyond the early brachiolaria stage during the experiment periods, although their survival rates were relatively high (Fig. 3-2). Most of larvae reared in 2 μm FSW and 0.45 μm FSW remained in bipinnaria stage and started regressing between Day 10 and Day 14.

In Batch 3, *A. planci* larvae developed to late brachiolaria stage in ALE, AAE and 25 μm FSW (Fig. 3-3). Development rates of larvae differed among these treatments, with slower rate being observed in 25 μm FSW (Table 3-1). Larvae reared in 2 μm FSW did not develop beyond bipinnaria stage.

The mean number of surviving larvae in ALE was similar to that in AAE treatment on Day 22 (Fig. 3-4). On the same day, however, the number in 25 μm FSW was significantly smaller than those in the other two treatments (ANOVA, $P < 0.05$).

In Batch 4, *A. planci* larvae reared in ALE developed to late brachiolaria stage between Day 10 and Day 14 (Fig. 3-3). A small number of larvae reared in AAE developed to late brachiolaria stage between Day 14 and Day 18 (Table 3-1). In contrast, larvae reared in 25 μm FSW did not develop beyond early brachiolaria stage. The mean number of surviving larvae in ALE was significantly larger than in AAE (t-test, $P < 0.005$, Fig. 3-4).

Table 3-2 summarises mean concentrations of eukaryotes, cyanobacteria and chlorophyll *a* in seawater during larval rearing experiments. The concentrations of eukaryotes and chlorophyll *a* in the coarse filtered seawater (0.214-0.437×10^3 cells mL^{-1} and 0.28-0.52 μg L^{-1} in 25 μm FSW treatments) were increased by enrichment of microalgae (3.246 - 4.053×10^3 cells mL^{-1} and 5.70-8.54 μg L^{-1} in ALE treatments) or reduced by further filtration (0.004-0.163×10^3 cells mL^{-1} and 0.08-0.25 μg L^{-1} in 2 μm FSW treatments). No eukaryotes were detected in duplicate 10 mL samples of 0.45 μm FSW. The concentration of cyanobacteria was significantly altered only in 0.45 μm FSW treatments.

The concentration of eukaryotes in the coarse filtered seawater in GBR (0.214×10^3 and 0.234×10^3 cells mL^{-1} in Batches 1 and 2) was almost half of that in Okinawan waters (0.437×10^3 and 0.336×10^3 cells mL^{-1} in Batches 3 and 4). However, total biomass of eukaryotes, in terms of the chlorophyll a concentration, was larger in GBR waters (0.40 μg L^{-1} and 0.52 μg L^{-1} in Batches 1 and 2) than in Okinawan waters (0.29 μg L^{-1} and 0.28 μg L^{-1} in Batches 3 and 4). The contribution of picoplankton (< 2 μm) to the chlorophyll a concentration in GBR (0.17 and 0.25 μg L^{-1}) was larger than in Okinawan waters (0.08 μg L^{-1}); consequently, biomass of nanoplankton (2-25 μm) was similar between these waters. The concentrations of cyanobacteria in GBR water (54.5-86.9 $\times$ 10^3 cells mL^{-1}) were an order of magnitude larger than in Okinawan water (6.40-7.10 $\times$ 10^3 cells mL^{-1}).

3.3.2 Larval rearing experiments using membrane filtered seawater, coarse filtered seawater and nutrient enriched seawater

A. planci larvae of Batches 5 and 6 reached late brachiolaria stage in 100 % NES and in 50 % NES treatments (Fig. 3-5). There were no significant differences in the rate of larval development between these treatments (Table 3-3), with nearly all surviving larvae completing their development by Day 14. *A. planci* larvae reared in 25 μm FSW developed to late brachiolaria in both batches, but their development rates were significantly slower than those in two NES treatments (Table 3-3). Larvae reared in 2 μm FSW did not develop beyond bipinnaria stage and started regressing between Day 10 and Day 14.

Changes in survival rates showed no trends, except for the rapid decrease beyond Day 14 in 25 μm FSW in Batch 5 (Fig. 3-6). The number of larvae completing development in 25 μm FSW was significantly smaller than those in 100 % NES and 50 % NES (ANOVA, $P < 0.01$). In Batch 6, there were no significant differences in number of larvae completing development among treatments of 100 % NES, 50 % NES and 25 μm FSW (ANOVA, $P > 0.1$).

The body length of larvae indicates the direction of growth. As shown in Figure 3-7, the body length of larvae reared in 2 μm FSW decreased gradually from Day 6. Apart from this, there were no clear differences in mean body length among all treatments or between batches. Body length decreased abruptly on Day 14 in 50 % NES in both batches and on Day 18 in 25 μm FSW for Batch 5, because the data included metamorphosing larvae, absorbing the larval body into the starfish primordium. In Batch 5, mean diameter of

settled juveniles in 25 μm FSW (0.44 ± 0.07 mm) was significantly smaller than those in two NES treatments (0.64 ± 0.09 mm for 100 % NES, 0.66 ± 0.05 mm for 50 % NES, t-test, P < 0.001).

Mean concentrations of eukaryotes, cyanobacteria, and chlorophyll a in two size fractions (total and < 2 μm) in each treatment are presented in Table 3-4. Nutrient enrichment enhanced the concentration of natural phytoplankton, particularly nanoplankton (2-25 μm). The concentration of eukaryotes in the nutrient enriched seawater (2.435 X 10^3 cells mL^{-1} in 50 % NES and 4.441 X 10^3 cells mL^{-1} in 100 % NES) were about 10 or 20 times larger than that in the coarse filtered seawater (0.207 X 10^3 cells mL^{-1} in 25 μm FSW). The total chlorophyll a concentrations in the nutrient enriched seawater (2.91 μg L^{-1} and 5.25 μg L^{-1}) were also increased 10-20 times compared to the coarse filtered seawater (0.28 μg L^{-1}), while the chlorophyll a concentration in a < 2 μm fraction in the nutrient enriched seawater (0.78 μg L^{-1} and 1.33 μg L^{-1}) increased about 4-7 times compared to the coarse filtered seawater (0.18 μg L^{-1}). Nutrient enrichment also enhanced the concentrations of cyanobacteria (202 X 10^3 cells mL^{-1} in 100 % NES and 142 X 10^3 in 50 % NES vs. 62 X 10^3 cells mL^{-1} in 25 μm FSW), but not to the same extent as for eukaryotes. In the membrane filtered seawater (2 μm FSW), the concentration of cyanobacteria was similar to that in the coarse filtered seawater (56 X 10^3 cells mL^{-1}), although the concentrations of eukaryotes and chlorophyll a were significantly reduced (0.004 X 10^3 cells mL^{-1} and 0.19 μg L^{-1}).

3.3.3 Larval rearing experiments using nutrient enriched seawater: rearing at ten different chlorophyll a concentrations

In Batch 7, A. planci larvae reared in 1.60 μg L^{-1} and 0.80 μg L^{-1} started developing to late brachiolaria stage between Day 14 and Day 16 (Fig. 3-8). There were no significant differences in the rate of larval development between these treatments. The mean number of days required to reach 50 % late brachiolariae and settlement was 21 days in both 1.60 μg L^{-1} and 0.80 μg L^{-1}. A few larvae developed to mid brachiolaria stage in 0.40 μg L^{-1}, but they eventually started regressing between Day 14 and Day 16. Larvae reared in 0.20 μg L^{-1} and 0.10 μg L^{-1} started regressing between Day 10 and Day 12, without developing beyond early brachiolaria or bipinnaria stage.

In Batch 8, A. planci larvae reared in 1.00 μg L^{-1} developed at the fastest rate, developing to late brachiolaria stage between Day 10 and Day 12 (Fig. 3-8). Larvae reared in 0.75 μg L^{-1} and 0.50 μg L^{-1} developed to late brachiolaria between Day 14 and Day 20. Their

development rates were significantly slower than those in 1.00 µg L^{-1} (Table 3-5). The number of days required to reach 50 % late brachiolaria and settlement in each treatment of 1.00 µg L^{-1}, 0.75 µg L^{-1} and 0.50 µg L^{-1} was 15, 22 and 47 days, respectively. Most *A. planci* larvae reared in 0.25 µg L^{-1} and 0 µg L^{-1} started regressing between Day 8 and Day 12.

Changes in mean body length of *A. planci* larvae of Batches 7 and 8 are shown in Figure 3-9. Steady increase in the body length of *A. planci* larvae was observed in 1.60 µg L^{-1} and 0.80 µg L^{-1} in Batch 7 and in 1.00 µg L^{-1} and 0.75 µg L^{-1} in Batch 8. The decrease in the mean body length on Day 20 in 0.8 µg L^{-1} in Batch 7 was due to absorption of the larval body into the starfish primordium. *A. planci* larvae reared in 0.50 µg L^{-1} in Batch 8 stopped growing beyond Day 12. The body length of larvae of both batches reared in chlorophyll *a* concentrations < 0.40 µg L^{-1} decreased in the latter part of experimental period.

3.4 Discussion

The development rate of *A. planci* larvae reared in coarse filtered seawater (25 μm FSW) varied widely among batches, regardless of chlorophyll *a* concentration (see the summary of rearing experiments in Table 3-6). This indicates that the availability of phytoplankton food is not the sole factor controlling larval development. Larval development was also influenced by concentration of supplementary amino acids. The relative importance of these two food sources can not be assessed from the results of this study. However, the growth, development and survival of *A. planci* larvae were significantly enhanced by elevating the concentrations of these food sources in seawater (Table 3-1, 3-3, 3-5, Fig. 3-1, 3-3, 3-5), indicating that *A. planci* larvae were food limited in coarse filtered seawater.

Reduction in size and growth rate of post-metamorphic juvenile due to food limitation during planktonic larval stages have been reported for several echinoderm species, including an asteroid (Paulay et al., 1985; Emlet, 1986; Fenaux et al., 1988; Basch, 1992a, b). The same observation was made in this study. The size of settled *A. planci* juvenile in the coarse filtered seawater was significantly smaller than size in the nutrient enriched seawater (0.44 ± 0.07 mm in 25 μm FSW treatment vs. 0.64 ± 0.09 and 0.66 ± 0.05 mm in 100 % NES and 50 % NES treatments in Batch 5, see 3.3.2). The smaller size of juvenile is likely to be a consequence of limited food supply to larvae in the coarse filtered seawater. It was only observed in one batch, but an important implication is that the effect of food limitation in *A. planci* larvae may extend to the post-metamorphic stage.

Phytoplankton concentrations in the coarse filtered seawater approximated those in natural seawater, suggesting food limitation in the field. The mean chlorophyll *a* concentration in coarse filtered seawater used in the experiments was 0.28-0.52 μg L^{-1} (Table 3-2, 3-4, 3-6). These values were similar to those reported for GBR water (0.09-0.64 μg L^{-1}, Ikeda et al., 1980; Bellamy et al., 1982; Furnas and Mitchell, 1984, 1986; Ayukai, unpubl. data) and Okinawan water (< 0.4 μg L^{-1}, Okinawa Prefectural Government, unpubl. data). However, the phytoplankton concentrations in the coarse filtered seawater fluctuated during the course of experiments (Table 3-6). This is problematic in determining the critical range of food concentration, because the effect of shortage in phytoplankton supplies on the growth and survival of asteroid larvae differs between developmental stages (Allison, 1994).

The results of rearing experiments at fixed phytoplankton concentrations give good indications on the critical food range. In Batch 7, the development rate of A. *planci* larvae did not differ between 1.60 μg L^{-1} and 0.80 μg L^{-1} (Fig. 3-8), indicating that larvae were not food limited between these chlorophyll *a* concentrations. There was no significant larval development below 0.40 μg L^{-1}. In Batch 8, larvae reared in 1.00 μg L^{-1} developed at optimal rates (Table 3-5, Fig. 3-8). The development rates were significantly slower in 0.75 μg L^{-1} and 0.50 μg L^{-1}, but there was modest larval survival at these chlorophyll *a* concentrations. These results suggest that, if total chlorophyll *a* concentration is used as an overall index of food availability to A. *planci* larvae, the critical range, where a modest survival to optimal survival through rapid development occur, is 0.5-0.8 μg L^{-1}.

Food limitation in A. *planci* larvae was not as severe as previously suggested. Lucas (1982) suggested that chlorophyll *a* concentrations above 0.4 μg L^{-1} are required for A. *planci* larvae to survive. Ayukai (1993) estimated that A. *planci* larvae can not meet their minimum energy requirement at a chlorophyll *a* concentration below 1.3-2.7 μg L^{-1}. In fact, however, A. *planci* larvae were able to develop through to metamorphosis in the coarse filtered seawater having mean chlorophyll *a* concentrations of 0.28-0.29 μg L^{-1}, although the development rates were significant slower than larvae reared under optimal food supplies (Batches 5 and 6, Table 3-6, Fig. 3-6). This inconsistency may be due to the difference in nutritional values between cultured microalgae and natural phytoplankton (Mortensen, 1938; Vance, unpubl., both cited in Strathmann, 1975). The possible importance of food sources other than phytoplankton also needs to be considered.

The consistent fact that A. *planci* larvae failed to develop in the membrane filtered seawater suggests that picoplankton and bacteria are unimportant food (0.45 μm FSW and 2 μm FSW treatments in Fig. 3-1, 3-3, 3-5). Cyanobacteria are the most abundant of picoplankton communities (cf. Stockner, 1988). In this study, the highest concentration of cyanobacteria in filtered seawater was 74.6 X 10^3 cells mL^{-1} (2 μm FSW treatment in Batch 2, Table 3-2). At this concentration, assuming that the equivalent spherical diameter (ESD) of a cyanobacterium is 1.5 μm (cf. Ayukai, 1994), the biomass of cyanobacteria is theoretically equivalent to the biomass of phytoplankton (assumed ESD of 5 μm) of about at 2,000 cells mL^{-1}. Concentrations of heterotrophic bacteria were not measured, but their biomass was possibly identical or larger than phytoplankton (Cho and Azam, 1990). If A. *planci* larvae could utilise these small particles as efficiently as phytoplankton, there should have been moderate larval development.

Ayukai (1994) reported that bacteria were not detected from the guts of *A. planci* larvae. Larvae were able to ingest cyanobacteria, but their clearance rate of cyanobacteria was one to two orders of magnitude lower than of cultured phytoplankton. Ayukai (1994) suggested from these results that bacteria were not utilisable for *A. planci* larvae due to their small size, although he did not deny the possible importance of colony-forming, detritus-attached bacteria. Similar results were reported for the larvae of the temperate asteroid, *Asterina miniata* (Pearse et al., 1991).

The complete failure of *A. planci* larvae to develop in filtered seawater makes it unlikely that DOM is a major food source. However, *A. planci* larvae are able to utilise dissolved free amino acids (DFAA), because DFAA enrichment significantly accelerated larval development (Fig. 3-2, Table 3-1). Transepidermal uptake of DFAA may provide a significant amount of energy to *A. planci* larvae at high substrate concentrations (Hoegh-Guldberg, 1994). DOM other than DFAA, i.e. sugars, fatty acids, acetate and nucleosides, are also possible foods for *A. planci* larvae (cf. Manahan, 1990). Concentrations of DOM in the coarse filtered seawater used in the experiments were possibly increased not only by breakdown of particulate organic matter (cf. Taylor et al., 1985), but also by *de novo* production by natural phytoplankton (Wilson, 1981). Hence, fluctuations of DOM concentration between experiments might be a cause of the variation in larval development in the coarse filtered seawater. Further investigation is needed to assess the possible importance of various kinds of DOM in the nutrition of *A. planci* larvae, but their role is not likely to be critical.

The results of this study agree with those obtained with larvae of an asteroid and several echinoid species in temperate waters. Basch (1992a, b and pers. comm.) reared larvae of the asteroid *Asterina miniata* in the laboratory using variously filtered seawater and seawater enriched with either DOM (not specified), bacteria or microalgae. While larvae developed rapidly in seawater enriched with microalgae, larvae did not develop in finely filtered seawater and seawater enriched with DOM nor bacteria. From San Juan Island, U.S.A., Paulay et al. (1985) reported that the larvae of the echinoid, *Dendraster excentricus*, reared in natural seawater developed significantly slower than those reared in natural seawater enriched with microalgae. Fenaux et al. (1994) obtained similar results with larvae of the echinoid, *Paracentrotus lividus*, in the Mediterranean. These authors came to the conclusion that echinoid larvae were usually food limited. Interestingly, the range of food concentration, where food limited growth and development occurred, differs between these two studies; 1-2 µg L^{-1} for *D. excentricus* and 0.1-0.4 µg L^{-1} for *P. lividus*. This is despite ecological and morphological similarities of these larvae.

This study suggests that the growth, development and survival of *A. planci* larvae are food limited in the field, but not to an extreme degree. A critical range for *A. planci* larvae of 0.5-0.8 μg L^{-1} total chlorophyll *a* is suggested as an index of food limitation. Although picoplankton (< 2 μm) seem unimportant, the relative importance of phytoplankton in different size fractions needs to be considered. The importance of DOM, particularly DFAA, as a potential food also needs further investigation.

Table 3-1 One-way ANOVA and Scheffe's test for the differences between mean proportions of the most advanced developmental stages of two batches (3 and 4) of *Acanthaster planci* larvae in three seawater treatments. ALE: alga enriched seawater, AAE: dissolved free amino acid mixture enriched seawater, 25 µm FSW: 25 µm mesh filtered seawater. Data were arcsine transformed when they were analysed.

Days	The most advanced stage	Mean proportion of larvae (%)				
		ALE		AAE		25 µm FSW
Batch 3						
10	Early brachiolaria	67	n.s.	55	$>^{***}$	9
14	Late brachiolaria	7	n.a.	0	n.a.	0
18	Late brachiolaria	33	$>^{***}$	9	n.a.	0
22	Late brachiolaria + Settled juvenile	63	n.s.	45	$>^{***}$	19
Batch 4						
10	Mid brachiolaria	22	n.a.	0	n.a.	0
14	Late brachiolaria + Settled juvenile	70	n.a.	0	n.a.	0
18	Late brachiolaria + Settled juvenile	84	$>^{***}$	19	n.a.	0
22	Late brachiolaria + Settled juvenile	89	$>^{***}$	17	n.a.	0

*$P<0.05$, **$P < 0.01$, ***$P < 0.005$
n.s.: not significant
n.a.: not analysed

Table 3-2 Mean concentrations of eukaryotic algae, cyanobacteria and chlorophyll *a* in four seawater treatments for *Acanthaster planci* larvae of batches 1-4. ALE: alga enriched seawater, 25 µm FSW: 25 µm mesh filtered seawater, 2 µm FSW: 2 µm membrane filtered seawater, 0.45 µm FSW: 0.45 µm glass fibre filtered seawater. Seawater used for rearing experiments with Batches 1, 2 and 3, 4 was, respectively, collected at Lizard Island (GBR waters) and at Chatan Reef, Okinawa Island (Okinawan waters). Values are mean ±1 S.D.

Treatment	Cell concentration (x10^3 cells mL^{-1})		Chlorophyll *a* (µg L^{-1})
	Eukaryotes	Cyanobacteria	
Batch 1			
ALE	3.246 ± 0.961	68.7 ± 37.7	8.06 ± 1.94
25 µm FSW	0.214 ± 0.112	64.8 ± 35.9	0.40 ± 0.20
2 µm FSW	0.004 ± 0.006	54.5 ± 35.5	0.17 ± 0.10
0.45 µm FSW	n.d.	24.7 ± 15.8	0.07 ± 0.03
Batch 2			
ALE	3.744 ± 0.109	86.9 ± 33.1	8.54 ± 2.60
25 µm FSW	0.234 ± 0.086	83.3 ± 30.3	0.52 ± 0.21
2 µm FSW	0.004 ± 0.004	74.6 ± 33.8	0.25 ± 0.11
0.45 µm FSW	n.d.	31.3 ± 16.2	0.08 ± 0.03
Batch 3			
ALE	4.053 ± 1.171	n.m.	7.48 ± 2.25
25 µm FSW	0.437 ± 0.222	7.10 ± 6.10	0.29 ± 0.10
2 µm FSW	0.163 ± 0.125	6.40 ± 5.90	0.08 ± 0.03
Batch 4			
ALE	3.360 ± 0.935	n.m.	5.70 ± 1.45
25 µm FSW	0.385 ± 0.178	6.70 ± 5.10	0.28 ± 0.08

n.d.: not detected
n.m.: not measured

Table 3-3 One-way ANOVA and Scheffe's test for the difference among mean proportions of the most advanced developmental stages of two batches (5 and 6) of *Acanthaster planci* larvae in three seawater treatments. 100 % NES: 100 % nutrient enriched seawater, 50 % NES: a mixture of 50 % nutrient enriched seawater and 25 μm mesh filtered seawater, 25 μm FSW: 25 μm mesh filtered seawater. Data were arcsine transformed when they were analysed.

Days	The most advanced stage	Mean proportion of larvae (%)				
		100 % NES		50 % NES		25 μm FSW
Batch 5						
6	Early brachiolaria	78	n.s.	79	>**	40
10	Late brachiolaria	24	n.s.	28	n.a.	0
14	Late brachiolaria + Settled juvenile	91	n.s.	99	>*	76[†1]
18	Late brachiolaria + Settled juvenile	100	n.s.	100	n.s.	91
22	Late brachiolaria + Settled juvenile	100	n.s.	100	>**	88
Batch 6						
6	Early brachiolaria	69	>**	24	n.a.	0
10	Late brachiolaria	17	n.s.	17	n.a.	0
14	Late brachiolaria + Settled juvenile	97	n.s.	96	>**	47
18	Late brachiolaria + Settled juvenile	100	n.s.	99	>*	81
22	Late brachiolaria + Settled juvenile	100	n.s.	99	n.s.	97

†1: no settled juveniles
*P < 0.05, **P < 0.005
n.s.: not significant
n.a.: not analysed

Table 3-4 Mean concentrations of eukaryotic algae, cyanobacteria, total chlorophyll a and chlorophyll a in a < 2 μm fraction in four seawater treatments for *Acanthaster planci* larvae of Batches 5 and 6. 100 % NES: 100 % nutrient enriched seawater, 50 % NES: a mixture of 50 % nutrient enriched seawater and 25 μm mesh filtered seawater, 25 μm FSW: 25 μm mesh filtered seawater, 2 μm FSW: 2 μm membrane filtered seawater. Values are mean ±1 S.D.

Treatment	Cell concentration ($\times 10^3$ cells mL^{-1})		Chlorophyll a (μg L^{-1})	
	Eukaryotes	Cyanobacteria	Total	< 2 μm
100 % NES	4.441 ± 0.989	202 ± 157	5.25 ± 2.32	1.33 ± 1.10
50 % NES	2.435 ± 0.564	142 ± 90	2.91 ± 1.35	0.78 ± 0.62
25 μm FSW	0.207 ± 0.077	62 ± 41	0.28 ± 0.10	0.18 ± 0.09
2 μm FSW	0.004 ± 0.002	56 ± 40	0.19 ± 0.10	0.20 ± 0.08

Table 3-5 One-way ANOVA and Scheffe's test for the difference among mean proportions of the most advanced developmental stages of *Acanthaster planci* larvae of Batch 8 in three chlorophyll *a* concentration treatments (1.00, 0.75 and 0.50 µg L^{-1}). Data were arcsine transformed when they were analysed.

Days	The most advanced stage	Mean proportion of larvae (%)				
		1.00 µg L^{-1}		0.75 µg L^{-1}		0.50 µg L^{-1}
8	Early brachiolaria	71	>***	35	n.s.	19
10	Mid brachiolaria	31	>*	13	n.a.	0
12	Late brachiolaria	8	n.a.	0	n.a.	0
14	Late brachiolaria	31	>**	5	n.a.	0
16	Late brachiolaria + Settled juvenile	67	>*	31[†1]	n.a.	0
20	Late brachiolaria + Settled juvenile	62	>*	39	>***	7

†1: no settled juveniles
*P < 0.05, **P < 0.01, ***P < 0.005
n.s.: not significant
n.a.: not analysed

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Table 3-6 Summary of rearing experiments with *Acanthaster planci* larvae reared in coarse filtered seawater (25 μm mesh filtered seawater, referred to as 25 μm FSW in the text). The mean concentrations and ranges (in parentheses) of chlorophyll *a* in the coarse filtered seawater for each batch, the most advanced developmental stages, and the mean number of days required to reach 50 % late brachiolariae or settled juveniles are presented. eB: early brachiolaria, lB+Jv: late brachiolaria and settled juvenile. "rg." indicates that larvae have regressed during the course of experiments.

Chlorophyll *a* concentration (μg L^{-1})	Most advanced developmental stages	Days to 50 % lB+Jv	Batch No.
0.28 (0.09 - 0.39)	eB (rg.)		4
0.28 (0.16 - 0.50)	lB+Jv	12.6	5
	lB+Jv	14.2	6
0.29 (0.14 - 0.47)	lB+Jv	26.0	3
0.40 (0.22 - 0.98)	eB		1
0.52 (0.34 - 0.98)	eB		2
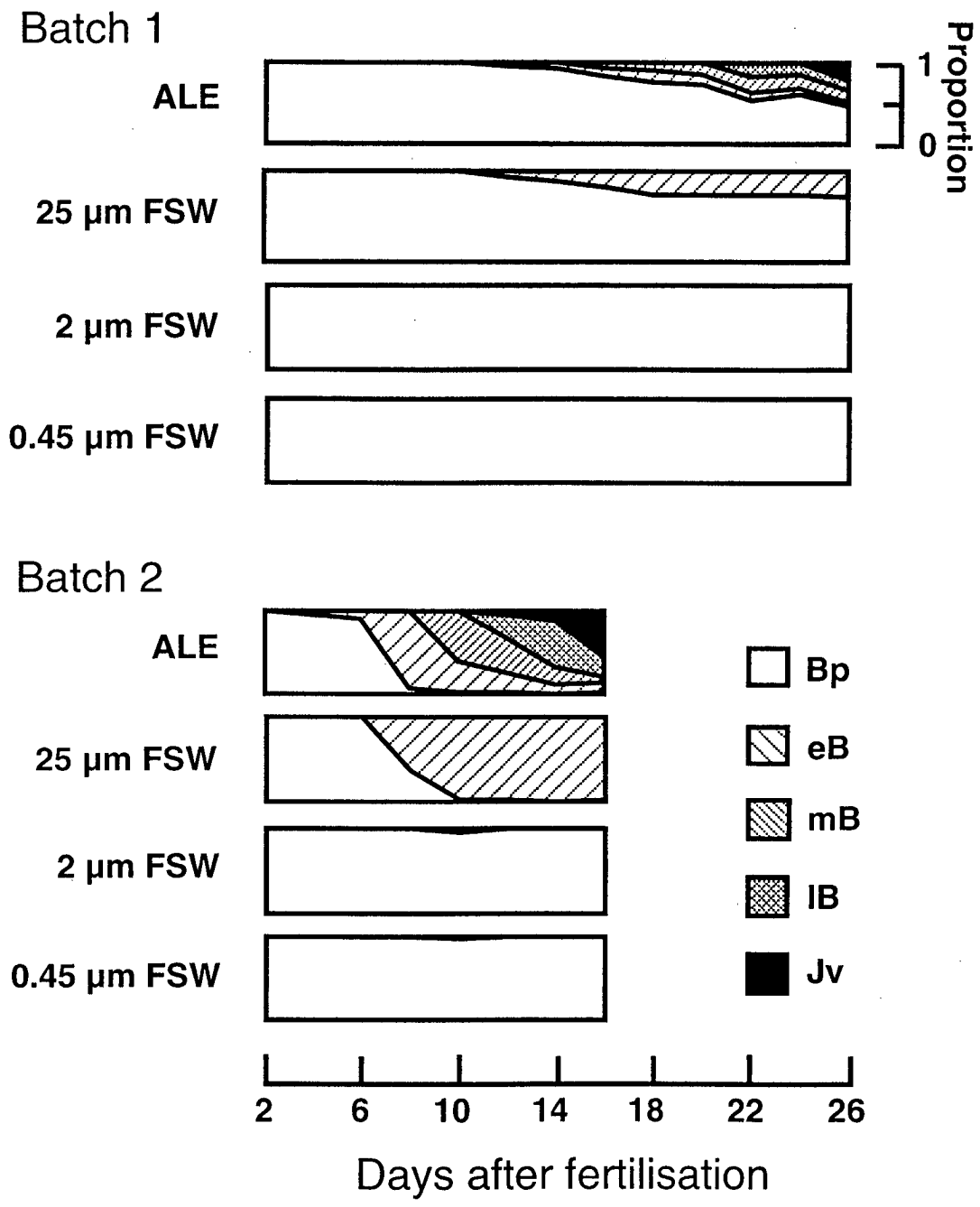
Figure 3-1 Development of two batches (1 and 2) of *Acanthaster planci* larvae reared in four different seawater treatments. ALE: alga enriched seawater, 25 μm FSW: 25 μm mesh filtered seawater, 2 μm FSW: 2 μm membrane filtered seawater, 0.45 μm FSW: 0.45 μm glass fibre filtered seawater. Bp: bipinnaria, eB: early brachiolaria, mB: mid brachiolaria, lB: late brachiolaria, Jv: metamorphosed juvenile.
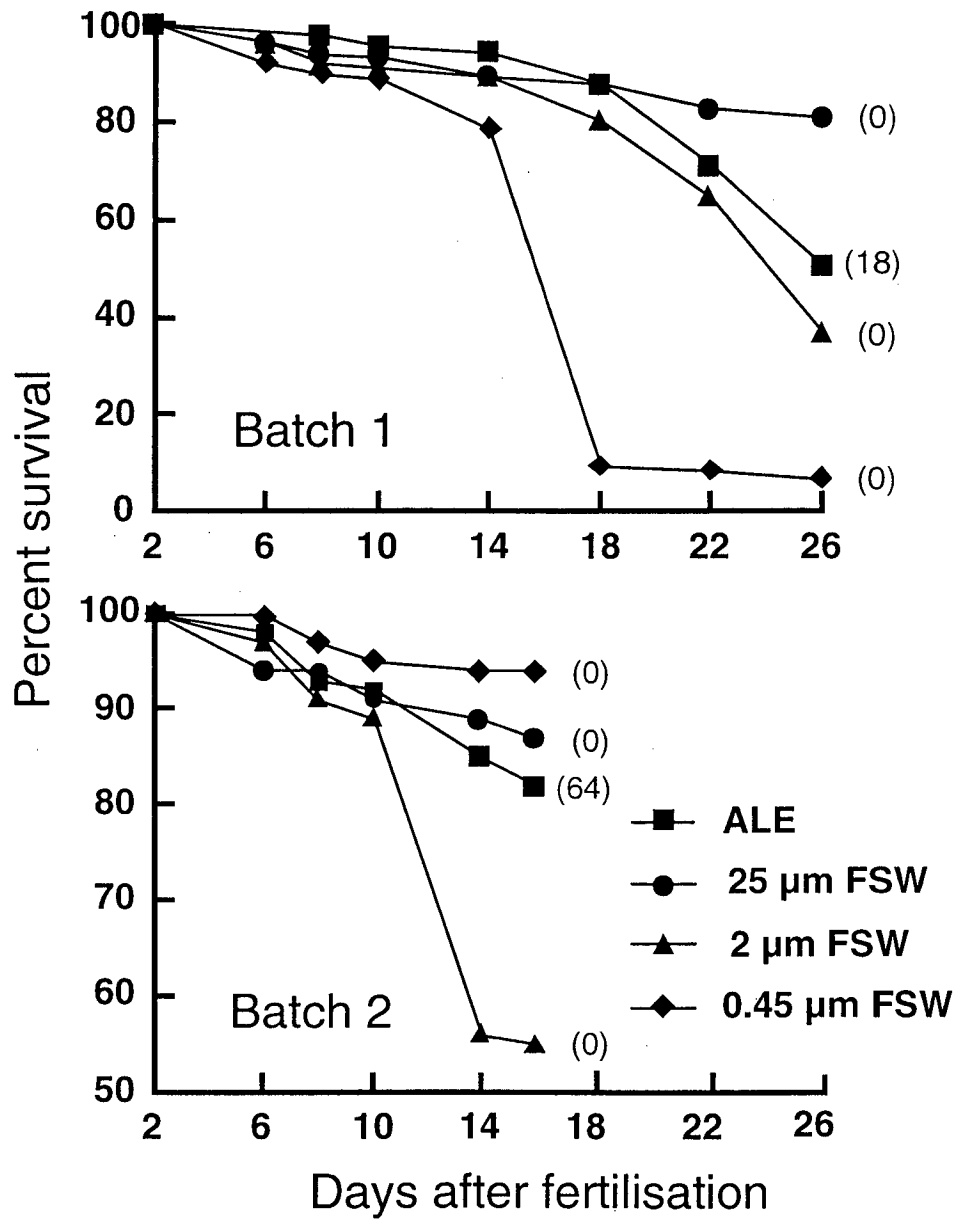
Figure 3-2   Changes in mean survival rate of two batches (1 and 2) of
Acanthaster planci larvae reared in four different seawater
treatments. ALE: alga enriched seawater, 25 μm FSW: 25 μm
mesh filtered seawater, 2 μm FSW: 2 μm membrane filtered
seawater, 0.45 μm FSW: 0.45 μm glass fibre filtered seawater.
Numbers in parentheses represent proportions of larvae that had
completed development.
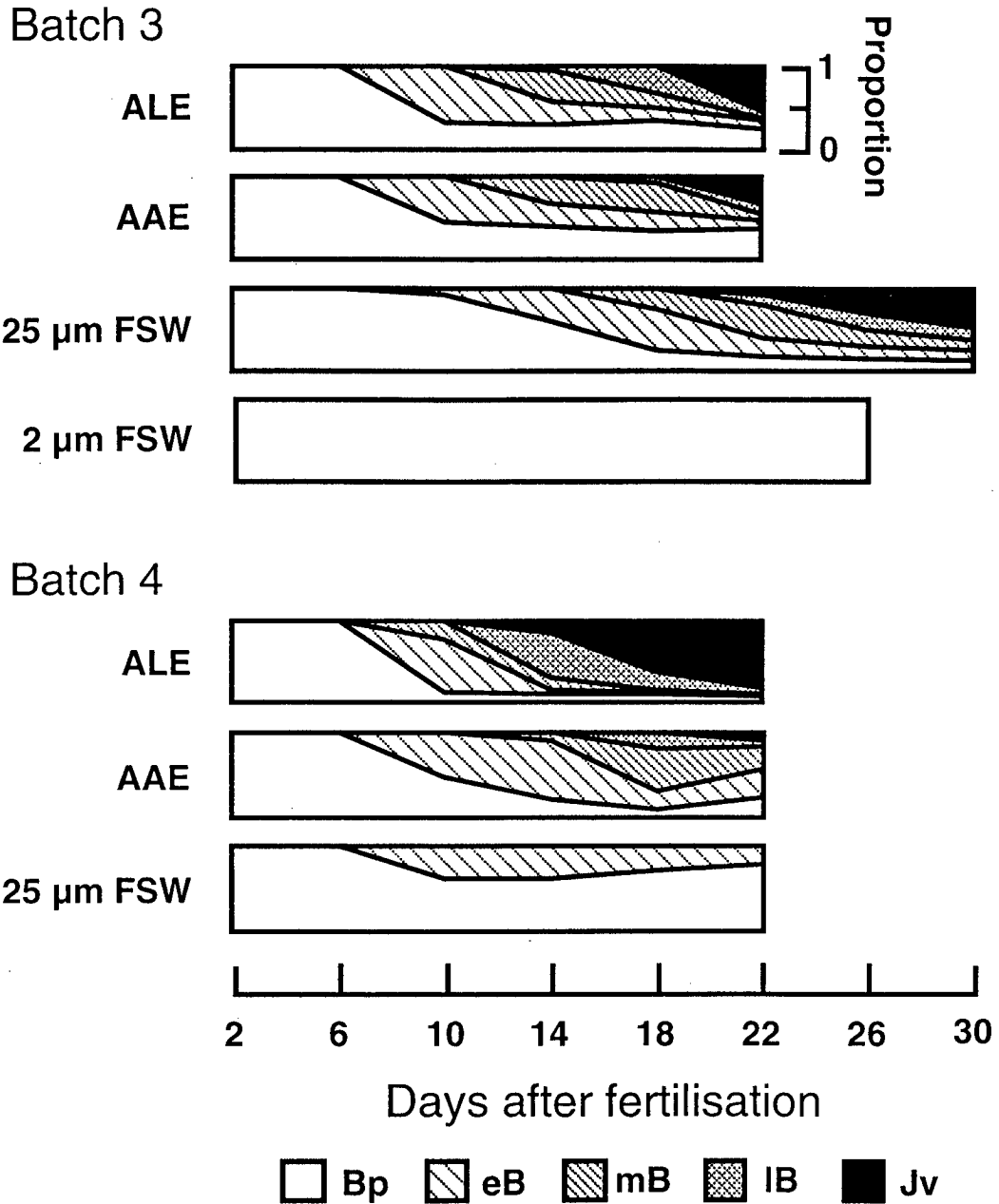
Figure 3-3 Development of two batches (3 and 4) of *Acanthaster planci* larvae
reared in four different seawater treatments. ALE: alga enriched
seawater, AAE: dissolved free amino acid enriched seawater, 25 μm
FSW: 25 μm mesh filtered seawater, 2 μm FSW: 2 μm membrane
filtered seawater. Bp: bipinnaria stage, eB: early brachiolaria, mB:
mid brachiolaria, lB: late brachiolaria, Jv: metamorphosed juvenile.
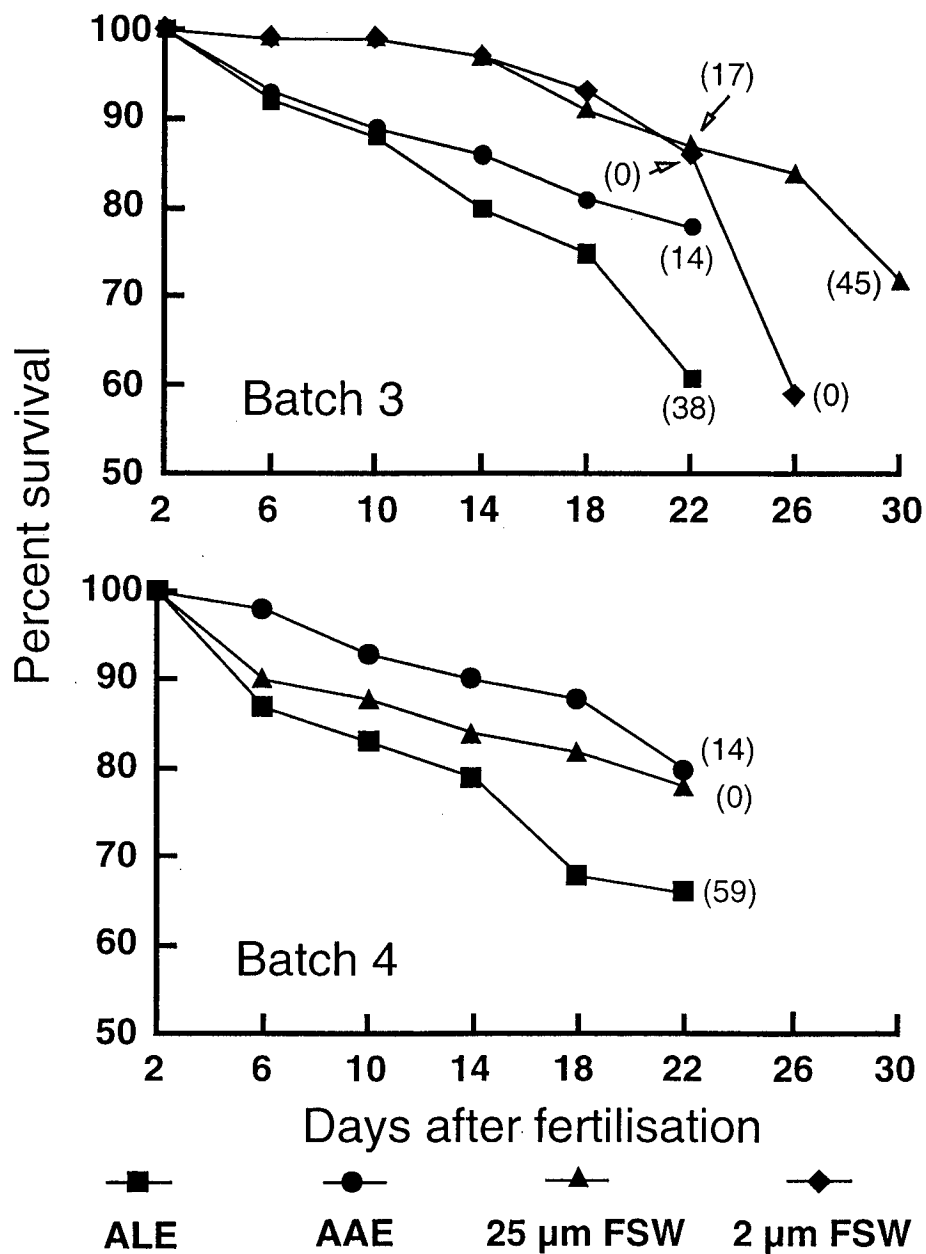
Figure 3-4 Changes in mean survival rate of two batches (3 and 4) of
Acanthaster planci larvae reared in four different seawater
treatments. ALE: alga enriched seawater, AAE: dissolved
free amino acid enriched seawater, 25 μm FSW: 25 μm
mesh filtered seawater, 2 μm FSW: 2 μm membrane filtered
seawater. Numbers in parentheses represent proportions of
larvae that had completed development.
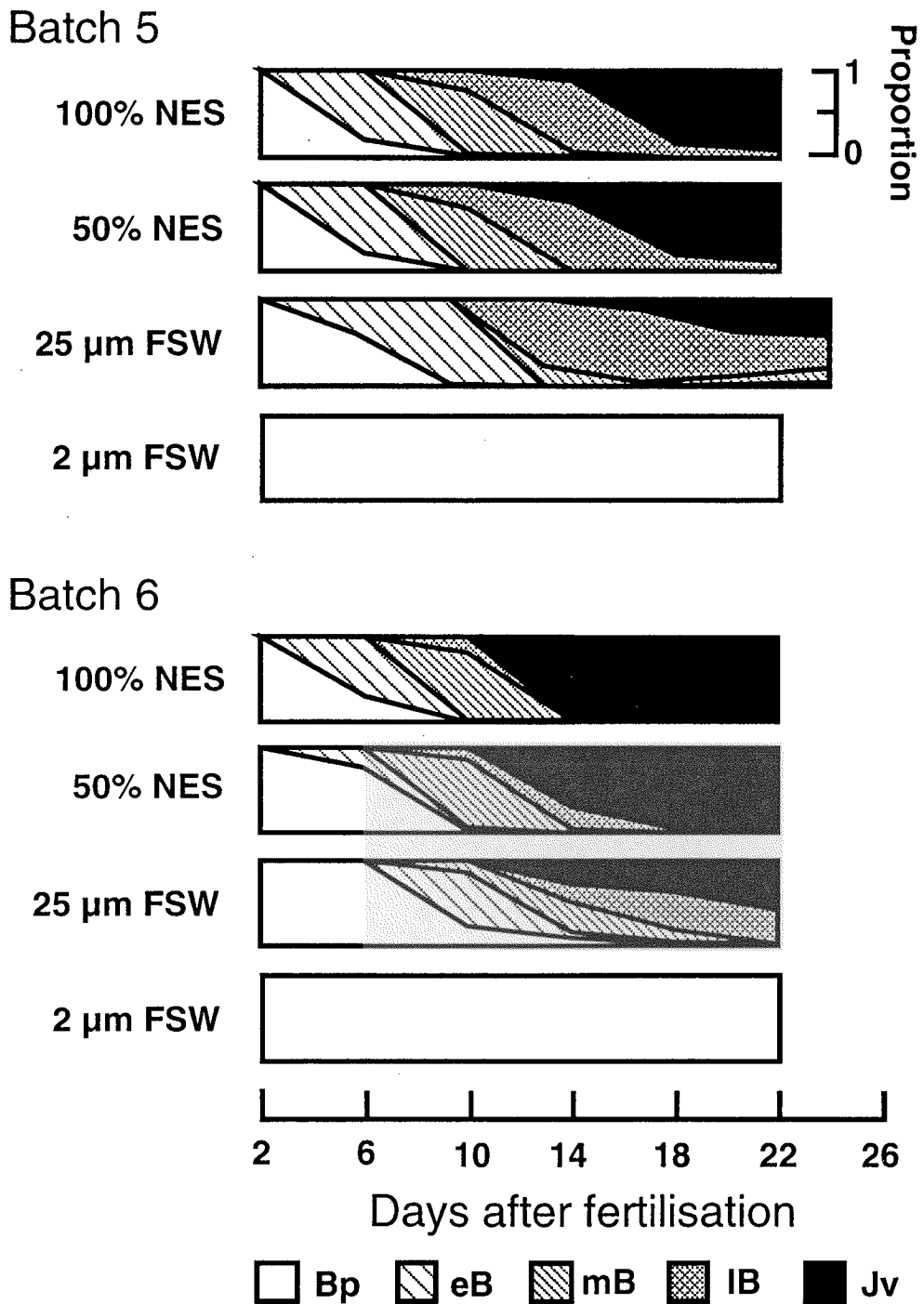
Figure 3-5 Development of two batches (5 and 6) of *Acanthaster planci* larvae reared in four different seawater treatments. 100 % NES: 100 % nutrient enriched seawater, 50 % NES: a mixture of 50 % nutrient enriched seawater and 50 % 25 μm mesh filtered seawater, 25 μm FSW: 25 μm mesh filtered seawater, 2 μm FSW: 2 μm membrane filtered seawater. Bp: bipinnaria stage, eB: early brachiolaria, mB: mid brachiolaria, lB: late brachiolaria, Jv: metamorphosed juvenile.
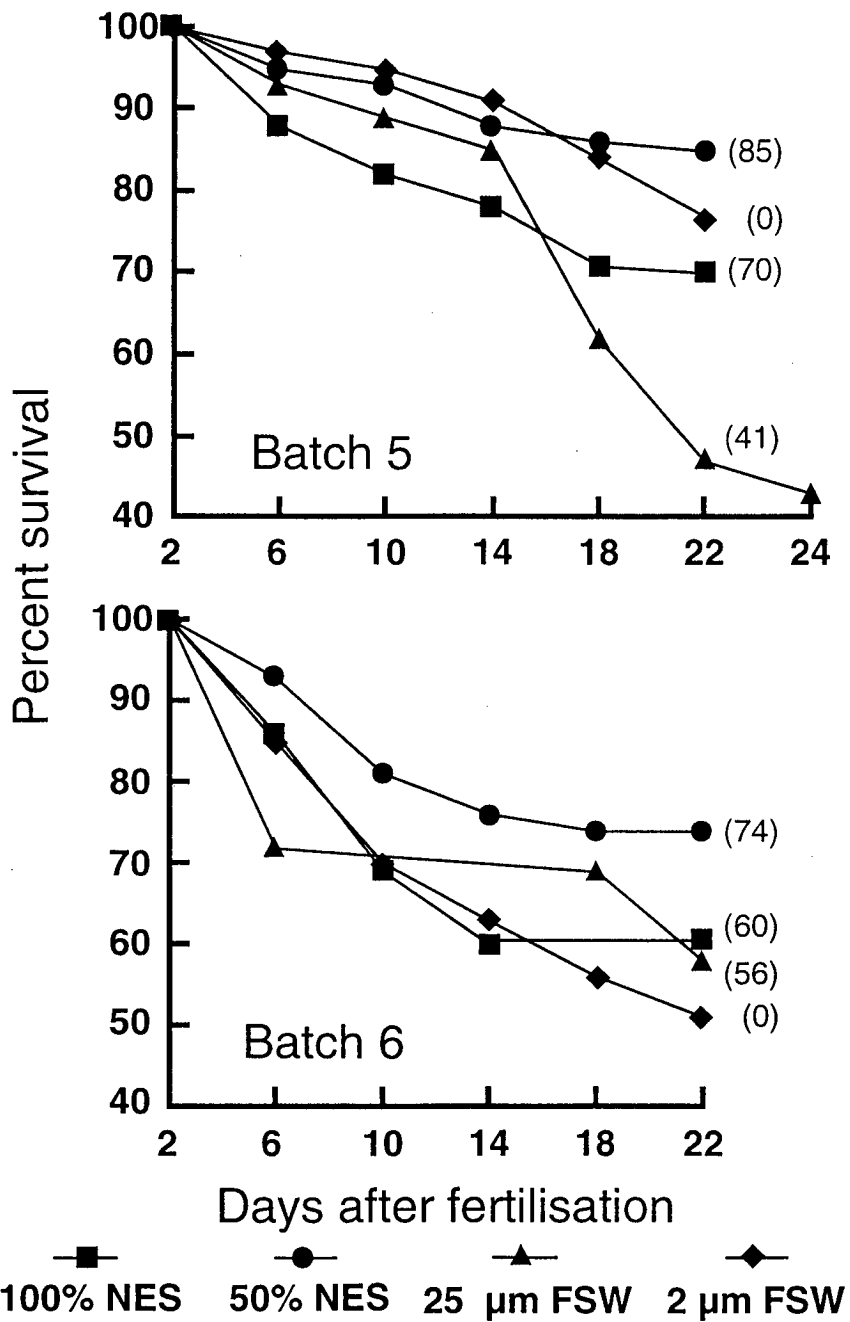
Figure 3-6 Changes in mean survival rate of two batches (5 and 6) of
Acanthaster planci larvae reared in four different seawater
treatments. 100 % NES: 100 % nutrient enriched seawater,
50 % NES: a mixture of 50 % nutrient enriched seawater
and 50 % 25 μm mesh filtered seawater, 25 μm FSW: 25
μm mesh filtered seawater, 2 μm FSW: 2 μm membrane
filtered seawater. Numbers in parentheses represent
proportions of larvae that had completed development.
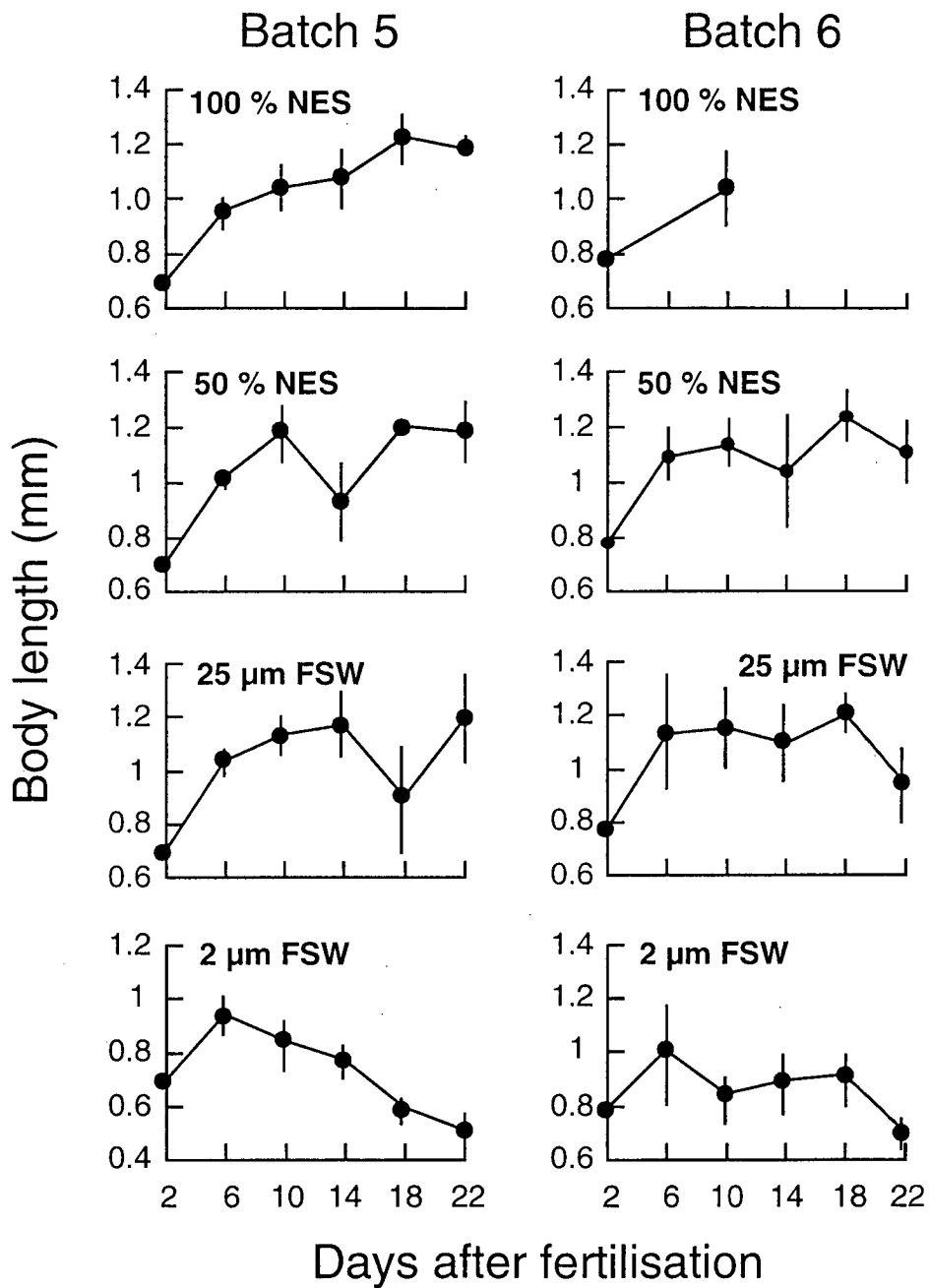
Figure 3-7 Changes in mean body length of two batches (5 and 6) of
Acanthaster planci larvae reared in four different seawater
treatments. 100 % NES: 100 % nutrient enriched seawater, 50
% NES: a mixture of 50 % nutrient enriched seawater and 50
% 25 μm mesh filtered seawater, 25 μm FSW: 25 μm mesh
filtered seawater, 2 μm FSW: 2 μm membrane filtered
seawater. Bars represent the ranges.
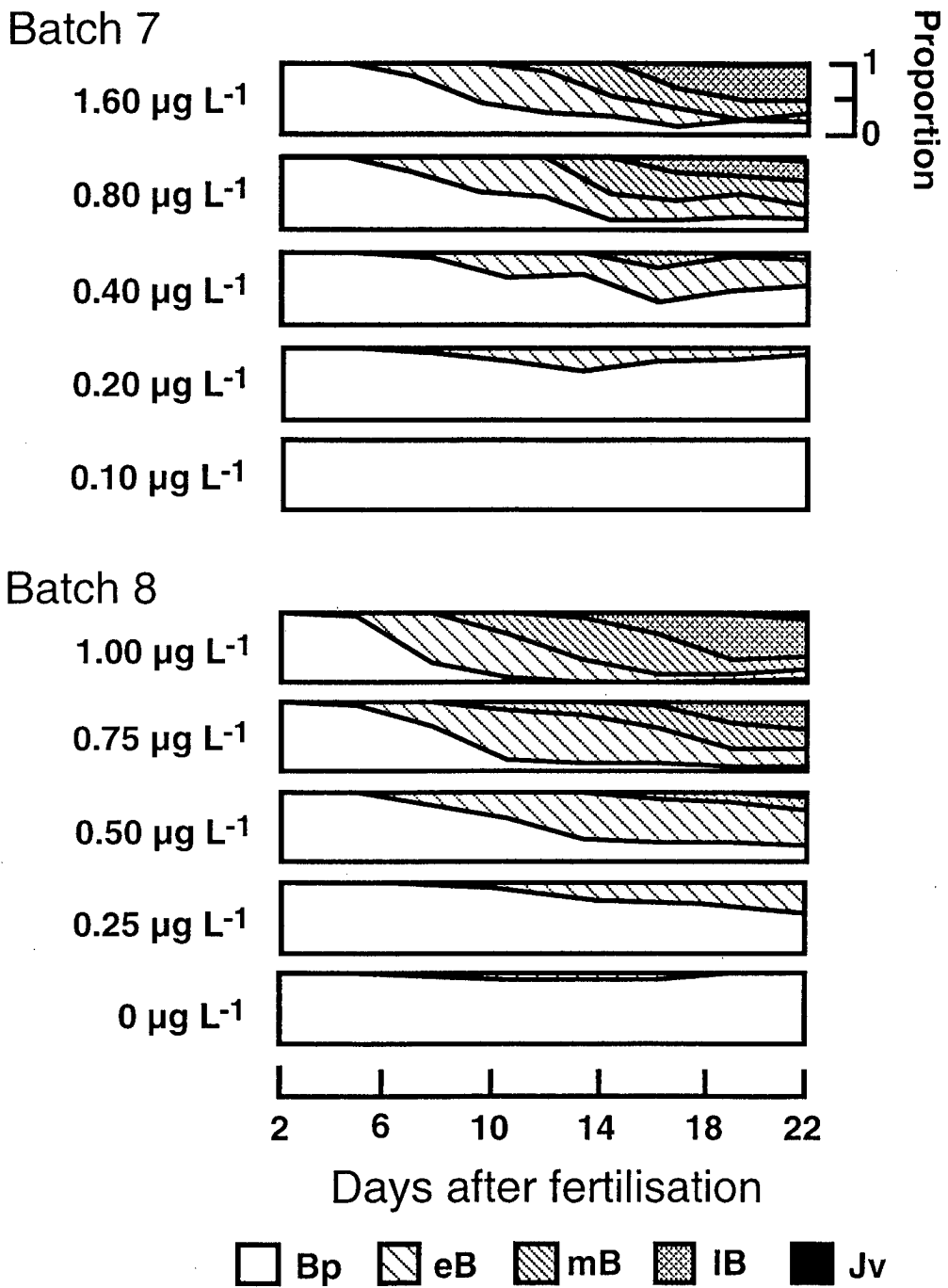
Figure 3-8 Development of two batches (7 and 8) of *Acanthaster planci* larvae reared under ten different chlorophyll *a* concentrations. Bp: bipinnaria stage, eB: early brachiolaria, mB: mid brachiolaria, lB: late brachiolaria, Jv: metamorphosed juvenile.
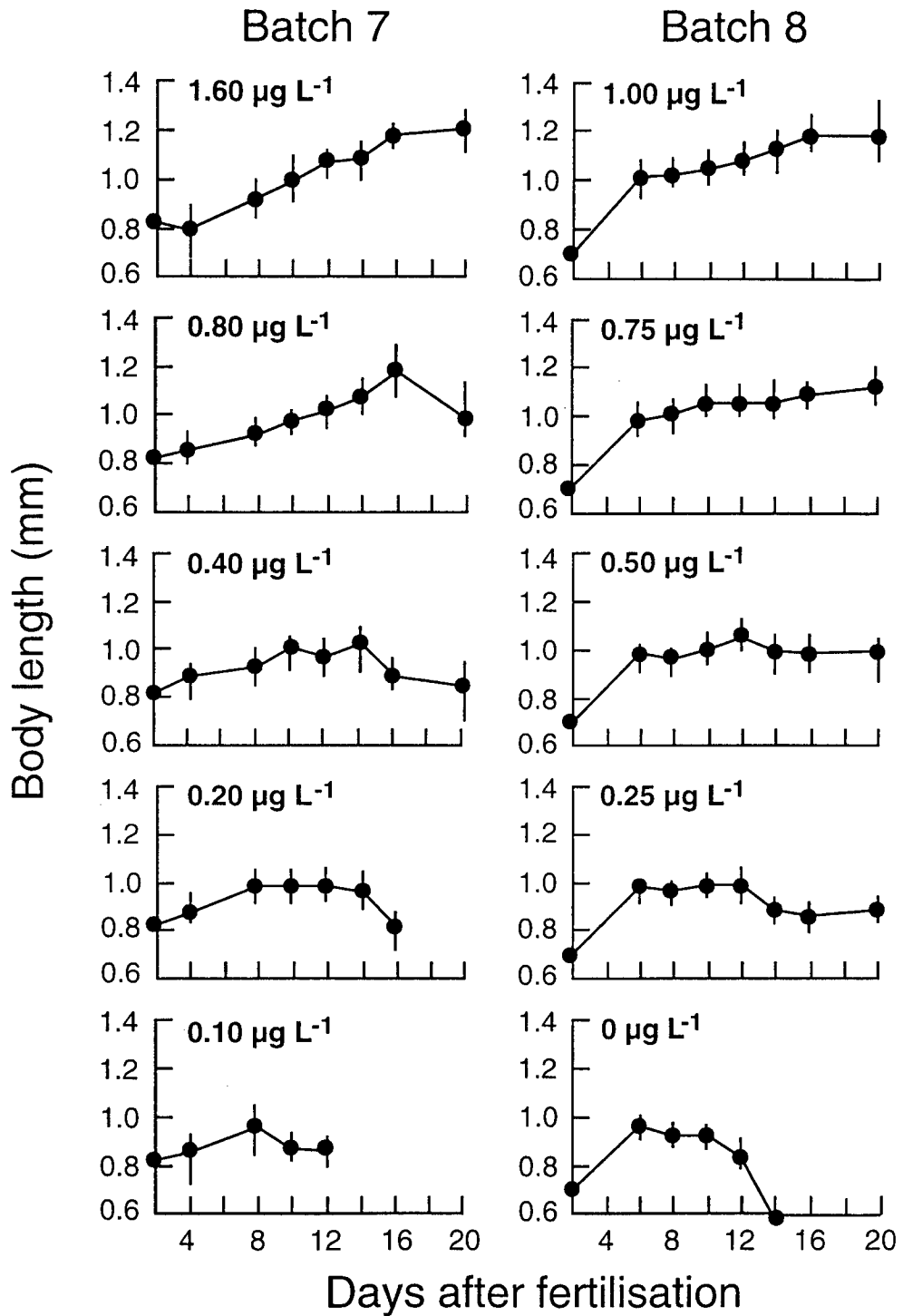
Figure 3-9 Changes in mean body length of two batches (7 and 8) of
Acanthaster planci larvae reared under ten different chlorophyll *a*
concentrations. Bars represent the ranges.

Chapter Four

Selective feeding by *Acanthaster planci* larvae

4.1 Introduction

Asteroid larvae create feeding currents using their ciliary band and capture food particles upstream from the band by the local reversal of ciliary beat (Strathmann, 1971; Gilmour, 1988). Strathmann (1971) observed that particles of ca. 1-2 μm were too small to induce the reversal of ciliary beat and are not efficiently captured by larvae. This observation would explain the inability of the larvae of *A. planci* and some temperate species to feed on bacteria (Pearse et al., 1991; Ayukai, 1994). There is, however, the report of bacterivory by larvae of antarctic species (Rivkin et al., 1986, 1991; Bosch et al., 1988). Given the different methods used in these studies, it is premature to conclude that the lower size limit of food particles for asteroid larvae differs between species.

To date, little attention has been paid to the diverse nature and variability of phytoplankton assemblages in the habitat of *A. planci* larvae. Photosynthetic picoplankton (0.2-2 μm) are the dominant component of phytoplankton assemblages in tropical waters (cf. Stockner, 1988). However, as phytoplankton biomass increases in response to event-associated nutrient injections, the size composition often shifts to larger size classes (cf. Malone 1971, 1980). Therefore, understanding size selective feeding of *A. planci* larvae is necessary to evaluate how such changes in phytoplankton biomass and size composition affect their nutrition.

The aim of this study was to measure the feeding rates on naturally-occurring food particles by *A. planci* larvae and to determine the dependency of feeding rates on particle size. *A. planci* larvae were kept in natural seawater for short periods and the number and size of phytoplankton in their guts were examined under an epifluorescence microscope. *A. planci* larvae were also incubated in suspensions of cultured microalgae (*Dunaliella tertiolecta*) and plastic beads of different sizes.

4.2 Materials and methods

4.2.1 Feeding experiments with naturally-occurring food particles

A. planci larvae were raised in the laboratory using the method described in 3.2.1 in Chapter 3. From 2 days after fertilisation, larvae were reared in natural or nutrient enriched (Guillard's f/2 solution, added at the volume ratio of 0.01 %) natural seawater in 4 L beakers. Temperature was maintained at approximately 28 °C using a water bath.

Seawater for feeding experiments was collected approximately 1 km offshore from the Lizard Island Research Station, filtered through a 25 μm mesh screen and brought back to the laboratory in a clean polycarbonate container. In each feeding experiment, duplicate 10 mL subsamples for enumeration of eukaryotes and cyanobacteria were withdrawn and filtered onto 0.45 μm Millipore black filters. Each filter was mounted on a slide glass using Zeiss immersion oil and examined under a Leitz epifluorescence microscope equipped with a blue excitation filter set (Leitz Code No. 513604).

Actively swimming larvae at the bipinnaria (4 day old) and the early brachiolaria (6 day old) stages (cf. Lucas, 1982) were harvested from the larval culture, gently sieved onto 60 μm mesh screen and washed with 0.2 μm syringe filtered seawater. They were transferred to a clean glass beaker (300 mL, < 150 larvae) filled with 0.2 μm membrane filtered seawater and kept for 24 hours to empty their guts.

Feeding experiments were started by pipetting 5-10 starved larvae with a small volume of seawater (< 0.5 mL) into plastic vials filled with 10 mL of natural seawater. After 5 or 15 minutes incubation,* a buffered glutaraldehyde solution was added to the vials to give a final concentration of 0.5 %. These samples were kept refrigerated until gut contents examination. Preserved larvae were sieved with 60 μm mesh screen, washed repeatedly

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*Ingestion and clearance rates were calculated on the assumption that no defaecation occurred during incubation. In a preliminary experiment, bipinnaria larvae were incubated for 5, 15, 30 and 45 minutes. The numbers of eukaryotes and cyanobacteria in the gut increased for the first 15 minutes and then became saturated or decreased (Fig. 4-1). This result suggested that ingestion and clearance rates could be underestimated in incubation beyond 15 minutes, because of the occurrence of defaecation.

with 0.2 μm syringe filtered seawater and filtered onto 0.45 μm Millipore black filters. These filters with larvae were mounted on glass slides and the numbers and sizes of cells in the gut of each larva were measured under an epifluorescence microscope.

Clearance rates of larvae (volume of seawater cleared per larva per unit time) were calculated by dividing ingestion rates (number of cells ingested per larva per unit time) by cell concentration in the natural seawater used. The volume of individual food cells was calculated by assuming an appropriate geometric shape. The equivalent spherical diameter (hereafter referred to as ESD) of individual cells was then calculated from the cell volume.

4.2.2 Feeding experiments with *Dunaliella tertiolecta* and fluorescently-labelled plastic beads

Twenty early brachiolaria larvae of *A. planci* were incubated for 3-5 minutes in 20 mL suspensions of either cultured *Dunaliella tertiolecta* or fluorescently-labelled plastic beads of three different sizes (1, 6 and 20 μm diameter, Fluorescebrite, Polysciences Inc.). There were six kinds of treatment: (1), *Dunaliella* suspensions (concentration range $5 \times 10^2 - 5 \times 10^3$ cells mL^{-1}); (2)-(4), three different sized bead suspensions of various concentrations ($5 \times 10^3 - 1 \times 10^5$ mL^{-1} for 1 μm bead and $5 \times 10^2 - 5 \times 10^3$ mL^{-1} for 6 and 20 μm beads); (5), mixed beads suspension (Mixture 1) that contained each of three different sized beads at the concentration of 1×10^3 mL^{-1}; and (6), mixed beads suspension (Mixture 2) that contained equal volumes (0.4189 nL) of three different sized beads (8×10^5 for 1 μm, 3.7×10^3 for 6 μm, 1×10^2 for 20 μm). There were no replicates. Suspensions with 1 μm beads were sonicated before use to prevent their aggregation.

The samples of *A. planci* larvae were processed using the same procedure as described in the previous section for examination of the gut contents.

4.3 Results

4.3.1 Clearance rates of naturally-occurring food particles

The blue excitation filter set that was used might not have the optimal resolution for examination of gut contents, since it illuminated the larvae themselves with weak yellow-green fluorescence. The yellow fluorescence of cyanobacteria, however, was intense enough for cell counts and size determinations. Although the red autofluorescence of eukaryotes was weak, the outline of each cell was clearly visible.

The results of four feeding experiments are summarised in Table 4-1. Concentrations of cyanobacteria in seawater ranged between 1.73×10^5 and 5.33×10^5 cells mL^{-1} and were 3 to 4 orders of magnitude higher than those of eukaryotes. Yet, even under these conditions, the numbers of cyanobacteria ingested by larvae were similar to or smaller than the numbers of eukaryotes ingested. Consequently, clearance rates of cyanobacteria by bipinnaria and early brachiolaria larvae were approximately 3 orders of magnitude lower than those of eukaryotes (0.035-0.349 μL larva^{-1} h^{-1} vs. 118-358 μL larva^{-1} h^{-1}). In four experiments, the size ranges of ingested cells by *A. planci* larvae were between 1 and 2 μm ESD for cyanobacteria and between 1 and 23.8 μm ESD for eukaryotes, respectively. The average size of ingested eukaryotes in four experiments ranged between 3.6 and 4.6 μm ESD. Assuming that the average ESD of a cyanobacterium is 1.5 μm, the calculated total volume of cyanobacteria ingested by larvae was less than 6 % of the total volume of all eukaryotes ingested.

4.3.2 Clearance rates of *Dunaliella tertiolecta* and fluorescently-labelled plastic beads

There were no significant differences in mean clearance rate between 6 and 20 μm plastic beads (one-way ANOVA, $P > 0.10$, Table 4-2). Clearance rates of 1 μm plastic beads, however, were three to four orders of magnitude lower than those of 6 and 20 μm plastic beads. Interestingly, clearance rates of *D. tertiolecta* (ca. 5 μm ESD) were significantly higher than those of 6 and 20 μm plastic beads (one-way ANOVA, $P < 0.01$), suggesting involvement of some chemostimulatory mechanism in feeding of larvae. Clearance rates of 1 μm plastic beads were reduced when they were offered in mixtures with larger plastic beads, being reduced to undetectable rates (Mixture 1) or about 10 % of the clearance rate when offered solely (Mixture 2) (Table 4-2).

4.4 Discussion

Cyanobacterial concentrations in these samples of seawater from near Lizard Island were 3-4 orders of magnitude higher than those of eukaryotes. Utilising such a dominant component of the phytoplankton is obviously advantageous for planktotrophic larvae (cf. Rassoulzadegan and Fenaux, 1979; Bell, 1991; Gallager et al., 1994). *A. planci* larvae, however, cleared eukaryotes from seawater at the rates more than 1,000 times greater than they did cyanobacteria. In terms of volume, cyanobacteria accounted for less than 6 % of phytoplankton ingested by *A. planci* larvae. The larvae were not able to take advantage of a major potential food source.

Ayukai (1994) used heat-killed, fluorescently-labelled cells (FLC) of five cultured phytoplankton to study feeding of bipinnaria and brachiolaria larvae of *A. planci*. Clearance rates were 5.76-14.0 μL larva^{-1} h^{-1} for cyanobacteria of 1-2 μm ESD and 230-394 μL larva^{-1} h^{-1} for eukaryotes of 5-17 μm ESD. His values for eukaryotes are in good agreement with those obtained in this study (118-358 μL larva^{-1} h^{-1}, Table 4-1). However, the values for cyanobacteria are two to three orders of magnitude larger than those obtained in this study (0.035-0.349 μL larva^{-1} h^{-1}). This may be partly due to differences in chemical properties between heat-killed and natural cyanobacteria. Also, *A. planci* larvae were kept in mono-specific FLC suspensions in the experiment of Ayukai (1994), whereas this study was conducted using natural seawater with particles of varying size, quantity and quality.

Size selective feeding of planktotrophic echinoderm larvae is explained, at least to an extent, by assuming cilia are not passive filters (Strathmann, 1971; Strathmann et al., 1972). Detailed studies using high speed cinematography have shown that a food capturing response by echinoderm larvae is elicited by detecting the physical disturbance caused by passing particles (Strathmann et al., 1972; Gilmour, 1988). There is also evidence to show that echinoderm larvae are able to distinguish between particles of similar sizes, but of different chemical properties (Rassoulzadegan et al., 1984; Appelmans, 1994). The difference in clearance rate between *A. planci* larvae feeding on *D. tertiolecta* and plastic beads of similar or larger size supports the involvement of chemosensory mechanisms in their feeding (Table 4-2).

Clearance rates of *A. planci* larvae on 1 μm plastic beads were much reduced when they were offered in mixtures with larger plastic beads (Table 4-2). This may indicate that the ingestion of small particles by *A. planci* larvae is regulated by the amount of larger

particles ingested. Such a regulatory mechanism would explain the discrepancy between Ayukai's study and this study with regard to clearance rate of cyanobacteria by A. *planci* larvae. It is also noteworthy that neither bacteria nor cyanobacteria were seen in the gut of A. *planci* larvae reared in *in situ* rearing chambers (Dixon, unpubl. cited in Birkeland and Lucas, 1990). Instead, Dixon found that the diet of A. *planci* larvae consisted mainly of dinoflagellates and diatoms, including large *Nitzschia* sp. Although there is a slight possibility that small cells like cyanobacteria were lost during sample preparation for electron microscopy, Dixon's observations also indicate that the feeding behaviour of A. *planci* larvae differs between natural seawater and mono-specific algal suspensions.

As a side line, this study showed that the food environment surrounding A. *planci* larvae is much more varied (Table 4-1) than anticipated from the variation in phytoplankton biomass. In the GBR, phytoplankton blooms during the summer are usually due to increases of phytoplankton in the >10 μm fraction (Furnas and Mitchell, 1986). Especially, pennate diatoms (10-20 μm) grow rapidly in response to nutrient inputs (Furnas, 1991). Under non-bloom conditions, picoplankton (< 2 μm) normally dominate over nanoplankton(2-10 μm) (Furnas and Mitchell, 1986). The biomass and composition of picoplankton also fluctuate considerably over short time scales (Ayukai, 1992). The solution for controversies over food limitation in A. *planci* larvae, as well as the differences in growth and development rates between larvae reared in different source of natural seawater (Chapter 3), may lie in understanding their feeding response to changing biomass and composition of phytoplankton, particularly in the nanoplankton size.

Table 4-1 Summary of four feeding experiments with naturally-occurring food particles. Concentrations of eukaryotes (Eu) and cyanobacteria (Cy) in 25 μm mesh filtered seawater (FSW), ingestion and clearance rates of eukaryotes and cyanobacteria by *Acanthaster planci* larvae and equivalent spherical diameter (ESD) of ingested eukaryotes. Values in parentheses are standard errors.

Developmental stage of larvae	Cell concentration in FSW (cells mL^{-1}) Eu	Cy ($\times 10^5$)	Incubation time (min.)	No. larvae examined	Mean no. cells ingested Eu	Cy	Clearance Rates (μL larva^{-1} h^{-1}) Eu	Cy	ESD of eukaryotes ingested (μm)*
Bipinnaria	239.9	1.73	5	13	7.2 (1.0)	0.9 (0.2)	358 (49)	0.065 (0.021)	3.6 (0.9)
Bipinnaria	239.9	1.73	15	10	10.0 (1.6)	1.5 (0.5)	167 (27)	0.035 (0.012)	4.0 (0.4)
Bipinnaria	55.7	5.10	15	22	1.6 (0.3)	1.3 (0.7)	118 (20)	0.103 (0.005)	3.6 (0.6)
Early brachiolaria	147.0	5.33	15	23	6.3 (0.8)	4.7 (0.8)	171 (98)	0.349 (0.056)	4.6 (0.4)

*ESD of cyanobacteria ranged between 1 and 2 μm.

Table 4-2 Clearance rates of *Dunaliella tertiolecta* and plastic beads of three different sizes by the early brachiolaria larvae of *Acanthaster planci*. Mixture 1 contained each of three different sized beads at the same concentration. Mixture 2 contained equivalent total volume (0.4189 nL) of three different sized beads. Data are mean values with standard errors and ranges in parentheses.

Suspensions	Particles	Concentration (No. mL^{-1})	Clearance rates (μL larva^{-1} h^{-1})
(1) *Dunaliella tertiolecta*		$5 \times 10^2 - 5 \times 10^3$	312 ± 58.4 (200 - 396)
(2) 1 μm		$5 \times 10^3 - 1 \times 10^5$	0.64 ± 0.54 (0 - 2.25)
(3) 6 μm		$5 \times 10^2 - 5 \times 10^3$	237 ± 61.9 (172 - 361)
(4) 20 μm		$5 \times 10^2 - 5 \times 10^3$	194 ± 53.3 (114 - 295)
(5) Mixture 1	1 μm	1×10^3	not detected
	6 μm	1×10^3	115 ± 17.7 (0 - 336)
	20 μm	1×10^3	188 ± 15.2 (60 - 300)
(6) Mixture 2	1 μm	8×10^5	0.06 ± 0.007 (0 - 0.11)
	6 μm	3.7×10^3	202 ± 20.8 (12 - 352)
	20 μm	1×10^2	270 ± 56.1 (0 - 750)
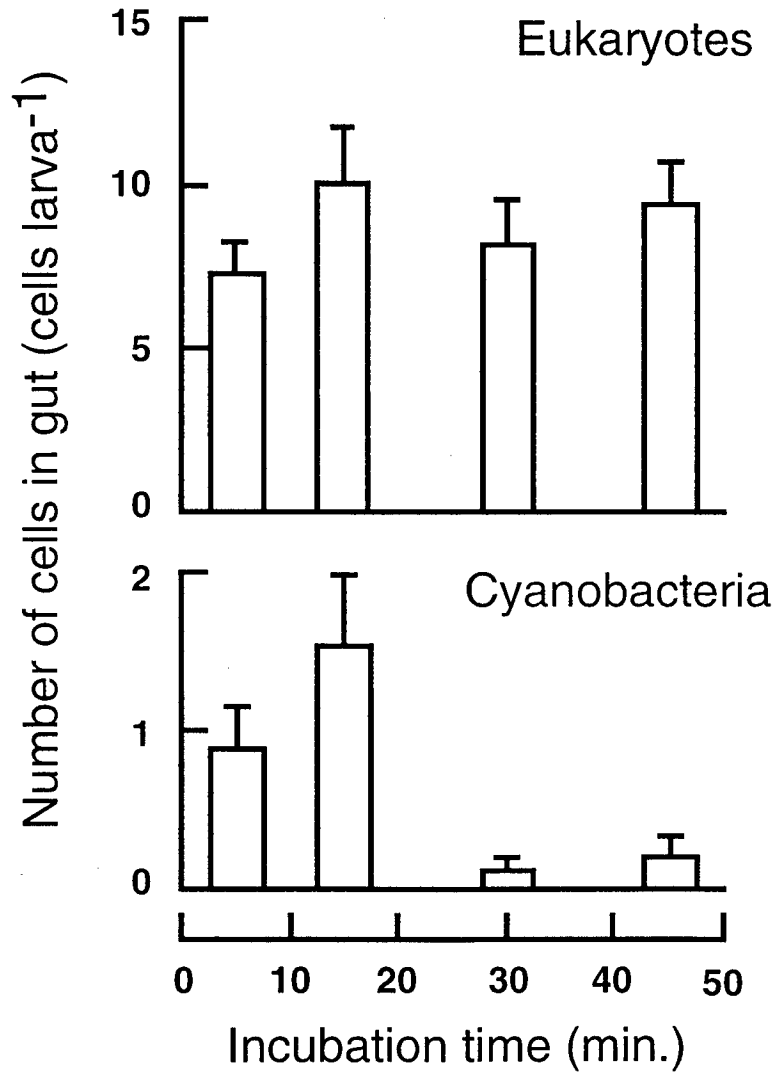
Figure 4-1 Time course variation in numbers of cells in the guts of
bipinnaria larvae of *Acanthaster planci* incubated in
natural seawater. Concentrations of eukaryotes and
cyanobacteria were 239.9 and 1.73×10^5 cells mL^{-1},
respectively. Data are means ± S.E.

Chapter Five

Net uptake of dissolved free amino acids by
Acanthaster planci larvae

5.1 Introduction

DOM is the largest organic carbon pool in the ocean, being about an order of magnitude greater than particulate organic matter (cf. Cauwet, 1978). DOM consists of a wide variety of organic matter with molecular weights ranging from less than 10^2 to over 10^5 daltons (Sharp, 1973; Ogura, 1974). Large molecular weight compounds are relatively inert and are not readily available to organisms (Taylor et al., 1985; Brophy and Carlson, 1989). DOM for which transepidermic uptake has been reported includes DFAA, sugars, fatty acids, acetate and nucleosides (cf. Stephens, 1988). These usually comprise less than 10 % of the total DOM pool (Williams, 1975) and, clearly, DOM is not an unlimited food source for marine organisms.

DOM has been suggested as a potential food for *A. planci* larvae (Lucas, 1982; Olson, 1987; Olson and Olson, 1989), and, recently, Hoegh-Guldberg (1993, 1994) measured transepidermal uptake rates of DOM by *A. planci* larvae using ^{14}C labelled glucose (sugar) and alanine, leucine and histidine (DFAA). *A. planci* larvae were able to take up both glucose and the three types of DFAA. The estimated kinetic parameters (the maximum influx and a half saturation constant) suggested that sugar was unimportant, but that DFAA could potentially contribute to a substantial part of the nutrition of larvae at high (> 1 µM) substrate concentrations. However, such estimations based on uptake rates determined with ^{14}C labelled substrates are not always reliable, because marine invertebrate larvae also release amino acids from their body during incubations (Johannes et al., 1969; Stephens, 1988). There are fluxes of DOM into and from the larval body.

The aim of this study was to measure the net uptake rate of DFAA by *A. planci* larvae and to estimate their potential importance in the larval nutrition. The net uptake rates of three amino acids, glutamic acid (acidic), arginine (basic) and alanine (neutral), were determined by measuring the change in concentrations of the substrates during incubations. Experiments used not only solutions of each amino acid, but also an equimolar mixture of three amino acids to test whether *A. planci* larvae selectively uptake DFAA.

5.2 Materials and methods

Early brachiolaria larvae of *A. planci* used for experiments were obtained from the mass-larval rearing facility in the Australian Institute of Marine Science (see Appendix I) and kept in 0.2 μm membrane and activated charcoal filtered seawater for 24 hours. Immediately before experiments, these larvae were concentrated on a 100 μm mesh screen and rinsed several times with filtered seawater. Then larvae were introduced into 20 mL scintillation vials filled with filtered seawater. The number of larvae in a vial ranged from 123 to 246. Vials with seawater and larvae were placed in tight-fit holes of an aluminium block to maintain experiment temperature at 29.5 °C.

Three amino acid solutions (100 μM), glutamic acid, arginine and alanine, were added to each vial to produce either equimolar mixtures of three amino acids or monospecific solutions of each amino acid. Initial concentrations of each amino acid ranged from 149 to 155 nM in equimolar mixtures, and from 148 to 501 nM in monospecific solutions.

A. planci larvae were incubated for 60 minutes. Triplicate 500 μL subsamples were withdrawn from each vial at 20 minutes intervals. Seawater in each subsample was gently passed through an acid washed 100 μm mesh screen to remove larvae and frozen for later DFAA analysis. As control experiments, amino acid solutions were added to identical vials that contained either no larvae or formalin-killed and washed larvae (N=153).

Concentrations of individual amino acids in each subsample were determined, using the pre-column derivatization method (Appendix II; Stanley et al., 1987; Ayukai et al., 1993). Because the concentrations used in this study were well below the half-saturation constant (K_t) of 2-3 μM (Hoegh-Guldberg, 1993), the rate of amino acid uptake by larvae in a vial was calculated from the rate of substrate depletion using the first-order depletion constant, "k", where $k = (\ln[S_0] - \ln[S_t])/t$ (Segel, 1976). The depletion constant k was calculated from the slope of a least-square linear regression analysis of ln-transformed amino acid concentrations with time. Values for the initial [S_0] and the final [S_t] concentration were calculated from the regression equation as trends at time 0 and time 1 (hour). Then the net uptake rate of amino acid by a larva (pmol amino acid larva^{-1} h^{-1}) was calculated by multiplying the amount of each amino acid present in the mixture by its respective depletion constant (k), and dividing each rate by the total number of larvae in the vial.

5.3 Results

There was no significant decrease for any of the three amino acids in the control experiments with no larvae and with formalin-killed larvae.

Net uptake of amino acid by *A. planci* larvae was observed only for alanine (Fig. 5-1). The uptake rates of alanine at the initial concentrations of 148 nM and 465 nM were 8.87 pmol larva^{-1} h^{-1} ($k = 0.437$, r$^2 = 0.999$, 152 larvae) and 36.87 pmol larva^{-1} h^{-1} ($k = 0.675$, r$^2 = 0.985$, 178 larvae), respectively. No significant changes in concentrations of glutamic acid and arginine during incubation were detected.

There was also no depletion in concentrations of glutamic acid or arginine after 1 hour incubation in the mixed solution of three amino acids (Fig. 5-2, 5-3). The concentration of alanine, however, decreased from 154 nM to 63 nM during incubation, indicating that *A. planci* larvae selectively took up alanine from seawater. The calculated net uptake rate of alanine was 15.40 pmol larva^{-1} h^{-1} ($k = 0.867$, r$^2 = 0.991$).

A considerable amount of glycine was detected in the ambient seawater in all experiments with *A. planci* larvae, but not in control experiments or blank seawater (see Fig. 5-3).

5.4 Discussion

A. planci larvae selectively took up alanine over glutamic acid or arginine. These results revealed limitations in the uptake capability of DFAA by *A. planci* larvae. Hoegh-Guldberg (1994) also reported that the transport rates of neutral amino acids (alanine and leucine) far exceeded those of basic amino acid (histidine). Selective uptake of neutral amino acids has also been reported for larvae of other echinoderm species (Manahan et al., 1983; Davis and Stephens, 1984; Manahan, 1990).

Generally, transport of one amino acid is only inhibited by other amino acids of the same charged group (Antia et al., 1991). The results of this study, therefore, suggest that the epithelium of *A. planci* larva lacks transport systems for acidic and basic amino acids. Alternatively, affinities of transport systems for acidic and basic amino acids in *A. planci* larvae were maintained at very low levels. Such a strategy may benefit *A. planci* larvae in conserving the energy cost for epidermal uptake to compensate the passive loss of acidic and basic amino acids from their intracellular DFAA pools (cf. Stephens, 1988). The concentrations of these amino acids are relatively low in natural seawater (Ayukai, unpubl. data).

The appearance of glycine in the ambient seawater shows substantial loss from *A. planci* larvae (Fig. 5-3). Glycine comprises up to 94 % of the intracellular DFAA pool in larvae of echinoderms (Stephens, 1988). Davis and Stephens (1984) observed that the larvae of the echinoid, *Dendraster excentricus*, lost glycine at a rate of 0.1-0.2 pmol larva^{-1} h^{-1} during the uptake of other DFAA. However, Stephens (1988) suggested that damage or death of larvae during incubations may result in liberating DFAA, primarily glycine, into solutions. The loss of glycine could be an artifact of laboratory circumstances.

The potential contribution of alanine uptake to the nutrition of *A. planci* larvae can be estimated by comparing the uptake rate to the instantaneous basic metabolism, measured as the rate of oxygen consumption. The alanine uptake was converted to the oxygen equivalent using the combustion ratio of 3 O^2:1 alanine (Gnaiger, 1983). The oxygen consumption rate of 6 day old larva was assumed to 200 pmol O^2 larva^{-1} h^{-1} (cf. Hoegh-Guldberg, 1993). The alanine uptake at the substrate concentration of 148 nM, 154 nM and 465 nM could account for 13.3 %, 23.1 % and 55.3 % of the basic metabolism, respectively.

Concentrations of DFAA in the pelagic waters normally ranged from a trace to 100 nM (Lee and Bada, 1975, 1977). In the central GBR waters (Magnetic Is.-Coral Sea), DFAA concentrations along a cross-shelf transect ranged from a trace to 174 nM, but they were usually below the limits of detection in samples taken at the shelf- or oceanic waters (Welborn and Hoegh-Guldberg, unpubl., cited in Ayukai and Hoegh-Guldberg, 1992). Ayukai et al. (1995) measured the DFAA concentration along two cross-shelf transects (Cairns and Cooktown) in the northern GBR waters. The mean DFAA concentrations for the two transects were 120 nM (range 64-229 nM) and 125 nM (range 45-230 nM), respectively. The proportion of neutral amino acids in the total DFAA concentration was approximately 70-85 % (Ayukai, pers. comm.). If an *A. planci* larva is assumed to take up all species of neutral amino acids from seawater at the same rate determined for alanine (cf. Manahan, 1989), at the high end of these ambient DFAA concentrations, the uptake of neutral amino acids could account for approximately 12 % of the basic metabolism of the larva (the combustion ratio 1pL O_2:1 pg amino acids mixture, Stephens, 1963).

DFAA do not seem to be the significant food sources for *A. planci* larvae, not only because of the low ambient concentration, but also because of the energy expenditure involved in their uptake. For example, the estimation of the potential energy contribution ignores the energy cost for carrier-mediated transport of amino acids by the epithelium of a larva (Stephens, 1968). In addition, the estimation does not take into account the extent to which amino acids may be incorporated into macromolecules rather than being used for larval metabolism (cf. Strathmann, 1975). Furthermore, amino acids acquired by epidermal uptake may not be translocated to deeper tissues due to the lack of a circulatory system in asteroid larvae and hence amino acids may be oxidised only at the superficial epithelium (Stephens, 1988).
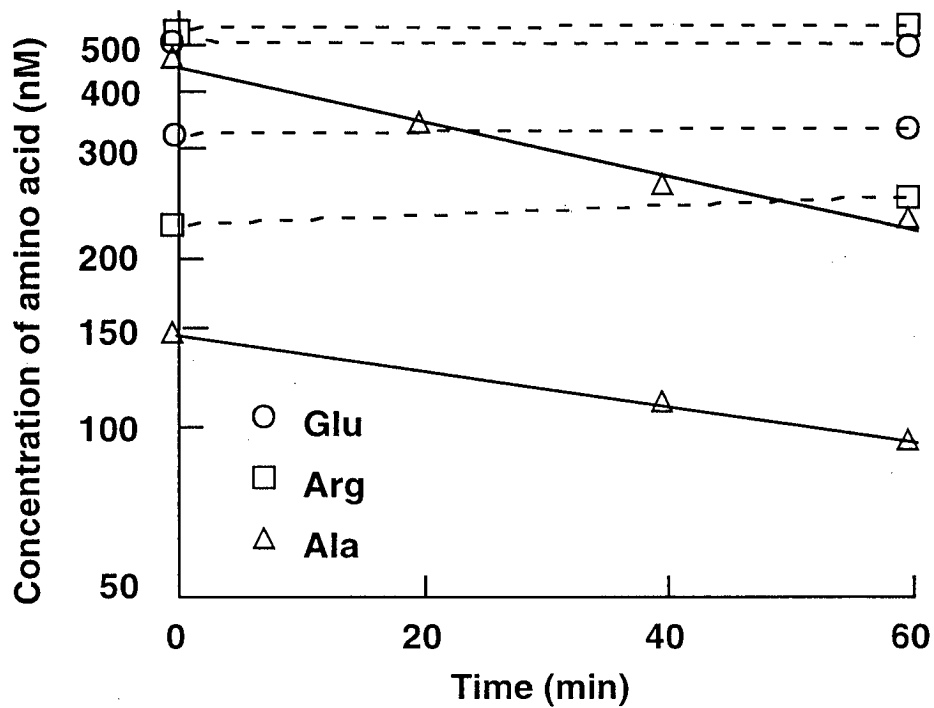
Figure 5-1 Changes in concentration of glutamic acid (Glu), arginine (Arg)
and alanine (Ala) in six solutions in the presence of
Acanthaster planci larvae. The significant changes in the
concentrations of glutamic acid and arginine were not detected.
The concentration of alanine decreased from 465 nM to 239
nM and from 148 nM to 96 nM. Each data point represents the
mean for three subsamples.
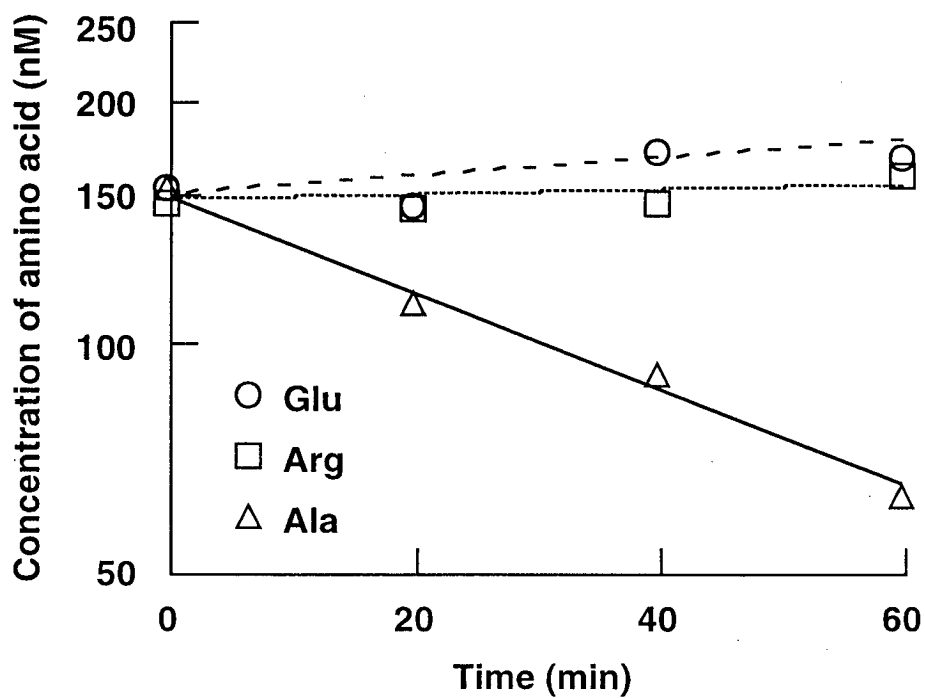
Figure 5-2 Changes in concentration of glutamic acid (Glu), arginine
(Arg) and alanine (Ala) in the equimolar mixture solution in
the presence of *Acanthaster planci* larvae. The initial
concentration of glutamic acid, arginine and alanine was 155
nM, 149 nM and 154 nM, respectively. There were no
depletion in the concentrations of glutamic acid and arginine,
while the concentration of alanine decreased from 154 nM to
63 nM. Each data point represents the mean for three
subsamples.
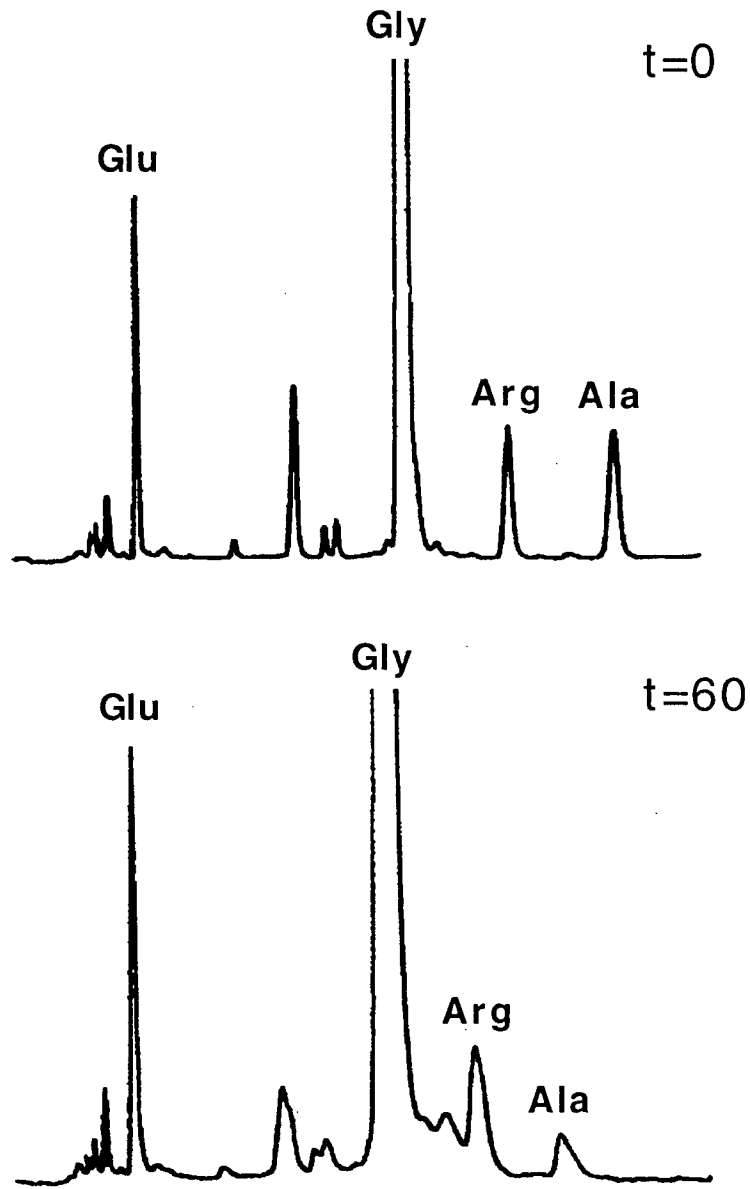
Figure 5-3 HPLC chromatograms showing the concentrations of amino acids at the start (t=0) and the end (t=60) of incubation of *Acanthaster planci* larvae. At t=0, glutamic acid (Glu), arginine (Arg) and alanine (Ala) were presented at 155, 149 and 154 nM. There were no depletion in the concentrations of glutamic acid and arginine, while the concentration of alanine decreased to 63 nM during 60 minutes incubation. Glycine (Gly) was probably released by *A. planci* larvae.

Chapter Six

Effects of different coralline algae on the growth of post-metamorphic *Acanthaster planci*

6.1 Introduction

Previous growth studies of *A. planci* showed that the post-metamorphic (alga-eating) juvenile stage is a period of slow absolute growth (Yamaguchi, 1973, 1974; Lucas, 1984; Zann et al., 1987; Habe et al., 1989). However, because the juveniles grow exponentially, their relative growth rate (instantaneous increment against size) is the largest in their life history (Yamaguchi, 1974, 1975). A small change in growth rate can substantially alter the duration of the post-metamorphic stage and, consequently, affect survivorship and time span to sexual maturity (cf. Yamaguchi, 1975; Lucas, 1984; Keesing and Halford, 1992a; see 1.2.2).

Post-metamorphic *A. planci* have been reported to feed on a wide range of crustose coralline algae (Habe et al., 1989). These coralline algae occur in various coral reef environments, from the reef flat down to 90 m (cf. Gordon et al., 1976; Adey et al., 1982). Their total coverage often exceeds 40 % of bottom substrates, dominating epilithic algal communities (e.g. Littler, 1973; Klumpp and McKinnon, 1989, 1992). The availability of coralline algae for juvenile *A. planci* is thus high. Species composition of coralline algal communities, however, varies depending on depth (Adey et al., 1982; Iryu and Matsuda, 1988). Settlement of *A. planci* larvae is thought to occur in various environments (Habe et al., 1989; Zann et al., 1987; Birkeland and Lucas, 1990; Johnson et al., 1991) and metamorphosed juveniles may thus encounter different types of coralline algae. However, their effects on growth rate are not known.

The aim of this study was to investigate the effect of different dietary coralline algae on the growth of juvenile *A. planci* in the laboratory and in the field. *A. planci* juveniles were reared in the laboratory with three different coralline algae as food and a large number of *A. planci* juveniles were placed in the field to measure the growth rate under natural conditions.

6.2 Materials and methods

6.2.1 Laboratory experiments

A. planci juveniles were obtained from the mass-larval rearing facility in the Australian Institute of Marine Science (Appendix II). About one hundred juveniles of various sizes were measured with an ocular micrometer to record their total diameter (initial size). The juveniles were separated into three size classes according to the number of arms: small (5 arms, 0.8-1.4 mm), medium (6-9 arms, 1.4-2.9 mm) and large (10-12 arms, 3.1-5.1 mm). Three juveniles, one of each size class, were caged with a few pieces of a coralline alga; either *Lithothamnium pseudosorum, Neogoniolithon clavacymosum* or *Lithophyllum insipidium.** Eleven cages were made for each of the three diet treatment groups (i.e. 33 juveniles in each group). The cages were placed in closed-circulation aquaria. All pieces of alga in each cage were checked once every 3-5 days to count surviving juveniles, . Simultaneously, the number of feeding scars on the surface of each piece of alga was counted to determine the feeding rate of juveniles. Seawater temperature was maintained between 26.5 and 28° C. Salinity was adjusted occasionally by adding distilled water to the aquaria. Photoperiod was set to 12 h light and 12 h dark. The surviving juveniles were measured after 36 days rearing period to record their total diameter (final size).

Growth rates of *A. planci* juveniles were calculated as follows:

$$\text{Growth rate} = \frac{\text{Growth increment}}{\text{Final size}}$$

The values were arcsine transformed and the mean for each of the three size classes was compared among diet treatment with One-way ANOVA (Zar, 1984).

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*Coralline algae were collected at various sites on Davies Reef and identified according to the description of Adey et al. (1982). *Lithothamnium pseudosorum* was collected in shaded crevices and in caves in reef bommies (3 - 8 m); *Neogoniolithon clavacymosum* was collected on the leeward reef slope (10 m); and *Lithophyllum insipidium* on the reef flat (1 m). The crusts of these coralline algae were individually examined under a dissecting microscope to remove fouled substrates, epiphytes and potential predators.

6.2.2 Field experiments

A. planci juveniles settled and metamorphosed on the crusts of the coralline alga, *Lithothamnium pseudosorum*, in closed-circulation aquaria of 15 L capacity in the mass-larval rearing facilities (see Appendix I). These juveniles were then reared in aquaria for 10 days (i.e. 0-month old), 2 months or 3 months after metamorphosis. Before experiments, each *Lithothamnium* crust was examined under a stereo microscope to count the number of juveniles. Simultaneously, the total diameters of randomly selected juveniles (N = 30 or 50) were measured with an ocular micrometer. Juveniles attached to *Lithothamnium* crusts were taken to the field in a 8 L plastic containers.

Juveniles were deployed at 10 m depth on the leeward reef slope of Davies Reef in May 1992 (3-month old juveniles), January 1993 (0- and 2-month old) and January 1995 (0-month old). The deployment site and technique were similar to those of Keesing and Halford (1992b). Two or three plastic crates (57 x 32 x 9 cm) were filled with natural coral rubble at the deployment site and brought back to the research vessel. *Lithothamnium* crusts with *A. planci* juveniles were gently placed on the coral rubble in the crates. Each crate was closed with a tight-fitting lid. Then the crates were returned to the site and buried to level its top with the bottom bed. The lids were gently removed about an hour after deployment.

After a period of deployment (32-92 days), the crates were retrieved with surrounding coral rubble to about 1 m^2. All coral rubble was examined by naked eye on the research vessel immediately after retrieval. The rubble was then brought back to the laboratory ashore to re-examine under stereo microscope. Recovered *A. planci* juveniles were measured to record their total diameter.

6.2.3 Estimating growth curves

Growth equations for *A. planci* juveniles in each of three algal diet groups and for juveniles deployed in the field were estimated by solving a difference equation derived from the regression of growth increments on the initial sizes (Yamaguchi, 1977).

When a linear relationship with a positive slope is observed between growth increments per unit time and initial sizes, the regression leads to a difference equation, in a general form such that:

$S_{t+1} - S_t = A\, S_t + B$

or

$S_{t+1} = (1+A)\, S_t + B$ Eq. (1)

where S_t is the initial size and S_{t+1} is the size at one unit time later, and A (> 0) and B are constants determined by the regression equation.

If we assume an exponential growth equation with respect to time such that:

$S_t = pe^{kt} + q$ Eq. (2)

where p, q and k are constants, the size after one unit time is $S_{t+1} = pe^{k(t+1)} + q$. By manipulating this equation, we obtain:

$S_{t+1} = (pe^{kt} + q)\, e^k + q\, (1 - e^k)$

$\qquad = e^k\, S_t + q\, (1 - e^k)$ Eq. (3)

Thus, Eq. (1) and Eq. (3) are equivalent and therefore the constants p and q in Eq. (2) are determined by $e^k = 1 + A$, and $q\, (1 - e^k) = B$ or $q = -B/A$. If the size at time 0 (the size immediately after metamorphosis) is designated as S_0, then the displacement constant in Eq. (2) $p = S_0 - q = S_0 + B/A$.

6.3 Results

6.3.1 The effect of coralline algae on the growth rate of *A. planci* juveniles

The number of surviving *A. planci* juveniles after 36 days rearing on each diet was 15 for *Lithothamnium* (Small 1, Medium 8, Large 6), 28 for *Neogoniolithon* (Small 9. Medium 11, Large 8) and 29 for *Lithophyllum* (Small 9, Medium 13, Large 7), respectively. Table 6-1 presents the ranges of initial size and growth increment, and the mean growth rate of juveniles reared on the three algae. One-way ANOVA for the difference in mean growth rates showed that juveniles reared on *Lithophyllum* grew significantly faster ($P < 0.005$) than those reared on *Neogoniolithon* or *Lithothamnium*. There were no significant differences in the mean growth rates of juveniles between *Neogoniolithon* and *Lithothamnium*.

The mean daily feeding rate on each coralline alga by individual *A. planci* juveniles (initial size range 3.4-5.2 mm) was 0.90 times for *Lithothamnium*, 1.13 for *Neogoniolithon* and 1.19 for *Lithophyllum*, respectively. However, these rates may be underestimated, because the number of feeding scars counted were limited to those with obvious outline. Feeding scars were often fuzzy and hardly distinguished from the background patterns on the surface of algae. Juveniles tended to move little on the algal surface and to feed on areas neighbouring where they fed previously. Due to this behaviour, the outline of feeding scars overlapped and became unclear.

6.3.2 Recovery of *A. planci* juveniles from the field

Table 6-2 summarises the results of the field experiments. For two groups of 0-month old juveniles, 2 out of 200 (1 %) and 108 out of 734 (15 %) individuals were recovered after 49 and 37 days deployment period, respectively. For 2- and 3-month old juveniles, 12 out of 53 (23 %) and 16 out of 150 (11 %) of individuals were recovered after 57 and 92 days deployment period, respectively. The calculated daily loss of juvenile was 9.0 % and 5.1 % for 0-month old juveniles, 2.6 % for 2-month old and 2.4 % for 3-month old, respectively.

Most of 0- and 2-month old *A. planci* juveniles recovered from the field were found attached to coral rubble. Feeding scars by *A. planci* juveniles were evident on the surface of three species of coralline algae, *Neogoniolithon clavacymosum*, *Porolithon onkodes* and *Hydrolithon* sp.

Among 16 juveniles recovered from the group of 3-month old ones, at least four juveniles were likely to be feeding on live corals. There were obvious feeding scars on the surface of juvenile coral colonies of the genera *Pocillopora*, *Seriatopora* and *Stylophora*, where these juveniles were found. The size of these juveniles ranged between 8.5 and 12.6 mm. Other twelve juveniles (7.5-11.8 mm) were found attached to coralline algae together with their feeding scars or on smooth rubble surface encrusted with epiphytes.

6.3.3 Growth curves for *A. planci* juveniles

Growth increments (per 30 days) of *A. planci* juveniles reared on each alga in the laboratory are plotted against their initial diameters in Figure 6-1. There were significant linear relationships between growth increments and initial sizes for each data set ($P < 0.05$, see Fig. 6-1 for regression equations). By extrapolating the arrays of the linear regression equations and the assumed juvenile size immediately after metamorphosis (0.50 mm, Yokochi and Ogura, 1987) to the curve-fitting method, the exponential growth equations were obtained:

Lithothamnium fed group	$D = 1.543 \, e^{\, 0.226t} - 1.043$
Neogoniolithon fed group	$D = 2.929 \, e^{\, 0.263t} - 2.429$
Lithophyllum fed group	$D = 2.666 \, e^{\, 0.449t} - 2.166$

where D is the total diameter (mm) at time t (month). The growth curves are shown in Fig. 6-2. As a means to indicating the substantial differences between these curves, the time spans required for juveniles to attain 8 mm were calculated: 3.0 months for the *Lithophyllum* fed group, 4.8 months for the *Neogoniolithon* fed group and 7.8 months for the *Lithothamnium* fed group.

For the field-deployed juveniles, growth increments were calculated from the mean initial and mean final diameter of deployed or recovered individuals. There was a significant linear relationship between growth increment and mean initial diameter ($P < 0.05$, see Fig. 6-1 for regression equation). The exponential growth equation was obtained:

Field-deployed juveniles	$D = 3.698 \, e^{\, 0.299t} - 3.198$

The growth curve is also shown in Fig. 6-2. The calculated time span required for juveniles to attain 8 mm was 3.7 months.

6.4 Discussion

A. planci juveniles feeding on *Lithophyllum insipidium* grew significantly faster than those feeding on *Neogoniolithon clavacymosum* or *Lithothamnium pseudosorum* (Table 6-1, Fig. 6-2). The basis for the difference in growth rates according to alga species is not clear. It is difficult to suggest that differences in growth rates are related to different feeding rates, because of uncertainty in measuring the feeding rates (see 6.3.1). One possible explanation is that the amounts of nutrients entering the juvenile body is controlled by digestability of each alga. Asteroid juveniles that feed on coralline algae remove the cell contents from the top surface, the epithallus and perithallus, without breaking the cell walls (Barker, 1979). The epithallus of *Lithothamnium* species is finely shielded with the specialised "*Lithothamnium*-type" cover cells, while the epithallus of other two algae is exposed (Adey et al., 1982). The perithallus of *Lithophyllum insipidium* has abundant secondary pit connections (Adey et al., 1982) and the digestive enzyme released by juveniles could readily reach the cell contents.

Consideration of food quality involves not only feeding rate and digestability, but also absorption efficiency and chemical composition of the food (Lawrence and Lane, 1982), for which there are no data here.

The release-recapture technique employed in this study may involve some error. The size of the recovered juveniles could be biased, because smaller juveniles can be more easily overlooked upon recovery. Alternatively, mobile larger juveniles might escape from the plastic boxes more frequently than smaller ones (cf. Keesing and Halford, 1992b). In addition, there is the slight possibility of *A. planci* juveniles naturally recruited into the plastic boxes. However, the estimated growth curve for *A. planci* juveniles in the field was similar to the growth rates reported in the previous studies. Yamaguchi (1973) reported that the diameter of *A. planci* juveniles increased exponentially from 0.5 mm to 11.1 mm in about 4.7 months; Lucas (1975) reported animals growing from 0.7 mm to ca. 5 mm about 3 months; while Habe et al. (1989) showed that the diameter of naturally-occurring juveniles increased from 4.3 mm to 6.6 mm in about one month. From the growth equation for the field-deployed juveniles, the calculated time spans required to grow from 0.5 to 11.1 mm, 0.7 to 5 mm and from 4.3 to 6.6 mm are 4.6, 2.7 and 0.9 months, respectively.

The effects of different coralline algae on the growth of *A. planci* juveniles are probably not evident in shallow water, because natural diets of juveniles appear to consist of a wide

variety of algae. Coralline algal communities are diverse and show a mosaic distribution over a centimetre scale in shaded microhabitats (Iryu and Matsuda, 1988), where naturally-recruited *A. planci* juvenile possibly occur (Yokochi and Ogura, 1987). Field observations made in this study, that *A. planci* juveniles fed on several species of coralline algae, agree with previous field and laboratory observations. Habe et al. (1989) reported that *A. planci* juveniles on reef slope fed on eleven species of coralline algae in the genera *Lithothamnium, Mesophyllum, Hydrolithon, Lithophyllum, Titanoderma* and *Amphiroa*. Yamaguchi (1973, pers. comm.), who reared juveniles with various bottom substrates, found that juveniles fed not only on coralline algae, but also on turf algae and benthic diatoms. The fact that the estimated growth rate of the field-deployed juveniles was similar to those obtained by Yamaguchi (1973) and Lucas (1975) in the laboratory and Habe et al. (1989) in the field indicates that growth rate of juveniles feeding on multi-specific natural foods does not differ much, irrespective of habitat in reef environments.

In the Great Barrier Reef, mass-recruitment of *A. planci* is thought to occur in deep water (Black and Moran, 1991; Johnson et al., 1991). This may be important due to the relatively slow growth rate of *A. planci* juveniles feeding on *Lithothamnium pseudosorum* (Fig. 6-2). Species diversity of coralline algal communities decreases with depth (Iryu and Matsuda, 1988). At depths greater than 30 m, *Lithothamnium pseudosorum* and *Lithothamnium* spp. become more abundant and often dominate the bottom beds in rhodolith form (Adey et al., 1982). *A. planci* juveniles recruiting in deep water are more likely to encounter *L. pseudosorum* and related species as their initial diet. Furthermore, *A. planci* larvae have a strong preference for settlement and metamorphosis on the surface of *L. pseudosorum* (Johnson et al., 1991). This is an interesting observation considering that *Lithothamnium pseudosorum* is not the optimal diet (cf. Birkeland and Lucas, 1990).

Habe et al. (1989) reported that *A. planci* juveniles in Okinawa switched their diet from algae to corals at 15 and 25 mm diameter. In this study, the size range of *A. planci* juveniles that were probably feeding on corals was between 8.5 and 12.6 mm (see 6.3.2). It is worth noting that feeding scars were left only on the colonies of Pocilloporidae corals (*Pocillopora* sp., *Seriatopora* sp. and *Stylophora* sp.). Habe et al. (1989) also reported that juveniles in an aquarium preferred *Pocillopora damicornis* when they were given access to several species of corals, including *Acropora* spp. Keesing and Halford (1992a) reported that the Pocilloporidae corals sustain high growth rates. Yamaguchi (1974) found that *A. planci* juveniles had to consume twice as much coral tissue of *Acropora nasuta* as of *Pocillopora damicornis* to gain the same amount of

weight. Small *A. planci* juveniles may be killed by the mesenteric filaments of *Acropora* sp., but not by *Pocillopora damicornis* (Yamaguchi, 1973; Lucas, 1984). Feeding on Pocilloporidae corals is advantageous for juveniles in acquiring nutrients with fewer hazards.

This study highlighted the influence of coralline algae on the growth rates and potential survivorship of *A. planci*: a poor diet will lead to a prolonged period of life at a small size and greater mortality (Birkeland, 1974; Keesing and Halford, 1992a; see 1.2.2). The estimated growth rate of *A. planci* juveniles in the field was relatively high and was similar to the previous growth data obtained with juveniles fed sufficient amount of foods in the laboratory or juveniles feeding on natural foods. Because a wide range of coralline algae is accessible to *A. planci* juveniles, food quality probably have little influence on growth rate and hence survivorship in relatively shallow reef environments. Dominance of poor diet may have a significant effect in reducing growth rate of juveniles in deep water due to the more limited range of coralline algae and this needs further investigation.

Table 6-1 The ranges of initial size and growth increment and the growth rate of *Acanthaster planci* juveniles of three size classes (Small, 5 arms; Medium, 6-9 arms; Large, 10-12 arms) reared in the laboratory on three dietary coralline algae, *Lithothamnium pseudosorum* (L.p.), *Neogoniolithon clavacymosum* (N.c.) and *Lithophyllum insipidium* (L.i.). Growth rates are mean $\pm$ 1 S.D. with ranges in parentheses.

Coralline alga	Size class	Initial size range (mm)	Growth increment range (mm)	Growth rate*
L.p.	S	1.4	0.6	0.32
	M	1.6 - 2.5	0.2 - 2.1	0.30 $\pm$ 0.13 (0.09-0.46)
	L	3.2 - 4.3	0.9 - 1.8	0.27 $\pm$ 0.05 (0.19-0.33)
N.c.	S	0.9 - 1.3	0.7 - 1.9	0.52 $\pm$ 0.09 (0.39-0.56)
	M	2.0 - 2.9	0.6 - 3.9	0.46 $\pm$ 0.13 (0.23-0.63)
	L	3.4 - 5.1	1.1 - 4.9	0.35 $\pm$ 0.11 (0.21-0.37)
L.i.	S	0.8 - 1.4	1.6 - 2.6	0.64 $\pm$ 0.03 (0.57-0.66)
	M	1.4 - 2.9	2.3 - 3.8	0.59 $\pm$ 0.06 (0.50-0.69)
	L	3.1 - 4.3	2.9 - 4.5	0.51 $\pm$ 0.05 (0.42-0.59)

*One-way ANOVA for the difference between mean growth rates for three size classes

Small   L.p. - N.c. : not analysed
N.c. - L.i. : $F = 15.4210 > F_{crit} \approx 4.4940$; $P < 0.005$

Medium   L.p. - N.c. : $F = 2.7740 < F_{crit} \approx 4.4513$; $P > 0.1$
N.c. - L.i. : $F = 22.1900 > F_{crit} \approx 4.3009$; $P < 0.0005$

Large   L.p. - N.c. : $F = 0.4892 < F_{crit} \approx 4.6672$; $P > 0.4$
N.c. - L.i. : $F = 11.6454 > F_{crit} \approx 4.6001$; $P < 0.005$

Table 6-2   Summary of the field experiments. The mean initial sizes, final sizes and the calculated growth increments per 30 days of four broods of *Acanthaster planci* juveniles and periods of deployment, the number of juveniles deployed and recovered are also presented. Mean values are presented with 1 S.D. and ranges in parentheses.

	No. deployed	Initial size (mm)	Deployment period (days)	No. recovered	Final size (mm)	Growth increment (mm mo^{-1})
0-month						
	200	0.6 ± 0.1 (0.5 - 0.7)	49	2	2.8 (2.5 and 3.0)	1.3
	734	0.7 ± 0.2 (0.4 - 1.0)	37	108	2.4 ± 0.5 (1.1 - 3.4)	1.4
2-month						
	53	1.2 ± 0.3 (0.9 - 2.0)**	57	12	4.2 ± 0.8 (3.4 - 5.6)	1.6
3-month						
	150	3.2 ± 0.2 (2.8 - 3.5)	92	16	10.0 ± 1.7 (7.5 - 12.6)	2.2

* :   Months after metamorphosis
** : Mean for 30 individuals, others are for 50 individuals
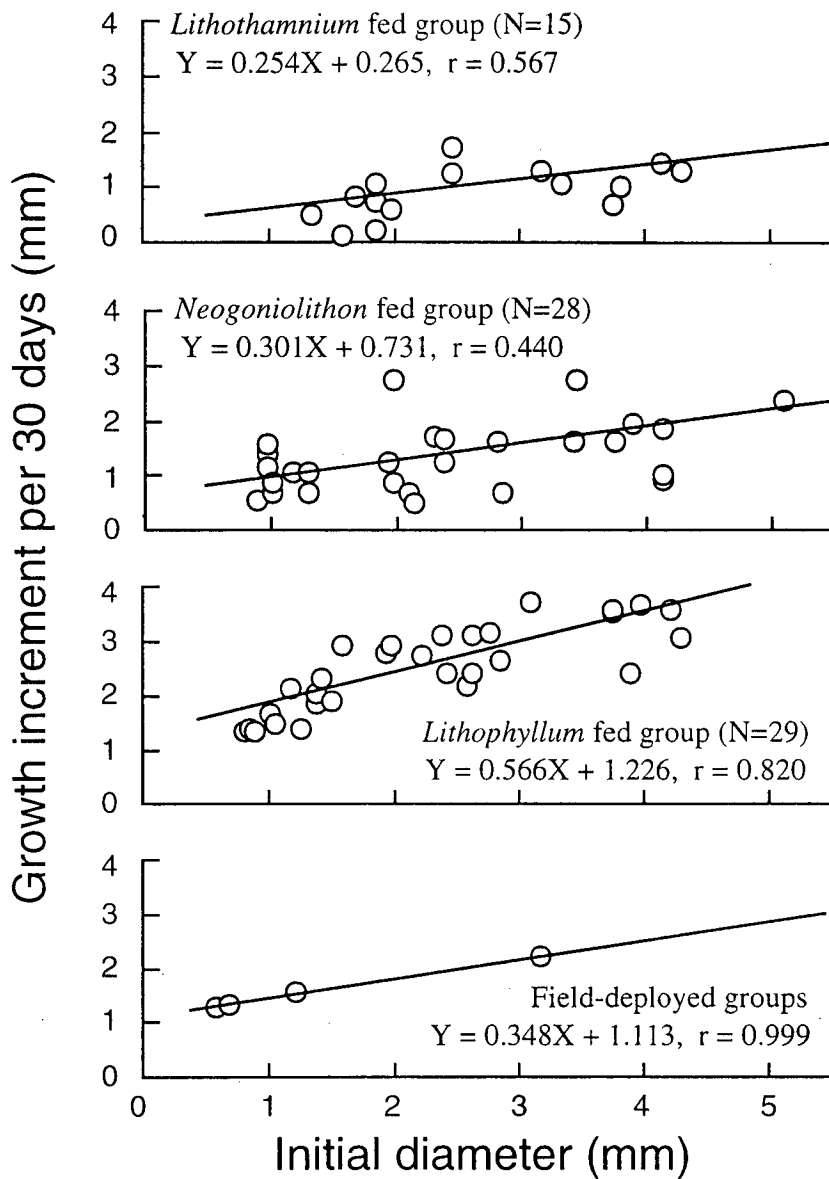
Figure 6-1 Growth increments per 30 days of *Acanthaster planci* juveniles
plotted against initial sizes. The growth data were obtained
from either individual juveniles reared in the laboratory with
three coralline algae (*Lithothamnium pseudosorum*,
Neogoniolithon clavacymosum and *Lithophyllum insipidium*)
or groups of juvenile deployed in the field. There was a
significant linear relationship between growth increments and
initial sizes in each of four data sets (P < 0.05).
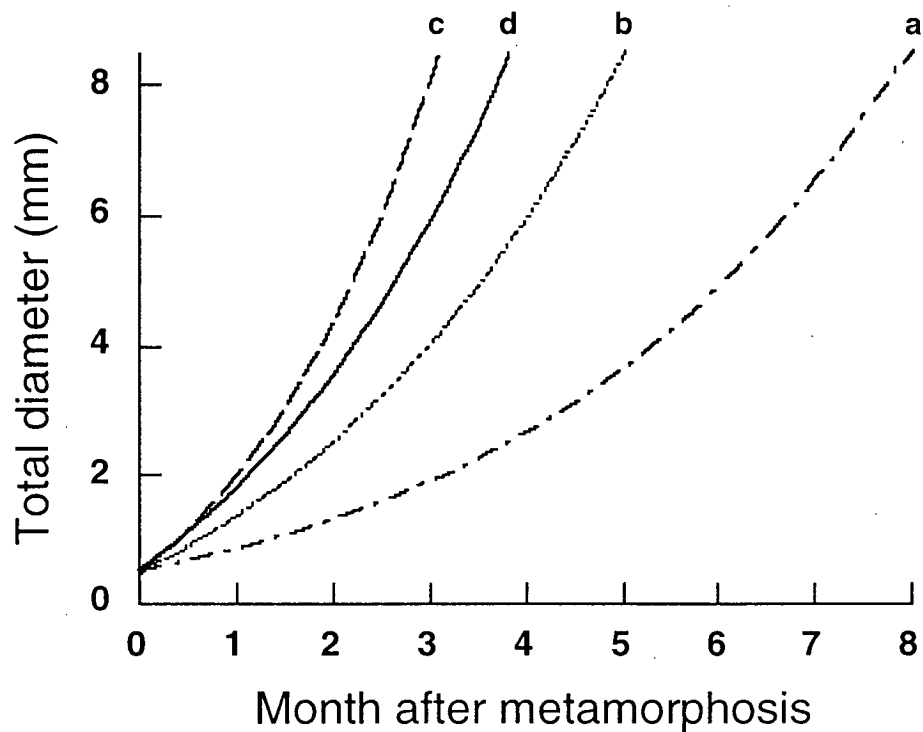
Figure 6-2 Fitted growth curves for *Acanthaster planci* juveniles reared in the laboratory with three different coralline algae and for juveniles deployed in the field. The growth equation for each group is:

$D = 1.543\ e^{0.226t} - 1.043$ for *Lithothamnium* fed group (a)

$D = 2.929\ e^{0.263t} - 2.429$ for *Neogoniolithon* fed group (b)

$D = 2.666\ e^{0.449t} - 2.166$ for *Lithophyllum* fed group (c)

$D = 3.698\ e^{0.299t} - 3.198$ for the field-deployed juveniles (d)

where D is the total diameter (mm) at time t (month after metamorphosis). The assumed size of juveniles immediately after metamorphosis (t=0) is 0.50 mm (Yokochi and Ogura, 1987).

Chapter Seven

General discussion

The aim of this chapter is to evaluate the importance of food limitation on the growth, development and survival of larval and juvenile *A. planci*, based on the results of this study and data on the availability of their food sources in GBR waters.

7.1 Food limitation in *Acanthaster planci* larvae

7.1.1 Potential food sources

Suspended particles

A. planci larvae ingest suspended particles of various sizes, ranging between 1 and 60 μm in diameter (Yamaguchi, 1973; Lucas, 1982; Uchida and Nomura, 1987; Dixon, unpubl. in Birkeland and Lucas, 1990; Ayukai, 1994; this study). Within this size range, relatively large phytoplankton (> 5 μm) such as dinoflagellates and pennate diatoms are undoubtedly utilisable foods for the growth and development of larvae, as shown by the previous and current larval rearing studies (Lucas, 1982; Uchida and Nomura, 1987; this study). The lower ends of the range, ultraplankton (< 5 μm), include two size categories, nanoplankton (> 2 μm) and picoplankton (< 2 μm). Flagellates are the majority of nanoplankton and potentially utilisable foods for larvae (Ayukai, 1994). Cyanobacteria are the dominant component of picoplankton (e.g. Stockner, 1988; Furnas and Mitchell, 1986), but their actual utilisation by larvae had not been fully understood. This was crucial in identifying the major foods for larvae, considering the large biomass of cyanobacteria in their putative habitats (see 4.1).

This study showed the very limited ability of *A. planci* larvae to feed on cyanobacteria. The clearance rates of larvae feeding on cyanobacteria were magnitudes lower that the rate for nanoplanktonic eukaryotes, even under conditions where cyanobacteria were overwhelmingly dominant (Table 4-1 in Chapter 4). This size selective feeding indicates that larvae are not only unable to utilise cyanobacteria efficiently, but also other picoplankton components. In fact, larvae consistently failed to develop in 2 μm filtered seawater (Fig. 3-1, 3-3, 3-5 in Chapter 3; see 3.4). These results show that potential particulate food sources for *A. planci* larvae are phytoplankton in a fraction > 2 μm, i.e. nanoplankton.

Total carbon quantity of particulate organic matter (POM) in the ocean is at least one order of magnitude greater than those of plankton (Cauwet, 1978). In coral reef waters, mucus aggregates produced by reef-building corals can be a dominant component of POM (Ducklow and Mitchell, 1979). Additional input of POM to coral reef waters occurs from algal detritus, such as fragments of epilithic turf algae (Gerber and Marshall, 1974; Hatcher, 1983). POM is usually associated with the substantial numbers of bacteria, which may account for up to 50 % of total bacterial biomass in coral reef waters (Sorokin, 1974; Moriarty, 1979).

POM has been studied as an alternative food source for marine herbivorous copepods (e.g. Roman et al., 1990), although nothing was known about its nutritional values (Poulet, 1983). Copepods are able to ingest and assimilate various kinds of POM, including freshly dead and decomposed phytoplankton, algal detritus (Poulet, 1983) and coral mucus (Gottfried and Roman, 1983). However, the presence of these non-living materials often disrupts saturation of feeding rates of the animals on living phytoplankton (Paffenhöfer and Strickland, 1970; Roman, 1984; Paffenhöfer and Van Sant, 1985). Ayukai (1987) reported that the defaecation rate of a copepod was greatly reduced when POM became a dominant component of suspended particles. These observations suggest that POM is not readily utilisable for copepods, despite its large quantity in seawater.

A. planci larvae certainly encounter POM and its associated bacteria during the planktonic period and ingest them, as other echinoderm larvae do (Strathmann, 1971). POM was not analysed in many studies in which *A. planci* larvae were reared using seawater from various sources. However, there was no suggestion of a major factor in addition to phytoplankton that was influencing larval development and survival.

Dissolved organic matter (DOM)

In common with other soft-bodied invertebrate larvae (Manahan, 1990), *A. planci* larvae have the ability to take up a wide variety of DOM (see 5.1). The transepidermal uptake of the major utilisable DOM species, i.e. DFAA (alanine, leucine and histidine) and a sugar (glucose), by *A. planci* larvae has been particularly studied (Hoegh-Guldberg, 1993, 1994; this study, see Chapter 5). The estimated kinetic parameters (the maximum influx and a half saturation constant) suggested that, while a sugar seems unimportant, DFAA potentially provide a significant amount of energy to the larvae at a substrate concentration above 1 µM (Hoegh-Guldberg, 1994). This is definitely true, as larvae developed at significantly faster rates in seawater enriched with DFAA mixtures (>5 µM) than in

untreated seawater (Fig. 3-1, Table 3-1 in Chapter 3). However, this study showed that the capability of larvae to take up an acidic and a basic amino acid is very limited (Fig. 5-1, 5-2 in Chapter 5). It is probable that neutral DFAA are a major component of utilisable DOM for *A. planci* larvae.

7.1.2 The relative importance of phytoplankton and neutral DFAA in the larval nutrition

Table 7-1 presents the potential contributions of nanoplankton and neutral DFAA to the minimum carbon requirement of bipinnaria larvae of *A. planci* in GBR waters. The reported ranges of concentrations of total chlorophyll *a* and neutral DFAA in GBR waters are 0.09-3.50 μg L^{-1} (Ikeda et al., 1980; Bellamy et al., 1982; Furnas and Mitchell, 1984; see Table 7-2) and 0-196 nM (Welborn and Manahan, unpubl., cited in Ayukai and Hoegh-Guldberg, 1992; Ayukai, unpubl. data, see 5.4). The rate of carbon intake was calculated based on the estimated rates of nanoplankton clearance and DFAA uptake obtained in this study (Chapters 4 and 5). The minimum carbon requirement of bipinnaria larvae of *A. planci* was estimated from their rate of oxygen consumption (cited in Ayukai, 1993).

It should be noted that the estimates involve many assumptions (see Table 7-1). For example, *A. planci* larvae are assumed to assimilate nanoplankton at a similar rate as that determined for mollusc larvae (Bayne, 1983), which probably have similar digestive processes to echinoderm larvae (cf. Strathmann, 1987). In addition, *A. planci* larvae are assumed to take up all species of neutral amino acids at the same rate determined for alanine. However, uptake of one amino acid usually inhibits uptake of other amino acids in the same charged group (Antia et al., 1991). In fact, Hoegh-Guldberg (1994) reported that the transport rate of leucine by *A. planci* larvae was slightly lower than alanine. Furthermore, it is dubious that amino acids acquired by epidermal uptake are 100 % oxidised and used for metabolism (Stephens, 1988; see 5.4). Thus, these estimates do not represent the net energy gain, but do suggest that *A. planci* larvae depend for the major part of their nutrition on nanoplankton in the field.

7.1.3 The critical range of phytoplankton (chlorophyll *a*) concentration

It is now seems appropriate to use chlorophyll *a* concentration as an index of food availability for *A. planci* larvae. Chlorophyll *a* concentration in the >2 μm fraction (i.e. nanoplankton) provides much information on food availability, but the temporal and spatial variability of phytoplankton communities in the GBR are still only investigated to a

preliminary extent (cf. Ayukai, 1992). Alternatively, total chlorophyll a concentration may be used, because more data sets for total chlorophyll a are available in GBR waters.

This study has proposed the critical range for $A.$ $planci$ larvae of 0.5-0.8 μg L^{-1} total chlorophyll a (Chapter 3). It has to be emphasised that, within this range, at least some level of larval survival may be supported. The problem is whether these values estimated under the laboratory conditions in this study can be directly extrapolated to the field data.

Food availability for larvae in culture containers was probably greater than that in ambient seawater having the same chlorophyll a concentration. According to the record of daily chlorophyll a measurements for nutrient enriched seawater, the average contribution of picoplankton (<2 μm) to the total phytoplankton biomass ranged from 6 to 64 % with the mean of 38 %. These values were presumably reflective of phytoplankton communities in the culture containers, as nutrient enriched seawater was merely diluted with filtered seawater to achieve appropriate chlorophyll a levels. Typically, the picoplankton contribution in GBR waters is about 50 % (cf. Furnas and Mitchell, 1986; see Table 7-2). The proportion of utilisable nanoplankton (>2 μm) in the culture containers was thus likely to be greater than in the ambient environments that $A.$ $planci$ larvae usually encounter. Also, concentrations of DOM in culture containers were probably elevated by breakdown of algal cells, algal excretions and seawater treatments (see 3.4). Particularly, neutral DFAA might have served as additional foods for larvae. Therefore, the proposed critical range should be regarded as conservative values.

There are obvious discrepancies in results regarding compensating and critical food concentrations for $A.$ $planci$ larvae between this study and previous studies (Lucas, 1982; Ayukai, 1993). Given the different methods employed in these studies, it is necessary to clarify how such discrepancies arose.

Lucas (1982) used cultured microalgae to make accurate quantification of the minimum food concentration required for $A.$ $planci$ larvae. In his experiments, a change from no survival to moderate survival through larval development occurred between 0.4 and 1.0 μg L^{-1} chlorophyll a. The low end of the range, i.e. the compensating concentration, is in good agreement with the results of this study. In contrast, it was observed in this study that larval development and survival reached an optimal level at about 0.8 μg L^{-1}. This difference is explained by the effectiveness of multi-specific natural foods on larval development (Mortensen, 1938; Vance, unpubl., both cited in Strathmann, 1975; see 3.4).

Further explanation is that A. *planci* larvae improve their efficiency of capturing nutritional particles by chemosensory feeding (see 4.4). Chemical properties of particles could provide specific information on nutritional quality to suspension feeders (Poulet and Marsot, 1978). Selective ingestion of particles flavoured with algal exudates by echinoid larvae has been reported (Rassoulzadegan et al., 1984; Appelmans, 1994). If larvae are given choice, such particle selection by chemical cues may increase the proportion of nutritious particles among all particles ingested and therefore enhance efficiency in acquiring nutrients (Appelmans, 1994). If A. *planci* larvae are able to take the advantage of the chemosensory feeding, their nutritional intake may be greater in multi-specific food environments than in mono-specific food environments.

Ayukai (1993) estimated a food concentration below which A. *planci* larvae are unable to compensate their basic metabolism from feeding. He used data on the rates of particle clearance and oxygen consumption. The compensating concentrations of phytoplankton for bipinnaria and brachiolaria larvae were about 1.3 μg L^{-1} and 2.7 μg L^{-1}, respectively. Clearly, these values do not reflect the observations of this study. Estimating compensating food concentrations is a basic approach in seeking to relate the feeding studies of larvae to food conditions in the field (e.g. Strathmann, 1975). Workers can obtain some clues on food availability to the larvae, but there are many restrictions in evaluating the extent of food limitation throughout larval periods with this approach. For instance, the estimation does not take into account the possible contribution of endogenous nutrients to the basic metabolism (cf. Fenaux, 1982). In addition, the estimation involves a number of assumption that are difficult to validate. Larvae are assumed to feed and assimilate natural phytoplankton at the same rates measured with cultured microalgae, but this assumption may not be valid for all types of phytoplankton as feeding rates of larvae vary with different food species (Strathmann, 1971).

7.1.4 Availability of phytoplankton in GBR waters

Data on phytoplankton (chlorophyll *a*) concentration in GBR waters are available from three oceanographic surveys conducted from 1975 to 1983 (Ikeda et al., 1980; Bellamy et al., 1982; Furnas and Mitchell, 1984), and unpublished data of Ayukai.

Table 7-2 presents the average and range of chlorophyll *a* concentration during the spawning season of A. *planci* (October - January, cf. Babcock and Mundy, 1993) in the central GBR waters. The data of Furnas and Mitchell (1984) included size-fractioned chlorophyll concentrations (>2 μm and <2 μm), showing that approximately half (41-67

%) of the total phytoplankton biomass consisted of picoplankton in shelf-waters. The mean chlorophyll a concentrations in the mid- and outer-shelf waters (0.09-0.46 µg L^{-1}) were usually below the suggested compensating level for $A.$ $planci$ larvae, but high ends of the range (ca. 0.5-0.6 µg L^{-1}) often extended to the level, at which larval survival may be supported. It is noteworthy that the chlorophyll a concentration in the GBR lagoon (0.56 and 0.64 µg L^{-1}) was within the critical range.

Ayukai (unpubl.) measured chlorophyll a concentration along five cross-shelf transects in the northern GBR area (Cairns - Cooktown) during the spawning season of $A.$ $planci$ from 1992 to 1995. Figure 7-1 shows the cross-shelf variation in chlorophyll concentrations for five transects. There was a tendency of decline from the lagoon to outer-shelf waters. The mean chlorophyll a concentration for the pooled data (N=387) was 0.36 µg L^{-1}. Chlorophyll a concentrations were mostly below 0.5 µg L^{-1}, but many values were in the critical range.

7.1.5 The importance of food limitation in $A.$ $planci$ larvae in GBR waters

The mean chlorophyll a concentrations in GBR waters are below or marginal to the critical range. This implies that $A.$ $planci$ larvae are always exposed to the risk of death from starvation. However, chlorophyll a concentration occasionally increases to the level at which larvae are able to survive for some time or to grow and develop moderately. Nothing is known of how long such conditions can persist, but it is suggested that $A.$ $planci$ larvae do not starve to an extreme extent. This study supports the basic concept of the larval starvation hypothesis (Lucas, 1982), that food availability is a major factor affecting development and survival of $A.$ $planci$ larvae in GBR waters, and advocates the importance of understanding the patterns of temporal and spatial variation in phytoplankton community structure and biomass.

The direct effect of food limitation in $A.$ $planci$ larvae reared in freshly-collected and coarse filtered seawater was apparent in batches of larvae that did not survive (25 µm FSW treatment, Fig. 3-1, 3-3 in Chapter 3). The indirect effect was extending larval periods (Fig. 3-3, 3-5). The difference in time to metamorphosis between unfiltered seawater treatments and enriched food treatments was 2-4 days. An instantaneous mortality rate of echinoderm larvae having a similar planktonic period (11-17 days, for the pluteus of $Strongylocentrotus$ $droebachiensis$ and $S.$ $purpuratus$) to $A.$ $planci$ larvae is in the range between 0.0625 and 0.2658 day^{-1} (Rumrill, 1990). With this mortality rate, 18-40 % of the remaining larvae may be lost during a 2 days extension to the larval

periods; 28-79 % of the remaining larvae may be lost during a 4 days extension. These estimates, however, are possibly rather conservative, because the mortality rate may not apply for coral reef environments, where a mass mortality of incoming plankton communities due to predation has been reported (Hamner et al., 1988). Several days of extension to larval periods can potentially cause several orders of magnitude variation in number of metamorphosed juveniles.

Food limitation may also affect survivorship in the post-metamorphic stage. In the larval rearing experiments, two batches of larvae were able to develop rapidly through to metamorphosis in coarse filtered seawater having similar chlorophyll a concentrations to the ambient level (range 0.16-0.50 μg L^{-1}, Batches 5, see Table 3-6 in Chapter 3). Although the development rates of the two batches of larvae were significantly slower than those reared under optimal food supplies (Table 3-3 in Chapter 3, see 3.4), the delay in time to settlement competency was only 2 days, which may lead one to suggest that the difference is not large (Olson, 1987). However, an important clue to the effect of food limitation appeared as size of metamorphosed juveniles (see 3.4). Small sized juveniles from these larvae is likely to be a consequence of limited nutrient supplies throughout the larval periods. The size of juveniles of the asteroid *Asterina miniata* was strongly affected by the level of larval nutrition (Basch, 1992a, b), but not by temporary starvation (Allison, 1994). Metamorphosis into small juveniles could lead to slow initial growth and hence an increase in mortality due to predation (Andrew and Choat, 1982; Emlet, 1986; cf. Keesing and Halford, 1992a, b; see 1.2.3).

Several factors are considered to be responsible for fluctuations of phytoplankton biomass in GBR waters. Terrestrial runoff is the major factor (Revelante and Gilmartin, 1982). Intrusion of sub-surface water from the Coral Sea and cyclone disturbance (rainfall, runoff and resuspending sediments) can also cause a large increase in plankton productivity over a wide area of GBR waters (Furnas and Mitchell, 1986; Furnas, 1989). These events are conspicuous, but there are more common, small scale events, including nanoplankton blooming inside semi-enclosed reef lagoons (Furnas et al., 1990).

Monitoring these events that cause fluctuations in phytoplankton biomass during the spawning season of *A. planci* is vital. Food limitation in larvae may be relaxed by a slight increase in chlorophyll a concentration, 0.3 μg L^{-1} or less, which is occasionally observed within a daily fluctuation (Ayukai, 1992). The possible link between nutrient-rich river runoff and outbreaks of *A. planci* through enhanced larval survival has received attention not only in GBR waters (Brodie, 1992), but also in Western Pacific (the

terrestrial runoff hypothesis, Birkeland, 1982). A proven link between these phenomena still seem to be missing; however, this study strongly supports further field research into the events associated with fluctuation in phytoplankton biomass and their effect on the nutrition and survival of *A. planci* larvae.

7.2 Food limitation in post-metamorphic *A. planci*

In the GBR communities the total coverage of coralline algae often dominates over epilithic algal communities (Klumpp and McKinnon, 1989, 1992). Their quantitative availability to *A. planci* juveniles is apparently high. This study revealed that quality of dietary coralline algae influenced the growth of *A. planci* juveniles. In shallow waters, however, the effect of algal food is probably not significant, because of the diverse nature of coralline algal communities in reef environments (Iryu and Matsuda, 1988). The growth rates of *A. planci* juveniles measured in the field in this study were relatively high and similar to those obtained previously with juveniles reared on natural substrates in the laboratory or naturally-occurring juveniles (Yamaguchi, 1973; Habe et al., 1989), suggesting that the ability of natural foods to sustain growth of juveniles is not substantially different among reef environment. It is suggested that food is not an important factor limiting growth and survivorship in shallow reef environments.

A. planci larvae settling in deep waters are more likely to encounter *Lithothamnium pseudosorum* or other *Lithothamnium* spp. as dominant coralline algae. These are unlikely to sustain rapid growth of juveniles. It is speculated that the growth rates of juveniles which metamorphosed on the surface of these algae will be limited and hence suffer the high predation pressure of small size for longer periods.

Table 7-1 Potential contribution of phytoplankton and neutral DFAA to the minimum carbon requirements of the bipinnaria larva of *Acanthaster planci* in GBR waters.

Food source	Food concentration[a]	Carbon intake rate[b] (ngC larva^{-1}h^{-1})	Percent contribution to metabolic requirements[c]
Phytoplankton	2.7 - 105.0	0.11 - 4.20	4.8 - 183.0
Neutral DFAA	trace - 196	ca. 0 - 0.27	0 - 11.8

a: Phytoplankton: μgC L^{-1} (carbon / chlorophyll *a* ratio of 30, Parsons et al., 1984)
   DFAA: nM

b: Assumptions made to calculate the carbon intake rate.

The percent contribution of picoplankton to the total phytoplankton carbon concentration (P_{total}, ngC mL^{-1}) is 50 % (A) (see Table 7-2).

The clearance rates of bipinnaria larvae feeding on naturally-occurring nanoplankton is 0.2 mL larva^{-1} h^{-1} (B) (this study, see Table 4-1 in Chapter 4).

The assimilation efficiency of invertebrate larvae feeding on nanoplankton is 40 % (C) (Bayne, 1983).

Carbon intake rate = P_{total} X A/100 X B X 40/100.

A. planci larvae take up all species of neutral amino acids at the rate determined for alanine (cf. Manahan, 1989; see 5.4).

Mean molecular weight of neutral amino acids is 141.87.

Combustion ratio of 1 pg amino acids = 1 pL O2 (Stephens, 1963).

c: Minimum amount of carbon required for compensating oxygen consumption (2.29 ngC larva^{-1} h^{-1}, cited in Ayukai, 1993).

Table 7-2 Summary of the available data on the chlorophyll a concentration (μg L^{-1}) in three areas of the central GBR (the GBR lagoon, mid- and outer-shelf waters) during the spawning season of *Acanthaster planci* (October - January). Values for chlorphyll a concentration are means with ranges in parentheses. Percentages in parentheses following the ranges indicate the contribution of picoplankton (< 2 μm) to the total chlorophyll a concentration. Sources of data are: (1) Ikeda et al. (1980); (2) Bellamy et al. (1982); (3) Furnas and Mitchell (1984).

Year	Depth	GBR Lagoon	Mid-shelf waters	Outer-shelf waters	Source
1975 - 1978	Surface (0 m)	0.64 (0.11 - 2.75)	0.36 (0.11 - 1.30)		(1)
	Sub-surface (7 m)	0.56 (0.10 - 3.50)			
1980 - 1981	Surface (0 m)		0.22 (0.12 - 0.64)	0.13 (0.01 - 0.57)	(2)
	Sub-surface (10 m)		0.22 (0.12 - 0.58)	0.14 (0.04 - 0.55)	
	Bottom (50 m or bottom)		0.41 (0.17 - 0.64)	0.30 (0.01 - 2.06)	
1983	Surface (0 m)		0.23 (0.06 - 0.53) (50 %)	0.11 (0.06 - 0.24) (59 %)	(3)
	Sub-surface (10 or 25 m)		0.23 (0.07 - 0.47) (52 %)	0.09 (0.06 - 0.15) (67 %)	
	Bottom (50 m or bottom)		0.46 (0.19 - 1.61) (41 %)	0.25 (0.09 - 0.54) (58 %)	
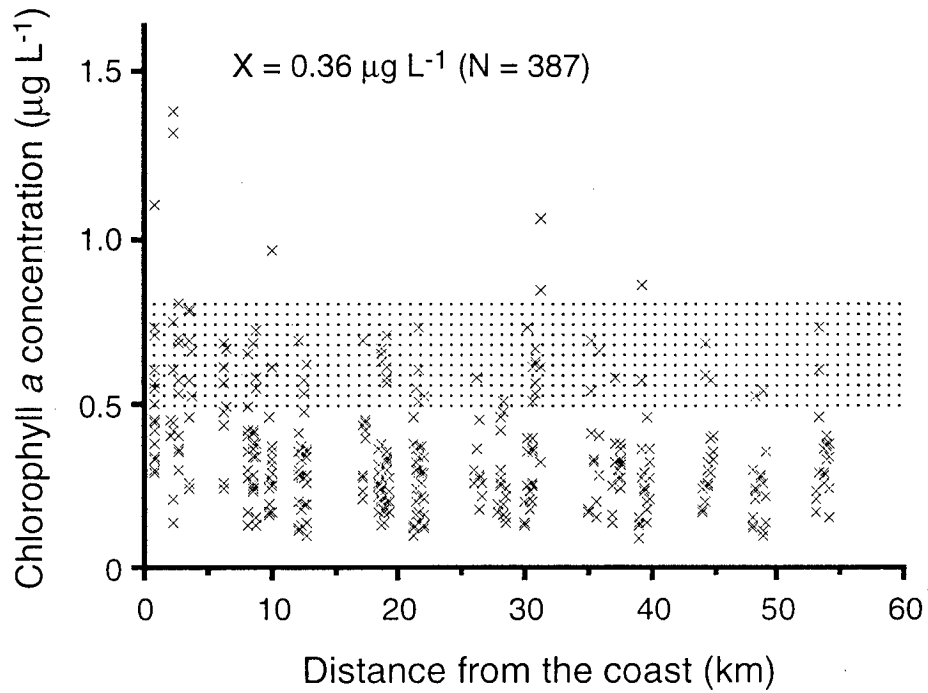
Figure 7-1 Cross-shelf variation in chlorophyll *a* concentration in the northern GBR area (Cairns - Cooktown) during the spawning season of *Acanthaster planci* from 1992 to 1995. The average for the pooled data (N = 387) is 0.36 μg L⁻¹. Hatched area indicates the critical range for *A. planci* larvae (0.5 - 0.8 μg L⁻¹). Data from Ayukai (unpubl.).

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Appendix I

Method of mass-larval rearing of *A. planci*

A large number of larvae and juveniles of *A. planci* were obtained from the mass-larval rearing facility in the Australian Institute of Marine Science from 1991 to 1995. The methods of larval rearing presented here are essentially similar to those described by Birkeland and Lucas (1990).

Adult individuals of *A. planci* (> 30 cm in diameter) were collected mostly at Davies Reef in the period between October and December. Individual starfish was sexed by biopsy after collection. Male and female starfish were kept separately in open-circulation aquaria of 1,000 L capacity.

Mature oocytes and spermatozoa were collected from a pair of adult starfish by injecting 10-15 mL of a 1×10^{-3} M 1-methyladenine solution. Spawned oocytes were placed into a 2 L clean plastic container filled with 0.5 μm filtered seawater. Two drops of a dense spermatozoa suspension were added to the container and gently mixed. Fertilised oocytes were poured into a 500 L glassfibre tank filled with 0.5 μm filtered seawater. Seawater was agitated by gentle aeration. Seawater temperature was maintained in the range between 27 and 29 °C.

A day after fertilisation (Day 1), aeration was stopped for about an hour to allow healthy gastrulae to swarm near the seawater surface of the rearing tank. Abnormal embryos and oocytes near the bottom were drained out with ≈100 L of seawater. The tank was then refilled with freshly filtered seawater (partial seawater exchange). On Day 2, seawater in a whole tank was drained slowly into a plastic container of which the bottom was covered with a 60-μm mesh screen. Early bipinnaria larvae retained on a screen were rinsed with filtered seawater and transferred to a freshly prepared rearing tank (complete seawater exchange). A 1:1 mixture of cultured microalgae, either *Dunaliella tertiolecta* or *D. primolecta* and *Phaeodactylum tricornutum*, was added to the tank to make a concentration about 5,000 cells mL^{-1}. Throughout larval development, partial and complete seawater exchange were conducted every alternate day. The mixture of cultured microalgae was added every day.

When brachiolaria larvae commenced their searching behavior and became competent to settle, they were transferred to either an open-circulation or closed-circulation (driven by air-lift) 15 L container with pieces of crustose coralline alga, *Lithothamnium pseudosorum.*

Appendix II

Analysis of dissolved free amino acids (DFAA) using high-performance liquid chromatography (HPLC)

1. Analysis technique

Concentrations of DFAA in seawater subsample were determined by the pre-column *o*-phthaldialdehyde derivatization technique with reverse phase high performance liquid chromatography. Analytical procedures were similar to those described by Ayukai et al. (1993) and Stanley et al. (1987), but the solvent system was modified in this analysis.

2. Reagents preparation

HPLC-grade tetrahydrofuran (THF) and methanol were obtained from FSE and BDH, respectively. Analytical-grade chemicals, amino acid standards (powder form), sodium acetate, sodium borate, *o*-phthaldialdehyde (OPA) and 2-mercaptoethanol (MCE) were obtained from SIGMA-Aldrich. Purified water (Millipore Super-Q) was used to prepare a buffer solution of 0.1 M sodium acetate and 0.4 M sodium borate.

Three amino acids, glutamic acid, arginine and alanine, were dissolved into 0.1 N HCl solution to produce an equimolar (250 µM each substrate) bulk solution. A standard solution containing each amino acid at the concentration of either 100 nM or 250 nM was prepared daily by a 1000-fold dilution of the bulk solution with 0.1 M sodium acetate solution.

Pre-column derivatizing reagent, *o*-phthaldialdehyde-2-mercaptoethanol (OPA-MCE), was prepared by dissolving 25 mg OPA into the mixture solution of 25 µL MCE, 500 µL methanol and 12 mL 0.4 M sodium borate solution.

HPLC solvent (solvent A) was prepared by mixing 0.1 M sodium acetate, methanol and THF at the volume ratio of 78:22:1. Another HPLC solvent (solvent B) was methanol.

3. The chromatography system

Two sets of the HPLC system were used in the analysis. Components of each system are presented in Table 1. Derivatives were separated with a Brownlee RP-18 column, which was operated at 30 °C in a column oven, and were eluted at the constant flow rate of 1 mL minute^{-1} using the following binary solvent gradient program.

Time	Solvent A (%)	Solvent B (%)
0 - 8	100	0
8 - 9	100 → 90	0 → 10
9 - 28	90	10
28 - 29	90 → 100	10 → 0
29 - 32	100	0

The program was set to end in 35 minutes. Solvents were degassed with helium. Derivatives were detected at the excitation wavelength of 330 nm and the emission wavelength of 440 nm.

4. Analytical procedures

Derivatization of amino acids contained in each of 500 μL subsample was conducted by adding 100 μL OPA-MCE solution into the sample. The solution was mixed, stood for two minutes and injected into the HPLC system. A standard solution (500 μL) was analysed by the same procedure at the beginning and after either three or six runs of subsample. A standard blank (0.1 M sodium acetate) and a seawater blank were also analysed in the same manner.

A peak area of each amino acid was measured with an integrater. Concentrations of each amino acid in a subsample was calculated by dividing the peak area by a peak standard, and then multiplying the concentration of the substrate in the standard. The mean experimental error in measuring the amount of each amino acid in standards was 9.3 % (range 0 - 34 %).

Table 1 Components of each of two HPLC systems used in the analysis.

Components	Manufacturer and production code	
	System 1 2	System
Solvent conditioner	Bromma LKB 2156	←
Pressure mixing valve	LKB 2040-203	-
Mixer driver	LKB 11360-2	-
System controller	LKB 2150 LC	-
Dual piston pump	LKB 2150	Shimadzu LC-10A[*]
Manual injection valve	Rheodyne 7125 with 50 μL loop	←
Guard column	Alltima C18, 5 μm guard cartridge	←
Column	Brownlee RP18, 5 μm particle size, 250 x 4.6 mm	
Column oven	Shimadzu CTO-10A	←
Spectrofluorometric detector	Shimadzu RF-551 with 12 μL flow cell	
Integrater	Bromma LKB 2221	Shimadzu C-R5A

* The pump was fitted with a pressure mixing valve, a mixer driver and a system controller