Growth and survival of juvenile barnacle *Balanus amphitrite*: interactive effects of cyprid energy reserve and habitat

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ABSTRACT: Using factorial laboratory and field-transplant experiments, we have examined the effects of cyprid energy reserve and habitat on the performance of juvenile *Balanus amphitrite*. The cyprid energy reserve (quantified as total lipid content) increased linearly with increasing food (the diatom *Chaetoceros gracilis*) concentrations, i.e. cyprids from nauplii fed the highest (1 × 10⁶ cells ml⁻¹), high (5 × 10⁵ cells ml⁻¹), low (1 × 10⁵ cells ml⁻¹) and the lowest (5 × 10⁴ cells ml⁻¹) food concentrations had the highest (0.81 µg cyprid⁻¹), high (0.65 µg cyprid⁻¹), low (0.45 µg cyprid⁻¹) and the lowest (0.18 µg cyprid⁻¹) energy reserves, respectively. These cyprids were induced to metamorphose in the laboratory and then transplanted to 4 field sites differing in food availability. Their performance (measured as growth and survival) was assessed on Day 6. Regardless of the site, both survival and growth were lower for juveniles obtained from cyprids with the lowest energy reserve compared with juveniles obtained from cyprids with low, high and the highest energy reserves. However, the magnitude of the effect of cyprid energy reserve on growth varied among sites. For example, juveniles obtained from cyprids with the lowest energy reserve grew larger (~2- to 3-fold) at sites with a high chlorophyll a and low C/N ratio (indicators of surplus and high-quality food) than their siblings at other sites. This was interpreted as the result of the interaction between the negative effects of the lowest energy reserve in cyprids and the positive effect of surplus food. Adverse effects of the lowest energy reserve in cyprids on juvenile growth, however, could not be completely compensated by surplus food. Regardless of the cyprid energy reserve, juvenile growth (not survival) increased as chlorophyll a increased. This study suggests that the *in situ* growth of juvenile barnacles may depend on both independent and interactive effects of cyprid energy reserve (larval nutritional condition) and habitat.

KEY WORDS: Barnacles · *Balanus amphitrite* · Cyprid energy reserve · Food availability · Juvenile growth · Juvenile survival · Larval nutritional condition

INTRODUCTION

The planktotrophic life of barnacle larvae is completed by a non-feeding stage, the cyprid stage. The cyprid stage is specialised for benthic habitat selection, and, once attached, they metamorphose to juveniles. The transition between the cyprid and the adult stage (settlement, metamorphosis and early juvenile growth) is recognised as a critical period in the life cycle of barnacles, yet the linkage between these stages has received little attention (see Jarrett 2003, Phillips 2004). Although the importance of juvenile performance for subsequent recruitment success has been recognised in many marine invertebrates (reviewed by Gosselin & Qian 1996, Moran 1999, Pechenik 1999), several aspects of juvenile performance in barnacles, e.g. their *in situ* growth response to intrinsic and extrinsic factors, have not been well documented.

Growth of barnacles is controlled by both endogenous and exogenous factors (e.g. Denley & Underwood 1979, Wethey 1983, Crisp & Bourget 1985, Walters & Wethey 1996). Among all potential endogenous fac-
tors, the energy reserve derived from cyprids and the age when cyprids metamorphose are frequently reported to have significant impacts on juvenile performance (e.g. Pechenik et al. 1993, Jarrett & Pechenik 1997). For example, juvenile barnacles obtained from nauplii that were fed high-food concentration grew larger than their siblings obtained from nauplii fed low-food concentration under laboratory conditions (Thiyagarajan et al. 2003b). In fact, the dependence of juvenile performance on larval nutritional history is not unique to barnacles and has been reported in several other marine organisms (reviewed by Pechenik 1999).

Many exogenous factors affect juvenile performance in barnacles. For example, variability in phytoplankton availability is a well-known growth-regulating factor for filter-feeding invertebrates (e.g. Sanford et al. 1994). Generally, the growth of juvenile barnacles increases as a function of food availability under laboratory conditions at optimal temperature (Thiyagarajan et al. 2003b). In contrast, field studies demonstrated that factors other than phytoplankton concentration determine spatial variation in barnacle growth (Sanford & Menge 2001). Nevertheless, the potential impact of food availability on juvenile barnacle growth under realistic field conditions has not been well studied. The studies available have focused primarily on the influence of either larval nutrition or age at metamorphosis on juvenile barnacle growth (Pechenik et al. 1993, Jarrett & Pechenik 1997, Jarrett 2000, 2003, Thiyagarajan et al. 2002a,b, 2003a,b) and have hardly investigated how cyprid energy reserves affect growth of juvenile barnacles in different habitats. However, previous studies have suggested a potentially complex relationship between larval nutritional condition (endogenous factor) and environmental variables (exogenous factor), which may consequently determine juvenile performance and, ultimately, the population dynamics of a given species (reviewed by Pechenik 1999). Thus, analysis of cyprid energy reserve and natural variability in phytoplankton at the juvenile habitat shall contribute to our understanding of how events occurring during the pelagic larval phase cope with food availability in juvenile habitat and affect subsequent recruitment success.

In this study, growth of juvenile barnacles was examined in relation to the variability of cyprid energy reserve and particular sites in subtropical waters of Hong Kong that differ in food availability. Two questions have been posed: (1) How does natural variation in food quantity (phytoplankton biomass) and/or quality (C/N ratio of suspended solids) influence the growth of juvenile Balanus amphitrite? And, (2) how do conditions during the pelagic larval phase and juvenile habitat interact to affect the growth of the juvenile barnacle?

MATERIALS AND METHODS

Cyprid energy reserve. Adult Balanus amphitrite were collected from the intertidal zone in Hong Kong. Nauplii obtained from several adult individuals were mass-reared to cyprid stage using the diatom Chaetoceros gracilis as food at 28°C, according to Thiyagarajan et al. (2002a). To investigate the effects of cyprid energy reserves on growth and survival of juvenile barnacles, nauplii were reared at 4 different food concentrations (feeding regimes): $5 \times 10^4$, $1 \times 10^5$, $5 \times 10^5$ and $1 \times 10^6$ cells ml$^{-1}$. These food concentrations were chosen because we previously found that cyprid energy reserves increased linearly with increasing food concentrations within this range (Thiyagarajan et al. 2003b). For each feeding regime, there were 5 replicate rearing tanks (volume 2 l). Water and food were changed every day, and under these culture conditions cyprids were harvested after 4 d. Cyprid energy reserve was quantified by total lipid contents and used as an index of larval nutritional condition (Anger 1998, Phillips 2002). Total lipids were extracted according to Mann & Gallager (1985) and quantified by the sulphuric acid-charring method (Marsh & Weinstein 1966), with tripalmitin as a standard. Five replicates (50 cyprids replicate$^{-1}$), corresponding to 5 rearing tanks, were used for each feeding regime.

Cyprid metamorphosis and early juvenile. All cyprids from replicate tanks within a feeding regime were mixed, allowed to attach, and metamorphosed individuals were used for the field outplant experiment. Same-age cyprids from the 4 feeding regimes were allowed to metamorphose on circular polystyrene plates (~25 cm$^2$) in the presence of a conspecific settlement factor (equivalent to 0.5 µg ml$^{-1}$ bovine serum albumin; Rittschof et al. 1984) within 24 h. During this period >80% of the cyprids metamorphosed to juveniles. Only completely metamorphosed juveniles were used for juvenile outplant experiments.

Study sites. Growth and survival of juveniles obtained from the 4 feeding regimes were examined at 4 sites (habitats): Peng Chau (PC), Old Airport (OA), Tung Lung Chau (TLC) and Hong Kong University of Science and Technology (UST) (Fig. 1). According to our preliminary study, each site had a different phytoplankton concentration (measured as chl a) and C/N ratio (indicator of food quality). However, water temperature and salinity remained the same during the study period. At each site, study locations with similar (for all sites) wave exposure and current speeds were chosen for the juvenile outplant experiments.

Analysis of food availability at the study sites. To evaluate the food availability, replicate water samples were collected every other day for 6 d (3 samples per day and 9 samples in total) at each study site during
the study period (early October 2004). Food availability at each study site was determined according to Shriver et al. (2002). Chl a was used as a measure of phytoplankton quantity available to juvenile barnacles, whereas the C/N ratio was used as a measure of food quality (Vahl 1980, Cranford et al. 1998, Sanford & Menge 2001). Chl a concentration was determined fluorometrically (Parson et al. 1984). The C/N ratio was analysed by a PerkinElmer 2400 Series II CHNS/O Analyser.

**Juvenile growth and survival.** The growth and survival of early juveniles were monitored on Day 6, according to Pechenik et al. (1993) and Jarrett (2003), at these 4 sites. Only individuals that did not experience crowding were included in the analysis. Growth was determined as the difference (growth increments) between initial and final shell basal diameter (rostrocarinal). Measurements were made with the help of an ocular scale (Olympus SZX12). Juvenile survival at each site was calculated as the percentage of juveniles that survived during the 6 d experimental period.

**Experimental design.** The factorial design of the experiment included 2 main factors: cyprid energy reserve (4 levels corresponding to 4 feeding regimes) and site (4 sites with different levels of phytoplankton availability). For each site, there were 3 frames (made of PVC: 40 × 40 cm) and each frame contained 4 replicate plates per cyprid energy reserve; in total there were 16 plates per frame (Fig. 1). These plates were arranged in 4 rows and 4 columns and separated by 3 cm, according to a orthogonal 4 × 4 Latin-square design (see Fig. 1). Each of the 4 cyprid energy reserves would appear exactly once in each of the 4 rows and in each of the 4 columns. Each replicate plate contained 10 to 25 juveniles, and there were 3 frames per site; thus, there were 12 replicates per site (3 frames × 4 replicates per frame). Three frames were placed (vertically) in the intertidal zone (~1 m above chart datum) of each site, and each frame was horizontally separated from the next by about 10 m. Growth increments and survival were measured as described in the previous section up to Day 6. The experiment was terminated on Day 6 because the effect of larval energy reserve on juvenile growth persists at least for the first 7 d after metamorphosis (Jarrett & Pechenik 1997, Thiyagarajan et al. 2003a,b). This experiment was conducted in October 2004, while all the preliminary experiments (including the 2 earlier repetitions of this experiment; data not shown) were conducted in August and September 2004.

**Data analysis.** Data were tested for homoscedasticity using Cochran’s test and normality assumptions were tested using the Kolmogorov-Smirnov test. The square-root-transformed chl a, C/N ratio and cyprid energy reserve data were analysed with 1-way ANOVA. Tukey’s test was used for post hoc comparison. A nested model ANOVA—cyprid energy reserve and site as fixed factors, with frame nested under the factor site—was used.

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**Fig. 1.** Map of Hong Kong showing the study sites (PC, Peng Chau; OA, Old Airport; TLC, Tung Lung Chau; UST, University of Science and Technology). Insert: a diagram of a frame showing the arrangements of polystyrene plates (marked as PC) with juveniles from cyprids of 4 different categories (VHEC, the highest energy cyprids; HEC, high energy cyprids; LEC, low energy cyprids; VLEC, the lowest energy cyprids) in a 4 × 4 orthogonal Latin-square design.
to ascertain differences in juvenile growth increments and survival. Initial size of the juveniles was not included as a co-factor in the above-mentioned ANOVA model, since their influence on juvenile barnacle growth increments was found to be insignificant in previous investigations (Jarrett & Pechnik 1997, Thiagarajan et al. 2003b). Due to a strong interaction between the factors of site and cyprid energy reserve (on growth increments), the effect of each factor was tested at a fixed level of the other factor using 1-way ANOVA and Tukey’s multiple comparison test. Prior to analysis, growth increment and survival data were square-root- and arcsine-transformed, respectively. The relationship between mean (n = 5) cyprid energy reserve and growth increment (n = 12) was analysed by Pearson product-moment correlation.

RESULTS

Cyprid energy reserve

The cyprid energy reserve increased linearly with increasing food concentrations. Cyprids from the larvae fed the highest (1 × 10^6 cells ml\(^{-1}\)), high (5 × 10^5 cells ml\(^{-1}\)), low (1 × 10^5 cells ml\(^{-1}\)) and the lowest (5 × 10^4 cells ml\(^{-1}\)) food concentrations had the highest, high, low and the lowest energy reserves, respectively (Fig. 2). A pair-wise multiple comparison procedure resulted in significant differences in energy reserves among feeding regimes. Therefore, cyprids from 4 feeding regimes were categorised into the highest energy cyprids (VHEC), high energy cyprids (HEC), low energy cyprids (LEC) and the lowest energy cyprids (VLEC), according to their lipid contents (Fig. 2). As we had expected, the larval feeding regimes adopted in this study produced cyprids with different energy reserves that allowed us to investigate the influence of cyprid energy reserve on juvenile performance.

Variation in food availability among sites. Chl \(\text{a}\) concentration varied with sites (Fig. 3A). The highest chl \(\text{a}\) concentrations were observed at PC and OA (ranging from 1.2 to 1.4 µg l\(^{-1}\)), while the lowest chl \(\text{a}\) concentrations were recorded at TLC and UST (ranging from 0.5 to 0.6 µg l\(^{-1}\)). Although PC and OA had similar chl \(\text{a}\) concentrations, the C/N ratios were markedly different. The surface water at PC contained a higher C/N ratio than that at other sites (Fig. 3B). The C/N ratio at OA was significantly lower than that at other sites. Over the study period, temperature and salinity varied from 23 to 26°C and 33 to 34‰, respectively. All the sites had moderate wave exposure, which was generated mainly by boat traffic and winds. Thus, the chosen sites served as a suitable platform for investigation of the impact of natural variation in food availability on juvenile performance.

Fig. 2. Mean (n = 5) cyprid energy reserve (quantified as total lipid content) of different categories of cyprids used in this study. Each replicate contained 50 cyprids. The 1-way ANOVA results are shown in the top right-hand corner of the figure. Means that are significantly different in Tukey’s test are indicated by different letters above the bars. See Fig. 1 for category abbreviations

Fig. 3. Mean (n = 9, SD) concentrations of (A) chl \(\text{a}\) and (B) C/N ratios at the study sites. The 1-way ANOVA results are shown in the top right-hand corner of each panel. Means that are significantly different in Tukey’s test are indicated by different letters above the bars. See Fig. 1 for site abbreviations
Juvenile growth increment. Both cyprid energy reserve and site had significant effects on juvenile growth increment (Table 1). There was a significant cyprid energy reserve × site interaction. According to mean square values in Table 1, the cyprid energy reserve appeared to cause more variation in growth increments than the site effect. The highest growth increment was recorded for juveniles obtained from VHEC (~880 to 920 µm in 6 d: Fig. 4A) at PC and OA, while the lowest growth was recorded for juveniles obtained from VLEC at TLC and UST (~93 to 105 µm in 6 d: Fig. 4A). There was no significant difference in growth increments between the juveniles obtained from HEC and LEC regardless of site (Table 2). Growth increments of juveniles obtained from VLEC at PC and OA were 2- to 3-fold higher than those of the siblings at TLC and UST (Fig. 4A, Table 2). Notably, the juveniles obtained from both HEC and LEC attained higher growth increments at OA than those at all other sites (Fig. 4A, Table 1). The mean growth increment increased consistently with increasing cyprid energy reserve regardless of the study sites (Fig. 5).

Juvenile survival. The ANOVA results showed a significant effect of cyprid energy reserve on survival, while the interaction between cyprid energy reserve and site was not significant (Table 1). Although juvenile survival did not differ significantly among the sites, it varied greatly among cyprid categories (energy reserves). For example, the juveniles obtained from VLEC showed low survival (about 30%) (Fig. 4B). In contrast, high survival rates (about 60 to 70%) were observed among the juveniles obtained from LEC, HEC and VHEC.

Discussion

Regardless of the variation of food availability in the juvenile habitat, both survival and growth were significantly lower for juveniles obtained from cyprids with...
the lowest energy reserve (i.e. larvae fed the lowest food concentration) compared to those obtained from cyprids with low, high and the highest energy reserves (i.e. larvae fed low, high and the highest food concentrations). However, juveniles obtained from cyprids with the lowest energy reserve grew slightly larger (about 2- to 3-fold) than their siblings when surplus and/or high-quality food was available. Our laboratory and field-transplant experiments suggest that the in situ growth of juvenile barnacles depends on both independent and interactive effects of cyprid energy reserve and habitat.

In this study, mean cyprid energy reserve (larval nutritional condition) was positively correlated with the mean growth increment of early juvenile barnacles (Fig. 5). This finding is consistent with previous studies on bryozoans (Wendt 1998), polychaetes (Qian & Pechenik 1998), molluscs (Miller 1993, Pechenik et al. 1996, Phillips 2002, 2004) and echinoderms (Basch & Pearse 1996, Emlet & Hoegh-Guldberg 1997). Similar patterns were also observed in reef fishes and ascidians (Searcy & Sponaugle 2001, Shima 2001, Marshall et al. 2003). The present study provides additional evidence for the consensus that the juveniles obtained from poorly fed larvae grow more slowly than those derived from well-fed larvae. Notably, the magnitude of the impact of cyprid energy reserve on juvenile growth appears to depend on the food availability of particular habitats. For example, the juveniles obtained from the highest energy reserve cyprids (VHEC) grew only 1.78-fold larger than the siblings obtained from the lowest energy reserve cyprids (VLEC) at OA, where surplus high-quality food was available. But, the growth increment was 2.78-, 2.76- and 5.91-fold at PC, TLC and UST, respectively (see Fig. 4A). At all these 3 sites, either food quantity (TLC and UST) or quality (PC) was lower than at OA (Fig. 2A,B). These results suggest that under favourable food availability, the negative effect of larval nutritional condition on juvenile growth is less pronounced. This was interpreted as the result of the interaction between the negative effects of the lowest energy reserve in cyprids and the positive effect of surplus food. Adverse effects of the lowest energy reserve in cyprids on juvenile growth, however, cannot be completely compensated by surplus food availability. The effect of cyprid energy reserve on juvenile growth,
However, is temporary in barnacles, i.e. significant negative cyprid energy effect on juvenile growth fades away after 7 to 12 d post-metamorphosis (Jarrett & Pechenik 1997, Thiyagarajan et al. 2003a,b). Nevertheless, several studies suggest that juvenile performance during the first few days after metamorphosis is critical for successful recruitment (e.g. Keough & Downes 1982, Gosselin & Qian 1996, Emlet & Hoegh-Guldberg 1997, Hunt & Scheibling 1997, Moran & Emlet 2001).

Juveniles obtained from LEC, HEC and VHEC grew slightly larger at UST than at TLC (Fig. 4A). Such significant growth difference between these 2 sites could not be explained by chlorophyll concentrations, yet differences in food quality may offer some explanation. The C/N ratio was lower at UST than at TLC, suggesting that food quality was inferior at TLC compared to at UST. Thus, this study implies that juvenile growth of barnacles may be affected by both quantity and quality of the food available at the sites, irrespective of the effects imposed by the cyprid energy reserve. Although our conclusions were largely based on the assumption that the study sites differed only in terms of food availability, the role of some other potential juvenile growth regulating factors (e.g. predation) on the outcome of this study cannot be completely ignored. Barnacles can also consume a wide variety of non-photosynthetic food particles, such as zooplanktons (Sanford & Menge 2001). Therefore, different non-photosynthetic plankton food concentrations at different juvenile habitats might have also affected our results in this study.

The most striking result of the present study is that variation in cyprid energy reserves, provided they were >0.45 µg lipid cyprid⁻¹, appeared to have less influence on juvenile growth (except at PC) and no effect on survival, suggesting that the lower threshold level for critical reserve effects falls somewhere between 0.18 and 0.45 µg lipid cyprid⁻¹. Although wild-caught cyprids have not been included in this experiment, our previous study showed that the lipid content of wild cyprids collected 3 times between March and July 2002 was >0.5 µg lipid cyprid⁻¹ (Thiyagarajan et al. 2003b). Therefore, it seems that wild cyprids (at least in subtropical Hong Kong waters) often accumulate energy reserves above this lower threshold level. Of course, this does not mean that wild juveniles are not stressed by energy reserve. In the field, the amount of cyprid energy reserve is determined by 2 processes. First, nauplii derive and accumulate energy reserve from phytoplankton; therefore, qualitative and quantitative variation in algal food can have a dramatic effect on cyprid energy reserve (Hentschel & Emlet 2000, Thiyagarajan et al. 2002b). Secondly, cyprids use substantial amounts of their stored energy reserve for dispersal, site selection and metamorphosis (Lucas et al. 1979, Miron et al. 2000). Therefore, it is difficult to determine the cause of variations in energy reserves in wild cyprids and their subsequent effect on juvenile performance (see Jarrett 2003). Though several possible explanations have been provided and discussed in recent papers (e.g. Phillips 2002, 2004, Jarrett 2003, Marshall & Keough 2003), the real physiological mechanisms behind the suppression of growth in juveniles obtained from larvae fed low food concentrations are not yet known. However, it is believed that slow growth of these juveniles might be caused by a reduced-feeding, digestive-capability, or food-assimilation efficiency (see Pechenik et al. 2002, Marshall et al. 2003).

Slowly growing juveniles may be more vulnerable to physical factors (Vermeij 1971), predation (Connell 1985) and competition (Bertness et al. 1991), and may consequently be wiped out of the population more readily than their fast-growing siblings. Therefore, we have hypothesised a lower survival in slow-growing juveniles obtained from VLEC compared with fast-growing juveniles obtained from HEC. This study proved our hypothesis. Although previous studies in barnacles have reported similar results, those studies determined juvenile mortality after 30 d post-metamorphosis (Jarrett & Pechenik 1997, Jarrett 2003). The results reported here suggest that high mortality in slow-growing juvenile Balanus amphitrite occurs within 6 d post-metamorphosis. Similarly, juvenile mussels obtained from larvae fed low food concentrations experience a higher mortality than their siblings from larvae fed high food concentrations (Phillips 2004). On the other hand, there was no significant difference in survival among the juveniles from LEC, HEC and VHEC, ~70% of them survived up to Day 6 at all sites (Fig. 4B). This result suggests that the energy reserve in juveniles from VLEC was sufficient to keep them alive at least for 6 d at the sites examined.

Lower survival of juveniles from VLEC compared with juveniles from LEC, HEC and VHEC should also be considered for the interpretation of our data. The interactive effect of nauplius feeding history and juvenile habitat might have been affected by the lower survival of juveniles from VLEC at least to some extent. Because we may not have fully quantified the effect of nauplius feeding history (or cyprid energy reserves) on juvenile growth when there was a lower survival of slow-growing juveniles from VLEC.

Several potential growth-regulating factors, such as wave exposure, have been excluded in this study. Nevertheless, the results presented here are of interest, because so far there have been few studies that link larval nutritional condition, food availability in the habitat and performance of juveniles for a single species. In the future, we will expand this study to test...
how seasonal changes in salinity, temperature and wave exposure influence the negative effect of low larval nutritional condition on early juvenile growth and, consequently, recruitment success.

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