Positive effects of large concentration in culture on the development of the lecithotrophic larvae of *Babylonia formosae* (Sowerby) (Prosobranchia: Buccinidae)

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**Abstract**

The negative effects of large larval concentration on larval development have been reported for many marine planktotrophic invertebrates. Comparable data for the lecithotrophic species are, however, not available. The present study was therefore undertaken to determine the influence of varying larval concentrations on the developmental performance of *Babylonia formosae* (Sowerby). The larvae of *B. formosae* were cultured at four concentrations: 1, 2, 4 and 8 larvae ml⁻¹. Over the 6-day experimental period, the cumulative percent settlement increased from 73, 83, 89 to 95%, and the mean settling time decreased from 3.88, 4.46, 3.62 to 3.21 days as the concentration increased from 1, 2, 4 to 8 larvae ml⁻¹, respectively. What was found was a positive correlation between larval concentration and cumulative percent settlement ($Y = 74.35 + 2.80X; R^2 = 0.61; p < 0.01$) as well as a negative correlation between larval concentration and mean settling time ($Y = 4.31 - 0.14X; R^2 = 0.59; p < 0.01$). It was hypothesized that lecithotrophic larvae accelerate their developmental rate to shorten the suboptimal planktonic period and to minimize substrate competition with increased larval concentration. When juveniles were reared at 0.2 individuals ml⁻¹ in one experiment, they all survived regardless of the concentration at which larvae were reared, and no significant difference was found in the cumulative increment of shell length on days 4, 8, 12 or 16. The mean growth rate was 39 μm d⁻¹. In a second experiment, however, juvenile mortality was 37 to 47% when juveniles were reared at higher concentrations (1 to 8 juveniles ml⁻¹). The juvenile growth rate also decreased from 29, 26, 23 to 18 μm d⁻¹ as the concentration increased from 1, 2, 4 to 8 juveniles ml⁻¹, respectively. The cumulative increment of shell length in different concentrations was significantly different on day 16 ($P < 0.05$), and there was a negative correlation between juvenile concentration and cumulative increment of shell length (mm) ($Y = 0.469 - 0.024X; R^2 = 0.12; P < 0.01$). Although juveniles clearly do better at low juvenile concentrations, the experiences of planktonic larval concentration may influence the rate of early juvenile growth was not indicated. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Many benthic marine invertebrates have a planktonic larval stage before settling and metamorphosing to juveniles. The duration of this planktonic period is a function of the developmental rate, which is influenced by such environmental factors as temperature, salinity, dissolved oxygen concentration, pH, pollutants, availability of food (Pechenik, 1987) and suitable substrates (Pechenik, 1990). In laboratory studies, larval crowding becomes an additional factor contributing to developmental plasticity (Loosanoff and Davis, 1963). Hinegardner (1969) suggested that one mature larva ml⁻¹ represents a comfortable concentration for the laboratory culture of sea urchins. Studies in invertebrate larval development have usually maintained their rearing ranges of 0.2–2 larvae ml⁻¹, for example with bryozoan (Wendt, 1996), asteroid (Basch, 1996), and gastropods (Miller, 1993; Pechenik et al., 1996). However, in some cases, the rearing concentration was in the range of 10–50 larvae ml⁻¹ or even higher, for example with barnacle (Pechenik et al., 1993), clams (Loosanoff et al., 1951; Hurley and Walker, 1996) and gastropod (Chaitanawisuti and Kritsanapuntu, 1997). In the aquaculture of Spisula solidissima similis and Babylonia areolata, 10 larvae ml⁻¹ has also been commonly used (Hurley and Walker, 1996; Chaitanawisuti and Kritsanapuntu, 1997).

The effects of high cultural concentrations (8–400 larvae ml⁻¹) on larval performance have been reported for many planktotrophic species. Laboratory study in oyster Crassostrea virginica indicated that the larval filtration rate was inversely proportional to larval concentration (Fritz et al., 1984). Low developmental and postmetamorphic growth rates accompanying high larval concentration were reported with surfclam Spisula solidissima similis (Hurley and Walker, 1996), hard clam Mercenaria mercenaria (Loosanoff and Davis, 1963), clam Scapharca broughtonii (Wang et al., 1993) and barnacle Balanus amphitrite (Pechenik et al., 1993).

The possible effects of larval concentration on feeding and development include: (1) reduced larval feeding efficiency, (2) physical interactions among larvae, and/or (3) accumulation of soluble wastes which lowers feeding times or and rates (Basch, 1996). In contrast, the effects mediated by feeding were excluded with the lecithotrophic species. Thus, suboptimal conditions are mainly contributed to physical interactions among larvae and the accumulation of soluble wastes. Under a suboptimal environment, it is expected that: (1) lecithotrophic larvae accelerate the developmental rate so as to reduce the planktonic period and to minimize substrate competition; and (2) lecithotrophic larvae experiencing high concentration have a slower postmetamorphic growth rate than those experiencing low concentration. However, data are not available at this time. Therefore, this study was undertaken to determine the influence of larval concentration on the development and postmetamorphic growth of the lecithotrophic species.

The neogastropod Babylonia formosae (Sowerby) was selected for this study. It is carnivorous and lives in the subtidal sandy or muddy bottoms at depths of 15 to 50 m.
The annual spawning season is from October to January (Chiu and Liu, 1994). Lecithotrophic eggs with a diameter of 0.52–0.57 mm are deposited in egg capsules. The number of eggs per capsule ranges from 0 to 60. Two to three weeks are required for veligers to hatch at temperatures between 25 and 27°C. From just a few days to up to one week after hatching, with no need for specific cues, free living veligers settle and metamorphose then benthic life starts.

2. Materials and methods

2.1. Obtaining and rearing animals

About eighty adult *B. formosae* were collected from Kaohsiung, Taiwan (Longitude 120°17′E; Latitude 22°38′N) in October, 1996. Snails were held in a 60-liter tank (60 × 30 × 35 cm) with recirculating aerated seawater and fed with frozen red-tailed shrimp, *Penaeus penicillatus* (2–4 cm in body length) every other day. Transparencies (29.6 × 21 cm) were provided on the bottom of the tank for the deposition of egg capsules. Laid egg capsules were gathered daily and transferred to a 30-liter tank for further culture. Developed embryos started to hatch on day 16. Hatched veligers were collected to study the effects of concentration on larval development. If larvae were not used on the actual collection day, they were discarded. Hence, larvae used in the experiments were all hatched within 24 h.

In all the experiments, larvae and juveniles were reared at 25±0.8°C and 30%oS. Seawater was autoclaved and fully aerated before use. During the experimental period, seawater was not aerated but was changed every day.

2.2. Effects of concentration on larval development

Concentration treatments were 1, 2, 4, and 8 larvae ml⁻¹, and each treatment was performed three times. Because of an insufficient supply of larval stock, three replicates were conducted on different days. In each treatment, larvae were put in a transparent acrylic box (15 × 7 × 4 cm) with 100 ml seawater. Larval development was observed with a dissecting microscope. The settlement and metamorphosis of larvae were determined from the observation of lost vela and from foot probing behavior. During the experimental period, the numbers of settling larvae were recorded daily. After all live larvae settled and metamorphosed, the experiments were terminated. The live juveniles from the final replicate were kept in their treated concentrations for another 15 days until the experiments on the effects of concentration on postmetamorphic growth were started.

The cumulative percent settlement and mean settling times were calculated. The mean settling time was obtained by dividing total settling time by the total number of settled larvae. Total settling time was the sum of daily settled larvae multiplied by their experimental periods (in days).
2.3. Effects of concentration on juvenile growth

Juveniles kept at previous experimental conditions were used in the follow-up experiments. Concentration treatments were 0.2 juvenile ml\(^{-1}\) in experiment I and 1, 2, 4 and 8 juveniles ml\(^{-1}\) in experiment II. In experiment I, 6-well tissue culture plates, with each well 3.4 cm in diameter and 1.5 cm in height, were used for juvenile cultures. Thirty juveniles from each of the previous larval concentrations were transferred to the culture plates with each well containing one juvenile in 5 ml seawater. In experiment II, a transparent acrylic box (15 × 7 × 4 cm) with 100 ml seawater was used as a container. To maintain the concentrations of 1, 2, 4, and 8 juveniles ml\(^{-1}\), a total of 100, 200, 400 and 800 juveniles respectively were added to each of the acrylic boxes. In each concentration treatment, juvenile growth was only measured for 30 individuals. These were held separately in 8 foamed polyester plastic boxes (25 × 31 × 3 mm) inside the acrylic box to trace individual growth. Each foamed polyester plastic box contained 3 to 4 juveniles. Fluid circulation within these boxes was through 112 uniformly rectangular holes (3 × 1 mm). During the experimental period, the juveniles were fed with frozen *Artemia salina* for one hour daily and the seawater was changed after feeding. Dead individuals were also removed.

The shell lengths of the juveniles were measured every four days until day 16. Initial, final and total increments of shell length were determined.

2.4. Data analyses

Data were analyzed by the Kruskal-Wallis nonparametric analysis of variance (ANOVA) and Dunns multiple comparisons test. General linear models were also used to examine the effects of concentration on larval development and juvenile growth.

3. Results

3.1. Effects of concentration on larval development

It was found that larval concentration accelerated the development of *Babylonia formosae*. Over the 6-day experimental period, all living larvae settled and metamorphosed. Less than 5% of the swimming larvae settled and metamorphosed on the first day (Fig. 1). The cumulative percent settlement among concentrations was significantly different between days 3 and 5 (\(P < 0.05\)). By day 3, the larvae were clearly settling sooner at progressively higher concentrations. However, on day 6, the cumulative percent settlements in 1, 2 and 4 larvae ml\(^{-1}\) were different but not significantly so. Over the 6-day experimental period, with increasing larval concentrations from 1, 2, 4 to 8 larvae ml\(^{-1}\), the cumulative percent settlement increased from 73, 83, 89 to 95%, respectively (Table 1). A positive correlation was observed between concentration and cumulative percent settlement (\(Y = 74.35 + 2.80X; R^2 = 0.61; P < 0.01\)). On the other hand, the mean settling time decreased from 3.88, 4.46, 3.62 to 3.21 days when concentrations increased from 1, 2, 4 to 8 larvae ml\(^{-1}\), respectively (Table 1). A
negative correlation between concentration and mean settling time was noted ($Y = 4.31 - 0.14X; R^2 = 0.59; P < 0.01$).

### 3.2. Effects of concentration on juvenile growth

Data from the growth experiments indicated that juveniles continued to grow during the experimental period (Table 2). In experiment I, juveniles from different larval concentration origins were reared individually at the concentration of 0.2 juvenile ml$^{-1}$. The mean juvenile initial shell length ranged from 1.09 to 1.27 mm. All juveniles were alive when the experiments were terminated. No significant difference in the cumulative

Table 1

<table>
<thead>
<tr>
<th>Concentration (larvae/ml)</th>
<th>Cumulative percent settlement and percent survival±SE</th>
<th>Mean settling time (day)±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>72.2±7.7 B</td>
<td>3.88±0.14 B</td>
</tr>
<tr>
<td>2</td>
<td>83.0±2.0 B</td>
<td>4.46±0.13 A</td>
</tr>
<tr>
<td>4</td>
<td>89.2±2.3 B</td>
<td>3.62±0.08 B</td>
</tr>
<tr>
<td>8</td>
<td>95.1±1.7 A</td>
<td>3.21±0.05 C</td>
</tr>
</tbody>
</table>

*Means differing significantly from each other are indicated by different letters.*
Table 2
Influence of cultural concentration on the shell growth of *Babylonia formosae*. Values are (mean±SD); sample sizes are in parentheses.

<table>
<thead>
<tr>
<th>Experiment I: Concentration (individual ml⁻¹)</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original larval concentration</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Juvenile concentration</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Initial shell length (mm)</td>
<td>1.27±0.28 (30)</td>
<td>1.09±0.13 (30)</td>
<td>1.20±0.17 (30)</td>
<td>1.18±0.20 (30)</td>
</tr>
<tr>
<td>Final shell length (mm)</td>
<td>1.89±0.53 (30)</td>
<td>1.66±0.36 (30)</td>
<td>1.91±0.34 (30)</td>
<td>1.78±0.44 (30)</td>
</tr>
<tr>
<td>Shell length cumulative increment (mm) on</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 4</td>
<td>0.17±0.11 (30)</td>
<td>0.17±0.15 (30)</td>
<td>0.20±0.10 (30)</td>
<td>0.18±0.10 (30)</td>
</tr>
<tr>
<td>day 8</td>
<td>0.33±0.16 (30)</td>
<td>0.27±0.19 (30)</td>
<td>0.33±0.13 (30)</td>
<td>0.30±0.15 (30)</td>
</tr>
<tr>
<td>day 12</td>
<td>0.47±0.25 (30)</td>
<td>0.41±0.25 (30)</td>
<td>0.49±0.18 (30)</td>
<td>0.44±0.21 (30)</td>
</tr>
<tr>
<td>day 16</td>
<td>0.62±0.29 (30)</td>
<td>0.57±0.29 (30)</td>
<td>0.71±0.23 (30)</td>
<td>0.60±0.28 (30)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment II: Concentration (individual ml⁻¹)</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original larval concentration</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Juvenile concentration</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Initial shell length (mm)</td>
<td>2.02±0.18 (30)</td>
<td>2.01±0.22 (30)</td>
<td>2.04±0.22 (30)</td>
<td>1.97±0.19 (30)</td>
</tr>
<tr>
<td>Final shell length (mm)</td>
<td>2.42±0.19 (19)</td>
<td>2.46±0.25 (17)</td>
<td>2.39±0.31 (18)</td>
<td>2.25±0.14 (16)</td>
</tr>
<tr>
<td>Shell length cumulative increment (mm) on</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 4</td>
<td>0.17±0.15 (20)</td>
<td>0.15±0.13 (19)</td>
<td>0.12±0.13 (23)</td>
<td>0.14±0.12 (21)</td>
</tr>
<tr>
<td>day 8</td>
<td>0.20±0.14 (20)</td>
<td>0.22±0.13 (19)</td>
<td>0.13±0.10 (22)</td>
<td>0.20±0.11 (19)</td>
</tr>
<tr>
<td>day 12</td>
<td>0.28±0.17 (19)</td>
<td>0.27±0.15 (17)</td>
<td>0.23±0.13 (18)</td>
<td>0.24±0.14 (16)</td>
</tr>
<tr>
<td>day 16</td>
<td>0.46±0.22 (19) A</td>
<td>0.42±0.16 (17) A</td>
<td>0.36±0.15 (18) A</td>
<td>0.29±0.14 (16) B</td>
</tr>
</tbody>
</table>

Means differing significantly from each other are indicated by different letters.

Increment of shell length (on days 4, 8, 12 and 16) was found among juveniles from different concentration origins, and the mean growth rate was 39 μm d⁻¹.

In experiment II, juveniles were kept at 1, 2, 4 and 8 juveniles ml⁻¹. Their mean initial shell length ranged from 1.97 to 2.04 mm (Table 2). Juvenile mortalities were between 37 and 47%. The difference in the cumulative increment of shell length was not significant for different concentrations on days 4, 8 and 12. On day 16, a negative correlation between concentration and the cumulative increment of shell length (mm) was found ($Y = 0.469 - 0.024X; R^2 = 0.12; P < 0.01$). The growth rate decreased from 29, 26, 23 to 18 μm d⁻¹ as concentrations increased from 1, 2, 4 to 8 juveniles ml⁻¹, respectively. By comparison, juvenile growth rates in experiment II were significantly lower than in experiment I ($P < 0.05$).

4. Discussion

In the results here, the positive effects of larval concentration on the development of *Babylonia formosae* were observed as larval crowding accelerated metamorphosis and increased postsettlement survival. The absence of a significant difference in the cumulative increment of shell length and survival among juveniles in experiment I indicated that the postmetamorphic performances of larvae experiencing high cultural concentrations during the planktonic stage are not affected in the early juvenile stage.

Although the concentration of invertebrate larvae in the field is usually low, high
concentrations from 10 to 40 larvae ml\(^{-1}\) of the snail *Thais haemostoma* and oyster *Crassostrea virginica* in Louisiana waters have been reported by St. Amant (1957). But, relationships between larval concentration and postmetamorphic performance have rarely been studied in the field.

Regarding the effects of larval concentration on development and postmetamorphic growth, the data here are comparable to those of the planktotrophic species. For example, sea star *Asterina miniata* larvae reared at 0.5 vs. 1.0 larva ml\(^{-1}\), showed a slow developmental rate in high larval concentration (Basch, 1996). Development and growth were also inversely related to concentrations in clams of *Spisula solidissima smiiilis* (10, 20, 30 and 50 larvae ml\(^{-1}\)) (Hurley and Walker, 1996), *Mercenaria mercenaria* (250, 500, 750, 1000 and 3000 eggs ml\(^{-1}\)) (Loosanoff and Davis, 1963) and *Scapharca broughtonii* (1, 8, 14, and 24 larvae ml\(^{-1}\)) (Wang et al., 1993). In marked contrast, it was found in this study that the larvae of *B. formosae* responded to high larval concentrations by metamorphosing precociously and its mortality was decreased. The effects of larval concentration on the development of this lecithotrophic species are quite different from the planktotrophic species, such as *S. solidissima smiiilis* and *M. mercenaria*. However, more studies are necessary to verify if the patterns observed in the current experiments are also present in other lecithotrophic species.

Increasing concentration from 1 to 2 larvae ml\(^{-1}\) had no significant effects on larval development. The most effective concentration in these experiments was 4 larvae ml\(^{-1}\) and above (Fig. 1). The mechanism through which high concentration provoked metamorphosis is unknown. The detection of ambient larval concentration and modification of the rate of development may be achieved by physical interactions among larvae (Basch, 1996) or by chemical responses to external cues, such as chemical substances emitted by larvae or soluble wastes excreted from organisms. The results here suggest larvae only respond to a change in concentration once it reaches a certain level. Nevertheless, it is not known whether the acceleration on the development would be greater if concentration increased above 8 larvae ml\(^{-1}\).

Rapid metamorphosis under high concentrations could be adaptive. In the presence of competitors, because of uncertainty in finding better habitats or depletion of energy reserves, larvae may choose to accelerate the developmental process and settle on the available habitats. In contrast, in low concentrations, the availability of space is unlimited. Prolonging its status in the plankton would make it possible to search for better habitats for future survival and successful reproduction. Roper et al. (1996) reared *Drosophila melanogaster* at two larval densities, i.e. 50 and 150 per vial, while the adult population density was standardized at the same level during their selected experiments. After 20 generations of selection, larval development time diverged, with longer larval development time and greater adult body size associated with lower larval density. Neither early adult fertility for females nor the lifespan differed in the two selected experiments. Even so, the late fertility of low density line females was significantly enhanced. These results suggest larval density does have an important impact on life history trade-offs in *D. melanogaster*. In *Crepidula fornicata*, larvae which had experienced temporary starvation showed reduced juvenile growth rates even though all of the juveniles were transferred to full ration immediately after metamorphosis (Pechenik et al., 1996, 1998). This also indicates that a trade-off is made between
adaptation for development under starvation and juvenile fitness in *C. fornicata*. The present findings of larval crowding reducing the developmental period in *B. formosae* is similar to *D. melanogaster*. Thus, it seems likely that the expression of trade-offs is in the pre-adult or adult period.

Based on this work for *B. formosae*, it appears that high concentration accelerates larval development and increases larval survival. Such results have implications in the molluscan mariculture of the lecithotrophic species. By means of artificial propagation, setting high concentrations in the planktonic stage and transferring settled juveniles to low concentrations, the mortality of the reared lecithotrophic species may indeed be reduced and its cultural period shortened.

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**References**


