JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 29, No. 1
March, 1998

# Ingestion Rate and Feeding Behavior of the Peppermint Shrimp *Lysmata wurdemanni* on *Artemia* Nauplii

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*Abstract.*—A laboratory study was conducted to examine the effects of food level and water temperature on the ingestion rate in the larvae of the peppermint shrimp *Lysmata wurdemanni*, a popular aquarium species. *Artemia* nauplii were used as the food item. Number of newly hatched *Artemia* nauplii consumed by the larvae (from zoea II to zoea VII, zoea I can metamorphose to zoea II without exogenous nutrition in about one day) was measured daily at four food concentrations (2, 5, 10 and 20 *Artemia* nauplii/mL) and two temperature levels (25 C and 28 C). The experiment was conducted in 500-mL beakers with three replicates for each treatment. The results show that the shrimp consumed significantly more food at 28 C than at 25 C ($P < 0.01$). Ingestion rate increased with increasing food concentration in all larval stages and with larval development except for the lowest food level (2 *Artemia* nauplii/mL) where insufficient food supply limited the ingestion rate to around 35 *Artemia* nauplii/larva per d after zoea IV stage. The larval development rate was significantly lower ($P < 0.05$) in the shrimp subjected to the two lowest food concentrations (2 and 5 *Artemia* nauplii/mL) in the 500-mL beakers. But in a separate experiment conducted in the 1,400-mL beakers, the results were reversed: both survivorship and development rate were significantly lower at the two higher food levels (6 and 12 nauplii/mL) than those at 3 nauplii/mL level. During molting period, ingestion rate decreased significantly, followed by a sharp rise to the normal rate the following day.

Food availability is an important limiting factor in survival of decapod larvae (Nakazawa 1912; Yatsuzuka 1960; Brick 1974; Bigford 1978; Paul et al. 1979; Paulay et al. 1985; Minagawa and Murano 1993). Increasing prey concentrations from 600 to 1600 *Artemia* nauplii per liter results in increasing survival rates for cultured zoeae of spider crab *Libinia emarginata* and mud crab *Scylla serrata* (Brick 1974; Bigford 1978). Modin and Cox (1967) found that larvae of the shrimp *Pandalus jordani* failed to survive if not fed soon after hatching. Similar results have been reported in many other decapod species (Anger and Dawirs 1981; Anger et al. 1981; Dawirs 1984; Mikami and Greenwood 1995).

A major problem facing aquaculturists is to understand suitable food and its optimal density for rearing larvae of cultured species. For example, successful rearing of penaeid shrimp larvae depends on providing different, suitable living food organisms as the development advances. In penaeid shrimp larval culture, *Artemia* nauplii were successfully used as larval food (Liao et al. 1983) owing to their high nutrition value and suitable size. Studies on ingestion rate in penaeid shrimp have laid a solid foundation for the successful shrimp larval rearing (Gopalakrishnan 1976; Emmerson 1980, 1984; Yufera et al. 1984; Yufera and Rodriguez 1985; Chu and Shing 1986; Loya-Javellana 1989).

*Lysmata wurdemanni* is one of the most popular aquarium ornamental shrimps. Limited efforts have been made to develop culture methods for the shrimp larvae using *Artemia* nauplii (Crompton 1992). However, mass-rearing of the larvae has not been achieved. A major reason is that optimal food supplies and feeding behavior of the shrimp on *Artemia* nauplii are poorly known.

The objective of the present study was to determine the ingestion rate, development

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TABLE 1. *Ingestion rate (nauplii/larva per d) of* Lysmata wurdemanni *zoeal larvae on* Artemia *nauplii (mean ± SD,* N = *3).*

| | Food concentration (nauplii/mL) | | | |
|---|---|---|---|---|
| | 2 | 5 | 10 | 20 |
| Temperature: 25 C | | | | |
| Zoea II | 29 ± 1 | 48 ± 3 | 62 ± 3 | 79 ± 5 |
| Zoea III | 30 ± 1 | 51 ± 3 | 65 ± 3 | 84 ± 4 |
| Zoea IV | 32 ± 2 | 53 ± 2 | 71 ± 3 | 87 ± 2 |
| Zoea V | 33 ± 1 | 57 ± 4 | 75 ± 5 | 87 ± 4 |
| Zoea VI | 34 ± 2 | 62 ± 4 | 71 ± 6 | 90 ± 4 |
| Zoea VII | 35 ± 0 | 64 ± 3 | 73 ± 5 | 93 ± 7 |
| Temperature: 28 C | | | | |
| Zoea II | 31 ± 2 | 57 ± 3 | 74 ± 6 | 94 ± 2 |
| Zoea III | 34 ± 1 | 59 ± 3 | 75 ± 5 | 96 ± 4 |
| Zoea IV | 36 ± 2 | 59 ± 4 | 79 ± 6 | 95 ± 3 |
| Zoea V | 36 ± 3 | 66 ± 6 | 82 ± 5 | 98 ± 6 |
| Zoea VI | 36 ± 1 | 65 ± 4 | 79 ± 4 | 99 ± 7 |
| Zoea VII | 37 ± 1 | 68 ± 5 | 87 ± 6 | 106 ± 5 |

rate, and survivorship of zoeal stage larvae of *L. wurdemanni. Artemia* nauplii were used as the food item because a previous study in our laboratory had shown that it resulted in better growth for the shrimp larvae than either rotifer or microalgae.

## Materials and Methods

The larvae used in the experiments were obtained from egg-bearing shrimp that were cultured at the Harbor Branch Oceanographic Institution. Newly hatched larvae were cultured in a 20-L plastic tank that was kept at 27–28 C, and fed with freshly hatched *Artemia* nauplii at a food concentration of 3–5 nauplii/mL. Daily complete water and food change were conducted.

### *Experiment 1: Effect of Developmental Stage, Temperature and Food Concentration on Food Ingestion Rate*

The shrimp larvae undergo seven zoeal stages (Crompton 1992). Ingestion rate of zoea II to zoea VII at the first day of each stage was investigated (Table 1). Zoea I larva can reach zoea II stage without food at 26 C in about 26 h after hatching. The ingestion rate experiment was carried out in 500-mL beakers each with 400 mL of gently aerated sterilized (with chlorine) sea water (31 ppt salinity). Each beaker contained 15–20 larvae collected from the stock culture tank. To keep all the experimental larvae at the same developmental stage, larvae with delayed development were replaced daily with the larvae from the stock culture tank. A three-way (development stage, temperature and food concentration) factorial experiment with three replicates was conducted. Temperature was controlled by water bath at two levels, 25 C and 28 C. There were four food concentration levels: 2, 5, 10, and 20 *Artemia* nauplii per mL. Food was renewed daily. Decrease in food density over the 24-h period was determined. The ingestion rate, $I$, was calculated using the equation (Paffenhofer 1971):

$$I = V(C_t - C_0)/nt$$

where $(C_t - C_0)$ is the decrease in concentration of nauplii within the experimental period, $t$ (24 h). $V$ is the water volume and $n$ is the number of shrimp larvae (the mean of the initial and final number of larvae in the 24-h period). The food concentration in each experimental beaker was determined by counting the number of nauplii in seven 1–5 mL samples collected by a 10-mL pipette. The samples with the highest and lowest concentrations (number/mL) were excluded. The average concentration of the remaining five samples was calculated to represent the food concentration.

### *Experiment 2: Effects of Food Density on Survival and Development*

The percentage of larvae with delayed development was measured from zoea IV to zoea V in Experiment 1. The larvae obviously delayed development at food levels of 2 and 5 nauplii/mL (Table 2). An experiment was conducted to confirm and further determine the effect of food concentration on the zoeal larval development and survival. Based on the results of Experiment 1, three food concentrations (3, 6, 12 *Artemia* nauplii/mL) were selected. The experiment was conducted in 1,400-mL beak-

TABLE 2. *Percentage of larvae with delayed development from zoea IV to zoea V stages at different food concentrations and temperatures (mean ± SD, N = 3).*

| Temperature | Food concentration (nauplii/mL) | | | |
|---|---|---|---|---|
| | 2 | 5 | 10 | 20 |
| 25 C | 25.9 ± 1.6 | 25.4 ± 2.9 | 13.9 ± 2.4 | 13.0 ± 3.3 |
| 28 C | 24.0 ± 0.9 | 24.9 ± 2.5 | 13.4 ± 2.8 | 14.0 ± 3.2 |

ers each with 1,000 mL gently aerated sterilized (with chlorine) sea water (31 ppt, 26.5–28.5 C). Initially 30 larvae were put in each beaker with four replicates for each food level. All water and food were changed daily. Survivorship and development rate (number of molts) were recorded every day.

### *Experiment 3: Effects of Molting on Food Ingestion Rate*

This experiment was to test the effect of molting on food ingestion rate. The zoeal larvae were placed in 50-mL beakers with 40 mL sterilized (with chlorine) water without aeration. The water temperature was aerated and maintained at 28 C, and food concentration at 5 *Artemia* nauplii/mL. Five shrimp, each in a beaker, were followed from zoea II to zoea VII. Food concentration was measured at 2230 h and 1030 h every day. Because most larvae molted between 2100 h to 2400 h, 2230 h was selected as the measurement time and all food was renewed at 1030 h.

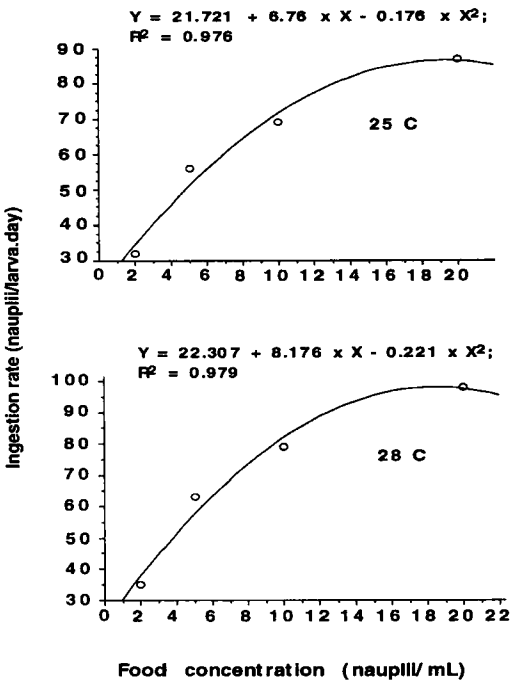


FIGURE 1. *Regression of ingestion rate on food concentrations at the temperatures of 25 C (upper) and 28 C (lower).*

Three-way ANOVA was used to analyze the effects of development stage, food concentration and temperature on ingestion rate. The Welsch step-up method (Sokal and Rohlf 1981) was used to compare the larval survivorship (Experiment 2) and percentage of larvae with delayed development (Experiment 1) among the different food concentrations. Difference in ingestion rate of the larvae between 50-mL and 500-mL beakers was analyzed by Student's *t* test.

## Results

The ingestion rate increased with increasing food concentration and slightly with increasing temperature for all developmental stages (Table 1). It also generally increased with larval development (Table 1). Mean (of all the stages) daily ingestion rate of zoeal larvae increased asymptotically with increasing food concentration in both temperature treatments (Fig. 1). Three-way ANOVA results show that effects of temperature, food concentration and development stage are all highly significant ($P < 0.01$). However, the only significant interaction term was between temperature and food concentration ($P < 0.001$).

In Experiment 1, some larvae began to delay development when metamorphosing

TABLE 3. *Average development time (d) at different food concentrations.*

| Stage | Food concentration (nauplii/mL) | | |
|---|---|---|---|
| | 3 | 6 | 12 |
| z-I–z-II | 1.2 | 1.2 | 1.2 |
| z-II–z-III | 2 | 2 | 2 |
| z-III–z-IV | 2 | 2 | 2 |
| z-IV–z-V | 2 | 2 | 2 |
| z-V–z-VI | 2 | 4 | 4 |
| z-VI–z-VII | 3 | 5 | 5 |
| Total | 14 | 18 | 18 |

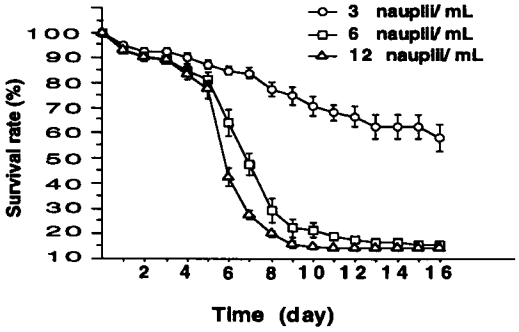


FIGURE 2. *Survivorship of the larvae cultured at different food concentrations (mean ± SE).*

from zoea IV to zoea V. There was no significant difference ($P > 0.05$) in percentage of larvae with delayed development (molt interval over 2 d) between food levels of 2 and 5 nauplii/mL, and between 10 and 20 nauplii/mL levels. The percentage of larvae with delayed development was significantly lower ($P < 0.005$) for the shrimp of higher food density levels (10 and 20 nauplii/mL (Table 2). Surprisingly, in Experiment 2, higher food concentrations (6 and 12 nauplii/mL) resulted in delayed larval development (Table 3).

The Experiment 2 also showed that food density affected the larval survivorship (Fig. 2). Average survivorship of zoeal larvae was higher at the food level of 3 nauplii/mL than that of 6 and 12 nauplii/mL. From day 6 to day 7, survivorship of the larvae at 6 and 12 nauplii/mL dropped sharply from about 80.0% to 64.2% and 42.5%, respectively. By the zoea VII stage (day 16), survivorship of the larvae at 3 nauplii/mL food level was 62.5%, but only 15.9% and 14.2% at 6 and 12 nauplii/mL food levels, respectively. All larvae cultured at the three food concentrations developed at the same rate (7.2 d from zoea I to zoea V) until they reached zoea V (Table 3). The larvae took an additional 9 d to reach zoea VII stage at the food levels of 6 and 12 nauplii/mL, but only 5 d at 3 nauplii/mL (Table 3).

In Experiment 3, each zoea stage (II to VII) lasts about 2 d and the molting to a new stage takes place on the second day. Table 4 shows that the ingestion rate was significantly lower ($P < 0.01$) at 12 h (1030 h of the 2nd day) before than that at 12 h after the molt (2230 h of the 2nd day). The ingestion rate increased sharply to the normal rate the following day (Table 4). For each of the larval stages from III to VII, the average ingestion rate on the first day (sum of the rates at 1030 h and 2230 h, Table 4) was significantly higher ($P < 0.05$) than that in the 5 nauplii/mL and 28 C treatment of the Experiment 1 (Table 1).

## Discussion

It is well known that there is a decrease in feeding rate before molt in adult decapods, and Penaeid shrimp aquaculturists have taken advantage of this phenomenon to save feed. However, there is little information on the effect of molt on larval feeding behavior. The present study shows that molting also resulted in decreased feeding rate in the larvae of all stages (Table 4).

TABLE 4. *Effect of molting on ingestion rate (nauplii/larva per 12 h) of the larvae (mean ± SD, N = 5). Most moltings occurred between 2100 h and 2400 h.*

| Stage/ time | 1st day | | 2nd day | |
|---|---|---|---|---|
| | 1030 h | 2230 h | 1030 h | 2230 h |
| Zoea II | | | 45 ± 5 | 19 ± 4 |
| Zoea III | 48 ± 7 | 43 ± 3 | 44 ± 3 | 25 ± 7 |
| Zoea IV | 50 ± 6 | 49 ± 3 | 46 ± 3 | 28 ± 8 |
| Zoea V | 50 ± 5 | 52 ± 5 | 48 ± 4 | 25 ± 7 |
| Zoea VI | 55 ± 6 | 51 ± 8 | 45 ± 5 | 30 ± 10 |
| Zoea VII | 53 ± 5 | 55 ± 7 | 47 ± 6 | 30 ± 5 |

The increase of temperature from 25 C to 28 C resulted in an increase of ingestion rate. Temperature is one of the most important factors that influences metabolic rate of organisms. Within a certain temperature range, metabolic rate increases with increasing temperature (Hochachka and Somero 1984; Prosser 1986).

Catch efficiency is an important consideration in developing a diet. The present study demonstrates that ingestion rate of *L. wurdemanni* zoeal larvae increased with increasing food concentration. A similar response has been reported for the larvae of many crustacean species (Gopalakrishnan 1976; Emmerson 1984; Yufera et al. 1984; Yufera and Rodriguez 1985; Chu and Shing 1986; Loya-Javellana 1989; Minagawa and Murano 1993). A study on freshwater prawn *Macrobrachium rosenbergii* larvae indicates that the principal form of food capture for this species is chance encounter (Moller 1978).

Higher food densities do not necessarily result in better growth or survivorship. In fact, an excess of food may have a negative effect on the survival of *Penaeus marginatus* (Gopalakrishnan 1976). A similar effect was observed at the present study. The survival (Fig. 2) and developmental rates (Table 3) were significantly lower at 6 and 12 *Artemia* nauplii/mL than those at 3 nauplii/mL. Survival rate dropped sharply at food levels of 6 and 12 *Artemia* nauplii/mL on the 7th day, maybe due to degraded water quality caused by an excess of food (Gopalakrishnan 1976) and/or bacteria explosion due to the availability of more substrate (higher density of *Artemia* nauplii).

Prey density also affects intermolt period and larval development. Numerous reports have shown that lower food concentrations resulted in longer intermolt periods (Welch and Sulkin 1974; Bigford 1978; Wienberg 1982; Heasman and Fielder 1983; Minagawa and Murano 1993). Little information on effect of high food concentration on decapod larval development has been reported. Experiment 1 shows that higher food density treatments resulted in a smaller percentage of larvae with delayed development (Table 2). Surprisingly, higher prey densities actually postponed larval development in Experiment 2. The different results between the two experiments may be due to different food densities, and/or larval source (broodstock). In this species, we have observed that there is an obvious difference in development rate among the individual larvae from the same batch.

The relationship between food density and ingestion rate of larvae is linear in some decapod species (Gopalakrishnan 1976; Yufera et al. 1984), but asymptotic in many others (Yatsuzuka 1960; Emmerson 1980; Yufera and Rodriguez 1985; Chu and Shing 1986; Crials and Anger 1986; Loya-Javellana 1989; Minagawa and Murano 1993; present study). The ingestion rate may increase linearly with increasing food concentration until it reaches a plateau at the saturation food concentration level, after which further increase in food concentration will not result in corresponding increase of ingestion rate.

Ingestion rate of the larvae at the 50-mL beakers (Experiment 3) was significantly higher ($P < 0.05$) than that at 500-mL beakers (Experiment 1) at the same food density (5 *Artemia* nauplii/mL) and temperature (28 C). This may be explained by the different rearing conditions. There was no aeration in the 50-mL beakers. *Artemia* nauplii and *L. wurdemanni* larvae were concentrated by phototaxis in certain areas. Therefore food density was much higher in those areas (200 *Artemia* nauplii for one larva) than those in the 500-mL beakers (2,000 *Artemia* nauplii for 15–20 larvae). Also higher food abundance was available to each larva in the 50-mL beakers. Interactions among the larvae may occur in the 500-mL beaker experiment (15–20 larvae per beaker), but absent in the 50-mL beaker experiment (1 larva per beaker). This influence could be seen from the 2 *Artemia* nauplii/mL treatment. From zoea IV to zoea VII stages, the feeding rate of the larvae did

not change significantly. Because the larvae have a strong swimming ability, absolute food availability may be more important than food density to the larvae. The results suggest that an optimal food concentration of about 3 *Artemia* nauplii/mL and 100–150 *Artemia* nauplii per day should be provided to each larva.

## Acknowledgments

This study was funded by Sea Grant, NOAA, Department of Commerce, USA under project number NA36RG-0700. We appreciate the useful comments made by an anonymous reviewer.

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