JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 29, No. 4
December, 1998

Effects of Food and Temperature on Survival and Development in the Peppermint Shrimp *Lysmata wurdemanni*

DONG ZHANG AND JUNDA LIN[1]

Department of Biological Sciences, Florida Institute of Technology, 150 West University Boulevard, Melbourne, Florida 32901-6988 USA

R. LEROY CRESWELL

Aquaculture Division, Harbor Branch Oceanographic Institution, Inc., 5600 US 1 North, Fort Pierce, Florida 34946 USA

Abstract.—The effects of four kinds of foods (*Artemia* nauplii, rotifer, and microalgae *Chaetoceros gracilis* and *Isochrysis galbana*) and four temperature regimes on survival and development of larval *Lysmata wurdemanni*, a marine ornamental shrimp, were determined. The larvae fed with *Chaetoceros* or *Isochrysis* only survived for a maximum of 17 d, before developing to zoea IV. The survivorship of the larvae fed with *Artemia* nauplii or rotifer from zoea II to postlarvae was 66.7% and 68.9%, respectively, without significant difference ($P > 0.05$). But larvae fed with *Artemia* nauplii grew significantly ($P < 0.05$) faster than those fed with rotifer. Larvae fed with *Artemia* nauplii reached postlarvae in 29–32 d, compared with 32–36 d in the rotifer treatment. *Artemia* nauplii are also suitable food for the postlarvae and juveniles of *L. wurdemanni*. The 30 postlarvae fed on *Artemia* nauplii all survived to reach sexual maturity in 50 to 70 d, growing from about 7 to 28 mm in total length. The effects of four temperature regimes on larval development of *L. wurdemanni* was also investigated. The duration (mean ± SD d) to the postlarvae at temperatures of 26 C (37.4 ± 5.4) and of 26–30 C daily (40.2 ± 5.8) was significantly ($P < 0.05$) longer than that at 28.5 C (29.3 ± 4.8) and at 27–29 C daily (28.7 ± 3.5).

The peppermint shrimp *Lysmata wurdemanni* occurs naturally along the Atlantic and Caribbean coasts of North and South America from New Jersey to Brazil (Williams 1984). It is commonly associated with hard coastal substrates such as jetties, rock outcropping, piers and buoys, and in association with tubular sponges, especially of the genus *Aplysina* (Sefton and Webster 1986). The genus *Lysmata* is among the many cleaner shrimps that are popular with aquarists, because of their coloration and the ease with which they can be maintained in captivity. Currently, all cleaner shrimps for aquarium industry are collected from the natural environment. The effects of removal of them from the coral reef ecosystem cause concern. The gap between supply and demand is expected to be reduced by aquaculture.

Efforts to culture *Lysmata* under artificial conditions have been made. Reproductive biology, broodstock nutrition, and development of different life stages in *L. debelius* and *L. ambionensis* have been studied (Fletcher et al. 1995; Simoes et al. 1998a, 1998b). Debelius (1984) reared the larvae of *L. seticaudata* to postlarvae. Blanchard (1992) reared *L. grabhami* larvae, but failed to culture them to postlarval stage. The larvae of *L. debelius* and *L. amboinensis* are able to feed on rotifers or small strain *Artemia salina* (Fletcher et al. 1995). *L. wurdemanni* larvae fed with rotifer (before day 14) and *Artemia* nauplii have also been cultured successfully under artificial conditions (Crompton 1992). The optimization of feeding regimes used during the shrimp larval rearing process is a major objective of the larviculture operation. Effect of different foods on the larval survival and development has not been tested for *L. wurdemanni*. To develop rearing techniques for the species, we conducted a study using four different diets, *Artemia* nauplii, rotifer *Brachionus plicatilis*, microalgae *Chaetoceros gracilis* and *Isochrysis galbana*. *Artemia* nauplii were also tested as a potential food for the postlarvae and juveniles.

It is well known that temperature affects

[1] Corresponding author.

growth rate and development in decapod larvae, but study on the effect of fluctuating temperature on the larval development is limited. In the present study, we also tested the effect of constant and fluctuating temperatures on larval development of the peppermint shrimp.

Materials and Methods

This study was conducted at Harbor Branch Oceanographic Institution, Inc., Fort Pierce, Florida, USA, between October, 1995 and October, 1996.

Larval Hatching

Reproductive biology of *L. wurdemanni* is similar to that of *L. ambionensis* and *L. debelius* (Fletcher et al. 1995; Simoes et al. 1998a). It is also a simultaneous hermaphrodite species. Fertilization occurs after molting and the eggs are retained under the adult's abdomen where they hatch as zoea larvae 10 to 12 d later.

The ovigerous shrimp were maintained in an indoor recirculating seawater system under 14 h light and 10 h dark. Temperature fluctuated about 1–1.5 C daily (between 26 and 29.5 C during the study period). The shrimp were fed in excess with frozen *Artemia* or squid once a day. Any shrimp that was going to hatch was moved to a 270-L conical fiberglass tank equipped with an internal standpipe with 53-μm mesh. After the shrimp had hatched and molted, it was returned to the broodstock tank. The newly hatched zoea I larvae were kept in the fiberglass tank (without feeding) for about 26 h, when they molted to zoea II, before being transferred to the experimental beakers for the food or temperature experiments.

Food Experiment

For the food experiment, sibling larvae were placed in 4-L bottles with 2.5 L of sea water (33–35 ppt salinity and 29 C temperature), with gentle agitation by bubbling. Each bottle contained 15 zoea II larvae. The light intensity was 5.3–6.9 μmol/s per m. Water exchange rate was 50% daily. Algae, *Artemia* nauplii, and rotifer were renewed everyday. The experiment included the following four treatments (each with three replicates): newly hatched *Artemia* nauplii, rotifer *Brachionus plicatilis*, microalgae *Chaetoceros gracilis* and *Isochrysis galbana*. The nauplii were obtained daily by hatching (26 C for 20 h) the cysts of small strain *Artemia franciscana* (grade 0 (platinum), Argent Chemical Laboratories). The rotifer (fed with *Chlorella*) and algae *Chaetoceros gracilis* and *Isochrysis galbana* used in the experiment were from the culture maintained by the Harbor Branch Oceanographic Institution. In all the treatments, food was provided in excess. The density for *Artemia* nauplii was 5–10/mL, rotifer 10–15/mL, *Chaetoceros* and *Isochrysis* 50,000–100,000 cells/mL. All experimental bottles were arranged randomly. Total length (TL) of 3 haphazardly selected larvae were measured (to the nearest 0.1 mm) using a micrometer under a dissecting microscope every 2 or 3 d starting at day two. Number of surviving larvae were counted once every 3 to 5 d.

Temperature Experiment

The temperature study was carried out in 200-mL beakers each containing 150 mL sterilized seawater of 30 ppt salinity and one larva. The larvae were fed with newly hatched *Artemia* nauplii. Complete water and food changes were conducted daily. The beakers were placed in a water bath. Two constant temperatures: 26 and 28.5 C (maintained by a water bath with submerged heaters in an indoor laboratory), and two fluctuating temperatures (between 27 and 29 C, and between 26 and 30 C day and night) (maintained by a large and small water bath with submerged heaters, respectively, in an outdoor laboratory) were tested. Seven replicate beakers were used for each temperature treatment. The TL of the shrimps was measured to the nearest 0.1 mm after each molting. Development time to postlarvae was recorded.

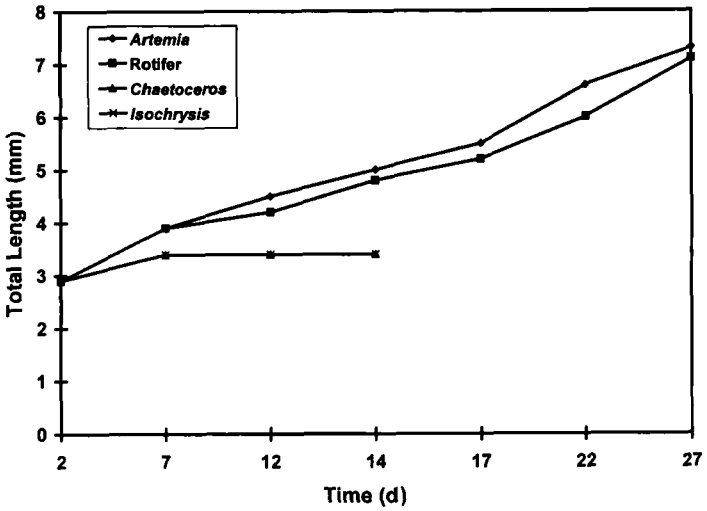

FIGURE 1. *Total length of* Lysmata wurdemanni *larvae fed with different foods.*

Culture of Postlarvae and Juveniles

Thirty postlarvae (mean ± SD TL: 7 ± 1 mm, measured using a ruler to the nearest 1 mm), fed with newly hatched *Artemia* nauplii since hatching, were put in a 25-L circular recirculate tank and were also fed with newly hatched *Artemia* nauplii (10–15/mL daily). The water (26.5–29 C, 30 ppt) was changed (25%) every other day. The shrimp were observed daily until they reached sexual maturity (indicated by the greenish developed gonad). Fifteen randomly selected shrimp were measured using a ruler (TL to the nearest 1 mm) at day 30 and day 60.

Data Analysis

One-way analysis of variance (ANOVA) was used to analyze the survivorship of the larvae fed with different diets, and the effects of temperature on development time. The T-method multiple comparison test was used to compare the means when ANOVA shows significant effect (Sokal and Rohlf 1995). Homogeneity of variance was assessed using Bartlett's test before ANOVA. Student's *t*-test was used to compare the percentages of *L. wurdemanni* larvae fed with *Artemia* and rotifer reaching postlarval stage.

Results

Food Experiment

Survivorship of larvae fed with *Artemia* nauplii or rotifer (86.7% and 88.9%, respectively) is significantly higher than that fed with *Chaetoceros* (15.6%) or *Isochrysis* (13.3%) on day 15. Similar percentages (66.7% and 68.9%, respectively) of the larvae fed with *Artemia* nauplii or rotifer reached the postlarval stage (*t*-test, $P > 0.05$). One way ANOVA results show that significant ($P < 0.001$) difference in survivorship occurred after day 10 among the treatments. There was no significant ($P > 0.05$) difference between the *Chaetoceros* and *Isochrysis* treatments, or between *Artemia* and rotifer treatments. However, survivorship in *Artemia* or rotifer treatment was significantly ($P < 0.01$) higher than that in *Chaetoceros* or *Isochrysis* treatment. A sharp decline in survival of larvae fed with the algae was observed after the 14th day. The larvae in the algal treatments stayed at zoea III stage until all died on day 17; at that time the larvae fed with *Artemia* nauplii or rotifer had reached mysis III stage.

The growth of larvae fed with different diets are shown in Fig. 1. The larvae fed with the algae did not grow after they became zoea III. Larvae fed with *Artemia*

TABLE 1. *Mean (±SD, N = 7) total length (mm) of different larval stages (Z = zoea, e.g., ZI = Zoea I; M = mysis, e.g., MI = Mysis I) of* Lysmata wurdemanni *reared at different temperature regimes.*

Temperature	ZI	ZII	ZIII	ZIV	ZV	ZVI
26.0 C	2.4 ± 0.7	2.7 ± 1.1	3.0 ± 0.8	3.2 ± 1.0	3.7 ± 1.1	4.2 ± 0.8
28.5 C[a]	2.4 ± 0.8	2.7 ± 0.8	3.0 ± 0.7	3.3 ± 1.1	3.7 ± 0.8	4.2 ± 0.7
27–29 C	2.4 ± 0.7	2.7 ± 0.9	3.0 ± 0.5	3.3 ± 1.0	3.7 ± 1.1	4.2 ± 0.8
26–30 C	2.4 ± 0.6	2.7 ± 0.7	3.0 ± 0.7	3.3 ± 0.8	3.7 ± 1.1	4.2 ± 0.9

[a] $N = 6$ after zoea VII.

nauplii developed faster, reaching postlarvae 3 to 4 d earlier than those fed with rotifer. At 29 C, larvae fed with *Artemia* nauplii and rotifer took 29–32 d and 32–36 d to reach postlarval stage, respectively. The larvae readily metamorphosed to postlarvae in the experimental bottles.

Temperature Experiment

All larvae developed to postlarvae at 27–29 C and 26 C. At 28.5 C, one larva died at zoea VII. At 26–30 C one larva did not metamorphose to postlarva until day 65, and one larva stayed at zoea until it died on day 41. Multiple comparisons test (T-method) revealed that the duration to postlarvae (mean ± 1 SD d) was not significantly ($P > 0.05$) different between 28.5 C (29.3 ± 4.8) and 27–29 C (28.7 ± 3.5), or between 26 C (37.4 ± 5.4) and 26–30 C (40.2 ± 5.8), but significantly ($P < 0.05$) different between the two groups (28.5 C and 27–29 C vs. 26 C and 26–30 C). The shortest development time to metamorphosis, 25 d, was recorded at 27–29 C: 13 d for zoeal stages (one d for zoea I to zoea II, then two d for each of the subsequent stages), and 12 d for the mysis stages (two d for each of the stages). Larvae with prolonged development underwent 7 to 11 molts during the mysis stages with an intermolt of 3 to 4 d. One larva did not reach postlarva at 26–30 C even after molting 17 times.

Although the rates of growth and development were affected by temperature, TL of the larvae was similar among the temperature treatments for each of the 13 larval stages (seven zoea and six mysis) (Table 1). In general, larvae metamorphosed to postlarvae before reaching 8 mm in TL.

Culture of Postlarvae and Juveniles

All the 30 postlarvae (7 ± 1 mm TL) survived to reach sexual maturity in 50 to 70 d when they reached about 28 mm in TL. The mean TL (±SD) of the shrimp at day 30 and day 60 was 20 (±2) and 28 (±2) mm, respectively.

Discussion

Food Experiment

Algae are used to feed zoeal larvae of penaeid shrimps (Gopalakrishnan 1976; Tobias-Quinitio and Villegas 1982; Wilkenfeld et al. 1984; Cao et al. 1990). Addition of algae also enable *L. grabhami* larvae to survive longer (Blanchard 1992). However, algae may not be appropriate food for *L. wurdemanni* larvae (Crompton 1992). *Artemia* nauplii and rotifers are suitable food for a variety of decapod larvae (McConaugha 1985), including newly hatched larvae of *Lysmata* species (Crompton 1992; Fletcher et al. 1995; present study). This is different from penaeid shrimp larvae which can only consume *Artemia* nauplii when they reach zoea III, even mysis I stage. The zoea I larvae of *L. wurdemanni* (2.4 mm TL) is equivalent in size to mysis I stage of penaeid shrimps. Rotifer can be used as food for *L. wurdemanni* larvae as well, although the larvae fed with rotifer or microalgae grew slower than those fed with *Artemia* nauplii. This may be due to smaller size and/or less suitable nutrition content of rotifers and microalgae. Food size is an important consideration in the larval rearing (Frost 1972), as shown in seven decapod species where small algae only occasionally support larval development compared to

TABLE 1. *Extended.*

ZVII	MI	MII	MIII	MIV	MV	MVI
4.7 ± 0.9	5.3 ± 1.1	5.8 ± 1.1	6.4 ± 1.0	6.6 ± 1.4	6.9 ± 1.1	7.1 ± 1.0
4.7 ± 0.8	5.4 ± 1.0	5.9 ± 1.0	6.4 ± 0.8	6.6 ± 1.1	6.9 ± 0.9	7.2 ± 1.1
4.7 ± 0.9	5.3 ± 1.0	5.8 ± 1.5	6.4 ± 0.9	6.6 ± 1.2	7.0 ± 1.3	7.2 ± 1.1
4.7 ± 0.7	5.4 ± 1.0	5.8 ± 1.4	6.4 ± 0.8	6.6 ± 1.7	6.9 ± 1.2	7.1 ± 1.5

larger algae (Harms and Seeger 1989). In decapod larvae, ingestion rate is normally low for small sized food (Grahame 1983). Larvae may be able to select food based on size. Larger animals and later development stages may take larger sized food (Grahame 1983). *Penaeus kerathurus* postlarvae did not ingest rotifiers when *Artemia* were present (Yufera et al. 1984).

Temperature Experiment

Temperature has been recognized as one of the main environmental factors affecting the development and growth of crustaceans (Abele 1982). Within optimal temperature range, larval development time decreases with increasing (constant) temperature (Sastry 1977). Customarily, optimal temperature for larvae is identified by subjecting larvae to different constant temperatures. However, crustacean larvae live in fluctuating temperature conditions in the natural environment. Larvae of the few crustaceans cultured at fluctuating temperature survived and developed better than those at constant temperatures (Costlow and Bookhout 1971; Sastry 1976, 1977). Our study also suggests that small fluctuating temperature (1.5–2.0 C daily) may be beneficial for the development of *L. wurdemanni* larvae. Poikilotherms are capable of temperature compensation. They frequently exhibit similar metabolic rates at widely different habitat temperatures. This process, however, consumes energy (Hazel and Prosser 1974). Therefore development and growth, even survival, are affected when temperature fluctuation is too high.

In crustacean larvae, growth is closely correlated with development. Criales and Anger (1986) found a trend of decreasing carapace length with increasing temperature in the shrimp *Crangon crangon*. However, zoeal larvae of a deep sea shrimp *Pandalus borealis* reach the same length at the same stage regardless of temperature (Wienberg 1982). Similar results were obtained in our study of *L. wurdemanni*.

L. wurdemanni passes through at least 13 larval stages (seven zoea and six mysis) before metamorphosing to the postlarval stage in about 25 to 40 d after hatching (present study), but can be as long as 67 d (Crompton 1992). Variations in time to metamorphosis has also been reported in *L. ensirostris* and *L. vittata* (Abele 1982), and in *L. debelius* (Fletcher et al. 1995). Temperature regime may play an important role in the larval development rate and may be used to shorten the larviculture duration. The larvae develop faster in *L. wurdemanni* than in the other two *Lysmata* species that have aquaculture potential: *L. debelius* (11–15 wk) and *L. ambionensis* (20 wk) (Fletcher et al. 1995).

Culture of Postlarvae and Juveniles

Artemia nauplii are also appropriate food for the postlarvae and juveniles of *Lysmata wurdemanni*, as the 30 peppermint shrimp all survived and reached sexual maturity in 50 to 70 d. These shrimp had been fed with *Artemia* nauplii since hatching. The high survivorship of different life cycle stages of *L. wurdemanni* fed on *Artemia* nauplii only and relatively short duration to sexual maturity are very encouraging for the mass culture prospect of the species.

Acknowledgments

This study was funded by Sea Grant, NOAA, Department of Commerce, USA

(grant number NA36RG-0700). Anonymous reviewers gave valuable comments on an earlier version of the manuscript.

Literature Cited

Abele, L. G. 1982. Larval morphology and diversity. Pages 43–100 *in* F. J. Vernberg and W. B. Vernberg, editors. The biology of crustacea, volume 2. Academic Press, New York, USA.

Blanchard, L. 1992. Ornamental shrimp culture: *Lysmata grabhami*. Technical report. Harbor Branch Oceanographic Institution, Inc., Fort Pierce, Florida, USA.

Cao, S., B. Xiang and H. Fang. 1990. Effect of several microagle on survival and development of *Penaeus chinensis*. Hai Yang Tong Bao, 9(3):27–33 (in Chinese).

Costlow, J. D. and C. G. Bookhout. 1971. The effect of cyclic temperature on larval development in the mud crab, *Rhithropanopeus harrisii*. Pages 211–220 *in* D.J. Crisp, editor. Fourth European Symposium on Marine Biology. Cambridge, Cambridge University Press, United Kingdom.

Criales, M. M. and K. Anger. 1986. Experimental studies on the larval development of the shrimps, *Crangon crangon* and *Crangon allmani*. Helgolander Meeresunters 40:241–265.

Crompton, W. D. 1992. Laboratory culture and larval development of the peppermint shrimp, *Lysmata wurdemanni* Gibbes (Caridea: Hippolytidae), Part I: Laboratory culture. Master's thesis, Corpus Christi State University, Corpus Christi, Texas, USA.

Debelius, H. 1984. Armored knights of the sea. Kernen Verlag, Essen, Germany and Quality Marine, Los Angeles California, USA.

Fletcher, D. J., I. Kotter, M. Wunsch and I. Yasir. 1995. Preliminary observations on the reproductive biology of ornamental cleaner prawns. International Zoo Yearbook 34:73–77.

Frost, W. B. 1972. Effects of size and concentration of food particles on the feeding behaviour of the marine planktonic copepod, *Calanus pacificus*. Limnology and Oceanography 17:805–815.

Gopalakrishnan, K. 1976. Larval rearing of red shrimp *Penaeus marginatus* (Crustacea). Aquaculture 9:145–154.

Grahame, J. 1983. Adaptive aspects of feeding mechanisms. Pages 65–107 *in* F. J. Vernberg and W. B. Vernberg, editors. The biology of crustacea, volume 8. Academic Press, New York, USA.

Harms, J. and B. Seeger. 1989. Larval development and survival in seven decapod species (Crustacea) in relation to laboratory diet. Journal of Experimental Marine Biology and Ecology 133:129–139.

Hazel, J. R. and C. L. Prosser. 1974. Molecular mechanisms of temperature compensation in poikilotherms. Physiological Review 54:620–676.

McConaugha, J. R. 1985. Nutrition and larval growth. Pages 127–154 *in* A. M. Wenner, editor. Larval growth. A. A. Balkema Press, Rotterdam, The Netherlands.

Sastry, A. N. 1976. Effects of constant and cyclic temperature regime on the pelagic larval development of a brachyuran crab. Pages 81–87 *in* G. W. Esch and R. W. McFarlane, editors. Thermal ecology II, ERDA Symposium Series 40, Technical Information Center, Energy Research and Development Administration, Oak Ridge, Tennessee, USA.

Sastry, A. N. 1977. Physiological adaptation of *Cancer irroratus* larvae to cyclic temperatures. Pages 57–65 *in* D. S. McLusky and A. J. Berry, editors. Physiology and behavior of marine organisms, Pergamon Press, Oxford, United Kingdom.

Sefton, N. and S. K. Webster. 1986. Caribbean reef invertebrates. Sea Challengers, Monterey, California, USA.

Simoes, F., I. Yasir and D. A. Jones. 1998a. Reproductive biology of *Lysmata debelius* (Bruce 1983) and *L. ambionensis* (De Man 1888) (Caridea, Hippolytidae) tropical marine cleaner shrimps important in the marine aquarium trade. Book of Abstracts, Aquaculture '98, February, 1998. World Aquaculture Society, Las Vegas, Nevada, USA.

Simoes, F., F. Ribeiro and D. A. Jones. 1998b. The effect of diet on the reproductive performance of marine cleaner shrimps *Lysmata debelius* (Bruce 1983) and *L. ambionensis* (De Man 1888) (Caridea, Hippolytidae) in capitivity. Book of Abstracts, Aquaculture '98, February, 1998. World Aquaculture Society, Las Vegas, Nevada, USA.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry, third edition. W. H. Freeman and Company, New York, USA.

Tobias-Quinitio, E. and C. T. Villegas. 1982. Growth, survival and macronutrient composition of *Penaeus monodon* Fabricius larvae fed with *Chaetoceros calcitrans* and *Tetraselmis chuii*. Aquaculture 29:253–260.

Wienberg, R. 1982. Studies on the influence of temperature, salinity, light and feeding rate on laboratory reared larvae of deep sea shrimp, *Pandalus borealis*. Meeresforsch 29:136–153.

Wilkenfeld, J. S., L. A. Lawrence and D. F. Kuban. 1984. Survival, metamorphosis and growth of penaeid shrimp larvae reared on a variety of algal and animal foods. Journal of World Mariculture Society 15:31–49.

Williams, A. B. 1984. Shrimps, lobsters, and crabs of the Atlantic coast of the Eastern United States, Maine to Florida. Smithsonian Institution Press, Washington, D. C, USA.

Yufera, M., A. Rodriguez, and L. M. Lubian. 1984. Zooplankton ingestion and feeding behavior of *Penaeus kerathurus* larvae reared in the laboratory. Aquaculture 42:217–224.