Phuket mar. biol. Cent. Res. Bull. **60**: 29-35 (1995)

THE LIFE CYCLE STAGES OF THE LEPADOMORPH BARNACLE, *OCTOLASMIS COR*, AND METHODS FOR THEIR LABORATORY CULTURE

By William B. Jeffries[1], Harold K. Voris[2], Sombat Poovachiranon[3] and Laurie C. Heil[1].

[1] Dickinson College, Carlisle, PA., U.S.A. [2] Field Museum of Natural History, Chicago, IL., USA, [3] Phuket Marine Biological Center, Phuket, Thailand.

ABSTRACT

Symbiotic barnacles, *Octolasmis cor* (Cirripedia, Thoracica), were removed live from the gills of mangrove crabs, *Scylla serrata*. They were cultured in filtered sea water and fed freshly hatched brine shrimp larvae at the Phuket Marine Biological Center, Thailand. Groups of up to 30 sexually mature *O. cor* growing in small glass bowls provided a reliable source of Nauplius 1 and 2 larvae. Methods are presented for culturing adults and the naupliar larval stages. Brief anatomic descriptions and drawings of the larval stages are provided as a guide to their recognition. A size comparison of the naupliar stages of *O. cor* is made with the larvae of three other octolasmid species: *O. aymonini geryonophila; O. forrestii*; and *O. mülleri*, previously reported in the literature.

INTRODUCTION

Decapod crustaceans are favored as food in many tropical countries and some may bear one to several symbiotic (*Sensu lato*, de Bary, 1879) lepadomorph barnacle species of the genus *Octolasmis*, frequently in large numbers and often to the hosts' disadvantage (Gannon and Wheatley, 1992). Wherever the host decapods are crowded as in tidal ponds, or held and fed in suspended cages to increase their weight and marketability, the barnacles are a potential problem. The octolasmids are spatial "parasites" that occupy gill surfaces of hosts, thereby diminishing the area available for normal gas exchange. Tidal ponds are currently used for crab aquaculture in the Philippines and there are floating-cage crab holding stations in the Straits of Johore, off Singapore.

Octolasmids are distributed worldwide in tropical and temperate seas, with most living on the exoskeleton of decapod Crustacea, *e.g.*, in Singapore waters twenty-seven of fifty-six decapod species examined hosted up to seven *Octolasmis* species each (Jeffries *et al.*, 1982).

The adults of more than thirty species of the lepadomorph barnacle *Octolasmis* (Poecilasmatidae) have been characterized since the genus was first described by Gray in 1825. The *Octolasmis* life cycle includes six nauplius (N1 to N6) and one cypris larval stages. All larval stages were reported for *Octolasmis mülleri* (Coker, 1902) and all but the cypris stage were described for *Octolamis forrestii* (Stebbing, 1894) by Lang (1976, 1979). Nauplius stages (N1 to N4) of *Octolasmis aymonini geryonophila* were reported by Colón-Urban *et al.* (1979).

The research reported here provides: outline drawings and general descriptions of *O. cor* (Aurivillius, 1892) nauplius and cypris larval stages which are useful as aids to recognition of specific larval stages in plankton; instructions for the laboratory cultivation of *O. cor* adults and larvae; and a time schedule of larval development.

MATERIALS AND METHODS

Sources of Live Crabs

Recently caught mangrove crabs, *Scylla serrata* (Forskål, 1755) used in our study of natural populations were purchased from fishermen in Phuket, Thailand (Jeffries *et al.*, 1992). In addition, several adult mangrove crabs were purchased at a fish market in Singapore en route to Phuket. These were the sources of the octolasmids used in the present study.

Sources of Live Barnacles

The mangrove crabs were sacrificed by removing the carapace. The eight gills were removed from each of the two gill chambers with forceps, placed in small glass bowls containing fresh sea water, and examined for adult octolasmids. Individual barnacles were removed from their attachment sites on the gill lamellae by grasping the barnacle peduncle base firmly with a jewelers' forceps, but without harmful pinching, while holding the gill in a fixed position. Barnacles were detached with an abrupt, sideways, shearing movement of the forceps. Whereas a few barnacles were destroyed by this procedure, with practice large numbers of intact living barnacles were obtained. Most had small pieces of gill lamellae attached to the peduncle base.

Culture of adult barnacles

Up to 30 adult barnacles were cultured in glass bowls 11 cm in diameter by 5 cm deep, in filtered sea water 1 to 3 cm deep. Freshly hatched brine shrimp, *Artemia salina* (Linnaeus, 1758), were supplied on alternate days as the only food. Typically, the food was concentrated by phototaxis and added at the end of the day so the barnacles would feed in the dark when the brine shrimp larvae moved irregularly, in the absence of light. The next morning, the barnacles were rinsed with fresh sea water to free them of excrement, exuviae, and the remaining brine shrimp larvae.

Following the above regimen, adult barnacles were kept alive, and they remained reproductively active for an indefinite period.

Harvest of nauplius larvae

Under laboratory conditions, liberation of larvae seemed to coincide with the presence of light and, upon release, the barnacle nauplii swam toward the light. Larvae to be used in the growth experiments were captured with a pipette from aggregations at the side of the dish closest to a source of light.

Culture of nauplius larvae

Nauplius larvae of *O. cor* thus obtained were placed in filtered (Nitex HC3-48) and aged (1 to 3 days) sea water recently seeded with actively growing green algae. The cylindrical glass culture jars were of two sizes: 2 L jars, 12 cm in diameter by 23 cm in height, and 8 L jars, 20.3 cm in diameter by 31 cm in height. Covered with clear plastic, the jars were maintained outdoors, in shaded concrete enclosures, partially immersed in flowing sea water. The temperature of the flowing sea water was recorded using a thermal data logger (Onset Instruments, Onset Computer Corp.).

The sea water in the cylinders was inoculated with algae (*Chlorella sp.*, *Tetraselmis sp.*, *Isochrysis galbana* (Parke, 1949), and *Platymonas sp.*) as monocultures or as mixtures. The algae were grown as monocultures in liter quantities on Enrichment f/2 medium (Guillard, 1975), in constant light, and with continuous bubbling of filtered air. Stock *Chlorella sp.* in monoculture proved adequate as a food source, to achieve viable larval cultures and to sustain the nauplius larval stages N2 to N6 to the cypris larval stage. Typically, 2 L and 8 L quantities of sea water were seeded with 5 to 50 ml of stock *Chlorella sp.* to achieve 67 to 670 cells per mm^3, 12 to 36 hours before the larvae were introduced. First and second naupliar stages (N1 and N2) of *O. cor* were introduced *en masse*, 300 to 1,000 per 2 L culture and 2,000 to 5,000 per 8 L culture, when possible, and in installments of smaller numbers over several days when fewer larvae were available.

The cultures of larvae were examined once or twice daily with the aid of a light beam passed through the cultures. Observation with this illumination and the unaided eye allowed approximate estimates of algal density as well as tentative identification of larval stages. Although subjective and imprecise, these observations were used successfully as an approximate, yet dependable, guide to determine the amount of algae to be added to support the growing larvae. More precise algal counts were obtained with a hemocytometer.

For identification, fresh larvae were examined microscopically using corn syrup (Anderson, 1992) in temporary slide mounts, with or without prior staining in fast green. Aqueous methyl cellulose also was used to slow the vigorous movements of live larvae thereby aiding observation, identification, and drawing.

Preservation and illustration

Larvae to be used later in detailed anatomic observations were fixed with 4% glutaraldehyde, postfixed with 2% osmium tetroxide, dehydrated and stored in 70% ethanol. Drawings were made using a camera lucida, measurements were made using an ocular micrometer, and photographs were taken with a Leitz compound microscope and a Minolta 35 mm camera using high contrast black and white film.

RESULTS AND DISCUSSION

Development of Ovigerous lamellae

"The ova, when excluded, remain in the sack [capitular] of the animal until the larvae are hatched", (Darwin, 1851). In octolasmids the first evidence of development is the appearance of two concave, roughly ovoid sheets, the ovigerous lamellae, within the capitulum.

In a study of reproduction and development with adult *O. cor* (n = 20) under controlled conditions at 25±1°C, an average of 9.05 days elapsed from the appearance of ovigerous lamellae in the capitular cavity until release of nauplius (N1) larvae.

Larval growth

In an experiment with *O. cor* nauplius larvae (N1) that had just been released by the parent barnacle, fourteen of 20 N1 larvae molted to the second naupliar stage (N2) within the first hour and, by the end of ten hours, all had molted to N2 (Fig. 1) at 29±1°C.

Under the described culture conditions, nauplius N2 larvae molted to the third naupliar stage (N3) on the third day. Similarly, the next molt, to the fourth naupliar stage (N4), transpired on day five, the fourth to the fifth naupliar stage (N5) on day seven, and the fifth to the sixth naupliar stage (N6) on day nine. Thus, 27 days elapsed from the first appearance of ovigerous lamellae until the first cypris larvae appeared, eighteen days after hatching. Table 1 summarizes development times from hatching to the cyprid stage.

The relationship between the host crab molt cycle and the life cycle of the dependent barnacles is presented diagrammatically in Fig. 2. The naupliar stages N1 to N6 are free in the water column and N2 through N6 feed on micro-plankton, primarily algae. The life cycles of the crab and barnacle are joined when the cypris larvae settle on the appropriate decapod host, *i.e. S. serrata.*

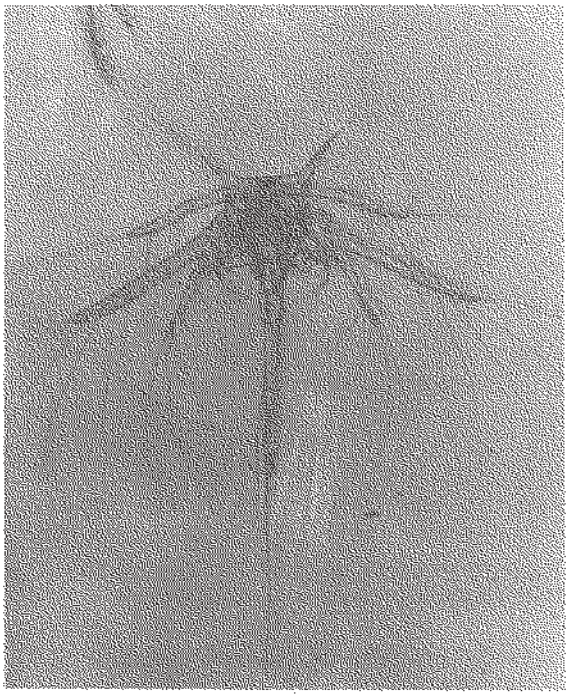

Figure 1. The second naupliar stage (N2) of *Octolasmis cor*.

Table 1. Development times for the larval stages of *Octolasmis cor*. The culturing conditions are given in the text. The times represent the minimums observed.

Stag	Duration (days)	Lapsed time(days)
Nauplius 1	1	1
Nauplius 2	2	3
Nauplius 3	2	5
Nauplius 4	2	7
Nauplius 5	2	9
Nauplius 6	9	18
Cyprid	>10	N.A.

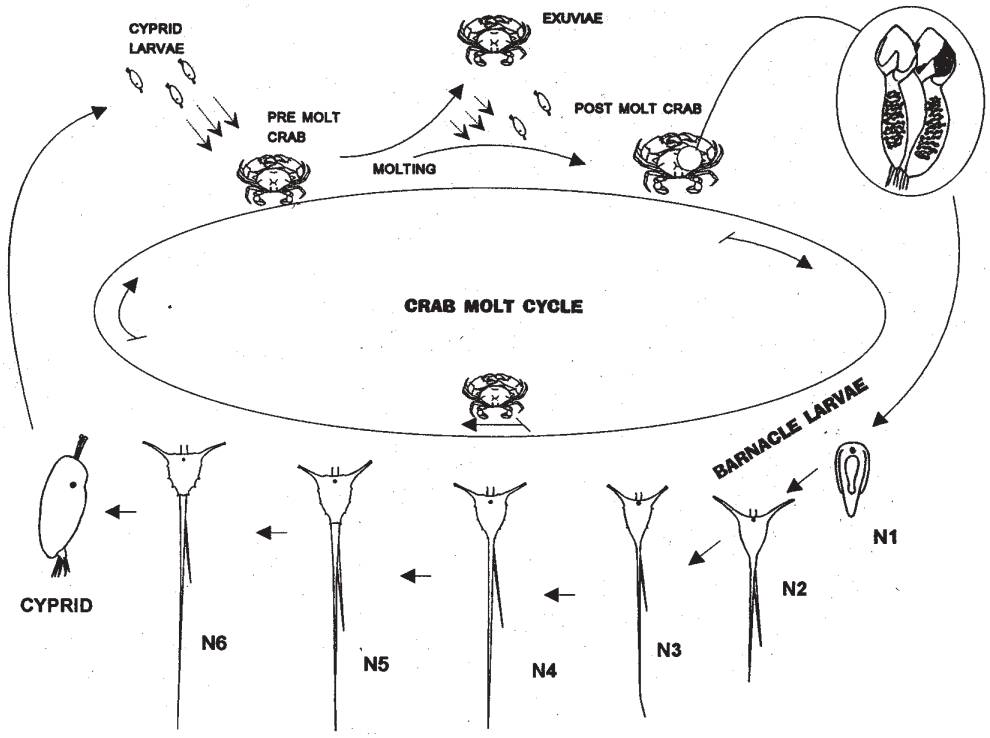

Figure 2. A composite drawing representing the symbiotic relationship between the mangrove crab *Scylla serrata* and the barnacle *Octolasmis cor*.

Introduction to larval stages N1 to cyprid

Outline drawings illustrating basic anatomic features sufficient for identification and for distinguishing the nauplius larval stages of *O. cor* with the aid of a microscope are provided in Fig. 3. An anterior median eye is a feature common to all the larval stages. Below are listed some distinguishing characters of each of the naupliar stages.

Nauplius 1 - paired, tapered frontal horns closely applied to the body and directed posteriad.

Nauplius 2 - paired, tapered frontolateral horns; two frontal filaments directed anteriad; cephalic shield outline smooth; prominent postero-dorsal thoracic spine; and a shorter abdominal process.

Nauplius 3 - paired, tapered frontolateral horns; two fontal filaments directed anteriad; cephalic shield with 2 pairs of lateral spines; prominent postero-dorsal thoracic spine; and a shorter abdominal process.

Nauplius 4 - paired, tapered frontolateral horns; two frontal filaments directed anteriad; cephalic shield with 1 pair of frontal spines and four pairs of lateral spines; prominent postero-dorsal thoracic spine; and a shorter abdominal process.

Nauplius 5 - paired, tapered frontolateral horns; two frontal filaments directed anteriad; cephalic shield with 1 pair of frontal spines, 4 pairs of lateral spines, and a clearly defined posterior margin; prominent postero-dorsal thoracic spine; and a shorter abdominal process.

Nauplius 6 - paired, tapered frontolateral horns; two frontal filaments directed anteriad; cephalic shield broad and robust, with 1 pair of frontal spines and 4 pairs of lateral spines (marginal gland spines, Lang, 1979), and a clearly defined posterior margin; prominent dorsal thoracic spine; and a shorter abdominal process.

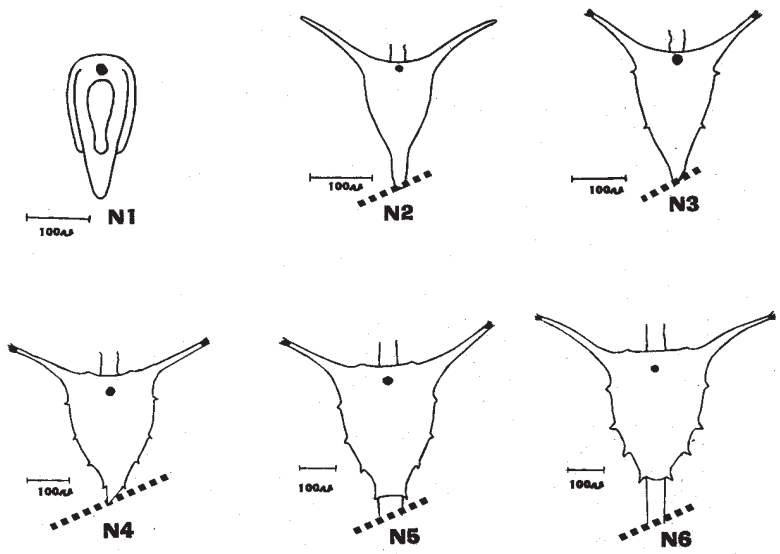

Figure 3. The nauplius stages N1 to N6 of *Octolasmis cor* illustrating the distinguishing features of each instar.

Cypris - laterally compressed, with enveloping bivalve carapace that is smooth in outline and tapered posteriorly; the integument is characterized by surface indentations; body and appendages generally obscure, but with antennules and nauplius eye visible anteriorly and paired thoracic appendages posteriorly.

Comparison with larvae of other species

A preliminary examination of the published descriptions of other *Octolasmis spp.* larval stages suggests they are anatomically similar to *O. cor* and limited comparisons of development times for larvae of the different species is feasible. For example, the minimum time for the larvae of *O. aymonini geryonophila* to grow from early nauplius 1 to early nauplius 6 was 14 days at 16°C (Colón-Urban *et al.*, 1979) whereas *O. cor* larvae took 9 days for this amount of development at 29°C and *O. forrestii* took 25 days at 24 to 27°C (Lang, 1979). *O. mülleri* took 14 days to develop from early nauplius 1 to the cypris stage at 24 to 29°C (Lang, 1976) whereas *O. cor* larvae took 18 days for this amount of development at 29° C.

Although direct comparison of developmental times for the larvae of these species can not be made at this time because of variation in conditions and methods of measurement, the evidence suggests that significant differences may occur and temperature is likely an important factor. A size comparison of *Octolasmis* larval stages is provided in Table 2.

Data reported on *O. mülleri* (Lang, 1976) showed that 6 of 10 cypris larvae introduced to a newly molted *Callinectes sapidus* (Rathbun, 1896) were attached to the gills at the end of 16 hours; following gill removal, 4 of the 6 continued metamorphosis to juveniles in 20 to 72 hours at room temperature (24 to 29°C). Using a minimum time of 14 days from hatching to cyprid and a minimum time of 20 hours for metamor-phosis from cyprid to juvenile, the development time from hatching would be 15 days, whereas with the maximum time of 18 days and 72 hours, respectively, the time would be 21 days.

Live cypris larvae of *O. cor* were not observed to attach, but if it is assumed that they are immediately competent to attach to a newly molted mangrove crab host and metamorphose, eighteen days after hatching, it seems likely that by day 19 to 21 they could be juvenile barnacles attached to a decapod host.

Table 2. A size comparison of the larval stages of several species of *Octolasmis*. The data for *O. aymonini geryonophila* (Colón-Urban, *et al.*, 1979), *O. forrestii* (Lang, 1979) and *O. mülleri* (Lang, 1976) were taken from the noted references.

	O. aymonini		*O. cor*		*O. forrestii*		*O. mülleri*	
	N	Length (μm)	N	Length (μm)	N	Length (μm)	N	Length (μm)
Nauplius 1	2	230.0	19	227.4	nd	259.5	nd	227.3
Nauplius 2	11	670.0	46	673.2	nd	787.2	8	814.0
Nauplius 3	10	910.0	18	1,231.3	nd	1,042.5	10	1,004.0
Nauplius 4	10	1,060.0	9	1,684.2	nd	1,446.8	9	1,270.0
Nauplius 5	nd	nd	18	2,033.7	nd	2,361.7	6	1,735.0
Nauplius 6	nd	nd	9	2,860.0	nd	2,829.7	9	2,659.0
Cypris	nd	nd	26	697.0	nd	nd	4	567.0

SUMMARY

1. Methods for the laboratory maintenance of *O. cor*, larvae and adults were described.

2. Outline drawings, size measurements, and brief anatomic descriptions of *O. cor* larval stages, N1 to N6 and cypris, were provided.

3. Comparisons were made of *O. cor* development with other *Octolasmis* species where information was available from the literature.

ACKNOWLEDGEMENTS

We thank the staff of the Phuket Marine Biological Center for their logistical support and the use of their facilities. In particular, we are grateful to the Director, Mr. Udom Bhatiyasevi, for his generous cooperation. We are most grateful to Mrs Porntip Na-Takuatung who supplied, and taught us how to culture, the tropical algae used to feed the barnacle larvae. We wish to thank Margaret and Ron for their interest and generous support of this research. Backing from the Dickinson College Faculty Research Fund, and the Field Museum of Natural History, made this investigation possible. We are pleased to acknowledge the assistance of Dickinson College students Melanie Hartman, Amy Hewitt, and Colleen Sculley.

REFERENCES

Anderson, D.T. 1992. Structure, function and phylogeny of coral-inhabiting barnacles (Cirripedia, Balanoidea). -Zoological Journal of the Linnean Society **106**:277-399.

Colón-Urban, R., P.J. Cheung, G.D. Ruggieri, and R.F. Nigrelli. 1979. Observations on the development and maintenance of the deep sea barnacle, *Octolasmis aymonini geryonophila* (Pilsbry). - International Journal of Invertebrate Reproduction **1**:245-252.

Darwin, C. 1851. A monograph on the sub-class Cirripedia. I. The Lepadidae: 1-400, 10 pls. (Ray Society London).

de Bary, A. 1879. Die Erscheinung Der Symbiose, pp. 1-30. Karl J. Trubner, Strassburg.

Gannon, A.T. and M.G. Wheatly. 1992. Physiological effects of an ectocommensal gill barnacle, *Octolasmis muelleri*, on gas exchange in the blue crab *Callinectes sapidus*. - Journal of Crustacean Biology **12**(1): 11-18.

Gray, J.W. 1825. A synopsis of the genera of cirripedes arranged in natural families with a description of some new species. - Ann. Phil. **10**: 97-107.

Guillard, R.L. 1975. 11: Culture of Phytoplankton for Feeding Marine Invertebrates. *In*: - W.L. Smith and H.H. Chanley (*eds*). *Culture of Marine Invertebrate Animals*. p 29-60. Plenum Press, New york.

Jeffries, W.B., H.K. Voris, and C.M. Yang. 1982. Diversity and distribution of the pedunculate barnacle *Octolasmis* in the seas adjacent to Singapore. - Journal of Crustacean Biology **2**(4): 562-569.

Jeffries, W.B., H.K. Voris, and S. Poovachiranon. 1992. Age of the mangrove crab *Scylla serrata* at colonization by stalked barnacles of the Genus *Octolasmis*. - The Biological Bulletin **182**(2): 188-194.

Lang, W.H. 1976. The larval development and metamorphosis of the pedunculate barnacle *Octolasmis müleri* (Coker, 1902) reared in the laboratory. - Biol. Bull., **150**: 255-267.

Lang, W.H. 1979. Larval development of shallow water barnacles of the Carolinas (Cirripedia: Thoracica) with keys to naupliar stages. - NOAA Techn. Rep. NMFS Circular **421**: 1-39.