LARVAL DEVELOPMENT OF BALANUS AMPHITRITE AMPHITRITE D. REARED IN THE LABORATORY

BY A. A. KARANDE

(Naval Chemical and Metallurgical Laboratory, Lion Gate, Bombay-1)

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ABSTRACT

Balanus amphitrite amphitrite D. is a common ship-fouling species encountered in many waters of the world. The larval metamorphosis of this species together with other Tetractitid and Chthamalid barnacles was examined under controlled laboratory conditions with a view to describing appropriately each of its six nauplii and cyprid stage. The naupliar characters such as setation of antennule, carapace sculpture, abdominal bulb and caudal process have been critically examined and their importance as the characters of diagnostic value has been discussed. The present study, carried out as it is on the live organisms, has helped to improve the descriptions of the larvae and has thus ensured their reliable identification in the plankton samples.

I. INTRODUCTION

Barnacle nauplius before achieving a settling cyprid stage undergoes six transformations within a period of about 5 to 15 days since its release from a parent body. Identification of each of these growth stages is of considerable interest to those who are engaged in studies on the marine zooplankton, the cirriped taxonomy and the marine fouling prevention. Faunastic lists of barnacles encountered along the Indian coasts have been earlier published by Nilsson Cantell (1938), Annandale (1909), Daniel (1956) and Karande and Palekar (1966). Observations on the larvae collected in the plankton and those reared in the laboratory have been made by Krishna Pillai (1958) and Karande and Thomas (1971) respectively.

Six common larviparous species encountered in Bombay waters are Balanus reticulatus, Balanus a. amphitrite D., Balanus a. variegatus D., Tetractitella karandei R., Chthamalus withersi P., and Chthamalus malayensis P. Larval metamorphosis of all these species except the first named barnacle 56
Balanus amphitrite amphitrite D. was examined under controlled laboratory conditions. The object of this work was to obtain a series of larvae and describe them so that their identification when encountered in planktonic samples becomes easier and reliable. This paper describes in details, the growth stages of the more common ship fouling species *B. a. amphitrite*.

II. MATERIALS AND METHODS

*B. a. amphitrite* is found both in inter-tidal and off-shore waters. The gravid adults settled on small rocks, molluscan shells or bakelite panels were maintained in small perspex tanks and the embryos or the first nauplii released by them were reared under controlled conditions to obtain their subsequent growth stages. A general outline of the rearing technique has been earlier reported by Karande and Thomas (1971).

The observations were made on the live larvae, the larvae immobilised by ethyl alcohol or the larvae preserved in formalin solution. All measurements were made on temporarily immobilised individuals laying in a natural position, ventral side up in cavity microslides. The size and shape of the carapace, setation of the antennule and the structure and spinulation of the abdominal process are the characters which adequately help the separation of one stage or species from that of the other.

III. RESULTS AND COMMENTS

(a) Morphology of Growth Stages

The first nauplius (Fig. 1) like many other *amphitrite* species has a pear-shaped body with anterior uniformly arched border. A full grown stage measures about 225 μ in length. 45 μ wide and 45 μ long abdominal area bears 30 μ long caudal spine. The anterior horns, closely appressed to the body, are about 50 μ long. Labrum measures about 60 μ in length. Median nauplius eye measures 23 μ in length and is 15 μ wide. Ninety per cent of the larvae transform into the II nauplius in about one hours time.

The second nauplius (Fig. 2) has well defined carapace, fronto-lateral horns, frontal filaments and well developed caudal and abdominal processes. The length of abdominal process is about half of the caudal process. Abdominal process at this stage bears only distal pair of spines and these spines have serrated margins. Its stem has a well defined furca with two slender ramii. Stem and ramii portions are demarkated by a ringlet of spinules. Preaxial seta of the antennule has not yet emerged.
The third nauplius resembles the second in many respects. A characteristic feature of this stage, however, is the emergence of a pre-axial seta on the antennule. Proximal pair of abdominal spines appears. Abdominal spine is still shorter than caudal one.

The fourth larva (Fig. 3) is very clearly different from the earlier one in that its carapace has lost its flat triangular appearance and has achieved a buoyant boat-like shape. Posterior margin of carapace bears two 30 μ long spines. The abdominal region becomes considerably bulky and both proximal and distal abdominal spines are well developed, the former being nearly one-third of the length of the latter. Abdominal spine continues to remain shorter than caudal spine. The latter loses its rigid appearance and some spinulation and tends to direct upwards. Two parallel rows of spinules along the long axis of the abdomen develop. Characteristic features of this stage are the presence of two pre-axial setae on antennule and the presence of posterior carapace spines. Fronto-lateral horns tend to show split free ends.

The fifth larva (Fig. 4) appears similar to the fourth larva generally. Caudal and abdominal spines grow subequal. Bulging abdomen is very clearly segmented and the cirriform appendages, now in the form of papillae, are seen under the exoskeleton. A terminal segment of the antennule becomes strikingly longer and a sub-terminal one becomes broader and bulbous. Measuring about 180 μ in length, it shows a setal pattern of 1-1-1-4-2-1-1-1. All three sub-apical setae have appeared. Distal and proximal pairs of abdominal spines have come closer.

The sixth nauplius (Fig. 5) retains all morphological features of the preceding stage and develops a few characteristic of its own. Abdominal appendages including the caudal appendage are observed below the exoskeleton. Appendages are cirriform. Three eyes are present. Antennule shows a setal pattern of 1-1-1-4-2-1-2-1, a typical of this stage.

The cyprid larva (Fig. 6) measuring 480 to 510 μ in length is fairly active and displays long jerky movements. A large number of droplets of about 10-12 μ size are seen under its carapace. A width of vacuum cup of the antennule is about 50 μ. Cyprid larva which undergoes “decortication” (Bernard and Lane, 1962) becomes a young barnacle any time within three days period (Fig. 7).
**Table I**

*Setation formulae of antennule of nauplii of Balanus amphitrite amphitrite*

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. of setae</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0 – 0 – 0 – 4 – 2 – 1 – 1 – 0</td>
</tr>
<tr>
<td>II</td>
<td>0 – 0 – 0 – 4 – 2 – 1 – 1 – 0</td>
</tr>
<tr>
<td>III</td>
<td>0 – 0 – 1 – 4 – 2 – 1 – 1 – 0</td>
</tr>
<tr>
<td>IV</td>
<td>0 – 1 – 1 – 4 – 2 – 1 – 1 – 0</td>
</tr>
<tr>
<td>V</td>
<td>1 – 1 – 1 – 4 – 2 – 1 – 1 – 1</td>
</tr>
<tr>
<td>VI</td>
<td>1 – 1 – 1 – 4 – 2 – 1 – 2 – 1</td>
</tr>
</tbody>
</table>

(b) *Characters of Diagonistic Interest*

(i) *Setation of antennule.—* Table I gives setation formulae of antennulae of various larval stages of *B. a. amphitrite*. Setation formulae of four species of *amphitrite* series, viz., *B. a. albicostatus*, *B. a. hawaiinsis*, *B. a. denticulata*, *B. a. communis* and of the temperate species like *Balanus balanus*, *B. crenatus*, *B. balanoides* and *B. hesperius* have been earlier reported. Table II gives comparative account of setation formulae of antennulae of the fifth and the sixth nauplii of some of these barnacles.

The setation pattern critically examined in the present species conforms with that of other species, in that there are no pre-axial setae at stages I and II and first pre-axial seta appears at stage III. There is addition of one each at stages IV and V. Earlier Krishna Pillai (1958) who examined plankton samples of *B. a. communis* (*B. a. amphitrite*) has shown only two pre-axial setae on antennulae of the fifth nauplius. Barnes and Costlow (1961) also have recorded two pre-axial setae in *B. balanus*. Critical examination of the laboratory grown fifth nauplius of *B. a. amphitrite* has however, unmistakably shown three pre-axial setae. It is further observed that the sixth nauplius antennule has six post-axial setae and not five as has been reported by Krishna Pillai (1958). See Table II, items 6 and 7. So far as present species is concerned, a number of pre-axial and post axial setae and their sequential emergence is of considerable help in identification of various
naupliar stages. For instance the second and the third nauplii which appear otherwise very similar can be separated from one another because of the presence of one pre-axial seta in the latter. Separation of the fourth and the fifth nauplii is possible because in the fourth larva there are two pre-axial setae whereas in the fifth larva there are three setae. Again, a freshly emerged sixth nauplius, possibly having poor eye pigmentation, can be separated from the fifth larva since the former has six post-apical setae and not five as is observed in the fifth nauplius.

**Table II**

*Setation formulae of antennule of the fifth and the sixth nauplii of some barnacles*

<table>
<thead>
<tr>
<th>No.</th>
<th>Species and Stage</th>
<th>Formulae</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>B. balanus</em></td>
<td>V 0-1-1-4-2-1-1-0 VI 1-1-1-4-2-1-1-1</td>
<td>Barnes and Costlow, 1961</td>
</tr>
<tr>
<td>2.</td>
<td><em>B. hesperius</em></td>
<td>V 1-1-1-4-2-1-1-1 VI 1-1-1-4-2-1-2-1</td>
<td>Barnes and Barnes, 1959b</td>
</tr>
<tr>
<td>3.</td>
<td><em>B. a. albicostatus</em></td>
<td>V 1-1-1-4-2-1-1-1 VI 1-1-1-4-2-1-2-1</td>
<td>Yagusi and Ishida, 1937</td>
</tr>
<tr>
<td>4.</td>
<td><em>B. a. hawaiinsis</em></td>
<td>V 1-1-1-4-2-1-1-1 VI 1-1-1-4-2-1-2-1</td>
<td>Hudinga and Kasa-hara, 1941</td>
</tr>
<tr>
<td>5.</td>
<td><em>B. a. denticulata</em></td>
<td>V 1-1-1-4-2-1-1-1 VI 1-1-1-4-2-1-2-1</td>
<td>Costlow and Book-hout, 1958</td>
</tr>
<tr>
<td>6.</td>
<td><em>B. a. amphitrite</em> (Planktonic)</td>
<td>V 0-1-1-4-2-1-1-0 VI 1-1-1-4-2-1-1-1</td>
<td>Krishna Pillai, 1958</td>
</tr>
<tr>
<td>7.</td>
<td><em>B. a. amphitrite</em> (Laboratory reared)</td>
<td>V 1-1-1-4-2-1-1-1 VI 1-1-1-4-2-1-2-1</td>
<td>Present work</td>
</tr>
<tr>
<td>8.</td>
<td><em>B. a. variegatus</em> (Laboratory reared)</td>
<td>V 1-1-1-4-2-1-1-1 VI 1-1-1-4-2-1-2-1</td>
<td>Karande (in press)</td>
</tr>
</tbody>
</table>

No variation of setation between individuals of any one stage is observed in *B. a. amphitrite*.

(ii) *Carapace spines.*—A distribution of spines and spinules on carapace edge is of help in separating the larvae of *B. a. amphitrite* from other
Figs. 1-5. The larval stages of *Balanus amphitrite*. I nauplius has no frontal filaments (Fig. 1). II nauplius has well developed caudal and abdominal spines (Fig. 2). IV nauplius differs from III in having a pair of carapace spines (Fig. 3). V nauplius (Fig. 4). VI nauplius has three eyes (Fig. 5).
Figs. 6-7. Cyprid larva (Fig. 6) after attachment and decortication loses its chitinous shell and two naupliar eyes (Fig. 7).
Balanus amphitrite amphitrite D.

balanid species. In *B. a. amphitrite* only one prominent spine is present on each side of the carapace. In *B. a. variegatus* an entire margin is spinulated. In two other barnacles, viz., *T. karandei* and *Ch. withersi* these spines are absent.

In *B. a. amphitrite*, *B. a. variegatus* and in *T. karandei* a distinct posterior border bearing a pair of spines emerges at stage IV and not at stage III as reported by Pyefinch (1948 a, b) who examined larvae of *B. balanoides* and *B. crenatus*. An emergence of posterior carapace spines at stage IV is not therefore a feature of only *Balanid* larvae (Jones and Crisp, 1954; Costlow and Bookhout, 1957, 1958; Barnes and Barnes, 1959 a, b) but is also observed in *Tetraclitid* barnacles. These spines, however, are absent in two *Chthamalid* barnacles studied in this laboratory.

(iii) *Caudal process.*—The spinulation on the caudal process has some value, if limited, in identification of the larvae of *Balanid*, *Tetraclitid* and *Chthamalid* barnacles. They are, however, of no assistance in separating one species from the other. Generally caudal process at stages I, II and III is longer than the abdominal process. At stage VI these processes grow almost subequal.

(iv) *Abdominal process.*—Abdominal process is comprised of two pairs of abdominal spines, the stem and its two ramii. The spines and spinulation, their number and the sequence of emergence or of disappearance is of considerable diagnostic value.

A distal pair of abdominal spines emerges at stage II and a proximal pair appears at stage III. These two pairs of spines are retained until late sixth nauplius. Krishna Pillai (1958) describes an emergence of a single median spine in between the members of the proximal pair in planktonic stage IV nauplius of *B. a. communis* (*B. a. amphitrite*). Similar median spine, he observes is also reported in the fourth nauplii of *B. perforatus*, *B. improvisus* and in *E. modestus*. A presence of this spine Pillai considers, is of diagnostic value. I have recorded the presence of a median spine in *B. a. variegatus*—but have failed to confirm its presence in the fourth nauplius of *B. a. amphitrite*. In both *amphitrite* species examined by me, no addition of a third pair of spines at stage V is made as is recorded for a temperate species *B. balanus* by Barnes and Costlow (1961).

The larvae of *B. a. amphitrite* like four other species, viz., *B. a. variegatus*, *Tetraclitella karandei*, *Ch. malayensis* and *Ch. withersi* take generally eleven
or twelve days to achieve a cypris stage. The first nauplius of *B. a. amphitrite*
may at times achieve cypris stage within five days period which has been
the shortest span of time hitherto recorded for any barnacle species.
The development amongst cirriped nauplii in general has been of uniform
pattern (Barnes and Barnes, 1959). The observations made on *B. a. amphitrite*
lend support to this view. Judging from the sequence of events with
respect to carapace growth, the abdomen, the caudal segment and the setation
of antennulæ, it is felt that the pattern of metamorphosis in *balanids* is more
comparable to *tretaclitids* than to *chthamalids*.

A setation pattern of antennulæ in this species is very similar not only
to *B. a. variegatus* but is also identical to that of many tropical and non-
tropical species studied by other workers. A number of setae for each stages
of growth is, however, specific and is therefore of considerable help in
separating one stage from the other. Emergence and growth of the distal and
the proximal pairs of abdominal spines follow the same pattern from one
stage to the next as has been recorded in many other species. A distal
pair of abdominal spines bears serrated margins at stage II nauplius.
Similar serration is noticed in *B. a. variegatus* and in *chthamalid* and *tretaclitid*
barnacles studied by us. The pattern of serration, however, is different in
each of these groups of barnacles.

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