Influence of temperature and salinity on larval development of *Balanus amphitrite*: implications in fouling ecology

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ABSTRACT: Recruitment of the barnacle *Balanus amphitrite* into a macrofouling community was examined in a semi-enclosed coastal environment (Hamana Bay, Japan), where water exchange with the adjacent open sea is very limited. The recruitment period of these barnacles was shorter than the period in which this species can breed and in which the cirripede larvae are present in the environment. Rearing of *B. amphitrite* larvae at different temperatures (15 to 30°C) and salinities (10, 20 and 30%) revealed that its development is euryhaline. The influence of temperature was found to be greatest on the second instar. Mortality rates at 15°C temperature ranged from 43% (30% salinity) to 99% (10% salinity). Rearing experiments indicated that larvae released to the environment during autumn and winter failed to establish themselves in the macrofouling community because of adverse environmental conditions. These results also show that loss of planktonic larvae through starvation and misrouting may well be the main cause of reproductive loss.

KEY WORDS: *Balanus amphitrite* · Biofouling · Cirripede larva · Cirripede ecology

INTRODUCTION

Macrofouling is an important aspect of biodeterioration in the marine environment and barnacles (Cirripedes) are one of its dominant components. Recruitment of barnacles into a macrofouling community is dependent on the surface characteristics of the substratum, and research in this area has been considerable. Recruitment has also been expressed as the link between adult and larval ecology (Olson & Olson 1989). Barnacles have planktotrophic larvae that remain planktonic for several weeks, sometimes up to 2 mo (Scheltema & Carlton 1984). This in turn permits their planktonic dispersal. From this perspective, study of the abundance of larvae and its influence on the adult community can be done only in enclosed conditions or in localities where larval dispersal is very restricted.

Hamana Bay, on the Pacific coast of Japan (Fig. 1), is semi-enclosed and divided into 2 parts (Hon Inlet and Shonai Inlet). The mouth of this bay is restricted to 200 m, and has a water volume of about $3.4 \times 10^9$ m³. Mazda (1984) reported that exchange with the open sea is very limited. Kajihara et al. (1976) observed that coastal water does not reach the central part of Shonai-Inlet. The semi-enclosed nature of this locality gave us a rare opportunity to study macrofouling dynamics with reference to the resident larval population. The investigation emphasized the cirripede ecology (Anil 1991) and this paper deals principally with *Balanus amphitrite*.

MATERIAL AND METHODS

Macrofouling community. The investigations on the macrofouling community in this inlet were carried out during May 1987 to April 1989. PVC pipes were exposed as substratum during the first year of investigation (May 1987 to April 1988) and acrylic panels
(15 × 10 × 0.3 cm) were used as substratum during the second year (May 1988 to April 1989).

Cirripede nauplii. Plankton samples collected once a month were preserved in formalin (5%). Vertical plankton hauls were made using a Kitahara net with a mesh size of 100 μm. Abundance of cirripede nauplii was determined as numbers m⁻³. First to sixth stage of cirripede nauplii (Balanomorpha), as described by Kado (1982), were observed for each station (Anil 1991).

Gonad condition. Balanus amphitrite, an acorn barnacle, is hermaphroditic (Charnov 1987). As egg development is easy to follow, it is often used for following the reproduction of cirripedes. Individuals of B. amphitrite were collected from the 3 inner stations during the second year to monitor their breeding. Differentiation of the gonad stages (0 to IV) was based on coloration and developmental condition of the ovary. At Stage 0, the ovary is absent. Stage I is a growing stage, where the ovary is granular but present only as a thin cream-colored layer of ovarian tissue on the basal membrane. At Stage II, the ovary fills about a third of the mantle (creamy yellow). At Stage III (mature stage), eggs in the ovary occupy most of the mantle (bright yellow). At Stage IV, nauplii with visible eye spots are present (dark brown).

Influence of temperature and salinity on larval development. The larval phase of Balanus amphitrite consists of 6 naupliar stages and a pre-settling cyprid stage. The first or second stage nauplii are readily released when gravid adults are reimmersed into seawater after being exposed to air for some time. The first stage nauplii do not feed, their instar duration is short and they molt into second stage within a few hours. Experiments on the influence of temperature and salinity were carried out until the larvae reached the cyprid stage.

The different salinities used in the experiment (10, 20 and 30%) were obtained by diluting filtered seawater with deionized water. The first stage nauplii were collected by filtering them through a plankton cloth and were placed into 200 ml beakers containing seawater (10, 20 and 30%). After 2 h, larvae belonging to the second stage were transferred from these beakers to rearing containers (24-well plates, Corning 25820-24) at the respective salinities. The rearing temperatures (15, 20, 23, 25, 27 and 30°C) and lighting conditions were controlled by placing the rearing containers in temperature-controlled chambers with three 20 W fluorescent lights on a 14:10 h light:dark cycle.

Cirripede nauplii from the second to sixth stages are phytoplanктotrophic and develop when fed a diet of unicellular algae (Scheltema & Williams 1982). In our study, Skeletonema costatum (1 × 10⁵ cells ml⁻¹) was used as the food. Each larva had 2 ml of media with an antibiotic dosage of 0.3 ml Crystamycin 1⁻¹ (300 mg Penicillin G and 500 mg Streptomycin in 4 ml of distilled water). Antibiotics were used to prevent the proliferation of bacteria and the dosage level of antibiotics used was adopted from Harms (1986).

The influence of temperature (t) on the duration of larval development (D) at different salinities has been calculated by using the power function $D = bt^m$ (b and m are constants). Harms (1984, 1986) used this equation to express the influence of temperature on the larval development of Eliminius modestus and Balanus balanoides. We only used data from larvae which successfully reached the cyprid stage in the analysis.

RESULTS

Macrofouling

Previously published details of this investigation (Anil et al. 1990, Anil 1991) revealed that recruitment to the macrofouling community is most active between May and October, but is dormant during winter and early spring. Active recruitment can be demarcated into outer and inner zones based on the composition of the macrofouling community, but such a zonation seems less significant during the dormant period. Megabalanus rosa, Balanus albicostatus, B. trigonus, B. eburneus and B. amphitrite were the 5 cirripede species present in the macrofouling assemblage (Anil 1991). The first 3 were restricted only to the outer zone (Stns A and B), influenced by marine environmental conditions. The remaining 2 were dominant in the inner zone with brackish condition (Stns C, D and E).

The settlement intensity of cirripedes on materials exposed for 1 mo is presented in Fig. 2. During the first year of investigation, the settlement of cirripedes...
peaked during June, decreased from September onwards and was absent from November to March. During the second year, settlement peaked in May 1988 and was reduced drastically from June 1988 onwards.

Cirripede nauplii

Cirripede nauplii were present in considerable numbers during May to November (Fig. 3), while during December to April their numbers were very much reduced, even reaching zero. During May to November the abundance followed no particular trend. Variation between the 2 years was not very conspicuous. However, during the first year, the number of larvae peaked during the latter half of the May to November period, whereas in the second year, such peaks occurred earlier (May to August). The inner stations often possessed dense concentrations of cirripede nauplii with values up to 55600 ind. m\(^{-3}\) (Stn E, June 1988). During the first year, maximum numbers of nauplii at Stns C, D and E were found in the September (17700 m\(^{-3}\)), November (22000 m\(^{-3}\)) and October (36700 m\(^{-3}\)) samples, respectively. First and second stages were dominant. At Stn A, stages beyond the third one were rarely encountered. At the other 4 stations, fourth to sixth stage nauplii were often encountered and their presence peaked between May and August (Anil 1991).

Gonad condition

The presence of individuals possessing fertilized ovaries (Stage III) and ovaries with nauplii inside (Stage IV) peaked in May 1988 (96%; Fig. 4). Mature gonads were dominant between May and September. The percentage of individuals with mature gonads during October to January ranged between 30 and 50%. During February to April, the percentage of individuals with mature gonads was negligible. The results indicate that this species has active reproduction capability between May and September and this activity continues at a reduced level until January.

Influence of temperature and salinity on larval development

The mortality rates at different temperature and salinity combinations are presented in Fig. 5. The nauplii of Balanus amphitrite were able to develop into the cyprid stage at all 3 tested salinities. At 10 and 20\%, the lowest mortality rates were found at 23°C (21 and 13\% respectively); at higher and lower temperatures than this, the mortality rate increased. The mortality rates at 15°C (10 and 20\% salinities) were found to be 99 and 58\% respectively, and the second and third stages contributed the most to the mortality.
The instar durations of Balanus amphitrite nauplii at different temperature and salinity combinations are presented in Fig. 6. At 15°C, the average duration at the second instar was the longest, followed by that of the sixth instar. At 20 and 30‰ (15°C), the second instar duration averaged 4.2 and 4.0 d, the maximum values noted for these salinities. In temperatures ranging from 23 to 30°C, variations in instar durations were not conspicuous, but the durations tended to be shorter at 25°C. The power function constants at different salinities (Table 1) indicated that the duration of the second instar at 10‰ salinity was influenced mostly by temperature \((m = -2.04)\). The influence of temperature on the duration of the fourth to sixth instars at these temperatures was less than those at 10 and 20‰, respectively. A comparison of the mortality rates at 15 and 20°C and 10 and 30‰ revealed a decrease in mortality from 99 to 43% and from 71 to 35%, respectively.

At 30‰ salinity combined with different temperatures (23 to 27°C), mortality rates ranged from 10 to 19% and increased at lower and higher temperatures. The mortality rates at these temperatures (15, 20 and 30°C) were less than those at 10 and 20‰. A comparison of the mortality rates at 15 and 20°C and 10 and 30‰ revealed a decrease in mortality from 99 to 43% and from 71 to 35%, respectively.

The total duration required by the nauplii of Balanus amphitrite to reach the cyprid stage is presented in Fig. 7. The duration was longest at 10‰, with the average ranging from 11.1 (30°C) to 22 d (15°C). At 20 and 30‰, the average duration ranged from 7.1 to
Table 1. *Balanus amphitrite*. Values of the power function \(D = bt^{m}\) for the influence of temperature \(t\) on the duration of larval development \(D\) for temperatures of 15 to 30°C at 10, 20 & 30% salinity

<table>
<thead>
<tr>
<th>Salinity (%)</th>
<th>b</th>
<th>m</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nauplius II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1471</td>
<td>-2.04</td>
<td>0.91</td>
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<tr>
<td>20</td>
<td>522</td>
<td>-1.78</td>
<td>0.90</td>
<td>&lt;0.001</td>
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<tr>
<td>30</td>
<td>305</td>
<td>-1.61</td>
<td>0.91</td>
<td>&lt;0.001</td>
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<tr>
<td>Nauplius III</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>308</td>
<td>-1.53</td>
<td>0.80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20</td>
<td>27</td>
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<td>0.55</td>
<td>&lt;0.001</td>
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<tr>
<td>30</td>
<td>27</td>
<td>-0.88</td>
<td>0.61</td>
<td>&lt;0.001</td>
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<tr>
<td>Nauplius IV</td>
<td></td>
<td></td>
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<tr>
<td>10</td>
<td>37</td>
<td>-0.85</td>
<td>0.62</td>
<td>&lt;0.02</td>
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<td>20</td>
<td>16</td>
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<td>31</td>
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<td>0.65</td>
<td>&lt;0.01</td>
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<tr>
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<tr>
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<td>8</td>
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<td>24</td>
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<td>0.78</td>
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<tr>
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<tr>
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<td>-0.86</td>
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<td>&lt;0.02</td>
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<tr>
<td>20</td>
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<td>Nauplius II to cypris</td>
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<tr>
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16.3 d. The minimum duration observed at 20 and 30% was 5 d.

**DISCUSSION**

Recruitment of cirripedes to the macrofouling community at this inlet showed that the duration and intensity of settlement during the second year were less than those of the first. One reason for this year-wise variation could be the difference in substratum used during the 2 years. Hui & Moyse (1987), while discussing settlement patterns and competition for space in cirripedes, proposed that the specificity of any particular species with regard to the physical and biological properties of substrata is also likely to be important. Southerland & Karlson (1972) noted that encrusting bryozoa can maintain surfaces free of fouling when alive. The successful thriving of *Membranipora* sp. over the exposed surface at this inlet during the peak cirripede settlement period could probably also be a reason for the reduced intensity and shorter period of cirripede settlement during the second year. It is also possible that the recruitment pattern itself changed. Changes in recruitment can arise from the influence of the environment on the reproductive cycles of adult cirripedes and on the metamorphosis of their larvae. *Balanus amphitrite* are strongly eurythermal and breed even at 15°C (Crisp & Costlow 1963, Iwaki 1981). The strong capacity to breed at low temperatures is not seen in *B. albicostatus* (Iwaki 1981) or *Megabalanus rosa* (Yamaguchi 1973). Observations of gonads during the second year revealed that *B. amphitrite* possessed mature ovaries during the early winter (Fig. 4).

Whether the community ecology at a site is governed by interactions among adults at the site or by limitations to supply of larvae reaching the site is determined by the regional pattern of circulation in coastal waters (Roughgarden et al. 1988). It has also been stated that isolation and integrity of estuarine populations are maintained by various hydrodynamic processes (Bousfield 1955, Dewolf 1973, Crisp 1984a). Martin & Foster (1986) showed that barnacle larvae are maintained within Mahurangi Harbor, New Zealand. The present investigation was carried out in a semi-enclosed bay where water exchange with the adjacent open sea is very limited (Mazda 1984). Since planktonic larvae are passively dispersed, the settlement of cirripedes in an embayment can be correlated to larval abundance with a reasonable degree of certainty. A similar view has been expressed by Foster (1987). At this inlet cirripede nauplii were present in plankton collected during September to November 1987, even peaking at some inner stations (Fig. 3). The observed settlement duration of cirripedes was, however, shorter than the period in which nauplii were encountered and drastically less than that in September (Fig. 2). Apparently larvae continued to be present in the inlet but failed to settle. In this context it is important to understand the metamorphosing capability of the larvae to ascertain the rationality of larval presence and failure in recruitment. In the present study, larvae of the
fourth to sixth stages were found mainly between May and August, and those found from November to April mainly belonged to the second and third stages, which in turn indicates the possibility of a failure in successful larval development. The environment during this period is characterized by lower water temperature and productivity, therefore this influence can also be important.

The nauplii present at the inner stations during late autumn and early winter months probably belong to *Balanus eburneus* and *B. amphitrite*. These are the only 2 species present in the inner inlet and they have the capability to breed over a wide range of temperatures. Rearing of *B. amphitrite* larvae, the most widely occurring barnacle species in this environment, at different temperature and salinity combinations was carried out to determine their influence on development and to get a perspective of larval ecology.

The methods for rearing cirripede larvae are well established. Hirano (1962) reared *Balanus amphitrite* larvae in mass culture and reported the influence of water temperature and food density. However, there are only a few published results which relate the influence of water temperature and salinity to the development of each naupliar stage of cirripedes (Scheltema & Williams 1982, Harms 1986).

Early stage nauplii were dominant between November and April at the inlet. The results of rearing experiments showed higher mortality at 15°C, especially at low salinities. Thus it is possible that a drastic reduction in actual settlement, despite the capacity to breed at low temperatures and the presence of nauplii in the environment, could be due to a drop in water temperature.

The total time required to reach the cyprid stage by the nauplii of *Balanus amphitrite*, obtained by applying the power function constants, is shown in Fig. 8. Other observations shown here are (1) the period of potential cirripede settlement, (2) the period during which *B. amphitrite* has mature ovaries and (3) the presence of cirripede nauplii in plankton. The continuous lines of these parameters indicate the peak period of settlement, the period when 50% of the adults possess mature ovaries and the period of maximum abundance of nauplii. The dotted portion of the lines show the period up to which settlement can be observed, the presence of mature ovaries in the adult population and the occurrence of cirripede nauplii in the plankton. The line representing the maturation of the ovary is divided into 2 periods; (1) up to the end of September when mature ovaries are found in more than 50% of the adults and (2) September to January when mature ovaries are at 15 to 30% and contain developing nauplii within the ovary.

It is possible to see in Fig. 8 that the period of settlement is shorter than the breeding period in *Balanus amphitrite* and the interval over which cirripede nauplii are present. Development to cyprid stage under field temperature conditions is shortest in the middle of the observed settlement period. From November onwards, the duration required increases as temperatures fell. Though the results of the rearing experiments are relevant to 15°C only, a tendency in the duration of larval development is shown for the December to March period at temperatures below 15°C (the average water temperature at Stn C during this period was found to range between 7.2 and 12.6°C). To summarize, the settlement period is shorter than the period in which the species can breed, a period during which the cirripede nauplii are present in the environment. The falling water temperature during November to March prolongs larval development duration.

The durations in Fig 8 are projected based on the rearing results where food supply was not a constraint. Larvae in the rearing experiment had a food supply of *Skeletonema costatum* at a concentration of $1 \times 10^5$ cells ml$^{-1}$. Such a concentration is nearly equal to that observed during red tide blooms during summer at this inlet (Watanabe et al. 1980). Even when food is not a constraint, high mortality occurs at 15°C. Scheltema & Williams (1982) found that at lower temperatures, increasing food concentrations can compensate for decreased feeding efficiency and result in increased survival and a shorter time to complete development to the cyprid stage. Barnes & Barnes (1958) concluded that the availability of food may be of fundamental importance in the development of planktonic larvae and may interact and compensate for the effect of tem-
temperature. Crisp (1984b) noted that though the loss of planktonic larvae through predation is widely believed to be the main cause of reproductive loss, starvation and misrouting may be even greater hazards. Perhaps the reduced availability of food during autumn and winter can pose restraints on larval development at this inlet. The potential to resist starvation not only depends on the species and on the particular larval stage considered, but also on environmental variables. Temperature, which controls metabolism and thus the rate of reserve utilization, can be important (Anger & Dawirs 1981). Olson & Olson (1989), assessing the food limitation of planktotrophic invertebrate larvae, stated that larval starvation is likely to be important to recruitment success in cirripedes. Lang & Marcy (1982), studying effects of early starvation on the survival and development of barnacle nauplii (*Balanus improvisus*), opined that it would be essential that nauplii are released into a food-rich environment, as the survival potential of starving stage II nauplii may decrease as soon as 24 h. Studies on specific mechanism of starvation sensitivity in the larval development of decapod crustaceans by Anger (1987) showed interaction between food availability and endogenous hormonal control of development. It has also been found that if an early larva is starved beyond a certain point, then its feeding ability will diminish and it will eventually experience irreversible damage, probably to both the mitochondria and hepatopancreas system (Storch & Anger 1983).

The other possible reasons for settlement failure during autumn and early winter, in spite of the breeding capabilities of *Balanus amphitrite* during that period and the presence of nauplii in the plankton samples, can be related to the quality of larvae released. In the rearing experiments, the nauplii of *B. amphitrite* hatched from the adults collected during late autumn and early spring (1988–1989) showed poor development capability in comparison to those collected during the summer months (Anil 1991). Perhaps the quality of larvae released determines the degree of successful development. There is every reason to believe this because the settlement of this species was also not found during field observations in that period. It has also been reported in bivalves *Mytilus edulis* and *Ostrea edulis* that lipid reserves in the egg seem to play an important role in the success of planktotrophic larvae and determine the energy present in offspring (Helm et al. 1973, Bayne et al. 1975). Lucas et al. (1979) studied the changes in neutral lipid levels of the cyprid *B. balanoides* and found that length of pelagic life depends critically on the initial levels of neutral lipids. Lucas & Crisp (1987) studied the energy metabolism of eggs during embryogenesis in *B. balanoides* and found that when starved adults retain their mature egg mass beyond the normal term, egg metabolism occurs largely at the expense of the remaining lipid reserves. The ability of adults to postpone hatching may therefore have important implications for the energy reserves and viability of the newly hatched nauplii. Whenever the retention period is reasonably short, each newly hatched larva contains enough energy to give it the required start. Energy metabolism of eggs thus seems to have a vital role in the larval ecology. Further studies detailing the influence of this factor on larval development may be able to answer the questions related to changes in the quality of larva during different seasons and further an understanding of macrofouling ecology of cirripedes.

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