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SOME FACTORS INFLUENCING THE SETTLEMENT OF BARNACLE LARVAE IN THE LABORATORY

N. KALYANASUNDARAM AND S. S. GANTI

Naval Science and Technological Laboratory, Visakhapatnam-3

ABSTRACT

Some factors influencing the metamorphosis and settlement of the larvae of the barnacle *Balanus amphitrite amphitrite* Darwin, in the laboratory were studied. The diatom *Nitzschia closterium* when used as food facilitated larval metamorphosis while the green alga *Dunaliella primolecta* was found unsuitable even in the early stages of metamorphosis. Larvae metamorphosed under normal daylight illumination, whereas they reached only up to V naupliar stage under total darkness. The addition of penicillin to the rearing vessels had no significant improvement in the total yield. Sterilised sea water medium, interestingly enough, did not support larval metamorphosis.

INTRODUCTION

INVESTIGATIONS on the rearing and settlement of barnacle larvae in the laboratory to aid studies on fouling problems have been receiving world-wide attention. The settlement of barnacles in the laboratory is influenced by several factors such as food, light, presence of bacteria, gregarious attraction, type of substrate etc., (Daniel, 1955 a, b, 1957, 1958a, 1963). In the past, several workers have employed different methods for rearing the local faunae. The barnacles studied by the various workers are *Balanus crenatus* B. (Herz, 1933), *B. balanoides*, *Chthamalus stellatus*, *Verrcua stroemia* (Bassindale, 1936), *B. amphitrite hawaiiensis* (Hudinaga and Kasahara 1941), *B. eburneus*, *B. a. denticulata* (Costlow and Bookhout, 1957 ; 1958), *B. a. variegatus*, *B. tintinnabulum tintinnabulum*, *Chthamalus stellatus stellatus* (Daniel 1958 a, b) ; *Elminius modestus* (Moyse, 1960 ; Wisely, 1960 ; Tighe-Ford, Power and Vaile, 1970) and *B. a. communis* (Karande and Thomas, 1971). Except Wisely (1960) who adopted a flowing seawater system for rearing the larvae, all others employed only static seawater condition. Freiberger and Cologer (1966) and Tighe-Ford *et al.*, (1970) transferred the larvae by filtration to fresh beakers periodically in order to eliminate the protozoans and waste products. Consequently, the yield of settlement has also been varying with different workers, the maximum on record being that by Moyse (1960) who obtained 80 to 90 % settlement of *E. modestus*. Wisely (1960) using the same species under flowing water conditions obtained only a very low yield 0.03%.

Balanus amphitrite amphitrite Darwin constitutes one of the most important fouling species in Indian waters. Except for the work of Karande and Thomas (1971) there is no report on the rearing of the larvae of *B. a. communis*. In the present investigation, some of the factors influencing the settlement, such as food, light, antibiotics and type of medium, have been examined.

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Material and Methods

Mature specimens of *B. a. amphitrite* settled on experimental bakelite, panels exposed at Naval Base Jetty, Visakhapatnam were brought to the laboratory for obtaining their larvae. The barnacles were then carefully screened for the presence of any other species, which were eliminated, when present. As a practice, the panels were always dried overnight in order to eliminate as far as possible copepode and protozoans. The panels were then kept in glass jars, containing sea water filtered through Whatman No. 1 filter paper. The larvae liberated in the glass jars were successively transferred to three intermediate 500 ml beakers before use in experiment, in order to further eliminate the possible presence of copepods and protozoans. Thirty larvae were then transferred to 100 ml beakers containing 60 ml of sea water and were subjected to the varying experimental conditions.

The following experimental conditions were independently examined for rearing the larvae. The role of diatom and green algal cultures in the metamorphosis and settlement of larvae was studied by the addition of 5 ml of cultures of *Nitzschia closterium* and *Dunaliella primolecta* in separate sets of beakers. The role of light in larval metamorphosis was studied by keeping sets of beakers with larvae in the normal day light conditions near the northern window and in total darkness. The effeet of penicillin in facilitating settlement by way of controlling bacterial growth was examined by addition of penicillin to the rearing beakers at a concentration of 800 units I.P./ml. Studies were also conducted in filtered sea water medium and sea water sterilized in autoclave at 15 lbs/sq. pressure for 30 mts. in order to determine the effect of the type of culture medium.

In the last three conditions, only the diet found suitable for larval growth was provided, as determined in the first set of experiments. The beakers were kept near the northern window for providing normal day light conditions, except in the experiments on the influence of illumination.

A daily record was maintained of survival of the larvae kept under different experimental conditions till the cyprids and their subsequent settlement and metamorphosis were observed.

Results

a. *Food*

The rate of survival of the larvae of *B. a. amphitrite* when fed on the species of algae is presented in Table 1. All the larvae remained active for the first two days. The survival rate gradually decreased on the 3rd day in beakers containing *D. primolecta* cultures and all the larvae were dead towards the end of 4th day in the IV naupliar stage. In beakers containing *N. closterium* the rate of mortality gradually increased with time and on 9th day when settlement was observed the total survival was 53%. The larvae from the beakers of *D. primolecta* when

TABLE 1. *Rate of survival of larvae of* Balanus amphitrite amphitrite *when fed on two species of algae*

Number of days	*Nitzschia* No. of larvae (Mean of 12 readings)	*Closterium* % survival	Stage of development	*Dunaliella Primolecta* No. of larvae (Mean of 12 readings)	% survival	Stage of development
1 ..	30	100	II Nauplius	30	100	II Nauplius
2 ..	30	100	III Nauplius	30	100	III Nauplius
3 ..	30	100	III Nauplius	27	90	III Nauplius
4 ..	30	100	IV Nauplius	15	50	IV Nauplius
5 ..	25	83	V Nauplius	..	..	..
6 ..	22	73	V Nauplius	..	..	..
7 ..	16	53	VI Nauplius	..	..	..
8 ..	16	53	Cypris	..	..	..
9 ..	16	53	Young spat	..	..	..

examined under microscope were found to have empty intestinal tracts, as compared to those in the beakers with *N. closterium*. The mortality of larvae on a diet of *D. primolecta* was probably due to starvation.

b. *Light*

The results of the effect of light and darkness on the larvae are presented in Table 2. The larvae up to IV stage were found to be apparently normal and healthy when kept in darkness. The percentage of mortality was also less in these stages than in later stages. However, the larvae failed to metamorphose beyond Vth stage and mortality was hundred per cent in the beakers kept in darkness on the 8th day. Larvae kept exposed to light showed a tendency to get entangled in the diatom film formed at the bottom of the beaker, accounting for a major portion of the mortality. The entanglement of this type was not observed in beakers kept in the dark, though some larvae were observed to get entangled in their exuviae towards the end of the 3rd day.

c. *Addition of antibiotics*

The addition of penicillin to sea water medium did not show any significant improvement in the survival of the larvae (Table 3). Although larvae have been reported to be affected by bacterial film in the absence of penicillin, in our experiments, no such film was recorded in both the sets viz., with or without penicillin. The protozoan fauna, which was originally present in sea water did not seem to affect larval development. In addition, though the concentration of penicillin was high (800 units I.P./ml), it did not seem to control protozoans in any way, as was evident from their presence in the beakers containing penicillin.

TABLE 2. *The effect of illumination on the metamorphosis of larvae*

Number of days	*Day light illumination*			*Total darkness*		
	No. of larvae (Mean of 12 readings)	% survival	Stage of development	No. of larvae (Mean of 12 readings)	% survival	Stage of development
1 ..	30	100	II Nauplius	30	100	II Nauplius
2 ..	25	83	III Nauplius	29	97	III Nauplius
3 ..	25	83	III Nauplius	27	90	III Nauplius
4 ..	22	73	IV Nauplius	27	90	IV Nauplius
5 ..	22	73	V Nauplius	27	90	IV Nauplius
6 ..	22	73	VI Nauplius	25	83	V Nauplius
7 ..	18	60	Cypris	15	50	V Nauplius
8 ..	12	40	Young spat	..	..	..

TABLE 3. *The effect of penicillin on the metamorphosis of the larvae*

Number of days	*With penicillin*			*Without penicillin*		
	No. of larvae (Mean of 12 readings)	% survival	Stage of development	No. of larvae (Mean of 12 readings)	% survival	Stage of development
1 ..	30	100	II Nauplius	30	100	II Nauplius
2 ..	30	100	III Nauplius	30	100	III Mauplius
3 ..	30	100	III Nauplius	30	100	III Nauplius
4 ..	25	83	IV Nauplius	30	100	IV Nauplius
5 ..	21	70	IV Nauplius	25	83	V Nauplius
6 ..	18	60	V Nauplius	22	73	V Nauplius
7 ..	14	47	VI Nauplius	16	53	VI Nauplius
8 ...	13	43	Cypris	15	50	Cypris
9 ..	13	43	Young spat	15	50	Young spat

d. *Type of sea water*

The larvae in the beakers containing sterilized sea water failed to show normal development (Table 4). It is evident from the results, that under sterile sea water conditions, the larvae failed to survive even on the second day. The results are

TABLE 4. *The role of sea water medium on the survival of larvae*

	Filtered sea water			*Sterilized sea water*		
Number of days	No. of larvae (Mean of readings)	% survival	Stage of development	No. of larvae (Mean of 12 readings)	% survival	Stage of development
1 ..	30	100	II Nauplius	30	100	II Nauplius
2 ..	30	100	III Nauplius	2	6	III Nauplius
3 ..	30	100	III Nauplius	..	..	..
4 ..	25	83	IV Nauplius	..	..	..
5 ..	22	73	V Nauplius	..	..	..
6 ..	21	70	V Nauplius	..	..	..
7 ..	18	60	VI Nauplius	..	..	..
8 ..	16	53	Cypris	..	..	..
9 ..	15	50	Yong spat	..	..	..

very interesting in the sense that the sterilized sea water definitely offered harmful effect to the larvae. This may probably be due to the break down of some sea water constituents during sterilization under pressure, resulting in the removal of certain beneficial effects.

DISCUSSION

It is observed from the results presented above that the metamorphosis and settlement of larvae of *B. a. amphitrite* is dependent on several factors.

Type of food plays a major role in successful metamorphosis. Among the two species of algae examined for support of larval growth it was observed that *D. primolecta* did not support good larval development ; the algae did not contribute to the dietary requirement of the species, and the larvae died primarily of starvation. On the other hand, *N. closterium* was found to facilitate larval metamorphosis, and settlement which took place in 9 day's time. Karande and Thomas (1971) have recorded at Bombay that *D. primolecta* supports larval growth and settlement of the same species of barnacle. It is evident therefore, that even in the same species of barnacle, the dietary requirement seems to be varying from place to place.

Costlow and Bookhout (1957) contended that a diet of animal origin should be provided in the later stages of development. They recorded normal growth and settlement of barnacle larvae when a diet of *Chlamydomonas sp.* was supplemented with fertilized eggs of *Arbacia puctulata*. However, food of animal origin has been found to be non-essential by subsequent workers (Moyse, 1960 ; Tighe-Ford *et al.*, 1970) and has been avoided in the present studies in order to minimise bacterial film formation.

The importance of varying intensity of light on the rate of growth of barnacle larvae have been emphasised by recent workers (Tighe-Ford *et al.*, 1970 ; Karande and Thomas, 1971). In the present investigation the larvae kept in darkness survived for five days with comparatively less mortality, but failed to metamorphose beyond stage V indicating that light is essential for normal growth. In addition, light facilitated normal growth and multiplication of algal species. Tighe-Ford *et al.* (1970) earlier observed that total darkness only retarded normal metamorphosis of larvae with respect to barnacle *E. modestus*, but did not inhibit complete metamorphosis. It has been observed in the present studies, that mortality of some larvae was partly due to their entanglement in the diatom film formed at the bottom of the beaker. The film formation by *N. closterium* was inhibited in dark, but normal metamorphosis of the larvae itself is affected due to darkness. It is felt that mortality of larvae could be considerably reduced if the film formation of the diatom species is effectively prevented under normal day light conditions.

In order to prevent the bacterial and protozoan contamination in the rearing vessels, addition of antibiotics has generally been recommended (Costlow and Bookhout, 1957 ; Wisely, 1960 ; Tighe-Ford *et al.*, 1970 ; Karande and Thomas, 1971). Our observations shows that addition of penicillin did not show any significant improvement in the yield. The incorporation of penicillin, further did not eliminate the protozoan population. No bacterial film formation was encountered in all the beakers, either with or without the addition of penicillin. Earlier Moyse (1960) also observed that addition of antibiotics was of no significance. However, it is felt that use of antibiotics in rearing may be essential in specific cases.

Larvae of *B. a. amphitrite* failed to survive even to the second day when kept in sterilized sea water. Autoclaving the sea water under pressure, appears to have altered the constitution of sea water in a manner so as to affect the larval development. Moyse (1960) had used only pasturised sea water heated up to 60°C which did not have any effect on the larval metamorphosis. Tighe-Ford *et al.* (1970) had sterilized sea water by irrediating the medium with ultra violet rays which did not seem to effect the sea water.

It may be concluded that when the larvae are provided with suitable food, light and the proper sea water medium, a yield of generally 45 to 50% Cyprides and settlement can be obtained. Any one of these factors acts as a limiting factor for the growth of the larvae, in addition to the factors known to influence larval growth and settlement like gregarious attraction, stagnation of water etc., (Daniel 1963). Although higher than 50% yield has been recorded by one worker (Moyse, 1960), most of the workers have been able to obtain only 50% or even less. It is apparent that other factors not yet known cause the mortality of larvae. Unless these factors are analysed improvement in the yield would not be possible.

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