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Effect of salinity and *p*H on the larval development and spat production of *Paphia malabarica*

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Abstract

Among the exploited bivalve resources of India, clams are the most widely distributed and abundant group. The major production centers are Vembanad and Ashtamudi lakes in Kerala, where, the Venerid clam, *Paphia malabarica* is exploited for export. Hatchery reared larvae of this clam were grown at salinities 10 – 40 ‰ range with an interval of 5 (± 1) and *p*H varying from 7.0 –8.5 to study their effect on growth, survival and number of days taken for settlement. The highest growth rate of spat was registered in the salinity range of 30 –35 ‰ and a *p*H of 8-9. The results of the investigations were tested statistically by one-way ANOVA for getting a comprehensive picture of the effect of these two parameters during larval rearing and spat production. These findings indicate scope for direct application in commercial clam rearing, and also for further understanding the key environmental factors that are involved.

Key words: Salinity, *p*H, larval development, spat production, *Paphia malabarica*

Introduction

Environmental conditions, besides algal diets, influence the growth and reproduction in bivalves. A wide range of fluctuations in the environmental conditions will also affect the survival and development of the larvae. The spawning season and development of larvae are usually synchronised with these favourable conditions. In natural environment, spawning and release of gametes take place during favourable conditions, for maximum larval survival and continuity of species (Sastry, 1965).

Among the various environmental parameters, the salinity, temperature and *p*H are the main factors affecting the development of bivalve larvae (Bayne, 1976). While, temperature influences survival and growth, the effect of salinity is more pronounced on the latter. Bayne (1976) studied the effect of temperature and salinity on bivalve larvae and their settlement in natural conditions. Numerous studies have been conducted on the effect of salinity on embryonic and larval development of temperate mussel, *Perna viridis* (Shau-Hwai Tan, 1997). The survival and behaviour of oysters in lower salinities have been studied by Loosanoff (1948, 1950, and 1952); Ingle and Dawson (1950); and Davis (1958). Aspari and Anshary (1997) studied the effect of temperature and salinity on the clam, *Tridacna gigas*.

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Important works from Indian waters are those related to the behaviour of clams *Meretrix meretrix* and *Katylesia opima* in low salinities (Ranade and Kulkarni, 1973); effect of temperature and salinity on growth and feeding rate of *Villorita cyprinoides* (Preetha and Nair, 1993) and on salinity tolerance in the adult yellow clam, *Paphia malabarica* (Ram Mohan and Velayudhan, 1998). However, no detailed works on such aspects have been carried out on clam larvae from Indian waters. An understanding of the fluctuations in salinity as well as *p*H level is highly essential for perfecting methods of spat production and culture techniques. Generally all the studies related to standardisation of hatchery protocol have been carried out as univariate experiments (Bayne, 1976; Loosanoff, 1950) with a single factor (at a time) or as a multivariate experiments (Davis, 2000) with two or more factors at a time. All the above results have been used to identify ideal rearing conditions. Considering the importance from the management point of view, the effect of salinity and *p*H on larval rearing of the clam, *Paphia malabarica* was studied in detail during the present work.

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Material and methods

The work was carried out at the Shellfish Hatchery Complex of the Tuticorin Research Centre of CMFRI in the south-east coast of India. Clams of 34.0 ± .8 mm length were collected from the Dalvapuram area in Ashtamudi Lake, and transported in wet packages to the hatchery. They were transferred to 100 l FRP tank containing fresh filtered seawater. Three natural spawning occurred in the hatchery tank. After spawning, the parent clams were removed and fertilised eggs were allowed to settle at the bottom. The supernatant water containing sperms and debris was decanted and fresh seawater was added. The free-swimming larvae were siphoned out to a 100-l FRP tank for rearing experiments in different salinities and *p*H. The effect of salinity and *p*H on growth and setting were studied individually and with different broods of larvae. *Nanochloropsis salina* at a density of 5 x 10^3 cells ml^{-1} was used as feed. The control temperature, salinity and *p*H were 27.5 ± 1.0 °C, 32.5 ± 0.7 $^{o}/_{oo}$ and 8.15 ± 0.20 respectively.

Effect of salinity on larval development

Seawater of the desired salinities was prepared, as follows, prior to water exchange.

$$\text{Desired salinity (ml)} = \frac{\text{Volume of desired salinity} \times 100}{\text{Volume of known salinity}}$$

Salinity was monitored using a salinometer. Larvae were acclimatised to the lower salinities gradually by a decrease of 5‰/day. They were exposed to salinity ranges of 10, 15, 20, 25, 30, 35 and 40‰ with 33‰ as control.

Effect of pH *on larval development*

Larvae were exposed to *p*H levels of 7.5, 8.0, 8.5, 9.0 and a mean control of 8.12 (8.10-8.14). For raising the value from ambient level of 8.12, Tris buffer of 7.0-9.0 was used. For bringing down the ambient level, a solution of citric acid was used. Seawater with desired *p*H level was prepared just prior to water exchange. Growth (antero- posterior measurement), growth rate, survival of umbo, and spat survival rate and number of setting days were taken as criteria to evaluate the best and optimum salinity and *p*H requirement during larval rearing of *P. malabarica*.

Results

Effect of salinity on larval development

Growth : The mean size of spat settled was significantly ($P \leq .01$) high in 30-33‰ than in the range of 10-25‰. The analysis of covariance showed no significance between salinities 30 and 33‰ ($P \geq 0.005$). The larvae did not survive beyond day 5 in higher salinity of 35 and 40‰ (Table 1).

Growth rate: The growth rate from umbo stage upto settlement showed a significant increase with such a trend in salinity. A high growth rate of 5.1 μm at 30‰ and 33‰ indicated faster growth. The overall growth rate (Table 2) showed significant difference between treatment ($P \geq 0.05$).

Survival : Maximum survival was observed during umbo stage in control salinity 33 ± 1 °/$_{oo}$. No larvae survived in higher salinities (Fig. 1). The same pattern of survival was observed in spat settle-

Table 1. *Mean size (μm) and length size frequency of larvae at different salinities during development*

	Salinity							
No. of days	10 °/$_{oo}$	15 °/$_{oo}$	20 °/$_{oo}$	25 °/$_{oo}$	30 °/$_{oo}$	35 °/$_{oo}$	40 °/$_{oo}$	Control
2	80 ± 0	80 ± 0	80 ± 0	80 ± 0	80 ± 0	80 ± 0	80 ± 0	80 ± 0
3	96 ± 2	96 ± 0	96 ± 0	96 ± 0	96 ± 0	96 ± 0	96 ± 0	96 ± 0
5	103 ± 3	110 ± 1	112 ± 2	106 ± 2	123 ± 2	96 ± 0	96 ± 0	128 ± 2
7	118 ± 2	115 ± 2	120 ± 3	121 ± 3	140 ± 3	-	-	138 ± 3
11	121 ± 2	115 ± 3	128 ± 3	128 ± 2	179 ± 2	-	-	179 ± 2
15	125 ± 2	125 ± 3	170 ± 4	169 ± 3	182 ± 3	-	-	182 ± 2
Size frequency								
80-100 μm	10	10	-	10	10	-	90	-
100-120 μm	20	20	10	10	-	90	10	-
120-140 μm	70	70	90	80	-	10	-	-
160-180 μm	-	-	-	-	20	-	-	10
180-200 μm	-	-	-	-	70	-	-	90

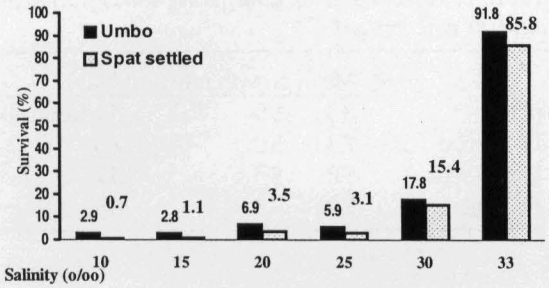

Fig. 1. *Percentage of survival of umbo and spat settlement in different salinities*

Table 2. *Growth rate (μm/day) of clam larvae reared at different salinities.*

Salinity (ppt) Stages	10	15	20	25	30	33
D-Umbo	5.4	5.1	5.1	5.9	6.3	7.2
Umbo-spat	0.8	1.3	1.0	1.8	5.1	5.5
D-spat	3.0	3.0	3.2	3.7	6.8	7.0

ment. 89 % of umbo settled at 30‰ while it was 93.4 % in control salinity. Settlement showed an increase with upto 33‰, beyond which survival got reduced (Fig. 2).

No. of days taken for spat settlement

The early initial settlement was on day 9 at 30 °/$_{oo}$ (Table 3). The complete settlement was observed by day 11. The initial settlement was high in the above salinity. Spat settlement was delayed in lower salnities. The initial settlement in salinities 25 ‰ and 20 ‰ was observed on day 11 and prolonged for another 4 days for complete settlement. This was clearly reflected in the development of larvae, growth and survival. In control salinity, the initial settlement was on day 10 and the process was completed the next day.

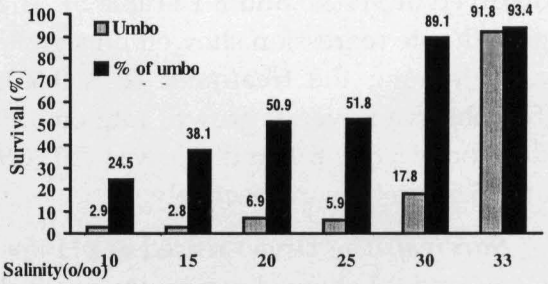

Fig. 2. *Percentage of umbo settled in different salinities*

Effect of p*H on larval development*

Growth. The larvae reared in *p*H 8.0 and ambient level of 8.1 showed a maximum mean size. The mean size of early umbo was observed as 139 μm in both *p*H levels. In a higher *p*H of 8.5, the observed mean size of umbo and spat was 134μm and 178 μm respectively. The larvae reared at lower and high *p*H did not develop and survive beyond *'D'* shape stage (Table 4). Analysis of Covariance showed no significance among various *p*H treatments

Table 3. *Effects of different salinities on clam larval setting*

Spat settlement	Salinity (‰)							
	10	15	20	25	30	35	40	33 (control)
Day of 1st setting	12	12	11	11	9	-	-	10
Final setting	15	15	15	15	11	-	-	11
Total No. of spat	285	275	350	310	1535	-	-	8580
Spat production (%)	2.85	2.75	3.5	3.1	15.4	-	-	85.8

($P \leq 0.05$). The larval size frequency distribution showed that 70% of spat of 180-200 μm settled in *p*H 8.1, 80 % in 8.0 and ambient level 8.1(Table 4). The size of larvae showed wide variation in *p*H 8.5.

Growth rate: The maximum growth rate of 7.3 μm/day in umbo stage was observed at *p*H 8.0 and 8.1 (Table 5). The growth rate regression showed no significance among the treatment ($P \leq 0.05$). The observed overall growth rate on 15th day for *p*H 8.0, 8.1 and 8.5 was 7.0, 6.9 and 6.5 μm/day respectively.

Survival: The larvae reared at *p*H levels 8.0 and 8.1 showed maximum survival of umbo on day 6, the survival rate being 73.4% and 84.5 % respectively (Fig. 3). 90 % of umbo further developed and settled in the *p*H range of 8.0 and 8.1 (Fig. 4). No larvae survived beyond '*D*' stage in *p*H 7.0, 7.5 and 9.0.

Table 5. *Growth rate of clam larvae during development in different* pH

	Mean growth rate (μm/day)			
*p*H	8.0	8.5	9.0	8.1 (control)
D–Umbo	7.3	5.0	-	7.3
Umbo-spat	5.8	5.5	-	5.7
D-spat	7.0	6.5	-	6.9

No. of days taken for spat settling

There was not much variation in settlement days in various *p*H levels (Table 6). In control, at *p*H 8.1, the larvae showed an initial settlement on day 12, which was completed on day 14. The inverse difference in the spat settlement in this experiment was due to low survival rate of spat.

Algal cell consumption

It was observed that with the development of larvae, the consumption of algae

Table 4. *Effects of different* pH *levels on larval mean size* (μm), *size frequency (%) and setting*

No. of days	*p*H				
	7.0	7.5	8.0	8.5	8.1 Control
	80 ± 0	80 ± 0	80 ± 0	80 ± 0	80 ± 0
2	80 ± 0	-	96 ± 1	96 ± 0	96 ± 0
3	-	-	122 ± 2	120 ± 2	125 ± 1
5	-	-	138 ± 2	134 ± 3	139 ± 2
7	-	-	180 ± 3	168 ± 2	182 ± 3
11	-	-	185 ± 2	178 ± 3	185 ± 2
15					
Size frequency					
80-100 μm	-	10	-	-	-
100-120 μm	-	90	-	-	-
120-140 μm	-	-	-	-	-
140-160 μm	-	-	10	10	5
160-180 μm	-	-	10	20	15
180-200 μm	-	-	80	70	80

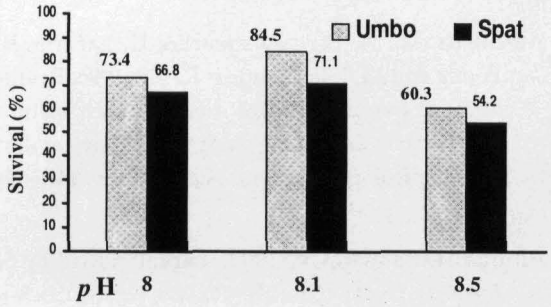

Fig. 3. *Percentage of survival of umbo during development and spat settled in different* pH *levels*

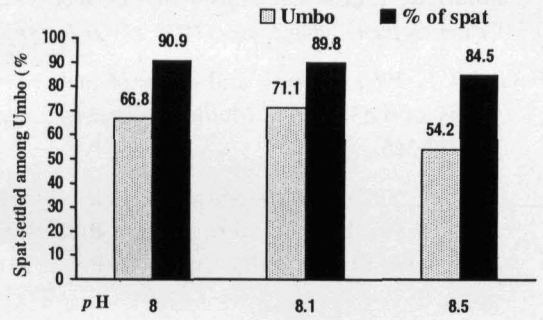

Fig. 4. *Percentage of umbo settled during development in different* pH *levels*

also increased in all the salinities (Table 7). The *D* larvae, umbo and spat in control salinity showed a maximum algal consumption. There was no significant variation in algal cell consumption in 8.0 and 8.1 *p*H levels (Table 8).

Table 6. *Effects of different* pH *levels on clam larval setting and spat production*

*p*H	7.5	8.0	8.5	8.1 (control)
Day of 1st setting	-	14	13	12
Final setting	-	15	15	14
Total No. of spat	-	6675	5415	7113
Spat production (%)	-	66.8	54.2	71.1

Table 7. *Algal cell consumption during larval rearing at different salinities*

	Algal cell consumption (%)							
Stage	10	15	20	25	30	35	40	33 control
D larvae	20	24	30	50	90	-	-	93
Umbo	28	28	38	70	90	-	-	90
Spat settled	35	35	58	70	85	-	-	82

Table 8. *Algal cell consumption during larval rearing at different levels of* pH

Stage	7.5	8.0	8.5	9.0	8.1 control
D larvae	-	85	67	-	88
Umbo	-	50	75	-	85
Spat settled	-	85	75	-	85

Discussion

Most species in temperate waters show seasonal restricted period of spawning unlike a prolonged one in tropical areas. In tropical conditions like Indian waters, spawning in bivalves is influenced by the changes in salinity over the animal beds (Ranade, 1973; Deshmukh, 1972). Naghabhushanam and Mane (1975) reported that increase in salinity initiated spawning in *Crassostrea madrasensis* from the east coast, and *Meretrix meretrix* and *Katelysia opima* from the southwest coast respectively.

Salinity profoundly influenced the growth and breeding of clams, *M. meretrix* and *K. opima* (Ranade and Kulkarni, 1973). Shau-Hwai Tan (1997) reported that larvae of *Perna viridis* could thrive in 16-30 $^{o}/_{oo}$. Similarly, responses were reported for the species *Mytilus viridis* (Lim, 1992; Tham *et al.*, 1972) and in *Mytilus edulis* (Bayne, 1965). It was reported that

a salinity range of 28-35‰ is good for normal development of larvae of *Mytilus californianus* (Young, 1941).

In the present study, the larvae reared and developed in lower salinities showed low survival rate. The larvae of *P. malabarica* reared in the ambient salinity 33‰ also showed high survival of 85 %. Walne (1965) observed lower growth of *Mytilus edulis* larvae at 15‰, slow growth at 24 % and a higher growth at 30-32‰. Anuradhakrishnan (1993) reported a survival rate of 70% in larvae of *Pinctada fucata* at 28-30‰. Albentosa *et al.* (1996) reported a high survival in clam *Venerupis decussatus* reared in salinity 27‰.

In the present study, the algae, *Nanochloropsis salina* was used. The mass culture of this species was carried out in the salinity range 28-30‰. It was observed that 90% algal cells was consumed in salinity 30‰ and only a marginal reduction occurred in low salinities. This algae being 2- 3 μm, was easily filtered and consumed by larvae for their development. Davis (1958) indicated the possibilities of algal death due to *p*H variations. Most of the marine species grew at *p*H range of 6.5 - 8.0 (Kinne, 1964). The algal species used in the present study were cultured in seawater with a *p*H range of 7.9 - 8.2. Algal consumption by larvae in this range showed normal development and settlement. The spat reared in these levels of *p*H also showed the same result. From the results of the experiments, it could be concluded that the larvae of the yellow clam *P. malabarica* could successfully be reared in salinity range 25-33‰ and *p*H 8.0 - 8.5.

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