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Reproductive performance of the blue swimmer crab, *Portunus pelagicus* in relation to salinity, pH and nature of substratum

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Abstract

Females of *P. pelagicus* of mean carapace width 159.86±3.04 mm collected from the Palk Bay were used to study the reproductive performance in relation to salinity, pH and nature of substratum. Each parameter was studied as a separate experiment (60 days) with three different treatments in triplicate. The results were analysed using one-way ANOVA and conclusions were drawn accordingly. In the trials where salinity was manipulated to study the effect on the reproductive performance, one-way ANOVA showed that the differences in average fecundity values among treatments were insignificant (P>0.01). However the average fecundity value was higher and spawning interval was lower at 35‰, when compared to 25 and 30‰. In pH manipulation studies, the average fecundity values of the treatments with pH 7.5±0.1, 8.1±0.1 and 8.5±0.1 did not show any significant difference (P>0.01). The highest average fecundity was obtained in the treatment with pH 8.1±0.1. In photoperiod regulation experiments, three regimens such as 18hL:6hD, 6hL:18hD and 12hL:12hD were studied. Though the differences in average fecundity values between treatments were insignificant (P>0.01), 6hL:18hD was observed to be the most suitable photoperiod regimen for better reproductive performance of *P. pelagicus* since it showed the highest average fecundity. In the study on the reproductive performance of the species with different kinds of substrata, sandy bottom was found to be highly favourable for broodstock development.

Keywords: Portunus pelagicus, reproductive performance, broodstock development.

1. Introduction

Marine blue swimmer crab, *Portunus pelagicus* is one of the candidate species for aquaculture because of its fast growth, attractive appearance and taste. Blue swimmer crab is available throughout the coast of India especially in the south-east and south-west region. Even though there are some established 'fattening' practices going on in some pockets in the coastal areas, there is no organized 'grow-out' of the species due to the difficulty in the availability of quality seeds.

As there are no commercial hatcheries for the species, the farmers are forced to depend on the wild for juvenile/younger crabs. Difficulty in obtaining juveniles or young ones from the wild for farming/fattening operations, their inferior quality and concerns of stock depletion due to over exploitation have encouraged research activities to frame out workable, cost effective and foolproof technology for producing baby crabs on a commercial basis. The primary requirement for evolving a hatchery technology is the availability of quality broodstock. So efforts are on to develop high quality broodstock under controlled conditions.

Temperature, salinity, pH, dissolved oxygen, stocking density, diet, water quality, photoperiod, light intensity, nature of tank bottom, colour of the tank, population density, organic load, nitrate levels, heavy metal concentrations, stress to which the brooder is exposed and size of the animal are some of the most significant factors affecting broodstock development in decapods (Primavera, 1985; Hansford *et al*., 1993; Ramos *et al*., 1995). The physico-chemical parameters under which the broodstock is maintained are of utmost importance as they can directly influence the egg and the embryo quality, which will ultimately reflect on the survival of larvae (Primavera, 1985).

The effect of salinity on oogenesis, embryogenesis and larval quality of south-western Atlantic estuarine grapsid crabs has been reported by Bas and Spivak (2000). The salinity conditions during embryogenesis have been observed to influence larval salinity tolerance and osmoregulatory capabilities in the estuarine crab, *Chasmagnathus granulata* also (Charmantier *et al*., 2002). Another important physico-chemical factor affecting the reproductive development in crustaceans is pH. Muthu *et al*. (1984) observed that a pH of 8.0 to 8.2 is inevitable for the complete development of ovary in unilateral-eyestalk ablated

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Indian white prawn, *Fenneropenaeus indicus*. According to Vijayan and Diwan (1995), pH has significant relationship with growth and moulting of *F. indicus*, which may reflect on the gonad development as well. Photoperiod, which is an important factor for gonadal growth, appears to act as an environmental cue for the commencement of reproduction in crustaceans and also controls feeding that may sequentially influence the brooder performance (Meusy and Payen, 1988). Photoperiodic regulation of ovarian development has been successfully demonstrated in the crayfish, *Procambarus simulans* by Perryman (1969). The nature of tank bottom is an important criterion influencing spawning of crabs. As per Campbell (1984), successful fastening of large number of ova in portunid crabs is found to occur only when the female has an opportunity to bury itself in the soft substratum like sand.

In this study, an attempt is made to understand the effects of salinity, pH, photoperiod and the nature of tank substratum on the reproductive performance of *P. pelagicus* through various reproductive indices like total number of spawning, spawning interval, fecundity and incubation period for each spawning.

2. Materials and methods

2.1. Collection and maintenance of animals

Mated females with spermatophores in the spermathecae (Robertson and Kruger, 1994) of mean carapace width 159.86±3.04 mm (±SD) were collected during November 2005 to December 2006 from Palk Bay off Mandapam (9°17′ N, 79 °9′ E). At a time, 20 animals were brought to the wet laboratory and acclimatized to controlled conditions by maintaining in black, oval FRP (Fibreglass Reinforced Plastic) tanks of 1 ton capacity for three days. Thereafter, healthy and active animals (nine animals for each experimental parameter) were selected and tagged by pasting red plastic tags with number on their carapace. The exact carapace width and weight of the animals were then measured and they were stocked in black, oval FRP tanks of 500 L capacity at the rate of one animal per tank under continuous aeration, for study. Monitoring of water quality parameters and water exchange (70%) were carried out every day. The animals were fed daily with raw cuttlefish (*Sepia pharaonis*) and clam (*Meritrix meritrix*) meat at a ratio 1:1, *ad libitum*. Feed waste was removed every morning after stopping the aeration. The experimental design was maintained the same throughout the study and the experimental period for each parameter was set as 60 days. All the experiments were carried out in triplicate with controls.

2.2. Assessment of fecundity and reproductive indices

The animals stocked were examined daily for berry development. Once the berry developed, the animals were weighed and twenty egg samples were taken from different regions of the light yellow berry of each animal to assess fecundity. When the berry attained dark grey colour, the animals were transferred to cylindro-conical FRP tanks of 500 L capacity for hatching. Once the eggs were hatched, the animals were weighed again and the difference in weight of the animal was taken as the berry mass to calculate fecundity. The reproductive performance indices such as total number of spawning, spawning interval, fecundity and incubation period at each spawning were also assessed timely.

2.3. Data analysis

The data are presented as the mean ± standard deviation (SD). The average of first and second spawning fecundity values of the treatments were analyzed using one-way ANOVA. Differences were considered significant at $P<0.01$. Inferences were drawn based on the results of the experiment and statistical analysis.

2.4. Parameters studied

In the experiment to study the reproductive performance in relation to salinity, the three values tried were 25‰ (treatment 1), 30‰ (treatment 2) and 35‰ (treatment 3/control). The temperature, pH and photoperiod were maintained at 28±1.0°C, 8.1±0.1 and 12hL:12hD (hL = hours light, hD = hours dark), respectively. The values selected to the study the reproductive performance in relation to pH were 7.5±0.1 (treatment 1), 8.5±0.1 (treatment 2) and 8.1±0.1 (treatment 3/control). The pH was adjusted by adding sodium carbonate or hydrochloric acid to increase or decrease, respectively. The temperature, salinity and photoperiod were maintained at 28±1.0°C, 35‰ and 12hL:12hD, respectively. The regimens tried in the experiment conducted to study the reproductive performance of *P. pelagicus* in relation to photoperiod were 18hL:6hD (threatment 1), 6hL:18hD (treatment 2) and 12hL:12hD (treatment 3/control). Tanks were covered with black sheets after the photophase and the light intensity maintained was 750 lux (Gardner and Maguire, 1998; Hoang *et al*., 2002). The temperature, salinity and pH were maintained at 28±1.0°C, 35‰ and 8.1±0.1, respectively.

In the investigation to study the effect of various types of tank substrata on the reproductive performance of *P. pelagicus*, the different types of treatments attempted were substratum with sand (treatment 1), substratum with seaweed (*Kappaphycus alvarezii*) (treatment 2) and without any material as substratum (treatment 3/control). For the study, beach sand was

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used after repeated washing with freshwater. Fresh seaweeds were collected from commercial seaweed farms nearby. The seaweeds were cut into comparatively smaller pieces and spread on the bottom. Temperature, salinity, pH and photoperiod were maintained at 28±1.0°C, 35‰, 8.1±0.1 and 12hL:12hD, respectively.

Temperatures in all the tanks for various experiments were maintained using titanium electronic aquarium heaters with thermostats (Azoo, USA). All the tanks except those to study the effect of the nature of substratum were provided with a sandy bottom of 4 inches thickness since earlier trials gave better reproductive response in tanks with sand as substratum.

3. Results

The body weight of the animals selected for the study ranged from 128 g to 154 g. Two spawnings occurred in all the experimental tanks under the parameters studied such as salinity, pH and photoperiod. Whereas, in the experiment to find out the most favourable substratum, two spawnings occurred in only those tanks with sand as substratum. Fecundity values of the second spawning were lesser than those of the first spawning in all the cases. The spawning intervals and incubation periods were comparable among the replicates of each parameter studied. The fecundities however, showed difference.

In the experiment where salinity was manipulated to understand the effect on reproductive performance, the incubation periods were 8 and 9 days for the first and second spawning, respectively in all the treatments (Table 1). The spawning intervals differed, recording 18, 17 and 16 days in two out of three replicates at 25, 30 and 35‰, respectively. The average of first and second spawning fecundity values showed a maximum at 35‰ salinity (Figure 1). One-way ANOVA showed that the average of first and second spawning fecundity values were not significantly different (P>0.01) among the treatments.

In the trials to study the effect of pH variation on the reproductive performance, the incubation periods under all the treatments continued to be 8 and 9 days for the first and second spawning, respectively (Table 2). The spawning interval was 16 days in two out of three replicates at all the pH values investigated. The average of the first and second spawning fecundity values were insignificantly different (P>0.01). The highest value was recorded at pH 8.0±0.1 (Figure 1).

In the study to trace out the most favourable photoperiod regimen, the incubation periods were 10 and 11 days in treatment 1 and, 9 and 10 days in treatments 2 and 3 for the first and second spawning, respectively (Table 3). The spawning intervals were 17 days in two out of three tanks in treatment 1 and 16 days in two out of three replicates in treatments 2 and 3. The average of the first and second spawning fecundity values were insignificantly different (P>0.01) and it recorded an increase at photoperiod regimen 6hL:18hD (Figure 1).

Out of the three treatments tried in the experiment to find out the most favourable tank substratum for *P. pelagicus*, the animals in tanks with sand developed berry twice during the experimental period. The tanks with seaweed as substratum and those without any material as substratum did not develop berry. The incubation periods were 8 and 9 days for the first and second spawning, respectively in all the replicates and the spawning interval was 18 days in two out of three tanks. The average fecundity at first and second spawning were 56,9186.7±41,632.2 and 44,0515.7±27,508.4, respectively.

Table 1. Reproductive performance indices of blue swimmer crab in different salinities (Statistical differences (P>0.01) were not observed among the treatments).

Performance indices (average values)	Treatments (T)					
	T1 (25‰)		T2 (30‰)		T3 (35‰)	
Spawning number	I	II	I	II	I	II
Fecundity ± SD	592857 ±12838.1	431960± ±7808.1	599404.66 ± 32566.4	441497 ±17346.6	611595 ±25547.4	436402.7 ±5695.7
Incubation period (days)	8	9	8	9	8	9
Spawning interval (days) ± SD	18.33±0.57735		17.33±0.57735		16.33±0.57735	

Table 2. Reproductive performance indices of blue swimmer crab in different pH (Statistical differences (P>0.01) were not observed among the treatments).

Performance indices (average values)	Treatments (T)					
	T1 (7.5±0.1)		T2 (8.5±0.1)		T3 (8.1±0.1)	
Spawning number	I	II	I	II	I	II
Fecundity ± SD	546511 ±10146.3	419965.7 ±17190.2	553896.7 ±24764.3	432965 ±36715.2	594494.7 ±7328.3	505186 ±21740.4
Incubation period (days)	8	9	8	9	8	9
Spawning interval (days) ± SD	16.33±0.57735		16.33±0.57735		15.66±0.57735	

Table 3. Reproductive performance indices of blue swimmer crab in different photoperiods (Statistical differences (P>0.01) were not observed among the treatments).

Performance indices (average values)	Treatments (T)					
	T1 (18hL:6hD)		T2 (6hL:18hD)		T3 (12hL:12hD)	
Spawning number	I	II	I	II	I	II
Fecundity ± SD	554767.33 ±22066.3	421360.66 ±9088.7	588205.33 ±1126.0	472466.66 ±2694.2	569778.33 ±11443.4	448205.33 ±8144.0
Incubation period (days)	10	11	9	10	9	10
Spawning interval (days) ± SD	16.66±0.57735		15.66±0.57735		15.66±0.57735	

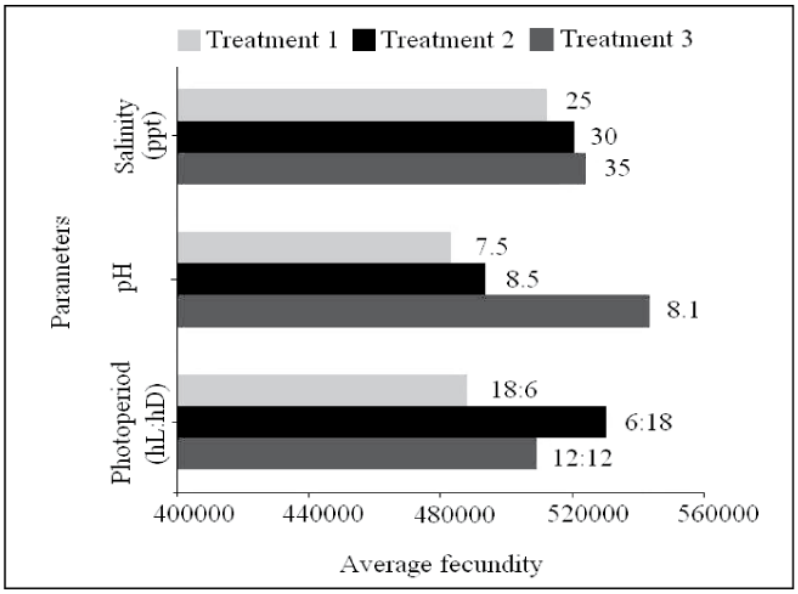

Figure 1. Effect of salinity, pH and photoperiod on the average fecundity (of first and second spawning) of blue swimmer crab.

3. Discussion

The reproductive characteristics of a species are the result of interaction between various endogenous and exogenous factors (Flores and Paula, 2002). Factors such as temperature, salinity, food availability, photoperiod and lunar cycles could determine the periodicity and extension of the reproductive period of a species, as well as its fecundity (Thurman, 1985). The parameters selected for the present study were salinity, pH, photoperiod and the nature of tank substratum.

Salinity conditions during embryogenesis were observed to influence larval salinity tolerance and osmoregulatory capabilities in the mud crab, *Rhithropanopeus harrisii* (Laughlin and French, 1989a) and *C. granulata* (Charmantier *et al*., 2002). It was found that low salinities during incubation rather than low temperatures, reduced zoeal dry weights by as much as 25% in *R. harrisii* (Laughlin and French, 1989b). In the present study, one way ANOVA of the average fecundity values of salinity treatments such as 25, 30 and 35‰ during the experimental period showed no significant difference (P>0.01). The other reproductive indices studied like incubation period and spawning interval also did not show much variation. So it is thought that all the three salinity values might have affected the reproductive character of the animals in a more or less similar way. Minor differences in the values of reproductive indices may not necessarily be attributed to salinity variation alone as there could be other physico-chemical and/or biological factors influencing the reproductive performance of the species. Since 35‰ treatment gave the highest average value for fecundity as shown in Figure 1, that value has been considered as the most favourable salinity for better reproductive performance of *P. pelagicus* under the present study. De Lestang *et al*. (2003) observed that *P. pelagicus* when exposed to salinity gradient was found to breed in waters of higher salinity. Giménez and Anger (2001) observed that in *C. granulata*, the larval biomass and, subsequently the early larval survival and growth were higher when the brooders were maintained at 32‰ salinity. However, salinity values beyond an optimum range are reported to be harmful for crustaceans by some authors. Vijayan and Diwan (1995) reported low growth rate and muscle necrosis in *F. indicus* exposed to low (5‰) and high (45‰) levels of salinity. Lakshmi *et al*. (1978) observed muscle necrosis in the brown shrimp, *Farfantepenaeus aztecus* exposed to sub-optimal and supra-optimal salinity ranges. They described this as an indication of stress. Since such harmful effects were not noticed in the present study, it is envisaged that the salinity values tried here are within the tolerable range of the species.

In the case of pH manipulation trials, the reproductive performance indices gave comparable results in all the treatments during the study period. Though there were differences in the average fecundity values, they were not statistically significant (P>0.01). So it is thought that the pH values tried might have influenced the reproductive performance of *P. pelagicus* in a similar manner. As 8.1±0.1 gave the highest average value for fecundity during the study period as shown in Figure 1, it was considered to be the most suitable pH for reproductive performance in this study. Besides, the lowest average spawning interval was also recorded at the same pH. Muthu *et al*. (1983) observed that all pH below 8.2 failed to induce gonadal growth even with eyestalk ablation in *F. indicus*. The results of the present study also agree to Cobo and Fransozo (2003), where a similar range of pH was found to be the optimum for ovary maturation and spawning in the red mangrove crab, *Goniopsis cruentata*. A concurrent pH range of 8.15 to 8.35 was adopted by Gandy (2004) for the broodstock development of *F. aztecus*. Similarly, Peixoto *et al*. (2004) maintained a pH of 8.05 for the successful maturation trials in the pink shrimp, *F. paulensis*. As per Vijayan and Diwan (1995), a pH of 8.0±0.2 gave fast moulting and highest growth increment in *F. indicus*. According to Havas and Hutchinson (1982), pH values below 7.0 are harmful to crustaceans. Mass mortality (Pillai *et al*., 1983) and impairment of osmoregulatory function (Allan and Maguire, 1992) were reported in crustaceans at low pH levels. In closed systems the decline of pH is one of the consequences of the oxidation of ammonia from animal excreta (Wickins, 1984).

The reproductive capability of crustaceans has been found to be influenced by the quantity, colour and intensity of light (Hoang *et al*., 2002). In the present investigation, there was no significant difference (P>0.01) between the average fecundity values of the different photoperiod regimens. This implies that the photoperiod regimens tried had a more or less similar influence on the reproductive performance of the animals. Vijayan and Diwan (1995) recorded a statistically insignificant difference between the photoperiod regimens 12hL:12hD, 24hL:0hD and 0hL:24hD in the reproductive performance of *F. indicus*. However, based on the highest average value for fecundity, 6hL:18hD was taken as the most suitable photoperiod regimen for reproductive performance in the present study (Figure 1). Similarly, Peixoto *et al*. (2004) adopted a photoperiod regimen with more dark hours (10hL:14hD) for the maturation of *F. paulensis*. Hamasaki *et al*. (2004) reported that 12 to 14 hours of darkness reduced the number of days to oviposition in the swimming crab, *P. trituberculatus*. A photoperiod regimen of 10hL:14hD was recommended by Hoang *et al*. (2002) for the successful maturation of pond reared penaeid prawn, *F. merguiensis*. Emmerson (1980) reported better reproductive performance of *F. indicus* held in tanks with black interiors in comparison with those in tanks with white interiors to the dark conditions inside. Although a profound effect of

photoperiod on reproductive activities has been illustrated in many animals, the precise mechanism by which it acts is meagrely known. Nagabhushanam and Farooqui (1981) have suggested that circadian rhythm or circa-lunadian rhythm of photosensitivity may be responsible for the effect whereas, Skinner and Graham (1974) have attributed fast moulting and better reproductive performance in animals kept under longer dark conditions to privacy.

The information on substratum preference for crustacean broodstock development is scanty. In the present study, it was found that the animals under treatments with seaweed as substratum and those without any substratum did not deposit egg. In broodstock experiments with the mud crab, *Scylla serrata*, Davis *et al.* (2004) observed that the berried females attached the eggs to the pleopods only when enough beach sand was provided as substratum, which otherwise resulted in a complete loss the eggs. Djunaidah *et al*. (2001) effectively used a mud-based medium for broodstock development in the same species. According to Hamasaki *et al*. (2008), who studied the ovipositional behaviour in *P. trituberculatus*, a sandy substratum is vital for successful egg attachment. The observations in the present study are in agreement with the above citations. According to Lavilla-Pitogo *et al.* (2001), provision of an optimum amount of substrata may not only reduce stress, but also reduce the build up of fouling organisms on the crabs. The present study also, showed the necessity of a sandy substratum for egg deposition in crabs. However, the preference shown by the spider crab, *Maja squinado* to areas of dense algal growth for spawning as reported by Sala *et al.* (1998) does not match with the present observations. This might be either due to the difference in bottom material preference from species to species or just that the seaweed might have acted as a shelter.

In the present study it was found that, the physico-chemical parameters selected like salinity, pH, photoperiod and tank substratum characteristics had varying degrees of influence on the reproductive performance of the animal. Though none of the values tried in any of the treatments could elicit a statistically significant difference, variations could be noticed in some of the reproductive indices like fecundity. The uniform decrease in fecundity exhibited during second spawning requires a detailed study to trace out the precise reasons. The importance of a sandy substratum for brood development in *P. pelagicus* is also demonstrated in the present study.

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