The effect of different pH and photoperiod regimens on the survival rate and developmental period of the larvae of *Portunus pelagicus* (Decapoda, Brachyura, Portunidae)

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Received: December 2011 Accepted: August 2012

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Keywords: *Portunus pelagicus*, Photoperiod, Larval rearing, Larval development, Larval survival.

Crustacean larval development occurs within a narrow range of environmental parameters (Sastry, 1983). Among the abiotic characteristics that influence the developmental period and survival rate of the larvae, pH and photoperiod are of great importance. High and low levels of pH are known to adversely affect the physiology of the animals due to the increased amounts of ammonia and nitrite (Oanh et al., 2000). High values and sudden fluctuations in pH are found to cause disease outbreaks (Cheng and Chen, 1998) and stress (Oanh et al., 2000), respectively in crustacean larvae. A pH around 8.5 was found to be the optimum for larval rearing of the mud crab *Scylla paramamosain* (Nghia et al., 2007). Another important parameter that decides the survival and developmental period of crustacean larvae is the period of exposure to light or darkness. Other aspects of behaviour and physiology that are influenced by lighting includes entrainment of physiological processes to circadian cycles (Dalley, 1980), endocrine control of metamorphosis (Eagles et al., 1986), initiation of ecdysis (Waddy and Aiken, 1991), cannibalism (Hecht and Pienaar, 1993) and swimming activity and hence metabolism (Gardner, 1996).

The effects of photoperiod on larval survival have been studied in several species such as the common shrimp *Crangon crangon* (Dalley, 1980), red king crab *Paralithodes camtschaticus* (Nakanishi, 1987), red frog crab *Ranina ranina* (Minagawa, 1994), spiny rock lobster *Jasus edwardsii* (Bermudes and Ritar, 2008) and Japanese spiny lobster *Panulirus japonicus* (Matsuda et al., 2012). Changes in survival rate and developmental period of decapod larvae in different lighting regimes are often simply attributed to effects on feeding. In the giant crab *Pseudocarcinus gigas*, it was observed that continuous light or dark regimes should be avoided for better overall larval survival (Gardner, 2001). In many decapod species, larvae are less severely affected by '0' hour light and are able to capture prey at a reduced rate as in *R. ranina* (Minagawa, 1994) or they may actually feed at a rate equal to or greater

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than in light as in the American lobster *Homarus americanus* (Eagles et al., 1986) and *P. camtschaticus* (Nakanishi, 1987). A 24 hour light period caused a relatively rapid development of larvae in *P. gigas* (Gardner and Maguire, 1998) and *P. camtschaticus* (Nakanishi, 1987). Similarly, longer light hours promoted smooth and regular metamorphosis, against delayed or suppressed metamorphosis from phyllosoma stage to puerulus stage caused by longer dark hours in *P. japonicus* (Matsuda et al., 2012). Though there are a number of studies on the effect of light on the activity, swimming or phototaxis of crab larvae (for example, Sulkin (1975); Gardner and Maguire(1998), those on the effect of pH and photoperiod on crab larval development period and survival are relatively less (Minagawa, 1994; Gardner and Maguire, 1998). The marine blue swimmer crab *Portunus pelagicus* is one of the candidate species for culture because of its fast growth, attractive appearance and taste. The species is available throughout the coast of India especially in the south-east and south-west region and breeds round the year (Pillai and Nair, 1973). Since commercial hatcheries are not available for the species (Soundarapandian and Tamizhazhagan, 2009), the farmers are forced to depend on the wild for juveniles for culture practices. Difficulty in obtaining juveniles from wild, their non-uniform size and concerns of stock depletion due to overexploitation have encouraged research activities to frame out a cost effective and foolproof technology for producing crab seeds on a commercial basis. Knowledge on the influence of abiotic characteristics like pH and photoperiod on the rate of survival and developmental period of the larvae of *P. pelagicus* is of extreme significance in standardising the hatchery technology and developing a conservation management strategy for the species. The studies on the effect of these parameters on the larvae of *P. pelagicus* from Indian waters are meagre. The present study attempts to trace out the effects of pH and photoperiod on the survival rate and developmental period of the larvae of *P. pelagicus* from the south-east coast of India. The study also attempts to find out the optimal range of these parameters for each larval stage.

Ten female *P. pelagicus* of size range 140 - 160 mm carapace width with light yellow berry were collected from the Gulf of Mannar off Mandapam (9°09' N, 78°44' E). The animals were brought live to the wet laboratory, given a prophylactic dip in 200 ppm formalin for 30 minutes (Parado-Estepa et al., 2002) and stocked in 1000 l FRP (Fibreglass Reinforced Plastic) tanks with black interior for acclimatization (2 days). Three of the most active animals were weighed (to the nearest 1 g) and stocked separately in 500 l FRP tanks for the study. The animals were fed to satiation with raw clam (*Meritrix meritrix*) and cuttlefish (*Sepia pharaonis*) meat (1:1). Excess feed, faeces, discarded eggs and other debris were siphoned out from the tank daily, accompanied by 10% water exchange. The temperature, salinity, pH and photoperiod were maintained at 28 ± 1 °C, 35 ‰, 8.1 ± 0.1 and 12hL:12hD, respectively. Dissolved oxygen level was

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maintained above 5 mg/l throughout the experiment. A sandy substratum (100 mm thickness) was provided in all the tanks for refuge and to prevent egg loss (Davis et al., 2004). The pH was adjusted by adding 1 normal solution (N) sodium carbonate or 1N hydrochloric acid when required. Tanks were covered with black sheets (200 µm thickness) after the photophase. Photoperiod adjustments were carried out using fluorescent lamps and the light intensity maintained was 750 lux. The berry colour was examined daily and at dark grey colour stage they were transferred to cylindro-conical FRP tanks of 500 l capacity with white interior for hatching.

Once the eggs were hatched, the zoea 1 (identified as per Shinkarenko, 1979) were collected using 150 µm mesh sieve and the most active ones (identified by their vigorous swimming activity in opposite direction in a whirl), from the same brood were stocked after rinsing in fresh seawater in 200 l rectangular FRP tanks at a density of 25 no l^{-1} (*i.e.* 5000 larvae per tank, as this stocking density gave better results in earlier trials). Dead larvae, feed waste and other debris were siphoned out prior to the daily water exchange (70%). Abiotic parameters were monitored regularly and continuous aeration was provided to maintain the dissolved oxygen level above 5mg/l in all the tanks throughout the experiment. The experiments were carried out in triplicate (n = 3) with controls (3 tanks were stocked initially). The survival rates at each of the five larval stages such as zoea 1 (Z1), zoea 2 (Z2), zoea 3 (Z3), zoea 4 (Z4) and megalopa (M) (identified as per Shinkarenko, 1979) were calculated by daily random sampling (10 samples were taken each time, twice daily, till the end of the experiment) (mortality due to any reason other than pH or photoperiod variation is assumed to be the same in all the replicates of the values tested under each parameter). At first crab instar (C1), they were harvested using 500 µm mesh nets and counted to estimate the final survival rate. The periods of development of the larvae (from Z1 to C1) for each replicate under the temperature and salinity treatments were also recorded.

Zoeae 1 and 2 were fed twice daily with *Skeletonema costatum* and *Brachionus plicatilis* (cell densities maintained at 50,000 no ml^{-1} and 25 no ml^{-1}, respectively). The larvae were also fed with formulated prawn feed (Frippak 2 CD - INVE, Belgium) at a rate of 0.5 g 1000 l^{-1} (4 times daily). Zoeae 3 and 4 were also fed with *S. costatum*, *B. plicatilis* and formulated prawn feed (at cell densities 50,000 cells ml^{-1}, 30 no ml^{-1} and at the rate 0.5 g 1000 l^{-1}, respectively). The frequency of feeding was maintained the same as in the case of Z1 and Z2. Megalopae were fed daily once with *Moina macrura* and newly hatched *Artemia* nauplii (cell densities maintained were 3 no ml^{-1} and 5 no ml^{-1}, respectively). They were also fed twice daily with freshly prepared egg custard at the rate of 0.5 g 1000 l^{-1}. Rotifers and *M. macrura* were collected from the wild and mass cultured in 1000 l FRP tanks on microalgae. Microalgal (*Chlorella* sp. and *S. costatum*) inoculums were obtained from the live feed laboratory of the Central Marine Fisheries Research Institute,

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Mandapam and scaled up in 1000 l FRP tanks.

Two parameters *viz.* pH and photoperiod were investigated separately. One set of experiment investigated the effect of three different pH values and another set investigated the effect of three photoperiod regimens. The pH values selected for the study were 7.5 ± 0.1 (T1/treatment 1), 8.1 ± 0.1 (T2/treatment 2/control) and 8.5 ± 0.1 (T3/treatment 3). For increasing or decreasing pH, 1N sodium hydroxide or 1N hydrochloric acid was added, when required. The temperature, salinity and photoperiod regimen maintained were 28.0 ± 0.1 ºC, 35 ‰ and 12hL:12hD, respectively. Temperatures were maintained by keeping the tanks in chilled room and then increasing the water temperature to the desired levels using aquarium heaters with thermostats (AZOO, USA, 100W).

The photoperiod regimens chosen for the experiment were 6hL:18hD (T1), 12hL:12hD (T2/control) and 18hL:6hD (T3). Tanks were covered with black sheets (200 µm thickness) after the photophase and the light intensity was maintained at 750 lux (Gardner and Maguire, 1998; Hoang et al., 2002). The temperature, salinity and pH maintained were 28.0 ± 0.1ºC, 35 ‰ and 8.1 ± 0.1, respectively. Observations on feeding, growth, cannibalism, activity and survival were recorded every morning and evening. The survival rates (stage wise and overall) and the overall larval developmental periods of each replicate under different pH and photoperiod treatments were statistically analyzed with one-way ANOVA. The data with significant difference ($P<0.01$) were then subjected to Post Hoc Test of multiple comparison using 'Fisher's Least Significant Difference (LSD)' (Payne et al., 1993). Among results, the overall survival rates were expressed as a percentage of the total larvae stocked and the larval developmental period in number of days.

Complete larval development took place in all the replicates under the parameters studied. The survival rate fell almost gradually with time and stage of the larvae in all the pH and photoperiod treatments studied. The differences in the average overall survival rates and developmental period among different pH treatments investigated were not significant ($P>0.01$). The overall survival rate at pH 7.5 ± 0.1, 8.0 ± 0.1 and 8.5 ± 0.1 were 6.28 ± 0.12%, 6.76 ± 00.91% and 5.59 ± 0.53%, respectively. When compared with the larval survival rates at the control pH 8.0 ± 0.1, the survival rates at other pH values such as 7.5 ± 0.1 and 8.5 ± 0.1, showed significant variance ($P<0.01$) at some of the larval stages (Table 1, Fig. 1). The average larval developmental period was 16 days.

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Table 1: Preferred pH and photoperiod values at each larval stage of *Portunus pelagicus* and the corresponding survival rates

Larval stages	Preferred pH	Corresponding survival rates (%) (±SD)	Preferred photoperiods (hL/hD)	Corresponding survival rates (%) (±SD)
Z1	7.5 ± 0.1	73.06 ± 02.57	12:12	73.87 ± 01.67
Z2	8.0 ± 0.1	84.81 ± 04.22	06:18	84.51 ± 02.95
Z3	8.5 ± 0.1	65.60 ± 04.68	18:06	85.10 ± 18.37
Z4	8.0 ± 0.1	42.47 ± 09.29	18:06	55.03 ± 11.52
M	7.5 ± 0.1	43.70 ± 07.61	12:12	39.04 ± 05.17

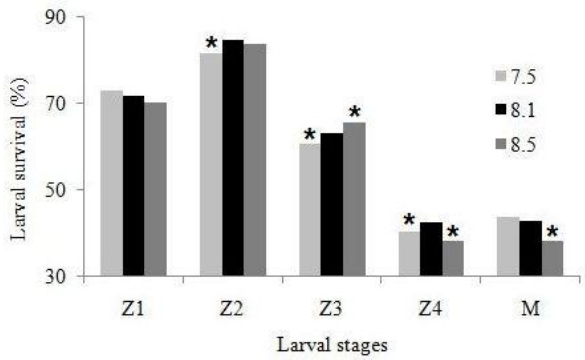


Figure 1: The survival rates of *P. pelagicus* larvae at each stage of development under different pH (Z1-zoea 1, Z2- zoea 2, Z3-zoea 3, Z4-zoea 4, M-megalopa) (*P<0.1)

The differences in the average overall survival rates and developmental period among the photoperiod treatments investigated were not significant (P>0.01). The overall survival rate at photoperiods 18hL:06hD, 12hL:12hD and 06hL:18hD were 5.55 ± 0.65%, 7.74 ± 0.37% and 6.63 ± 0.97%, respectively. When compared with the larval survival rates at the control photoperiod regimen 12hL:12hD, the survival rates at other photoperiod regimens such as 18hL:06hD and 06hL:18hD, showed significant variance (P<0.01) at some of the larval stages (Table 1, Fig. 2). The average larval developmental period was 16 days.

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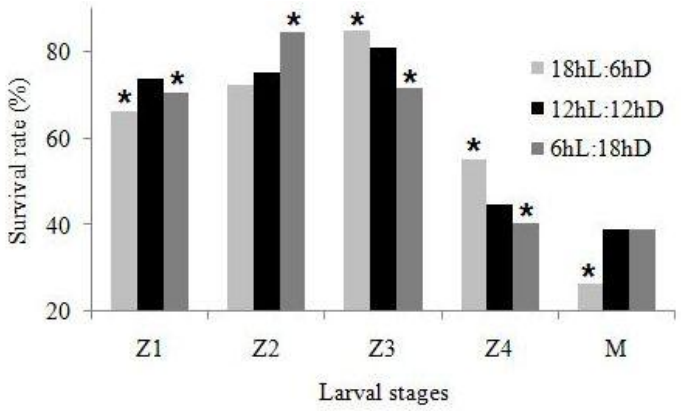


Figure 2: The survival rates of *P. pelagicus* larvae at each stage of development under different photoperiods (Z1-zoea 1, Z2- zoea 2, Z3-zoea 3, Z4-zoea 4, M-megalopa) (*P<0.1)

Rearing techniques, disease and nutrition are the three main areas of research that have supported commercial production of marine fish and crustacean larvae (Sorgeloos and Leger, 1992). One of the serious consequences of ambient pH variation is the change in the ammonia level, which is the principal excretory product in crustaceans (Kinne, 1976). In the present study, the pH values investigated the influence of larval survival rate in an almost similar manner. However, since the highest average survival was obtained at 8.0 ± 0.1, that pH was considered as the most suitable for larval rearing in the present study. A similar range of pH was found to be the optimum for larval rearing in the mangrove crab *Ucides cordatus*, (Ventura et al., 2008) and white leg shrimp *Litopenaeus vannamei* (Van-Tri and Hoa, 2004). Furthermore, commercial seed production of *P. pelagicus* was attained with an overall survival rate of 4.3% at a pH range of 7.5 - 8.0 (Soundarapandian et al., 2007). The survival rates under a particular pH varied significantly with stage. This indicates that the influence of a particular pH varies considerably from stage to stage.

Low values of pH are considered to be harmful for crustacean larvae. A pH below 5 has been found to cause decreased activity, gill damage, moulting difficulty and death in *Macrobrachium rosenbergii* larvae (New and Singholka, 1985). Such harmful effects were not observed in any of the replicates under the pH treatments followed here. Sudden fluctuations in pH and rearing in low pH levels have lead to mass mortality due to stress in the larvae of blue crab *Callinectes sapidus* (Sandoz and Rogers, 1944) and *M. rosenbergii* (Oanh et al., 2000). However, even the lowest pH tested (7.5 ± 0.1) did not cause any significant adverse effect in the present study.

Intensity variations, spectral composition, polarization and photophase duration are some of the characteristics of light that influence the physiological activities and behaviour of animals (Dalley, 1980). Among these, the daily cycle of light and darkness, and the seasonal changes in the proportions of light-dark period are of more significance in the life of crustaceans (Dalley, 1980). In the present study, the different photoperiod regimens investigated

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had a statistically insignificant influence on the survival rate and developmental period of *P. pelagicus* larvae. Concurrent insignificant influence was shown also in the first three states of phyllosoma larvae in *J. edwardsii* when reared under photoperiod regimes with 0, 6, 12, 18 or 24 h light (Bermudes and Ritar, 2008). Likewise, the survival rates of juvenile *F. merguiensis* did not show significant difference (P>0.01) when reared under photoperiod regimens 12hL:12hD and 10hL:14hD (Hoang et al., 2003).

However, since 12hL:12hD showed the highest survival rate and lowest developmental period, that regimen is the most favourable photoperiod for *P. pelagicus* larval rearing here. Similar photoperiod regimens were reported to be optimum for larval rearing in the tiger shrimp *Penaeus monodon* (Khalik et al., 1992), mud crab *Scylla serrata* (Quinitio et al., 2001) and palaemonid shrimp *Palaemonetes argentinus* (Diaz et al., 2003). The significant stage-specific preference for photoperiod observed here shows that the most ideal photoperiod values, where the survival rate is expected to be the highest and the moulting rate optimum, do change with larval stage.

Zoeal stages of *P. pelagicus* in the present study showed phototaxis as generally shown by zoeae of many other crabs (e.g. Sulkin et al., 1979; Gardner, 1996), but an apparent movement towards light at megalopae stage was absent as against the reports in the portunid crab *Macropipus* sp. and common shore crab *Carcinus maenas* (Rice, 1966). In the present study, feeding was found to be normal and consequently the growth was somewhat parallel in all the replicates studied. Decapod larvae use methods other than sight to spot prey (Kumlu, 1999), probably this is the reason why the larval growth was not adversely affected by more dark hours in the 6hL:18hD regimen. In *P. argentinus*, long light hours increases moulting rate subsequently resulting in lower biomass incorporation due to the high expenditure of energy for moulting (Diaz et al., 2003). However, marked or abrupt differences in growth were not observed in the present study.

Cannibalism is another characteristic that is known to be influenced directly by light. A significant increase in cannibalism, proportional to the length of light hours was reported in the zoeae *P. gigas* (Gardner and Maguire, 1998). The authors also opined that in dim light, *P. gigas* larvae might have difficulty in sighting small feed like *Artemia* sp. and therefore would switch over to younger zoeae, which was displayed by an increase in cannibalism. In *M. rosenbergii*, the larvae aggregated in the brighter parts of the tank were more vulnerable to cannibalism (Kutty et al., 2000). In the present study, the larvae were provided with enough feed in terms of both variety and quantity. In addition, the larvae might have used some method other than sight for spotting prey during dim light conditions. So cannibalism for the purpose of feeding is doubtful. However, an enhanced rate in cannibalism was indeed noticed in the brighter regions of the tank where the larvae aggregated. Genetic factor appears to be the reason for this tendency.

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Acknowledgements

We express our sincere thanks to Dr. G. Syda Rao, Director, Central Marine Fisheries Research Institute (CMFRI), Cochin, for his guidance and encouragement. Acknowledgement is also due to Dr. G. Gopakumar, Scientist-in-Charge, Regional Centre of CMFRI, Mandapam, for the facility provided.

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