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Influence of Temperature, Salinity, pH and Light on Molting and Growth in the Indian White Prawn *Penaeus indicus* (Crustacea: Decapoda: Penaeidae) under Laboratory Conditions

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Abstract

The effects of temperature (26, 31, 32.5 and 35°C), salinity (5, 15, 25, 35 and 45 ppt), pH (7±0.2, 8±0.2 and 9±0.2) and light (24hL:00hD, 12hL:12hD and 24hD:00hL) on the molt cycle and growth of early juveniles of Indian white prawn *Penaeus indicus* were investigated independently under laboratory conditions. Temperature, salinity and pH significantly ($P<0.01$) influenced the molting and growth of the prawn. The influence by photoperiod was not significant ($P>0.05$). The optimal levels of temperature, salinity and pH, which gave the fast molt with highest growth increment in *P. indicus* were 31°C, 15 ppt and pH 8±0.2, respectively. Outside the optimum level, more molting did not produce more growth. Extreme levels of temperature, salinity and pH affected feed intake and normal molting resulting in poor tissue growth.

Introduction

The Indian white prawn *Penaeus indicus* is a popular species in shrimp culture fields of the Indian subcontinent and Southeast Asia. The environment of the region where the species is farmed is often subjected to rapid and wide fluctuations in environmental factors. Prawns move in space and time through these abiotic factors, including temperature, salinity, pH and light, which significantly influence their growth process (Aiken 1980; Boyd 1990; Dall et al. 1990). Knowledge about the growth pattern and molting of a particular species to different levels of environmental variables could serve as a reliable indicator of its environmental limitations as a potential aquaculture species. Molting frequency and size increase may be influenced independently by changes in environmental conditions, hence to best determine growth rate of prawns, both molting

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frequency and growth over the period need to be measured. However, most of the studies that deal with environmental limiting factors of penaeid prawns have measured changes in survival or growth only (review by Lester and Pante 1992). Studies that examine penaeid growth and molting are limited (Zein-Eldin and Griffth 1966; Lakshmi et al. 1978; Wickins 1984; Allan and Maguire 1992). In the present study, the independent influence of selected environmental factors, viz. temperature, salinity, pH and light, on the molting frequency and growth of *P. indicus* was determined under laboratory conditions. Objectives of the study were: 1) to determine the optimum environmental regime in which growth is highest, 2) to study the environmental influence on molting frequency; and 3) to test the hypothesis 'more molting means more growth.'

Materials and Methods

Experiments were conducted at the marine prawn hatchery laboratory at Narakkal, Cochin, India. Early juveniles of *P. indicus* belonging to the same brood (26-27 mm in total length and 70-80 mg wet weight) were selected from the laboratory stock populations (temperature 29±1°C, salinity 20±2 ppt, pH 8±0.2). Prawns were reared in four experimental groups to test temperature, salinity, pH and light; the details are given in Table 1. Prawns were not sexed, as male and female penaeids grow at similar rates in the size range used (Motoh 1981). The range of environmental factors were chosen to encompass those in shrimp culture fields of India and Southeast Asia.

Table 1. Experimental design used to test the effects of temperature, pH, salinity and light on molting and growth of *Penaeus indicus*.

	Temperature (°C)	pH	Salinity (ppt)	Light/dark (h)
Group I in relation to temperature (n=12)	26	8 ± 0.2	15	12L : 12D
	31			
	32.5			
	35			
Group II in relation to to pH (n=17)	31	7 ± 0.2	15	12L : 12D
		8 ± 0.2		
		9 ± 0.2		
Group III in relation to salinity (n=17)	31	8 ± 0.2	5	12L : 12D
			15	
			25	
			35	
			45	
Group IV in relation to light (n=12)	31	8 ± 0.2	15	24hL:00hD
				12hL:12hD
				24hD:00hL

Prawns were allowed to acclimate to the selected treatment of temperature, salinity, pH and light for 72 h prior to experiment initiation. Separate animals were used for the four different experiments. Prawns were held individually in 5-l cylindrical plastic jars with 3-l test solutions to examine the growth and molting of each prawn. At the start and end of each experiment, prawns were blotted dry and weighed in a Metler electronic balance. Total length (tip of rostrum to tip of telson) was measured using a vernier caliper. Prawns were fed to satiation twice daily in the morning and evening on a pelletized shrimp feed (40% crude protein). Based on visual observation of leftover feed and fecal matter removed from each container, feeding rate was recorded as poor, average and good. Presence of exuvia indicated the event of molt. Every morning, each container was checked to record molting. Molt cycle duration was expressed as average time between successive molts of each prawn during the experimental period of 20 d.

$$\text{Molt cycle duration of individual prawn (h)} = \frac{\text{Total time duration of the completed molt cycles (h)}}{\text{Number of molts}}$$

Three-fourths of the medium was replaced once daily. Each experiment was continued for 20 d. Growth was recorded as the length and wet weight gain during the experimental period.

Temperature

Temperature levels selected and simulated for the experiment are given in Table 1. Four separate water baths were used at a time for the four different treatments. Groups of 12 prawns, kept individually in plastic containers, were arranged in each waterbath set at temperatures of 26, 31, 32.5 and 35°C with a temperature control unit comprising a submersible heater regulated by a thermostat. The temperature of the waterbath was made uniform by continuous stirring and bubbling. Parameters other than the experimental ones were maintained at constant levels during the experiment.

Salinity

Selected salinities for the study are given in Table 1. Test salinities were prepared either by diluting seawater with stored and aerated tapwater or by partial freezing of seawater. During the salinity experiment other parameters were kept constant.

pH

Different pH media levels (Table 1) were prepared in a 250-l fiberglass tank, and kept at least 3 d to stabilize. Every day pH was adjusted to the required level. Media for lower ranges of pH were prepared by using 1N HCL, and higher ranges were prepared by using a 1N NaOH solution. The freshly prepared media of pH 9±0.2 were turbid and not clear. In such cases, solutions were allowed to settle completely and only the clear supernatant was used for the experiment. Air stones were not used for aeration purposes to avoid their possible interaction with the pH media; instead aeration was given directly through plastic aeration tubes. The pH in the containers was checked at 4-h intervals; and variation from the desired pH levels was compensated by adding acid or alkali. During the experiment, parameters other than pH were kept constant.

Light

The three light regimes selected for the experiment are given in Table 1. The circular photoperiodism chamber made of rigid PVC sheet (100 x 80 cm) was covered with black canvas. A 40 W daylight fluorescent bulb produced mean illumination of 400-600 lux at the water surface in the chamber during the light phase, and light was cut off during the dark phase. The light periods were set by standard time switches. Other parameters were maintained at constant levels.

Statistical analysis

Mean and standard deviation were calculated from the experimental data. The significance of the difference among the treatments under each experiment was tested using one-way analysis of variance (ANOVA). The comparison of individual means of the different treatments was made using the Student-Neuman-Keuls multiple range test (Zar 1984).

Results

Temperature

Higher temperature levels were found to accelerate the molt cycle and growth of *P. indicus* (Table 2). In the case of molting, the increase was linear from 26 to 35°C. The longest molt cycle duration of 138±10.8 h was recorded at 26°C. At temperatures of 31 and 32.5°C, molts occurred at a faster rate with a reduced molt cycle duration of 96 h. The shortest molt cycle of 84 h was recorded among prawns exposed to 35°C. But the fast molt cycle, coupled with a corresponding growth gain in terms of length and weight, was observed only up to a temperature of 31°C. Beyond 31°C, the molt cycle was faster but did not produce much increase in length or weight.

A maximum gain in length of 11.9 mm was recorded at 31°C. The minimum increment of length (4.4 mm) was recorded at 35°C. In terms of wet weight, the highest increment of weight (204.7 mg) was recorded at 31°C. For other ranges of temperature, both lower and higher, growth was significantly lower than at 31°C. The lowest growth in terms of weight was recorded at the

Table 2. Effects of temperature on molting and growth in *P. indicus* juveniles.

		Temperature (°C)				
		26	31	32.5	35	F value
Increase in length (mm)	N	12	12	12	12	138.2*
	$\bar{X}$	6.9^a	11.9^b	8.33^c	4.4^a	
	±SD	(1.2)	(0.8)	(0.9)	(0.8)	
Increase in wet weight (mg)	$\bar{X}$	162.3^a	204.7^b	159.9^a	56.4^c	2,882.1*
	±SD	(7.0)	(2.6)	(2.4)	(3.0)	
Molt cycle duration (h)	$\bar{X}$	138.0^a	96.0^b	96.0^b	84.0^b	97.95*
	±SD	(10.8)	(0.0)	(0.0)	(12.5)	
Feeding rate		average	good	good	average	

highest temperature of 35°C. Rate of feed intake was good at temperatures of 31 and 32.5°C, and average at 26 and 35°C. When duration of molt was considered with growth increment, the most favorable temperature observed was 31°C. ANOVA showed a significant difference (P<0.01) in molting period and growth due to temperature change.

Salinity

At salinities of 15 and 25 ppt, prawns molted normally with a shortest molt cycle period of 96 and 98.1 h, respectively; at a lower salinity of 5 ppt, and at higher salinities of 35 and 45 ppt, prawns showed extended molt periods (Table 3).

The animals exposed to 5, 35 and 45 ppt salinities developed muscle necrosis, and two prawns from 35 ppt and six prawns from 45 ppt died in the course of the experiment. The dead animals were found in half exuviated condition. The fastest molt cycle along with highest growth was recorded at 15 ppt.

Table 3. Effects of salinity on molting and growth in *P. indicus* juveniles.

		Salinity (%)					
		5	15	25	35	45	F Value
Increase in length (mm)	N	17	17	17	15**	11**	301.7*
	$\bar{X}$	7.5^a	11.8^b	9.6^a	4.6^c	2.9^c	
	±SD	(0.8)	(0.6)	(0.8)	(0.7)	(1.0)	
Increase in wet weight (mg)	$\bar{X}$	133.4^a	212.5^b	181.7^c	75.7^d	29.5^e	496.89*
	±SD	(8.4)	(5.8)	(23.7)	(6.0)	(4.8)	
Molt cycle duration (h)	$\bar{X}$	104.5^a	96^a	98.1^a	160.8^b	130.9^b	28.48*
	±SD	(11.8)	(0.0)	(4.7)	(41.8)	(16.5)	
Feeding rate		good	good	good	average	poor	

*Significant at 1% level (P<0.01)
**2 prawns and ***6 prawns died during the course of the experiment
Values with different superscripts in the horizontal row are significantly different (P<0.01).

In terms of length and weight, the highest growth of 11.8 mm and 212.5 mg, respectively, was recorded among the prawns exposed to 15 ppt salinity, followed by 25 ppt with a wet weight of 181.7 mg and length of 9.6 mm. At the highest experimental salinity (45 ppt), length and weight gain recorded were lowest, 2.9 mm and 29.5 mg, respectively. Growth attained at salinities of 5 and 35 ppt was less than at 15 and 25 ppt. Feeding was very poor at 45 ppt, average at 35 ppt, and good at salinities of 5, 15 and 25 ppt. Significant changes were observed in the molt cycle duration and growth of the prawns subjected to different salinity levels ($P<0.01$).

pH

At the acidic pH of 6±0.2, 90% of the prawns did not survive the experimental period of 20 d, hence the group was discarded. At pH 7±0.2, six prawns died, and at pH 9±0.2, five prawns died. Prawns exposed to pH 6 and 9 were very weak and feeding was poor. Abnormal swimming pattern and muscle necrosis were common in these animals. About 80% of the dead animals were found in half exuviated condition. At pH 8±0.2 and 7±0.2, all experimental animals molted successfully and were healthy and active with good feed intake; at pH 9±0.2 feed intake was poor. Average molt cycle duration for pH 8±0.2 was 96 h. In higher pH 9±0.2, an extended molt cycle period of 109.3 h was recorded. The molt cycle duration observed at pH 7±0.2 was lowest at 91.6 h (Table 4).

The maximum length increment of 10.9 mm and weight increment of 194.9 mg was recorded in prawns exposed to pH 8±0.2, while the lowest length and weight increments of 3.1 mm and 48.7 mg, respectively, were noted among the prawns kept at pH 9±0.2. Although the prawns exposed to pH 7±0.2 underwent normal and fast molting, growth attained was considerably lower than that of prawns exposed to pH 8±0.2. The highest growth, in terms

Table 4. Effects of pH on molting and growth in *P. indicus* juveniles.

		pH			
		7 ± 0.2	8 ± 0.2	9 ± 0.2	F value
Increase in length (mm)	N	11**	17	12***	345.9*
	$\bar{X}$	4.9^a	10.9^b	3.1^c	
	±SD	(0.8)	(0.9)	(0.3)	
Increase in wet weight (mg)	$\bar{X}$	155.5^a	194.9^b	48.7^c	4,541.9*
	±SD	(2.6)	(0.39)	(4.5)	
Molt cycle duration (h)	$\bar{X}$	91.6^a	96.0^a	109.3^a	12.77*
	±SD	(9.7)	(0.0)	(12.6)	
Feeding rate		good	good	poor	

*Significant at 1% level ($P<0.01$)
**6 and ***5 prawns died during the course of the experiment
Values with different superscripts in the horizontal row are significantly different ($P<0.01$).

of length, weight and fast molt cycle, was obtained at pH 8±0.2. Prawns exposed to different pH levels showed significant variation in molt cycle duration and growth (P<0.01).

Light

The different light regimes tested in the present study had no significant effect over the molt cycle of the prawn (P>0.05). Prawns kept in complete darkness showed a slightly accelerated molt period of 96 h (Table 5), which was not significant (P>0.05) compared to the other light regimes: 12hL:12hD (96 h) and 24hL:00hD (100 and 102 h). Growth increment obtained under different light regimes also did not reveal any significant variation.

Table 5. Effect of light on molting and growth in *P. indicus* juveniles.

		Light (h)			
		24	12	0	F value
Increase in length (mm)	N	12	12	12	4.406**
	$\bar{X}$	9.0^a	9.2^a	10.1^a	
	±SD	(0.9)	(1.0)	(1.0)	
Increase in wet weight (mg)	$\bar{X}$	180.7^a	181.3^a	183.3^a	1.962**
	±SD	(3.5)	(3.2)	(3.4)	
Molt cycle duration (h)	$\bar{X}$	102.0^a	100.0^a	96.0^a	1.638**
	±SD	(10.9)	(9.34)	(0.0)	
Feeding rate		good	good	good	

**Values of 'F' are not significant at 5% level (P>0.05)
Values with different superscripts in the horizontal row are significantly different (P<0.01).

Discussion

Temperature, one of the important environmental factors, directly affected the molt cycle and growth of *P. indicus*. Molting frequency increased (decreased molt cycle duration) with corresponding increases in temperature. But the relationship was not linear in terms of the tissue growth of the animal. A coordinated increase of molting frequency and tissue growth was noted only up to 31°C. Beyond this temperature, no corresponding tissue growth was recorded, although the molt cycle duration was reduced. Present observation agrees with Zein-Eldin and Griffth (1966), who observed an increase in growth rate over a temperature range of 25-32.5°C, and a precipitous drop at 35°C in *P. aztecus*. Reduced growth of *P. serratus* in low as well as in high temperatures was reported by Richard (1978). According to Conan (1985), in crustaceans, within a specific temperature range, intervals between ecdysis are usually shortened with a corresponding increase in tissue growth until a threshold level of temperature is reached. Beyond this threshold temperature, molting becomes rather erratic. This is true in the case of *P. indicus* where the temperature threshold was up to 31°C. Above this temperature, significant tissue growth was not achieved despite a shortened molt cycle. The differential effect of temperature on growth might be due to its direct effect on body metabolism mediated through endocrine secretions (Aiken and Waddy 1975) or by rate of feeding. In *P. indicus* the optimum temperature for molting and growth recorded was 31°C which is also the threshold temperature of the animal.

In the present study, molting and growth in juvenile *P. indicus* were significantly influenced by varying levels of salinity. *P. indicus* exposed to lower (5 ppt) and higher (45 ppt) levels of salinity had lower growth rates and developed muscle necrosis. Lakshmi et al. (1978) observed muscle necrosis in *P. aztecus* exposed to sub-optimal and supra-optimal salinity ranges. They described the development of muscle necrosis as an indication of stress on the animal. Low feed intake in the prawns maintained at 35 and 45 ppt could be due to physiological stress in extremely saline conditions. Lester and Pante (1992) pointed out that salinities below 5 ppt and above 35 ppt usually have a negative effect on prawn growth. These observations show that, although these penaeids can survive in a wide range of salinities, best growth takes place only in the optimum range. Outside the optimum range, the growth increment attained was comparatively low. In the extreme range of salinities, prawns must expend considerable energy for osmoregulation at the expense of other processes, such as growth. In *P. indicus*, molting occurred at a faster rate with highest growth increment at 15 ppt compared to all other salinities tested.

The pH was found to have a direct effect over the molting process of *P. indicus*. In the present study, both lower (acidic) and higher (alkaline) pH were limiting factors for the process of molting and growth. Low pH is reported to be harmful to crustaceans (Leivestad and Muniz 1976; Havas and Hutchinson 1982). In line with the present study, Allan and Maguire (1992) recorded depressed growth of *P. monodon* in pH 5.5 compared with those grown in pH 7.8. Pillai et al. (1978) reported the mass mortality of crustaceans in Vembanad lake due to low pH. The effect of reduced pH on mineralization in *P. monodon* was studied by Wickins (1984) who observed that sub-optimal pH in the acidic range adversely affected body mineralization in the prawn. He also noted reduced growth in *P. monodon* and *P. occidentalis* at a low pH of 6.4. Allan and Maguire (1992) suggested an impairment of osmoregulatory function at low pH levels. In the present study, stress on the prawns was more evident in the alkaline pH of 9±0.2. This can be due to the higher alkalinity resulting from the interaction between NaOH and seawater. In culture ponds, the stress to prawns is less, as the pH increases gradually due to CO_2 removal from the water by algae without forming alkaline conditions. The present study clearly indicated that an optimum pH of 7.8-8.2 provides ideal conditions for successful molting and best growth in *P. indicus*. Outside this optimum range, both higher and lower pH adversely affected growth rate.

The influence of light-dark regimes on molting in tropical penaeids is yet to be investigated in detail. In the present investigation, the photoperiod did not show any significant effect on molting period or growth. Skinner and Graham (1974) noticed precocious molting in animals kept in darkness when compared with those exposed to light. But they attributed the effect to privacy rather than to amount of exposure to darkness. In the present case, the slightly accelerated molt period among the prawns under complete darkness was not significant. The effect of photoperiod in crustacean growth is generally inconclusive and contradictory in nature. Forster and Beard (1973) reported enhanced growth rate in continuous darkness in *Palaemon serratus*; while Chittleborough (1975) reported inhibition of growth in darkness in *Panulirus longipus*. In the present study, no correlation was obtained between the light/dark period on molting and growth of *P. indicus*. Observations of Bishop and Herrnkind (1976), Nakamura (1988) and Justo et al. (1991) agree with the current study that molting and growth of prawns would not be affected by changes in photoperiod.

The feeding pattern observed in the current study is worth mentioning. Feeding rates were low outside the optimum range of temperature, salinity and pH. In extreme levels of temperature, salinity and pH, prawns appeared lethargic with poor feed intake. Hence, in semi-intensive farming conditions, when shrimp farms face extreme conditions of temperature, salinity and pH, farmers may reduce feeding rate to prevent feed wastage and spoilage in pond water.

The current study showed that best growth performance of the species was achieved only in optimum conditions of temperature, salinity and pH. Outside the optimum range, and under adverse conditions, the prawns survived, but with decreased growth. The concept that 'more molting means more growth' in penaeids is true only in the optimum levels of environmental variables. Otherwise, increase in molting frequency did not necessarily enhance growth.

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