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The Influence of Water Renewal Rates on the Reproductive and Molting Cycles of *Penaeus paulensis* in Captivity

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ABSTRACT

The present study analyzed the reproduction of wild-caught Penaeus paulensis in relation to its molt cycle. The experimental design consisted of two treatments (continuous water flow and batch renewal) with two replicates. The stocking density in experimental tanks (1.50 x 0.96m) was approximately 7 animals/m^2, resulting in 4 males (23.9 ± 2.3 g) and 6 females (52.0 ± 5.5 g) per tank. Shrimp had their uropods cut for individual marking and female maturation was induced through the unilateral eyestalk-ablation. The intermolt period of females and males (17.4 ± 3.2 and 17.8 ± 4.6 days, respectively), number of days between molting and the first spawn (6.9 ± 2.8 days) and number of spawns in the intermolt period (1.4 ± 0.5), presented no significant differences (P>0.05) between treatments. No influences on molt cycle or reproductive performance parameters could be related to differences in water quality parameters, especially nitrogenous compounds, suggesting a trend towards reduce water exchange in shrimp maturation. However, a relative decrease in the number of eggs per spawn was observed. This possibly was due to the smaller maturation tanks. The results of P. paulensis molt cycle could be useful for accompaniment and better planning of the reproduction in captivity.

Key words: Molt, maturation, penaeid

INTRODUCTION

The pink shrimp *Penaeus paulensis* is distributed from Ilhéus (14^{o}50'S), Brazil to Mar del Plata (38^{o}30'S), Argentina (D'Incao, 1991). As most penaeid shrimps, *P. paulensis* presents two different phases in its life cycle: an oceanic one, marked by the reproduction and larval development, and another represented by growth in estuarine areas (D'Incao, 1991). Since *P. paulensis* is a closed-thelicum species, mating takes place between males in their intermolt period and recently molted (soft cuticle) females (Dall et al., 1990). After the hardening of the exoskeleton, sexually mature females may develop their gonad and spawn a few times before their next molt regardless of mating success. Brisson (1986) described in detail the mating behavior of *P. paulensis*, which is marked by the male turning upside down below the female, rapidly rotating perpendicularly, squeezing the female and then transferring the spermatophore.

Although several studies have already dealt with the reproduction of this species under field (Zenger and Agnes, 1977; Iwai, 1978) and laboratory conditions using wild and captive broodstock (Marchiori and Boff, 1983; Beltrame and Andretta, 1991; Marchiori and Cavalli, 1993; Vinatea et al., 1993; Petersen, 1996; Cavalli et al., 1997; Peixoto, 2000), not much information on the

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fundamental aspects of the reproductive biology of this species is available.

Generally high water exchange rates (100-400% per day) are adopted in the maturation of penaeid shrimps (Bray and Lawrence, 1992; Ogle, 1991), mainly to reduce the levels of nitrogenous compounds. However, lower water exchange might result in a more stable environment, since no further effects of ammonia on molting and reproductive performance of *P. paulensis* were detected under long-term exposure (Cavalli et al., 1998). Accordingly, the present study describes the influence of water renewal on the reproductive and molt cycles of a wild-caught broodstock. These information may lead to a better understanding of the processes involved on the reproduction of *P. paulensis*.

MATERIAL AND METHODS

Wild shrimp were captured off shore in southern Brazil (27°30'S) at depths of 40-60 meters by 30-min long otter trawls. They were then transported to the laboratory and acclimated in 12-ton tanks for 20 days. After the acclimation period, shrimp were randomly transferred to four rectangular tanks (1.50 x 0.96 m) with a working volume of 700 liters. The stocking density was approximately 7 animals/m^2, and the male-female ratio was 1:1.5 (4 males and 6 females per tank). The distribution of the females (mean wet weight ± SD = 52.0 ± 5.5 g) and males (23.9 ± 2.3g) was performed so as to obtain biomass of approximately 280 g/m^2 in all tanks. Temperature was maintained at around 27°C through immersion heaters with thermostat. Photoperiod was set at 15-h light and 9-h dark, and continuous aeration was provided. Feeding was offered in four daily portions (9:00, 12:00, 15:00 and 18:00 h), composed of fresh frozen fish (various species), shrimp (*Artemesia longinaris*), squid (*Illex* sp.) and crab (*Callinectes* sp.), respectively. Maturation was induced through the unilateral eyestalk ablation of the females (Marchiori and Boff, 1983). At the same time, shrimp had their uropods cut for individual marking (Fig. 1).

The experiment consisted of two treatments with two replicates and lasted for 45 days. Two experimental tanks received a continuous flow of pre-heated seawater, which promoted from 1.5 to 2.0 turnovers a day.

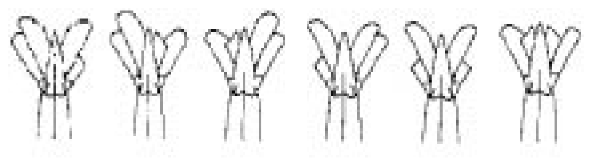

Figure 1 - Dorsal view of the differentiated uropods cuts in the *Penaeus paulensis* females and respective letters. For the males the same pattern was applied from A to D.

In the other tanks, 50% of the water was renewed daily in one single operation. The withdrawal of food remains and faeces, and the inspection of exuviae were also carried out daily. At the same time, samples for the determination of water quality were taken. Analyses of total ammonia (TAN; $NH_4^- + NH_3$) followed Solorzano (1969) and Unesco (1983), while the estimations of unionized ammonia (NH_3) were based on Whitfield (1974). Nitrite (NO_2) concentrations were established according to Bendschneider and Robinson (1952).Values of pH, temperature and salinity were also recorded daily.

After eyestalk ablation, the experimental tanks were monitored three times a week (Sundays, Tuesdays and Thursdays) with the purpose of selecting females ready to spawn. Females with ripe (green-colored) ovaries were transferred to individual 180-litre square shaped tanks for spawning. Partially spent and unspawned females were maintained in the spawning tanks for another night and only then returned to their original tanks. The number of eggs per spawn was estimated by subsampling. The water in the spawning tank was stirred and three 100-ml aliquots were collected for counting. Fertilization rates were determined by microscopic analysis of at least 30 eggs. Hatching rates were estimated by placing random samples of 100 eggs from each spawn into two 1-litre beakers with moderate aeration. After 24 hours, nauplii and dead eggs were counted.

Analysis of variance (ANOVA) was used to detect significant differences ($P<0.05$) between replicates. Once no differences were found, replicate data were pooled and the Student's t-test was then applied at 0.05 significance level. Percentage data were arcsine/square root transformed for analysis, but only untransformed values are presented.

RESULTS

Water quality parameters monitored during the experimental period are presented in Table 1. The concentrations of ammonia, unionized ammonia and nitrite were significantly different between treatments. Higher levels of these compounds were found in the tanks with batch renewal. On the other hand, mean pH levels were lower on the batch renewal system. Temperature and salinity did not vary significantly among treatments.

The number of spawns per female initially stocked, eggs/spawn and the total number of eggs were not significantly different between treatments (Table 2). The intermolt period of females and males, number of days between molting and the first spawn, number of spawns in the intermolt period and the interval of days among spawns presented no significant differences (P>0.05) between the treatments and therefore were pooled (Table 3).

Table 1 - Mean (± SD) chemical and physical water quality parameters in the continuous water flow and batch renewal treatments of *Penaeus paulensis* reproduction system. Means within a row with a different superscript letter are significantly different (P<0.05).

	Continuous flow	Batch renewal
Ammonia (mg/l TAN)	0.36 ± 0.30^a	2.21 ± 1.03^b
Unionized Ammonia (mg/l NH_3)	0.02 ± 0.02^a	0.09 ± 0.04^b
Nitrite (mg/l NO_2)	0.06 ± 0.07^a	0.62 ± 1.22^b
PH	8.05 ± 0.08^a	7.85 ± 0.09^b
Temperature (°C)	27.6 ± 0.6	27.6 ± 0.5
Salinity (‰)	31 ± 1	31 ± 1

Table 2 - Mean (± SD) reproductive performance parameters of *Penaeus paulensis* under laboratory conditions with continuous flow and batch renewal. Means within a row with a different superscript letter are significantly different (P<0.05).

	Continuous flow	Batch renewal
Number of spawns	23	20
Spawns/female*	2.3 ± 1.2	2.2 ± 1.0
Eggs/female*	216,400 ± 126,400	231,540 ± 150,927
Eggs/spawn	84,579 ± 31,979	101,856 ± 46,761
Total egg production	2,686,000	2,315,400

* Spawns or eggs per females initially stocked.

Table 3 - Mean (± SD) duration of the intermolt period (days), interval between molting and first spawn (days), number of spawns per intermolt period and interval between spawns (days) of *Penaeus paulensis* under laboratory conditions.

	Females	Males
Number of molts	41	21
Intermolt period	17.4 ± 3.2	17.8 ± 4.6
Interval molting/1st pawn	6.9 ± 2.8	--
Spawns/Intermolt period	1.4 ± 0.5	--
Interval between spawns	4.4 ± 2.6	--

DISCUSSION

The higher ammonia levels found in the batch renewal system might be originating from the excretion by shrimp (Chen and Nan, 1994; Wasielesky et al., 1994), the feed supplied in excess (Ostrensky et al., 1992) and/or bacterial mineralisation (Spotte, 1979), which were diluted in the continuous circulation treatment.

Sub-lethal concentrations of ammonia have been shown to decrease growth and molting rates of penaeid juveniles (Chen and Kou, 1992; Wasielesky et al., 1994). However, under long-term exposure to ammonia concentrations (up to 6.86 mg/l), no influence were detected on *P. paulensis* molting or reproductive performance, affecting only survival of females and growth of males (Cavalli et al., 1998). In accordance, no influences on molt cycle or reproductive performance could be related to ammonia concentration or any of the water quality parameters. The present results reinforced a trend towards reduced water exchange for shrimp reproduction and maintenance of a more stable environment, which might result in reduction of labour and overall water requirements (Cavalli et al., 1998) and less stress to the breeding population (Bray and Lawrence, 1992).

The only fertilized spawns were those obtained at the beginning of the experimental period (first week). This could be attributed to the impregnation of females in the ocean and/or in the larger acclimation tanks. The lack of mating during the experimental period may be related to the small size of the tanks, shrimp stocking density and/or the absence of substratum. However, *P. paulensis* female stay on the bottom and do not swim around before or during mating (Brisson, 1986). In accordance, Marchiori and Boff (1983), working in similar tanks as here, but at a lower stocking density (3 females and 3 males) and a 50mm sediment layer, reported the courtship ritual and obtained success in nauplii production. The mean number of eggs per spawn was relatively low (16,000-40,000 eggs/spawn) compared with those usually obtained when larger maturation tanks are applied.

Although the potential fecundity of *P. paulensis* reported by Iwai (1978) under field (Southeast Brazilian off-shore areas) was up to 500,000 eggs per spawn, when eyestalk ablated wild females were maintained in captivity for at least 45 days, the mean number of eggs per spawning event ranged from 16,000 to 160,000 (Marchiori and Boff, 1983; Cavalli et al., 1997; Cavalli et al., 1998), while the number of nauplii per spawn ranged from 60,000 to 140,000 (Beltrame and Andreatta, 1991; Marchiori and Cavalli, 1993; Vinatea et al., 1993; Petersen et al., 1996; Reis et al., 1998). In the present study, the number of eggs per spawning event was in agreement with those usually observed for wild broodstock maintained in larger circular tanks, but again a relative decrease was observed in the number of eggs per spawn in this tank shape/size. Reis et al. (1998) found no significant differences in hatching rate and nauplii per spawn of *P. paulensis* broodstock, comparing different maturation tank size (2.5 and 4m diameter) and volumes\shapes of spawning tanks (140 up to 210 liters square shape and conical-cylinder shape). However, Crocos and Kerr (1986) observed for *Penaeus esculentus* a higher number of matings and spawns in larger tanks. Similarly, a direct relationship between the number of eggs per spawn and the size of the spawning tanks was observed for *Penaeus vannamei* (Lotz and Ogle, 1994). It is, therefore, possible to speculate that the number of eggs per spawn may be reduced in smaller maturation tanks.

The reproductive performance traits could be affected by many factors such as different species, broodstock source and size (Menasveta et al., 1994; Cavalli et al., 1997; Palacios et al., 1999), nutrition (Harrison, 1990; Sangpradub et al., 1994), genetics (Benzie, 1997; Benzie, 1998) and environmental conditions (Hansford and Marsden, 1995; Crocos and Coman, 1997). Therefore, these findings must be considered to compare reproductive performance reported in different studies.

When considering the molt cycle of penaeids in captivity, some environmental aspects and other common maturation procedures such as the females eyestalk-ablation, must be considered. Previous studies demonstrated that especially at lower temperatures, *Penaeus indicus* ablated females had shorter molt cycles than unablated counterparts, but as temperature increased, molt cycle duration decreased until there was approximately the same duration for ablated and unablated wild females (Emmerson, 1980). The increase in molting rates at higher temperatures reflected a general acceleration of shrimp metabolism, but since molting was under endocrine control, the sinus glands and many of the neurosecretory cells in the eyestalk exerted a strong influence on the molting process (Dall et al., 1990). Browdy and Samocha (1985) reported that the molt cycle was more frequent in ablated *Penaeus semisulcatus* females (22.1 ± 2.4 days) than in unablated ones (23.8 ± 2.0 days). Cruz et al. (1998) also reported an increase in the molt cycle length in mature *Macrobrachium amazonicum* females, but no significant differences were found between males (11.9 ± 2.4days) and females (11.4 ± 2.8 days). The results in the present study are in accordance with other shrimp under laboratory conditions, however further studies analyzing the effects of size, temperature and eyestalk ablation on molt cycle of *P. paulensis* are still required.

The results involving molt cycle and reproductive performance relationship could improve the outlook for better accompaniment and planning of the *P. paulensis* reproduction system in captivity. Therefore, even without using uropod markers, we recommended daily observations of the molts occurred in the maturation tanks, which could be associated with the mating behavior and amount of spawns expected during the reproduction period.

RESUMO

O presente estudo foi proposto para analisar a reprodução de um estoque selvagem de *Penaeus paulensis* em relação ao seu ciclo de mudas. O desenho experimental foi composto por dois tratamentos em duplicata (fluxo contínuo e com renovação). A densidade de estocagem nos tanques experimentais (1,50 x 0,96m) foi de aproximadamente 7 camarões/m^2, resultando em 4 machos (23,9 ± 2,3 g) e 6 fêmeas (52,0 ± 5,5 g) por tanque. Os camarões tiveram seus urópodos cortados para marcação individual e a maturação das fêmeas foi induzida por ablação unilateral do pedúnculo ocular. O período intermuda de fêmeas e machos (17,4 ± 3,2 e 17,8 ± 4,6 dias, respectivamente), número de dias entre a muda e primeira desova (6,9 ± 2,8 dias) e número de desovas no período intermuda (1,4 ± 0,5), não apresentaram diferenças significativas (P>0,05) entre os tratamentos. Nenhuma influência no ciclo de muda ou nos parâmetros de performance reprodutiva foram relacionadas a diferenças na qualidade de água, principalmente no que se refere aos compostos nitrogenados, sugerindo uma redução nas taxas de renovação de água em sistemas de maturação. Contudo, uma diminuição no número de ovos por desova foi observado. Este fato parece estar associado as pequenas dimensões dos tanques utilizados. Os resultados do ciclo de muda de *P. paulensis* podem ser úteis para o acompanhamento e melhor planejamento da reprodução desta espécie em cativeiro.

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