BOLETIM DO INSTITUTO DE PESCA
ISSN 1678-2305 online version
Scientific Note

MOULTING CYCLE STAGES IN *Macrobrachium rosenbergii* BY SETOGENESIS METHOD

Marcos Antônio Sinésio da Silva[1]
Bianca de Oliveira Ramiro[1]
Marino Eugênio de Almeida Neto[2]
Ricardo Romão Guerra[1]

[1]Universidade Federal da Paraíba – UFPB, Centro de Ciências Agrárias, Programa de Pós-graduação em Zootecnia, Rodovia PB-070, s/n, CEP 58397-000, Areia, PB, Brasil. E-mail: ricardo@cca.ufpb.br (corresponding author).

[2]Universidade Federal da Paraíba – UFPB, Centro de Ciências Humanas, Sociais e Agrárias, Departamento de Ciência Animal, Rua João Pessoa, s/n, CEP 58220-000, Bananeiras, PB, Brasil.

Received: July 20, 2018
Approved: December 21, 2018

ABSTRACT

This study had the objective of evaluating the duration of the moulting cycle stages of *Macrobrachium rosenbergii*, using the method of setogenesis. The experiment was conduced in the Prawn Farming Laboratory of the Federal University in Paraiba, in the town of Bananeiras, Paraiba, Brazil. Fifteen prawns (5.7 ± 0.3 g) were distributed in 3 aquariums (60 x 30 x 40 cm), maintained in natural photoperiod (12 x 12 hours), at a controlled temperature (28 °C), and feed was offered 3 times a day (8 a.m., 12 and 16 p.m.), throughout the experimental period. For identification, the animals were marked with colored silicone rings on the ocular peduncle and with colored plastic discs fixed on the carapace. From the setogenesis method, the prawns were classified into stage A, B, C, D0, D1, D2 or D3 of the moulting cycle. Setogenesis was observed stereomicroscopically every day, at the same time, 7 a.m., from the first ecdysis to the next ecdysis. The duration of the moulting cycle was 27.7 ± 3.2 days, and the intermoult stage (C) was the longest (8.0 ± 2.38 days). Stage A was the shortest (1 day), and the pre-moult stage (stages D) lasted 12.98+1.65 days. We concluded that juvenile of *M. rosenbergii* have a diecdysis type moulting cycle with shorter intermoult than pre-moult periods.

Key words: prawn farming; growth; ecdysis.

ESTÁGIOS DE MUDA EM *Macrobrachium rosenbergii* PELO MÉTODO DE SETOGÊNESE

RESUMO

O presente estudo teve como objetivo avaliar a duração dos estágios de muda do *Macrobrachium rosenbergii*, utilizando o método da setogênese. O experimento foi realizado no Laboratório de Carcinicultura da Universidade Federal da Paraíba, em Bananeiras, Paraíba, Brazil. Quinze camarões (5,7 ± 0,3 g) foram distribuídos em 3 aquários (60 x 30 x 40 cm), mantidos em fotoperíodo natural, temperatura controlada (28 °C) e ração ofertada 3 vezes ao dia (8 a.m., 12 e 16 p.m.), durante todo o período experimental. Para identificação, os animais foram marcados com anéis coloridos de silicone no pedúnculo ocular e com discos plásticos coloridos fixados à carapaça. Os camarões foram classificados em estágio A, B, C, D0, D1, D2 ou D3 do ciclo de muda, através do método da setogênese. A setogênese foi observada estereomicroscopicamente todos os dias, às 7 a.m., no mesmo horário, a partir da primeira ecdise até a ecdise seguinte. Como resultado, a duração do ciclo de muda foi 27,7 ± 3,2 dias, com o estágio de intermuda (C) sendo o mais duradouro (8,00 ± 2,38 dias). O estágio A foi o mais breve (1 dia), e a fase de pré-muda (os estágios D) durou 12,98 ± 1,65 dias. Conclui-se que juvenis de *M. rosenbergii* tem o ciclo de muda do tipo diecdise com o período de intermuda menor que o de pré-muda.

Palavras-chave: carcinicultura; crescimento; ecdise.

INTRODUCTION

Within aquaculture activity, freshwater prawn farming has had a prominent position, with high growth worldwide, mainly with the production of *Macrobrachium* prawn genus (FAO, 2014). In this scenario, *Macrobrachium rosenbergii* is the main representative species cultivated, with a production of 220,254 tons per year (FAO, 2014). In Brazil, the introduction of *M. rosenbergii* occurred in 1977 through the Department of Oceanography of the Federal University in Pernambuco (UFPE), which was able to import post-larvae from Hawaii, USA. From the introduction into the country, the species

showed an excellent ability to adapt to the climatic conditions (Valenti and Cavalcanti, 1998). Afterwards, much research was developed with this species, mainly in the 1980s. However, in the 1990s the intensity of scientific studies and advances reduced significantly with increased cultivation of marine species.

One of the areas of study on *M. rosenbergii* prawn that has advanced little is on the physiology of their moulting cycle. Moulting, or ecdysis, is one of the most important physiological aspects of crustacean life (Corteel et al., 2012). This physiological process directly or indirectly impacts the lives of these animals, mainly feeding, reproduction, metabolism, behavior and sensitive acuity (Passano, 1960; Santos et al., 2014; Barbieri et al., 2017). Moulting for crustaceans represents the possibility of completing normal growth processes. This occurs cyclically every time the organism is prepared to increase in length and weight. At that moment, the old exoskeleton is discarded, giving place to the new exoskeleton, which was already ready below the previous one, but still very flexible and vulnerable (Saravanan and Kamalam, 2008; Corteel et al., 2012). The new exoskeleton will then stiffen until it has the consistency and hardness of the previous exoskeleton. During this process the body of the prawn tends to absorb water to promote the expansion of the new exoskeleton, which when stiffening will shape a body slightly larger than the one existing before the ecdysis. Finally, the water absorbed will give rise to tissue synthesized by the animal in the following moments, consolidating the increase of volume and weight of the animal (Chang, 1995; Hayd et al., 2008; Barbieri et al., 2013).

Once the new exoskeleton of shrimp is formed before discarding the old one, for all the effects that the moult cycle exerts on these animals, it was observed the necessity and importance of predicting and average length of the moulting cycle (Corteel et al., 2012). One of main moulting cycle effects on the prawn culture systems is on feed consumption. By the time the molt occurs, begins the physiological abstinence. That biological phenomenon implies in reasonable feed leftovers on the culture systems, deteriorating the water quality and raising the production costs. Therefore, the predictability of when the most of the cultivated population will be in molting could bring important technological advances to prawn culture (Almeida Neto and Freire, 2007). Thus, several methods have been developed to observe the duration and stages of the moultling cycle (Saravanan and Kamalam, 2008; Corteel et al., 2012), for example: histological observation of the integument (Silva et al., 2018), measurement of gastroliths and/or regeneration of pereiopods and observation of the setal development of the appendices (setogenesis). The most used and widespread moulting cycle observation method is the setogenesis method, mainly because it is fast and neither cause stressful situations to the animal nor mutilations, even with repeated observations. The setogenesis is based on the observation of the degree of development of the setal structures, such as the setae themselves (external protuberances in appendages such as uropods, pleopods and antigens), setae cones, setae bases, and setae nodules, as well as the formation of the new exoskeleton, as the new epicuticle and the epidermal line of the appendices (Robertson et al., 1987; Chan et al., 1988; Rusaini and Owens, 2011).

In setogenesis method, the moultling cycle was initially divided into five main stages: Stage A (Recent postmoult), Stage B (Late postmoult), Stage C (Intermoult), Stage D (Premoult) and Stage E (Ecdysis) (Passano, 1960; Smith and Dall, 1985). Later, Chan et al.(1988) described the setogenesis in penaeid shrimp *Litopenaeus vannamei* by subdividing it into seven stages: Stage A (Recent postmoult), Stage B (Late postmoult), Stage C (Intermoult), Stages D0 and D1 (Recent premoult) and Stages D2 and D3 (Late premoult), and stage E (Ecdysis) was not described, since it is an event that lasts a few seconds.

Among the biological aspects of prawn that are affected by the moulting cycle, two main ones, feeding and reproduction, can be highlighted. Regarding feeding, some authors, such as Smith and Dall (1985) and Chan et al. (1988) reported that there are moments in the life of prawn in which food consumption stops or decreases. This is extremely important in the producer management of the species. This cyclical process of non-feeding of prawns was called physiological abstinence, and may be related to the fact that in the process of exoskeleton detachment, some structures such as the mouth, esophagus and part of the stomach cease to be completely functional (Almeida Neto and Freire, 2007). These organs have a layer of chitin in continuation to the outer layers, which detaches along with the old exoskeleton at the moment of the moult, preventing the organs to continue performing their normal functions (Ceccaldi, 1987). On the other hand, prawn reproduction is also strongly affected by the moulting cycle. In *L. vannamei*, copula, and consequent transfer of spermatophores to the female, is only effective when it occurs at moments of intermoulting, with the exoskeleton rigid (Ostrensky and Barbieri, 2002). While in *M. rosenbergii* it is the reverse, the copula and consequent transfer of spermatophore to the female telic only occurs after the nuptial moult, in the moments of recent postmoult, with the flexible exoskeleton (Valenti and Cavalcanti, 1998).

As mentioned above, the molting cycle interferes with several faults in the production system, such as feeding, breeding and development. Therefore, the present study aimed to evaluate the duration of the moult cycle, and its stages, in juveniles of *M. rosenbergii*, through the setogenesis method. Thus, it can subsidize studies aimed at adopting new strategies in food management, as well as generating basic information for studies on the digestive, reproductive and behavioral physiology of the species.

MATERIAL AND METHODS

Experimental design

The experiment was carried out in the Laboratory of Prawn Farming of the Agricultural College "Vidal de Negreiros" (CAVN), Center for Human, Social and Agricultural Sciences (CCHSA), Federal University of Paraíba (UFPB), in the municipality of Bananeiras, Paraíba, Brazil, and at the Histology Department of the UFPB, Areia, Paraíba, Brazil. Fifteen juvenile *Macrobrachium rosenbergii* were used with 5.7 ± 0.3 g. The prawns were distributed in 3 aquariums (60 cm x 30 cm x 40 cm). Each aquarium had the bottom covered by biological plaques, a layer of fiberglass wool and 5 cm of fine sand, from bottom up. And each one had a small pump blowing

air constantly through porous stones. Above each aquarium a biofilter was installed with a layer of fiberglass wool, one 5 cm layer of coarse sand and one 3 cm layer of fine sand, respectively. The water circulation through the biofilters was through suction pumps submerged in each experimental aquarium. This way, the aquarium water was circulated continuously throughout the biofilters at the rate of 150 L h^{-1}. The temperature was maintained at 28 °C through thermostats inserted into each aquarium. The ammonia analysis was carried out every 3 days with the ALCON Ltda kit for the analysis of toxic ammonia. The aquariums were kept under natural photoperiod, 12 x 12 hours and the prawns were fed 3 times a day throughout the experimental period (8:00 a.m., 12:00 and 4:00 p.m.) at a rate of 30% of the biomass per day; with commercial feed for marine prawns containing 30% crude protein. One hour after feeding, the leftover feed was removed from the aquariums by siphoning.

Observations on the moulting stages

For identification, the animals were marked with colored silicone rings on the ocular peduncle and with colored plastic discs fixed on the carapace. The first moult of each prawn was awaited, and then certified when the exoskeleton with the marking of the carapace released in the water was observed. This moment was considered as day "0" for this prawn, and, from that day on, it was observed daily, always at 2:00 p.m. This same procedure was repeated for the other prawns under experimental conditions.

Thus, through the method of setogenesis, the animals had their stage of the moult cycle characterized and determined daily stereomicroscopically according to Corteel et al. (2012) adapted methodology. With the next moult, after day "0", the animals were removed from the aquarium and this was counted as the last day of observation in this animal. That is, each prawn had its stage of moulting cycle characterized daily, by a complete cycle of moult, from one ecdysis to another, with 15 moulting cycles observed in 15 different prawns.

Statistical analysis

At the end of the experimental period and tabulation of data (days), statistical analysis was carried out by using the statistical software program Statistic version 13.0. The data were submitted to a descriptive statistical analysis to evaluate the means, variances, deviations and standard errors of the total duration of the moult cycle and its stages.

RESULTS

From the observations of setogenesis in the endopods of the uropods, we were able to identify and characterize seven stages of the moulting cycle for juvenile freshwater prawn *Macrobrachium rosenbergii*: A, B, C, D0, D1, D2 or D3 (Figure 1). In stage A, immediately after moulting, the setal structures were still in formation; with the absence of the setal cones in most setae; absence of the inner cone in all setae; setal bases not very evident and the epidermis completely filling the setal bases (Figure 1B). Stage B was characterized by the setal cones in most setae, as well as the development of the inner cone in some setae; the setal bases were more evident with denser setal nodules and partial filling of the setal base by the epidermis (Figure 1C). In the intermoult stage (stage C), all structures of the exoskeleton were already formed and evident, with emphasis on the internal cone present in all setae and the epidermal line formed parallel to the sternum nodules (Figure 1D). The Stage D0, first stage of prawn pre-moult, was identified by the maximum retraction of the epidermis and its detachment from the old exoskeleton (apolysis) (Figure 1E). Stage D1, was marked by the appearance of the new setae ("setogenesis") (Figure 1F). For stage D2, we noticed the presence of the new epicuticle, with the new more visible setae (Figure 1G). Finally, stage D3, immediately before ecdysis, was characterized by the complete formation of the new (invaginated) setae and the high proximity of the new epicuticle with the old setal nodules (Figure 1).

The duration of the complete moulting cycle was 27.7 ± 3.2 days on average, and the intermoult stage (C) was the longest (8.00 ± 2.38 days). The post-moult phase (adding stages A and B) was 6.76 ± 1.96 days, while the pre-moult phase (adding stages D) lasted 12.98 ± 1.65 days (Table 1).

Table 1. Moulting cycle stages in *Macrobrachium rosenbergii* by setogenesis method.

Variables	Average ± Standard deviation (days)	Variance	Standard error	CV* (%)
Intermould duration	27.76 ± 3.29	10.85	0.9139	11.85
Intermoult stage - A	1.00 ± 0.00	0.00	0.0000	0.00
Intermoult stage - B	5.76 ± 1.96	3.85	0.5440	34.02
Intermoult stage - C	8.00 ± 2.38	5.66	0.6602	29.75
Intermoult stage - D0	4.69 ± 1.65	2.73	0.4583	35.18
Intermoult stage - D1	3.92 ± 0.95	0.91	0.2646	24.23
Intermoult stage - D2	2.30 ± 0.48	0.23	0.1332	20.86
Intermoult stage - D3	2.07 ± 0.27	0.07	0.0769	13.04

*CV= coefficient of variation.

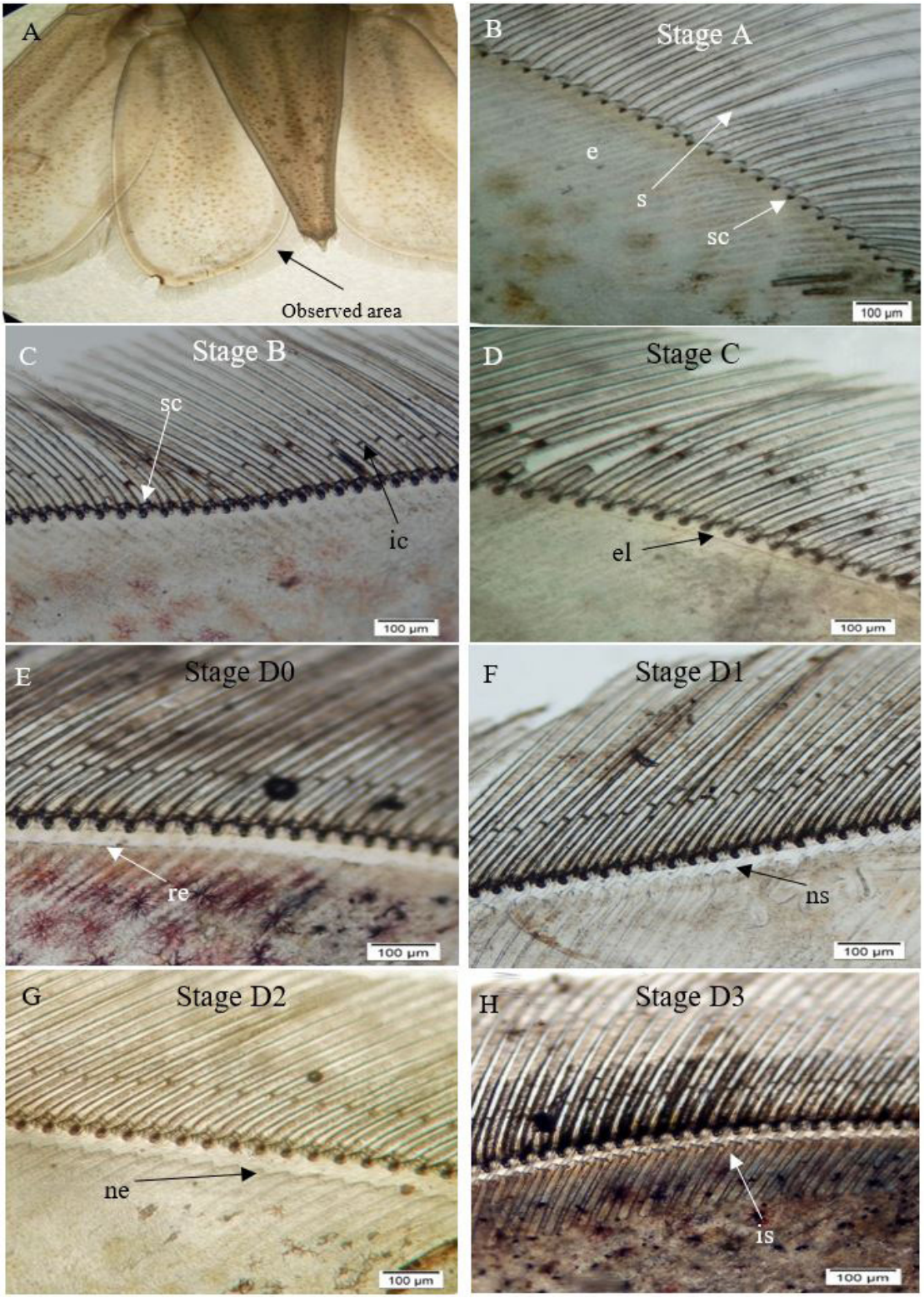

Figure 1. Phomicrografies of the moulting cycles from *Macrobrachium rosenbergii*, by setogenesis method. (A) Uropods of *M. rosenbergii*, showing the observation place, the left endopods; (B) Characteristics of A moulting cycle, showing the setae (s), epidermis (e) and setal cone (sc); (C) Stage B, showing the setal cone (sc) and the internal cone (ic); (D) Stage C, formation of epidermal line (el); (E) Stage D0, showing the maximal retraction of the epidermis (re); (F) Stage D1, showing the development of new setae (ns) "setogenesis"; (G) Stage D2, showing the formation of a new epicuticle (ne); (H) Stage D3, showing the complete formation of the new invaginated setae (is).

DISCUSSION

Throughout the experimental period, we observed that in stage A (recent post-moult), the prawns had a flaccid exoskeleton texture; thus, the absence of the setal structures can be explained. According to Almeida Neto and Freire (2007), the correlation between the post-moult stage A and the soft texture can be explained by the fact that at this stage the exoskeleton is semi-calcified and with high permeability, which makes it soft. In this stage, the calcified layers of the exoskeleton (epicuticle, pigmented layer of the endocuticle and, mainly, the endocuticle) of the prawn are still malformed.

In stage B (late post-moult), the texture of the exoskeleton was harder, but still not very flexible. However, the appearance of the internal cone in some setae was observed as the main characteristic to determine the stage, corroborating the results found by Peebles (1977) and Gastelú et al. (2011) in studies with *Macrobrachium rosenbergii* and *Macrobrachium acanthurus*, respectively. Probably, the appearance of this internal cone is inherent to the species of the *Macrobrachium* genus, although in studies performed by Almeida Neto and Freire (2007) and Promwikorn et al. (2007) with species of penaeid shrimp; *Litopenaeus vannamei* and *Penaeus monondon*, respectively, did not observe the existence of such structure.

At the intermoult stage (stage C), we observed that the texture of the exoskeleton was completely rigid, presumably with all the exoskeleton structures formed. According to Smith and Dall (1985) and Chan et al. (1988), in the intermoult stage the hard texture of the exoskeleton is due to its complete calcification. Barnabé (1996) emphasizes that the completion of the new integument and the growth of muscle tissue occurs in the stage of intermoult.

The pre-moult stages (stages D) were characterized mainly by the formation of the new exoskeleton. According to Chang (1995), for the prawn to increase in size and weight, it is necessary for the organism to develop a new exoskeleton underneath the previous one and later release the old exoskeleton, a process characterized as moulting.

Almeida Neto and Freire (2007) observed that the duration of the moulting cycle in juvenile *L. vannamei* prawn in fattening nurseries was on average from 10.47 to 13.07 days, being smaller than in *Macrobrachium rosenbergii* (27.7 ± 3.2 days). Besides the fact that they are distinct species, the great difference in the duration of the complete moulting cycle may have been influenced by environmental factors, since our experiment was developed in laboratory conditions, while this other one was in natural conditions of an excavated nursery. Barnabé (1996) describes that in crustaceans, the duration of the moulting cycle, besides being different among species, is also different within the same species due to factors such as: size, age, temperature, photoperiod and ocular peduncle ablation. (Almeida Neto and Freire, 2007).

Even though the moulting cycle is regulated by endogenous, mainly hormonal factors, this physiological process can be influenced directly by environmental factors. According to Chen and Chen (2003), moulting in decapods is affected by factors such as temperature, salinity, pH, light intensity, pollutants and also nutrition. These same authors observed that the frequency of moulting in *M. rosenbergii* juveniles at a pH of 6.8, 6.2 and 5.6 was significantly lower than at a higher pH (7.4 and 8.2). Instudies with *Macrobrachium amazonicum* larvae, Hayd et al. (2008) observed that the duration of the moulting cycle was 1-2 days and 3-4 days with a temperature of 29 to 21°C, respectively. For juvenile *M. rosenbergii* this is the first study that brings moulting cycle stages by setogenesis method, not having studies with different temperatures or pH. With the results, it was observed that soon after the molting the animals were with all the structures of the new exoskeleton formed, however still very flaccid due to non-calcification, however, in the following stages already the complete formation of the new exoskeleton is observed. Thus, we can confirm the hypothesis that the molt interferes directly in the feeding, especially in the early stages, and the exoskeleton is not fully calcified, since the animal cannot crush the food with the maxillary appendages.

CONCLUSION

It was concluded that it is possible to apply the method of setogenesis to freshwater prawns *Macrobrachium rosenbergii*, making it possible to predict the effective moment of the moult in this prawn, and that the intermediate stage is the longest in relation to the stages that characterize the preparation for molting, the stages D, overlapping, in duration, the others.

REFERENCES

Almeida Neto, M.E.; Freire, A.G. 2007. Avaliação de consumo alimentar e textura do exoesqueleto do camarão marinho *Litopenaeus vannamei* (crustacea: penaeidae) em cultivo comercial, durante o ciclo de muda. Boletim do Instituto de Pesca, 33(2): 147-156.

Barbieri, E.; Branco, J.O.; Santos, M.C.F.; Hidalgo, K.R. 2013. Effects of Cadimium and Zinc on Oxygen consumption and ammonia excretion of the sea-bob shrimp, according to temperature. Boletim do Instituto de Pesca, 39(3): 299-309. **http://dx.doi.org/10.20950/1678-2305.2013v39n3p299**.

Barbieri, E.; Ferreira, A.C.; Rezende, K.F.O. 2017. Cadmium effects on shrimp ammonia exetion (Farfantepenaeus paulensis) at differents temperatures and levels. Pan-American Journal of Aquatic Sciences, 12(3): 176-183.

Barnabé, G. 1996. Bases biológicas y ecológicas de la acuicultura. Barcelona: Acribia Editorial. 566p.

Ceccaldi, H. 1987. La digestión en los crustáceos. In: Monteros, J.E.; Labarta, U. Nutrición en acuicultura. Madrid: Industrias Gráficas, p. 67-84.

Chan, S.M.; Rankin, S.M.; Keeley, L.L. 1988. Characterization of the molt stages in *Penaeus vannamei*: Setogenesis and hemolymph levels of total protein, ecdysteroids, and glucose. The Biological Bulletin, 175(1): 185-192. **http://dx.doi.org/10.2307/1541558**.

Chang, E.S. 1995. Physiological and biochemical changes during the molt cycle in decapod crustaceans: an overview. Journal of Experimental Marine Biology and Ecology, 193(1): 1-14. **http://dx.doi.org/10.1016/0022-0981(95)00106-9**.

Chen, S.M.; Chen, J.C. 2003. Effects of pH on survival, growth, molting and feeding of giant freshwater prawn *Macrobrachium rosenbergii*. Aquaculture (Amsterdam, Netherlands), 218(1): 613-623. **http://dx.doi.org/10.1016/S0044-8486(02)00265-X**.

Corteel, M.; Dantas-Lima, J.J.; Wille, M.; Alday-Sanz, V.; Pensaert, M.B.; Sorgeloos, P.; Nauwynck, H.J. 2012. Moult cycle of laboratory-raised *Penaeus (Litopenaeus) vannamei* and *P. monodon*. Aquaculture International, 20(1): 13-18. **http://dx.doi.org/10.1007/s10499-011-9437-9**.

FAO – Food and Agriculture Organization of the United Nations. 2014. FIGIS – Fisheries Statistics – Aquaculture. [online] URL: <http://www.fao.org>

Gastelú, J.C.; Oliveira, J.M.; Brito, L.O.; Oliveira, A.; Moreira, M.G.B.S. 2011. Efeito da temperatura, maturação ovariana e muda na variação da taxa respiratória de fêmeas do camarão *Macrobrachium acanthurus*. Revista Portuguesa de Ciências Veterinárias, 106(577-580): 81-86.

Hayd, L.A.; Anger, K.; Valenti, W.C. 2008. The moulting cycle of larval Amazon River prawn *Macrobrachium amazonicum* reared in the laboratory. Nauplius, 16(2): 55-63.

Ostrensky, A.; Barbieri, R.C. 2002. Camarões marinhos – engorda. 2ª ed. Viçosa: Aprenda Fácil. 351p.

Passano, L.M. 1960. Molting and its Control. In: Waterman. T. The physiology of crustacea: metabolism and growth. Cambridge: Academic Press. p. 473-536. **http://dx.doi.org/10.1016/B978-0-12-395628-6.50021-X**.

Peebles, J.B. 1977. A rapid technique for molt staging in live *Macrobrachium rosenbergii*. Aquaculture (Amsterdam, Netherlands), 12(2): 173-180. **http://dx.doi.org/10.1016/0044-8486(77)90185-5**.

Promwikorn, W.; Kirirat, P.; Intasaro, P.; Withyachumnarnkul, B. 2007. Changes in integument histology and protein expression related to the molting cycle of the black tiger shrimp, *Penaeus monodon*. Comparative Biochemistry and Physiology, 148(1): 20-31. **http://dx.doi.org/10.1016/j.cbpb.2007.04.009**. PMid:17601758.

Robertson, L.; Bray, W.; Leung-Trujillo, J.; Lawrence, A. 1987. Practical molt staging of *Penaeus setiferus* and *Penaeus stylirostris*. Journal of the World Aquaculture Society, 18(3): 180-185. **http://dx.doi.org/10.1111/j.1749-7345.1987.tb00437.x**.

Rusaini, R.; Owens, L. 2011. A simple technique to stage the moult of *Penaeus monodon*. Asian Fisheries Science, 24(1): 1-11.

Santos, D.B.; Barbieri, E.; Bondioli, A.C.; Melo, C.B. 2014. Effects of lead in white shrimp (*Litopenaeus schmitti*) metabolism regarding salinity. O Mundo da Saúde, 38(1):16-23.

Saravanan, S.; Kamalam, B.S. 2008. Moulting and Behaviour Changes in Freshwater Prawn. The Fish Site. [online] URL: <https://thefishsite.com/articles/moulting-and-behaviour-changes-in-freshwater-prawn>

Silva, M.A.S.; Almeida Neto, M.E.; Ramiro, B.O.; Santos, I.T.F.; Guerra, R.R. 2018. Histomorphologic characterization of the hepatopancreas of freshwater prawn *Macrobrachium rosenbergii* (De Man, 1879). Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 70(5): 1539-1546. **http://dx.doi.org/10.1590/1678-4162-10497**.

Smith, D.M.; Dall, W. 1985. Moult staging the tiger prawn *Penaeus esculentus*. In: Rothlisberg, P.C.; Hill, B.J.; Staples, D.J. In: Australian National Prawn Seminar, 2, Australia, 1985. Proceedings… Camberra, Cleveland: FRDC. p. 85-95.

Valenti, W.C.; Cavalcanti, L.B. 1998. Carcinicultura de Água Doce. Brasília: IBAMA. p. 19-25.