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Original Article

Acute toxicity of total ammonia to *Macrobrachium rosenbergii* postlarvae at different salinity levels

Toxicidade aguda da amônia total para pós-larvas de *Macrobrachium rosenbergii* em diferentes níveis de salinidades

J. H. S. Motta[a]*, L. C. Santos[b], F. M. Dutra[b], A. B. Souza[c], M. F. Polese[c], L. S. Glória[d], A. P. Oliveira[b] and E. L. C. Ballester[b]

[a]Universidade Federal do Oeste do Pará – UFOPA, Monte Alegre, PA, Brasil
[b]Universidade Federal do Paraná – UFPR, Palotina, PR, Brasil
[c]Instituto Federal do Espírito Santo – IFES, Piúma, ES, Brasil
[d]PIC Pig Improvement Company – Genus plc, Hendersonville, TN, United States

Abstract

Nitrogen compounds, particularly ammonium, nitrite and nitrate, are a major problem in shrimp production systems. These compounds can accumulate in the aquatic environment and reach harmful or even lethal levels. Thus, monitoring the levels of nitrogenous compounds such as ammonia and studying their effects on the animals are essential. One tool used for this purpose is acute toxicity testing based on the evaluation of LC50 values. Furthermore, tools that can help improve the performance of aquatic organisms in culture are needed. The present study aimed to evaluate the effect of salinity on the toxicity of total ammonia to postlarvae of the freshwater prawn *Macrobrachium rosenbergii*. For this purpose, acute toxicity testing (LC50-96h) was performed using 540 postlarvae with a mean weight of 0.13 g and a mean total length of 2.47 cm, divided into 54 experimental units of two liters each. A completely randomized design in a 3×6 factorial scheme was used, combining three salinities (0, 5, and 10 $g.L^{-1}$) and six total ammonia concentrations (0, 8, 16, 32, 64, and 128 $mg.L^{-1}$), with three replicates per combination. The LC50 values for *M. rosenbergii* postlarvae at 24, 48, 72, and 96 h and their respective confidence intervals (95%) were estimated using the trimmed Spearman-Karber method. The results showed that salinities of 5 or 10 $g.L^{-1}$ did not reduce the acute toxicity of total ammonia.

Keywords: aquaculture, shrimp, freshwater, water chemistry, nitrogen compounds.

Resumo

Compostos nitrogenados, particularmente amônia, nitrito e nitrato, são um grande problema nos sistemas de produção de camarão. Esses compostos podem se acumular no meio aquático e atingir níveis nocivos ou mesmo letais. Assim, monitorar os níveis de compostos nitrogenados como a amônia e estudar seus efeitos nos animais são essenciais. Uma ferramenta utilizada para este fim são os testes de toxicidade aguda baseados na avaliação dos valores de CL_{50}. Além disso, são necessárias ferramentas que possam ajudar a melhorar o desempenho dos organismos aquáticos em cultura. O presente estudo teve como objetivo avaliar o efeito da salinidade na toxicidade da amônia total para pós-larvas do camarão de água doce *Macrobrachium rosenbergii*. Para tanto, o teste de toxicidade aguda (CL_{50}-96h) foi realizado utilizando-se 540 pós-larvas com peso médio de 0,13 g e comprimento total médio de 2,47 cm, divididas em 54 unidades experimentais de dois litros cada. O delineamento experimental utilizado foi o inteiramente casualizado, em esquema fatorial 3×6, combinando três salinidades (0, 5 e 10 $g.L^{-1}$) e seis concentrações de amônia total (0, 8, 16, 32, 64 e 128 $mg.L^{-1}$), com três repetições por combinação. Os valores de CL_{50} para pós-larvas de *M. rosenbergii* em 24, 48, 72 e 96 h e seus respectivos intervalos de confiança (95%) foram estimados pelo método de Spearman-Karber aparado. Os resultados mostraram que salinidades de 5 ou 10 $g.L^{-1}$ não reduziram a toxicidade aguda da amônia total.

Palavras-chave: aquicultura, camarão, água doce, química da água, compostos nitrogenados.

*e-mail: motta.henri@gmail.com
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1. Introduction

Several species of freshwater prawn have the potential for aquaculture (Sampaio et al., 2007; Pantaleão et al., 2014; Lima et al., 2014). Among them, the Malaysian giant freshwater prawn (*Macrobrachium rosenbergii*) is a species native to the Indo-Pacific region (western Indo-Pacific, Pakistan to Vietnam, Philippines, New Guinea, and northern Australia) (Chan, 1998; New and Nair, 2012). It naturally inhabits rivers, lakes, and reservoirs that communicate with brackish waters (Ling and Merican, 1961). This species is of global importance, with its production exceeding 290,000 tons, corresponding to the fifth most produced crustacean species in the world (FAO, 2022).

Regarding the production of aquaculture species, there is concern about the accumulation of toxic compounds, especially nitrogenous compounds (Biudes et al., 2011; Henares and Camargo, 2014). Among these compounds, ammonia accumulates in aquaculture production units, mainly as a result of the metabolism of feed protein supplied to the organisms produced (Mayzaud and Conover, 1988; Green and Hardy, 2002).

In view of the lethal potential of ammonia and the consequent significant economic losses, studies evaluating the effect of this compound on aquatic animals and its toxicity levels are needed (Dutra et al., 2016). According to Hodgson (2004), one approach to obtain this information is acute toxicity testing based on the evaluation of LC_{50} values (Lethal Concentration for 50% of the exposed population).

Furthermore, tools to minimize the toxicity of ammonia are needed. Extrinsic factors such as temperature and salinity can affect the absorption and excretion of ammonia in shrimp (Regnault, 1987; Randall and Tsui, 2002). Animals that inhabit freshwater and brackish water environments have developed mechanisms to regulate hemolymph concentrations of Na^+ and Cl^- and, consequently, the uptake of ions. Thus, mechanisms of active ammonia excretion through synergistic stimulation of ammonia and Na^+ absorption sites, catalyzed by the enzyme Na^+/K^+ATPase, may have been selected in some animals exposed to habitats with different salinity gradients (Leone et al., 2014).

Since part of the life cycle of *M. rosenbergii* occurs in brackish water and there are reports of successful creation of the species in salinized waters, the present work aimed to identify the ammonia tolerance levels of the species and to analyze the feasibility of using salt as a mitigating agent of ammonia toxicity.

2. Material and Methods

The experiment, a 96 h trial, was conducted at the Shrimp Farming Laboratory of the Center for Research and Development in Sustainable Aquaculture, Federal University of Paraná – Palotina Campus (NPDA/UFPR-Palotina). The pos-larvae were obtained by reproduction and larviculture carried out in the laboratory. The methodology described by Peltier (Peltier, 1978) based on LC_{50} values within 96 h (LC_{50}-96h; acute toxicity test) was used to evaluate the direct relationship between salinity and the toxicological effect of total ammonia.

In the bioassays, 540 *M. rosenbergii* postlarvae (0.14 ± 0.06 g and 2.47 ± 0.35 cm) were stored in 54 experimental units of two liters. The animals were housed individually in perforated plastic subunits to avoid competition and cannibalism. In each unit, the larvae were maintained under constant aeration in an air conditioning-controlled environment within the ideal range for the species, respecting a density of 5:1 (individuals per liter) and a photoperiod of 12 h. In addition, the animals were fasted during the experiment.

A completely randomized design in a 3×6 factorial scheme was used, combining three salinities (0, 5, and 10 $g.L^{-1}$) and six concentrations of total ammonia (0, 8, 16, 32, 64, and 128 mg $N\text{-}NH_3.L^{-1}$), with three replicates per combination. The total ammonia concentrations used in the experiment were based on the LC_{50}-96h values determined by Armstrong et al. (1978).

The total ammonia concentrations chosen for the experiment were obtained by preparing a stock solution of 1,000 $mg.L^{-1}$ of total ammonia, diluting 3.819 $g.L^{-1}$ of ammonium chloride P.A (ANIDROL) in distilled water and later in previously dechlorinated tap water. A stock solution of 40 liters was prepared for each saline concentration (5 and 10 $g.L^{-1}$) by diluting 200 and 400 g of salt (SALT: FISH ONLY – NUTRZOO), respectively. The salt was weighed on a precision scale (MARTE/SHIMADZU MOD AY-220 – CAP 220g X 0,01g). These 40-liter stock solutions were then used to supply the experimental units.

The mortality of prawns was evaluated based on the lack of response to mechanical stimulation with a glass stick and change in the normal color of the animal, which becomes whitish after its death. The postlarvae were observed every hour over the first 8 hours of the experiment. After this period, observations were made every 3 hours, totaling 96 hours. The 3-hour period was adopted to avoid decomposition of the animal and changes in water quality due to the release of nitrogenous compounds.

Dissolved oxygen (Microprocessed oximeter ALFAKIT AT-160), temperature (digital thermometer Inconterm) and pH (pHmetro Luca 210) were evaluated daily to determine whether the water quality variables remained within the appropriate range for the species. To confirm that the total ammonia concentrations were within the proposed range, 200 mL water was collected from each experimental unit into plastic bottles at the beginning and end of the experiment and sent to the Laboratory of Water Quality and Limnology of UFPR-Palotina. Total ammonia concentrations were measured by the indophenol colorimetric method (Grasshoff et al, 1999) and alkalinity by titration following the method proposed by Macêdo (2005).

Before storage in the experimental units, a sample of the population was submitted to biometrics to obtain the total weight with a precision scale (MARTE/SHIMADZU MOD AY-220 – CAP 220g X 0,01g) and the total length (TL = measured between the anterior end of the rostrum and the posterior end of the telson) using a caliper (ZAAS), as described by Oliveira et al. (2014). The median lethal concentrations for *M. rosenbergii* postlarvae at 24, 48, 72, and 96 h and their respective confidence intervals (95%) were estimated using the trimmed Spearman-Karber method (Hamilton et al., 1977).

Survival was estimated based on a binomial distribution using generalized linear models. For this purpose, the number of animals that survived each observation was counted in relation to the total number of individuals per replicate at the beginning of the experiment. The GLIMMIX procedure of the Statistical Analysis System was used and, in case of a significant difference, the Tukey test was applied.

3. Results

For treatments 0, 8, 16, and 32 mg.L^{-1}, the total ammonia concentrations during the experiment remained close to the concentrations determined for the experiment. For treatments 64 and 128 mg.L^{-1}, the total ammonia concentrations were slightly above the proposed. The dissolved oxygen, temperature, pH, and alkalinity values remained within the recommended range for the production of the species throughout the experimental period (Table 1).

During the course of the experiment, mortality was 100% within less than 24 h among postlarvae of *M. rosenbergii* exposed to the total ammonia concentration of 128 mg.L^{-1} at a salinity of 10 g.L^{-1}. At the same ammonia concentration, a significant difference ($p < 0.05$) in survival was observed between postlarvae maintained at a salinity of 10 g.L^{-1} and those maintained at salinities of 0 and 5 g.L^{-1} (Table 2).

For the ammonia concentration of 64 mg.L^{-1}, salinity also significantly influenced ($p < 0.05$) the survival of *M. rosenbergii* postlarvae. Survival was lower at a salinity of 10 g.L^{-1} compared to salinity of 0.0 mg.L^{-1} (Table 2).

The time to death of 50% of the larvae was also influenced by both ammonia concentration and salinity (Table 2). Regarding this variable, the average survival was 56.67% for the treatment with 64 mg N-NH_3.L^{-1} at a salinity of 10 g.L^{-1}. It is important to note that the confidence interval was lower than 50% (38.83%) in this treatment; thus, during production, survival may be less than 50% when this treatment is used.

The mortality rates observed at each salinity were used to estimate the median lethal concentration (LC_{50}-96h) of total ammonia at 24, 48, 72 and 96 h, the confidence interval, and the safety level. The results are summarized in Table 3.

Table 1. Means (± SD) of the water quality variables of *Macrobrachium rosenbergii* postlarvae exposed to different levels of total ammonia and salinity for 96 h.

Treatment		OTA (mg.L^{-1})	Temperature (°C)	pH	DO (mg.L^{-1})	Alkalinity (mg.L^{-1}$CaCO_3$)
Salinity (g.L^{-1})	PTA (mg.L^{-1})					
0	0	1.31 ± 0.30	25.58 ± 0.38	8.24 ± 0.1	6.54 ± 0.68	72.67 ± 8.33
	8	8.59 ± 0.49	25.50 ± 0.3.6	8.06 ± 0.08	6.65 ± 0.81	59.17 ± 4.75
	16	16.32 ± 1.40	25.66 ± 0.33	7.96 ± 0.06	6.52 ± 0.88	52.67 ± 12.26
	32	30.74 ± 1.77	25.53 ± 0.39	7.74 ± 0.09	6.62 ± 0.83	60.67 ± 45.16
	64	74.92 ± 11.23	24.92 ±0.54	7.07 ± 0.94	6.89 ± 0.53	43.83 ± 26.51
	128	166.51 ± 16.35	25.51 ± 0.43	7.50 ± 0.56	6.73 ± 0.51	42.67 ± 27.75
5	0	1.38 ± 0.31	25.53 ± 0.43	8.21 ± 0.09	6.17 ± 0.79	107.83 ± 39.82
	8	8.51 ± 0.89	25.58 ± 0.4	8.08 ± 0.13	6.27 ± 0.87	92.83 ± 19.49
	16	15.92 ± 2.4	25.43 ± 0.43	8.01 ± 0.06	6.53 ± 0.79	76.17 ± 17.07
	32	27.57 ± 2.96	25.38 ± 0.37	7.82 0.13	6.23 ± 0.91	70.67 ± 27.14
	64	73.77 ± 7.92	25.38 ± 0.25	7.48 ± 0.38	6.84 ± 0.45	51.83 ± 35.24
	128	155.37 ± 24.31	25.33 ± 0.43	7.23 ± 0.59	7.05 ± 0.5	49.00 ± 35.13
10	0	1.21 ± 0.36	25.50 ± 0.39	8.23 ± 0.07	6.86 ± 0.51	134.83 ± 39.09
	8	8.42 ± 0.80	25.63 ± 0.43	8.15 ± 0.06	6.75 ± 0.47	106.50 ± 8.02
	16	17.32 ± 1.2	25.54 ± 0.33	8.12 ± 0.04	6.90 ± 0.49	92.17 ± 19.83
	32	29.69 ± 1.52	2559 ± 0.4	7.90 ± 0.09	6.77 ± 0.41	83.83 ± 19.74
	64	73.32 ± 8.51	25.54 ± 0.21	7.28 ± 0.72	7.05 ± 0.52	59.67 ± 45.28
	128	167.56 ± 7.92	25.34 ± 0.43	7.41 ± 0.55	7.05 ± 0.34	87.83 ± 16.88

DO = dissolved oxygen; PTA = proposed total ammonia; OTA = observed total ammonia.

Table 2. Survival and time to death (hours) of *M. rosenbergii* postlarvae maintained at different levels of ammonia and salinity.

Treatment		Survival (%)	Standard error (%)	Confidence interval (%)	
Salinity (‰)	PTA (mg.L^{-1})			Lower	Upper
0	0	93.33^{a}	4.554	76.9	98.33
	8	96.67^{a}	3.277	79.76	99.53
	16	100.00^{a*}	N/A	–	
	32	100.00^{a*}	N/A	–	
	64	83.33^{a}	6.804	65.66	92.89
	128	6.67^{c}	4.554	1.671	23.09
5	0	100.00^{a*}	N/A	–	
	8	100.00^{a*}	N/A	–	
	16	100.00^{a*}	N/A	–	
	32	100.00^{a*}	N/A	–	
	64	73.33^{ab}	8.074	55.02	86.08
	128	13.33^{c}	6.206	5.09	30.62
10	0	100.00^{a*}	N/A	–	
	8	93.33^{a}	4.554	76.91	98.33
	16	96.67^{a}	3.277	79.76	99.53
	32	93.33^{a}	4.554	76.9	98.33
	64	56.67^{b}	9.047	38.83	72.93
	128	0.00^{d**}	N/A	–	

PTA = proposed total ammonia; N/A = not applicable.
*For means of 100% there is no standard error;
**For 0% means there is no standard error. Different letters in the same column indicate a significant difference ($p < 0.05$) between treatments.

Table 3. Ammonia LC_{50} (mg N-NH_3.L^{-1}) values for *M. rosenbergii* postlarvae and their respective confidence intervals (N/A - not applicable).

Salinity (‰)	Hours	LC_{50}	Confidence interval LC_{50}	Level of security (mg N-NH_3.L^{-1})
0	24	-	-	N/A
	48	90.51	84.63 - 96.79	9.05
	72	86.80	77.41 - 97.33	8.68
	96	84.54	75.00 - 95.30	8.45
5	24	97.01	87.20 - 107.92	9.70
	48	82.35	68.68 - 98.73	8.23
	72	82.35	68.68 - 98.73	8.23
	96	82.23	68.69 - 98.45	8.22
10	24	70.20	61.48 - 80.15	7.02
	48	64.00	55.59 - 73.69	6.40
	72	62.54	53.96 - 72.48	6.25
	96	51.41	36.96 - 71.51	5.14

4. Discussion

Researchers have investigated the toxicity of total ammonia to shrimp at varying levels of salinity but the knowledge base is incomplete. Schuler et al. (2010), studying the acute toxicity of ammonia at 10 $g.L^{-1}$ salinity to Pacific white shrimp (*Penaeus vannamei*), observed mortality rates of 4.2, 25 and 46% at 20, 30 and 40 $mg.L^{-1}$ of total ammonia, respectively, after 48 h.

On the other hand, Barbieri (2010), who studied the acute toxicity of ammonia to *Penaeus schmitti* at different salinity concentrations, found the following mortality rates after 96 h of exposure to 5 ‰ salinity: 6.66% at 5 $mg.L^{-1}$ of ammonia, 20% at 10 $mg.L^{-1}$, 26.6% at 20 $mg.L^{-1}$, and 100% at 30, 40, 60 and 80 $mg.L^{-1}$. However, when salinity was increased to 20 ‰, the author observed mortality rates of 0.0% at 5 and 10 $mg.L^{-1}$ of ammonia, 13.3% at 20 $mg.L^{-1}$, 66.6% at 30 $mg.L^{-1}$, and 100% at 60 and 80 $mg.L^{-1}$ after 96 h of exposure. The results reported by Barbieri (2010) demonstrate that, unlike what was observed in the present experiment, the increase in salinity reduces the toxicity of ammonia. Different conditions such as the shrimp species and developmental stage, as well as water quality parameters, are important to understand the relationship between total ammonia toxicity and salinity.

The LC_{50}-96h values of total ammonia obtained in the present study were higher than those found by Chen and Lin (1992) for *Penaeus chinensis* also exposed to salinity of 10 $g.L^{-1}$. The LC_{50} increased from 28.18 to 51.41 $mg.L^{-1}$ at a salinity of 10 $g.L^{-1}$. However, comparison of the LC_{50}-96h values at 0 and 10 $g.L^{-1}$ in the present study showed a reduction from 84.54 to 51.41 $mg.L^{-1}$, indicating that the increase in salinity was detrimental to *M. rosenbergii* as it increased the toxicity of ammonia.

Jiang et al. (2000) showed that, as salinity increases, ammonia excretion decreases from 66.82% at 10 $g.L^{-1}$ to 61.93% at 40 $g.L^{-1}$. The reduction of ammonia excretion by aquatic organisms leads to the accumulation of this metabolite in tissues and, consequently, mortality due to its toxicity (Randall and Tsui, 2002). This may explain the results obtained in the present experiment, where in the treatments with ammonia concentration of 64 and 128 $mg.L^{-1}$ without salinity (0 $g.L^{-1}$) ammonia toxicity was lower compared to treatment with salinity 10 $g.L^{-1}$, because increased mortality was observed when salinity was increased. Therefore, at higher salinities, the excretion of ammonia was probably lower, with the compound accumulating and reaching toxic levels. This result also corroborates the findings of Chen and Chia (1996) who demonstrated an inverse relationship between ammonia excretion and salinity in the mud crab *Scylla serrata*.

Studies conducted with other shrimp species report that ammonia excretion increases when the animals are hyper-regulated, *i.e.*, in an environment with low ion concentration (such as $NaCl^{-}$) (Chen et al., 1994; Lee and Chen, 2003), and decreases when they are hypo-regulated (in environments with high ion levels). High ammonia levels exert toxic effects on the metabolism of shrimp and can also cause immunosuppression, resulting in a series of physiological dysfunctions such as ionic imbalance, molting complications, growth delay, nervous system disorders, difficulties in respiratory metabolism and, eventually, a significant increase in mortality (Li et al., 2023).

The combination of the lower rate of ammonia excretion (which accumulates in the body) and the high basal metabolic rate for osmoregulation when submitted to a salinity of 10 $g.L^{-1}$ resulted in harmful environment for *M. rosenbergii*. Although salinity exerts a considerable effect on the internal concentration of ammonia, sodium (Na^{+}) has a lower affinity than NH_4^{+} for the enzyme responsible for active transport to the intracellular environment; hence, the absorption sites do not interfere much with the toxicity of ammonia (Eddy, 2005).

Taken together, the results of the present study demonstrated that salinity concentrations of 5 or 10 $g.L^{-1}$ did not reduce the acute toxicity of ammonia to *M. rosenbergii*. Indeed, the opposite occurred, with the increase in salinity being harmful to the animal, increasing the toxicity of ammonia. Therefore, increasing salinity should not be adopted as a strategy to minimize the effects of acute ammonia toxicity on the production of *M. rosenbergii* postlarvae.

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References

ARMSTRONG, D.A., CHIPPENDALE, D., KNIGHT, A.W. and COLT, J.E., 1978. Interaction of ionized and un-ionized ammonia on short-tem survival and growth of prawn larvae, *Macrobrachium rosenbergii. The Biological Bulletin*, vol. 154, no. 1, pp. 15-31. http://dx.doi.org/10.2307/1540771. PMid:29323956.

BARBIERI, E., 2010. Acute toxicity of ammonia in White shrimp (*Litopenaeus schmitti*) (Burkenroad, 1939, Crustacea) at different salinity levels. *Aquaculture (Amsterdam, Netherlands)*, vol. 306, no. 1-4, pp. 329-333. http://dx.doi.org/10.1016/j.aquaculture.2010.06.009.

BIUDES, J.F.V., CAMARGO, A.F.M. and HENARES, M.N.P., 2011. Impact of maintenance of *Macrobrachium rosenbergii* De Man, 1879 (Crustacea, Decapoda, Palaemonidae) broodstock on the water used in culture ponds. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 71, no. 4, pp. 857-863. http://dx.doi.org/10.1590/S1519-69842011000500006.

CHAN, T.Y., 1998. Shrimps and prawns. In: K.E. CARPENTER and V.H. NIEM, eds. *FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific*. Rome: FAO, vol. 2, pp. 851-972.

CHEN, J.-C. and CHIA, P.-G., 1996. Hemolymph ammonia and urea and nitrogenous excretions of Scylla serrata at different temperature and salinity levels. *Marine Ecology Progress Series*, vol. 139, pp. 119-125. http://dx.doi.org/10.3354/meps139119.

CHEN, J.-C. and LIN, C.-Y., 1992. Lethal effects of ammonia on *Penaeus chinensis* Osbeck juveniles at different salinity levels. *Journal of Experimental Marine Biology and Ecology*, vol. 156, no. 1, pp. 139-148. http://dx.doi.org/10.1016/0022-0981(92)90021-2.

CHEN, J.-C., CHEN, C.-T. and CHENG, S.-Y., 1994. Nitrogen excretion and changes of hemocyanin, protein and free amino acid levels in the hemolymph of Penaeus monodon exposed to different concentrations of ambient ammonia-N at different salinity levels. *Marine Ecology Progress Series*, vol. 110, pp. 85-85. http://dx.doi.org/10.3354/meps110085.

DUTRA, F.M., FORNECK, S.C., BRAZÃO, C.C., FREIRE, C.A. and BALLESTER, E.L.C., 2016. Acute toxicity of ammonia to various life stages of the Amazon river prawn, *Macrobrachium amazonicum*, Heller, 1862. *Aquaculture (Amsterdam, Netherlands)*, vol. 453, pp. 104-109. http://dx.doi.org/10.1016/j.aquaculture.2015.11.038.

EDDY, F.B., 2005. Ammonia in stuaries and effects on fish. *Journal of Fish Biology*, vol. 67, no. 6, pp. 1495-1513. http://dx.doi.org/10.1111/j.1095-8649.2005.00930.x.

FOOD AND AGRICULTURE ORGANIZATION - FAO, 2022. *The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation*. Rome: FAO. http://dx.doi.org/10.4060/cc0461en.

GRASSHOFF, K., KREMLING, K. and EHRHARDT, M., 1999. *Methods of seawater analysis, third completely revised and extended edition*. 3rd ed. New York: Wiley-VHC, 634 p. http://dx.doi.org/10.1002/9783527613984.

GREEN, J.A. and HARDY, R.W., 2002. The optimum dietary essential amino acid pattern for rainbow trout (*Oncorhynchus mykiss*), to maximize nitrogen retention and minimize nitrogen excretion. *Fish Physiology and Biochemistry*, vol. 27, no. 1/2, pp. 97-108. http://dx.doi.org/10.1023/B:FISH.0000021878.81647.6e.

HAMILTON, M.A., RUSSO, R.C. and THURSTON, R.V., 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. *Environmental Science & Technology*, vol. 11, no. 7, pp. 714-719. http://dx.doi.org/10.1021/es60130a004.

HENARES, M.N.P. and CAMARGO, A.F.M., 2014. Treatment efficiency of effluent prawn culture by wetland with floating aquatic macrophytes arranged in series. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 74, no. 4, pp. 906-912. http://dx.doi.org/10.1590/1519-6984.10413. PMid:25627602.

HODGSON, E., 2004. *A textbook of modern toxicology*. Hoboken: John Wiley & Sons. http://dx.doi.org/10.1002/0471646776.

JIANG, D., LAWRENCE, A.L., NEILL, W.H. and GONG, H., 2000. Effects of temperature and salinity on nitrogenous excretion by *Litopenaeus vannamei* juveniles. *Journal of Experimental Marine Biology and Ecology*, vol. 253, no. 2, pp. 193-209. http://dx.doi.org/10.1016/S0022-0981(00)00259-8. PMid:11033364.

LEE, W.-C. and CHEN, J.-C., 2003. Hemolymph ammonia, urea and uric acid levels and nitrogenous excretion of *Marsupenaeus japonicus* at different salinity levels. *Journal of Experimental Marine Biology and Ecology*, vol. 288, no. 1, pp. 39-49. http://dx.doi.org/10.1016/S0022-0981(02)00597-X.

LEONE, F.A., BEZERRA, T.M.S., GARÇON, D.P., LUCENA, M.N., PINTO, M.R., FONTES, C.F.L. and MCNAMRA, J.C., 2014. Modulation by K+ plus NH4+ of microsomal (Na+, K+)- ATPase activity in selected ontogenetic stages of the diadromous river shrimp *Macrobrachium amazonicum* (Decapoda, Palaemonidae). *PLoS One*, vol. 9, no. 2, pp. e89625. http://dx.doi.org/10.1371/journal.pone.0089625. PMid:24586919.

LI, Y., TONG, R., LI, Z., ZHANG, X., PAN, L., LI, Y. and ZHANG, N., 2023. Toxicological mechanism of ammonia-N on haematopoiesis and apoptosis of haemocytes in *Litopenaeus vannamei*. *The Science of the Total Environment*, vol. 879, pp. 163039. http://dx.doi.org/10.1016/j.scitotenv.2023.163039. PMid:36966842.

LIMA, J.F., GARCIA, J.S. and SILVA, T.C., 2014. Natural diet and feeding habits of a freshwater prawn (*Macrobrachium carcinus*: Crustacea, Decapoda) in the estuary of the Amazon River. *Acta Amazonica*, vol. 44, no. 2, pp. 235-244. http://dx.doi.org/10.1590/S0044-59672014000200009.

LING, S.W. and MERICAN, A.B.O., 1961. Notes on the life and habits of the adults and larval stages of *Macrobrachium rosenbergii*. *Proceedings of the Indo-Pacific Fisheries Council*, vol. 9, no. 2, pp. 55-60.

MACÊDO, J.A.B., 2005. *Métodos laboratoriais de análises físico-químicas e microbiológicas*. 3. ed. Belo Horizonte : Conselho Regional de Química.

MAYZAUD, P. and CONOVER, R.J., 1988. O:N atomic ratio as a tool to describe zooplankton metabolismo. *Marine Ecology Progress Series*, vol. 45, no. 3, pp. 289-302. http://dx.doi.org/10.3354/meps045289.

NEW, M.B. and NAIR, M., 2012. Review Article: global scale of freshwater prawn farming. *Aquaculture Research*, vol. 43, no. 7, pp. 960-969. http://dx.doi.org/10.1111/j.1365-2109.2011.03008.x.

OLIVEIRA, V.S., RAMOS-PORTO, M., SANTOS, M.C.F., HAZIN, F.H.V., CABRAL, E. and ACIOLE, F.D., 2014. Características biométricas, distribuição e abundância relativa do camarão Plesionika edwardsii na costa nordeste do Brasil. *Boletim do Instituto de Pesca*, vol. 40, no. 2, pp. 215-222.

PANTALEÃO, J.A.F., HIROSE, G.L. and COSTA, R.C., 2014. Ocurrence of male morphotypes of *Macrobrachium amazonicum* (Caridea, Palaemonidae) in a population with an entirely freshwater life cycle. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 74, no. 3, suppl. 1, pp. S223-S232. http://dx.doi.org/10.1590/1519-6984.03713. PMid:25627389.

PELTIER, W.H., 1978. *Methods for measuring the acute toxicity of effluents to aquatic organisms*. 2nd ed. Cincinnati: Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, EPA-600/4-78-012.

RANDALL, D.J. and TSUI, T.K.N., 2002. Ammonia toxicity in fish. *Marine Pollution Bulletin*, vol. 45, no. 1-12, pp. 17-23. http://dx.doi.org/10.1016/S0025-326X(02)00227-8. PMid:12398363.

REGNAULT, M., 1987. Nitrogen excretion in marine and freshwater crustacea. *Biological Reviews of the Cambridge Philosophical Society*, vol. 62, no. 1, pp. 1-24. http://dx.doi.org/10.1111/j.1469-185X.1987.tb00623.x.

SAMPAIO, C.M.S., SILVA, R.R., SANTOS, J.A. and SALES, S.P., 2007. Reproductive cycle of *Macrobrachium amazonicum* females (Crustacea, Palaemonidae). *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 67, no. 3, pp. 551-559. http://dx.doi.org/10.1590/S1519-69842007000300022. PMid:18094840.

SCHULER, D.J., BOARDMAN, G., KUHN, D.D. and FLICK, G.J., 2010. Acute toxicity of ammonia and nitrite to Pacific White Shrimp, *Litepennaeus vannamei*, at low salinities. *Journal of the World Aquaculture Society*, vol. 41, no. 3, pp. 438-446. http://dx.doi.org/10.1111/j.1749-7345.2010.00385.x.