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MORTALITY OF PACIFIC WHITE SHRIMP SUBMITTED TO HYPOTHERMIC AND HYPOSALINIC STRESS

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ABSTRACT

Abrupt changes in water quality parameters affect strongly the growth, survival and resistance to disease of farmed marine shrimps. However, unlike the determination of the toxic levels of substances affecting 50% of the population, standard protocols to nontoxic stressors tests are often neglected. The main objective of this work was to establish the lethal temperature (LT_{50}) and salinity (LS_{50}) for 50% of the population. Juvenile shrimps weighting from 10 g to 12 g and 17 day-postlarvae reared at 28 °C temperature and 32‰ salinity were submitted to hypothermic stress for one hour at temperatures of 7°C, 10°C, 11.5°C, 13°C and 16°C (juveniles), or for 72 hours at temperatures of 11°C, 12°C, 13°C and 14°C (postlarvae). Besides hypothermic stress, juveniles were submitted to 24 hours hyposaline stress in water having 0‰, 3‰, 6‰ and 9‰ salinities, and the postlarvae for 72 hours in water having 0‰, 1.5‰, 3‰, 4.5‰ and 6‰ salinities. Mortality rates were determined after those periods. The LT_{50} were 11.7 °C for juveniles and 12.9°C for postlarvae, and the LS_{50} were 2.4‰ and 1.8‰ for the juveniles and postlarvae, respectively.

Key words: *Litopenaeus vannamei*; lethal temperature; lethal salinity.

MORTALIDADE DO CAMARÃO BRANCO DO PACÍFICO SUBMETIDO AO ESTRESSE HIPOTÉRMICO E HIPOSSALINO

RESUMO

Alterações abruptas nos parâmetros da qualidade da água têm forte influência no crescimento, sobrevivência e resistência às doenças dos camarões marinhos cultivados. Entretanto, ao contrário da determinação dos níveis tóxicos de substâncias que afetam 50% da população, protocolos padrão para testes de estressores não tóxicos são muitas vezes negligenciados. O objetivo deste trabalho foi estabelecer a temperatura (TL_{50}) e salinidade (SL_{50}) letal para 50% da população. Camarões juvenis pesando entre 10 e 12 g e pós-larvas de 17 dias cultivados à temperatura de 28 °C e salinidade de 32‰ foram submetidos ao estresse hipotérmico por uma hora nas temperaturas de 7°C, 10°C, 11,5°C, 13°C e 16°C (juvenis), ou 72 horas nas temperaturas de 11°C, 12°C, 13°C e 14°C (pós-larvas). Além do estresse hipotérmico, juvenis foram submetidos por 24 horas ao estresse hipossalino em águas contendo salinidade de 0‰, 3‰, 6‰ e 9‰, e pós-larvas por 72 horas em água contendo salinidade de 0‰, 1,5‰, 3‰, 4,5‰ e 6‰. As taxas de mortalidade foram determinadas após estes períodos. A TL_{50} calculada foi de 11,7°C para os juvenis e 12,9°C para as pós-larvas, e a SL_{50} calculada foi de 2,4‰ e 1,8‰ para juvenis e pós-larvas, respectivamente.

Palavras-chave: *Litopenaeus vannamei*; temperatura letal; salinidade letal.

INTRODUCTION

Marine shrimp farming is one of the most important activities in world aquaculture, generating more than 23.5 billion dollars; the production of the *Litopenaeus vannamei* species alone is responsible for 18.5 billion dollars (FAO, 2016). This species is largely farmed in tropical areas, but the farms have also spread to subtropical areas (KRUMMENAUER *et al*., 2011).

L. vannamei, as tropical species reaches optimum growth between 28°C to 32°C (VAN WYK and SCARPA, 1999). Highest survival rates are found between 20°C to 30°C (PONCE-PALAFOX *et al*., 1997). However, low temperatures, as well as salinity

fluctuations can produce mortality in shrimp farms (PEIXOTO JUNIOR *et al*., 2003; PAN *et al*., 2007; ROY *et al*., 2010). These two parameters are important immunosuppressive factors that favor the occurrence of diseases (WANG and CHEN, 2005; QIU *et al*., 2011). Temperature and salinity are also considered the most important physical factors affecting marine organisms, with complex biological actions (LIU *et al*., 2012).

The calculation of lethal temperatures can predict, for example, the temperature which an organism can withstand before dying, the period which the animal can survive in a determined temperature, or the temperature where a percentage of population can survive in one period of time. These indexes can be effective for physiological comparisons (FRY *et al*., 1946). Studies employing lethal temperatures or salinities to 50% of the animals have been used in aquaculture to check the efficiency that several treatments might have regarding the resistance of the organisms to these parameters (VILLEGAS, 1990; ATWOOD *et al*., 2003; INENO *et al*., 2005).

However, unlike the determination of the toxic levels of substances affecting 50% of the population, standard protocols to nontoxic stressors tests are often neglected (SMIT *et al*., 2008). VRIES *et al*. (2008) had analyzed data related to lethal temperatures to 50% of several aquatic animals, and found substantial variation in the applied methodology. The period of test varied in general between 24 to 96 hours, but it could also be short, lasting only 30 minutes. The acclimation period varied also between 6 to 204 hours. These variations make comparisons difficult, and so, standard protocols should be established. Same variations are found in the tests for lethal salinity calculation (ZHANG *et al*., 2009; EMERENCIANO *et al*., 2011). This work therefore intended to establish a methodology and determine the lethal temperature and salinity to 50% of the postlarvae and juvenile *L. vannamei* shrimps.

METHODS

Juveniles and postlarvae of *Litopenaeus vannamei* of high health lineage, SPEEDLINE HB12, were purchased from AQUATEC (Rio Grande do Norte State, Brazil) and cultivated in super-intensive biofloc system at Marine Shrimp Laboratory (LCM) of Federal University of Santa Catarina (UFSC). For the experiments, juveniles from 10 g to 12 g and postlarvae with 16 days (PL_{16}) were transferred from the main biofloc tanks and acclimated for 72 h and 24 h respectively, in clear water at 28°C, 32‰ salinity, constant aeration and daily water exchange. During the acclimation period, animals were fed 4 times a day with commercial feed (35% raw protein and 7.5% ethereal extract). After the stress, they were not fed anymore.

For hypothermic stress experiments, 300 juveniles having 10 to 12 grams were distributed in 15 aquaria (40 L) and acclimated according to conditions described before. After 72 h, short shocks of one hour were made moving animals to aquaria with the same salinity but at temperatures of 7 °C, 10 °C, 11.5 °C, 13 °C and 16 °C, controlled by ice and thermostats. The mortality rate was calculated 24 hours after by the following equation:

$$\text{Mortality } (\%) = \left[(\text{LAS} - \text{LAE}) \, . \, \text{LAS}^{-1} \right] . \, 100 \quad (1)$$

Where:

LAS = living animals at the start.

LAE = living animals at the end.

Regarding postlarvae hypothermic stress, two thousand postlarvae were placed in a 30 L aquarium for acclimation. After 24 hours, they were moved into 1 liter Erlenmeyers in groups of 50 postlarvae (PL17) each, filled with water in the same condition of acclimation. The Erlenmeyers were placed randomly into coolers filled with water up to half of them. Each cooler corresponded to one treatment: 11°C, 12°C, 13°C and 14°C, with 4 Erlenmeyers each (n=4). The water temperature inside coolers was decreased according to SAMOCHA *et al*. (1993) with modifications, 1°C each 15 minutes until 18°C, and each 30 minutes until the treatment temperature, controlled by ice and thermostats. After 72 h, the mortality rate was calculated.

In relation to hyposaline stress experiments, 360 juveniles having also 10 to 12 grams were distributed in 12 aquaria (60 L) and acclimated. After acclimation period, shrimps were transferred directly to others aquaria at the same temperature but with salinities of 0‰, 3‰, 6‰ and 9‰ for 24 hours. Salinities were adjusted with filtered and sterilized seawater, and filtered fresh water. All treatments were made in triplicates. After this period, the mortality rate was calculated as before.

Hyposaline stress was also evaluated in postlarvae following procedures similar to those applied to hypothermic stress. Two thousand *L. vannamei* PL_{16} were separated in one 30 L aquarium in acclimation conditions. After 24 hours, they were moved directly into 1 liter Erlenmeyers in groups of 50 PL_{17} each, containing water at 28°C with salinities of 0, 1.5‰, 3‰, 4.5‰ and 6‰. Each treatment had 4 replicates (n=4). Erlenmeyers were placed randomly into 3 aquaria, which were filled with water up to the half of them. Temperature was kept at 28°C with the help of heaters. Mortality rate was calculated 72 hours after.

The shrimps were considered dead when they returned to the temperature of 28°C and did not move under mechanical stimulus. The dead shrimps were removed from the aquaria. Temperature and salinity were checked every day and the pH was checked immediately before stress (YSI, model Profissional Plus). The pH for low salinities treatments were increased until 7.5 using sodium hydroxide (1M NaOH).

The mortality rates were plotted against the temperature or salinity of exposure and the regression analysis was performed on the results with the software Statistica 13, as well as the calculation of lethal temperature and salinity for 50% of the shrimp populations (respectively LT_{50} and LS_{50}). Among the available regression models, the mathematical model that best fit the data was chosen, using the highest coefficient of determination (R^2) as the criterion for better function.

RESULTS

Results show a strong relation between the increase of mortality and reduction of temperature or salinity ($p<0.01$). Following the highest coefficient of determination, the linear regression analysis was made for hypothermic stress and the geometric regression analysis for hyposaline stress for juveniles and postlarvae. In all experiments, the lowest parameters utilized, that is, the lowest temperature in thermal stress and the lowest salinity in the saline stress for juveniles as well as for postlarvae produced 100% death. Figure 1 shows the regression analysis and the calculated lethal temperature for 50% (LT_{50}) of juveniles and postlarvae during 24 and 72 hours of exposure, respectively, under hypothermic stress.

Higher temperatures produced an average mortality rate of 1.7% and 24.5% respectively for juveniles and postlarvae. Mortality increased significantly with temperature decreasing in both cases: from 20% to 70% in 13°C and 11.5°C, respectively for juveniles, and from 41% to 74.5% in 13°C and 12°C, respectively for postlarvae.

Figure 2 shows the hyposaline stress regression analysis and and the calculated lethal salinity for 50% (LS_{50}) of juveniles and postlarvae during 24 and 72 hours of exposure, respectively, under hyposalinity stress. Mortality varied from 100% to 17.8% among juveniles and from 100% to 12% among postlarvae. In this case, significant increasing in the average mortality was also observed between two consecutive treatments: from 41.1% to 100% in 3‰ and 0‰, respectively among juveniles and from 26.5% to 75% in

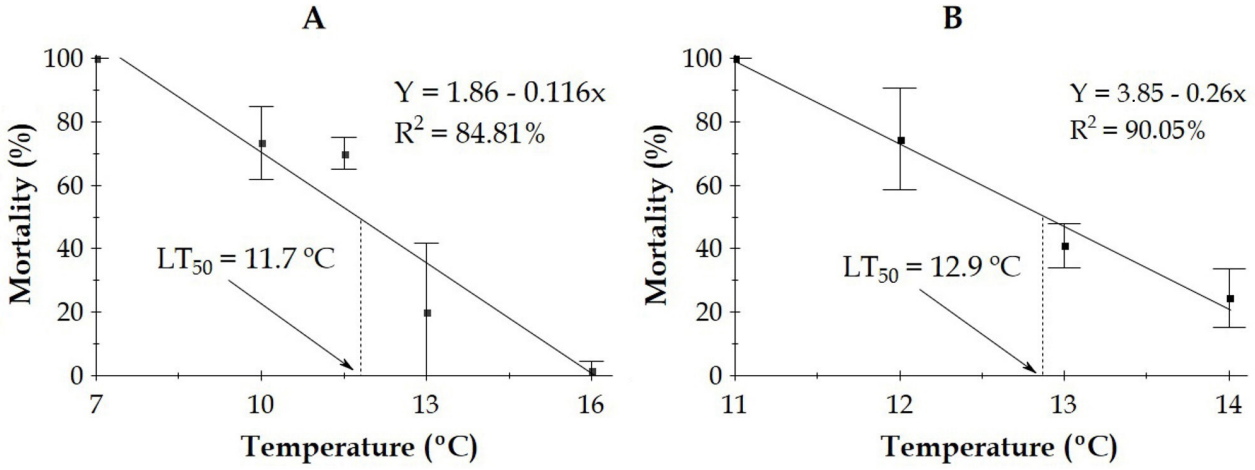

Figure 1. Regression analysis of *L. vannamei* mortality under hypothermic stress. A – Juveniles. B – Postlarvae. LT_{50} = lethal temperature for 50% of the population.

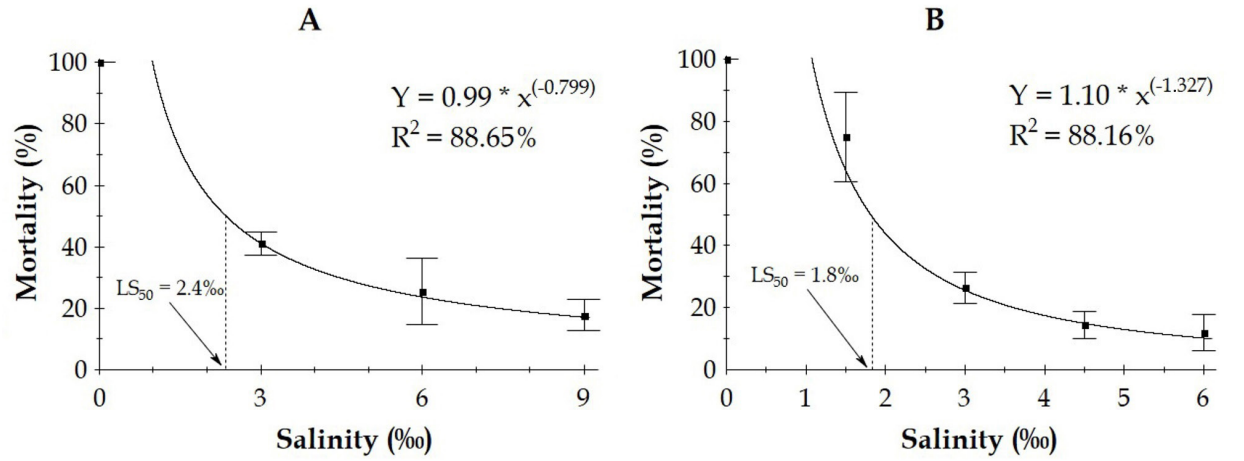

Figure 2. Regression analysis of *L. vannamei* mortality under hyposalinic stress. A – Juveniles. B – Postlarvae. LT_{50} = lethal salinity for 50% of the population.

3‰ and 1.5‰, respectively among the postlarvae. The pH of the marine water (32‰) varied between 7.6 and 7.8 before the stress.

DISCUSSION

Determination coefficients were high in all experiments, which mean a strong relation between mortalities and the analyzed parameters, reflected by an increase of the mortality when temperature and salinity decreased. Average mortality among juveniles increased 50% in the 1.5°C gap between 11.5°C and 13°C; the same gap between 10°C and 11.5°C treatments changed the average mortality to only 3%. In the first temperature range (11.5°C and 13°C), probably the limit to the *L. vannamei* tolerance to stress occurs. The regression analysis also indicated a LT_{50} in this interval, in which a small change in temperature can produce strong influence in the animal mortality, notwithstanding the fact that the LT_{50} is very close to the temperature that produced an average mortality of 70%.

Juveniles ceased moving when placed into cold water, but moved again when returned to the temperature of 28°C. Those alive started moving again few minutes after, and survived until the end of experimental period. This shows that those shrimps which did not survive returned already dead from hypothermic shock, or else died few minutes after the return.

Postlarvae showed an interval close to that observed in the juveniles, with LT_{50} (72) in 12.5°C. The same way as the juveniles, the postlarvae ceased moving when placed into cold water; the living ones starting to move soon after they were returned to the ambient temperature.

HOANG *et al*. (2002) calculated the critical thermal minima (CTMin) for *Penaeus merguiensis* at different acclimation temperature (15°C, 18°C, 21°C and 24°C) and cooling rate of 1°C and 3°C *per* hour. These last authors observed that both acclimation temperature and cooling rate influenced CTMin; higher acclimation temperatures showed higher CTMin, and the 1°C *per* hour cooling rate presented higher CTMin than the 3°C cooling rate suggesting that regarding the cold water tolerance for this species, the chronic effect is as important as the acute effect.

L. vannamei is apparently more sensible to low temperatures than other penaeids (KUMLU *et al*., 2010a). KUMLU *et al*. (2010b) established that the thermal tolerance zone for juveniles would be between 7.5°C and 11°C, being necessary to keep the water over 12°C to avoid mortality, corroborating with the results obtained by the present work, considering the use of a sudden variation regarding the juveniles.

Temperature has direct influence on crustaceans metabolism, producing strong effect on the growth and survival of penaeids shrimps (PARADO-ESTEPA, 1998). However, there are few studies related to the effects of low temperature on metabolism, and the most part of them being made in temperatures above 20°C (XINGQIANG *et al*., 2010). HOANG *et al*. (2002) and KUMLU and KIR (2005) suggest that the survival is not determined only by temperature, and that other factors could bring influence to the cold tolerance of the penaeid shrimps. Among these factors are the acclimation temperature, cooling rate, salinity and diet (CHIM *et al*., 2001; KIR and KUMLU, 2008a, 2008b). Temperature can affect physiological responses within the biokinetic range in the organisms (REYNOLDS, 1977). Low temperatures produce deleterious effect upon the structure and function of the crustaceans cell membranes, which: increases the membrane thickness; reduces the fluidity by changing the proportion of the saturated and unsaturated fatty acids, increasing unsaturation; affects the activity of the enzymes associated to the membrane and changes the proportion of the different phospholipid groups (PRUITT, 1990).

Regarding salinity, mortality in juveniles as well as postlarvae occurred only in treatments with zero salinity. In both cases, a sudden salinity change of 32‰ to 3‰ was not sufficient to cause death to 50% of the animals. The difference in the average mortality is lower between intervals with high salinity than intervals with low salinity, which results a nonlinear regression analysis. As for the juveniles, no treatment was made between 0‰ and 3‰, and an increase of almost 60% was observed between average mortalities of these treatments. Most of the juveniles that could not resist to the treatments died in the hours following the shock application.

As for the postlarvae, there was an increase of almost 50% in the mortality rate between treatments at 1.5‰ and 3‰, being limiting for survival. The calculated LS_{50} (72) remained close to 1.5‰ treatment, whose mortality rate was near 75%. At 6‰ salinity, on the other hand, mortality was very low. Even remaining in low salinity water for more time, postlarvae showed resistance to hyposaline stress higher than the juveniles. Osmoregulation in crustaceans develops from the last metamorphosis. In general, adults have lesser osmoregulation capacity (CHARMANTIER, 1998), while the postlarvae shrimps tend to quickly increase their resistance to hypo-osmotic conditions with their development (SAMOCHA *et al*., 1998).

The penaeid shrimps are known to be euryhaline species growing in a wide range of salinities. Juveniles of *Penaeus monodon* presents high survival rate when cultivated in salinity of 10‰ (YE *et al*., 2009). Postlarvae of *Farfantepenaeus subtilis* were transferred from 30‰ to low salinities without acclimation and the LS_{50} was estimated at 12‰ to 96 hours of exposure (SILVA *et al*., 2010). The shrimp *Farfantepenaeus paulensis* presented the LS_{50} of 13.3‰ and 10.3‰ for juveniles with 49 and 78 mm, respectively, after 24 hours of exposure (BARBIERI *et al*., 2014). But *L. vannamei* is more tolerant to low salinities and it has high survival rates when transferred from salinity 30‰ to salinities smaller than 5‰, even without acclimation (JAYASANKAR *et al*., 2009).

PAZ *et al*. (2011) found 35%, 43% and 72% survival rates for *L. vannamei* postlarvae at 20 days cultivation respectively in 1, 2 and 4‰ salinities for 48 hours. These data corroborate those obtained in the present work. Tolerance to saline stress increases with the postlarva age. Nevertheless, acclimation of postlarvae with age less than 15 days in salinities lower than 4‰ is not recommended, and older postlarvae can resist to 1‰ salinities for as much as two days (McGRAW *et al*., 2002). Regarding juveniles of the same species having approximately 7 grams, ZHANG *et al*. (2009) reported that the lethal salinity for 50% of the population was 7.02‰ in 24 hours of exposure, lower value than the one used in the present work. However, the rearing

temperature was 20°C, which could have reduced the resistance to hyposaline stress, since the resistance of the penaeids to low salinities seems to decrease at lower temperatures (TSUZUKI *et al*., 2000; HERNÁNDEZ-RODRÍGUEZ *et al*., 2006).

Among other factors that may influence the resistance of shrimp to low salinities are the density, pH, food composition, acclimation and concentration of ions such as potassium and magnesium (McGRAW *et al*., 2002; ARANEDA *et al*., 2008; ROY *et al*., 2007; HUAI *et al*., 2009; XIE *et al*., 2014). It is necessary to further explore the physiological mechanisms on the response to low salinity to understand some results regarding survival and growth (LI *et al*., 2008). *L. vannamei* isosmotic point occurs at 24.7‰ salinity (CASTILLE JUNIOR and LAWRENCE, 1981). Among crustaceans, osmoregulation is a complex physiological factor involving Na^+ and Cl^- ions, besides sodium-potassium pump. When exposed to low salinity, the body permeability decreases and the water excretion through renal organs increase, while organic osmolytes are regulated to reduce the osmotic pressure difference between the environment and hemolymph. The renal organ reabsorbs Na^+ and Cl^-, and divalent ions such as Mg^{2+}, Mn^{2-} e SO_4^{2-} are regulated by antennal gland (PÉQUEUX, 1995). The gill is an important organ responsible for the Na^+ e Cl^- osmoregulation, where the K^+ plays an essential role in this process (LUCU and TOWLE, 2003).

CONCLUSION

The lethal temperature to 50% of the *L. vannamei* is 11.7°C for the juveniles and 12.9°C for the postlarvae. The lethal salinity for 50% of the *L. vannamei*, is 2.4‰ for the juveniles and 1.8% for the postlarvae, in accordance with the methodology proposed in this experiment.

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