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Effects of water temperature on oxidative stress parameters in the pink shrimp *Farfantepenaeus brasiliensis* during transport

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ABSTRACT

Farfantepenaeus brasiliensis juveniles (5.53 ± 1.20 g) were subjected to different temperatures to evaluate the optimal temperature for transport. The shrimp were acclimated in a 4000 L tank at 22.4 °C and salinity of 22‰. The shrimp were then transported for 12 h at different temperatures (16.0, 19.3, 22.4 (control), 25.0 or 28.0 °C) in transparent plastic bags containing one-third water (10 L) and two-thirds pure oxygen, with three repetitions for each treatment. Analysis was performed at the beginning and the end of the transport. The hemolymph from five shrimp in each plastic bag was collected to measure catalase (CAT), glutathione-*S*-transferase (GST) and total antioxidant capacity against peroxyl radicals. The results showed that shrimp transported in lower temperatures presented higher antioxidant competence and the best water quality parameters. Therefore, the temperature we recommend for transporting *F. brasiliensis* is 19.3 °C, which enhanced activities of CAT and GST and provided better water quality conditions.

1. Introduction

Worldwide aquaculture production has grown substantially during the last two decades (FAO, 2010). The penaeid shrimp *Farfantepenaeus brasiliensis* is widely distributed from North Carolina (USA) to the coast of Rio Grande do Sul (D'Incao, 1995, 1999) and lives in a broad range of water temperatures. In Brazil, it is one of the most common species in the live bait trade, especially in the coastal states of Rio de Janeiro, São Paulo, Paraná and Santa Catarina (Beccato, 2009; Preto et al., 2009).

The transport and storage of live crustaceans are becoming increasingly common and have been made possible through technological advances such as specially designed tanks and transport vehicles equipped with aeration and/or oxygenation inputs (Fotedar and Evans, 2011). Moreover, aquatic animals in Brazil are frequently transported in plastic bags (Golombieski et al., 2003).

The transportation of live organisms generally does not take into account the animal's health and welfare and, in most cases, results in high mortality. This mortality occurs because transport is a traumatic procedure involving a succession of adverse stimuli, including the capture, transport and storage of the animals (National Research Council, 2006; Robertson et al., 1988). Moreover, transportation for long periods may result in water quality deterioration, which can be exacerbated by increases in the temperature and in the ammonia excretion rate in a closed system (Golombieski et al., 2003; Jiang et al., 2000; Teo et al., 1989).

The effects of environmental stressors are also studied in aquatic organisms to elucidate the oxidative damage and physiological effects resulting from transport. Several techniques have been developed to maximize health and survival and to reduce stress during transport, including the addition of cold water inside the plastic bags (Fotedar and Evans, 2011).

Stress responses have been studied in many vertebrate and invertebrate species to identify the mechanisms involved (Barton, 2002; Barton and Iwama, 1991; Buchanan, 2000; Moberg, 1985; Selye, 1973). In general, crustaceans vary their tolerance to environmental perturbations (e.g., temperature fluctuations, high levels of ammonia and varying salinity). Therefore, the recommended practices are species specific (Fotedar and Evans, 2011). However, no literature is available for *F. brasiliensis* concerning oxidative stress and antioxidant systems.

Various protocols have been established to provide technological guidelines in the handling and transportation of live crustaceans (APEC, 1999; Aquatic Animal Welfare Guidelines, 2005; Codex Alimentarius, 1983). Contradictory results have been presented concerning the capacity of crustaceans to feel pain (Sample, 2007); however, recent reports suggest that crustaceans do, indeed, feel pain (Barr et al., 2008; Parodi et al., 2012; RSPCA, 2001; Yue, 2009). Although

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they do not have the classical pain receptors of higher order vertebrates, it is nevertheless important to minimize the possibility of pain by maintaining good husbandry and care (Bennison, 2000).

Temperature is a controlling factor in the physiology of poikilothermic species (Dove et al., 2005), and in temperate regions like the South of Brazil, water temperatures range from 12 °C in the winter to 28 °C in the summer (Garcia et al., 2008). Water temperature changes can stress living organisms and adversely affect their health (Yu et al., 2009). Stress induced by water temperature changes has also been associated with enhanced generation of reactive oxygen species (ROS) and oxidative stress (Lushchak and Bagnyukova, 2006a).

Oxidative stress is defined as an imbalanced state between elevated concentrations of pro-oxidants over antioxidants that results in the generation of ROS (Matés et al., 1999). ROS are naturally produced during oxidative metabolism. These ROS include superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (HO), and singlet oxygen (Livingstone, 2001). The antioxidant defense system of aerobic organisms includes enzymes such as catalase (CAT) and glutathione-*S*-transferase (GST), which are important for ROS scavenging and/or the prevention of oxidative damage.

Catalase is involved in the detoxification of hydrogen peroxide (H_2O_2) (Guemouri et al., 1991). Glutathione-*S*-transferases are a family of dimeric multifunctional enzymes involved in several processes including: (1) detoxification of xenobiotics; (2) protection from oxidative damage; and (3) intracellular transport of hormones, endogenous metabolites and exogenous chemicals in diverse organisms (Eaton and Bammler, 1999; Frova, 2006; Goto et al., 2009; Sheehan et al., 2001).

Total antioxidant scavenging capacity assay against peroxyl radicals quantifies the capacity of animals to neutralize ROS, thus providing an index of biological resistance to oxidative stress (Winston et al., 1998). This assay has been used to evaluate the ROS scavenging capacity of several marine invertebrate species (Regoli and Winston, 1998; Regoli et al., 2000). In recent years, a new technique based on the fluorescence detection of ROS using a peroxyl radical generator has been proposed (Amado et al., 2009). The main objective of this study was to evaluate the best temperature for transporting *F. brasiliensis* in terms of antioxidant competence and water quality parameters.

2. Material and methods

2.1. Experimental design

F. brasiliensis juveniles (5.53 ± 1.20 g) were obtained from the Marine Station of Aquaculture, Universidade Federal do Rio Grande — FURG, Rio Grande do Sul State (RS), Southern Brazil. Shrimp were acclimated in tanks with 4000 L of filtered water at 22.4 °C and 22‰ salinity. After acclimatization, the animals were transported for 12 h at five different temperatures (16.0, 19.3, 22.4, 25.0 or 28.0 °C), with three replicates of each treatment. The control temperature was that of the sample collected before starting the experiment at initial time (22.4 °C). Feeding was suspended 12 h before beginning the experiment. Every 30 min the plastic bags of all treatments except the initial time were scrambled to simulate the vehicle movement.

The shrimp were transported in 50 L transparent plastic bags at a density of three shrimp per liter (30 shrimp per bag) containing one-third water (10 L), two-thirds pure oxygen and tied with rubber strings. The water temperature inside the plastic bags was slowly adjusted in tanks to match the temperature tested (16.0 ± 0.2, 19.3 ± 0.3, 22.4 ± 0.2, 25.0 ± 0.3 or 28.0 ± 0.4 °C), which was maintained until the end of transport. To maintain the different water temperatures, five 200 L tanks (useful volume 120 L) containing the experimental units were used as water baths. The water in these tanks (where the plastic bags remained during the experimental period) was heated by submerged heaters with thermostats and cooled with ice. The experiment started, after 3 h, when all treatments reach the temperature tested. Moreover, it was repeated three times. Each tank corresponds to one treatment that received one plastic bag. Every 12 h the samples were collected (five shrimp per treatment) and the plastic bags with other animals were changed, in order to replicate the experimental conditions.

Before sealing, a transparent hose was inserted into the bags through which 60 mL water samples were collected every 2 h to monitor the water quality during transport. The water quality parameters (temperature, salinity, dissolved oxygen and pH) were monitored every 2 h using a multi-parameter YSI 556 (Yellow Springs Instruments, Yellow Springs, OH, USA). Total ammonia and alkalinity were also monitored every 2 h according to the methods of UNESCO (1983) and Baumgarten et al. (1996), respectively.

2.2. Enzymatic assay

The hemolymph of five shrimp from each plastic bag was collected at the beginning (initial time) and at the end of transport. The samples were obtained directly from the heart of each shrimp using sterile syringes containing an anticoagulant solution (glucose 0.1 M, sodium citrate 30 M, citric acid 0.026 M, NaCl 0.45 M, EDTA 0.02 M and pH adjusted to 7.4) (Söderhall and Smith, 1983), transferred to 1.5 mL polyethylene tubes and stored at −80 °C in an ultra-freezer. For protein quantification and antioxidant enzyme analysis, the hemolymph was centrifuged twice at 500 and 900 ×*g* at 4 °C for 35 and 15 min, respectively, to obtain a pellet. After centrifugation, the pellet was re-suspended in a cold 4 °C buffer solution containing Tris base (20×10^{-3} M), EDTA (1×10^{-3} M), dithiothreitol (1×10^{-3} M), KCl (150×10^{-3} M), and PMSF (0.1×10^{-3} M), with the pH adjusted to 7.6. All biochemical determinations were performed at least in triplicate.

The total antioxidant capacity against peroxyl radicals was analyzed by quantifying ROS in the hemolymph as described by Amado et al. (2009). The catalase and GST activity measurements were based on McCord and Fridovich (1969) and Habig et al. (1974), respectively.

All results were expressed as enzyme units except the total antioxidant capacity, which was expressed as the relative areas between the difference in the same sample with and without the addition of 2,2′-azobis-2-methylpropionamidine dihydrochloride (ABAP) and standardized to the ROS area without ABAP (background area). The relative difference between the ROS area with and without ABAP was considered a measurement of the total antioxidant capacity. A large area difference corresponded to a low antioxidant capacity because high fluorescence levels were obtained after the addition of ABAP, indicating low competency in neutralizing peroxyl radicals. The total fluorescence production was calculated by integrating the fluorescence units (FU) along the time (30 min) and measurement after adjusting the FU data to a second order polynomial function (Amado et al., 2009).

One CAT unit represents the amount of enzyme needed to degrade 1 μmol of H_2O_2 per min and per mg of total proteins present in the homogenates, at 30 °C and pH 8.00. One GST unit is the amount of enzyme necessary to conjugate 1 μmol of 1-chloro-2,4-dinitrobenzene (Sigma-Aldrich) per min and per mg of total protein present in the homogenates, at 25 °C and pH 7.00.

2.3. Statistical analysis

The statistics were expressed as the mean ± standard error. Assumptions of normality and homogeneity were previously verified (Levene test). All tests of enzyme activities and water quality parameters were performed by analysis of variance (one-way ANOVA) followed by Newman–Keuls post-hoc comparisons with a significance level of 0.05.

3. Results

3.1. Water quality and survival

The physicochemical water quality parameters are shown in Table 1. Water quality parameters analyzed during the experimental period