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Lipid peroxidation and antioxidant capacity in *Peckoltia oligospila* (Günther, 1864) submitted to transport under different concentration of dissolved oxygen

Thamyres Vanessa Nascimento da Silva[a], Luis André Luz Barbas[b], Marcelo Ferreira Torres[b], Luís André Sampaio[c], José María Monserrat[a,d,*]

[a] Laboratório de Bioquímica Funcional de Organismos Aquáticos (BIFOA), Pós-Graduação em Aquicultura, Universidade Federal do Rio Grande - FURG, Instituto de Oceanografia, CP 474, 96201-900 Rio Grande, RS, Brazil
[b] Laboratório de Aquacultura de Espécies Tropicais, Instituto Federal de Educação, Ciência e Tecnologia do Pará - IFPA Castanhal, 68740-970 Castanhal, PA, Brazil
[c] Laboratório de Piscicultura Estuarina e Marinha, Programa de Pós-Graduação em Aquicultura, Universidade Federal do Rio Grande - FURG, Instituto de Oceanografia, CP 474, 96201-900 Rio Grande, RS, Brazil
[d] Instituto de Ciências Biológicas (ICB), FURG, Brazil

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ABSTRACT

It was analyzed antioxidant competence and lipid peroxidation in juveniles of *Peckoltia oligospila* submitted to transport in plastic bags under different concentrations of dissolved oxygen for up to 24 h. Oxidative balance of fish transported for 3, 6, 12, and 24 h in normoxic (NX), moderate hyperoxia (MH), and severe hyperoxia (SH) was evaluated. For this, it was determined the total antioxidant capacity against peroxyl radicals (ACAP) and lipid peroxidation levels (TBARS) in liver, gills, muscle, and brain. After 24 h and, regardless of water quality, dissolved oxygen concentrations, and duration of transport, no mortality occurred for any of the treatments. The antioxidant competence of *P. oligospila* in the different organs remained high, except for some occasional decrements. Because of this, TBARS concentration remained low in the different organs, irrespective of transport time or initial oxygen concentrations. According to the results, *P. oligospila* antioxidant system was efficient enough for the prevention and/or suppression of products derived from lipid peroxidation. Therefore, the oxidative status was not severely affected in the assessed organs over transport time in hyperoxic conditions. Furthermore, *Peckoltia oligospila* can be safely transported in plastic bags without an extra supply of oxygen for up to 24 h, since survival was not affected.

1. Introduction

Brazil is home to a wide variety of freshwater fish (Buckup et al., 2007) and ranks at the 9th position among the most important exporter countries of ornamental fish (ONU, 2014). The Brazilian Amazon basin is one of the main areas from which valuable commercial ornamental fish species are captured and subsequently shipped to distribution centres worldwide, mainly to the United States, Japan, and Germany (Chao, 2001).

Popularly known as "bola pleco", *Peckoltia oligospila* fish belongs to the Loricariidae family, and is distributed in South America, notably in the draining of Tocantins (3° 57′ 26.715″ S and 49° 36′ 41.44″ W), Capim (1° 44′ 45.129″ S and 47° 47′ 34.55″ W), and Guamá (1° 31′ 59.147″ S and 48° 3′ 21.296″ W) rivers (Armbruster, 2008). Colours and morphology of Loricariidae are appealing features to the ornamental fish market (Ramos et al., 2013). *P. oligospila* inhabits tropical freshwater environments, with pH ranging from 6.5 to 7.2, is an omnivorous species, feeding preferentially on wood chips, microalgae, and small aquatic invertebrates (Fisch-Muller, 2003).

The shipping of live fish is frequently carried out in closed systems, such as double-layered polyethylene bags, filled with a third of its volume with water and two-thirds with pure oxygen. After sealed, bags are placed inside polystyrene boxes to protect fish from light and prevent changes in temperature during the transport (Berka, 1986; Lim et al., 2003). High mortality rates after transport have been associated with exposure to a variety of stressors, such as handling, packaging, loading, and unloading (Dhanasiri et al., 2013; Manuel et al., 2013; Tacchi et al., 2015). Moreover, long-term transport as well stoking

* Corresponding author at: Laboratório de Bioquímica Funcional de Organismos Aquáticos (BIFOA), Centro de Biotecnologia e Doenças de Organismos Aquáticos (CBD), Universidade Federal do Rio Grande - FURG, CP 474, 96201-900 Rio Grande, RS, Brazil.
E-mail address: monserrat_jm@furg.br (J.M. Monserrat).

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density impacts water quality, which is especially aggravated by gradual decreases in dissolved oxygen concentrations and the build-up of carbon dioxide (CO_2) and ammonia (Harmon, 2009; Sampaio and Freire, 2016).

Supersaturating water with oxygen guarantees the supply of oxygen for long periods of transport (Harmon, 2009). On the other hand, empirical protocols for the transport of live fish are frequently used, whereby injection of pure oxygen is performed without a systematic and qualified methodology. These aspects constitute potential problems for the shipping of live fish and producers are not aware of the physiological consequences of hyperoxia (Ritola et al., 2002; Hermes-Lima and Zenteno-Savín, 2002; Hermes-Lima, 2004).

Studies on the deleterious effects of water temperature and dissolved oxygen on physiological responses and oxidative balance of aquatic organisms submitted to transport indicate that a species-specific approach must be used for the elaboration of transport protocols (Bubner et al., 2009; Souza et al., 2013). However, reports on the oxidative status in fish affected by hyperoxia are rather limited, mainly with regard to transport in hyperoxic water (Lushchak and Bagnyukova, 2006; Barbas et al., 2017). A few studies, however, indicate that exposure to hyperoxia up to 180% saturation generates a pro-oxidant condition that induces antioxidant enzymes activity either during hyperoxia or after the return to normoxia (Lushchak et al., 2005; Lushchak and Bagnyukova, 2006).

The onset of oxidative stress occurs because of the unbalance between the concentrations of pro-oxidants (including reactive oxygen species - ROS) and antioxidants, causing damage to macromolecules (Lushchak, 2014; Sies, 2015). During aerobic metabolism, ROS are produced by cells via reductive reactions of molecular oxygen (O_2), accompanied by simultaneous generation of several toxic and reactive intermediates, namely radical superoxide ($O_2^{\bullet-}$), hydroxyl radicals ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2) (Hermes-Lima, 2004). Therefore, to minimize the deleterious effects of ROS, aerobic organisms have developed both enzymatic and non-enzymatic antioxidant defence systems for the elimination of ROS and/or oxidative damage prevention (Urso and Clarkson, 2003; Comporti, 2010).

Both antioxidant systems form complex defence webs (Lushchak, 2011), in which some enzymes, e.g. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase (GST) are known to have major antioxidant activity (Hermes-Lima, 2004; Vasconcelos et al., 2007). The non-enzymatic protection system comprises dietary substances, such as α-tocopherol, ascorbic acid, β-carotene, flavonoids and lipoic acid, that enhance protection of tissues against ROS (Lykkesfeldt and Svendsen, 2007; Lobo et al., 2010; Kütter et al., 2014).

Measurements of antioxidant activity, e.g. through assessment of total antioxidant scavenging capacity against peroxyl radicals, quantifies the organism capacity to neutralize this type of ROS (Amado et al., 2009). Nevertheless, when ROS production progresses beyond the preventive and detoxifying capacity of the antioxidant system (Halliwell and Gutteridge, 2015), molecules are oxidized and loss of homeostatic regulation with loss of biological functions take place, resulting in lipid peroxidation and oxidized proteins and deoxyribonucleic acid (DNA) (Jones, 2006; Dogan et al., 2011; Lushchak, 2011; Vinagre et al., 2012). Lipid peroxidation generates important consequences for biological systems. Polyunsaturated fatty acids, for example, are the most susceptible to oxidation when excessive production of ROS occurs. The intensity of lipid peroxidation is determined by measuring levels of primary products, conjugated dienes and lipid peroxides, and/or by-products of lipid peroxidation, such as malondialdehyde and other aldehydes, which are assayed with thiobarbituric acid and expressed as thiobarbituric acid-reactive substances (TBARS) (Lushchak and Bagnyukova, 2006; Halliwell and Gutteridge, 2015).

Oxidative status of *P. oligospila* exposed to hyperoxia during transport in closed systems is still unknown. In this study, we hypothesized that the supersaturation of water of transport using a pure oxygen-supply protocol, will render the environment pro-oxidant, predisposing the animals to suffer with increased ROS, with consequent lipid damage, this response being dependent of the antioxidant competence of each organ. Thus, the objective of this study was to investigate total antioxidant capacity and lipid peroxidation in organs of juvenile *P. oligospila* submitted to different transport times in plastic bags, under distinct initial concentrations of dissolved oxygen in the water for up to 24 h. Additionally, parameters of water quality post-transport were also evaluated.

2. Material and methods

2.1. Experimental animals and design

P. oligospila juveniles (30.39 ± 3.70 g) were obtained from a trade company of ornamental fish in the State of Pará, Northern Brazil and were acclimated in 60 L tanks. Filtered water, under constant aeration was maintained under the following conditions: 27.5 ± 1.2 °C (temperature), 7.53 ± 0.13 mg O_2 L^{-1} (dissolved oxygen), 20.53 ± 0.91 mg CO_2 L^{-1}, 6.5 ± 0.8 (pH), 18.33 ± 3.33 mg $CaCO_3$ L^{-1} (alkalinity), and 18 ± 2.0 mg $CaCO_3$ L^{-1} (hardness). After acclimation, trials were carried out and treatments consisted of fish being transported for 3, 6, 12, and 24 h under moderate hyperoxia (MH), severe hyperoxia (SH), and normoxia (NX) (control group). Concentrations of dissolved oxygen were classified based on the solubility of oxygen in the water as described by Boyd and Tucker (1998). All treatments were performed in triplicate and prior to the beginning of the experiment, fish were fasted for 24 h. The methodologies applied in this experiment were approved by the Ethical and Animal Welfare Committee of the Universidade Federal do Rio Grande - FURG (protocol number Pq007/2015).

For the transport trials, 30 L transparent plastic bags were used and filled with 3 L of water from the acclimation tanks. Fish were packed at a density of two fish per litre, i.e., 6 fish per bag (60 g/L), which corresponds to the density used for the transport of this species in the Amazon region. Different dissolved oxygen (DO) concentrations were injected into the plastic bags according to preliminary tests, which allowed for the setting-up of a standardized methodology. Following oxygen injection, bags were sealed with rubber strings and placed in polystyrene boxes (170 L), after which were loaded on a motor vehicle for transportation. Every 60 min were traveled around 4 km by car, to simulate a transport situation. During the whole experiment, a total of 24 trips were conducted. Three bags for each oxygen concentration and transport time were employed (18 fish). Biochemical measurements (see below) were performed on 15 fish for each treatment.

Three DO concentrations were tested (see below) for the treatments NX, MH and SH, respectively. To achieve the desired DO concentrations in the transport water, pure oxygen and/or atmospheric air were injected (air compressor Vortex Blower - Hailea 290G, maximum flow: 350 L/min, max pressure: 0.009 MPa) into the plastic bags as follows (data expressed as mean ± 1 standard error of the mean):

a) NX (OD = 7.66 ± 0.15 mg L^{-1}): 3 L of water and the remaining volume of the bags filled with atmospheric air (n = 10).
b) MH (OD = 12.42 ± 0.97 mg L^{-1}): 3 L of water followed by the injection of pure oxygen and atmospheric air at a ratio of 1:2 (5 and 8 s for the injection of pure oxygen and atmospheric air, respectively) (n = 10).
c) SH (OD = 23.35 ± 0.8 mg L^{-1}): 3 L of water followed by the injection of atmospheric air and pure oxygen at a ratio of 1:2 (5 and 8 s for the injection of atmospheric air and pure oxygen, respectively) (n = 10).

Preliminary test were conducted to train and standardize the three different oxygen concentrations employed in the experiment. After bags were sealed and 10 min had elapsed, DO was measured with a portable