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Euryhalinity of the estuarine copepod *Pseudodiaptomus richardi* and its high potential to be employed as live food in aquaculture

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ABSTRACT

The use of copepods in aquaculture has been raising great interest in the last years, especially as live food for larviculture of marine/estuarine fish. The copepod *Pseudodiaptomus richardi* is a euryhaline species, extremely promising for culture in a vast array of salinities. The survival of *P. richardi* was thus here evaluated 24 h after its abrupt transfer to higher salinities (15 and 30), from control salinity 5. Besides survival, body fluid osmolality and body hydration were also evaluated upon these same hyper-osmotic shocks, but after 5 and 30 min, 1 and 24 h. The whole body activity of the Na^+/K^+-ATPase was evaluated after 48 h of acclimation to these same salinities. Adult copepods displayed 100% survival upon both hyper-osmotic shocks after 24 h; copepodites and nauplii had some mortality in salinity 30 (16–22%). Body hydration decreased in both salinities (78 to 66–70%). In salinity 15 body hydration was maintained after 5 min, and recovered after 24 h. In the highest salinity (30) the hyper-osmotic shock induced immediate (5 min) water loss, coherent with their loss of swimming activity, and there was no trend of recovery, even after 24 h. The reduction in body hydration does not necessarily reflect volume reduction, but increases in dry mass, from salt uptake. Copepods transiently lose water when submitted to salinity 30, quickly showing a decrease in body hydration – given the strong outward osmotic gradient – but probably recover volume after intra-and extra-cellular solute (inorganic and organic) concentrations increase, and water uptake. This result is consistent with their recovery of normal swimming behavior after a few hours in salinity 30. Body fluid osmolality at all salinities was always ~200–300 mOsm/kg H_2O above water osmolality. Na^+/K^+-ATPase specific activity of adult copepods acclimated to the salinities of 5, 15, or 30 was stable, of ~2 nmol ADP/μg protein·h. *P. richardi* is thus a hyper-conformer which keeps a constant rate of salt uptake to remain hyper-osmotic at all salinities. In conclusion, it can be safely used as live food for fish larvae grown in salinities 5–30. In salinities approaching full-strength seawater, it shows a transient osmoregulatory disturbance when abruptly exposed, but it is fully able to acclimate, survive and reproduce even in these higher salinities.

1. Introduction

Aquaculture is growing drastically in production rates globally, especially in developing countries, with the perspective of overcoming more traditional animal protein production sources in the next decades (FAO, 2012). However, marine aquaculture productivity (19.3 million tons) is still well below the productivity reached by inland aquaculture (44.3 million tons, FAO, 2012). One of the reasons for this relatively low throughput is the reduced success of larviculture using traditional live food, rotifers and *Artemia* spp. These organisms do not meet the nutritional needs of most cultured marine fish (Schipp et al., 1999; Støttrup, 2000, 2003).

Copepods seem to be a valuable alternative, as they are a relevant part of the natural diet of larvae of many species of fishes and invertebrates (Chew and Chong, 2011; Hobbs et al., 2006). Copepods are nutritionally very complete in terms of amounts and proportions of polyunsaturated fatty acids (Drillet et al., 2006; McKinnon et al., 2003; Sargent et al., 1997; Watanabe et al., 1983), and display swimming activity which attracts the attention of fish larvae (Buttino et al., 2012; Delbare et al., 1996; Olivotto et al., 2008, 2009; Payne et al., 2001). Successful larviculture of several fish species has fostered increased interest in the use of copepods as live food in the last years (Barroso et al., 2013; Payne et al., 2001; Schipp et al., 1999).

Among Copepoda, the order Calanoida is especially relevant for this purpose of serving as live food in fish culture systems, as they are predominantly holoplanktonic, remaining constantly available to the larvae (Støttrup et al., 1986). However, despite the growing interest in the use of copepods in marine pisciculture, the studies reporting optimum conditions for their intensive production are still scarce, and detailed and

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complete protocols for their use are still not available, actually as for most copepod species (Ajiboye et al., 2011; Drillet et al., 2011).

One aspect that hinders copepod culture is the scarce knowledge on the physiological requirements of coastal species. The main parameter to be considered is salinity, which is especially variable in coastal areas, and which strongly affects survival, feeding, swimming, and reproduction of aquatic organisms in general, and copepods in particular (Calliari et al., 2008; Castro-Longoria, 2003; Cervetto et al., 1999; Chen et al., 2006; Peck and Holste, 2006; Tester and Turner, 1991). More specifically, variations in salinity affect ammonia excretion rates (Farmer and Reeve, 1978), the energetic cost of growth (Calliari et al., 2006), thus being highly relevant for the production and maintenance in large scale of these organisms.

The copepod *Pseudodiaptomus richardi* is reported to be euryhaline and eurythermic, thus being vastly distributed in tropical and subtropical coastal and estuarine regions of the globe (Kaminski et al., 2009; Lopes, 1994; Magalhães et al., 2006; Montú, 1980; Montú and Gloeden, 1986). The species occurs in various estuarine regions of Brazil, such as, for example: the Patos Lagoon estuary (Kaminski et al., 2009; Montú, 1980; Montú and Gloeden, 1986), Paranaguá Bay estuarine complex (Lopes et al., 1998) in Southern Brazil; Guaraú river estuary in Southeastern Brazil (Lopes, 1994), and Caeté river estuary — Amazon Region in Northern Brazil (Magalhães et al., 2006). The species has been shown to withstand high stocking densities, displaying rapid population increment under intensive culture conditions, showing viability for aquaculture applications (Kaminski et al., 2007). However, basic knowledge on its physiology, especially with respect to salinity, is still poor. Such knowledge will certainly foster the development of detailed protocols for the culture of this copepod and its use as live food for fish larvae, especially estuarine fish or marine fish that breed in brackish water.

Copepods go through several developmental stages, performing successive molts until they reach the adult stage, when molting ceases. They display 12 stages, divided into 3 developmental phases: 6 nauplii stages, 5 copepodite stages, and the adult stage (Björnberg, 1981; Mauchline, 1998). The developmental stages of *P. richardi* have been investigated by Kaminski et al. (2009). Females carry their eggs and liberate nauplii to the water. Nauplii are 100–192 μm wide (average of ~132 μm), and 183–450 μm long (average of ~318 μm); copepodites are 133–350 μm wide (average of ~236 μm), and 350–950 μm long (average of ~624 μm); adults are 242–433 μm wide (average of ~329 μm) and 709–1108 μm long (average of ~913 μm). Given these size differences along development, copepods can be potentially used in distinct phases of development of fish larvae: nauplii can be offered as the first feed of small fish larvae, while copepodites and adults can be offered to more developed and larger larvae, as a supplement or replacement for the use of rotifers and *Artemia*, respectively.

The occupation of dilute waters by crustaceans is associated with a high activity of the Na^+,K^+-ATPase in interface epithelia, and a good osmoregulatory capacity, also termed "anisosmotic extracellular regulation" (Freire et al., 2008b; Kirschner, 1991; Péqueux, 1995). Animals that live in freshwater, as a rule, need another "pump" to power salt uptake, the V-H^+-ATPase, thus being named "strong osmoregulators" (Charmantier et al., 2009; Freire et al., 2008a; Kirschner, 1991, 2004). Marine osmoconformers, in their turn, do not hold significant osmotic gradients between the extracellular fluid and the external medium, and thus display very low Na^+,K^+-ATPase expression and activity (Charmantier et al., 2009; Foster et al., 2010; Kirschner, 1991; Péqueux, 1995). Besides the capacity for anisosmotic extracellular regulation, survival in an osmotically challenging environment is also strongly dependent on the capacity for isosmotic extracellular regulation, or cell volume regulation (Charmantier et al., 2009; Foster et al., 2010; Freire et al., 2008b).

This study aimed at gathering physiological information about *P. richardi*: survival, fluid osmolality, body water content, and Na^+, K^+-ATPase activity, in its habitat salinity (5), and in increased salinities (15 and 30), values commonly used in the culture of marine and estuarine species.

2. Materials and methods

2.1. Establishment of stock cultures

2.1.1. Microalgae culture

Microalgae cultures were established in order to feed the copepods, thus assuring a good nourishing of the cultivated animals. Inoculi of the microalgae *Chaetoceros muelleri* and *Isochrysis galbana* were obtained from the Center of Production and Propagation of Marine Organisms (CPPOM, Guaratuba, Paraná, Brazil), and those of *Thalassiosira weissflogii*, from the Laboratory of Phytoplankton and Marine Microorganisms of the Federal University of Rio Grande (FURG, Rio Grande, Rio Grande do Sul, Brazil, www.aquicultura.furg.br/index.php/pesquisa/laboratorios/210-laboratorio-de-ecologia-de-fitoplancton-e-de-microorganismos-marinhos.html). Microalgae were replicated according to the *Batch* culture method (FAO, 1996; Guillard, 1975), and were used to feed the copepods, both in stocks and during the salinity change experiments. Microalgae which were used to feed copepods were undergoing their exponential growth phase (FAO, 1996; Lourenço, 2006).

2.1.2. Copepod culture

Copepods were collected through horizontal surface tows using 50-cm diameter, 300 μm mesh zooplankton nets. This mesh size was chosen because it favors the capture of adult copepods, making it easier to sort and identify the species to be used. Hauls were performed in the estuary of Paranaguá Bay, in water of salinity ~5, next to the mouth of the Guaraguaçu River (Fig. 1). The collected material retained in the net cod end was immediately transferred to two 20-liter gallons with whole water from the site and was then taken, along with two extra 20-liter gallons containing only whole water, to the laboratory (~1-hour speedboat ride).

Upon arrival in the laboratory, the whole water was filtered (0.45 μm) and transferred to clean gallons. After that, hundreds of adult individuals (males and females) of *P. richardi* were identified and sorted under a stereomicroscope and then stocked in four 20-liter culture tanks containing filtered whole water (salinity 5). These copepods were daily fed a mixture of the three cultivated microalgae, which were offered in excess. Appropriate volumes of the algae cultures were offered to the copepods, sufficient to keep minimum concentrations of 80,000 cells/mL of *I. galbana* (∅ 4.7 μm), 70,000 cells/mL of *C. muelleri* (∅ 7.3 μm), and 5000 cells/mL of *T. weissflogii* (∅ 13.2 μm). The goal was to reach approximately 1 mg C/L, a concentration considered sufficient to assure a good nutritional status to copepods (Kiørboe et al., 1985). The algae densities were calculated using the equivalent biovolume approach (Hillebrand et al., 1999). Continuous and gentle aeration was provided. The tanks were kept in an experimental room with fixed temperature (20 ± 2 °C) and natural photoperiod (~12 h light:12 h dark). Thousands of nauplii released 24 h later were then separated using a 200 μm mesh and were transferred to new 20-liter tanks where they were kept at the same conditions for 12–15 days, when they reached adulthood. These individuals constituted the reproduction tanks, which originated several other batches of nauplii, which, in their turn, after growing, were then used in the experiments. For each sampling event in the field (total of five events), 4–6 stock/reproduction tanks were prepared, each one generating 4–5 new batches of copepods which were used in the experiments. Adults collected from the environment were never used, only their offspring. The batches of nauplii obtained from the stock/reproduction tanks were observed daily, for quick detection of the last molt at the last stage of copepodites to adults. Adults and last-stage copepodites (copepodites V) were then concentrated and sorted to be used in the experiments.