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Salinity and temperature variations reflecting on cellular PCNA, IGF-I and II expressions, body growth and muscle cellularity of a freshwater fish larvae

Y.S. Martins [a], R.M.C. Melo [a], P.H.A. Campos-Junior [a], J.C.E. Santos [b], R.K. Luz [c], E. Rizzo [a], N. Bazzoli [d,*]

[a] Departamento de Morfologia, Instituo de Ciências Biológicas, Universidade Federal de Minas Gerais, P.O. Box 486, Antônio Carlos 6627, Belo Horizonte, MG 30161-970, Brazil
[b] Hidrobiology and Hatchery Station of Três Marias CODEVASF, P.O. Box 11, Três Marias, MG 39205-000, Brazil
[c] Laboratório de Aquacultura da Escola de Veterinária da Universidade Federal de Minas Gerais, P.O. Box 567, Antônio Carlos 6627, Belo Horizonte, MG 30123970, Brazil
[d] Programa de Pós-Graduação em Zoologia de Vertebrados da PUC Minas, 500, prédio 41, Belo Horizonte, MG 30525-610, Brazil

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ABSTRACT

The present study assessed the influence of salinity and temperature on body growth and on muscle cellularity of *Lophiosilurus alexaxdri* vitelinic larvae. Slightly salted environments negatively influenced body growth of freshwater fish larvae and we observed that those conditions notably act as an environmental influencer on muscle growth and on local expression of hypertrophia and hypeplasia markers (IGFs and PCNA). Furthermore, we could see that salinity tolerance for NaCl 4 g l^{-1} diminishes with increasing temperature, evidenced by variation in body and muscle growth, and by irregular morphology of the lateral skeletal muscle of larvae. We saw that an increase of both PCNA and autocrine IGF-II are correlated to an increase in fibre numbers and fibre diameter as the temperature increases and salinity diminishes. On the other hand, autocrine IGF-I follows the opposite way to the other biological parameters assessed, increasing as salinity increases and temperature diminishes, showing that this protein did not participate in muscle cellularity, but participating in molecular/cellular repair. Therefore, slightly salted environments may provide adverse conditions that cause some obstacles to somatic growth of this species, suggesting some osmotic expenditure with a salinity increment.

1. Introduction

Larval development is remarkable because it is a critical stage in fish culture. This life period is more susceptible to pathogenic infections, contaminants and environmental changes than other stages of development and growth. Thus, some therapeutic products have been applied to the larviculture of freshwater fish to ensure better survival and growth rates than in natural conditions. Of them, common salt (NaCl) is remarkable because it does not show toxic properties at low concentrations to both the environment and fish. In larviculture, treatment with slightly salted freshwater (1–12 g NaCl l^{-1}) has permitted a higher survival rate for larvae of some freshwater fish species than larvae reared in non-salted freshwater (Likongue et al., 1996; Wang et al., 1997; Borode et al., 2002; Beux and Zaniboni Filho, 2007; Luz and Portella, 2002; Luz et al., 2008; Luz and Santos, 2008a,b). Moreover, these treatments provided improvement to energetic expenditure and hormonal balance during larval development (Boeuf and Payan, 2001), and they are recommended for decreasing osmotic stress between internal fish and external environments during transport of fingerlings and young fish (Carneiro et al., 2007). Those hormones that act in somototrophic axis such as TH, GH, IGF group, prolactin, cortisol, among others are also important in osmorregulation process and they are sensible to salinity variation of external environment. Besides, food conversion capacity is influenced by external salinity concentration (Boeuf and Payan, 2001). Those thresholds and trade-off experienced by fish due to hormonal balance and external and internal NaCl levels have complex molecular and physiological connections that changes between developmental stage, environment and species thus acting on survival and growth, and this subject is over studied by aquaculture science due to its economic importance (Deane and Woo, 2009). In fish, somatic growth is directly also correlated to temperature variation (Deane and Woo, 2009). Therefore, information on species-specific salinity tolerances and their interaction with other environmental parameters during ontogenetic development is useful for maximising growth rates, conditioning and development rates (O'neil et al., 2011).

* Corresponding author.
E-mail address: bazzoli@pucminas.br (N. Bazzoli).

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The skeletal muscle is a post-mitotic tissue, and its post-embryonic growth is dependent on mononuclear myogenic progenitor cells that proliferate and differentiate to provide a source of nuclei for myotube formation and proliferation (Koumans and Akster, 1995). Muscle cells assessed in starved fish have been shown to differ extensively in terms of their size, morphology and proliferation rate to those extracted from fed fishes (Fauconneau and Paboeuf, 2000). The number and size of fibres is referred to as muscle cellularity and this is thought to be an important determinant of characteristics of flesh texture (Johnston, 1999; Fauconneau and Paboeuf, 2000). Perhaps, the main interest in muscle growth from an aquaculture perspective is the genotypic and phenotypic variation observed in muscle cellularity (Johnston, 1999). The number of muscle fibres recruited to reach a given size varies between species, different strains and between different groups reared in different conditions and is influenced by genetic load, diet, exercise training, temperature and other important parameters involved in fish culture (Johnston, 1999; Fauconneau and Paboeuf, 2000). Consequently, analysis of environmental conditions that could influence muscle growth in the larval phase of commercial fish species is very important for larviculture, which aims at best final fish size and good quality flesh. Nevertheless, few studies have investigated the mechanisms and conditions of larval muscle growth in wild populations reared under different environmental conditions.

A unique characteristic in fish when compared to other vertebrates is that hypertrophy and hyperplasia are both remarkably important in post-hatching (or postnatal) muscle growth, while in most other vertebrates post-hatching growth is through hypertrophy only (Mommsen and Moon, 2001; Johnston, 2006). Because of the economic importance of fish skeletal muscle, regulation mechanisms of hypertrophy and hyperplasia in this tissue have been thoroughly studied in fish (Mommsen and Moon, 2001; Johnston, 2006; Johnston et al., 2011).

There are many regulatory endocrine–paracrine peptides and hormones that promotes muscle growth in fish, such as IGFs, IGFBPs, GH, among others (Johnston, 2006; Carnevalli et al., 2006; Olivotto et al., 2008, 2010, 2011; Johnston et al., 2011), whereas other ones seems to have an antagonistic role in that process, such as myostatin, cortisol, prolactin and others (Boeuf and Payan, 2001; Carnevalli et al., 2006; Olivotto et al., 2010, 2011). One important group of regulatory growth peptides are the insulin growth-factors (IGFs), and these have multiple functions that are necessary for normal development and survival and are controlled via the endocrine and autocrine/paracrine pathways (Bower et al., 2008). The regulation of fibre mass is thought to be controlled by signalling pathways involving insulin-like growth factors such as IGF-I and IGF-II. IGF-I activates the Akt-mTor signalling pathway, which has a central role in regulating protein synthesis and degradation in muscle, while the autocrine IGF-II is related to skeletal myocyte differentiation and myosin synthesis (Johnston, 2006). Myogenic progenitor cells, which are undifferentiated interstitial myotomal cells, are responsible for differentiation and proliferation of muscle fibres. These cells are known PCNA positive cells when active (Johnston, 2006). PCNA is a DNA polymerase associated peptide which is upregulated and controlled by positive and negative signalling pathways, and it is related to DNA repair and cell proliferation mechanisms (Brodeur et al., 2003). Remarkably these regulatory factors have a plastic expression in skeletal muscle with environmental changes (Johnston, 2006), and, therefore, IGFs and PCNA could be good biomarkers of hypertrophy and hyperplasia for muscle growth.

The bumblebee catfish *Lophiosilurus alexandri* Steindachner, 1876 can reproduce in captivity without any hormonal stimuli. Furthermore, it has a high market value because of an absence of intra-bones in lateral muscle and the good flavour of its flesh, and, for these reasons, *L. alexandri* experiences a huge fishery pressure, necessitating important developments in larviculture and nursery technologies of *L. alexandri* (Bazzoli and Godinho, 1997; López and Sampaio, 2000; Pedreira et al., 2008, 2009; Luz et al., 2011; Melilo-filho et al., in press). Growth, survival, cannibalism, production and non-ionised ammonia levels have been significantly altered between different treatments of salinity and density of *L. alexandri* larvae, showing the greatest improvement at 2% salinity and a density of 20 larvae l^{-1} (Luz and Santos, 2008a; Santos and Luz, 2009). Luz and Santos (2008b) showed that *L. alexandri* larvae do not tolerate high salinity levels (6–8 g NaCl l^{-1}), presenting high rates of functional deformity and mortality. Even though knowledge about the influence of salinity on larval growth of *L. alexandri* is documented, the interaction between temperature and salinity on growth is unknown for this species. Moreover, the combined influence of salinity and temperature on muscle cellularity is not well documented in scientific literature, nor is the expression of regulatory markers on larval muscle growth of reared freshwater fish regarding both these parameters. Considering this, the present study assessed the influence of temperature and salinity on body growth and muscle cellularity of *L. alexaxdri* vitelinic larvae.

2. Materials and methods

2.1. Experimental drawing

The experiments were carried out at the Hydrobiology and Hatchery Station of Três Marias, Brazil – CODEVASF. Recently-hatched larvae of *L. alexandri* were stocked in 500 ml artificially aerated plastic tanks (DO > 5 mg l^{-1}). In each tank, 96.00 ± 8.23 larvae per salinity/temperature treatment combination were stocked. The following salinities (S) and temperatures (T), which allowed for an acceptable range of growth and survival, were tested (Luz and Santos, 2008a,b): S_0 = freshwater (pH 7.15), S_2 = 2 g NaCl l^{-1} (pH 7.19), S_4 = 4 g NaCl l^{-1} (pH 7.21), T_{25} = 25.5 ± 0.6 °C and T_{30} = 30.0 ± 0.8 °C. Then, 6 treatments were assessed (S_0T_{25}, S_2T_{25}, S_4T_{25}, S_0T_{30}, S_2T_{30} and S_4T_{30}). The experiment started immediately after hatching; daily inspections were conducted; but only at 96 h post hatching did we find dead larvae, when they were removed. Before fixation, larvae were anaesthetised and sacrificed with carnation oil, following the ethic rules and procedures stated by COBEA (Brazilian College of Animal Experimentation) (COBEA, 1991). Around 50 larvae were removed for a 50 ml becker with water, and carnation oil was titrated with the use of pasteur pipette gradually up to achieve a concentration of 0.03 ml L^{-1}.

2.2. Biometric analysis

Larvae samples (8–14 individuals per treatment) were randomly collected each 24 h over 7 days and then fixed in 10% buffered formalin to record the following biometric data: Total length (TL) and yolk sac volume (SV = $4/3\pi ab$, where "*a*" is yolk sac length and "*b*" is yolk sac width). Biometric data were obtained through the use of stereomicroscopy. For each treatment, the length increment index (LI) was calculated which is expressed by the equation $LI = \left(x\frac{TL}{SV}\right)_i - \left(x\frac{TL}{SV}\right)_f$, where x is the arithmetic mean of TL/SV ratio; i is this mean of all treatments at the first day of experiment; and f is this same mean at final day of experiment in each treatment.

2.3. Histology and histometry

Samples (3–4 individuals per tratment) of fourth day post hatching larvae (when mortality started occurring) were fixed in