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Rearing temperature induces changes in muscle growth and gene expression in juvenile pacu (*Piaractus mesopotamicus*)

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ABSTRACT

Pacu (*Piaractus mesopotamicus*) is a fast-growing fish that is extensively used in Brazilian aquaculture programs and shows a wide range of thermal tolerance. Because temperature is an environmental factor that influences the growth rate of fish and is directly related to muscle plasticity and growth, we hypothesized that different rearing temperatures in juvenile pacu, which exhibits intense muscle growth by hyperplasia, can potentially alter the muscle growth patterns of this species. The aim of this study was to analyze the muscle growth characteristics together with the expression of the myogenic regulatory factors MyoD and myogenin and the growth factor myostatin in juvenile pacu that were submitted to different rearing temperatures. Juvenile fish (1.5 g weight) were distributed in tanks containing water and maintained at 24 °C (G24), 28 °C (G28) and 32 °C (G32) (three replicates for each group) for 60 days. At days 30 and 60, the fish were anesthetized and euthanized, and muscle samples (n = 12) were collected for morphological, morphometric and gene expression analyses. At day 30, the body weight and standard length were lower for G24 than for G28 and G32. Muscle fiber frequency in the <25 μm class was significantly higher in G24, and the >50 μm class was lower in G24. MyoD gene expression was higher in G24 compared with that in G28 and G32, and myogenin and myostatin mRNA levels were higher in G24 than G28. At day 60, the body weight and the standard length were higher in G32 but lower in G24. The frequency distribution of the <25 μm diameter muscle fibers was higher in G24, and that of the >50 μm class was lower in G24. MyoD mRNA levels were higher in G24 and G32, and myogenin mRNA levels were similar between G24 and G28 and between G24 and G32 but were higher in G28 compared to G32. The myostatin mRNA levels were similar between the studied temperatures. In light of our results, we conclude that low rearing temperature altered the expression of muscle growth-related genes and induced a delay in muscle growth in juvenile pacu (*P. mesopotamicus*). Our study provides a clear example of thermally induced phenotypic plasticity in pacu fish and shows that changing the rearing temperature during the juvenile stage can have a considerable effect on gene expression and muscle growth in this species.

1. Introduction

Fish skeletal muscle is predominantly composed of white muscle, which never comprises less than 70% of the bulk of myotomal muscle (Zhang et al., 1996). White muscle exhibits glycolytic metabolism and fast-contracting fibers (Driedzic and Hochachka, 1976; Zhang et al., 1996). Increasing body size in fish is mainly due to the growth of white muscle fibers (Zimmerman and Lowery, 1999).

Fish muscle growth involves a population of proliferative satellite cells (Johnston et al., 2011), which provide nuclei that are essential for the continuous production of muscle fibers (hyperplasia) and an increase in fiber size (hypertrophy) (Koumans and Akster, 1995). These muscle growth mechanisms are controlled by several molecular pathways, and complex interspecies differences exist but are not well understood in fish.

The MyoD family of muscle transcription factors plays an important role during development and muscle growth (Palmer and Rudnicki,

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2003). The primary myogenic regulatory factors (MRFs), MyoD and Myf5, direct the commitment and proliferation of myogenic precursor cells (MPCs) towards a myogenic lineage, and the secondary MRFs, myogenin and MRF4, control the terminal differentiation and fusion of myoblasts to form myotubes that mature into new fibers. The other MPCs are absorbed into existing muscle fibers to maintain the nuclear to cytoplasmic ratio as the fibers increase in diameter during growth (Koumans et al., 1991).

Muscle growth is also controlled by the expression of myostatin, which is a member of the transforming growth factor-β (TGF-β) family (McPherron et al., 1997). Myostatin, which is mostly expressed in skeletal muscle, was first identified in mice (McPherron et al., 1997) and has been demonstrated to negatively regulate skeletal muscle growth in several mammalian species. In contrast to what was observed in mammals, the role of myostatin in fish may not be restricted to muscle growth regulation and may have other possible functions in other tissues where it is expressed (Østbye et al., 2001; Rodgers et al., 2001; Acosta et al., 2005; Patruno et al., 2008; Lee et al., 2009). In vitro studies in mammals have shown that myostatin inhibits the proliferation and differentiation of MPCs by decreasing the level and activity of MyoD and myogenin (Thomas et al., 2000; Ríos et al., 2001; Langley et al., 2002) through a physical interaction between either MyoD or myogenin and the transcription factor Smad3/4, which is activated by myostatin (Liu et al., 2001; Langley et al., 2002).

Most fish species have a wide range of thermal tolerance. However, the environmental comfort range that provides ideal conditions for growth is specific for each species (Emerson, 1986). Several environmental factors, such as water quality, oxygen availability and temperature, can affect the physiological systems and growth rate of fish (Brett, 1979; Johnton, 2006). The thermal regime during early developmental stages can influence the rate of myogenesis, muscle fiber cellularity and the expression of genes that are related to muscle development and growth pattern in adults (Assis et al., 2004; Johnton, 2006; Alami-Durante et al., 2007; Macqueen et al., 2008; Campos et al., 2013).

The pacu, *Piaractus mesopotamicus* (Holmberg, 1887, Characidae, Serrasalminae), is a neotropical freshwater fish that is commercially important because it is extensively cultivated in Brazilian aquaculture programs (Urbinati and Gonçalves, 2005), and it is one of the most important food species that is farmed in the Pantanal wetland areas of the Parana–Paraguay basin (Oliveira et al., 2004). The pacu is a fast growing fish and can reach 1.2 kg in its first culture year (Bernardino and Colares de Melo, 1989). This fast growth depends on hyperplastic and hypertrophic muscle growth mechanisms that are controlled by transcriptional and growth factors (Dal Pai et al., 2000; Almeida et al., 2008, 2010).

Because temperature is an important environmental factor that influences fish growth rates (Assis et al., 2004; Campos et al., 2013), it can be considered an important parameter for the success of aquaculture systems and is directly related to muscle plasticity and growth (Johnston et al., 2011). In the present work, we hypothesize that changes in cultivation temperature during the pacu juvenile stages, which represent intense muscle growth by hyperplasia, can potentially alter the muscle growth pattern of this species (Assis et al., 2004; Almeida et al., 2008). The aim of this study was to analyze the muscle growth characteristics and expression of the MRFs, MyoD and myogenin, and the growth factor myostatin in juvenile pacu that were submitted to different rearing temperatures.

2. Materials and methods

2.1. Fish samples

This experiment was approved by the Ethics Committee of the Biosciences Institute, UNESP, Botucatu, SP, Brazil (Protocol no. 332).

The experiment was performed in the Agência Paulista de Tecnologia dos Agronegócios (APTA), Presidente Prudente, SP, Brazil and in the Skeletal Muscle Biology Laboratory (LBME), Department of Morphology, UNESP, Botucatu, SP, Brazil.

Juvenile fish (1.5 g weight) were distributed into nine tanks (initial density of 0.450 kg/m^3) under three separate recirculation systems whose temperatures were maintained at 24 °C (G24), 28 °C (G28) and 32 °C, (G32) (three replicates for each group) during the entire experiment (60 days). The fish were fed three times a day with a commercial food containing 40% crude protein until apparent satiation. During the experimental period, the following parameters of tank water quality were measured: temperature, dissolved oxygen, nitrite, nitrate and ammonia. The averages that were obtained were as follows: in G24, 23.65 °C, 7.33 mg/L, 1.2 mg/L, 20 mg/L, and 0 mg/L; in G28, 27.18 °C, 7.14 mg/L, 1.2 mg/L, 20 mg/L, and 0 mg/L; and in G32, 32.09 °C, 6.61 mg/L, 1.4 mg/L, 30 mg/L, and 0 mg/L.

At days 30 and 60, the fish were euthanized by an overdose of the anesthetic benzocaine in water, individually weighed (g) and measured (cm). Epaxial white muscle samples below the dorsal fin were collected for morphological and morphometric analyses ($n = 6$) and for the gene expression analysis ($n = 6$).

2.2. Morphological and morphometric analysis

White muscle samples were fixed in 10% buffered formalin and preserved in 70% ethanol. The samples were dehydrated with a series of ethanol concentrations (80, 90 and 95%) and soaked in resin (Historesin–Leica Instruments GmbH, Germany) according to the manufacturer's recommendations. Transverse sections (4 μm) were obtained using a glass-razor microtome and stained using the hematoxylin–eosin (HE) method.

The individual fiber area was measured using a compound microscope that was attached to a computerized imaging analysis system (Leica Qwin, Wetzlar, Germany) (Dubowitz and Brooke, 1973). We used 6 fish per experimental group and measured all fibers in random fields of image samples until those taken were 200 fibers per animal and 1200 fibers per group. The fiber diameter (D) was indirectly estimated using the individual fiber area (A) and the formula $D = 2A^{0.5}\ \pi^{-0.5}$ (Valente et al., 1999).

For each group, the muscle fiber diameters were grouped into three classes <25 μm, 25–50 μm and >50 μm, based on Almeida et al. (2008). The muscle fiber frequency was expressed as the number of fibers from each diameter class relative to the total number of fibers that were measured.

2.3. MyoD, myogenin and myostatin mRNA expression

White muscle samples were frozen in liquid nitrogen, and total RNA was isolated using TRIzol Reagent (Life Technologies Corporation, Carlsbad, CA, USA) according to the manufacturer's protocol. The RNA-extracted samples were quantified using a NanoVue Plus Spectrophotometer (GE Healthcare Life Sciences, Piscataway, NJ, USA), and the RNA purity was ensured after obtaining a 260/280 nm OD ratio of approximately 2.0. The RNA integrity was assessed using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and by calculating the RNA integrity number (RIN). The average RIN value for all samples was 8.95 ± 0.49 (scale 1–10), which indicated high-quality RNA with minimal degradation.

Total RNA (2 μg) was treated with Amplification Grade Deoxyribonuclease I according to the protocol provided by Invitrogen (Life Technologies). Purified total RNA was reverse transcribed using random hexamer primers and a High-Capacity cDNA Archive Kit (Life Technologies) in a volume of 100 μL according to the manufacturer's protocol. A sample without reverse transcriptase (NoRT) was used to verify DNase treatment efficiency.

MyoD, myogenin and myostatin gene expression levels were detected by quantitative real-time polymerase chain reaction (RT-qPCR) using an ABI 7300 Real Time PCR System (Life Technologies). A sample without

cDNA template (NTC) was used to verify that the master mix was free from contaminants. Two microliters of cDNA (20 ng/μL) were amplified using the Power SYBR Green PCR Master Mix 2× (Life Technologies) and 400 nM of each primer (Table 1) to yield a final volume of 25 μL. The real-time conditions were as follows: 10 min at 95 °C, 40 cycles of denaturation at 95 °C for 15 s, and annealing/extension at 60 °C for 1 min. The β-actin gene was used as a reference to normalize the quantification of the mRNA target.

Primer pairs for MyoD, myogenin, myostatin and β-actin were designed based on cDNA sequences from *P. mesopotamicus* (GenBank accession numbers: MyoD—FJ686692; myogenin—FJ810421; myostatin—HM561964.1; and β-actin—sequence not published) using Primer Express 3.0 software (Life Technologies) (Table 1).

An amplification plot graphically displayed the fluorescence that was detected over the number of cycles that were performed. The signal baseline and the threshold were set manually for each detector (MyoD, myogenin, myostatin and β-actin), which generated a threshold cycle (Ct) for each sample.

The PCR efficiency of each individual assay was determined by creating standard curves for the targets and the reference gene. The standard curves were obtained by using serial dilutions (1:10) of the sample cDNA and were represented by semi-log regression line plots of the Ct value vs. the logarithm of the starting amount of cDNA. The standard curve slope showed acceptable values between −3.8 and −3.3, as reported by Patruno et al., 2008.

The difference between the Ct values was calculated for each target gene by subtracting the mean Ct of the duplicate reactions for the reference gene from the mean Ct of duplicate reactions (ΔCt = Ct target gene − Ct reference gene). All samples were then compared to the ΔCt value of a calibrator sample to obtain a ΔΔCt value (ΔCt target −ΔCt calibrator). The animal that showed the lowest gene expression at G24 was chosen as the calibrator sample to evaluate the putative differential mRNA expression of the target gene relative to the sample.

Therefore, by using the $2^{-\Delta\Delta Ct}$ method (Ct comparative method), the data were recorded as the fold-change in gene expression that was normalized to the reference gene and relative to the calibrator sample (Livak and Schmittgen, 2001). The specificity of each primer was confirmed by the presence of a single peak in the dissociation curve analysis.

2.4. Statistical analysis

ANOVA complemented with the Multiple Comparisons Test (Tukey) was utilized to analyze the body weight and standard length data (Zar, 2009). The muscle fiber diameter data were expressed as the frequency percentage, as analyzed by the Goodman test between and within the multinomial population (Goodman, 1965). The relative gene expression data were analyzed using the Kruskal–Wallis test complemented with the Dunn multiple comparisons test (Zar, 2009). The statistical significance level was set at $p < 0.05$ for all analyses.

3. Results

3.1. Anatomical data

During the experimental period, the fish showed an increase in body weight and standard length that was independent of the rearing temperature. At day 30, the body weight and standard length were lower in G24 compared to those of G28 and G32. These parameters were similar between G28 and G32. At 60 days, the body weight and standard length were higher in G32 than in G28 and were higher in G28 than in G24 (Table 2).

3.2. Morphological and morphometric analysis

HE staining showed that white skeletal muscle comprised most of the muscle mass at all temperatures that were studied. This muscle consisted of round and polygonal fibers that were separated by a fine septum of connective tissue, the endomysium. Thicker septa of connective tissue separated the muscle fibers into fascicles that composed the perimysium. Muscle fibers were distributed into a mosaic pattern that was characterized by fibers of different diameters (Fig. 1).

The frequency distribution of the white muscle fibers in the <25 μm diameter class was significantly different at all studied temperatures. At 30 days, this fiber class was significantly higher in G24 than in G28 and G32. G28 presented a significantly higher percentage of fibers in the <25 μm class than G32.

In this time period, the frequency distribution of the muscle fibers in the 25–50 μm diameter class was significantly higher in G32 than in G24 and G28. In the >50 μm class, G28 and G32 showed similar results, and these were higher than those of G24 (Table 3).

At the end of the experimental period (60 days), the frequency distribution of muscle fiber <25 μm diameter class was higher in G24 than in G28 and G32; and, at these temperatures, the frequency distribution of the muscle fiber <25 μm diameter class was similar. In the 25–50 μm class, the muscle fiber frequency distribution was similar at all studied temperatures, and in the >50 μm class, G32 showed a higher percentage than G24.

3.3. MyoD, myogenin and myostatin mRNA expression

The RT-qPCR results showed changes in gene expression in fish that were cultivated at different temperatures. After 30 days of cultivation, MyoD gene expression was higher in G24 than in G28 and G32. In G24, the myogenin and myostatin mRNA levels were higher than those in G28 but were similar to those observed in G32. Moreover, this expression was different between G28 and G32.

At 60 days, the MyoD mRNA levels were higher in G24 than in G28 but were similar to that in G32. In G28 and G32, the MyoD expression was similar. The myogenin mRNA levels were similar between G24

Table 1
Oligonucleotide primers used for RT-qPCR amplification of the genes MyoD, myogenin, myostatin and reference gene β-actin.

Gene	Sequence (5′ → 3′)	Size of amplified fragment (bp)
MyoD[a]	S: TACTACCCCGTCCTGGATCA	140
	A: GTCAGAGCCGCTTCAGTGTC	
Myogenin[b]	S: AGCGCAGCACATTGATGAAC	57
	A: TCCTCAGGATTTCCACTTTTGG	
Myostatin[c]	S: GACGGAAGGAGGCACATAAGAA	60
	A: CGTGACCCCAGCGTTCAC	
β-actin[d]	S: TCACAGAGGCTCCCCTGAAC	64
	A: CTCAAACATGATCTGGGTCATCT	

S: primer sense; A: primer antisense; pb: bases pairs.
GenBank accession numbers: a: FJ686692; b: FJ810421; c: HM561964; d: partial sequence not published.

Table 2
Mean and standard deviation (SD) of the body weight (BW) (g) and standard lengths (LS) (cm) of juvenile pacu (*Piaractus mesopotamicus*) in G24, G28 and G32 at 30 and 60 days.

Groups					
Period variable		G24	G28	G32	Value (p)
30 days	BW	7.80 (2.35)[a]	11.00 (1.99)[b]	11.17 (3.59)[b]	P < 0.001
30 days	LS	5.81 (0.61)[a]	6.46 (0.34)[b]	6.52 (0.62)[b]	P < 0.001
60 days	BW	20.40 (5.26)[a]	32.30 (7.36)[b]	43.00 (13.45)[c]	P < 0.001
60 days	LS	8.08 (0.66)[a]	9.54 (0.73)[b]	10.50 (1.10)[c]	P < 0.001

Values with the same letters are not statistically significant (P < 0,001, Tukey–Multiple Comparison).

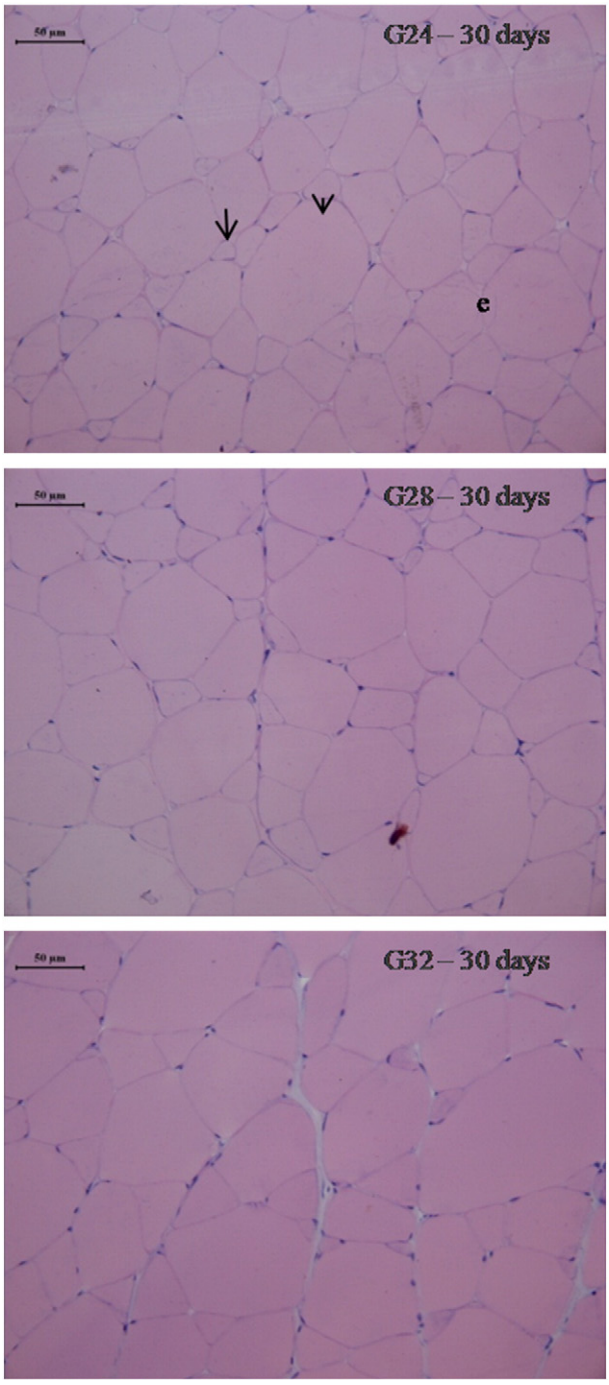

Fig. 1. Transverse sections of pacu (*Piaractus mesopotamicus*) white skeletal muscle in G24, G28 and G32 at 30 days. A mosaic pattern of the different muscle fiber diameters that are comprised of small (arrows) to large (arrowhead) fibers can be observed. Muscle fibers are separated by the endomysium (e). Hematoxylin–eosin stain. Scale bars: 50 μm.

and G28 and between G24 and G32, but these values were higher in G28 than in G32. The myostatin mRNA levels were similar among the studied rearing temperatures (Fig. 2).

4. Discussion

We demonstrate that differences in cultivation temperature alter the expression of muscle growth-related genes and muscle growth characteristics in pacu (*P. mesopotamicus*) juveniles.

Muscle development in teleosts is sensitive to thermal conditions and could result in different phenotypes regarding growth pattern (Johnton, 2006). Changes in water temperature could influence behavior, metabolic processes, reproduction and growth (Fry, 1971). Higher temperatures increase fish metabolism and food intake, resulting in greater growth (Kieffer et al., 2013). This fact may, in part, explain the results that were observed in our study, where, at 30 and 60 days, the body weight and total length were higher in G32 (32 °C) than in G24 (24 °C). Macqueen et al. (2008) also observed direct temperature effects on muscle development growth rates of juvenile and adult *Salmo salar* L. These authors observed low body mass in fish that were reared at a lower temperature (2 °C).

At 30 and 60 days of cultivation, G24 showed a high frequency of fibers in the <25 μm class when compared to G28 and G32. The presence of <25 μm diameter fibers indicates intense muscle fiber recruitment in juvenile pacu that were reared at this temperature (Valente et al., 1999). Assis et al. (2004) studied muscle growth in pacu eggs that were incubated at 25, 27 and 29 ºC and noticed that, 25 days post-hatching with rearing at room temperature, the percentage of fibers in the < 20 μm class was higher at 25 ºC than at 27 and 29 °C, and this phenotype was associated with low total length. Campos et al. (2013) observed that, 30 days post-hatching, larvae from *Solea senegalensis* that were incubated at 15 °C presented a majority of fibers with small diameters, and the total number of fibers was lower at this temperature compared with fish that were cultivated at higher temperatures (18 °C and 21 °C). Interestingly, Campos et al. (2013) also observed lower body weight and standard length in larvae that were reared at a lower temperature (15 °C). Therefore, it is possible that low temperature during the initial growth phase could influence muscle fiber recruitment and development and, therefore, influence the final size of the fish (Johnton and McLay, 1997; Macqueen et al., 2008). In our study, pacu juveniles reared at low temperature (24 °C) grew significantly less than those of the other groups, which was confirmed by low body weight and standard length. Considering these data with our morphometric analysis, we could infer that a low rearing temperature for pacu juveniles was sufficient to induce a delay in the muscle-growth process, which could influence the growth rate, development and ultimate body size.

During muscle growth by hypertrophy, fibers expand and absorb satellite cell nuclei to maintain a relatively constant ratio between the nucleus and the cytoplasm of fiber (Koumans et al., 1994). In the analyzed juvenile pacu, muscle fiber hypertrophy was the main process that was observed in G28 and G32, as demonstrated by the observation that the highest muscle fiber frequency occurred in the 25–50 and >50 μm classes.

Muscle growth mechanisms are regulated by the sequential expression of the MRFs family, which includes MyoD, Myf5, myogenin and MRF4 (Watabe, 1999; Watabe, 2001). Studies in fish have shown that variations in rearing temperature can alter the gene expression of MRFs and modify muscle plasticity and cellularity (Fernandes et al., 2006; Macqueen et al., 2007; Campos et al., 2013). During early developmental stages (pre-metamorphosis and metamorphosis), eggs of *S. senegalensis* that were incubated at low temperature (15 °C) showed higher Myf5 and MyoD2 mRNA expression, and the diameter and total number of fibers was lower than in eggs cultivated at higher temperatures (18 and 21 °C) (Campos et al., 2013). After hatching, the larvae were reared at the same temperature (20.8 ± 0.1 °C) for 30 days, and, although gene expression was similar between groups, the diameter and total number of fibers remained lower at 15 °C. The authors concluded that high MRF gene expression was most likely related to early myoblast commitment to a myogenic progenitor cell, and incubation of *S. senegalensis* eggs at a low temperature delayed embryonic development and produced smaller larvae with fewer and smaller fibers throughout growth compared with those incubated at 18 and 21 °C.

In the present study, we observed that at 30 and 60 days of cultivation, MyoD gene expression was higher in G24, although, at 60 days, its expression was significantly different only when compared with G28.

Table 3
The frequency distribution (%) of white muscle fiber diameter in classes: <25 μm, 20–50 μm and >50 μm of juveniles pacu (*Piaractus mesopotamicus*) in G24, G28 and G32, at 30 and 60 days of the experiment.

Diameter classes (μm)					
Time period group		<25	25–50	>50	Total fibers
30	G24	719 (59.9)^{c}	468 (39.0)^{a}	13 (1.1)^{a}	1200
30	G28	602 (50.2)^{b}	532 (44.3)^{a}	66 (5.5)^{b}	1200
30	G32	516 (43.0)^{a}	633 (52.8)^{b}	51 (4.3)^{b}	1200
60	G24	508 (42.3)^{b}	553 (46.1)^{a}	139 (11.6)^{a}	1200
60	G28	407 (33.9)^{a}	629 (52.4)^{a}	164 (13.7)^{ab}	1200
60	G32	423 (35.3)^{a}	573 (47.8)^{a}	204 (17.0)^{b}	1200

Frequency distribution of white muscle fibers of juvenile of pacu (*Piaractus mesopotamicus*) in diameter classes (μm): class <25; class 25–50 and class >50 at 30 and 60 days of the experiment. Small letters in the vertical line compare the frequency of fibers between the temperatures. Values with the same letters are not statistically significant ($P < 0.05$, Goodman test).

During muscle growth, MyoD controls satellite cell proliferation (Megeney and Rudnicki, 1995; Watabe, 2001; Kuang and Rudnicki, 2008), and these cells provide nuclei for new muscle fiber formation (hyperplasia) and hypertrophy (McCarthy et al., 2011). The mechanism underlying the increased MyoD mRNA expression found in G24 could be related to the satellite cell proliferation rate and could be responsible for the control of muscle fiber recruitment that occurred during the analyzed experimental periods. Additionally, during the same periods of cultivation, G24 showed a higher frequency of fibers in the <25 μm class than was observed in G28 and G32 and a lower body weight/length standard. In spite of the influence of temperature on muscle growth and cellularity in teleosts being shown to be highly dependent upon the species and developmental stage (Alami-Durante et al., 2007; Macqueen et al., 2008), according to the results that were obtained by Campos et al. (2013), and although we did not analyze the total number of fibers, we can infer that low-rearing temperature in pacu juveniles could influence the muscle cellularity, cell number and ultimate size of this species.

After 30 days of cultivation, G24 showed higher myogenin mRNA expression than that in G28, and, at 60 days, G28 showed a higher myogenin mRNA expression than that found in G32. During muscle growth, myogenin controls the terminal differentiation and fusion of myoblasts to form myotubes that mature into new fibers (Koumans et al., 1991). According to Wilkes et al. (2001), rearing temperature can change the timed expression of MRFs during the early stages of fish muscle development and modulate the proliferation and differentiation of myoblasts. The authors observed an increase in MyoD and myogenin gene expression in larvae of trout that were reared at a lower temperature (4 °C) compared to fish that were reared at a higher temperature (8 °C). Based on the myogenin gene expression that we observed at 60 days, we suggest that this MRF could influence muscle differentiation and the size of muscle fibers during the juvenile stage and that these changes have the potential to alter the total muscle fiber numbers during growth.

At 30 days of cultivation, myostatin gene expression was higher in G24 compared to G28. At 60 days, the myostatin gene expression was similar at all temperatures that were evaluated.

In mammals, several studies have investigated the actions of myostatin in skeletal muscle development and growth, and it is well established that this growth factor controls muscle growth (McPherron et al., 1997; Lee and McPherron, 2001) by inhibiting proliferation and differentiation of myogenic cells in vitro (Joulia et al., 2003; Trendelenburg et al., 2009). Indeed, myostatin inhibits satellite cell proliferation by activating the cyclin-dependent kinase inhibitor p21, which forces the withdrawal of the cells from the cell cycle and maintains them in a quiescent and undifferentiated state (Thomas et al., 2000; Manceau et al., 2008).

In fish, although a few studies have shown enhanced muscle growth after the inhibition of myostatin (Lee et al., 2009; Medeiros et al., 2009), others have not shown a strong effect of this growth factor on muscle mass (Acosta et al., 2005; Sawatari et al., 2010). Moreover, strong myostatin expression in non-muscle tissue such as the eyes, brain, testis and spleen (Østbye et al., 2001; Radaelli et al., 2003; Garikipati et al., 2006, 2007) and the presence of differentially expressed paralogs (Rescan et al., 2001) strongly suggest that myostatin may have additional functions aside from muscle growth regulation.

Seiliez et al. (2012), who used trout satellite cell cultures treated with human myostatin, observed that this growth factor did not affect MyoD and myogenin protein expression. To our knowledge, only a few studies related to muscle myostatin expression and rearing temperature have been reported. As reported by Campos et al. (2013), myostatin was up-regulated and MyoD and myogenin were highly expressed at low temperature. These results are, in part, in agreement with the results observed in pacu at 30 days in G24. This finding indicates a possible lack of inhibitory effect of myostatin on myoblast proliferation in the skeletal muscle of juvenile pacu that are reared at low temperature. In this condition, high myostatin also did not inhibit the differentiation of myoblasts that was detected by high myogenin mRNA expression. A similar myostatin gene expression pattern was observed in the groups at 60 days and did not directly influence the muscle phenotypic plasticity by modulating cell proliferation and differentiation activity. According to Gabillard et al. (2013), the function of myostatin in fish is not restricted to a specific tissue but is mainly involved in the control of tissue mass by inhibiting cell proliferation and cell growth. In this context, the fact that myostatin expression in fish is rarely associated with muscle growth strongly suggests that if any action of myostatin in muscle exists, it would be limited. According to this hypothesis, the term “myostatin” appears to be not appropriate with regard to the role of the protein in fish, and we propose that its previous name, GDF-8, should be used.

5. Conclusion

According to our results, we conclude that low rearing temperature changed the expression of muscle growth-related genes and induced a delay in muscle growth in juvenile pacu (*P. mesopotamicus*). Our study provides a clear example of thermally induced phenotypic plasticity in pacu fish and shows that changing the rearing temperature during the juvenile stage can have a considerable effect on gene expression and muscle growth in this species.

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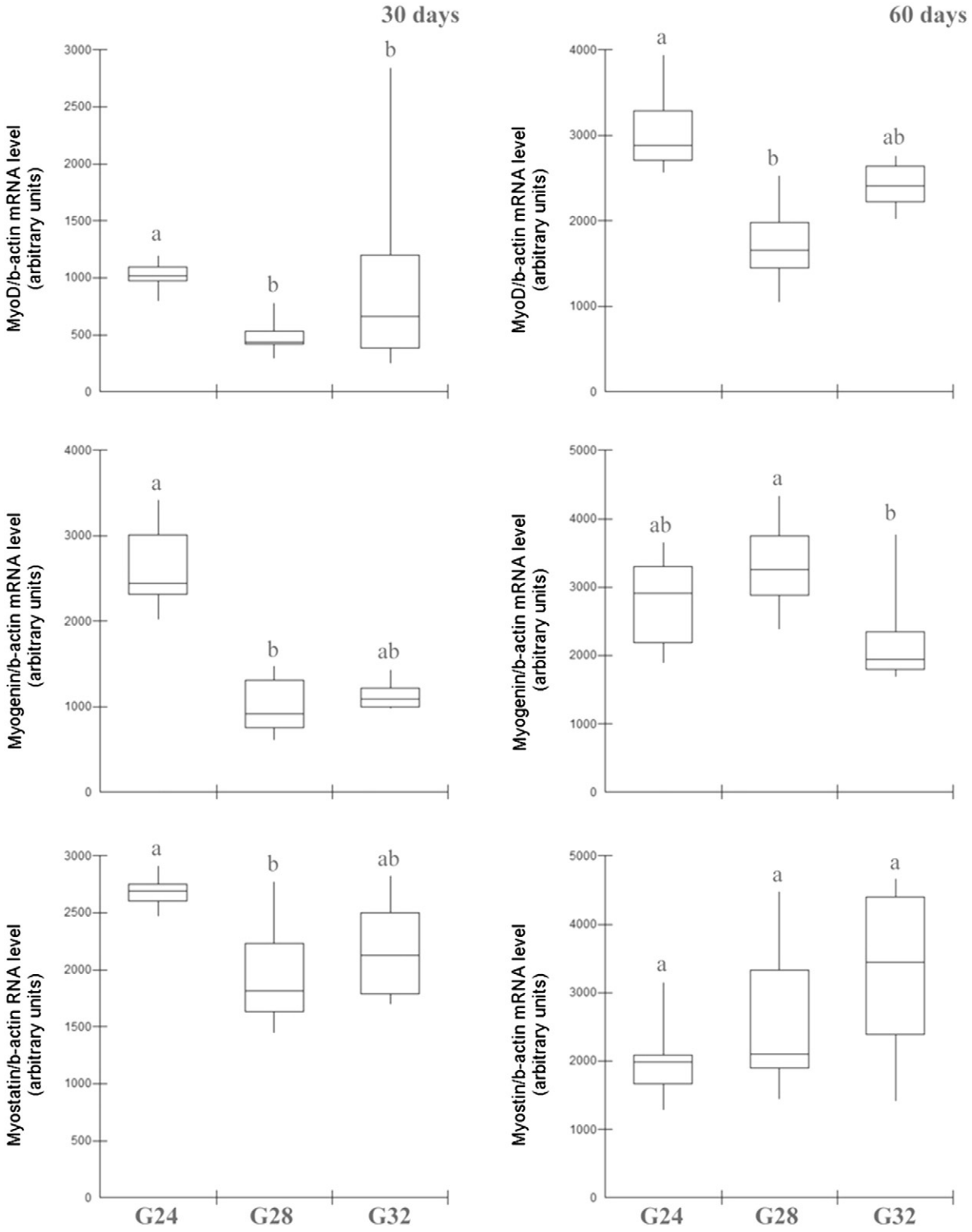

Fig. 2. Real-time RT-PCR quantification of MyoD (a), myogenin (b) and myostatin (c) mRNA expression in the white skeletal muscle of pacu (*Piaractus mesopotamicus*) in G24, G28 and G32 at 30 and 60 days. Data were expressed as the minimum, 1st quartile, median, 3rd quartile and maximum values. Values with the same letters are not statistically different ($P < 0.05$, Kruskal–Wallis test complemented by the Dunn Multiple Comparisons Test).

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