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Effect of salinity on survival, growth and biochemical parameters in juvenile Lebranch mullet *Mugil liza* (Perciformes: Mugilidae)

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Teleost fish growth may be improved under isosmotic condition. Growth and metabolic performance of juvenile *Mugil liza* (isosmotic point: 12‰) were evaluated after 40 days in different salinities (0, 6, 12 and 24‰). Tests were performed in quadruplicate (30 fish/tank; 0.48 ± 0.1 g body weight; 3.27 ± 0.1 cm total length) under controlled water temperature (28.2 ± 0.1°C) and oxygen content (>90% saturation). Fish were fed on artificial diet (50% crude protein) four times a day until apparent satiation. Results showed that salinity influenced juvenile mullet growth. Fish reared at salinity 24‰ grew better than those maintained in freshwater (salinity 0‰). Gill Na^+,K^+-ATPase activity and whole body oxygen consumption showed an U-shape-type response over the range of salinities tested, with the lower values being observed at the intermediate salinities. Although no significant difference was observed in liver glycogen content at different salinities, it tended to augment with increasing salinity. These findings indicate that energy demand for osmorregulation in juvenile *M. liza* can be minimized under isosmotic condition. However, the amount of energy spared is not enough to improve fish growth. Results also suggest that *M. liza* is able to alternate between different energy-rich substrates during acclimation to environmental salinity.

O crescimento de peixes teleósteos pode ser melhorado em condição isosmótica. O crescimento e o desempenho metabólico de juvenis da tainha *Mugil liza* (ponto isosmótico: salinidade de 12‰) foram avaliados após 40 dias de cultivo em diferentes salinidades (0, 6, 12 e 24‰). Os testes foram realizados em 4 réplicas (30 peixes/tanque; 0,48 ± 0,1 g de peso corporal; 3,27 ± 0,1 cm de comprimento total) em condições controladas de temperatura (28,2 ± 0,1°C) e conteúdo de oxigênio (>90% saturação). Os peixes foram alimentados quatro vezes ao dia com dieta artificial (50% de proteína bruta) até a saciedade aparente. Os resultados mostraram que a salinidade influenciou o crescimento dos juvenis da tainha. Os peixes cultivados na salinidade 24‰ cresceram melhor que aqueles mantidos na água doce (salinidade 0‰). A atividade da Na^+,K^+-ATPase branquial e o consumo corporal de oxigênio mostraram uma resposta do tipo em forma de U, na faixa de salinidade testada, com os menores valores sendo observados nas salinidades intermediárias. Apesar de não ter sido observada diferença significativa no conteúdo de glicogênio entre os peixes mantidos nas diferentes salinidades, este parâmetro tendeu a aumentar com o incremento da salinidade. Estes achados indicam que a demanda energética para osmorregulação em juvenis de *M. liza* podem ser minimizados em condição isosmótica. Entretanto, a quantidade de energia poupada não é suficiente para melhorar o crescimento. Os resultados também sugerem que *M. liza* é capaz de alternar entre diferentes substratos ricos em energia durante a aclimatação à salinidade da água.

Keywords: Glycogen, Growth, Metabolism, Na^+, K^+-ATPase.

Introduction

Aquaculture seeks to provide an ideal and healthy environment to maximize fish growth in a shorter period of time. Among the environmental parameters affecting fish growth, salinity is one of the most extensively studied. In fact, it is a determinant factor for growth of fishes, including mullets (Jobling, 1994; Boeuf & Payan, 2001; Nordlie, 2009; O'Neill *et al*., 2011; Pérez-Robles *et al*., 2012; Fazio *et al*., 2013).

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Teleost fish are able to maintain the ionic and osmotic homeostasis of their body fluids across environmental salinities by using osmoregulatory mechanisms, which are energy demanding processes (Sampaio & Bianchini, 2002). In turn, growth is the net positive result from the energy provided by food ingestion and the metabolic expenditure (Jobbling, 1994). Considering the osmoregulatory cost as being proportional to the osmotic gradient existing between the fish body fluids and the external medium (Handeland *et al*., 1998), it is reasonable to expect that growth would be maximized when fish is reared in water of salinity near the isosmotic condition. In teleosts, the plasma isosmotic point generally corresponds to the water salinity of 12‰ (Boeuf & Payan, 2001; Tsuzuki *et al*., 2007; Herrera *et al*., 2009; Nordlie, 2009). However, the significance and fate of the energy spared under isosmotic conditions remain controversial (Boeuf & Payan, 2001; Kidder III *et al*., 2006). In addition, optimal salinities for growth and metabolic rates are influenced by other factors, including species and developmental status (Morgan & Iwama, 1991).

The metabolic energy demand is met by energy-rich macronutrients such as carbohydrates, lipids and proteins, which can either be used directly as fuel (respiratory substrates) or be stored in the body. Although carbohydrates contain less energy than lipids, they are preferentially used by cells as fuel (Jobling, 1994). Although glucose is the main energy source used in cell metabolism, fish can change the metabolic energy production and utilization to improve its energy supply by using other metabolic substrates when subjected to stressing environmental conditions (Tseng & Hwang, 2008).

Euryaline fish can live in a wide range of environmental salinities due to their ability to synthesize new salt-transporting proteins as they move from salt to fresh water and vice versa (Kidder III *et al*., 2006). Therefore, water from different sources can be used to cultivate euryaline fish.

The Lebranche mullet *Mugil liza* (Valenciennes, 1836) is an euryaline species, being an important fishing resource in the Southern Atlantic coast (Vieira, 1991; Reis & D'Incao, 2000). Its low position in the food web (Oliveira & Soares, 1996) and easy handling have attracted the attention of fish farmers. Therefore, many studies have been developed to ascertain the aquaculture potential of this fish species. Briefly, studies conducted so far were directed to artificial fertilization (Godinho *et al*., 1993), larviculture (Galvão *et al*., 1997), optimal stocking density (Sampaio *et al*., 2001), optimal temperature (Okamoto *et al*., 2006), and protein requirement (Carvalho *et al*., 2010). Regarding salinity, no mortality of juvenile *M. liza* was observed after 96 h of abrupt transfer from salt water (30‰) to dilute salinities down to 5‰ (Fonseca Neto & Spach, 1998). It is also known that tolerance to ammonia and nitrite is lower in juvenile mullets maintained in fresh water than in those kept at intermediate and high salinities (Sampaio *et al*., 2002). However, little is known about the effect of salinity on growth of juvenile Lebranche mullet.

In light of the background described above, the main objective of the present study was to evaluate the effect of water salinity on survival, growth, feed efficiency (FE), condition factor (CF), gill Na^+,K^+-ATPase activity (NKA), whole-body oxygen consumption rate (OCR), and liver glycogen content in juvenile mullet *M. liza*. Water salinities tested corresponded to 0, 50, 100 and 200% of that described as being isosmotic with the body fluids of the studied species.

Material and Methods

Fish acclimation and experimental design. Juvenile *Mugil liza* were captured at Cassino Beach (Southern Brazil) and transferred to the laboratory of Marine Fish Culture. Acclimation was made in two 1,000-L tanks with fresh water in recirculation systems equipped with mechanical and biological filters, UV sterilization, and temperature controllers. Room temperature (25°C) and photoperiod (14L:10D) were fixed. Fish were fed four times a day (9:00, 13:00, 17:00 and 21:00 h) with commercial diet (NRD 50% crude protein, INVE) until apparent satiation. These conditions were also maintained during the experimental period. Fish feces were siphoned out daily, when at least 50% of the water was renewed.

After 20 days of acclimation, 480 fish (body wet weight: 0.48 ± 0.01 g; total length: 3.27 ± 0.01 cm) were randomly stocked in 16 tanks (50 L). Tanks were kept using recirculating aquaculture systems equipped with mechanical and biological filters, UV sterilization, and temperature controllers. Experimental salinities tested were 0, 6, 12 and 24‰, which correspond to 0, 50, 100 and 200% of the isosmotic salinity for most teleost fish. Acclimation to the desired experimental salinity was performed by a daily increase in 6 units of water salinity from 0 up to 24‰. Experimental salinities were obtained by a mixture of dechlorinated tap water with natural sea water. Dissolved oxygen concentration (oximeter, YSI), water temperature (oxymeter YSI Model), salinity (refractometer, Atago), pH (pH meter, Quimis) and total ammonia (American Public Health Association (APHA), 2005) were measured daily over the 40-days experimental period.

Survival, growth and zootechnical parameters. Fish survival (*S*) was expressed as percentage of total fish tested and calculated according to the following equation: $S = [(Ni\text{-}Nf)/Ni]\ x100$, where *Ni* is the number of fish stocked in the tank at the beginning of the experiment while *Nf* corresponds to the number of living fish after 48 h of experiment.

All fish were weighed to the nearest 0.01 g every 20 days using an electronic balance. Feeding was stopped 24 h before fish weight measurement. Specific growth rate (*SGR*) was expressed in percentage and calculated according to the following equation: $SGR = [(\ln Wf\text{-}\ln Wi)/T]x100$, where *Wf* and *Wi* is the fish body mass at the beginning and the end of the experiment, respectively.

Feed efficiency (*FE*) was calculated based on the following equation: $FE = (BG/DFO)$, where *BG* is the biomass gain (g) and *DFO* is the dry feed offered (g).

Weight coefficient variation (*WCV*) was calculated using the following expression: $WCV = (SD/MW)x100$, where *SD* is the standard deviation and *MW* is the mean weight (g).

Condition factor (*CF*) was calculated using the following equation: $CF = (BW/L^3)$, where *BW* is the body weight (g) and *L* is the fish length (cm).

Whole-body oxygen consumption. At the end of the 40-days experimental period, whole body oxygen consumption was measured following procedures described by Cunha *et al*. (2009). Water oxygen concentration was measured every 15 min with an oximeter (YSI model Hexis 55). Measurements were made at oxygen saturation >70% to avoid any possible influence of the water oxygen concentration on fish oxygen consumption. Oxygen consumption rate (*OCR*) was calculated using the following equation: $OCR = [(Oi-Of)xV/(TxB)]$, where *Oi* and *Of* correspond to the water oxygen content (mg O_2 L^{-1}) at the beginning and the end of the measurement period, respectively; *V* is the tank volume (L); *B* is the fish biomass (g) and *T* is the measurement duration (h). Results were expressed in mg O_2 g wet body $mass^{-1}$ h^{-1}.

Tissue sample collection and biochemical analyses. After the oxygen measurement, fish were anesthetized (50 ppm benzocaine) and immediately killed by spinal section. The second gill arch at the left side and the liver were immediately dissected, frozen in liquid nitrogen and stored in ultrafreezer (-80°C) for further measurement of gill NKA and liver glycogen content, as described below.

Gills were homogenized in 300 µl of cold buffer (pH 7.3) containing 150 mM sucrose, 10 mM ethylenediaminetetraacetic acid, 50 mM imidazole, and 11.5 mM sodium deoxycholate, and centrifuged (10,000 x *g*) at 4°C for 30 s. The supernatant was used for NKA measurement following the protocol described by McCormick (1993). Sample absorbance was measured using a spectrophotometer (ELX 800 Universal Microplate Reader/Bio-Teck Instruments, Winooski, Vermont, USA). The protein concentration in the supernatant was determined using a commercial reagent kit based on the Biuret reagent (Doles, Goiânia, GO, Brazil). Enzyme activity was expressed in µmoles ADP mg $protein^{-1}$ h^{-1}.

Liver samples were weighed and homogenized in 100 mM sodium citrate solution (10% weight/volume). Glycogen concentration was determined using an adaptation of the method described by Carr & Neff (1984). In this case, glucose concentration was measured using a commercial reagent kit based on the glucose-oxidase method (Doles, Goiânia, GO, Brazil). Sample absorbance was measured using a spectrophotometer (ELX 800 Universal Microplate Reader/Bio-Teck Instruments, Winooski, Vermont, USA). Results were expressed in mg g $wet\ tissue^{-1}$.

Data presentation and analysis. Results were expressed as mean ± standard error. Mean values were compared using One-Way analysis of variance (ANOVA) followed by the Tukey test. Mean values were considered significantly different when $p<0.05$. Correlation between OCR and NKA was evaluated using the Pearson correlation index (*R*).

Results

Mean water temperature and NH_3-N concentration did not differ among treatments over the experimental period ($p>0.05$). Mean values corresponded to 28.2 ± 0.1°C and 0.02 ± 0.00 mg L^{-1}, respectively, while dissolved oxygen remained above 90% saturation. Mean water pH was similar among salinities 6, 12 and 24‰ (general mean = 8.20 ± 0.02) ($p>0.05$), but lower in fresh water (6.93 ± 0.01) ($p<0.05$).

There was no effect of water salinity on fish survival, which was close to 100% in all treatments ($p>0.05$). Initial body weight was similar among treatments ($p>0.05$). However, final body weight and SGR were significantly affected by water salinity. They increased with increasing salinity, being significantly higher in salinity 24‰ than in fresh water ($p<0.05$). In turn, WCV, FE and CF were similar in all salinities ($p>0.05$) (Table 1). Also, gill NKA (Fig. 1), OCR (Fig. 2), and liver glycogen (Fig. 3) content did not differ among salinities ($p>0.05$). However, mean values of NKA showed the "U-shaped" type response across the range of salinities tested, being lower near the isosmotic salinity (6-12‰) and higher at salinities 0 and 24‰ (Fig. 1). OCR followed a similar pattern to that described for gill NKA (Fig. 2). In fact, a significant and positive correlation was observed between gill NKA and OCR ($OCR = 0.049 + 0.119\ x\ NKA$; $R = 0.942$). Also, no significant difference was observed in liver glycogen content among salinities ($p>0.05$). However, a tendency of higher glycogen content was observed with increasing salinities (Fig. 3).

Table 1. Body weight, weight coefficient variation (WCV) specific growth rate (SGR), feed efficiency (FE) and condition factor (CF) in juvenile Lebranche mullet *Mugil liza* maintained in different salinities for 40 days[1]. [1]Data are expressed as mean ± standard error (n = 20). Different letters denote significant difference among treatments for the same parameter ($p < 0.05$).

Parameter	Salinity (‰)			
	0	6	12	24
Initial body weight (g)	0.48 ± 0.00[a]	0.46 ± 0.02[a]	0.48 ± 0.00[a]	0.49 ± 0.01[a]
Final body weight (g)	6.16 ± 0.17[a]	6.50 ± 0.12[ab]	6.77 ± 0.08[b]	7.22 ± 0.05[b]
WCV	0.20 ± 0.01[a]	0.22 ± 0.01[a]	0.21 ± 0.01[a]	0.18 ± 0.01[a]
SGR (g/day)	6.39 ± 0.06[a]	6.44 ± 0.07[ab]	6.76 ± 0.06[ab]	6.78 ± 0.07[b]
FE (gain/g dry feed)	0.79 ± 0.04[a]	0.80 ± 0.03[a]	0.82 ± 0.02[a]	0.80 ± 0.03[a]
CF	2.36 ± 0.07[a]	2.44 ± 0.03[a]	2.51 ± 0.02[a]	2.62 ± 0.02[a]

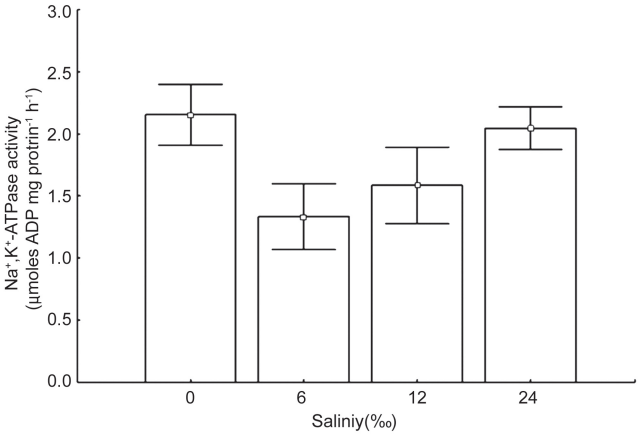

Fig. 1. Gill Na^+- K^+- ATPase activity in juvenile Lebranche mullet *Mugil liza* (n = 6) after acclimation to different salinities for 40 days. Data are expressed as mean ± standard error.

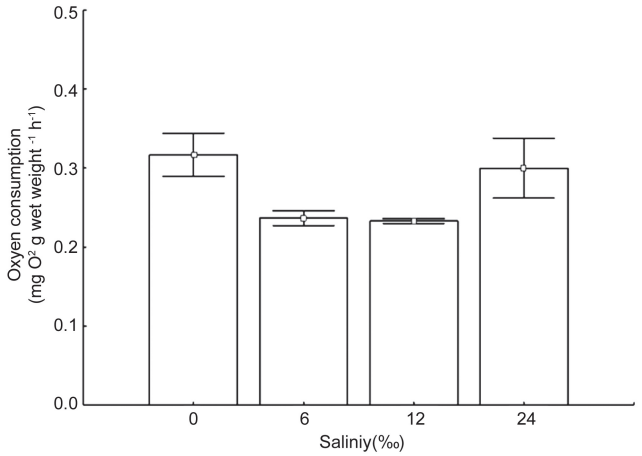

Fig. 2. Whole-body oxygen consumption in juvenile Lebranche mullet *Mugil liza* (n = 6) after acclimation to different salinities for 40 days. Data are expressed as mean ± standard error.

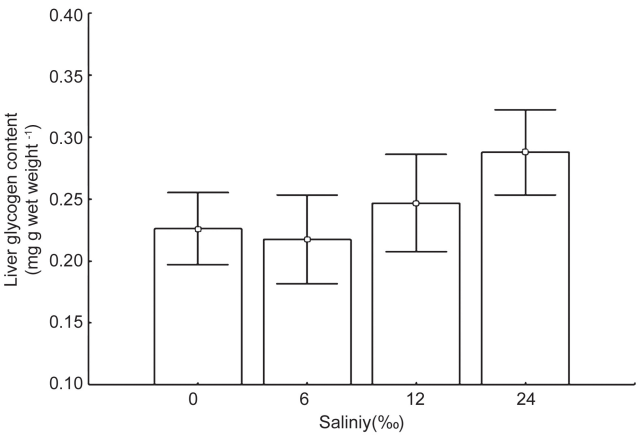

Fig. 3. Liver glycogen content in juvenile Lebranche mullet *Mugil liza* (n = 6) after acclimation to different salinities for 40 days. Data are expressed as mean ± standard error.

Discussion

Fish held in hypo or hyperosmotic environments would show additional energy requirements for osmoregulation that could hamper growth when compared to those kept at an isosmotic environment (Boeuf & Payan, 2001; Tsuzuki *et al*., 2007; Herrera *et al*., 2009; Pérez-Robles *et al*., 2012). Energy cost associated with osmoregulation may vary at the different ontogenetic developmental stages. In addition, response to salinity has shown to be highly variable among fish species. This variation is likely associated with the interaction between osmoregulatory mechanisms with other physiological processes (Jobling, 1994; Imsland *et al*., 2002) and fish ecology (O´Neill *et al*., 2011).

Among all parameters analyzed in the juvenile mullet *M. liza* only growth measured as wet body mass was affected by water salinity, with fish acclimated to fresh water showing a lower growth than those maintained in salt water (24‰). These results are in agreement with findings of Sampaio & Bianchini (2002). The lower growth in fresh water could be due to an increased gill NKA activity and, consequently, to a higher energy expenditure associated with osmoregulation. Effectively, the osmoregulatory energy demand is usually directly correlated to gill NKA (Laiz-Carrión *et al*., 2005). In turn, OCR is often used to estimate the metabolic status of fish subjected to different water salinities (Sampaio & Bianchini, 2002; da Silva-Rocha *et al*., 2005). This parameter was significantly and positively correlated with the gill NKA in juvenile *M. liza* over the range of salinities tested. This finding clearly indicate that the osmotic comfort zone for juvenile *M. liza* corresponds to water salinities in or around the isosmotic environmental condition. Actually, the response of juvenile *M. liza* gill NKA in the range of salinities tested corresponded to the "U-shaped" marine euryhaline model reported by Jensen *et al*. (1998), Boeuf & Payan (2001) and Varsamos *et al*. (2005). Jensen *et al*. (1998) highlighted that this pattern could represent an adaptive mechanism to improve the energy use since it allows fish to maintain a low gill NKA when facing a wide salinity gradient.

The lower growth observed in juvenile *M. liza* maintained in fresh water seems to be related to a higher energy cost associated with osmoregulation under this environmental condition. Although no significant difference was observed in gill NKA, OCR and liver glycogen content among salinities, the trends of higher gill NKA and OCR combined with the trend of lower liver glycogen content observed in fish maintained in fresh water support this idea. However, it is possible that the lower growth observed in fresh water is likely caused by an increased energy demand for osmoregulation and maintenance of other physiological processes. This statement is based on the fact that trends of increase in gill NKA and OCR were also observed in juvenile *M. liza* maintained in salt water (24‰), but no decrease in liver glycogen content was observed. Under this osmotic condition, even a trend of increase in liver

glycogen content was observed. The possible use of an alternative source of energy other than carbohydrates by fish maintained in fresh water, as discussed below, cannot be ruled out. In fish, plasma glucose level necessary to meet the metabolic energy requirement can be maintained based on glycogen reserves (Baldisserotto *et al*., 2007; Pérez-Robles *et al*., 2012). However, as previously described in *Mugil cephalus* Linnaeus (Fazio *et al*., 2013), stress related to changes in water salinity can induce alterations in metabolic energy production and utilization. Therefore, energy supply during acclimation to environmental salinity can be fulfilled by other metabolic substrates than carbohydrates, such as proteins and lipids (Tseng & Hwang, 2008). Effectively, Woo & Kelly (1995) reported that the growth improvement observed in *Sparus sarba* (Forsskål) at the isosmotic salinity could be assigned to either a reduction in metabolism when fish is maintained under an isosmotic condition or a metabolic reorganization in order to prioritize the use of carbohydrates and lipids while proteins are spared and used for growth.

The "U-shape" pattern observed for gill NKA and OCR over the range of salinities tested indicates that the energy demand associated with osmoregulation is reduced at intermediate salinities. Therefore, it would be expected that liver glycogen content could be higher under this osmotic condition if only carbohydrates were being used to fulfill the energy demand associated with osmoregulation. However, the higher content of liver glycogen was observed in juvenile *M. liza* maintained at the higher salinity tested (24‰). This finding clearly indicates that the additional energy demand imposed by exposure to high salinity is being supplied, at least in part, by other energy substrates than liver glycogen. In fact, lipids are also reported as an important source of energy during salinity acclimation in the mullet *M. cephalus*. Therefore, it seems that *Mugil liza* is able to reorganize its metabolism in order to prioritize the metabolic use alternative energy-rich substrates such as proteins and/or lipids when exposed to 24‰, as already reported for other euryhaline fish (Woo & Kelly, 1995, Tseng & Hwang, 2008). Considering the facts that *M. liza* is an iliophagus fish (Oliveira & Soares, 1996) and that the optimal dietary level of proteins required by this fish corresponds to 35% crude protein (Carvalho *et al*., 2010), no protein shortage for fish growth would be expected to occur since the feed employed in the present study had 50% crude protein.

The best mean increment in growth was observed when juvenile *M. liza* was maintained in a water salinity corresponding to the double of the relative value considered as isosmotic for teleost fish. This finding clearly indicates that the isosmotic salinity is not necessarily the optimal condition for growth of juvenile *M. liza*, as previously reported for other marine fish (O´Neill *et al*., 2011). Despite that a lower growth performance of juvenile *M. liza* was observed in fresh water, the result obtained was better than that found for other Brazilian marine fish such as the fat snook *Centropomus parallelus* (Tsuzuki *et al*., 2007), the flatfish *P. orbignyanus* (Sampaio & Bianchini, 2002) and the cobia *Rachycentron canadum* (Resley *et al*., 2006) under similar experimental conditions.

In conclusion, data reported here show that energy demand for osmoregulation in juvenile *M. liza* can be minimized in water salinity close to the isosmotic condition. However, the amount of energy spared under this condition is not enough to improve growth in juvenile mullets. In addition, our findings suggest that *M. liza* is able to alternate the use of energy-rich substrates according to the environmental salinity.

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