

## Effect of Salinity on the Oxygen Consumption of Larvae of the Silversides *Odontesthes hatcheri* and *O. bonariensis* (Osteichthyes, Atherinopsidae)

Mônica Yumi Tsuzuki<sup>2\*</sup>, Carlos Augusto Strüssmann<sup>1</sup> and Fumio Takashima<sup>1</sup>

<sup>1</sup>Department of Marine Biosciences; Faculty of Marine Science; Tokyo University of Marine Science and Technology (formerly Tokyo University of Fisheries); Tokyo 108-8477; Minato, Konan 4-5-7; Japan. <sup>2</sup>Laboratório de Piscicultura Marinha; Departamento de Aqüicultura; CCA; Universidade Federal de Santa Catarina; C.P.: 476; mtsuzuki@cca.ufsc.br; 88040-970; Florianópolis - SC - Brazil

### ABSTRACT

Starved larvae of the silversides *O. hatcheri* (2- and 5-days-old) and *Odontesthes bonariensis* (5-days-old) were used to compare the oxygen consumption rates at 0, 5, 10, 20 and 30 ppt salinity. Oxygen consumption of *O. hatcheri* and *O. bonariensis* was minimal at 0 and 10 ppt, respectively, salinities close to those encountered in areas inhabited by these fishes. In both species, oxygen consumption rates thereafter increased with increasing salinity, and then abruptly decreased at 30 ppt. Lower consumption at extreme salinities might be a result of reduced activity, which in itself was salinity-modulated. Differences in activity may explain the fact that oxygen consumption rates of 5-day-old larvae were higher than 2-day-old larvae, which still possess yolk-sac. In this case, starved larvae incurred in higher metabolic demand due to the continuous swimming in the search for food.

**Key words:** Salinity, respiratory metabolism, euryhaline fish, silversides, *Odontesthes hatcheri*, *Odontesthes bonariensis*

### INTRODUCTION

Members of the Atherinopsidae family display various degrees of salinity tolerance and, as a result they have radiated into a wide range of environments (Hubbs et al., 1971; Bamber and Henderson, 1988; Middaugh et al., 1990). One representative of this family, the silverside *Odontesthes bonariensis*, also known as pejerrey, is an important commercial species naturally found in the temperate and sub-tropical inland waters of South America. Pejerrey has been introduced to several countries as a game fish or as a candidate for freshwater aquaculture (Bonetto and Castello, 1985). The congeneric *O. hatcheri*,

from the fresh waters of Patagonia, is also a potential species for cultivation in temperate areas (Strüssmann et al., 1997). Although *O. bonariensis* and *O. hatcheri* are classified as fluvial euryhaline species (Marty, 1992), and are capable of tolerating different salinities (Tsuzuki et al., 2000a,b), the metabolic costs for osmoregulation are not understood in these fishes.

Oxygen consumption has been used as an indirect indicator of metabolism in fish (Cech, 1990), and its measurement at different salinities has been employed in an attempt of assessing the energetic cost of osmoregulation in several species (Farmer and Beamish, 1969; Iwama et al., 1997; Morgan et al., 1997; Kim et al., 1998; Da Silva Rocha et al.,

\* Author for correspondence

2005). Respiratory responses at different salinities seem to differ widely among teleost species. Low oxygen consumption rates were obtained at the isosmotic salinity with the Nile tilapia *Oreochromis niloticus* acclimated to various salinities (Farmer and Beamish, 1969), rainbow trout *Oncorhynchus mykiss* (Walbaum), (Rao, 1968), and sea bream *Sparus sarba* (Woo and Kelly, 1995). On the other hand, Morgan and Iwama (1991) found low oxygen consumption rates in fresh water, and the consumption increased with the increase in salinity with juvenile rainbow and steelhead trout *O. mykiss* and fall chinook salmon *O. tshawytscha*. Ron et al. (1995), studying 20-month fresh and seawater reared Mozambique tilapia *O. mossambicus* from yolk-sac fry, showed that the fish in sea water had significantly lower oxygen consumption rates compared to those reared in fresh water.

The energetic cost of ionic and osmotic regulations seems to play a significant role in growth rates (Boeuf and Payan, 2001). Some studies support the idea of growth enhancement arising from reduced metabolic cost for osmoregulation (Febry and Lutz, 1987; Woo and Kelly, 1995). De Silva and Perera (1976) suggested higher energy/protein requirement in high salinities, an effect that possibly reflects an elevated metabolic cost of osmoregulation in such salinities (Johnson and Katavic, 1986). It has been proposed that energetic cost associated with the ionic and osmotic regulations was minimal in the environment that was natural for a particular species and life-history stage (Morgan and Iwama, 1991).

The metabolic responses during brackish- or seawater acclimation process have never been studied in the atherinidae fish. Therefore, the present trial was to examine the respiratory changes associated with the salinity adaptation in starved larvae of *Odontesthes hatcheri* and *O. bonariensis*.

## MATERIALS AND METHODS

### Source of Materials and Experimental Set Up

Fertilized eggs of *Odontesthes bonariensis* (approximately the 10<sup>th</sup> generation after introduction into Japan) and *O. hatcheri* (3<sup>rd</sup> generation) were obtained by natural spawning of broodstock maintained at the Inland Water

Fisheries Experimental Station, Kanagawa Prefecture Fisheries Research Center, Japan.

Water temperature during the experiments varied between 19.4 and 20.5°C. Salinity levels were obtained by mixing dechlorinated tap water to artificial sea water (Van't Hoff, 1903) and adjusted using an optical refractometer (Atago) to the nearest 1 ppt. Oxygen concentration in the rearing containers was maintained near saturation through constant aeration. Until the start of the oxygen consumption measurements, water was exchanged daily by siphoning the bottom of the containers and adding 70% clean water. Water quality was monitored before and throughout the experiment. The unionised ammonia nitrogen (FAN) level was  $0.02 \pm 0.0$  mg L<sup>-1</sup> (mean  $\pm$  standard error). Water pH varied from 7.3 to 7.6. The photoperiod was controlled at 12 h: 12 h darkness.

### Oxygen Consumption of Larvae At Different Salinities

Starved larvae of *O. hatcheri* (2- and 5-days-old) and *O. bonariensis* (5-days-old) that were incubated and hatched at 0, 5, 10, 20 and 30 ppt were used to estimate the oxygen consumption rates. Four larvae were stocked in each of the stop-flow respirometers (200-mL glass bottles) at the experimental salinities. Fish were allowed to acclimate to the bottles for 12 h, when aeration was provided to maintain dissolved oxygen near saturation. After the acclimation period, 100% of the water was changed with minimum disturbance and the bottles were sealed. The bottles were then kept under constant illumination and isolated from external sources of disturbance. The incubation period of 18 h was determined in pre-trials as the minimum period necessary to detect any difference between the groups before the water dissolved oxygen content dropped to below 50% of the saturation level. Bottles without larvae for each salinity level were used as controls.

Larvae were measured (standard and total lengths) to the nearest 0.01 mm and wet-weighed to the nearest 0.1 mg. The dry weight of the larvae was taken after 24 h at 60°C. The oxygen concentration was determined by Winklers' titration method (Strickland and Parsons, 1972). Oxygen consumption was expressed as weight-specific respiration rate:  $WSR = 1,000 \times V \times (O_{ct} - O_{ex}) / T \times W$  (mL O<sub>2</sub> g<sup>-1</sup> fish dry weight h<sup>-1</sup>), where  $O_{ct}$  and  $O_{ex}$  are the control and experimental O<sub>2</sub> concentrations (mLO<sub>2</sub> L<sup>-1</sup>), respectively,  $V$  is the

volume of the bottles (L), T is the incubation time (h), and W is the dry weight of the fish (g).

### Statistical Analysis

All the treatments were run in triplicate. Differences between the replicates and the treatments were analysed by one-way analysis of variance (ANOVA) with subsequent Tukey test. Statistical significance was assumed at  $P < 0.05$ .

## RESULTS AND DISCUSSION

The oxygen consumption of starved larvae of *O. hatcheri* increased from 0 to 20 ppt and then decreased at 30 ppt (Table 1). The oxygen

consumption of *O. bonariensis* seemed to follow a sigmoidal distribution starting with high consumption rates.

Aside from 30 ppt, oxygen consumption by larvae was minimal at 0 and 10 ppt for *O. hatcheri* and *O. bonariensis*, respectively (see below comments about consumption at 30 ppt). Studies with other species have also revealed cases where the minimum consumption rates occurred in the hypotonic conditions (Moser and Hettler, 1989; Morgan and Iwama, 1991) such as in *O. hatcheri* or near isotonic conditions (Rao, 1968; Farmer and Beamish, 1969) such as in *O. bonariensis* (it was assumed that the osmotic pressure for both species were around 300 mOsm  $\text{kg}^{-1}$ ; Strüssmann et al., 1994).

**Table 1** - Oxygen consumption rates<sup>1</sup> (mL O<sub>2</sub> g<sup>-1</sup> fish dry weight h<sup>-1</sup>) of *Odontesthes hatcheri* and *O. bonariensis* starved larvae at different salinities.

Salinity (ppt)	<i>O. hatcheri</i>		<i>O. bonariensis</i>
		5-days-old <sup>3</sup>	5-days-old <sup>4</sup>
0	0.35±0.12 <sup>a</sup>	0.70±0.02 <sup>a</sup>	1.04±0.03 <sup>a</sup>
5	0.51±0.02 <sup>b</sup>	1.05±0.20 <sup>bc</sup>	0.83±0.00 <sup>b</sup>
10	0.63±0.01 <sup>c</sup>	1.00±0.00 <sup>bc</sup>	0.76±0.00 <sup>c</sup>
20	0.86±0.01 <sup>d</sup>	1.17±0.01 <sup>c</sup>	0.94±0.01 <sup>ab</sup>
30	0.75±0.01 <sup>e</sup>	0.49±0.02 <sup>d</sup>	0.56±0.01 <sup>d</sup>

<sup>1</sup> Data presented as mean ± standard error (n=3). Means in the same column followed by different letters differ significantly ( $P < 0.05$ ).

<sup>2</sup> 2.4±0.0 mg wet weight, 8.9±0.1 mm total length.

<sup>3</sup> 1.8±0.0 mg wet weight, 8.7±0.1 mm total length.

<sup>4</sup> 0.9±0.0 mg wet weight, 7.1±0.1 mm total length.

Morgan and Iwama (1991) reviewed the studies on the metabolic responses of several species of fishes from fresh-, brackish-, and saltwater. They concluded that low metabolic rates are most often associated to the water salinity in which the species are most commonly found and, therefore, most physiologically adapted to, at a particular life stage. This seems to be in agreement with the natural environment in which *O. hatcheri* and *O. bonariensis* are distributed. *Odontesthes hatcheri* occurs in rivers that seem to be poor in minerals (Luchini, 1981; Marty, 1992). In contrast, 0 ppt does not seem to be the usual salinity level encountered in the areas inhabited by *O. bonariensis*. Pampasic lakes are peculiar by the high content of Na, Cl and other elements (Mac Donagh, 1934; Ringuelet et al., 1967) and this seems to be also the condition of the ground water in this region (Saravia et al., 1987).

In both species, the oxygen consumption rates thereafter increased with the increase in salinity and then abruptly decreased at 30 ppt. Swanson (1996) also observed that the oxygen consumption paradoxically decreased at salinities far above that of the seawater in the milkfish (*Chanos chanos*), and concluded that consumption rates were not necessarily related to the magnitude of the osmotic gradient between the organism and the surrounding water. It was suggested that the low oxygen consumption at extremely high salinities was rather a result of the reduced activity, which in itself was salinity-modulated. This probably explains the results at 30 ppt since fish at this salinity were visibly less active than those at lower salinities. Differences in activity also explain the fact that oxygen consumption rates of 5-day-old starved larvae were higher than those of 2-day-old starved larvae with yolk-sac (Table 1). Starved larvae were probably subjected to high metabolic

demand due to the continuous swimming in the search for food (Hunter, 1981).

The present study shows that the oxygen consumption rates are minimal at salinities similar to the ones encountered in the natural habitat of the species, which corroborates with the studies with juveniles and sub-adults that showed that *O. hatcheri* presents higher survival and growth rates, and better adaptability to fresh water than *O. bonariensis* (Tsuzuki, 1999; Tsuzuki et al., 2000a,b).

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## RESUMO

As larvas em inanição de peixe-rei *O. hatcheri* (2 e 5 dias de idade) e *Odontesthes bonariensis* (5 dias de idade) foram usadas para comparar as taxas de consumo de oxigênio em salinidades de 0, 5, 10, 20 e 30 ppt. As taxas de consumo de oxigênio de *O. hatcheri* e *O. bonariensis* foram mínimas a 0 e 10 ppt, respectivamente, salinidades próximas aquelas encontradas nas áreas onde estes peixes habitam. Em seguida, em ambas as espécies, as taxas de consumo de oxigênio aumentaram com o incremento da salinidade, e abruptamente caíram a 30 ppt. As taxas de consumo mais baixas em salinidades extremas podem ser resultado da atividade reduzida, sendo portanto modulada pela salinidade. Diferenças na atividade possivelmente explicam o fato das taxas de consumo de oxigênio de larvas de 5 dias de idade serem maiores em comparação a larvas de 2 dias, ainda com saco vitelínico. Neste caso, larvas em inanição impõem um maior gasto metabólico devido a natação contínua em busca de alimento.

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PÁGINA  
EM  
BRANCO