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Salinity effects on osmoregulation and growth of the euryhaline flounder *Paralichthys orbignyanus*

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Abstract

The flounder, *Paralichthys orbignyanus*, is found in coastal and estuarine waters of the Western South Atlantic Ocean. It is being considered for aquaculture due to its high market price and wide tolerance to environmental factors such as salinity, pH, and nitrogenous compounds. The objective of this study was to characterize the ionic and osmotic regulation of *P. orbignyanus* over the range of its tolerated ambient salinities (0–40 ‰) and to evaluate the survival and growth in freshwater (0 ‰) and seawater (30 ‰) over 90 days. After 15 days of exposure to different salinities (0 ‰, 10 ‰, 20 ‰, 30 ‰ and 40 ‰), plasma osmolality and ionic (Na^+, Cl^-, K^+ and Ca^{2+}) concentrations slightly increased with salinity. The isosmotic point was estimated as 328.6 mOsm kg^{-1} H_2O and corresponded to 10.9 ‰ salinity. After 90 days, survival was similar in freshwater and seawater, but osmo- and ionoregulation was significantly affected in freshwater and flounders reared in this medium showed a lower growth rate than those reared in seawater. Based on the results from this study, *P. orbignyanus* can be characterized as a marine/estuarine euryhaline teleost capable of hyper/hypo iono- and osmoregulation over the fluctuating salinity regime faced by this species in the environment. Furthermore, results suggest that the lower growth rate exhibited by *P. orbignyanus* in freshwater could be due, at least partially, to a higher energy expenditure associated to a higher branchial Na^+, K^+-ATPase activity in this environment.

Keywords: Aquaculture; Flounder; Ionoregulation; Osmoregulation; *Paralichthys orbignyanus*; Salinity

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1. Introduction

Wu and Woo (1983) consider estuaries suitable for aquaculture because they are enclosed and sheltered, but the varying salinity conditions faced by fishes living in this type of environment might impose ionic and osmotic stress.

The flounder *Paralichthys orbignyanus* occurs in estuarine and coastal waters of Brazil, Uruguay and Argentina (Lema et al., 1980), and constitutes an important fishing resource in these areas. Improvements towards its use in aquaculture are being achieved due to its high market price and wide tolerance to environmental factors. For example, it has been demonstrated that *P. orbignyanus* has a high tolerance to salinity (Wasielesky et al., 1995), nitrogenous compounds (Bianchini et al., 1996), and acid stress (Wasielesky et al., 1997). Furthermore, induced spawning in *P. orbignyanus* has been successfully obtained by Cerqueira et al. (1997).

Because salinity is variable in estuarine regions and osmoregulation is an energy demanding process, certain ambient salinities might help maximize growth and/or reproduction by decreasing osmoregulatory energy expenditure. Thus, the ability of estuarine fishes to deal with fluctuating salinity constitutes an important factor when trying to maximize fish growth and reproduction in captivity. In this context, the objective of this study was to characterize the iono- and osmoregulation in *P. orbignyanus* over the environmental salinity range (0–40 ‰) tolerated by the species (Wasielesky et al., 1995) and to evaluate the effect of hypo- (freshwater) and hyperosmotic (seawater) conditions on survival and growth.

2. Materials and methods

2.1. Osmo- and ionoregulatory studies

Flounders (average weight = 176.0 ± 19.5 g) were collected at the Cassino Beach in Southern Brazil (32°S, 52°W), transferred to the laboratory, and held for 5 days in 1000-l tanks filled with water of the same salinity as the collection site (33 ‰). Throughout the experiment, temperature was kept within 22 ± 1 °C and the photoperiod was fixed at 12 L/12 D. After this acclimation period, the flounders were actively preying upon living *Mugil platanus* juveniles given as feed source.

Water at different salinities were prepared using dechlorinated tap water (0 ‰) or concentrated seawater (50 ‰), which was obtained by heating (50 °C) the seawater and providing strong aeration to facilitate evaporation. Flounders acclimated to 33 ‰ salinity were then transferred to 300-l tanks (six fish per tank) and progressively acclimated during 3 days to the desired experimental salinities (0 ‰, 10 ‰, 20 ‰, 30 ‰ and 40 ‰). They were maintained in these salinities for 15 days. During this period, they were fed ad libitum living *M. platanus* juveniles. Feces were siphoned out once daily and at least 50% of the water was renewed.

After the 15-day experimental period, all the fish were actively feeding. They were anaesthetized (50 ppm benzocain) and blood (1 ml) was then sampled by blind cardiac puncture using heparinized syringes and immediately centrifuged. Plasma was stored in

liquid nitrogen for further osmotic and ionic analysis. Samples of each experimental salinity were also taken for osmotic and ionic analysis. A semi micro-osmometer (Knauer; Germany) was used to determine osmolality (mOsm kg^{-1} H_2O). Plasma cations (Na^+, K^+ and Ca^{2+}) and anion (Cl^-) concentrations (mEq l^{-1}) were determined by flame photometry (Digimed NK-2004; Brazil) and titration (Jenway PLM3; England), respectively.

The isosmotic and isoionic points were estimated as the intersect of the isosmotic or isoionic lines and the regression lines between plasma and water osmolality or ionic concentrations.

2.2. Growth studies

Flounders were captured and transferred to the laboratory as described above. They were tagged with colored plastic rings (Ø2 mm) attached to the fish operculum with a nonabsorptive silk thread in order to determine the individual growth rate over a 90-day experimental period. Flounders were then acclimated to seawater (30 ‰) in 500-l tanks for 1 week. During the acclimation period, they were also conditioned to feed ration (Lansy Dynamics; INVE). Flounders were randomly divided into four groups (14 flounders per group). Two groups were maintained in seawater (30 ‰) and the other two were gradually acclimated to freshwater (0 ‰), for 1 week.

During the two acclimation periods as well as the experimental one, the water temperature was kept within 22 ± 1 °C and the photoperiod was fixed at 12 L/12 D. Fish were fed ration in excess by hand at least four times a day. Feces were siphoned out once daily and 30% of the water was renewed.

After acclimation to seawater or to freshwater, individual growth was evaluated over 90 days. At the start of the experiment and every 30 days, flounders were anaesthetized (50 ppm benzocain), and standard length and wet weight were measured. No mortality was associated with this procedure.

Specific growth rate (SGR) was calculated as $SGR=[(\ln w_f - \ln w_i)/t \times 100]$, where w_f = final weight (g), w_i = initial weight (g), and t = time (days).

At the end of the experiment, blood from five flounders of each replicate ($n = 10$ per salinity) was sampled and plasma was obtained as described above. These flounders were then sacrificed and the second gill arch at the left side was dissected. Plasma and gill

Table 1
Osmolality and ionic composition of the experimental media employed

Salinity (‰)	Osmolality (mosM kg^{-1} H_2O)	Na^+ (mEq l^{-1})	Cl^- (mEq l^{-1})	K^+ (mEq l^{-1})	Ca^{2+} (mEq l^{-1})
0	3 ± 0.2^a	nd	2.2 ± 0.3^a	nd	nd
10	332 ± 5.6^b	172 ± 2.3^a	169 ± 3.8^b	2.0 ± 0.1^a	3.4 ± 0.1^a
20	565 ± 7.8^c	361 ± 5.9^b	311 ± 4.9^c	4.4 ± 0.1^b	7.4 ± 0.2^b
30	910 ± 8.9^d	552 ± 5.7^c	469 ± 4.2^d	6.8 ± 0.2^c	10.7 ± 0.4^c
40	1080 ± 15.2^e	778 ± 8.1^d	606 ± 7.6^e	10.5 ± 0.3^d	14.6 ± 0.7^d

Data are expressed as mean ± S.E. ($n = 10$). Same letter indicates statistically equal means within the column ($P > 0.05$). nd = not detected.

samples were frozen in liquid nitrogen until analysis. Plasma osmolality and ionic composition (Na^+ and Cl^-) were determined as described above. The gill filaments were dissected from the frozen gill sample and homogenized in 2 ml of an ice-cold medium containing 250 mM sucrose, 20 mM EDTA and pH 7.4 adjusted with Tris–HCl buffer. Homogenization was performed with a glass Teflon Potter homogenizer and a

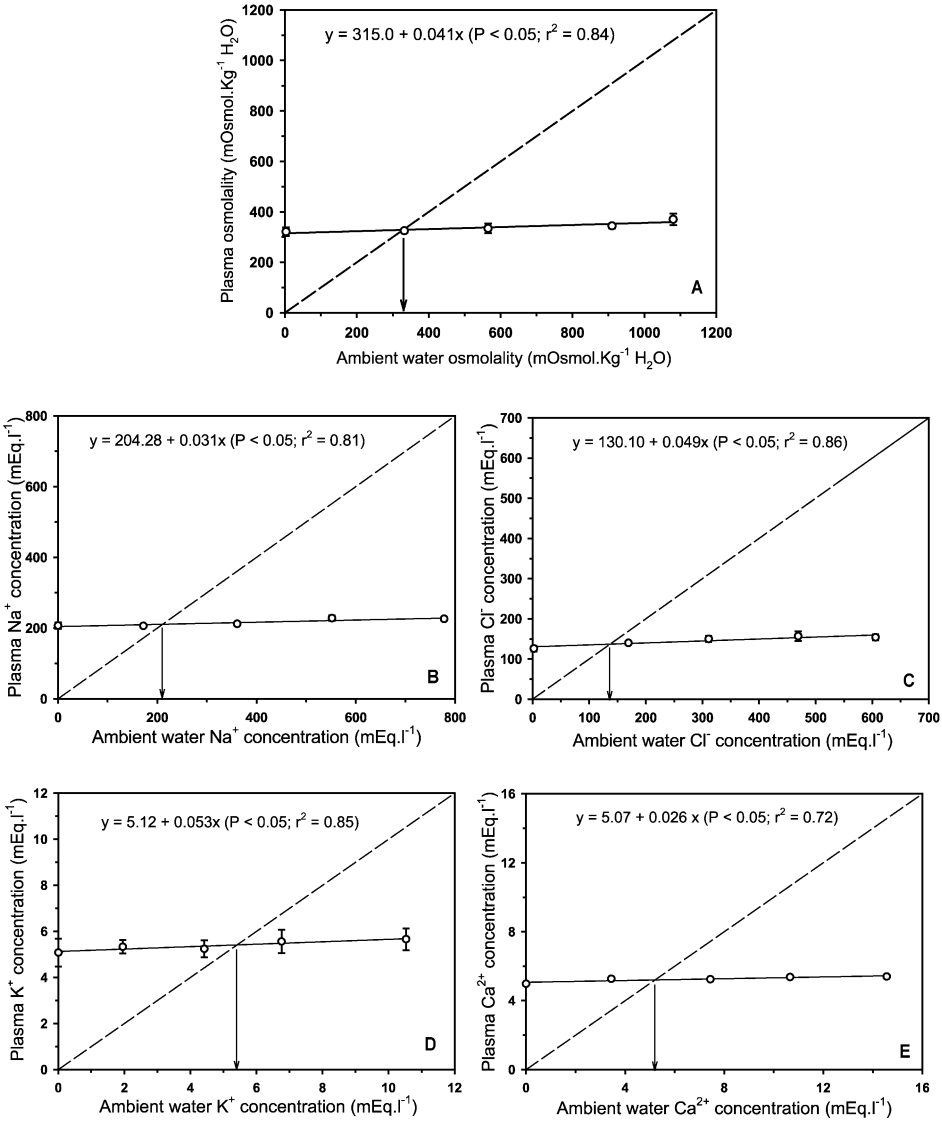

Fig. 1. (A) Plasma osmolality and (B) Na^+, (C) Cl^-, (D) K^+, and (E) Ca^{2+} concentrations of *P. orbignyanus* as a function of ambient water osmolality and ion concentration. Data are means ± S.E. ($n = 6$). The arrow indicates the isosmotic or the isoionic point.

supernatant was obtained by centrifugation (3 °C) at 1000 × *g* for 20 min. Gill Na^+,K^+-ATPase was determined following the method described by Bianchini and Castilho (1999) and Castilho et al. (2001).

Data from growth studies in freshwater and seawater were compared using the Student's *t*-test for two samples. Data normality and homogeneity of variances were previously checked using Kolmogorov–Smirnof and Levene tests, respectively. The significance level adopted was 95% ($\alpha = 0.05$).

3. Results

3.1. Osmo- and ionoregulatory studies

Osmolality and ionic composition of the experimental media are shown in Table 1. A significant positive linear regression was exhibited between water salinity and each variable measured ($P < 0.001$, $r^2 = 0.99$).

Plasma osmolality and ionic composition of flounders acclimated for 15 days to different salinities showed a slightly, but significant, positive linear relationship over the salinity range tested (Fig. 1). The isosmotic point was estimated as 328.6 mOsm kg^{-1} H_2O, which corresponded to 10.9‰ salinity. The isoionic points for Na^+, Cl^-, K^+, and Ca^{2+} were estimated as 210.8, 136.8, 5.41 and 5.21 mEq l^{-1}, which corresponded to 11.6‰, 8.4‰, 22.6‰ and 15.7‰ salinity, respectively.

3.2. Growth studies

There were no significant differences between replicates for both flounder survival and growth over the 90-day test ($P > 0.05$). Thus, data from replicates for the same experimental salinity were pooled and analyzed together.

Table 2
Standard length, wet weight and specific growth rate (SGR) of *P. orbignyanus* reared in freshwater and seawater over 90 days

	Time (days)	Freshwater	Seawater
Length (cm)	0	13.78 ± 0.19^a	14.30 ± 0.22^a
	30	14.65 ± 0.24^a	15.29 ± 0.21^b
	60	15.63 ± 0.26^a	16.83 ± 0.22^b
	90	16.23 ± 0.26^a	17.46 ± 0.25^b
Weight (g)	0	25.95 ± 1.24^a	29.19 ± 1.36^a
	30	33.32 ± 1.70^a	38.85 ± 1.86^b
	60	46.04 ± 2.18^a	56.58 ± 2.36^b
	90	51.75 ± 2.48^a	65.32 ± 2.68^b
SGR (%/day)	30	0.89 ± 0.05^a	0.98 ± 0.11^a
	60	0.85 ± 0.05^a	0.96 ± 0.05^a
	90	0.39 ± 0.03^a	0.49 ± 0.04^b

Data are expressed as mean ± S.E. ($n = 26$). Same letter indicates statistically equal means in freshwater and seawater.

Table 3
Plasma osmolality and ionic composition and gill Na^+,K^+–ATPase activity of *P. orbignyanus* reared in freshwater and seawater for 90 days

	Freshwater	Seawater
Osmolality (mOsm kg^{-1})	261 ± 6^a	320 ± 3^b
Na^+ (mEq l^{-1})	226 ± 4^a	232 ± 3^a
Cl^- (mEq l^{-1})	131 ± 3^a	160 ± 3^b
Na^+,K^+-ATPase (μmol P_i mg^{-1} h^{-1})	29.9 ± 1.7^a	20.6 ± 1.2^b

Data are expressed as mean ± S.E. ($n = 10$). Same letter indicates statistically equal means in freshwater and seawater.

Survival was not significantly affected by salinity ($P > 0.05$), since, at the end of the 90-day test, it was 92.9% in both freshwater and seawater.

At the end of the 90-day test, length and weight of flounders maintained in seawater were significantly higher than those in freshwater. Specific growth rate was also significantly higher in flounders maintained in seawater than those in freshwater (Table 2).

Osmolality and Cl^- plasma concentration of flounders reared in freshwater for 90 days were significantly lower than in those maintained in seawater ($P < 0.001$). However, no significant difference ($P > 0.05$) was detected in the plasma Na^+ concentration of flounders reared in freshwater and seawater (Table 3).

Gill Na^+, K^+-ATPase activity of flounders reared in freshwater was significantly higher ($P < 0.003$) than those registered for flounders reared in seawater (Table 3).

4. Discussion

Plasma osmolality of the euryhaline flounder *P. orbignyanus* was slightly influenced by salinity in the range tested. There was a significant positive linear relationship between plasma and ambient water osmolality. However, the slope was found to be very low ($b = 0.041$). According to Franklin et al. (1992), an osmotic imbalance is considered a symptom of 'non-adaptation'. The fact that salinity only slightly affected both plasma osmolality and ion concentrations of *P. orbignyanus* could indicate that this species is 'adapted' to face ambient water salinity ranging from 0‰ to 40‰ at least for a short period of time (15 days).

The isosmotic point of *P. orbignyanus* was estimated as 328.6 mOsm kg^{-1} H_2O and corresponded to 10.9‰ salinity. This value is similar to those estimated for other euryhaline teleost fishes (Table 4). At this point, it is interesting to note that the plasma osmolality of stenohaline marine teleosts ranges from 370 to 480 mOsm kg^{-1} H_2O, while for stenohaline freshwater teleosts, it ranges from 260 to 330 mOsm kg^{-1} H_2O (Jobling, 1995). When *P. orbignyanus* was acclimated to 40‰ salinity, its plasma osmolality (370 mOsm kg^{-1} H_2O) was equivalent to the lower limit of the range for marine teleosts. On the other hand, when acclimated to freshwater, its plasma osmolality (321 mOsm kg^{-1} H_2O) was close to the upper limit of the range for freshwater fishes. Thus, the osmoregulatory performance showed by *P. orbignyanus* over the salinity range tested for a short period of time (15 days) seems to confirm its physiological euryhalinity.

Table 4
Isosmotic salinity (IS) of selected euryhaline teleost fishes from different habitats

Species	IS (‰)	Habitat	Reference
Fundulus kansae	11.2	FW	Stanley and Fleming, 1977
Jordanella floridae	11.6	FW	Nordlie and Walsh, 1989
Dormitator maculatus	10.2	FW/EST	Nordlie and Haney, 1993
Floridichthys carpio	12.7	EST	Nordlie and Walsh, 1989
Cyprinodon variegatus	11.1	EST	Nordlie, 1985
Poecilia latipinna	11.3	EST	Nordlie et al., 1992
Gillichthys mirabilis	10.5	EST	Yoshikawa et al., 1993
Adinia xenica	12.1	EST/SW	Nordlie, 1987
Sparus aurata	11.6	EST/SW	Tort et al., 1994
Scophthalmus maximus	11.4	EST/SW	Gaumet et al., 1995
Paralichthys orbignyanus	10.9	EST/SW	Present study

Values were estimated from data presented by the respective authors. Preferential habitat: FW – freshwater; EST – estuarine; and SW – seawater.

Euryhalinity can be defined in terms of physiological and ecological adaptations to the environment (Woo and Chung, 1995). Besides its physiological euryhalinity, *P. orbignyanus* also seems to be the one among other flounder species occurring in the Western South Atlantic Ocean coast that most resembles the concept of ecological euryhalinity. This statement is based on the fact that it can be found in freshwater in upper estuaries as well as in full seawater (Fabré and Astarloa, 1996).

Results from ionoregulatory studies in *P. orbignyanus* are also in agreement with those described for other teleost fishes (Cataldi et al., 1995; Gaumet et al., 1995; Woo and Chung, 1995). For example, a survey on plasma Na^+ concentration of euryhaline teleosts is summarized in Table 5. It can be observed that it ranges from 138 to 226 mEq l^{-1}, independently of the salinity considered, i.e., from freshwater to seawater.

Table 5
Plasma Na^+ concentration (mEq l^{-1}) of selected euryhaline teleost fishes

Species	Salinity			Reference
	FW	ISO	SW	
Oncorhynchus kisutch	138	143	150	Morgan and Iwama, 1998
Oncorhynchus clarki	142	*	197	Morgan and Iwama, 1996
Oreochromis mossambicus	145	145	138	Morgan et al., 1997
Leptatherina wallacei	154	164	191	Thompson and Withers, 1992
Mugil cephalus	157	173	203	Nordlie and Leffler, 1975
Oncorhynchus tshawytscha	158	161	156	Morgan and Iwama, 1991
Gadus morhua	163	167	168	Dutil et al., 1992
Sparus aurata	181	*	182	Mancera et al., 1993
Pomacanthus imperator	*	122	163	Woo and Chung, 1995
Scophthalmus maximus	*	156	158	Gaumet et al., 1995
Paralichthys orbignyanus	207	206	226	Present study

Salinity: FW – freshwater (0 – 7 ‰); ISO – isosmotic salinity (10 – 14 ‰); and SW – seawater (25 – 43 ‰). * indicates data not available.

Despite the fact that salinity only slightly affected the osmo- and ionoregulation in *P. orbignyanus* after a short-time exposure (15 days) to extreme salinities (0‰ and 40‰), we must consider that flounder exposure to these salinities for longer periods of time could induce more severe effects on plasma osmotic and ionic balance. In fact, our results from the long-term studies (90 days) show that *P. orbignyanus* exposure to extremely low salinity (freshwater) induced a significant decrease in both plasma osmolality and Cl^- concentration. Similar response was also observed when other euryhaline fish species were exposed to extreme salinities for long periods of time (Nordlie, 1985; Venturini et al., 1992; Cataldi et al., 1995). Some authors consider this response as a symptom of 'nonadaptation', as proposed by Franklin et al. (1992). Based on Morgan and Iwama (1996), such kind of response could indicate that fish is subjected to a stressing salinity condition.

It is described that environmental stressors such as salinity can affect fish growth (Borski et al., 1994). The fact that *P. orbignyanus* growth was significantly reduced by exposure to extremely low salinity (freshwater) over 90 days indicates that this species is actually stressed under such salinity condition. At this point, it is interesting to remind that gills are the main site of active transport of Na^+ and Cl^- from the water into the extracellular fluid of the fish and that branchial Na^+, K^+-ATPase activity is directly related to the Na^+ and Cl^- uptake across the gills (Flik et al., 1997; Perry, 1997). The latter fact characterizes the osmoregulation as an energy-demanding process. In light of the above, we can consider that some ambient extreme salinities such as freshwater might induce reduced growth and/or reproduction rates in euryhaline teleost fishes by increasing osmoregulatory energy expenditure. Therefore, the lower growth rate of *P. orbignyanus* observed in freshwater when compared to that registered in seawater could be explained, at least in part, by an increased osmoregulatory energy expenditure associated to the higher branchial Na^+, K^+-ATPase activity observed in freshwater. This higher enzyme activity is necessary to maintain the plasma osmo- and ionoregulatory balance in such extremely low salinity.

Despite the lower growth rate observed in freshwater, our results indicate that *P. orbignyanus* can survive and grow in freshwater as well as saltwater. This fact is not exclusive of *P. orbignyanus*, but has also been described for other known *Paralichthys* species, such as the southern flounder, *P. lethostigma*, and the summer flounder, *P. dentatus*. For example, salinity tolerance of the southern flounder increases with age, and this species can be grown in salinities as low as 0‰ within days after completing metamorphosis without affecting growth or survival (Daniels and Borski, 1998; Smith et al., 1999). The summer flounder tolerance to salinity also changes with age and reaches a maximum at the juvenile stage, when this species develops tolerance to both low (5‰) and high salinity (50‰) (Schreiber and Specker, 1999).

In summary, our results indicate that the flounder *P. orbignyanus* can be characterized as a marine/estuarine euryhaline teleost capable of carrying hyper/hypo iono- and osmoregulation over the fluctuating salinity regime faced by this species in the environment. However, they show that long-term exposure to freshwater induces a reduction in growth rate of *P. orbignyanus*, possibly due to an increase in energy expenditure associated to the osmo- and ionoregulation under this salinity condition.

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