

The significance of cortisol on acclimation to salinity in pejerrey *Odontesthes bonariensis*

[Importância do cortisol na aclimação a salinidade em peixe-rei *Odontesthes bonariensis*]

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ABSTRACT

The role of cortisol on the osmoregulation of pejerrey *Odontesthes bonariensis* at different salinities was investigated in adult fish injected with 0.7mg hydrocortisone per 100g body weight of fish, and transferred to 0, 5 and 20ppt of NaCl. Blood cortisol was 566ng/ml at the beginning of the experiment (0h) but surged to 1250ng/ml within 3h in cortisol-injected fish. Cortisol levels were influenced not only by treatment but also by time, being higher at 3h compared to 24h. Salinity level, time of exposure and their interaction, but not cortisol treatment, significantly affected plasma osmolality and the concentration of ions Cl⁻ and Na⁺. This study showed that exogenous cortisol does not seem to play a significant role on the regulation of plasma osmolality and concentration of individual ions in pejerrey.

Keywords: pejerrey, *Odontesthes bonariensis*, cortisol, osmoregulation, salinity

RESUMO

Investigou-se a participação do cortisol na osmoregulação de peixe-rei *Odontesthes bonariensis*, em diferentes salinidades, em peixes adultos injetados com 0,7mg hidrocortisona por 100g de peso corporal, e transferidos para 0, 5 e 20ppt de NaCl. No início do experimento (0h), o cortisol encontrado no plasma foi de 566ng/ml, aumentando para 1250ng/ml em 3h em peixes injetados com cortisol. A concentração de cortisol foi influenciada não somente pelo tratamento, mas também pelo tempo, sendo maior 3h após a inoculação, comparada à 24h. A salinidade, o tempo de exposição e a interação desses dois fatores, mas não o tratamento com o cortisol, afetaram significativamente a osmolaridade e a concentração dos íons Cl⁻ e Na⁺ do plasma. Este estudo mostrou que, o cortisol exógeno não influi significativamente na regulação da osmolaridade e da concentração de íons Na⁺ e Cl⁻ no plasma em peixe-rei.

Palavras-chave: peixe-rei, *Odontesthes bonariensis*, cortisol, osmorregulação, salinidade

INTRODUCTION

The pejerrey, *Odontesthes bonariensis* is an economically important atherinid fish from South America. Pejerrey is considered as a freshwater species because of its natural

occurrence in inland waters, and introductions domestically and to other countries have been made almost entirely in fresh water. However, recent studies revealed better performance, in particular higher survival and growth rates, at salinities of 5 to 20 parts per thousand (ppt) compared to 0ppt (Tsuzuki et al., 2000a; 2001).

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It was also shown that pejerrey held in 0ppt presents basal levels of cortisol several-fold higher than those of typical freshwater species such as carp or tilapia (Tsuzuki et al., 2000b; 2001). Furthermore, pejerrey maintained in fresh water after stressful conditions such as transportation or handling had marked increases in blood cortisol, glucose and hematocrit values, and decreases in blood ions and osmolality, whereas fish transferred to higher salinity levels showed the opposite responses (Tsuzuki et al., 2001).

In most fish, cortisol has been found to be the most important corticosteroid in the circulation, playing an important role in the osmoregulatory process (Assem and Hanke, 1981; Morgan and Iwama, 1996; Sakamoto et al., 2001), and in the respiratory and intermediary metabolism (Chan and Woo, 1978; Boon et al., 1991; Morgan and Iwama, 1996). Cortisol has been regarded as a stress-related hormone (Schreck, 1981) as well as a seawater-adapting hormone, improving the hypoosmoregulatory ability in some teleost fish (Bisbal and Specker, 1991; Hwang and Wu, 1993). Cortisol stimulates Na^+ - K^+ -ATPase activity and influences water and electrolyte exchange in tissues (Forrest et al., 1973; Abo Hegab and Hanke, 1984; Madsen, 1990).

In studies with pejerrey, as previously cited (Tsuzuki et al., 2000b; 2001), cortisol levels were possibly affected not only by the stress of husbandry procedures, but also by salinity, making it impossible to establish a causal relationship between the changes in cortisol with those observed in glucose, ions and osmolality. Artificial elevation of blood cortisol levels has been regarded as an effective tool to isolate the effect of stress and investigate the role of cortisol in metabolism and osmoregulation in fish (Assem and Hanke, 1981; Hwang and Wu, 1993; Specker et al., 1994). Improvement of the hypoosmoregulatory ability occurred in the coastal cutthroat trout, *Oncorhynchus clarki clarki* parr (Morgan and Iwama, 1996), in the Atlantic salmon *Salmo salar* (Bisbal and Specker, 1991) and the rainbow trout, *Oncorhynchus mykiss* (Madsen, 1990). Cortisol injection increased ATPase activity and reduced the increases in plasma Na^+ in the stenohaline common carp, *Cyprinus carpio*, after transfer to a salinity of 15ppt, whereas in the euryhaline tilapia, *Oreochromis mossambicus*

(=*Sarotherodon mossambicus*) exposed to 27ppt, it inhibited enzyme activity and had no effect on the Na^+ concentration (Abo Hegab and Hanke, 1984). In yearling coho salmon, *Oncorhynchus kisutch*, and in channel catfish, *Ictalurus punctatus*, cortisol treatment did not prevent changes in plasma osmolality and electrolyte concentrations in fresh water or during seawater acclimation (Redding et al., 1984; Eckert et al., 2001). Therefore, cortisol influence on the osmoregulatory process seems to differ widely among fish. No study on the physiology of cortisol has ever been conducted in atherinid fish.

Hence, the objective of the present study was to investigate the role of cortisol on the osmoregulation of adult pejerrey.

MATERIAL AND METHODS

Hatchery-reared adults (mean \pm SEM body weight of 237.6 \pm 2.3g and total length of 31.6 \pm 0.1cm) were purchased from a commercial pejerrey fish-farm¹. Fish were raised and maintained in fresh water until the beginning of the experiment, except for a brief exposure to 5ppt during and immediately after transport from the farm to the laboratory at the Tokyo University of Marine Science and Technology, Japan. Fish were then allowed to acclimate to the experimental tanks (180l), at a density of 67fish/m³ (16kg/m³) for one week, at 0ppt with constant flow. Fresh water was obtained by dechlorination of Tokyo city tap water through an activated charcoal filter. After filtration the water had the following characteristics: dissolved oxygen = 7.5mg/l, pH=7.8, $\text{NH}_4\text{-N}$ <0.1mg/l, alkalinity = 44.4mg/l, total hardness = 70.1mg/l, non detectable levels of heavy metals (Cu, Cd, Hg, Se, Cr, Zn, Fe, Pb, As), Na^+ = 16.2mg/l, Cl^- = 23.8mg/l, and free chlorine = 0.01mg/l.

Experimental salinities in the tanks were produced by dissolving NaCl in dechlorinated tap water and adjusted with an optical refractometer² to the nearest 1ppt. The rearing water during the experiment was daily exchanged at a make up rate of 50%; this procedure ensured that unionised ammonia nitrogen was kept below 0.04mg/l. Constant temperature (20.4 \pm 0.1°C; mean \pm SEM) and

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natural photoperiod were used. Aeration was provided to maintain dissolved oxygen near saturation levels throughout the experiment.

Fish were fed to satiation once a day with a commercial diet for ayu. Feeding was discontinued 48h prior to and during the experiment. Groups of control and cortisol-treated fish within the same salinity were distinctively tagged with subcutaneous implants of charcoal ink and stored in a communal tank to avoid differential handling- and tank-related bias. Fish were rapidly anesthetized with 0.7ml/l 2-phenoxyethanol for measurement, tagging and injection.

Cortisol was administered as a single injection bellow the pelvic fin since this form of administration elevated cortisol to desirable levels and resulted in less stress than abdominal pellet implantation in adult pejerrey (Tsuzuki, 2000)³. A dosage of 0.7mg hydrocortisone⁴ per 100g of body weight of fish was delivered using 1% saline solution (SS) as a vehicle. It was found in preliminary trials that this dosage caused a surge in plasma cortisol consistent with that of stressed animals transferred to fresh water, i.e. maximum levels around 1200ng/ml, approximately 3h after transfer (Tsuzuki et al., 2001). Injection volumes in all treatments were 0.2ml of vehicle per 100g body weight.

Fish injected with SS only (control) and SS plus cortisol were immediately transferred to 0, 5 and 20ppt in 180l plastic tanks at a density of 16kg/m³. In order to verify if the injection itself had any effect on the parameters studied, additional groups of fish were sham-injected (only needle perforation) and non-injected (only handled) and then transferred to 0ppt.

Blood samples were taken from three to four fish in each group before, and at 3 and 24h after injection and transfer to different salinities. Total blood (usually between 2-4ml) was drawn from caudal vessels into lithium-heparinized syringes. Plasma was extracted from the blood by centrifugation for 10min at 3,000rpm and stored at -85°C until analysis. Plasma cortisol levels were determined by a radioimmunoassay

procedure (I¹²⁵ radioimmunoassay SPAC-S cortisol kit)⁵. Plasma osmolality was measured by a vapour pressure osmometer (5500 vapour pressure osmometer)⁶ and ions Cl⁻ and Na⁺ by ionmeter (CIM-104A ionmeter)⁷.

Data were analysed using a PROC MIXED in SAS (SAS/STAT software version 9, SAS Institute) by multifactorial analysis of variance (three-way ANOVA) to assess the significance of changes with cortisol treatment (yes or no), time of observation (3 and 24h), and salinity (0, 5 and 20ppt) and their interactions. The significance of the differences between the group means was assessed by t-test (P<0.05). Results are expressed as mean±SEM.

RESULTS AND DISCUSSION

In the present study, mortality or differential behaviour was not observed in any of the cortisol or salinity treatments.

Plasma cortisol, osmolality, Cl⁻ and Na⁺ in sham- and non-injected (only handled) fish showed the same trends as the control SS fish (Fig. 1 and 2) (P>0.05). Cortisol level, which was 566ng/ml before the beginning of the experiment (0h), increased to about 1250ng/ml within 3h in cortisol-injected fish regardless of salinity, and decreased at 24h (Fig. 1). Only cortisol treatment and time affected blood cortisol levels (Tables 1 and 2).

However, while plasma cortisol attained the desired levels in cortisol-injected pejerrey, it had no noticeable effect on osmolality and ion levels at any of the salinities. Plasma osmolality and the concentration of ions Cl⁻ and Na⁺ in general showed similar trends, being affected by salinity, time and the interaction of salinity and time, but not by cortisol (Table 1). Osmolality values were higher at 20ppt, compared to 0 and 5ppt, at 3 and 24h. At 5 and 20ppt, values at 3 and 24h were statistically different, with maximum value at 24h (P<0.001) The same trend was found for Cl⁻ and Na⁺, although differences between 0 and 5ppt were obtained at 24h for these ions (Fig. 1; Table 3).

³Tsuzuki, M.Y., preliminary results, 2000 - Tokyo University of Marine Science and Technology, Tokyo, Japan.

⁴Sigma-Aldrich Co.- Tokyo, Japan.

⁵Daiichi Radioisotope - Chiba, Japan.

⁶Wescor Inc. - Utah, USA.

⁷Shimadzu - Kyoto, Japan.

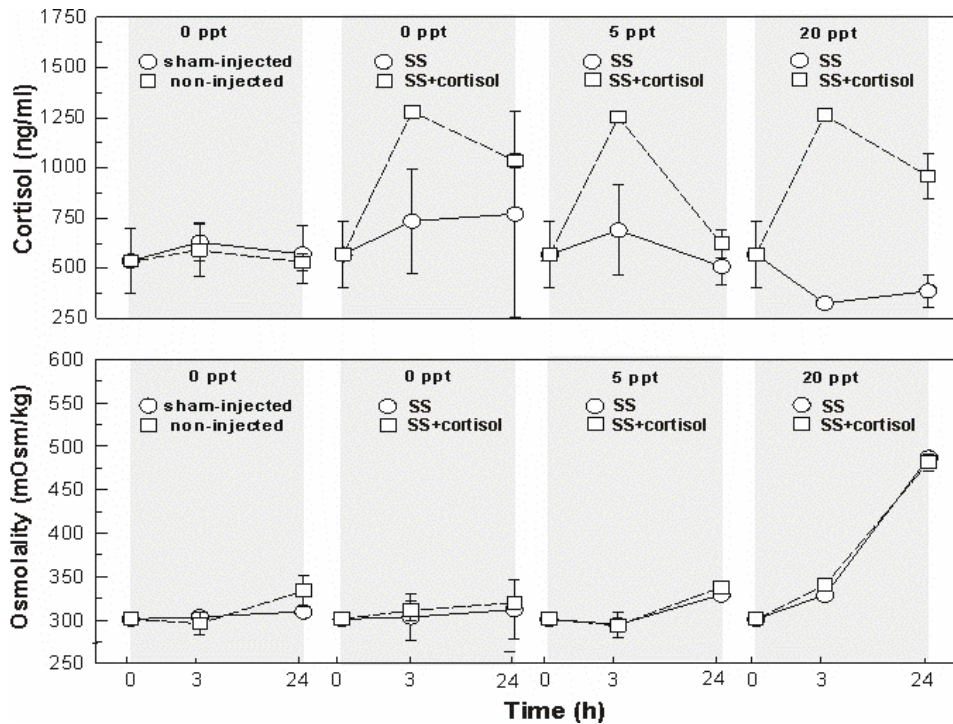


Figure 1. Plasma cortisol and osmolality levels in saline solution (SS) and cortisol solution injected, sham-injected and non-injected pejerrey transferred to 0, 5 and 20ppt. Each point represents the mean±SEM.

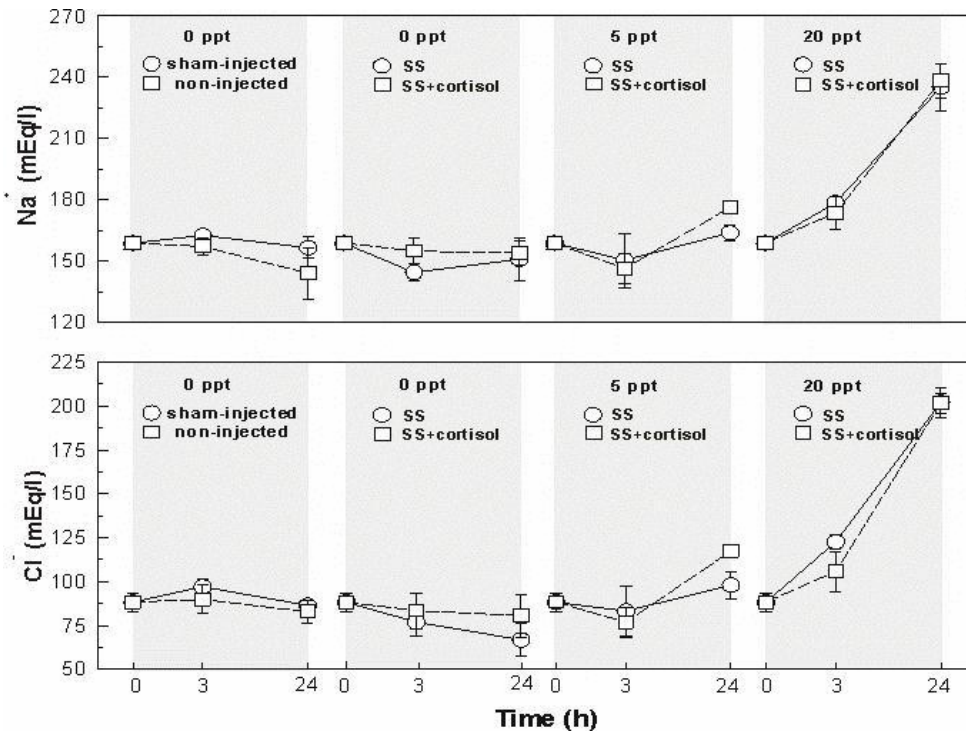


Figure 2. Plasma Cl⁻ and Na⁺ levels in saline solution (SS) and cortisol solution injected, sham-injected and non-injected pejerrey transferred to 0, 5 and 20ppt. Each point represents the mean±SEM.

The significance of cortisol...

Table 1. Summary of the three-way analysis of variance for blood cortisol, osmolality and ions Cl⁻ and Na⁺ levels in pejerrey *Odontesthes bonariensis*

Effect	Cortisol (ng/ml)		Osmolality (mOsm/kg)		Cl ⁻ (mEq/l)		Na ⁺ (mEq/l)	
	F-value	Pr>F	F-value	PR>F	F-value	Pr>F	f-value	Pr>F
Salinity	2.42	0.1125	82.28	<0.0001	73.82	<0.0001	55.13	<0.0001
Time	5.85	0.0243	83.49	<0.0001	39.90	<0.0001	37.38	<0.0001
Cortisol	32.14	<0.0001	0.81	0.3772	0.22	0.6467	0.52	0.4799
Salinity and time	1.18	0.3264	29.60	<0.0001	22.85	<0.0001	13.51	0.0002
Salinity and cortisol	2.21	0.1334	0.07	0.9314	1.00	0.3838	0.24	0.7894
Time and cortisol	4.18	0.0530	0.01	0.9182	2.09	0.1626	0.37	0.5506
Salinity, time and cortisol	0.08	0.9256	0.36	0.6985	0.22	0.8063	0.57	0.5719

Table 2. Test for mean differences among time (h) and cortisol treatment for blood cortisol in pejerrey *Odontesthes bonariensis*

Time (h)	Cortisol treatment	DLSM ¹	Probability ²
3 X 24		204.9	<0.0001
	no X yes	-527.7	<0.0001

¹DLSM = differences of least squares means; ²Probability value for DLSM among time or cortisol treatment in blood cortisol level.

Table 3. Test for mean differences among time (h) and salinity (ppt) for blood osmolality, Cl⁻ and Na⁺ in pejerrey *Odontesthes bonariensis*.

Salinity	Time	Osmolality		Cl ⁻		Na ⁺	
		DLSM ¹	Probability ²	DLSM	Probability	DLSM	Probability
0	3 X 24	-9.1	0.3806	-5.2	0.5994	-2.8	0.7157
5	3 X 24	-38.9	0.0003	-26.0	0.0134	-20.7	0.0105
20	3 X 24	-149.8	<0.0001	-87.8	<0.0001	-60.7	<0.0001
0 X 5	3	13.0	0.1433	0.1	0.9887	1.2	0.8624
0 X 5	24	-16.7	0.1020	-31.0	0.0056	-16.7	0.0437
0 X 20	3	-27.3	0.0068	-34.1	0.0011	-26.3	0.0011
0 X 20	24	-168.0	<0.0001	-127.0	<0.0001	-84.2	<0.0001
5 X 20	3	-40.3	0.0001	-34.2	0.0011	-27.6	0.0007
5 X 20	24	-151.3	<0.0001	-96.0	<0.0001	-67.5	<0.0001

¹DLSM = differences of least squares means. ²Probability value for DLSM among levels of salinity or time for osmolality, Cl⁻ or Na⁺.

The lower cortisol levels at 5 and 20ppt after stress of transport or crowding of adult pejerrey as observed in previous studies (Tsuzuki et al., 2001) were possibly due to a natural preference for higher salinity levels, resulting in decreased cortisol secretion, or increased clearance rates, as pointed out by Goswami et al. (1983), Redding et al. (1984) and Nichols and Weisbart (1985). Data on the survival and growth of pejerrey larvae and adults at different salinities seemed to support the first hypothesis as both were shown to be euryhaline and to perform better at salinities close to 20ppt, especially compared to 0ppt (Tsuzuki et al., 2000a). This notion was reinforced by information on the natural distribution of this species, which, although continental, coincides with areas where surface

and ground water have substantial salinity (Mc Donagh, 1934; Ringuet et al., 1967; Saravia et al., 1987).

However, in these studies with pejerrey, cortisol levels were possibly affected by a synergic effect of the stress of husbandry procedures and salinity, making it difficult to evaluate the effect of cortisol on individual ions and osmolality. In this way, one hypothesis tested here was that the artificial elevation of the blood cortisol level would prevent or at least mitigate the increases in plasma osmolality and individual electrolyte concentrations at higher salinity levels, such as 20ppt. Cortisol implantation has been used to demonstrate that cortisol potentiates hypoosmoregulatory ability in some salmonids,

tilapia and medaka (Bisbal and Specker, 1991; Hwang and Wu, 1993; Sakamoto et al., 2001). Single injection of cortisol at similar doses used in this study, also influenced the respiratory and intermediary metabolism in other species (Abo Hegab and Hanke, 1984; Chan and Woo, 1978).

In the present experiment, it is possible that other hormones are involved in the osmoregulation of *O. bonariensis* as cortisol treatment did not affect plasma osmolality and individual ions, fact also observed in channel catfish transferred to hyperionic salt water (Davis and Simco, 1976; Eckert et al., 2001) and in coho salmon during seawater acclimation (Redding et al., 1984). These last authors suggested that other factors in addition to cortisol might be required to activate ion transport mechanisms in coho salmon. Subsequently, physiological roles for catecholamine, thyroxine, cortisone and growth hormone during saltwater acclimation have been proposed in other species (Mazeaud and Mazeaud, 1981; Dangé, 1986; Patiño et al., 1987; Madsen, 1990). For instance, increased demineralization caused by catecholamine action on ionic transfer across the gills was observed in mullet subjected to salt water stress (Pic et al., 1975). Likewise, Dangé (1986) observed that the ability of cortisol to stimulate branchial $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in tilapia was augmented by thyroxine in hyperosmotic conditions whereas Madsen (1990) indicated that cortisol and growth hormone are synergistically involved in the transition to hypoosmoregulation in sea trout parr, *Salmo trutta trutta*.

In contrast to the lack of an effect on osmolality and ions in this experiment, previous studies have shown a close association of glucose and cortisol levels in pejerrey (Tsuzuki et al., 2000b; 2001) in which endogenous cortisol was measured. Cortisol is assigned to have a stimulatory effect on liver gluconeogenesis, elevating blood sugar levels (Wedemeyer et al., 1990; Boon et al., 1991), possibly providing readily accessible energy for osmoregulation (Assem and Hanke, 1979a,b; Abo Hegab and Hanke, 1982; 1984). It is not clear if this is the case with pejerrey since, as discussed above, there were no noticeable changes in ionic and osmotic parameters that could be associated with cortisol and hence with glucose, nor did the cortisol/glucose availability prevent the surge in blood ions at 20ppt. Thus, it appears more likely

that the elevated glucose levels observed as a result of elevations in cortisol levels are associated with other stressful situations such as predator avoidance, counter current swimming and other behaviours where glucose is necessary for muscle activity. This could be a further indication that cortisol, at least alone, is not directly implicated in osmoregulation in pejerrey as discussed previously.

In conclusion, this study showed that exogenous cortisol does not seem to play a significant role on the regulation of plasma osmolality and concentration of individual ions. It is suggested that other factor(s) (perhaps, other hormones acting in conjunction with cortisol) may be required to activate ion transport mechanisms.

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The significance of cortisol...

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