

# Growth, food intake regulation and metabolic adaptations in goldfish (*Carassius auratus*) exposed to different salinities

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## Abstract

The aim of the present study was to investigate the effects of different salinities (0, 2, 4, 6, 8 and 10‰) on food consumption, growth, metabolic resources, and several stress indicators in goldfish. Possible changes in feeding regulators, brain neuropeptide Y, circulating ghreline, and the hypothalamic monoaminergic transmission were also examined. Salinities up to and including 6‰ did not affect weight gain, standard growth and feed conversion rates. The goldfish showed good adaptation to these salinities in terms of metabolic resources (lipids and glycogen content in liver and muscle) after 21 days of salinity exposure. The unaltered haematocrit, haemoglobin, glycemia and plasma cortisol levels indicated that salinities up to and including 6‰ do not produce significant stress in goldfish. Higher salinities (8 and 10‰) produced significant muscle dehydration, significant increases in circulating cortisol, and adverse effects on growth, food intake and food conversion rate. Although this salt-induced reduction in food intake does not appear to involve either central (neuropeptide Y) or peripheral (ghrelin) potent orexigenic regulators for this species, a possible role for the hypothalamic serotonergic system cannot be discarded. Diurnal locomotor activity was significantly lower in all goldfish exposed to salinity compared to FW fish. In conclusion, *Carassius auratus*, a freshwater stenohaline fish exhibits good growth and no signs of stress in saline waters up to 6‰ salinity. These results demonstrate that using such salinities to reduce the incidence of diseases and mortality does not produce significant physiological alterations in this species.

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## 1. Introduction

The capacity of fish to osmoregulate in the face of changing environmental salinity has been thoroughly investigated in species that migrate between freshwater and seawater during their life cycle, or that live in estuaries (McCormick and Bradshaw, 2006). Although less studied, the physiological responses of freshwater stenohaline species to saline environments are attracting increased interest, particularly with respect to using saline water for the optimization of aquaculture practices.

Salt addition has been demonstrated to optimize embryonic and larval growth and development of some freshwater fish (Luz and Portella, 2002; Fashina-Bombata and Busari, 2003). Moreover,

beneficial effects of salinity have been reported in the prevention of some diseases in juvenile and adult fish (Barton and Zitzon, 1995; Altinok and Grizzle, 2001a). Reports have described the efficacy of salts to mitigate the effects of stress during handling, crowding and transport (Wurts, 1995; Carneiro and Urbinati, 2001) and in post-stress recovery (Tsuzuki et al., 2001).

One of the physiological functions clearly influenced by water salinity in fish is growth (Boeuf and Payan, 2001; Engström-Öst et al., 2005). Thus, larval culture at low salinities produces higher growth and survival rates than in freshwater conditions in some freshwater species (Britz and Hecht, 1989; Luz and Portella, 2002; Luz et al., 2004). However, the effect of salinity on growth of juvenile and adult stenohaline freshwater species has received less attention, and a great deal of variation exists between those species studied (Maceina and Shireman, 1980; Wang et al., 1997; Heyward et al., 1995; Altinok and Grizzle, 2001b; Engström-Öst et al., 2005). Moreover, the

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Table 1  
Water pH and osmolality at the different salinities tested

	Salinity (‰)					
	0	2	4	6	8	10
pH	7.08±0.10 <sup>a</sup>	6.99±0.07 <sup>ab</sup>	6.93±0.11 <sup>ab</sup>	6.85±0.05 <sup>b</sup>	6.87±0.11 <sup>b</sup>	6.84±0.08 <sup>b</sup>
Osmolarity (mOsm/l)	3.33±0.51 <sup>c</sup>	70.0±2.48 <sup>d</sup>	139.0±2.48 <sup>c</sup>	195.3±11.85 <sup>bc</sup>	250.3±5.24 <sup>ab</sup>	284.0±33.24 <sup>a</sup>

Values are mean±S.E.M.; (n=4/group). Different letters indicate statistically significant differences.

underlying physiological mechanisms involved in the relationship between salinity and growth remain unknown to date.

Goldfish (*Carassius auratus*) is a freshwater stenohaline cyprinid, popular in ornamental aquarium industry, where the use of salts is common practice. In fact, low salinity (1‰) exposure reduces mortality of goldfish experimentally challenged with bacterial pathogens (Altinok and Grizzle, 2001a). However, there is no information on the physiological mechanisms underlying the benefits of salt addition. In fact, negative effects of salinity on growth performance in juvenile goldfish have been reported by Altinok and Grizzle (2001b), where growth rate and feed conversion ratio were adversely affected by salinity (1, 3 and 9‰). However, to date no studies have analyzed the possible changes in feeding, metabolism, and resources underlying such effects. This lack of information and our previous experience on food intake regulation in goldfish, led us to perform the present study. Our main objective was to elucidate the physiological and behavioural adaptations to changes in salinity in the goldfish. Our interest focuses on food consumption and on some key regulators of feeding, and on possible effects on growth performance and metabolic resources. Moreover, locomotor activity and some stress indicators, such as haematological parameters, plasma glucose and cortisol were also studied to obtain further information on the physiological responses to salinity exposure in a stenohaline freshwater teleost.

## 2. Materials and methods

### 2.1. Animals

Goldfish (*C. auratus*) provided by a commercial supplier in Madrid were maintained in holding aquaria (50 l) with a constant flow of filtered freshwater (FW) at 18.5±1.3 °C and 12 L:12D photoperiod (lights on at 0700 h). They were fed twice daily with a 2% body weight (bw) ration of floating pellets (Sera Biogram) at 0900 and 1500 h. Animals were acclimated to these conditions for at least 30 days prior to the experimental use, showing a normal feeding pattern during this acclimation period. All the experiments in the present study were performed in accordance with the UFAW Handbook on the Care and Management of Laboratory Animals and complied with the Spanish legal requirements.

### 2.2. Effect of salinity on food intake, growth and metabolism (Experiment 1)

Goldfish (n=72, 20.06±3.32 g bw) were placed in 5 l-aquaria (3 goldfish/aquarium) at 18.5±1.3 °C, where continuous aeration was provided to maintain dissolved oxygen near saturation levels. In each aquarium, 75% of water volume was renewed daily. Salinity was gradually raised 2‰ per 24 h by adding marine salt to reach the following salinity groups: 0 (FW), 2, 4, 6, 8 and 10‰, and then goldfish were exposed to these salinities for 21 days. These treatments were tested in four replicates. The water pH values were measured by a pHmeter (Crison, GLP 21, Barcelona, Spain), and osmolality was determined with a micro-osmometer (13DR-autocal, Medical Equipment Company, Worcester-

shire, United Kingdom) at the beginning of the experiments. The feeding timetable remained unchanged (twice per day) throughout the experimental period, but the total daily ration was increased to 3% bw. Any food remaining 30 min after feeding was collected and quantified as described below. At the end of experimental period, blood (1 ml) was withdrawn from the caudal vein using a 1 ml sterile plastic heparinized syringe and a 0.5 mm×16 mm Microlance needle. Plasma samples obtained by centrifugation were immediately frozen and stored at -80 °C for the analyses described below. Fish were killed at midday (12:00–14:00 h). Total liver was carefully dissected, and muscle tissue samples (0.5 g) were taken from both sides below the dorsal fin. Liver and muscle samples were stored frozen at -80 °C until glycogen, total lipids and proteins were determined as described below.

#### 2.2.1. Biometric indexes

Fish were individually weighed once per week (days 1, 7, 14 and 21) throughout the experimental period. The body weight gain (WG, g) was calculated as the increase in total biomass at the end of the experimental period,  $WG = W_f - W_i$ , where  $W_f$  and  $W_i$  are the final and initial body weights, respectively. The specific growth

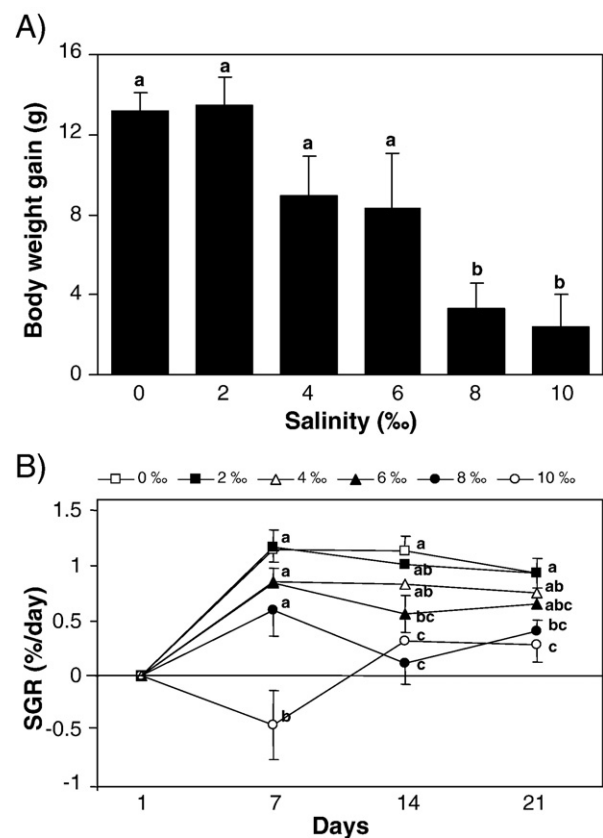


Fig. 1. Effects of chronic exposure (21 days) to different salinities on body weight gain (A) and specific growth rate, SGR, (B) in goldfish (*Carassius auratus*). Values are mean±S.E.M (n=4/group). Different letters indicate significant differences between salinity treatments (ANOVA, Duncan test  $p < 0.05$ ).

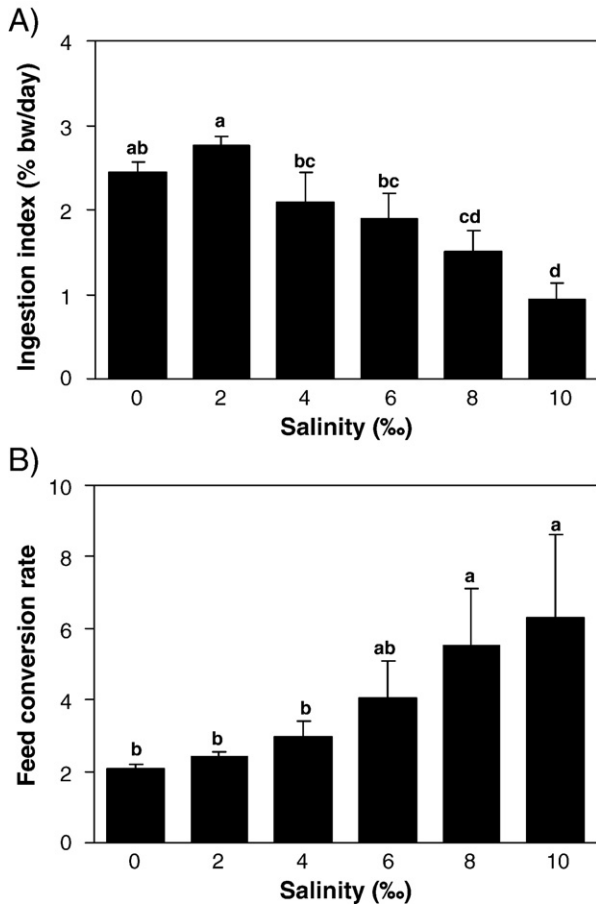


Fig. 2. Ingestion index (A) and feed conversion rate (B) in goldfish (*Carassius auratus*) after chronic exposure (21 days) to different salinities. Values are mean  $\pm$  S.E.M ( $n=4$ /group). Different letters indicate significant differences between salinity treatments (ANOVA, Duncan test  $p<0.05$ ).

rate (SGR, %/day) was calculated according to the formula  $(\ln W_f - \ln W_i) \times 100 / \Delta t$ ; where  $\Delta t$  is the time interval (in days) between  $W_i$  and  $W_f$  measurements. Food intake (FI) was measured daily in each aquarium (3 fish/aquarium) at 30 min after food supply and was calculated as follows:  $FI = W_i - (W_f \times F)$  where  $W_i$  = initial dry food weight,  $W_f$  = remaining dry food weight and  $F$  = correction factor (De Pedro et al., 1997). The ingestion index was defined as  $(\% \text{ bw/day}) = FI \times 100 / W_i$ . The food conversion ratio (FCR) was calculated as total food consumption (g)  $\times$  weight gain<sup>-1</sup>(g) over the experimental period (21 days). Two samples of muscle tissue (0.5 g) were weighed to the nearest milligram immediately upon removal and dried at 80 °C for 24 h and reweighed every 24 h until constant weight was obtained. Water content in muscle was expressed as (wet weight–dry weight)/wet

weight)  $\times 100$ . The hepatosomatic index (HSI) was calculated as (wet liver weight/body weight)  $\times 100$ .

### 2.2.2. Haematology and blood biochemistry

Haematocrit was determined using a microhaematocrit reader (Compur M1100, Lab-Center, Madrid, Spain) and the values were expressed as the percentage of erythrocytes. The haemoglobin concentration was estimated by spectrophotometry (540 nm) using the cyanomethaemoglobin method with Drabkin's reagent (Blaxhall and Daisley, 1973). Plasma glucose and triglycerides were determined using enzymatic/colorimetric methods (Glucose Trinder and GPO-Trinder, respectively, Sigma Diagnostics). Total plasma proteins were measured by the Lowry method (Lowry et al., 1951) using serum bovine albumin as standard.

### 2.2.3. Metabolic resources and cortisol determination

Liver and muscle glycogen contents were quantified by spectrophotometry (Dubois et al., 1956) after extraction with ethanol and previous digestion with KOH (Montgomery, 1957). Total lipids in liver and muscle samples were extracted with chloroform:methanol (2:1) according to Folch et al. (1957), with modifications (Gujarro et al., 2003), and the total amount of lipids was determined by spectrophotometry (505 nm) using triolein as standard. Protein content in liver and muscle was determined by the Lowry method. Cortisol plasma levels was measured in duplicates by radioimmunoassay (RIA) using a commercial kit, (Cortisol RIA-1635, DSL 2000, Diagnostic Systems Labs, Sinsheim, Germany). The validity of this RIA for goldfish plasma has been previously reported (De Pedro et al., 1997).

### 2.2.4. Locomotor activity

Locomotor activity was automatically recorded by one infrared sensor (Omron Corporation, E3 S-AD12, Tokyo, Japan) located on one side of the aquaria, as described by Sánchez-Vázquez and Madrid (2001). Briefly, every time a fish interrupted the light beam, it generated an output signal that was transferred via an interface to a computer that recorded and stored the data at 10-min intervals throughout 24-h cycles. Total daily activity (TAct) was determined individually in each aquarium as the total number of light beams broken throughout the 24-h cycle. The percentages of diurnal (DAct) and nocturnal (NAct) activities corresponded to the activities registered during the photophase (0700–1900 h), and the scotophase (1900–0700 h) of daily photocycle, respectively. The food-anticipatory activity (FAA) was quantified as the sum of the number of light beam interruptions during the 2 h intervals before both daily meals.

## 2.3. Effect of salinity on food intake regulators (Experiment 2)

Goldfish ( $n=24$ ,  $35.03 \pm 4.27$  g bw) were divided in three groups ( $n=8$ /group) and distributed in 5 l-aquaria (2 fish/aquarium) at  $18.5 \pm 1.3$  °C with continuous aeration. The animals were exposed to 0, 2, and 8‰ salinity for 28 days. Each treatment had four replicates. The salinity was gradually raised as described above until the appropriate experimental salinities were achieved. Daily feeding timetable and ration was the same as described for the previous experiment. At the end of the experimental period, blood samples were collected and plasma was stored at  $-80$  °C until ghrelin levels were quantified. The

Table 2  
Haematological and plasma biochemical parameters in goldfish (*Carassius auratus*) after exposition to different salinities

	Salinity (‰)					
	0	2	4	6	8	10
Haematocrit (%)	25.79 $\pm$ 1.28	22.91 $\pm$ 1.17	24.54 $\pm$ 2.80	25.33 $\pm$ 1.97	21.92 $\pm$ 3.16	24.67 $\pm$ 6.67
Haemoglobin (g/100ml)	21.77 $\pm$ 2.20	20.89 $\pm$ 2.49	22.17 $\pm$ 1.81	21.85 $\pm$ 2.57	25.17 $\pm$ 3.91	26.47 $\pm$ 6.06
Osmolality (mOsm/l)	262.8 $\pm$ 4.92 <sup>c</sup>	274.2 $\pm$ 6.53 <sup>c</sup>	271.8 $\pm$ 5.41 <sup>c</sup>	265.3 $\pm$ 12.5 <sup>c</sup>	296.7 $\pm$ 3.02 <sup>b</sup>	351.6 $\pm$ 10.93 <sup>a</sup>
Triglycerides (mg/dl)	349.1 $\pm$ 23.38	317.4 $\pm$ 56.78	277.7 $\pm$ 33.43	353.9 $\pm$ 31.97	342.4 $\pm$ 31.39	331.4 $\pm$ 102.09
Glucose (mg/dl)	36.25 $\pm$ 6.45	28.09 $\pm$ 3.55	32.86 $\pm$ 2.77	26.63 $\pm$ 2.85	25.38 $\pm$ 1.24	32.21 $\pm$ 7.22
Total proteins (mg/ml)	72.63 $\pm$ 4.06 <sup>a</sup>	70.12 $\pm$ 15.86 <sup>a</sup>	57.45 $\pm$ 4.62 <sup>ab</sup>	51.69 $\pm$ 3.41 <sup>b</sup>	52.06 $\pm$ 5.64 <sup>b</sup>	54.61 $\pm$ 1.64 <sup>b</sup>
Cortisol ( $\mu$ g/100ml)	0.81 $\pm$ 0.36	0.98 $\pm$ 0.30 <sup>b</sup>	0.62 $\pm$ 0.27 <sup>b</sup>	0.68 $\pm$ 0.19 <sup>b</sup>	1.03 $\pm$ 0.25 <sup>b</sup>	2.66 $\pm$ 0.71 <sup>a</sup>

Values are mean  $\pm$  S.E.M ( $n=4$ /group). Different letters indicate statistically significant differences ( $p<0.05$ ).

Table 3  
Hepatosomatic index (HSI), liver and muscle metabolic resources in goldfish (*Carassius auratus*) after exposition to different salinities

		Salinity (‰)					
		0	2	4	6	8	10
Liver	HSI (%)	3.57±0.45	3.89±0.16	3.71±0.11	3.39±0.32	3.47±0.40	2.56±0.48
	Proteins (µg/mg)	326.1±47.66 <sup>b</sup>	384.2±17.10 <sup>b</sup>	383.77±2.29 <sup>b</sup>	378.83±4.15 <sup>b</sup>	554.2±6.91 <sup>a</sup>	556.2±90.22 <sup>a</sup>
	Lipids (µg/mg)	0.87±0.16	0.69±0.12	0.59±0.08	0.55±0.05	0.61±0.09	0.70±0.15
	Glycogen (µg/mg)	102.6±13.75	107.7±16.05	74.9±11.53	92.7±7.78	75.8±8.61	79.8±19.26
Muscle	Moisture (%)	78.82±0.50 <sup>a</sup>	78.65±0.32 <sup>a</sup>	78.32±0.60 <sup>a</sup>	77.91±0.30 <sup>ab</sup>	77.87±0.34 <sup>ab</sup>	76.01±1.07 <sup>b</sup>
	Proteins (µg/mg)	521.8±39.11 <sup>b</sup>	519.8±43.82 <sup>b</sup>	494.2±50.97 <sup>b</sup>	681.9±82.67 <sup>ab</sup>	902.3±34.01 <sup>a</sup>	837.3±124.02 <sup>a</sup>
	Lipids (µg/mg)	0.35±0.06	0.48±0.10	0.32±0.07	0.31±0.08	0.25±0.07	0.29±0.05
	Glycogen (µg/mg)	38.75±2.82	30.71±1.55	28.89±4.94	32.56±4.60	27.57±3.49	27.48±2.64

Values are mean±S.E.M ( $n=4$ /group). Different letters indicate statistically significant differences.

telencephalon and the half of hypothalamus were placed in 250 µl and 125 µl, respectively, of 2 M acetic acid containing aprotinin (200 µg/ml) to prevent proteolysis, and immediately stored at  $-80$  °C until analysis. The remaining half of hypothalamus was directly frozen at  $-80$  °C until monoamines were quantified.

### 2.3.1. Ghrelin and NPY determinations

Ghrelin plasma levels were determined in duplicate by radioimmunoassay (RIA) using commercial kits, (Ghrelin RIA S-2227, Bachem, Peninsula Laboratories, Inc.) previously validated for goldfish plasma. For the analysis of hypothalamic and telencephalic NPY content, half of the hypothalamus and full telencephalon, respectively, were incubated for 10 min at 100 °C in 125 µl of 2 M acetic acid containing 200 µg/ml aprotinin. After sonication, the homogenates were centrifuged (13,000 rpm for 3 min) and the supernatants used to determine the hypothalamic and telencephalic content of NPY by RIA using a commercial kit (Bachem S-2029, Peninsula Laboratories, Inc.) previously validated (De Pedro et al., 2006).

### 2.3.2. Hypothalamic monoamines

The monoamines analyzed were epinephrine (E), norepinephrine (NE), dopamine (DA) and its metabolite, dihydroxyphenylacetic acid (DOPAC), and serotonin (5-HT) and its metabolite, 5-hydroxyindol-3-acetic acid (5-HIAA). Hypothalamic content of these compounds was quantified by high-performance liquid chromatography (HPLC) with coulometric detection (Coulochem II, ESA), as previously described (De Pedro et al., 2001). Briefly, half of hypothalamus was homogenized by sonication in 125 µl of cold 0.2 N perchloric acid containing 0.2 mM sodium bisulphite, 0.4 mM EDTA and 100 nM of isoproterenol as an internal standard. The homogenate was centrifuged (13,000 rpm for 1 min) and the supernatant injected into the HPLC system. The HPLC system consisted of a Water pump (Model 590), a pulse dampener and an Agilent automatic injector with a C18 reversed phase column (125×4.6 mm ID, 5 µm particle size). The potentials of the electrodes were +50 and +300 mV for the analytical cell nos. 1 and 2, respectively. The mobile phase (flow rate=1 ml/min) consisted of 10 mM  $H_3PO_4$ , 0.1 mM EDTA, 0.4 mM sodium octanesulphonic acid and 3.5% acetonitrile (pH 2.84). The amount of monoamines in the samples was calculated as the area under the peaks, and expressed as ng/mg proteins. Protein content in hypothalamus was determined by the Lowry method (Lowry et al., 1951).

### 2.4. Statistical analyses

Data were analyzed by an analysis of variance (ANOVA) followed by the post-hoc Duncan multiple range test. A probability level of  $p<0.05$  was considered statistically significant. The values were expressed as the means±S.E.M (the standard error of the mean).

## 3. Results

The addition of marine salt to freshwater significantly reduced pH values from 7.08 in FW to 6.84 at 10‰, and the water osmolarity increased in parallel to salinity increases (Table 1). No mortality occurred during the 21- (Experiment 1) and 28-days (Experiment 2) of exposure

to any of the tested salinities. Fig. 1 shows the body weight gain (g) and SGR (%/day) during 21-days exposure to different salinities (Experiment 1). A reduction trend in the body weight gain is observed with salinity exposure, with the reduction being statistically significant at 8 and 10‰ salinity (Fig. 1A). The SGR (%/day) was statistically similar in all groups during the first week of salinity exposure, except in goldfish kept at 10‰, which showed a negative growth rate ( $p<0.05$ ). During the second and third weeks of the experiment, the SGR was significantly lower in goldfish exposed at 8 and 10‰ salinities. A

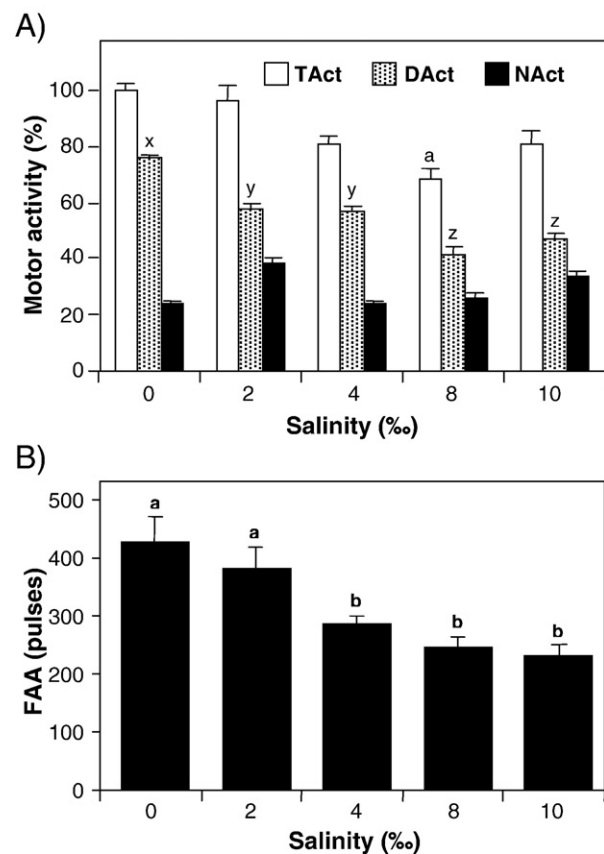


Fig. 3. Daily locomotor activity of goldfish (*Carassius auratus*) after chronic exposure (21 days) to different salinities. (A) Total daily activity (TAct) is expressed as the percentage of activity (24 h) with respect to activity exhibited by freshwater (0‰) goldfish. DAct and NAct correspond to diurnal (photophase, 0700–1900 h), and nocturnal activities (scotophase, 1900–0700 h) of daily photocycle. (B) Food anticipatory activity (FAA) in goldfish after chronic exposure (21 days) to different salinities.

Table 4  
Hypothalamic and telencephalic NPY content, and plasma ghrelin in goldfish (*Carassius auratus*) exposed to different salinities

	Salinity (‰)		
	0	2	8
Hypothalamic NPY (ng/hyp.)	6.91±1.08	7.62±0.55	7.26±0.42
Telencephalic NPY (ng/tel.)	45.69±3.67	52.46±1.56	47.96±2.79
Plasma ghrelin (pg/ml)	121.45±25.49	131.93±20.36	170.67±39.60

Values are mean±S.E.M (n=8/group).

significant recovery of growth rate was found in fish exposed to 10‰ during the second week (Fig. 1B).

The ingestion index (% bw/day) and feed conversion rate in this Experiment 1 are shown in Fig. 2. The daily food intake is significantly modified by salinity, and a direct correlation between salinity increases and feeding reductions can be observed (Fig. 2A). Similarly, the feed conversion rate increased in parallel with salinity, being significantly worse (high values) at the higher salinities (8‰ and 10‰) (Fig. 2B). Table 2 summarizes the haematological and biochemical parameters in goldfish from Experiment 1. Neither haematocrit nor haemoglobin were altered significantly by exposure to the different salinities. Plasma osmolarity increased significantly (p<0.05) at the two highest salinities, 8‰ and 10‰. The plasma triglycerides and glucose levels

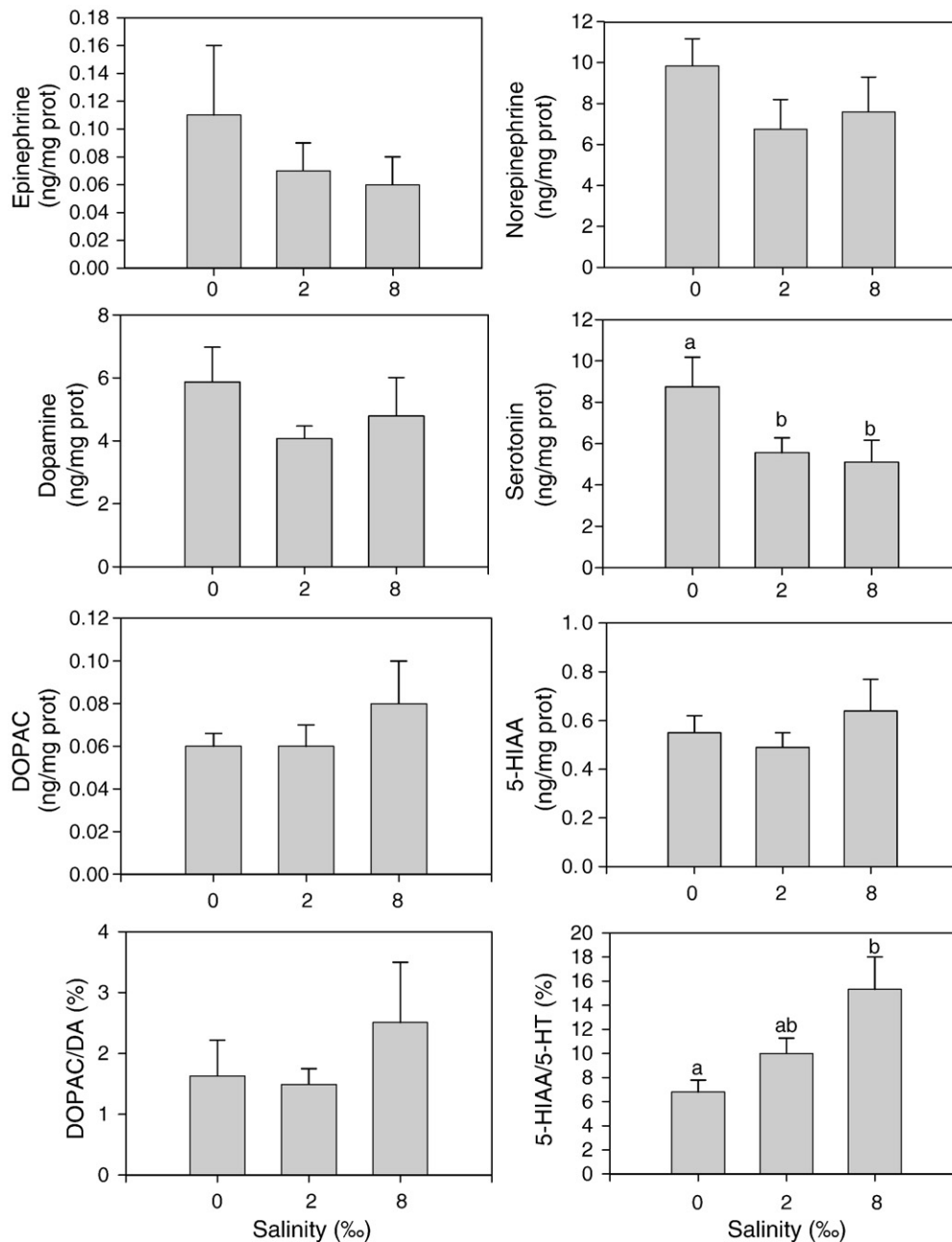


Fig. 4. Hypothalamic content of epinephrine, norepinephrine, dopamine (DA), dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT), 5-hydroxyindol-3-acetic acid (5-HIAA) and the turnover DOPAC/DA, and 5-HIAA/5-HT in goldfish (*Carassius auratus*) after chronic exposure (28 days) to salinities of 0, 2 and 8‰. Values are mean±S.E.M (n=8/group). Different letters indicate significant differences between salinity treatments (ANOVA, Duncan test p<0.05).

remained unchanged in salinity-exposed fish, but total protein content in plasma was significantly reduced at salinities greater than 6‰. Plasma cortisol levels remain unchanged with respect to FW goldfish in the different salinities tested except in the case of the highest salinity (10‰), where circulating cortisol increased significantly ( $p < 0.05$ ).

The metabolic resources in liver and muscle at the end of experimental period (21 days) are shown in Table 3. The hepatosomatic index was statistically similar in all the goldfish. Total protein content in liver and muscle were significantly higher ( $p < 0.05$ ) in goldfish exposed to 8‰ and 10‰ salinities compared to the other groups. Neither lipids nor glycogen was significantly modified by salinity exposure compared to FW goldfish, but a tendency towards lower glycogen contents in both tissues in salt-exposed goldfish was observed. Salinities of 10‰ produced a significant ( $p < 0.05$ ) muscle dehydration.

The changes in locomotor activity elicited by salinity exposure in goldfish are summarized in Fig. 3. Locomotor activity data from goldfish exposed to 6‰ salinity were not obtained due to failure of these infrared photocells during the experiment. Lower values of daily total motor activity can be observed at salinities  $\geq 4$ ‰, but this reduction in total activity was statistically significant only in the 8‰ group ( $p < 0.05$ ). Diurnal activity, however, was significantly lower in all goldfish exposed to different salinities compared to FW fish. By contrast, no effect of salinity was observed on nocturnal activity (Fig. 3A). The food-anticipatory activity (FAA) was significantly reduced at salinities of 4‰ and greater ( $p < 0.05$ ) (Fig. 3B).

The effects of salinity exposure (0, 2 and 8‰) on food intake and growth in the second experiment were very similar to those above described in the first experiment. In fact, body weight gain and the ingestion index were significantly lower at 8‰ salinity compared to FW and 2‰ salinity (data not shown). The feed conversion rate was significantly higher at 8‰ salinity compared to FW and 2‰ salinity (data not shown), as occurred in Experiment 1.

Table 4 shows the effects of goldfish exposure to 0, 2 and 8‰ salinities on two feeding regulators, brain NPY content and circulating ghrelin (Experiment 2). The NPY content in hypothalamus and telencephalon was not significantly modified in goldfish by salinity exposure. No significant changes in circulating ghrelin were detected in any of the studied groups.

Fig. 4 summarizes data from the study of hypothalamic monoaminergic transmission in goldfish exposed to FW and salinities of 2 and 8‰. The hypothalamic epinephrine and norepinephrine content showed similar values in all the goldfish. Similarly, there were no significant differences in both the hypothalamic content of dopamine, and its metabolite, the DOPAC, but the dopaminergic turnover (DOPAC/DA ratio) showed a higher (but not significant) value at high salinity than that observed in both FW and low salinity (2‰). The hypothalamic serotonergic transmission was altered in fish exposed to 2 and 8‰ salinity, with lower hypothalamic 5-HT content compared to FW fish. Consequently, a statistically significant increase ( $p < 0.05$ ) in the serotonergic turnover (5-HIAA/5-HT rate) was observed by salinity exposure.

#### 4. Discussion

The present results indicate that goldfish exhibit a high tolerance to low salinities, representing a good model to investigate the physiological responses to salinity in a stenohaline freshwater fish. Compared to other freshwater teleosts, the salinity tolerance exhibited by goldfish is similar to that found for the common carp (*Cyprinus carpio*) (Wang et al., 1997), higher than that of silver carp (*Hypophthalmichthys molitrix*) (Von Oertzen, 1985) and

lower than that of grass carp (*Ctenopharyngodon idella*) (Maceina and Shireman, 1980). Our results show that the biometric indexes of weight gain, SGR and FCR in goldfish are not significantly affected by salinities up to (and including) 6‰. In contrast, Altinok and Grizzle (2001b) found that the SGR and FCR of juvenile goldfish (mean mass around 2 g) were adversely affected at 3‰ salinity. This difference could be related to fish size, as the tolerance of freshwater fish to different salinities appears to be dependent on size and developmental stage (Watanabe et al., 1985; Britz and Hecht, 1989; Fashina-Bombata and Busari, 2003). Body weight changes observed in our study are well correlated with food intake, and FW and a low salinity environment (2‰) were the most stimulatory to feeding. A similar result was reported in grass carp at 3‰, but in contrast to goldfish, the grass carp growth was lower in a 3‰ saline environment than in FW (Maceina and Shireman, 1980).

One interesting result from our study is the good performance observed at 2‰ salinity, with feeding, growth rate and feed conversion efficiency similar to FW conditions. These results support previous reports emphasizing the positive effect of using low salinities in freshwater fish juveniles (Wang et al., 1997) and larvae (Britz and Hecht, 1989; Luz and Portella, 2002; Luz et al., 2004). By contrast, higher salinities (8 and 10‰) adversely affected the goldfish growth. In fact, the half of replicates did not exhibit a positive rate of growth during the first week of exposure to these salinities, coincident with a reduced food intake. This salt-induced reduction in food intake does not appear, at least at 8‰ salinity, to be linked to either central (NPY) or peripheral (ghrelin) potent orexigenic regulators for this species (De Pedro and Björnsson, 2001). Similar negative effects of high salinities have been reported in other cyprinids, such as the grass carp (Maceina and Shireman, 1980) and the common carp (Wang et al., 1997). Nevertheless, in the present study, the SGR partially recovered during the second and third weeks of experimental period, indicating a relatively high adaptation capacity of this species to high salinity exposure. Data from metabolic resources support such capacity, since neither lipids nor glycogen content in liver or muscle is significantly altered by salinity exposure for 21 days. A lipid depletion is produced during salinity acclimation in salmonids (Sheridan, 1989), and in the sturgeon (*Acipenser brevirostrum*) (Jarvis and Ballantyne, 2003), where lipids are mobilized to meet the metabolic costs of salinity acclimation. Such response is not observed in goldfish, which exhibit a good adaptation in terms of metabolic resources without higher energy demands after 21 days of salinity exposure.

Plasma osmolarity increases, as found in goldfish in the current study, is a common response of stenohaline species to salinity changes (De Boeck et al., 2000; Eckert et al., 2001; Tam et al., 2003; Benli and Yilds, 2004). Nevertheless, the tolerance limits of stenohaline freshwater fish to saline water appear to be determined by both the inability of the processes that regulate dehydration to adjust for the loss of water, and by the inability of cells to function with increasing osmotic concentrations. The significant increase in plasma osmolarity observed at 8 and 10‰ salinity suggests that the adaptation limit to salinity in goldfish is around these values. In fact, the exposure to a 10‰ salt concentration causes slight muscle dehydration in goldfish,

as occurs in grass carp (Maceina and Shireman, 1979) and in common carp (Van der Linden et al., 1999). Such osmotic stress is noticeable in the increase in liver and muscle protein content in goldfish exposed to 10‰ salinity, according to previous data in the common carp (De Boeck et al., 1997).

The primary stress response in teleost fish includes increases in circulating cortisol (Wendelaar-Bonga, 1997). The unaltered plasma cortisol levels in our study indicate that salinity up to 8‰ does not produce significant stress, and suggests the high tolerance to salinity in goldfish. On the other hand, keeping in mind the key role of cortisol as a seawater-adapting hormone, the significant increase in plasma cortisol levels in goldfish at 10‰ salinity is evidence of such a role for this hormone in osmoregulatory processes (Tsuzuki et al., 2001; Boeuf and Payan, 2001). The unchanged plasma glucose, haematocrit, and haemoglobin in goldfish exposed to the different salinities also support the absence of stress in our fish. An increase in the number of red cells (haemoconcentration) is of significance when freshwater fish are stressed in salt water. Salinity increased haematocrit and haemoglobin have been described in stenohaline species, such as the tilapia (*Sarotherodon melanotheron*) (Lea Master et al., 1990), and the grass carp (Yildiz and Uzbilek, 2001), but not in euryhaline species such as the Atlantic cod (*Gadus morhua*) (Mugil and Sayer, 2004) and tilapia (Verdegem et al., 1997). Two main factors can contribute to the absence of stress in the present study in goldfish. On one hand, salinity was gradually increased, which reduces the osmotic stress and facilitates the acclimation to different salinities. On the other hand, cortisol, glucose and haematological parameters were analyzed after a long period of salt exposure (21 days). Thus, our results regarding haematological parameters and cortisol plasma levels corroborated the data discussed above on growth performance, and pointed to successful acclimation of goldfish to these saline conditions.

The diurnal activity pattern exhibited by freshwater goldfish in the present study disappears in salinities higher than 4‰, due to significant reductions in both, diurnal activity and FFA. These are the first data, to our knowledge, on the effects of salinity on motor activity in a teleost species. Bearing in mind the direct relationship between feeding and motor activity (Sánchez-Vázquez and Madrid, 2001), it could be suggested that the lower food intake observed at these high salinity environments could be linked to the lower diurnal activity in these fish. The similar values of food intake and motor activity in both FW and 2‰ salinity support this proposal.

The exposure of goldfish to 8‰ salinity altered hypothalamic serotonergic transmission with an elevation in the rate of 5-HT metabolism, in agreement with results reported in the common carp (De Boeck et al., 1996). In some teleosts, certain stressful conditions elevate brain serotonergic activity, and from our data in goldfish and previously in the carp, salinity could be considered one such stress agent. Nevertheless, the higher 5-HIAA/5-HT turnover in our study appears to be a consequence of a low 5-HT production, which does not suggest an additional activation of the 5-HT-CRF-cortisol axis. The hypothalamic monoaminergic system plays a pivotal role in the complex regulation of food intake in goldfish (De Pedro et al., 1997,

2001, 2003), and the low contents of both, DA and 5-HT, in goldfish at 8‰ could indicate the involvement of monoamines in feeding regulation during the salinity acclimation.

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