Mar Biol (2008) 153:661–671
DOI 10.1007/s00227-007-0840-8

RESEARCH ARTICLE

Interaction of short-term testosterone treatment with osmotic acclimation in the gilthead sea bream *Sparus auratus*

Francisco J. Arjona · Susana Sangiao-Alvarellos · Sergio Polakof · Angel García-López · María P. Martín del Río · Gonzalo Martínez-Rodríguez · José L. Soengas · Juan M. Mancera

Received: 28 June 2007 / Accepted: 3 October 2007 / Published online: 23 October 2007

Abstract To assess the interaction between testosterone (T) treatment and acclimation to different salinities, seawater-acclimated gilthead sea bream (*Sparus auratus*) were implanted with slow-release coconut oil implants alone (control) or containing T (5 μg/g body mass). After 5 days, eight fish of control and T-treated groups were sampled. The same day, eight fish of each group were transferred to low salinity water (LSW, 6 ppt, hypoosmotic test), seawater (SW, 38 ppt, control test) and high salinity water (HSW, 55 ppt, hyperosmotic test) and sampled 9 days later. Gill Na^+, K^+-ATPase activity increased in HSW-acclimated fish with respect to SW- and LSW-acclimated fish in both control and T-treated groups. Kidney Na^+, K^+-ATPase activity was also enhanced in HSW-acclimated fish, but only in T-treated group. From a metabolic point of view, most of the changes observed can be attributed to the action of salinity and T treatment alone, since few interactions between T treatment and osmotic acclimation to different salinities were observed. Those interactions included in treated fish: in the liver, decreased capacity in using glucose in fish acclimated to extreme salinities; in the gills, decreased capacity in using amino acids in HSW; in the kidneys increased capacity in using amino acids in extreme salinities; and in the brain, decreased glycogen and acetoacetate levels of fish in LSW.

Abbreviations

Ala-AT	Alanine aminotransferase (EC. 2.6.1.2.)
Asp-AT	Aspartate aminotransferase (EC. 2.6.1.1.)
HK	Low Km hexokinase (EC. 2.7.1.11.)
G6Pase	Glucose 6-phosphatase (EC. 3.1.3.9.)
G3PDH	α-Glycerophosphate dehydrogenase (EC. 1.1.1.8.)
G6PDH	Glucose 6-phosphate dehydrogenase (EC. 1.1.1.49)
GDH	Glutamate dehydrogenase (EC. 1.4.1.2.)
GK	Glucokinase (EC. 2.7.1.2.)
HOAD	3-Hydroxiacil-CoA-dehydrogenase (EC. 1.1.1.35.)
LDH-O	Lactate dehydrogenase-oxidase (EC. 1.1.1.27.)
PFK	6-Phosphofructo 1-kinase (EC. 2.7.1.11.)
PK	Pyruvate kinase (EC. 2.7.1.40.)

Introduction

In addition to their role in reproduction, in fish, sex steroids are involved in other physiological processes, such as growth (Sparks et al. 2003), digestion and food utilization (Ince et al. 1982), gut transport (Reshkin et al. 1989), shifts in body composition (Haux and Norberg 1985) and osmoregulation and energy metabolism (see below).

In salmonids, an inverse relationship between sexual maturation (and/or treatment with exogenous sex steroids)

Communicated by S.A. Poulet.

F. J. Arjona · M. P. Martín del Río · J. M. Mancera (✉)
Departamento de Biología,
Facultad de Ciencias del Mar y Ambientales,
Universidad de Cádiz, 11510 Puerto Real, Cádiz, Spain
e-mail: juanmiguel.mancera@uca.es

S. Sangiao-Alvarellos · S. Polakof · J. L. Soengas
Laboratorio de Fisioloxía Animal,
Departamento de Bioloxía Funcional e Ciencias da Saúde,
Facultade de Bioloxía, Universidade de Vigo,
36310 Vigo, Spain

A. García-López · G. Martínez-Rodríguez
Instituto Ciencias Marinas de Andalucía, CSIC,
11510 Puerto Real, Cádiz, Spain

and seawater (SW) adaptability has been demonstrated (McCormick and Naiman 1985; McCormick 1995; LeFrançois and Blier 2000). In non-salmonid species, treatment with 17β-estradiol (E_2) decreased gill Na^+, K^+-ATPase activity and hypoosmotic capacity in tilapia (Vijayan et al. 2001) and *Fundulus* (Mancera et al. 2004), whereas an increase occurred in gilthead sea bream (Guzmán et al. 2004). There are few studies regarding the influence of testosterone (T) on osmoregulatory capacity of non-salmonid species (Sunny and Oommen 2000, 2002). In a previous study, we have observed that T treatment enhanced gill and kidney Na^+, K^+-ATPase activities in SW-acclimated gilthead sea bream suggesting an hypoosmoregulatory role for T (Sangiao-Alvarellos et al. 2006).

As for energy metabolism, T treatment is known to increase oxygen consumption (Sparks et al. 2003; Ros et al. 2004), and to induce anabolic changes at the hepatic level (Peter and Oommen 1989; Woo et al. 1993; Singh and Gupta 2002; Sunny et al. 2002). In gilthead sea bream kept in SW, T treatment increased levels of plasma metabolites (glucose, lactate, protein and triclyceride), and induced several metabolic changes in the liver, gills, kidneys and brain (Sangiao-Alvarellos et al. 2006).

Gilthead sea bream is a partially euryhaline species for which gonadal development occurs in estuarine areas (brackish water), whereas spawning always occurs in SW (Arias 1976). In this species, gill Na^+, K^+-ATPase activity is lower in isosmotic environments (estuarine areas) and higher in hyperosmotic environments (seawater of open sea; Laiz-Carrión et al. 2005). Furthermore, several metabolic changes occur in osmoregulatory (gills and kidneys) and non-osmoregulatory (liver and brain) tissues during acclimation to hyperosmotic and hypoosmotic environments (Sangiao-Alvarellos et al. 2003, 2005a). Similar changes in the activity of enzymes involved in energy metabolism have been observed in other marine fish species during acclimation to LSW or HSW (Nakano et al. 1998; Kelly and Woo 1999; Kelly et al. 1999; Le François et al. 2004). Since we have demonstrated significant effects of T treatment on both energy metabolism and osmoregulation in SW-acclimated gilthead sea bream, the aim of the present study was to assess the possible interaction of T treatment with osmotic acclimation in gilthead sea bream transferred from SW to hyperosmotic or hypoosmotic environments.

Materials and methods

Fish

Sexually inmature male gilthead seabream (*Sparus auratus* L.; 150 ± 10 g body mass) were provided by Planta de Cultivos Marinos (CASEM, Universidad de Cádiz, Puerto Real, Cádiz, Spain) and transferred to the laboratories of the Faculty of Marine and Environmental Sciences (Puerto Real, Cádiz). Inmature specimens of approximately 1-year old were used in order to eliminate the possible interference of endogenous androgens (the first sexual maturation in males occurs at a fish size of approximately 400 g; Zohar et al. 1984). Fish were acclimated to seawater (SW, 38 ppt salinity, 1,103 mOsm/kg H_2O) in 500 l aquaria in an open system. During the experimental period (June–July 2004), fish were maintained under natural photoperiod and constant temperature (21°C), and fed with commercial dry pellets (Dibaq-Diprotg SA, Segovia, Spain) at a daily rate of 1% body weight. Fish were fasted for 24 h before sampling. The experiments described comply with the European Union Council (86/609/EU) and the Spanish Government (RD 1201/2005) guidelines for the use of animals in research (Consejería de Agricultura y Pesca de la Junta de Andalucía animal facilities, refs. CA/3/U and CA/4/CS).

Experimental design

SW-acclimated fish were injected intraperitoneally (5 µl/g body mass) with slow-release coconut oil alone (control) or containing T (5 µg/g body mass). This dose has been previously reported in this species to induce a chronic increase in T levels for a week (Sangiao-Alvarellos et al. 2006). After 5 days, eight fish of oil-implanted group and eight fish of T-implanted group were sampled. The same day, eight fish of each group were transferred to low salinity water (LSW, hypoosmotic test), SW (transfer test) and high salinity water (HSW, hyperosmotic test). After 9 days, all groups were sampled (see below). One group of untreated fish ($n = 8$) served as non-injected control. LSW (6 ppt salinity, 178 mOsm/kg H_2O) was obtained by mixing SW with dechlorinated tap water in a recirculating system. HSW (55 ppt salinity, 1368 mOsm/kg H_2O) was obtained by mixing full SW with natural marine salts (Unionsal, Cádiz, Spain) in a recirculating system. The system in the tanks containing SW was also recirculated to be comparable with that of LSW and HSW. Once the systems became recirculated, the common water quality criteria and salinity were checked every day.

Sampling

Fish were anaesthetized with 2-phenoxyethanol (0.1% v/v) and weighed. A blood sample was collected from the caudal vein using ammonium-heparinized syringes. The plasma, obtained by centrifugation (1 min at 10,000*g*), was divided into two aliquots. One aliquot was immediately frozen in liquid nitrogen and stored at −80°C until further assay of T and protein levels and osmolality. The other aliquot, for the assessment of metabolites was deproteinized

immediately (using 6% perchloric acid), neutralized (using 1 mol l^{-1} potassium bicarbonate), frozen in liquid nitrogen and stored at −80°C until further assay. To assess Na^+, K^+-ATPase activity, 3–5 filaments from the second branchial arch (cut just above the septum with fine point scissors) and a portion of the caudal kidney were taken, placed in 100 µl of ice-cold SEI buffer (150 mmol l^{-1} sucrose, 10 mmol l^{-1} EDTA, 50 mmol l^{-1} imidazole, pH 7.3), and frozen at −80° C. Brain, liver, the remaining kidney and the remaining branchial arches were also removed, weighed, freeze-clamped in liquid nitrogen and stored at −80°C until assay.

Analytical techniques

Plasma glucose, lactate and triglyceride levels were measured using commercial kits (Spinreact, Spain) adapted to microplates. Plasma protein concentration was quantified using the bicinchoninic acid method with bovine serum albumin as standard. Plasma osmolality was assessed with a vapor pressure osmometer (Fiske One-Ten Osmometer, Fiske, VT, USA). Plasma levels of T were quantified by ELISA following the method described by Rodríguez et al. (2000). Gill and kidney Na^+, K^+-ATPase activity were assessed using the microassay method of McCormick (1993) adapted for use in gilthead sea bream (Mancera et al. 2002).

Frozen liver, brain, kidneys, and gills were finely minced on a chilled Petri dish to very small pieces, which, still frozen, were divided into two different (but relatively homogeneous) portions to assess enzyme activities and metabolite levels. For the assessment of metabolite levels, frozen tissue was weighed and homogenized immediately by ultrasonic disruption with 7.5 volumes of ice-cooled 6% perchloric acid, neutralized with 1 mol l^{-1} potassium bicarbonate, centrifuged, and the supernatant assayed. Tissue lactate and triglyceride levels were determined spectrophotometrically using commercial kits (Spinreact, Spain). Tissue glycogen levels were assessed using the method of Keppler and Decker (1974). Glucose obtained after glycogen breakdown (after subtracting free glucose levels) was determined with a commercial kit (Biomérieux, Spain). Tissue total α-amino acids level was assessed colorimetrically using the nynhidrin method of Moore (1968) with modifications to adapt the assay to a microplate format. For the assessment of enzyme activities (HK, GK, PK, PFK, G6Pase, G6PDH, G3PDH, GDH, Ala-AT, Asp-AT, LDH, and HOAD), frozen tissue was homogenized by ultrasonic disruption with 10 volumes of ice-cold stopping buffer containing (in mmol l^{-1}) 50 imidazole-HCl (pH 7.5), 1 mercaptoethanol, 50 NaF, 4 EDTA, 250 sucrose and a protease inhibitor cocktail (Sigma, P-2714). The specific conditions for enzyme assays were described previously (Sangiao-Alvarellos et al. 2003, 2006).

Statistics

Data were statistically analyzed by two-way ANOVA in which treatment (control and T) and salinity (LSW, SW, and HSW) were the main factors. When significant effects were observed for any factor, a one-way ANOVA followed by a Student–Newman-Keuls test was carried out. Differences were considered to be statistically significant at $P < 0.05$.

Results

The parameters assessed did not show any differences between untreated fish (day 0) and fish implanted with coconut oil alone (control; data not shown). Plasma T levels increased in controls in parallel with salinity, showing significant differences when comparing HSW- with LSW-acclimated fish. In fish treated with T for 5 days, plasma T levels increased significantly. At 9 days post-transfer (14 days post-implant), T-treated group maintained in all experimental conditions (LSW, SW and HSW) showed higher T levels than those of controls. In this group, T levels remained higher in LSW- or HSW-acclimated fish compared with SW-acclimated fish (Fig. 1). No differences were observed in gonadal differentiation after two weeks of exposure to T (data not shown).

Plasma osmolality displayed in both control and T-treated fish significant differences depending on salinity, with higher levels in HSW- than in LSW- and SW-acclimated fish (Fig. 2a). Gill Na^+, K^+-ATPase activity showed

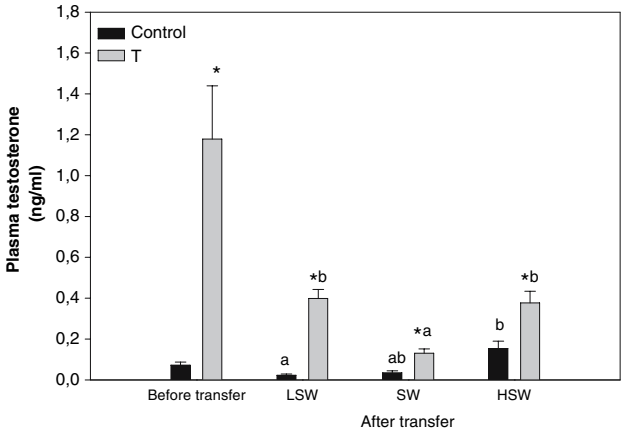

Fig. 1 Effects of implantation of coconut oil alone (control) or containing T (5 µg g^{-1} body mass) on plasma T levels before and after salinity transfer from SW (38 ptt) to LSW (6 ptt), SW or HSW (55 ptt). Fish were intraperitoneally implanted and kept in SW for 5 days. One group was sampled before transfer and the other groups were transferred to new environmental salinities and sampled after 9 days. Values are the means ± SEM ($n = 8$).*Asterisks* indicate significantly different ($P < 0.05$) from fish injected with coconut oil alone (control) under the same experimental conditions. *Different letters* indicate significant differences ($P < 0.05$) among groups (LSW, SW or HSW) within each treatment (control and T)

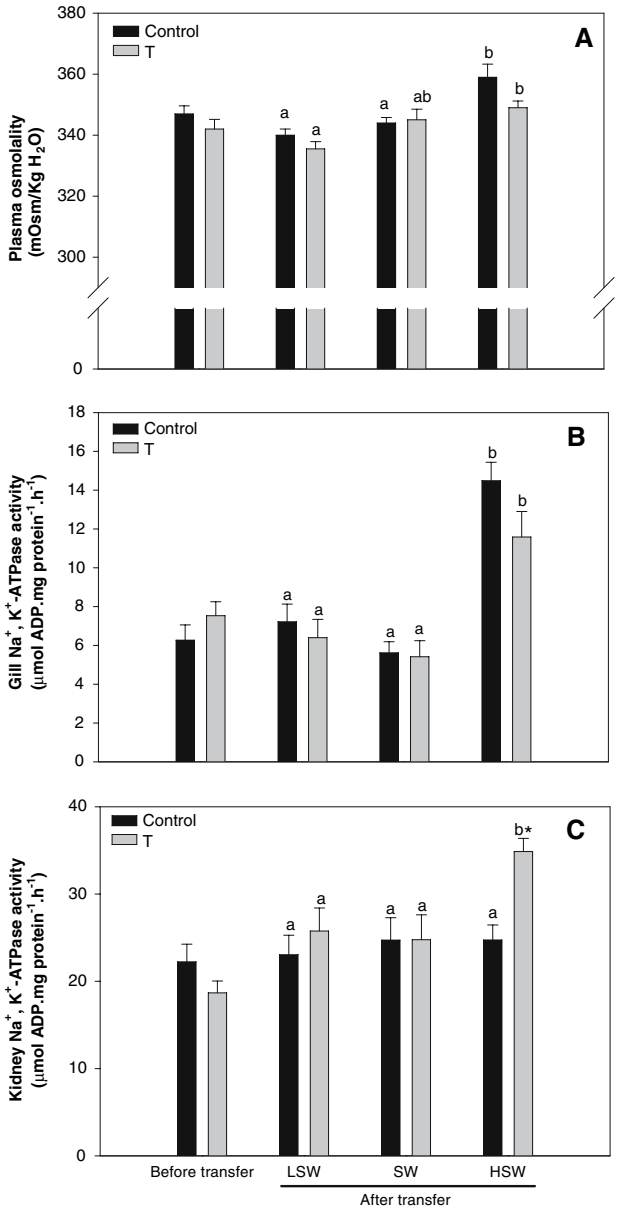

Fig. 2 Plasma osmolality (**a**), gill (**b**) and kidney (**c**) Na^+, K^+-ATPase activities in *S. auratus*. Further details as in legend of Fig. 1

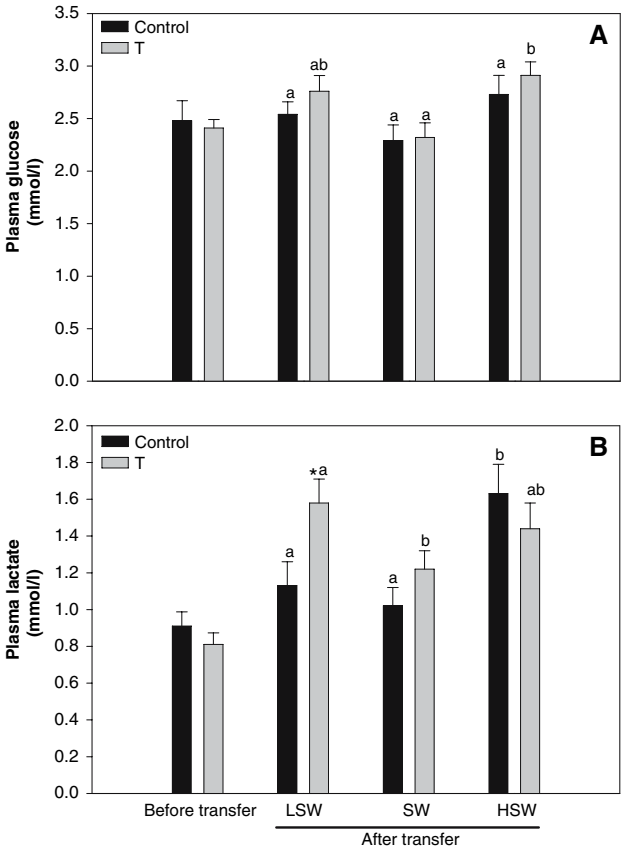

Fig. 3 Plasma glucose (**a**) and lactate (**b**) levels in *S. auratus*. Further details as in legend of Fig. 1

in both groups a significant increase in HSW- with respect to SW- and LSW-acclimated fish (Fig. 2b). Kidney Na^+, K^+-ATPase activity was also higher in HSW-acclimated fish, but only in T-treated group (Fig. 2c).

Plasma glucose levels increased in HSW- and LSW- with respect to SW-acclimated fish (Fig. 3a), whereas in T-treated group, differences were significant between HSW- and SW-acclimated fish. (Fig. 3a). Plasma lactate levels increased in controls submitted to hyperosmotic conditions (Fig. 3b). Plasma levels of triglycerides decreased in controls acclimated to extreme salinities, although the differences were not significant (data not shown). Plasma protein levels did not change (data not shown).

The changes in metabolite levels and enzyme activities in liver are displayed in Table 1. T treatment before transfer increased lactate and triglyceride levels, and G6Pase activity while decreased HK, G6PDH, GDH, Ala-AT and Asp-AT activities. After transfer, glucose and amino acid levels, and G6Pase, G6PDH and Asp-AT activities in control fish showed significant effects of salinity. In T-treated fish transferred to LSW, decreased levels of glycogen, glucose, amino acid and triglyceride, and HK and G6Pase activities as well as enhanced levels of lactate and PFK (optimal) activity were noticed. In this group transferred to SW, lower HK and G6PDH, and higher PFK (activation ratio), G6Pase, G3PDH and Asp-AT activities were noticed. Finally, T-treated fish transferred to HSW showed with respect to controls a decrease in glucose levels, and HK, G6Pase, GDH and Asp-AT activities and an increase in acetoacetate levels. No significant changes were noticed for PFK (activity ratio), FBPase and HOAD activities (data not shown).

In the gills (Table 2), T treatment before transfer elicited decreased levels of glycogen and amino acid, and GDH and LDH-O activities. After transfer, significant differences were noticed among controls for glycogen and lactate

Table 1 Liver levels of metabolites and enzyme activities in SW-acclimated gilthead sea bream after 5 days of intraperitoneal implantation of coconut oil alone (control) or containing T (5 μg g^{-1} body mass), followed by 9 days of acclimation to LSW (6 ppt), SW (38 ppt) or HSW (55 ppt)

Parameter	Treatment	Before transfer	After transfer		
			LSW	SW	HSW
Metabolite levels					
Glycogen (μmol glycosyl units g^{-1} wet wt.)	Control	598.0 ± 48.6	637.3 ± 58.9a	660.5 ± 58.0a	541.6 ± 58.3a
	T	576.1 ± 47.2	455.9 ± 25.7*a	589.4 ± 57.9a	562.4 ± 52.9a
Glucose (μmol g^{-1} wet wt.)	Control	52.5 ± 2.40	69.0 ± 5.16a	57.0 ± 2.68b	70.8 ± 4.53a
	T	54.0 ± 4.84	53.7 ± 4.16*a	52.8 ± 1.76a	57.9 ± 2.73*a
Lactate (μmol g^{-1} wet wt.)	Control	0.25 ± 0.05	0.20 ± 0.02a	0.36 ± 0.06a	0.30 ± 0.03a
	T	0.45 ± 0.09*	0.94 ± 0.10*a	0.53 ± 0.10b	0.30 ± 0.05b
α-amino acid (μmol g^{-1} wet wt.)	Control	76.8 ± 3.50	116.8 ± 9.94a	70.5 ± 4.90b	82.9 ± 5.24b
	T	74.6 ± 2.11	74.9 ± 4.29*a	74.5 ± 4.72a	85.5 ± 4.52a
Triglyceride (μmol g^{-1} wet wt.)	Control	3.86 ± 0.49	4.65 ± 0.62a	4.59 ± 0.48a	4.79 ± 0.59a
	T	5.94 ± 0.59*	3.13 ± 0.24*a	7.17 ± 1.53b	4.47 ± 0.55ab
Acetoacetate (μmol g^{-1} wet wt.)	Control	3.76 ± 0.25	3.86 ± 0.29a	3.56 ± 0.17a	3.60 ± 0.20a
	T	3.65 ± 0.23	3.95 ± 0.13a	3.54 ± 0.28a	4.30 ± 0.15*a
Enzyme activities					
Carbohydrate metabolism					
HK activity (U mg^{-1} protein)	Control	0.17 ± 0.013	0.18 ± 0.016a	0.14 ± 0.01a	0.20 ± 0.026a
	T	0.11 ± 0.015*	0.12 ± 0.013*a	0.23 ± 0.03*b	0.08 ± 0.01*a
PFK activity					
Optimal activity (U mg^{-1} protein)	Control	1.24 ± 0.07	1.25 ± 0.15a	1.40 ± 0.11a	1.56 ± 0.12a
	T	1.32 ± 0.15	1.80 ± 0.15*a	1.64 ± 0.16a	1.59 ± 0.04a
Fructose 2,6-P_2 activation ratio (%)	Control	49.5 ± 10.4	40.9 ± 8.8a	46.3 ± 9.21a	63.0 ± 9.01a
	T	92.7 ± 21.9	59.4 ± 6.96a	101.0 ± 14.7*b	57.7 ± 2.80a
G6Pase activity (U mg^{-1} protein)	Control	11.4 ± 1.60	39.0 ± 4.52a	18.1 ± 2.29b	36.9 ± 5.15a
	T	18.9 ± 1.85*	18.8 ± 2.80*,a	27.9 ± 2.18*a	18. 1 ± 1.87*a
G6PDH activity (U mg^{-1} protein)	Control	1.52 ± 0.15	1.22 ± 0.07a	1.88 ± 0.19b	1.87 ± 0.28ab
	T	0.96 ± 0.10*	1.42 ± 0.13a	1.28 ± 0.12*a	1.57 ± 0.13a
G3PDH activity (U mg^{-1} protein)	Control	1.27 ± 0.13	1.63 ± 0.12a	1.44 ± 0.15a	1.94 ± 0.16a
	T	1.32 ± 0.06	1.72 ± 0.07a	1.95 ± 0.09*a	1.72 ± 0.08a
Amino acid metabolism					
GDH activity (U mg^{-1} protein)	Control	3.20 ± 0.20	2.68 ± 0.12a	2.72 ± 0.12a	3.03 ± 0.24a
	T	2.40 ± 0.12*	2.77 ± 0.11ab	3.26 ± 0.26a	2.56 ± 0.06*b
Ala-AT activity (U mg^{-1} protein)	Control	5.98 ± 0.36	4.42 ± 0.47a	5.14 ± 0.32a	5.09 ± 0.53a
	T	4.50 ± 0.40*	4.40 ± 0.29a	4.93 ± 0.35a	4.71 ± 0.46a
Asp-AT activity (U mg^{-1} protein)	Control	6.18 ± 0.49	6.84 ± 0.33ab	6.27 ± 0.31a	8.15 ± 0.48b
	T	3.56 ± 0.77*	6.90 ± 0.41a	8.53 ± 0.73*b	7.02 ± 0.11*ab

Values are the means ± SEM (n = 8 fish per group)

* Significantly different ($P < 0.05$) from fish implanted with vegetable oil alone (control) under the same experimental conditions

Different letters indicate significant differences ($P < 0.05$) among groups (SW, LSW, and HSW) within each treatment (control and T)

levels, and HK, G6PDH, LDH-O and HOAD activities. In T-treated fish, transfer to LSW decreased HK activity. However, transfer to SW increased glycogen and lactate levels, and PK (optimal) activity, while decreased activities of GDH and Asp-AT. Finally, transfer of T-treated fish to HSW decreased G6PDH and Asp-AT activities and increased glycogen and lactate levels, and PK (optimal) activity. No significant changes were noticed for glucose levels, and PK (activity and activation ratios) and G3PDH activities (data not shown).

Metabolic changes observed in the kidneys are shown in Table 3. T treatment before transfer increased glucose and triglyceride levels and G3PDH and Ala-AT activities, while decreased HK, PK (optimal) and GDH activities. In control

Table 2 Gill levels of metabolites and enzyme activities in SW-acclimated gilthead sea bream after 5 days of intraperitoneal implantation coconut oil alone (control) or containing T (5 μg g^{-1} body mass), followed by 9 days of acclimation to LSW (6 ppt), SW (38 ppt) or HSW (55 ppt). Further details as in legend of Table 1

Parameter	Treatment	Before transfer	After transfer		
			LSW	SW	HSW
Metabolite levels					
Glycogen (μmol glycosyl units g^{-1} wet wt.)	Control	0.66 ± 0.06	1.15 ± 0.07a	1.04 ± 0.06b	1.14 ± 0.12a
	T	0.50 ± 0.005*	1.23 ± 0.15a	1.41 ± 0.2*ab	2.36 ± 0.46*b
Lactate (μmol g^{-1} wet wt.)	Control	0.29 ± 0.03	0.39 ± 0.06a	0.26 ± 0.03b	0.29 ± 0.02ab
	T	0.23 ± 0.03	0.37 ± 0.16a	0.37 ± 0.03*a	0.42 ± 0.07*a
α-amino acid (μmol g^{-1} wet wt.)	Control	17.4 ± 0.85	36.2 ± 1.64a	42.7 ± 3.93a	35.6 ± 2.23a
	T	38.7 ± 3.36*	40.3 ± 2.11a	39.9 ± 2.68a	34.2 ± 2.39b
Enzyme activities					
Carbohydrate metabolism					
HK activity (U mg^{-1} protein)	Control	0.68 ± 0.07	0.97 ± 0.09a	0.65 ± 0.067b	0.88 ± 0.06ab
	T	0.91 ± 0.05*	0.49 ± 0.05*a	0.62 ± 0.07ab	0.77 ± 0.07b
PK activity					
Optimal activity (U mg^{-1} protein)	Control	4.37 ± 0.38	5.07 ± 0.45a	4.63 ± 0.39a	4.84 ± 0.28a
	T	4.31 ± 0.27	5.77 ± 0.68a	6.45 ± 0.73*a	5.82 ± 0.27*a
G6PDH activity (U mg^{-1} protein)	Control	1.93 ± 0.14	2.77 ± 0.28a	1.86 ± 0.14b	1.80 ± 0.14b
	T	1.70 ± 0.23	2.51 ± 0.30a	1.63 ± 0.15b	1.47 ± 0.04*b
Amino acid metabolism					
GDH activity (U mg^{-1} protein)	Control	5.00 ± 0.27	5.20 ± 0.31a	4.54 ± 0.17a	5.08 ± 0.29a
	T	4.15 ± 0.23*	5.64 ± 0.56a	3.93 ± 0.34*b	5.01 ± 0.26b
Asp-AT activity (U mg^{-}1 protein)	Control	0.81 ± 0.06	0.87 ± 0.10a	0.79 ± 0.07a	1.10 ± 0.10a
	T	0.79 ± 0.07	0.77 ± 0.08ab	0.55 ± 0.07*b	0.79 ± 0.08*a
Lactate metabolism					
LDH-O activity (U mg^{-1} protein)	Control	0.47 ± 0.06	0.53 ± 0.054a	0.33 ± 0.05b	0.33 ± 0.03b
	T	0.16 ± 0.02*	0.61 ± 0.048a	0.30 ± 0.03b	0.33 ± 0.04b
Lipid metabolism					
HOAD activity (U mg^{-1} protein)	Control	0.28 ± 0.015	0.39 ± 0.017a	0.27 ± 0.027b	0.45 ± 0.044a
	T	0.37 ± 0.026*	0.48 ± 0.03*a	0.31 ± 0.025b	0.32 ± 0.031*b

fish, significant effects of salinity transfer were observed in glycogen, glucose, amino acid and triglyceride levels and G3PDH and Ala-AT activities. In T-treated fish transferred to LSW, lower glycogen and higher amino acid levels and Ala-AT activity were noticed. In treated fish transferred to SW, higher glucose levels and HK and LDH-O activities, as well as lower amino acid levels, were noticed. Finally, transfer to HSW decreased glucose levels and PK (activation ratio) activity, while increased PK (optimal) and GDH activities with respect to controls. No significant changes were observed for lactate levels and G6Pase, G6PDH and Asp-AT activities (data not shown).

In the brain (Table 4), T treatment before transfer decreased glucose and triglyceride levels and PFK (activity ratio) activity. After transfer to different salinities in controls, significant effects were noticed in the levels of glycogen and HOAD activity. In T-treated fish transferred to LSW, significant decreases in glycogen and glucose, acetoacetate and triglyceride levels, as well as increased PFK (optimal) activity, were observed. In treated fish, transfer to SW decreased PFK activity (activation ratio) compared with controls. Finally, transfer to HSW decreased lactate and amino acid levels and PFK (activity ratio), while increased triglyceride levels and HOAD activity. No significant changes were observed for amino acid levels, HK, PFK (activation ratio), G6PDH, Ala-AT, Asp-AT and GDH activities (data not shown).

Discussion

Testosterone levels

Testosterone-treated fish displayed higher T levels than controls with values of 0.3–1 ng ml^{-1} close to those observed in gilthead sea bream during maturation and

Table 3 Kidney levels of metabolites and enzyme activities in SW-acclimated gilthead sea bream after 5 days of intraperitoneal implantation of coconut oil alone (control) or containing T (5 μg g^{-1} body mass), followed by 9 days of acclimation to LSW (6 ppt), SW (38 ppt) or HSW (55 ppt). Further details as in the legend of Table 1

Parameter	Treatment	Before transfer	After transfer		
			LSW	SW	HSW
Metabolite levels					
Glycogen (μmol glycosyl units g^{-1} wet wt.)	Control	2.02 ± 0.43	2.41 ± 0.30a	2.08 ± 0.18a	1.19 ± 0.17b
	T	1.85 ± 0.28	3.18 ± 0.33*a	2.61 ± 0.12ab	1.60 ± 0.15b
Glucose (μmol g^{-1} wet wt.)	Control	2.37 ± 0.19	2.73 ± 0.10a	2.10 ± 0.13b	2.84 ± 0.21a
	T	3.53 ± 0.29*	2.83 ± 0.10ab	3.15 ± 0.32*a	2.15 ± 0.23*b
α-amino acid (μmol g^{-1} wet wt.)	Control	8.80 ± 0.69	5.49 ± 0.37a	14.5 ± 2.04b	10.0 ± 1.52ab
	T	8.62 ± 1.53	8.00 ± 0.97*a	9.31 ± 0.86*a	10.7 ± 1.67a
Triglyceride (μmol g^{-1} wet wt.)	Control	0.51 ± 0.04	0.54 ± 0.09a	0.75 ± 0.09ab	1.64 ± 0.51b
	T	1.11 ± 0.23*	1.11 ± 0.33a	0.95 ± 0.20a	0.68 ± 0.08a
Enzyme activities					
Carbohydrate metabolism					
HK activity (U mg^{-1} protein)	Control	1.06 ± 0.12	0.83 ± 0.22a	1.01 ± 0.26a	0.94 ± 0.15a
	T	0.61 ± 0.14*	1.00 ± 0.27a	1.61 ± 0.18*a	1.01 ± 0.12a
PK activity					
Optimal activity (U mg^{-1} protein)	Control	4.29 ± 0.42	4.15 ± 0.33a	3.96 ± 0.37a	3.20 ± 0.28a
	T	3.08 ± 0.22*	3.91 ± 0.16a	4.03 ± 0.42a	4.63 ± 0.55*a
Activity ratio (%)	Control	19.7 ± 1.79	14.0 ± 1.79a	11.5 ± 1.48a	11.5 ± 1.16a
	T	18.3 ± 2.05	12.9 ± 1.72ab	17.4 ± 2.26a	9.82 ± 1.51b
Fructose 1,6-P_2 activation ratio (%)	Control	62.3 ± 5.11	37.6 ± 4.89a	41.4 ± 7.52a	48.4 ± 6.92a
	T	61.4 ± 7.16	33.6 ± 3.80a	60.7 ± 9.49b	30.8 ± 4.42*a
G3PDH activity (U mg^{-1} protein)	Control	0.80 ± 0.05	0.93 ± 0.04a	1.17 ± 0.05b	0.98 ± 0.10ab
	T	0.96 ± 0.05*	1.03 ± 0.07a	0.98 ± 0.12a	1.10 ± 0.08a
Amino acid metabolism					
GDH activity (U mg^{-1} protein)	Control	5.32 ± 0.19	5.43 ± 0.14a	5.48 ± 0.58a	5.45 ± 0.19a
	T	4.61 ± 0.22*	5.48 ± 0.33a	7.14 ± 1.63a	6.80 ± 0.29*a
Ala-AT activity (U mg^{-1} protein)	Control	3.04 ± 0.42	2.01 ± 0.25a	4.04 ± 0.51b	2.31 ± 0.25a
	T	4.78 ± 0.52*	3.17 ± 0.42*a	3.87 ± 1.04a	3.03 ± 0.49a
Lactate metabolism					
LDH-O activity (U mg^{-1} protein)	Control	0.67 ± 0.06	0.54 ± 0.05a	0.48 ± 0.05a	0.54 ± 0.05a
	T	0.60 ± 0.03	0.47 ± 0.04a	0.81 ± 0.10*b	0.74 ± 0.07*b

spawning (Gothilf et al. 1997; Chaves Pozo et al. 2007). Those values also agree with those reported previously in gilthead sea bream submitted to a similar T treatment (same dose, vehicle and time of exposition; Sangiao-Alvarellos et al. 2006). Modifications in clearance rate and/or dynamic of T release from coconut oil implant could explain differences observed in T plasma levels among fish acclimated to different environmental salinities (LSW, SW and HSW).

After transfer, plasma T levels increased in parallel with salinity in control group and were significantly different among HSW- and LSW-acclimated fish. Haddy and Pankhurst (2000) studied the effects of salinity on plasma T levels in black bream *Acanthopagrus butcheri*, demonstrating that in males, those levels were higher in fish held at 35 ppt than in fish held at 20 ppt or 5 ppt, although the differences were not significant. Our results in the control group could suggest an osmoregulatory role of this hormone during hyperosmotic acclimation in gilthead sea bream (see below).

Osmoregulatory parameters

We have previously reported in gilthead sea bream that treatment with 2 μg T/g body mass for 7 days enhanced gill Na^+, K^+-ATPase activity, whereas the dose of 5 μg T/g body mass (same used in the present study) was ineffective (Sangiao-Alvarellos et al. 2006). These results agree with those reported by Sunny and Oommen (2000), who

Table 4 Brain levels of metabolites and enzyme activities in SW-acclimated gilthead sea bream after 5 days of intraperitoneal implantation of coconut oil alone (control) or containing T (5 μg g^{-1} body mass), followed by 9 days of acclimation to LSW (6 ppt), SW (38 ppt) or HSW (55 ppt). Further details as in legend of Table 1

Parameter	Treatment	Before transfer	After transfer		
			LSW	SW	HSW
Metabolite levels					
Glycogen (μmol glycosyl units g^{-1} wet wt.)	Control	1.37 ± 0.11	0.99 ± 0.13a	1.78 ± 0.21b	1.12 ± 0.15a
	T	1.10 ± 0.11	0.64 ± 0.07*a	1.84 ± 0.20b	0.83 ± 0.20a
Glucose (μmol g^{-1} wet wt.)	Control	1.90 ± 0.22	2.01 ± 0.18a	2.67 ± 0.33a	2.04 ± 0.39a
	T	1.26 ± 0.11*	0.38 ± 0.06*a	2.37 ± 0.24b	1.78 ± 0.31b
Lactate (μmol g^{-1} wet wt.)	Control	3.16 ± 0.13	3.68 ± 0.26a	3.41 ± 0.34a	4.28 ± 0.33a
	T	3.03 ± 0.21	4.85 ± 0.47*a	3.57 ± 0.30b	3.38 ± 0.21*b
Acetoacetate (μmol g^{-1} wet wt.)	Control	8.30 ± 0.49	8.58 ± 0.07a	8.88 ± 0.16a	8.94 ± 0.19a
	T	8.63 ± 0.12	3.46 ± 0.37*a	9.28 ± 0.25b	9.09 ± 0.27b
Triglyceride (μmol g^{-1} wet wt.)	Control	3.87 ± 1.01	2.25 ± 0.30a	2.16 ± 0.20a	1.28 ± 0.41a
	T	0.86 ± 0.07*	0.40 ± 0.08*a	2.31 ± 0.44b	3.06 ± 0.55*b
Enzyme activities					
Carbohydrate metabolism					
PFK activity					
Optimal activity (U mg^{-1} protein)	Control	2.70 ± 0.21	2.41 ± 0.14a	2.92 ± 0.26a	2.71 ± 0.29a
	T	2.89 ± 0.24	3.27 ± 0.33*a	3.02 ± 0.30a	2.94 ± 0.33a
Activity ratio (%)	Control	24.1 ± 2.67	13.3 ± 1.52a	17.4 ± 2.33a	16.7 ± 1.22a
	T	14.4 ± 1.44*	18.2 ± 2.92a	15.2 ± 1.23ab	10.1 ± 1.68*b
Lipid metabolism					
HOAD activity (U mg^{-1} protein)	Control	0.17 ± 0.01	0.24 ± 0.2a	0.18 ± 0.02ab	0.16 ± 0.02b
	T	0.19 ± 0.02	0.19 ± 0.03a	0.21 ± 0.02a	0.22 ± 0.01*a

observed a higher increase of gill Na^+, K^+-ATPase activity after treatment with intermediate T doses. As suggested in gilthead sea bream submitted to different hormonal treatments (cortisol: Laiz-Carrión et al. 2003; 17β-estradiol: Guzman et al. 2004) including T (Sangiao-Alvarellos et al. 2006), a down regulation of T receptors in chloride cells could occur thus explaining the differential stimulation of gill Na^+, K^+-ATPase activity by T treatment observed in the present study. The existence of T receptors has not been demonstrated in gilthead sea bream, though T receptors have been described in the gills of Atlantic salmon (Jakobsson et al. 1997). Therefore, further studies will be necessary to test this hypothesis. Moreover, kidney Na^+, K^+-ATPase activity did not change in specimens treated with T for 5 days, suggesting the lack of influence of T on this enzymatic activity.

In gilthead sea bream, a partially euryhaline species, the relationship between gill Na^+, K^+-ATPase activity and environmental salinity is expressed as a clear "U-shaped curve" (Laiz-Carrión et al. 2005; Sangiao-Alvarellos et al. 2005a). A similar pattern has been reported for other euryhaline species (Jensen et al. 1998; Kelly et al., 1999). In this study, we have also observed an increase in gill Na^+, K^+-ATPase in LSW- with respect to SW-acclimated fish, but differences were not significant. In the kidneys of control fish transferred to different salinities, no changes were observed in Na^+, K^+-ATPase activity in contrast with the direct relationship between environmental salinity and kidney Na^+, K^+-ATPase activity reported in black sea bream (Kelly et al. 1999) and gilthead sea bream (Sangiao-Alvarellos et al. 2003). However, this species showed two clear stages in the time course of kidney Na^+, K^+-ATPase activity after hypersalinity transfer: a first stage (day 1) in which enzyme activity increased followed by a second stage with a decline of this activity to levels comparable to those of SW-acclimated fish (Sangiao-Alvarellos et al. 2005a). Thus, we may suggest that transferred fish (after 9 days) in the present study were in the second stage.

Nine days after transfer to HSW, gill Na^+, K^+-ATPase activity increased significantly in control fish in agreement with previous data reported in gilthead sea bream submitted to similar hyperosmotic transfer (Laiz-Carrión et al. 2005; Sangiao-Alvarellos et al. 2005a). In T-treated group, this parameter also showed a significant increase in HSW- with respect to SW- and LSW-acclimated fish. In the kidneys, we have observed interactive effects of T treatment with osmotic acclimation in Na^+, K^+-ATPase activity, which increased in T-treated group acclimated to HSW, but not in

fish maintained in SW. These results suggest that, in addition to T, another factor stimulated by increased salinity (or even salinity itself) may be involved in inducing the hypoosmoregulatory action of T (see McCormick 1996). This increased Na^+, K^+-ATPase activity in T-treated fish submitted to HSW could induce a reduction in urine production and/or an increase in ion excretion in the kidney of HSW-fish.

Metabolic parameters

The changes in the tissues of T-treated fish before transfer agree in general with those previously reported in SW-acclimated specimens of gilthead sea bream treated with 5 μg T/ body mass for 3–7 days (Sangiao-Alvarellos et al. 2006). The different response of several parameters (i.e., lack of changes in glucose and lactate levels) could be attributed to the different time course used: in the former experiment those changes were apparent after 7 days, a time period longer than the 5 days used in the present study.

Moreover, changes observed in the metabolic parameters in tissues (liver, gills, kidneys and brain) of control fish acclimated to different salinities (LSW, SW and HSW) were also similar to those previously described in the same species under similar salinity conditions (Sangiao-Alvarellos et al. 2003, 2005a).

Since no significant interactions of T treatment with osmotic acclimation were noticed in plasma metabolite levels, the next paragraphs will therefore be devoted to the interactions observed in tissues. It is important to remark that there are no studies available in literature in which T treatment and osmotic acclimation had been assessed together before. For this reason, this part of the discussion lacks supporting references.

Metabolite levels and enzyme activities assessed were representative of major pathways involved in energy metabolism in fish, such as those related to (1) carbohydrates, like glucose and glycogen levels, and activity of enzymes involved in glucose use (HK and GK), glucose release (G6Pase), glycolysis (PK, PFK, G3PDH), gluconeogenesis (FBPase) and synthesis of pentoses (G6PDH), (2) amino acid levels and activity of amino acid catabolising enzymes (GDH, Ala-AT, Asp-AT), (3) triglyceride levels and activity of an enzyme of β-oxidation (HOAD), and (4) monocarboxylates, like lactate and acetoacetate levels, and the activity of the enzyme involved in lactate use (LDH-O).

In the liver, HK activity of T-treated fish displayed lower values in LSW- and HSW- compared with SW-acclimated fish suggesting that T treatment decreased the liver capacity for using exogenous glucose in fish acclimated to extreme salinities. This is also reflected in a concomitant decrease in glucose levels and G6Pase activity. In other parameters (i.e., glycogen, amino acid and triglyceride levels), this decrease was evident in LSW- but not in HSW-acclimated specimens.

In the gills, T treatment increased glycogen and lactate levels in SW- and HSW-acclimated fish in parallel with increased glycolytic potential in the same salinities. In contrast, those specimens displayed a decreased capacity for oxidation of amino acids (based on changes observed in GDH and Asp-AT activities). These results suggest that T treatment in gills decreased the capacity of using amino acids as fuel compared with control fish, whereas increased their capacity of synthesizing glycogen. However, considering that no significant changes were noticed in glucose levels or in the capacity of using exogenous glucose, the changes observed in glycogen levels appear to be marginal.

In the kidneys, amino acid levels in controls decreased in LSW- compared with SW-acclimated fish, while levels were similar in T-treated fish acclimated to both salinities. Interestingly, the response of Ala-AT activity to different salinities in controls (decreased in extreme salinities) disappeared in T-treated fish that showed the same activity in the three salinities assessed. This allows us to suggest that the metabolism of amino acids in the kidneys was enhanced under T treatment only in fish acclimated to extreme salinities, probably due to the competition for energy resources between the effects of T treatment and osmotic acclimation. Only marginal interactive effects were noticed between osmotic acclimation and energy metabolism in other parameters assessed, since most changes can be attributed to the action of T alone. In this way, the increase observed in glycogen levels of treated fish in SW agrees with that previously observed (Sangiao-Alvarellos et al. 2006).

In the brain, the main interactions observed were the decreased levels of glycogen, glucose, triglyceride and acetoacetate in LSW-acclimated fish treated with T. It seems that the brains of gilthead sea bream acclimated to LSW demand more metabolites, suggesting that the energy demand raised by osmotic acclimation (Sangiao-Alvarellos et al. 2003, 2005a) is higher than the effects of T treatment alone, since this hormone induced in the same tissue increased levels of those metabolites in SW-acclimated specimens (Sangiao-Alvarellos et al. 2006, this study).

In summary, the parallel increase of plasma T levels with salinity in the control group allow us to suggest an osmoregulatory role for T during hyperosmotic acclimation in gilthead sea bream, which needs further research. T treatment alone induced in SW-acclimated gilthead sea bream osmoregulatory and metabolic changes similar to those previously described (Sangiao-Alvarellos et al. 2006). When those fish were transferred to extreme osmotic conditions (LSW and HSW), several interactions occurred in several parameters. At the osmoregulatory level, those interactions included enhanced kidney Na^+, K^+-ATPase activity in

HSW-acclimated fish with no differences observed in plasma osmolality with respect to SW-acclimated fish. From a metabolic point of view, interactions observed addressed that changes produced by transfer to extreme salinities (LSW and HSW) were of higher magnitude thus masking those produced by T treatment alone resulting in less changes due to T treatment, when fish were transferred to extreme salinities. This is almost the converse to that observed in the same species treated with E_2 (Sangiao-Alvarellos et al. 2005b) in which E_2-treated fish transferred to extreme salinities displayed more metabolic changes than non-treated fish transferred to the same salinities.

Acknowledgments This study was partly supported by grants VEM2003-20062 (Ministerio de Ciencia y Tecnología and FEDER, Spain) and PGIDT04PXIC31208PN (Xunta de Galicia, Spain) to J.L.S., and grant BOS2004-04439-C02-01B (Ministerio de Educación y Ciencia and FEDER, Spain) to J.M.M. The authors wish to thank Planta de Cultivos Marinos (CASEM, Universidad de Cádiz, Puerto Real, Cádiz, Spain) for providing the experimental fish. The experiments described comply with the European Union Council (86/609/EU) and the Spanish Government (RD 1201/2005) guidelines for the use of animals in research (Consejería de Agricultura y Pesca de la Junta de Andalucía animal facilities refs. CA/3/U and CA/4/CS).

References

Arias A (1976) Sobre la biología de la dorada, *Sparus aurata* L., de los esteros de la provincia de Cádiz. Inv Pesq 40:201–222

Chaves-Pozo E, Liarte S, Vargas-Chacoff L, Garcia-Lopez A, Mulero V, Meseguer J, Mancera JM, Garcia-Ayala A (2007) 17β-Estradiol ttriggers postspawning in spermatogenically active gilthead seabream (*Sparus aurata* L.) males. Biol Reprod 6:142–148

Gothilf Y, Meiri I, Elizur A, Zohar Y (1997) Preovulatory changes in the levels of three gonadotropin-releasing hormone-encoding messenger ribonucleic acids (mRNAs), gonadotropin beta-subunit mRNAs, plasma gonadotropin, and steroids in the female gilthead seabream, *Sparus aurata*. Biol Reprod 57:1145–1154

Guzmán JM, Sangiao-Alvarellos S, Laiz-Carrión R, Míguez JM, Martín del Río MP, Soengas JL, Mancera JM (2004) Osmoregulatory action of 17β-estradiol in the gilthead sea bream *Sparus auratus*. J Exp Zool 301A:828–836

Haddy JA, Pankhurst NW (2000) The effects of salinity on reproductive development, plasma steroid levels, fertilisation and egg survival in black bream *Acanthopagrus butcheri*. Aquaculture 188:115–131

Haux C, Norberg B (1985) The influence of estradiol-17β on the liver content of protein, lipids, glycogen, and nucleic acids in juvenile rainbow trout, *Salmo gairdneri*. Comp Biochem Physiol 81B:275–279

Ince BW, Lone KP, Matty AJ (1982) Effect of dietary protein level, and an anabolic steroid, ethylestrenol on the growth, food conversion efficiency and protein efficiency ratio of rainbow trou (*Salmo gairdneri*). Br J Nutr 47:615–624

Jakobsson S, Mayer I, Borg B (1997) Androgen binding in the gills of atlantic salmon, *Salmo salar*, mature male parr, and immature male smolts. J Exp Zool 278:391–394

Jensen MK, Madsen SS, Kristiansen K (1998) Osmoregulation and salinity effects on the expression and activity of Na^+, K^+-ATPase in gills of European sea bass, *Dicentrarchus labrax* (L.). J Exp Zool 282:290–300

Kelly SP, Woo NYS (1999) Cellular and biochemical characterization of hypoosmotic adaptation in a marine teleost, *Sparus sarba*. Zool Sci 16:505–514

Kelly SP, Chow INK, Woo NYS (1999) Haloplasticity of black seabream (*Mylio macrocephalus*): hypersaline to freshwater acclimation. J Exp Zool 283:226–241

Keppler D, Decker K (1974) Glycogen. determination with amyloglucosidase. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Academic, New York, pp 1127–1131

Laiz-Carrión R, Guerreiro PM, Fuentes J, Martín del Río MP, Canario AVM, Mancera JM (2005) Branchial osmoregulatory response to environmental salinities in the gilthead sea bream, *Sparus auratus*. J Exp Zool 303A:563–576

Laiz-Carrión R, Martín del Río MP, Míguez JM, Mancera JM, Soengas JL (2003) Influence of cortisol on osmoregulation and energy metabolism in gilthead sea bream *Sparus aurata*. J Exp Zool 298A:105–118

Le François N, Blier P (2000) Branchial Na^+, K^+-ATPase activity in brook charr (*Salvelinus fontinalis*): effect of gonadal development in hypo- and hyperosmotic environmentes. J Exp Zool 286:647–655

Le François NR, Lamarre SG, Blier PU (2004) Tolerance, growth and haloplasticity of the Atlantic wolfish (*Anarhichas lupus*) exposed to various salinities. Aquaculture 236:659–675

Mancera JM, Laiz-Carrión R, Martín del Río MP (2002) Osmoregulatory action of PRL, GH and cortisol in the gilthead seabream (*Sparus auratus* L.). Gen Comp Endocrinol 129:95–103

Mancera JM, Smolenaars M, Laiz-Carrión R, Martin del Rio MP, Wendelaar Bonga SE, Flik G (2004) 17β-estradiol affects osmoregulation in *Fundulus heteroclitus*. Comp Biochem Physiol 139B:183–191

McCormick SD (1993) Methods for nonlethal gill biopsy and measurement of Na^+, K^+-ATPase activity. Can J Fish Aquat Sci 50:656–658

McCormick SD (1995) Hormonal control of gill Na^+, K^+-ATPase and chloride cell function. In: Wood CM, Shuttleworth TJ (eds) Hormonal control of gill Na^+, K^+ATPase and chloride cell function. Academic, San Diego, pp 285–315

McCormick SD (1996) Effect of growth hormone and insulin-like factor I on salinity tolerance and gill Na^+, K^+-ATPase in atlantic salmon (*Salmo salar*): interaction with cortisol. Gen Comp Endocrinol 101:3–11

McCormick SD, Naiman RJ (1985) Hypoosmoregulation in an anadromous teleost: influence of sex and maduration. J Exp Zool 234:193–198

Moore S (1968) Amino acid analysis: aqueous dimethyl sulfoxide as solvent for the nynhidrin reaction. J Biol Chem 1242:6281–6283

Nakano K, Tagawa M, Takemura A, Hirano T (1998) Temporal changes in liver carbohydrate metabolism associated with seawater transfer in *Oreochromis mossambicus*. Comp Biochem Physiol 119B:721–728

Peter MCS, Oommen OV (1989) Oxidative metabolism in a teleost, *Anabas testudineus* Bloch: effect of testosterone and estradiol-17β on hepatic enzyme activities. Fish Physiol Biochem 6:377–385

Reshkin SJ, Grover ML, Howerton RD, Grau EG, Ahearn GA (1989) Dietary hormonal modification of growth, intestinal ATPase, and glucose transport in tilapia. Am J Physiol 256:E610–E618

Rodríguez L, Begtashi I, Zanuy S, Carrillo M (2000) Development and validation of an enzyme inmunoassay for testosterone: effects of photoperiod on plasma testosterone levels and gonadal development in male sea bass (*Dicentrarchus labrax*, L.) at puberty. Fish Physiol Biochem 23:141–150

Ros AFH, Becker K, Canario AVM, Oliveira RF (2004) Androgen levels and energy metabolism in *Oreochromis mossambicus*. J Fish Biol 65:895–905

Sangiao-Alvarellos S, Laiz-Carrión R, Guzmán JM, Martín del Río MP, Míguez JM, Mancera JM, Soengas JL (2003) Acclimation of *S. aurata* to various salinities alters energy metabolism of osmoregulatory and nonosmoregulatory organs. Am J Physiol 285:R897–R907

Sangiao-Alvarellos S, Arjona FJ, Martín del Río MP, Míguez JM, Mancera JM, Soengas JL (2005a) Time course of osmoregulatory and metabolic changes during osmotic acclimation in *Sparus auratus*. J Exp Biol 208:4291–4304

Sangiao-Alvarellos S, Guzmán JM, Laiz-Carrión R, Míguez JM, Martín del Río MP, Mancera JM, Soengas JL (2005b) Actions of 17β-estradiol on carbohydrate metabolism in liver, gills, and brain of gilthead sea bream *Sparus auratus* during acclimation to different salinities. Mar Biol 146:607–617

Sangiao-Alvarellos S, Polakof S, Arjona FJ, García-López A., Martín del Río MP, Martínez-Rodríguez G, Míguez JM, Mancera JM, Soengas JL (2006) Influence of testosterone administration on osmoregulation and energy metabolism of gilthead seabream *Sparus auratus*. Gen Comp Endocrinol 149:30–41

Singh C, Gupta RC (2002) Influence of testosterone propionate and methyl testosterone on RNA, DNA, glycogen and protein contents in liver and ovary of fresh water catfish, *Clarias batrachus* (Bloch). J Exp Zool India 5:41–44

Sparks R, Shepherd BS, Ron B, Richman III NH, Riley LG, Iwama GK, Hirano T, Grau EG (2003) Effects of environmental salinity and 17α-methyltestosterone on growth and oxygen consumption in the tilapia, *Oreochromis mossambicus*. Comp Biochem Physiol 136B:657–665

Sunny F, Oommen OV (2000) Steroid hormones regulate enzymes of osmoregulation in a freshwater fish *Oreochromis* (tilapia) *mossambicus*. J Endocrinol Reprod 4:63–73

Sunny F, Oommen OV (2002) Rapid action of testosterone and diethylstilbestrol on enzymes of osmoregulation in a freshwater fish *Oreochromis mossambicus*. Endocrine Res 28:69–81

Sunny F, Jacob A, Oommen OV (2002) Sex steroids regulate intermediary metabolism in *Oreochromis mossambicus*. Endocrine Res 28:175–188

Vijayan MM, Takemura A, Mommsen TP (2001) Estradiol impairs hypoosmoregulatory capacity in the euryhaline tilapia, *Oreochromis mossambicus*. Am J Physiol 281:R1161–R1168

Woo NYS, Chung ASB, Ng TB (1993) Influence of oral administration of estradiol-17β and testosterone on growth, digestion, food conversion and metabolism in the underyearling red sea bream, *Chrysophrys major*. Fish Physiol Biochem 10:377–387