Available online at www.sciencedirect.com
ScienceDirect
General and Comparative Endocrinology 149 (2006) 30–41
GENERAL AND COMPARATIVE ENDOCRINOLOGY
www.elsevier.com/locate/ygcen

Influence of testosterone administration on osmoregulation and energy metabolism of gilthead sea bream *Sparus auratus*

Susana Sangiao-Alvarellos [a], Sergio Polakof [a], Francisco J. Arjona [b], Angel García-López [c], María P. Martín del Río [b], Gonzalo Martínez-Rodríguez [c], Jesús M. Míguez [a], Juan M. Mancera [b], José L. Soengas [a,*]

[a] *Laboratorio de Fisioloxía Animal, Departamento de Bioloxía Funcional e Ciencias da Saúde, Facultade de Bioloxía, Universidade de Vigo, 36310 Vigo, Spain*
[b] *Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, 11510 Puerto Real, Cádiz, Spain*
[c] *Instituto de Ciencias Marinas de Andalucía, CSIC, 11510 Puerto Real, Cádiz, Spain*

Received 15 November 2005; revised 21 February 2006; accepted 2 May 2006
Available online 12 June 2006

Abstract

The osmoregulatory and metabolic role of testosterone (T) in the euryhaline teleost *Sparus auratus* was examined. Fish were implanted with a slow-release coconut oil implant alone (control) or containing T (2 or 5 $\mu g\,g^{-1}$ body weight) and sampled 1, 3, and 7 days after implantation. Gill Na^+,K^+-ATPase activity increased in fish treated with the lower dose of T after 7 days of treatment. Kidney Na^+,K^+-ATPase activity enhanced at first day post-implantation in the group treated with the higher dose of T but the values diminished by day 3. Plasma levels of metabolites (glucose, lactate, triglyceride, and protein) increased after T treatment. This higher availability of plasma metabolites was reflected in several metabolic changes within different tissues of T-treated fish such as (i) increased glycogen levels and capacity for gluconeogenesis, ketogenesis, glucose exporting, and amino acid catabolism in the liver, (ii) enhanced lipogenic capacity in the gills, (iii) increased glycogen levels and capacity for oxidizing amino acids in the kidney, and (iv) enhanced levels of glycogen, aceotacetate, glucose and triglycerides, and higher capacity of phosphorylating glucose in the brain. These results provide evidence regarding an osmoregulatory and metabolic role for T in *S. auratus* that could be related to changes in both processes during sexual maturation.

Keywords: Gilthead sea bream; *Sparus auratus*; Testosterone; Osmoregulation; Energy metabolism

1. Introduction

The most important androgens in fish are 11-ketotestosterone (11-KT)[1] and testosterone (T), being both involved in the control of spermatogenesis, and development of the secondary sexual characters (Borg, 1994; Weltzien et al., 2004). The changes in plasma levels of both androgens during the reproductive cycle of male gilthead seabream have been recently described (Chaves-Pozo, 2005). 11-KT levels increased during spermatogenesis peaking (ca. 0.6 $ng\,ml^{-1}$) at the end of such process (just prior spawning) indicating the clear implication of this androgen in driving the spermatogenetic development in male teleost (Borg, 1994). During the rest of the reproductive cycle, 11-KT

* Corresponding author. Fax: +34 986812556.
E-mail address: jsoengas@uvigo.es (J.L. Soengas).

[1] *Abbreviations used:* 11-KT, 11-ketotestosterone; Ala-AT, alanine aminotransferase (EC. 2.6.1.2.); Asp-AT, aspartate aminotransferase (EC. 2.6.1.1.); E_2, 17β-estradiol; FBPase, fructose 1,6-bisphosphatase (EC. 3.1.3.11.); G3PDH, α-glycerophosphate dehydrogenase (EC. 1.1.1.8.); G6Pase, glucose 6-phosphatase (EC. 3.1.3.9.); 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.); HK, hexokinase (EC. 2.7.1.1.); 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.); SEI, sucrose-EDTA-imidazole; T, testosterone.

0016-6480/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2006.05.003

levels were kept low (< 0.3 ng ml^{-1}). As T levels are concerned, they showed little changes during spermatogenesis (0.4–0.5 ng ml^{-1}), peaked during post-spawning (ca. 1 ng ml^{-1}), and kept relatively high (ca. 0.7 ng ml^{-1}) during resting. The significance of peak T levels at post-spawning is unclear. Possible mechanisms would involve the gonadotroph cell development of the pituitary (Cavaco et al., 2001), the inhibition of subsequent spermatogenetic processes to initiate the resting period (T inhibits spermatogenesis induced by 11-KT; Cavaco et al., 2001), and/or the initial step in the preparation of the gonad for the next reproductive cycle (Prat et al., 1990). In addition to their role in reproduction, sex steroids are known to be involved in growth (Sparks et al., 2003), digestion and food utilization (Ince et al., 1982), gut transport (Reshkin et al., 1989), and shifts in body composition (Dasmahapatra and Medda, 1982;Haux and Norberg, 1985).

It is well established that sexual maturation and treatment with exogenous sex steroids have a negative effect on the adaptability of several salmonids to seawater (McCormick and Naiman, 1985; McCormick, 1995; Le François and Blier, 2000). In non-salmonid species, there are few studies focused on the role of sex steroids on the adaptability to different salinities, the majority of them dealing with estrogen effects. Negative influence of 17β-estradiol (E_2) treatment on gill Na^+,K^+-ATPase activity and hypoosmotic capacity have been reported in tilapia (Vijayan et al., 2001) and mummichog (Mancera et al., 2004). However, in gilthead sea bream *Sparus auratus* a clear stimulatory effect of E_2 on gill Na^+,K^+-ATPase activity was observed after long-term treatment (Guzmán et al., 2004). To our knowledge, the evidence about a direct effect of T on gill Na^+,K^+-ATPase activity has been only reported in tilapia (Sunny and Oommen, 2000, 2002) in which gill Na^+,K^+-ATPase and Ca^{+2}-ATPase activities were significantly enhanced by as early as 30 min after T treatment and also after 5 days of treatment.

Gonadal steroids exert significant effects on intermediary metabolism in vertebrates. In fish, there are several studies regarding effects of estrogens on fish energy metabolism (Mommsen and Walsh, 1988) generally addressing a metabolic reallocation of reserves from liver to the gonad. In this way, in a previous study we showed several changes in the liver, gills and brain carbohydrate metabolism of E_2-treated gilthead sea bream (Sangiao-Alvarellos et al., 2005b). Considering that changes in androgen levels during the reproductive season have been shown to correlate with changes in energy allocation (Leonard et al., 2002), a role for androgens in energy metabolism seems to be reasonable. Accordingly, treatment with T in fish increased oxygen consumption (Sparks et al., 2003; Ros et al., 2004), and induced several changes in intermediary metabolism suggesting an anabolic role for this hormone in the liver (Peter and Oommen, 1989; Woo et al., 1993; Singh and Gupta, 2002; Sunny et al., 2002a). A possible metabolic role in other tissues has been less studied (Gupta et al., 1993). Therefore, the precise role of androgens in energy metabolism of teleosts remains to be thoroughly investigated.

Our group has analysed the influence of different hormones in the adaptation of gilthead sea bream to hyperosmotic and hypoosmotic environments (Mancera et al., 2002, 1994; Laiz-Carrión et al., 2002, 2003, 2005; Sangiao-Alvarellos et al., 2005a). In these studies, we have also analysed the osmoregulatory and metabolic action of estrogens like E_2 (Guzman et al., 2004; Sangiao-Alvarellos et al., 2005b), but to date there are no information about the possible osmoregulatory and metabolic role of T in such processes in gilthead sea bream. This species can develop gonads in estuarine areas, characterised by the presence of brackishwater but spawning always happen in seawater (Arias, 1976). It is known that in gilthead sea beam there is a "U-shaped" relationship between environmental salinity and gill Na^+,K^+-ATPase activity, with lower activities at isosmotic environments (estuarine areas) and higher at hyperosmotic environments (sea water of open sea) (Laiz-Carrión et al., 2005). Therefore, high levels of T necessary to stimulate gonadal development are present once gilthead sea bream enters seawater where osmoregulatory (increased gill Na^+,K^+-ATPase activity and hypoosmoregulatory capacity) and metabolic changes occur. Therefore, increasing levels of T observed during last phases of gonadal development take place when gilthead seabream is in seawater where osmoregulatory (increased gill Na^+-K^+-ATPase activity and hypoosmoregulatory capacity) and metabolic changes occur. Therefore, we aimed to assess a possible relationship between increased T levels and osmoregulatory and metabolic changes. This situation simulating the increased levels of this hormone occurring in male gilthead seabream during spawning in seawater.

2. Materials and methods

2.1. Fish

Sexually immature male gilthead sea bream (*Sparus auratus* L.; 81 ± 2 g body weight) 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 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 male gilthead sea bream occurs when fish reach approximately 400 g of weight; Zohar et al., 1984). Fish were acclimatised to seawater (SW, 38 p.p.t. salinity, 1103 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), being fed on 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 here described comply with the European Union Council (86/609/EU) and the University of Cádiz (Spain) Guidelines for the use of laboratory animals.

2.2. Experimental design

Fish were caught by netting, lightly anaesthetized with 2-phenoxyethanol (0.05% v/v; Sigma), weighed, and injected intraperitoneally (5 μl g^{-1} body weight) with a slow-release coconut oil implant alone (control) or containing T (2 or 5 μg g^{-1} body weight). Fish ($n = 9$ per group and time) were sampled after 1, 3, and 7 days post-implantation (see below).

Thereafter, one group of untreated fish ($n = 9$) was sampled serving as uninjected control at time 0 days. The dose of T and the administration procedure were similar to others previously described in literature (Bjerkeng et al., 1999; Sunny et al., 2002b).

2.3. Sampling

Fish were deeply 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 for plasma testosterone, protein levels, and osmolality. The other aliquot for the assessment of plasma metabolites concentration, 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 the Na^+,K^+-ATPase activity, 3–5 filaments from the second branchial arch (cut just above the septum with a 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, and the remaining kidney and branchial arches were removed in few seconds, freeze-clamped in liquid nitrogen, and stored at −80 °C until assay.

2.4. Analytical techniques

Plasma glucose, lactate, and triglyceride levels were measured using commercial kits from Spinreact (Spain) adapted to microplates. Plasma protein concentration was quantified using the bicinchoninic acid method with BCA protein kit (Pierce, Rockford, USA) for microplates, with bovine serum albumin as standard. Plasma Cl^- levels were measured with the Chloride Sigma kit (No. 461–3). Plasma osmolality was assessed with a vapor pressure osmometer (Fiske One-Ten Osmometer, Fiske, VT, USA).

Plasma levels of T were quantified by enzyme-linked immunosorbent assay (ELISA) following the method described by Rodríguez et al. (2000). Steroids were extracted from 3.5–8 μl plasma in 1–1.5 ml methanol. T standard was purchased from Sigma–Aldrich (Madrid, Spain). Mouse anti-rabbit IgG monoclonal and specific anti-steroid antibodies and enzymatic tracer (steroid acetylcholinesterase conjugate) were obtained from Cayman Chemical Company (Michigan, USA). Microtiter plates (MaxiSorp™) were purchased from Nunc (Roskilde, Denmark). A standard curve from 6.1×10^{-4} to 2.5 ng ml^{-1} was established in all assays. Standards and extracted plasma samples were run in duplicate. The lower limit of detection (90% of binding, ED90) was 19 pg ml^{-1} plasma (0.06 pg $well^{-1}$). The inter-assay coefficient of variation at 50% of binding (100 × standard deviation × $mean^{-1}$ of points corresponding to 50% of binding) was 11.5% ($n = 6$) whereas the intra-assay coefficient of variation (calculated from samples duplicates) was 4.0 ± 0.3%. Details on cross-reactivity for T specific antibody were given by the supplier.

Gill and kidney Na^+,K^+-ATPase activity were determined using the microassay method of McCormick (1993) adapted for its use in gilthead sea bream (Mancera et al., 2002).

Frozen liver, brain, kidney, and gill were finely minced on a chilled Petri dish to very small pieces that, still frozen, were divided into two different (but relatively homogeneous) aliquots to assess enzyme activities and metabolite levels. For the assessment of metabolite levels, frozen tissue was homogenized immediately by ultrasonic disruption with 7.5 vol 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 ninhydrin method of Moore (1968) with modifications to adapt the assay to a microplate format. For the assessment of enzyme activities, frozen tissue was homogenized by ultrasonic disruption with 10 vol of ice-cold stopping-buffer containing 50 mmol l^{-1} imidazole–HCl (pH 7.5), 1 mmol l^{-1} 2-mercaptoethanol, 50 mmol l^{-1} NaF, 4 mmol l^{-1} EDTA, 250 mmol l^{-1}sucrose, and a protease inhibitor cocktail (Sigma, P-2714). The homogenate was centrifuged and the supernatant used in the enzyme assays. Enzyme activities were determined using a microplate reader (Spectrafluor, Tecan). Reaction rates of enzymes were determined by the increase or decrease in absorbance of NAD(P)H at 340 nm. The reactions were started by the addition to the wells of homogenates (15 μl), at a pre-established protein concentration, omitting the substrate in control wells (final volume 295 μl), and allowing the reactions to proceed at 20 °C for pre-established times (3–10 min). No changes were found in tissue protein levels in any of the groups studied (data not shown), and therefore enzyme activities are expressed in terms of milligrams protein. Protein was assayed in homogenates using microplates following the bicinchoninic acid method with bovine serum albumin (Sigma, USA) as standard. The specific conditions for enzyme assays were described previously (Laiz-Carrión et al., 2002, 2003; Sangiao-Alvarellos et al., 2003, 2005a,b).

2.5. Statistics

Data were statistically analysed by two-way ANOVA in which treatment (control, and different doses of T) and time (1, 3, and 7 days) were the main factors. Post hoc comparisons were made using a Student–Newman–Keuls test, with the differences considered to be statistically significant at $P < 0.05$.

3. 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). *P*-values resulting from the two-way ANOVA of all parameters analysed are displayed on Table 1.

Plasma T levels increased in treated fish at day 1 after implant and decreased throughout the experiment. However, plasma T levels always showed higher values in treated fish compared with controls and presented a lineal relationship with doses of T implanted (Fig. 1). Hormonal implant did not affect hepatosomatic index (data not shown).

After 7 days of treatment, gill Na^+,K^+-ATPase activity showed a significant increase in fish treated with the lowest dose of T (2 μg g^{-1} body weight), but not with the higher dose (5 μg g^{-1} body weight) (Fig. 2A). Kidney Na^+,K^+-ATPase activity only showed increased values at first day post-implantation in the group treated with the higher dose of T (Fig. 2B). There were no differences in plasma osmolality between treatments or days (Fig. 2C).

Plasma glucose and lactate levels presented a similar pattern (Figs. 3A and B), with the T-treated groups showing higher values than controls after 7 days of treatment. T treatment increased plasma triglyceride levels at days 1 and 3 post-implantation, but levels decreased to values similar to those of controls at 7 days (Fig. 4A). Plasma protein increased at day 7 in fish treated with the higher dose of T (Fig. 4B).

In the liver (Table 2), T treatment increased glycogen and triglyceride levels after 3 and 7 days. Lactate values displayed a dose-dependent increase throughout the experiment whereas acetoacetate levels also enhanced after 7 days of treatment with the higher dose of T. As for

Table 1
P-Values from two-way ANOVA of parameters measured in plasma, liver, gills, kidney, and brain of *S. auratus* after 1–7 days of intraperitoneal implantation of coconut oil alone (control) or containing T (2 and 5 μg g^{-1} b.w.), Treatment and time are the main factors. ns, no significant

Tissue	Parameter	Treatment	Time	Treatment × Time
Plasma	Testosterone levels	<0.001	<0.001	<0.001
	Glucose levels	0.006	<0.001	ns
	Lactate levels	0.06	<0.001	ns
	Protein levels	ns	ns	0.048
	Triglyceride levels	0.004	<0.001	ns
	Osmolality	ns	0.001	ns
Liver	Glycogen levels	0.01	ns	ns
	Glucose levels	ns	ns	0.01
	Lactate levels	0.017	<0.001	0.022
	Triglyceride levels	0.023	0.015	ns
	α-Amino acid levels	ns	ns	ns
	Acetoacetate levels	<0.001	0.005	0.018
	GK activity	ns	ns	ns
	PFK activity (optimal)	ns	ns	0.005
	PFK activity (activity ratio)	ns	ns	ns
	PFK activity (activation ratio)	ns	0.046	ns
	FBPase activity	0.021	0.027	ns
	G6Pase activity	<0.001	<0.001	<0.001
	G6PDH activity	ns	ns	ns
	G3PDH activity	ns	<0.001	<0.001
	GDH activity	ns	ns	ns
	Asp-AT activity	<0.001	0.038	ns
	Ala-AT activity	<0.001	<0.001	ns
	HOAD activity	0.042	<0.001	<0.001
Gills	Na^+,K^+-ATPase activity	ns	0.006	ns
	Glycogen levels	ns	ns	ns
	Glucose levels	ns	ns	ns
	Lactate levels	0.025	<0.001	0.01
	α-Amino acid levels	ns	0.02	ns
	HK activity	ns	ns	ns
	PK activity (optimal)	0.026	<0.001	0.03
	PK activity (activity ratio)	ns	ns	ns
	PK activity (activation ratio)	ns	<0.001	ns
	G6PDH activity	0.022	<0.001	0.038
	G3PDH	ns	ns	ns
	GDH activity	ns	0.014	ns
	Asp-AT	ns	ns	ns
	HOAD activity	0.033	ns	<0.001
	LDH-O activity	<0.001	0.018	<0.001
Kidney	Na^+,K^+-ATPase activity	ns	ns	0.028
	Glycogen levels	0.048	ns	ns
	Glucose levels	ns	ns	ns
	Lactate levels	ns	0.02	ns
	Triglyceride levels	ns	0.029	ns
	α-Amino acid levels	ns	ns	ns
	HK activity	ns	0.002	ns
	PK activity (optimal)	ns	<0.001	ns
	PK activity (activity ratio)	0.034	0.016	ns
	PK activity (activation ratio)	0.006	<0.001	ns
	G6Pase activity	0.038	ns	<0.001
	G6PDH activity	ns	ns	ns
	G3PDH activity	ns	0.032	ns
	GDH activity	ns	ns	0.035
	Ala-AT activity	ns	0.004	<0.001
	LDH-O activity	ns	ns	ns
Brain	Glycogen levels	0.05	ns	ns
	Glucose levels	0.002	<0.001	<0.001
	Lactate levels	ns	ns	ns
	Triglyceride levels	0.016	0.014	ns
	α-Amino acid levels	ns	ns	ns

(*continued on next page*)

Table 1 (*continued*)

Tissue	Parameter	Treatment	Time	Treatment × Time
	Acetoacetate levels	0.021	0.035	ns
	HK activity	<0.001	<0.001	ns
	GK activity	ns	ns	ns
	PFK activity (optimal)	ns	ns	ns
	PFK activity (activity ratio)	ns	ns	ns
	PFK activity (activation ratio)	ns	ns	ns
	G6PDH activity	ns	ns	ns
	G3PDH activity	ns	ns	ns
	GDH activity	ns	ns	ns
	Asp-AT activity	ns	ns	ns
	Ala-AT activity	ns	ns	ns

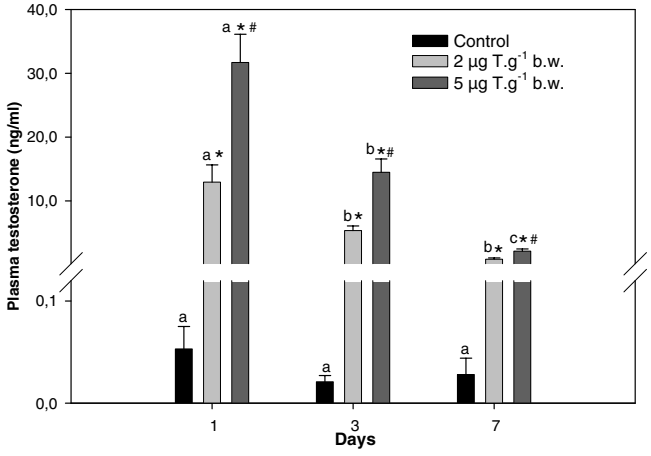

Fig. 1. Plasma testosterone levels in *S. auratus* after 1, 3, and 7 days of intraperitoneal implantation of coconut oil alone (control) or containing (2 or 5µg g^{-1} body weight). Values are the means ± SE ($n = 9$ fish per group). ∗, significantly different ($P < 0.05$) from control under the same experimental conditions. #, significantly different ($P < 0.05$) from fish treated with 2 µg T g^{-1} body weight under the same experimental condition. Different letters indicate significant differences ($P < 0.05$) among sampling times within each treatment.

enzyme activities, T treatment increased FBPase, GDH, Ala-AT, and Asp-AT activities after 3 and 7 days. In addition, treatment with the higher dose of T decreased G3PDH activity after 3 days but increased at 7 days post-implant compared with controls. A dose-dependent increase was detected in G6Pase activity after 7 days of T treatment. No significant changes were noticed for glucose and amino acid levels as well as GK, PK, and G6PDH activities (data not shown).

Gills lactate levels increased after 7 days of treatment with the higher dose of T (Table 3). However, PK total activity and activity ratio enhanced at this time but only with the lower dose of T. At both doses, G6PDH activity increased after 1 day of treatment, while in HOAD activity this increase was observed at 3 and 7 days post-implant. Finally, LDH-O activity enhanced after 7 days of treatment with the higher dose of T. There were no differences in glucose and amino acid levels as well as HK, G3PDH, GDH, and Asp-AT activities between treatments or days (data not shown).

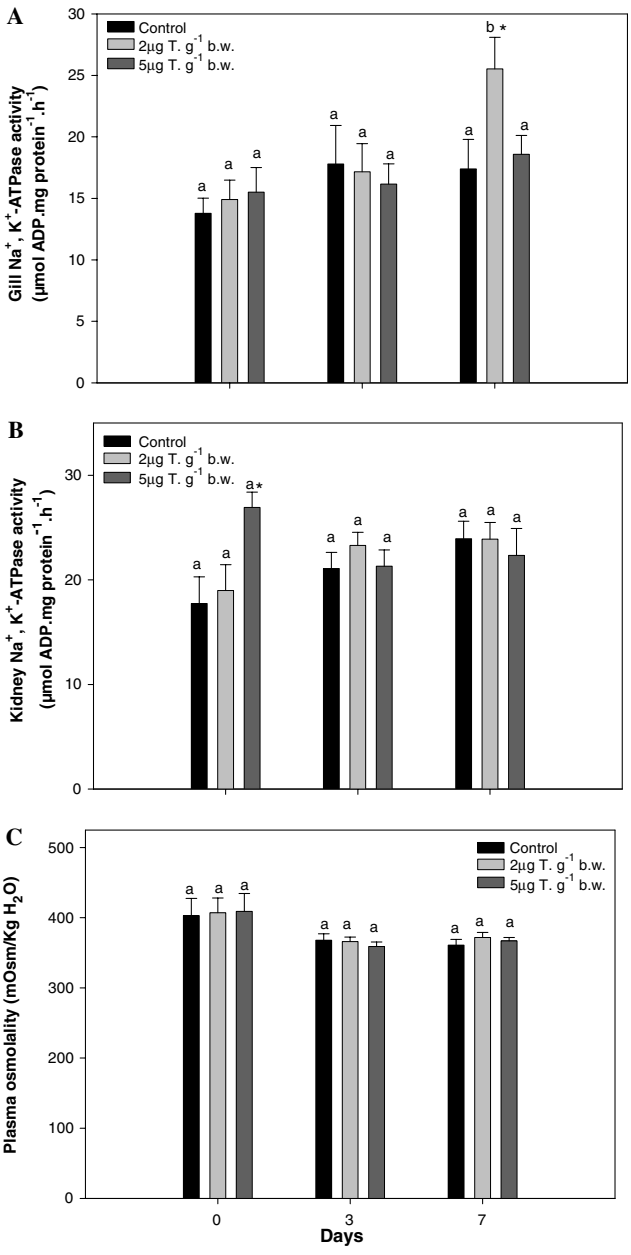

Fig. 2. Na^+,K^+-ATPase activity in gills (A) and kidney (B), and plasma osmolality (C) in *S. auratus*. Further details in legend of Fig. 1.

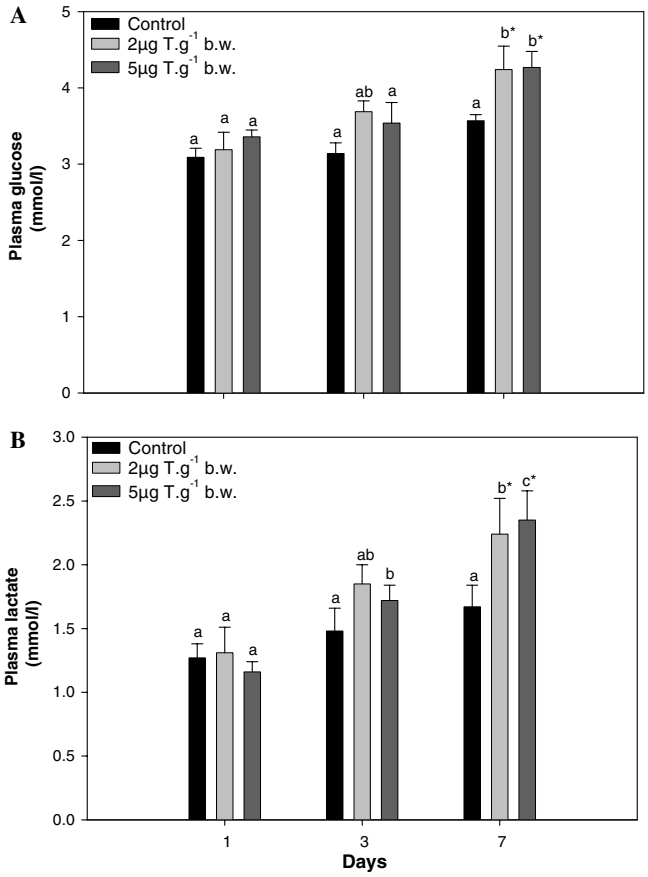

Fig. 3. Plasma glucose (A) and lactate (B) levels in *S. auratus*. Further details in legend of Fig. 1.

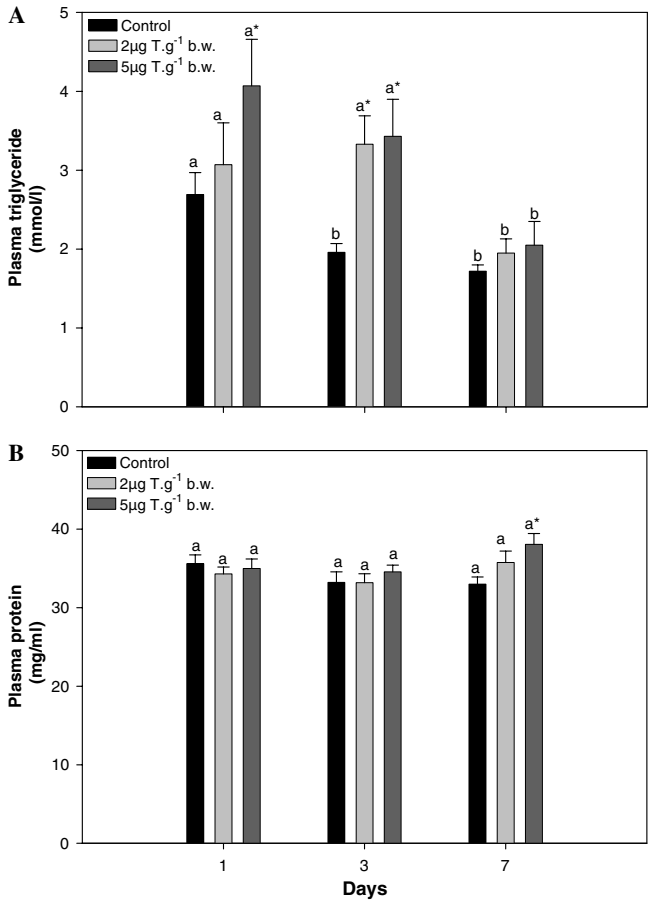

Fig. 4. Triglyceride (A) and protein (B) levels in *S. auratus*. Further details in legend of Fig. 1.

Metabolic changes observed in the kidney are shown in Table 4. Fish treated with the higher dose of T enhanced glycogen levels throughout the experiment. Amino acid levels decreased after 3 days with both doses of T. The activity and activation ratios of PK increased with both doses of T. Hormonal administration diminished G6Pase activity being the differences significant after 7 days. On the other hand, Ala-AT activity enhanced in treated fish in a dose-dependent way only at 7 days post-implant. No significant modifications were observed for glucose, lactate, and triglyceride levels as well as G6PDH, GDH, and LDH-O activities (data not shown).

In brain (Table 5), HK activity and glycogen levels increased showing significant differences after 3 and 7 days of treatment with both doses of T. In addition, glucose and triglyceride levels also enhanced but only after 7 days. Acetoaceate levels displayed a dose-dependent increase after 3 days of hormonal administration. No significant changes were noticed for lactate and amino acid levels as well as GK, G6PDH, GDH, Asp-AT, and Ala-AT activities (data not shown).

4. Discussion

Implants of coconut oil plus T elevated chronically plasma levels of this hormone, which decreased slightly with time but being always significantly higher than controls. The levels observed in treated fish after 1–3 days (10–30 ng ml^{-1}) were higher than those observed in gilthead sea bream during its sexual cycle, ca. 0.4–0.5 ng ml^{-1} during spermatogenesis and spawning and, around 1 ng ml^{-1} at post-spawning (Gothilf et al., 1997; Meiri et al., 2002). However, levels observed in treated fish after 7 days were around 1 ng ml^{-1} i.e. similar to those observed after spawning. Accordingly, a possible pharmacological action of T cannot be discarded, especially for the effects observed after 1 and 3 days of treatment.

The first objective of this study was to evaluate the possible action of T on the osmoregulatory system of gilthead sea bream. Treatment with the lower dose of T (2 µg g^{-1} body weight), but not with the higher (5 µg g^{-1} body weight), increased gill Na^+,K^+-ATPase activity at 7 days post-implantion. These data are in agreement with those obtained by Sunny and Oommen (2000) who reported that administration of T (0.125, 0.25, and 0.5 µg g^{-1} body weight) for 5 days significantly enhanced gill Na^+,K^+-ATPase activity in tilapia. It has been suggested that sex steroids affect the osmoregulatory system through two ways: (i) a direct one by binding to their receptors in gills, and (ii) an indirect pathway through modifications in the levels of different osmoregulatory

Table 2
Liver levels of metabolites and enzyme activities in *S. auratus* after 1, 3, and 7 days of intraperitoneal implantation of coconut oil alone (control) or containing T (2 and 5 μg g^{-1} b. w.)

Parameter	Treatment (μg g^{-1} b.w.)	Time (days)		
		1	3	7
Metabolite levels				
Glycogen (μmol glycosyl units g^{-1} wet wt.)	Control	560 ± 44.8a	578 ± 31.4a	558 ± 44.2a
	2 μg T	648 ± 34.1a	716 ± 18.6*a	773 ± 32.4*a
	5 μg T	636 ± 27.4a	741 ± 18.9*a	763 ± 21.9*a
Lactate (μmol g^{-1} wet wt.)	Control	0.49 ± 0.07a	0.46 ± 0.03a	0.46 ± 0.04a
	2 μg T	0.76 ± 0.08*ab	0.59 ± 0.05b	0.92 ± 0.14*a
	5 μg T	0.48 ± 0.03a	0.70 ± 0.05a	1.27 ± 0.24*b
Triglyceride (μmol g^{-1} wet wt.)	Control	3.37 ± 0.59a	3.34 ± 0.24a	3.23 ± 0.27a
	2 μg T	3.52 ± 0.32a	4.51 ± 0.29*b	4.38 ± 0.39*b
	5 μg T	3.45 ± 0.39a	4.41 ± 0.21*b	4.97 ± 0.38*b
Acetoacetate (μmol g^{-1} wet wt.)	Control	1.43 ± 0.14a	1.59 ± 0.12b	1.57 ± 0.22b
	2 μg T	1.29 ± 0.10a	1.35 ± 0.15a	1.38 ± 0.17a
	5 μg T	1.58 ± 0.06a	1.57 ± 0.22a	2.28 ± 0.16*#b
Enzyme activities				
Carbohydrate metabolism				
FBPase (U mg^{-1} protein)	Control	0.94 ± 0.08a	0.97 ± 0.08a	0.90 ± 0.09a
	2 μg T	1.22 ± 0.06a	1.26 ± 0.08*a	1.66 ± 0.10*b
	5 μg T	1.42 ± 0.07*a	1.31 ± 0.06*a	2.15 ± 0.12*#b
G6Pase (U mg^{-1} protein)	Control	2.15 ± 0.19a	2.26 ± 0.26a	2.22 ± 0.47a
	2 μg T	1.91 ± 0.20a	2.54 ± 0.36a	7.33 ± 1.36*b
	5 μg T	2.48 ± 0.36a	1.79 ± 0.32a	9.43 ± 1.12#b
G3PDH (U.mg–1 protein)	Control	3.59 ± 0.10a	4.03 ± 0.25a	2.85 ± 0.17b
	2 μg T	4.30 ± 0.28a	3.44 ± 0.15b	3.42 ± 0.19a
	5 μg T	4.10 ± 0.19a	2.95 ± 0.17*b	3.74 ± 0.32*a
Amino acid metabolism				
GDH (U mg^{-1} protein)	Control	6.05 ± 0.24a	6.72 ± 0.27a	6.21 ± 0.44a
	2 μg T	7.04 ± 0.29a	8.47 ± 0.26*a	7.72 ± 0.34a
	5 μg T	6.49 ± 0.32a	9.50 ± 0.31*b	9.18 ± 0.45*b
Asp-AT (U mg^{-1} protein)	Control	5.20 ± 0.17a	5.33 ± 0.21a	5.70 ± 0.38a
	2 μg T	5.75 ± 0.27a	5.36 ± 0.16a	7.93 ± 0.26*b
	5 μg T	5.36 ± 0.28a	7.63 ± 0.32*#b	8.41 ± 0.38*b
Ala-AT (U mg^{-1} protein)	Control	5.82 ± 0.33a	5.79 ± 0.38a	5.03 ± 0.36a
	2 μg T	7.07 ± 0.28a	7.33 ± 0.44*a	8.02 ± 0.30*a
	5 μg T	6.37 ± 0.44a	7.74 ± 0.37*a	9.74 ± 0.52*a
Lipid metabolism				
HOAD (U mg^{-1} protein)	Control	0.17 ± 0.03a	0.15 ± 0.04a	0.14 ± 0.02a
	2 μg T	0.26 ± 0.02a	0.14 ± 0.06a	0.06 ± 0.01b
	5 μg T	0.18 ± 0.01a	0.08 ± 0.01b	0.11 ± 0.02b

Values are the means ± SE ($n = 9$ fish per group). ∗, significantly different ($P < 0.05$) from control under the same experimental conditions. #, significantly different ($P < 0.05$) from fish treated with 2 μg T g^{-1} under the same experimental condition. Different letters indicate significant differences ($P < 0.05$) among sampling times within each treatment.

hormones. Accordingly, Jakobsson et al. (1997) observed receptor-like binding of T in the cytosolic and nuclear fractions of Atlantic salmon gills, which suggested that the effect of androgens on osmoregulation may be exerted directly at the gill level, rather than by an indirect effect involving other endocrine systems. However, these two possibilities are not mutually exclusive. No data are currently available on the presence of T receptors in gill mitochondria-rich cells of *S. auratus*. Nevertheless, and according to the results of Jakobsson et al. (1997) it can be hypothesized a direct effect of T on mitochondria-rich cells, which would increase Na^+,K^+-ATPase activity improving the hypoosmoregulatory capacity of this species. Clearly, further studies are necessary in order to elucidate the presence of T receptors in the osmoregulatory organs (i.e. gills and kidney) of gilthead sea bream.

Another interesting result is the ineffectiveness of the higher T dose used in the present study (5 μg g^{-1} body weight) for enhancing gill Na^+,K^+-ATPase activity in *S. auratus*. This finding agree with that obtained by Sunny and Oommen (2000) who showed that the maximum effective dose of T for increasing that enzyme activity was 0.25 μg g^{-1} body weight. This situation could be explained by a downregulation of T receptors in gills (if present in this tissue) due to T treatment. A similar situation has been reported for other steroids like cortisol in other species (Shrimpton and Randall, 1994) including *S. auratus* (Laiz-Carrión et al., 2003).

Table 3
Gills levels of metabolites and enzyme activities of *S. auratus* after 1, 3, and 7 days of intraperitoneal implantation of coconut oil alone (control) or containing T (2 and 5 μg g^{-1} b.w.). Further details in legend of Table 2

Parameter	Treatment (μg g^{-1} b.w.)	Time (days)		
		1	3	7
Metabolite levels				
Glycogen (μmol glycosyl units g^{-1} wet wt.)	Control	1.56 ± 0.19a	1.39 ± 0.19a	1.47 ± 0.23a
	2 μg T	1.57 ± 0.16a	0.87 ± 0.34a	1.43 ± 0.19a
	5 μg T	1.32 ± 0.15a	1.45 ± 0.26b	1.79 ± 0.26b
Lactate (μmol g^{-1} wet wt.)	Control	0.37 ± 0.04a	0.36 ± 0.04a	0.37 ± 0.07a
	2 μg T	0.38 ± 0.04a	0.46 ± 0.07a	0.53 ± 0.10a
	5 μg T	0.31 ± 0.03a	0.49 ± 0.06b	0.84 ± 0.07*c
Enzyme activities				
Carbohydrate metabolism				
PK optimal activity (U mg^{-1} protein)	Control	3.48 ± 0.37a	3.56 ± 0.22a	3.78 ± 0.40a
	2 μg T	5.23 ± 0.42*a	3.38 ± 0.21b	3.12 ± 0.23b
	5 μg T	4.64 ± 0.31a	4.46 ± 0.52a	3.85 ± 0.25a
Activity ratio (%)	Control	10.3 ± 1.50a	10.8 ± 1.59a	8.3 ± 0.72a
	2 μg T	10.5 ± 1.06a	12.8 ± 0.83a	13.3 ± 1.27*a
	5 μg T	11.6 ± 1.81a	10.9 ± 1.01a	9.9 ± 1.44a
Activation ratio (%)	Control	12.9 ± 0.93a	15.3 ± 3.72a	17.8 ± 2.08a
	2 μg T	12.0 ± 1.28a	20.4 ± 2.67b	23.4 ± 2.30b
	5 μg T	15.0 ± 1.40a	16.2 ± 1.24a	17.2 ± 1.56a
G6PDH (U mg^{-1} protein)	Control	1.31 ± 0.28a	1.22 ± 0.11a	1.24 ± 0.11a
	2 μg T	2.56 ± 0.07*a	1.48 ± 0.23b	1.27 ± 0.06b
	5 μg T	2.38 ± 0.12*a	1.58 ± 0.10b	1.46 ± 0.15b
Lipid metabolism				
HOAD (U mg^{-1} protein)	Control	0.28 ± 0.03a	0.25 ± 0.02a	0.24 ± 0.02a
	2 μg T	0.20 ± 0.01a	0.33 ± 0.03*b	0.31 ± 0.03*b
	5 μg T	0.21 ± 0.02a	0.36 ± 0.04*b	0.34 ± 0.02*b
Lactate metabolism				
LDH-O (U mg^{-1} protein)	Control	0.49 ± 0.08a	0.45 ± 0.04a	0.55 ± 0.06a
	2 μg T	0.57 ± 0.07a	0.63 ± 0.07a	0.47 ± 0.10a
	5 μg T	0.52 ± 0.09a	0.66 ± 0.10a	1.15 ± 0.10*#b

Fish treated with the higher dose of T, but not with the lower, showed a rapid enhancement of kidney Na^+,K^+-ATPase activity at day 1. It is interesting to remark that at this time gill Na^+,K^+-ATPase activity was not affected by hormonal treatment, suggesting the existence of specific tissue sensitivity to T treatment. In addition, a similar increase in kidney Na^+,K^+-ATPase activity, without any increase of this activity in the gills has been reported in gilthead sea bream 1 day after abrupt transfer from sea water to high salinity water (Sangiao-Alvarellos et al., 2005a) suggesting a possible involvement of T in that change. However, at 1 day post-implantation T levels are very high, and a pharmacological action of T cannot be discarded.

It is well established that sexual maturation, as well as treatment with sex steroids (estrogens), impairs smolting of several salmonid species as indicated by a suppression of morphological smolt characteristics, reduction of gill Na^+-K^+-ATPase activity, and hypoosmoregulatory capacity (McCormick and Naiman, 1985;McCormick, 1995). However, contradictory results have been reported by Järvi et al. (1991), who were unable to show any direct negative correlation between gill Na^+,K^+-ATPase activity and plasma levels of androgens in pre-smolts and early maturing Atlantic salmon males. In addition, Guzmán et al. (2004) indicated a clear stimulatory effect of E_2 on gill Na^+,K^+-ATPase activity in gilthead sea bream after a long-term treatment. Our results showed a stimulatory effect of T treatment on gill and kidney Na^+,K^+-ATPase activity in sea water-acclimated fish, as previously reported for T-treated tilapia (Sunny and Oommen, 2000). These studies reveal that sex steroids do not always have a negative effect on hypoosmoregulatory capacity and their influence is probably species-specific. Since gilthead seabream always need to go to seawater for spawning (Arias, 1976), a mechanism impairing final maturation and spawning in hypoosmotic environments could be suggested. Therefore, increasing levels of T could be necessary not only to stimulate the final phases of gonadal development but also to increase gill Na^+-K^+-ATPase activity and hypoosmoregulatory capacity in seawater. In this way, it will be interesting to study the modification of osmoregulatory system of T-treated fish submitted to hypoosmotic and hyperosmotic transfer.

Hematocrit value of T-treated fish was not different from controls (data not shown) and, accordingly, the changes observed in plasma metabolites were not the result of hemoconcentration or hemodilution and can be attributed directly to T action. Treatment with this hormone increased at different doses (2 and 5 μg g^{-1} body weight) and times (1, 3, and 7 days) plasma levels of glucose, lactate, triglyceride, and protein. In red sea bream, T treatment

Table 4
Kidney levels of metabolites and enzyme activities of *S. auratus* after 1, 3, and 7 days of intraperitoneal implantation of coconut oil alone (control) or containing T (2 and 5 μg g^{-1} b.w.). Further details in legend of Table 2

Parameter	Treatment (μg g^{-1} b.w.)	Time (days)		
		1	3	7
Metabolite levels				
Glycogen (μmol glycosyl units g^{-1} wet wt.)	Control	1.69 ± 0.33a	1.78 ± 0.34a	1.96 ± 0.32a
	2 μg T	2.25 ± 0.32a	2.38 ± 0.33a	1.62 ± 0.48a
	5 μg T	2.94 ± 0.25*a	2.71 ± 0.37*a	3.79 ± 0.75*#b
α-Amino acid (μmol g^{-1} wet wt.)	Control	43.0 ± 3.05a	47.5 ± 2.70a	47.2 ± 3.05a
	2 μg T	38.3 ± 3.28a	28.0 ± 2.84*b	34.8 ± 4.35a
	5 μg T	38.8 ± 5.16a	34.6 ± 2.58*a	39.8 ± 7.51a
Enzyme activities				
Carbohydrate metabolism				
HK (U mg^{-1} protein)	Control	2.74 ± 0.20a	3.08 ± 0.19a	2.85 ± 0.17a
	2 μg T	2.24 ± 0.11a	3.06 ± 0.13b	2.61 ± 0.10a
	5 μg T	2.64 ± 0.26a	2.62 ± 0.11a	2.31 ± 0.07a
PK optimal activity (U mg^{-1} protein)	Control	5.17 ± 0.37a	4.76 ± 0.65a	4.58 ± 0.30a
	2 μg T	5.39 ± 0.51a	5.37 ± 0.54a	4.63 ± 0.36a
	5 μg T	5.36 ± 0.48a	4.89 ± 0.51a	4.15 ± 0.18a
Activity ratio (%)	Control	3.64 ± 0.55a	3.67 ± 0.66a	3.95 ± 1.55a
	2 μg T	4.58 ± 1.13a	6.38 ± 0.65*b	8.12 ± 1.19*c
	5 μg T	7.04 ± 0.74*#a	9.11 ± 1.32*a	7.32 ± 2.00*a
Activation ratio (%)	Control	9.68 ± 4.34a	12.4 ± 2.12a	12.9 ± 2.40a
	2 μg T	18.9 ± 2.27*a	27.9 ± 2.84*a	24.9 ± 1.84*a
	5 μg T	24.7 ± 2.56*a	27.5 ± 1.98*a	25.0 ± 2.32*a
G6Pase (U g^{-1} protein)	Control	1.59 ± 0.12a	1.76 ± 0.21a	1.84 ± 0.19a
	2 μg T	0.96 ± 0.23a	1.15 ± 0.24a	0.53 ± 0.15*a
	5 μg T	0.96 ± 0.27a	0.89 ± 0.17a	0.47 ± 0.11*a
G3PDH (U mg–1 protein)	Control	1.44 ± 0.07a	1.22 ± 0.13a	1.21 ± 0.11a
	2 μg T	1.62 ± 0.12a	1.54 ± 0.09a	1.20 ± 0.09b
	5 μg T	1.45 ± 0.08a	1.14 ± 0.05b	1.41 ± 0.32a
Amino acid metabolism				
Ala-AT (U mg^{-1} protein)	Control	0.77 ± 0.42a	0.76 ± 0.13b	0.64 ± 0.09a
	2 μg T	1.63 ± 0.32a	0.86 ± 0.14b	1.04 ± 0.11*b
	5 μg T	0.86 ± 0.12#a	1.12 ± 0.11a	2.04 ± 0.30*#b

increased plasma levels of glucose and triglycerides while no effect was observed in those of protein (Woo et al., 1993). In another study performed in red grouper, Ng et al. (1984) did not find any changes in plasma glucose levels in T-treated fish. Altogether, the results of these reports indicate a species–specific response to T treatment.

At hepatic level, glycogen increased in T-treated fish as previously reported in singi fish (Dasmahapatra and Medda, 1982), red grouper (Ng et al., 1984), red sea bream (Woo et al., 1993), and catfish (Singh and Gupta, 2002). This could be the result of: (i) increased entry of exogenous glucose and/or (ii) increased gluconeogenesis. However, the finding that GK activity did not show significant changes suggests that an enhanced use of exogenous glucose did not take place in the liver of T-treated fish. In contrast, the increase in FBPase activity suggests an enhancement of gluconeogenic potential, similarly to that observed in T-treated red sea bream (Woo et al., 1993). This higher liver gluconeogenesis could be the result of increased conversion of amino acids into glucose as suggested the enhanced Ala-AT activity in red sea bream (Woo et al., 1993) and tilapia (Sunny et al., 2002a,b). In the present study not only liver Ala-AT but also Asp-AT and GDH activities increased in T-treated fish reinforcing the hypothesis of stimulated amino acid catabolism in treated fish. Liver G6Pase activity was also increased by hormonal treatment, which may indicate that part of the glucose produced by gluconeogenesis was released into the circulation and exported to other tissues. A similar increase in G6Pase activity was also observed in other teleosts treated with T (Woo et al., 1993; Sunny et al., 2002a,b). The higher liver capacity for exporting glucose could be responsible for the hyperlycemia observed in T-treated fish. However, considering that T treatment is also known to stimulate glucose absorption in fish intestine (Reshkin et al., 1989) we cannot discard that at least part of the increased plasma glucose could be the result of higher absorption from the gut. The lack of changes in the remaining enzymes assessed in the liver of T-treated fish is in agreement with other studies which showed no effects of T treatment on glycolysis and pentose phosphate pathway (Peter and Oommen, 1989; Woo et al., 1993). The increased liver acetoacetate levels observed in T-treated fish could be the result of enhanced liver ketogenesis from non-esterified fatty acids. Considering that in other processes, like food deprivation, both gluconeogenesis and ketogenesis are simultaneously activated in gilthead sea

Table 5
Brain levels of metabolites and enzyme activities of *S. auratus* after 1, 3, and 7 days of intraperitoneal implantation of coconut oil alone (control) or containing T (2 and 5 μg g^{-1} b.w.). Further details in legend of Table 2

Parameter	Treatment (μg g^{-1} b.w.)	Time (days)		
		1	3	7
Metabolite levels				
Glycogen (μmol glycosyl units g^{-1} wet wt.)	Control	0.54 ± 0.10a	0.47 ± 0.07a	0.52 ± 0.06a
	2 μg T	0.48 ± 0.06a	0.74 ± 0.10*a	0.65 ± 0.02*a
	5 μg T	0.71 ± 0.12a	0.74 ± 0.11*a	0.73 ± 0.04*a
Glucose (μmol g^{-1} wet wt.)	Control	2.25 ± 0.40a	2.57 ± 0.44a	2.46 ± 0.29a
	2 μg T	2.55 ± 0.38a	2.26 ± 0.32a	5.89 ± 0.31*b
	5 μg T	2.27 ± 0.22a	2.07 ± 0.70a	5.99 ± 0.70*b
Triglyceride (μmol g^{-1} wet wt.)	Control	1.34 ± 0.24a	1.59 ± 0.32a	1.46 ± 0.37a
	2 μg T	1.77 ± 0.25ab	1.44 ± 0.26a	2.04 ± 0.34*b
	5 μg T	1.58 ± 0.30a	2.72 ± 0.47ab	3.38 ± 0.52*b
Acetoacetate (μmol g^{-1} wet wt.)	Control	0.74 ± 0.09a	0.77 ± 0.09a	0.76 ± 0.08a
	2 μg T	0.94 ± 0.12a	1.10 ± 0.06*a	1.13 ± 0.05*a
	5 μg T	0.87 ± 0.15a	1.40 ± 0.105*#b	1.95 ± 0.11*#a
Enzyme activities				
Carbohydrate metabolism				
HK (U mg^{-1} protein)	Control	1.63 ± 0.06a	1.51 ± 0.11a	1.42 ± 0.05a
	2 μg T	1.84 ± 0.10a	2.34 ± 0.10*a	1.98 ± 0.12a
	5 μg T	2.09 ± 0.10*a	2.26 ± 0.05*a	2.45 ± 0.10*#a
PFK optimal activity (U mg^{-1} protein)	Control	4.85 ± 0.27a	4.21 ± 0.31a	4.47 ± 0.40a
	2 μg T	4.79 ± 0.34a	4.07 ± 0.43a	4.23 ± 0.35a
	5 μg T	4.37 ± 0.44a	4.45 ± 0.38a	4.84 ± 0.25a
Activity ratio (%)	Control	12.5 ± 1.05a	12.4 ± 1.36a	12.5 ± 0.98a
	2 μg T	13.4 ± 0.99a	13.2 ± 1.44a	13.5 ± 1.15a
	5 μg T	15.5 ± 1.15a	15.2 ± 0.98a	11.4 ± 1.19b
Activation ratio (%)	Control	13.0 ± 0.78a	13.5 ± 0.88a	13.4 ± 0.79a
	2 μg T	14.5 ± 1.16a	14.9 ± 0.87a	13.7 ± 1.24a
	5 μg T	15.1 ± 2.01a	16.6 ± 1.17a	12.5 ± 0.91a
G3PDH (U mg–1 protein)	Control	0.22 ± 0.02a	0.21 ± 0.03a	0.24 ± 0.04a
	2 μg T	0.18 ± 0.03a	0.19 ± 0.02a	0.21 ± 0.03a
	5 μg T	0.12 ± 0.03a	0.28 ± 0.05b	0.24 ± 0.04b

bream liver (Sangiao-Alvarellos et al., 2005c), an increased ketogenesis under this experimental conditions where gluconeogenesis is also activated seems reasonable. However, it will be necessary to test this hypothesis in further experiments assessing ketogenic capacity.

In the gills, T treatment seems to affect only lipid metabolism since treatment with this hormone increased the lipogenic capacity on day 1 after treatment (based on increased G6PDH activity) and enhanced the capacity for oxidizing fatty acids on days 3 and 7.

In the kidney, the more important change observed after T treatment was the increase of glycogen levels. As in the case of the liver, the origin of the higher number of glycosyl units used to synthesize glycogen could be due to either the enhanced entry of exogenous glucose or to the higher endogenous production. Since no changes were observed in HK activity in T-treated fish, we may suggest again a gluconeogenic origin for the glycosyl units. In addition, the increase observed in Ala-AT activity reinforces the hypothesis of an enhanced conversion of amino acids into glucose in kidney of T-treated fish.

Brain is an important target for androgens in fish (Weltzien et al., 2004), however there are only few reports in literature regarding effects of T treatment on brain energy metabolism. Thus, increased MDH activity was detected in T-treated catfish (Gupta et al., 1993). In our study, brain glycogen, glucose, triglyceride, and acetoacetate levels increased after T treatment. This increase in acetoacetate and triglyceride levels is in agreement with that observed simultaneously in the liver. These results suggest that the enhanced liver production could result in increased plasma values of ketone and triglycerides, and therefore the higher levels in brain of T treated fish can be due to increased uptake of such metabolites. This could be attributed, similarly to the increase observed in glycogen and glucose levels, to an enhanced capacity of exogenous glucose phosphorylation based on the increased activity of HK in T-treated fish.

In summary, higher plasma T levels in gilthead sea bream elicited not only an increased hypoosmoregulatory capacity but also several metabolic changes in plasma and different tissues, mainly liver and brain. These changes may be related to the energy repartitioning process occurring in nature during osmotic adaptation to different environmental salinities of male maturing fish.

Acknowledgments

This study was partly supported by Grants VEM2003-20062 (Ministerio de Ciencia y Tecnologı´a and FEDER,

Spain), and PGIDT04PXIC31208PN (Xunta de Galicia, Spain) to J.L.S., and Grant BFU2004-04439-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.

References

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

Bjerkeng, B., Johnsen, K., Mayer, I., Storebakken, T., Nilssen, K.J., 1999. Influence of 11-ketotestosterone, 17ß-estradiol, and 3,5,3'-triiodo-L-thyronine on distribution and metabolism of carotenoids in Arctic charr, *Salvelinus alpinus* L.. Fish Physiol. Biochem. 21, 353–364.

Borg, B., 1994. Androgens in teleost fishes. Comp. Biochem. Physiol. 109C, 219–245.

Cavaco, J.E.B., Bogerd, J., Goos, H., Schulz, R.W., 2001. Testosterone inhibits 11-ketotestosterone-induced spermatogenesis in African catfish (*Clarias gariepinus*). Biol. Reprod. 65, 1807–1812.

Chaves-Pozo, E., 2005. Interacción inmuno-reproductora en el testículo de dorada (*Sparus aurata* L.) Ph.D. Thesis, Universidad de Murcia, Murcia, Spain.

Dasmahapatra, A.K., Medda, A.K., 1982. Effect of estradiol dipropionate and testosterone propionate on the glycogen, lipid, and water contents of liver, muscle, and gonad of male and female (vitellogenic and non-vitellogenic) singi fish (*Heteropneustes fosssilis* Bloch). Gen. Comp. Endocrinol. 48, 476–484.

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.

Gupta, B.L., Ansari, M.A., Sinha, I.P., Gupta, S.P., 1993. In vitro effect of testosterone propionate on lactate dehydrogenase and malate dehydrogenase in brain and gonads of *Channa punctatus*. Biochem. Mol. Biol. Int. 29, 85–91.

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

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, B.W., Lone, K.P., Matty, A.J., 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.

Järvi, T., Lofthus, R., Sigholt, T., 1991. On growth and smoltification in Atlantic salmon parr-the effect of sexual maturation and competition. Nord. J. Freshwater Res. 66, 72–88.

Keppler, D., Decker, K., 1974. Glycogen. Determination with amyloglucosidase. In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis. Academic Press, New York, pp. 1127–1131.

Laiz-Carrión, R., Guerreiro, P.M., Fuentes, J., Martín del Río, M.P., Canario, A.V.M., Mancera, J.M., 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, M.P., Míguez, J.M., Mancera, J.M., Soengas, J.L., 2003. Influence of cortisol on osmoregulation and energy metabolism in gilthead sea bream *Sparus aurata*. J. Exp. Zool. 298A, 105–118.

Laiz-Carrión, R., Sangiao-Alvarellos, S., Guzmán, J.M., Martín del Río, M.P., Míguez, J.M., Soengas, J.L., Mancera, J.M., 2002. Energy metabolism in fish tissues related to osmoregulation and cortisol action. Fish Physiol. Biochem. 27, 179–188.

Leonard, J.B.K., Iwata, M., Ueda, H., 2002. Seasonal changes of hormones and muscle enzymes in adult lacustrine masu (*Oncorhynchus masou*) and sockeye salmon (*O. nerka*). Fish Physiol. Biochem. 25, 153–163.

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.

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

Mancera, J.M., Pérez-Fı´gares, J.M., Fernández-Llebrez, P., 1994. Effect of cortisol on brackish water adaptation in the euryhaline gilthead sea bream (*Sparus aurata* L.). Comp. Biochem. Physiol. 107A, 397–402.

Mancera, J.M., Smolenaars, M., Laiz-Carrión, R., Martin del Rio, M.P., Wendelaar Bonga, S.E., Flik, G., 2004. 17β-estradiol affects osmoregulation in *Fundulus heteroclitus*. Comp. Biochem. Physiol. 139B, 183–191.

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

McCormick, S.D., 1995. Hormonal control of gill Na^+,K^+-ATPase and chloride cell function. In: Wood, C.M., Shuttleworth, T.J. (Eds.), Hormonal control of gill Na^+K^+ATPase and chloride cell function. Academic press, San Diego, CA, pp. 285–315.

McCormick, S.D., Naiman, R.J., 1985. Hypoosmoregulation in an anadromous teleost: influence of sex and maduration. J. Exp. Zool. 234, 193–198.

Meiri, I., Gothilf, Y., Zohar, Y., Abigail, Z., 2002. Physiological changes in the spawning gilthead seabream, *Sparus aurata*, succeding the removal of males. J. Exp. Zool. 292, 555–564.

Mommsen, T.P., Walsh, P.J., 1988. Vitellogenesis and oocyte assembly. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology, vol. 11A. Academic Press, New York, pp. 347–406.

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

Ng, T.B., Woo, N.Y.S., Tam, P.P.L., Au, C.Y.W., 1984. Changes in metabolism and hepatic ultrastructure induced by estradiol and testosterone in immature female *Epinephelus akaara* (Teleostei, Serranidae). Cell Tissue Res. 236, 651–659.

Peter, M.C.S., Oommen, O.V., 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.

Prat, F., Zanuy, S., Carrillo, M., De Mones, A., Fostier, A., 1990. Seasonal changes in plasma levels of gonadal steroids of sea bass, *Dicentrarchus labrax* L. Gen. Comp. Endocrinol. 78, 361–373.

Reshkin, S.J., Grover, M.L., Howerton, R.D., Grau, E.G., Ahearn, G.A., 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 o fan enzyme inmunoassay for testosterona: effects of photoperiod on plasma testosterona levels and gonadal development in male sea bass (*Dicentrarchus labrax*, L.) at puberty. Fish Physiol. Biochem. 23, 141–150.

Ros, A.F.H., Becker, K., Canario, A.V.M., Oliveira, R.F., 2004. Androgen levels and energy metabolism in *Oreochromis mossambicus*. J. Fish Biol. 65, 895–905.

Sangiao-Alvarellos, S., Laiz-Carrión, R., Guzmán, J.M., Martín del Río, M.P., Míguez, J.M., Mancera, J.M., Soengas, J.L., 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, F.J., Martín del Río, M.P., Míguez, J.M., Mancera, J.M., Soengas, J.L., 2005a. Time course of osmoregulatory and metabolic changes during osmotic acclimation in *Sparus auratus*. J. Exp. Biol. 208, 4291–4304.

Sangiao-Alvarellos, S., Guzmán, J.M., Laiz-Carrión, R., Míguez, J.M., Martín del Río, M.P., Mancera, J.M., Soengas, J.L., 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.

Shrimpton, J.M., Randall, D.J., 1994. Downregulation of corticosteroid receptors in gill of coho salmon due to stress and cortisol treatment. Am. J. Physiol. 267, R432–R438.

Singh, C., Gupta, R.C., 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, B.S., Ron, B., Richman III, N.H., Riley, L.G., Iwama, G.K., Hirano, T., Grau, E.G., 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, O.V., 2000. Steroid hormones regulate enzymes of osmoregulation in a freshwater fish *Oreochromis* (tilapia) *mossambicus*. J. Endocrinol. Reprod. 4, 63–73.

Sunny, F., Oommen, O.V., 2002. Rapid action of testosterone and diethylstilbestrol on enzymes of osmoregulation in a freshwater fish *Oreochromis mossambicus*. Endocr. Res. 28, 69–81.

Sunny, F., Jacob, A., Oommen, O.V., 2002a. Sex steroids regulate intermediary metabolism in *Oreochromis mossambicus*. Endocr. Res. 28, 175–188.

Sunny, F., Lakshmy, P.S., Oommen, O.V., 2002b. Rapid action of cortisol and testosterone on lipogenic enzymes in a fresh water fish *Oreochromis mossambicus*: short-term in vivo and in vitro study. Comp. Biochem. Physiol. 131B, 297–304.

Vijayan, M.M., Takemura, A., Mommsen, T.P., 2001. Estradiol impairs hypoosmoregulatory capacity in the euryhaline tilapia, *Oreochromis mossambicus*. Am. J. Physiol. 281, R1161–R1168.

Weltzien, F.A., Andersson, E., Andersen, O., Shalchian-Tabrizi, K., Norberg, B., 2004. The brain-pituitary-gonad axis in male teleosts, with special emphasis on flatfish (*Pleuronectiformes*). Comp. Biochem. Physiol. 137A, 447–477.

Woo, N.Y.S., Chung, A.S.B., Ng, T.B., 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.

Zohar, Y., Billard, R., Weil, C., 1984. La reproduction de la daurade) (*Sparus aurata*) et du bar (*Dicentrarchus labrax*): connaissance du cycle sexuel et controle de la gametogenese et de la ponte. In: Barnabe, G., Billard, R. (Eds.), L'aquaculture du Bar et des Sparides. INRA Press, Paris, pp. 3–24.