Seasonal and sex-related variations in serum steroid hormone levels in wild and farmed brown trout *Salmo trutta* L. in the north-west of Spain

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ABSTRACT

Serum steroid profiles were investigated in order to evaluate the potential use of circulating sex steroid levels as a tool for sex identification in brown trout. Changes in the serum concentrations of testosterone (T), progesterone (P), 17-β-estradiol (E2), and cortisol (F) in wild and farmed mature female and male brown trout, *Salmo trutta* L., were measured in each season (January, May, July, and October) in six rivers and four hatcheries located in the north-west of Spain. Serum cortisol levels in farmed brown trout were significantly higher and showed a seasonal pattern opposite to that found in wild trout. Because levels of the hormones under study can be affected by disruptive factors such as exposure to phytoestrogens (which alters the hypothalamic–pituitary–gonadal axis) and infection with *Saprolegnia parasitica* (which alters the hypothalamic–pituitary–adrenal axis), both factors are taken into account.

Key words | 17-β-estradiol, brown trout, cortisol, hop cultures, progesterone, *Saprolegnia*, testosterone

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INTRODUCTION

Brown trout, *Salmo trutta* L., is a species with a growth rate that does not make it competitive in the field of commercial salmonid culture. In the field of sport fishing, however, the brown trout is highly regarded internationally (Elliott 1989). In Spain, as in other countries, there are numerous hatcheries engaged in periodically releasing stock of this species into various watercourses. In order to manage their reproduction correctly, it is essential to know the endocrine status of the fish. Furthermore, wild brown trout populations are a very valuable genetic resource that can be used as broodfish in hatcheries. Several studies in salmonid fish, including brown trout, have correlated gonad development and gametogenesis within the annual reproductive cycle to seasonal fluctuations in sex steroid hormone profiles (Breton *et al.* 1983; Fostier *et al.* 1983; Kagawa *et al.* 1983; Fostier & Jalabert 1986; Tam *et al.* 1986; Norberg *et al.* 1989; Estay *et al.* 2003). However, individual levels can vary under the influence of body size, nutritional condition, gonadal maturity, and certain environmental conditions (Onuma *et al.* 2003). A further feature of the understanding of salmonid endocrinology is that the information available on hormone status in wild fish is limited. This is of particular concern, because hormone levels may be affected by growing conditions that expose fish to a variety of stressors, including adverse water quality, hypoxia, handling, and overcrowding (Pickering *et al.* 1987; Kubokawa *et al.* 1999).

On the other hand, aquatic xenoestrogens, such as phytoestrogen 8-prenylnaringenine from hop (whose cultivation is significant on the banks of the rivers of León (469 ha, 1,000 t/year), can also modify the estrogenic activity

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(Milligan *et al.* 2002; Clotfelter & Rodriguez 2006; Kelly *et al.* 2010). Phytoestrogens may alter the feedback loops in the hypothalamic–pituitary–gonadal axis by mimicking the effects of estrogens and triggering their specific receptors or they may bind to hormone receptors and block the action of estradiol, progesterone, and testosterone.

In a concomitant way, the hypothalamic–pituitary–interrenal axis of the brown trout is activated in response to most forms of environmental stress, including pollution, leading to an increase in blood cortisol levels. Chronic high cortisol levels cause *Saprolegnia parasitica* infection and suppress several of the endocrine processes which control sexual maturation, thus resulting in a significant reduction of the gonads in both male and female fish (Pickering 1989).

Saprolegniosis is a fungal disease of fish and fish eggs most commonly caused by the *Saprolegnia* species called 'water molds'. Saprolegniosis in salmonid fish is normally associated with a range of predisposing factors such as skin damage, smoltification, sexual maturation, stress, and other infections (Pickering 1994). Sexual maturation in both sexes of salmonid fish is associated with a marked increase in susceptibility to *S. parasitica* although male fish appear to be more vulnerable than females (Richards & Pickering 1978; Aller-Gancedo & Fernández-Díez 1987). There is some evidence which suggests that the increase in susceptibility in male fish is related, at least in part, to high androgen levels (Cross & Willoughby 1989).

In the present study, the seasonal variations in four steroid hormones, testosterone (T), progesterone (P), 17-β-estradiol (E2), and cortisol (F), in the serum of male and female brown trout were studied. A comparison was made of serum steroid profiles in wild and farmed brown trout and in relation to the presence of *S. parasitica* infection or contamination by phytoestrogens. Levels of sex steroids (T, P, and E2) were investigated to evaluate the potential use of circulating sex steroid levels as a tool for sex identification in this species.

MATERIALS AND METHODS

Fish and samples

The study was conducted in four provinces of the Castile and Leon region in the north-west of Spain between October 2010 and July 2011 in six rivers (the Esla, Omaña, Órbigo, and Porma rivers in the Province of Leon; the Arlanzón river in the Province of Burgos and the Carrión river in the Province of Palencia) and four hatcheries owned by the Castile and Leon Regional Government (Vegas del Condado in the Province of Leon, Quintanar de la Sierra in the Province of Burgos, El Soto in the Province of Palencia, and Ucero in the Province of Soria). A particularity of the Porma and Órbigo rivers' banks is the widespread cultivation of hops.

Brown trout, both healthy and, when possible, infected with *S. parasitica* were collected in January, May, July, and October. In the region where the study was conducted, brown trout spawn between November and January. Wild brown trout were captured by electro-fishing at different sampling points on the rivers mentioned above. Only fish 300 to 500 g in weight and 22 to 24 cm in length were considered. In the hatcheries, blood samples were taken from male and female broodfish, kept separately in different raceways situated in the open air. These fish were between 3 and 5 years old (512 ± 236 g in weight and 35.3 ± 5.8 cm in length). The water temperature varied over the seasons from 4 to 16 °C at the different sampling points on the rivers and from 7 to 14 °C in the hatcheries. In total, 192 blood samples were investigated (107 from wild brown trout and 85 from broodfish), of which 36 were from fish infected with *S. parasitica*. Details of the distribution of samples are shown in Table 1. All the trout were anesthetized with 50 mg kg^{-1} of MS 222 (tricaine methane sulfonate). Individual blood samples were then obtained by puncturing the caudal vein. All trout were returned alive to the water with the exception of those trout that were suffering from saprolegniosis. After clotting overnight at 4 °C the blood samples were centrifuged at 1,000 g for 45 min to obtain the serum. The sera were stored for 2 to 3 years at −20 °C until used.

Cortisol analyses (192 samples, 100%) were performed with an automated AxSYM® system utilizing a fluorescence polarization immunoassay method (FPIA) from Abbott Laboratories (North Chicago, IL, USA). Sex steroids (P, T, and E2) concentrations were also measured using an automated two-step chemiluminescent magnetic microparticle immunoassay (CMIA) on an Abbott ARCHITECT® diagnostic analyzer. These clinical chemistry tests are very frequently

Table 1 | Distribution of the samples of brown trout *Salmo trutta* L. used in this study

Health status	Origin	Hormonal sex	Province	River or fish farm	Sampling period
Free from saprolegniosis (156)	Wild (99)	Female (53)	Leon (82)	Esla (7)	January (15)
		Male (45)	Burgos (8)	Omaña (18)	May (55)
		Unrecorded (1)	Palencia (9)	Órbigo (27)	July (19)
				Porma (30)	October (10)
				Arlanzón (8)	
				Carrión (9)	
	Farmed (57)	Female (20)	Leon (32)	Vegas del Condado (32)	January (12)
		Male (37)	Burgos (6)	Quintanar (6)	May (35)
			Palencia (9)	El Soto (9)	July (5)
			Soria (10)	Ucero (10)	October (5)
Infected with *S. parasitica* (36)	Wild (8)	Female (4)	Leon (8)	Esla (3)	January (7)
		Male (4)		Porma (5)	July (1)
	Farmed (28)	Male (28)	Leon (28)	Vegas del Condado (28)	January (28)

used by veterinarians for the measure of analytes found in very low proportions in biological fluids (the sensitivity of the assays for cortisol, P, T, and E2 is less than 0.1 ng/dL). The accuracy of both FPIA and CMIA methods is very high, due to the fact that they use antibodies to bind with high affinity and specificity to the analytes. All the different assays utilize a four-parameter logistic curve fit method (4PLC, Y weighted) to generate the standard calibration curves. The number of samples of P analyzed was 168 (138 from trout free of saprolegniosis and 30 from fish infected by *S. parasitica*). For T, the corresponding total was 165 (135 and 30) and for E2, 172 (42 and 30).

Statistics

Values were presented as the mean ± the standard deviation, the number of samples being given in brackets. Statistical differences in the mean values for serum concentrations of the four steroids analyzed in relation to sex (male or female), season (January, May, July, or October), origin (wild or farmed fish), and *S. parasitica* infection (fish with or without saprolegniosis) were assessed by the parametric analysis of variance (ANOVA) or non-parametric Kruskall–Wallis tests, depending on whether the conditions for a classical analysis of variance were fulfilled or not. In the case of differences between trout with or without saprolegniosis the comparison was made only for the samples taken in January and only from males because all of the 36 *S. parasitica*-infected fish, except one, were found at that time and 32 were male (Table 1). The aforementioned statistical tests were performed with the Epi Info™ software for Windows (Version 3.3), while $p < 0.05$ was taken as the level of significance. Principal components and factor analysis was performed with IBM – SPSS Statistics v.20 package.

RESULTS

Serum concentrations of the four steroids varied greatly between individuals. This meant that in several cases the differences found in relation to sex, season, origin, and health status were not statistically significant, because of the wide dispersion of the data. The data from fish showing *S. parasitica* infection were removed from the main data sets and treated separately.

The serum profiles for sex steroids in both sexes showed seasonal variations (Figure 1). In females, P and E2 concentrations showed their lowest values in January and May and increased in July and October; in contrast, T levels decreased from January to July, followed by a rapid rise reaching a peak value in October. In males, both P and E2 concentrations were low and similar in all four seasons, but T levels showed the same patterns as in females, except for a small rise observed in May (Figure 1). Seasonal

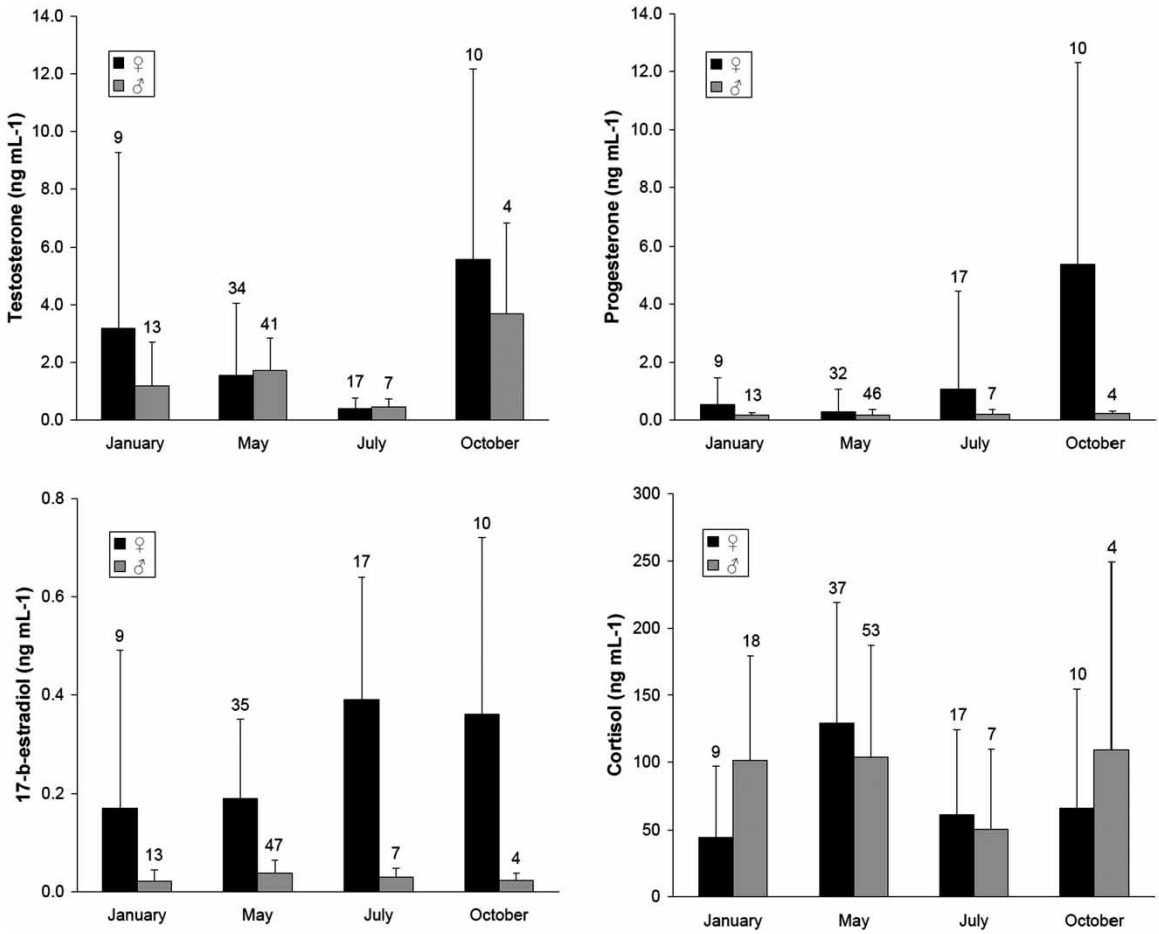

Figure 1 | Seasonal variations in serum steroids levels in wild and farmed mature female and male brown trout *Salmo trutta* L. Data are shown as the mean and the standard deviation for the number of samples indicated.

differences were statistically significant for T in females ($p = 0.02$) and in males ($p = 0.04$), but for P ($p = 0.001$) and E2 ($p = 0.01$) only in females. Differences between females and males in the concentration of sex steroids were significant only for P in October, but for E2 in all seasons (Figure 1). Seasonal differences in C levels were significant ($p = 0.004$) only in females, showing higher levels of C in May than in the other seasons (Figure 1). Males showed equal concentrations of F in January, May, and October but lower levels lower in July. No significant differences in F levels between females and males were observed, although females showed higher levels than males in May and July, but lower in January and October.

Although similar seasonal differences in sex steroid profiles were found between wild and farmed fish, these differences were significant only for E2 in January and May (Table 2). However, serum F levels were significantly ($p = 0.006$) higher in farmed brown trout (120.8 ± 75.9 ng mL^{-1}, 57) as compared to wild trout (82.4 ± 86.6 ng mL^{-1}, 99) and showed opposite seasonal patterns (Figure 2). In farmed fish, the highest concentrations were observed in October, the lowest in May, whereas in wild fish the highest levels were recorded in May and the lowest in October. The differences observed between wild and farmed fish were statistically significant in all seasons with the exception of May, when wild fish showed significantly ($p < 0.001$) higher F levels than in the other seasons.

With regard to saprolegniosis, trout infected with *S. parasitica* showed lower levels of sex steroids (T, P, and E2), but higher levels of F, than uninfected trout. However, the differences were statistically significant only for T ($p = 0.02$) and for P ($p = 0.04$) (Table 3).

Table 2 | Differences in serum sex steroids concentrations between wild and farmed brown trout *Salmo trutta* L. Data are shown as the mean ± the standard deviation, with the number of samples being given in brackets

Steroid	Origin	January	May	July	October
(a) Females					
Testosterone (ng mL^{-1})	Wild	2.22 ± 5.14 (6)	1.66 ± 2.83 (26)	0.40 ± 0.39 (14)	7.28 ± 7.32 (7)
	Farmed	5.07 ± 8.60 (3)	1.09 ± 0.84 (8)	0.37 ± 0.05 (3)	1.62 ± 0.90 (3)
Progesterone (ng mL^{-1})	Wild	0.25 ± 0.20 (6)	0.34 ± 0.94 (23)	1.27 ± 3.73 (14)	3.98 ± 6.83 (7)
	Farmed	1.06 ± 1.69 (3)	0.11 ± 0.06 (9)	0.15 ± 0.09 (3)	8.60 ± 7.42 (3)
17-β-Estradiol (ng mL^{-1})	Wild	0.09 ± 0.06 (6)	0.22 ± 0.18 (24)	0.33 ± 0.22 (14)	0.42 ± 0.42 (7)
	Farmed	0.34 ± 0.57 (3)	0.14 ± 0.11 (11)	0.64 ± 0.27 (3)	0.22 ± 0.14 (3)
(b) Males					
Testosterone (ng mL^{-1})	Wild	1.40 ± 1.71 (9)	1.51 ± 1.22 (27)	0.39 ± 0.29 (5)	6.04 ± 2.79 (2)
	Farmed	0.68 ± 0.96 (4)	2.06 ± 0.95 (14)	0.57 ± 0.40 (2)	1.33 ± 0.13 (2)
Progesterone (ng mL^{-1})	Wild	0.16 ± 0.08 (9)	0.12 ± 0.09 (26)	0.13 ± 0.12 (5)	0.20 ± 0.04 (2)
	Farmed	0.23 ± 0.09 (4)	0.24 ± 0.27 (20)	0.33 ± 0.23 (2)	0.23 ± 0.19 (2)
17-β-Estradiol (ng mL^{-1})	Wild	0.01 ± 0.01 (9)	0.04 ± 0.02 (26)	0.03 ± 0.01 (5)	0.03 ± 0.01 (2)
	Farmed	0.04 ± 0.03 (4)	0.05 ± 0.03 (21)	0.04 ± 0.04 (2)	0.02 ± 0.02 (2)

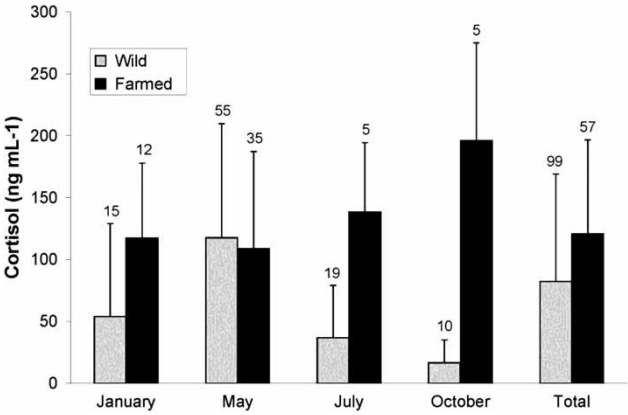

Figure 2 | Seasonal and total differences in serum cortisol concentrations between wild and farmed brown trout *Salmo trutta* L. Data are shown as the mean and the standard deviation for the number of samples indicated.

The checking of a presumable modification of the estrogenic activity in trout due to water contamination by phytoestrogens from hop cultures on the banks of the rivers in León region led us to observe higher levels of E2 in trout from the rivers Porma and Órbigo than those registered in trout from unaffected rivers, such as those in Palencia and Burgos provinces (Table 4).

Although concentrations of E2, T, and P fluctuated widely between individual fish, the three steroids taken together might be usable as a tool for sex identification in brown trout. Females showed levels of E2 greater than 0.078 ng mL^{-1}, of T less than 1.92 ng mL^{-1}, and of P greater than 0.14 ng mL^{-1}. In cases of doubt, the determination with the greatest descriptive value was T, followed by P and E2. When these sex steroid levels were considered, a good correlation (*kappa* value =0.88) was obtained between the hormonal sex assigned and gonadal sex. All the females were correctly identified (31 out of 31) but seven gonadal males (out of 92) were classed as females on the basis of their sex hormone concentrations.

Finally, with regard to the principal components and factor analysis, we found that KMO (Kaiser–Meyer–Olkin) was around 0.6. We also found that 53% of the variance

Table 3 | Differences in serum steroids levels in wild and farmed mature brown trout *Salmo trutta* L. with and without saprolegniosis

Health status	Testosterone (ng mL^{-1})	Progesterone (ng mL^{-1})	17-β-Estradiol (ng mL^{-1})	Cortisol (ng mL^{-1})
Free from saprolegniosis	1.18 ± 1.51 (13)	0.18 ± 0.08 (13)	0.021 ± 0.02 (13)	101.2 ± 78.3 (18)
Infected with *S. parasitica*	0.37 ± 0.94 (26)	0.12 ± 0.09 (26)	0.015 ± 0.02 (26)	162.2 ± 159.3 (32)

Data are shown as the mean ± the standard deviation, the number of samples being given in brackets. Only data for males sampled in January are included.

Table 4 | Differences in serum steroids levels in wild mature brown trout *Salmo trutta* L. with and without presumable contamination by phytoestrogens

Origin	Testosterone (ng mL^{-1})	Progesterone (ng mL^{-1})	17-β-Estradiol (ng mL^{-1})	Cortisol (ng mL^{-1})
Rivers of Leon province (presumably contaminated)	1.31 ± 1.16 (9)	0.09 ± 0.03 (9)	0.082 ± 0.062 (9)	110 ± 78 (9)
Rivers of Palencia province (not contaminated)	1.59 ± 0.94 (9)	0.17 ± 0.09 (9)	0.027 ± 0.021 (9)	92 ± 79 (10)
Rivers of Burgos province (not contaminated)	1.65 ± 1.16 (9)	0.10 ± 0.03 (8)	0.020 ± 0.062 (8)	98 ± 62 (10)

Data are shown as the mean ± the standard deviation, the number of samples being given in brackets. Only data for males sampled in May are included.

can be explained by one factor, 77% by two factors, and 100% by three factors. Three-dimensional (3D) scatter plot of principal components (Figure 3) led to two clearly separated clusters for the variables: one consists of five variables related to the results for farmed fish and the other to those that are in relationship to wild fish. The distance between the cluster borders evidences the differences in serum sex steroids concentrations between wild and farmed brown trout *Salmo trutta* L. 3D factorial analysis plot (Figure 4) unmistakeably shows the statistical differences in the mean values for serum concentrations of the four steroids analyzed in relation to season (January, May, July, or October).

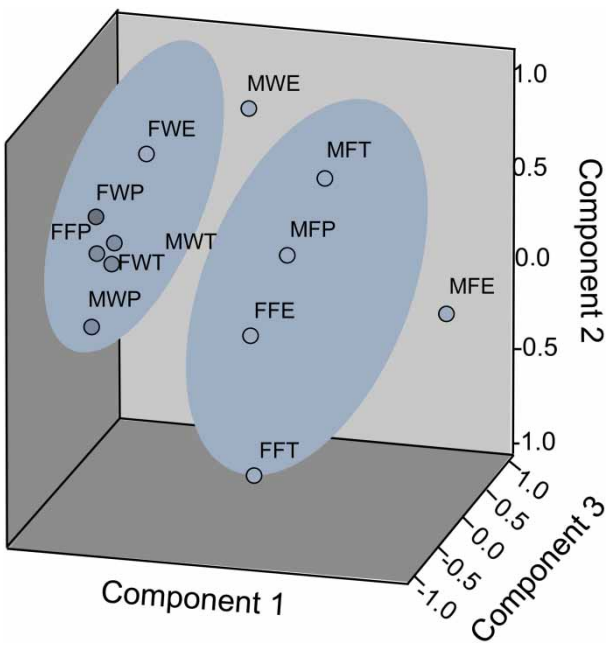

Figure 3 | 3D scatter plot of principal components: *Left cluster*: MWE = male (wild) estradiol, FWE = female (wild) estradiol, FWP = female (wild) progesterone, MWT = male (wild) testosterone, FWT = female (wild) testosterone, MWP = male (wild) progesterone. *Right cluster*: MFT = male (farmed) testosterone, MFP = male (farmed) progesterone, FFE = female (farmed) estradiol, MFE = male (farmed) estradiol, FFT = female (farmed) testosterone. *Unexpected location*: FFP = female (farmed) progesterone.

DISCUSSION

It is noteworthy that great individual differences were found in the serum concentrations of the four steroids studied. This might be related to various factors, including body size, age, nutritional condition, gonadal maturity, dominance status, and also certain environmental conditions. Thus, Onuma *et al.* (2003) showed year-on-year differences in plasma levels of steroid hormones and in sexual maturation in pre-spawning chum salmon (*Oncorhynchus keta*), some of which were influenced by year-to-year variations in sea surface temperatures. Cardwell *et al.* (1996) reported higher steroid levels in dominant male rainbow trout (*O. mykiss*) than in subordinate fish.

In the present study, the serum profiles for sex steroids in both sexes showed seasonal variations that tracked the natural reproductive cycle. In females, E2 and P concentrations showed their lowest values in January and May and increased in July and October, but T levels decreased

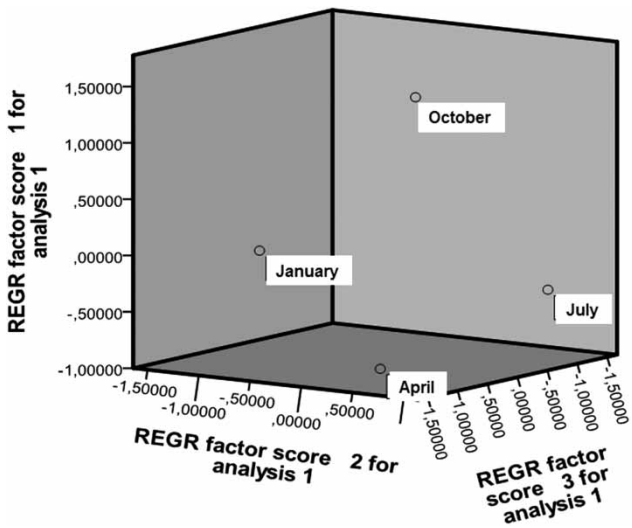

Figure 4 | 3D factorial analysis plot.

from January to July, followed by a rapid rise, reaching their peak value in October. Levels of E2 and P were consistent with the general pattern described in salmonids, in which functional significance is given to the regulatory role of E2 during the vitellogenesis process and of P during final maturation and ovulation (Fostier *et al.* 1983). In almost all teleost fish species studied so far, T has been detected in the plasma of females during the reproductive season. Although the precise role of this steroid in females still remains unclear, it has been suggested that high T concentrations may have a vitellogenic action on the liver and may also be involved in final oocyte maturation, in addition to the fact that T is a precursor for the synthesis of E2 by aromatization in the ovary (Fostier *et al.* 1983; Kagawa *et al.* 1983; Norberg *et al.* 1989). In males, both E2 and P concentrations were similar in all four seasons and remained very low, but T levels showed the same pattern as in females, in accordance with the physiological role of T during the spermatogenesis process in males (Fostier *et al.* 1983).

The seasonal pattern of sex steroid levels observed in the present study for brown trout during the course of their reproductive cycle were, in general, similar to the patterns described for other salmonids (Fitzpatrick *et al.* 1986; Fostier & Jalabert 1986; Feist *et al.* 1990; Estay *et al.* 1998), but lower concentrations were found than had been reported previously. Thus, for female brown trout, Breton *et al.* (1983) reported mean levels of E2 between 0.42 and 10.5 ng mL^{-1}; Norberg *et al.* (1989) found mean levels of 0.5 to 22 ng mL^{-1} for E2 and of between 2 and 65.2 ng mL^{-1} for T; and Estay *et al.* (2003) reported mean maximum levels of up to 65.2 ng mL^{-1} for E2 and 90 ng mL^{-1} for T during the course of the annual reproductive cycle. These differences may be related to the different methodologies used, to the geographical zone studied, or to the various lineages of the trout analyzed. Norberg *et al.* (1989) also found that plasma levels of both E2 and vitellogenin were considerably higher in farmed female brown trout than in wild females, but levels of 17α,20β-dihydroxy-4-pregnen-3-one were higher in the wild trout. They concluded that these differences between wild and farmed females might be due to differences in stress susceptibility, to environmental conditions, to nutritional status, or to genetic divergences between the strains. In the present study, the overall patterns of sex steroids were similar in both wild and farmed brown trout and their differences possibly were caused by difference in maturation (ovulation and spermiation) period. For example, plasma T levels of wild females peaked in October while the peak levels of hatchery females appeared in January (Table 2). This suggests the possibility that there was a difference in maturation period between wild and hatchery fish. With respect to the significantly higher serum F levels found in farmed brown trout than in wild fish (Figure 2), it might be expected that wild brown trout would be more stressed than farmed trout, owing to the acute stress caused by capture, confinement, and bleeding. Under aquaculture conditions, fish may be exposed to a range of stressors, including handling and overcrowding, that can cause a long-term rise in F, which may explain the higher levels found in the farmed brown trout. In contrast to the differences between wild and farmed brown trout, no significant differences were found in F levels between males and females. Kubokawa *et al.* (1999) studied the effect of acute stress on plasma F, sex steroid hormones, and glucose levels in male and female sockeye salmon (*O. nerka*) during the breeding season, finding different levels of F in females and males (higher in females), both under natural conditions and as a response to acute artificial stress caused by their confinement. These authors also showed that the rise in F levels caused a decrease in circulating androgens and confirmed the results previously obtained by Pickering *et al.* (1987), who found that both acute stress (capture, netting, and confinement for one hour) and chronic stress (confinement for one month in small aquaria) caused a significant rise in plasma F, but lowered the levels of plasma T and 11-ketotestosterone in sexually mature male brown trout. The levels of F found in the present study suggest that in same cases the fish were stressed at the time of sampling and this fact might be a factor in the low levels of gonadal steroids reported.

The findings reported here of higher levels of F in brown trout infected with *S. parasitica* are in accord with Pickering & Christie (1981). They are on the same general lines as the results reported by Pickering & Duston (1983) for brown trout and Pottinger & Day (1999) for rainbow trout. These authors demonstrated that oral administration or intraperitoneal implants of cortisol resulted in chronically high blood F levels and increased the susceptibility of fish to *S. parasitica* infection. Trout infected with *S. parasitica* also showed lower levels of sex steroids (Table 3), possibly

because of the suppressant effect of the higher F levels mentioned above or related to the hemodilution associated with saprolegniosis (Richards & Pickering 1979; Hatai & Hoshiai 1994).

In connection to a presumable modification of the estrogenic activity in trout due to water contamination by phytoestrogens, our results are in agreement with the aforementioned predictions (Milligan *et al.* 2002), which suggest that 8-prenylnaringenin from hop cultures (Table 4) enhances E2 levels. The observed differences are moderate, as expected from the environmentally acceptable levels (<200 ng/L) obtained for overall phytoestrogens (8-prenylnaringenin and related hop flavonoids), according to the procedure detailed in Rong *et al.* (2000), in the Órbigo River.

Finally, it is of interest to note that the concentrations of E2, T, and P taken together so as to allow for individual variations, were capable of being used to distinguish between the sexes in brown trout. Thus, with the cut-off levels used in the present study, all the females and 92% of the males were correctly predicted. The issue of identifying sex by non-destructive methods in wild salmonids, Atlantic salmon and sea trout has recently been addressed (Pottinger *et al.* 2005). These authors concluded that a high degree of uncertainty was created when attempting to ascribe the sex based only on E2 levels while the measurement of vitellogenin was the most accurate and reliable marker of sex with clear divergence between males and females.

CONCLUSION

Changes in the serum concentrations of testosterone, progesterone, 17-β-estradiol, and cortisol in wild and farmed mature female and male brown trout, *Salmo trutta L.*, were measured in each season in several rivers and hatcheries located in the north-west of Spain. Serum profiles for sex steroids in both sexes showed seasonal variations that tracked the natural reproductive cycle, similar to those described for other salmonids, but with lower concentrations than those previously reported. The overall patterns were similar in both wild and farmed brown trout, except for serum cortisol levels, which were significantly higher in farmed brown trout and in those individuals infected with *S. parasitica*. No significant differences were found in cortisol levels between males and females. In connection to a presumable modification of the estrogenic activity in trout due to water contamination by phytoestrogens, our results suggest that, although faint, an endocrine impact can be presumed.

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