

# **Contrasting seasonal modulation of the stress response in male and female rainbow trout**

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Running head: Divergent stress responsiveness in rainbow trout

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**Abstract:** Between June and September the magnitude of the plasma cortisol response of maturing male and female rainbow trout to confinement was indistinguishable. A progressive increase in confinement-induced cortisol levels in both sexes occurred during the period May to September, associated with the seasonal rise in water temperature. Between September and January there was a more than 50% reduction in the magnitude of the cortisol response to confinement in male fish which coincided with declining water temperature and significant increases in plasma 11-ketotestosterone and elevated plasma testosterone levels. In contrast, female trout showed no evidence of a decline in cortisol responsiveness to confinement during the period September to January. Plasma estradiol-17 $\beta$  levels were significantly greater in females than males throughout the study period and this difference was maximal between September and January. However, plasma testosterone was also elevated in females during this period and levels overall were higher than those in male fish. Previous studies have shown estradiol-17 $\beta$  and testosterone to have diametrically opposed effects on stress responsiveness in trout, with the former enhancing, and the latter suppressing, the cortisol response to a stressor. The relative roles of androgens, estrogen and water temperature in modulating the stress responsiveness of rainbow trout is discussed.

Key words: stress, cortisol, androgen, estrogen, rainbow trout, sexual maturity

## INTRODUCTION

The neuroendocrine stress response of fish is primarily concerned with protecting, or re-establishing, homeostasis under challenging conditions. The magnitude and/or duration of elements of this response are known to vary with species (Davis & Parker, 1986; Waring *et al.*, 1992), strain (Pottinger & Moran, 1993), and with environmental temperature (Sumpter *et al.* 1985; Pickering & Pottinger, 1987) although the functional significance of this variation is uncertain. In sockeye salmon (*Oncorhynchus nerka* Walbaum; Donaldson & Fagerlund, 1970, 1972; Fagerlund, 1970; Fagerlund & Donaldson, 1969), brown trout (*Salmo trutta* L.) and rainbow trout (*O. mykiss* Walbaum; Sumpter *et al.*, 1987; Pottinger *et al.*, 1995) the dynamics and magnitude of the corticosteroid response to stress is influenced by sexual maturity. As is the case for mammals, in which sex-related modulation of stress-responsiveness is observed (Pottinger, 1999), the functional significance of this phenomenon has yet to be established.

In both brown and rainbow trout, mature male fish respond to a standardised stressor with a significantly lower elevation of blood cortisol than is the case for immature fish. Plasma adrenocorticotropin (ACTH) levels during stress are also reduced in mature male fish suggesting that the suppression of responsiveness occurs at the level of the brain, hypothalamus or pituitary (Pottinger *et al.*, 1995). Gonadal steroids have been implicated in the control of maturity-related modulation of stress responsiveness in mature male fish. The administration of both testosterone (T) and 11-ketotestosterone (11KT) to immature brown and rainbow trout resulted in a significantly lower plasma ACTH and cortisol response to confinement stress than was observed in control fish (Pottinger *et al.*, 1996). However, there is no substantive

account of effects of maturation on stress responsiveness in mature female fish, most of the information available on modulation of the stress response by gonadal steroids concerns male fish. Those studies which have examined cortisol dynamics in mature female fish (Donaldson & Fagerlund, 1970; Fagerlund, 1970) fail to clarify whether the stress response of female salmonids is modified by the onset of maturity, although plasma levels of cortisol in unstressed fish are reported to be higher in mature female salmonids than in males (Donaldson & Fagerlund, 1970; Pickering & Christie, 1981).

The administration of the female-specific estrogen, estradiol-17 $\beta$  (E<sub>2</sub>), to immature rainbow trout increased the stress-induced elevation of blood ACTH and cortisol relative to control fish (Pottinger *et al.*, 1996) suggesting that, in common with mammals, sexually mature female salmonids may display an enhanced stress responsiveness. However, unlike mammals, in addition to high plasma levels of E<sub>2</sub> during the reproductive period female salmonid fish also have high blood T levels (Scott & Sumpter, 1983) which may exceed levels in male fish (Scott *et al.*, 1980). A measurement of stress-induced cortisol levels in mature female rainbow trout on a single occasion during the reproductive cycle revealed that levels of cortisol were not elevated in females compared to pre-maturational levels (Pottinger *et al.*, 1996). This study was carried out to determine whether the stress responsiveness of female rainbow trout is modified during the reproductive cycle. The cortisol response to a standardised stressor, and blood levels of gonadal steroids, were measured at intervals during the reproductive period. Characterising the effect of sexual maturity on stress-responsiveness in female trout will contribute to our understanding of the functional significance of maturity-related modification of the hypothalamic-pituitary-interrenal axis in fish.

## MATERIALS AND METHODS

### *Experimental design*

During March 1997 twelve female rainbow trout (New Mills Trout Farm, Brampton, Cumbria; age 2+ years; mean weight  $\pm$  SEM;  $748 \pm 54$  g) and sixteen male rainbow trout (Trent Fish Culture Co., Mercaston, Derbyshire; age 2+ years;  $400 \pm 21$  g) were transferred to two 1500 l outdoor circular glassfibre tanks, each supplied with a constant flow of lakewater ( $30 \text{ l min}^{-1}$ , temperature range during the experimental period  $6^{\circ}\text{C} - 17^{\circ}\text{C}$ ). A third group of mixed-sex fish (Stirling strain; age 1+ years) were treated similarly to provide a non-maturing group. However, almost all these fish matured during the course of the study and were not included in the analyses. The fish were fed pelleted food (Trouw Standard Expanded 60) three times weekly at  $0.6 - 1.6 \text{ g } 100 \text{ g}^{-1} \text{ body weight day}^{-1}$ . During May, the fish were netted from their holding tanks and transferred to 50 l polypropylene tanks, each supplied with a constant flow of lakewater ( $15 \text{ l min}^{-1}$ ). Four fish were confined in each of seven tanks. After 1 h, the fish in trough 1 were transferred into anaesthetic (2-phenoxyethanol, 1:2000) and a 1.0 ml blood sample was removed from the Cuvierian sinus into a heparinized syringe. Blood samples were transferred to tubes and placed on ice until being centrifuged. Plasma was stored frozen ( $-70^{\circ}\text{C}$ ) until required for assay. Each fish was weighed and measured, and a passive integrated transponder (PIT) tag (FishEagle Co.) was inserted into the dorsal musculature to allow the subsequent identification of individual fish. The fish were then returned to their holding tank to recover. This confinement and blood-sampling procedure was repeated on a further seven occasions between May and January.

### *Analytical procedures*

Cortisol, testosterone, 11-ketotestosterone and estradiol-17 $\beta$  were measured in the plasma samples by established radioimmunoassay procedures (Pickering *et al.*, 1987; Pottinger & Pickering, 1985; Pottinger & Pickering, 1990).

### *Statistical analysis*

The data were analysed using analysis of variance (ANOVA, Genstat 5, Lawes Agricultural Trust) with individual fish, sex, and time (date of confinement) as factors. Significant differences were determined using the estimated standard error of the differences between means. Where mean and variance were found to be interdependent, the data were log-transformed prior to analysis.

## **RESULTS**

### *Somatic data*

Throughout the experimental period, female fish remained larger than male fish (Fig. 1b) while displaying similar patterns of growth and change in condition (Fig. 1c). Specific growth rate of both male and female fish declined slightly, but significantly ( $P < 0.05$ ) between May and September (Fig. 1d) before dropping precipitously between October and January with weight loss occurring between November and January. During the experimental period, water temperature increased from 9.2°C in May to a maximum of 17.1°C in September before declining to 6.7°C in December (Fig. 1a).

### *Gonadal steroids*

Plasma levels of gonadal steroids were low in May (Fig. 2) and during the subsequent months displayed sex-specific profiles characteristic of gonadal recrudescence. Plasma T levels were significantly higher at all subsequent samples in both sexes compared to values in May ( $P < 0.001$ ). Overall, T levels were significantly higher in female than male fish ( $P < 0.001$ ) and this was most pronounced in December (Fig. 2a) when levels in females reached a maximum of  $129 \text{ ng ml}^{-1}$  and levels in male fish declined to  $23 \text{ ng ml}^{-1}$ . Plasma E2 levels remained low in male fish throughout the experimental period ( $< 2 \text{ ng ml}^{-1}$ ; Fig. 2b). In female fish, plasma E2 levels in July were significantly higher than those in May and continued to increase to reach a maximum in November of  $23.3 \text{ ng ml}^{-1}$ , before declining in December. Plasma 11KT levels (Fig. 2c) remained low ( $\leq 3 \text{ ng ml}^{-1}$ ) in female fish throughout the experimental period while levels in male fish increased from September to reach a peak of  $50.3 \text{ ng ml}^{-1}$  during November.

### *Plasma cortisol levels following confinement*

There were significant differences in the response of the rainbow trout to confinement stress related to sex ( $P < 0.001$ ), and time of year ( $P < 0.001$ ) and there was a significant sex\*time interaction ( $P < 0.001$ ). Although there was a significant difference in post-confinement plasma cortisol levels between male and female fish in May, there were no sex-related differences between May and September, the response of both male and female fish to confinement was indistinguishable (Fig. 3). During this period there was a significant ( $P < 0.001$ ) increase in the mean post-confinement plasma cortisol level from  $\sim 35 \text{ ng ml}^{-1}$  to  $\sim 100 \text{ ng ml}^{-1}$ . Between September and January, the cortisol levels in mature female trout following confinement were sustained and

displayed no significant change with time (September: 106 ng ml<sup>-1</sup>; December: 129 ng ml<sup>-1</sup>). In contrast, the cortisol levels in mature male fish post-confinement showed a significant ( $P<0.001$ ) decline over this period, from 110 ng ml<sup>-1</sup> in September to 38 ng ml<sup>-1</sup> in December.

## DISCUSSION

These data are the first to show modulation of the magnitude of the stress response in relation to changes in the levels of gonadal steroids in a population of naturally maturing salmonid fish. During the period June to September the response of male and female fish to confinement was indistinguishable, with post-stress plasma cortisol levels of between 50 and 100 ng ml<sup>-1</sup>. The significant progressive increase in post-confinement cortisol levels which was observed in both sexes during the period May to September is likely to be related to water temperature, which increased during this period from 9°C to 17°C. The magnitude of the stress response has previously been demonstrated to be temperature-related in salmonid fish (Sumpter *et al.* 1985; Pickering & Pottinger, 1987). There is no clear explanation for the significant difference in stress-induced cortisol levels between male and female fish in May; it is possible this may reflect persistence of differences in responsiveness arising from the preceding maturational cycle.

Between September and January there was a more than 50% reduction in the magnitude of the cortisol response to confinement in male fish, consistent with previous observations (without accompanying plasma steroid levels) that sexually



mature male salmonid fish display an attenuated response to stressors (Sumpter *et al.*, 1987; Pottinger *et al.*, 1995). This has been demonstrated to be an androgen-dependent phenomenon (Pottinger *et al.*, 1996). In the present study it is clear that the onset of decline in responsiveness of the male fish occurs between September and October and coincides with increasing plasma 11KT levels. Plasma T levels were also increasing in males during this period. Both androgens suppressed the ACTH and cortisol response to confinement when administered to immature rainbow trout (Pottinger *et al.*, 1996) although 11KT was more consistent in its effects.

Water temperature decreased during the period September to December and it might reasonably be suggested that the decline in responsiveness of the male fish was driven by declining environmental temperature rather than alterations in the gonadal steroid profile. Although trends in water temperature undoubtedly contribute to the seasonal modulation of stress responsiveness in fish, previous studies have clearly demonstrated a difference in the cortisol response to stressors of mature male and immature rainbow trout (Pottinger *et al.*, 1995; 1996) and brown trout (Sumpter *et al.*, 1987), which is independent of water temperature. Nor do we think that the data indicate that the mature male fish are acclimating to the repeated stressor. The short duration of the stressor, coupled with the almost monthly intervals between testing, would render acclimation unlikely. We have observed no evidence of acclimation in similar groups of mature rainbow trout exposed to episodes of confinement at monthly intervals during the period March – July (Pottinger & Carrick, 1999). In addition, there was no evidence that alteration of stress responsiveness in the males was related to changes in body weight, coefficient of condition, or specific growth rate during this period.

In female rainbow trout, in contrast to the male fish, no evidence of a decline in responsiveness to confinement was apparent during the period September to December. The magnitude of the female response to confinement in September was indistinguishable from that in December. In mammals there is a sexual dimorphism in the glucocorticoid response to stress, with females being more responsive than males (Kant *et al.*, 1983; Spinedi *et al.*, 1994; Aloisi *et al.*, 1994). Estrogen treatment of gonadectomised male rats results in an enhanced corticosterone response to stress (Handa *et al.*, 1994a) and, similarly, treatment of immature rainbow trout with estrogen results in a cortisol response to confinement which is enhanced compared to that of sham-implanted fish (Pottinger *et al.*, 1996). Plasma E2 levels were significantly greater in females than males throughout the study period and these differences were maximal between September and January. However, plasma T levels were also elevated in females during this period and overall, were higher than T levels in male fish. A previous study has demonstrated clearly that E2 and T have diametrically opposed effects on stress responsiveness in trout, with E2 enhancing and T suppressing, the cortisol response to a stressor (Pottinger *et al.*, 1996). This previous study also showed that naturally maturing female fish and immature fish had similar increments in blood cortisol in response to a single episode of confinement during December. There are therefore at least three factors present during the reproductive period which may influence the female cortisol response to a stressor. Elevated E2 levels will enhance responsiveness, while elevated T levels, together with declining water temperature, will tend to drive the response downwards. The observed response of the female, which maintains a level of responsiveness to confinement consistent with that reached during September, must represent an amalgamation of

these opposing factors. As was the case for the males, there was no evidence that body weight, coefficient of condition or specific growth rate were related to stress responsiveness in the female fish.

It is not known whether a similar maturity-related modulation of stress responsiveness occurs in other species of fish. Nor has the functional significance of the sex difference in responsiveness to stress been established. It has been suggested that in males androgen-induced suppression of responsiveness may be directed at reducing the potentially deleterious effects of stress on reproductive processes (Handa *et al.*, 1994b) and in females, enhanced/sustained responsiveness to stress may represent a mechanism by which environmental conditions unfavourable to reproduction can inhibit reproductive processes (Viau & Meaney, 1991). Both of these hypotheses await testing.

### **Acknowledgement**

The authors thank Dr A. P. Scott (CEFAS, Lowestoft) for his kind gift of radiolabelled 11KT. This work was funded by the Natural Environment Research Council of the UK.

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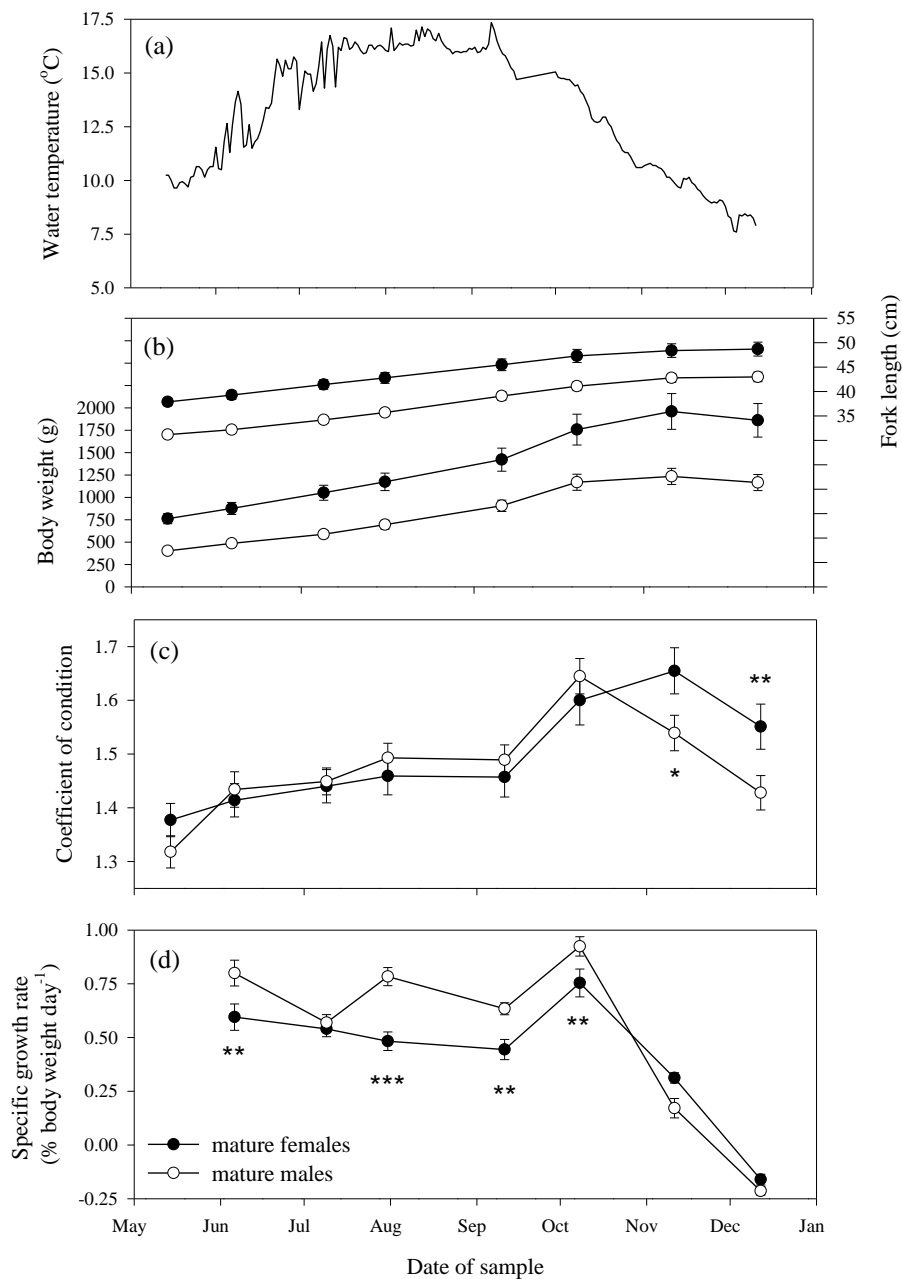
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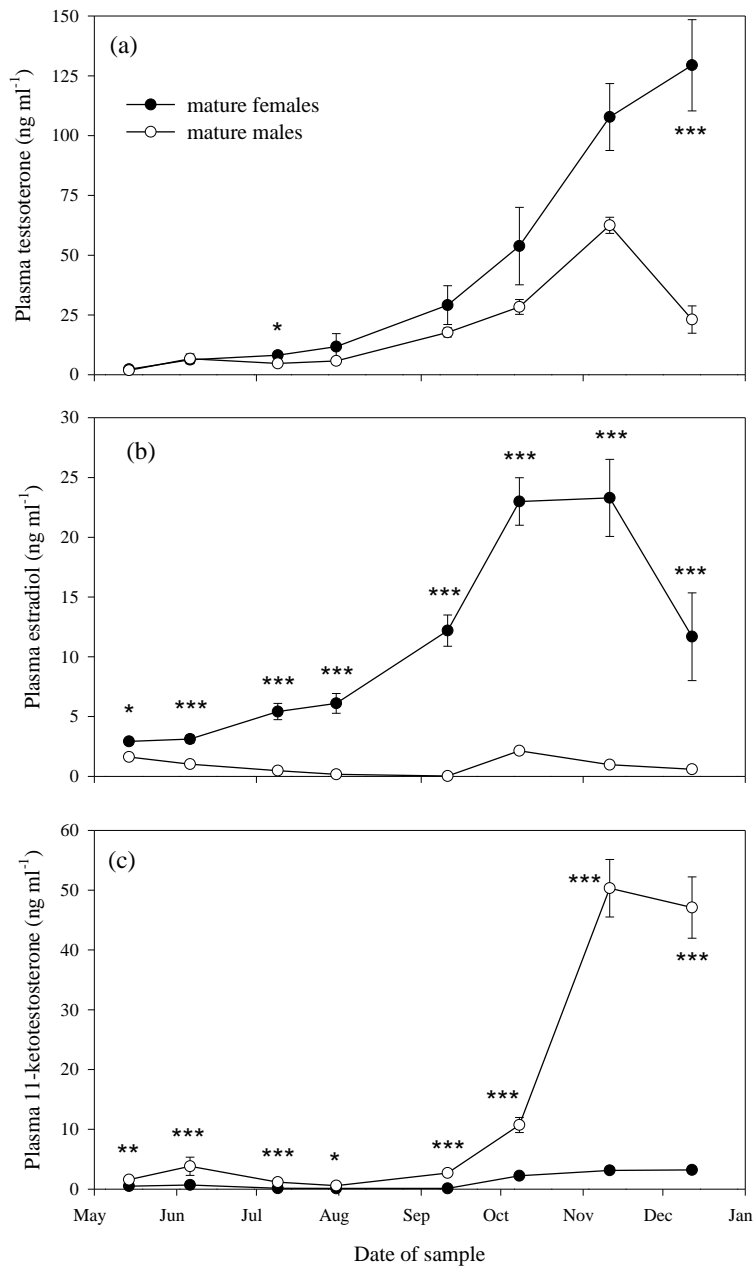
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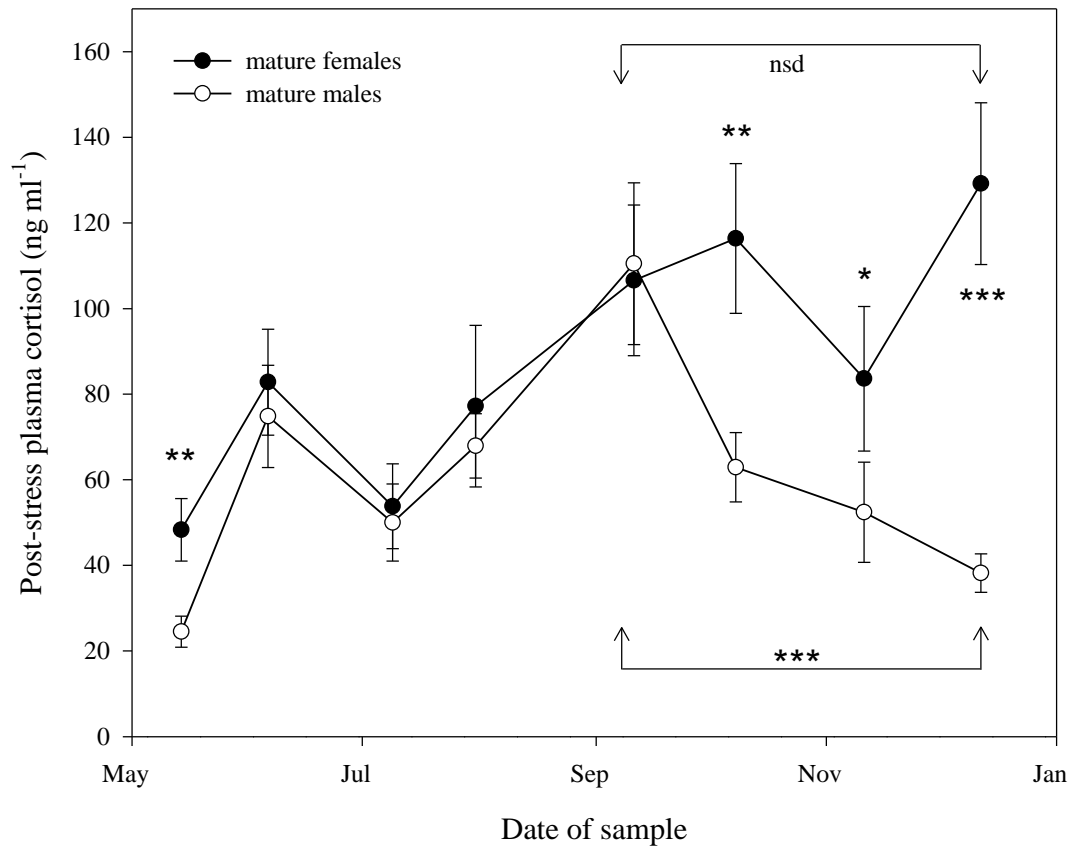




**Figure 1.** (a) Water temperature during the period May to January, and (b) body weight and fork length, (c) coefficient of condition, and (d) specific growth rate of male (○) and female (●) rainbow trout. Each point is the mean  $\pm$  SEM ( $n = 12-16$ ). In some cases the symbol obscures the error bars. Significant differences between male and female fish at specific times are denoted by \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .



**Figure 2.** (a) Plasma testosterone, (b) plasma estradiol-17 $\beta$ , and (c) plasma 11-ketotestosterone in male ( $\circ$ ) and female ( $\bullet$ ) rainbow trout. Each point is the mean  $\pm$  SEM (n = 12-16). In some cases the symbol obscures the error bars. Significant differences between male and female fish at specific times are denoted by \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .



**Figure 3.** Plasma cortisol levels in male (○) and female (●) rainbow trout following a 2 h period of confinement at intervals between May and January. Each point is the mean  $\pm$  SEM (n = 12-16). Significant differences between male and female fish at specific times are denoted by \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ . Comparisons over time within males or females are depicted by the lower and upper arrowed bars respectively. nsd: no significant difference.