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High blood cortisol levels and low cortisol receptor affinity: is the chub, *Leuciscus cephalus*, a cortisol-resistant teleost?

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Page head: cortisol resistance in a teleost fish

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ABSTRACT

In contrast to the relatively minor intra- and inter-species differences in blood cortisol levels reported for salmonid species, there is a more pronounced distinction between cortisol levels among the Salmonidae and Cyprinidae, with both basal and stress-induced cortisol levels markedly higher in the latter. This study shows that in the chub, *Leuciscus cephalus*, a widely distributed European cyprinid, mean blood cortisol levels during stress (1500 ng ml^{-1}) exceeded those reported for most other species of fish and even in unstressed chub, cortisol levels (50 – 100 ng ml^{-1}) were within the range known to cause immunosuppression, growth retardation and reproductive dysfunction in salmonid fish. The chub appears to be atypical only with respect to plasma cortisol levels; the levels of plasma glucose and plasma lactate in unstressed and stressed chub are similar to those reported for other species. Plasma levels of 11-ketotestosterone in males and 17β-estradiol in females are lower than reported for salmonids but similar to other cyprinid species and display clear stress-induced reduction. Comparative analysis of the binding characteristics of the trout and chub gill cortisol receptor revealed that the total number of binding sites in gill tissue for each species was similar (B_{max}; ~50 – 100 fmol mg^{-1} protein). However, the affinity of the binding site for cortisol displayed an 8-fold difference between the species (rainbow trout: K_d ~ 6 nM; chub: K_d ~ 50 nM). Therefore, the potentially adverse effects of high circulating levels of cortisol found both at rest and under conditions of stress in chub may be offset by the lower affinity of the cortisol receptor, rather than the abundance of target-tissue receptor sites. This strategy is similar to that reported for some glucocorticoid-resistant rodent species and New World primates.

Key words: stress, chub, rainbow trout, cortisol, glucose, lactate, 11-ketotestosterone, 17β-estradiol, cortisol receptor, glucocorticoid resistance, salmonid, cyprinid, *Oncorhyncus mykiss*, *Leuciscus cephalus*

INTRODUCTION

The steroid hormone cortisol plays a pivotal role in the teleost stress response but relatively few species have been studied in depth with respect to the levels of cortisol in the blood under different stages of development and environmental conditions. The Family Salmonidae (Order Salmoniformes) are best represented but even within this group only a limited range of species have been examined, to varying degrees; the rainbow trout (*Oncorhynchus mykiss*), Pacific salmon (*Oncorhynchus* spp.), brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) (Barton and Iwama, 1991; Gamperl *et al*., 1994; Mommsen *et al*., 1999). In unstressed rainbow trout, plasma cortisol levels are consistently reported as #10 ng ml^{-1} while exposure to stressors is rarely reported to elevate levels beyond 200 ng ml^{-1} (Barton and Iwama, 1991; Gamperl *et al*., 1994). This is similar to levels reported for other salmonid species although relatively minor inter-species differences in the magnitude of response to stressors have been reported (Pickering and Pottinger, 1989; Ruane *et al*., 1999). In addition, the level of cortisol in the blood of both unstressed and stressed fish is subject to modulation by a number of internal and external factors such as sexual maturity (Sumpter *et al*., 1987; Pottinger *et al*., 1995), genetic pedigree (Pottinger and Carrick, 1999), water temperature (Sumpter *et al*. 1985) and salinity adaptation (Shrimpton *et al*., 1994).

In contrast to the relatively minor intra- and inter-species differences in blood cortisol levels reported for salmonid species, there appears to be a more pronounced distinction between cortisol levels among the Salmonidae and those species grouped in the Family Cyprinidae (Order Cypriniformes). The available data are limited to only a few species but in these cyprinids at least, both basal and stress-induced cortisol levels are markedly higher than those reported for salmonids. Cortisol levels in unstressed common carp (*Cyprinus carpio*) have been reported to be –50 - 150 ng ml^{-1}, rising to –300 – 450 ng ml^{-1} during exposure to stressors (Dabrowska *et al*., 1991; van Dijk *et al*., 1993; Pottinger, 1998). In another cyprinid species, the roach (*Rutilus rutilus*), baseline cortisol levels are similar to those observed in salmonid fish (#10 ng ml^{-1}) while exposure to a stressor increases plasma cortisol to levels as high as –700 ng ml^{-1} (Pottinger *et al*., 1999). There are limited data for a third cyprinid species, the goldfish (*Carassius auratus*) in which cortisol levels in ostensibly unstressed fish are reported as ranging between 5 – 300 ng ml^{-1} (Spieler and Noeske, 1984), 7 – 110 ng ml^{-1} (Paxton *et al*., 1984) and 25 – 50 ng ml^{-1} (Singley and Chavin, 1975).

Preliminary studies on the chub, *Leuciscus cephalus*, a widely distributed European cyprinid, indicated that blood cortisol levels during stress exceeded those reported for most other species of fish (T. G. Pottinger, T. R. Carrick, W. E. Yeomans, unpublished) and even in unstressed chub, cortisol levels were within the range known to cause immunosuppression, growth retardation and reproductive dysfunction in salmonids (Pickering and Pottinger, 1989). However, no pathological or adverse effects were evident in the population of chub under study. Further work, reported here, was carried out to confirm the preliminary observations on the corticosteroid

response of chub to acute and chronic stressors, to evaluate the normality or otherwise of stress-induced alterations in gonadal steroids and in indicators of carbohydrate metabolism, and to assess whether cortisol target-tissue receptivity, in terms of B_{max} or K_d, reflected the presence of high circulating levels of cortisol and might be accountable for the lack of adverse effects.

MATERIALS AND METHODS

Effects of chronic and acute stressors on plasma cortisol, glucose and lactate levels

The experimental fish (chub, *Leuciscus cephalus*) were obtained from the Environment Agency Calverton Fish Farm (67 ± 1 g; 16.8 ± 0.1 cm; mean ± SEM, n = 224) and were maintained in twenty partially covered 1500 l outdoor circular glassfibre tanks, 50 fish per tank, each supplied with a constant flow of lake water (15 l min^{-1}, density –3.4 g l^{-1}). The fish were acclimated for 5 months before use and were fed once daily, five days per week (Trouw Excel 23). During this study (June 1998, water temperature 10.2 – 15.9 ^{o}C), eight tanks were denoted controls and remained undisturbed prior to sampling (sampled at 0, 1, 4, 24, 48, 72, 168, 336 h). Ten tanks provided fish for the acute stressor time-course (sampled at 0, 0.5, 1, 2, 4, 24, 48, 72, 168, 336 h). After the sampling of 8 fish from a single tank at time 0 h all remaining fish in the 9 acute tanks were netted and transferred to buckets for 5 mins before being returned to their holding tanks. This constituted the acute stressor. A further two tanks provided fish for the chronic stressor time-course. After removal of a 0 h control group, 72 fish were transferred in batches of eight to stainless steel wire mesh baskets suspended one in each of nine 50 l confinement tanks, each supplied with a constant

flow of lake water (10 l min^{-1}, density –30.5 g l^{-1}). The fish from a single confinement tank were sampled at each interval (0.5, 1, 2, 4, 24, 48, 72, 168, 336 h). To accommodate the sampling schedule several operators were required and the start of each time-course was staggered. At each sample point eight chub were netted into a bucket containing an anaesthetic solution in lake water (2-phenoxyethanol; 1:2000) and a blood sample was removed from the caudal vessels into a heparinized syringe. Blood was stored on ice until being centrifuged. Plasma samples were stored frozen (-20^{o}C) until analysis. Fish were killed by a blow to the head and the weight, length, and sex of each fish was recorded. For each sample, the concentrations of cortisol, glucose and lactate were determined.

Effects of a chronic stressor on plasma cortisol, 11-ketotestosterone and 17β-estradiol levels

Four hundred and eighty chub (128 ± 3.5 g; 20.6 ± 0.2 cm; mean ± SEM, n = 144) were distributed between 12 holding tanks (40 fish per tank, conditions as above, density –5 g l^{-1}) during June 1999. After a one month acclimation period, at time 0 h 16 fish were sampled from two tanks, 8 fish per tank (undisturbed controls). From a further two tanks, 64 fish were transferred in batches of eight to stainless steel wire mesh baskets suspended one in each of eight 50 l tanks (flow rate 10 l min^{-1}, holding density –58 g l^{-1}). At 4, 24, 48 and 168 h after the initial disturbance a further 16 fish were sampled from the confinement tanks and from control tanks (8 per tank). Fish were anaesthetized, blood sampled, and processed as described above. Water temperature during the study period was 14.1 – 18.7 ^{o}C. For each sample, the concentrations of cortisol, 11-ketotestosterone and 17β-estradiol were determined.

Relative binding affinity and capacity of trout and chub gill cortisol receptor

Gill cytosols were prepared from five chub and five immature rainbow trout in order to compare the binding characteristics of the gill cortisol receptor in each species. Gill tissue was collected and processed according to the method of Pottinger and Moore (1997). Fish were netted from their holding tank into a bucket containing anaesthetic (2-phenoxyethanol, 1:2000). The fish were killed by a blow to the head and the gill arches were removed by dissection. Tissue was placed directly in homogenization buffer (0.2 M tris-HCl, pH 7.4, 12 mM monothioglycerol, 1.0 mM EDTA, 10.0 mM sodium molybdate, 20% glycerol) on ice. The tissue was rinsed, filaments were separated from the arches and wet weighed, and fresh buffer was added in the ratio 2.5:1 (volume:weight). The tissue was homogenised, on ice (Ultra-Turrax TP 18/10) and the homogenate was transferred to 13.5 ml polycarbonate centrifuge tubes and centrifuged at 30,000 g for 60 mins at 4^{o}C. The resultant cytosol was dispensed in aliquots into capped polypropylene tubes and frozen at -70^{o}C until required. The protein concentration in the chub and trout gill cytosols were 18 and 13 mg ml^{-1} respectively. The dissociation constant (K_d) and maximum number of specific binding sites (B_{max}) were determined by saturation analysis. Aliquots of gill cytosol (200 µl) were incubated (for 2 h at 4^{o}C) in duplicate together with 100 µl of homogenisation buffer containing [1,2,6,7-^{3}H]cortisol (Amersham, 2.48 TBq mmol^{-1}) at concentrations of 0.7, 1.4, 2.8, 5.6, 11.2, 22.4 nM both in the absence and presence of a 1000-fold excess of inert cortisol. After incubation, a 500 µl aliquot of dextran-coated charcoal (1.25% activated charcoal, 0.125% dextran, in homogenization buffer) was added to each tube, the tubes were mixed, allowed to stand on ice for 10 mins and then centrifuged at 2200 g and 4^{o}C for 10 mins. A 500 µl aliquot of supernatant was transferred to a 5.0 ml scintillation vial together with 4.0 ml Ecoscint

A (National Diagnostics), mixed, and counted under standard ^{3}H conditions in a Packard 1900 TR liquid scintillation counter. B_{max} and K_d were derived by non-linear regression (Sigmaplot 5; SPSS Inc.) from a plot of specifically bound cortisol (fmol mg protein^{-1}) against free cortisol (nM), described by the equation $y = (a.x)/(b+x)$ where $a = B_{max}$, $b = K_d$, $y = [Bs]$, $x = [Free]$.

Assays

Plasma cortisol, 11-ketotestosterone and 17β-estradiol were measured using established radioimmunoassay procedures (Pickering *et al*., 1987a; Pottinger & Pickering, 1985; Pottinger & Pickering, 1990). Assay of chub plasma to which known amounts of cortisol were added revealed that no systematic under- or over-estimation of cortisol occurred. Plasma glucose and lactate were determined colourimetrically (Sigma Diagnostics procedures nos. 510 and 735). Concentrations of protein in gill cytosol were determined by the method of Ohnishi and Barr (1978).

Statistical analysis

Time-course data were subjected to analysis of variance (Genstat 5, Lawes Agricultural Trust) with treatment and time 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

Effects of chronic and acute stressors on plasma cortisol, glucose and lactate levels

Plasma cortisol levels in the three groups of unstressed chub sampled at time 0 were 95 ± 16, 110 ± 35 and 102 ± 21 ng ml^{-1}, rising within 1 h to a mean of 1408 ± 154 ng ml^{-1} in acutely stressed fish and 810 ± 181 ng ml^{-1} in chronically stressed fish (P<0.001; Fig. 1). In the acutely stressed fish mean plasma cortisol returned to levels indistinguishable from control, undisturbed, fish within 24 h of the initial disturbance while chronically confined fish required up to 72 h to return to baseline.

Prior to exposure to the stressors there was a significant difference (P<0.001, Fig. 2) in plasma glucose levels between the designated control fish (2.2 ± 0.2 mmol l^{-1}) and those from the acute (3.0 ± 0.3 mmol l^{-1}) and chronic (2.9 ± 0.19 mmol l^{-1}) groups Plasma glucose levels in control fish progressively increased during the course of the study rising to 4.0 ± 0.2 mmol l^{-1} after 336 h (P<0.001). Exposure to both acute and chronic stressors elevated plasma glucose levels significantly (P<0.001) to maxima of 7 – 8 mmol l^{-1}. Levels in acutely stressed chub returned to baseline more rapidly (4 – 24 h) than those in chronically stressed fish (48-72 h). After 72 h no further significant differences were apparent between the three groups.

Plasma lactate levels in chub exposed to acute and chronic stressors rose rapidly from –4.7 mmol l^{-1} to maximum values of –10 mmol l-1 within 30 – 60 mins (P<0.001; Fig.3). Lactate levels in both stressed groups returned to baseline within 2 h of the onset of disturbance and continued to decrease to levels significantly (P<0.001) lower than those of control fish by 4 h. After 24 h no further significant differences between

the three groups were apparent.

The inclusion of sex as a covariate in the ANOVA revealed gender to be a significant factor in determining plasma cortisol (P = 0.053) and glucose levels (P = 0.021) in chub. Levels of plasma cortisol and glucose were higher overall in female than male (cortisol: ♂, 357 ± 34 ng ml^{-1}, n = 123; ♀, 266 ± 31 ng ml^{-1}, n = 101; glucose: ♂, 4.71 ± 0.12 mmol l^{-1}, n = 123; ♀, 4.31 ± 0.15 mmol l^{-1}, n = 101).

Effects of a chronic stressor on plasma cortisol, 11-ketotestosterone and 17β-estradiol levels

In contrast to the preceding experiment, no significant effect of gender on cortisol levels was detected using sex as a covariate. Confinement elevated plasma cortisol levels from 50 ± 12 ng ml^{-1} to 537 ± 53 ng ml^{-1} within 4 h (P<0.05; Fig. 4a). Cortisol levels in confined fish declined to 264 ± 44 ng ml^{-1} within 48 h and remained at approximately this level until the end of the study. Plasma cortisol levels in control fish remained within the range 50 – 100 ng ml^{-1} throughout the experimental period.

In male chub, plasma 11-ketotestosterone (11KT) levels prior to confinement were 29.6 ± 7 ng ml-1 (Fig. 4b). Confinement rapidly depressed plasma 11KT levels to 3.5 ± 0.7 ng ml-1 (P<0.001) and in confined fish mean 11KT levels remained within the range 0.4 – 5.3 ng ml^{-1} throughout the study period. Mean 11KT levels in control fish remained within the range 17 – 32 ng ml^{-1}.

In unstressed female chub, mean plasma 17β-estradiol (E2) levels were within the range 2.5 – 5.0 ng ml^{-1} (Fig. 4c). Plasma E2 levels in confined female chub were

significantly lower (P<0.01) than those in control fish within 24 h of the onset of confinement and remained significantly lower for the remainder of the study.

Relative binding affinity and capacity of trout and chub gill cortisol receptor

Specific binding of ^{3}H-cortisol was obtained for both rainbow trout and chub gill cytosols (n = 5). Fitting a single rectangular hyperbola to the mean specifically bound cortisol at each of 6 concentrations of total ligand, against the free cortisol at each concentration of ligand, provided estimates for B_{max} for trout and chub of 48 ± 0.7 and 82 ± 15 fmol mg^{-1} protein respectively and for K_d of 6.2 ± 0.2 and 49.8 ± 12.3 nM respectively (Fig. 5a). The data are also presented as a Scatchard plot for comparative purposes (Fig. 5b).

DISCUSSION

The levels of plasma cortisol in unstressed chub and the response of the chub to acute and chronic stressors have not previously been reported. These data show that levels of cortisol in unstressed chub (50 – 100 ng ml^{-1}) are of a similar order of magnitude to, or higher than, those in other cyprinid species such as the common carp (Dabrowska *et al*., 1991; van Dijk *et al*., 1993; Pottinger, 1998) and are far in excess of cortisol levels in unstressed salmonid fish (#10 ng ml^{-1}; Barton and Iwama, 1991). Following the imposition of a stressor, cortisol levels in chub are elevated approximately 15-fold, to a mean level of almost 1500 ng ml^{-1} (the highest individual level recorded in a stressed fish during the present study was 1927 ng ml^{-1}). The magnitude of this stress-induced increase, in terms of a proportional elevation above

basal levels, is similar to that seen in salmonid fish. However, in absolute terms, the levels of cortisol observed in even unstressed chub far exceed the levels of cortisol known to elicit adverse effects on growth, reproduction and the immune system in salmonid fish (Pickering *et al*., 1989; Carragher *et al*., 1989). Inclusion of sex as a covariate during the analysis of the cortisol data revealed that, during the first experiment, cortisol levels were higher overall in stressed female chub than in stressed male chub. Given that these fish were sexually mature it is possible that these results reflect a situation similar to that reported for salmonid fish, and other vertebrates, in which males exhibit an androgen-dependent reduction in the corticosteroid response to a stressor (Pottinger *et al*., 1996; Pottinger and Carrick, 2000). It is unclear why a similar relationship between gender and cortisol elevation was not observed during the second experiment although this might be related to the relative timing of the experiments – in relation to season, the second study took place a month later than the first.

In terms of the limited range of endocrine and metabolic parameters examined in this study, the chub appears to be atypical only with respect to plasma cortisol levels. Although there are no previously published data for chub with which to compare these results, the levels of plasma glucose and plasma lactate observed in unstressed chub and the changes in these metabolites which were observed in stressed chub during the present study are similar to those reported for related species, the carp (Hertz *et al*., 1989; Dabrowska *et al*., 1991; van Raaij *et al*., 1996) and roach (Pottinger *et al*., 1999). There was again evidence of a sexually dimorphic response to a stressor in that glucose levels were significantly related to sex, being higher overall in female than male chub.

Similarly, the plasma levels of 11KT (20 – 30 ng ml^{-1}) and E2 (2.5 – 5 ng ml^{-1}) in sexually mature male and female chub in the present study are consistent with levels reported for other cyprinid species (Venkatesh *et al*., 1989; Barry *et al*., 1990; Kestemont *et al*., 1999). Exposure to a confinement stressor resulted in a marked reduction in levels of both E2 and 11KT for the duration of the study, an effect which has previously been observed in only one other cyprinid, the roach (Pottinger *et al*., 1999), and was most pronounced for 11KT in male fish. Unstressed chub are clearly able to maintain "normal" levels of gonadal steroids in the blood even in the presence of concentrations of cortisol (–100 ng ml^{-1}) which would, in salmonid fish, be associated with a significant reduction in circulating gonadal steroid levels (Pickering *et al*., 1987b) although it should be noted that levels of gonadal steroids in these chub were considerably lower than levels in salmonid fish at a similar stage in the reproductive cycle (Scott *et al*., 1980a, b).

The question arises: how does the chub tolerate circulating levels of cortisol which in other species of fish would be highly immunosuppressive? Corticosteroid / glucocorticoid resistance (/tolerance of, or insensitivity to, high circulating levels of hormone) has been well-documented in mammalian species, both as a pathological abnormality in human patients (Arai and Chrousos, 1995) and as a non-pathologic characteristic of certain primate and rodent species (Brandon *et al*., 1991; Taymans *et al*., 1997). Target-tissue sensitivity to steroid hormones may be modulated in a number of ways. These mechanisms include alterations in the abundance of receptors within target-tissues (Iida *et al*., 1985), modification of the affinity of the receptor for the ligand (Brandon *et al*., 1991), or changes in the processing of the activated

receptor after formation of the receptor-ligand complex (Rhouma *et al*., 1997). In addition, levels of cortisol-binding globulins in the plasma may be important in regulating target-tissue availability of cortisol (Alexander and Irvine, 1998). However, there is no firm evidence that fish possess a cortisol-binding globulin which is directly analogous to that found in mammals (Mommsen *et al*., 1999).

Preliminary comparative analysis of the binding characteristics of the trout and chub gill cortisol receptor revealed that, although the total number of binding sites in gill tissue for each species was similar, the affinity of the binding site for cortisol displayed an 8-fold difference between the species. The estimate of affinity obtained for rainbow trout gill cytosolic cortisol binding (K_d – 6 nM) is similar to that obtained for coho salmon gill cytosol (*Oncorhynchus kisutch*; Maule and Schreck, 1990) but higher than that reported elsewhere for rainbow trout and Atlantic salmon in which triamcinolone acetonide was used as ligand (Shrimpton and McCormick, 1998, 1999). However, this cortisol analogue is acknowledged to display a higher affinity for cortisol-binding sites than the native ligand. The maximum number of cortisol-binding sites (B_{max}; –50 – 100 fmol mg^{-1} protein) detected in trout and chub gill cytosols during the present study was similar to that previously reported for rainbow trout (Shrimpton and McCormick, 1999) and is also consistent with the only other report of corticosteroid binding in gill tissue from a cyprinid fish in which specific binding of cortisol to gill cytosol from carp displayed a B_{max} of 112 fmol mg^{-1} protein (Kloas *et al*., 1998). However, in marked contrast to rainbow trout gill cytosol, a considerably higher dissociation constant (K_d – 50 nM) was determined for specific cortisol binding in chub gill cytosol in the present study. This also is consistent with the data of Kloas *et al*. (1998) who determined a K_d of 31 nM for carp gill cytosol.

This suggests that the potentially adverse effects of high circulating levels of cortisol found both at rest and under conditions of stress in chub may be offset by the lower affinity of the cortisol receptor, rather than the abundance of target-tissue receptor sites which were similar in both chub and trout. Only one other study has examined receptor-like cortisol binding in tissues of a cyprinid fish; Weyts *et al*. (1998) reported a dissociation constant for specific binding of cortisol to carp leucocytes of – 4 nM. This is considerably lower than that reported in the present study for chub, and is also lower than the K_d determined by Kloas *et al*. (1998). However, these authors report that basal cortisol levels in the strain of carp from which this K_d was derived were in the range 5 – 20 ng ml^{-1}, more akin to levels found in salmonid fish than those observed in chub during the present study. While inadequate periods of acclimation, or sub-optimal conditions, can result in chronically elevated blood cortisol levels in fish the chub employed in the present study displayed consistent resting and stress-induced levels of cortisol over an 18-month period. We are confident that the higher blood cortisol levels measured during this study are a true reflection of the normal physiology of the chub and are not an artefact.

In conclusion, plasma cortisol levels in both undisturbed and stressed chub are markedly higher than reported for other teleost fish. Cortisol levels in salmonid species are at least ten-fold lower than those in chub. Cortisol levels in other cyprinid species, although overall higher than those in salmonid fish, do not equal those measured in chub. Preliminary measurements of specific cortisol binding in gill cytosol, a cortisol target-tissue, suggest that chub avoid adverse effects of sustained levels of cortisol in excess of – 100 ng ml^{-1} because their cortisol receptor has a lower affinity for cortisol than that of species with lower resting levels of plasma cortisol.

This strategy is akin to that reported for the some glucocorticoid-resistant rodent species and New World primates in which atypically high resting corticosterone or cortisol levels are accompanied by lower receptor affinities than are evident in species displaying low circulating glucocorticoid levels (Taymans *et al*., 1997; Reynolds *et al*., 1997; Hastings *et al*., 1999).

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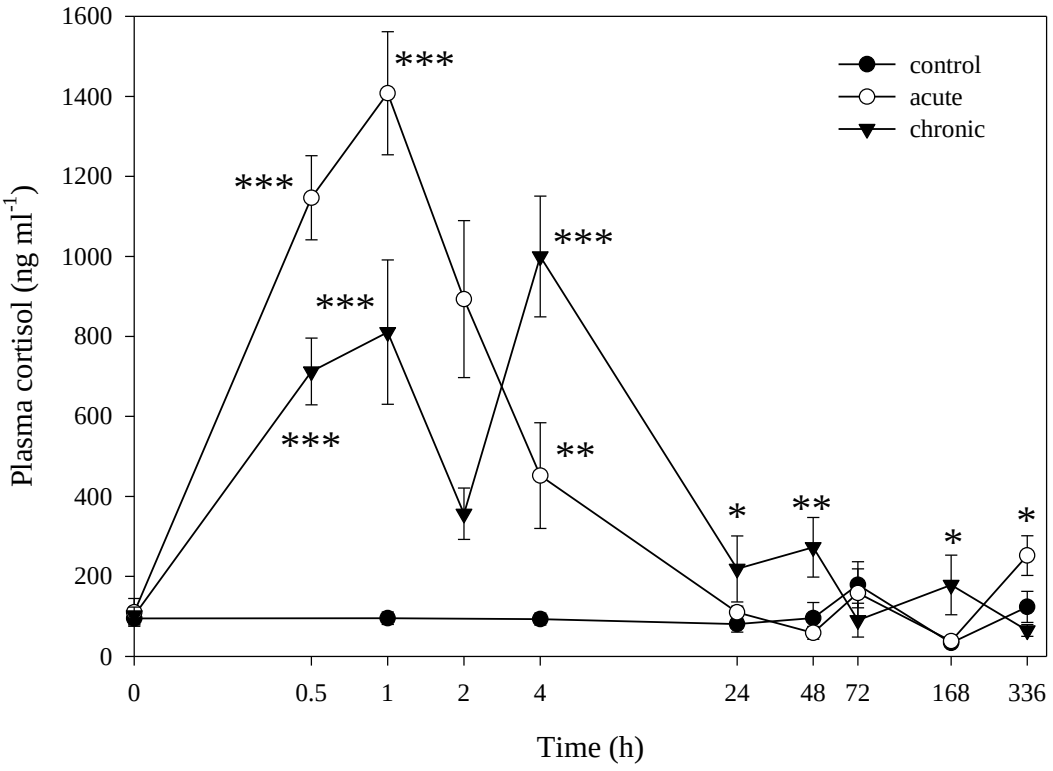

FIG. 1. Plasma cortisol levels in undisturbed (□), acutely stressed (Φ; 5 mins handling and disturbance), and chronically stressed (□; continuous confinement) chub at intervals following the onset of disturbance. Each point is the mean ± SEM, n = 8. Significant differences from control (undisturbed) values at each time point are denoted by * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

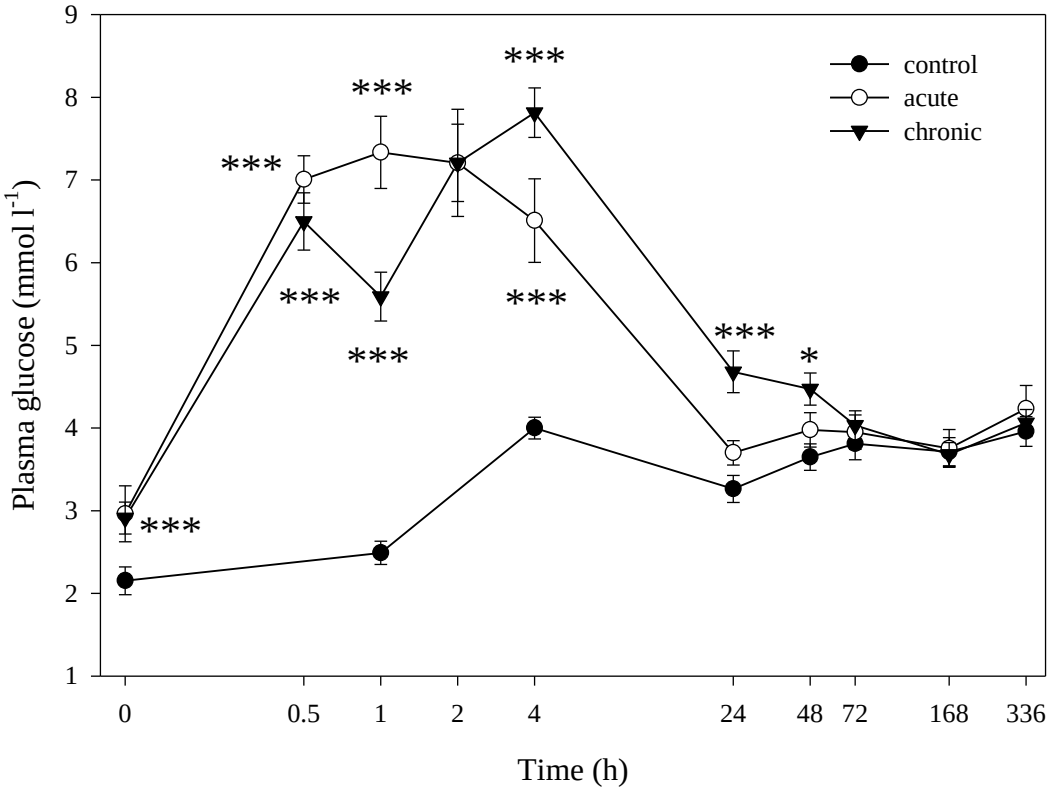

FIG. 2. Plasma glucose levels in undisturbed (□), acutely stressed (Φ; 5 mins handling and disturbance), and chronically stressed (□; continuous confinement) chub at intervals following the onset of disturbance. Each point is the mean ± SEM, n = 8. Significant differences from control (undisturbed) values at each time point are denoted by * $P<0.05$; *** $P<0.001$.

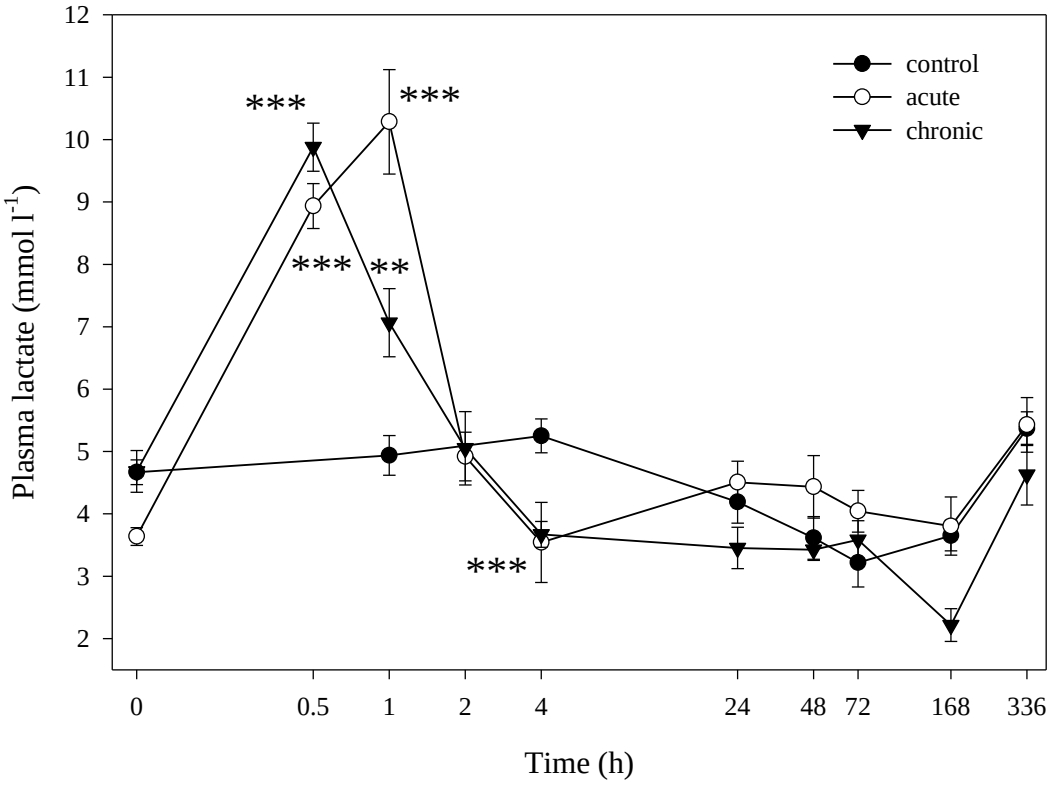

FIG. 3. Plasma lactate levels in undisturbed (□), acutely stressed (Φ; 5 mins handling and disturbance), and chronically stressed (□; continuous confinement) chub at intervals following the onset of disturbance. Each point is the mean ± SEM, n = 8. Significant differences from control (undisturbed) values at each time point are denoted by ** $P<0.01$; *** $P<0.001$.

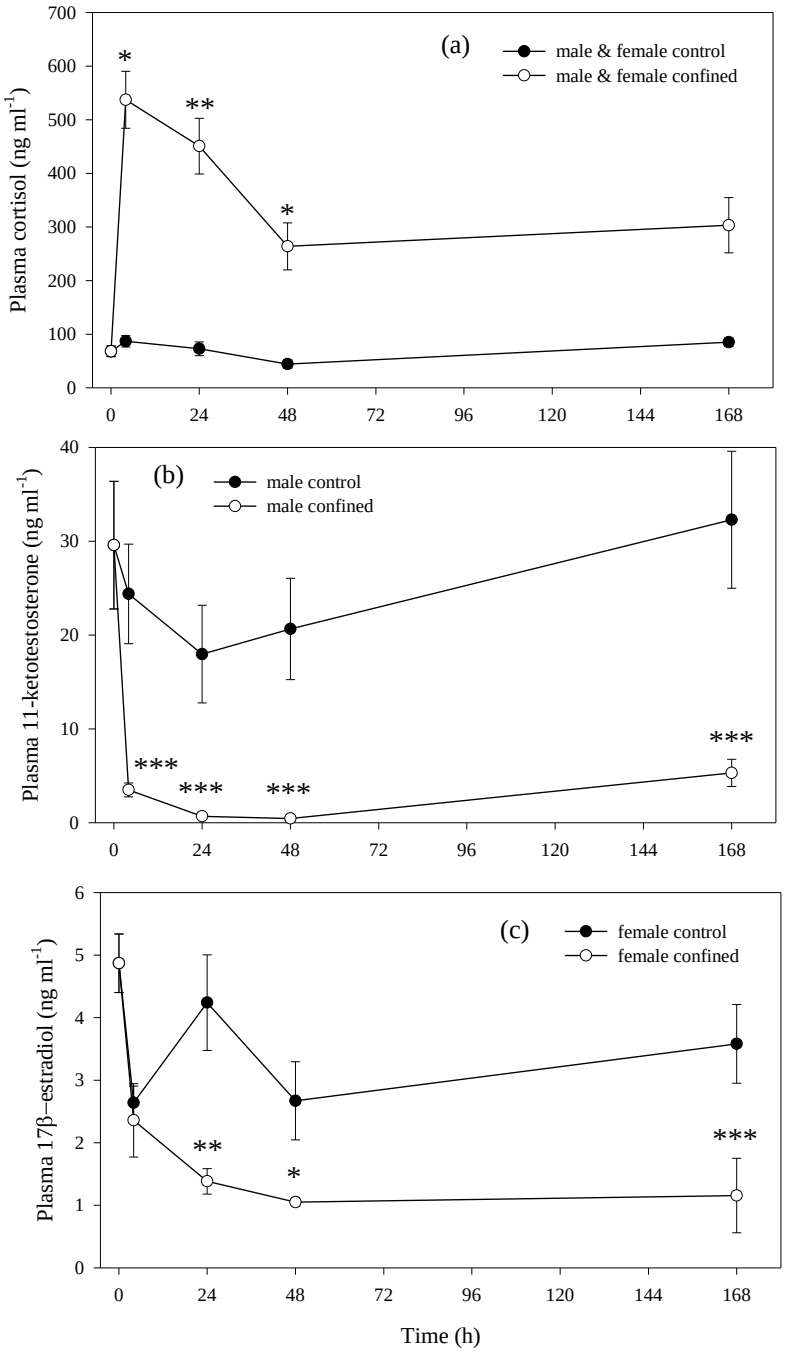

FIG. 4. (a) plasma cortisol levels in male and female chub, (b) plasma 11-ketotestosterone levels in male chub and (c) plasma 17β-estradiol levels in female chub, undisturbed (□) and chronically confined (Φ), at intervals following the onset of disturbance. Each point is the mean ± SEM, n = 5 - 11. Significant differences from control (undisturbed) values at each time point are denoted by * $P<0.05$; ** $P<0.01$;

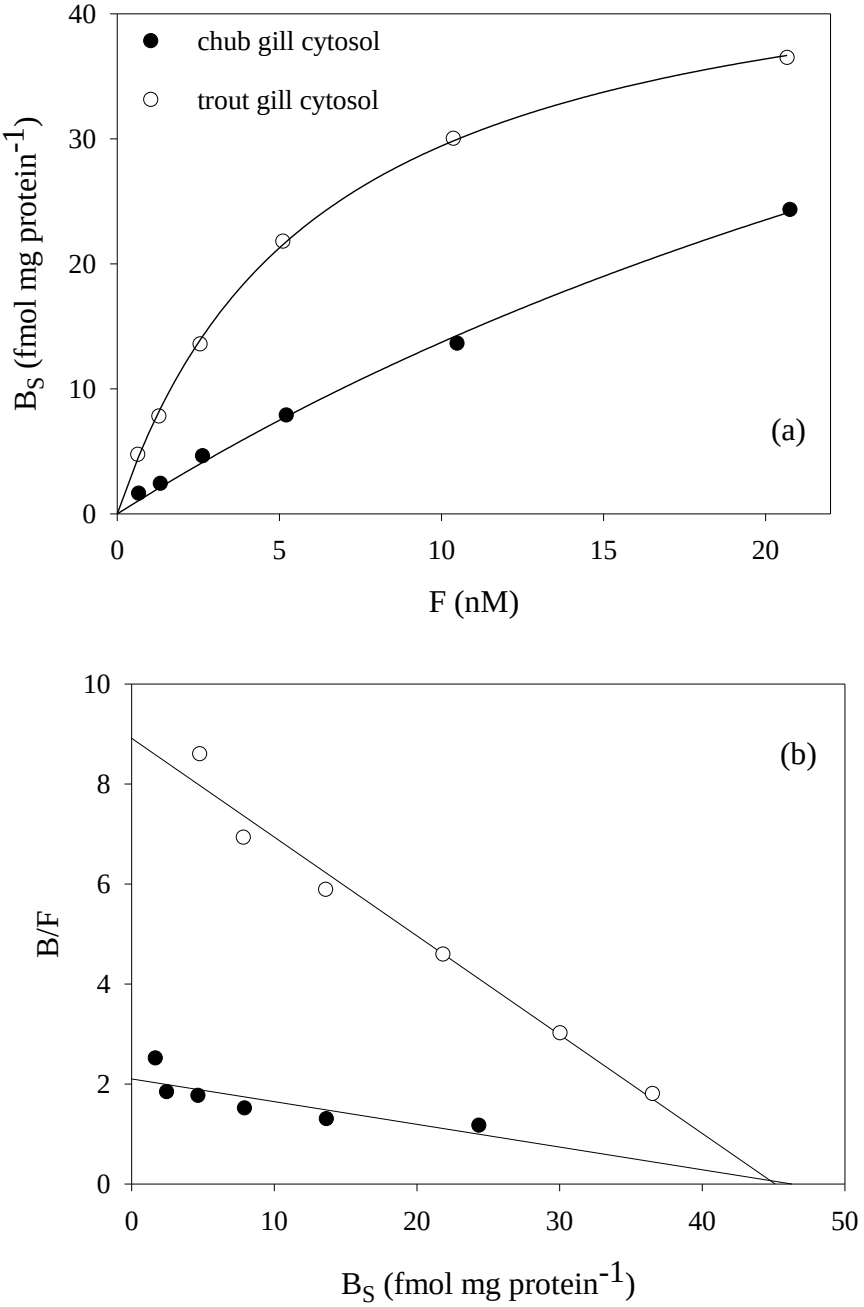

FIG. 5. (a) The specific binding (B_s) of ^{3}H-cortisol, at increasing concentrations of ^{3}H-cortisol, to gill cytosol from trout (Φ) and chub (□) plotted against the concentration (nM) of free ^{3}H-cortisol at each concentration. A single rectangular hyperbola was fitted by non-linear regression to each data set and used to derive K_d

and B_{max}. Trout: r^2 = 0.999; B_{max}: 48 fmol mg^{-1} protein; Kd: 6.2 nM; Chub: r^2 = 0.996; B_{max}: 82 fmol mg^{-1} protein; Kd: 49.8 nM. (b) A Scatchard plot of the data presented in (a). The differences in K_d for the two species derived from this plot (trout: 5.1 nM; chub: 25 nM) are clearly indicated by the different gradients of the best-fit lines. The common intercept indicates that B_{max} is similar for the two species (trout: 45.2 fmol mg^{-1} protein; chub: 52.5 fmol mg^{-1} protein). The non-linear fit used in (a) provides parameter estimates with greater reliability than the linear fit of a line to transformed data (b) accounting for the apparently identical B_{max} for the two species indicated by the Scatchard plot.