The Effects of Chronic Plasma Cortisol Elevation on the Feeding Behaviour, Growth, Competitive Ability, and Swimming Performance of Juvenile Rainbow Trout

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ABSTRACT

Plasma cortisol elevation, a common consequence of stress, occurs in salmonids of subordinate rank; these fish acquire a smaller share of available food and grow more slowly. This study examined the role of cortisol itself in these phenomena. Cortisol implants, with parallel sham and control treatments, were used to create a chronic threefold elevation in plasma cortisol levels in juvenile rainbow trout, and the individual feeding patterns of the fish were evaluated using X-ray radiography. The three treatment groups were (1) held alone and fed to satiation, thereby providing a measure of voluntary appetite, or mixed together in equal proportions and fed to either (2) satiation or (3) half-satiation, thereby allowing assessment of the additional effects of competitive interaction and food limitation. Chronic plasma cortisol elevation had significant negative effects on individual appetite, growth rate, condition factor, and food conversion efficiency, independent of whether the fish were held under unmixed or mixed conditions. Under the latter, mean share of meal was reduced and fin damage increased in cortisol-treated fish; negative growth effects were more severe with food limitation, but the response patterns were otherwise unchanged. Even in the absence of other groups, cortisol-treated fish showed more variable feeding patterns. When compared at the same individual ration levels, cortisol-treated fish had lower growth rates, reflecting a higher "cost of living." Cortisol treatment had no effect on aerobic swimming performance. These results suggest that the structure of the feeding hierarchy may not be determined solely by competitive ability but may also be greatly influenced by differences in the feeding behaviour of unstressed fish versus stressed fish caused by cortisol elevation in the latter.

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Introduction

Cortisol is the most active and abundant corticosteroid in fish blood, and its structure has been highly conserved in all of the vertebrate species in which it is found (van der Boon et al. 1991). In fish, cortisol production and secretion occur in the interrenal tissue and are regulated by the pituitary primarily via stimulation by adrenocorticotropic hormone (ACTH) and α-melanophore-stimulating hormone (α-MSH; van der Boon et al. 1991; Wendelaar Bonga et al. 1995). The primary targets of cortisol action are the gills, intestine, and liver, which reflect the two main adaptive functions of cortisol identified to date: osmoregulation and the maintenance of a balanced energy metabolism (Wendelaar Bonga 1997). With respect to the latter, cortisol plays an important role in mobilizing fuels such as glucose, lipids, and fatty acids for the maintenance of homeostasis and exerts direct and indirect effects on intermediary metabolism, particularly in response to stress (van der Boon et al. 1991).

Plasma cortisol levels are known to cycle diurnally (Holloway et al. 1994; Reddy and Leatherland 1995) and to change according to season (McLeese et al. 1994). However, it is the rapid and often severe elevation of cortisol levels that occurs in response to different stressors (e.g., handling, confinement, poor water quality, toxicants) that has made cortisol the most commonly used indicator of stress in fish (Wendelaar Bonga 1997).

It is also well established that plasma cortisol levels are related to social rank when fish are held together under conditions that promote competitive interaction, with subordinate fish having elevated cortisol titres relative to dominant individuals (Ejike and Schreck 1980; Schreck 1981; Pottinger and Pickering 1992; Fox et al. 1997). Morphological differences in the relevant cells of the adenohypophysis (Boddingius 1976) and interrenal gland (Noakes and Leatherland 1977) have been similarly correlated with social rank in salmonids. Under such conditions, subordinate fish acquire a smaller share of available food (McCarthy et al. 1992, 1993) and grow more slowly (Metcalfe et al. 1992) than dominant fish. However, even when ration is

controlled, subordinate fish still grow more slowly (Abbott and Dill 1989), an effect probably related to their greater metabolic energy expenditure per unit ration (Li and Brocksen 1977; Metcalfe 1986). In addition, cortisol treatment alone is known to depress growth rate (Barton et al. 1987).

It is therefore unclear to what degree cortisol elevation alone—independent of social (or other) stress—affects feeding behaviour, competitive ability, and, therefore, social rank. In other words, Is cortisol elevation a cause or a consequence of subordinate rank? Does cortisol elevation itself alter appetite and performance? We attempt to elucidate the relative importances of these complex interacting factors.

Coconut oil implants (Specker et al. 1994) were employed to elevate cortisol titres within the physiological range. An X-ray radiography technique that allows the measurement of feeding performance in individual fish (McCarthy et al. 1992, 1993; Gregory and Wood 1998) was used to assess appetite and social dominance. The feeding performance and growth rates of cortisol-treated fish were compared with those of appropriate sham and control groups under conditions where the three treatment groups were held alone and fed to satiation, thereby allowing a measurement of voluntary appetite. In addition, the same measurements were performed under conditions where these same treatment groups were held together in a competitive situation at two different ration levels. The relative influences of cortisol treatment on fin damage (Abbott and Dill 1989) and aerobic swimming performance, the latter employing the standard U_{crit} test (Brett 1964), were also determined. The results provide some important new insights into the effects of stress and cortisol on individual feeding physiology and behaviour in salmonid fish.

Material and Methods

Juvenile (~10 g) rainbow trout (*Oncorhynchus mykiss*) were obtained from Humber Springs Trout Hatchery in Orangeville, Ontario, and held in dechlorinated Hamilton, Ontario, tap water (see Alsop and Wood [1997] for composition) at 15° ± 1°C in 60-L tubs covered by fabric mesh and shaded by opaque plastic lids. The tanks had constant aeration and flow through at a rate of ~1 L min^{-1} and were subjected to a photoperiod of 12.5L : 11.5D.

Treatment Groups

Fish were anaesthetized individually in 0.1 g L^{-1} tricaine methanesulfonate (MS-222) set to a pH of ~8.0 with 3.0 M NaOH, weighed, and then placed briefly on ice. The fish were then injected peritoneally with a mixture of 25 mg cortisol mL^{-1} in melted coconut oil (~27°C) at a ratio of 10 μL g^{-1} of fish, resulting in an overall dose of 250 mg cortisol kg^{-1} of fish (cortisol = 11β,17α,21-Trihydroxypregn-4-ene-3,20-dione; Sigma Chemical Co., St. Louis, Mo.). This dose was selected on the basis of a preliminary experiment using a variety of cortisol concentrations so as to achieve an approximate tripling of baseline cortisol concentrations relative to control fish (G. DeBoeck and C. M. Wood, unpublished results). Immediately after injection, the fish were placed on ice for roughly 1 min to promote solidification of the coconut oil and then were placed in a large, aerated recovery tank. This treatment group is hereafter referred to as the "cortisol" group. A second group ("sham") was treated in the same way but was injected only with coconut oil free of cortisol. A third group ("control") received no treatment whatsoever. The fish were allowed to recover overnight in separate tanks according to treatment before being reanaesthetized, weighed (to 0.1 g), measured (fork length to 0.1 cm), and marked for individual identification with a PanJet ink injector and Alcian Blue 8GX dye. After being marked, the fish were placed alternately in one of seven identical tanks.

Series 1: Unmixed Tanks

Three tanks were used, each containing 24 fish from one of the three treatment groups. There were no significant differences in mean initial mass in these tanks ($P > 0.37$; Table 1). The fish in these tanks were fed to satiation daily.

Series 2: Mixed Tanks

Four tanks were used in this series, with 24 fish placed in each tank (eight from each treatment group). The fish in two tanks were fed to satiation once per day, while those in the other two were fed a half-satiation diet calculated on a mass-specific basis relative to the feeding of the fish in the satiation tanks in this series (Gregory and Wood 1998). Fish in both series were fed a diet consisting of repelleted commercial fish feed (Ziegler Bros., Gardners, Pa.) prepared as in Gregory and Wood (1998). All food was consumed during feeding. There were no significant differences in mean initial mass between any of the tanks used in this series ($P > 0.97$; Table 2) or between those in series 1 and series 2 ($P > 0.88$).

X-Ray Radiography

The fish were allowed to recover from treatment for a period of 1 wk before being X-rayed. On the day of X-raying, the fish were fed food prepared as above but containing 8.5 grade (0.400–0.455-mm) lead glass ballotini beads (Jencons USA, Bridgeville, Pa.) at a ratio of 6% by dry mass of food. The presence of the beads did not affect the palatability of the food, as judged from the total meal size. The fish were given at least 1 h after feeding to settle in order to avoid regurgitation of the food. The fish were then anaesthetized as above and X-rayed in groups of eight in a divided glass grid using a General Electric AMX-110 mobile X-ray machine at 70 kVP (kilovolts peak)

Table 1: Mean values ± SE (*n*) of mass, condition factor (CF), coefficient of variation (CV) in feeding, and mortality in the control, sham, and cortisol fish from the unmixed tanks of series 1

Variable	Control	Sham	Cortisol
Initial mass (g)	9.52 ± .52 (24)[a]	10.02 ± .63 (24)[a]	8.86 ± .60 (24)[a]
Final mass (g)	13.91 ± .61 (24)[a]	13.98 ± .98 (23)[a]	10.26 ± 1.06 (13)[b]
CF (100 × g cm^{-3})	1.32 ± .02 (24)[a]	1.29 ± .02 (23)[a]	1.09 ± .05 (13)[b]
CV (%)	41.09 ± 5.18 (24)[a,b]	38.19 ± 3.50 (23)[a]	53.60 ± 6.37 (13)[b]
Mortality (%)	0 (0/24)[a]	4.2 (1/24)[a]	45.8 (11/24)[b]

Note. Numbers in parentheses are *n*'s. Groups with different letters (a, b) are significantly different from one another ($P < 0.05$). Differences in sample size are due to mortality.

and 150 mAs (milliampere seconds) at 61 cm. In only three cases were fish seen to regurgitate food, and examination of the X-ray images suggested that no food had been cleared before X-raying. The fish were X-rayed in this manner three times, with 1-wk recovery periods during which the normal feeding regime was maintained. A sample of more than 200 food pellets was X-rayed, and less than 5% of the pellets were found to be devoid of beads. At the time of X-raying, the fish were weighed and their dorsal and caudal fins examined, and the fin score of each fish (a measure inversely proportional to damage) was calculated as in Gregory and Wood (1998).

Specific growth rates (SGR in % d^{-1}; McCarthy et al. 1992) and condition factors (CF; 100 × mass × $length^{-3}$) were calculated for each fish at the end of the experiment. Mean share of meal (MSM; the average percentage of the three X-rayed meals obtained by each individual fish) and coefficient of variation (CV; a measure of the variability in food consumption from meal to meal for each individual fish) were calculated for each fish based on food consumption ascertained by counting the number of beads found in the digestive tracts of the fish from the X-ray images (McCarthy et al. 1992; Gregory and Wood 1998). Average food consumption ("appetite" in % body mass [BM] $meal^{-1}$) was also calculated for each fish based on its individual mass-specific food intake over the three X-rayed meals. Conversion efficiencies (CE, in percentage) were also calculated for each fish using estimated total food consumption (dry mass) based on the X-ray measurements and change in wet mass throughout the experiment (CE = [final mass − initial mass]/[mean food consumption × number of days] × 100%).

Plasma Sampling

A third series of fish was kept in two identical tanks and fed to satiation daily. This series comprised a mixture of all three treatment groups for use in regular plasma sampling. On days 3, 7, and 14, at least six fish from each treatment were killed in 3.0 g L^{-1} MS-222, a concentration that is sufficiently high to prevent the induction of a cortisol response (Beitinger and McCauley 1990). A blood sample was taken using a haematocrit tube following caudal severance. The blood was spun for 5 min at 5,000 *g*, and the plasma was extruded and frozen in liquid nitrogen before being stored at −70°C. Plasma cortisol concentrations were determined using an ImmuChem Coated Tube Cortisol ^{125}I Radioimmunoassay Kit (ICN Biomedicals, Costa Mesa, Calif.). The fish were also dissected at the time of sampling in order to establish the presence of an implant in the cortisol- and sham-treated fish. All fish from series 1 and 2 (except those used in the swimming trials) were killed and sampled in the same manner at the end of the experiment (day 23), and any fish that died during the course of the study also were dissected for implant confirmation.

Swimming Performance

After the conclusion of the three X-raying periods, satiation-fed fish from all three treatment groups were used in the determination of aerobic swimming performance (U_{crit}). One control fish was taken from each of the two satiation-fed mixed tanks of series 2 and one from the unmixed control tank of series 1 (also satiation-fed). Three sham- and cortisol-treated fish were selected in the same manner from the appropriate satiation-fed tanks of series 1 and 2 and were put together into a single group of nine fish. Three such groups were formed, each containing three satiation-fed fish from each treatment group. Each group of nine fish was swum together in a 100-L Beamish-type respirometer, as described in Gregory and Wood (1998), using velocity increments of 0.75 BL s^{-1} (body lengths per second) and 40-min time intervals. U_{crit} values (in BL s^{-1}) were calculated as in Brett (1964). There were no significant differences in the body lengths of the fish from the three treat-

Table 2: Mean values ± SE (*n*) of mass, condition factor (CF), coefficient of variation (CV) in feeding, and mortality in the satiation- and half-satiation-fed control, sham, and cortisol fish from the mixed tanks of series 2

	Satiation			Half-Satiation		
Variable	Control	Sham	Cortisol	Control	Sham	Cortisol
Initial mass (g)	8.89 ± .63 (16)[a]	10.25 ± .70 (16)[a]	8.70 ± .70 (16)[a]	9.69 ± .72 (16)[a]	9.42 ± .70 (16)[a]	8.95 ± .61 (16)[a]
Final mass (g)	12.11 ± .80 (16)[a,b]	14.62 ± 1.25 (14)[a]	9.14 ± .94 (12)[b]	12.54 ± 1.21 (11)[a]	11.86 ± 1.23 (14)[a]	8.68 ± .70 (11)[b]
CF (100 × g cm^{-3})	1.34 ± .02 (16)[a,*]	1.35 ± .05 (14)[a]	1.09 ± .03 (12)[b]	1.22 ± .04 (11)[a,*]	1.26 ± .04 (14)[a]	1.03 ± .02 (11)[b]
CV (%)	49.64 ± 6.48 (16)[a]	51.43 ± 9.77 (14)[a]	72.13 ± 10.07 (12)[a]	35.26 ± 4.97 (11)[a]	35.97 ± 9.09 (14)[a]	53.31 ± 8.10 (11)[a]
Mortality (%)	0 (0/16)[a,*]	12.5 (2/16)[a,b]	25.0 (4/16)[b]	31.3 (5/16)[a,*]	12.5 (2/16)[a]	31.3 (5/16)[a]

Note. Numbers in parentheses are *n*'s. Different letters (a, b) indicate significant differences according to treatment within either the satiation or the half-satiation diet group ($P < 0.05$). Differences in sample size are due to mortality.

[*] Indicates a significant difference according to diet (i.e., mixed satiation vs. half-satiation) between members of the same treatment group ($P < 0.05$).

ment groups used in the swimming trials ($P > 0.14$). The fish were fasted for at least 48 h before swimming and were killed and dissected as above after the completion of swimming trials.

Statistical Analyses

Correlation and regression analyses consisted of Pearson correlations and least-squares regression, and comparisons of regression slopes and elevations were carried out using analysis of covariance and the Tukey test of multiple comparison (Zar 1996). Differences in group variance were evaluated using *F*-tests. Comparisons of group means were conducted using two-sample *t*-tests, paired *t*-tests, *z*-tests of proportion, or one-way ANOVA followed by Tukey tests where appropriate, and all average values are reported as mean ± SE (*n*).

Results

Plasma Cortisol

Only 6% of the treated fish were found to be without implants at the time of dissection. At all sampling periods, cortisol treatment resulted in a threefold increase in plasma cortisol (to ~200 ng mL^{-1}) relative to controls (all $P < 0.05$), whereas sham treatment did not result in an elevation in cortisol at any sampling period (all $P > 0.12$; Fig. 1). The elevated levels are within the physiological range of highly stressed fish (e.g., Barton et al. 1980; Schreck 1981; Holloway et al. 1994; Pagnotta et al. 1994).

Plasma cortisol in control (29.8 ± 9.9 ng mL^{-1}) and sham (12.4 ± 3.6 ng mL^{-1}) fish held in the unmixed tanks of series 1 were significantly lower than those of control (66.2 ± 9.6 ng mL^{-1}) and sham (63.2 ± 10.4 ng mL^{-1}) fish in the mixed tanks of series 2 (all $P < 0.04$; all $n \geq 8$). There were no significant differences in mean plasma cortisol between fish in the satiation and half-satiation tanks in either the control or the sham groups (all $P > 0.22$). There were no differences between the cortisol levels of the sham and control fish in any of the tanks in either series (all $P > 0.11$).

Appetite

In order to evaluate the influence of cortisol on voluntary feeding, independent of competition with fish in other treatment groups, we compared the levels of feeding in the satiation-fed unmixed (series 1) and mixed (series 2) tanks. In both series, cortisol-treated fish exhibited significantly lower appetites than either control or sham-treated fish ($P < 0.001$). Furthermore, mean food consumption in cortisol-treated fish was not significantly different in unmixed versus mixed tanks ($P > 0.15$; Fig. 2). Under unmixed conditions, there were no significant differences in appetite between the control and the sham fish ($P > 0.44$). Sham-treated fish had slightly lower mean consumption rates than did controls in the mixed tanks ($P < 0.02$), although they were much higher than those of the cortisol-treated fish ($P < 0.001$).

Feeding Rank

In order to evaluate the effect of cortisol on the relative feeding performances of individual fish, the mean share of meal (MSM) values were compared between fish in the different mixed treatment groups under conditions of high (satiation) and low (half-satiation) food availability (series 2). There were no significant differences in MSM between the control and the sham fish under either satiation or half-satiation conditions (all $P > 0.17$). However, the cortisol-treated fish showed much lower

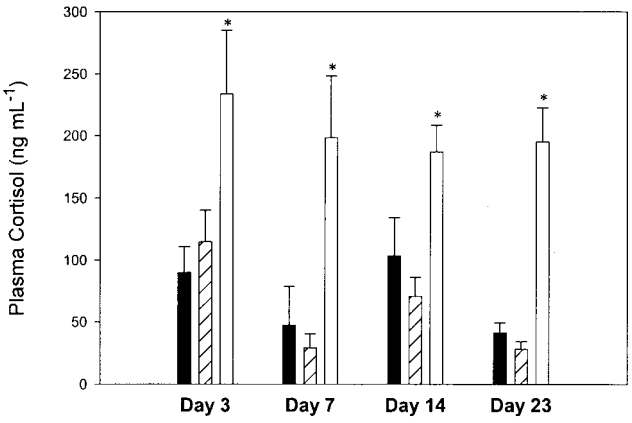

Figure 1. The mean values of plasma cortisol levels in fish from the three treatment groups sampled on days 3, 7, 14, and 23. Sample sizes were as follows: control (*solid bars*): $n = 4, 6, 10, 29$; sham (*hatched bars*): $n = 5, 6, 5, 26$; cortisol (*empty bars*): $n = 6, 6, 9, 7$. Differences in sample size are due primarily to mortality, although some are due to problems with sampling small fish. The asterisk indicates values significantly different from those of the control fish ($P < 0.05$). Error bars represent SE.

MSM values under both feeding regimes (all $P < 0.001$; Fig. 3). The mean MSM values of the control fish in the satiation versus half-satiation mixed tanks were not significantly different ($P > 0.05$; Fig. 3). The MSM of the sham- and cortisol-treated fish in series 2 did not differ significantly when grouped according to ration (all $P > 0.61$).

Because the average MSM of each tank must be the same (by definition), there were no differences in MSM among the unmixed treatments of series 1. However, the cortisol-treated fish showed significantly higher variance in MSM than both the control and the sham fish in this series (all $P < 0.03$). There was no significant difference in variance in MSM between the control and the sham fish in this series ($P > 0.05$).

Coefficient of variation (CV), which describes the day-to-day variability in feeding of each individual fish, is another commonly used indicator of feeding rank, with subordinate fish showing more variable feeding patterns (i.e., higher CV). In the unmixed tanks of series 1, the cortisol-treated fish showed a significantly higher mean CV over the three X-rayed meals than did the sham-treated (but not the control) fish ($P < 0.03$). There was no significant difference between the mean CV of the sham and the control fish in this series ($P > 0.64$; Table 1). There were also no significant differences in mean CV in any of the mixed tanks (satiation and half-satiation) of series 2, either according to treatment or ration (all $P > 0.11$; Table 2).

There were no significant differences in mean fin score between any of the unmixed treatment groups in series 1 ($P > 0.09$; Fig. 4). In both the satiation and the half-satiation groups of series 2, however, the cortisol-treated fish showed significantly lower fin scores (i.e., more damage) than both the control and the sham fish (all $P < 0.002$; Fig. 4). There were no significant differences in fin score between the control and the sham fish in any of the tanks (all $P > 0.23$). Neither ration level nor mixing of fish had any effect on the fin scores of the fish within any of the treatment groups (all $P > 0.22$).

Growth Rate

Both treatment and ration were found to have a significant effect on the growth rates of individual fish (Fig. 5). In all cases, the cortisol-treated fish showed significantly lower mean growth rates than both the control and the sham groups (all $P < 0.0001$). There were also significant differences in mean SGR between the control and the cortisol (but not the sham) fish in the satiation group of series 2 compared to those in the half-satiation group (all $P < 0.05$; Fig. 5*A*).

Fish from the different treatment groups were paired according to similar food consumptions (to within 0.1% BM meal^{-1}), and differences in SGR were evaluated using paired *t*-tests. Cortisol fish showed significantly lower growth rates than both the control and the sham fish even in the cases where their food consumption was nearly identical (all $P < 0.001$). No significant difference in SGR was found between control and sham fish with similar food consumption ($P > 0.34$). In all cases, fish in the cortisol treatment group showed significantly

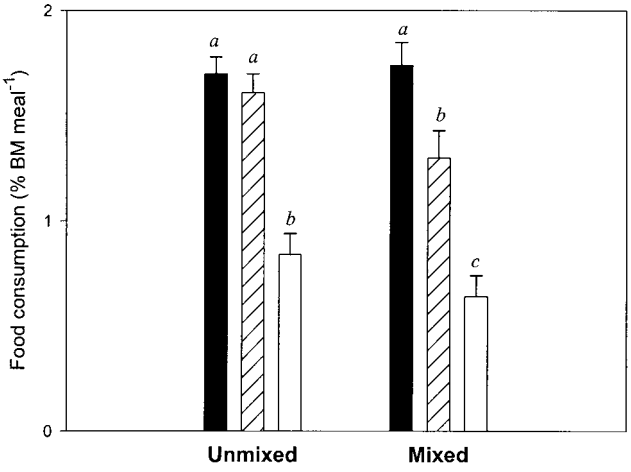

Figure 2. The average individual food consumptions ("appetites" in % body mass [*BM*] per meal) of the satiation-fed control (*solid bars*), sham (*hatched bars*), and cortisol (*empty bars*) fish from the unmixed (series 1) and mixed tanks (series 2). Different letters (*a*, *b*, *c*) indicate significant differences between treatments within the mixed or unmixed tanks ($P < 0.05$). There were no significant differences in mean food consumption between comparable treatment groups of unmixed and mixed tanks (all $P > 0.15$). All sample sizes $\geq n$ reported for corresponding groups in Tables 1 and 2. Error bars represent SE.

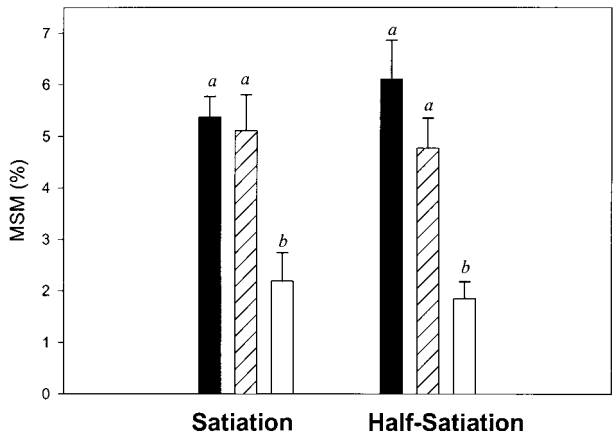

Figure 3. The average values of mean share of meal (*MSM* in percentage) for fish in the satiation and half-satiation tanks from the control (*solid bars*), sham (*hatched bars*), and cortisol (*empty bars*) treatment groups of series 2. Different letters (*a*, *b*) indicate significant differences between treatments within the diet groups ($P < 0.05$). There were no significant differences in MSM between comparable treatment groups of satiation and half-satiation-fed tanks (all $P > 0.05$). All sample sizes $\geq n$ reported for corresponding groups in Tables 1 and 2. Error bars represent SE.

lower conversion efficiencies than the control and the sham fish (all $P < 0.0001$) and in some cases showed negative mean conversion efficiencies (Fig. 5*B*). There were no significant differences in conversion efficiencies between the control and the sham fish in any of the tanks (all $P > 0.51$). In addition, half-satiation-fed fish in all three treatment groups had significantly lower conversion efficiencies than their counterparts in the satiation-fed tanks (all $P < 0.03$; Fig. 5*B*).

In each of the unmixed tanks, SGR was positively correlated with appetite (all $r \geq 0.6042$; all $P < 0.002$). A significant correlation between appetite and SGR was found only among sham-treated fish in the satiation-fed mixed tanks ($r = 0.5752$; $P < 0.04$). Among the mixed, half-satiation-fed fish, there were positive correlations between SGR and food consumption in the sham ($r = 0.7519$; $P < 0.002$) and cortisol treatment groups ($r = 0.8719$; $P < 0.001$), but not among control fish ($P > 0.15$).

When the data for all fish were pooled together, a positive correlation was found between SGR and food consumption in all three treatment groups (all $r \geq 0.2828$; all $P < 0.05$; Fig. 6). The slope of the regression line for this relationship was significantly higher in the sham fish as compared to the control fish ($P < 0.005$), but there were no significant differences in slope between the cortisol-treated fish and either the control or the sham fish (all $P > 0.20$). Notably, the control and sham regressions both showed significantly higher elevations than that of the cortisol fish (all $P < 0.001$).

Among the cortisol fish, SGR was negatively correlated with CV in both the satiation ($r = -0.7189$; $P < 0.001$) and half-satiation ($r = -0.7994$; $P < 0.001$) mixed tanks, but not in the unmixed tank ($P > 0.13$). With the exception of the satiation-fed sham fish in series 2 ($r = -0.7311$; $P < 0.001$), no such correlations existed in the control or the sham fish in either series (all $P > 0.06$).

As with SGR, the cortisol-treated fish of both series 1 and 2 showed significantly lower condition factors than the control and sham fish (all $P < 0.001$; Tables 1 and 2). The satiation-fed control fish of series 2 demonstrated significantly higher condition factors than the half-satiation-fed control fish ($P < 0.01$), but no other such ration-related differences were found (Table 2).

Mortality

In series 1 none of the control fish died, while only one individual in the sham group died. Cortisol-treated fish exhibited significantly higher levels of mortality in this series (Table 1). Similar trends were seen in series 2, with higher levels of mortality observed in all treatment groups under conditions of restricted ration (Table 2). Some of the mortality was attributed to aggression, as evidenced by large amounts of fin damage noticeable before the deaths of the fish.

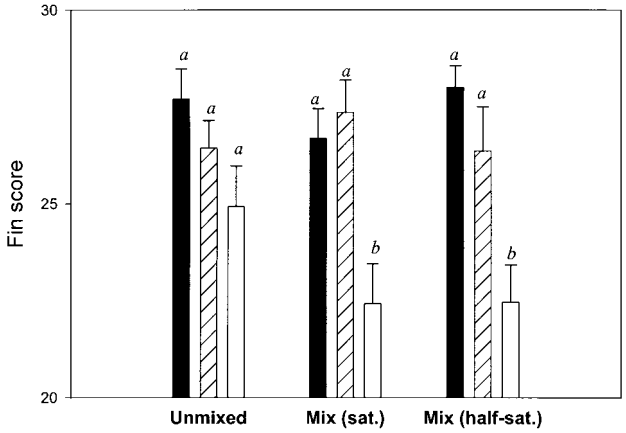

Figure 4. The average individual fin scores of the control (*solid bars*), sham (*hatched bars*), and cortisol (*empty bars*) fish from the unmixed (series 1) and mixed tanks (series 2). Different letters (*a*, *b*) indicate significant differences between treatments within the mixed or unmixed tanks ($P < 0.05$). There were no significant differences in mean fin score between comparable treatment groups of the unmixed, mixed satiation, or mixed half-satiation tanks (all $P > 0.10$). All sample sizes $\geq n$ reported for corresponding groups in Tables 1 and 2. Error bars represent SE.

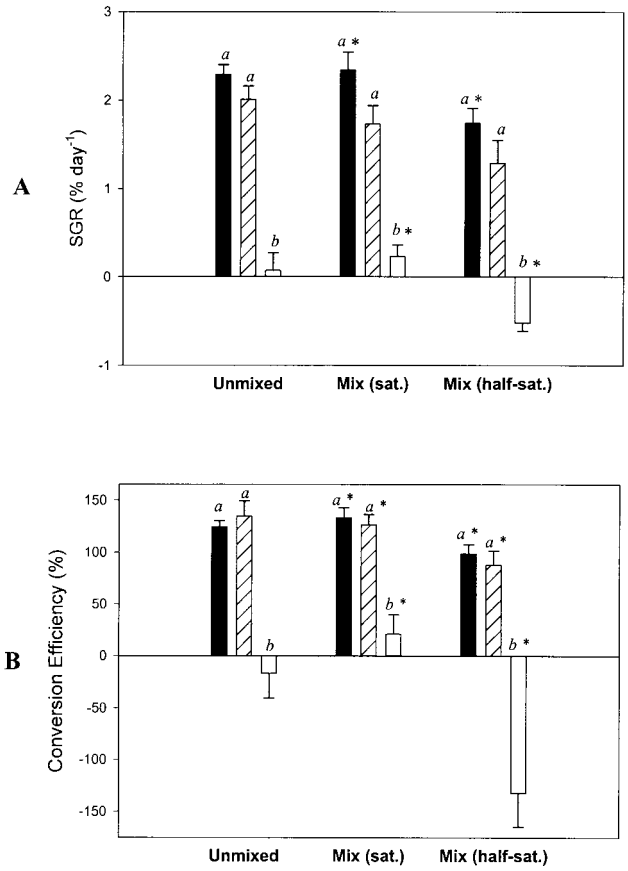

Figure 5. The average (*A*) specific growth rates (*SGR* in % d^{-1}) and (*B*) conversion efficiencies (%) of fish in the unmixed (series 1), mixed satiation (series 2), and mixed half-satiation (series 2) tanks from the control (*solid bars*), sham (*hatched bars*), and cortisol (*empty bars*) treatment groups. Different letters (*a*, *b*) indicate significant differences according to treatment within the unmixed or mixed diet groups ($P < 0.05$). An asterisk indicates a significant difference according to diet (i.e., mixed satiation vs. half-satiation) between members of the same treatment group ($P < 0.05$). All sample sizes $\geq n$ reported for corresponding groups in Tables 1 and 2. Error bars represent SE.

Swimming Performance

There were no significant differences among the mean U_{crit} values (in BL s^{-1}) of the three mixed swimming groups ($P > 0.24$). The swimming performances of these fish were grouped according to treatment (nine fish per treatment), and no significant differences in mean U_{crit} were found among fish in the three treatment groups (control: 4.89 ± 0.13 BL s^{-1}, sham: 5.25 ± 0.13 BL s^{-1}, cortisol: 5.06 ± 0.31 BL s^{-1}; $P > 0.47$).

Discussion

Elevated levels of plasma cortisol significantly reduce the appetites of individual rainbow trout, even when these fish are not in competition with untreated conspecifics and are fed to satiation (Fig. 2). This finding is consistent with those of previous studies on channel catfish (*Ictalurus punctatus*; Davis et al. 1985) and rainbow trout (Barton et al. 1987). Cortisol is known to elevate the levels of glucose and/or amino acids in the blood of fish (reviewed by Andersen et al. [1991]), which may represent the proximate stimuli acting to suppress appetite. When fish are acutely stressed, such as by the presence of predators, a delay in feeding behaviour may be of substantial adaptive significance.

The cortisol-treated fish in the mixed tanks of series 2 consumed much less food than the control and sham fish, both in terms of weight-specific consumption (Fig. 2) and mean share of meal (Fig. 3). The portion of the meal obtained by a fish is generally used as an indicator of position in the feeding hierarchy, with dominant fish typically receiving a larger fraction of the meal (e.g., McCarthy et al. 1992, 1993). It is possible that fish with elevated cortisol levels assume a position of subordinance and that the higher MSM obtained by control and sham fish reflects this fact. However, the voluntary appetites of cortisol-treated fish were lower even in the absence of competition from untreated fish (Fig. 2). Furthermore, a decrease in ration, which is expected to impose a more rigid dominance hierarchy (McCarthy et al. 1992), did not affect the mean share

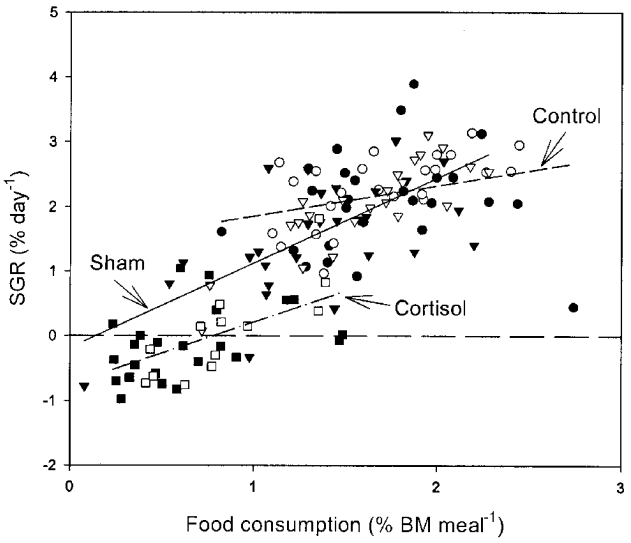

Figure 6. The relationships between mean food consumption (in % body mass [*BM*] per meal) and specific growth rate (*SGR* in % d^{-1}) for control (*circles*), sham (*triangles*), and cortisol (*squares*) fish. Open symbols represent data from fish in the unmixed tanks of series 1, while filled symbols represent data from fish in the mixed tanks of series 2. Regressions were as follows: control (*dashed line*): $r^2 = 0.0800$, $P < 0.05$, $n = 51$, $y = 0.47x - 0.19$; sham (*solid line*): $r^2 = 0.5291$, $P < 0.0001$, $n = 51$, $y = 1.31x - 0.19$; cortisol (*dotted dashed line*): $r^2 = 0.3412$, $P < 0.0001$, $n = 36$, $y = 0.95x - 0.74$. Differences in sample size are due to mortality.

of meal obtained by fish treated with cortisol (Fig. 3). It therefore seems more likely that control fish receive more food in the mixed tanks simply because the reduced appetites of the cortisol-treated fish make them less likely to compete for food.

The variability in the amount of food consumed from meal to meal, measured as CV, is another method used to quantify the ranks of individual fish (McCarthy et al. 1992, 1993). In the unmixed tanks of series 1, the cortisol fish had significantly higher mean CV values than those treated with sham implants (Table 1). They also exhibited greater variance in MSM among individuals. This suggests that even when not mixed with unstressed fish, those treated with cortisol show more variable feeding patterns both within and among individuals. This indicates that more variable patterns of feeding result not only from the superior competitive ability of dominant fish but also simply as a by-product of elevated cortisol levels. In the mixed tanks of series 2, there were no significant differences in mean CV between the cortisol and the sham or the control fish (Table 2). In addition, there was no significant difference in mean CV between cortisol fish in the unmixed tanks and those held with untreated fish, indicating that the presence of "unstressed" fish did not lead to more variable feeding patterns among individuals with chronic cortisol elevation. Once again, this suggests that stressed fish may acquire less food and show more variable feeding patterns than other fish, primarily due to the effects of cortisol and not necessarily as a result of obligate subordination.

Fin score was also used as a potential measure of dominance and aggression. There were no significant differences in mean fin score between any of the treatment groups in the unmixed tanks of series 1 (Table 1), but in series 2, cortisol-treated fish showed lower fin scores (i.e., had suffered more damage) than members of the other treatment groups (Table 2). There were no significant differences in average fin score between the cortisol-treated fish of series 1 and those of series 2, indicating that fish with elevated cortisol did not necessarily experience more aggression when mixed with untreated fish.

Chronic elevation of cortisol also resulted in significantly lower growth rates regardless of holding conditions or group ration (Fig. 4). In addition, individual cortisol-treated fish grew more slowly than control and sham fish even in the cases when they received the same amount of food. This is consistent with other studies on cortisol-treated fish (Davis et al. 1985; Barton et al. 1987), although this phenomenon previously has been reported only for whole groups of fish rather than individuals. This depression of growth undoubtedly results from two primary factors: reduced food intake and lower conversion efficiency, both of which we found (Figs. 5*B*, 6). The latter reflects a higher cost of living at any given food intake, as shown by the significant differences in elevation of the regression lines in Figure 6. This higher cost of living may result from a combination of several interacting effects, including a chronic increase in metabolic rate associated with the stress response (Metcalfe et al. 1995), reduced absorption of food material in the gut (Barton et al. 1987), the promotion of cellular atrophy or the delay of cell proliferation in certain tissue types (Wendelaar Bonga 1997), and/or increased levels of protein and lipid catabolism (Davis et al. 1985). The relevance of this last phenomenon is further emphasized by the significant reduction in condition factors seen in cortisol-treated fish (Tables 1 and 2; Barton et al. 1987).

In the unmixed tanks of series 1, there were significant correlations between appetite and SGR that were sometimes absent in series 2. This result suggests that when fish of different treatments are mixed together, factors other than food intake (e.g., the effects of social interactions) may play a role in determining growth. In this regard, the higher plasma cortisol levels of both sham and control fish in the mixed tanks of series 2, relative to the unmixed levels of series 1, may be an important complicating factor.

There was no correlation between SGR and indicators of rank such as CV when cortisol-treated fish were held alone (series 1). However, when the cortisol fish were kept in contact with untreated fish (series 2), they showed a highly significant negative correlation between SGR and CV. It may be, therefore, that the rank of cortisol-treated fish relative to others within their own treatment group may be an important determinant of growth rate when these fish are forced to interact with "unstressed" fish.

Despite its deleterious effects on feeding behaviour and growth reported here, as well those on immunity and reproduction reported elsewhere (Pickering et al. 1987; Pickering and Pottinger 1989; Fox et al. 1997; reviewed by Wendelaar Bonga [1997]), chronic cortisol elevation had no effect on the swimming performances of individual trout. Similarly, handling stress, which is well known to elevate cortisol levels, recently has been shown to have no effect on the U_{crit} values of juvenile rainbow trout (Peake et al. 1997). In our study, any negative effects of cortisol on general health may have been compensated by reduced investment in growth, which has been shown to positively influence swimming performance in several species (Kolok and Oris 1995; Farrell et al. 1997; Gregory and Wood 1998). In addition, neither muscle glycogen metabolism (Soengas et al. 1992) nor haematocrit (Sørensen and Weber 1995), both potential determinants of swimming performance (Gallaugher et al. 1995; Gregory and Wood 1998), are negatively influenced by plasma cortisol levels. In fact, haematocrit may even become elevated by chronic cortisol treatment (Barton et al. 1987). Given that swimming (i.e., escape) is the predominant behavioural reaction to stressors in fish, it seems appropriate that the primary stress hormone not impair this strategy (Peake et al. 1997).

The results of this study reinforce the view that chronic plasma cortisol elevation, such as that induced by stressors like pollution and overcrowding, can have serious deleterious effects on the growth, physical condition, feeding behaviour, and survivability of individual fish, although swimming performance

appears to be unaffected by chronic stress. The ability of fish to compete with conspecifics may also be influenced by high levels of stress, with stressed fish consuming less food, growing more slowly, and perhaps suffering more intense intraspecific aggression relative to unstressed fish. More important, the elevation of cortisol in itself, independent of any other effects of chronic stress, may negatively influence individual behaviour and would be independent of the actions of dominant or unstressed fish. This phenomenon may have been interpreted mistakenly in previous studies as a sign of subordinate rank. In any event, our study emphasizes the value of investigating the effects of stress on behaviour as well as on physiology and the importance of exploiting, rather than suppressing, the previously underutilized resource of individual variation.

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Literature Cited

Abbott J.C. and L.M. Dill. 1989. The relative growth of dominant and subordinate juvenile steelhead trout (*Salmo gairdneri*) fed equal rations. Behaviour 108:104–113.

Alsop D.H. and C.M. Wood. 1997. The interactive effects of feeding and exercise on oxygen consumption, swimming performance, and protein usage in juvenile rainbow trout (*Oncorhynchus mykiss*). J Exp Biol 200:2337–2346.

Andersen D.E., S.D. Reid, T.W. Moon, and S.F. Perry. 1991. Metabolic effects associated with chronically elevated cortisol in rainbow trout (*Oncorhynchus mykiss*). Can J Fish Aquat Sci 48:1811–1817.

Barton B.A., R.E. Peter, and C.R. Paulencu. 1980. Plasma cortisol levels of fingerling rainbow trout (*Salmo gairdneri*) at rest, and subjected to handling, confinement, transport, and stocking. Can J Fish Aquat Sci 37:805–811.

Barton B.A., C.B. Schreck, and L.D. Barton. 1987. Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. Dis Aquat Org 2:173–185.

Beitinger T.L. and R.W. McCauley. 1990. Whole-animal physiological processes for the assessment of stress in fishes. J Gt Lakes Res 16:542–575.

Boddingius J. 1976. The influence of social rank on adenohypophysial cell activity in *Salmo irideus*. Cell Tissue Res 170:383–414.

Brett J.R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J Fish Res Board Can 21:1183–1225.

Davis K.B., P. Torrance, N.C. Parker, and M.A. Suttle. 1985. Growth, body composition and hepatic tyrosine aminotransferase activity in cortisol-fed channel catfish, *Ictalurus punctatus* Rafinesque. J Fish Biol 27:177–184.

Ejike C. and C.B. Schreck. 1980. Stress and social hierarchy rank in coho salmon. Trans Am Fish Soc 109:423–426.

Farrell A.P., W. Bennett, and R.H. Devlin. 1997. Growth-enhanced transgenic salmon can be inferior swimmers. Can J Zool 75:335–337.

Fox H.E., S.A. White, M.H.F. Kao, and R.D. Fernald. 1997. Stress and dominance in a social fish. J Neurosci 17: 6463–6469.

Gallaugher P., H. Thorarensen, and A.P. Farrell. 1995. Hematocrit in oxygen transport and swimming in rainbow trout (*Oncorhynchus mykiss*). Respir Physiol 102:279–292.

Gregory T.R. and C.M. Wood. 1998. Individual variation and interrelationships between swimming performance, growth rate, and feeding in juvenile rainbow trout (*Oncorhynchus mykiss*). Can J Fish Aquat Sci 55:1583–1590.

Holloway A.C., P.K. Reddy, M.A. Sheridan, and J.F. Leatherland. 1994. Diurnal rhythms of plasma growth hormone, somatostatin, thyroid hormones, cortisol and glucose concentrations in rainbow trout, *Oncorhynchus mykiss*, during progressive food deprivation. Biol Rhythm Res 25:415–432.

Kolok A.S. and J.T. Oris. 1995. The relationship between specific growth rate and swimming performance in male fathead minnows (*Pimephales promelas*). Can J Zool 73:2165–2167.

Li H.W. and R.W. Brocksen. 1977. Approaches to the analysis of energetic costs of intraspecific competition for space by rainbow trout (*Salmo gairdneri*). J Fish Biol 11:329–341.

McCarthy I.D., C.G. Carter, and D.F. Houlihan. 1992. The effect of hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Biol 41: 257–263.

McCarthy I.D., D.F. Houlihan, C.G. Carter, and K. Moutou. 1993. Variation in individual food consumption rates of fish and its implications for the study of fish nutrition and physiology. Proc Nutr Soc 52:427–436.

McLeese J.M., J. Johnsson, F.M. Huntley, W.C. Clarke, and M. Weisbart. 1994. Seasonal changes in osmoregulation, cortisol, and cortisol receptor activity in the gills of parr/smolt of steelhead trout and steelhead-rainbow trout hybrids, *Oncorhynchus mykiss*. Gen Comp Endocrinol 93:103–113.

Metcalfe N.B. 1986. Intraspecific variation in competitive ability and food intake in salmonids: consequences for energy budgets and growth rates. J Fish Biol 28:525–531.

Metcalfe N.B., A.C. Taylor, and J.E. Thorpe. 1995. Metabolic

rate, social status and life-history strategies in Atlantic Salmon. Anim Behav 49:431–436.

Metcalfe N.B., P.J. Wright, and J.E. Thorpe. 1992. Relationships between social status, otolith size at first feeding and subsequent growth in Atlantic salmon (*Salmo salar*). J Anim Ecol 61:585–589.

Noakes D.L.G. and J.F. Leatherland. 1977. Social dominance and interrenal cell activity in rainbow trout, *Salmo gairdneri* (Pisces, Salmonidae). Environ Biol Fishes 2:131–136.

Pagnotta A., L. Brooks, and L. Milligan. 1994. The potential regulatory roles of cortisol in recovery from exhaustive exercise in rainbow trout. Can J Zool 72:2136–2146.

Peake S., C. Barth, and R.S. McKinley. 1997. Effect of recovery parameters on critical swimming speed of juvenile rainbow trout (*Oncorhynchus mykiss*). Can J Zool 75:1724–1727.

Pickering A.D. and T.G. Pottinger. 1989. Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. Fish Physiol Biochem 7:253–258.

Pickering A.D., T.G. Pottinger, J. Carragher, and P. Sumpter. 1987. The effects of chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, *Salmo trutta* L. Gen Comp Endocrinol 68:249–259.

Pottinger T.G. and A.D. Pickering. 1992. The influence of social interaction on the acclimation of rainbow trout, *Oncorhynchus mykiss* (Walbaum) to chronic stress. J Fish Biol 41: 435–447.

Reddy P.K. and J.F. Leatherland. 1995. Influence of the combination of time of feeding and ration level on the diurnal hormone rhythms in rainbow trout. Fish Physiol Biochem 14:25–36.

Schreck C. 1981. Stress and compensation in teleostoan fishes: responses to social and physical factors. Pp. 295–321 in A.D. Pickering, ed. Stress and Fish. Academic Press, London.

Soengas J.L., P. Rey, G. Rozas, M.D. Anrés, and M. Aldegunde. 1992. Effects of cortisol and thyroid hormone treatment on the glycogen metabolism of selected tissues of domesticated rainbow trout, *Oncorhynchus mykiss*. Aquaculture 101: 317–328.

Sørensen B. and R.E. Weber. 1995. Effects of oxygenation and the stress hormones adrenaline and cortisol on the viscosity of blood from the trout *Oncorhynchus mykiss*. J Exp Biol 198: 953–959.

Specker J.L., D.M. Portesi, S.C. Cornell, and P.A. Veillete. 1994. Methodology for implanting cortisol in Atlantic salmon and effects of chronically elevated cortisol on osmoregulatory physiology. Aquaculture 121:181–193.

van der Boon J., G.E.E.J.M. van den Thillart, and A.D.F. Addink. 1991. The effects of cortisol administration on intermediary metabolism in teleost fish. Comp Biochem Physiol A 100:47–53.

Wendelaar Bonga S.E. 1997. The stress response in fish. Physiol Rev 77:591–625.

Wendelaar Bonga S.E., P.H.M. Balm, and A.E. Lamers. 1995. The involvement of ACTH and MSH in the stress response in teleost fish. Neth J Zool 45:103–106.

Zar J.H. 1996. Biostatistical Analysis. 3d ed. Simon & Schuster, Upper Saddle River, N.J.