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# Repeated acute stress reduces growth rate of Atlantic salmon parr and alters plasma levels of growth hormone, insulin-like growth factor I and cortisol

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**Abstract**

Atlantic salmon (*Salmo salar*) parr were subjected to acute handling stresses and growth-monitored for at least 30 days. In fish stressed twice daily, growth rate in weight was 61% lower than controls after 11 days (1.00 vs. 2.57% $day^{-1}$) and over a 30 day period it was 50% lower than controls (1.53 vs. 3.07% $day^{-1}$). In fish stressed once daily, growth rate was 18% lower than controls after 10 days (2.17 vs. 2.63% $day^{-1}$) and over a 30-day period it was 34% lower than controls (1.71 vs. 2.59% $day^{-1}$). In fish stressed once daily, food consumption was reduced by 62% and 37% after 17 and 37 days, respectively. At the end of 40 days of acute stress once daily, control and stressed fish were sampled 1 h prior to, 3 and 7 h after a stress event. Plasma growth hormone levels were significantly higher in the stressed group than in the controls prior to and 7 h after stress. Plasma insulin-like growth factor I (IGF-I) levels were higher in the stressed group only 3 and 7 h after stress. Plasma cortisol levels were lower in the stressed group prior to and 3 h after stress. The results indicate that acute stressors decrease growth of Atlantic salmon parr, with increasing frequency of stress having a more rapid and greater effect. 

*Keywords:* Atlantic salmon; Growth rate; IGF-I; Cortisol; Handling stress

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## 1. Introduction

It is widely accepted that stress can reduce growth in fish (Pickering, 1990). Documentation of these effects, however, is limited and provides conflicting results. This may be due in part to the wide variety of disturbances incorporated in the term stress. Crowding, which includes several stressors as well as physical differences in the rearing environment, has been shown to decrease growth in several salmonid species (Wedemeyer, 1976; Fagerlund et al., 1981; Pickering and Stewart, 1984). Surprisingly, there is little information on the effect of acute stressors like handling on fish growth. Pickering et al. (1982) observed that a single handling stress of 2-year-old brown trout (*Salmo trutta*) resulted in several physiological changes lasting up to 2 weeks, but did not affect growth rate. Barton et al. (1987) found that a daily acute handling stress for 10 weeks did not decrease growth of juvenile rainbow trout (*Oncorhynchus mykiss*).

There are a several pathways through which stress may affect growth. In reviewing the endocrine pathways affected by stress, Pickering (1993) suggests that most of the anabolic and catabolic hormones are involved in regulating stress-induced alterations in growth. Cortisol levels rise following acute and chronic stress and this has been strongly linked to higher plasma glucose and energy mobilization following stress (Schreck, 1981; Gamperl et al., 1994). Cortisol is probably important for normal growth, but increases above 'resting' levels that occur following stress may reduce growth (Schreck, 1993). Cortisol administration reduces growth in rainbow trout and channel catfish (*Ictalurus punctatus*)(Davis et al., 1985; Barton et al., 1987), providing a direct link between this hormone and stress-related changes in growth. Growth hormone, in concert with insulin-like growth factor I (IGF-I) is the major endocrine promoter of growth in salmonids as in other vertebrates (for review, see Björnsson, 1996). The present knowledge on the effects of stress on this growth-promoting endocrine system in salmonids is limited to studies on rainbow trout. Acute stress increases plasma growth hormone levels in the first 30 min (Kakisawa et al., 1995), but decreases it over several hours (Farbridge and Leatherland, 1992). Long-term chronic stress appears to suppress plasma growth hormone levels (Pickering et al., 1991). When rainbow trout have been chased or forced to swim to exhaustion, growth hormone levels are at preexercise levels (Barrett and McKeown, 1989; Kakisawa et al., 1995), whereas they are elevated during sustained exercise (Barrett and McKeown, 1989). Only a few studies have examined the response of growth hormone to stress under conditions when growth is known to be altered (Pickering et al., 1991; Takahashi et al., 1991), and no data on the response of IGF-I to stress in fish is available.

Atlantic salmon (*Salmo salar*) are of major importance in aquaculture, sea ranching and restoration efforts. Often, the juveniles are of sea-run (undomesticated) parentage and thus the degree of domestication is much less than for rainbow trout in aquaculture. As salmon are usually released into the wild or net-pens as smolts, it is the juvenile stage which is at the greatest risk for exposure to stressors including handling, crowding, transportation and predation. Domesticated and wild salmonids are known to respond differently to stressors, such as predator presence (Johnsson et al., 1996). The aim of the present study was thus to elucidate how stress affects growth performance of juvenile Atlantic salmon of undomesticated parentage, and how this relates to food consumption

as well as the endocrine regulation of growth. For this purpose, two series of experiments were carried out on juvenile Atlantic salmon, where the effects of repeated handling stress on growth rate, food consumption and plasma levels of cortisol, growth hormone and IGF-I were monitored.

## 2. Materials and methods

### *2.1. Rearing conditions*

Juvenile (0 + ) Atlantic salmon of Connecticut River strain, sea-run parentage were transferred from the White River National Fish Hatchery (Bethel, VT) to the Conte Anadromous Fish Research Center (Turners Falls, MA) several months prior to initiation of the experiments and reared in 1.5 m diameter tanks. The fish were fed #4 feed (Zeigler Bros., Gardners, PA) to satiation three times daily. The experiments were conducted in isolated, side-by-side rooms with identical 1 m tanks with one tank for each treatment. Water inflow, aeration and overhead lighting were carefully matched. Overhead lighting was supplied by overhead fluorescent lights providing natural day-length spectrum (500 lx at tank surface), and photoperiod was adjusted twice weekly to achieve a simulated natural photoperiod. The top of each tank was partially covered so that fish could not be disturbed by the person restocking the automatic feeders. There was no entry into these rooms except for restocking feeders and administration of the stress protocol. The animals were maintained in 1 m diameter tanks at $18 \pm 0.2$°C in experiment 1 and $16 \pm 0.2$°C in experiment 2 with a flow rate of 4 l $min^{-1}$ with water supplied from a common header tank. Fish were acclimated to tank conditions for at least 2 weeks before experiments began. The daily ration for each experiment was based on hatchery tables designed to give maximum growth for a given body size and temperature. Uneaten food was observed in each group in each experiment, indicating that the ration levels used were at or near the maximum.

### *2.2. Experiment 1 (twice daily stress)*

Parr (4 months post-hatch) were randomly separated into two groups of 20 and placed in tanks in separate rooms, one for the experimental group and one for the controls. Feed was administered at 4.9% body weight $day^{-1}$ by automatic feeders set to run from 06:00 to 17:30 h with two 1 h breaks beginning at 08:00 and 15:00 h to accommodate two separate stress events each day. Stress events conducted on the experimental group consisted of either chasing, crowding or draining the tank. Chasing entailed rapidly and randomly chasing the fish around the tank with a length of PVC pipe for 15 min. Crowding involved netting all of the fish and placing them in a cylindrical plastic mesh enclosure (8 l) within their rearing tank for 15 min. Draining the tank occurred over approximately 5 min until the dorsal fins of the fish were exposed. The tank was then allowed to refill at 4 l $min^{-1}$. The fish in the control tanks received no such stressors. Inventories on growth parameters were conducted on days 0 (August 3), 11, 20, and 30 during which the fish were anesthetized (87.5 mg $l^{-1}$ MS-222) and weight in 0.1 g and

fork length in millimeters were recorded. No stress events were conducted on these days.

### 2.3. Experiment 2 (once daily stress)

Parr (6 months post-hatch) were randomly separated into two groups of 33–34 and placed in tanks in separate rooms. Water was supplied from a common header tank. Feed was delivered at 4.1% body weight $day^{-1}$ using automatic feeders set to run from 06:00 to 17:30 h with a 2 h break each day between 09:00 and 11:00 h to accommodate one single stress event. The daily stress event occurred at 10:00 h and followed the same stressor protocols as in experiment 1. Fish were anesthetized (87.5 mg $l^{-1}$ MS-222) and weight in 0.1 g and fork length in millimeters were recorded on days 0 (September 27), 10, 21, 30, and 40. As above, stress events were not conducted on these days.

Food consumption was assessed twice during experiment 2 (days 17 and 37) by hand-feeding to satiation (until 1–2 pellets were left uneaten on the bottom of the tank) at hourly intervals throughout the day. Weight gain for that day was estimated from the growth rate for that interval. The percentage dry weight of food and Atlantic salmon parr was determined to be 94.4% and 22%, respectively, by drying to a constant weight at 60°C for 24 h. These values were used to convert wet to dry weight food consumptions.

On day 42 at 08:00 h (0 h) 10–11 fish were rapidly removed from each tank with no handling and minimal disturbance to the remaining fish. The removed fish were anesthetized (200 mg $l^{-1}$ MS-222), blood samples were drawn from the caudal vessels into heparinized syringes, and placed on ice. Blood samples were taken in 8 min or less to ensure that basal cortisol levels could be measured (Biron and Benfey, 1994). Plasma was separated by centrifugation and stored at $-80$°C for later analysis of hormone concentrations. One hour after the initial sampling, the remaining fish in the experimental group received a crowding stress and the control group was not stressed. Two additional samplings followed at 4 h ($n = 11$–12) and 8 h ($n = 10$–11) after the initial sampling (3 and 7 h after stress).

### 2.4. Hormone analyses

Plasma cortisol levels were measured by a validated direct enzyme immunoassay as outlined in Carey and McCormick (1997). Sensitivity as defined by the dose–response curve was 1 to 400 ng/ml. The lower detection limit was 0.30 ng/ml. Using a pooled plasma sample, the average intraassay variation was 5.5% ($n = 10$) and the average interassay variation was 8.8% ($n = 10$). Plasma growth hormone levels were measured by a radioimmunoassay validated for Atlantic salmon (Björnsson et al., 1994). Typical measuring range was 0.1 to 50 ng/ml with $ED_{50} = 2.2$ ng/ml and an average intraassay and interassay variation of 5.4% ($n = 9$) and 3.9% ($n = 9$), respectively. Plasma IGF-I was measured by homologous radioimmunoassay as described by Moriyama et al. (1994). Sensitivity as defined by the dose–response curve was 1 ng/ml to 250 ng/ml. The lower detection limit was 0.20 ng/ml. Using a pooled plasma sample, the average intraassay variation was 7% ($n = 5$) and the average interassay variation was 6.5% ($n = 5$).

## 3. Calculations and statistics

Condition factor was calculated as $(\text{wt} \times l^{-3}) \times 100$, where wt = body weight in grams and $l$ = fork length in cm. Instantaneous growth rate in body weight (% day$^{-1}$) was calculated as $((\ln \text{wt}_2 - \ln \text{wt}_1) \times (t_2 - t_1)^{-1}) \times 100$; wt is the mean weight at a given time interval and $t$ = time in days. Instantaneous growth rate in length (% day$^{-1}$) was calculated as $((\ln l_2 - \ln l_1) \times (t_2 - t_1)^{-1}) \times 100$; $l$ is the mean length at a given time interval. Two-way analysis of variance (ANOVA) was used to compare control and stressed groups over time. If a significant effect of stress was detected ($P < 0.05$), control and stress groups were compared at each time point (one-way ANOVA, $P < 0.05$). Due to the large variation in plasma cortisol levels, one- and two-way ANOVA's were conducted on ranks (nonparametric test).

## 4. Results

### *4.1. Experiment 1 (twice daily acute stress)*

Length and weight increased throughout the experiment in both groups, with much more rapid increases in the control group (Fig. 1). Statistically significant differences in length and weight were observed after 11 days ($P < 0.05$). Growth rate in wet body weight of controls increased from 2.57% day$^{-1}$ in the first 11 days to 3.38 and 3.34% day$^{-1}$ in the next two growth intervals (Fig. 1). After 11 days, growth rate of the stressed group was 1.00% day$^{-1}$, remained about the same after 20 days (1.13% day$^{-1}$), and recovered slightly to 2.45% day$^{-1}$ after 30 days. In the three sequential measurement intervals, growth rate of stressed fish was 61, 66 and 27% lower than controls. Growth rate in length of stressed fish was 46, 55, and 27% lower than controls in the three sequential measurement intervals.

Condition factor of controls increased during the experiment from 1.13 to 1.25, whereas condition factor of stressed fish decreased from 1.13 to 1.05 (Fig. 1). Condition factor of stressed fish was significantly lower than that of controls after 11 days and remained significantly lower through the remainder of the study.

### *4.2. Experiment 2 (once daily acute stress)*

Length and weight increased throughout the experiment in both groups, with more rapid increases in the control group (Fig. 2). Statistically significant differences in length and weight were observed after 40 and 30 days, respectively. Growth rate of body weight remained relatively constant in the control group over the 40 day period (2.39–2.72% day$^{-1}$; Fig. 2). After 10 days growth rate of the stressed group was 2.17% day$^{-1}$, decreased progressively to 1.26% day$^{-1}$ after 30 days and recovered slightly to 1.52% day$^{-1}$ after 40 days. In the four sequential measurement intervals growth rate of stressed fish was 18, 39, 47 and 40% lower than controls. Growth rate in length of stressed fish was 8, 32, 40 and 25% lower than controls in the four sequential measurement intervals.

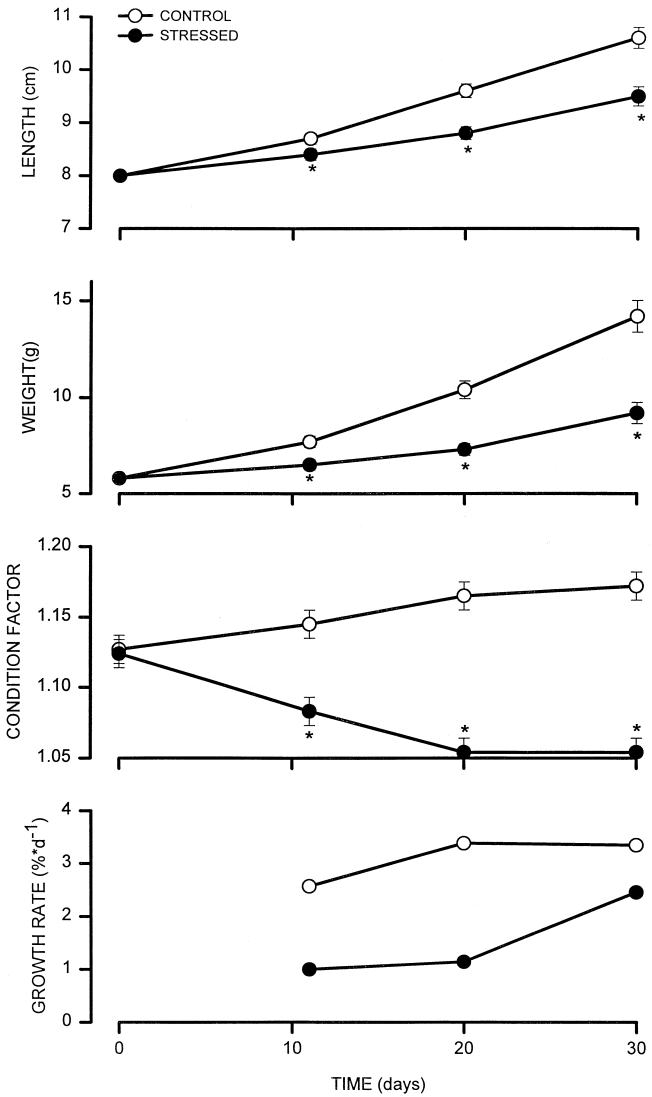


Fig. 1. Length, weight, condition factor and growth rate in weight of Atlantic salmon parr subjected to a twice daily acute stress. Values are mean ± SE ($n = 12$ per group). There was a significant effect of stress and significant changes over time for length, weight and condition factor ($P < 0.05$, two-way ANOVA). There was a significant interaction effect only for condition factor. Asterisk indicates a significant difference from the control group (one-way ANOVA, $P < 0.05$).

Condition factor of controls increased throughout the experiment from 1.06 to 1.25, whereas condition factor of stressed fish remained relatively constant between 1.07 and 1.11 (Fig. 2). Condition factor of stressed fish was significantly lower than controls after 10 days and remained significantly lower through the remainder of study.

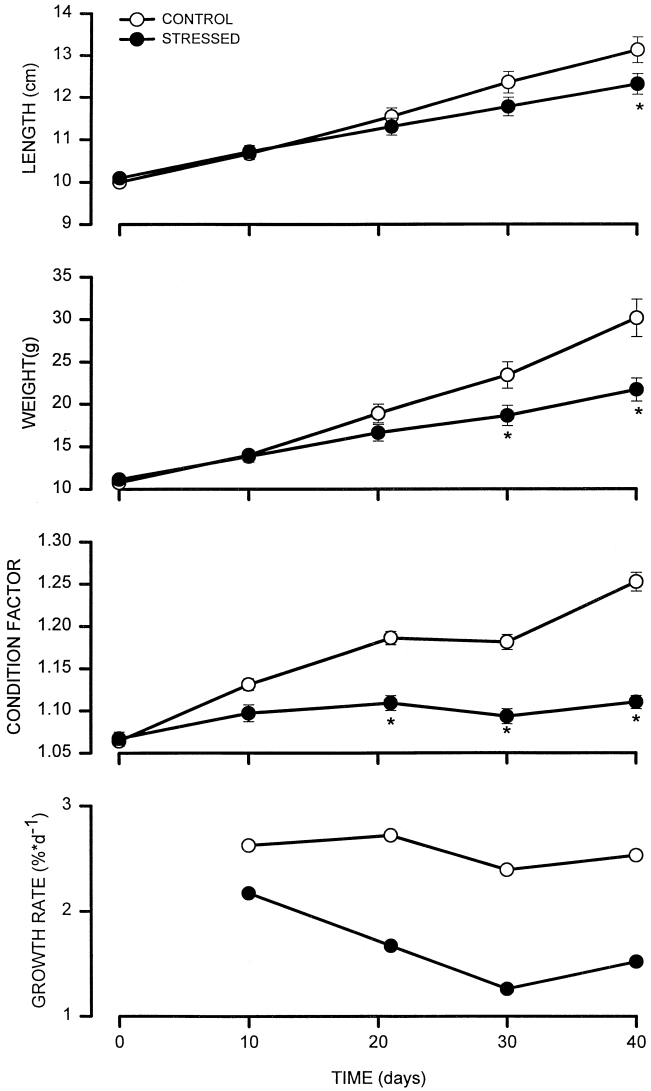


Fig. 2. Length, weight, condition factor and growth rate in weight of Atlantic salmon parr subjected to a daily acute stress. Values are mean ± SE ($n = 33$–$34$ per group). There was a significant effect of stress and significant changes over time for length, weight and condition factor ($P < 0.05$, two-way ANOVA). There was a significant interaction effect only for condition factor. Asterisk indicates a significant difference from the control group (one-way ANOVA, $P < 0.05$).

After 17 days, food consumption based on dry weight was 5.4% $day^{-1}$ in controls and 2.1% $day^{-1}$ in stressed fish, indicating a 61% reduction in food intake of stressed fish. After 37 days, food consumption was 6.2 and 4.6% $day^{-1}$ in control and stressed fish, respectively, indicating a 37% reduction in food consumption of stressed fish.

### 4.3. Endocrine profiles

Forty-two days after a once daily acute stress (experiment 2), fish were sampled 1 h prior to, and 3 and 7 h after exposure to the stress (Fig. 3). Plasma growth hormone levels of the stressed group were significantly higher than that of controls, both prior to and 7 h after the daily stress ($P < 0.05$). There was an apparent rise in plasma growth

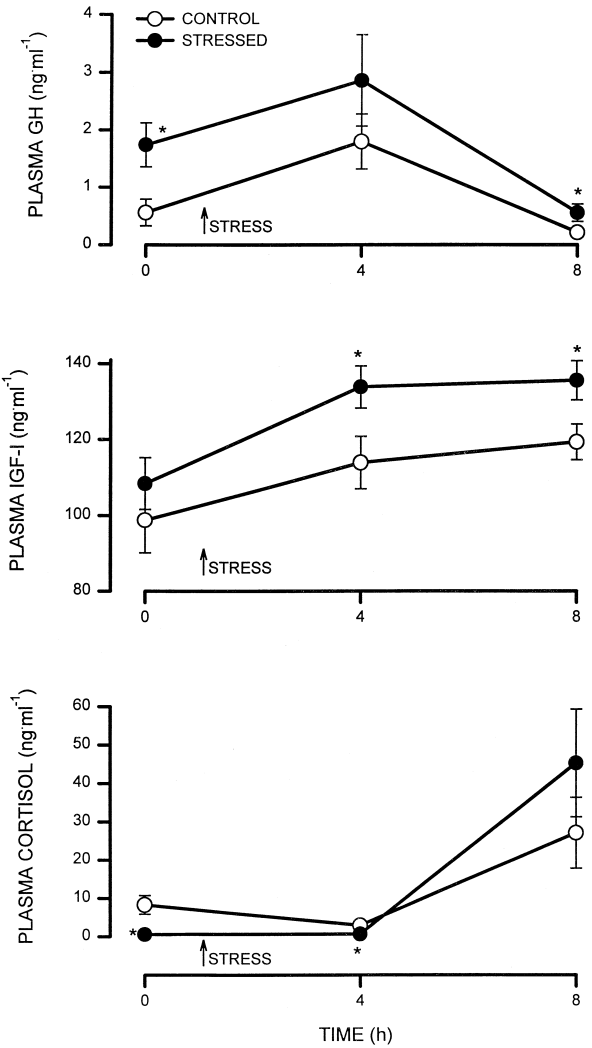


Fig. 3. Plasma growth hormone, insulin-like growth factor I and cortisol in Atlantic salmon parr subjected to a daily acute stress for 42 days. Fish were sampled 1 h prior to, 3 and 7 h after an acute (15 min) stress. The control group was not subjected to stress. Values are mean ± SE ($n = 10$ per group at each sampling point). There was a significant effect of stress and significant changes over time for all three hormones ($P < 0.05$, two-way ANOVA). There was a significant interaction effect only for plasma cortisol. Asterisk indicates a significant difference from the control group (one-way ANOVA, $P < 0.05$).

hormone levels in both groups 4 h after the first sampling, followed by a decline to levels that were lower than at the initial sampling. There was a significant effect of stress and significant changes over time in plasma growth hormone levels ($P < 0.05$, two-way ANOVA) and no significant interaction. Significant changes over time occurred in control and stress groups when analyzed separately ($P < 0.001$, one-way ANOVA).

Plasma IGF-I levels of the stressed group were not significantly different from that of the control group prior to initiation of the daily stress. Three and seven hours after stress, the plasma IGF-I levels were significantly higher than in controls. There was a general rise in plasma IGF-I levels in both groups during the day. There was a significant effect of stress and significant changes over time in plasma IGF-I levels ($P < 0.05$, two-way ANOVA) and no significant interaction. Significant changes over time occurred in the stress group ($P = 0.006$) but not in controls ($P = 0.21$) when analyzed separately (one-way ANOVA).

Plasma cortisol levels were significantly lower in the stressed group compared with the control group, both prior to and also 3 h after initiation of the daily stress. Plasma cortisol increased substantially at the last sampling period, but did not differ significantly between the groups at this time. There was a significant effect of stress and significant changes over time in plasma cortisol levels, and a significant interaction ($P < 0.05$, two-way ANOVA). Significant changes over time occurred in control and stress groups when analyzed separately ($P < 0.01$, one-way ANOVA).

## 5. Discussion

The acute handling stresses in the present study resulted in reduced growth rate of juvenile Atlantic salmon. For the first 20 days of each experiment, growth rate and growth reduction relative to controls was greater in twice daily than once daily stressed groups, indicating increased growth depression with increased frequency of stress. This relationship was less clear at the end of each experiment, where there was a slight recovery in growth rate in the stressed groups, perhaps the result of an habituation to the repeated stress. In addition to the greater overall impact, twice daily stress resulted in more rapid effects; in this group the lowest growth rate occurred in the first 11 days, whereas in the once daily group there was a progressive decrease in growth rate through the first 30 days. Although these two studies were conducted sequentially, we cannot rule out the possibility that the advancing season or 2°C lower temperature also played a role in these differences.

The adverse effects of a daily acute stress on growth of juvenile Atlantic salmon differs from the findings of Barton et al. (1987) in which comparable daily acute stress of similarly sized juvenile rainbow trout had no effect on growth. Although a variety of differences in experimental conditions could play a role, it seems probable that the different findings are the result of species differences, especially differences in genetic background. The present study used a strain of Atlantic salmon with little or no selection for fast growth and hatchery conditions, whereas Barton et al. (1987) examined domesticated rainbow trout. One of the primary goals of domestication has been to achieve maximum growth under hatchery conditions which include frequent stressors.

Domesticated brown trout (*S. trutta*) show a lesser antipredator response and have a higher weight growth than wild trout in the presence of a predator (Johnsson et al., 1996). Genetic differences in the stress response of different strains of several salmonids including rainbow trout and Atlantic salmon have been demonstrated (Fevolden et al., 1991; Pottinger et al., 1992; Heath et al., 1993; Pottinger and Moran, 1993). Domestic rainbow trout are more aggressive feeders and are less responsive to external changes than Atlantic salmon (McCormick, unpublished observation). The relatively greater sensitivity of Atlantic salmon growth to external conditions is exemplified by the work of Pickering et al. (1987) who found that providing overhead cover significantly increased growth of Atlantic salmon but not rainbow trout. Direct comparison of the intensity of the primary and secondary stress response of Atlantic salmon and rainbow trout have yielded conflicting results (Davis and Parker, 1983; Fevolden et al., 1993). Differences in stress response at different developmental stages may also be important in determining the impact of stress on growth (Barton et al., 1985; Carey and McCormick, 1997). This affects our ability to directly compare studies on difference species with different life history patterns, such as anadromous Atlantic salmon and nonanadromous rainbow trout.

Although stress decreases both weight and length growth rate, these two are not affected to the same extent as indicated by the consistently greater effect of stress on growth in weight and the progressive increased differences in condition factor. Thus, the stressed fish become leaner while they appear to strive to keep length growth at a normal rate. A similar response occurs when fish receive food after a period of starvation (Farbridge et al., 1992).

In the present study the measurement of food consumption by hand feeding to satiation was probably an underestimate of the amount normally consumed by the fish in the rest of the study when automatic feeders were used. The results indicate, however, that there was substantial effect of daily acute stress on food consumption, with reductions of 61% and 37% relative to controls after 17 and 37 days, respectively. Throughout the study, food was withheld from both groups during, and 45 min after, each stress event to ensure that the disturbance itself did not affect food consumption. The decrease in food consumption observed in the present study is therefore a direct response of fish to acute stress. Reduced feeding following acute stress has been observed in other studies (Pickering et al., 1982), and may be the primary means by which stress affects growth rate.

Reductions in food consumption of 61% in stressed fish relative to controls early in the study and by 37% later on indicates a possible decrease in the effect of stress on food consumption with time. This may be related to the slight increases in growth rate of stressed fish in the last interval of both experiments, further indication of a possible accommodation or compensation to stress with time. Food consumption of stressed fish early in the study (day 17) was decreased to a much greater extent than their growth rate (61% vs. 39%), suggesting that conversion efficiency may have been greater in the stressed fish. Previous studies have shown that conversion efficiency generally increases with decreasing ration (Brett and Groves, 1979), and the apparently higher conversion efficiency of stressed fish may be a function of this relationship. In the final interval, the effect of stress on food consumption and growth was similar (37% vs. 40% reductions,

respectively, relative to controls), possibly reflecting physical conditioning or habituation to stress.

Plasma growth hormone levels of the unstressed controls are consistent with other studies demonstrating low growth hormone levels during the parr stage of Atlantic salmon (McCormick and Björnsson, 1994) after which they increase rapidly during the parr–smolt transformation (reviewed in Björnsson, 1996). In contrast to previous studies in which long-term continuous stress suppresses plasma growth hormone levels (Pickering et al., 1991), the present study indicates that repeated acute stress results in elevated plasma growth hormone. The 'prestress' endocrine status of the stressed group, with growth hormone levels significantly higher (and IGF-I levels somewhat higher) than the controls, may also be interpreted as an action of growth hormone in energy mobilization (Sheridan, 1986) to accommodate physical activity and/or any putative energetic demands of stress. Increased plasma growth hormone levels may also be a response to reduced food intake, since growth hormone is elevated during prolonged starvation in several salmonids (Barrett and McKeown, 1989; Kakisawa et al., 1995; Johnsson et al., 1996). Growth hormone levels increased transiently after the first sampling in both control and stressed groups. This may be due in part to a diel rhythm of growth hormone which in rainbow trout has been shown to be responsive to the timing of feeding (Boujard and Leatherland, 1992).

Recent studies show that growth hormone treatment of rainbow trout causes an elevation of circulating IGF-I levels (Moriyama et al., 1995), and that there is a positive correlation between the seasonal plasma profiles of growth hormone and IGF-I in Atlantic salmon (McCormick et al., 1996). In the present study, both growth hormone and IGF-I levels are elevated in both groups (ANOVA $P < 0.05$) 3 h after the initiation of stress, whereas growth hormone levels but not IGF-I levels have declined 4 h later. These data support the view that growth hormone is a major regulator of circulating IGF-I levels in salmonids, and that plasma IGF-I levels may be elevated for some time after growth hormone stimulation of IGF-I secretion.

Several studies have shown that growth hormone increases during starvation and that growth rate of fish is inversely related to circulating levels of growth hormone (Björnsson, 1996). The present finding of increased growth hormone associated with lower growth rates in stressed fish is therefore not surprising. However, plasma IGF-I has been shown to be positively correlated with food intake and growth rate (Moriyama et al., 1994; Perez-Sanchez et al., 1994) and the present finding of no difference in plasma IGF-I in stressed fish (sampled before the daily stress) that have low growth rates and reduced food intake is surprising. It should be noted that the changes in plasma growth hormone and IGF-I may be a response to stress and/or reduced intake rather than causal to increased growth rate. Further examination of temporal changes in response of plasma growth hormone and IGF-I to stress and reduced food intake would be of value in resolving the apparent differences in these studies.

Perhaps the most interesting finding for plasma cortisol in the present study is that levels prior to the daily stress event were significantly lower in the stressed group than in the controls. This finding suggests that repeated acute stress (in this case for 42 days) results in lower 'basal' plasma cortisol. Barton et al. (1987) found no differences in basal cortisol between control rainbow trout and those given a daily acute stress for 10

weeks. Although there is evidence that fish under chronic stress can 'compensate' (or become desensitized) and exhibit lower plasma cortisol with time (1–8 weeks; Strange et al., 1978; Pickering and Stewart, 1984; Pottinger and Pickering, 1992), similar evidence for acute stress is lacking. Redding et al. (1984) have shown that long-term stress (5 days of confinement) can increase the clearance rate of cortisol. A similar response to repeated acute stress could explain the lower plasma cortisol levels of stressed fish in the present study.

It is somewhat surprising that there was low plasma cortisol in the stressed group 3 h after the acute stress. Although most studies in which Atlantic salmon were subjected to an acute stress found that cortisol remains elevated for three or more hours (Fevolden et al., 1991; Fevolden et al., 1993; Carey and McCormick, 1997), it is possible that cortisol has increased and returned to basal levels within 3 h of the acute stress in the present study (e.g., Biron and Benfey, 1994). The primary stress response of smolting salmonids is much greater in smolts than in parr (Barton et al., 1985; Carey and McCormick, 1997), and the absence of observed changes in plasma cortisol found in the present study may relate the use of parr as experimental subjects. It is also possible that the sensitivity of the fish to stress decreases with time. Barton et al. (1987) found a decrease in the 1 h post-stress levels of plasma cortisol in rainbow trout after 4 and 8 weeks of exposure to a daily acute stress. Similarly, Schreck et al. (1995) found exposure of chinook salmon (*Oncorhynchus tshawytscha*) to repeated acute stresses reduced the increases in plasma cortisol and glucose that accompanied a 2 h transport stress. The increase in plasma cortisol in both groups after 7 h may be the result of multiple disturbances due to prior cohort sampling in the tanks. Multiple disturbances have been shown to result in cumulative increases in plasma cortisol in salmonids (Barton et al., 1986), though it is difficult to imagine that the minor disturbance of sampling cohorts would evoke a greater response than the severe stress experienced by the stressed group.

Pickering and Stewart (1984) found that chronic stress (crowding) in brown trout resulted in higher plasma cortisol for the first 25 days, but from 39 to 110 days there were no differences from controls. Growth rate was lower in chronically stressed fish throughout the study, especially in the last 40 days. The authors concluded that growth suppression due to crowding was not mediated by high circulating cortisol. Similarly, the present study does not provide evidence for the involvement of cortisol in stress-related suppression of growth. In spite of substantially lower growth in the stressed group, plasma cortisol in the stressed group was significantly lower than controls prior to and 3 h after stress.

## 6. Unlinked References

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