The effects of environmental stress on the physiology of growth in rainbow trout, *Salmo gairdneri* Richardson

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The effects of environmental stress on the physiology of growth in rainbow trout, *Salmo gairdneri* Richardson.

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C O N T E N T S

SUMMARY

1. Plasma growth hormone (GH) levels were measured in rainbow trout using a radioimmunoassay developed against chinook salmon growth hormone.
2. Slow-growing strains of trout had higher growth hormone levels than faster-growing strains reared under similar conditions.
3. Starvation resulted in a marked and sustained rise in plasma GH levels in both 0+ and 1+ rainbow trout. This effect was independent of any activation of the hypothalamic-pituitary-interrenal axis.
4. Acute handling stress followed by short-term (24 h) confinement had little effect on plasma GH levels in 0+ rainbow trout although there was some evidence of GH suppression in 1+ fish.
5. Chronic confinement and prolonged overcrowding caused an elevation of blood growth hormone levels. This was particularly apparent when blood cortisol levels were also elevated in these chronically stressed fish. When acclimation ultimately occurred, both cortisol and GH levels returned to normal.
6. No evidence of a suppression of GH levels was found in chronically stressed fish, even though growth was suppressed in these fish and our initial working hypothesis that growth suppression in stressed trout may be caused by a suppression of pituitary growth hormone secretion was rejected.
7. A strong inverse correlation was found in several of the experiments between plasma growth hormone levels and the coefficient of condition of individual fish.
8. Corticosteroid implantation to elevate blood cortisol levels, within the physiological range of otherwise unstressed fish, did not affect blood GH levels at 7, 16 or 22 days post-implantation.
9. It is concluded that growth hormone treatment would not overcome problems of stress-induced growth suppression and that further work is now needed on the mechanism of action of the hormone in salmonid fish.
10. These findings are discussed in relation to the existing literature on growth hormone physiology in both fish and higher vertebrates and with regard to future developments within the aquaculture industry.

IMPLICATIONS OF THE STUDY FOR THE AQUACULTURE INDUSTRY

There is little doubt that both mammalian and teleost growth hormones can accelerate growth and increase food conversion efficiency in all commonly-reared species of salmonid fish. The failure to exploit this technology is largely related to two factors:-

- the prohibitive cost of growth hormone preparations
- industry and consumer resistance to all forms of hormone treatment.

Rapid progress is currently being made in the field of recombinant DNA technology with salmonid growth hormone genes having been expressed in bacterial hosts and it seems likely, therefore, that salmon and trout growth hormone will become both cheaper and more readily available. Industry and consumer resistance to hormone treatment is largely based on the bad publicity given to the mis-use of steroid hormones, which are readily absorbed by the human alimentary canal. Growth hormone is a proteinaceous hormone which is destroyed by heat (cooking) and which is rapidly broken down in the digestive tract. Thus, the problems related to steroid hormones are not applicable to growth hormone.

Despite the constant pressure to increase the growth rate of cultivated salmonid fish, it would seem that the problem of stress-induced growth suppression is unlikely to be solved by growth hormone treatment. From the present investigation it is clear that chronically stressed, poorly-growing rainbow trout have elevated levels of immunologically measurable growth hormone in their blood. This is also true for poor-growing strains of fish when compared with some of the faster growing strains. These data are consistent with several other studies on salmonids and also with work on domesticated strains of poultry. It seems likely, therefore, that if poor growth in fish is related to growth hormone physiology, the solution to this problem will lie in a study of growth hormone activity, target tissue sensitivity (including growth hormone receptor studies) and the role of somatomedins or growth factors in poorly-growing or chronically-stressed fish. Undoubtedly from the aquacultural point of view, avoidance of the environmental conditions responsible for growth suppression is still the best policy.

INTRODUCTION

In those vertebrates that have been closely studied (predominantly mammals), the pituitary hormone somatotropin (GH or growth hormone) is a prime determinant of somatic growth. The hormone stimulates protein biosynthesis and tissue growth, enhances lipid utilization and lipid release from the adipose tissues (a protein-sparing effect) and suppresses the peripheral utilization of glucose. Its effects may be mediated by a secondary molecule(s), somatomedin, synthesized primarily in the liver and kidney. GH secretion from the pituitary gland is controlled by two hypothalamic hormones, GRF (growth hormone releasing factor) and SRIF (somatotropin release-inhibiting factor or somatostatin).

It has been known for more than 30 years that hypophysectomy of teleost fish results in a reduction or cessation of growth (Pickford, 1953a) and that this effect can be reversed by injections of mammalian growth hormone (Pickford, 1953b). Since this pioneering work, many of the features of the mammalian system have now been identified in teleosts. Immunocytochemical studies have identified acidophilic cells in the proximal pars distalis of the pituitary gland as the somatotropes (Ingleton & Stribley, 1975; Nagahama et al., 1981; Wagner & McKeown, 1983) and growth hormone has been isolated and purified from the pituitary glands of several teleost species (Farmer et al., 1976; Wagner et al., 1985; Kawauchi et al., 1986; Kishida et al., 1987). Mammalian somatostatin inhibits GH secretion both in vitro (Fryer et al., 1979) and in vivo (Cook & Peter, 1984; Sweeting & McKeown, 1986) in fish and evidence for the presence of somatostatin in the brain, hypothalamus and pituitary gland of teleosts is accumulating (Dubois et al., 1979; King & Millar, 1979; Olivereau et al., 1984). Moreover, lesions in certain areas of the hypothalamus cause a stimulation of the somatotropes and an elevation of blood GH levels in the goldfish, Carassius auratus (Fryer, 1981; Cook & Peter, 1983). To date, however, little is known about the possible stimulatory (GRF) activity of the teleost hypothalamus on somatotrope activity and nothing is known about the role of somatomedin(s) in facilitating the effects of GH on teleost metabolism.

Aquacultural interests in growth hormone physiology have centred around two major areas:

1. Growth promotion of fish by means of exogenous GH.
2. The role of GH in smoltification and seawater adaptation in salmonid fish. Numerous studies have demonstrated the growth-promoting effects of mammalian and teleost GH, either alone or in combination with other anabolic hormones (reviewed by Donaldson et al., 1979; Weatherley & Gill, 1987). The increased appetite and improved food conversion efficiency in GH-treated fish

has stimulated considerable interest in the aquacultural possibilities of GH-induced growth promotion in salmonids (see, for example, Higgs et al., 1978; Gill et al., 1985). However, the high cost of GH preparations at the current time together with industry and consumer resistance to most types of hormonal treatment, has so far limited the application of this form of technology.

The osmoregulatory influences of GH in teleost fish were first demonstrated by Smith (1956) who showed that mammalian GH increased the survival rate of brown trout, Salmo trutta, during seawater adaptation. Since this early study, several workers have shown that GH is a major factor in the control of smoltification and seawater adaptation in salmonid fish (Komourdjian et al., 1976; Clarke et al., 1977; Brewer & McKeown, 1978; Miwa & Inui, 1985; Sweeting et al., 1985; Bolton et al., 1987a).

Despite these advances in our knowledge of the role of GH, a major limitation to progress has been the lack of suitable techniques for the measurement of growth hormone in the pituitary gland and blood of teleost fish. Heterologous radioimmunoassays (using antisera to mammalian GH) are of limited value because of the wide differences in immunological properties of teleost and mammalian GH. Thus, although mammalian growth hormones are effective growth-promoters in fish (see above), teleost growth hormones show little cross-activity with antisera to mammalian somatotropins (Hayashida & Lagios, 1969) and are relatively ineffective as growth promoters in the rat tibia bioassay, the standard mammalian bioassay for GH (Hayashida, 1971; Farmer et al., 1976). A homologous radioreceptor assay was developed by Fryer (1979) for tilapia, Oreochromis (≡ Sarotherodon) mossambicus, growth hormone but this approach has not been widely adopted. Since the isolation and purification of teleost growth hormones (see above), specific radioimmunoassays have been developed for the carp, Cyprinus carpio (Cook et al., 1983) and for the chum salmon, Oncorhynchus keta (Bolton et al., 1986; Wagner & McKeown, 1986). The degree of cross-reactivity within the salmonid group is sufficient to allow the chum salmon RIA's to be used for several different salmonid species, including the rainbow trout, Salmo gairdneri. Indeed, it has been recently shown that the predicted amino acid sequence of coho salmon, Oncorhynchus kisutch, GH is 97% homologous with chum salmon GH (Nicoll et al., 1987) and is identical with the predicted amino acid sequence of rainbow trout GH (Agellon & Chen, 1986).

This recent availability of specific and sensitive teleost growth hormone RIA's has further enhanced interest in the role of GH in controlling the physiology of salmonid fish. A prerequisite for future studies is an understanding of the relationship between stress responses (sometimes the unavoidable consequences of experimental manipulation) and the physiology of

growth. Growth suppression in teleost fish is a consequence of many forms of environmental stress and, in view of the marked effects of stress on the secretion of several other teleost pituitary hormones (ACTH - Sumpter et al., 1986; α-MSH and endorphin - Sumpter et al., 1985; gonadotropin - Pickering et al., 1987a), it is reasonable to propose that growth suppression in stressed fish may be caused by a suppression of pituitary growth hormone secretion. Existing information on this point is extremely limited for teleost fish. Cook & Peter (1984) suggested that an increase in serum GH levels in the goldfish approximately 24 h after blood sampling may have been due to the effects of stress, whereas Wagner & McKeown (1986) concluded that handling stress had no effect on blood GH levels in the rainbow trout. The mammalian picture offers little by the way of further clarification. Physical and psychological stresses stimulate GH secretion in humans (Muller, 1974), suppress GH secretion in rats (Terry et al., 1977) and have no obvious effects on GH secretion in sheep (Moberg, 1985).

An almost ubiquitous response of most vertebrates to both acute and chronic stress is an elevation of plasma corticosteroid levels (see Donaldson, 1981 for a review of the teleost literature). The catabolic properties of this group of hormones makes the corticosteroids growth suppressants in their own right. For example, the catabolic action of cortisol (the principal corticosteroid in this group of fish) causes a marked reduction in the coefficient of condition of the brown trout when the hormone is administered at physiological levels (Pickering & Duston, 1983). However, in addition to any possible direct effects of corticosteroids on growth (see also Loeb, 1976) studies with mammals indicate that corticosteroids can also suppress GH secretion although the response is complex, with both stimulatory and suppressive components (Ceda et al., 1987; Nakagawa et al., 1987).

Very little is known about the possible interaction between corticosteroids and GH secretion in fish. Olivereau & Olivereau (1968) found that adrenalectomy stimulated somatotrope activity in the eel, Anguilla anguilla, pituitary gland although they interpreted this as a non-specific stress response. Higgs et al. (1977) found that bovine GH injections increased interrenal cell nuclear diameter in coho salmon but no hormonal measurements were attempted. More recently, Nishioka et al. (1985) showed that cortisol stimulated the release of GH from tilapia pituitaries in vitro.

In the light of this fragmentary information, the current research was established as a joint project between the Freshwater Biological Association and Brunel University to examine the effects of environmental stress on plasma growth hormone levels in the rainbow trout. The research was commissioned by

MAFF for the period December 1986 - March 1987 and extended (with funding from the FBA's Science Vote) for the period April - December 1987. Plasma cortisol levels were monitored; firstly to assess the magnitude and duration of the stress response and secondly, to look for possible interactions between the hypothalamic-pituitary-interrenal axis and growth hormone secretion. Plasma growth hormone was measured using a radioimmunoassay developed by P.Y. Le Bail (INRA, Rennes) against chinook salmon, Oncorhynchus tshawytscha, GH and validated for use with other salmonid fish, including the rainbow trout (Le Bail et al., in preparation). It is likely that, as salmonid growth hormone becomes more cheaply available (as a result of recombinant DNA technology - see Agellon & Chen, 1986; Nicoll et al., 1987), interest in its growth-promoting potential for aquaculture will increase. The present study is a prerequisite for future work on growth hormone physiology in salmonids and should contribute to our understanding of the mechanisms of growth suppression in stressed fish.

MATERIALS AND METHODS

For the reader's convenience, the detailed protocol for each experiment is given in the Results section of the report and this Materials and Methods section will be reserved for those procedures common to all experiments.

Maintenance of experimental fish

This investigation consisted of seven separate experiments on hatchery-reared rainbow trout carried out at the FBA's Windermere hatchery during the period December 1986 to December 1987. All the fish were reared in large (1500 l), outdoor, fibreglass tanks each supplied with a constant flow of Windermere lake water (35 l min^{-1}, temperature range during the study period 3-17.5°C - see Fig. 6a). Fish were fed once daily with commercial trout pellets at the rates recommended by the manufacturers (exact rate dependent upon fish size and water temperature). Oxygen levels in each tank were maintained close to saturation throughout the study, with the exception of the crowded fish in Experiment 6 when O_2 levels dropped at certain times to as low as 30% saturation.

Blood sampling

At each sampling time fish were rapidly anaesthetized in phenoxyethanol (1:2000) and blood samples obtained from the caudal vessels by means of heparinized syringes; with two operators the whole procedure normally took less than 2 minutes. Following centrifugation at 4°C, plasma samples were stored at -70°C until assayed for growth hormone and/or cortisol. After blood sampling each fish was then killed by a blow to the head, weighed, measured (fork length) and sexed.

Cortisol radioimmunoassay

Plasma cortisol levels were determined by radioimmunoassay using previously validated techniques (Pickering & Pottinger, 1983; Pickering et al., 1987b). Aliquots (200 µl) of plasma were extracted with 1 ml Aristar ethyl acetate. After thorough mixing and centrifugation 20- to 200-µl aliquots of the organic supernatant were pipetted into assay tubes together with 20,000 dpm [1, 2, 6, 7- ^{3}H] cortisol (86 Ci $mmol^{-1}$, Amersham International). A range of standard tubes (0-800 pg cortisol $tube^{-1}$) containing 20,000 dpm [^{3}H] cortisol $tube^{-1}$ was prepared in duplicate from a stock solution of unlabelled cortisol in ethyl acetate. All tubes were evaporated to dryness under vacuum at 35°C. BSA-saline (100 µl, 0.1% bovine serum albumin in 0.9% NaCl) was added to each tube followed by 100 µl of antiserum (Steranti anti-cortisol-3-

(CMO)HSA) at a concentration sufficient to bind ≈ 50% of the [^{3}H] cortisol in the absence of inert steroid. After vortex mixing and incubation for 4-18 h at 4°C, 100 µl of chilled dextran-charcoal suspension (0.5% charcoal, 0.1% dextran, 0.9% NaCl) was added to each tube. After further mixing, tubes were incubated on ice for 5 min and centrifuged at 1200 g for 5 min at 4°C. Aliquots (200 µl) of supernatant were added to scintillation vials containing 5 ml Unisolve 1 liquid scintillation fluid (Koch-Light) and counted under standard ^{3}H conditions. A standard curve was constructed and unknowns were read from this curve.

a) Assay characteristics

Aliquots of ethyl acetate-extracted plasma diluted parallel to the standard curve over the complete range 0-800 pg tube^{-1}. The smallest amount of cortisol statistically distinguishable from 0 was 10 pg tube^{-1} (≡ 0.2 ng ml^{-1} plasma). The following values for between-assay variation were obtained from 10 separate assays of three plasma pools: low pool 1.4 ± 0.1 ng ml^{-1} ($\bar{x}$ ± SEM), coefficient of variation (CV) = 20.8%; medium pool 7.6 ± 0.2 ng ml^{-1}, CV = 7.1%; high pool 37.2 ± 1.8 ng ml^{-1}, CV = 15.5%. The following values for within-assay variation were obtained from eight determinations of the three plasma pools: low pool 1.3 ± 0.1 ng ml^{-1}, CV = 21.2%, medium pool 6.7 ± 0.14 ng ml^{-1}, CV = 6.2%, high pool 39.1 ± 1.2 ng ml^{-1}, CV = 8.9%. Regression analysis of measured cortisol against cortisol added to stripped plasma gave a correlation coefficient (r) of 0.98 ($p < 0.01$) and a gradient of 0.9.

Development of a growth hormone radioimmunoassay

Initial attempts to measure growth hormone (GH) in rainbow trout plasma were made using a radioimmunoassay, developed in Japan, based on chum salmon GH (Bolton et al., 1986). This assay was chosen because it was the first published RIA for GH in salmonid fish, although soon after its publication details of another, separate growth hormone radioimmunoassay (also based on chum salmon GH) appeared in the press (Wagner & McKeown, 1986). As reported, the GH RIA developed by Bolton et al. (1986) appeared acceptable; it did not appear to cross-react with other pituitary hormones such as gonadotropin (GTH) and prolactin (PRL). Moreover, plasma from most salmonids (including the rainbow trout) cross-reacted well in the assay although species from other genera of fish (eel, tilapia, goldfish and carp) showed little or no cross-reactivity. In its published form the assay was not very sensitive (limit of detection 3-4 ng ml^{-1}) but reports suggested that GH levels in salmonid fish were generally greater than 10 ng ml^{-1} indicating, therefore, that sensitivity would not be a limiting factor.

The ingredients for the assay were obtained, via Dr J.P. Bolton, from Tokyo and the assay was established at Brunel University. However, it became immediately apparent that the plasma GH levels in rainbow trout reared at the FBA were substantially lower than those reported by other groups for salmonid fish (Wagner & KcKeown, 1986; Sweeting & McKeown, 1986; Bolton et al., 1986) and that the lack of sensitivity of the original assay was, indeed, a major limiting factor. Consequently, an attempt was made to increase the sensitivity of the assay by:-

a) improving the iodination techniques for the labelled GH, including increasing the specific activity from 2-5 $\mu Ci\ \mu g^{-1}$ to 20-50 $\mu Ci\ \mu g^{-1}$.

b) altering the assay protocol by delaying the addition of the label.

c) adding less labelled GH (5000 dpm instead of 20,000 dpm).

d) using a lower concentration of antibody (1:20,000 instead of 1:5,000).

Together, these changes increased the assay sensitivity to give a limit of detection of ≈ 0.75 ng ml^{-1}.

However, a further problem with the assay appeared when we attempted to measure GH levels in the plasma of sexually maturing fish. The "GH" levels correlated extremely well with GTH levels measured in the same plasmas with an independent gonadotropin radioimmunoassay (Pickering et al., 1987a). On testing the cross-reactivity of the GH assay with highly-purified chinook salmon GTH (kindly provided by Dr B. Breton) we observed a 30% cross-reaction. This observation was then independently confirmed by Dr J.P. Bolton who, at that time, was working at the INRA laboratory at Rennes. After considerable correspondence and cross-checking between ourselves and J.P. Bolton, P.Y. Le Bail and P. Prunet (Rennes) and T. Hirano and H. Kawauchi (Japan) the following conclusions were drawn:-

1. The GH RIA as established and reported by Bolton et al. (1986) showed no cross-reaction with GTH and was GH specific.

2. Later preparations of purified chum salmon GH (from Japan), which replaced the original preparation (used up in the earlier stages of assay development), were contaminated with GTH (probably less than 5%).

3. The greater ease of iodination of GTH compared with GH (which is reluctant to take up ^{125}I) exaggerated the degree of cross-reactivity of the assay to $\approx$ 30%.

Five different GH preparations from Kawauchi's laboratory (received via T. Hirano) all produced assays with serious cross-reactivity with GTH and were unacceptable. A method was developed to separate labelled GTH from labelled GH by gel-filtration on Sephacryl S300, which resulted in a much purer GH label. This purified iodinated GH was then used to develop a GH-specific assay.

However, the gel-filtration step was tedious, as it required a 1 metre column with an elution time of 2 days. Moreover, the sensitivity of this modified assay was little improved (limit of detection $\approx$ 0.75 ng ml^{-1}).

At this stage it was decided to abandon Bolton's GH RIA and collaborate with P. Le Bail (Rennes) who was independently developing a salmonid GH RIA based on chinook salmon growth hormone.

Le Bail provided purified chinook salmon GH (free of GTH contamination), raised a rabbit antiserum to this purified chinook GH and, using these ingredients, a radioimmunoassay was developed in collaboration with Le Bail. The chinook GH preparation was soluble at pH7 (the Japanese material was insoluble at this pH), iodinated readily, and the labelled preparation was stable for relatively long periods (in excess of 8 weeks). The antibody was of a higher titre than that supplied by J.P. Bolton and produced a more sensitive standard curve. The limit of detection of this assay was $\approx$ 0.2 ng ml^{-1}. The specificity of the assay depends primarily on the purity of the labelled GH - with the original chinook preparation the cross-reactivity with salmonid GTH and prolactin was less than 0.01%. Brown trout, rainbow trout, Atlantic salmon (Salmo salar), pink salmon (Oncorhynchus gorbuscha), chum salmon, coho salmon and sockeye salmon (O. nerka) all cross-react well in this RIA (i.e. blood and pituitary homogenates diluted parallel to the chinook salmon standard curve). The antiserum does recognise salmonid GTH to some extent but this is not a problem in the assay provided that pure GH label is used. In view of the increased sensitivity of the assay developed using materials supplied by Le Bail, this assay was used for all the experiments reported here. Full details of the assay protocol, its characteristics, and the evidence validating the assay for the measurement of GH in salmonid plasma are being prepared for publication (Le Bail et al., in preparation). However, as evidence of the validity of the assay, Fig. 1 compares the displacement of labelled GH from the antiserum with purified chinook GH and purified chinook GTH. EVen at very high concentrations, well above the highest blood levels reported, GTH shows no displacement of iodinated GH under the assay conditions routinely adopted during the entire study.

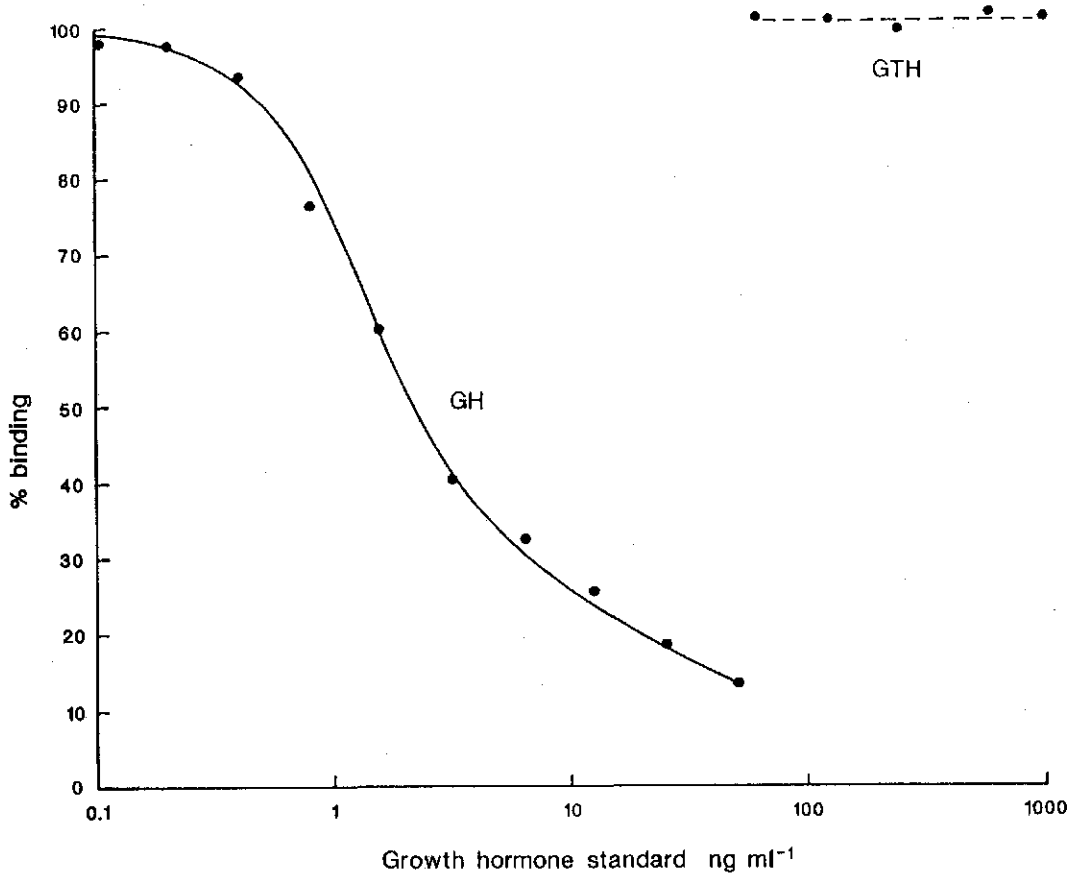

Fig. 1. A comparison of the displacement of labelled GH from the antiserum with purified chinook GH (continuous line) and purified chinook GTH (broken line). There is no cross-reaction between the antiserum and GTH even at a GTH concentration of 1 μg ml^{-1}.

Statistical analyses

For each experiment, body weight, length, K factor, plasma cortisol concentration and plasma growth hormone concentration were separately analysed by analysis of variance (ANOVA, Genstat) with treatment (stressed, unstressed) time and number (sequence within each sample) as factors. Tanks and fish were used as blocking effects to produce a nested error structure with which to assess the significance of the factors and their interactions. From a plot of residuals against fitted values, appropriate transformations were selected, where necessary, to improve homogeneity of variance. Levels of significance given in this report are derived from these analyses but, for ease of presentation, data are given as arithmetic means ± SEM. Linear regression was used to correlate plasma GH levels with plasma-cortisol levels in Experiment 4, and to correlate $\log_e$ plasma growth hormone levels with the coefficient of condition of the fish in several experiments.

RESULTS

Experiment 1. A comparison of the basal plasma growth hormone levels in different strains of rainbow trout.

a) Experimental design

At the time of this study, several different strains of rainbow trout were being reared at the FBA's hatchery (Table 1). These fish constituted much of the basic material for subsequent experimental studies and it was important, therefore, to have some knowledge of the variability in plasma GH levels prior to experimentation. Blood samples were taken from at least eight fish per strain.

In addition, a study of the effects of choice of anticoagulant on measurable growth hormone levels was undertaken using a single strain of rainbow trout. Heparin-treated, EDTA-treated and untreated blood samples were obtained from 36 New Zealand strain rainbow trout (12 fish per treatment) and plasma/serum samples were then prepared and stored as described in the Materials and Methods section.

b) Results

Statistically significant differences were found between the mean growth hormone levels of the seven strains of rainbow trout examined in this experiment (Fig. 2).

At one extreme, 2+ fish of the Annandale strain had mean plasma GH levels of less than 0.5 ng ml^{-1} whereas 1+ fish of the New Zealand strain had GH levels of almost 3.5 ng ml^{-1}. Although the correlation with size (and hence growth rate) was not absolute, it is interesting that the fish with the slowest growth rate and the poorest coefficient of condition (New Zealand strain, Table 1) were also the fish with the highest GH levels. A negative correlation between GH levels and K factor was evident in several of the subsequent experiments.

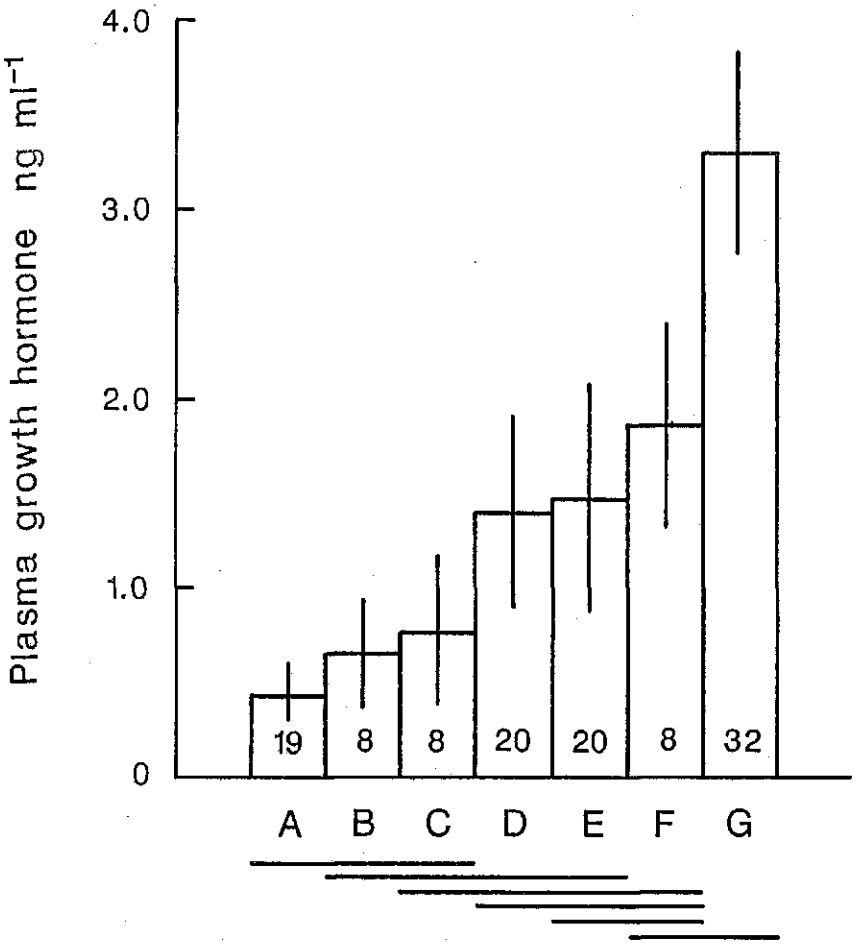

Fig. 2. Mean plasma growth hormone levels of seven strains of rainbow trout (A-G, see Table 1 for details). Each value is the mean ± SEM, the number of replicates is indicated at the base of each column. Strains sharing the same horizontal line are not significantly different ($p > 0.05$).

Table 1

	Strain	Age	Mean wt	Mean length	Mean K factor
		years	g	cm	
A	Annandale	2+	862.4	37.5	1.62
B	Butley	1+	183.0	24.3	1.24
C	Home	1+	89.0	18.8	1.24
D	Annan	1+	269.9	27.0	1.36
E	Cloan	1+	216.5	25.6	1.27
F	Caribou	1+	166.4	24.7	1.16
G	New Zealand	1+	61.6	17.5	1.14

Table 1. Meristic details of the seven strains of rainbow trout used in Experiment 1 for the comparison of basal plasma growth hormone levels.

In view of the comparatively high plasma GH levels in New Zealand rainbow trout, this strain was selected for a study of the effects of anti-coagulant on measured GH levels. The mean serum (i.e. no anticoagulant) GH level was 2.5 ± 0.5 (12) ng ml^{-1} (mean ± SEM (n)), the mean plasma level from EDTA-treated blood samples was 3.4 ± 0.7 (12) ng ml^{-1} and the mean plasma level from heparinized blood samples was 3.8 ± 1.2 (12) ng ml^{-1}. These differences were not statistically significant (analysis of variance) and it was concluded that these anticoagulants did not interfere with the GH RIA. Heparin was used as anticoagulant for all subsequent experiments.

Experiment 2. The effect of starvation on plasma growth hormone levels in 1+ rainbow trout.

a) Experimental design

Five hundred and sixty 1+ rainbow trout (Butley strain, mean body weight 295 g were distributed into four outdoor rearing tanks (see above for details) so that each tank contained 140 fish. The fish were fed once daily with commercial trout pellets at the rate recommended by the manufacturers, left for a period of two weeks to recover from the effects of handling stress (see Pickering et al., 1982) and then 10 fish were sampled from each tank (0 h sample). Food was then withdrawn from two of the four tanks (starved group) and further samples of 10 fish per tank were taken at 1, 2, 4 and 6 weeks. During the course of this experiment, the water temperature varied between 12.1 and 16.5°C.

b) Results

Marked individual variation in size at each sampling time masked any effect of starvation on body weight until 6 weeks after food withdrawal, when the starved fish had a significantly ($p < 0.05$) lower body weight than the fed fish (Fig. 3a). This difference in mean weight at 6 weeks was not accompanied by any difference in body length (Fig. 3b).

However when the coefficient of condition was considered (K factor = 100 W/L^3, a measure of shape which is relatively, although not totally, independent of body size), it was clear that starvation had a marked and highly significant ($p < 0.001$) suppressive effect within 2 weeks, an effect which was maintained for a further 4 weeks (Fig. 3c). Thus by the end of the experiment the mean coefficient of condition of the fed fish was 1.68 compared with 1.22 for the starved fish (i.e. the starved fish were significantly thinner ($p < 0.001$) than the fed fish).

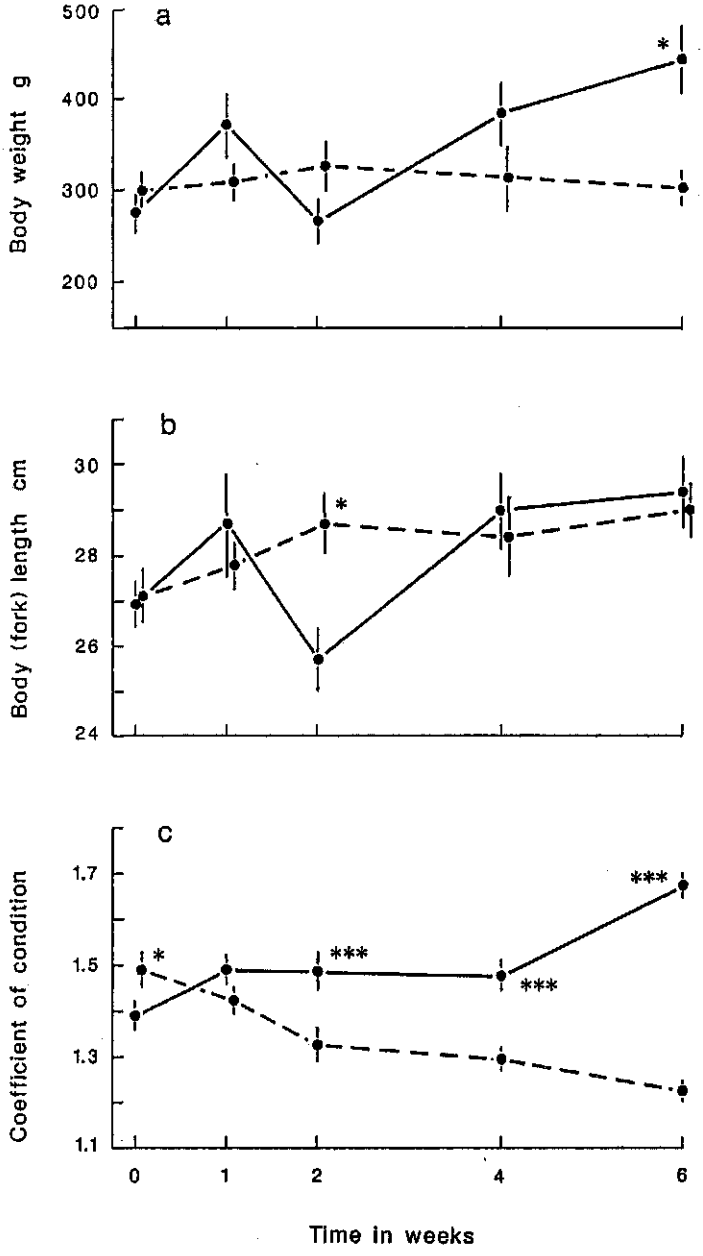

Fig. 3. Changes in a) body weight b) body length and c) coefficient of condition of 1+ rainbow trout during 6 weeks of starvation. Broken lines represent the starved fish, continuous lines the fed, control fish. Each point is the mean ± SEM (n = 20). Asterisks denote significant differences between fed and starved fish at each sampling time (* $p < 0.05$, *** $p < 0.001$).

Blood cortisol levels were not affected by the period of starvation and remained below 1 ng ml^{-1} in both groups of fish (Fig. 4a).
Inspection of the raw data for plasma GH revealed that seven out of the total of 560 fish were extremely small (less than 100 g), had a coefficient of condition of < 1.0 and had highly elevated GH levels (10 - 100 ng ml^{-1} cf. 0.1 - 10 ng ml^{-1} for the remaining 553 fish). These small, stunted fish (which were dark in appearance and had been identified as 'runts' prior to any

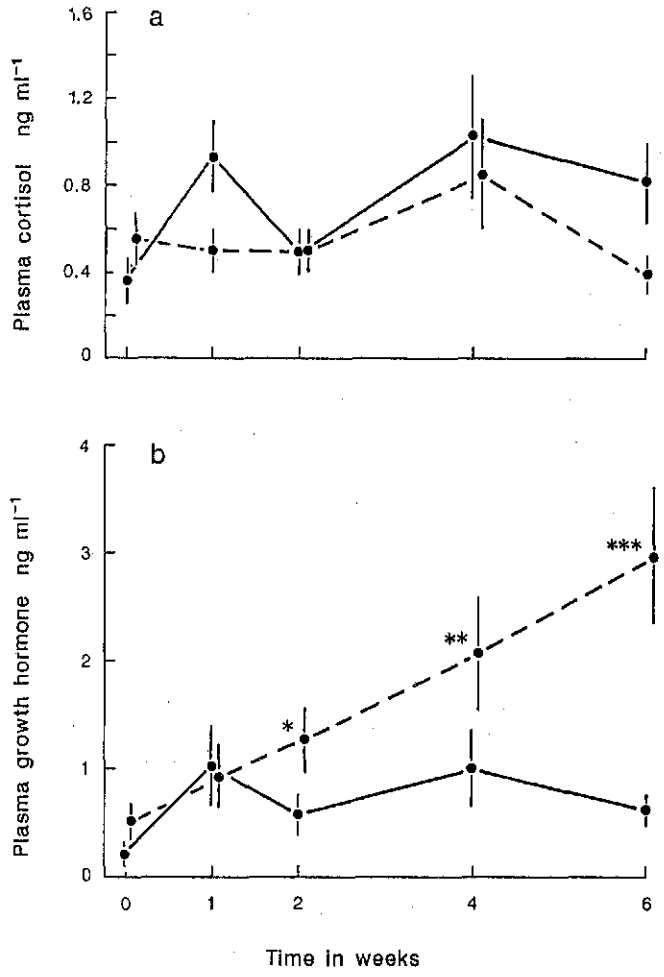

Fig. 4. Changes in a) plasma cortisol and b) plasma GH levels of 1+ rainbow trout during 6 weeks of starvation. Broken lines represent the starved fish, continuous lines the fed, control fish. Each point is the mean ± SEM (n = 20). Asterisks denote significant differences between fed and starved fish at each sampling time (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

hormone determinations) were found in both the fed and starved groups and were eliminated from the statistical analysis in an attempt to improve homogeneity of variance. Analysis of the data for the remaining fish (Fig. 4b) showed that starvation had a clear stimulatory influence on plasma GH levels so that by the end of the experiment GH levels had increased from ≈ 0.5 ng ml^{-1} to 3 ng ml^{-1} ($p < 0.001$). The fed fish showed no such increase during the course of the experiment (Fig. 4b).

Experiment 3. The effects of starvation and chronic confinement on growth hormone levels in 0+ rainbow trout

a) Experimental design

A sample of 12 fish (0 h sample) was taken from each of four outdoor rearing tanks each containing one thousand 0+ rainbow trout (Stirling strain,

mean body weight 25g). A further two hundred and forty rainbow trout of the same age, strain and stocking density were taken from an additional rearing tank and distributed equally into sixteen fry troughs (80 x 40 x 20 cm), each supplied with a constant flow of Windermere lake water (20 l min^{-1}, 11.5 - 16.5°C). This treatment constituted a chronic confinement stress. Food was then withheld from two of the four outdoor rearing tanks and from eight of the sixteen fry troughs the remaining tanks and troughs receiving food, once daily, at the recommended levels.

At 1, 2 and 4 weeks, twelve fish were sampled from each of the four rearing tanks (unconfined fish). At these times, twelve fish were also sampled from each of four different troughs of confined fish (two fed, two unfed) so that no trough was sampled more than once during the course of the experiment. The limited volume of blood obtained from each fish made it necessary to pool samples from groups of four fish, thus giving six pooled plasma samples per treatment at each of the sampling times (three pooled samples from each of two duplicate tank/troughs).

b) Results

Starvation of unconfined fish resulted in a cessation of weight increase (Fig. 5a) and a reduction in the rate of increase in length (Fig. 5c), resulting in a dramatic decrease in the coefficient of condition (Fig. 5e). In the confined fish, both fed and starved 0+ trout showed little increase in weight (Fig. 5b) and a reduced rate of increase in length (Fig. 5d). No significant differences between fed and starved fish in either weight or length could be demonstrated at any time during the four weeks of confinement although the data suggest that the fed, confined fish were just beginning to increase in weight by the end of the experiment (Fig. 5b).

The coefficient of condition of both groups of confined fish fell significantly ($p < 0.001$ in each case) during the first half of the experiment but, by the end of the study, the K factor of the fed fish had clearly begun to recover (Fig. 5f). By comparison, the confined, starved fish showed a continuous decrease in K factor, similar to that of the unconfined, starved fish (cf. Fig. 5e and 5f). Taken together, these data indicate that starvation produced a suppression of growth throughout the experimental period whereas confinement caused growth suppression during the first two weeks but, as the fish acclimated to the conditions, growth recommenced during the second two weeks in those confined fish with access to food.

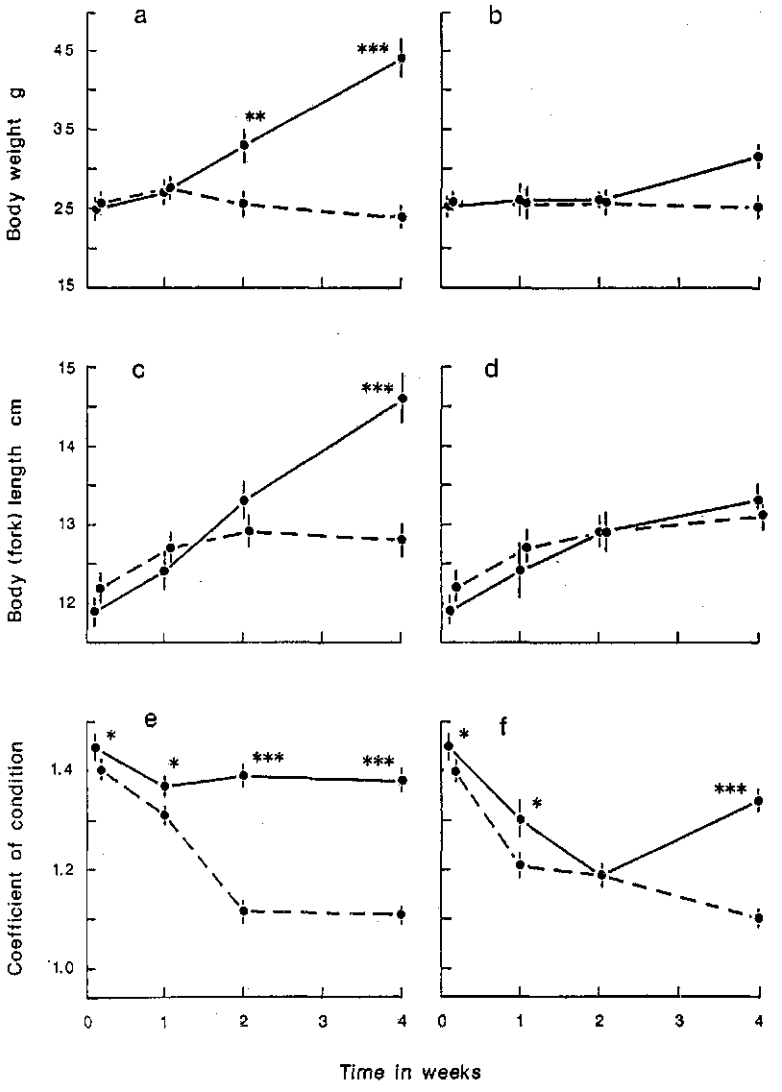

Fig. 5. Changes in a) and b) the body weight, c) and d) the body length, e) and f) the coefficient of condition of unconfined and confined 0+ rainbow trout during 4 weeks of starvation. Broken lines represent the starved fish, continuous lines the fed, control fish. Each point is the mean ± SEM (n = 24). Asterisks denote significant differences between fed and starved fish at each sampling time (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

In contrast to the results of Experiment 2 on the effects of starvation on 1+ rainbow trout, starvation of unconfined 0+ rainbow trout caused a significant elevation of plasma cortisol levels ($p < 0.01$) for at least two weeks after food withdrawal (Fig. 6a). Plasma growth hormone levels of the starved fish also increased, from a mean of 1.3 ng ml^{-1} to almost 8 ng ml^{-1} ($p < 0.001$), whereas in the fed fish GH levels stayed at ≈ 1 ng ml^{-1} (Fig. 6c).

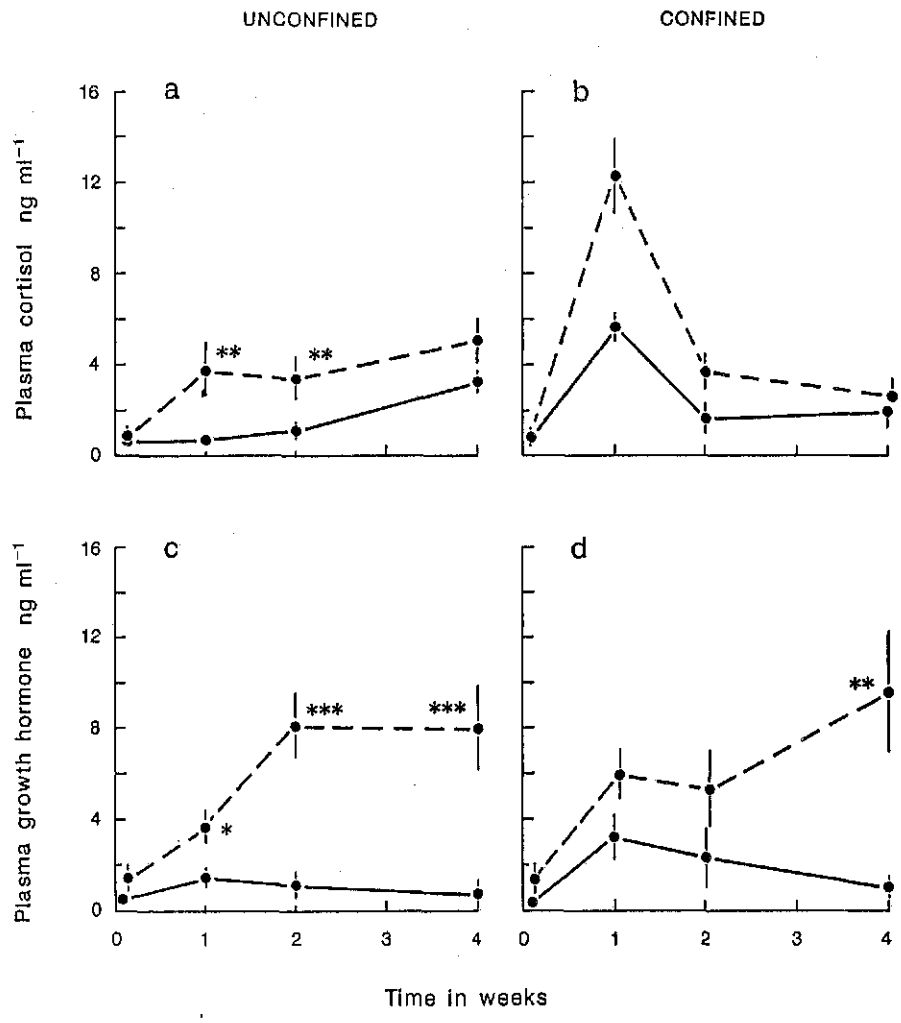

Fig. 6. Changes in a) and b) plasma cortisol levels and c) and d) plasma GH levels of 0+ rainbow trout during 4 weeks of starvation. Broken lines represent the starved fish, continuous lines the fed, control fish. Each point is the mean ± SEM (n = 24). Asterisks denote significant differences between fed and starved fish at each sampling time (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Confinement caused a significant elevation of blood cortisol levels after one week in both the starved and fed fish ($p < 0.001$ and < 0.01 respectively, Fig. 6b). In both groups, cortisol levels then returned to basal levels. This pattern of acclimation to the stress of chronic confinement supports the interpretation of the growth data for this part of the experiment (see above). Plasma GH levels in the confined, starved fish showed a progressive increase during the course of the study, similar to that shown by the unconfined, starved fish (cf. Fig. 6c and 6d). In the fed, confined fish, plasma GH showed an initial increase (to ≈ 3 ng ml^{-1}) during the first week of confinement ($p < 0.05$) but this then declined to basal levels (≈ 1 ng ml^{-1}) as the fish acclimated to the stress of chronic confinement and resumed growth.

Experiment 4. The effect of chronic crowding stress on growth hormone levels in 1+ rainbow trout

a) Experimental design

Four outdoor tanks were each stocked with 650 1+ rainbow trout (Annandale strain, mean body weight 150 g) to give an initial stocking density of 70 g l^{-1}. The fish were left for a period of 3 weeks to acclimate to the new conditions and then eight fish were sampled from each of the four tanks (December 1986). After this initial sample, fish were removed from two of the tanks to give a final stocking density of 25 g l^{-1} (uncrowded) and fish were added to the remaining two tanks to give a final stocking density of 100 l^{-1} (crowded). The stocking densities were then maintained close to these levels (Fig. 7b) by periodic adjustments to the number of fish in each tank during the next 9 months. The fish were fed once daily with commercial trout pellets at the rates recommended by the manufacturers (exact rate dependent upon fish size and water temperature - see Fig. 7a) and mortalities were recorded on a daily basis. Oxygen levels within each tank were measured at several different times during this study using a Yellow Springs Instrument oxygen electrode. At approximately monthly intervals, from December 1986 to September 1987 a sample of eight fish was taken from each of the four tanks.

b) Results

By means of periodic adjustments to the number of fish and the volume of water in each tank the mean stocking density of the control tanks was maintained at 25 g l^{-1}, the chronically-crowded tanks at a density within the range 100-120 g l^{-1} (Fig. 7b). Mortality rates in the control fish were very low (≈ 0.5% per month) whereas the crowded fish had a background mortality rate of ≈ 2% per month (Fig. 7c). However, the cumulative mortality of the crowded fish was punctuated by two instances of major overnight mortality (indicated by the arrows on Fig. 7c).

The first of these occurred at the end of May when a sudden 3°C rise in water temperature, as a result of seiche movements in the lake caused by strong winds, increased the oxygen consumption of the fish and reduced the oxygen carrying capacity of the water. Oxygen levels in the crowded tanks went down to below 30% saturation, causing an overnight mortality of ≈ 13% of the population. Oxygen levels in the uncrowded control tanks were ≈ 50% saturation. Because of this increase in water temperature, aeration devices were installed in all four tanks for the remainder of the study period. Unfortunately, in early August a failure in the air-compressor supplying both crowded and uncrowded tanks caused a further overnight mortality (≈ 10% of the remaining population of crowded fish) as O_2 levels again fell to less than

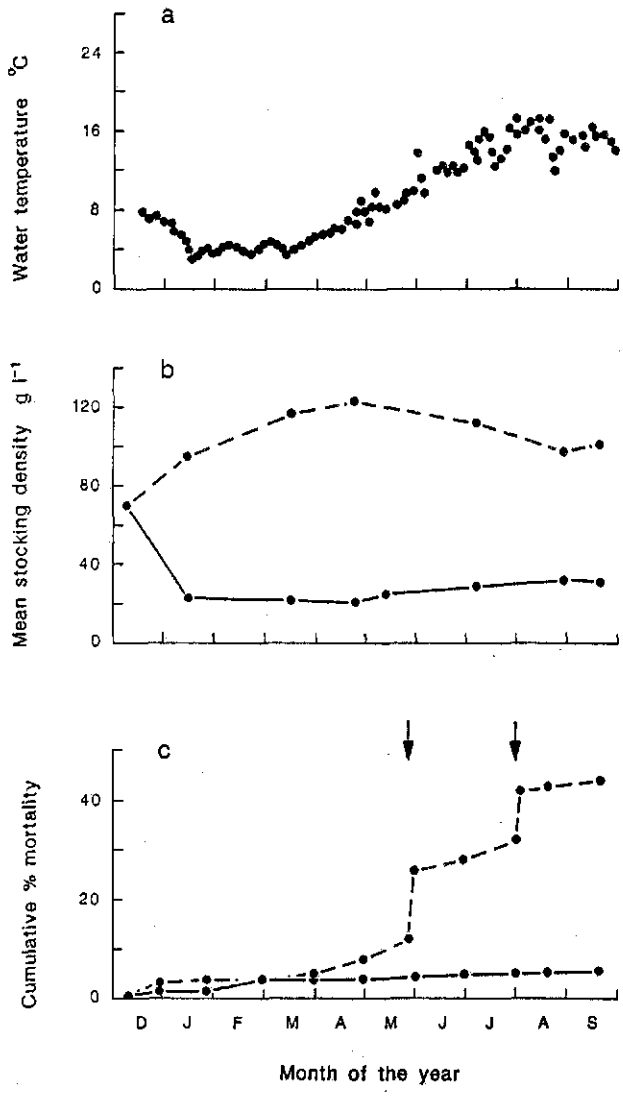

Fig. 7. Changes in a) water temperature b) mean stocking density and c) cumulative % mortality of 1+ rainbow trout during a 10 month period of chronic crowding. Broken lines represent the crowded fish, continuous lines the uncrowded, control fish. The two arrows on Fig. 7c indicate periods of oxygen deficiency (see text for details).

30% saturation in the crowded tanks. Oxygen levels in the uncrowded control tanks were over 60% saturation and no mortalities occurred. The overall effect of these problems was a final cumulative mortality (after correction for sampling losses) of 45% in the crowded tanks compared with only 5% in the uncrowded controls.

Both groups of fish grew during the 10 month study period (Fig. 8a) although the crowded fish showed evidence of a check in growth around the time of the first instance of oxygen deficiency (early May). By September, fish in the uncrowded tanks were significantly heavier than those in the crowded tanks (Fig. 8a, $p < 0.01$). Thus, chronic crowding caused growth suppression despite

both crowded and uncrowded fish having access to identical rations. The data for body length showed very similar characteristics to the weight data (cf. Fig. 8a and 8b).

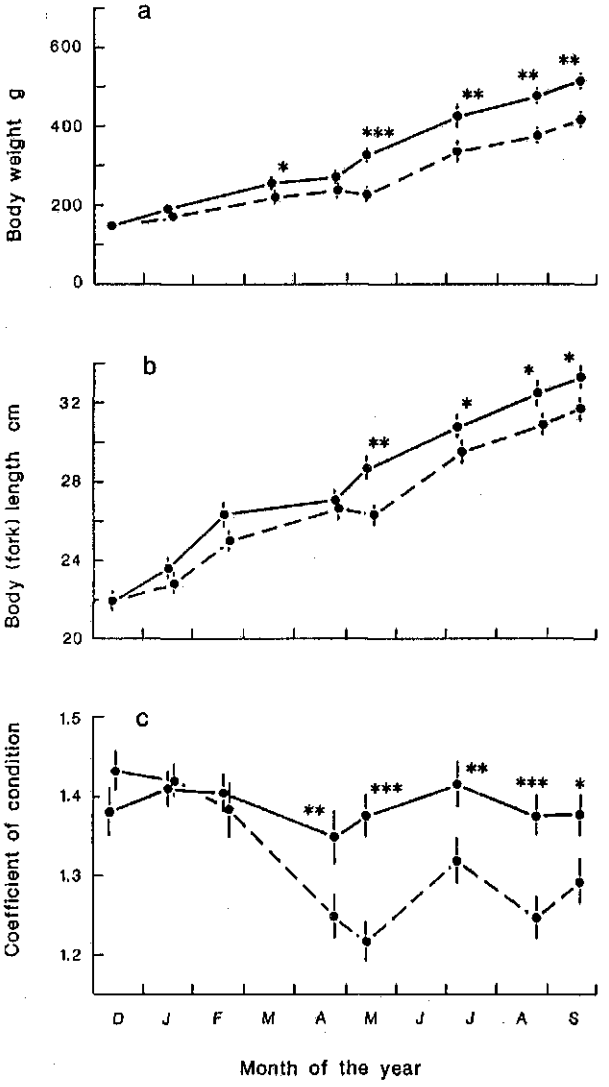

Fig. 8. Changes in a) body weight, b) body length and c) coefficient of condition of 1+ rainbow trout during a 10 month period of chronic crowding. Broken lines represent the crowded fish, continuous lines the uncrowded, control fish. Each value is the mean ± SEM (n = 16). Asterisks denote significant differences between crowded and uncrowded fish at each sampling time (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

The coefficient of condition of the uncrowded, control fish remained relatively constant at ≈ 1.4 throughout the study whereas in the crowded tanks the mean K factor dropped progressively to a low of ≈ 1.2 in May followed by a slight rise to 1.3 at the end of September (Fig. 8c). The differences in K factor between the fish from crowded and uncrowded tanks were significantly different from

April onwards. Thus, the growth suppression of the crowded fish (as indicated by changes in weight and length) was accompanied by a general reduction in the coefficient of condition.

At the start of the experiment, blood cortisol concentrations were at basal levels (< 1 ng ml^{-1}) but increased significantly in both crowded and uncrowded fish until late April ($p < 0.001$ in each case, Fig. 9a). It was not

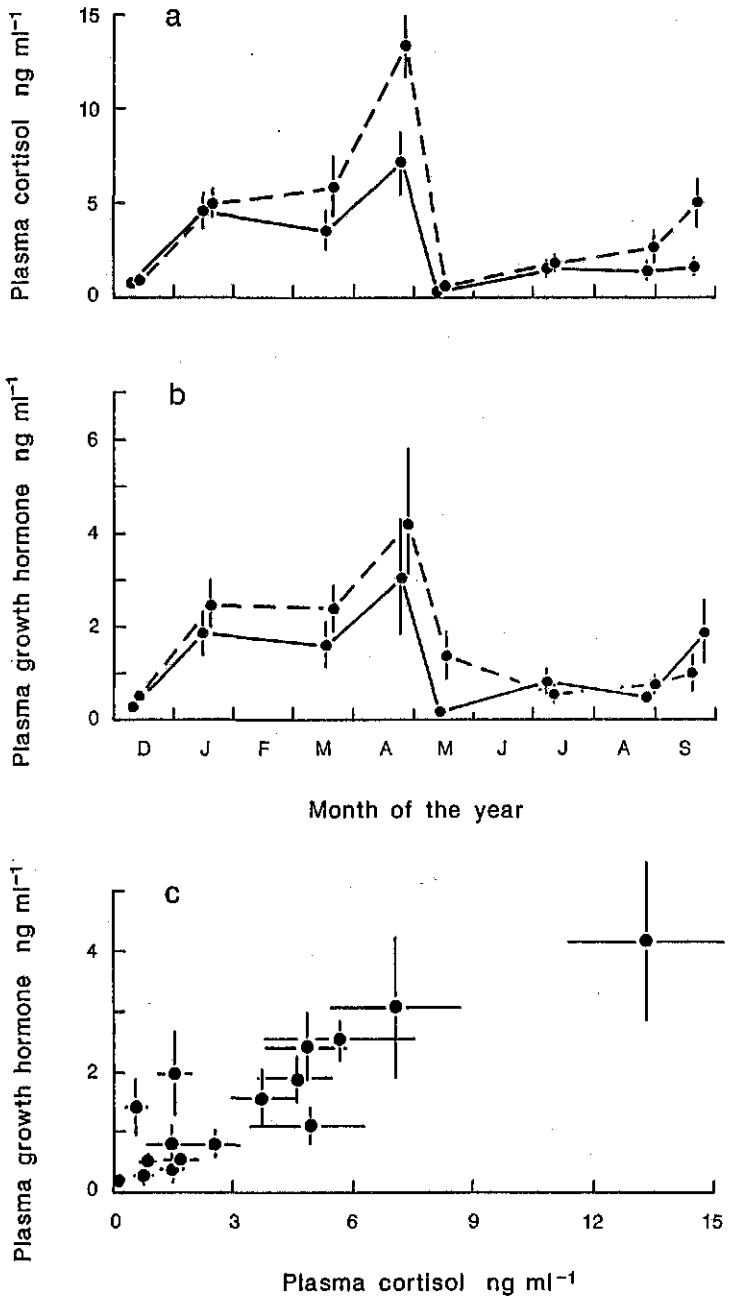

Fig. 9. Changes in a) plasma cortisol and b) plasma growth hormone levels in 1+ trout during a 10 month period of chronic crowding. Broken lines represent the crowded fish, continuous lines the uncrowded, control fish. Each value is the mean ± SEM (n = 16). c) illustrates the strong, positive correlation between mean plasma cortisol and growth hormone levels in a) and b). Linear regression $y = 0.291x + 0.461$, $p < 0.001$, $r^2 = 77.6\%$.

possible to demonstrate significant differences in cortisol levels between the two treatments at any one sampling time although the peak cortisol levels in late April were almost twice as high in the crowded fish as in the controls (13.4 cf. 7.1 ng ml^{-1}).

It seems likely that the cortisol elevation during the first half of this study reflects a degree of chronic stress in both groups of fish caused by reduced oxygen levels in the tanks as water temperature rose during the spring. This interpretation is strengthened by the fact that, as soon as aeration was provided, cortisol levels in both groups returned to basal values and remained low during the rest of the study. Thus, the fish appeared to be fully acclimated to the stress of crowding per se but both groups (crowded and uncrowded) were subsequently stressed by the deterioration in water quality.

Interestingly, the pattern of change in plasma growth hormone levels matched, almost perfectly, the changes in plasma cortisol (cf. Fig. 9a and 9b). As a result, a strong and highly significant ($p < 0.001$) positive correlation was found between mean plasma cortisol and mean plasma GH levels (Fig. 9c). It is concluded, therefore, that chronic stress during the first half of this study, probably as a result of water quality deterioration, caused an elevation of both plasma cortisol and growth hormone levels. Once the oxygen levels in the tanks were improved by means of additional aeration, both cortisol and GH levels returned to basal values.

Experiment 5. The effect of acute handling/confinement stress on plasma growth hormone levels in 0+ rainbow trout

a) Experimental design

In June 1987 six outdoor rearing tanks were stocked with rainbow trout fry (Stirling strain, mean weight 0.5 g) at a density of 1000 fish $tank^{-1}$. The fish were fed with commercial trout food at the rates recommended by the manufacturers and reared under these conditions until mid-August, by which time the fish had increased in weight to ≈ 10 g. An initial sample (unhandled controls) of twenty-four fish was then taken from one of the five rearing tanks and, at the same time, a further one hundred and sixty fish were evenly distributed into eight small (80 x 40 x 20 cm) troughs each supplied with a constant flow of Windermere lake water (20 l min^{-1}, 16.7°C). At 1, 4, 7 and 24 h post-stress twelve fish were sampled from duplicate confinement troughs (stressed fish) so that no trough was sampled more than once. Thus, the stress consisted of the initial handling/netting followed by confinement for a period of up to 24 h. At each sampling time twenty-four fish were also taken from one of the four remaining stock tanks (unstressed controls) so that each tank was

only sampled once. This experimental design avoided repeated disturbance due to handling, and permitted duplicate confinement troughs to be sampled at each time post-stress. However, there were insufficient facilities available to allow the use of duplicate rearing tanks at each sampling time. In view of the small size of the fish, blood samples were pooled from each of four fish (i.e. six pooled samples for both stressed and control fish at each of the four sampling times post-stress).

b) Results

Acute stress caused an immediate (1 h) increase in the plasma cortisol levels of 0+ rainbow trout from 1.5 ng ml^{-1} to ≈ 9 ng ml^{-1} (Fig. 10a, p 0.001). These elevated cortisol levels were then maintained for the next 24 h,

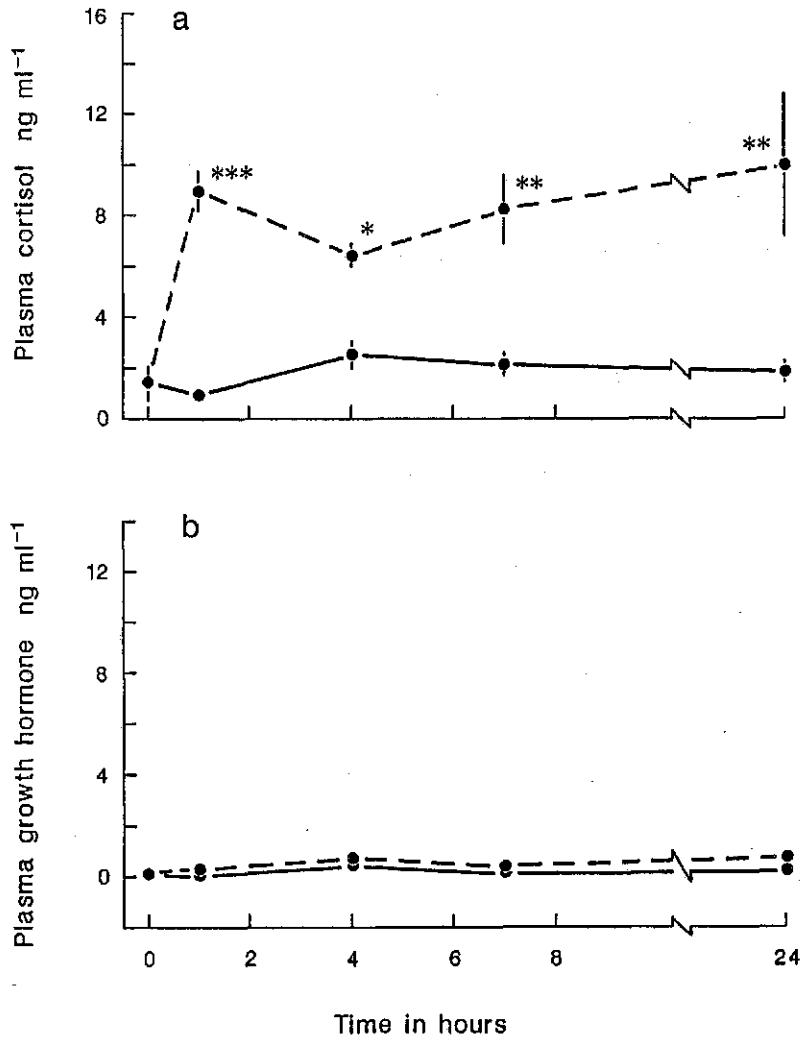

Fig. 10. Changes in a) plasma cortisol and b) plasma growth hormone levels in 0+ rainbow trout during acute handling/confinement stress. Broken lines represent the stressed fish, continuous lines the unconfined, control fish. Each value is the mean ± SEM (n = 24). Asterisks denote significant differences between stressed and unstressed fish at each sampling time (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

at which time the experiment was terminated. By comparison, cortisol levels in the unstressed control fish remained at basal levels (1-2 ng ml^{-1}) throughout the study. Growth hormone levels in both groups of fish were low (< 1 ng ml^{-1}) and at no time was it possible to demonstrate any significant difference between the stressed and unstressed fish (Fig. 10b).

Experiment 6. The effect of acute handling/confinement stress on plasma growth hormone levels in 1+ rainbow trout

a) Experimental design

In view of the very limited cortisol response and lack of a growth hormone response in acutely stressed 0+ rainbow trout (see Results for Experiment 5) a further experiment was designed to examine the effects of acute stress on older (1+) rainbow trout. In previous studies with such fish we have consistently observed a much more marked cortisol response to acute stresses such as handling and short-term confinement than was observed in the 0+ fish in Expt. 5. Thirty-six outdoor rearing tanks were each stocked with forty-six 1+ rainbow trout (Home strain, mean weight 207 g) and left for two weeks for the fish to overcome the effects of handling and to acclimate to the new conditions. The fish from eighteen randomly selected tanks were stressed by transferring them to small (80 x 40 x 20 cm) confinement tanks for a period of 1 h and then returned to their own rearing tanks. Each confinement tank was supplied with a constant flow of Windermere lake water (20 l min^{-1}, 9.3°C). The remaining eighteen rearing tanks served as unstressed controls. Six fish were sampled from each of two tanks of stressed fish and two tanks of control fish at 0 h (pre-stress controls), 0.5 h (midway through the confinement period), 1 h (immediately post-confinement) and at 2, 4, 8, 24, 48 and 96 h post-stress. With this experimental design duplicate tanks were used for each treatment at each sampling time and no tank was sampled more than once.

b) Results

Acute handling stress resulted in a rapid elevation of plasma cortisol levels in 1+ rainbow trout, from basal levels of 2 ng ml^{-1} to a peak of 80 ng ml^{-1}, 30 min after the fish were handled and confined (Fig. 11a). When the fish were returned to their rearing tanks (after 1 h of confinement) the cortisol levels dropped during the next 24 h. Cortisol levels in the unstressed control fish remained at basal levels (≈ 2 ng ml^{-1}) throughout the 96 h study period.

Plasma growth hormone levels in both groups of fish were low (< 2 ng ml^{-1}) although analysis of variance revealed a statistically significant effect of stress ($p < 0.01$) which took the form of an overall suppression of

plasma GH levels in the stressed fish (0.66 ± 0.09 ng ml^{-1}, n = 108) compared with the unstressed controls (1.26 ± 0.16 ng ml^{-1}, n = 108). However, no significant treatment - time interaction could be demonstrated (Fig. 11b).

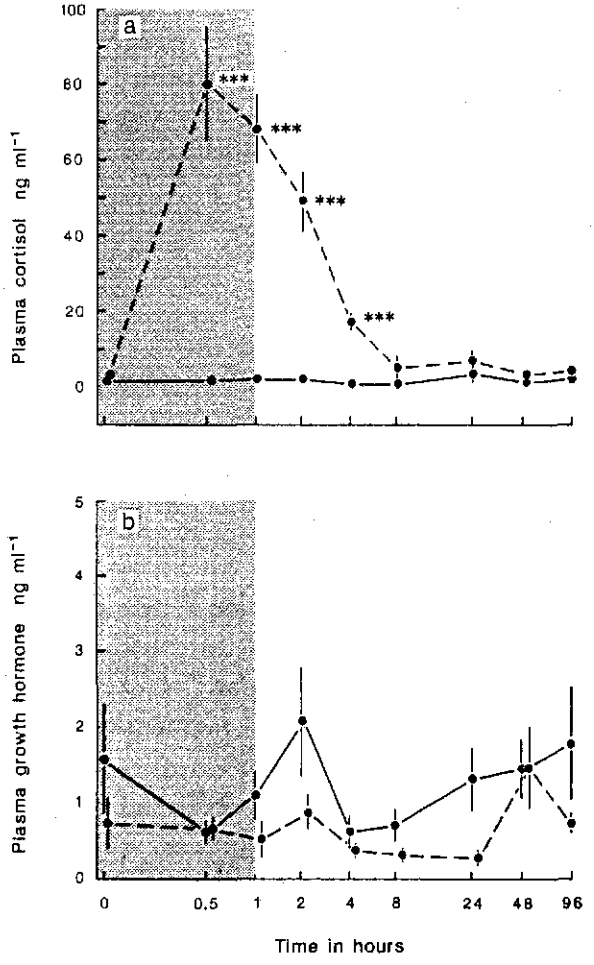

Fig. 11. Changes in a) plasma cortisol and b) plasma growth hormone levels in 1+ rainbow trout during and after acute handling/confinement stress. Broken lines represent the stressed fish, continuous lines the unconfined, control fish. Each value is the mean ± SEM (n = 12). Asterisks denote significant differences between stressed and unstressed fish at each sampling time (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). The shaded area indicates the period of confinement.

Experiment 7. The effect of chronic cortisol implantation on plasma growth hormone levels in 1+ rainbow trout

a) Experimental design

One hundred and twenty 1+ rainbow trout (Annandale strain, mean weight 300 g) were anaesthetized in phenoxyethanol (1:2000) and given an intraperitoneal injection of 10 mg cortisol suspended in 1 ml molten cocoa butter at 35 °C. This produces a solid, slow-release implant in the body cavity which is capable

of maintaining a chronic elevation of plasma cortisol levels over a period of several weeks (Pickering & Duston, 1983). The cortisol-implanted fish were then divided equally between two outdoor rearing tanks, each supplied with a constant flow of Windermere lake water (35 l min^{-1}, 12.5-15.3°C). A further 120 fish were given an intraperitoneal implant consisting of 1 ml cocoa butter only (sham-implanted controls) and, similarly, were divided equally into two outdoor rearing tanks. The fish were then fed, once daily, with commercial trout pellets at the rates recommended by the manufacturers and a pulse of malachite green (2.27 ppm) was administered on a daily basis to prevent fungal infection. At 7, 12, 16 and 22 days post-implantation, five fish were sampled from each of the four tanks (i.e. 10 fish per treatment).

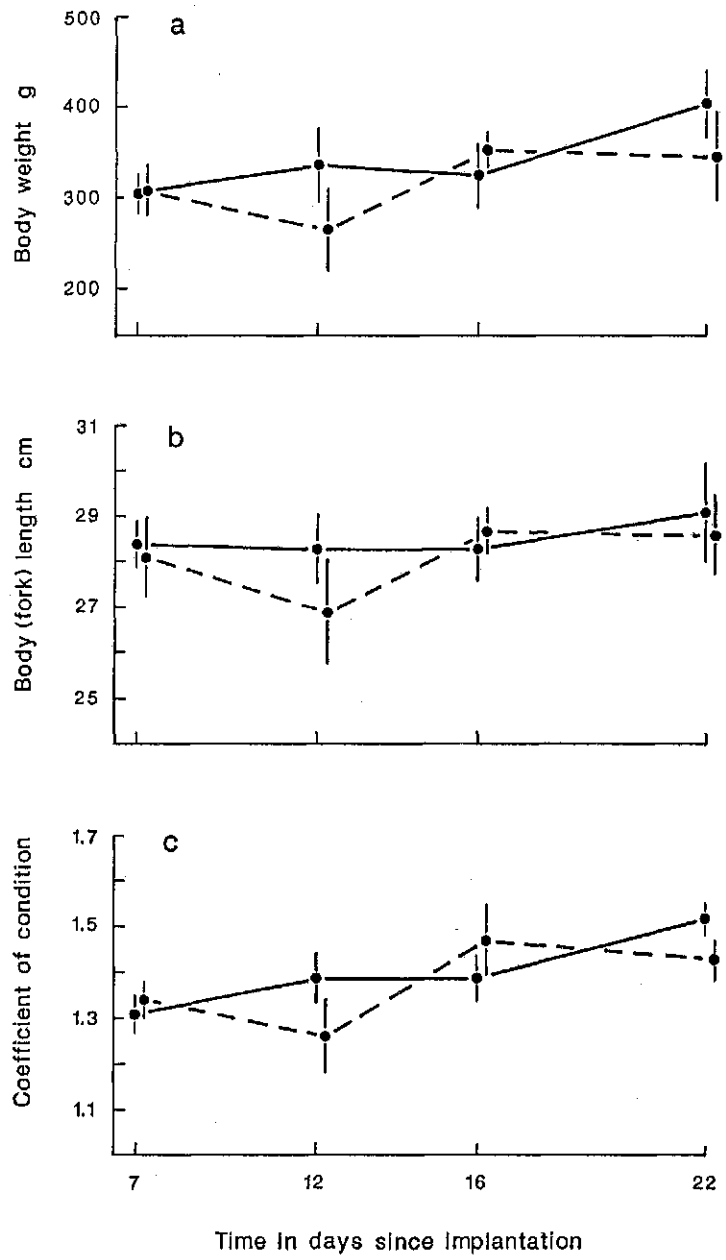

Fig. 12. Changes in a) body weight, b) body length and c) coefficient of condition of 1+ rainbow trout following cortisol implantation. Broken lines represent trout given a 10 mg intraperitoneal implant of cortisol, continuous line the sham-implanted control fish. Each value is the mean ± SEM (n = 10).

b) Results

Intraperitoneal implantation of 10 mg cortisol did not alter the body weight, body length or coefficient of condition of 1+ rainbow trout during 22 days post-implantation (Fig. 12a,b,c).

Cortisol implantation did, however, result in a highly significant ($p < 0.001$), and stable elevation of plasma cortisol levels to ≈ 15 ng ml^{-1}. By comparison, sham-implanted control fish had mean plasma cortisol levels of ≈ 2 ng ml^{-1} (Fig. 13a). A treatment - time interaction ($p < 0.05$) was found in the growth hormone data which appeared as a significant ($p < 0.001$) elevation of plasma GH levels in the cortisol-implanted fish, from < 2 ng ml^{-1} to ≈ 25 ng ml^{-1}, at 12 days post-implantation (Fig. 13b).

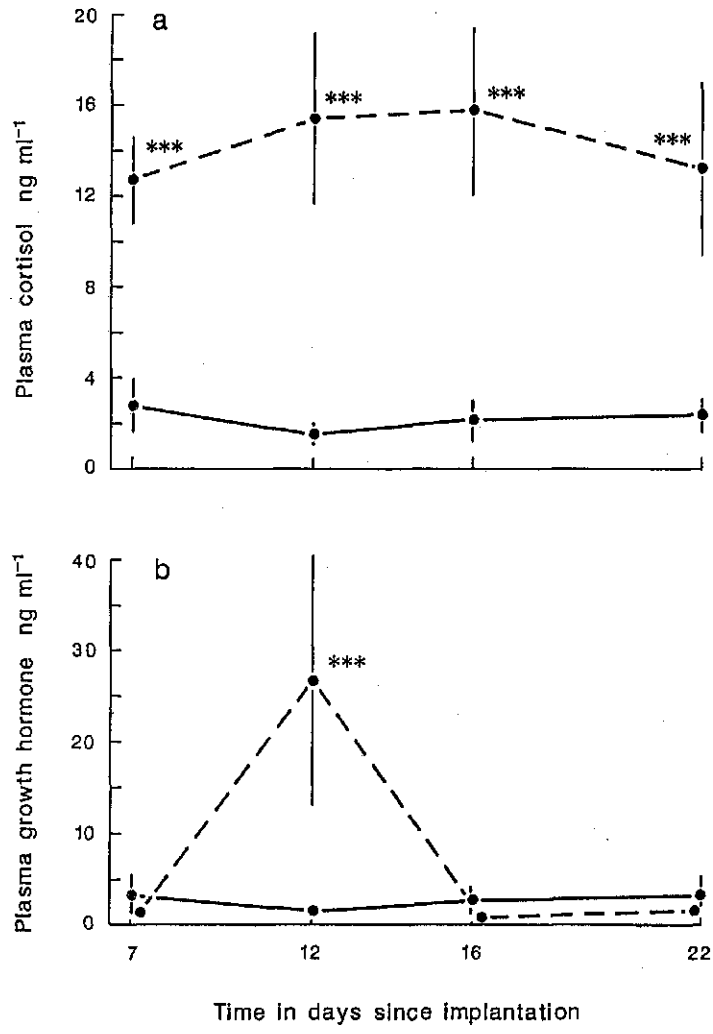

Fig. 13. Changes in a) plasma cortisol and b) plasma growth hormone levels of 1+ rainbow trout following cortisol implantation. Broken lines represent trout given a 10 mg intraperitoneal implant of cortisol, continuous lines the sham-implanted control fish. Each value is the mean ± SEM (n = 10). Asterisks denote significant differences between cortisol-treated and control fish at each sampling time (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

However, no differences in GH levels were detected between either treatment at 7, 16 or 22 days post-implantation (Fig. 13b), thus making it difficult to interpret the elevated GH levels in cortisol-treated fish at 12 days post-implantation. Visual inspection of the raw data showed that the elevation at 12 days was primarily caused by very high GH levels in 3 out of the 10 cortisol-implanted fish sampled at this time (plasma GH 95, 100 and 25 ng ml^{-1}). Interestingly, these 3 fish had K factors of 0.97, 1.06 and 1.10 respectively, all considerably lower than the mean K factor of 1.36 for the 1+ rainbow trout used in this experiment. In view of the significant inverse correlation between GH and K factor observed in several of the experiments (see below), the coefficient of condition was used as a covariate in the analysis of variance to see whether the elevation of GH levels at 12 days post-implantation was explicable in terms of the K factor of the fish sampled at this particular time. Although this covariate increased the probability value (i.e. decreased the level of significance) of both the treatment*time interaction and the difference between means at 12 days post-implantation, both remained at less than 0.05.

It is concluded that experimental elevation of plasma cortisol levels (from 2 to 15 ng ml^{-1}) by means of intraperitoneal implantation did not alter plasma GH levels in 1+ rainbow trout at 7, 16 and 22 days post-implantation. Elevated GH levels in cortisol-implanted fish at 12 days post-implantation were caused, in part, by three abnormal fish (very low K factor, very high GH levels) out of a sample of ten. However, the possibility that chronically-elevated plasma cortisol levels caused a temporary elevation of plasma GH levels cannot be discounted.

Correlation between plasma growth hormone levels and the coefficient of condition

In several of the experiments undertaken during the course of this investigation it was observed that occasional slow-growing, stunted fish with very low coefficients of condition were characterized by extremely high plasma growth hormone levels (up to 100 ng ml^{-1}, see for example Experiments 2 and 7). This prompted a reanalysis of the data to see whether this apparent inverse association between growth hormone levels and K factor was an overall feature of the population or whether it was simply a characteristic of the few obvious 'runts' in each experiment. Regression analysis revealed a significant inverse correlation between the logarithm of the growth hormone concentration and the coefficient of condition in five of the experimental populations used in this study (Figs 14 and 15).

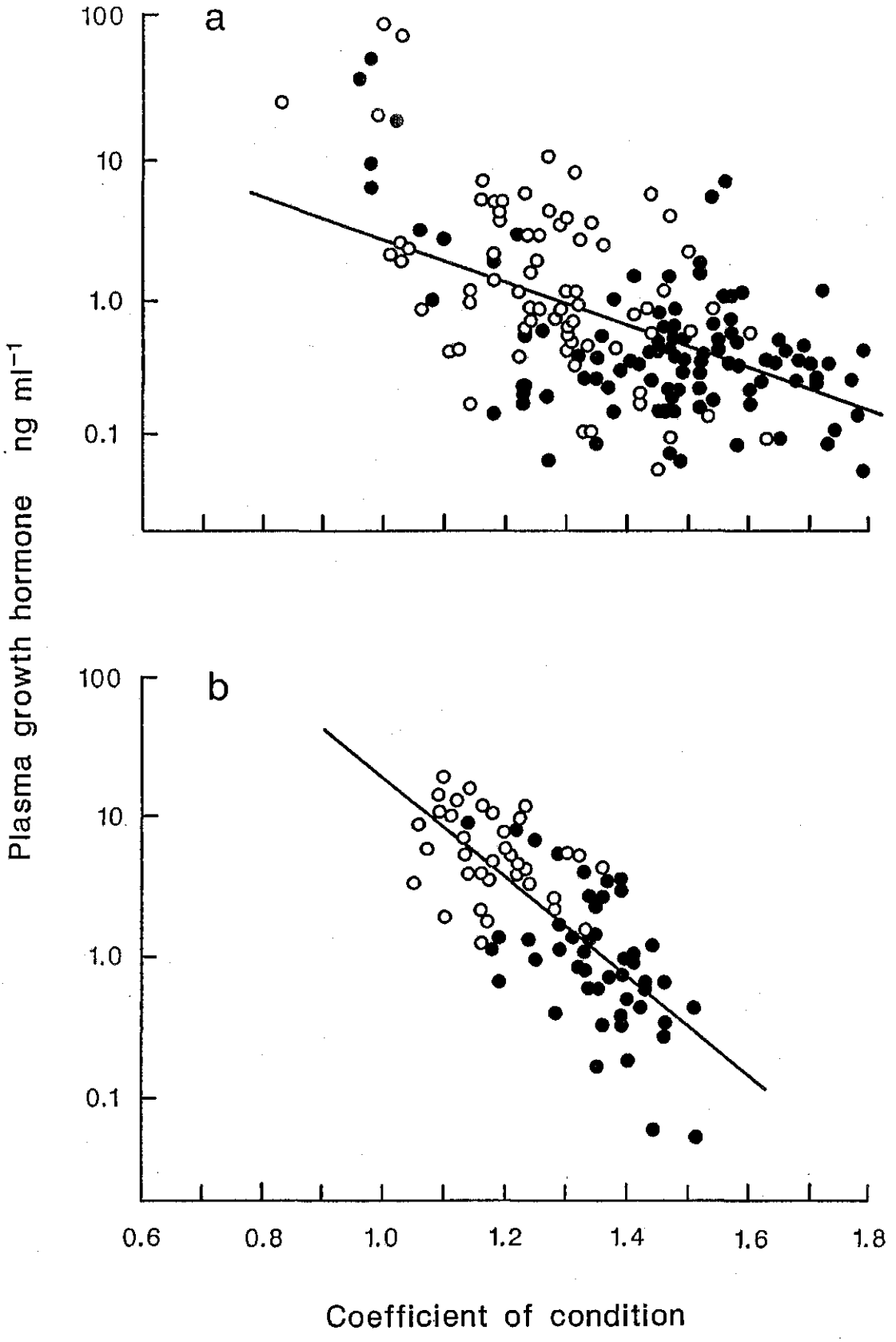

Fig. 14. Inverse correlation between the logarithm of plasma growth hormone concentration and coefficient of condition of a) starved and fed 1+ rainbow trout from Experiment 2 and b) starved and fed unconfined 0+ rainbow trout from Experiment 3. Fed fish are represented by the solid circles, starved fish by the open circles. The following regression equations were obtained:-

a) LogGH = -3.65 KF + 4.68 r^2 = 25.5%, $p < 0.001$
b) LogGH = -8.26 KF + 11.2 r^2 = 54.3%, $p < 0.001$

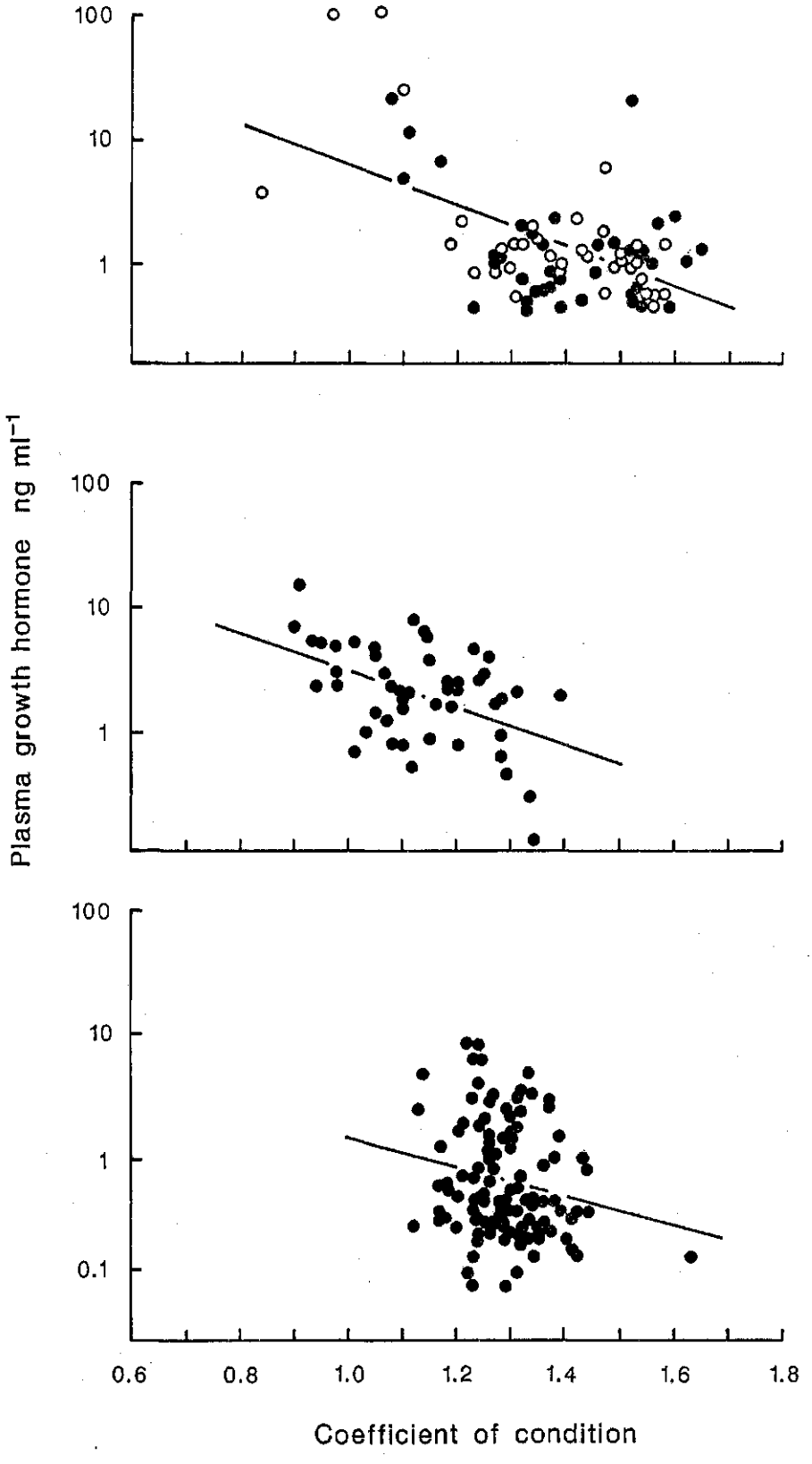

Fig. 15. Inverse correlation between the logarithm of plasma growth hormone concentration and coefficient of condition of a) cortisol-implanted and control 1+ rainbow trout from Experiment 7, b) 1+ rainbow trout (New Zealand strain) from Experiment 1 and c) unstressed, control 1+ rainbow trout from Experiment 6. Solid circles represent unstressed, control fish, open circles represent rainbow trout given a 10 mg intraperitoneal implant of cortisol. The following regression equations were obtained:-

a) LogGH = -3.70 KF + 5.50 r^2 = 28.1%, $p < 0.001$
b) LogGH = -3.37 KF + 3.37 r^2 = 20.7%, $p < 0.001$
c) LogGH = -2.72 KF + 3.12 r^2 = 3.9%, $p < 0.05$

DISCUSSION

One of the first facts to emerge from this study was the relatively low concentration of growth hormone in the blood plasma of ostensibly healthy rainbow trout (range of mean plasma GH levels in seven different strains of trout 0.5–3.5 ng ml^{-1}). These levels are approximately an order of magnitude lower than those measured by Cook & Peter (1984) in goldfish serum (15–30 ng ml^{-1}), by Wagner & McKeown (1986) in rainbow trout plasma (20–85 ng ml^{-1}), by Bolton et al. (1987) in the blood of coho salmon smolts (≈ 40 ng ml^{-1}) and by Bjornsson et al. (in press) in Atlantic salmon smolts (17 ng ml^{-1}). However in a very recent paper, Barrett & McKeown (in press) reported growth hormone levels, of only 6 ng ml^{-1} in fed, control steelhead trout, Salmo gairdneri. For several reasons it seems unlikely that the low GH levels found during the present study resulted from problems when applying a radioimmunoassay developed against chinook salmon (Oncorhynchus) GH to rainbow trout (Salmo) plasma. Firstly, Wagner & McKeown (1986), using an RIA developed against chum salmon (Oncorhynchus) GH, measured high GH levels in rainbow trout plasma. Secondly, there is a remarkable degree of homology between salmonid growth hormone molecules (see Agellon & Chen, 1986; Nicholl et al., 1987). Thirdly, application of Le Bail's chinook GH assay to coho salmon (Oncorhynchus) plasma revealed similar low GH levels to those reported in the present study for rainbow trout (J.P. Sumpter, unpublished results). We suggest that these low plasma GH levels reflect the degree of purity of the GH standard used in the assay, and, probably, the nutritional status of the fish and are more likely to represent the true situation for unstressed, well-fed fish than are some of the higher levels reported by other workers. Full details of the protocol and validation of the chinook GH assay can be found in Le Bail et al. (in preparation).

In mammals and birds, GH secretion may be markedly episodic (see e.g. Terry et al., 1977; Valilatos-Younken & Zarkower, 1987). It is not possible from the present study to determine whether episodic GH release is characteristic of the rainbow trout (to do this would require cannulation of the blood vessels of individual fish). However, if episodic GH secretion was occurring in our experimental fish, we might have expected to see a greater degree of variability in the data. Much of the individual variation that did occur could be related to the condition of the fish and did not appear to be the result of episodic release of the hormone. Further studies are needed to resolve this question.

Marked and highly significant differences in plasma GH levels were found between the different strains of rainbow trout used during our initial survey.

Overall, there was a broad inverse correlation between plasma growth hormone levels and the mean growth rate of the fish. On the face of it this observation might seem somewhat surprising. However, the most rapid phase of body growth in young rats occurs during a time when both pituitary and plasma levels of GH are lowest (Muller, 1975) and faster-growing lines of chickens have been consistently found to have lower circulating concentrations of GH than slower-growing lines (Burke & Marks, 1982; Stewart & Washburn, 1983). Further studies on target tissue sensitivity and hormone utilization rates might well explain this paradox.

Food withdrawal caused a sustained and highly significant rise in the plasma growth hormone levels of both 0+ and 1+ rainbow trout. This is consistent with the work of Wagner & McKeown (1986) and Barrett & McKeown (in press) and supports the earlier cytological and ultrastructural studies of Olivereau (1970) and Gas (1975) on the somototropes of starved carp. However McKeown et al. (1975), using a heterologous GH radioimmunoassay developed against bovine growth hormone, were unable to detect any changes in plasma "growth hormone" levels in starved, fingerling kokanee salmon, Oncorhynchus nerka.

In humans, a slow plasma GH rise has been reported during prolonged fasting but, according to Muller (1975) "the great variability in GH levels observed in a careful metabolic and hormonal evaluation of prolonged starvation in the human casts some doubt on its importance as a metabolic regulator during fasting". It is apparent that the effects of GH on metabolism in mammals are complex and that many of the available data are often contradictory (see review by Davidson, 1987). Nevertheless, an important component of the normal metabolic adaptation to prolonged starvation is accelerated lipolysis, and the administration of mammalian growth hormone preparations to salmonid fish decreases muscle lipid content (Higgs et al., 1976) and increases muscle free fatty acids (McKeown et al., 1975). The precise role(s) of growth hormone during prolonged starvation in fish awaits further elucidation.

Food withdrawal during the present study caused, as expected, an immediate cessation of increase in weight but the rate of increase in length did not slow down for a period of a week or two. This continued growth (with respect to length), during a period of rising plasma growth hormone levels, resulted in a rapid decline in the coefficient of condition of the fish. Interestingly, a similar increase in length relative to weight can be seen when feeding fish are treated with exogenous GH (Chartier-Baraduc, 1959; Komourdjian et al., 1976; Higgs et al., 1976, 1978; Markert et al., 1977; Gill et al., 1985). Thus, the decrease in coefficient of condition during starvation may be exaggerated by elevated blood growth hormone levels.

Acute stress, in the form of handling and short-term confinement, had relatively little effect on plasma GH levels of 0+ rainbow trout and 1+ rainbow trout. Twenty four hours of confinement did not significantly alter the GH levels of 0+ rainbow trout (which remained below 1 ng ml^{-1} throughout the whole experimental period) despite a measurable stress response, in terms of blood cortisol levels, in these fish. Acute handling followed by 1 h confinement caused a slight but significant suppression of blood GH levels of 1+ rainbow trout during the next 24 h. However, the results of this second experiment on the effects of acute stress were somewhat equivocal in that we were unable to demonstrate any significant treatment * time interaction in the plasma GH levels despite a highly significant treatment * time interaction for the cortisol data from these fish. Certainly, in neither of the acute stress experiments was there any evidence of an increase in growth hormone secretion in response to the stress. This contrasts with the conclusions of Cook & Peter (1984) who found that the stress of injection caused an increase in serum GH at 24 h post-stress in the goldfish. However, Wagner & McKeown (1986) did not find any effects of handling stress on the circulating growth hormone levels in rainbow trout although sustained exercise for a period of 24 h caused an increase in plasma GH levels of both fed and starved steelhead trout (Barrett & McKeown, in press). Acute stress can result in an increase in blood GH levels in primates (Muller, 1975) but suppresses GH release in rats (Terry et al., 1977). Thus, there are marked species differences in the response of the pituitary somatotropes to environmental stress and further studies with other groups of teleost fish are indicated.

Chronic stress, in the form of long term (4 wk) confinement of 0+ rainbow trout and severe crowding (9 month) of 1+ rainbow trout, did significantly alter plasma GH levels. In both cases this took the form of an increase in circulating growth hormone levels which was correlated with elevated cortisol levels and coincided with suppressed growth and a reduced coefficient of condition. Moreover, a significant inverse correlation between growth hormone levels and coefficient of condition was demonstrated with the control groups of fish for several of the experiments in this investigation. The experimental design did not allow us to control food intake in individual fish although both chronically stressed and unstressed fish were given identical rations. Thus, it was not possible to determine whether growth rate suppression was mediated by the hormonal changes or whether these changes were consequent to reduced food intake and suppressed growth. However, from a consideration of the effects of starvation on growth and GH levels in otherwise unstressed fish, it seems probable that food intake was reduced in the chronically stressed fish

and that this then resulted in the elevated plasma GH levels. The catabolic effects of elevated cortisol levels in the stressed fish almost certainly exacerbated the problems of reduced food intake. In both chronic stress experiments the fish eventually acclimated to the conditions and cortisol levels came down. At the same time, blood GH levels returned to normal. There was no evidence whatsoever that chronic stress caused any prolonged suppression of blood growth hormone levels and, therefore, our original working hypothesis that "growth suppression in stressed fish may be caused by a suppression of pituitary growth hormone secretion" must be rejected. Indeed, it would seem that in chronically stressed fish, particularly when blood cortisol levels are elevated, blood growth hormone levels are likely to be higher than in the unstressed control fish.

A similar correlation between reduced growth rate and blood GH elevation has been observed in the phenomenon of 'stunting' in both coho and Atlantic salmon smolts (Bolton et al., 1987b; Bjornsson et al., in press). Premature transfer of potential smolts to seawater results in a prolonged inhibition of somatic growth which is coupled with markedly elevated circulating GH levels. If such fish are then returned to fresh water, they recommence growth and blood GH levels return to normal. It has been proposed, therefore, that the problem of suppressed growth in such stunts is related to target tissue sensitivity rather than the rate of pituitary GH secretion, a suggestion which is supported by the observation that GH binding in membrane preparations from the liver and gills of coho stunts was deficient when compared with similar preparations from normal smolts (Fryer & Bern, 1979). Clearly, much more information is now needed concerning the mechanisms of action of salmonid growth hormone in both normal and chronically-stressed fish and the possible role of insulin-like growth factors or somatomedins.

The close correlation between mean plasma cortisol and mean plasma growth hormone levels in chronically stressed 1+ rainbow trout calls for some comment on the possible links between the hypothalamic-pituitary-interrenal axis and hypothalamic-somatotrope activity in fish. In general, data for this area of teleost endocrinology are sparse and fragmentary. Olivereau & Olivereau (1968) found that adrenalectomy caused an activation of the somatotropes in the eel pituitary gland but, because similar responses were found in sham-operated fish, they concluded that the somatotrope response was a non-specific response to the stress of surgery. Ball & Hawkins (1976) found that mammalian GH preparations could elevate blood cortisol levels in hypophysectomized Poecilia but the purity of the hormone preparations was questionable. Similarly, Higgs et al. (1977) showed that bovine GH increased the interrenal nuclear diameter

of coho salmon. Cortisol increases the *in vitro* secretion of GH from tilapia pituitaries (Nishioka *et al.*, 1985), a result similar to that reported for human pituitary cell monolayers (Nakagawa *et al.*, 1985) and for incubated rat pituitary glands (Nakagawa *et al.*, 1987). However, the bulk of the evidence from *in vivo* studies suggests that corticosteroids suppress GH levels in mammals although their actions are complex and biphasic, with both stimulatory and suppressive components (Ceda *et al.*, 1987; Casanueva *et al.*, 1988). In the present investigation, chronic implantation of cortisol to produce elevated levels of the hormone within the physiological range ($\approx$ 15 ng ml^{-1}) did not alter plasma GH levels in 1+ rainbow trout at 7, 16 and 22 days post-implantation. Thus, it is unlikely that the elevated levels of plasma GH in chronically stressed fish were caused by increased blood cortisol levels.

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