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Effects of Acute and Chronic Stress on the Levels of Circulating Growth Hormone in the Rainbow Trout, *Oncorhynchus mykiss*

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The acute stress of handling followed by confinement for a period of 1 or 24 hr caused a typical stress response in rainbow trout (elevation of plasma ACTH and cortisol) and a significant reduction in the concentration of circulating growth hormone. The chronic stress of low oxygen levels in both crowded and uncrowded tanks of fish caused a significant elevation of circulating GH levels, an effect which was abolished by the provision of additional aeration to the rearing tanks. This chronic elevation of GH levels was closely correlated with an elevation of plasma cortisol in the same fish. These findings are discussed in relation to stress-induced growth suppression and to the links between the hypothalamic–pituitary–interrenal axis and somatotrope activity.

Growth suppression in teleost fish is a consequence of most forms of environmental stress (Pickering, 1990), but little is known about the physiological and endocrinological mechanisms behind this response. An almost invariable reaction of fish to environmental stress is the secretion of catecholamines and corticosteroids into the blood stream, hormones responsible for the mobilization of energy reserves as the fish attempts to avoid or overcome the immediate threat (see Mazeaud and Mazeaud, 1981; Donaldson, 1981). Undoubtedly, the catabolic effects of these two groups of hormones (the so-called 'stress-hormones') are responsible for some of the observed growth suppression. Indeed, cortisol treatment causes a clear suppression of somatic growth in the rainbow trout (Barton *et al.*, 1987) and in the channel catfish, *Ictalurus punctatus* (Davis *et al.*, 1985). However, of the other hormones known to influence fish growth, pituitary growth hormone (GH) is one of the most potent. Natural or recombinant forms of mammalian, avian, and teleost GH's are all extremely effective stimulators of growth in fish (see review by Weatherley and Gill, 1987) and, therefore, the possibility arises that growth suppression in stressed fish may also be mediated by reduced GH secretion and/or increased clearance leading to a lowering of plasma GH levels. This hypothesis is not without its support from the literature because Terry *et al.* (1977) have shown that GH secretion in the rat may be suppressed for up to 5 hr following a 30-min stress (forced swimming).

The recent development and validation of teleost GH radioimmunoassays (Cook *et al.*, 1983; Bolton *et al.*, 1986; Wagner and McKeown, 1986; Le Bail *et al.*, 1991) now opens up this field of stress physiology in teleost fish to practical investigation. The present study was designed to investigate the effects of both acute and chronic stresses on the circulating levels of GH in the rainbow trout, the magnitude of the

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stress response being determined by the measurement of plasma ACTH and cortisol.

MATERIALS AND METHODS

Experimental fish. All the fish used in this study were 1+ rainbow trout (i.e., fish in their second year of growth) which had been reared from ova at the IFE's experimental fish hatchery. It was not practicable to use the same strain of fish throughout the investigation and details of the various strains used are given for each experiment. The fish were reared in large (1500 liter), outdoor, fibreglass tanks each supplied with a constant flow of Windermere lake water (35 liters min^{-1}) and, with the exception of Experiment 3, oxygen levels in each tank were maintained close to saturation. 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).

Experiment 1: The effect of 1 hr handling/confinement stress. Thirty-six rearing tanks were each stocked with forty-six 1+ rainbow trout (Home strain, mean weight 207 g) and left for 2 weeks for the fish to overcome the effects of handling (Pickering *et al.*, 1982) and to acclimate to the new conditions. During this experiment, the water temperature was in the range 9.1 to 10.2°. The fish from 18 randomly selected tanks were stressed by transferring them to small (80 × 40 × 20 cm) confinement tanks for a period of 1 hr and then returned to their own rearing tanks. Each confinement tank was supplied with a constant flow of lake water (20 liters min^{-1}). The remaining 18 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 hr (prestress controls), 0.5 hr (midway through the confinement period), 1 hr (immediately postconfinement), and at 2, 4, 8, 24, 48, and 96 hr poststress. With this experimental design, duplicate tanks were used for each treatment at each sampling time and no tank was sampled more than once. At each sampling time the fish were rapidly anaesthetized (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 min. Each fish was then killed by a blow to the head, weighed, measured, and sexed. Plasma samples were prepared by centrifugation at 4° and stored at −70° until analysed for cortisol and GH.

Experiment 2: The effect of 24 hr handling/confinement stress. Twenty-four rearing tanks were each stocked with 40 1+ rainbow trout (Annandale strain, all female stock, mean weight 473 g) and left for two weeks to overcome the initial handling stress and to acclimate to the new conditions. During this experiment, the water temperature was in the range 15.1–17.0°. Six fish from each of 12 randomly selected rearing tanks were then confined in small tanks (see above for details) for periods of 0, 1, 4, 8, 12, and 24 hr. At the end of each confinement period, the fish were rapidly anaesthetized and plasma samples were prepared as in Experiment 1. However, EDTA was used as the anticoagulant because plasma ACTH levels were also measured in these fish and heparin is known to interfere with the ACTH radioimmunoassay (Sumpter and Donaldson, 1986). At each sampling time, 6 unstressed control fish were also taken from 2 of the remaining 12 rearing tanks. Thus, duplicate tanks were used at each sampling time and no tank of fish was sampled more than once.

Experiment 3: The effect of chronic crowding stress. Four outdoor rearing tanks were each stocked with 650 1-year-old rainbow trout (Annandale strain, mixed sexes, mean initial body weight 150 g) to give an initial stocking density of 70 g/$liter^{-1}$. The fish were left for a period of 3 weeks to acclimate to the new conditions and then 8 fish were sampled from each of the four tanks (December). After this initial sample, fish were removed from two of the tanks to give a final stocking density of 25 g/$liter^{-1}$ (uncrowded) and fish were added to the remaining two tanks to give a final stocking density of 100 g/$liter^{-1}$ (crowded). These densities were then maintained by periodic adjustments to the number of fish in each tank during the next 9 months, to compensate for fish growth, mortalities, and sampling losses. 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 (3.5–17.1°) and mortalities were recorded on a daily basis. At approximately monthly intervals from December to September, a sample of 8 fish was taken from each of the four tanks. The fish were rapidly anaesthetized, plasma samples were prepared (see above) for cortisol and GH determination, and the fish were then killed by a blow to the head, weighed, measured, and sexed.

Hormone measurements. All hormones were measured using well-established and validated radioimmunoassays. The plasma levels of ACTH, cortisol, and GH were determined according to the techniques of Sumpter and Donaldson (1986), Pickering *et al.* (1987), and Le Bail *et al.* (1991), respectively.

Statistical analyses. For all experiments, plasma cortisol, GH, and ACTH (Experiment 2 only) were separately analysed by analysis of variance (ANOVA, Genstat) with treatment (stressed, unstressed) and time as factors. For Experiment 3, body weight, length, and K factor ($100\ W/L^3$) were also analysed by the same techniques. 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. Appropriate transformations were selected, from a plot of residuals against fitted values, to improve homogeneity of variance. Cortisol data

were analysed after log transformation; GH and ACTH data were analysed after square root transformation. Levels of significance 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 3.

RESULTS

Experiment 1: The effect of 1 hr of handling/confinement stress. This acute stress resulted in a rapid elevation of plasma cortisol, 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. 1a). When the fish were returned to their rearing tanks (after 1 hr of confinement) the cortisol levels dropped during the next 24 hr. Cortisol levels in the unstressed control fish remained at basal levels throughout the 96-hr study period. Plasma growth hormone levels in both groups of fish were low (<2 ng ml^{-1}) although analysis of variance revealed a significant effect of stress ($P < 0.01$) which took the form of an overall suppression of plasma GH levels in the stressed fish (Fig. 1b). The mean plasma GH level for all the stressed fish was 0.66 ± 0.09 (108) ng ml^{-1} (mean ± SEM(n)) compared with 1.26 ± 0.16 (108) ng ml^{-1} for the unstressed controls.

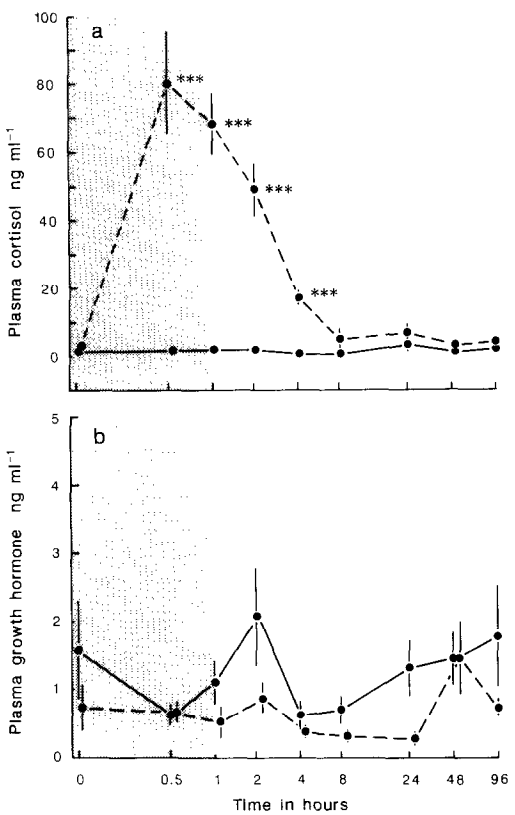

FIG. 1. Changes in (a) plasma cortisol and (b) plasma GH levels in 1+ rainbow trout during and after acute handling/confinement stress. Broken lines represent the stressed fish, continuous lines the unstressed controls. Each value is the mean ± SEM (n = 12). Asterisks denote significant differences at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

Experiment 2: The effect of 24 hr of handling/confinement stress. Confinement resulted in a highly significant ($P < 0.001$) and persistent elevation of plasma ACTH levels (Fig. 2a). ACTH was elevated from basal levels of approximately 20 pg ml^{-1} by the time of the first sample (1 hr) and remained high throughout the period of confinement. The change in plasma cortisol levels paralleled that of the ACTH levels (Fig. 2b), rising from less than 2 ng ml^{-1} in the unstressed controls to approximately 60 ng ml^{-1} throughout the period of confinement. The plasma cortisol level after 1 hr of confinement was 73.2 ± 8.8 (12) ng ml^{-1}, very similar to the value of 68.0 ± 8.9 (12) after a similar period of confinement in Experiment 1. Plasma GH levels in both stressed and unstressed fish were low (generally less than 1 ng ml^{-1}) but again, there was an overall suppression ($P < 0.01$) of GH levels in the stressed fish (Fig. 2c). Indeed, growth hormone was undetectable in many of the plasma samples from the stressed fish. At the present time, we can give no explanation for the low GH levels in the control fish at 12 hr.

Experiment 3: The effect of chronic crowding stress. During the first 5 months of this study, the seasonal increase in water temperature (3.2 to 13.9°) caused a gradual deterioration in water quality (oxygen levels) in both crowded and uncrowded tanks, the result of increased oxygen consumption

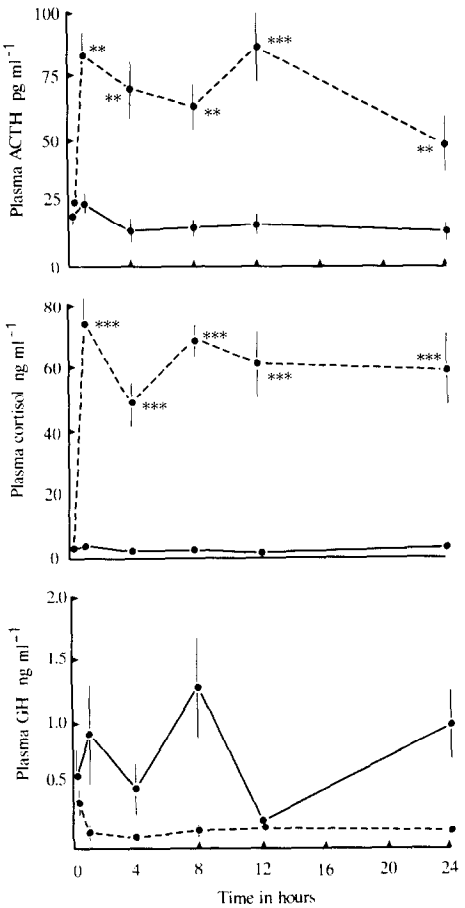

FIG. 2. Changes in (a) plasma ACTH, (b) plasma cortisol, and (c) plasma GH levels in 1+ rainbow trout during 24 hr of confinement stress. Broken lines represent the stressed fish, continuous lines the unstressed controls. Each value is the mean ± SEM (n = 12). Asterisks denote significant differences at each sampling time (**$P < 0.01$, ***$P < 0.001$).

by the fish and reduced oxygen carrying capacity of the water. Mortality rates in the uncrowded control fish during this period were low (≈0.5% per month), whereas the mortality rate in the crowded fish was ≈2% per month. During early May, a series of wind-driven seiche movements within the lake supplying the experimental hatchery resulted in short-term fluctuations in water temperature, culminating in a rapid 3° rise. This caused an overnight mortality of 13% in the crowded tanks. The oxygen levels (measured with a VSI oxygen probe) within each of the crowded tanks was less than 30% saturation compared with ≈50% for each of the uncrowded controls. Because of this deterioration of water quality in both groups of fish, aeration devices were then installed in all four tanks for the remainder of the study. Subsequent oxygen levels in all tanks were greater than 70% saturation.

Both groups of fish grew during the 10-month study period (Figs. 3a and 3b), although the crowded fish showed evidence of a growth check around the period of low

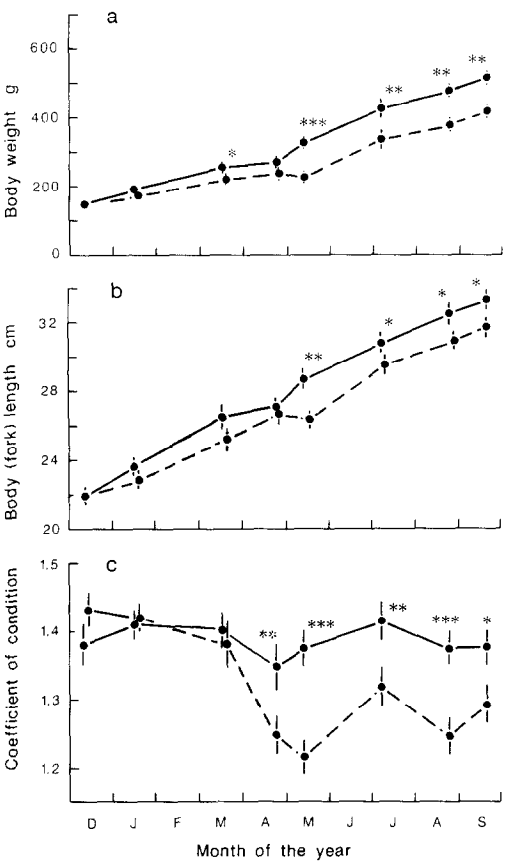

FIG. 3. Changes in (a) body weight, (b) body length, and (c) coefficient of condition of 1+ rainbow trout during a 9-month period of chronic crowding. Broken lines represent the crowded fish, continuous lines the uncrowded, control fish. Each value is the mean ± SEM (n = 16). Asterisks denote significant differences at each sampling time (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

oxygen levels. By the end of the study, the fish in the uncrowded tanks were significantly heavier than those in the crowded tanks ($P < 0.01$), despite both groups of fish having access to similar rations per capita. The data for body length showed similar trends to the weight data (cf. Figs. 3a and 3b). The coefficient of condition of the uncrowded control fish remained relatively constant at 1.4 whereas the K factor of the crowded fish dropped to 1.2 by late April and remained low thereafter (Fig. 3c). 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 during the spring months ($P < 0.001$ in each case, Fig. 4a). However, the peak cortisol levels in late April were almost twice as high in the crowded fish than in the controls (13.4 cf. 7.1 ng ml^{-1}). Once aeration had been installed into the tanks, the blood cortisol levels of both groups dropped to basal values and remained low for the rest of the study. Interestingly, the pattern of change in plasma GH levels matched, almost perfectly, the changes in plasma cortisol (cf. Figs. 4a and 4b). As a result, a strong and highly significant ($P < 0.001$) positive correlation was found between the mean plasma cortisol and mean plasma GH levels (Fig. 4c).

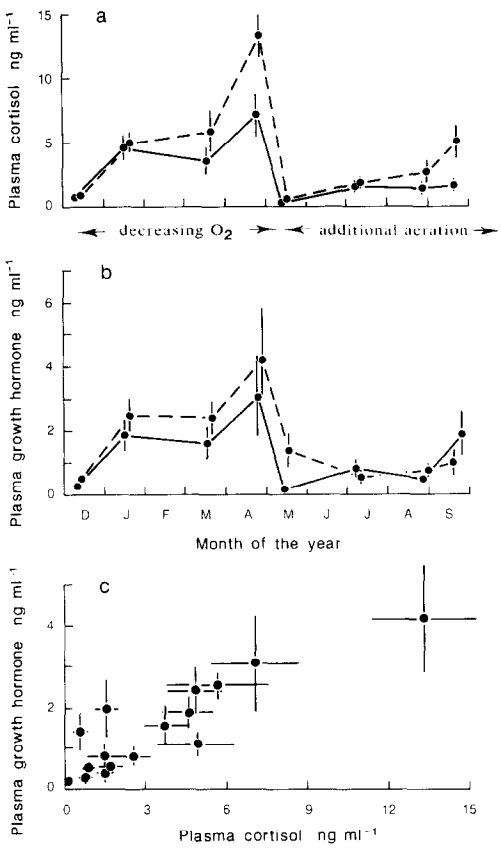

FIG. 4. Changes in (a) plasma cortisol and (b) plasma GH levels in 1+ rainbow trout during a 9-month period of chronic crowding. Broken lines represent the crowded fish, continuous lines the uncrowded, control fish. Each value is the mean ± SEM ($n = 16$). c illustrates the strong, positive correlation between mean plasma cortisol and GH levels in a and b. Linear regression: $y = 0.291 + 0.461$, $P < 0.001$, $r^2 = 77.6\%$.

DISCUSSION

The stress of handling and confinement produced a typical stress response in 1+ rainbow trout, in the form of an activation of the hypothalamic–pituitary–interrenal axis (HPI). In addition, both 1 and 24 hr of confinement resulted in a significant suppression of circulating GH levels. It was not possible, however, to discern any clear time course in the development of this response and, in view of the very low GH levels in the unstressed controls, it is suggested that an increase in assay sensitivity would help clarify the details of this response (even though the assay we used is considerably more sensitive than the other published assays capable of measuring GH in salmonid fishes; see Discussion in Le Bail *et al.*, 1991). Nevertheless, both confinement experiments produced essentially the same result—a significant ($P < 0.01$) reduction in circulating GH levels in the acutely stressed fish. This contrasts with

the observations of Cook and Peter (1984) who concluded that the stress of injection caused an increase in serum GH at 24 hr poststress in the goldfish, *Carassius auratus*, and with those of Wagner and McKeown (1986) who were unable to find any effects of handling stress on circulating GH levels in rainbow trout. 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. The type of environmental stress may also affect the response. Although low, it seems likely that variations in GH levels of the magnitude observed in the present investigation (up to 10-fold differences between individual control and stressed fish) are of physiological significance to the fish. The basal level of GH in the blood of rapidly growing trout is of a similar level to that observed here (see Sumpter *et al.*, 1991, for data on rapidly growing diploid and triploid rainbow trout). A further factor worth taking into account when considering the results of Experiments 1 and 2 is that of the mode of GH release. As already discussed (Le Bail *et al.*, 1991), GH secretion may be pulsatile, in which case stress could inhibit the pulsed release of GH whilst not affecting steady-state secretion. This mode of secretion may also account for some of the variability in GH levels in unstressed fish. Further work in this area is now required.

Plasma GH levels in both crowded and uncrowded rainbow trout changed significantly during the 9-month study period. This took the form of an *increase* in plasma GH which coincided with suppressed growth rates and a reduction in the coefficient of condition, and was closely correlated with an elevation of plasma cortisol levels. It seems likely that the elevation of GH levels was related to changes in water quality rather than to crowding per se because it occurred in both crowded and uncrowded tanks at a time when the oxygen levels were low and disappeared as soon as additional aeration was supplied. Unfortunately, it was not possible to monitor food *intake* (although both crowded and uncrowded fish were given identical rations) and we were unable, therefore, to determine whether this elevation of plasma GH occurred as a direct response to changes in water quality or whether it was a consequence of reduced food intake (see Sumpter *et al.*, 1991). It is interesting, however, that VanHelder *et al.* (1987) found a significant correlation between GH levels and the oxygen demand/availability ratio in humans, with GH levels increasing as oxygen demand increases and/or oxygen availability decreases. Similar mechanisms may exist in salmonid fish because Barrett and McKeown (1988) have shown that sustained exercise (and, therefore, increased oxygen demand) also increases plasma GH levels in two species of salmonid fish. Further studies are now needed to resolve the link between hypoxia and plasma GH levels in teleost fish.

The close correlation between plasma cortisol and mean plasma growth hormone levels in the fish from the chronic crowding experiment calls for some comment on the possible links between the HPI axis and hypothalamic–somatotrope activity in fish. In general, data for this area of teleost endocrinology are sparse and fragmentary. Olivereau and Olivereau (1968) found that adrenalectomy caused an activation of the somatotropes of the eel, *Anguilla anguilla*, but, because similar responses were found in sham-operated fish, they concluded that the somatotrope response was a nonspecific response to the stress of surgery. Ball and Hawkins (1976) found that mammalian GH preparations could elevate blood cortisol levels in hypophysectomized *Poecilia latipinna* 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, *Oncorhynchus kisutch*, and Young (1988) found that ovine GH, either *in vivo* or *in vitro*, enhanced the interrenal response of coho salmon to ACTH. 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 present investigation demonstrates that handling and confinement stress elicits a *reduction* in plasma GH levels concomitant with increased HPI activity. There is, therefore, no concensus of opinion on the link(s) between the pituitary somatotropes and the HPI axis in teleosts. The bulk of evidence from *in vivo* mammalian studies suggests that corticosteroids suppress GH levels although their actions are complex and biphasic, with both stimulatory and suppressive components (Ceda *et al.*, 1987; Casanueva *et al.*, 1988).

One of the original aims of the present study was to assess the potential role of GH in the well-known phenomenon of stress-induced growth suppression in fish. The GH suppression in response to an acute confinement stress is certainly one pathway which could lead to growth suppression, but the elevation of circulating GH in chronically stressed fish (exposed to poor water quality) would seem to be at variance with the concept of growth suppression via reduced GH secretion. 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, *Salmo salar*, smolts (Bolton *et al.*, 1987; Björnsson *et al.*, 1988). However, the problem of suppressed growth in such stunts is thought to be related to target-tissue sensitivity rather than the rate of pituitary GH secretion (Fryer and 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 in mediating these effects.

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