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Circulating growth hormone, cortisol and thyroxine levels after 24 h seawater challenge of yearling coho salmon at different developmental stages

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Abstract

The response of the endocrine system to short-term exposure to sea water (SW) was examined before, during and after the parr-smolt transformation of coho salmon. Five transfers of yearling coho salmon (*Oncorhynchus kisutch*) from fresh water (FW) to FW, or from FW to SW for 24 h were carried out between March and September. Condition factor declined significantly in late April, and gill Na^+, K^+-ATPase activity was elevated between late March and early June. Increases in plasma osmolality after transfer to SW were high in early March, low from late March – early June and slightly higher in September. In fish transferred from FW to FW, plasma levels of thyroxine (T_4) were elevated in late March–late April, and plasma levels of cortisol were high in late March–early June; both hormones declined to early March levels thereafter. Growth hormone (GH) levels increased in late March and remained elevated for the duration of the study. A significant linear correlation existed between plasma cortisol and growth hormone levels in FW to FW transferred fish. After SW transfer, T_4 levels were significantly lower than values in FW controls in early March and were significantly higher than FW control levels in early June and early September. Cortisol levels increased significantly after each transfer except in late April. However, compared with the early March and early September transfers, the magnitude of the elevation was reduced in late March. GH levels were significantly elevated above FW control levels after seawater transfer in late April only, despite a trend for mean GH levels to be higher after transfer in June and September. Thus, minimal increases in cortisol, and increases in GH occur after SW transfer of functional (optimally hypo-osmoregulating) smolts.

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1. Introduction

Enhanced hypo-osmoregulatory ability of freshwater (FW) juveniles during the salmonid parr-smolt transformation has been linked, both developmentally and experimentally, to increases in thyroid hormones, cortisol and growth hormone (GH) (see Hoar, 1988). Information on short-term changes in circulating levels of these hormones after seawater (SW) exposure and their significance is less complete, and the literature contains several points of conflict. In some studies, GH levels were found to increase after SW exposure of smolts (Sweeting et al., 1985; Boeuf et al., 1989; Yada et al., 1991), whereas in others there was no change (Young et al., 1989a; Rydevik et al., 1990). Similarly, a number of authors have reported increased levels of circulating cortisol of varying magnitude in coho salmon after SW exposure (Redding et al., 1984b; Young et al., 1989a; Avella et al., 1990), but conflicting reports are available on Atlantic salmon (Langdon et al., 1984; Nichols and Weisbart, 1985; Langhorne and Simpson, 1981, Langhorne and Simpson, 1981; cf. Bisbal and Specker, 1991). Comparison of responses of juvenile coho salmon at two developmental stages (smolt and post-smolt) suggested that the magnitude of the elevation in cortisol after SW transfer depended on developmental stage (Young, 1985; Avella et al., 1990). Similar data are available for chinook and sockeye salmon (Franklin et al., 1992a).

The response of the thyroid to SW also appears to depend on developmental stage (see Specker, 1988), with premature transfer of coho salmon to SW resulting in depressed thyroid activity. However, little is known about the response of the thyroid to SW challenges during the course of the parr-smolt transformation and during the later reversion of smolts to a more parr-like condition.

These previous studies have often been restricted to the examination of only one or two hormones at one or two time points during development, which, with species differences, may partly explain some of the discrepancies in the literature. This study was undertaken to resolve the relationship between developmental stage and the response of the endocrine system of coho salmon to short-term exposure to SW. Coho salmon at different developmental stages were subjected to a 24 h SW challenge (Clarke and Blackburn, 1977; Clarke, 1982) after which circulating levels of thyroxine, GH and cortisol, and several physiological indicators of development were measured. We report that developmental stage influences the magnitude of the response of these components of the endocrine system to increased salinity.

2. Materials and methods

2.1. Animals

Yearling coho salmon obtained from the Californian Department of Fish and Game's Iron Gate Hatchery were maintained in an outdoor 1200 l circular stock tank supplied with recirculating, temperature-controlled (12–14°C) FW for 4 weeks before experimentation. They were subjected to natural photoperiod and were fed to satiation twice daily.

2.2. *Seawater exposure*

Two 1200 l circular tanks were divided into two compartments with mesh dividers. One tank was supplied with FW, as above, while the other contained artificial SW at 32‰. Eight fish were rapidly transferred by handnet into each compartment of the SW and FW challenge tanks and were sampled 24 h later.

2.3. *Sampling*

In order to minimise stress-induced elevations in cortisol levels, fish in one of the compartments in each of the challenge tanks were rapidly netted and killed in buffered tricaine methanesulfonate (200 mg l^{-1}; MS-222; Sigma Chemical Co., St. Louis, MO). Blood obtained from the caudal vessels using heparinized (ammonium heparin) syringes was centrifuged, and the resulting plasma was frozen on dry ice and stored at −80°C until analysis for cortisol and GH. Fish remaining in the other compartment of each tank were rapidly netted and killed by cranial concussion. Plasma from these fish was used for thyroxine and osmolality determinations, and gills were dissected out, frozen on dry ice in sucrose-EDTA-imidazole buffer, and stored at −80°C.

2.4. *Assays*

Osmolality was determined using a vapor pressure osmometer. Cortisol (Young, 1986), GH (Björnsson et al., 1994), thyroxine (Dickhoff et al., 1982; Specker and Kobuke, 1987) and gill Na^+, K^+-ATPase (McCormick and Bern, 1989) were measured using established assays validated for coho salmon.

2.5. *Statistics*

Data on hormone levels and osmolality after transfer to FW or SW were analysed by two-way ANOVA (sampling time × salinity) to determine whether the seasonal pattern of plasma levels was different in samples from FW and SW animals. Differences between levels after transfer to FW or SW at each sampling time were analysed by one-way ANOVA. Data on seasonal changes in hormone levels of FW coho salmon were analysed by one-way ANOVA and the Student–Newman–Keuls multiple range test. Before analysis, data displaying heterogeneity of variance were log-transformed.

3. Results

3.1. *Body weight, length and condition factor*

Over the course of the study, fish increased in average weight from 22 g to 44 g and in length from 12.5 to 16 cm (data not shown). Condition factor (Fig. 1) dropped markedly

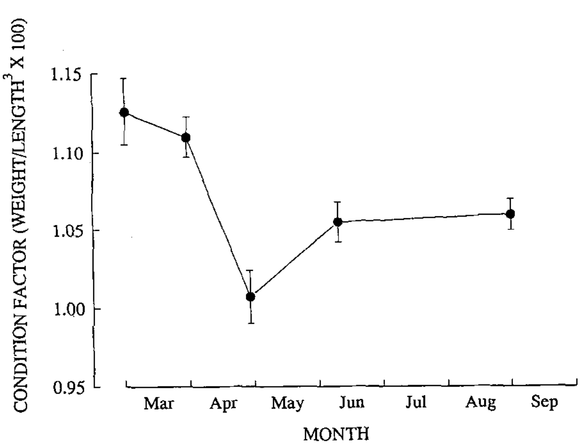

Fig. 1. Changes in condition factor of yearling coho salmon. Each point represents mean ± S.E. ($n=8$).

($P<0.01$) between late March and late April, and then increased ($P<0.05$) to intermediate values for the remainder of the study.

3.2. *Plasma osmolality and gill Na^+, K^+-ATPase activity*

In animals transferred from FW to FW for 24 h, plasma osmolality remained constant at 290–295 mOsm kg^{-1} throughout the study (Fig. 2). Increases of approximately 50 mOsm kg^{-1} above FW control values occurred after fish were transferred to SW for 24 h in early March ($P<0.01$). Best hypoosmoregulatory performance occurred in animals transferred in late March, late April and early June, with increases of 15–20 mOsm kg^{-1} over FW control values ($P<0.01$, late March, early April; $P<0.05$, late June) Osmolality in these animals after 24 h in SW was significantly ($P<0.05$) lower than in animals transferred in early March. Fish transferred in early September displayed intermediate hypoosmoregula-

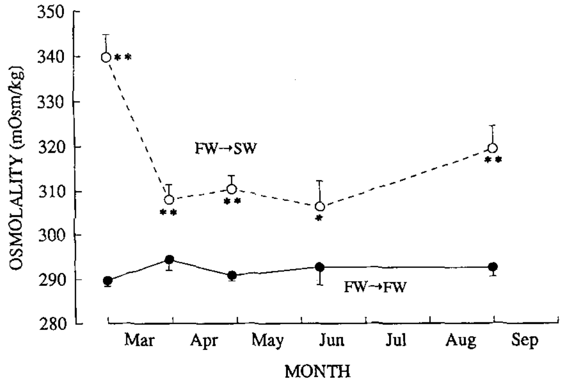

Fig. 2. Changes in plasma osmolality after transfer of yearling coho salmon from fresh water to either fresh water (FW→FW; ●) or seawater (FW→SW; ○) for 24 h. Each point represents mean ± S.E. ($n=8$). $^*P<0.05$, $^{**}P<0.01$, compared to FW control.

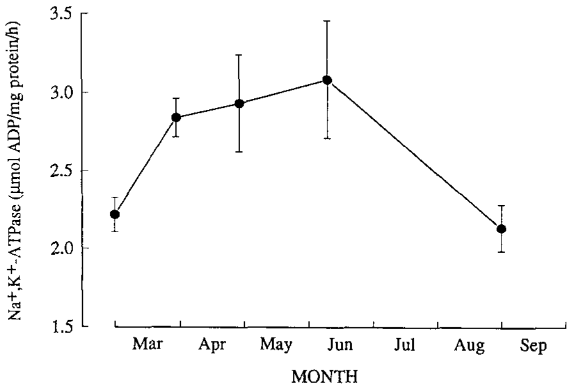

Fig. 3. Changes in gill Na^+, K^+-ATPase activity of yearling coho salmon transferred from fresh water to fresh water for 24 h. Each point represents mean ± S.E. ($n = 8$).

tory ability, with increases of about 30 mOsm kg^{-1} over values of FW controls ($P < 0.05$).

Gill Na^+, K^+-ATPase activity in fish transferred from FW to FW increased approximately 33% ($P < 0.05$) from the early March sample to levels which were maintained from April to early July but which decreased significantly ($P < 0.05$) in the early September sample (Fig. 3). Activity was quite variable in late April and June. Gill Na^+, K^+-ATPase activity in fish transferred to SW for 24 h was not significantly different from activity of FW control fish at any time point (data not shown).

3.3. *Plasma thyroxine*

Plasma thyroxine levels (Fig. 4a) varied significantly with sampling time and with salinity ($P < 0.001$; 2-way ANOVA). The significant interaction ($P < 0.001$) between these factors indicated that the seasonal pattern of thyroxine levels varied significantly between animals transferred to FW or SW. In fish transferred from FW to FW, plasma thyroxine levels were significantly higher ($P < 0.01$) in late March and late April compared to those in early March. Thyroxine then declined ($P < 0.05$) to levels seen in the initial sample. A small but significant decline ($P < 0.05$) in circulating thyroxine occurred after SW exposure in early March. In late March and late April, there was no significant difference in levels between FW and SW-transferred fish, but in early June ($P < 0.01$) and early September ($P < 0.05$), plasma thyroxine was significantly elevated in fish after SW exposure.

3.4. *Plasma cortisol*

Plasma cortisol levels (Fig. 4b) varied significantly with sampling time ($P < 0.005$; 2-way ANOVA) and with salinity ($P < 0.001$; 2-way ANOVA). The significant interaction ($P < 0.005$) between these factors indicated that the seasonal pattern of cortisol levels varied significantly between animals transferred to FW or SW. Cortisol rose significantly ($P < 0.05$) from initial levels of approximately 10 ng ml^{-1} in FW control fish in early March to a plateau of 23–30 ng ml^{-1} from late March to early June. Levels in September

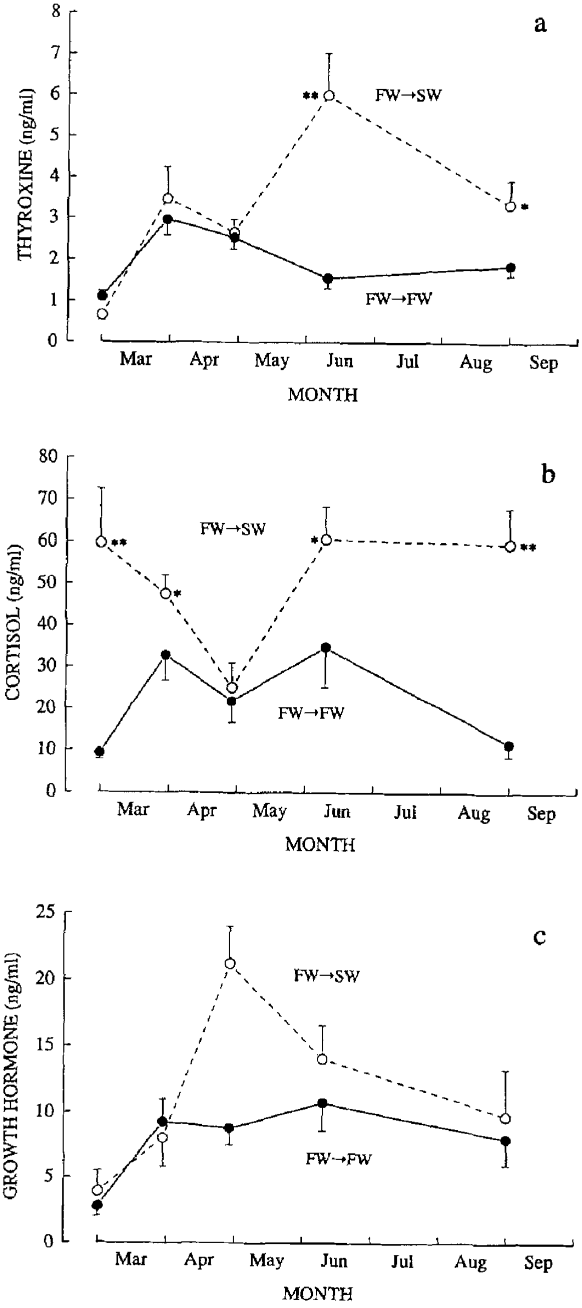

Fig. 4. Changes in (a) plasma thyroxine, (b) cortisol and (c) growth hormone levels after transfer of yearling coho salmon from fresh water to either fresh water (FW → FW; ●) or seawater (FW → SW; ○) for 24 h. Each point represents mean ± S.E. ($n = 8$). $^{*}P < 0.05$, $^{**}P < 0.01$, compared to FW control.

were similar to those in early March. Transfer of fish to SW in early March elicited a significant increase ($P<0.01$) of about 50 ng ml^{-1} over FW control values. In the late March transfer, levels in SW fish were only about 20 ng ml^{-1} higher ($P<0.05$) than those of FW controls, and in late April, there was no significant difference between values of FW and SW-transferred fish. In the June ($P<0.05$) and September ($P<0.01$) transfers, levels in SW fish were significantly higher than those in FW control fish, rising to concentrations similar to those seen after SW transfer in early March.

3.5. *Plasma growth hormone*

Plasma GH levels varied (Fig. 4c) significantly with sampling time ($P<0.001$; 2-way ANOVA) and with salinity ($P<0.02$; 2-way ANOVA). The significant interaction ($P<0.05$) between these factors indicated that the seasonal pattern of GH levels varied significantly between animals transferred to FW or SW. GH levels in FW controls increased significantly ($P<0.001$) from 2.5 ng ml^{-1} in early March to a plateau of approximately 8–9 ng ml^{-1} between late March and early June. Levels were lower (but not significantly so) in early September. The only significant difference between levels in FW controls and those exposed to SW was in late April, where levels in the latter doubled to 20 ng ml^{-1} ($P<0.01$). Levels in SW fish in June averaged approximately 13.5 ng ml^{-1} but were not significantly different from levels of 9 ng ml^{-1} in FW controls.

3.6. *Relationship between plasma hormones*

A significant linear correlation ($r^2=0.59$; $n=40$; $P<0.001$; $\mathrm{GH}=4.609+0.149\times\mathrm{cortisol}$) existed between GH and cortisol levels in FW controls but there was no significant correlation between levels after SW exposure (data not shown). No significant correlations existed between levels of other hormones after transfer to FW or to SW.

4. Discussion

Results of this study indicate that the response of several components of the endocrine system of juvenile coho salmon to increased salinity varies with development. Developmental status of the animals used in this study was assessed by measuring condition factor, plasma osmolality after SW transfer, gill Na^+, K^+-ATPase activity and hormone levels.

Condition factor, the classical variable to measure to assess the progression of the parr-smolt transformation, underwent a striking reduction in coho sampled in late April. Lowered condition factor presumably reflects the catabolic nature of the smolt's metabolism (McCormick and Saunders, 1987; Sheridan, 1986) and increased linear growth (Winans and Nishioka, 1987).

The 24 h SW challenge test (Clarke and Blackburn, 1977; Clarke, 1982) has been widely employed to assess the extent of the preparatory increase in hypoosmoregulatory ability prior to downstream migration. Time course studies on coho salmon (Richman and Zaugg, 1987; Richman et al., 1987) are in accord with Clarke and Blackburn's observation (Clarke and Blackburn, 1977) that a single sampling at 24 h or 48 h after transfer to SW is sufficient to evaluate SW tolerance in Pacific salmon. On the basis of elevations in plasma osmolality after exposure to sea water for 24 h, coho salmon in this study had achieved maximal hypoosmoregulatory ability in the period from late March to early June, coincident with elevated gill Na^+, K^+-ATPase activity, and in agreement with other studies on this stock (see Young et al., 1989a). In September, fish in the post-smoltification period were still able to regulate plasma osmolality reasonably well, despite a decline in gill Na^+, K^+-ATPase activity. This may reflect, at least in part, the doubling in weight of animals by this time, compared to earlier samples, and the consequent reduction in surface area: volume ratio. Increase in size represents a potential confounding influence in interpretation of changes in hypoosmoregulatory ability during long-term developmental studies on the parr-smolt transformation. However, all other indicators measured suggest that animals in September had reverted to a parr-like condition (desmoltification).

The seasonal changes in plasma levels of GH, thyroxine and cortisol in coho transferred from FW to FW were similar in pattern and magnitude to those reported previously for this stock of coho salmon (Young, 1986; Young et al., 1989a) and for other stocks (Dickhoff et al., 1978; Dickhoff et al., 1982; Specker and Schreck, 1982; Barton et al., 1985; Sweeting et al., 1985) and other species (Dickhoff et al., 1982; Björnsson et al., 1989a; Boeuf et al., 1989; Prunet et al., 1989; Franklin et al., 1992a). Although control fish were transferred from FW to FW, and were thus exposed to a very brief handling stress, it is probable that hormone levels in these controls are representative of the developmental pattern in undisturbed fish. Handling stress in salmonids does not affect thyroid hormone levels (Laidley and Leatherland, 1988) and cortisol levels return to normal within 4–8 h (Pickering et al., 1982). Less is known about the response of GH to a handling stress, although acute confinement for 1 h or 24 h resulted in a small but significant decrease in GH levels in rainbow trout (Pickering et al., 1991). Increased circulating levels of these hormones generally coincided with enhanced seawater adaptability in most studies. Levels of GH were not correlated with increased SW adaptability in landlocked amago and sockeye salmon (Yada et al., 1991), although it is notable that the latter species did not display a distinct seasonal increase in SW adaptability, unlike that seen in studies on another landlocked sockeye stock (Franklin et al., 1992a).

Based on these criteria, juvenile salmon in the present study seem to have become functional smolts in the period of late March–late April (when wild smolts migrate in north California), and possibly beyond, depending on the criteria used: although gill Na^+, K^+-ATPase activity and thyroxine levels were reduced in fish sampled in early June, both hypoosmoregulatory ability and plasma cortisol levels remained high.

The effect of transfer to SW for 24 h on circulating hormone levels seen in this study suggest that post-transfer hormone levels may provide additional criteria by which to judge the progression of the parr-smolt transformation, since the direction and magnitude of the response were related to the date of transfer. Circulating thyroxine levels were depressed after transfer in early March, were at least as high as levels in FW controls in late March and late April, and were significantly higher than FW control values in June and early September. It is already well-recognised that the response of the coho thyroid to SW is a development-dependent phenomenon (Specker, 1988). By measuring circulating thyroid hormone levels and/or the response to thyrotropin, several studies have shown that coho thyroid activity decreases when parr or incompletely smoltified ('early smolt') fish are transferred to SW, but is maintained or increases after smolts are exposed to SW (Dickhoff et al., 1982; Folmar et al., 1982; Specker and Schreck, 1982; Redding et al., 1984a; Specker and Kobuke, 1987; Specker, 1988). Similar observations have been made on Atlantic salmon, (Boeuf et al., 1989; Specker et al., 1989; McCormick and Saunders, 1990). We are unaware of other studies on the effects of salinity on thyroid activity in animals in the late smolt and post-smolt stages. Clearly, further work on thyroid activity after transfer of post-smolts is warranted before depressed thyroid activity can be considered a characteristic of animals transferred to sea water too early and too late in the developmental process. The role of thyroid hormones in sea water adaptation, if any, remain obscure, even though increased thyroid activity is a central feature of the parr-smolt transformation (Hoar, 1988). Depression of thyroid activity after incorrectly-timed transfer of salmon to sea water is associated with growth retardation (e.g. Young et al., 1989b).

In this study, cortisol levels, 24 h after transfer, exhibited a distinct relationship to developmental stage, with animals in late April or late May (functional smolts on the basis of criteria discussed above) showing little or no elevation in cortisol after SW exposure. Short-term elevations in plasma cortisol have been reported after SW exposure of coho salmon (Redding et al., 1984b; Young, 1985; Young et al., 1989a; Avella et al., 1990), chinook salmon (Strange and Schreck, 1980; Franklin et al., 1992a) and sockeye salmon (Franklin et al., 1992b). Reports on Atlantic salmon are inconsistent. Earlier studies reported no increase (Langdon et al., 1984; Nichols and Weisbart, 1985; Langhorne and Simpson, 1986) but Bisbal and Specker (1991) found distinct increases. The magnitude and the duration of the elevation appear to be variable in coho salmon. In accord with the present data, two studies comparing cortisol levels of two groups of juveniles (smolts and post-smolts) have suggested that increases are moderate and relatively transitory in smolts of good hypoosmoregulatory ability, and the increased magnitude and duration of the response in animals of limited hypoosmoregulatory ability may reflect a general stress response which could eventually stimulate hypoosmoregulatory mechanisms (Young, 1985; Avella et al., 1990). Franklin et al. (1992a) and Franklin et al. (1992b) have shown that the magnitude and duration of elevated cortisol levels were lowest in hypo-osmoregulating sockeye and chinook salmon smolts. Developmental changes in metabolic clearance rate (Redding et al., 1984a; Nichols and Weisbart, 1985) may also influence the extent of the elevation in plasma cortisol after SW transfer.

Several in vivo and in vitro studies have shown that, under appropriate conditions, exogenous cortisol can stimulate hypoosmoregulatory ability and/or gill Na^+, K^+-ATPase activity in Pacific salmon (Richman and Zaugg, 1987; McCormick and Bern, 1989; McCormick et al., 1991a), Atlantic salmon (Bisbal and Specker, 1991), sea trout (Madsen, 1990b) and non-anadromous rainbow trout (Madsen, 1990a; Madsen, 1990c), in addition to several non-salmonid species (e.g. Pickford et al., 1970; Epstein et al., 1971; Forrest et al., 1973; Hirano et al., 1975). The extent and duration of cortisol increases after SW transfer may therefore be related in part to the length of time osmoregulatory organs of juvenile salmon have been exposed to the development-associated increase in plasma cortisol while still in FW, i.e. whether animals are fully pre-adapted, through the action of cortisol and other osmoregulatory hormones, for SW life. Little is known about the mechanisms underlying the loss of hypoosmoregulatory ability in 'desmoltifying' salmon. If an extended increase in circulating cortisol after SW transfer has a specific effect on hypoosmoregulatory mechanisms, this may be reflected in the further increase in gill Na^+, K^+-ATPase activity that generally develops 1–2 weeks after SW exposure (e.g. Björnsson et al., 1989b).

Results from the present study suggest that increased plasma GH in juvenile coho salmon after 24 h of seawater exposure is a development-related phenomenon, occurring at a time of peak hypoosmoregulatory ability and when other development-related hormones (thyroxine, cortisol) are high in FW fish. Although significant elevations in GH were only seen in one transfer, late in April, the overall pattern in Fig. 4c suggests a tendency towards increased GH levels after SW exposure in the June and September samples as well. However, juveniles in late March and June displayed good hypoosmoregulatory ability in the absence of significant elevations of GH after SW transfer and the functional significance of the increase in late April is therefore unclear.

Various experimental studies indicate that in addition to its growth-promoting and metabolic role, GH functions as a SW-adapting hormone in salmonids (reviewed by Sakamoto et al., 1993; Boeuf et al., 1994). In addition to development-associated increases in GH levels in some species (discussed above), relatively short-term increases in plasma GH levels after SW transfer have been reported for juvenile chum salmon and rainbow trout (Hasegawa et al., 1987; Sakamoto et al., 1990), for Atlantic salmon (Boeuf et al., 1989) and in some studies on coho salmon (Sweeting et al., 1985; Sakamoto et al., 1991) but not others (Young et al., 1989a). Recent studies on juvenile amago and sockeye salmon (Yada et al., 1991) and Atlantic salmon (Rydevik et al., 1990) have led to diverging opinions on the physiological significance of increased GH levels after SW transfer. Yada et al. (1991) found GH levels increased only in juveniles with good hypoosmoregulatory ability. Notably, precociously mature amago juveniles with poor hypoosmoregulatory ability did not display increased GH levels after SW transfer. In contrast, in a single time-point experiment, GH levels of Atlantic salmon smolts did not increase after SW transfer, but significant increases were observed in mature male parr (Rydevik et al., 1990). The latter authors suggested that the increase in GH levels in mature male parr represented part of a mechanism to increase hypoosmoregulatory ability in fish not pre-adapted for SW existence by exposure to GH during development. The apparent species difference in developmental pattern of GH during the parr-smolt transformation, where increases correlate with enhanced hypoosmoregulatory ability in Atlantic salmon, but not in amago or sockeye salmon, also appears to extend to the response to increased salinity.

Schmitz et al. (1994) have shown that juveniles of two Baltic stocks of Atlantic salmon do not display increases in GH during FW residence but exhibit a season-dependent increase in plasma GH levels in response to a SW challenge: plasma GH levels increased after SW exposure at the time that fish displayed increased hypo-osomoregulatory ability. These authors have suggested that low temperatures during spring may retard the physiological processes that prepare juvenile salmon for SW residence, including the developmental increase in GH; further, increased environmental salinity may trigger the full development of hypo-osmoregulation, through a mechanism that involves increased GH secretion after SW exposure. Boeuf et al. (1989) reported peaks in plasma GH during the parr-smolt transformation of Atlantic salmon, followed by sustained high levels in the post-smolt period. Relatively prolonged (7–10 days) increases in GH occurred after three SW transfers of smolts of good hypoosmoregulatory ability.

The recent examination of GH kinetics after SW transfer of coho salmon smolts clearly demonstrates increased GH secretion rate and plasma levels after 2 days in seawater, and elevated secretion rate, metabolic clearance rate and plasma levels after 2 weeks residence in SW (Sakamoto et al., 1991). Studies examining possible development-related changes in secretion rate and metabolic clearance rate of GH, and tissue levels of GH receptors (Sakamoto et al., 1993, Sakamoto et al., 1994) may help the interpretation of some of the conflicting data on plasma GH levels after SW transfer. It is conceivable that transient increases in hypo-osmoregulating fish in late March and early June in this study may have been missed by a single sampling 24 h after seawater exposure. It is also possible that the 24 h SW challenge test may not have been sufficient to identify relatively subtle variances in hypoosmoregulatory ability. Possible differences between fish we classified as good hypo-osmoregulators may be reflected in the difference in GH response of fish to a SW challenge.

In summary, the developmental events associated with the parr-smolt transformation of coho salmon include changes in the response of several components of the endocrine system to increased external salinity. The minimal changes in plasma cortisol in optimally hypo-osmoregulating smolts probably reflect the prior action of cortisol and GH in preparing juvenile salmon for a hyperosmotic environment; a reduced cortisol response is indicative of the reduced stress that SW imposes at this developmental stage. The relative importance of an increase in circulating GH after transfer still remains unclear since hypo-osmoregulating fish do not always exhibit such an increase in GH levels. However, recent work (reviewed by Sakamoto et al., 1993) shows that acclimation to increased salinity is accomplished through the interaction of at least four hormones: cortisol, GH, triiodothyronine (Leloup and Lebel, 1993) and insulin-like growth factor-1 (McCormick et al., 1991b). The significant correlation between plasma cortisol and GH in juvenile FW coho salmon in this study is further evidence of a close functional relationship between these hormones (Sakamoto et al., 1993)

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