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Endocrine and physiological changes in Atlantic salmon smolts following hatchery release

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Abstract

Physiological and endocrine changes during smolt development were examined in Atlantic salmon (*Salmo salar*) reared and released as part of a restoration program on the Connecticut River and its tributaries. Fish were reared in a cold water hatchery in Pittsford, VT and released into the Farmington River, CT (a major tributary of the Connecticut River) or into 'imprint ponds' fed by the Farmington River. Smolts were recaptured 10–20 days after their release at a smolt bypass facility 16 km downstream of their release site. Fish sampled at the hatchery from January to May had only moderate smolt development based on salinity tolerance, gill Na^+,K^+-ATPase activity and hormone profiles. In contrast, smolts released into the river or imprint ponds had higher salinity tolerance, gill Na^+,K^+-ATPase activity, plasma growth hormone, insulin-like growth factor I (IGF-I) and thyroxine than smolts that remained in the hatchery. These physiological and endocrine changes were nearly identical to those of smolts that had been released into the river 2 years earlier as fry and were captured as active migrants at the same bypass facility (stream-reared smolts). The stomach contents as a percent of body weight (primarily aquatic insects) varied greatly among individuals and were greater in hatchery-reared fish than stream-reared smolts. Results from the rearing of hatchery fish at temperatures similar to that of the Farmington River indicate that some of the physiological changes may be due to increased temperature after release, though other factors may also be involved. The results indicate that substantial physiological smolt development can occur after hatchery release, coincident with downstream migration.

Keywords: Atlantic salmon; Smolts; Hatchery

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1. Introduction

Artificial rearing and release of anadromous salmonids has been a widely adopted strategy for restoration and population enhancement. Previous research has shown that the timing of release can dramatically affect survival in the ocean, and that this effect is due in part to developmental changes in salinity tolerance and other physiological and endocrine changes that occur during the parr–smolt transformation (Virtanen et al., 1991; Staurnes et al., 1993). When hatchery smolt development is compared with fish in the wild, it has been shown that hatchery fish often have altered or compromised smolt development and that these differences may explain the relatively poor return rates of hatchery-reared fish (McCormick and Björnsson, 1994; Shrimpton et al., 1994; Sundell et al., 1998).

The altered developmental patterns of hatchery-reared fish suggests that there may be important physiological changes that occur after the smolts are released into the wild. Currently, there is very limited information on post-release changes in smolts and how these relate to survival during downstream migration and ocean entry. Ugedal et al. (1998) found that salinity tolerance of hatchery-reared brown trout (*Salmo trutta*) was higher in fish recaptured during migration compared to fish at the time of release from the hatchery. Chinook salmon (*Oncorhynchus tshwytscha*), coho salmon (*O. kisutch*) and steelhead trout (*O. mykiss*) smolts released into the Columbia River had substantially higher gill Na^+,K^+-ATPase activity relative to fish that remained in their hatchery of origin (Zaugg et al., 1985). An understanding of the changes that occur after hatchery release will be important to any attempt to improve current hatchery rearing and release methods. To our knowledge, there are no published studies on changes in physiology or endocrinology of Atlantic salmon after their release as smolts.

The present study was undertaken to determine what smolt-related endocrine and physiological changes occur in captive fish after release from their hatchery environment. In the first year (1999), fish were examined during hatchery rearing and after release into imprint ponds. In the second year (2000), fish were released into imprint ponds and into a major tributary of the Connecticut River. The latter group was recaptured during their downstream migration along with fish that had been released 2 years earlier as fry. The results indicate that important physiological changes occur after hatchery-reared fish are released, and that these changes are likely to affect their osmoregulatory capacity, imprinting and survival during downstream migration and ocean entry.

2. Materials and methods

2.1. Fish rearing and sampling

Juvenile Atlantic salmon (*Salmo salar*) were raised at the Pittsford National Fish Hatchery (Pittsford, VT, USA) as part of a restoration program, and were the progeny of adults returning to the Connecticut River. Throughout the study, fish were maintained in raceways supplied with stream water under natural photoperiod and fed to satiation daily with automatic feeders and hand feeding. In the autumn of 1998, large 0+ fish (>10 cm) were graded out and placed in separate raceways. These fish were sampled approximately

monthly beginning in January 1999 (January 19: 4.6 °C, February 23: 2.9 °C, March 24: 2.7 °C, April 27: 5.8 °C) and included both parr (< 11 cm fork length) and 1+ smolts (>12 cm fork length). The smaller grade fish were destined to be 2+ smolts and were sampled in 2000 (see below). On April 6, 1999, 1+ parr and smolts were transferred to 10-m diameter imprint ponds located at the Rainbow dam (Windsor, CT) that were fed by the Farmington River. On April 29, fish in imprint ponds were crowded using a seine and sampled as described below.

Two-year-old smolts were sampled five times from January to May 2000 (January 27: 0.1 °C, March 2: 2.2 °C, March 30: 2.6 °C, April 13: 5.8 °C, May 2: 7.6 °C). On April 14, 2000, 2+ hatchery smolts were transferred to either the imprint ponds or directly into the Farmington River, 16 km upstream of Rainbow Dam. Construction of a sampler at the smolt bypass structure at Rainbow dam allowed sampling of actively migrating hatchery-reared fish on April 26 (10.2 °C) and May 8 (20.7 °C). Smolts in imprint ponds were sampled on April 26 and released the next day. Fish that had been stocked as fry 1 or 2 years earlier (stream-reared parr and smolts, respectively) were captured prior to migration on April 7 by electrofishing on West Salmon Brook, a tributary of the Farmington River. Parr and smolts were clearly distinguishable at this time based on size and appearance. Actively migrating stream-reared smolts were also sampled at the smolt bypass on April 26 and May 8. Fish were sampled immediately after removal from the bypass and imprint ponds as described below, within 10 min of first being disturbed. This time course did not allow for measurement of undisturbed levels of cortisol, and thus this hormone was not measured in the present study.

In order to examine the effect of temperature on physiological changes after release, smolts from Pittsford National Fish Hatchery were transferred to the Conte Anadromous fish Research Center on March 30. They were kept in 1000-l circular tanks supplied with 4 l min^{-1} Connecticut River water under natural photoperiod conditions and fed ad libitum twice daily. One tank was kept at 5.0 ± 0.5 °C and the other at 11 ± 0.5 °C and both sampled on May 4.

Sampled fish were anesthetized with 200 mg l^{-1} tricaine methane sulphonate (neutralized and buffered with sodium bicarbonate, pH 7.0) and length (l) and weight (w) were measured. Blood was collected in heparinized syringes from the caudal vasculature, stored on ice for less than 30 min, centrifuged at $3000 \times g$ for 5 min, then plasma removed and frozen on dry ice. A gill biopsy (approximately six to eight primary gill filaments) was taken and placed in 100 μl of SEI (250 mM sucrose, 10 mM Na_2EDTA, 50 mM imidazole, pH 7.3) on ice for determination of Na^+,K^+-ATPase activity. Samples were frozen on dry ice within 30 min and stored at − 80 °C until analysis.

On May 8, hatchery-reared and stream-reared smolts were also sampled for stomach fullness by opening the body cavity, removing all prey items in the stomach and placing them in alcohol. The samples were subsequently dried to a constant weight at 60 °C for 24 h and expressed as mg dry weight/g wet body weight of the whole fish.

2.2. Seawater challenge

Fish were transported from the hatchery, imprint pond or collection facility in a 200-l insulated transport tank with continuous aeration in water obtained at the site. Temperature

changed less than 1 °C during transport to the Conte Anadromous Fish Research Center. Upon arrival, fish were transferred to 1.5-m diameter closed-system tanks with biological and charcoal filtration maintained at 30 ± 0.2 ‰ and 8 ± 0.5 °C. Due to high river temperatures on May 8 (20.7 °C), temperature of the seawater challenge tanks was changed to 12 °C. After 24 h in seawater, fish were anesthetized, bled and plasma stored as described above. Plasma was analyzed for sodium concentration using an ion selective electrode with an AVL analyzer (Roswell, GA, USA) using external standards.

2.3. Gill Na^+,K^+-ATPase activity

Gill Na^+,K^+-ATPase activity was measured according to the microassay protocol of McCormick (1993). Gill filaments were homogenized in SEI buffer containing 0.1% sodium deoxycholate. Following centrifugation ($3000 \times g$ for 0.5 min) to remove large debris, Na^+,K^+-ATPase activity was determined by linking ATP hydrolysis to the oxidation of nicotinamide adenine dinucleotide (NADH), measured at 340 nm for 10 min at 25 °C, in the presence and absence of 0.5 mM ouabain. Protein content in the gill homogenate was measured using a bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL, USA) and specific activities were expressed as μmol ADP mg^{-1} of protein h^{-1}.

2.4. Plasma hormones

Plasma GH levels were measured using a specific double-antibody salmon GH radioimmunoassay as outlined in Björnsson et al. (1994). The GH assay requires duplicate 50-μl samples and there was insufficient plasma to measure plasma GH in parr in 1999. Plasma insulin-like growth factor I (IGF-I) and thyroxine (T_4) were measured in duplicate 10 μl samples by radioimmunoassays as outlined in Moriyama et al. (1994) and Dickhoff et al. (1978), respectively.

2.5. Statistics

All values are reported as mean and standard error. Differences between parr and smolt and changes over time (1999) were examined by two-way ANOVA. Differences between fish that remained in the hatchery and fish released into the river or imprint ponds (1999 and 2000) were examined by one-way ANOVA followed by Newman–Keuls test ($P < 0.05$). Due to the lack of normality and unequal variance, stomach fullness of hatchery-reared and stream-reared smolts was compared using the Mann–Whitney *U*-test.

3. Results

3.1. Hatchery rearing and release into imprint ponds (1999)

Gill Na^+,K^+-ATPase activity of smolts at the hatchery increased 75% between January and March and then remained stable through April (Fig. 1). Gill Na^+,K^+-ATPase of parr

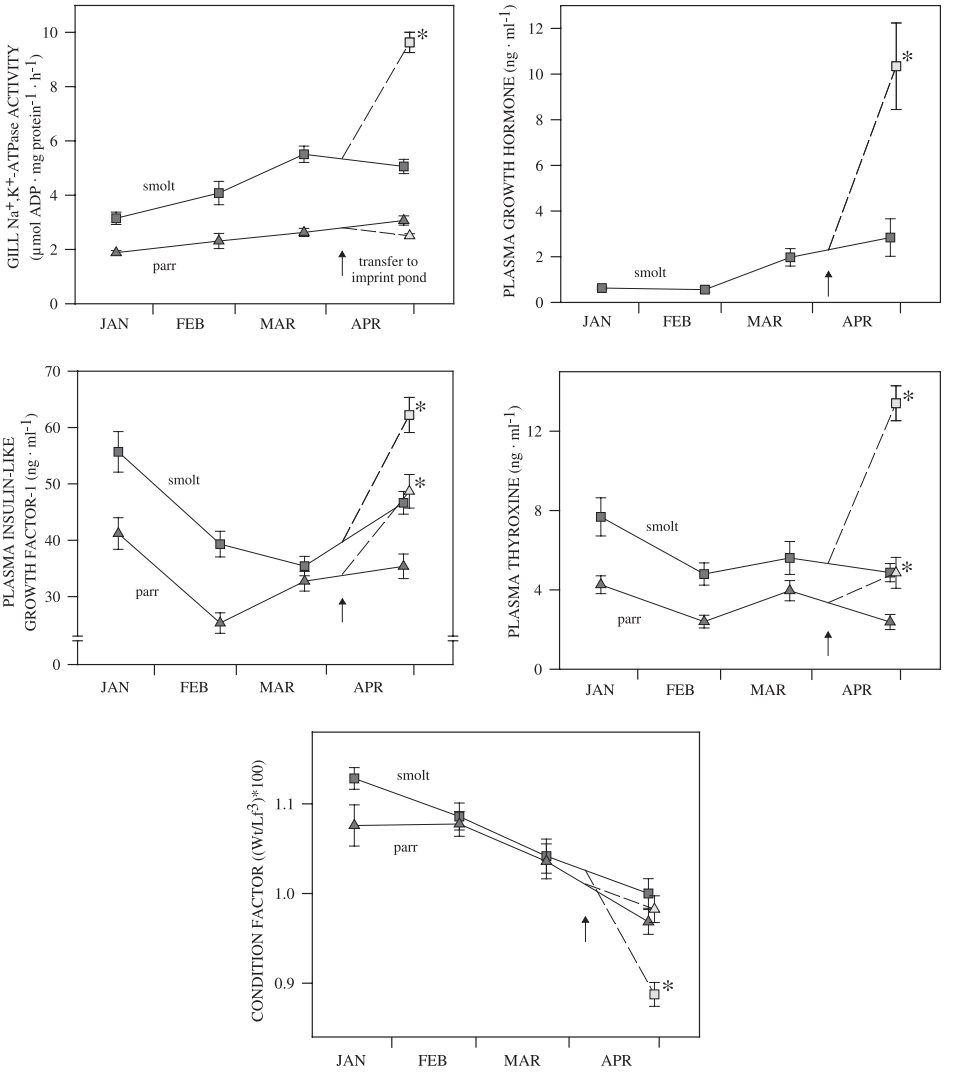

Fig. 1. Physiological and endocrine changes in juvenile Atlantic salmon during hatchery rearing and after release into imprint ponds in 1999. At least 10 fish were sampled at each time interval for each group. There was a significant effect of time for all parameters ($P < 0.05$, two-way ANOVA), a significant effect of group (parr vs. smolt) for all parameters except condition factor and a significant interaction between time and group for all parameters except plasma IGF-I. Asterisk indicates a significant difference of released fish from fish that remained at the hatchery.

was lower than smolts in January and increased slowly but continuously through April (40% increase). Following release into imprint ponds, gill Na^+,K^+-ATPase activity of smolts was 90% higher than smolts remaining in the hatchery, whereas gill Na^+,K^+-ATPase activity of released parr had decreased.

Plasma GH was low (<1 ng ml^{-1}) in hatchery smolts in January and February, then increased to 2–3 ng ml^{-1} in March and April. Plasma GH increased 2.6-fold (>10 ng ml^{-1}) in smolts released into imprint ponds compared to smolts retained in the hatchery.

Plasma IGF-I was high in hatchery smolts in January, decreased progressively through March and then increased again in April. There was an even greater increase in April for fish released into imprint ponds (33% higher than hatchery smolts). A similar pattern of plasma IGF-I was seen for hatchery parr, though levels were generally lower than in smolts. Parr released into imprint ponds had 38% higher plasma IGF-I than fish that remained in the hatchery.

Plasma thyroxine of hatchery smolts decreased slightly between January and April but rose substantially (2.8-fold) in smolts released into imprint ponds. Plasma thyroxine of hatchery parr remained stable from January to April and was generally lower than smolts. Plasma thyroxine of parr in imprint ponds was 2.0-fold higher than parr that remained in the hatchery.

Condition factor of hatchery smolts decreased progressively from January to April, with an even greater decrease for smolts released into imprint ponds. Condition factor of hatchery parr was similar to that of hatchery smolts throughout the study, but condition factor of parr did not decrease following release into imprint ponds.

3.2. Hatchery rearing and release into imprint ponds and the river (2000)

Gill Na^+,K^+-ATPase activity of smolts at the hatchery increased 96% between January and April and then decreased slightly in early May (Fig. 2). Following release into imprint ponds, gill Na^+,K^+-ATPase activity was 82% higher than smolts remaining in the hatchery. Hatchery-reared smolts recaptured as active migrants on April 26 and May 8 had gill Na^+,K^+-ATPase activity that was 63% and 2.5-fold higher, respectively, than fish that remained in the hatchery. These levels were lower than stream-reared smolts migrating on April 26, but nearly identical on May 8.

Plasma GH of smolts at the hatchery increased 2.6-fold between January and April and then decreased slightly in early May (Fig. 2). Following release into imprint ponds, plasma GH was 10.7-fold higher than smolts remaining in the hatchery. Hatchery-reared smolts recaptured as active migrants on April 26 and May 8 had plasma GH that was 8.3- and 13.1-fold higher, respectively, than fish that remained in the hatchery. These levels were lower than stream-reared migrating fish on April 26, but nearly identical on May 8.

Plasma IGF-I of smolts at the hatchery increased 73% between January and March, decreased in April and rose again in early May (Fig. 2). Plasma IGF-I of fish released into imprint ponds was nearly identical to smolts remaining in the hatchery. Although hatchery-reared and stream-reared smolts recaptured as active migrants on April 26 and May 8 had slightly higher levels of plasma IGF-I than fish that remained in the hatchery, there was no significant difference among these groups.

Plasma thyroxine of smolts at the hatchery increased slightly between January and April, and then rose steeply in early May (Fig. 2). Although plasma thyroxine of fish released into imprint ponds and captured as active migrants was much higher then when they were released in mid-April, the levels were not significantly different from fish

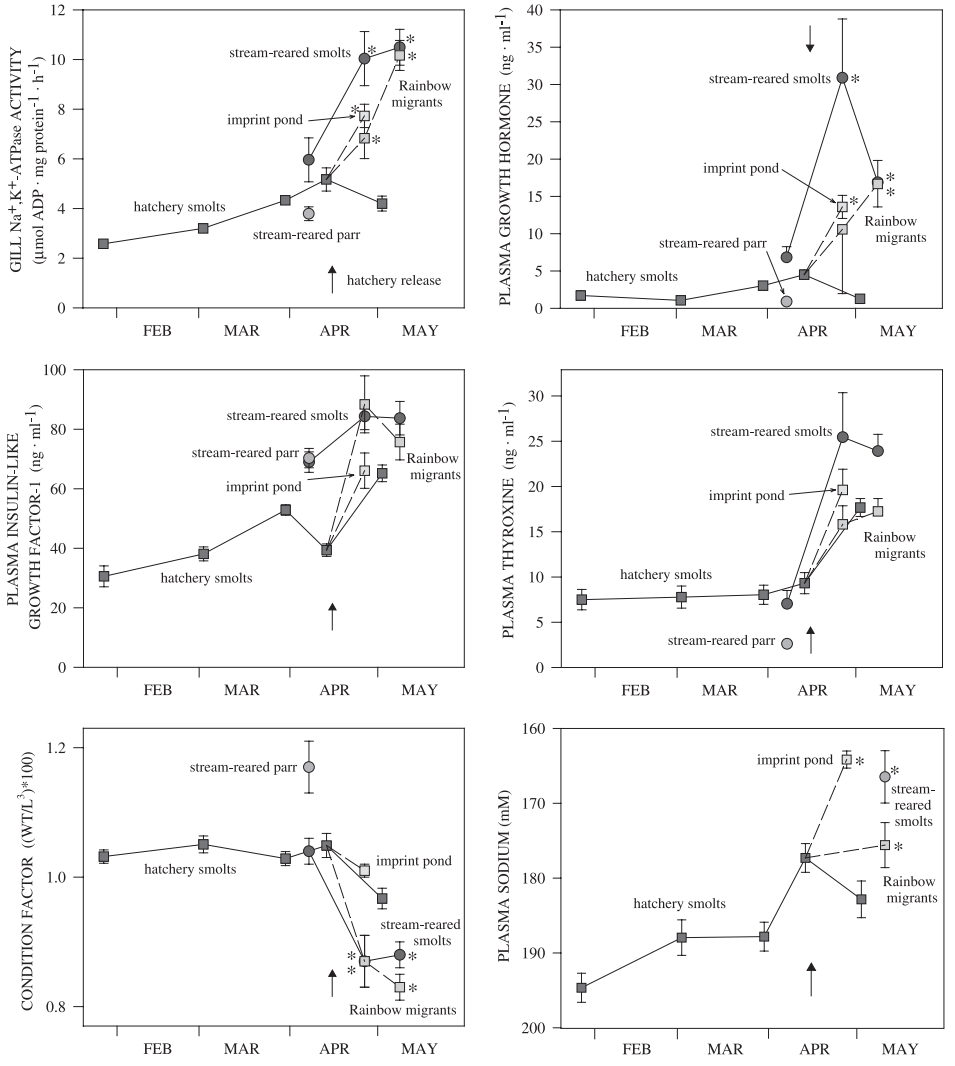

Fig. 2. Physiological and endocrine changes in juvenile Atlantic salmon during hatchery rearing and after release into imprint ponds and into the Farmington River in 2000. At least 10 fish were sampled at each time interval for each group. Stream-reared fish were those that had been released as fry 1 year earlier (parr) or 2 years earlier (smolts). Smolts in imprint ponds were sampled on April 26. Stream-reared parr and smolt were captured prior to migration on April 7 by electrofishing on West Salmon Brook, a tributary of the Farmington River, and as actively migrating smolts at a smolt bypass on the Farmington River on April 26 and May 8, at the same time that hatchery-reared fish were recaptured (Rainbow migrants). Asterisk indicates a significant difference of released fish from fish that remained at the hatchery and sampled on May 2.

remaining in the hatchery and sampled in early May. Plasma thyroxine of stream-reared smolts captured as active migrants on April 26 and May 8 was higher than hatchery-reared smolts but did not differ significantly.

Condition factor of hatchery smolts remained relatively constant from January to April and then dropped slightly in early May (Fig. 2). Following release into imprint ponds, condition factor was similar to that of smolts remaining in the hatchery. Hatchery-reared smolts recaptured as active migrants on April 26 and May 8 had condition factor that was significantly lower than that of fish remaining in the hatchery. These levels were similar to those of migrating stream-reared smolts on April 26 and May 8.

Salinity tolerance as measured by changes in plasma sodium after 24 h exposure to 30‰ seawater increased progressively in hatchery fish between January and April and then decreased slightly in early May (Fig. 2). Following release into imprint ponds, salinity tolerance was significantly higher than that of smolts remaining in the hatchery. Hatchery-reared smolts recaptured as active migrants on May 8 also had significantly higher salinity tolerance, though these were lower than migrating stream-reared smolts.

For the mean values presented in Fig. 2, there was a significant correlation of gill Na^+,K^+-ATPase activity and plasma GH ($r^2 = 0.96$), IGF-I ($r^2 = 0.76$) and thyroxine ($r^2 = 0.68$). There were significant negative correlations between plasma sodium after seawater challenge and gill Na^+,K^+-ATPase activity ($r^2 = 0.70$), plasma GH ($r^2 = 0.66$), IGF-I ($r^2 = 0.62$) and thyroxine ($r^2 = 0.67$). The three endocrine parameters were also positively correlated with one another with r^2 values between 0.62 and 0.85.

There was substantial individual variability in stomach fullness of actively migrating smolts sampled on May 8 (0.00–4.18 mg dry weight/g wet body weight). One of 11 hatchery fish and 6 of 12 stream-reared fish had no discernible stomach contents. Mean relative stomach fullness was significantly greater for hatchery-reared than stream-reared smolts (1.06 ± 0.27 and 0.49 ± 0.35 mg dry weight/g wet body weight, respectively, $P = 0.012$). Larval and adult stages of aquatic and terrestrial insects were the major prey items in all smolts examined. There was a significantly positive correlation between stomach fullness and gill Na^+,K^+-ATPase activity ($r^2 = 0.18$), but no significant correlation of stomach content with any endocrine parameter.

3.3. Effect of temperature on smolt development

Fish subjected to warm temperature treatment (11 °C) had 22% higher gill Na^+,K^+-ATPase activity than fish under the cold temperature treatment (5 °C), but these difference were not significantly different ($P = 0.086$, Table 1). Salinity tolerance also did not differ

Table 1
Physiological changes in fish reared at Pittsford National Fish Hatchery and transferred to the Conte Lab where they were given temperature treatments characteristic of the hatchery (5 °C) or the Farmington River (11 °C)

	5 °C treatment	11 °C treatment	P-level
Gill Na^+,K^+-ATPase	6.12 ± 0.47	7.46 ± 0.59	0.0865
Plasma GH (ng ml^{-1})	4.09 ± 0.52	9.43 ± 1.79	0.0121
Plasma IGF-I (ng ml^{-1})	47.8 ± 2.7	58.3 ± 2.6	0.0116
Plasma T_4 (ng ml^{-1})	22.0 ± 2.1	8.8 ± 1.0	< 0.0001
Condition factor	1.04 ± 0.02	1.0 ± 0.02	0.0182
Plasma Na (mM)	166.9 ± 0.6	168.5 ± 1.4	0.2646

Fish were transferred on March 30 and sampled on May 4. Plasma Na values are those 24 h after transfer to 30 ppt seawater. Values are mean ± standard error.

between warm and cold temperature treatments. Plasma GH increased 2.3-fold and IGF-I increased 22% in the warm treatment, whereas plasma thyroxine was 2.5-fold higher in cold than warm treatment. Condition factor was slightly but significantly lower in the warm temperature treatment.

4. Discussion

In each of the 2 years studied, smolts in imprint ponds had higher levels of gill Na^+,K^+-ATPase activity and higher plasma GH than fish kept in the hatchery. Salinity tolerance was also measured in year 2000 and was significantly greater in smolts released into imprint ponds. Similar increases were found in hatchery-reared fish that were recaptured as active migrants, and the gill Na^+,K^+-ATPase activity, condition factor and plasma GH levels were similar to that of stream-reared smolts (those that had been released as fry 2 years earlier and were recaptured as smolts). These results indicate that substantial and physiologically relevant changes can occur in hatchery-reared fish after they are released from the hatchery. There was an especially strong correlation between gill Na^+,K^+-ATPase activity and plasma GH ($r^2 = 0.96$). Previous research has shown that exogenous GH can increase both salinity tolerance and gill Na^+,K^+-ATPase activity in Atlantic salmon (McCormick, 1996) and is likely to be the most important environmental cue for smolting (Björnsson, 1997). Thus, the post-release changes in GH of hatchery-reared fish may have had a causal role to the increased salinity tolerance and gill Na^+,K^+-ATPase activity observed in these fish.

Releases into imprint ponds in year 1999 caused plasma thyroxine levels to increase relative to fish remaining in the hatchery and this increase was much larger in smolts than in parr. Plasma thyroxine levels rose to even higher levels in imprint ponds in year 2000, but the effect of release was not as clear, because plasma thyroxine in this year increased in the hatchery in early May, whereas it had remained constant in 1999. Nonetheless, in all instances, there was an increase in plasma thyroxine of hatchery smolts after release into imprint ponds and in the wild to levels that were much higher than when the fish were released in mid-April. Although thyroid hormones have at least a supporting role in osmoregulatory changes during smolting, they have been even more strongly implicated in the changes in migratory behavior and imprinting that occur during smolting (Iwata, 1995; McCormick et al., 1998). If the surge in thyroxine does cause imprinting, then the higher thyroid levels observed in fish in imprint ponds and actively migrating hatchery-reared smolts suggests that these fish will have imprinted to the Farmington River.

The inclusion of both parr and smolt in imprint ponds provides an opportunity to evaluate whether post-release changes are specifically related to smolt development. Increased gill Na^+,K^+-ATPase activity and decreased condition factor in imprint ponds occurred only in smolts. Although plasma thyroxine increased significantly in both parr and smolts, the relative increase and absolute levels were much higher in smolts. Plasma IGF-I levels increased in both groups and was slightly higher in smolts than in parr. It would therefore appear that the increase in gill Na^+,K^+-ATPase activity, decrease in condition factor and large increases in plasma thyroxine that occur after hatchery release are directly related to the parr–smolt transformation. Although we were unable to test it in

the present study, the large increases in plasma GH that occurred in hatchery-reared smolts after release (Fig. 2) are also likely to be dependent on developmental stage. Parr in the wild (McCormick and Björnsson, 1994) and in hatcheries (Shrimpton et al., 2000) do not show significant increases in plasma GH during mid to late spring.

For the most part, there was a good correspondence between changes in gill Na^+,K^+-ATPase activity and salinity tolerance. The moderate increases in gill Na^+,K^+-ATPase activity in hatchery fish was accompanied by moderate decreases in plasma sodium after seawater challenge (increased salinity tolerance). In fish released into imprint ponds and actively migrating stream-reared smolts, both gill Na^+,K^+-ATPase activity and salinity tolerance were higher than in fish in the hatchery. The one exception was in hatchery fish released upstream of Rainbow dam and recaptured as actively migrating smolts (Fig. 2). These fish had high levels of gill Na^+,K^+-ATPase activity but only moderate salinity tolerance. Because the salinity-challenged fish are subjected to handling and transport prior to the test, it is possible that this stress affected their performance. The stress may have been greater in the hatchery fish relative to stream-reared fish as the former had been transported 2 weeks prior to their recapture. Previous research on coho salmon (*O. kisutch*) smolts has shown that stress effects can be cumulative (Barton et al., 1986). In addition, there was a greater temperature differential between fresh water and seawater at the last sampling period. These are inherent limitations in the seawater challenge test under field conditions. For these reasons, gill Na^+,K^+-ATPase activity may be a more unbiased measure of osmoregulatory smolt development.

Hatchery-reared smolts in the present study did not complete smolting by late April or early May, even when held after other fish had been released. Other hatcheries using the same genetic stock of salmon have much greater smolt development occurring in the hatchery by this time (Shrimpton et al., 2000). This difference may be due to the relatively cold (near ambient) winter and spring temperatures of Pittsford National Fish Hatchery, compared with the warmer conditions at many other hatcheries. Several studies have shown that certain aspects of the parr–smolt transformation are delayed or compromised in hatchery-reared fish (McCormick and Björnsson, 1994; Shrimpton et al., 1994; Sundell et al., 1998). Leonard and McCormick (2001) have recently shown that several important physiological indicators of smolt development can be advanced in a hatchery where warm winter conditions prevail and hypothesized that this differential in developmental events may have negative consequences for survival after release. The apparently incomplete or delayed development in the hatchery examined in the present study may actually be advantageous if it allows fish to complete development after release with a timing that is more similar to wild fish.

While it is clear that there are important changes that occur after release from the hatchery, the environmental cues that direct these changes are less clear. The goal of the laboratory temperature study was to mimic the temperature differential between the hatchery and fish released into the wild. These studies indicate that temperature could account for some of the observed changes, particularly in plasma GH and IGF-I, which increased significantly in the high temperature treatment (Table 1). Previous laboratory studies have also suggested that higher rearing temperatures can advance smolt-related increases in gill Na^+,K^+-ATPase activity, plasma GH and IGF-I (McCormick et al., 2000). In contrast, plasma thyroxine decreased with increased temperature in the present study,

suggesting that the increased temperature experienced in the imprint ponds and in the wild is unlikely to be responsible for the large increases in plasma thyroxine in these fish. Novel water, increased flow and turbidity have been suggested to affect thyroid hormones in smolting salmonids (Iwata, 1995; McCormick et al., 1998). Specker et al. (2000) found that plasma thyroxine increased in Atlantic salmon smolts within hours of exposure to increased turbidity caused by cleaning of hatchery ponds. When and under what conditions smolts are stocked may play a role in their subsequent physiological responses, and further investigation of the factors that affect these responses is warranted.

Actively migrating fish recaptured in this study are a subset of the original population and the possibility that this is not an unbiased sample of the hatchery population that was originally released cannot be ruled out. It is likely that some fish have succumbed to predators and others may not have migrated. In a study of hatchery-reared brown trout, Ugedal et al. (1998) found that only 50% of the fish had high salinity tolerance at the time of release (plasma chloride <160 mM, 72 h after exposure to after 34‰ seawater), whereas almost all of the migrating fish had high salinity tolerance. Since only 34% of the fish were found to migrate after release, the recaptured fish may have represented a subset of the originally released population. It was observed that the average fin condition of recaptured fish was superior to that of fish at the hatchery, yet the period of time seemed too short for substantial fin regeneration. Thus, the possibility cannot be ruled out that some of the observed differences were due to the fact that recaptured fish were a subset of the original population, perhaps those with superior smolt development and fin condition. However, such unbiased sample cannot explain all of the observed differences. Fish sampled in imprint ponds had many of the same physiological changes observed after release, yet were a random sample of the original hatchery populations. In addition, the changes observed for some of the physiological changes such as gill Na^+,K^+-ATPase activity and plasma GH were several fold higher in recaptured smolts and it seems unlikely that a sampling artifact alone could explain such large differences.

The large individual variation in stomach fullness in both hatchery- and stream-reared smolts in the present study is notable. Although several fish had empty stomachs, most of these fish had at least a small amount of material in the intestine, suggesting they were not in a state of starvation. These results may also have been influenced by the relatively high temperatures just prior to capture May 8 (temperatures had increased rapidly over the previous several days due to an early heat wave and low water flows). Given the widely held notion that hatchery fish adapt poorly to conditions in the wild, it is somewhat surprising that a greater proportion of the hatchery-reared smolts had food in their stomachs and had greater mean stomach fullness than fish reared in the wild. Munakata et al. (2000) found that hatchery-reared honmasu salmon (*O. rhodurus* × *masou*) released as parr into a stream began feeding within 1 day and had similar stomach fullness as wild fish after 2 weeks. Plasma growth hormone of released hatchery parr was elevated for at least a month after release relative to wild fish. This indicates that hatchery fish have the capacity to successfully forage within 3 weeks after release from the hatchery.

The present study has several implications for survival of hatchery-reared fish that are released into the wild. Maximum ocean survival is thought to depend on ocean entry coinciding with maximum smolt development, particularly those developmental events associated with hypoosmoregulation, as fish that are kept in fresh water past maximum

smolt development may have poor ocean survival (Virtanen et al., 1991; Duston et al., 1991; Staurnes et al., 1993; McCormick et al., 1999). The capacity of hatchery-reared fish to complete smolting after release into the river and to have similar timing of developmental events to that of stream-reared fish indicates that these fish may have near optimal developmental timing. Prior to ocean entry, the process of imprinting to the stream of origin may be important to population structure and overall reproductive success. Previous research has implicated thyroid hormones in the imprinting process (McCormick et al., 1998), and the large increases in plasma thyroxine levels may indicate that fish are imprinting after release and during their downstream migration. While this proposed scenario is attractive, further research will be necessary to determine the survival advantage conferred by the endocrine and physiological changes after hatchery release observed in the present study.

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References

Barton, B.A., Schreck, C.B., Sigismondi, L.A., 1986. Multiple acute disturbances evoke cumulative physiological stress responses in juvenile Chinook salmon. Trans. Am. Fish. Soc. 115, 245–251.

Björnsson, B.Th., 1997. The biology of salmon growth hormone: from daylight to dominance. Fish Physiol. Biochem. 17, 9–24.

Björnsson, B.Th., Taranger, G.L., Hansen, T., Stefansson, S.O., Haux, C., 1994. The interrelation between photoperiod, growth hormone, and sexual maturation of adult Atlantic salmon (*Salmo salar*). Gen. Comp. Endocrinol. 93, 70–81.

Dickhoff, W.W., Folmar, L.C., Gorbman, A., 1978. Changes in plasma thyroxine during smoltification of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 36, 229–232.

Duston, J., Saunders, R.L., Knox, D.E., 1991. Effects of increases in freshwater temperature on loss of smolt characteristics in Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 48, 164–169.

Iwata, M., 1995. Downstream migratory behavior of salmonids and its relationship with cortisol and thyroid hormones: a review. Aquaculture 135, 131–139.

Leonard, J.B.K., McCormick, S.D., 2001. Metabolic enzyme activity in stream- and hatchery-reared Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 58, 1585–1593.

McCormick, S.D., 1993. Methods for non-lethal gill biopsy and measurement of Na^+,K^+-ATPase activity. Can. J. Fish. Aquat. Sci. 50, 656–658.

McCormick, S.D., 1996. Effects of growth hormone and insulin-like growth factor I on salinity tolerance and gill Na^+,K^+-ATPase in Atlantic salmon (*Salmo salar*): interactions with cortisol. Gen. Comp. Endocrinol. 101, 3–11.

McCormick, S.D., Björnsson, B.Th., 1994. Physiological and hormonal differences among Atlantic salmon parr and smolts reared in the wild, and hatchery smolts. Aquaculture 121, 235–244.

McCormick, S.D., Hansen, L.P., Quinn, T.P., Saunders, R.L., 1998. Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 55, 77–92.

McCormick, S.D., Cunjak, R.A., Dempson, B., O'Dea, M.F., Carey, J.B., 1999. Temperature-related loss of smolt characteristics in Atlantic salmon in the wild. Can. J. Fish. Aquat. Sci. 56, 1649–1658.

McCormick, S.D., Moriyama, S., Björnsson, B.Th., 2000. Low temperature limits photoperiod control of smolting in Atlantic salmon through endocrine mechanisms. Am. J. Physiol. 278, R1352–R1361.

Moriyama, S., Swanson, P., Nishii, M., Takahashi, A., Kawauchi, H., Dickhoff, W.W., Plisetskaya, E.M., 1994. Development of homologous radioimmunoassay for coho salmon insulin-like growth factor-I. Gen. Comp. Endocrinol. 96, 149–161.

Munakata, A., Björnsson, B.Th., Jonsson, E., Amano, M., Ikuta, K., Kitamura, S., Kurokawa, T., Aida, K., 2000. Post-release adaptation processes of hatchery-reared honmasu salmon parr. J. Fish Biol. 56, 163–172.

Shrimpton, J.M., Bernier, N.J., Iwama, G.K., Randall, D.J., 1994. Differences in measurements of smolt development between wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) before and after saltwater exposure. Can. J. Fish. Aquat. Sci. 51, 2170–2178.

Shrimpton, J.M., Björnsson, B.Th., McCormick, S.D., 2000. Can Atlantic salmon smolt twice? Endocrine and biochemical changes during smolting. Can. J. Fish. Aquat. Sci. 57, 1969–1976.

Specker, J.L., Eales, J.G., Tagawa, M., Tyler, W.A., 2000. Parr–smolt transformation in Atlantic salmon: thyroid hormone deiodination in liver and brain and endocrine correlates of change in rheotactic behavior. Can. J. Zool. 78, 696–705.

Staurnes, M., Lysfjord, G., Hansen, L.P., Heggberget, T.G., 1993. Recapture rates of hatchery-reared Atlantic salmon (*Salmo salar*) related to smolt development and time of release. Aquaculture 118, 327–337.

Sundell, K., Dellefors, C., Björnsson, B.Th., 1998. Wild and hatchery-reared brown trout, *Salmo trutta*, differ in smolt related characteristics during parr–smolt transformation. Aquaculture 167, 53–65.

Ugedal, O., Finstad, B., Damsgard, B., Mortensen, A., 1998. Seawater tolerance and downstream migration in hatchery-reared and wild brown trout. Aquaculture 168, 395–405.

Virtanen, E., Soderholmtana, L., Soivio, A., Forsman, L., Muona, M., 1991. Effect of physiological condition and smoltification status at smolt release on subsequent catches of adult salmon. Aquaculture 97, 231–257.

Zaugg, W.S., Prentice, E.F., Waknitz, F.W., 1985. Importance of river migration to the development of seawater tolerance in Columbia river anadromous salmonids. Aquaculture 51, 33–47.