ENDOCRINE CONTROL OF SMOLTING:

AN OVERVIEW

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EXTENDED ABSTRACT ONLY DO NOT CITE

Environmental entrainment of the parr-to-smolt transformation is mediated by the endocrine system. Smolting is associated with changes in plasma levels of a constellation of hormones, including insulin, growth hormone (GH), insulin-like growth factor-I (IGF), thyroid hormones, and cortisol, among others (Fig. 1). Plasma insulin is often the first to become elevated in spring-smolting fish. Plasma GH, IGF, thyroxine and cortisol become elevated almost simultaneously during spring concomitant with a decline in plasma insulin. GH may indirectly be responsible for elevated blood cortisol since GH has been shown to enhance cortisol production by interrenal tissue in response to adrenocorticotropin (ACTH). Increased IGF production may be due to direct stimulation by GH. Enhanced IGF production may also derive from elevated insulin and thyroid hormones.

Functions of the hormones in controlling smolt physiology are becoming established. GH not only regulates growth and behavior, but also influences seawater tolerance in cooperation with cortisol and IGF (see Th. Bjornsson, this symposium). Thyroid hormones clearly promote body silvering, among other actions, and also synergize in actions of other hormones.

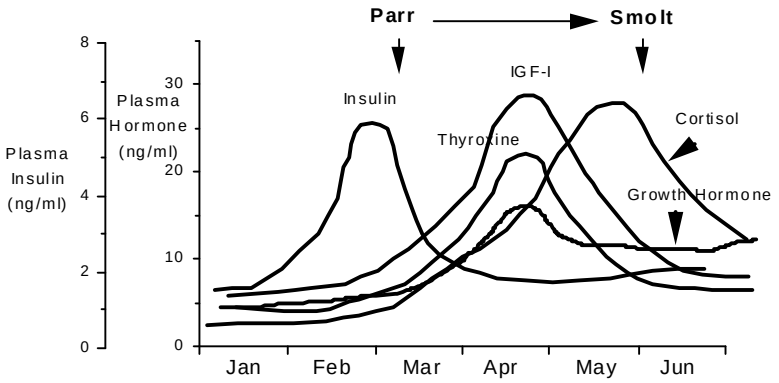

Figure 1. Composite diagram of plasma hormone levels during smoltification of coho salmon (after Dickhoff et al., 1997). Significant departure from the pattern of hormonal change shown may be observed during smolting of fish in varied environmental conditions.

Vernal increase in daylength, temperature, and feeding activity may be the primary zeitgebers for spring smolting. Increasing photoperiod is probably the primary stimulator of GH production. Temperature and feeding activity may enhance production of insulin, IGF, and thyroid hormones (Dickhoff et al., 1997).

The endocrine regulation of smolting is complex and interactive. The possibility for direct actions of GH (independent of IGF), GH stimulated local or systemic production of IGF, interaction of IGF-I (or IGF-II) with insulin receptors complicate interpretation of results (Fig. 2). Injection or implantation of hormones into parr near the onset of smoltification is an informative experimental approach to examine possible physiological actions of hormones during smoltification. However, such injection experiments may give misleading results. For example, injection of GH may result in hyperglycemia, which would be followed by a surge in plasma insulin, thus complicating the interpretation of GH action. More graded or gradual changes in plasma GH levels as observed during the normal process of smolting may not produce hyperglycemia and related hyperinsulinemia. The role of each hormone, receptor specificity, receptor regulation, ligand binding proteins (IGFBPs) and their modifying enzymes, their response to environmental factors, and their

actions on smolt physiology and behavior need to be defined more precisely for a coordinated view of the endocrine control of smoltification.

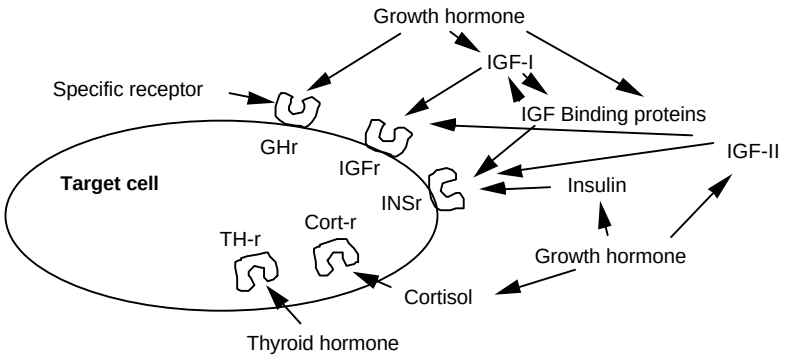

Figure 2. Potential actions of hormones on target cell regulation. All of the different routes of action have been shown in vertebrates or in smolting salmonids.

Most studies of smoltification have been conducted on juvenile salmonids during the spring. Many of the endocrine changes coincident with smolting during spring are annual changes also observed in older fish in seawater and in maturing adults. Changes in insulin, GH, and IGF during the spring are undoubtedly controlling seasonal metabolic adjustments favoring increased protein anabolism during the spring and summer growing season. It is difficult to separate metabolic endocrine changes from specific smoltification processes during the spring. Comparative studies of autumn-smolting species, for example, chinook salmon, should provide an opportunity to separate smolt-specific endocrine functions from general metabolic actions ocurring during the spring.

Reference

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PROBABILITY OF SEAWARD MIGRATION IN PREVIOSLY MATURE ATLANTIC SALMON (*SALMO SALAR*) MALE PARR

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Early sexual maturation is commonly observed among wild and hatchery reared male Atlantic salmon (*Salmo salar*) parr. Even if mature male parr may smoltify successfully the following spring, there is evidence from natural stocks that they do not migrate to sea in the same numbers as sexually immature fish of the same age. Also, previously mature male parr from hatchery stocks released into the river during the smolt migration are less likely to migrate to the sea, and they may contribute significantly less to the sea-catch of salmon than do previously immature males. The conclusions concerning the effects of early sexual maturation on smolting in male parr are, however, still contradictory. Some studies have reported similar smolt performance in previously mature males compared with immature fish, other studies have reported poorer performance in previously mature. This paper summarises important findings on smolting in previously mature males reported in published papers and provides new information from a study on wild salmon on Island.

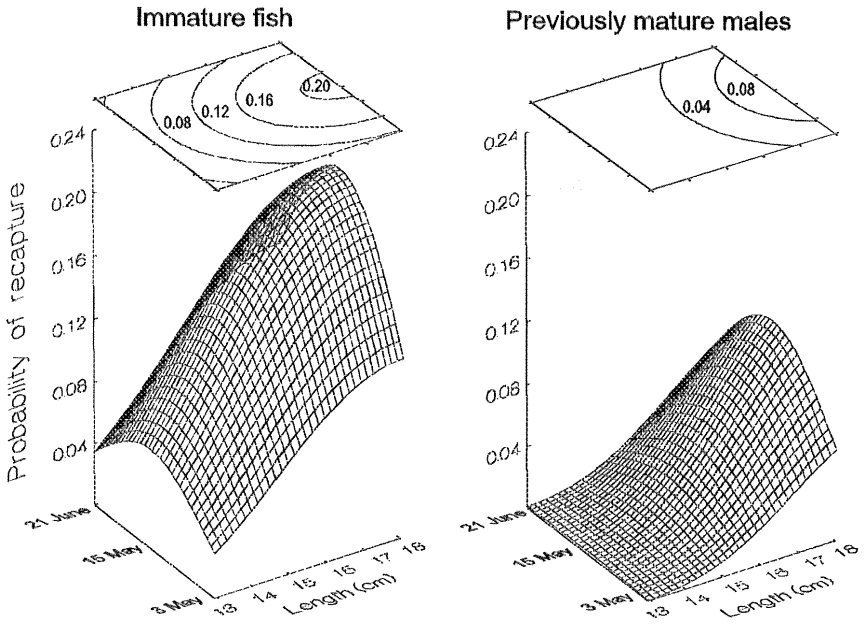

Figure 1. Predicted probabilities of adult recapture rates for releases of immature fish and previously mature male parr in relation to fish size and release date. Probabilities were obtained by probit regression (model likelihood ratio of fit, $\chi^2 = 849$; df = 5; $p < 0.001$) using data on length at tagging and adult recapture incidence of individual fish. (Adopted from Berglund et. al 1992)

Berglund et al. (1992) released tagged previously mature male parr and immature fish of hatchery origin and evaluated recapture rates in the sea as a function of length at tagging and release date. It was found that the recapture rate of mature parr was lower than for immature fish and that the depression was higher for smaller fish (Fig. 1). The recapture rate of mature males were ca. 40% and 7% of that of immature fish for 17 and 13 cm fish, respectively. It was concluded, from complementary studies seawater adaptability and migratory behaviour, that the main reason for the observed pattern was that previously mature males had a lower likelihood to go through the parr-smolt transformation

In natural rivers, the decreased probability of smoltification in previously mature parr, compared with immature parr, is related to increased winter mortality (Myers 1984) and to a lower likelihood to go through the parr-smolt transformation (Berglund et. al 1992). In a study on wild salmon in Little Codroy River, Myers (1984) concluded that the probability of smolting in the second year for a mature male parr was approximately 13% of that of a female.

In order to get more information on the size-specific probability of smolting in wild salmon parr, and to test the generality of Myer's findings, we studied smolting of individually identified mature male and immature parr in River Ulfarsa on Iceland. In the autumn 1994, 1995 and 1996, in total 4534 parr were caught by electrofishing and tagged with PIT-tags. Fish were measured, assigned as mature or immature, and the probability of smolting was calculated by using data from a smolt trap operated in spring.

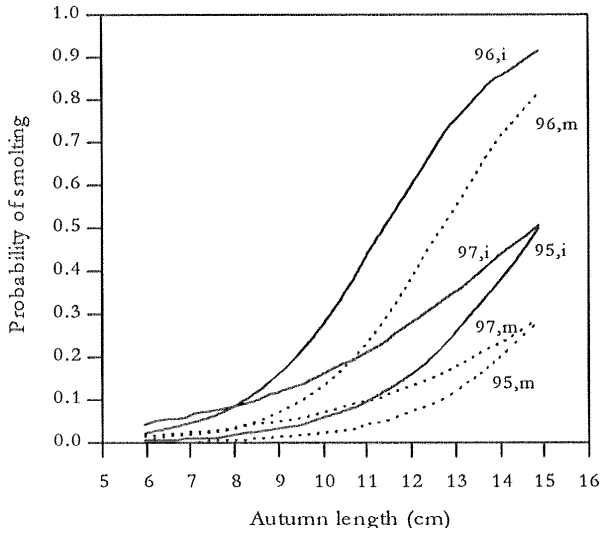

Figure 2. Predicted probabilities of smolting of immature fish (solid lines) and previously mature male parr (broken lines) in relation to fish size in the autumn prior to migration in spring and smolt year. Probabilities were obtained by logistic regression (model likelihood ratio of fit, $\chi^2 = 589$; df = 6; $p < 0.001$) using data on length at tagging or recapture in autumn and smolt trap recapture incidence of individual fish. Smolt year and

state of maturity at tagging is indicated in the figure. (Arnason et. al unpublished).

The results showed a pattern similar to that observed for adult recapture rates of hatchery fish (Fig. 1). Previously mature males had a substantially decreased probability of smolting compared with immature fish (Fig. 2). In general the probability of smolting for mature male parr was approximately 50% of that of immature fish. In good smolt years, however, the difference seems to be much smaller for large parr.

It is concluded that, in general, the probability of smolting of a mature male parr is substantially lower than that of immature fish, but that the difference between the two categories vary both with geographical location and among years within a river. It seems probable that climatic conditions affect both the winter survival of a mature male parr and its likelihood to transform into a smolt.

The Swedish Forestry and Agricultural Research Council (SJFR) supported this study.

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SMOLTING-RELATED CHANGES

IN THE SALMONID VISUAL SYSTEM:

OBSERVATIONS AND ENDOCRINOLOGY

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Abstract

Anadromous salmonids undergo the parr-smolt transformation in preparation for a seawater existence. In addition to all the classic smolting-related physiological changes, the visual system undergoes a shift from a "long wavelength"-sensitive visual pigment (porphyropsin) to a "short wavelength"-sensitive visual pigment (rhodopsin). These ocular changes optimizes the sensitivity of the retina to correspond with changes from the "red-dominated" light environment found in freshwater to the "blue-dominated" marine light environment.

Thyroid hormones are known to play a significant role in smolting, and they have also been shown to play a significant role in visual pigment changes. Generally, smolting-related peaks in 3,5,3'-triiodo-L-thyronine (T_3) result in a shift from a porphyropsin-dominated retina to a rhodopsin-dominated retina. Recent investigations have indicated that T_3 acts directly on the tissue responsible for visual pigment changes, the retinal pigment epithelium (RPE). These investigations on the RPE have demonstrated the presence of T_3 receptors; 5'-deiodinase enzyme activity (the ability to convert T_4 to T_3); and *in vitro* responses to T_3-enhanced medium consistent with visual pigment shifting.

Introduction

The parr—smolt transformation process transforms anadromous salmonids from stream-dwelling parr to seawater-adaptable smolts. Timely changes in metabolism, growth, osmoregulation (reviewed by Hoar, 1988) behaviour, and olfaction (Scholz *et al*. 1985; White *et al.* 1990) are directed by an orchestration of growth hormone (GH), cortisol (Richman and Zaugg, 1987) prolactin (PRL) and thyroid hormones (TH) (Dickhoff *et al.* 1978). Thyroid hormones play perhaps the greatest role in this transformation as they have been shown to affect body silvering, behaviour, osmoregulation, growth and learning ability (Higgs *et al*. 1982; Dickhoff and Sullivan, 1987; Morin *et al*. 1989).

The salmonid visual system also shows profound changes during the parr—smolt transformation. Parr salmonids have been shown to have photoreceptors that are dominated by a long wavelength-sensitive visual pigment based on vitamin A_2 (porphyropsin), while smolt salmonids, which have undergone 4–5 months of physiological changes since the parr stage, exhibit photoreceptors that are dominated by a short wavelength-sensitive visual pigment based on vitamin A_1 (rhodopsin). These kind of changes permit salmonids to adjust the sensitivity of their visual system to accomodate the spectral characteristics of freshwater and marine environments.

Research by Bridges (1972) and Tsin (1979) have indicated that thyroid hormones are involved in changing the visual pigment composition of salmonids and that they might actually have a direct role in these changes. We investigated the role of thyroid hormones in visual pigment shifting by examining the various facets that comprise the "classic" view of the hormone-tissue response paradigm. These facets are as follows: First, observations in the wild of tissue changes occurring in a timely fashion to changes in hormone levels. Second, under a laboratory setting designed to lessen external factors: manipulation of hormone levels and observing tissue responses. Third, *in vitro* experiments to determine if the tissue actually has receptors for the hormone in question. Fourth, the determination of any local (intracellular) mechanisms that may regulate the levels or effects of the hormone. Finally, isolated tissue experiments to determine *in vitro*, if the hormone, in the absence of other endocrine factors, can effect tissue responses that are appropriate to observations seen in the unaltered, intact organism.

Changes In Visual Pigment Compostion During Smolting

Juvenile yearling coho salmon (*Oncorhynchus kisutch* Walbaum) were sampled weekly (21 January to 14 July 14, 1992) from outdoor raceways at Capilano Hatchery at Vancouver, British Columbia, Canada. Fish were challenged with 30‰ seawater for 24 h under darkroom conditions and all subsequent manipulations of animals and retinal samples were performed under darkroom conditions.

Fish were netted and killed by a sharp blow to the head and blood samples were obtained by trans-section of the caudal peduncle and collected into ammonium heparin-coated microhaematocrit tubes. Circulating 3,5,3'-triiodothyronine (T_3) hormone levels were determined via the use of radioimmunoassay (RIA) and plasma sodium levels (indicating hypo-osmoregulatory ability *viz*. state of smoltification) were determined by flame spectroscopy (Pye-Unicam model SP 191).

Visual pigments were obtained by removing the eyes after blood collection and removing the retinae and associated pigment epithelia and placing in a potassium alum solution. For extraction and analysis, the solution was decanted, the tissue was washed with double-distilled water and centrifuged lightly. The tissue was then placed in an ice-cold 15 ml rotary glass homogenizer and the visual pigments were extracted with a 0.4 ml sample of 2% digitonin prepared as described by Bridges (1977). After homogenization, the homogenate was poured into 1.5 ml ultracentrifuge tubes (Beckman) and incubated at 23 C for 1 h. After centrifugation (24,000 *g*) for 15 min at 4 C, the supernatant was removed with a pasteur pipette buffered with saturated sodium borate (1/10 v/v) and the extracts were transferred by pasteur pipette to 1.5 ml microcuvettes for analysis. Prior to spectrophotometric analysis, each sample received 0.05 ml of freshly prepared 0.2 M neutralized hydroxylamine sulphate. Retinal samples were analyzed using the bleaching method described by Munz and Beatty (1965), Beatty (1966;1969) and Tsin (1979). Samples were placed into a temperature-controlled cell holder (23±0.3 C) in a computer-driven Spectronic 3000 array recording spectrophotometer (Milton Roy). The optical density of the samples before bleaching were determined from 360 to 650 nm. The samples were exposed(12 min) to a 40 W quartz halogen lamp placed 40 cm above the cuvettes then returned to the spectrophotometer and bleached spectra were obtained. Referring to templates from Munz and Beatty (1965), the percent porphyropsin was estimated by calculating from the normalized difference spectrum of each extract.

Plasma sodium and visual pigment changes can be seen in Figure 1.

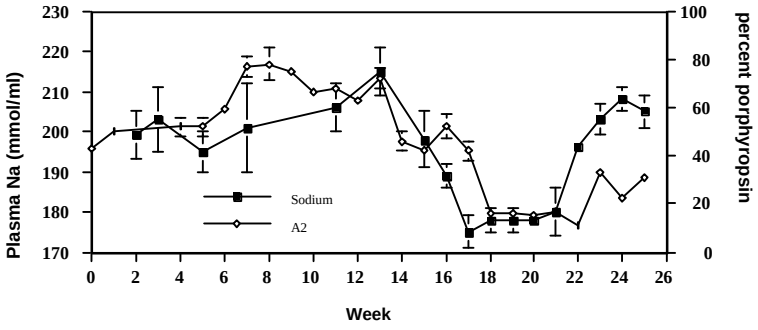

Figure 1. Plots of mean plasma sodium concentrations levels of coho salmon transferred into 30‰ seawater for 24 h molar percentage porphyropsin in the retinae of smolting coho salmon. Plasma sodium concentration indicate that between Weeks 17 to 21, the coho can be considered to be smolts. The trends in hypo-osmoregulatory ability and visual pigment composition are evident and correlation analysis of the mean values indicate a significant correlation ($P<0.005$) between plasma sodium and molar percentage porphyropsin.

Changes in circulating T_3 are seen in Fig. 2.

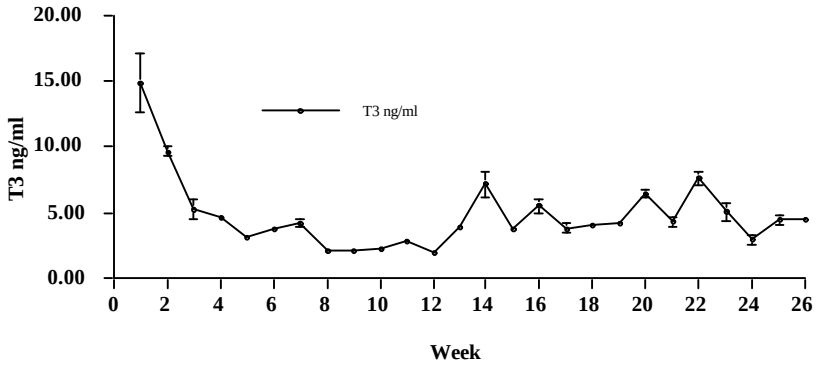

Figure 2. Plasma T_3 titres found in blood samples from smolting coho salmon. Data points are mean values ± s.e. (N=10). Values marked by an asterisk are significantly different ($P<0.05$) from the preceding point.

As seen in Fig. 1., changes in the visual pigment composition of the fish were significantly correlated ($P < 0.005$) with their ability to hypo-osmoregulate. Using hypo-osmoregulatory ability as a measure of smolt-status, it is evident that the period of time where the fish can be considered to be smolts corresponds with the time that the visual system is dominated by rhodopsin, the visual pigment that is most suited for the marine light environment. In concordance with the other prepatory changes seen in coho salmon while they are still in freshwater, the visual pigment conversion process and the acquisition of rhodopsin-dominated retinae may be considered to be a feature of smolting.

Thyroid hormones may have influenced the changes in visual pigment composition of the fish.. Over the 8 weeks after the T_3 maximum that was seen in week 1 (Fig. 2), there was an increase in the molar percentage of porphyropsin. Thyroid hormone studies by Beatty (1969) demonstrated an 80% increase in porphyropsin 3–4 weeks after T_4 (L-thyroxine) injections in kokanee salmon. The initial high levels of T_3, which were observed in this study, can thus be implicated in the rise of porphyropsin content of the retinae as these increases occurred in the 8 weeks following the T_3 maximum. Although the retinae may respond immediately to T_3-directed production of porphyropsin, complete turnover of photoreceptor visual pigment content has been shown to take 4–7 weeks in rainbow trout (Tsin, 1979).

As smoltification progressed, where there was a steady shift to rhodopsin-dominance in the retinae, the role of T_3 is not quite as evident. During the 10 week shift from a porphyropsin- to a rhodopsin-dominated retina (week 9–19), although there was a minor elevation in T_3 levels in week 14 and rhodopsin dominance was seen 4 weeks later, a cause and effect relationship cannot confidently be inferred. There is the possibility, however, that the retinae may respond differently to elevations of T_3 levels and while early responses to T_3 result in shifting to porphyropsin dominance, responses to T_3 during a later stage of development may result in rhodopsin dominance.

The Effect of T_3 and T_3-blockers On Visual Pigment Composition

By manipulating the visual pigment composition of juvenile coho salmon by using different light and temperature regimes and treating fish with both thyroid hormone and thyroid hormone-blockers, an investigation was made of the role of thyroid hormones in controling the visual pigment composition of their retina.

Yearling juvenile coho salmon were obtained from Capilano Hatchery and placed in 100*l* fiberglass "bath-tub" tanks which were divided into two temperature/light regimes: 5 C/dim and 15 C/bright (referred to in this paper as 5 C and 15 C conditions). The "dim" and "bright" tanks showed water-surface illuminance of 9.1×10^{-7} lx and 2.6×10^{-6} lx, respectively. A timer was used to control the lights and the fish were exposed to a L:D 12:12 photoperiod. During different phases of the experiment, the light arrangement and corresponding water supply was switched to the opposite tanks to obviate possible tank effects. The thyroid hormone-treatment fish (T_3) were fed with feed supplemented with T_3 (12 $\mu gm \cdot gm^{-1}$ feed) (Fig. 3) whereas methimazole (1-methyl-2-mercapto-imidazole) was used as an anti-thyroid agent and administered via the feed in the proportions of 6 $mg \cdot gm^{-1}$ feed. To determine the effect of thyroid hormone-blocker on visual pigment shifting, the fish were treated with methiamazole and then transferred to their opposite light/temperature regimes for 4–5 weeks before analysis (Figs. 4 & 5). The procedures involved in visual pigment analysis followed those described in the previous section.

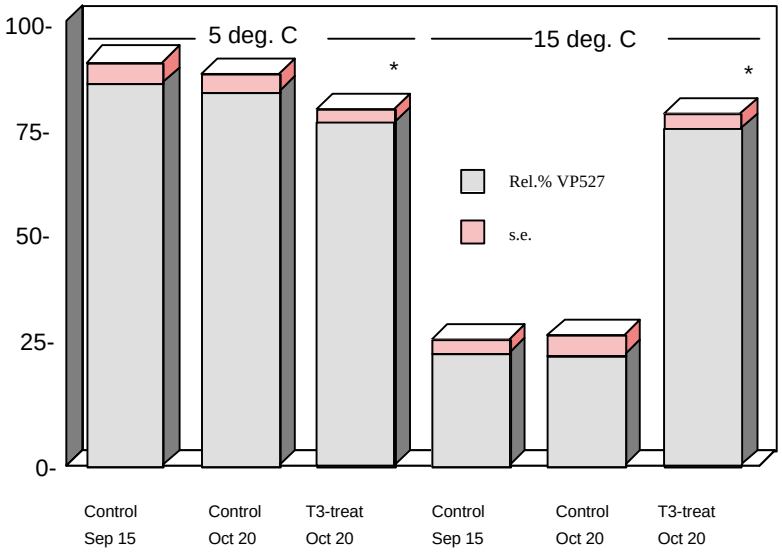

Figure 3. Changes in relative percent porphyropsin in the retinae of coho salmon that were reared at acclimation temperatures of 5 and 15° and treated with T_3 (cross-hatched columns) over a 5 week time period. The asterisk (*) indicates porphyropsin levels that were significantly different ($P < 0.05$) from both initial and 5 week controls. (N=8–10).

The effects of T_3 on rhodopsin-dominated retina are dramatic and are similar to what has been observed by other researcher, however, the response of the porphyropsin-dominated retina, that is a subtle shift to increases in rhodopsin content, indicates that T_3 can have an effect on visual pigment content that results in changes in either direction.

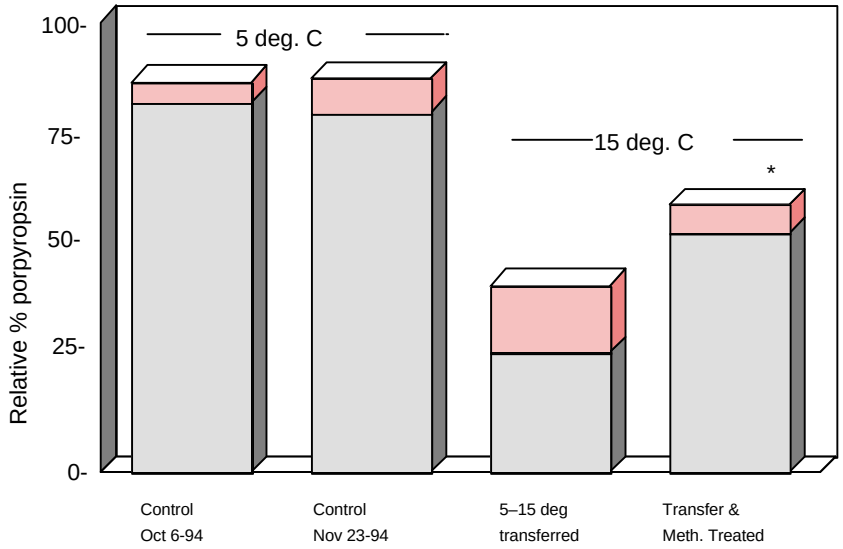

Figure 4. Changes in relative percent porphyropsin in the retinae of coho salmon that were initially acclimated to 5° C and then transferred to 15°C conditions and treated with methimazole or vehicle for 6 weeks. The transfer to 15° C yielded significant reductions in percent porphyropsin however, within this group the methimazole treated group had significantly ($P< 0.05$) higher levels of porphyropsin. (N=8–10).

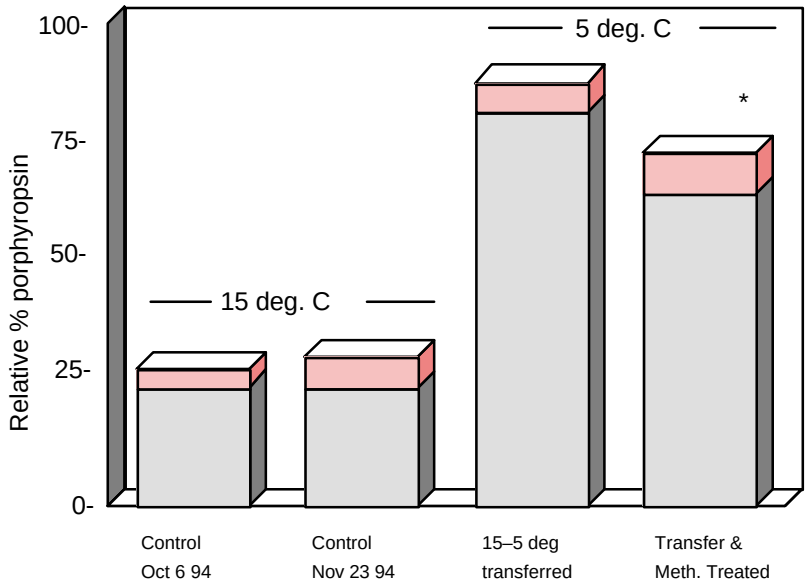

Figure 5. Changes in relative percent porphyropsin in the retinae of coho salmon that were initially acclimated to 15° C and then transferred to 5°C conditions and treated with methimazole or vehicle for 6 weeks. The transfer to 5° C yielded significant increase in percent porphyropsin however, within this group the methimazole treated group had significantly ($P< 0.05$) lower levels of porphyropsin. (N=8–10).

When treated with thyroid hormone-blocker and transferred to conditions that would usually result in a shift in visual pigment composition, it is evident in Figs. 4 and 5 that there was an impairment of visual pigment shifting. The conclusions of this experiment would be that the administration of T_3 can result in visual pigments shifting in on direction or the other, depending on which visual pigment is already dominant, and also gleaned from this experiment is the observation that the presence of thyroid hormone is necessary for the full effect of visual pigment shifting to take place.

Thyroid Hormone Receptors and 5' deiodinase Activity in the RPE

Thyroid hormones, which are the ligands which interact with the T_3-receptors, have demonstrated the ability to direct changes in the visual pigment compostion of salmonids; changes which are associated with smoltification. Since the visual pigment composition of the salmonid eye is dependent on the relative proportions of retinal or didehydroretinal that is found in the photoreceptors and stored in retinal pigment epithelial (RPE) cells. These cells, located at the back of the eye and interdigitating with the photoreceptors, are intimately involved in providing the Vitamin A_1-based retinal chromophore (retinal) or the Vitamin A_2-based chromophore (3,4-didehydroretinal) to the photoreceptors. For the scotopic visual system, these chromophores will form rhodopsin and porphyropsin, respectively. In order for thyroid hormones to have a direct effect on the visual pigment composition, the RPE has to demonstrate the presence of nuclear T_3-receptors.

In addition, in teleost fish, L-thyroxine (T_4) is the predominant hormone produced by the thyroid (Grau *et al*., 1986) however, it is T_3 (3,4,3'-triiodo-L-thyronine) which is considered to be the active hormone acting at the cellular level (Shields and Eales, 1986). Circulating T_4 can be extrathyroidally monodeiodinated to its more physiologically potent derivative via a 5'-deiodinase enzyme ($T_4$5'D or 5'D). This deiodination generally takes place in the liver, which is the primary tissue that determines plasma T_3 levels (Eales and MacLatchy, 1989). There are, however, several nonhepatic tissues have also demonstrated the ability to deiodinate T_4 including: the kidney (Leatherland, 1981), muscle, gill (MacLatchy and Eales, 1992; Sweeting *et al*., 1994) and brain (Frith and Eales, 1996). Extrahepatic deiodination may provide the opportunity for the respective tissues to provide themselves with T_3, independent of hepatic 5'D activity and concomittant circulating T_3 levels (Sweeting *et al*., 1994), therefore, if the RPE demonstrates local 5'D activity, it provides the RPE the opportunity to undergo or complete thyroid hormone-directed developmental changes. Such a finding would also further substantiate the RPE as a target tissue for thyroid hormones with visual pigment changes being a consequence of thyroid hormone actions.

Thyroid hormone receptor assay and 5'D activity was determined by using methodology outlined by Bres and co-workers (1994). After 24 h of dark adaptation, the eyes were enucleated and placed into a lightproof beaker

containing an ice-cold buffer solution. The eyes were removed from the light-proof beaker and placed onto a porcelan tile that contained a number of semi-hemispherical wells (20 mm dia.; 10 mm depth). This tile was placed upon a bed of ice and the wells were $^2/_3$ filled with buffer solution. The eye was hemisected and after the retina was removed, the RPE cells were gently aspirated from the posterior chamber of the eye. After all possible RPE cells were removed, they were transferred into a 1.5 ml Wheaton teflon-glass homogenizer. The homogenate was then lightly centrifuged to obtain the microsomal fraction in the supernatant while the pellet was resuspended in buffer solution. The homogenized RPE cells of 8–10 eyes were pooled for the next part of this procedure which entailed layering 10 mls of the RPE homogenate over 5 mls of a second buffer in a 20 ml. tapered ultracentrifuge tube. The layered suspension was centrifuged at 109'000 *g* for 45 min at 4°C, the supernatant was poured out for further manipulation to obtain the microsomal fraction for 5'D analysis. The isolated nuclei left in the tube were resuspended. The supernatant from the initial homogenized RPE cell described above was ultracentrifuged at 110'000 *g* for 1hr at 4°C. The microsomal pellet was resuspended in 1.5 mls of buffer for 5'D enzyme assay.

The nuclei obtained from the isolation procedure were analysed by using Scatchard analysis where the Bound/Free ratio ([*HR*/*H*]) is plotted against the bound fraction ([*HR*]). The molarity of the total binding sites in the tube ([*R*]; $pmol \cdot L^{-1}$) is represented by the *x*-intercept being divided by the amount of DNA in the assay tube and the dissociation constant (K_d) is represented by the negative reciprocal of the regression line. 5'D activity was measured by measuring the production of labelled iodine from a radiolabelled T_4 precursor.

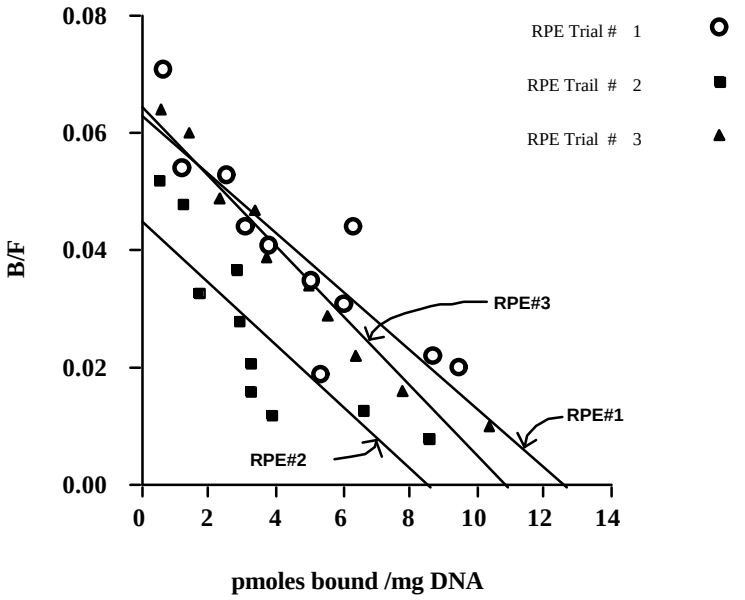

Figure 6. Multiple Scatchard plots of the three trials of [^{125}I]T_3 bounding to nuclear extracts of RPE tissue.

Analysis of the RPE nuclei (Fig. 6) yielded a MBC of 240 fmol·mg^{-1} DNA and the K_d of 1.74 x 10^{-10} (RPE #1), a MBC of 170 fmol·mg^{-1} DNA and a K_d of 1.80 x 10^{-10} (RPE #2) and a MBC of 220 fmol·mg^{-1} DNA and a K_d of 1.56 x 10^{-10} (RPE #3). Lineweaver-Burk double-reciprocal plot analysis of microsomal fractions (see Fig. 7 for an example) indicated that the 5'D enzyme in the RPE had a mean K_m value of 0.24 ± 0.04 nM and a mean V_{max} value of 0.47 ± 0.04 pmol T_4·h^{-1}·mg. prot^{-1}.

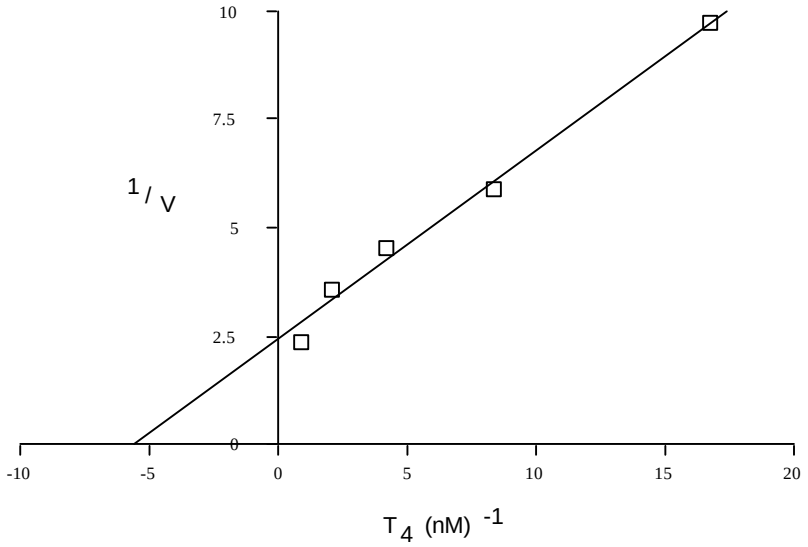

Figure 7. Lineweaver-Burk double-reciprocal plot of $T_4$5'D activity in RPE microsomal preparations measured over low T_4 substrate levels (0.06 — 1.2 nM).

The results of this experiment indicate that the RPE of juvenile coho salmon demonstrate the presence of T_3 receptors and are thus able to act as target tissue for thyroid hormones. In addition, the RPE also demonstrate the ability to deiodinate T_4 into the physiologically more potent T_3 to allow tissue specific responses. These observations indicate that visual pigment changes that occur during smolting may indeed respond to circulating T_3 during smolting, and may in fact, be able to self-direct visual pigment changes during smolting via its own 5'D enzyme system.

The Effect Of T_3 on Retinoid Metabolism In Isolated RPE Cells

The retinal pigment epithelial (RPE) cells play a critical rôle in maintaining the visual cycle and spectral sensitivity of coho salmon. Changes in spectral sensitivity are brought about due to the conversion of A_1 retinoids to A_2

retinoids, resulting in altering the relative proportions of the retinoid pools in the RPE and ultimately an alteration of the visual pigment complement. Experiments on salmonids that involved the manipulation of thyroid hormones to bring about visual pigment changes suggest that thyroid hormones may have some effect on a putative terminal ring dehydrogenase which would bring about the A_1- to A_2-retinoid conversion in order to effect changes in spectral sensitivity. These thyroid hormone experiments have involved *in vivo* treatment by the external or internal administration of thyroid hormones, however, it is unclear if thyroid hormones may actually be affecting some other agent which may be acting at the level of the RPE to bring about visual pigment changes.

We undertook a study to determine if the physiologically active thyroid hormone, T_3 (3,4,3'-triiodo-L-thyronine), can work at the level of the RPE in affecting changes in visual pigment compostion.

By isolating coho salmon RPE tissue and incubating these cells with ^{3}H-labelled retinol and incubating with and without exogenous thyroid hormone, this *in vitro* experiment can demonstrate the effects of T_3 on the production of didehydroretinoids. RPE isolation and incubation procedures involved sterile technique, and briefly, isolated RPE cells of both eyes were placed into inoculated into the wells of 24-well multiwell plate (Cell Wells, Corning NY; 15 mm dia. x 15 mm depth) containing sterile-filtered Leibovitz culture medium (L-15; Sigma). These wells then transferred to a light-proof and humidified incubation chamber at acclimation temperature (12°C). The effects of 3,5,3'-tri-iodothyronine (T_3) on the metabolic fate of ^{3}H-labelled retinol was investigated by incubating with [11,12-^{3}H]All-*trans*-retinol, 35.2 Ci/mmol and the presence or absence of T_3. 24 h control, 48 h control, formalin-killed, 24 h T_3 exposure and 48 h T_3 exposures were investigated. After the appropriate incubation times, the retinoids were extracted and analysed using a synthesis of procedures utilized by Tsin and co-workers (1985), Vahlquist and co-workers (1990), Rollman and co-workers (1993), and Andersson and co-workers (1994). Under dim red-light conditions, the RPE tissue was homogenized and the retinoids were extracted with acetone. A 5% water-deactivated alumina column was used to obtain retinyl ester and neutral retinoid fraction. The retinyl ester fraction was saponified, hydrolyzing the esterified retinoids into free alcohols. The retinoids were analyzed using HPLC. Chromatographic analysis was performed using a Nucleosil ODS (C18) column (150 x 3.2 mm, 5 µm particle size; Alltech, Deerfield IL) and eluting at 0.4 ml·min^{-1} with acetonitrile/water

(82:18). Elution was monitored at 354 nm with the eluate collected every 20 sec. The eluate was then counted in a scintillation counter to measure the tritiated retinoids.

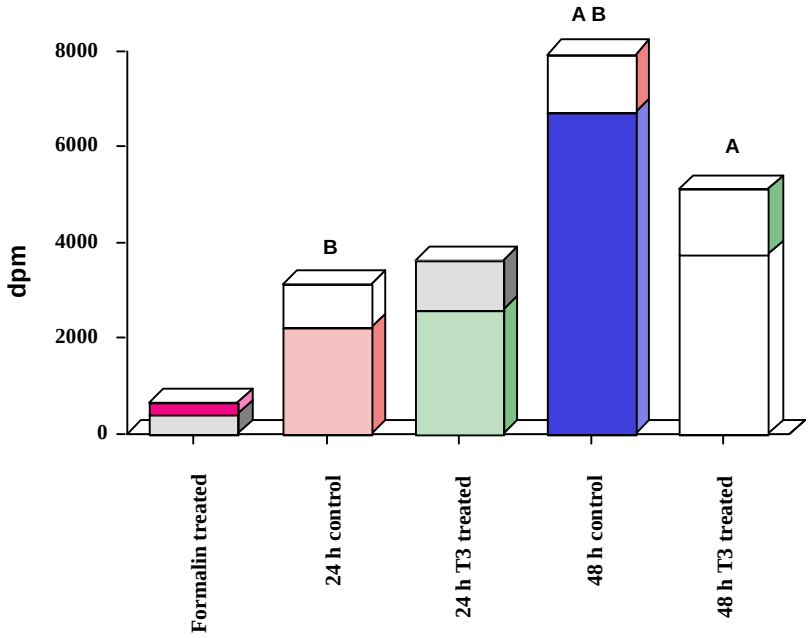

Figure 8. Comparative effects of T_3 on the production of [^{3}H]-labelled all-*trans* 3,4-didehydroretinol eluted from the neutral retinoid fractions. A & B: significant difference between groups ($P < 0.05$)

As can be seen in Fig. 8, isolated RPE cells, when incubed for 48 hours with a mixture of [^{3}H]-labelled all-*trans* retinol and T_3, there was a reduction in the production of [^{3}H]-labelled all-*trans* 3,4-didehydroretinol. *In vivo*, this would result in a relative reduction of the amount of porphyropsin formation and thus a shift towards rhodopsin formation. This action is consistent with what is seen in smolting salmonids, that is, after exposure to thyroid hormones, a shift away from porphyropsin to rhodopsin dominance is seen in the retina, shifting the spectral sensistivity towards the shorter wavelengths seen in the marine

environment. This particual experiment indicates that T_3 can act, by itself, directly on RPE cells and result in changes in retinoid metabolism similar to what is seen *in vivo*.

Summary of Findings

The experiments described above, have shown that the RPE of juvenile coho salmon exhibit the presence of T_3 receptors and a 5'deiodinase enzyme system, thus permitting the eye to be directly responsive to circulating T_3 or self-directed production of T_3. The *in vitro* experiments demonstrate that T_3, in the relative absence of other endocrine factors, can direct the metabolism of retinoids in salmonid RPE tissue. These experimental observations, when coupled with observations seen in smolting salmonids in wild or hatchery settings, indicate that the thyroid hormones seen during the parr—smolt transformation are probably acting directly on the salmon's visual system to bring about changes in spectral sensitivity that would pre-adapt this aspect of the salmonids sensory system as it does all the other aspects of the salmonid sensory and physiological systems.

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EFFECT OF MATURATION ON PARR GROWTH AND SMOLTING OF ATLANTIC SALMON

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EXTENDED ABSTRACT ONLY DO NOT CITE

Parr maturation, a ubiquitous life history feature in male Atlantic salmon (*Salmo salar*), is of great importance to the dynamics of salmon populations. Parr maturation has been associated with a decreased probability of smolting and thus increasing incidences of mature parr may result in decreases in anadromous males. Maturation may inhibit smolting if mature parr fail to achieve sufficient fall size to undergo the parr-smolt transformation or if mature parr exhibit higher mortality than non-maturing parr during the presmolt winter.

In the present study, we determine the influence of maturation on parr growth and smolting of Atlantic salmon stocked as fry as part of the effort to restore populations to the Connecticut River, USA. The primary goal of the fry stocking program is to produce "wild-reared" smolts to improve survival and thus enhance adult returns. Because parr maturation may conflict with smolt production, we were interested in the following questions:

1) How abundant are mature parr in populations supported through fry stocking?;

2) How does parr size/growth relate to mature parr abundance; and

3) How does maturation influence parr growth and recruitment to smolt?

We addressed these questions by completing population-level and individual-based analyses of parr growth and maturation and mark-recapture studies to test the effect of parr maturity on smolt recruitment.

Mature parr were abundant in the populations sampled, ranging between 25 and 52% mature at age-1 and between 34 and 67% mature at age-2. Percent mature at age-2 was significantly greater than percent mature at age-1 ($\chi^2 = 21.0$; $p < 0.001$). The variation in percent age-1 mature in October/November was largely explained by mean total length of age-1 parr the preceding June. As mean total length of age-1 parr in June increased, percent maturing the following October/November significantly increased (regression; $p = 0.016$; $r^2 = 0.60$). The probability of parr maturing in October increased with increasing June length and weight (logistics regression: length: $p = 0.052$; weight: $p = 0.014$). No parr < 100 mm TL in June were recaptured as mature in October, while parr as small as 90 mm TL in June were recaptured as immature in October. Data from parr individually tagged in June and recaptured in October showed immature parr exhibited two-fold greater individual growth than maturing parr and thus in October/November, immature age-1 parr were consistently greater in mean length than mature age-1 parr (ANCOVA; $p < 0.001$).

Condition factor of maturing and non-maturing parr was very similar in June (ANOVA; $p = 0.62$), but differed significantly by October (mature > immature; ANOVA; $p < 0.0001$). Large differences in condition factor among maturing and immature parr that arose between June and October were primarily driven by large declines in condition factor of immature parr.

Smolt recruitment was highly dependent upon state of maturity the preceding fall. Relative to the fall parr sample that was approximately equally divided between mature and immature individuals, the smolt sample was three to one in favor of previously immature individuals (fall parr vs. smolt: $\chi^2 = 16.6$; $p = 0.001$; $N = 1369$), whereas the remaining parr sample was nearly four to one in favor of previously mature individuals (fall parr vs. remaining parr: $\chi^2 = 8.7$; $p = 0.003$; $N = 1131$).

Smolt age was significantly dependent upon previous state of maturity ($\chi^2 = 8.4$; $p = 0.0037$): only 18% of age-2 smolts ($N = 67$) were previously mature, whereas five of the seven age-3 smolts were previously mature, suggesting a positive association between maturation and older age at smolting.

By coupling fall mark and spring recapture results with smolt production data, it was determined that the reduced contribution of mature parr to the smolt migration resulted primarily from a one-third probability of smolting for mature parr rather than higher mortality between fall and spring. The delayed age at

smolting resulting from the reduced probability of smolting for mature age-1 parr may result in significant (~ 20%) losses in potential smolt production when maturation percentages approach the ~ 45% maximum estimated in this study.

This study showed, over a broad spatial scale, that variation in incidence of maturation is largely explained by variability in parr size among tributaries and river reaches and further empirically demonstrates a direct negative effect of maturation on presmolt parr growth and recruitment to smolt. Parr maturation is therefore an important consideration for the enhancement and/or restoration of Atlantic salmon populations through fry stocking.

CONTROL OF DRINKING AND OSMOREGULATION AND

ROLE OF THE GUT MIGRATORY SALMONIDS

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Control of drinking is essential in fish moving from fresh water to sea water and vice versa. Osmoregulatory processes in fresh water are focused on ionic uptake and water removal and drinking is usually at a low level except in larvae where there may be links with calcium uptake or feeding (Tytler *et al*, 1990). Upon entry to sea water removal of excess salts and replacement of water lost through dehydration, mainly via the gills are essential for homeostasis of the body fluids. The rate of drinking and efficiency of water absorption in the gut are of major importance in determining hydration in fish.

The renin angiotension system (RAS) has an important role as a cardiovascular and electrolyte regulator in vertebrates and all the components have been identified in fish (Olson, 1992). Drinking in fish is dependent on an intact RAS and stimulation of the endogenous RAS by increases in salinity, elevation of blood plasma ionic concentration, dehydration, blood volume depletion or lowered blood pressure are major stimuli for increased drinking, which can be inhibited by angiotension converting enzyme (ACE) inhibitors, in both freshwater and marine fish (Fuentes and Eddy, 1997).

The control of drinking rate and the role of the RAS in fish have now been studied on many occasions but there is little information on water absorption in the gut, and regulation of this important process. The aim of the study was to examine the role of a major component of the RAS, angiotension II (AII), in gut motility and implications for absorption of water in the gut of rainbow trout (*Oncorhynchus mykiss*).

Pieces of intestine from freshwater rainbow trout (200-500 g) as strips or rings were suspended in trout saline gassed with 95% O_2, 5% CO_2 at 20^oC, equilibrated to 0.5 g using a force transducer. Contraction responses to administration of AII at 10^{-10} M to 10^{-5} M, acetylcholine (Ach) at 10^{-8} M to 10^{-2} M, and co-administration of both were recorded. Responses of a more sensitive preparation under similar conditions were also tested. The pressure within a 5-8 cm sac of rainbow trout intestine was set to 50 mm Hg using a saline filled syringe attached by polythene tubing at one end, and responses recorded using a pressure transducer attached to the other end of the sac. Results obtained were similar to those for gut strips or rings.

Gut contraction response to Ach was as expected, as has been shown previously. However there was no significant response to AII alone, or when it was co-administered with Ach. A representative response from the various preparations mentioned is shown in Fig.1.

On many occasions it has been shown that increased circulatory levels of AII achieved either by administration of AII, or stimulation of the endogenous RAS increases drinking in both freshwater and marine fish (Fuentes and Eddy, 1997b). Presumably as in mammals a major element of drinking regulation is via the hypothalamus, but there is little information about the role of AII in the circulation and target tissues. Does this peptide have a role in the physiology of the gut in salt and water absorption, of digestion, and is gut motility important in these processes? The results show that motility of the gut preparations, in response to acetylcholine was as expected, but there was no response to AII. From this we conclude that gut motility, in response to the RAS is unlikely to be an important factor in osmoregulatory processes. However there may well be other factors involved such as the effect of the RAS on water absorption across the gut, and how these systems interact when food is present in the gut.

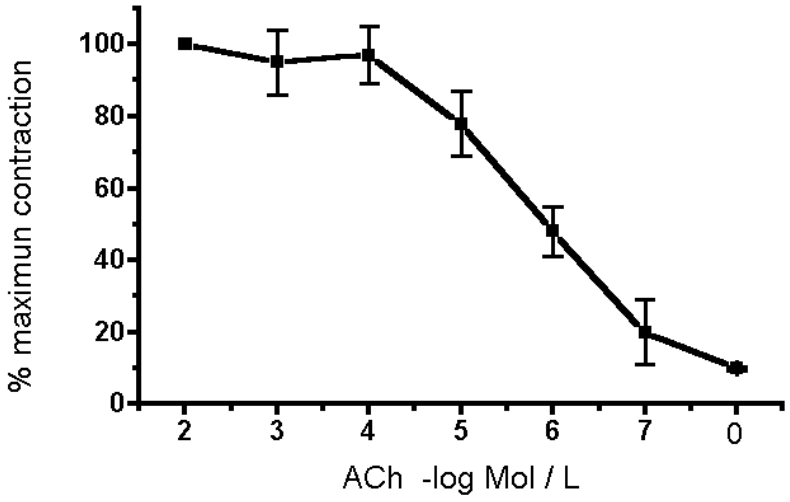

Figure 1. Contraction of rainbow trout intestine in response to acetylcholine or angiotensin II. Values are expressed as present maximum contraction, and are representative of the preparations described in the text. Administration of angiotensin II (Ans^1, Val^5) as acetate (Sigma) at 10^{-10} to 10^{-5} Molar to similar preparations did not result in significant contractions. Co-administration of acetylcholine (10^{-6} M) and angiotensin II (10^{-6} M) was without significant effect.

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ENERGETIC ASPECTS OF IONIC REGULATION IN FISH

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Discussion

There is a significant discrepancy between the theoretical estimates of how much energy, as measured by oxygen consumption in different salinities, should be consumed by the active transport of ions between body fluids and the environment in fish, and the experimental data for that cost as reported in the literature. The theoretical estimates are in the order of 1-3% of total body oxygen consumption that should be attributed to active ion transport, while the experimental evidence ranged from 7-20%. Furthermore, one might expect on theoretical grounds that the water salinity in which fish would have the minimum energetic demand for active ion and osmotic regulation would be the isotonic salinity, since the blood to water gradients would be at their minimum at that salinity.

In studying this discrepancy, we have arrived at two major conclusions. One is that the energy consumed by the ion transport tissues of the teleost gill and the rectal gland of the shark comprise approximately 1-2% of total body oxygen consumption (Morgan and Iwama, in prep; Morgan et al. 1997a); a value that is in remarkable agreement with the theoretical estimates. However, our data also confirm previous reports in that the metabolic response of the whole animal to

changes in water salinity is large, and in the order of 10-25% of resting oxygen consumption (e.g., Morgan et al. 1997b). It is our opinion that there is a significant energetic cost to a state of stress that may be present in the fish in the laboratory, but which is not always detected by classical indicators of stressed states in fish (eg. increased plasma cortisol and glucose concentrations; see Barton and Iwama 1991). Our experiments with artificially elevating circulating cortisol levels with implants resulted in signifcantly increased oxygen consumption rates. However, Davis and Schreck (1997) found exogenous cortisol alone did not increase oxygen consumption rates in juvenile coho salmon. The assessment of the energetic cost of stress warrants further study.

The second major conclusion is that the metabolic response of fish to salinity likely depends on the developmental stage and life cycle for a particular species, such that the minimum energy expended for ionic/osmotic regulation will be the water salinity that is normal for that life stage for that species. It is generally not at the isotonic water salinity. Thus for the pre-smolt juvenile salmonids (*Oncorhynchus sp.*), for example, fresh water would be the normal salinity, and therefore you would expect the minimum oxygen consumption to be in fresh water. This is supported by our experimental data, and most of the data reported in the literature (see Morgan and Iwama 1991). The fish, therefore, does not behave as a passive permeable epithelial bag, but seems to be specifically selected for minimal energy expenditure in the water salinity that is normal for a particular life stage.

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BIOCHEMICAL ANALYSES OF THE OLFACTORY SYSTEM IN MASU SALMON DURING THE PARR-SMOLT TRANSFORMATION

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Introduction

It is now widely accepted that some specific odorant information of the natal stream is imprinted into the olfactory system of juvenile salmon during downstream feeding migration, and that adult salmon evoke this information during upstream reproductive migration. Since the olfactory imprinting hypothesis for salmon homing was proposed by Hasler and Wisby *(1951)*, many behavioral and electro-physiological studies have demonstrated the importance of olfactory function (reviewed by Hasler and Scholz, 1983; Hara, 1994; Ueda and Yamauchi, 1995; Dittman and Quinn, 1996). However, few attempts have been made to investigate the biochemical aspects of the olfactory function in any salmonid species. Recently, several biochemical analyses of the olfactory system (olfactory epithelium, olfactory nerve, and olfactory bulb) in masu salmon *(Oncorhynchus masou)* during the parr-smolt transformation (smolting) have been carried out in our laboratory. *In* this paper, we briefly review biochemical changes of olfactory function in connection with the imprinting phenomena in masu salmon.

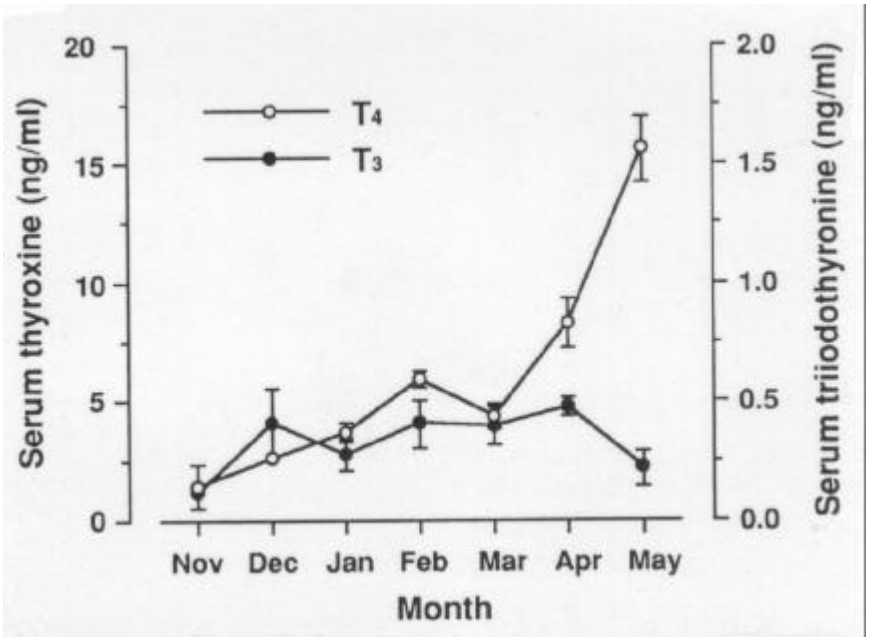

Figure 1. Changes in serum thyroxine (T4; o) and triiodothyronine (T3; •) levels of wild masu salmon during smolting.

Fish

Yearling wild masu salmon were collected monthly from the Shakotan River, Hokkaido, Japan from November to May. Parr marks were evident from November to March ~arr stage), obscured in April (pre-smolt stage), and completely absent in May (full-smolt stage). Serum thyroid hormone concentrations were measured by radio-immunoassay. Thyroxine ~4) levels were low until March, increased from April, and peaked in May. Triiodothyronine (T3) levels were constantly low, gradually increased to April, and dropped in May (Fig. 1).

Thyroid hormone receptors (TRs)

Thyroid hormone-specific binding Sites *(i.e.* thyroid hormone receptors) in the olfactory system of masu salmon during smolting were detected by newly developed ~ *vifro* autoradiography with frozen sections (Kudo *et* a'., 1994). A saturation experiment with the brain indicated the presence of a single class of binding sites with high affinity. T3-specific binding was detected in the olfactory epithelium, the telencephalon, the optic tectum, and the cerebellum, but not in the olfactory bulb. The T3-specific biding value in the olfactory epithelium was higher than in all other regions of the brain. This binding value in the olfactory epithelium increased at the full-smolt stage (Fig. 2).

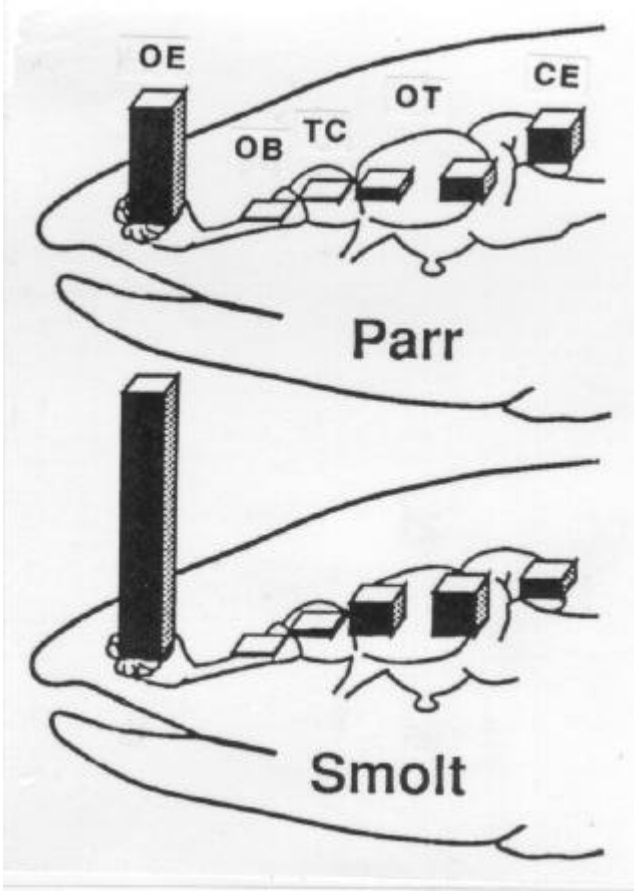

Fig. 2. Comparison of TRs levels of the olfactory epithelium (OE), olfactory bulb (OB), telencephalon (TC), optic tectuin (01), and cerebellum (CB) in wild masu salmon parr and smolt.

Olfactory system-specific protein (N24)

Using sodium dodecyl sulfate-polyacrylamid gel electrophoresis (SDS-PAGE), an olfactory system-specific 24 kDa protein (N24) was identified in kokanee sockye salmon *(0. nerka)* by the electrophoretic comparison of proteins restricted to the olfactory system with those found in other parts of the brain (Shimizu et a'., 1993). By Western blotting analysis, a specific polyclonal antiserum to N24 recognized only the 24 kDa protein in the olfactory system of masu salmon, and the immuno-reactivity of N24 in fish at the parr stage was stronger than at the flill-smolt stage (Fig. 3). The molecular biological analysis of protein and nucleotide sequencing revealed that N24 had a remarkable homology to glutathione 5-transferase enzymes (GSTs). By Northern blotting analysis, the expression of N24 mRNA in the olfactory epithelium was the highest in March (Kudo *et aL,* in preparation).

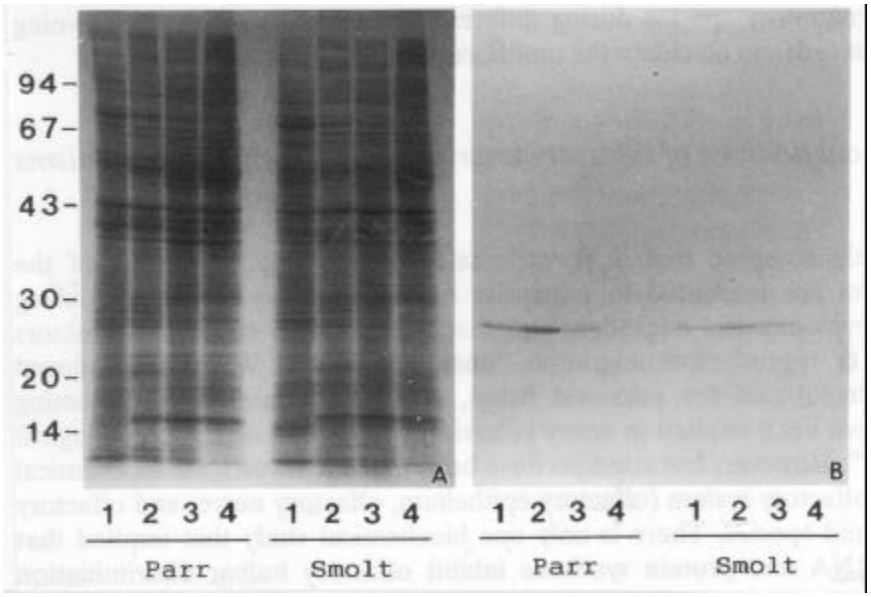

Fig. 3. SDS-PAGE~~~ (A~an~½dvvWev5~tern bl6ttii~g (B) of soluble extracts of the olfactory epithelium (1), olfactory nerve (2), olfactory bulb (3), and telencephalon (4) in wild masu samon parr and smolt.

Cytosolic proteins of the olfactory system

Cytosolic proteins of the olfactory system in masu salmon were analysed by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) during smolting (Shimizti *et* a'., 1995). One protein spot in part icul& with an estimated molecular weight of 27kDa and isoelectric point of 5.6 (~7) disappeared in common with the olfactory system during smolting. In the olfactory epithelium, three protein spots of 23, 53 and 54 kDa (all p1 *5.2)* increased their visibility in February and May (Fig. 4).

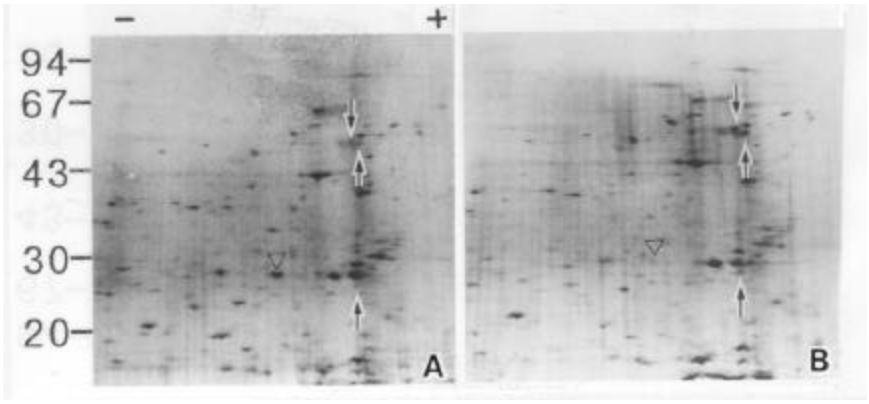

Fig 4. 2D-PAGE of soluble extracts of the olfactory epithelium in wild masu salmon parr (A) and smolt (B). Arrows and an open arrowhead indicate the protein which appear and disappear during smolting, respectively.

Conclusion

The present paper briefly describes several biochemical changes of olfactory function in masu salmon during smolting. The increase of serum T4 levels as well as the enrichment of TRs in the olfactory epithelium at the full-smolt stage may have important roles on neural development and differentiation in the olfactory system. The decrease of N24 immuno-reactivity in the olfactory system at the full-smolt stage indicates a negative correlation with the changes of T4 and TRs. Since thyroid hormone is a potent Inhibitor of GSTs, this hormone may inhibit N24 activity in the olfactory system during smolting. The appearance and disappearance of cytosolic proteins of the olfactory system may also be regulated by thyroid hormone. Although thyroid hormone definitely regulates various physiological functions during smolting in salmonids, the roles of this hormone in the olfactory system are still indistinct. Further biochemical and molecular biological analyses of the salmon olfactory system during smolting should be done to clarify the olfactory imprinting mechanism in salmon. Furthermore, *Oncorhynchus* species whose juvenile make their downstream migration immediately alter emergence also imprint the natal river odorant without smolting. The comparative biochemical &id molecular biological studies on the olfactory system in juvenile salmon with or without smolting are now in progress in our laboratory.

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GROWTH, MOVEMENT AND MATURITY

OF INDIVIDUAL ATLANTIC SALMON PARR

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Introduction

Age and size at smolting are plastic demographic variables in Atlantic salmon (*Salmo salar*)(Saunders & Schom, 1985); depending on developmental rates, smolt ages can range from one to six (Metcalfe & Thorpe, 1990). Within any one river system smolt ages are much less variable and appear to depend on growth rates (Thorpe, 1986; Duston & Saunders, 1997), dominance interactions (Metcalfe et al., 1990) and male parr maturation (Saunders et al., 1994). Smolt age and size have clear demographic consequences and understanding the operation of the factors governing smolt age is an important part of assessing the recruitment dynamics of salmonids. Much of the work identifying mechanisms regulating smolt age has been conducted in the laboratory, largely due to the ease of tracking the developmental decisions and fate of individual fish in the laboratory.

Methods

We present a field study in which we tagged and resampled individual Atlantic salmon parr in a small stream (1 km study section of West Brook, Whately, MA, USA) from May 1997 to March 1998. The stream is part of the Connecticut River Atlantic salmon restoration effort and is stocked with fry ($50 \cdot 100$ m^{-2}) each Spring. There is no natural reproduction in the stream. From these data we have developed a record of the growth, movement and maturity of over 550 individual age-1+ fish and have migration timing information from a subset of these.

From 5/14/97 to 4/1/98 we tagged 568 age-1 parr during nine sampling sessions. We used electroshocking to sample the study stream the first, seventh, eighth and ninth sessions and used one-person hand-held seines at night during the other sessions, except for sample 2 where we used seines during the day. During sampling, we recorded the location of each fish (±2.5 m). Untagged fish were tagged with PIT tags and sampled for scales after anesthetizing with MS-222; all fish were measured for length, wet mass, maturity status (milt expression) and a digital photograph was taken. Upon recovery, each fish was returned to its approximate sampling location.

Results

We present mean lengths and weights for each sample and we calculated growth rates between samples using data from the subset of individual fish that were captured in both consecutive samples. Because fish were individually-tagged, we analyzed sizes and growth rates retrospectively for fish that eventually made a particular life history decision. Here, we focus on differences between parr that matured and parr that were never observed to mature.

Over 350 fish were caught during the electrofishing samples 1 and 7 (Fig. 1) and about 250 fish were caught during each of the night seining samples (2-5). Sampling was less efficient using seining during the day (sample 2) and during winter (samples 8 and 9). By the seventh sample, 96.7 percent of the parr sampled were recaptures and the rate remained high for samples 8 and 9 (Fig. 1).

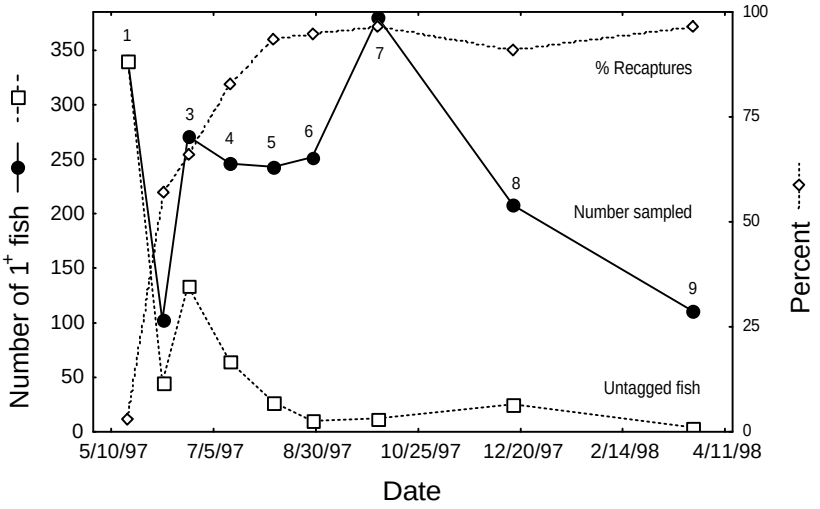

Figure 1. Total number of fish sampled, number of untagged fish and percent recaptures for the nine sample sessions. Numbers indicate sample numbers from text.

Out of the nine possible recapture events (samples), individual fish were captured on average four times. Fish were caught one to five times with equal frequency (15%) and six or more times less frequently (12-2%). Using fish from sample eight, an ANOVA revealed insignificant differences in length ($F_{7,177}$=1.2, p=0.30) and mass ($F_{7,177}$=1.2, p=0.27) among fish that were captured between two and eight times, suggesting that our repeat sampling had no effect on fish sizes. Use of a Jolly-Seber mark-recapture model indicated that the 1-km study section contained approximately 500 age-1 fish and that survival from sample to sample over samples 2 to 7 (late Spring through late Summer) was 96%. Survival estimates over the Winter are not yet available.

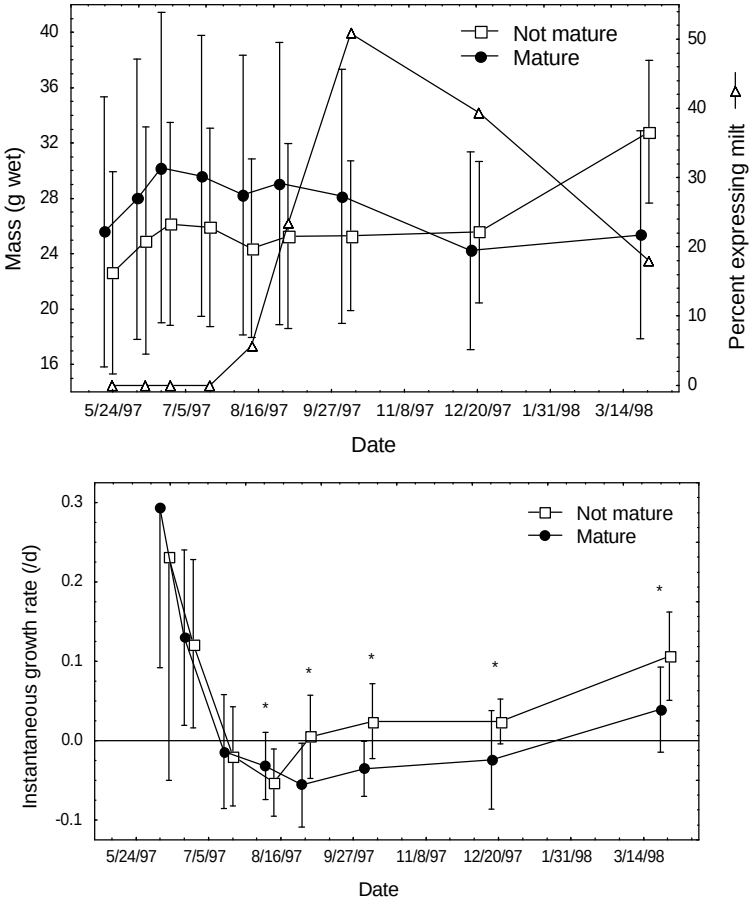

Figure 2. Average (±std) mass and percent of all fish expressing milt (above), and growth rates (below) for the nine sample sessions for fish that matured at some point (males) during sampling or never matured (males or females). Growth rates are presented for fish that were caught in both of two consecutive sample sessions are plotted on the date of the second sample (e.g. the first point is growth from sample 1 to sample 2). Stars indicate significant differences between mature and not mature growth rates based on paired t-tests ($p<0.005$).

On average, fish grew rapidly during the early summer (0.28 d^{-1}), most fish lost mass (-0.03 d^{-1}) during the summer and early fall, and growth was again relatively rapid (0.07 d^{-1}) in late Winter. The first mature fish were observed on 8/6, and by 9/29 50.8 percent of all the fish in the stream were mature (expressing milt). In Spring, fish that eventually matured were 13 percent heavier (25.6 g vs. 22.6 g) than fish that were not observed to mature, but by fall maturing fish grew more slowly and were 5% lighter than non-maturing fish (Fig. 2). Near the end of March (sample 9), fish that had matured were 29% lighter than fish that had not matured. Differences in growth corresponded with the onset of maturity, growth rate differences between mature and non-mature fish were insignificant before the end of August, but non-mature fish grew significantly faster following the end of August through March (Fig 2).

Movement of individual fish was minimal from sample 1 to sample 9 and appeared to vary with maturity status section (chi-square=7.49, p=0.0062). For the fish that were captured 5-9 times (N=147), 70 out of 79 (0.89) not mature and 48 out of 68 (0.71) mature fish were captured each time in the same 20-m.

Acknowledgment:

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LOSS OF SMOLT CHARACTERS

IN JUVENILE ATLANTIC SALMON –

ENERGETIC CONSIDERATIONS AND EFFECTS OF SALINITY

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EXTENDED ABSTRACT ONLY DO NOT CITE

The present study was done in order to describe long-term consequences of restricted feeding in Atlantic salmon (*Salmo salar*) during the critical early post-smolt phase. Groups of underyearling Atlantic salmon smolts were transferred to duplicate seawater tanks, and subjected to five different ration levels, 0 % (starved), 25 %, 50 %, 75 % or 100 % (full fed). In order to avoid unneccessary negative consequences for the fish, restricted rations were discontinued and all groups were re-fed in excess as soon as significant physio-logical consequences of food-deprivation on hypo-osmoregulatory ability were observed (after 6 weeks).

After 6 weeks of starvation or feeding 25% ration, plasma Cl^+ levels were elevated as compared with groups fed 50-100% rations (Table 1), indicating osmotic disturbance. No differences in branchial Na^+,K^+-ATPase activity were observed among groups at this time, suggesting that the starved and 25% fed groups were unable to maintain long-term hydro-mineral balance in sea water. Re-feeding had a significant effect on branchial Na^+,K^+-ATPase activity in the starved group (Table 1), and a concurrent reduction in plasma Cl^+ levels. Plasma

Cl^- levels were still slightly elevated at week 8, whereas after 16 weeks levels were similar in all groups.

Plasma Cl^- levels (mM)					
Weeks	0%	25%	50%	75%	100%
0	132.2 (0.6)				
6	153.3^{a}(1.5)	143.6^{b}(1.9)	137.1^{c}(1.3)	139.9^{bc}(1.2)	139.5^{bc}(1.1)
8	144.4^{a}(1.7)	141.8^{ab}(0.6)	140.4^{b}(0.9)	139.1^{b}(0.8)	137.7^{b}(0.7)
16	131.3^{a}(0.3)	132.3^{a}(0.4)	134.4^{a}(1.0)	134.0^{a}(0.4)	130.3^{a}(0.9)
Branchial Na^+,K^+-ATPase activity (umol ADP·mg $prot^{-1}$·h^{-1})					
0	7.1 (0.6)				
6	22.5^{a}(1.6)	21.7^{a}(1.2)	21.7^{a}(1.9)	24.3^{a}(1.7)	25.8^{a}(2.5)
8	33.8^{a}(3.3)	20.9^{b}(1.3)	18.0^{b}(0.9)	23.8^{b}(1.6)	24.8^{b}(2.4)
16	16.9^{a}(1.1)	17.3^{a}(1.4)	12.6^{a}(0.7)	17.7^{a}(1.6)	15.4^{a}(2.7)

Table 1. Plasma Cl^- levels and branchial Na^+,K^+-ATPase activity (mean, sem) of post-smolt Atlantic salmon fed different ration levels for 6 weeks in seawater. Week 0 is day of transfer, before feeding regimes were established. different letters within dates indicate statistical differences (ANOVA, SNK, $p<0.05$).

As expected, the restricted rations significantly influence growth and condition factor of Atlantic salmon post-smolts (Table 2). Growth rate decreased systematically with ration levels, causing significant reductions in length. With exception of the 75 % ration, no compensatory growth was observed following re-feeding, hence size differences which were established during restricted feeding were maintained after 10 weeks of feeding (Table 2).|

Fork length (cm)					
Weeks	0%	25%	50%	75%	100%
0	20.9 (0.2)				
6	21.6^{a}(0.2)	21.6^{a}(0.4)	21.9^{ab}(0.2)	22.7^{ab}(0.4)	23.0^{b}(0.4)
8	21.7^{a}(0.2)	22.1^{a}(0.1)	22.5^{a}(0.2)	23.3^{b}(0.2)	23.9^{b}(0.3)
16	24.9^{a}(0.3)	25.7^{ab}(0.2)	26.2^{b}(0.4)	27.9^{c}(0.3)	28.1^{c}(0.3)
Condition factor ($100W \cdot L^{-3}$)					
0	0.95 (0.01)				
6	0.83^{a}(0.01)	0.96^{b}(0.01)	1.02^{c}(0.02)	1.06^{c}(0.01)	1.07^{c}(0.01)
8	0.89^{a}(0.01)	0.97^{b}(0.01)	1.06^{c}(0.01)	1.11^{d}(0.02)	1.13^{d}(0.01)
16	1.11^{a}(0.02)	1.16^{b}(0.02)	1.19^{b}(0.01)	1.18^{b}(0.02)	1.19^{b}(0.01)

Table 2. Fork length and condition factor (mean, sem) of post-smolt Atlantic salmon fed different ration levels for 6 weeks in seawater. Week 0 is day of transfer, before feeding regimes were established. Different letters within dates indicate statistical differences (ANOVA, SNK, $p<0.05$).

There are few studies on long-term consequences of food-deprivation on hypo-osmoregulatory ability in fish with which to compare our results. Studies of rainbow trout (Jürss *et al*., 1983;1987) and tilapia (Jürss *et al*.,1984; Kültz and Jürss, 1991) have shown a reduction in branchial Na^{+},K^{+}-ATPase activity after several weeks of fasting, both in fresh water and sea water. Further, Kültz and Jürss (1991) indicated higher plasma osmolality 52 hours after transfer to sea water in starved than fed tilapia. Similar findings are also reported by Vijayan *et al*. (1996), who found higher plasma Cl^{+} levels after 3 days in sea water in tilapia which had been straved for two weeks pre-transfer. In line with the present data, Vijayan *et al*. (1996) demon-strated high branchial Na^{+},K^{+}-ATPase activity in fed and fasted tilapia after transfer to seawater.

Our results suggest that nutritional factors and/or energy levels are critical for the maintenance of hydro-mineral balance of salmon post-smolts. Our findings further suggest a critical role of gill Na^{+},K^{+}-ATPase in long term hypo-osmoregulation of salmon smolts in seawater. Our results do not support the concept of rapid (compensatory) growth in post-smolt Atlantic salmon during periods of food-deprivation and re-feeding (for review, see Jobling, 1994).

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BRAIN PLASTICITY AND

SALMON PARR-SMOLT TRANSFORMATION:

PUTATIVE ROLES BY NEURONAL NITRIC OXIDE

AND THYROID HORMONES

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Discussion

Neurodevelopment and reorganization of neural circuits in the brain is likely to be an important stage during parr-smolt transformation (smoltification) of anadromous salmonids. Neural development features cell proliferation followed by neuronal and glial differentiation, nerve fiber growth and formation of synaptic connections along with cell apoptosis and selective fiber elimination, i.e. “parcellation” (Ebbesson 1980). During smoltification, changes in morphology and chemistry of neural/neuroendocrine circuits direct the changes in physiology and behavior. The sequence and timing of interactions between environmental cues and epigenetic factors at cellular and molecular levels are crucial for such changes.

For the smoltification process, light conditions, photoperiod and regulation of pituitary functions are crucial, emphasizing a central role for visual (pineal organ, retina) and neurosecretory circuitries (Holmqvist 1993). During smoltification, reorganization has been shown in retinal and pineal axonal projections. Neurochemical changes include transient serotonergic neurons in the habenular nucleus and telencephalon, transient total levels of various neurotransmitter/hormones, such as catecholamines, indolamines, GABA, and glutamate (Ebbesson et al., 1996a), and quantitative and distributional changes of mu- and kappa-opioid receptors (Ebbesson et al., 1996b) (see Table 1). All mentioned transmitters have been shown to influence neural development in other vertebrates.

Thyroid hormones' (TH) influence on brain development is well documented. The differentiated timing and distribution of receptor subtype expression indicates variations in TH functions during neural development, and preliminary studies in coho salmon indicate relatively high triiodothyronine binding in discrete brain regions and pituitary during smoltification (see Table 1). Recent data stress that the timing of TH availability clearly precedes the characteristic thyroxine peak, and coincides with the norepinephrine peak (which increases the conversion of thyroxine to functionally active triiodothyronine) and noted brain changes (Ebbesson et al., 1998; L. Ebbesson unpublished observations). To further elucidate the timing and mechanisms of TH in neural development during smoltification, the temporal and spatial expression pattern of TH receptor subtypes will be investigated, in relation to the indicated interactions with nitric oxide (NO).

In biological tissue, NO is produced by three major nitric oxide synthase (NOS) isotypes: neuronal, endothelial, and inducible, all present in the brain. Being a diffusible messenger molecule, NO can act at multiple target sites and over large areas. Among other proposed actions, NO is the principal regulator of cyclic GMP. NO was recently discovered to possess multifuntional properties, including in development, neuroregulation of physiological homeostasis and neural/neuroendocrine plasticity. So far, NO brain functions are relatively poorly studied and only in a few mammalian species. NO and TH share several functional properties, and TH status influences NOS expression in the brain.

In Atlantic salmon parr, NOS isotypes are present in all major brain regions (Holmqvist et al., 1994). The highest amount is located in neuronal circuits (Holmqvist and Ekström 1997), and a partial cDNA sequence encoding the

neuronal NOS isotype (nNOS) was recently isolated from adult salmon cerebellum (Öyan A., Goksoyr A. and Holmqvist B., in prep.). The brain distribution of nNOS mRNA transcripts recently confirmed the specific neuronal association, and revealed the spatial and temporal expression pattern from early embryonic stages to adults (Holmqvist et al 1998). In the embryo, nNOS is expressed in brain regions shown to undergo the first neural and transmitter phenotype differentiation in teleosts. In general, a relatively high nNOS expression is indicated prior to smoltification. In parr, nNOS is expressed abundantly in discrete hypophysiotrophic and visual circuits, including the hypothalamic optic nucleus (or suprachiasmatic nucleus, comprising a retinorecipient and supraoptic/chiasmatic subnucleus that innervates the pituitary), proliferative zones and in regions exhibiting neurological changes during smoltification. Neuronal NOS is highly associated with the neurotransmitter/hormones shown to fluctuate during smoltification (Table 1). The distribution of nNOS in the brain suggests that NO is an important messenger molecule in the light-neuroendocrine-pituitary axis. Thus, NO is likely to play a major role in smoltification processes, specifically in the development and regulation of central mediators of sensory information including osmotic, light/visual, olfactory, viscero-sensory stimuli and neuroendocrine activity.

The knowledge of chemoarchitectonically and functionally characterized neural circuits in the salmon brain, together with the observed brain changes and new data on TH, enables us to identify the specific neural/neuroendocrine circuits likely to be central for smoltification, and to study the mechanisms of neuroplasticity. In our view, this provides a basis for future interdisciplinary studies to gain more knowledge about the biology of the smoltification process.

Brain structure	Brain changes during salmon smoltification	nNOS localization	TH actions	TH receptors
Pineal organ	Transient pineal projections	fb, M		S
Retina	UV photosensitivity dissapears (TH)	yes (cb)	S,M	M
Visual centers:	Transient retinal			
such as	projections	yes (cb,fb		
HON/SOC, SCN,		yes (cb,fb		M
Ppp,		yes (cb)		
optic tectum	Increase in μ- and κ-opiate receptors			
Olfactory bulb	Olfactory imprinting	yes (fb)	S,M	M
Telencephalon	Transient increase in 5-HTir fibers	yes (cb)	M	M
	Increase in μ- and κ-opiate receptors			
hippocampus M	Learning and memory	yes?, M	M	M
Habenula	Transient 5-HTir neurons (right)	yes (cb, right)		M
	Increase in κ-opiate receptors			
Neurosecretory nuclei:	Neurohormone influence on pituitary			
NPP, NPO, SOC.	activity	yes (cb)	M	S,M
v.hyp. ,CSFc	Increase in μ- and κ-opiate receptors	yes (cb)		
Neurohormone systems:				
catecholamines		yes (cb)	S,M	M
indolamines		yes (cb)	S,M	
peptides	Transient catecholamine levels (total)	yes (cb)		
	Transient 5-HTir neurons (in LPOA)			
Cerebellum	Increase in μ- and κ-opiate receptors	yes (cb)	M	M

Table 1. Morphological and chemical brain changes in salmonids, the presence of neuronal nitric oxide synthase (NOS), actions of thyroid hormones (TH), and the presence of TH receptors shown in salmonids (S) and/or corresponding structure of mammals (M). cb= cell bodies, fb= fibers

(data on salmon brain circuitry changes from Ebbesson et al (1988) *Cell Tissue Res.* **252,** 215-218; Ebbesson et al (1992) *Cell Tissue. Res* **268,** 389-392; Holmqvist et al (1994) *Aquaculture* **121**, 1-12, Ebbesson et al, unpublished observations).
Abbreviated structures: CSFc= cerebro-spinal fluid contacting system, HON= hypothalamic optic nucleus, NPO = magnocellular preoptic nucleus, NPP= parvocellular preoptic nucleus, SCN = suprachiasmatic nucleus, SOC = supraoptic/chiasmatic nucleus, PON = periventricular pretectal optic nucleus, v. hyp. = ventral hypothalamus.

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IS GROWTH HORMONE A REGULATOR

OF THE SMOLTIFICATION PROCESS?

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The temporal control of the smoltification process may have a basis in a circannual endogenous rhythm although this has not been conclusively established (Eriksson and Lundqvist 1982). What has been extensively demonstrated is that environmental information, especially photoperiod, is of critical importance for the commencement of the process, and photoperiod manipulation can be used to inhibit smoltification as well as to produce out-of-season smolts.

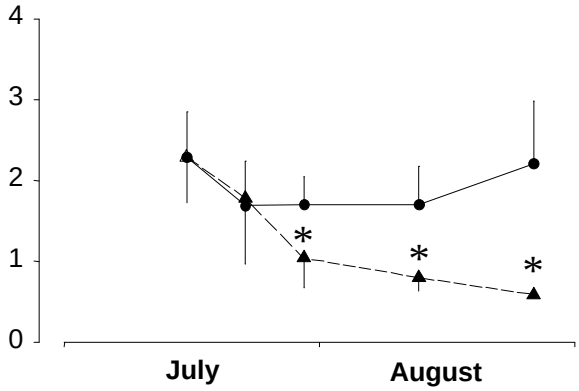

Figure 1. Growth hormone levels of 0+ Atlantic salmon parr on 24L:0D photoperiod (•) compared with a group moved onto 12L:12:D (▲) on July 15th (Björnsson, Hamre, Bjørnevik and Hansen, unpublished)

Changes in plasma growth hormone (GH) levels are intimately tied to changes in photoperiod. Increase in photoperiod will increase plasma GH levels in salmon parr, both within and outside the normal period of smoltification (Björnsson *et al* 1995; McCormick *et al* 1995). Recently, a clear evidence was obtained showing that a decrease in photoperiod will induce a decrease in circulating GH levels in Atlantic salmon parr (Figure 1). While changes in daylength thus induce changes in the GH secretory system, constant daylength (including continuous light) over an extended period appears to suppress GH levels (Björnsson *et al* 1995; Björnsson, Hansen *et al*, unpublished). Temperature plays a modulating role in the GH response to photoperiod so that when daylength is increased, the GH increase appears sooner in fish kept at higher temperature (McCormick, Björnsson *et al*, unpublished).

Due to the sensitivity of the GH-secretory system in salmon to light, it is of great importance for any work, experimental or practical, which involves changes in daylength, to consider the consequences for GH secretion and the physiological development of the fish. In salmon, GH is now recognized to be a multifunctional hormone, participating in the regulation of growth (including shape), metabolism, osmoregulation and behaviour (Björnsson 1997). All these functions have not been studied directly during the smoltification process. However, the changes in growth, shape and metabolism that take place during smoltification are fully consistent with the known physiological effects of GH in salmon, where GH mobilizes fat, builds protein and has a primary stimulatory effect on skeletal length growth, creating a lean fish with a great potential for subsequent weight growth. Also, the stimulation of hypoosmoregulatory ability through induction of Na^+,K^+-ATPase activity in gills as well as in the intestine has been well demonstrated to be an important GH function during the smoltification process.

Then, how strong is the evidence supporting the idea that GH is a major regulator of the smoltification process? The question is warranted as the bulk of the evidence is circumstantial in nature, of two types: GH causes a number of smoltification-like changes, and the GH-IGF-I system activated during the smoltification process. A Devil's advocate could argue that a GH treatment does not make salmon smoltify, and if the smoltification process is ruled by an endogenous rhythm set to occur during spring while the GH-IGF-I system is activated by increased daylength, the two events would occur concurrently, even if they were not functionally related. However, when all data are taken together

the case for GH being a major regulator of the parr-smolt transformation process still appears solid, but it is also clear that other endocrine systems are also involved. A number of previous studies have provided multiple hormonal profiles during smoltification (e.g. Young *et al* 1989, 1995) allowing speculations on functional relationships between growth hormone and other hormonal systems during the smoltification process. Recent studies have started to give insights into the mechanistic basis for such endocrine interrelationships. For example, in juvenile coho salmon, growth hormone has been found to increase corticosteroid receptor abundance (Shrimpton *et al* 1995), and the goitrogen propylthiouracil has been shown to suppress not only thyroid hormones levels, but also growth hormone levels (Ebbeson *et al* 1998). At present, it is of major importance to continue the research into such functional relationships among the endocrine systems involved, in order to obtain a clear picture of the endocrine regulation of the parr-smolt transformation process.

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CAN ATLANTIC SALMON SMOLT TWICE?

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Introduction

Juvenile salmon show seasonal increases in their ability to migrate from fresh water to seawater. A number of morphological and physiological changes occur during the parr-smolt transformation. An increase in gill Na^+K^+ATPase activity is correlated with the increase in saltwater tolerance. In Atlantic salmon, bimodal growth distributions are common in hatchery and laboratory-reared fish and have also been documented in wild populations. In hatchery fish the larger, faster growing upper mode (UM) will smolt in their first spring as 1+ juveniles, whereas the smaller, slower growing lower mode (LM) require an additional year of growth before smolting as 2+ juveniles. To date most of the laboratory studies examining physiological changes associated with smolting in Atlantic salmon have focused on UM fish in their first year. The limited number of studies that have focused on LM fish, however, indicate that these fish also exhibit seasonal rhythms associated with smolting, but the magnitude of the changes are significantly less than that seen for UM fish (Shrimpton and McCormick 1998).

Increases in salinity tolerance that occur during smolting are known to be reversible. Fish that are maintained in fresh water beyond the period of normal spring migration lose their elevated capacity to osmoregulate in seawater (Duston et al. 1991). Research on Atlantic salmon reared in the wild indicates that loss of smolt characteristics also occurs under some conditions in naturally

migrating smolts (McCormick et al. 1997). It is believed that fish that lose smolt characteristics cease migration and continue to reside in the river. Fish that physically appear to be smolts have been captured behind dams in the Connecticut River with low levels of gill Na^+K^+ATPase activity. It is not known whether or not these fish that have undergone the parr-smolt transformation, will repeat the morphological and physiological changes associated with smolting the following spring.

Methods

To evaluate their ability to smolt twice, Atlantic salmon were held at the White River National Fish Hatchery in Bethel VT for two years. At monthly intervals, we examined the growth, physical appearance, physiological changes and endocrine levels of the fish.

Results

Bimodal growth distribution was evident by the first October post hatch. LM fish were 9.9±0.5 g and 9.7±0.2 cm and the UM fish were 19.9±1.7 g and 12.0±0.4 cm. By the first spring, both UM and LM fish had shown significant growth (Figure 1). In May LM and UM fish were 23.4±2.0 g, 12.9±0.4 cm and 71.5±5.1 g, 19.1±0.5 cm, respectively. At the end of the study the following May, LM fish were 159±8 g and 25.3±0.5 cm and the UM fish were 828±65 g and 42.1±0.9 cm. In their first April, UM fish developed silver colouration and dark fin margins characteristic of smolts. The silver colouration of UM fish remained until the end of the study, one year later. LM fish did not lose parr marks during the first spring, but developed the silver colouration characteristic of smolts during the second spring.

Seasonal changes in gill Na^+K^+ATPase activity are shown in Figure 2. In the first spring, UM and LM fish showed significant increases in gill Na^+K^+ATPase activity. The increase in enzyme activity, however, was threefold greater in the UM fish than that observed for the LM fish. Gill Na^+K^+ATPase activity was highest in April, May and June. Gill Na^+K^+ATPase activity declined after this time and remained low in both groups until the next spring. Both UM and LM fish showed significant increases in gill Na^+K^+ATPase activity in the second spring. Although, the levels of gill Na^+K^+ATPase in the UM fish were approximately 70% of LM fish, they were clearly higher than the small increase

in observed in the LM fish the previous spring. Na^+K^+ATPase activity in the gills of LM fish during the second spring were similar to those of the UM during the first spring.

Growth hormone (GH) and cortisol are known to stimulate gill Na^+K^+ATPase activity and increase saltwater tolerance (Madsen 1990; McCormick 1996). GH and cortisol showed seasonal rhythms that varied in magnitude between the two years. In the first year of the study the endocrine changes were dominated by a large increase in plasma GH. In the spring of 1995, plasma GH of UM fish increased 8-fold (to 4.2±0.7 ng ml^{-1}), whereas plasma GH of LM fish increase only 47% ($p>0.05$). In the spring of 1996, however, changes in GH in both UM and LM were smaller than the previous year. UM and LM fish showed a 51% and a 44% increase in plasma GH, respectively. In contrast to the changes in GH observed, plasma cortisol increased relatively little in the spring of 1995; levels increased from less than 2 ng ml^{-1} to 8.8±2.4 ng ml^{-1} (UM) and 3.1±2.5 ng ml^{-1} (LM). In the spring of 1996, plasma cortisol increased significantly to 41.5±16.3 ng ml^{-1} and 13.7±6.8 ng ml^{-1} in UM and LM, respectively. GH and cortisol increase gill Na^+K^+ATPase activity individually, but also act synergistically when administered together (Madsen 1990; McCormick 1996). An endogenous increase in these two hormones concomitantly would result in the greatest increase in gill Na^+K^+ATPase activity. This pattern is seen in the UM fish during the spring of 1995, but not during 1996. We do not know whether the differences observed between the two years are due to a differential response to environmental stimuli between the two years, or ontogenic differences.

Gill Na^+K^+ATPase activity shows seasonal variation in both UM and LM Atlantic salmon. The rise in Na^+K^+ATPase activity in LM fish during the first spring indicates that parr undergo seasonal changes in osmoregulatory physiology, but the extent of the change is much greater in smolts. Our findings indicate that UM Atlantic salmon are capable of undergoing the physiological changes associated with smolting during two successive years.

Acknowledgements

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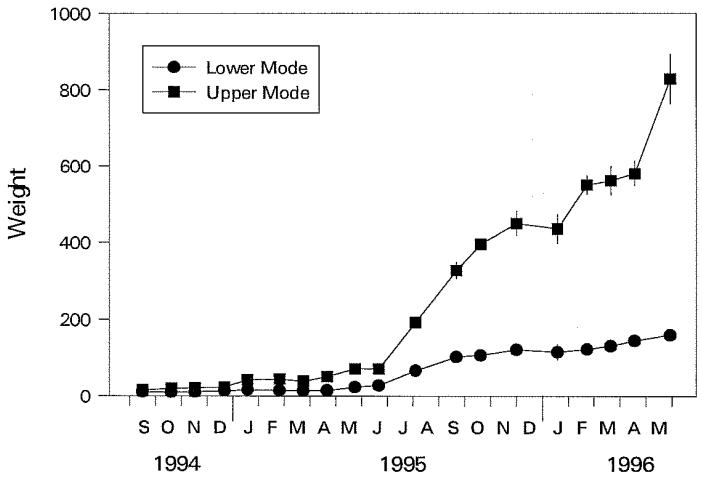

Figure 1. Mean weight of UM and LM juvenile Atlantic salmon sampled at monthly intervals from the White River National Fish Hatchery at Bethel, VT.

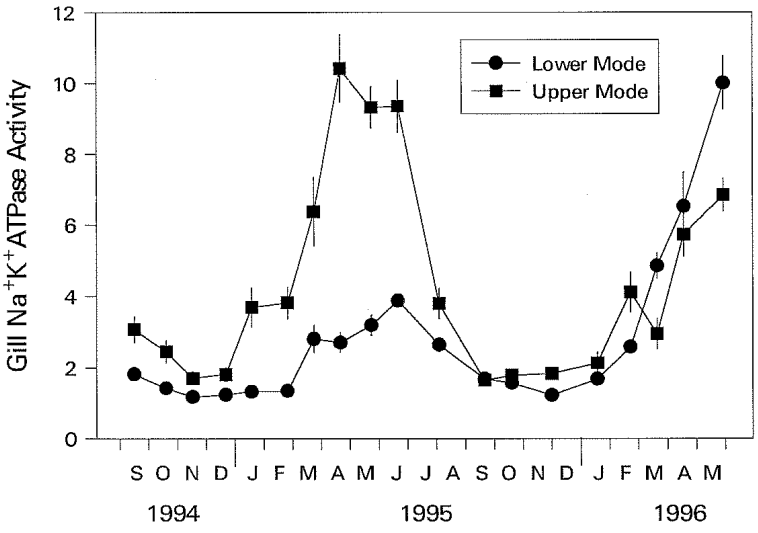

Figure 2. Gill Na^+K^+ATPase activity of UM and LM juvenile Atlantic salmon sampled at monthly intervals from the White River National Fish Hatchery at Bethel, VT.

INTESTINAL FLUID ABSORPTION

DURING PARR-SMOLT TRANSFORMATION

OF ATLANTIC SALMON, *SALMO SALAR*.

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EXTENDED ABSTRACT ONLY DO NOT CITE

Salmonids show a wide spectrum of seawater-adaptive patterns in which the anadromous Atlantic salmon, together with for example steelhead trout and coho salmon, are placed intermediate on a scale predicting the degree of developmental preadaptation of hypo-osmoregulatory ability (McCormick and Saunders 1987). During springtime, these fish undergo complex physiological, morphological and behavioral transformations in fresh water (FW) to become ready for entry into seawater (SW). This process, the parr-smolt transformation, includes changes in the osmoregulatory mechanisms from a fresh water to a seawater state. In SW, the intestine is of major importance in maintaining internal water balance.

In this abstract we briefly review the changes in intestinal fluid absorption that we have observed in the gut of Atlantic salmon during the parr-smolt transformation and the regulation of this process by hormones. Furthermore, we present our recent work on the mechanisms underlying changes in intestinal ion and water absorption.

Atlantic salmon often show a bimodal distribution in growth during their first year, resulting in a group of lower mode fish that remain as parr and a group of

upper mode fish that undergo parr-smolt transformation. The rate of intestinal fluid absorption (J_v), measured across the posterior intestine *in vitro*, was elevated in springtime in the fish undergoing parr-smolt transformation, whereas it remained unchanged in lower mode salmon (Veillette et al 1993). Smolts transferred to SW exhibited increased intestinal J_v over their FW counterparts. This demonstrates that increased intestinal J_v is development-dependent and preparatory for osmoregulation in SW. We have recently documented, in chinook salmon, seasonal increases in intestinal Na^+,K^+-ATPase activity, which may also be part of the pre-adaptive changes that occur in the salmon intestinal tract (PA Veillette and G Young, unpublished).

The parr-smolt transformation is accompanied by a complex pattern of changes in endocrine factors, of which cortisol and growth hormone (GH) may mediate the pre-adaptive increase in hypoosmoregulatory ability (McCormick et al 1991). Therefore, we examined next the effects of cortisol implants and GH

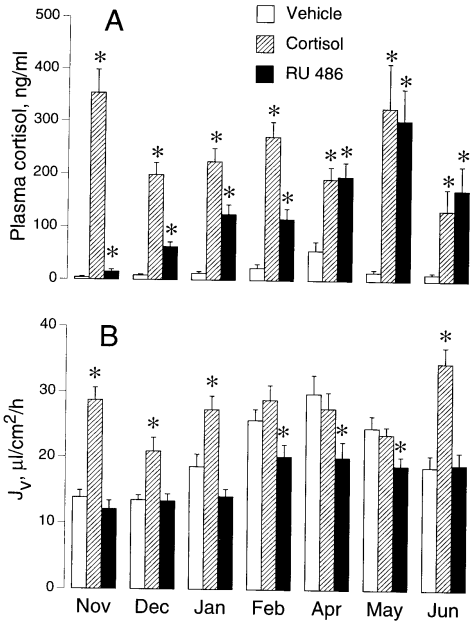

Fig.1. From Veillette *et al* 1995

injections, alone or together, on intestinal J_v of FW post-smolts (Cornell et al 1994). Cortisol increased the intestinal fluid transport, whereas GH was without effect.

To explore further the effects of cortisol on intestinal fluid absorption, we examined the responsiveness of J_v to cortisol and the corticosteroid antagonist RU-486 at different developmental stages during parr-smolt transformation (Veillette et al 1995). Juvenile Atlantic salmon in FW were implanted with cortisol, RU-486 or vehicle alone (control) once-a-month from November through June. Eight days after implantation, J_v and plasma cortisol levels were measured. As shown in Fig. 1, both plasma cortisol levels and J_v of the control fish peaked in April and were positively correlated over time. The cortisol implants increased J_v only in the parr (Nov-Jan) and post-smolt (Jun) stages when J_v of the controls was low. Conversely, RU-486 inhibited J_v only during the peak smolt period (Feb – May) when the J_v of the controls was elevated. Thus, we conclude that cortisol is a necessary endocrine signal mediating the developmental change in intestinal J_v during the parr- smolt transformation.

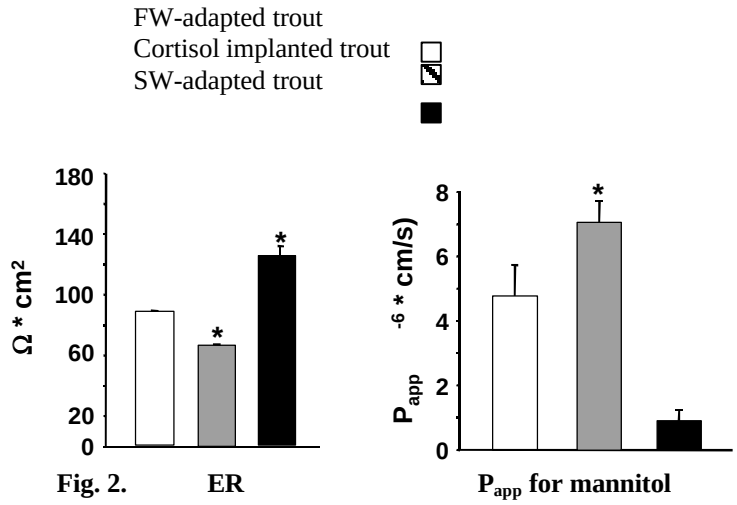

Fig. 2. ER **P_{app} for mannitol**

The ion transporting mechanisms underlying intestinal fluid transport of SW fish are to a large extent a combination of Na^+,K^+-ATPase activity and Na^+,K^+,Cl^--co-transport (Loretz 1995). In a study of FW-adapted rainbow trout, we examined the effects of cortisol implants and GH injections, alone or together, on J_v and intestinal Na^+,K^+-ATPase activity. Treatment with cortisol and GH

together resulted in an increase of both parameters. In this study, we showed for the first time a positive correlation between intestinal J_v and intestinal Na^+,K^+-ATPase activity.

In addition to the ion transporting mechanisms, the permeability of the epithelium is also important in controlling the rate of intestinal fluid absorption. Stripped intestinal preparations from rainbow trout adapted to FW and SW were mounted in a modified Ussing chamber and transepithelial potential (TEP), transepithelial resistance (TER) and shortcircuit current (SCC) were measured. Additionally, the preparations were exposed to several hydrophilic markers and their apparent permeability coefficients (P_{app}) were calculated. SCC showed a higher absolute value in SW-adapted trout compared with FW-adapted trout, indicating higher ion transport activity in SW. As shown in Fig. 2, SW-adapted trout also showed a marked elevation in TER compared with trout in FW. P_{app} was lower for all markers in SW compared with FW-adapted trout and decreased with increasing molecular size. On the other hand, FW-adapted trout implanted with cortisol for 7 days showed an increased paracellular permeability as demonstrated by a decreased TER and increased P_{app} for mannitol. Furthermore, a more serosa-negative TEP and a higher absolute value of SCC were demonstrated in cortisol-implanted fish compared with controls. Thus, the paracellular permeability decreased in SW-adapted rainbow trout, whereas cortisol implants increased the permeability of the tight junctions. Both treatments resulted in a higher ion transporting activity as indicated by the higher absolute values of SCC.

In conclusion, cortisol mediates the increased fluid absorption rates seen during the parr-smolt transformation, probably by increasing intestinal Na^+,K^+-ATPase activity and paracellular permeability. When the fish enters SW, the increased ion transporting activity remains, but the paracellular permeability decreases.

Acknowledgements

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THE GH - IGF-I ENDOCRINE AXIS, SMOLTIFICATION AND LIFE HISTORY PLASTICITY IN CHINOOK SALMON

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Discussion

Juvenile salmonids undergo a developmental process termed smoltification, which stimulates fish to make a transition from freshwater rearing habitats to the ocean. As a species, chinook salmon show a variety of juvenile life histories, undergoing smoltification and entering the ocean at ages varying from 1-month to greater than one year post-emergence (Figure 1).

Recent work has shown that smoltification may be plastic within a population and suggests that this plasticity is related to growth rate - faster growing fish smolting in the fall with slower growing fish smolting in the succeeding spring (Beckman and Dickhoff, submitted). Evidence is accumulating that smoltification, and the plasticity associated with it, is strongly influenced by activity of the growth hormone (GH) - insulin-like growth factor I (IGF-I) endocrine axis. The GH - IGF-I axis appears to have a dual role, integral to both smoltification and growth (Komourdjian et al. 1976, Dickhoff et al. 1997). In this work we tested whether we could modulate smoltification of spring chinook

salmon in the fall through changing activity of the GH - IGF-I axis by altering nutritional status.

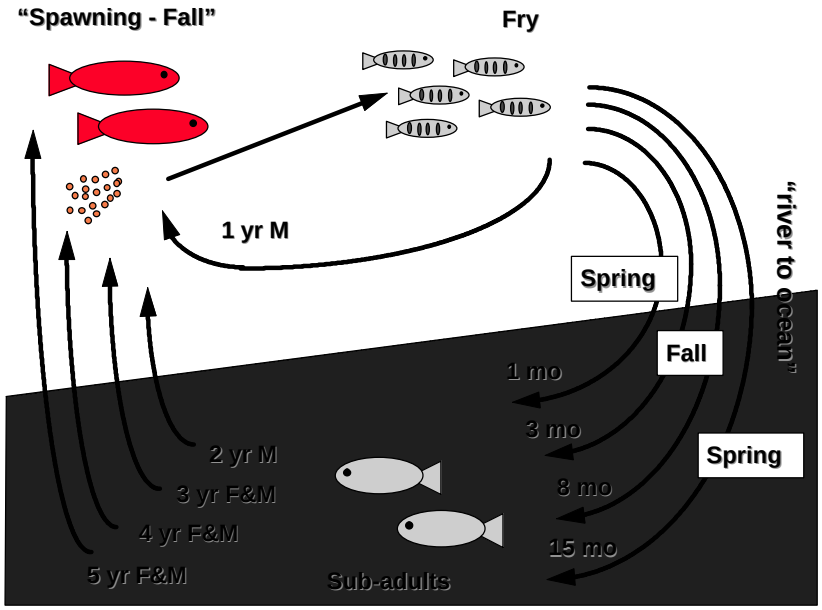

Figure 1. Diagram illustrating life history plasticity of chinook salmon. Smoltification may occur from 1 to 15 months post-emergence; while, maturation may occur from age 1 to 5.

Specifically, a group of Quilcene chinook salmon were size graded in July into large and small size categories. Subsequently, each size category was subdivided into two groups whose growth rate was modulated by varying feeding rate, resulting in four groups: Big-HiFeed, Big-LoFeed, Small-HiFeed, Small-LoFeed (Figure 2).

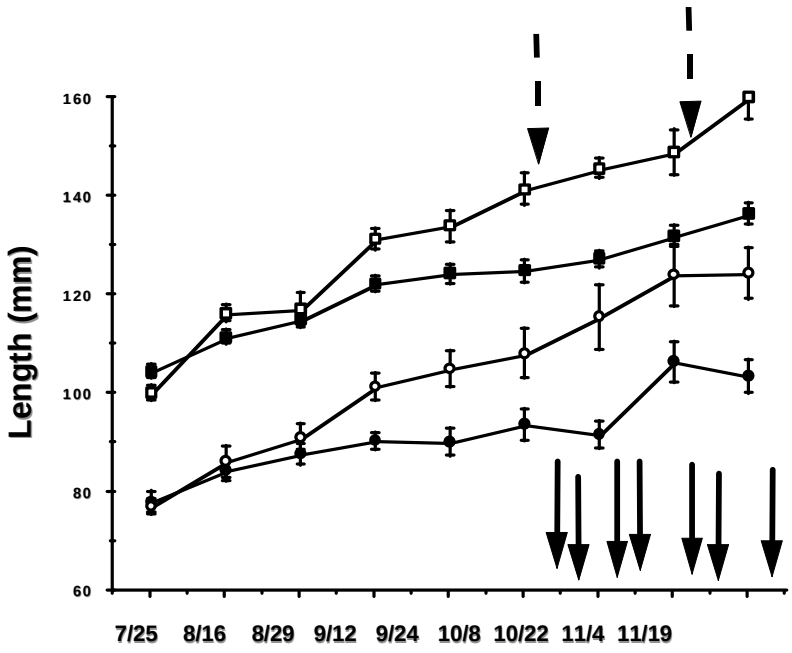

Figure 2. Size of chinook salmon juveniles in four experimental groups : Big-HiFeed (open square), Big-LoFeed (solid square), Small-HiFeed (open circle), Small-LoFeed (closed circle). Physiological samples were taken on all dates where size was measured, solid arrows indicate dates where behavior was observed, dashed arrows indicate dates where seawater challenges were conducted.

Size, plasma, pituitaries, and gill tissue were collected at two week intervals from July through November. Behavior was observed on seven dates in October and November. Two seawater challenges were conducted, one in October and one in November. Growth differed significantly between groups and resulted in four distinctly different size groups by October.

Significant differences in behavioral activity were observed, with fish from HiFeed groups (higher growth rate) displaying higher activity levels (suggestive of “migratory urge”) than fish from LoFeed groups in a modified adult holding

raceway. Significant differences in plasma IGF-I and gill Na^+ K^+ ATPase activity were also found between treatment groups. Plasma IGF-I increased significantly in Small-HiFeed fish within two weeks of increased feeding rate. In contrast, plasma IGF-I levels in Big-LoFeed fish did not show a significant decrease until 8 weeks after feeding rate was decreased. Gill Na^+ -K^+ ATPase activities found in Small-LoFeed fish were significantly less than found in other treatment groups through August and September.

Overall, significant differences in physiology and behavior were found between treatment groups; however, these differences did not correspond neatly to either differences in size or growth rate. Our results suggest that organismal "decisions" about smolting may be made prior to the time we began our experimental manipulations; yet, our modulation of growth rate in late summer and fall did appear to modify some of the physiological and behavioral facets of smoltification.

Acknowledgments:

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ALTERATIONS IN CONTAMINANT FATE DURING SMOLTIFICATION IN JUVENILE COHO SALMON, *ONCORHYNCHUS KISUTCH*

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Abstract

The reported increased sensitivity of smolts to contaminants may be in part due to alterations in the toxicokinetics of pollutants resulting from morphological, physiological or biochemical changes naturally occurring in these fish at this time. The objective of the present study, therefore, was to determine the effects of smoltification on the uptake, distribution, and metabolism of the model PAH, benzo[a]pyrene in juvenile coho salmon. These studies revealed that the uptake rate of benzo[a]pyrene increased up to five-fold during the smoltification period in coho, which is likely attributable to higher measured oxygen consumption rates and ventilation volumes in smolts. Alterations in the disposition of benzo[a]pyrene were also noted in fish sampled throughout this period. Biotransformation enzyme levels and activities also change significantly during this period, however, the overall metabolism and excretion of benzo[a]pyrene is not significantly different between parr and smolts. Examinations such as these of the biological and environmental factors affecting salmonid sensitivity to xenobiotics will lead to a more comprehensive understanding of the fate of contaminants in fish and lead to a better understanding of the environmental significance of aquatic pollutants.

Introduction

At an ever increasing frequency, juvenile salmonids are encountering contaminated environments during their out migrations. Prior to, or accompanying the seawater migration of juvenile coho, a period of development known as smoltification occurs, which consists of a spectrum of simultaneous or consecutive morphological, physiological and biochemical changes which culminate in the adaptations necessary for a marine existence (Hoar, 1976). This transition has profound effects on, and leads to substantial adaptations of, the physiology and metabolism of anadromous salmonids (Wedemeyer, Saunders and Clark, 1979; Folmar and Dickhoff, 1980).

Early developmental stages in fish tend to be more sensitive to the toxic effects of xenobiotics than are juveniles or adults (Stegeman and Hahn, 1994). During outmigration, young fish may be more sensitive to certain xenobiotics during smoltification or when exposed to chemicals in seawater which may effect marine survival. For example, exposure of smolts to water polluted from sulfite pulp mills, municipal sewage and agricultural runoff have reduced seawater adaptability, increased infestation of trematode parasites, and lowered disease resistance in the Upper Grays Harbor estuary of the Chehalis River in Washington State (NRC, 1996). Our own studies have shown that the smoltification process can affect the sensitivity of salmonids to various metal and organic contaminants, with smolts being predominantly more sensitive than parr.

There is a growing body of evidence suggesting that environmental and physiological factors (Kennedy 1995) can effect the toxicokinetics (including absorption, distribution and excretion) of xenobiotics in fish. The increased sensitivity of smolts to contaminants may be in part due to alterations in the toxicokinetics of pollutants resulting from morphological, physiological or biochemical changes naturally occurring in these fish at this time. The objective of this study, therefore, was to determine the effects of smoltification on the uptake, distribution, metabolism and excretion of the model polycyclic aromatic hydrocarbon, benzo[a]pyrene, in juvenile coho salmon.

Materials and Methods

Fish

Yearling coho salmon, *Oncorhynchus kisutch*, were obtained weekly from Capilano Hatchery in North Vancouver, B.C. in February until June when the fish were released from the hatchery. Fish were maintained at seasonal temperatures (4-8¡C) and under natural photo periods for several days until an experiment was started.

Chemicals

Unlabelled benzo[a]pyrene (B[a]P) was purchased from Sigma Chemicals (St. Louis, MO.) and [1,3,6-^{3}H]- benzo[a]pyrene (52 Ci/mmol) was purchased from NEN Research Products (DuPont, Canada Ltd., Mississauga, ON). B[a]P metabolite standards were obtained from the National Cancer Institute Chemical Repository, Midwest Research Institute (Kansas City, MO.).

Smolt status

Gill tissue was analyzed for Na+/K+-ATPase activity using methods (Seubert and Kennedy 1997). Fish were examined for morphological and color changes indicative of smoltification.

Benzo[a]pyrene uptake

Individual fish at each sampling time were placed in an aquarium containing [^{3}H]BaP at an initial concentration of 5 µg BaP/L. BaP uptake rates were estimated as described by Lemke and Kennedy (1997) as the inverse rate of disappearance of BaP-derived radioactivity from the water. Oxygen consumption measurements were also at each sampling period using standard respirometry (Cech 1990).

Biotransformation Enzyme Analysis

The baseline activities and levels of several Phase I and II biotransformation enzymes of coho were measured biweekly starting in February until June. Microsomal fractions were prepared according to Förlin and Andersson (1985). Total cytochrome P450 content was determined by the method of Omura and Sato (1964). Ethoxyresorufin O-deethylase (EROD) activity was measured

using the method of Burke and Mayer (1974). Glutathione S-transferase (GST) in cytosol was measured spectrophotometrically as described by Habig et al. (1974).

Exposure to Benzo[a]pyrene in distribution, metabolism, and excretion studies

Each month from February to June, fish were injected intraperitoneally with 10 mg (1 µCi)/kg of [^{3}H]-B[a]P to determine the effects of smoltification on chemical disposition, metabolism and elimination. Potential confounding effects on B[a]P uptake may have occurred if fish were exposed to B[a]P in water, therefore, fish were exposed to the chemical by i.p. injection to ensure that all fish through the study received the same dose.

Tissue Sampling and Radioactivity Determination

Tissues were homogenized and oxidized to determine the amount of ^{3}H-B[a]P derived radioactivity. Oxidized homogenates and bile was counted by LSC.

Biliary Metabolite Analysis

The method used by Lemke and Kennedy (1997) was followed for the quantitation of B[a]P and its metabolites in bile. Bile was extracted three times with ethyl acetate (5:1) to separate Phase I metabolites. The remaining aqueous layer then underwent a series of enzymatic incubations and extractions to separate various Phase II metabolites which were quatitated by LSC.

Phase I metabolites were separated by HPLC using a Hewlett Packard 1050 series liquid chromatograph equipped with a Perkin-Elmer HC-ODS SIL-X reverse phase column (0.26 x 25 cm), a HP 1046A fluorescence detector and an HP Integrator (excitation 263 nm and emission 370 nm). For metabolite separation, the methods of Elnenaey and Schoor (1981) were followed.

Elimination of Benzo[a]pyrene

The radioactivity recovered from the oxidized tissues; carcass, liver, kidney and fat were totaled for each fish to obtain the amount dpms remaining in each organism. The elimination of [^{3}H]-B[a]P was calculated by subtracted the recovered radioactivity in the organism from the amount injected.

Results

Smolt status

Gill Na+/K+-ATPase activities are shown in Figure 1. The characteristic freshwater parr marks (dark pigmented bars on the lateral surface, perpendicular to the lateral line) were present at the start of the sample period. They remained distinct until May when there was a progressive disappearance until the characteristic silver appearance of the transformed smolt appeared at the end of the sample period in June.

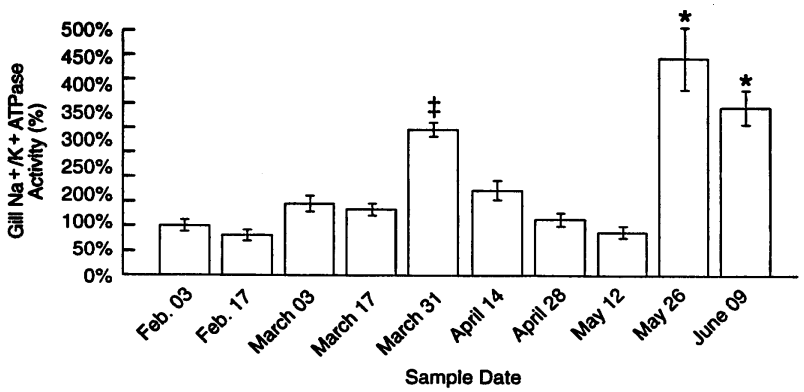

Figure 1. Na+/K+ ATPase activity in gills of coho sampled from February to June. Values are represented as the percent change from activity measured on the first sampling date, Feb. 3 (100%). Values with common symbols are not statistically different at $p<0.05$.

Uptake of benzo[a]pyrene and oxygen consumption rates

Uptake rates of BaP appear to increase with time from February to June (Figure 2). Although the results could not be compared statistically, oxygen consumption rates on February 18, March 16, April 19, May 13, and June 15 were: 130, 99, 188, 236 and 308 mg O2/g/h, respectively.

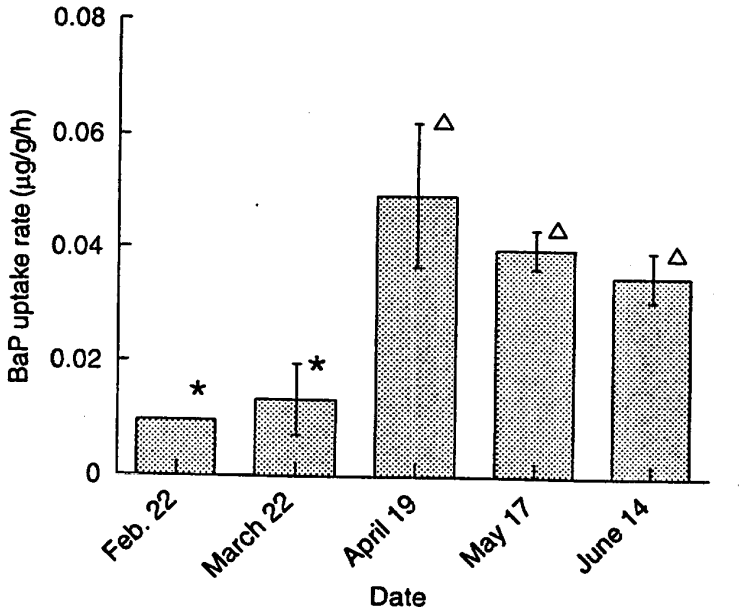

Figure 2. BaP uptake rates in coho sampled from February to June. Values with common symbols are not significantly different at $p<0.05$.

Biotransformation Enzymes

Liver cytochrome P450, EROD, glutathione S-transferase activities and levels in fish sampled from February to June are shown in Figure 4. Significant changes occurred in all measured levels and activities during smoltification.

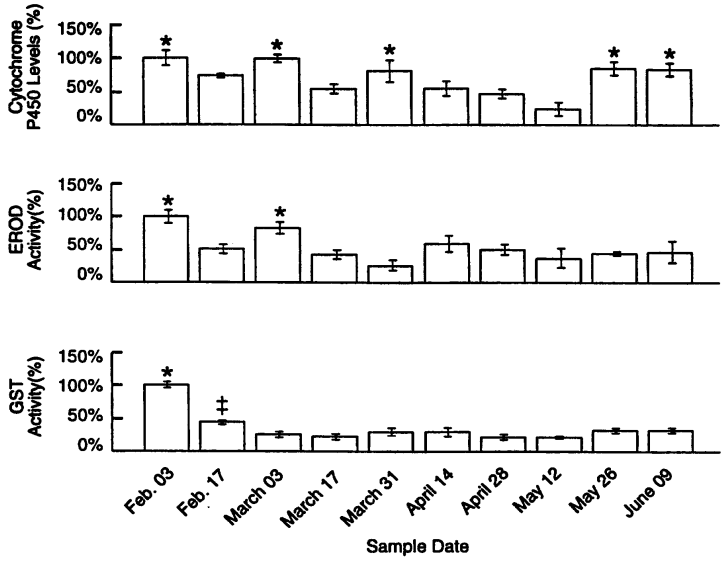

Figure 3. Biotransformation enzyme levels and activities during smoltification. Values are expressed as the percent change from enzyme levels measured on the first sampling day, Feb.3(100%). Values with common symbols are not statistically different at $p<0.05$.

Tissue Distribution of Benzo[a]pyrene

Following an i.p. injection of [^{3}H]-B[a]P, B[a]P-derived radioactivity was found in all tissues sampled. Figure 4 shows the percent body burden of B[a]P derived radioactivity in tissues sampled from February to June (1 and 7 days) after receiving an i.p. injection. The general trend of the body burden of B[a]P derived radioactivity in tissues over the experimental period was

liver>fat>kidney>carcass. The highest levels of recovered radioactivity were found in the liver on all the sample days for every month over the experimental period, except in the month of June where fat had the higher percent body burden.

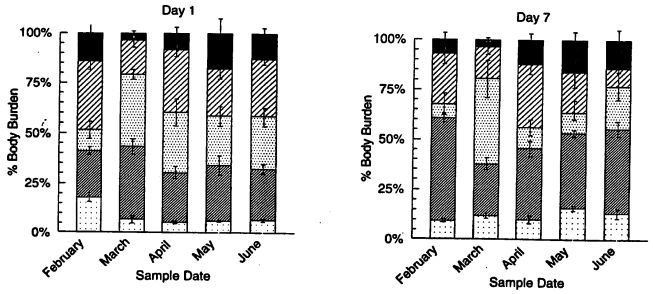

Figure 4. Percent body burden of BaP-derived radioactivity in the kidney (■), liver (▨), bile (⊡), fat (▧) and carcass (□).

Biliary Metabolites

Juvenile coho salmon metabolized B[a]P to both Phase I and II metabolites (Table 1). HPLC analysis of bile collected at each sampling period revealed that <10% of the radioactivity recovered was parent compound, 55 to 60% as extractable Phase I metabolites, 16 to 24% were glucuronic acid conjugates, 8 to 10% were sulfate conjugates, 5 to 9% were unknown conjugates and the remaining 3 to 7% of the radioactivity recovered was an unknown water soluble metabolite(s). There were no significant differences in the proportions of these metabolite groups from February to June.

	Metabolite groups				
	Organic soluble	Glucuronide	Sulfate	Unknown conjugates	Aqueous soluble
Month					
February	62.9 ± 2.8	16.2 ± 2.7	8.2 ± 0.3	7.1 ± 0.4	5.7 ± 0.7
March	64.5 ± 4.5	18.5 ± 3.4	8.5 ± 1.2	5.5 ± 0.5	3.1 ± 0.5
April	57.2 ± 3.7	20.0 ± 0.8	9.9 ± 1.3	6.0 ± 1.4	7.0 ± 1.7
May	59.4 ± 1.9	18.0 ± 0.7	8.3 ± 0.7	8.7 ± 0.8	5.6 ± 0.2
June	54.7 ± 1.3	23.7 ± 0.9	8.3 ± 1.9	7.8 ± 0.6	5.4 ± 0.8

Values are the means ± SEM for 5 fish. There were no significant differences between months in the percents within classes of metabolites at a significance level of $p<0.05$.

Table 1. Percent of total radioactivity as metabolite classes in the bile of coho. No significant differences were noted at $p<0.05$.

Chromatographic separation of B[a]P and organic soluble metabolites by HPLC revealed a variety of tentatively identified Phase I metabolites which triols/tetrols, diols, quinones and phenols. Quinones and phenols compromised the majority of Phase I metabolites: 20-40% and 20-30%, respectively. The identified phase I metabolite which accumulated in the greatest quantity was r-7,t-8,9,c-10-tetrahydrotetrol.

Elimination of benzo[a]pyrene

The elimination of B[a]P derived radioactivity from juvenile coho salmon is shown in Figure 3. The majority of the injected B[a]P was eliminated 24 hours following i.p. injection and ranged from 71 to 94% of the total injected dose. The lowest amount eliminated (71% of the total dose) occurred in March.

Discussion

During smoltification a variety of morphological, physiological and biochemical changes occur which may alter the sensitivity of juvenile salmonids to toxicants. In this study, the effects of smoltification on the uptake, distribution, metabolism and elimination of benzo[a]pyrene (BaP) in coho salmon were examined. It can be concluded from the results that the hatchery fish utilized in this study showed characteristic freshwater adapted (parr) traits at the beginning of the experiments followed by a characteristic transformation into seawater adapted (smolt) fish by the end of the experimental period.

The uptake of [^{3}H]BaP from water was rapid. This has been attributed to the highly lipophilic nature of these chemicals which cross biological membranes by

passive diffusion (Kennedy, 1995). The increasing rates of BaP uptake through smoltification may be related to changes in fish respiration rates and oxygen utilization by the fish. Alterations in the amount of water flowing past the gills by changes in ventilation rate or volume have been shown to affect the uptake of hydrophobic compounds such as BaP which are ventilation-limited (Schmieder and Weber, 1992). The fatty acid composition of epithelial membrane in the gill changes during smoltification, however, it is unknown whether these changes in the structure of the membrane significantly affect the diffusion of hydrophobic chemicals.

Various factors, including pre-exposure to xenobiotics, seasonal variations, reproductive status, developmental stage, species differences and fish migration can modulate the biotransformation system. In general, with all of the enzymes examined, peak enzyme activities occurred in February and March and then declined through to June. As well, a shift in energy utilization may play a crucial role as energy is required for the synthesis of new enzymes and many biotransformational reactions. There are several changes in energetics during the 'parr-smolt' transformation process, which results in a shift from anabolic to catabolic metabolism (Wedemeyer, Saunders and Clark, 1979; Folmar and Dickhoff, 1980). The development of hepatic enzymes is under complex ontogenic control and generally do not develop gradually (Ronis and Cunny, 1994). The observed changes in biotransformation enzyme activities through the smoltification process may have several explanations, including hormonal modulation.

The largest shifts in tissue distribution of B[a]P derived radioactivity occurred within the first two months of the experiment coinciding with significant changes in protein and enzyme activities and levels. There are several possible reasons which may cause an altered distribution of B[a]P which include alterations in detoxification rates, changes in the amounts of tissue lipids and osmoregulatory adjustments involving maintenance of water and ion balance.

Juvenile coho salmon were capable of metabolizing B[a]P to both unconjugated (Phase I) and conjugated (Phase II) forms at a relatively rapid rate compared to many other xenobiotics. The larger percentage of organic soluble metabolites over other metabolites extracted suggest the phase II system of juvenile coho salmon is operating at lower capacity than other teleost species, possibly attributed to the developmental state of the organism rendering them unable to conjugate compounds effectively during this period of their life cycle. The chromatographic separation of B[a]P and organic soluble metabolites by HPLC

revealed tetrols, diols, quinones and phenols, and the major identified phase I metabolite of B[a]P in juvenile coho was 7,8,9,10-tetrol. The metabolites recovered from the bile of juvenile coho salmon indicate a metabolic pathway in coho towards the metabolic activation of B[a]P to carcinogenic metabolites capable of inducing cytotoxicity and tumor production (Varanasi et al, 1989). The large percentage of triols and tetrols in organic soluble extractions in the present study, provides further support of the presence of the metabolic pathway leading to bioactivation of B[a]P. Although biotransformation enzyme activities were altered during smoltification, there were few significant changes in metabolite groups produced over this period.

The elimination of a xenobiotic, whether it be the parent compound or its metabolite, reduces the potential toxic effects to the organism. In the present study, the vast majority of the administered B[a]P was excreted into the bile of the fish 24 hours following chemical administration. The relatively fast elimination of B[a]P by coho may be a reflection of the biotransformational capabilities of these fish which possess an efficient excretion system for B[a]P. Therefore, smoltification did not appear to affect the ultimate elimination of this PAH.

This study begins to assess the effects of smoltification on the toxicokinetics of the model carcinogen B[a]P and to determine the causes of altered susceptibility to chemicals during this developmental period in juvenile coho. Significant changes in xenobiotic uptake, metabolizing enzyme activities and chemical distribution occurred during this period, however, there were no significant changes in the classes of metabolites produced or in the total elimination of the chemical. This research illustrates the complex modulatory effects of developmental processes on chemical toxicokinetics in aquatic organisms. It also points out the importance of increasing the knowledge base regarding biological and environmental modulation of chemical fate and the associated underlying principles, if accurate predictions and assessments of contaminant effects are to be made in aquatic ecosystems.

Acknowledgments

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INFLUENCE OF TEMPERATURE

ON SMOLT DEVELOPMENT AND REVERSION

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EXTENDED ABSTRACT ONLY DO NOT CITE

Several experiments were conducted to determine the influence of temperature on development and loss of smolt characteristics in Atlantic salmon. The interaction of photoperiod and temperature on smolt development was examined by rearing Atlantic salmon juveniles at a constant temperature of 10 ^{o}C or ambient temperature (AMB: 1-3 ^{o}C from January to April followed by seasonal increase) under simulated normal daylength (LDN). At 10 ^{o}C an increase in daylength (LD 16:8) in February resulted in advanced increases in gill Na^{+},K^{+}-ATPase activity, whereas fish at AMB did not respond to increased daylength. Increases in gill Na^{+},K^{+}-ATPase activity under normal photoperiod occurred later at AMB than at 10 ^{o}C. Plasma GH and IGF-I increased within 7 days and remained elevated after LD 16:8 at 10 ^{o}C but did not respond at AMB. Plasma cortisol increased transiently following LD 16:8 at both temperatures. Plasma thyroxine was consistently higher at AMB but increased transiently following LD 16:8 at 10 ^{o}C. Plasma triiodothyronine was initially higher in the 10 ^{o}C group than at AMB, but there was no response to LD 16:8 in either group. The results indicate that low temperature limits the ability of photoperiod to advance the parr-smolt transformation in Atlantic salmon, and provide strong evidence that GH, IGF-I and cortisol are involved in the response of the parr-smolt transformation to changes in temperature and photoperiod.

To examine the ability of temperature to act as a zeitgeber for smolt development, Atlantic salmon juveniles were reared under simulated normal photoperiod (LDN) or short days (LD 8:16). In each photoperiod there were 2

temperature treatments: AMB (ambient: 1-3 ^{o}C in winter then normal temperature increases in April) and ADV (advanced: 1-3 ^{o}C in winter then advanced temperature increases in February). Under LDN, ADV temperature treatment resulted in greater rate of increase in gill Na^{+},K^{+}-ATPase activity than under AMB, but the timing of initiation (early March) and peak levels (late April) of gill Na^{+},K^{+}-ATPase activity was not altered. Similarly, under LD 8:16, ADV temperature treatment resulted in greater rate of increase in gill Na^{+},K^{+}-ATPase activity in March, but the timing of initiation was not affected. Although peak levels were earlier in the LD 8:16, ADV temperature group, this was primarily due to lower peak levels (half that of the LDN groups). The results indicate that temperature plays a role in the timing primarily by affecting the rate of smolt development, and is unlikely to act as a zeitgeber.

A combination of laboratory and field experiments were used to examine the influence of temperature on the loss of smolt characteristics in Atlantic salmon. Atlantic salmon that had previously been released as fry in tributaries of the Connecticut River were captured from 1993-1997 during their normal spring smolt migration 198 km from the mouth of the river. Smolts had high levels of gill Na^{+},K^{+}-ATPase activity and salinity tolerance early in migration. Significant decreases in gill Na^{+},K^{+}-ATPase activity and salinity tolerance were seen in smolts at the end of the migratory period in all years sampled. Loss of smolt characteristics was earlier and more severe when temperatures were high during the migratory period. The reduction in gill Na^{+},K^{+}-ATPase activity was directly related to the degree days of river temperature during the migratory period (r^{2}=0.75). Migrating smolts were also compared in several other rivers along the east coast of North America. Reduced gill Na^{+},K^{+}-ATPase activity was found at the end of migration in warmer, southern rivers (Connecticut River and Penobscot River, Maine), but not in northern rivers (Catamaran Brook, New Brunswick and Conne River, Newfoundland). Both hatchery and stream-reared fish held in the laboratory exhibited a more rapid loss of physiological smolt characteristics when held at higher temperature. There is a direct relationship between degree days experienced by fish and loss of physiological smolt characteristics, indicating that increased temperature shortens the period of physiological preparedness in smolts. Late migrants in warm, southern rivers lose physiological smolt characteristics, probably as a result of high temperatures experienced by these fish during migration. Delays in migration such as those that may occur at dams will have negative impacts on smolt survival in southern rivers.

Three major effects of temperature were found in these studies. First, low temperature limits the ability of photoperiod to advance the parr-smolt transformation in Atlantic salmon. Second, temperature affects smolt development primarily by affecting the rate rather than the timing of development, and is unlikely to act as a zeitgeber. Finally, increased temperature (degree days) shortens the period of physiological preparedness for seawater entry of smolts.

GROWTH AND PARR-SMOLT TRANSFORMATION IN STRAINS OF WILD ATLANTIC SALMON AT DIFFERENT TEMPERATURES

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EXTENDED ABSTRACT ONLY DO NOT CITE

Introduction

In order to describe the consequences of low freshwater temperatures on survival and growth of juvenile Atlantic salmon (*Salmo salar* L.), a comparative study was performed with Atlantic salmon from rivers Suldalslågen (regulated), Årdal (regulated) and Stryn (natural). All rivers are located in Western and South-Western Norway, with spawning populations consisting mostly of MSW salmon.

Methods

Juvenile salmon from all strains were hatched and first fed in spring of 1995. After first feeding (approximately 1g body weight) the fish were distributed among four different temperature regimes (4°C, 5.5°C, 7°C and a simulated

natural temperature regime, SNT, for river Suldalslågen). Temperature profile for SNT decreased from 11°C in late summer to <4°C during January-April, reaching 6°C in late May. Photoperiod was simulated natural (60'25"N) throughout.

Results

Mortality was low in all strains at all temperatures. Growth was significantly influenced by freshwater temperature, with a reduction in overall growth rate at 5.5°C and 4°C compared with 7°C (Table 1). Size distribution in May 1996 was unimodal at 4°C, with a change to a bimodal distribution, representing potential 1+ and 2+ smolts, at 5.5°C, 7°C and SNT (Table 1). Salmon from river Stryn had the highest growth rate at all constant temperature regimes, while fish from river Årdal showed the lowest growth rate. River Suldalslågen strain was intermediate between the other two strains. At the SNT regime, however, juvenile salmon from the Suldalslågen strain had the highest growth rate, followed by strains Stryn and Årdal (Table 1).

Temp	4.0°C		5.5°C		7.0°C		SNT	
Strain	LM	UM	LM	UM	LM	UM	LM	UM
Stryn	6.4^{a}(1.0)	-	7.7^{c}(0.9)	9.6^{a}(1.4)	8.3^{b}(3.5)	12.0^{a}(3.0)	7.9^{c}(1.5)	9.6^{b}(1.8)
Suldal	6.4^{a}(1.0)	-	7.3^{d}(1.1)	9.2^{b}(1.4)	7.8^{b}(1.9)	11.1^{a}(2.5)	7.5^{c}(1.1)	10.8^{a}(1.6)
Årdal	5.9^{b}(0.9)	-	7.1^{d}(1.6)	-	7.7^{b}(2.4)	11.5^{a}(3.2)	7.5^{c}(1.7)	10.8^{a}(3.2)

Table 1. Final length (cm, mean, sem) on 26 May 1996 of upper and lower mode juvenile Atlantic salmon from three river strains reared at four different temperature regimes. Different letters within dates indicate statistical differences (ANOVA, SNK, $p<0.05$).

Smolts from all strains at SNT showed a significant increase in branchial Na^{+},K^{+}-ATPase activity until early May, with a subsequent reduction in late May (Table 2). There were significant differences among strains in peak branchial Na^{+},K^{+}-ATPase activity in early May, with the Suldal strain showing the highest overall activity, followed by strains Stryn and Årdal (Årdal significantly different from Suldal).

Strain			
Date	Stryn	Suldal	Årdal
16 March	$3.8^{a}(0.5)$	$4.6^{a}(0.9)$	$2.8^{a}(0.6)$
16 April	$5.2^{a}(0.7)$	$3.8^{a}(0.5)$	$4.6^{a}(0.5)$
10 May	$7.6^{ab}(0.8)$	$9.0^{a}(0.7)$	$5.2^{b}(1.0)$
28 May	$4.9^{a}(0.4)$	$6.9^{a}(0.7)$	$4.1^{a}(0.8)$

Table 2. Branchial Na^{+},K^{+}-ATPase activity (mean, sem) during spring 1996 of upper mode juvenile Atlantic salmon from three river strains reared at SNT. Different letters within dates indicate statistical differences (ANOVA, SNK, $p<0.05$).

The present study demonstrates strain differences in freshwater growth potential of juvenile Atlantic salmon at low and moderate temperatures. Our results are in line with those of Jensen and Johnsen (1986) that growth rate declines continuously with decreasing temperature until a strain-specific lower threshold temperature is reached. Our results suggest that the river Årdal strain is not as well adapted to low temperatures, and consequently can be expected to have a higher temperature optimum for growth. A further consequence of such characteristics is the unimodal size distribution even at 5.5°C, with no 1+ smolts from this strain (Table 1). Our results support the traditional view that salmon strains differ in biological characteristics between rivers, as adaptations to their local river conditions (see Shearer, 1992). River Årdal generally drains a lowland catchment area, with higher summer and winter temperatures, while rivers Stryn and Suldal drain high mountain areas (both rivers) and glaciers (Stryn) with lower overall water temperatures. Temperature profile of SNT simulated the Suldal river, reaching only 6°C in late May, corresponding with spring temperatures in river Stryn (4-5°C in mid-June, own observations).

Branchial Na^{+},K^{+}-ATPase activity was lower in the Årdal strain than in the other two strains at SNT (Table 2), suggesting that the low temperatures during April and May (3.5-6.0°C) delayed or inhibited the expected smolt-related increase in enzyme activity. Since no further increase was observed in late May, it is suggested that the Årdal strain never developed full branchial Na^{+},K^{+}-ATPase activity under this temperature regime.

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EFFECTS OF FASTING

ON GROWTH PERFORMANCE AND SMOLTING

OF BROWN TROUT, *SALMO TRUTTA* L.

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Introduction

Smolting is a process occurring in most salmonid fish species. This complex process is preceeded by a juvenile stream phase and followed by a marine growing phase. The length of the juvenile stream phase, as well as the marine phase, can vary considerably, depending on the species and growth conditions (Groot, 1996). In a sibling population of Atlantic salmon (*Salmo salar*) juveniles it has been observed that under good growing conditions a bimodal size distribution is established during the first autumn (Thorpe, 1977). The upper modal group fish will smolt and emigrate during the following spring and the lower modal group fish stay in the river at least for another year. Whether an individual salmon keeps growing over the autumn and subsequently enters the upper mode of the size distribution, is determined by an individual threshold for growth, which must be exceeded. If that threshold is not exceeded the fish will become anorexic and stop growing (Metcalfe et al. 1992). Consequently, it has been proposed that there should be a sensitive period for the smolting decision in salmon, and that decision is taken at the end of July and the beginning of August (Thorpe, 1989; Metcalfe et al., 1992). It also has been shown in Atlantic salmon that the greater the growth opportunity during that period the greater the proportion of salmon maintaining rather than arresting growth (Thorpe et al., 1989).

In Finland most hatchery reared brown trout (*Salmo trutta*) smolt at the age of 2+. Pirhonen and Forsman (1998) found that even if the growth of brown trout is reduced by decreasing the feeding frequency during their second summer, the proportion of smolting fish could not be affected. However, in that experiment all the fish grew at relatively high rates during August (the expected sensitive period for the smolting decision), possibly influencing the high incidence of smolting in all the groups. In the present experiment we wanted to test whether we could affect the proportion of smolting trout in a hatchery population by decreasing the growth opportunity both during the first and second summer. During the second summer the fish were periodically totally deprived of food. The hypothesis for being tested was that the brown trout would put off smolting if the growth opportunity was decreased during August.

Materials and Methods

The experiment was carried out between 20.6.1995 and 15.10.1996 at the Finnish Game and Fisheries Institute's Laukaa Fisheries Research and Aquaculture (62°27'N, 25°55'E). The fish were one-year old brown trout of the freshwater migrating Rautalampi strain. The experimental fish had experienced four different feeding frequencies during the previous growth period between 28.7.1994 and 2.11.1994. During that period 12 groups of fish were cultivated in black 0.28 m^2 experimental tanks (volume: 0.084 m^3), giving triplicate groups of fish for each feeding frequency treatment. Initial number of trout in each tank was 100 (wet weight 2.5g, dry weight 0.54g). Feeding frequencies were 4 h twice a day (FR1), 4 h every other day (FR2), 30 min twice a day (FR3) and 30 m day^{-1} (FR4). On 2.11.1994 wet weights (treatment means ± s.d., n=3) were, respectively, 12.6±1.00, 9.1±0.44, 12.5±0.25 and 10.3±0.65. There were no statistical differences between FR1 and 3 groups or between FR2 and 4, but FR1 and 3 were significantly bigger than FR2 and 4. The same differences in size still existed on 8 February, 1995, but by 19 June those differences had disappeared and the size range in mean wet weight was 21.4g (FR4) - 23.0g (FR1).

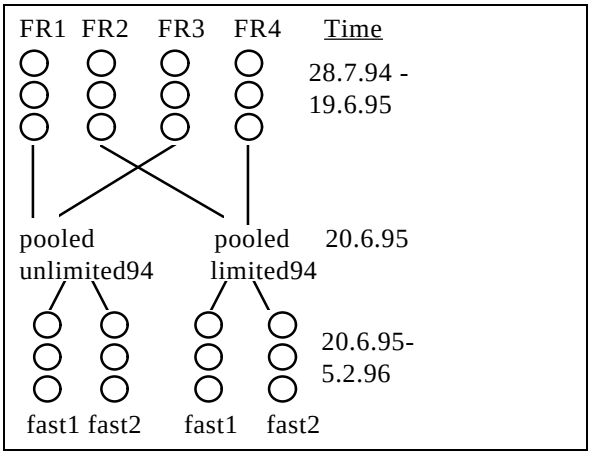

Figure A. Experimental setup during the growth periods.

On 20 June, 1995, the fish from FR1 and FR3 groups were pooled and later on this group is referred to as unlimited94, and also FR2 and FR4 were pooled (limited94). These fishes were divided into 12 green 0,72 m^2 experimental tanks (volume: 0.29 m^3), 6 tanks for each of the 94 treatment groups. At the beginning of the experiment the number of individuals per tank was 55-57. Three tanks from each treatment groups were deprived of food for the following three weeks (designated as fast1) and the other tanks were fasted for three weeks from 9 August (fast2) (Fig 1).

When not starved the fish were fed 4 hours in the morning and 4 hours in the evening a commercial pelleted dry feed by belt feeders. Feeding time was decreased to 3 + 3 h day^{-1} on 26 September and down to 4 h day^{-1} in mid-November.

A certain amount of uneaten food on the tank bottom suggested that the fish were always being fed in excess. Simulated natural photoperiod was used throughout the experiment and the light intensity at the surface of the tank was about 100 lx. Water temperature was recorded daily (not during the weekends) to the nearest 0.1°C. Temperature ranged mainly between 13°C and 15°C from the beginning of July until mid-September but reached a maximum of 16.0°C on 7 August. Temperature fell constantly below 10°C in mid-October. Constant

winter temperature (2.1-2.3°C) was reached in mid-November. Total length and wet weight was measured at two to three week intervals until the beginning of October and then once in November and February. On 5 February 1996, 10 immature individuals and all mature males from each tank were implanted with PIT-tags (Destron/IDI, 400 kHz) behind the dorsal fin just below the skin so that the tag remained parallel to the long axis of the fish. These tagged individuals were used in smolt behaviour studies from the end of March, 1996.

Feed intake was measured 5 times by using the X-radiographic method (Talbot and Higgins, 1983), and a sample of 30-35 fish per tank was X-rayed on each occasion. Diet used for feed intake measurement was prepared from the normal feed by grinding and incorporation of a known quantity of X-ray dense glass balls (ballotini size 9; Jencons Ltd.) followed by compression into pellets and re-drying at 40-45°C. Then the pellets were ground coarsely again and the fraction appropriate for the size of fish was sieved out. Standard curves were then prepared by X-raying known weights of the marked feed and counting the numbers of ballotinis present. Thus, the amount of ballotini in a unit mass of feed could be calculated for later estimation of feed intake by the fish. All feeds were stored at 5°C prior to use. Feed intake measurements were made by providing the marked feed during the morning feeding period, followed immediately by anaesthetisation of fish (MS-222), X-raying (Siemens Nanodor X-ray machine, Agfa Structurix D7 film), weighing (to 0.1 g) and measurement of total length (to 0.1 cm). X-ray plates were then developed and the amounts of feed consumed by the individual fish were estimated from the numbers of ballotinis in the gastrointestinal tract.

4 individuals from each tank (12 per treatment) were taken for proximate body water, fat and protein content after each fasting period and two times thereafter. The water inlet hose into one of the unlimited94 fast1 tanks was unfastened on 13 July and caused the death of 38 fish. After this composition samples were not taken from that tank. Samples were first dried for 24 h at 105°C and then ground into a fine powder. Sub-samples were then taken for the analysis of lipid (Soxhlet method) and protein (as Kjeldahl-N x 6.25). Condition factor was calculated as $100W.L^{-3}$, where L was the total length in cm.

The effect of fasting on smolting was evaluated in terms of movement behaviour with the help of PIT-technique. The monitoring of behaviour was started at the end of March 1996 and was continued until 15 October 1996 in a semi-natural stream, inside a green house to avoid avian and mammalian predation and to shelter the equipment. The stream was built in an earth pond, which was 18 m

long and 5 m wide. The bottom of the stream was covered with 2 - 10 cm diam. rocks. A bright metal wall was mounted in the middle of the stream to divide the tank into two 2.5x18 m sections. One side of the tank was then covered with two 7m long black plastic sheets to offer shelter for the fish. Water was introduced from four points (two on each side, one at the beginning and one in the middle) to the surface through perforated tubes to give unidirectional current to the water body. The water outlet was from the surface in the middle of the stream. One belt feeder on each side, right behind the first water inlet, provided food at 3 h intervals for 15 minutes, throughout the day. At both ends of the stream there was a 20cm diam and 50 cm long acrylic tube mounted in a waterproof acrylic box at right angles to the central wall. Through these tubes the fishes were able to change the side of the tank and therefore to swim around the whole stream. Around one of the tubes there were two PIT-tag detecting antennae sending the data (date, time, tag code and antenna number) of the moving individuals directly to the computer file. This system allowed us to estimate the individual migration activity from the number of antennae passages (see Pirhonen et al., 1998, for details). Water depth was 25 cm at both ends of the flume where the tubes were placed (i.e 5cm above the tube), and at its deepest about 70cm beside the central wall. Water temperature was recorded daily by a digital thermometer, which gave the highest and lowest temperature of the measuring period. A flow meter was mounted in one of the water inlet tubes and the actual flow rate was recorded once a day. The movement data were handled and individual sums were calculated for each day and for each month. The data were also compared within maturity status.

The effect of food restriction during 1994 and fasting in 1995 on maturity was calculated from the numbers of mature males in the cultivation tanks in November 1995. Mature individuals were fin clipped for future recognition. At the end of the experiment in October 1996 all the fishes were killed and sexed. The number of those mature males which were not mature during the previous autumn was compared to the number of immature males.

Statistics were performed using SYSTAT statistical software, with possible differences among treatments being tested using a two-way ANOVA model. Arcsine transformation of the composition data was used before computations. *Post-hoc* comparisons between sample means were tested by Tukey's test. Student's *t*-test was used to compare the movement activity between the maturity groups.

Results

Food intake

Food intake was elevated at least for one month after each fasting period in 1995 (Fig. 2 and Table 1). Food intake decreased to very low levels when the temperature decreased towards winter. Food restriction during the summer 1994 did not affect food intake during the following summer, but on 8 August an occasional interaction between 94 and 95 treatments was observed (Table 1).

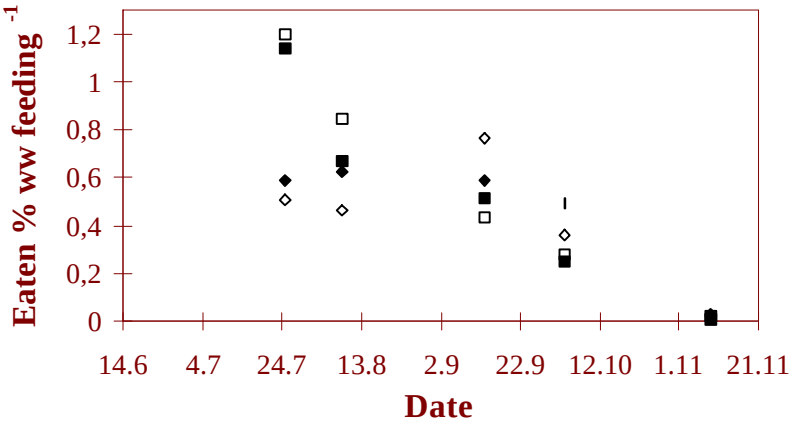

Figure 2. Amount of food eaten (% ww feeding^{-1}) on five occasions during the summer and autumn 1995. Open symbols refer to the limited94 groups and filled symbols to the unlimited94 groups. Squares refer to the groups fasted in June-July 1995 (fast1) and diamonds to the groups fasted in August 1995 (fast2).

Wet weight and condition factor (CF)

During the fasting periods mean wet weight decreased only slightly in all the fasted groups (Fig. 3) but because the fed groups kept growing, significant differences in size were observed at the end of each fasting period. The groups fasted in August caught up the size of the fed groups much faster than the groups fasted in June-July (Fig. 3 and Table 1). At the end of the growing

period in November (1995) there were no significant differences in wet weight between the treatments (Table 1).

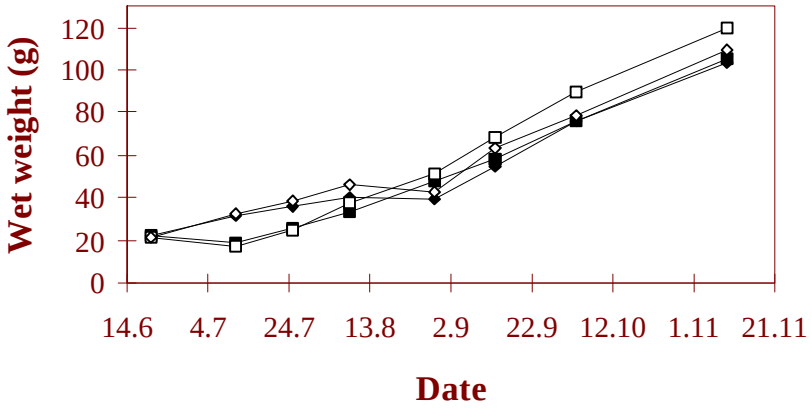

Figure 3. Wet weights of the trout during 1995. Symbols as in Fig. 2.

Fasting affected significantly also the CF by decreasing it by 12 % in unlimited94 fast2 groups and by about 16 % in the other groups. When feeding resumed after fasting CF quickly returned to the level of unfasted groups (Fig. 4). CF tended to be significantly higher in the groups fed restricted rations over the previous summer (Fig. 4 and Table 1).

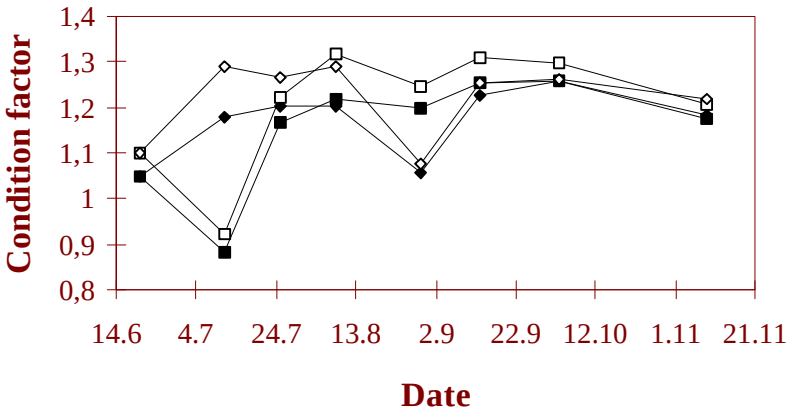

Figure 4. Condition factors of the trout during 1995. Symbols as in Fig. 2.

	11.7.95	25.7.95	8.8.95	29.8.95	13.9.95	3.10.95	9.11.95	15.2.96
Weight								
Pooled SE	1.04	1.63	2.22	1.85	2.56	3.27	4.61	6.14
94	NS	NS	<0.05	NS	<0.05	NS	NS	<0.05
95	<0.001	<0.01	<0.01	<0.05	NS	NS	NS	NS
94*95	NS	NS	NS	NS	NS	NS	NS	NS
CF								
Pooled SE	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01
94	<0.01	<0.01	<0.001	NS	<0.05	NS	<0.05	<0.05
95	<0.001	<.0.05	NS	<0.001	<0.05	NS	NS	NS
94*95	<0.05	NS	NS	NS	NS	NS	NS	NS
Eaten % ww								
Pooled SE		0.08	0.06		0.05	0.03	0.01	
94		NS	NS		NS	NS	NS	
95		<0.001	<0.01		<0.05	<0.05	NS	
94*95		NS	<0.05		NS	NS	NS	

Table 1. Statistical significances of food restriction during 1994 or fasting during 1995 and their interaction on total length, wet weight, condition factor (CF) or amount or food eaten (% ww) on eight occasions in

brown trout. Food intake was not measured on three of these occasions.

Body composition

The effect of fasting on the body composition of trout was most pronounced after the first fasting period, when protein and moisture contents were higher and fat content lower in fasted than in fed groups (Table 2). Fasting for three weeks in August did not affect fat content, but protein content of fasted groups was significantly higher than in groups fed during August. After the June-July fasting period the limited94 groups had a higher fat content and lower protein content than the unlimited94 groups (Table 2). Otherwise no differences were observed in body composition, except once in protein content in February 1996.

	11.07.95			29.08.95			10.11.95			15.02.96		
	prot	fat	moist	prot	fat	moist	prot	fat	moist	prot	fat	moist
L94 fast1	61.3	27.1	78.7	56.1	32.6	72.8	53.9	36.0	70.5	54.6	37.5	70.2
L94 fast2	57.9	31.4	74.4	58.7	31.8	72.7	55.1	35.1	71.0	54.7	37.0	70.7
U94 fast1	65.5	22.8	77.9	57.1	31.6	72.8	54.7	35.9	69.9	57.1	34.1	71.1
U94 fast2	61.6	27.8	75.0	60.4	29.3	73.8	54.6	34.4	70.6	56.5	35.2	71.5
pooled SE	1.0	1.4	0.4	0.6	0.7	0.9	0.5	1.0	0.9	0.6	0.8	0.2
94	<.01	<.01	NS	NS	NS	NS	NS	NS	NS	<.05	NS	NS
95	<.01	<.01	<.001	<.01	NS	NS	NS	NS	NS	NS	NS	NS
94 * 95	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 2. Mean percentages of fat and protein (of dry weight) and moisture of brown trout on four occasions in 1995 - 1996 and the statistical significances of 94 and 95 treatments, and their interaction. L94=limited94, U94=unlimited94, fast1=fasted in June-July 1995, fast2=fasted in August 1995.

Sexual maturation

In November 1995 the proportion of mature males of all the individuals was higher in the groups fasted in August than in June-July. In both fasting groups

that proportion was also higher in the groups fed unlimited rations in 1994. There was no clear indication of the effects of previous food restriction or fasting on sexual maturation of previously immature males after the third growing period, when the fishes were not handled (Table 3).

Table 3. The proportions (%) of mature males in November 1995 or in October 1996. The values for Nov. 1995 were calculated from all the individuals and values for Oct. 1996 were calculated from males, which were immature in November 1995. Fast1= fasted in June - July 1995, Fast2 = fasted in August 1995.

		Fast1	Fast2
November 1995	Limited 94	1.4	5.0
	Unlimited 94	3.2	5.4
October 1996	Limited 94	36.4	50.0
	Unlimited 94	38.5	25.0

Smolting

The general pattern of movement behaviour was very similar in all the groups (Fig. 5). The activity increased significantly at the beginning of May, peaked in mid May and finally almost ceased at the end of May. Over the rest of the summer the movement activity was relatively low. At the beginning of October a new rise in the activity was observed, which was not as pronounced as in May (Fig. 5). The autumnal activity was caused by mature males and was directed mainly against the current while the vernal direction of movement was with the current.

There were no differences in the movement behaviour between the treatments, neither in daily nor in monthly sums of antennae passages. The only difference which was observed was in October 1996: at that time immature fish from the unlimited94 groups accounted for significantly higher number of passages (individual mean sum was 6.1 passages) than the immature fish from the limited94 groups (2.2). In comparison, mature males accounted for 24.1 and 57.7 passages respectively during October. Total fasting in 1995 had no effect

on the number of antennae passages irrespective of the maturity status in the autumn 1996.

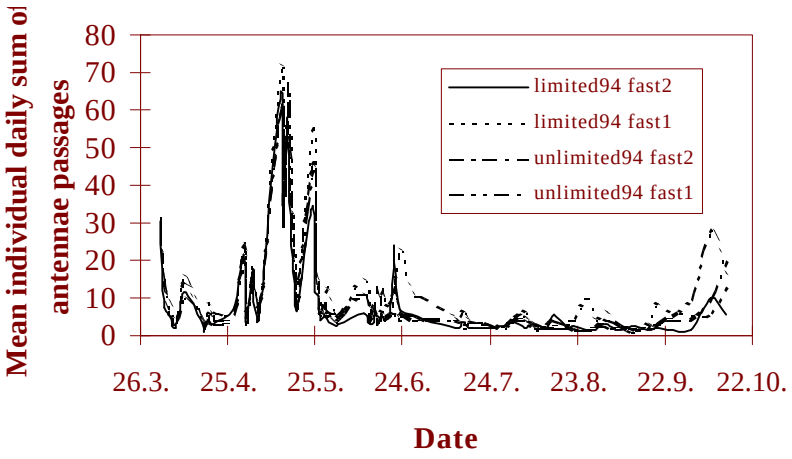

Figure 5. Movement behaviour of brown trout in a semi-natural stream in 1996 expressed as mean individual sum of antennae passages, counted from the individuals which have moved on the specific day.

When looking at the data by maturity status, we observed that mature males moved significantly less during May than did immature fishes (both males and females), when counted from the total monthly number of antennae passages. During other months no differences were observed, except in October when mature males moved significantly more than immature fishes. 18 individuals were already mature in 1994, and all of them developed milt during the following autumn also. The movement behaviour of those individuals was compared to the behaviour of fishes maturing for the first time in 1995 (19 individuals). However, no differences were observed when individual sums of monthly activities were compared.

Discussion

The trout which were fed restricted rations during their first summer (1994) were able to compensate for the decreased growth but not before the spring 1995. This result confirms that compensation hardly occurs at low temperature (Pirhonen and Forsman, 1998). Dobson and Holmes (1984) reported that compensatory growth occurs throughout the year in rainbow trout (*Oncorhynchus mykiss*) even though the compensation was relatively small at temperatures below 9°C. Our trout were able to compensate for the loss of weight by increasing food intake significantly after each fasting period in 1995 over the month following food deprivation. Quinton and Blake (1990) have shown that rainbow trout are usually able to compensate the lost weight gain of a three week food deprivation during the following three week period under liberal feeding conditions. When considering both the condition factor and the food intake it seems that the greatest compensation occurs during the first two weeks after re-feeding.

During the first summer the fish fed for four hours twice a day did not differ in size from the trout fed for only 30 minutes twice per day. This result can be explained by the fact that small fish can be satiated in a relatively short time. Elliott (1975) showed that the time needed to satiate brown trout increased with fish size and temperature, and a 10 g trout at 15°C needed less than 10 minutes to be satiated. Results of Pirhonen and Forsman (1998) suggest that feeding for 30 min day^{-1} is not enough for a 50 g trout if fed only once a day.

We don't know why the fish fed restricted rations during 1994 tended to constantly have higher condition factors than their unrestricted counterparts. It might be that the fish were "prepared" for another period of undernutrition by accumulating body energy reserves. However, differences in body composition do not necessarily support that idea because body fat content was significantly higher in limited94 groups during 1995 only in July. On the other hand, condition factor does not necessarily reflect the energy reserves because condition factor can not predict muscle lipid content (Johansen and Jobling, 1998).

It seems likely that the fasting period during August was not proportionally as severe for the trout as the fasting period in June-July. Mean decrease in condition factor in August was somewhat smaller than in June-July, and no significant difference in body fat content was observed in August while in June-

July there were differences in fat, protein and moisture contents between fed and fasted groups. Therefore it is possible that the effect of duration of fasting depends on fish size, and caution should be taken in comparing and drawing conclusions between experiments with different sizes of fish.

The proportion of mature males was very low in all the groups in November 1995, but the incidence of maturation was somewhat lower in the groups fasted in June-July. In Atlantic salmon it has been shown that feed restriction during early spring can suppress sexual maturation in a certain proportion of the population (Rowe and Thorpe, 1990), but similar suppression was not observed in Arctic charr (Jobling et al., 1993). The present result suggests that it could be possible to decrease the maturation in brown trout by feed restriction during early summer.

On the basis of the present experiment it seems likely that the decision for smolting in brown trout should be taken at a different time than in salmon, because the decrease in growth opportunity did not affect the smolt migration in any significant manner. Pirhonen and Forsman (1998) have shown that restricted feeding over the summer and autumn did not affect smolting of trout during the following spring. The present result confirms that even negative growth, because of total food deprivation for three weeks during summer months, does not affect smolting of trout. The fact that mature and maturing males moved significantly less than immature fishes is in accordance with the knowledge that maturation suppresses smolting of brown trout (Dellefors and Faremo, 1988; Pirhonen et al., 1998) and salmon (Fängstam et al., 1993; Thorpe, 1994). The present result that also early sexual maturation prevents smolting in trout is accordance with the observations of Dellefors and Faremo (1988).

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ENVIRONMENTAL CONTROL OF SMOLT (*SALMO SALAR*)

DOWNSTREAM MIGRATORY BEHAVIOR:

A DIRECT EVALUATION

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EXTENDED ABSTRACT ONLY DO NOT CITE

There is little direct evidence concerning environmental control of downstream migratory behavior of salmonids. Environmental factors regulate physiological changes, but do the same factors also regulate behavioral changes? The effect of some environmental factors on the physiology of Atlantic salmon has been studied in the laboratory, and correlates of migratory behavior have been examined in the field. Photoperiod seems to be the primary regulator of physiological changes associated with smolting. Although increasing mean daily temperature can advance smolting it is not necessary for the completion of smolting. Increasing photoperiod and temperature probably act together to advance smolting and temperature could direct smolting in the absence of photoperiod cues. Behavioral studies of salmon smolts have not included examination of the effect of various environmental conditions on individual behavior during downstream migration. Most previous studies have used indirect measures to examine downstream migratory behavior. This study evaluated the effects of temperature on the behavior of Atlantic salmon parr and smolts in the laboratory.

Based on previously established methods for examining the physiology of smolting we have developed a protocol for examining the influence of environmental factors on behavior of smolts. In the laboratory we continuously monitored downstream migratory behavior from 12 April - 24 July 1997. Temperature conditions were manipulated in each of eight tanks to examine the influence of varying temperature conditions on migration. Blood samples and gill biopsies were taken prior to and following experimentation to relate behavioral and physiological changes. Experimental conditions proved satisfactory for examining behavior of salmon parr (non-migratory salmon) and smolts (migratory salmon).

Changes in daily activity rhythms were evident and downstream movements of parr were two-orders of magnitude lower than of smolts. An advanced temperature regime (1^{o}C increase every 3 d) induced increased activity earlier in the season than the ambient (control, 1^{o}C increase every 4 d) temperature regime. A delayed regime (1^{o}C increase every 9 d) delayed behavioral and physiological activity relative to the ambient regime. The number of downstream passages of smolts peaked on 9 May, 18 May, and 7 May in the advanced, ambient, and delayed treatments, respectively. Fish maintained under ambient and advanced conditions ceased activity by mid-June. Fish exposed to the delayed temperature regime sustained downstream movements through July. Through the migratory season, gill Na^{+}, K^{+} -ATPase activity (an index of osmoregulatory physiology) decreased in smolts maintained under ambient and advanced regimes, whereas it remained elevated in the delayed group. In April enzyme activity levels averaged 3.0 ± 0.3, 3.9 ± 0.4, and 2.8 ± 0.3 Tmol $ADP{+}mg^{-1}$ $prot{+}h^{-1}$ for the advanced, ambient, and delayed groups, respectively. Whereas in July, levels averaged 1.7 ± 0.2, 1.6 ± 0.1, and 4.1 ± 0.4 Tmol $ADP{+}mg^{-1}$ $prot{+}h^{-1}$, respectively. Changes in osmoregulatory physiology associated with the parr-smolt transformation were coincident with changes in migratory behavior.

Field analyses of downstream migratory behavior were initiated in the spring of 1997. A preliminary test of an antennae monitoring system was conducted on Mill Brook, Hawley, MA. Seventeen hatchery and sixteen wild smolts were tagged and released approximately 0.5 mi above the antennae on 22 May. Twenty smolts were read by the antennae system. Hatchery smolts migrated earlier (24 May) than wild smolts (27 May). In the fall of 1997, 80 parr were tagged and released above the Mill Brook antennae to monitor winter activity of parr as well as downstream migration in the spring. In April 1998 a fish trap was constructed in Mill Brook downstream of the PIT antennae system to capture

those fish which successfully overwintered and smolted in Mill Brook. Also, in the spring of 1998 wild and hatchery fish will be exposed to two temperature treatments in the laboratory and released into Mill Brook to monitor differences in migratory behavior in the field.

Temperature regimes can strongly influence smolt physiology and behavior and are likely to affect migratory timing and success. Naturally fluctuating water temperatures and the resulting affect on successful migration must be considered when contemplating smolt releases in salmon restoration programs.

PREDATION ON ATLANTIC SALMON SMOLTS

IN NEW ENGLAND RIVERS

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Abstract

Restoration of extirpated populations of Atlantic salmon (*Salmo salar*) in New England has been a slow process with mixed results. Survival between the smolt stage and adult return has been extremely low in southern New England rivers and only somewhat higher in Maine waters. One source of mortality is riverine predation on migrating smolts by fishes and birds. Recent predation studies in New England provide evidence of significant mortality in the smolt stage, prior to reaching the ocean. Known predators of Atlantic salmon smolts in rivers of New England and eastern Canada include at least 6 species of birds and as many as 11 species of freshwater fishes. The results of predation studies in New England are reviewed with particular emphasis on recent studies in Maine. Field studies confirm bioenergetics model predictions that chain pickerel (*Esox niger*) are potentially major predators during the early portions of smolt runs, when water temperatures are lower. Overall, smallmouth bass (*Micropterus dolomieu*) may not be significant predators, except during warmer water temperatures or below artificial structures where smolts are especially vulnerable. Predation of smolts by double-crested cormorants (*Phalacrocorax auritus*) along the Penobscot River was highest near dams; an average of 7.3% of hatchery smolts may be consumed by cormorants each year in the River. A

recent study is discussed that may result in reduced predation on stocked fry and smolts. This involves reinforcing the anti-avian predator response while in the hatchery. Fish experiencing regular startle responses and accessibility to, at least, limited types of cover exhibited greater predator-avoidance behavior once stocked in a stream.

Introduction

It has long been known that smolts of Atlantic salmon (*Salmo salar*) suffer mortality from several sources during their downstream migration in the spring. In addition to direct and delayed mortality associated with dams and other human-built structures (Stier and Kynard 1986), smolts also are prey for aquatic birds, freshwater fishes, and--when they enter tidally-influenced areas at the river mouth--marine mammals and species of marine fishes.

Such predator-prey relationships have been documented in New England and eastern Canada rivers for decades (e.g., White, 1939; Elson, 1962, 1975). Anthony (1994) listed over 50 types of predators of Atlantic salmon at different life stages throughout its world range. But, in recent years, researchers have begun to estimate the extent of such predation. These recent studies suggest that predation during out-migration of smolts may be high and reflective, not only of natural biological processes, but of the effects of human activities that concentrate smolts above and below dams, where they are more easily captured by predators. The objective of this discussion is to summarize studies of predation on Atlantic salmon smolts in New England and elaborate on some recent research that attempts to define the scope of such mortality..

Sources of Mortality in Atlantic Salmon Smolts

Double-crested Cormorants and Other Aquatic Birds

Aquatic birds are the most visible type of predator on smolts. Birds can be counted, their behavior observed, and their prey analyzed. For the latter, regurgitation of food brought to young birds by adults can be collected (Blackwell et al., 1995) or adults can be lethally collected along rivers and their stomachs removed intact (Blackwell et al., 1997). As a result, although predation by freshwater fishes is often out of view of researchers, the feeding activities of aquatic birds have received more attention.

Several species of birds have been documented as predators of Atlantic salmon smolts in rivers of eastern Canada (Table 1), and even more in Europe. But, in New England, the most effective avian predator appears to be the double-crested cormorant (*Phalacrocorax auritus*). Cormorants have long been known to prey upon Atlantic salmon smolts. However, populations in New England only began to multiply once offshore islands were vacated by human settlers and birds were placed under Federal protection (Krohn et al., 1995). The rapidly increasing populations of double-crested cormorants in New England eventually coincided with increasing efforts to restore extirpated populations of Atlantic salmon. For over 30 years, this salmon restoration has largely been facilitated by stocking (Moring et al., 1995).

To address this increasing conflict between cormorants and fisheries, several status and synopsis reports were issued at regular intervals (Meister and Gramlich, 1967; Cormorant Study Committee, 1982; Dube and Godin, 1987). This led to a recent study by Blackwell (1996) that examined double-crested cormorants on the Penobscot River, Maine, and derived estimates of predation on smolts. Blackwell found that cormorants are predators of opportunity, utilizing species of abundance, then easily shifting to new species of abundance--those that can be captured in quantity. Birds foraging in the river above tidal influence consumed 11 animal species, while those foraging below tidal influence consumed 27 freshwater species as well as seasonally-available marine species.

Salmon were not found in stomachs of double-crested cormorants in the lower Penobscot River in April, were rare in the first week in June, and were absent thereafter. But in May, Atlantic salmon smolts were among the five highest ranking prey taxa in each of three river sections.

Table 1. Known predators of Atlantic salmon fry, parr, and smolts in rivers of eastern Canada and New England, USA.

Common name	**Scientific name**	**Reference**
Double-crested cormorant	*Phalacrocorax auritus*	White (1937)
-		White (1939)
-		Meister and Gramlich (1967)
-		Cormorant Study Committee (1982)
-		Dube and Godin (1987)
-		Blackwell et al. (1995)
-		Blackwell (1996)
-		Blackwell and Krohn (1997)
-		Blackwell et al. (1997)
Common merganser	*Mergus merganser*	White (1937)
-		White (1939)
-		Elson (1962)
-		Elson (1975)
-		Anderson (1986)
Common murre	*Uria aalge*	Montevecchi et al. (1988)
Northern gannet	*Sula bassanus*	Montevecchi et al. (1988)
Osprey	*Pandion haliaetus*	Blair (1956)
Belted kingfisher	*Megaceryle alcyon*	White (1937)
-		Elson (1962)
Chain pickerel	*Esox niger*	Cooper (1941)
-		Barr (1962)
-		Warner et al. (1968)
-		van den Ende (1993)
Northern pike	*Esox lucius*	McCrimmon (1954)
Striped bass	*Morone saxatilis*	Schulze (1996)
American eel	*Anguilla rostrata*	Elson (1941)
-		Godfrey (1957)
Largemouth bass[a]	*Micropterus salmoides*	Warner (1972)
Smallmouth bass	*Micropterus dolomieu*	Warner (1972)
-		Fay (pers. comm.)
Yellow perch[a]	*Perca flavescens*	Warner at al. (1968)
Burbot[a]	*Lota lota*	Warner et al. (1968)
Brook trout	*Salvelinus fontinalis*	McCrimmon (1954)
Creek chub	*Semotilus atromaculatus*	McCrimmon (1954)
Common shiner[b]	*Luxilus cornutus*	McCrimmon (1954)

[a]Predator on landlocked Atlantic salmon.

[b]Predator in laboratory experiments.

Note: other species, such as brown trout, have been documented as predators on Atlantic salmon in European waters.

Assuming smolts were all of hatchery origin (wild fish are but a small portion of smolt numbers), Blackwell estimated that double-crested cormorants consumed an average of 7.3% (+/- 1.2%) of the total number of salmon smolts stocked each year in the Nation's largest Atlantic salmon river. Most predation was associated with dams along the river, presumably where smolts were concentrated in surface waters of forebays, or below dams, where smolts were disoriented from passing over spillways or through turbines (Blackwell, 1996; Blackwell and Krohn, 1997).

Marine Mammals

Considerable subjective opinion has suggested that predation on smolts by marine mammals has been increasing in the past two decades. Actual information supporting such conclusions is scarce and, obviously, is difficult to measure when dealing with Federally-protected animals. Some of this predation may be a consequence of osmotic stress experienced by smolts as they enter saline environments (Järvi, 1989), but this has not been tested in New England waters. Similarly, most of the documented studies of mammal predation on Atlantic salmon smolts have been conducted in Europe (Svärdson, 1955, 1957; Anthony, 1994) or have dealt with predation on adult salmon.

However, some studies in North America have identified marine mammal predators. Gray seals (*Halichoerus grypus*), for example, are known predators of maturing salmon held in coastal aquaculture pens, and numbers of marine mammals have been expanding rapidly. The population of gray seals along Sable Island, in the Gulf of St. Lawrence, increased 13% annually between 1962 and 1990 (Anthony, 1994). Coastal populations in Maine also increased 13% annually during the past two decades. Harbor seal (*Phoca vitulina*) populations doubled in Massachusetts between 1972 and 1989 and more than doubled along the Maine coast between 1986 and 1993 (Kenney and Gilbert, 1994).
Despite the outcry from anglers and other casual observers, there is no conclusive evidence that the diet of marine mammals along the New England coast includes Atlantic salmon--of any stage--as a significant component (Benoit and Bowers, 1990; Anthony, 1994).

Freshwater Fishes

Numerous species of freshwater fishes have been shown to prey upon Atlantic salmon, either as fry, parr, or smolts (Table 1). However, documenting the extent of such predation in a large river system is a difficult task that has been attempted only recently (van den Ende, 1993; Schultze, 1996). Logistically, field sampling is extremely difficult--even dangerous--in the spring in large New England rivers. However, Spicer et al. (1995) were able to track the downstream migratory patterns of Atlantic salmon smolts in the Penobscot River during this season. Individual routes and periods of cessation by radio-tagged smolts suggested that movements were not continuous and that predation by freshwater fishes may be more than incidental.

While smallmouth bass (*Micropterus dolomieu*) were observed gorging themselves on smolts exiting a self-release pond near Enfield, Maine (C. Fay, Penobscot Indian Nation, Old Town, ME, unpublished data), van den Ende (1993) was unable to document such feeding in the main stem of the Penobscot River. He concluded that low metabolic requirements, along with reduced water temperatures, kept smallmouth bass relatively inactive during the initial part of the smolt migration.

Chain pickerel (*Esox niger*), however, were quite active during the entire smolt migration period, and predation by chain pickerel was significant. Although assumptions had to be broad because of limited data used for predictive models, van den Ende (1993) was able to make preliminary estimates of smolt losses from chain pickerel. Even with relatively wide confidence limits, he concluded that this species may be a significant predator on smolts. Consumption in the lower Penobscot River could average 276 - 646 smolts each day, depending on water temperature. Highest consumption rates were at water temperatures of 10^{o} C (based on laboratory feeding experiments and application of a bioenergetics model), a point where smallmouth bass were beginning to become active as well.

Warner and Kynard (1986) provided evidence that young striped bass congregate below dams, preying on Atlantic salmon smolts, especially those killed or disoriented by passage over spillways or through turbines. More recently, Schulze (1996) used predictive models to estimate the extent of such predation by striped bass. Most spawning populations of striped bass were extirpated decades ago from New England waters, and numbers of fish born

elsewhere that move into New England waters in summer were extremely depressed in the 1980s and early 1990s. In recent years, however, those populations have rebounded and there has been success at reintroducing spawning striped bass to some waters in New England. The management concern is that such success with striped bass comes into direct conflict with efforts at restoring Atlantic salmon in the United States as well as establishing sustainable populations of brown trout (*Salmo trutta*) in lower portions of coastal rivers.

Predator Avoidance

Hatchery fish typically suffer higher mortality from bird predators than do wild fish (Fenderson and Carpenter, 1971; Sosiak, 1978; Kennedy and Greer, 1988). As a consequence, Knudsen et al. (1992) and Hockett (1994) theorized that any anti-predator avoidance response inherent in young salmon is lost in the hatchery environment. Hockett's hypothesis was that any startle reaction during hatchery rearing can not be completed with a move to cover--cover generally does not exist in hatchery raceways. Since a startle response never results in predation (or any detrimental consequence), young salmon eventually lose the ability to recognize threats and avoid predators, which can be fatal when salmon eventually are stocked in a river.

Hockett (1994) designed a 3 X 2 experiment that tested the reactions of "trained" and "untrained" Atlantic salmon parr to disturbance by a model of a kingfisher. Using two levels of habitat complexity and three levels of disturbance (none, human disturbance, bird model disturbance), parr were maintained for 47 days, then tested and videotaped in a large observation chamber.

Fish accustomed to human disturbance showed declining use of cover when startled, while fish trained by regular disturbance from a bird model showed significantly greater use of cover when startled by the overhead presence of a bird. Salmon accustomed to the availability of cover (PVC pipes or black-colored substrate) quickly moved to such cover when disturbed, while fish not experienced with cover did not ($P<0.001$). Hockett's experiments suggest that the anti-avian response may be retained in hatchery-cultured Atlantic salmon if the fish are regularly exposed to simulated "disturbance" by a bird model and have some type of habitat complexity available as cover. This may be a feasible culture technique that could increase survival after fish are stocked.

Summary

It is becoming increasingly apparent that mortality during the riverine phase of smolt migration may be a major influence on low return rates of adult Atlantic salmon. Predation by birds, fish and, to a lesser extent, marine mammals may seem little more than natural selection in a biological community. However, this is not necessarily the case. It is natural selection, but in an altered ecosystem. Smallmouth bass, for example, are not native to New England. They were introduced about the time of the Civil War, coinciding with the period of rapid exploitation of Atlantic salmon, extensive pollution in major rivers, and the construction of numerous dams. Chain pickerel, although native to southern New England and southern Maine, exist in most of the salmon rivers of today as a result of widespread distribution by humans in the twentieth century. These esocids--apparently a significant predator of Atlantic salmon smolts in the Penobscot River, never co-existed with the salmonid species when runs were abundant and wild.

In addition, populations of double-crested cormorants have increased almost exponentially in many areas of the Northeast. The species now has established breeding colonies in areas never utilized decades ago. Their feeding on smolts can be considered a natural event. Yet, the species is Federally-protected, with numbers of breeding pairs increasing well into the 1980s. The number of suitable nesting sites on islands along the coast now appears to be fully utilized in some areas of New England, and the natural feeding on smolts in rivers is concentrated today at sites where dams are present--another human influence that was never a factor prior to the eighteenth and nineteenth centuries.
Future management of Atlantic salmon must have, as a major objective, the examination of the smolt migration period as a principal component in rehabilitation and restoration of Atlantic salmon in rivers of New England.

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SMOLTIFICATION, DISEASE

AND SALTWATER-ENTRY BEHAVIOR

OF JUVENILE CHINOOK SALMON

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EXTENDED ABSTRACT ONLY DO NOT CITE

Saltwater challenge and preference experiments were conducted from 14 February to 30 March 1997 on four groups of juvenile spring chinook as part of a larger study investigating the interactions of smoltification, growth and behavior of fish raised in commercial net pens in Young's Bay, OR. On each date fish were transported to Oregon State University's Oak Creek Laboratory and assigned to either saltwater challenge or preference tanks. Fish were given either a 24 hour saltwater challenge or served as a control group.

In saltwater preference experiments, fish were placed into each of two windowed tanks. A saltwater gradient was introduced from the bottom over the course of one hour and fish position was recorded periodically. No fish died in the challenge experiments.

The preference experiments found that on the first three dates, more than 50% of the fish were holding in saltwater within 35 minutes of introduction, and 75-100% preferred saltwater after 24 hours. On the last date, 50% saltwater preference occurred only after 24 hours, and only 64% of the fish showed saltwater preference. Morphological and physiological indices suggested that the fish were more smolted throughout the experimental timecourse.

Additionally, by the last sampling date, fish were highly infected with bacterial kidney disease.

These results indicate that while juvenile chinook may appear to be smolted and able to tolerate and enter salt water, other factors may influence their willingness to do so.

MIGRATORY BEHAVIOUR OF JUVENILE SALMONIDS AT SIMPLE AND COMPLEX DAMS: THE POSSIBLE APPLICATION OF THE MAMMALIAN MODEL FOR LOCOMOTOR BEHAVIOUR DURING STRESS

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EXTENDED ABSTRACT ONLY DO NOT CITE

Juvenile salmonids in the Columbia River encounter a series of dams during their downstream migration. During passage through the dams a significant stress response occurs. Since 1992 we have studied the behaviour of radiotagged fish released at 3 dams (Bonneville, The Dalles, and John Day). The migration routes taken by juveniles below the dam generally follow the thalwag of the river. Migration rates were variable and were influenced by the hydrology and topography of the river channel as well as flow. The three dams studied represent a range of simple (John Day) to complex (Bonneville and The Dalles) hydrology and channel geomorphology. Below John Day fish have little opportunity to affect the rate or route of their migration. At The Dalles however, the complex hydrology and bottom topography strongly influenced migration rates and routes.

In mammals and amphibians administration of corticotropin releasing hormone CRH, has effects on numerous behaviours including locomotor activity. Rats tested in novel, and presumably stressful environments animals show a significant decrease in activity. In contrast in familiar environments locomotor

activity increase. Both these trends can be reversed by administration of a CRH antagonist.

We suggest that stress, possibly acting via elevated levels of CRH, due to passage through the dams, may be acting to modify migratory behaviour in juvenile salmonids, in situations where the fish can choose, or has the capability to hold. We have some field observations that tend to support this idea and laboratory investigations are being conducted to determine if the mammalian model can be applied to teleosts.

THE RIVERINE, ESTUARINE AND COASTAL MIGRATORY BEHAVIOUR OF WILD ATLANTIC SALMON SMOLTS

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Introduction

Atlantic salmon *Salmo salar* (L.) smolts move seaward through river estuaries and into coastal waters during their spring migration. The estuarine and coastal phases of the smolt migration are considered to be periods of highest mortality, during which time the smolts encounter increasing salinities, variable tidal currents and novel prey and predators. The timing of this migration has been suggested to be important to the survival of the smolts within the marine environment and their subsequent return as adult fish (Hansen and Jonsson, 1989). This abstract describes the results of studies in England and Wales on the migratory behaviour of wild Atlantic smolts during their emigration from freshwater to the marine environment.

Materials and Methods

Smolts were trapped at night in freshwater as they migrated seawards and implanted with miniature 300 kHz acoustic transmitters (Moore *et al.*, 1990). The movement of the smolts within the river and estuary were monitored using an array of 300 kHz acoustic signal relay buoys (Moore *et al.*, 1995). Migration in the open sea was studied by actively tracking individual fish from a small research vessel immediately they left the estuary mouth.

Results and Discussion

Migration in the nocturnal although there was a seasonal change in this pattern of movement. Nocturnal migration early in the smolt run switched to movement during both freshwater sections of all four rivers was predominantly day and night as the season progressed (Figure 1). This change in the migratory pattern of behaviour was reflected in a significant seasonal change in the residency time of the smolts, with fish tagged later in the season spending less time in freshwater before migrating into coastal waters. As a consequence, a significant proportion of the tagged smolts migrated out of the estuaries and into coastal waters over a brief 5-10 day period. This period may represent the optimal time or window of opportunity for smolts to migrate from the freshwater and into the marine environment. The nocturnal pattern of migration would appear to be the result of an endogenous rhythm of swimming activity which results in fish moving into the upper water column after dusk and migrating downstream. This hypothesis is supported by a laboratory based behaviour study which suggested that the light dark cycle may act as a *zeitgeber* (Moore *et al.* 1995). The nocturnal pattern of migration which was not evident in later-running smolts suggests a modification to the clock component controlling swimming behaviour.

Departure from the predominantly nocturnal pattern of migration in later-run smolts may be related to ensuring that they reach coastal waters at the optimal time.

The movement of smolts through the estuaries was indicative of a nocturnal selective ebb tide transport pattern of migration. All the smolts migrated seawards on an ebb tide close to the surface and within the fastest moving section of the water column. Smolt migration in the lower portion of the estuaries was indicative of active directed swimming with the fish moving at speeds in excess of the current and often against the direction of flow. The significant change in the smolts behaviour from moving passively with the current to actively swimming seawards could have been initiated when the fish encountered a particular salinity threshold. There was no apparent period of acclimation required when moving from fresh to saltwater, and a physiological requirement to move to a saline environment may therefore be an important cue in initiating smolt migration.

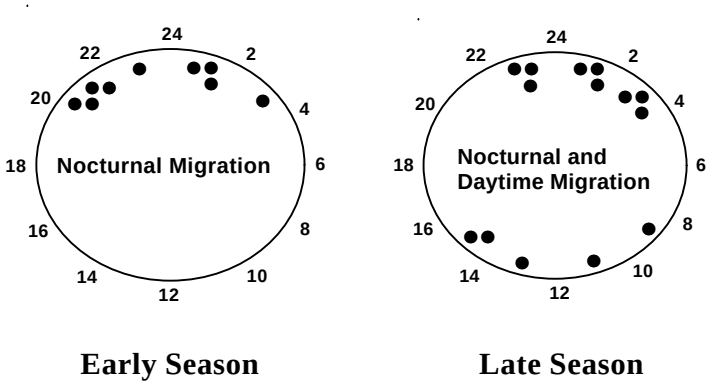

Figure 1. The seasonal migration of Atlantic salmon smolts in the River Conwy in relation to the time of day. ● indicates the movement of a single smolt detected by a sonar buoy situated in the freshwater section of the

Migration within coastal waters occurred during both day and night. Fish migrated close to the surface, and there was a strong tidal component to the speed and direction of movement (Figure 2). As a result smolts migrated rapidly during the initial stages of their marine life cycle.

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River Conwy.

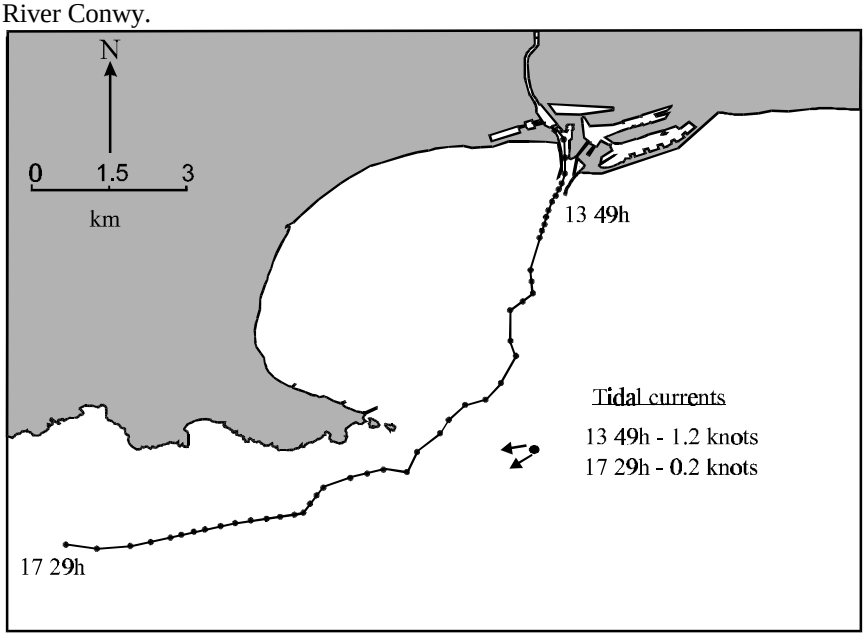

Figure 2. Track of a salmon smolt within the coastal zone. The smolt (145mm in length) had previously been trapped and tagged in the freshwater section of the River Tawe. The speed over the ground of the smolt during the track was 57 cm s^{-1}. ●----● indicate a 2 minute period.

SMOLT MIGRATION IN WILD ATLANTIC SALMON:

EFFECTS OF EARLY SEXUAL MATURATION, TAGGING AREAS

AND AMBIENT TEMPERATURE ON RUN-TIMING

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EXTENDED ABSTRACT ONLY DO NOT CITE

During the years 1995–1997 the smolt migration of Atlantic salmon were studied in a small river on the southwest coast of Iceland. The river is 18 km long from its origin to the sea and drains a lake 10.4 km from the estuary. In autumn during the years 94, 95 and 96 salmon parr of sizes greater than 6.0 cm in fork length were captured by electrofishing on four different locations in the river. They were all tagged with Passive Integrated Transponder (PIT) tags. In total 3354 immature parr (both sexes) and 1180 early mature male parr were tagged. During spring in all years 1995 - 1997, approximately eight months after tagging, a smolt trap was operated upriver about 600 meter from the estuary. An automatic PIT-tag monitor system was placed in the trap to enable registration of the PIT-tagged fish when they passed the monitoring unit on their downstream swimming. For each tagged fish were the individual PIT-tag code, date and time of entry noted and stored as computer files. All fish entering the trap was also measured on length and weight.

In total 10561 salmon smolts were trapped during the three-year study period. 5.7 % (n = 601) of the total number of smolts trapped had previously been PIT-tagged. The proportion of tagged smolts varied from 3.8 % in 1995 (n = 99), 6.7

% in 1996 (n = 332) to 5.7 % in 1997 (n = 170). Of the total number of tagged smolts entering the trap 505 and 96 individuals had been classified as immature and mature parr, respectively, in the autumn at tagging. The smolt run was dominated by two year classes, 2-and 3-summer-old fish. Only few 4-summer-old smolt entered the trap.

There was a large difference in mean length of parr from different tagging areas in the river. Highest mean length of each year classes was observed from areas in the lower part of the river (about 3.5 km from the estuary) and fish from area highest up in the river (about 9.5 km from the estuary) had the lowest mean length in each year class. This difference in fork length at tagging was also observed in the smolt run, eight months from tagging, where the largest mean length in both 2^+ and 3^+ age-classes were observed in smolts originated from the lower tagging site in the river. Areas located lower in the river had also a higher proportion (64.3 %) of two-summer-old smolts than areas located high in the river (11.6 %).

Smolts from the lower areas entered the trap earlier in the spring than smolts originating from upstream areas in the system. Fish entering the trap early in the smolt run were larger in size at time of tagging eight months before the smoltrun. This was true both for immature parr and previously mature male parr.

We thank our colleagues at the Institute of Freshwater Fisheries on Iceland for valuable discussions and help during the fieldwork. Grants from the Swedish Agricultural and Forestry Research council (SJFR) made this study possible.

MIGRATION OF JUVENILE CHINOOK SALMON AND

EFFECTS OF DEVELOPMENT, STRESS, PATHOGENS

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We evaluated migratory behavior in yearling spring chinook salmon, *Oncorhynchus tshawytscha*, through the lower 200 km of the Columbia River and through the freshwater portion of the estuary to determine identify threats to successful ocean entry. Our goal is to understand those factors affecting performance of fish transported in barges or those migrating to the sea after passing a total of eight major hydroelectric dams. We used a variety of clinical indices to ascertain the stress of fish associated with passage of dams, collection for transport, and during the 35 hr barge trip. We also assessed smolt development by measuring gill Na^+/K^+-ATPase activity and the infection level with the causative organism for bacterial kidney disease (BKD). Evaluation of

these factors in fish from throughout the run suggests that there is considerable variation between fish sampled on different dates and between years. At the uppermost dam the emigrants may differ in level of stress, degree of smolt development, and in severity of BKD infection. Variation in these factors may have been a function of time, hydrologic condition or biological variables such interaction with steelhead trout, *Oncorhynchus mykiss*.

Radiotelemetry of barged and fish migrating down the entire Snake-Columbia system showed that nearly all of the fish successfully migrated over 135 km from their release site or from the lowermost dam, respectively, to the estuary. While many migrants remained in the main channel in the estuary, others assumed different routes through small channels over and around sand bars and structure. Migration rates in the river were rather consistent through time, but early-run fish seemed to have a greater abundance of slower individuals; migration in the estuary was closely correlated with tidal cycles with fish ceasing to move during incoming and high tides. Once the fish reached the estuary, they were exposed to significant avian predation in amounts of 5-35% of each study population.

We speculate that fish that are well developed (smolted) and in good condition readily make the transition from fresh to salt water and hence are less vulnerable to predation from above. Stress associated with passage of numerous dams or during certain times of the collection and transport process could retard development and delay seawater entry or exacerbate the effects of pathogens. Fish infected with bacteria at levels indicative of disease could also be more vulnerable to predation or have lower fitness in salt water. We are using a population-based simulation model that incorporates life history stages and habitat variables to ascertain the importance of smoltification, stress and disease in chinook in the estuary of major river systems. Our hope is to relate indices of fish phenotype at the estuary to fitness.

CHANGES IN GILL GLUCOCORTICOID RECEPTOR TRANSCRIPT LEVELS DURING SMOLTIFICATION AND EFFECTS OF CORTISOL ON ITS LEVELS IN MASU SALMON, *ONCORHYNCHUS MASOU*

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In salmonids, cortisol plays a central role in development of seawater adaptability during smoltification (McCormick, 1995). Cortisol action is mediated by the glucocorticoid receptor (GR). Previous data on salmon GR indicates that GR transcripts and their translation products are localized in gill chloride cells in chum salmon (Uchida *et al*, 1998) and that the number of gill cortisol receptors changes during smoltification of coho salmon (Shrimpton *et al*.,1994a). However, the direction and magnitude of the changes in gill GR transcript levels during smoltification of salmonids remain unclear. In order to address this issue, we examined changes in gill GR transcript levels and serum cortisol concentrations during smoltification in wild masu salmon (Exp.1). Although a large number of studies have been performed on hormonal regulation of GR transcription in mammals, this area has hardly been explored in fish. The only findings to date show that cortisol (Shrimpton *et al*.,1994b) or growth hormone (GH) treatment (Shrimpton *et al*.,1995) induces changes in the number of gill cortisol receptors in coho salmon. In Exp.2, the effects of cortisol on gill GR transcript levels were therefore investigated experimentally to elucidate hormonal effects on gill GR transcript levels in masu salmon.

In Exp.1, yearling wild masu salmon were caught from the Kenichi River, Hokkaido, Japan, from January to May 1996. Following anesthesea, gills and blood were collected. Total RNA was extracted from gills and used to measure

GR transcript levels by competitive PCR which was recently developed in our laboratory (Mizuno *et al*, in preparation). Collected blood was used to determine serum cortisol concentrations using radioimmunoassay. Consequently, gill GR transcript levels were found to be very low from January to February. However, the levels increased dramatically during smoltification, reaching their highest value in April to subsequently decline in May (Fig. 1). Serum cortisol concentrations were maintained at low levels from January to March (6.85 ± 1.75 - 7.66 ± 0.77 ng_ml^{-1}). In April, a maximum (18.0 ± 2.32 ng_ml^{-1}) was observed and thereafter maintained in May (13.6 ± 4.40 ng_ml^{-1}). This data demonstrates that gill GR transcript levels are closely related to serum cortisol levels. The assumption that GR is situated in the cytosol, and is translocated to the nucleus upon binding to its ligand (Gorski and Gannon, 1976), is widely accepted. Moreover, the cortisol binding assay cited above (Shrimpton *et al*., 1994a) revealed that a reduction in the concentration and affinity of GR was observed in the gill cytosolic fraction during smoltification, when plasma cortisol levels surge. These and the present results raise the hypothesis that gill GR transcript levels may increase to synthesize new cytosolic receptors in order to obviate the lack of receptors in cortisol target cells when plasma cortisol levels rise.

In Exp.2, hatchery-reared masu salmon parr and pre-smolts were intraperitoneally injected with cortisol (10μg/g BW) every 2 days, totalling 6 times. Two days following the last injection, the fish were sampled and gills collected to measure GR transcript levels. Our data show that cortisol treatment induced an increase in gill GR transcripts in both parr and pre-smolts (Fig.2), again suggesting that gill GR transcription may be positively regulated by cortisol. In a previous study (Shrimpton *et al*.,1994b), cortisol treatment induced a decrease in the concentration and affinity of GR in gill cytosolic preparations as demonstrated by a cortisol binding assay. From our data, the increase in gill GR transcript levels after cortisol treatment may have resulted from a decrease in the number of free cytosolic receptors. Experimental data correspond to those from wild fish, whose GR transcript and serum cortisol levels both peak in April.

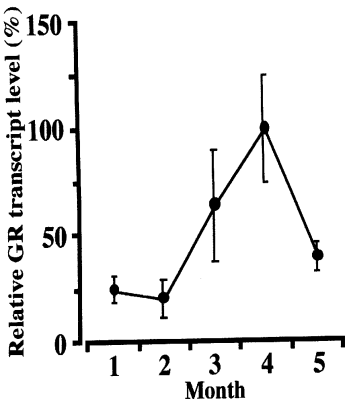

Figure 1. Changes in gill GR transcript levels during smoltification of wild masu salmon. Vertical bars indicate standard errors of the means of triplicate measurements of one sample. The value in April was arbitrarily set at 100%.

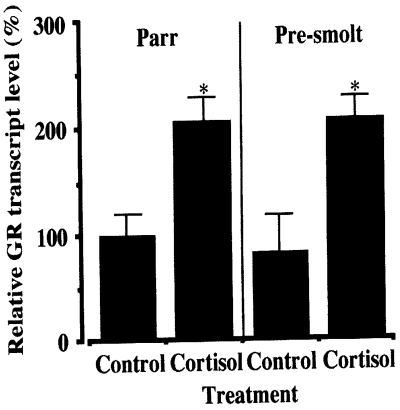

Figure 2. Effects of cortisol on gill GR transcript levels in masu salmon parr and pre-smolt.Vertical bars indicate standard errors of the means of triplicate measurements of one sample. The value in control parr was arbitrarily set at 100%.
*$p < 0.05$, significantly different from the value of control

These present findings demonstrate that gill GR transcript levels increase during smoltification of wild masu salmon. The increase is accompanied by that of serum cortisol of suggesting that GR transcription is positively regulated by cortisol. At present, we analyze the effects of growth hormone on gill GR transcription in masu salmon, since a previous study indicated that growth hormone treatment induced increases in the number of gill cortisol receptors in coho salmon (Shrimpton *et al*. 1995).

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EFFECTS OF GROWTH HORMONE AND CORTISOL ON GILL NA^+, K^+-ATPASE MRNA LEVELS IN MASU SALMON

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The anadromous salmonids undergo a parr-smolt transformation (smoltification) before migrating to the sea. The transition from fresh water to seawater necessitates an alteration in osmoregulatory mechanisms. The activity of gill Na^+, K^+-ATPase increases in smolts prior to seawater entry through endocrine stimulation. During smoltification, circulating concentrations of many hormones are known to increase. Among those hormones, it is generally accepted that growth hormone and cortisol play important roles in the development of seawater tolerance (Bern and Nishioka, 1993).

Na^+, K^+-ATPase is composed of two different protein subunits, α and β, both of which exist in several distinct isoforms (Fambrough *et al*., 1986). In teleosts including salmonids, a large number of investigations has focused on the hormonal regulation of Na^+, K^+-ATPase activity, chloride cell development, and ion flux in the gill (McCormick, 1995), but little is known about hormonal effects on gene expression of Na^+, K^+-ATPase α- and β-subunits. Only one report has been published recently on hormonal regulation of Na^+, K^+-ATPase gene expression by Northern blot analysis using *Xenopus* α-subunit cDNA in brown trout (Madsen *et al*., 1995), whereas data on hormonal regulation of Na^+, K^+-ATPase β-subunit mRNA levels is entirely lacking. Knowledge of endocrine factors that mediate the expression of the Na^+, K^+-ATPase subunits is needed to clarify the development of hypo-osmoregulatory ability in salmonids.

In the present study, we investigated the effects of growth hormone and cortisol on gill Na^+, K^+-ATPase α- and β-subunit mRNA levels in masu salmon.

Fish at the parr and pre-smolt stage, maintained in fresh water, were injected intraperitoneally with ovine growth hormone (oGH: 3μg/g), cortisol (F: 10μg/g), a combination of oGH (3μg/g)+F (10μg/g), or saline as control every other day for a total of 6 injections. After sacrifice, gills were frozen in liquid N_2 and stored at -80°C until assay for Na^+, K^+-ATPase activity and Northern blot analysis. Poly $(A)^+$ RNA was isolated from gills and analysed by Northern blotting using masu salmon Na^+, K^+-ATPase α- or β-subunit cDNAs as probe.

Effects of oGH, F and oGH+F on Na^+, K^+-ATPase activity in the gill are shown in figure 1A. In parr, gill Na^+, K^+-ATPase activity was 0.18 0.06 μmol Pi/mg protein/hr in controls. Treatment with oGH or oGH+F resulted in significantly higher activity, i.e., 0.85 0.29 and 0.85 0.28 μmol Pi/mg protein/hr, respectively ($p<0.05$, compared to the control). The F treatment had no effect on enzyme activity. In pre-smolts, Na^+, K^+-ATPase activity was 0.18 ±0.02 μmol Pi/mg protein/hr in control, while treatment with oGH, F, or oGH+F yielded significantly higher values of 0.40 ±0.04, 0.34 ±0.05 and 0.69 ±0.05 μmol Pi/mg protein/hr, respectively ($p<0.05$, compared to the control).

Changes in gill Na^+, K^+-ATPase α- and β-subunit mRNA levels after treatment with oGH, F or oGH+F are shown in figure 1B and C. In the gill of all the treatment groups, one main α-subunit mRNA band corresponding to 3.3 kb, and one β-subunit mRNA band corresponding to 2.3 kb were detected. In both the parr and pre-smolt stages, the levels of Na^+, K^+-ATPase α- and β-subunit mRNA were significantly ($p<0.05$) higher in fish treated with F or oGH+F relative to control fish (1.3-2.7 fold), whereas oGH was without effect.

In our previous hormone treatment experiment in masu salmon, extensive tubular systems were observed throughout the cytoplasm of chloride cells after oGH treatment (Mizuno *et al*., unpublished data). In fish treated with F, the development of tubular systems was not observed. Instead, elaboration of rough endoplasmic reticulum occurred after treatment with F (Mizuno *et al*., unpublished data). These results suggest the following model for the endocrine control of development of hypo-osmoregulatory ability: synthesis of Na^+, K^+-ATIPase α- and β-subunits may be controlled mainly by cortisol, whereas the increase in Na^+, K^+-ATPase activity is associated with the development of

tubular systems in chloride cells and this appears to be controlled mainly by growth hormone and/or its mediator, insulin-like growth factor-I.

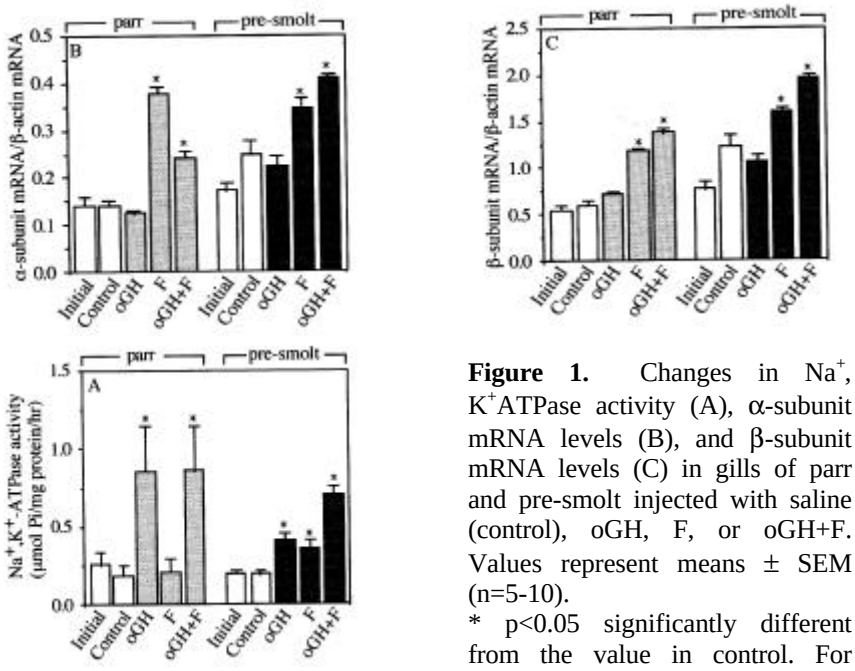

Figure 1. Changes in Na^+, K^+ATPase activity (A), α-subunit mRNA levels (B), and β-subunit mRNA levels (C) in gills of parr and pre-smolt injected with saline (control), oGH, F, or oGH+F. Values represent means ± SEM (n=5-10).
* $p<0.05$ significantly different from the value in control. For abbreviations see text.

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