Metabolic enzyme activity during smolting in stream- and hatchery-reared Atlantic salmon (*Salmo salar*)

Jill B.K. Leonard and Stephen D. McCormick

Abstract: To evaluate the metabolic differences between Atlantic salmon (*Salmo salar*) parr and smolts and the effect of rearing environment, we examined metabolic enzyme activity in white muscle, liver, and heart in stream- and hatchery-reared juveniles. Spring increases in gill Na^+,K^+-ATPase (3.5-fold) and cardiosomatic index (37–69%) and decreases in condition factor (~17%) occurred in smolts, but not in parr. White muscle phosphofructokinase (PFK) increased during spring and was 3.6-fold higher in smolts than in parr by late spring. There were seasonal increases in liver citrate synthase (CS) (~42%), liver β-hydroxyacyl-coenzyme A dehydrogenase (HOAD) (~60%), and heart CS (~23%) and decreases in liver lactate dehydrogenase (LDH) (~28%) in parr and smolts. Activity of liver HOAD was greater in stream-reared smolts (~18%) than in parr or hatchery smolts. Heart PFK activity increased during spring in wild-reared parr and smolts, while it decreased in hatchery-reared smolts. White muscle LDH and PFK increased earlier in spring in hatchery- than in stream-reared smolts. Our results suggest that increased heart size and high white muscle PFK occur during smolting and may be adaptive for downstream and ocean migration. Hatchery- and stream-reared Atlantic salmon differ in the timing of metabolic changes during smolting, which may impact their long-term survival.

Résumé : Un examen de l'activité des enzymes métaboliques du muscle blanc, du foie et du coeur chez des jeunes Saumons de l'Atlantique (*Salmo salar*) élevés en ruisseau et en pisciculture a été entrepris pour évaluer les différences métaboliques entre les tacons et les saumoneaux et les effets du milieu d'élevage. Au printemps, la Na^+,K^+-ATPase des branchies augmente de 3,5 fois et l'indice cardiosomatique de 37–69%, alors que le coefficient d'embonpoint diminue (~17%) chez les saumoneaux, mais pas chez les tacons. La phosphofructokinase (PFK) du muscle blanc augmente au printemps et elle est 3,6 fois plus élevée chez les saumoneaux que chez les tacons à la fin du printemps. Il y a des augmentations saisonnières de la citrate synthase (CS) du foie (~42%) et du coeur (~23%) et de la β-hydoxyacyl-coenzyme A déshydrogénase (HOAD) du foie (~60%) chez les tacons et les saumoneaux, ainsi qu'une diminution de la lactate déshydrogénase (LDH) du foie (~28%). L'activité de la HOAD du foie est plus grande (~18%) chez les saumoneaux de ruisseau que chez les tacons ou les saumoneaux de pisciculture. L'activité de la PFK du coeur augmente au cours du printemps chez les tacons et les saumoneaux sauvages, mais elle diminue chez les saumoneaux de pisciculture. La LDH et la PFK du muscle blanc augmentent plus tôt chez les saumoneaux de pisciculture que chez les saumoneaux de ruisseau. Nos résultats indiquent que, durant la saumonification, la taille du coeur s'accroît et la PFK augmente dans le muscle blanc, ce qui pourrait être des adaptations pour la migration vers l'aval et l'océan. Les Saumons de l'Atlantique élevés en pisciculture et en ruisseau diffèrent dans la phénologie de leurs changements métaboliques durant la saumonification, ce qui peut affecter leur survie à long terme.

[Traduit par la Rédaction]

Introduction

Alteration of metabolism during the parr–smolt transformation has been found in several salmonid species. In Atlantic salmon (*Salmo salar*), standard and active metabolic rates are 50% higher in smolts than in parr (Maxime et al. 1989). Maxime et al. (1989) suggested that this elevation of metabolic rate in smolts might be due to elevated respiratory enzyme activity and mitochondrial proliferation resulting from an increase in thyroid hormone levels. Metabolism may also increase due to the metabolic demands of development and differentiation (McCormick and Saunders 1987). Atlantic salmon smolts have decreased available liver and muscle glycogen compared with parr (Fontaine and Hatey 1953;

Received May 15, 2000. Accepted April 26, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on July 11, 2001.
J15761

J.B.K. Leonard[1,2] and S.D. McCormick. Conte Anadromous Fish Research Center, Biological Resources Division, U.S. Geological Survey, Turners Falls, MA 01376, U.S.A., and Department of Biology, University of Massachusetts, Amherst, MA 01003, U.S.A.

[1]Corresponding author (e-mail: jileonar@nmu.edu).
[2]Address for correspondence: Department of Biology, Northern Michigan University, 1401 Presque Isle Avenue, Marquette, MI 49855, U.S.A.

 DOI: 10.1139/cjfas-58-8-1585

Wendt and Saunders 1973) as well as elevated levels of blood glucose (Wendt and Saunders 1973). In coho salmon (*Oncorhynchus kisutch*) smolts, decreased glycogen is caused by a combination of decreased glycogen synthesis and increased glycogenolysis in the liver (Sheridan et al. 1985). Lipid metabolism is also altered during the parr–smolt transformation such that total body and muscle lipid decreases in smolts (Saunders and Henderson 1970, 1978). Evidence from chinook (*Oncorhynchus tshawytscha*) (Cowley et al. 1994) and coho salmon (Sheridan et al. 1985; Sheridan 1986) indicates that lipid depletion is caused by increased lipolysis and decreased fatty acid synthesis. There is also some evidence that there is a reorganization of amino acid distribution during smoltification; however, there was no net change in the total amino acid content of smolts (Fontaine and Marchelidon 1971). It has been suggested that this reorganization is linked to guanine and hypoxanthine deposition in the skin and scales (smolt silvering, Fontaine and Marchelidon 1971) as well as possible changes in cellular osmoregulatory gradients (McCormick and Saunders 1987). There is also evidence that there is an elevation of activity of respiratory enzymes in the gill (Chernitsky and Shterman 1981; Langdon and Thorpe 1985; McCormick and Saunders 1987) and liver (Blake et al. 1984) of Atlantic salmon smolts.

The work described above concentrated on laboratory studies using hatchery-reared fish as experimental animals. While it is likely that these changes also occur in the wild, it is unknown if there are substantial differences in smolt-associated metabolic changes between hatchery-reared and stream-reared salmon. Possible differences in smolt development due to rearing environment are of particular interest due to the usually poor return rates of hatchery fish compared with wild fish (briefly reviewed by McCormick et al. 1998). Recently, there has also been an increased use of fry rather than smolt releases to restore depleted salmonid stocks (e.g., Rideout and Stolte 1988), and there is little knowledge of smolt development in salmonids released as fry.

Evidence suggests that there are substantial differences in development that occur between salmon reared in the hatchery environment and salmon reared in the wild. Brauner et al. (1994) demonstrated an effect of rearing environment on the swimming ability of coho salmon smolts. McDonald et al. (1998) also showed an effect of rearing habitat on swimming capacity in Atlantic salmon juveniles that was difficult to overcome even in modified hatchery rearing environments. Shrimpton and co-workers found significant differences in cortisol dynamics, including receptor level differences and circulating hormone concentrations, between stream- and hatchery-reared coho salmon smolts (Shrimpton et al. 1994*a*, 1994*b*), while McCormick and Bjornsson (1994) found differences in thyroid hormone and growth hormone profiles between hatchery- and wild- reared Atlantic salmon juveniles.

We are interested in further examining the physiological differences between fish that undergo smoltification in the wild and those that smolt in a hatchery environment in an effort to understand the underlying basis for potential differences in survival of smolts reared in hatcheries and in the wild. In this study, we focus on differences in the capacity of metabolic enzymes between parr and smolts (as determined by Na^+,K^+ ATPase activity) and the impact of rearing environment on these developmental changes. Specifically, we have focused on several enzymes critical to glycolysis (phosphofructokinase (PFK)), fatty acid metabolism (β-hydroxyacyl-coenzyme A dehydrogenase (HOAD)), aerobic metabolism (citrate synthase (CS)), and lactate regulation (lactate dehydrogenase (LDH)).

Materials and methods

Fish

Stream-reared Atlantic salmon were collected by electrofishing in West Salmon Brook, Connecticut, on March 15 (3.0°C), April 5 (5.0°C), and June 2 (14.5°C, parr only), 1994. There is no natural reproduction above any mainstem dam in the Connecticut River, and since all smolt releases occur in the mainstem of the river, stream-reared fish were easily identified. Migrating stream-reared smolts were captured at downstream bypass structures at hydroelectric facilities on the Farmington River (15 km downstream of West Salmon Brook) on May 20 and 25 and at Cabot Station on the Connecticut River on May 11 and 20. The size distributions in March and April showed a clear distinction between 1-year-old parr and 2-year-old smolts. One-year-old fish from the White River National Fish Hatchery (Bethel, Vt.) were sampled on March 21 (2.8°C), April 6 (3.0°C), and May 11 (11.9°C). Those captured on May 11 had been released from the hatchery on April 14 and subsequently captured at Cabot Station and thus were active migrants. Hatchery-reared smolts were easily distinguished because all fish released from the hatchery as smolts are adipose fin clipped. Due to the natural temperature changes occurring over the period of the study, fish could not be sampled at the same temperature. While this has the potential to confound our results, it was unavoidable in a field-based study. It should be emphasized that seasonal effects found in this study may well include the effect of temperature on the juvenile salmon, along with other seasonally linked parameters such as photoperiod or increasing wild food availability; however, since all groups were experiencing similar temperatures over the period of sampling, rearing effects (between groups) are unlikely to be a function of temperature per se.

Sampling

Captured fish were anaesthetized with 100 $mg \cdot L^{-1}$ tricaine methansulfonate (MS-222, pH 7.0). Fish were measured for mass and fork length and then bled from the caudal vessels. The heart was removed by cutting posterior to the bulbus arteriosus and anterior to the sinus venosus. The heart was then blotted dry to remove internal blood, weighed, and frozen immediately on dry ice. The liver was also removed, weighed, and frozen. A sample of white muscle was excised from the left side of the fish dorsal to the midline, anterior to the dorsal fin, weighed, and frozen. A gill biopsy was taken from the second gill arch. Approximately three to five gill filaments were cut above the septa and placed in 100 μL of SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) and frozen. All samples were then returned to the laboratory where they were stored at –80°C for later assay.

Enzyme assays

We measured four metabolic enzymes in heart, liver, and white muscle: CS (Guderley et al. 1986; McCormick et al. 1989), HOAD (Bradshaw and Noyes 1975; Guderley and Gawlicka 1992), PFK (Ling et al. 1966; Guderley and Gawlicka 1992), and LDH (Vassault 1983; Guderley and Gawlicka 1992). Samples were homogenized in a buffer containing 50 mM imidazole, 2 mM EDTA, 5 mM $MgCl_2$, and 1 mM glutathione at pH 7.5. Tissues were homogenized using a ground glass homogenizer in 20 volumes of ice-cold homogenization buffer. Homogenates were then centri-

fuged at 3000 × *g* for 5 min and the supernatant was immediately assayed kinetically at 25°C in 96-well microplates using the following specific conditions.

CS (EC 4.1.3.7): 50mM Tris, 0.78 mM 5,5′-dithiobis-(2-nitrobenzoic acid), 0.17 mM acetyl coenzyme A, 0.57 mM oxaloacetic acid (omitted for control), pH 8.1. Read at 412 nm.

PFK (EC 2.7.1.1): 75 mM Tris–HCl, 200 mM KCl, 6 mM $MgCl_2$, 1 mM KCN, 2 mM AMP, 1.75 mM ATP, 0.16 mM NADH, 4 units of triose phosphate isomerase, 4 units of 3-phosphoglycerol dehydrogenase, 4 units of aldolase, 5 mM fructose-6-phosphate (omited for control), pH 8.0. Read at 340 nm.

LDH (EC 1.1.1.27): 100 mM potassium phosphate, 0.4 mM pyruvate, 0.16 mM NADH, pH 7.0. Read at 340 nm. This assay was routinely run without control, since preliminary experiments showed extremely low activity (<2% of total) in the absence of substrate (pyruvate) in all tissues.

HOAD (EC 1.1.1.35): 100 mM triethanolamine HCl, 5 mM EDTA, 1 mM KCN, 0.23 NADH, 0.15 acetoacetyl coenzyme A (omitted for control), pH 7.0. Read at 340 nm.

All metabolic enzyme assays were run in duplicate and the activities expressed in international units (i.e., micromoles of substrate transformed to product per minute per gram wet mass tissue.

Gill Na^+,K^+-ATPase (EC 3.6.1.3): Na^+,K^+-ATPase activity was determined with a kinetic assay run in 96-well microplates at 25°C and read at a wavelength of 340 nm for 10 min (McCormick 1994). Gill tissue was homogenized in 125 µL of SEID (SEI buffer and 0.1% deoxycholic acid) and centrifuged at 5000 × *g* for 30 s. Ten-microlitre samples were run in two sets of duplicates: one set containing assay mixture and the other assay mixture and 0.5 mM ouabain. The resulting ouabain-sensitive ATPase activity is expressed as micromoles of ADP per milligram of protein per hour. Protein concentrations were determined using the BCA (bicinchoninic acid) protein assay (Pierce, Rockford, Ill.).

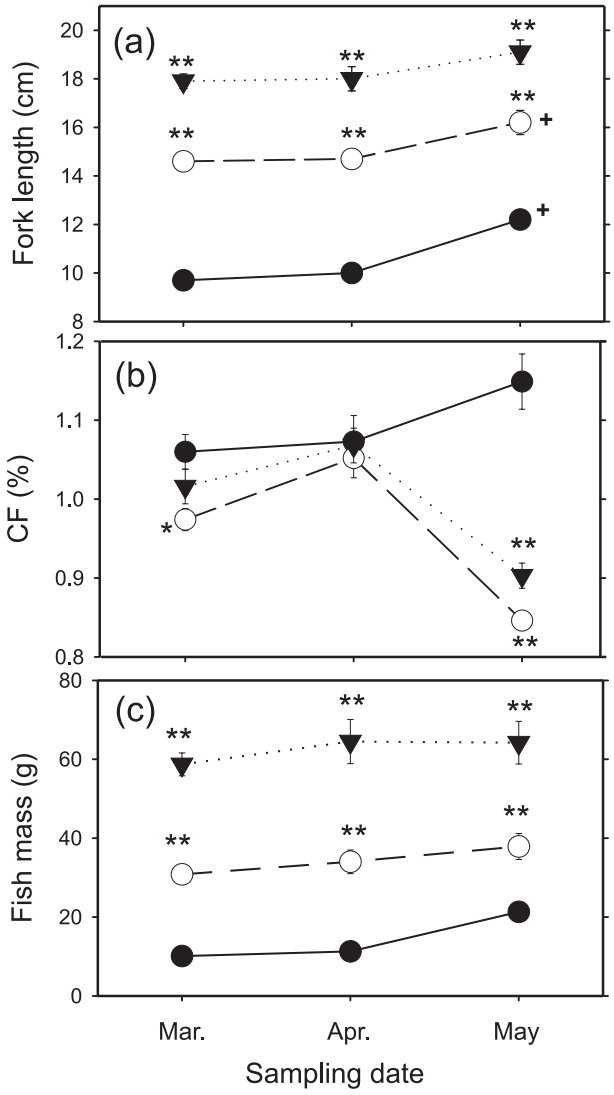
Statistics and calculations

Cardiosomatic index (CSI) was calculated as (heart mass·total mass $^{-1}$) × 100. Hepatosomatic index (HSI) was calculated as (liver mass·total mass $^{-1}$) × 100. Condition factor was calculated as (total mass·length^{-3}) × 100. Length, weight, CSI, HSI, Na^+,K^+-ATPase, and condition factor data were analyzed using analysis of variance (ANOVA) and analysis of covariance (ANCOVA) with length as a cofactor. All metabolic enzyme data were also analyzed using multivariate analysis of variance (MANOVA) followed by post hoc testing using ANOVA. Further post hoc testing of all ANOVA results used the least square means general linear models procedure after testing for the appropriate statistical assumptions using SAS (SAS Institute Inc., Cary, N.C.) for each significant factor.

Results

There was no significant difference in gill Na^+,K^+-ATPase levels (*t* test, $p = 0.55$) between stream-reared smolts captured in the Farmington and Connecticut rivers, suggesting that these fish were at similar smolt and migratory stages. As a result, data for all stream-reared smolts were pooled and comparisons presented are between parr, stream-reared smolts, and hatchery-reared smolts at three time periods. At all sampling times, hatchery smolts were always longer and heavier than stream-reared smolts and all smolts were larger than parr ($p = 0.001$). Parr and stream-reared smolts were significantly longer (ANOVA, $p = 0.001$) in May (note: data from June sampling are included in this group) than in March (Fig. 1*a*), whereas hatchery smolts were not significantly longer at the end of the study. There was no significant increase in weight over the course of the study within any of the groups when all groups were analyzed together ($p = 0.11$) (Fig. 1*c*). Parr were significantly larger in May when ANOVA was performed on parr data alone ($p = 0.001$). Condition factor differed between the groups and over time ($p = 0.001$); condition factor of parr increased over the course of the study, while smolts had decreased condition factor in May (Fig. 1*b*).

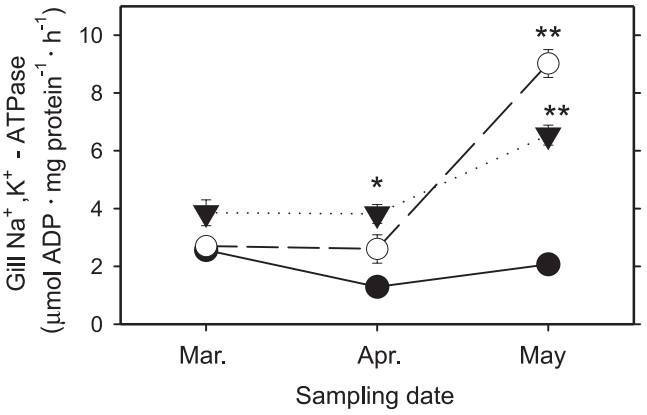
Fig. 1. (*a*) Fork length, (*b*) condition factor, and (*c*) fish mass of Atlantic salmon parr (●), stream-reared smolts (○), and hatchery-reared smolts (▼) sampled during March, April, and May–June 1994 in the Connecticut River watershed. Data are means ± SE. A plus sign indicates a significant increase in length within a juvenile salmon group over March values. A single asterisk indicates a significant difference from parr activity at a given sampling time and a double asterisk indicates a significant difference from the other two juvenile salmon groups at a given sampling time. Statistics indicate ANOVA and post hoc testing with $\alpha = 0.05$.



Gill

Gill Na^+,K^+-ATPase activity was below 4 µmol·mg protein^{-1}·h^{-1} in all groups during March and April. In May, both

Fig. 2. Gill Na^+,K^+-ATPase of Atlantic salmon parr (●), stream-reared smolts (○), and hatchery-reared smolts (▼) sampled during March, April, and May–June 1994 in the Connecticut River watershed. Data are means ± SE. A single asterisk indicates a significant difference from parr activity at a given sampling time and a double asterisk indicates a significant difference from the other two juvenile salmon groups at a given sampling time. Statistics indicate ANOVA and post hoc testing with $\alpha = 0.05$.



stream- and hatchery-reared smolts showed significantly elevated Na^+,K^+-ATPase activity, with stream-reared smolts showing the greatest elevation (3.5-fold) over earlier levels (Fig. 2). There was no change in gill Na^+,K^+-ATPase of parr in May. Na^+,K^+-ATPase was not significantly affected by fish length. During May, all groups were significantly different from one another, with Na^+,K^+-ATPase of hatchery- and stream-reared smolts being 3.2-fold and 4.3-fold higher, respectively, than that of parr.

Metabolic enzymes

The overall MANOVA showed significant effects of fish stages ($p < 0.001$), sampling date ($p < 0.001$), and the interaction between these factors ($p < 0.001$) on combined metabolic activity (all enzymes in all tissues). We will further describe these results in more detail, incorporating results of tissue- and enzyme-specific ANOVA. There was no affect of length on any of the following parameters as adjudged by ANOVA with length as a covariate.

Heart

CSI remained constant in parr but increased significantly in smolts in May (Fig. 3*a*). CSI in May was significantly different in all groups and was elevated by 37 and 69% for hatchery- and stream-reared smolts, respectively, over parr values.

Heart CS increased in activity over the course of the study in all groups by approximately 23% (MANOVA, $p < 0.001$), and there was a marginally significant difference between the groups ($p = 0.052$) (Fig. 3*c*). There was a significant interaction (ANOVA, $p = 0.03$) between sampling time and group, which was a result of a slightly lower CS activity in hatchery smolts in May.

Heart HOAD differed between the groups of juvenile salmon (MANOVA, $p = 0.009$), but did not differ between sampling times ($p = 0.39$) (Fig. 3*d*).

Heart LDH did not change significantly during the study (MANOVA, $p = 0.44$), nor did the three groups vary significantly ($p = 0.86$). There was a significant interaction between group and sampling date ($p = 0.003$) resulting from a tendency for stream-reared smolts to have decreased activity in April, while hatchery smolts tended to have slightly higher activity (Table 1).

Heart PFK differed significantly with sampling time (MANOVA, $p = 0.03$) and there was a significant interaction between time and group (ANOVA, $p = 0.001$), although MANOVA showed no significant difference between juvenile groups ($p = 0.77$). In March, heart PFK of hatchery smolts was elevated over that of both parr and stream-reared smolts (Fig. 3*b*). In April, hatchery smolt heart PFK did not significantly differ from that of the other groups. In May, PFK levels of both parr and stream-reared smolts were elevated over initial levels and were significantly higher than those of hatchery smolts.

Liver

HSI differed significantly (ANOVA, $p = 0.0001$) (Fig. 4*a*) between the groups of juvenile salmon, and although there was no significant difference between sampling times ($p = 0.41$), there was a significant interaction between group and sampling time ($p < 0.01$). On average, hatchery smolt HSI was 35% higher than that of parr, while there was no difference between parr and stream-reared smolts. The interaction is an indication of a tendency for hatchery smolts to have a slightly elevated HSI in the early sampling times, while stream-reared smolts and parr tended to increase HSI slightly over time.

Liver CS differed significantly between groups (MANOVA, $p < 0.001$) and sampling times ($p < 0.001$). Stream-reared smolts had consistently higher liver CS activities (~13% higher than parr) than the other groups (Fig. 4*c*). All three groups had increased liver CS activity over the course of the study (~42%).

Similar to liver CS, liver HOAD showed a pattern of increased activity with time (~60%; $p < 0.001$) (Fig. 4*d*). Stream-reared smolts had a significantly higher enzyme activity level (18% higher than parr) than the other two groups ($p = 0.001$).

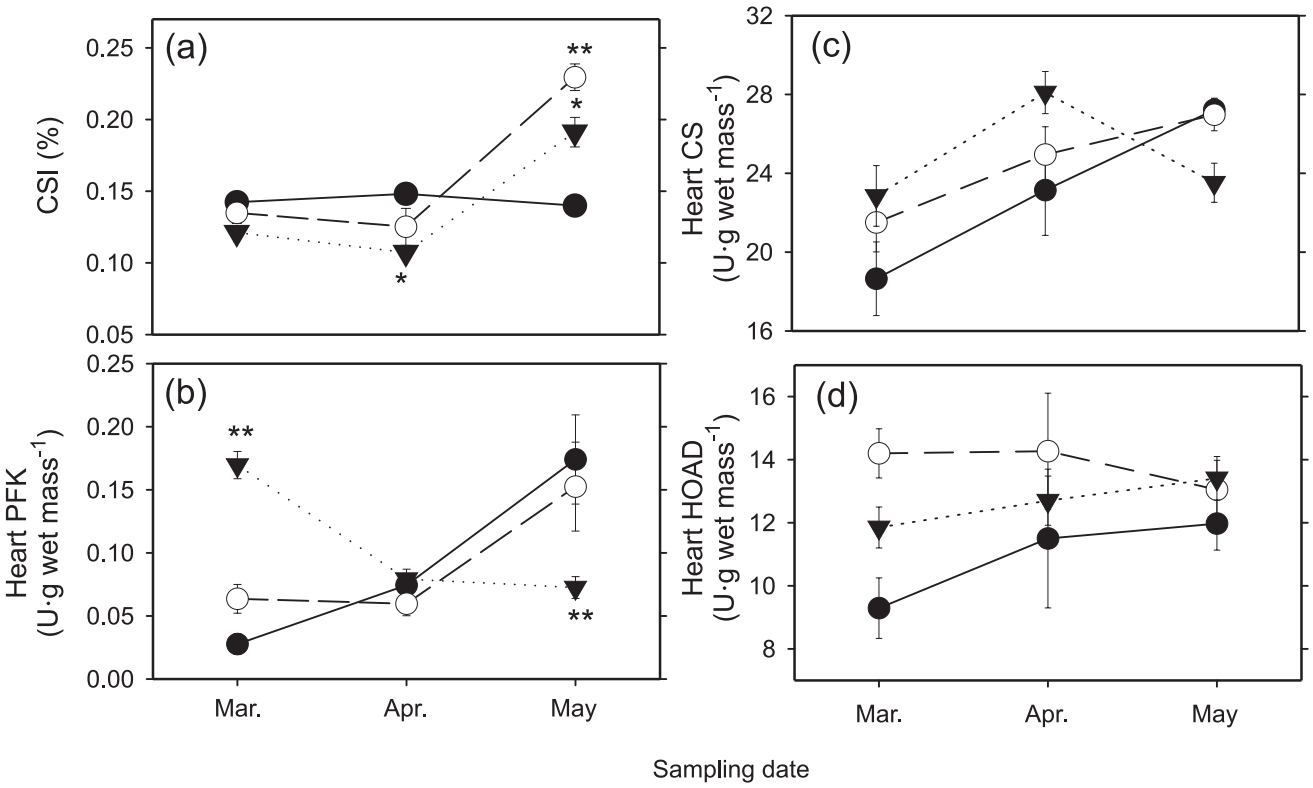
Liver LDH (Fig. 4*b*) differed significantly with sampling date (MANOVA, $p < 0.001$) and juvenile group ($p < 0.001$), and there was a significant interaction between sampling date and group (ANOVA, $p = 0.001$). Liver LDH activity of hatchery- and stream-reared smolts decreased progressively from March to May. Liver LDH activity of parr decreased from March to April, but then increased in May and was almost double that of smolts in May.

Liver PFK did not differ significantly with sampling date (MANOVA, $p = 0.52$) or juvenile salmon group ($p = 0.88$) (Table 1). There was a slight, but significant, interaction between sampling date and group (ANOVA, $p = 0.02$).

White muscle

White muscle CS differed significantly between groups (MANOVA, $p < 0.001$) and sampling dates ($p = 0.01$), and there was a significant interaction between sampling date and group (ANOVA, $p = 0.03$). There was no significant difference in white muscle CS activity in hatchery smolts over

Fig. 3. (*a*) CSI, (*b*) heart PFK activity, (*c*) heart CS activity, and (*d*) heart HOAD activity of Atlantic salmon parr (●), stream-reared smolts (○), and hatchery-reared smolts (▼) sampled during March, April, and May–June 1994 in the Connecticut River watershed. Data are means ± SE. A single asterisk indicates a significant difference from parr activity at a given sampling time and a double asterisk indicates a significant difference from the other two juvenile salmon groups at a given sampling time. Statistics indicate ANOVA and post hoc testing with $\alpha = 0.05$.



the course of the study (Fig. 5*b*). In contrast, both parr and stream-reared smolts had elevated white muscle CS early in the study followed by lower levels in May. Parr showed the greatest decrease in activity (–43%) between April and May.

White muscle HOAD did not change significantly during the study (MANOVA, $p = 0.31$) (Table 1), nor were the juvenile salmon groups significantly different ($p = 0.56$).

White muscle LDH significantly differed between sampling dates (MANOVA, $p < 0.001$) and groups ($p < 0.001$), and there was a significant interaction between sampling date and group (ANOVA, $p = 0.001$). LDH activity in hatchery smolts was significantly higher ($p = 0.001$) in April than in March or May (Fig. 5*c*). Both parr and stream-reared smolts had low LDH activity in March and April but had significantly higher LDH activities in May (85 and 56% increases, respectively; $p = 0.001$).

White muscle PFK differed significantly between sampling dates (MANOVA, $p = 0.001$) and juvenile groups ($p < 0.001$), and there was a significant interaction between sampling date and group (ANOVA, $p < 0.001$). Parr white muscle PFK did not change significantly over the course of the study when analyzed using ANOVA that included smolt data; however, when the parr were analyzed separately from the smolts, there was a significant increase in activity in May over earlier levels ($p = 0.001$) (Fig. 5*a*). Both groups of smolts showed increased white muscle PFK during the study; hatchery-reared smolt white muscle PFK was highest in April (2.7-fold increase), while stream-reared smolt PFK was highest in May (3.6-fold increase).

Discussion

The large increases in gill Na^+,K^+-ATPase activity and decreases in condition factor in hatchery- and stream-reared smolts indicate that these fish, which were selected as putative smolts based on their size and appearance, were indeed smolting in the spring. Smolts captured in May–June were active migrants, which further supports their classification as smolt-stage juveniles. Increased gill Na^+,K^+-ATPase activity is frequently used as an indicator of smolting in salmonids and results from the increased number of mitochondrial-rich cells in the gills that function to increase salinity tolerance in fish (Hoar 1988; Uchida et al. 1996). In contrast with smolts, there were no significant increases in gill Na^+,K^+-ATPase activity in wild-reared parr captured between March and May, while condition factor increased in parr during spring as expected with increasing food availability.

Seasonal effects

In addition to the characteristic increase in gill Na^+, K^+-ATPase activity, a number of other enzymes changed in activity level over the course of the study. While we refer here

Table 1. Mean values (±SE) of biochemical indices (U·g wet mass^{-1}) measured in Atlantic salmon parr and stream- and hatchery-reared smolts in March, April, and May–June of 1994.

	Parr			Stream-reared smolts			Hatchery-reared smolts		
	March	April	May–June	March	April	May–June	March	April	May–June
N	8	9	10	10	5	20	12	11	11
Heart LDH	118.5 (21.87)	100.06 (5.29)	123.72 (2.90)	127.82 (8.72)	91.64 (2.39)	113.07 (3.73)	103.98 (3.63)	124.08 (9.01)	94.19 (5.42)
Liver PFK	0.90 (0.24)	1.33 (0.18)	0.75 (0.07)	0.82 (0.17)	1.05 (0.28)	0.88 (0.07)	0.82 (0.11)	0.73 (0.12)	1.11 (0.11)
White muscle HOAD	1.27 (0.17)	1.24 (0.18)	1.25 (0.12)	1.05 (0.12)	1.00 (0.10)	1.47 (0.16)	1.02 (0.06)	1.25 (0.14)	1.18 (0.07)

Note: *N* is number of individuals.

to these as seasonally affected parameters, it should be noted that they are actually being affected by a variety of season-linked factors, likely including temperature, photoperiod, and food availability. Heart and liver CS increased in all three juvenile salmon groups throughout the spring. This suggests an overall increase in metabolic respiratory capacity in the two tissues, probably a reflection of increased mitochondrial volume (Blake et al. 1984; Moyes 1996). Liver HOAD also increased in all three groups over the course of the study; however, there were no changes in this enzyme in the other tissues examined. This suggests an increased capacity to mobilize fatty acids (elevated lipolysis) in the liver, as has been demonstrated for coho salmon smolts (Sheridan et al. 1985). Liver LDH activity decreased in all three groups, particularly smolts, over the course of the study, suggesting a decreased reliance on lactate–pyruvate conversion as spring progresses. Liver LDH has been shown to decrease during smolting and has been implicated in low concentrations of blood glucose and liver glycogen via decreased gluconeogenesis (Plisetskaya et al. 1994; Ji et al. 1996).

CSI increased in smolts over the course of the study, while parr CSI remained unchanged. This is in accord with Poupa et al. (1974) who found that heart growth is accelerated (relative to whole-body growth) during salmon smolting. During this phase, the cardiac compact shell rapidly increases in mass, while the spongy cardiac tissue growth rate remains unchanged relative to parr and ocean-stage salmon growth rates. It may be that the increased heart mass of smolts is necessary for the cardiac output demands of the constantly swimming, schooling life history that is characteristic of salmonids at sea.

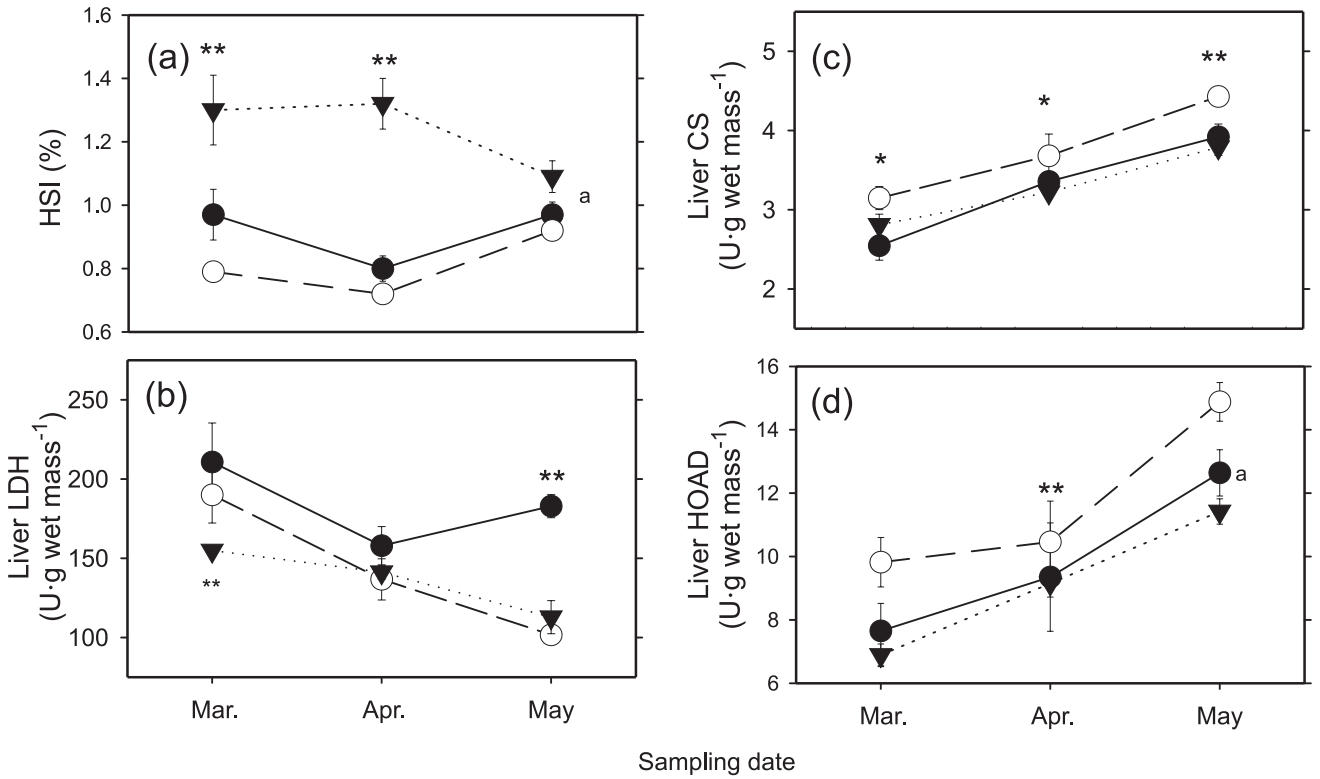
Influence of hatchery rearing

In several of the parameters measured, we found significant differences between smolts reared in the hatchery and those that underwent smolting in the wild. Both liver CS and HOAD activities were higher in stream-reared smolts than in hatchery-reared smolts. However, these lower enzyme activities in hatchery-reared smolts may be offset by their larger livers (higher HSI than in stream-reared smolts). Sheridan et al. (1985) suggested that the increased lipolysis occurring in smolts is a response to the metabolic demands of smolting exceeding food input. If this is the case, then the elevated levels of lipolysis suggested by our data for stream-reared smolts may reflect the difference in food availability between stream- and hatchery-reared smolts with subsequent biochemical, and perhaps behavioral, consequences that may occur when hatchery smolts are released into the wild (as in our May samples).

Hatchery- and stream-reared smolts showed reversed patterns of heart PFK activity. In hatchery smolts, heart PFK was highest in March, while in stream-reared smolts (and parr), the enzyme activity was low early in the spring and was highest in May. The common pattern between parr and stream-reared smolts suggests that the increasing heart PFK activity may be influenced more by natural-stream rearing than by life history stage.

Heart HOAD and mass also differed between the two smolt groups. CSI in stream-reared smolts was consistently higher than in hatchery-reared smolts. Exercise has been shown to influence heart size in some species (Farrell et al.

Fig. 4. (*a*) HSI, (*b*) liver LDH activity, (*c*) liver CS activity, and (*d*) liver HOAD activity of Atlantic salmon parr (●), stream-reared smolts (○), and hatchery-reared smolts (▼) sampled during March, April, and May–June 1994 in the Connecticut River watershed. Data are means ± SE. A single asterisk indicates a significant difference from parr activity at a given sampling time and a double asterisk indicates a significant difference from the other two juvenile salmon groups at a given sampling time. Statistics indicate ANOVA and post hoc testing with $\alpha = 0.05$.

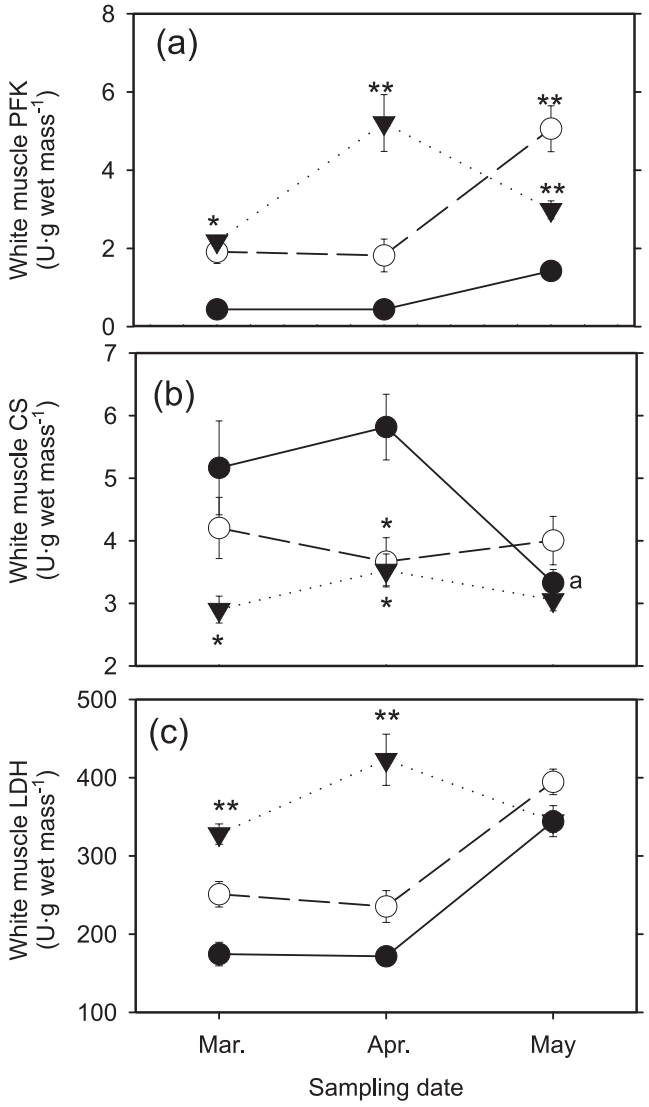


1991), and it is possible that the differences that we observed were a function of the greater activity regime that is probable for stream-reared fish. However, it should be noted that increased heart weight with exercise is not a consistent phenomenon in fishes (Davison 1997), and the potential impact of exercise on heart weight in juvenile Atlantic salmon should be investigated. The difference observed in the present study may have developed soon after release into the wild as fry and been maintained thereafter. Additionally, the increasing heart size (CSI) of smolts has the effect of exaggerating the PFK elevation in wild-reared smolts, if these data are considered on a whole-organ basis, further emphasizing the differences between wild- and hatchery-reared smolts.

The patterns of PFK and LDH activities in white muscle, which differ in the two smolt groups, suggest a temporal shift in smolt enzyme regulation. In both white muscle PFK and white muscle LDH, there is a peak in hatchery smolt activity in April, followed by a decline in May. In stream-reared smolts the elevation in activity occurs in May. In the case of white muscle LDH, despite the early elevation of activity in hatchery smolts, the increase in activity may be a seasonal response common to all groups, since parr also show an elevation of activity in May. This again suggests that the wild-rearing habitat differs from that of the hatchery in its effect on metabolic parameters. In the case of white muscle PFK, however, the elevation in enzyme activity also appears to be related to life stage. Parr show an increase in white muscle PFK during May, but it is not as great as that seen in the smolt groups, and the level of activity in parr is much lower than in the smolts. It may be that the peak in white muscle PFK is related to the cortisol peak that is characteristic of the parr–smolt transformation (Hoar 1988; Shrimpton et al. 1994*b*). Cortisol is involved in stimulating or maintaining increased plasma glucose in response to stress. Atlantic salmon smolts exhibit higher plasma cortisol and glucose than parr when given an identical handling stress (Carey and McCormick 1998). PFK is a key enzyme in glycolysis and its elevation may indicate an upregulation of the glycolytic pathway in order to facilitate glucose utilization and homeostasis during smolting. Shrimpton et al. (1994*b*) found a temporal displacement between the cortisol concentration peaks in hatchery- and wild-reared Atlantic salmon smolts similar to that found in our study for white muscle PFK. McCormick and Bjornsson (1994) found higher levels of plasma cortisol in migrating stream-reared smolts than in smolts reared and kept in a hatchery. It is possible that the increased white muscle PFK of smolts is part of an adaptation for increased burst swimming ability that evolved due to increased mortality from swimming predators during downstream migration and in the ocean.

In conclusion, our data suggest that, although they undergo some of the same metabolic changes as stream-reared smolts, Atlantic salmon smolts produced in hatcheries respond to a different degree, in a temporally different manner, or show an altered pattern of metabolic change compared with fish reared in the wild. While the implications for long-

Fig. 5. White muscle (*a*) PFK activity, (*b*) CS activity, and (*c*) LDH activity of Atlantic salmon parr (●), stream-reared smolts (○), and hatchery-reared smolts (▼) sampled during March, April, and May–June 1994 in the Connecticut River watershed. Data are means ± SE. A single asterisk indicates a significant difference from parr activity at a given sampling time, a double asterisk indicates a significant difference from the other two juvenile salmon groups at a given sampling time, and "a" indicates a significant difference between smolt groups, neither of which is different from parr, at a given sampling time. Statistics indicate ANOVA and post hoc testing with $\alpha = 0.05$.



term survival and reproductive success are unclear, our findings suggest that smolts reared in hatcheries differ substantially in their physiology from smolts reared in the wild. If some or all of these metabolic changes are indeed adaptive, perhaps observable in the differing body form or swimming capacities of parr and smolts, then altered metabolic patterns occurring under hatchery conditions may be involved with reduced survival or return rates of hatchery-reared fish. More investigation of the physiological effects of hatchery rearing on fitness is warranted. White muscle PFK and CSI exhibited differences as a function of both smolting and environment and may be especially useful for further examination of the impact of rearing and release on smolt development and survival.

Acknowledgements

The authors would like to thank Michael O'Dea, Joseph Zydlewski, Mark Shrimpton, Judy Carey, Steve Gephard, and Bruce Williams for their help in collecting and sampling fish. Mark Shrimpton also made many helpful comments in review and Ted Castro-Santos provided valuable statistical insight. We also thank the White River National Fish Hatchery, U.S. Fish and Wildlife Service, for donating hatchery fish and helping with sampling at the hatchery.

References

Blake, R.L., Roberts, F.L., and Saunders, R.L. 1984. Parr–smolt transformation of Atlantic salmon (*Salmo salar*): activities of two respiratory enzymes and concentrations of mitochondria in the liver. Can. J. Fish. Aquat. Sci. **41**: 199–203.

Bradshaw, R.A., and Noyes, B.E. 1975. L-3-Hydroxyacyl coenzyme A dehydrogenase from pig heart muscle. *In* Methods in enzymology. *Edited by* J.M. Lowenstein. Academic Press, London, U.K. pp. 122–128.

Brauner, C.J., Iwama, G.K., and Randall, D.J. 1994. The effect of short-duration seawater exposure on the swimming performance of wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) during smoltification. Can. J. Fish. Aquat. Sci. **51**: 2188–2194.

Carey, J.B., and McCormick, S.D. 1998. Atlantic salmon smolts are more responsive to an acute handling and confinement stress than parr. Aquaculture, **168**: 237–253.

Chernitsky, A.G., and Shterman, L. Ya. 1981. Peculiarities of osmoregulation in the migrating juvenile Atlantic salmons. Vopr. Ikhtiol. **21**: 497–502.

Cowley, D.J., Sheridan, M.A., Hoffnagle, T.L., Fivizzani, A.J., Barton, B.A., and Eilertson, C.D. 1994. Changes in lipid metabolism and plasma concentrations of thyroxine, cortisol, and somatostatin in land-locked chinook salmon, *Oncorhynchus tshawytscha*, during smoltification. Aquaculture, **121**: 147–155.

Davison, W. 1997. The effects of exercise training on teleost fish, a review of recent literature. Comp. Biochem. Physiol. A, Comp. Physiol. **117**: 67–75.

Farrell, A.P., Johansen, J.A., and Suarez, R.K. 1991. Effects of exercise training on cardiac performance and muscle enzymes in rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem. **9**: 305–312.

Fontaine, M., and Hatey, J. 1953. Contribution à l'étude du métabolisme glucidique du saumon (*Salmo salar* L.) à diverses étapes de son développement et de ses migrations. Physiol. Comp. Oecol. **3**: 36–52.

Fontaine, M., and Marchelidon, J. 1971. Amino acid contents of the brain and the muscle of young salmon (*Salmo salar* L.) at the parr and smolt stages. Comp. Biochem. Physiol. A, Comp. Physiol. **40**: 127–134.

Guderley, H., and Gawlicka, A. 1992. Qualitative modification of muscle metabolic organization with thermal acclimation of rainbow trout, *Oncorhynchus mykiss*. Fish Physiol. Biochem. **10**: 123–132.

Guderley, H., Blier, P., and Richard, L. 1986. Metabolic changes during the reproductive migration of two sympatric coregonines, *Corgonus artedii* and *Coregonus clupeaformis*. Can. J. Fish. Aquat. Sci. **43**: 1859–1865.

Hoar, W.S. 1988. The physiology of smolting salmonids. *In* Fish physiology. Vol. IXB. *Edited by* W.S. Hoar and R.J. Randall. Academic Press, New York. pp. 275–343.

Ji, H., Bradley, T.M., and Tremblay, G.C. 1996. Lactate-dependent gluconeogenesis and atractyloside-sensitive flux through pyruvate carboxylase are reduced during smoltification of Atlantic salmon (*Salmo salar*). J. Exp. Zool. **276**: 375–386.

Langdon, J.S., and Thorpe, J.E. 1985. The ontogeny of smoltification: developmental patterns of gill Na^+,K^+-ATPase, SDH and chloride cells in juvenile Atlantic salmon, *Salmo salar* L. Aquaculture, **45**: 83–96.

Ling, K.H., Paetkau, V., Marcus, F., and Lardy, H.A. 1966. Phosphofructokinase *In* Methods in enzymology. *Edited by* W.A. Wood. Academic Press, London, U.K. pp. 425–429.

Maxime, V., Boeuf, G., Pennec, J.P., and Peyraud, C. 1989. Comparative study of the energetic metabolism of Atlantic salmon (*Salmo salar*) parr and smolts. Aquaculture, **82**: 163–171.

McCormick, S.D. 1994. Ontogeny and evolution of salinity tolerance in anadromous salmonids: hormones and heterochrony. Estuaries, **17**(1A): 26–33.

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

McCormick, S.D., and Saunders, R.L. 1987. Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth, and metabolism. Am. Fish. Soc. Symp. **1**: 211–229.

McCormick, S.D., Saunders, R.L., and MacIntyre, A.D. 1989. Mitochondrial enzyme and Na+-K+-ATPase activity, and ion regulation during parr–smolt transformation of Atlantic salmon (*Salmo salar*). Fish Physiol. Biochem. **6**: 231–241.

McCormick, S.D, Hansen, L.P., Quinn, T.P., and Saunders, R.L. 1998. Movement, migration, and smolting. Can. J. Fish. Aquat. Sci. **55**(Suppl. 1): 77–92.

McDonald, D.G., Milligan, C.L., McFarlane, W.J., Croke, S., Currie, S., Hooke, B., Angus, R.B., Tufts, B.L., and Davidson, K. 1998. Condition and performance of juvenile Atlantic salmon (*Salmo salar*): effects of rearing practices on hatchery fish and comparison with wild fish. Can. J. Fish. Aquat. Sci. **55**: 1208–1219.

Moyes, C.D. 1996. Cardiac metabolism in high performance fish. Comp. Biochem. Physiol. A, Comp. Physiol. **113**: 69–75.

Plisetskaya, E.M., Moon, T.W., Larsen, D.A., Foster, G.D., and Dickhoff, W.W. 1994. Liver glycogen, enzyme activities, and pancreatic hormones in juvenile Atlantic salmon (*Salmo salar*) during their first summer in seawater. Can. J. Fish. Aquat. Sci. **51**: 567–576.

Poupa, O., Gesser, H., Jonsson, S., and Sullivan, L. 1974. Coronary-supplied compact shell of ventricular myocardium in salmonids: growth and enzyme pattern. Comp. Biochem. Physiol. A, Comp. Physiol. **48**: 85–95.

Rideout, S.G., and Stolte, L.W. 1988. Restoration of Atlantic salmon to the Connecticut and Merrimack rivers. *In* Present and future atlantic salmon management. *Edited by* R.H. Stroud. Atlantic Salmon Federation, Ipswich, Mass. pp. 67–81.

Saunders, R.L., and Henderson, E.B. 1970. Influence of photoperiod in smolt development and growth of Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. **27**: 1295–1311.

Saunders, R.L., and Henderson, E.B. 1978. Changes in gill ATPase activity and smolt status of Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. **35**: 1542–1546.

Sheridan, M.A. 1986. Effects of thyroxine, cortisol, growth hormone, and prolactin on lipid metabolism of coho salmon, *Oncorhynchus kisutch*, during smoltification. Gen. Comp. Endocrinol. **64**: 220–238.

Sheridan, M.A., Woo, N.Y.S., and Bern, H.A. 1985. Changes in the rates of glycogenesis, glycogenolysis, lipogenesis, and lipolysis in selected tissues of the coho salmon (*Oncorhynchus kisutch*) associated with the parr–smolt transformation. J. Exp. Zool. **236**: 35–44.

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

Shrimpton, J.M., Bernier, N.J., and Randall, D.J. 1994*b.* Changes in cortisol dynamics in wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) during smoltification. Can. J. Fish. Aquat. Sci. **51**: 2179–2187.

Uchida, K., Kaneko, T., Yamauchi, K., and Hirano, T. 1996. Morphological analysis of chloride cell activity in the gill filaments and lamellae and changes in Na^+K^+-ATPase activity during seawater adaptation in chum salmon fry. J. Exp. Zool. **276**: 193–200.

Vassault, A. 1983. Lactate dehydrogenase *In* Methods in enzymatic analysis. *Edited by* H.U. Bergmeyer. Academic Press, London, U.K. pp. 118–126.

Wendt, C.A.G., and Saunders, R.L. 1973. Changes in carbohydrate metabolism in young Atlantic salmon in response to various forms of stress. Int. Atl. Salmon Found. Spec. Publ. Ser. **4**: 55–82.