Journal of Fish Biology (2002) **61,** 1294–1304
doi:10.1006/jfbi.2002.2156, available online at http://www.idealibrary.com on IDEAL®

Seasonal changes in androgen levels in stream- and hatchery-reared Atlantic salmon parr and their relationship to smolting

J. M. Shrimpton* and S. D. McCormick

USGS, Leetown Science Center, Conte Anadromous Fish Research Center, One Migratory Way, P.O. Box 796, Turners Falls, MA 01376, U.S.A. and Department of Biology, University of Massachusetts, 221 Morrill Science Center, Amherst, MA 01003, U.S.A.

(*Received 13 November 2001, Accepted 8 October 2002*)

In stream-reared Atlantic salmon *Salmo salar*, plasma androgens were significantly greater in mature male parr than immature males and females in October, but had declined by January and did not differ significantly from immature fish throughout the spring. Immature fish in March were significantly larger and had greater gill Na^+,K^+-ATPase activity than their previously mature counterparts. Bimodal growth distribution was seen in hatchery-reared Atlantic salmon and a proportion of the male fish in the lower mode matured. Plasma testosterone (T) and 11-ketotestosterone (11-KT) were significantly elevated from September to December in mature male (1+ year) parr. In January, plasma androgens had declined in mature males and did not differ significantly from immature fish. By May all the hatchery fish were large enough to smolt and a proportion of the previously mature males had increased gill Na^+,K^+-ATPase activity. Therefore elevated androgens in the previous autumn do not prevent smolting. Parr with higher plasma T and 11-KT in April and May, that are presumably beginning to mature, had lower gill Na^+,K^+-ATPase activity, indicating that future maturation and associated increases in androgens may inhibit smolting.

Key words: testosterone; 11-ketotestosterone; Atlantic salmon; smolting; maturation.

INTRODUCTION

Early maturation of male parr and smolting have been considered to be in developmental conflict (Thorpe, 1987). For example, ‘ precocious ’ maturation has been documented to impair smolting (Foote *et al*., 1991). Despite the potential developmental conflict, some fishes that mature in the autumn are capable of smolting the following spring (Saunders *et al*., 1994; Berglund, 1995). The mechanism for the conflict between smolting and maturation has been suggested to be mediated by androgens, which interfere with development of hypoosmoregulatory ability (Lundqvist *et al*., 1989).

Variation in Atlantic salmon *Salmo salar* L. life history strategies can have a dramatic effect on timing of the parr-smolt transformation and survival, and is therefore of importance for development of Atlantic salmon enhancement strategies. An increased emphasis on fry release programmes for Atlantic

*Author to whom correspondence should be addressed at present address: Biology Program, University of Northern British Columbia, 3333 University Way, Prince George, B.C., Canada V2N 4Z9. Tel.: +1 250 960 5991; fax: +1 250 960 5539; email: shrimptm@unbc.ca

0022–1112/02/111294+11 $35.00/0

salmon necessitates knowledge of the interaction between the development of seawater tolerance and maturation for formulating effective enhancement efforts. Stream-reared Atlantic salmon were sampled to determine size, gill Na^+,K^+-ATPase activity, and plasma androgens during the autumn, in mid-winter and early spring. Additionally hatchery fish were sampled to evaluate the potential impact of maturation on smolting. Hatchery-reared upper mode (UM) and lower mode (LM) Atlantic salmon were sampled at approximately monthly intervals over 15 months, to examine growth, physical appearance, physiology and endocrinology as assessed by changes in gill Na^+,K^+-ATPase activity, plasma testosterone (T), and plasma 11-ketotestosterone (11-KT) levels.

MATERIALS AND METHODS

STREAM-REARED FISH AND SAMPLING CONDITIONS

Fish currently present in the Connecticut River watershed are the progeny of sea-run and F1 domestic fish that have been stocked as fry. These stream-reared fish were sampled from Ball Mountain Brook (43°05′ N; 72°48′ W) and Winhall River (43°08′ N; 72°51′ W), both tributaries of the West River in southern Vermont. Fish were captured by electrofishing in mid-October, late January, late February and late March. After the fish were stunned in the electric field, they were immediately transferred to a bucket containing 200 mg l^{-1} tricaine methane sulphonate (neutralized and buffered with sodium bicarbonate, pH 7·0).

HATCHERY FISH AND REARING CONDITIONS

Juvenile Atlantic salmon were raised at the White River National Fish Hatchery (Bethel, VT, U.S.A.). Throughout the study, fish were maintained in 4 m circular concrete ponds supplied with well water while under natural photoperiod and fed to satiation daily with automatic feeders and hand feeding. Beginning when the fish were 1 year old (1 year after hatching), fish were sampled from two circular ponds at approximately monthly intervals. Fish were not fed the morning of sampling, which occurred between 1000 and 1300 hours Eastern Standard Time. Eight to 10 fish were captured from a single pond and rapidly transferred to a bucket containing 200 mg l^{-1} tricaine methane sulphonate (neutralized and buffered with sodium bicarbonate, pH 7·0). Following sampling, fish from the second pond were sampled in the same manner. Growth of fish showed a bimodal distribution with significant differences in size between UM and LM fish in each pond (Shrimpton *et al.*, 2000). Saunders *et al.* (1994) showed there was little movement between modal groups throughout rearing. Throughout the study the difference in size between the modal groups was so large that it is unlikely that fish could have moved between the modal groups.

Mature and immature fish were differentiated by morphological differences at capture, but final group designation was based on visual inspection of gonads following dissection. Immature males were characterized by clear gonads that were <1 mm in width. Mature male gonads were opaque and noticeably thickened (2 to 16·1 mm in width). Gonads of previously mature males never returned to looking like immature testes.

SAMPLING PROCEDURES

For both stream- and hatchery-reared fish fork length (L_F), body mass (M) and testes mass (M_G) were measured after anaesthetization. Blood was collected in heparinized syringes from the caudal vasculature within 5 min of first disturbing the fish. Blood was stored on ice for <30 min, centrifuged at 3000 ***g*** for 5 min, plasma removed and frozen on dry ice. A gill biopsy (*c.* six to eight primary gill filaments) was taken and placed in 100 µl of SEI (150 mM sucrose, 10 mM Na_2EDTA, 50 mM imidazole, pH 7·3) on ice for

determining Na^+,K^+-ATPase activity. Samples were frozen on dry ice within 30 min. All samples were stored at -80° C until analysis.

ANALYSIS OF GILL Na^+,K^+-ATPase ACTIVITY

Gill Na^+,K^+-ATPase activity was measured according to the microassay protocol of McCormick (1993). Gill filaments were homogenized in SEI buffer containing 0·1% sodium deoxycholate. Following centrifugation (3000 ***g*** for 0·5 min) to remove large debris, Na^+,K^+-ATPase activity was determined by linking ATP hydrolysis to the oxidation of nicotinamide adenine dinucleotide (NADH), measured at 340 nm for 10 min at 25° C, in the presence and absence of 0·5 mM ouabain. Protein content in the gill homogenate was measured using a bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL, U.S.A.). Specific activities were expressed as μmol ADP mg^{-1} of protein h^{-1}.

DETERMINATION OF PLASMA TESTOSTERONE AND 11-KETOTESTOSTERONE

Plasma T and 11-K were analysed on ether extracted plasma samples according to the protocol of Sower & Schreck (1982) as modified by Fitzpatrick *et al.* (1986). For the assay, 25 μl and 50 μl of plasma were used for T and 11-KT, respectively. Antibodies for T and 11-KT were obtained from Endocrine Sciences Products, Calabasas Hills, CA, U.S.A. The cross-reactivity for the T antibody was 4·7% with 11-KT, and for the 11-KT antibody was 1·0% with T (Fitzpatrick *et al.*, 1986).

CALCULATIONS AND STATISTICAL ANALYSIS

Condition factor (K) and gonado-somatic index (I_G) were calculated as $K=100\ M\ L_F^{-3}$ and $I_G=100\ M_G\ M^{-1}$. For stream-reared fish seasonal changes in L_F, M, K, gill Na^+,K^+-ATPase activity, plasma T and plasma 11-KT, a two-way analysis of variance (ANOVA) was used to determine whether time of sampling or group had a significant effect on these variables. The seasonal data for hatchery fish were divided into three temporal groups: March to June in the first year, and August to March and April to May in the second year. Two-way ANOVA was conducted on: LM males, LM females and UM time interval 1; LM, LM mature and UM time interval 2; LM, LM mature (non-smolts), LM mature S (putative smolts) and UM for time interval 3. When factors were found to be statistically significant, Tukey's test was used to determine differences between the individual groups and time interval. Statistical significance was taken at a level of $P<0·05$. All values are expressed as means ± 1 S.E.

As sexual maturation was determined *post hoc*, sample size of groups varied for both stream-reared and hatchery fish. For the stream-reared mature fish, sample size was 10, seven, six and six, and for immature fish, sample size was 10, one, six and eight, in October, January, February and March, respectively. For hatchery-reared fish sample size varied between four and eight for each group, except for LM mature in August ($n=1$), September ($n=3$), and the second March ($n=3$), LM immature in October ($n=3$), and LM mature S in the second May ($n=3$).

RESULTS

SEASONAL CHANGES IN STREAM-REARED FISH

Stream-reared fish did not differ significantly in length ($P=0·87$) and mass ($P=0·10$) (Table I). Fish showed little growth over the winter and in early spring immature fish were significantly larger and had significantly higher K than mature fish ($P<0·05$).

Plasma T and 11-K were significantly elevated ($P<0·001$) in autumn in mature fish compared to immature fish, but did not differ between these two groups at any other time of the year (Fig. 1). Gonads of mature male parr were enlarged

TABLE I. Mean ± 1 S.E. size (L_F and mass) and condition factor for immature and mature stream-reared Atlantic salmon parr during the autumn and spring. *Value is significantly different from immature fish from the same date. ‡Value is significantly different from October mature value ($P<0{\cdot}05$)

Date	Group	L_F (cm)	Mass (g)	K
October	Immature	12·4 ± 0·5	18·8 ± 0·6	0·98 ± 0·02
	Mature	12·5 ± 0·5	21·4 ± 2·8	1·04 ± 0·01
March	Immature	13·4 ± 0·4	22·6 ± 1·9	0·94 ± 0·03
	Mature	12·2 ± 0·8*	17·4 ± 4·4*	0·90 ± 0·02‡

in October and mature parr could be easily stripped of milt. Gonads of mature parr were also significantly larger than immature parr (I_G values) at all sampling intervals, except for the final early spring sample (Fig. 1). The decline in I_G appeared linear over time.

Gill Na^+,K^+-ATPase activity was low in the autumn and showed a gradual increase over the year in both immature and mature parr (Fig. 1). There were significant differences over time ($P<0{\cdot}0001$) and between mature and immature fish ($P<0{\cdot}0005$). Gill Na^+,K^+-ATPase activity was significantly greater by the end of February compared to the October sample in both immature and mature fish. Gill Na^+,K^+-ATPase activity did not differ between immature and mature fish until the last sample interval at the end of March ($P<0{\cdot}005$).

SEASONAL CHANGES IN HATCHERY FISH

Considerable variation in growth was seen in the Atlantic salmon used in this study. UM fish were significantly larger than LM fish at every sampling interval. The L_F and mass of LM males that became sexually mature in the first autumn did not differ significantly from LM immature fish, although K differed significantly (Table II). Gonads of male parr began to change in appearance and become opaque in the first April of sampling, without an increase in size (Fig. 2). In August, one male parr was sampled that had appreciably developed gonads. The maximum I_G was found in September. In October and November, mature males could be easily stripped of milt (running), but milt could not be stripped from any male after the November sample. After this point, there was a consistent decline in I_G to the end of the study. The decline in testes mass was slower after January. At the last two sample intervals, the gonads were <2% body mass for the LM mature fish, but significant differences in K existed (Table II).

In the first April, UM fish developed silvering and dark fin margins characteristic of smolts, and these fish remained silver until the end of the study. LM fish did not lose parr marks during the first spring, but many developed the silver colouration characteristic of smolts during the second spring. Visual inspection of the gonads indicated that some of the LM males that had matured the previous autumn were silver in the second spring, whereas others retained parr marks and darker pigmentation characteristic of parr.

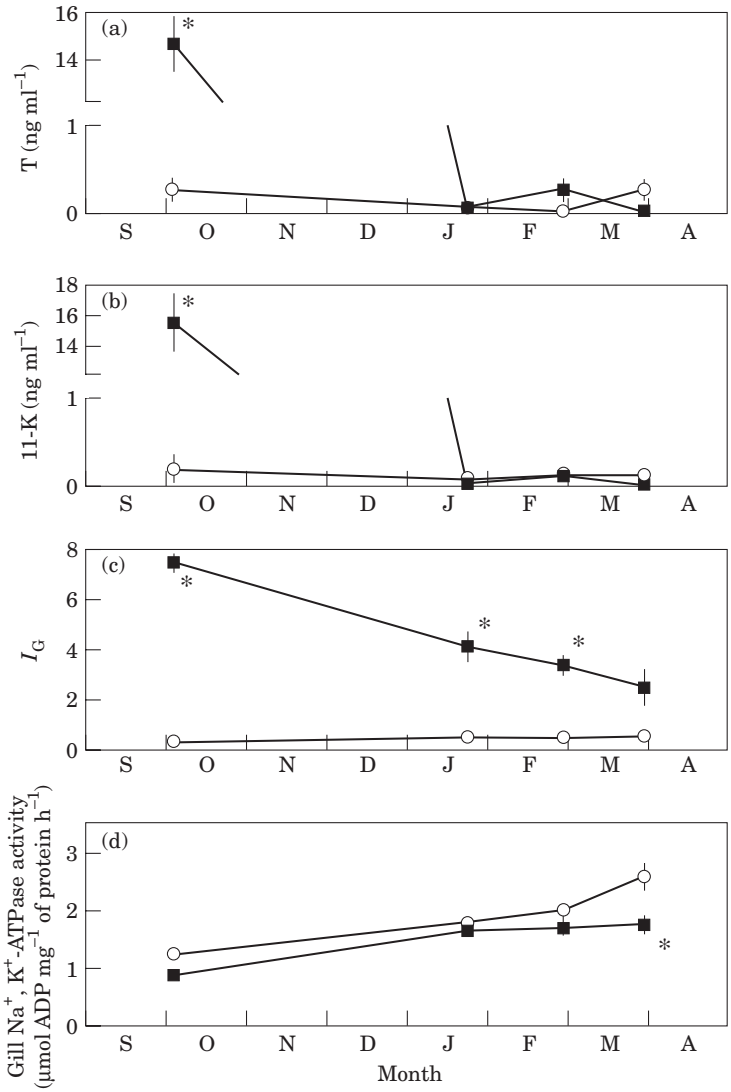

FIG. 1. Seasonal changes in (a) plasma testosterone, (b) plasma 11-ketotesosterone, (c) gonado-somatic index and (d) gill Na^+,K^+-ATPase activity for immature (○) and mature (●) Atlantic salmon parr sampled from Ball Mountain Brook and Winhall River in southern Vermont. Data from the two rivers were pooled for each sample date. *Value is significantly different from mature fish compared to immature fish sampled at the same date. Values are mean ± S.E.

Although little change was seen in androgens from March to June in UM and LM fish (Fig. 2), LM males had significantly greater T (P<0·05) and 11-KT (P<0·001) levels than LM females and UM fish (Fig. 2). LM mature fish showed significant increases in plasma T (P<0·001) and 11-KT (P<0·001) from September to November. In January, plasma T and 11-KT dropped significantly in the LM mature fish to levels below that of the immature UM and LM fish (Fig. 2). In the second spring, both T and 11-KT showed slight increases in some LM mature fish. LM mature fish were divided into two groups *post-hoc* based on

TABLE II. Mean ± 1 S.E. size (L_F and mass) and condition factor for hatchery-reared lower mode (LM) immature and mature, and upper mode (UM) Atlantic salmon parr during the autumn and spring ($P<0{\cdot}05$). LM mature S, putative smolts (see text). *Value is significantly different from LM immature fish from same date ($P<0{\cdot}05$). Data are pooled for the two months indicated

Date	Group	L_F (cm)	M (g)	K	Plasma cortisol (ng ml^{-1})
October–November	LM	22·4 ± 0·2	122 ± 3	1·09 ± 0·01	0·6 ± 0·4
	LM mature	21·0 ± 0·6	116 ± 9	1·25 ± 0·03*	18·5 ± 6·6*
	UM	31·6 ± 1·3*	392 ± 43*	1·23 ± 0·04*	2·0 ± 0·9
April–May	LM	24·7 ± 0·4	152 ± 7	1·00 ± 0·02	16·7 ± 7·5
	LM mature	22·6 ± 1·1	154 ± 25	1·27 ± 0·02*	1·0 ± 0·8*
	LM mature S	24·5 ± 1·5	149 ± 24	1·00 ± 0·07	19·2 ± 10·3
	UM	40·0 ± 1·3*	703 ± 75*	1·07 ± 0·04	23·6 ± 14·2

gill Na^+,K^+-ATPase activity; fish with high gill Na^+,K^+-ATPase activities were designated LM mature S, putative smolts. T was significantly greater in LM mature than LM immature, LM mature S, or UM in April and May ($P<0{\cdot}01$), but 11-KT did not differ significantly ($P=0{\cdot}062$). In April and May, there was no correlation between plasma androgens and gonad mass. Plasma cortisol also showed seasonal changes that differed between the groups of fish examined. LM mature males had significantly elevated plasma cortisol in the autumn, but were the only group that showed no increase during the spring (Table II).

In the second April and May, gill Na^+,K^+-ATPase activity, ranged from 1·5 to 12·1 μmol mg of protein^{-1} h^{-1}. From this wide range in values of gill Na^+,K^+-ATPase activity, it appeared that some of the previously mature fish had smolted and some had not. For fish sampled in April and May, gill Na^+,K^+-ATPase activity was found to be negatively correlated with plasma T concentration ($P<0{\cdot}001$) (Fig. 3), but not 11-KT ($P=0{\cdot}066$). Gill Na^+,K^+-ATPase activity was correlated with K ($P<0{\cdot}001$) (Fig. 3), but not L_F ($P=0{\cdot}409$).

DISCUSSION

In the present study the time course of changes in circulating androgens in hatchery- and stream-reared Atlantic salmon juveniles was established. Immature stream- and hatchery-reared fish showed little change in plasma T and 11-KT over the duration of the study. During the first spring of sampling, however, LM males exhibited slight, but significant elevations in T and 11-KT over LM females and UM fish (Fig. 2). Differences in androgens between males and females during the spring have been reported in coho salmon *Oncorhynchus kisutch* (Walbaum) (Patiño & Shreck, 1986). Sexual dimorphism for T, 11-KT and androstenedione have been shown even earlier in development prior to gonad differentiation in coho salmon (Feist *et al.*, 1990). The greater variation and higher levels of androgens in LM male Atlantic salmon in the present study, therefore, may be linked to future maturation of these individuals.

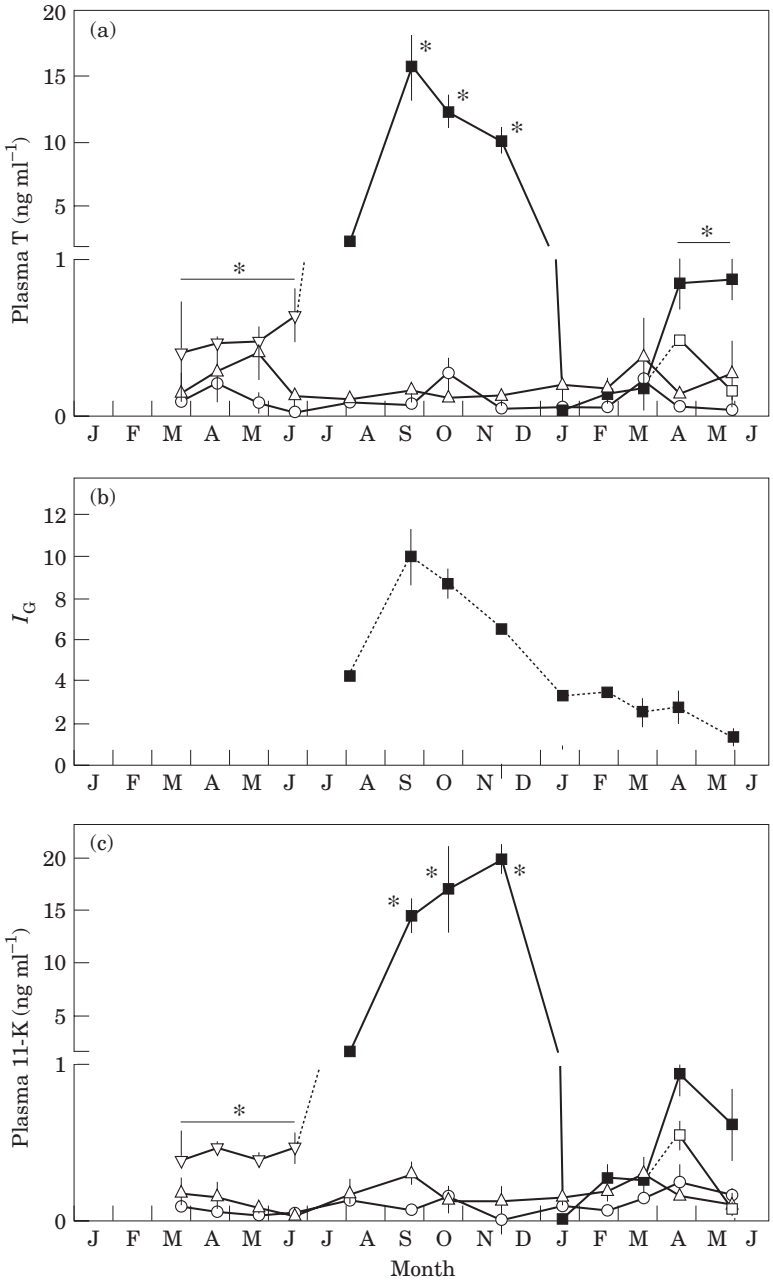

FIG. 2. Seasonal changes in (a) plasma testosterone, (b) gonado-somatic index and (c) 11-ketotestosterone concentration in juvenile Atlantic salmon reared at the White River Nation Fish Hatchery in Bethel, VT, U.S.A. UM (△) are Atlantic salmon that smolt after 1 year, LM (○) are Atlantic salmon that smolt after 2 years, and LM mature (■) are Atlantic salmon that mature as parr. From March to June LM males (▽) were separated from the females; after June LM includes immature males and females. □, are putative smolts (see text). *Value is significantly different from the LM for the same sampling interval. Values are mean ± S.E.

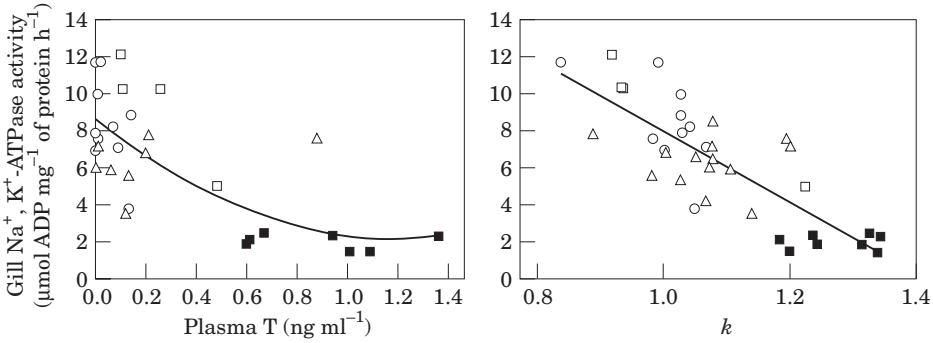

FIG. 3. Correlations for gill Na^+,K^+-ATPase activity with plasma testosterone and condition factor for fish sampled in April and May of their second year. Symbols as described in Fig. 2.

Plasma levels of T and 11-KT were highest during the autumn when the mature male parr were running and could be easily stripped of milt. Mean plasma levels in the autumn for both androgens were similar for stream- and hatchery-reared mature and immature parr (Figs 1 and 2). The values are similar to those reported by previous authors for mature Atlantic salmon parr (Berglund *et al.*, 1995; Moore & Waring, 1999), but two- to three-fold greater than values given by Mayer *et al.* (1990). The androgen titres found in mature parr, however, are half the values reported for mature adult salmon (Sower & Schreck, 1982; Fitzpatrick *et al.*, 1986; Scott & Sumpter, 1989). The reason for the differences in circulating androgen concentration between mature adult salmon and sexually mature parr is not clear. It is possible that the mature parr in this and previous studies lack external stimuli (i.e. adult females) that enhance the endocrine response as demonstrated by Moore & Waring (1999). Both stream- and hatchery-reared salmon from this study were not exposed to any chemicals or sounds associated with spawning adults.

A peak in plasma T has been shown to occur before spermiation, whereas 11-KT reaches a maximum with spermiation in several species of salmonids (Scott *et al.*, 1980; Kime & Manning, 1982). This is consistent with the present findings, where T levels increased first and 11-KT levels peaked coincident with milt expression in males. Plasma cortisol levels were also elevated during the autumn in mature hatchery-reared fish (Table II). Increases in plasma cortisol occur during migration and spawning in adult salmon (Donaldson & Fagerlund, 1972). These authors postulated that this elevation in cortisol may be linked to greater olfactory acuity or postspawning ‘programmed death’ in adults, although the function of an increase in mature parr is not known.

The condition factor of mature hatchery-reared fish remained high throughout the study (Table II), despite significant decreases in I_G (Fig. 2). In April and May, K of the LM mature fish diverged into two groups (Table II), consistent with groupings based on gill Na^+,K^+-ATPase activity (Fig. 3). The lower K in LM mature fish that smolted and the strong positive correlation between K and gill Na^+,K^+-ATPase activity among both mature groups (Fig. 3), therefore, may be associated with the parr-smolt transformation in this group.

Smolting and increased gill Na^+,K^+-ATPase activity were consistently characterized by silver colouration in immature fish. In contrast, increased gill

Na^+,K^+-ATPase activity associated with smolting in the second spring in LM fish that had previously matured was not correlated with silver colouration. Some LM mature fish were dark with visible parr marks, but showed elevated gill Na^+,K^+-ATPase activity. Colouration in the LM mature fish, therefore, did not appear to be associated with smolting. The mechanism for the differences in colouration between the two groups of mature fish may be associated with other endocrine changes that occur during the spring. Rydevik *et al.* (1989) found circulating levels of thyroid hormones to be highly variable in previously mature fish during the spring. Thyroid hormones have been shown to promote changes characteristic of smolting such as silvering (Miwa & Inui, 1985). Thyroid hormones may have been high in some of the previously mature fish in this study, leading to the differences in colouration observed.

A possible consequence of maturation is that smolting is turned off by androgens. Alternatively, lack of smolting is a consequence of different life history trajectories and the ability to smolt is determined much earlier. In the present study, many of the hatchery-reared fish and all of the stream-reared fish that matured the previous autumn did not smolt the following spring based on low levels of gill Na^+,K^+-ATPase activity. Lack of smolting in previously mature stream-reared fish may be a consequence of the size-dependent nature of smolting. Although similar in size to immature fish in autumn, mature parr were significantly smaller in early spring. These fish may have been too small or lacked sufficient energy reserves for the parr-smolt transformation due to maturation the previous autumn. Smolting in previously mature male parr, however, has been shown to occur if fish are of sufficient size (Saunders *et al.*, 1994). In this study all of the hatchery fish were large enough to smolt, but there was over a five-fold difference in gill Na^+,K^+-ATPase activity within the LM mature fish, indicating that some of the previously mature fish did not smolt. These results indicate that factors other than just size determine whether previously mature parr will smolt (for the first time) or mature a second time.

High levels of plasma androgens have been shown to impair seawater tolerance in Atlantic salmon (Lundqvist *et al.*, 1989; Berglund *et al.*, 1992). The high levels of T and 11-KT in the autumn, however, are not likely to have a direct effect on smolting in the spring as androgens decreased to very low levels in January (Figs 1 and 2). Later in the spring (April and May) a slight but significant increase in plasma T was measured in some LM mature fish which was negatively correlated with gill Na^+,K^+-ATPase activity (Fig. 3). Whether such low levels of T are sufficient to inhibit smolting and impair saltwater tolerance is not known. Berglund *et al.* (1995) demonstrated that silastic capsules releasing low levels of T enhanced testes growth and spermiation, whereas higher levels of T were inhibitory. Low androgen concentrations, therefore, may be sufficient to elicit a physiological response such as depressed gill Na^+,K^+-ATPase activity. 11-KT has been shown to decrease basal levels of ACTH (Pottinger *et al.*, 1996) and suppress interrenal activity (Young *et al.*, 1996), although T has been found to have less of an effect. A decrease in interrenal activity by androgens has also been linked to a decreased stress response (Pottinger *et al.*, 1996) and may be a mechanism for lower plasma cortisol levels in the spring (Table II) and lack of an increase in gill Na^+,K^+-ATPase activity in LM mature fish that do not smolt (Fig. 3).

Two lines of evidence indicate high androgens associated with prior maturation will not affect smolting. First, androgen levels have decreased by early winter, well in advance of all of the major manifestations of spring smolting. Second, it can be argued that the high androgens seen in autumn could impact smolting by affecting developmental events (e.g. brain and hypothalamic changes) in autumn. If this were the case, it could be predicted that all fish that previously matured and had high androgens would not smolt the following spring, and this is not the case. The present data does suggest, however, that spring increases in androgens associated with future maturation could impact smolting, since slight elevations in androgens in spring were associated with low gill Na^+,K^+-ATPase activity. There are at least two possible explanations for these results. One is that the spring elevations in androgens and low gill Na^+,K^+-ATPase activity are the result of a developmental decision taken earlier to mature and not to smolt, and that the connection between the two is therefore related but not causal. Alternatively, the small but significant increases in androgens seen in spring may be directly causal to the decision not to smolt.

We thank the staff of the White River National Fish Hatchery, U.S. Fish and Wildlife Service for providing and maintaining the fish used throughout the study. We also thank J. Carey, T. Dubreuil, J. Leonard, M. O'Dea and J. Zydlewski for their assistance sampling the fish. We express our gratitude to C. Slater and C. Schreck for their assistance and supplies for measuring plasma testosterone and 11-ketotestosterone. We thank two anonymous reviewers for their comments that improved the manuscript. Financial support for this work was provided by an Atlantic Salmon Restoration Ecology and Management Research Grant from the Northeastern Research Station of the USDA Forest Service.

References

Berglund, I. (1995). Effects of size and spring growth on sexual maturation in 1+ Atlantic salmon (*Salmo salar*) male parr: interaction with smoltification. *Canadian Journal of Fisheries and Aquatic Sciences* **52,** 2682–2694.

Berglund, I., Mayer, I. & Borg, B. (1992). Effects of sexual maturation, castration, and androgen implants on growth in one- and two-year old parr in a Baltic Atlantic salmon (*Salmo salar* L.) stock. *Journal of Fish Biology* **40,** 281–292.

Berglund, I., Antonopoulou, E., Mayer, I. & Borg, B. (1995). Stimulatory and inhibitory effects of testosterone on testes in Atlantic salmon male parr. *Journal of Fish Biology* **47,** 586–598.

Donaldson, E. M. & Fagerlund, U. H. M. (1972). Corticosteroid dynamics in Pacific salmon. *General and Comparative Endocrinology* (Suppl.) **3,** 254–265.

Feist, G., Schreck, C. B., Fitzpatrick, M. S. & Redding, J. M. (1990). Sex steroid profiles of coho salmon (*Oncorhynchus kisutch*) during early development and sexual differentiation. *General and Comparative Endocrinology* **80,** 219–313.

Fitzpatrick, M. S., Van Der Kraak, G. & Schreck, C. B. (1986). Plasma profiles of sex steroids and gonadotropin in coho salmon (*Oncorhynchus kisutch*) during final maturation. *General and Comparative Endocrinology* **62,** 437–451.

Foote, C. J., Clarke, W. C. & Blackburn, J. (1991). Inhibition of smolting in precocious male chinook salmon, *Oncorhynchus tshawytscha. Canadian Journal of Zoology* **69,** 1848–1852.

Kime, D. E. & Manning, N. J. (1982). Seasonal patterns of free and conjugated androgens in the brown trout, *Salmo trutta. General and Comparative Endocrinology* **48,** 222–231.

Lundqvist, H., Borg, B. & Berglund, I. (1989). Androgens impair seawater adaptability in smolting Baltic salmon (*Salmo salar*). *Canadian Journal of Zoology* **67,** 1733–1736.

Mayer, I., Lundqvist, H., Berglund, I., Schmitz, M., Schulz, R. & Borg, B. (1990). Seasonal endocrine changes in Baltic salmon, *Salmo salar*, immature parr and mature parr. I. Plasma levels of five androgens, 17α-hydroxy-20β-dihydroprogesterone, and 17β-estradiol. *Canadian Journal of Zoology* **68,** 1360–1365.

McCormick, S. D. (1993). Methods for nonlethal gill biopsy and measurements of Na^+,K^+-ATPase activity. *Canadian Journal of Fisheries and Aquatic Sciences* **50,** 656–658.

Miwa, S. & Inui, Y. (1985). Effects of L-thyroxine and ovine growth hormone on smoltification of amago salmon (*Oncorhynchus rhodurus*). *General and Comparative Endocrinology* **4,** 41–52.

Moore, A. & Waring, C. P. (1999). Reproductive priming in mature male Atlantic salmon parr exposed to the sound of redd cutting. *Journal of Fish Biology* **55,** 884–887. doi:10.1006/jfbi.1999.1034

Patiño, R. & Schreck, C. B. (1986). Sexual dimorphism of plasma sex steroid levels in juvenile coho salmon, *Oncorhynchus kisutch*, during smoltification. *General and Comparative Endocrinology* **61,** 127–133.

Pottinger, T. G., Carrick, T. R., Hughes, S. E. & Balm, P. H. M. (1996). Testosterone, 11-ketotestosterone, and estradiol-17β modify baseline and stress-induced interrenal and corticotropic activity in trout. *General and Comparative Endocrinology* **104,** 284–295.

Rydevik, M., Bergström, E., Lundqvist, H. & Eriksson, L. O. (1989). Seasonal changes in thyroid hormones and osmoregulation in mature male parr and 1- and 2-year-old immature Baltic salmon (*Salmo salar* L.) parr. *Canadian Journal of Zoology* **67,** 942–947.

Saunders, R. L., Duston, J. & Benfey, T. J. (1994). Environmental and biological factors affecting growth dynamics in relation to smolting of Atlantic salmon, *Salmo salar* L. *Aquaculture and Fisheries Management* **25,** 9–20.

Scott, A. P., Bye, V. I., Baynes, S. M. & Springate, J. R. C. (1980). Seasonal variations in plasma concentrations of 11-ketotestosterone and testosterone in male rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology* **17,** 495–505.

Scott, A. P. & Sumpter, J. P. (1989). Seasonal variations in testicular germ cell stages and in plasma concentrations of sex steroids in male rainbow trout (*Salmo gairdneri*) maturing at 2 years old. *General and Comparative Endocrinology* **73,** 46–58.

Shrimpton, J. M., Björnsson, B. Th. & McCormick, S. D. (2000). Can Atlantic salmon smolt twice? Endocrine and biochemical changes during smolting. *Canadian Journal of Fisheries and Aquatic Sciences* **57,** 1969–1976.

Sower, S. A. & Schreck, C. B. (1982). Steroid and thyroid hormones during sexual maturation of coho salmon (*Oncorhynchus kisutch*) in seawater or fresh water. *General and Comparative Endocrinology* **47,** 42–53.

Thorpe, J. E. (1987). Smolting versus residency: developmental conflict in salmonids. *American Fisheries Society Symposium* **1,** 244–252.

Young, G., Thorarensen, H. & Davie, P. S. (1996). 11-Ketotestosterone suppresses interrenal activity in rainbow trout (*Oncorhynchus mykiss*). *General and Comparative Endocrinology* **103,** 301–307.