Evidence for cumulative temperature as an initiating and terminating factor in downstream migratory behavior of Atlantic salmon (*Salmo salar*) smolts

Gayle Barbin Zydlewski, Alex Haro, and Stephen D. McCormick

Abstract: Temperature control of Atlantic salmon (*Salmo salar*) smolt migration was tested using a novel technique allowing nearly continuous monitoring of behavior with complete control over environmental conditions. Parr and presmolts were implanted with passive integrated transponder tags, placed in simulated streams, and monitored for upstream and downstream movements. Beginning 18 April, temperature was increased 1 °C every third day (advanced), fourth day (ambient), and tenth day (delayed). Smolt downstream movements were initially low, peaked in mid-May, and subsequently declined under all conditions. Parr downstream movements were significantly lower than those of smolts in all treatments (0.8 ± 0.5 movement·day^{-1} versus 26.5 ± 4.5 movements·day^{-1}, mean ± SE) and showed no increase. At delayed temperatures, smolts sustained downstream movements through July; those under ambient and advanced conditions ceased activity by mid-June. Initiation and termination of downstream movements occurred at significantly different temperatures but at the same number of degree-days in all treatments. Physiological changes associated with smolting (gill Na$^+$,K$^+$-ATPase activity and plasma thyroxine) were coincident with behavioral changes. This is the first evidence of a behavioral component to the smolt window. We found that temperature experience over time is more relevant to initiation and termination of downstream movement than a temperature threshold.

Résumé : Une nouvelle technique qui permet un suivi presque continuel du comportement des saumoneaux de salmons atlantiques (*Salmo salar*) et un contrôle total des conditions environnementales nous a permis d'évaluer le contrôle exercé par la température sur la migration. Nous avons muni des tacons et des pré-saumoneaux d'étiquettes à transpondeur passif intégré, les avons placés dans des cours d'eau artificiels et avons suivi leurs déplacements vers l'amont et vers l'aval. À partir de 18 avril, nous avons augmenté la température de 1 °C à tous les trois (conditions anticipées), quatre (conditions ambiantes) ou dix jours (conditions retardées). Au départ, les déplacements des saumoneaux vers l'aval sont faibles; ils atteignent un sommet à la mi-mai pour diminuer ensuite dans toutes les conditions. Les déplacements des tacons vers l'aval sont significativement moins importants que ceux des saumoneaux dans toutes les conditions ($0,8 \pm 0,5$ déplacement·jour^{-1} par rapport à $26,5 \pm 4,5$ déplacements·jour^{-1}; moyenne ± erreur type) et ne subissent aucun accroissement. Aux températures retardées, les saumoneaux continuent leurs déplacements vers l'aval jusqu'à la fin de juillet, alors qu'aux conditions ambiantes et anticipées, les déplacements cessent à la mi-juin. Le début et la cessation des déplacements vers l'aval se font à des températures différentes, mais à la même sommation de chaleur en degrés-jours, dans les diverses conditions. Les changements physiologiques associés à la transformation en saumoneau (l'activité des la Na$^+$,K$^+$ adénosine triphosphatase et la thyroxine du plasma) coïncident avec la modification des comportements. Il s'agit de la première fois qu'on démontre l'existence d'une composante comportementale à la fenêtre temporelle de la possibilité de transformation en saumoneau. Nous avons trouvé que l'exposition à la température sur une période de temps explique mieux le début et la fin des déplacements vers l'aval qu'un seuil thermique.

[Traduit par la Rédaction]

Introduction

Before spring emigration, juvenile salmon undergo a dramatic change in preparation for life in the ocean: the parr–smolt transformation (reviewed by Hoar 1988). Changes associated with the transformation have been shown or speculated to be influenced by seasonal changes in environmental conditions, especially daylength (photoperiod) and

G. Barbin Zydlewski,[1,2] A. Haro, and S.D. McCormick. US Geological Survey, Conte Anadromous Fish Research Center, Turners Falls, MA 01376, USA.

[1]Corresponding author (e-mail: gayle.zydlewski@umit.maine. edu).
[2]Present address: School of Marine Sciences, University of Maine, 5741 Libby Hall, Room 204A, Orono, ME 04469, USA.

temperature, which both increase in the spring.

Although there is substantial evidence that photoperiod and temperature control smolt physiology, we have only correlative and indirect evidence for the environmental factors controlling downstream migratory behavior of smolts. Field studies have been used to relate environmental factors and population-level downstream movements of salmonids. Downstream migration of Atlantic salmon (*Salmo salar*) smolts has been reported to be initiated at a threshold temperature, 10 °C or slightly above (White 1939; Osterdahl 1969; Solomon 1978). There is also evidence that a temperature threshold does not exist for migratory release of smolts (Jonsson and Ruud-Hansen 1985; Hvidsten et al. 1995). Still other evidence indicates an intermediate role of temperature: where temperature did not seem to have a significant effect on the initiation of migration but seemed to control the subsequent decrease in migratory activity of steelhead trout (*Oncorhynchus mykiss*) (Zaugg and Wagner 1973). Wagner (1974) reported that photoperiod appears to act as a synchronizer of the parr–smolt transformation and when photoperiod is out of phase with temperature, the smolt migration period can be extended or shortened (Wagner 1974). Also, short-term changes in temperature and water flow have been correlated with increased downstream migration in the field (Jonsson et al. 1991; Erkinaro et al. 1998; Aarestrup et al. 2002).

Behavioral differences between parr and smolts include changes in swimming activity, aggressiveness, rheotropism (Godin et al. 1974), schooling behavior (Gibson 1983), diurnal swimming patterns (Thorpe et al. 1988), and critical holding velocities (Graham et al. 1996). Laboratory studies of the parr–smolt transformation have concentrated on differences in behavior between parr and smolts in the spring but have seldom measured downstream migration directly and continuously. Further, changes in behavior between parr and smolt have rarely been studied under controlled environmental conditions.

While there has been no direct experimental evidence of the role of seasonal temperature changes as they affect behavior during the parr–smolt transformation, seasonal increases in daylength and temperature are hypothesized to synchronize physiological and behavioral changes necessary for successful migration to the sea (McCormick et al. 1998). When fish reach the appropriate size-related developmental stage, springtime increases in temperature and photoperiod begin to play a role. Once parr have reached approximately 10–13 cm in autumn, they typically undergo transformation the following spring (Elson 1957). There is a great deal of evidence that some of the physiological factors related to smolting, particularly osmoregulatory changes, can be manipulated by altering photoperiod and temperature. Abrupt increases in daylength result in advances in maximum salinity tolerance and gill Na^+,K^+-ATPase activity (Saunders et al. 1989; McCormick et al. 1996a). Warm rearing temperatures significantly advance the date of peak gill Na^+,K^+-ATPase activity with an upper temperature limit for successful smolting being species dependent (McCormick et al. 1996b). Duston et al. (1991) also indicated that increases in fresh water temperature accelerate the loss of some smolt characters, notably gill Na^+,K^+-ATPase activity.

There is substantial evidence that the environmental control of both physiological and behavioral changes during smolting is mediated by several endocrine systems (Bern 1978). Plasma thyroid hormones are typically elevated during the transformation (Hoar 1988) and have been associated with behavioral changes such as phototaxis (Iwata et al. 1989) and salinity preference (Iwata et al. 1990). There is some evidence, from electrofished and angled Atlantic salmon smolts (McCormick and Björnsson 1994), trap-captured biwa salmon (*Oncorhynchus rhodurus*) fry (Fujioka et al. 1990), and Arctic char (*Salvelinus alpinus*) smolts (Högasen and Prunet 1997), that plasma thyroxine concentration is elevated in migrants compared with non-migrants. There is also evidence that thyroid hormone levels do not always increase concurrently with downstream migration in Atlantic salmon smolts (Youngson et al. 1989). The direct relationship between circulating thyroid hormone levels and downstream movements of individuals remains unclear.

Due to the ambiguity involved with correlating natural migration with environmental factors and physiological condition, we developed a novel laboratory approach to continuously examine downstream migration under completely controlled environmental conditions. The objectives of this study were to (*i*) continuously quantify daily downstream movements of individuals undergoing the parr–smolt transformation under controlled conditions, (*ii*) manipulate environmental conditions to determine whether a temperature threshold exists for initiation of migratory behavior of smolts, and (*iii*) assess the link among osmoregulatory physiology (gill Na^+,K^+-ATPase activity), endocrine status (plasma thyroid hormone levels), and behavior that occur under varying environmental conditions.

Materials and methods

Fish maintenance

Atlantic salmon parr from the White River National Fish Hatchery in Vermont were transported in February 1997 to the Conte Anadromous Fish Research Center on the Connecticut River in Turners Falls, Massachusetts. Fish were kept in flow-through 1.5-m-diameter tanks at 4–6 °C until the beginning of behavioral experimentation. Photoperiod was maintained at natural daylength for the latitude of the laboratory (42°30′N). Fish were fed pelleted Zeigler Brothers feed ad libitum before and during experimentation.

Between 7 and 10 March 1997, fish were anesthetized with 100 mg MS-222·L^{-1} buffered with sodium bicarbonate (pH = 7.0). Fork length and weight were recorded for 220 Atlantic salmon juveniles. Fish of 8.5- to 11.9-cm fork length were classified as putative parr (*n* = 52). Fish of greater than 12.0 cm fork length were classified as putative smolts (*n* = 168). These lengths and classifications were chosen because parr reaching or exceeding 10.0 cm in length at the end of the first growing season (the previous fall) are most likely to become smolts the next spring (Elson 1957). All fish were tagged internally with a 23-mm-long, 3.4-mm-diameter, 0.6-g passive integrated transponder (PIT) tag with a unique eight-digit code (Texas Instruments). A small incision (approximately 4.0 mm) was made on the ventral surface of the fish between the pectoral fins and a tag was placed intraperitoneally. For fish smaller than 10.0 cm, one suture was made in the incision to ensure closure. Prelimi-

nary studies revealed that the incision would close and heal without suturing in fish larger than 10.0 cm and fish of less than 8.5 cm fork length were too small to carry these tags. Fish were allowed to recover for 1 month before being placed into experimental conditions.

Behavioral experimentation and physiological sampling

On 2 April 1997, length, weight, and PIT tag number were recorded for 75 previously tagged fish. Eighteen putative smolts and seven putative parr were placed in three 1.5-m-diameter circular tanks designed to record upstream and downstream movements of individually marked fish (Fig. 1). Similarly, on 4 April 1997, 120 salmon were split between three 2.4-m-diameter circular tanks (32 putative smolts and eight putative parr in each) designed as above. On both dates, nonlethal gill biopsies were taken for analysis of gill Na^+,K^+-ATPase activity.

Each circular tank had a barrier in the middle and a pump circulated water around the barrier. Water supply to the tanks was flow-through. Water velocity through the narrow portion of the tank was maintained at 50 cm·s^{-1} and ranged from 10 to 40 cm·s^{-1} around the wide portion of the tank depending on distance from the outside wall. Gravel and cobble substrate was placed in the widened portion of the tank and black plastic was attached to the top of the tank to provide shade. Two antennas were built to fit in the narrow portion of each tank. The antennas were 0.3 m apart in the 1.5-m tanks and 0.5 m apart in the 2.4-m tanks. The two antennas were wired to two TIRIS S2000 readers with a different reader energizing each antenna. When in the field of an antenna, a tag would be energized and the reader number, date, time, and tag number were recorded on a computer. The readers were programmed to sequentially switch between pairs of antennas in each of four tanks every 5 min. Tag detections at each antenna were recorded for 2.5 min (12–13 reads·s^{-1}) during every 20-min period of the day. Behavioral recordings began on 11 April 1997 and continued until 24 July 1997, except for 20 June when there was a power outage. On 18 April, the maintenance temperature of 4–6 °C was terminated and three temperature regimes were initiated: ambient, advanced, and delayed (Fig. 2). Treatments were achieved by changing the amount of chilled, heated, and ambient Connecticut River water sources into the tank but maintaining constant overall flow rate for all tanks. Based on spring temperature changes over the previous 5 years, the ambient group experienced approximately a 1 °C increase every fourth day until 26 May when the temperature reached 16 °C. The advanced group experienced approximately a 1 °C increase every third day until 10 May when the temperature reached 16 °C; this rate of increase is rare but does occur at this latitude. The delayed group experienced approximately a 1 °C increase every tenth day; this rate of increase simulates a late spring at this latitude or a more typical spring at a northern latitude. Each temperature regime was simulated in two tanks, one 1.5-m and one 2.4-m tank.

On 19 June length, weight, PIT tag number, nonlethal gill biopsies, and nonlethal blood samples were taken from approximately eight smolts in each tank. Gill biopsies were used to determine gill Na^+,K^+-ATPase activity and plasma samples were used to determine thyroxine (T_4) and 3,5,3′-

Fig. 1. Schematic representation of the circular stream tank used to monitor movements of individual Atlantic salmon. A barrier to water flow is shown as a cross-hatched structure in the center of the tank. The barrier helped produce unidirectional water motion throughout the tank. Gravel and cobble substrate was on the bottom of the tank in the widened portion. Two antennas, encircling the water column in the narrowed region of the tank, were wired to readers and a datalogging computer.

triiodo-L-thyronine (T_3) concentrations. All fish were returned to their original tanks to analyze behavior subsequent to physiological sampling. Physiological sampling of the fish was not conducted before 19 June in an effort to maintain the integrity of the behavioral data being collected.

Behavioral experiments were terminated on 24 July. Length, weight, PIT tag number, gill biopsies, and blood samples were taken from all fish. Sex was determined for all fish. Based on morphological appearance (presence–absence of parr marks, degree of silvering, darkening of fin margins) and gill Na^+,K^+-ATPase activity levels, it was evident that some of the "putative" parr underwent the parr–smolt transformation. Rather than reclassifying these fish as smolts a posteriori, they were removed from the analyses. Similarly, some fish less than 13.0 cm fork length did not demonstrate physiological and morphological smolt characteristics. Therefore, parr and smolts were more conservatively classified as fish between 8.5 and 10.5 cm fork length in April (9.1 ± 0.2 cm, 7.5 ± 1.0 g) and fish greater than 13.0-cm fork length in April (14.5 ± 0.1 cm, 30.2 ± 0.7 g), respectively. These criteria better fit the description of parr–smolt transformation reported by Elson (1957). Only fish meeting these size criteria were used in the data analysis. Twelve fish died in mid-June when temperatures in the advanced and ambient tanks were approaching 24 °C. There was 100% tag retention during the experimental period. Final sample sizes were 15 parr and 26 smolts in the ambient group, 14 parr and 30 smolts in the advanced group, and 12 parr and 26 smolts in the delayed group.

Length, weight, and condition factor were compared using a nested (tank nested within treatment) repeated-measures ANOVA general linear model (GLM) for treatment, developmental state (parr versus smolt), and tank effects. Least square means (LSMeans) tests were performed for pairwise comparisons. Tests were also conducted for smolts alone to test for treatment and tank effects. Specific growth rate from 2 or 4 April to 24 July were compared using a nested ANOVA (GLM) for treatment and tank effects.

Fig. 2. Seasonal daily movements (upstream and downstream) of Atlantic salmon parr and smolts maintained under three temperature regimes: (*a*) ambient, (*b*) advanced, and (*c*) delayed. The *x* axis of each panel indicates the day of the year; each indicated date represents the 2-week period used for statistical analyses (periods 1–7). The *y* axis of each panel is the mean ± SE of the number of movements per individual parr or smolt. Solid bars, positive values, are daily downstream movements and open bars, negative values, are daily upstream movements. The second *y* axis and associated line on each graph indicate the temperatures experienced in the treatment groups. Data are shown separately for parr and smolts from each treatment group. Asterisks and pound signs indicate when values are significantly (ANOVA (GLM) on individual data, $p < 0.05$) greater or less than, respectively, movements in the ambient group. The arrowed line indicates values significantly greater than movements in the ambient group for the period of time indicated by the line.

72

Can. J. Fish. Aquat. Sci. Vol. 62, 2005
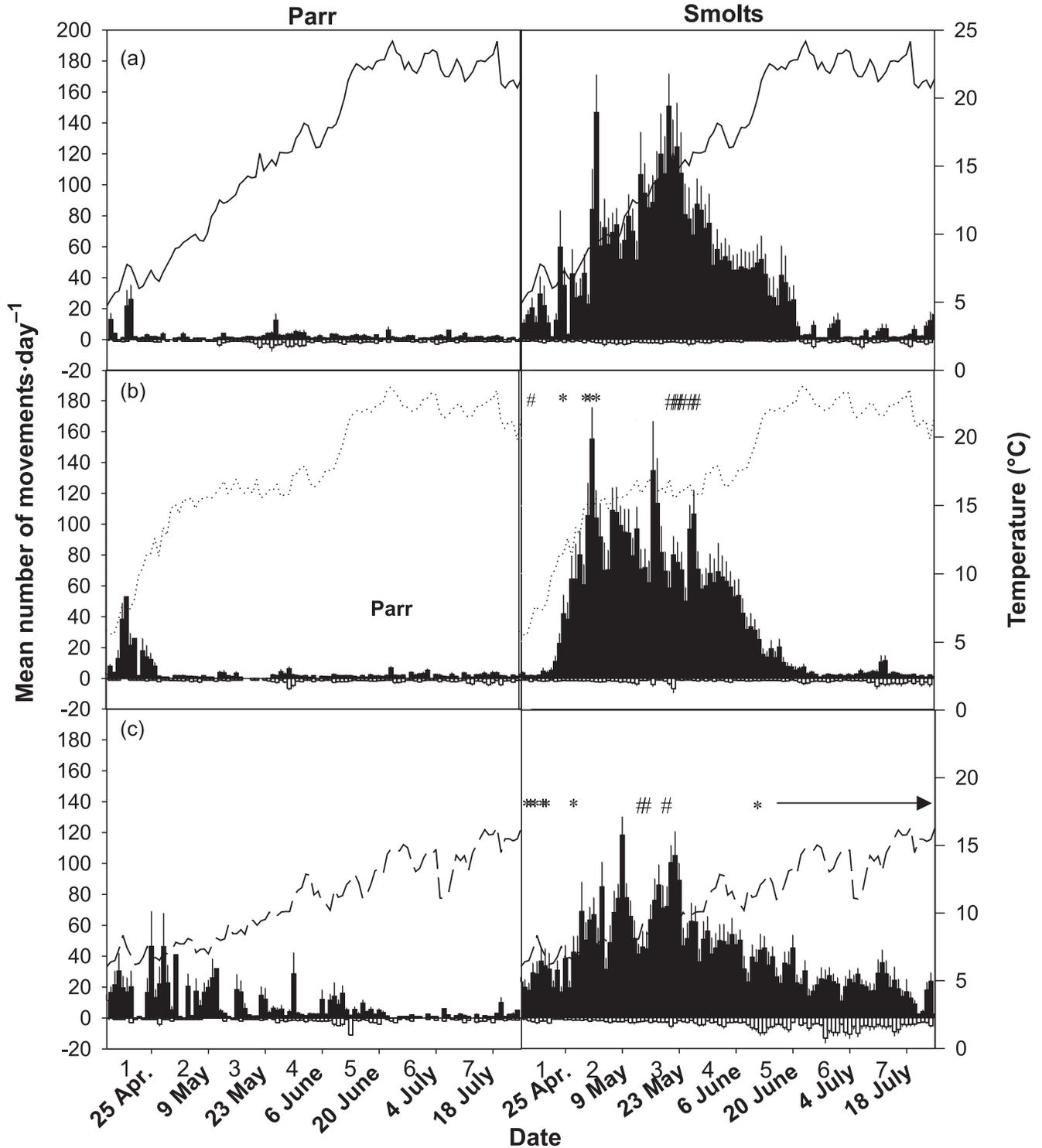
Behavioral quantification

Movements of parr and smolts were quantified to examine seasonal and daily changes. The frequency distribution of tag detections revealed a cluster of alternating reads below 0.3 s and one above 0.3 s. Those between 0.3 and 3 s were actual movements between antennas, and those below 0.3 s were crosstalk between the antennas and were eliminated from the data set. If the time taken to move between antennas was greater than 3 s, the movement was considered to be around the widened portion of the tank and not direct movement between the antennas in the narrow portion of the tank and removed from the data set. To swim around the inner perimeter of the 1.5-m tanks in 3 s, a 20-cm fish would have to swim at 3.5 body lengths·s^{-1}. Fängstam et al. (1993), with a similar apparatus, reported movements of 2.3 body lengths·s^{-1} during peak migration for 15- to 19-cm Baltic salmon (*S. salar*). Individual daily number of movements (for the 3 h of the day that recordings were made, 7.5 min·h^{-1}; not extrapolated to a full day) was used to examine seasonal patterns of behavior. Individual variation is reported as mean ± SE.

To assess treatment (ambient, advanced, delayed), developmental (parr versus smolt), and tank effects on movements while maintaining individual variation (Ruohonen 1998), a nested (tank within treatment) repeated-measures ANOVA (GLM) was used. Data were not normally distributed and were therefore ranked (lowest to highest using Statistica; StatSoft, Inc.) to conduct the following analyses. The ANOVA design included date as a repeated measure because all individual movements were quantified per day throughout the study. The number of downstream and upstream movements conducted by each individual was summed for each day and used in the repeated-measures analysis. Net movement of individuals was calculated by subtracting the number of upstream movements from the number of downstream movements. Seasonal patterns of downstream movements, particularly for smolts, were not linearly related to time. Therefore, the migratory season was broken into 2-week periods, each of which approached linearity, and separate ANOVAs were run on seven 2-week periods. Nested repeated-measures ANOVAs (GLM) were run for each behavioral period for comparisons of treatment, developmental, and tank effects. When developmental effects were significant, treatment effects were examined for smolts only. Reported significant differences among treatment and developmental groups take into account any tank effects, where differences among treatments or developmental stages are significant above and beyond differences between tanks within treatment group (therefore, possible differences between 1.5- and 2.4-m tank replicates were accounted for in the analyses). LSMeans tests were performed for pairwise comparisons between treatment groups and developmental groups.

To examine the average treatment (temperature) effect on smolt migration, the 7-day running average of the daily number of downstream movements was calculated for smolts in each treatment group. Degree-days were calculated as the cumulative mean daily temperature experienced by the fish since 1 January 1997. To analyze the relationship between degree-days and downstream movement, the transition period from low to elevated downstream activity was defined as the date where smolt downstream movements exceeded the 95th percentile of parr movements (20 downstream movements per day). This was defined as the date that the "behavioral" smolt window opened. For each treatment regime, an endpoint to the window was specified as when smolt downstream activity returned to within the 95th percentile of parr movements. The date that the smolt window opened and closed, the temperature and degree-days experienced on open and close, and the number of days that the "behavioral" smolt window remained open were compared for all three treatment groups. Kruskal–Wallis and Kolomogorov–Smirnov paired tests (when appropriate) were used because some of the data were not normally distributed and did not have homogeneous variances.

Physiological measurements

Approximately two primary gill filaments taken from fish in April, June, and July were placed in individual tubes with 100 µL of ice-cold SEI buffer (250 mmol sucrose·L^{-1}, 10 mmol $Na_2EDTA·L^{-1}$, 50 mmol imidazole·L^{-1}, pH = 7.3) and frozen within 1 h at –80 °C. Gill Na^+,K^+-ATPase activity was measured using the microassay method of McCormick (1993).

Plasma samples collected in June and July were assayed for T_4 and T_3. Concentrations were assayed by a direct radioimmunoassay (Dickhoff et al. 1978) as modified by McCormick and Naiman (1984). Intra- and interassay coefficients of variation for these assays were 2.9–10.6% and 12.4–16.2%, respectively.

A two-way ANOVA was used to test for treatment and date effects on gill Na^+,K^+-ATPase activity and plasma thyroid hormone levels. When appropriate, LSMeans post hoc tests were used for multiple comparisons. Data did not meet the assumptions of the ANOVA and were ranked (lowest to highest using Statistica; StatSoft, Inc.) to run the analyses.

Results

Upstream and downstream movements of parr and smolts were continuously quantified from early spring into summer. At the beginning of the experiment (11 April), smolts demonstrated little movement in either the upstream or the downstream direction. Net movement of smolts subsequently became downstream in all groups with an average net movement per day (over the entire experiment) of 26.5 ± 4.5 (*n* = 82). Net downstream movement of smolts ended in the ambient and advanced treatments by 23 June when levels reached the low levels demonstrated by parr. Parr net movement was also downstream but at significantly lower levels than smolts: 0.82 ± 0.52 (*n* = 42) (ANOVA, *p* = 0.0001, df = 1, 116). Parr and smolts differed significantly in the daily number of downstream movements in all behavioral periods, with smolts demonstrating more downstream movements than parr (ANOVA, *p* < 0.0005 for all cases, df = 1, 116).

At ambient temperature, downstream movements increased around 24 April, with a peak occurring during the week of 14 May (Fig. 2*a*). Thereafter, the number of downstream movements declined to reach parr levels by 23 June. The increase in downstream movements far exceeded changes in upstream movements. At advanced temperature, smolts behaved similarly to those in ambient temperature: increased numbers of downstream movements around 16 April with a

Fig. 3. Seven-day running average of the number of downstream movements of Atlantic salmon smolts in the associated temperature treatment groups (mean ± SE). Values for gill Na^{+},K^{+}-ATPase activity are provided for the 3 days when gill biopsies were taken from individuals. Asterisks and pound signs indicate when delayed-group downstream movement values are significantly (ANOCA (GLM) on individual data, $p < 0.05$) greater or less than, respectively, movements in the ambient group. Plus and less than signs indicate when advanced-group downstream movement values are significantly greater or less than, respectively, movements in the ambient group. The arrowed line indicates values in the delayed group significantly greater than movements in the ambient group for the period of time indicated by the line.
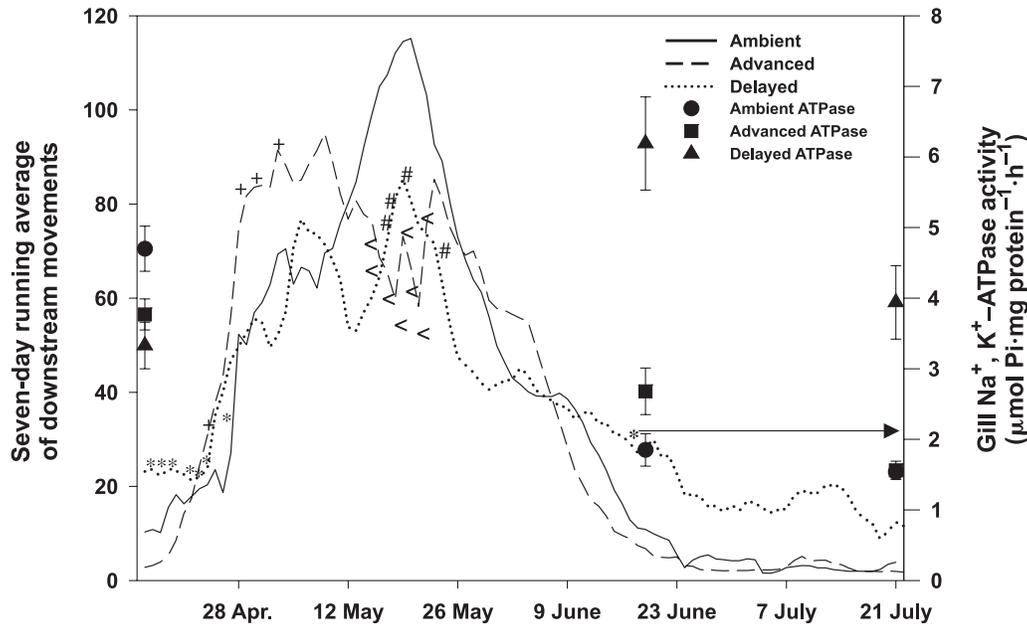
peak during the week of 8 May and a decline to parr levels by 15 June (Fig. 2b). At delayed temperature, smolt behavior was very different from that of those in ambient and advanced temperatures: increased numbers of downstream movements around 28 April with a peak during the week of 10 May followed by a slow decline that never decreased to parr levels or smolt levels in the other two temperature groups (Fig. 2c).

Temperature treatments resulted in different migratory behavior, as expressed by net movement and downstream movements. The effect of temperature treatment on net movements was significant for all days tested (ANOVA, $p = 0.0013$, df = 2, $n = 116$). Temperature effects on net movements were significant during periods 1, 3, and 5–7 (ANOVA, $p < 0.005$, df = 2, $n = 116$) but not periods 2 (weeks 3 and 4) or 4 (weeks 7 and 8) (ANOVA, $p > 0.05$, df = 2, $n = 116$). The same effects were seen for downstream movements; temperature effects were significant in all periods except for periods 2 and 4. When the complete distribution of downstream movement data (mean and standard error) was analyzed, there were no significant differences between temperature treatments (ANOVA, $p = 0.118$ and $p = 0.090$). However, LSMeans significant differences between the advanced and ambient groups are seen in periods 2 and 4 (Figs. 2 and 3) but do not contribute enough to result in significant differences among all three treatment groups. Significant differences during the last two periods were due to the significantly higher number of downstream movements maintained by smolts in the delayed temperature compared with the low numbers demonstrated by smolts in the ambient and advanced temperatures (LSMeans, $p < 0.05$). Using the

7-day running average of downstream activity, the large difference in the duration of activity in the delayed group was obvious (Fig. 3). Smolts in the delayed group remained above the 95th percentile of parr downstream movement until 3 July, while smolts in the ambient and advanced groups declined to the 95th percentile of parr downstream movement levels by 15 and 10 June, respectively.

The duration of downstream activity was significantly longer under delayed temperature than at other temperatures (ANOVA treatment effect in periods 5–7, $p = 0.001$, df = 2, $n = 116$). This sustained period of downstream movement is evident in the differences between parr and smolt behavior at this time. During the last two periods of the experiment, smolts at ambient and advanced temperatures did not differ from parr in number of downstream movements (ANOVA developmental effect, $p > 0.50$). Smolts experiencing delayed temperature had sustained downstream movements above parr levels during both of these periods (ANOVA developmental effect, $p < 0.0001$, df = 1, $n = 116$).

The absolute temperature at which downstream activity increased differed significantly among the different temperature treatments, indicating that a single temperature does not initiate downstream movement (Fig. 4). Downstream movements increased at 9.1 °C for smolts at ambient temperatures, at 12.5 °C for smolts at advanced temperatures, and at 7.2 °C for smolts at delayed temperatures (Table 1) (Kruskal–Wallis, $p < 0.0001$). However, cumulative degree-days since 1 January was a better predictor of downstream migratory behavior than absolute temperature alone. At 649–700 degree-days, all smolts initiated downstream movements and there were no significant differences among the groups

Fig. 4. Seven-day running average of the number of downstream movements conducted by Atlantic salmon smolts in the associated temperature treatment groups (circles, ambient; squares, advanced; triangles, delayed) shown as a function of (a) temperature and (b) cumulative degree-days since 1 January.
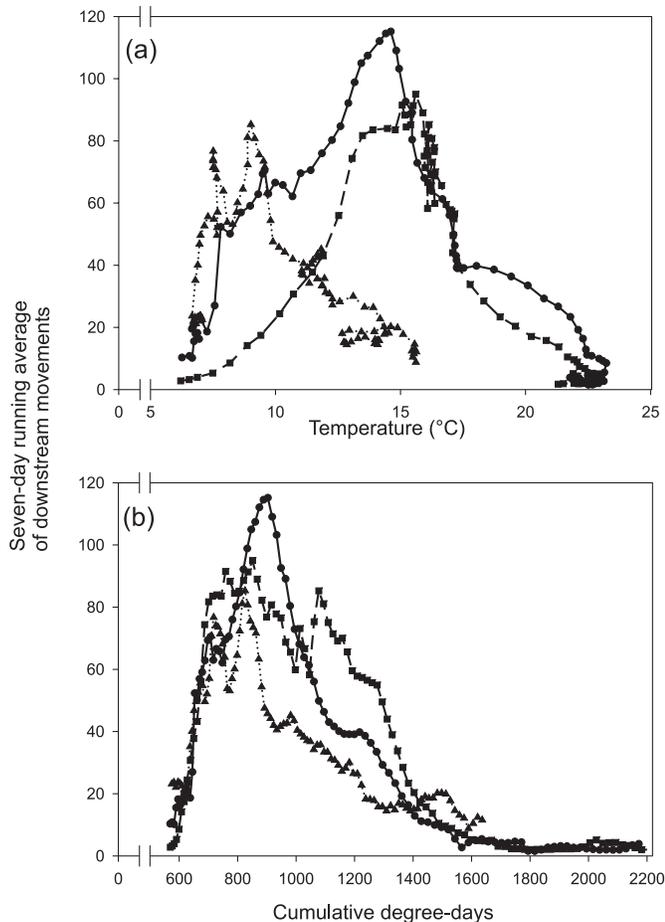
in the degree-days at downstream initiation (Table 1) (Kruskal–Wallis, $p = 0.09$, df = 2).

Loss of downstream activity at ambient and advanced temperatures occurred during the first week in June, when temperatures reached approximately 16 °C. A decline, but not a complete loss, is also seen in the delayed group just over 1 week later. Cumulative degree-days also seems to be important when determining the termination of migration (Table 1). There was no difference among groups for the degree-days experienced when the behavioral smolt window closed, 1132–1256 (Kruskal–Wallis, $p = 0.3129$, df = 2), but there were differences for the absolute temperature on closure, 11.8–18.8 °C (Kruskal–Wallis, $p < 0.0001$, df = 2).

Based on morphological appearance in July (silvering, loss of parr marks, and dark fin margins), fish larger than 13 cm in April underwent the parr–smolt transformation. Final length, weight, condition factor, and specific growth rates differed significantly between parr (initial length = 10.5 cm) and smolts (initial length = 13 cm) within treatments. Temperature treatment had a substantial effect on growth dynamics associated with smolting. This effect is evident as lower final weight (ANOVA treatment effect, $p = 0.0065$, df = 3, $n = 56$), condition factor (ANOVA treatment

effect, $p = 0.002$, df = 3, $n = 56$), and specific growth rates (ANOVA treatment effect, $p = 0.01$, df = 3, $n = 56$) of smolts experiencing a delayed temperature regime (Table 2).

Temperature treatment had a significant effect on gill Na^+,K^+-ATPase activity (Fig. 3), plasma T_3 levels, and plasma T_4 levels (Fig. 5). Significant differences (two-way ANOVA, treatment effect: $p < 0.0001$, df = 2, $n = 167$; date effect: $p < 0.0001$, df = 2, $n = 167$; interaction: $p < 0.001$) were due to significantly higher gill Na^+,K^+-ATPase levels for smolts at delayed temperatures during the last two sampling periods (LSMeans, $p < 0.0001$, $n = 167$). On the individual level, there was a positive correlation between gill Na^+,K^+-ATPase activity and number of downstream movements of smolts recorded on the day before samples were taken in June (Pearson, $r^2 = 0.35$, $p < 0.0001$, $n = 44$) and in July (Pearson, $r^2 = 0.19$, $p = 0.0001$, $n = 69$). Temperature treatment effects were observed for plasma T_4 levels (Fig. 5a) (two-way ANOVA, treatment effect: $p < 0.001$, df = 2, 102; date effect: $p < 0.001$, df = 1, 102; interaction: $p < 0.001$). In June, smolts experiencing delayed temperature demonstrated the highest levels of plasma T_4 (LSMeans, $p < 0.005$, df = 2, $n = 44$). In July, smolts at advanced temperature demonstrated higher levels of plasma T_4 than those at ambient temperature (LSMeans, $p < 0.02$, df = 2, $n = 44$). Temperature treatment also affected plasma T_3 levels (Fig. 5b) (two-way ANOVA, temperature effect: $p < 0.01$, df = 2, $n = 102$; date effect: $p < 0.001$, df = 1, $n = 102$; no interaction: $p = 0.43$). On 19 June, smolts experiencing ambient temperature had significantly lower levels of plasma T_3 than those at advanced (LSMeans, $p < 0.01$, df = 2, $n = 108$) and delayed (LSMeans, $p < 0.001$, df = 2, $n = 108$) temperatures. Although there were no pairwise differences (among treatments) on 21 July, the same trends are apparent, with the smolts at delayed temperatures having the highest levels of plasma T_3.

Discussion

This study provides the first direct evidence that different temperature regimes, particularly temperature change experienced over time (degree-days), can modify downstream movement of Atlantic salmon smolts. Under identical environmental conditions, smolts expressed significantly more downstream movements than parr. Smolts that experienced an early and rapid increase in temperature (advanced group) expressed an early peak in downstream movements, but the termination of the migratory season was similar to that of those experiencing a normal temperature increase (ambient group). Most notably, the fish experiencing a late and slower increase in temperature (delayed group) in the spring expressed a similarly timed peak in movement but a longer migratory period. Therefore, an early increase in temperature resulted in an early increase in downstream movement of smolts, whereas sustained cold spring temperatures resulted in a sustained migratory period. Degree-days is a better indicator of the initiation and termination of downstream movement than is absolute temperature. This is in opposition to the idea that there is a temperature threshold for initiation of downstream movement.

Few laboratory studies have examined smolt downstream migratory behavior directly, and no studies have examined the

Table 1. Opening and closing dates, temperatures, and calculated degree-days (mean ± SE) for the "behavioral" smolt window of Atlantic salmon smolts maintained under three temperature treatments in simulated stream conditions in 1997.

Behavioral window	Ambient	Advanced	Delayed
Open date	30 April ± 1.9	27 April ± 1.5	25 April ± 1.8
Close date	5 June ± 2.4	1 June ± 2.9	12 June ± 5.1
Temperature on open (°C)	9.1±0.5a	12.5±0.5b	7.2±0.1c
Temperature on close (°C)	18.8±0.7a	18.4±0.5a	11.8±0.5b
Degree-days on open	688±17.7	700±20.5	649±13.2
Degree-days on close	1196±44.7	1256±50.1	1131±59.1
Days open	36±3.5	35±3.5	48±6.0

Note: Different letters indicate statistical differences ($p < 0.05$) among treatment groups.

Table 2. Initial and final size, condition factor, and growth (mean ± SE) of Atlantic salmon smolts maintained under three temperature treatments in simulated stream conditions in 1997.

Growth information	Ambient	Advanced	Delayed
Initial length (cm)	14.8±.2	14.4±.2	14.3±.3
Final length (cm)	20.6±.3	21.2±.3	20.7±.3
Initial weight (g)	31.9±1.6	29.2±1.5	29.3±1.7
Final weight (g)	104.5±4.3ab	115.1±4.0a	97.7±4.7b
Initial condition factor ($100 \times$ g·cm^{-3})	0.968±0.010	0.966±0.010	0.990±0.011
Final condition factor ($100 \times$ g·cm^{-3})	1.18±0.018a	1.18±0.017a	1.10±0.020b
Specific growth (g·day^{-1})	0.0089±0.0005a	0.0109±0.0005b	0.0091±0.0006a

Note: Different letters indicate statistical differences ($p < 0.05$) among treatment groups.

influence of manipulated environmental factors on smolt downstream movements under completely controlled conditions. Wagner (1974) examined downstream movement of steelhead trout. He found that smolts exposed (in the laboratory) to changing temperature were captured at a higher rate in a downstream trap after release than those exposed to constant temperature. Fängstam et al. (1993) examined the effect of early maturation on downstream movement of Baltic salmon using a similar experimental apparatus as used in the current study. Berglund et al. (1994) examined the effect of steroids on downstream migration of smolts of Baltic salmon and found that injection of the androgen 11-ketoandrostenedione resulted in reduced downstream movement of smolts. Lundquist and Eriksson (1985) examined the annual cycle of swimming behavior in previously mature male Baltic salmon with a similar apparatus. They found an increase in downstream movements during spring with a reversal of swimming behavior in late summer under constant photoperiod and temperature, suggesting a circannual rhythm of smolting.

Field studies of smolting salmonids have shown patterns of migration similar to those found in the present study (Jonsson and Ruud-Hansen 1985; Veselov et al. 1998; Antonsson and Gudjonsson 2002). Specifically, Whalen et al. (1999) have shown that peak migration occurs later in the season for tributaries with lower temperature. Also, McCormick et al. (1999) has shown that annual differences in the time to 50% migration of Atlantic salmon smolts from the Connecticut River are related to annual temperature differences. In a field study using release of laboratory-reared fish, Wagner (1974) found that when the temperature cycle was out of phase and behind the photoperiod cycle, the smolt migratory period of steelhead trout was extended (as seen in our delayed treatment), and when temperature was

out of phase and ahead, the migratory period was shortened (as seen in our advanced treatment).

Our study indicates that absolute temperature alone is not a regulator of downstream migration because downstream movement is initiated at significantly different temperatures for each of the temperature treatment groups. The temperature effects on migration observed in our study are best explained through the expression of temperature as cumulative degree-days because downstream movement is initiated at the same number of degree-days when fish experience varying temperature regimes. Various environmental factors have been suggested as regulators of migratory release. Some authors have suggested that there is a temperature threshold for the release of migration (White 1939; Fried et al. 1978; Veselov et al. 1998). If field data (particularly Veselov et al. 1998; Antonsson and Gudjonsson 2002) were reinterpreted using degree-days, the link between degree-days and downstream movement may be discovered.

The effect of the interaction between temperature and time, expressed as degree-days, on both the physiology and behavior of smolts may indicate that some feature experienced over time interacts with temperature to regulate smolting. Perhaps the idea that temperature modifies the rate of response to photoperiod for physiological changes (reviewed by Hoar 1988) is also true for behavioral changes associated with smolting. Timing associated with the loss of behavioral smolt characters (i.e., smolt downstream movements returning to parr levels) also had a dependence on degree-days. At the same time, physiological indicators of smolting, particularly gill Na$^+$,K$^+$-ATPase activity, returned to low levels late in the season. While reviewing the relationship of smolt physiology and temperature, McCormick et al. (1996b) concluded that decreased gill Na$^+$,K$^+$-ATPase activity was di-

Fig. 5. Mean and SE of (*a*) plasma T_4 and (*b*) plasma T_3 levels measured on Atlantic salmon smolts from simulated streams maintained at three temperature treatments throughout the migratory season (solid bars, ambient; hatched bars, advanced; open bars, delayed). Statistical differences within each date are indicated by different letters above the bars.
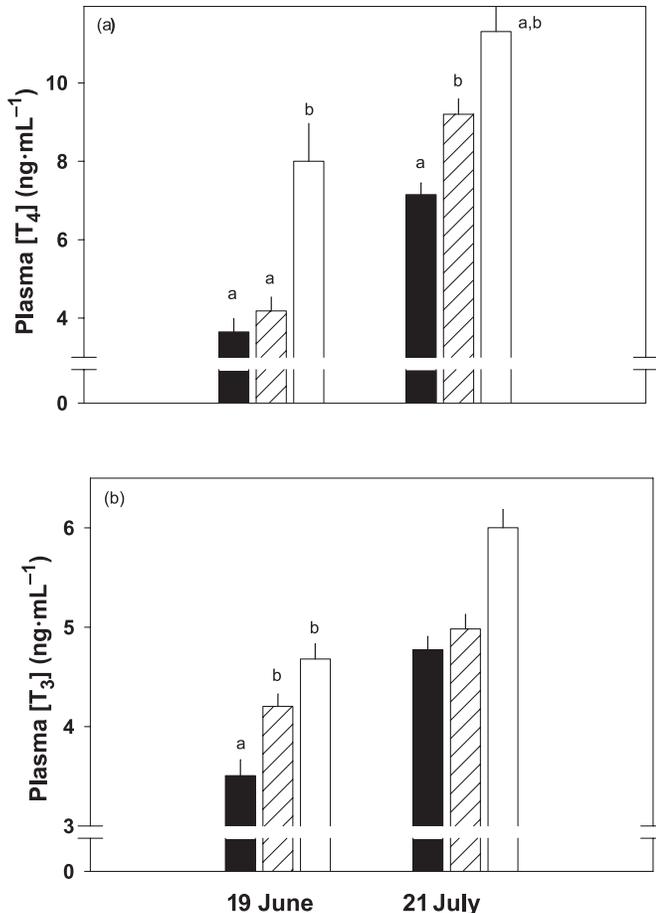
rectly related to degree-days.

Changes in smolt physiology such as gill Na^+,K^+-ATPase activity and salinity tolerance show the same pattern of increase and decline when smolts are maintained in fresh water at high water temperatures (Duston et al. 1991; McCormick et al. 1996*b*) as those demonstrated behaviorally in the present study. From the physiological data, an optimal time frame for migration, controlled by the area's environmental conditions, has been implicated as a physiological smolt window (McCormick et al. 1998). The current study shows that there is not only a physiological limit to the timing of migration but also a limit to behavioral motivation under various environmental conditions that may play a role in the ultimate success of migration to seawater, a behavioral smolt window. Furthermore, as with the opening of the behavioral smolt window, the closure is also likely to be regulated through some interaction of temperature over time. The occurrence and possible relationship between the physiological and behavioral smolt windows have implications for the duration of migration available for seawater entry. For example, if smolts are delayed at dams or kept at hatcheries for

extended periods before release, then even if released past dams or into streams, they are not likely to express downstream movements in fresh water at high temperatures or after a certain number of degree-days. Thus, the observed behavioral smolt window indicates that delays in migration may decrease smolt survival based on both physiological and behavioral parameters.

There seems to be a close, although not necessarily causal, relationship between downstream movement and osmoregulatory physiology, as had been previously speculated (Hoar 1988; McCormick et al. 1998). Typically, gill Na^+,K^+-ATPase activity peaks in early to mid-May, depending on annual temperature (McCormick et al. 2000). The patterns of gill Na^+,K^+-ATPase activity in our study followed the same pattern as the behavior. In this study, physiological measurements were not made during the behavioral peak to maintain the integrity of behavioral quantification. While maintaining cooler than normal temperatures in the delayed group, the gill Na^+,K^+-ATPase activity remained high; this relationship was also reported by Sigholt et al. (1998). This is particularly interesting in light of the fact that the number of downstream movements remained high as well, whereas the physiological and behavioral parameters declined in both the ambient and the advanced groups. There was a clear relationship between gill Na^+,K^+-ATPase activity and downstream migratory behavior at the individual and treatment level. Pirhonen and Forsman (1998), examining brown trout and sea trout (*Salmo trutta*) behavior and gill Na^+,K^+-ATPase activity, found a relationship at the population level but not at the individual level. The discrepancy at the individual level may be related to species differences or differences in experimental conditions, as has been seen for brown trout (Nielsen et al. 2004).

Thyroid hormones have been implicated in behavioral changes associated with smolting (Iwata 1995). Although the relationship between changes in T_3 and T_4 levels, temperature, and the continuous expression of downstream movement are first documented here, the relationship between plasma levels and movement as well as the effect of exogenous T_3 and T_4 on behaviors associated with migration have been shown in other salmonids. Exogenous T_4 treatments resulted in decreased aggression of sea-run brown trout, steelhead trout, and masu salmon (*Oncorhynchus masou*), suggesting that T_4 elevation reduces behaviors associated with maintenance of position and thus might also promote downstream migration (Hutchison and Iwata 1998). In the present study, both plasma T_3 and T_4 levels showed similar changes relative to temperature treatment. Furthermore, there was a strong positive correspondence between behavior and plasma thyroid hormones, especially plasma T_4. The present results provide further indirect, correlative evidence for a relationship between thyroid hormones and downstream migratory behavior. Further work will be necessary to provide more direct evidence of the role of thyroid hormones in smolt migration.

Morphological appearance and behavioral characters of parr and smolt seem to correspond, where morphological characters or downstream movement could be used to identify fish as parr or smolt at the beginning of the migratory season. There were distinct behavioral differences between parr and smolts from the end of April until the beginning of

June. Depending on temperature regime experienced, late-season differences in morphology and behavior were not present due to the apparent reversion to parr-level nonmigratory status. In the future, behavioral measures can be used in conjunction with physiological and morphological measures to identify smolt status and potentially to estimate appropriate timing for release of smolts in restoration and enhancement programs.

We have demonstrated that these novel methods can be used to examine questions relative to migration timing as they relate to environmental conditions and critical salmonid life history stages, particularly release and termination of downstream movements of smolts. Previously, these questions have been examined using field data that are correlative and subject to sampling bias (Zaugg and Wagner 1973; Solomon 1978; Muir et al. 1994). Now, questions relating to potential behavioral differences between species, stocks, or smolts of differing physiological condition can be probed using the described laboratory methods.

Physiological parameters associated with smolting have been used to predict survival to seawater entry; however, seawater survival requires a number of specific behavioral expressions, including downstream migration. While indirect field methods have been used to examine the environmental control of behavior, this is the first study to show that the time frame for smolt migration is affected by temperature experience. Our results suggest that in cooler climates and during cooler springs, the smolt migration is likely to last significantly longer than in warmer climates or early springs. This study also provides the first direct demonstration that the expression of downstream movement at the individual level is related to the physiological parameters associated with smolting, connecting the environment, through the neuroendocrine system, to behavioral changes. An important next step to better understand this connection will be manipulation of circulating levels of hormones while monitoring downstream movements. It will also be of interest to examine the genetic component of temperature effects on downstream migration, in particular to examine whether there are river-specific or latitudinal differences in the effect of temperature on smolt migratory behavior.

Acknowledgements

References

Aarestrup, K., Nielsen, C., and Koed, A. 2002. Net ground speed of downstream migrating radio-tagged Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.) smolts in relation to environmental factors. Hydrobiologia, **483**: 95–102.

Antonsson, T., and Gudjonsson, S. 2002. Variability in timing and characteristics of Atlantic salmon smolt in Icelandic rivers. Trans. Am. Fish. Soc. **131**: 643–655.

Berglund, I., Lundquist, H., and Fängstam, H. 1994. Downstream migration of immature salmon (*Salmo salar*) smolts blocked by implantation of the androgen 11-ketoandrostenedione. Aquaculture, **121**: 269–276.

Bern, H.A. 1978. Endocrinological studies on normal and abnormal salmon smoltification. *In* Comparative endocrinology. *Edited by* P.J. Gaillard and H.H. Boer. Elsevier/North Holland Biomedical Press, Amsterdam, Netherlands. pp. 77–100.

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

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

Elson, P.F. 1957. The importance of size in change from parr to smolt in Atlantic salmon. Can. Fish Cult. **21**: 1–6.

Erkinaro, J., Julkunen, M., and Niemelä, E. 1998. Migration of juvenile Atlantic salmon *Salmo salar* in small tributaries of the subarctic River Teno, northern Finland. Aquaculture, **168**: 105–119.

Fängstam, H., Berglund, I., Sjoberg, M., and Lundqvist, H. 1993. Effects of size and early sexual maturity on downstream migration during smolting in Baltic salmon (*Salmo salar*). J. Fish Biol. **43**: 517–529.

Fried, S.M., McCleave, J.D., and Labar, G.W. 1978. Seaward migration of hatchery reared Atlantic salmon *Salmo salar* smolts in the Penobscot River estuary, Maine, USA, riverine movements. J. Fish. Res. Board Can. **35**: 76–87.

Fujioka, Y., Fushiki, S., Tagawa, M., Ogasawara, T., and Hirano, T. 1990. Downstream migratory behavior and plasma thyroxine levels of biwa salmon *Oncorhynchus rhodorus*. Nippon Suisan Gakkaishi, **56**: 1773–1779.

Gibson, R.J. 1983. Water velocity as a factor in the change from aggressive to schooling behaviour and subsequent migration of Atlantic salmon smolt (*Salmo salar*). Nat. Can. **110**: 143–148.

Godin, J.-G., Dill, P.A., and Drury, D.E. 1974. Effects of thyroid hormones on behavior of yearling Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. **31**: 1787–1790.

Graham, W.D., Thorpe, J.E., and Metcalfe, N.B. 1996. Seasonal current holding performance of juvenile Atlantic salmon in relation to temperature and smolting. Can. J. Fish. Aquat. Sci. **53**: 80–86.

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

Högasen, H.R., and Prunet, P. 1997. Plasma levels of thyroxine, prolactin, and cortisol in migrating and resident wild Arctic char, *Salvelinus alpinus*. Can. J. Fish. Aquat. Sci. **54**: 2947–2954.

Hutchison, M.J., and Iwata, M. 1998. Effect of thyroxine on the decrease of aggressive behavior of four salmonids during the parr–smolt transformation. Aquaculture, **168**: 169–175.

Hvidsten, N.A., Jensen, A.J., Vivas, H., Bakke, O., and Heggeberget, T.G. 1995. Downstream migration of Atlantic salmon smolts in relation to water flow, water temperature, moon phase and social interaction. Nord. J. Freshw. Res. **70**: 38–48.

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

Iwata, M., Yamanome, T., Tagawa, M., Ida, H., and Hirano, T. 1989. Effects of thyroid hormones on phototaxis of chum and coho salmon juveniles. Aquaculture, **82**: 329–338.

78

Can. J. Fish. Aquat. Sci. Vol. 62, 2005

Iwata, M., Yamauchi, K., Nishioka, R.S., Lin, R., and Bern, H.A. 1990. Effects of thyroxine, growth hormone and cortisol on salinity preference of juvenile coho salmon (*Oncorhynchus kisutch*). Mar. Behav. Physiol. **17**: 191–201.

Jonsson, B., and Ruud-Hansen, J. 1985. Water temperature as the primary influence on timing of seaward migration of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **42**: 593–595.

Jonsson, B., Jonsson, N., and Hansen, L.P. 1991. Differences in life history and migratory behaviour between wild and hatchery-reared Atlantic salmon in nature. Aquaculture, **98**: 69–78.

Lundquist, H., and Eriksson, L. 1985. Annual rhythms of swimming behavior and seawater adaptation in young Baltic salmon, *Salmo salar*, associated with smolting. Environ. Biol. Fishes, **14**: 259–267.

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

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

McCormick, S.D., and Naiman, R.J. 1984. Osmoregulation in the brook trout, *Salvelinus fontinalis*. I. Diel, photoperiod and growth related physiological changes in freshwater. Comp. Biochem. Physiol. A Comp. Physiol. **79**: 7–16.

McCormick, S.D., Björnsson, B.Th., Sheridan, M., Eilertson, C., Carey, J.B., and O'Dea, M. 1996*a*. Increased daylength stimulates plasma growth hormone and gill Na$^+$,K$^+$-ATPase in Atlantic salmon (*Salmo salar*). J. Cell. Physiol. **165**: 245–254.

McCormick, S.D., Shrimpton, J.M., and Zydlewski, J.D. 1996*b*. Temperature effects on osmoregulatory physiology of juvenile anadromous fish. *In* Global warming: implications for freshwater and marine fish. *Edited by* C.M. Wood and D.G. McDonald. Cambridge University Press, Cambridge, UK. pp. 279–301.

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

McCormick, S.D., Cunjak, R.J., Dempson, B., O'Dea, M.F., Carey, J.B. 1999. Temperature-related loss of smolt characteristics in Atlantic salmon (*Salmo salar*) in the wild. Can. J. Fish. Aquat. Sci. **56**: 1–10.

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

Muir, W.D., Giorgi, A.E., and Coley, T.C. 1994. Behavioral and physiological changes in yearling chinook salmon during hatchery residence and downstream migration. Aquaculture, **127**: 69–82.

Nielsen, C., Aarestrup, K., Norum, U., and Madsen, S.S. 2004. Future migratory behaviour predicted from premigratory levels of gill Na$^+$/K$^+$-ATPase activity in individual wild brown trout (*Salmo trutta*). J. Exp. Biol. **207**: 527–533.

Osterdahl, L. 1969. The smolt run of a small Swedish river. *In* Salmon and trout in streams. *Edited by* T.G. Northcote. MacMillan, Vancouver, B.C. pp. 205–215.

Pirhonen, J., and Forsman, L. 1998. Relationship between Na$^+$,K$^+$-ATPase activity and migration behavior of brown trout and sea trout (*Salmo trutta* L.) during the smolting period. Aquaculture, **168**: 41–47.

Ruohonen, K. 1998. Individual measurements and nested designs in aquaculture experiments: a simulation study. Aquaculture, **165**: 149–157.

Saunders, R.L., Specker, J.L., and Komourdjian, M.P. 1989. Effects of photoperiod on growth and smolting in juvenile Atlantic salmon (*Salmo salar*). Aquaculture, **82**: 103–117.

Sigholt, T., Åsgård, T., and Staurnes, M. 1998. Timing of parr–smolt transformation in Atlantic salmon (*Salmo salar*): effects of changes in temperature and photoperiod. Aquaculture, **160**: 129–144.

Solomon, D.J. 1978. Migration of smolts of Atlantic salmon (*Salmo salar* L.) and sea trout (*Salmo trutta* L.) in a chalkstream. Environ. Biol. Fishes, **3**: 223–229.

Thorpe, J.E., Morgan, R.I.G., Prestwell, D., and Higgins, P.J. 1988. Movement rhythms in juvenile Atlantic salmon, *Salmo salar* L. J. Fish Biol. **33**: 931–940.

Veselov, A.Je., Sysoyeva, M.I., and Potutkin, A.G. 1998. The pattern of Atlantic salmon smolt migration in the Varzuga River (White Sea Basin). Nord. J. Freshw. Res. **74**: 65–78.

Wagner, H.H. 1974. Photoperiod and temperature regulation of smolting in steelhead trout. Can. J. Zool. **52**: 219–234.

Whalen, K.G., Parrish, D.L., and McCormick, S.D. 1999. Migration timing of Atlantic salmon smolts relative to environmental and physiological factors. Trans. Am. Fish. Soc. **128**: 289–301.

White, H.C. 1939. Factors influencing descent of Atlantic salmon smolts. J. Fish. Res. Board Can. **4**: 323–326.

Youngson, A.F., Hansen, L.P., Jonsson, B., and Naesje, T.F. 1989. Effects of exogenous thyroxine or prior exposure to raised waterflow on the downstream movement of hatchery-reared Atlantic salmon smolts. J. Fish Biol. **34**: 791–797.

Zaugg, W.S., and Wagner, H.H. 1973. Gill ATPase activity related to parr–smolt transformation and migration in steelhead trout (*Salmo gairdneri*): influence of photoperiod and temperature. Comp. Biochem. Physiol. B Comp. Biochem. **45**: 955–965.