Temperature-related loss of smolt characteristics in Atlantic salmon (*Salmo salar*) in the wild

Stephen D. McCormick, Richard A. Cunjak, Brian Dempson, Michael F. O'Dea, and Judith B. Carey

Abstract: Atlantic salmon (*Salmo salar*) that had previously been released as fry in tributaries of the Connecticut River were captured from 1993 to 1997 during their normal spring smolt migration 198 km from the mouth of the river. Smolts had peak levels of gill Na^+,K^+-ATPase activity and salinity tolerance early in migration (early May), indicating physiological readiness to enter seawater. Significant decreases in gill Na^+,K^+-ATPase activity (29–66%) and salinity tolerance were seen in smolts at the end of the migratory period (late May and early June). Reduced gill Na^+,K^+-ATPase activity occurred earlier in warm years and was directly related to the degree-days during migration ($r^2 = 0.75$). Reduced gill Na^+,K^+-ATPase activity was found at the end of migration in warmer, southern rivers (Connecticut River and Penobscot River, Maine) but not in northern rivers (Catamaran Brook, New Brunswick, and Conne River, Newfoundland). Both hatchery- and stream-reared fish held in the laboratory exhibited a more rapid loss of physiological smolt characteristics when held at higher temperature. The results indicate that late migrants in southern rivers lose physiological smolt characteristics due to high temperatures during spring migration. Delays in migration, such as those that occur at dams, may have negative impacts on smolt survival in warmer rivers.

Résumé : Les saumons atlantiques (*Salmo salar*), libérés à l'état d'alevin dans les affluents du fleuve Connecticut, ont été capturés entre 1993 et 1997, pendant leur migration normale au printemps à l'état de smolt, à 198 km de l'embouchure du fleuve. Au début de la migration (début mai), l'activité Na^+,K^+-ATPasique et la tolérance à la salinité atteignaient un pic, ce qui est une indication qu'ils sont prêts à pénétrer en eau salée. Des baisses importantes de l'activité Na^+,K^+-ATPasique (29–66%) et de la tolérance à la salinité ont été observées chez les smolts à la fin de la période de migration (fin mai et début juin). Une réduction de l'activité Na^+,K^+-ATPasique a été observée plus tôt les années chaudes, et elle était directement liée aux degrés-jours pendant la migration ($r^2 = 0{,}75$). Cette réduction a été constatée à la fin de la migration dans les eaux chaudes des cours d'eau du sud (fleuve Connecticut et rivière Penobscot, dans le Maine), mais pas dans les rivières du nord (ruisseau Catamaran, au Nouveau-Brunswick, et rivière Conne, à Terre-Neuve). Les poissons d'élevage et les poissons sauvages maintenus au laboratoire perdaient plus rapidement les caractéristiques physiologiques des smolts lorsqu'ils étaient exposés à une température plus élevée. Les résultats montrent que les migrants tardifs des rivières du sud perdent les caractéristiques physiologiques du smolt à cause de la température élevée pendant la migration printanière. Les retards dans la migration, comme ceux attribuables aux barrages, peuvent avoir des effets négatifs sur la survie des smolts dans les cours d'eaux chaudes.

[Traduit par la Rédaction]

Introduction

At the time of downstream migration in spring, smolts develop salinity tolerance and undergo other physiological and behavioral changes in preparation for seawater entry. Increased salinity tolerance is the result of changes in the gill, gut, and kidney, including higher gill Na^+,K^+-ATPase activity that provides ionic and electrical gradients used for Na^+ and Cl^- transport. These developmental changes are reversible; when prevented from entering seawater, hatchery-reared smolts lose their migratory urge, salinity tolerance, and the underlying osmoregulatory changes (Duston et al. 1991; see Hoar 1988 for review). Fish that have lost their smolt characteristics and are subsequently released have substantially reduced adult return rates (Virtanen et al. 1991; Staurnes et al. 1993). It is not currently known, however, whether loss of physiological smolt characteristics occurs in migrating salmon in the wild. Loss of these characteristics

S.D. McCormick,[1] M.F. O'Dea, and J.B. Carey. Conte Anadromous Fish Research Center, Biological Resources Division, U.S. Geological Survey, P.O. Box 796, Turners Falls, MA 01376, U.S.A.
R.A. Cunjak. Department of Biology, Faculty of Forestry and Environmental Management, University of New Brunswick, Fredericton, NB E3B 6E1 Canada.
B. Dempson. Department of Fisheries and Oceans, Northwest Atlantic Fisheries Center, St. John's, NF A1C 5X1, Canada.

[1]Author to whom all correspondence should be addressed. e-mail: mccormick@umext.umass.edu

1650

Can. J. Fish Aquat. Sci. Vol. 56, 1999

may be particularly important when migration is delayed, as might occur at dams and impoundments. Salmon inhabiting rivers with higher spring temperatures, such as those in the southern portion of their natural distribution, may have a greater risk of losing smolt characteristics because higher temperatures result in a more rapid loss of salinity tolerance and gill Na^+,K^+-ATPase activity in smolting salmonids (Adams et al. 1973; Zaugg and McLain 1976; Zaugg 1981; Duston and Saunders 1990).

Southern New England is historically the geographical southern limit of the distribution of Atlantic salmon (*Salmo salar*) in North America. Atlantic salmon in the Connecticut and several other large New England rivers were extirpated in the early 1800s following dam construction (Moffitt et al. 1982). A restoration effort begun in 1967 originally involved release of smolts from a variety of North American stocks (Rideout and Stolte 1988). A more recent strategy for restoration of Atlantic salmon to rivers of New England involves hatchery spawning of returning adults and release of progeny into tributaries as recently hatched fry (McMenemy 1995; Orciari and Leonard 1996). Although adult returns to the Connecticut river have been relatively stable, returns to other New England rivers have experienced substantial declines in the last decade.

The present study was undertaken to determine whether loss of salinity tolerance occurs in Atlantic salmon in nature and to examine the potential environmental factors involved. McCormick and Björnsson (1994) made physiological comparisons between wild- and hatchery-reared fish in the Connecticut River; however, they examined migrating fish at only a single time point and therefore could not resolve any differences that might occur over the migratory period. In the present study, we examine physiological changes in migrating smolts over the entire migratory period from several rivers with different spring temperature regimes. We hypothesized that if temperature-related loss of smolt characteristics occurs in nature, they would be detected in warmer, southern rivers and to a lesser extent or not at all in cooler, northern rivers. We also hypothesized that year-to-year variation in spring temperatures would affect the loss of smolt characteristics in the wild. Finally, stream-reared smolts were subjected to controlled temperature experiments in order to verify the results of earlier studies with hatchery-reared smolts, which demonstrated a temperature-related loss of smolt characteristics (Duston et al. 1991).

Materials and methods

Sampling sites

The Connecticut River is the largest river in New England, draining a 40 000-km^2 watershed and emptying into Long Island Sound. For the last 25 years, a restoration program released hatchery-produced smolts that originated primarily from the Penobscot River (Maine) but also included several other rivers (Rideout and Stolte 1988). Since 1987, more than a million fry have been released annually into tributaries of the Connecticut River. Progeny of Connecticut River returns comprise the bulk of current releases, but introductions from outside stocks (primarily Penobscot River) continued until 1995. Atlantic salmon released as fry reside in the streams for 2–3 years prior to smolt migration. From 1993 to 1997, these stream-reared fish were captured as smolts at a bypass facility at Cabot Station (a dam on the Connecticut River at Turners Falls, Mass., 198 km from the river mouth; Fig. 1). This facility

was designed to facilitate movement of fish around the dam and contained a dewatering screen and water table for immediate capture and sampling of fish.

In 1995, smolts on the Penobscot River (drainage area = 22 000 km^2) were sampled at a bypass facility at Weldon Dam, 104 km from the river mouth. In 1995, smolts from Catamaran Brook, New Brunswick, were sampled at a fish counting fence located 0.2 km from the confluence with the Little Southwest Miramichi and 95 km from the estuary. Catamaran Brook (drainage area = 52 km^2) is a third-order tributary of the Miramichi River system and the site of a long-term salmon research project. Atlantic salmon are the most common species; smolts typically emigrate at age 3 and annual numbers range between 500 and 2500 (see Cunjak and Therrien 1997 for details). Due to high water, sampling on the Penobscot River and Catamaran Brook in other years was possible only for a small portion of the migratory period. From 1995 to 1997, smolts were captured from the Conne River, located on the south coast of Newfoundland, at a counting fence 12 km from the river mouth (see Dempson and Stansbury 1991 for details). Conne River has a drainage area of 602 km^2, and over the period 1987–1997 the annual production of wild smolts ranged from 55 000 to 100 000 (Dempson et al. 1998).

Migratory smolt sampling

In each of the rivers, sampling of smolts began as soon as at least 10 fish per day had migrated (less than 1% of the smolt run had passed before this date); sampling occurred weekly thereafter until migration ceased. On the Connecticut River, fish were sampled as soon as they appeared in the water table (maximum time of 15 min since entering the bypass). In the Penobscot River, Catamaran Brook, and Conne River, fish were removed from the capture box each morning. Routinely, 10–12 fish selected at random were sampled immediately after capture as described below. In some years at the end of the migratory period (June), fish numbers decreased and fewer than 10 fish were sampled at a given time point.

In addition to immediate sampling of smolts, 80 smolts from the Connecticut River were captured at the first sampling period in 1993 and 1994 and brought to the Conte Anadromous Fish Research Center (Turners Falls) so that physiological changes under known temperature conditions could be monitored. Fish were held in 1.8-m-diameter tanks supplied with river water at about 4 L·min^{-1} with aeration and fed commercial salmon pellets twice a day. Lighting was by daylight through overhead windows supplemented by natural-spectrum fluorescent lights controlled by timers to give a normal seasonal increase in daylength. At weekly intervals, 10 fish were sampled for gill biopsy and 10 fish were subjected to a seawater challenge. In 1996, Atlantic salmon juveniles in a power canal on the Deerfield River were sampled by angling or seining from June 17 to 20. The Deerfield River is a major tributary of the Connecticut River and the power canal was located 13 km from Cabot Station. These fish were large enough and had the appearance of smolts but had apparently stopped migrating and taken up residence in the power canal. On June 26, 1996, Atlantic salmon parr were captured by electrofishing on the Sawmill River, Montague, Mass., 9.6 km from Cabot Station. These fish were used to determine gill Na^+,K^+-ATPase activity in parr for comparison with smolts that were losing physiological smolt characteristics.

Temperature studies

Hatchery- and stream-reared smolts from the Connecticut River were subjected to different controlled temperature regimes in order to verify previous studies on the effect of temperature on loss of smolt characteristics in hatchery fish from more northern regions. Fish were reared from eggs at the White River National Fish Hatchery in Bethel, Vermont. On May 20, 1996, smolts (14–20 cm) were transferred to the Conte Anadromous Fish Research Center. Fish were randomly divided into two 1-m-diameter tanks

Fig. 1. Water temperature of the Connecticut river at Cabot Dam (arrow on map) and gill Na$^+$,K$^+$-ATPase activity (mean ± SE) in migrant, stream-reared Atlantic salmon smolts in 1993–1997 captured throughout the normal migratory period. Arrowheads on each temperature profile indicate the dates when 50, 75, and 95% of the total number of smolts have migrated (data from Northeast Utilities Service Company, Downstream Passage of Atlantic Salmon Smolts, 1993–1997). An asterisk indicates a significant difference from peak levels of gill Na$^+$,K$^+$-ATPase activity for that year (first or second sampling period; Kruskal–Wallis test, $P < 0.01$). In 1996, juvenile nonmigrant Atlantic salmon (17–21 cm fork length, triangle) were captured in a power canal on the Deerfield River, 13 km from Cabot Station. Degree-days in May for each year are shown in the lower left corner.

CONNECTICUT RIVER
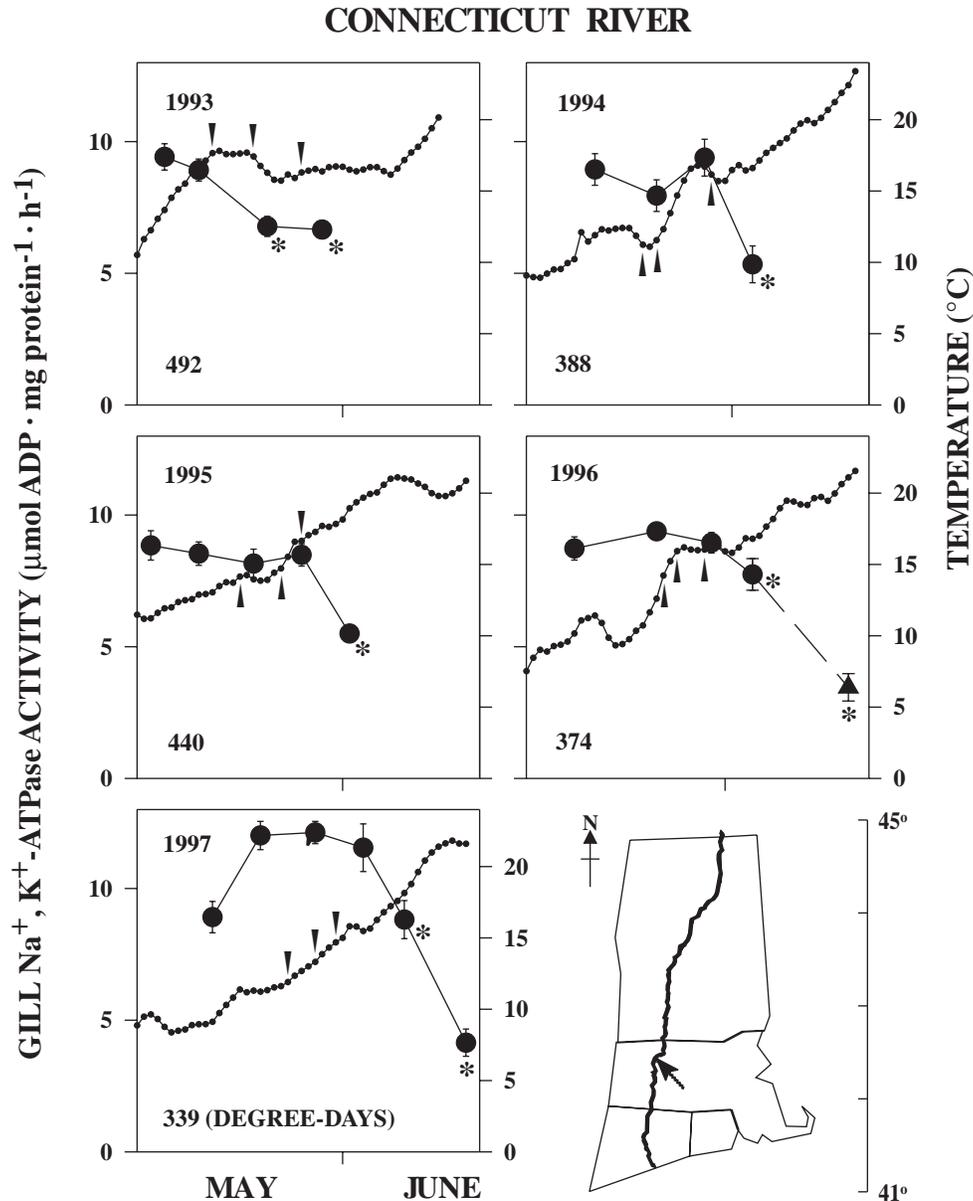
containing about 60 fish each and kept at 10–13°C for 3 days. Thereafter, one group was maintained on ambient river water (increasing from 11.0 to 20.0°C) and the second on chilled river water (11.0–13.0°C), each with a flow rate of 4 L·min^{-1}. Both groups were fed to satiation twice daily. Lighting was supplied by overhead natural-spectrum fluorescent lights and a simulated natural photoperiod was maintained by adjusting the on–off cycle twice a week. Feed was withheld for 24 h prior to sampling, which occurred at 10:00–11:00 EST. Fish were sampled monthly in the hatchery and every 10 days in the laboratory. On May 31, 1996, 10 fish from each group were subjected to a seawater challenge (35‰).

On May 27, 1997 (river temperature 13.9°C), 30 smolts were captured at Cabot Station and randomly divided into two groups.

Both groups were held in 1.8-m-diameter tanks supplied with river water at about 4 L·min^{-1} with aeration and fed commercial salmon pellets twice a day. One group was supplied with river water at ambient temperature and the other with chilled river water (9.7–10.5°C). Two weeks after capture (June 11, 1997), fish were sampled for a gill biopsy and then subjected to a seawater challenge the following day.

Sampling methods

Fish were anesthetized (100 mg MS 222·L^{-1}, pH 7.0) and fork length and weight recorded. For gill Na$^+$,K$^+$-ATPase, a nonlethal biopsy was taken as described in McCormick (1993). In several experiments, blood was drawn from the caudal vessels into a 1-cm^3

1652

Can. J. Fish Aquat. Sci. Vol. 56, 1999

Fig. 2. Percent decrease in gill Na^+,K^+-ATPase activity as a function of degree-days in Atlantic salmon smolts migrating in the Connecticut River in 1993–1997. The change in gill Na^+,K^+-ATPase activity and degree-days was calculated from the peak levels of gill Na^+,K^+-ATPase activity for a given year (first or second sampling date).
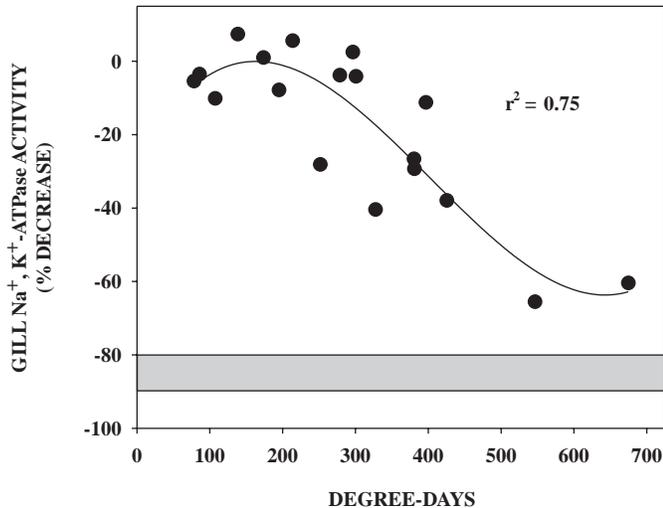
heparinized syringe and spun at $3000 \times g$ for 5 min at 4°C and plasma stored at –80°C until analysis.

Seawater challenge

Fish were transferred to 1-m-diameter tanks containing 40‰ seawater (artificial sea salt added to dechlorinated tap water) maintained at 10°C. Paper and charcoal filtration and continual aeration maintained low ammonia and high oxygen levels. After 24 h the surviving fish were anesthetized and sampled as described above. Plasma chloride and osmolality were used as indicators of the capacity to regulate ions following seawater exposure. In an effort to reduce mortality, 35‰ was used for seawater challenges in 1994 and subsequent years.

Gill Na^+,K^+-ATPase activity and plasma chloride

Four to six primary gill filaments were severed above the septum, placed in 100 µL of ice-cold SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3), and frozen at –80°C within 30 min. Na^+,K^+-ATPase activity was measured by the method outlined in McCormick (1993). Plasma chloride was measured by coulometric titration (Labconco, Kansas City, Mo.).

Statistical analysis

Condition factor was calculated as (weight/length^3) $\times$ 100, with wet weight in grams and fork length in centimetres. Degree-days were calculated as the cumulative mean daily temperature. The statistical significance of changes over time in gill Na^+,K^+-ATPase activity and plasma ions in each of the stream-reared groups (migrant and captive) was examined by the nonparametric Kruskal–Wallis test; if significant differences occurred ($P < 0.05$), then nonparametric pairwise comparisons were made to the initial sampling date ($P < 0.01$). Comparisons of mortality in seawater challenge tests were performed using Fisher's exact test ($P < 0.05$). Influence of thermal regimes on gill Na^+,K^+-ATPase activity and plasma ions after seawater challenge was analyzed by two-way analysis of variance (ANOVA); if significant temperature effects were found ($P < 0.05$), a one-way ANOVA was used to compare groups at the same time point.

Results

Migrating smolts in the Connecticut River

From 1993 through 1997, Atlantic salmon smolts were captured during their normal migratory period at Cabot Station. Temperature differed substantially between years (Fig. 1). For example, the dates at which temperatures first reached 15°C were May 7, 1993, May 24, 1994, May 23, 1995, May 22, 1996, and May 30, 1997, and there was considerable variation in the number of degree-days.

Gill Na^+,K^+-ATPase activity was relatively high (8–10 µmol ADP·mg protein^{-1}·h^{-1}) at the first sampling period in all years (Fig. 1). The only year in which significant increases occurred after the first sampling was 1997, the coolest of the five years. In every year, a significant decrease in gill Na^+,K^+-ATPase activity occurred toward the end of the migratory period. In the warmest year (1993), significant decreases were found in May, whereas in all other years, significant decreases were not found until the very end of the migratory period in early June. In 1996, 17- to 21-cm fish that had the morphological appearance of smolts were captured in a power canal on the Deerfield River on June 17–20 (after the normal migratory period). These fish had a gill Na^+,K^+-ATPase activity of 3.5 ± 0.5 µmol ADP·mg protein^{-1}·h^{-1}, 63% lower then peak smolt levels (Fig. 1). Parr (9–13 cm) collected in the Sawmill River (a tributary of the Connecticut River) on June 26 had a gill Na^+,K^+-ATPase activity of 2.0 ± 0.2 µmol ADP·mg protein^{-1}·h^{-1}.

Degree-days from peak levels could explain 75% of the variation in percent change from peak levels of gill Na^+,K^+-ATPase activity over the five years of sampling (Fig. 2). Several independent variables were tested for their ability to predict changes in absolute and percent change in gill Na^+,K^+-ATPase activity from peak levels. Using best subsets and forward stepwise regression, degree-days from the peak levels was chosen over date, days from May 1, degree-days from May 1, temperature, and number of days over 15°C.

In 1993 and 1994, 10 fish at each sampling period were subjected to a seawater challenge. In both years, salinity tolerance was greatest at the beginning of migration (Tables 1 and 2). Significant decreases in salinity tolerance (increases in plasma chloride and mortality after seawater challenge) occurred in the latter part of the migratory period in both years; decreases were detected as early as May 22 in 1993 and May 27 in 1994. Significant decreases in gill Na^+,K^+-ATPase activity and salinity tolerance also occurred in captive fish that were maintained in ambient river water in both years.

In 1993 and 1994, smolts captured at the beginning of the migratory period were smaller than those later in the run (Tables 1 and 2), and condition factor remained constant throughout the migration in both years. Similar patterns were observed in 1995–1997 (data not shown). In 1993, plasma chloride of migrants was highest in early May and was slightly but significantly lower on May 10 and 28. In 1994, there was no significant difference in plasma chloride over the course of the migration.

Migrating smolts in other North American rivers

In 1995, wild migrating smolts were sampled during their

Table 1. Physiological changes in migrant and captive stream-reared juvenile Atlantic salmon captured at a bypass of Cabot Station, Turners Falls, on the Connecticut River during normal migration in 1993.

	Group	May 5	May 10/14	May 20/22	May 28/June 1
Length (cm)	Migrant	15.6±0.3	15.9±0.3	17.5±0.3*	18.2±0.6*
	Captive	—	14.2±0.3*	15.4±0.6	15.7±0.5
Weight (g)	Migrant	33.4±1.8	36.2±2.7	48.2±2.8*	54.2±6.3*
	Captive	—	22.8±1.2*	29.8±3.8	32.1±3.5
Condition factor (g·cm^{-3} × 100)	Migrant	0.87±0.01	0.89±0.02	0.89±0.02	0.88±0.03
	Captive	—	0.80±0.01*	0.79±0.02*	0.81±0.03
Gill Na$^+$,K$^+$-ATPase (µmol ADP·mg protein^{-1}·h^{-1})	Migrant	9.4±0.5	8.9±0.4	6.8±0.4*	6.7±0.7*
	Captive	—	8.7±0.2	3.6±0.3*	4.6±0.5*
Plasma chloride (mM)	Migrant	136±1	128±1*	133±4	129±7*
	Captive	—	136±3	134±2	124±6
	Migrant SW	166±7	159±7	180±11*	202±16*
	Captive SW	—	182±8*	189±7*	183±10*
Mortality (%)	Migrant SW	0	0	40	0
	Captive SW	—	0	30	20

Note: Captive fish were those initially captured on May 5 and maintained in flowing river water in 1.8-m-diameter tanks. Values are mean ± SE (n = 10–15 per group). SW fish were those subjected to a 24-h seawater challenge. An asterisk indicates a significant difference from the May 5 migrants (Kruskal–Wallis test, P < 0.01). The first date represents the migrant group and the second the captive group.

Table 2. Physiological changes in migrant and captive stream-reared juvenile Atlantic salmon captured at a bypass of Cabot Station, Turners Falls, on the Connecticut River during normal migration in 1994.

|	Group	May 11	May 20	May 27	June 3
Length (cm)	Migrant	14.9± 0.6	17.4±0.6*	18.4±0.6*	16.8±0.5
	Captive	—	15.2±0.3	14.9±0.4	15.7±0.4
Weight (g)	Migrant	29.8±4.3	48.5±4.7*	54.8±4.5*	41.0±3.6
	Captive	—	29.1±1.9	27.0±3.0	36.0±3.4
Condition factor (g·cm^{-3} × 100)	Migrant	0.85±0.01	0.89±0.01	0.87±0.03	0.85±0.02
	Captive	—	0.82±0.02	0.79±0.03	0.92±0.05
Gill Na$^+$,K$^+$-ATPase (µmol ADP·mg protein^{-1}·h^{-1})	Migrant	8.9±0.6	8.0±0.6	9.4±0.7	5.3±0.7*
	Captive	—	8.4±0.3	4.8±0.6*	3.5±0.5*
Plasma chloride (mM)	Migrant	127±1	128±2	123±2	126±2
	Captive	—	127±2	133±2*	127±1
	Migrant SW	133±1	135±4	150±3*	138±3
	Captive SW	—	135±5	150±6*	157±4*
Mortality (%)	Migrant SW	0	50	40	70
	Captive SW	—	0	0	20

Note: Captive fish were those initially captured on May 11 and maintained in flowing river water in 1.8-m-diameter tanks. Values are mean ± SE (n = 10 per group). SW fish were those subjected to a 24-h seawater challenge. An asterisk indicates a significant difference from the May 11 migrants (Kruskal–Wallis test, P < 0.01).

normal migratory period on the Penobscot River, Catamaran Brook, and Conne River. River temperatures and changes in gill Na$^+$,K$^+$-ATPase activity for these rivers and the Connecticut River in 1995 are shown in Fig. 3. Gill Na$^+$,K$^+$-ATPase activity in smolts from the Penobscot River, Catamaran Brook, and Conne River was initially lower than the levels seen in the Connecticut River. Smolts from the Penobscot River had increased gill Na$^+$,K$^+$-ATPase activity in the middle of the run and then subsequently had significantly lower levels in June. In contrast, gill Na$^+$,K$^+$-ATPase activity in smolts from Catamaran Brook and the Conne River increased steadily throughout the migratory period, with no decrease at the end of the migratory period. This pattern of low initial gill Na$^+$,K$^+$-ATPase activity with steadily increasing values throughout migration occurred in all three years of sampling on the Conne River (Fig. 4).

Temperature studies

Hatchery-reared fish were subjected to two temperature regimes (ambient and chilled) beginning on May 20, 1996 (Fig. 5). Gill Na$^+$,K$^+$-ATPase activity in hatchery-reared smolts on ambient river water decreased rapidly and significantly from peak levels of 10.0 µmol ADP·mg protein^{-1}·h^{-1} to 4.9 µmol ADP·mg protein^{-1}·h^{-1} after 10 days and to 2.0 µmol ADP·mg protein^{-1}·h^{-1} after 22 days. In contrast, there was no significant change in gill Na$^+$,K$^+$-ATPase activity in smolts maintained on chilled river water (11–13°C) after 10 days and a significant decrease to 5.4 µmol ADP·mg protein^{-1}·h^{-1} after 22 days. Gill Na$^+$,K$^+$-ATPase activity in fish on chilled river water was significantly greater than in fish on ambient river water at both sampling periods. Salinity tolerance on May 31, 1996, was significantly lower in fish on ambient river water (plasma chloride = 175 ±

Fig. 3. Water temperature and gill Na$^+$,K$^+$-ATPase activity (mean ± SE) in migrating Atlantic salmon smolts in four rivers in North America in 1995. CT, Connecticut River (circles); PEN, Penobscot River (squares); CAT, Catamaran Brook (inverted triangles); CON, Conne River (righted triangles). Degree-days in May were 440 (CT), 340 (PEN), 187 (CAT), and 309 (CON). Arrowheads on each temperature profile indicate the dates when 50, 75, and 95% of the total number of smolts have migrated (Conne River on x-axis). An asterisk indicates a significant decrease from peak levels of gill Na$^+$,K$^+$-ATPase activity for a particular river (first or second sampling period; Kruskal–Wallis test, $P < 0.01$).
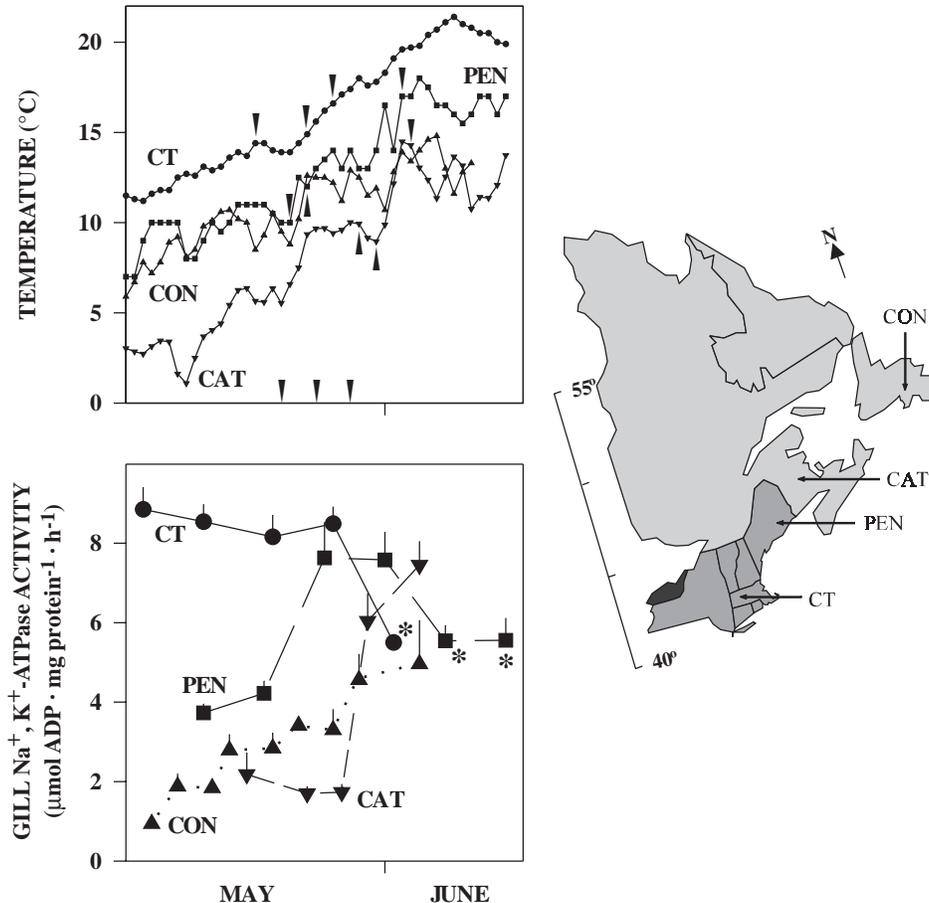
5.3 mM) than in fish on chilled river water (plasma chloride = 151 ± 2.1 mM).

Stream-reared fish captured at Cabot Station on May 27, 1997, were subjected to two temperature regimes, ambient river temperature (increasing from 13.9 to 19.2°C) and 10°C (9.7–10.5°C). Gill Na$^+$,K$^+$-ATPase activity upon capture was 12.1 ± 0.4 μmol ADP·mg protein^{-1}·h^{-1} ($n = 12$). After 14 days at ambient temperature (258 degree-days), gill Na$^+$,K$^+$-ATPase activity had decreased by 75% to 3.1 ± 0.3 μmol ADP·mg protein^{-1}·h^{-1} ($n = 13$). Gill Na$^+$,K$^+$-ATPase activity in smolts held at 10°C (148 degree-days) had decreased to 6.8 ± 1.0 μmol ADP·mg protein^{-1}·h^{-1} ($n = 12$) but was more than twofold higher than that in fish maintained at ambient temperature (Kruskal–Wallis test, $P < 0.001$). Plasma chloride after seawater challenge was significantly greater in smolts at ambient temperature (179 ± 4.9 mM) than in smolts at 10°C (158 ± 2.6 mM).
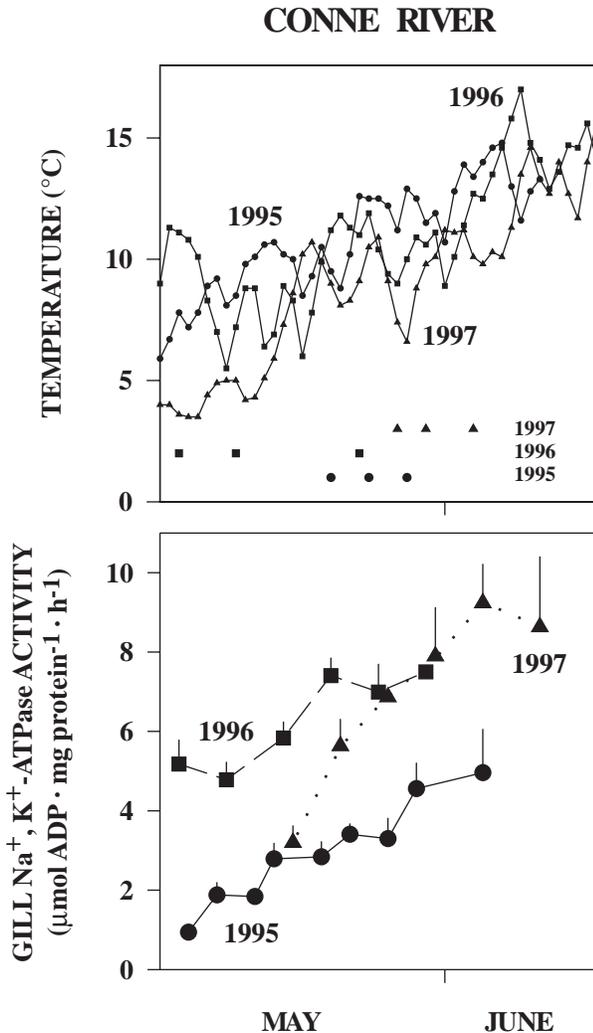
To examine whether holding the temperature of seawater challenges constant throughout the study while varying freshwater-rearing temperature affected measurement of salinity tolerance, two experiments were conducted with hatchery fish at seasonally varying temperatures in late winter (2°C) and late spring (16°C). Fish in freshwater at 2°C

were transferred to 35‰ at 2 and 10°C; plasma chloride after 24 h was 168 ± 4 and 177 ± 2 mM, respectively (mean ± SE, $n = 10$ per group, Student t test, $P = 0.06$,). Fish in freshwater at 16°C were transferred to 35‰ at 16 and 10°C; plasma chloride after 24 h was 165 ± 4 and 158 ± 4 mM, respectively ($n = 10$ per group; Student t test, $P = 0.19$). In each case, isothermal seawater transfer was not significantly different from those at 10°C.

Discussion

Lower gill Na$^+$,K$^+$-ATPase activity and salinity tolerance were seen at the end of the migratory period in all years that fish were sampled in the Connecticut River. The first appearance of decreases and the degree of decrease were directly related to the temperature regime (Figs. 1 and 2). In the Penobscot River, which had relatively warm temperatures in late spring, smolts also had significantly lower gill Na$^+$,K$^+$-ATPase activity at the end of the run (Fig. 3). In contrast, the more northern Catamaran Brook and Conne River had no loss of gill Na$^+$,K$^+$-ATPase activity at the end of the migratory period. The pattern of increases seen in Catamaran Brook in 1995 and the Conne River in 1995–
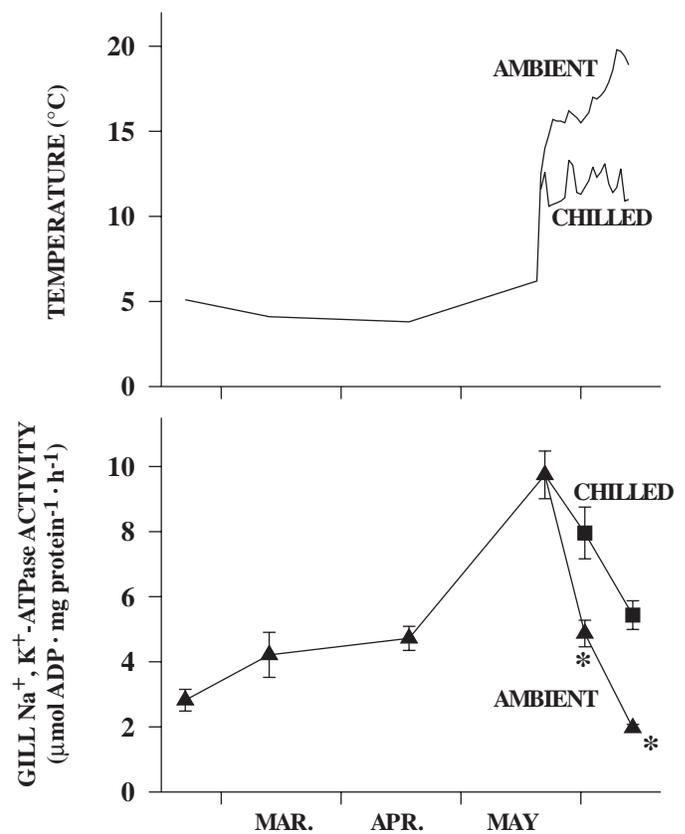
Fig. 4. Water temperature and gill Na$^+$,K$^+$-ATPase activity (mean + SE) in wild migrant Atlantic salmon smolts in the Conne River sampled in 1995 (circles), 1996 (squares), and 1997 (triangles). Degree-days in May were 309 (1995), 290 (1996), and 223 (1997). In the upper panel, symbols for each year at the bottom indicate the time of 50, 75, and 95% of the smolt migration.

CONNE RIVER

Fig. 5. Water temperature and gill Na$^+$,K$^+$-ATPase activity (mean ± SE, n = 10 per group) in Atlantic salmon reared at a hatchery and exposed to ambient (triangles) or chilled (sqaures) river water. Ambient river temperatures in May and June are nearly identical to those experienced by fish in the wild. An asterisk indicates a significant difference from the chilled group (Kruskal–Wallis test, $P < 0.01$).

1997 was very similar, with low levels initially followed by increases throughout the migratory period (Figs. 2 and 3).

A temperature-related loss of smolt characteristics is the most likely explanation for the observed patterns of physiological changes in wild and stream-reared Atlantic salmon smolts. Laboratory studies of hatchery- and stream-reared fish in the present study indicate that reducing temperature below ambient in spring reduces the rate of loss of physiological smolt characteristics. This finding agrees with previous research demonstrating that for several species of smolting salmonids the loss of salinity tolerance and other smolt characteristics in hatchery- or laboratory-reared fish occurs more rapidly at higher temperatures (Adams et al. 1973; Zaugg and McLain 1976; Zaugg 1981; Duston et al. 1991). That temperature is the primary determinant is demonstrated by the clear and direct relationship between degree-days (cumulative daily temperature) and loss of smolt characteristics under both constant and increasing temperature in

captive populations (Duston et al. 1991; McCormick et al. 1997) and fish in the wild (Fig. 2). This relationship between temperature (degree-days) and loss of smolt characteristics can explain most of the observed patterns in loss of smolt characteristics in naturally migrating populations of Atlantic salmon in the Connecticut and Penobscot rivers.

There is substantial evidence that smolt survival and subsequent adult returns are dependent on the timing of smolt migration and the physiological preparedness of smolts. Release of hatchery-reared fish has shown sharp seasonal differences in adult return rates that are strongly correlated with smolt development, including very low return rates associated with loss of smolt physiology (Virtanen et al. 1991; Staurnes et al. 1993). The present study lends further support to the concept of a physiological "smolt window" in which there is a limited time for successful migration of smolts. This smolt window is controlled by environmental and biotic factors regulating the onset, development, and subsequent loss of salinity tolerance and other smolt characteristics (see Hoar 1988; McCormick et al. 1998). There is very limited information on the influence of run timing on smolt survival in wild populations. Hansen (1987) found that lake-reared smolts that migrated down the River Imsa (Norway) in late spring and throughout the summer had reduced

1656

Can. J. Fish Aquat. Sci. Vol. 56, 1999

survival compared with stream-reared fish that migrated primarily in May. In Catamaran Brook the average smolt-to-grilse return rate from 1990 to 1996 was 8.5% (Cunjak and Therrien 1997). The grilse return rate from the 1992 smolt run was only 4.6% and may have been due to the very late migration of many smolts (20% migrated after June 21) that was probably due to unusually low spring discharge. In the Conne River, years with later smolt migration subsequently have lower adult (grilse) return rates (Dempson et al. 1998). It should be noted that factors other than smolt physiology will also be important in determining changes in smolt survival during the normal migratory period. McCormick et al. (1998) suggested that there is both a physiological and an ecological smolt window that may have independent periods for high smolt survival. There may also be genetic factors that affect the interaction of temperature and loss of smolt characteristics. Loss of smolt characteristics has, however, been observed in hatchery populations derived from stocks from many latitudes in both Europe and North America (Whitesel 1993; McCormick et al. 1997). It is possible that the original stocks of the Connecticut River and elsewhere in New England were adapted to avoid these losses, through either early migration or resistance to temperature-induced loss of smolt physiology.

Loss of smolt characteristics in Atlantic salmon in the Connecticut River occurred after the peak of migration in all years. Nonetheless, in some years, a significant portion of the population will be affected by loss of smolt characteristics. The warmest spring of the years studied was in 1993, and decreased gill Na^+,K^+-ATPase activity was detected earlier (as both absolute date and time since the beginning of the migratory period) in 1993 than in any other year. Significant decreases in gill Na^+,K^+-ATPase activity were detected on May 20, 1993, and about 20% of the run occurred after this date. The fact that these changes were detected 198 km from the mouth of the Connecticut River indicates that the additional time needed to reach the ocean will result in even greater loss of smolt characteristics and in a larger portion of the population being affected. The proportion of the population affected by loss of smolt characteristics will depend on the timing of migration of the population and the temperature experienced throughout the migratory period. Dams and their impoundments will increase the problem of lost smolt characteristics by delaying migration and increasing water temperatures. In regulated river systems, loss of smolt characteristics can be reduced by ensuring that smolts have the opportunity to maximize migratory rate, by ensuring safe, efficient, and timely passage of fish over or through dams. To increase the effectiveness of resource management, information on loss of smolt characteristics and reduced smolt survival could be combined with adaptive management strategies. Such management actions may be particularly important in years with unusually rapid rising or high spring temperatures or in mitigating the effects of global climate change.

The strong correspondence between the loss of salinity tolerance and decreased gill Na^+,K^+-ATPase activity suggests a mechanism by which temperature may exert its influence on salinity tolerance. Gill Na^+,K^+-ATPase activity is located primarily in chloride cells, which are responsible for ion secretion in seawater (Karnaky et al. 1976; Foskett and Scheffey 1982). Chloride cell density increases along with gill Na^+,K^+-ATPase activity during smolting (Langdon and Thorpe 1985; Lubin et al. 1991), and the decrease in gill Na^+,K^+-ATPase after the peak of smolting indicates that the number of chloride cells may also decrease. Higher temperatures may result in higher "turnover" or cell death of chloride cells, decreased renewal of chloride cells, or both. The effect of temperature may also be less direct, perhaps differentially affecting endocrine systems promoting or inhibiting the maintenance of chloride cells and salinity tolerance.

Some of the variability that we observed in salinity tolerance may have been due to stress caused by transport of stream-reared fish and their exposure to a laboratory environment. Such stress may be inherent to studies in which fish from the wild are tested under laboratory conditions. We have found that laboratory-reared fish subjected to handling and confinement stress do not show decreased salinity tolerance (S.D. McCormick, unpublished results). This stress, however, may not be similar to that experienced by fish from the wild that have never been subjected to handling or confinement. Gill Na^+,K^+-ATPase activity in fish sampled immediately upon capture will not be affected by stress and may be a more accurate representation of changes in smolt development.

There is little information on the degree to which migratory behavior is lost and how it is affected by high temperature or how tight is the coupling between smolt physiology and behavior. Greenstreet (1992) found that movement rates of individually tagged Atlantic salmon smolts migrating through a fish ladder increased with increasing temperature up to 16°C but decreased slightly at 16–18°C. Studies of Pacific salmon show a close correspondence between physiological smolt development and migratory behavior (Zaugg et al. 1985; Zaugg 1989; Ewing et al. 1994), although these studies did not explicitly examine loss of smolt characteristics. If there is a strong link between migratory behavior and gill Na^+,K^+-ATPase activity in Atlantic salmon, migratory behavior will decrease in late spring and will be lost more rapidly at high temperatures. If the link is not strong, smolts with reduced gill Na^+,K^+-ATPase activity may continue to migrate to the estuary. It is likely that they will experience substantial osmotic disequilibrium or spend substantially more time acclimating to seawater than normal smolts, making them more likely to suffer higher predation rates (Handeland et al. 1996).

Gill Na^+,K^+-ATPase activity was low in smolts early in migration in northern rivers (Catamaran Brook and the Conne River), intermediate in smolts from the Penobscot River, and highest in smolts from the Connecticut River, roughly corresponding to the early spring temperature regimes for the four rivers. There are also temperature-related differences within rivers. In the Conne River the greatest gill Na^+,K^+-ATPase activity at the first sampling period occurred in 1995, the warmest of the three years examined. In the Connecticut River the only year in which significant increases occurred early in migration was 1997, which had the coolest spring temperatures. Smolts sampled in tributaries of the Connecticut River have lower gill Na^+,K^+-ATPase activity than smolts at Cabot Station (S.D. McCormick, unpublished observation), indicating that the peak levels are reached in the mainstem. Previous research has shown that

the timing of increased salinity tolerance and other aspects of smolting are most strongly influenced by changes in daylength and that temperature plays a less important role in the onset of physiological smolt characteristics (Johnston and Saunders 1981; Clarke et al. 1985; Duston and Saunders 1990). The present findings suggest that temperature may play a role in the initial (early spring) phase of smolt physiology.

In spite of the fairly large differences in early spring temperatures among the rivers, there was general similarity in the migratory period of Atlantic salmon. Almost all of the migration in each year began after May 1 and was completed by the first week of June. In most years the date of 50% migration was quite similar among the rivers. The importance of photoperiod in initiating migration may account for the similarity in run timing among these rivers. There was variation in run timing within rivers that was correlated with spring temperatures. In the Connecticut River, the earliest and latest days of 50% migration were in 1993 (May 12) and 1997 (May 23), the warmest and coolest springs, respectively (Fig. 1). Similarly, the date of 50% completion of the smolt migration in the Conne River was May 3, 1996, when there was an early increase in temperatures, whereas a cool spring the subsequent year was associated with 50% migration on May 26 (Fig. 3).

We have demonstrated that migrating Atlantic salmon smolts have initially high salinity tolerance and gill Na^+,K^+-ATPase activity and that reductions in these physiological smolt characteristics occur at the end of the normal migratory period in southern populations that experience high temperatures in late spring. Loss of physiological smolt characteristics did not occur in more northern rivers. Because the loss of smolt characteristics is related to degree-days and not just absolute temperature, time of migration will also be a factor in determining how many smolts will be affected. Thus, factors other than just temperature, such as hydrographic and climatic conditions and barriers to migration such as dams, will also be important. In warm southern rivers, significant delays in migration will have negative impacts on the capacity of smolts to survive in seawater and return as adults.

Acknowledgments

References

Adams, B.L., Zaugg, W.S., and Mclain, L.R. 1973. Temperature effect on parr–smolt tranformation in steelhead trout (*Salmo gairdneri*) as measured by gill sodium–potassium stimulated ATPase. Comp. Biochem. Physiol. A, Comp. Physiol. **44**: 1333–1339.

Clarke, W.C., Lundqvist, H., and Eriksson, L.O. 1985. Accelerated photoperiod advances seasonal cycle of seawater adaptation in juvenile Baltic salmon, *Salmo salar* L. J. Fish Biol. **26**: 29–35.

Cunjak, R.A., and Therrien, J. 1997. Inter-stage survival of wild juvenile Atlantic salmon, *Salmo salar* L. Fish. Manage. Ecol. **5**: 209–223.

Dempson, J.B., and Stansbury, D.E. 1991. Using partial counting fences and a two-sample stratified design for mark–recapture estimation of an Atlantic salmon smolt population. N. Am. J. Fish. Manage. **11**: 27–37.

Dempson, J.B., Furey, G., and Bloom, M. 1998. Status of Atlantic salmon in Conne River, SFA 11, Newfoundland, 1997. Can. Stock Asses. Secr. Res. Doc. 98/28.

Duston, J., and Saunders, R.L. 1990. The entrainment role of photoperiod on hypoosmoregulatory and growth-related aspects of smolting in Atlantic salmon (*Salmo salar*). Can. J. Zool. **68**: 707–715.

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.

Ewing, R.D., Barratt, D., and Garlock, D. 1994. Physiological changes related to migration tendency in rainbow trout (*Oncorhynchus kisutch*). Aquaculture, **121**: 277–287.

Foskett, J.K., and Scheffey, C. 1982. The chloride cell: definitive identification as the salt-secretory cell in teleosts. Science (Washington, D.C.), **215**: 164–166.

Greenstreet, S.P.R. 1992. Migration of hatchery reared juvenile Atlantic salmon, *Salmo salar* L., smolts down a release ladder. 1. Environmental effects on migratory activity. J. Fish Biol. **40**: 655–666.

Handeland, S.O., Jarvi, T., Ferno, A., and Stefansson, S.O. 1996. Osmotic stress, antipredator behaviour, and mortality of Atlantic salmon (*Salmo salar*) smolts. Can. J. Fish. Aquat. Sci. **53**: 2673–2680.

Hansen, L.P. 1987. Growth, migration and survival of lake reared juvenile anadromous Atlantic salmon *Salmo salar* L. Fauna Norv. Ser. A, **8**: 29–34.

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.

Johnston, C.E., and Saunders, R.L. 1981. Parr–smolt transformation of yearling Atlantic salmon (*Salmo salar*) at several rearing temperatures. Can. J. Fish. Aquat. Sci. **38**: 1189–1198.

Karnaky, K.J., Kinter, L.B., Kinter, W.B., and Stirling, C.E. 1976. Teleost chloride cell. Autoradiographic localization of gill Na,K-ATPase in killifish *Fundulus heteroclitus* adapted to low and high salinity environments. J. Cell Biol. **70**: 157–177.

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

Lubin, R.T., Rourke, A.W., and Saunders, R.L. 1991. Influence of photoperiod on the number and ultrastructure of gill chloride cells of the Atlantic salmon (*Salmo salar*) before and during smoltification. Can. J. Fish. Aquat. Sci. **48**: 1302–1307.

McCormick, S.D. 1993. Methods for nonlethal gill biopsy and

1658

Can. J. Fish Aquat. Sci. Vol. 56, 1999

measurement of Na$^+$,K$^+$-ATPase activity. Can. J. Fish. Aquat. Sci. **50**: 656–658.

McCormick, S.D., and Björnsson, 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., Shrimpton, J.M., and Zydlewski, J.D. 1997. 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, New York. pp. 279–301.

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

McMenemy, J.R. 1995. Survival of Atlantic salmon fry stocked at low density in the West River, Vermont. N. Am. J. Fish. Manage. **15**: 366–374.

Moffitt, C.M., Kynard, B., and Rideout, S.G. 1982. Fish passage facilities and anadromous fish restoration in the Connecticut River basin. Fisheries (Bethesda), **7**: 2–11.

Orciari, R.D., and Leonard, G.H. 1996. Length characteristics of smolts and timing of downstream migration among three strains of Atlantic salmon in a southern New England. N. Am. J. Fish. Manage. **16**: 851–860.

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.

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

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

Whitesel, T.A. 1993. Comparison of juvenile Atlantic salmon (*Salmo salar*) reared in a hatchery and introduced into a stream: a two-size-threshold model of smoltification. Can. Spec. Publ. Fish. Aquat. Sci. No. 118. pp. 239–247.

Zaugg, W.S. 1981. Advanced photoperiod and water temperature effects on gill Na$^+$-K$^+$ adenosine triphosphatase activity and migration of juvenile steelhead (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. **38**: 758–764.

Zaugg, W.S. 1989. Migratory behaviour of underyearling *Oncorhynchus tshawytscha* and survival to adulthood as related to prerelease gill (Na$^+$-K$^+$)-ATPase development. Aquaculture, **82**: 339–353.

Zaugg, W.S., and McLain, L.R. 1976. Influence of water temperature on gill sodium, potassium-stimulated ATPase activity in juvenile coho salmon (*Oncorhynchus kisutch*). Comp. Biochem. Physiol. A, Comp. Physiol. **54**: 419–421.

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