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[Article]

Migratory Urge and Gill Na^+,K^+-ATPase Activity of Hatchery-Reared Atlantic Salmon Smolts from the Dennys and Penobscot River Stocks, Maine

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Abstract.—Hatchery-reared Atlantic salmon *Salmo salar* smolts produced from captive-reared Dennys River and sea-run Penobscot River broodstock are released into their source rivers in Maine. The adult return rate of Dennys smolts is comparatively low, and disparity in smolt quality between stocks resulting from genetic or broodstock rearing effects is plausible. Smolt behavior and physiology were assessed during sequential 14-d trials conducted in seminatural annular tanks with circular flow. "Migratory urge" (downstream movement) was monitored remotely using passive integrated transponder tags, and gill Na^+,K^+-ATPase activity was measured at the beginning and end of the trials to provide an index of smolt development. The migratory urge of both stocks was low in early April, increased 20-fold through late May, and declined by the end of June. The frequency and seasonal distribution of downstream movement were independent of stock. In March and April, initial gill Na^+,K^+-ATPase activities of Penobscot River smolts were lower than those of Dennys River smolts. For these trials, however, Penobscot River smolts increased enzyme activity after exposure to the tank, whereas Dennys River smolts did not, resulting in similar activities between stocks at the end of all trials. There was no clear relationship between migratory urge and gill Na^+,K^+-ATPase activity. Gill Na^+,K^+-ATPase activity of both stocks increased in advance of migratory urge and then declined while migratory urge was increasing. Maximum movement was observed from 2 h after sunset through 1 h after sunrise but varied seasonally. Dennys River smolts were slightly more nocturnal than Penobscot River smolts. These data suggest that Dennys and Penobscot River stocks are not markedly different in either physiological or behavioral expression of smolting.

Populations of Atlantic salmon *Salmo salar* in New England rivers have declined since the 1980s and remain critically low (USASAC 2008). Populations in the Dennys River and seven other Maine rivers were listed as endangered under the federal Endangered Species Act in 2000, and the Penobscot River was added in 2009. Most adults returning to Maine rivers are derived from stocked hatchery smolts. The smolt-to-adult return rates, however, can vary widely among drainages. In the Penobscot River, over 500,000 smolts are stocked annually (mean ± SE return rate = 0.172 ± 0.036%, 2001–2005; USASAC 2008). Penobscot River-origin smolts released in the Merrimack River, Massachusetts, during the same period had a comparable return rate (0.176 ± 0.040%; USASAC 2008). However, Dennys River smolts stocked into their river of origin had a return rate that was more than an order of magnitude lower (0.012 ± 0.019%, 2001–2002; USASAC 2008).

It is unclear whether the disparity in survival rates is a function of environmental differences. The Dennys River is a small (342 km^2), relatively undeveloped watershed without main-stem dams (Figure 1). The Penobscot River is large (20,109 km^2 above head of tide), with widespread riparian development and multiple dams. Predator assemblages (e.g., smallmouth bass *Micropterus dolomieu*, harbor seal *Phoca vitulina*, and double-crested cormorant *Phalacrocorax auritus*) are similar in both watersheds, but dams on the Penobscot River impede fish passage and increase smolt vulnerability to predation (Blackwell et al. 1997; Moring et al. 1999). Estuary and bay smolt mortality is hypothesized to be higher in the Dennys River than in the Penobscot River. Unlike the Penobscot River, the Dennys River estuary has a convoluted geomorpholo-

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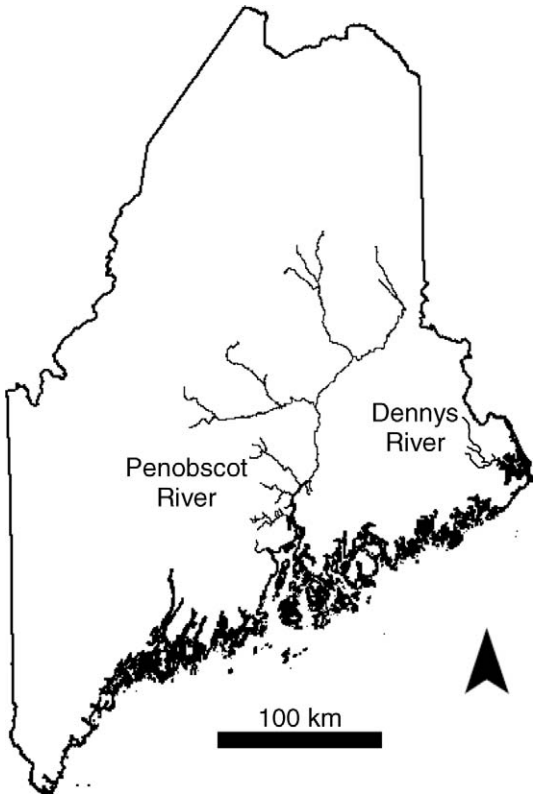

FIGURE 1.—Map of the state of Maine, showing the Penobscot and Dennys rivers.

gy, extreme tidal ranges (5.7 m), and chaotic flow patterns (Brooks 2004). Numerous Atlantic salmon aquaculture cages also exist in Dennys Bay, and these may impact wild Atlantic salmon populations as they pass through this area (Naylor et al. 2005).

Alternatively, low returns of Dennys River smolts may be due to poor smolt quality resulting from current and past management. Despite decades of interbasin stock transfers between the Penobscot and Dennys rivers, the stocks are genetically separate (Baum 1997; Spidle et al. 2003). The source of genetic differences (e.g., adaptive or anthropogenic) is unknown, but the stocks have been exposed to decades of hatchery selection. Due to the scarcity of wild Penobscot River broodstock, most hatchery smolts (86%) have been produced from hatchery (sea-ranched) broodstock or through wild × hatchery crosses (13%; P. Santavy, U.S. Fish and Wildlife Service [USFWS], unpublished data). Dennys River adult returns are so low that hatchery production relies exclusively on captive broodstock. Age-1 and age-2 parr are collected in the Dennys River and are reared to maturity in freshwater; their progeny are stocked as fry. It is therefore likely that most broodstock parr originate from previously stocked fry and may not experience a smolt migration for multiple generations (USASAC 2008). Negative domestication effects have been observed in salmonids after only one generation in captivity (Kostow 2004; Pearsons et al. 2007).

Differences in stock quality, if present, may be most apparent during critical periods such as smolting. Smolting is a period of behavioral and physiological development in preparation for migration and life at sea (McCormick et al. 1987; Hoar 1988). Smolting is stimulated by increases in day length and changes in temperature (Hoar 1988; Muir et al. 1994) that define a smolt "window" when the developmental state is optimal for migration (McCormick et al. 1987). A shift from territorial to migratory behavior (particularly at night) occurs coincident with physiological changes (Greenstreet 1992).

Smolts gain the ability to tolerate full-strength seawater in part through proliferation of mitochondrion-rich cells in the gills (Richman et al. 1987). These cells are rich in Na^+,K^+-ATPase (enzyme code 3.6.1.36; IUBMB 1992), and enzyme activity peaks during smolting. As a result, gill Na^+,K^+-ATPase is often used as an indirect indicator of seawater tolerance (McCormick et al. 1987). If smolts remain in freshwater beyond the period of migration, a reversal of physiological and behavioral traits occurs (Stefansson et al. 1998). Cessation of migratory urge (Zydlewski et al. 2005), decreased gill Na^+,K^+-ATPase activity (McCormick et al. 1998), decreased seawater tolerance (McCormick et al. 1998; Handeland et al. 2004), and reversals of hormonal shifts (e.g., growth hormone and cortisol; McCormick et al. 1997) are observed as these fish undergo desmoltification.

The objectives of this laboratory study were to compare smolt development of hatchery-reared Atlantic salmon of the Dennys and Penobscot River stocks. Changes in "migratory urge" (as indicated by downstream movement) and gill Na^+,K^+-ATPase activity during smolting were assessed. These characteristics are associated with successful transition to the marine environment and, by extension, may provide insight into the overall performance and survival of these stocks.

Methods

Study animals.—First filial generation (F_1) sea-run Penobscot River broodstock were collected at a Penobscot River fishway (river kilometer 48) between May and September 2005. The fish were then trucked (45 km) to the USFWS Craig Brook National Fish Hatchery in Orland, Maine, and were held there until spawning that fall. Dennys River broodstock captured

as parr in the Dennys River were captive reared at the same hatchery until they were spawned as mature (age-3–5) adults in fall 2005.

Penobscot and Dennys River eggs were transferred at the eyed egg developmental stage to a smolt production facility, the USFWS Green Lake National Fish Hatchery (GLNFH) in Ellsworth, Maine. Eggs were incubated for 6 months until hatch and then were reared for 12 months (to age-1 smolts) using identical but stock-specific circular production pools (6- and 9-m diameter).

On February 25, 2005, study animals (hereafter termed "smolts") were nonselectively netted from production pools and anesthetized using tricaine methanesulfonate (MS-222) at a concentration of 100 mg/L (buffered with 20-mmol $NaHCO_3$, pH = 7.0). Each fish was measured (fork length [FL] and mass) and tagged with a Model TX1411L passive integrated transponder (PIT) tag (134.2 kHz, 2 × 12 mm, 0.1 g; Destron Fearing, St. Paul, Minnesota). Each tag was implanted intraperitoneally through a 2-mm incision in the smolt's ventral surface (Gries and Letcher 2002). Tag retention was 100% during the study.

To minimize size differences, the length frequency distribution of Penobscot River smolts was matched to that of Dennys River smolts (<5% of Penobscot River candidate smolts were excluded). After processing, Dennys River smolts ($N = 281$) and Penobscot River smolts ($N = 286$) were transferred to separate 2- × 2-m square holding tanks with rounded corners. Standard hatchery water (filtered and ultraviolet sterilized) was supplied (flow-through) at a rate of 38 L/min using a spray bar. The ambient temperature varied seasonally (2.7–19.7°C). Smolts were held under a simulated natural photoperiod for the hatchery (latitude: 44°35′9″N) and were fed to satiation once daily (3-mm pellets; Corey Hi-Pro, Friona, Texas).

Experimental conditions.—Three test tanks (Figure 2) were constructed at the GLNFH based on Zydlewski et al. (2005). Water within each 1.5-m-diameter test tank was circulated around a 77-cm-diameter cylindrical partition to simulate a unidirectional-flowing stream. The partition was positioned to create a narrow, 15-cm-wide raceway against one tank wall and a 58-cm-wide resting zone on the opposite side of the tank. Two rectangular PIT tag antennas (15 × 60 cm) were tightly fitted at the upstream and downstream ends of the raceway (33 cm apart). Each antenna was connected to a Model FS2001-F-ISO transceiver (Destron Fearing), which sent data to a computer. The resting zone was shaded with black plastic, and its floor was covered with mixed cobble (2–10 cm) to provide cover away from the antennas. Flow was generated by two submerged, 6.4-mm inlets positioned upstream of the raceway (Figure 2); these inlets supplied hatchery water at a rate of 38 L/min. Velocity was not uniform; lower flows (10–30 cm/s) were maintained in the resting zone, and higher flows (30–50 cm/s) were maintained in the raceway. Tank water depth was maintained at 0.3 m.

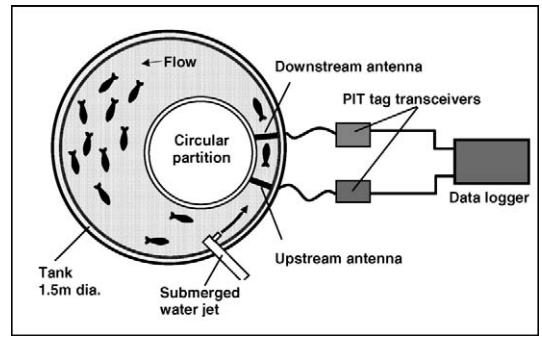

FIGURE 2.—Schematic of a behavioral evaluation tank (1.5-m diameter) at the Green Lake National Fish Hatchery, Ellsworth, Maine. Water circulated around the center partition to produce a unidirectional flow. Passive integrated transponder (PIT) antennas were positioned in the narrow tank section and were configured to quantify "upstream" and "downstream" movements of PIT-tagged Atlantic salmon smolts.

One 50-W, clear-glass, full-spectrum incandescent light bulb was centered 1.6 m above the water surface in each tank. A programmable timer was used to maintain photoperiod. The three tanks were arranged in a triangle, approximately 2 m apart on center, and each tank received indirect light from adjacent tank lights. A temperature logger (Stowaway XT108–05; Onset Computer Corporation, Bourne, Massachusetts) set to record temperature at 1-h intervals was deployed within the center partition of one tank.

Behavioral experiments.—Seven sequential 14-d trials started on March 23 and ended on June 29, 2005. For each trial, 30 Dennys River smolts and 30 Penobscot River smolts were netted from their respective holding tanks, anesthetized, measured (FL and mass), biopsied (gill; see below), and allowed to recover. These fish were then transferred to the three test tanks (20 fish/tank; 10 fish of each stock). Smolt movements were monitored by recording PIT tag detections on the antennas at 1-s intervals during the entire trial. Smolts were not fed. Disturbance was limited to a brief midday check when smolts were least active, and this action had no detectable effect on smolt movement. At the end of each trial, smolts were measured and a second gill biopsy was collected. Previously untested smolts were used for each trial.

Gill Na^+,K^+-ATPase assay.—The distal 2–3 mm of three to four gill filaments from each smolt were cut

from the first gill arch (left side at the start of a trial and right side at the end of a trial). The tissue was placed in a 500-μL microcentrifuge tube containing 100 μL of sucrose–EDTA–imidazole solution (250-mM sucrose, 10-mM EDTA, 50-mM imidazole, pH = 7.3) and was immediately frozen on dry ice. Biopsies were stored at −80°C for a maximum of 120 d prior to assay. The V_{max} (the maximum velocity that the reaction is catalyzed by the enzyme in the absence of other limiting factors) of Na^+,K^+-ATPase activity in gill homogenate was determined in duplicate as change in NADH at 340 nm with and without ouabain inhibitor (McCormick 1993). Protein concentration was determined in triplicate using the bicinchoninic acid (BCA) method (Smith et al. 1985; BCA protein kit; Pierce, Rockford, Illinois). Activity of gill Na^+,K^+-ATPase is expressed as micromoles of ADP per milligram of protein per hour (μmol ADP·mg protein^{-1}·h^{-1}). Assay data were rejected if the coefficient of variation of V_{max} or protein exceeded 15% between replicate values (resulting in the omission of 16% of assays).

Analysis of behavioral data.—Criteria described by Zydlewski et al. (2005) were used to classify and enumerate PIT tag detections as directional movements ("downstream" or "upstream"). Successive detections of a unique PIT tag on both antennas within a 3-s interval were classified as a directional movement (Figure 2). If a detection interval was greater than 3 s, the event was excluded because it is plausible that the smolt reversed direction and circled the tank from the opposite direction. Multiple detections on one or both antennas within 1-s intervals were classified as duplicates or "crosstalk" and were discarded. Antenna efficiency was not quantitatively characterized; we assumed each fish in a single tank had the same probability of successful detection. In this study, downstream movements of smolts were used as a measure of migratory urge.

Frequent movements (both directions) were observed during the initial 6 d of each trial and are attributed to a period of acclimation (see also Zydlewski et al. 2005). Thus, only data from trial days 7–13 (inclusive) were used for analyses of smolt movements. For each smolt, average daily movements were calculated for both directions and these data were analyzed separately. Daily movement means were nonnormally distributed (Shapiro–Wilk test). Data were tested using a three-way analysis of variance (ANOVA) on ranked data, with stock, tank, and trial as factors. When factors or interactions were significant, the data were compared post hoc with a Tukey's honestly significant different (HSD) multiple comparison test. The cumulative distribution of mean daily movements was compared between stocks using a Kolmogorov–Smirnov (KS) two-sample test.

To examine diel patterns, average number of downstream movements for each smolt during each 1-h interval of the day was calculated. Hourly averages were nonnormally distributed (Shapiro–Wilk test). Movement was compared with a two-way ANOVA on ranked data using trial and stock as factors. When factors or interactions were significant, data were compared post hoc using Tukey's HSD multiple comparison test. To compare diel patterns, movements were categorized as "day" (lights on) or "night" (lights off). The 1-h bins during which lights were turned on or off were excluded from analysis. The proportion of movement during the day was compared using two-way ANOVA on ranked data using trial and stock as factors. Data were compared post hoc using Tukey's HSD multiple comparison test.

Analysis of gill Na^+,K^+-ATPase activity and physical measures.—Data were analyzed using a *t*-test (using ranked data when nonnormally distributed; Shapiro–Wilk test). Gill Na^+,K^+-ATPase activity, condition factor ($K = 100 \times [\text{mass}/\text{FL}^3]$, where mass is in g and FL is in cm), and body mass were nonnormally distributed; FL was normally distributed. The FLs measured at the start and end of a trial were similar within each stock and trial (*t*-test). Lengths were averaged by stock and trial to derive mean FLs. Relationships between gill Na^+,K^+-ATPase activity, FL, mass, *K*, and migratory urge were tested using regression analysis of ranked data. For all analyses, differences were considered significant at *P*-values less than 0.05.

Results

Seasonal Behavior

Dennys and Penobscot River smolts displayed similar patterns of movement over the course of this study. Movement was predominately downstream (>96% of average daily movements; Figure 3A). The cumulative distributions of upstream and downstream movements were independent of stock (KS test), and directional movements did not differ between stocks within trials. Downstream movements remained low through late April (<15 movements/d), increased 10-fold by early May, and continued to increase through mid-May, exceeding April levels by 20-fold. Downstream movements remained high (Dennys River smolts: 431 movements/d; Penobscot River smolts: 449 movements/d) through mid-June, declining by half by the end of June (trial 7; Figure 3A). During mid-May (trial 4), downstream movements averaged 476 movements/d for Penobscot River smolts versus 332 movements/d for Dennys River smolts, but the

difference was not significant (Tukey's test: $P = 0.11$). A tank effect was observed in all trials and was attributed to differences in antenna efficiency (two-way ANOVA: df = 2, $P < 0.001$). Significant tank × stock interactions were not observed (df = 2, $P = 0.35$), indicating that there was no tank-related bias for stock-specific detectability.

Diel Activity

Downstream movements occurred mostly at night for both stocks, but there was seasonal variation (Figure 4). In March and early April (trials 1–2), when smolt movements were low, only 20% of downstream movements occurred at night. In late April and early May, downstream movements increased and were predominately at night (trial 4; Dennys River smolts: 83%, Penobscot River smolts: 73%). The proportion of daytime movements was highest (up to 40%) for both stocks at the end of peak migration. Daily movements typically peaked 2 h after sunset and remained high until at least 1 h after sunrise (Figure 4). Through trial 4, the frequency of movements was lowest at midday and remained low until after sunset. While the overall patterns of movement were similar between stocks, movements of Penobscot River smolts in trials 4 and 5 persisted further into early morning, resulting in more daytime movements than were exhibited by Dennys River smolts (Figure 4).

Gill Na^+,K^+-ATPase Assay

Both stocks exhibited a seasonal pattern of gill Na^+,K^+-ATPase activity associated with smolting. Enzyme activity was low in March, peaked in late April, and declined to the lowest observed levels by the end of June (Figure 3B). Gill Na^+,K^+-ATPase activities did not differ between stocks at the end of each trial, but Dennys River smolts had higher enzyme activity than Penobscot River smolts at the beginning of trials 2 and 3 (Tukey's test: $P = 0.003$ and $P < 0.001$; Figure 3B).

Patterns of change in gill Na^+,K^+-ATPase activity differed between stocks. Activity level measured at the start of each trial in Dennys River smolts increased nearly twofold between late March and late April (trials 1–3), while the Na^+,K^+-ATPase activity level of the Penobscot River smolts remained unchanged. Enzyme activity in Dennys River smolts then declined by 35% in early May (at the start of trial 4), while activity in Penobscot River smolts increased by 25%. Unlike Dennys River smolts, Penobscot River smolts had greater changes in activity level within a trial (start versus end) than between trials during trials 1–3.

Peak gill Na^+,K^+-ATPase activities occurred in advance of peak movements for both stocks. By June (trial 6), gill Na^+,K^+-ATPase activity had decreased to March levels (trial 1), while downstream movements remained sixfold higher for both stocks (Figure 3). There were no direct relationships between gill Na^+,K^+-ATPase activity and the frequency of movement within a trial for Penobscot River smolts. The same was true for Dennys River smolts for all trials except trial 4, where a weak positive relationship with downstream movements was observed ($R^2 = 0.132$, $P = 0.032$).

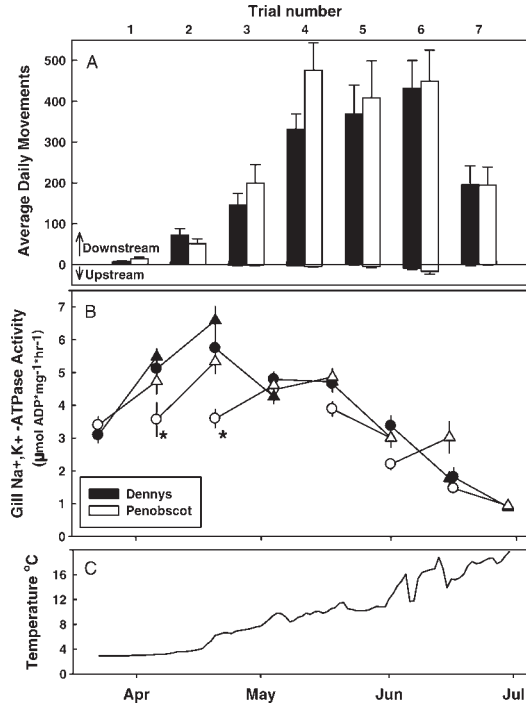

FIGURE 3.—Seasonal mean (±SE) movements and gill Na^+,K^+-ATPase activity of Penobscot and Dennys River Atlantic salmon smolts assessed during 14-d behavioral trials: **(A)** upstream (–) and downstream (+) movements, **(B)** gill Na^+,K^+-ATPase activity (μmol ADP•mg protein^{-1}•h^{-1}) at the start (circles) and end (triangles) of each behavioral trial, and **(C)** temperature profile over the seven trial periods.

Physical Comparisons

The mean FL of smolts did not change within any 14-d trial but increased between trials. Length differed between stocks only in trial 1 (Dennys River smolts were shorter; *t*-test: $P = 0.043$) and trial 7 (Dennys River smolts were longer; *t*-test: $P = 0.003$; Table 1). Similarly, mass measured at the start of each trial increased throughout the 3-month study, differing between stocks only for trial 1. During each 14-d trial, the unfed smolts decreased in body mass; the greatest losses occurred in trials 4–7, when mean daily water

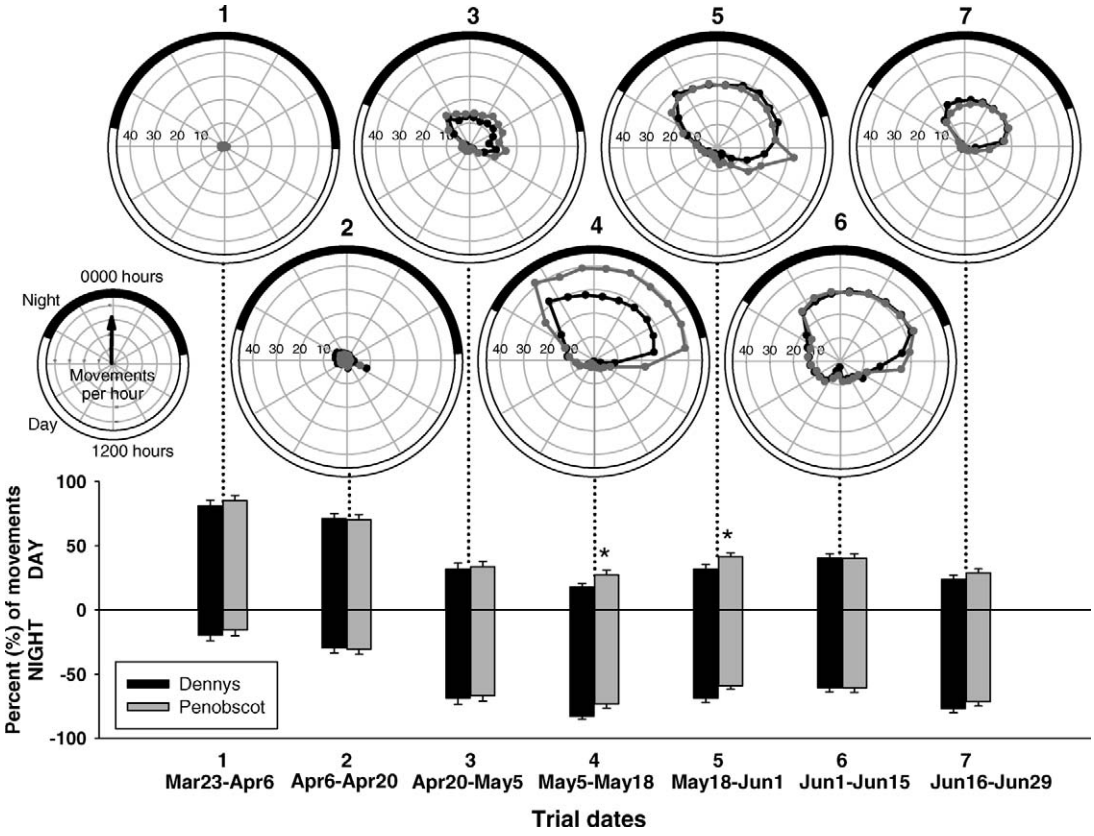

FIGURE 4.—Mean diel downstream movements of Penobscot and Dennys River Atlantic salmon smolts assessed in 14-d behavioral trials. Circular "radar plots" (top graphs) represent movement data in twenty-four 1-h bins such that distance from the center indicates magnitude and the position on the circle indicates time of day (0000 hours EST at the top, 1200 hours EST at the bottom). The concentric ring on each graph indicates night (black) and day (white). The lower bar graph shows the mean (±SE) percentage of movements occurring during night (–) and day (+). Asterisks indicate significant differences between stocks within a trial.

TABLE 1.—Descriptive statistics for Dennys (D) and Penobscot (P) River Atlantic salmon smolts used in 14-d behavioral trials. Range of mean daily water temperatures (temp) during experimental trials and mean (±SE) fork length (FL), mass, and condition factor (K) are shown. Asterisks indicate significant differences between stocks within a trial ($P \leq 0.05$).

					Mass (g)		K	
Trial	Dates	Temp (°C)	Stock	FL (mm)	Start	End	Start	End
1	Mar 23–Apr 6	2.9–3.2	D	170 ± 1.8	53 ± 1.8	52 ± 1.8	1.07 ± 0.00	1.04 ± 0.00
			P	175 ± 1.7*	59 ± 1.7*	59 ± 1.8*	1.09 ± 0.01	1.06 ± 0.01
2	Apr 6–Apr20	3.2–6.2	D	173 ± 1.8	53 ± 1.7	52 ± 1.7	1.02 ± 0.01	1.00 ± 0.01
			P	174 ± 1.6	55 ± 1.6	55 ± 1.6	1.06 ± 0.01*	1.02 ± 0.01
3	Apr 20–May 4	6.2–9.4	D	177 ± 1.7	55 ± 1.8	54 ± 1.7	1.02 ± 0.01	0.96 ± 0.01
			P	174 ± 1.7	56 ± 1.6	53 ± 1.5	1.05 ± 0.01*	0.99 ± 0.01*
4	May 4–May18	9.4–10.5	D	181 ± 1.6	57 ± 1.6	53 ± 1.4	0.98 ± 0.01	0.90 ± 0.01
			P	177 ± 1.8	55 ± 1.8	52 ± 1.7	1.00 ± 0.01	0.92 ± 0.01
5	May 18–Jun 1	10.5–12.1	D	182 ± 1.6	56 ± 1.5	52 ± 1.5	0.94 ± 0.01	0.87 ± 0.01
			P	180 ± 1.7	56 ± 1.6	53 ± 1.5	0.96 ± 0.01*	0.89 ± 0.01
6	Jun 1–Jun 15	12.1–13.9	D	185 ± 1.6	57 ± 1.4	54 ± 1.3	0.90 ± 0.01	0.86 ± 0.01
			P	185 ± 2.0	58 ± 2.0	55 ± 1.9	0.92 ± 0.01	0.86 ± 0.01
7	Jun 16–Jun 29	15.3–19.0	D	194 ± 1.7	65 ± 2.2	61 ± 2.1	0.90 ± 0.01	0.83 ± 0.01
			P	186 ± 1.6*	61 ± 2.0	58 ± 1.9	0.93 ± 0.01*	0.87 ± 0.01*

temperatures ranged from 9.4°C to 19.0°C (Table 1). Patterns of loss were similar for both stocks, although Dennys River smolts had a greater loss (1.5%; *t*-test: $P = 0.01$) in trial 1, and Penobscot River smolts had a greater loss (1.3%; *t*-test: $P = 0.02$) in trial 2. The *K* for both stocks declined throughout the study (Table 1). The *K*-value was greater for Penobscot River smolts than for Dennys River smolts at the beginning of four trials (2, 3, 5, and 7) and remained higher at the conclusion of two of those trials (3 and 7). For all trials and for both stocks, *K* declined between the start and end of a trial.

Correlations between physical characteristics (FL and *K*) and gill Na^+,K^+-ATPase activity or migratory urge were absent or weak. There was no relationship between FL and gill Na^+,K^+-ATPase activity or movements in any trial for either stock. Gill Na^+,K^+-ATPase activity and *K* were negatively correlated for Dennys River smolts in trial 6 ($R^2 = 0.248$, $P = 0.040$) and positively correlated for Penobscot River smolts in trial 3 ($R^2 = 0.122$, $P = 0.033$). There was no relationship between downstream movements and *K* for Penobscot River smolts in any trial, but these measures were negatively correlated in trial 2 ($R^2 = 0.286$, $P = 0.002$) and trial 4 ($R^2 = 0.130$, $P = 0.034$) for Dennys River smolts.

Discussion

Both Penobscot and Dennys River Atlantic salmon smolts exhibited physiological and behavioral changes characteristic of the parr–smolt transformation. Smolts exhibited an increase in migratory urge and gill Na^+,K^+-ATPase activity, followed by a return to parr-like levels in response to containment in freshwater. Qualitatively, these changes are well characterized for Atlantic salmon smolts and are consistent with the results of a plethora of studies (e.g., McCormick et al. 1998, 1999; Whalen et al. 1999; Zydlewski et al. 2005). Observed differences between stocks were minor but noteworthy.

Stocks differed in their physiological response to the test tank during early trials (1–3). Unlike Dennys River smolts, the gill Na^+,K^+-ATPase activity of Penobscot River smolts increased only after exposure to the test tank environment (Figure 3B). Measures of smolting are sensitive to environmental stimuli, and large poststocking increases in gill Na^+,K^+-ATPase activity have been documented (Zaugg 1982; Zaugg et al. 1985; Beeman et al. 1994; McCormick et al. 2003). Natural rearing environments are also associated with heightened growth hormone and seawater tolerance (e.g., steelhead *Oncorhynchus mykiss* smolts; Zydlewski et al. 2003). In this study, tested smolts experienced cobble substrate and encountered higher-velocity flows than their respective cohorts in the holding tanks. It is possible that the difference between stocks indicates diminished responsiveness to environmental factors in Dennys River smolts or an advance in the timing of Penobscot River smoltification mediated by test conditions. More likely, however, is that this difference reflects the higher initial enzyme activities for Dennys River smolts than for Penobscot River smolts (Figure 3).

While migratory urge among Penobscot River smolts was 44% higher than that of Dennys River smolts at the peak of movements, there were no statistical differences. Individual data varied considerably, and larger sample sizes might have revealed existing differences. Because the Penobscot River is three times the length of the Dennys River, an earlier onset of migration for Penobscot River smolts might be expected. Timing of smoltification has a heritable component (Nielsen et al. 2001; Olsen et al. 2004; Garcia de Leaniz et al. 2007), and smolts from headwater stocks may migrate earlier than those of downriver stocks (Stewart et al. 2006).

Downstream movements occurred predominately at night, though modest differences were observed in this pattern over time and between stocks. Movements in the early daylight hours increased towards the end of smolting, particularly for Penobscot River smolts (Figure 4). Nocturnal smolt migration is typical of many Atlantic salmon populations (Greenstreet 1992; Moore et al. 1995; Carlsen et al. 2004), although smolts migrate during the day in some rivers (e.g., sub-Arctic River Utsjoki; Davidsen et al. 2005). Nocturnal migration is thought to reduce predation risk, particularly early in the smolt run at low temperatures (Metcalfe et al. 1999). Later in the run, the risk of diurnal migration may be offset by enhanced foraging opportunity, growth, and performance (Valdimarssons et al. 1997; Metcalfe et al. 1999). For these fish, the cost of failing to reach seawater in synchrony with physiological development may be higher than the risks associated with daytime migration.

These small differences in physiology and behavior may reflect adaptive characteristics or incongruity in broodstock rearing. Although high survival in hatchery programs minimizes selective loss of genetic material (Doyle 1983), domestication can contribute to the fixation of deleterious alleles (Lynch and O'Hely 2001) and can induce phenotypic changes (e.g., Kostow 2004). Additionally, in teleosts, the experiences of parents can influence gamete quality and the phenotype of the progeny (Eskelinen 1989; Swain and Foote 1999; Izquierdo et al. 2001). This has also been observed in other taxa (e.g., lizards; Sorci and Clobert 1997). Such is the case for these two Atlantic salmon stocks, which exhibit markedly different survival to the

eyed egg stage in the hatchery (mean ± SE = 88 ± 0.03% for Penobscot River stock versus 76 ± 0.05% for Dennys River stock, 2003–2007; D. Buckley, USFWS, unpublished data).

Many factors (including domestication) can influence the migratory behavior of smolts. Differences in the run timing of hatchery smolts produced from wild and captive broodstock of masu salmon *O. masou* were apparent after only three generations (Koyama et al. 2007). The control of smolt migration timing is a complex synthesis of endogenous and exogenous factors. The interaction of photoperiod and water temperature is thought to be the primary driver of seasonal gill Na^+,K^+-ATPase trajectories (McCormick et al. 1987, 1999; Handeland et al. 2004). Timing of smoltification can also be influenced by smolt size, smolt age (Jutila et al. 2006), variation in water temperature, and flow (Greenstreet 1992; Hvidsten et al. 1995; Whalen et al. 1999; Zydlewski et al. 2005), but those factors were controlled in the current study. Cohabitation of multiple fish (and stocks) within study tanks may have homogenized behavior through a "follow-the-leader" effect (Hansen and Jonsson 1985). Experiments addressing the influences of stock interactions could provide additional information but would impose obvious logistical challenges.

Food deprivation in the test tanks may also have influenced movements. An inverse relationship between food uptake and gill Na^+,K^+-ATPase activity has been observed in brown trout *S. trutta* smolts (Pirhonen and Forsman 1998a), but the relationship of food intake to smolt behavior is unclear (Pirhonen and Forsman 1998b). Metabolic requirements could prompt increases in downstream movements if smolts were energy limited. This scenario is unlikely, however, as upstream movements were highest (trial 6) and downstream movement declined (trial 7) coincident with elevated temperatures and maximum loss of body mass.

The absence of a clear relationship between gill Na^+,K^+-ATPase and migratory urge, while unexpected in this study, has been previously reported in salmonids (Pirhonen and Forsman 1998; Aarestrup et al. 2002). A temporal offset in physiology and behavior could reflect a natural condition. For example, smolts of Chinook salmon *O. tshawytscha* migrate from headwater areas prior to an increase in gill Na^+,K^+-ATPase activity (Ewing et al. 1980). Alternately, the methodological approach of this study may have caused an apparent decoupling. The peak migration of Dennys and Penobscot River hatchery smolt cohorts released into their appropriate rivers occurred in mid-May 2005 (P. Ruksznis, Maine Department of Marine Resources, unpublished data; J. Hawkes, National Oceanic and Atmospheric Administration Fisheries, unpublished data). If the tested smolts had not been confined, the observed movement pattern would have resulted in rapid entry into seawater coincident with peak gill Na^+,K^+-ATPase and, presumably, high seawater tolerance (McCormick et al. 1987; Nilsen et al. 2003; Bystriansky et al. 2006).

Elevated migratory urge of test smolts persisted past the peak of migration observed in the field. Such persistence in "migratory restlessness" (zugunrhue) has been observed in controlled studies with migratory birds (Berthold 2001; Gwinner and Czesschlik 2001). Maintaining migratory urge over a 2-month period may be advantageous for smolts that are impeded or delayed during migration. Even past the peak of physiological preparedness, postsmolts retain the ability to acclimate to seawater (Arnesen et al. 2003). Such an outcome may be preferable to remaining in a warming freshwater environment as a result of an incomplete migration.

Pending increases in the abundance of sea-run Atlantic salmon, captive-reared broodstock will be required to support stock enhancement measures in the Dennys River and elsewhere. Based on the results of this study, it is clear that the innate migratory urge and freshwater physiological development of Denny River Atlantic salmon smolts is intact relative to Penobscot River smolts. It seems unlikely that the modest differences observed would cause the drastic differences observed in adult return rates. This unsatisfying conclusion is complicated by the fact that genetic and rearing environment effects could not be isolated in this study. Characterizing these effects should be a management priority where Atlantic salmon enhancement programs rely on captive-reared broodstock. Paired releases, such as smolts produced by captive-reared and sea-run parents, would provide a direct and meaningful comparison of smolt performance.

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