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Gill Na^+,K^+-ATPase of Atlantic salmon smolts in freshwater is not a predictor of long-term growth in seawater

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ABSTRACT

Gill Na^+,K^+-ATPase activity is a widely used measure of osmoregulatory preparedness in salmonid smolts. The degree to which this measure may predict long term performance is uncertain. In order to assess the relationship of this enzyme to long term growth and ion homeostasis, a cohort of Atlantic salmon hatchery smolts was used in a controlled environment with no salinity perturbations. In May 2006, gill Na^+,K^+-ATPase activity from 940 individually PIT tagged, Penobscot River smolts (USFWS, Green Lake National Fish Hatchery, Maine, United States) was measured immediately prior to isothermal transfer from freshwater to 32 ppt seawater. From the observed range of activities, individuals were classified as having "low", "middle", or "high" enzyme activity levels. Individual size (fork length and mass) was recorded on days 0, 1, 3, and 14 and monthly for four months. Growth rates over four time periods were calculated for individual fish maintained until the end of the experiment. Gill Na^+,K^+-ATPase activities were also measured from a subset of sampled fish. All groups effectively osmoregulated as evidenced by minor perturbations in plasma osmolyte levels. Apart from initial weight loss on transfer, fish grew throughout the experiment, however, there were no differences (fish size, growth rate, and gill Na^+,K^+-ATPase activity in seawater) among groups with initially different gill Na^+,K^+-ATPase activities (prior to seawater entry). While gill Na^+,K^+-ATPase activity may be predictive of performance during the acute phase of acclimation (first few days), typical variation in this enzyme, expressed in freshwater at the peak of smolting, does not appear to be predictive of long-term growth in seawater.

1. Introduction

At the stage of emigration from freshwater, juvenile salmonids must be prepared for the osmoregulatory challenge of entering seawater. Osmoregulatory performance can be assessed as survival in seawater, growth in seawater, and by using multiple physiological measures. Traditionally, juvenile salmonids are isothermally transferred from fresh to salt water and maintained in salt water anywhere from 24 to 96 h (Clarke and Blackburn, 1977 and others). After 24–96 h in salt water, survival and physiological measures of osmoregulatory performance (e.g. plasma osmolality and plasma ions) are assessed. Fish surviving these tests and demonstrating normal plasma osmolality and ion levels are assumed to have good long-term survival in the ocean.

Elevated gill Na^+,K^+-ATPase activity in salt water has been observed in a number of teleost species (Epstein et al., 1980; Kamiya and Utida, 1968) including anadromous salmonids (Hoar, 1988). This measure has been a powerful tool in assessing smolt development, and has served as an indirect indicator of osmoregulatory competence (McCormick, 1993). The interaction of photoperiod and water temperature is thought to be the primary driver of seasonal Na^+,K^+-ATPase trajectories in salmon (Handeland et al., 2004; McCormick et al., 1987, 2002).

In natural systems, smolt preparedness for seawater is typically assessed in freshwater. At this time, it is relatively easy to capture naturally migrating smolts and take morphological and physiological measurements. In the short term, it is assumed that gill Na^+,K^+-ATPase activity of a salmon captured migrating out of freshwater will be indicative of its ultimate performance upon entering seawater, such that individuals that are physiologically prepared will perform better, and as a result incur less mortality upon seawater entry (Moser et al., 1991). Such preparation may also allow juveniles to pass through the estuary more quickly (Schreck et al., 2006) and lower in the water column than those less prepared (Price and Schreck, 2003). Consistent with this hypothesis is the observation that steelhead smolts (*Oncorhynchus mykiss*) with elevated gill Na^+,K^+-ATPase had a decreased risk of avian predation in the Columbia River estuary (Kennedy et al., 2007).

It is tempting to consider average gill Na^+,K^+-ATPase activities from a cohort in freshwater to indicate how well a population of fish

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may perform, but gill Na^+,K^+-ATPase activity is highly variable during smolting (e.g. Zydlewski et al., 2010). Long-term seawater performance (growth and survival) is rarely quantified, and individual based information is sparse. In this study, we sought to assess the long-term performance of Atlantic salmon upon seawater entry as smolts. We used initial (freshwater) gill Na^+,K^+-ATPase activities to determine if variation in this enzyme's activity resulted in differences in growth scope and ionic homeostasis in seawater.

2. Materials and methods

2.1. Research animals

First filial (F1) sea-run Penobscot River, Maine Atlantic salmon broodstock were captured in a trap at the top of a fishway at the first dam on the river (river km 48) between May and September 2004. The fish were then trucked (45 km) to USFWS (United States Fish and Wildlife Service) Craig Brook National Fish Hatchery (CBNFH) in Orland, Maine and held there until spawning that fall. 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 hatched, reared in circular tanks (2 m diameter) before being transferred to production pools (6 m diameter) and reared as age 1+ smolts. Tanks and pools were flow through freshwater with simulated natural photoperiod and natural lighting, respectively.

On 12 April 2006, 1013 juveniles were non-selectively netted from a single production pool and anesthetized using 100 mg/L MS-222 (buffered with 20 mmol $NaHCO_3$, pH = 7.0). Each fish was measured (fork length, mm and mass, g) and PIT tagged with a Destron Fearing (St. Paul, MN) TX1411L tag (134.2 kHz, 2×12 mm, 0.1 g). The tags were implanted intra-peritoneally through a 2 mm surgical incision on the smolt's ventral surface (Gries and Letcher, 2002) and placed into the production pool for recovery. These fish (hereafter termed "smolts" for the purposes of this study) remained in the production pool and were maintained by the staff at GLNFH as part of normal rearing practices until 10 May 2006. Based on the natural variability in gill Na^+,K^+-ATPase activity for Atlantic salmon maintained at GLNFH observed in 2005 (Zydlewski et al., 2010) we estimated the peak of physiological smolting to be on or about 10 May.

On 10 May 2006, 940 smolts were anesthetized, scanned for a PIT tag, measured (as above) and a non-lethal gill biopsy was taken for subsequent gill Na^+,K^+-ATPase activity measurement (see Section 2.4). Smolts were then transported to the University of Maine Aquaculture Research Center (ARC; 68 km) in two aerated 1000 L transport tanks. Fish were divided into five 1000 L tanks maintained as a single re-circulating seawater (SW; 32 ± 2 ppt) system. This system was maintained with one particle filter, two chiller systems and biological filters seeded and established 6 weeks prior to the initiation of the study. Water quality (total ammonia, nitrite, pH, dissolved oxygen, and temperature) was monitored daily. Target total ammonia levels were dependent on temperature, varying from 0.5 to 0.7 ppm (total ammonia never rose above 0.78 ppm). Water flow to the tanks was adjusted to maintain low levels of total ammonia. With the exception of fasting 24 h in advance of all sampling, smolts were fed by hand to satiation (until fish stopped striking the food hitting the surface) 2–4 times daily with standard diets (Corey Aquafeeds, Friona, TX, Hi-Pro—Parr and Smolt Feed for Atlantic Salmon and Trout, 3 mm).

2.2. Maintenance of environmental conditions in seawater

Water temperature was 8.5 °C (approximately isothermal with the pond at GLNFH), and initial photoperiod was simulated natural photoperiod (SNP) for the GLNFH (latitude: 44° 35′ 9″ N). Throughout the study, temperature and photoperiod regimes were maintained at those reflective of the theoretical migratory path of an Atlantic salmon smolt leaving the Penobscot River and migrating to the Labrador Sea by September (as estimated from Friedland et al., 1999). Approximate latitude and longitude were determined along the migratory path from the Penobscot River on 10 May, at Gulf of Maine (GOM) GoMOOS buoy F (44° 33′ 20″ N) on 17 May, GoMOOS Buoy I (Eastern Maine shelf; 44° 86′ 21″ N) on 24 May, GoMOOS Buoy M (Jordan Basin; 43° 29′ 27″ N) on 31 May, Scotian Shelf (44° 04′ 47″ N) on 14 Jun, the Laurentian Channel (45° 03′ 40″ N) on 5 July, the Grand Banks (45° 01′ 44″ N) on 26 July, and the Labrador Sea (51° 44′ 22″ N) on 16 August. From this, day length was determined from the NOAA photoperiod calculator. Target temperatures were based on those retrieved from buoys or satellite data from the respective regions from 2005 (GoMOOS and SeaWifs; www.mar.dfo-mpo.gc.ca/science/ocean/ias/seawifs/seawifs_4.html). Initial temperatures (10 May–14 May) were 1 °C higher than Penobscot River temperatures to accommodate isothermal transfer of fish from the hatchery to the University of Maine tanks. Photoperiod and temperature regimes are shown in Fig. 1.

2.3. Freshwater gill Na^+,K^+-ATPase activity designation

In order to designate groups based on gill Na^+,K^+-ATPase activity, on 11 May 2006, 940 gill biopsies from day 0 were processed as described below (Section 2.5). Gill Na^+,K^+-ATPase activity ranged from 0.6 to 10.8 μmol ADP · mg protein^{-1} · h^{-1} (Fig. 2). Based on these results all individuals were categorized by their freshwater ATPase (FWATP) level as "low", "middle", or "high". Low levels ranged from 0.6 to 4.3; middle ranged from 4.9 to 5.7; and high ranged from 6.5 to 10.8 μmol ADP · mg protein^{-1} · h^{-1}. FWATP groups were haphazardly divided among the holding tanks. On day 14 fish biomass was equalized among tanks (13.6, 13.3, 13.8, and 13.1 kg of fish in each).

2.4. Sampling protocol

On day 0 (10 May), sixty PIT tagged individuals were lethally sampled prior to transport and SW transfer of study smolts. All fish were measured and gill biopsied as above (Section 2.1). Additionally,

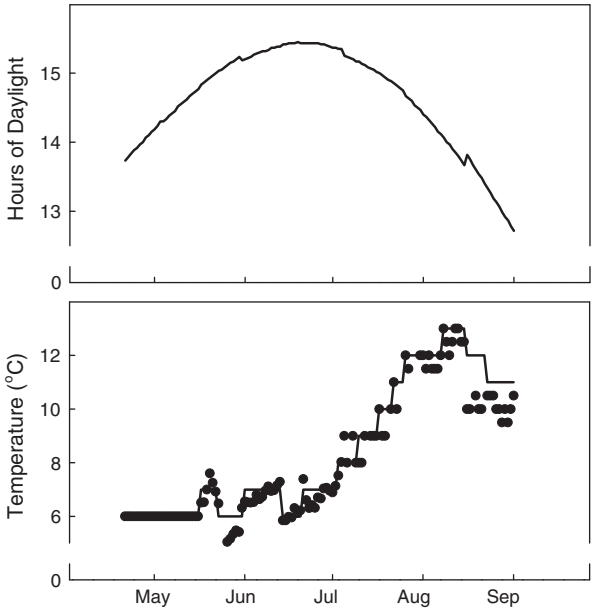

Fig. 1. Photoperiod (top) and temperature (bottom) experienced by Atlantic salmon smolts held in seawater. Conditions were calculated to match those experienced by naturally migrating smolts in the Atlantic Ocean. Target water temperature is indicated by the solid line while actual temperatures are indicated by solid circles.

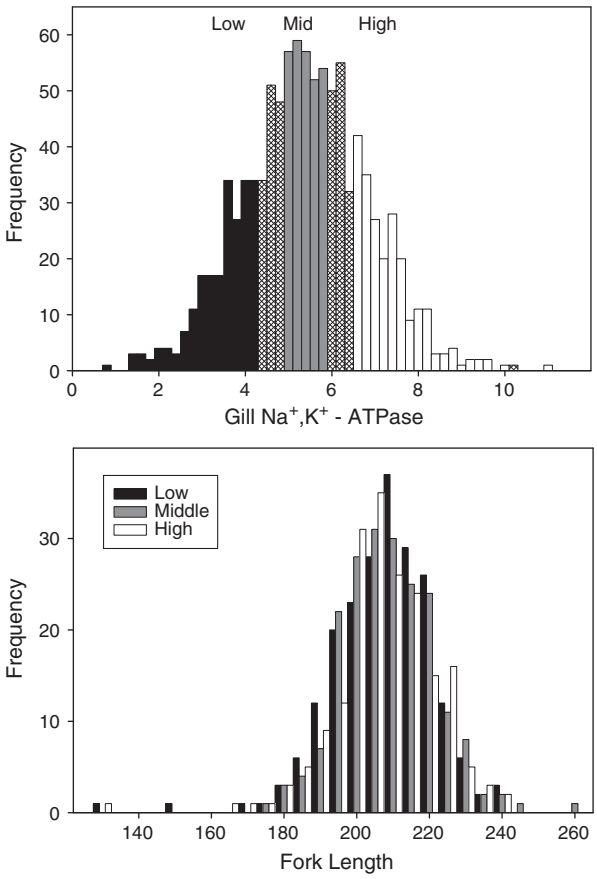

Fig. 2. (Top) Gill Na^+,K^+-ATPase activity (μmol ADP · mg $protein^{-1}$ · h^{-1}) for 940 Atlantic salmon sampled to assign as "low", "middle" or "high" enzyme activity prior to seawater transfer (n = 200). (Bottom) Fork length (mm) frequency histogram, grouped by enzyme activity, for 600 Atlantic salmon smolts maintained to determine long-term performance in seawater.

a blood sample was collected from the caudal vein into a 1 mL ammonium heparinized syringe. The needle was removed and the blood expelled into a 1.8 mL centrifuge tube. The blood was spun at 2000 × gravity (*g*) for 5 min and the plasma removed into a 0.5 mL tube, frozen and stored at −80 °C for subsequent analysis. Similarly, sixty smolts were sampled on days 1, 3, 14, 44, 70, 98, and 132 (May 11, 13, and 24, and, thereafter, approximately monthly on June 23, July 19, August 16 and September 19), twenty fish from each FWATP designation ("low", "middle", and "high"). Smolts from each category were sampled evenly from all tanks. There is no gill Na^+,K^+-ATPase activity data from day 98.

On days 14, 44, 70 and 98, all smolts were non-lethally sampled. Smolts were scanned for PIT identification, length and mass measured and returned to their respective tanks for recovery. On days 3, 6, 21, and 44 non-target fish (based on FW Na^+,K^+-ATPase activity designation) were removed to maintain rearing density.

2.5. Gill Na^+,K^+-ATPase activity measurement

The distal 2–3 mm of three to four gill filaments from each smolt were excised from the left side first gill arch, placed in a 500 μL microcentrifuge tube containing 100 μL of SEI solution (250 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3), and immediately frozen on dry ice. Biopsies were stored at −80 °C for a maximum of 120 days prior to assay. V_{max} of Na^+,K^+-ATPase activity of gill homogenate was determined in duplicate as change in [NADH] at 340 nm with and without ouabain (McCormick, 1993). Protein concentration was determined in triplicate using the bicinchoninic acid (BCA) method (Smith et al., 1985; BCA Protein kit, Pierce, Rockford, IL, USA). Activity of gill Na^+,K^+-ATPase is expressed as μmol ADP · mg $protein^{-1}$ · h^{-1}.

2.6. Plasma analyses

Plasma potassium, sodium, and chloride ion concentrations were measured using an EasyLyte electrolyte analyzer (Medica Corporation, Bedford, MA, USA) with internal calibration and two point external standard verification for sodium and chloride (100 and 200 mM NaCl). Plasma osmolality was measured using an Advanced Instruments 3200 (Norwood, MA, USA) freezing point depression osmometer with 50, 290 and 850 mOsm external standards.

2.7. Statistical analyses

For day 0, length and mass data for each of the FWATP groups were compared. Length, mass, and condition factor for all individuals were also related to freshwater gill Na^+,K^+-ATPase activity (FWATP) using linear regression. Condition factor was calculated as $100 \cdot g \cdot cm^{-3}$. Changing fork length and mass were recorded for all FWATP groups through time. Within each sampling date a one-way ANOVA (or Kruskal–Wallis if the data did not meet assumptions of ANOVA) was run to examine differences in fork length and mass for the three FWATP groups.

Specific growth rates for those fish maintained until day 98 (n = 305; 103 low; 102 middle; 100 high) were calculated for growth periods 0–14 days, 15–44 days, 45–70 days, and 71–98 days. Specific growth was calculated as $100 * (\ln(mass_2) - \ln(mass_1)) \cdot \Delta time^{-1}$, with time measured as days. A two-way ANOVA (factors: FWATP and date) was used to analyze differences. The relationship between gill Na^+,K^+-ATPase activity levels and specific growth rate was analyzed using linear regression. Similarly, the relationship between freshwater gill Na^+,K^+-ATPase activity and final gill Na^+,K^+-ATPase activity was analyzed using linear regression for each lethal sampling date. Plasma sodium, chloride, osmolality and gill Na^+,K^+-ATPase activity differences associated with FWATP and date were tested with a two-way ANOVA. Where data were not normally distributed they were ranked before analysis and *post hoc* analyses were carried out with the Holm–Sidak method. Otherwise, parametric multiple comparisons were carried out with the Bonferroni method.

3. Results

3.1. Initial measures of FWATP groups

Initial FW sampling on 10 May (day 0) resulted in a normal distribution of gill Na^+,K^+-ATPase activity among the sample population. By design, gill Na^+,K^+-ATPase activity for these FWATP groups (n = 200) differed significantly ($p < 0.001$, Kruskal–Wallis; Figs. 2 and 3). Size (length and mass) did not differ among the three FWATP groups sampled on day 0 ($p = 0.841$, $p = 0.560$, respectively; Fig. 4). For all smolts, median fork length was 206 mm (198 mm and 215 mm, 25th to 75th percentiles; Fig. 2) and the median mass was 90.5 g (80 g and 102.6 g, 25th to 75th percentiles). Gill Na^+,K^+-ATPase activity was not related to length ($p = 0.429$, $r^2 = 0.0006$), mass ($p = 0.596$, $r^2 = 0.00028$) or condition factor ($p = 0.194$, $r^2 = 0.0017$).

3.2. Tank effects

All data (length, mass and gill Na^+,K^+-ATPase activity) were analyzed for the effects of fish being reared in different tanks. No tank effects were found for length and mass (ANOVA, $p > 0.40$ for tank effects within each date of sampling). For gill Na^+,K^+-ATPase activity, tank effects of activities were found on days 3 and 14.

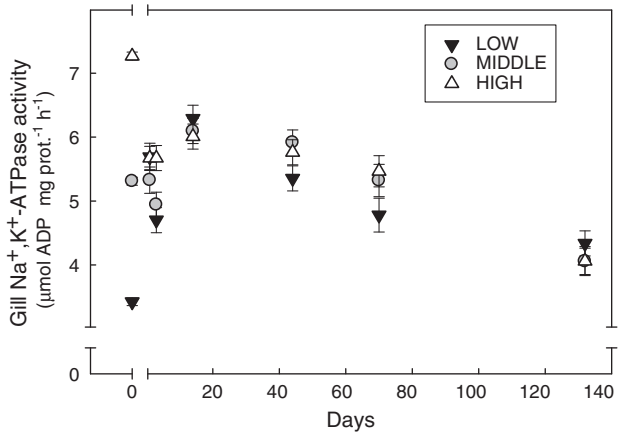

Fig. 3. Gill Na^+,K^+-ATPase activity (μmol ADP · mg protein^{-1} · h^{-1}) for Atlantic salmon smolts transferred to seawater. Initial Na^+,K^+-ATPase activities (in FW) indicated to the left of the x axis break. Data are mean ± SE. Note that activities were not available on day 98.

3.3. Growth and survival in SW

No seawater-induced mortalities were recorded during the experiment. When examined in a full two-way ANOVA (factors: date and FWATP) all analyses (length, mass, and specific growth rate) resulted in a significant effect of date ($p>0.001$) but not FWATP, and there were no interaction effects between the factors. There were no differences within dates for fork length or mass on any of the sampling dates ($p>0.40$). While all groups had increasing growth rates throughout the study (date factor in two-way ANOVA, $p<0.001$), there was no effect of FWATP group ($p=0.866$) and no interaction between the factors ($p=0.972$). Specific growth rate was not related to gill Na^+,K^+-ATPase activity at any intervals except between days 45 and 70 ($p=0.025$), however the explanatory power was low ($r^2=0.0845$, $n=55$).

Smolts increased in both length and mass over the entire study; however smolts did not increase in mass until 70 days after transfer. From 0 to 3 days, smolt mass declined. This trend is described by negative specific growth from 0 to 14 days, becoming positive by 14–44. Specific growth and mass then increased for the remaining weeks of the study.

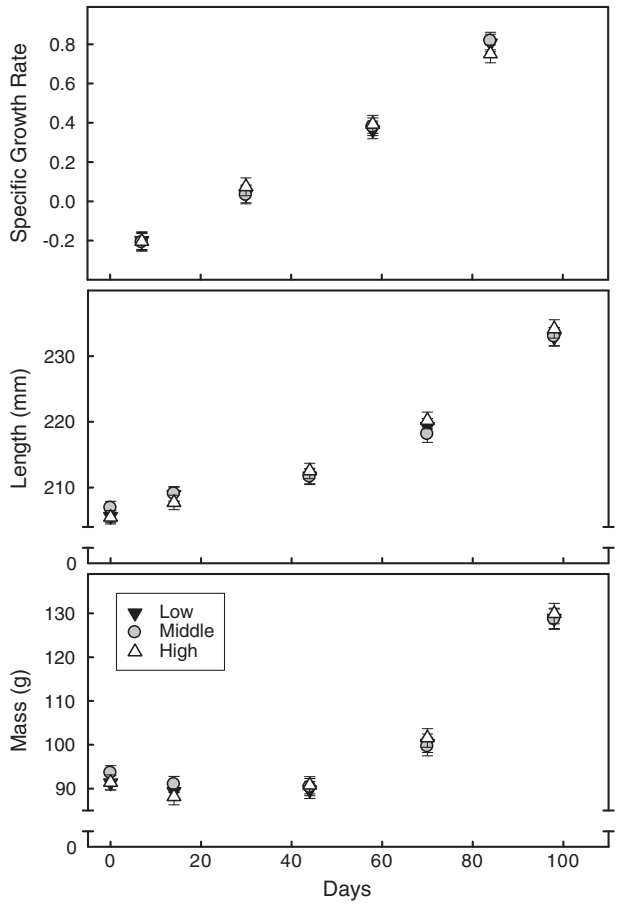

Fig. 4. Specific growth rate ($100\times(\ln(mass_2)-\ln(mass_1))/(t_2-t_1)$), fork length (mm), and mass (g) of Atlantic salmon smolts transferred to 32 ppt seawater; data are grouped by gill Na^+,K^+-ATPase activities prior to seawater transfer. Data are mean ± SE.

3.4. Gill Na^+,K^+-ATPase activity and plasma osmolytes

When examined in a full two-way ANOVA (factors: date and FWATP) both factors and their interaction had significant effects on enzyme activity ($p>0.001$). Subsequent to seawater transfer, gill Na^+,K^+-ATPase activities converged by day 7, only differing among groups on day 3 ($p=0.034$, Kruskal–Wallis). On day 3 the high group was elevated with respect to each of the other groups ($p<0.05$, Tukey test). There were no differences within dates for any other sample dates ($p>0.10$). Gill Na^+,K^+-ATPase at sampling was generally not related to initial gill Na^+,K^+-ATPase activities, except on day 3 when ATPase activities were positively related to day 0 ($p=0.025$, $n=60$, $r^2=0.0838$).

When plasma sodium, chloride and osmolality were examined in a two-way ANOVA with no interactions (factors: date and FWATP), date was significant for both plasma osmolality ($p>0.001$) and plasma chloride ($p>0.001$), but not sodium (Table 1). While variable, plasma chloride stabilized after day 44. Similarly, slightly higher osmolalities were observed in the first month of the study and stabilized after day 44.

4. Discussion

Based on this study, it is unlikely that small scale variation in freshwater gill Na^+,K^+-ATPase activity levels expressed during the peak of smolting are predictive of short or long-term osmoregulatory performance in seawater. Gill Na^+,K^+-ATPase activity levels measured in this study are consistent with the range of values reported for salmon smolts at the GLNFH (Zydlewski et al., 2010; Stephen McCormick, personal communications). The 'low', 'middle' and 'high' groups spanned the normal range of values that are associated with fish that are smolting (Fig. 2). Regardless of group, smolts in this study exhibited only slight differences in osmolytes over time, indicating competence for seawater entry. The slight changes in both plasma chloride and osmolality over time likely reflect shifts in ionic homeostasis rather than osmotic perturbation.

The adaptive advantage to smolting is the ability to enter seawater with minimal osmotic perturbation. For poorly prepared fish, this "acute adaptive phase" (Holmes and Donaldson, 1969) is marked by a rapid elevation and partial recovery of plasma ions followed by a "chronic regulatory phase" which gradually establishes a new ionic homeostasis (Evans, 1984; Jacob and Taylor, 1983; McCormick et al., 1989). In this study, gill Na^+,K^+-ATPase activity levels in seawater converged after day 3 during this acute adaptive phase. By seven days after seawater entry, initial differences in gill Na^+,K^+-ATPase activities were no longer evident, with all groups having stabilized to a level similar to the initial values of the middle group. An increase in gill Na^+,K^+-ATPase activity associated with reestablishment of ion homeostasis after seawater entry corresponds to the chronic regulatory phase of seawater acclimation; this was observed in the low group.

Table 1
Plasma osmolytes of Atlantic salmon smolts (grouped as 'low', 'middle' and 'high' by initial gill Na^+,K^+-ATPase activity) transferred to seawater on day 0. Data reported are means ± SE. There are no significant differences among groups at any time point for plasma sodium, chloride or osmolality.

Day	Plasma [Na^+] (mmol)			Plasma [Cl^-] (mmol)			Plasma osmolality (mOsm/kg)		
	Low	Middle	High	Low	Middle	High	Low	Middle	High
1	160.2 ± 0.71 (17)	161.8 ± 1.31 (18)	162.2 ± 0.86 (22)	150 ± 0.65 (17)	151.1 ± 1.03 (18)	150 ± 0.78 (22)	328 ± 6.1 (16)	336 ± 6.7 (17)	343 ± 7.0 (21)
3	155.8 ± 2.21 (16)	156 ± 1.38 (20)	157.2 ± 1.28 (19)	152.8 ± 1.49 (16)	152 ± 1.11 (20)	151.8 ± 1.10 (19)	366 ± 7.9 (16)	345 ± 3.6 (18)	347 ± 3.9 (19)
14	153.1 ± 1.24 (17)	154.7 ± 0.99 (20)	155.4 ± 1.08 (18)	150.5 ± 1.08 (17)	150.8 ± 0.75 (20)	151.2 ± 0.76 (19)	320 ± 4.8 (16)	322 ± 2.9 (19)	321 ± 2.6 (17)
44	162.7 ± 1.41 (19)	161.5 ± 0.91 (20)	161.0 ± 0.93 (17)	160 ± 1.20 (20)	158.6 ± 0.65 (19)	158.3 ± 0.76 (17)	353 ± 7.9 (18)	343 ± 3.0 (19)	345 ± 5.1 (16)
70	160.1 ± 1.13 (20)	158.9 ± 0.94 (20)	159.0 ± 1.09 (19)	158.3 ± 0.87 (20)	157.9 ± 0.66 (20)	157.9 ± 0.81 (19)	338 ± 2.03 (18)	328 ± 5.7 (20)	329 ± 2.0 (19)
98	161.1 ± 0.55 (74)	159.4 ± 1.00 (69)	160.5 ± 0.56 (71)	155.2 ± 0.55 (74)	153.4 ± 1.33 (69)	154.6 ± 0.52 (71)	333 ± 1.6 (81)	334 ± 2.2 (79)	330 ± 1.5 (76)
132	–	–	–	–	–	–	337 ± 2.7 (19)	336 ± 3.0 (19)	331 ± 2.0 (16)

Smolts that are less physiologically prepared for seawater entry may suffer from osmotic perturbations (Stagg et al., 1989) and high mortality (Berg et al., 1995) upon transfer to seawater. In a natural system, such fish may choose freshwater in stratified environments, avoiding higher salinities (Flagg and Smith, 1982; Iwata, 1995; Price and Schreck, 2003). Osmotic perturbations can make smolts more vulnerable to predation (Handeland et al., 1996) and the estuary is indeed an area of high smolt mortality; in the Penobscot River, 10–30% of acoustic tagged Atlantic salmon smolts are lost in this region (Holbrook, 2007). However no such disadvantage in seawater tolerance was observed for the low FWATP group, short or long term.

Of interest is the fact that enzymatic activity levels of the high group actually decreased after seawater transfer. One could hypothesize that these fish had "extra" activity. This is an important consideration as osmoregulation and associated machinery may have considerable energetic costs (Eddy, 1975, 1982; Farmer and Beamish, 1969; Morgan et al., 1997). Such osmoregulatory 'freeboard' during downstream migration would not come without short term energetic consequences and may affect growth. Alternately, in a natural environment, this activity may provide additional osmoregulatory capacity necessary for performance that was not tested through static seawater transfer in this laboratory setting. McCormick et al. (2009) showed that different isomers of gill Na^+,K^+-ATPase have different roles in fresh and seawater, but it remains unclear how changes in these isoforms affect measured enzyme activities.

Regardless of any potential differences during the acute acclimation phase, growth assessed through the chronic regulatory phase did not differ among any of the three groups. In all groups, the first 14 days in seawater was marked by a decrease in mass (negative specific growth) which could be attributed to multiple causes, e.g., osmotic loss or interruption of feeding. This period encompasses the period associated with acclimation to seawater when lower (albeit not negative) growth rates have been observed (Duston and Saunders, 1997). It is also reasonable to assume that handling and transfer to seawater would have influenced short term feeding through a stress response (Carey and McCormick, 1998; Gadomski et al., 1994). Additionally, rearing in an elevated salinity would likely result in lower water content in muscle tissue, potentially reducing mass through tissue dehydration rather than energy stores (Blackburn and Clarke, 1987). All of these factors likely contributed to the negative growth rates observed in the first two weeks of this study. Subsequent to this, there was a clear increasing trend in specific growth rate. This is consistent with the period of high growth observed in salmon during spring and summer months (Mørkøre and Rørvik, 2001; Torstensen et al., 2008). The marked increases in size and growth rate between days 44 and 98 can be explained by the increased temperature and maximal day length at this time. Growth scope is clearly greater with increased temperature (Austreng et al., 1987). There was an apparent increase in food intake at this time as well.

Gill Na^+,K^+-ATPase is an extremely useful and widely used measure of smolt development (e.g. Aarestrup et al., 2000; McCormick et al., 2002; Zaugg and Wagner, 1973) and responsiveness to endocrine factors (e.g. Seidelin and Madsen, 1997; Madsen et al., 2004). In all cases, however, there was no relationship between long term growth and ionic homeostasis with respect to gill Na^+,K^+-ATPase. It seems likely that any advantage of elevated enzyme activity in preparation for seawater entry would be experienced during the initial days after seawater entry, but likewise, none was observed.

For the short term, if gill Na^+,K^+-ATPase is a good indirect indicator of hypoosmoregulatory ability, why were no differences observed in this study? First, it is clear that transfer into 32 ppt seawater, while practically relevant based on Gulf of Maine salinities during this time of year, may not be a challenge sufficient to elicit a short term perturbation during this period of smolting. In assessing short term performance, higher strength seawater challenges may be of greater use in assessing performance. Secondly, within cohort variability is high. Even with relatively high sample sizes, differences among highly differentiable FWATP levels at day 0 were not distinguishable after a short time (24 h) in seawater. High levels of variability also contributed to resultant low sensitivity of most of the tests conducted. The power of statistical analyses was generally below 0.100 for all tests (except those tests for date, which had power near or above 0.8). Thirdly, Na^+,K^+-ATPase is one enzyme in a suite of transport proteins that are required to change in response to changing osmotic conditions (Hiroi and McCormick, 2007). If mechanistic changes in osmoregulatory system components are not in parallel, the relationship between enzyme activity and performance may be blurred. This may also contribute to studies where seawater tolerance and increased gill Na^+,K^+-ATPase are not strongly associated (e.g. Saunders and Harmon, 1990).

The recent work of Richards et al. (2003) has made clear that seawater acclimation is not simply an up-regulation of gill Na^+,K^+-ATPase proteins. The characterization of α-isoforms in rainbow trout (*O. mykiss*) and Atlantic salmon (McCormick et al., 2009) has identified α1a and α1b isoforms as having different roles in fresh and seawater. As a result, shifts in salinity likely elicit different isoform expressions of this enzyme, such that seawater entry may result in a theoretical decline in α-subunit expression. Indeed, these isoforms exhibit reciprocal expression in the gills of salmonids (Bystriansky

et al., 2006). The utility of measured gill Na^+,K^+-ATPase activity will be greatly informed by an understanding of the relative contributions of different isoforms to *in vitro* activity.

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