The loss of hyperosmoregulatory ability in migrating juvenile American shad, *Alosa sapidissima*

Joseph Zydlewski and Stephen D. McCormick

Abstract: Investigations on juvenile American shad (*Alosa sapidissima*) revealed several physiological changes associated with downstream migration. Plasma chloride decreased 20% in wild juvenile shad during the autumn migration. Migrants had lower condition factor and hematocrit than non-migrant shad captured by beach seining. Gill Na^+,K^+-ATPase activity of migrant shad was higher than non-migrant; a 2.5-fold increase was observed in 1993, while a 57% increase was observed in 1994. Similar changes were observed in laboratory studies of shad maintained in fresh water under simulated natural temperature and photoperiod. Plasma chloride dropped 68% and gill Na^+,K^+-ATPase activity increased 3-fold over a 3-month period. Decreased plasma chloride was associated with increased mortality. Increases in gill Na^+,K^+-ATPase activity decreases in plasma chloride and osmolality, and incidence of mortality were delayed and moderated, but not eliminated, in shad maintained at constant temperature (24°C). Shad did not survive in fresh water past December regardless of temperature regime. In seawater, all shad survived and showed no perturbation of plasma chloride at 24°C or simulated natural temperature (above 4°C). The decline in hyperosmoregulatory ability, as influenced by declining temperatures, may serve as a proximate cue for autumnal migration.

Résumé : Des études portant sur l'alose savoureuse (*Alosa sapidissima*) à l'état juvénile ont révélé la présence de plusieurs changements physiologiques associés à la migration vers la mer. La teneur plasmatique en chlorures a diminué de 20 % chez les aloses juvéniles sauvages durant la migration automnale. Les poissons migrants présentaient un coefficient de condition et un hématocrite moins élevés que les poissons non migrants capturés à la senne de plage. L'activité de Na^+,K^+-ATPase dans les branchies était plus élevée chez les aloses migratrices que chez les sédentaires; un accroissement de 2,5 fois a été observé en 1993 et une augmentation de 57 % l'année suivante. Des changements semblables ont été observés lors d'études de laboratoire portant sur des aloses maintenues en eau douce, avec simulation de la température et de la photopériode du milieu naturel. La teneur plasmatique en chlorures a chuté de 68 % et l'activité de la Na^+,K^+-ATPase dans les branchies a augmenté de trois fois au cours d'une période de 3 mois. Une diminution de la teneur plasmatique en chlorures a été associée à un accroissement de la mortalité. Des augmentations de l'activité de Na^+,K^+-ATPase dans les branchies, des diminutions de la teneur plasmatique en chlorures et de l'osmolalité, ainsi que l'incidence de la mortalité, ont été retardées et limitées, mais pas éliminées chez les aloses maintenues à température constante de 24°C. Les aloses n'ont pas survécu en eau douce après décembre, peu importe le régime thermique maintenu. En eau salée, toutes les aloses ont survécu, et leur teneur plasmatique en chlorures n'a présenté aucune perturbation à 24°C ou à une température naturelle simulée (supérieure à 4°C). Le déclin de la capacité hyperosmorégulatoire, liée à une diminution des températures, pourrait constituer un indice immédiat du début de la migration automnale.
[Traduit par la Rédaction]

Introduction

Seaward migrating juvenile anadromous fish must maintain or develop hypo-osmoregulatory ability prior to entry into seawater (SW). For anadromous salmonids, the ability to survive in SW develops during the parr–smolt transformation. This well-studied developmental change includes alterations in morphology, behavior, and physiology, including increased hypo-osmoregulatory ability and salinity preference (Baggerman 1960; McCormick and Saunders 1987; Hoar 1988). Increased SW tolerance during migration is correlated with increased gill Na^+,K^+-ATPase activity (Zaugg and McLain 1970; Zaugg and Wagner 1973; Hart et al. 1981; McCormick et al. 1987), which establishes ionic and electrical gradients for salt secretion by gill chloride cells.

Some physiological changes associated with the development of salinity tolerance in salmonids may result in ion and water imbalances in fresh water (FW) prior to seaward migration (Langdon and Thorpe 1985). Several workers have observed decreases in plasma chloride concentration (Koch et al. 1959; Houston and Threadgold 1963) and muscle chloride content (Fontaine 1951). Other studies, however, offer no supportive evidence for a change in plasma ion concentration (see Folmar and Dickhoff 1980). Primmett et al. (1988) reported no significant change in plasma sodium in Atlantic salmon

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(*Salmo salar*) but did observe significant changes in whole body transepithelial Na^+ exchange in FW during smolting. It has been hypothesized that changes in ion and water balance could be related to migratory behavior of diadromous fish (Fontaine 1975).

Information on the osmoregulatory physiology of seaward migrating American shad (*Alosa sapidissima*) is limited. In contrast to salmonids, American shad develop SW tolerance during larval–juvenile metamorphosis, preceding the peak of autumnal migration by up to 3 months (Zydlewski and McCormick 1997). While SW tolerance in shad is linked to gill development and increased gill Na^+,K^+-ATPase activity at metamorphosis, gill Na^+,K^+-ATPase activity was shown to also increase in autumn without a change in ability to survive in SW. Howey (1985) observed high mortality in juveniles maintained in FW past the period of migration, but the causes have not been examined.

The seaward migration of shad, while largely uncharacterized, is correlated with declining autumnal temperatures. Though there is evidence for a size-dependent component for migration (Limberg 1996) size does not appear to be a determining factor in the timing of migration (Stokesbury and Dadswell 1989). The peak of migration occurs in autumn when river temperatures fall to 16–9°C (Leggett and Whitney 1972; O'Leary and Kynard 1986). Behavioral studies have demonstrated avoidance of temperatures below 8°C, and other sub-lethal and lethal effects at lower temperatures in FW (Chittenden 1972). As decreasing temperature is correlated with migratory behavior, temperature may elicit changes in osmoregulatory physiology.

We investigated changes in the osmoregulatory physiology of juvenile shad from their river residence through the period of downstream migration in the field. Fundamental measures of osmoregulation including plasma ion levels and gill Na^+,K^+-ATPase activity were assessed in juvenile shad prior to their migration (non-migrants) and during their subsequent migration (migrants) during the summer and autumn of 1993 and 1994. To corroborate field observations, non-migrant shad were captured, acclimated to laboratory conditions, and sampled through the period of migration. Juvenile shad were subjected to altered temperature and salinity regimes to determine the effects of temperature and salinity on the osmoregulatory physiology of shad during the migratory period. We also tested the ability of SW-acclimated shad to osmoregulate in FW after the period of migration.

Materials and methods

Field sampling

Non-migratory and migratory juvenile shad were sampled during the summer and autumn of 1993 and 1994. Juveniles captured in a cove area 2 km north of the Turners Falls Dam on the Connecticut River were defined as non-migratory juveniles. The area is 300 m from the channel and is a rearing site for young American shad and other fish. A 90-m beach seine (2 m deep, 3.2-mm mesh) was used to capture juvenile shad. Efforts were made to capture non-migrant fish from July to September in 1993 and 1994. Attempts to capture non-migrants as early as July were successful in 1993 but failed until August 30 in 1994. In both 1993 and 1994, non-migrants could not be captured after mid-September because of their downstream migration.

Setting and hauling the seine took 15–30 min. Two sequential samples of twelve fish were removed from the net and anesthetized in 100 $mg \cdot L^{-1}$ MS-222 (tricaine methanesulfonate; pH 7.0) for gill and blood sampling. Fork length and mass were measured, then the caudal peduncle was severed and blood was collected in heparinized capillary tubes. The tubes were kept on ice for less than 1 h prior to centrifugation for 5 min at 13 500 × *g*. Hematocrit was measured and plasma separated. For 1993 samples, the plasma was removed into 250-µL microcentrifuge tubes and stored at –80°C prior to analysis. In 1994, plasma samples were analyzed immediately after centrifugation.

Actively migrating juvenile shad were captured on the Connecticut River at Turners Falls, MA at a by-pass structure at the Cabot Station hydroelectric facility operated by Northeast Utilities (198 km from the ocean). Sampling dates for migrant shad were dictated by the dates on which the Cabot bypass was operated by Northeast Utilities personnel. Shad were captured on all sampling attempts; thus, an absolute beginning or end of migration cannot be reported. The peak of migration in 1993 occurred during October (RMC Environmental Services, Inc. 1994) when water temperatures were between 10.7°C and 15.0°C. Quantitative data for the fall migration in 1994 is not available, though migrating shad were abundant between the last week of September and the first week of November. Early and late migrants are defined as those shad captured before October 1 and after November 1, respectively. In general, migrants were observed in the dam bypass in the evening (1800–2100 EST) early in the run and during the peak of the run. Late migrants would also pass during daylight hours.

The downstream bypass pours over a dewatering grate, which diverts fish into a 9.4 m long, 36 cm wide flume (with a depth of 1.1–1.4 m) and delivers them onto a sorting table (5.8 m long and 38 cm wide; depth was adjusted to approximately 25 cm). Efforts were made to minimize the possible influence of stress on shad by sampling fish that spent less than 10 min in the structure. This was accomplished by clearing all fish out of the delivery channel at 10 min intervals. A maximum of 12 juvenile shad were sampled at once. A total of 24 fish were sampled at each time point. Sampling was carried out as described for non-migrant fish.

Temperature and salinity study

Before the migratory period, juvenile shad were captured on August 18–20, 1994, by beach seining as previously described. Fish were transported to the Conte Anadromous Fish Research Center (Turners Falls, MA) and divided into four 1.5 m diameter 1100-L tanks maintained as flow-through systems with unfiltered river water (60 fish per tank). All groups were maintained under a natural photoperiod regime (sunlight supplemented with Na/Hg illumination) throughout the experiment (automatic timers were set to turn on after sunrise and before sunset; 350 lx at water surface). Shad began feeding 2 days after introduction to the laboratory (No. 2 salmon feed, Zeigler Bros., Gardners, PA) Four fish from each tank were sampled on August 31, prior to any treatment. After this sampling the tanks were converted to closed systems with biological and particle filtration and temperature control. Over the next 10 days the salinities of two tanks were elevated in parallel to 32 ppt salinity (8 $ppt \cdot day^{-1}$ for 2 days and 2 $ppt \cdot day^{-1}$ until 32 ppt was reached; Forty Fathoms Marine Mix, Marine Enterprises International Inc., Baltimore, MD). The remaining two tanks were maintained at 0 ppt (river water; 5.9 ppm Na^+, 2.1 ppm Cl^-, conductivity 185 $\mu S \cdot cm^{-1}$).

One SW and one FW tank were maintained at 24°C for the length of the experiment. The temperatures of the other two tanks were maintained within 0.5°C of the daily measured river temperature (simulated natural temperature; SNT). Over the course of the study, corresponding FW and SW groups did not differ by more than 1°C. Mortalities were recorded daily. Sampling was performed at approximately 4-week intervals and included 10 fish from each treatment. The timing of the last sampling points was dictated by survival in each treatment. Fish were anesthetized with MS-222 (100 $mg \cdot L^{-1}$; pH 7.0), length and mass were measured, and blood was taken as described

above. Sampling of each group was completed within 10 min. Ten microlitres of plasma was immediately used for chloride ion analysis. For the December 1 time point, surplus plasma after chloride analysis was stored at –80°C in 250-μL vials.

Osmotic challenges

To determine whether changes in osmoregulatory ability at the time of migration (see Results) persist, juvenile shad were maintained in 12 ppt SW past the period of migration and subjected to FW exposure 6 months later. Juvenile shad were reared from eggs in the laboratory as described in Zydlewski and McCormick (1997). On September 25, 1993, juveniles were placed into a 1.5 m diameter 1100-L circular tank in a recirculating system with particle and biological filtration. Dechlorinated city water was adjusted to 12 ppt using sea salt. Temperatures were maintained at 13–14°C. Fish were held under a natural photoperiod regime (described above). No mortalities were observed for several months prior to beginning the experiment. Average fork length and mass were 9.9 cm and 8.7 g, respectively. On March 19, 1994, 10 fish were killed (100 $mg \cdot L^{-1}$ MS-222, pH 7.0), and gill tissue and blood were collected as described above. Immediately after sampling, two groups of juveniles were transferred to independently recirculating 1.5 m diameter 1100-L circular tanks maintained at 13–14°C. Salinities of the receiving tanks were 24 and 0 ppt (dechlorinated city water; 10.2 ppm Na^+, 18.0 ppm Cl^-, conductivity 169 $\mu S \cdot cm^{-1}$). After 24 h, mortalities were counted, and surviving fish in each group were sampled as described above.

Gill Na^+,K^+-ATPase activity

The first gill arch (or multiple arches in fish less than 4 g) was removed and immediately immersed in 100 μL of ice-cold SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3). Samples were kept on ice for less than 1 h before being stored at –80°C. Gill samples were assayed for Na^+,K^+-ATPase activity using the microplate method described by McCormick (1993) as validated for shad by Zydlewski and McCormick (1997). Gill samples were thawed immediately prior to assay and homogenized in 125 μL of 0.1% sodium deoxycholate in SEI buffer. The gill arch was removed from the tube, and the homogenate was centrifuged to remove other insoluble material. Specific activity of Na^+,K^+-ATPase was determined in duplicate by measuring ATPase activity with and without 0.5 M ouabain in a solution containing 4 $U \cdot mL^{-1}$ lactate dehydrogenase, 5 $U \cdot mL^{-1}$ pyruvate kinase, 2.8 mM phosphoenolpyruvate, 0.7 mM adenosine triphosphate (ATP), 0.22 mM nicotinamide adenine dinucleotide (reduced) (NADH), 50 mM imidizole, 45 mM NaCl, 2.5 mM $MgCl_2$, 10 mM KCl (pH 7.5). Kinetic analysis of ATP hydrolysis (decreased [NADH]) was measured at 340 nm using a 96-well plate reader (Molecular Devices Corporation, Menlo Park, CA). ADP standards were used to routinely (daily) test the assay mixture; NADH standards were used for calculating enzyme activity. Reported coefficients of variation for the assay for a single homogenate and multiple samples of an individual fish are 4.7 and 11%, respectively (McCormick 1993). Protein concentration of the gill homogenate was determined in triplicate using the bicinchoninic acid (BCA) method (Smith et al. 1985). The Pierce BCA protein kit (Pierce, Rockford, IL) was used with bovine albumen as standard. Activity of gill Na^+,K^+-ATPase is expressed as micromoles ADP per milligram protein per hour.

Plasma ions and protein

Plasma chloride concentration analysis was performed using a Labconco model 442-5000 digital chloridometer (Kansas City, MO). Osmolality was measured in a Wescor 5500 vapor pessure osmometer (Logan, UT). Plasma sodium concentration was determined by diluting plasma 1:1000 in deionized water and analyzed using an Instrument Laboratories AA/AE model 551 (Thermo Jarrell Ash Corporation, Franklin, MA, U.S.A.) atomic absorption spectrometer at 589 nm. Appropriate external standards were used for the above analyses. Plasma protein concentration was measured in triplicate from a 1:33.3 dilution using the BCA method described above.

Statistics

Where p values are not reported, significance of statistical analysis is judged at the $p < 0.05$ level. One-way analysis of variance (ANOVA) was used for multiple group analysis. If significant differences were observed by one-way ANOVA, Newman–Keuls multiple comparison tests were used for post-hoc analysis. In the analysis of the effect of temperature on risk of mortality, only data from the FW SNT group in the temperature and salinity experiment are included. Daily risk is expressed as a 7-day average for the previous week leading up to a given date. Where presented, average daily temperature change is given for the same 7-day period.

Results

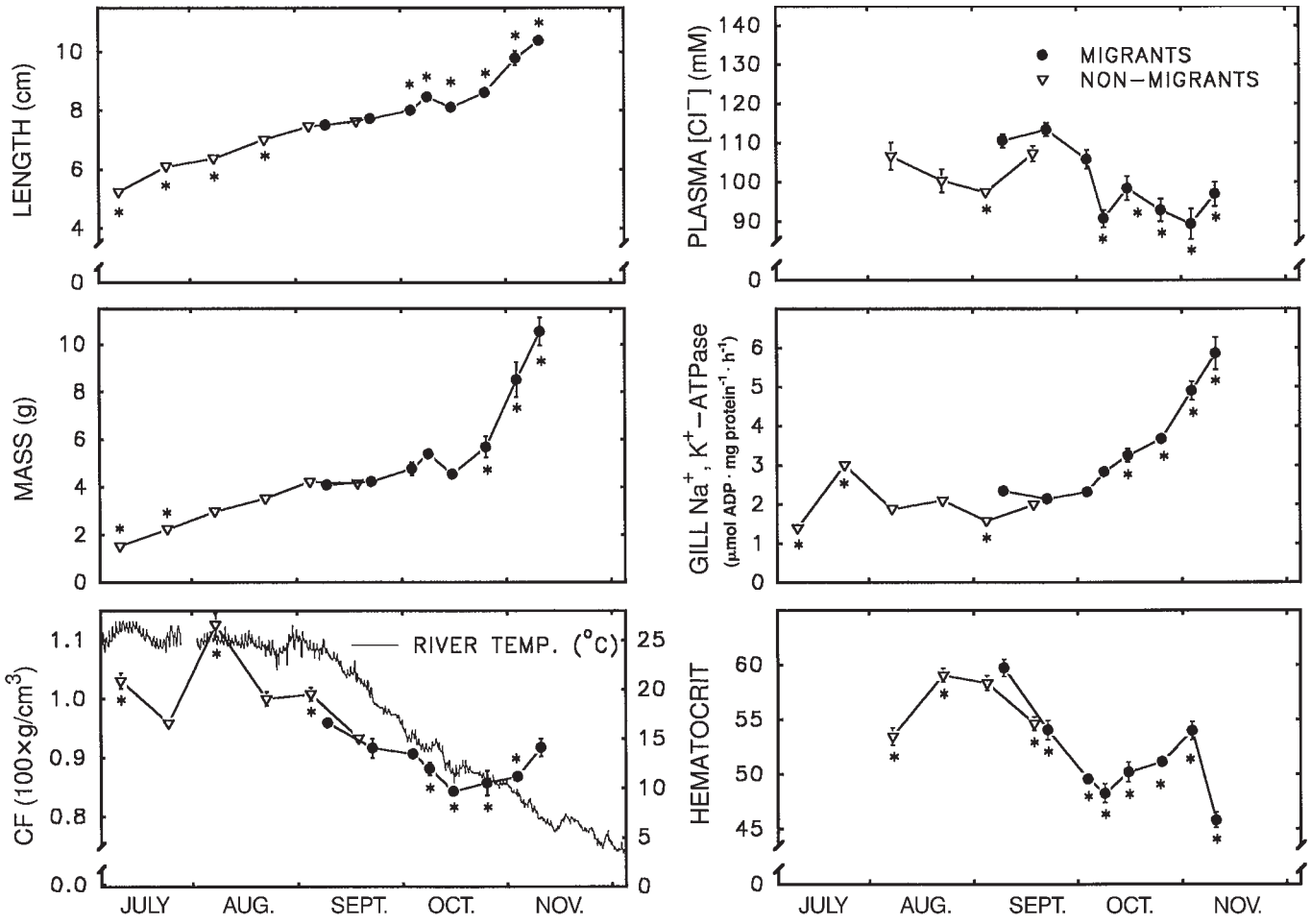
Field sampling

Length and weight increased in both non-migrant and migrant fish over time (July 11 – September 19 and September 10 – November 12, 1993, respectively) (Fig. 1). Non-migrant and migrant fish sampled within the 3-week period when both could be captured did not differ in length or mass. Late migrants were significantly larger than early migrants. Those fish sampled in November, well after the peak of migration, were 30% longer and 2.3-fold heavier than those captured at the peak of the run (10.3 cm and 10.5 g vs. 8.1 cm and 4.5 g). Non-migrant fish had higher condition factor (CF; calculated as $100 \times \text{mass (g)}/\text{length}^3$ (cm)) than did migrant fish; CF was lowest at the peak of migration. River temperature at the peak of migration ranged from 10.7 to 15.0°C.

Plasma chloride levels of non-migrant fish were not significantly different from early migrants (Fig. 1). Plasma chloride of migrant fish declined from 113 mM on September 23 to 90 mM on October 10 (a 20% reduction) and remained low thereafter. Gill Na^+,K^+-ATPase activity was in the range of 1.4–3.0 μmol ADP·mg $protein^{-1} \cdot h^{-1}$ for non-migrant and early migrant fish. During the migration there was a significant 2.5-fold increase in gill Na^+,K^+-ATPase activity as temperatures fell below 12°C. Non-migrant and early migrant hematocrits were between 53 and 60%. Hematocrit declined during the migration, and low levels (48–50%) occurred during the peak of migration when river temperatures were between 11 and 14°C. The lowest hematocrit occurred when river temperatures fell below 6°C on November 11.

As in 1993, non-migrant and migrant shad increased in length and mass over the sampling period in 1994 (Fig. 2). Non-migrant and migrant fish sampled within a 1-week interval did not differ in length or mass. Non-migrant fish captured in late August had the highest CF. The CF of migrant shad declined significantly when river temperatures first fell below 12°C on October 13. No mean CF values greater than 1.0 were observed in 1994. Plasma chloride levels of non-migrant fish captured in late August and early September 1994 were lower than those of early migrants (August 30 and September 15; Fig. 2). Plasma chloride declined from 132 mM on August 22 to 106 mM on October 27 (a decline of 20%) and remained low thereafter. Migrants captured during November had plasma chloride levels of 106–110 mM. The magnitude of decline in plasma chloride was the same as in 1993; however, values were 15–20 mM higher in 1994.

Fig. 1. Length, mass, CF, plasma chloride concentration, gill Na^+,K^+-ATPase activity, and hematocrit of field-captured juvenile shad from 1993 ($n = 24$ at each date). An asterisk indicates a significant difference from the first migrant sample on September 10 (one-way ANOVA; Newman–Keuls post-hoc test). Connecticut River temperature is shown in the lower left graph. Values are mean ± SE.



In 1994, gill Na^+,K^+-ATPase activity of non-migrants did not differ from early migrants. Gill Na^+,K^+-ATPase activity of migrant shad increased from 2.8 to 4.4 µmol ADP·mg protein^{-1}·h^{-1} (57% increase) from September 22 to October 20. Activity peaked when temperatures were 12–14°C, declined in early November, and reached peak levels again in late November. The increase in gill Na^+,K^+-ATPase levels observed in 1994 was of lesser magnitude than was observed in 1993; initial and peak levels of activity in 1994 were 33% higher and 35% lower, respectively, than in 1993. Hematocrit of non-migrants did not differ from early migrants. Hematocrit ranged between 49 and 52% from late September to early November and dropped to 42% in late November when river temperature had fallen below 6°C.

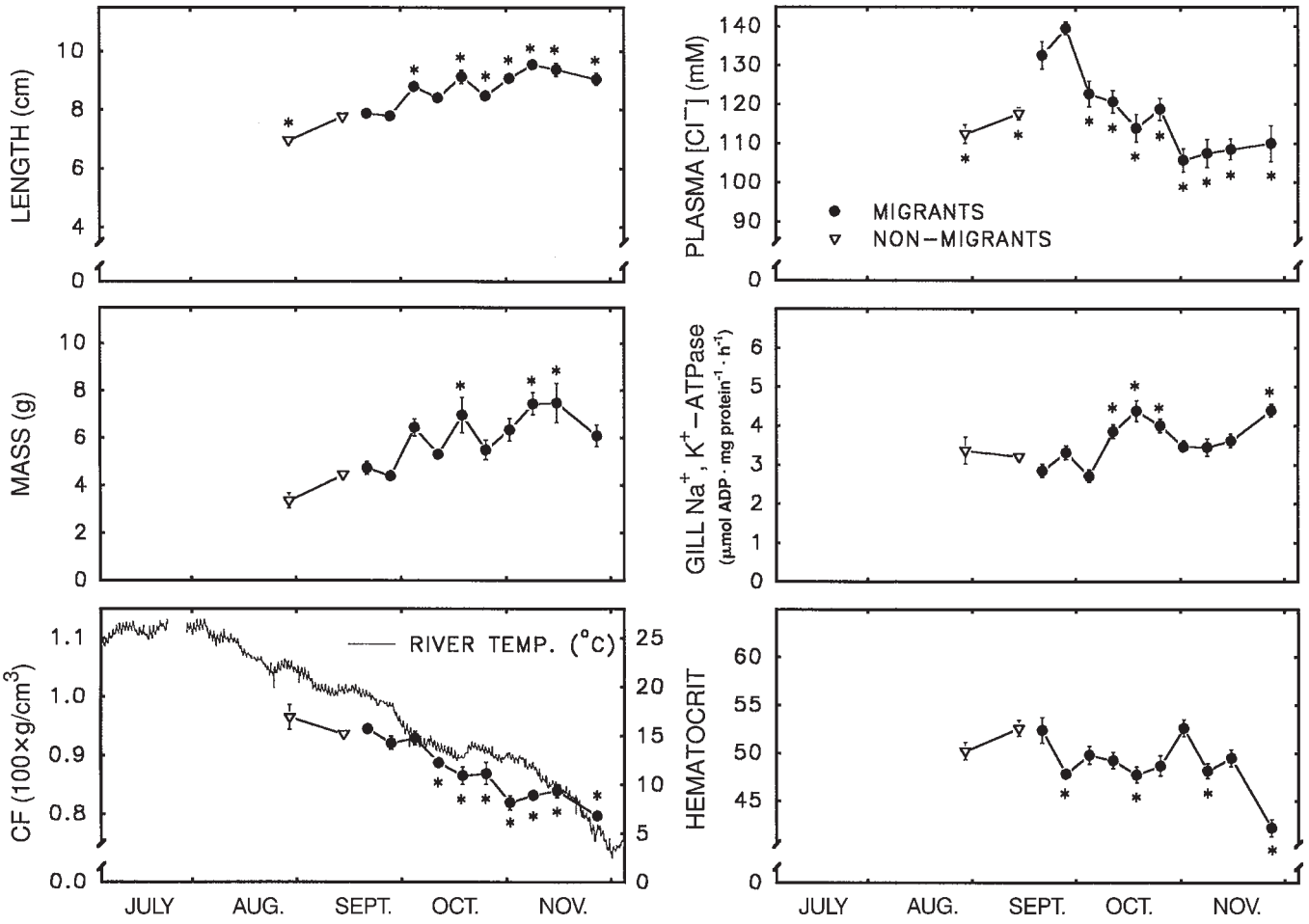
Temperature and salinity study

Among juvenile shad exposed to different temperature and salinity regimes, mortality was greatest in the SNT group in FW (Fig. 3). Risk of death in the FW SNT group was greatest (3%·day^{-1}) at low temperatures (<10°C). Risk was negatively correlated with the slope of temperature change (7-day average), though this only explains 9% of the variation ($R^2 = 0.09$; $p < 0.005$). Mortalities occurred in the FW 24°C treatment, though half of these occurred in early December. No such pattern was apparent in either SW treatment. Mortalities were observed in the SW SNT group but only when temperatures fell below 4°C, and no deaths occurred throughout the experiment in the SW group maintained at 24°C. Dead shad had no obvious signs of physical damage from disease or injury.

Length and mass did not differ between FW and SW groups on the same temperature regimes prior to and on December 1 (Fig. 4). Differences were observed in the 24°C groups on December 16 (after the FW 24°C group had slowed and finally ceased feeding activity on December 3). Both length and mass of the groups on SNT were significantly lower than the 24°C groups on December 1 but did not differ from one another. FW SNT slowed and ceased feeding at 13°C (November 7), and SW SNT ceased feeding at 6°C (November 29). CF was lowest at the onset of the experiment, possibly because of acclimation to laboratory conditions and recovery after transport. By October, CF had increased to 1.09–1.17 in all groups, and there was no significant difference among the groups. There was a significant decline in CF during autumn in all groups. CF of the fish in SNT fell to 0.92 and 0.95 for FW and SW groups, respectively. The decline in CF of the 24°C groups was more moderate but was significant in both groups.

Significant decreases in plasma chloride occurred in both FW groups (Fig. 4). The FW SNT group decreased from an

Fig. 2. Length, mass, CF, plasma chloride concentration, gill Na^+,K^+-ATPase activity, and hematocrit of field captured juvenile shad from 1994 ($n = 24$ at each date). An asterisk indicates a significant difference from the first migrant sample on September 22 (one-way ANOVA; Newman–Keuls post-hoc test). Connecticut River temperature is shown in the lower left graph. Values are mean ± SE.



initial level of 117 to 38 mM (a 68% decline) over a 2-month period. Over the same 2 months, plasma chloride levels in the 24°C group declined from 127 to 93 mM, further declining to a value of 57 mM (55% decrease) on December 16. Plasma chloride levels in the SW groups remained between 140 and 150 mM from October to December. The SW groups differed only at the last date (December 16) when the SW SNT (2.1°C) group had slightly (5%) but significantly higher plasma chloride. Hematocrit declined significantly in three of the four groups during the period of acclimation (August 31 – October 4) but remained unchanged in the FW 24°C group. Hematocrit decreased to 33% when temperature fell below 5°C in the SW SNT group.

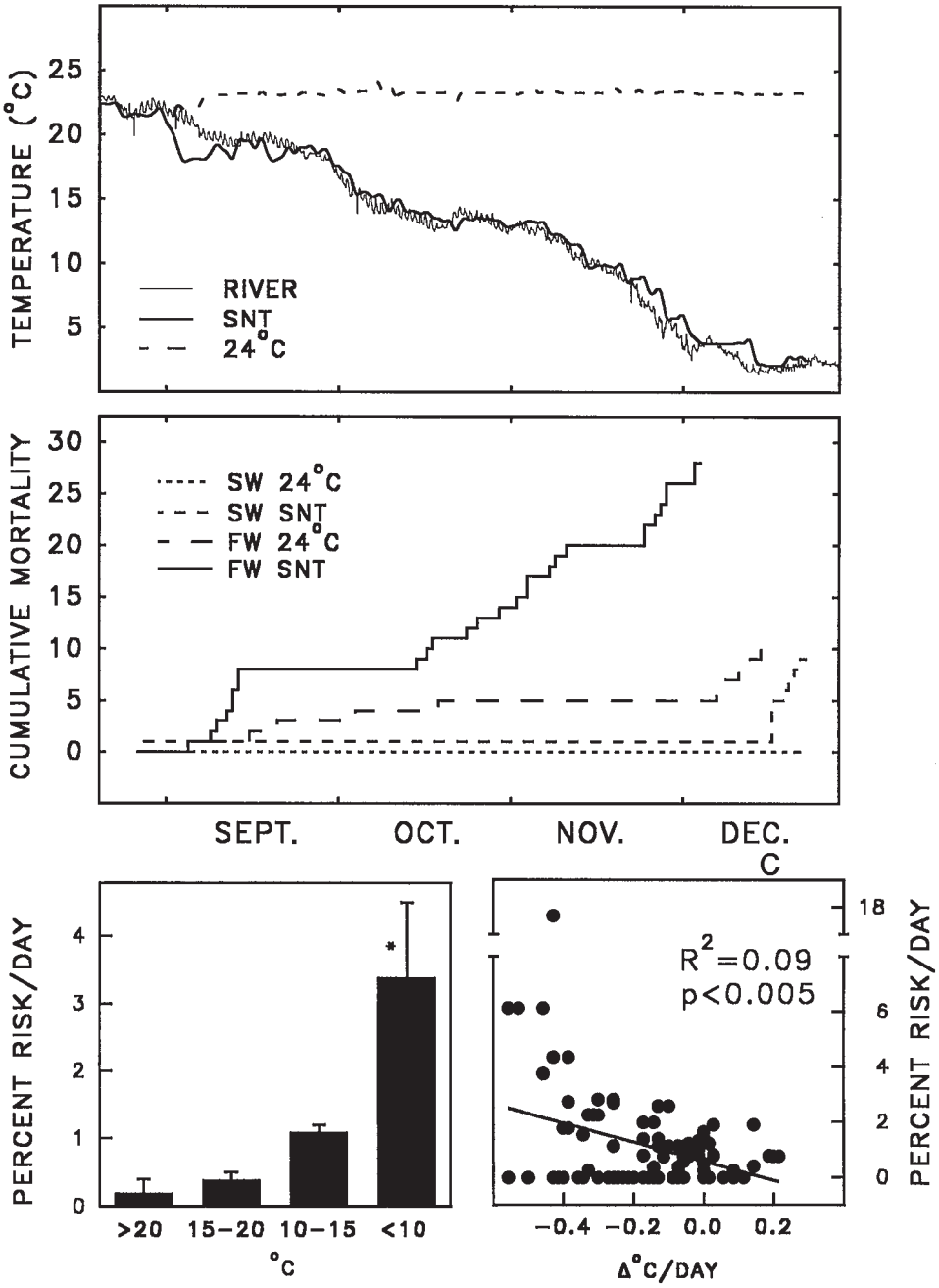
Plasma osmolality of the FW SNT treatment was significantly lower than the SW groups (Table 1). Plasma osmolality was correlated with plasma chloride concentration ($R^2 = 0.68$, $p < 0.0001$), but differences in osmolality between treatments were not of the magnitude observed in plasma chloride levels. While chloride had declined to 38 mM in the FW SNT treatment (73% lower than SW), plasma osmolality was 288 mmol·kg^{-1} (16% lower than SW). Plasma osmolality of the FW 24°C group was significantly higher than the SNT FW groups and lower than the SW SNT but not the SW 24°C group. The FW groups had 26–27% higher plasma protein levels than did corresponding SW groups. The FW 24°C group had a higher hematocrit than did the SW groups.

Gill Na^+,K^+-ATPase activity increased in all groups over the course of the experiment. Activity in the FW SNT group increased more than threefold from initial levels, peaking at 7.0 µmol ADP·mg $protein^{-1}·h^{-1}$ on October 27. In the FW 24°C group, activity peaked at 5.6 µmol ADP·mg $protein^{-1}·h^{-1}$ on December 1. The rate and extent of increase in gill Na^+,K^+-ATPase activity was lower in the FW 24°C group than the FW SNT group. Gill Na^+,K^+-ATPase activity increased threefold within 1 month of acclimation to SW. Gill Na^+,K^+-ATPase activity of the SW groups did not change significantly during the remainder of the experiment, and there was no difference in activity between the two SW groups at any time.

Osmoregulatory challenge

Fish from the three groups of juvenile shad subjected to an increase or decrease of 12 ppt salinity for 24 h (12 (control), 0, and 24 ppt) were of similar size and mass (one-way ANOVA, $p = 0.071$ and 0.122 for length and mass, respectively). Average fork length and mass for all fish was 9.9 ± 0.11 cm and 8.7 ± 0.34 g, respectively. Transfer of shad from 12 to 0 ppt resulted in 50% mortality after 24 h (Table 2). For the surviving fish in 0 ppt, plasma sodium dropped by 32%,

Fig. 3. Upper panels: Temperature of 24°C and SNT treatments (heavy lines) and the Connecticut River (fine lines). For clarity, only the temperature profiles for the two SW groups are shown; profiles of the corresponding FW groups are nearly identical. Lower panels: Average daily risk of death (7-day average) for the FW SNT group. Left graph shows risk versus absolute temperature; right graph shows risk versus change in temperature (7-day average).



plasma chloride dropped by 75%, and plasma osmolality by 29%. Plasma protein and hematocrit remained unchanged (Table 2). The Na^+/Cl^- ratio increased 53% to 1.55. No mortality occurred in the fish transferred to 24 ppt. There was no perturbation in sodium or chloride ions of fish transferred to 24 ppt SW and the Na^+/Cl^- ratio did not change. Plasma protein, osmolality, and hematocrit remained unchanged.

Discussion

In the laboratory and in the field, plasma chloride concentration of juvenile shad declined over the period of migration. Low plasma chloride levels of juvenile shad were associated with increased mortality in the laboratory. Decreasing temperature is clearly associated with the loss of hyperosmoregulatory ability as evidenced by the decrease in plasma chloride from 117 to 38 mM in the FW SNT group and the high mortality observed in this group. The effect of temperature on mortality appears to have two components: absolute temperature and rate of temperature decline (Fig. 3). Decreasing temperature is not the only factor affecting this change in physiology, however, as mortality and plasma chloride declines are slowed but not

Fig. 4. Length, mass, CF, gill Na^+,K^+-ATPase activity, plasma chloride, and hematocrit for FW and SW (32 ppt) 24°C and SNT groups. Light vertical lines indicate homogenous subsets within a sampling date (one-way ANOVA, followed by Newman–Keuls test; where there are only two groups, *t*-test comparison was used). Values are mean ± SE.

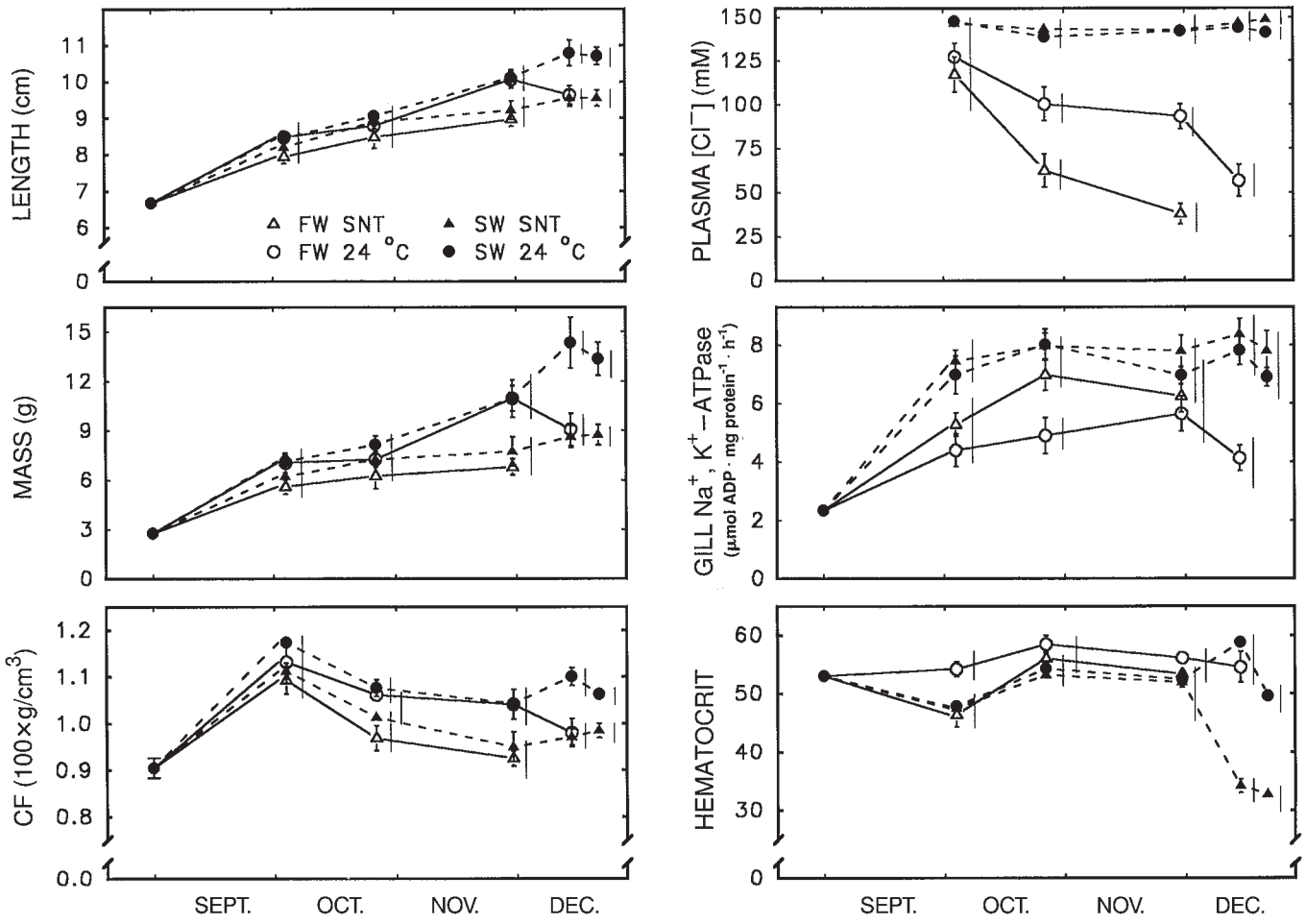


Table 1. Plasma chloride, protein, osmolality, and blood hematocrit for December 1 (shown in Fig. 4).

Treatment	Plasma $[Cl^-]$ (mM)	Plasma protein ($mg \cdot mL^{-1}$)	Osmolality ($mosmol \cdot kg^{-1}$)	Hematocrit (%)
SW 24°C	142±1.3*a* (10)	29.3±1.01*a* (10)	335±4.0*ab* (10)	52±1.3*a* (10)
SW SNT	143±2.4*a* (10)	28.1±1.75*a* (4)	344±4.2*a* (9)	52±1.0*a* (10)
FW 24°C	93±7.1*b* (10)	37.2±1.23*b* (10)	318±4.8*b* (9)	56±0.9*b* (10)
FW SNT	38±5.7*c* (10)	35.5±2.25*b* (3)	288±9.1*c* (8)	53±0.5*ab* (10)

Note: Values are mean ± SE, with number of samples given in parentheses. Values followed by different letters are significantly different at the $p < 0.05$ level (one-way ANOVA, Newman–Keuls test).

Table 2. Survival, plasma chloride, sodium, protein, osmolality, and hematocrit of juvenile shad before and after transfer from 12 ppt SW to either 0 or 24 ppt salinity SW for 24 h.

	Survival (24 h)	Plasma $[Na^+]$ (mM)	Plasma $[Cl^-]$ (mM)	$[Na^+]/[Cl^-]$ ratio	Plasma protein ($mg \cdot mL^{-1}$)	Osmolality ($mosmol \cdot kg^{-1}$)	Hematocrit (%)
12 ppt (0 h)	—	158±3.8*a* (10)	137±2.1*a* (10)	1.01±0.03*a* (10)	31.5±1.7*a* (10)	352±7.1*a* (10)	47±1.4*a* (10)
0 ppt (24 h)	50%	117±5.0*b* (6)	62±3.7*b* (7)	1.55±0.05*b* (6)	29.4±2.7*a* (6)	251±14.7*b* (6)	49±2.3*a* (6)
24 ppt (24 h)	100%	157±2.1*a* (14)	137±2.0*a* (15)	0.99±0.02*a* (14)	32.0±0.8*a* (14)	352±3.2*a* (15)	48±0.8*a* (15)

Note: Values are mean ± SE, with number of samples given in parentheses. Values followed by different letters are significantly different at the $p = 0.05$ level (one-way ANOVA, Newman–Keuls test).

prevented when juveniles were maintained at constant temperature (24°C). Mortalities continued in the FW 24°C group while photoperiod was decreasing. Photoperiod has been shown to strongly affect the migratory physiology of salmonids (Hoar 1988) and may also play a role in shad physiology.

The ability to maintain normal physiological function with severely depressed plasma chloride levels suggests a wide tolerance range for plasma chloride concentration. Plasma osmolality appears to be more effectively regulated. While plasma osmolality is depressed in shad with low plasma chloride in the temperature and salinity study, the absolute difference in plasma osmolality between SW and FW (56 mosmol·kg^{-1}) is less than the absolute difference in plasma chloride (105 mM) (Table 1). The moderate decline in plasma osmolality must be due to compensation (increases) of plasma osmolytes other than chloride. It seems unlikely that this compensation is through increases in plasma sodium. Zydlewski and McCormick (1997) observed no change in plasma sodium over the period of migration in laboratory-reared shad while plasma chloride decreased 19%. Additionally, sodium decreased 26% following an abrupt transfer from 12 ppt SW to FW (Table 2). Major cations such as potassium, magnesium, and calcium are also unlikely to compensate for chloride, as these are relatively minor plasma components and would further increase the electrical imbalance caused by low chloride. Inorganic anions other than chloride are also relatively minor components in the plasma. Bicarbonate concentrations may partly compensate for the low levels of plasma chloride. Acid–base regulation is perturbed in goldfish (*Carassius auratus*) held in chloride-free environment, resulting in alkylosis (De Renzis and Maetz 1973). Similarly, hypercapnia in catfish (*Ictalurus punctatus*) results in a significant decrease in plasma chloride (Cameron and Iwama 1988). Under severe hypercapnia (P_{CO_2} = 56 Torr (1 Torr = 133.3 Pa)), bicarbionate levels reached 20–50 mM. Under ambient P_{CO_2} levels, however, the contribution of this anion is 5 mM, a level typical of bony fish (Holmes and Donaldson 1969). Bicarbonate may increase significantly in shad but is not likely to be the only contributing osmolyte. Elevated plasma protein and free amino acids may also moderate the drop in osmolality as plasma chloride decreases in FW. The osmotic contribution of protein in teleost plasma is difficult to determine and is usually small (less than 10 mosmol· kg^{-1}; Cameron and Iwama 1988). In long-term acclimation of shad to FW, where there was compensation of osmolality, plasma protein concentrations were 26% higher than in shad acclimated to SW (Table 1). We do not know the degree to which this increase contributes to total plasma osmolality. However, plasma protein does not differ after transfer from 12 to 0 ppt when the decrease in osmolality is more consistent (not compensated) with the observed decline in plasma chloride (101 mosmol·kg^{-1} vs. 75 mM; Table 2).

Care was taken to minimize the possible influence of capture methods on physiological measurements of wild fish. However, the process of beach seining for non-migrant shad lasted up to 30 min; significant ion perturbations are observed in juvenile shad subjected to confinement stress within this time frame (J. Zydlewski and S.D. McCormick, unpublished data). Plasma chloride of migrant shad was less likely to be affected by sampling stress, as no fish spent more than 10 min in the bypass structure before sampling. Therefore, we may have underestimated the differences between plasma chloride concentrations of migrant and non-migrant shad. In all laboratory studies, sampling was carried out quickly so that handling stress is not likely to have influenced our measurements. While shad showed no obvious signs of rearing stress, we cannot rule out the possibility that chronic stress of rearing conditions may have influenced some measurements.

In other anadromous fish, salinity tolerance and gill Na^+,K^+-ATPase activity increase concurrently during or just prior to seaward migration (Zaugg and McLain 1970; McCormick et al. 1987). The increase in gill Na^+,K^+-ATPase activity during the migratory period in shad does not appear to be related to increased salinity tolerance. Previous work has shown that salinity tolerance of shad develops at the larval–juvenile metamorphosis, and there is no detectable increase in salinity tolerance at the time of migration (as determined by 35 ppt SW challenges; Zydlewski and McCormick 1997). If a change in hypo-osmoregulatory ability occurs during migration, it is quite subtle.

An alternative explanation is that increased gill Na^+,K^+-ATPase activity during migration is an acclimation response to decreasing temperatures due to impaired hyperosmoregulatory ability. While the role of this enzyme is well substantiated in ion excretion, the precise role and relative importance of Na^+,K^+-ATPase for ion uptake in FW is unclear. Current models for ion uptake through the gill, however, rely on Na^+,K^+-ATPase to establish an electrochemical gradient (Evans et al. 1982; Evans 1993). Evidence exists in other teleosts for an increase in gill Na^+,K^+-ATPase activity during cold acclimation. Goldfish (*Carassius auratus*) acclimated to 1°C FW have enzyme activities 2-fold higher than those held in 20°C (Paxton and Umminger 1983); roach (*Rutilus rutilus*) and Arctic char (*Salvelinus alpinus*) have 1.5- to 2.0-fold increases in gill Na^+,K^+-ATPase activity at 5 versus 15°C (Schwarzbaum et al. 1991), and in a marine fish, the Atlantic cod (*Gadus morhua*), a 1.7-fold increase was measured in fish acclimated to 1 versus 8°C (Staurnes et al. 1994). Similarly, in the closely related alewife (*Alosa pseudoharengus*), a cold acclimation effect on gill Na^+,K^+-ATPase activity was observed in FW (McCormick et al. 1997). These results are consistent with our observation that the highest gill Na^+,K^+-ATPase activities observed in FW occurred in the FW SNT group at low temperatures. Low temperatures may decrease hyperosmoregulatory ability by its effect on ion transport kinetics, and increases in the number of pump sites would compensate.

This cold acclimation effect is not, however, observed in all species (Paxton and Umminger 1983). Increases in gill Na^+,K^+-ATPase activity may occur in some species while other compensatory mechanisms, such as membrane fluidity and permeability, might be employed in others. Different mechanisms may also be used in a single species. In contrast to the effect of temperature on gill Na^+,K^+-ATPase activity in FW-acclimated shad, no difference in activity was observed in SW-acclimated shad at 24 and 5°C. Likewise, in SW-acclimated alewife, decreased temperature caused no increase in gill Na^+,K^+-ATPase activity (McCormick et al. 1997). For these fish, different mechanisms may be used to compensate for the effect of temperature on ion uptake and ion excretion, respectively.

As with the changes observed in plasma chloride, temperature cannot be the sole influence on changes in gill Na^+,K^+-ATPase activity in juvenile shad during the migratory period.

Juvenile shad maintained at constant temperature (24°C) in FW also exhibited an increase in gill Na^+,K^+-ATPase, though of less magnitude. Developmental state and (or) other environmental factors must also influence this and other physiological changes in juvenile shad.

A lower limit of thermal tolerance of 4°C was found in this study; attempts to maintain shad in SW below 4°C resulted in mortality (Fig. 3). Fish acclimated in SW below 6°C did not feed and hematocrit decreased to 33%. A similar decrease in hematocrit was observed in field-captured migrant shad at low temperatures. While plasma chloride of juvenile shad in SW at 5°C was significantly perturbed with respect to the 24°C group, the increase was slight (5%). The lower thermal limit of juvenile shad in SW is not likely to be restricted by osmoregulatory ability but by other physiological limitations.

Yearly temperature cycles are correlated with the timing and dynamics of the seaward migration of juvenile shad (Leggett and Whitney 1972; O'Leary and Kynard 1986). This study has demonstrated an effect of decreasing fall temperatures on juvenile shad physiology in the laboratory. Such effects may explain yearly variations in the physiology of fish captured in the wild. In 1993 the first sustained decline in river temperature below 25°C occurred in the second week of September, and temperatures steadily fell to 10°C by November 1 (a period of 6 weeks, and an average rate of $0.33°C \cdot day^{-1}$). The decline in 1994 began much earlier. The river temperature dropped below 25°C for the last time in the first week of August reaching 10°C only by the second week of November (a period of more than 12 weeks, an average rate of $0.15°C \cdot day^{-1}$). The faster decline in 1993 may have provided a more succinct and cogent influence on migration, a later onset of physiological changes and less variability over the course of migration. (Mortality of shad in the laboratory was also affected by the rate of temperature decline.) A slower and more gradual decline in river temperature, as observed in 1994, may be related to a more moderate decline in hyperosmoregulatory ability observed over the course of migration. Higher average gill Na^+,K^+-ATPase activity in early migrants of 1994 may have been caused by an earlier decrease in temperature, possibly a more important influence on this parameter.

Decreased CF occurred in both wild and captive shad during the period of migration in autumn. CF of field-captured shad declined steadily in migrants from September through October in both 1993 and 1994. Declines in CF were also observed in fish reared in the laboratory at SNT and 24°C (FW and SW), without a change in feeding regime. This observation indicates a possible endogenous change in the relative rates of growth in length and mass over the period of migration that is independent of food availability. A decrease in CF is observed in salmonids and is associated with changes in lipid content during the parr–smolt transformation (Hoar 1976, 1988). While decreased CF in salmonids is hypothesized to be an adaptation to life at sea, the significance of this change in body form is poorly understood.

Based on thermal tolerance alone, downstream migration is required for the survival of American shad spawned in a river that cools to temperatures below 4°C in the winter. Juvenile shad avoid temperatures below 8°C (Chittenden 1972), and the thermal preference range of juveniles may be reflected by the 13–18°C isotherms followed by adult shad in the Atlantic Ocean (Leggett and Whitney 1972). Feeding ceased altogether at 13°C in FW, and plasma chloride had fallen below 63 mM; fish in SW continued to feed until temperatures fell to 6°C. The loss of hyperosmoregulatory ability and cessation of feeding observed in this study at temperatures considerably higher than the lethal limit of 4°C implies a substantial physiological cost of remaining in FW at these temperatures.

There is considerable individual variation in the ability of juvenile shad to survive in FW through the period of migration. Mortalities in FW in the laboratory occurred over several months. We have also captured late migrant shad in the last week of November at temperatures below 5°C in the Connecticut River. These fish had difficulty orienting in a strong current but appeared normal in all other respects. In rare instances shad can be reared past the migratory period in FW at temperatures above 10°C (R.M. Ross, Research and Development Laboratory, Wellsboro, PA, personal communication). The degree to which hyperosmoregulatory ability is lost and the degree to which this is controlled by environmental conditions may vary between and within populations.

The loss of hyperosmoregulatory ability extends past the period of migration. In shad acclimated to 12 ppt SW, direct transfer to FW resulted in 50% mortality after 24 h. Plasma chloride and sodium decreased 55 and 26%, respectively, causing the Na^+/Cl^- ratio to increase from 1.01 to 1.55. Plasma sodium and chloride of juvenile shad transferred from 12 to 24 ppt SW showed no perturbation. Poor hyperosmoregulatory ability in postmigratory shad is also observed when transfer to FW is not abrupt. Juvenile shad previously held in 12 ppt had reduced plasma chloride and high mortality in salinities less than 1 ppt even when acclimated over a period of 2 weeks (15–16°C) (J. Zydlewski and S.D. McCormick, unpublished data).

The loss of hyperosmoregulatory ability in juvenile shad may hinder the formation of landlocked populations and reflect the marine origin of clupeids (Svetovidov 1952). To our knowledge, a single landlocked population of shad exists in a reservoir of the San Joaquin River (Lambert et al. 1980). This area is outside the indigenous range of American shad where winter temperatures are mild; no landlocked populations have been reported on the Atlantic coast. There appears to be variability in the degree to which marine residence is obligatory within the subfamily Alosinae. Two anadromous alosine species indigenous to the Atlantic coast (blueback herring, *Alosa aestivalis*, and alewife) have formed prominent landlocked populations. In contrast to shad, alewife acclimated to SW can be re-acclimated to FW without mortality at 20°C (McCormick et al. 1997). Because of the probable marine origin of this group, obligatory marine residence is likely to be a shared ancestral trait. The ability of some species of alosine fish to reside in FW past the period of migration probably represents a derived pattern in osmoregulatory physiology.

The decrease and subsequent loss of hyperosmoregulatory ability in shad may serve as a proximate cue for downstream migration. A similar hypothesis has been suggested for salmonids (Fontaine 1975) though the evidence for ion perturbations associated with migration has been equivocal (see Folmar and Dickhoff (1980) and Introduction). A decline in hyperosmoregulatory ability may occur in all migrant juvenile shad though not detectable by measurement of plasma chloride concentration. Compensatory mechanisms may mask modest declines in the ability to regulate ions.

Because of impaired hyperosmoregulatory ability, delays in downstream migration, as might occur at dams, could significantly affect survival of migrants, particularly late migrants. It is likely that the observed changes in osmoregulatory physiology are part of a suite of changes (e.g., temperature and salinity preferences, decreased CF, rheotactic behavior, etc.) that occur at the time of migration. This set of changes has been shown to be strongly affected by temperature but must be affected by other environmental and (or) ontogenic factors. It will be important to investigate the interaction of temperature with factors such as photoperiod and food availability for their influence on the physiological changes that occur during migration.

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