The ontogeny of salinity tolerance in the American shad, *Alosa sapidissima*

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Abstract: Larval and juvenile American shad (*Alosa sapidissima*) raised from eggs in the laboratory were subjected to biweekly 24-h seawater (35 ppt) challenges. There was no survival in seawater before 36 days post-hatch, and most mortalities occurred within 2 h of transfer. Twenty-four hour survival reached 89% in seawater at 45 days post-hatch (when larval–juvenile metamorphosis occurred), 96% at 58 days post-hatch, and 92–100% from 58 to 127 days post-hatch. Survival in seawater for 24 h was a good indicator of long-term survival and growth. Seawater tolerance was associated with gill development and increased gill Na^+,K^+-ATPase activity, which occurred at the onset of the larval–juvenile metamorphosis (3 months prior to the peak of migration). Gill Na^+,K^+-ATPase activity increased threefold in juvenile shad acclimated to 35-ppt seawater, reached peak levels 5 days after transfer, and remained elevated with respect to controls. Plasma sodium and chloride increased 12 and 11%, respectively, within 48 h of seawater exposure. Plasma sodium recovered to initial levels and plasma chloride stabilized at a level 10% higher than initial levels after 5 days. Ionic perturbations that occurred at elevated salinities stabilized when gill Na^+,K^+-ATPase activity increased.

Résumé : Des aloses savoureuses (*Alosa sapidissima*) larvaires et juvéniles élevées depuis l'oeuf dans le laboratoire ont été soumises à des stimulations par l'eau de mer (35 parties par millier) pendant 24 h deux fois par semaine. On n'a pas enregistré de survie dans l'eau de mer avant 36 jours après l'éclosion et la plus grande partie de la mortalité est survenue dans un délai de 2 h après le transfert dans l'eau de mer. La survie pendant 24 h a atteint 89% dans l'eau de mer 45 jours après l'éclosion (lorsque la métamorphose larve–juvénile se produit), 96% à 58 jours après l'éclosion et 92–100% entre 58 et 127 jours après l'éclosion. La survie dans l'eau de mer pendant 24 h était un bon indicateur de la survie et de la croissance à long terme. La tolérance à l'eau de mer a été liée au développement des branchies et à une activité Na^+,K^+-ATPasique accrue qui survient au début de la métamorphose larve–juvénile (3 mois avant la migration de pointe). L'activité Na^+,K^+-ATPasique a triplé chez l'alose juvénile acclimatée à l'eau de mer (35 parties par millier), a atteint son maximum 5 jours après le transfert et est demeurée élevée par rapport à la situation observée chez les témoins. Les concentrations de sodium et de chlorure plasmatiques ont augmenté de 12 et 11% dans un délai de 48 h après l'exposition à l'eau de mer. La concentration de sodium plasmatique est revenue à sa valeur initiale et celle du chlorure s'est stabilisée à une concentration supérieure de 10% à la valeur initiale après 5 jours. Les perturbations ioniques qui sont survenues à des salinités élevées se sont stabilisées lorsque l'activité Na^+,K^+-ATPasique a augmenté.
[Traduit par la Rédaction]

Introduction

The American shad (*Alosa sapidissima*) is an abundant anadromous teleost indigenous to eastern North America. This economically and ecologically important species has also been introduced to the Columbia and Sacramento rivers on the west coast. Reproductive adults migrate into coastal rivers in the spring when river temperatures are between 14 and 20°C (Leggett and Whitney 1972). Spawning occurs in open water beyond tidal influence, and young generally remain in fresh water until autumn. The downstream migration of juvenile American shad appears to be strongly correlated with autumnal temperature and peaks when declining river temperatures reach 9–16°C (Leggett and Whitney 1972; O'Leary and Kynard 1986). Migration does not appear to be size dependent (Stokesbury and Dadswell 1989).

A physiological necessity of anadromy is the ability to hypoosmoregulate at the time of seawater entry. Developmental changes in hypoosmoregulatory ability have been examined in detail in anadromous salmonids (see Hoar 1988). The development of salinity tolerance in smolting salmonids occurs during the parr–smolt transformation, a process encompassing behavioral, morphological, and physiological changes, at the time of migration. This development of hypoosmoregulatory abilities has been strongly linked to increased gill Na^+,K^+-ATPase activity and other changes in the osmoregulatory organs (Zaugg and McLain 1970; Boeuf et al. 1985; McCormick et al. 1987; Hoar 1988).

In contrast to other anadromous species such as salmonids, information on the osmoregulatory physiology of juvenile American shad is sparse. Eggs and yolk-sac larvae can survive in brackish water but are unable to develop in salinities significantly greater than iso-osmotic (Milner 1876; Leim 1924). There is no information available pertaining to free-feeding larvae through recently metamorphosed juveniles. Tagatz (1961) reported high mortality of juvenile shad (5.6–7.9 cm fork length) in isothermal direct transfers to 33-ppt seawater at 21°C and observed increased mortality when the transfer was accompanied by a decrease in temperature. Chittenden (1973), however, found 100% survival in isothermal (17°C) transfers of juvenile shad (4.4–6.1 cm) into seawater. These

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limited and somewhat conflicting results indicate that the ontogeny of salinity tolerance is still unclear. This study sought to determine the ontogeny of salinity tolerance in American shad by following a cohort reared in the laboratory. Gill Na^+,K^+-ATPase activity, gill development, and plasma ions of this cohort are reported. Associated growth studies are included to demonstrate that laboratory conditions allowed for growth comparable with that of wild fish. Changes in osmoregulatory physiology that accompany seawater acclimation of juvenile shad were also investigated.

Methods

Rearing of American shad

Adult American shad were captured by gill netting on June 6, 1993, in the Connecticut River approximately 2 km south of the Turners Falls Dam (198 km from the ocean). Eggs from seven females were fertilized with milt from three males and transported to the laboratory (Conte Anadromous Fish Research Center, Turners Falls, Mass.). Average egg diameter was 3.4 mm, and total number was estimated at 91 000 (von Bayer 1910). Filtered Connecticut River water (16.6–19.0°C during incubation) was supplied to an upwelling jar (Eagar Inc., North Salt Lake, Utah). Every 2nd day eggs were treated with a 1:600 dilution of formalin for 10 min to prevent fungus. Hatching occurred on June 13, 1993; this date is defined as day 0 for post-hatch ages reported. Yolk-sac larvae were evenly distributed between three identical 1 m diameter (300 L) tanks supplied with unfiltered river water. The fish were maintained on simulated natural photoperiod (325 lx at the water surface; Phillips Colortone 50 fluorescent bulbs). Brine shrimp nauplii were made available from days 1 to 42 at 5-min intervals via automatic dispensers (Argentemia Platinum Grade, Argent Chemical Laboratories, Redmond, Wash., from days 1 to 17; Argentemia Silver Grade from days 18 to 42). Density of nauplii in the tanks was maintained at greater than 2000 nauplii/L through the day, the recommended optimal prey density for clupeid larvae (Saksena and Hounde 1972).

Shad were fed Biokyowa fry feed (Biokyowa, Chesterfield, Mo.) from day 1 through to the end of the study (B recipe, 250–400 μm, from days 1 to 48; C recipe, 700 μm, from days 38 to 62; 1000 μm from day 49 and thereafter). Number 2 salmon feed (Zeigler Bros., Gardners, Penn.) was provided from day 69 onward. Total tank biomass was estimated at each sampling through the study, and feeding rate of dry food was adjusted to approximately 5% total mass per day. Mortalities and uneaten food were siphoned from the rearing tanks daily.

The transition from endogenous to exogenous feeding occurred between days 3 and 6. Free-feeding larvae metamorphosed between days 26 and 45 (described by Leim 1924). The process of metamorphosis includes changes in coloration, formation of scales, and modification of the skeletal system. Postmetamorphic fish, referred to as juveniles, have coloration and morphology similar to that of adults.

Seawater challenges

The ontogeny of salinity tolerance was determined by performing a series of seawater challenge experiments, which commenced at day 18 and were continued until day 127. The first five experiments (days 18–58) were performed in 65-L, 0.5 m diameter circular tanks. The freshwater (control) tank was maintained as a flow-through system with unfiltered river water. The seawater (experimental) tank was a closed system (total volume of 1100 L) with both paper and charcoal filtration. Densities were maintained below 1 g/20 L to ensure that total ammonia levels remained below 10^{-5} M. Temperature was regulated to match river temperature to within ±0.5°C. Seawater was mixed to 35 ppt (Forty Fathoms Marine Mix, Marine Enterprises International Inc., Baltimore, Md.) in river water. Flow rates for these tanks were matched at 1 L/min. Identical 1-m (300 L) circular tanks were used for the experimental and control groups after day 58. Water sources and temperature were as described above. Flows in both the experimental and control tanks were matched at 10 L/min.

Fish were transferred by water brailing to ensure that they were not dewatered at any time during the transfer. Mortalities were counted and removed at 1, 2, 4, 6, 8, and 24 h after transfer. Food (of appropriate types described above) was made available to the surviving fish at each time point. At the end of the 24-h test period, survivors of the experimental and test groups were terminally anesthetized (100 mg/L tricaine methanesulfonate (MS-222), pH 7.0). Fork length and weight were measured. Fish transferred at day 45 (both control and experimental) were held for 7 days to assess long-term condition, feeding, and mortality. Shad maintained in this experiment were fed by hand five times per day and sacrificed after 7 days. Twenty-four fish were used in each treatment group for all challenges except for days 100 and 127 when 12 fish were used.

Growth and sampling

The same rearing cohort of shad used for seawater challenge experiments was also sampled weekly ($n = 24$) from day 18 to day 75 and then approximately biweekly through to day 145 (November 5). Fish were anesthetized with 100 mg/L MS-222 (pH 7.0), and fork length and weight (beginning on day 36) were measured. Gill tissue samples were taken for measurement of Na^+,K^+-ATPase activity beginning on day 18. For fish sampled on days 18 and 26 (premetamorphic shad), all gill arches were excised. For fish less than 4 g, multiple gill arches were removed to collect adequate tissue. For 4- to 8-g shad, the first arch was removed and for shad greater than 8 g, approximately the upper third of the ceratobranchial of the first gill arch was removed for analysis. The tissue was immediately immersed in 100 μL of ice-cold SEI buffer (150 mM sucrose, 10 mM ethylenediaminetetraacetic acid (EDTA), 50 mM imidazole, pH 7.3). Samples were kept on ice for less than 1 h before being stored at –80°C.

Blood was collected beginning on day 89 by severing the caudal peduncle and collecting in heparinized microhematocrit capillary tubes. Tubes were kept on ice for 30–45 min prior to being spun in a hematocrit centrifuge for 5 min at 13 500 × g, and hematocrit was measured. Personal observations demonstrate that hematocrit increases slightly but significantly over the course of 1 h, with most of the increase occurring within 15 min. While we were unable to process the samples immediately, all samples were treated similarly. Hence, hematocrit measurements are slightly elevated, but differences among groups are unaffected. The plasma layer was removed into 250-μL microcentrifuge tubes and stored at –80°C prior to analysis of sodium, chloride, and protein.

Seawater acclimation

Prior to rearing shad in the laboratory, changes in the osmoregulatory physiology of shad during seawater acclimation were studied. Wild shad juveniles were captured in August 1992 by beach seining in the Connecticut River at a site approximately 2 km north of the dam at Turners Falls. These fish were subjectively classified as nonmigratory as the capture site is a bay area 300 m from the main river channel. The juveniles were transported to the Conte Anadromous Fish Research Center and acclimated to laboratory conditions in fresh water for 10 days in a 1.5 m diameter (1100 L) circular tank maintained with river water (20.3–23.0°C) at 15 L/min. The juveniles began feeding (Zeigler No. 4 salmon feed) within 1–2 days. On September 27, 16 fish from the acclimation tank were sacrificed (day 0 group) and 160 fish were divided into 1 m diameter (300 L) circular tanks with either fresh water (control) or 35-ppt seawater (experimental). Temperatures of the experimental and control tanks were maintained at 20.0 ± 1.0°C. Flows were matched at 10 L/min. Seawater was mixed to 35 ppt using dechlorinated city water. The control tank was maintained as a flow-through system with river water; dechlorinated city water was mixed in to regulate temperature. Fish were fed

daily but were starved 24 h prior to initial transfer and all sampling. Sixteen fish were sampled at the beginning of the experiment, and eight shad from each group were sampled 1, 2, 3, 5, 7, 14, and 21 days after transfer. Length, weight, gill, and blood were measured and sampled as described above.

Gill development

To estimate gill development, gill arches were removed and placed into freshly mixed Champy–Maillet's fixative (0.2% osmium tetraoxide, 25 mg/mL iodine, and saturated metallic zinc) for 16 h (Avella et al. 1987). The tissue was rinsed with deionized water and dehydrated to 70% ethanol for storage. The gill tissue was examined using a Zeiss STEMI SR dissecting microscope at 1.2–4× magnification. The longest primary filament of the first gill arch was measured by obtaining a digital image via a CCD video camera (MTI CCD-72, Michigan City, Ind.) and applying a computerized image analyzing system (Image-1, Universal Imaging, West Chester, Pa.). A stage micrometer was used for calibration. Gill index was defined as the length of the longest primary filament divided by fork length.

Na^+,K^+-ATPase assay

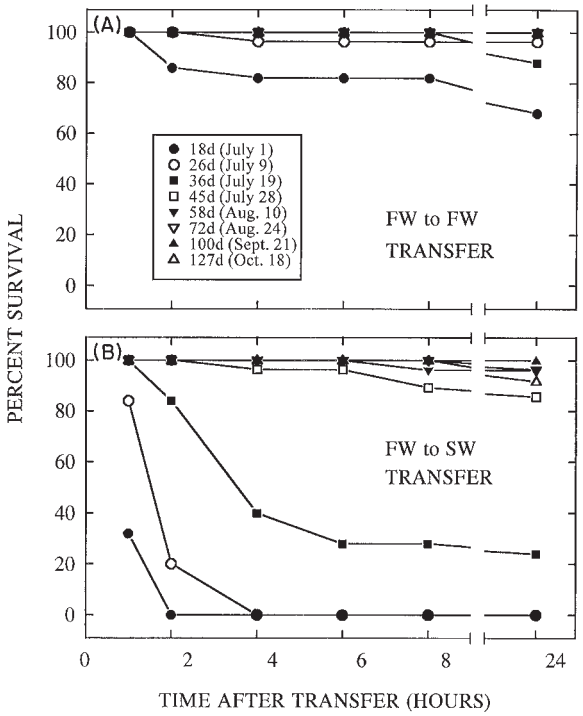
Gill Na^+,K^+-ATPase activity was determined as described by McCormick (1993). This assay was validated for American shad as outlined in McCormick and Bern (1989), and optimal assay conditions for Na^+,K^+-ATPase activity were identical to those for salmonids. Routinely, gill samples were thawed immediately prior to assay and homogenized for 10–15 s in 125 μL of 0.1% sodium deoxycholate in SEI buffer with a motorized pestle (Kontes, Vineland, N.J.), and most of the cartilage was removed from the tube. The homogenate was then centrifuged at 3750 × *g* for 30 s at 4°C to remove remaining 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/mL lactate dehydrogenase, 5 U/mL pyruvate kinase, 2.8 mM phosphoenolpyruvate, 0.7 mM adenosine triphosphate (ATP), 0.22 mM nicotinamide adenine dinucleotide (reduced; NADH), 50 mM imidazole, 45 mM NaCl, 2.5 mM $MgCl_2$, and 10 mM KCl (pH 7.5). Kinetic analysis of the decline in NADH was measured at 340 nm for 10 min (readings every 10 s) using a 96-well plate reader (Molecular Devices Corporation, Menlo Park, Calif.). Protein concentration of the gill homogenate was determined in triplicate using the bicinchoninic acid method (Smith et al. 1985) using bovine serum albumin as standard. Ouabain-sensitive Na^+,K^+-ATPase activity is expressed as micromoles ADP per milligram protein per hour.

Shad gill tissue samples included the gill arch (see above) because it was necessary to take tissue from multiple arches from young juveniles and exclusion of the arch at this step was found to be impractical. For consistency, the arch was included in all samples during homogenization. Inclusion of the gill arch was shown to result in slightly lower gill Na^+,K^+-ATPase activity than that of gill tissue without the arch (by approximately 10%), but values were proportional to tissue samples without the arch.

Plasma analyses

Important osmolytes (chloride, sodium, protein) and total osmolality were measured where possible. Plasma chloride concentration was determined using a Labconco model 442-5000 digital chloridometer (Labconco Corp., Kansas City, Mo.). Plasma sodium concentration was determined by diluting the plasma 1:1000 in deionized water. Samples for the laboratory-reared shad were analyzed using an Instrument Laboratories AA/AE model 551 spectrometer at 589 nm (Thermo Jarrell Ash Corp., Franklin, Mass.). Samples from the seawater acclimation study were analyzed using a Spectra AA10-7042250 atomic absorption spectrometer at 589 nm (Mulgrave, Victoria, Australia). Plasma protein concentration was determined by diluting the plasma 1:33.3 and measuring protein concentration using the Pierce kit described above. Osmolality was measured on a Wescor 5500 vapor pressure osmometer (Wescor Inc., Logan, Utah). In several instances, plasma volumes were insufficient to measure all parameters.

Fig. 1. Development of salinity tolerance in larval and juvenile American shad as determined by isothermal transfers from fresh water to 35-ppt seawater. Each plot represents the percent survival at 1, 2, 4, 6, 8, or 24 h after transfer to fresh water (A) or seawater (B; experimental) (n = 24 fish per group except for days 100 and 127, n = 12). Shad on days 18–26 were premetamorphic and had completed metamorphosis by day 45. Note that all symbols are not visible because of overlap. Refer to Fig. 2 (24-h time point) for clarity.



Statistics

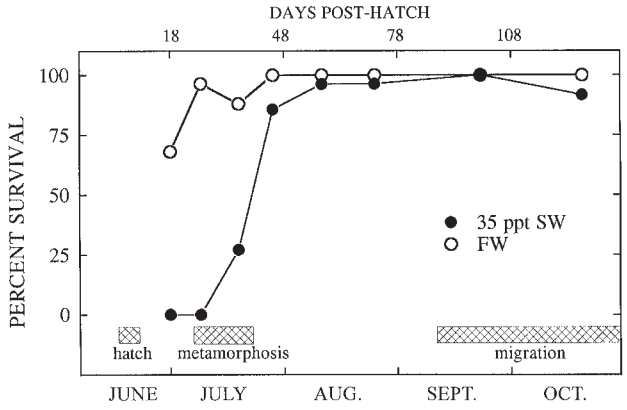
Statistical analyses described in the text and figures were carried out using Crunch 4.0 software (Crunch, Berkeley, Calif.). Where p values are not reported, significance of statistical analysis is at the $p < 0.05$ level. Two-way analysis of variance (ANOVA) was used for comparison of freshwater and seawater groups over time in the seawater acclimation study. If factors or interactions were significant, one-way ANOVA was used for multiple group analysis. In all multiple group analyses, significance with one-way ANOVA was followed by Tukey's post-hoc test. All statistical intervals for measurements are reported as mean ± SE.

Results

Seawater challenges

When larval shad were exposed to 35-ppt seawater on days 18 and 26 after hatching, high mortalities occurred within 2 h and all fish died within 4 h of transfer (Fig. 1). At day 36, survival in the seawater after 24 h was 25% and reached 89% survival by day 45. This rapid increase in seawater tolerance from day 36 to day 45 was coincident with completion of larval–juvenile metamorphosis in most fish (Fig. 2). Fish in both the control and experimental groups fed within 8 h of transfer, and

Fig. 2. Survival of American shad 24 h after isothermal transfer to 35-ppt seawater ($n = 24$ per group for the first six challenges and $n = 12$ for the remaining challenges). Cross-hatched bars mark the time of hatching and metamorphosis as observed in laboratory-reared fish. The cross-hatched bar indicating migration corresponds to the migration of wild fish in the Connecticut River.



neither group showed any behavioral indication of stress after transfer from day 45 onward. Survival in freshwater control groups was higher than or equal to survival in seawater at all time points. From day 58 to day 127, survival in the seawater challenge remained high (92–100%) and did not differ from the freshwater control by more than 8%. No change in seawater tolerance as measured by survival in 35-ppt seawater was discernible during the period of fall migration.

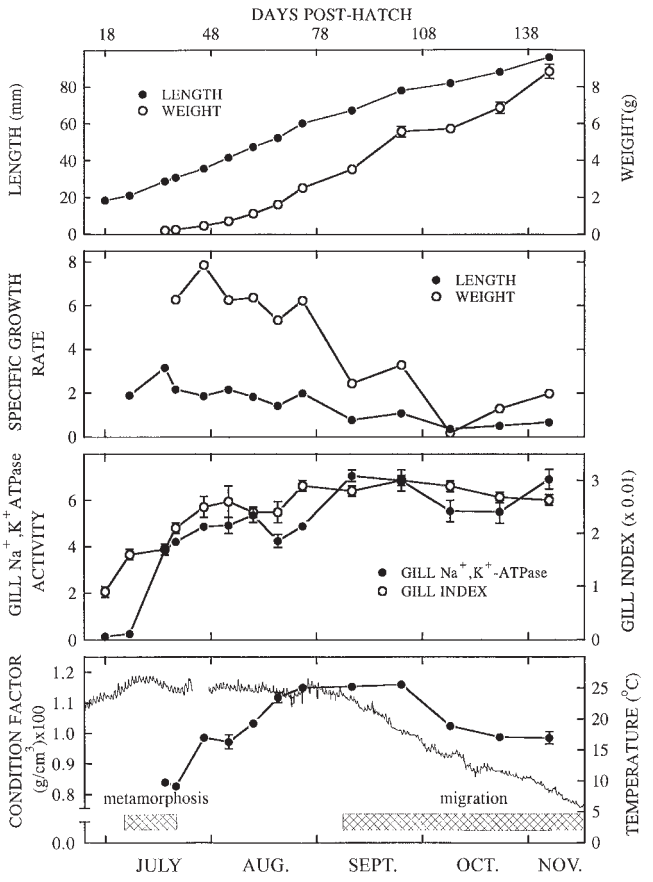
Survival at 24 h in seawater was shown to reflect long-term survival and growth. The day 45 groups were held for 7 days in fresh water or seawater. After the initial 24-h period there were no mortalities in the seawater group and one mortality in the freshwater control over the next 6 days. Both the seawater and freshwater groups fed well over the 7-day experiment. After 7 days, seawater and freshwater groups did not differ significantly in either weight or length (data not shown).

Growth and freshwater physiology

Length and weight of shad sacrificed for physiological sampling increased over the course of the study (Fig. 3). Specific growth rate in weight peaked at the conclusion of metamorphosis and gradually declined in autumn. Condition factor increased from 0.84 in July to 1.12–1.16 in September. Coincident with declining temperatures in autumn there was a significant reduction in condition factor to 0.99 (one-way ANOVA, Tukey's test).

The first measurement of gill index on day 18 (July 1) was the lowest observed (0.009) (Fig. 3). Gill index increased sharply during the period of metamorphosis, reaching 0.025 by day 45 (a 2.8-fold increase), and remained elevated through the study (0.024–0.030). The onset of salinity tolerance during metamorphosis was preceded by a twofold increase in gill index and was coincident with a 10-fold increase in gill Na^+,K^+-ATPase activity (Fig. 3). Survival for 24 h in seawater was strongly correlated with both gill index and gill Na^+,K^+-ATPase activity ($r = 0.93$, $p < 0.001$ and $r = 0.92$, $p < 0.001$, respectively). There was a gradual increase in gill Na^+,K^+-ATPase activity after metamorphosis, which reached its highest levels between mid-September and November.

Fig. 3. Length, weight, specific growth rate, gill Na^+,K^+-ATPase activity, gill index (see text), condition factor, and temperature profile for laboratory-reared shad. Gill Na^+,K^+-ATPase is expressed in μmol ADP·mg protein^{-1}·h^{-1}. Values are given as the mean ± SE for each time point ($n = 24$ except for gill index for which $n = 8$).



Blood samples were taken beginning on day 89 because of the inability to collect sufficient quantities of blood from younger fish. Analyses of plasma sodium, chloride, and protein concentrations and hematocrit are shown in Table 1. Plasma sodium levels did not change with time whereas chloride levels were lowest at days 131 and 145. The sodium/chloride ratio increased at day 131 and was significantly greater at day 145. Total plasma osmolality was correlated to plasma chloride ($r = 0.73$, $n = 31$, $p < 0.0001$) and to plasma sodium levels, though less strongly ($r = 0.64$, $n = 31$, $p < 0.0001$) (data not shown). Total plasma protein concentration did not differ over time. Hematocrit values did not significantly change from day 89 to day 145 and the range of means was 51–54.

Seawater acclimation

Transfer of juvenile shad to 35-ppt seawater caused an increase in plasma sodium from 174 to 196 mM after 24 h (Fig. 4). Plasma sodium levels returned to initial levels 5 days after transfer. Control levels of plasma sodium did not change significantly over the 21-day experiment. The plasma chloride concentration, 122 mM on day 0, was not elevated 24 h after transfer to seawater but increased to 143 mM on day 2. The plasma chloride concentration in seawater stabilized within 5 days at 130–135 mM. Plasma chloride in controls fell

Table 1. Plasma sodium and chloride concentrations, sodium/chloride ratio, protein concentration, and hematocrit in laboratory-reared juvenile shad from day 89 to day 145.

	Day 89 (Sept. 10)	Day 103 (Sept. 24)	Day 117 (Oct. 8)	Day 131 (Oct. 22)	Day 145 (Nov. 5)
Sodium (mM)	121±2.0*a* (6)	130±3.5*a* (15)	132±2.5*a* (21)	131±3.2*a* (24)	130±2.5*a* (23)
Chloride (mM)	113±1.8*abc* (16)	123±2.0*bc* (22)	125±2.0*c* (24)	111±5.1*ab* (24)	101±4.2*a* (23)
Sodium/chloride ratio	1.03±0.02*a* (6)	1.04±0.02*a* (15)	1.05±0.03*a* (21)	1.23±0.06*ab* (24)	1.33±0.05*b* (23)
Protein (mg/mL)	32.8±2.28*a* (3)	34.5±1.19*a* (9)	33.2±0.60*a* (14)	33.8±0.91*a* (22)	35.3±1.23*a* (2)
Hematocrit	53±1*a* (23)	54±1*a* (24)	53±1*a* (23)	51±1*a* (24)	53±1*a* (24)

Note: Values are given as the mean ± SE, with *n* given in parentheses. Means followed by different letters are significantly different (one-way ANOVA and Tukey's test, $p < 0.05$).

significantly from initial levels of 122 to 94 mM on day 7 then returned to initial levels by day 21.

In fish transferred to seawater, hematocrit decreased gradually from an initial level of 54 to 49 over 3 days. Hematocrit of control fish increased from 54 to 61 within 24 h and fell to initial levels 48 h after transfer. Control fish had significantly higher hematocrit than seawater fish at all time points except at day 14.

Acclimation of juvenile shad to seawater brought about a gradual threefold increase in gill Na^+,K^+-ATPase activity, leveling off after 5 days in seawater. Although gill Na^+,K^+-ATPase activity increased steadily for the first 48 h after seawater exposure, control and seawater groups were statistically indistinguishable until 3 days after transfer. Gill Na^+,K^+-ATPase activity of fish in seawater remained elevated with respect to controls through the remainder of the 21-day study. Gill Na^+,K^+-ATPase activities of control fish sampled on day 2 were significantly higher than those of fish sampled on day 14.

There was no mortality in either the seawater or freshwater control groups over the 21-day study. The lengths and weights of shad sampled at the beginning of the experiment were 8.0 ± 0.1 cm and 6.0 ± 0.3 g, respectively ($n = 16$). Lengths and weights of fish sampled on day 21 were 8.6 ± 0.2 cm and 6.8 ± 0.7 g for the seawater group ($n = 8$) and 8.7 ± 0.2 cm and 7.5 ± 0.5 g for the control group ($n = 8$). Length and weight of fish in freshwater and seawater groups did not differ, whereas length and weight both increased significantly over time ($p < 0.03$ and 0.03).

Discussion

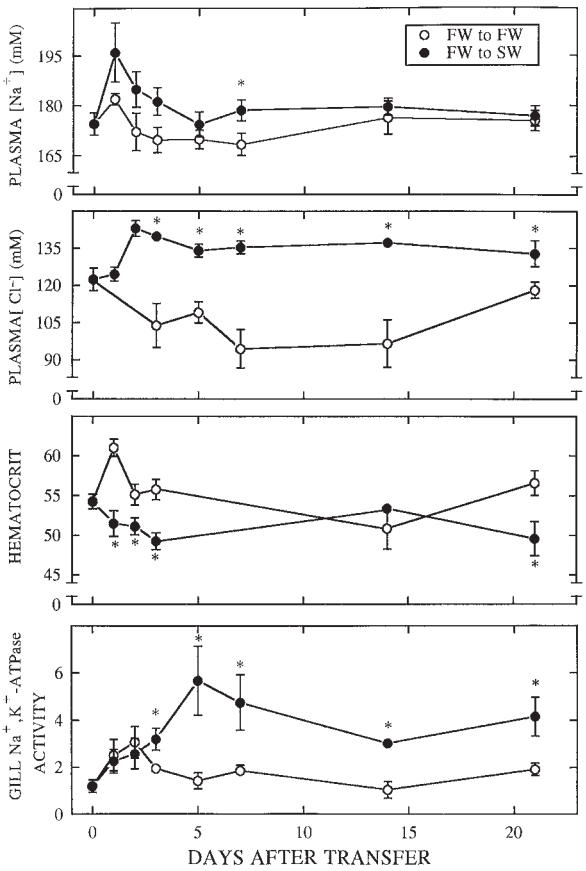
This study demonstrates that American shad develop salinity tolerance at the onset of metamorphosis, between 26 and 45 days post-hatch. This contradicts earlier observations (Tagatz 1961) of high mortalities in 5.6- to 7.9-cm juvenile shad in 33-ppt seawater. Chittenden (1973), however, observed high tolerance of 4.4- to 6.1-cm juvenile shad in full-strength seawater. The likely explanation for the discrepancy, as Chittenden suspected, was the stress of handling in conjunction with the density of fish in the tanks used for the tests. Whereas Tagatz (1961) used 10 fish in a 19-L tank for the experiments, Chittenden used a 227-L tank for 6–28 fish. We have observed that juvenile shad are very sensitive to handling stress. Shad captured in the field (6.4–7.2 cm in length) have high mortality when placed into seawater immediately after transport. Once acclimated to laboratory conditions, however, fish from the same group have low mortality in full-strength seawater and acclimate readily (J. Zydlewski and S.D. McCormick, unpublished data).

Larval shad can survive for several days in salinities of up to 22.5 ppt (Leim 1924). Limburg and Ross (1995) present evidence that mortality and growth rates of shad larvae (16 and 35 days old) do not differ in 0-, 10-, and 20-ppt seawater and suggest that salinity does not restrict shad larvae to freshwater habitat. The present study indicates that larval shad are physiologically unable to survive in salinities typical of coastal, nonestuarine environments until after metamorphosis. Attempts to gradually acclimate larval shad to full-strength seawater also result in high mortality when salinity reaches 20–24 ppt (Milner 1876). Though larvae can survive in salinities that are moderately hyperosmotic, inability to osmoregulate in full-strength seawater appears to be an important factor limiting the early life history of the shad to freshwater and low-salinity estuaries.

Survival in 24-h seawater challenges was shown to be a good test of long-term survival in 45-day-old laboratory-reared shad. There were no further mortalities after 7 days in seawater than had occurred within 24 h of transfer. Both freshwater and seawater groups fed within 8 h of transfer, and length and weight of fish in freshwater and seawater did not differ after 7 days. Similarly, in the 21-day seawater acclimation study, there was no difference in the length or weight of freshwater and seawater fish 21 days after transfer whereas both groups increased in size over time. Though these experiments were not specifically designed to determine growth rates in fresh water and seawater, the results support the hypothesis that growth rates in fresh water and seawater do not differ.

The significance of developing salinity tolerance early in the life history of the shad is uncertain as the downstream migration of juvenile shad is largely uncharacterized. Seaward migration appears to be triggered by declining autumnal temperatures (peaking when temperatures fall to 9–16°C) and northern stocks emigrate earlier than southern stocks (Leggett and Whitney 1972; O'Leary and Kynard 1986). Downstream migration past dams does not appear to be size dependent (Stokesbury and Dadswell 1972; O'Leary and Kynard 1986), although the possible impact of dams on the migration of juvenile shad is unknown. Marcy (1972) argued that the largest juvenile shad are the first to leave the river and observed some juveniles entering the estuary as early as July. Whether or not these fish are migrating into the ocean or remain in the estuary until autumn is unknown. Young shad spawned nearer to the mouth of the river (as close as 50–83 km in the Connecticut River; Glebe and Leggett 1981) may find themselves in elevated salinities because of passive movement or competition before juveniles in the upper reaches begin their seaward migration in the autumn. The ability to hypoosmoregulate may serve to augment nursery areas during years of high juvenile

Fig. 4. Plasma sodium and chloride concentrations, hematocrit, and gill Na^+,K^+-ATPase after transfer of freshwater-acclimated juvenile shad (field captured) to fresh water or 35-ppt seawater at 20°C. Gill Na^+,K^+-ATPase is expressed in μmol ADP·mg protein^{-1}·h^{-1}. Fish were sampled 1, 2, 3, 5, 7, 14, and 21 days after transfer. By two-way ANOVA analysis, salinity had a significant effect on all parameters; time was a significant factor only in plasma sodium concentration and hematocrit; and the interaction of time and salinity was significant for chloride concentration, hematocrit, and gill Na^+,K^+-ATPase. By one-way ANOVA, significant differences within seawater-acclimated fish occurred in plasma sodium concentration ($p < 0.03$), plasma chloride concentration($p < 0.007$), hematocrit ($p < 0.02$), and gill Na^+,K^+-ATPase ($p < 0.004$); within freshwater controls, differences occurred in plasma chloride concentration ($p < 0.04$), hematocrit ($p < 0.001$), and gill Na^+,K^+-ATPase ($p < 0.04$). An asterisk signifies differences between freshwater and seawater groups (one-way ANOVA). Values are given as the mean ± SE for each time point ($n = 16$ at the initial sampling and $n = 8$ at all other sampling points).



populations (Crecco et al. 1983). Early development of salinity tolerance may provide an advantageous flexibility for the timing of seaward migration.

Gill development and increased gill Na^+,K^+-ATPase activity were associated with increased salinity tolerance at metamorphosis. The formation of gill filaments and secondary lamellae begins in late premetamorphic shad (Shardo 1995) and gill index (primary filament length/fork length) measured on day 18 was the lowest observed (0.009). Gill index increased 2.8-fold during metamorphosis and remained high thereafter. Metamorphosis was also marked by a rapid 10-fold increase in gill Na^+,K^+-ATPase activity. Increases in gill index preceded the rise in gill Na^+,K^+-ATPase activity and may reflect the time course of cellular proliferation followed by differentiation of Na^+,K^+-ATPase-rich chloride cells. The increase in salinity tolerance at day 36 coincided with the rapid increase in Na^+,K^+-ATPase but occurred after the early proliferation of gill tissue. A detailed morphological study on the ontogeny of mitochondria-rich cells would be helpful in determining the precise role of the gills during the development of salinity tolerance.

Although gill Na^+,K^+-ATPase activity increases concurrent with the development of salinity tolerance, an additional increase in gill Na^+,K^+-ATPase activity is observed in juvenile shad at the time of migration in both laboratory-reared and field-captured shad (J. Zydlewski and S.D. McCormick, unpublished results). Increased gill Na^+,K^+-ATPase activity during migration does not appear to be linked to increased hypoosmoregulatory capabilities. We cannot, however, rule out the possibility that hypoosmoregulatory ability increases in autumn but cannot be detected using 24-h survival in seawater as a measure.

Wild fish captured prior to the migratory period have lower gill Na^+,K^+-ATPase activities than fish reared in the laboratory (Fig. 3), as shown by the control group in the seawater acclimation study (Fig. 4). Premigratory wild fish generally have Na^+,K^+-ATPase levels between 1 and 2 μmol ADP·mg protein^{-1}·h^{-1} (J. Zydlewski and S.D. McCormick, unpublished results), whereas the laboratory-reared fish in this study had basal levels of 4 μmol ADP·mg protein^{-1}·h^{-1}. These higher levels may be an artifact of long-term rearing in a laboratory environment.

The size of shad reared in the laboratory was not different from that of field-captured shad in the Connecticut River, and growth rates of laboratory and wild fish are probably similar. In general, specific growth rate in weight was highest after metamorphosis and gradually declined into the fall. Howey (1985) also observed a peak in growth rate just after metamorphosis and a decrease in growth rate after 75 days post-hatch. Similar to salmonid smolts, a decline in condition factor occurs during the period of natural migration. As juvenile shad do not go through a striking morphological change as do salmonids, it is unclear if this drop is the effect of decreased feeding owing to decreasing water temperatures or possibly an adaptive change in form.

The plasma sodium/chloride ratio of juvenile shad in fresh water increased significantly over the period of September to November from 1.03 to 1.33. This change in the ion ratio was caused by a decrease in plasma chloride levels while the plasma sodium levels remained unperturbed. Such increases in plasma sodium/chloride ratio have been interpreted as indicating that sodium is more tightly regulated than chloride (Nordlie 1987). The higher ratio is similar to that calculated for adult shad in fresh water from the data of Leggett and O'Boyle (1976) (sodium/chloride = 1.40).

The response of plasma ions in freshwater-acclimated juvenile shad transferred to seawater is similar to that described for salmonids (Koch et al. 1959; Holmes and Donaldson 1969). Entry into seawater is immediately followed by an acute adaptive phase (Holmes and Donaldson 1969) marked by a rapid elevation and partial recovery of plasma ions followed by a

chronic regulatory phase, which gradually establishes a new ionic homeostasis. Similar patterns of ionic perturbation and subsequent recovery have been described for other teleosts (Jacob and Taylor 1983; Evans 1984). While plasma sodium levels in the juvenile shad transferred to seawater reached a new steady state close to initial levels, plasma chloride remained elevated with respect to initial levels. Shad transferred to seawater in this study had no mortality, but the perturbations of plasma sodium and chloride concentrations were significant and were stabilized when gill Na^+,K^+-ATPase increased (3–5 days after transfer).

The increase in gill Na^+,K^+-ATPase activity and reestablishment of ion homeostasis after transfer to seawater corresponds to the chronic regulatory phase of seawater acclimation. Although more rapid changes in gill Na^+,K^+-ATPase activity have been reported for the killifish, *Fundulus heteroclitus*, and the tilapia, *Oreochromis mossambicus* (Towle et al. 1977; Hwang et al. 1989), the time course of increased gill Na^+,K^+-ATPase activity during seawater acclimation in juvenile shad is consistent with most other euryhaline teleost species. In general, an increase in gill Na^+,K^+-ATPase activity and stabilization of plasma ions is observed 3–7 days after transfer to seawater (Jacob and Taylor 1983; Evans 1984). The correspondence between increased gill Na^+,K^+-ATPase activity and recovery of plasma ions in seawater is further evidence for the involvement of this enzyme in ion homeostasis.

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