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The effect of migration distance and timing on metabolic enzyme activity in an anadromous clupeid, the American shad (*Alosa sapidissima*)

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Abstract

The American shad (*Alosa sapidissima*) is a common anadromous fish species with ecological and economic importance on the east coast of North America. This iteroparous species undergoes an energetically costly upriver spawning migration in spring. To evaluate metabolic changes associated with this migration, we assessed the maximum activity of five metabolic enzymes (citrate synthase (CS), phosphofructokinase (PFK), lactate dehydrogenase (LDH), β-hydroxyacyl coenzyme A dehydrogenase (HOAD), alanine aminotransferase (GPT)) in liver, red muscle and white muscle during upstream migration in two successive years in the Connecticut River. For aerobic capacity (CS), glycolytic capacity (LDH) and utilization of stored lipid and protein energy (HOAD and GPT), there is a general pattern of increasing activity with a subsequent decline at the most upriver sites. Red muscle CS activity increased by as much as 40% during the migration while white muscle CS activity was 120% higher in the river than in the ocean. In contrast, muscle anaerobic capacity, indicated by PFK, was low as fish entered the river and then increased 5-fold at the most upriver sites. White muscle HOAD increased ~30% while red muscle HOAD and muscle GPT increased as much as 60%. There were interannual and sex-associated differences in enzyme activity during upstream migration and through time at a single location. In some cases interannual differences can be larger than those seen during upriver migration as in the case of red muscle CS where sampling years differed by 125%. These interannual differences may be a result of differing river conditions that affect migratory effort. We have demonstrated that American shad use tissue and sex-specific regulation of enzyme activity during migration and we suggest that American shad metabolically acclimate to upstream migration.

Introduction

Animal migration is often energetically costly (Dingle 1996) and these costs may be at least partially incurred from locomotion (Brett 1973), reproductive development (Lambert and Dodson 1990; Smith et al. 1990) and acclimation to a new environment (Leggett and O'Boyle 1976; Kirschner 1993). Modification of the metabolic enzyme systems to maximize the effectiveness of metabolism can occur during migration (Bishop et al. 1995; Guderley et al. 1986; Mommsen et al. 1980). These changes may occur in response to locomotory demands, or may arise before needed by the animal as an anticipatory adaptation to migration as is seen in the smolting juvenile Atlantic salmon (McCormick and Saunders 1987). Changes in metabolic enzymes have been documented in migratory fishes during the spawning migration, including coregonid fishes (Guderley et al. 1986) and sea lamprey, *Petromyzon marinus* (LeBlanc et al. 1995), but general patterns of metabolic change during fish migration are still unclear because of the small number of species examined to date.

The American shad (*Alosa sapidissima*) is the most abundant anadromous species found in the eastern United States. American shad typically migrate

upriver during the winter or spring to spawn. The species is semelparous in southern populations and iteroparous in northern rivers. Mid-Atlantic populations exhibit a clinal change in iteroparity. In the Connecticut River, as many as 40% of migrating shad are not first-time spawners (Glebe and Leggett 1981). American shad spawn in the main-stem of the river. Juveniles hatch in early summer and remain in the river until fall when they migrate downstream. Connecticut River fish typically remain at sea for three to five years before returning on their first spawning migration (Glebe and Leggett 1981).

The Connecticut River is 675 km long and stretches from northern New Hampshire to its mouth at Old Lyme, CT where it enters Long Island Sound. The river drains approximately 25,370 km^2 and is subject to large runoff and high flow conditions during the early spring when the snow melt occurs ($>$50,000 cfs at US Geological Survey Montague Gauging Station). This period is coincident with the beginning of upriver American shad migration. Temperature also varies considerably during the migratory period ranging from 10 to 24 °C and high temperatures have been suggested as the proximate cause for the end of the run (Leggett and Whitney 1972; Shoubridge and Leggett 1978). In addition, migratory fish must surmount a number of barriers to migration, including small, natural waterfalls and manmade obstacles such as the hydroelectric power dams that block the main-stems of many rivers. Fish successfully using fish ladders or fish elevators can accomplish passage past these structures.

Upstream migration of fish is frequently limited by a variety of physiological and environmental factors. While these factors likely vary with species, they include temperature, photoperiod, water flow, energy reserves and reproductive state. In the case of American shad, temperature is probably the greatest delimiter of this migratory window. Shad have been shown to move along the coast during their ocean period coincident with the 13 to 18 °C isotherm (Leggett and Whitney 1972). Shad move into their natal rivers when temperatures are 10 to 13 °C (Leggett and Whitney 1972), suggesting a lower behavioral thermal limit to migration of approximately 6–8 °C. There is also evidence that high water flow conditions may be an important limitation to migratory activity (Quinn and Adams 1996) and high flow would normally be encountered at the beginning of the migratory run as the winter snow melt occurs in New England. The end of the migratory period may also be limited by temperature, as well, since shad have been shown to stop migrating at approximately 22 °C with peak migratory activity occurring near 18 °C (Leggett and Whitney 1972). There may be additional regulating factors such as photoperiod, however, that could also limit migration.

This paper describes the metabolic differences, as measured by changes in maximal activity of several key metabolic enzymes, that are seen between American shad captured in the ocean, at the beginning of their upriver migration (estuary) and at three upriver locations (139, 198, 228 km). We discuss how the observed changes in enzyme activity relate to successful migration. Further, this study characterizes differences in metabolic enzyme systems between early, mid-run and late migrant American shad in the Connecticut River. In order to accomplish these goals, we have chosen to examine the activities of five enzymes which are located in various metabolic pathways in an effort to gain insight into broad metabolic changes occurring during migration. Citrate synthase is a regulatory citric acid cycle enzyme that indicates mitochondrial abundance and aerobic capacity (Somero and Childress 1980; Couture and Guderley 1990; Moyes 1996). β-hydroxyacyl coenzyme A dehydrogenase also indicates general aerobic metabolism as well as lipolytic activity (Couture and Guderley 1990; Davison 1997). Glycolytic capacity is indicated by the regulatory enzyme phosphofructokinase (Couture and Guderley 1990 Soengas et al. 1996) and lactate dehydrogenase (anaerobic terminatus; Somero and Childress 1980). Metabolic incorporation of alanine, as an index of protein catabolism, is indicated by the activity of alanine aminotransferase which is a major enzyme in the glucose/alanine cycle (Mendez and Wieser 1993).

Materials and methods

Fish

Migration distance effects

Fish were captured and sampled in 1993 and 1994 at four sites along the main-stem of the Connecticut River (Figure 1): Old Lyme, CT (1 km upriver); Holyoke Dam (Northeast Utilities), Holyoke, MA (139 km upriver); Cabot Station (Northeast Utilities), Turners Falls, MA (198 km upriver); Vernon Dam (New England Power Co.), Vernon, VT (228 km upriver). Vernon Dam represents the farthest upriver site where significant numbers of shad are successfully

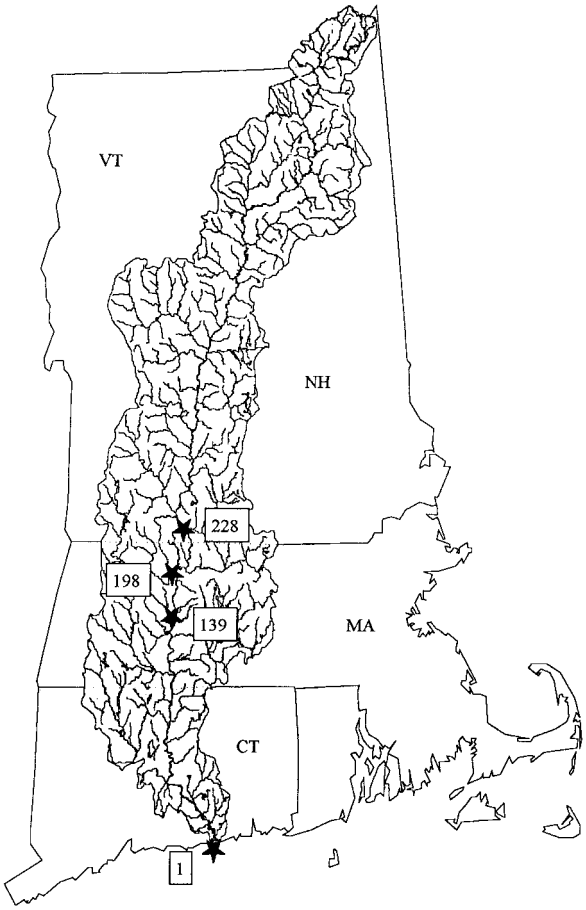

Figure 1. Map of the Connecticut River showing sampling sites (★) for adult American shad sampled within the river in 1993 and 1994. Numbers indicate the site distance (km) from the mouth of the river. Sites are as follows: Old Lyme, CT – km 1; Holyoke, MA – km 139; Turners Falls, MA (Cabot Station) – km 198; Vernon, VT – km 228. The ocean sampling site off Barnagut Light, NJ is not shown.

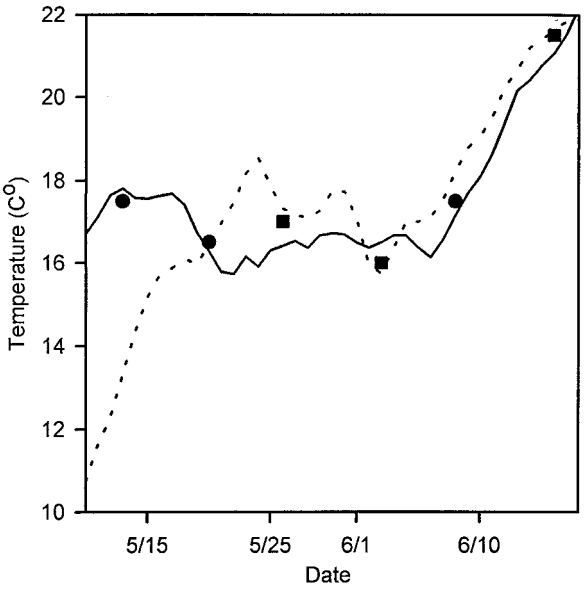

Figure 2. Ambient mean daily canal temperature (°C) for 1993 and 1994 and sampling dates when adult American shad were captured at the Cabot Station fish ladder for the migration timing experiment. ● 1993, ■ 1994. _____ Turners Falls canal temperature 1993, ... Turners Falls canal temperature 1994.

passed through fish passage structures. Additionally, American shad were sampled in the ocean (33 ppt) off Barnagut Light, NJ in May 1995. Fish sampled at Old Lyme and off Barnagut Light (ocean) were captured *via* gill net (5" and 6" stretch mesh) and sampled immediately. At all other sites, fish were taken from fish trapping devices built into fish passage structures at dam facilities. At the river sites, efforts were made to capture the fish within 3 days of their appearance at the site in order to minimize the confounding effects of sampling fish that had spent variable amounts of time in the river. By sampling the first fish arriving at each site, we assume that the animals ascended the river in a directed manner and arrived at each site as soon as was possible. At each site approximately 20 fish were taken during each year (10 male, 10 female). The fish were immediately anesthetized using MS-222 (50 mg l^{-1}, pH 7.0) and bled from the caudal vessels with heparinized syringes. The fish were then placed on ice (0.25–3 h) until they could be dissected for tissue sampling (liver, gonad, red muscle and white muscle). Tissues sampled were then immediately frozen at −80 °C.

Temperatures at the ocean and estuarine sampling sites (both 1993 and 1994) were similar while there were increases in temperature at upriver sampling sites during both years. Temperatures and sampling dates were as follows: oceanic site, Barnagut Light, NJ 5.7.95 (10.6 °C); Old Lyme, CT (km1) 4.27.93 (10.4 °C), 4.28.94 (10.0 °C); Holyoke Dam, Holyoke, MA (km 139) 5.6.93 (15.5 °C), 5.9.94 (12.0 °C); Cabot Station, Turners Falls, MA (km 198) 5.13.93 (18.0 °C), 5.26.94 (17.0 °C); Vernon Dam, Vernon, VT (km 228) 5.27.93 (17.5 °C), 6.10.94 (19.4 °C). Temperature was more variable in 1994 (Figure 2). There was a greater amount of time between the samplings of Holyoke Dam and Cabot Station in 1994 because the opening of the fish ladder at Cabot Station was delayed.

Migration time effects

Adult American shad were captured and sampled in 1993 and 1994 at Cabot Station (km 198) as previously described. Approximately 20 fish (10 male, 10 female) were taken at each of three sampling dates corresponding to early, mid-run and later periods in the shad migration each spring. The early sampling date was chosen to be 7–10 days after the opening of the fish ladder (Figure 2). The mid-run sampling occurred 7–10 days later when there was an abundance of fish passing through the facility. The late sampling occurred when there were few fish passing through the ladder (approximately 14 days after the mid-run sampling date). In both sampling years, the fish captured from Cabot Station during the early period also served as the experimental animals for the migration distance study.

Fish ladder effects

In the spring of 1993, 20 fish (10 male, 10 female) were captured from the tailrace region of the Cabot Station facility at the base of the fish ladder using a snagging hook and line. Fish were captured less than one minute after initial hooking. These fish were compared to the fish captured at the top of the ladder on the same day as previously described.

Enzyme assays

Muscle and liver samples were assayed for activity of citrate synthase (CS), phosphofructokinase (PFK), lactate dehydrogenase (LDH), alanine aminotransferase (GPT) and β-hydroxyacyl coenzyme A dehydrogenase (HOAD). The assays were optimized for American shad tissue and are based on the following methods: CS (Srere 1969; Guderley et al. 1986; McCormick et al. 1989), PFK (Ling et al. 1966; Guderley and Gawlicka 1992), LDH (Vassault 1983; Guderley and Gawlicka 1992), HOAD (Bradshaw and Noyes 1975; Guderley and Gawlicka 1992), GPT (Wroblewski and LaDue 1956; Guderley et al. 1986). Tissue was homogenized in buffer containing 50 mM imidazole, 2 mM EDTA, 5 mM $MgCl_2$ and 1 mM glutathione. Tissue samples were homogenized on ice and then sonicated on ice for 2×5 s bursts at 100% power using a needle probe (B. Braun Instruments, South San Francisco, CA). Samples were then centrifuged at 2000 *g* for 5 min at 4 °C and then immediately assayed in a microplate spectrophotometer equipped with temperature control and automatic agitation using 96 well round bottom microplates at 25 °C with appropriate dilutions for the range of the assay. Specific conditions for each assay were as follows:

Citrate synthase (CS; E.C. 4.1.3.7): 50 mM Tris, 0.78 mM 5,5′ dithiobis-(2-nitrobenzoic acid), 0.17 mM acetyl Coenzyme A, 0.57 mM oxaloacetic acid (omitted for control), pH 8.1. Read at 412 nm.

Phosphofructokinase (PFK; E.C. 2.7.1.11): 75 mM Tris HCl, 200 mM KCl, 6 mM $MgCl_2$, 1 mM KCN, 2 mM AMP, 0.75 mM ATP, 0.16 mM NADH, triose phosphate isomerase in excess, 3-phosphoglycerol dehydrogenase in excess, aldolase in excess, 5 mM fructose-6-phosphate (omitted for control), pH 8.0. Read at 340 nm.

Lactate dehydrogenase (LDH; E.C. 1.1.1.27): 100 mM potassium phosphate, 0.4 mM pyruvate, 0.16 mM NADH, pH 7.0. Read at 340 nm. This assay was routinely run without control since preliminary experiments showed extremely low activity without the presence of the substrate (pyruvate) for all tissues.

β-hydroxyacyl coenzyme A dehydrogenase (HOAD; E.C. 1.1.1.35): 100 mM triethanolamine HCl, 5 mM EDTA, 1 mM KCN, 0.23 NADH, 0.15 mM acetoacetyl coenzyme A (omitted for control), pH 7.0. Read at 340 nm.

Alanine aminotransferase (GPT; E.C. 2.6.1.2): 1040 mM L-alanine, 104 mM potassium phosphate, 0.29 mM NADH, lactate dehydrogenase in excess, 23.4 mM α-glutaric acid (omitted for control), pH 7.4. Read at 340 nm.

All assays were run in duplicate and the activities were expressed in international units (*i.e.*, μmol of substrate transformed to product min^{-1}) $\times$ g^{-1} wet mass tissue. CS, HOAD and GPT were performed on samples from both years. PFK and LDH were assayed only in 1994.

Proximate Analysis

Total lipid was determined after extraction of lipids from a known amount of tissue using spectrophotometer analysis (Sheridan et al. 1983). Briefly, lipids were extracted in 20 vol of chloroform:methanol (2:1 v:v) in the manner of Folch et al. (1957). Aliquots of extracted lipid were then analyzed using the sulpho-phospho-vanillin reaction described by Frings et al. (1972) using extracted and gravimetrically quantified cod liver oil as a standard. Total protein was determined in tissue after homogenization and sonication (2×5 s bursts) in 0.1 N NaOH. Protein was then determined by spectrophotometric analysis using the method of Lowry et al. (1951) using bovine serum

albumin as a standard. Plasma glucose was determined based on the hexokinase enzymatic colorimetric method of Stein (1963) (Sigma #16HK-UV).

Statistics

Data were analyzed using analysis of covariance (ANCOVA) with fish fork length as the covariate using the SAS statistical package (SAS Institute Inc., Cary, NC) with $\alpha = 0.05$ (Tables 1 and 2). ANCOVA was selected because American shad display a length dimorphism between the sexes where males are consistently smaller than females. The oceanic fish were sampled in a different year than river migrants and we do not conclusively know that these fish were destined to enter the Connecticut River. Therefore, we do not include this sampling site in our analyses of river migrant parameters. Instead, a separate ANCOVA analysis was performed using the oceanic samples and the samples from km 1 (both years) to determine if the ocean samples differed significantly from those taken at the beginning of the riverine portion of the migration.

Results

Migration distance effects

Females were larger than males (males 42 $\pm$ 0.2 cm; females 48 $\pm$ 0.2 cm fork length) and there was a slight decrease in length at the most upriver sites (Table 1). The ocean site was unusual in that there was little difference in length between the sexes and lengths were intermediate to those seen in the river migrants.

White muscle CS activity in the river shad was much higher than that in the shad sampled in the ocean and there was a subsequent decrease in activity at the most upriver sampling sites (Figure 3). Males had a higher CS activity than females. LDH levels were low in fish sampled in the ocean, higher in most riverine sampled fish and then again lower in the fish sampled at km 228. For fish sampled within the river, males had greater LDH activity than females. HOAD activity was variable, however, there was a general pattern of increasing activity in the early portions of migration followed by a decrease at the most upriver sites. Males also tended to have greater white muscle HOAD activity than females. GPT activity increased with sampling site distance from the ocean with greater increases being observed in males. There was a decrease in GPT activity in both sexes at the most upriver sampling site. PFK activity levels were relatively high in the ocean, dropped at the riverine sites closest to the river mouth and tended to rise as fish moved farther upriver.

Red muscle enzymes showed generalized increases in enzyme activity during migration relative to oceanic levels except for PFK which decreased (Figure 4). With the exception of PFK, regardless of initial enzyme activity levels, there was higher activity during the migratory phase, but activity was lower at the most upriver sampling site. Specifically, red muscle CS was intermediate at the oceanic sampling site. 1993 riverine levels were lower than that of the oceanic fish and decreased further during migration, whereas 1994 levels were higher than oceanic levels at the beginning of the migration and continued to increase until there was a slight drop at km 228. LDH was low in the oceanic fish, increased at the initial riverine sites and then decreased at the most upriver sites. Males generally had higher levels of LDH activity than females during the riverine migration. Red muscle HOAD followed a similar pattern to CS with moderate oceanic levels, high 1994 activities and lower 1993 enzyme levels. GPT also showed an increase in activity at more upriver sampling sites with a suggestion of a similar decrease at km 228. Red muscle PFK was high in the ocean, low at the mouth of the river and increased over the course of migration.

Liver enzyme activity levels vary depending on the enzyme assessed (Figure 5). CS levels were highest in the shad captured in the ocean. 1993 levels decreased from this activity throughout migration. In 1994, the CS activity was slightly lower than oceanic levels, but was relatively stable throughout migration. Liver LDH was highly variable in the ocean. Within the river, LDH levels tended to be high near the mouth of the river and decrease at more upriver sites, although there is an apparent increase at the most upriver site. Liver HOAD was highest in the ocean. Activity at the mouth of the river was low, but rose at farther upstream sites until there was again a decrease at the most upriver sites. Activity levels were significantly higher in 1994 than in 1993. Liver GPT was lowest in the ocean and increased in the river to a maximum at km 228. Changes in PFK activity were equivocal with a tendency toward little change in males, but a decrease in females from high oceanic levels to lower levels in the river.

Lipid levels were highest in the ocean samples in all three tissues examined (Table 1). In both years in all three tissues there was significantly less stored lipid

Table 1. Proximate composition (protein, lipid; mg g^{-1} wet mass), length (cm), gonadosomatic index (GSI,%) and plasma glucose (GLU; mM) for American shad captured at four different river locations (estuary – km 1; Holyoke Dam – km 139; Cabot Station – km 198; Vernon Dam – km 228) and at an oceanic site (Barnagut Light, NJ)

Site	A[1]	B	1995 Ocean		1993 Males				1994 Males				1993 Females				1994 Females			
			male	female	1	139	198	228	1	139	198	228	1	139	198	228	1	139	198	228
N			7	11	10	10	13	13	8	9	11	11	18	10	10	7	11	11	10	10
length	l(a,a,b,c)[2],sl,y,sy	s',sg	45.6	46.9	43.2	42.7	42.0	43.0	44.5	43.4	40.2	37.6	48.3	48.0	46.9	45.6	49.8	48.5	47.6	45.5
			(0.7)[3]	(0.5)	(0.4)	(0.4)	(0.3)	(0.3)	(0.9)	(1.0)	(1.1)	(0.7)	(0.6)	(0.7)	(0.8)	(0.7)	(0.8)	(0.5)	(0.5)	(0.8)
total weight	l(a,b,c,d),s,sy	g(ab,a,b),s', sg	1550	1678	1219	1103	987	1028	1445	1151	908	679	1781	1660	1533	1269	2088	1775	1602	1285
			(40)	(54)	(38)	(44)	(23)	(39)	(104)	(73)	(69)	(51)	(60)	(70)	(103)	(82)	(89)	(61)	(50)	(73)
GSI	l(ac,b,ab,c),s,sl	s'	6.82	12.79	7.53	8.30	6.62	6.30	8.09	8.14	6.94	5.87	13.42	16.31	17.97	13.70	15.18	18.64	16.89	14.39
			(0.59)	(0.98)	(0.30)	(0.36)	(0.2)	(0.37)	(0.32)	(0.31)	(0.35)	(0.33)	(0.55)	(0.86)	(1.48)	(1.60)	(0.53)	(1.19)	(1.24)	(1.01)
GLU	l(a,b,c,c),s,y,ly				6.3	5.0	8.7	8.7		7.3	8.6	8.2	5.0	4.7	7.9	8.3	7.0	5.8	8.7	8.3
			–	–	(0.8)	(0.5)	(0.3)	(0.7)	–	(0.4)	(0.5)	(0.3)	(0.3)	(0.5)	(0.6)	(0.6)	(0.3)	(0.3)	(0.3)	(0.2)
liver lipid	l(a,b,c,d),s,sl,ly	g(a,b, b),s'	115.6	60.7	61.5	29.7	42.5	28.1	60.3	37.0	41.5	18.9	25.9	16.5	19.8	17.3	41.3	26.0	31.6	9.4
			(17.2)	(8.1)	(7.5)	(4.8)	(4.8)	(1.8)	(13.6)	(3.4)	(2.6)	(3.0)	(3.1)	(2.5)	(4.0)	(0.9)	(3.0)	(2.1)	(1.7)	(1.4)
liver protein	l(a,b,ac,c),s,y,ly	g(a,b,a),s'	167.7	142.7	183.5	187.7	178.0	169.0	156.6	171.6	147.4	147.2	154.1	195.0	162.7	139.3	143.4	150.7	132.6	137.6
			(7.2)	(3.9)	(10.4)	(3.0)	(2.7)	(5.1)	(1.6)	(3.7)	(4.1)	(3.0)	(4.5)	(8.6)	(2.5)	(5.2)	(4.3)	(3.3)	(3.0)	(3.2)
red lipid	l(a,b,c,c)	g(a,b, b)	324.4	289.5	123.8	97.8	66.3	78.6	129.0	114.6	89.3	64.9	118.2	103.0	68.6	77.7	134.2	100.0	88.2	71.1
			(32.4)	(29.2)	(12.7)	(5.1)	(3.5)	(5.0)	(6.0)	(11.2)	(7.8)	(9.5)	(8.1)	(10.7)	(5.4)	(6.0)	(4.8)	(6.4)	(8.8)	(9.9)
red protein	l(ac,a,a,b), y,ly	g(a,b, a)	147.6	143.3	161.9	168.8	168.0	192.9	147.7	138.3	133.4	129.7	153.4	163.0	156.9	189.0	140.3	117.6	121.6	119.7
			(2.8)	(3.9)	(5.1)	(5.5)	(5.1)	(4.7)	(5.4)	(8.9)	(3.1)	(2.0)	(5.1)	(4.5)	(4.4)	(9.1)	(5.2)	(2.0)	(2.1)	(3.7)
white lipid	f,l(a,b,c,b),y,ly	g(a,b, b)	240.8	191.2	100.1	75.0	72.1	50.8	85.0	43.9	54.9	41.8	109.1	74.8	102.9	53.0	90.9	51.9	60.1	50.4
			(12.1)	(28.1)	(8.3)	(12.2)	(7.5)	(4.0)	(7.0)	(6.0)	(9.9)	(6.2)	(9.4)	(13.5)	(7.0)	(5.0)	(6.6)	(7.0)	(8.8)	(10.1)
white protein	l(a,b,c,c),y	g(a,b,a)	127.5	133.9	149.5	211.8	195.9	181.1	120.6	150.0	140.5	133.9	147.6	204.6	183.4	175.8	124.5	132.0	118.2	126.2
			(4.3)	(4.2)	(3.7)	(4.3)	(2.1)	(4.4)	(3.6)	(2.2)	(4.5)	(3.0)	(5.4)	(4.7)	(4.7)	(5.5)	(5.7)	(4.2)	(8.6)	(5.27)

Values are mean (standard error); [1]column A indicates the results of analysis of covariance (for length and weight only, analysis of variance) and multiple comparisons analysis of riverine data while column B indicates the results of similar analyses comparing the ocean and estuarine sites. '**y**' indicates a significant effect of year, '**s**' a significant effect of sex for riverine sites, '**l**' a significant effect of location for riverine sites, '**f**' a significant effect of length for riverine sites, '**sl**' a significant interaction between sex and location for riverine sites, '**sy**' a significant interaction between sex and year for riverine sites, '**ly**' a significant interaction between location and year for riverine sites, '**g**' a significant difference between sites for the ocean-estuary comparison, '**s'**' a significant effect of sex on the ocean-estuary comparison, '**sg**' a significant interaction between location and sex for the ocean-estuary comparison and '**f'**' a significant effect of length for the ocean-estuary comparison on a given parameter as indicated by analysis of covariance ($\alpha = 0.05$); [2]parenthetical letters after '**l**' and '**g**' indicate the results of multiple comparisons testing for riverine location (0,139,198,228 km respectively) and ocean-estuary groups (ocean, estuary 1993, estuary 1994 respectively); parenthetical numbers are 1 SEM.

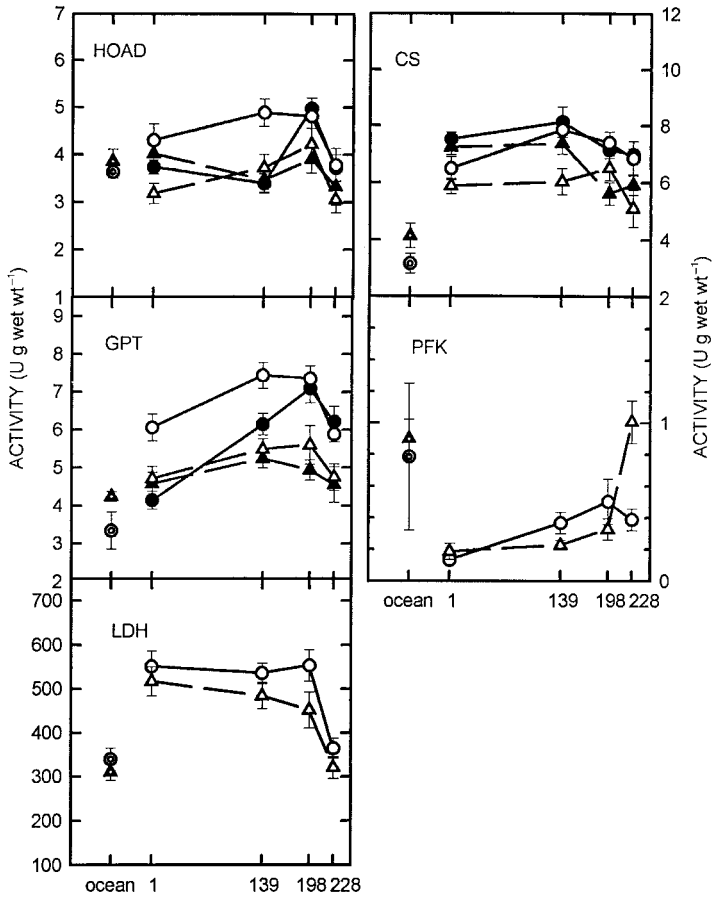

Figure 3. Enzyme activity (U g wet weight^{-1}) and sampling site location for white muscle of adult American shad. β-hydroxyacyl coenzyme A dehydrogenase (HOAD); citrate synthase (CS); alanine aminotransferase (GPT); phosphofructokinase (PFK); lactate dehydrogenase (LDH). ● males 1993; ○ males 1994; ▲ females 1993; △ females 1994; ⊙ oceanic males (1995); ◬ oceanic females (1995). Values are means ± 1 SEM. Significant results of ANCOVA on white muscle enzymes (significance at $\alpha = 0.05$) at riverine sites (km 1, 139, 198, 228) are as follows: GPT – location (a,b,b,c), sex, year, sex × location; LDH – location (a,a,a,b); PFK - location(a,ab,b,c), sex × location; HOAD – length, location (a,a,b,a), sex, year, sex × year, location × year; CS – location (a,b,a,a), sex, year, location × year. Significant results of ANCOVA comparison (significance at $\alpha = 0.05$) of ocean and estuary samples (estuary '93, estuary '94, ocean '95) are as follows: GPT – group (a,a,b), sex × group; LDH – length, group (a,–,b); PFK - group (a,–,b); HOAD – sex × group; CS – group (a,b,c).

at the mouth of the river than the ocean and levels decreased further with distance upstream. Levels were higher in the white muscle in 1994. Males showed a greater amount of lipid in the liver than females. Total protein levels were relatively low in the oceanic samples in red and white muscle (Table 1). In both tissues, there was little change in protein content during 1994 while in 1993 there was an apparent increase in protein over the course of migration. Liver protein was variable, but generally there was more protein in male livers with a tendency toward lower protein levels in females at the most upriver sites. Plasma glucose data were not available for fish captured in the ocean or for males captured in the estuary in 1994. Available data shows a significant effect of location, sex and sampling year (Figure 6, Table 1). There was little change between the estuarine and Holyoke (km 139) sampling sites. Samples from the two upriver sites, however, had significantly higher concentrations of plasma glucose in both males and females in both years.

Migration time effects

Fork length between the two sexes differed significantly with females averaging 46 cm and males 41 cm (Table 2). There was no effect of sampling time or year. Gonadosomatic index (GSI) was calculated as (gonad weight/somatic weight) × 100. GSI was significantly larger in females (16.6) than males (6.3). Fish

Table 2. Mean absolute enzyme activities (U g^{-1} wet mass) for American shad captured at Cabot Station in Turners Falls, MA at three different times during the migratory period: early (E), mid-run (M) and late (L). Enzymes measured are citrate synthase (CS), β-hydroxyacyl coenzyme A dehydrogenase (HOAD), phosphofructokinase (PFK), lactate dehydrogenase (LDH) and alanine aminotransferase (GPT)

		1993 Males			1994 Males			1993 Females			1994 Females		
Time	A[1]	E	M	L	E	M	L	E	M	L	E	M	L
N		13	11	11	11	12	9	10	9	8	10	8	9
liver CS	f,t(a,b, b)[2],s,y,ty	3.3 (0.2)[3]	5.2 (0.7)	3.8 (0.2)	6.3 (0.4)	5.6 (0.3)	4.3 (0.5)	3.7 (0.2)	4.7 (0.6)	3.2 (0.1)	6.4 (0.5)	5.3 (0.3)	4.9 (0.2)
liver HOAD	f,t(a,a, b),s,y,ty	4.7 (0.2)	5.1 (0.3)	7.1 (0.4)	8.9 (0.6)	7.8 (0.5)	7.7 (1.0)	6.5 (0.2)	6.2 (0.3)	7.8 (0.3)	9.8 (0.8)	8.6 (0.6)	11.1 (0.7)
liver PFK	t(ab,a,b)	–	–	–	0.34 (0.06)	0.46 (0.09)	0.22 (0.08)	–	–	–	0.27 (0.09)	0.30 (0.13)	0.12 (0.03)
liver LDH	t(a,b, a),s,ts	–	–	–	12.5 (1.8)	5.8 (0.4)	8.5 (0.6)	–	–	–	6.1 (0.7)	4.7 (0.4)	6.7 (0.5)
liver GPT	f,y,ty	43.0 (2.2)	41.1 (3.2)	47.2 (3.3)	45.7 (3.9)	59.5 (3.4)	46.9 (5.2)	39.6 (1.0)	38.5 (3.6)	39.4 (1.3)	44.5 (2.7)	53.6 (3.0)	50.8 (2.2)
red CS	t(a,b, c),y,ty	34.3 (1.5)	30.6 (1.7)	28.8 (2.2)	69.7 (2.6)	66.0 (3.5)	52.9 (3.7)	31.2 (2.1)	29.9 (1.0)	28.1 (2.1)	70.1 (4.0)	63.3 (3.3)	57.8 (4.4)
red HOAD	t(a,b, b),y	29.2 (2.9)	22.6 (1.6)	18.3 (2.0)	45.0 (2.4)	34.4 (2.4)	29.5 (2.6)	25.1 (2.0)	24.1 (1.5)	19.4 (1.8)	40.3 (2.5)	31.3 (2.1)	33.3 (2.8)
red PFK	t(a,a, b),s	–	–	–	0.24 (0.02)	0.30 (0.03)	0.15 (0.03)	–	–	–	0.29 (0.08)	0.29 (0.03)	0.22 (0.05)
red LDH	f,s	–	–	–	131.4 (9.6)	116.2 (5.3)	106.1 (12.8)	–	–	–	115.2 (6.1)	93.3 (5.4)	125.7 (30.9)
red GPT	f,t(a,b, b),y,ts,ty	12.6 (0.7)	4.9 (0.4)	6.6 (0.5)	18.7 (1.2)	16.7 (1.1)	12.8 (0.7)	9.3 (0.8)	4.4 (0.5)	5.0 (0.2)	13.4 (1.0)	14.6 (0.9)	14.6 (1.9)
white CS	f,t(a,a, b),s,ty	7.2 (0.3)	6.1 (0.3)	9.8 (0.7)	7.4 (0.4)	7.1 (0.2)	6.5 (0.5)	5.6 (0.4)	4.7 (0.6)	8.3 (0.5)	6.5 (0.4)	6.7 (0.4)	4.8 (0.5)
white HOAD	f,t(a,b, b),s	5.0 (0.2)	4.3 (0.3)	3.7 (0.3)	4.8 (0.3)	4.0 (0.2)	3.8 (0.4)	3.9 (0.3)	3.3 (0.4)	3.7 (0.3)	4.2 (0.3)	3.8 (0.1)	2.9 (0.3)
white PFK		–	–	–	0.50 (0.14)	0.61 (0.13)	0.39 (0.08)	–	–	–	0.33 (0.07)	0.76 (0.16)	0.58 (0.17)
white LDH	t(a,a,b)	–	–	–	553.2 (36.1)	475.4 (15.6)	373.0 (31.3)	–	–	–	451.9 (41.4)	486.8 (28.6)	429.6 (29.1)
white GPT	t(a,b, b),s,y, ts,ty	7.1 (0.4)	4.7 (0.3)	5.7 (0.3)	7.3 (0.3)	6.3 (0.3)	5.8 (0.4)	4.9 (0.3)	4.0 (0.1)	4.8 (0.3)	5.6 (0.5)	5.9 (0.3)	4.8 (0.3)

[1]Column A indicates the results of analysis of covariance (for length only, analysis of variance) and multiple comparisons analysis. '**y**' indicates a significant effect of year, '**s**' a significant effect of sex, '**t**' a significant effect of migration time, '**f**' a significant effect of length, '**ts**' a significant interaction between sex and time, '**sy**' a significant interaction between sex and year, '**ty**' a significant interaction between time and year (α = 0.05); [2]parenthetical letters after '**t**' indicate the results of multiple comparisons testing for migration time (E, M, L respectively); [3]parenthetical numbers are 1 standard error of the mean (SE).

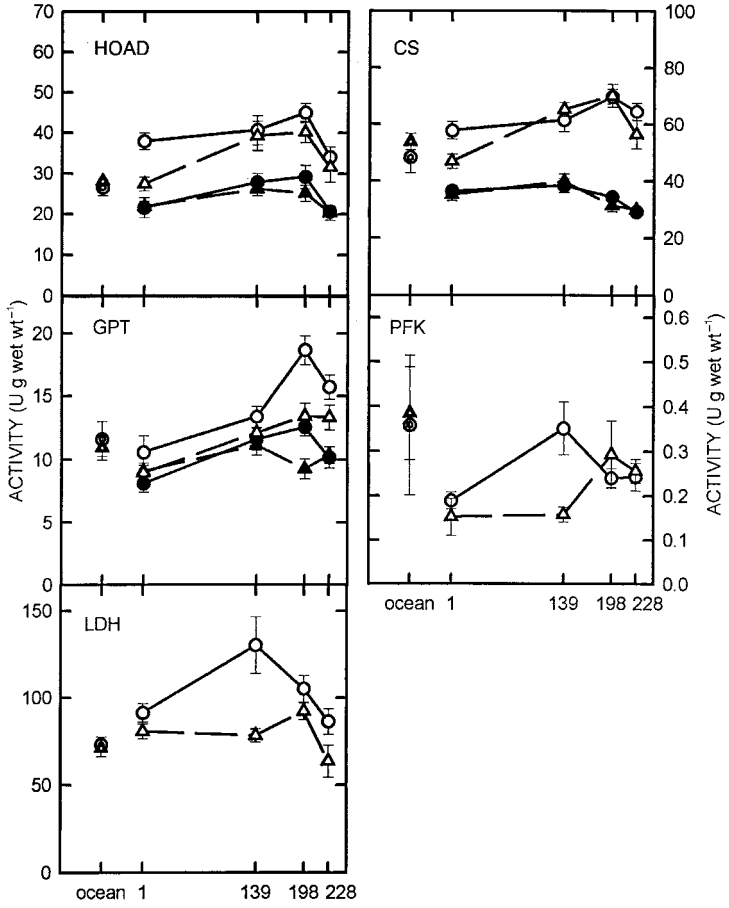

Figure 4. Enzyme activity (Ug wet weight^{-1}) and sampling site location for red muscle of adult American shad. β-hydroxyacyl coenzyme A dehydrogenase (HOAD); citrate synthase (CS); alanine aminotransferase (GPT); phosphofructokinase (PFK); lactate dehydrogenase (LDH). ● males 1993; ○ males 1994; ▲ females 1993; △ females 1994; ◎ oceanic males (1995); ◬ oceanic females (1995). Values are means ± 1 SEM. Significant results of ANCOVA on red muscle enzymes (significance at $\alpha = 0.05$) at riverine sites (km 1, 139, 198, 228) are as follows: GPT – length, location (a,bc,b,c), year, sex × length; LDH – length (ac,b,ab,c), sex × location; PFK – sex × location; HOAD – location (a,b,b,a), year; CS – location (a,b,b,a), year, location × year. Significant results of ANCOVA comparison (significance at $\alpha = 0.05$) of ocean and estuary samples (estuary '93, estuary '94, ocean '95) are as follows: GPT – length, group (a,b,ab); LDH – group (a,–,b); PFK – group (a,–,b); HOAD – group (a,b,a), sex × group; CS – group (a,b,a).

sampled during the late portion of the migration also had significantly lower (17%) GSI in both sexes. There were no differences between sampling years (Table 2).

White muscle enzymes demonstrated decreased activity in HOAD, LDH and GPT (males) while CS increased in the latter portions of the run in 1993, but not 1994 (Table 3). White muscle GPT decreased by 15% and HOAD by 19%. Sampling year was only a significant factor in GPT activity. Sex was a significant factor in CS, HOAD and GPT with males displaying generally higher levels of activity in all cases (length adjusted means). Length was a significant covariant for CS and HOAD.

Red muscle enzymes consistently showed a lower enzyme activity in four (CS, HOAD, GPT, PFK) of the five enzymes measured as migration progressed (Table 3). For example, GPT decreased by 30% and HOAD decreased by 27%. Enzyme activities in all three enzymes measured in both years (CS, HOAD, GPT) were significantly higher in both sexes in 1994 at all time points. Sex was a significant factor in PFK and LDH with females having higher enzyme activity (comparison of adjusted means). Length was a significant covariant for LDH and GPT.

Liver enzymes showed no clear pattern of change, however, time of sampling was significant in CS, HOAD, PFK and LDH (Table 3). Sampling years were

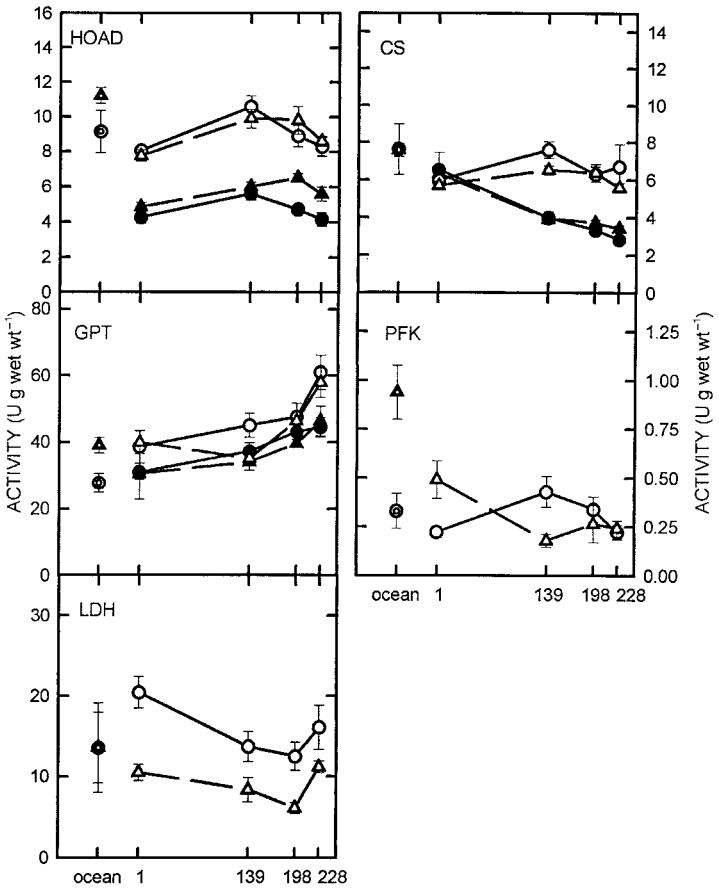

Figure 5. Enzyme activity (Ug wet weight^{-1}) and sampling site location for liver of adult American shad. β-hydroxyacyl coenzyme A dehydrogenase (HOAD); citrate synthase (CS); alanine aminotransferase (GPT); phosphofructokinase (PFK); lactate dehydrogenase (LDH). ● males 1993; ○ males 1994; ▲ females 1993; △ females 1994; ◎ oceanic males (1995); ◬ oceanic females (1995). Values are means ± 1 SEM. Significant results of ANCOVA on liver enzymes (significance at $\alpha = 0.05$) at riverine sites (km 1, 139, 198, 228) are as follows: GPT – length, location (a,a,b,c), year; LDH – location (a,bc,b,ac), sex; PFK – sex × location; HOAD – location (a,b,b,a), sex, year; CS – location(a,a,b,b), year, location × year. Significant results of ANCOVA comparison (significance at $\alpha = 0.05$) of ocean and estuary samples (estuary '93, estuary '94, ocean '95) are as follows: GPT – group (ab,a,b); PFK – sex; HOAD - group (a,b,c); CS – group (a,b,b).

significantly different for CS, HOAD, and GPT while the sexes differed in CS, HOAD, and LDH. Females showed higher CS and HOAD activity while males showed higher LDH activity when means adjusted for length were compared. Generally there was a tendency for enzyme activity to be higher in 1994 in the enzymes measured in both sampling years. Length was a significant covariant for CS, HOAD and GPT.

Total lipid decreased in white muscle and liver at the late sampling time (Table 2). Red muscle showed high total lipid levels at the mid-run sampling point. Sampling year was a significant factor in all three tissues and there were sex differences in liver and red muscle. Total protein increased in red muscle (Table 2), but showed little change in liver and white muscle. Protein content was consistently different between the sampling years in all three tissues. Plasma glucose was not statistically significantly different between sexes, years or sampling days (Table 2).

Fish ladder effects

For the experiment comparing fish sampled from the bottom and top of the Cabot Station fish ladder, there was no significant difference between the two locations in the following parameters: length, red muscle CS, HOAD, GPT, protein or lipid, white muscle CS, HOAD, GPT, protein or lipid, liver CS, HOAD, GPT, protein or lipid, plasma glucose (data not shown).

Table 3. Proximate composition (protein, lipid; mg/g wet mass), length (cm), gonadosomatic index (GSI,%) and plasma glucose (GLU; mM) for American shad captured at Cabot Station in Turners Falls, MA at three different times during the migratory period: early (E), mid-run (M) and late (L)

	A	1993 Males			1994 Males			1993 Females			1994 Females		
Time		E	M	L	E	M	L	E	M	L	E	M	L
N		13	11	11	11	12	9	10	9	8	10	8	9
length	s	41.9	42.5	40.6	40.2	39.2	40.6	46.9	46.6	46.2	47.6	46.4	45.0
		(0.3)[3]	(0.5)	(0.7)	(1.1)	(1.0)	(1.0)	(0.7)	(0.6)	(0.6)	(0.5)	(0.9)	(1.0)
total weight	t(a,ab, b)[2],s	987 (23)	1015 (47)	868 (50)	908 (69)	833 (62)	812 (69)	1533 (103)	1502 (92)	1449 (64)	1602 (50)	1484 (91)	1292 (115)
GSI	t(a,a,b),s	6.62 (0.19)	6.43 (0.34)	6.19 (0.28)	6.90 (0.40)	6.64 (0.30)	4.92 (0.34)	17.97 (1.48)	16.88 (0.99)	16.20 (0.76)	16.89 (1.24)	17.89 (1.18)	13.83 (1.67)
GLU	ts	8.7 (0.3)	8.4 (0.4)	8.1 (0.5)	8.6 (0.5)	8.9 (0.2)	8.5 (0.4)	7.9 (0.6)	7.8 (0.5)	9.8 (0.7)	8.7 (0.3)	8.6 (0.6)	9.5 (0.4)
liver lipid	t(a,b,b),s, y,ty	42.5 (4.8)	33.5 (5.4)	30.3 (3.4)	41.5 (2.6)	22.6 (2.6)	17.9 (4.4)	22.6 (3.2)	26.1 (7.0)	27.2 (4.9)	31.6 (1.7)	12.0 (2.4)	8.4 (0.8)
liver protein	s,y,ts, sy	178.0 (2.7)	201.0 (5.8)	193.5 (4.9)	147.4 (4.1)	129.3 (2.9)	123.9 (6.9)	162.7 (2.5)	174.9 (4.2)	183.2 (4.5)	132.6 (3.0)	128.5 (2.3)	128.1 (5.4)
red lipid	f,t(a,b,a), s,y, ty,sy	66.3 (3.5)	99.1 (12.2)	66.4 (8.2)	89.3 (7.9)	119.5 (9.0)	66.4 (7.2)	68.6 (5.4)	125.2 (7.4)	83.4 (6.8)	88.2 (8.8)	125.0 (9.9)	60.5 (5.8)
red protein	f,t(a,b,b), y,ty	168.0 (5.1)	174.6 (5.3)	185.7 (4.2)	133.4 (3.1)	144.7 (7.8)	132.2 (1.9)	156.9 (4.4)	168.3 (3.8)	183.7 (3.2)	121.6 (2.1)	140.6 (6.9)	139.3 (3.1)
white lipid	f,t(a,b,b), y,sy	72.1 (7.5)	49.9 (7.4)	47.3 (6.8)	54.9 (9.9)	47.9 (6.6)	49.5 (7.4)	102.9 (7.0)	82.4 (10.0)	76.1 (10.8)	60.1 (8.8)	48.5 (7.4)	49.5 (10.9)
white protein	f,t(a,b,a), y	195.9 (2.1)	208.8 (5.6)	197.3 (3.1)	140.5 (4.5)	142.4 (2.2)	134.5 (2.7)	183.4 (4.7)	206.5 (5.0)	189.5 (6.3)	118.2 (8.6)	133.8 (5.6)	138.1 (4.7)

[1]Column A indicates the results of analysis of covariance (for length and weight only, analysis of variance) and multiple comparisons analysis. '**y**' indicates a significant effect of year, '**s**' a significant effect of sex, '**t**' a significant effect of migration time, '**f**' a significant effect of length, '**ts**' a significant interaction between sex and time, '**sy**' a significant interaction between sex and year, '**ty**' a significant interaction between time and year ($\alpha = 0.05$); [2]parenthetical letters after '**t**' indicate the results of multiple comparisons testing for migration time (E, M, L respectively); [3]parenthetical numbers are 1 standard error of the mean (SE).

Discussion

Effects of migration distance

The pattern of activity of metabolic enzymes is generally different during the oceanic and riverine phases of the adult American shad life history and there are further modifications of activity during the course of migration. The activity of CS is increased in both red and white muscle after river entry indicating an increased aerobic capacity associated with migration. The higher activity is present at the mouth of the river, particularly in white muscle, suggesting that increases in activity may occur prior to river entry. There is an increase in CS activity during migration, perhaps in direct response to the increased locomotor activity of migration. Migration could be modelled as a prolonged exercise event given the extended periods of increased locomotor activity inherent in the behavior. Exercise training has been shown to increase CS activity in heart, red muscle and white muscle in rainbow trout, *Oncorhynchus mykiss* (Farrell et al. 1991) and in red muscle in coalfish, *Pollachius virens*, (Johnston and Moon 1980). The increase in red muscle capacity in American shad is of similar magnitude to exercised fish (~40%) and is likely a result of the increased aerobic needs of this tissue during migra-

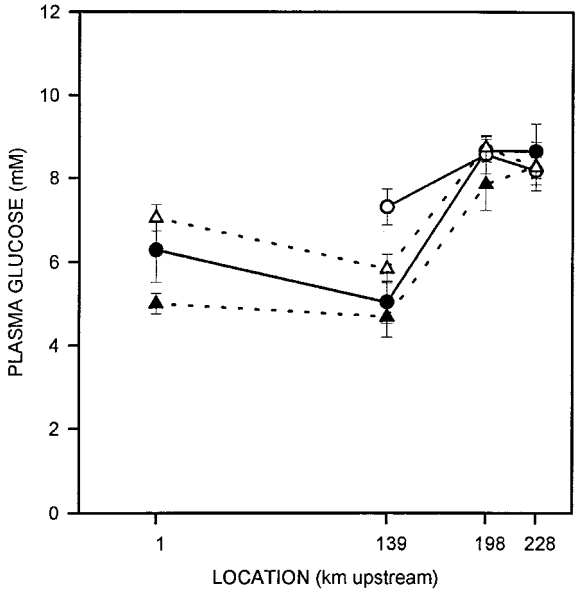

Figure 6. Plasma glucose and sampling site location for adult migratory American shad. ● males 1993; ○ males 1994; ▲ females 1993; △ females 1994. Values are means ± 1 standard error (S.E.) Statistical significance is indicated in Table 1.

tion given the tissue's function in sustained swimming. The increased activity in the white muscle of American shad suggests that there is also an increased need for aerobic energy production in this tissue during migration. This could be a result of the demands of oxygen debt repayment associated with burst swimming which may increase during riverine migration. It should be noted, however, that the experimental response of metabolic enzymes to training is not always of this magnitude (Davison 1989). Relatively large changes in metabolism can be met through regulation of enzyme activity rather than increases in enzyme concentration (Groen et al. 1982; Newsholme and Crabtree 1986). This suggests that changes in maximal enzyme activity that occur concurrently with exercise training, and perhaps migration, could be a result of factors other than energy demand itself.

The activity of the enzymes associated with liberation of stored energy forms (HOAD and GPT) is also increased over the course of migration in somatic muscle. It is interesting to note that these increases do not occur between the ocean and the mouth of the river, implying that these systems are not upregulated prior to river entry. It may be that these systems are only upregulated after the fish stop feeding upon river entry (Chittenden 1976) and begin to rely completely on stored lipid and protein to power their migration.

Changes in glycolytic activity are somewhat contradictory. LDH activity in both muscle types increased during the migration while PFK decreased in the white muscle. This suggests that the glycolytic pathway itself probably does not need to be upregulated during migration. The rise in PFK activity at the end of the migration may be a response to increases in plasma glucose at the upriver sites (Figure 6) or perhaps to undocumented changes in the enzyme's regulatory cofactors. The rise in glucose may be tied to increased stress toward the end of migration or with the attainment of a certain total energy expenditure (*i.e.*, distance travelled). It is possible that the rise results from the fact that the fish sampled at these two sites had just successfully ascended a fish ladder; however, we have shown that there is no difference in plasma glucose between fish captured at the bottom of a fish ladder and those sampled after passage. Therefore, it is more likely that the change in glucose is associated with migration distance and might be triggered by increased levels of cortisol produced during more stressful stages of the migration (Biron and Benfey 1994; Milligan 1996). Plasma cortisol levels and other indicators of stress have not yet been measured during the upriver migration in American shad.

The observed patterns of enzyme activity in migrating American shad are quite different from those described for upstream migrating sockeye salmon (*Oncorhynchus nerka*). Mommsen et al. (1980) demonstrated decreases in activity of white muscle LDH, CS and HOAD as well as liver GPT, HOAD, CS and LDH. Red muscle enzyme activities were largely unchanged in that species. Our results generally show an increase in aerobic enzyme activity. This increase is particularly striking in our study because we are comparing migration enzyme activities to levels seen in ocean captured animals. Generally, the pattern of sockeye salmon enzyme activity is more similar to that of an animal undergoing a starvation regime while American shad patterns are more characteristic of the results of extended exercise studies (Foster and Moon 1991; Johnson and Moon 1980). These metabolic differences between an iteroparous and a semelparous anadromous species may be crucial to our understanding of how these two strategies of migration evolved and under what constraints they currently exist.

In a number of the enzymes monitored, there is a tendency for a reversal of the riverine pattern at the most upriver sampling site (Vernon, VT). For example, in enzymes that tend to increase during migration (e.g., white muscle GPT and LDH), there is a drop in

activity at this site. Vernon Dam represents the most upriver location where large numbers of American shad are counted during the migration and is close to the putative historical extent of the range of this species (Bellows Falls, VT – km 280; Stevenson 1898; Moffitt et al. 1982). The changes in enzyme activity patterns may indicate a return to oceanic levels when fish near the end of their migratory journey. This suggests a modulation to oceanic metabolic organization prior to the actual return to the sea. This hypothesis is appealing given that downstream migration probably occurs rapidly relative to upstream migration and it may be advantageous to be prepared for oceanic life and resumption of feeding immediately upon seawater reentry. In Atlantic salmon, *Salmo salar* (Soengas et al. 1996), carp, *Cyprinus carpio* (Blasco et al. 1992) and roach, *Rutilus rutilus* (Mendez and Wieser 1993) metabolic recovery from starvation requires two weeks or more after the resumption of feeding. Post-spawning shad migrate up the Atlantic coast toward their summer feeding grounds in the Gulf of Maine, the Bay of Fundy and the Gulf of St. Lawrence following their return to the ocean (Newes and Depres 1979) and their capacity to reach these areas may be linked to the time needed to recover from their spawning migration, although we currently have no information on the metabolic state of these animals. It is important to recognize that the changes in muscle LDH and CS are different from those typically seen in starvation studies where the activity of these enzymes is decreased along with PFK (Foster and Moon 1991; Mendez and Wieset 1993), despite the fact that American shad do not feed during migration (Chittenden 1976). The decreases in CS, HOAD and GPT activity at the end of migration could be associated with the increases in glucose and PFK. The functional linkage of these associations is not clear since enzymes involved in aerobic and anaerobic metabolism, as well as stored energy liberation, are all being downregulated from peak migration levels. It is possible that the decreases in enzyme activities merely indicate the complete exhaustion of the fish at the migratory terminus, although this is somewhat difficult to support given the elevation of PFK activity. Clearly the modulations of enzyme activity occurring at the end of the migration are complex and will require further study to elucidate their functional significance.

The liver enzymes analyzed also varied with migration. With the exception of GPT, there is a generalized decrease in enzyme activity after river entry. Much of the work of the liver with respect to reproductive development (e.g., vitellogenin production) has already occurred prior to the fish entering the river since there is little increase in gonadosomatic index after river entry (Glebe and Leggett 1981). The decreasing liver enzyme activities may reflect a shift in organ priority with more energy being committed to the locomotory systems. The decrease in liver CS, LDH and HOAD activities are consistent with those seen in food deprivation studies (Foster and Moon 1991).

Year-to-year variation in enzyme activity is in some cases quite large. This variability is particularly evident in the red muscle and liver in HOAD and CS (Figures 3 and 4). In red muscle CS, for example, there is a 125% difference in activity between the two years sampled. There is no evidence to suggest that this is an effect of the assay procedures used since all tissues for a given year were analyzed at the same time and there is little year-to-year effect on white muscle activity. There are no obvious explanations for this effect since the variation in temperature between the years would probably not cause this pattern, based on temperature acclimation studies in a variety of other fish species (e.g., Guderley and Blier 1988; Pelletier et al. 1993). Further, if temperature were causing the differences between enzyme activities in different years, then there should be no difference in activity at km 1 (Old Lyme, CT) where the temperature was the same during the two sampling years. We suggest that there are other factors playing a role such as variation in flow regime or delays at river barriers which would change the total energy demand of the migration and might be reflected in varying enzyme capacity. River discharge was greater ($\sim$50%) during the period of American shad migration in 1994 than in 1993 (U.S. Geological Survey Montague City gauging station data, station #01170500). The higher flow may have necessitated greater expenditure of stored energy during the migration, as indicated by lower lipid levels in 1994. We suggest that this increased energetic requirement resulted in upregulation of some enzyme's capacities and may be the reason for the year-to-year differences seen in enzymatic activity.

The effect of annual variation makes it difficult to assess the importance of our data from the fish captured in the ocean since they were captured in a different year from the river migrants. We do not know the magnitude of enzyme activities in the river during 1995 and the ocean sampling site must therefore be assessed with caution. Further, it is unknown if the Connecticut River was the destination of the fish cap-

tured in the ocean. They were clearly going to make a spawning run into a river, as evidenced by their reproductive condition, however, and the Connecticut and Hudson Rivers are the most likely destinations for these fish, given the sampling date and the relative American shad populations of the northeastern rivers. Despite the limitations to this data, however, the oceanic site is considerably different from riverine sites (specifically the estuary) in both enzyme activity, including those enzymes that do not show great annual variability (e.g., white muscle CS, GPT), and in local lipid stores. These data highlight the necessity of sampling migratory fish before they begin their migratory run in order to fully appreciate the effect of migration on the physiology of the animal. Efforts must continue to be made to develop an understanding of the physiological differences between oceanic and freshwater migratory adult stages.

Scaling of physiological parameters with body size is a well documented occurrence (Somero and Childress 1980; Taylor et al. 1982; Goolish 1991). Metabolic enzymes in fishes are no exception, with aerobic mass-specific respiration rate decreasing with size while anaerobic effects are more equivocal (Somero and Childress 1980; Goolish 1991). In this study, there are effects of scaling (length) on several metabolic enzymes, including GPT and HOAD. Typically, the scaling that we have observed shows a similar directional effect to previous work (Goolish, 1991) where aerobic enzymes (GPT, HOAD) tend to decrease in activity with increasing size (if they are affected by size). Only two instances of scaling in anaerobic-linked enzymes were seen (white muscle LDH in upriver sampling and red muscle LDH in the migration timing study) and in both cases smaller fish had higher enzyme activity levels. That these effects are documented in individuals of roughly the same age (3–5) and at the same ontogenetic stage is unusual and results from the secondary sexual dimorphism of length of the species. We have found that shad exhibit sex effects based on this size dimorphism (e.g., liver and red muscle GPT) and sex effects that are independent of length (e.g., white muscle CS, liver HOAD) demonstrating the complexity of the differential sex-associated metabolic characteristics in this species. For example, in the upriver samples, the variation in unadjusted red muscle GPT is largely explained by length differences (multiple regression, $\beta = -0.48$, $p < 0.001$) although both year and sampling location are highly significant factors ($\beta_{\text{year}} = 0.37$, $p_{\text{year}} < 0.001$; $\beta_{\text{location}} = 0.15$, $p_{\text{location}} = 0.03$). Despite the close correlation between sex and length in this data set (significant correlation coefficient = 0.73), sex is not a significant factor. On the other hand, some of the enzymes measured are unaffected by length yet still show differences between the sexes, as is the case for white muscle CS sampled at different riverine sampling sites. The greatest explanatory power of white muscle CS variation is sex (multiple regression, $\beta = -0.37$, $p = 0.001$) while year ($\beta = -0.16$, $p = 0.02$) and sampling location ($\beta = -0.20$, $p = 0.02$) are also significant. Since the goal of our study has been to investigate metabolic changes occurring during the migratory period, we have tried to minimize the effects of scaling in our analyses by using statistical techniques which adjust for length. It is important to remember that scaling effects can be substantial, however, when evaluating raw enzyme activity data. Given this caveat, however, most of the tissue enzymes measured in this study do not show a significant length scaling effect (Table 2, Figures 3–5) suggesting that the size range sampled is not large enough to demonstrate scaling effects or that other factors changing during this life history stage are much greater than scaling effects.

Migration time effects

The timing of seasonal animal migration within a year is usually tied to changes in the environment and resource availability, which impact potential reproductive success (Dingle 1996). The migration of animals is, however, often temporally extended and individuals may embark on their migrations at different times. In birds, this may mean that juveniles leave the summer feeding grounds at a different time than adults (Dingle 1996) while in anadromous fish species there are different groups of individuals entering a river throughout the period of migration (Glebe and Leggett 1981a). There may be population level advantages to the temporal dispersion of migration within the limits of the migratory season, including preventing short-term catastrophic events from impacting the entire migratory population and facilitating an extended hatching period. Individuals may also be predisposed to greater success under differing migration conditions given a high level of individual variation frequently. If this is the case, there may be several different periods within the migratory season that are selected by individuals that lead to increased migratory success.

In this study, the timing of migration with respect to temperature varied between the two sampling years

(Figure 1). 1994 was characterized by an increase in river temperature in mid-May followed by a brief period of stable temperature followed by increasing temperatures. The shad migration was fairly typical during 1994 with early migrants arriving at relatively cool temperatures (13–14 °C) and migration coming to a close during the final, rapidly warming period (18–20 °C). 1993 was somewhat different, however. The river increased in temperature early (mid-late April) such that temperatures were already at 16 °C for the earliest migrants through the Holyoke Dam facility (km 139). Conversely, Cabot Station was not operated until May 17 in 1994 so that the earliest fish through Holyoke Dam were likely delayed in their migration at Cabot Station. Although our sampling points in both years took place approximately one week after the Cabot Station ladder opening, the 1994 early sampling point probably contained a lower proportion of actual early migrants than the 1993 sampling point because the delays in 1994 allowed later migrants to catch up with early fish below Cabot Station. While this has the potential of confounding our between year comparisons, it demonstrates the occurrence of migratory delay in this species and highlights the year to year variability in river conditions that the animals are forced to deal with.

The decrease in enzyme activity level over the course of our sampling indicates a decreased metabolic capacity, at least in the somatic muscle, as the migratory period progresses. The decrease is primarily in aerobic capacity and in the capacity to access stored lipid and protein. In red muscle, CS, HOAD, GPT and PFK decreased indicating that capacity for aerobic and anaerobic metabolism may be decreasing, suggesting that the late migrant fish may be more metabolically challenged than earlier migrants. Similarly in white muscle, there is a decrease in HOAD and GPT activity, indicating a decreased ability to mobilize local lipid and amino acid stores in later migrants. It is interesting to note that there is no change in white muscle PFK over time suggesting that the anaerobic burst swimming associated with migration is not overtaxing the anaerobic machinery in this tissue. It will be necessary to document other parameters in the glycolytic chain, however, to fully test this hypothesis. The changes in white muscle CS are, however, equivocal. In 1994 there appears to be no increased demand for aerobic capacity while late in migration in 1993 there does seem to be a need to increase white muscle aerobic capacity. This would presumably be a response to the demands of repaying oxygen debt incurred by anaerobic activity (Goolish 1991).

It is possible that the observed enzyme variation over time could be an effect of different acclimation temperatures when the enzymes are assayed at a constant temperatures. However, this seems unlikely given that the temperature changes seen by migratory shad are frequently very rapid and it is unlikely that the fish become acclimated to any given temperature. If the groups of shad sampled in this study actually are distinct groups of fish that come into the river at different times, however, the potential for acclimation to different ocean or estuarine temperatures would exist. Five of our 6 sampling periods (early, peak and late 1993, and early and peak 1994) all occurred at 17–18 °C which suggests that temperature variability is probably not the primary cause of the temporal variability in the enzymes sampled and that they may be more closely linked to locally stored substrates. If the temperature changes were responsible for the enzyme variation seen in this study, the patterns of enzyme change should more closely follow those of river temperature.

Sex differences in enzyme activity reveal several patterns. Females have a greater aerobic capacity in the liver and more glycolytic capacity in red muscle than males. The liver in females may still be producing, or geared to produce, vitellogenin which would require greater energy resources than in males. The need for higher glycolytic capacity for red muscle in females is unclear. The greater aerobic potential in white muscle in males over that seen in females suggests that males may have a greater need to support oxygen debt repayment than females, probably due to greater lactate accumulation. This would suggest that males are spending more time burst swimming during their migration or that they are more adapted to the metabolism of lactate in general.

In both the liver and red muscle, there are considerable year-to-year differences in enzyme activity. The differences in enzyme activity correspond well with stored lipid and protein. Generally in 1994 the enzyme activities are high and the energy stores are low; in 1993 the enzyme activities are low and the energy stores are higher. This is particularly true of HOAD and GPT, suggesting that lower stores are the result of an upregulation of the mobilization of energy.

This study demonstrates that remodeling of the metabolic enzyme systems does occur in American shad during riverine migration. For aerobic capacity (CS), glycolytic capacity (LDH) and utilization

of stored lipid and protein energy (HOAD and GPT) there is a general pattern as the fish moved upstream of increased activity with a subsequent decline at the most upriver locations. Such a remodeling may be important to ultimate migratory success and demonstrates a responsiveness of the metabolic system to the changing demands of migratory behavior. Our work also suggests a preparatory ability by American shad to change enzyme maximal capacity prior to migration into the river and prior to returning to the sea which may increase the lifetime reproductive success of these iteroparous, anadromous fish.

There are differences in metabolic capacity, both aerobic and anaerobic, that occur in American shad captured at different periods of their migration. Whether these differences demonstrate a differentiation between groups of migrant fish or reflect a response to varying conditions during the migratory period is unknown. We do know, however, that survival is higher in fish migrating in the early and middle portions of the run (Shoubridge and Leggett 1978) and this may confer an increase in reproductive success for these animals if they are able to spawn again in subsequent years.

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