Effects of migration distance on whole-body and tissue-specific energy use in American shad (*Alosa sapidissima*)

Jill B.K. Leonard and Stephen D. McCormick

Abstract: We examined total and tissue-specific energy content of upstream-migrating American shad (*Alosa sapidissima*) in the Connecticut River. Total energy depletion over the course of the 228-km migration ranged from 35 to 60%. The approximate contributions of different tissues to energy use during migration were white muscle 57%, subdermal fat 27%, red muscle 8%, viscera 6%, and liver 2%. American shad preferentially use energy stores in the skin and its subdermal fat layer (depleted by 63%) while sparing red muscle protein. Both lipid and protein were used as energy sources throughout migration, although lipids were depleted to a greater extent (e.g., white muscle lipid decreased 48% and protein 30%). Large fish expended 2–21% more energy during migration than small fish. Migrating to upriver sites (198–228 km) is 50–100% more energetically expensive than to lower river sections for females. This suggests that upriver range expansion may be limited by females in that they may have reached a threshold level of energy expenditure in this upriver area. American shad may possess physiological mechanisms for tissue-specific energy use allowing maintenance of critical tissues necessary for postspawning survival.

Résumé : Nous avons étudié les réserves énergétiques totales et de divers tissus chez l'alose savoureuse (*Alosa sapidissima*) en montaison dans le fleuve Connecticut. La baisse totale des réserves énergétiques au cours de cette migration de 228 km variait de 35 à 60%. Les contributions approximatives des différents tissus à la dépense d'énergie durant la migration étaient les suivantes : muscles blancs 57%, graisse sous-dermique 27%, muscles rouges 8%, viscères 6% et foie 2%. L'alose savoureuse utilise préférentiellement les réserves énergétiques de la peau et de la couche de graisse sous-dermique (appauvrie de 63%) et économise les protéines de ses muscles rouges. Des lipides et des protéines ont été utilisés comme sources d'énergie durant la migration, mais ce sont les teneurs en lipides qui ont le plus diminué (p. ex., les teneurs des muscles blancs en lipides ont baissé de 48%, tandis que pour les protéines, la diminution est de 30%). Les gros poissons ont dépensé 2 à 21% plus d'énergie que les petits poissons durant la migration. La migration vers les sites d'amont (198–228 km) est 50 à 100% plus coûteuse énergétiquement que celle vers les secteurs du cours inférieur pour les femelles. Cela laisse penser que l'expansion de l'aire de l'espèce plus en amont pourrait être limitée par les femelles, qui pourraient avoir atteint leur plafond de dépense énergétique. L'alose savoureuse pourrait disposer de mécanismes physiologiques lui permettant d'utiliser sélectivement les réserves énergétiques de certains tissus et assurant ainsi la protection de tissus critiques pour la survie post-fraye.

[Traduit par la Rédaction]

Introduction

It is commonly believed that fish migration is energetically costly given the locomotor demands of long-distance travel (Gross et al. 1988; Roff 1988; Dingle 1996). Therefore, energy availability has the potential to be a limiting factor in migration, particularly in those species that do not feed during migration. There have been numerous studies describing the energetic costs of migration in fish (e.g., Idler and Bitners 1958, 1959; Glebe and Leggett 1981*a*, 1981*b*; Crawford et al. 1986; Lambert and Dodson 1990*a*; Reid et al. 1993; Hatano et al. 1995; Jorgensen et al. 1997). Most of these studies have concentrated on whole-body energy use over the course of migration or have described an in-depth examination of energy usage in a single tissue. These approaches provide limited insight into any potentially adaptive physiological mechanisms of energy utilization that may result from differential liberation of energy from different tissues or storage forms throughout the animal. Further, most of this work, with the exception of Glebe and Leggett (1981*a*, 1981*b*), Crawford et al. (1986), and Lambert and Dodson (1990*a*), has focused on salmonids, particularly semelparous Pacific species. These fish do not need to conserve stored energy in order to power the return downstream migration as iteroparous fishes presumably must. Lambert and Dodson (1990*a*, 1990*b*) have demonstrated correlations between costs of migration, overwintering condition, postspawning survival, and lifetime fecundity in iteroparous coregonines (cisco (*Coregonus artedi*) and lake whitefish (*Coregonus clupeaformis*)). More recently, Jonsson et al. (1997) have evaluated energy use in Atlantic salmon (*Salmo salar*) and have shown that iteroparous populations can sus-

Received June 25, 1998. Accepted March 4, 1999.
J14669

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tain energetic losses of 60–70% during upstream migration. Berg et al. (1998) have also demonstrated differences in energy accumulation and survivorship between virgin and repeat-spawning brown trout (*Salmo trutta*), suggesting an impact of energetic constraints on repeat-spawning ability. However, physiological mechanisms for tissue-specific energy conservation by iteroparous fishes remain unexplored.

The American shad (*Alosa sapidissima*) is an abundant, anadromous clupeid ranging from Florida to Atlantic Canada. American shad display a clinal variation in postspawning survival from completely semelparous populations in the south to largely iteroparous populations in the north (Glebe and Leggett 1981*b*). In the Connecticut River, American shad are iteroparous, with about 30–40% of returning fish having spawned in a previous year (Glebe and Leggett 1981*b*). In the Connecticut River, spawning occurs in May and June at water temperatures between 10 and 22°C. While this is an apparently large thermal window, May–June is a period of rapid temperature increase in the river, with migration and spawning lasting only 6–8 weeks each year. Fish migrate as much as 300 km upriver during this period and may spawn several times during this journey. Further, American shad have been impacted by human modification of rivers throughout their range, leading to reduced populations in many river systems, although restoration efforts are currently underway in some of these rivers (Rulifson 1994; St. Pierre 1994). Mitigation efforts frequently include fish ladders that permit passage past dams. In the Connecticut River, American shad pass through as many as four mainstem hydroelectric power dams, each of which is equipped with a fish passage facility (one fish elevator, three fish ladders of varied design).

There has been considerable work addressing energy use in migrating American shad in previous studies. Notably, Leggett and Trump (1978) and Glebe and Leggett (1981*a*, 1981*b*) have provided a groundwork for our research. We have chosen to expand upon these studies for several reasons. Firstly, it is unknown whether there are mechanisms available to American shad by which they can spare certain tissue energy sources while still utilizing others during upstream migration. Tissues where energy sparing occurs could presumably be used to power downstream migration and (or) enhance recovery from migration while in the ocean. Knowledge of these mechanisms is critical to understanding energy management in migratory fishes, particularly iteroparous species. Secondly, the work of Leggett and collaborators was done in the 1970's at a period when shad were not successfully passing over the first hydroelectric power dam (Holyoke Dam, km 139). Fish now migrate past a facility 280 km (Bellows Falls Dam) from the mouth of the river, with 42–63% of the population migrating above the Holyoke Dam in recent years (data from 1990–1997, Connecticut River Coordinators Office, personal communication). We wish to understand whether energy use has been altered since the installation of fish passage facilities, whether fish traveling far up the river may be unable to successfully return to the ocean because of excessive energy depletion, or if other factors are involved in the upstream range expansion of this population.

In this study, we document differential use of energy derived from different substrates and energy storage areas. We also examine the impact of migration distance, fish size, and sex on total and tissue-specific energy use. This allows us to assess the overall cost of migration in American shad and to gain an understanding of how the increased metabolic costs of migration are apportioned between different tissues and energy supplies.

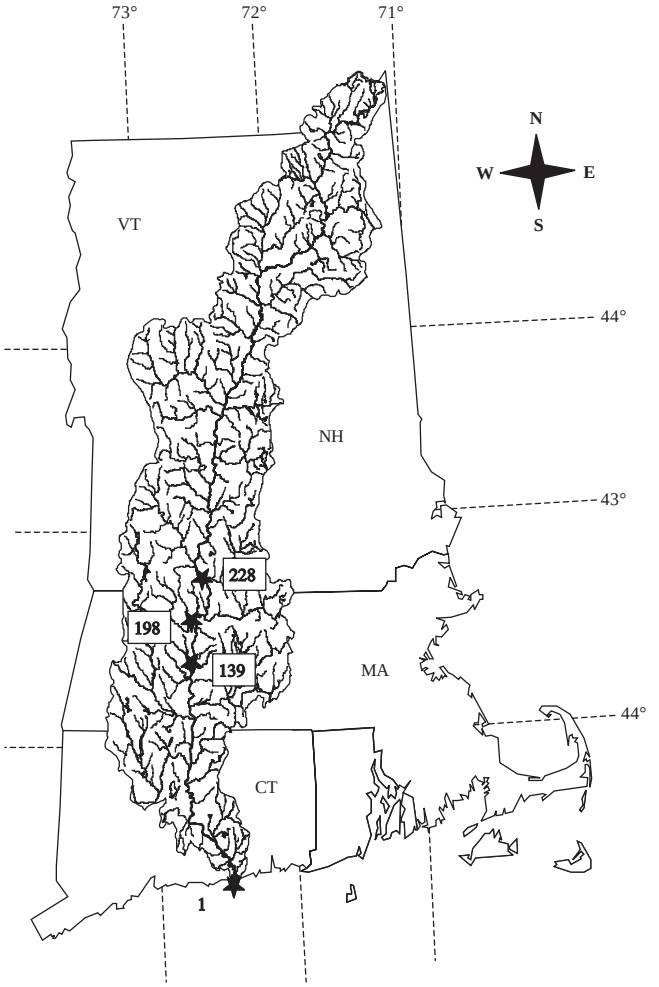


Fig. 1. Map showing the Connecticut River drainage area including the four river sampling sites (stars) for American shad located at km 1 (Old Lyme, Conn.), km 139 (Holyoke, Mass.), km 198 (Turners Falls, Mass.), and km 228 (Vernon, Vt.).

Materials and methods

Fish

Adult migratory American shad were sampled at four sites on the Connecticut River (Old Lyme, Conn. (km 1), Holyoke Dam, Holyoke, Mass. (km 139), Cabot Station, Turners Falls, Mass. (km 198), and Vernon Dam, Vernon, Vt. (km 228)), during spring in 1993 and 1994 (Fig. 1). Efforts were made to sample fish within 1 week of the arrival of the first migratory cohort at each location to minimize the confounding effects of variable amounts of time spent in the river prior to sampling. It is difficult to know precisely when fish first enter the estuary, and fish ladders are not necessarily operated with the arrival of the fish, but rather in compliance with federal regulations based on river discharge. Therefore, there may be some variation that we could not control in our sampling time relative to the arrival of cohorts at each location. American

shad were also sampled at an ocean site off Barnagut Light, N.J., in the spring of 1995. At the ocean site and at Old Lyme (km 1), fish were captured using 14- and 15-cm stretch mesh gill nets. At other sites, fish were captured at fish-trapping facilities built into fish passageways at hydroelectric dams. At each location, about 10 males and 10 females were sampled.

Fish were removed from the net or trap and immediately anesthetized with tricaine methanesulfonate (MS 222) at 50 $mg \cdot L^{-1}$ (buffered with 0.24 mM $NaHCO_3$ and adjusted to pH 7.0 with NaOH). Fish were bled from the caudal vessels using heparinized syringes and then placed on ice until tissue samples could be removed (<2 h). Gonad, liver, and remaining viscera were removed, weighed, and frozen at –80°C. The remaining body was then frozen and later sliced to produce a series of cross sections posterior to the head. Each cross section was then dissected on the left side to separate white muscle, red muscle, and the skin including the subdermal fat layer (SDF). This resulted in all the somatic tissue from one half of the carcass being separated by tissue type. For each fish, all left-side red muscle, white muscle, and skin/SDF were weighed and frozen separately. Samples of red and white muscle tissue were also taken from the right side of the body anterior to the dorsal fin for determination of muscle moisture content by drying to a constant weight at 60°C for 24 h.

Proximate analysis

Frozen gonad, liver, red and white muscle, and skin/SDF were homogenized with one volume of deionized water. The resulting homogenates were divided into aliquots for total lipid, total protein, and glycogen determination. Total lipid aliquots were extracted immediately, while protein and glycogen aliquots were frozen for later assay. Total lipid was determined using the protocol of Sheridan et al. (1983). Lipids were extracted from the homogenate (1 mL) using 20 volumes of 1:1 chloroform–methanol (Folch et al. 1957). Extracted lipid was spectrophotometrically determined using extracted and gravimetrically quantified cod liver oil as a standard (Frings et al. 1972). The aliquot for protein analysis was thawed, resuspended, and sonicated (two 5-s bursts) in 0.1 N NaOH (1:20 w/v). Protein was then quantified using the methods of Lowry et al. (1951) with bovine serum albumin as a standard. Glycogen was estimated in thawed aliquots after resuspension in 100 mM sodium citrate buffer (pH 5.0, 1:10 w/v) using the methods of Carr and Neff (1984) and Busacker et al. (1990). Samples were digested with amyloglucosidase (125 $\mu g \cdot mL$ $homogenate^{-1}$) for 2 h at 55°C and compared with undigested controls. Glucose in digested and undigested samples (glucose control) was assayed in a spectrophotometer using the glucose oxidase – *o*-dianisidine – peroxidase reaction (Raabo and Terkildsen 1960).

All values of mass of lipid, protein, and glycogen were transformed into their energetic content using the energetic equivalents of 35.52 $kJ \cdot g^{-1}$ for extracted lipid and 23.63 $kJ \cdot g^{-1}$ for total protein (Craig et al. 1978) and 17.2 $kJ \cdot g^{-1}$ for glycogen (Brafield 1985). Note that the conversion factor used for lipid is lower than is typical of many earlier studies because Craig et al. (1978) suggested that this is a more realistic conversion factor for samples extracted using the chloroform–methanol method employed in this study.

In order to obtain estimates of total tissue and total body energy content, caloric equivalents (kilojoules per gram) of protein, lipids, and glycogen from each tissue aliquot were extrapolated to the total mass of the tissue (in the cases of white and red muscle and skin/SDF, this value was doubled to account for the unsampled tissue on the right side of the fish). Total body content was then calculated as the total of all the tissue contents (white muscle, red muscle, skin/SDF, gonad, liver, remaining viscera). Note that this method of calculating total body energy content does not include the head, spinal column and ribs, or fins. The reported values are therefore underestimates of true content, although it is unlikely that energy can be easily mobilized from these excluded areas of the body.

Gonadosomatic index (GSI) was calculated as gonad mass/(total mass – gonad mass) × 100.

Statistics

Data were analyzed using the general linear models analysis of covariance (ANCOVA) with a three-way classification with fish fork length as the covariate using the SAS statistical package (SAS Institute Inc., Cary, N.C.) at $\alpha = 0.05$ followed by least squares means comparisons (as needed). Use of the length covariate was chosen because American shad display a sexual size dimorphism where spawning males are consistently smaller than same-age females. This analytic technique generates adjusted means for the tested groups based on the length regressions for all fish (either all within-river individuals or the estuary/km 1 groups) sampled including all sites, sexes, and years. These adjusted means were then tested for homogeneity in a manner similar to that employed by analysis of variance (ANOVA). The primary assumption of this analysis is that the slopes of the regressions of a measured variable on length for all site–sex–year combinations are homogeneous. Other test assumptions include significance of the relevant regressions and linearity of the response as well as ANOVA assumptions of randomness, homoscedasticity, and normality (Snedecor and Cochran 1989; Sokal and Rohlf 1995). All these assumptions were satisfactorily met in this study.

Actual values of energy content for fish captured at the different locations are presented as well as the least squares means corrected for length (adjusted means). All statistics presented take variation in fish size into account (Tables 1 and 2). Our intent is to clarify our data with respect to the sexual length dimorphism while still detailing the absolute costs of migration in the species.

Results

Length differed between males and females at all sites except the ocean sampling location (Table 1). There was a significant decrease in the size of females from both years and males from 1994 at the most upriver site.

Total energy was depleted by 35–61% from estuarine levels over the course of the 228-km upriver migration and differed significantly between the sexes and between years (Table 1; Fig. 2). Total energy was also significantly higher in the ocean (1995) than in the estuary (Old Lyme, km 1) in 1993 and 1994 (Table 1).

In white muscle, both lipid and protein were utilized over the course of migration in both sexes (Figs. 3 and 4; Table 1). Overall, lipid-based energy was used more extensively (48% depletion over 228 km for length-corrected means) than protein-based energy (30% depletion). Both lipid-derived energy and protein-derived energy were utilized from the earliest portions of the migration. As expected, relatively more energy (26% calculated over all river sites) is available in female white muscle than in male white muscle given the generally larger size of females. When size is taken into account, however, there was no difference in white muscle energy at a given site between the sexes. Levels of energy available from lipid in the ocean were higher than levels seen in the estuary in 1993 and 1994. Oceanic protein-derived energy content of white muscle was higher than the estuary samples in 1993 but not in 1994. Greater energy depletion occurred in 1994 than in 1993.

Table 1. Mean absolute energy values (kJ) for muscle and cumulative energy content in American shad captured at four river locations, estuary (km 1), Holyoke Dam (km 139), Cabot Station (km 198), and Vernon Dam (km 228), and at an oceanic site, Barnagut Light, N.J.

	A	B	1995 males	1995 females	1993 males			
Site (km)			Ocean	Ocean	1	139	198	228
N			7	11	10	10	13	13
Length	s, l (a, a, b, c) sy, ly	s′, gs′	45.6 (0.7)	46.9 (0.5)	43.2 (0.4)	42.7 (0.4)	42.0 (0.4)	43.0 (0.3)
Total mass	l (a, b, c, d), s, sy	g (ab, a, b), s′, sg	1 550 (40)	1 678 (54)	1219 (38)	1103 (44)	987 (23)	1028 (39)
Total	f, l (a, b, b, c), y, sy, ly	f′, g (a, b, b)	11 807 (471)	12 327 (899)	8316 (651)	7502 (703)	5264 (474)	4959 (346)
White total	f, l (a, a, b, c), y, sy, ly	f′, g (a, b, b)	7 516 (377)	7 907 (681)	4916 (411)	5252 (469)	3471 (350)	3064 (214)
White protein	f, l (a, b, c, d), y, sl, ly	f′, g (a, b, a)	3 027 (152)	3 085 (107)	2087 (1060)	2211 (129)	1645 (151)	1835 (92)
White lipid	f, l (a, a, b, c), y, sy, ly	f′, g (a, b, b)	4 489 (304)	4 822 (587)	2830 (320)	3042 (357)	1826 (360)	1229 (202)
White glycogen			0.7 (0.1)	0.8 (0.0)	0.0 (0.0)	0.0 (0.0)	0.4 (0.3)	0.0 (0.0)
White % H_2O	l (a, b, b, b)	g (a, b, b)	52.7 (4.5)	52.5 (3.6)	65 (1)	67 (2)	71 (1)	71 (2)
Red total	f, l (a, bc, ab, c), y, sy, ly	f′, g (a, b, a)	718 (31)	733 (51)	847 (95)	794 (59)	643 (60)	585 (46)
Red protein	f, s, y, sy	f′	315 (15)	332 (21)	290 (24)	307 (18)	248 (21)	247 (13)
Red lipid	f, l (a, bc, a, c), y, sy, ly	f′, g (a, b, a)	402 (20)	402 (34)	557 (62)	487 (44)	395 (26)	338 (36)
Red glycogen		f′, s′	0.1 (0.0)	0.1 (0.0)	0.2 (0.1)	0.0 (0.0)	0.0 (0.0)	
Red % H_2O	l (a, b, c, b), y, ly	g (a, b, c)	28.4 (1.4)	28.3 (1.3)	89 (1)	69 (3)	67 (1)	68 (1)

Note: Also included are data for fork length (cm), total wet mass (g), and muscle moisture content (% mass). Parenthetical numbers are 1 SE of the mean. Column A indicates the results of ANCOVA (for length only, ANOVA) and multiple comparisons analysis of riverine data; 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, "gs" 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 ANCOVA ($\alpha = 0.05$). Parenthetical letters after "l" and "g" indicate the results of multiple comparisons testing for riverine location (km 0, km 139, km 198, and km 228, respectively) and ocean–estuary groups (ocean, estuary 1993, and estuary 1994, respectively).

White muscle moisture increased significantly after American shad entered freshwater.

Utilization of lipid-based energy (32%), but not protein, is seen in red muscle (Figs. 3 and 4; Table 1). There was no difference between the ocean site and the estuary in protein-based energy of red muscle, and the difference between ocean and estuary lipid-based energy was only significant in 1993. There was a significant effect of sampling sex and year on energy utilized from protein but not energy from lipid. Red muscle energy content was length dependent. Sex was only a significant factor in riverine lipid content depletion. Red muscle energy content was initially lower and more rapidly used in 1994 than in 1993. Red muscle moisture increased sharply after American shad entered freshwa-

Table 1 (*concluded*).

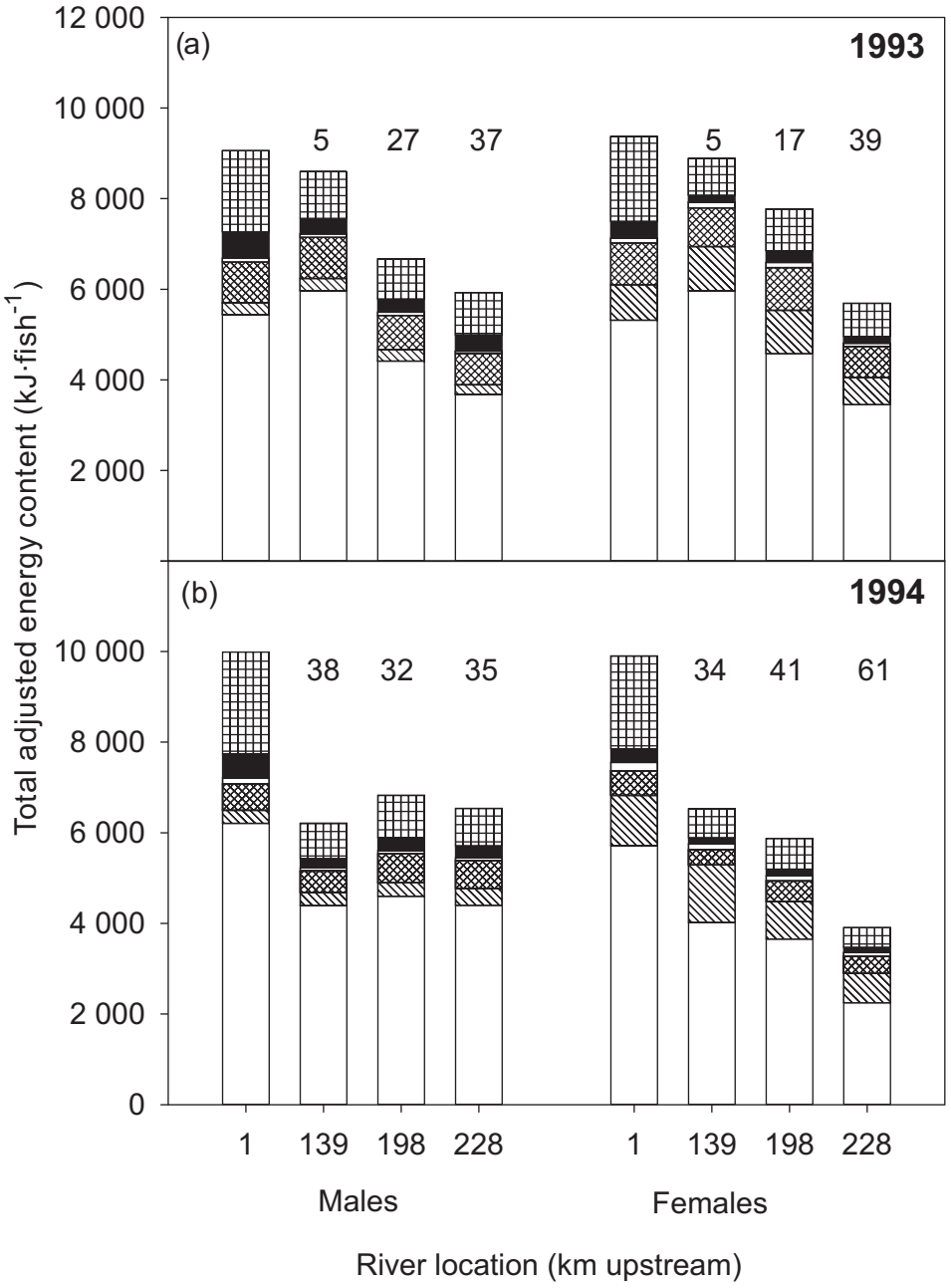
1994 males				1993 females				1994 females			
1	139	198	228	1	139	198	228	1	139	198	228
8	9	11	11	18	10	10	7	11	11	10	10
44.5	43.4	40.2	37.6	48.3	48.0	46.9	45.6	49.8	48.5	47.6	45.5
(0.9)	(1.0)	(1.1)	(0.7)	(0.6)	(0.7)	(0.7)	(0.7)	(0.8)	(0.5)	(0.5)	(0.7)
1445	1151	908	679	1 781	1 660	1533	1269	2 088	1775	1602	1285
(104)	(73)	(69)	(51)	(60)	(70)	(103)	(82)	(89)	(61)	(50)	(73)
9818	5503	4485	2931	11 167	10 531	8836	6405	12 461	8441	7328	4280
(1213)	(471)	(489)	(388)	(585)	(1042)	(1197)	(817)	(572)	(441)	(343)	(552)
6097	3928	3073	2058	6 477	7 037	5274	4015	7 389	5267	4603	2493
(682)	(334)	(375)	(275)	(445)	(732)	(869)	(549)	(461)	(275)	(254)	(491)
3010	2217	1712	1321	3 087	2 928	2319	1886	3 627	2927	2666	1597
(226)	(187)	(155)	(134)	(155)	(229)	(233)	(205)	(203)	(129)	(94)	(266)
3087	1711	1360	737	3 390	4 109	2955	2129	3 762	2340	1937	895
(471)	(183)	(248)	(147)	(318)	(534)	(668)	(355)	(276)	(159)	(185)	(258)
0.2	0.1	0.1	0.0	0.6	0.1	0.1	0.0	0.2	0.0	0.4	0.1
(0.1)	(0.0)	(0.0)	(0.0)	(0.6)	(0.0)	(0.1)	(0.0)	(0.1)	(0.0)	(0.2)	(0.1)
64	71	67	65	65	70	67	69	64	69	68	72
(2)	(1)	(1)	(7)	(1)	(2)	(1)	(1)	(1)	(2)	(1)	(1)
567	405	401	234	1 100	1 020	1051	716	799	526	602	423
(56)	(42)	(49)	(29)	(80)	(111)	(113)	(64)	(44)	(36)	(34)	(45)
279	226	205	128	386	383	375	318	360	292	313	235
(27)	(18)	(30)	(14)	(25)	(41)	(42)	(23)	(26)	(18)	(17)	(20)
287	178	196	106	714	637	676	398	437	234	289	188
(36)	(29)	(26)	(16)	(59)	(74)	(72)	(45)	(26)	(22)	(20)	(27)
0.7	0.1	0.0	0.0	0.3	0.1	0.2	0.1	2.6	0.1	0.1	0.0
(0.2)	(0.1)	(0.0)	(0.0)	(0.1)	(0.0)	(0.1)	(0.0)	(2.3)	(0.1)	(0.1)	(0.0)
61	67	63	66	90	66	64	69	59	67	64	68
(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)	(1)

ter. After river entry, a generalized increase was seen at more upriver sites, although moisture content was extremely high in both sexes in 1993 in the estuary.

In the liver, both lipid-based energy and protein-based energy are utilized (76 and 38%, respectively) with lipid providing a greater proportion of the fuel for migration (Fig. 3; Table 2). The liver in general contributes a proportion of its available energy similar to that of red and white muscle; however, because of its small mass, it only accounts for about 2% of the energy consumed during migration (Fig. 3). Stored, lipid-based energy was significantly higher in the ocean fish than in the estuary samples. Fork length and sex were both significant factors in total liver energy use, primarily because of the effect of length and sex on female liver protein.

Visceral stores of both lipid-based energy and protein-based energy were depleted (57 and 34%, respectively) during migration (Fig. 3; Table 2), with more energy being pro-

Fig. 2. Total adjusted mean energy content for male and female adult American shad captured at four sites (km 1, km 139, km 198, and km 228) in the Connecticut River in (*a*) 1993 and (*b*) 1994. Bars indicate the contribution from white muscle (open), gonad (diagonal hatch), red muscle (cross-hatch), liver (horizontal hatch), viscera (solid), and skin (squares) at each site. Values above bars indicate the percent depletion of total energy at that site relative to total content of fish captured at km 1 based on adjusted means.



vided by lipid than by protein in both sexes. Much of this depletion occurred during the first phases of migration between the estuary and the first dam (km 139). Visceral energy was also significantly affected by both fork length and sex, primarily because males have higher visceral stores than females.

GSI was higher in females than in males except in the ocean (Table 2). There was a small increase in GSI from the estuary to Holyoke Dam (km 139). Thereafter, there was little change in GSI as the fish moved upriver until they reached the most upriver site where there was a significant decrease. Gonads had decreased lipid- and protein-based energy in both sexes over the course of migration (Table 2). Gonad energy content differed with sex (female > male), fork length (large > small), and year (1994 > 1993).

Protein could not be measured in raw skin/SDF homogenates because of the high lipid content in the tissue. The portion of tissue unhomogenized was largely the skin itself (dermis and epidermis), which is unlikely to be mobilized as an energy source during migration, given its relative insolubility (Whitear 1984). Lipid-based energy was extremely high on a per gram basis, with skin/SDF lipid providing about 33% of the total energy available from total body lipid (Table 2). Only white muscle has a larger lipid store.

Fig. 3. Adjusted mean tissue energy content for migrating (*a*) male and (*b*) female adult American shad in 1993 sampled in (1) the estuary (km 1), (2) Holyoke Dam (km 139), (3) Cabot Station (km 198), and (4) Vernon Dam (km 228). Bars indicate energy from protein (solid) and lipid (open). Values above the bar sets indicate the percent total energy depletion for the indicated tissue between km 1 and km 228 based on adjusted means. Values in parentheses indicate the percent contribution of the indicated tissue to the whole-body energy depletion between km 1 and km 228 for the groups shown.

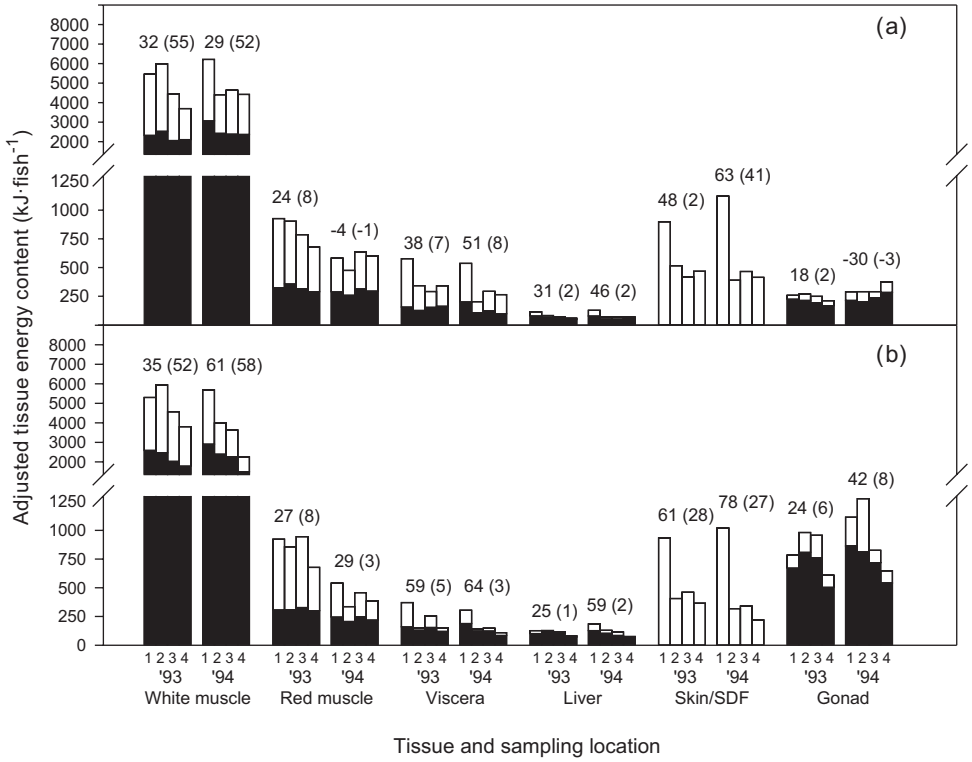
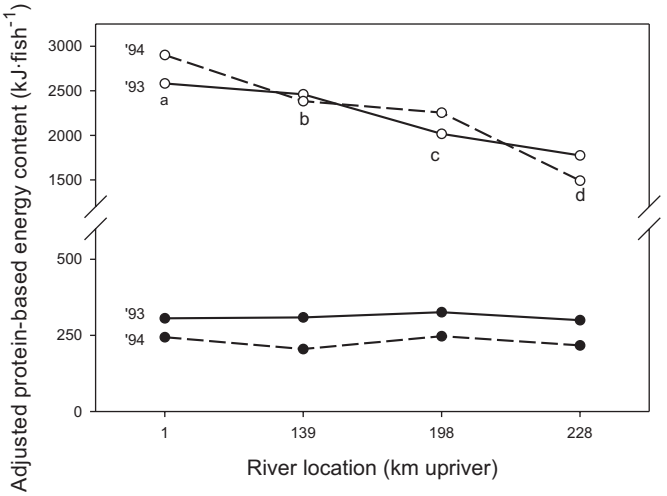


Fig. 4. White (open circles) and red (solid circles) muscle adjusted mean protein-based tissue energy content in female American shad sampled at four different sites in the Connecticut River in 1993 (solid line) and 1994 (broken line). For white muscle, different letters indicate significantly different energy contents. There were no differences in red muscle protein between the sites.



Skin/SDF energy was depleted throughout migration by 48–80% (Fig. 3; Table 2). Skin/SDF energy content was significantly affected by fish length such that larger fish depleted this source more rapidly than smaller fish.

In all tissues examined, glycogen was marginally detectable and contributed very little energy (<1%) relative to lipid and protein (Tables 1 and 2).

Discussion

Our results parallel and expand upon those of Glebe and Leggett (1981*a*), who also worked with the Connecticut River American shad population. They found that somatic tissue contributed over 90% of the energy required for migration, including the downstream migration. In our study, somatic tissues (red and white muscle and skin/SDF) provided about 90% of the energy for upstream migration. The studies differ, however, in that Glebe and Leggett (1981*a*) reported an approximately 26–30% depletion of the somatic stores in the first 139 km of the migration. Our work found an approximately 29–61% depletion (Fig. 3) in the white muscle stores alone over the entire 228-km upstream migration. When Glebe and Leggett (1981*a*) gathered their data, km 139 (Holyoke Dam) was the functional upriver extent of the migration. Currently, American shad are able to move upstream more than 228 km, an increase of 64%. Estimates suggest that a substantial portion of the population utilized this upstream portion of the range (42–63% for 1990–1997, Connecticut River Coordinators Office, personal

Table 2. Mean absolute energy values (kJ) for tissue energy content in American shad captured at four river locations, estuary (km 1), Holyoke Dam (km 139), Cabot Station (km 198), and Vernon Dam (km 228), and at an oceanic site, Barnagut Light, N.J.

	A	B	1995 males	1995 females	1993 males			
Site (km)			Ocean	Ocean	1	139	198	228
Viscera total	f, s, l (a, b, b, b), y	f′, s′	556 (61)	235 (30)	553 (84)	309 (62)	248 (39)	312 (159)
Viscera protein	f, l (a, b, b, b), ly	f′, g (a, a, b)	189 (15)	146 (11)	149 (12)	116 (11)	140 (9)	153 (64)
Viscera lipid	s, l (a, b, b, b), y	f′, s′	366 (49)	89 (27)	405 (72)	193 (56)	108 (34)	159 (86)
Viscera glycogen	l (a, b, abc, c)	f′	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Liver total	f, s, l (a, b, b, c), y, ly	f′, g (a, b, a)	181 (19)	152 (13)	109 (14)	71 (12)	57 (5)	49 (4)
Liver protein	f, s, l (a, a, b, c), sl, sy	f′	89 (9)	93 (6)	73 (6)	58 (11)	42 (3)	39 (12)
Liver lipid	l (a, b, b, c), y, ly	g (a, b, c)	92 (14)	59 (8)	35 (5)	12 (2)	15 (2)	10 (1)
Liver glycogen	f, l (a, b, b, b), y, sl, ly	g (a, b, a)	0.0 (0.0)	0.1 (0.1)	1.1 (0.4)	0.0 (0.0)	0.2 (0.1)	0.0 (0.0)
Skin total	f, l (a, b, b, b), ly	f′, g (a, b, a)	2634 (130)	2430 (208)	1693 (114)	890 (132)	707 (77)	815 (89)
Skin lipid	f, l (a, b, b, b), ly	f′, g (a, b, a)	2634 (130)	2430 (208)	1693 (114)	890 (132)	707 (77)	815 (89)
Gonad total	f, s, l (a, b, a, c), y, sl, ly	f′, s′, g (a, a, b), gs	203 (15)	891 (71)	198 (13)	185 (11)	138 (8)	134 (10)
Gonad protein	f, s, l (a, a, a, b), y, sl	f′, s′, g (a, a, b)	155 (12)	753 (59)	176 (10)	149 (7)	110 (7)	109 (7)
Gonad lipid	f, s, l (a, b, ac, c), y, sl, sy, ly	f′, s′, g (a, a, b), gs	48 (6)	138 (14)	23 (4)	36 (5)	28 (4)	25 (4)
GSI	s, sl, l (ac, b, ab, c)	s′, gs	11.4 (1.5)	9.9 (0.5)	7.5 (0.3)	8.3 (0.4)	6.6 (0.2)	6.3 (0.4)

Note: Also included are data for GSI (%). Parenthetical numbers are 1 SE of the mean. Column A indicates the results of ANCOVA (for length only, ANOVA) and multiple comparisons analysis of riverine data; 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, "gs" 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 ANCOVA ($\alpha = 0.05$). Parenthetical letters after "l" and "g" indicate the results of multiple comparisons testing for riverine location (km 0, km 139, km 198, and km 228, respectively) and ocean–estuary groups (ocean, estuary 1993, and estuary 1994, respectively).

communication). The data show that, at the maximum upriver site in both studies, fish had similar levels of energy depletion. It is not clear why this occurs, although it could be the result of behavioral changes in the migration of American shad over time leading to changes in migration speed.

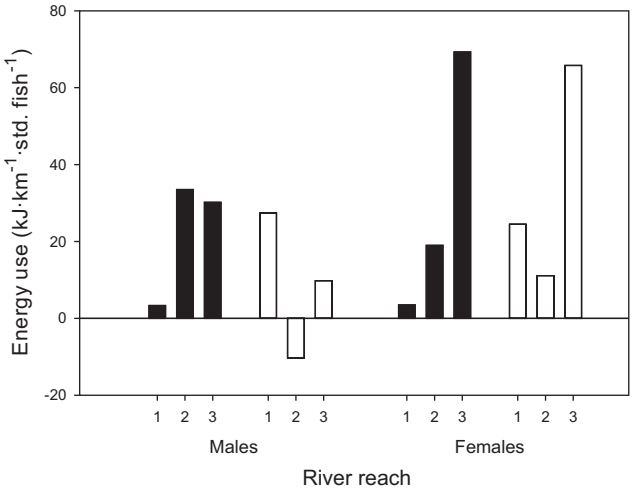
If we compare, instead, the energy depleted to reach km 139 in both our study and Glebe and Leggett (1981*a*), we find differences that highlight annual effects on migration. In our study, white muscle energy content in 1993 did not change substantially between km 1 and km 139, while in 1994, there was a decrease in white muscle energy content (29.1% males, 32.8% females) between km 1 and km 139, similar to the expenditure (26–30% somatic) seen by Glebe and Leggett (1981*a*). This suggests that there are large year-to-year differences affecting energy expenditure, including the expenditure of energy prior to the first mainstem passage obstacle (Holyoke Dam, km 139). This expenditure between km 1 and km 139 (1994), combined with the large female expenditures between km 198 and km 228, is respon-

Table 2 (*concluded*).

1994 males				1993 females				1994 females			
1	139	198	228	1	139	198	228	1	139	198	228
530	182	225	162	423	198	285	162	378	195	191	119
(140)	(15)	(23)	(27)	(38)	(12)	(57)	(19)	(55)	(15)	(19)	(11)
198	101	103	65	173	144	164	122	210	139	135	88
(14)	(4)	(8)	(8)	(9)	(6)	(17)	(11)	(20)	(8)	(10)	(3)
332	82	122	97	250	54	122	40	168	56	56	32
(129)	(11)	(18)	(20)	(32)	(8)	(44)	(10)	(43)	(9)	(10)	(10)
0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0
(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.1)	(0.1)	(0.0)
127	62	46	31	148	145	129	85	212	149	129	79
(28)	(5)	(6)	(4)	(14)	(15)	(13)	(6)	(13)	(11)	(8)	(6)
76	47	32	26	115	128	108	72	147	118	96	72
(9)	(3)	(4)	(3)	(12)	(13)	(10)	(5)	(9)	(7)	(6)	(6)
50	15	13	5	30	17	21	14	64	31	34	7
(19)	(2)	(2)	(1)	(4)	(3)	(5)	(1)	(6)	(4)	(2)	(1)
0.4	0.0	0.0	0.0	3.2	0.0	0.2	0.3	1.2	0.2	0.0	0.1
(0.2)	(0.0)	(0.0)	(0.0)	(0.7)	(0.0)	(0.1)	(0.1)	(0.3)	(0.1)	(0.0)	(0.0)
2222	690	629	355	2097	1024	1059	781	2373	882	867	490
(324)	(128)	(64)	(62)	(104)	(188)	(138)	(122)	(58)	(104)	(78)	(74)
2222	690	629	355	2097	1024	1059	781	2373	822	867	490
(324)	(18)	(64)	(62)	(104)	(188)	(138)	(122)	(58)	(104)	(78)	(74)
276	236	111	91	922	1107	1038	646	1310	1421	937	677
(26)	(19)	(7)	(13)	(47)	(57)	(94)	(108)	(85)	(151)	(88)	(52)
203	161	101	68	775	904	823	526	1012	924	802	562
(19)	(12)	(8)	(6)	(40)	(49)	(75)	(83)	(68)	(100)	(76)	(43)
73	75	11	24	147	203	216	120	298	497	135	114
(9)	(9)	(2)	(7)	(11)	(11)	(20)	(45)	(21)	(54)	(13)	(13)
8.1	8.1	6.9	5.9	13.4	16.3	18.0	13.7	15.2	18.6	16.9	14.4
(0.3)	(0.3)	(0.4)	(0.3)	(0.6)	(0.9)	(1.5)	(1.6)	(0.5)	(1.2)	(1.2)	(1.0)

sible for the very large (61%) total energy depletion seen in females in 1994. The source of year-to-year variation is somewhat unclear, although the two sampling years in our study were quite different, particularly in water flow; 1994 was characterized by high river discharge during the migration (25% greater than in 1993 as measured by the U.S. Geological Survey gauging station at Montague, Mass.), indicating that high water flow may be involved in the greater energy expenditure of fish migrating in 1994 compared with 1993. This is particularly relevant given that, in both males and females, fish sampled in 1994 from the estuary (km 1) had a greater energy reserve than those migrating in 1993. This type of large variation in energy availability was also seen by Glebe and Leggett (1981*a*), who saw much larger energy reserves in fish migrating through the estuary in 1972 (compared with 1973). These data suggest that not only is annual variation large and likely critical to migratory success, but annual effects may be acting prior to river entry (energy storing) as well as during the upstream migration (energy expending).

Fig. 5. Energy expenditure per kilometre of river traversed per reach of river sampled: (1) Old Lyme to Holyoke Dam (km 1 to km 139), (2) Holyoke Dam to Cabot Station (km 139 to km 198), and (3) Cabot Station to Vernon Dam (km 198 to km 228). Bars indicate data from 1993 (solid) and 1994 (open) Values are based on differences between standardized means for each sex at two adjacent sites. When values are pooled across years (based on a priori individual standardization), there is a significant difference between female energy expenditure in reach 3 and all other male and female reach expenditures (ANOVA, $p < 0.05$).



Glebe and Leggett (1981*a*) found that smaller American shad (males) consumed greater proportions of their somatic muscle than did larger shad. In our study, large fish (females) expended a slightly larger amount of energy (using length-corrected values for comparison) than did small fish (males), but this effect varied strongly between years. In 1993, females expended 39% of total energy, while males expended 37%. In 1994, however, females expended 60% of total energy, while males expended only 35%. Our data are in agreement with the observations of Jonsson et al. (1997), who found that migrating large Atlantic salmon of either sex expended more energy than small fish, but it is difficult to definitively separate the effects of sex and length in American shad. The differences between our study and Glebe and Leggett (1981*a*) may be the result of annual variability in energy use, perhaps resulting from differences in river discharge or temperature. Based on comparison of American shad from semelparous and iteroparous populations, Glebe and Leggett (1981*b*) suggested that populations of fish expending more than 60% of their total energy during migration would tend to be semelparous. The Connecticut River American shad population is partially semelparous, and they may represent a threshold population that encounters a high level of energy expenditure in intermittent years or only in one sex. The use of the 60% energy expenditure threshold is, however, subjective, as pointed out by Jonsson et al. (1997). They demonstrated that Atlantic salmon can endure 60–70% energy expenditure in iteroparous populations, although greater than 62% energy depletion resulted in less than 15% postspawning survival. Perhaps threshold levels of energy expenditure leading to semelparity are species dependent. Threshold values are only useful, however, when the premigratory condition of the fish is known and invariable. We have very little information on oceanic condition of American shad, or other anadromous fishes, and no knowledge of how premigratory condition might relate to postspawning survival or migratory success. The relationship between energy expenditure and iteroparity must continue to be examined in a variety of species before generalizations can be accepted.

Differences in mean energy content were adjusted for distance between the sampling sites and the adjusted means plotted versus the region of the river between the sampling sites (Fig. 5). The cost of migration on a per distance traveled basis for American shad (about 25 $kJ \cdot km^{-1} \cdot fish^{-1}$) in our study is similar to that proposed for alewife (*Alosa pseudoharengus*) and blueback herring (*Alosa aestivalis*) in Nova Scotia (Crawford et al. 1986) as well as to that demonstrated for American shad in the lower reaches of the Connecticut River (Glebe and Leggett 1981*a*). In the present study, the areas between the estuary and Holyoke Dam (km 139) and between Holyoke Dam and Cabot Station (km 198) were not different in energy utilization (when the years were pooled and the individual-based means tested for differences using ANOVA). However, female American shad use a consistently higher amount of energy between Cabot Station (km 198) and Vernon Dam (km 228) than earlier in the migration and higher than that used by males (ANOVA, $p < 0.05$) in the same river reach. The cause of this difference is unclear because there are no known costs that would apply only to females in the upper river reaches. Spawning might be more energetically expensive for females than for males, but spawning also occurs below km 198, as evidenced by decreasing GSI and gonadal condition (this study; Glebe and Leggett 1981*a*), and we see no evidence of sex-differential energy usage below km 198. It is possible that there are behaviors inherent in traversing either fish ladders or the power canal in this river reach that make these structures more challenging to females. Regardless of the cause of this increased energy expenditure, it may explain the greater number of males than females that passed over the Vernon Dam (km 228).

Despite the energetic expense of migration in the last reach of the river sampled (between Cabot Station and Vernon Dam), fish captured at Vernon Dam (km 228) still have a considerable store of energy. While skin/SDF energy was depleted by about 63%, total white muscle energy was depleted by about 41%. Overall standardized energy use by adult American shad was about 44% over the 228-km migration distance. It seems likely that some of the remaining energy could be used to return to the sea, although the downstream migration is aided by the river current. It is unclear how the balance between returning successfully to the sea and making advances farther upriver is determined in these fish or how much further these animals could successfully expand their range in the river. The 35–40% energy expenditure seen in American shad (with the exception of 1994 females) may represent a threshold for iteroparity in this population. Perhaps greater energy expenditure depletes the stored energy resource to the point where postspawning survival is minimal. The balance between the advantage of further upriver migration after this depletion relative to postspawning mortality would then be crucial in determining the proportion of semelparity in the population.

We assume that our sampling was from a single cohort of animals moving upriver together, and our sampling protocol of capturing the earliest fish arriving at a passage facility was intended to facilitate this assumption. Several aspects of our data suggest that we may not have been entirely successful. The higher white muscle energy reserves in fish captured in 1993 at km 139 (relative to km 1) suggest that these fish may be a specific subset of the population that chooses to migrate upriver past the spawning sites nearest the ocean (km 50–137, Glebe and Leggett 1981*a*). The males in 1994 also suggest that there may be different cohorts of fish moving through the upstream areas, since there is no increase in total depletion of energy above km 139, suggesting that an approximately 35% depletion is typical and individuals (at least males) are unlikely to expend more than this amount of energy. Passage data through Holyoke Dam (km 139), Cabot Station (km 198), and Vernon Dam (km 228) suggest that about 10% of fish passing Holyoke Dam will ascend the Cabot Station ladder and only about 1% of Holyoke Dam fish will pass Vernon Dam (D. Pugh, Massachusetts Fish and Wildlife Cooperative Unit, personal communication). We were sampling a dwindling subset of the population as we moved upstream, but whether these fish are substantially different from the rest of the population remains unknown.

Tissue-specific energy expenditure patterns may reveal some of the metabolic control mechanisms used by American shad during their migration. Our data demonstrate large differences in the patterns of energy utilization between tissues and between lipid and protein energy sources. Although all tissues examined contributed significantly to the energy used during migration, white muscle and the skin/SDF contributed the most energy (Figs. 3 and 4). Skin/SDF lipid contributed about 21% to the entire energy pool in American shad, despite the fact that skin/SDF comprises less than 5% of the whole-animal weight. White muscle lipid contributed about 35% and white muscle protein about 24% of the energy in the whole body. White muscle lipid was consistently lower (Table 1) between the ocean and the estuarine sampling site, suggesting that river entry itself is powered by white muscle lipid stores. White muscle protein was not used prior to freshwater entry, nor were red muscle energy stores. The large contribution of subdermal lipid stores to migration is unusual for those anadromous fishes that have been studied, and skin/SDF can be thought of as a specialized tissue for energy storage in American shad. The SDF is part of the hypodermal area of the integumentary region and is enlarged in some other fishes, including Atlantic mackerel (*Scomber scombrus*) (Whitear 1984), bogue (*Boops boops*) (Kapoulas and Miniadis-Meimaroglou 1985), and cyclostomes. Sea lampreys (*Petromyzon marinus*) use lipid from this area (the fat column or myeloid region) to provide energy for upstream migration (Beamish et al. 1979). The SDF storage site differs from that of salmonid species that typically mobilize lipid from muscle and viscera during migration (Sheridan 1988), although recent evidence from Arctic char (*Salvelinus alpinus*) suggests that some salmonids may also use lipid stored in the skin (Jorgensen et al. 1997).

There were no significant differences in protein content of red muscle (length adjusted) between any of our samplings (Fig. 4), yet significant utilization of protein occurred in all other tissues examined. This suggests that energy sparing of red muscle occurs in American shad during migration. It is unknown whether sparing occurs in other species, since red muscle is generally not separated from white muscle in other energetic studies. Integrity of the protein structure of red muscle may be crucial to the aerobic swimming capacity that is necessary for continued upstream migration and return to the ocean. Either white muscle can sustain a reduction in protein while retaining function (burst swimming) or the animal sacrifices white muscle function in exchange for energy. Extensive white muscle depletion has been shown to compromise swimming ability in salmonids (Williams and Brett 1987). If white muscle function is compromised in American shad, the importance of burst swimming to total reproductive success may be lower than sustained swimming. Salmonids, however, may use white muscle more extensively during the process of nest building and spawning (Hendry 1998) than American shad, since American shad are nonnesting, broadcast spawners.

There are few absolute energetic differences between the sexes that cannot be attributed to a length effect based on the sexual length dimorphism (females larger than males) seen in this species. An effect of length on proximate composition is to be expected, given the known effects of scaling on metabolic parameters. Aerobic pathways scale such that relative aerobic costs decrease with increasing size (Goolish 1991). This suggests that upstream migration would be more costly for smaller individuals (Weihs 1977). However, both Jonsson et al. (1997) and our study found that larger fish expended more energy during migration. There was some suggestion from Somero and Childress (1980) that, in fish, anaerobic metabolism scales such that larger fish have greater costs for anaerobic activity, and this may be partially responsible for the length-dependent patterns seen in our study. However, we have also measured metabolic enzyme changes in American shad and found few sex-independent, length-dependent effects on either aerobic or anaerobic enzymes during migration (Leonard and McCormick 1999). The mechanisms behind the larger energy expenditure of only slightly larger migratory individuals of the same species remain unclear, although individual behavioral choices could play a significant role.

Sex differences that are independent of length are only seen in liver protein content, visceral lipid content, and red muscle protein (Tables 1 and 2). Elevated liver protein levels in females may be the result of vitellogenin production in the liver during reproductive maturation. This function was also suggested as the basis for elevated lipid levels in female landlocked *Alosa fallax lacustris* (Luzzana et al. 1996), and yet we did not see this difference in liver lipid in this study. Increased visceral lipid in males (also found by Glebe and Leggett 1981*a*) may be a result of relatively smaller gonads, which are probably energetically less costly to maintain, allowing males to use the visceral space taken up in females by large gonads for fat storage. It is unclear why males should have relatively more protein in their red muscle than females, although this relationship could play a role in the greater migration distances achieved by males in the Connecticut River.

American shad use lipid- and protein-based energy throughout the course of their migration, although the relative use of lipid is greater. The contemporaneous use of these energy

sources is somewhat unusual, since several other fishes have been shown to use lipid first and then use protein later in migration. Protein is typically mobilized after lipid in sea lamprey (Beamish et al. 1979). Sockeye salmon (*Oncorhynchus nerka*) use both lipid and protein during their migration, but lipid plays a much larger role early in migration, while protein is more important later in migration and during spawning (Idler and Bitners 1958, 1959). Crawford et al. (1986) demonstrated that migrating alewife and blueback herring use lipid, but not protein, as their energy source for upstream migration in the LeHave and Margaree rivers in Nova Scotia. This suggests that within the alosines, there is a difference in the pattern of energy source mobilization, since American shad use protein in the early stages of migration. The migrations characterized in Crawford et al. (1986), however, were relatively short (<35 km) and do not rule out the use of protein as an energy source for longer migration in these species.

Clearly, upstream anadromous migration is energetically expensive for American shad. White and red muscle, SDF, liver, and viscera all contribute to energy use during migration. However, we have shown that white muscle and SDF store most of the energy that powers migration. Red muscle protein is spared during migration. Additionally, American shad are probably not energetically exhausted at the end of their current maximum migratory distance in the Connecticut River, since 40% energy depletion is common, but 60% depletion is possible. Differential use of energy between the sexes in the last region of the river encountered may indicate a sex-specific limitation on migration that may represent an energetic constraint on the extent of migration.

Acknowledgements

The authors would like to thank Dr. Mark Sheridan, the Massachusetts Fish and Wildlife Cooperative Research Unit (especially Don Pugh), the New Jersey Department of Environmental Protection (especially Russ Allen), the Vermont Department of Fish and Wildlife (especially Ken Cox), the Connecticut River Coordinators Office, Northeast Utilities, New England Electric Co., Harry Root, the crew of the FV *Compromise*, and particularly Todd Dubreuil and the students and staff of the S.O. Conte Anadromous Fish Research Center for their help in conducting this study. We would also like to acknowledge the assistance of Drs. Kevin Whalen, Joseph Zydlewski, and Andrew Hendry and two anonymous reviewers for their careful evaluation of previous versions of the manuscript.

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