Anadromous fish as marine nutrient vectors

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Abstract—The tidal freshwater of Virginia supports anadromous herring (*Alosa* spp.) spawning runs in the spring; however, their importance as nutrient delivery vectors to the freshwater fish food web remains unknown. The stable isotope signatures of fishes from 21 species and four different guilds (predators, carnivores, generalists, and planktivores) were examined in this study to test the hypothesis that marine derived nutrients (MDNs) brought by anadromous fish would be traced into the guilds that incorporated them. Spawning anadromous fish were ^{13}C and ^{34}S-enriched ($\delta^{13}C$ and $\delta^{34}S$ of approximately 18‰ and 17.7‰, respectively) relative to resident freshwater fish. Of the guilds examined, only predators showed ^{13}C and ^{34}S-enrichment similar to the anadromous fish; however, some generalist catfish also showed enriched signatures. Specific fatty acid $\delta^{13}C$ signatures for gizzard shad (*Dorosoma cepedianum*), blue catfish (*Ictalurus furcatus*), and alewife (*Alosa pseudoharengus*), show a 10‰ range among fishes, clearly reflecting isotopically distinct dietary sources. The $\delta^{13}C$ and $\delta^{34}S$ distribution and range among the freshwater fishes suggest that both autochthonous and allochthonous (terrestrial C3 photosynthetic production and MDN) nutrient sources are important to the tidal freshwater fish community.

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Streams in which anadromous fish spawn are often nutrient poor and the spawning anadromous fish may be an important source of nutrients to them (Kline et al., 1993; Wipfli et al., 2003). Sometimes spawning anadromous fish even fertilize near-stream terrestrial environments (Ben-David et al., 1998; Koyama et al., 2005). The spawning fish are frequently semelparous and deliver marine derived nutrients (MDN) to the freshwater as moribund biomass, excreted ammonium ion (NH_4^+), or through gamete release (Cederholm et al., 1989; Browder and Garman, 1994; Wipfli et al., 2003). Several studies in Alaska and the Pacific Northwest of North America have demonstrated the importance of marine nutrients brought to freshwater streams by anadromous salmonids (Bilby et al., 2003; Kline et al., 1993; Francis et al., 2006). In the Gulf of Mexico, migrating Gulf menhaden (*Brevoortia patronus*) transported estuarine nutrients into inshore environments (Deegan, 1993), and returning salmon contributed to the productivity of Lake Ontario tributaries (Rand et al., 2002). However, less work has been done on the East Coast of the United States where coastal development has been much more intense and the dominant anadromous species (*Alosa* spp.; herring (*A. aestivalis*), American shad (*A. sapidissima*), and alewife (*A. pseudoharengus*)) are often not highly abundant (Deegan, 1993; Garman and Macko, 1998). Although the *Alosa* spp. on the east coast tend towards an iteroparous life cycle rather than a semelparous one, they do experience heavy postspawning mortality (alewife postspawning mortality has been measured as 41% (Havey, 1961) and between 39% and 57% (Durbin et al., 1979)). Because tidal freshwater streams receive nutrients from marine and freshwater primary productivity at different times, the incorporation of these nutrients by consumers may be different depending on feeding guilds. Fish found in the same area in a stream may derive nutrition from local or translocated productivity. In nutrient poor systems, such as East Coast United States tidal freshwater areas, it is important to understand nutrient sources to different feeding guilds (e.g., predators, carnivores, generalists, and planktivores).

For more than 20 years now, carbon and nitrogen stable isotopes (re-

ported as a ratio of heavy to light isotopes and given δ notation with units of ‰, see *Materials and methods* section for more detail) have been used to determine the importance of MDN in freshwater systems, and to characterize the trophic structure within those systems (Kline, et al., 1993; Vander-Zanden et al., 1999). For example, carbon and nitrogen isotopes have shown that anadromous Pacific salmon (*Oncorhynchus* spp.) were a significant source of allochthonous nitrogen to coastal streams where spawning occurs (Kline et al., 1993).

Hesslein et al. (1991) used sulfur isotopes to differentiate freshwater migratory and non-migratory fishes in the Mackenzie River Basin, Canada. On the East Coast of the United States, anadromous river herring (*Alosa* spp.) retain their marine isotope signal after spending part of the spring spawning in freshwater, and that some freshwater piscivores are ^{34}S and ^{13}C-enriched after preferentially consuming migrating *Alosa* spp. during the spawning run (Garman and Macko, 1998; MacAvoy et al., 2000).

An additional tool for determining origins and transformations of organic material from different sources is the stable isotope ratio of specific compounds. Isolating a specific compound, or class of compounds, then measuring the isotope ratio on those compounds, may offer a more robust technique to trace biologically significant compounds (such as fatty or amino acids) than would be possible from bulk isotope analysis alone. For example, examining the carbon isotopic composition of fatty acids from an animal, particularly essential fatty acids, allows the direct determination of dietary sources that contribute to the fatty acid pool of that animal (Stott et al., 1997). Although bulk isotope analysis can be an effective nutrient tracer in systems with isotopically distinct nutrient sources (Peterson et al., 1985), the isotopes of specific fatty acids may provide more confidence in identifying sources (Canuel et al., 1997).

Carnivorous heterotrophs are unable to synthesize fatty acids longer than 18-carbons, nor can they desaturate carbon-carbon bonds between the ninth and terminal methyl carbon, therefore, these essential fatty acids must be obtained from diet (Olsen 1999). Because essential fatty acids are not influenced by subsequent metabolism within a eukaryotic heterotroph, they retain their original isotope composition (Stott et al., 1997). Fatty acids synthesized by marine plankton and incorporated into marine fish would be highly enriched in ^{13}C relative to those produced by freshwater primary producers or C3 photosynthesis. Additionally, short chain fatty acids, used as precursors in the biosynthesis of unsaturated or longer chain saturated fatty acids, should be ^{13}C enriched in relation to biosynthesized fatty acid products (Murphy and Abrajano, 1994). In this study, the fatty acid nomenclature used is carbon number:number of double bonds. For example, 18:2 is an 18-carbon fatty acid with two points of unsaturation. The desaturation of 16:0 to 16:1 and 18:0 to 18:1–18:2 occurs by a systematic fractionation of roughly 2‰ per desaturation (DeNiro and Epstein, 1977; Monson and Hayes, 1982). Also, studies have shown that the elongation of fatty acids by *de novo* synthesis results in a 2‰ per 2-carbon acetyl group addition. These fractionations allowed the identification of fatty acids that were directly incorporated from symbiotic bacterial sources in mussels as opposed to those obtained through *de novo* synthesis (Murphy and Abrajano, 1994).

In this study we compared the $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$ of bulk tissues, plus the $\delta^{13}C$ of specific fatty acids among four guilds of fish plus anadromous *Alosa* spp. in a tidal freshwater stream on the East Coast of the United States. Our objective was to determine if anadromous fish, captured more than 40 km from the salt-wedge, were isotopically distinct from freshwater residents, and to determine if freshwater guilds showed the incorporation of marine allochthonous organic material.

Materials and methods

Field collections by boat electrofisher were made in the tributaries and main-stem of the Rappahannock River, VA (within a 40-mile area between Fredericksburg and Tappahannock, VA) during March and May 1997 and 1998 (Fig. 1). The Rappahannock River is tidal in this region (tidal range: 0.1 to 1 meter) and shares many physicochemical characteristics with other tidal freshwater rivers in the region (Garman and Nielsen, 1992). Fishes were collected and placed on ice in the field, transported back to the laboratory, and muscle tissue samples were taken, which were then dried for later analysis. Analysis of the sulfur and compound specific fatty acid samples took several years and were completed by 2002.

The fishes were placed into four different guilds based on feeding strategies taken from Jenkins and Burkhead's (1993) seminal work on Virginia freshwater fishes, plus an anadromous life cycle group (Table 1).

Bulk isotope tissue analysis, elemental analyzer, and isotope ratio mass spectrometry

Samples of dorsal muscle tissue were dried at 60°C for three days and homogenized in preparation for analysis. The tissues were then lipid extracted by refluxing them in dichloromethane for 35 minutes (Knoff et al., 2002), except for those samples selected for compound specific analysis, which were soxlet extracted (see below; gas chromatography-mass spectrometry (GC-MS) and compound specific stable isotope analysis (CSIA)). One milligram (mg) of dried, lipid-extracted muscle was used for $\delta^{13}C$ and $\delta^{15}N$ analysis. Six mg was used for $\delta^{34}S$ analysis. A Carlo Erba elemental analyzer (EA) (Fisons/VG/Micromass, Manchester, UK) coupled to a Micromass Optima isotope ratio mass spectrometer (IRMS) (Fisons/VG/Micromass, Manchester, UK) was used to obtain $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values. The $\delta^{13}C$ and $\delta^{15}N$ were obtained concurrently, and $\delta^{34}S$ was determined during separate analytical runs.

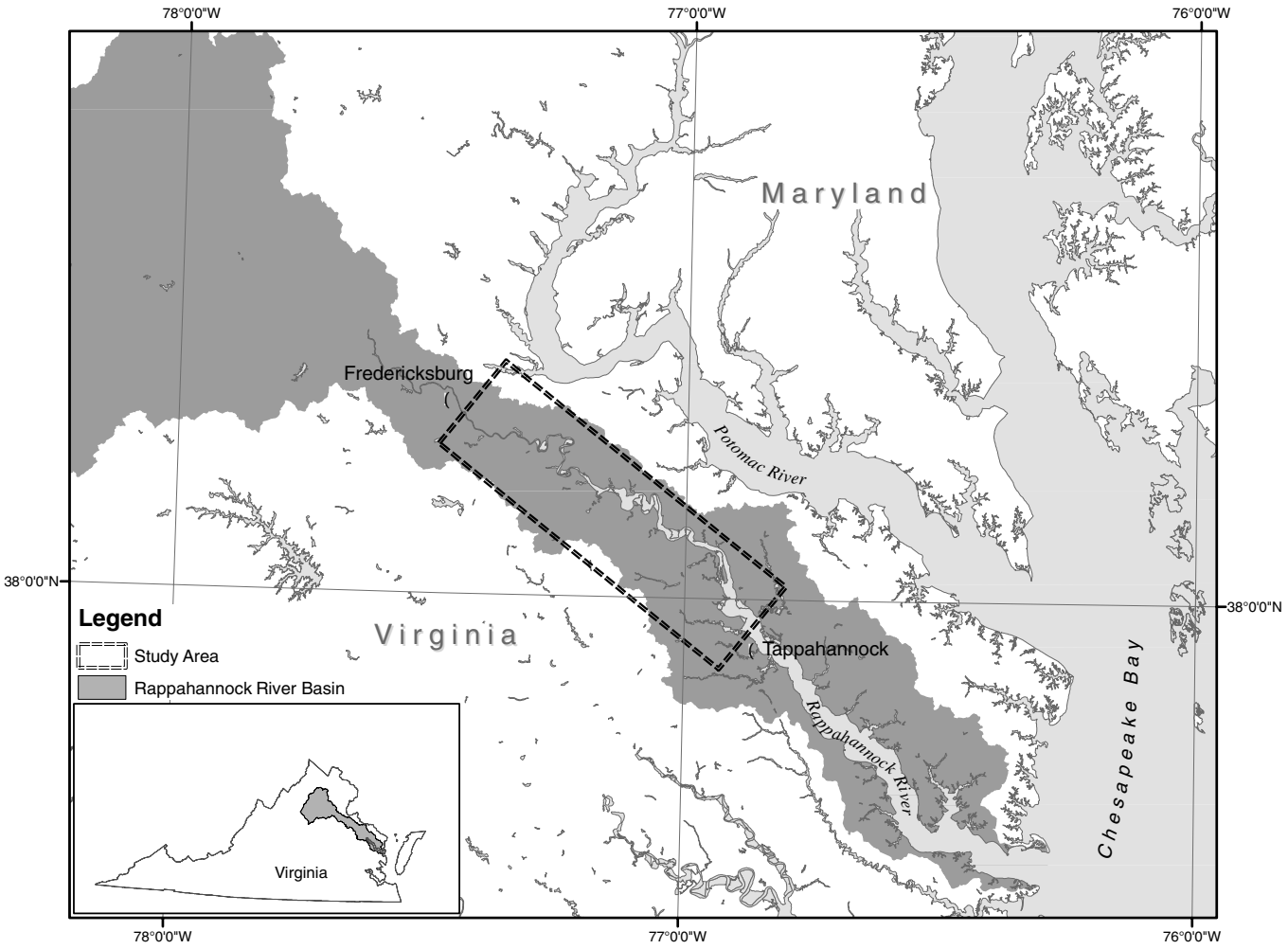

Figure 1

The boxed area indicates the section of the Rappahannock River, Virginia, between the towns of Fredericksburg and Tappahannock, where all fish were captured to determine the role of anadromous fish as marine nutrient vectors to the freshwater environment. Boat electrofishing was conducted between February and May 1997 and 1999. Sampling was conducted so that fish were captured before, during and after the spring spawning run of the anadromous *Alosa* spp.

The isotope compositions are reported in relation to standard material and follow the same procedure for all stable isotopic measurements, as follows:

$$\delta^x E = [({}^xE/{}^yE)\text{sample}/({}^xE/{}^yE)\text{standard}] - 1) \times 1000, \quad (1)$$

where E = the element analyzed (C, N, or S);
x = the molecular weight of the heavier isotope; and
y = lighter isotope (x=13, 15, 34, and y=12, 14, 32 for C, N, and S, respectively).

The standard materials to which the samples are compared are Pee Dee Belemnite for carbon, air N_2 for nitrogen, and Canyon Diablo Triolite for sulfur. Reproducibility of all measurements was typically 0.2‰ or better. Between every 12 samples, a laboratory standard was analyzed. In a typical run of 60 samples (+5 standards, 65 measurements total) the standard deviations for $\delta^{15}N$ and $\delta^{13}C$ were <0.2‰. For $\delta^{34}S$, standard deviations were <0.3‰.

Gas chromatograph-mass spectrometer (GC-MS)

Once dried, muscle samples selected for compound specific isotope analysis (CSIA) were lipid extracted (Soxhlet method from Ballentine et al., 1996) and the fatty acids had a methyl group added to the carboxyl end (derivitized) so they could be characterized by gas chromatography (GC). This was done by heating with BF_3CH_3OH for eight minutes (Ballentine et al., 1996). The fatty acid methyl esters (FAME) were analyzed by GC-MS using a Hewlett Packard 5890 Series II gas chromatograph (Palo Alto, CA) interfaced to a Hewlett Packard 5971A mass sensitive detector (Palo Alto, CA), with helium gas as the carrier. A 60-meter J&W DB-5

column (J&W Scientific, Folsom, CA) was used for FAME separation. The GC oven temperature program used was as follows: 100°C for 2 minutes, ramp at 3°C/min. to 210°C, hold for 20 min, ramp 1°C/min. to 220°C, hold for 10 min.

Compound specific stable isotope analysis (CSIA)

The FAMEs were analyzed for their stable carbon isotope compositions using a Hewlett Packard 5890 Series II gas chromatograph interfaced through a combustion furnace with a VG Isoprime IRMS (Fisons/VG/Micromass, Manchester, UK). The GC was equipped with the same column that was used for the GC-MS analysis and helium was the carrier gas. The GC oven temperature program was identical to that used for the GC-MS FAME identification. Time elution was used to identify peaks. The CO_2 combustion products of the fatty acids eluting from the column were introduced into the mass spectrometer after passing through a water trap.

All FAME $\delta^{13}C$ values were corrected for the addition of the methyl group to the original fatty acid. The derivatization of the fatty acids to their methyl esters results in a predictable and reproducible isotope effect (Ballentine et al., 1996; Uhle et al., 1997). Adding a methyl group to the fatty acid alters its isotope signature. However, if the isotopic ratio of the methanol (in this case $\delta^{13}C$=–46‰, measured by injecting the methanol into the mass spectrometer through the GC) and the fatty acid methyl ester are known, then the isotopic signature of the original fatty acid can be determined using a mass balance Equation 2.

$$\delta^{13}C_{\ FAME} = f_{FA}\delta^{13}C_{\ FA} + f_{Methanol}\,\delta^{13}C_{\ Methanol} \qquad (2)$$

where $\delta^{13}C_{FAME}$, $\delta^{13}C_{FA}$, and $\delta^{13}C_{\ Methanol}$ = the carbon isotope signatures of the FAME, the underivatized fatty acid, and the methanol, respectively; and

f_{FA} and $f_{Methanol}$ = the fractions of carbon in the FAME due to the underivatized fatty acid and methanol, respectively (Ballentine et al., 1996; Uhle et al., 1997).

Each sample was injected four to eight times (depending on the reproducibility of the analysis). Only $\delta^{13}C$ values that were within 1.5‰ of each other were considered to reflect the $\delta^{13}C$ of the FAME (MacAvoy et al., 2002). Therefore, the $\delta^{13}C$ reported for each FAME identified is represented by an average value and a standard deviation. Every sixth sample injected was an internal, laboratory standard (naphthalene-d, $\delta^{13}C$–25.7‰) to insure consistent performance of the GC, oxidation furnace, and mass spectrometer.

Table 1

Fish species examined by guild (including an anadromous group) from the Rappahannock River to assess the role of marine fish as nutrient vectors. Guild assignments are based on diet as reported in Jenkins and Burkhead (1993).

Guild	Species name	Common name
Predator	*Ictalurus furcatus*	blue catfish
	Lepisosteus osseus	longnose gar
Carnivore	*Micropterus salmoides*	largemouth bass
	Lepomis gibbosus	pumpkinseed
	Hybognathus regius	eastern silvery minnow
	Notemigonus crysoleucas	golden shiner
	Lepomis macrochirus	bluegill
	Perca flavescens	yellow perch
Generalist	*Anguilla rostrata*	American eel
	Ameiurus catus	white catfish
	Ameiurus nebulosus	brown bullhead
	Ictalurus punctatus	channel catfish
Planktivore	*Menidia beryllina*	inland silverside
	Dorosoma cepedianum	gizzard shad
	Erimyzon oblongus	creek chubsucker
Anadromous	*Alosa aestivalis*	blueback herring
	Alosa pseudoharengus	alewife
	Alosa sapidissima	American shad
	Morone saxatilis	striped bass
	Morone americana	white perch

Statistical analysis

Kruskal-Wallis nonparametric procedures were used to test for differences in isotopic values among anadromous fish and the different guilds (predators, carnivores, generalists, and planktivores, (α=0.05)). The Dunn procedure was used to examine differences between groups (Rosner, 1990). Statview SE + Graphics (Abacus Concepts, Inc., Cary, NC), JMP In (SAS, Cary, NC) and Microsoft Excel version 5.0 (Microsoft, Inc., Redmond, WA) were used for statistical tests. The Dunn procedure reduces the risk of type-1 error inherent in multiple comparison techniques. It does so by increasing the Z-score needed to reject the null hypothesis as the number of individual groups being compared increases. In the present study, a Z-score of ±3.02 (0.9975 confidence) was needed for a difference to be significant.

Results

The first objective of this study was to establish that the spawning anadromous fish retained the marine isotope signal more than 40 km upstream from saline waters. This was the case for all three isotopes examined.

Table 2

Isotope values for all fish used in this study seperated by Family. "A" indicates anadromous, * indicates euryhaline range. Guild assingments are based on diet as reported in Jenkins and Burkhead (1993). "C" indicates a group with some isotope data derived from MacAvoy et al. (2000). White perch (*Morone americana*) shows elevated ^{13}C content is probably not marine protein given the low $\delta^{34}S$ ratio; *M. americana* is a secondary carnivore and the high $\delta^{13}C$ reflect this. Standard deviation is given after the ± and N is in parentheses.

Family and Species	Common name	Guild: food types	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Anguillidae					
Anguilla rostrata	American eel	generalist: insects, snails, fish, clams	–24.7±0.7 (3)	11.2±0.8 (3)	0.9±2.4 (3)
Atherinidae					
Menidia beryllina	inland sliverside	planktivore	–23.8±0.9 (3)	15.5±0.2 (3)	10.0±0.9 (3)
Catostomidae					
Erimyzon oblongus[C]	creek chubsucker	planktivore: planktonic crustaceans	–28.1 (1)	10.9 (1)	5.1 (1)
Centrarchidae					
Micropterus salmoides	smallmouth bass	carnivore	–23.0±1.9 (5)	14.5±1.3 (5)	7.6±3.2 (5)
Lepomis gibbosus	pumpkinseed	carnivore: insects, worms	–25.4±1.1 (8)	13.1±1.3 (8)	6.5±2.3 (9)
Lepomis macrochirus	bluegill	carnivore: insects, worms	–23.7±2.2 (5)	14.7±1.8 (5)	4.7±2.0 (5)
Clupeidea					
Alosa pseudoharengus[A, C]	alewife spawning	anadromous: copepods, diatoms, ostracods, shrimp, fish	–17.4±1.1 (7)	12.8±0.8 (7)	17.9±0.8 (6)
Alosa aestivalis[A, C]	blueback herring spawning	anadromous: copepods, cladocerans	–19.0±0.6 (7)	13.2±0.3 (7)	17.5±0.4 (7)
Alosa sapidissima[A, C]	juvenile American shadspawning	anadromous: copepods, small invertebrates	–20.2±0.6 (4)	12.6±0.4 (4)	8.0±2.2 (4)
Dorosoma cepedianum	gizzard shad	planktivore: filter feeder	–20.2±2.1 (7)	14.0±0.9 (7)	7.8±2.5 (7)
Cyprinidae					
Hybognathus regius	eastern silvery	minnowcarnivore: diatoms, algae, ooze detritus	–23.0±2.1 (6)	12.4±3.4 (6)	6.5±2.5 (6)
Notemigonus crysoleucas	golden shiner	carnivore: microcrustaceans insects	–24.8±1.1 (5)	13.1±1.6 (5)	2.5±1.7 (5)
Ictaluridae					
Ictalurus furcatus[C]	blue catfish	carnivore/piscivore	–21.6±1.9 (43)	15.4±2.0 (43)	9.2±3.0 (43)
Ictalurus punctatus	channel catfish	opportunistic generalist	–20.5±2.0 (3)	13.4±1.2 (3)	8.5±3.2 (3)
Ameirus nebulosus	brown bullhead	generalist/omnivorous	–24.0±0.8 (3)	13.2±0.5 (5)	5.3±1.6 (5)
Ameirus catus	white catfish	generalist/omnivorous	–21.2±2.7 (10)	15.8±2.3 (10)	8.7±4.7 (10)
Lepisosteidae					
Lepisosteus osseus	longnose gar	predator, piscivore	–23.1	16.8	8.34
Moronidae					
Morone saxatilis[A]	striped bass	generalist, piscivorous	–25.0±2.3 (2)	13.3±2.4 (2)	3.4±4.3 (2)
Morone americana[A*]	white perch	carnivorous: worms, shrimp, fish	–20.7±1.2 (5)	16.7 ±1.4 (5)	7.5±3.9 (5)
Percidae					
Perca flavescens[C]	yellow perch	carnivore: insects small fish	–25.1±2.1 (6)	14.3±2.2 (6)	6.9±1.6 (6)

The second objective was to test whether the different guilds of fish showed the incorporation of the marine isotope signal brought to the tidal freshwater by the anadromous fishes. This was observed, but largely limited to the predator guild.

Of the groups examined, the anadromous fish were the most ^{13}C-enriched, with mean values of approximately –19‰, followed by predators and planktivores (means –21.8‰ and –22.0‰, respectively), which were not significantly different from each other. This suggests that, of the remaining two guilds, carnivores were significantly ^{13}C-depleted relative to generalists (mean –24.1‰ and –23.5‰, respectively; Table 2). There was approximately a 10‰ range in $\delta^{13}C$ among the exclusively freshwater guilds (Table 2, Fig. 2).

Anadromous fish have elevated $\delta^{15}N$ values relative to freshwater fish with similar feeding strategies. However, the trophic enrichment and diet-tissue discrimination associated with $\delta^{15}N$ signatures make using nitrogen a less effective tracer for source than carbon or sulfur. In this study there was less variability within the guilds $\delta^{15}N$ signatures, relative to $\delta^{13}C$, although the range (‰)

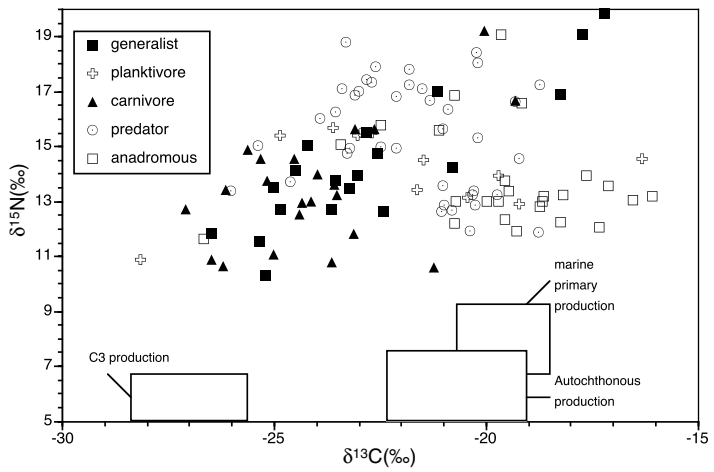

Figure 2

δ^{15}N vs. δ^{13}C values for the four guilds and anadromous *Alosa* species, showing that most resident freshwater fishes are approximately two trophic levels above primary producers (C3 or autochthonous production), in contrast to the *Alosa* spp., whose δ^{15}N reveals that they are one trophic level above marine primary production. Boxes indicate the isotope signature of C3 terrestrial plant primary production, freshwater autochthonous production, and marine primary production. *Alosa* spp. are ^{13}C-enriched relative to most freshwater residents, reflecting marine primary production.

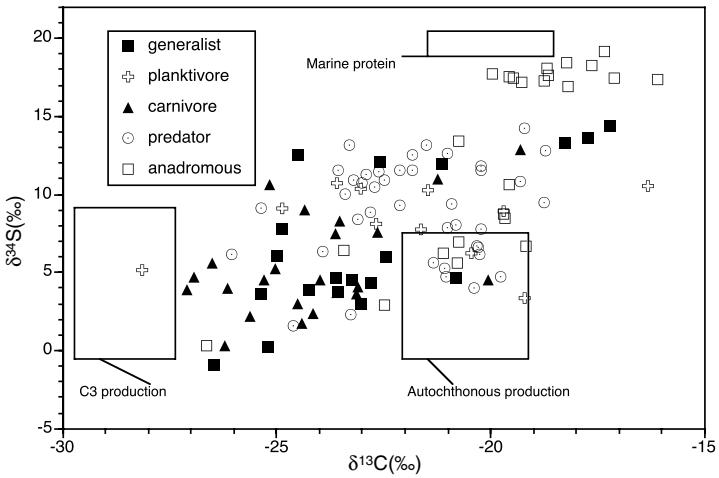

Figure 3

δ^{34}S vs. δ^{13}C values for the four guilds and anadromous *Alosa* species, with boxes to indicate the isotope signature of C3 terrestrial plant primary production, freshwater autochthonous production, and marine primary production. *Alosa* spp. are highly ^{34}S-enriched relative to most freshwater residents, reflecting marine sulfate (which becomes incorporated into primary producers and *Alosa* spp. while they grow in the Atlantic Ocean). Predators are the only guild showing elevated δ^{34}S, indicating the incorporation of marine protein derived from *Alosa* spp.

of δ^{15}N values among all fishes was similar to that observed for δ^{13}C (10‰). The anadromous fish had the lowest δ^{15}N values and generally grouped between 12‰ and 13‰; however, their values were not lower than generalists or carnivores. The predators were the most ^{15}N-enriched of any group (Table 2). There were no significant differences among the δ^{15}N values for carnivores, generalists, and planktivores (Table 2).

Sulfur isotopes were hypothesized to be the most useful for tracing marine protein into freshwater, owing to extreme differences between the δ^{34}S of marine plankton and various sulfur sources in freshwater. Predator fishes and anadromous *Alosa* spp. showed elevated ^{34}S signals relative to other resident freshwater fishes, indicating that the predators incorporated *Alosa* spp. sulfur (protein). The range of δ^{34}S values among all the fish captured was from approximately 0‰ to 20‰, a considerably larger range than observed for the other two isotopes (Table 2, Fig. 3). Significant differences were observed in δ^{34}S among several of the separate groups. Anadromous species were highly ^{34}S-enriched relative to all resident freshwater fish (Table 2, Fig. 2), although the striped bass (40 cm total length (TL)) had values between 0.3‰ and 6.4‰, the lowest of the anadromous δ^{34}S values. Predators were the most ^{34}S-enriched of the resident fish, followed by planktivores (a trend also observed for δ^{13}C). Carnivores and generalists were the most ^{34}S-depleted of the guilds and were not significantly different from each other (Table 2). Sulfur was the only stable isotope that completely separated the anadromous *Alosa* spp. from the full time freshwater residents. All of the *Alosa* spp. individual values were ^{34}S-enriched and outside the ranges observed in the other groups (Table 2).

Fatty acid analysis

Fatty acid (FA) isotope values show that some predators derive fats from anadromous fish and that there is a large variation among FA isotope values. FA δ^{13}C values were determined for one alewife (anadromous), one gizzard shad (*Dorosoma cepedianum,* a native freshwater planktivore), and two blue catfish (*Ictalurus furcatus*, an introduced piscivorous predator). For the blue catfish bulk δ^{13}C and δ^{34}S values from muscle tissue showed that one individual (A in Table 3) was significantly ^{13}C and ^{34}S-depleted relative to the other. This was also the case for the respective δ^{13}C values of their individual FAs. The anadromous alewife and the more ^{13}C-enriched blue

Table 3

Fatty acid (FA) $\delta^{13}C$ values for Rappahannock River fish. Means ± 1 Standard Deviation. (n=3). Values are corrected for CH4OH derevitization. FAs show that carbon from anadromous fish has been incorporated by *Ictalarus furcatus* but not by other resident fishes. Bulk isotope values show trends similar to the FAs and are as follows: alewife *A. pseudoharengus*, $\delta^{13}C$ –19.3‰, $\delta^{15}N$ 11.9‰, $\delta^{34}S$ 17.1‰; blue catfish *Ictalarus furcatus* (A) $\delta^{13}C$ –26.0‰, $\delta^{15}N$ 13.3‰, $\delta^{34}S$ 6.1‰; *I. furcatus* (B) $\delta^{13}C$ –19.3‰, $\delta^{15}N$ 16.6‰, $\delta^{34}S$ 10.8‰; gizzard shad *Dorosoma cepedianum* $\delta^{13}C$ –21.5‰, $\delta^{15}N$ 14.5‰, $\delta^{34}S$ 10.2‰.

Fatty acid	*Alosa pseudoharengus* alewife (‰)	*Ictalurus furcatus* blue catfish (‰)	*A Ictalurus furcatus* blue catfish (‰)	*B Dorosoma cepedianum* gizzard shad (‰)
12:0	–22.4 (0.4)	–28.5 (0.5)	–22.5 (0.9)	–27.4 (1.0)
14:0	–27.4 (1.8)	–33.6 (0.9)	–26.9 (0.6)	–25.5 (1.4)
16:1	–26.8 (0.8)	–35.4 (0.6)	–25.6 (0.7)	–27.4 (0.6)
16:0	–22.1 (0.1)	–30.3 (0.2)	–23.3 (0.3)	–25.7 (0.6)
18:1	–23.3 (0.6)	–30.5 (0.6)	–24.5 (0.7)	–28.7 (0.4)
18:0	–19.9 (1.8)	–28.8 (0.7)	–20.4 (1.1)	–23.5

catfish (B) had $\delta^{13}C$ FA values that, for the most part, overlapped with each other. Their 16 and 18 carbon length FAs were generally ^{13}C-enriched relative to the gizzard shad and the second blue catfish (A) (Table 3). For all fish, except gizzard shad, the saturated 12:0, 16:0, and 18:0 FAs were more enriched (2‰ to 6‰) than the 14:0, 16:1 and 18:1 FAs. 14:0 FAs are not elongated to 16 or 18 carbons in animals, which is why they are ^{13}C-depleted relative to saturated 16:0 and 18:0 (see Discussion). For the gizzard shad, the 12:0 FAs were 2‰ depleted relative to the 14:0 FAs. The blue catfish (B) with low $\delta^{13}C$ and $\delta^{34}S$ bulk values, generally had more ^{13}C-depleted FAs than other fishes. There was up to a 10‰ range among the FAs within an individual fish, with unsaturated FAs ^{13}C-depleted relative to saturated, and longer saturated chains being generally ^{13}C-depleted relative to shorter chain FAs (Table 3).

Discussion

The fact that the anadromous *Alosa* spp. were the most ^{13}C-enriched of the groups examined was expected because they retain the ^{13}C-enriched (relative to freshwater) signal of marine carbon fixation (Garman and Macko, 1998, MacAvoy et al., 2000, Hoffman et al., 2007). High $\delta^{13}C$ in freshwater systems with anadromous fish does not necessarily indicate trophic status (Garman and Macko, 1998; MacAvoy et al., 2000; Gregory-Eaves et al., 2007). The ^{13}C-enriched predators (mostly piscivorous catfish) show a wide range in $\delta^{13}C$, from –16 to –27‰ (white perch also show elevated $\delta^{13}C$ relative to most resident freshwater fish, but they also are ^{34}S-depleted, indicating that their carbon signature reflects their status as a secondary carnivore, not marine carbon). The most ^{13}C-enriched of the predators reflect the consumption of marine material, probably spawning adult *Alosa* spp., which had the most ^{13}C-enriched values of any prey item found. A number of predators, however, clearly derive very little carbon from marine migrants; they are strictly freshwater feeders, as shown by their ^{13}C-depleted carbon isotope values. Among the remaining three guilds, the planktivores (within which the anadromous *Alosa* spp., mainly filter feeders, were not included) were the most ^{13}C-enriched, driven largely by the migratory and filter-feeing gizzard shad (Jenkins and Burkhead, 1993). Gizzard shad ^{13}C enrichment probably reflects consumption of autochthonous production and not marine derived nutrients, because the gizzard shad $\delta^{34}S$ are too low to reflect substantial marine material (Table 2 and see below). The $\delta^{13}C$ range among the resident freshwater fishes suggest, not surprisingly, that both autochthonous and allochthonous production contribute to carbon fixation in this tidal freshwater stream. Indeed, in the York River estuary, a few kilometers south of the Rappahannock River, Raymond and Bauer (2001) estimate that between 38% and 56% of dissolved organic carbon was derived from internal (autochthonous) sources.

Only a small percent of the residents show an exclusive allochthonous signal in the region of the Rappahannock River examined, and most of the resident freshwater fish show an autochthonous $\delta^{13}C$ signature, which is characteristic of small tributaries close to the main stem of a large piedmont river. The $\delta^{13}C$ range of allochthonous productivity in Virginia tidal freshwater streams is between –25‰ and –28‰ (Garman and Macko, 1998; Hoffman et al., 2007). Because CO_2 solubility is limited in water, systems dominated by autochthonous production tend to be ^{13}C-enriched relative to C3 plants that appear in small streams dominated by C3 allochthonous production (Michener and Schell, 1994). Garman and Neilson (1992) note that the presence of gizzard shad and detritivores in Virginia tidal freshwater suggest that autochthonous production is important in these systems relative to non-tidal areas upstream, where fishes primarily consume terrestrial arthropods (Garman, 1991). Most of the guilds examined in this study reflected the predominance of autochthonous production and have $\delta^{13}C$ values that are lower

than would be expected for a C3 dominated system. The anadromous *Alosa* spp. were also ^{13}C-enriched relative to other guilds. All of their $\delta^{13}C$ values cluster between –22‰ and –16‰, whereas all other guilds range to approximately –28‰ range (the most ^{13}C-depleted values reflecting allochthonous production). This ^{13}C enrichment in *Alosa* spp. is not due to incorporating autochthonous freshwater production. The ^{13}C-enrichment is a signal from the marine environment from which the *Alosa* spp. biomass was derived. This interpretation is supported by the markedly ^{34}S-enriched values of the *Alosa* spp., which are in most cases 7‰ greater than any other fish in this study ($\delta^{34}S$ value of sulfur fixed from marine SO_4 in the ocean at present is highly enriched relative to freshwater [Kaplan et al., 1963]). Therefore, the ^{13}C enrichment of the *Alosa* spp. biomass (and other anadromous fishes) is due to a marine influence, not an autochthonous influence.

Of the guilds examined, predators show the highest $\delta^{34}S$ value after the *Alosa* spp., but are not significantly enriched in ^{13}C relative to other guilds. The elevated ^{34}S in predators (many of whom are piscivores) shows that more marine sulfur is incorporated by this guild relative to others. The predator's elevated $\delta^{15}N$ values place them at the top of the fish food web, although some smaller individuals (blue catfish), feed at lower trophic levels while young (Jenkins and Burkhead, 1993).

The link between anadromous *Alosa* spp. and the predators is also supported by the fatty acid carbon isotope signatures. *Alosa* spp. 16 and 18 carbon FAs were generally the most ^{13}C-enriched of the fish examined (Table 3). The two large (53cm TL) blue catfish show two very different FA isotope profiles. One blue catfish (B in Table 3) had a series of highly ^{13}C-enriched FAs (bulk muscle tissue $\delta^{13}C$ and $\delta^{34}S$ are also enriched in this individual) and the other had FAs with isotope signatures similar to allochthonus primary production (also consistent with bulk muscle tissue $\delta^{13}C$ and $\delta^{34}S$). Shorter chain (12 carbon) and more saturated FAs reveal the original $\delta^{13}C$ of the fats in the diet. Longer chain and unsaturated FAs can be subject to *de novo* transformations, which result in well established fractionations as chain length is systematically increased or as a double bond between carbons is created (making a point of unsaturation in a saturated FA). Generally, there is a 2‰ depletion in $\delta^{13}C$ arising from each unsaturation and another 2‰ depletion for each two carbon acetyl group addition (Deniro and Epstein, 1977). The most conservative tracer of dietary FAs, are the enriched precursors to long chain and unsaturated FAs. Among the FAs analyzed, the 12:0, 16:0, and 18:0 yield the best $\delta^{13}C$ estimate for dietary FAs, which clearly show distinct isotope signals depending on the carbon sources listed below: 1) ^{13}C-enriched marine isotope signals (represented by alewife and blue catfish B), 2) allochthonus production (represented by blue catfish A), or 3) a mix of autochthonous and allochthonus production, with the possibility of marine influences (represented by gizzard shad, although their $\delta^{34}S$ values do not reflect the typical marine signal).

The $\delta^{13}C$ and $\delta^{34}S$ distribution and range among the freshwater fishes suggest, not surprisingly, that both autochthonous and allochthonous nutrient sources, with the allochthonous sources being terrestrial C3 vegetation and marine primary production inwelling to this tidal freshwater stream, more than 40 km from the Chesapeake Bay. Unlike streams on the West Coast of the United States, where marine derived nitrogen and carbon can be an important nutrient source to inland ecosystems (Kline et al., 1993; Bilby et al., 2003; Chaloner et al., 2002), for all fish guilds in the study reported here, except the predators, there was not significant marine nutrient uptake. Several West Coast studies have shown that marine derived nitrogen, and some marine derived carbon, contributed to invertebrates (Francis et al., 2006; Hicks et al., 2005), primary producers, and juvenile fish within or near the sites receiving the spawning anadromous fish (Bilby et al., 2003; Koyama et al., 2005). For example, Bilby et al. (1996) found that 17% and 30% of the nitrogen in collector-gathers and juvenile coho salmon *(Oncorhynchus kisutch)* in Washington, were derived from spawning salmon. Ben-David et al. (1998) found that salmon carcasses may have contributed to the nitrogen incorporated by some terrestrial plants, as well as deer mice, squirrels, and voles; and Wipfli et al. (2003) found that salmon carcasses fueled increased growth rates among young salmonids. However, those studies show that only some material from decaying salmon makes its way into invertebrates and riparian vegetation (Bilby et al., 1996, 1998; Francis et al., 2006). There is strong evidence however, that the nutrients deposited as a result of the postspawning death of anadromous adults did significantly sustain fry the following year (Bilby et al., 1996, 1998).

In the East Coast stream examined here, carnivores and generalists, which consume benthic invertebrates as part of their diet, did not show a marine signal. Compared with anadromous salmonids on the West Coast, East Coast herring have a lower postspawning mortality and their runs have less biomass. Both of these facts indicate that a limited amount of marine protein and nitrogen maybe be delivered to spawning streams unless it is consumed directly by predatory fish. This is consistent with findings suggesting benthic insects in *Alosa* spp. spawning streams do not accumulate large amounts of marine derived material, even if they are living closely with post-spawning anadromous fish carcasses (Francis et al., 2006; Garman, 1992). It should be noted that in West Coast streams associated with spawning salmon, invertebrate uptake can be substantial (Hicks et al., 2005; Chaloner et al., 2002). Unlike most West Coast streams however, some tidal streams in Virginia have large piscivorous fish (introduced from Texas, Louisiana, or Mississippi in the 1970s) and these fish clearly incorporate marine material. So, while salmon (and presumably herring) on the West Coast import nutrients to the base of the food web (terrestrial autotrophs, young-of-the-year fish, and some invertebrates), in the steams examined here the marine material enters the top of the aquatic food web

where spawning adult anadromous fish are consumed by piscivorous fish. In order to fully understand the importance of a migratory or transitory nutrient source to consumers, the time required for that nutrient to be incorporated must be understood, thereby allowing a temporal evaluation of ecosystem structure. While the results of this study suggest that marine material does not form a substantial nutrient source to most of the fish community, more work needs to be done to investigate marine inputs derived from spawning anadromous fish, to other, lower order components of East Coast United States tidal freshwater systems.

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