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# Feeding habitats of the Gulf sturgeon, *Acipenser oxyrinchus desotoi*, in the Suwannee and Yellow rivers, Florida, as identified by multiple stable isotope analyses

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**Abstract** Stable  $^{13}\text{C}$ ,  $^{15}\text{N}$ , and  $^{34}\text{S}$  isotopes were analyzed to define the feeding habitats of *Acipenser oxyrinchus desotoi* in the Suwannee and Yellow River populations. For the majority (93.9%) of Suwannee subadults and adults,  $^{13}\text{C}$  and  $^{34}\text{S}$  signatures indicate use of nearshore marine waters as primary winter feeding habitat, probably due to the limiting size of the Suwannee Sound estuary. In the Yellow River population,  $^{13}\text{C}$  isotope signatures indicate that adults remain primarily within Pensacola Bay estuary to feed in winter, rather than emigrating to the open Gulf of Mexico. A minor Suwannee River subset (6% of samples), comprised of juveniles and subadults, displayed  $^{13}\text{C}$  signatures indicating continued feeding in freshwater during the spring immigration and fall emigration periods. This cannot be interpreted as incidental feeding since it resulted in a 20.5% turnover in tissue  $\delta^{13}\text{C}$  signatures over a 1–3 month period. Cessation of feeding in the general population does not coincide with high river water temperatures.

The hypothesis of reduced feeding in freshwater due to localized prey depletion as a result of spatial activity restriction is not supported by the present study. Instead, Suwannee River *A. o. desotoi* appear to follow two trophic alternatives; 1) complete cessation of feeding immediately upon immigration in spring, continuing through emigration 8–9 months later (the predominant alternative); 2) continued intensive feeding for 1–3 months following immigration, switching to freshwater prey, selected primarily from high trophic levels (i.e., large prey). Stable  $\delta^{34}\text{S}$  data verifies that recently immigrated, fully-anadromous *A. o. desotoi* adults had fed in nearshore marine waters, not offshore waters.

**Keywords** Food habits · Freshwater feeding · Stable isotopes · Carbon · Nitrogen · Sulfur · Suwannee River · Florida

## Introduction

Only limited investigations of the food sources and feeding chronology of the Gulf sturgeon (also referred to as the Gulf of Mexico sturgeon) *Acipenser oxyrinchus desotoi* (AOD) have been undertaken. The Gulf sturgeon is an anadromous fish species inhabiting Gulf of Mexico marine waters and coastal rivers, and following an ontogenetic progression and a seasonal migratory cycle. From spring through fall, Suwannee River adults ( $\sigma^{\text{♂}}$  >1,350 mm;  $\omega^{\text{♀}}$  >1,500 mm TL) subadults (>1000 mm TL, <1350–1500 mm TL; fully

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migratory, but lacking maturing gonads), and juveniles (<1000 mm TL) migrate to congregate within a small number of deep, spatially-confined holding areas (Foster and Clugston 1997) located in the middle (rkm 34–109) and upper river (rkm 204). However, mark and recapture tagging and recent telemetry studies have demonstrated frequent movement of individuals from one holding area to another during midsummer, covering distances as great as 50–140 rkm (Sulak et al. 2007). Holding areas appear to have hydraulic characteristics that minimize energy expenditure during a period of fasting in freshwater (Mason and Clugston 1993; Gu et al. 2001). Seasonal occupancy of holding areas is most probably related to energy conservation (Sulak et al. 2007).

Beginning in September–October, all non age-0 Suwannee River AOD migrate downstream (Foster 1993; Carr et al. 1996; Foster and Clugston 1997; Parkyn et al. 2007). Throughout winter, juveniles feed in the Suwannee estuary (Sulak and Clugston 1999; Sulak et al. 2009). However, fully anadromous sub-adults and adults emigrate first to the estuary, then to nearshore feeding grounds (Foster 1993; Foster and Clugston 1997; Edwards et al. 2003, 2007; Parkyn et al. 2007), and ultimately to foraging areas in the open Gulf of Mexico (Edwards et al. 2003).

Understanding utilization of marine, versus estuarine, versus freshwater feeding habitats has remained problematic in AOD. Moreover, Gulf of Mexico winter feeding areas have remained poorly defined. Boat-tracking of acoustically-tagged Pearl River and Pascagoula River AOD has suggested that feeding is restricted to shallow, nearshore areas (Ross et al. 2009). However, limited acoustic surveying has been accomplished in the open Gulf of Mexico. Recent information from data-logging receivers also indicates that feeding is restricted to nearshore waters (Edwards et al. 2007). For AOD populations inhabiting rivers that empty into bays (e.g., Choctawhatchee Bay, Pensacola Bay) and sounds (e.g., Mississippi Sound) such semi-enclosed basins may serve as the primary winter feeding habitat (Fox et al. 2002). Use of the open gulf may be limited to adult females needing high energy intake to complete ovarian maturation (Fox et al. 2000). However, Suwannee Sound is probably too small to sustain the trophic needs of the large Suwannee population and too shallow (<2 m deep) to protect against winter cold kill. Only juveniles

feed within the Suwannee estuary throughout winter (Sulak et al. 2009). The rest of the population moves first into nearshore waters (Harris et al. 2005; Parkyn et al. 2007), then disperses into the open gulf (Edwards et al. 2007).

A pattern of winter weight gain, alternating with spring to fall weight loss has been reported for AOD (Wooley and Crateau 1985; Mason and Clugston 1993; Foster and Clugston 1997; Clugston et al. 1995; Sulak and Clugston 1999). Winter weight gains up to 80–120% have been documented (Wooley and Crateau 1985; Sulak and Clugston 1999), in contrast to summer weight loss.

Based on stomach content analysis Mason and Clugston (1993) concluded that AOD (excluding age-0 fish) cease feeding in freshwater, fasting for 8–10 months. These authors speculated that feeding cessation may be due to limiting high water temperatures, but did not explain why feeding ceases in springtime at temperatures well below 20°C. Subsequently, Chapman and Carr (1995) and Carr et al. (1996) advanced the more involved thermal barrier hypothesis that AOD are restricted from feeding in summer due to confinement within putative thermal refugia where prey become depleted and unavailable. Gu et al. (2001) conducted a limited stable carbon isotope analysis of 36 Suwannee River AOD (580–1820 mm TL) from two short rounds of sampling, concluding that AOD “...do not feed significantly in fresh waters”.

The primary goal of the present study is to utilize multiple stable isotope data to define the fundamental carbon sources and primary feeding habitats of the Suwannee River population of AOD. A secondary goal is to evaluate differences in feeding habitats between Suwannee River and Yellow River AOD. The Suwannee River empties into Suwannee Sound, a shallow (<2 m), muddy sand substrate, mesohaline oyster-bar limited estuary of rather small area (81 km<sup>2</sup>). Winter re-locations of AOD in Suwannee nearshore areas coincide with concentrations of marine benthic prey (Fox et al. 2002; Edwards et al. 2003; Brooks and Sulak 2005; Harris et al. 2005; Sulak et al. 2007; Ross et al. 2009; Sulak et al. 2009). In contrast, the Yellow River empties into the relatively vast (377 km<sup>2</sup>) mesohaline Pensacola Bay, which may provide ample AOD winter feeding habitat and prey resources. Thus, migration into the open Gulf of Mexico may be unnecessary, as has been suggested for the Choctawhatchee Bay AOD population (Fox et al. 2000, 2002).

This study analyzes the  $^{13}\text{C}$  stable carbon isotope to identify feeding habitats based on isotope signatures that tend to be highly conserved in fin and muscle tissues (Hesslein et al. 1993; MacAvoy et al. 2001; Harvey et al. 2002). To evaluate the trophic level of prey consumed,  $^{15}\text{N}$  stable nitrogen isotope data were analyzed according to stepwise enrichment typical of  $^{15}\text{N}$  up the food chain (DeNiro and Epstein 1978; Fry et al. 1984; Owens 1987). Finally,  $^{34}\text{S}$  stable sulfur isotope evidence from Suwannee River and Yellow River AOD was used to distinguish between nearshore-estuarine versus offshore marine winter feeding habitats, given the distinctive  $^{34}\text{S}$  signature of oceanic sulfate (Peterson and Howarth 1987), and as an independent test of freshwater versus estuarine-marine feeding. To ground-truth AOD feeding habitat, means of  $^{13}\text{C}$ ,  $^{15}\text{N}$  and  $^{34}\text{S}$  data were compared with mean isotopic values obtained for 19 taxa of North-eastern Gulf of Mexico (NEGOM) freshwater, estuarine, and marine consumers.

The current paradigm for AOD juvenile through adult trophic behavior upon entry into the Suwannee River in February–April is that feeding ceases in freshwater, resuming again when the population emigrates back into saline waters in October–November. This is based on stomach content analyses (Huff 1975; Mason and Clugston 1993; Carr et al. 1996) and on limited  $^{13}\text{C}$  analyses (Gu et al. 2001). An initial thermal refuge hypothesis (Mason and Clugston 1993) proposed that AOD feeding stopped due to thermal limitation of feeding activity in the river in summer. A more involved thermal barrier hypothesis (Chapman and Carr 1995; Carr et al. 1996; Gu et al. 2001) proposed that reduced feeding in freshwater was due to spatial confinement to putative thermal refugia (summer holding areas), where prey resources become exhausted. Sulak et al. (2007) hypothesized that AOD feeding ceased in freshwater since the energetic cost of foraging in the oligotrophic Suwannee River exceeded the trophic benefit obtained.

The rapid and dramatic weight gains documented for AOD indicate intense feeding in estuarine-marine habitats (Heard et al. 2000; Fox et al. 2002; Edwards et al. 2003; Brooks and Sulak 2005; Harris et al. 2005; Sulak et al. 2007; Ross et al. 2009; Sulak et al. 2009). Prey-rich soft substrates occur in shallow coastal bays and nearshore waters (1–10 m depth), as well as in deep offshore waters (>30 m depth). Thus, Suwannee River AOD either remain within shallow nearshore

areas (<10 m depth), or they migrate to deeper (30–200 m) offshore areas.

Using multiple stable isotope methods (Peterson and Howarth 1987; Sullivan and Moncreiff 1990), this study addresses these fundamental questions regarding AOD feeding habitat utilization: 1) Beyond age-0, does the population utilize freshwater prey while in the Suwannee River? 2) Do migratory AOD adults utilize primarily shallow nearshore versus deeper offshore habitat in winter? 3) Is the growing Suwannee population experiencing a paradigm shift in feeding habitat use?

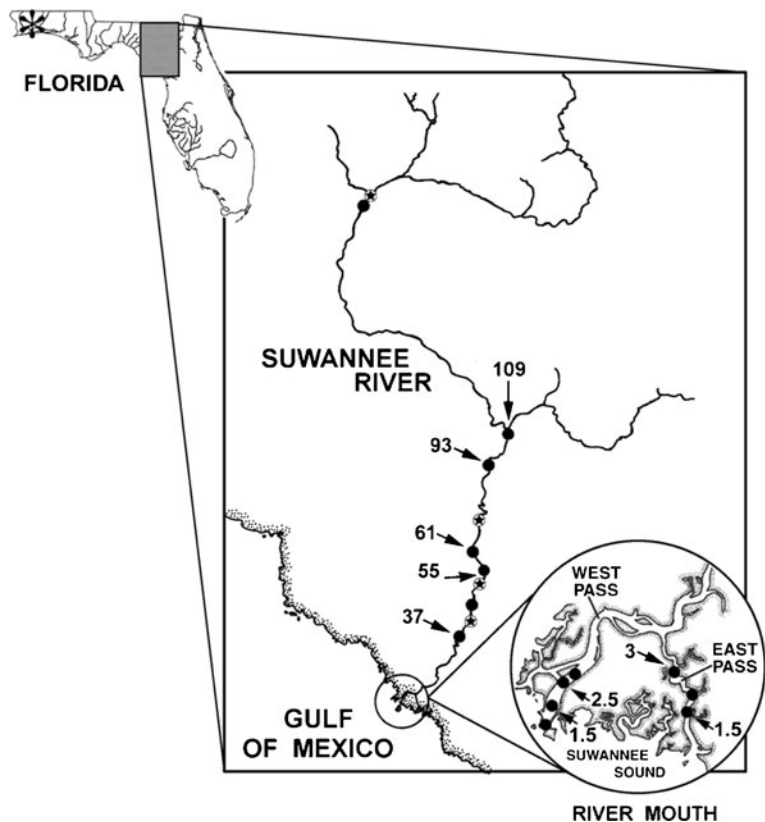
## Materials and methods

### Study areas and samples

A total of 262 tissue samples were obtained from 260 individuals collected in the Suwannee River in 1998–2002. Two individuals were sampled twice in repeat captures. Two age-0 individuals (94 and 256 mm TL) were included. Most specimens ( $N=240$  specimens, 365–1998 mm TL, yielding 242 samples) were collected in gill nets between rkm 1.5 and 109 (Fig. 1) and from all months except December and January. Relatively few samples were available from July and August when tissue sampling was minimized to avoid handling stress. Nineteen additional juveniles (575–882 mm TL), preserved frozen at  $-10^{\circ}\text{C}$  until analyzed, were available from pre-1996 U.S. Fish and Wildlife Service (USFWS) gill net collections. Three AOD (1138–1528 mm TL) were 1999–2000 recaptures of hatchery-reared individuals released into the Suwannee River in 1992. Additionally, ten adults (1766–2010 mm TL) from the Yellow River (Fig. 1) (Sturgeon Lake, rkm 13, Lat.  $30.5823^{\circ}\text{N}$ , Long.  $-86.9119^{\circ}\text{W}$ , May 2002) were used to enable a basic AOD inter-population trophic habitat use comparison.

Other taxa representative of freshwater, estuarine and marine feeding habitats were also collected for comparative isotope analyses. Included were six comparative fish and invertebrate consumer species from the Suwannee River, six estuarine-nearshore fish species collected in Suwannee estuarine waters and seven fish and invertebrate taxa from deep (75 m) offshore NEGOM marine waters (Sulak et al. 2010). Tissue samples for analysis were taken

**Fig. 1** Map of the Suwannee River showing the main *Acipenser oxyrinchus desotoi* sampling locations (solid dots). The primary locations for specimens used in the present study are denoted with arrows and rkm labels. Asterisk on the Florida outset denotes the location of the Yellow River sampling area



either immediately upon collection, or in the laboratory from fresh specimens placed on ice in the field. All study specimens are summarized in Table 1.

#### Sample processing

The trauma and potential infection risk associated with invasive sampling of muscle tissue from live AOD for stable isotope samples was avoided. Instead, for live net-collected specimens, marginal dorsal or pectoral fin tissue was utilized. Using sterilized surgical scissors, a 15-mm long by 2-mm wide strip of tissue (ca 0.5 g), sufficient for  $^{13}\text{C}$ ,  $^{15}\text{N}$  and  $^{34}\text{S}$  analyses, was excised from the trailing edge of the fin for most specimens. For age-0 specimens only a 5-mm by 1-mm sample was taken, providing sufficient mass for  $^{13}\text{C}$  and  $^{15}\text{N}$  analysis only. The broad-spectrum antibiotic Panalog<sup>®</sup> was applied to the fin clip area prior to release. For frozen USFWS pre-1996 specimens, marginal fin tissue had been degraded by oxidation during long storage. Thus, for these specimens, 1.0–2.0 g of white muscle

tissue was excised from the anterior dorso-lateral musculature below the epidermis. White muscle tissue was also sampled from comparative fish species since the thin fin membranes of most teleosts were unsatisfactory for tissue sampling. Although skin (epidermal/dermal) tissue has a somewhat slower turnover rate than metabolically more active white muscle (Pinnegar and Polunin 1999), turnover rates for all fish tissues are similar (Hesslein et al. 1993; MacAvoy et al. 2001). White muscle in juvenile and adult fishes preserves isotope signatures for 3–15 months (Hesslein et al. 1993; MacAvoy et al. 2001; Harvey et al. 2002), a sufficiently long time interval to conserve signatures of prey consumed prior to river immigration and dietary switches after immigration (Monteiro et al. 1981). Soft body tissues were obtained from three comparative invertebrate species (Table 1). Fresh, mechanically-cleaned (scales, bone, exoskeleton, and shell removed), tissue samples were rinsed in de-ionized water in the field and/or in the laboratory, placed in labeled plastic snap-cap vials or aluminum foil packets, and frozen

**Table 1** Multiple stable isotope data (mean ± SD) for *Acipenser oxyrinchus desotoi* and comparative species analyzed in the present study (supplemented by selected data from the literature). Habitat key: EB = estuarine benthic, MB = marine benthic, ME = marine epipelagic, RB = riverine (freshwater) benthic, RP = riverine (freshwater) pelagic. Sampling area key: GM = Gulf of Mexico outer continental shelf, SE = Suwannee estuary, SR = Suwannee River, WI = Wisconsin, YR = Yellow River. The quantity 'N' refers to samples for simultaneous  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analyses. The quantity 'n' refers to samples used for  $\delta^{34}\text{S}$  analysis. Species 2-letter abbreviations in parentheses are those used in figures

Species	TL (mm)	Habitat	Area	'N'	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{34}\text{S}$ (‰)
<b>Gulf Sturgeon:</b>							
<i>A. o. desotoi</i> main SR subset	365–1998 mm	RB	RB	244(C), 243(N)	-18.2±0.9	+10.5±0.7	+13.4±1.9 (14)
<i>A. o. desotoi</i> minor SR subset	565–1300 mm	RB	SR	16	-22.9±1.5	+11.6±1.1	+13.5±1.4 (10)
<i>A. o. desotoi</i> SR age-0	94–256 mm	RB	SR	2	-29.8, -32.1	+8.5, +11.6	
<i>A. o. desotoi</i> YR set	1766–2010 mm	RB	YR	10	-15.8±0.8	+12.5±0.5	+13.3±1.4 (10)
<i>A. o. desotoi</i> (Gu et al. 2001)	580–1820 mm	RB	SR	36	-18.5±0.9		
<i>Acipenser fulvescens</i> Lake Sturgeon (Stelzer et al. 2008)	>910 mm	Lake Winnebago	WI	62 (muscle)	-27.9	+12.7	
<b>Comparative Suwannee Sound and Cedar Key Estuarine Taxa:</b>							
<i>Arius felis</i> (AF) Hardhead Catfish		EB	SE	21	-19.7±1.6	+10.1±1.0	
<i>Bairdiella chrysoura</i> (BC) Silver Perch		EB	SE	29	-18.0±1.7	+12.1±1.6	+11.6±1.0 (10)
<i>Bagre marina</i> (BM) Gafftopsail Catfish		EB	SE	16	-17.2±1.7	+12.1±1.1	
<i>Cynoscion nebulosus</i> (CN) Spotted Seatrout		EB	SE	19	-17.2±1.9	+12.4±1.8	+11.0±2.8 (10)
<i>Lagodon rhomboides</i> (LR) Pinfish		EB	SE	6	-19.1±2.4	+ 9.6±1.8	
<i>Micropogonias undulatus</i> (MU) Atlantic Croaker		EB	SE	4	-20.3±0.8	+14.1±0.3	
Comparative Suwannee Sound and Cedar Key Estuarine Taxa - range in means					-17.2 to -20.3	+9.6 to +14.1	+11.0 to +11.6
<b>Comparative Suwannee River Freshwater Taxa:</b>							
<i>Corbicula fluminea</i> <sup>a</sup> ≤20 mm (CF) Asian Clam		RB	SR	31	-33.1±1.2	+7.7±0.4	
<i>Corbicula fluminea</i> <sup>a</sup> >20 mm (CF) Asian Clam		RB	SR	17	-30.6±0.7	+7.3±0.3	
<i>Lepomis auritus</i> (LA) Redbreast Sunfish		RB	SR	11	-27.3±0.7	+8.5±0.4	
<i>Notropis harperi</i> (NI) Redeye Chub		RB	SR	14	-27.0±0.2	+10.5±0.2	
<i>Poecilia latipinna</i> (PL) Sailfin Molly		RP	SR	23	-25.9±0.3	+7.3±0.5	-4.2±0.7 (9)
<i>Trinectes maculatus</i> <sup>b</sup> (TM) Hogchoker (freshwater juveniles)		RB	SR	16	-27.8±1.5	+8.8±1.0	
Comparative Suwannee River Freshwater Taxa - range in means					-25.9 to -33.6	+7.3 to +10.5	-4.2
<b>Comparative Offshore GM Marine Taxa:</b>							
<i>Hemanthias vivanus</i> (HV) Red Barbier		MB	GM	30(C), 29(N)	-17.6±0.4	+11.0±0.4	
<i>Parques umbrosus</i> (PU) Cubbyu		MB	GM	30	-16.6±0.4	+14.5±1.1	
<i>Paroxcoetus brachypterus</i> (PB) Sailfin Flyingfish		ME	GM	26	-17.2±0.4	+8.3±0.8	
<i>Pristigymys alta</i> (PA) Short Bigeye		MB	GM	10	-17.5±0.9	+12.2±0.8	



Table 1 (continued)

Species	TL (mm)	Habitat	Area	'N'	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{34}\text{S}$ (‰)
<i>Promotgrammus martinicensis</i> (PM) Roughtongue Bass		MB	GM	25	-17.7±0.4	+11.8±0.4	+19.9±0.6 (10)
<i>Nicella</i> sp. <sup>c</sup> (NI) (red gorgonian)		MB	GM	25	-15.0±2.2	+9.4±0.5	
<i>Argopecten gibbus</i> <sup>a</sup> (AG) Calico Scallop		MB	GM	23	-17.6±0.5	+7.2±0.3	
Comparative Offshore GM Marine Taxa - range in means					-15.0 to -17.7	+7.2 to +14.5	+19.9
Oceanic Fish (Hesslein et al. 1993)							+17 to +18

<sup>a</sup> filter-feeding pelecypod

<sup>b</sup> freshwater juvenile phase of estuarine fish species

<sup>c</sup> sessile particulate-feeding gorgonian

pending laboratory processing. Muscle tissue samples from pre-1996 frozen specimens were similarly prepared. All samples were subsequently dried at 50° C for a minimum of 24 h and ground to powder with a mortar and pestle. From each powdered sample, a 100±1.0 µg subsample for <sup>13</sup>C and <sup>15</sup>N analyses was placed in a metallic foil capsule in a numbered multi-well analysis plate designed for processing stable isotope samples. For <sup>34</sup>S analysis a 500±1.0 µg subsample of powdered dry tissue was prepared. All samples were sent to specialized stable isotope service laboratories for analysis, accomplished within 1–6 mo of collection and sample preparation (except samples from the USFWS pre-1996 frozen specimens).

#### Stable isotope analysis

For  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  determinations, all prepared samples were analyzed at the University of California at Davis Stable Isotope Laboratory in 1998–2002. Two carbon and nitrogen reference standards (Pee Dee Belemnite and atmospheric nitrogen, respectively) were analyzed per each set of 12 tissue samples analyzed. Analytical precision based on 276 such reference standards run at the same time as test samples was ±0.06‰ for  $\delta^{13}\text{C}$  and ±0.17‰ for  $\delta^{15}\text{N}$ . Additionally, samples from 24 Suwannee River AOD, ten Yellow River AOD, and specimens of five comparative taxa (species representative of the freshwater riverine fauna of the Suwannee River, were subsequently analyzed for  $\delta^{34}\text{S}$  at the U.S. Geological Survey (USGS) Western Region Center stable isotope laboratory, Menlo Park, CA. Specimens for sulfur isotope analysis were selected from among those displaying <sup>13</sup>C signatures less enriched than -21‰ (i.e., those potentially indicative of freshwater feeding), and from the largest adults available (i.e., the most likely candidates for feeding in open marine waters), particularly individuals collected in spring and early summer (i.e., potentially retaining isotope signatures from recent winter marine feeding). Canyon Diabolo troilite was the reference standard utilized for sulfur isotope analyses. Analytical precision was <0.02‰.

#### Stable isotope data interpretation

Based on the literature, with focus on Gulf of Mexico information (Table 2), a  $\delta^{13}\text{C}$  value of -25‰ was

**Table 2** Stable  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  data (means  $\pm$  SD) reported in the literature for carbon sources in the northeastern Gulf of Mexico (NEGOM) region (supplemented with data from other regions where NEGOM data are limited). Key: DOC = dissolved organic carbon, GOM = Gulf of Mexico region, NWGOM = northwestern Gulf of Mexico, POM = particulate organic matter

Carbon source	N	Size range (mm)	$\delta^{13}\text{C} \pm \text{SD}$ (‰)	$\delta^{15}\text{N} \pm \text{SD}$ (‰)	$\delta^{34}\text{S} \pm \text{SD}$ (‰)
Marine holoplankton (NEGOM and comparative regions)					
POM and mesoplankton, NEGOM, sea surface over ~75 m bottom depth (Sulak et al. 2010)	32	>125 $\mu\text{m}$ <335 $\mu\text{m}$	-19.4 $\pm$ 1.2	+8.1 $\pm$ 0.7	
POM NEGOM, sea surface over 90 m bottom depth (Thayer et al. 1983)		>125 $\mu\text{m}$	-21.8 $\pm$ 1.8		
POM NEGOM (Eadie and Jeffrey 1973)		0.3 $\mu\text{m}$	-19.6 to -20.5		
Mesoplankton NEGOM, sea surface over 90 m bottom depth (Thayer et al. 1983)		>500 $\mu\text{m}$	-21.5 $\pm$ 0.5		
POM NWGOM (Macko et al. 1984)			-21.0 $\pm$ 1.4	+7.5 $\pm$ 0.8	
Mesoplankton NWGOM (Macko et al. 1984)			-19.2 $\pm$ 0.7	+8.9 $\pm$ 0.9	
POM GOM (Calder and Parker 1968)			-22.2		
POM GOM (Fry and Sherr 1989)	87		-21.7 $\pm$ 1.6		
Marine plankton (Peterson et al. 1985)			-21.3 $\pm$ 1.1	+8.6 $\pm$ 1.0	+18.0 $\pm$ 0.6
			<b>-19.2 to -22.2</b>	<b>+7.5 to +8.9</b>	<b>+18.0</b>
<b>RANGE FOR MARINE SOURCES FROM CITED LITERATURE</b>					
Marsh & Estuarine Plants					
<i>Spartina</i> spp., Barataria Basin marshes, LA (Chmura et al. 1987)			-11.7 to -13.6		
<i>Spartina alterniflora</i> , Sapelo Island, GA (Peterson and Howarth 1987)			-12.9 $\pm$ 0.5	+6.0 $\pm$ 2.1	+0.9 $\pm$ 5.2
<i>Spartina alterniflora</i> , Sapelo Island, GA (Haines 1976)			-12.3 to -13.4		
<i>Spartina</i> , Graveline Bay Marsh, MS (Sullivan and Moncreiff 1990)			-13.2 $\pm$ 0.1	+5.2 $\pm$ 0.5	+1.4 $\pm$ 2.2
<i>Spartina alterniflora</i> , NJ (Currin et al. 2003)			-12.3 to -13.2	+11.3 to +12.4	+13.2 to +15.1
Edaphic microalgae, Graveline Bay Marsh, MS (Sullivan and Moncreiff 1990)			-20.6	+6.1	+14.3
Salt marsh diatoms, Bay of Mont Saint Michel, France (Créach et al. 1997)			-14.4 to -20.1	+5.9 to +6.0	
Estuarine macroalgae, <i>Ulva</i> and <i>Gracilaria</i> (Simenstad and Wissmar 1985)			-10.5 to -20.5		
4 species of Caribbean seagrasses (Fry et al. 1982)			-4.0 to -13.2		
Estuarine surface sediment, Kukjuktuk Bay, Alaska (Hesslein et al. 1991)			-23.3	+7.1	+13.0
Salt-marsh zooplankton, Graveline Bay Marsh, MS (Sullivan and Moncreiff 1990)			-4.0 to -23.3	<b>+5.2 to +12.4</b>	<b>+0.9 to +15.1</b>
					<b>+10.7 to +14.3 (excl. <i>Spartina</i>)</b>
<b>RANGE FOR ESTUARINE &amp; SALT MARSH SOURCES FROM CITED LITERATURE</b>					
Terrestrial Plants (C-3 photosynthesis)					
Upland oaks and pines, Sapelo Island, GA (Peterson and Howarth 1987)			-29.3 $\pm$ 1.4	+4.0 $\pm$ 0.9	+1.8 $\pm$ 1.0
Upland C-3 plants (Peterson et al. 1985)			-28.6 $\pm$ 1.3	-0.6 $\pm$ 1.2	+4.7 $\pm$ 0.9



Table 2 (continued)

Carbon source	N	Size range (mm)	$\delta^{13}\text{C} \pm \text{SD}$ (‰)	$\delta^{15}\text{N} \pm \text{SD}$ (‰)	$\delta^{34}\text{S} \pm \text{SD}$ (‰)
Upland fir, maple and alder (Simenstad and Wissmar 1985)			-29.2 to -31.0		
Terrestrial DOC, Mississippi River (Eadie et al. 1978)			-26.0, -28.0		
Terrestrial plant POM in GOM estuaries (Shultz and Calder 1976)			-26.0		
<b>RANGE FOR TERRESTRIAL SOURCES FROM CITED LITERATURE</b>			<b>-26.0 to -31.0</b>	<b>-0.6 to +4.0</b>	<b>+1.8 to +4.7</b>

accepted as an a priori threshold to differentiate a freshwater diet based on less enriched ( $\delta^{13}\text{C} < -25\text{‰}$ ) terrestrial plant C-3 carbon from a diet based on more enriched ( $\delta^{13}\text{C} > -25\text{‰}$ ) marine or estuarine phytoplankton, C-4 marsh plants, edaphic marsh microalgae and/or estuarine macroalgae (Table 2). Based on Fry and Sherr (1989), a  $\delta^{13}\text{C}$  value of  $-21\text{‰}$  was accepted as a second threshold to differentiate a diet including mixed freshwater-estuarine carbon sources ( $\delta^{13}\text{C} > -21\text{‰}$ ) from a diet of mixed estuarine-marine sources ( $\delta^{13}\text{C} < -21\text{‰}$ ). Thayer et al. (1983) determined that marine plankton was the major carbon source for marine neritic food webs in the NEGOM. Data from comparative regions are similar,  $-19.2\text{‰}$  to  $-22.2\text{‰}$ , contrasting with signatures for typical salt marsh primary producers,  $-4\text{‰}$  to  $-20.6\text{‰}$  (Table 2), and confirming a marine versus estuarine  $^{13}\text{C}$  threshold at  $-21$  to  $-22\text{‰}$ . Values of  $\delta^{13}\text{C}$  for strictly upland terrestrial plants fueling freshwater rivers range from  $-26.0\text{‰}$  to  $-31.0\text{‰}$  (Table 2), indicating an estuarine-marine versus terrestrial  $^{13}\text{C}$  threshold of  $-25\text{‰}$ .

For  $\delta^{34}\text{S}$ , a threshold of  $+8\text{‰}$  was selected to differentiate sulfate of terrestrial/freshwater origin ( $+2\text{‰}$  to  $+8\text{‰}$ , Peterson and Howarth 1987) versus estuarine origin (generally  $> +8\text{‰}$  to  $< +17\text{‰}$ , excluding refractory *Spartina*). A  $\delta^{34}\text{S}$  threshold of  $+17\text{‰}$  was selected to identify sulfate from terrestrial sources based on a range of  $+16.6\text{‰}$  to  $+20.3\text{‰}$  previously reported for marine ecosystems (Tables 1 and 2). No  $\delta^{34}\text{S}$  data appear to be available for NEGOM marine plankton at the base of the food chain. However, one trophic step higher on the carbon food chain, a  $\delta^{34}\text{S}$  value of  $+19.9 \pm 0.6\text{‰}$  has been reported by Sulak et al. (2010) for a NEGOM site-attached, planktivorous deep-reef fish. This value is just outside the range of  $+17\text{‰}$  to  $+18\text{‰}$  reported by Hesslein et al. (1993) for 'oceanic fish', but contrasts with the range of  $+4\text{‰}$  to  $+16\text{‰}$  for Mississippi salt marsh fishes (Sullivan and Moncreiff 1990). Tissue levels of  $\delta^{34}\text{S}$  in fishes tend to be closely tied to the sulfate source, with no enrichment up the food chain (Hesslein et al. 1991).

#### Statistical analyses

*Descriptive Statistics* Means  $\pm$  SD in univariate isotopic data were calculated for sets and subsets of AOD samples assembled based on the selected  $\delta^{13}\text{C}$  thresholds, and by river origin. Means  $\pm$  SD

were also calculated for comparative taxa. Plots of  $\delta^{15}\text{N}$  versus  $\delta^{13}\text{C}$  means, and  $\delta^{34}\text{S}$  versus  $\delta^{13}\text{C}$  values were prepared for visual contrast with reference to  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  thresholds in order to identify primary AOD feeding habitats for the comparative sample sets and subsets.

*Analysis of Similarity (ANOSIM)* The ANOSIM utility in Primer 6<sup>®</sup> software (Clarke and Gorley 2006) was used to independently test the null hypothesis that no significant differences exist among the threshold-determined sets and subsets of AOD samples in multivariate matrices of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data, or  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  isotope data. The Excel<sup>®</sup> raw data set of stable isotope values for AOD samples was converted into a Bray-Curtis coefficient resemblance matrix and analyzed via a one-way ANOSIM, allowing for a maximum of 999 permutations of the data. Individual 'R' statistic values were plotted against the determined 'Global R' statistic to evaluate significant departures from the null hypothesis. Since only positive data values can be utilized in ANOSIM,  $\delta^{13}\text{C}$  values in the raw data matrix (all negative) were first converted to absolute values. Values for  $\delta^{34}\text{S}$  (a mix of negative and positive values) were brought into a positive range by addition of the constant 10 to each value. Units for converted values for all three stable isotope variables ranged from ones to tens, avoiding disproportionate weighting of scores for any individual variable in the resultant resemblance matrix. Approximate equivalency in range of scores obviated the need for further standardization or transformation.

*Two-Sample Contrasts* Following ANOSIM substantiation that statistically significant differences ( $p < 0.05$ ) existed among sets and subsets of AOD samples, two-sample contrasts were undertaken. SigmaStat 2.03<sup>®</sup> software was used to analyze univariate stable isotope data. When comparative data passed an equal variance test, a parametric contrast ( $t$ -test on means) was applied. When data failed an equal variance test, a non-parametric contrast (Mann–Whitney Rank Sum test on medians) was applied.

*Cluster Analysis* Cluster analysis was used to independently group AOD samples to test the integrity of sets and subsets determined by  $\delta^{13}\text{C}$  thresholds. The untransformed resemblance matrix of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data for all AOD samples was analyzed using the

Bray-Curtis coefficient and the group-average linking strategy. Statistically significant ( $p < 0.05$ ) groups were identified using the SIMPROF utility in Primer 6 based on 999 permutations of the data in the Bray-Curtis resemblance matrix. SIMPROF identified the percent resemblance level at which all major significant group separations occurred.

*Ordination* Non-metric multi-dimensional scaling (MDS) was employed, using Primer 6 software, as a second independent multivariate method for statistically grouping individual samples by spatial proximity in MDS space, based on analysis of the Bray-Curtis similarity matrix for multiple stable isotopes. This provided an objective test of the reality of sets and subsets of AOD samples originally assembled based on empirical  $\delta^{13}\text{C}$  thresholds. The resemblance matrices used for MDS were based on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, or  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values, determined for individual samples of AOD and comparative species. MDS, which is considered as one of the most effective ordination methods for ecological analyses (Everitt 1978; Kenkel and Orloci 1986), maps the similarity or dissimilarity of samples in non-metric space (Clarke and Warwick 2001). The goodness-of-fit of MDS ordination plots was evaluated by the calculated Kruskal model-1 'stress' value, a chi-square-like statistic (Gotelli and Ellison 2004). A minimum stress value of 0.00 (methodologically set to 0.01) indicates no departure of data points (no scatter) from a perfect fit to the Shepard diagram regression line. Stress values  $\ll 0.20$  indicate data trends that have high non-parametric statistical rigor. All MDS analyses utilized 50 iterations of the data.

#### Isotope-size and isotope-season relationships

Stable  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data were plotted against specimen total length (mm) to elucidate potential trophic relationships correlated with specimen size. Stable  $\delta^{13}\text{C}$  data were plotted against month, and against a backdrop of mean temperature by month for middle reaches of the Suwannee River, to elucidate potential trophic relationships correlated with season and water temperature. Stable  $\delta^{15}\text{N}$  data were also plotted against month to test the null hypothesis that means per month did not differ among themselves. A Kruskal-Wallis one-way analysis of variance on ranks test was utilized.

## Results

### Empirically-defined sets and subsets

Based on habitat-specific  $\delta^{13}\text{C}$  isotope threshold values, the set of Suwannee River AOD samples was separated into three subsets (Fig. 2): 1) a main subset of 244 samples in the estuarine-marine range ( $\delta^{13}\text{C} > -21\text{‰}$ ); 2) a minor subset of 16 samples in the mixed estuarine-marine and freshwater range ( $\delta^{13}\text{C} < -21\text{‰}$  to  $> -25\text{‰}$ ), and a final subset of two YOY samples in the wholly freshwater range ( $\delta^{13}\text{C} < -25\text{‰}$ ). All ten Yellow River samples comprised a set that fell into the estuarine-marine range.

### Descriptive statistics

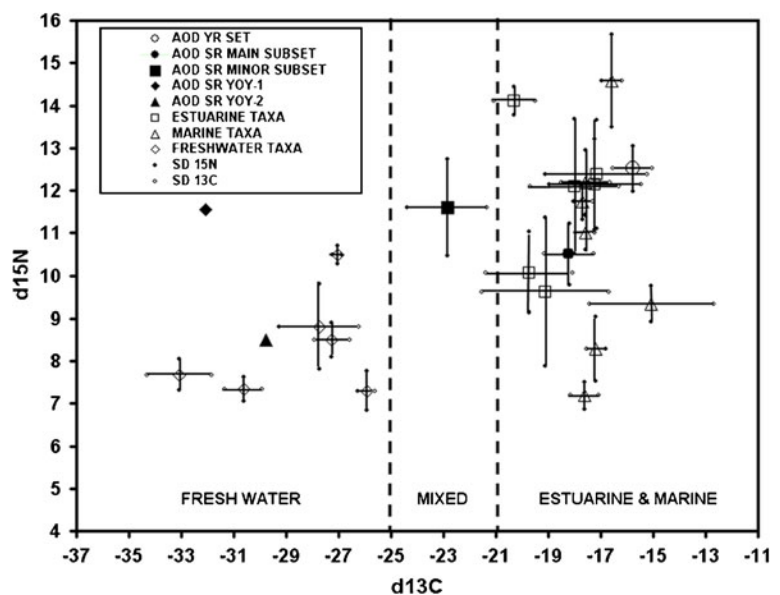
Means  $\pm$  SD for sets and subsets of AOD determined, based on stable isotope thresholds and river origin, are given in Table 1. The mean in  $\delta^{13}\text{C}$  values ( $-18.2 \pm 0.9\text{‰}$ ) for the main Suwannee River AOD subset from all months and river reaches sampled fell within the range of means for six comparative resident Suwannee Sound - Cedar Key estuarine taxa, distinctly outside the range of means of six comparative Suwannee River freshwater taxa (Table 1, Fig. 2). However, the  $\delta^{13}\text{C}$  mean ( $-22.9 \pm 1.5\text{‰}$ ) for the minor Suwannee River subset (16 juveniles and subadults) fell intermediate between means for comparative marine versus freshwater taxa (Table 1).

Values in  $\delta^{13}\text{C}$  for the final subset comprising two age-0 Suwannee River individuals ( $-29.8\text{‰}$ ,  $-32.1\text{‰}$ ) fell within the range of means for all comparative freshwater taxa analyzed (Table 1), but were slightly more negative than comparative literature values for terrestrial carbon (Table 2). The mean in  $\delta^{13}\text{C}$  values ( $-15.8 \pm 0.8\text{‰}$ ) for the Yellow River set (10 adults) fell intermediate between the means for comparative estuarine and marine taxa analyzed (Table 1).

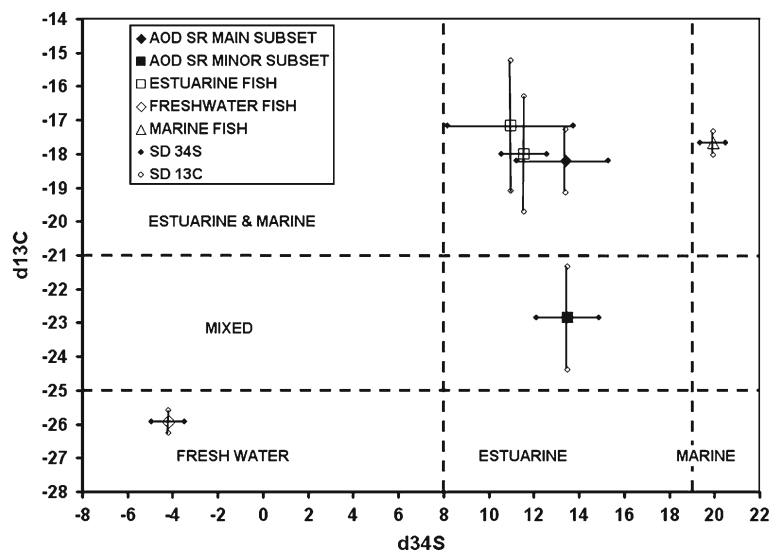
Means  $\pm$  SD in  $\delta^{15}\text{N}$  values for the Suwannee River main and minor AOD subsets, and the Yellow River set, ranged from  $+10.5\text{‰}$  to  $+12.5\text{‰}$  (Table 1), approximately 1–2 trophic steps (assuming 1.8‰ to 3.0‰ per step) enrichment (DeNiro and Epstein 1978; Fry et al. 1984; Owens 1987) versus the range reported for Gulf of Mexico marine plankton,  $+8.1\text{‰}$  to  $+8.9\text{‰}$  (Table 2). Means for these AOD sets and subsets fell within the range of  $+9.6\text{‰}$  to  $+14.1\text{‰}$  for six comparative Suwannee Sound-Cedar Key estuarine fish taxa, but above the range of  $+7.3\text{‰}$  to  $+10.5\text{‰}$  for three Suwannee River freshwater fish taxa analyzed (Table 1). The mean in  $\delta^{15}\text{N}$  ( $+12 \pm 0.5\text{‰}$ ) for the Yellow River AOD set was approximately one trophic step higher than the mean for the Suwannee main subset ( $+10 \pm 0.7\text{‰}$ ).

Means  $\pm$  SD in  $\delta^{34}\text{S}$  values for all AOD sets and subsets were confined between the  $+8\text{‰}$  and  $+17\text{‰}$  thresholds representing the lower and upper limits typically bracketing sulfate sources in Gulf of Mexico estuarine ecosystems (Fig. 3, Table 2). Means for AOD sets

**Fig. 2** Plot of  $\delta^{13}\text{C}$  (‰) versus  $\delta^{15}\text{N}$  (‰) data (means  $\pm$  SD) for *Acipenser oxyrinchus desotoi* (AOD) compared with data for other Suwannee River freshwater, Suwannee-Cedar Key estuarine, and Gulf of Mexico offshore marine species. *SR* Suwannee River; *YR* Yellow River; *YOY* age-0 AOD; *SD* standard deviation. Dashed lines denote  $\delta^{13}\text{C}$  carbon source thresholds



**Fig. 3** Plot of  $\delta^{13}\text{C}$  (‰) versus  $\delta^{34}\text{S}$  (‰) data (means  $\pm$  SD) for 24 Suwannee River (SR) and 10 Yellow River (YR) *Acipenser oxyrinchus desotoi* (AOD) compared with data for one Suwannee River freshwater species, two Suwannee-Cedar Key estuarine fish species, and one Gulf of Mexico offshore marine species. Dashed horizontal lines denote  $\delta^{13}\text{C}$  carbon source thresholds; dashed vertical lines denote  $\delta^{34}\text{S}$  source thresholds



and subsets (+13.3‰ to +13.5‰) were within the *Spartina* marsh cordgrass range (+13.2‰ to +15.1‰) reported by Currin et al. (2003), but distinct from less enriched *Spartina* values reported elsewhere (Table 2). Means were below the marine plankton value (+18.0‰) reported by Peterson et al. (1985), below the general range (+16.6‰ to +20.3‰) reported in the literature for marine ecosystems (Table 2), and below the value (+19.9‰) determined herein for one comparative offshore NEGOM fish species (Table 1). However, means for AOD sets and subsets were slightly higher than values (+11.0‰ to +11.6‰) determined for two comparative Suwannee estuary fish species (Table 1).

ANOSIM multivariate pair-wise tests

In a one-way ANOSIM test of the AOD  $\delta^{13}\text{C}/\delta^{15}\text{N}$  data among all sets and subsets of samples, a ‘Global R’ statistic of 0.909 was determined (Table 3). Pair-wise contrasts of AOD sets and subsets were significant at the  $p < 0.001$  level for four of six contrasts using 999 permutations, and at the  $p < 0.05$  for the remaining two contrasts where sample size limited the number of permutations that could be performed to fewer than 200 (Table 3). No individual ‘R’ statistic exceeded the ‘Global R’ statistic (Table 3).

**Table 3** One-way ANOSIM results for pair-wise contrasts among all sets and subsets of *Acipenser oxyrinchus desotoi* samples based on a Bray-Curtis resemblance matrix of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data. Key: SR = Suwannee River, YR = Yellow River, YOY = age-0 fish

ANOSIM sample statistic (‘Global R’): 0.909  
 Significance level of sample statistic:  $p=0.001$   
 Number of permutations: 999  
 Number of permuted statistics  $\geq$  ‘Global R’: 0  
 ANOSIM pair-wise tests

Groups	$N_1$ vs $N_2$	‘R’	p	Permutations
SR main subset vs SR minor subset	244 vs 16	0.911	<0.001	999
SR main subset vs SR YOY subset	244 vs 2	1.000	<0.001	999
SR minor subset vs SR YOY subset	16 vs 2	0.992	<0.007	153
SR main subset vs YR set	244 vs 10	0.879	<0.001	999
SR minor subset vs YR set	16 vs 10	0.996	<0.001	999
SR YOY subset vs YR set	2 vs 10	1.000	<0.015	66

## Two-sample contrasts

Results of two-sample tests contrasting sets and subsets of AOD samples are presented in Table 4. All  $\delta^{13}\text{C}$  and all  $\delta^{15}\text{N}$  pair-wise contrasts were statistically significant ( $p < 0.05$ ). No comparable  $\delta^{34}\text{S}$  contrasts were significant.

## MDS ordination

*$\delta^{13}\text{C}/\delta^{15}\text{N}$  matrix, data for AOD and all comparative taxa* A 3-dimensional MDS ordination analysis of distance data for the  $\delta^{13}\text{C}/\delta^{15}\text{N}$  Bray-Curtis similarity matrix for all samples from all taxa resolved three distinctive (stress=0.04) trophic groups (Fig. 4): 1) a large group containing the Suwannee River main AOD subset, the Yellow River AOD set, and all comparative estuarine and marine taxa, 2) a large group containing all comparative freshwater taxa, as well as the two age-0 AOD, and 3) a small group containing all samples in the Suwannee River minor AOD subset, spatially intermediate between the estuarine-marine group and the freshwater group.

*$\delta^{13}\text{C}/\delta^{15}\text{N}$  matrix, data for AOD* A 2-dimensional MDS ordination analysis of distance data for the  $\delta^{13}\text{C}/\delta^{15}\text{N}$  Bray-Curtis similarity matrix for AOD samples alone ( $N=261$  paired data points, after elimination of one data pair due to an aberrant  $\delta^{15}\text{N}$  value), labeled as per a priori determined sets and subsets, indicated substantive group structure (stress=0.06) among samples in MDS space. That is, there was no spatial overlap of data points among the MDS-defined sets and subsets of data points (Fig. 5). A Bray-Curtis cluster (untransformed  $\delta^{13}\text{C}/\delta^{15}\text{N}$  multivariate data, group-average linkage) overlain upon the same 2-dimensional MDS ordination plot resolved four statistically-significant groups of AOD samples (SIMPROF test, 999 data permutations,  $p < 0.05$ ; MDS stress=0.06) (Fig. 5): Cluster group 1) a Suwannee River main group comprised of 250 samples with estuarine-marine  $\delta^{13}\text{C}$  signatures (including nine previously assigned to the Suwannee River minor subset); Cluster group 2) a Suwannee River minor group comprised of eight samples with mixed freshwater-estuarine-marine  $\delta^{13}\text{C}$  signatures, seven from the original Suwannee River minor subset, one from the original main subset; Cluster group 3) a second Suwannee River minor group comprised of

two age-0 individuals with strictly freshwater  $\delta^{13}\text{C}$  signatures; and Cluster group 4) a final group with enriched  $\delta^{13}\text{C}$  signatures suggesting wholly estuarine feeding, including all Yellow River samples and one re-assigned sample from the Suwannee River main subset. All four cluster-MDS overlay resolved groups occupied nearly exclusive positions in MDS space (i.e., almost no overlap of respective data points among the four cluster groups) based on combined  $\delta^{13}\text{C}/\delta^{15}\text{N}$  data (Fig. 5). The groups thus redefined by cluster-MDS congruence are also largely congruent with the AOD sets and subsets determined empirically based upon  $\delta^{13}\text{C}$  source thresholds (Fig. 2).

*$\delta^{13}\text{C}/\delta^{15}\text{N}/\delta^{34}\text{S}$  matrix, data for AOD and selected comparative taxa.* A 2-dimensional MDS ordination analysis of distance data for the  $\delta^{13}\text{C}/\delta^{15}\text{N}/\delta^{34}\text{S}$  Bray-Curtis similarity matrix for AOD samples alone resolved four groups (stress=0.04) (Fig. 6). One large group contained the main Suwannee River AOD subset, Yellow River set, and the two comparative estuarine fish taxa. Two comparative single-taxon marine and freshwater fish groups stood apart from the large marine-estuarine group. The minor Suwannee River AOD subset (specimens with  $\delta^{13}\text{C}$  values indicating freshwater or mixed freshwater and estuarine-marine feeding) occupied MDS space intermediate between the comparative marine group and the freshwater group, but was much closer to the marine group, also partially overlapping the estuarine group.

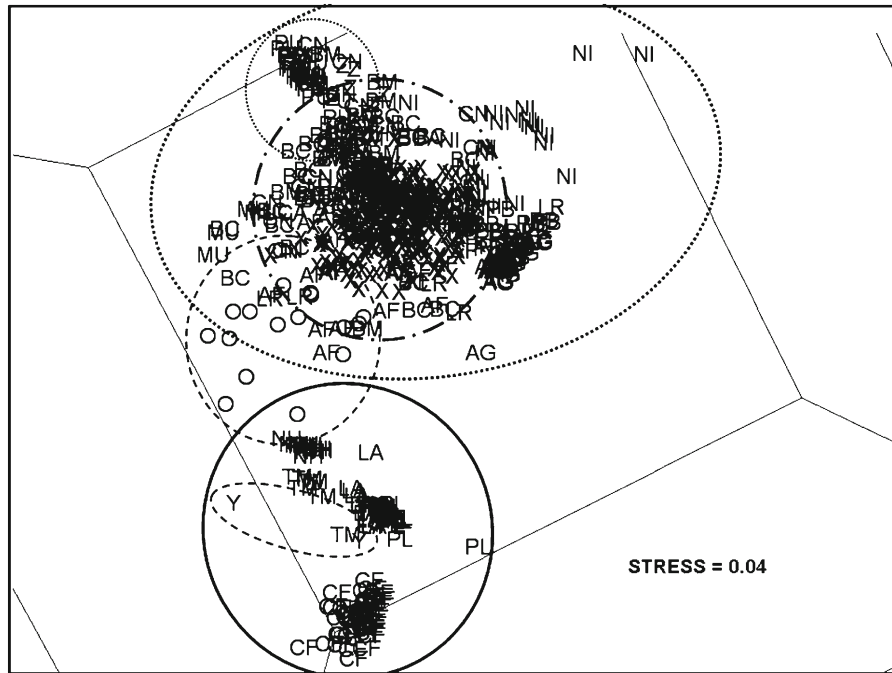
## Isotope-size and isotope-season relationships

A plot of  $\delta^{13}\text{C}$  data against AOD total length (mm) revealed that specimens with  $\delta^{13}\text{C}$  values in the freshwater range ( $< -25\text{‰}$ ) and in the mixed freshwater—estuarine-marine range ( $> -25\text{‰}$  to  $< -21\text{‰}$ ) were all juveniles and subadults ( $< 1350$  mm TL) (Fig. 7a, open boxes). A plot of  $\delta^{15}\text{N}$  data against AOD total length (Fig. 7b) indicated that several of these individuals (including seven of eight individuals in Cluster group 2) had  $\delta^{15}\text{N}$  levels elevated above those of the general AOD population (i.e., AOD Cluster group 1), suggesting feeding at a higher trophic level than for the general population (i.e., selection of prey from higher in the food chain, and probably of large size, equating to selection of larger packets of energy while feeding in freshwater).

**Table 4** Results of t-tests on means, or Mann–Whitney rank sum (MWRS) tests on medians, of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  data for comparative sets and subsets (except age-0 subset) of *Acipenser oxyrinchus desotoi* samples. Key as for Table 3

Set or Subset	SR main subset		SR minor subset		SR main subset		SR minor subset		YR set		YR set	
	13C ‰	15N ‰	13C ‰	15N ‰	13C ‰	15N ‰	13C ‰	15N ‰	13C ‰	15N ‰	13C ‰	15N ‰
Mean (parametric)	-18.21	10.50	-22.86	11.60	0.88	10.50	-18.21	12.51	0.57	12.51	-15.77	12.51
Median (non-parametric)	-18.10	10.52	-22.66	11.45	244	10.52	-18.10	12.51	10	12.51	-15.77	12.51
Variance	244	243	16	16	244	243	16	16	10	10	24	24
N	244	243	16	16	244	243	16	16	10	10	24	24
df	244	243	16	16	244	243	16	16	10	10	24	24
t Statistic (t-test)												
T Statistic (MWRS)			136.00									
Significant at $p < 0.05$			YES						YES			YES
p			0.001						0.001			0.001
Power (at $p = 0.05$ )									1.000			1.000
<b>Set or Subset</b>	<b>SR main subset</b>		<b>SR minor subset</b>		<b>SR main subset</b>		<b>SR minor subset</b>		<b>YR set</b>		<b>YR Set</b>	
Isotope	15N	15N	15N	15N	15N	15N	15N	15N	15N	15N	15N	15N
Mean (parametric)	10.50	10.50	11.60	11.60	10.50	10.50	12.51	12.51	12.51	12.51	12.51	12.51
Median (non-parametric)	10.52	10.52	11.45	11.45	10.52	10.52	12.51	12.51	12.51	12.51	12.51	12.51
Variance	243	243	16	16	243	243	16	16	10	10	24	24
N	243	243	16	16	243	243	16	16	10	10	24	24
df	243	243	16	16	243	243	16	16	10	10	24	24
t Statistic (t-test)												
T Statistic (MWRS)			3150									
Significant at $p < 0.05$			YES						YES			YES
p			0.001						0.001			0.027
Power (at $p = 0.05$ )									1.000			0.529
<b>Set or Subset</b>	<b>SR main subset</b>		<b>SR minor subset</b>		<b>SR main subset</b>		<b>SR minor subset</b>		<b>YR set</b>		<b>YR set</b>	
Isotope	34S	34S	34S	34S	34S	34S	34S	34S	34S	34S	34S	34S
Mean (parametric)	13.42	13.42	13.49	13.49	13.42	13.42	13.27	13.27	13.27	13.27	13.27	13.27
Variance	3.56	3.56	1.91	1.91	3.56	3.56	1.85	1.85	1.85	1.85	1.85	1.85
N	14	14	10	10	14	14	10	10	10	10	10	10
df	14	14	10	10	14	14	10	10	10	10	10	10
t Statistic (t-test)			22				22		22		18	18
T Statistic (MWRS)			-0.0924				0.280		0.280		0.344	0.344
Significant at $p < 0.05$			NO				NO		NO		NO	NO
p			0.927				0.837		0.837		0.735	0.735
Power (at $p = 0.05$ )			0.050				0.050		0.050		0.050	0.050





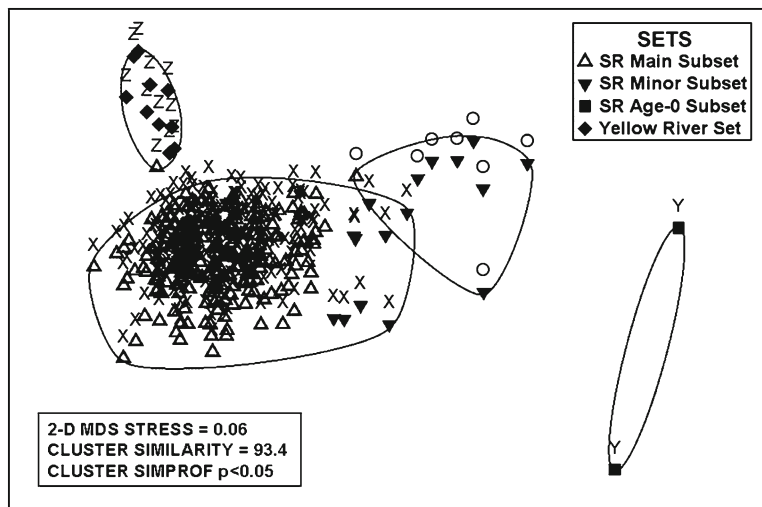
**Fig. 4** MDS 3-dimensional plot of distance data for Bray-Curtis  $\delta^{13}\text{C}/\delta^{15}\text{N}$  similarity matrix for *Acipenser oxyrinchus desotoi* (AOD) and comparative aquatic taxa characteristic of freshwater or estuarine-marine habitats. Key to groups: Large dotted circle = universe of comparative estuarine-marine species (abbreviations as per Table 1); dot-dash circle = main Suwannee River (SR) AOD

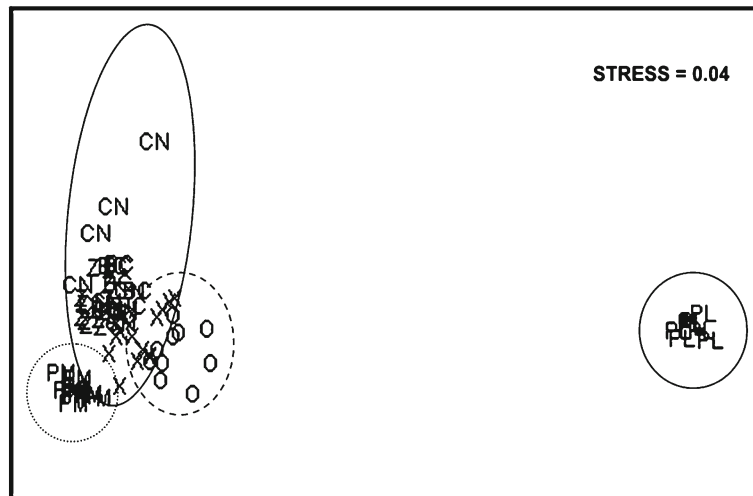
subset (X); small dotted circle = Yellow River AOD set (Z); dashed circle = minor SR AOD subset (O); solid circle = universe of comparative freshwater taxa (abbreviations as per Table 1); dashed ellipse = subset of SR age-0 AOD (Y). Circles and ellipses have been inserted to facilitate visualization of empirical definition versus MDS analysis of group membership

A plot of  $\delta^{13}\text{C}$  data and long-term mean Suwannee River water temperature against month shows that AOD specimens displayed depleted  $\delta^{13}\text{C}$  values indicative of freshwater or mixed freshwater and estuarine-marine carbon signatures (Fig. 8, open

boxes) primarily in February – April and October – November. In contrast, specimens in the general AOD population (main AOD subset) displayed no  $\delta^{13}\text{C}$  values  $< -21\%$ , i.e., no indication of feeding in freshwater, regardless of month or water temperature.

**Fig. 5** *Acipenser oxyrinchus desotoi* trophic groups re-defined via overlay of two multivariate analyses from  $\delta^{13}\text{C}/\delta^{15}\text{N}$  Bray-Curtis resemblance matrix.: Statistically-significant (SIMPROF, 93.4% similarity,  $p > 0.05$ ) cluster groups (ellipses) overlaid upon 2-dimensional MDS plot. Letter labels indicate cluster group membership re-assignments: X = main SR group; O = minor SR group; Y = SR age-0 group; Z = Yellow River group





**Fig. 6** MDS 2-dimensional plot of distance data for Bray-Curtis  $\delta^{13}\text{C}/\delta^{15}\text{N}/\delta^{34}\text{S}$  similarity matrix for *Acipenser oxyrinchus desotoi* (AOD) and comparative aquatic taxa analyzed. Key (refer to Table 1 for key to taxa abbreviations): Large solid line ellipse = main Suwannee River (SR) AOD subset (X) +

Yellow River AOD set (Z) + two comparative estuarine fish species (CN and BC); small dashed line ellipse = minor SR AOD subset; dotted circle = the comparative marine fish PM; circle with solid line = the freshwater fish PL

A plot of mean  $\delta^{15}\text{N}$  values versus month for the main Suwannee River AOD subset displayed a trend of increasing values from February through October (Fig. 9). A Kruskal-Wallis one-way ANOVA on ranks found significant differences in  $\delta^{15}\text{N}$  means among months ( $H=42.188$ ,  $df=8$ ,  $p<0.001$ ). Dunn’s pairwise contrasts found significant differences ( $p<0.05$ ) between February versus August ( $Q=3.518$ ), February versus October ( $Q=3.256$ ), March versus August ( $Q=4.624$ ), and March versus October ( $Q=4.370$ ).

**Discussion**

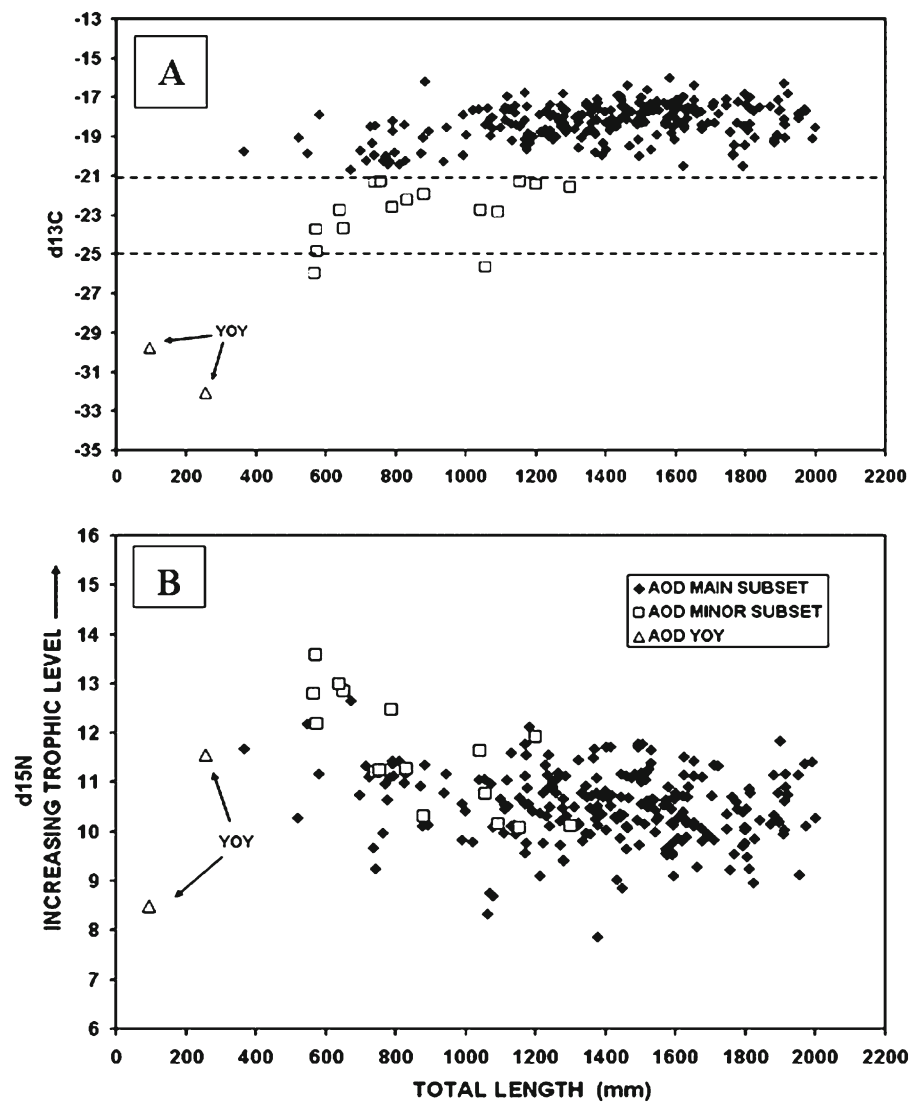
Question 1: Beyond age-0, does the AOD population utilize freshwater prey while in the Suwannee River?

Mason and Clugston (1993) concluded that AOD stores sufficient energy reserves during feeding in saline waters to become ‘indifferent’ to feeding upon riverine prey. Nonetheless, they proceeded to speculate that seasonal fasting is a response to elevated water temperatures, drawing a parallel from the striped bass, *Morone saxatilis*, a temperate-latitude species in which feeding ceases when water temperatures are high. This parallel is inappropriate to AOD, a taxon endemic to and well adapted to summer conditions in Gulf Coast rivers, and one which remains active in

mid-summer temperatures up to 28–33°C (Sulak et al. 2007). The hypothesis of seasonal exhaustion of prey within putative thermal refugia (Chapman and Carr 1995; Carr et al. 1996; Gu et al. 2001) has been refuted by Sulak et al. (2007), who alternatively hypothesized that AOD cease feeding in oligotrophic freshwater rivers because the energetic cost of foraging generally exceeds the trophic benefit. The more effective trophic strategy in the anadromous AOD is seasonally-focused intensive feeding in food-rich estuarine and marine benthic habitats (Heard et al. 2000; Fox et al. 2002; Edwards et al. 2003; Brooks and Sulak 2005; Harris et al. 2005; Sulak et al. 2007; Ross et al. 2009; Sulak et al. 2009).

In the present study, application of empirical thresholds to  $\delta^{13}\text{C}$  data indicated that the majority of Suwannee AOD sampled ( $N=244$ , 93.9%) ceased to feed in freshwater, displaying isotopic signatures indicative of estuarine-marine carbon only ( $\delta^{13}\text{C} > -21\text{‰}$ ) (Table 1, Fig. 2). Fasting among this major subset was upheld by a statistically-significant increasing trend in  $\delta^{15}\text{N}$  over the February-October period of trophic dormancy in the river (Fig. 9). A slow but significant rise in  $\delta^{15}\text{N}$  in GS epithelial tissue during a period of catabolism of stored energy is consistent with the pattern reported for fish muscle by MacNeil et al. (2006). The mean  $\delta^{13}\text{C}$  ( $-18.2\pm 0.9$ ) for the main Suwannee River AOD subset mirrors that determined

**Fig. 7** Values (‰) of  $\delta^{13}\text{C}$  (a) and  $\delta^{15}\text{N}$  (b) versus total length for individual Suwannee River *Acipenser oxyrinchus desotoi* (AOD). YOY denotes two age-0 fish. Dashed lines in (a) denote  $\delta^{13}\text{C}$  boundaries separating estuarine-marine ( $>-21\text{‰}$ ), mixed ( $<-21$  to  $>-25\text{‰}$ ), and freshwater ( $<-25\text{‰}$ ) carbon sources

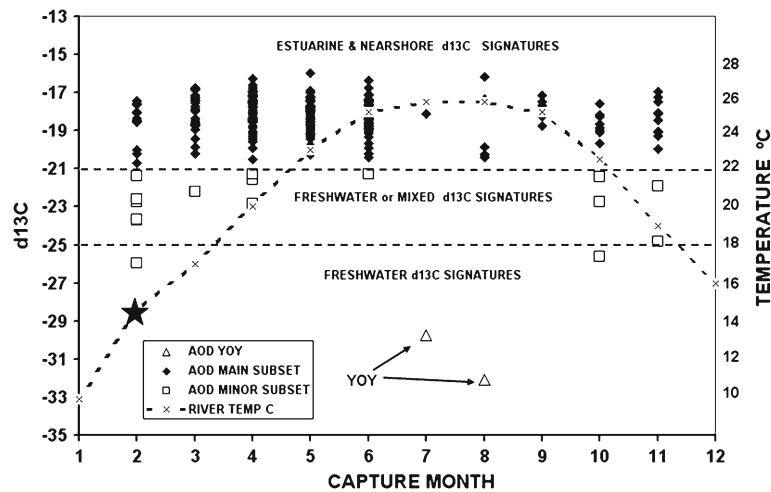


by Gu et al. (2001) for 36 Suwannee River AOD, 580–1820 mm TL ( $-18.5\text{‰}$ ; range  $-19.5\text{‰}$  to  $-17.6\text{‰}$ ). However, present results for the minor Suwannee River AOD subset ( $\delta^{13}\text{C} = -22.9 \pm 1.5$ ) challenge the conclusions of reduced or no freshwater feeding by AOD (Gu et al. 2001).

Whether within the context of comparative taxa analyzed (Fig. 4), or of all AOD samples analyzed following immigration into the Suwannee River (Figs. 2 and 7), a small group of juvenile and subadult AOD fed consistently on prey containing terrestrial isotopic signatures. Results are statistically significant in terms of either univariate  $\delta^{13}\text{C}$  data (Fig. 2) or multivariate  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic data (Figs. 4 and 7). Freshwater feeding occurred to an extent sufficient

to result in a depletion of nearly  $5\text{‰}$  (i.e., a 20.5% carbon turnover) in tissue  $\delta^{13}\text{C}$  signatures among 16 AOD. It is inaccurate to describe feeding as reduced or incidental among the general population while in freshwater. Instead, the alternatives appear to be either complete feeding cessation for 8–9 months (main AOD subset), or continued substantive feeding for 3–4 months after switching to freshwater prey (minor AOD subset), and/or resumption of feeding 1–2 months prior to emigration. Anecdotal information confirms feeding activity in the AOD while in freshwater (Burgess 1963; Berg 2004).

Like other sturgeon species, AOD is very plastic in its behavior, with a small percentage of the population (6% in the present study) often behaving differently



**Fig. 8** Individual Suwannee River *Acipenser oxyrinchus desotoi* (AOD)  $\delta^{13}\text{C}$  values by capture month. YOY denotes two age-0 AOD. Carbon source boundaries are indicated by horizontal dashed lines. Curved line is a plot of 30-year mean monthly river water temperatures for the

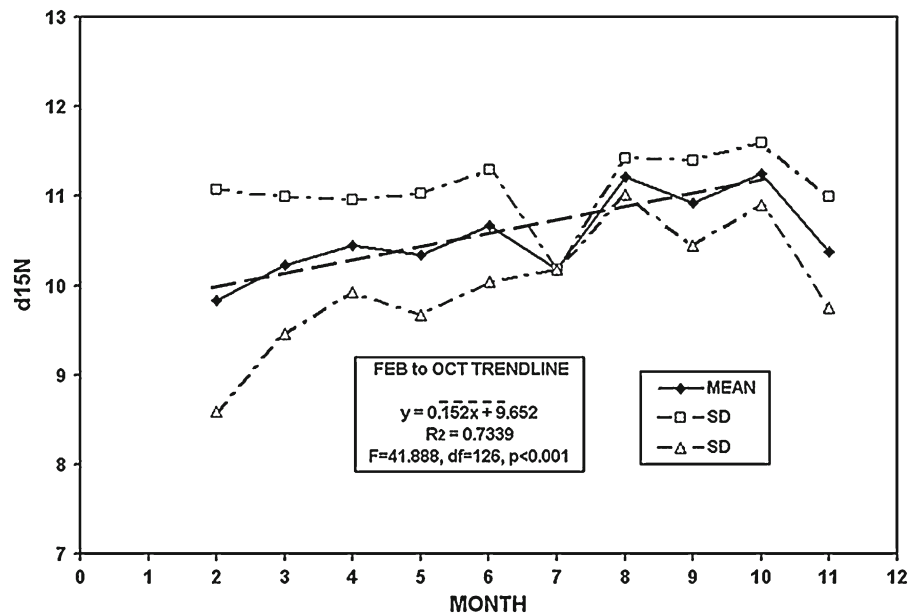
middle Suwannee River (adapted from Chapman and Carr 1995). Star denotes mean temperature (14°C) at which AOD begin to migrate into the river in spring, the same time that the majority of individuals cease to feed

from the general population. That a small number of Suwannee River AOD individuals continue to feed in freshwater is consistent with the concept of intra-population variation in fish behavior (Hammerschlag-Peyer and Layman 2010) and in sturgeon feeding behavior (Stelzer et al. 2008). The mean  $\delta^{13}\text{C}$  value for the Suwannee River minor subset, intermediate between the  $-21\text{‰}$  and  $-25\text{‰}$  thresholds, indicates mixed

feeding, most parsimoniously interpreted as switching from estuarine-marine feeding in winter to substantive freshwater feeding in the river (Fig. 8). Incidental consumption of freshwater food items would not suffice to realize the observed  $\delta^{13}\text{C}$  depletion.

General cessation of feeding in the river is unsurprising given the energetic expense of swimming against the river current in order to forage for macrofaunal prey

**Fig. 9** Means ( $\pm$ SD) in  $\delta^{15}\text{N}$  (‰) for the Suwannee River AOD main subset ( $N=244$ ) versus capture month. Linear regression of means upon month (dashed line) displays a significant increase in  $\delta^{15}\text{N}$ , February through October (i.e., across the period of seasonal residence in freshwater), followed by a 1.0‰ drop in November when exogenous feeding resumes



that is four times less abundant, and six times lower in biomass, per unit area in freshwater reaches, than in the saline river mouth estuary (Sulak et al. 2007). Most individuals are trophically dormant from May through September (Fig. 8), a seasonal period of energy conservation while residing within and occasionally moving among a small number of holding areas.

For the main Suwannee subset,  $\delta^{13}\text{C}$  data indicate that feeding stops immediately upon immigration in February–April when river water temperatures averaged only 14°C (Fig. 8), close to the mean minimum annual temperature of ~10°C experienced by AOD (versus >25°C in mid-summer). Thus, high temperatures cannot be the trigger that initiates trophic dormancy. Indeed, Mason and Clugston (1993) found only marine prey in spring-caught AOD from the Suwannee River. Carr et al. (1996) also reported that 57% of 157 spring-caught AOD had empty stomachs, while 43% contained marine prey. However, in the minor AOD subset, feeding in freshwater must continue with sufficient intensity and duration (Monteiro et al. 1981; Hansson et al. 1997) to significantly deplete tissue isotopic signatures well outside of the  $\delta^{13}\text{C}$  estuarine-marine range of the main AOD subset (Table 1).

The mean  $\delta^{15}\text{N}$  signature for the minor subset of Suwannee River AOD (+11.6±1.1‰) is slightly but not significantly enriched (Table 4) versus the main Suwannee River subset (+10.5±0.7‰). Nonetheless, the 1.1‰ enrichment for the minor subset suggests that AOD that do feed in freshwater select prey representing higher trophic levels, i.e., potentially larger prey than the dominant chironomids (Mason 1991). Mason and Clugston (1993) found that the stomachs of six subadult AOD collected in middle reaches of the Suwannee River in summer–fall 1988 contained such larger prey: ‘...a few aquatic insects and numerous oligochaetes, snails and gastropods’. Selective feeding on larger prey in an oligotrophic blackwater river with limited benthic prey density (Sulak et al. 2007) would represent an energetically advantageous foraging strategy among individuals immigrating in suboptimal condition.

The statistically-significant mean  $\delta^{13}\text{C}$  depletion in the minor subset of Suwannee River AOD ( $\delta^{13}\text{C}=-22.9‰$ ) versus the main subset ( $\delta^{13}\text{C}=-18.2‰$ ) (Table 4) represents a 20.5% contribution of isotopically-light terrestrial carbon following a switch from a diet of estuarine-marine prey. In the slow-growing Canadian broad whitefish, *Coregonus nasus*, Hesslein et al. (1993) determined a

mean  $\delta^{13}\text{C}$  turnover rate in white muscle of 0.18% per day. At such a rate, the observed 20.5% depletion in AOD signatures would require 114 days. However, based on the remarkable rate of weight gain reported in AOD (Wooley and Crateau 1985; Sulak and Clugston 1999)  $\delta^{13}\text{C}$  turnover in AOD may be more rapid. Nonetheless, to realize the observed  $\delta^{13}\text{C}$  depletion in 1–3 months would require consistent intake of wholly freshwater prey for an extended period.

Present  $\delta^{34}\text{S}$  data appear equivocal in addressing the matter of freshwater versus estuarine-marine carbon sources contributing to the diet. The means for both the main and minor Suwannee River subsets are statistically identical (Table 4). The  $\delta^{34}\text{S}$  turnover rate may be too low during a period of arrested growth to reveal a switch to freshwater feeding. Furthermore, uncertainty remains regarding the interpretation of  $\delta^{34}\text{S}$  ratios for estuarine detrital-based food webs with variable contribution of  $^{34}\text{S}$ -enriched sulfate versus  $^{34}\text{S}$ -depleted sulfide (Peterson 1999).

If AOD continued to feed in freshwater, monthly mean  $\delta^{15}\text{N}$  values should remain constant, reflecting continued intake of preferred low trophic level prey. However, the main Suwannee River AOD subset displayed a statistically-significant (Dunn’s test) trend of increasing monthly mean  $\delta^{15}\text{N}$  values from February–March (initiation of immigration) through August–October (initiation of emigration) (Fig. 9). An increase in  $\delta^{15}\text{N}$  during a period of cessation of exogenous feeding is consistent with catabolism of internal muscle mass, which simulates feeding exogenously at a higher trophic level.

The mean  $\delta^{13}\text{C}$  value of -18.2‰ for the Suwannee River main AOD subset indicates that marine phytoplankton carbon is the main AOD carbon source. In turn, the primary feeding habitat of subadults and adults lies in high-salinity marine waters, either near-shore or offshore. In contrast, the mean  $\delta^{13}\text{C}$  value of -15.8‰ for the Yellow River is 4–5  $^{13}\text{C}$  trophic steps removed from the signature of marine phytoplankton, but is only 1–2 steps enriched versus the typical signatures of *Spartina* cordgrass (Table 2). This suggests that the Yellow River adults analyzed did not migrate into polyhaline waters in winter to feed, but remained in the mesohaline estuary. Thus, where expansive mesohaline bays and sounds are available, they may serve as the primary winter feeding habitat for subadult and adult AOD (Fox et al. 2002; Ross et al. 2009).

Question 2: Do migratory AOD adults utilize primarily shallow nearshore versus deeper offshore habitat in winter?

Present  $\delta^{34}\text{S}$  data resolve the question of the nearshore versus offshore location of AOD feeding grounds. Comparative  $\delta^{34}\text{S}$  means for the main and minor Suwannee River subsets, and the Yellow River set (Fig. 3), are statistically identical (Table 4). They are slightly more enriched (+13.3‰ to +13.5‰) than two comparative estuarine fish species (+11.0‰ to +11.6‰), but substantially less enriched than one offshore fish species (+19.9‰), and also substantially less enriched than NEGOM marine plankton (Table 2). Thus,  $\delta^{34}\text{S}$  data indicate that AOD do not migrate to deep offshore waters to feed, but remain in shallow nearshore waters, corresponding with earlier telemetry results (Edwards et al. 2007; Ross et al. 2009).

The hypothesized greater utilization of estuarine prey among adult AOD in the Yellow River corresponds with the much larger extent of feeding habitat in Pensacola Bay than in Suwannee Sound. Mesohaline sounds and bays support high benthos density (Heard et al. 2000; Ross et al. 2009) and provide waters deep enough to guard against winter cold-kill. The comparative  $\delta^{15}\text{N}$  means for the small Yellow River AOD sample set (+12.5±0.5‰) and the larger Suwannee River main AOD subset (+10.5±0.7‰) were not significantly different. However, the elevated Yellow River mean suggests that prey from a higher trophic level (i.e., prey of large size) are selected compared to those utilized by Suwannee River AOD. Heard et al. (2000) estimated that large ghost shrimp were sufficiently abundant in Choctawhatchee Bay to sustain the entire Choctawhatchee River AOD population during winter.

Telemetry studies confirm that, except for the Suwannee population, a substantial proportion of subadults and adults in other AOD populations remain in coastal sounds and bays to feed (Fox et al. 2002; Ross et al. 2009). In contrast, in the comparatively smaller and shallower Suwannee Sound only juveniles remain (Sulak et al. 2009), while subadults and adults migrate into the open Gulf of Mexico (Edwards et al. 2003, 2007).

Question 3: Is the growing Suwannee population experiencing a paradigm shift in feeding habitat use?

What is known about the life history of North American sturgeons derives largely from study of anthropogenically

severely depleted populations inhabiting highly altered ecosystems. These populations have not been limited by intrinsic population density effects. Thus, knowledge of ‘standard’ feeding habitat choice for any sturgeon species may be rather flawed. It can be hypothesized that anadromy in sturgeons arose as an adaptive mechanism to enable exploitation of rich marine food resources, despite increased predation risk and the increased energetic cost of osmoregulation in fishes whose internal physiology and early life history bear the stamp of their freshwater chondrosteian ancestors. Mason and Clugston (1993) have speculated that the relative ease of rapid caloric intake in marine habitats during the winter is sufficient to sustain the entire annual cost of AOD metabolism, growth and reproduction. Thus, it may generally be cost-ineffective for AOD to feed in summer on sparse prey of small size in oligotrophic Gulf Coast rivers (Sulak et al. 2007). However, this may not prove true as density-dependent factors ultimately come into play for recovering sturgeon populations experiencing Malthusian increase. As protected populations increase in numbers and mean biomass per fish, it can be hypothesized that AOD feeding behavior and trophic habitat use will be altered due to competition for limited resources. Since initiation of harvest prohibition in Florida in 1984, the Suwannee River AOD population (fish >1000 mm TL) has increased five-fold from 2000 to 3000 individuals in the early 1990s (Carr et al. 1996; Chapman et al. 1997) to 10 000–15 000 in 2006–2007; mean biomass per fish has simultaneously increased from 17 kg to 28 kg (M. Randall and K. Sulak, USGS, unpubl. data). Feeding in freshwater, documented herein via  $^{13}\text{C}$  isotopic evidence among just 6% of the population, is a life history option that may grow in importance. As AOD population density increases toward carrying capacity and as benthic prey availability in marine feeding habitats is more rapidly depleted in winter, fewer individuals may return to the river in prime condition each spring. This may necessitate a feeding activity and habitat paradigm shift, i.e., broader adoption of continued feeding in freshwater after immigration.

Similarly, feeding habitat use may change during population booms. For example, age-1 and age-2 juveniles normally feed in the river mouth estuary in winter (Sulak et al. 2009), then fast in holding areas from spring to fall. However, such juveniles (386–690 mm TL) were captured in May–September in river mouth (rkm 0–2.5, Fig. 1) gill net sampling in substantial numbers in 1993 ( $N=39$ ) and 1994 ( $N=69$ ) for



the first time ever following the bumper spawning years of 1991 and 1992 (USGS unpubl. data). Increases in weight for recaptured individuals indicated they had been feeding during summer.

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