

Evaluating the effect of environmental disturbance on the trophic structure of Florida Bay, U.S.A.: Multiple stable isotope analyses of contemporary and historical specimens

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Abstract

Widespread seagrass and sponge mortality and increased water column turbidity caused by phytoplankton blooms and suspended sediments occurred throughout Florida Bay from 1987 to the mid 1990s. This disturbance led to the hypothesis that Florida Bay was shifting from dependence on benthic production to dependence driven primarily by water-column production. We tested this hypothesis by (1) evaluating the current trophic structure of Florida Bay and then (2) comparing it to a reference site in Biscayne Bay populated by dense seagrass beds with no recent history of large-scale seagrass mortality. Additionally, we (3) compared the current trophic structure of Florida Bay to a prior trophic structure recorded in historical specimens collected before the onset of the environmental disturbance. No evidence was found to support the occurrence of a large-scale shift from a seagrass-dominated to a plankton-dominated system. The $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ signatures of macroinvertebrates and fishes collected from sites in Florida Bay expressed the strong dominance of benthic production and were similar to values obtained from the Biscayne Bay reference site. Highly mobile apex predators that effectively integrate the signatures of their prey over longer temporal and larger spatial scales had $\delta^{13}\text{C}$ signatures that ranged from -13‰ to -11‰ and $\delta^{34}\text{S}$ signatures that ranged from $+2\text{‰}$ to $+6\text{‰}$, indicating that the food web of the bay is currently dominated by strong benthic–pelagic coupling. A comparison between Florida Bay's historic communities (preserved fish from 1956–1966) and its contemporary communities also supports the conclusion that the fundamental character of the bay remains relatively unchanged.

Ecosystem-level disturbances occurred throughout Florida Bay from 1987 to the mid 1990s. These disturbances—including widespread seagrass and sponge mortality and increased water column turbidity caused by phytoplankton blooms and suspended sediments—have been linked to intensive human modification of the freshwater flow regime of south Florida over the last 100 yr, coupled with multiyear droughts (Butler et al. 1995; Fourqurean and Roblee 1999; Fourqurean et al. 2003). Persistent, large-scale seagrass die-off events that occurred during this time apparently led to shifts in the Bay's community structure; specifically, densities of canopy-dwelling species (e.g., rainwater killifish, *Lu-*

cania parva; silver jenny, *Eucinostomus gula*; goldspotted killifish, *Floridichthys carpio*; and dusky pipefish, *Sygnathus floridae*) declined and densities of plankton-feeding and benthic species less dependent on seagrass cover (e.g., anchovy, *Anchoa mitchilli*; and gulf toadfish, *Opsanus beta*) increased (Matheson et al. 1999; Thayer et al. 1999); Boesch et al. (1993) suggested that Florida Bay as a whole might be shifting from a system that is dependent on benthic production (i.e., seagrass, seagrass epiphytes, benthic algae, or sediment organic matter) to one driven primarily by water-column production (i.e., phytoplankton). Because Florida Bay functions as an essential nursery for many species important to fisheries, such a change could have a devastating impact on the ecological and economic productivity of the entire system. To test the hypothesis of Boesch et al., this article (1) evaluates the current trophic structure of Florida Bay and then (2) compares it to that of a reference site in Biscayne Bay populated by dense seagrass beds with no recent history of large-scale seagrass mortality and (3) compares the current trophic structure of Florida Bay to a prior trophic structure recorded in historical specimens collected before the onset of the environmental disturbances in the late 1980s.

Contemporary trophic dynamics

For aquatic ecosystems, carbon and nitrogen isotope ratios are often considered adequate tools for identification of forage location, types of food items included in diet, and trophic level. Unfortunately, in estuarine and coastal systems, interpretation of stable isotope data is often confounded by:

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lack of separation in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of primary producers across regions; local variability in signatures within species or functional groups of primary producers related to differences in biogeochemical and physical factors; and variability among individuals of the same species who consume the same diet. A further complication in these systems is that estuaries generally support a relatively diverse fauna with complex feeding relationships (e.g., opportunistic feeding strategies, omnivory), making separation between trophic levels indistinct.

In estuaries such as Florida Bay, the $\delta^{13}\text{C}$ values of most types of primary producers overlap. In these systems, the average value of $\delta^{13}\text{C}$ for epiphytic algae (seagrass-associated algae) is $-18\text{‰} \pm 2\text{‰}$ (Wainwright et al. 2000; Moncreiff and Sullivan 2001; Chanton and Lewis 2002); for epipelagic (sediment-associated) algae, $-16\text{‰} \pm 2\text{‰}$ (Moncreiff et al. 1992; Currin et al. 1995; Wainwright et al. 2000); for phytoplankton, $-21.5\text{‰} \pm 4.5\text{‰}$ (Chanton and Lewis 1999; Wainwright et al. 2000); and for organic matter derived from mangroves, $-26\text{‰} \pm 3\text{‰}$ (Harrigan et al. 1989; Fry et al. 1999). The use of $\delta^{15}\text{N}$ is also problematic in resolving diets of mobile, upper-trophic level predators in these systems because the $\delta^{15}\text{N}$ signatures of primary producers and lower trophic levels are affected at local and regional scales by agricultural and urban inputs (in northeastern Florida Bay and lower Biscayne Bay) and by enhanced denitrification (in restricted basins throughout Florida Bay; Corbett et al. 1999), and most of the predators are highly opportunistic (feeding on a variety of invertebrates and other fishes).

Although the $\delta^{13}\text{C}$ signatures of many of the dominant primary producers are similar, the $\delta^{34}\text{S}$ signatures are markedly different. Epiphytes and phytoplankton derive sulfur from seawater sulfate, which has a $\delta^{34}\text{S} \approx +21\text{‰}$, while epipelagic algae derive sulfur in part from sediment sulfides, which may be as much as 30‰ depleted relative to seawater sulfate. The $\delta^{34}\text{S}$ of mangroves is also depleted, probably reflecting both reducing sediment conditions as well as the influence of terrestrial (precipitation-derived) sulfate ($\approx +2$ – $+8\text{‰}$). Additionally, in contrast to carbon and nitrogen, sulfur is essentially a conservative natural tracer allowing evaluation of possible food sources without the complication of considering metabolic- and trophic-level fractionation factors. So in ecosystems such as Florida Bay, where $\delta^{13}\text{C}$ signatures of food sources overlap but $\delta^{34}\text{S}$ signatures diverge, sulfur stable isotopes can be used to effectively resolve diet histories (Fry et al. 1999).

Given the considerations outlined above, we used multiple stable isotope analyses ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$) to investigate the relative dependence of consumers (i.e., invertebrates and fish collected from 1997–1999) on different types of primary production and to establish an understanding of natural spatial variability in stable isotopic signatures of the biota in Florida Bay relative to variability forced by possible shifts in feeding patterns.

Historical trophic dynamics

Although extensive collections of fish have been archived by museums, government agencies, and universities, these

preserved specimens—some dating back to the early 20th century—have not routinely been utilized for stable isotope studies. For decades it was widely accepted that preservation of organic tissue with formalin and/or ethanol would confound dietary signatures of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by incorporating isotopically light carbon from formalin into muscle tissue or by leaching organic constituents from tissue (Hobson et al. 1997; Bosely and Wainwright 1999; Kaehler and Pakhomov 2001). However, recent studies indicate that preservation does not preclude successful interpretation of trophic status. Although characterizations of preservation effects differ between species within studies and between studies in general (Arrington and Winemiller 2002; Edwards 2002; Sarakinos et al. 2002), all of these studies indicate that preservation and storage effects on stable isotope composition of muscle tissue are relatively small and predictable. Isotopic shifts in fish stored for up to 2 yr in buffered formalin, as well as fish fixed in buffered formalin and then stored in 70% ethanol (ETOH), were on the order of -1‰ for $\delta^{13}\text{C}$, $+0.5\text{‰}$ for $\delta^{15}\text{N}$, and were not significant for $\delta^{34}\text{S}$ (Ogawa et al. 2001; Chasar 2002; Edwards et al. 2002). These offsets in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are well within ranges of intrasite variability and trophic level fractionation in fish and occur rapidly (on the order of days to weeks) with no subsequent depletion with storage over long periods of time. Edwards et al. (2002) demonstrated that specimens fixed in 10% buffered formalin and stored for 12–15 yr in ETOH expressed a ^{13}C -depletion of approximately 1‰ relative to frozen specimens.

Based on this recent evidence, we evaluated the trophic dynamics of historic, or predisturbance, Florida Bay using a multiple stable isotope analysis ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$) approach. Our historical construct is derived from fish specimens collected from Florida Bay and Biscayne Bay from 1956–1966 and preserved and archived by the Florida Museum of Natural History.

Study area

The five primary stations for this study are in Florida Bay (Fig. 1), a large (2,200 km²), shallow (average depth 1 m), subtropical lagoonal estuary characterized by carbonate mud banks, small mangrove islands, and seagrass beds. Seagrass beds cover >80% of the mud banks and basins throughout the bay and are dominated by turtle grass (*Thalassia testudinum*), shoal grass (*Halodule wrightii*), and manatee grass (*Syringodium filiforme*) (Hall et al. 1999; Fourqurean et al. 2001). Monospecific beds of turtle grass are particularly dense in the interior basins, while shoal grass is locally dominant in areas of physical disturbance or areas exposed to salinity and temperature stress. Manatee grass occurs in regions where light is limiting to the other species (i.e., the deeper water channels and along the Gulf edge of the Bay) (Zieman et al. 1999).

These stations represent an impact gradient across Florida Bay in terms of conditions related to seagrass die-off events, specifically increased salinity, turbidity, large-scale microalgal blooms, and shifts in the relative abundances of canopy-dwelling and pelagic fish species (Fourqurean and Robblee 1999; Matheson et al. 1999; Thayer et al. 1999). Stations

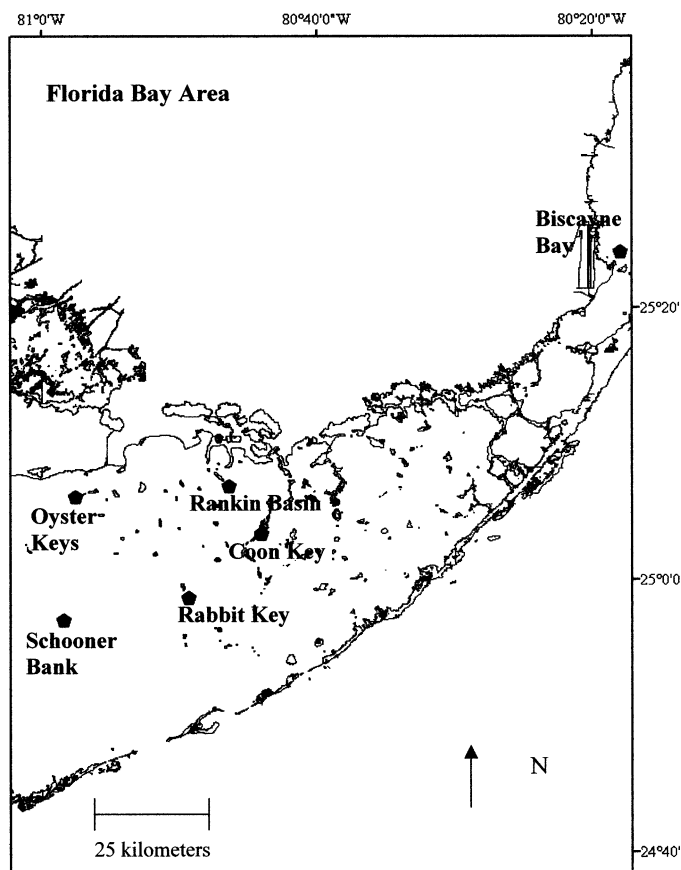


Fig. 1. Map of study area. Outer Gulf stations are Schooner Bank and Oyster Keys; interior stations are Rabbit Key, Coon Key, and Rankin Basin; reference station is in lower Biscayne Bay.

range from the least-impacted western, or Gulf, edge of the bay to its interior. Gulf sites include Schooner Bank, 24°57.04'N 80°58.38'W, and Oyster Keys, 25°06.17'N 80°57.52'W; interior sites include Rabbit Key, 24°58.69'N 80°49.31'W, Coon Key, 25°03.40'N 80°44.01'W, and Rankin Basin 25°06.90'N 80°46.37'W (see Roblee et al. 1991). An additional station in Biscayne Bay, 25°24.10'N 80°17.97'W, was selected as a reference site because it is populated by dense seagrass beds with no recent history of large-scale seagrass mortality; however, central and lower Biscayne Bay have been affected by urban and agricultural contaminants (Lietz 1999), so this station is not considered pristine.

Materials and methods

Collections of seston, seagrasses, seagrass epiphytes, invertebrates, and fishes were conducted at Gulf, interior, and Biscayne Bay stations during three consecutive years: 30 September–02 October 1997; 18–20 May 1998; and 30 August–01 September 1999. Samples were collected from a variety of habitats at each station, including seagrass beds, open (unvegetated) areas, channels, and fringes of mangrove islands.

Seston was collected with plankton nets (10-min tows with 64-, 88-, and 150- μ m mesh) at each station during the

1997 and 1998 sampling events. The products of the individual tows were rinsed with ambient seawater through a 150- μ m mesh nitex sieve to remove larger zooplankton and debris and were concentrated on a 10- μ m mesh sieve. Samples were then rinsed with ambient seawater into 60-ml glass sample jars for stable isotope analysis and 20-ml scintillation vials for taxonomic identification of phytoplankton. Samples for isotopic analysis were placed on ice for transport to Florida State University (FSU). The contents of the scintillation vials were preserved with approximately 0.1 ml of Lugol's solution. Whole (unfiltered) water samples were also collected in 1997 and 1998 for the characterization of phytoplankton. These samples were collected in brown 1-liter high density polyethylene (HDPE) bottles (two samples per station); contents were preserved with 5 ml of Lugol's solution. All preserved samples were transported to the FSU Department of Biology for processing.

Seagrasses were collected in bulk from all stations in all years; grasses were placed in 4-liter Ziploc bags and placed on ice for transport to FSU. Upon return to the laboratory, blades were clipped free of roots and rhizomes, carefully rinsed with deionized (DI) water, and dried at 60°C. Epiphytes were separated from seagrasses by gently brushing the dried blades and sieving to remove seagrass debris. Seagrasses and epiphytes were coarsely ground.

Macroinvertebrates and fishes were collected from all stations in all years using a variety of gear types, including roller-frame trawl, bottom scrape, gill net, hook and line, and pole spear or Hawaiian sling. In addition to conducting a general survey of biota, we targeted apex predators (e.g., several sharks, *Carcharhinus leucas* and *Carcharhinus limbatus*; gray and lane snappers, *Lutjanus griseus* and *Lutjanus synagris*, respectively; and jack crevalle, *Caranx hippos*) and those species noted in the literature to have changed in relative abundance in conjunction with seagrass die-off events, including anchovy (*Anchoa* sp.), which increased, and rain-water killifish (*Lucania parva*), which decreased (Matheson et al. 1999).

Invertebrates and teleosts (bony fish) were sorted by species and placed in Ziploc bags. Sharks and rays were sacrificed, identified, and measured to the nearest centimeter total length (TL) in the field, and muscle tissue was sampled (~10 g). All specimens were placed on ice for transport to FSU for processing. In the laboratory, macroinvertebrates were processed by dissecting muscle tissue free of carapace and viscera, with the exception of caridean shrimp, which were pooled and processed whole. Fish were measured (TL, nearest cm) and weighed and identified to the species level. For fish less than approximately 6–8 cm TL, head and guts were removed, and specimens were pooled by species for each site. Specimens larger than approximately 6–8 cm TL were filleted and axial muscle processed for individual fish. When a large number ($\gg 6$) of the same species of fish were collected at a specific station, both individuals and pooled samples were processed for analysis (e.g., gray snapper, gulf toadfish, pinfish). All samples were stored at -20°C until processed for stable isotope analysis.

Taxonomic enumeration and identification of plankton and epiphytes were conducted using both light microscopy and scanning electron microscopy (Prasad et al. 2001, 2002). Al-

Table 1. Station comparison of stable isotope values for seston, epiphytes, and seagrasses. Lack of sample (e.g., seston was not collected in 1999) or sample size insufficient for isotopic determination is noted by "i.s."

		Seston				Epiphytes			Seagrasses		
		$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{34}\text{S}$ (‰)		$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{34}\text{S}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{34}\text{S}$ (‰)
Schooner Bank	1997	-17.5	5.7	4.5	Turtle grass	i.s.	i.s.	i.s.	-9.2	4.5	10.0
	1998	-20.1	6.1	i.s.	Turtle grass	-17.4	5.8	17.2	-8.5	4.8	7.1
	1999	i.s.	i.s.	i.s.	Turtle grass	-15.3	3.3	16.2	-10.9	2.7	5.7
Oyster Keys	1997	-18.8	5.3	i.s.	Turtle grass	-16.2	6.0	i.s.	-9.0	3.5	13.8
	1998	-21.1	7.8	i.s.	Turtle grass	-15.9	3.7	16.0	-12.2	2.2	14.6
	1999	i.s.	i.s.	i.s.	Turtle grass	-15.2	3.1	i.s.	-11.3	2.1	13.8
					Shoal grass	i.s.	i.s.	i.s.	-12.5	-1.5	10.6
Rabbit Key	1997	-12.4	2.7	i.s.	Turtle grass	-15.9	5.0	i.s.	-11.4	2.1	6.1
	1998	-16.0	5.5	i.s.	Turtle grass	-15.1	5.6	i.s.	-9.3	2.3	5.3
	1999	i.s.	i.s.	i.s.	Turtle grass	-15.6	i.s.	8.3	-9.5	2.4	4.6
					Shoal grass	i.s.	i.s.	i.s.	-12.5	2.1	9.6
Coon Key	1997	-15.7	4.3	i.s.	Turtle grass	i.s.	i.s.	i.s.	-8.6	4.1	-4.4
	1998	-17.4	5.2	i.s.	Turtle grass	-15.7	3.3	13.3	-10.1	6.5	-2.6
	1999	i.s.	i.s.	i.s.	Turtle grass	-16.0	2.6	i.s.	-10.3	4.0	i.s.
Rankin Basin	1997	-17.4	6.0	i.s.	Turtle grass	i.s.	i.s.	i.s.	-11.8	5.2	0.2
	1998	-17.2	4.0	i.s.	Shoal grass	-14.7	5.0	i.s.	-11.9	3.7	6.0
	1999	i.s.	i.s.	i.s.	Shoal grass	-14.7	i.s.	i.s.	-11.9	2.3	6.0
Biscayne Bay	1997	-15.9	7.2	-1.0	Turtle grass	-16.2	10.5	i.s.	-11.7	5.8	3.5
	1998	-14.6	6.1	i.s.	Turtle grass	-16.2	8.0	17.7	-9.8	5.0	1.4
	1999	i.s.	i.s.	i.s.	Turtle grass	-16.1	5.9	15.9	-11.1	4.5	0.4

though we had originally planned to evaluate carbon (C), nitrogen (N), and sulfur (S) stable isotopes for each of the individual size fractions of seston collected, it was necessary to composite all size fractions from each site to provide enough material for stable isotope analysis. For $\delta^{13}\text{C}$ analysis, subsamples of plant and animal tissues were acidified with 10% hydrochloric acid (HCL) to remove carbonates (Chanton and Lewis 1999); all acidified (for $\delta^{13}\text{C}$) and unacidified tissue samples (for $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$) were rinsed three times in DI water, freeze-dried, and then pulverized using a Crescent Wig-I-bug. Samples for carbon and nitrogen analysis were sent to Isotope Services (Los Alamos, New Mexico) and analyzed on a Carlo-Erba NA 1500 elemental analyzer and an IRMS. Samples for sulfur analysis were sent to Coastal Science Laboratories (Austin, Texas), where they were combusted and run on a dual inlet IRMS. Blind standards were sent with each sample run. Precision for stable isotope analysis was 0.2‰ for carbon and nitrogen and 0.5‰ for sulfur.

Historical samples—The Florida Museum of Natural History allowed us to dissect approximately 1–2 g of axial muscle tissue from fish collected from 1956–1966 in Florida Bay and Biscayne Bay; nine species were found in the museum's collection that were well-represented for the same geographic regions represented in our 1997–1999 collections. Muscle tissue from these specimens was soaked in DI water for 3 d (changing water every 24 h) to remove preservatives and then processed for C, N, and S stable isotopes as described in the preceding section. Based on recent research (Arrington and Winemiller 2002; Chasar 2002; Edwards et al. 2002),

stable C isotope values for all preserved fish were corrected by adding 1‰.

Results

Water-column primary production—The results of stable C, N, and S isotope analyses for material collected from net-tows are presented in Table 1. Sample size was insufficient at most sites to conduct S isotope analysis. The 1997 stable C and S isotope results indicate that seston samples included suspended sediment-associated algae, seagrass detritus, and/or epiphytes (i.e., $\delta^{13}\text{C}$ values were $>$ [more ^{13}C -enriched than] -18‰ , and the limited $\delta^{34}\text{S}$ values (Schooner Bank and Biscayne Bay) were $< +5\text{‰}$, whereas values for phytoplankton representative of well-flushed coastal and open-ocean systems have average values of $\delta^{13}\text{C} \approx -21\text{‰}$ and $\delta^{34}\text{S} \approx +20\text{‰}$ (Chanton and Lewis 2002). In 1998, seston collected from the Gulf stations produced $\delta^{13}\text{C}$ values typical of phytoplankton (i.e., $\delta^{13}\text{C}$ of -20‰ at Schooner Bank and $\delta^{13}\text{C}$ of -21‰ at Oyster Keys). In 1997 and 1998, $\delta^{13}\text{C}$ values were generally more depleted at Gulf stations (-17.5‰ to -21.2‰) than interior stations (-12.4‰ to -17.4‰); and of the interior stations, Rankin Basin and Coon Key were consistently more ^{13}C -depleted than Rabbit Key. For both 1997 and 1998, interior sites were ^{15}N -depleted relative to Gulf sites.

Table 2 lists the predominant algal species occurring across all sites in 1997 and 1998; cells are described as being planktonic (P), benthic (B), or planktonic/benthic (PB; ambiguous; e.g., morphology indicative of benthic form but

Table 2. Site-specific occurrence of predominant algal species (>10% relative abundance for particular site) in whole-water, net, and epiphyte samples. Cells are classified as diatom (D), dinoflagellate (DN), or red algae (RA); types are listed as planktonic (P), benthic (B, epipelagic or epiphytic), or planktonic/benthic (PB).

Species	Class	Type	Whole water	Net	Epiphyte
<i>Amphora</i> spp.	D	B			Rabbit Key
<i>Amphora ventricosa</i> Gregory	D	B	Coon Key		Schooner Bank, Biscayne Bay
<i>Ardissonia crystallina</i> var? <i>bacillaris</i> (Grun.) Grun.	D	B			
<i>Ardissonia formosa</i> (Hantz.) Grun.	D	B		Schooner Bank	
<i>Bacillaria paxillifer</i> (Müller) Hendey	D	P		Schooner Bank, Oyster Key	
<i>Chaetoceros simplex</i> Ostenf.	D	P	Schooner Bank		
<i>Chaetoceros</i> spp.	D	P		Coon Key, Biscayne Bay	
<i>Climacosphenia monilgera</i> Ehrenb.	D	B		Schooner Bank	
<i>Cocconeis dirupta</i> Gregory	D	B			Coon Key
<i>Cocconeis scutellum</i> Ehrenb.	D	B			Schooner Bank, Coon Key, Rankin Basin
<i>Cocconeis</i> sp.	D	B		Coon Key	
<i>Cyclotella choctawhatcheeana</i> Prasad	D	P	Coon Key, Rankin Basin		
<i>Cyclotella</i> sp.	D	P	Rankin Basin		
<i>Cyclotella</i> sp. (w/cross bar)*	D	P	Coon Key	Coon Key, Rankin Basin	
<i>Cylindrotheca closterium</i> (Ehrenb.) Lewis & Reiman	D	P/B	Schooner Bank, Oyster Key		
<i>Entomoneis</i> spp.	D	B/P	Oyster Key		
<i>Fosliella atlantica</i> (Foslie) Taylor	Red	B			
<i>Grammatophora marina</i> (Lyngb.) Kutz	D	B			
<i>Haslea ostrearia</i> (Gaillon) Simonsen	D	B			
<i>Hyalosira delicatula</i> Kützing	D	B			Schooner Bank
<i>Hyalosynedra laevigata</i> (Grun.) Williams & Round	D	B	Oyster Key	Rabbit Key, Rankin Basin, Biscayne Bay	Oyster Key, Rabbit Key
<i>Hyalosynedra laevigata</i> var. <i>angustata</i> (Grunow)	D	B	Rabbit Key, Rankin Basin, Biscayne Bay		Oyster Key, Rabbit Key
<i>Hyalosynedra</i> spp.	D	B	Oyster Key, Rabbit Key, Biscayne Bay		Oyster Key
<i>Mastogloia angulata</i> Lewis	D	B	Schooner Bank		Oyster Key
<i>Mastogloia binotata</i> (Grun.) Cl.	D	B		Biscayne	Schooner Bank
<i>Mastogloia cribrosa</i> Grun.	D	B			Schooner Bank, Rabbit Key, Rankin Basin, Biscayne Bay
<i>Mastogloia crucicula</i> (Grun.) Cl.	D	B			Rabbit Key, Biscayne Bay
<i>Mastogloia ovalis</i> A. Schmidt	D	B			Rabbit Key
<i>Mastogloia ovata</i> Grun.	D	B			Biscayne Bay
<i>Mastogloia</i> spp.	D	B			
<i>Microtabella interrupta</i> (Ehrenb.) Round	D	B	Schooner Bank, Biscayne Bay	Rabbit Key, Coon Key	
<i>Navicula</i> spp.	D	B	Biscayne Bay	Oyster Key	
<i>Neosynedra provincialis</i> (Grunow) Williams & Round	D	B	Rankin Basin		
<i>Nitzschia longissima</i> (Breb.) Ralfs	D	B			
<i>Psammodyctyon panduriformis</i> (Gregory) D. G. Mann	D	B			
<i>Prorocentrum mexicanum</i> Tafall	DN	B/P		Schooner Bank, Oyster Key	
<i>Prorocentrum micans</i> Ehrenb.	DN	P	Coon Key	Rankin Basin	
<i>Pseudonitzschia</i> sp.	DN	P	Schooner Bank		
<i>Pyrodinium bahamense</i> Plate	DN	P	Rabbit Key, Coon Key, Rankin Basin	Coon Key, Rankin Basin, Biscayne Bay	Oyster Key
<i>Rhabdonema adriaticum</i> Kützing	D	B	Oyster Key		Schooner Bank
<i>Rhopalodia gibberula</i> (Ehr.) O. Müller	D	B			Schooner Bank, Oyster Key
<i>Striatella unipunctata</i> (Lyngb.) Ag.	D	B	Rabbit Key, Coon Key, Rankin Basin, Biscayne Bay		Rabbit Key, Coon Key
<i>Reimerothrix floridensis</i> Prasad et al. 2001†	D	B			
<i>Thalassiosira</i> sp. (5–9- μ m diameter)	D	P			
<i>Thalassiosira</i> sp. (8- μ m diameter)	D	P	Schooner Bank		
<i>Taxarium hennedyanum</i> Grunow	D	B	Schooner Bank	Schooner Bank, Rabbit Key	

* Soon to be described as a new species.

† Published as a new genus and species in *Phycologia* 40: 35–46 (2001).

Table 3. Comparison of net and whole-water phytoplankton samples. Relative abundances of cell types are % planktonic (P), % benthic (B), % planktonic/benthic (PB), % epiphyte (E). %E represents the proportion of benthic species documented both in water-column and epiphyte samples for a particular station.

	Net				Whole water			
	%P	%B	%PB	%E	%P	%B	%PB	%E
1997								
Schooner Bank	28	58	14	53	54	7	39	68
Oyster Keys	31	63	6	28	60	7	33	36
Rabbit Key	1	96	3	43	37	58	5	58
Coon Key	71	28	0.2	82	62	37	0.3	18
Rankin Basin	29	70	0.5	61	33	66	1	10
Biscayne Bay	28	67	4	59	16	74	10	38
1998								
Schooner Bank	6	86	8	38	42	56	0	34
Oyster Keys	3	96	1	87	10	90	0	59
Rabbit Key	32	68	0	49	6	85	9	23
Coon Key	95	5	0	67	70	28	2	45
Rankin Basin	34	62	1	35	58	40	2	31
Biscayne Bay	3	88	9	54	2	97	1	29

documented to date only in plankton samples). Total cell counts, relative abundances of planktonic versus benthic cell types, and species assemblages in material from net-tows and whole-water samples differed from 1997 to 1998 (Table 3). In general, there is some disparity in the relative abundances of cell types between net (integrated over a large surface area) and whole-water (discrete 1-liter grabs) samples, a factor of both heterogeneous distribution of seston and mesh size of plankton nets and sieves. For most sites, benthic algae were more dominant in net-tows, while planktonic and planktonic/benthic were more dominant in grab samples. For all stations and all sampling dates, both net and whole-water samples contained large amounts of benthic algae, a characterization that includes both epipelagic (sediment-associated) and epiphytic (seagrass-associated) algae. Although epiphytic and epipelagic algae may have species in common, Moncreiff et al. (1992) found that sediment-associated and seagrass communities were typically composed of different taxa; our data indicate that roughly 50% of the benthic algae in the water column were suspended epiphytic cells (the average of %E for all whole-water and net samples in Table 3), and 50% were suspended sediment-associated cells (average of $[100 - \%E]$).

Benthic primary production—Turtle grass was collected for all years from all stations except Rankin Basin. At Rankin Basin, turtle grass was collected only in 1997; shoal grass was collected in 1998 and 1999 (Table 1). Cross-plots of $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ (Fig. 2a) and $\delta^{13}\text{C}$ versus $\delta^{34}\text{S}$ (Fig. 2b) values for epiphytes and seagrasses across the period of study illustrate that throughout Florida Bay and in Lower Biscayne Bay, epiphytes were consistently depleted in ^{13}C relative to seagrasses. With the exception of enriched $\delta^{15}\text{N}$ values for epiphytes in Biscayne Bay, there was no consistent difference in $\delta^{15}\text{N}$ between epiphytes and seagrasses. Epiphytes were consistently enriched in ^{34}S relative to sea-

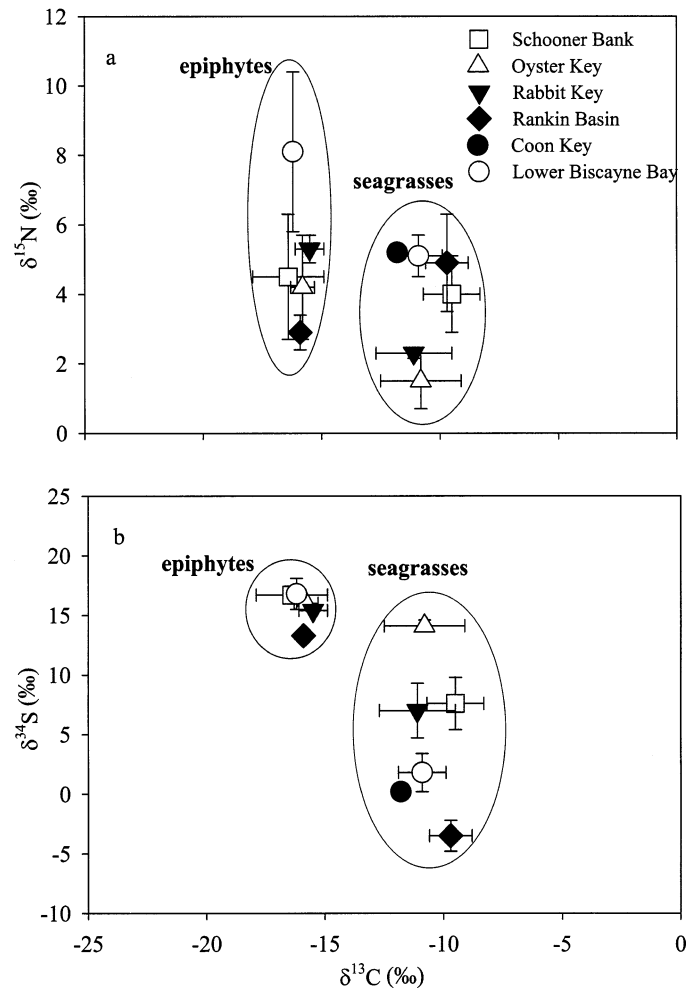


Fig. 2. Cross-plot of (a) $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ and (b) $\delta^{13}\text{C}$ versus $\delta^{34}\text{S}$ for epiphytes and seagrasses for 1997–1999. Points represent mean and standard deviation for turtle grass-associated epiphytes across all sampling years.

grasses. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of epiphytes and seagrasses evidenced no apparent regional gradient (i.e., from interior to Gulf). And while there was also little difference in $\delta^{34}\text{S}$ values for epiphytes among stations, $\delta^{34}\text{S}$ values for seagrasses collected from interior stations—Rankin Basin, Coon Key, and Biscayne Bay—were depleted relative to seagrasses collected from the Rabbit Key and the Gulf stations. Seagrasses from Oyster Key were depleted in ^{15}N and enriched in ^{34}S relative to all other stations for all sampling dates.

Consumers—Forty-five species (invertebrates and fishes) are represented in our collections from 1997–1999 (Web Appendix 1 at http://www.aslo.org/lo/toc/vol_50/issue_4/1059a1.pdf). Although ubiquitous and locally common species (pink shrimp, pinfish, gulf toadfish, pigfish, silver jenny, white grunt, gray snapper, and crevalle jack) were present in our collections for the entire study period, total and relative abundances in the collections varied greatly both by station and by year. Analysis of variance (ANOVA) and multiple comparison procedures (Tukey's and Dunnett's) ($\alpha = 0.05$,

power >0.8 for all results reported as significant) were used to evaluate (1) patterns of stable C, N, and S isotopes for invertebrates and fish specific to site and year; (2) patterns in stable C, N, and S for these organisms pooled across all years (1997–1999), at both local (site) and regional levels; (3) a comparison of these contemporary consumers to historical consumers (1956–1966), at both local and regional scales (Gulf, interior, and Biscayne Bay).

Interannual differences in consumer stable C, N, and S isotopes—Only a few species recurred frequently enough in collections from any particular site to allow valid assessment of site-specific interannual trends; these included gray snapper, crevalle jack, and pinfish (Table 4). The $\delta^{13}\text{C}$ values for gray snapper did not change significantly from 1997 to 1998 at Schooner Bank, Rabbit Key, and lower Biscayne Bay. For lower Biscayne Bay, although $\delta^{13}\text{C}$ values for 1997 versus 1998 and 1998 versus 1999 ($p = 0.121$) are not significantly different, $\delta^{13}\text{C}$ values for 1999 were ^{13}C -enriched relative to those of 1997. Gray snapper were also collected from Coon Key in both 1998 and 1999; Kruskal–Wallis ANOVA on ranks supports that specimens collected from Coon Key in 1998 and 1999 were not significantly different (median values -9.6 and -11.6 , respectively, $p = 0.111$). There were no significant interannual differences in $\delta^{13}\text{C}$ for crevalle jack from Oyster Key and Rankin Basin or for pinfish from Rankin Basin.

Interannual shifts in $\delta^{15}\text{N}$ values for gray snapper were significant only for Schooner Bank (more ^{15}N -enriched in 1998) and lower Biscayne Bay (more ^{15}N -enriched in 1999); the $\delta^{15}\text{N}$ values for crevalle jack from Rankin Basin were significantly more enriched in 1998 than 1997. All other interannual comparisons of $\delta^{15}\text{N}$ for these species, including Coon Key, were not significant ($p > 0.05$). No year effect for $\delta^{34}\text{S}$ was apparent at any site for gray snapper, crevalle jack, or pinfish.

Spatial trends in consumer stable C, N, and S isotopes—To evaluate spatial trends in stable C, N, and S isotopes for contemporary Florida Bay fauna, data for each species were integrated over the entire study period, from 1997 to 1999 (Web Appendix 1). Dual-isotope plots of $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ versus $\delta^{34}\text{S}$ for some of these individual species (crevalle jack, lemon shark, gray snapper, pinfish, penaeid shrimp, and gulf toadfish) are presented in Fig. 3. Fish from the interior (Rabbit Key, Rankin Basin, and Coon Key) stations were enriched in ^{13}C and depleted in ^{34}S relative to fish of the same species collected from the Gulf stations, Schooner Bank, and Oyster Key. In general, we found that mobile apex predators (e.g., crevalle jack and lemon and bull sharks) expressed less regional association than other consumers.

Across Florida Bay, $\delta^{15}\text{N}$ values for lower trophic levels were relatively consistent (pooled across year and station): $\delta^{15}\text{N} = 6.3\text{‰} \pm 1.7\text{‰}$ for caridean shrimp; $\delta^{15}\text{N} = 6.3\text{‰}$ for Atlantic bay scallop; and $\delta^{15}\text{N} = 8.1\text{‰} \pm 0.6\text{‰}$ for penaeid shrimp, blue crab, spiny lobster, pipefishes (syngnathids), scrawled cowfish, and fringed filefish; the majority of all mid- to upper-trophic-level consumers sampled during this study fell within a narrow range of $\delta^{15}\text{N}$ values, with a mean of $11.4\text{‰} \pm 1.2\text{‰}$. Several species collected from Bis-

Table 4. Interannual comparison of mean (standard deviation in parentheses) stable C, N, and S isotope values for crevalle jack (*Caranx hippos*), gray snapper (*Lutjanus griseus*), and pinfish (*Lagodon rhomboides*). ANOVA/Tukey's results are listed as significant (s) or nonsignificant (ns), with significant results in bold.

	<i>n</i>	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{34}\text{S}$
Gray snapper (<i>Lutjanus griseus</i>)				
Schooner Bank				
1997	4	-13.60 (1.30)	9.45 (0.54)	6.53 (1.29)
1998	4	-13.20 (1.27)	10.85 (0.79)	4.97 (1.53)
<i>p</i>		ns 0.676	s 0.027	ns 0.215
Rabbit Key				
1997	4	-10.47 (0.17)	11.25 (0.41)	2.57 (0.90)
1998	11	-10.98 (0.79)	11.03 (0.66)	2.53 (1.71)
1999	1	-9.82 (0.00)	12.11 (0.00)	1.80 (0.00)
<i>p</i>		ns 0.238	ns 0.542	ns 0.971
Lower Biscayne Bay				
1997	10	-13.09 (0.80)	12.37 (0.96)	4.12 (1.12)
1998	8	-12.55 (0.69)	12.27 (0.67)	4.90 (1.16)
1999	3	-11.48 (0.82)	14.18 (0.51)	5.13 (1.17)
<i>p</i>		s 0.013	s 0.009	ns 0.462
Crevalle jack (<i>Caranx hippos</i>)				
Oyster Key				
1997	3	-12.36 (0.89)	13.14 (0.67)	2.31 (1.96)
1998	1	-13.70 (0.00)	13.40 (0.00)	4.60 (0.00)
1999	11	-12.17 (0.29)	12.40 (0.17)	3.67 (1.07)
<i>p</i>		ns 0.724	ns 0.087	ns 0.279
Rankin Basin				
1997	4	-13.65 (0.44)	11.45 (0.62)	2.27 (0.91)
1998	2	-12.90 (0.28)	14.95 (0.64)	4.80 (1.56)
<i>p</i>		ns 0.089	s 0.003	ns 0.058
Pinfish (<i>Lagodon rhomboides</i>)				
Rankin Basin*				
1997	3	-14.07 (0.25)	9.77 (0.58)	2.30 (1.30)
1998	2	-13.25 (0.64)	9.65 (1.34)	1.50 (1.40)
<i>p</i>		ns 0.124	ns 0.897	ns 0.667

* *n* values are pooled for pinfish from Rankin Basin.

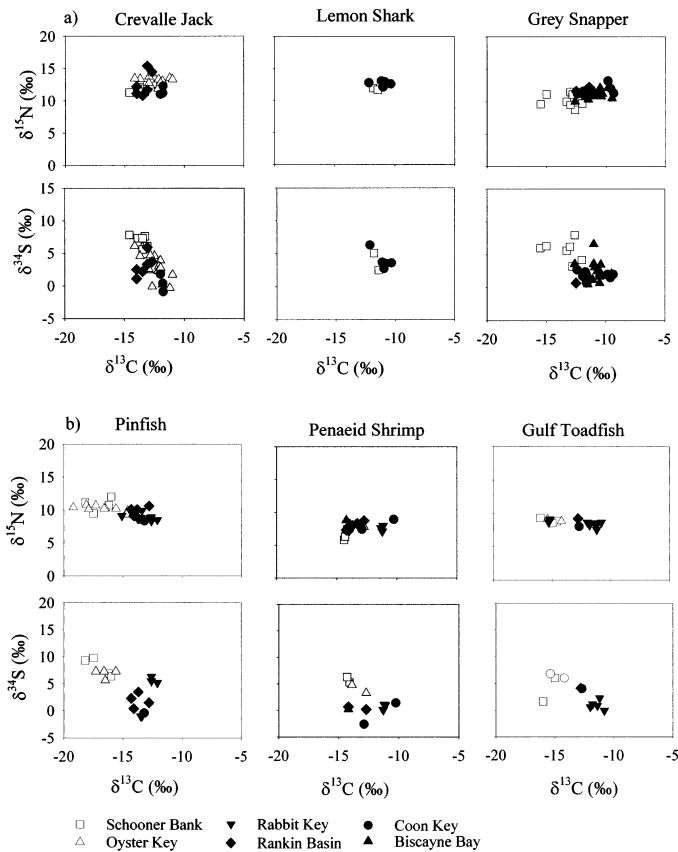


Fig. 3. Comparisons of $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ versus $\delta^{34}\text{S}$ for individual species across Florida Bay: (a) three tertiary carnivores; and (b) an omnivore (pinfish), a primary carnivore (penaeid shrimp), and secondary carnivore (gulf toadfish).

cayne Bay and Oyster Key—pinfish, gray snapper, gulf toadfish, and crevalle jack—were significantly ($p < 0.001$) enriched in ^{15}N relative to other stations.

In a broader approach to the evaluation of large-scale spatial trends, fauna were grouped according to estimated trophic level and geographic region. For these comparisons, trophic level was derived from Livingston et al. (1997), and consumers were grouped by region into Gulf (Schooner Bank and Oyster Key), interior (Rabbit Key, Rankin Basin, and Coon Key), and reference (lower Biscayne Bay), as described previously. Although differences were not always significant, fauna were ^{13}C -depleted for Gulf sites relative to interior and reference stations; ^{15}N -enriched for Gulf and reference relative to interior stations; and ^{34}S -enriched for Gulf relative to both interior and reference stations (Table 5). Differences in stable C and S isotope values between the interior of Florida Bay and the Biscayne Bay reference station were not significant for omnivores and primary and secondary carnivores.

Historical fish samples—Table 6 presents a species-specific comparison of mean C, N, and S results by region between the preserved fish from the Florida Museum of Natural History and the contemporary collections. The basic trend and character of the Bay appear relatively unchanged

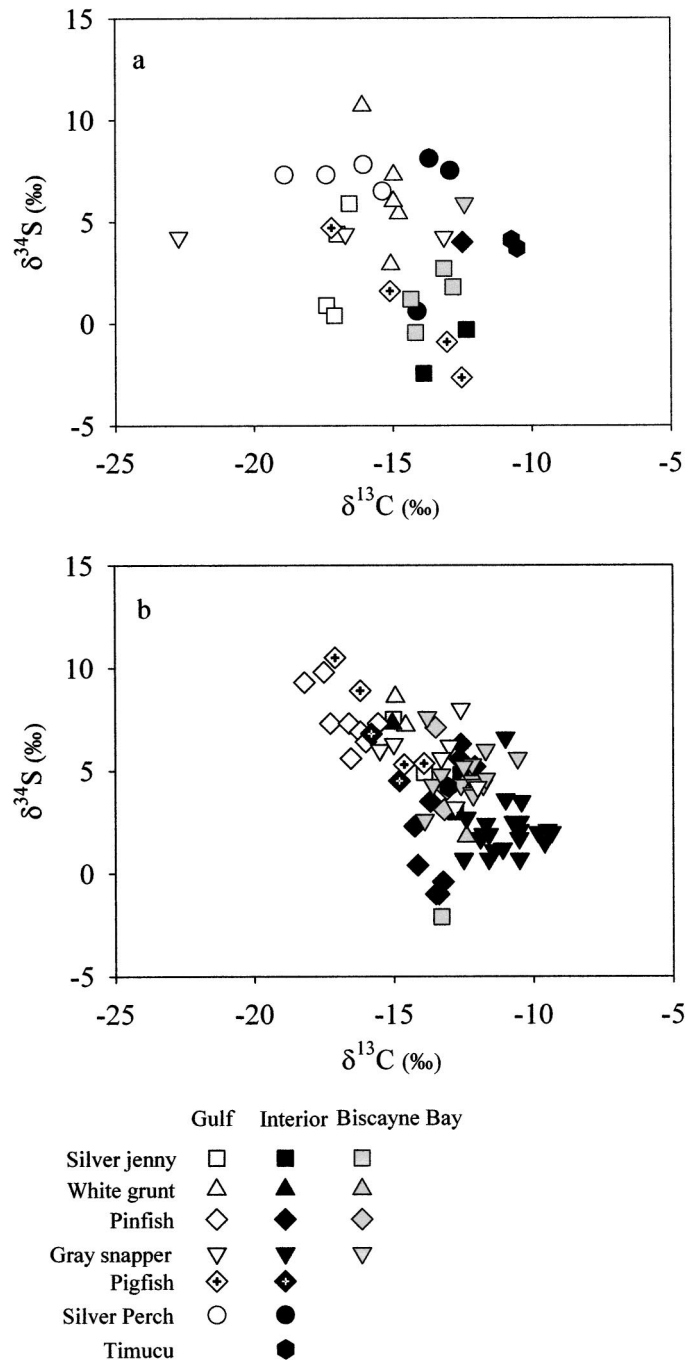


Fig. 4. Comparison of (a) preserved specimens from Florida Museum of Natural History, 1956–1963, and (b) current field specimens from Florida Bay and Biscayne Bay, 1997–1999. $\delta^{13}\text{C}$ for museum specimens is adjusted for preservation effects (+1‰); all museum specimens were fixed in formalin and stored in ethanol.

(i.e., Gulf specimens were depleted in ^{13}C and enriched in ^{34}S relative to interior stations and lower Biscayne Bay) (Fig. 4). A comparison of all species pooled by region indicates that there are no significant ($p > 0.05$) differences in $\delta^{13}\text{C}$ of contemporary and historical fish (Table 7). For all regions, the $\delta^{15}\text{N}$ signatures of museum specimens are more enriched ($p < 0.05$) than those of contemporary specimens; and for

Table 5. Regional comparison of mean (standard deviation in parentheses) stable C, N, and S isotope values for different trophic levels (omnivore, primary carnivore, secondary carnivore, and tertiary carnivore). Results of ANOVA/Tukey's test are listed as significant (s) or nonsignificant (ns); data for any region significantly different from other regions are in bold.

Regional	Omnivores						Primary carnivores					
	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{34}\text{S}$		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{34}\text{S}$	
Gulf	-16.3	(0.33)	10.00	(0.23)	7.51	(0.63)	-14.19	(0.38)	11.41	(0.17)	5.83	(0.82)
Interior	-13.32	(0.37)	9.10	(0.26)	2.25	(0.66)	-13.45	(0.52)	9.43	(0.23)	3.32	(1.12)
Reference	-13.27	(0.60)	11.54	(0.42)	4.32	(1.05)	-13.892	(0.49)	11.32	(0.21)	0.33	(0.73)
<i>p</i>	s	s	s	s	ns	ns	s	s	s	s	s	s
<i>p</i>	<0.001	≤0.040	≤0.040	≤0.040	0.529	0.529	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
<i>p</i>	ns	ns	ns	ns	>0.997	>0.997	ns	ns	ns	ns	ns	ns
<i>p</i>	>0.997	>0.997	>0.997	>0.997	≥0.240	≥0.240	≥0.948	≥0.948	≥0.948	≥0.948	≥0.092	≥0.092
Regional	Secondary carnivores						Tertiary carnivores					
	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{34}\text{S}$		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{34}\text{S}$	
Gulf	-15.09	(0.32)	10.87	(0.20)	6.26	(0.44)	-13.12	(0.17)	12.08	(0.18)	5.30	(0.30)
Interior	-12.93	(0.48)	9.00	(0.30)	2.34	(0.64)	-11.56	(0.18)	11.80	(0.17)	2.57	(0.32)
Reference	-12.82	(0.56)	11.42	(0.35)	3.03	(0.79)	-12.66	(0.24)	12.85	(0.22)	4.80	(0.48)
<i>p</i>	s	s	s	s	s	s	s	s	s	s	s	s
<i>p</i>	≤0.003	≤0.003	≤0.003	≤0.003	≤0.003	≤0.003	≤0.001	≤0.001	≤0.016	≤0.016	≤0.001	≤0.001
<i>p</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>p</i>	≥0.991	≥0.991	≥0.991	≥0.991	≥0.775	≥0.775	≥0.264	≥0.264	≥0.449	≥0.449	≥0.264	≥0.264

the Gulf, $\delta^{34}\text{S}$ signatures of museum specimens are significantly ($p < 0.001$) more depleted than are those of contemporary specimens, while $\delta^{34}\text{S}$ values for interior and Biscayne samples were not different.

Discussion

Spatial trends in current communities—Primary production: Although there were no apparent spatial trends in $\delta^{13}\text{C}$ of seagrasses or epiphytes within Florida Bay during the period of study, seagrasses from the interior of the bay were enriched in ^{15}N and depleted in ^{34}S relative to the western Gulf edge of the bay, indicating that suboxic microbial respiration (i.e., denitrification, sediment sulfide production) rates were higher at interior sites. Within-region variability and large-scale west-to-east enrichment in $\delta^{15}\text{N}$ of macrophytes have been documented across Florida Bay in the recent past (Corbett et al. 1999; D. Evans unpubl. data) and attributed to progressive denitrification within the bay and inputs of ^{15}N -enriched water to the northeastern region of the bay. West-east depletion in $\delta^{34}\text{S}$ signatures for seagrasses is indicative of a well-mixed region on the outer Gulf edge of the bay, in contrast to poorly flushed interior basins with more reducing conditions in sediments.

In lower Biscayne Bay, seagrasses are ^{15}N -enriched compared to all Florida Bay sites and ^{34}S -depleted relative to Gulf sites, which may indicate a combined influence of microbial respiration and inflow of anthropogenic N (Leitz 1999; Fry 2000; Dillon and Chanton in press) from the Miami-Dade metropolitan area.

Consumers: Chanton and Lewis (2002) noted a strong antipathetic shift in the $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ of consumers (macroinvertebrates and fishes) in estuaries along a gradient from seagrass beds toward more open marine environments. Such trends result primarily from a shift in the dependence of

consumers from benthic (enriched in ^{13}C , depleted in ^{34}S) to planktonic (depleted in ^{13}C , enriched in ^{34}S) production (Harrigan et al. 1989; Deegan and Garritt 1997; Wainright et al. 2000). When $\delta^{13}\text{C}$ versus $\delta^{34}\text{S}$ for Florida Bay consumers are pooled and plotted together with similar values in other consumers collected from intermediate grass beds (well-flushed, nutrient-rich, relatively high water column production) and estuarine sites dominated by water-column production (Chanton and Lewis 2002), the gradient in stable isotopic signatures across the bay indicates a shift from a strong benthic signal in lower Biscayne Bay and interior Florida Bay stations (Rabbit Key, Rankin Basin, and Coon Key) to an increasingly water-column-based, or phytoplankton, signal at the Gulf edge of the bay (Fig. 5). As described above, this gradient is forced in part by the increasing importance of planktonic production, as well as by regional differences in stable isotopic signatures of seagrasses and benthic algae (including both epiphytic and sediment-associated signatures) related to sediment conditions. A simple linear regression of the $\delta^{34}\text{S}$ of seagrasses (a proxy for influence of sediment sulfides) versus $\delta^{34}\text{S}$ of consumers (individual data for all species, all years) indicates a correlation between the two ($y = 2.63 + 0.229x$; $r = 0.41$; $p < 0.001$). A plot of $\delta^{34}\text{S}$ of seagrass versus mean $\delta^{34}\text{S}$ of all consumers combined for each site (Fig. 6) illustrates that as much as 40% of the variability in consumer $\delta^{34}\text{S}$ could be related to reducing conditions in the sediments (Carlson and Forrest 1982; Trust and Fry 1992; Currin et al. 1995); additional variability would then be attributable to dietary shifts, or shifts in community structure.

Relative importance of seagrasses, benthic algae, and phytoplankton—Our data indicate that seagrasses, seagrass detritus, and epipelagic algae are the dominant sources of organic carbon to the contemporary Florida Bay and lower

Table 6. Species-specific regional comparison of mean C, N, and S stable isotope signatures (with standard deviation in parentheses) for museum and contemporary fish.

		Museum (1956–1963)	Current (1997–1999)
<i>Silver jenny (Eucinostomus gula)</i>			
Gulf	$\delta^{13}\text{C}$	–17.0 (0.3)	–14.4 (0.8)
	$\delta^{15}\text{N}$	12.5 (0.4)	12.3 (0.1)
	$\delta^{34}\text{S}$	2.9 (2.7)	6.2 (1.8)
Interior	$\delta^{13}\text{C}$	–13.1 (1.1)	–12.5 (0.3)
	$\delta^{15}\text{N}$	12.0 (0.7)	9.3 (1.1)
	$\delta^{34}\text{S}$	–1.4 (1.5)	3.9 (1.2)
Biscayne	$\delta^{13}\text{C}$	–13.6 (0.7)	–13.3
	$\delta^{15}\text{N}$	12.7 (0.7)	10.5
	$\delta^{34}\text{S}$	1.3 (1.3)	–2.1
<i>White grunt (Haemulon plumieri)</i>			
Gulf	$\delta^{13}\text{C}$	–15.2 (0.6)	–14.8 (0.1)
	$\delta^{15}\text{N}$	13.0 (0.4)	10.8 (1.1)
	$\delta^{34}\text{S}$	6.5 (2.4)	7.9 (1.0)
<i>Pinfish (Lagodon rhomboides)</i>			
Interior	$\delta^{13}\text{C}$	–12.5	–13.3 (0.6)
	$\delta^{15}\text{N}$	11.9	8.9 (0.7)
	$\delta^{34}\text{S}$	4.0	1.5 (1.7)
<i>Gray snapper (Lutjanus griseus)</i>			
Gulf	$\delta^{13}\text{C}$	–14.9 (2.4)	–13.4 (1.2)
	$\delta^{15}\text{N}$	12.8 (0.1)	10.1 (1.0)
	$\delta^{34}\text{S}$	4.3 (0.1)	5.6 (1.5)
Biscayne	$\delta^{13}\text{C}$	–12.4	–12.6 (0.9)
	$\delta^{15}\text{N}$	15.2	12.6 (1.0)
	$\delta^{34}\text{S}$	5.9	4.7 (1.1)
<i>Pigfish (Orthopristis chrysoptera)</i>			
Gulf	$\delta^{13}\text{C}$	–14.5 (2.1)	–15.0 (2.0)
	$\delta^{15}\text{N}$	12.1 (1.3)	10.5 (0.5)
	$\delta^{34}\text{S}$	0.7 (3.2)	7.5 (2.2)

Table 7. Comparison of mean (with standard deviation in parentheses) C, N, and S stable isotope signatures for all species combined by region for museum (1956–1963) and contemporary (1997–1999) fish specimens.

		n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{34}\text{S}$
<i>Gulf</i>					
Recent 1997–1999	42	–15.17 (1.68)	10.47 (0.98)	7.12 (1.84)	
Museum 1956–1966	20	–16.12 (2.24)	13.12 (1.15)	4.43 (3.27)	
p		ns 0.067	s <.001	s <.001	
<i>Interior</i>					
Recent 1997–1999	61	–12.55 (1.91)	10.28 (1.22)	2.63 (2.08)	
Museum 1956–1966	8	–12.59 (1.38)	12.98 (1.06)	3.15 (3.70)	
p		ns 0.959	s <.001	ns 0.571	
<i>Reference</i>					
Recent 1997–1999	31	–12.73 (0.83)	12.26 (1.10)	4.29 (1.89)	
Museum 1956–1966	7	–13.47 (1.44)	13.31 (1.33)	2.64 (2.52)	
p		ns 0.074	s 0.034	ns 0.072	

Biscayne Bay ecosystems. The cross-plots of $\delta^{13}\text{C}$ versus $\delta^{34}\text{S}$ presented in Fig. 7 illustrate the relative importance of phytoplankton (estimated range of values), seagrass epiphytes, seagrasses, and epipelagic algae (estimated range of values) on the trophic character of each site. The estimated ranges of values for phytoplankton and epipelagic algae provided in Fig. 7 are broad enough to allow a conservative comparison of consumers to primary production— $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ for phytoplankton include values from estuarine, near-shore, and open-ocean environments; the range for epipelagic algae includes values of $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ from several estuarine and coastal zones, including muddy, reducing saltmarsh sediments (Wainright et al. 2000; Moncrieff and Sullivan 2001).

Because the consumer data points represent means for each individual species occurring at a site, and the species are classified as ubiquitous (Loftus 2000), these cross-plots should represent a fairly accurate picture of the production-dependence of the food web as a whole. Consumers from Oyster Key, Rabbit Key, Rankin Basin, Coon Key, and Biscayne Bay all expressed a very strong benthic signal characteristic of the combined influence of seagrasses and epipelagic algae. Consumer values at Schooner Bank appear to range from seagrass and epipelagic algae toward epiphytes and plankton.

Highly mobile apex predators express little site association in either $\delta^{13}\text{C}$ or $\delta^{34}\text{S}$ (e.g., see Fig. 3). They effectively integrate the signatures of their prey over longer temporal and larger spatial scales than do smaller forage species. As integrators, these predators should provide an accurate view of the trophic character of system(s) within their range. For tertiary carnivores collected across Florida Bay, $\delta^{13}\text{C}$ sig-

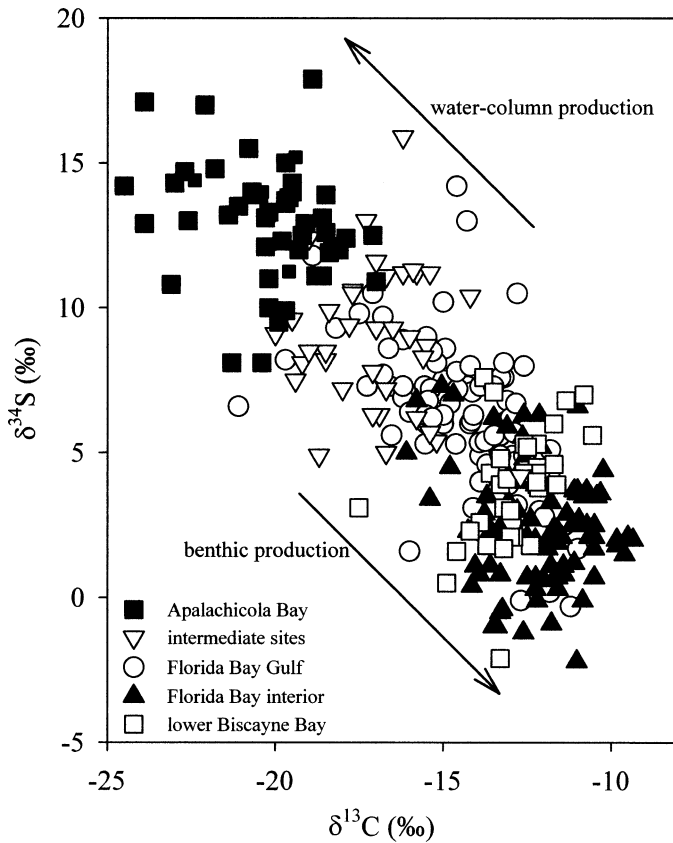


Fig. 5. Summary comparison of consumer tissue $\delta^{13}\text{C}$ versus $\delta^{34}\text{S}$ from several Florida coastal ecosystems. Data illustrate the gradient in stable isotope values as trophic dependence shifts from seagrass beds “benthic” ($\delta^{13}\text{C}$ -enriched, $\delta^{34}\text{S}$ -depleted) to the water-column (“planktonic”) ($\delta^{13}\text{C}$ -depleted, $\delta^{34}\text{S}$ -enriched) (Chanton and Lewis 2002).

natures range from -13‰ to -11‰ , and $\delta^{34}\text{S}$ signatures range from $+2\text{‰}$ to $+6\text{‰}$. These values indicate that the food web of the bay is currently characterized by strong benthic–pelagic coupling and that seagrasses, seagrass detritus, sedimentary organic matter, and epipellic algae are the dominant sources of organic carbon to the system.

Historical samples—A review of research on preservation effects on avian muscle tissue, bird eggs, marine invertebrates, and fish reveals that storage in formalin and ethanol (with storage times ranging from 3 weeks to 8 yr) may result in ^{13}C depletion ranging from 0.6‰ to 2‰ (Mullin et al. 1984; Hobson et al. 1997; Bosley and Wainright 1999). Results of recent studies specific to fish indicate that the impact of preservation on the stable C isotope ratio of fish tissue is predictable, discrete (i.e., an initial depletion at the onset of preservation, with no cumulative isotopic shift), and small ($\Delta\delta^{13}\text{C} \approx -1\text{‰}$) (Arrington and Winemiller 2002; Chasar 2002; Edwards 2002). Results were similar for nitrogen, with ^{15}N enrichment related to formalin and ethanol small and predictable, on the order of 0‰ to 1.5‰ (Ogawa et al. 2001; Arrington and Winemiller 2002; Edwards 2002). These differences are on the same order as intraspecific variability in

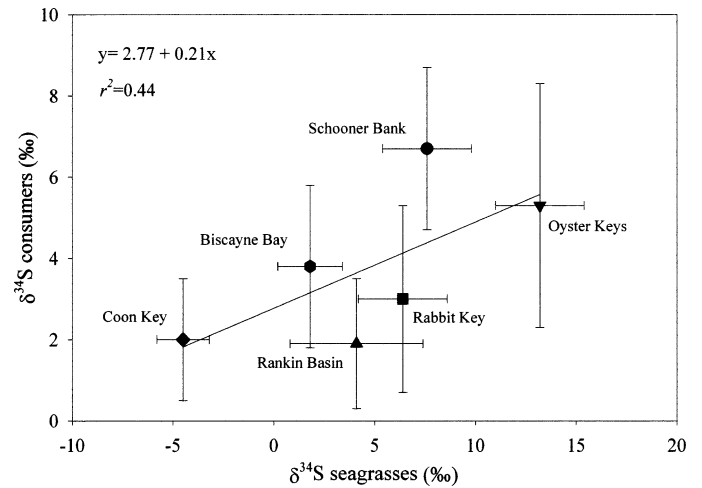


Fig. 6. Regression of means for seagrass $\delta^{34}\text{S}$ versus consumer tissue $\delta^{34}\text{S}$. Means represent all specimens combined by site across all years.

fish (or same functional feeding groups) within a given ecosystem.

Examples of utilization of fluid-preserved museum specimens for dietary studies based on stable isotopes are rare.

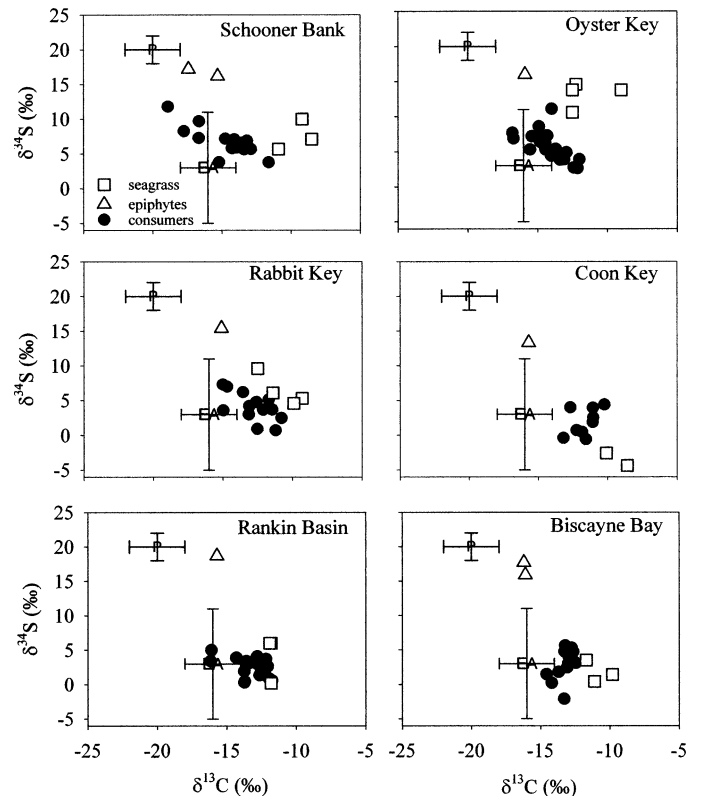


Fig. 7. Site-specific comparisons of $\delta^{13}\text{C}$ versus $\delta^{34}\text{S}$ for seagrasses, seagrass epiphytes, and consumers. Ranges of values for phytoplankton (P) and epipellic algae (EA) are estimated from published values for similar estuarine and coastal settings. Consumer data points represent the means for each of the species occurring at a given site.

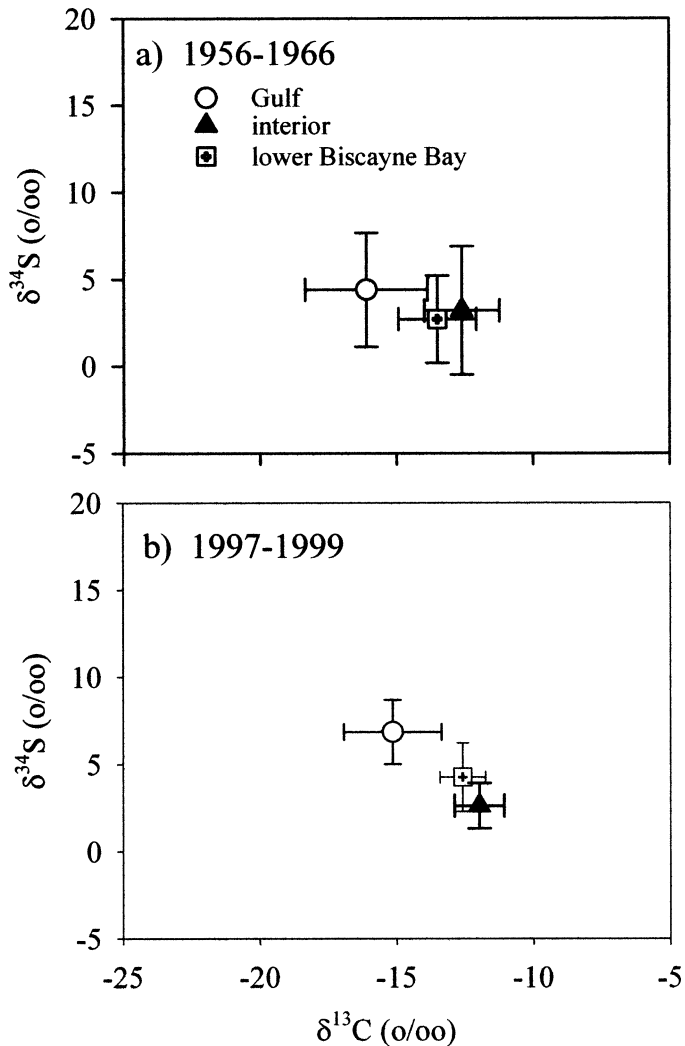


Fig. 8. Comparison of (a) preserved, and (b) current fish specimens from Florida Bay. $\delta^{13}\text{C}$ values for museum specimens were adjusted for preservation effects (+1‰); all museum specimens were fixed in formalin and stored in ethanol, and collection dates ranged from 1956 to 1963.

After demonstrating that formalin fixation and storage in ETOH for up to 117 weeks induced only slight changes in $\delta^{15}\text{N}$ in muscle tissue of gobiid fishes ($\Delta\delta^{15}\text{N} < 0.2\text{‰}$), Ogasawara et al. (2001) evaluated the history of eutrophication in Lake Biwa, Japan, during the 20th century by comparing $\delta^{15}\text{N}$ values in preserved (formalin-fixed, stored in ETOH) fishes (1916–1994) to $\delta^{15}\text{N}$ values of sediments in cores (0–9 cm, representing 1900–1995). In this study, preservation effects were assumed to be small and not significant, and no correction factor was applied.

For our study, based on demonstrated significant but predictable preservation effects on stable C isotopic composition of muscle tissue ($\Delta\delta^{13}\text{C} \approx -1\text{‰}$), we corrected $\delta^{13}\text{C}$ of archived fish and compared these historical specimens to contemporary collections. When this was done for Florida Bay and Biscayne Bay in the context of the benthic to pelagic gradient illustrated in Fig. 8, we find that the overall trophic character of the bay has not changed over the years

and is strongly driven by benthic production (seagrasses, benthic algae, and possibly sedimentary organic matter). Small differences in stable isotopic signatures for historical versus recent Gulf consumers could be related to natural species-specific interannual variability (on the order of 1–2‰ over the course of study, 1997–1999, Web Appendix 1); differences could also be related to influence of greater water delivery to the western edge of Florida Bay from Shark River Slough (e.g., terrestrial and mangrove sulfur) and more seagrass (*Thalassia testudinum*) coverage in this region during the 1950s to 1970s (Hall et al. 1999; Lorenz et al. 1999).

No evidence was found to support the occurrence of a large-scale shift in Florida Bay from a historically seagrass-dominated to a plankton-dominated system. The $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ signatures of macroinvertebrates and fishes collected from several sites in Florida Bay expressed a strong dominance of benthic production. And although intermediate consumers from Gulf stations (Oyster Key and Schooner Bank) expressed depletion in $\delta^{13}\text{C}$ and enrichment in $\delta^{34}\text{S}$ relative to interior stations (Rabbit Key, Coon Key, and Rankin Basin), reflecting the stronger influence of epiphytes and phytoplankton, this could reflect natural spatial variation that has existed historically. This spatial variation is probably the result of gradients in sediment redox conditions and higher epiphyte and phytoplankton abundances for Gulf stations (Frankovitch and Fourqurean 1997)—primary and secondary consumers often express a shift in the relative importance of specific dietary/prey items based on availability (Deegan and Garritt 1997; Wainright et al. 2000; Moncrieff and Sullivan 2001).

The lack of a catastrophic shift in the trophic character of the bay from 1984 to 1995 could be attributable to the spatial and temporal extent of seagrass mortality. Although short-shoot densities of *Thalassia* decreased locally by as much as 65% in central Florida Bay, comparative surveys conducted in 1984 and 1994 indicate that for the bay as a whole, decreases were more modest, with decreases in short-shoot density and standing stock of approximately 22% and 28%, respectively (Hall et al. 1999; Thayer et al. 1999). During this time, turtlegrass remained the dominant species, was consistently present throughout the bay (Hall et al. 1999), and continued to support high faunal abundances similar to predisturbance levels (Sheridan et al. 1997). And while there were periodic cyanobacterial blooms in the north-central portion of the bay (Phlips et al. 1999) and increases in the relative abundances of pelagic fish species (e.g., *Anchoa mitchilli*; Thayer et al. 1999), by 1997, seagrasses were beginning to recover, and anchovy were rare at all of our stations. Our stable isotope data corroborate the limited community response described above and indicate that even though mid- and upper-trophic-level consumers may have included more pelagic species in their diet temporarily, most continued to feed preferentially in and around the seagrass canopy and did not experience a complete or irreversible shift in their forage base.

This does not mean that Florida Bay is safe from future compromises to ecosystem functioning—several scientists have proposed that persistent, low-level, external disturbances to natural systems will result in loss of resilience and eventually force an alternate (negative) stable state (Scheffer

et al. 2001; Poff et al. 2002; Oechel et al. 1993). Livingston (1984) conducted a 9-yr study of seagrass communities in Apalachicola Bay, Florida, and found that even extreme natural variations (e.g., fire, flood, hurricanes) resulted in negligible shifts in the bay's trophic structure, whereas habitat alterations forced by human influences resulted in rapid shifts in community structure. And although Livingston documented recovery in Apalachicola Bay, Scheffer et al. (2001) propose that ecosystems suffering chronic long-term environmental disturbance could experience catastrophic shifts in functioning with the continued application of stressors.

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