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Trophic Niche Metrics Reveal Long-Term Shift in Florida Bay Food Webs

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Abstract

Seagrass beds in Florida Bay are home to many ecologically and economically important species. Anthropogenic press perturbation via alterations in hydrology and pulse perturbations such as drought can lead to hypersalinity, hypoxia, and sulfide toxicity, ultimately causing seagrass die-offs. Florida Bay has undergone two large-scale seagrass die-offs, the first in the late 1980s and early 1990s and the second in 2015. Post-die-off events, samples were collected for stable isotope analysis. Using historical (1998–1999) and contemporary (2018) stable isotope data, we examine how food webs in Florida Bay have changed in response to seagrass die-off over time by measuring contributions of basal sources to energy usage and using trophic niche analysis to compare niche size and overlap. We examined three consumer species sampled in both time periods (*Orthopristis chrysoptera, Lagodon rhomboides, and Eucinostomus gula*) in our study. Seagrass production comprised the majority of source usage in both datasets. However, contemporary consumers had a mean increase of 18% seagrass usage and a mean decrease in epiphyte usage of 7%. The shift in trophic niche from epiphyte usage (green pathway) toward seagrass usage (brown pathway) may indicate that food web browning is occurring in Florida Bay.

Key words: Stable isotopes; Hypervolumes; Seagrass; Coastal ecosystems; Resource use; Seagrass die-off..

Highlights

- Contemporary consumers use less epiphyte and more seagrass production
- Resource niche spaces between historical and contemporary food webs do not overlap
- The shift in resource usage indicates a change in energy flow in seagrass food webs

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Introduction

Food web function—that is, the production of individuals and the transformation and movement of energy across a community network, is a crucial ecosystem function that long-term press perturbations can substantially alter (Bartley and others 2019; D'Alelio and others 2019; Beauchesne and

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others 2021). Two examples of major alterations to food webs are food web greening and browning, which refer to the pathways primary production enters the food web (Hayden and others 2017, 2019; Leech and others 2018). Primary production can enter the food web by consumption of live resources, typically referred to as green pathway, or as detritus, referred to as brown pathway. A food web that experiences a shift from direct consumption of live primary production to detrital energy pathways is described as undergoing food web browning, while food webs that shift from detrital pathways to direct consumption of primary production are described as experiencing food web greening (Hayden and others 2017, 2019; Leech and others 2018). Shifts in the way primary production enters a food web can alter food web structure and the efficiency of energy transfer within a system (Scheffer and Carpenter 2003; Hayden and others 2017, 2019). Detrital trophic pathways typically have slower turnover and are less energetically efficient than green pathways (Rooney and others 2006; Blanchard and others 2011). Additionally, greening or browning of a food web may affect overall community composition by favoring generalists and pelagic organisms versus benthic organisms, respectively (Hayden and others 2019).

Studies of oligotrophic, subarctic lakes have documented food web greening, attributed to eutrophication and increased temperature (Hayden and others 2017, 2019). Cold, clear lakes were compared to warmer, murky lakes along a temperature and productivity gradient to predict the interactive effects of nutrient enrichment and climate change (Hayden and others 2017, 2019). Increasing temperature and productivity were accompanied by a shift in trophic pathways from primarily benthic (brown) production to pelagic (green) production. Hayden and others (2019) also noted that the shift from benthic to pelagic pathways was caused by a shift in the diet of benthic primary consumers to pelagic production and not by increased pelagic consumption by higher consumers. Leech and others (2018) found similar shifts in trophic structure and food web greening in lakes exhibiting eutrophication across the continental USA.

Evidence for browning or increases in the detrital channel of food webs is much more sparse. Most cases of increased detrital inputs were observed with substantial changes, gain or loss, in allochthonous inputs to the food web. In subarctic grasslands, a reduction in allochthonous inputs from ephemeral streams resulted in greater dependence on local detrital inputs to the food web (Hoekman and others 2019). Woody debris additions, typically used in forested stream restoration, increase detrital inputs into forest food webs (Entrekin and others 2020; Rosi-Marshall and Wallace 2002). Similar trends have been observed in other aquatic systems, where browning of food webs can occur following restoration, as detrital pools of resources build up over time (Rezek and others 2017; James and others 2020). In restored habitats, food web browning shifted food web structure to more closely resemble food webs in natural, un-restored habitats (Rezek and others 2017; James and others 2020).

We know much less about the food web response of coastal systems to long-term system change. Greening has been observed in marine food webs in seagrass-dominated systems altered by anthropogenic stressors, including eutrophication and overfishing (Tewfik and others 2005). A long-term study of the San Francisco Bay showed the gradual greening of the food web in response to changes in nutrient concentrations and stoichiometry over time (Glibert and others 2011). However, studies in saltmarsh ecosystems have shown muted food web responses to long-term changes in detrital inputs or nutrient additions (Buchsbaum and others 2009; Nelson and others 2019). As the need to respond to climate change becomes more urgent and more extensive restoration efforts are proposed in coastal systems, it is critical to understand how coastal systems and their food webs respond to long-term press perturbations. Energetic shifts signaled by food web greening or browning may provide insight into how anthropogenic disturbances are affecting coastal food webs. Understanding structural responses, like browning and greening, can aid in setting reasonable and achievable restoration and conservation targets to ensure coastal systems continue to deliver the important services they provide.

Florida Bay, a productive seagrass environment, has experienced various press and pulse perturbations with unknown implications for the food web function it provides. Florida Bay is a shallow estuary located at the southern tip of the Florida peninsula. The estuary supports large seagrass beds comprised primarily of *Thalassia testudinum* (Fourqurean and Robblee 1999), which provide critical habitat to many ecologically and economically important species, including a lucrative recreational fishery (Kelble and others 2013; Brown and others 2018; Stainback and others 2019). Florida Bay is hydrologically connected to the Everglades and upland waterways of the Florida Peninsula.

The upland freshwater flow has been highly engineered since the middle part of the twentieth century to sustain development, control flooding, and support agriculture (Light and Dineen 1994; McPherson and Halley 1996; Renken and others 2005).

Consequently, the water flow regimes to Florida Bay have been drastically altered for decades, with an estimated 2–3 times less freshwater flow between 1990 and 2000 than at the end of the nineteenth century (Marshall and others 2020). The reduction in freshwater input coupled with drought has caused chronic seasonal hypersalinity in the central region of the bay (Marshall and others 2020). Hypersalinity, combined with increasing temperatures, has led to hypoxic stress, sulfide toxicity, and ultimately large-scale seagrass die-offs in Florida Bay regions (Koch and others 2007). These conditions have led to two large-scale seagrass die-offs. The first began in 1987, fully recovering in 2010 (Robblee and others 1991; Hall and others 2021), and a second of similar size that started in 2015 (Hall and others 2016, Figure 1). Areas affected by the 2015 seagrass die-off have begun showing signs of recovery, but basins have not fully recovered (Rodemann and others 2021).

The drought-induced seagrass die-off in the late 1980s and early 1990s inspired several studies to assess the cause and the ecological consequences of the loss of seagrass habitats and the source of primary production in the Florida Bay shallow coastal system. For instance, Chasar and others (2005) used stable isotope analysis (SIA) to determine if the large-scale mortality of seagrasses in Florida Bay had caused a basal shift of the bay's food web from primarily benthic trophic pathways to pelagic pathways. Despite the large-scale seagrass die-off and subsequent years of persistent algal blooms and increased turbidity, shifts in the trophic structure of the Florida Bay system were not observed (Chasar and others 2005). After the 2015 large-scale seagrass mortality event (also drought-induced), additional sampling and SIA were performed. Food web data collected approximately 20 years apart under similar post seagrass die-off conditions provide a unique opportunity to reexamine the trophic structure of the Florida Bay system, determine how food web function may have been altered by continued press and pulse perturbations, and compare trophic responses to both events.

Stable isotope analysis has allowed ecologists to develop niche metrics that use the variability in isotope values to estimate niche widths (Bearhop and others 2004) that can be mapped and explored in space using areas calculated by convex hulls, standard ellipses, or kernel density estimations (Blonder and others 2014; Layman and others 2007; Jackson and others 2011). More recently, stable isotope niche metrics have allowed researchers to compare trophic niches for consumers along productivity gradients in seagrass ecosystems (Lesser and others 2020), between restored and unrestored habitats (James and others 2020), and to determine how variation in individual movement alters resource use (Rezek and others 2020). By trophic niches, we refer to how organisms assimilate energy from the production channels available in their environment (Lesser and others 2020). These metrics use variation in resource use as determined by Bayesian mixing models to visualize and quantify trophic niche space as well as the degree of overlap among niche spaces (Lesser and others 2020). Here, we compared the trophic niche of post seagrass die-off Florida Bay consumers from samples taken in 1997–1999 (historic data; Chasar and others 2005), and recently collected data fol-

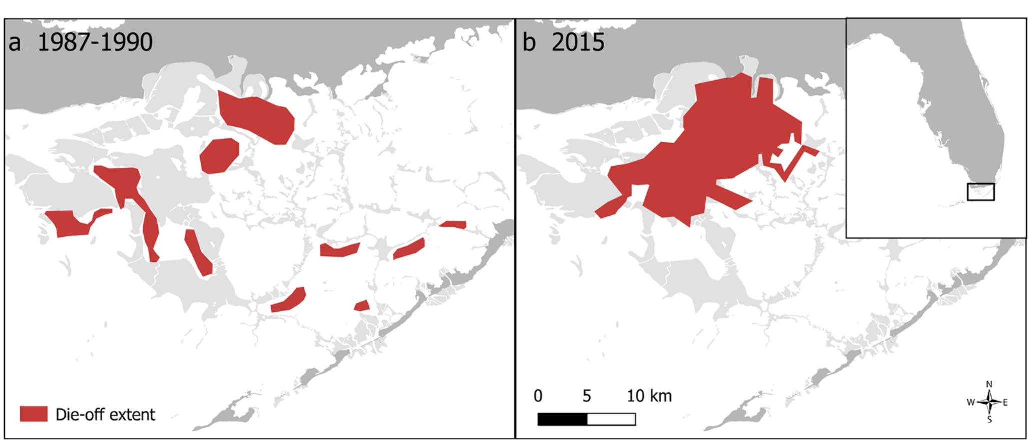

Figure 1. Map of Florida Bay historical (**a**) and contemporary (**b**) seagrass die-offs. Extent of die-off is represented in red.

lowing the 2015 die-off (contemporary data; James and others 2022) to determine if the trophic structure of the ecosystem has shifted over time. We also used these datasets to determine if the food web response to the two seagrass die-off events is trophically similar. Our primary objectives were to quantify the niche space for three dominant consumers in both data sets and determine the overall changes in resource use in the Bay between 1998 and 2018. Given the chronic loss of seagrass habitat, we hypothesized that the food web as a whole would shift to be dominated by green resources. This shift in resource usage would be evident in the trophic niches of consumers with an increased reliance on epiphytes and macroalgae (green) and decreased reliance on seagrass and mangrove (brown) production. Alternatively, a food web-wide shift toward brown resources and increased usage of seagrass and mangrove production in trophic niches would indicate food web browning. To address these hypotheses, we applied mixing models to quantify shifts in basal resource use of consumers collected from Florida Bay in 1998, informed by Chasar and others (2005), and again in 2018. Additionally, we used hypervolume niche analysis to compare the differences between historical and contemporary trophic niches.

Materials and Methods

Collection Methods

Contemporary consumer and basal source (primary producer) samples were collected using the methods in James and others (2022). Briefly, consumers at low and intermediate trophic levels, including Silver Jenny mojarra (*Eucinostomus gula*), pigfish (*Orthopristis chrysoptera*), and pinfish (*Lagodon rhomboides*), were collected in the wet season (September) of 2018 (3 years post the 2015 seagrass die-off event) via otter trawl. Five individuals of each species collected at each sampling location were pooled to generate one composite sample (for example, $n = 1$ pooled sample/species/site). In preparation for SIA, whole individuals were dried at 50 °C for 48 h and ground together to create composite samples. Digestive systems of individuals were not removed prior to processing, which would potentially allow some dietary items to be included in the ground samples, although these contributions would be minimal compared to the mass of the whole animal (James and others 2022).

Likewise, live primary producers including seagrasses (*Halodule wrightii and T. testudinum*), macroalgae species (for example, *Halimeda* spp., *Caulerpa* spp., *Penicillus* spp., *Batophora oerstedi*), and mangrove leaves were collected by hand concurrent with the nekton collection (James and others 2022). Blades of seagrass were rinsed with deionized (DI) water and then, scraped to remove sediment and epiphytes. Both seagrasses and macroalgae were acid washed with 10% HCl and rinsed with DI water prior to being dried at 50 °C for 48 h. Mangrove leaves were rinsed with DI water and dried under the same conditions. These primary producers were then ground and sent for SIA. Macroalgal species were prepared and sent off to be analyzed individually. Gastropod grazers (*Turbo castanea*) that specialize in feeding on epiphytes were used as proxies to calculate the isotope values of epiphytes by correcting the gastropod isotope value one trophic level (Frankovich and Zieman 2005; James and others 2022). Trophically corrected isotope values from primary consumers have commonly been used to acquire isotope values of primary producers in instances where collecting sufficient organic matter for SIA is difficult (Frankovich and Zieman 2005; James and others 2022). The gastropods were collected at the same time as other primary producer samples. In preparation for SIA, gastropods were removed from their shells, rinsed with DI water, and dried at 50 °C for 48 h. All primary producer and nekton samples were analyzed at the Washington State University Stable Isotope Core Facility for C, N, and S stable isotopes. Analytical error, measured as the standard deviation of replicate samples measured across all runs, was 0.4‰ for δ^{13}C, 0.5‰ for δ^{15}N, and 0.9‰ for δ^{34}S. The isotopic data were calculated using a multi-point normalization from at least two internal standards or certified reference materials (Washington State University 2023). Ten percent of samples were run in duplicate to confirm accuracy and reproducibility of the measurements. The average offset between the standard sample and duplicate for each isotope is -0.1 ± 0.4, -0.3 ± 0.4, and 0.2 ± 0.3 for δ^{13}C, δ^{15}N, and δ^{34}S, respectively (average offset ± SD). The mean and standard deviation of all contemporary consumer and producer isotope values are found in Table 1.

Historic consumer samples from Chasar and others (2005) used in this study were collected from Rankin Basin, Rabbit Key, and Schooner Bank in 1997, 1998, and 1999 (6–10 years post the start of the 1987 seagrass die-off event). Pigfish (*Orthopristis chrysoptera)*, pinfish (*Lagodon rhomboides)*, and mojarra (*Eucinostomus gula*), among other species, were collected, dried, and wrapped for stable C and N at Isotope Services in Los Alamos, New Mexico and S isotopic analysis at Coastal

Table 1. Mean and Standard Deviation Isotope Values for Sources and Consumers

Species	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$			
Benthic algae	− 18.6 ± 3.8	2.6 ± 2.5	19.6 ± 3.3			
Epiphytes	− 13.6 ± 2.2	0.4 ± 0.5	10.1 ± 2.6			
Seagrass	− 11.3 ± 1.6	− 0.5 ± 3.1	4.0 ± 4.0			
Mangroves	− 27.7 ± 1.1	1.1 ± 1.5	− 13.5 ± 3.8			
	Historic	*Contemporary*	*Historic*	*Contemporary*	*Historic*	*Contemporary*
O. chrysoptera	− 15.0 ± 2.0	− 13.5 ± 0.5	10.5 ± 0.5	7.5 ± 1.0	7.5 ± 2.2	2.1 ± 0.9
L. rhomboides	− 13.8 ± 0.5	− 13.1 ± 1.2	9.7 ± 0.9	6.8 ± 0.8	2.0 ± 1.4	5.0 ± 1.8
E. gula	− 12.5 ± 0.3	− 12.3 ± 1.3	9.3 ± 1.0	7.1 ± 0.9	3.9 ± 1.2	1.7 ± 1.3

Scientific Laboratories in Austin, Texas (Chasar and others 2005). Analytical error for SIA of these historical samples was 0.2‰ for δ^{13}C and δ^{15}N and 0.5‰ for δ^{34}S. Additional collection details can be found in Chasar and others (2005). The museum specimen values analyzed by Chasar and others (2005) were not included in the present analysis due to the potential influence of preservatives on the tissues used for SIA.

Resource Contributions

The historic basal source values given by Chasar and others (2005) did not include mangroves as a source, nor were they thoroughly analyzed with mixing models, and therefore did not fully represent the mixing space for the food web in Florida Bay. Mixing space refers to the isotopic distributions of major basal resources available to consumers in our system. Additionally, there are no other stable isotope datasets within our timeframe and system that include sulfur isotopes; therefore, we use only the contemporary resource values when parameterizing the mixing model (Table 1). Although there may be variation in the isotope values of the sources over time within the source types, this variation will not impact our ability to distinguish between source types. This is because the variation within a source, even over time, is smaller than the variation between sources that is caused by the distinct biogeochemical processes used by each production type (Fry 2006). In short, the variation within seagrasses over time would not be so drastic that it would cause overlap with mangroves, algae, or epiphytes in isotopic space.

Bayesian mixing models were run in R version 4.0.3 (R Core Team 2020) using the package MixSIAR (v 3.1.12, Stock and others 2018) to determine the relative basal source contributions to each historic and modern consumer species. Each model was run with a Markov chain Monte Carlo algorithm that consisted of three chains, chain length of 1,000,000, burn-in of 500,000, and thin of 500 to ensure model convergence. Algal species could not be distinguished isotopically from one another and were combined in analyses. Basal resources were averaged across space to serve as the source values for the mixing models, and since spatial variation was not a hypothesis being tested and was shown to be small by James and others (2022). We use two trophic steps to represent an average consumer trophic level and fully enclose the mixing space. Using two trophic steps to represent an average consumer trophic level accounts for the number of fractionations that take place between a source and a given consumer and uses that information to estimate the relative trophic position of the consumer (Lesser and others, 2020). The trophic enrichment (mean and standard deviation) for each element was $C = 2.5 \pm 0.3$‰, $N = 7.25 \pm 1$‰, and $S = 1 \pm 0.2$‰ (Nelson and others 2015, 2019).

Niche Metrics

We used hypervolume niche analysis to assess changes in species trophic niche over time for the species that occurred in both data sets (*O. chrysoptera*, *L. rhomboides,* and *E. gula)*. Due to small sample sizes from both data sets, the output from the mixing models and the R package truncnorm (v 1.0.8, Mersmann and others 2018) were used to randomly generate 100 points from the mean and standard deviation between the 2.5% and 97.5% confidence intervals for the estimated contribution of each basal resource to each consumer's diet (James and others 2020). This approach ensured that we had an adequate number of points for the hypervolume analysis (Blonder and others 2014) and incorporated the uncertainty from the mixing models in the hypervolume analysis (James and others 2020). The mean value for each source contribution to each consumer was z-transformed before hypervolume analysis to allow for stan-

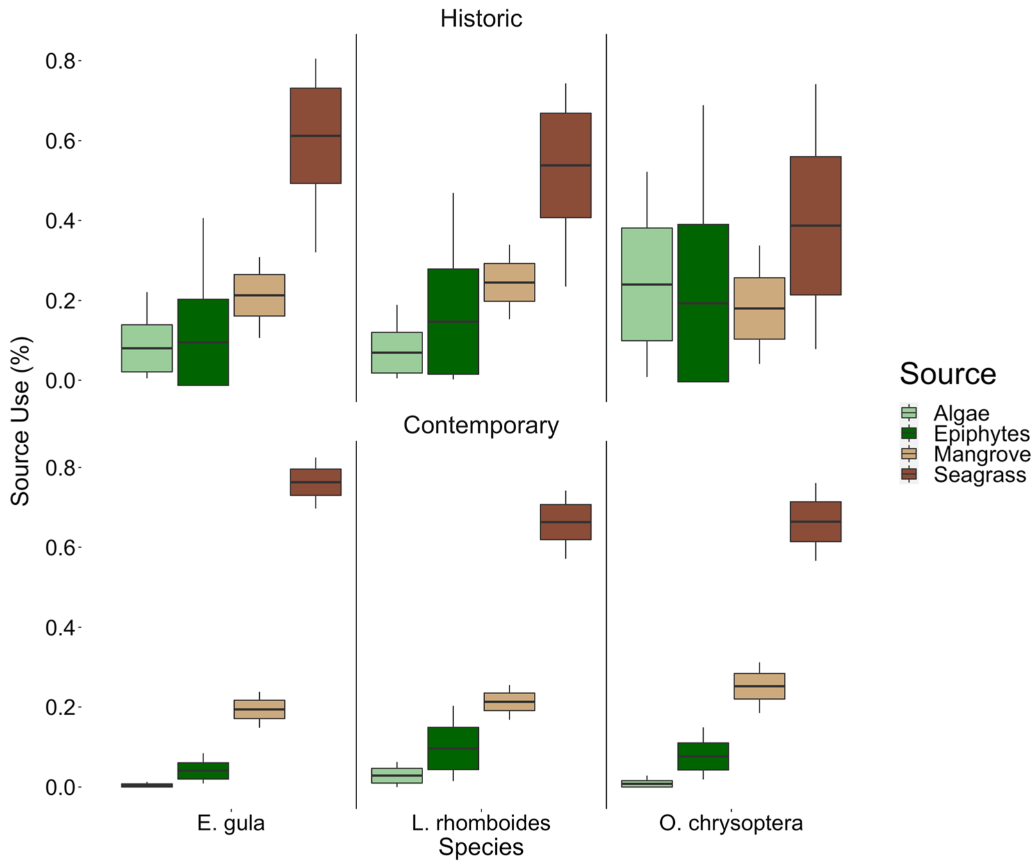

Figure 2. Proportion of primary production use by historic (top) and contemporary (bottom) consumer species.

dardized, comparable axes in *n*-dimensional space (Blonder and others 2014; Lesser and others 2020). We used the Hypervolume R package (v 2.0.12) to seed a Gaussian kernel density estimation that generated a cloud of points based on the distribution of the *z*-scored values along the 4 resource axes that define the multidimensional trophic niche of the species (Wilson and others 2017; Lesser and others 2020). The quantile threshold used was 0.05, so that each hypervolume included 95% of the total probability density of resource use (Blonder and others 2014). To determine the variation in hypervolume metrics, the entire process was repeated 100 times (that is, random points and hypervolumes were generated 100 times per species in each time period) (James and others 2020). The fraction of unique hypervolume space for consumer species is reported for both the historic (1997–1999) and contemporary (2018) food webs as well as the percentage overlap between hypervolumes (Sorenson similarity; Blonder and others 2014).

Results

Resource Use

Resource use differed between historic and contemporary food webs, but the importance of seagrass production as the major basal resource contributor was common in both time points and across species (Figure 2). Contributions of algae production were low overall, with epiphyte and mangrove contributions differing between species and over time (Figure 2). Most of the changes of the basal resources integrated by consumers occurred due to the shift in seagrass and epiphyte contribution.

Among *L. rhomboides*, *O. chrysotera*, and *E. gula*, epiphyte use from historic to present decreased by an average of 7% (Figure 2). This shift was accompanied by a mean increase in reliance in seagrass resources of 18% between the two time periods. In contrast, the mangrove and algae contributions’ direction of change was species-specific. Historically, seagrasses made up 54% of *L. rhomboides* resource use. This was followed by epiphytic

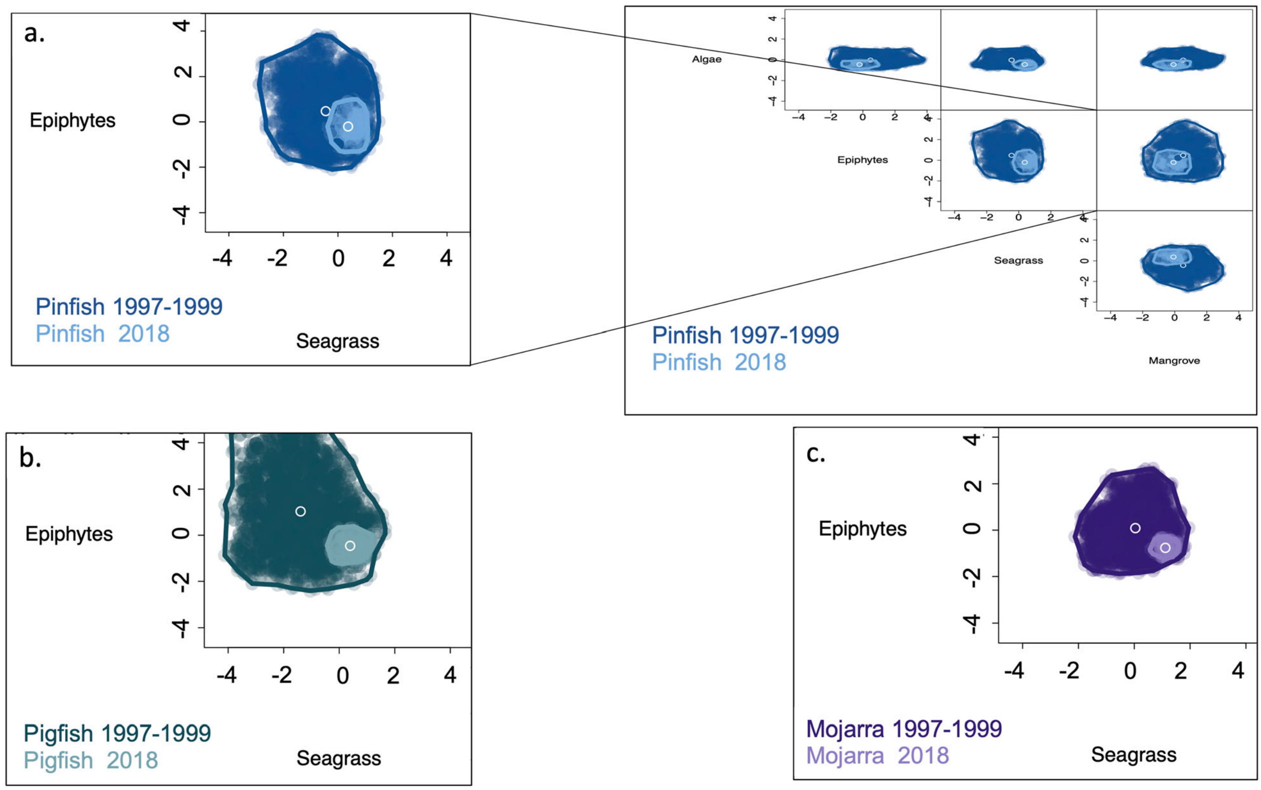

Figure 3. Resource use niches for epiphyte and seagrass usage in pinfish (*L. rhomboides*; a), pigfish (*O. chrysoptera*; b), and mojarra (*E. gula*; c) in collection years 1997–1999 and 2018. Areas in color represent trophic niches, and size of the niches represents the variation of resource use within the population. Axes are z-scored source contributions, with values greater than zero indicating relatively more resource use than the global mean and values less than zero indicating relatively less. Resource use niches including all sources are located in Figure S1. Hypervolumes are multidimensional representations of niche space and therefore, can appear to be overlapped when displayed in two dimensions, when mathematically they are not.

and mangrove contributions at 15% and 24%, respectively (Figure 2a; Table S1). Today, *L. rhomboides* source 66% of their energy from seagrasses, 21% from mangroves, 10% from epiphytes, and 3% from algae (Figure 2b; Table S1). The changes observed in *O. chrysoptera* diet over time include an increase in seagrass contributions from 39 to 66%, a shift in mangrove contributions from 18 to 25%, a decrease in epiphytic contributions from 19 to 8%, and a decrease in algal contributions from 24 to 1% (Figure 2; Table S1). For *E. gula*, seagrass contributions increased from 61 to 76%, mangrove contributions decreased from 21 to 19%, epiphytic contributions decreased from 10 to 4%, and algal contributions decreased from 8 to 0% (Figure 2; Table S1).

Niche Metrics

Niche hypervolume sizes decreased in each intraspecies comparison over time. *L. rhomboides* niche size decreased from 102.9 to 2.54 (97.5% change, Figure 3a; Table 2), *O. chrysoptera* from 870.18 to 1.10 (99.9% change, Figure 3b; Table 2), and *E. gula* from 93.13 to 0.15 (99.8% decrease, Figure 3c; Table 2). The Sorensen overlap index between the historic and contemporary food web was 0. Overlap in each intraspecies comparison over time was also 0 for *E. gula* and *O. chrysoptera*, but approximately 4% overlap was present in *O. chrysoptera* (Figure 3; Table 2). It is important to note that hypervolumes are multidimensional representations of niche space and therefore, when displayed in two dimensions, can appear to be overlapped when mathematically they are not. The shifts in resource usage between historical and contemporary niches account for the relative movement of the centroids within niche space (Figure 3). The distance between centroids was greatest for *O.* chrysoptera at 3.74 and ranged between 1.52 and 1.71 for *L. rhomboides* and *E. gula*, respectively (Table 2). The shift in centroid and the decrease in niche size both contribute to the absence of overlap in all the intraspecies comparisons.

Table 2. Niche Size, Percentage Overlap and Centroid Distance of *O. chrysoptera, L. rhomboides, and E.gula* in 1997–99 and 2018

Species	Niche Size		Niche overlap	Centroid distance
	1997–1999	2018		
O. chrysoptera	870.18(508.35–1348.13)	1.10(0.53–1.95)	0.00(0.00–0.01)	3.74(3.44–4.04)
L. rhomboides	102.90(59.71–156.47)	2.54(1.24–4.19)	0.04(0.02–0.07)	1.52(1.17–1.82)
E. gula	93.19(50.41–166.02)	0.15(0.07–0.25)	0.00(0.00–0.00)	1.71(1.43–2.02)

Values are mean (2.5–97.5% CI)

Discussion

Our analysis demonstrates the overall importance of seagrass beds to the food webs in Florida Bay. The mixing models indicate that, for both historical and contemporary food webs, direct inputs of seagrass production represent the majority of energy assimilated by consumers. However, contemporary consumers increased usage of seagrass energy relative to historic consumers and decreased reliance on epiphytes than their historical counterparts (Figure 2; Table S1). The shift from epiphyte usage toward seagrass usage may indicate that the food webs in Florida Bay are undergoing food web browning.

Direct consumption of living seagrass is typically thought to be limited to large vertebrate grazers such as waterfowl, sea turtles, and manatees; however, smaller grazers such as omnivorous and herbivorous fishes and invertebrates also directly consume seagrass leaves (Valentine and Duffy 2006). Parrotfishes, in particular, have been noted as direct consumers of seagrasses in the Florida Keys and around the world (Kirsch and others 2002; Valentine and Duffy 2006). However, seagrass production typically enters the food web through detritus (Fourqurean and Schrlau 2003). Aside from direct detrital consumption via invertebrates and benthic fishes, particulate organic matter (POM) produced by seagrass beds is also an important carbon source for bacteria (Williams and others 2009). According to a stable isotope and fatty acid analysis of pelagic, epiphytic, and sediment bacteria in Florida Bay by Williams and others (2009), seagrass-derived carbon contributed 13–67% of total bacterial carbon. Small invertebrates consume the seagrass carbon processed through bacterial pathways and pass that energy on to higher trophic levels like fishes (Azam and others 1983; Pomeroy and others 2007).

The decrease in epiphyte contribution to the diets of *O. chrysoptera*, *L. rhomboides*, and *E. gula* between time periods may be indirectly caused by the loss of seagrass cover in Florida Bay. The mass seagrass die-offs observed in Florida Bay are thought to be caused by a cascade of stressors that ultimately lead to an imbalance of O_2 concentrations in the system (Koch and others 2007). Seagrass habitats are susceptible to hypersalinity through hypoxia and direct and indirect effects of sulfide toxicity (Koch and others 2007). Initially, a lack of substrate, associated with seagrass mortality, may have reduced epiphyte availability; however, seagrass beds have recovered, albeit slowly, to pre-disturbance concentrations following the 1987 seagrass die-offs (Hall and others 2021). Persistent algal blooms and re-suspended sediment limited recovery of Florida Bay seagrasses for the first 8–10 years after the 1987 die-off, but recovery was observed 16–17 years post-die-off (Hall and others 2021). Sample collection of both the historic and contemporary data used in the present study fall within the 8–10 year frame of limited recovery for seagrasses, which would suggest that substrate availability for epiphytes could be low. However, because both datasets occur during this timeframe of recovery, it is unlikely that seagrass substrate availability over time is a driving factor of decreased epiphyte usage.

A more likely driver of epiphyte concentrations during recovery periods is the succession of seagrass species that occur after a die-off. Initial recovery of seagrass beds is typically populated by *Halodule wrightii*, a species of seagrass that has smaller blades than *T. testudinum* (Hall and others 2021). The smaller blades of *H. wrightii* would provide less substrate for epiphyte attachment even at similar coverage concentrations to that of *T. testudinum*. Depending on the severity of the die-off, coverage of *T. testudinum* and *H. wrightii* were similar in patchy loss areas and *H. wrightii* had higher percentage cover in severely impacted areas during 1997–1999 when Chasar and others (2005) were sampling (Hall and others 2021). During the 2018 sampling, *H. wrightii* was the main species present (pers. comm., W. Ryan James). These slight differences in seagrass succession and species coverage during the sampling periods could have influenced the concentration of epiphytes and ultimately their availability to consumers.

Hypersaline conditions in Florida Bay during the summer months could also be causing lower epiphyte densities. Studies in Shark Bay, Australia found diversity and densities of seagrass epiphytes decreased significantly as hypersalinity increased (Harlin and others 1985; Kendrick and others 1988). A similar reduction in epiphytic diatoms was observed across a hypersalinity gradient in southern Texas (Jewett-Smith 1991). Frankovich and Fourqurean (1997) noted that species of epiphytes in Florida Bay changed along a salinity gradient from primary producers in areas with lower and more stable salinity to epiphytic molluscs in areas of variable salinity or hypersalinity. However, recurrent hypersalinity events were not measured or considered in their study (Frankovich and Fourqurean 1997). In another study performed in Florida Bay, Frankovich and others (2009) found that nutrient enrichment, especially *P*, can affect community structure of epiphytes, which may lead to increases of unpalatable or toxic species. However, other contributing factors (such as salinity) have a greater impact on structuring epiphyte communities in marine systems than nutrient enrichment alone (Snoeijs 1999; Frankovich and others 2009). It is not apparent that hypersalinity conditions were present during either of the sampling periods for the data used here; however, hypersalinity events were measured in the time in between the historic and contemporary data collections (Kelbe and others 2007; Glibert and others 2021). Frequent hypersalinity events and eutrophication could be contributing to the decrease in epiphyte use within Florida Bay.

The absence of overlap in the historical and contemporary diets of *O. chrysoptera*, *L. rhomboides*, and *E. gula* indicates a shift in resource usage within Florida Bay. In all cases, there is a shift away from epiphyte consumption toward the benthic primary production of seagrasses suggesting a possible browning of the food web. This shift is evident in the relative movement of the hypervolume centroids in niche space. The positional movement of the niches is primarily driven by the decreased usage of epiphytes and increased usage of seagrasses. Additionally, *O. chrysoptera* and *E. gula* decreased consumption of algae, which also supports the idea that the food web is shifting to one more dominated by benthic production. Detrital pathways often have slower turnover rates (that is, production:biomass ratios) and lower energy transfer efficiency than primary producer pathways (Rooney and others 2006; Blanchard and others 2011). A shift toward more benthic energy pathways could increase stability and resiliency of the food web, as described by Rooney and others (2006) and Blanchard and others (2011). However, food web browning in Florida Bay may impact community composition by favoring benthic organisms and their consumers over pelagic species (Hayden and others 2017, 2019).

Lastly, the difference in niche size between historical and contemporary food webs in Florida Bay may indicate a shift in seagrass productivity over time. Lesser and others (2020) used the same hypervolume metric to measure niche size in *L. rhomboides* and *O. chrysoptera* across a production gradient of seagrass beds in the Big Bend region of Florida. When compared, niche sizes in systems with less dense stands of seagrass were larger than those in highly dense systems (Lesser and others 2020). Lesser and others (2020) postulated that in highly productive seagrass habitats, generalist fishes like *L. rhomboides* and *O. chrysoptera* can consume preferred diet items instead of eating a broader range of less preferred diet items in less productive systems. Santos and others (2022) found similar results, as *L. rhomboides* in Biscayne Bay seagrass ecosystems displayed decreases in niche size in areas that had more seagrass. If this is true for the food webs in Florida Bay, it may suggest that regardless of concentrations, epiphytes are not a preferred diet item for our species of interest and the contemporary recovery of seagrass beds enabled *L. rhomboides* and *O. chrysoptera* to consume preferred diet items that rely on seagrass production pathways.

As both historical and contemporary datasets used in the present analysis were collected during recovery periods after seagrass die-offs, it is difficult to determine if the observed shifts in trophic niches are a characteristic of the stage of seagrass recovery or a new baseline for the ecosystem. The absence of long-term stable isotope sampling in Florida Bay, including sulfur isotopes, prevents us from delineating how the trophic shift fits into the history of Florida Bay seagrass bed habitats. Long-term datasets, like those used by Nelson and others (2015), allow for better clarity when examining the drivers of trophic structure in a system. Monitoring programs in Florida Bay, such as those that track juvenile fish abundances and seagrass coverage (NOAA/SFER 2019), could include samples for SIA so that changes to trophic niches could be tracked through time and in conjunction with environmental data collection.

Additionally, our approach of using published historical SIA data could be applied to other ecosystems to track changes in trophic structure over time and in the face of anthropogenic impacts. The limitations of using historical SIA data include that those we encountered in this project: limited or no access to raw stable isotope data to run stable isotope mixing models and inconsistencies with identifying all basal sources within an ecosystem. The methods we used to overcome these limitations, namely creating distributions based on the outputs of mixing models from published stable isotope data and using contemporary source data to better define the mixing space, are not perfect solutions but still allow us to make meaningful observations about changes to energy cycling in our system. The insight provided by a historical timepoint can be valuable in developing plans for restoration and conservation.

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DATA AVAILABILITY

All data available at https://github.com/wryanjames/James_etal_ICES_JMS.

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