



Reef fish community structure along the southeastern US Atlantic continental shelf break and upper slope appears resistant to increasing lionfish (*Pterois volitans/miles*) density

¹ Southeast Fisheries Science Center, National Marine Fisheries Service, 101 Pivers Island Drive, Beaufort, North Carolina 28516

² Southeast Fisheries Science Center, National Marine Fisheries Service, 3500 Delwood Beach Road, Panama City, Florida 32408

³ 6 Sorghum Lane, Savannah, Georgia 31411

* Corresponding author email: <nate.bachelor@noaa.gov>

Nathan M Bachelor ^{1*}
Christina M Schobernd ¹
Stacey L Harter ²
Andrew W David ²
George R Sedberry ³
G Todd Kellison ¹

ABSTRACT.—Temperate reefs host diverse fish communities along the southeast United States Atlantic coast (SEUS), yet the sustainable management of reef fishes faces myriad challenges. One challenge has been the introduction of Indo-Pacific lionfish (*Pterois volitans/miles*; hereafter “lionfish”), which have spread quickly throughout the SEUS since their introduction in the late 1900s. We analyzed long-term (2001–2019) video data along the continental shelf break and upper slope (45–125 m deep) of the SEUS to assess changes in lionfish densities over time, characterize reef fish community structure, and determine if native reef fish community structure has changed due to lionfish. Lionfish densities increased substantially during the study, from zero individuals observed in 2001 to approximately 1.2 individuals observed per 100 m³ (and present in all included transects) by 2019, yet no fish community metrics were negatively related to lionfish density. Demersal habitat influenced fish community structure more than any other variable examined, with more individuals and different fish communities occurring on high-relief compared to low-relief hardbottom habitats. The effects of latitude, depth, and bottom temperature on reef fish community structure were generally weak or nonexistent. Although previous empirical work has found that lionfish negatively affect native fishes at small scales (<30 km²), it is unclear why we did not find similar results in our larger-scale study. It may be related to vagaries of the spatial scale of observation, lionfish effects being primarily limited to high-relief habitats, time lags, or lionfish densities not being high enough yet to cause observable ecological effects.

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Various species have invaded ecosystems by range expansion or human introductions, and many invasive species are a major threat to native ecosystem functioning and biodiversity (Bax et al. 2003). In the aquatic realm, most high-profile invasions have been invertebrate species introduced into lakes or estuaries via ballast water used in global shipping (Carlton 2001), such as zebra mussels (*Dreissena polymorpha*) introduced into the Great Lakes (Mills et al. 1993). Fish introductions, and in particular predatory fish introductions, can be especially damaging to native aquatic ecosystems due to loss of biodiversity, displacement of native species, and altered community structure (Goldschmidt et al. 1993, Beisner et al. 2003, Pine et al. 2007).

The Indo-Pacific lionfish (*Pterois volitans* and *Pterois miles*; hereafter “lionfish”)—a generalist piscivore native to coral reef habitats in the Indian and Pacific oceans—has become one of the most successful invasive species in the Atlantic Ocean (Whitfield et al. 2002). Lionfish have established populations in the western Atlantic Ocean, Caribbean Sea, Gulf of Mexico, and Mediterranean Sea (Whitfield et al. 2002, Betancur-R. et al. 2011, Bariche et al. 2017) following introductions from the aquarium trade in the 1980s (Schofield 2009). Their fast growth rates, high fecundity, generalist foraging habits, broad patterns of habitat use, large home ranges, and few natural predators have led to a rapid increase in density throughout the western Atlantic and elsewhere (Morris et al. 2011, Côté et al. 2013, Green et al. 2021). Lionfish are now a likely permanent component of demersal fish assemblages in continental shelf and shelf-break waters of the western Atlantic, including from Cape Hatteras, North Carolina, to the Florida Keys (Whitfield et al. 2006, Ruttenberg et al. 2012, Bacheler et al. 2016a). By 2010, lionfish had already surpassed densities of 1 individual per 100 m² in some locations off North Carolina (Whitfield et al. 2014). While highly rugose natural or artificial reefs are considered primary lionfish habitat (Schofield 2009, Bejarano et al. 2015), they also use other complex habitats like mangroves and seagrasses (Claydon et al. 2012). There are biologically diverse fish communities that associate with these same reef habitats (Schobernd and Sedberry 2009, Bacheler et al. 2019) that are already facing a variety of threats including fishing pressure, habitat loss, and fluctuating environmental conditions. Lionfish can influence native fish communities via predation (Muñoz et al. 2011), as well as competition with native predator fishes (Eddy et al. 2020).

It is unclear if or how invasive lionfish have influenced native fish community structure at a regional scale (i.e., 100s of km²). Experimental studies have shown that lionfish can clearly reduce recruitment of native prey fish at small scales (<30 km²; Albins and Hixon 2008, Green et al. 2014, Albins 2015). For instance, in a localized reef in the Bahamas, lionfish density increased rapidly during the 2000s, which coincided with a 65% decline in biomass of potential lionfish prey species between 2008 and 2010 (Green et al. 2012). Similarly, Albins (2013) showed that invasive lionfish had a much stronger influence on a coral reef fish community than a native predatory grouper, reducing native coral reef fish abundance by 94% and species richness by a mean of five species per patch reef. Moreover, Dahl et al. (2016) documented declines of small demersal reef fishes at some artificial reefs in the northern Gulf of Mexico after the lionfish invasion. The biggest limitation of most studies that have examined lionfish effects on fish community dynamics, however, is that the spatial scale of these studies has been relatively small, mostly occurring over areas less than 30 km². In contrast, Ballew et al. (2016) showed that increasing invasive lionfish densities

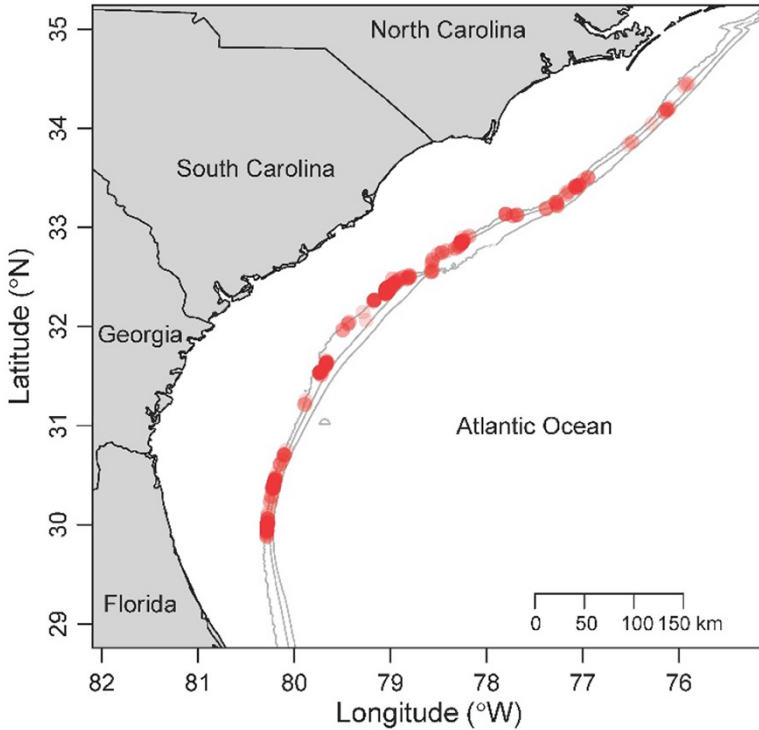


Figure 1. Map of the study region showing the distribution of underwater video samples collected between North Carolina and Florida along the southeast United States continental shelf break, 2001–2019. Each red dot represents an underwater video sample collected in this study, and the darker the symbol, the greater overlap among sampling points. Three depth isobaths are shown: 50 m, 100 m, and 200 m.

coincided with a 45% decline in the abundance of one species, tomtate (*Haemulon aurolineatum*), on the southeast United States continental shelf (about 100,000 km²). Other studies have found no regional influence of lionfish on native fish species' density or richness (Elise et al. 2015, Hackerott et al. 2017).

Here we examined whether lionfish have influenced reef fish community structure along the southeast United States Atlantic (hereafter, "SEUS") continental shelf break and upper slope (45–125 m deep; about 5° latitudinal range) over the past two decades. There were three objectives for our study: (1) to assess changes in lionfish density over time along the SEUS continental shelf break and upper slope, focusing on temporal changes, spatial hotspots, and habitat preferences; (2) to characterize fish communities along the SEUS continental shelf break and upper slope; and (3) to determine if fish community structure has been affected by increasing lionfish densities.

METHODS

STUDY AREA.—In the SEUS, the continental shelf break is the strip of seafloor that separates the broad, flat continental shelf from the steeper continental slope. In our study, we analyzed data collected in depths ranging from 45 to 125 m between Cape

Hatteras, North Carolina, and Cape Canaveral, Florida (Fig. 1), which encompassed the continental shelf break and the shallowest part of the upper continental slope in the SEUS. Reef fishes along the continental shelf and upper slope in the SEUS generally associate with scattered patches of rocky substrates that are quite variable, ranging from flat limestone pavement to high-relief (>15 m) rocky ledges (Schobernd and Sedberry 2009). The position and strength of the Gulf Stream also influences the reef fish community in the SEUS via nutrient availability and bottom water temperature dynamics (Lee et al. 1991, Bane et al. 2001, Hyun and He 2010, Whitfield et al. 2014).

DATA COLLECTION.—We sampled demersal habitats along the continental shelf break and upper slope in the SEUS using a video-based transect approach. Hardbottom reef habitats were targeted for transect sampling, and specific locations were chosen using four potential data sources: (1) known reef fish spawning locations (Sedberry et al. 2006, Schobernd and Sedberry 2009); (2) bathymetry and backscatter information from multibeam sonar maps shared with us or created on previous or current research cruises; (3) reef locations provided by colleagues; and (4) sites discovered during previous surveys led by one or more of the authors of this manuscript. Sampling occurred haphazardly throughout the SEUS each year when weather conditions and water currents allowed, but attempts were made to disperse sampling each year across multiple locations in the study area. Transects typically followed depth contours.

Reef-associated fish species were surveyed along transects using video collected from submersibles or remotely operated vehicles (ROVs). Submersible video was collected in 2001–2004, while ROV video was collected in 2004–2019. Bachelier et al. (2016b) showed that the detectability of fishes was comparable from submersibles and ROVs, so video data from these two vehicles were combined. Two submersibles were used to collect data in our study: CLELIA (2001) and JOHNSON SEA LINK II (2002–2004), both owned and operated by the Harbor Branch Oceanographic Institute. A tracking system linked to the ship's navigation system was used to determine the continuous position and speed (range = 0.1–2.2 ms⁻¹; mean = 0.5 ms⁻¹) of the submersible. The video camera was mounted at a 45° downward angle on the forward end of each submersible. Depending on the altitude of the vehicle, the camera was 2–3 m above the bottom allowing consistent observation of fish and substrate. Both submersibles were outfitted with xenon arc lights that illuminated bottom habitats and fishes for easier identification and Sea-Bird Sealogger (SBE-19 and SBE-25) conductivity-temperature-depth (CTD) profilers to continuously record bottom temperature and depth. Three ROVs were used in our study: PHANTOM S-2 (2004, 2007–2019), HELA (2006), and MOHAWK (2014). The umbilical of each ROV was attached to a down weight to keep the vehicles near the bottom during dives while allowing a 30-m scope of movement. As with the submersibles, the geographic position and speed (range = 0.3–0.8 ms⁻¹; mean = 0.4 ms⁻¹) of the ROVs were recorded continuously during each dive using a tracking system linked to the ship's Global Positioning System. Video cameras were attached to a tilt bar on the front of each ROV, allowing cameras to be moved between forward-looking and down-looking orientations. The PHANTOM and HELA ROVs were equipped with two 250-W tungsten-halogen lights (Deep Sea Power and Light), while the MOHAWK used LED lights, and all ROVs included a Sea-Bird Sealogger (SBE-39) CTD to provide continuous water temperature and depth information.

VIDEO READING.—Our video reading protocol was adapted from Bacheler et al. (2016b). Each submersible or ROV dive transect was divided into multiple partial transects (i.e., “subtransects”) determined by habitat type and distance surveyed. Each subtransect consisted of a single habitat type, and new subtransects began when a different habitat type was encountered. If any habitat-specific subtransect was less than 50 m long, it was removed from the analyses due to the relatively small amount of information contained in short subtransects. Subtransect volume (m^3) was calculated as the product of the subtransect width, height, and length. Subtransect width and height were estimated using two forward-aiming laser beams that were separated by a known distance, which was 25 cm for submersible videos and 10 cm for ROV videos. Subtransect length was determined by measuring the distance along the submersible or ROV path from the subtransect start position to the subtransect end position (using ESRI ArcMap 10.3). For our study, the experimental unit was the “transect,” which we define as the combination (i.e., sum) of subtransects of the same habitat type in a single dive. Thus, transects are the sum of all fish counts and sampling volume across all subtransects (at least 50 m long) of the same habitat type within a single dive.

Habitat types were defined empirically based on the presence or absence of consolidated (hard) sediments and the relief of the substrate. Five habitat types were identified in our study: (1) sand (relief = 0 m, hardbottom absent); (2) pavement (PAV; relief <0.3 m, hardbottom present); (3) low-relief outcrop (LRO; relief = 0.3–1.0 m, hardbottom present); (4) moderate-relief outcrop (MRO; relief = 1.1–3.0 m, hardbottom present); and (5) high-relief ledge (HRL; relief >3.0 m, hardbottom present; Fig. 2). All sand transects were removed prior to analysis to focus on reef-associated fish species, and substrate relief was estimated using the lasers visible in videos. No lionfish, and very few other fishes, were observed on sand transects.

Underwater videos were examined to determine habitat type and identify and enumerate fishes. Individual fish were identified to the lowest taxonomic level possible from the start to end of each subtransect, and a tally system was used to determine counts for each taxon in each subtransect. Videos were often stopped and rewound frame by frame when multiple species were present and fish counts were high. Individuals were recorded as “unknown” if they could not be confidently identified. Moreover, we estimated counts of individuals in large schools (over approximately 250 individuals). Fish attraction to, and schooling around, underwater vehicles has been observed for some fish species (Stoner et al. 2008), which we attempted to ameliorate in our study by avoiding recounts of individuals that we could uniquely identify using color patterns or body markings.

OBJECTIVE 1: ASSESS CHANGES IN LIONFISH DENSITY OVER TIME.—We addressed the first objective of our study by including lionfish density as the response variable in a generalized additive model (GAM). GAMs are semiparametric regression models that relate a response variable to predictor variables using smoothed, nonlinear functions (Hastie and Tibshirani 1990). GAMs can fit a variety of statistical distributions to underlying data, can uncover hidden patterns in the data using flexible predictor functions, and are easy to interpret (Wood 2011). We used GAMs instead of nominal analyses because it was important to standardize transect densities of lionfish by predictor variables that might influence their density or detection

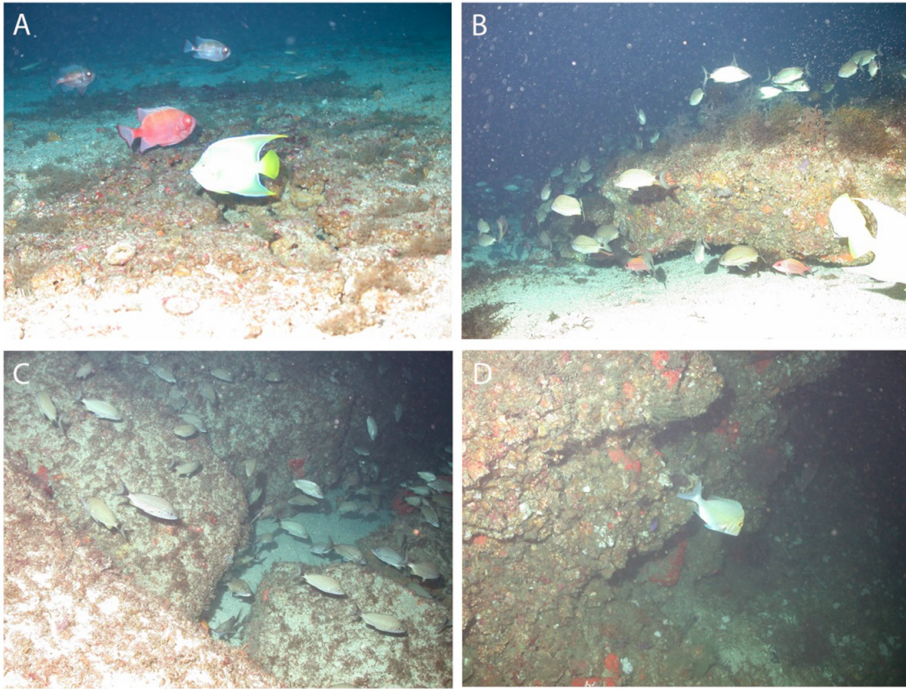


Figure 2. Four demersal habitat types observed from underwater video sampling along the south-east United States Atlantic continental shelf break and upper slope and included in analyses: (A) pavement, (B) low-relief outcrop, (C) moderate-relief outcrop, and (D) high-relief ledge.

(Maunder and Punt 2004). We were most interested in how lionfish densities may have changed over time after standardizing for predictor variable effects.

The GAM related lionfish transect counts to six predictor variables as:

$$\hat{y} = s_1(\text{year}) + s_2(\text{lat}) + s_3(\text{depth}) + s_4(\text{temp}) + f_1(\text{habitat}) + \text{offset}(\log(\text{vol})), \quad \text{Eq. 1}$$

where \hat{y} is lionfish count per transect, *year* is the year of the sample, *lat* is latitude (°N), *depth* is bottom depth (m), *temp* is bottom water temperature (°C), *habitat* is the habitat type, *vol* is volume of water sampled in the transect, f_1 is a categorical function, and s_{1-3} are cubic spline functions. The “offset” term accounts for variable transect volume and converts lionfish counts to density. Transect volume was log-transformed before inclusion to remove heteroscedasticity. We included latitude alone, not as a combined latitude × longitude position variable, because our sampling observations occurred along a small strip of longitude that encompassed the continental shelf break and upper slope habitat. No predictor variables exhibited multicollinearity given that the variance inflation factors were less than two for all covariates (Neter et al. 1989). Effective degrees of freedom for each smoothed predictor variable were chosen automatically by a built-in algorithm in the *mgcv* library, but we verified that the basis dimension for each smoothed predictor was set high enough to prevent over-smoothing. GAMs were coded, run, and analyzed in R v3.6.3 (R Core Team 2020) using the *mgcv* library 1.8-31 (Wood 2011).

We evaluated numerous data transformations and distributions for our response variable based on data type and model fit (using the “gam.check” function in the *mgcv* library). Best fit was achieved for lionfish density using a log transformation and a Gaussian distribution. This GAM fit well and met assumptions of normality and constant variance. We then compared our full GAM (i.e., the model including all predictor variables) to a variety of reduced models containing fewer predictor variables using Akaike’s information criterion (AIC; Burnham and Anderson 2002). AIC identifies the best predictive model relative to other models by balancing the number of parameters of the model with its fit. Models with the lowest AIC values were considered best, and here we used Δ AIC values, which is a measure of each model relative to the best model in the set. Therefore, the best model had a Δ AIC value of zero.

OBJECTIVE 2: CHARACTERIZE REEF FISH COMMUNITY STRUCTURE.—We used a combination of univariate and multivariate analytical approaches to characterize and describe reef fish community structure in the SEUS. For univariate analyses, we created five additional GAMs that were coded similar to Equation 1 except for two differences. First, the predictor variable *year* was replaced with lionfish density (*lion*; number of lionfish per 100 m³) to specifically and directly quantify the influence of increasing lionfish densities on fish community structure (see Objective 3 below). Note that habitat type, latitude, depth, and bottom temperature were also included in these models based on previous research (Bacheler and Smart 2016, Bacheler et al. 2016b). Second, five different response variables were used in GAMs that described various aspects of the fish community, namely species richness, Shannon diversity, the number of individuals of small demersal species, the number of individuals of fishery-targeted species, and the number of individuals of non-fishery-targeted species. Species richness was defined as the number of unique taxa observed on each transect (excluding “unknown” taxa). Shannon diversity measured both the number of taxa present as well as the relative abundance of each taxa (i.e., evenness) and was calculated as:

$$\text{Shannon Diversity} = - \sum_{i=1}^S p_i \ln p_i, \quad \text{Eq. 2}$$

where S is the total number of taxa observed in the transect and p_i is the proportion of S made up of the i th taxa. The number of individuals of small demersal species was included as an additional response variable because they were presumably the most vulnerable to lionfish predation (Dahl et al. 2016). The total number of individuals from taxa targeted by fishers and the total number of individuals from taxa not targeted by fishers were each included as separate response variables to disentangle fishing effects (which can be large in the SEUS; Shertzer et al. 2009) on the fish community from lionfish effects. If lionfish were negatively influencing the reef fish community, we would expect significant negative relationships between these five fish community metrics and lionfish density.

We evaluated numerous data transformations and distributions for each of these five response variables. Species richness was count data, so we evaluated Poisson, negative binomial, and Tweedie distributions. The negative binomial distribution outperformed other distributions based on model diagnostics and AIC. The Shannon diversity response variable was continuous, and the best fit was a Gaussian

distribution with no data transformations. Best fits for the remaining three response variables were achieved using a log transformation and a Gaussian distribution.

We also characterized the reef fish community in the SEUS using multivariate analyses. Specifically, we used nonmetric multidimensional scaling (nMDS) to visualize if and how fish community structure varied across five predictor variables: habitat type, depth, year, latitude, and bottom water temperature. We used the univariate analyses above because they are easy to visualize and interpret, but one potential drawback is that community composition could change over time and space and not be detected using our univariate response variables if, for instance, some species have substituted for others over time, which is better tested for using multivariate statistics. For multivariate analyses, we used densities of each taxa per transect (number observed per m^3) to standardize for variable transect volume. Only taxa comprising 0.01% or more of the total density were included in the multivariate analyses to down-weight the influence of rare species that may be observed due to chance; this resulted in 71 taxa that made up 99.69% of the total fish density being included in the multivariate analyses (Clarke and Warwick 2001). Moreover, highly abundant taxa tend to strongly influence multivariate analyses, so we square-root transformed all fish density data to allow taxa of intermediate densities to have some influence on the results (Clarke and Warwick 2001). We used PRIMER-E software v6 to conduct all multivariate analyses.

The main goal of this multivariate analysis was to determine if fish community structure in the SEUS varied significantly across habitats, space, time, depth, or water temperature. nMDS is a gradient analysis approach that produces a rank-based ordination on a similarity matrix, where points closer together are more similar than points farther away. We visualized interrelationships among replicates using nMDS plots that were based on Bray–Curtis similarity coefficients, which were zero-adjusted to be less erratic with sparse samples (Clarke et al. 2006). Each nMDS plot included a stress value that reflects the accuracy of the representation of community structure. Lower stress values are reflective of higher accuracy, and values <0.20 generally indicate that plots are providing a good representation of the data. The stress values in our nMDS plots were all 0.16, which indicates that our plots represent a good fit of the fish community structure data.

We included the same predictor variables in our multivariate analyses that were included in the univariate analyses, except transect volume was not included here because we used densities instead of count data for multivariate analyses, and year was included instead of lionfish density. nMDS requires predictor variables to be categorical, however, so we binned our predictor variables as follows. Habitat type was included the same way as in our univariate analyses, with four levels: pavement, low-relief outcrop, moderate-relief outcrop, and high-relief ledge. Depth was included with two levels, with the shallow depth zone (45–65 m) representing the continental shelf break and the deep depth zone (66–125 m) representing the upper slope (Schobernd and Sedberry 2009). Year was included with three levels containing approximately even sampling effort (2001–2008, 2009–2014, and 2015–2019). Latitude was included with three similarly sized latitude bins (29.5–30.9°N, 31.0–32.9°N, and 33.0–34.5°N), and bottom temperature was included with two levels containing approximately the same sampling effort (10.7–20.0 °C and 20.1–27.9 °C). Note that categorizing depth, year, latitude, and bottom temperature in different ways had no discernable influence on our results.

The nMDS plots provide a visualization of potential differences in fish community structure among groups, but do not provide any indication of statistical significance. Therefore, we also developed a series of one-way analysis of similarity (ANOSIM) tests, one for each predictor variable. When the global ANOSIM test was significant, we further analyzed all pairwise level comparisons within each categorical variable. ANOSIM provides two metrics to determine significance: *P* values and *R* statistics. In our study, all *P* values were significant at an alpha value of 0.05, which often happens when sample sizes are large. Therefore, we focused on the *R* statistic of each test, which reflects the absolute difference (effect size) in community structure between groups. The *R* statistic ranges from 0 to 1, where 0 indicates identical community structure among groups and 1 indicates complete separation among groups. For our study, an *R* statistic greater than 0.2 for global or pairwise tests was considered sufficiently large biological separation among groups, following the recommendation of Clarke (1993). When sufficiently large separation was found in fish communities using the ANOSIM test, we further applied a SIMPER technique (Similarity of Percentages) to determine which taxa were contributing most to the dissimilarity between groups within a predictor variable.

OBJECTIVE 3: HAVE LIONFISH INFLUENCED REEF FISH COMMUNITY STRUCTURE.— To determine whether lionfish have had a measurable influence on reef fish community structure in the SEUS, we evaluated the sign and significance of the lionfish density predictor variable in the five univariate GAMs described above for Objective 2. If lionfish influenced the native fish community, we would expect to observe significant negative relationships between our univariate fish community metrics and lionfish densities. We further evaluated Objective 3 by examining whether fish community structure varied by time period in the multivariate analyses. Ideally, we would have tested for differences in community structure across different lionfish densities, but lionfish density was strongly confounded with habitat type, so instead we tested for differences in fish community structure over time. We would expect a significant year effect if lionfish influenced native fish community structure in the SEUS.

RESULTS

A total of 274 submersible or ROV dives were conducted on the continental shelf break and upper slope between North Carolina and northern Florida (Fig. 1). Dives occurred each year from 2001 to 2019 except in 2005 and 2011 (Table 1). Within these dives, 493 transects were completed over hardbottom and included in our analyses, ranging from a low of three transects in 2003 to a high of 81 transects in 2010 (Table 1). Mean latitude sampled across all years was 31.9°N, but sampling overall ranged from 29.9°N (St. Augustine, Florida) to 34.5°N (Cape Lookout, North Carolina). Mean depth sampled by year was fairly consistent, ranging from 51 to 66 m (overall mean = 61 m), and overall depths sampled in our study ranged from 45 to 125 m (Table 1). There were some differences in the relative proportion of habitats surveyed across years (Table 1) and latitudes in our study (Table 2). The total volume of water sampled in our study was 2,758,590 m³ and mean transect volume was 5596 m³ (range = 108–77,404).

Table 1. Information for each of the 17 years of underwater video sampling data from the southeast United States continental shelf break and upper slope and included in the analyses. Habitat classes are defined as: PAV = pavement, LRO = low-relief outcrop, MRO = moderate-relief outcrop, and HRL = high-relief ledge.

Year	Dives	Transects	Mean latitude (°N; range)	Mean depth (m; range)	Mean date (range)	Habitat (%)			
						PAV	LRO	MRO	HRL
2001	4	9	31.6 (31.5–31.6)	66 (63–70)	9/7 (9/7–9/8)	37	60	2	0
2002	9	25	31.6 (29.9–32.9)	51 (47–55)	7/31 (7/28–8/4)	15	31	24	29
2003	2	3	31.5 (31.2–32.1)	60 (50–79)	8/9 (8/7–8/10)	74	26	0	0
2004	19	31	31.9 (29.9–33.5)	64 (48–95)	5/10 (4/17–8/30)	32	11	11	45
2005	0	0	---	---	---	---	---	---	---
2006	7	8	32.2 (30.0–33.1)	64 (48–72)	6/9 (6/8–6/11)	77	0	14	9
2007	16	22	32.5 (29.9–33.5)	61 (47–84)	8/20 (8/17–8/23)	31	41	10	17
2008	10	15	32.0 (29.9–33.4)	57 (50–78)	7/20 (7/19–7/22)	29	46	5	21
2009	9	14	31.7 (30.0–33.1)	55 (48–66)	11/17 (11/17–11/19)	16	53	19	12
2010	34	81	31.3 (29.9–33.1)	56 (50–70)	8/17 (5/5–10/8)	18	31	31	20
2011	0	0	---	---	---	---	---	---	---
2012	29	62	32.3 (29.9–33.4)	58 (45–90)	7/12 (7/7–7/18)	11	41	23	25
2013	27	66	32.4 (29.9–34.5)	66 (50–119)	7/5 (7/2–7/11)	17	50	19	14
2014	21	25	32.4 (30.4–32.9)	65 (49–109)	6/22 (6/19–6/26)	18	23	27	31
2015	13	16	31.9 (30.4–32.9)	60 (49–74)	6/22 (6/19–6/26)	25	19	34	22
2016	11	13	31.6 (30.1–33.4)	57 (47–82)	6/13 (6/12–6/15)	2	27	23	48
2017	23	37	31.9 (30.0–33.4)	66 (50–125)	6/27 (6/23–7/4)	10	22	14	54
2018	17	25	31.6 (29.9–32.9)	60 (47–75)	5/18 (5/12–5/23)	8	21	29	41
2019	23	41	32.1 (30.0–33.9)	64 (48–118)	6/13 (6/8–6/17)	6	49	21	24
Total	274	493	31.9 (29.9–34.5)	61 (45–125)	7/11 (4/17–11/18)	29	34	22	15

A total of 214 fish taxa were observed in our study (Online Supplementary Material), of which 91 were fishery-targeted taxa, 123 were nontargeted taxa, and 57 were small demersal species. Taxa most commonly observed by transect were reef butterflyfish (*Chaetodon sedentarius*; 91.7% of transects), Labridae (89.9%), Pomacentridae (87.6%), and tattler (*Serranus phoebe*; 85.8%; Online Supplementary Material). A total of 16 taxa were observed on at least 50% of transects, 54 were observed on at least 10% of transects, and 160 taxa were observed on less than 10% of transects. Taxa with the highest mean densities (number observed per 100 m³) were tomtate (15.71), vermilion snapper (*Rhomboplites aurorubens*; 5.77), and Pomacentridae (1.88). A total of 3.1% of individual fish observed across all years could not be identified to at least the family level.

OBJECTIVE 1: ASSESS CHANGES IN LIONFISH DENSITY OVER TIME.—We related lionfish transect densities to six predictor variables, and the full GAM including all predictor variables was selected over all reduced models (Table 3). The full model explained a large amount of the model deviance (50.2%) and was 7.8 AIC points better than the next best model that excluded bottom water temperature. The full model indicated that mean standardized lionfish density increased from nearly zero in 2001

Table 2. Percentage of each habitat type surveyed by underwater video within three latitude bands along the southeast United States continental shelf break and upper slope, 2001–2019. PAV = pavement, LRO = low-relief outcrop, MRO = moderate-relief outcrop, HRL = high-relief ledge.

Latitude bin	PAV	LRO	MRO	HRL
29.5°N – 30.9°N	20	27	3	50
31.0°N – 32.9°N	17	36	28	19
33.0°N – 34.5°N	8	33	21	37

Table 3. Model selection for generalized additive models relating six different response variables (i.e., lionfish, species richness, Shannon diversity, small demersal individuals, fishery-targeted individuals, and nontargeted individuals) to potential predictor variables from underwater video samples collected along the southeast United States continental shelf break and upper slope, 2001–2019. The best two models (based on Δ AIC) are shown for each response variable. Degrees of freedom are shown for factor (*f*) terms, effective degrees of freedom are shown for smoothed terms (*s*), “ex” means that variable was excluded from the model, and “NA” means the variable was not applicable to that particular model. Asterisks denote significance at the following alpha levels: *0.05, **0.01, ***0.001. Δ AIC is the difference in Akaike information criterion between that particular model and the best model in the set (indicated by 0.0), and %Dev is the percent deviance explained by the model.

Model	Δ AIC	%Dev	<i>s</i> (lion)	<i>s</i> (lat)	<i>s</i> (depth)	<i>s</i> (temp)	<i>f</i> (hab)	<i>s</i> (year)
Lionfish								
Full	0.0	50.2	NA	6.5***	1.0***	2.6*	3***	4.5***
Full – <i>temp</i>	7.8	49.0	NA	6.8***	1.0***	ex	3***	4.8***
Species richness								
Full	0.0	20.6	2.6***	6.5***	3.2**	3.7***	3	NA
Full – <i>hab</i>	0.1	19.7	2.6***	6.3***	3.2**	3.6**	ex	NA
Shannon diversity								
Full	0.0	26.9	1.7	7.5*	1.0**	2.5*	3***	NA
Full – <i>lion</i>	2.6	26.1	ex	7.6*	1.0**	2.5*	3***	NA
Small demersal individuals								
Full	0.0	33.9	2.6***	4.7***	4.7**	5.0***	3***	NA
Full – <i>depth</i>	7.6	29.8	2.7***	4.0***	ex	4.4***	3***	NA
Fishery-targeted individuals								
Full	0.0	51.8	3.1***	8.3***	5.8**	3.1***	3***	NA
Full – <i>depth</i>	13.7	49.4	3.3***	8.2***	ex	3.6***	3***	NA
Nontargeted individuals								
Full	0.0	53.6	3.6***	5.4*	4.9*	1.0***	3***	NA
Full – <i>depth</i>	8.7	51.7	3.7***	4.7**	ex	1.0***	3***	NA

to a high of 1.2 lionfish per 100 m³ in 2019 (Fig. 3). The percentage of transects in which lionfish were observed also displayed a large increase during the study time frame (Fig. 3). For instance, lionfish were observed on less than 20% of transects before 2005, but were observed on greater than 80% of transects each year from 2012 through 2019 and 100% of transects in 2014, 2015, 2016, and 2019 (Fig. 3).

The lionfish GAM also indicated the species was influenced by each of the predictor variables included in the model. There was a strong relationship between standardized lionfish density and habitat type, with standardized density of lionfish being almost twice as high on high-relief ledge transects (0.60 lionfish per 100 m³) compared to pavement transects (0.30 lionfish per 100 m³; Fig. 4). Standardized lionfish density also varied across latitudes, being lowest in the southern portion of the study area (i.e., Florida) and higher in Georgia, South Carolina, and especially North Carolina. Moreover, standardized lionfish density was negatively related to depth, with density an order of magnitude greater at 45–50 m deep compared to 125 m deep (Fig. 4). Last, lionfish density was positively related to bottom water temperature up until 20 °C, after which standardized density appeared invariant or negatively related to temperature (maximum temperature observed in our study = 27.9 °C).

OBJECTIVE 2: CHARACTERIZE REEF FISH COMMUNITY STRUCTURE.—For each of the five univariate GAMs describing fish community structure, full models were preferred over all reduced models using AIC (Table 3). The most competitive runner-up model excluded habitat from the GAM of species richness (Δ AIC = 0.1), while Δ AIC values for runner-up models for the other four GAMs were larger, ranging

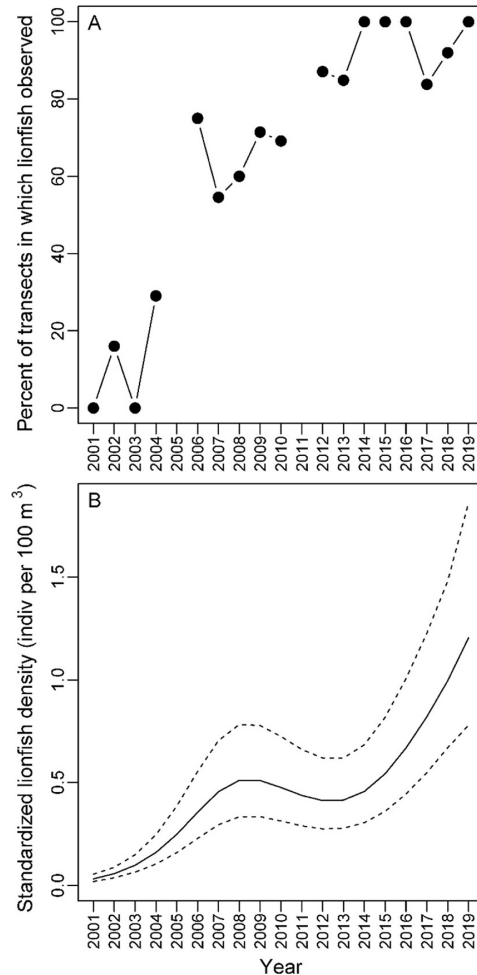


Figure 3. (A) Percent of transects in which lionfish were observed from submersible and ROV video sampling along the southeast United States continental shelf break and upper slope, 2001–2019. (B) Mean lionfish (*Pterois* spp.) densities (individuals per 100 m³) over time from underwater video sampling using generalized additive models. Filled circles show standardized densities (mean values at average values of all other model covariates) and dashed lines indicate 95% confidence intervals.

from 2.6 (Shannon diversity) to 13.7 (fishery-targeted individuals). Final (full) GAMs explained a substantial amount of the model deviance, ranging from 20.6% for species richness to 53.6% for nontargeted individuals (Table 3).

Species richness, small demersal individuals, total fishery-targeted individuals, and total nontargeted individuals showed similar trends across habitat types, latitude, depth, and bottom water temperature (Fig. 5). These response variables were mostly positively related to the relief of hardbottom, with generally higher values on high-relief ledges compared to pavement habitats. Species richness, small demersal individuals, and total nontargeted individuals were also positively related to latitude, with higher values off North and South Carolina compared to Florida (Fig. 5). Total fishery-targeted individuals was somewhat negatively related to latitude, but was

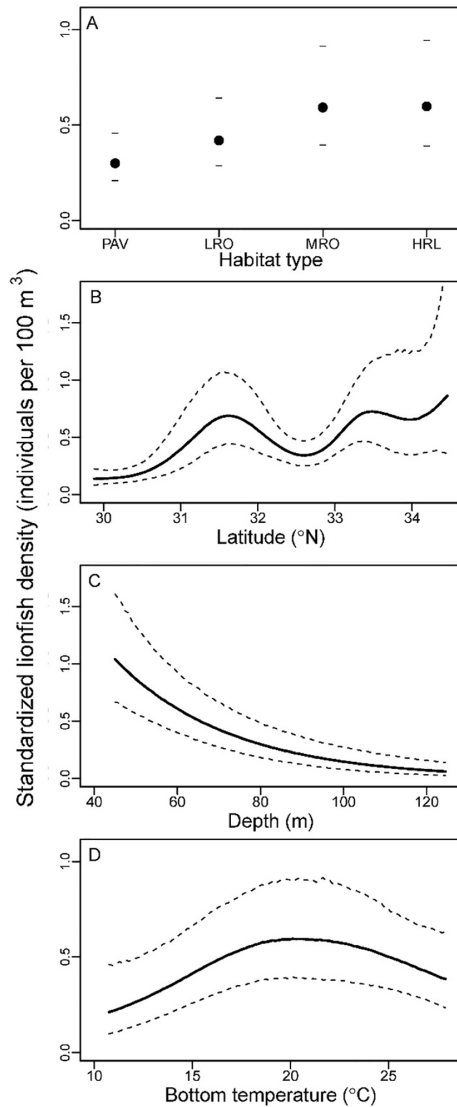


Figure 4. Mean lionfish (*Pterois* spp.) densities (individuals per 100 m³) related to four predictor variables from generalized additive models based on underwater video samples along the southeast United States continental shelf break and upper slope, 2001–2019. Filled circles or solid black lines show standardized densities (mean values at average values of all other model covariates) and dashed lines indicate 95% confidence intervals.

also quite variable. Each of these four response variables were negatively related to depth until about 100 m deep, at which point there appeared to be an inflection point and these four response variables appeared to increase. All four response variables were positively related to bottom water temperature, except that species richness plateaued beyond about 18 °C (Fig. 5).

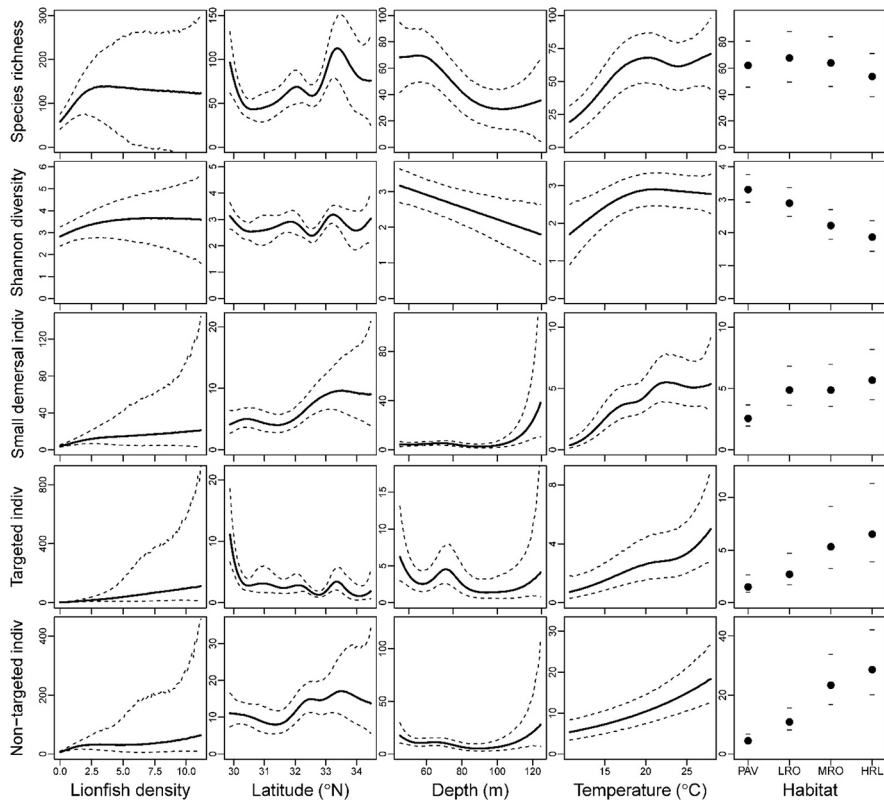


Figure 5. Standardized response variables (i.e., species richness, Shannon diversity, small demersal individuals, fishery-targeted individuals, and nontargeted individuals) related to five predictor variables from generalized additive models based on underwater video samples along the southeast United States continental shelf break and upper slope, 2001–2019. Units for the species richness and Shannon diversity plots are values per mean transect volume, while units for total individuals, targeted individuals, and nontargeted individuals are mean densities (individuals per 100 m³). Lionfish density is the number of individuals per 100 m³. Filled circles or solid black lines show standardized densities (mean values at average values of all other model covariates) and dashed lines indicate 95% confidence intervals.

Shannon diversity was related to predictor variables in somewhat different ways than the four other response variables. Shannon diversity was lowest for high-relief ledges and highest for low-relief outcrop habitat types, and there appeared to be a weak relationship with latitude. Shannon diversity was negatively related to depth and positively related to bottom water temperatures until about 20 °C, after which it appeared to plateau (Fig. 5).

Using multivariate analyses, it appeared that fish community structure varied significantly by habitat but not by depth, year, latitude, or bottom water temperature. For instance, fish community structure appeared to differ across habitat types based on nMDS plots (Fig. 6), and the global ANOSIM *R* value for habitat type was significant at 0.32 (Table 4). Moreover, fish community structure varied significantly across each pairwise comparison of higher-relief habitats (MRO or HRL) with lower-relief habitats (PAV or LRO), with the highest pairwise *R* values being HRL-PAV (0.64),

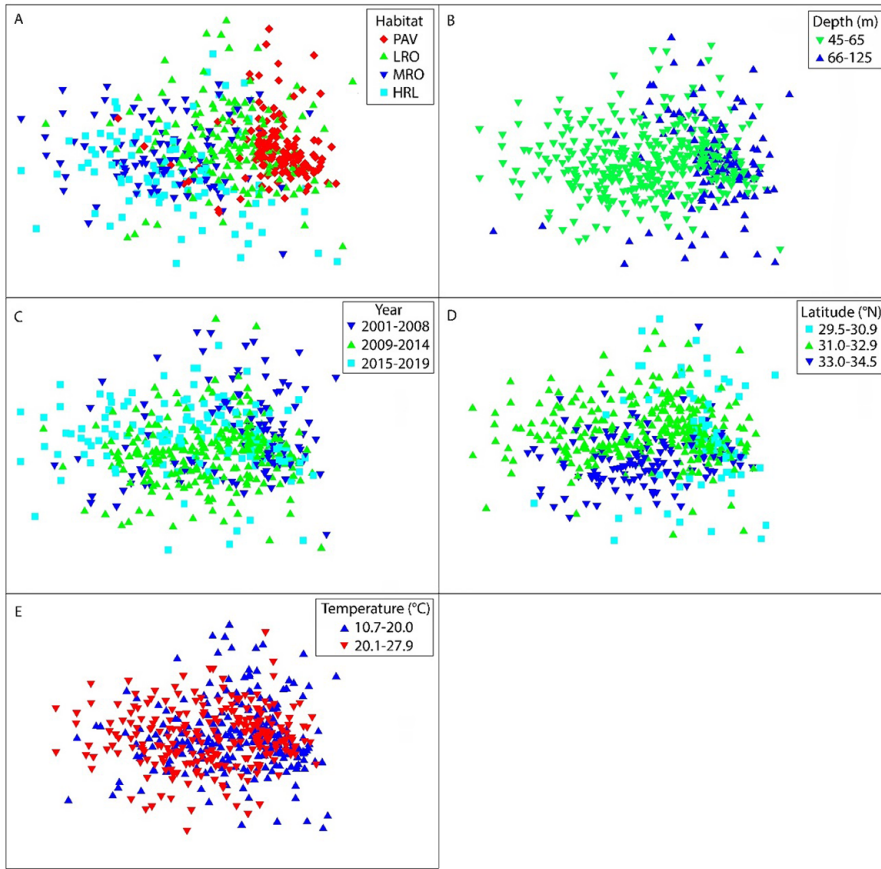


Figure 6. Nonmetric multidimensional scaling ordination of fish community data from the southeast United States continental shelf break and upper slope (2001–2019) by (A) habitat type, (B) depth, (C) year, (D) latitude, and (E) temperature. Ordinations were derived from the Bray–Curtis similarity matrix using square-root transformed fish densities. Two-dimensional stress was 0.16 for all plots. Habitat classes are defined as: PAV = pavement, LRO = low-relief outcrop, MRO = moderate-relief outcrop, and HRL = high-relief ledge.

MRO-PAV (0.64), HRL-LRO (0.27), and MRL-LRO (0.21). No other predictor variable had a significant global R value, with the highest being 0.18 for depth, followed by 0.15 for year, 0.11 for latitude, and 0.04 for temperature (Table 4). The nMDS plots appeared to be consistent with ANOSIM results (Table 4, Fig. 6). SIMPER revealed that separation in fish communities among different habitat types was primarily driven by three highly-abundant taxa (tomtate, vermilion snapper, and Pomacentridae), which all had greater densities on higher relief habitats compared to lower relief habitats (Table 5).

OBJECTIVE 3: HAVE LIONFISH INFLUENCED REEF FISH COMMUNITY STRUCTURE.—

None of the univariate or multivariate analyses suggested a lionfish effect on fish community structure in the SEUS. The five univariate response variables describing the fish community were either positively related or unrelated to increasing lionfish densities (Fig. 5), the opposite of what would be expected if lionfish were having measurable community-level affects. Fish community structure did not change

Table 4. Global and pairwise R statistics and P values for the one-way analysis of similarity tests comparing shelf-break fish communities in the southeast United States across each of the five predictor variables: habitat, depth, year, latitude, and temperature. Global comparisons tested for differences in fish communities across all levels of each predictor variable, while pairwise comparisons tested for differences in fish communities between each pairwise combination of levels of each predictor variable (and are only shown for variables when the global R statistic was ≥ 0.20). Bold rows were considered to be statistically significant based on R values.

Variable	Comparison	R	P
Habitat	Global	0.32	0.001
Habitat	HRL-PAV	0.64	0.001
Habitat	MRO-PAV	0.64	0.001
Habitat	HRL-LRO	0.27	0.001
Habitat	MRO-LRO	0.21	0.001
Habitat	LRO-PAV	0.15	0.001
Habitat	HRL-MRO	0.03	0.003
Depth	Global	0.18	0.001
Year	Global	0.15	0.001
Latitude	Global	0.11	0.001
Temperature	Global	0.04	0.001

significantly over the course of our study (global R for year = 0.15), suggesting a weak or nonexistent lionfish effect on reef fish community structure. We also experimented with a wide variety of yearly groupings, and the global R value was not significant for all groupings.

DISCUSSION

Temperate rocky reef habitats along the southeast United States Atlantic continental shelf break and upper slope host a biologically and functionally diverse reef fish community (Schobernd and Sedberry 2009, Bacheler et al. 2019). These fish species are influenced by a mosaic of demersal habitats, fluctuating environmental conditions, significant fishing pressure, and, over the last two decades, an invasion

Table 5. Output from SIMPER analysis showing the three main taxa driving mean density (individuals per 100 m³) differences across the four habitat types sampled at the shelf break and upper slope in the southeast United States. “% contribution” shows the percent dissimilarity explained by each of the taxa across habitats, and “cumulative % contribution” shows the percent dissimilarity explained by the three main taxa combined across habitats.

	Habitat comparisons			
	HRL-PAV	MRO-PAV	HRL-LRO	MRO-LRO
<i>Haemulon aurolineatum</i>				
Density on habitat 1	0.44	0.50	0.44	0.50
Density on habitat 2	0.03	0.03	0.14	0.14
% contribution	18.1%	21.1%	16.6%	19.4%
<i>Rhomboplites aurorubens</i>				
Density on habitat 1	0.29	0.23	0.29	0.23
Density on habitat 2	0.02	0.02	0.07	0.07
% contribution	12.1%	9.7%	11.6%	9.9%
<i>Pomacentridae</i>				
Density on habitat 1	0.14	0.15	0.14	0.15
Density on habitat 2	0.08	0.08	0.12	0.12
% contribution	5.4%	5.8%	5.2%	5.2%
Cumulative % contribution	35.6%	36.6%	33.4%	34.5%

of predatory lionfish (Whitfield et al. 2002, Bacheler and Smart 2016). Lionfish are thought to be opportunistic, generalist predators at invaded reefs, feeding on a wide range of mainly fish prey (about 20–80 mm total length) as adults (Muñoz et al. 2011), thus competing with native predatory fishes (Eddy et al. 2020). There are numerous empirical examples of lionfish negatively influencing reef fish species throughout their introduced range (e.g., Albins and Hixon 2008, Green et al. 2014, Albins 2015), but, while important and informative, most of these studies have taken place at relatively small spatial and temporal scales. We tested for lionfish-induced changes in the reef fish community along the southeast US Atlantic continental shelf break and upper slope from 2001–2019, examining a variety of univariate and multivariate fish community response variables. While lionfish density dramatically increased during our study, simultaneous changes in the reef fish community were not apparent, suggesting relatively minor ecological effects of lionfish over large spatial and temporal scales in the region.

Unlike most previous lionfish-focused studies, we did not observe a significant negative influence of lionfish on the native reef fish community in the SEUS. We suggest four potential explanations for this discrepancy. First, the influence of lionfish on reef fish may be dependent on the spatial scale of observation. Most previous studies have examined lionfish effects on prey species over small spatial scales (<30 km²), whereas our study tested for the influence of lionfish on the reef fish community at a much broader spatial scale (hundreds of km²). Generally, predation effects on prey are stronger at small spatial scales compared to larger spatial scales due to differences in prey movement and recruitment, changes in prey behavior, and availability of refugia that are nonscalable (Englund 1997, de Roos et al. 1998). Thus, it is possible that lionfish reduce prey densities strongly at small spatial scales but more weakly at large spatial scales, consistent with the results of Elise et al. (2015) and Hackerott et al. (2017). However, the observation by Ballew et al. (2016) that tomtate has declined regionally due to lionfish predation conflicts with this explanation.

Second, a regional effect of lionfish on prey species may not be expected given that lionfish are not uniformly spread throughout the study region, instead occurring primarily around high-relief hardbottom habitats in shallower water (<70 m) north of Florida. These high-relief habitats are fairly rare in the SEUS (Kendall et al. 2008, Fautin et al. 2010), suggesting that lionfish predation may be high in a few patchy high-relief locations and low in the vast majority of remaining habitats, potentially resulting in an overall negligible effect. However, our results were unchanged even when examining relationships between fish community metrics and lionfish density exclusively in high-relief habitats (global $R = 0.12$). Our study focused on continental shelf-break and upper-slope habitats, but some evidence suggests that lionfish may be more common in shallower depths (Whitfield et al. 2014, Bacheler et al. 2016a, *but see* Andradi-Brown et al. 2017). Therefore, lionfish may be having a stronger impact on prey fish community dynamics in shallower, continental shelf waters (e.g., Ballew et al. 2016) compared to the deeper shelf-break and upper-slope habitats examined in our study.

A third potential explanation is that lionfish densities may not have increased enough in the region to have a significant influence on fish community structure. In our study, lionfish densities were highest in the last five years of the study (2015–2019), averaging 0.9 lionfish individuals per 100 m³ of hardbottom habitat, which was similar to lionfish densities in invaded Bahamian waters (about 1.0 fish per 100

m²; Darling et al. 2011) and the Gulf of Mexico (about 0.5 fish per 100 m²; Dahl and Patterson 2014). Whitfield et al. (2014) documented lionfish densities up to 4.4 fish per 100 m² on the continental shelf (38–46 m deep) of North Carolina in 2007–2010, suggesting lionfish can potentially reach much higher densities than we documented in our study. Most experimental studies suggest that lionfish densities must be relatively high to elicit a negative response on native species (e.g., Green et al. 2014, Benkwitt 2015). If lionfish densities along the continental shelf break and upper slope continue to increase to levels observed in Whitfield et al. (2014) or Dahl et al. (2016), community-level effects from lionfish predation may be more likely to be observed.

The last potential reason why we may not have observed a lionfish effect on reef fish community structure is that our methodology may not have been robust or powerful enough to detect an effect (Cohen 1988). For this hypothesis to be correct, lionfish must have had a significant effect on the fish community over the last two decades that our analyses failed to detect. Our analyses did not appear to lack statistical power: confidence intervals of our univariate analyses were relatively narrow, all predictor variables were included in the final univariate models, and a habitat effect (but not lionfish effect) was significant in univariate and multivariate analyses. Ultimately, however, statistical power cannot be determined after a study is completed (Hoenig and Heisey 2001), so it is impossible to rule out whether low statistical power contributed to our specific research findings.

Habitat type was by far the most important variable influencing reef fish community structure in our study. We found that more fish occurred on high-relief hard-bottom habitats compared to lower-relief or pavement habitats, which is consistent with most previous studies in the SEUS and Gulf of Mexico (Barans and Henry 1984, Sedberry and Van Dolah 1984, Parker and Ross 1986, Sluka et al. 2001, Schobernd and Sedberry 2009, Bachelier et al. 2016a, Garner et al. 2019). Moreover, fish community structure was influenced by habitat type in our multivariate analyses, suggesting that not only more fish but different fish communities inhabit high-relief compared to low-relief hardbottom habitats. Similarly, Paxton et al. (2017) found distinct fish communities in habitats with different reef morphologies in shallower water on the continental shelf, and the highest species richness was found at intermediate levels of reef complexity, which agrees with our findings.

Univariate fish community variables in the SEUS were also influenced by depth. Most fish community variables were generally negatively related to depth, being higher in shallower water (45–60 m) in our study compared to deeper water (80–125 m). These results are broadly consistent with previous work within and outside of the SEUS (Moore et al. 2010, Fitzpatrick et al. 2012, Bachelier et al. 2016a, 2019, Geraldini et al. 2019a,b). It is unclear why depth has strong effects on reef fish communities, but it is likely due to the ways in which biotic and abiotic factors vary across depths, including predation (Jordan et al. 2012), fishing pressure (Pereira et al. 2018), habitat (Schobernd and Sedberry 2009, Wilborn et al. 2018), and environmental conditions such as bottom water temperature (Whitfield et al. 2014).

There was a strong latitudinal effect in our study, with most univariate response variables being positively related to latitude. Changes in the fish community across latitude, however, appeared to be a matter of magnitude and not a structural change. For instance, more individuals and species were observed off North and South Carolina compared to Florida, similar to results from studies primarily occurring on the continental shelf and shelf break in the SEUS (Bachelier et al. 2019, Geraldini et al.

2019b). The makeup of species, however, did not significantly change across the range of latitudes examined in the SEUS based on our multivariate analyses. Bacheler et al. (2019) showed that, on the continental shelf, snapper were more common off Florida and grouper were more common off North and South Carolina, suggesting that fish community structure may be more variable in shallower compared to deeper waters across latitude in the SEUS. One possible explanation is that fish communities in shallower water may be more influenced by seasonal bottom temperature fluctuations across the range of latitudes in the SEUS than deeper-water fish communities (Whitfield et al. 2014).

There were some shortcomings of our study. First, we employed an observational study design to examine lionfish effects on the fish community, and related broad-scale temporal or spatial trends in lionfish density to changes in native fish community structure. However, observational studies cannot determine causation (Hilborn 2016), which requires experimental study designs. It is challenging to quantify regional effects of lionfish using experimental designs, which is why we chose an observational study design similar to Ballew et al. (2016). Second, like all sampling gears, underwater video is selective and does not provide a perfectly representative sample of the true fish community. For instance, underwater video has been shown to miss small (i.e., juvenile), secretive, and camouflaged species (Mallet et al. 2014, Bacheler et al. 2017), suggesting that our results only apply to the portion of the fish community available to video sampling. Third, our study was based on a combination of surveys from several distinct studies and survey efforts, and therefore lacked a cohesive sampling design that might have resulted in an increased ability to detect changes in lionfish density and reef fish community structure. Last, there may be a time lag between lionfish reaching high densities in the SEUS and declines in native fish densities if, for instance, lionfish primarily prey on small juveniles and limit native fish recruitment. If there is a time lag between lionfish densities increasing and native fish densities decreasing, it would be more difficult for us to detect a lionfish effect on native fishes.

There are many threats facing reef fish communities inhabiting continental shelf-break and upper-slope waters in the SEUS including climate change, habitat loss, and fishing pressure (Fautin et al. 2010). The introduction of lionfish in the Atlantic is yet another threat to reef fish biodiversity in the SEUS and elsewhere, including the Gulf of Mexico, Caribbean, southwest Atlantic, and Mediterranean Sea (Betancur-R et al. 2011, Bariche et al. 2017). While a number of studies have implicated lionfish in native fish declines and additional cascading ecological effects (Albins and Hixon 2008, Lesser and Slattery 2011, Green et al. 2012, Ballew et al. 2016), we did not document significant changes in the reef fish community in the SEUS over the last two decades. Given conflicting results of previous work, more research is needed to determine if and how lionfish are influencing reef fish communities in the SEUS and elsewhere at regional spatial scales.

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