The following supplements accompany the article

Diet of invasive lionfish on hard bottom reefs of the Southeast USA: insights from stomach contents and stable isotopes

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Supplements 1–5. Distribution of sample sizes, additional methodological details and analyses, and selection of U.S. South Atlantic fishes with potential dietary overlap with *Pterois volitans*.

Supplement 1. Table S1. Distribution of sample sizes of *Pterois volitans* by study site and by year, study site characteristics, and distribution of prey surveys. Stomach samples do not include empty stomachs

Site	2004 Stomachs	2006 Stomachs	2004 Isotopes	Depth (m)	Habitat[a]	Prey Survey[b]
18 Fathom	31	3	24	38.1	wreck	X
Bobby6	2	0	2	43.9	low	
Capdan99	2	0	2	39.6	medium	
CF001	7	2	10	41.2	high	X
CF14	6	0	6	39.6	medium	
City of Houston	3	0	4	30.5	wreck	X
Dan's spot	2	8	2	39.6	high	X
Gerry's wreck	11	5	12	45.4	low	X
Kenny1	2	10	1	42.7	medium	X
Kenny3	0	0	1	42.7	medium	
Lobsterrocksth3	9	23	12	42.7	high	X
Lobsterrocknorth1	5	7	4	36.6	low	X
Lobsterrocksth1	5	0	10	41.2	high	
Lobsterwreck	3	0	4	38.1	wreck	
Naecobow	6	0	7	42.7	wreck	
Naecostern	3	19	6	42.7	wreck	X
SETower	7	0	8	41.2	medium	X
Woo2	0	2	0	39	high	X

Total	104	79	115

[a] Wreck sites were characterized by vertical relief >1 m heavily colonized by sessile invertebrates and macroalgae, with the wreck structure providing ample overhangs and undercuts for shelter purposes. Low relief hard bottom sites were characterized by vertical relief <0.25 m sparsely colonized by sessile invertebrates and macroalgae, with no overhangs. Available shelter consisted of holes eroded/scoured into the mostly flat surfaces. Medium relief hard bottom sites consisted of vertical relief >0.25 m but < 1 m. Sessile invertebrates and macroalgae tended to occupy greater percent cover than on low relief sites and shelter consisted of eroded/scoured holes. High relief hard bottom sites provided vertical relief >1 m in the form of ledges or rocky outcrops, were densely colonized by sessile invertebrates and macroalgae, and contained overhangs or undercuts as shelter. Riggs et al. (1998) provides further details of benthic community processes on hard bottoms and Kendall et al. (2009) provides justification for ledge height classifications.

[b] X indicates a site was surveyed with a visual prey census.

Supplement 2. *Pterois volitans* — cumulative prey analysis. We constructed cumulative prey curves for each year separately and combined to determine the adequacy of sample sizes of *Pterois volitans* and plotted the average number of new prey items found in each stomach versus the total number of stomachs analyzed (Ferry & Caillet 1996). We used PRIMER to permute the order of stomachs analyzed 999 times and considered that an adequate number of stomachs had been analyzed if an asymptote was reached (Ver. 6; Warwick 1993, Warwick & Clarke 2001). We also calculated five different estimates of prey items (Chao 1, Chao 2, jacknife 1, jacknife 2, bootstrap) to determine how many prey types were potentially missed by sampling. These additional estimators differ in exact method of computation but tend to utilize rare prey items to estimate the true number of prey in the diet (Colwell & Coddington 1994). To provide a standard measure of sample size precision and for comparison among diet studies, we calculated the mean coefficient of variation (CV = [standard deviation /mean] × 100) of the mean cumulative number of prey taxa generated for the final four stomach samples (Bizzarro et al. 2007). Values of < 10% CV are a general benchmark for adequate precision in age and growth studies (Campana 2001, Bizzarro et al. 2007). For both years combined, the cumulative number of prey categories (18) recorded from these specimens had neared an asymptote and additional estimates of prey items indicated that only 1.5–3 prey categories may have been missed by sampling. In addition, the mean CV of the mean cumulative number of prey taxa generated for the final four stomach samples was 0.59%, suggesting that characterizing the diet of lionfish with these samples was sufficiently precise. For 2004, 14 prey categories were recorded, only 0.4–2 prey categories may have been missed by sampling, and the mean CV for the final four stomach samples was 1.1%. For 2006, 15 prey categories were recorded, only 2–4 prey categories may have been missed by sampling, and the mean CV for the final four stomach samples was 1.5%. Figure S1 shows the mean cumulative number of prey categories per stomach sample for *Pterois volitans*. Prey categories are from Table 1 in the main article with the addition of unidentified stomach contents. Mullidae and Echinodermata (items regurgitated from multiple fish while in a common holding tank) were not included because they could not be assigned to a single or multiple stomachs.

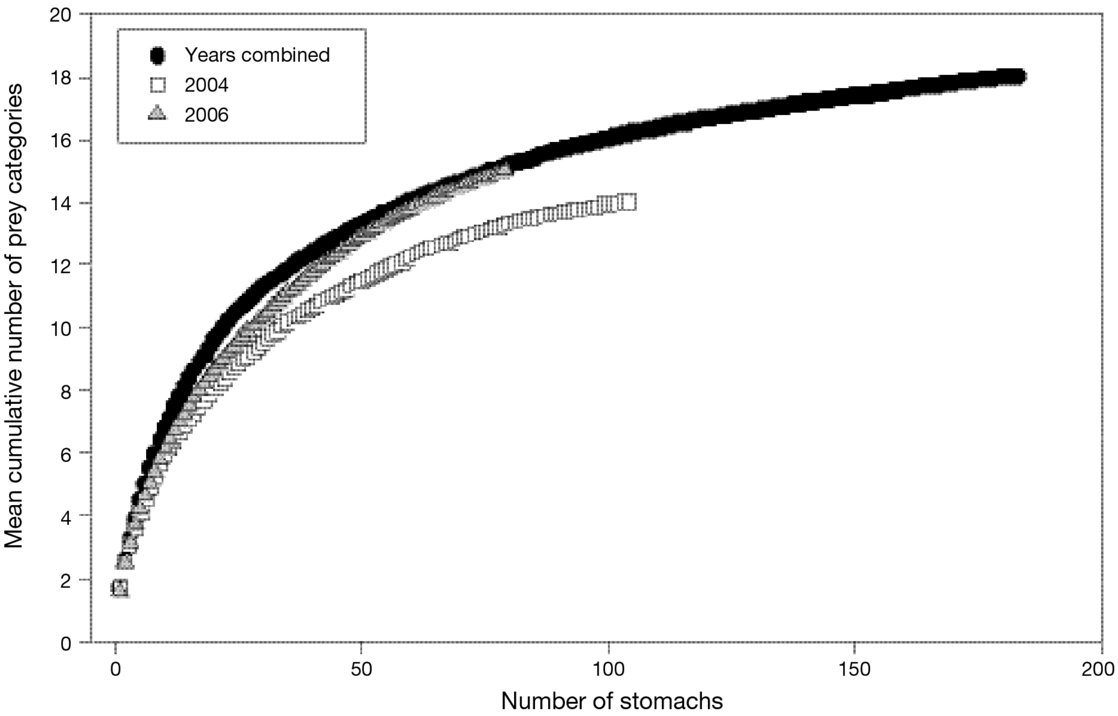

Fig. S1. Mean cumulative number of prey categories per stomach sample for *Pterois volitans*

Supplement 3. Methodological details of 1-way Analysis of Similarities (ANOSIM) for *Pterois volitans* stomach contents.

For ANOSIM of prey categories, each prey category was treated as a variable (species) and the percent weight of each category per stomach examined between years. For prey lengths, we generated a frequency distribution of all prey lengths across years and prey length bins were then treated as variables and the number of prey in each length bin examined between years. For prey volume, we generated a frequency distribution of total prey volume per stomach across years and total prey volume per stomach bins were then treated as variables and the distribution of total stomach volume examined between years. For prey number, we generated a frequency distribution of total number of prey per stomach (without regards to prey category) across years and total number of prey per stomach bins were then treated as variables and the distribution of total number of prey per stomach examined between years. Note that the ANOSIM of prey categories examines differences between years by accounting for the identity and number of prey consumed per category, while the ANOSIM of prey number differs by examining differences between years in the physical prey processing characteristics of lionfish (i.e., the total number of prey per stomach without regards to prey category).

Supplement 4. Two-way ANOSIM pairwise tests (Table S2) and similarity percentages (SIMPER) analyses (Table S3) comparing changes in *Pterois volitans* diet with size while accounting for any differences between years.

Table S2. Two-way ANOSIM pairwise tests

Size class (TL, cm)	R statistic	p
15 – 19.9 vs 20 – 24.9	0.133	0.045
15 – 19.9 vs 25 – 29.9	0.212	0.094
15 – 19.9 vs 30 – 34.9	0.345	0.001
15 – 19.9 vs ≥35	0.223	0.028
20 – 24.9 vs 25 – 29.9	0.094	0.195
20 – 24.9 vs 30 – 34.9	0.120	0.099
20 – 24.9 vs ≥35	0.072	0.192
25 – 29.9 vs 30 – 34.9	–0.0290	0.766
25 – 29.9 vs ≥35	–0.0250	0.655
30 – 34.9 vs ≥35	0.022	0.326

Table S3. Two-way similarity percentages (SIMPER) analyses comparing changes in *Pterois volitans* diet with size while accounting for any differences between years. SIMPER results between years were similar to one-way results already reported so those data are not presented. Results presented following two-way ANOSIM significant pairwise tests for size classes (S3.1) 15 – 19.9 cm TL vs 20 – 24.9 cm TL, (S3.2) 15 – 19.9 vs 30 – 34.9, and (S3.3) 15 – 19.9 vs ≥35. Abundance[a] is average percent volume of recognizable prey in each size class. Percent contribution is the contribution of each prey category to the observed dissimilarity. Prey categories contributing < 3% to the observed dissimilarity are not listed

Table S3.1

	Size Class Abundance[a]		
Prey Category	15 – 19.9 cm TL	20 – 24.9cm TL	Percent Contribution
Crustacea	5.73	0.83	28.28
Haemulidae	0.91	2.29	17.42
Pomacentridae	0.91	1.42	12.92
Carangidae	0	1.63	11.29
Blennidae	0.91	1.80	10.03
Acanthuridae	0	0.83	6.24
Serranidae	0.87	0.75	5.58

[a]Values presented are square root transformed as in SIMPER analysis.

Table S3.2

Prey Category	Size Class Abundance[a]		Percent Contribution
	15 – 19.9 cm TL	30 – 34.9cm TL	
Crustacea	5.73	0.32	29.92
Haemulidae	0.91	2.35	20.2
Serranidae	0.87	3.14	10.0
Carangidae	0	1.26	6.75
Gobiidae	0.91	0.3	6.51
Pomacentridae	0.91	0.12	5.06
Blennidae	0.91	0.38	5.05
Scaridae	0	1.65	3.2
Labridae	0.02	0.53	3.02
Bothidae	0	0.34	3.01

[a] Values presented are square root transformed as in SIMPER analysis.

Table S3.3

Prey Category	Size Class Abundance[a]		Percent Contribution
	15 – 19.9 cm TL	≥35 cm TL	
Crustacea	5.73	0.88	31.31
Haemulidae	0.91	1.62	17.9
Serranidae	0.87	3.2	11.18
Scaridae	0	1.75	9.45
Bothidae	0	0.96	7.67
Pomacentridae	0.91	0.59	5.34
Carangidae	0	0.39	3.77
Gobiidae	0.91	0	3.77

[a] Values presented are square root transformed as in SIMPER analysis.

Supplement 5. Table S4. Selection of U.S. South Atlantic fishes with potential dietary overlap with *Pterois volitans*.[a]

Species	Fishes as % of diet	Sample size	Prey taxa overlap[b]
sand diver, *Synodus intermedius*	94.5	18	Y
inshore lizardfish, *S. foetens*[d]	76 – 100	3 – 603	Y
red lizardfish, *S. synodus*	100	2	?
green moray, *Gymnothorax funebris*[e]	?[c]	3	?
spotted moray, *G. moringa*	100	6	Y
purplemouth moray, *G. vicinus*	62.5	4	Y
spotted snake eel, *Ophichthus ophis*	50	4	Y
coronetfish, *Fistularia tabacaria*	100	2	?
trumpetfish, *Aulostomus maculatus*	73.5	69	Y
scamp, *Mycteroperca phenax*[f]	“most important”	91	Y
gag, *M. microlepis*[g]	95.1 – 99.8	979	Y
black grouper, *M. bonaci*[h]	76.7 – 100	4 – 72	Y
yellowmouth grouper, *M. interstitialis*	100	5	Y
tiger grouper, *M. tigris*	100	34	Y
yellowfin grouper, *M. venenosa*	95.3	51	Y
graysby, *C. cruentata*	66.2	26	Y
Nassau grouper, *Epinephelus striatus*	54	153	Y
schoolmaster, *Lutjanus apodus*	60.7	58	Y
cubera snapper, *L. cyanopterus*	100	11	Y
dog snapper, *L. jocu*	60.7	56	Y
mahogany snapper, *L. mahogoni*	75	8	Y
peacock flounder, *Bothus lunatus*	85.7	7	Y
longlure frogfish, *Antennarius multiocellatus*	75	5	Y
splitlure frogfish, *A. scaber*	100	5	Y

[a] Taxa are listed if they were determined to have at least 50% fish prey in stomach contents and if they are believed to have a benthic feeding strategy while occupying the mesocarnivore trophic level.

[b] Prey taxa overlap with Y indicates that species listed had consumed the same species or genus of prey as lionfish; ? = unknown.

[c] Green moray were included because they are the largest western Atlantic moray and because Gudger (1929) (cited in Randall 1967) reported fish in the stomachs of three specimens, although their % contribution to the diet is not known.

References: Dietary data from Randall (1967) except as indicated; [d]Randall (1967) and Cruz-Escalona et al. (2005); [e]Randall (1967) and Gudger (1929); [f]Matheson et al. (1986); [g]Naughton & Saloman (1985); [h]Randall (1967) and Brulé et al. (2005).

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