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Habitat-specific feeding by cownose rays (*Rhinoptera bonasus*) of the northern Gulf of Mexico

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Abstract Past studies have suggested that increases in cownose ray (*Rhinoptera bonasus*) abundance may pose problems for fisheries management due to their specialized diet of exploitable mollusks. However, more recent work has identified cownose rays as opportunistic generalists, consuming mainly non-commercial prey (e.g. soft-bodied invertebrates) most abundant in their locale. To better assess the generalist vs. specialist foraging behaviors of cownose rays, including their impact to commercial shellfish of the north-central Gulf of Mexico, we conducted gut content analysis on 201 individuals from coastal Alabama. Prey items were analyzed for frequency of occurrence and percent composition by weight, which were used to develop an index of importance. Our diet analysis indicated a minimal impact of cownose rays to exploitable shellfish species of coastal Alabama. Further, we observed important spatial and ontogenetic diet variability: adult diets were dominated by crustaceans along barrier islands and open waters of the Gulf of Mexico, whereas juvenile and young-of-the-year individuals almost exclusively consumed bivalves in riverine and estuarine areas. Individual cownose ray diets were primarily monotypic (54.3%) and appeared selective for amphipods (i.e. *Haustorius* sp.) when hyperabundant along gulf barrier islands and bivalves at estuarine sites regardless of ambient densities. While this study highlights the importance of locality in determining dietary composition of cownose rays, we do not suggest this species is opportunistic or completely mollusk-specialized. Alternatively, we propose cownose rays exhibit a continuum of foraging behaviors between specialization and generalization, depending on locale and prey availability.

Keywords Elasmobranch · Feeding · Foraging · Gulf of Mexico · Batoid · Cownose ray · Opportunism · Diet

Introduction

Recent meta-analyses have revealed the potential for declines of marine top predators (e.g. large >2 m sharks) to cascade down to losses in benthic shellfish resources. These effects are predicted for various marine ecosystems via increases in populations of intermediate ("meso") predators that exclusively consume these shellfish (Myers et al. 2007) and/or changes in habitat use by these species with the loss of behaviorally-mediated interactions (Heithaus et al. 2008). However, limited information on habitat use and feeding ecology exist for many of these meso-predators throughout their range (e.g. batoids and small sharks), which reduces our ability to predict the overall consequences of their hypothesized release from predation. Some species implicated in trophic

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cascades, such as the cownose ray (*Rhinoptera bonasus*), are assumed specialist predators (Myers et al. 2007) despite growing evidence of opportunistic feeding habits (Collins et al. 2007a). Given the impact diet heterogeneity on suppressing trophic cascades (Strong 1992), detailed information on mesopredator diets across space and ontogeny is necessary to fully understand the future of community dynamics in marine ecosystems.

Myliobatid rays (Chondrichthyes: Myliobatidae) have been implicated as nuisance predators of several economically important mollusks across the world. In the Caribbean, the spotted eagle ray *Aetobatus narinari* is a prime predator of the gastropod *Strombus gigas* (Iversen et al. 1986), and thus a pest to conch farmers of the Turks and Caicos Islands (C. Dyer, Caicos Conch Farm, pers. comm.). In Ariake Sound (Japan), *Aetobatus flagellum* heavily consumes commercially important bivalves *Ruditapes philliparum* and *Atrina pectinata* (Yamaguchi et al. 2005). In Chesapeake Bay, cownose rays have negatively impacted surf clam *Mya arenaria* (Smith and Merriner 1985) and restoration of eastern oyster *Crassostrea virginica* (Merriner and Smith 1979), while damaging ecologically important eelgrass (*Zostera marina*) habitat (Orth 1975). Off North Carolina, cownose rays have decimated bay scallops (*Argopecten irradians*) clustered in high density areas (Peterson et al. 2001; Myers et al. 2007; Powers et al. 2011).

Negative impacts of cownose rays to restored shellfish habitats have led to the development of predator control programs that encourage ray harvest (e.g. Virginia Sea Grant). As with other K-selected elasmobranch species, cownose rays may not be sustainable for these types of fishing practices due to their low fecundity (Neer and Thompson 2005). Moreover, rays may even provide important indirect benefits to commercial benthic resources should their diet reveal consumption of other shellfish-consuming species (e.g. brachyuran crabs) as demonstrated with *Myliobatis californica* in Tomales Bay, CA (Gray et al. 1997). There is some evidence that cownose rays may actually prefer soft-bodied invertebrate prey over hard-shelled mollusks (Collins et al. 2007a). Thus, detailed quantification of the diet is imperative prior to implementation of management controls on cownose rays, especially because diets may vary greatly across their range.

The general ecology of the cownose ray is virtually unknown in the north-central Gulf of Mexico (NCGOM), particularly off the Alabama coast. Limited evidence suggests cownose rays are common to abundant in this region (Shipp 1986; Parsons 2006), which is an area also characterized by productive oyster fisheries (Kilgen and Dugas 1989). Past aerial surveys have shown that cownose rays are capable of forming aggregations of thousands of individuals in the NCGOM, and that these schools are often seen generating sediment plumes characteristic of ray foraging (Rogers et al. 1990). Moreover, rays appear strongly associated with regions of high riverine input and hypoxic bottom waters (Craig et al. 2010), which also describe the Mobile Bay and Perdido Bay estuaries of the Alabama coast. Despite evidence of cownose ray occurrence and valuable shellfish resources of coastal Alabama, there have been no studies of cownose ray diet from these areas.

We examined the gut content of individuals across all maturity stages from nearshore Gulf of Mexico waters to inshore estuarine regions of the Alabama coast to better describe cownose ray foraging behavior and potential impacts to exploitable shellfish species. Where possible, dietary composition of cownose rays was compared to either simultaneous or concurrent benthic samples, or published macroinvertebrate densities from the collection areas to better understand how prey availability influenced foraging.

Methods

Specimen collection

Cownose rays were collected from monthly year-round gillnet surveys along coastal Alabama from 2007 to 2010. The gear utilized was 300 m (3.0 m depth) monofilament gillnets with alternating 50 m panels of 10 cm and 15 cm stretched mesh. In a few cases, animals were also taken opportunistically by spear and longline gear (e.g. open waters of the Gulf of Mexico) where gillnets could not efficiently collect rays. Surveys spanned multiple habitat types (seagrass, oyster, sand, and mud bottom) and covered the breadth of coastal Alabama waters including Mississippi Sound, Mobile Bay and Perdido Bay (Fig. 1). All collected animals were sexed, weighed and measured for disk width (distance between the tips of pectoral fins) to the nearest millimeter. Guts (stomachs and intestines) were removed immediately upon capture after overdosing

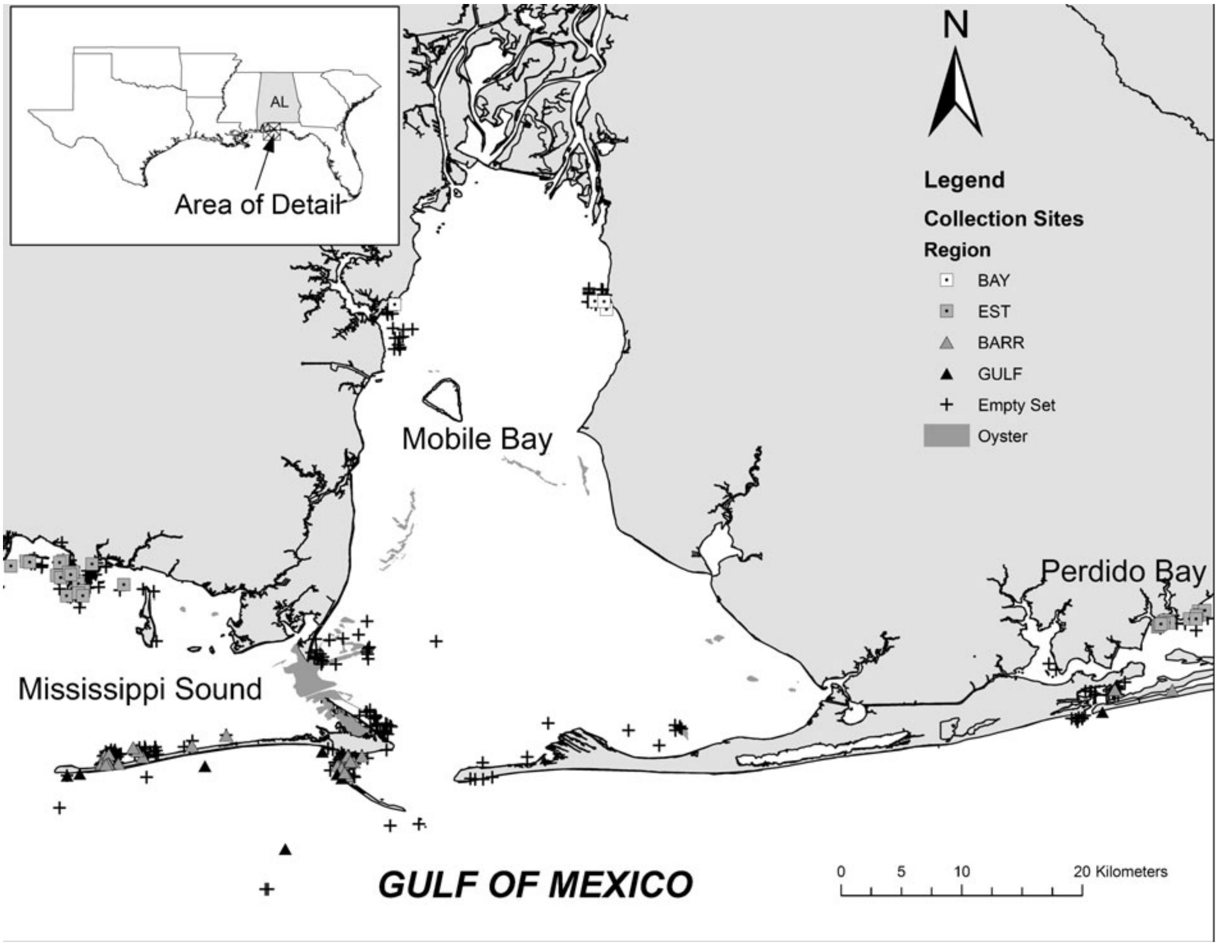

Fig. 1 Map of cownose ray collection effort across coastal Alabama. Empty sets where no rays were caught, or rays had empty gut content are represented by a plus sign. Sites where rays were caught with food in their guts are classified by major region: *BAY* Mobile Bay Sites (*white squares*); *EST* Estuarine sites (*light gray squares*); *BARR* Barrier Island sites (*dark gray triangles*); *GULF* Gulf of Mexico sites (*black triangles*). Sites of known oyster habitat are highlighted in *mid-gray*

the rays with Tricaine methanesulfonate (MS-222). Guts were then placed in containers with 10% buffered formalin and transferred to ethanol after 48 h.

Several metrics were used to assess the importance of various taxonomic groups to cownose ray diet. During gut content analysis all prey items were enumerated (if possible), wet weighed and identified to the lowest possible taxon (LPT). If gut material was too digested for identification, the material was weighed and placed into an "unidentified material" category. For each prey item, we calculated percent frequency of occurrence: $\%F_a = \frac{100\times S_a}{S}$ where S_a is the number of stomachs containing food group a, and S is the total number of stomachs containing food (Hyslop 1980). Using this metric and the percentage of total weight (%W), we utilized an Index of Importance (IOI): $IOI_a = \frac{100\times HI_a}{\sum_{a=1}^{n} HI}$ where $HI_a = \%\mathrm{F}_a + \%\mathrm{W}_a$ (Gray et al. 1997).

Sample classification

Prior to analyzing the impacts of various factors on cownose ray diet, samples were grouped by region and maturity state. Rays were separated into four regions of capture based on dominant bottom habitat and salinity. Collections sites from north Mississippi Sound, lower Mobile Bay and Perdido Bay estuaries were characterized as "EST" regions, due to the dynamic nature of environmental conditions (large salinity range; 9–32 ppt) and mainly mud and oyster dominated benthos. Collections from upper Mobile Bay were grouped into the “BAY” region, due to lower salinity levels (mean=10.7 ppt). Sites from the north side of barrier islands were grouped into a “BARR” region, and represented higher saline (mean=24.3 ppt) sandflats. All collection sites south of barrier islands were grouped into a “GULF” category where >29 ppt seawater was always encountered and bottom sediments were comprised of mixed sand and fine shell-hash (Fig. 1). Maturity states of rays were divided among three main categories: mature (>650 mm DW), juvenile (500–650 mm DW) and young-of-the-year (YOY; <500 mm DW) individuals following Neer and Thompson (2005).

Sample size sufficiency across ontogeny and space was assessed with cumulative prey curves (Ferry and Caillet 1996). Curves were created in PRIMER v6 and determined the cumulative number of prey taxa across samples (Sobs) based on the LPT level of identification. To remove the effect of sampling chronology on curve smoothness, the order was randomized across 999 permutations. Curves approaching an asymptote were considered sufficient in explaining dietary breadth, whereas those that did not asymptote were inadequate measures of the full diversity of prey items.

Feeding strategy diagrams and prey specific weight

To examine the dietary importance of a particular prey item on an individual basis, as well as further elucidate the foraging behavior of cownose rays, we created a feeding strategy diagram (Amundsen et al. 1996). These diagrams provided a visual assessment tool for examining niche width contribution, importance and strategy (i.e. specialization vs. generalization) of different prey items using occurrence and prey-specific weight. Prey-specific weight was determined by dividing the total weight of a particular prey item across samples by the cumulative amount of total gut content weight from all individuals possessing that prey item (i.e. excludes individuals not possessing the prey type).

Multivariate statistical design

Spatial and ontogenetic variation in cownose ray diet was examined using multivariate analyses on all individuals with guts containing food items (n=138). Prey group weights were standardized by dividing each group by individual body weight of the fish (Arthur et al. 2009). Standardized prey group weights were then imported into PRIMER, fourth-root transformed, and used to develop a Bray-Curtis similarity matrix. Two-way crossed permutational multivariate analysis of variance (PERMANOVA) examined the effects of region and maturity state (factors) on the standardized weight contribution of various LPTs. PERMANOVA used a population-wide dissimilarity metric to evaluate differences in the dietary composition among the various maturity states and regions (Anderson 2001). All tests utilized Bray-Curtis dissimilarity as the metric and were permutated 999 times under a reduced model. Significant factors were further analyzed using a PERMANOVA pairwise comparison, and similarity of percent contribution (SIMPER) analysis was employed to examine the prey categories most responsible for the separation among factors (Clarke 1993). We accompanied our analysis with PERMDISP to determine possible reasons for the rejection of the null hypothesis (no differences in standardized weight contribution of dietary groups across sites) by PERMANOVA as this test is known to be sensitive to sample dispersion (Anderson et al. 2006). Non-metric multidimensional scaling (NMDS) was employed to visually assess this dispersion of samples.

Benthic sampling

We collected benthic samples of invertebrate abundance concurrently with cownose ray collections to characterize prey availability (Fig. 2). The first site was Point aux Pins (PAP), an estuarine location characterized by shallow mud and seagrass habitat in north Mississippi Sound. At PAP, a 750 m^2 grid with 9 fixed stations was sampled monthly using hand cores (0.01 m^2) from December 2008 to December 2009. A second benthic sampling site was located off Pelican Island, a barrier island due south of Dauphin Island. In spring of 2009 (February–May), we sampled this benthic sand flat community also using hand cores (0.01 m^2). On a given sampling day, five cores from 10 randomly selected points (50 samples) were taken with simultaneous 1 h gillnet soaks. Similar core sampling was conducted in September 2010 off Perdido Beach. Benthic samples were also taken offshore of the west and east ends of Dauphin

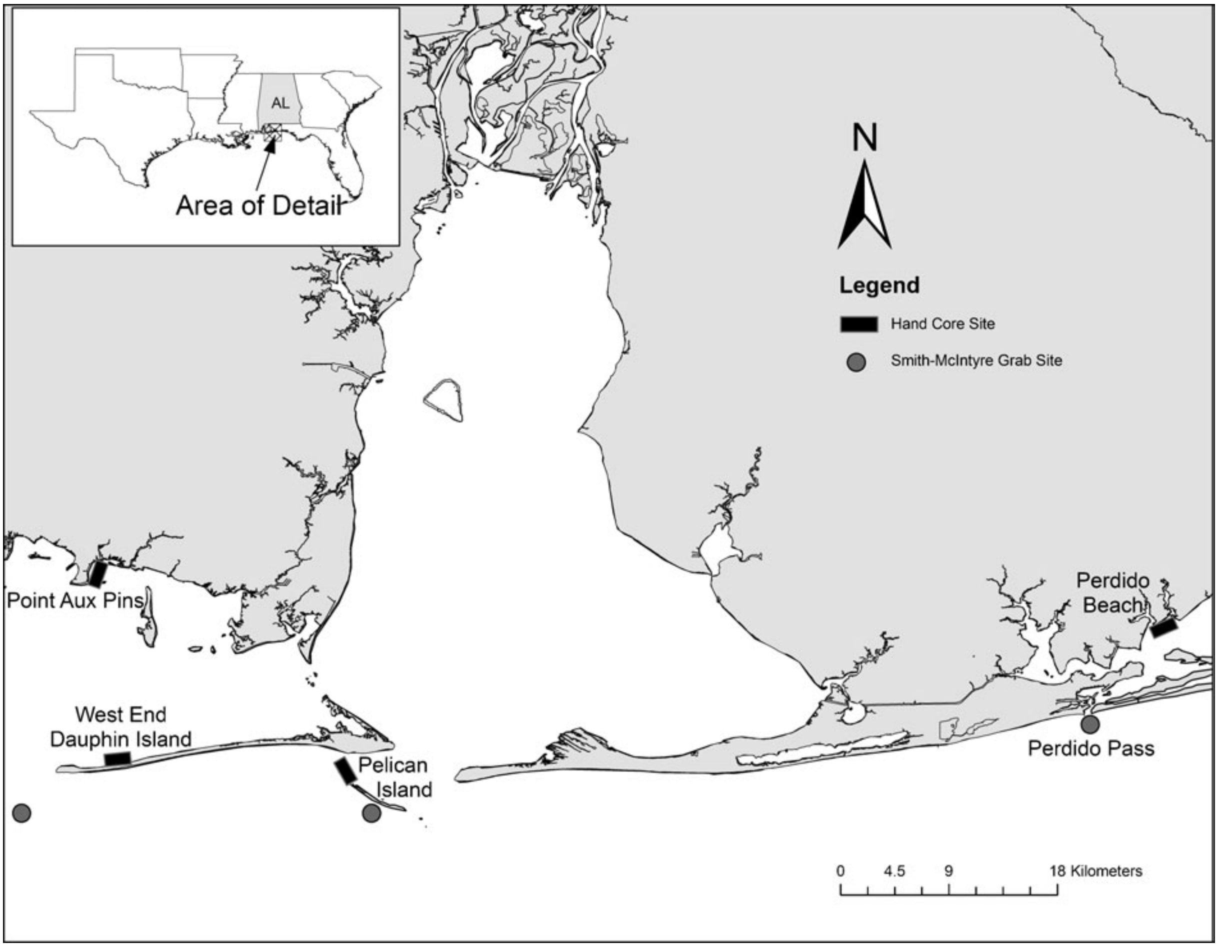

Fig. 2 Map of benthic sampling sites across coastal Alabama from 2007 to 2010. *Black rectangles* represent inshore core sampling regions. *Gray circles* represent Smith-McIntyre grab sites conducted in "GULF" waters

Island and south of Perdido Pass (GULF sites) using a Smith-McIntyre benthic grab (0.1 m^2). The contents of the grab were sub-sampled using five hand cores (0.01 m^2).

For all locations, we compared composition of taxa within the diet of rays with densities of benthic invertebrates from core sampling. Given the more recent association of cownose rays as opportunistic generalists (e.g. Collins et al. 2007a), we hypothesized that there would be no difference between rank of importance of benthic taxa in the diet and the rank of average density in the environment. A true electivity index could not be quantified as some prey in the diet could not be enumerated effectively (e.g. pulverized bivalves), thus, our analysis of diet versus prey availability was qualitative.

Results

Diet analysis

Of 201 guts examined, 154 contained food items (23.4% overall vacuity index) and 138 contained a portion of food items that could be resolved to LPT and/or BTG. Gut vacuity ranged from 12.5 to 34.6% from 2007 to 2009, and appeared generally lower in the summer months when samples were dominated by YOY individuals (Table 1). Guts with identifiable diet samples came predominantly from YOY (*n*=61) and mature (*n*=54) individuals, with lesser amounts of juveniles (*n*=23). A scatterplot of percent body weight contribution of total food items vs. disk width displayed a negative ontogenetic trend (Fig. 3).

Table 1 Sample size distribution across years, season and maturity state. Due to logistics, sampling was restricted in 2010. Seasonal abbreviations are as follows: *SU* summer; *F* Fall; *W* Winter; *SP* spring. *YOY* young-of-the-year. Mature animals dominated the catch in spring and variably in the fall, whereas YOY individuals dominated the summer catch across most years

Year	2007		2008				2009				2010				
Season Maturity	SU	F	W	SP	SU	F	W	SP	SU	F	W	SP	SU	F	TOTAL
YOY	10	3	7	5	19	10	2	1	25	10	0	0	0	1	93
Juvenile	3	0	2	4	9	5	2	0	5	1	0	0	1	0	32
Mature	0	7	5	17	4	11	10	4	2	3	0	1	3	9	76
Total	13	10	14	26	32	26	14	5	32	14	0	1	4	10	201
Vacuity Index (%)	23.1	20.0	28.6	34.6	25.0	26.9	21.4	20.0	12.5	28.6		0.0	25.0	10.0	23.4

Though variable, immature animals (YOY and juvenile) were found to contain greater relative amounts of total ingested material (~6% BW) than mature animals (generally <3% BW).

Thirty prey categories were identified from gut content analyses (Table 2). Taxon richness was influenced by both maturity state and region. Cumulative prey curves for mature and YOY individuals both approached an asymptote, whereas JUV individuals were clearly far from asymptotic (Fig. 4a). Overall, taxon richness in mature adults was much higher than JUV and YOY individuals and higher in BARR/GULF sites than BAY/EST sites (Fig. 4b). With all individuals pooled, cownose ray diet was dominated by bivalves (IOI_{ALL}=39.11%), with tellinid (6.02%) and venerid clams (6.38%) representing the most commonly identified families (Table 2). Crustaceans (i.e. malacostracans) were the second most important dietary item across all individuals (IOI_{ALL}=18.22%) and were mainly dominated by amphipods (9.01%). Crustaceans were then followed by echinoderms (IOI_{ALL}=7.34%), polychaetes (IOI_{ALL}=6.80%), gastropods (IOI_{ALL}=3.12%) and nematodes (IOI_{ALL}=0.21%) in terms of dominance (Table 2).

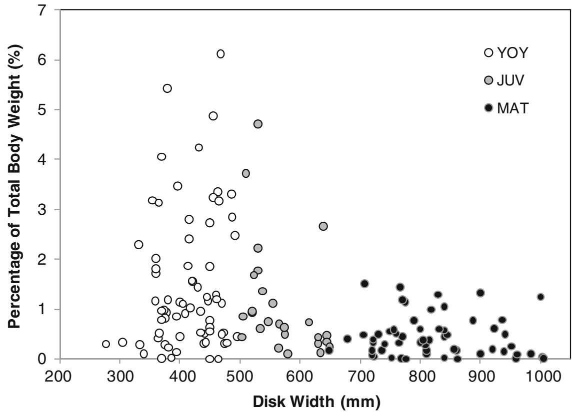

Fig. 3 Scatterplot of gut contents weight (expressed as a proportion of the total body weight) vs. Disk Width (distance between wing tips of the ray)

Diet was observed to vary ontogenetically (Table 2, Fig. 5a). For YOY individuals, gut content was dominated by bivalves (IOI_{YOY}=63.98%), followed by polychaetes (5.83%), gastropods (5.31%), crustaceans (3.91%) and echinoderms (1.75%). Juvenile ray diets were also mainly composed of bivalves (IOI_{JUV}=47.49%), but had higher proportions of polychaetes (7.53%), crustaceans (4.90%), and echinoderms (4.34%). In addition, juvenile guts were noted to contain plant material (3.68%). Adult diets were found to be the most heterogeneous with the greatest dominance of crustaceans (IOI_{MAT}=30.68%) echinoderms (10.89%) polychaetes (7.79%), and plant material (8.45%) and the lowest IOI for bivalves (23.15%).

Collection location (i.e. region; Table 2, Fig. 5b) also significantly influenced diet. For individuals collected from "BAY" sites in northern Mobile Bay, the diet was nearly monotypic with mactrid clams (e.g. *Mulinia lateralis*; IOI_{BAY}=80.29%). In estuarine locations, veneroid bivalves were most important (IOI_{EST}=66.81%), comprised of tellinid (10.17%) and venerid (4.85%) clams (Table 2). Estuarine diets also differed from bay sites with the inclusion of polychaetes (4.76%), crustaceans (4.21%), echinoderms (1.61%) and gastropods (2.64%). Diets from barrier island locations were dominated by crustaceans (IOI_{BARR}=34.67%), then bivalves (18.80%), echinoderms (11.13%), polychaetes (10.47%), and gastropods (3.80%). Guts from animals collected in the open waters

Table 2 Cownose ray dietary composition by percent frequency of occurrence (%FO), percent weight (%W), and percent index of importance (%IOI).%IOI values for major prey groupings are highlighted in bold

		ALL			Maturity state -%IOI			Region -%IOI			
Taxonomic group	Subcategory	%FO	%W	%IOI	YOY	JUV	MAT	BAY	EST	BARR	GULF
PLANT MATERIAL		0.12	0.02	**4.61**	**0.00**	**3.68**	**8.45**	**0.00**	**1.64**	**5.92**	**9.18**
NEMATODA		0.01	0.00	**0.21**	**0.00**	**0.00**	**0.45**	**0.00**	**0.00**	**0.55**	**0.00**
POLYCHAETA	Sum			**6.80**	**5.83**	**7.53**	**7.79**	**0.00**	**4.76**	**10.47**	**3.62**
	unidentified	0.16	0.03	5.95	5.25	7.53	5.99	0.00	4.22	8.83	3.62
	Capitellidae	0.02	0.00	0.64	0.57	0.00	1.35	0.00	0.54	1.10	0.00
	Chaetopteridae	0.01	0.00	0.21	0.00	0.00	0.45	0.00	0.00	0.55	0.00
GASTROPODA	Sum			**3.12**	**5.31**	**0.00**	**2.74**	**0.00**	**2.64**	**3.59**	**3.75**
	unidentified	0.06	0.00	1.99	2.53	0.00	2.28	0.00	1.05	2.33	3.75
	Nassaridae	0.01	0.00	0.22	0.00	0.00	0.46	0.00	0.54	0.00	0.00
	Marginellidae	0.02	0.01	0.91	2.78	0.00	0.00	0.00	1.04	1.26	0.00
BIVALVIA	Sum			**39.11**	**63.98**	**47.49**	**23.15**	**95.40**	**65.81**	**19.74**	**19.78**
	Veneroida	0.38	0.32	22.59	41.34	36.96	8.94	6.81	48.46	8.34	2.47
	Tellinidae	0.15	0.04	6.02	7.66	5.95	4.99	0.00	10.17	4.75	2.41
	Donax sp.	0.03	0.04	2.36	0.00	0.00	4.34	0.00	0.00	1.40	8.27
	Veneridae	0.06	0.01	2.38	5.49	1.39	0.45	0.00	4.85	1.14	0.00
	Gemma gemma	0.01	0.01	0.72	1.73	1.34	0.00	0.00	1.31	0.55	0.00
	Macrocallista nimbosa	0.02	0.00	0.70	0.00	0.00	1.46	0.00	0.00	2.39	0.00
	Mactridae	0.01	0.01	0.65	2.01	0.00	0.00	8.30	1.00	0.00	0.00
	Mulinia lateralis	0.05	0.02	2.23	5.74	1.85	0.00	80.29	0.00	0.00	0.00
	Arcidae	0.03	0.00	0.99	0.00	0.00	2.02	0.00	0.00	0.01	5.43
	Nuculidae	0.01	0.00	0.46	0.96	0.00	0.00	0.00	0.00	1.17	0.00
	Geuksenia demissa	0.01	0.00	0.01	0.56	0.00	0.00	0.00	0.00	0.00	1.21
ECHINODERMATA	Sum			**7.34**	**1.72**	**4.34**	**10.89**	**0.00**	**1.61**	**11.13**	**9.62**
	Ophiuroidea	0.04	0.00	1.30	1.16	1.34	1.39	0.00	1.61	1.15	1.21
	Echinoidea	0.02	0.00	0.63	0.00	0.00	1.36	0.00	0.00	0.55	2.41
	Mellita sp.	0.08	0.08	5.41	0.56	3.00	8.15	0.00	0.00	9.43	6.01
CRUSTACEA	Sum			**18.22**	**3.91**	**5.36**	**30.68**	**0.00**	**4.21**	**25.03**	**34.25**
	unidentified	0.04	0.00	1.28	0.56	0.00	2.28	0.00	0.53	1.67	2.43
	Xanthidae	0.03	0.00	1.08	0.00	1.33	1.97	0.00	0.00	2.23	1.24
	Panaeidae	0.04	0.00	1.27	0.00	1.33	2.26	0.00	0.00	1.09	4.85
	Callinectes sp.	0.04	0.00	1.27	0.56	0.00	2.26	0.00	0.53	0.55	4.85
	Pinnixa sp.	0.06	0.00	1.93	0.00	0.00	4.10	0.00	0.00	1.10	8.56
	Callianassa sp.	0.04	0.00	1.32	0.00	0.00	2.79	0.00	0.00	0.00	7.48
	Amphipoda	0.13	0.15	9.01	2.79	2.69	12.74	0.00	3.16	17.84	0.00
	Isopod	0.03	0.00	1.06	0.00	0.00	2.26	0.00	0.00	0.55	4.85
UNIDENTIFIED		0.38	0.25	**20.59**	**17.75**	**31.61**	**16.81**	**4.60**	**19.35**	**23.55**	**19.77**

of the Gulf of Mexico were also mainly comprised of crustaceans (IOI_{GULF}=34.67%), followed by bivalves (18.80%), echinoderms (9.74%), and polychaetes (3.67%). Gulf diets were also found to possess the highest amounts of grasses (9.29%) of all locations.

Multivariate analyses

The impact of temporal factors (year, season) on the standardized weights of cownose ray dietary assemblage was insignificant (PERMANOVA, $p>0.05$),

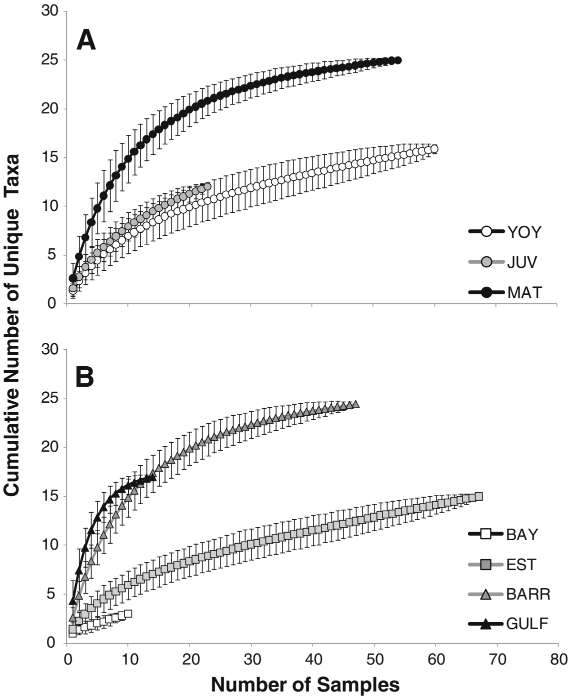

Fig. 4 Cumulative Prey Curves for all samples with identifiable contents (n=138). **a** Samples grouped by Maturity State (*YOY* young-of-the-year; *JUV* juvenile; *MAT* mature). **b** Samples grouped by region (*BAY* Upper Mobile Bay, *EST* estuary; *BARR* Barrier Island; *GULF* Gulf of Mexico). *Error bars* represent standard deviations around a resampled mean after 999 iterations

thus we elected to remove these factors to eliminate analyses of third-order interaction terms and to assist with interpreting pairwise comparisons. Moreover, multiple life stages were rarely captured within the same region and season, which prevented meaningful pairwise comparisons. Two-way crossed PERMANOVA on standardized weights of LPT found maturity state alone did not significantly influence diet in cownose rays (PERMANOVA, p=0.101; Table 3). However, region was found to be a significant factor (PERMANOVA, p=0.001; Table 3), as well as the interaction between maturity state and region (PERMANOVA, p=0.049; Table 3). Pairwise tests found all regions to be significantly different from one another, with the exception of GULF and BARR (p=0.148; Table 4). PERMDISP found that these regional differences could be partially explained by heterogeneity in sample dispersion (PERMDISP, p=0.001), with all regions having significantly lower mean deviations from centroids than BARR sites. The PERMDISP findings were supported by the NMDS plot of regional sample classification (2D Stress = 0.13), which showed a strong clustering of BAY samples within an array of EST samples (Fig. 6). The remaining EST samples were sparsely dispersed throughout multidimensional space (Fig. 6), and GULF and BARR samples were generally distributed throughout the plot with little evidence of clustering.

Because collections were limited to primarily single life stages at BAY and GULF sites (YOY and MAT, respectively), this reduced the number of unique permutations carried out by the PERMANOVA for pairwise tests of the interaction term. Thus, not all regions could be compared within a single maturity state. The diet of YOY individuals was significantly different from MAT diet at BARR sites (p=0.007, Table 5), though no differences were detected between maturity states at all other sites. Juvenile diets were found to significantly differ between EST and BARR sites (p=0.015; Table 5). YOY diets also varied significantly between EST and BARR sites, and also between EST and BAY sites and BARR and BAY sites. Mature diets were also found to vary significantly across all regions, though no mature animals were sampled from BAY sites and thus precluded any comparisons (Table 5).

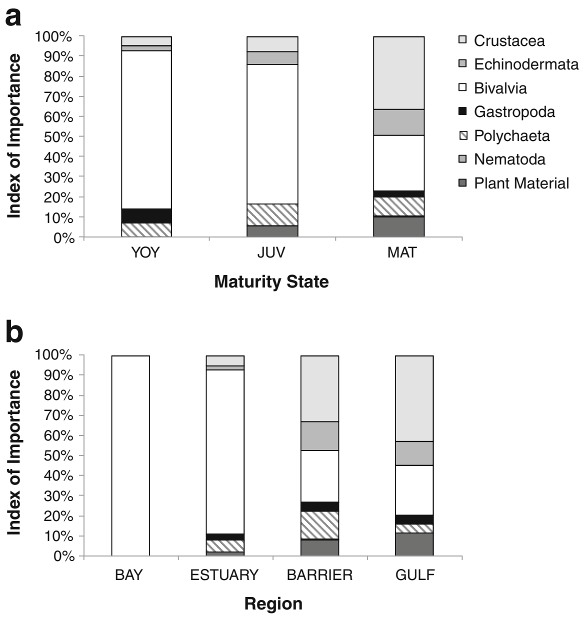

Fig. 5 Stacked bar charts of % Index of Importance of major prey categories for all individuals separated by life stage (**a**: *YOY* young-of-the-year; *JUV* juvenile, *MAT* mature adult), and region (**b**)

Table 3 Results from two-way crossed PERMANOVA of cownose ray dietary data

PERMANOVA

Source	df	SS	MS	Pseudo-F	*P*(perm)	Unique perms
Maturity	2	9867	4933.3	1.4161	0.101	999
Region	3	47258	15753.0	4.522	**0.001**	999
Maturity × Region	5	23704	4740.9	1.361	**0.049**	995
Residual	127	442420	3483.6			
Total	137	582860				

Pairwise tests *P*-value based on PERMANOVA (perm) or Monte Carlo (MC) methods depending upon the number of unique permutations.* - denotes a test with few unique permutations (i.e. low sample size). Items in bold represent significant pairwise comparisons

SIMPER analysis showed the main contributor to the dissimilarity between EST and GULF sites was the greater abundance of veneroid and tellinid bivalves in the diet of EST samples and greater abundance of *Mellita* sp. (sand dollars) in GULF samples (Table 6a). Veneroid and tellinid clam abundance also largely contributed to dissimilarity between EST and BARR samples, though BARR samples were found to have higher abundance of polychaetes and amphipods (Table 6b). BAY samples differed from all other regions mainly due to the high abundance of the sole identified species, *Mulinia lateralis*.

SIMPER analysis for interactions found that the highest contributors to dissimilarity between YOY and mature diets at BARR sites were polychaetes and marginellid gastropods for YOY individuals and amphipods for mature animals (Table 7a). As with the SIMPER analysis of regional dissimilarities, all life stages were generally found to consume higher amounts of veneroid clams at EST sites and contain higher amounts of polychaetes (YOY, JUV) or amphipods (MAT) at BARR sites (Table 7b–f). Mature individuals were also found to consume higher amounts of *Mellita* sp. than immature individuals at

Table 4 Pairwise tests are shown for significant factors: Region

PAIR-WISE TEST (Region)

Region 1	Region 2	*t*	*P*(perm)	Unique perms
EST	GULF	1.6642	**0.018**	998
EST	BARR	2.6137	**0.001**	999
EST	BAY	2.4678	**0.002**	998
GULF	BARR	1.1645	0.148	998
GULF	BAY	1.6441	**0.005**	999
BARR	BAY	1.8609	**0.001**	998

Pairwise tests *P*-value based on PERMANOVA (perm). Items in bold represent significant pairwise comparisons

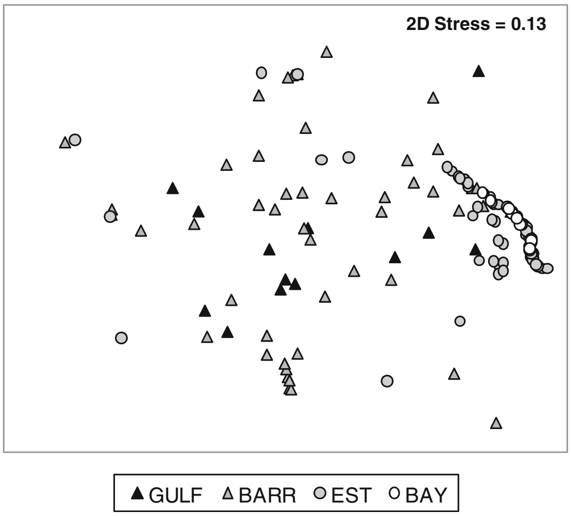

Fig. 6 Non-metric multi-dimensional scaling (*NMDS*) plot of the cownose ray diet assemblages based on the broad taxonomic group (*BTG*) classification. Samples are based on Bray-Curtis dissimilarity measure and are represented from the four collection regions across Alabama waters (*GULF* Gulf of Mexico samples, *black triangles*; *BARR* Barrier Island samples, *gray triangles*; *EST* Estuary samples, *gray circle*; and *BAY* Upper Mobile Bay samples; *white circles*). Distances between points represent how similar (points close together) or different (points far apart) samples are from one another. Only samples with identifiable food items were considered in this ordination (n=138)

Table 5 Pairwise tests are shown for significant factors: Maturity State × Region

PAIR-WISE TESTS (Maturity × Region)					
Grouping factor	Comparison	*t*	*P*(perm)	Unique perms	P(MC)
BAY*	JUV, YOY	0.44771	0.788	9	0.859
EST	JUV, YOY	1.1442	0.234	999	0.235
EST	JUV, MAT	1.1679	0.231	989	0.222
EST	YOY, MAT	1.2218	0.153	999	0.19
GULF*	JUV, MAT	1.0525	0.387	13	0.367
GULF*	YOY, MAT	1.2084	0.228	13	0.207
BARR	JUV, YOY	1.1966	0.118	311	0.235
BARR	JUV, MAT	1.1216	0.219	998	0.267
BARR	YOY, MAT	**1.7149**	**0.007**	**997**	**0.005**
JUV*	EST, GULF	1.4772	0.065	17	0.095
JUV	EST, BARR	**1.7109**	**0.015**	**978**	**0.021**
JUV*	EST, BAY	1.5382	0.064	17	0.083
JUV*	GULF, BARR	0.90435	1.000	5	0.543
JUV*	GULF, BAY	No test			
JUV*	BARR, BAY	1.0049	0.668	2	0.444
YOY*	EST, GULF	1.1337	0.380	44	0.26
YOY	EST, BARR	**2.6058**	**0.001**	**999**	**0.001**
YOY	EST, BAY	**3.2741**	**0.001**	**999**	**0.001**
YOY*	GULF, BARR	1.2719	0.436	6	0.226
YOY*	GULF, BAY	1.6785	0.270	9	0.073
YOY	BARR, BAY	**2.6461**	**0.001**	**793**	**0.003**
MAT	EST, GULF	**1.6155**	**0.004**	**979**	**0.012**
MAT	EST, BARR	**1.3884**	**0.036**	**997**	**0.054**
MAT	GULF, BARR	**1.942**	**0.001**	**999**	**0.002**

Pairwise tests *P*-value based on PERMANOVA (perm) or Monte Carlo (MC) methods depending upon the number of unique permutations.* - denotes a test with few unique permutations (i.e. low sample size). Items in bold represent significant pairwise comparisons

BARR sites, and greater amounts of plant material and *Donax* sp. from GULF sites (Table 7c). Guts from BAY samples (YOY only) were found to have a large contribution of *Mulinia lateralis*, which was absent in all guts from other regions (Table 7g–h).

Feeding strategy

In the feeding strategy diagram, we observed multiple taxa clustered in the upper left quadrant, where prey had high between phenotype contribution to niche width. These included a series of bivalves (*Donax* sp., *Gemma gemma*, Tellinidae), sand dollars, haustorid amphipods and margin shells, depending on region (Fig. 7). Only one prey type occurred in samples from BAY sites, the bivalve *Mulinia lateralis*, which was thus represented as a dominant prey category in the diagram (upper right portion; Fig. 7). The prey categories represented in the lower left quadrant were rare occurrences and contributed very little to the diet in terms of prey-specific weight. Items falling into this rare category included polychaetes, decapod crustaceans and brittle stars (Fig. 6).

Benthic sampling

Benthic sampling revealed variable results when matching the importance of certain prey items in the diet versus available items (Table 8). Overall, despite a polychaete dominated benthos across many sites, cownose rays generally consumed other invertebrate species of lower relative abundance. For example, at Point Aux Pins (PAP) veneroid bivalves were of highest importance to rays, despite their densities being considerably lower than amphipods and polychaetes at this site. In nearshore Gulf of Mexico waters, rays more commonly consumed echinoderms (e.g. *Mellita* sp.) and bivalves, despite low overall

Table 6 Results from two-way crossed similarity of percent (SIMPER) analyses for significant regional effects. Species contributions that summed cumulatively to >75% are shown. Average abundances are multiplied by 1×10^5

a)						
EST vs. GULF						
Average dissimilarity = 92.09	EST	GULF				
Taxon	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Veneroida	7.77	0.24	28.69	1.01	31.16	31.16
Tellinidae	1.29	0.03	14.16	0.55	15.38	46.54
Mellita sp.	0.00	1.05	9.47	0.59	10.29	56.83
Plant material	0.11	0.79	8.79	0.49	9.55	66.38
Gastropoda	0.01	0.38	7.88	0.72	8.56	74.93
Donax sp.	0.00	1.01	3.04	0.24	3.31	78.24
b)						
EST vs. BARR						
Average dissimilarity = 96.45	EST	BARR				
Taxon	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Veneroida	7.77	0.61	35.26	1.07	36.55	36.55
Tellinidae	1.29	0.17	12.73	0.54	13.20	49.75
Polychaeta	0.11	0.92	11.99	0.68	12.43	62.18
Amphipoda	0.06	1.91	6.83	0.40	7.08	69.26
Plant material	0.11	0.33	5.90	0.34	6.11	75.38
c)						
EST vs. BAY						
Average dissimilarity = 95.15	EST	BAY				
Taxon	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Mulinia lateralis	0.00	7.99	38.68	1.49	40.65	40.65
Veneroida	7.77	0.72	35.35	1.27	37.15	77.80
d)						
GULF vs. BAY						
Average dissimilarity = 96.66	GULF	BAY				
Taxon	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Mulinia lateralis	0.00	7.99	41.16	1.72	42.58	42.58
Gastropoda	0.38	0.00	20.62	2.49	21.33	63.91
Veneroida	0.24	0.72	14.38	2.16	14.87	78.78
e)						
Groups BARR & BAY						
Average dissimilarity = 100.00	BARR	BAY				
Taxon	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Mulinia lateralis	0.00	7.99	49.15	1.73	49.15	49.15
Polychaeta	0.92	0.00	16.72	1.12	16.72	65.87
Marginellidae	0.22	0.00	8.21	0.44	8.21	74.08
Gastropoda	0.24	0.00	7.45	0.41	7.45	81.52

densities of these items from cores. The abundance of cownose rays showed a positive correlation with amphipod abundance at the Pelican Island sampling site (Fig. 8). During late winter, rays consumed large quantities of haustorid amphipods in this area while seemingly ignoring available polychaetes and coquinas (*Donax* sp.) and switched to polychaetes when amphipod densities had dropped to relatively low levels in the

Table 7 Similarity of percent (SIMPER) contribution tables for significant interaction effects. Cumulative species contributions that summed to at least 50% are shown. Average abundances are multiplied by 1×10^5

a)

BARR: MAT vs. YOY

Average dissimilarity = 94.13	Group MAT	Group YOY				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Polychaeta	0.58	2.38	24.97	1.12	26.53	26.53
Amphipoda	2.47	0.14	17.19	0.73	18.27	44.80
Marginellidae	0.00\	1.69	11.44	0.46	12.16	56.95

b)

JUV: EST vs. BARR

Average dissimilarity = 95.80	Group EST	Group BARR				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Veneroida	6.97	0.11	46.98	1.34	49.04	49.04
Polychaeta	0.32	1.67	15.90	0.66	16.60	65.64

c)

MAT: GULF vs. BARR

Average dissimilarity = 94.93	Group GULF	Group BARR				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Amphipoda	0.00	2.47	19.47	0.67	20.51	20.51
Mellita sp.	0.87	0.97	14.78	0.66	15.57	36.08
Plant material	0.92	0.17	9.50	0.92	10.01	46.09
Donax sp.	1.18	0.16	8.21	0.41	8.65	54.75

d)

MAT: GULF vs. EST

Average dissimilarity = 96.62	Group GULF	Group EST				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Veneroida	0.04	3.13	23.09	0.74	23.89	23.89
Tellinidae	0.04	2.29	20.36	0.68	21.08	44.97
Plant material	0.92	1.21	16.24	0.74	16.81	61.78

e)

MAT: BARR vs. EST

Average dissimilarity = 94.50	Group BARR	Group EST				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Veneroida	0.78	3.13	23.51	0.83	24.88	24.88
Tellinidae	0.18	2.29	19.22	0.71	20.34	45.22
Amphipoda	2.47	0.00	16.33	0.66	17.28	62.50

f)

YOY: EST vs. BARR

Average dissimilarity = 98.21	Group EST	Group BARR				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Veneroida	8.67	0.00	41.18	1.24	41.93	41.93
Polychaeta	0.05	2.38	16.98	1.00	17.29	59.22

g)

YOY: EST vs. BAY

Average dissimilarity = 94.95	Group EST	Group BAY				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Mulinia lateralis	0.00	8.00	37.98	1.47	40.00	40.00
Veneroida	8.67	0.80	35.38	1.26	37.26	77.26

Table 7 (continued)

h)

YOY: BARR vs. BAY

Average dissimilarity = 100.00	Group BARR	Group BAY				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Mulinia lateralis	0.00	8.00	47.28	1.67	47.28	47.28
Polychaeta	2.38	0.00	17.34	1.23	17.34	64.62

late spring (Table 8). Cownose rays off the west end of Dauphin Island (WEDI) also consumed haustorids above polychaetes and bivalves in this area. At Perdido Bay (PD) in summer 2010, bivalve abundance (*Gemma gemma*) exceeded that of polychaetes, and rays were found to solely consume *Gemma gemma.*

Discussion

Overall, bivalves represent important resources to cownose rays from the Alabama coast (Table 2). Moreover, bivalves were preferably consumed even when present at low ambient densities at many

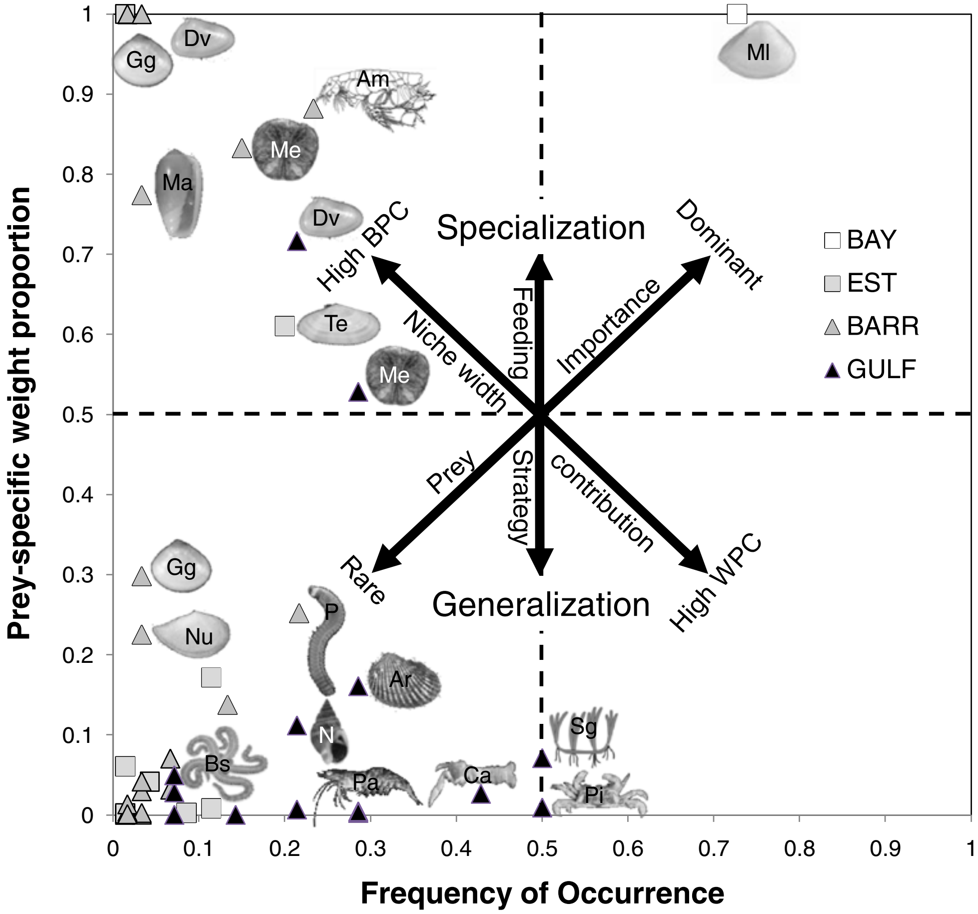

Fig. 7 Redrawn feeding strategy diagram from Amundsen et al. (1996) incorporating identified prey taxa of cownose rays and their feeding strategy classification across regions of Alabama. Images of select items are offset to the right of symbols for visual interpretation. Abbreviations are as follows: *Am* Amphipod; *Ar* Arcidae; *Bs* brittle star (Ophiuroid); Ca = *Callianassa* sp.; Dv = *Donax variabilis*; Gg = *Gemma gemma*; Ma = Marginellidae; Me = *Mellita* sp. (Sand dollar); Ml = *Mulinia lateralis*; *N* Nassaridae; *Nu* Nuculidae; *Pa* Panaeidae; *Pi Pinnixa* sp.; *Sg* Seagrass (plant material)

Table 8 Benthic Sampling table showing dominant taxa for each site and their densities. Also shown are the important taxa in the diet of rays taken from the same sites (based on %IOI), and their rank of numerical abundance based on the benthic data. A rank of 1 would be the item theoretically most available, whereas rankings of greater number represent less abundant prey. For instances where the dominant items in the ray guts were identified to lower taxonomic level than the benthic samples, they are placed in parentheses. Abbreviation for sites: *GULF* Gulf of Mexico, *PAP* Point Aux Pins, *PB* Pelican Bay, *WEDI* West End of Dauphin Island, and *PD* Perdido Bay. Abbreviations for seasons: *F* Fall, *W* winter, *SP* spring and *SU* summer

Year	Site	Region	Season	Dominant Benthic Taxa	Density (Individuals/m^2)	Important Taxa (Ray Diet)	Rank
2007	GULF	GULF	F	Crustaceans	1475.0	Bivalves (Arcidae)	3
				Polychaeta	700.0	Crustaceans (Xanthidae)	1
				Bivalves	420.0		
2008	GULF	GULF	SU	Polychaeta	1900.0	*Mellita* sp.	N/A
						Ophiuroida	N/A
2008	PAP	EST	F	Polychaeta	264.1	Bivalves (Veneroida)	3
				Amphipods	11.7	Polychaeta	1
				Bivalves	3.2	Ophiuroida	N/A
2009	PB	BARR	W	*Haustorius* sp.	4066.7	*Haustorius* sp.	1
				Polychaeta	66.7		
				Donax sp.	50.0		
2009	PAP	EST	W	Polychaeta	207.8	Bivalves (Tellinidae)	3
				Amphipods	23.4		
				Bivalves	12.2		
2009	PB	BARR	SP	*Haustorius* sp.	406.8	Polychaeta	2
				Polychaeta	100.0		
				Donax sp.	83.3		
2009	PAP	EST	SU	Polychaeta	200.0	Bivalves (Veneroida)	3
				Amphipods	29.5	Gastropod	4
				Bivalves	5.1		
				Gastropods	2.7		
2010	WEDI	BARR	SP	*Haustorius* sp.	1008.3	*Haustorius* sp.	1
				Donax sp.	33.3	*Donax* sp.	2
				Pinnixa sp.	30.6		
2010	PD	EST	SU	*Gemma gemma*	54.8	*Gemma gemma*	1
				Polychaeta	21.6		

locations (Table 8). Bivalve predation has been demonstrated in the cownose ray throughout its Atlantic range (Smith and Merriner 1985, 1987; Blaylock 1993), however, recent studies have suggested bivalves are less important to cownose rays of the Gulf of Mexico (Collins et al. 2007a; Craig et al. 2010). We found evidence of bivalve consumption across maturity states, though bivalves were considerably more important to the diet of YOY and JUV individuals. Immature rays in the northern regions of Mobile Bay appeared to exclusively consume the bivalve *Mulinia lateralis*. Smith and Merriner (1985) also reported consumption of *M. lateralis* by mature and immature cownose rays in the Potomac River, VA (Smith and Merriner 1985). We did not record the presence of *M. lateralis* in adult ray guts, potentially due to low relative use of the upper estuaries (M.J. Ajemian, unpubl. data). Populations of *M. lateralis* are abundant and encountered year-round in northern Gulf of Mexico estuaries (Flemer et al. 1998), and are probably an important and reliable food item for faster-growing immature individuals. At these younger ages, individuals ingest larger amounts of food relative to body weight (Fig. 3). For these reasons, the estuarine regions of coastal Alabama may serve as important nursery grounds for the cownose ray.

Other bivalves known to be numerically dominant in the benthos of estuaries and bays of the northern

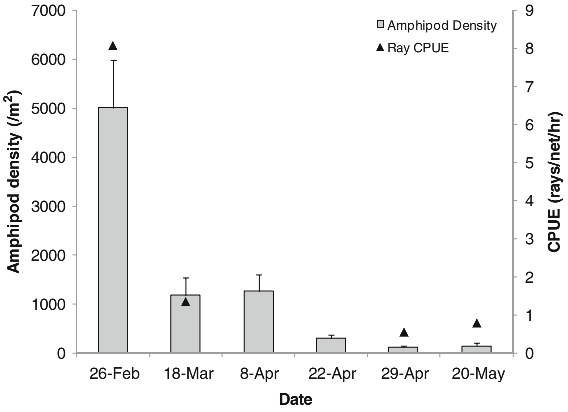

Fig. 8 Dual plot of amphipod density (*gray vertical bars*) and cownose ray average abundance (*black triangles*) over time at Pelican Island. Amphipod density scale is shown on the left y-axis and CPUE on the right y-axis

Gulf of Mexico include the tellinid *Macoma mitchelli* and the venerids *Gemma gemma* and *Anomalocardia auberiana* (Flemer et al. 1998). It is likely that these species also comprised the majority of gut contents from estuarine regions (e.g. Perdido Bay, northern Mississippi Sound) but were too pulverized for identification to lower taxonomic levels. Our diet analysis revealed that polychaetes were relatively unimportant to cownose rays feeding in these regions (IOI_{BAY}=0.00%; IOI_{EST}= 4.76%) when compared to bivalves (IOI_{BAY}=95.40%; IOI_{EST}=66.81%). Concurrent benthic sampling at Perdido Beach with cownose ray collection sites revealed high abundances of amethyst gem clams *Gemma gemma* (54.8 individuals/m^2) and lower average abundance of polychaetes (21.6 individuals/m^2) (Table 8). While the abundance of amethyst gem clams was 2.5× that of polychaetes, polychaetes were practically non-existent in cownose ray diets from this region. Thus, cownose rays may not be feeding opportunistically from inshore estuaries, despite having been proposed to by other studies of cownose ray foraging behavior (Smith and Merriner 1985; Collins et al. 2007a; Craig et al. 2010). Alternatively, rays may selectively feed on dominant bivalves, which maybe more important for faster growing YOY and juvenile rays (see above).

The prey-specific importance of haustorid amphipods in the diet of cownose rays captured off the barrier islands (e.g. Pelican Island) was supported by benthic core data from these sites (Table 8, Fig. 8). Several individuals caught in this region had diets comprised of nearly 100% haustorid amphipods, suggesting potential selection for these crustaceans. In examining stomachs of cownose rays from eelgrass (*Zostera marina*) habitat in Chesapeake Bay, Smith and Merriner (1985) also reported several amphipods. However, these authors considered amphipods as “incidentally ingested with *Mya* during feeding activity” (Smith and Merriner 1985). Heavy consumption of amphipods has also been reported for cownose rays in Charlotte Harbor, Florida (Collins et al. 2007a) and by pompano (*Trachinotus carolinus*) along northern Gulf of Mexico barrier islands, including Dauphin Island (Wheeler et al. 2002). In late winter and spring 2009, densities of *Haustorius* sp. reached a maximum coincident with an abundance peak of cownose rays to the region (Fig. 8). This peak in cownose ray densities likely represented a return from offshore waters with temperatures inshore nearing 15.5°C (M. J. Ajemian, unpubl. data). As abundant and dominant benthic macrofauna along beaches and barrier islands of the NCGOM (Rakocinski et al. 1991), haustorid amphipods likely provide an important food resource for cownose rays (particularly adults) returning to inshore waters from overwintering grounds. Moreover, the correlation between ray abundance and numerically dominant prey (i.e. *Haustorius* sp.) maybe related to a threshold density, as seen in related eagle ray *Myliobatis tenuicaudatus* (Hines et al. 1997). Ray abundance dropped nearly 10-fold concomitant with a decrease in amphipod densities (Fig. 8). Ray abundance along barrier island sand flats may thus be driven by benthic prey availability, though further simultaneous coring and diet sampling is needed to confirm this relationship.

A recent diet analysis of trawled cownose rays from offshore waters of the northwestern Gulf of Mexico found polychaetes to be the most frequently occurring prey item, as well as the most commonly occurring benthic infauna from trawl sites (Craig et al. 2010). Similarly, off the immediate coast of Alabama, polychaetes numerically dominated the benthos (Table 8). However, polychaetes made up a relatively small percentage of the dietary importance (IOI_{GULF}=3.62%) when compared to crustaceans (34.25%), bivalves (19.78%), and echinoderms (9.62%). Cownose rays along coastal and inshore waters of Alabama do not appear to exhibit a heavy reliance on polychaetes as seen in animals collected off Louisiana shelf waters. While generalist foraging may appear to characterize rays collected from GULF waters (Fig. 7), individuals still appear to avoid dominant polychaetes.

Trawl data from coastal Alabama showed that five-slotted sand dollar (*Mellita quinquiensperforata*) was the most numerically abundant epifaunal species in this area (J. Mareska, AL Marine Resources Division) during the sampling period. Off nearby NW Florida, past clam dredges and quadrat surveys have indicated *M. quinquiesperforata* to be the most common macroorganism from the surf zone to 15-m depth (Salsman and Tolbert 1965; Jolley 1972). *Mellita* sp. were relatively important to animals captured along the barrier islands and gulf sites of Alabama (Table 2) and contributed a relatively large prey-specific weight (Fig. 7). Consumption of *Mellita* sp. by cownose rays was also reported by Collins et al. (2007a) in the eastern Gulf of Mexico, and along the Outer Banks of North Carolina by Smith and Merriner (1985). Other than cownose ray, consumption of sand dollars has not been previously reported in elasmobranchs, though we also found remains of *Mellita* sp. in two benthic foraging teleosts (*Pogonias cromis* and *Sciaenops ocellatus*) and they are a preferred foraging resource for gray triggerfish (*Balistes capristus*) from offshore locales (Kurz 1995). When compared to other benthic invertebrates, echinoids are known to produce among the lowest energetic equivalents (Steimle Jr. and Terranova 1985), making their periodic and heavy consumption by cownose rays rather unexpected. However, these low energetic values of sand dollars could be matched with hyper-abundance as observed in historical benthic sampling in the northern Gulf of Mexico.

Spatial/ontogenetic differences in diet

Cownose rays off coastal Alabama displayed strong spatial and marginal ontogenetic variation in diet. Surprisingly, this is the first known diet study of *R. bonasus* where site was considered as a factor in the quantitative diet analysis. Previous diet studies of rays have lumped individuals from various sites together, or were only concerned with the ontogenetic component of diet variation (e.g. Collins et al. 2007a). Thus, it is possible that previous diet studies of myliobatid rays have confused ontogenetic variation with spatial differences, as many species of elasmobranchs are known to exhibit habitat partitioning by life stage (Grubbs 2010). For cownose rays off coastal Alabama, immature animals were more commonly caught in fresher and more turbid environments, whereas adults were more abundant along barrier islands and more saline gulf waters (M.J. Ajemian, unpubl. data). In coastal areas, diet is more diverse, evidenced by the significantly higher dispersion in dissimilarity along BARR and GULF sites (Fig. 6). These differences in habitat maybe linked to the need for immature individuals to utilize more consistently productive estuaries and bays for consumption of higher quality benthic prey (e.g. *M. lateralis*) during their peak growth (see above), or for protection against large predators more commonly encountered in offshore waters. Mature adult cownose rays, on the other hand, may afford to utilize less productive and more diverse coastal habitats with their higher mobility (Collins et al. 2007b) and larger gape. Though relatively unimportant in the overall diet, the bivalves consumed by mature individuals included larger and thicker shelled species not observed in immature guts such as the venerid clam *Macrocallista nimbosa* and ark clams (arcidae). These species are associated with higher saline waters of the northern Gulf of Mexico (Jolley 1972), and probably require considerably greater force to consume than inshore tellinid and mactrid clams. Thus, juvenile and YOY rays maybe limited to their estuarine habitat also due to their inability to excavate coarser sediments (sand, shell hash) and crush larger bivalves associated with barrier island sandflats and coastal Gulf of Mexico waters.

Across all regions, cownose ray guts were dominated by a single prey item (54.7%), potentially supporting specialization for high density prey patches (Hines et al. 1997; Peterson et al. 2001; Yamaguchi et al. 2005; Collins et al. 2007a). However, multiple prey categories were observed across the specialist space of the feeding strategy diagram (Fig. 7), suggesting cownose ray specialization on prey was variable. Individual cownose rays thus appeared to specialize on different resource types as each food category became available (Amundsen et al. 1996). The overall dietary diversity (i.e. generalism) observed in adults, on the other hand, maybe representative of greater habitat heterogeneity of larger and more mobile individuals. In conclusion, cownose ray diet from coastal Alabama represented a continuum of feeding strategies (i.e. variation along y-axis of feeding strategy diagram), perhaps exhibiting generalist foraging across all regions, but specialization when high quality resources become episodically available. We therefore propose that cownose rays as neither mollusk-specialized nor opportunistic generalists, but rather display habitat-specific foraging behaviors.

Management implications

Cownose rays off coastal Alabama were not found to consume commercially harvested eastern oyster *Crassostrea virginica*. Ray abundance was lower in oyster regions of Alabama (i.e. SW Mobile Bay) compared to other habitats during the study period, and any animals collected from these areas typically had empty guts (Fig. 1). We opportunistically sacrificed black drum *Pogonias cromis* from oyster reefs of Mobile Bay and found remains of eastern oyster in the gut contents, indicating oysters were available for consumption during the survey period. However, if live oysters were only present as shell matrices on high relief reefs, they may have only been available to more dexterous predators like black drum (Cave 1978; Sutter et al. 1988; George 2007).

We propose three possible reasons for the lack of cownose ray predation on oysters: (1) Oysters were located in unsuitable habitat (low salinity, high turbidity, high relief reef matrix) for large adults capable of crushing through shell; (2) Oyster populations were at such low densities during the study period that they were not detected by schools of rays migrating through the area; (3) Cownose rays do not preferentially consume eastern oyster, even when available. Despite the negative associations of cownose rays with eastern oyster consumption, there is little to no evidence in the scientific literature of natural predation on eastern oyster by cownose rays. Though sample sizes were relatively low (n=40), Smith and Merriner (1985) only reported a single eastern oyster consumed by cownose rays from Chesapeake Bay and was relatively unimportant when compared to other reported bivalve species in the diet (0.3% by number, 0.1% by volume). More recently, a study that examined feeding preferences in captive cownose rays found that soft-shell (*Mya aernaria*) and hard(*Mercenaria mercenaria*) clams were actually preferred over eastern oyster (Fisher et al. 2011). Thus, the reported negative impacts of cownose rays to eastern oyster seeding programs (Merriner and Smith 1979) maybe a response to the rays to artificially elevated levels of naturally less abundant resource. We suggest continued sampling of cownose rays across the inshore sector of Alabama, with an emphasis on regions where oyster restoration may involve small, single-shell seeding programs. These types of programs are probably at the highest potential risk to cownose ray impacts, especially if planting occurs during peak periods of cownose ray abundance and if densities of oyster exceed those of the most abundant (and available) bivalves or crustaceans within the region.

The diet variability in Gulf of Mexico cownose rays, likely influenced by prey availability and diversity at various locales, complicates assessing the consequences of eliminating top predators (e.g. sharks) in marine ecosystems. Most trophic cascades have demonstrated strong direct linkages between top and intermediate predators, and intermediate predators and their resources (Pace et al. 1999). Moreover, in marine ecosystems, these cascades have been disproportionately evidenced in hard substrate environments such as coral reefs and the rocky intertidal zone (Pinnegar et al. 2000). Though we demonstrated periodic specialization for certain prey types (including mollusks) by cownose rays, the inconsistent foraging across mainly unstructured bottom habitat may impede transmission of cascading effects from overexploited top predators down to benthic resources (Strong 1992; Polis and Strong 1996; Polis et al. 2000). Our study highlights the critical need for empirical dietary data prior to implementing population control measures on fishes with low productivity like cownose rays (Stevens et al. 2000). Quantitative diet studies such as the present can thus serve as an important tool to food web ecologists and managers of benthic resources.

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