HABITAT USE BY SEAHORSES AND PIPEFISHES (FAMILY SYNGNATHIDAE) IN BISCAYNE NATIONAL PARK, A MARINE PROTECTED AREA IN SOUTH FLORIDA, USA.

by

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Abstract

Seahorses and their relatives, the pipefishes, (family Syngnathidae) are a group of charismatic marine fishes found in coastal habitats including estuaries, mangroves, seagrasses and coral reefs. Knowledge of habitat use by species of conservation concern is important when evaluating the relative contribution of a marine protected area to recovery efforts. This study presents the results of underwater visual surveys of broadly-defined habitats (continuous Submerged Rooted Vegetation (SRV), discontinuous SRV, and reefs) conducted in Biscayne National Park (BNP), a 720 km^2 marine protected area in Florida, USA. Syngnathids were more likely to be found inside the sheltered waters of Biscayne Bay at sites characterized by fine sediment, reduced horizonal visibility, 30-70% seagrass cover (predominantly *Thalassia testudinum*) and lower % coverage of reef-associated benthic invertebrates (sponges, corals, gorgonians) and turf algae. The most abundant syngnathids in BNP were the Dwarf Seahorse (*Hippocampus zosterae*), the Gulf Pipefish (*Syngnathus scovelli*), and the Dusky Pipefish (*S. floridae*). Large seahorses (*Hippocampus erectus* and *H. reidi*) were poorly represented in my surveys. Syngnathid species assemblage varied by major habitat type, however only *Syngnathus floridae* was significantly more likely to be found in continuous SRV habitats. Discriminant function analysis (DFA) revealed that relative to habitats occupied by *H. zosterae* and S. *scovelli*, those occupied by *S. floridae* had higher % coverage *Thalassia*, and higher salinity. The analysis further revealed that habitats occupied by *H. zosterae* are associated with relatively deeper sediments, lower % coverage of sponges, and higher % cover drift algae compared to habitats used by *S. scovelli*. Sediment type emerged as the most important predictor of occurrence for *H. zosterae*, *S. scovelli*, and syngnathids generally and is an important parameter to consider for conservation and management of syngnathid habitat. It is likely that the sheltered waters of Biscayne Bay provide

important habitat for syngnathids within BNP, but also that Biscayne Bay is exposed to greater environmental stressors resulting from its proximity to the mainland and the effects commercial bait-shrimp trawling. Implementation of the no-trawl-zone proposed in the 2014 Fisheries Management Plan for Biscayne National Park and improving water quality would benefit syngnathid habitat.

Lay Summary

Biscayne National Park (BNP) protects a unique combination of habitats including mangrove coastlines, shallow, clear-water bays, seagrass meadows, and coral reefs. My research team and I surveyed 79 sites throughout BNP and found eight species (two seahorses and six pipefishes) in the park. The most common species were the Dwarf Seahorse (*Hippocampus zosterae*), the Gulf Pipefish (*Syngnathus scovelli*) and the Dusky Pipefish (*S. floridae*). Large seahorses were poorly represented. As a group, seahorses and pipefishes were more likely to be found in sheltered, shallow-water habitats with fine sediment and 30-70% seagrass cover. The habitats used by the three most common species differ by seagrass coverage, sediment depth, salinity, and coverage of sponges and drift algae. Sediment type strongly predicts the occurrence of seahorses and pipefishes generally. Seahorse and pipefish habitat is affected by habitat degradation. Improvements in water quality and a no-trawl zone in BNP would likely improve habitat.

Preface

All work presented in the current thesis is original work. Chapter 2 was planned and designed by Emilie Stump within the Project Seahorse research program led by Dr. Amanda Vincent, Principal Investigator. Additional mentorship was provided by Dr. Lindsay Aylesworth. Field work was performed by Emilie Stump with assistance from Jane Carrick, Cate Gelston, Rachel Plunkett, and Chelsea Bennice. Logistic support regarding project finances was provided by Scott Finestone. Analysis of data was performed under the supervision of Dr. Sally Otto and Dr. Jordan Rosenfeld, with additional support provided by scientists at the National Marine Fisheries Service Southeast Fisheries Science Center, Protected Species Unit, including Dr. Joan Browder, Dr. Ian Zink, and, Dr. Joe Serafy.

Work with live animals adhered to Animal Ethics Protocol A12-0288: Creating Momentum for Global Seahorse Populations.

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f = frequency of occurrence

List of Abbreviations

BNP – Biscayne National Park

CBD – Center for Biological Diversity

CPCe – Coral Point Count with Excel

CPUE – catch per-unit effort

cm – centimeters

ESA – Endangered Species Act

FFWCC – Florida Fish and Wildlife Conservation Commission

GIS – Geographic Information System

GPS – Global Positioning System

IUCN – International Union for the Conservation of Nature

m – meters

MPA – Marine Protected Area

PVC – Polyvinyl chloride

ppt – parts per thousand

SCUBA – Self Contained Underwater Breathing Apparatus

SCHEME – System of Classification of Habitats in Estuarine and Marine Environments

SD – standard deviation

SRV – Submerged Rooted Vegetation

Acknowledgements

I would like to acknowledge Dr. Amanda Vincent, upon whose work this project builds, who supervised the initial two years of my MSc. Dr. Vincent taught me the importance of sharing science beyond the walls of academia and for pushing the boundaries of what I thought I could accomplish. In addition to Dr. Vincent, I would like to acknowledge the staff of Project Seahorse, Scott Finestone, Regina Bestbier, and Lily Stanton, for their collaboration on "Seahorses: Magical Creatures in our Backyard", a conservation outreach campaign created on behalf of Biscayne National Park, and for additional logistic and communications support.

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I would also like the thank the Vancouver Ultimate League for making me a part of their community for one year and giving me a healthy outlet during times of stress.

Dedication

I would like to dedicate this thesis to the millions of public servants who enable the scientific enterprise to exist. I hope that the work I do now and in the future honors the sacrifices you have made and helps to build a more just, balanced and equitable world.

I would also like to dedicate this thesis to my mother and father, who taught me the importance of commitment, honor, hard work, perseverance, and integrity, my sister, the human being I am most proud of in all the world, and my two nieces and nephew growing up in Virginia.

Chapter 1: Introduction

1.1 Habitat use by fishes

Habitat use by fishes is influenced by several factors including the degree of habitat structural complexity, the level of interspecific competition and the perceived risk of predation (Werner and Hall, 1979; Savino and Stein, 1989; Utne *et al.* 1993; Jordan *et al.* 1998; Munday *et al.* 2001; Schofield, 2003). Habitat complexity plays a key role in providing refuges from predators, moderating physical disturbance, and mediating competitive interactions through niche partitioning (Flynn and Ritz 1999; Morberg and Folke 1999; Werner 1979). Basic habitat attributes, like water depth, velocity, and clarity influence prey abundance as well as foraging strategy of predators (Dill 1983). For instance, fish are often strongly adapted to foraging in either sheltered environments with low velocities, or habitats with strong currents and wave action (Harding *et al.* 1998). While habitat use by commercial species is relatively well researched, for many marine fishes only general, descriptive knowledge of habitat use is available. For threatened fish species, gathering and analyzing habitat use in a quantitative manner is important to guide conservation efforts.

1.2 The family Syngnathidae

Seahorses and their relatives, the pipefishes, sea dragons, and pipehorses (Syngnathidae) are ecologically, economically, medicinally and culturally important in many regions (Ahnesjö and Craig 2011; Vincent *et al.* 2011) and are widely studied. Syngnathids are predominantly distributed in temperate, sub-tropical and tropical coastal waters, with a distribution from about 71°N to 56°S, and they inhabit a wide variety of predominantly marine habitats (Dawson 1985). Many species occur in shallow coastal habitats, particularly in seagrass beds but also among corals, macroalgae, mangrove roots, estuaries, or lagoons (Dawson 1985; Lourie 2004). They often mimic vegetation in color, shape and behavior (Howard and Koehn 1985; Kendrick and Hyndes 2003) and are important predators of mobile benthic invertebrates (Tipton and Bell 1988; Bologna, 2007). Due to their presence in coastal areas subject to human activity, syngnathid populations are experiencing anthropogenic stressors throughout much of their respective ranges.

1.2.1 Anthropogenic stresses to syngnathid populations

Syngnathid populations are subject to direct harvest (Vincent 1996), the lethal and sub-lethal effects of incidental harvest (bycatch; Baum *et al.* 2003), and habitat changes including degraded physical habitat structure and water quality. Physical damage to habitat can be caused by dredging, siltation, and loss of vegetation or benthic invertebrates, often related to activities such as port development (Masonjones *et al.* 2010) or recreational boating (Bell *et al.* 2002). Water quality can include changes in chemistry (hypoxia, altered pH, eutrophication caused by excessive nutrients) or clarity of the water associated with suspended particulates or eutrophication (Lotze *et al.* 2006). These chemical and clarity alterations can also lead to loss of vegetation or benthic invertebrates (Burkholder *et al.* 2007) or may simply be outside the range of tolerance of some syngnathids, causing stress, disease, or mortality (Ripley and Foran 2007). Many syngnathids are dependent on the most threatened of marine habitats including mangroves (Polidoro *et al.* 2010), seagrasses (Short *et al.* 2011), coral reefs (Carpenter *et al.* 2008), and estuaries (Blaber *et al.* 2000). As such, many syngnathids are listed under national or regional endangered species legislation in many countries (examples in Vincent *et al.* 2011).

1.2.2 Regulatory and legislative protections for syngnathid fishes

As species of conservation concern, syngnathids, especially seahorses, are subject to varied protections from a suite of regulatory measures including trade legislation, fishery-specific management plans, dedicated protected areas, endangered species legislation, and the tangential benefits of occurring within larger Marine Protected Areas (MPAs), which conserve essential habitat. Marine Protected Areas are one among a suite of management tools used for the conservation of marine habitats and vulnerable marine species (Juffe-Bignoli *et al.* 2014). By limiting human activities such as fishing, development, and resource extraction, MPAs have demonstrated efficacy in restoring benthic habitats (Pandolfi *et al.* 2003; Mumby *et al.* 2007; Diaz-Pulido *et al.* 2009; Mumby and Harbone, 2010) and fisheries (e.g. Polunin and Roberts, 1993; Mosquera *et al.* 2000; McClanahan and Arthur, 2001; Halpern, 2003; Aburto-Oropeza *et al.* 2011; Chirico *et al.* 2017).

1.2.2.1 MPAs as tools for conservation of syngnathid habitats

Knowledge of habitat use by species of conservation concern is important when evaluating the relative contribution of a MPA to recovery efforts and to guide further population monitoring. For instance, field studies have shown that many syngnathids are consistently associated with specific habitats, or exhibit preferences for specific locations in the landscape (Smith *et al.* 2008; Malavasi *et al.* 2007; Diaz-Ruiz *et al.* 2000; Bell *et al.* 2002; Dias and Rosa 2003; Moreau and Vincent 2004). Placement of MPAs with the goal of maximizing fish biodiversity may also be informed by considering syngnathids, as the diversity and density of syngnathid fishes may be indicative of larger ecosystem health in seagrass and estuarine systems (Shokri *et al.* 2008).

1.3 Project and site description

This thesis investigates the distribution and habitat use of syngnathid fishes of Biscayne National Park, a MPA in Florida, with the goal of better understanding habitat use, contribution to local biodiversity, and potential sensitivity to habitat change. Biscayne National Park (BNP) is a 728km^2 predominantly marine U.S. National Park and MPA located off the coast of Miami-Dade County, southeastern Florida (Figure 1).

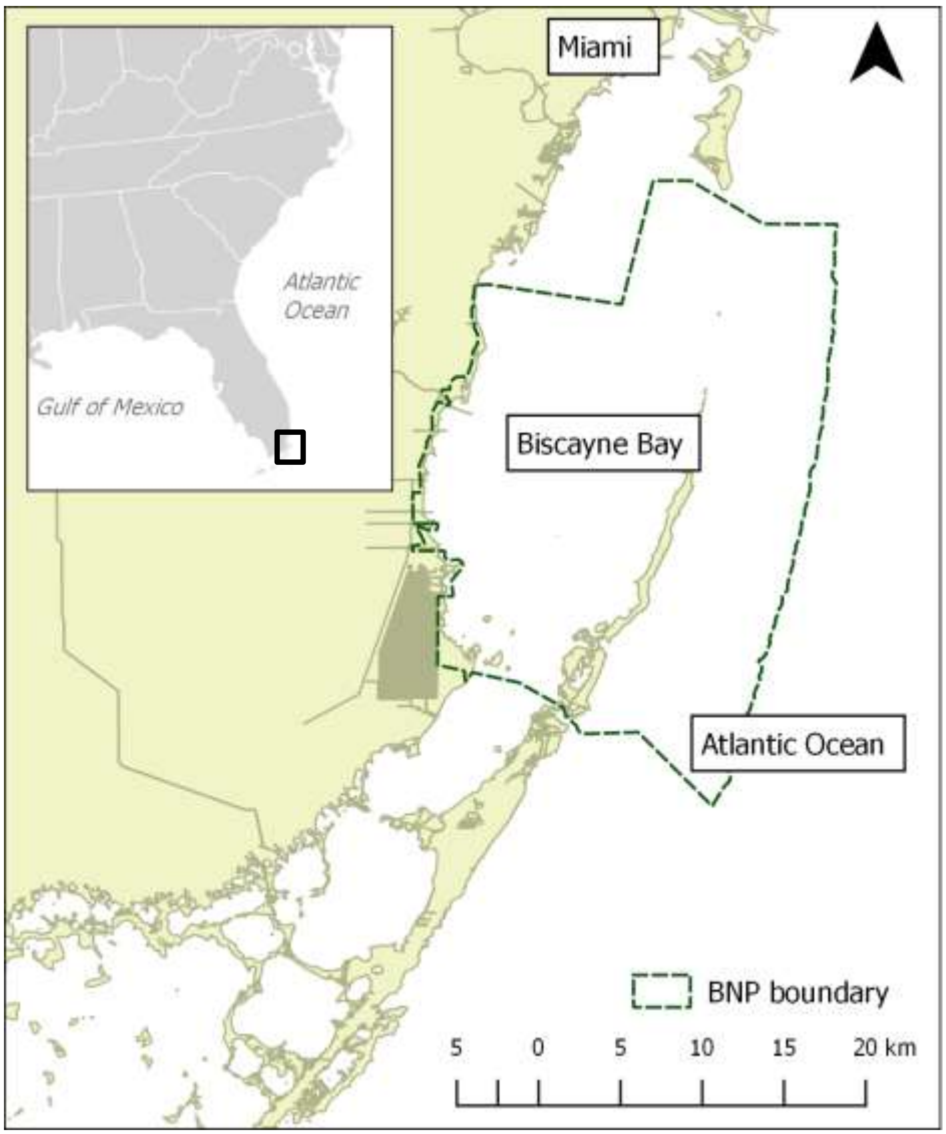

Figure 1. Biscayne National Park in southeastern Florida, USA, south of Miami.

This MPA preserves the longest remaining continuous stretch of mangrove on the eastern coast of Florida, extensive seagrass meadows in the southern portion of Biscayne Bay, and adjacent coral reefs at the northern extent of the Florida Keys (Ault *et al.* 2001), all of which are potential habitats for syngnathid fishes.

1.3.1.1 Syngnathids of Biscayne National Park

Fifteen species of syngnathids have been recorded in Biscayne National Park (Appendix A, Table S1), including three species of conservation concern (Table 1; Ault *et al.* 2001).

Table 1. Syngnathids of conservation concern in Biscayne National Park with conservation status under the International Union for the Conservation of Nature (IUCN) and the United States Endangered Species Act (ESA).

Common name	Scientific Name	Habitat	Conservation status
Lined Seahorse	*Hippocampus erectus*	seagrass, mangrove, sponges, sargassum, estuaries, saltmarshes	IUCN Vulnerable
Longsnout seahorse	*Hippocampus reidi*	seagrass, coral, mangrove, sargassum	IUCN Near Threatened
Dwarf Seahorse	*Hippocampus zosterae*	seagrass	ESA – ongoing status review Recent de-listing

1.3.1.2 Project Objectives

The specific objectives of this study were: 1) to assess the relative abundance of different syngnathid species in BNP and compare it to previous studies in BNP; 2) to determine the distribution and habitat associations of syngnathids with respect to major habitat types and environmental gradients; and 3) to determine the environmental variables that best discriminate habitat use among syngnathid species, with the goal of developing predictive models for habitat management. My results are interpreted in the context of information needs and challenges to identifying, managing, and protecting syngnathid habitat within a conservation area that is influenced by a myriad of internal and external environmental stressors (Browder *et al.* 2005), including commercial trawling and ongoing urbanization of the surrounding landscape.

Chapter 2: Habitat use by Seahorses and Pipefishes of Biscayne National Park

2.1 Introduction

Habitat use by fishes is influenced by several factors including the degree of habitat structural complexity, the level of interspecific competition and the perceived risk of predation (Werner and Hall, 1979; Savino and Stein, 1989; Utne *et al.* 1993; Jordan *et al.* 1998; Munday *et al.* 2001; Schofield, 2003). Habitat complexity plays a key role in providing refuges from predators, moderating physical disturbance, and mediating competitive interactions through niche partitioning (Flynn and Ritz 1999; Morberg and Folke 1999; Werner 1979). For many marine fishes only general, descriptive knowledge of habitat use is available. For fishes of conservation concern gathering habitat use data and providing quantitative descriptions and predictive tools is important to guide conservation efforts. This study explores habitat use by seahorses and pipefishes (family Syngnathidae) in Biscayne National Park, a 728km^2 predominantly marine U.S. National Park and MPA located off the coast of Miami-Dade County, southeastern Florida. Syngnathids are a group of predominantly marine fishes which are found in coastal habitats such as corals, macroalgae, mangrove roots, estuaries, or lagoons (Dawson 1985; Lourie 2004). Due to their presence in coastal areas subject to human activity, syngnathid populations are experiencing anthropogenic stressors in parts of their respective ranges. The methods outline protocols for survey methodology and data analysis (non-parametric multi-dimensional scaling, discriminant function analysis, logistic regression, bycatch estimation). Results include the characterization of major habitat types in BNP, relative abundance of syngnathid species, syngnathid distribution and environmental use, descriptive analysis of occupied habitats for three relatively abundant species (*Syngnathus scovelli*, *S. floridae*, and *H. zosterae*), and single-variable predictive models of occurrence. These results are discussed in the context of the three principal objectives of this study: 1) to assess the relative abundance of different syngnathid species in BNP; 2) to determine distribution and habitat use in syngnathids with respect to major habitat types and environmental gradients; and 3) to determine the environmental variables that best discriminate habitat use among syngnathid species, with the goal of developing predictive occupancy models for habitat management.

2.2 Methods

2.2.1 Survey and site characterization

2.2.1.1 Determination of survey sites

Survey sites were selected using random sampling stratified by major habitat type. A total of 79 sites were surveyed from May–September 2016. The open-source Geographic Information System (GIS) platform QGIS (QGIS development team 2016) was used to overlay random points atop habitat layers available from the United Florida Reef Tract Map (Florida Fish and Wildlife Conservation Commission-Fish and Wildlife Research Institute, St Petersburg, Florida). Habitat was stratified using three broad habitat types previously mapped in BNP: Continuous Submerged Rooted Vegetation (SRV), discontinuous SRV, and reef/hardbottom (Madley *et al.* 2002). These habitats differ primarily in terms of substrate and the extent of vegetative coverage. Continuous SRV was defined by Madley *et al.* as the presence of continuous beds of any shoot density that cover 10-100% of the substrate, while Discontinuous SRV was defined as areas of rooted vegetation with breaks in coverage. Reef/hardbottom habitats were characterized by hardened substrate of unspecified relief formed by exposed bedrock with variable coverage associated with benthic plants or animals, or by reefs created by the bio-deposition of calcium carbonate (e.g. coral)(Madley *et al.* 2002; Estep *et al.* 2017). For more detailed description of categories, please see (Madley *et al*. 2002). My surveys aimed for a representation of 50% of sites in Continuous SRV, 40% of sites in Discontinuous SRV, and 10% of sites in Reef/Hardbottom habitat (Figure 2, reproduced from figure 4b in Ault *et al.* 2001), which roughly matches the relative area of these habitat types within BNP. To confirm the original map-based habitat classifications, *in situ* classifications into the three major habitat categories were also made by observers in the field.

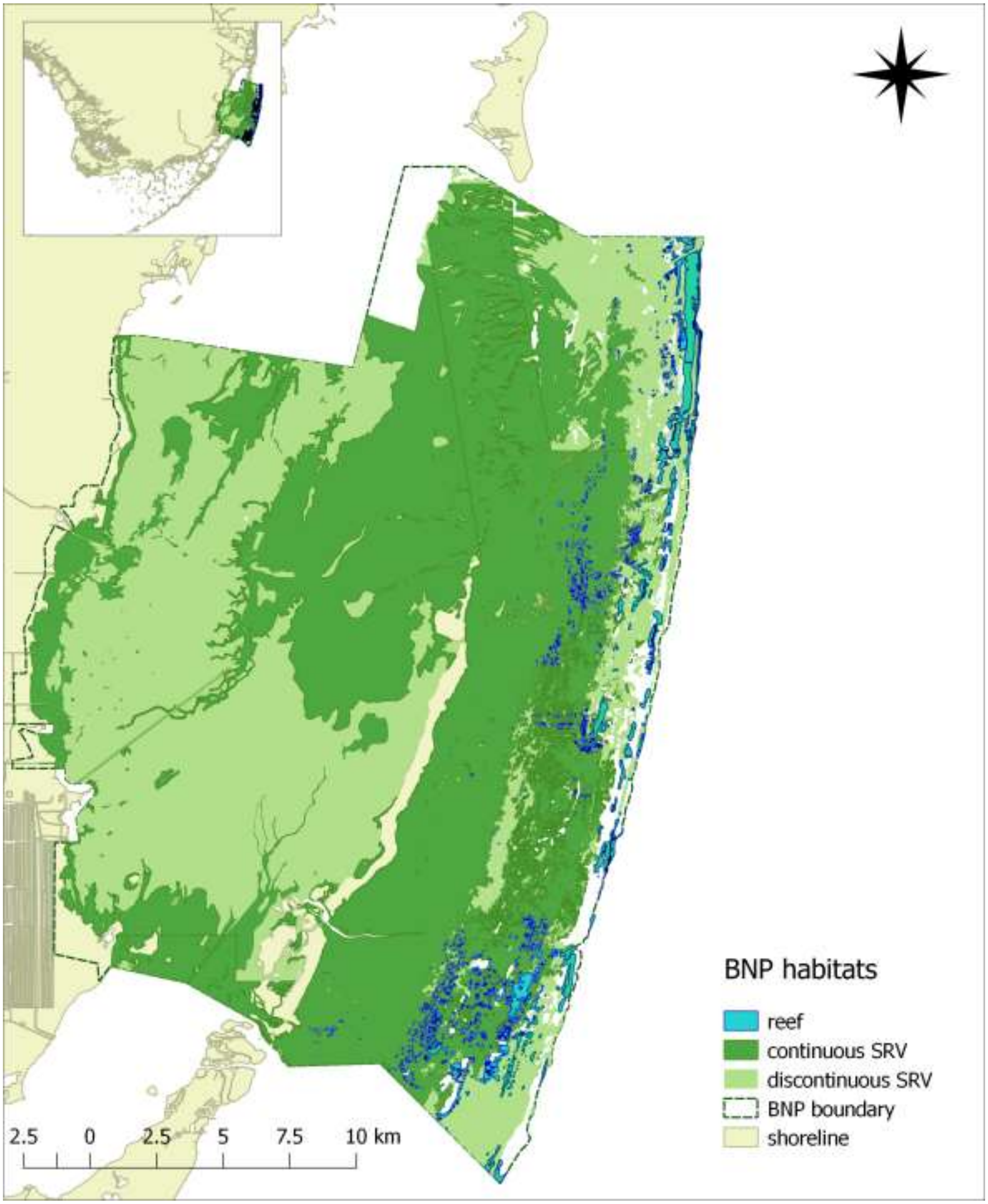

Figure 2. Map of Biscayne National Park showing major habitat types. GIS habitat layers created by Florida Fish and Wildlife Conservation Commission Fish and Wildlife Research Institute. Discontinuous SRV includes map class "unconsolidated sediment".

2.2.1.2 Survey methodology

The team performed 70-minute timed swims at each site. Paired SCUBA divers conducted four non-overlapping 20-minute timed swims in each of four cardinal directions originating from a central node. The last of these four directions was always with the current and shortened to 10 minutes to attempt to standardize the distance travelled for all timed swims. Search time was constrained to 70 minutes per site to fully sample sites at varying depths without limiting search time at deeper depths due to air restrictions at depth. This search plan was modified for patch reefs to accommodate restricted size and/or non-uniform dimensions of the patches. The length of each replicate transect was recorded by towing a GPS unit behind one of the paired divers. Variation in distance is attributable to several environmental factors including current velocity, rugosity, and habitat type. Tracks from GPS indicated the mean total distance travelled per site was 417 m (SD+/-171 m). Distance travelled for non-reef sites was 399 m (SD +/- 166 m). Distance travelled per site was highest for reef sites (mean = 525+/- 165 m), which are generally subject to strong oceanic currents. Effective lateral visual search area for each diver varied due to habitat type and conditions, and is estimated at to 0.5 m.

2.2.1.3 Fish counts and observations

Timed swims were paused when a syngnathid was encountered. Syngnathids were video-recorded before attempting capture to verify species identity and estimate size. If capture was successful, syngnathids were measured following methods outlined in Lourie *et al.* (2004) and Dawson (1985).

2.2.1.4 In-situ habitat characterization

Substrate characteristics and habitats were classified in the field by E.S. using the System of Classification of Habitats in Estuarine and Marine Environments described in Madley *et al.* (2002). Habitat characters measured include depth (m), salinity (ppt), horizontal visibility (m), sediment depth (cm), dominant seagrass species (if applicable), and blade length (cm). Sediment type was estimated at each site and assigned a qualitative descriptor based on categories modified from Madley *et al.* (2002) (Appendix B, Table S2). Water samples were collected at depth near the substrate and brought to the surface, where salinity was then measured using a refractometer. Horizontal visibility was measured as the horizontal distance in meters at which a Secchi disk, towed by one of the divers, was no longer visible to a stationary diver. Sediment depth was measured as the depth in centimeters to which a 1” diameter PVC pipe could be pressed into the substrate. Percent coverage of different substrate types per site was calculated using data from eight approximately 1m^2 photographic quadrats taken at each site approximately 1 m above the substrate.

2.2.2 Data analysis

2.2.2.1 Video and image processing and estimation of percent coverage of substrate

Digital photographs were analyzed using the random point count image analysis software (CPCe “Coral Point Count with Excel” v.3.4) to determine percent composition of habitat-forming components of the benthos. Twenty-five points were randomly overlaid on each photograph, and the underlying feature was classified. The proportion of points falling on each habitat category was divided by the total number of valid points to generate percent coverage data for each habitat feature. Photoquadrats were also used to verify in-situ major habitat and substrate designations.

2.2.2.2 Nonparametric tests comparing habitat types and occupied vs. unoccupied sites

All statistical analyses were performed in XLStat statistical software (XLStat 2017). Statistical analyses were performed with a Type 1 error criterion of $\alpha = 0.05$. Nonparametric tests were used because normality and equality of variance assumptions necessary for parametric tests were usually violated. The Mann-Whitney U Test was used to examine differences in the means of measured variables sub-sampled by location (inside and outside Biscayne Bay), major habitat type (continuous SRV, discontinuous SRV, and reef sites), and occupancy status (presence/absence).

2.2.2.3 Discriminant function analysis to differentiate habitat use of syngnathid species

For three species (*S. scovelli*, *H. zosterae* and *S. floridae*) enough individuals were sampled to compare their habitat use. A descriptive discriminant function analysis was performed to investigate how habitats used by the three species (*S. scovelli*, *H. zosterae* and *S. floridae*) differ at the macrohabitat scale. Discriminant function analysis is a descriptive and classificatory technique developed by R.A. Fisher in 1936 to describe characteristics that contribute most to the distinction amongst a priori defined groups (three syngnathid species in the current context). Brown and Wicker (2000) recommend that the total sample size should be at least ten times the number of discriminator variables. While observations need not be distributed evenly across the groups, Brown and Wicker (2000) further recommend that the number of cases in each group be at least equal to the number of variables. The total sample size for the development of the discriminant functions was 54 observations (N= 19, 14, and 21 observations for *H. zosterae*, *S. floridae* and *S. scovelli*, respectively), and Itherefore selected a maximum of 5 discriminator variables (see below). Quadratic rather than linear equations were used to account for inequality of the covariance matrices (Box's statistic, $p<0.0001$).

2.2.2.3.1 Variable selection

Consultation with regional experts with extensive field experience in South Florida were considered to select variables with the greatest potential to distinguish among the three species. Variables selected were salinity, sediment depth (cm), % coverage *Thalassia* (%*Thalassia*), % coverage sponges, and % coverage drift algae (Appendix B, Table S3).

Salinity, % *Thalassia*, and sediment depth exhibited pairwise correlations somewhat greater than 0.3 (range of 0.07-0.44; Appendix C, Table S6), which may hamper model fitting because highly intercorrelated variables are likely to load on the same function and thus not contribute significantly in a unique way to group discrimination (Brown and Wicker 2000). However, each variable was retained for the following reasons: Salinity was retained for the analysis because previous work has suggested this variable drives fish assemblage structure in the Biscayne Bay system (Serafy *et al*. 1997). The percent coverage of *Thalassia* (%*Thalassia*) was retained because habitat partitioning in some syngnathids is thought to occur along gradients of seagrass coverage (Curtis and Vincent 2005; Franco *et al.* 2006; Malavasi *et al.* 2007). Sediment depth can be an indicator of current velocity, which may differentiate habitats occupied by species exhibiting relatively high mobility from those with low mobility (Masonjones *et al.* 2010). Finally, drift algae was selected due to its potential role as a method of dispersal for *H. zosterae* (Fedrizzi *et al.* 2015; Masonjones *et al.* 2010). Finally, the maximum collinearity among variables of 0.44 is also relatively low and would not be expected to seriously bias the analysis.

2.2.2.3.2 Model validation and cross-validation

I performed a cross-validation of the discriminant functions using the hold-out method (Brown and Wicker 2000). Two-thirds of the observations were used as a "developmental sample" for fitting the model, and the remaining one third as a "cross-validation" sample for testing the model fit. The functions derived from the developmental sample were then used to classify observations in the cross-validation sample. My analysis correctly classified 57% of 54 cases in the development sample, and 46% of 26 cases in the cross-validation sample. Validation results of the developmental and cross-validation sample are available in Appendix C (Table S6 and S7).

2.2.2.4 Logistic regression

As an alternative approach to a discriminant function analysis I used logistic regression to quantify the relationship between occupancy and select environmental variables. Logistic regression is a simple way of modelling animal distribution and is used when the dependent variable follows a binomial distribution (e.g., presence vs. absence at a site).I used the logit model (Equation 1) to link probability of presence to the explanatory variables.

$$p = \frac{\exp(\beta X)}{1 + \exp(\beta X)}$$

Equation 1. logit function

2.3 Results

2.3.1 Characteristics of major habitat types in BNP

Based on *in situ* assessment, 38 sites were classified continuous SRV, 28 sites as discontinuous SRV, and 13 sites were classified as reef/hardbottom. Reef sites were unique in exhibiting significantly deeper depths, lower % coverage *Thalassia*, lower % coverage drift algae and higher % coverage turf algae and benthic invertebrates (corals, gorgonians, and sponges). Reef sites differed from discontinuous SRV, but not continuous SRV, in having significantly higher salinity and lower % coverage of macroalgae (Table 2).

Continuous SRV and discontinuous SRV sites exhibited some significant differences in measured environmental variables. Continuous SRV sites had higher salinity, greater horizontal visibility, longer blade lengths, higher % coverage of *Syringodium*, higher % coverage of *Thalassia*, and lower percent coverage of macroalgae. The mean number of syngnathid species recorded per site did not differ significantly between continuous SRV and discontinuous SRV habitats (Table 2).

Table 2. Mean biotic and abiotic habitat characteristics in continuous SRV, discontinuous SRV, and reef habitats. Values accompanied by different superscript letters differ significantly from one another in a row (Mann-Whitney U-test, $p<0.05$).

Variables	Continuous SRV	Discontinuous SRV	Reef
depth (m)	3.28[a]	3.23[a]	6.33[b]
salinity (ppt)	27.97[a]	25.92[b]	28.22[a]
horizontal visibility (m)	10.05 [a]	8.18 [b]	14.80[c]
sediment depth (cm)	12.23	8.91	7.00
blade length (cm)	26.03[a]	19.54[b]	NA
% *Halodule*	0.25	0.02	NA
% *Syringodium*	7.25[a]	2.41[b]	NA
% *Thalassia*	64.28[a]	32.36[b]	0.24[c]

% Drift algae	2.81[a]	1.02[b]	0.35[c]
% macroalgae	2.75[a]	8.89[b]	3.40[a]
% turf algae	0.28[a]	0.47[a]	13.63[b]
% scleractinians	0.04[a]	0.05[a]	0.55[b]
% sponge	0.20[a]	0.57[a]	3.58[b]
% gorgonian	0.08[a]	0.83[a]	22.44[b]
% coral	0.06[a]	0.09[a]	3.46[b]
mean # species/site	1.50[a]	1.44[a]	0.00[b]

2.3.2 Characteristics of sites inside and outside of Biscayne Bay

Of the 79 surveyed sites, 38 were located inside Biscayne Bay, and 41 were located outside of Biscayne Bay. Two sites were sampled slightly outside of BNP but were retained for analysis. Sites inside Biscayne Bay were significantly shallower, had reduced horizontal visibility, and lower salinities (Table 3).

Table 3. Mean abiotic variable measurements at sites inside and outside Biscayne Bay. Values in bold indicate significant difference in the distribution of measurements associated with the respective variable (Mann-Whitney U-test, p<0.05).

	salinity	SD salinity	horizontal visibility (m)	sediment depth (cm)	blade length (cm)	depth (m)
in	**26.30**	2.89	**8.62**	11.57	22.58	**3.06**
out	**28.00**	1.80	**11.36**	8.36	24.14	**4.50**

Sites inside Biscayne Bay exhibited significantly higher mean % coverage of macroalgae (8.2% vs 2.0%; two-tailed Mann-Whitney U-test, p=0.03), lower percent coverage of turf algae (0.06% vs 8.16%; two-tailed Mann-Whitney U-test, p=<0.0001), corals (0.06% vs 1.18% two-tailed Mann-Whitney U-test; p=0.001), and gorgonians (0.42% vs 7.65%; two-tailed Mann-Whitney U-test, p=0.007). Although there was also a difference in scleractinian corals, the difference was not significant (0.00% vs 0.24%, two-tailed Mann-Whitney U-test, p=0.17). Sites within the Bay were more likely to be characterized by fine rather than coarse sediments (Fisher's exact tests, $p<0.05$) and exhibited greater variation in salinity as evidenced by higher standard deviation in salinity measurements.

2.3.3 Characteristics of occupied sites

Forty-nine of 79 sites (62%) were occupied by one or more species of syngnathid during the study period. Sites occupied by syngnathids were significantly more likely to exhibit shallower depths and reduced horizontal visibility (Table 4).

Table 4. Mean abiotic variable measurements at sites occupied and unoccupied by syngnathids. Values in bold indicate significant difference in the distribution of measurements associated with the respective variable (Mann-Whitney U-test, $p<0.05$).

	salinity	depth (m)	horizontal visibility (m)	sediment depth (cm)	blade length (cm)
occupied	27.43	**3.12**	**8.49**	10.78	23.94
unoccupied	27	**4.94**	**13.08**	9.10	21.25

Additionally, sites occupied by syngnathids exhibited significantly higher % coverage of *Thalassia*, and significantly lower percent coverage of turf algae and benthic invertebrates such as sponges, corals, and gorgonians (Figure 3).

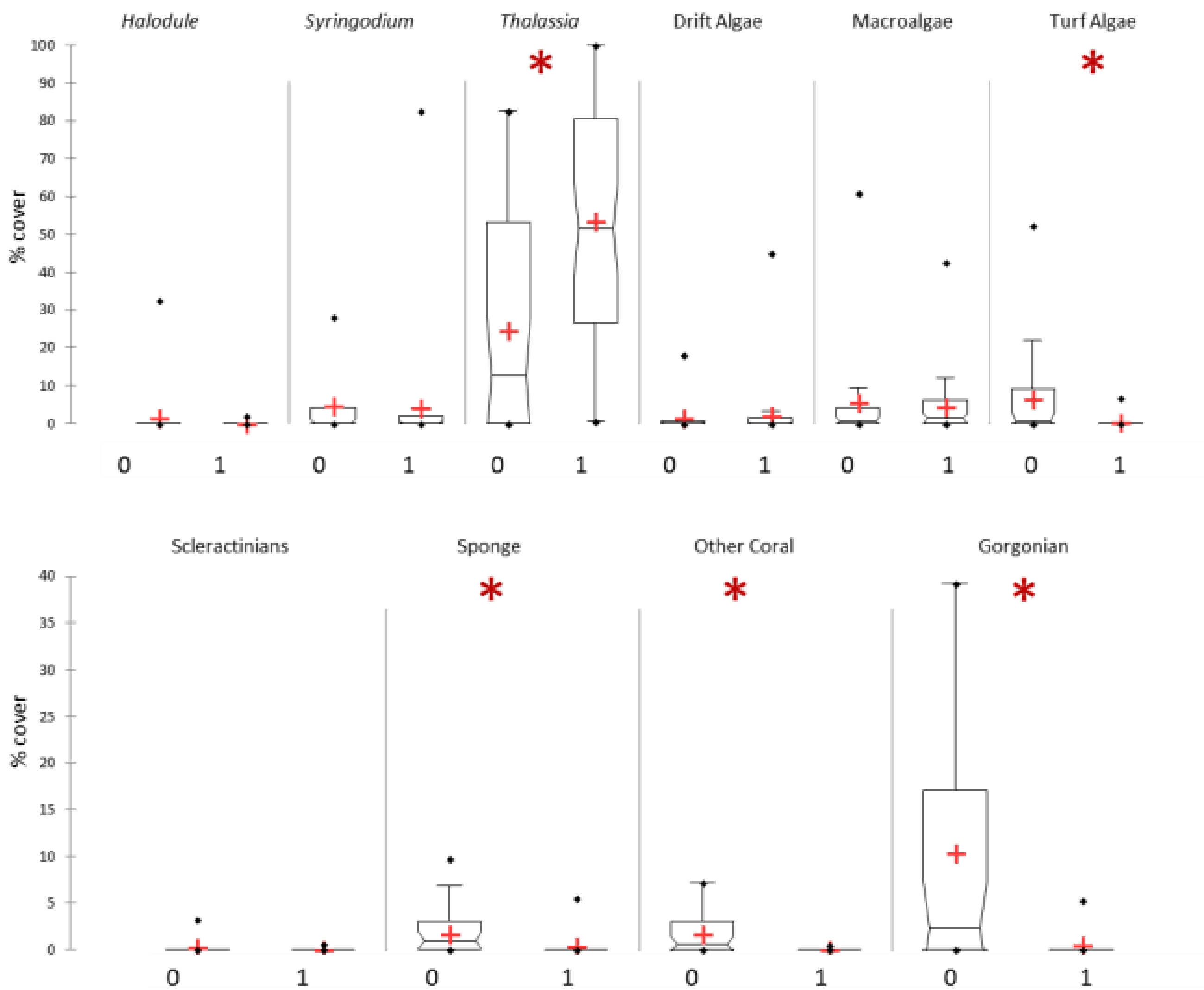

Figure 3. Boxplots of percent coverage at sites occupied (1) and unoccupied (0) by syngnathids. Red cross indicates the mean, notches indicate the median, the box represents the 1st to 3rd quartile range, points indicate maximum and minimum observations, and whiskers indicate 10th and 90th percentiles. A significant difference between % coverage measurements at occupied vs. unoccupied sites is indicated by an asterisk (Mann-Whitney U-test, p<0.05).

Syngnathids were 44% more likely to occupy sites located inside Biscayne Bay (Chi-square test, p=0.04)(Figure 4).

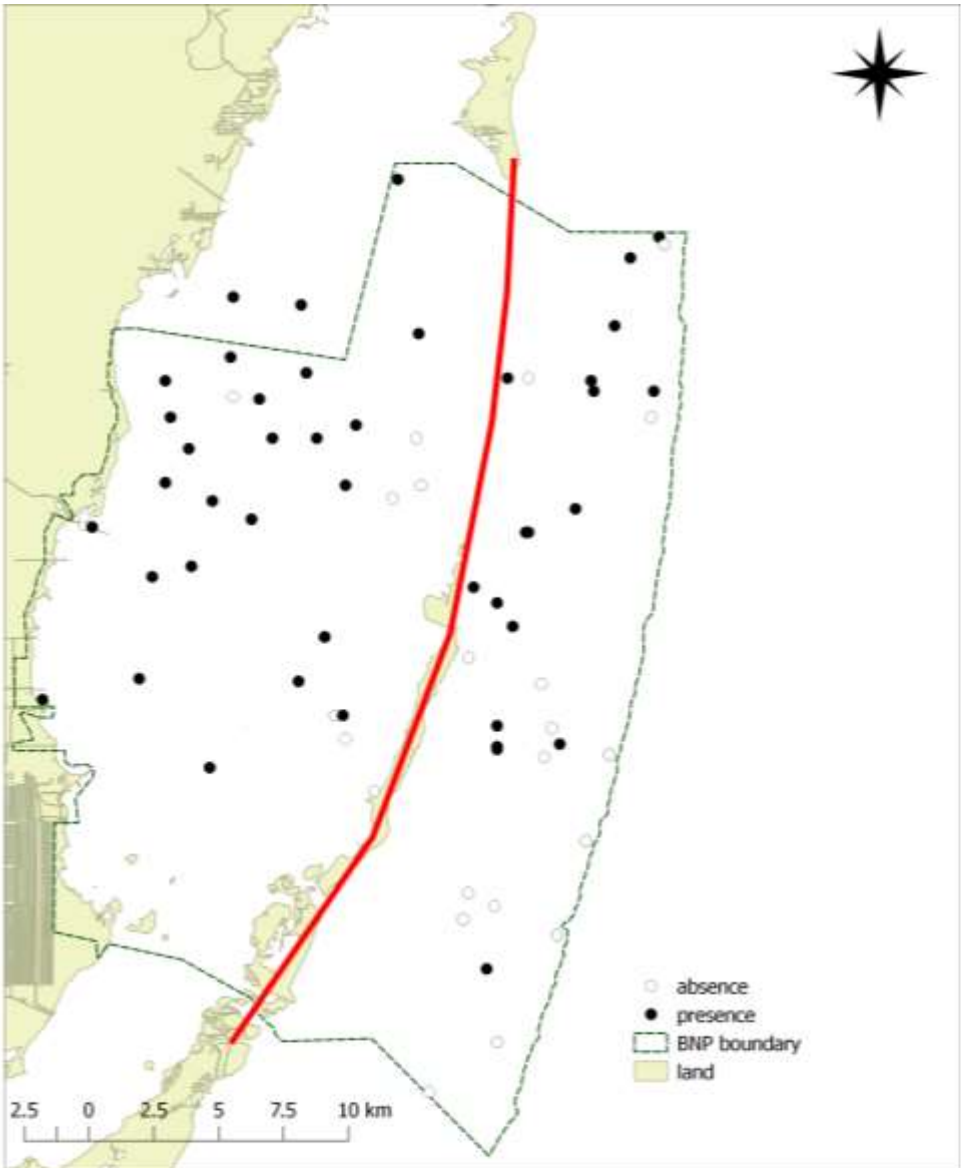

Figure 4. Map of sample sites color coded by syngnathid occupancy. The approximate outer border of Biscayne Bay is visible as the red line. Areas to the left of the line are inside Biscayne Bay.

Within Biscayne Bay the only significant difference in measured variables between sites occupied by syngnathids and unoccupied sites was higher mean % *Halodule* coverage at unoccupied sites (0.75% vs 0.077%; two-tailed Mann-Whitney U-test, $p<0.0001$). Outside of

Biscayne Bay, sites that were occupied by syngnathids tended to share characteristics with sites located inside the Bay: these occupied sites tended to be shallower, exhibit reduced horizontal visibility, greater % coverage of *Thalassia*, and reduced coverage of turf algae and benthic invertebrates (sponge, coral, gorgonians) (two-tailed Mann-Whitney U-tests, $p<0.05$) and were characterized by finer sediments, such as silt/clay and fine sand, rather than coarse sands, coral rubble, or hardbottom.

All syngnathids were observed in either continuous SRV or discontinuous SRV, and there were no syngnathids observed at reef sites. Additionally, syngnathids were more likely to be found inside Biscayne Bay, rather than outside Biscayne Bay. A two-way unbalanced ANOVA with interactions was performed to examine the relative contribution of location (inside or outside of the bay), major habitat type, and the interaction of the two to syngnathid occurrence. The model was significant ($F_4 = 0.284$, $p = 0.837$). Most of the variance in the model was attributable to major habitat category ($F_2 = 13.963$, $p<0.0001$), rather than location inside/outside the bay ($F_1= 0.024$, $p=0.878$) or their interaction ($F_1=0.377$, $p=0.542$). Model parameters indicate that of the three major habitat categories, continuous SRV and discontinuous SRV had a comparable positive influence on syngnathid presence.

The two-way unbalanced ANOVA with interactions was repeated for n=66 sites, excluding (analysis excluded reef sites) to test the hypothesis that presence/absence of syngnathids was equal inside/outside the bay and among the two major habitat categories (continuous SRV and discontinuous SRV) excluding reef sites. The model was not significant ($F_3 = 0.284$, $p = 0.837$). Neither location inside/outside the Bay ($F_1 = 0.02$, $p= 0.889$), nor major habitat category ($F_1 =$

0.316, p= 0.576), nor their interaction, (F_1 = 0.316, p = 0.567) was found to have significant effects on presence/absence of syngnathids generally.

In summary major habitat category is a stronger predictor of syngathid occurrence than location inside or outside the bay, or the interaction of major habitat category and location.

2.3.4 Relative abundance of syngnathid species

A total of 143 syngnathids were recorded during the study, and identification to species was possible for 123 individuals observed. Twenty-seven syngnathids were juveniles that could not be identified to species in the field (Table 5). The most commonly observed species, in decreasing order, were dwarf seahorse (*Hippocampus zosterae*), gulf pipefish (*Syngnathus scovelli*), and dusky pipefish (*Syngnathus floridae*).

Table 5. Relative frequency of syngnathids (number of individuals and number of occupied sites) and frequency of occurrence at 79 sites in Biscayne National Park, FL.

Species	# of individuals	# of sites	frequency of occurrence (*f*)
Hippocampus zosterae	36	22	0.28
Syngnathus scovelli	32	19	0.24
Syngnathus floridae	32	17	0.22
Anarchopterus criniger	6	4	0.05
Cosmocampus albirostris	3	3	0.04
Cosmocampus brachycephalus	6	4	0.05
Syngnathus. louisianae	4	4	0.05
Hippocampus erectus	3	1	0.01
Syngnathus pelagicus	1	1	0.01
no ID	27		

Syngnathid species composition varied by major habitat category. The most frequently recorded species in continuous SRV were *H. zosterae* (n=15 sites) and *S. floridae* (n= 14 sites), while the most frequently recorded species in the discontinuous SRV were *S. scovelli* (n=10 sites) and *H. zosterae* (n=7 sites). Occupancy by habitat category differed significantly only for *S. floridae*, which was more likely to be found in continuous SRV (present at 37% of sites) than at discontinuous SRV habitat (present at 10% of sites; Table 6, Chi-squared test, p=0.03).

Table 6. Occurrence and species composition of syngnathids identified to species at continuous and discontinuous SRV (in-situ classification) sites surveyed from May–September 2016 in Biscayne Bay, FL.

	# sites (% total)	**# occupied (% occupied)**	**#syn.**	**# species**	**species (#ind.; #sites)**
Continuous SRV	38 (48%)	27 (71%)	74	7	*H. zosterae* (23; 15) *S. floridae* (27; 14) * *S. scovelli* (10;8) *S. louisianae* (4;3) *A. criniger* (5;3) *H. erectus* (3;1) *C. albirostris* (1;1)
Discontinuous SRV	28 (35%)	22 (58%)	51	7	*S. scovelli* (22;10) *H. zosterae* (13;7) *C. brachycephalus* (6;3) *S. floridae* (5;3) *C. albirostris* (2;1) *A. criniger* (1;1) *S. louisianae* (1;1)

* Significant difference in occupancy between continuous and discontinuous SRV sites (Chi-squared test, p=0.03)

2.3.5 Differences in habitat use among syngnathid species

2.3.5.1 Discriminant function analysis

Two functions separating the habitats used by each of the three species were identified by the discriminant analysis (Table 7). Function 1 (F1) was significant (p=0.001) and explained 77% of the variance. The second function (F2) was marginally insignificant (p = 0.085) and explained 23.5% of the variance in the data (Table 7, Figure 5).

Table 7. Eigenvalues, % variance explained, canonical correlations, Barlett's statistic, and p values of discriminant functions.

Function	Eigenvalue	% variance explained	Canonical correlation	Bartlett's Statistic	p
1	0.59	76.50	0.61	30.974	0.001
2	0.18	23.49	0.39	8.181	0.085

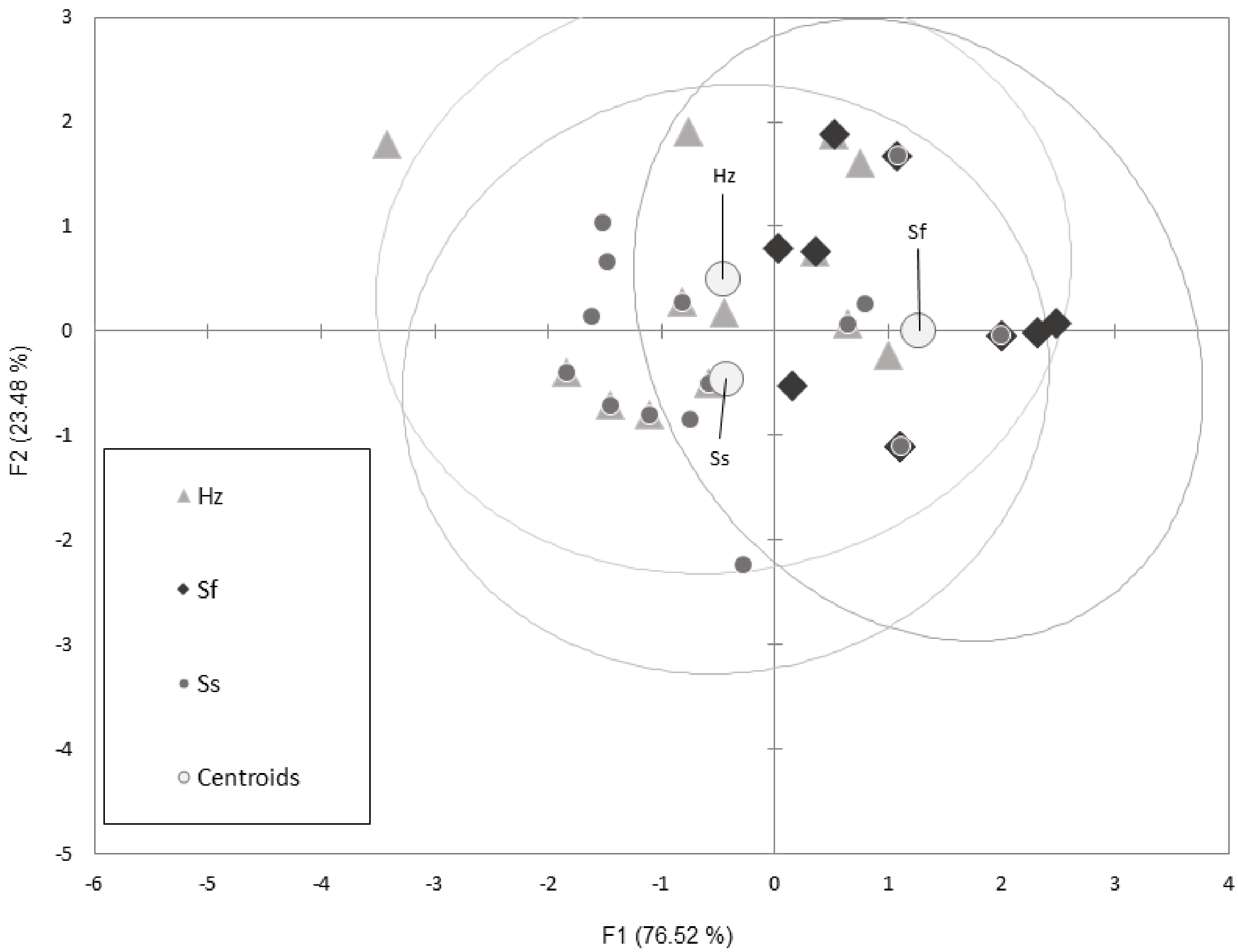

Figure 5. Biplot of quadratic discriminant analysis of habitats occupied by individuals of three species of syngnathids: *Hippocampus zosterae* (Hz), *Syngnathus floridae* (Sf), *Syngnathus scovelli* (Ss).

Group centroids indicate that F1 discriminates habitats occupied by *S. floridae* from those occupied by *H. zosterae* and *S. scovelli* (Figure 5), with *S. floridae* scoring towards the positive end of the spectrum. Standardized discriminant coefficients were examined to determine the relative contribution of discriminator variables to the function (Table 7). Standardized discriminant coefficients have been converted to z scores to eliminate scaling differences among the discriminator variables (Brown and Wicker 2000). Discriminant function 1 (F1) is primarily defined by high % *Thalassia* and shallow sediment depth (Table 8). Relative to habitats occupied by *H. zosterae* and *S. scovelli*, those occupied by *S. floridae* tend to have shallower sediment, a greater percent coverage of *Thalassia*, and higher salinities.

Table 8. Standardized and unstandardized discriminant function coefficients for two functions (1 and 2) distinguishing among three species of syngnathids.

	Standardized function		Unstandardized function	
	F1	F2	F1	F2
salinity	0.312	0.051	0.113	0.019
sediment depth	-0.779	0.548	-0.131	0.092
% *Thalassia*	0.921	0.255	0.035	0.010
% sponge	0.083	-0.497	0.058	-0.348
% drift algae	-0.231	0.469	-0.077	0.157
Intercept			-3.437	-1.745

Group centroids indicate that F2 discriminates habitats occupied by *H. zosterae* from those occupied by *S. scovelli*, although the discriminating ability of this function was marginally significant (p=0.085, Figure 5). Standardized discriminant coefficients indicate that F2 is primarily defined by sediment depth and % sponge, with sediment depth contributing positively to the function and % sponge contributing negatively to the function (Table 8). Thus, habitats occupied by *H. zosterae,* which had relatively high discriminant scores on F2, are characterized by relatively deeper sediments and a lower % coverage of sponges, compared to those sites occupied by *S. scovelli.* Classification success of the discriminant model averaged 57.4% across the three species (Table S7).

2.3.5.2 Logistic regression to define habitat occupancy

Logistic regression generated significant predictors of occurrence for all syngnathids combined, and included % coverage *Thalassia*, % coverage sponge, and sediment type (Figure 6). Significant predictors of occurrence for *H. zosterae* include % coverage *Thalassia*, % coverage sponge, sediment depth and sediment type. The only significant predictor of occurrence for *S. scovelli* was sediment type. Salinity, % coverage *Thalassia*, % coverage sponge, and sediment type were significant predictors of occurrence in *S. floridae* (Figure 6, Table 9).

My predictive logistic regression models indicate the probability of occurrence of any syngnathid increases with increasing % coverage *Thalassia* and decreases with increasing % coverage sponge and increasing coarseness of sediment (Figure 6). The probability of occurrence of *H. zosterae* increases with increasing % coverage *Thalassia*, deeper sediments, finer sediment, and decreasing % coverage sponge. *S. scovelli* is more likely to be found in environments with fine sediments. Finally, the probability of occurrence of S. floridae increases with increasing salinity, % coverage *Thalassia,* and finer sediments, and decreases with increasing % coverage sponge (Figure 6, Table 9).

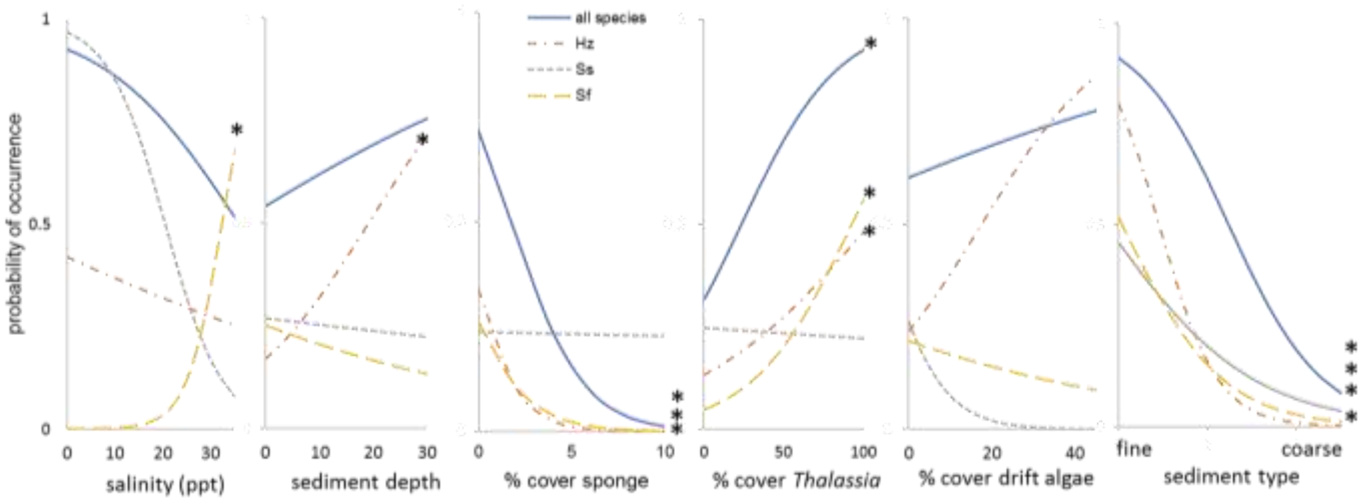

Figure 6. Logistic regression of selected predictor variables against presence/absence of all syngnathids, *H. zosterae*, *S. scovelli*, and *S. floridae*. See Table 8 for goodness-of-fit and significance. Asterisks represent significant relationships between the variable and occupancy.

Table 9. Goodness of fit (Nagelkerke R^2) and measure of significance (-2 Log(Likelihood)) for single-variable logistic regressions against presence/ absence for all syngnathid species combined, and *H. zosterae*, *S. scovelli*, and S. *floridae* separately.

	all species		*H. zosterae*		*S. scovelli*		*S. floridae*	
	R^2	p	R^2	p	R^2	p	R^2	p
salinity	0.009	0.5	0.001	0.84	0.049	0.133	**0.092**	**0.045**
sediment depth (cm)	0.017	0.376	**0.109**	**0.023**	0.001	0.835	0.009	0.553
sediment type	**0.278**	**<0.0001**	**0.287**	**<0.0001**	**0.085**	**0.033**	**0.134**	**0.008**
% *Thalassia*	**0.254**	**<0.0001**	**0.088**	**0.03**	0.001	0.869	**0.216**	**0.001**
% sponge	**0.181**	**0.001**	**0.085**	**0.009**	0	0.947	**0.078**	**0.048**
% coverage drift algae	0.003	0.693	0.042	0.139	0.033	0.197	0.003	0.711

Full model outputs for single variable logistic regressions available in Appendix D.

2.4 Discussion

2.4.1 Relative abundance and distribution of syngnathids in Biscayne National Park

My first objective was to report the relative abundance of syngnathids and compare my findings to previous studies in south Florida. The most frequently encountered syngnathid species in underwater visual surveys were *H. zosterae* (n=36 individuals, frequency of occurrence (*f*)= 0.28), *S. scovelli* (n=32 individuals, *f*=0.24) and *S. floridae* (n=32 individuals, *f* = 0.22). Serafy *et al.* (1997) presented a list of nearshore fish species caught in roller-frame trawl surveys conducted at eight sites in Biscayne Bay in 1993 and 1994. Six of the eight sites surveyed were located north of the Biscayne National Park boundary; the two sites that were located within Biscayne National Park (one at Black Point, and one at Turkey Point) showed relative abundance of sygnathids that was very different from that observed in this study: *C. albirostris* was the most abundant with four individuals collected, followed by *H. erectus* (three individuals), *S. scovelli* (two individuals), and *S. floridae* (one individual). *Hippocampus zosterae* was absent from the two sites surveyed in BNP (Serafy *et al.* 1997). Interpretation of these data needs to be strongly tempered by the very small numbers of sygnathids collected. Ault *et al.* (2001), however, also sampled the relative abundance of nearshore fishes in randomized roller trawl surveys conducted from 1996-2001, at sites located both north of BNP and within BNP proper, and obtained similar results with a much larger sample size. The two most abundant species of syngnathids were *H. erectus* (n=253) and *C. albirostris* (n=180), which were not collected at any of my sites within Biscayne Bay, while *H. zosterae* (n=12) ranked low in abundance.

Both previous surveys were conducted using roller-frame trawls, and it is possible that the low numbers of *H. zosterae* in those surveys are due to low-catchability of smaller species, including sygnathids in this gear type (Baum *et al.* 2003). Comparison of my results with these earlier studies demonstrates the need for standardized, long-term monitoring of syngnathid assemblages as it is difficult to compare relative abundance across differing survey methods. However, the absence of the two largest seahorses, *H. erectus* and *H. reidi*, from the Biscayne Bay portion of BNP where they had been previously recorded is notable. Both *H. erectus* and *H. reidi* are relatively large seahorses reaching a maximum height of 19 cm and 17 cm, respectively (Lourie *et al.* 2004). Consequently, had either species occurred in high abundance at the surveyed sites, underwater visual census would have detected them relatively easily, and detection in those species would have been easier than in *H. zosterae*, which reaches a maximum adult height of only 2.5 cm (Lourie *et al.* 2004). This strongly suggests that these species have either declined since the earlier surveys were completed, that they are extremely patchily distributed, or that their habitat requirements are now outside of the current conditions in Biscayne Bay.

A recent study focusing on the community and population structure of syngnathids in the seagrass beds of Tampa Bay, on the western coast of Florida (Masonjones *et al.* 2010) found *S. scovelli* occurred most frequently (f = 0.79) and was followed by *H. zosterae* (f = 0.16) and *S. louisianae* (f = 0.03). These surveys were conducted using modified pushnets, which are highly effective at surveying small fishes in seagrass beds. (Kirk *et al.* 1954). The similarity between syngnathid communities in Tampa Bay and those sampled in my BNP surveys also suggests that differences in community structure between roller-frame trawl surveys and underwater visual surveys in BNP may in part be due to selective gear sampling, particularly regarding the low abundance of *H. zosterae* observed in the roller-frame trawl surveys. The higher relative abundance of *S. louisianae* and absence of *C. albirostris* in the Tampa Bay surveys suggests that species assemblages do indeed differ between BNP and Tampa Bay, as there is a well-studied bio-geographic break at the Florida Keys that separates Atlantic and Gulf of Mexico populations (Avise 1992).

Comparing my results to patterns of relative abundance in other areas of Florida is also informative. A recent study focusing on the community and population structure of syngnathids in the seagrass beds of Tampa Bay, on the western coast of Florida (Masonjones *et al.* 2010), found *S. scovelli* occurred most frequently (f = 0.79) and was followed by *H. zosterae* (f = 0.16) and *S. louisianae* (f = 0.03). These surveys were conducted using modified pushnets, which are highly effective at surveying small fishes in seagrass beds. (Kirk *et al.* 1954). The similarity between syngnathid communities in Tampa Bay, sampled using fine-meshed pushnets, and the communities sampled in my BNP surveys also suggests that differences in community structure between roller-frame trawl surveys and my underwater visual surveys in BNP may in part be due to selective gear sampling, particularly regarding the low abundance of *H. zosterae* observed in the roller-frame trawl surveys. However, there are known differences in species assemblage on the west coast versus east coast of Florida. The higher relative abundance of *S. louisianae* and absence of *C. albirostris* in the Tampa Bay surveys suggests that species assemblages do indeed differ between BNP and Tampa Bay, as there is a well-studied biogeographic break at the Florida Keys that separates Atlantic and Gulf of Mexico populations (Avise 1992).

2.4.2 Habitat use by syngnathids

My second objective was to determine the distribution and habitat use of syngnathids with respect to major habitat types and environmental gradients**.** Reef sites were characterized as relatively high-energy environments occurring at greater mean depths, with greater horizontal visibility, greater percent coverage of benthic invertebrates, and coarse rather than fine sediments. Reef sites were not found to be occupied during by syngnathids during my surveys, however to ensure proportional allocation of survey effort, only 13 reef sites were surveyed. Additionally, while the underwater visual survey methodology detected syngnathids in less rugose habitats, it is likely that the complexity of reef habitats limited the effectiveness of searching using this technique.

The remaining two major habitat types (continuous SRV and discontinuous SRV) were more similar in their profile of environmental variables, reflecting the reality that habitats often exist as gradients in the undersea landscape. However, some biologically meaningful differences emerged in the variables salinity, % coverage *Thalassia*, % coverage rhyzophytic macroalgae, blade length. Perhaps correspondingly, there emerged differences in species assemblage and relative abundance of species between the two habitats. Among the three most abundant species (*S. scovelli*, *H. zosterae* and *S. floridae*), *H. zosterae* was more commonly associated with continuous SRV habitat while *S. scovelli* was more commonly associated with discontinuous SRV habitat, however differences in occupancy were not significant for either species. In contrast, *S. floridae* disproportionately utilized continuous SRV habitat, as revealed by a statistically greater frequency of occurrence of *S. floridae* within continuous SRV, and by the similarity between variables important in defining continuous SRV and those identified as important in describing habitats occupied by *S. floridae* using discriminant function analysis and logistic regression.

Sediment type was the most significant predictor of occurrence across syngnathid species, with the probability of occurrence decreasing with larger grain size. Sediments with small grain sizes are usually associated with depositional environments that are protected from wind and wave energy, such as sheltered bays and lagoons, while coarse sediments are more likely to be found in areas exposed to wind and wave energy which causes silts and clays to be removed (Madley *et al.* 2002). Seahorses and pipefishes are not powerful swimmers (Ashley-Ross 2002) and would be less likely to be found in high-energy environments, as confirmed by my data. In addition to sediment type, my analyses revealed that syngnathids generally were more likely to be found in shallow sites, with relatively reduced horizontal visibility, higher % coverage of *Thalassia* and lower % coverage of reef-associated benthic invertebrates (sponges, corals, gorgonians) and turf algae. This suite of characteristics is more commonly found in protected sites located inside, rather than outside, Biscayne Bay, and likely reflects the overall preference of syngnathids for low-energy environments (Masonjones *et al.* 2010).

2.4.3 Modelling occupancy and differences among species

My third objective was to determine which environmental variables best discriminate among the most abundant species of syngnathids: *H. zosterae*, *S. scovelli* and *S. floridae*. Discriminant function analysis resulted in a well-supported function that distinguished habitats occupied by *S. floridae* from those occupied by *H. zosterae* and/or *S. scovelli* based on the variable % coverage of *Thalassia*, which also emerged as the strongest single-variable predictor of occurrence for *S. floridae*. This finding is consistent with Masonjones *et al.* (2010), who found that *S. floridae* was rarely observed in beds with relatively low blade heights and blade densities and was most abundant at deeper sites with seagrass blade lengths, possibly due to greater protection from predators (Masonjones *et al.* 2010). This hypothesis is supported by my field observations of camouflage in *S. floridae* in both coloration and behavior. When observed in the field, *S. floridae* was typically motionless, oriented vertically with the head facing upwards, with bright green coloration, well camouflaged amongst blades of seagrass (Figure 7).

Figure 7. Adult *Syngnathus floridae* in a bed of *Thalassia testudinum* seagrass. Notice evidence of camouflage in behavior (vertical orientation of the body) and coloration.

Of the three species, *H. zosterae* was the most strongly associated with depositional areas characterized by relatively deep and fine sediments. Seahorses and pipefishes are broadly considered to be slow swimmers (Ashley-Ross 2002). The strong association between *H.*

zosterae and fine sediment/low energy environments could reflect the idea that *H. zosterae* is the least powerful swimmer amongst syngnathids and *H. zosterae* may rely entirely on passive dispersal through drifting on floating vegetation (Mason-Jones *et al.* 2010; Fedrizzi *et al.* 2015). Within appropriate low-energy environments, *H. zosterae* is a seagrass-associated species that occupies a broader niche within the seagrass landscape than *S. floridae*. This is potentially reflective of tolerance to a wider range of salinities (or variation in salinity) on the part of *H. zosterae*, as denser *Thalassia* beds with longer blade lengths are correlated with higher, more stable salinities, as well as deeper water (Lirman and Cropper 2003). *Hippocampus zosterae* also exhibited a stronger positive association with drift algae (*Laurencia* spp.) than either *S. scovelli* or *S. floridae*, however only the difference between *S. scovelli* and *H. zosterae* in terms of mean % coverage of drift algae was significant. Drift algae was a commonly observed holdfast for *H. zosterae* during my study, and in other studies (Masonjones *et al.* 2010). It has also been hypothesized that dispersal primarily occurs via rafting on drift algae (Fedrizzi *et al.* 2015) as has been proposed for other syngnathids (Abe *et al.* 2002).

My description of *H. zosterae* habitat supports previous studies. Matheson *et al.* (1999) described the habitat most frequently occupied *by H. zosterae* in nearby Florida Bay as a mixed, relatively lush seagrass bed, although it was found at other sites which varied in their proximity to freshwater inputs. Masonjones *et al.* (2010) stated that *H. zosterae* was found to be a generalist in the seagrass landscape, occurring across a gradient of seagrass species, macroalgal abundance, and distance to open water.

Discriminant analysis revealed that sites occupied by *S. scovelli* exhibited relatively shallower sediment depths and a higher % coverage of sponges as compared to those occupied by *H. zosterae*. This habitat description applies well to in-bay hardbottom habitats, which are characterized by a foundation of oolitic limestone coverage by a thin sediment layer and populated with a variety of soft coral and sponge species, with sparse colonization by seagrasses (Ault *et al.* 2001). Sponges are filter-feeding organisms that require some water flow for feeding on suspended particulates (Vogel 1977). It is possible that lower percent coverage of sponges in habitats occupied by *H. zosterae* is also indicative of relatively low flow environments and that despite many habitat use similarities, *S. scovelli* can expand its niche into higher-energy habitats colonized by sponges. Additionally, *S. scovelli* was found in relatively open areas, sparsely colonized by seagrasses and was the only species for which increasing % coverage of *Thalassia* inversely predicted occurrence (although this result not significant). My finding that *S. scovelli* was more likely to be found in areas sparsely colonized by seagrasses is supported by the work of Bell *et al.* (2001), who compared the abundances of *S. scovelli* between *Thalassia* beds that were heavily fragmented due to boat propeller scarring and continuous reference sites that were unaffected by scarring. *S. scovelli* was consistently found in higher abundances in the fragmented beds. These results suggest that while *S. scovelli* is associated with seagrass ecosystems, some fragmentation does not inhibit retention or recruitment in this species (Bell *et al.* 2002). *Syngnathus scovelli* is a euryhaline species, which is known to enter fresh water and occupy low-salinity environments (Targett 1984; Bolland and Boettcher 2005). It is possible that my sampling, which was restricted to waters >1m depth and distant from sources of freshwater input with mean salinities of 25ppt (+/-3 ppt), did not fully reflect the breadth of habitats occupied by *S. scovelli*.

Although syngnathids as a group occur in sheltered low-energy environments, my study demonstrates that they may be well-differentiated in their habitat use within their broader range of occupancy. Comparison of current syngnathid abundance and community structure with historical surveys suggests that the absence of larger syngnathids like *H. erectus* and *H. reidii* in my surveys may be in part due to bycatch from roller-from trawls. This strongly suggests a need to establish no-trawl zones in BNP to protect snygnathids and other species that may be vulnerable to bycatch in fisheries.

Chapter 3: Conclusions

Mangrove forests (Polidoro *et al.* 2010), seagrass beds (Short *et al.* 2011), and coral reefs (Carpenter *et al.* 2008) are among the most biodiverse and ecologically important coastal marine habitats on Earth. My study further emphasizes the importance of preserving vulnerable coastal habitats. Below, I outline some specific conclusions and recommendation regarding the conservation of these resources in BNP.

3.1 Model limitations

My study employed discriminant function analysis primarily as a descriptive rather than a predictive tool, however the functions generated by the analysis can be used in a predictive manner (Brown and Wicker 2000) by future researchers and resource managers. Validation quantifies confidence in predictions produced from future application of the created model. Resubstituting of the data, a common practice in aquatic literature in which the same data are used for both model construction and prediction, can produce highly biased estimates of correct classification rates (Olden *et al.* 2002). Should the functions generated in the discriminant function analysis be used predictively, validation results are provided using the resubstitution method (Appendix B, Table S7) and, more appropriately (Olden *et al.* 2002), cross-validated using the hold-out method (Appendix B, Table S8; Brown and Wicker 2000), which validates the model using observations which were not used in the construction of the original model (Olden *et al.* 2002).

Confidence in future predictions based on my logistic regression model outputs should be tempered by the knowledge that the models have only been validated using resubstitution.

3.2 Historical and ongoing stressors in BNP

Biscayne National Park and its resident animals and plants are subject to multiple stressors due to their proximity to a major metropolis, historical modifications to the natural hydrology of south Florida (Browder *et al.* 2005), commercial and recreational resource use (Ault *et al.* 2001), and climate change (Obeysekera *et al.* 2011). These stressors are likely to interact in the BNP system, which can lead to additive, antagonistic, or synergistic effects on the system, or any of its components (Crain *et al.* 2008).

3.2.1 Development and land use

Biscayne National Park is flanked to the north and west by Miami-Dade County, the most populous county in Florida, home to a growing population of nearly 2.8 million people (US Census Bureau 2018). Urban infrastructure development projects, such as the expansion of US Highway 1, have caused algal blooms and seagrass die-offs in southern BNP by releasing excess nutrients and sediments into the water column (Rudnick 2007. Presentation to the Water Resources Advisory Committee, South Florida Water Management District). In addition to ecological stressors resulting from increasing urban infrastructure, Miami-Dade County also includes large areas of land used for agriculture, run-off from which also introduces both nutrients and sediments to BNP (Carey *et al.* 2011).

3.2.2 Water management

Biscayne National Park has been affected by historical changes to natural hydrology and flow regimes. South Florida is currently the site of the largest hydrologic restoration project ever attempted in the United States, the Comprehensive Everglades Restoration Plan (CERP). This project was authorized by the US Congress in 2000 to restore south Florida's ecosystem following over 100 years of human alteration to accommodate development and the increasing demands for land and freshwater resources. Freshwater delivery to the BNP's Biscayne Bay was historically diffuse, entering through a system of creeks fed by low topography channels in the Everglades as well as groundwater seepage (Davis 1943; Kohout 1967). Today freshwater enters BNP through a human-engineered system of canals, impoundments, levees, and water pumping stations. The canal zone along the western shore of Biscayne Bay experiences large fluctuations in salinity and concentrated nutrient inputs, while other parts of Biscayne Bay are maintained at near-oceanic salinities that are higher than historical mesohaline conditions. These salinity differences lead to structural differences in organismal assemblages between stable-salinity habitats and those adjacent to freshwater canals (Serafy *et al.* 1997).

3.2.3 Commercial trawling

Biscayne Bay has historically supported a large commercial fishery for both bait and food shrimp, targeting pink shrimp, *Farfantepenaeus duorarum*. Additionally, there is a recreational shrimp fishery which remains uncharacterized (Johnson *et al.* 2012). The commercial bait shrimp fishery of Biscayne Bay began to operate in the 1960s, south of the Rickenbacker Causeway and expanded operations southwards into what is now BNP with the construction of Black Point Marina. This fishery operated nearly every night of the year, principally over areas with muddy sand bottoms and high organic content, in waters deeper than approximately 1m (Johnson *et al.* 2012).

Roller-frame trawls, the principal gear used in the bait shrimp fishery, affect major habitat types in BNP to varying degrees. While this gear type causes minimal damage to seagrass beds, substantial damage is inflicted on less flexible benthic organisms, including sponges, gorgonians, corals, and in-bay hardbottom habitats generally (Ault *et al.* 1997). Additionally, many non-target species are taken as bycatch in this gear type (Serafy *et al.* 1997; Ault *et al.* 2001).

3.2.4 Recreational impacts

Recreational boating is a popular activity in BNP, which can lead to propeller scaring of seagrass beds. Propeller scars are characterized by narrow paths within which seagrasses and other organisms have been dislodged from the sediment. Seagrasses that are restricted to areas <2 m deep are particularly susceptible, and in areas where boating activities are locally intense, propeller scaring can be a major source of habitat destruction or act synergistically with other sources of environmental stress (Bell *et al.* 2001). Additional habitat damage can be caused by improper moorings, marine debris from monofilament fishing line, and damage to sensitive habitats (coral reefs) caused by inexperienced SCUBA divers or snorkelers.

3.3 Species Impacts

3.3.1 Relative vulnerability of focal species to seagrass loss

The three focal species of this research, *H. zosterae*, *S. scovelli*, and *S. floridae*, are predicted to vary in their response to localized environmental changes in the BNP system. I expect that *S.*

scovelli populations are the least likely to be affected by potential loss of seagrass habitat, and may be somewhat resilient to other environmental stressors, such as degradation of water quality. Among the three species, *S. scovelli* was most often found in areas that could be described as "highly impacted" – it may favor low salinity areas within Biscayne Bay (Targett 1984), and my surveys confirmed that it was found in areas with relatively low seagrass coverage. Low-salinity areas are the most likely to be exposed to shore-based stressors including salinity fluctuations due to pulsed freshwater discharges (Serafy *et al.* 1997) and localized increases in nutrients and pollutants (Carey *et al.* 2011). Due to its distribution among a broader range of seagrass densities, I suggest that *H. zosterae* will be moderately affected by stressors acting on the BNP system.

Among the three focal species, *S. floridae* is most likely to be restricted to relatively dense, healthy *Thalassia* beds and I expect that due to this apparent preference, it is the most likely to be affected by potential loss of seagrass habitat in Biscayne Bay. Abundances of *S. floridae* declined with loss of seagrass in neighboring Florida Bay (Matheson *et al.* 1999). However, as a relatively large pipefish, *S. floridae* may prefer deeper habitats with longer seagrass blades that create more protection from predators and facilitate possible open ocean migrations (Lazzari and Able 1990; Masonjones *et al*. 2010). These deeper seagrass beds may be located further from the coast and be less susceptible to disturbances.

3.3.2 Vulnerability of non-focal species

The absence of the two largest seahorses, *H. erectus* and *H. reidi* inside Biscayne Bay during my surveys is notable. Fisheries-independent roller-frame trawl surveys conducted in BNP from

1996 to 2000 indicated that *H. erectus* may have formerly been among the most abundant syngnathids in BNP and that both *H. erectus* and *H. reidi* were more common than *H. zosterae* (Ault *et al.* 2001). These surveys must be interpreted with caution, as the spatial extent of these surveys extends beyond the BNP boundary to the north, and published studies indicate that, in the case of *H. erectus*, abundance may be higher in northern Biscayne Bay, outside of the BNP boundary (Serafy *et al.* 1997). Additionally, the relatively low proportion of *H. zosterae* in the fisheries independent surveys is likely due to low catchability due to its small size (Baum *et al.* 2003). My findings are, however notable given the relatively high catchability of large seahorses like *H. erectus* and *H. reidi* in roller-frame trawls (Baum *et al.* 2003) and may indicate currently reduced population numbers due to high bycatch removal rates from the system in the past and/or to degradations in habitat quality.

To address the idea of bycatch affecting syngnathid populations, I estimated the total number of individual syngnathids of eight species removed from Biscayne National Park by the commercial bait shrimp fishery from 2008-2016 (Figure 8).

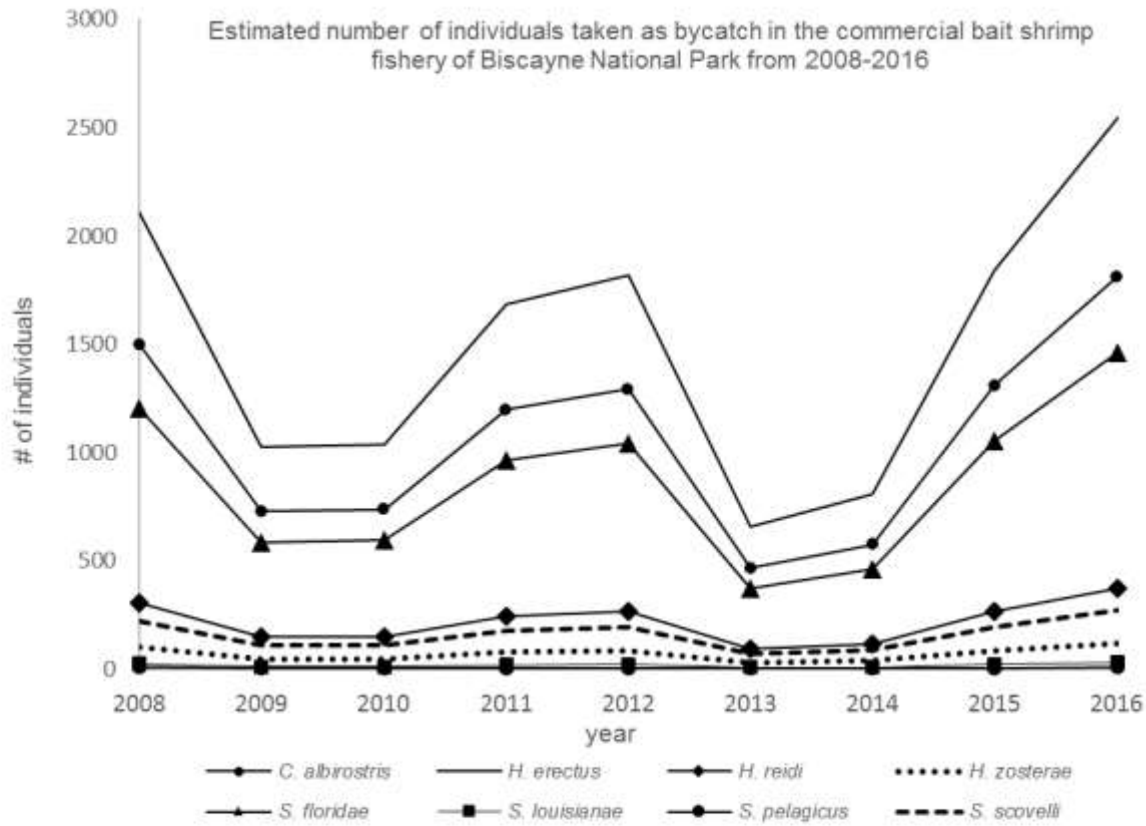

Figure 8. Estimated number of syngnathids removed by the commercial bait shrimp fishery of Biscayne National Park from (2008-2016).

These estimates assumed a fixed catch-per-unit-effort (CPUE) for each species over time, which is based on fisheries-independent survey data collected from 1996-2000 throughout the entirety of Biscayne Bay. Estimates have been scaled to account for the total area trawled, but do not account for spatial heterogeneity in trawl effort (Ault *et al*. 1997) nor for changes in CPUE since 1996-2000. For more information, please see Appendix E.

The number of commercial bait shrimp trips fluctuated during the study period, ranging from a minimum of 417 trips in 2013, to a maximum of 1621 trips in 2016. The mean number of trips taken per year was 956 (SD±388).

Hippocampus erectus was the most common species taken as bycatch by roller-frame trawls in Biscayne Bay, with a mean of 1105 (SD±609) individuals removed as bycatch per year. This was followed by *C. albirostris* (1068±434), *S. floridae* (860±349), *H. reidi* (220±89), *S. scovelli* (160 ±65), *H. zosterae* (71±29), *S. louisianae* (18±7) and *S. pelagicus* (6±2) (Ault *et al.* 2001).

Given that some syngnathids, particularly seahorses, tend to be patchily distributed and to naturally occur in low abundances (Foster and Vincent 2004) my estimated bycatch removal rates suggest the need for further analyses to better quantify the potential impact of roller-frame trawl fishing in Biscayne Bay. Additional analyses should include estimates of natural mortality and population growth parameters. Repeat surveys using the same methodology would provide updated CPUE and inform calculations of changes in population size over time.

It is also possible that degraded habitat resulting from roller-frame trawl operation in Biscayne Bay accounts for the absence of larger seahorses in my surveys. Ault *et al.* (1997) estimated that roller-frame trawls of the bait shrimp fishery sweep the entire shallow-bottom habitat (depth = 1.4-1.8 m) of south Biscayne Bay up to four times per year. While designed to minimally damage seagrass beds, roller-frame trawls cause physical damage to other less flexible components of the benthos, such as invertebrate communities (Ault *et al.* 2001). I observed several *H. erectus* individuals at a single site located outside of Biscayne Bay, in an area that is not subjected to roller-frame trawling. Individuals used tall (20-30 cm) stalks of *Udotea* sp. macroalgae, which were colonized by benthic invertebrates such as tunicates, sponges, and corals, as holdfasts (Figure 9). It is possible that damage to suitable holdfasts in Biscayne Bay may have contributed to the absence of large seahorses such as *H. erectus* and *H. reidi* during my surveys.

Figure 9. Adult *H. erectus* using *Udotea* sp. macroalgae as a holdfast in a bed of *Thalassia testudinum*.

3.4 Conservation implications

3.4.1 *Hippocampus zosterae* Endangered Species Act Status Review Process

Hippocampus zosterae is currently undergoing Status Review for listing under the US Endangered Species Act, following submission of a petition by the Center for Biological Diversity (CBD 2011) to the U.S. Secretary of Commerce. The findings of this study should inform the ongoing status review process.

My surveys revealed that *H. zosterae* was a relatively abundant member of the syngnathid species assemblage of BNP. *Hippocampus zosterae* varies in abundance throughout its global range. Previously published studies from Biscayne Bay/Biscayne National Park (Serafy *et al.* 1997; Ault *et al.* 2001) suggested that *H. zosterae* was relatively uncommon. Previous authors (Baum *et al.* 2003) suggest that the small size of *H. zosterae* leads to low catchability in roller-frame trawls. High relative abundance of *H. zosterae* observed in my study supports the observations of Baum *et al.* and provides a novel description of *H. zosterae* as a relatively abundant species in the study area.

I confirm habitat use descriptions by previous researchers suggesting that *H. zosterae* is a generalist in the seagrass landscape. However, descriptions of suitable habitat, or estimations of Area of Occupancy (IUCN 2012), which are used for conservation assessment and planning should account for *H. zosterae*'s apparent inability to occupy high-energy seagrass environments, as indicated by multiple observations of poor swimming ability and strong correlations between occupancy and fine sediment, by including current velocity (or appropriate surrogates, such as sediment particle size) as a variables in habitat models.

3.4.2 Additional research and synthesis needed for species of conservation concern

Given the relatively higher potential vulnerability of larger seahorses, such as *H. erectus* and *H. reidi*, to roller-frame trawl gear I recommend a review of all available literature concerning the abundance, population trends, habitat use, range size, and other potential range-wide threats to these species. The spatial patterns of roller-frame trawl fisheries should be considered. The release of such a review might prompt further release, analysis, and synthesis of existing data at local and/or regional scales and generate funding opportunities for additional research on population status, if necessary.

3.5 Recommendations for management

3.5.1 Habitat mapping and identification of suitable habitat

Mapping of seagrass beds and the sheltered low-energy environments that are important for many species of syngnathids should be a priority. These maps would enrich understanding of the current distribution and abundance of available habitat. Similarly, research to develop predictive occupancy models for all syngnathids in BNP would support a better understanding of species conservation status with respect to environmental factors, and the potential consequences of habitat change and management interventions.

3.5.2 Seahorses as flagship species for Biscayne Bay

Flagship species are charismatic species that attract public support, sympathy, raise environmental awareness and can invoke protection for at-risk habitats and less charismatic species under the umbrella of their larger habitat requirements (Caro and O'Doherty 1999; Lambeck 1997). Local culture, perception and value of different species is important to consider when choosing an effective flagship species that resonate with local communities (Bowen-Jones and Entwistle 2002). I propose that the seahorse may be particularly effective as a flagship species in Miami, also known as the "Magic City". Seahorses are steeped in mythology and have long appealed to artists as symbols and subjects around the world. The Miami metropolitan area has a thriving visual arts culture, as evidenced by the area's support for public art, investment in architecture by the public and private sector, the yearly Art Basel art show, which hosts 250 of the world's leading galleries and draws over 70,000 visitors each year (https://www.artbasel.com/).

The potential of the seahorse as a flagship species for Miami waterways has already been realized by organizations such as Miami Waterkeeper, which uses the seahorse as a central motif in its logo, and individuals, such as environmental artist Xavier Cortada (Figure 10).

Figure 10. Xavier Cortada, "Seahorse | Seagrass," 60″ x 36″, acrylic on canvas, 2014. This painting was created to commemorate the 40th anniversary of the Biscayne Bay Aquatic Preserve.

3.5.3 2014 Biscayne National Park Fisheries Management Plan – No Trawl Zone

Due to the lack of detailed data on distribution and abundance for most species in any given area, conservation planners often rely on surrogate species, groups of species, or environmental attributes to inform the placement of conservation areas with the goal of extending protection to a maximum number of species (Reid 1998). Syngnathids have demonstrated efficacy as surrogate species for the conservation of fish assemblages in estuarine seagrass beds (Shokri *et al.* 2009). Shokri *et al.* (2009) argued that syngnathids could be used as an efficient surrogate group to select MPAs for other fish within a single estuarine system. Seagrass MPAs that were selected to maximize density and assemblage variation of syngnathids included more non-syngnathid species than a random selection of locations. I recommend that managers consider using syngnathids as surrogate species to inform the placement of the no-trawl zone within the Biscayne Bay portion of BNP. This no-trawl zone is listed as an action point in the Selected Alternative (Alternative 4 "Rebuild/conserve Park Fisheries Resources") in the 2014 BNP Fisheries Management Plan.

Bibliography

Aburto-Oropeza, O., Erisman, B., Galland, G.R., Mascareñas-Osorio, I., Sala, E., and Ezcurra, E. (2011). Large recovery of fish biomass in a no-take marine reserve. PLoS ONE *6*, e23601.

Ault, J., Smith, S., Meester, G., Juo, J., and Bohnsack, J. (2001). Site characterization for Biscayne National Park: assessment of fisheries resources and habitats. NOAA Technical Memorandum NMFS-SEFSC-468

Ahnesjö, I., and Craig, J.F. (2011). The biology of Syngnathidae: pipefishes, seadragons and seahorses. Journal of Fish Biology *78*, 1597–1602.

Avise, J.C. (1992). Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos *63*, 62.

Ashley-ross, M.A. (2002). Mechanical properties of the dorsal fin muscle of seahorse (*Hippocampus*) and pipefish (*Syngnathus*). Journal of Experimental Zoology *293*, 561–577.

Baum, J.K., Meeuwig, J.J., and Vincent, A.C. (2003). Bycatch of lined seahorses (*Hippocampus erectus*) in a Gulf of Mexico shrimp trawl fishery. Fishery Bulletin *101*, 721–731.

Bell, S.S., Hall, M.O., Soffian, S., and Madley, K. (2002). Assessing the impact of boat propeller scars on fish and shrimp utilizing seagrass beds. Ecological Applications *12*, 206–217.

Blaber, S. (2000). Effects of fishing on the structure and functioning of estuarine and nearshore ecosystems. ICES Journal of Marine Science *57*, 590–602.

Bolland, J., and Boettcher, A. (2005). Population structure and reproductive characteristics of the gulf pipefish, *Syngnathus scovelli*, in Mobile Bay, Alabama. Estuaries *28*, 957–965.

Bologna, P.A.X. (2007). Impact of differential predation potential on eelgrass (*Zostera marina*) faunal community structure. Aquatic Ecology *41*, 221–229.

Browder, J.A., Alleman, R., Markley, S., Ortner, P., and Pitts, P.A. (2005). Biscayne Bay conceptual ecological model. Wetlands *25*, 854–869.

Brown, M. T., and Wicker, L. R. (2000). Discriminant analysis. In *Handbook of applied multivariate statistics and mathematical modeling* (pp. 209-235).

Burkholder, J.M., Tomasko, D.A., and Touchette, B.W. (2007). Seagrasses and eutrophication. Journal of Experimental Marine Biology and Ecology *350*, 46–72.

Carey, R.O., Migliaccio, K.W., and Brown, M.T. (2011). Nutrient discharges to Biscayne Bay, Florida: Trends, loads, and a pollutant index. Science of The Total Environment *409*, 530–539.

Carpenter, K.E., Abrar, M., Aeby, G., Aronson, R.B., Banks, S., Bruckner, A., Chiriboga, A., Cortes, J., Delbeek, J.C., DeVantier, L., *et al.* (2008). One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science *321*, 560–563.

Chirico, A.A.D., McClanahan, T.R., and Eklöf, J.S. (2017). Community- and government-managed marine protected areas increase fish size, biomass and potential value. PLOS ONE *12*, e0182342.

Crain, C.M., Kroeker, K., and Halpern, B.S. (2008). Interactive and cumulative effects of multiple human stressors in marine systems. Ecology Letters *11*, 1304–1315.

Dawson, C.E. (1985). Indo-Pacific Pipefishes (Red Sea to the Americas) (Ocean Springs, Missis: Gulf Coast Research Library).

Dill, L.M. (1983). Adaptive flexibility in the foraging behavior of fishes. Canadian Journal of Fisheries and Aquatic Sciences *40*, 398–408.

Estep, A., Waara, R.J., Lee, J.R., Feeley, M.W., Patterson, M.E., Davis, A.D., Miller, J., Witcher, B.D., Patterson, J.M., and Vargas, R.M. Biscayne National Park offshore benthic habitat mapping project: Drafting, accuracy assessment and revisions. Natural Resource Report NPS/SFCN/NRR—2017/1390.

Fedrizzi, N., Stiassny, M.L.J., Boehm, J.T., Dougherty, E.R., Amato, G., and Mendez, M. (2015). Population Genetic Structure of the Dwarf Seahorse (Hippocampus zosterae) in Florida. PLOS ONE *10*, e0132308.

Flynn, A.J., and Ritz, D.A. (1999). Effect of habitat complexity and predatory style on the capture success of fish feeding on aggregated prey. Journal of the Marine Biological Association of the UK *79*, 487–494.

Halpern, B.S. (2003). The impact of marine reserves: do reserves work and does reserve size matter? Ecological Applications *13*, 117–137.

Harding, J.M., Burky, A.J., and Way, C.M. (1998). Habitat preferences of the Rainbow Darter, *Etheostoma caeruleum*, with regard to microhabitat velocity shelters. Copeia *1998*, 988.

Howard, R.K., and Koehn, J.D. (1985). Population dynamics and feeding ecology of pipefish (Syngnathidae) associated with eelgrass beds of Western Port, Victoria. Marine and Freshwater Research *36*, 361–370.

Jordan, F., Babbitt, K.J., and Mclvor, C.C. (1998). Seasonal variation in habitat use by marsh fishes. Ecology of Freshwater Fish *7*, 159–166.

Johnson, D.R., Browder, J.A., Brown-Eyo, P. and Robblee, M.B., 2012. Biscayne Bay commercial pink shrimp, *Farfantepenaeus duorarum*, fisheries, 1986-2005. *Marine Fisheries Review*, *74*(4), pp.28-43.

Juffe-Bignoli, D., Burgess, N.D., Bingham, H., Belle, E.M.S., De Lima, M.G., Deguignet, M., Bertzky, B., Miliam, A.N., Martinez-Lopez, J., Lewis, E., *et al.* (2014). Protected planet report (U.K.: Cambridge).

Kendrick, A., and Hyndes, G. (2003). Patterns in the abundance and size-distribution of syngnathid fishes among habitats in a seagrass-dominated marine environment. Estuarine, Coastal and Shelf Science *57*, 631–640.

Lambeck, R.J. (1997) Focal species: a multi-species umbrella for nature conservation. Conservation Biology 11: 849–856.

Lazzari M.A., Able K.W. (1990). Northern pipefish *Syngnathus fuscus* occurrences over the Mid-Atlantic Bight continental shelf: Evidence of seasonal migration. Environmental Biology of Fishes. 27: 177–185.

Leysen, H., Dumont, E.R., Brabant, L., Van Hoorebeke, L., and Adriaens, D. (2011). Modelling stress in the feeding apparatus of seahorses and pipefishes (Teleostei: Syngnathidae): Mechanical stress in syngnathid skulls. Biological Journal of the Linnean Society *104*, 680–691.

Lirman, D., and Cropper, W.P. (2003). The influence of salinity on seagrass growth, survivorship, and distribution within Biscayne Bay, Florida: field, experimental, and modeling studies. Estuaries *26*, 131–141.

Lotze, H.K. (2006). Depletion, degradation, and recovery potential of estuaries and coastal seas. Science *312*, 1806–1809.

Lourie, S.A., Foster, S.J., Ernest, W.T., and Vincent, A.C.J. (2004). A guide to the identification of seahorses (Project Seahorse and TRAFFIC North America).

Madley K.A., Sargent B., Sargent F.J. Development of a system for classification of habitats in estuarine and marine environments (SCHEME) for Florida. Unpublished report to the US Environmental Protection Agency, Gulf of Mexico Program (Grant Assistance Agreement MX-97408100), Florida Marine Research Institute, Florida Fish and Wildlife Conservation Commission, St. Petersburg, 43pp. 2002.

Masonjones, H.D., Rose, E., McRae, L.B., and Dixson, D.L. (2010). An examination of the population dynamics of syngnathid fishes within Tampa Bay, Florida, USA. Current Zoology *56*, 118–133.

McClanahan, T.R., and Arthur, R. (2001). The effect of marine reserves and habitat on populations of East African coral reef fishes. Ecological Applications *11*, 559–569.

Moberg, F., and Folke, C. (1999). Ecological goods and services of coral reef ecosystems. Ecological Economics *29*, 215–233.

Molloy, P.P., McLean, I.B. and Côté. I.M. "Effects of marine reserve age on fish populations: a global meta-analysis." Journal of applied Ecology 46.4 (2009): 743-751.

Mosquera, I., Côté, I.M., Jennings, S., and Reynolds, J.D. Conservation benefits of marine reserves for fish populations. 12.

Mumby, P.J., Harborne, A.R., Williams, J., Kappel, C.V., Brumbaugh, D.R., Micheli, F., Holmes, K.E., Dahlgren, C.P., Paris, C.B., and Blackwell, P.G. (2007). Trophic cascade facilitates coral recruitment in a marine reserve. Proceedings of the National Academy of Sciences *104*, 8362–8367.

Mumby, P.J., and Harborne, A.R. (2010). Marine reserves enhance the recovery of corals on caribbean reefs. PLoS ONE *5*, e8657.

Munday, P.L., Jones, G.P., and Caley, M.J. (2001). Interspecific competition and coexistence in a guild of coral-dwelling fishes. Ecology *82*, 2177–2189.

IUCN, 2012. IUCN Red List Categories and Criteria: Version 3.1. Second Edition. Gland IUCN, Switzerland and Cambridge, UK (2012)

Obeysekera, J., Irizarry, M., Park, J., Barnes, J., and Dessalegne, T. (2011). Climate change and its implications for water resources management in south Florida. Stochastic Environmental Research and Risk Assessment *25*, 495–516.

Olden, J.D., Jackson, D.A., and Peres-Neto, P.R. (2002). Predictive models of fish species distributions: a note on proper validation and chance predictions. Transactions of the American Fisheries Society *131*, 329–336.

Pandolfi, J.M. (2003). Global trajectories of the long-term decline of coral reef ecosystems. Science *301*, 955–958.

Polidoro, B.A., Carpenter, K.E., Collins, L., Duke, N.C., Ellison, A.M., Ellison, J.C., Farnsworth, E.J., Fernando, E.S., Kathiresan, K., Koedam, N.E., *et al.* (2010). The loss of species: mangrove extinction risk and geographic areas of global concern. PLoS ONE *5*, e10095.

Polunin, N., and Roberts, C. (1993). Greater biomass and value of target coral-reef fishes in two small Caribbean marine reserves. Marine Ecology Progress Series *100*, 167–176.

Qin, G., Zhang, Y., Huang, L., and Lin, Q. (2014). Effects of water current on swimming performance, ventilation frequency, and feeding behavior of young seahorses (*Hippocampus erectus*). Journal of Experimental Marine Biology and Ecology *461*, 337–343.

Rudnick, D. (2007). Algal Blooms in Eastern Florida Bay and Southern Biscayne Bay. Presentation to the Water Resources Advisory Committee. (Everglades Division. South Florida Water Management District).

Ripley, J.L., and Foran, C.M. (2009). Direct evidence for embryonic uptake of paternally-derived nutrients in two pipefishes (Syngnathidae: Syngnathus spp.). Journal of Comparative Physiology B *179*, 325–333.

Savino, J.F., and Stein, R.A. (1989). Behavior of fish predators and their prey: habitat choice between open water and dense vegetation. Environmental Biology of Fishes *24*, 287–293.

Schofield, P.J. (2003). Habitat selection of two gobies (*Microgobius gulosus, Gobiosoma robustum*): influence of structural complexity, competitive interactions, and presence of a predator. Journal of Experimental Marine Biology and Ecology *288*, 125–137.

Serafy, J.E., Valle, M., Faunce, C.H., and Luo, J. (2007). species-specific patterns of fish abundance and size along a subtropical mangrove shoreline: an application of the Delta Approach. Bulletin of Marine Science *80*, 16.

Short, F.T., Polidoro, B., Livingstone, S.R., Carpenter, K.E., Bandeira, S., Bujang, J.S., Calumpong, H.P., Carruthers, T.J.B., Coles, R.G., Dennison, W.C., *et al.* (2011). Extinction risk assessment of the world's seagrass species. Biological Conservation *144*, 1961–1971.

Strawn, K. (1954). The Pushnet, a one-man net for collecting in attached vegetation. Copeia *1954*, 195.

Targett, T.E., 1984. A breeding population of Gulf pipefish (*Syngnathus scovelli*) in a Georgia estuary. Contributions in Marine Science 27:169-174.

Tipton, K., and Bell, S.S. (1988). Foraging patterns of two syngnathid fishes: importance of harpacticoid copepods. Marine Ecology Progress Series *47*, 31–43.

Utne, A.C.W., Aksnes, D.L., and Giske, J. (1993). Food, predation risk and shelter: an experimental study on the distribution of adult two-spotted goby *Gobiusculus flavescens* (Fabricius). Journal of Experimental Marine Biology and Ecology *166*, 203–216.

Vogel, S., 1977. Current-induced flow through living sponges in nature. *Proceedings of the National Academy of Sciences*, *74*(5), pp. 2069-2071.

Vincent, A.C.J. (1996). The international trade in seahorses (Cambridge, U.K: Traffic International).

Vincent, A.C.J., Foster, S.J., and Koldewey, H.J. (2011). Conservation and management of seahorses and other Syngnathidae. Journal of Fish Biology *78*, 1681–1724.

Werner, E.E., and Hall, D.J. (1979). Foraging Efficiency and Habitat Switching in Competing Sunfishes. Ecology *60*, 256–264.

Appendix A Review of habitat use of syngnathids recorded in BNP

Table 1: Global habitat associations of syngnathids recorded in Biscayne National Park

Scientific name	*Anarchopterus criniger*	*Bryx dunckeri*	*Cosmocampus albirostris*	*Cosmocampus brachycephalus*	*Cosmocampus elucens*
common name	Fringed Pipefish	Pugnose Pipefish	Whitenose Pipefish	Crested Pipefish	Shortfin Pipefish
max size (cm)	10	7.5	20.8	10	?
max depth	5	30	40	10	345
seagrass	•	•	•		•
coral			•		
mangrove					
algae	•	•	•		•
rock/rubble		•	•		
oysters					
sponges					
open substrate	•				
pelagic					
habitat	seagrass, mud banks, and floating algae[1]	estuaries, seagrass, algae, rock [2,3]	coral, seagrass, rubble, algae[2,5]	seagrass[2]	seagrass, algae[2,6]

Table 1 continued: Global habitat associations of syngnathids recorded in Biscayne National Park

Scientific name	*Hippocampus zosterae*	*Micrognathus crinitus*	*Microphis brachyurus*	*Syngnathus floridae*	*Syngnathus louisianae*
common name	Dwarf Seahorse	Insular Pipefish	Opossum Pipefish, Shorttailed Pipefish	Dusky Pipefish	Chain Pipefish
max size (cm)	2.5	15	19.5	25*	38.1
max depth	5	7	10		38
seagrass	•	•		•	•
coral		•			
mangrove			•		
algae		•	•		•
rock/rubble		•			
oysters					
sponges					
open substrate		•			
pelagic					
habitat	seagrass [7]	sand, coral, seagrass, algae, rock, sea fans [3, 12]	sargassum, mangroves[2], freshwater [13]	seagrass [19]	estuaries, seagrass, sargassum [18]

Table 1 continued: Global habitat associations of syngnathids recorded in Biscayne National Park					
Scientific name	*Hippocampus erectus*	*Syngnathus springeri*	*Hippocampus reidi*	*Syngnathus pelagicus*	*Syngnathus scovelli*
common name	Lined Seahorse	Bull Pipefish	Longsnout Seahorse	Sargassum Pipefish	Gulf Pipefish
max size (cm)	19	38	17.5	20	18.5
max depth	73	18-128	55	73	6
seagrass	•		•		
coral		•	•		
mangrove	•		•		
algae	•	•	•	•	
rock/rubble	•				
oysters	•		•		
sponges	•		•		
open substrate					
pelagic		•			
habitat	seagrass, sponges, sargassum[7], mangroves, channels, near saltmarshes, oysters, weedy banks [8,9,10,11]	coral, pelagic, sargassum [14, 15, 16, 17]	mangrove, seagrass, algae, oysters, coral, sponges, gorgonian corals, sargassum [7]	sargassum[2]	estuaries[19]

Appendix B Description of variables

Table S2. Generalized sediment categories qualifiers, adapted from Madley *et al.* 2002.

Sediment type	**Description:**
silt/clay	Fine particulate sediments <0.0625mm in size comprise more than 50% of the sediment.
fine sand	Fine sand with particles ranging in size from 0.0625mm to 0.5mm greater than 50% of the sediment. May be mixed with finer particles (silt/clay).
Coarse and mixed coarse	Particles ranging in size from 0.5mm to 2mm comprise more than 50% of the sediment. Particles >2mm may be present.
Coral rubble	Mixed large particles (>50mm)
hardbottom	Consolidated substrate of bedrock comprises greater than 50% of the substrate.

Table S3. description of variables measured during the study

variable	**name**	**description**	**type**
Time	time	Total time committed to surveying site	continuous
Distance	Distance	Distance of each replicate transect within site	
Area	Area	Area surveyed in site [to be calculated]	
Presence in Biscayne Bay		is the site located inside or outside Biscayne Bay	binary
Horizontal visibility (m)	H_vis	Horizontal distance, recorded within 1m of the substrate, at which a secchi disk is no longer readily identifiable.	continuous
Depth (m)	depth	Depth recorded by depth gauge	continuous
Salinity (ppt)	sal	Water sample collected within 1 m of the substrate, measured by refractometer	continuous
Sediment depth (cm)	sed_depth	Distance a PVC pipe can be pushed into the substrate, until it can no longer be pushed	continuous
Dominant seagrass	dom_sg	Visual observation of most dominant seagrass species	categorical
Continuous SRV	cont	Visual observation; if a seagrass bed is continuous [define this more clearly] =1; if a seagrass	binary

		bed is classed as discontinuous [define more clearly] = 0.	
Blade length (cm)	blade_length	Mean of 5 length measurements of the dominant species of seagrass measured at the point nearest to center of site (start point)	continuous
Sediment type	sed_type	Qualitative description of dominant sediment type at point nearest to center of site (start point)	ordinal
Major category	cat	Visual observation; site classing based on FWC schema	categorical
Mapped category	Map_cat	GPS coordinates of each site overlaid on FWC habitat maps [more description needed, name of maps, etc]	categorical
Percent coverage	**SD** DCR MD mud rock rubble sand **SG** HW SY TT **INV** SCLE Sponge CRL GORG **AL** DA MAC TA	5-8 photoquadrats taken at each site; CPCE used to process generate percent coverage of various components of the substrate.	continuous

Appendix C Discriminant analysis supplementary material

Table S4. Sample sizes for developmental and cross-validation sample

	n	Ss	Sf	Hz	total
developmental	54	21	14	19	54
cross-validation	26	10	7	9	26
total	80	31	21	28	80

Table S5. Descriptive Statistics for variables selected for use in Discriminant Function Analysis

Variable	Min	Max	Mean	SD
Salinity	19.000	32.000	26.648	2.908
Sed_depth (cm)	1.000	25.000	9.093	6.181
%*Thalassia*	0.670	100.000	47.455	29.626
% Sponge	0.000	5.500	0.740	1.453
% drift algae*	0.000	12.570	1.288	2.962

Table S6. Correlations between variables chosen for Discriminant Analysis

Variables	sal	sed_depth	TT	sponge	MAC
Salinity	**1.000**	0.071	0.441	0.025	-0.029
Sed_depth (cm)	0.071	**1.000**	0.336	-0.292	-0.200
%Thalassia	0.441	0.336	**1.000**	-0.257	0.006
% Sponge	0.025	-0.292	-0.257	**1.000**	0.025
% drift algae	-0.029	-0.200	0.006	0.025	**1.000**

Table S7. Percent of correct classification of the development sample for Discriminant Analysis

	Predicted group membership				
Actual group membership	*H. zosterae*	*S. floridae*	*S. scovelli*	Total	% correct
H. zosterae	8	2	9	19	42.11%
S. floridae	3	9	2	14	64.29%
S. scovelli	3	4	14	21	66.67%
Total	14	15	25	54	57.41%

Table S8. Percent of correct classification of the cross-validation samples for Discriminant Analysis.

	Predicted group membership				
Actual group membership	*H. zosterae*	*S. floridae*	*S. scovelli*	Total	% correct
H. zosterae	3	2	4	9	33.33%
S. floridae	5	1	1	7	14.29%
S. scovelli	2	0	8	10	80.00%
Total	10	3	13	26	46.15%

Appendix D Logistic Regression Model Summaries

Single-variable logistic regression model summaries

D.1 all syngnathids

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	2.544	2.903	0.768	0.381	-3.146	8.233			
salinity	-0.070	0.106	0.442	0.506	-0.278	0.137	0.932	0.757	1.147

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	0.928	0.288	10.378	0.001	0.364	1.493			
% sponge	-0.524	0.199	6.928	0.008	-0.915	-0.134	0.592	0.401	0.875

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	2.360	0.563	17.594	< 0.0001	1.257	3.463			
sediment type	-0.957	0.259	13.606	0.000	-1.465	-0.448	0.384	0.231	0.639

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	0.170	0.439	0.149	0.699	-0.691	1.031			
sediment depth	0.032	0.037	0.759	0.384	-0.040	0.103	1.032	0.961	1.109

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	0.454	0.247	3.381	0.066	-0.030	0.938			
% Drift Algae	0.017	0.046	0.142	0.706	-0.073	0.108	1.018	0.930	1.114

D.2 Hippocampus zosterae

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	-0.316	2.920	0.012	0.914	-6.038	5.407			
salinity	-0.022	0.107	0.041	0.839	-0.232	0.189	0.978	0.793	1.207

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	-0.674	0.289	5.431	0.020	-1.241	-0.107			
% sponge	-0.738	0.426	2.992	0.084	-1.573	0.098	0.478	0.207	1.103

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	1.378	0.713	3.732	0.053	-0.020	2.776			
sediment type	-1.525	0.517	8.696	0.003	-2.538	-0.511	0.218	0.079	0.600

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	-1.584	0.507	9.781	0.002	-2.577	-0.591			
sediment depth	0.083	0.038	4.814	0.028	0.009	0.157	1.086	1.009	1.170

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	-1.155	0.280	17.056	< 0.0001	-1.703	-0.607			
% Drift Algae	0.066	0.052	1.621	0.203	-0.035	0.167	1.068	0.965	1.182

D.3 Syngnathus scovelli

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	3.436	3.034	1.283	0.257	-2.510	9.383			
salinity	-0.167	0.113	2.182	0.140	-0.389	0.055	0.846	0.678	1.056

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	-1.166	0.301	14.992	0.000	-1.756	-0.576			
sponge	-0.005	0.148	0.001	0.974	-0.296	0.286	0.995	0.744	1.331

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	-0.182	0.568	0.103	0.749	-1.296	0.932			
sediment type	-0.609	0.327	3.474	0.062	-1.250	0.031	0.544	0.286	1.032

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	-0.995	0.489	4.141	0.042	-1.953	-0.037			
sediment depth	-0.008	0.040	0.043	0.836	-0.086	0.070	0.992	0.918	1.072

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	-1.029	0.292	12.421	0.000	-1.601	-0.457			
% Drift Algae	-0.138	0.156	0.788	0.375	-0.443	0.167	0.871	0.642	1.182

D.4 Syngnathus floridae

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	-8.816	4.133	4.551	0.033	-16.917	-0.716			
salinity	0.272	0.147	3.404	0.065	-0.017	0.561	1.312	0.983	1.752

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	-1.038	0.308	11.336	0.001	-1.643	-0.434			
% sponge	-0.532	0.373	2.029	0.154	-1.263	0.200	0.588	0.283	1.221

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	0.072	0.649	0.012	0.912	-1.199	1.343			
sediment type	-0.896	0.421	4.535	0.033	-1.721	-0.071	0.408	0.179	0.931

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	-1.095	0.519	4.454	0.035	-2.112	-0.078			
sediment depth	-0.026	0.044	0.339	0.560	-0.113	0.061	0.974	0.893	1.063

Source	Value	Standard error	Wald Chi-Square	Pr > Chi²	Wald Lower bound (95%)	Wald Upper bound (95%)	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Intercept	-1.289	0.294	19.186	< 0.0001	-1.866	-0.712			
% Drift Algae	-0.021	0.062	0.115	0.735	-0.144	0.101	0.979	0.866	1.107

Appendix E Estimation of bycatch removal rates for Biscayne National Park

To understand the potential role of bycatch in limiting syngnathids in BNP, I estimated the total number of individual syngnathids of eight species removed from Biscayne Bay by the commercial bait shrimp fishery from 2008-2016 (data provided by Florida Fish and Wildlife Conservation Commission) by multiplying the total number of commercial bait shrimp trips taken per year by CPUEs from fishery-independent TRAWL survey data using the same gear (Ault *et al.* 2001).Ithen scaled the result to the estimated total area trawled by the commercial bait shrimp fleet (Ault *et al.* 1997) (Equation 2)   .

$$\#species\ x\ _y = \#trips_y \times CPUE_{species\ x} \times multiplier$$

Equation 2

We excluded trips taken as part of the commercial food shrimp fishery, which uses wing-net gear to skim shrimp from the surface of Biscayne Bay (Johnson *et al.* 2012). CPUEs were derived from surveys conducted in Biscayne Bay, both within BNP and north of BNP (Ault *et al.* 2001).

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Table S10. Catch, effort, and CPUE of eight species of syngnathids in fishery-independent survey of Biscayne Bay (Ault *et al.* 2001)

species	# individuals	effort (#trips)	CPUE (#ind./trip)
C. albirostris	180	983	0.183
H erectus	253	983	0.257
H reidi	37	983	0.038
H zosterae	12	983	0.012
S. floridae	145	983	0.148
S. louisianae	3	983	0.003
S. pelagicus	1	983	0.001
S. scovelli	27	983	0.027

Table S11. Equation parameters for calculation of bycatch removal rates for BNP

Area of BNP (km^2)	720
Estimated Area of Biscayne Bay portion of BNP (km^2)	360
area trawled in 1 year (X4)(Ault *et al.* 1997)	1440
area trawled in 5 years (km^2)	7200
multiplier	6.10

Table S12. Estimates of the number of individual syngnathids removed from BNP by the commercial bait shrimp trawl fishery

year	trips	*C. albirostris*	*H. erectus*	*H. reidi*	*H. zosterae*	*S. floridae*	*S. louisianae*	*S. pelagicus*	*S. scovelli*	*TOTAL*
2008	1,341	1498	2105	308	100	1207	25	8	225	5476
2009	653	729	1025	150	49	588	12	4	109	2666
2010	660	737	1036	152	49	594	12	4	111	2695
2011	1,071	1196	1681	246	80	964	20	7	179	4373
2012	1,157	1292	1816	266	86	1041	22	7	194	4724
2013	417	466	655	96	31	375	8	3	70	1703
2014	514	574	807	118	38	462	10	3	86	2099
2015	1172	1309	1840	269	87	1055	22	7	196	4786
2016	1621	1811	2545	372	121	1459	30	10	272	6619
AVERAGE	956	1068	1501	220	71	860	18	6	160	
SD	388.164	434	609	89	29	349	7	2	65	
TOTAL	8,606	9613	13511	1976	641	7744	160	53	1442	