Habitat associations of seahorses and pipefishes (Syngnathidae) in Biscayne National Park, Florida, USA

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Habitat associations of seahorses and pipefishes (Syngnathidae) in Biscayne National Park, Florida, USA

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Habitat degradation and loss are key drivers of population decline in some species of seahorses, pipefishes, and their relatives (family Syngnathidae). To better understand the distribution and habitat associations of syngnathids in Biscayne National Park (BNP), a coastal Marine Protected Area (MPA) of international conservation significance in southeastern Florida, we conducted underwater visual surveys for syngnathids at 79 sites stratified into three dominant habitat categories (Reef, Continuous Submerged Rooted Vegetation (CSRV), and Discontinuous Submerged Rooted Vegetation (DSRV)). Non-parametric statistical tests indicate that sites where syngnathids occurred were characterized by fine/muddy sediment, reduced horizontal visibility, relatively high seagrass coverage, and low coverage of sessile benthic invertebrates. No syngnathids were recorded at Reef sites, despite reef-associated species being previously reported in BNP. Logistic regression indicated that the presence of fine sediment (an indicator of low-velocity conditions) and relatively high seagrass coverage were significant predictors of syngnathid presence. Variation in habitat use among the three most abundant sympatric
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syngnathids (*Hippocampus zosterae*, *Syngnathus scovelli*, and *Syngnathus floridae*) suggests niche partitioning based on salinity and/or local hydrodynamic regime. Many conservation assessments assume that, for seagrass associated syngnathids, all available seagrass area is suitable habitat, however this study suggests that sheltered, low-energy seagrass beds characterized by fine sediment may provide a more suitable habitat for the most common syngnathids of BNP. Identification of habitat associations that maximize occurrence within apparently suitable habitat is a useful approach for guiding conservation of data-poor species, especially in the context of ongoing regional and global habitat loss.

Keywords: Biscayne National Park, habitat use, conservation, occurrence, Logistic regression
1. INTRODUCTION

Assessment of habitat quality or importance – the relative ability of a habitat to support individual growth and population persistence – is often inferred from patterns of habitat use in natural systems (Werner et al. 1977, Garshelis 2000, Manly 2002). Both abiotic (i.e., water depth, velocity, and salinity) and biotic (i.e., vegetation type, percent coverage of benthic invertebrates) attributes can influence habitat use and selection. For instance, many fishes are often strongly adapted to foraging in either sheltered environments with low velocities, or habitats with strong currents and wave action in coastal habitats (Harding et al. 1998). Habitat associations influence survival, growth, and reproduction of all animals but may be especially important for relatively sedentary species that do not readily change locations (Caldwell & Gergel 2013, Seitz et al. 2014). More mobile species may repeatedly sample different habitats, moving among them to select suitable locations. In contrast, less mobile species may be more constrained in their ability to track optimal environmental conditions (Abrahms et al. 2021) and may remain close to the habitat where they first settle, coping with the conditions they find (Tischendorf & Fahrig 2000, Caldwell & Gergel 2013).

Seahorses and their relatives, the pipefishes, sea dragons, and pipehorses (family Syngnathidae) are relatively sedentary species that are globally distributed in predominantly shallow temperate, sub-tropical and tropical waters (Dawson 1985). They are associated with a multitude of habitats, including seagrasses (Bell et al. 2002, Malavasi et al. 2007, Masonjones et al. 2010), corals (Perante et al. 2002), mangroves (Aylesworth et al. 2015, Rosa et al. 2007), macroalgae (Strawn 1958, Moreau & Vincent 2004) and sponges (Kuiter 2001). Many syngnathids are associated with sensitive coastal habitats that are threatened including mangroves (Polidoro et al. 2010),
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seagrasses (Short et al. 2011), coral reefs (Carpenter et al. 2008), and estuaries (Blaber et al. 2000); in these areas, they are vulnerable to changes in both habitat extent and quality (Vincent et al. 2011). Furthermore, intrinsic biological characteristics including small home ranges, low population densities, and complex reproductive sociality may contribute to increased vulnerability (Foster & Vincent 2004). Most syngnathid species studied to date have bipartite life histories, with planktonic young and adults that exhibit site fidelity and are camouflaged to match the local habitat (Harasti et al. 2014, Vincent et al. 2005, Foster & Vincent 2004). Indeed, adults may have home ranges of only a few square metres for months or years on end (Harasti et al. 2014, Vincent et al. 2005, Foster & Vincent 2004), although at least some species do appear to also have seasonal migrations (Power & Atrill, 2003, Bolland & Boettcher 2005, Monteiro et al. 2006).

The loss and/or degradation of essential habitat is a notable threat to many syngnathids worldwide and is a leading factor in the threatened status of species as diverse as *Hippocampus whitei* from estuaries in New South Wales, Australia, to *Hippocampus capensis* in South African lagoons (Claassens et al. 2020, Foster & Vincent 2004, Harasti 2016, Vincent et al. 2011). Population declines have been recorded following physical degradation of habitat associated with coastal development (Harasti 2016, Masonjones et al. 2010), fishery impacts (Marcus et al. 2007), pollution (Livingston 1984), and eutrophication (Ripley & Foran 2007). Consequently, syngnathids are listed under national or regional endangered species legislation in many countries (Stanton et al. 2021). Conservation measures include protected areas, fishery-specific management plans, endangered species legislation, and export regulations. Marine Protected Areas (MPAs) are among the suite of management tools used for the conservation of marine
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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 protecting and restoring benthic habitats and fisheries (Halpern 2003, Mumby et al. 2007, Pandolfi et al. 2003). While MPAs are often established to protect sensitive species like seahorses, the habitat dependencies of these species are often poorly understood. For example, conservation efforts for seahorses in the Philippines have led to the establishment of 35 new MPAs (Marcus et al. 2007, Project Seahorse Foundation for Marine Conservation) despite a limited understanding of seahorse habitat needs (Yasué et al. 2012, Vincent et al. 2011). In adopting such conservation measures, it would clearly help to understand patterns of habitat use of resident seahorses and their relatives.

To this end, we conducted systematic underwater visual surveys to determine syngnathid habitat associations across 79 sites in Biscayne National Park, an MPA in southeastern Florida that is hydrologically connected to the internationally significant Greater Everglades terrestrial and freshwater ecosystem (Browder et al. 2005, Gunderson & Light 2006, Sklar et al. 2005). BNP 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, NPS 2015). Unfortunately, the park is positioned to the south and east of one of the most rapidly developing urban centers in the United States, Miami-Dade County, and has experienced decades of anthropogenically-induced change including decreasing freshwater inputs caused by watershed management, downstream effects of rapid coastal development in neighboring areas, and disturbances from commercial and recreational boating and fishing (Caccia & Boyer 2005, Crain et al. 2008). These and other
stressors have resulted in the loss and fragmentation of coastal seagrasses (Browder et al. 2005, Santos et al. 2011, Serafy et al. 1997) and extensive declines in coral cover within the park (Dupont et al. 2008).

Even though 15 species of syngnathid have been recorded in BNP (Table S1), two of which are species of conservation concern (IUCN 2022), their habitat associations remain poorly understood. In this study we investigated (1) the distribution and associations of syngnathids with respect to major habitat types and environmental correlates, (2) the relative abundance of different syngnathid species in BNP, and (3) the environmental variables that best predict occurrence and discriminate habitat use among the most abundant syngnathid species.
2. MATERIALS & METHODS

Study site and species

Biscayne National Park (BNP) is a 728 km$^2$ predominantly marine U.S. National Park and
marine protected area (MPA) located in the Atlantic Ocean, off the coast of southeastern Florida
(Figure 1). Designated a U.S. National Park in 1980, the Biscayne National Park MPA preserves
the longest remaining continuous stretch of mangrove on the eastern coast of Florida, extensive
seagrass meadows in Biscayne Bay, and coral reefs at the northern extent of the Florida Keys
(Ault et al. 2001, Estep et al. 2017). BNP is roughly divided on a north-south axis into relatively
shallow, sheltered Biscayne Bay (average depth 3 m) and a deeper, more exposed offshore area
that may experience stronger currents (Fig. 1, Ault et al. 2001, Estep et al. 2014). Although many
activities that will directly harm habitat are regulated within BNP (e.g., coral removal or bottom
trawling), protection offered by the MPA is limited because recreational, commercial, and
subsistence fishing are permitted in many areas of the park (NPS 2015).

Twelve syngnathid species have been recorded in published surveys of BNP (Roessler et al.
2002, Serafy et al. 1997, Ault et al. 2001), while an additional three species have been reported
in the grey literature (Supporting Information, Table S1). Three of the recorded syngnathids are
seahorses, of which one is Near Threatened and one is Threatened /Vulnerable (IUCN 2022,
Table S1).

Survey site selection

Survey sites to determine syngnathid habitat associations were selected using random sampling
stratified by three major habitat types codified in Madley et al. (2002): (1) Continuous
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Submerged Rooted Vegetation (CSRV – predominantly seagrass communities comprised of *Thalassia testudinum, Syringodium filiforme,* and/or *Halodule wrightii*), (2) Discontinuous Submerged Rooted Vegetation (DSRV – mixed seagrass and hardbottom), and (3) Reef (Ault et al. 2001, Madley et al. 2002, Rohmann 2008). The three dominant habitat categories differed primarily in terms of sediment characteristics, the extent of vegetative cover, and associated shelter from currents for benthic dwelling animals. CSRV was defined by Madley et al. (2002) as the presence of continuous beds of any shoot density covering ≥ 90% of the substrate. DSRV was defined as areas of rooted vegetation covering 10% to <90% of the substrate with breaks in coverage resulting in isolated patches. Reef habitats, which included live and dead coral cover, were characterized by hardened substrate formed by exposed bedrock with variable coverage by benthic plants or animals, or by reefs created by the bio-deposition of calcium carbonate (e.g. coral) and includes a composite of coral-reef associated features including aggregate reef, patch reef, pavement, and reef rubble (Estep et al. 2017, Madley et al. 2002).

A total of 79 sites were surveyed from May–September 2016. Thirty-eight sites were located inside and 41 were located outside Biscayne Bay (Figure 1). Two sites inside Biscayne Bay were sampled slightly north of the BNP boundary but were retained for analysis. The open-source Geographic Information System (GIS) platform QGIS (QGIS development team 2016) was used to overlay sites and data atop publicly available habitat layers (FWRI 2016) for visualization purposes.

Our surveys aimed for a representation of 50% CSRV, 40% DSRV, and 10% Reef habitat, roughly corresponding to the relative area of these habitat types within BNP boundaries (Ault et
To confirm the original map-based habitat classifications, *in situ* classifications were also made at each site, resulting in 38 sites (48%) classified as CSRV, 28 as DSRV (35%), and 13 as Reef (16%). Concordance of field classification with preliminary map-based habitat designations was 82% for CSRV sites, 57% for DSRV and 61% for Reef sites. Discrepancies likely reflect the coarse spatial scale of maps used to design the study. References to habitat category henceforth refer to categories assigned using field data.

**Survey methodology**

Fish were counted during 70-minute timed swims at each site. Timed swims use an unrestricted search area and have been found to be more effective than belt transects in locating cryptic, data-poor species such as seahorses (Aylesworth et al. 2017). Paired SCUBA divers conducted non-overlapping 20-minute timed swims in each of four cardinal directions (i.e., a cross) originating from a central node marked by the location of the sampling boat anchor. The last transect survey at each site was performed in the direction of the prevailing current and shortened to 10 minutes to better standardize the distance travelled for all timed swims. Distance travelled was recorded on a GPS unit towed behind one of the paired divers, and time was recorded using an underwater watch. GPS tracks were used to confirm non-overlapping searches for each site. We constrained all searches to 70 minutes per site to standardize search effort. Many patch reefs were not large enough to accommodate a full four transects; the search plan was therefore modified to a non-overlapping “U” pattern for patch reefs to accommodate the restricted size and/or non-uniform patch dimensions of these features while maintaining the 70-minute search time. In addition to passive visual observations, divers also actively used their hands to search through vegetation or debris to ensure high detection rates when cover was present; this resulted in ~20% slower
search rates (area/time) in vegetated patches with heavy cover relative to more open reef sites. Divers were instructed to maintain an effective lateral visual search area of approximately 0.5 m on all transects. The mean area searched per site was 224.4 m (SD = 98.9 m).

Fish counts and observations
Timed swims were paused when a syngnathid was encountered. Syngnathids were video recorded before attempting capture to verify species identification, sex, and to estimate size. Due to variable capture efficiency, size and sex data were excluded from analyses. All research and fish handling were executed in accordance with the University of British Columbia's Animal Care Committee permit A12-0288.

Measured habitat variables
We measured variables related to both water quality and benthic habitat attributes to quantify mean habitat conditions at each site. Variables included measures of water quality (salinity, visibility), benthic habitat (sediment depth, substrate type), and percent coverage of seagrass, invertebrates, and algae (Table 2). Water samples were collected within 30 cm of the bottom and brought to the surface where salinity (ppt) was measured using a refractometer. 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 2.5 cm diameter PVC pipe could be pressed into the sediment by hand. To characterize benthic habitat, we classified benthic habitat features according to 15 microhabitat attributes, which were expressed as percent of bottom cover in survey quadrats.
Percent cover for each microhabitat attribute was estimated by taking eight approximately 1 m² photographic quadrats at each site approximately 1 m above the substrate (2 photos from each of the four transects, taken at the beginning and the end of each transect). Photoquadrats were later analyzed to identify habitat features and generate percent coverage data (list of percent coverage variables available in Table 2). Five seagrass blade lengths (cm) of the predominant seagrass species were directly measured with a tape at the survey origin, and total water depth was measured with a digital depth sounder.

Data analysis

Percent coverage data

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 benthic features. Twenty-five points were randomly overlaid on each photograph and classified according to the underlying microhabitat feature. The proportion of points falling within each habitat category was divided by the total number of points to generate percent coverage data for each microhabitat type. To avoid including estimates of percent mud, sand, and rock/rubble cover as redundant predictor variables, sites were classified into one of three categories (A = mud, B = sand, C = rock) according to the dominant sediment type.

Pairwise comparisons of occupied and unoccupied habitat

Binary presence/absence data were analyzed as frequencies using contingency tables and the chi-square statistic (performed in XLStat 2017) to test the null hypothesis (H₀) that occurrence was independent of 1) major habitat type and 2) location inside/outside Biscayne Bay (Quinn &
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Pairwise comparisons were used to assess differences in habitat attributes between sites where syngnathids were present and absent, among the three major habitat types (CSRV, DSRV, Reef), and among the three most abundant syngnathid species. Continuous microhabitat data were analyzed using nonparametric statistical tests (Mann-Whitney U tests performed in XLStat, 2017) because assumptions of normality and equality of variance were violated for most variables. To account for multiple comparisons, inflation of Type 1 error across multiple pair-wise tests was controlled by applying a Bonferroni correction to adjust a-priori significance level from $\alpha = 0.05$ to $\alpha = 0.0003$ ($n = 155$ tests).

Prediction of syngnathid presence using logistic regression

We also used environmental predictors to develop a multi-variable logistic regression model of syngnathid presence. Candidate variables were initially screened by fitting univariate logistic regression models with a logit link function using the “glm” function in {R} (R Core Team 2021). Only independent variables with a univariate significance $\leq 0.25$ were retained for subsequent inclusion in the multivariate logistic regression (Hosmer & Lemeshow 2000, Table S3). Collinearity and coefficient inflation were reduced by examining correlations and relationships among continuous and categorical predictor variables. For continuous variables, Pearson correlation coefficients were calculated in {R} using the “corr” function in the boot package (Canty & Ripley 2019). Variable combinations resulting in significant ($p < 0.05$) Pearson correlation coefficient absolute values $> 0.40$ were excluded from the analysis (Zuur et al. 2010, Tables S4, S5). Nominal logistic regression was used to examine relationships between paired continuous/categorical predictor variables at $p$-value of $p = 0.05$. Variance inflation factors (VIFs) were calculated using the “vif” function in the “car” package (Fox & Weisberg...
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2019) for all variables used in multivariate models (Table S6). In addition to examining possible
collinearity and resulting coefficient inflation, standardized residual and Cook’s distance plots
were assessed to confirm the absence of influential observations.

This initial full model was reduced to a main effects model (Hosmer et al. 2013), henceforth “the
model”, by assessing the p-value of the Wald statistic for each covariate and eliminating
variables above a threshold significance value of 0.05. Previously excluded variables were singly
added to the model to examine their influence on the significant coefficient estimates. Variables
that changed coefficient estimates by > 20% were considered to provide a potential needed
adjustment in the presence of the main effects model’s significant covariates (Hosmer et al.
2013). Interactions between variables were not significant and were therefore not included in the
final model. Standardized residual and Cook’s distance plots were assessed to confirm the
absence of influential observations.

Model fit was evaluated using likelihood ratio tests (“lmtest” in the {R} package “lmtest”;
Zeileis & Hothorn 2002), where a significant likelihood ratio test indicates that the more
complex model provides a significantly better fit. A repeated k-fold cross validation procedure (n
= 10, k = 5) was performed using the “train” function in the “caret” package (Kuhn 2008) to
derive model performance metrics (model specificity, sensitivity, and classification accuracy;
Agresti 2007). The final model is presented with unstandardized log-odds and odds ratio
coefficient estimates.
3. RESULTS

Syngnathid species encountered in Biscayne National Park

A total of 146 individual syngnathids were recorded at 49 of the 79 sites surveyed ($f =$ frequency of occurrence $= 0.62$, Table 1). Of these, 123 individual syngnathids were positively identified in the field as one of nine species; twenty-three individuals, mostly juveniles, could not be reliably identified in the field (Table 1). The most frequently recorded species were the dwarf seahorse (*Hippocampus zosterae*, 36 individuals recorded at 22 sites, $f = 0.28$), gulf pipefish (*Syngnathus scovelli*, 32 individuals recorded at 18 sites, $f = 0.23$), and dusky pipefish (*Syngnathus floridai*, 32 individuals recorded at 17 sites, $f = 0.22$). Less frequently encountered species include *Anarchopterus criniger, Cosmocampus albirostris, Cosmocampus brachycephalus, Syngnathus louisianae, Hippocampus erectus*, and *Syngnathus pelagicus*, all of which occurred at 5 or fewer sites ($f < 0.06$). Search rates for syngnathids tended to be non-significantly slower at syngnathid-occupied sites, likely due to the shorter distances covered while slowly hand searching through the seagrass in the 70-minute timed swim interval (Table 2). The mean search rate at sites occupied by *S. scovelli* were, however, significantly higher than at sites occupied by *H. zosterae* (0.99 SD 0.87 vs 0.52 SD 0.55, $p = 0.005$)

General habitat characteristics of occupied sites

Sites where syngnathids occurred generally had lower horizontal visibility, relatively high seagrass coverage, and reduced coverage of sessile benthic invertebrates (Table 2). Additionally, there was a significant association between sediment type and syngnathid presence ($X^2 = 23.4$, df $= 2$, $p < 0.0001$), with syngnathids occurring significantly more frequently at sites with muddy sediment (Fisher’s exact test, $p < 0.0001$) and less frequently at sites with rocky sediment.
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(Fisher’s exact test, p < 0.0001). Higher occurrence of syngnathids at sites with fine/muddy sediment corresponded with a greater frequency of occurrence at sites with seagrass cover (p < 0.0001), and low coverage of rock-associated benthic invertebrates, including sponges (p < 0.0001), corals (p < 0.0001), gorgonian corals (p < 0.0001), and reef-associated turf algae (p < 0.0001, Table 2).

Landscape scale habitat associations (inside vs. outside Biscayne Bay)

The frequency of habitat types and sediment categories differed inside vs. outside of Biscayne Bay. Significantly more fine sediments, such as silts and muds, were recorded at sites inside Biscayne Bay (Fishers exact test, p < 0.0001), while coarser sediments, including coarse sands and coral rubble, were found predominantly outside the Bay (Fisher’s exact test, p < 0.0001). Reef sites were only present outside the Bay, and correspondingly the mean coverage of reef-associated features was higher outside the Bay, particularly for scleractinian corals (1.47 % vs 0.06 %, p < 0.0001) and turf algae (4.93 % vs 0.06 %, p < 0.0001).

The proportion of occupied sites was higher inside than outside Biscayne Bay (74 % vs 49 % occupied) but the effect was not significant after applying the Bonferroni correction ($\chi^2 = 13.1$, df = 1, p = 0.04). Differences in the mean number of species (1.69 vs. 1.63, p = 0.65) and individuals (3.21 vs. 2.71, p = 0.28) within syngnathid-occupied sites inside and outside Biscayne Bay were less pronounced and not statistically significant.

Three syngnathid species dominated the BNP area, but their abundances differed inside and outside the Bay. *S. scovelli* ($f = 0.66$) was the most abundant inside the Bay, although *H. zosterae*...
(f = 0.53) and *S. floridae* (f = 0.47) also occurred with high frequency. In contrast, *S. scovelli* (f = 0.17) was least abundant outside the Bay, where *H. zosterae* (f = 0.39) and *S. floridae* (f = 0.34) occurred with comparable frequency. Among the less-commonly encountered syngnathids, two species (*Anarchopterus criniger* and *C. brachycephalus*) were more abundant or only recorded inside Biscayne Bay while four species (*Syngnathus louisianae*, *Cosmocampus albirostris*, *H. erectus*, and *S. pelagicus*) were more abundant or only recorded outside Biscayne Bay.

Collectively, these distributions suggest a general sensitivity of syngnathids to an offshore gradient of environmental conditions.

**Major habitat associations**

Environmental conditions differed significantly among major habitat categories (Table S2). Measured environmental conditions at Reef sites were the most distinct (Table S2), however we recorded no syngnathids at Reef sites. CSRV sites had a mean of 72 % ± 24 % seagrass coverage, while DSRV had a significantly lower mean coverage of 36 % ± 28 % (p < 0.0001). Additional differences in salinity, horizontal visibility, search rate, seagrass blade length, and gorgonian coverage between CSRV and DSRV sites were not significant with a Bonferroni correction (Table S2).

Significant correlations between syngnathid presence and major habitat type ($\chi^2 = 25.803$, df = 2, p < 0.0001) were driven by the absence of syngnathids at Reef sites. Syngnathids occurred with similar frequencies at CSRV and DSRV sites (71 and 79% occurrence, respectively; $\chi^2$ =0.477, df = 1, p = 0.490; Figure 1, Table 3). A total of nine and seven syngnathid species were recorded at CSRV vs. DSRV sites, respectively (Table 3), but neither species richness or the mean number
of observed individuals differed significantly between CSRV and DSRV sites (p = 0.87 and p = 0.99, respectively).

*S. floridae* were dominant at CSRV sites (*f* = 0.71; *H. zosterae* *f* = 0.61, *S. scovelli* *f* = 0.26) but were less frequent at DSRV sites where *S. scovelli* was most abundant (*f* = 0.79; *H. zosterae* *f* = 0.46, *S. floridae* *f* = 0.18, and *C. brachycephalus* *f* = 0.18, Table 3). The lined seahorse (*H. erectus*) and *S. pelagicus*, both rarely recorded in our surveys, were uniquely found at CSRV sites (Table 3).

Predicting syngnathid occurrence - Logistic regression

Univariate logistic regressions indicated significant (p < 0.05) relationships between the occurrence of syngnathids and depth, visibility, sediment type, and the % cover of seagrass, *Thalassia*, invertebrates, sponge, coral, gorgonian, and turf algae (Table S3). Excluding variable pairs with Pearson correlations greater than absolute value of 0.40 to minimize collinearity in regression (Zuur et al. 2010) reduced the initial suite of 10 candidate variables to four (Table S4). Only depth, sediment type, % coverage seagrass, and % coverage turf algae were retained in the full model (Hosmer et al. 2013), and in combination exhibited adjusted VIFs of < 1.5 (Table S6).

The four-term logistic regression model did not provide a significantly better fit than either a three-term model with turf algae removed (likelihood ratio (LR) test, *X*²(-1, N = 70) = 2.26, p = 0.13) or the more parsimonious two-term final model ((LR test, *X*²(-2, N = 70) = 4.60, p = 0.10, Table S7)), which retained only sediment type and % seagrass coverage, with mud sediment and seagrass both having positive effects on occurrence without interaction. Differences in overall
accuracy between the four, three, and two variable logistic regression models were minor (Table S8).

The final two-variable logistic regression model (including sediment type and continuous seagrass cover) explained 50% of the variance in syngnathid presence (Tjur’s $R^2$, Table S8), with a pooled sensitivity (correct classification of presence) of 60%, pooled specificity (correct classification of absence) of 88%, and overall correct classification of 78% ± SD 12%.

Coefficient estimates suggest the strongest significant effects are for sediment type (rocky sediments and sandy sediments, followed by muddy sediment), followed by % seagrass cover (Table 4). Muddy sediment and increasing seagrass coverage increased the probability of syngnathid presence, with the effect of muddy sediment being roughly 74% greater than the effect of seagrass (odds ratios of 1.79 and 1.03, Table 4). Coarser sediments, such as predominantly sand or rock, had significantly lower probability of occurrence of syngnathids (odds ratio of 0.07 and < 0.00, respectively). The exponentiated difference in substate type parameter estimates between muddy sediments and sandy sediments (17.12) suggests that the odds of encountering a syngnathid on muddy sediments are roughly 17 times greater than on sandy sediments, and that the probability of encountering syngnathids over rocky sediments is extremely low (Table 4).

Model predictions (Figure 2) suggest that the probability of encountering syngnathids in areas with muddy sediment is, on average, 64% (95% with confidence interval (CI) of 35%–87%), increasing to 93% (CI 47% – 100%) at sites with 50% seagrass and nearly 100% (CI 60% –
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100 %) at sites with maximum seagrass coverage. In contrast the probability of encountering a syngnathid over sandy sediments averages 5 % (CI 0 % – 50 %) at sites without seagrass and 67 % (CI 0 % – 100 %) at sites with 100 % seagrass. Predicted occurrence over rock sediment is effectively zero, reflecting the fact that syngnathids were never collected in association with this microhabitat.

Differentiation of habitat use among *S. scovelli*, *S. floridae* and *H. zosterae*

The three most common syngnathids encountered in this study showed strong differences in habitat associations on multiple habitat attributes, including site depth, salinity, sediment depth, and benthic invertebrate cover (Figure 3). The most conspicuous patterns of habitat association among the three species were the contrasts between *S. scovelli*, found at significantly lower salinity sites with low seagrass coverage, short blade lengths and high macroalgal cover, and the congeneric *S. floridae* found at sites with significantly higher salinities, greater seagrass coverage, longer blade lengths, and less macroalgae cover (Figure 3), likely corresponding to contrasting environmental conditions between nearshore sites within Biscayne Bay and sites further removed from freshwater inputs (Figure 1). Conditions at sites occupied by *H. zosterae* were generally intermediate (Figure 3). A contingency table analysis found no significant differences among species in sediment type associations; all three species were most frequently recorded at sites with predominantly silt, clay, or mud sediments.
4. DISCUSSION

Our findings on how syngnathid fishes are associated with marine habitats in Biscayne National Park advance understanding of habitat use by the region’s syngnathids as a family, as well as for three of the most abundant species in the park. Syngnathids displayed associations with discrete habitat types, implicating habitat as a major correlate of presence and abundance. In particular, the presence of fine sediment and high seagrass coverage were significant predictors of syngnathid presence in BNP. Fine grain sizes directly influence ecosystem (Erftemeijer 1994, Mateo et al. 2006) and population (Jumars & Nowell 1984, Koch et al. 2006) dynamics and can also serve as an indicator of low-energy hydrodynamic conditions (Madley et al. 2002, van Duin et al. 2001). The effect of sediment type was nearly 75% greater than the effect of seagrass coverage, indicating that sheltered, low-energy seagrass beds characterized by fine/muddy sediment may provide a more suitable habitat for syngnathids than seagrass beds in areas with coarse sediments. Sediment coarseness generally increased with offshore distance in BNP; the finest sediments, such as muds, silts, and clays, were found in protected Biscayne Bay, while the coarsest sediments were found in deeper water exposed to stronger hydraulics outside of Biscayne Bay. Such distribution means that most syngnathids were found in the Bay.

Although the nine syngnathid species that we observed in our BNP surveys (Table 1) were present in earlier (1968 – 1973) biodiversity assessments of southern Biscayne Bay and Card Sound (Brook 1977, Roessler et al. 2002) we did not detect the previously recorded (large and charismatic Longsnout Seahorse, *Hippocampus reidi* (Ault et al. 2001, Lourie et al. 2004) or two smaller pipefishes, the Insular Pipefish (*Anarchopterus tectus*) and the Pugnose Pipefish (*Bryx dunckeri*, as *Syngnathus dunkeri*) recorded by Serafy et al. (1997). This discrepancy in species
detection and abundances could be an artefact of differences in survey location or gear type, e.g. throw traps vs. otter trawls vs. our visual underwater surveys (Bobsien & Brendelberger 2006).

The Longsnout Seahorse (*Hippocampus reidi*) and the Whitenose Pipefish (*Cosmocampus albirostris*) are generally associated with reef habitats (Kuiter 2009, Lourie et al. 2004, Rosa et al. 2007, Table S1). Although neither species was recorded at the Reef sites we surveyed in BNP, it is possible that increased survey effort could have resulted in sightings of these syngnathids. Alternatively, observed declines in the coverage and quality of coral reefs in BNP (Dupont et al. 2008) may account for the absence of these syngnathids from our surveyed Reef sites. Cross-calibrating different gear types to better understand temporal trends would be valuable for *H. reidi* as the species is assessed globally as Near Threatened on the IUCN Red List (Oliveira & Polom 2017), while the others are all considered to be of Least Concern.

Sheltered, low-velocity waters may be a shared habitat preference for many syngnathids (Ashley-Ross 2002, Fedrizzi et al. 2015, Lourie et al. 2005, Sogard et al. 1989; however, see Harasti et al. 2012), which tend to be relatively weak swimmers (Ashley-Ross 2002). Low currents also improve the deposition, retention, and accumulation of organic matter and nutrients (Fonseca & Bell 1998, Koch et al. 2006), influence larval recruitment (Bell & Westoby 1986, Bertola et al. 2020), mobility (Ashley-Ross 2002, Vogel 1994), the abundance of prey and feeding rates (Bell et al. 1994), and the composition of the epiphyte community that forms the base of seagrass food webs (Koch et al. 2006, Heck et al. 2008). Grain size can serve as an indicator of current velocity; fine sediment particles are more likely to become suspended and transported in the water column with greater wind, current, or wave energy, leaving behind exposed coarse sediments that are too heavy to transport (Stokes Law, van Duin et al. 2001,
Syngnathid Habitat Use in BNP

Madley et al. 2002). If fine grain sizes correlate to low-velocity hydrodynamic conditions, our modelling suggests that sheltered, low energy seagrass beds characterized by fine sediment may provide a more suitable habitat for the most common syngnathids than structurally comparable seagrass beds in more energetic settings characterized by coarse sediment. Field studies have confirmed that, in the absence of sheltering effects, some fishes that are typically associated with seagrass habitats are entirely absent or present at low densities (Hovel et al. 2002, Nagelkerken et al. 2002). Syngnathid occurrence may be directly mediated by an upper current velocity threshold that is lower than the upper threshold for seagrass occurrence (Fonseca & Bell 1998, Koch et al. 2006). Below this hypothetical velocity threshold associations with local habitat features may then determine the relative abundance of different syngnathids.

Our modelling indicates an elevated probability of syngnathid occurrence with increasing seagrass coverage; sites occupied by syngnathids had roughly double (60% vs. 30%) the seagrass coverage of unoccupied sites. Many studies report higher fish density with greater seagrass density (Ault et al. 2001, Gillanders 2006, Turner et al. 1999), and changes in syngnathid abundance have often been linked to the extent and coverage of seagrass (Fourqurean & Robblee 1999, Gillanders 2006, Masonjones et al. 2010, Matheson et al. 1999, Rose et al. 2019, Sheridan et al. 1997). Seagrass beds with higher percent coverage may harbor high abundances of copepods and other syngnathid prey (Brook 1977, Lewis 1984, Manning et al. 2019, Virnstein et al. 1983), potentially facilitating more effective ambush predation by syngnathids (Kendrick & Hyndes 2003) while providing refuge from larger predators (Heck & Orth 2006, Nelson 1979).
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Although low frequency of occurrence precluded analysis of species-specific habitat associations for most syngnathid taxa, *H. zosterae*, *S. scovelli*, and *S. floridae* were sufficiently abundant to allow some level of statistical inference concerning habitat selection. The strongest pattern of habitat differentiation appeared to be an offshore gradient of elevated depth and salinity where *S. floridae* increased and *S. scovelli* decreased in abundance, supporting the hypothesis of niche partitioning between these pipefishes (Brook 1977). *S. floridae* was generally found in deeper waters and its narrower observed salinity range is consistent with a minimum reported salinity tolerance of 28 ppt (Mobley & Jones 2007, Masonjones et al. 2010), compared to the lower (0–25 ppt) and substantially more variable salinity ranges reported for the euryhaline *S. scovelli* (Lirman et al. 2016, Partridge et al. 2007, Santos et al. 2011, Whatley 1969).

While each species was associated with fine sediments, *H. zosterae*, was most likely to be found in areas with significantly deeper sediment. Deep, fine sediment can indicate areas of lower velocities with high rates of deposition of particulate and/or organic material (Koch et al. 2006). *Hippocampus zosterae* is a small ambush predator with relatively limited mobility (Masonjones et al. 2019) and, like many seahorses, is hypothesized to have a small home range size (Foster and Vincent 2004, Masonjones et al. 2019). Researchers have speculated that *H. zosterae* may rely primarily on local currents as a means of passive dispersal and maintaining population connectivity (Fedrizzi et al. 2015, Masonjones et al. 2010). Our findings suggest that persistent *H. zosterae* populations may be more frequently found in areas with reduced exposure to high current velocity. Despite this apparent preference for low energy habitats, Masonjones et al. (2010) described *H. zosterae* as a generalist in the seagrass landscape, occurring across a wide
range of seagrass densities within sheltered Tampa Bay. This is broadly consistent with our observation that *H. zosterae* was caught at more sites than any other species.

**Implications for syngnathid conservation**

Our study points to the value of identifying and safeguarding remaining sheltered seagrass beds and lower velocity habitats with fine substrate for securing syngnathid populations within BNP. Habitat loss and degradation are key drivers of population declines in many syngnathids (Harasti 2016, Masonjones et al. 2010, Matheson et al. 1999, Vincent et al. 2011); Indeed, of the 20 Endangered, Vulnerable, or Near Threatened syngnathid species, habitat loss or degradation was listed as a threat for 19 of them (Pollom et al. 2021, IUCN 2022). The three species whose habitat associations we could explore specifically are assessed as Least Concern on a global scale, but the status of their BNP populations is not known. Furthermore, very low catches of less common species, including the globally threatened *Hippocampus erectus* and *Hippocampus reidi* (Pollom et al. 2021, IUCN 2022) prevented quantitative assessment of their habitat associations. We do know, however, that critical marine habitats in BNP have been degraded by stressors including salinity fluctuations caused by freshwater management practices that alter or divert estuarine flows (Browder et al. 2005, Lirman & Cropper 2003, Santos et al. 2011), regional water quality declines (Millette et al. 2019), an increased frequency of harmful algal blooms associated with eutrophication (Boyer et al. 2009, Collado-Vides et al. 2013, Millette et al. 2018), sedimentation (LaPointe & Clarke 1992), fishing, and the direct physical impacts of boating (Ault et al. 1997, Bell et al. 2002, Dupont et al. 2008). Sustained attention to protecting water quality and the health of the more sheltered habitat areas in light of ongoing habitat degradation would likely pay dividends for syngnathid fishes.
Many existing status assessment and conservation tools rely on estimates of range size derived from available habitat use information (IUCN 2012, Masonjones et al. 2017, Carlson et al. 2019). Range size is assumed to be correlated to population size (Mace 1994, IUCN 2022), and changes in range are interpreted as indicative of population trends (Corsi et al. 2000). These methods typically assume a uniform distribution of both habitat quality and the focal species across the landscape, and may badly overestimate the occupied range for species with specific uncaptured habitat associations (i.e., a preference for low-velocity or mesohaline waters within the seagrass landscape), or species – like syngnathids – with intrinsic characteristics including low mobility and/or low dispersal potential that render them more sensitive to changes in the amount and fragmentation of suitable habitat (Caldwell & Gergel 2013, Fedrizzi et al. 2015). To be most effective, long-term conservation of relatively data-poor species should identify the intrinsic and environmental thresholds that limit occurrence within apparently suitable habitat, especially in the context of ongoing regional and global habitat loss.
Acknowledgements

This is a contribution from Project Seahorse. We are grateful to Jane Carrick, Cate Gelston, Dr. Chelsea Bennice, Rachel Plunkett, Colin Foord, Dr. Heather Masonjones and Dr. Sarah Foster for their participation in field work, and to Victoria Burdett-Coutts for her excellent training in scientific diving. Tremendous gratitude is extended to Dr. Sarah (Sally) Otto for her exceptional leadership and guidance in the drafting of this manuscript. We additionally acknowledge with appreciation the kind engagement of the following individuals: Dr. Lindsay Aylesworth, Dr. Joan Browder, Tom Jackson, and Dr. Les Kaufman. The following colleagues generously reviewed the manuscript: Dr. Eric Taylor, Clayton Manning, and Dr. Angela Stevenson. We would also like to thank the captain and crew of the F/V Gladiator and the F/V Warrior who kindly provided galley table space and access to expensive onboard Wi-Fi in the Bering Sea during the revision process. We sincerely thank the Herbert W. Hoover Foundation for generously funding the field work and manuscript production. We also thank Guylian Chocolates Belgium for its ongoing support through a partnership for marine conservation with Project Seahorse.
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Contributions

E. Stump contributed to design and conception, collected the data, analyzed the data, interpreted the data, and wrote the paper. J. Rosenfeld provided advice on data analysis and edited various versions of the paper. A. Vincent contributed to developing methods to study syngnathids, obtained funding, provided guidance during field work, and edited various versions of the paper.
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Syngnathid Habitat Use in BNP


https://doi.org/10.3354/meps243011


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Syngnathid Habitat Use in BNP


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for the Oceans and Fisheries, Fisheries Centre Research Reports volume 29, issue 2. Vancouver, Canada.


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### Table 1. Occurrence and relative frequency of syngnathids at 79 sites surveyed in Biscayne National Park from May – September 2016. Study-wide occurrence is compared to surveys conducted by Roessler et al. 2002 (surveys conducted from 1968 – 974), Serafy et al. 1999, and Ault et al. 2001.

<table>
<thead>
<tr>
<th>Species</th>
<th>IUCN Red List status</th>
<th># ind.</th>
<th># sites</th>
<th>frequency of occurrence (f)</th>
<th>Roessler et al. 2002</th>
<th>Serafy et al. 1997 *</th>
<th>Ault et al. 2001 **</th>
</tr>
</thead>
<tbody>
<tr>
<td>all Syngnathidae</td>
<td></td>
<td>146</td>
<td>49</td>
<td>0.62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hippocampus zosterae</em></td>
<td>LC</td>
<td>36</td>
<td>22</td>
<td>0.28</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><em>Syngnathus scovelli</em></td>
<td>LC</td>
<td>32</td>
<td>18</td>
<td>0.23</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><em>Syngnathus florides</em></td>
<td>LC</td>
<td>32</td>
<td>17</td>
<td>0.22</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Anarchopterus criniger</td>
<td>LC</td>
<td>6</td>
<td>4</td>
<td>0.05</td>
<td>x¹</td>
<td>x²</td>
<td>X</td>
</tr>
<tr>
<td><em>Cosmocampus albirostris</em></td>
<td>LC</td>
<td>3</td>
<td>3</td>
<td>0.04</td>
<td>x</td>
<td>x</td>
<td>X</td>
</tr>
<tr>
<td><em>Cosmocampus brachycephalis</em></td>
<td>LC</td>
<td>6</td>
<td>5</td>
<td>0.06</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Syngnathus louisianae</em></td>
<td>DD</td>
<td>4</td>
<td>4</td>
<td>0.05</td>
<td>x</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td><em>Hippocampus erectus</em></td>
<td>VU</td>
<td>3</td>
<td>1</td>
<td>0.01</td>
<td>x</td>
<td>x</td>
<td>X</td>
</tr>
<tr>
<td><em>Syngnathus pelagicus</em></td>
<td>LC</td>
<td>1</td>
<td>1</td>
<td>0.01</td>
<td>x</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Syngnathid Habitat Use in BNP

* Serafy et al. (1997) additionally recorded *Bryx dunckeri* (as *Syngnathus dunckeri*) and *Anarchopterus tectus*

** Ault et al. 2001 additionally recorded *Hippocampus reidi*

1 as *Micrognathus crinigerus* 2 as *Micrognathus crinitus* using earlier nomenclature
Table 2. Means (SE) and standard deviation (SD) of continuous variables, and frequencies of categorical variables (sediment type) at sites unoccupied and occupied by syngnathids in Biscayne National Park. Values in bold indicate significant differences in either means (Mann-Whitney U test for continuous variables) or frequencies (Fisher’s exact test) with a Bonferroni correction adjusting the critical α from 0.05 to 0.0003 (n = 155 tests)

<table>
<thead>
<tr>
<th>variable</th>
<th>absent (n= 30)</th>
<th>present (n = 49)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rate (m² / min)</td>
<td>1.26 (0.17)</td>
<td>0.94 (0.12)</td>
<td>0.035</td>
</tr>
<tr>
<td>depth (m)*</td>
<td>5.15 (0.48)</td>
<td>3.2 (0.28)</td>
<td>0.002</td>
</tr>
<tr>
<td>horizontal visibility (m)</td>
<td>13.33 (1.05)</td>
<td>8.45 (0.43)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>27.41 (0.44)</td>
<td>26.93 (0.44)</td>
<td>0.994</td>
</tr>
<tr>
<td>sediment depth (cm)</td>
<td>9.48 (1.66)</td>
<td>10.57 (1.10)</td>
<td>0.393</td>
</tr>
<tr>
<td>% seagrass</td>
<td>29.23 (6.27)</td>
<td>57.95 (4.57)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>% Halodule</td>
<td>1.38 (1.14)</td>
<td>0.04 (0.04)</td>
<td>0.278</td>
</tr>
<tr>
<td>% Syringodium</td>
<td>4.58 (1.54)</td>
<td>4.11 (2.01)</td>
<td>0.667</td>
</tr>
<tr>
<td>% Thalassia</td>
<td>24.39 (5.12)</td>
<td>53.41 (4.45)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>blade length (cm)</td>
<td>21.27 (1.3)</td>
<td>24.09 (1.27)</td>
<td>0.352</td>
</tr>
<tr>
<td>% invertebrates</td>
<td>14.13 (3.2)</td>
<td>0.82 (0.23)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>% sponge</td>
<td>1.75 (0.46)</td>
<td>0.39 (0.15)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>% coral</td>
<td>2.02 (0.47)</td>
<td>0.05 (0.03)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>% gorgonian</td>
<td>10.28 (2.52)</td>
<td>0.49 (0.18)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>% algae</td>
<td>13.53 (3.38)</td>
<td>6.59 (1.54)</td>
<td>0.315</td>
</tr>
<tr>
<td>% drift algae</td>
<td>1.42 (0.71)</td>
<td>1.94 (0.99)</td>
<td>0.422</td>
</tr>
</tbody>
</table>
**Syngnathid Habitat Use in BNP**

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>% rhyzophoric macroalgae</td>
<td>5.71 (2.36)</td>
<td>12.49</td>
</tr>
<tr>
<td>% turf algae</td>
<td>6.54 (2.19)</td>
<td>11.60</td>
</tr>
</tbody>
</table>

| Sediment type: MUD | 9 (p < 0.0001) | NA | 39 (p < 0.0001) |
| Sediment type: SAND | 14 (p = 0.008) | NA | 8 (p = 0.004) |
| Sediment type: ROCK | 6 (p = 0.004) | NA | 0 (p < 0.0001) |

**Table 3.** Occurrence and species composition of syngnathids identified to species at continuous and discontinuous submerged-rooted vegetation (CSRV and DSRV sites, respectively) surveyed from May–September 2016 in Biscayne National Park, Florida.

<table>
<thead>
<tr>
<th></th>
<th># occupied</th>
<th>SW diversity</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># sites (% total)</td>
<td>(% occupied)</td>
<td># ind.</td>
</tr>
<tr>
<td>CSRV 38 (48%)</td>
<td>27 (71%)</td>
<td>74</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSRV 28 (35%)</td>
<td>22 (79%)</td>
<td>49</td>
<td>7</td>
</tr>
</tbody>
</table>
Syngnathid Habitat Use in BNP

- *H. zosterae* (0.46)
- *C. brachycephalus* (0.18)
- *S. floridae* (0.18)
- *C. albirostris* (0.07)
- *A. criniger* (0.04)
- *S. louisianae* (0.04)
Syngnathid Habitat Use in BNP

**Table 4.** Coefficients (as unstandardized log-odds) and odds ratios with 95% confidence interval and p-value of the Wald Statistic the final model of occurrence for all syngnathids in Biscayne National Park, Florida in 2016.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Coefficient</th>
<th>95% CI</th>
<th>Odds Ratios</th>
<th>Odds Ratio 95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>B0 (sediment type: mud)</td>
<td>0.15</td>
<td>-0.89 – 1.20</td>
<td>1.79</td>
<td>0.54 – 6.54</td>
<td>0.348</td>
</tr>
<tr>
<td>sediment type: sand</td>
<td>-2.69</td>
<td>-4.39 – -1.33</td>
<td>0.03</td>
<td>0.00 – 0.15</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>sediment type: rock</td>
<td>-18.48</td>
<td>NA – 123.28</td>
<td>NA</td>
<td>NA</td>
<td>0.993</td>
</tr>
<tr>
<td>% seagrass</td>
<td>0.03</td>
<td>0.01 – 0.06</td>
<td>1.04</td>
<td>1.01 – 1.08</td>
<td>0.009</td>
</tr>
</tbody>
</table>
Figure 1. Major habitat types overlaid by 79 surveyed sites in Biscayne National Park, a marine protected area located in southeastern Florida, USA, south of Miami. Sample sites are color-coded by syngnathid absence (0) or presence (1).
Figure 2. Probability of occurrence of syngnathids in BNP on muddy and sandy substrates across a gradient of seagrass coverage. Dashed lines and shaded regions indicate 95% confidence intervals.
Syngnathid Habitat Use in BNP

Figure 3. Boxplot of environmental variables associated with individual occurrences of *Hippocampus zosterae* (n = 36 individuals), *Syngnathus floridiae* (n = 32 individuals), and *Syngnathus scovelli* (n = 32 individuals). Lower and upper box boundaries mark the 25th and 75th percentiles, respectively. The line inside box is the median and the lower and upper error lines represent 10th and 90th percentiles, respectively. Filled circles are data falling outside 10th and 90th percentiles. Asterisks indicate significance at p < 0.05 (*), p < 0.01 (**), and p < 0.0001 (***) using the Mann-Whitney U test.