

Fine-scale habitat partitioning facilitates sympatry between two octopus species in a shallow Florida lagoon

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ABSTRACT: Species coexistence is a critical determinant of biodiversity and community structure, yet resource partitioning mechanisms that facilitate coexistence remain understudied for many taxa, including cephalopods. *Octopus vulgaris* and *Macrotritopus defilippi* cohabit a shallow-water lagoon in South Florida. Temporal and spatial distribution as well as habitat association were examined as potential resource partitioning mechanisms to facilitate coexistence between these species. Methods included *in situ* visual observations, marking locations of octopus-occupied dens, and photoquadrats of octopus dens and surrounding habitats. Den locations were marked year-round for 3 yr to determine consistency of spatial distribution and temporal trends. Octopus abundance was highest during spring and lowest during fall for both species, indicating that temporal partitioning (in terms of seasonality) is likely not a mechanism of coexistence. *O. vulgaris* and *M. defilippi* had the highest densities of occupied dens in the same general shallow areas of the lagoon; thus there was no evidence of spatial partitioning. Although octopuses spatially overlapped in the same general area, multiple substrate categories were available, and each species' den and surrounding habitat was associated with different substrates. *O. vulgaris* was associated mostly with hard bottom and inhabited hard-structured dens while *M. defilippi* was associated with soft sandy bottom and inhabited burrows in the sand as dens. Fine-scale habitat partitioning is made possible by this lagoon's heterogeneous microenvironments, which aid in explaining coexistence of these 2 octopus species.

KEY WORDS: Resource partitioning · Cephalopods · Spatial distribution · Seasonal abundance · Den ecology · Habitat heterogeneity

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1. INTRODUCTION

Underlying mechanisms of species coexistence are often complex and remain a central topic in ecology. Two or more species often occur in sympatry; thus, identifying mechanisms of coexistence is crucial for understanding biodiversity and community structure (Vieira & Port 2007). Closely related sympatric species tend to exhibit similar morphology and ecological requirements, therefore exploiting similar niche requirements. The competitive exclusion theory posits

that stable coexistence of competing species within a community is only possible when limited resources are partitioned (Gordon 2000).

Mechanisms of resource partitioning can include (1) habitat partitioning, (2) temporal partitioning, (3) diet partitioning, and (4) morphological partitioning. Habitat partitioning can result from spatial partitioning and/or structural partitioning (i.e. habitat heterogeneity) of the habitat and abiotic factors (e.g. water velocity, depth of water) (Bergeron & Bourget 1986, Weir et al. 2009, Connan et al. 2014). Temporal parti-

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tioning may refer to 2 species feeding at different times during the day, a change in seasonal abundance of each species, or changing diets depending on seasonal food availability (Gutman & Dyan 2005, Camisa et al. 2017, Fossette et al. 2017). Diet partitioning may result from differences in foraging strategies or feeding biology, which allows species to forage in different habitats or at different trophic levels (Weir et al. 2009, Albo-Puigserver et al. 2015). These mechanisms of niche partitioning can occur alone or in combination with others, or coexistence could occur by having high overlap in one niche dimension and low overlap in another (i.e. niche-complementarity hypothesis; Jimenez et al. 1996, Barnes 2002, Vieira & Port 2007).

Resource partitioning has been studied for a wide range of taxa; however, certain taxa have received little attention, including cephalopods (coleoids: cuttlefishes, squids, and octopuses). Related field research has focused on adaptive life history traits or strategies that influence trophic interactions (e.g. Mather & O'Dor 1991, Staudinger et al. 2011, 2013), behavioral ecology (e.g. Forsythe & Hanlon 1988, Leite et al. 2009a,b, Hanlon & Messenger 2018), and camouflage (e.g. Hanlon et al. 2009, Allen et al. 2014). Most cephalopod studies focus only on a single species; however, more than one octopus species can often be found inhabiting an area. Cephalopods play important roles as consumers of shelled mollusks, polychaetes, and crustaceans, and serve important predator and prey roles in food webs of different ecosystems (Ambrose 1986, Smale 1996, Boyle & Rodhouse 2005). It is useful to understand the underlying mechanism(s) of cephalopod habitat coexistence because of their importance in many marine food webs, and because such information can inform conservation strategies.

Similar morphology and ecological requirements among various species of shallow-water octopuses suggest that competition for resources (e.g. habitat, prey, and space for den sites) influence their distribution, density, and overall coexistence. For example, 3 sympatric species of shallow-water Hawaiian octopus may persist due to differences in temporal spacing and microhabitat preferences (Houck 1982). Activity rhythms of the 3 species were examined in a laboratory and compared to field observations; however, the number of field observations was not expressed and only descriptions of activity levels and microhabitats were given. Coexistence of *Octopus vulgaris*, *O. briareus*, *Callistoctopus macropus*, and *Amphioctopus burryi* on Caribbean coral reefs was documented from descriptive observations of differ-

ent foraging behaviors, prey, den types, and temporal spacing (Hochberg & Couch 1971, Hanlon 1988). A study examining feeding morphologies in deep-sea octopuses demonstrated diet partitioning thereby allowing increased octopus species diversity (Voight 2013). Three sympatric, sand-dwelling mimic octopus species (*Thaumoctopus mimicus*, 'Blandopus' or 'white V octopus, *Octopus* sp. 18', and *Wunderpus photogenicus*) were observed to overlap greatly in their niche requirements (foraging, habitat, activity period) (Norman et al. 2001, Hanlon et al. 2008); however, their mechanisms of coexistence have not been thoroughly examined. These octopus coexistence studies were either conducted in the laboratory or based on descriptive field observations of potential resource partitioning mechanisms with little empirical data (with the exception of Voight 2013, who investigated the mouth and musculature of 2 deep-sea octopus species). Another study mentioned multiple octopus species in one location, but did not investigate mechanisms of species coexistence (Anderson et al. 2008). Thus, robust *in situ* studies that analyze octopus resource partitioning mechanisms would help advance the understanding of species' coexistence.

In studies concerned with the coexistence of similar sympatric species, the importance of analyzing habitat type and spatial distribution patterns has been emphasized (Edington & Edington 1972, Crow et al. 2010, Connan et al. 2014). Also, information about relative abundance and fluctuations in biomass are important for understanding the ecology of species (Gordon 2000, Kaiser et al. 2011). Here, we examined coexistence of 2 octopus species (*O. vulgaris* and *Macrotritopus defilippi*) in a South Florida shallow-water environment for 3 consecutive years.

O. vulgaris is found in sand, rock, rubble, seagrass, and coral reef environments. It generally makes its home or 'den' in a hole in the substrate littered with shells or by excavating sand under a boulder (Woods 1965, de Beer & Potts 2013, Guerra et al. 2014). *O. vulgaris* is known to feed mainly on crustaceans, bivalves, and gastropods (depending on geographical location) and has varying activity patterns in different habitats (Woods 1965, Meisel et al. 2013, Hanlon & Messenger 2018). Off the east coast of South Africa, laboratory and field observations of *O. vulgaris* suggested that mating, gonad maturation, and egg laying lack seasonality; however, there was a suggestion for seasonality in female maturation and sex ratio (Smale & Buchan 1981). *M. defilippi* is known to inhabit sandy plains, making a new or pre-existing sand burrow its den, but substrate information about

this species' den and microhabitat have never been quantified. It can also bury directly into the sand and use flounder mimicry to escape predators (Hanlon 1988, Hanlon et al. 2010). These species overlap in geographical range; however, their habitat coexistence has not been studied. The coexistence of *O. vulgaris* and *M. defilippi* (and 2 others: *O. filiosus* and *O. briareus*) was documented in Bonaire, but the focus of that study was the diet of *O. vulgaris* (Anderson et al. 2008). Although there are habitat differences between these 2 species, there is potential habitat overlap because *O. vulgaris* is found in an array of environments. The habitat of *O. vulgaris* has not been studied at our South Florida lagoon site and has never been studied in the presence of *M. defilippi*. Also, there have been few studies of *M. defilippi*, and little is known about the ecology (spatial distribution, activity time, diet, foraging behaviors) and intra- and interspecific interactions of this species.

We posed the following questions: (1) Do the 2 species overlap temporally? (2) Do they overlap spatially? (3) If the latter, are there differences in habitat associations between the 2 species? If species are abundant at different times of the year, we hypothesize the potential use of temporal partitioning to lessen competitive interactions. If species are abundant during the same time of year, we hypothesize within-species aggregation, but also between species over-dispersion (spatial partitioning). If species overlap spatially, we hypothesize species to associate with different habitat compositions (habitat partitioning).

2. MATERIALS AND METHODS

2.1. Description of species

Octopus vulgaris has a world-wide geographical distribution that includes subtropical and tropical waters (Warnke et al. 2004). It is a medium to large sized octopus species that can weigh up to 5 kg. The average mantle length (ML) of *O. vulgaris* is 250 mm. This species has stout arms (equal length and thickness) with an arm length of 3–5 times its ML. It has highly variable body patterns often exhibiting a reddish-brown reticulated pattern (Hanlon 1988, Humann & DeLoach 2013). *Macrotritopus defilippi* has been documented in the Caribbean, Atlantic Ocean, and Mediterranean (Hanlon et al. 2010, Crocetta et al. 2015). *M. defilippi* is a small to medium sized octopus with a ML of approximately 90 mm. A specimen collected in the Canary Islands weighed 50 g with a ML 41 mm (Guerra et al. 2013). This species has long,

slender arms with an arm length up to 6 times its ML. Its most distinguishing characteristics are dark bars and white spots down each arm, a long narrow mantle, very long arms, and a small head with protruding eyes (Hanlon 1988, Humann & DeLoach 2013). These characteristics were used to aid in species identification. From field observations, ML size was estimated to range from 12 to 178 mm for *O. vulgaris* and 12 to 90 mm for *M. defilippi*. The majority of octopuses observed were not at the extreme ends of reported size estimates.

2.2. Study site

Spatial distribution, habitat association, and relative abundance were examined at Blue Heron Bridge (BHB), within the Phil Foster Park portion of the Lake Worth Lagoon, Riviera Beach, FL, USA (see Fig. 1). The study site has a heterogeneous benthic environment, including mainly sandy plains, but also rock, shell, rubble substrate, anthropogenic materials (e.g. glass bottles, cans, cement blocks, pipes, and sunken boats), and has a mean depth of 3 m. Water visibility at BHB is heavily influenced by the tidal cycle because of its close proximity to the Atlantic Ocean via the Palm Beach Inlet. The majority of octopus observations took place within 1 to 2 h of high slack tide to minimize tidal current (which often peaked at 0.77 m s^{-1}) and maximize water visibility, and thus, opportunities of locating these cryptic animals. In a few rare cases, observations were done at low tide if water visibility was $\geq 3 \text{ m}$ to increase chances of locating octopuses. Observations were made between 06:00 and 21:00 h.

2.3. Spatial distribution

Visual census during SCUBA dives was used to locate occupied dens and collect habitat association data. This method is commonly used to measure abundance of benthic animals and other ecological aspects of various octopus species (Aronson 1986, Forsythe & Hanlon 1997, Katsanevakis & Verriopoulos 2004a,b). For maximal search coverage, the study site was divided into 3 areas relative to the direction of BHB: southwest, south, and southeast (see Fig. 1). Each dive was designated to one of these areas, and north–south swim paths were used to survey the entire area for octopus-occupied dens. The numbers of dives in each area were kept approximately the same to ensure equal amounts of search time. Search

time or dive hours were kept approximately the same for each dive. Between 2 and 4 dives wk^{-1} were conducted year-round between January 2014 and December 2016. Spatial distribution of occupied dens was recorded over 3 yr (2014, 2015, 2016) to determine if intra- and interspecific patterns were consistent across years.

Once an occupied den was located, the latitudinal and longitudinal coordinates (decimal degrees) were recorded on an eTrex® 10 (Garmin) GPS device that was kept in a dive float. ArcGIS 10.2 (ESRI) was used to overlay occupied octopus den GPS points for visual representation of octopus spatial distribution. Spatial data on occupied dens were analyzed using base functions and the 'spatstat' package (Baddeley et al. 2015) in R version 3.3.1 (R Core Team 2017). First, kernel density maps were created as a data exploration method to depict any areas of high or low octopus density for both species. Next, for each year, univariate Ripley's K functions (Ripley 1981) were used to test for non-random spatial patterns (intraspecific aggregation or over-dispersion) up to 30 m for each octopus species independently, and bivariate Ripley's K functions were used to test for significant spatial interactions between species (interspecific aggregation or over-dispersion). Ripley's K is a standard second-order spatial statistic that evaluates the number of points within a certain distance (r) of a randomly chosen point relative to expectations based on the density of points in the study area. Significant deviations of the K -statistic indicate either regularity or aggregation at scale r in a spatial point pattern data set. To stabilize variance and aid in interpretation, K -statistics were square-root transformed to L -statistics (Besag 1977), as is common practice. Monte Carlo permutation procedures ($N_{\text{sim}} = 999$; Ripley edge correction) were implemented to generate simulation envelopes that allowed for detection of non-random univariate and bivariate den spatial patterns.

2.4. Habitat association

After locating and recording an occupied den, a photoquadrat was used to collect substrate composition data for each octopus species' den and surrounding habitat. The quadrat (0.13 m^2) was first placed directly over the octopus den, which was defined as 'den habitat'. A Powershot D20 (Canon) camera was used to record the substrate within the quadrat. In addition to the single den photoquadrat, 8 additional photoquadrats were collected around the den ($\sim 1 \text{ m}^2$) and that area was defined as the 'surrounding habi-

tat' of the octopus (total of 9 photoquadrats). A total of 30 occupied dens were sampled for each species to determine possible significant differences in habitat association between *O. vulgaris* and *M. defilippi* for den and surrounding habitats. Photoquadrat samples were recorded from octopuses of similar size. Along with comparing habitats between species, we also wanted to determine if the octopus' habitat differed from the general substrate composition of the BHB study site. Therefore, 30 random samples of the BHB study site, defined as 'BHB', were collected using the same methodology as den (1 photoquadrat) and surrounding (9 photoquadrat) octopus habitat. Random GPS locations for BHB sampling were generated in ArcGIS 10.2 (ESRI) and were made sure to be distributed throughout the study site to allow the most accurate representation of BHB's general substrate composition.

Percent substrate composition was calculated using Coral Point Count with Excel extensions (CPCe) software for each species' den (1 photoquadrat sample $^{-1}$), surrounding habitat (9 photoquadrats sample $^{-1}$), and BHB random substrate samples (1 and 9 photoquadrats sample $^{-1}$) (Kohler & Gill 2006). To determine percent substrate category of each species' habitat and BHB, 4 major substrate categories were defined for CPCe code: hard bottom (rock, rubble, shells), soft bottom (sand), human debris (referring to anthropogenic materials such as aluminum cans, glass bottles, cement blocks, pipes) and fauna and flora (e.g. algae, sponges, hydroids, seagrasses). Each photo was overlaid with 50 random points, for substrate coding to achieve an accurate frequency for each substrate category for den habitat, surrounding habitat, and BHB (Pante & Dustan 2012). Each random point was coded with a specific substrate category, and a percentage for each substrate category was calculated. For surrounding habitat, the percent substrate category was averaged for the 9 photos. This was also done for the 9 photos at each of the 30 BHB random substrate sample locations.

All analyses for octopus habitat association were conducted in the ecological software program PRIMER-7 (Clarke & Gorley 2015). Similarity between octopus species' habitat association (den habitat and surrounding habitat) and substrate composition of BHB were analyzed using a resemblance-based permutation test analysis of similarities (ANOSIM) due to data not meeting multivariate analysis of variance (MANOVA) test assumptions. ANOSIM was applied using a Bray-Curtis similarity coefficient for the pairwise similarity matrix. Data were square-root transformed because samples were dominated by 2 sub-

strate categories (soft bottom and hard bottom). The square-root transformation allows the less common categories (human debris, fauna and flora) to also contribute to the similarity metric. Since the ANOSIM test is sensitive to heterogeneity of multivariate dispersion, a square-root transform was also appropriate to decrease heterogeneity of dispersion (Anderson & Walsh 2013). Homogeneity of multivariate dispersion was met for den habitat and surrounding habitat similarity data (PERMDISP; $p = 0.634$ and $p = 0.139$, respectively). ANOSIM provides a global permutation-based test that reports both overall probabilities of differences between groups (*O. vulgaris*, *M. defilippi*, BHB) and probabilities between group pairs. For groups that were significantly dissimilar, an analysis of similarity percentages (SIMPER) was conducted to determine the relative contribution of each substrate type to dissimilarity between groups and similarity within groups.

2.5. Octopus abundance

Octopus-occupied den counts were used as an estimate of octopus abundance to determine if there was seasonal variation of octopus abundance within and between species. Octopus-occupied den counts were grouped into 1 of 4 seasonal categories, defined as winter (December, January, February), spring (March, April, May), summer (June, July, August), and fall (September, October, November). We did not resample the same den location twice in any year and assumed the recorded dens were by different individuals. A chi-squared statistic (significance level set at $\alpha = 0.05$) of a 2×4 contingency table (octopus species \times seasonal categories) was used to determine seasonal variation in octopus abundance within and between species for each year (2014–2016).

3. RESULTS

In total, 249 SCUBA dives (371.4 dive hours) were conducted over 3 yr (2014–2016). The average (\pm SD) number of dives was 7.0 ± 2.3 dives mo^{-1} . The number of monthly dives was lower when there was poor water visibility, poor water quality (high levels of indicator bacteria), or severe weather conditions (tropical storms or hurricanes). Although total dive hours varied from year to year, diving remained relatively consistent between seasons for each year (see Table 1 for a breakdown of dive hours). In total, 434 occupied dens were found: 296 for *Octopus vulgaris*

Table 1. Number of dive hours spent searching for octopuses in the Blue Heron Bridge study site each season (winter, spring, summer, fall) over a 3 yr period (2014–2016). Seasons were defined as: winter (December, January, February), spring (March, April, May), summer (June, July, August), and fall (September, October, November)

	Dive hours			Total
	2014	2015	2016	
Winter	21.1	27.9	33.6	82.6
Spring	19.5	35.8	43.3	98.6
Summer	25.4	45.6	37.0	108.0
Fall	21.7	39.0	21.5	82.2
Total	87.7	148.3	135.4	371.4

and 138 for *Macrotritopus defilippi*. For breakdown by year, in 2014 a total of 80 *O. vulgaris* and 23 *M. defilippi* occupied dens were recorded. For 2015, 134 *O. vulgaris* and 44 *M. defilippi* dens were recorded, and for 2016 there were 82 *O. vulgaris* and 71 *M. defilippi* occupied dens recorded. Unoccupied octopus dens were not counted because they could be used by a variety of other species. Also, other species make similar homes (e.g. mantis shrimp holes in the sand) that could be mistaken for an octopus den if an octopus was not present.

3.1. Spatial distribution

Pooled across years, most occupied dens for both species were found at the southwest and southeast areas of BHB (Fig. 1). *M. defilippi* had more occupied dens scattered throughout the south sandy area than *O. vulgaris*. Kernel density maps illustrate the fine-scale distributions and densities of occupied dens for each year and for each species (Fig. 2). Occupied den density for *O. vulgaris* across the study area ranged from 0.0 to 13.8 dens 1000 m^{-2} in 2014, from 0.0 to 18.1 dens 1000 m^{-2} in 2015, and from 0.0 to 15.0 dens 1000 m^{-2} in 2016. Occupied den density for *M. defilippi* across the study area ranged from 0.0 to 4.8 dens 1000 m^{-2} in 2014, 0.0 to 6.6 dens 1000 m^{-2} in 2015, and 0.0 to 14.7 dens 1000 m^{-2} in 2016. Over 3 yr, there was a visual trend of higher occupied den density in southwest and southeast areas for both species.

For all 3 yr, occupied *O. vulgaris* dens were significantly aggregated at scales ≥ 2.0 m, especially on the southwest end. Conversely, the spatial patterns of occupied *M. defilippi* dens were statistically random except in 2016 where *M. defilippi* dens were significantly aggregated at scales ≥ 2.0 m. There was no evidence of significant interspecific aggregation or

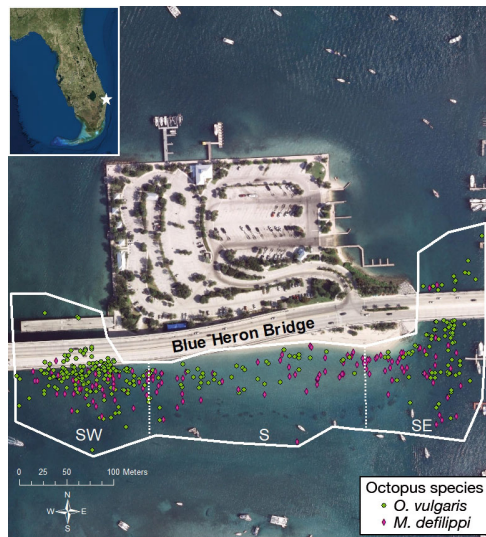


Fig. 1. Blue Heron Bridge (BHB), Phil Foster Park (26.7843° N, 80.0427° W) located within the Lake Worth Lagoon, FL, USA. Location within Florida indicated by star in inset image. Study location (outlined in white, ~62 000 m²) was in BHB waters and was divided into 3 survey areas (white-dashed lines) relative to BHB: southwest (SW), south (S), and southeast (SE). Occupied den locations for *Octopus vulgaris* (n = 296) and *Macrotritopus defilippi* (n = 138) were recorded via GPS for years 2014–2016

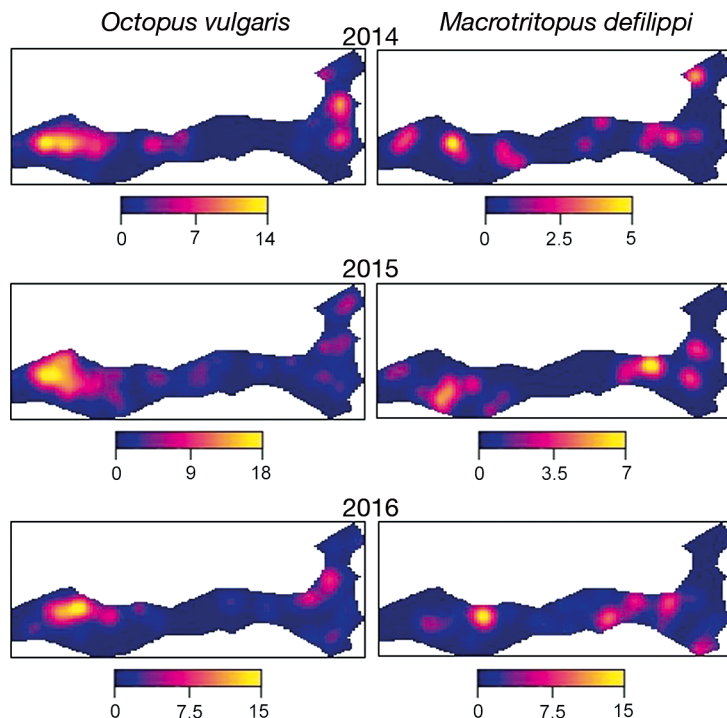


Fig. 2. Octopus kernel density maps at Blue Heron Bridge based on occupied den GPS data for individual years 2014–2016 and octopus species *Octopus vulgaris* and *Macrotritopus defilippi*. Kernel density map scale: occupied octopus dens per 1000 m². Blue-purple colors represent low occupied den densities, pink-red colors represent medium occupied den densities and orange-yellow colors represent high occupied den densities

over-dispersion between occupied dens of the 2 species in any year.

3.2. Habitat association

Den habitat for *O. vulgaris* was composed of all 4 substrate categories and had a larger percentage of hard bottom, human debris, and fauna and flora than den habitat for *M. defilippi*. Den habitat for *M. defilippi* was mainly soft bottom with a low (or zero) percentage of the other substrate categories (Fig. 3). In areas inhabited by both species, there were patches of both sand and hard substrate. Although the main substrate category for both species' den habitat and general substrate composition of BHB was soft bottom, there was a significant difference between groups (octopus species' den habitat and BHB) (ANO-SIM; $R = 0.149$, $p = 0.001$). Pairwise tests revealed significant differences between *O. vulgaris* and *M. defilippi* for den habitat ($R = 0.264$, $p = 0.001$) and between *O. vulgaris* den habitat and BHB ($R = 0.221$, $p = 0.001$). There was no significant difference between *M. defilippi* den habitat and BHB ($R = -0.017$, $p = 0.913$). Soft bottom was the largest contributor for similarity among the 3 groups (SIMPER; *O. vulgaris* 60 %, *M. defilippi* 93 %, BHB 88 %).

Hard bottom was the largest contributor of dissimilarity (37 %) between the species' den habitats and between *O. vulgaris* and BHB (36 %) (SIMPER; Fig. 3).

Similar trends were seen for both species' surrounding habitat, with *O. vulgaris* associated with a larger percentage of hard bottom, human debris, and fauna and flora than *M. defilippi*. Yet for *O. vulgaris*' surrounding habitat, there was a decrease in hard bottom and human debris and an increase in soft bottom. Surrounding habitat for *M. defilippi* still had a larger percentage of soft bottom (Fig. 4). Results for general BHB substrate composition (9 photoquadrats per BHB sample) remained almost the same as previously reported (1 photoquadrat per BHB sample). There was a significant difference between groups for octopus species' surrounding habitat and BHB (ANOSIM; $R = 0.131$, $p = 0.001$). Pairwise tests revealed significant differences between *O. vulgaris* and *M. defilippi* for surrounding habitat ($R = 0.253$, $p = 0.001$) and *O. vulgaris* surrounding habitat and BHB ($R = 0.172$, $p = 0.001$). There was no significant difference between *M. defilippi*

Fig. 3. Mean (\pm SE) percent substrate category for den habitat of *Octopus vulgaris* (stippled), *Macrotritopus defilippi* (striped), and general substrate composition for Blue Heron Bridge (BHB) (grey). Photos represent the den habitat for each octopus species and BHB substrate composition; white arrow indicates octopus in photo. There was a significant difference between *O. vulgaris* and *M. defilippi* (ANOSIM; $R = 0.264$, $p = 0.001$) den habitats and *O. vulgaris* den habitat and BHB (ANOSIM; $R = 0.221$, $p = 0.001$). Percent contribution of dissimilarity (SIMPER) between *O. vulgaris* and *M. defilippi* den habitats and between *O. vulgaris* den habitat and BHB is listed above the respective bracket for each substrate category

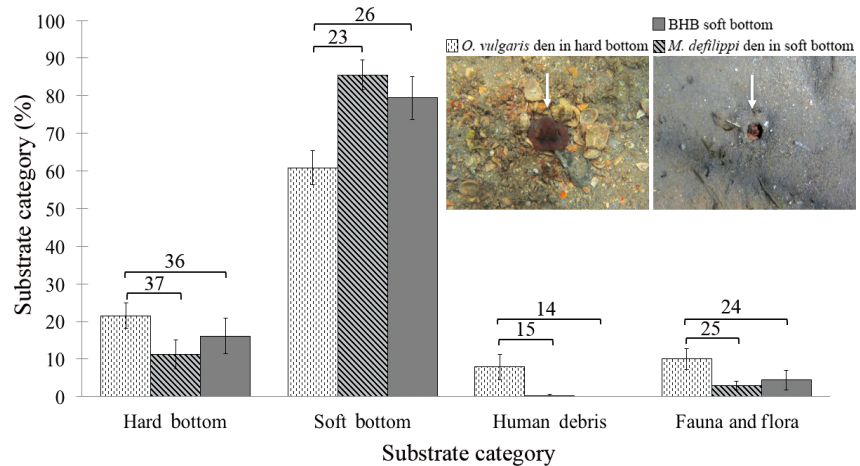
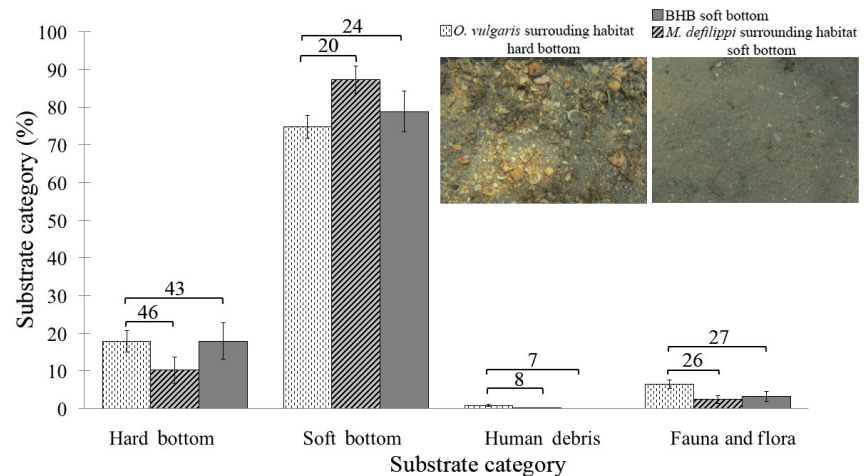


Fig. 4. Mean (\pm SE) percent substrate category for surrounding habitat for *Octopus vulgaris* (stippled), *Macrotritopus defilippi* (striped), and general substrate composition for Blue Heron Bridge (BHB) (grey). Photos represent surrounding habitat for each octopus species and BHB substrate composition. There was a significant difference between *O. vulgaris* and *M. defilippi* (ANOSIM; $R = 0.253$, $p = 0.001$) surrounding habitats and *O. vulgaris* surrounding habitat and BHB (ANOSIM; $R = 0.172$, $p = 0.001$). Percent contribution of dissimilarity (SIMPER) between *O. vulgaris* and *M. defilippi* surrounding habitats and between *O. vulgaris* surrounding habitat and BHB is listed above the respective bracket for each substrate category



surrounding habitat and BHB ($R = -0.003$, $p = 0.480$). Again, soft bottom was the major contributor of similarity for each group (SIMPER; *O. vulgaris* 67%, *M. defilippi* 89%, BHB 85%) and hard bottom contributed most (46%) to differences in surrounding habitat between octopus species and between *O. vulgaris* and BHB (43%) (SIMPER; Fig. 4).

3.3. Octopus abundance

Both species showed a seasonal trend of highest abundance during spring, then decreasing abundance through summer and fall, followed by an increase again during the winter (Fig. 5). There was among-season variation in octopus abundance between years, yet the trend was similar for all 3 yr. There was no difference in seasonal octopus abundance between species for years 2014 ($\chi^2 = 4.804$, $p = 0.187$) and 2016 ($\chi^2 = 5.098$, $p = 0.165$) and only a slight difference in

2015 ($\chi^2 = 8.548$, $p = 0.036$). A seasonal change in octopus abundance within species was detected for *O. vulgaris* and *M. defilippi* in 2014 ($\chi^2 = 43.900$, $p <$

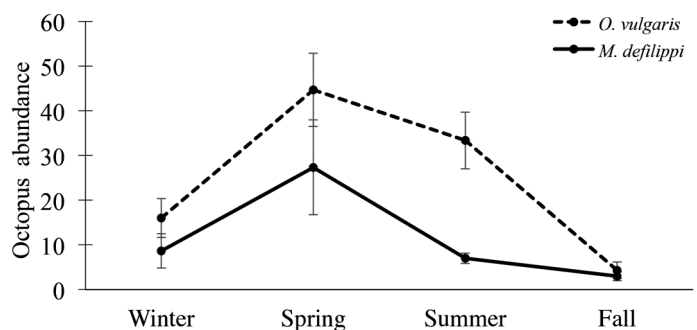


Fig. 5. Average (\pm SE) seasonal octopus abundance across 3 yr (2014–2016) for *Octopus vulgaris* (dashed line) and *Macrotritopus defilippi* (solid line). Seasons were defined as winter (December, January, February), spring (March, April, May), summer (June, July, August), and fall (September, October, November)

0.0001; $\chi^2 = 15.9783$, $p = 0.0013$, respectively), 2015 ($\chi^2 = 44.806$, $p < 0.0001$; $\chi^2 = 15.000$, $p = 0.0011$, respectively), and 2016 ($\chi^2 = 39.444$, $p < 0.0001$; $\chi^2 = 71.704$, $p < 0.0001$, respectively). During the spring, there were single 1 to 2 h dives where 5 to 12 *O. vulgaris* and 5 to 7 *M. defilippi* were recorded. These high numbers of octopus were never observed on a single dive during another season.

4. DISCUSSION

Given that sympatric species do not necessarily partition all their critical resources, focusing on one resource may underestimate the importance of resource partitioning mechanism(s) responsible for coexistence (Limbourn et al. 2007). This study examined temporal distribution (seasonal variation in abundance), spatial distribution of occupied dens, and habitat association as potential resource partitioning mechanisms responsible for the coexistence of 2 octopus species.

We have provided the first report on spatial distribution and densities for these sympatric species. Both *Octopus vulgaris* and *Macrotritopus defilippi* showed intraspecific aggregation of occupied dens at the BHB ecohabitat. Aggregation, clumping, or overlapping of den distribution have been reported in previous studies for *O. vulgaris* (Mather & O'Dor 1991, Guerra et al. 2014) and *Octopus insularis* (Leite et al. 2009b). These spatial patterns were seen with *O. insularis* densities ranging from 1.2 to 8.8 ind. 1000 m⁻² in Brazil (Leite et al. 2009b) and 3.8 to 3.9 ind. 1000 m⁻² in NW Spain (Guerra et al. 2014). Katsanevakis & Verriopoulos (2004a) reported the density of *O. vulgaris* in Greek coastal waters on soft sediment to range from 0 to 6.9 ind. 1000 m⁻² and Aronson (1986) reported a mean density of *O. briareus* to be 7.9 ind. 1000 m⁻² in a saltwater lake on Eleuthera Island, The Bahamas, which he termed high density. Densities in our study for *O. vulgaris* and *M. defilippi* in South Florida were similar to these previous studies, suggesting that BHB can support high densities of these 2 octopus species. This South Florida lagoon is the only location known at which *O. vulgaris* and *M. defilippi* coexist in high densities. Since both species were most abundant during the same season (spring), we suggest temporal partitioning does not facilitate coexistence.

Benthic octopuses are typically known as solitary animals not living near each other (Boal 2006, Guerra et al. 2014). There was evidence of intraspecific aggregation for both species. Due to the high density

of both octopus species, we anticipated species to aggregate in different areas at BHB; however, this was not the case and both species aggregated in the same general area at BHB. There was no evidence of interspecific aggregation (spatial overlap) or overdispersion (spatial partitioning) between species. If the 2 species were actively positioning their dens at certain distances (near or far) from dens of the other species, the signal in the spatial data would have been much stronger. Instead, our results suggest that spatial partitioning (in terms of den location) is not a resource partitioning mechanism that facilitates coexistence between these 2 species.

In high densities, den spatial distribution is a compromise because it is crucial for these soft-bodied invertebrates to have shelter for survival. The importance of habitat heterogeneity for species coexistence has been documented in other taxa and is gaining support in cephalopods. Substrate type and den availability are 2 factors responsible for octopus distribution in multiple octopus species (Mather 1982, Leite et al. 2009b, Guerra et al. 2014) and octopus coexistence (Hochberg & Couch 1971, Houck 1982). This is also the first study for *M. defilippi* that has quantified associated habitat (den and surrounding) and compared it to the associated habitat of a sympatric octopus species (*O. vulgaris*).

Octopus species coexist in the same general areas of BHB (i.e. southwest and southeast areas) due to this lagoon's fine-scale habitat heterogeneity. The substrate category that contributed to habitat (den and surrounding) dissimilarities between species was hard bottom.

O. vulgaris can be found in many sub-habitats on and around coral reefs and seagrass beds throughout Florida and the Caribbean, and only inhabits sand plains when they are adjacent to substrates that contain natural dens of hard materials (Hanlon 1988, Katsanevakis & Verriopoulos 2004a,b). This species requires hard objects for their dens and such hard objects are absent from the open sand plains, which dominate BHB habitat (especially in the south area; Fig. 1). The majority of hard and 3-dimensional substrates (rock, rubble, fauna and flora, and human debris) were concentrated at the southwest and southeast areas of BHB. The distribution of these materials is a factor of den selection and therefore is most likely responsible for the aggregate den distribution of *O. vulgaris*.

This spatial pattern was stronger for *O. vulgaris* occupied dens than for *M. defilippi* occupied dens likely due to their differences in habitat association. *M. defilippi* is a sand-dwelling species similar to the

octopuses in the 'long-armed sand octopus' clade, which includes the Indo-Pacific mimic octopuses (*Thaumoctopus mimicus*, *Wunderpus photogenicus*, white 'V' octopus, and Hawaiian long-armed sand octopus), that require sand habitat (Hanlon et al. 2008, Huffard et al. 2010). Since the general substrate composition of BHB is sand, this could explain the weaker aggregate den distribution for *M. defilippi*. However, lack of aggregated den distribution for *M. defilippi* for years 2014 and 2015 could also be due to fewer occupied dens recorded. By recording occupied den locations over a 3 yr period we were able to detect if spatial distribution trends were consistent. In the case of *M. defilippi*, although there was a weak trend, 2016 showed evidence of intraspecific aggregation in the same general locations of BHB as *O. vulgaris*. Even though *M. defilippi* were sometimes found in near proximity to *O. vulgaris*, they were exclusively on sand substrates; thus, we use the term 'fine scale' habitat partitioning to explain species coexistence.

The combination of high octopus abundance, accessibility to the study location, and extensive dive time permitted us to conduct the first 3 yr study on octopus resource partitioning mechanisms. These species exhibit spatial-temporal overlap by occurring in high densities in the same general area during the same season. Coexistence could occur by having high overlap in previously mentioned niche dimensions and low overlap in latter niche dimension-habitat type (i.e. niche-complementarity hypothesis, Jimenez et al. 1996).

Sympatric species may also be partitioning other resources at varying degrees to facilitate coexistence (Jimenez et al. 1996, Barnes 2002, Vieira & Port 2007). Food abundance and availability are factors reported to influence octopus density and distribution (Guerra et al. 2014). Diet, activity period(s), and foraging strategies should be examined for these coexisting species to determine if they assist in coexistence. The diet of *M. defilippi* has never been reported and we are unaware of reports on these aforementioned topics as resource partitioning mechanisms for octopuses under natural conditions.

Many ecological coexistence studies (including this study) assume that species coexist due to partitioning of resources, thus lessening competitive interaction. However, alternative explanations for species coexistence may not be from competitive pressure, but from environmental preferences and tolerances of each species, relating to dispersal and establishment of each species, or source and sink resource dynamics (Gordon 2000, Kirol et al. 2015). Therefore, similar

species may have increased chances of coexisting because of these shared environmental and ecological attributes.

Many of the octopuses observed during the spring were juveniles and are likely responsible for the high density of octopuses during spring months. BHB's close proximity to the Palm Beach Inlet would make dispersal/migration and recruitment possible. Recruitment of juvenile octopuses has been reported to peak in spring and summer (Aronson 1986, Katsanevakis & Verriopoulos 2004a) and was responsible for the overall increase in the octopus population. It appears that water temperature is one parameter that is correlated with octopus density. Small octopuses may prefer shallow, warm water to achieve a greater growth rate and shorten the period in which they are most vulnerable to predation (Forsythe 1993). Once they become adults, medium sized and larger octopuses have been reported to abandon warm waters for deeper, cooler waters to reduce the energy cost of a higher metabolism (Rees 1950, Voight 1992, Katsanevakis & Verriopoulos 2004a). After using this shallow warm water habitat to speed growth, medium to large sized octopuses could migrate to deeper, cooler waters and then potentially return to mate. Mating events (both species) and females with eggs (only *O. vulgaris*) have been observed; therefore, the lagoon may function as a nursery and mating habitat (e.g. source habitat acting as a population refuge). More observations are needed to determine the reproductive period of these 2 species in the western Atlantic and octopus size-class recordings to confirm BHB as a nursery/recruitment habitat.

We encourage future studies on ecological coexistence of cephalopods to measure additional resource partitioning mechanisms mentioned (i.e. diet, activity time, foraging strategies) and the influence of abiotic factors on octopus' spatial distribution and abundance. Since this is a shallow area, heavily influenced by tidal flow and freshwater input, temperature and salinity should be measured. Salinity was reported to influence octopus presence; low salinities are associated with octopuses being absent or their restriction to areas of normal salinity (Hartwick et al. 1984). Octopus tracking would be instrumental to determine if these species have a seasonal migration pattern to and from BHB via the Palm Beach Inlet. By further identifying mechanisms of coexistence, we can provide insight into cephalopod coexistence, and conservation strategies to maintain or increase cephalopod diversity, an important group in many marine food webs.

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