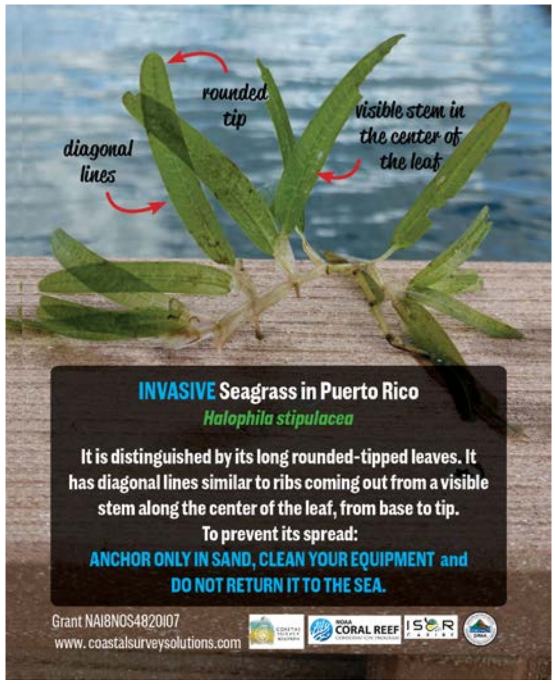
Assessing the impacts of the invasive seagrass, *Halophila stipulacea*, and two major hurricanes on the distribution of native seagrass communities of Culebra Island, Puerto Rico



October 23, 2019

Prepared by: Coastal Survey Solutions LLC
Award Number: NA18NOS4820107

This report was made possible with the support from NOAA's Coral Reef Conservation Program

Assessing the impacts of the invasive seagrass, *Halophila stipulacea*, and two major hurricanes on the distribution of native seagrass communities of Culebra Island, Puerto Rico

Stacey M. Williams[1,2], Jorge Sabater[1], Yasmin Detres[2], Ricardo Colón-Rivera[3], William J. Hernandez[4], Dorimar Ortiz[5]

1 Coastal Survey Solutions LLC, PO Box 1362, Lajas PR 00667-1362
2 Institute for Socio-Ecological Research, PO Box 3151, Lajas PR 00667-3151
3 Departmento de Recursos Naturales y Ambientales, PO Box 366147, San Juan PR 00936
4 Environmental Mapping Consultants, 3033 Sect Cobo Apt 3, Aguadilla PR 00603
5 Ciencias Brava, 180 Carr 194, La Loma Box 136, Fajardo PR 00738

Principal investigators
Stacey M. Williams, PhD-- (stcmwilliams@gmail.com)
Jorge Sabater, MS-- (jorge.sabater.clavell@gmail.com)
Yasmin Detres, PhD-- (ydetres@gmail.com)
Ricardo J. Colón-Rivera, PhD-- (ricardojcolon@gmail.com)

Contents

List of Figures

List of Tables

Executive Summary

This study was funded by the Coral Reef Conservation Program of the National Oceanic and Atmospheric Administration to better understand the distribution of the invasive seagrass, *Halophila stipulacea*, in Culebra Island, Puerto Rico. A total of 200 sites were randomly surveyed for the presence of seagrass. At each site, biological factors such as seagrass composition, cover (Braun Blanquet) and condition were recorded and estimated. Also, environmental parameters like conductivity, temperature and dissolved oxygen were recorded. Out of the 200 sites sampled, only 40 sites were absent of seagrass. There were three species of seagrass identified at the sites surveyed, and these were *Thalassia testudinum*, *Syringodium filiforme*, and *H. stipulacea*. The community structure of seagrass species significantly varied between locations and depth ranges. *T. testudinum* contributed 59.6% to the seagrass communities in shallower waters (>10m), followed by *H. stipulacea* 28.7%, and *S. filiforme* (11.8%). Mid-water (10-20m) seagrass communities were dominated by *H. stipulacea* with a contribution of 73.93%, and *S. filiforme* (23.9%). The seagrass community structures in water >20m were mostly characterized by *H. stipulacea* (82.5% contribution). Spatially, seagrass communities were overall significantly different between locations. The only areas with similar seagrass assemblages were within protected areas, like bays, Ensenada Honda, and Bahía de Almodóvar. *T. testudinum* was the dominant seagrass species in these bays, contributing between 50 to 70% of the overall seagrass communities.

Out of the 160 sites with seagrass present, *H. stipulacea* was present at 97 sites. The overall mean cover (± SE) of *H. stipulacea* in Culebra was 1.59 ± 0.08% based on the Braun Blanquet (BB) method, which corresponds to any number of individuals

covering 5-25% of the area. High cover of *H. stipulacea* was observed at 43 sites, and these areas included Cayo Norte and Carlos Rosario. On the other hand, we did not observe *H. stipulacea* in areas between Playa Sardinas and Playa Tamarindo, and southside of Luis Peña. High cover of *H. stipulacea* was observed at shallower depths (average 7.66 ± 0.78 m), ranging from 1.2m to 18.9m. *H. stipulacea* was mostly absent at the deeper sites (20-30m), with the exception at two sites (23m), where average densities reached as high as 1.67 ± 0.33 (Braun Blanquet ± SE).

Nine sessile- and five motile-benthic species were identified in the seagrass habitats. Sponges were the most frequent and diverse benthic category in seagrass, following echinoderms. Given the low abundance of benthic organisms, it was challenging to distinguish patterns between the different seagrass species. Seagrass beds with a mix of *H. stipulacea* and *S. filiforme* were characterized with a high species richness. Further research needs to happen to examine the ecological impacts on the native benthic and fish communities.

There was a significant negative relationship between the cover of *H. stipulacea* and other native seagrasses, especially with *T. testudinum*. H. stipulacea was absent in areas with dense *T. testudinum*. Other factors affecting the cover of H. stipulacea were depth, dissolved oxygen and conductivity. Depth was the most influential factor, contributing 45% of the model's variation.

In addition to developing geographical maps of the occurrence and cover of *H. stipulacea*, a risk assessment map was also produced. Given the results of the mapping and statistical analyses, the factors affecting *H. stipulacea* invasion were identified as boat activity, *H. stipulacea* presence, continuous cover of native seagrass, optimal depth for *H. stipulacea*, habitat availability, and within a Marine Protected Area. There were only a few areas that were at low risk of *H. stipulacea* invasion. One such area was between Tamarindo and Luis Peña. This area is within the Luis Peña Natural Reserve and is protected from fishing and anchoring is limited to small sand patched when mooring buoys are occupied. This protection may be one of the factors limiting the spread of slow colonization of *H. stipulacea*, since anchoring is one of the primary vectors of its invasion. Other possible factors probably are the continuous cover of native seagrass and the environmental nature of the area (strong currents). Additional monitoring should occur in high risk areas (4-5 index) with undocumented *H. stipulacea*; as there is a potential for *H. stipulacea* invasion in these areas given the depth, habitat availability, and boat traffic.

Through the use of satellite imagery, we were able to identify benthic habitats, especially seagrass areas affected by the passing of two major hurricanes, Irma and Maria, in Culebra Island. From the supervised classification of the two Sentinel 2 images, we estimated that the cover of seagrass decreased by 232.02ha. Seagrass loss was concentrated between Punta Tamarindo and Punta Melones and western, northwestern side of Luis Peña. There were benthic anomalies identified, one of which being seagrass cover seemed to increase in deeper waters. These anomalies could be based on a number of factors, spatial resolution of the sensors (10m), sunglint areas that obscure the benthic features, similarities in the spectral signatures of various substrates (e.g. seagrass, algae), confused pixels due to lack of ground validation point density training samples from the classifier, and water clarity to resolve the benthic features. Ground-truthing should be collected to verify the estimates in change of benthic composition.

The final goal of this project was to increase awareness about the threat, transmission, and mitigation of *H. stipulacea*. There were four educational tools produced: a tabloid, waterproof identification cards, waterproof stickers, and magnets. We focused the outreach on the east side of the island of Puerto Rico, Fajardo, Culebra Island and Vieques Island. Tabloids, ID cards and other materials were distributed to marinas, marine shops, educational groups and government entities. They were also shared on social media and will be available on both the Coastal Survey Solutions and Institute for Socio-Ecological Research's website.

Introduction

Seagrasses are common colonizers of lagoonal and lower intertidal sediment substrates (Perry and Beavington-Penney 2005), and globally have an estimated cover of 0.6 x 10^6 km^{-2}, which is equivalent to 10% of the coastal ocean surface (Charpy-Roubaud and Sournia 1990, Hemminga and Duarte 2000). Their global cover is comparable to that covered by corals reefs and mangroves. Seagrasses are one of the most ecologically and economically valuable ecosystems on Earth (Duarte 2002) and provide ecological services that are directly beneficial to humans (Terrado and Borum 2004). They regulate the oxygen and nutrients in the water column and sediments, stabilize sediments, protect shorelines, provide an important food source and habitats for other marine organisms and organic carbon production (Duarte 2002, Orth et al. 2006, Connolly 2009). They are the basis of an important detrital food chain (Short and Wyllie-Echeverria 1996).

Seagrass beds contribute to the oceanic carbonate lime mud production (Enríquez and Schubert 2014) by providing a substrate on which a range of epiphytic calcareous faunas occur (Perry and Beavington-Penney 2005) and efficiently act as sediment traps. Calcium carbonate produced by the epiphytes contribute to the local sediment budget and therefore play an important role in the oceanic carbon cycle. The dominant calcifying epibionts on *Thalassia testudinum* leaves are encrusting Mg-calcite red algae, followed by a minor contribution from bryozoans and serpulids (Enríquez and Schubert 2014). In tropical lagoon and shelf settings, the calcium carbonate production rates by encrusting epiphytes range from 180g $CaCo_3$ m^{-2} $year^{-1}$ in Jamaica (Land 1970) to 2800g $CaCo_3$ m^{-2} $year^{-1}$ in Barbados (Patriquin 1972). Calcium carbonate production rates by epiphytes vary spatially, especially across environmental gradients (Walker and Woelkerling 1988, Bosence 1989).

Seagrass presence and survival are dependent on several environmental factors: light, temperature, dissolved carbon dioxide, nutrients, salinity, moderate levels of wave exposure and a suitable substrate for anchoring (Green and Short 2003). Biological competition from other species, such as fleshy macroalgae may also influence the distribution and growth of seagrasses. Natural (hurricanes) and anthropogenic disturbances have impacted seagrass habitats for centuries. Significant declines in seagrass biomass and growth have been linked to some anthropogenic influences (Udy et al. 1999). The primary cause of seagrass biomass loss is the reduction in water clarity, from increased nutrient loading and increased turbidity (Duarte et al. 2004).

There is a growing threat to native seagrasses in the Caribbean, the invasive seagrass, *Halophila stipulacea*. *H. stipulacea* originates from the Indian Ocean and the Red Sea. In 2002, *H. stipulacea* was first discovered in the Caribbean in Grenada, West Indies (Ruiz and Ballantine 2004) and since then it has been observed in many other islands (Willette et al. 2014). The rapid spread of this invasive seagrass has been aided by human intervention via fishing traps and anchors (Ruiz and Ballantine 2004, Willette and Ambrose 2009, 2014). From recent studies (Willette and Ambrose 2012, Steiner and Willette 2015a), *H. stipulacea* has major negative ecological and functional impacts on Caribbean seagrasses. *H. stipulacea* can expand laterally at a high rate (up to 6 cm d^{-1}), and can displace *Syringodium filiforme* in as little as 10-12wks (Willette and Ambrose 2012). Furthermore, *H. stipulacea* is reported growing up to and within coral reefs

(Steiner and Willette 2015b) with preliminary data showing significantly lower herbivore rates of the invasive seagrass by reef-associated organisms (Willette, unpublished data).

During the 2016 field sampling for NOAA NCRMP, *H. stipulacea* was observed at a number of sites in Culebra Island and Ceiba, Puerto Rico. *H. stipulacea* was observed colonizing around coral reef areas as deep as 80ft, and within the Luis Peña Marine Reserve in Culebra Island. There has been little published information on the distribution and effects of *H. stipulacea* colonization in Puerto Rico. Therefore, we do not know the exact extent of this invasive species and its effects on the native seagrass habitats in Puerto Rico.

In September 2017 two major hurricanes, Irma and Maria, made landfall one week apart in Puerto Rico. The impacts of these two storms on land were pronounced, however the impact on the marine communities is unknown. Hurricanes can produce strong surge and waves, which can physically damage seagrass by the tearing, stripping, and breakage of leaves and shoots (Michot et al. 2002). Also, seagrass beds can be partially or completely buried by sediment that is resuspended, deposited by rivers, and/or eroded from the land. Also, the physical damage from the surge and wave could have assisted in the dispersal of *H. stipulacea*. Therefore, the distribution of this invasive seagrass may have increased as it may have colonized new areas.

Our study focuses on seagrass habitats, which are an Essential Fish Habitat and also designated as a critical habitat for green turtles. We assessed the extent of the invasive seagrass, *H. stipulacea,* at 200 random points around Culebra Island. We also examined the impacts of two major hurricanes on the coverage seagrass species around Culebra Island. The major goals of this project are listed below.

Goals

1. Create a geographically referenced map indicating the occurrence and coverage of *Halophila stipulacea*. Also, these maps will identify areas at risk for *H. stipulacea* colonization, by integrating the physiological limits (conductivity, temperature, depth) of this seagrass.
2. Identify highly impacted seagrass areas affected by both hurricanes from satellite data and field observations. Give suggestions of possible sites for seagrass restoration.
3. Collect information that could aid in the management and removal *of H. stipulacea* and in the conservation of fisheries and coral reef habitats.
4. Increase the awareness about the threat, transmission, and mitigation of *H. stipulacea*.

Materials and Methods

Site

In 2015, the Northeast Reserve Marine Ecological Corridor and Culebra Island were designated as a NOAA Habitat Blueprint Focus Area. The ecological and economic value of the Northeast Reserve and Culebra Island has led NOAA to identify these areas as a coral reef conservation priority by Puerto Rico’s marine resource management community.

Culebra Island is a $72km^2$ volcanic island located 27km off eastern Puerto Rico. Canal Luis Peña no-take Natural Reserve (CLNR), located on the west coast of Culebra Island, was established on September 30, 1999 as the first No-Take Marine Fishery Reserve in territorial waters of Puerto Rico (Pagán et al. 1999). This marine reserve covers an area of 4.75km^2. High diversity of sessile-benthic invertebrates and fish communities characterizes the coral reefs within this reserve (Pagán-Villegas et al. 1999, Hernández-Delgado et al. 2000). Recently, a Watershed Management Plan was produced to reduce the land runoff pollution at Playa Tamarindo, located within the CLNR.

In 2015, NOAA's National Centers for Coastal Ocean Science developed a detailed benthic habitat map of shallow-water habitats in the northeast and Culebra Island, Puerto Rico. The map covers 744km^2 of shallow-water habitats and at high spatial resolutions (100m^2). Also, a GeoTIFF was created to model the bathymetry of the seafloor in northeast Puerto Rico, specifically of the Northeast Ecological Reserve. The model was created by integrating soundings from several different sources, which included LiDAR, NOAA Single-beam and multibeam bathymetry, and historical lead line soundings. The bathymetry models consist of three different resolutions: 4m, 20m, and 100m.

In 2016, Coastal Survey Solutions was contracted by NOAA to assess the coral reef state in Culebra Island and other parts of the Northeast Reserve, using the NRCMP protocol. We identified and marked the occurrence of *Halophila stipulacea* at multiple sites in Culebra Island and Ceiba. *H. stipulacea* was present in seagrass habitats identified in benthic habitats by NOAA (Figure 1). The depth of the invasive seagrass ranged from 15m to 24m, and it was observed to grow within *Thalassia testudinum* beds.

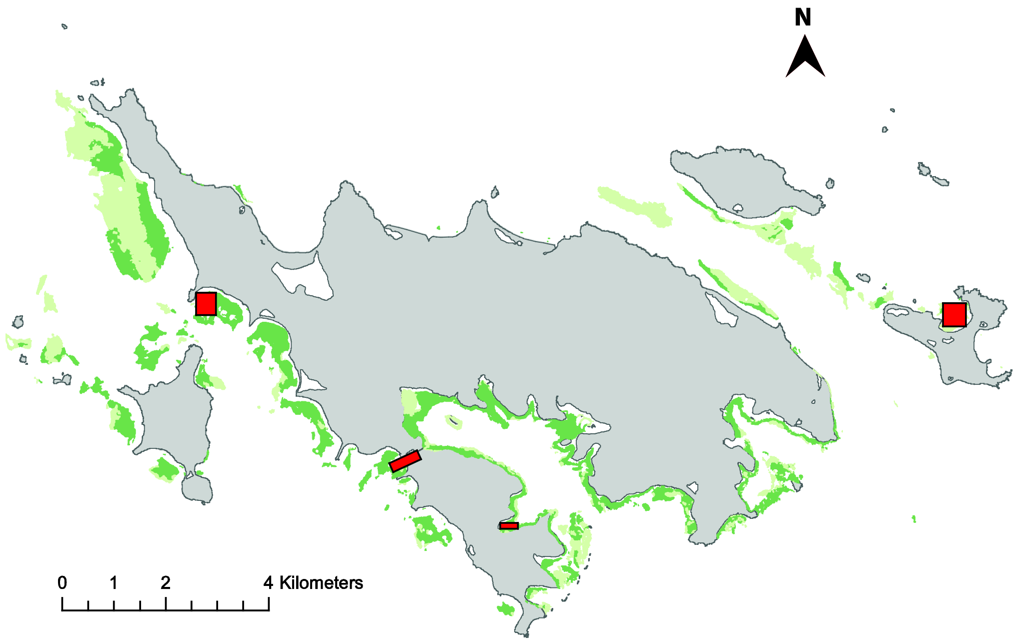


Figure 1 Map of Culebra Island. The light green represents areas of sparse seagrass habitat, while the dark green denotes areas of continuous seagrass as represented by NOAA's Benthic Habitat map. Red box areas represent locations where *Halophila stipulacea* was recorded during NCRMP surveys in 2016.

Sampling

In this project, we focused surveys in seagrass habitats identified in the NOAA's benthic habitat map and areas no greater than 30m in depth. Based on these criteria, a base map of the survey area was created, which included the NOAA habitat benthic map seagrass polygons segregated by depth ranges with the use of a combination of imagery, including lead line, single beam, multibeam, and Light Detection and Ranging (LiDAR) (Fig. 2).

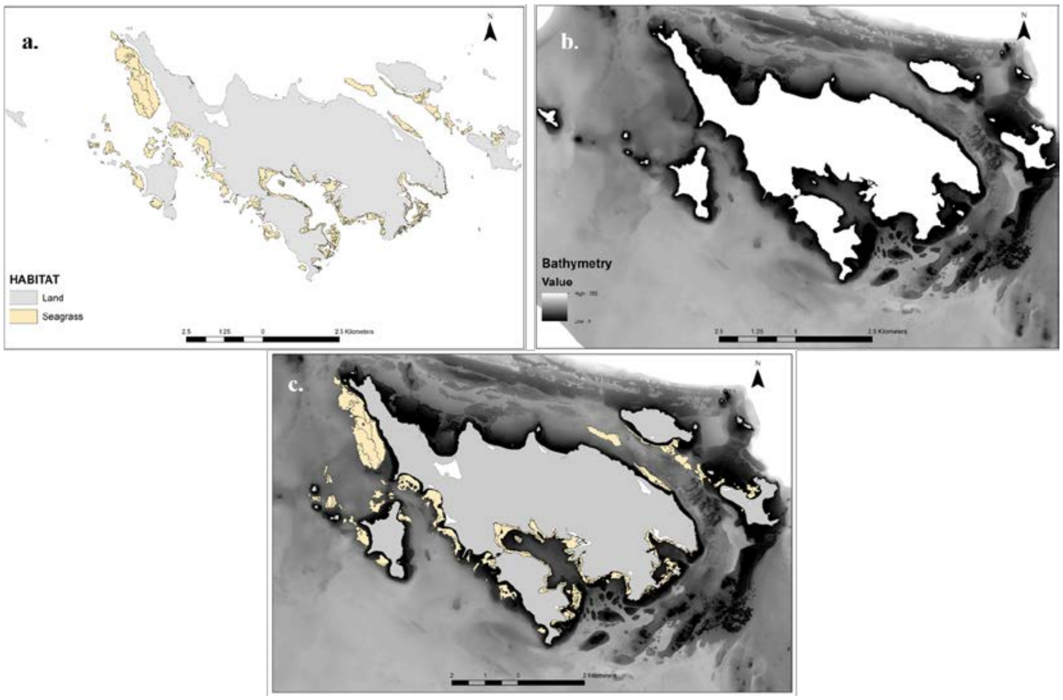


Figure 2 The base maps of Culebra Island with the overlaid seagrass polygons from the NOAA Habitat map (a), bathymetry created from historical hydrographic data including lead line, single beam, multibeam, and LiDAR imagery (b), and both the seagrass polygons and bathymetry (c).

The base map was created and 150 sites were randomly selected by stratified depths ranges: 1-10m, 11-20m, and 21-30m within the seagrass polygons (Fig. 3). ArcGIS was used to select random points within depth contours. The number of random points created was dependent on the size of the polygon. In addition to the 150 random chosen points, another 50 sites were haphazardly surveyed for seagrass. These sites were surveyed because they are targeted by boat users (mooring buoys) and are highly trafficked. See Index 1 for coordinates of each site location.

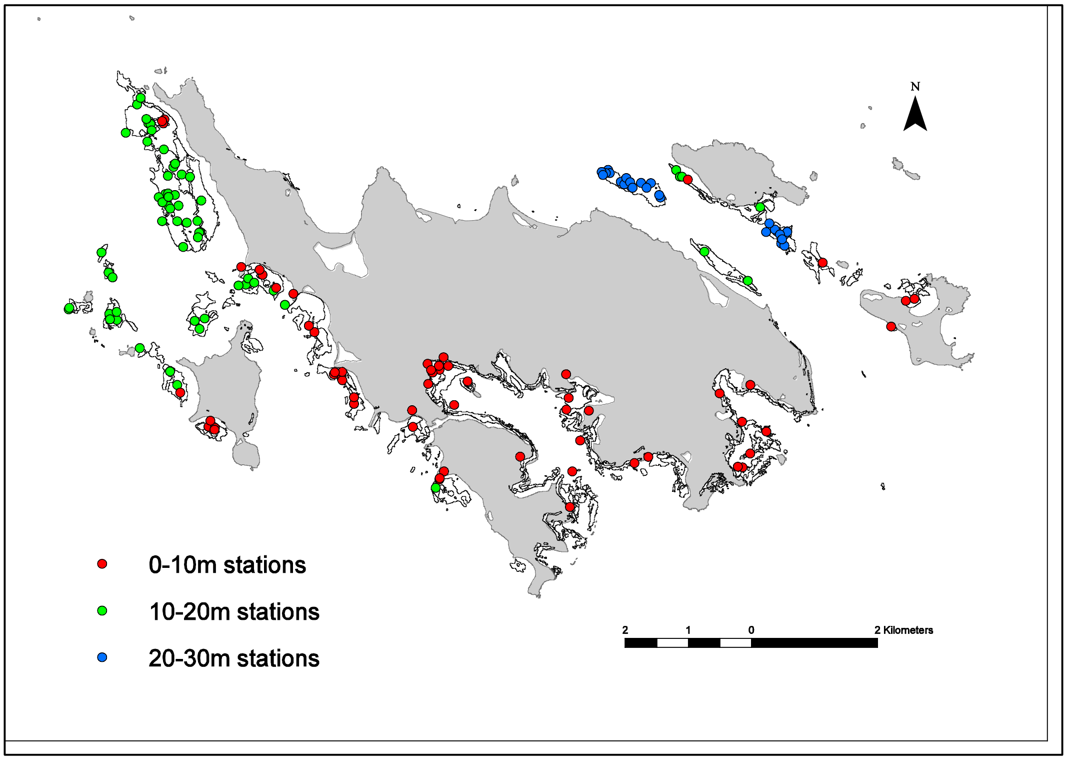


Figure 3 Random points (150 in total) selected within the seagrass polygons at depth ranges from 0-10m, 10-20m, and 20-30m in Culebra Island, Puerto Rico.

At each site, videos were recorded with a Hero5 GoPro camera mounted on a weighted frame in the vertical position (Fig. 4). The base of the frame was the size of a $0.25m^2$ quadrat, allowing for the standardization of seagrass densities and cover. At each site the frame was dropped haphazardly three times within a 10m radius of the station's waypoint. Environmental parameters like conductivity, temperature and dissolved oxygen were recorded with YSI Pro2030 Plus Multimeter at the site. Depending on the depth of the site, these parameters were measured from 0.5m to 20m. Turbidity was also measured at each site with a Secchi disk.

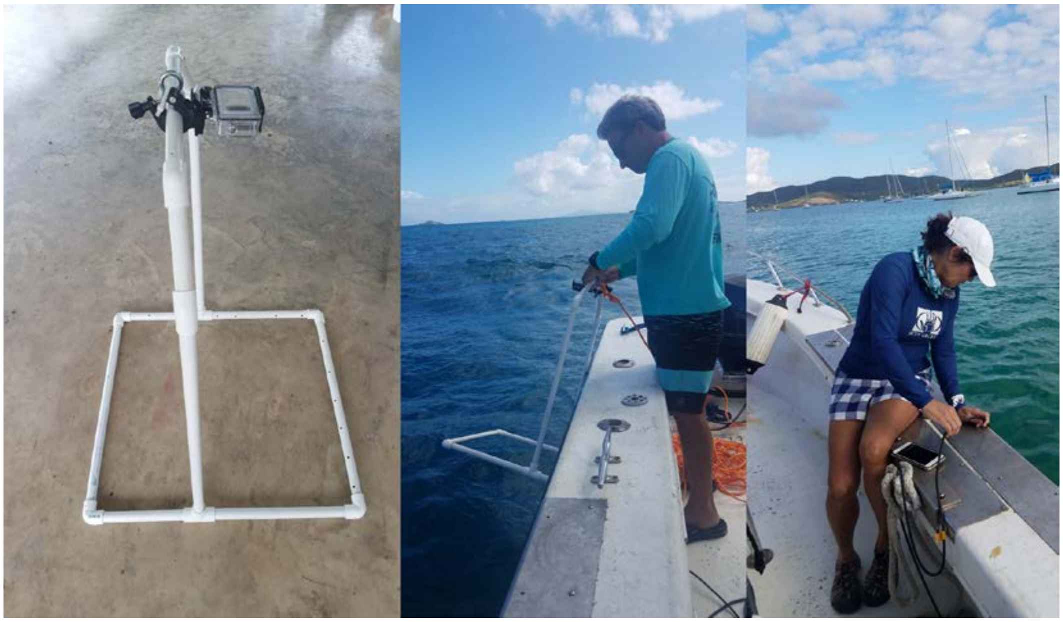


Figure 4 Photographs of the camera frame used to sample seagrass and the team surveying the seagrass and environmental parameters at each random site.

In the laboratory, three captures were sampled from each video (3 replicates). Figure 5 shows an example of three captures from Site 63 located south of Cayo Norte. All captures were analyzed and seagrass cover and composition were estimated.

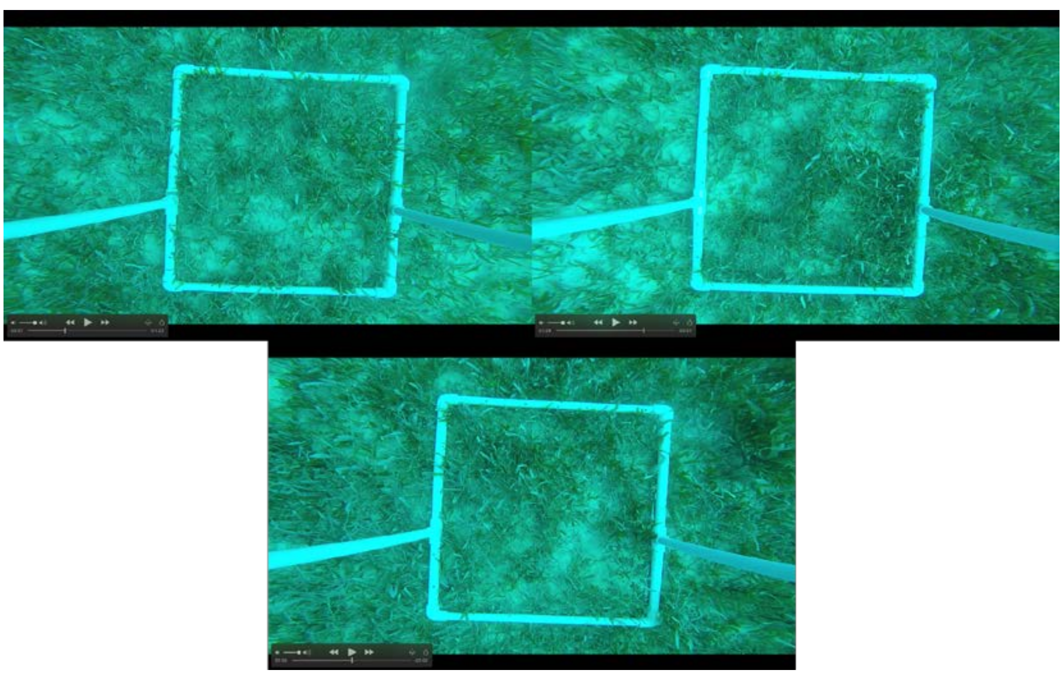


Figure 5 Three replicate photograph captures taken from a video recorded at a random site in Culebra Island, Puerto Rico.

We used the Braun Blanquet (values range from 0-5) method to assess seagrass cover (Table 1). The type of substrate, presence of invertebrates, and condition of seagrass (breakage or covered in sediment) were also estimated and recorded in each capture.

Table 1 The cover of seagrass based on the Braun Blanquet estimate.

Score	Cover
0	Taxa absent from quadrat
0.1	Taxa represented by a solitary shoot, <5% cover
0.5	Taxa represented by a few (<5) shoots, >5% cover
1	Taxa represented by many (>5) shoots, <5% cover
2	Taxa represented by many (>5), 5 - 25% cover
3	Taxa represented by many (>5),25 - 50% cover
4	Taxa represented by many (>5), 50 - 75% cover
5	Taxa represented by many (>5), 75 - 100% cover

Statistics

Distribution of seagrass

A two-way Permutational Multivariate Analysis of Variance (PERMANOVA) based on Bray-Curtis similarity measures was performed to examine the presence/absence and composition of seagrass species between locations (Culebrita, Cayo Norte, Cabeza de Perro, Bahía de Almodóvar, Sardineras, Ensenada Honda, Tamarindo, Carlos Rosario, Luis Peña), and depth ranges (0-10m, 10-20m, 20-30m). Similarity Percentages (SIMPER) tests were performed to identify the contribution of each seagrass species to the observed similarity or dissimilarity between regions and depth ranges.

A two-way PERMANOVA based on Euclidean distance measures was carried out to compare the percent cover (Braun Blanquet) of *H. stipulacea* between locations and depth ranges. We performed a PERMANOVA based on Euclidean distance, given we did not meet the parameters of normality. Euclidean distance measured for univariate

PERMANOVA analyses produces sums-of-squares estimates equivalent to parametric Analysis of Variance (Anderson 2001).

Invertebrate composition

A one-way PERMANOVA was performed to measure the differences in invertebrate composition present between the seagrass assemblage (presence or absence). Seagrass assemblages were as followed, monospecific beds of *T. testudinum*, *Syringodium filiforme* and *H. stipulacea* and mixed assemblages, included *H. stipulacea* + *S. filiforme*, *T. testudinum* + *S. filiforme*, and *T. testudinum* + *H. stipulacea* + *S. filiforme*. All PERMANOVA and SIMPER procedures were performed using PRIMER-E and PERMANOVA software (Anderson et al. 2008).

Environmental Factors

A Generalized Additive Model was performed (GAM) to examine the relationship between *H. stipulacea* cover and the cover of native seagrasses and the environmental parameters (depth, temperature, conductivity, turbidity, and dissolved oxygen). Habitat type was not included in the analysis because the majority of the sites were surveyed in unconsolidated sediment. *H. stipulacea* cover was the dependent factor, and the environmental parameters and native seagrass (*T. testudinum* and *S. filiforme*) cover were the independent factors. A smoothness treatment was fitted by maximum likelihood through the Laplace approximation. Best fit models were chosen based on the lowest Akaike Information Criteria (AIC) score. The analysis was performed in R v.3.1.1 using package mgcv v1.8-0. (Wood 2006) for GAM.

Risk Assessment Map

A risk assessment map was developed to visualize areas in Culebra Island at risk of *H. stipulacea* transmission. The parameters selected for the assessment are based on the data collected and analyzed during this study, the bathymetric data available, and the benthic habitats maps created by NOAA. The data collected from all sampling stations was analyzed and cross referenced to the presence or absence of *H. stipulacea* in order to identify the values most conducive to the colonization of the invasive seagrass. A total of six parameters were identified as possible key in determining conditions for the successful colonization and spread of *H. stipulacea*: a) available substrate, b) depth, c) native seagrass density, d) *H. stipulacea* presence, e) boat anchorage, and f) marine reserve. Individual shapefiles for each parameter were created and classified using a binary weight system (0 or 1) to categorize the vulnerability of the area within each parameter, where zero (0) represents less risk of a specific parameter and one (1) represents more risk for that parameter (Fig. 6).

Areas classified as sand in the benthic habitat map were identified as most vulnerable and thus given a value of 1. For depth, sampling data suggested the range between 4m and 15 m was the most vulnerable and areas within these depths were also given a value of 1. Regarding the presence of native seagrasses, data suggests *H. stipulacea* was less likely to be found in dense native seagrass patches. Seagrass habitats from the NOAA habitat map were classified as 0 in dense areas (>75%) and 1 in sparse areas (<75%). For the fourth parameter, *H. stipulacea* presence, point sampling stations where *H. stipulacea* was observed were converted to polygon shapefiles utilizing a 25m buffer and assigned a value of 1. Polygons of areas known to be frequently visited by boaters or areas of short- and long-term anchorage were also created and assigned a value of 1. Finally, for the six parameter, marine reserve, we observed the occurrence of *H. stipulacea* to be lower in the Luis Peña Natural Reserve, when compared to adjacent areas outside. The area within the reserve was given a value of 0 whereas the rest was assigned as 1. All six shapefiles were then intersected together into a single map with the resulting combined polygons. These new polygons were then reclassified individually with the sum of their corresponding vulnerability indices.

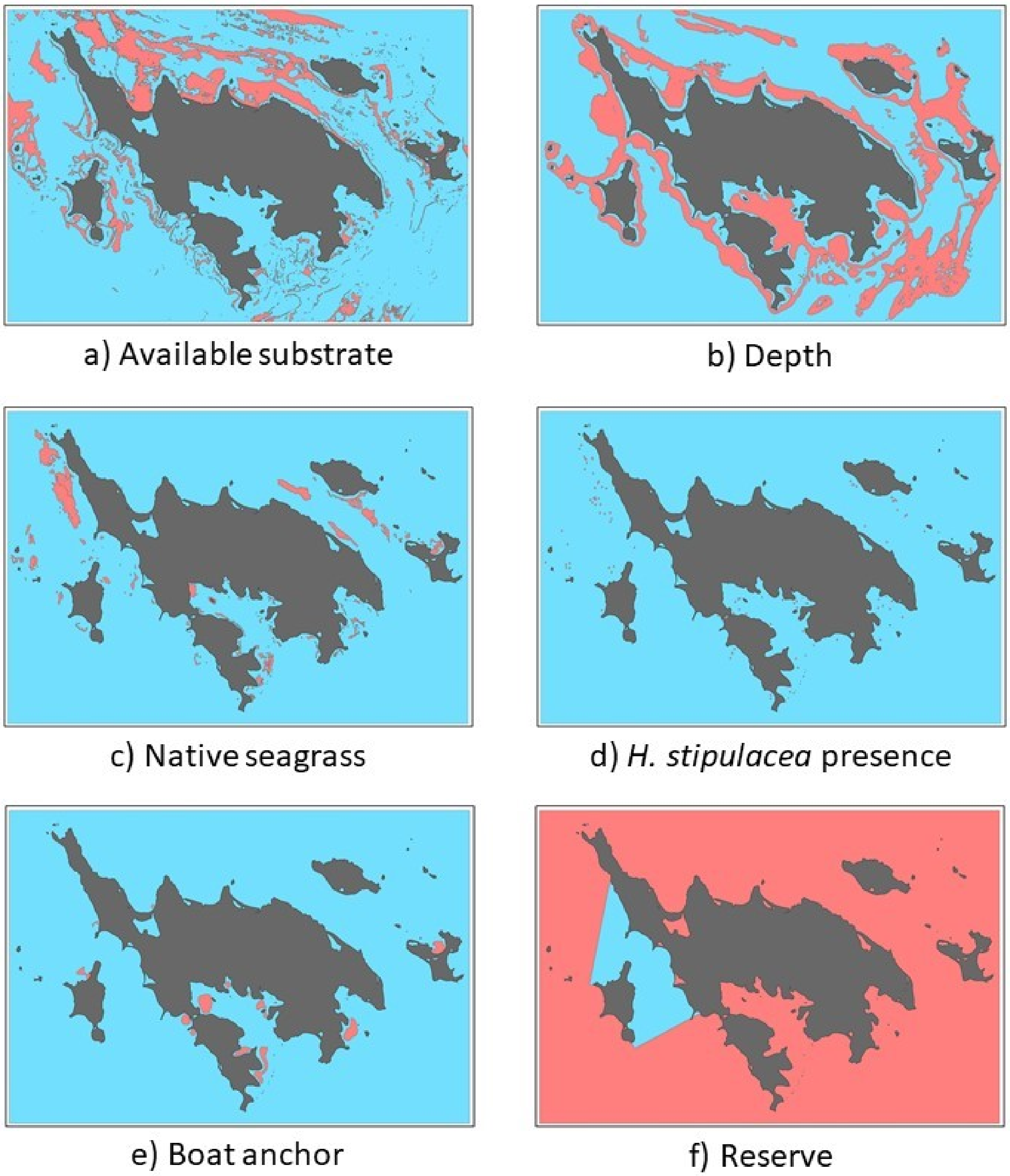


Figure 6 Shapefile for each individual risk parameter for *Halophila stipulacea* transmission in Culebra Island, Puerto Rico. Red represents high risk (1) and blue represents low risk (0).

Hurricane impacts

In order to identify highly impacted seagrass areas affected by both hurricanes from satellite data, Sentinel 2 satellite imagery multi-spectral instrument (MSI) data were downloaded free of charge from the Copernicus Open Access Hub website (https://scihub.copernicus.eu/dhus/#/home). This sensor provides high resolution (10m) imagery from RGB and near-IR bands that can be used for benthic classification. The images selected were prioritized based on coverage of the area of interest, cloud-free images, and images before and after the hurricane. Based on these criteria two images were obtained, from February 24, 2017 and December 19, 2017. An atmospheric correction was performed to the images using the SEN2COR processing routine (ESA S2-PDGS-MPC-L2A-SUM-V2.8, 2019) to remove the atmospheric effects and obtain a surface reflectance image. An additional step was performed to remove sunglint effects from the imagery based on Hedley et al., (2005).

The images were cropped to the area of interest and co-registered. A landmask was applied before the benthic classification was performed. An initial unsupervised classification (pixel-based) was applied to evaluate image quality, data gaps and water column effects on the imagery. After this evaluation, areas with data gaps were masked and/or removed. A supervised classification (Maximum Likelihood) was performed to the images. Supervised classification creates training areas, signature files and segments the images based on pixel information from known classes (Richards 2013). The training features were based on the updated benthic habitat map for the Northeast Puerto Rico and Culebra Island BioMapper (Kågesten et al., 2015). In addition, benthic surveys completed for this project were also used to improve the training samples. The classification was based primarily on habitat type (Table 2) and classes that had multiple type (e.g. seagrass continuous, seagrass patchy) were combined into one class. The final products after the supervised classification were two benthic habitat maps, one from February 24, 2017, before hurricanes Irma and Maria, and one from December 19, 2017, after the hurricanes. These images were chosen because cloud cover and sunglint were relatively low compared to other images. The area of each benthic categories were calculated for each image. Benthic areas were compared between the two images to assess change.

Table 2 Adapted from Kågesten et al (2015) habitat classes criteria used for the classification and new classes merged.

Habitat Overview Attribute	Definition	New Class
Coral Reef (High Relief, High Coral)	Coral Reef and Hardbottom; Coral Cover >10%; Moderate-Very High Topographic Complexity	Coral Reefs
Coral Reef (High Relief, Low Coral)	Coral Reef and Hardbottom; Coral Cover <10%; Moderate-Very High Topographic Complexity	Coral Reefs
Coral Reef (Low Relief, High Coral)	Coral Reef and Hardbottom; Coral Cover >10%; Very Low - Low Topographic Complexity	Coral Reefs
Coral Reef (Low Relief, Low Coral)	Coral Reef and Hardbottom; Coral Cover <10%; Very Low - Low Topographic Complexity	Coral Reefs
Seagrass (Continuous)	Sand, Mud or Reef Rubble; Seagrass (Continuous (90% - 100%)	Seagrass
Seagrass (Patchy)	Sand, Mud or Reef Rubble; Seagrass (Patchy (10% - <90%))	Seagrass
Algae (Continuous)	Sand, Mud or Rhodoliths; Algae (Continuous (90% - 100%))	Algae
Algae (Patchy)	Sand, Mud or Rhodoliths; Algae (Patchy (10% - <90%))	Algae
Sand	Sand; No Biological Cover / Unknown Cover	Sand
Mud	Mud; No Biological Cover / Unknown Cover	Mud
Unknown	Unknown Area (Deepwater, Sunglint)	Unknown

Results and Discussion

Seagrass assemblage

A total of 200 sites were surveyed for the presence of seagrass. The site locations were based on the classification designated by NOAA in the benthic habitat maps. Out of the 200 sites sampled, only 40 sites were absent of seagrass (Fig. 7). These sites with no seagrass present were mostly located in the deeper areas (20-23m), close to Cayo Norte, which is located to the northeast of Culebra Island.

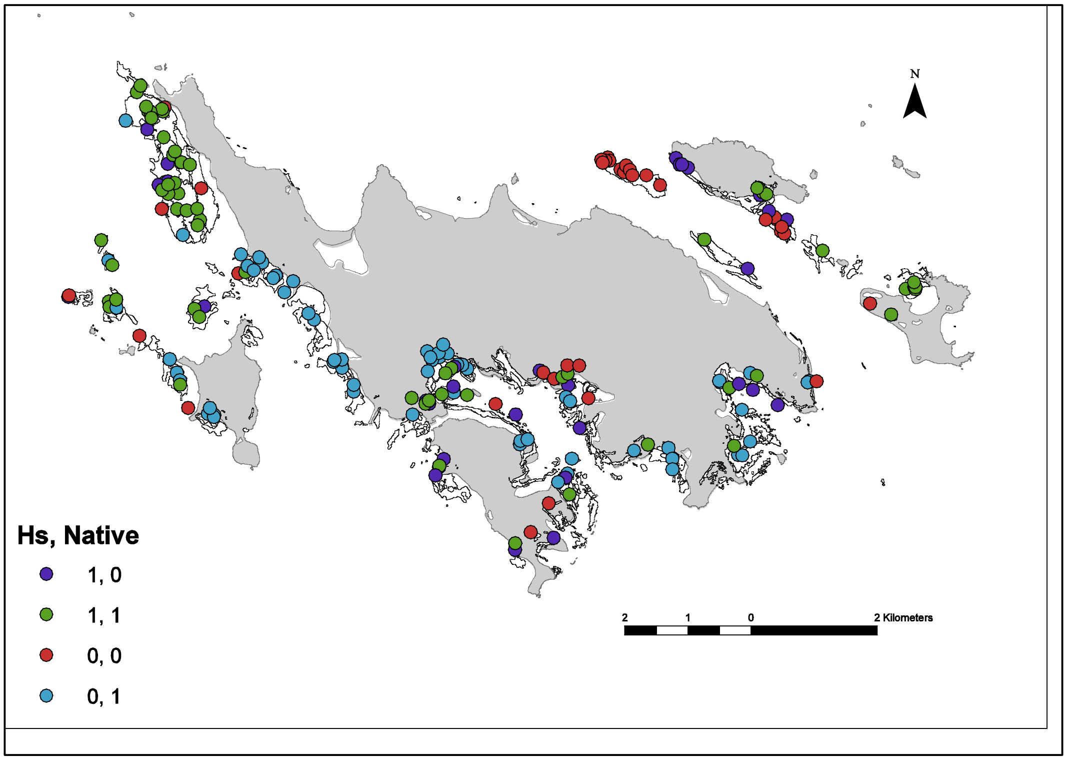


Figure 7 Sites (total 200) surveyed from the random arrangement in Culebra Island, Puerto Rico. The presence of *Halophila stipulacea* (represented by 1) and other seagrass, in this case *Syringodium filiforme* and *Thalassia testudinum* (represented by 1) at the random sites surveyed in Culebra Island, Puerto Rico. Zero represents no seagrass was present at the site.

There were three species of seagrass identified at the sites surveyed, and these were *Thalassia testudinum*, *Syringodium filiforme*, and *Halophila stipulacea*. *S. filiforme* was the most common seagrass observed (62% sites), followed by *H. stipulacea* (61%), and *T. testudinum* (46%) (Fig. 7). *Halodule wrightii* was not observed in this study, even though it is present in Culebra (Hernandez et al. 2017). Hernandez et al. (2017) reported a decline of *H. wrightii* cover due to the passing of the hurricanes in 2017 and by indirect impacts of environmental stress gradients (land-source pollution). The highest mean cover (~4%) of *H. wrightii* reported in their surveys was about 4% in Bahia Linda. Given the low abundance of *H. wrightii* and the sampling design of this study (random points), we might have missed locating this species.

The community structure of seagrass species significantly varied between locations and depth ranges (Table 3). *T. testudinum* contributed 59.6% to the seagrass communities in shallower waters (>10m), followed by *H. stipulacea* 28.7%, and *S. filiforme* (11.8%). Mid-water (10-20m) seagrass communities were dominated by *H. stipulacea* with a contribution of 73.93%, and *S. filiforme* (23.9%). The seagrass community structures in water >20m were mostly characterized by *H. stipulacea* (82.5% contribution). Spatially, seagrass communities were overall significantly different between locations. The only areas with similar seagrass assemblages were within protected areas, like bays, Ensenada Honda, and Bahía de Almodóvar. *T. testudinum* was the dominate seagrass species in these bays, contributing between 50 to 70% of the overall seagrass communities.

Table 3 The results of the two-way Permutational Multivariate Analysis of Variance examining the differences of seagrass assemblages between locations and depth ranges in Culebra Island, Puerto Rico.

Source	df	SS	MS	Pseudo-F	P(perm)
Lo	8	1.98 E+05	24753	14.37	0.001
De	2	6.22E+04	31098	18.05	0.001
De x Lo	6	32189	5364.8	3.11	0.001

Out of the 160 sites with seagrass present, *H. stipulacea* was present at 97 sites. At sites where *H. stipulacea* was present, the average cover ranged from 0.07 to 5 (BB). The difference in cover between locations and depth ranges was significant (Table 4). The overall mean cover (± SE) of *H. stipulacea* in Culebra was 1.59 ± 0.08% based on the Braun Blanquet (BB) method, which corresponds to any number of individuals covering 5-25% of the area. High cover (4-5 BB) of *H. stipulacea* was observed at 43 sites (Fig. 8). As seen in Figure 8, these areas were located on the east side of Culebra Island, especially south of Cayo Norte. Also, there were relatively high densities of *H. stipulacea* (3-4) north of Playa Tamarindo. *H. stipulacea* was absent or not as prevalent in areas between Playa Sardinas and Playa Tamarindo, and on the south side of Luis Peña. Hernandez et al. (2017) also confirmed the absence or low presence of *H. stipulacea* from Bahia Tamarindo to Punta Melones. These areas were characterized by mixed stands of mostly *T. testudinum* and *S. filiforme* (Hernandez et al. 2017). Future studies of the physical and environmental dynamics of this area (Bahia Tamarindo to Punta Melones) might give some insight on how to mitigate the spread of the invasive seagrass. We have noticed relatively high currents in this area, which may inhibit the rhizome pieces from settling and colonizing. Coincidentally, this area also lies within the Luis Peña Natural Reserve, where fishing is prohibited.

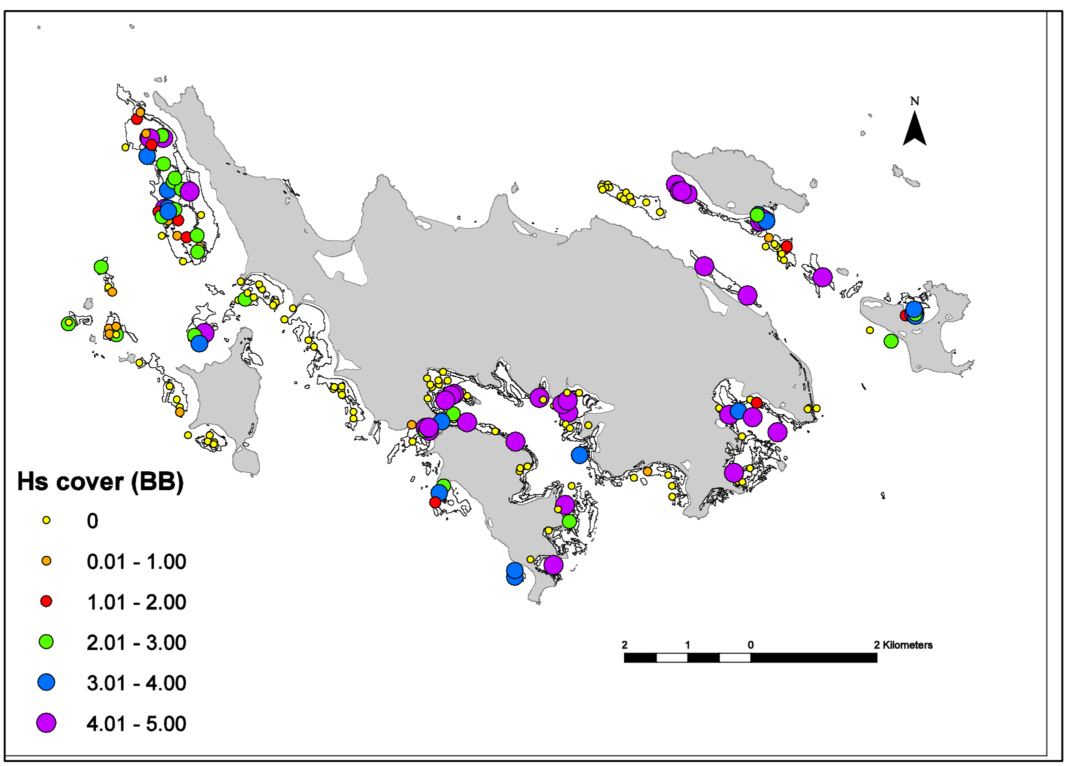


Figure 8 The mean cover of *Halophila stipulacea* based on Braun Blanquet at the random sites (200 in total) in Culebra Island, Puerto Rico.

High cover of *H. stipulacea* was observed at shallower depths (average 7.66 ± 0.78m), ranging from 1.2m to 18.9m. *H. stipulacea* was mostly absent at the deeper sites (20-30m), with the exception at two sites (23m), where average densities reached as high as 1.67 ± 0.33 (Braun Blanquet ± SE). Leaf blades were small and sparse at the deeper sites when compared to shallower sites. *H. stipulacea* at deeper sites were either young, or morphological differences could be due to environmental factors, like low light penetration in deeper waters. Further monitoring of this area is necessary to measure the exact coverage and how quickly it is spreading at these deeper sites since uncolonized sand is the dominant substrate in this area. Uncolonized sandy areas favor the spread of this invasive seagrass (Steiner and Willette 2015).

Table 4 The results of the two-way Permutational Multivariate Analysis of Variance examining the differences of the cover of *Halophila stipulacea* based on Braun Blanquet between locations and depth ranges in Culebra Island, Puerto Rico.

Source	df	SS	MS	Pseudo-F	P(perm)
Lo	8	1.98E+05	24753	14.369	0.001
De	2	62197	31098	18.052	0.001
Lo x De	6	32189	5364.8	3.1142	0.001

Monospecific beds of *H. stipulacea* were recorded at 26 sites. The majority of the sites where represented by mixed seagrass stands. The most common mixed stand was comprised of *H. stipulacea* and *S. filiforme*. *S. filiforme* was common around Culebra. *S. filiforme* can develop into dense meadows. However, in Culebra the cover of this seagrass never reached 4 or 5. An inverse relationship was observed between the densities of *H. stipulacea* and *S filiforme* (GAM, $p<0.0001$). The sparse cover of *S. filiforme* has possibly allowed for the invasion of *H. stipulacea*. The chances of *H.*

stipulacea displacing *S. filiforme* is high, given the results of other published studies in the Caribbean (Steiner and Willette 2015, Willette and Ambrose 2012).

T. testudinum, also known as turtle grass, is a climax species and one of the most dominant seagrasses in the Caribbean (Williams 1990). It can form thick meadows, which provides valuable functions such as food source, refuge and coastal protection (Williams 1990). In this study, *T. testudinum* was not as common as the other seagrasses. High densities of *T. testudinum* were observed in sheltered bays, such as Bahía de Almodóvar and Ensenada Honda and along the coast between Punta Tamarindo and Punta Melonas. *H. stipulacea* was absent in areas where *T. testudinum* was highly dense. There was a significant negative relationship between *H. stipulacea* and *T. testudinum* densities (GAM, $p<0.0001$). Steiner and Willette (2015) observed that same negative pattern between the two species and attributed the lack of invasion to the thick rhizome layer produced by *T. testudinum* and the shading effects of its benthic shoot cover. Space limitation for fragment colonization and/or the shading effect of the long *T. testudinum* blades, could be why there was a paucity of *H. stipulacea* invasion at these areas.

Environmental factors

The adaptability to a variation of physiological conditions has allowed the invasion of *H. stipulacea* to a wide range of habitats. Environmental factors measured during sampling were as followed, the temperature ranged from 26.2 to 29.1°C, conductivity from 53.5 to 60.2mg/cm, dissolved oxygen from 3.92 to 7.93mg/l, and turbidity 0.51 to 14.01m. Environmental factors were re-sampled at the sites throughout the study period, but no major variance was observed between sampling units. The lack in variance was to be expected given Culebra Island does not have any rivers.

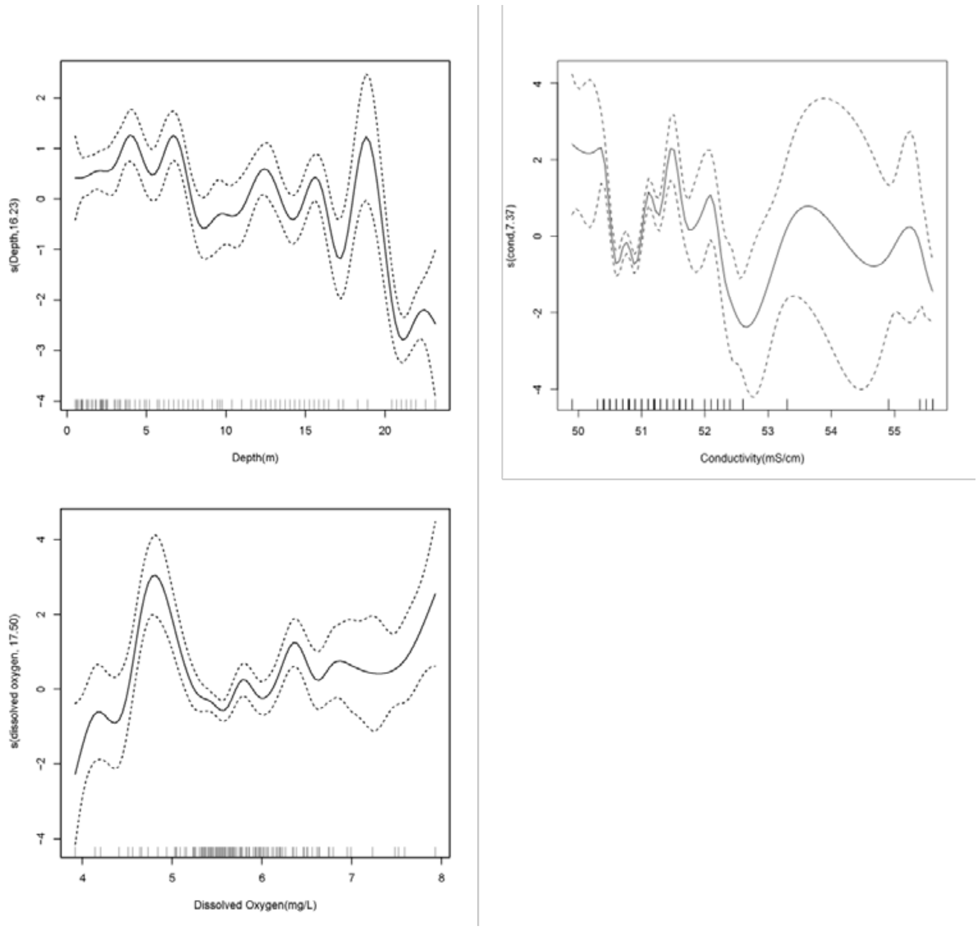


Figure 9 The plots of the Generalized Additive Model examining the relationship between *Halophila stipulacea* cover and depth (meters), conductivity (mS/cm), and dissolved oxygen (mg/L).

As seen in the GAM (Table 5), the ideal model for assessing *H. stipulacea* density included independent factors, such as the densities of native seagrasses, depth (m), dissolved oxygen (mg/L), and conductivity (mS/cm). This model explained 62.1% variance of the *H. stipulacea* distribution. Temperature and turbidity were not included in the optimal model. The cover of *T. testudinum* and *S. filiforme* was negatively associated with *H. stipulacea* cover. All of the environmental factors that were included in the model were significantly associated with the cover of *H. stipulacea*. As seen in the GAM plots (Fig. 9) the relationships are nonlinear. Depth was more influential on the cover of

H. stipulacea than other environmental factors. Depth contributed 46.5% of the model's variation

Table 5 The variation of *Halophila stipulacea* cover between the cover of native seagrass species, *Thalassia testudinum* and *Syringodium filiforme*, and environmental parameters, depth (meters), conductivity (mS/cm, and dissolved oxygen (mg/L). The model in Table 5 represents the best-fit model using a Generalized Additive Model analysis.

	Estimate	**SE**	**t value**	**p value**
Intercept	2.64	0.09	28.75	<0.0001
T. testudinum	-0.53	0.05	-11.55	<0.0001
S. filiforme	-0.37	0.07	-5.29	<0.0001
Smooth terms	edf	Ref.df	F	p value
s(Depth)	16.23	18.39	15.33	<0.0001
s(Conductivity)	7.37	8.26	6.67	<0.0001
s(DO)	17.5	19.16	4.3	<0.0001
R-sq (adj)	0.447			
AIC	1680.18			

Benthic communities

The presence of benthic organisms identified in seagrass habitats was low. We identified nine sessile- and five motile-benthic species (see Table 6). Benthic organisms were only present at 18% of the seagrass sites (total 160). Sponges were the most frequent and diverse benthic category in seagrass, with six known and two unidentified species. The most recurrent sponge was *Clathria curacaoensis*, a sponge common in seagrass habitats.

Table 6 The abundance of sessile- and motile-benthic organisms observed at the different seagrass assemblages. Hs= *Halophila stipulacea*, Sf= *Syringodium filiforme,* Tt=*Thalassia testudinum*.

	Hs	**Hs + Sf**	**Tt**	**Tt + Sf**	**Tt + Hs + Sf**
Sessile-benthic					
Sponge					
Amphimedon compressa		1			
Clathria curacaoensis	5	5			
Desmapsamma anchorata		1			
Dysidae janiae	1				1
Neopetrosia subtriangularis?		2			
Smenospongia sp.	1				
Unknown sponge sp. 1		1		2	
Unknown sponge sp. 2		1		1	
Hard Coral					
Manicina areolata				1	1
Motile-benthic					
Crustacean					
Lobatus gigas	1	3		1	
Anemone					
Cassiopea sp.	3	1		3	
Echinoderm					
Oreaster reticulatis		1			
Holothuria mexicana	3	3	1	1	
Diadema antillarum	1	2			
Species Richness	7	11	1	6	2

A small scleractinian coral, *Manicina areolata*, was observed at two sites. Out of the motile invertebrates, echinoderms, conch (*Lobatus gigas*) and anemones (*Cassiopea* sp.) were the most commonly recorded. For the echinoderms, these include the sea star, *Oreaster reticulates*, sea cucumber, *Holothuria mexicana*, and several recruits of the sea urchin, *Diadema antillarum*, were observed.

Given the low abundance of benthic organisms, it was challenging to distinguish patterns between the different seagrass species, as seen in the PERMANOVA (Table 7). However, habitats with a mix of *H. stipulacea* and *S. filiforme* were characterized with a high richness of benthic species, followed by monospecific beds of *H. stipulacea*.

Monospecific beds of *T. testudinum* had the lowest benthic richness, with only the donkey dung sea cucumber (*H. mexicana*) observed.

Table 7 The results of the one-way Permutational Multivariate Analysis of Variance examining the differences of benthic invertebrate assemblages between seagrass assemblages in Culebra Island, Puerto Rico.

Source	df	SS	MS	Pseudo-F	P(perm)
Assemblage	5	13011	2168.4	0.86638	0.606

Risk assessment

We defined an area at "risk," as an area vulnerable to *H. stipulacea* invasion or areas where spread is highly expected because *H. stipulacea* is present at the site or in the vicinity (25m radius). The factors affecting *H. stipulacea* invasion were identified as depth, available substrate, native seagrass density, *H. stipulacea* presence, boat anchorage, and marine reserve. Given the high abundance and wide distribution of *Halophila stipulacea* in Culebra, we believe shallow areas surrounding the island are vulnerable to its invasion. The only area not as vulnerable to *H. stipulacea* invasion, which was shaded blue (area 261.48 ha, see Table 8), was between Tamarindo and Luis Peña (see Fig. 10). This area is within the Luis Peña Natural Reserve and is protected from fishing and anchoring is limited to small sand patches when mooring buoys are occupied. This protection may be one of the factors limiting the spread of slow colonization of *H. stipulacea*, since anchoring is one of the primary vectors of its invasion. Other possible factors maybe are the continuous cover of native seagrass and the environmental nature of the area. There are dense *T. testudinum* beds along Tamarindo, which may inhibit the invasion of *H. stipulacea*. Also, there are strong currents between Tamarindo and Luis Peña which may not allow for the settlement and colonization of the invasive seagrass. This area should be monitored because we did observe young stands of *H. stipulacea* around patch reefs along the southeast of Luis Peña. A more intensive survey needs to be carried out around patch reefs in Culebra in order to understand the potential impacts this seagrass may have on the reef communities.

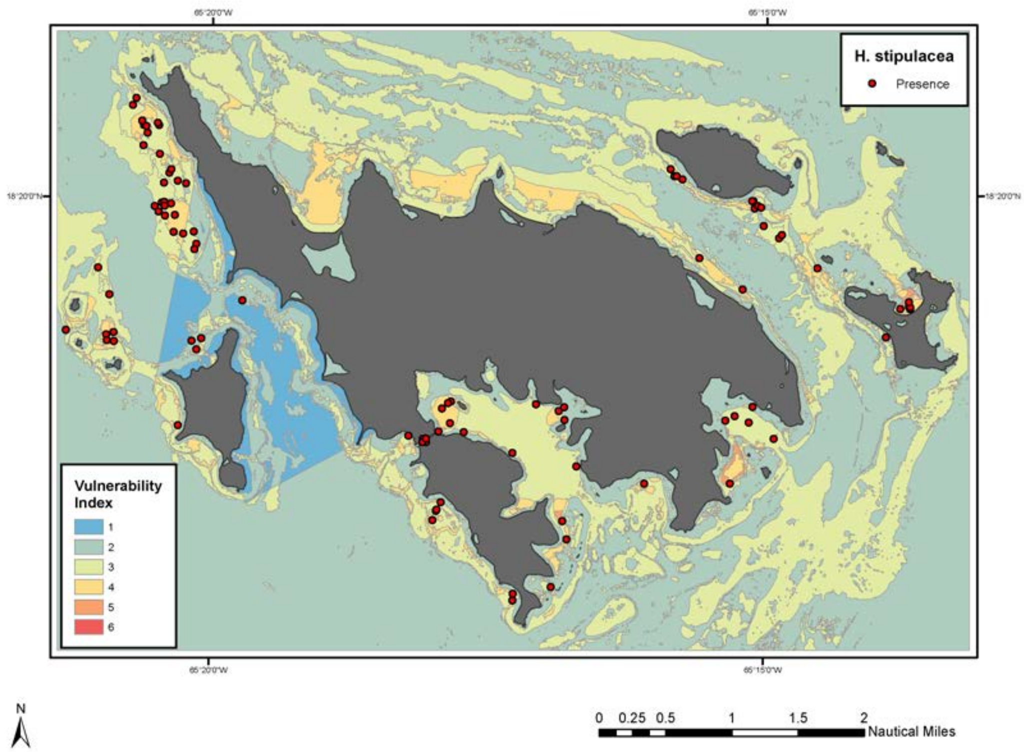


Figure 10 Risk assessment map showing the degree of vulnerability to the colonization of *Halophila stipulacea* in Culebra Island, Puerto Rico. Areas with a higher index have a higher probability of being invaded by *Halophila stipulacea*.

Additional monitoring should occur in medium to high risk areas (3-5 index) with undocumented *H. stipulacea*, and these include Playa Flamenco, Playa Resaca, and Playa Brava, which are all located on the north coast. Based on the NOAA benthic habitat map, these areas are all sandy, with not seagrass formation. However, given the fast growth and colonization of *H. stipulacea*, there is a potential for it to spread to these areas given the depth, habitat availability, and boat traffic. We did not observe any areas to have a vulnerability index of 6 (Table 8).

Table 8 The total area (hectares) for the vulnerability calculations from the risk assessment of *Halophila stipulacea* invasion in Culebra Island, Puerto Rico.

Vulnerability Index	Area (Ha)	% of Total
0	261.48	3.07
1	5336.20	62.57
2	2579.25	30.25
3	337.23	3.95
4	13.39	0.16
5	0.19	0
6	0	0

Hurricane impacts

As mentioned before, the images selected were prioritized based on coverage of the area of interest, cloud-free images, and images before and after the hurricane. Based on these criteria two images were obtained, from February 24, 2017 and December 19, 2017. However, the images were heavily affected by sunglint even after applying the corrections to remove these effects (Hedley et al. 2005). To overcome these limitations, a subset of the images was created to remove the heavily sunglinted areas and focus the benthic classification on priority areas identified in the field surveys (Fig. 11). These five areas were analyzed and classified into six main benthic categories (sand, mud, algae, seagrass, coral reef, and land) through supervised classification of the two Sentinel 2 images.

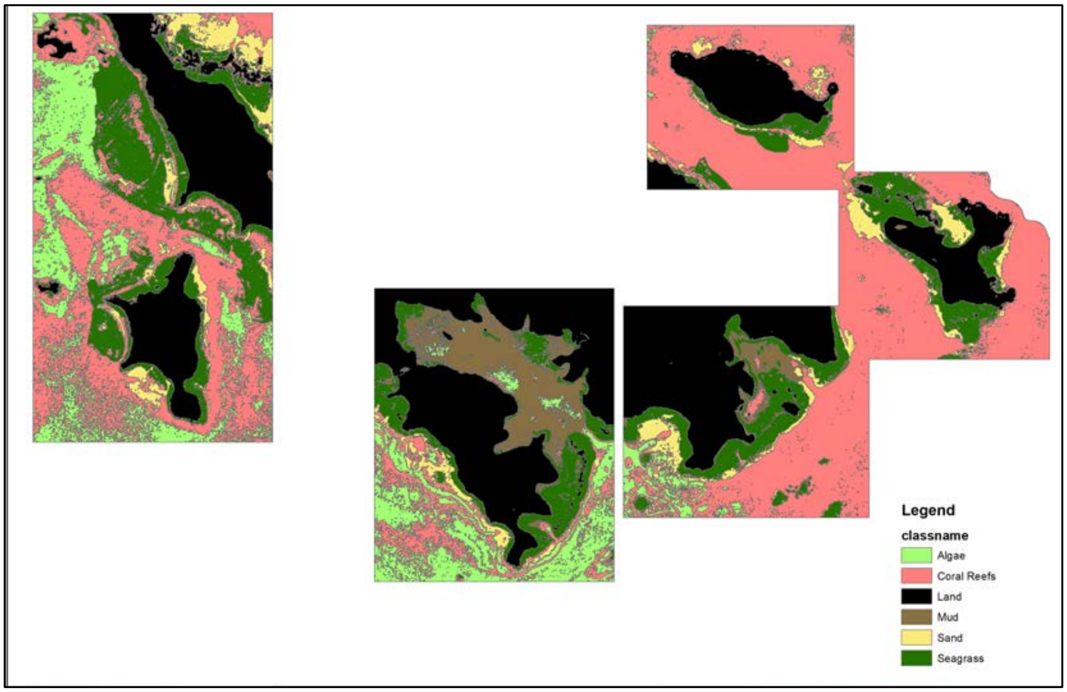


Figure 11 Benthic habitat map in a subset of images in Culebra Island, Puerto Rico before the two hurricanes 2017 (February 24, 2017). Six benthic categories were developed from supervised classification of the two Sentinel 2 images.

As seen in Table 9 the cover of seagrass decreased by 232.02 ha, and the loss of seagrass was concentrated between Punta Tamarindo and Punta Melones and western, northwestern side of Luis Peña. Other areas of seagrass loss was located on the southwestern side of Cayo Norte, and inside Bahía de Almodóvar. Both hurricanes entered Puerto Rico waters from the south east. Therefore, it is plausible that the greatest loss of seagrass occurred in areas more exposed to waves and wind, which tend to be south facing. As reported in Hernandez et al. (2017), the passing of these two major hurricanes resulted in significant declines of seagrass cover, mostly due to the burial and suffocation of sediment. However, during these surveys, we did not observe any direct impacts of the hurricanes on seagrass beds, such as places where seagrass was buried with sediment or signs of physical damage to the leaves and/or rhizomes. This was also confirmed in Hernández et al. (2017) study, which they also reported that physical disruption of the seagrass habitat matrix was not common during their post-hurricane surveys. There were other habitat categories that declined in areal coverage after the hurricanes, and these were coral reefs, mud, and sand.

Table 9 The area (hectares) for each of each of the benthic categories before and after the hurricane.

Category	Area (ha) Before	Area (ha) After
Algae	686.25	1852.61
Coral Reefs	1974.01	1184.69
Land	1613.04	1632.66
Mud	259.09	171.84
Sand	280.17	202.78
Seagrass	881.81	649.79

Benthic Mapping limitations

There were some anomalies of the benthic categorization when assessing the hurricane impacts. These anomalies are based on the cumulative effects of various factors that include: spatial resolution of the sensors (10m) to resolve the benthic features, sunglint areas that obscure the benthic features, similarities in the spectra signatures of various substrates (e.g. seagrass, algae), confused pixels due to lack of ground validation point density training samples from the classifier, and water clarity to resolve the benthic features (Kågesten et al. 2015, Purkis and Roelfsema 2015, Schweizer et al. 2005). As seen in Figure 12, seagrass and sand cover increased close to the most northwestern point of Culebra. The seagrass increase was located in deeper waters, deeper than >50ft. Given the depth of the area and the physiological limits of the native seagrass, it can be assumed that this area might be characterized by a dense, monospecific bed of *H. stipulacea*.

Another assumption was that it could be misidentified and might be algae, given the spectral signatures of algae and seagrass are very similar and many times hard to distinguish, especially in deeper areas. In addition, sunglint could have affected the supervised classification of the two images which could have resulted in overestimating the cover of coral reefs and misclassified other features. Field surveys are needed to confirm these assumptions.

Even with the limitations in the benthic mapping, these provided a good estimate of the changes in benthic cover after the hurricanes. Higher-resolution sensors with multiple bands (>4 bands) combined with a field campaign focused on the ground validation for benthic classification is recommended to provide a more detailed assessment of these changes (e.g. Kågesten et al. 2015).

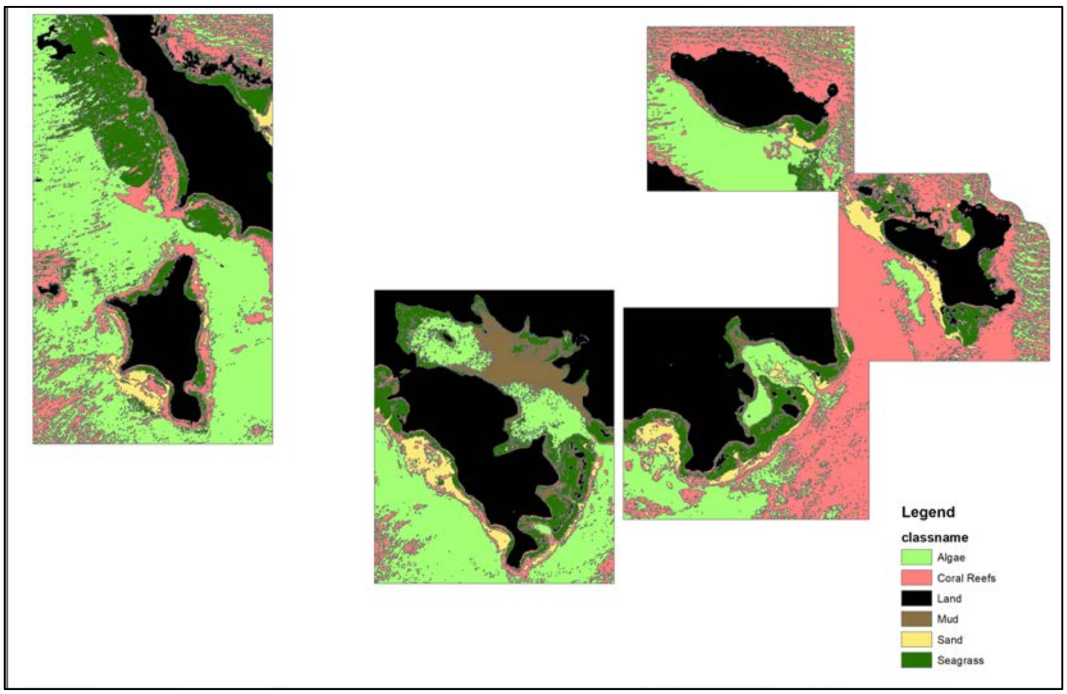


Figure 12 Benthic habitat map in a subset of images in Culebra Island, Puerto Rico after the two hurricanes 2017 (December 19, 2017). Six benthic categories were developed from supervised classification of the two Sentinel 2 images.

Educational outreach

The last goal of this project was to increase awareness about the threat, and transmission of *H. stipulacea*. Given the abundance of *H. stipulacea* identified in this study and the amount of floating rhizomes observed in Culebra Island, we wanted to reiterate to boaters to anchor in sand. We also emphasized to remove any *H. stipulacea* floating in the water or in an anchor. Pieces should not be thrown back to the water. The first educational tool produced was a tabloid. The tabloid highlighted four main points, 1) the importance of seagrasses in general, 2) what is *H. stipulacea* and how to identify it, 3) how is *H. stipulacea* spreading, and 4) what can be done to mitigate its spread (Fig. 13). Laminated identification cards (English and Spanish), magnets and stickers were also produced to aid in the identification of the invasive seagrass (Fig. 13).

Figure 13 Photographs of the educational material, which includes a tabloid (left) and identification card (right). Educational material highlights the importance of seagrass, and how to identify and mitigate the spread of *Halophila stipulacea*.

We focused the outreach on the east side of the island of Puerto Rico, Fajardo, Culebra Island and Vieques Island. Tabloids and identification cards were distributed to six marinas (Puerto del Rey, Puerto Chico, Villa Marina, Sunbay Marina, Dos Marinas, Marina Sardinera), four marine shops (The Marine Store, Boat Tech Performance, The Skipper Shop and West Marine in Fajardo), and six marine educational organizations

(University of Puerto Rico at Humacao, Coalición Pro Corredor Ecológico del Noreste, Centro Cultural Multidisciplinario de Juan Martín, Biblioteca University Interamericana, Para La Naturaleza in Cabezas de San Juan Reserve, Puerto Rico Sea Grant) (see Fig. 14). Digital copies will be available on Coastal Survey Solutions's website, and Institute for Socio-Ecological Research's website and Facebook page, and among other environmental group's Facebook pages, such as Corales del Este, Friend of the Luis Peña Channel Natural Reserve, Defendemos El Corredor Ecológico del Noreste, etc. We also distributed copies of all materials to the Department of Natural and Environmental Resources.

Figure 14 Photographs taken distributing the educational material to the local marinas, marine stores, organizational organizations, and local government agencies.

Conclusions

There are no confirmed records of when *Halophila stipulacea* first colonized Culebra Island. However, the spread of this invasive seagrass is pronounced, and could potentially have long-lasting effects on the native seagrass communities and patch coral reefs of Culebra. As of our last surveys in the winter of 2018, *H. stipulacea* was observed at 61% of the seagrass sites. Depth does not seem to be a factor limiting the colonization of this species, as it was observed down to 23m. Just recently, we observed *H. stipulacea* in large, dense monospecific beds off the coast of Vieques and Ceiba in 27m of depth. The east coast is not the only area where *H. stipulacea* has been witnessed. In September 2019, we observed *H. stipulacea* in Salinas and La Parguera, Puerto Rico. It can, therefore, be assumed that *H. stipulacea* may be along the east and south coast of Puerto Rico. Below are recommendations for the next steps and how to manage the spread of *H. stipulacea*.

1. Protect and conserve the native seagrass habitats around Puerto Rico, especially *Thalassia testudinum*. Dense *T. testudinum* beds have limited the colonization of the invasive seagrass. Restoration activities should focus on increasing native cover and especially in areas where damage has occurred to native seagrass beds.
2. Identify potential herbivores of *H. stipulacea*. There have been limited reports of some turtles eating the invasive (Becking et al. 2014), and potentially some echinoderms (Scheibling et al. 2018). Therefore, management officials might want to protect and possibly restore herbivores in areas where *H. stipulacea* is present.
3. The invasion and rapid spread of *H. stipulacea* have been attributed by the transport of the plant material by boats and possible fishing gear (Ruiz and Ballantine 2004, Vera et al. 2014, Willette et al. 2014). An outreach program to boaters and fishers is needed to educate them about this species and ways to limit its spread. Educational campaigns should also target other essential stakeholders, such as government and educational institutions, and local diving shops.

4. Further research is needed in Puerto Rico. The ecological impacts of the invasion are not fully understood. Given native seagrass are Essential Fish Habitats, how do the invasive seagrass impact fish and benthic communities.

References

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32-46

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: Guide to software and statistical methods. PRIMER-E: Plymouth, UK

Becking LE, van Brussel T, DeBrot AO, Christianen MJA (2014) First record of a Caribbean green turtle (*Chelonia mydas*) grazing on invasive seagrass (*Halophila stipulacea*). Caribb J Sci 48:162-163

Bosence D (1989) Surface sublittoral sediments of Florida Bay. Bull Mar Sci 44:434-453

Charpy-Roubaud C, Sournia A (1990) The comparative estimation of phytoplankton and microphytobenthic production in the oceans. Mar Microb Food Webs 4:31-57

Connolly RM (2009) Seagrass. In A marine climate change impacts and adaptation report card for Australia 2009, eds. Ploczansha ES, Hobday AJ, Richardson AJ. NCCARF Publication 05/09

Duarte CM (2002) The future of seagrass meadows. Environ Conserv 29:192-206

Duarte CM, Alvarez E, Grau A, Krause-Jensen D (2004) Which monitoring strategy should be chosen? In European Seagrasses: An introduction to monitoring and management. Ed Borum J, Duarte CM, Krause-Jensen D, Greve TM.

Enríquez S and Schubert N (2014) Direct contribution of the seagrass *Thalassia testudinum* to lime mud production. Nat Commun 5:1-12

Green EP, Short FT (2003) World Atlas of Seagrasses Prepared by the UNEP World Conservation Monitoring Centre. University of California Press, Berkeley, USA

Hemminga M, Duarte CM (2000) Seagrass Ecology, Cambridge Univ Press, Cambridge, UK

Hernández-Delgado EA, Alicea-Rodriguez L, Toledo CG, Sabat AM (2000) Baseline characterization of coral reefs and fish communities within the proposed Culebra Island Marine Fishery Reserve, Puerto Rico. Proc Gulf Caribb Fish Inst 51:537-555

Hernández-Delgado EA, Toledo-Hernández C, Ruíz-Díaz C, Gómez-Andújar NX, Medina-Muñiz JL, Suleimán-Ramos SE. (2017) Seagrass Rapid Assessment of Hurricane Maria impacts-Northeast Reserves System Habitat Focus Area (NER-HFA), Culebra Island, Puerto Rico. Final Report Department of Homeland Security, Federal Emergency Management Agency, and Department of Interior.

Kågesten, G, Sautter W, Edwards K, Costa B, Kracker L, Battista T (2015) Shallow-water benthic habitats of Northeast Puerto Rico and Culebra Island. NOAA Technical Memorandum NOS NCCOS 200. Silver Spring, MD. 112 pp. http://dx.doi.org/10.7289/V5Z899FH

Michot TC, Burch JN, Arrivillaga A, Rafferty PS, Doyle TW, Kremmerer (2002) Impacts of Hurricane Mitch on Seagrass beds and associated shallow reef communities along the Caribbean coast of Honduras and Guatemala: USGS Open File Report 3-181, 65p.

Land LS (1970) Carbonate mud: production by epibiont growth on *Thalassia testudinum*. J Sedim Petrol 40:1361-1363

Orth RJ, Carruthers TJB, Dennison WC, Duarte CN, et al. (2006) A global crisis for seagrass ecoystems. Bioscience 56:987-996

Pagán-Villegas, IM, Hernández-Delgado EA, Vicente VP (1999) Documento de designación de la Reserva Natural del Canal Luis Peña, Departamento de Recursos

Patriquin DG (1972) The origin of nitrogen and phosphorous for growth of the marine angiosperm *Thalassia testudinum*. Mar Biol 15:35-46

Perry CT, Beavington-Penney SJ (2005) Epiphytic calcium carbonate production and facies development with sub-tropical seagrass beds, Inhaca Island, Mozambique. Sed Geol 174-161-176

Purkis S, Roelfsema C (2015). Remote Sensing of Submerged Aquatic Vegetation and Coral Reefs. 10.1201/b18210-15

Richards JA (2013) Remote Sensing Digital Image Analysis. An introduction. Fifth Edition. Springer.

Ruiz H, Ballantine DL (2004) Occurrence of the seagrass *Halophila stipulacea* in the tropical west Atlantic. Bull Mar Sci 75:131

Scheibling RE, Patriquin DG, Filbee-Dexter K (2018) Distribution and abundance of the invasive seagrass *Halophila stipulacea* and associated benthic macrofauna in Carriacou, Grenadines, Eastern Caribbean. Aqua Bot 144:1-8

Schweizer D, Armstrong RA, Posada J (2005) Remote sensing characterization of benthic habitats and submerged vegetation biomass in Los Roques Archipelago National Park, Venezuela, International Journal of Remote Sensing, 26:12, 2657-2667, DOI: 10.1080/01431160500104111

Short FT, Wyllie-Echeverria S (1996) Natural and human-induces disturbances on seagrasses. Environ Conserv 23:17-27

Steiner SCC, Willette DA (2015a) The expansion of *Halophila stipulacea* (Hydrocharitaceae Angiospermae) is changing the seagrass landscape in the Commonwealth of Dominica, Lesser Antilles. Caribb Natur 22:1-19

Steiner SCC, Willette DA (2015b) Dimming sand halos in Dominica and the expansion of the invasive seagrass *Halophila stipulacea*. Reef Encounter 30:43-45

Terrados J, Borum J (2004) Why are seagrasses important? Goods and services provided by seagrass meadows. In Eurpoean seagrasses: an introduction to monitoring and management. Eds Borum J, Duarte CM, Krause-Jensen D, Greve TM. Monitoring and Managing of European Seagrasses.

Udy JW, Dennison WC, Long WJL, McKenzie LJ (1999) Responses of seagrass to nutrients in the Great Barrier Reef, Australia. Mar Ecol Prog Ser 185:257-271

Vera B, Collado-Vides L, Moreno C, van Tussenbroek BI (2014) *Halophila stipulacea* (Hydrocharitaceae): a recent introduction to the continental waters of Venezuela. Caribb J Sci 48:66-70

Walker DI, Woeklkerling WMJ (1988) Quantitative study of sediment contribution by epiphytic coralline red algae in seagrass meadows in Shark Bay, Western Australia. Mar Ecol Prog Ser 43:71-77

Willette DA, Ambrose RF (2009) The distribution and expansion of the invasive seagrass *Halophila stipulacea* in Dominica, West Indies, with a preliminary report from St. Lucia. Aquat Bot 91:137-142

Willette DA, Ambrose RF (2012) Effects of the invasive seagrass *Halophila stipulacea* on the native seagrass, *Syringodium filiforme*, and associated fish and epibiota communitites in the Eastern Caribbean. Aquatic Botany 103:74-82

Willette DA, Chaifour J, Debrot AO, Engel S, Miller J, Oxenford HA, Short FT, Steiner SCC, Vedie F (2014) Continued expansion of the trans-Atlantic invasive marine angiosperm *Halophila stipulacea* in the Eastern Caribbean. Aquat Bot 112:98-102

Williams SL (1990) Experimental studies of Caribbean seagrass bed development. Ecol Monogr 60:449-469

Wood SN (2006) Generalized Additive Models: An Introduction with R. Boca Raton, FL

Index

Index 1 The coordinates and depths of each random site surveyed for seagrass cover and composition in Culebra Island, Puerto Rico.

Sites	Latitude	Longitude	Depth (m)
1	18.3023	-65.2770	0.91
2	18.2997	-65.3033	3.96
3	18.2965	-65.2527	0.52
4	18.3079	-65.2994	2.50
5	18.2949	-65.2700	7.01
6	18.3029	-65.2972	3.05
7	18.3432	-65.3411	9.15
8	18.2885	-65.2796	2.20
9	18.2993	-65.3339	8.54
10	18.3075	-65.3005	2.16
11	18.3068	-65.3141	5.18
12	18.2922	-65.2992	7.62
13	18.3130	-65.3182	4.88
14	18.3062	-65.3140	5.18
15	18.3041	-65.2800	3.66
16	18.2956	-65.2872	3.66
17	18.3074	-65.3140	2.99
18	18.3020	-65.3035	2.32
19	18.2958	-65.2680	7.01
20	18.3041	-65.3382	3.96
21	18.3186	-65.2297	3.96
22	18.2980	-65.2782	7.01
23	18.2992	-65.3330	5.79
24	18.3185	-65.3215	5.79
25	18.3084	-65.2981	3.29
26	18.2934	-65.2986	6.71
27	18.2989	-65.3330	6.10
28	18.3063	-65.2952	0.88
29	18.3193	-65.3241	8.23
30	18.3211	-65.3261	8.54
31	18.3188	-65.2284	3.66
32	18.3148	-65.2318	1.83
33	18.3087	-65.3012	1.28
34	18.2936	-65.2793	2.32
35	18.2945	-65.2545	0.73
36	18.3070	-65.3153	7.93
37	18.3024	-65.2803	0.61
Sites	Latitude	Longitude	Depth (m)

Sites	Latitude	Longitude	Depth (m)
38	18.3084	-65.2995	1.80
39	18.3426	-65.3413	11.89
40	18.3239	-65.2422	7.62
41	18.3028	-65.3122	5.79
42	18.3355	-65.2626	9.76
43	18.3078	-65.3007	2.44
44	18.3219	-65.3266	7.32
45	18.2945	-65.2538	0.64
46	18.3139	-65.3191	7.62
47	18.3050	-65.2574	1.22
48	18.3222	-65.3293	9.45
49	18.2924	-65.2992	7.32
50	18.3038	-65.3122	4.57
53	18.3058	-65.3011	2.07
54	18.3063	-65.2528	1.52
56	18.3002	-65.3337	5.18
57	18.3010	-65.2540	1.52
58	18.3073	-65.3151	8.23
59	18.3097	-65.2989	0.88
60	18.3429	-65.3415	11.59
61	18.3287	-65.3390	12.20
62	18.3144	-65.3479	17.07
63	18.3368	-65.2643	15.55
64	18.3325	-65.3408	14.63
65	18.3389	-65.3412	14.33
66	18.3400	-65.3436	15.24
67	18.3326	-65.3404	14.63
68	18.3252	-65.2599	13.11
69	18.3453	-65.3453	16.16
70	18.3148	-65.3347	13.72
71	18.3212	-65.3492	16.16
72	18.3271	-65.3356	13.41
73	18.3158	-65.3551	18.60
74	18.3309	-65.3389	14.02
75	18.3197	-65.3286	14.02
76	18.3196	-65.3297	16.46
77	18.3426	-65.3435	12.20
78	18.3317	-65.2517	13.41
79	18.3359	-65.2638	18.90
80	18.3144	-65.3362	15.24
81	18.3153	-65.3490	13.41
82	18.3104	-65.3444	16.16

Sites	Latitude	Longitude	Depth (m)
83	18.3351	-65.3405	11.89
84	18.3250	-65.3381	14.33
Sites	Latitude	Longitude	Depth (m)
86	18.3317	-65.3355	15.55
87	18.3161	-65.3550	17.07
88	18.3364	-65.3397	10.37
89	18.3189	-65.3244	9.15
90	18.3462	-65.3447	13.41
91	18.3320	-65.3419	17.07
92	18.3285	-65.3376	12.50
93	18.3425	-65.3432	11.89
94	18.3211	-65.2534	13.11
95	18.3145	-65.3489	14.33
96	18.3133	-65.3355	10.37
97	18.3308	-65.3403	13.41
98	18.3264	-65.3359	12.80
99	18.3353	-65.3384	15.55
100	18.3313	-65.3413	15.55
101	18.3156	-65.3479	14.33
102	18.3412	-65.3469	17.38
103	18.3169	-65.3227	13.41
104	18.3206	-65.3283	14.33
106	18.3359	-65.2634	15.85
107	18.3369	-65.3394	15.24
108	18.3324	-65.3394	14.63
109	18.3240	-65.3503	17.38
110	18.3286	-65.3413	13.72
111	18.3350	-65.3372	15.55
112	18.3432	-65.3438	12.50
113	18.3200	-65.3274	11.89
114	18.3416	-65.3430	11.59
115	18.3287	-65.3360	12.50
116	18.3052	-65.3387	11.59
117	18.3071	-65.3398	11.59
118	18.2910	-65.2998	10.37
119	18.3205	-65.3486	15.85
120	18.3321	-65.3404	14.02
121	18.3278	-65.2480	22.56
122	18.3366	-65.2748	21.34
123	18.3368	-65.2746	21.95
124	18.3276	-65.2485	23.17
125	18.3283	-65.2492	22.56

Sites	Latitude	Longitude	Depth (m)
126	18.3347	-65.2712	21.95
127	18.3363	-65.2743	20.73
128	18.3352	-65.2720	21.65
129	18.3351	-65.2726	20.73
130	18.3345	-65.2711	20.73
Sites	Latitude	Longitude	Depth (m)
132	18.3363	-65.2749	21.65
133	18.3347	-65.2721	21.95
134	18.3282	-65.2476	21.95
135	18.3356	-65.2718	21.65
136	18.3265	-65.2485	20.73
137	18.3262	-65.2480	21.95
138	18.3364	-65.2755	21.34
139	18.3285	-65.2494	21.34
140	18.3350	-65.2712	20.73
143	18.3343	-65.2687	21.04
144	18.3329	-65.2667	20.43
145	18.3342	-65.2708	20.73
146	18.3360	-65.2753	21.95
148	18.3294	-65.2503	21.34
149	18.3282	-65.2507	21.34
150	18.3271	-65.2483	21.95
151	18.3327	-65.2521	4.88
152	18.3327	-65.2519	4.88
153	18.3320	-65.2512	6.71
154	18.3318	-65.2507	7.01
155	18.3051	-65.2440	1.52
156	18.3050	-65.2442	1.22
157	18.3051	-65.2428	7.62
158	18.3164	-65.2350	5.18
159	18.3185	-65.2282	2.44
160	18.3188	-65.2282	3.05
161	18.3195	-65.2284	4.27
162	18.3058	-65.2518	2.13
163	18.3048	-65.2573	0.61
164	18.2958	-65.2551	3.35
165	18.3012	-65.3008	3.35
166	18.3016	65.3006	1.52
167	18.3016	-65.3010	1.52
168	18.3016	-65.3013	1.52
169	18.3012	-65.3014	1.52
170	18.2960	-65.2871	1.52

Sites	Latitude	Longitude	Depth (m)
171	18.2963	-65.2860	3.96
172	18.2998	-65.2878	4.88
173	18.3013	-65.2909	8.54
174	18.3025	-65.2952	4.27
175	18.3037	-65.2972	7.93
176	18.3026	-65.2990	2.13
177	18.3068	-65.2959	0.95
178	18.3068	-65.2967	2.13
Sites	Latitude	Longitude	Depth (m)
179	18.3065	-65.2971	5.00
180	18.3063	-65.2976	6.40
181	18.3056	-65.2985	7.93
182	18.3061	-65.2843	5.03
183	18.3059	-65.2838	3.96
184	18.3050	-65.2821	3.96
185	18.3053	-65.2809	2.44
186	18.3058	-65.2801	1.52
187	18.3069	-65.2802	1.52
188	18.3069	-65.2784	0.91
189	18.3018	-65.2797	1.83
190	18.2953	-65.2649	3.20
191	18.2938	-65.2643	3.72
192	18.2922	-65.2643	3.84
193	18.3040	-65.2558	6.71
194	18.3046	-65.2545	6.10
195	18.3037	-65.2523	7.01
196	18.3016	-65.2486	12.20
197	18.2915	-65.2800	0.91
198	18.2909	-65.2803	3.66
199	18.2902	-65.2813	2.26
200	18.2872	-65.2827	2.56
201	18.2823	-65.2819	6.71
202	18.2830	-65.2854	2.20
203	18.2805	-65.2877	7.01
204	18.2814	-65.2877	5.67
205	18.3017	-65.3008	1.22
206	18.3001	-65.3369	10.37
207	18.3034	-65.3382	10.37
208	18.3156	-65.3479	1.52
209	18.3144	-65.3479	1.52