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***“Impact of the invasive sea vine *Halophila stipulacea* on native seagrasses,
invertebrates and fish assemblages”***

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I. INTRODUCTION

Seagrass meadows are important marine ecosystems as they render several economic, social and ecological services, in addition to goods essential to humankind. For instance, above- and below-ground biomass of seagrass meadows accretes sediments, protecting coastal communities from hazards such as beach erosion (De Falco et al 2000; Christianen et al 2013). Likewise, sediment accretion and stabilization decrease water depth, which in turn attenuate wave action (Gillis et al 2014). In addition, leaf, stems and roots from seagrasses may also increase water friction, reducing water velocity and attenuating wave energy, also reducing coastal erosion (Koch et al 2009). Seagrasses also provide important sites that support multiple recreational activities such as snorkeling, scuba diving, kayaking, and swimming, generating annual revenues estimated on the order of millions of dollars (Puerto Rico Sea Grant College Program Strategic Plan 2018-2021). Furthermore, due to their sensitivity to environmental changes, seagrasses are used as pollutant indicators such as in the case of the US National Estuarine Eutrophication Assessment (Orth et al 2006).

From an ecological standpoint, seagrass meadows increase seawater pH, promoting calcification in nearby coral reefs (Gillis et al 2014; Guannel et al 2016). In addition, seagrass meadows provide essential habitats to a myriad of juvenile reef fish and invertebrate species, some of which are commercially important such as juveniles of the yellowtail snapper (*Ocyurus chrysurus*) and spiny lobster (*Panulirus argus*). They also provide habitat to other species which are listed under the Endangered Species Act including the green turtle (*Chelonia mydas*), and the West-Indian manatee (*Trichechus manatus*). In fact, seagrasses from Culebra Island are designated as Resource Category 1 habitat and as Designated Critical Habitat for *C. mydas*.

However, seagrass meadows around the globe are on decline. Approximately 29% of seagrass meadows worldwide have been lost in the last 127 years, at a rate of 27 km² yr⁻¹ (Waycott et al 2009), and since 1990s the percent of decline have been estimated at 7% per year (Gillis et al 2014). Yet, government agencies, the public, and most stakeholders seem to have little awareness or interest on this fact and therefore little attention has been given to seagrass decline when compared to other submerged ecosystems such as coral reefs.

Several factors acting at a global, regional and local scales including water temp, nutrient enrichment, sedimentation, and water pollution among others, have been mentioned as having potential negative impacts on seagrass meadows (Waycott et al., 2009). Yet in the Caribbean, the recent introduction of *Halophila stipulacea* has creating an addition stress to native seagrass beds.

H. stipulacea, a native seagrass from the western Indian Ocean and Red Sea, was first observed in Grenada in 2002 and since then has spread to at least 19 Caribbean Islands, including Puerto Rico, and South America (Willette et al 2012; Willette & Ambrose 2012; Vera et al 2014). The circumstances in which this seagrass expanded its range are unknown, yet it is likely to have expanded through vegetative propagules after breaking by anchoring action and further transportation via commercial and recreational vessel traffic (Willette et al 2012, 2014). *H. stipulacea* is a dioecious tropical flowering seagrass with a rather impressive physiological capacity to acclimate to a wide range of environmental conditions including variation in temperature, salinity, nutrient loads, and light intensity (van Tussenbroek et al 2016; Oscar et al 2018). Since its arrival to the Caribbean, studies other than documenting *H. stipulacea* presence and range expansion, have been scarce. Yet, those few studies focusing on determining the effects of the invasive seagrass on native seagrass meadows agree on the negative impact *H. stipulacea* could have, in particular those seagrass meadow dominated by native Caribbean seagrasses such as *Halodule wrightii*, *H. decipiens* and *Syringodium filiforme* (Willette and Ambrose 2012; Steiner & Willette 2013; Scheibling et al 2017). Other studies have also highlighted the capacity of *H. stipulacea* of restructuring the juvenile fish community associated to seagrass meadows (Olinger et al 2017). However, no long-term studies, addressing the potential impact of *H. stipulacea* on Caribbean seagrass demersal faunal assemblages have been conducted.

Culebra Island provided one of the healthier seagrass meadows in Puerto Rico. The best-conserved seagrass beds are concentrated next to primary sub-tropical dry forests, which have been under great urban development and truisitic pressures in recent years. Hence, the impacts of these activities on the seagrass meadows are of great concern. However, there is a lack of long-term data on the species distribution and conditions of these beds, making it difficult to assess their status in relation to the rapid urban development. In this study, we monitored, for one year, four seagrass meadows along the southern coasts of Culebra, with contrasting environmental conditions and *H. stipulacea* invasive pressure.

The general goals of this proposal are to: **1)** Understand how seagrass meadows under contrasting environmental conditions and threatened by rapidly spreading *H. stipulacea* respond to seasonal changes in water temperature, light intensity, sediment load and water current fluctuations, as a proxy to understand how seagrass meadows will adapt to the undergoing climate changes and human activities; **2)** Assess the effects of *H. stipulacea* dominance on seagrass-associated demersal fish and invertebrate assemblages; **3)** Experimentally assess the growth dynamics of *T. testudinum* under a competitive interaction with *H. stipulacea*. Through this field experiment we will gain technical skills and experience as to develop cost-effective and efficient seagrass farming procedures for establishing future strategy to enhance populations of native seagrasses. These goals concord with the Program's priority (5) "*Local and Management Needs*".

This report will summarize the research outcomes for October 1, 2019, to April 30, 2022. During these period, we performed five monitoring periods, i.e., winter 2020, summer 2020, fall 2020, winter 2021, and spring 2021. The accomplished activities consisted of:

1. Estimating seagrass cover, canopy height, and shoot density (as described in Aim1: *Characterize the spatio-temporal variation in percent cover, above and below ground biomass, and shoot density of native and invasive seagrasses in Culebra, Puerto Rico under contrasting environmental conditions and across seasons (fall, winter, spring, and summer) during a period of one*).
2. Assessing the fish community and demersal seagrass invertebrate and cryptic fish fauna (as defined in Aim2: *Characterize the spatio-temporal variation of demersal fish and invertebrate assemblages associated to native and invasive seagrasses under contrasting environmental conditions during a period of one year*).
3. Prepared and monitored the seagrass competition experiment (as defined in Aim3: *Assess the effect of H. stipulacea on the percent cover, above and below ground biomass, shoot density and growth of T. testudinum through a competitive interaction field experiment.*).

II. METHODOLOGY

Study sites: This study was conducted in the Island municipality of Culebra, Puerto Rico. Culebra Island is located 28Km, northeast of Puerto Rico and is part of the Northeast Reserves System Habitat Focus Area (NER-HFA), a top priority site for NOAA's Habitat Blueprint Program and the PR Department of Natural and Environmental Resources (PRDNER), Figure 1.

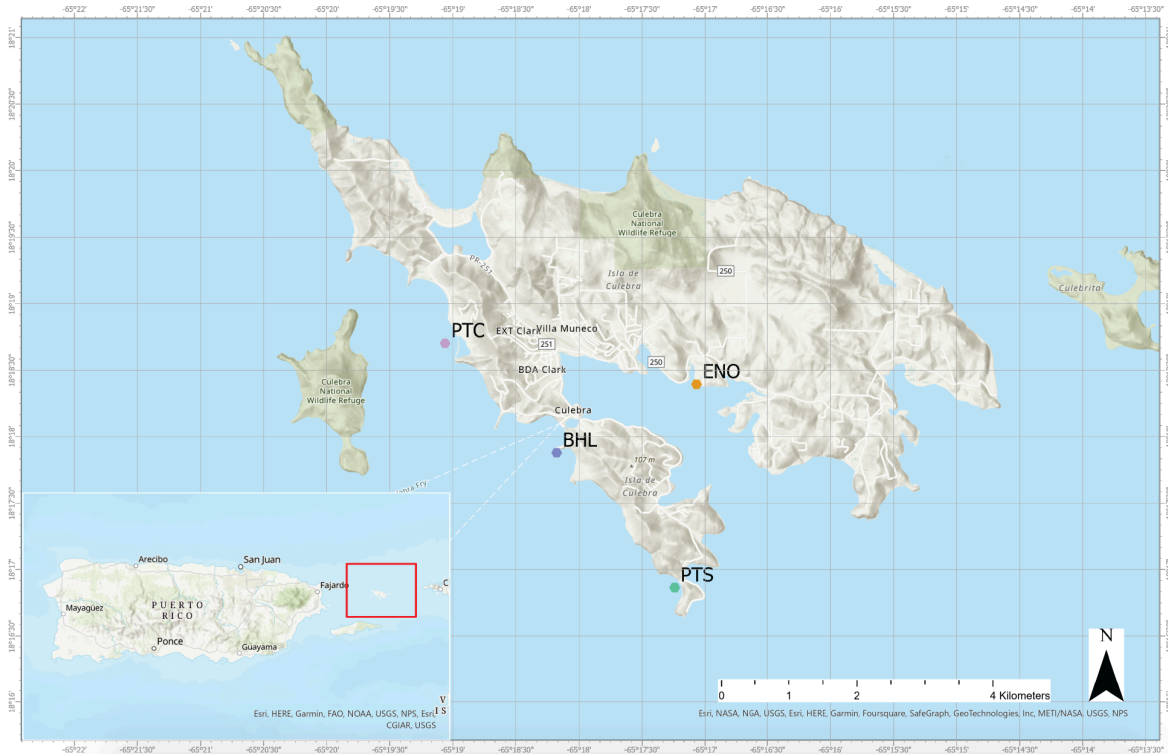


Figure 1: Study sites in the municipality of Culebra, Puerto Rico. The colored points represent the studied seagrass meadows: Punta Tamarindo Chico (PTC), Ensenada Onda (ENO), Bahia Linda (BHL), Punta Soldado (PTS).

Four sites along the southern coast of Culebra were selected based on the human-derived stresses observed and the level of invasion by *H. stipulacea*, Figure 1 and Figure 2. Punta Tamarindo Chico (PTC; 18°18'42.1 "N 65°19'03.7 "W) is located within the Canal Luis Peña No-take Natural Reserve, Figure 1 & 2D. As such, the seagrass meadows at this site exhibit relatively low human impact, as most of the human activities here are ecologically friendly (i.e., kayaking, snorkeling, and scuba diving), and consequently, water visibility is often over 25m with relatively low sedimentation. Overall, this site exhibit over 80% of seagrass cover, primarily dominated by the native seagrass *T. testudinum* and, to a less extent, by other native seagrasses such as *H. wrightii* and *S. filiforme*. Percent cover of *H. stipulacea* is estimated at 5% or less (Hernández-Delgado et al. 2018). This meadow also supports a resident population of green turtles. Ensenada Onda (ENO; 18°18'23.6"N 65°17'04.1"W) is located within Ensenada Onda Bay and next to one of two of the Puerto Rico Ports Authority's Facilities, Figure 1 & 2B.

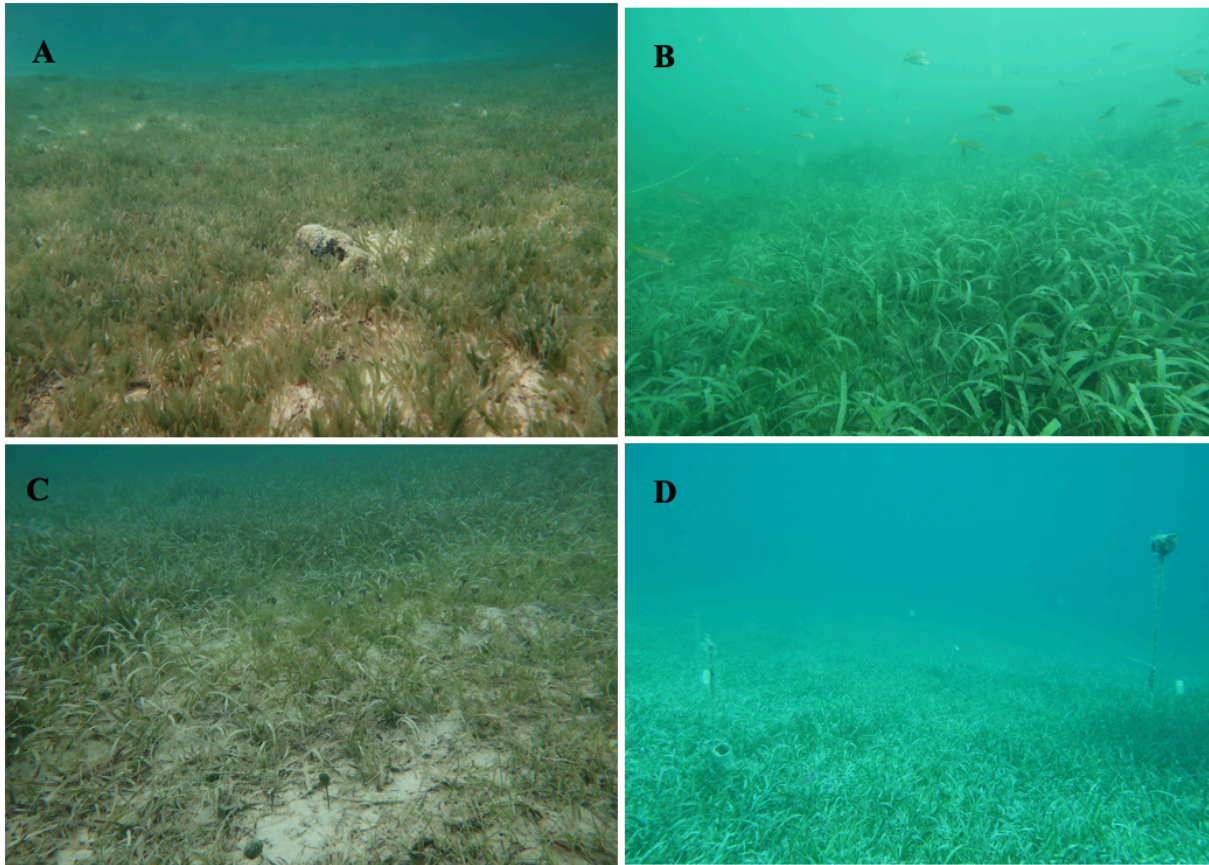


Figure 2: Panoramic of the fourth study site. A) Punta Soldado (PTS), B) Ensenada Onda (ENO), C) Bahía Linda (BHL) and D) Punta Tamarindo (PTC).

This site is subjected to frequent disturbances, including recurrent sediment-laden runoff pulses, boat traffic, and occasional anchoring. Consequently, sediment resuspension is high. At shallow water (0.5 - 4m), ENO is dominated by *T. testudinum*. However, below the 4m depth, *H. stipulacea* is the dominant seagrass and in a less extent by green and brown algae. Bahía Linda (BHL; 18°17'52.7 "N 65°18'10.6 "W) is located next to the Puerto Rico Ports Authority's main docking facilities, Figure 1 & 2C. This site is also constantly under pressure by anchoring from recreational vessels, especially during the weekends. In addition, this area is subjected to chronic turbidity mainly due to sediment and nutrient loads from an adjacent steep dirt road and Canal de la Lobina, which drains nearby. This site exhibits a 68% cover of *T. testudinum* and nearly 29% by the invasive sea vine *H. stipulacea*. Punta Soldado (PTS; 18°16'51.8 "N 65°17'14.5 "W) exhibits relatively low human impact, and human activities are mostly ecologically friendly (i.e., snorkeling and scuba diving) Figure 1 & 2A. Water visibility and sedimentation are comparable

to PTC, Figure 2. Before hurricanes Irma and María, much of the bottoms deeper than 2m were covered by corals and sand, with small and scattered patches of a mixed assemblage of native seagrass bed in the shallower areas (<1m water depth). However, after the hurricanes, *H. stipulacea* rapidly colonized the area and currently covers most mid to deep sandy bottom, i.e., > 4m of depth.

At each site, three transects, 10m long each, were established by hammering one aluminum rod bar at both ends of the transects. Transects were set parallel to the coast at 4m water depth. Within each transect, we surveyed the following seagrass metrics: **Percent cover of seagrass meadows** including seagrasses, algal functional groups, cyanobacteria, and open substrate by placing five, 1m² quadrants subdivided into a hundred, 1cm² squares alongside each transect. **Shoot density** (# of shoot/m²) by counting the number of shoots within a 30x30cm section of the 1m² quadrat. **Canopy height** by measuring the length of 10 to 20 seagrass blades of each seagrass occurring within each 1m² quadrant. **Above and below biomass** by collecting ten samples using a core sampler of 15cm diameter. Once collected, the core samples were placed in labeled plastic bags, and in the boat, we cleaned the root from sediment and stored it at 4°C for further analysis. In the laboratory, the vegetative material was classified into above-ground biomass (i.e., leaf, shoots, including sheath material and flowers), Figure 3A and below-ground biomass (i.e., rhizomes and roots) per seagrass species, Figure 3B. After classification, we dried the material at 60°C for 24 hours and then weighed it. Finally, we converted the weighted material into g/cm³ of above or below ground. All seagrass metrics were conducted every three months (summer, fall, winter, and spring).

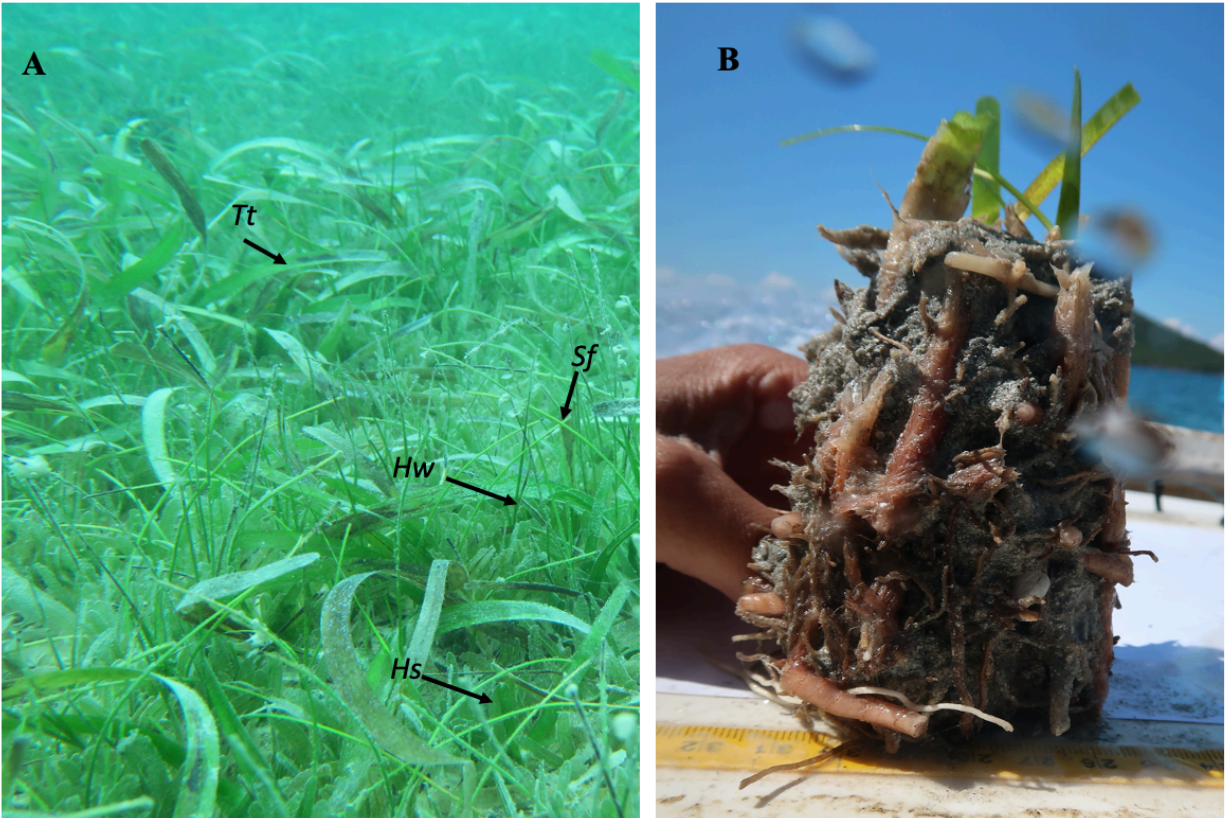


Figure 3: Marine seagrass from above and below ground. A) Image of the four seagrasses in Bahia Linda (BHL). B) A core sample for biomass analysis of *Thalassia testudinum* from Punta Tamarindo Chico (PTC). *Thalassia testudinum* (*Tt*), *Syringodium filiforme* (*Sf*), *Halodule wrightii* (*Hw*), and *Halophila stipulacea* (*Hs*)

We also survey the juvenile reef-fish assemblages, the demersal seagrass invertebrates, and the cryptic fish fauna associated with the different sites. To survey the juvenile reef-fish assemblages, we used pound-nets (50x50x50cm) with 0.75 cm square mesh made from "vinyl-coated wire," as described by Olinger et al. (2017). We selected this fishing art because, unlike other fishing arts, which may select some species of fish over others this one target juvenile fish in a non-biased fashion. Three pound-nets were deployed during each sampling effort at least 15m apart from each other near the transects. As different fish species may be present in the seagrass meadows during different periods, *i.e.*, day and night, pound-nets were first deployed during the morning (*i.e.*, 7-8 am) and retrieved during the afternoon of the next day, *i.e.* (7-8 am). Once recovered, trapped fish and any other organism were identified to the nearest taxonomical group either *in situ* or through digital images taken at the time of collection. Afterward, captured fish were released back to the seagrass meadows. Traps were deployed once every three months (summer, fall, winter, and spring) for one year.

We used the drop traps technique described by Holmquist (1997) to determine the demersal seagrass invertebrate and cryptic fish fauna. Box traps were made of sheet aluminum of 0.25 x 0.25 m, lacking a solid top or bottom. Drop traps were deployed at meters 0, 5, and 10 of each transect, and invertebrates or any other organism within the drop trap were caught using a 0.25 m wide framed and handled net (bar seine) with 0.5 mm square mesh. The collected organisms were placed in plastic bags and on the boat, in buckets filled with water for further identification to the lowest taxon possible, photographed and released back to the seagrass meadows, Figure 4.

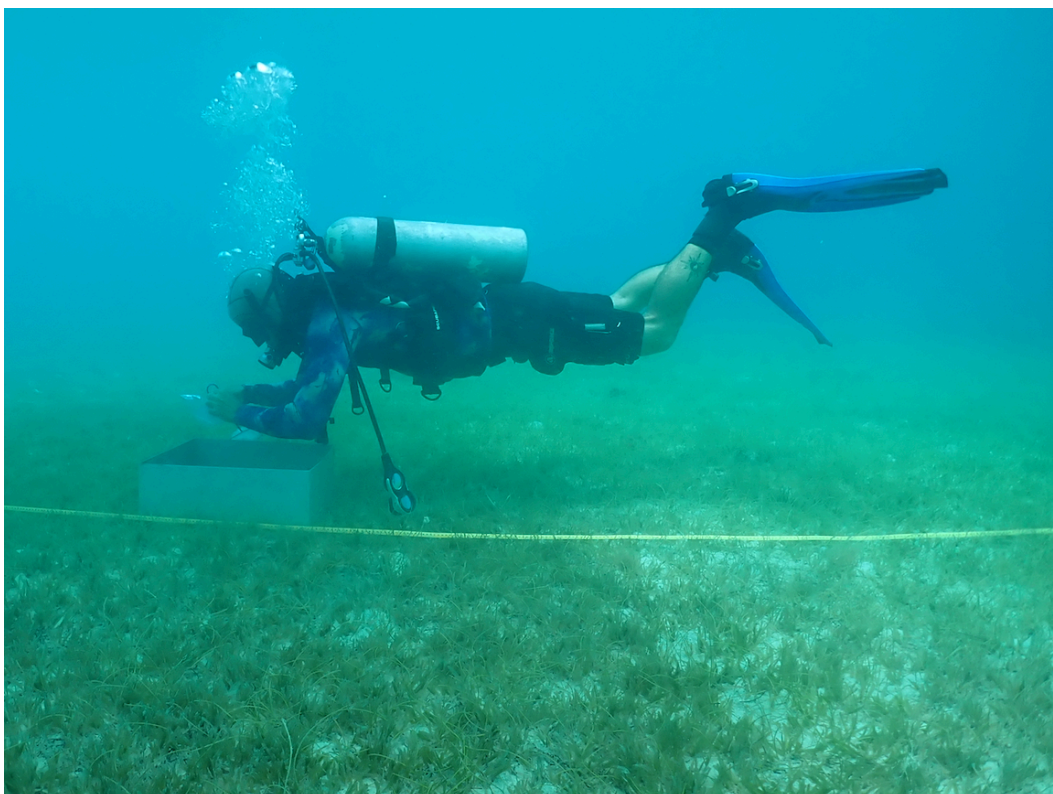


Figure 4: Diver conducting the invertebrates survey along a transept using the Drop trap techniques.

Environmental assessment: Four environmental parameters were measured: water temperature (WT), light intensity (LI), sedimentation rate (SR), and water motion (WM), Figure 5. These devices were installed on environmental stations at depths ranging from 4 - 6m from December 2019 to September 2020. Each environmental station (one per surveyed site) consisted of six stainless-steel rods, each 1.5m in length and 1.3mm in diameter, hammered 1.0m into the ground, so 0.5m of each rod was above the ground. Each stainless-steel rod was 2m apart from each other. *WT/LI measurements:* to measure WT/LI at each study site, we set one Hobo Pendant/light data logger 64k-UA-002-64 (Onset Company) device attached to the end of one of the rods, Figure 5A.

This device was programmed to record the temperature at 15 minutes intervals. Light intensity data were obtained every 15 minutes. However, LI we only used the first ten days after deployment, as seaweeds overgrowing the device affect the sensor's ability to detect light (CPRD pers. obs). Temp/light devices were replaced at three-month intervals for the one-year study duration.

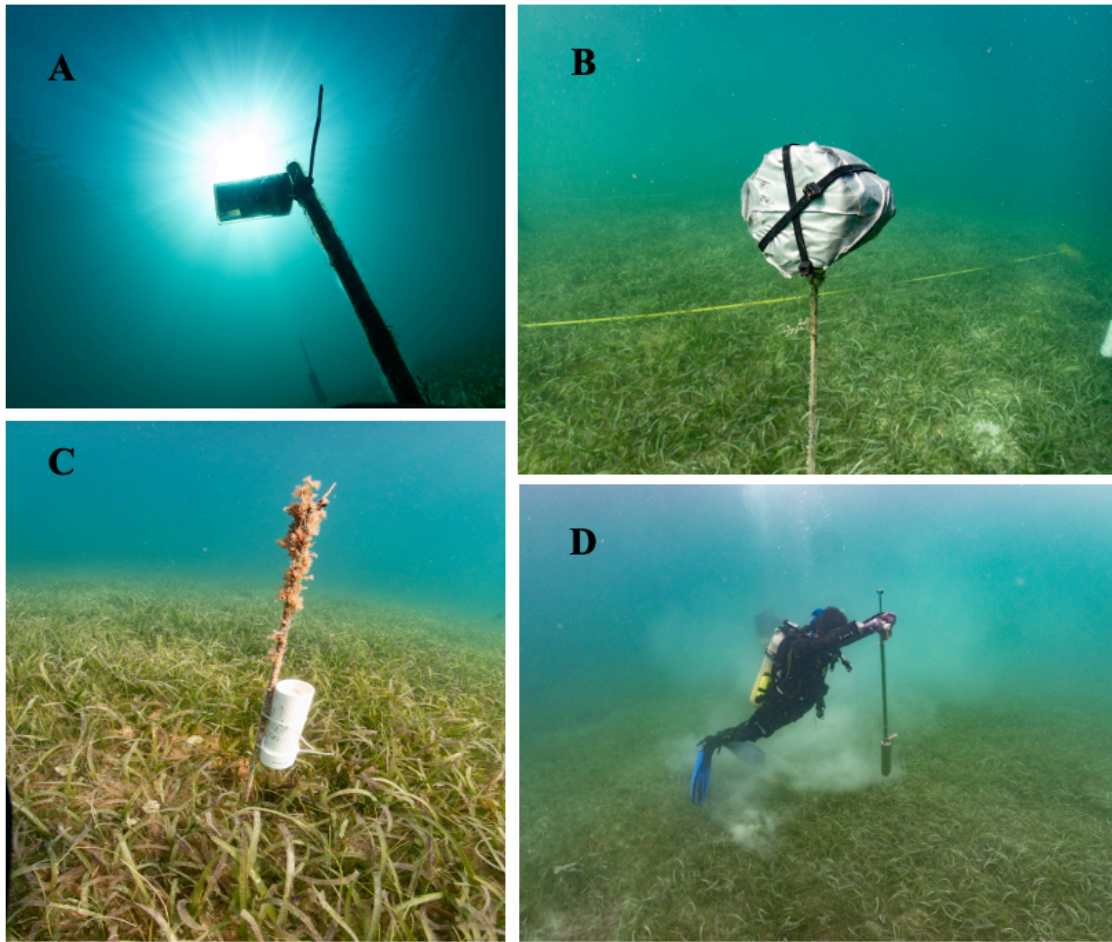


Figure 5: Sensors and core sampling. A) Temperature and Light Intensity sensor, B) Water motion sensor inside the adapter, C) Sedimentation trap and D) Core sampling for biomass estimation.

SR measurements: Sedimentation rate was estimated as explained in Toledo-Hernández et al. (2007). Briefly, four PVC pipes of 5.0 mm x 30.0 mm were attached to the end of four rods not previously used, with the pipe opening 0.3m above ground. Sediment traps were retrieved and replaced at three months intervals. Once collected, the content was washed, filtered with pre-weighed Whitman Nitrate Cellulose filter membranes, 47 mm in diameter and 45 mm in pore size, dried to constant weight at 65°C, and data were converted to mg cm⁻² day⁻¹, Figure 5C.

Water motion (WM): water motion was monitored using the Hobo Pendant G acceleration/tilt data loggers & sensor UA-004-64 (Onset Company), Figure 5B. At each site, a WM device was placed following the recommendations of Ruiz-Diaz 2014. In short, one WM device was inserted inside a sphere of polystyrene of 30cm diameter covered with waterproof tape and attached to the end of a rod not previously used using stainless steel braided 30cm long. Water motion data were monitored every 15 minutes. Each device was replaced every three months (at seasonal intervals) for one year.

Competition experiment: To assess the effect of *H. stipulacea* on the growth of *T. testudinum*, an *in situ* competitive interaction experiment was conducted in PTS. This site was selected because it is extensively dominated by *H. stipulacea*, thereby plug collection of *H. stipulacea* was accessible. On the other hand, *T. testudinum* was selected because it is the dominant native seagrass across Culebra and, in general, the wider Caribbean region. To accomplish this experiment, a table subdivided into 20 cubicles, each of 20x20x20, was constructed Figure 6. The table was elevated 1m above the ground to reduce the grassing from fish and invertebrates. Five of the twenty cubicles were planted with plugs of *H. stipulacea* and five with *T. testudinum*. These were considered the control cubicles. The remaining ten cubicles were planted with equal amounts of *H. stipulacea* and *T. testudinum* plugs. The table was visited at three-month intervals for eight months, where we measured the number of shoots and canopy height of each seagrass.



Figure 6: Panoramic view of competition experiment table between *Thalassia testudinum* and *Halophila stipulacea*.

III. RESULTS

We utilized eighteen people during this period; the three co-authors and fifteen volunteers, i.e., one high schooler, two permanent residents of Culebra, and 12 college students, three graduate and nine undergraduate students. A total of 945.5 hours were required to complete the proposed aims. Of these total hours, 438 were paid, whereas 508 were donated. We utilized \$12,791 during winter monitoring period (December 2019 and February 2020). Cost for the summer 2020 activities (June, and August 2020) were \$18,676.42. To complete the actions performed from October 7-12, 2020, January 27 and February 2, 2021 (Fall 2020 and Winter 2021 monitoring), we utilized \$16,066.45. To complete the activities conducted from March 30 and September 30, 2021 (Spring 2021), we used \$7,730.16. Finally, from October 1, 2021, to March 2022, we utilized \$11,258.76. The total amount requested equals \$66,522.79.

AIM 1: “*Understand how seagrass meadows under contrasting environmental conditions and threatened by rapidly spreading *H. stipulacea* react to seasonal changes in water temperature, solar radiation, sediment load and water current fluctuations, as a proxy to understand how seagrass meadows will adapt to the undergoing climate changes and human activities*”.

The seagrass metrics estimates varied by seagrass species, sites, and time. Therefore, for convenience purposes, we are presenting the results by seagrass species.

a. *Thalassia testudinum*

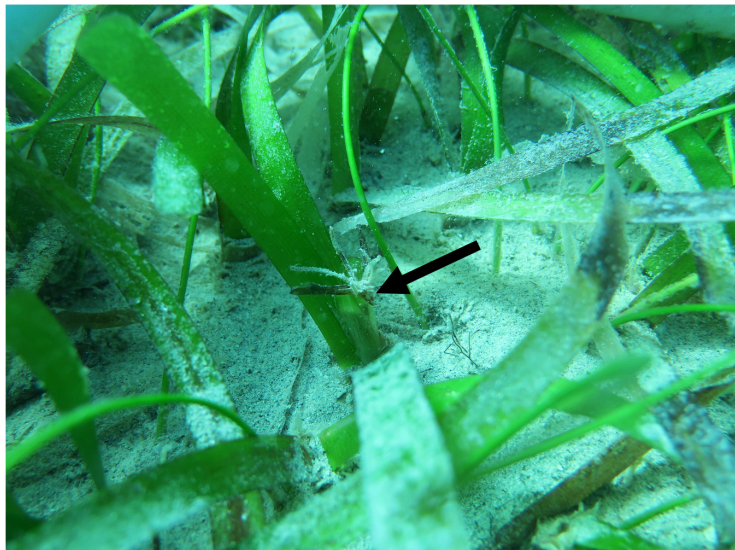


Figure 7: Image of a female flower of *T. testudinum* within a transept at Punta Tamarindo Chico (PTC), October 8, 2020.

i. Percent Cover

T. testudinum was present in three of the four selected sites, e.g., ENO, BHL, and PTC, Figure 7. However, at these sites, % covered varied significantly ($F = 98.28$; $P < 0.0001$). For instance, the mean % cover at PTC was 87.27%, whereas at BHL and ENO was 47% and 53%, respectively (Figure 8). Furthermore, the percent cover of *T. testudinum* was significantly variable when contrasted across the monitoring period ($P < 0.001$). Percent cover at PTC varied cyclically across monitoring periods, with lower mean cover ($\approx 83\%$ cover) during January and November 2020 and April 2021 and higher % cover ($\approx 90\%$ cover) in June 2020 and January 2021 (Figure 8). *T. testudinum* cover at the ENO and BHL showed different patterns of variation to that observed in PTC, although their variation across time was similar (Figure 8). At these sites, the peak percent cover was recorded during January 2020 (75% at ENO and 68% at BHL) but then steadily declined in June and November 2020 to nearly 55% at ENO and 45% at BHL. However, in January 2021, the % cover increased again to nearly 65% in ENO and 56% at BHL. Finally, at both sites, *T. testudinum* percent cover declines below the 50% cover in April 2021 (Figure 8). At BHL, the statistical analyses revealed differences between Apr 21, Jan 20, Jun 20, Nov 20, and Jan 21. Meanwhile, at ENO and PTC, the statistical analyses showed no differences among months. Temperature has a significantly positive association with *T. testudinum* % cover ($R = 0.0962$; $P = 0.018$; Figure 9). However, light intensity (LI) showed an even stronger positive association with % cover than temperature ($R = 0.213$; $P < 0.000$; Figure 9). Sedimentation on the other hand, showed a positive, yet no significant association with % cover. On the contrary, water motion exhibited a significantly negative interaction with % cover ($R = 0.225$; $P < 0.000$; Figure 9).

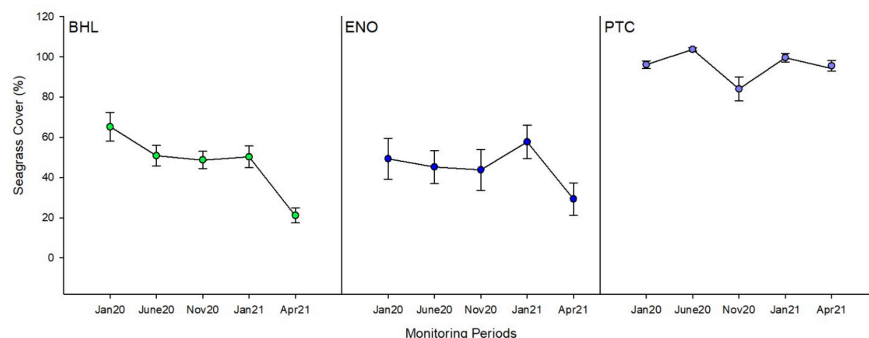


Figure 8. Mean *Thalassia testudinum* cover (in percent) by study sites estimated by monitoring periods. The study sites are Bahía Linda (BHL); Ensenada Onda (ENO); Punta Tamarindo Chico (PTC). Whisker represents standard error.

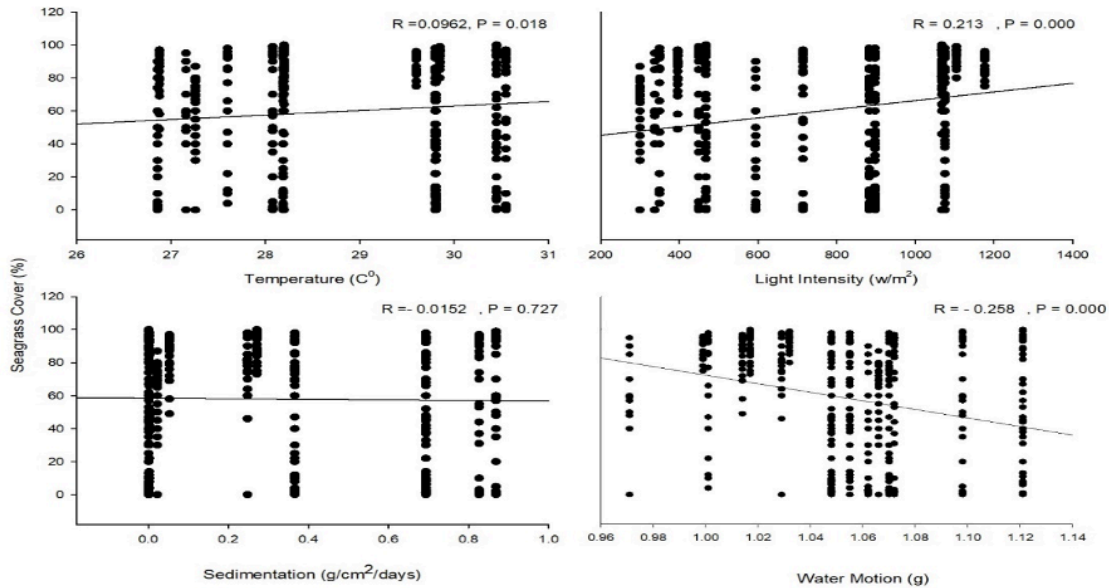


Figure 9. Correlation analyses between environmental parameters and *Thalassia testudinum* percent cover.

ii. Shoot Density

Shoot density varied significantly across sites, with PTC showing significantly higher shoot density than BHL and ENO ($F = 73.57$; $P < 0.01$; Figure 10). The Tukey HDS analysis showed that shoot density at PTC (*mean*: 369 shoot/m²) was significantly higher than ENO (151 shoot/m²) and BHL (149 shoot/m²; $P < 0.001$). Nevertheless, no differences between ENO and BHL were detected. Shoot density at BHL, ENO, and PTC showed a significant variation across time ($P < 0.001$), although the variation across time at these sites exhibited a similar pattern (Figure 10). For instance, at all sites, the highest peak occurred in January 2020 and decreased in June and November 2020. However, shoot density gradually increased from January to April 2021, although at ENO, shoot density slightly decreased in April 2021. At BHL, the statistical analyses showed differences between June 20 and Jan 20, Nov 20, Jan 21, and Apr 21. Meanwhile, at ENO, the periods with no statistical differences were Apr 21 - Jan 21, Apr 21 - Jun 21, Jun 20 - Jan 21, and Nov 20 - Jan 21. Finally, statistical differences were only detected at PTC between Jan 20 - Jan 21, Apr 21, and Jan 21 – Jun 20 and Nov 20.

Temperature, sedimentation, and water motion exhibited a significantly negative association with *T. testudinum* shoot density ($R = -0.132$; $P = 0.001$; $R = -0.230$; $P = 0.000$ & $R = 0.343$; P

< 0.000 respectively; Figure 11). On the other hand, light intensity (LI) showed a slightly positive yet significant association with shoot density ($R = 0.0877$; $P = 0.039$; Figure 11).

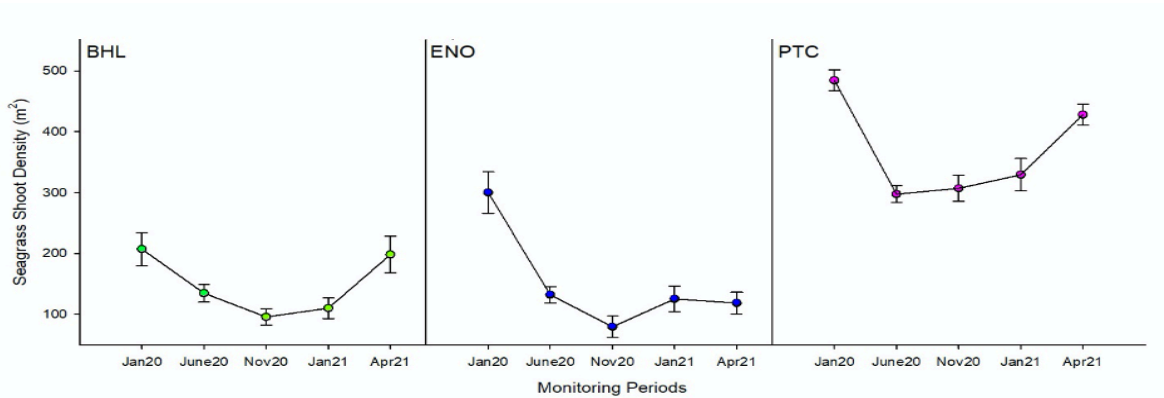


Figure 10. Mean *Thalassia testudinum* shoot density estimated by study sites and through monitoring periods. The study sites are Bahía Linda (BHL); Ensenada Onda (ENO); Punta Tamarindo Chico (PTC). Whisker represents standard error.

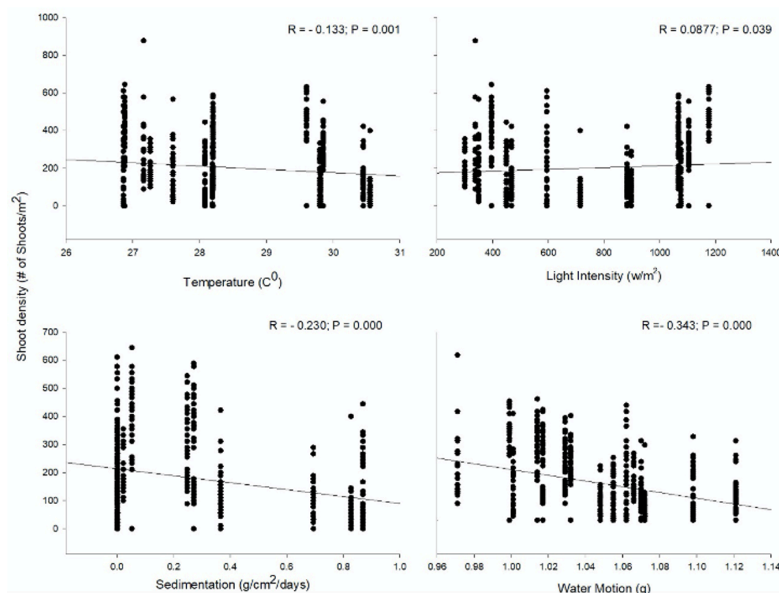


Figure 11. Correlation analyses between environmental parameters and *T. testudinum* shoot density.

iii. Canopy Height (leaf length)

The study sites' canopy heights were statistically different ($P < 0.000$; Figure 12). Overall, leave length was significantly higher in ENO (27.3 cm) compared with PTC (22.19) and BHL (19.49; $F = 57.01$; $P < 0.001$). Leave length also was statistically different across time ($P < 0.001$). For instance, at ENO, canopy height ranged from 23cm in November 2020 to 33cm in January 2020,

while at PTC, leave length varied from 16cm in November 2020 to 31cm in January 2020. Leave length at BHL varied from 15cm on Jan20 to 25cm on Nov 20. However, at BHL, only Jun 20 was statistically different from Jan 20, Jan 21, and Apr 21. At ENO, statistical differences were detected between Jan 20 and Nov 20, Jan 21 and Apr 21, and between Nov 20 and Jun 20. At PTC, statistical differences were detected among all months except Jun 20 and Apr 21 and Nov 20 and Jan 21. Neither temperature nor sedimentation or water motion exhibited a significantly association with *T. testudinum* canopy height (Figure 13). Light intensity (LI), on the other hand, showed a significantly negative association with canopy height ($R = -0.225$; $P = 0.000$; Figure 13).

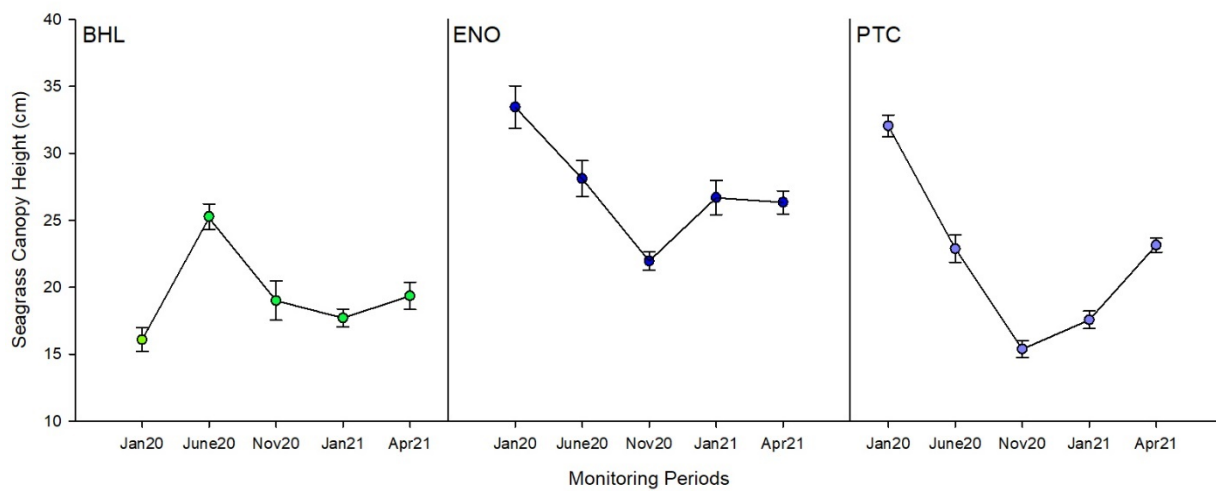


Figure 12. Mean *Thalassia testudinum* canopy height estimated by study sites and through monitoring periods. The study sites are Bahía Linda (BHL); Ensenada Onda (ENO); Punta Tamarindo Chico (PTC). Whisker represents standard error.

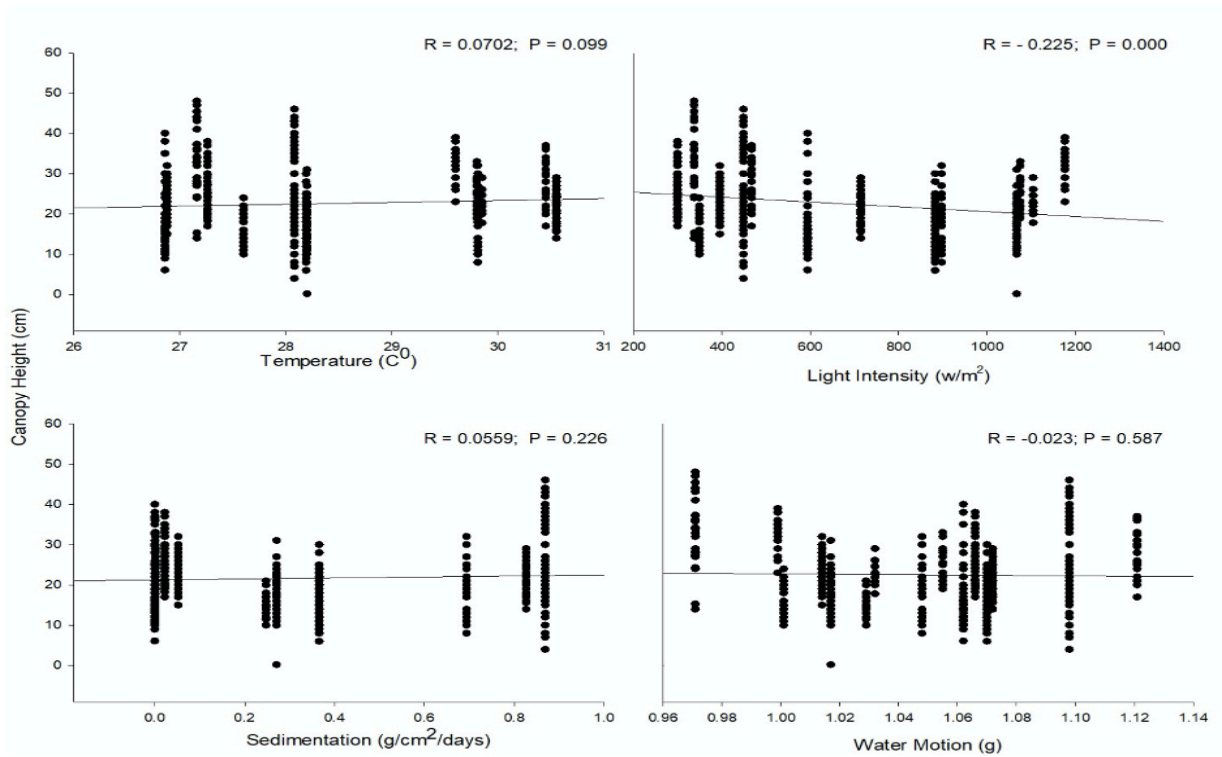


Figure 13. Correlation analyses between environmental parameters and *T. testudinum* canopy height.

iv. Above-ground Biomass (AGB)

Overall, the AGB estimated for *T. testudinum* at ENO, PTC & BHL varied across sites and monitoring periods (Figure 14). When compared among sites, the above biomass was significantly higher at PTC (191g/cm², followed by BHL (132 g/cm²) and ENO (107 g/cm²; P=0.001). No clear patterns were observed when data were analyzed across the monitoring period. For instance, aboveground biomass was the lowest in November 2020 at PTC, whereas at ENO and BHL in Jun 20. Meanwhile, April 2021 showed the highest aboveground biomass at PTC and ENO. However, at BHL, the period with the highest aboveground biomass was November 2020. However, no statistical analyses were detected across time at BHL and ENO. At PTC, only Jan 20 and Nov 20 were statistically different.

Temperature and sedimentation exhibited a slightly negative yet nonsignificant association with *T. testudinum* AGB (Figure 15). Light intensity (LI) also showed a nonsignificant yet positive association with AGB. However, water motion exhibited a negative and significant interaction with AGB (R = 0.362; P < 0.000, Figure 8).

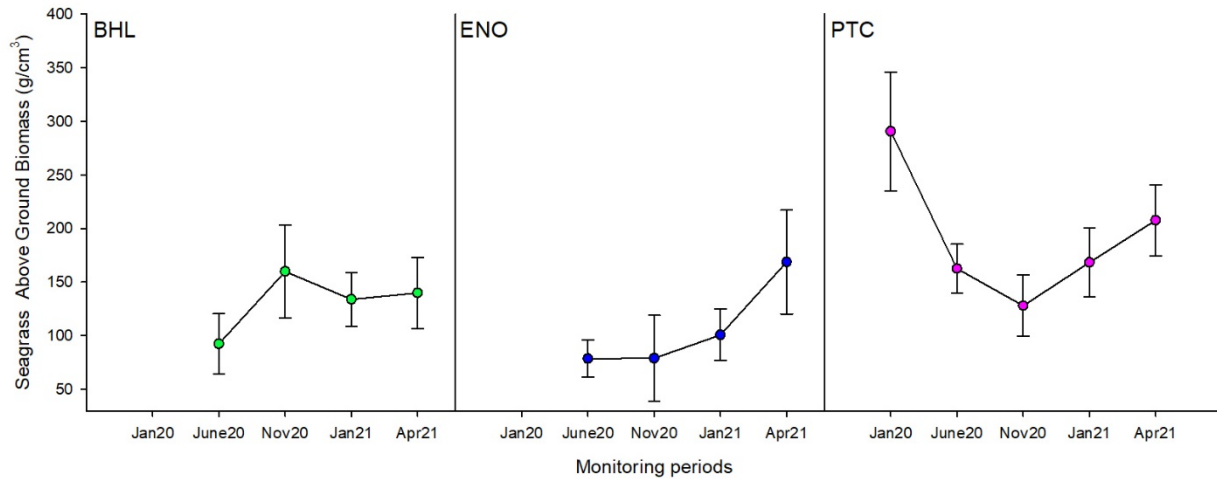


Figure 14. Mean *Thalassia testudinum* Above-ground biomass estimated by study sites and through monitoring periods. The study sites are Bahía Linda (BHL); Ensenada Onda (ENO); Punta Tamarindo Chico (PTC). Whisker represents the standard error.

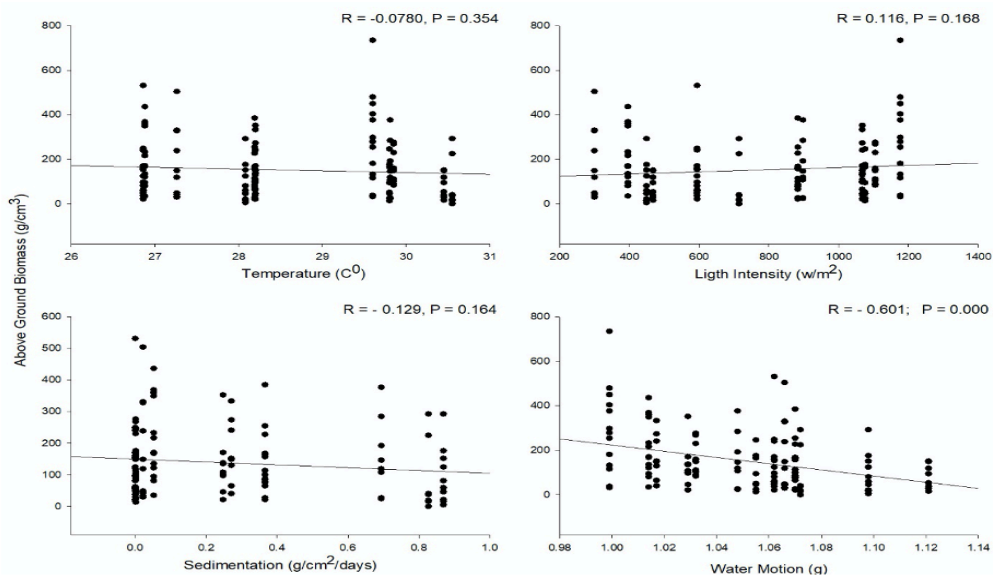


Figure 15. Correlation analyses between environmental parameters and *T. testudinum* Above Ground Biomass.

v. Below-ground Biomass (BGB)

Like AGB, BGB varied significantly across sites ($P < 0.0001$; Figure 9). PTC exhibited the highest BGB (1145 g/cm^2), followed by BHL (419 g/cm^2) and, lastly, ENO (217 g/cm^2), Figure 16. Furthermore, at PTC and ENO, below-ground biomass increased with time from January 2020 to April 2021. At BHL, however, below-ground biomass increased from January to November 2020

but decreased in January 2021 and increased again in April 2021. A BHL statistical differences were only detected between Jun 20 and Nov 20. By contrast, ENO exhibited significant differences across all monitoring periods except Nov 20 and Apr 21. Meanwhile, at PTC, Jan 20 was statistically different from Nov 20, Jan 21, and Apr 21.

Temperature, sedimentation, and water motion exhibited a significantly negative association with *T. testudinum* BGB ($R = 0.254$; $P = 0.002$, $R = -0.243$, $P = 0.005$, & $R = 0.601$; $P < 0.000$) respectively). On the other hand, light intensity (LI) showed a positive, though nonsignificant, association with BGB (Figure 17).

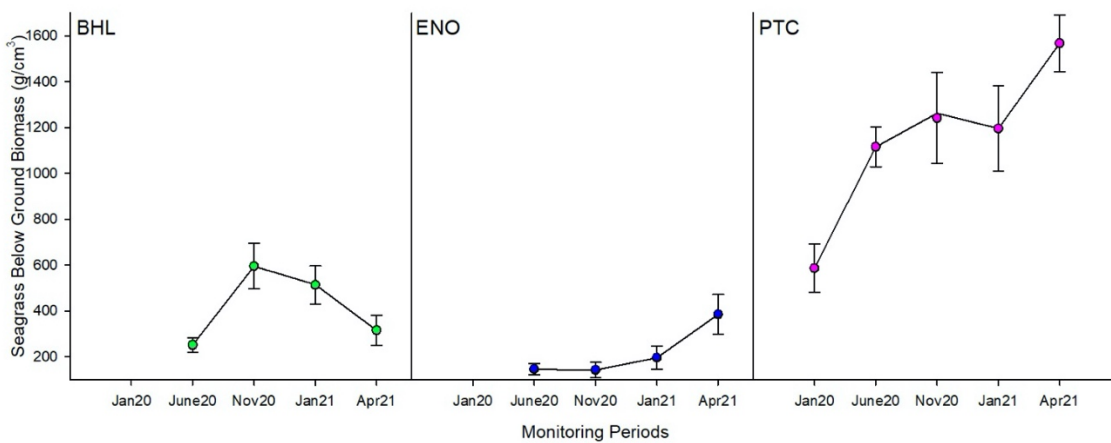


Figure 16. Mean *Thalassia testudinum* Below Ground Biomass (BGB) estimated by study sites and through monitoring periods. The study sites are Bahía Linda (BHL); Ensenada Onda (ENO); Punta Tamarindo Chico (PTC). Whisker represents the standard error.

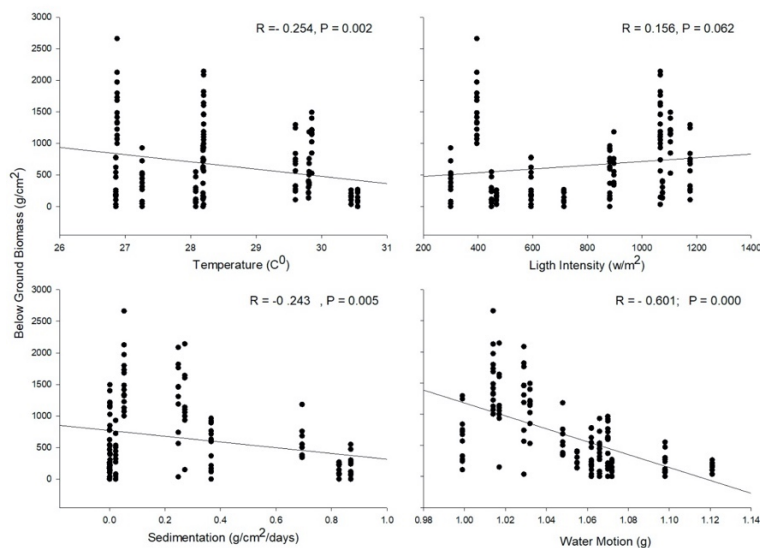


Figure 17. Correlation analyses between environmental parameters and *T. testudinum* Below Ground Biomass.

b. *Halophila stipulacea*

i. Percent Cover

H. stipulacea was present in three out of the four selected sites. These sites were ENO, PTS, and BHL. Percent cover showed significant variation across sites ($P < 0.001$; Figure 17). Mean % cover across sites was higher at PTS (e.g., 60%), followed by BHL (e.g., 32% cover) and lastly ENO (28% cover). Shoot density also varied through time. For instance, Nov 20 showed the highest percent cover at PTS and ENO (85% and 42%, respectively), whereas the percent cover at BHL was recorded in Apr 21. Meanwhile, Jan 20 showed the lowest percent cover at PTS and ENO (44% and 27%, respectively). The lowest percent cover at BHL was recorded on Jan 21. The variation in % cover through time was statistically significant ($P < 0.001$). For instance, at BHL, significant differences were detected between Apr 21 and Jan 20, Nov 20, and between Jan 20 and June 20, Nov 20 and Nov 21. At ENO, significant differences were detected between Apr 21 and Jan 20, Nov 20, and between Jan 20 and June 20, Nov 20 and Jan 21. At PTS, statistical significances were detected between Apr 21 and Jan 20 and Nov 20, between Jan 20 and June 20, Nov 20, Jan 21, and between Nov 20 and June 20 and Jan 21.

Temperature and sedimentation, both showed negative associations with *H. stipulacea* % cover; yet only sedimentation showed a significant association ($R = -0.101$; $P = -0.020$; Figure 18). Meanwhile, Light intensity (LI), showed a strong significantly positive association with *H. stipulacea* cover ($R = 0.454$; $P = 0.000$), whereas water motion showed a significantly negative interaction ($R = 0.112$; $P < 0.008$; Figure 18).

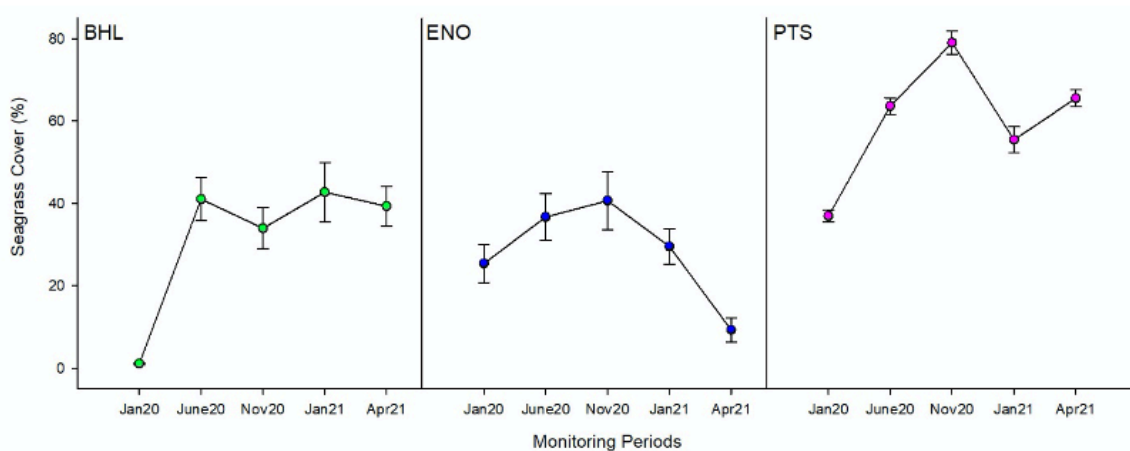


Figure 18. Mean *Halophila stipulacea* cover (in percent) across study sites and monitoring periods. The study sites are Bahía Linda (BHL); Ensenada Onda (ENO); Punta Soldado (PTS). Whisker represents standard error.

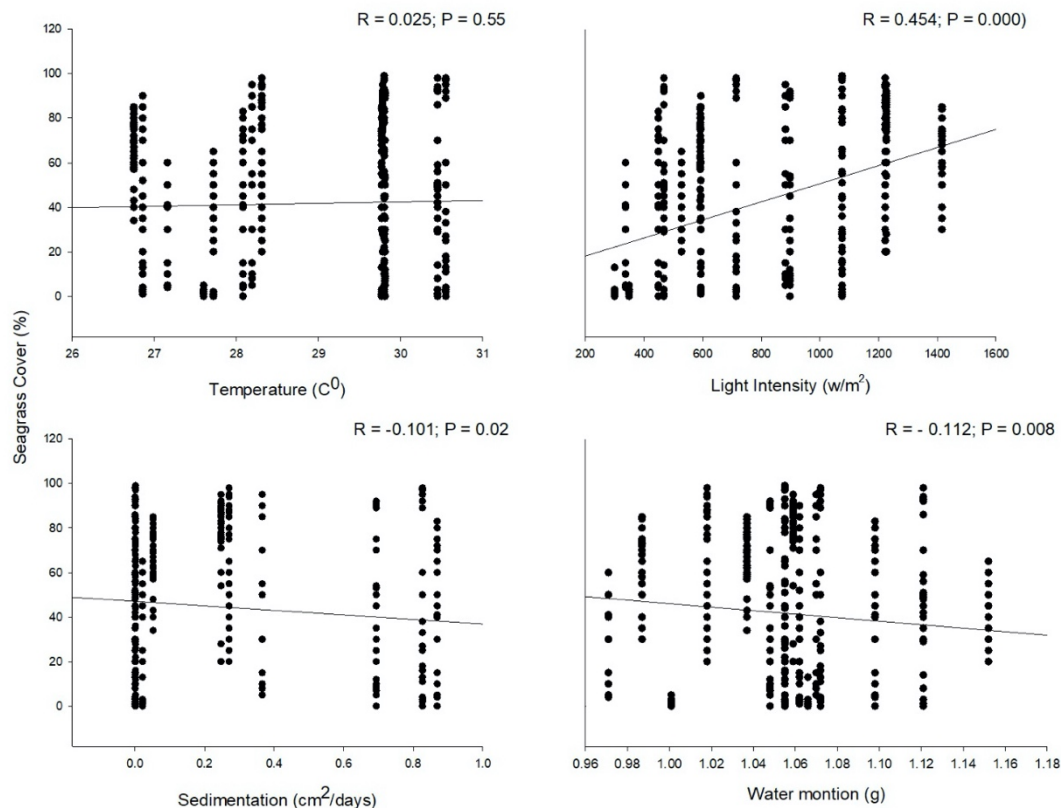


Figure 19. Correlation analyses between environmental parameters and *Halophila stipulacea* percent cover.

ii. Shoot Density

As previously described in percent cover, shoot density varied across sites (Figure 20). Overall, shoot density was higher in PTS with an overall mean density of 1189 shoot/m², followed by ENO with 583 shoots/m² and BHL with 463 shoots/m². The difference in shoot density across sites was statistically different ($P < 0.001$). Shoot density also varied through time. For instance, the lowest shoot density at BHL was recorded in Nov20 (e.g., 39 shoot/m²), while the highest was recorded in Apr 21 (e.g., 735 shoot/m²). January 2020 showed the highest shoot density at PTS and ENO (1700 and 910 shoot/m², respectively). The lowest shoot density at PTS was recorded in June 2020, with 1300 shoots/m². In contrast, the lowest shoot density at ENO was recorded in April 2021 (100 shoot/m²). Shoot density varied significantly across the monitoring period. For instance, at BHL, significant differences were detected between Jan 20 and Nov, June 20, Jan 21, and Apr 21, and

between Nov 20 and Jun 20 and Jun 21. Meanwhile, at ENO, significant differences were detected between Apr 21 and Jan 20, Jun 20 and Jan 21, and Jan 20 and Jan 21. At PTS, significant differences were detected between Apr21 and Jan20 and Nov 20, Jan 20 and June 20, and Nov 20 and Jan 21.

Temperature and sedimentation exhibited significantly negative associations with *H. stipulacea* shoot density ($R = -0.112$; $P = 0.000$ & $R = -0.227$; $P = 0.000$, respectively; Figure 21). Meanwhile, Light intensity (LI), showed a significantly positive association with shoot density ($R = 0.094$; $P = 0.030$). Water motion did not show a significant interaction (Figure 21).

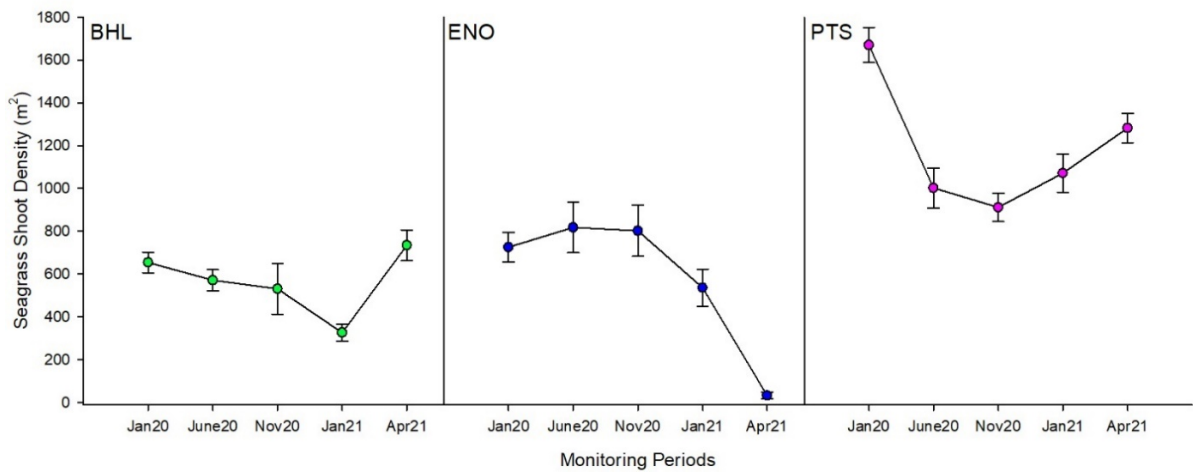


Figure 20. Mean *Halophila stipulacea* shoot density across study sites and monitoring periods. The study sites are Bahía Linda (BHL); Ensenada Onda (ENO); Punta Soldado (PTS). Whisker represents standard error.

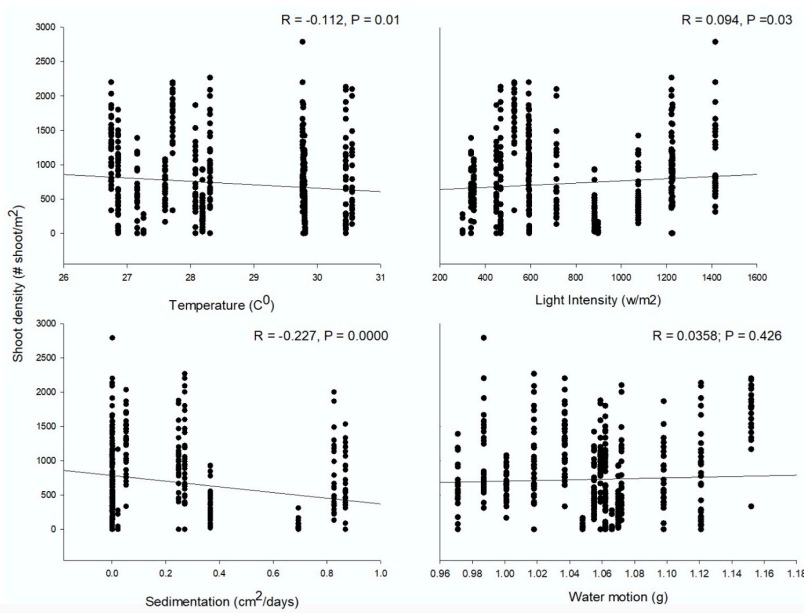


Figure 21. Correlation analyses between environmental parameters and shoot density from *Halophila stipulacea*.

iii. Canopy Height (leave length)

Canopy height was highly variable across sites (Figure 22). Overall, the canopy height ranged from 5 to 7.6cm. However, it was higher at ENO with a mean leave length of 6cm, followed by BHL and PTS with 4 cm (Figure 22). These differences were statistically significant ($P < 0.000$). Variation through time was also statistically significant ($P < 0.000$). For instance, at BHL, significant differences were detected between Nov20 and Jan20 and Jan21 and Jan21 and Apr21. At ENO, significant differences were detected between June 20 and Nov 20 and Jan 21 and between Nov 20 and Jan 21. Finally, significant differences were detected at PTS between Nov20 and Apr 21 and between Jan21 and Apr21.

Temperature, sedimentation, and water motion exhibited a significantly positive association with *H. stipulacea* canopy height ($R = 0.265$; $P = 0.000$; $R = 0.338$; $P = 0.000$, & $R = 0.135$; $P < 0.001$ respectively; Figure 23). Light intensity (LI), on the other hand, showed a significantly negative association with ($R = 0.338$; $P = 0.000$; Figure 23).

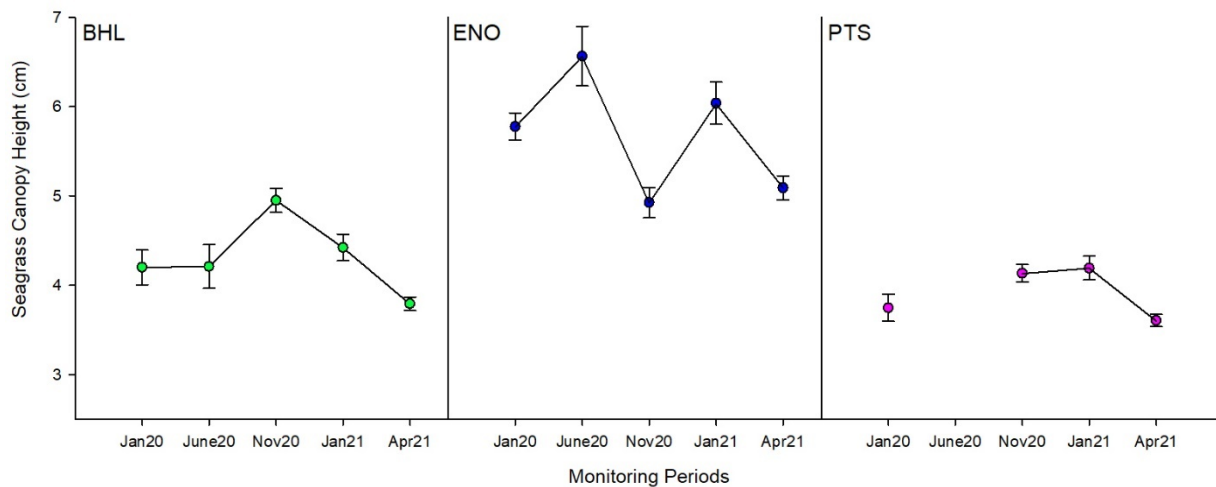


Figure 22. Mean *Halophila stipulacea* Canopy Height across sites and monitoring periods. The study sites are Bahía Linda (BHL); Ensenada Onda (ENO); Punta Soldado (PTS). Whisker represents standard error.

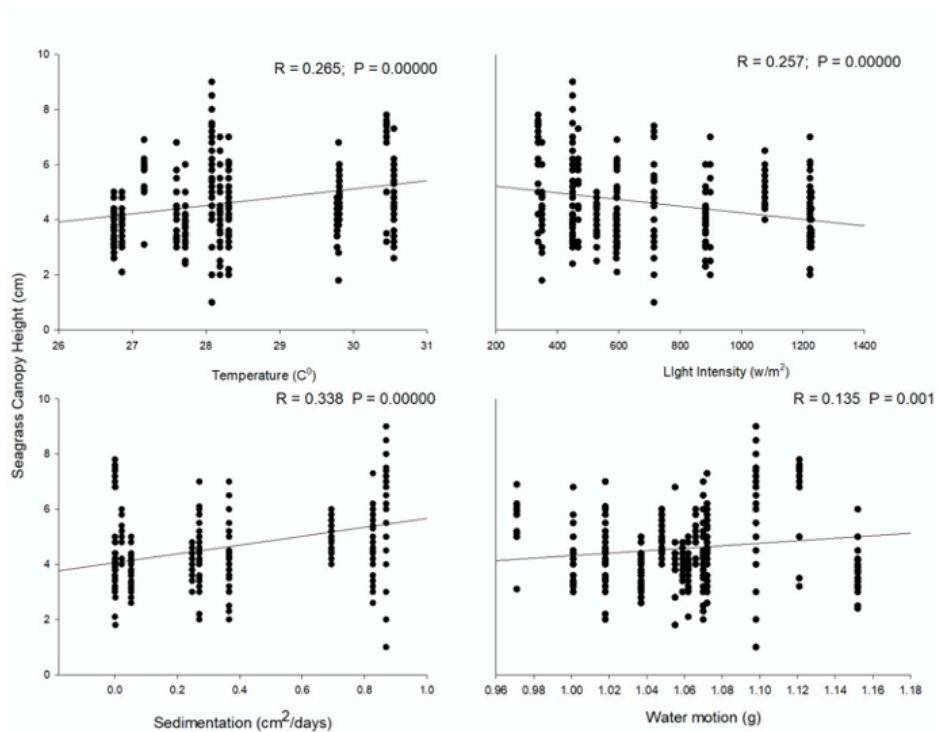


Figure 23. Correlation analyses between environmental parameters and Canopy Height from *Halophila stipulacea*.

iv. Above-ground Biomass (AGB)

Aboveground biomass (AGB) of *H. stipulacea* significantly varied across sites ($P < 0.02$; Figure 24). For instance, AGB was higher at PTS and ENO, with a mean of 73 and 71 g/cm^2 , respectively (Figure 24). BHL showed a mean AGB of 41 g/cm^2 . AGB also varied through time. Apr 21 was the month with the highest AGB at PTS, ENO, and PTC. Nov 2020 was the month with was higher AGB in BHL. The lowest AGB at PTS was recorded in Jun 2020, whereas ENO and BHL were in January 2021. Meanwhile, at PTC, the lowest AGB was recorded on Nov 20. Statistical differences at BHL were detected between Nov 20 and Jan 21. Meanwhile, significant differences were detected at PTS between Jun 20, Jan 21 and Apr 21. No statistical differences through time were detected at ENO.

Neither temperature, sedimentation, light intensity (LI) nor water motion exhibited significant interactions with AGB.

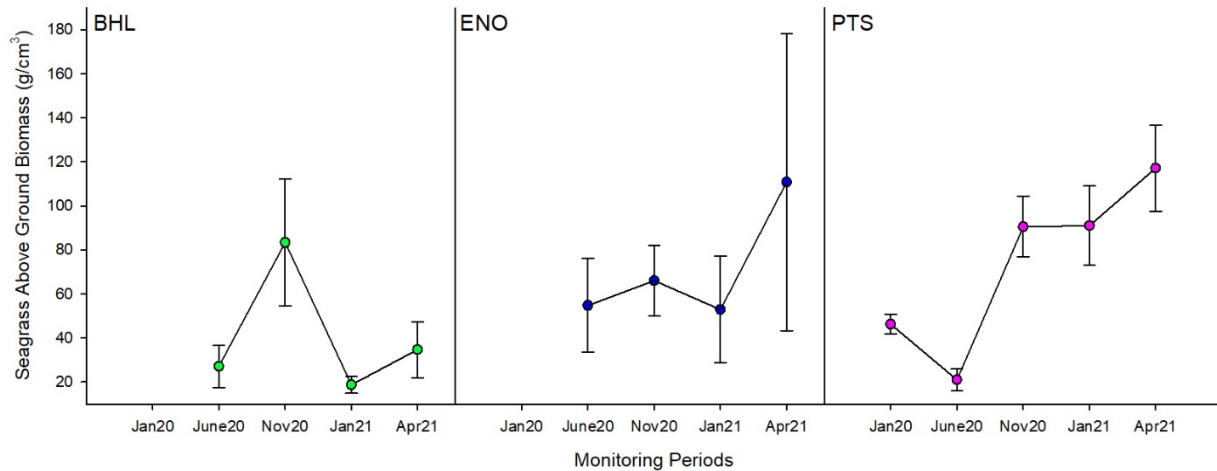


Figure 24. Mean Above Ground Biomass of *Halophila stipulacea* across sites and monitoring periods. The study sites are Bahía Linda (BHL); Ensenada Onda (ENO); Punta Tamarindo Chico (PTC). Whisker represents standard error.

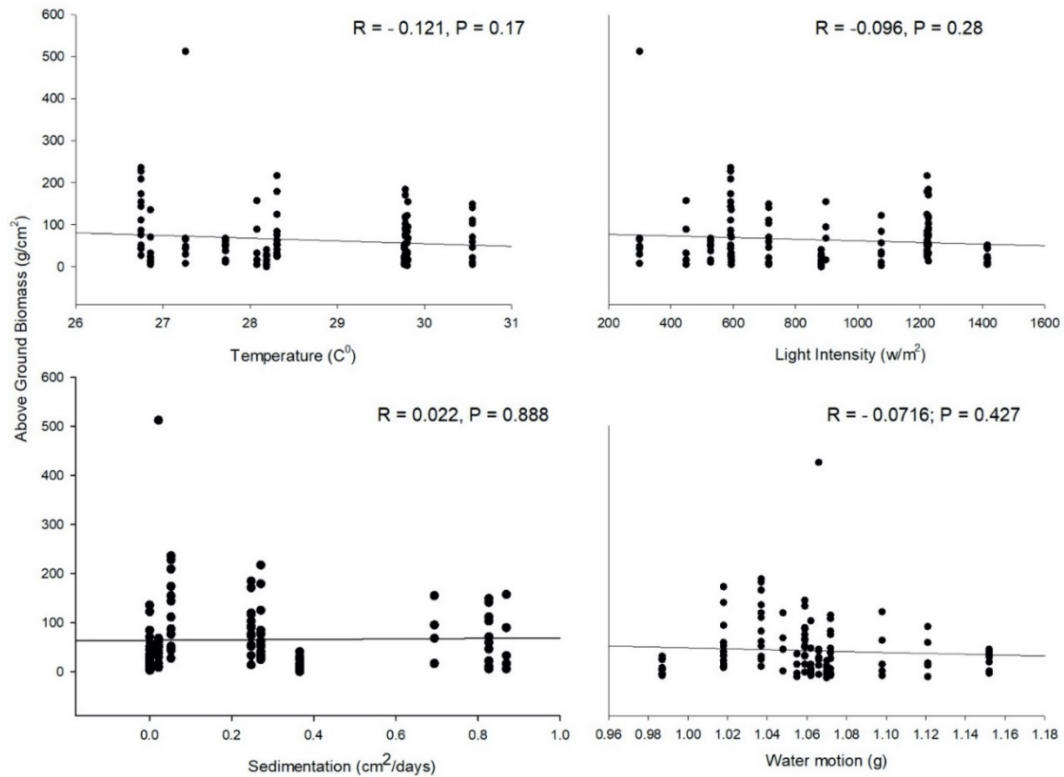


Figure 25. Correlation analyses between environmental parameters and Above Ground Biomass of *Halophila stipulacea*.

v. Below-ground Biomass (BGB)

Below-ground biomass (BGB) varied in a similar mode as AGB. However, no statistical differences were detected across sites or time. PTC showed the highest mean BGB of 132g/cm²,

followed by BHL with 129g/cm² and ENO with 87 g/cm² (Figure 26). When compared through time, PTS showed the highest BGB in Jan 21. While at ENO and BHL, the highest BGB was recorded in Jun and Nov 20, respectively, whereas at PTC, the highest BGB was recorded in Apr 2021. The lowest BGB at PTS was recorded in Jun 2020, while ENO and BHL were recorded on Jan 21. The lowest BGB at PTC was recorded on Nov 20.

Neither temperature, light intensity nor sedimentation showed significant interactions with BGB. Meanwhile, water motion showed strong negative and significant interaction with BGB (Figure 27).

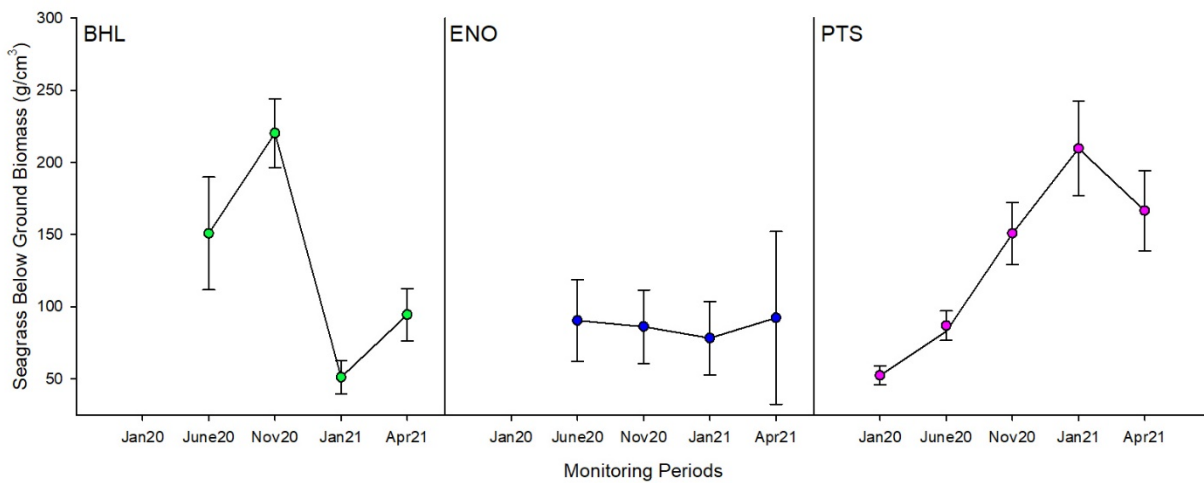


Figure 26. Mean below-ground biomass of *Halophila stipulacea* across sites and monitoring periods. The study sites are Bahía Linda (BHL); Ensenada Onda (ENO); Punta Soldado (PTS). Whisker represents standard error.

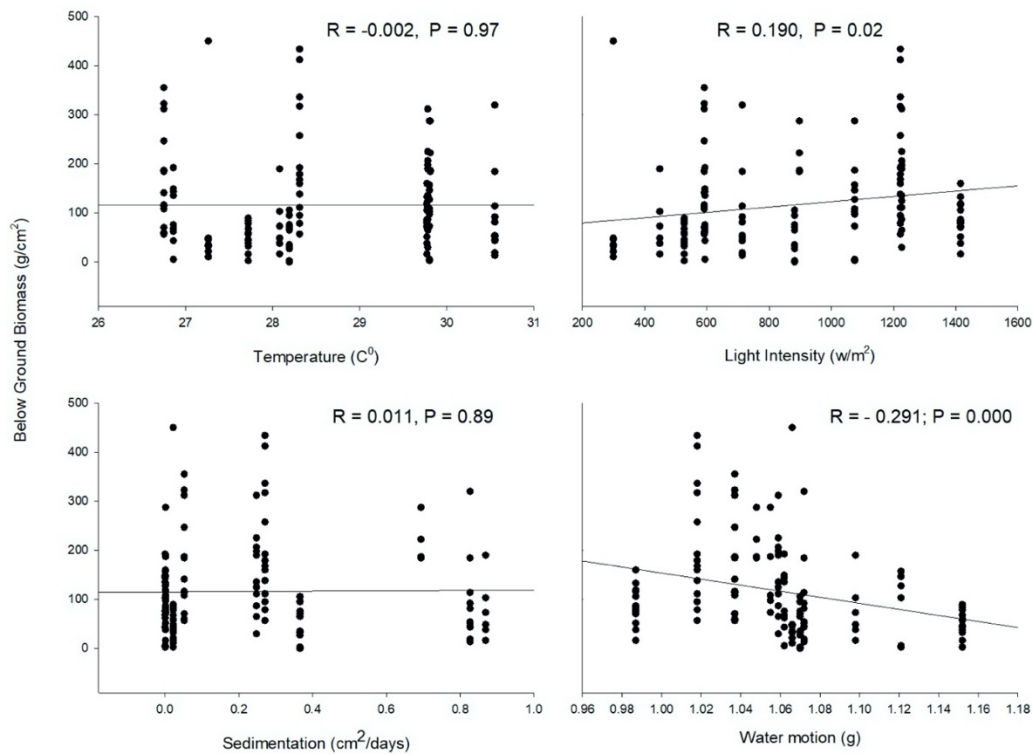


Figure 27. Correlation analyses between environmental parameters and Below Ground Biomass of *Halophila stipulacea*.

c. *Syringodium filiforme*

a. Percent Cover

S. filiforme was present at BHL, PTS, and PTC. However, during the first monitoring period, it was absent from PTS. Afterward, its presence at this site steadily increased. *S. filiforme* was absent from ENO throughout the study period. Overall, the % cover of *S. filiforme* was higher at BHL ($\approx 19\%$ cover), followed by PTC with 5% cover and PTS with 3% cover. Differences in *S. filiforme* cover were statistically significant ($P < 0.000$).

Percent cover was also variable across time, yet no clear pattern between sites was detected, (Figure 28). For instance, at BHL, the highest % cover was detected on Jan 20 ($\approx 32\%$) and Jan 21 (23%), whereas the lower % cover was detected on Nov 20 (15%) and Apr 21 (9%). Meanwhile, at PTS, the % cover varied from 0 on Jan 20 to 9% on Apr 21. Furthermore, the occurrence of *S. filiforme* was aggregated in the quadrants where the grass was present. A similar low % cover across time was also observed in PTC, with a lower % cover in Jun 20 (2% cover) to 11% cover

in Nov 20. At BHL, significant differences were detected between Jan 20 and Apr 21. However, at PTS, significant differences were detected between Apr 21 and Jan 20, Jun 20, Nov 20, and Jan 21. Finally, significant differences were detected at PTC between Nov 20 and Jan 20, Jun 20, Jan 21, and Apr 21.

Neither temperature, light intensity, nor water motion showed significant interactions with % cover. However, sedimentation exhibited a positive and significant interaction with % cover ($R = -0.106$; $P < 0.017$), (Figure 29).

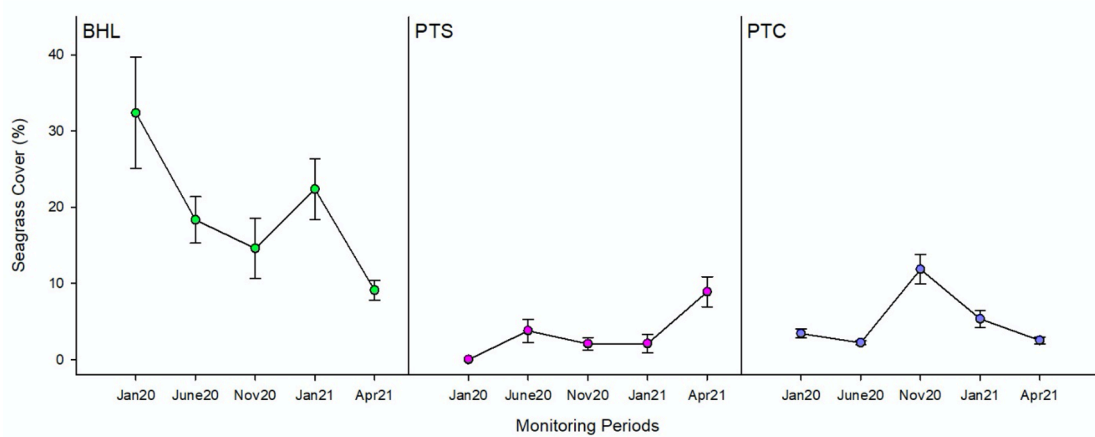


Figure 28. Mean *Syringodium filiforme* cover (in percent) across study sites and monitoring periods. The study sites are Bahía Linda (BHL); Punta Soldado (PTS); Punta Tamarindo Chico (PTC). Whisker represents standard error.

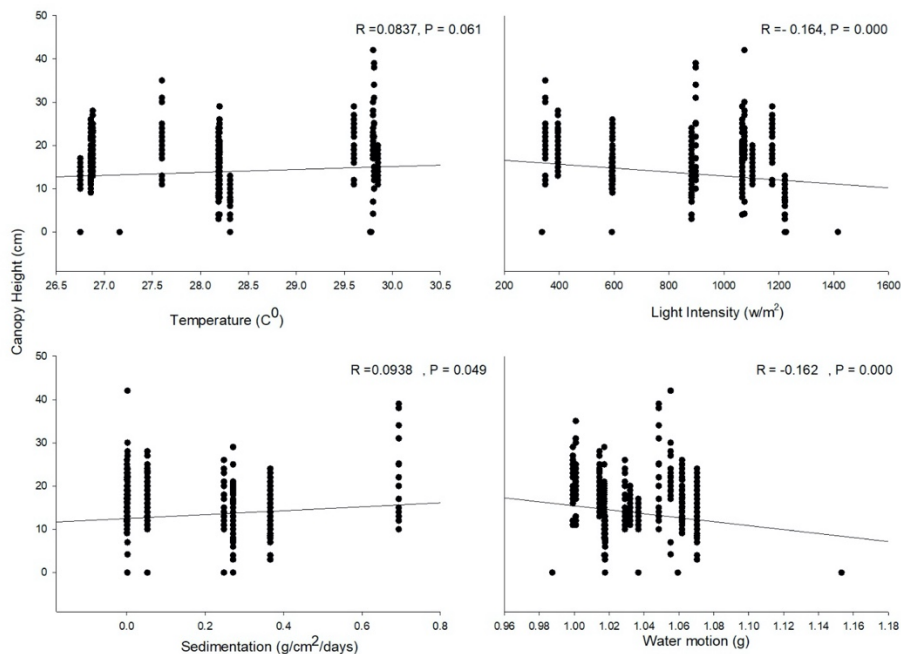


Figure 29. Correlation analyses between environmental parameters and *Syringodium filiforme* canopy height.

ii. Shoot Density

Shoot density for *S. filiforme* varied significantly among study sites ($P < 0.000$; Figure 30). Overall, BHL showed the highest shoot density (212.13 shoot/m²), followed by PTC (190 shoot/m²) and PTS (102 shoot/m²). However, shoot density by sites/time did not exhibit a clear pattern. For example, at BHL, the highest densities were recorded on Jan 20 and Apr 21 (450 and 210 shoots/m²), while the lowest densities were recorded from Jun20-Jan21. Meanwhile, at PTS, the shoot density was lower on Jan 20 (0 shoots) and higher on Jun 20 (225shoot/m²). From Nov 20 to Apr 21, shoot density ranged from 78 to 111 shoot/m². At PTC, shoot density ranged from 262 shoot/m² on Jan 20 to 132 shoot/m² on Jan 21. The statistical analyses revealed significant differences between Jan 20 and Jun 20, Nov 20, Jan 21, and Apr 21. Meanwhile, at PTS, significant differences were only detected between Jan 20 and Jun 20. At PTC, significant differences were detected between Jan 20 and Jun 20, Nov 20 and Jan21, Jun 20 and Jan 21, and Jan 21 and Apr 21.

Neither temperature nor light intensity showed significant interactions with shoot density. However, sedimentation and water motion both exhibited negative and significant interactions ($R = -0.140$; $P < 0.003$ & $R = -0.299$; $P < 0.000$, respectively), (Figure 31).

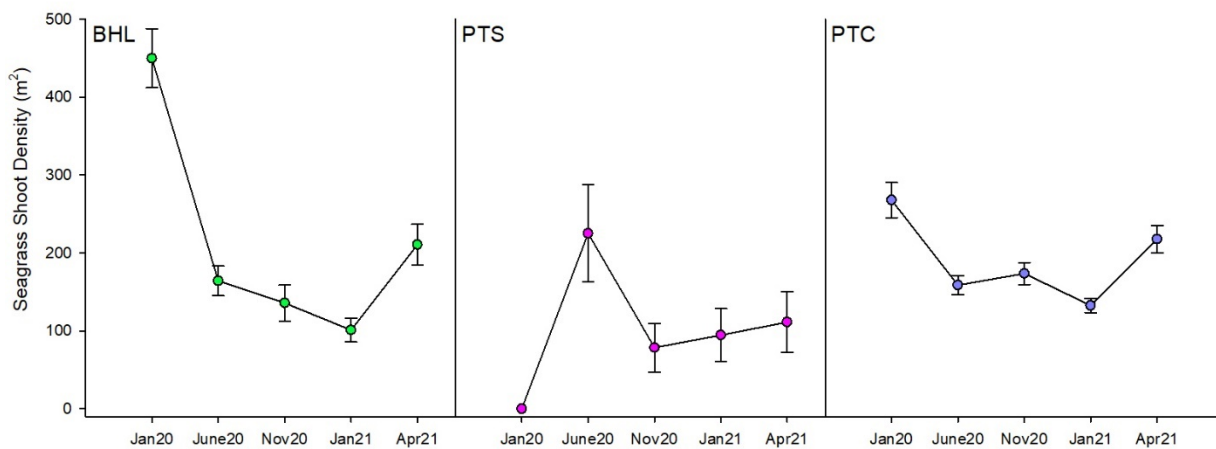


Figure 30. Mean *Syringodium filiforme* shoot density across study sites and monitoring periods. The study sites are Bahía Linda (BHL); Punta Soldado (PTS); Punta Tamarindo Chico (PTC). Whisker represents standard error.

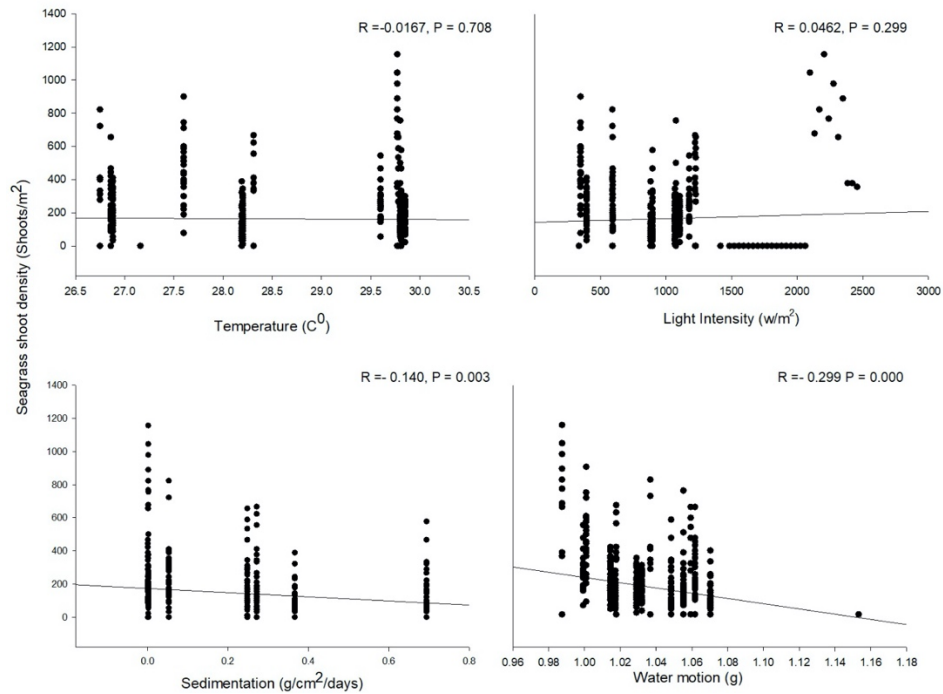


Figure 31. Correlation analyses between environmental parameters and *Syringodium filiforme* shoot density

iii. Canopy Height (leaf length)

Compared to the other metrics, canopy height slightly varied between BHL and PTC (20cm and 18cm, respectively). However, canopy height was minimal at PTS, with a mean leaf length of 2cm. Differences between PTS, PTC, and BHL were statistically significant ($P < 0.000$; Figure 32). Meanwhile, statistical differences were detected when leaf length was compared across time by sites. For instance, at BHL, leaf length from Jan 20 to Nov 20 ranged from 21cm to 23 cm. We observed a slight decrease in mean length on Jan 21 (15cm) but on Apr 21 spiked again to a mean height of 17cm. At PTC, leaf lengths were similar from Jun 20 to Jan 21, ranging from 15 to 16cm. Peaks were observed on Jan 20 (22cm) and Apr 21 (20cm). At PTS, leaf length ranged from 4.1 to 4.3cm from Jan 21 to Apr 21. At BHL, significant differences were detected between Apr 21 and Jun 20 and Nov 20, Jan 20 and Jun 20, Nov 20 and Jun 21, and Jan 21 and Jun 20 and Nov 20. Meanwhile, at PTS, significant differences were detected between Apr 21 and Jun 20, Nov 20 and Jan 20, Jan 20 and Jan 21, Jan 21 and Jun 20, and Nov 20. At PTC, significant differences were detected between Apr 21 and Jun 20, Nov 20 and Jan 21; and between Jan 20 and Jun 20, Nov 20 and Jan 21.

Temperature showed slight positive, though no significant interaction with Canopy height. By contrast, light intensity and water motion showed significant and negative interaction with Canopy height ($R = -0.164$; $P < 0.000$ & $R = -0.162$; $P < 0.000$, respectively). Sedimentation, on the other hand, showed a positive and significant interaction with Canopy height ($R = 0.938$; $P < 0.049$; Figure 33).

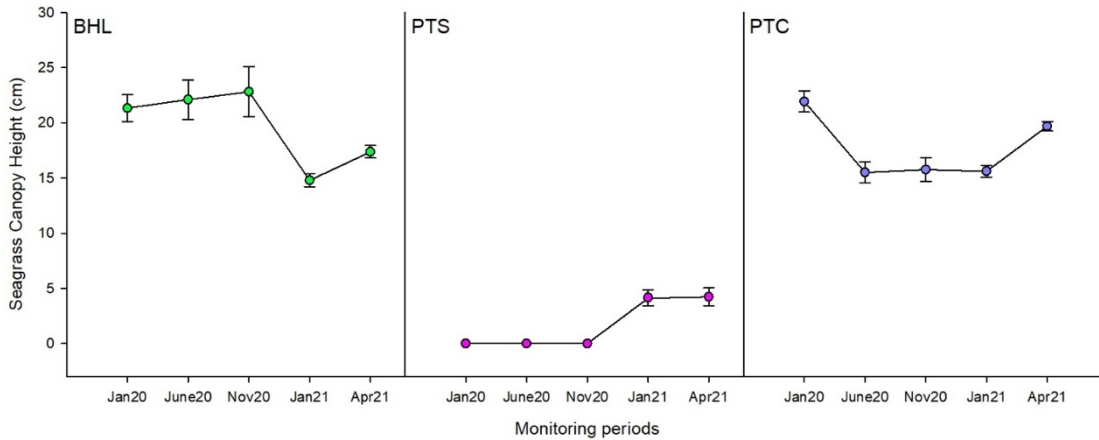


Figure 32. Mean *Syringodium filiforme* Canopy Height across sites and monitoring periods. The study sites are Bahía Linda (BHL); Punta Soldado (PTS); Punta Tamarindo Chico (PTC). Whisker represents standard error.

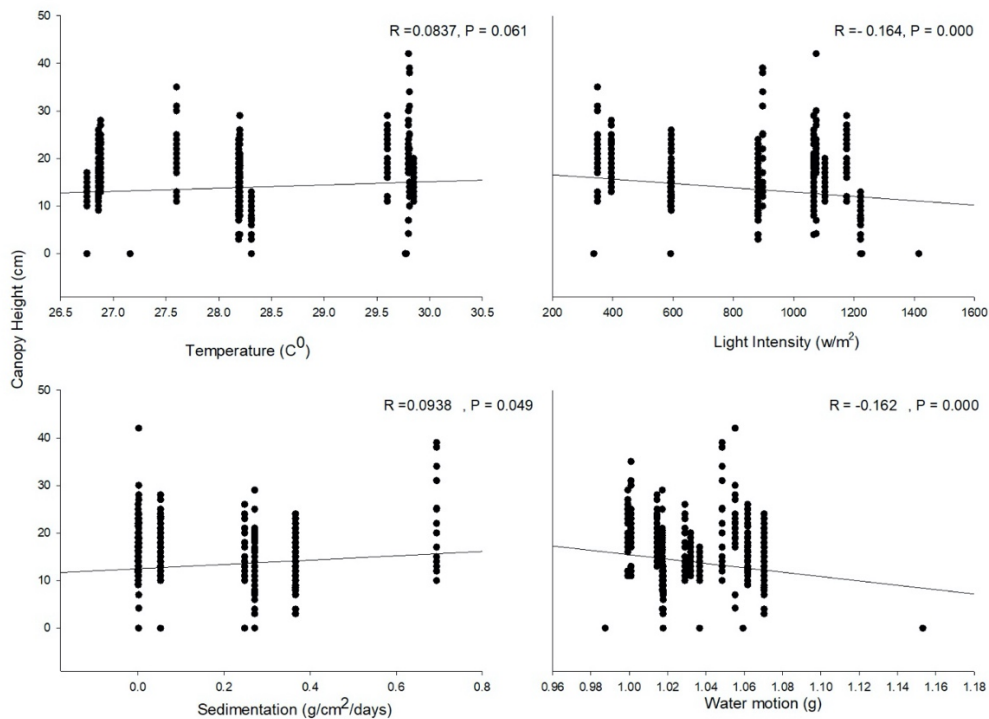


Figure 33. Correlation analyses between environmental parameters and *Syringodium filiforme* Canopy height.

iv. Above-ground Biomass (AGB)

AGB varied across sites and time, yet these differences were not statistically significant (Figure 34). BHL ($14\text{g}/\text{cm}^2$) showed higher AGB than PTS and PTC combined (4 and $5\text{ g}/\text{cm}^2$, respectively). However, BHL exhibited significantly higher variation across time than PTS and PTC. For instance, at BHL, the highest AGB (i.e., $57.87\text{g}/\text{cm}^2$) was observed in Nov 20, whereas the lowest (i.e., no AGB) was recorded on Jan 20 and Jun 20. Meanwhile, the highest AGB at PTS ($10\text{g}/\text{cm}^2$) was recorded in Nov 20, while the highest at PTC was recorded in Jan 20. The lowest AGB at PTS was recorded in Jan 20 and Jun 20, whereas at PTC, the lowest was recorded in Apr 21 ($3\text{g}/\text{cm}^2$). At BHL, significant differences were detected.

Neither Temperature, light intensity, nor water motion showed significant interactions with AGB. Meanwhile, sedimentation showed a slightly positive yet significant interaction with AGB (Figure 35).

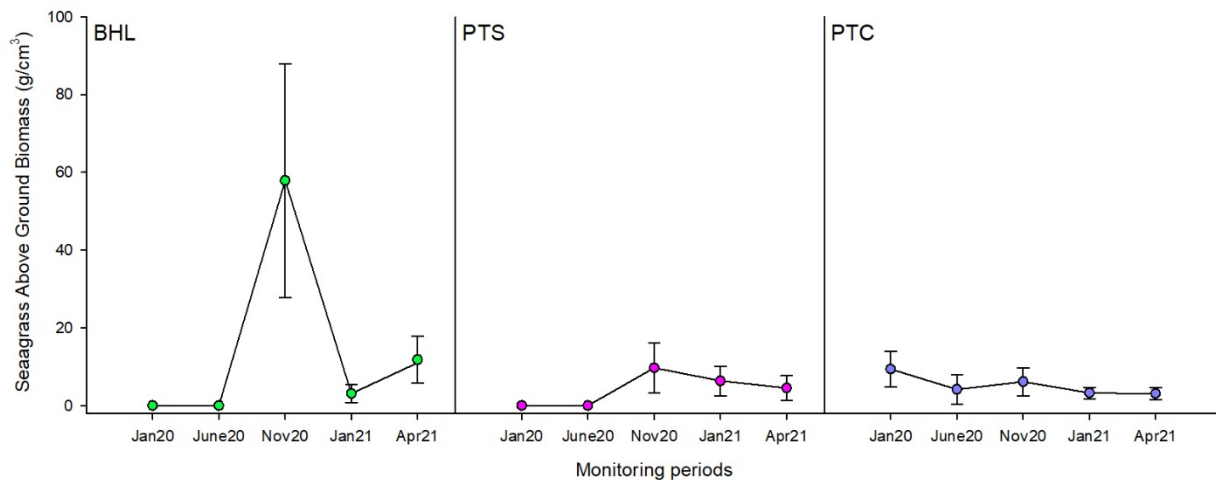


Figure 34. Mean *Syringodium filiforme* Above Ground Biomass across sites and monitoring periods. The study sites are Bahía Linda (BHL); Punta Soldado (PTS); Punta Tamarindo Chico (PTC). Whisker represents standard error.

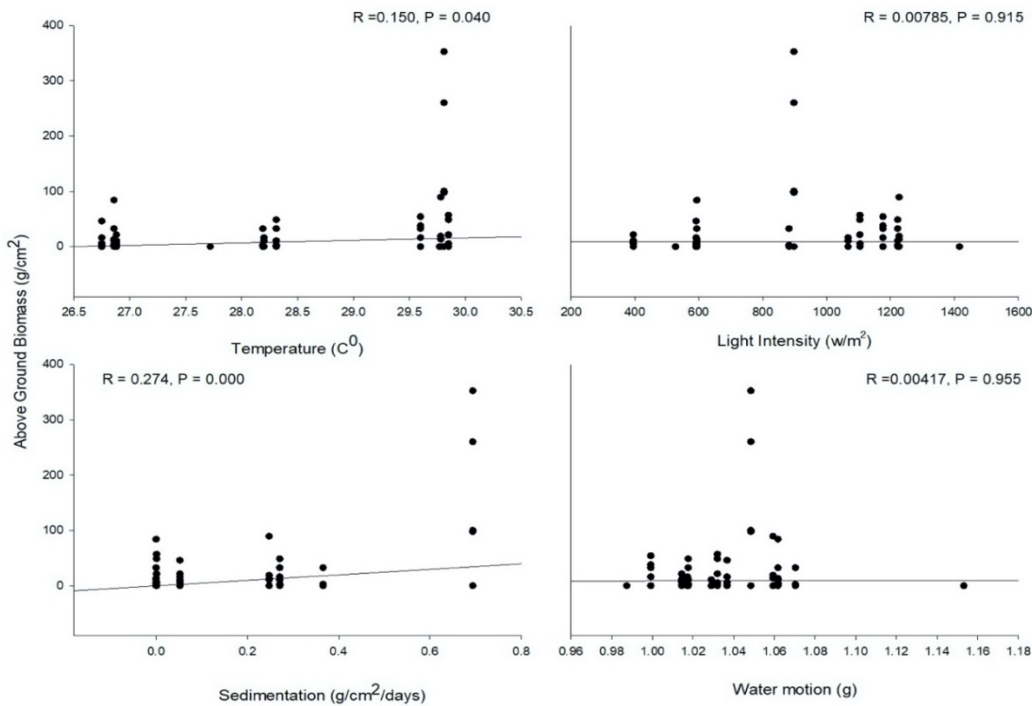


Figure 35. Correlation analyses between environmental parameters and *Syringodium filiforme* Above Ground Biomass.

v. Belowground biomass (BGB)

BGB also varied across sites and time, although differences were not significantly different (Figure 36). However, in contrast to AGB, the mean BGB was higher at PTC (44g/cm^2), followed by BHL (32g/cm^2) and PTS (26g/cm^2). Variation across time was similar between BHL and PTS, as both showed no BGB detection in Jan 20 and Jun 20, and then both showed mean BGB increments of 63 and 73g/cm^2 , respectively, in Nov20. From Jan 21 to Apr 21, BHL showed mean BGB of 25 and 68g/cm^2 , respectively, whereas at PTC, mean BGB for these same months were 21 and 36g/cm^2 . The highest BGB (e.g., 136g/cm^2) was observed at PTC on Jan 20. After that decreased with BGB ranging from 24 to 15g/cm^2 . No significant interaction was detected between Temperature, light intensity, sedimentation, and water motion with AGB.

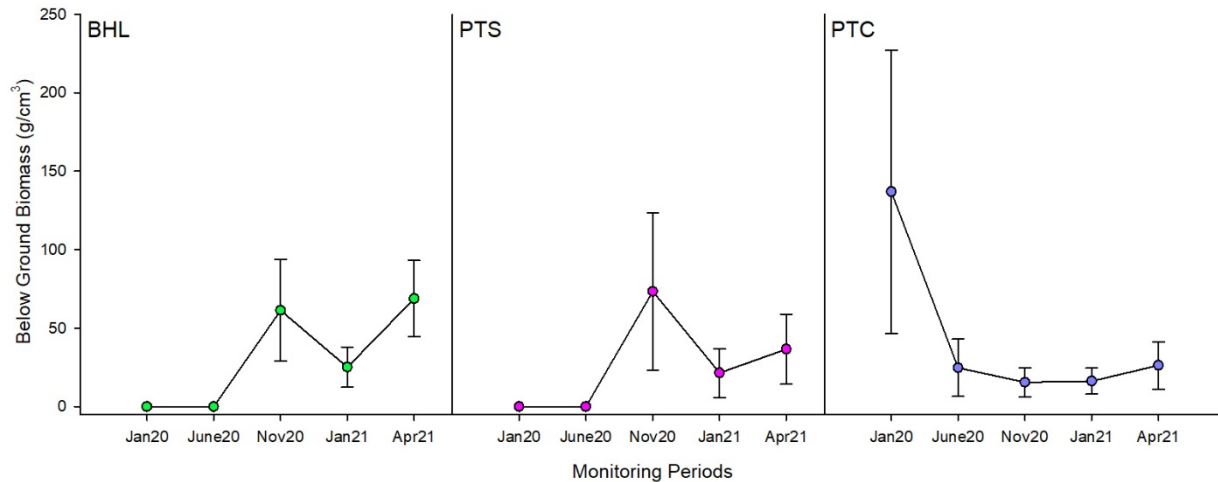


Figure 36. Mean *Syringodium filiforme* Below Ground Biomass across sites and monitoring periods. The study sites are Bahía Linda (BHL); Punta Soldado (PTS); Punta Tamarindo Chico (PTC). Whisker represents standard error.

AIM 2: “Assess the effects of *H. stipulacea* dominance on seagrass-associated demersal fish and invertebrate assemblages”.

d. FISH ABUNDANCE & DIVERSITY

Overall, 21 reef fish species and one invertebrate species were captured across the study sites and monitoring period using the pound net technique (Table 1 and Figure 37). The reef fish most frequently caught was the yellowtail *Ocyurus chrysurus* (nine captures). However, seven different reef-fish species were captured only once, while four reef species were captured twice. Meanwhile, ENO was the site with the highest number of captures with 31, followed by PTS with 23, BHL with 11, and PTC with nine captures. Nov 20 was the month with the highest number of captures with 30 individuals, followed by Jan 20 with 17 captures, Jan 21 with 14 captures, Apr 21 with ten captures, and Jun 20 with three captures.



Figure 37: Fish species collected using the pound net technique A) *Holocentrus adscensionis*, B) *Archosargus rhomboidalis*, C) *Aulostomus maculatus*, D) *Haemulon aurolineatum*, E) *Sphoeroides spengleri*, F) *Halichoeres bivittatus*, G) *Acanthostracion quadricornis*, H) *Ocyurus chrysurus*, I) *Acanthurus tractus*, & J) *Haemulon plumierii*.

Overall, eleven functional groups were captured across the study sites and throughout the study using the drop trap technique (Table 2 and Figure 38). The functional group most frequently caught were gastropods with 1934 individuals, followed by Bivalves with 908 individuals, crustaceans with 228, and Urchins with 138. Other functional groups such as mollusk and Tunicates were also captured but in fewer numbers (<11 individuals). PTS was the site with the highest number of capture with 1539 individuals, 50% of which were gastropods. PTC followed with 757 individuals (60% of which were gastropods). The site with the least captures was ENO, with 339 individuals. Jan21 was the period with the highest number of captures, with 1109 individuals. During the remaining monitoring periods, the number of captures ranged from 402- to 464.

Table 1. Total number of reef fish and invertebrates caught using the Pound net technique. The study sites are Bahía Linda (BHL); Ensenada Onda (ENO); Punta Tamarindo Chico (PTC).

Species	Jan20				Jun20				Nov20				Jan21				Apr21			
	PTS	BHL	ENO	PTC	PTS	BHL	ENO	PTC	PTS	BHL	ENO	PTC	PTS	BHL	ENO	PTC	PTS	BHL	ENO	PTC
<i>Holocentrus rufus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
<i>Aulostomus maculatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
<i>Ocyrus chrysurus</i>	0	0	2	1	0	0	0	0	0	1	1	0	0	0	0	4	0	0	0	0
<i>Lutjanus spp.</i>	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Callinectes sp.</i>	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Archosargus rhomboidalis</i>	0	0	4	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
<i>Haemulon aurolineatum</i>	1	0	1	0	3	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
<i>Haemulon flavolineatum</i>	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
<i>Haemulon plumieri</i>	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	1	0	1	0
<i>Haemulon chrysargyreum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Haemulon sciurus</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
<i>Sphaeroides spengleri</i>	2	0	0	0	0	0	0	0	0	0	0	0	3	1	2	0	0	0	0	0
<i>Octopus spp.</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Odontoscion dentex</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>Chaetodon capistratus</i>	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0
<i>Lutjanus synagris</i>	0	0	0	0	0	0	0	0	0	0	5	0	0	0	2	0	0	0	0	0
<i>Haemulon boschmae</i>	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
<i>Eucinostomus lefroyi</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>Halichoeres maculipinna</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Halichoeres bivittatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
<i>Pseudopeneus maculatus</i>	0	0	0	0	0	0	0	0	3	1	0	1	0	0	0	0	0	0	0	0
<i>Sargocentron vexillarium</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

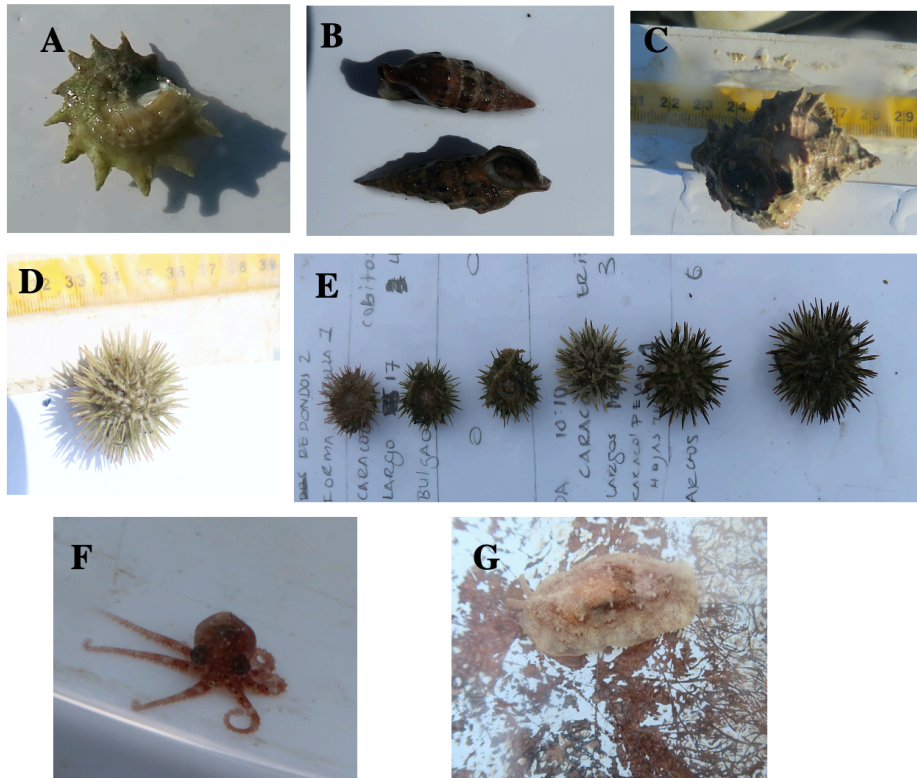


Figure 38: Fish species collected using pound net technique A) *Astraea* spp. B) *Cerithium* spp.,) *Phyllonotus* spp., D) *Tripneustes ventricosus*, E) *Lytechinus variegatus*, F) *Octopus* spp., G) *Discodoris* spp

Table 2: Total number of captures the study sites and monitoring using drop technique

Functional Groups	Jan20				Jun20				Nov20				Jan21				Apr21			
	PTS	BHL	ENO	PTC	PTS	BHL	ENO	PTC	PTS	BHL	ENO	PTC	PTS	BHL	ENO	PTC	PTS	BHL	ENO	PTC
Bivalves	1	8	0	14	7	11	9	165	6	6	2	13	634	10	1	17	0	3	0	1
Gastropods	48	54	78	144	71	46	52	0	442	71	82	116	0	104	69	146	153	113	91	54
Urchins	0	0	11	0	0	0	1	29	1	0	14	0	64	1	14	1	0	0	2	0
Crustaceans	5	26	0	11	47	13	2	0	19	6	1	8	0	21	2	23	33	8	1	2
Molluks	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Sponges	0	0	0	0	0	0	4	3	0	0	1	1	0	0	0	0	0	0	0	0
Tunicates	0	0	0	0	0	0	0	0	1	0	0	8	0	0	1	1	0	0	0	0
Fish	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scorpanidea fish	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cosmocampus	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chiton	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

AIM 3. “Assess the effect of *H. stipulacea* on the percent cover, above and below ground biomass, shoot density and growth of *T. testudinum* through a competitive interaction field experiment.”

The competition experiment was set to start on Feb 21. During this period, 20 experimental plugs of *T. testudinum* were collected and planted in individual cubicles. Five additional plugs of *H. stipulacea* were also planted during that period. The mean number of *T. testudinum* shoots per TEU was 29; for *H. stipulacea* was 26 shoots per plug. By Mar 21, we recorded 100% mortality in all *H. stipulacea* plugs. Subsequent *H. stipulacea* plugs planted in Mar 21, Jun 21, and Jul 21 failed to survive. Therefore, we could not complete the competition experiment due to the high *H. stipulacea* plugs mortality. By contrast, *T. testudinum* plugs showed 100% survival from Feb 21 to Nov 21 (Figure 39). The average number of shoots from Mar 21 to Nov 21 steadily increased from 30 ± 9.15 (SE) to 33 ± 9.15 (SE). The mean leave length of *T. testudinum* from Jun 21 to Nov 21 was 9.7cm. However, the total length of *T. testudinum* was variable across months. For instance, we recorded the highest leave length (i.e., 10.66 cm) in Jun 21. After that, the leaf length decreased (Figure 40). Since we could no longer visit the experimental setting after Jul 21, the table was dismantled on Aug 21, at the *T. testudinum* plugs were replanted in the same collection area.

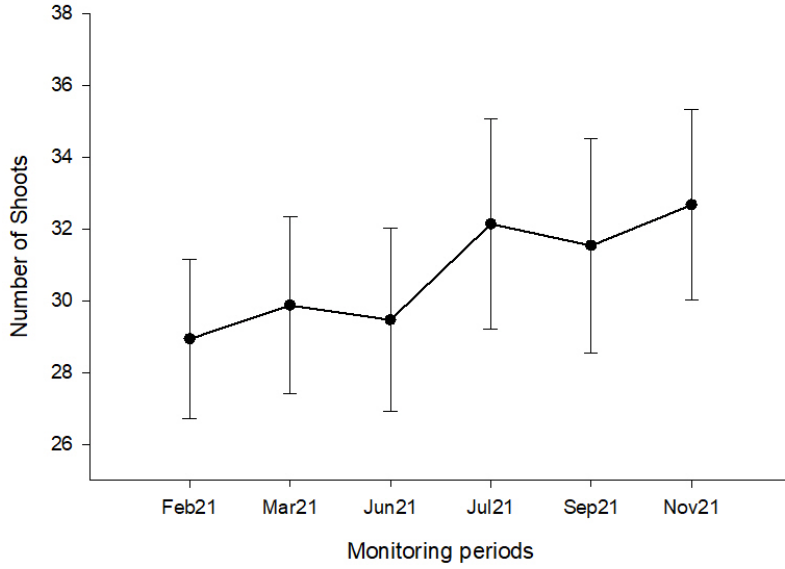


Figure 39. Mean number of shoots across monitoring periods from *Thalassia testudinum* experimental transplant units. Whisker represents standard error.

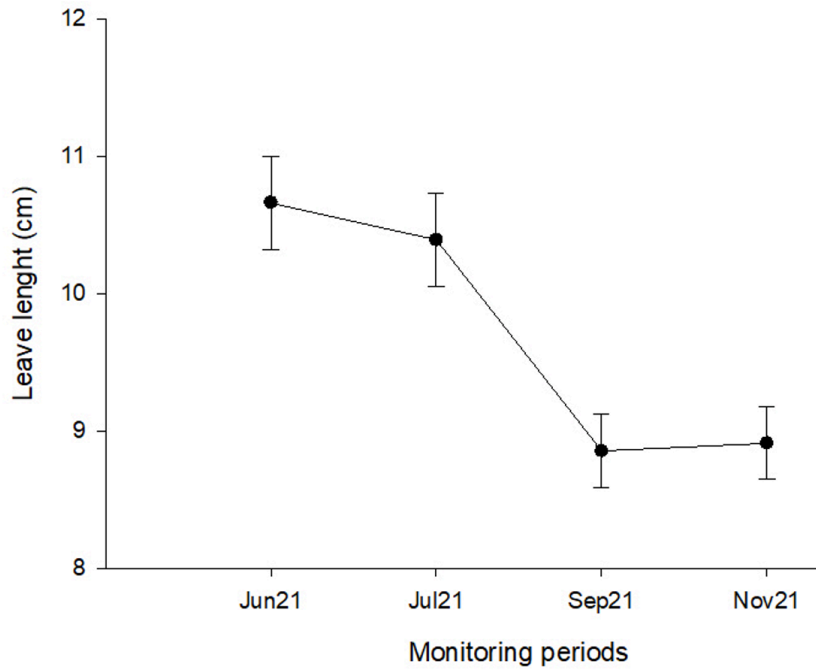


Figure 40. Mean leaf length across monitoring from *Thalassia testudinum* experimental units.

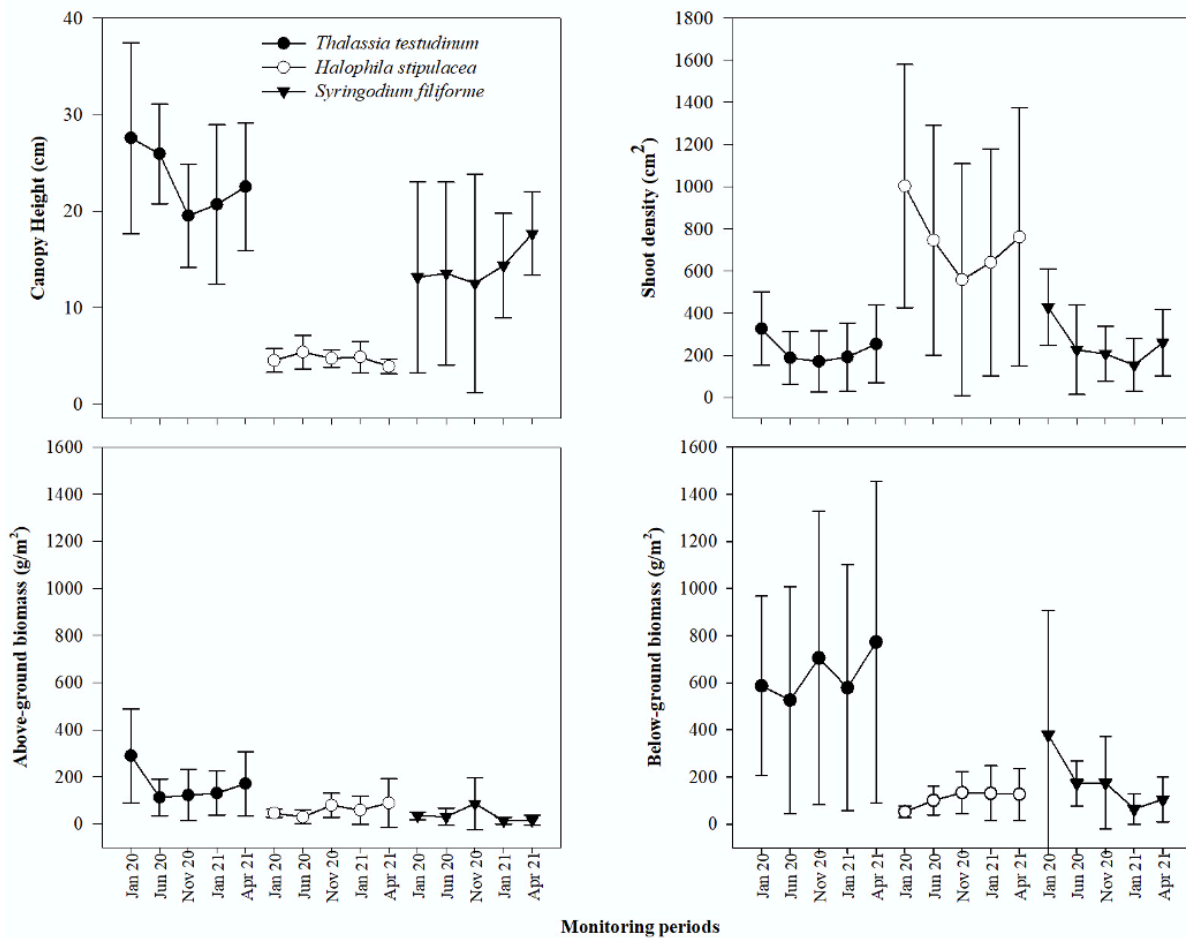


Figure 42. Summary of seagrasses metric. Whisker represents standard error

DISCUSSION

Overall, the distribution and abundance of seagrasses in Culebra were mainly influenced by site specific conditions. That is, sites with low human/environmental perturbation showed the highest stable seagrass community (i.e., PTC), whereas the sites with the highest human or environmental impact showed less seagrass stability (BHL and PTS). Yet a tendency between seasonal variation of environmental conditions and physical metrics of seagrasses was observed (Figure 42). This is contrary to other studies in tropical meadows where physical metrics such as leaf length, shoot density and biomass have shown marked seasonal variation (Medina-Gomez et al 2016). Therefore, longer monitoring is needed to clearly understand these effects in Culebra.

Seagrass meadows at Culebra are under multiple threats. Some of these threats, i.e., seawater temperature and light intensity have global scales distribution, therefore are virtually impossible

to manage. Others are local in origin i.e., water sedimentation. These are far easier to manage and prevent. In our study, the local threats have a greater impact on seagrass meadows than global scale threats. Yet local threats varied in intensity by site.

Collectively, four seagrasses i.e., *Thalassia testudinum*, *Syringodium filiforme*, *Halodule wrightii* and *Halophila stipulacea* were persistently observed throughout the study periods. However, their distribution and dominance varied over sites. For example, at PTC *T. testudinum* was clearly the dominant seagrass (highest in cover, shoot density and above-below biomass), followed by *S. filiforme*. *H. wrightii* and *H. stipulacea* were also present at this site but not within the transects. Instead, these seagrasses were associated with some blowholes. Considering that *T. testudinum* is a climax species (Furman et al., 2019), our data suggest that PTC has been under low environmental and human-derived stress in recent years.

At PTS, the dominant seagrass was *H. stipulacea*, followed by *S. filiforme*. Therefore, for most of the study, seagrass cover, shoot density, and above-below biomass was higher at *H. stipulacea* than *S. filiforme*. We did not observe *S. filiforme* at PTS during the first monitoring period, but its abundance increased afterward. However, during the last monitoring period (e.g., April 2021), *H. stipulacea* dominance dramatically decreased due to a mysterious growth of what we believed was a consortium of microorganisms that resemble a mat that smothered the invasive seagrass. This mat growth had little effect on *S. filiforme* as their leaf grew above the mat. However, many gastropods died because of this mat. It is enigmatic to us what causes this extensive mat growth at PTS; however, similar mats have also been observed in small patches dominated by *H. stipulacea* at PTC. Given the high-water quality in PTS the microbial mats were the most important factor influencing the seagrass meadow at PTS. Likewise, it is unknown to us why *T. testudinum* is yet to colonize PTS. Perhaps, the substrate characteristics at PTS may play a role, given that no extreme environmental event (except for Hurricanes Irma and Maria) nor human-derived stressor have been noticed by our group at PTS during and after this study.

Seagrasses at BHL were the most diverse compared to the other sites, as the four species were reported within the transects. From these *T. testudinum* and *H. stipulacea* showed the highest dominance, followed by *S. filiforme* and, to a lesser extent, *H. wrightii* (<1%). Furthermore, the four seagrasses were observed co-existing within several quadrants, with *H. stipulacea* growing underneath the canopy of the other three long-leaf seagrasses. Nonetheless, the presence of two pioneer species, i.e., *S. filiforme* and *H. wrightii* (Gallegos et al., 1994), in addition to the invasive

H. stipulacea, suggests this site has been exposed to recurrent physical disturbance i.e., vessel anchoring in years previous to this study.

ENO is the site with the lowest water quality for seagrass, so only two seagrass species were recorded, *T. testudinum* and *H. stipulacea*. Within the transects and in the shallower areas (<4.5m), *T. testudinum* showed a higher presence. However, in deeper water (>4.5m), *H. stipulacea* was the only seagrass recorded. Furthermore, the canopy height from both seagrasses was tallest than at any other site. Arguably, this may be a strategy to optimize photosynthesis in a low-light environment. On the other hand, a dramatic decrease in the abundance of seagrasses recorded during April 2021 monitoring resulted from a grounding cargo vessel, which wiped off all seagrasses in one of our transects and nearby areas. However, further visits have shown relatively rapid re-colonization of *H. stipulacea* in the impacted area. In contrast, *T. testudinum* has not recolonized areas previously occupied by it.

In Culebra, physical metrics range similarly to other studies. For instance, when pooling the *T. testudinum*, our mean leaf height is within the range of those reported in Medina-Gomez et al. (2016) but higher than Schurbert et al. (2015). Meanwhile, shoot density was below that reported for *T. testudinum* beds on Mexican Caribbean coasts and Carrie Bow Cay in Belize (Enriquez and Pantoja-Reyes). Being the larger seagrass in Culebra, *T. testudinum* showed the highest above-below ground biomass.

On the other hand, some of the metrics estimated for *H. stipulacea* are comparable to other sites in the Red Sea. For instance, leaf length in our study sites ranged from 3 to 5.5 cm, whereas the Gulf of Aqaba (northern Red Sea) ranged from 3.5cm to 5cm (Mejia et al., 2016; Rotini et al., 2017). Meanwhile, our estimated shoot density was remarkably lower (200- 1,600 shoot m⁻²) compared to other sites such as the harbor of Palinuro, Italy (10,500 shoots m⁻²; Gambi et al., 2009) and Grace (3132 to 13148 shoot m⁻²; Apostoloki et al 2019), yet our results were similar to those reported at the eastern coast of Tunisia (Brahim et al., 2019). We did not find comparable records of the above-below biomass. However, biomass in the studies conducted in Italy and Grace is likely to be higher than ours. The contrasting density and biomass may be due to nutrient availability and hydrodynamic conditions. That is, areas with higher shoot densities and biomass may be exposed to fewer wave actions and higher nutrients.

In general, *S. filiforme* is a versatile seagrass co-existing with *T. testudinum* as part of the climax, submerged vegetation in the meadow with relatively low human perturbation. At the same time,

co-occupying meadows with high human perturbation. did not show a typical behavior of a pioneer seagrass species, with higher abundance in the disturbed area. Yet, in either location, *S. filiforme* was the dominant seagrass, further showing a low capacity to arrest succession, avoiding the entrance of climax or invasive species.

OUTREACH

From October 2019 to April 2021, four major outreach activities to present the project’s outcome were presented. These were:

1. April 2021: Invited to Ocean World lecture series “*Impacts of Halophila stipulacea seagrass in the Caribbean.*” By Zoom and FB live SAMPR.ORG



2. April 2021. Impacted (750 persons; persons: school students, undergraduates, graduates, professors, and teachers). Invited to the second virtual symposium, “*The Power of Women in Science,*” University of Puerto Rico at Rio Piedras”
3. December 2021: Program Bridge to the Doctorate- Cohorte XIII. Puerto Rico Louis Stokes Alliance for Minority Participation A Consortium of the University of Puerto Rico (Impacted 20 persons).



4. June and July 2021 Mentoring activity for the “Marine Internship Pre-college Program 2021.



Figure 33. Marine internship Precollege Program 2021 fieldtrip images. A. Field trip to Cabezas de San Juan to collect seagrasses samples. B) Sample collection by Co-PI and internship students C) PI showing two interns how to identify seagrasses based on their unique features. D) Final Presentation.

- a. Two high schooler students Figure 33.
 - i. Activities
 - 1. Two field trips (Escambrón bay and Seven Seas Bay seagrasses areas in North and Northeast of Puerto Rico, impacted six students
 - 2. Conference of Seagrass ecosystem
 - 3. Oral presentation of the final project Internship student, Impacted 30 students.

SIDE PROJECTS: two side research projects were developed. These research projects are entitled:

- 1. *“Differences in the phyllosphere microbiota between invasive and native seagrasses in the Caribbean”*
 - a. This project has been developed in collaboration with the Dra. Filipa Godoy and Nataliya Chorna (University of Puerto Rico, School of Medicine)
 - b. Results from the project will be presented in Oral presentation ISME18; International Society for Microbial Ecology, Aug 14-19, 2022, Lausanne, Switzerland by the undergraduate student Diego A Perez Morales
- 2. *“The effects of native and invasive seagrasses on the nutrient composition of marine sediments”*
 - a. This project has been developed in collaboration with the Dr. Jose Dumas (Agricultural Research Station, University of Puerto Rico, Mayaguez Campus).

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