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Short communication

New sighting of seagrasses in the Eastern Tropical Pacific (Bahía Potrero, Costa Rica)

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ABSTRACT

Seagrass meadows provide a multitude of ecosystem services, yet they are currently threatened and declining worldwide due to anthropogenic impacts. Current knowledge of seagrass presence in the Eastern Tropical Pacific (ETP) is scarce, in part due to challenges in finding the small seagrass species found and their dynamic and possibly ephemeral nature, with only very limited reports of currently extant meadows. Here, we characterize seagrasses at a new location, Bahía Potrero, on the northern Pacific coast of Costa Rica. Seagrasses were sighted at this location on four occasions between 2015 and 2017. Two seagrass species were found, *Halophila baillonis* and *Halodule beaudettei*. Seagrasses were present at ~ 3–6 m depth at mean sea level (local tidal range ~ 3 m). Biomass and sediment cores were collected in 2017, total biomass was 3.9 ± 4.0 g DW m^{-2} and density 569 ± 493 shoots m^{-2}. Leaf length was 1.1 ± 0.29 cm, width 0.5 ± 0.08 cm, and area 1.4 ± 0.67 cm^2. Above ground biomass $\delta^{13}C$ was -11.7 ± 0.8‰ and $\delta^{15}N$ 5.4 ± 0.8‰, while below ground was -11.7 ± 1.3‰ and 5.3 ± 0.8‰. Sediment $\delta^{13}C$ was -21.2 ± 0.6‰ and $\delta^{15}N$ was 8.6 ± 0.4‰. Sediments were dominated by very fine sand, with 1.6 ± 0.3% organic carbon content and 23.0 ± 7.9% inorganic carbon. Carbon content standardized to 10 cm sediment depth was 20.5 ± 5.1 Mg OC ha^{-1} and 295.1 ± 109.8 Mg IC ha^{-1}. Given the numerous threats that seagrasses are under and the lack of information on seagrasses in the ETP it is critical to increase our knowledge on seagrasses in this region for adequate management and conservation initiatives.

1. Introduction

Seagrass meadows are coastal ecosystems which provide a multitude of ecosystem services (Orth et al., 2006; Nordlund et al., 2016). Seagrasses in the Eastern Tropical Pacific (ETP) are part of the Tropical Atlantic bioregion, according to Short et al. (2007). The species of seagrasses reported for the ETP are *Ruppia maritima*, *Halophila baillonis*, *Halodule beaudettei,* and *Halodule wrightii* (Cortés, 2001; Van Tussenbroek et al., 2010; Samper-Villarreal et al., 2014, 2018b). Seagrasses in the ETP have been reported previously at limited locations from Mexico to Panama (Short et al., 2007; Van Tussenbroek et al., 2010). However, seagrass meadows in the ETP can be more difficult to find than their Caribbean counterparts as they are formed by smaller species, they tend to occur in more turbid waters, at a larger tidal range (3 m ETP; 0.5 m Caribbean), and they can be ephemeral (Samper-Villarreal et al., 2018b). As such, the only reports of current seagrass presence in the ETP are on the Pacific coast of Costa Rica (Samper-Villarreal et al., 2014, 2018b), Nicaragua (Cortés-Núñez et al., 2012), and El Salvador (Ramírez et al., 2017).

Seagrasses are currently threatened and are declining worldwide due to anthropogenic impacts (Waycott et al., 2005; Orth et al., 2006). In the ETP a meadow previously reported from the Pacific coast of Costa Rica consisting of *H. baillonis* and *R. maritima* disappeared in the mid-1990s following a severe storm (Cortés, 2001). Given the dynamic nature of seagrass meadows in the ETP, scarce seagrass sightings, our current limited knowledge, and the threat to seagrass meadows worldwide further research on these meadows is currently warranted.

Here, we characterize seagrasses found at a new location in the ETP, Bahía Potrero in the northern Pacific coast of Costa Rica, providing further information on seagrass distribution in the ETP and providing information on sediment, carbon and environmental conditions during sampling.

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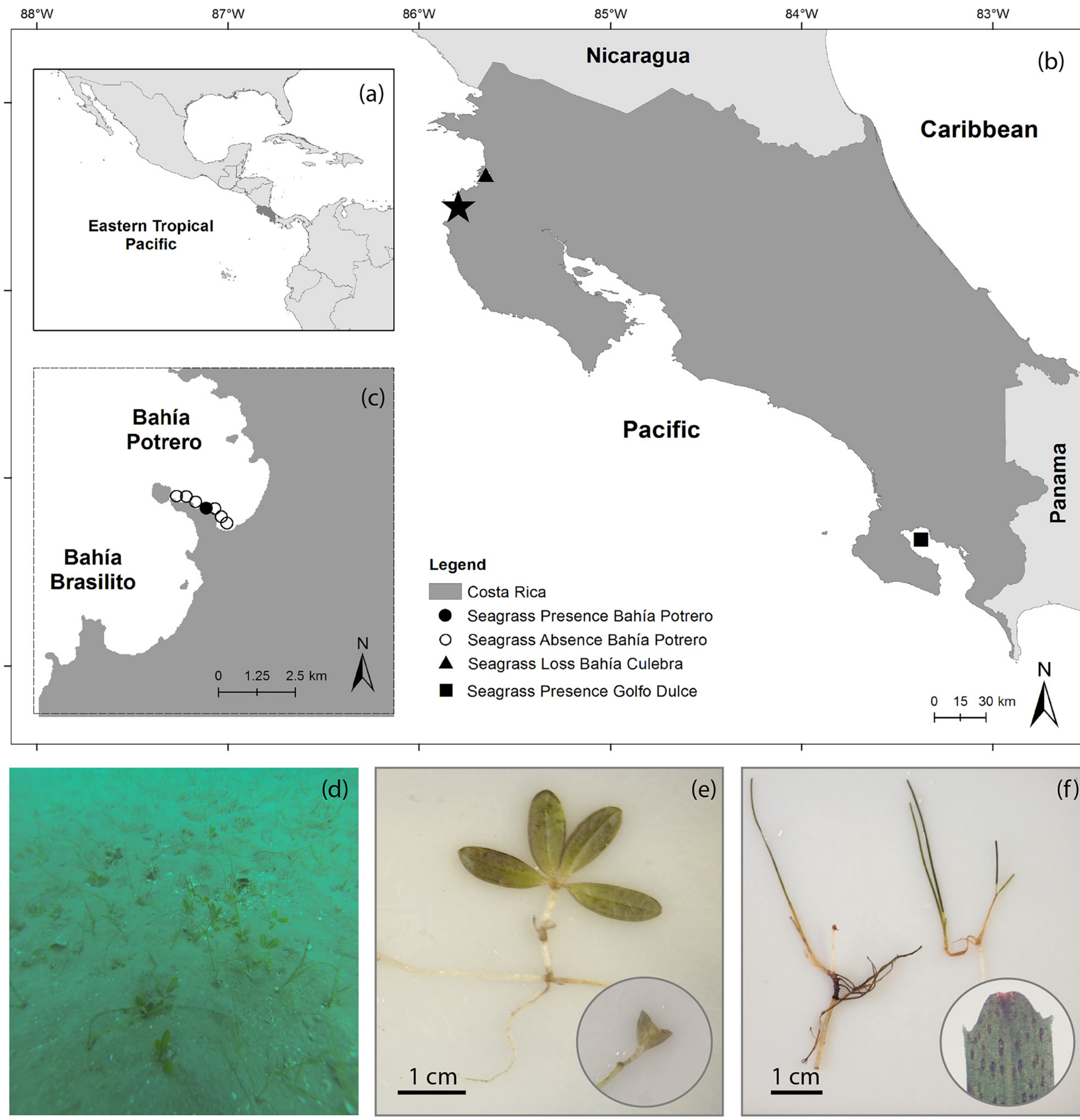

Fig. 1. Seagrasses at Bahía Potrero in the Eastern Tropical Pacific. (a) Location of Costa Rica in the region; (b) study site (star) at Bahía Potrero on the northern Pacific coast of Costa Rica; (c) close up of Bahía Potrero with seagrass survey areas and seagrass location; (d) typical cover of seagrasses at Bahía Potrero; (e) *Halophila baillonis* with close up of herbivory on leaf; and (f) *Halodule beaudettei* with close up of typical leaf tip.

2. Methods

2.1. Study site

The southern section of Bahía Potrero, on the Pacific coast of Costa Rica (10°26′33.85″N, 85°47′23.30″W; Fig. 1), was initially surveyed in 2015 as part of an environmental assessment for the intended re-activation of a local marina. Seagrasses were first sighted at one location on 1 March 2015, then sighted again at this location and species identified on 18 July 2015. Subsequently, seagrass presence was surveyed preliminarily on 22 February 2016, and seagrasses were characterized on 17–18 March 2017. Semidiurnal tidal range on the northern Pacific coast of Costa Rica is approximately 3 m (Samper-Villarreal et al., 2012).

2.2. Seagrass survey

Seagrass presence and absence was surveyed on February 2016 by dividing the southern section of Bahía Potrero, which is an area of similar environmental conditions to the location of the initial seagrass sighting, into seven similar sized areas. Within each area, the GPS coordinates of the central point was noted, ca. 100 m from the coastline (Fig. 1). At each point water depth and Secchi depth were recorded. A 60 mL subsuperficial water sample was collected at each point and salinity measured using a manual refractometer. From the central point at each location haphazard transects of approximately 75 m length and spot checking were carried out in all directions ≤ 5 m depth at low tide by a group of three spotters to verify presence or absence of seagrasses. The adjacent Isla Plata was not accessible due to weather conditions at the time of sampling.

2.3. Seagrass and sediment characterization

In March 2017, the maximum width (parallel to the coast line) and length (perpendicular to the coast line) of the area with seagrass presence were measured using a 50 m field tape measure. Within this area, Secchi (m) was measured parallel to the sea floor four times, once towards each cardinal point. Six subsuperficial 60 mL water samples were collected and salinity measured using a manual refractometer. Si x 50 mL subsuperficial water samples were collected in the field, kept cold and filtered within 12 h using glass microfiber filters. Filtered water was frozen until further processing. Water nutrient concentrations (μmol L^{-1}) of ammonium, nitrite, nitrate, phosphate, and silicate were determined using an autoanalyzer (Strickland and Parsons, 1972).

Based on the total area with seagrass presence found in 2017, three transects of 75 m length were carried out perpendicular from the coastline, with 15 m horizontal distance between each transect and the next. Along each transect, a sampling point was selected every 15 m, for a total of five points per transect. At each point water depth was measured and calibrated to mean sea level (MSL). A biomass sample, a sediment sample for grain size and a sediment sample for carbon content were collected at each point. Biomass samples were collected using a PVC corer (8 cm diameter *x* 15 cm depth) and sieved through a 1 mm mesh bag to remove sediment. Samples were kept cold in the field, then frozen until processed in the lab. Species were identified based on general and region specific seagrass taxonomic identification keys (Kuo and Den Hartog, 2001; Van Tussenbroek et al., 2010). Number of shoots per species per core were noted and biomass was separated into above and below ground biomass per species and dried at 60 °C. Leaf area (m^2) was estimated using ImageJ (Schneider et al., 2012) from photographs of the leaves from a maximum of three shoots in each sample with seagrass biomass. Epiphytes were scraped off when present. Leaf Area Index (LAI, m^2 m^{-2}) was estimated from the mean leaf area and density in each sample.

Sediment samples for grain size consisted of ~500 g total sediment, which was dried at 60 °C. A subsample of 100 g of sediment was sifted through various sieve sizes at 90 rpm for 15 min on an automatic sieve shaker, separating sediment according to particle diameter > 4, 4, 2, 1, 0.5, 0.25, 0.125, < 0.062 mm. Sediment samples for carbon content were collected using a 60 mL plastic syringe in the field, 20 mL of sediment volume were sampled. Sediment samples were kept cold in the field, then dried at 60 °C and weighed (g). Dry bulk density was estimated for each sample as the dry sediment weight divided by the volume sampled (g mL^{-1}). Dried sediment samples were homogenized using a mortar and pestle. Carbon content was estimated by Loss on Ignition (LOI) (Heiri et al., 2001). Organic matter content was estimated by placing preweighed crucibles in a muffle furnace at 550 °C for 4 h allowing to cool to room temperature in a desiccator and reweighed. Inorganic carbon was measured by placing crucibles at 950 °C for 2 h, allowing to cool overnight to 60 °C and then allowing to cool to room temperature in a desiccator and reweighed. The difference in crucible weight was used to estimate content of organic (OC) and inorganic (IC) carbon (Fourqurean et al., 2012; Howard et al., 2014). Results are presented as mean ± standard deviation.

Stable isotopes (δ^{13}C and δ^{15}N) were measured for unacidified above and below ground seagrass biomass of *H. baillonis* from the six cores with enough material for analysis. δ^{13}C was measured for acidified seagrass sediment and δ^{15}N for non-acidified sediment from seven cores. Acidification of seagrass sediment was carried out by adding 11 mL of HCl 10% to approximately 1 mL of sediment, allowing to react at room temperature over a 24 h period. Samples were then centrifuged at 3500 rpm for 3 min and the supernatant removed, rinsed with 14.5 mL of distilled water twice, centrifuging between rinsings. Acidified sediments were then dried at 60^{0}C and rehomogenised for stable isotope analysis. All samples were loaded onto tin capsules and isotopic analysis was carried out at the UC Davis Stable Isotope Facility (http://stableisotopefacility.ucdavis.edu/).

3. Results

3.1. Environmental variables

Seagrasses in Bahía Potrero were found from a depth of 3.3 to 6.3 m (MSL). Secchi depth doubled from February 2016 (1.8 ± 0.5 m, n = 7) to March 2017 (4.1 ± 0.1 m, n = 4). Salinity was slightly lower in February 2016 (32 ± 0 ppt, n = 7) than March 2017 (35 ± 0 ppt, n = 6).

Nutrient concentrations in the water column were as follows: silicate (11.3 ± 2.1 μmol L^{-1}, n = 6); ammonium (2.4 ± 0.37 μmol L^{-1}, n = 6); nitrite (1.7 ± 0.13 μmol L^{-1}, n = 6); nitrate (1.6 ± 0.29 μmol L^{-1}, n = 6); phosphate (0.52 ± 0.03 μmol L^{-1}, n = 5). Phosphates were under the detection limit in one sample.

Sediment carbon content at Bahía Potrero was 1.6 ± 0.3%OC and 23.0 ± 7.9%IC (n = 15). Carbon content standardized to 10 cm sediment depth was 20.5 ± 5.1 Mg OC ha^{-1} and 295.1 ± 109.8 Mg IC ha^{-1}. Dry sediment bulk density was 1.3 ± 0.2 g mL^{-1}. Sediments were dominated by very fine sand (44 ± 12%), followed by fine sand (14 ± 12%), then coarse sand (10 ± 5%), very coarse sand (9 ± 5%), medium sand and silt clay (both 8 ± 3%), very fine pebbles (5 ± 2%), and pebbles (3 ± 2%). Sediment δ^{13}C was -21.2 ± 0.6‰ and δ^{15}N was 8.6 ± 0.4‰ (n = 7).

3.2. Seagrass presence at Bahía Potrero

Seagrasses were sighted at Bahía Potrero on four occasions between March 2015 and March 2017. Seagrasses were only present in one of the seven areas surveyed in Bahía Potrero in 2016 (Fig. 1). Seagrass cover in the field is considered to be low (Fig. 1). On March 2017, seagrasses were present in an area with a maximum length of 76 m perpendicular to the coast and a 51 m wide band parallel to the coast, for a total estimated area of 3876 m^2. Two seagrass species were found, *Halophila baillonis* and *Halodule beaudettei*. Species identification of *Halodule* was based on leaf tip morphology (Kuo and Den Hartog, 2001; Van Tussenbroek et al., 2010), exhibiting a longer median tooth typical of *H. beaudettei* (Fig. 1). While *H. baillonis* was found on all sightings, *H. beaudettei* was only found on February 2016. A dried herbarium sample of each species was deposited at the Herbario Nacional de Costa Rica (CR).

3.3. Seagrass morphometrics

From the 15 cores systematically sampled, seagrasses were only present in seven of the cores. Results on seagrass morphometrics are based on the seagrass cores and they are summarized in Table 1. Leaf area of *H. baillonis* in 2017 was twice the size of one shoot measured in 2016 (Table 1). Biomass above ground to below ground ratio was 0.9 ± 0.5. There was evidence of herbivory on 25% of leaves, with elliptical portions of the leaves missing (Fig. 1). Above ground biomass carbon content of *H. baillonis* was 33.4 ± 2.6%, and nitrogen was 2.9 ± 0.7% (n = 6). Below ground biomass carbon content was 27.6 ± 4.4% and nitrogen 1.1 ± 0.3% (n = 6). Above ground biomass δ^{13}C was -11.7 ± 0.8‰ and δ^{15}N 5.4 ± 0.8‰ (n = 6). Below ground biomass δ^{13}C was -11.7 ± 1.3‰ and δ^{15}N 5.3 ± 0.8‰ (n = 6).

4. Discussion

Seagrass presence in the ETP is scarce, with ephemeral or opportunistic seagrass species reported (*H. baillonis*, *H. beaudettei*, *H. wrightii*, and *R. maritima*) from a limited number of locations (Van Tussenbroek et al., 2010; Samper-Villarreal et al., 2018b). Seagrass presence has been more recently reported from the Pacific coasts of El Salvador (Ramírez et al., 2017), Nicaragua (Cortés-Núñez et al., 2012), and Costa Rica (Samper-Villarreal et al., 2014, 2018b). At Bahía Potrero we found

Table 1
Seagrass parameters at Bahía Potrero, northern Pacific coast of Costa Rica on February 2016 and March 2017, and those reported for Golfo Dulce on the southern Pacific coast of Costa Rica.

Species Component Subcomponent	Bahía Potrero (26.Feb.2016) (n)	Bahía Potrero (18-19.Mar.2017)[a]	Golfo Dulce
Halodule beaudettei			–
Leaves			–
Length (cm)	2.5 ± 1.5 (13)	–	–
Width (cm)	0.05 ± 0.01 (13)	–	–
Area (cm^2 $shoot^{-1}$)	0.2 ± 0.1 (7)	–	–
Foliar shoots			
Density (shoots m^{-2})	–	0.0 [b]	0.0 [b] 1,266.7 ± 905.5 [c]
Biomass			
Total	–	0.0 [b]	0.0 [b] 115.7 ± 63.8 [c]
Halophila baillonis			
Leaves			
Length (cm)	1.5 ± 0.03 (4)	1.1 ± 0.2	1.0 ± 0.4 [b]
Width (cm)	0.6 ± 0.01 (4)	0.5 ± 0.1	0.4 ± 0.1 [b]
Area (cm^2 $shoot^{-1}$)	3.6 (1)	1.4 ± 0.5	1.3 ± 0.8 [b]
Petiole length (cm)	0.18 ± 0.02 (4)	0.2 ± 0.1	–
Foliar shoots			
Stem height (cm)	1.3 (1)	1.4 ± 0.3	–
Density (shoots m^{-2})	–	569 ± 493	4,841 ± 3433 [b] 1,284 ± 534 [c]
Rhizomes and roots			
Root length (cm)	–	2.0 ± 0.5	–
Rhizome internode length (cm)	–	1.6 ± 0.4	–
Rhizome diameter (cm)	–	0.1 ± 0.0	–
Total rhizome length (m m^{-2})	–	22.3 ± 12.1	164 ± 73 [b]
Rhizome growing tips (GT m^{-2})	–	313 ± 253	1,631 ± 1042 [b]
Biomass			
AG biomass (g DW m^{-2})	–	1.9 ± 2.6	9.0 ± 6.8 [b]
Rhizome biomass (g DW m^{-2})	–	1.4 ± 1.2	7.4 ± 3.5 [b]
Root biomass (g DW m^{-2})	–	0.6 ± 0.5	14.4 ± 8.7 [b]
Total (g DW m^{-2})		3.9 ± 4.0	30.7 ± 16.3 [b] 36.0 ± 14.8 [c]

[a] n = 7 cores. AG = Above ground.
[b] Rincón de Osa (Samper-Villarreal et al., 2014).
[c] Playa Colibrí (Sarmento de Carvalho, 2013).

H. baillonis and *H. beaudettei*. The specimens of *H. beaudettei* reported here presented a typical acute median tooth, differing from *H. wrightii* (Kuo and Den Hartog, 2001; Van Tussenbroek et al., 2010). While in El Salvador only shoalgrass (*H. wrightii)* was reported (Ramírez et al., 2017) both *H. beaudettei* and *H. wrightii* are considered to be potential synonyms, as their taxonomy is based on leaf-tip morphology (Phillips, 1967; Kuo and Den Hartog, 2001; Van Tussenbroek et al., 2010). Until genetic studies are carried out to clarify the taxonomic standing of *Halodule* species the current taxonomic keys separate these species by leaf tip morphology (Kuo and Den Hartog, 2001; Van Tussenbroek et al., 2010). Further south on the Pacific coast of Costa Rica, both *H. baillonis* and *H. beaudettei* dominate the meadows in Golfo Dulce (Samper-Villarreal et al., 2014, 2018b) (Fig. 1). North of Bahía Potrero, at Bahía Culebra (Fig. 1), a meadow dominated by *R. maritima* with some *H. baillonis* disappeared in the mid-1990s following a severe storm (Cortés, 2001); we did not find *R. maritima* at Bahía Potrero.

The area with seagrasses at Bahía Potrero (~3870 m^2) is within the size range of meadows on the Pacific coast of Costa Rica (< 100–900,000 m^2) (Samper-Villarreal et al., 2018b). Seagrasses in Bahía Potrero were found from a depth of 3 to 6 m (MSL). Seagrasses at Golfo Dulce have been found slightly shallower at 2.5–4.5 m (MSL) (Sarmento de Carvalho, 2013; Samper-Villarreal et al., 2014). The depth at which seagrasses occur is most likely related to variations in light availability, which is closely linked to water quality, mainly suspended matter and chlorophyll concentrations. In Golfo Dulce, vertical Secchi depth measured during the rainy season was 3.8 ± 0.1 m (Samper-Villarreal et al., 2014); which is at the higher values measured at Bahía Potrero during the dry season of 2017. Further studies on the seasonal variability, the effect of water clarity and salinity on seagrass dynamics in the ETP are needed.

Seagrass sediment organic carbon content fell within worldwide mean values of 2.5 ± 0.1%OC (Fourqurean et al., 2012). Sediment inorganic carbon is higher than reported at other seagrass locations with a mean of 10.15 ± 0.04%IC (Campbell et al., 2015). Dry sediment bulk density fell within mean values for seagrasses, 1.03 ± 0.02 g DW mL^{-1} (Fourqurean et al., 2012). Seagrass biomass $\delta^{13}C$ was within known ranges for *Halophila* species (Hemminga and Mateo, 1996); while sediment $\delta^{13}C$ was more depleted, indicating potential allochthonous carbon sources (Samper-Villarreal et al., 2016; Reef et al., 2017). Biomass and sediment $\delta^{15}N$ was enriched (McClelland and Valiela, 1998; Samper-Villarreal et al., 2016) and similar to nutrient loaded mangroves and bivalves from Golfo Dulce (Samper-Villarreal et al., 2018a), despite relatively low nutrient concentrations in the water column compared to others on the Pacific coast of Costa Rica (Palter et al., 2007; Samper-Villarreal et al., 2018a). This indicates potential nutrient loading at this location which needs to be further studied.

Seagrass biomass, foliar shoot density, rhizome length and number of growing tips of *H. baillonis* were much lower at Bahía Potrero than those reported for Golfo Dulce (Table 1). Above ground to below ground ratio (AG:BG) of *H. baillonis* at Bahía Potrero was ~ 1:1; while in Golfo Dulce below ground biomass was much higher than above ground biomass (Samper-Villarreal et al., 2014). In contrast, *H. baillonis* leaf length, width and area were similar to Golfo Dulce (Table 1). Very limited abundance of *H. beaudettei* at Bahía Potrero coincides with seagrasses at one location in Golfo Dulce, though this species is abundant at another location within that gulf (Table 1). The factors leading to variations between Bahía Potrero and Golfo Dulce are yet to be identified and further research on the variability of seagrasses in the ETP is needed.

Grazers appear to be biting the leaves of *H. baillonis* at Bahía Potrero. This type of grazing evidence on *H. baillonis* leaves has also been observed in Golfo Dulce (Samper-Villarreal et al., 2014). The organisms that are consuming this species remain unknown, and may well include much larger organisms such as sea turtles which would consume the entire foliar shoot (Valentine and Duffy, 2006); with seagrass material previously found in sea turtle gut content in Golfo Dulce (Sarmento de Carvalho, 2013). Consumption by marine megaherbivores would lead to variations in shoot density and biomass that would not be identified by the methods used at Bahía Potrero.

Bahía Potrero seagrasses are not part of a marine protected area of the National System of Conservation Areas of Costa Rica (SINAC). *Halophila baillonis* is listed as Vulnerable in the IUCN Red List (Short et al., 2011). Given the disappearance in the 1990s of a seagrass meadow nearby (Cortés, 2001) and our limited understanding of the ecological dynamics of this critically important coastal habitat in the ETP, and applying the precautionary principle, we recommend protection and further research of this coastal habitat on the Pacific coast of Costa Rica.

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References

Campbell, J., Lacey, E., Decker, R., Crooks, S., Fourqurean, J., 2015. Carbon storage in seagrass beds of Abu Dhabi, United Arab Emirates. Estuaries Coasts 38, 242–251.
Cortés, J., 2001. Requiem for an eastern Pacific seagrass bed. Revista de Biología Tropical 49 (Suppl. 2), 273–278.
Cortés-Núñez, J., Breedy-Shadid, O., Sánchez-Noguera, C., Pacheco-Solano, C., 2012. Los Ecosistemas Marinos del Refugio de Vida Silvestre La Flor y del Corredor Biológico Paso del Istmo, Rivas, Nicaragua. Proyecto Investigación Marino-Costero: Asegurando las Bases Científicas y Educativas para la Protección y Manejo de Tortugas Marinas en los RVS La Flor y Río Escalante-Chococente. CIMAR, Paso Pacífico, DANIDA, Managua, Nicaragua.
Fourqurean, J.W., Duarte, C.M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M.A., Apostolaki, E.T., Kendrick, G.A., Krause-Jensen, D., McGlathery, K.J., 2012. Seagrass ecosystems as a globally significant carbon stock. Nat. Geosci. 5, 505–509.
Heiri, O., Lotter, A.F., Lemcke, G., 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. J. Paleolimnol. 25, 101–110.
Hemminga, M., Mateo, M., 1996. Stable carbon isotopes in seagrasses: variability in ratios and use in ecological studies. Mar. Ecol. Prog. Ser. 140, 285–298.
Howard, J., Hoyt, S., Isensee, K., Pidgeon, E., Telszewski, M. (Eds.), 2014. Coastal Blue Carbon: Methods for Assessing Carbon Stocks and Emissions Factors in Mangroves, Tidal Salt Marshes, and Seagrasses. Conservation International, Intergovernmental Oceanographic Commission of UNESCO, International Union for Conservation of Nature, Arlington, Virginia, USA.
Kuo, J., Den Hartog, C., 2001. Seagrass taxonomy and identification key. In: Short, F., Coles, R. (Eds.), Global Seagrass Research Methods. Elsevier Science B.V, The Netherlands, pp. 31–58.
McClelland, J.W., Valiela, I., 1998. Changes in food web structure under the influence of increased anthropogenic nitrogen inputs to estuaries. Mar. Ecol. Prog. Ser. 168, 259–271.
Nordlund, L.M., Koch, E.W., Barbier, E.B., Creed, J.C., 2016. Seagrass ecosystem services and their variability across genera and geographical regions. PLoS One 11, e0163091.
Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck Jr, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., 2006. A global crisis for seagrass ecosystems. Bioscience 56, 987–996.
Palter, J., León Coto, S., Ballestero, D., 2007. The distribution of nutrients, dissolved oxygen and chlorophyll a in the upper Gulf of Nicoya, Costa Rica, a tropical estuary. Revista de Biología Tropical 55, 427–436.
Phillips, R., 1967. On species of the seagrass, *Halodule*, in Florida. Bull. Mar. Sci. 17, 672–676.
Ramírez, E., Menjívar, J., Cerén, G., Rivera, A., Henríquez, A.V., Liles, M.J., 2017. Shoalgrass *Halodule wrightii* (Ascherson, 1868) meadows in El Salvador: distribution and associated invertebrates at the estuary complex of Bahía de Jiquilisco. Lat. Am. J. Aquat. Res. 45, 864–869.
Reef, R., Atwood, T.B., Samper-Villarreal, J., Adame, M.F., Sampayo, E.M., Lovelock, C.E., 2017. Using eDNA to determine the source of organic carbon in seagrass meadows. Limnol. Oceanogr. 62, 1254–1265.
Samper-Villarreal, J., Cortes, J., Benavides-Varela, C., 2012. Description of the Panamá and Iguanita mangrove stands of Bahía Culebra, North Pacific coast of Costa Rica. Revista de Biología Tropical 60 (Suppl. 2), 109–120.
Samper-Villarreal, J., Bourg, A., Sibaja-Cordero, J.A., Cortés, J., 2014. Presence of a *Halophila baillonii*Asch. (Hydrocharitaceae) seagrass meadow and associated macrofauna on the Pacific Coast of Costa Rica. Pac. Sci. 68, 435–444.
Samper-Villarreal, J., Cortés, J., Polunin, N.V., 2018a. Isotopic evidence of subtle nutrient enrichment in mangrove habitats of Golfo Dulce, Costa Rica. Hydrol. Process. 32, 1956–1964.
Samper-Villarreal, J., Van Tussenbroek, B.I., Cortés, J., 2018b. Seagrasses of Costa Rica: from the mighty Caribbean to the dynamic meadows of the Eastern Tropical Pacific. Rev. Biol. Trop. 66 (Suppl. 1), S53–S65.
Samper-Villarreal, J., Lovelock, C.E., Saunders, M.I., Roelfsema, C., Mumby, P.J., 2016. Organic carbon in seagrass sediments is influenced by seagrass canopy complexity, turbidity, wave height, and water depth. Limnol. Oceanogr. 61, 938–952.
Sarmento de Carvalho, M., 2013. Análisis Descriptivo de las Condiciones Ambientales y Estado de Salud de la Tortuga Negra (*Chelonia mydas agassizii*) en un Sitio de Forrajeo en el Golfo Dulce, Costa Rica. Instituto Internacional en Conservación y Manejo de Vida Silvestre. Universidad Nacional, Heredia, Costa Rica.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671.
Short, F.T., Carruthers, T., Dennison, W., Waycott, M., 2007. Global seagrass distribution and diversity: a bioregional model. J. Exp. Mar. Biol. Ecol. 350, 3–20.
Short, F., Polidoro, B., Livingstone, S.R., Carpenter, K.E., Bandeira, S., Bujang, J.S., Calumpong, H.P., Carruthers, T.J., Coles, R.G., Dennison, W.C., Erftemeijer, P.L., Miguel, D.F., Freeman, A.S., Jagtap, T., Kamal, A.H.M., Kendrick, G.A., Kenworthy, W.J., La Nafie, Y.A., Nasution, I.M., Orth, R.J., Prathep, A., Sanciangco, J.C., van Tussenbroek, B., Vergara, S.G., Waycott, M., Zieman, J.C., 2011. Extinction risk assessment of the world's seagrass species. Biol. Conserv. 144, 1961–1971.
Strickland, J.D., Parsons, T.R., 1972. A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada, Ottawa, Canada.
Valentine, J.F., Duffy, J.E., 2006. The central role of grazing in seagrass ecology. In: Larkum, A., Orth, R., Duarte, C. (Eds.), Seagrasses: Biology, Ecology and Conservation. Springer, The Netherlands, pp. 463–501.
Van Tussenbroek, B., Santos, M.G.B., Wong, J.G.R., Van Dijk, J.K., Waycott, M., 2010. A Guide to the Tropical Seagrasses of the Western Atlantic. Universidad Nacional Autónoma de México, México D.F.
Waycott, M., Longstaff, B.J., Mellors, J., 2005. Seagrass population dynamics and water quality in the Great Barrier Reef region: a review and future research directions. Mar. Pollut. Bull. 51, 343–350.