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Effect of temperature and salinity on the seagrass *Halophila baillonii* (Hydrocharitaceae) under aquarium conditions

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ABSTRACT

Introduction: *Halophila baillonii*, also known as “clover grass”, is a rare seagrass species found in tropical waters off the American continent. This is a small and ephemeral species classified as Vulnerable in the IUCN Red List.
Objective: To determine how variations in temperature and salinity affect this seagrass.
Methods: *H. baillonii* was collected in the southern Pacific coast of Costa Rica either by hand or with a corer (8 cm diameter). Two experiments with three treatments each were carried out in aquaria. Each treatment was applied to three aquaria, for a total of nine aquaria per experiment. The temperature treatments consisted of 23 °C (Low), 28 °C (Control), 33 °C (High), with a constant salinity of 25 over 51 days. Salinity treatments were 15 (Low), 25 (Control), 35 (High) with a constant minimum temperature of 28 °C over 31 days. Five plant performance parameters were measured: 1) foliar shoot survival; 2) increase in the number of foliar shoots; 3) horizontal rhizome elongation; 4) rhizome internodal length; and 5) leaf area.
Results: *H. baillonii* survival rates were higher when collected manually rather than using a corer. All plant performance parameters were higher at 28 °C temperature (control). In contrast, variables of plant performance were similar in all salinity treatments, except that the seagrass presented smaller leaves at higher salinities. Female flowers were found towards the end of the experiments, being the first report of flowering of this species under aquaria conditions.
Conclusion: *H. baillonii* has a wide salinity tolerance, thus enabling plant survival during dry or rainy seasons. In contrast, *H. baillonii* appears to be more sensitive to lower and higher temperatures than 28 ºC. This is the first study reporting the response of this threatened species to experimentally induced fluctuations of temperature and salinity.

Key words: Eastern Tropical Pacific; Golfo Dulce; climate change; environmental factors; seagrass condition.

RESUMEN

Efecto de la temperatura y salinidad sobre el pasto marino *Halophila baillonii* en condiciones de acuario

Introducción: *Halophila baillonii*, también conocido como “pasto de trébol”, es una especie poco común de pasto marino que se encuentra en aguas tropicales del continente americano. Esta es una especie pequeña y efímera clasificada como Vulnerable en la Lista Roja de la UICN.
Objetivo: Determinar cómo las variaciones en la temperatura y salinidad afectan a este pasto marino.

Métodos: Se colectó *H. baillonii* en el sur de la costa Pacífica de Costa Rica por medio de colecta manual o con nucleadores (8 cm diámetro). Se realizaron dos experimentos con tres tratamientos cada uno en acuarios. Cada tratamiento se aplicó a tres acuarios, para un total de nueve acuarios por experimento. Los tratamientos en el experimento de temperatura fueron 23 °C (Bajo), 28 °C (Control), 33 °C (Alto), con una salinidad constante de 25 durante 51 días. Los tratamientos del experimento de salinidad fueron 15 (Bajo), 25 (Control), 35 (Alto) con una temperatura mínima constante de 28 °C durante 31 días. Se midieron cinco parámetros del rendimiento de las plantas: 1) supervivencia de los haces foliares; 2) incremento en el número de haces foliares; 3) elongación del rizoma horizontal; 4) longitud internodal del rizoma; y 5) área foliar.

Resultados: Las tasas de supervivencia de *H. baillonii* fueron mayores cuando fueron colectadas manualmente en lugar de colectadas con un nucleador. El rendimiento de las plantas fue mejor en condiciones de 28 °C temperatura (control). En contraste, no hubo variación en el rendimiento de la planta en los tratamientos de salinidad, excepto por hojas más pequeñas en el pasto a mayores salinidades. Se encontraron flores femeninas en los acuarios hacia el final de los experimentos, siendo este el primer reporte de floración para esta especie en condiciones de acuario.

Conclusión: *H. baillonii* tuvo una amplia tolerancia a la salinidad, así permitiéndole a la planta sobrevivir tanto en la época seca como lluviosa. En contraste, *H. baillonii* parece ser más sensible a temperaturas menores o mayores a 28 °C. Este es el primer estudio que reporta la respuesta de esta especie amenazada a fluctuaciones en temperatura y salinidad en condiciones experimentales.

Palabras clave: Pacífico Tropical Oriental; Golfo Dulce; cambio climático; factores ambientales; condición de pastos marinos.

INTRODUCTION

Seagrasses are flowering plants that can live and reproduce while submerged in brackish and marine water. The “clover grass”, *Halophila baillonii* Asch. is a small seagrass species and its foliar shoot is composed of a cluster of approximately four leaves on a vertical shoot (van Tussenbroek et al., 2010). This seagrass is dioecious, with female or male flowers found in the centre of the leaf cluster (van Tussenbroek et al., 2010). In the IUCN Red List, *H. baillonii* is listed as a Vulnerable seagrass species (Short et al., 2010). Overall, not much is known about the relative abundance of *H. baillonii*. It is mostly considered to be a rare species in much of its range, mostly forming fragmented populations in the Caribbean Sea and Atlantic, with few populations in the Eastern Tropical Pacific (ETP) (Magalhães et al., 2025; Samper-Villarreal et al., 2018b; Samper-Villarreal, 2024; Short et al., 2010).

On the Pacific coast of Costa Rica, *H. baillonii* was previously found in Bahía Culebra. However, that meadow disappeared after a severe storm in 1996 (Cortés, 2001). Following its disappearance there were no reports of seagrass presence on the Pacific coast of Costa Rica until 2010, when a monospecific meadow of *H. baillonii* was found in Golfo Dulce, on the Southern Pacific coast of Costa Rica (Samper-Villarreal et al., 2014). Soon after, in 2011, a larger seagrass meadow containing *H. baillonii* was found nearby within Golfo Dulce (Sarmento de Carvalho, 2013). More recently, *H. baillonii* was found on the Pacific coast of Costa Rica at Bahía Potrero (Samper-Villarreal et al., 2018a), El Jobo and Matapalito (Samper-Villarreal et al., 2020), and Sámara (Samper-Villarreal et al., 2022). The biggest meadow including this species on the Pacific coast of Costa Rica is found at Playa Colibrí, Golfo Dulce (Samper-Villarreal et al., 2018b). Water temperature and salinity at Playa Colibri vary over time. Furthermore, seagrasses in this meadow show temporal variability of density and spatial distribution linked to changes in environmental conditions (Barquero Chanto, 2018).

Our understanding of seagrasses in general in the ETP is limited (Samper-Villarreal, 2024). Information on the ecology, reproduction, physiology and population dynamics of *H. baillonii* in the ETP and within its distribution range is scarce (Samper-Villarreal, 2024; Samper-Villarreal et al., 2018b, Short et al., 2010). Therefore, enhancing knowledge of seagrasses in the ETP, particularly *H. baillonii*, an understudied yet critical species, is essential for advancing seagrass management and conservation efforts. Here, we assessed the effect of temperature and salinity fluctuations on *H. baillonii* plant performance under controlled aquarium conditions. We hypothesized that *H. baillonii* would have optimal growth under temperature and salinity conditions similar to those found in the field.

MATERIALS AND METHODS

Field collection site: Samples for this experiment were collected at Playa Colibrí in Golfo Dulce, on the southern Pacific coast of Costa Rica (8° 40’ 12.43” N, 83° 26’ 35. 27” W). The sample collection site was at a meadow within the gulf, located at ~100 m from the coastline and ~1 m depth at low tide. The Pacific coast of Costa Rica has a dry season (December-April) and a rainy season (May-November). Samples were collected at the beginning of the dry season (January and February).

Sample collection: Samples were collected on two separate dates, January 27th and February 13th of 2020, using two different sampling methods: (1) a corer and (2) manual collection. Cores were collected using an 8 cm diameter PVC corer inserted into the sediment to a depth of 5 cm to extract the plants and associated sediment intact. The cores were placed in plastic containers and filled with seawater from the site. Manual collection entailed carefully extracting the seagrass plants with roots and rhizomes attached by hand and gently rinsing them free of associated sediment while making sure the rhizome contained an apical meristem. Rhizome sections collected with either method had a minimum of one and a maximum of ten foliar shoots. Manually collected samples were stored inside a sealed zip lock bag with paper towel dampened with sea water from the site to maintain humidity levels. Floral buds were not seen in the foliar shoots at the time of collection. Samples were transported inside a portable cooler without additional temperature controls to the facilities of CIMAR, University of Costa Rica, San José.

Experimental setup: Each aquarium (39 cm long, 19 cm wide, and 20 cm high) was filled with 10 L of artificial seawater (Red Sea Salt), and when necessary, reverse Osmosis De-Ionized (RO/DI) water was added to compensate increases in salinity from evaporation. The water was recirculated without blowing additional bubbles into the aquarium with a submerged 2W water pump (Aquatic Pond). A thermostat with heater (Dophin Heater 50 W) was used to maintain the water at a constant temperature (Fig. 1). Every two weeks, 10 % of the water was replaced with new artificial seawater, making sure the salinity was consistent due to evaporation.

The aquaria for the temperature experiment were filled with ~3 cm height of natural sediment previously collected from seagrass meadows from both coasts of Costa Rica, cleaned with water and NaClO to sterilize the sediment, and finally dried and sieved through a 2 mm sieve to exclude any potential remnants of macro-invertebrates, shells, and stones. For the salinity experiment, the sediment height in the aquaria was ~3 cm and consisted of unprocessed sediment collected at the same time and site as the seagrass samples. There were nine aquaria in total for each experiment, and one core-collected and one manually-collected seagrass sample was planted in each aquarium using randomly generated numbers.

During the acclimation and experimental period, aquaria were monitored daily, measuring salinity and temperature manually using a portable ACT refractometer and a digital aquarium thermometer. Additionally, a HOBO data logger was introduced randomly in one aquarium per treatment, logging temperature at 30-minute intervals for at least one week in each experiment. Water pH was measured using commercially available water alkalinity strips for aquaria and remained between 8.5 and 9.0 during the experiments.

Experimental treatments: Average ambient conditions at the sampling site were

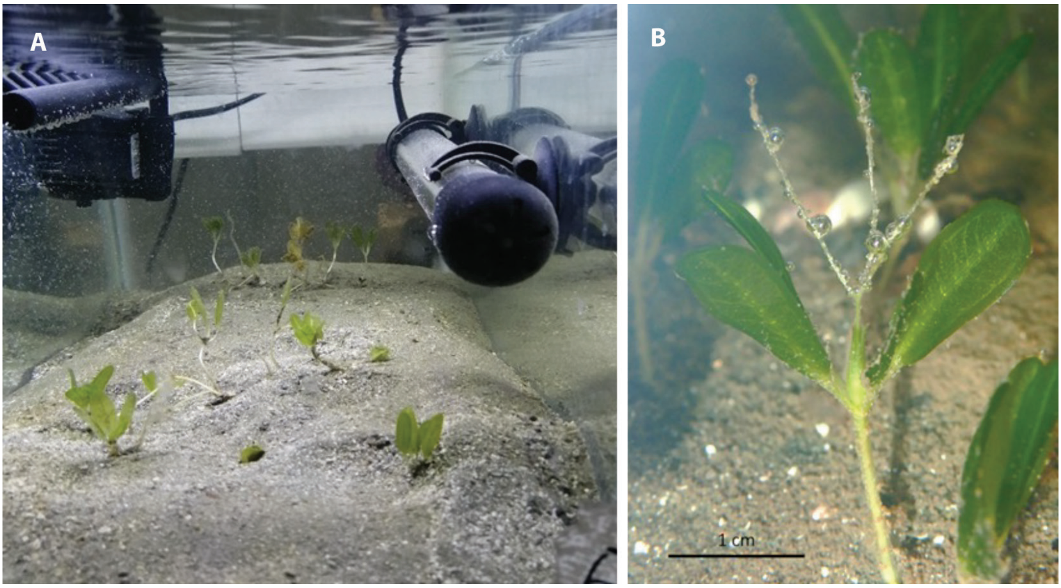

Fig. 1. *Halophila baillonii* under aquarium conditions. **A)** Experimental set up. **B)** Female flower at the center of the leaf pseudo whorl of a foliar shoot.

25 salinity and 28 °C temperature, measured as part of a long-term seagrass monitoring program at Colibrí based on four samplings per year over the two years prior to sample collection (Samper-Villarreal et al., *In prep*). These average ambient conditions were therefore selected as the control values for each of the experiments. A one-year monthly study, between March 2016 and March 2017, at a site within this meadow found that water temperature was highest in July (31.6 ± 1.1 ºC) and lowest in October (27.1 ± 0.05 ºC) (Barquero Chanto, 2018). As maximum temperatures reported in the study were ~ 5 units above the average, temperature treatment variation was selected as ± 5 ºC compared to the control. In the monthly study, salinity was highest in April (33.8 ± 0.4) and lowest in October (19.6 ± 1.4) (Barquero Chanto, 2018). As maximum salinities were almost 10 units above average conditions at the sampling site the salinity treatment range was selected to be ± 10.

Temperature treatment: Aquaria were set up indoors applying an artificial LED light of 1600 lumens (LED- MS60 16W) set to 12:12 h light:dark cycle with air temperature at 23°C and salinity at 25. Seagrasses were planted in each of the nine aquaria and acclimated for eight days under control conditions. Conditions in each aquarium were modified according to each treatment on day one of the experiment. The experimental setup consisted of three temperature treatments (T): *Control* (CT 28 °C), *Low* (LT 23 °C), and *High* (HT 33 °C). Thermostats with heaters were used to increase water temperature for treatments requiring temperatures higher than 23 °C. Plants were exposed to the treatments for 51 days. Temperature and salinity variations between treatments during the experiment is presented in Table 1.

Salinity treatment: Aquaria were set up in a roofed area outdoors with natural sunlight and a global radiation of 2 MJ m^{-2} (Instituto Meteorológico Nacional, 2020). Seagrasses were planted in each of the nine aquaria and acclimated for 10 days under control conditions. Conditions in each aquarium were modified for each treatment on the first day of the experimental time period. The experimental setup consisted of three salinity treatments (S):

Table 1
Water temperature and salinity conditions during experiments (nine aquaria per experiment, three aquaria per treatment) and number of leaves at the end of the experiments per *Halophila baillonii* foliar shoot.

Experiment	Temperature (°C) mean ± SD	Salinity mean ± SD	Number of leaves per shoot mean ± SD (n)
Temperature			
Low (LT)	24.2 ± 1.1	28.9 ± 1.1	4 ± 0 (9)
Control (CT)	28.5 ± 0.8	28.7 ± 1.0	2 ± 0 (3)
High (HT)	32.9 ± 1.4	28.9 ± 1.1	3 ± 1 (9)
Salinity			
Low (LS)	25.4 ± 2.7	16.8 ± 2.0	4 ± 1 (6)
Control (CS)	25.9 ± 1.6	25.1 ± 1.6	3 ± 1 (6)
High (HS)	26.2 ± 2.7	34.2 ± 2.7	4 ± 0 (11)

Control (CS 25), *Low* (LS 15), and *High* (HS 35). Water temperature conditions were maintained at a minimum of 28 °C with thermostat heaters. Plants were exposed to treatments for a total of 31 days.

Seagrass response: Plant performance was monitored five times a week. The plant performance parameters were: 1) foliar shoot survival; 2) increase in the number of foliar shoots; 3) horizontal rhizome elongation; 4) rhizome internodal length (length of rhizome between two consecutive foliar shoots); and 5) leaf area. Additionally, the number of leaves per foliar shoot were counted for leaf area samples and the number of flowering shoots in each aquarium noted if present. Foliar shoots were considered alive from the moment they began to emerge from the sediment with pale green leaves until the leaves lost their darker green color, indicating leaf senescence. Foliar shoot increase was the number of shoots that were alive at the end of the experiment minus the number of shoots that were alive at the beginning of the experiment.

Rhizome elongation (mm week^{-1}) and internodal length (cm) were estimated by taking subsequent perpendicular and scaled photographs of foliar shoots of each plant. The image analysis software ImageJ (Schneider et al., 2012) was used to estimate rhizome length between foliar shoots on subsequent days. A negative rhizome elongation refers to the change between subsequent days caused by the death of the rhizome tissue previously present and not compensated by growth of new rhizome in that time period. Leaf area (cm^2) was measured once the experiments were finalized. Three shoots were randomly selected and photographed perpendicularly flat over a white surface with the leaves separated and a scale added. Length, width and area were measured for each leaf using ImageJ and the surface area (cm^2) per shoot was calculated.

Data analysis: The effect of salinity and temperature on plant performance was analyzed using one-way analysis of variance (ANOVA). When differences among treatments were found, post hoc Tukey HSD was used to identify variation between treatments. Normality of the data was tested with the Kolmogrov-Smirnov test and homogeneity was tested with Bartlett. When data did not show normality and transformation of data was not possible the Wilcoxon non-parametric test was used. All statistical analyses were done using R v.3.5.1. (R Core Team, 2020).

RESULTS

Collection method: During the experiments, hand-collected *H. baillonii* had higher shoot survival rates (55 %) than those collected with a corer extraction method (22 %) (Wilcoxon test, P = 0.02).

Temperature experiment plant performance: Rhizome elongation varied among temperature treatments (ANOVA, $F_{2, 80} = 70.2$, $P < 0.001$) and was highest in the CT treatment (Fig. 2). Rhizome internodal length also showed differences among treatments. Plants at 33 °C (HT) had shorter internodal lengths (4.2 mm) compared those at 28 °C (CT 15.3 mm) and 23 °C (LT, 16.4 mm) (ANOVA, $F_{2, 24} = 7.2$, $P < 0.05$). Foliar shoot increase (Fig. 2) also varied among treatments (ANOVA, $F_{2, 7} = 5.3$, $P < 0.05$). Shoots increased in 50 % of the rhizomes sampled in CT and LT, while they only increased in 17 % of the HT rhizomes. CT showed the highest increase in the number of shoots, while HT showed the least shoot increase. Similarly, surface area per leaf varied among treatments (ANOVA, $F_{2, 66} = 46.9$, $P < 0.05$). CT had a higher leaf surface area, with 0.7 ± 0.2 cm^2 compared to 0.4 ± 0.1 cm^2 in HT and 0.4 ± 0.09 cm^2 in LT. There were also differences among treatments for leaf length ($F_{2, 66} = 26.4$, $P < 0.05$) and width ($F_{2, 66} = 57.6$, $P < 0.05$). For both leaf length and width, controls differed from the rest of the treatments, while HT and LT had no significant differences between them. Thus, both leaf length and width were larger at 28 °C (CT) than at 23 °C (LT) or 33 °C (HT). Number of leaves per foliar shoot varied among temperature treatments (Wilcoxon test, $P < 0.001$). There were less leaves per shoot in HT than CT yet the number of shoots analyzed per treatment was imbalanced due to very low number of HT shoots available (Table 1).

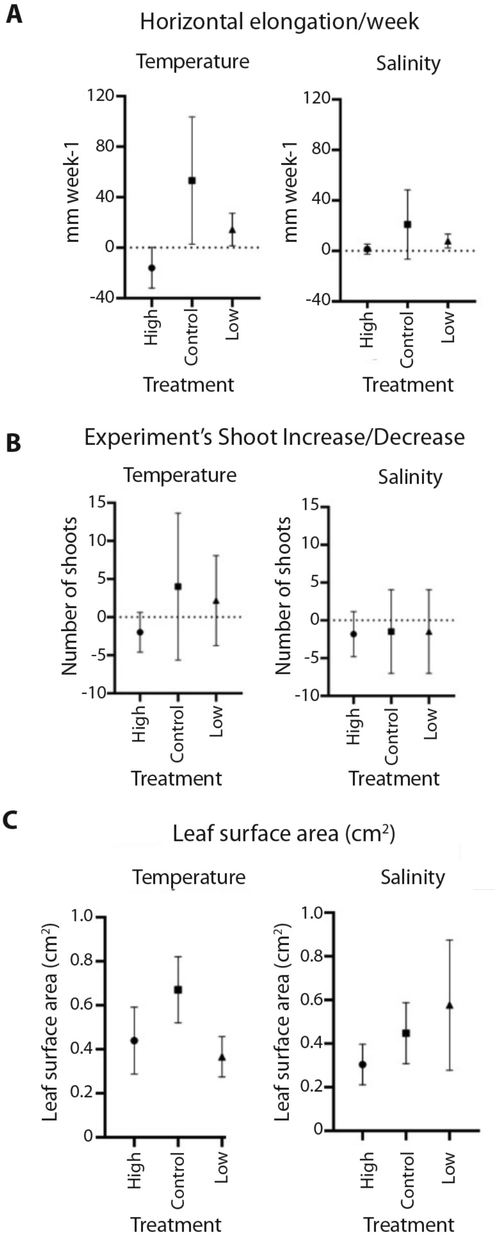

Fig. 2. *Halophila baillonii* plant performance (mean ± standard deviation, n = 3) per temperature and salinity treatment **a)** Horizontal rhizome elongation (mm $week^{-1}$). **b)** Foliar shoot increase/decrease. **c)** Leaf surface area (cm^2). Note: Refer to Table 1 for temperature and salinity values for each treatment.

Salinity experiment: There were no differences among salinity treatments in regards to rhizome elongation (ANOVA, $F_{2, 54} = 1.8$, $P = 0.2$), internodal length (ANOVA, $F_{2, 32} = 1.2$, $P = 0.3$) or number of foliar shoots (ANOVA, $F_{2, 15} = 0.01$, $P = 1$; Fig. 2). In contrast, there was variation among treatments in regards to leaf surface area (ANOVA, $F_{2, 86} = 7.1$, $P < 0.05$; Fig. 2), leaf length ($F_{2,86} = 6.2$, $P < 0.05$), and width ($F_{2,86} = 15.3$, $P < 0.05$). Leaf surface area was 0.4 ± 0.1 cm^2 in the control salinity treatment (CS 25), 0.5 ± 0.3 cm^2 in the low salinity treatment (LS 15), and 0.3 ± 0.1 cm^2 in the high salinity treatment (HS 35). For both length and width, there were differences between HS and the rest of the treatments, while CS and LS did not differ between them. This indicates that leaf length and width were smaller at higher salinities; while leaf length and width were similar at control and lower. There was no variation in the number of leaves per foliar shoots among treatments (ANOVA, $F_{2, 21} = 1.9$, $P = 0.2$; Table 1)

Flowering: It was noted that at the end of the experiment when plants were placed again at control temperature (28°C) and salinity (25), 50 % of the *H. baillonii* foliar shoots produced flowers. Only female flowers were seen within the aquaria (Fig. 1). No flowers were found during the acclimation periods or when plants were exposed to experimental treatment conditions in either of the experiments.

DISCUSSION

This is the first study reporting the response of the threatened species *H. baillonii* to experimentally induced fluctuations of temperature and salinity. We report that this species has a wide salinity tolerance while it appears to be more sensitive to temperatures below or over 28 °C. Other species of the *Halophila* genus have been grown under controlled aquaria conditions, studying, for example, the flowering, thermal tolerance, and simulated climate change conditions for *Halophila stipulacea* (Forssk.) Asch. (McMillan, 1980; Moses & Fredrick, 2017; Nguyen et al., 2020; Wesselmann et al., 2020). Furthermore, temperature, pH, hyposalinity stress, and salinity reduction has been studied for *Halophila johnsonii* Eiseman (Gavin & Durako, 2012; Griffin & Durako, 2012; Torquemada et al., 2005). Likewise, light deprivation, substrate, salinity, light, and CO_2 enrichment effects in *Halophila ovalis* (R. Br.) Hook. have been previously assessed (Bujang et al., 2008; Longstaff et al., 1999; Sidik et al., 2010; Wong, 2016). *Halophila decipiens* Ostenf. was also grown in experimental conditions, discovering it is the first seagrass known to need exogenous vitamins (Bird et al., 1998) and the role of light and salinity on seed germination for this species (McMillan, 1988). The effect of light on seed germination was also studied for *Halophila engelmannii* Asch., as well as its high salinity tolerance and flowering (McMillan, 1976; McMillan, 1987; McMillan & Moseley, 1967). To the best of our knowledge this constitutes the first experiment under aquarium conditions and to report flowering in aquaria for *H. baillonii*.

Plants collected by hand had a higher survival rate than those collected with a corer in our study. This finding was unexpected, because of the potential alteration to root and rhizomes and their microbiome in manually collected samples versus intact ones in cores (Wang et al., 2021). The lower survival of core samples is not likely due to a lower number of apical meristems of the rhizomes in corer samples. A previous study at a nearby site within Golfo Dulce, which collected samples using corers of the same diameter (5 cm) as the ones used in this study, reported rhizome growing tip densities of 1 630 m^2, there were also on average 10 rhizome sections per core, half of which had foliar shoots, and one foliar shoot per rhizome section on average (Samper-Villarreal et al., 2014). Thereby, it is assumed that both cored and manually collected samples had growing rhizome tips and a similar number of shoots. Potentially, the transportation method could have played a role in the sample survival as manually collected samples were stored in plastic bags with a wet tissue while cored samples were transported covered with water. However, the reason for differences in sampling technique survival remain unclear at this time and further study on the most effective sampling and transport methods for this species is needed particularly given implications for potential restoration initiatives.

Treatment values were selected based on the variation between average and maximum temperatures and salinities reported at the site prior to the beginning of the experiment (Barquero Chanto, 2018; Samper-Villarreal et al., *In Prep*). The lower treatments (15 salinity and 23 °C temperature) fell slightly below reported values at the time and to date (19 salinity and 26 °C temperature Samper-Villarreal, 2024). *H. baillonii* plants performed best at the CT temperature treatment (28 ºC) compared to the other treatments. Nonetheless, plants still survived at LT (23 ºC) and HT (33 ºC), indicating a certain tolerance for higher temperatures, although its performance was poor. Seagrasses can tolerate temperature changes (Thorhaug et al., 1978) yet their tolerance to high temperature for long periods is less studied for tropical seagrasses (Campbell et al., 2006). Additionally, short-term exposures at seawater temperatures as high as 40°C could cause irreparable effects and damage seagrass physiology (Campbell et al., 2006). *Halophila stipulacea* was able to perform over a wide range of temperatures (8 – 38 ºC) (Wesselmann et al., 2020), similar to other tropical seagrasses (Campbell et al., 2006). Other species such as *Halophila ovalis, Zostera capricorni* Asch., and *Syringodium isoetifolium* (Asch.) Dandy showed low tolerance for short-period thermal stress (1 – 4 h at 35 – 45 °C) (Campbell et al., 2006). Temperature and salinity tolerance thresholds at small incremental intervals in experimental conditions for this species should be identified and further understanding of environmental conditions in the field for this species is needed.

Plant performance of *H. baillonii* appeared to be mostly unaffected by salinity fluctuations. Even so, the aquarium experiments conducted here suggest that *H. baillonii* tolerates the low salinities used better than it does the higher salinity conditions it was exposed to in the experiment. The high salinity treatment (HS 35) plants were exposed to in this experiment was 10 units above average salinity at the site (CS 25); however, it is important to keep in mind that average sea water salinity is roughly 35. *H. baillonii* was been reported in salinities of 40.6 ± 1.5 in semiarid conditions in Brazil (Barros et al., 2014), yet most seagrasses are thought to be more sensitive to hypersaline conditions (Adams & Bate, 1994; Biebl & McRoy, 1971; Doering & Chamberlain, 1999; Kamermans et al., 1999; Ogata & Matsui, 1965; Van Katwijk et al., 1999). Diminished performance for other seagrass species has also been described for hypo- and hyper salinities (Kamermans et al., 1999; McMillan & Moseley, 1967; Walker, 1985; Walker & McComb, 1990). *H. baillonii* is also found naturally in salinities of 25.1 during the rainy season and up to 32.8 in the dry season in Honduras (Carrasco & Caviedes, 2013; Carrasco & Caviedes, 2015).

In this study, the response of *H. baillonii* to temperature and salinity fluctuations was only studied during a short time (51 and 31 days respectively) and under experimental aquaria conditions. Therefore, *H. baillonii* response in the field may vary and further studies are needed. The plant's performance varied between the two experiments. This may be due to potential differences in light intensity between both experiments, as the temperature experiment had consistent artificial lighting while the salinity experiment had natural fluctuating lighting.

This experimental aquarium study provides a first estimate of horizontal elongation rate for this species to the best of our knowledge. The highest average horizontal rhizome elongation for *H. baillonii* in this aquarium study was 137 cm year^{-1}. This appears to be within the range of horizontal elongation rates reported for other *Halophila* species. *H. ovalis* has the highest horizontal elongation rate for the genera growing 356 cm year^{-1}. *H. decipiens* has a horizontal elongation of 215 cm year^{-1} while *Halophila hawaiiana* Doty & Stone rhizomes grow 89 cm year^{-1} (Marbà & Duarte, 1998). At optimal control conditions, the internodal length of *H. baillonii* in this study was 15.3 ± 6.2 mm. Other *Halophila* species were found to have an internodal length between 10 and 17 mm from samples collected in their natural habitat (Marbà & Duarte, 1998), comparable to the ones in this study.

During the experiments, only female flowers were found in this aquarium study. Recent studies have indicated that *H. baillonii* may be a recent introduction into the Eastern Tropical Pacific (Van Dijk et al., 2023). On the Pacific coast of Costa Rica, only < 1 % of 1 300 shoots had flowers and all flowers found were female (Samper-Villarreal 2025). It is thought that *H. baillonii* may be solely propagating by clonal growth on the Pacific coast of Costa Rica. This could explain why 100 % of our samples that flowered were female.

This study reports the successful culture of *H. baillonii* in aquaria under controlled conditions and may help understand how the plant will behave in the future, such as under increased sea water temperatures and variations in freshwater input linked to climate change. The experimental findings from this study forewarn a potential decline of *H. baillonii* in Golfo Dulce if marked fluctuations of water temperature and salinity occurred in the future, with the addition of the exceptional conditions of Golfo Dulce as a tropical fjord (Morales-Ramírez et al., 2015). *Halophila baillonii* is an important food source for manatees (*Trichechus manatus* L.) in Belize (Ramos et al., 2024; Short et al., 2006). In Golfo Dulce it has been reported as a key food source for the green sea turtle (*Chelonia mydas* L.) and parrotfish (Bessesen & Guido, 2012; Samper-Villarreal & Cortés, 2020). Thereby, negative impacts on seagrass meadows in Golfo Dulce's ecosystem may also impact other marine life.

Ethical statement: the authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

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REFERENCES

Adams, J. B., & Bate, G. C. (1994). The ecological implications of tolerance to salinity by *Ruppia cirrhosa* (Petagna) Grande and *Zostera capensis* Setchell. *Botanica Marina*, *37*, 449–456. https://doi.org/10.1515/botm.1994.37.5.449

Barros, K., Costa, F., & Rocha-Barreira, C. (2014). A *Halophila baillonis* Ascherson bed on the semiarid coast of Brazil. *Feddes Repertorium*, *125*, 93–97. https://doi.org/10.1002/fedr.201400033

Barquero Chanto, J. E. (2018). *Caracterización y recomendaciones de manejo de una pradera de pastos marinos en playa Colibrí, Golfo Dulce, Costa Rica* [Tesis de maestría]. Universidad Nacional de Costa Rica, Costa Rica.

Bessesen, B. L., & Guido, S. R. (2012). Tropical fiord habitat as a year-round resting, breeding, and feeding ground for East Pacific green sea turtles (*Chelonia mydas*) off Costa Rica. *Herpetogical Review*, *43*(4), 539–541.

Biebl, R., & McRoy, C. P. (1971). Plasmatic resistance and rate of respiration and photosynthesis of *Zostera marina* at different salinities and temperatures. *Marine Biology*, *8*, 48–56. https://doi.org/10.1007/BF00349344

Bird, K. T., Johnson, J. R., & Jewett-Smith, J. (1998). In vitro culture of the seagrass *Halophila decipiens*. *Aquatic Botany*, *60*(4), 377–387. https://doi.org/10.1016/S0304-3770(97)00093-4

Bujang, J. S., Huat, L. L., Zakaria, M. H., Arshad, A., & Ogawa, H. (2008). Laboratory culture of the seagrass *Halophila ovalis* (R. Br.) Hooker f. *Marine Research in Indonesia*, *33*(1), 1–6. https://doi.org/10.14203/mri.v33i1.500

Campbell, S. J., McKenzie, L. J., & Kerville, S. P. (2006). Photosynthetic responses of seven tropical seagrasses to elevated seawater temperature. *Journal of Experimental Marine Biology and Ecology*, *330*(2), 455–468. https://doi.org/10.1016/j.jembe.2005.09.017

Carrasco, J. C., & Caviedes, V. (2013). *Ecología del sistema fluvio lagunar Chachaguala: énfasis en la diversidad y distribución de las comunidades de peces a escala espacial. Omoa, Honduras. Parque Nacional Cuyamel Omoa* [Reporte técnico]. Fundación para la Investigación y la Conservación de los Ecosistemas y la Biodiversidad, Honduras.

Carrasco, J. C., & Caviedes, V. (2015). *Taxonomía, ecología y estructura de las comunidades de peces de la Laguna de Chachaguala, Omoa, Honduras: con énfasis en pesquerías* [Reporte técnico]. Federación Nacional de Pescadores Artesanales de Honduras (FENAPESCAH) y Cuerpos de Conservación de Omoa (CCO).

Cortés, J. (2001). Requiem for an eastern Pacific seagrass bed. *Revista de Biología Tropical*, *49*(Suppl. 2), 273–278. https://doi.org/10.15517/rbt.v49i2.26334

Doering, P., & Chamberlain, R. (1999). Experimental studies on the salinity tolerance of Turtle Grass, *Thalassia testudinum*. In S. A. Bortone (Ed.), *Seagrasses: Monitoring, Ecology, Physiology and Management* (pp 99–106). CRC Press. https://doi.org/10.1201/9781420074475.ch6

Gavin, N. M., & Durako, M. J. (2012). Localization and antioxidant capacity of flavonoids in *Halophila johnsonii* in response to experimental light and salinity variation. *Journal of Expxperimental Marine Biology and Ecology*, *416*, 32–40. https://doi.org/10.1016/j.jembe.2012.02.006

Griffin, N. E. & Durako, M. J. (2012). The effect of pulsed versus gradual salinity reduction on the physiology and survival of *Halophila johnsonii* Eiseman. *Marine Biology*, *159*, 1439–1447. https://doi.org/10.1007/s00227-012-1923-8

Instituto Meteorológico Nacional. (2020). Instituto Meteorológico Nacional. San José, Costa Rica. Environmental data requested on 1 June 2020. Station 84 139 CIGEFI.

Kamermans, P., Hemminga, M. A., & De Jong, D. J. (1999). Significance of salinity and silicon levels for growth of a formerly estuarine eelgrass (*Zostera marina*) population (Lake Grevelingen, The Netherlands). *Marine Biology*, *133*, 527–539. https://doi.org/10.1007/s002270050493

Longstaff, B. J., Loneragan, N. R., O'Donohue, M. J., & Dennison, W. C. (1999). Effects of light deprivation on the survival and recovery of the seagrass *Halophila ovalis* (R.Br.) Hook. *Journal of Expxperimental Marine Biology and Ecology*, *234*(1), 1–27. https://doi.org/https://doi.org/10.1016/S0022-0981(98)00137-3

Magalhães, K. M., Lima, T. H. A., Barcellos, R. L., & Souza, A. M. C. (2025). *Halophila baillonii*'s hidden distribution range: Rediscovery and conservation of a rare seagrass in the Tropical Atlantic. *Aquatic Botany*, *196*, 103828. https://doi.org/10.1016/j.aquabot.2024.103828

Marbà, N., & Duarte, C. M. (1998). Rhizome elongation and seagrass clonal growth. *Marine Ecology Progress Series*, *174*, 269–280. https://doi.org/10.3354/meps174269

McMillan, C. (1976). Experimental studies on flowering and reproduction in seagrasses. *Aquatic Botany*, *2*, 87–92. https://doi.org/10.1016/0304-3770(76)90011-5

McMillan, C. (1980). Flowering under controlled conditions by *Cymodocea serrulata*, *Halophila stipulacea*, *Syringodium isoetifolium*, *Zostera capensis* and *Thalassia hemprichii* from Kenya. *Aquatic Botany*, *8*, 323–336. https://doi.org/10.1016/0304-3770(80)90062-5

McMillan, C. (1987). Seed germination and seedling morphology of the seagrass, *Halophila engelmannii* (Hydrocharitaceae). *Aquatic Botany*, *28*(2), 179–188. https://doi.org/10.1016/0304-3770(87)90039-8

McMillan, C. (1988). Seed germination and seedling develo-pment of *Halophila decipiens* Ostenfeld (Hydrocharitaceae) from Panama. *Aquatic Botany*, *31*(1–2), 169-176. https://doi.org/10.1016/0304-3770(88)90046-0

McMillan, C., & Moseley, F. N. (1967). Salinity tolerances of five marine spermatophytes of Redfish Bay, Texas. *Ecology*, *48*(3), 503–506. https://doi.org/10.2307/1932688

Morales-Ramírez, Á., Acuña-González, J., Lizano, O., Alfaro, E., & Gómez, E. (2015). Rasgos oceanográficos en el Golfo Dulce, Pacífico de Costa Rica: una revisión para la toma de decisiones en conservación marina. *Revista de Biología Tropical*, *63*(Suppl. 1), 131–160. http://dx.doi.org/10.15517/rbt.v63i1.23100

Moses, J. S., & Fredrick, E. J. (2017). Flowers of the intertidal seagrass *Halophila stipulacea* (Forsskl) Ascherson: a new record from tropical coast of Tanzania, Indo-Pacific. *African Journal of Plant Science*, *11*(7), 294–297. https://doi.org/10.5897/ajps2017.1573

Nguyen, H. M., Yadav, N. S., Barak, S., Lima, F. P., Sapir, Y., & Winters, G. (2020). Responses of invasive and native populations of the seagrass *Halophila stipulacea* to simulated climate change. *Frontiers in Marine Science*, *6*, 812. https://doi.org/10.3389/fmars.2019.00812

Ogata, E., & Matsui, T. (1965). Photosynthesis in several marine plants of Japan as affected by salinity, drying and pH, with attention to their growth habitats. *Botanica Marina*, *13*, 199–217. https://doi.org/10.1515/botm.1965.8.2-4.199

R Core Team. (2020). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ramos, E. A., Tellez, M., Castelblanco-Martinez, N., & May-Collado, L. (2024). Antillean manatees feed on floating *Halophila baillonii* in Placencia Lagoon, Belize. *Latin American Journal of Aquatic Mammals*, *19*(1), 133–140. https://doi.org/10.5597/lajam00322

Samper-Villarreal, J. (2024). Seagrasses in the Eastern Tropical Pacific: species, distribution, ecology, blue carbon, and threats. *Latin American*

Journal of Aquatic Research, *52*, 336–349. https://doi.org/10.3856/vol52-issue3-fulltext-3167

Samper-Villarreal, J. (2025). Rare occurrence of only female flowers suggests a lack of sexual reproduction and potential clonality of the seagrass *Halophila baillonii* Asch. on the Pacific coast of Costa Rica. *Aquatic Botany*, *196*, 103822. https://doi.org/10.1016/j.aquabot.2024.103822

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. *Pacific Science*, *68*(3), 435–444 https://doi.org/10.2984/68.3.10

Samper-Villarreal, J., Cambronero Bolaños, R., Heidemeyer, M., Mora Vargas, M., & Mora Vargas, R. (2020). Characterization of seagrasses at two new locations in the Eastern Tropical Pacific (El Jobo and Matapalito, Costa Rica). *Aquatic Botany*, *16*, 103237. https://doi.org/10.1016/j.aquabot.2020.103237

Samper-Villarreal, J., & Cortés, J. (2020). Seagrass characterization on the southern Pacific coast of Costa Rica: history, vegetation, and environment. *Botanica Marina*, *63*(5), 429–438. https://doi.org/10.1515/bot-2020-0022

Samper-Villarreal, J., Moya-Ramírez, J. & Cortés, J. (2022). First characterization of seagrasses at Sámara Bay, Pacific coast of Costa Rica. *Aquatic Botany*, *178*, 103486. https://doi.org/10.1016/j.aquabot.2021.103486

Samper-Villarreal, J., Rojas-Ortega, G., Vega-Alpízar, J. L., & Cortés, J. (2018a). New sighting of seagrasses in the Eastern Tropical Pacific (Bahía Potrero, Costa Rica). *Aquatic Botany*, *151*, 25–29. https://doi.org/10.1016/j.aquabot.2018.07.010

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. *Revista de Biología Tropical*, *66*(Suppl. 1), S53–S65. https://doi.org/10.15517/rbt.v66i1.33260

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 Years of Image Analysis. *Nature Methods*, *9*, 671–675. https://doi.org/10.1038/nmeth.2089

Short, F., Carruthers, T., Van Tussenbroek, B., & Zieman, J. (2010). *Halophila baillonii* [Web page]. The IUCN Red List of Threatened Species 2010. Version 3.1. http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T173382A7004500.en

Short, F. T., Fernandez, E., Vernon, A., & Gaeckle, J. L. (2006). Occurrence of *Halophila baillonii* meadows in Belize, Central America. *Aquatic Botany*, *85*(3), 249–251. https://doi.org/10.1016/j.aquabot.2006.04.001

Sidik, B. J., Harah, Z. M., Fakhrulddin, I. M., Anuar, M. K., & Arshad, A. (2010). Growth performance of Malaysian's spoongrass, *Halophila ovalis* (R.Br.) Hooker *f.* under different substrate, salinity and light regime. *Coastal Marine Science*, *34*(1), 103–107.

Thorhaug, A., Blake, N., & Schroeder, P. B. (1978). The effect of heated effluents from power plants on seagrass (*Thalassia*) communities quantitatively comparing estuaries in the subtropics to the tropics. *Marine Pollution Bulletin*, *9*(7), 181–187. https://doi.org/10.1016/0025-326X(78)90175-3

Torquemada, Y. F., Durako, M. J., & Lizaso, J. L. S. (2005). Effects of salinity and possible interactions with temperature and pH on growth and photosynthesis of *Halophila johnsonii* Eiseman. *Marine Biology*, *148*, 251–260. https://doi.org/10.1007/s00227-005-0075-5

Van Dijk, K., Waycott, M., Biffin, E., Creed, J., Albertazzi, F., & Samper-Villarreal, J. (2023). Phylogenomic Insights into the Phylogeography of *Halophila baillonii* Asch. *Diversity*, *15*, 111. https://doi.org/10.3390/d15010111

Van Katwijk, M. M., Schmitz, G. H. W., Gasseling, A. P., & Van Avesaath, P. H. (1999). Effects of salinity and nutrient load and their interaction on *Zostera marina*. *Marine Ecology Progress Series*, *190*, 155–165. https://doi.org/10.3354/meps190155

Van Tussenbroek, B. I., Santos, M. B., Wong, J. G. R., Van Dijk, K. & Waycott, M. (2010). *A guide to the tropical seagrasses of the Western Atlantic*. Universidad Nacional Autónoma de México.

Walker, D. I. (1985). Correlations between salinity and growth of the seagrass *Amphibolis antarctica* (labill.) Sonder & Aschers., In Shark Bay, Western Australia, using a new method for measuring production rate. *Aquatic Botany*, *23*(1), 13–26. https://doi.org/10.1016/0304-3770(85)90017-8

Walker, D. I., & McComb, A. J., (1990). Salinity response of the seagrass *Amphibolis antarctica* (Labill.) Sonder et Aschers.: an experimental validation of field results. *Aquatic Botany*, *36*(4), 359–366. https://doi.org/10.1016/0304-3770(90)90052-M

Wang, L., English, M., Tomas, F., & Mueller, R. (2021). Recovery and community succession of the *Zostera marina* rhizobiome after transplantation. *Applied and Environmental Microbiology*, *87*(3), e02326–20. https://doi.org/10.1128/AEM.02326-20

Wesselmann, M., Anton, A., Duarte, C. M., Hendriks, I. E., Agustí, S., Savva, I., Apostolaki, E. T., & Marbà, N. (2020). Tropical seagrass *Halophila stipulacea* shifts thermal tolerance during Mediterranean invasion. *Proceedings of the Royal Society B*, *287*(1922), 20193001. https://doi.org/10.1098/rspb.2019.3001

Wong, S. (2016). *The ecophysiological effects of CO2 enrichment on the seagrass Halophila ovalis* [Doctoral dissertation]. Murdoch University, Australia.