

Growth and morphological responses of *Halophila beccarii* to low salinity

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Abstract. *Halophila beccarii* Ascherson is classified as a threatened seagrass species by IUCN because of the reductive tendency of its distribution area. This seagrass is considered a euryhaline species adapted to a wide range of salinities from freshwater and brackish water to marine water. Previous studies showed that the species tends to grow better under low salinity; however, its optimum salinity has not been determined. In Vietnam, *H. beccarii* grows in habitats with low salinity (0–20 ppt). The results show that salinity affects the growth, survival rate, shoot density, biomass, and morphological characteristics of the grass. The leaf dimension is more prolonged and broader; the petiole and shoot length are longer at 10 ppt salinity. In contrast, both the number of shoots and biomass peak at 5 ppt and decrease at lower and higher salinities. The study reveals that *H. beccarii* can grow better under mesohaline conditions than freshwater and hypersaline conditions with an optimum salinity at 5–10 ppt. These findings would explain the species' distribution dynamics in coastal environments and be helpful information for conserving the seagrass populations in habitats with fluctuating salinity as coastal lagoons in Central Vietnam.

Keywords: *Halophila beccarii*, brackish, lagoon, salinity, seagrass

1 Introduction

Seagrasses are flowering plants that adapt to life in wholly submerged saline environments, belonging to the order Alismatales in the class Monocots; they distribute on the tropical and temperate coasts of the globe [1, 2]. Seagrass, mangrove and coral reef are important closely-related ecosystems. Above-ground biomass of seagrasses can improve water clarity and stabilize sediment. Furthermore, seagrasses are a vital component of coastal ecosystems, being the ideal habitat for aquatic animals by providing food, shelter, and nursery grounds [3-5]. Unfortunately, seagrass beds have been seriously declining around the globe because of various natural and

anthropogenic disturbances [4, 6]. As a result, seagrass meadows are declining rapidly, with 14% of seagrass species facing extinction risks [4]. The loss of seagrass meadows causes negative impacts on coastal ecosystems (e.g., estuaries, coastal lagoons, coral reefs, and mangroves) and human communities living on coastal resources. Therefore, practical activities to prevent this loss on a global scale are extremely necessary for conserving and managing seagrass meadows [7].

Salinity fluctuations can affect seagrasses' growth in natural habitats [8-10] as well as culture conditions [11-14]. Adverse effects of salinity on seagrasses were recorded in some previous studies. The salinity levels exceeding the tolerant ecological threshold, like hypersaline conditions,

reduce the growth of seagrasses [12-16] or increase mortality [12-14]. These high salinities adversely affect biochemical activities such as metabolic intensity [17], carotenoid concentration, and dark respiration or decrease plant photosynthesis [11, 16, 18, 19]. Under natural conditions, salinity is considered one of the environmental factors that play an essential role in the distribution change of seagrass meadows [20].

Among 72 species of seagrasses recorded worldwide, *Halophila* (Hydrocharitaceae) is the largest genus consisting of 17 species observed in five of the six seagrass ecoregions in the Indo-Pacific and tropical Atlantic region [4]. As a member of *Halophila* genus, *Halophila beccarii* is widely present in the Indo-Pacific region [20-23]. *H. beccarii* meadows have seriously decreased, and the species has been listed as a threatened seagrass species (Vulnerable – VU) in the International Union for Conservation of Nature (IUCN) categories [1, 4, 24]. However, *H. beccarii* beds have still distributed quite commonly in some countries in Southeast Asia, such as Vietnam [20, 21, 25], India [26, 27], the Philippines [28], Bangladesh [23], Sri Lanka [29], Malaysia [30], and Thailand [31]. *H. beccarii* has been recorded on the sandy bottom, sand mixed with clay and silt in estuaries, lagoons, mangroves, bays, and sheltered shallow tidal areas [20, 27, 32-34]. The species can grow as an annual or perennial plant, depending on the specific distribution environment [20, 28, 29, 31]. It can grow to form mono-species meadows. However, it also grows with other plants, such as salt marsh (*Porteresia coarctata*), mangroves (*Avicennia alba* and *A. marina*), seaweeds (*Ulva intestinalis*, *Ulva* sp., *Dictyota* sp.) [23], or seagrass species as *Halophila ovalis*, *Halophila ovata*, *Halodule uninervis* to form mixed specific meadows [23, 29, 31]. *H. beccarii* is a euryhaline species, having a wide range of salinity tolerance [20, 31]. The salinity

tolerance of the species has been reported under experimental and natural conditions. Seagrasses can survive at a salinity range of 0–45 ppt under culture conditions, although the leaf dimension becomes smaller than under hypersaline conditions [35]. In the natural environment, *H. beccarii* distributes in a salinity range of 0–37 ppt [20, 23, 27, 31, 32, 34, 36]. Seagrasses spread in shallow coastal waters with a depth from 0.5 to 2 m [20, 26, 28]. Some previous studies reported that environmental factors, such as salinity, water temperature, light, water depth, and water turbidity, affected the distribution and abundance of the seagrass, and salinity was one of the factors that played a vital role in the distribution change of this species [20, 31, 37, 38]. Under culture conditions, seagrasses are affected by salinity in terms of morphological characteristics (leaf length, leaf width, shoot length, and the number of leaves) [35, 39], the shoot growth rate [13], and mortality [12]. The seagrass *H. beccarii* has a capacity for survival and growth in both increasing salinity (25–45 ppt) and decreasing salinity (0–25 ppt), but it grows better under low salinity conditions [35].

In Vietnam, *H. beccarii* is commonly found in the lagoons and estuaries in Central Vietnam with a 0.2–20 ppt salinity range [20, 21, 40] and is documented as one of the dominant seagrasses in the Tam Giang–Cau Hai Lagoon system [20, 41]. However, the species distribution in the field is not stable [20], which could be due to the salinity changes. Therefore, in this study, we carried out a mesocosm experiment to examine the growth capacity and morphological responses of *H. beccarii* against low salinity conditions in Cau Hai Lagoon (Thua Thien Hue).

2 Materials and methods

Collecting plants and sediments

The materials used for the experiment were collected in Cau Hai Lagoon (16°19'22" N, 107°50'59" E), belonging to the Tam Giang–Cau Hai Lagoon system. The lagoon is influenced by the tropical monsoon climate with a high average temperature and rainfall (25.2 °C and 2,744 mm) [42, 43]. There are two distinct seasons: the dry season (March to August) and the rainy season (September to February), with an average temperature of 38–41 and 20–22 °C and a rainfall of 75% [43]. Cau Hai Lagoon is relatively shallow (average depth of 1–1.5 m) [41]. Its salinity varies spatially and seasonally, ranging from 0.2 to 29.9 ppt [37, 44]

H. beccarii rhizome fragments and sediments were collected at the same place from a shallow area where the *H. beccarii* meadow is dense and abundant. At the sampling site (water depth 0.8 m, salinity 11 ppt), surface sediment (5-cm deep) was taken and placed in plastic boxes (26 × 20 × 18 cm); the rhizome fragments were sampled and kept in coolers with a small volume of water. Both the rhizome fragments and sediments were transported to the outdoor experimental area at the University of Sciences, Hue University, within 24 hours.

Experimental design and data collection

The experiments were performed for 12 weeks (20 March to 20 June 2019) in the outdoor culture

system with a transparent roof and natural light. The experimental design consists of five glass aquarium tanks (70 × 40 × 44 cm), 20 plastic boxes (26 × 20 × 18 cm), and five water filters for filtering and circulating water. Freshwater (tap water) and sea salt were used to prepare the water for different salinity treatments [45]. Based on the salinity where the seagrass is distributed in the field [20, 27, 31, 32, 41], we tested five salinity levels: 0, 5, 10, 15, and 20 ppt. The salinity was determined by using a multi-parameter water quality monitoring device (Horiba U50, Japan). A 0.5 mm mesh diameter sieve was used to remove coarse gravel and plant materials from the sediments. They were then put in plastic boxes to make a 5-cm substrate layer. The *H. beccarii* rhizome fragments were carefully selected and planted immediately. Twelve rhizome fragments of a similar size and vitality, bearing four shoots, were planted in each plastic box. Four planting boxes were placed in each glass aquarium tank for salinity treatments. The morphological characteristics were determined after eight weeks. The characteristics are the number of leaves per shoot and the number of shoots, leaf length and width, petiole and shoot length, and growth rate. The lengths were measured with a Digital Caliper (Minutolo 530-104, Japan, 0–150 mm, accuracy: ±0.05 mm) [46]. The number of survival rhizome fragments was counted after the first week. The growth elongation was tracked and measured with small bamboo sticks in the first eight weeks (Fig. 1).

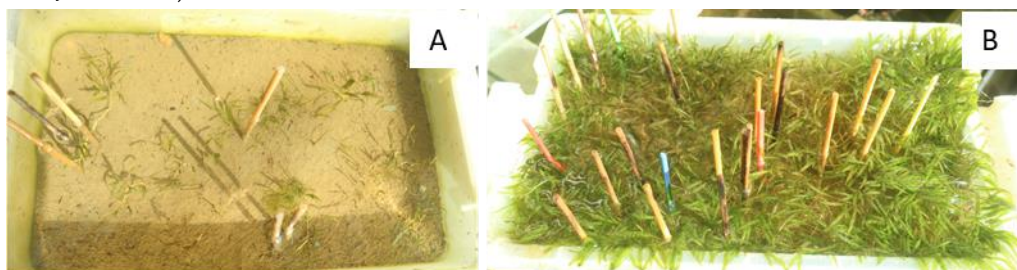


Fig. 1. Rhizome elongation of *H. beccarii* after eight weeks, marked with small bamboo sticks: at 0 ppt (A) and 5 ppt (B)

The salinity was kept constant and checked every three days; any fluctuations were adjusted immediately; the water column was maintained 25 cm high in all tanks during the experiment. The experiment was conducted outdoors under a transparent roof with air temperatures from 25.5 to 37.8 °C and light intensity at 39,700 lux (Handy Lux Meter, HTC, India).

After 12 weeks, the seagrasses were harvested to determine the number of shoots and biomass. After cleaning carefully with fresh water to remove sediment, epiphyte, and salt, the plants were oven-dried at 60 °C to constant weight and then weighed to determine biomass [47].

Statistical analysis

All variables were examined for the normal distribution prior to the analysis by using the Shapiro-Wilk test. The significant difference of variables was tested with one-way ANOVA, and the Tukey post hoc test or Friedman ANOVA and Wilcoxon matched pairs that depended on variables' normality. Data were analyzed with the IBM SPSS statistic software, v.20.

3 Results and discussions

3.1 Survival and growth rate of rhizome fragments

Our observations indicate that *H. beccarii* rhizome fragments could survive and grow at salinity from 0 to 20 ppt. However, the salinity significantly affected the survival rate ($F = 14.04$, $p < 0.0001$), lowest at 0 ppt with $33.3 \pm 3.4\%$ and highest (100%) at 5 ppt and 10 ppt. This rate decreased gradually to 81.3 ± 2.08 and $66.7 \pm 34\%$ at 15 and 20 ppt (Fig. 2).

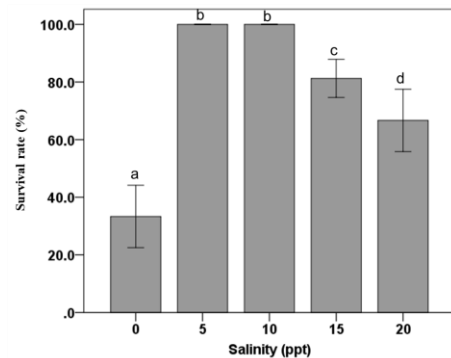


Fig. 2. Survival rate of *H. beccarii* rhizome fragments after first week at different salinities (mean \pm SD, $n = 4$). The same letter a, b, c, or d indicates no statistical difference between columns.

The growth rate increased rapidly during the first 2–5 weeks and decreased significantly in the following weeks at all salinity treatments (except 0 ppt). The rate differs significantly among salinity treatments [FA , $\chi^2(4, n = 128) = 134.1$, $p < 0.0001$]. It peaked at 10 ppt (4.4 ± 0.2 mm·day⁻¹) at week five and decreased to a minimum at 0 ppt salinity (0.9 ± 0.1 mm·day⁻¹) (Fig. 3).

The results indicate that *H. beccarii* could survive and maintain its growth in the salinity range from 0 to 20 ppt. The highest survival rate was observed at 10 ppt. The species could survive but grew poorly at 0 ppt during eight weeks. The tolerance to low salinity was also reported for several other seagrass species, such as *Zostera noltii*, *Amphibolis antarctica*, and *Posidonia sinuosa* [13, 48]. In another study, Fakhrulddin et al. revealed that *H. beccarii* collected from a Malaysian estuary could tolerate the salinity range of 0–45 ppt with no mortality during 303 days of experiment [35]. This difference might be due to the gradual change in treated salinity, from 25 ppt to 0 ppt and from 25 ppt to 45 ppt at two-week intervals so that the plants would gradually adapt to salinity; as a result, there was no shoot mortality. On the other hand, in our study, the species suffered from sudden salinity changes from 11 ppt (in the field) to tested salinities (0, 5, 10, 15, and 20 ppt), reducing the survival rate of the species.

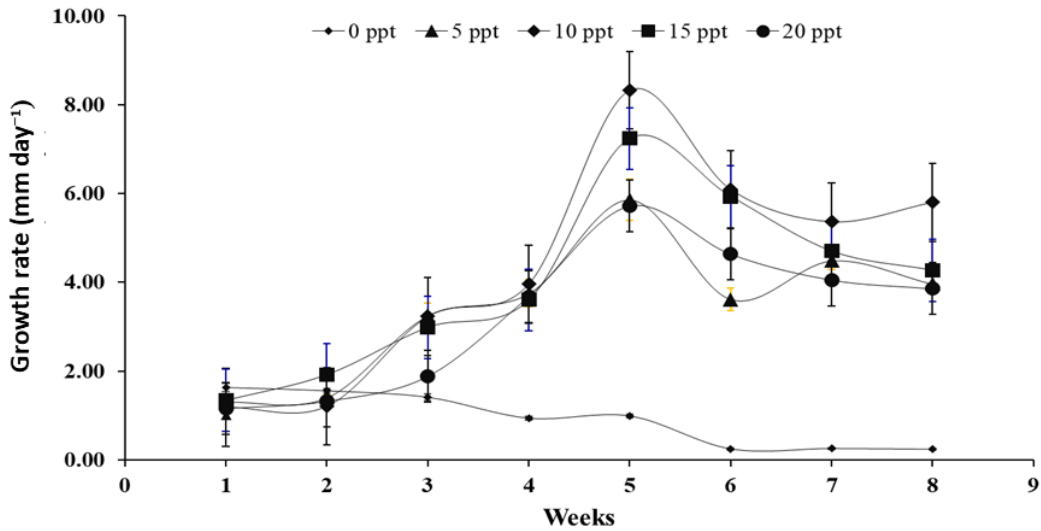


Fig. 3. Growth rate of *H. beccarii* rhizome at different salinities during eight weeks (mean \pm SD, $n = 48$)

3.2 Response of morphological characteristics

There were significant differences of leaf length ($F = 60.9$, $p < 0.0001$), petiole length ($F = 35$, $p < 0.0001$), leaf width ($F = 23.5$, $p < 0.0001$), and shoot length ($F = 92.7$, $p < 0.0001$) among salinity

treatments. Leaf length, petiole length, and leaf width peaked in both salinity 10 and 15 ppt, but it was the lowest at 0 ppt; shoot length was highest at 5 and 10 ppt and lowest at 0 ppt (Fig. 4A-D).

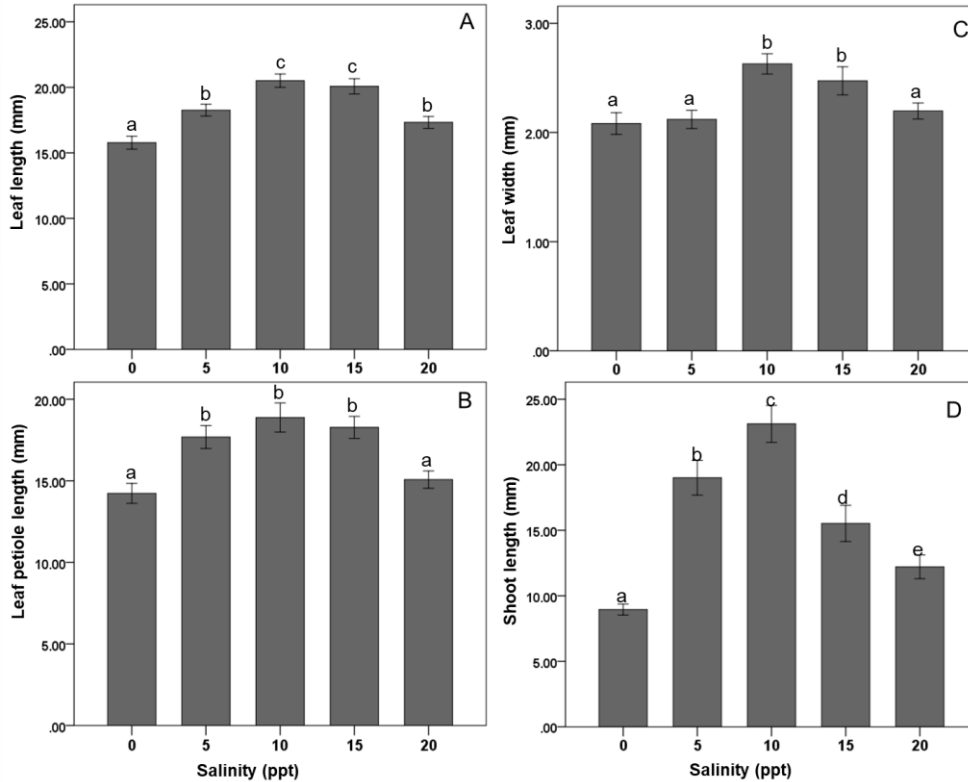


Fig. 4. Morphological characteristics of *H. beccarii* growing at different salinities after eight weeks. A. leaf length; B. petiole length; C. leaf width; and D. shoot length (mean \pm SD, $n = 40$). Different letters denote significant differences among salinity treatments ($p < 0.05$).

H. beccarii formed new shoots 2–3 days after planting. These shoots carried 3–4 leaves in the first week. The number of leaves per shoot ranged from 2 to 12 (7.2 ± 0.1). There was a significant variation in the number of leaves among salinity treatments [$FA, \chi^2(4, n = 40) = 87.2, p < 0.0001$]. The number of leaves reached the maximum at 10 ppt salinity (8.7 ± 0.2), decreased significantly with the increase or decline in salinity, and became the lowest at 0 ppt salinity (5 ± 0.2) (Fig. 5).

The salinity had a substantial effect on the morphological responses of *H. beccarii*. The number of leaves, leaf length, leaf width, and petiole length peaked at 10 and 15 ppt, while shoot length peaked at 5 and 10 ppt, and these characteristics decreased significantly at 0 ppt and 20 ppt salinity. The above results differed from those of *H. beccarii* from Malaysia, i.e., the leaf size and shoot length tended to be larger at 20–25 ppt salinity [35]. Furthermore, the effect of salinity on leaf elongation was also found in *Cydomocea nodosa*, which peaked at 30–39 ppt salinity [13]. Therefore, the responses of morphological characteristics of *H. beccarii* in this study reflected

a better growth under lower salinity conditions (5–15 ppt).

Unlike natural conditions, culture conditions provide *H. beccarii* with higher values of leave number per shoot, leaf dimension, and petiole length (Table. 1). It is possible that the cultural conditions were more advantageous for the growth of the species.

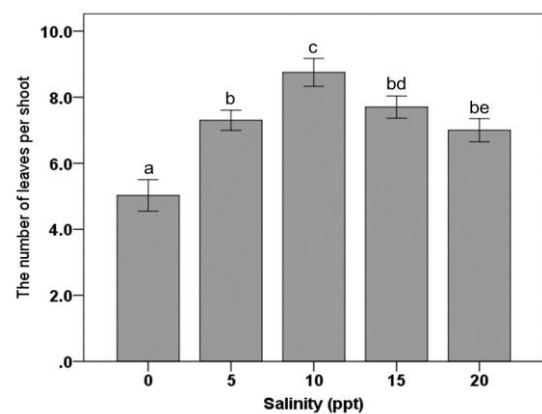


Fig. 5. Number of leaves per shoot of *H. beccarii* at different salinities after eight weeks (mean \pm SD, $n = 40$). The same letter a, b, c, d, or e indicates no statistical difference between columns.

Table 1. Comparison of morphological characteristics of *H. beccarii* between this study and previous studies

Habitat, country	Number of leaves/shoot	Leaf length, (mm)	Leaf width, (mm)	Petiole length (mm)	References
Estuary, India	3–10	12	1.5	4–13	[34]
Mangrove, estuary, lagoon, Singapore	10	8–15	1–2	–	[49]
Coastline, Myanmar	–	5.5–7.5	1.5–2	8–14	[50]
Estuary, Bangladesh	4–8	10–25	2	30	[23]
Estuary, Malaysia (cultural conditions)	6–11	–	–	–	[35]
Islands, India	2–6	4.7–7.6	0.8–3.9	5.7–12.7	[27]
Lagoon, Vietnam (cultural conditions)	2–12	10.2–23.9	1.3–3.3	11.4–25.2	This study

3.3 Number of shoots and biomass

In the eighth week, we observed a significant difference in the number of shoots of *H. beccarii* at the salinity treatments ($F = 26.4, p < 0.0001$). The number of *H. beccarii* shoots was highest at 5 ppt and 10 ppt salinity (466 ± 50.1 and 351.1 ± 30.6) and lowest at 0 ppt and 20 ppt salinity (43 ± 6.3 and 170 ± 31.1) (Fig. 6). At the end of the experimental period (twelfth week), the number of shoots also peaked at 5 ppt (955 ± 131.2) and lowered significantly at 0, 10, 15 ppt with $25.8 \pm 7.5, 224.5 \pm 77.4, 74.5 \pm 36.7$, respectively. The *H. beccarii* shoots decayed, and the number had a minimum value at 20 ppt salinity (10.5 ± 7.1) (Fig. 7B, Fig. 6).

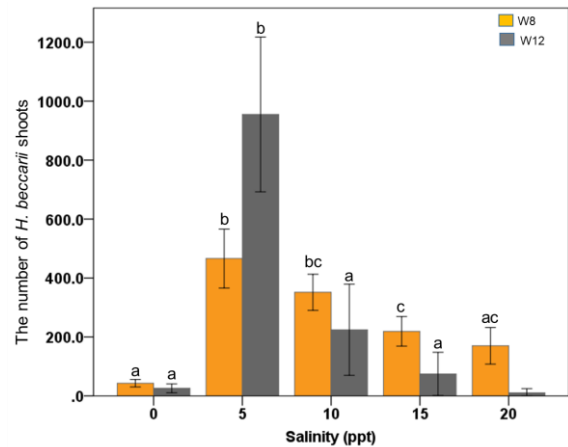


Fig. 6. Number of *H. beccarii* shoots at different salinities in the eighth week (w8) and twelfth week (w12), mean \pm SD, $n = 4$. The same letter a, b, or c indicates no statistical difference between columns.

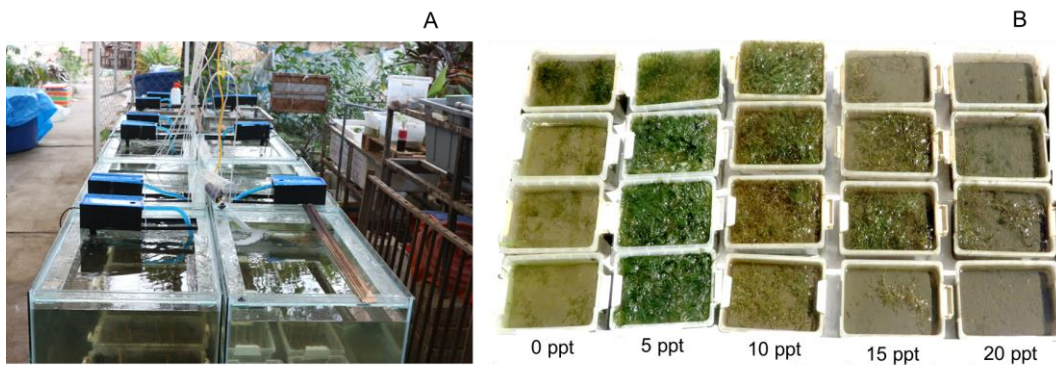


Fig. 7. Growth of *H. beccarii* in the experiment. **A:** Experimental setup; **B:** *H. beccarii* at different salinity treatments after 12 weeks (B)

The salinity strongly affected the biomass of *H. beccarii*. The one-way ANOVA revealed a significant difference in biomass among salinity treatments ($F = 19.2, p < 0.0001$). The biomass of *H. beccarii* had a maximum value at 5 ppt (4.7 ± 0.5 g) and the lowest at 0 ppt and 20 ppt with 0.2 ± 0.1 and 0.6 ± 0.3 g (Fig. 8).

The salinity treatments had remarkable effects on the number of shoots and biomass of *H. beccarii*. Both of them peaked at 5 ppt salinity and decreased significantly with increasing or declining salinity. Unfavourable salinity prolongation could disturb the metabolism of cells, reduce photosynthetic performance, and increase ageing and fading, which might reduce

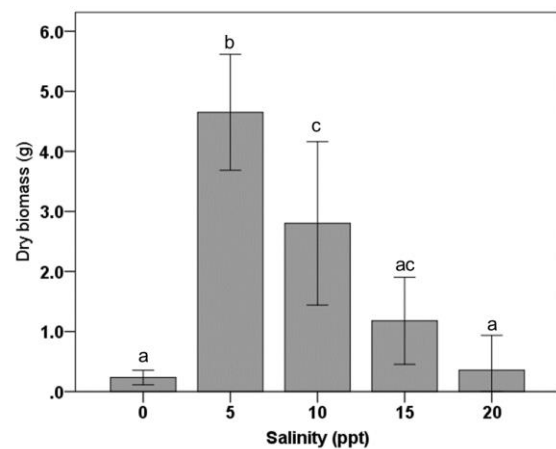


Fig. 8. Biomass of *H. beccarii* in different salinities after 12 weeks (mean \pm SD, $n = 4$)

the number of shoots and biomass of the seagrass [16, 51-53]. The results help explain the instability of salinity in coastal lagoons and estuaries that have influenced the temporal and spatial distribution dynamic of the seagrass *H. beccarii* in the field [20, 37].

Each seagrass species has an optimum salinity for survival. We found that the optimum salinity of the seagrass *H. beccarii* was at 5 and 10 ppt (equivalent to oligohaline and mesohaline water). This salinity is lower than that for other seagrasses, such as *Zostera capensis* (15–35 ppt) [15], *Posidonia oceanica* (25–39 ppt) [12], *Zostera marina* (10–25 ppt) [54], *Cymodocea nodosa* (30–41 ppt) [13], *Ruppia cirrhosa* (15–35 ppt) [15], and *Thalassia testudinum* (30–40 ppt) [55]. This result is possibly an adaptation of the species to a brackish water lagoon environment, such as Cau Hai Lagoon, where the salinity is from freshwater to polyhaline and frequently fluctuates with the inflow of rivers.

Studies in India, Myanmar, Bangladesh, Malaysia, and Thailand reported that *H. beccarii* in the field was abundant at a salinity of 16–37 ppt [23, 31, 32, 34, 36]; meanwhile, studies in Vietnam showed that the species was abundant at lower salinity (<21 ppt) [21, 41, 56, 57]. Specifically, in Cau Hai Lagoon, *H. beccarii* obtained high coverage and biomass at a salinity of 8–14 ppt [20, 44, 58], which may explain the low optimum salinity of the species in this study.

4 Conclusion

The study shows that *H. beccarii* could survive and continue growing in the range of tested salinity of 0–20 ppt. Salinity significantly affected the survival rate, growth rate, morphological characteristics, number of shoots, and biomass accumulation of *H. beccarii*. The survival rate, growth rate, dimension of leaf, shoot length, the number of shoots, and biomass reached the

highest values at 5 ppt and 10 ppt salinity. It suggested that the optimal salinity for the growth of the seagrass *H. beccarii* was at 5–10 ppt. The species could survive under freshwater conditions (ca. 0 ppt); however, it hardly grew during the experimental period. The seagrass *H. beccarii* from Cau Hai Lagoon could better tolerate the mesohaline conditions than the freshwater and hypersaline conditions. The species tended to adapt to brackish water. This information is helpful for conserving the seagrass *H. beccarii* populations in estuaries and coastal lagoons with high salinity fluctuations in Central Vietnam.

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