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## ***Halophila beccarii* Aschers (Hydrocharitaceae) Responses to Different Salinity Gradient**

<sup>1</sup>I.M. Fakhruddin, <sup>1</sup>B. Japar Sidik and <sup>2</sup>Z. Muta Harah

<sup>1</sup>Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, Nyabau Road, 97008 Bintulu, Sarawak, Malaysia

<sup>2</sup>Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

*Corresponding Author: B. Japar Sidik, Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia Tel: +6086-855202*

### **ABSTRACT**

*Halophila beccarii* Aschers., a small monoecious seagrass with rosette leafy shoot and well-developed rhizome or underground stem that acts as anchors occurs in environment with salinity fluctuation e.g., brackish coastal water of mangrove system, lagoon and marine coastal areas. Shoots represent most of the above-ground component of the plant and in direct contact to the ever changing salinity but it is not known how they respond to such fluctuations. Therefore, this study examined the effects of salinity variations on above-ground response variables; number of leaves per shoot, leaf length, leaf width and petiole length of the seagrass *Halophila beccarii*. Survival and above-ground response variables to salinity were characterized in culture-experiments in which plants were exposed to increased salinity (from 25-30, 45 psu) and decreased salinities (from 25-25, 20, 15, 10, 6, 4, 2, 0.9, 0.52 and 0 psu) treatments at two-week intervals. No plants mortality and significant changes in number of leaves per shoot were observed with increased or decreased salinity treatments. Plants continued to survive even in hypotonic environment to 0 psu. *Halophila beccarii* was significantly affected by increased or decreased salinity altering the dimensions of leaves. Leaf length and leaf width became shorter and narrower at lower (4-0.52 psu) and higher (45 psu) salinity. These results suggest that the seagrass *H. beccarii* could be negatively affected by hypo- or hypersalinity conditions, although salinity changes did not alter the tolerance of this species. *Halophila beccarii* tolerated hyposaline conditions better than hypersaline conditions.

**Key words:** Salinity, *Halophila beccarii*, outdoor, culture, morphological changes, seagrass, tolerance

### **INTRODUCTION**

Variation in salinity are common in near shore coastal wetlands e.g., lagoons, rivers and estuaries where seagrasses formed the conspicuous vegetation. These variations in salinity are either natural, resulting from rains during monsoon season, drought and, the ebb and flow tides, or man-induced e.g., water management project (Muta Harah *et al.*, 1999). These events whether natural or man-made may occurred relatively rapid and impact on salinity may last from days to weeks or even months. Field investigation by several researchers reported that, *Halophila beccarii* Aschers. belonging to genus *Halophila* are known to inhabit lagoon and rivers areas with wide

salinity variation, 0-35 psu (Muta Harah *et al.*, 1999; Muta Harah *et al.*, 2003) and mangroves, 16.0-35.0 psu (Abu Hena *et al.*, 2007; Abu Hena and Short, 2009). Although, in some studies (Untawale and Jagtap, 1977; Jagtap, 1991) salinity range were not mentioned where *H. beccarii* occurred e.g., river estuaries and mangrove areas, these areas are subjected to tidal influence and flow of water from inland and hence under the influence of ever changing salinity. The fact that *H. beccarii* continue to inhabit, propagate and colonize these areas indicated that they are able to adapt and respond to fluctuation in salinity.

*Halophila beccarii* is often considered as rare species in Malaysia (Den Hartog, 1970; Japar Sidik, 1994), however it is known to occur in many places (Muta Harah *et al.*, 1999, 2002a, b, 2003) and is thus a common seagrass in East Coast of Peninsular Malaysia. Plants are able to survive under exposure to 0 psu (Muta Harah *et al.*, 2002a), attributed to daily freshwater release from nearby paddy field water gate and a salinity as high as 35 psu (Muta Harah *et al.*, 1999; Muta Harah *et al.*, 2003; Abu Hena *et al.*, 2007; Abu Hena and Short, 2009). However, no quantitative understanding of the responses of *H. beccarii* to the gradient of salinity was investigated. Shoots represent most of the above-ground component of the plant and in direct contact to the ever changing salinity but it is not known how they respond to such fluctuations. Therefore, this study examined the plants' above-ground response variables i.e., number of leaves per shoot, length and width of leaves on the shoots and petiole length of leaves against the tested gradient of salinity in the laboratory cultures.

## MATERIALS AND METHODS

**Plant material and substrate:** *Halophila beccarii* plant ramets consisting of 4 nodes intact with roots and shoots were collected from Pantai Sari (040°57.195' N, 115°24.394' E), Punang-Sari-Lawas River Estuary, Sarawak, Malaysia during low tide in late August 2008 and subsequently cleaned and, grown and maintained in a glass tank filled with artificial seawater of salinity 25 psu in shaded outdoor condition at the Research Centre, Universiti Putra Malaysia Bintulu Sarawak Campus from August 2008 to February 2010. Plant from the field were processed to grow them in the laboratory. Ramets were harvested using hand to avoid damage and ensure intact rhizome fragment, washed with seawater to remove any adherent debris before they were placed in a plastic box and immediately transported to a laboratory at a research centre located at Universiti Putra Malaysia Bintulu Sarawak Campus, Bintulu, Sarawak, Malaysia. Thirty individual ramets were measured for the number of leaves per shoot, length and width of longest leaves on the shoots and petiole length of leaves by using Mitutoyo Vernier Calliper and grown in two 120×45×45 cm glass tanks filled with 25 cm depth of 25 psu artificial seawater (prepared from commercial sea salt Marinemix, Marine Enterprises International Inc., Baltimore, USA i.e., a formulation containing all essential major, minor and trace elements) with the bottom spread of 5 cm thick loamy sand substrate collected from Tg. Batu Beach (3°13' 46.37" N, 113°3' 52.19" E), Bintulu, Sarawak. The culture system was fitted with an external filter and a submersible pump to provide filtration and circulation of water inside the tank. The Marinemix artificial seawater provides the nutrients to sustain *H. beccarii* growth and development while in culture. The culture system was kept under a shaded out-door natural light regimes that fluctuated from 160  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  (10% of the ambient light intensity outside) to ~240  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  (15% of the ambient light intensity outside) recorded between 12.00 noon to 2.00 pm. Water temperature and pH were recorded as 24-29°C and 7.5-8.4, respectively. Water level height of 25 cm (the water salinity maintained) in each of the

tank was maintained by adding distilled water. The ramets propagated and colonized the substrate and they formed the stock plant propagules for experiments to examine *H. beccarii* tolerance and morphological changes when exposed to salinity gradient as performed below.

**Setup of salinity experiment:** Four individual ramets (Fig. 1a) of *H. beccarii* harvested from the stock plant propagules above were planted in a plastic tray (33×26×9 cm) filled with loamy sand substrate. The planted ramets occupied only half of the substrate surface to allow plants colonizing the remaining substrate surface. Four of such plastic trays were immersed in two separate 122×45×45 cm glass tanks filled with 25 cm depth of 25 psu artificial seawater (Marinemix) as shown in Fig. 1b. Plants in the plastic trays in the two tanks were allowed to grow and propagate for eight weeks. For each plastic tray, the planted and the initial bare area of the substrate colonized by new shoots of *H. beccarii* was marked, recorded and measured for following: the number of leaves per shoot, length and width of longest leaves on the shoots and petiole length of leaves. Leaf length, width and petiole length were measured using Mitutoyo Vernier Calliper. This is the baseline conditions of *H. beccarii* plants at salinity of 25 psu before two separate experiments

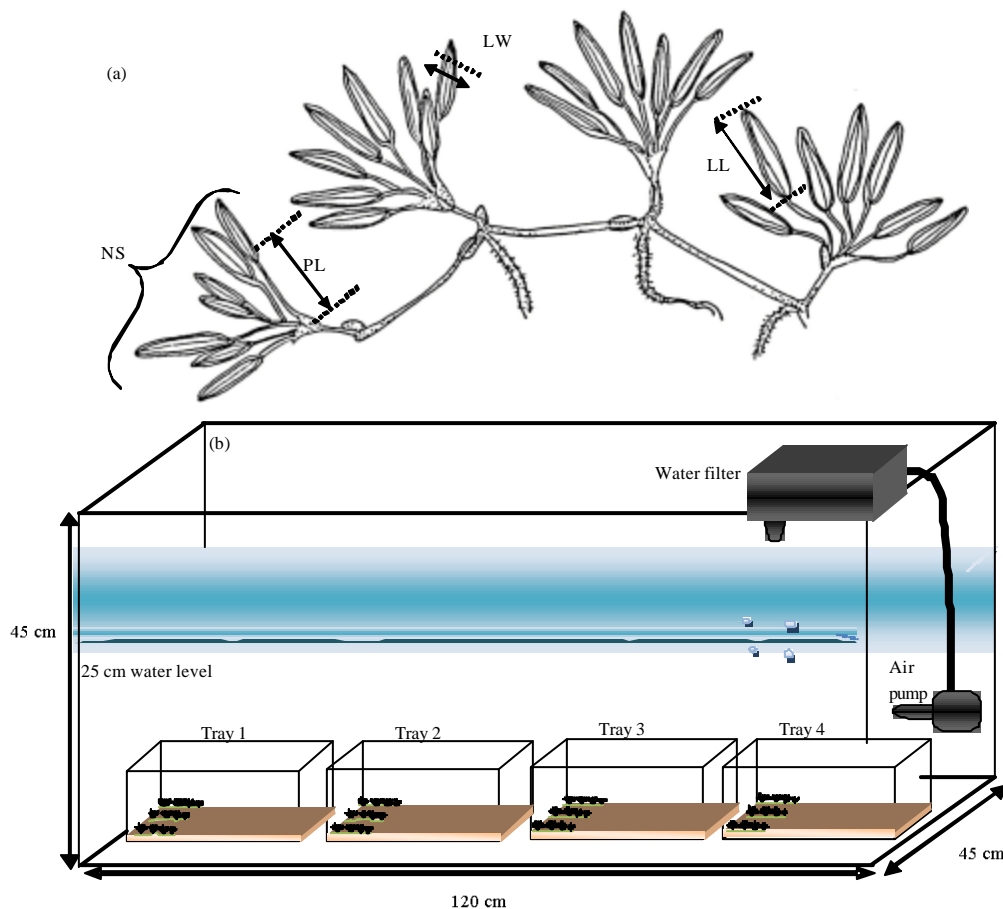


Fig. 1(a-b): (a) General morphology of a ramet consisting of 4 shoots with 7-8 leaves used as a planting material; number of leaves per shoot (NS), leaf length (LL), leaf width (LW) and petiole length (PL) and (b) A culture system for growing *Halophila beccarii* in different salinities



Fig. 2: All shoots on the ramet were initially marked by placing small wooden pegs beside the shoots

were performed to test how salinity affected *H. beccarii* performance where; (1) tank-1: salinity at 25 psu were consecutively increased to 30 and 45 psu (above the level reported in the literature) after two weeks intervals and, (2) tank-2: salinity at 25 psu were decreased consecutively 20, 15, 10, 6, 4, 2, 0.90, 0.52 psu at 2 weeks interval and 0 psu where the plants were left to continue to grow. All shoots on the ramets were initially marked by placing a small wooden peg beside them (Fig. 2). This is to ensure the newly produced shoot corresponded to the shoot without marks. The number of leaves per shoot, length and width of longest leaves on the shoots and petiole length of leaves from new established shoots were recorded and measured just prior to the target salinity treatment. The maintenance of the culture system basically followed the general procedure described above.

**Analysis:** The effects of salinity on the response variables i.e., number of leaves per shoot, length and width of longest leaves on the shoots and petiole length of leaves were subsequently analysed using One-Way ANOVA. Duncan's new multiple range comparison test (DNMRT,  $p < 0.05$ ) was used to identify means that differed from each other. The original data from four response variables from each experiment were scatter plotted against the different salinities and Pearson correlation coefficients were obtained using SAS 9.0. Principle Component Analysis (PCA) was carried out using Primer 5, version 5.2.8 to obtain the relationship between the response variables and the treated salinities.

## RESULTS AND DISCUSSION

**Survival and production of shoot and leaves:** There was no shoot mortality after immediate exposure till the end of the tested salinity. Irrespective of salinity gradient either increased or decreased salinities, planted ramets produced newly formed shoot with leaves. Young shoots were produced within 2-3 days, bearing 6-11 leaves after 5-7 days. Old leaves ceased to grow and detached from the shoot. The number of leaves per shoot varied markedly among replicate plastic trays within each salinity (Fig. 3a, b), tended to be high (13.0-18.3%) at low salinity (2-6 psu) and freshwater, respectively. At salinities above 25 psu the variations were (12.7-14.4%). The number of leaves per shoot, although variable, was not correlated with decreased ( $r = -0.061$ ,  $p > 0.05$ ) or increased ( $r = -0.23$ ,  $p > 0.05$ ) salinities. Under laboratory conditions, number of leaves per

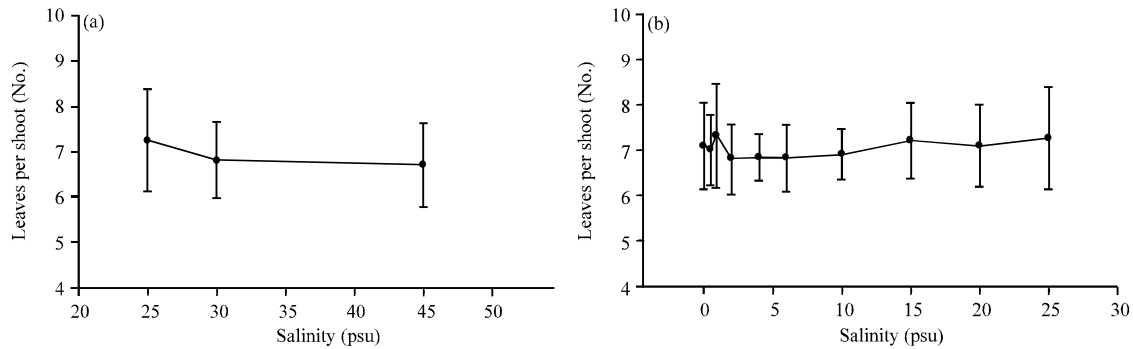


Fig. 3(a-b): *Halophila beccarii* responses, number of leaves per shoot after exposure to different levels in salinity at 2-week intervals, (a) Increased salinity from 25-45 psu and (b) Decreased salinity from 25-0 psu

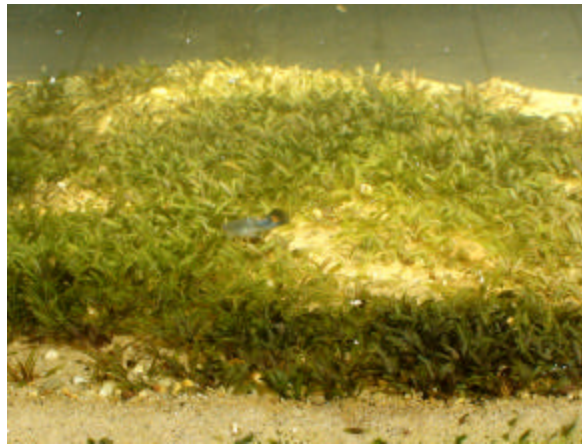


Fig. 4: *Halophila beccarii* grown in freshwater and continue to propagate via rhizome. Under this condition the plant propagated vegetatively

shoot were within the range values recorded in the field (6-11 leaves) and other locations e.g., 4 (Ascherson, 1871 cited from Den Hartog, 1970), 6-10 (Den Hartog 1957, 1970; Hodgkiss and Morton, 1978; Menez *et al.*, 1983), 6-11 leaves (Parthasarathy *et al.*, 1988), 4-12 leaves (Muta Harah *et al.*, 2003). A study conducted by Hillman *et al.* (1995) on *Halophila ovalis*, showed plants only survived 7 days when exposed to low salinity (10 psu), while Benamina *et al.* (1999) reported *H. ovalis* leaves became smaller and shorter after exposed to low salinity before deceased. In contrast *H. beccarii* survived and continue to propagate at low salinity until the level of freshwater. The ability to grow under 0 psu more than 303 days is first reported under laboratory culture. To date the adapted plants is still being grown in our laboratory. *Halophila beccarii* demonstrated its tolerance to salinity gradient manipulation and continue to survive in freshwater (Fig. 4). The plant ability to survive indefinite to freshwater may involve combinations of dynamic cell wall elasticity and adjustment of cells cytoplasm osmotic gradient which was observed in other seagrass e.g., *Ruppia maritima* (Touchette, 2007).

**Responses of *H. beccarii* to different salinity:** The longest leaf length under increased salinities (Fig. 5a) and decreased (Fig. 5b) were both shorter and attained maximum values

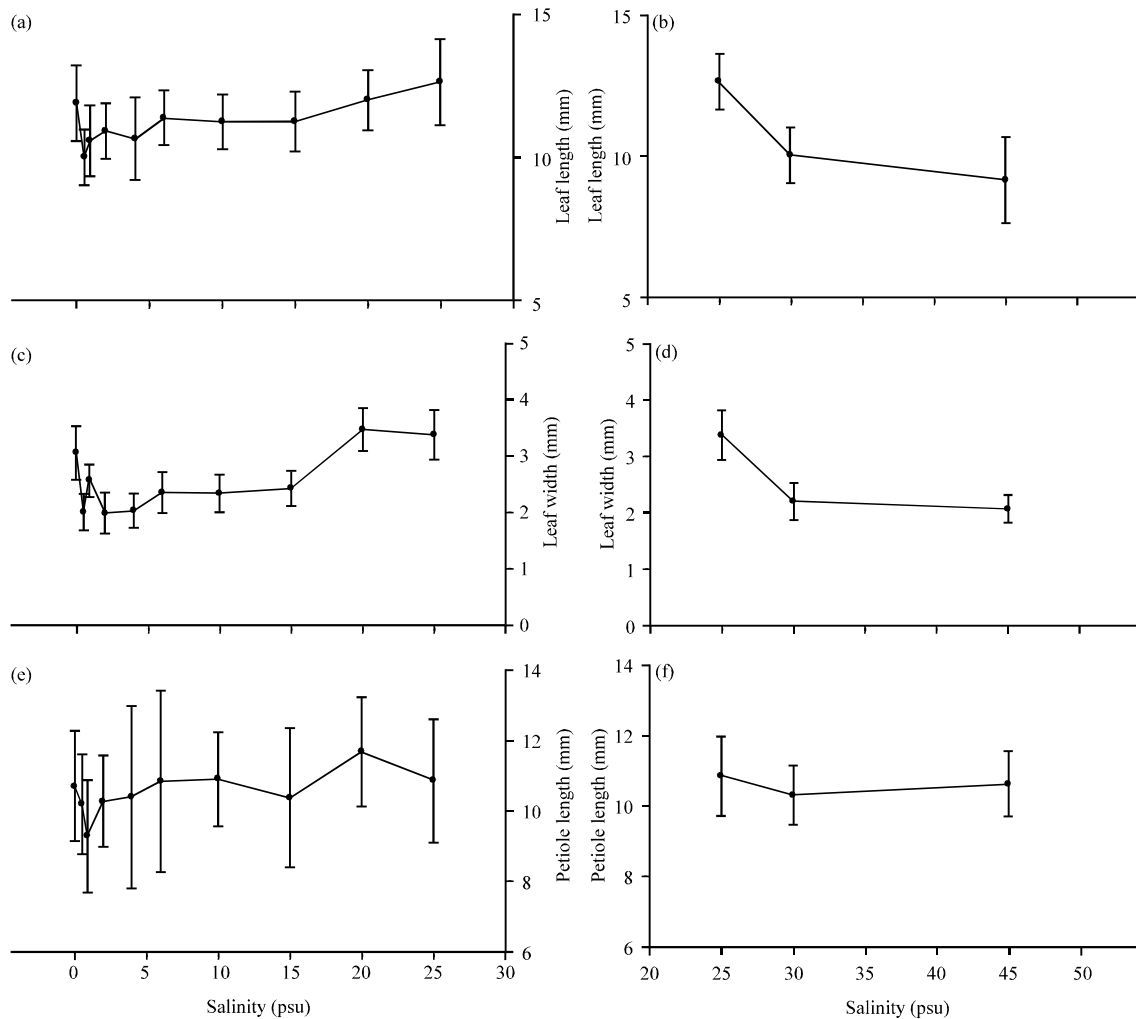


Fig. 5(a-f): *Halophila beccarii* response variables after exposure to increased (from 25-45 psu) and increased (from 25-0 psu) salinity, (a, b) Leaf length, (c, d) Leaf width and (e, f) Petiole length

between 20 and 25 psu. Leaves tend to be longer at freshwater level. There were discernible trends in leaf length with increased or decreased salinities. Leaf length became shorter and negatively correlated with increased (Fig. 5a) or decreased ( $p < 0.05$ ,  $r = -0.159$ , Fig. 5b) salinities. Similar responses were shown by leaf width under increased (Fig. 5c) and decreased (Fig. 5d) salinities. There were no obvious trends in leaf petiole length with salinity (Fig. 5e, f).

Factor analysis of the data from the increased salinity (from 25-45 psu) experiments resulted in three principal components with eigenvalues approximately one or greater than one that together explained about 93% of the total variance in the original response variables (Table 1). The loadings (correlations) of each response variable on the two components (Table 1) showed that leaf length and leaf width contributed almost equally to PC1, with all parameters being positively correlated with PC1. In contrast the number of leaves per shoot and petiole length had the greatest loading on the positive and negative site of PC2, respectively. PC1 varied significantly with salinity, leaf length (ANOVA,  $p < 0.0001$ ,  $F = 133.35$ ) and leaf width (ANOVA,  $p < 0.0001$ ,  $F = 242.95$ ). PC1

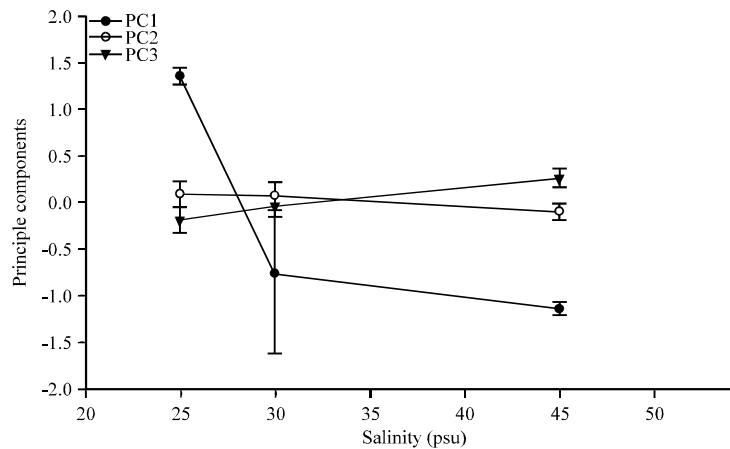


Fig. 6: *Halophila beccarii* responses and increased salinity: principal components 1 (PC1), 2 (PC2) and 3 (PC3) summarizing most of the original variation (ca. 93%) in the data set. All values are Means±SE (25 psu, n = 69; 30 psu, n = 38; 45 psu, n = 56)

Table 1: The increased salinity (25-45 psu) experiments. Principal components (PC1, PC2 and PC3), their eigenvalues, the amount of variation explained by each component and loading of each response variable on the three principal components explaining more than 93% of the total variance in the data set

Component	PC1	PC2	PC3
Eigenvalues	1.783	1.002	0.927
Variance explained (%)	44.563	25.060	23.169
Loading			
Leaves length	0.889	0.049	-0.261
Leaves width	0.911	-0.031	-0.148
Petiole length	0.328	-0.607	0.722
Leaves per shoot	0.231	0.764	0.562

was significantly higher at 25 psu, than all other salinity and lowest of PC1 was observed at 45 psu (Fig. 6). The experiments showed increased salinity gave effects toward leaf length and leaf width where the leaves observed to be shorter and narrower at maximum salinity of 45 psu. PC2 was mainly contributed by number of leaves per shoot (ANOVA,  $p = 0.96 > 0.05$ ,  $F = 0.04$ ) which was not affected by salinity. PC3 (petiole length) (ANOVA,  $p = 0.12 > 0.05$ ) also does not showed any effect toward increasing in salinity. PC2 and PC3 remain constants toward the increased salinity.

Factor analysis on the parameters observed from decreased salinity (25-0 psu) experiments resulted in three main principle components with eigenvalues approximately one or greater than one together explained about 85% of total variance in the original response variable (Table 2). The loadings (correlations) of each response variable on the three components (Table 2) showed leaf length and leaf width contributed almost equally with all parameters being positively correlated with PC1. PC1 varied significantly with salinity, leaf length (ANOVA,  $p < 0.0001$ ,  $F = 23.55$ ) and leaf width (ANOVA,  $p < 0.0001$ ,  $F = 130.05$ ). PC1 significantly higher at 25 and 20 psu the lowest of PC1 was observed within range of 4-0.52 psu (Fig. 7). There was a sudden decreased in the PC1 at the salinity from 20-15 psu. At 0 psu, PC1 showed good respond by having longer leaf length and wider leaf length, respectively. PC2 which was mainly contributed by number of leaves per shoot was not affected by salinity (ANOVA,  $p = 0.83 > 0.05$ ,  $F = 0.55$ ). PC3 (petiole length) was also affected by salinity (ANOVA,  $p < 0.0001$ ,  $F = 5.65$ ) being significantly shorter at 25 psu compared to other salinities (Fig. 7).



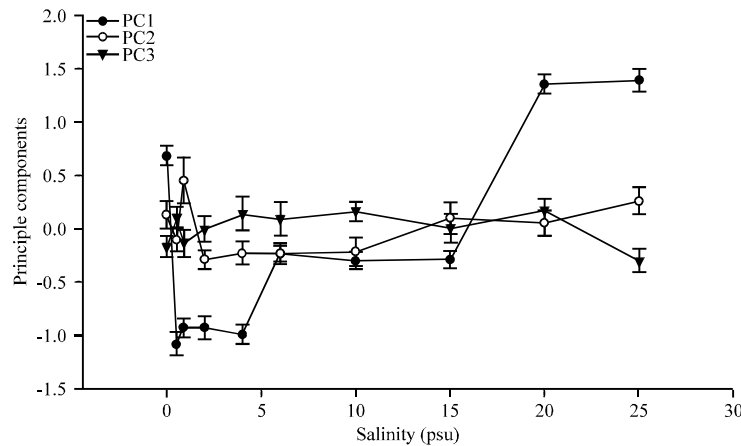


Fig. 7: *Halophila beccarii* responses and decreased salinity: principal components 1 (PC1), 2 (PC2) and 3 (PC3) summarizing most of the original variation (ca. 85%) in the data set. All values are Means $\pm$ SE (25 psu, n = 69; 20 psu n = 64; 15 psu, n = 54; 10 psu, n = 54; 6 psu, n = 56, 4 psu, n = 59; 2 psu, n = 35; 0.90 psu, n = 40; 0.52 psu, n = 31; 0 psu, n = 69)

Table 2: The decreased salinity (25-0 psu) experiments. Principal components (PC1, PC2 and PC3), their eigenvalues, the amount of variation explained by each component and loading of each response variable on the three principal components explaining more than 85% of the total variance in the data set

Component	PC1	PC2	PC3
Eigenvalues	1.442	1.031	0.923
Variance explained (%)	36.038	25.785	23.071
Loading			
Leaves length	0.778	-0.012	-0.358
Leaves width	0.807	0.187	-0.099
Petiole length	0.430	-0.378	0.814
Leaves per shoot	0.023	0.924	0.349

Despite the hyposaline conditions, *H. beccarii* leaf dimensions remained at a relatively stable and within the range of those from the fields. Our results suggest that *H. beccarii* tolerated hyposaline conditions better than hypersaline conditions. According to Jagtap and Untawale (1981), gradual increase in salinity from 0-35 psu (when the monsoon ends) have no harmful effect on the growth of *H. beccarii*. It was also observed that new growth occurred when salinity was reduced to minimum by precipitation and flowering detected when salinity was 5.38 psu. Most seagrasses are thought to be more sensitive to increased salinity (Ogata and Matsui, 1965; Biebl and McRoy, 1971; Zieman, 1975; Adams and Bate, 1994; Kamermans *et al.*, 1999; Van Katwijk *et al.*, 1999). The cause of these effects may be due to toxicity by salt excess (Zhu, 2001), elevated metabolic cost to maintain internal ionic balance (Sibly and Calow, 1989), or negative alterations in the photosynthetic and respiratory rates (Ogata and Matsui, 1965; Biebl and McRoy, 1971; Kraemer *et al.*, 1999). The species may therefore be similar to *Ruppia*, *Zannichellia* and *Potamogeton pectinatus* that can tolerate very sudden and very large fluctuations in salt content (Den Hartog, 1970). Although, *H. beccarii* has wide salt tolerance and can penetrate purely marine environment (salinity >30 psu, Muta Harah *et al.*, 1999; Abu Hena *et al.*, 2007; Abu Hena and Short, 2009), it is generally restricted to mangroves (Den Hartog, 1970; Jagtap and Untawale, 1981) and brackish water areas (Van Tien, 1998; Muta Harah *et al.*, 2003).

## CONCLUSION

*Halophila beccarii* survived and continue to propagate at high salinity (i.e., 45 psu beyond the high field salinity of 35 psu) and low salinity until the level of freshwater. The respond of above ground parts e.g., leaf dimensions remained at a relatively stable and within the range of those from the fields. The results of the culture experiments suggest *H. beccarii* tolerated hyposaline conditions better than hypersaline conditions. The ability to grow under 0 psu or freshwater condition for more than 303 days is first reported under laboratory culture.

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