Vietnam Academy of Science and Technology

Vietnam Journal of Marine Science and Technology

journal homepage: vjs.ac.vn/index.php/jmst

Response to salinity of the submerged aquatic vegetation species *Najas indica* (Willd.) Cham.

Dang Thi Le Xuan[1,*], Phan Thi Thuy Hang[2], Ton That Phap[2], Hoang Cong Tin[2], Luong Quang Doc[2]

[1]*Department of Biology, Hue University of Education, Thua Thien Hue, Vietnam*
[2]*Faculty of Biology, Hue University of Science, Hue University, Thua Thien Hue, Vietnam*
[*]E-mail: bplexuan@gmail.com

Received: 17 May 2021; Accepted: 30 September 2021

ABSTRACT

Najas indica (Willd.) Cham is known as a freshwater species of submerged aquatic vegetation. However, this species is widely distributed in both freshwater and brackish environments. This study examined the survival, growth rate and morphological performance of *N. indica* collected from the Cau Hai lagoon (Thua Thien Hue) against different salinity treatments in a mesocosm experiment to determine the optimal salinity for the species. The results showed significant effects of different salinities on survival rates, growth, biomass, and the morphological characteristics of *N. indica*. The species could survive and continue growing at 0–15 ppt but died completely at 20 ppt and 25 ppt after the first week of the 8-week experiment. Leaf length tended to be shorter in higher salinity. Shoot length, the number of internodes and branches per shoot, biomass reached the highest values at 5 ppt and 10 ppt. These suggested that the optimal salinity of the *N. indica* was at a range of 5–10 ppt. Study results were informative to explain the distribution change of the freshwater originated hydrophyte *N. indica* in lagoon environments in Vietnam.

Keywords: *Najas indica*, salinity, lagoon, submerged aquatic vegetation.

Citation: Dang Thi Le Xuan, Phan Thi Thuy Hang, Ton That Phap, Hoang Cong Tin, and Luong Quang Doc, 2022. Response to salinity of the submerged aquatic vegetation species *Najas indica* (Willd.) Cham. *Vietnam Journal of Marine Science and Technology*, *22*(1), 29–35. https://doi.org/10.15625/1859-3097/16072

INTRODUCTION

Submerged aquatic vegetations (SAV) include vascular and non-vascular plants growing submerged entirely in the water environment. SAV communities are critical components, supplying habitats, foods, shelters, and nursery grounds for numerous animals (invertebrates, shellfishes, fishes, and waterfowl). Therefore, they enhance biodiversity for coastal ecosystems [1, 2]. In addition, SAV joins in the nutrient cycle, promotes sediment stabilization, and prevents erosion from wave activities and storms [2]. Unfortunately, the area of distribution of SAV meadows has seriously declined worldwide, with 65% of seagrass species and 48% of other SAV species being lost [3]. The primary reasons were environmental pollution, eutrophication, physical impacts created by anthropogenic activities, and natural disasters [4, 5]. *Najas indica* (Willd.) Cham. is one of the freshwater-originated submerged aquatic vegetation. The species are distributed commonly in estuaries, rice fields, aquaculture ponds, and lagoons of Vietnam, India, Sri Lanka, and Burma [6–9]. *N. indica* showed a wide salinity tolerance, i.e., growing in freshwater and brackish water bodies. However, salinity change was known as one of the major factors affecting its population distribution [10]. The salinity fluctuation also affects morphological characters of SAV, e.g., seagrass *Halophila ovalis* [11]. Some plants have a salt removal mechanism by increasing salt accumulation in older leaves and promoting the aging process and defoliation [12], whereas *N. indica* copes with salinity stresses through means that enhance proline concentration [13] and activity of antioxidant enzymes [14].

Previous studies showed that *N. indica* is distributed in estuaries and coastal lagoons with a wide range of salinity from 0 ppt to 21 ppt [6, 8, 10, 15], for example, in the Tam Giang lagoon, Cau Hai lagoon (Thua Thien Hue, Vietnam), and Chilka lagoon (India). However, the responses of *N. indica* against salinities were not determined. Therefore, this study examined the survival, growth rate and morphological performance of *N. indica* collected from Cau Hai lagoon (Thua Thien Hue) in a mesocosm experiment against different salinities from freshwater to polyhaline corresponding to lagoon environmental conditions of Vietnam. The findings could explain the distribution fluctuations of this species in the natural environments.

MATERIALS AND METHODS

Collecting plants and sediments

N. indica plants and sediments for the experiment were collected in the Cau Hai lagoon (16°19’22”N, 107°50’59”E) belonging to the Tam Giang - Cau Hai lagoon system (Thua Thien Hue province). The lagoon has a salinity range of 0.2–29.9 ppt, a water temperature range of 27.4–36.1°C, and a water depth range of 0.45–2.4 m [8].

N. indica shoots and sediments were collected in the same area where *N. indica* meadow was dense and abundant in the Cau Hai lagoon. The selected sampling site has a depth of 1.1 m (measured by Handy Depth Sounder Hondex PS-7) and a salinity of 8.7 ppt (measured by Horiba U-50, Japan). *N. indica* plants with adventitious roots on shoots were collected and kept in a plastic box with a little lagoon water. The sediment of about 5 cm surface layer was taken by a spoon sampler and placed in plastic containers (L26 × W20 × H18 cm). All plant and sediment materials were transported to the outdoor mesocosm experimental area in Hue University of Sciences within 24 h for further experiment.

Experimental design and data collection

The outdoor mesocosm experiment was performed for eight weeks (20 March to 20 May 2019) in the outdoor culture system with a transparent roof and natural light conditions. The experimental system consisted of 6 glass aquarium tanks (L70 × W40 × H44 cm), 24 plastic boxes (L26 × W20 × H18 cm), and six water filters for filtering and circulating water. The freshwater (tap water) and sea salt were used to prepare the water for different salinity treatments. Based on the distribution of *N. indica* in the field [6, 8, 9, 15], there were six salinity treatments, including 0 ppt (freshwater), 5 ppt (oligohaline), 10 ppt, 15 ppt (mesohaline), and 20 ppt, 25 ppt (polyhaline) (Fig. 1).

Sediments were sieved to a mesh diameter of 0.5 mm to remove coarse gravels and plant materials. The sediments were the left in plastic boxes to make a 5 cm substrate layer. *N. indica* shoots were carefully selected and planted immediately without an acclimatization period. Thirty *N. indica* intact shoots (approximate 6 cm in length) bearing adventitious roots were chosen and grown into the plastic box. Four replicated planted boxes of *N. indica* shoots were placed in each glass aquarium tank and maintained ambient salinity treatment. The morphological parameters, including the number of internodes per shoot, branches per shoot, shoot length, and leaf length, were determined every week. The number of internodes and branches per shoot were counted; leaf length and shoot length were measured using a Digital Caliper (Minutolo 530-104, Japan, 0–150 mm, accuracy: ±0.05 mm) [16]. The number of survival shoots was counted in the first week. The growth rate of shoots was determined by measuring the main shoot length every week using a ruler (mm).

Salinity treatments were kept constantly by checking every three days using a multi-parameter water quality monitoring device (Horiba U50, Japan) and adjusting immediately; the water column was maintained at 40 cm height in all tanks during the experiment. The experiment was conducted in outdoor condition with a transparent roof; thus, air temperature and light intensity depended on nature; air temperature from 25.5°C to 37.8°C, and the light intensity measured at noon reached 39,700 lux (by Handy Lux Meter).

After eight weeks of the experiment, *N. indica* was harvested and transported into the laboratory to measure the biomass. After cleaning carefully with fresh water to remove sediment, epiphyte, and salt, the plants were oven-dried at 60°C to constant weight, then weighed to determine biomass [17].

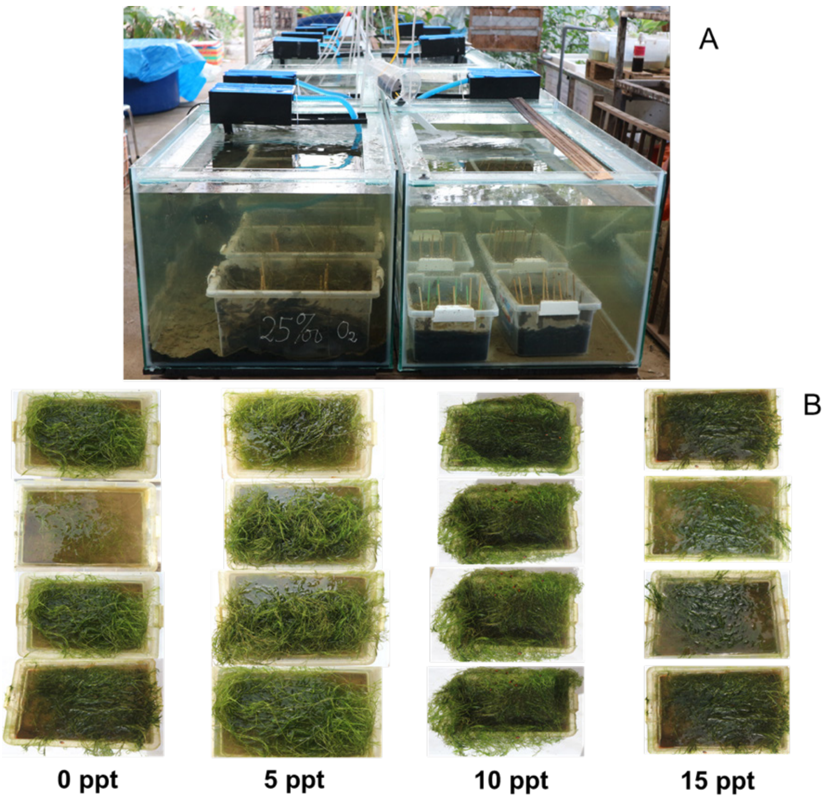

Figure 1. The applied culture system (A); and the *N. indica* shoots developed in salinity treatments after eight weeks (B)

Data analysis

Analyses were performed using IBM SPSS 20.0. First, all variables were checked for normality by the Shapiro-Wilk Test. Then, the significant difference of variables was tested by the Friedman ANOVA test and Wilcoxon matched-pairs post hoc test or One-Way ANOVA and Tukey post hoc test depending on the variable's normality.

RESULTS AND DISCUSSION

Survival and growth rates of *Najas indica* in different salinity conditions

The survival of *N. indica* over time in experimental salinities differed significantly among salinity treatments (ANOVA, $F = 189.7$, $p < 0.0001$). After the first week, plants died off in 20 ppt and 25 ppt salinity treatments but survived in the remaining salinities. The survival rate was 75.5 ± 2.5%, 70.7 ± 5.3%, 65.5 ± 2.1%, and 23.8 ± 1.4% in the salinities 0, 5, 10 and 15 ppt, respectively, (Fig. 2). The survival rate of *N. indica* shoots tended to decrease with increasing salinity.

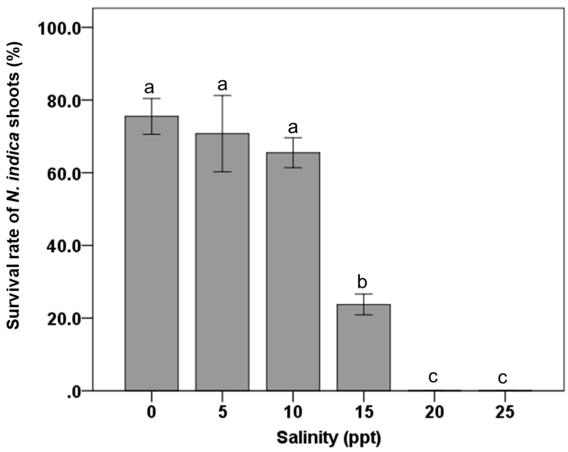

Figure 2. The survival rate of *N. indica* shoots in the salinity treatments after eight weeks (mean ± *SE*, $n = 4$)

N. indica is a freshwater species that has distributed and adapted to the brackish water environment in the Cau Hai lagoon. Compared to another freshwater SAV species, *Vallisneria americana* also commonly found in estuaries and brackish lagoon [18], *N. indica* was able to adapt to the higher salinity conditions. This result showed that the *N. indica* plants only survived from 0 ppt to 15 ppt at low salinity conditions.

Most plants were more susceptible to high salinity than low salinity. The high salinity condition could lead the plant to stop the growth and even die [12]. Our experiment recorded that the *N. indica* shoots ultimately died at salinities 20 ppt and 25 ppt after the first week while growing well at the range of salinity from 0 ppt to 15 ppt.

The growth rate of *N. indica* showed a significant difference between salinity treatments. It was highest at 10 ppt (13.2 ± 0.7 mm day^{-1}) and lowest at 15 ppt (8.9 ± 0.6 mm day^{-1}), [*FA*, χ^2 (*df*3, $n = 240$) = 44,299, $p < 0.0001$]. However, the growth of the *N. indica* in salinities tended to increase rapidly after 2–4 weeks and decrease significantly in the weeks followed, Fig. 3.

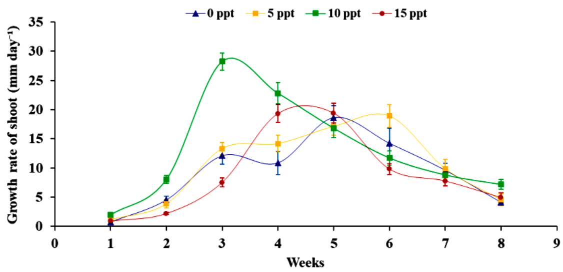

Figure 3. The growth rate (mm day^{-1}) of *N. indica* shoots in the salinity treatments during eight weeks (mean ± *SE*, $n = 240$)

The survival and growth rates of *N. indica* in this experiment showed that the species favored salinity from 0–15 ppt. However, in the field, *N. indica* was likely to be tolerant of relatively higher salinity, below 20 ppt (in Cau Hai lagoon) [9, 10], even up to 21 ppt (in Thuy Tu lagoon) [15], possibly the salinity in the field is gradually increasing, so the plant can adapt little by little. On the other hand, the distribution of *N. indica* recorded in the area at a high salinity of about 20 ppt is very rare with low biomass and coverage. Therefore, the narrower favorable range of saltiness in this study could help explain the distribution and abundance of *N. indica* in nature, making it more effective for monitoring and navigating *N. indica* meadows in similar ecological water bodies.

Morphological characters

The results showed that there were significant differences of the morphological characteristics of the *N. indica* in salinity treatments about shoot length (ANOVA, $F = 67.4$, $p < 0.0001$), leaf length (ANOVA, $F = 20.5$, $p < 0.0001$), the number of branches per shoot (ANOVA, $F = 23.9$, $p < 0.0001$), and the number of internodes per shoot (ANOVA, $F = 34.5$, $p < 0.0001$), Figs. 4A–4D).

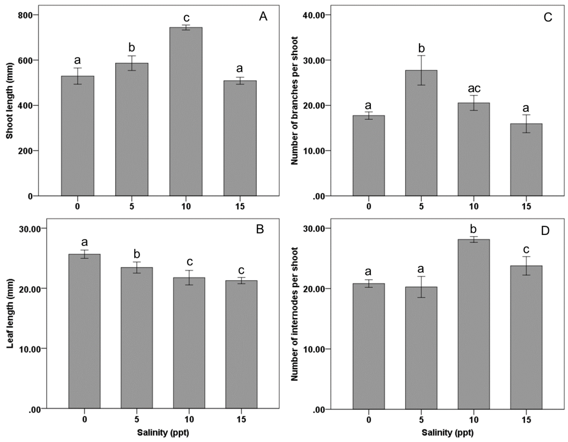

Figure 4. (A) shoot length; (B) leaf length; (C) number of branches per shoot; (D) number of internodes per shoot of *N. indica* in the salinity treatments after eight weeks
(mean ± SE, $n = 30$)

Shoot length was highest at salinity 10 ppt (529.2 mm) and lowest at 0 ppt and 15 ppt (508.6–586.1 mm). The number of branches per shoot was highest at 5 ppt salinity (27.7) and lowest at 0 ppt and 5 ppt salinity (15.9–17.7), while the number of internodes per shoot was highest at 10 ppt (28.1) and lowest at 0 ppt and 5 ppt (20.3–20.8). Leaf length tended to decrease gradually with salinity, reaching its highest at 0 ppt (25.6 mm) and lowest at 10 ppt and 15 ppt (21.3–22.7 mm).

Thus, both shoot length and the number of internodes per shoot peaked at the salinity 10 ppt while the number of branches per shoot reached the highest value at 5 ppt. The shoot length of *N. indica* in this experiment was significantly shorter than the recorded in the natural environment. In Cau Hai lagoon, *N. indica* meadows are dense like an "underwater tropical forest" with shoot lengths up to 156 cm [9, 10]. The experimental period was probably conducted for only 8 weeks with the limited living space of the glass tanks in the culture system. Therefore, the growth of the *N. indica* might be restricted. However, the species still exhibited a range of suitable salinity for growth, which is consistent with the information recorded in natural conditions.

Biomass accumulation

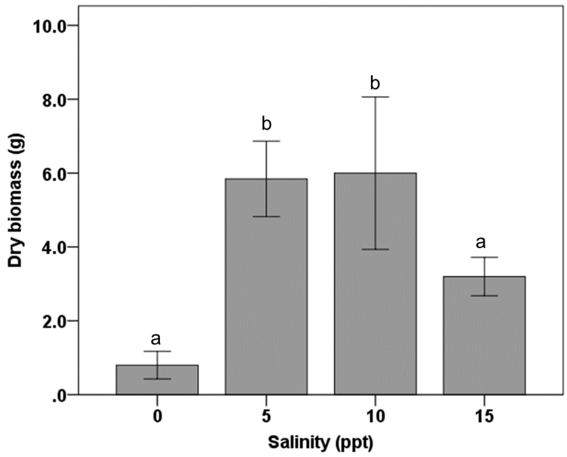

Figure 5. Biomass of *N. indica* in the salinity treatments after eight weeks

Biomass accumulation reflects the capacity of development and growth of plants, which is one of the essential indicators used to evaluate primary productivity and abundance of population. At the end of the experiment, we determined dry biomass of *N. indica* at the salinity treatments of 0, 5, 10, and 15 ppt; results were 0.8 ± 0.2 g, 5.8 ± 0.5 g, 5.9 ± 1.1 g, and 3.1 ± 0.3 g, respectively, Fig. 5. There was a significant difference in biomass between salinity treatments (ANOVA, $F = 17$, $p < 0.0001$). The biomass of *N. indica* obtained the highest values at 5 ppt and 10 ppt and the lowest at 0 ppt. These results were consistent with distribution records of the species in the natural environment; for instance, *N. indica* meadows in the Cau Hai lagoon had high coverage and biomass in the salinity range of 4–15 ppt [9, 10].

CONCLUSION

The freshwater-originated submerged aquatic plant *N. indica* from the Cau Hai lagoon could survive and continue growing at the salinity of 0–15 ppt, but ultimately died at the salinities 20 ppt and 25 ppt in mesocosm condition. The responses of *N. indica* in survival and growth differed significantly between salinity treatments from freshwater to polyhaline. The species' growth rate, biomass accumulation, and morphological characteristics (shoot length, number of branches, number of internodes) performed best at 5 ppt and 10 ppt. The optimal salinity of the *N. indica* was at a range of 5–10 ppt. The experimental results clarify the distribution and abundance of the *N. indica* meadows in the brackish lagoons, which may be impacted if salinity increases.

REFERENCES

[1] Carpenter, S. R., and Lodge, D. M., 1986. Effects of submersed macrophytes on ecosystem processes. *Aquatic botany*, *26*, 341–370. https://doi.org/10.1016/0304-3770(86)90031-8

[2] Short, F., Carruthers, T., Dennison, W., and Waycott, M., 2007. Global seagrass distribution and diversity: a bioregional model. *Journal of Experimental Marine Biology and Ecology*, *350*(1–2), 3–20. https://doi.org/10.1016/j.jembe.2007.06.012

[3] Lotze, H. K., Lenihan, H. S., Bourque, B. J., Bradbury, R. H., Cooke, R. G., Kay, M. C., Kidwell, S. M., Kirby, M. X., Peterson, C. H., and Jackson, J. B., 2006. Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science*, *312*(5781), 1806–1809. doi: 10.1126/science.1128035

[4] Duarte, C. M., 2002. The future of seagrass meadows. *Environmental Conservation*, *29*(2), 192–206. https://doi.org/10.1017/S0376892902000127

[5] Short, F. T., Koch, E. W., Creed, J. C., Magalhaes, K. M., Fernandez, E., and Gaeckle, J. L., 2006. SeagrassNet monitoring across the Americas: case studies of seagrass decline. *Marine Ecology*, *27*(4), 277–289. https://doi.org/10.1111/j.1439-0485.2006.00095.x

[6] Shaw, B., Rout, N., Barman, B., Choudhury, S., and Rao, K., 2000. Distribution of macrophytic vegetation in relation to salinity in the Chilka lake, a lagoon along east coast of India. *Indian Journal of Marine Sciences*, *29*(2), 144–148.

[7] Triest, L., 1988. A revision of the genus Najas L. (Najadaceae) in the Old World (Najadaceae). *Koninklijke Academie voor Overzeese Wetenschappen. Klass voor Natuur-en Geneeskundige Wetenschappen. Verhandelingen in-8. Nieuwe Reeks*.

[8] Phan, T. H., Stiers, I., Nguyen, T. H., Pham, T. T., Ton, T. P., Luong, Q. D., and Triest, L., 2018. Spatial and temporal distribution of submerged aquatic vegetation in a tropical coastal lagoon habitat in Viet Nam. *Botanica Marina*, *61*(3), 213–224. https://doi.org/10.1515/bot-2017-0107

[9] Phan, T. T. H., 2018. Submerged Aquatic Vegetation in a Tropical Coastal Lagoon Environment: Dynamics and Resilience Strategy.

[10] Dang, T. L. X., Truong, T. H. T., Hoang, L. T. L., Tran, T. T. S., Ton, T. P., Phan, T. T. H., Luong, Q. D., 2020. Morphological characteristics and distribution of *Najas indica* (Wild.) Cham. in Cau Hai lagoon, Thua Thien Hue province. *Hue University Journal of Science: Natural Science*, *129*(1A), 107–114. https://doi.org/10.26459/hueuni-jns.v129i1A.5638

[11] Benjamina, K. J., Walker, D. I., McComb, A. J., and Kuo, J., 1999. Structural response of marine and estuarine plants of *Halophila ovalis* (R. Br.) Hook. f. to long-term hyposalinity. *Aquatic Botany*, *64*(1), 1–17. https://doi.org/10.1016/S0304-3770(98)00103-X

[12] Zhu, J. K., 2001. Plant salt tolerance. *Trends in Plant Science*, *6*(2), 66–71. https://doi.org/10.1016/S1360-1385(00)01838-0

[13] Rout, N. P., Tripathi, S. B., and Shaw, B. P., 1997. Effect of salinity on chlorophyll and proline contents in three aquatic macrophytes. *Biologia Plantarum*, *40*(3), 453–458. https://doi.org/10.1023/A:1001186502386

[14] Tripathi, R. D., Singh, R., Tripathi, P., Dwivedi, S., Chauhan, R., Adhikari, B., and Trivedi, P. K., 2014. Arsenic accumulation and tolerance in rootless macrophyte *Najas indica* are mediated through antioxidants, amino acids and phytochelatins. *Aquatic Toxicology*, *157*, 70–80. https://doi.org/10.1016/j.aquatox.2014.09.011

[15] Tran, N. Q. A., Luong, Q. D., 2012. Current status of submerged aquatic vegetation in Con Chim aquaculture protected area, Tam Giang - Cau Hai lagoon system. *Hue University Journal of Science*, *73*(4), 9–17.

[16] Short, F. T., and Duarte, C. M., 2001. Methods for the measurement of seagrass growth and production. *Global Seagrass Research Methods*, *2001*, 155–198.

[17] Short, F. T., Duarte, C. M., Shorts, F. T., Coles, R., and Short, C. A., 2001. Methods for the measurements of seagrass abundance and depth distribution. *Global Seagrass Research Methods*, *155*, 182.

[18] Doering, P. H., Chamberlain, R. H., and Haunert, D. E., 2002. Using submerged aquatic vegetation to establish minimum and maximum freshwater inflows to the Caloosahatchee Estuary, Florida. *Estuaries*, *25*(6), 1343–1354. https://doi.org/10.1007/BF02692229