Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(1): 1443 – 1454 (2024)
www.ejabf.journals.ekb.eg

Improvement of the Performance of *Enhalus acoroides* Seedling in Field Using Bamboo Boxes: Short Communication

Aditya Hikmat Nugraha[*1], Tri Apriadi[2], Jelita Rahmahidayati[1], Anifa Yuniasri[1], Zuhrotul Lailiah[1]

[1]Department of Marine Science, Faculty of Fisheries and Marine Sciences, Raja Ali Haji Maritime University, Indonesia

[2]Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, Raja Ali Haji Maritime University, Indonesia

[*]Corresponding Author: adityahn@umrah.ac.id

ARTICLE INFO

Article History:
Received: May 28, 2023
Accepted: Dec. 29, 2023
Online: Feb. 14, 2024

Keywords:
E.acoroides,
Generative,
Restoration,
Seagrass,
Seed

ABSTRACT

The restoration of the seagrass ecosystem is one of the solutions to address the decline in the seagrass ecosystems worldwide. The restoration of seagrass ecosystems using generative techniques has several advantages including protecting seagrass diversity in nature. The use of seagrass seeds in ecosystem restoration using generative techniques has several challenges including the low survival rate due to the hydrodynamic activity and predation of seagrass seeds. In this study, an attempt was made to use *E. acoroides* seeds from the fruit to be planted in seagrass ecosystems with bamboo boxes for seed protection. Seagrass nurseries were carried out in the field in two locations with different environmental characteristics. The first was a sand substrate, while the second was a sandy mud substrate. The results showed that all the seeds planted in the field grew at the start of planting. The survival rate of seagrass seedlings produced in this study was better than previous studies that carried out seagrass seeding directly in the field. Seagrass seeds planted in the second location had better growth and morphometrics than the first station. Based on the study's results, it was deduced that storing seagrass seeds in boxes can be used as an alternative method for survival rate improvement in the seagrass seedling process.

INTRODUCTION

Seagrass ecosystems are part of the coastal zone and have an essential ecosystem service supporting life processes **(Cullen-Unsworth *et al.*, 2014)**. Some marine organisms depend on seagrass ecosystems, such as fish, shellfish, gastropods and dugongs. The ecosystem services of seagrass ecosystems include being a habitat for marine biota, a provider of food sources, a source of blue carbon and other roles **(Kawaroe *et al.*, 2016; Nugraha *et al.*, 2019; Nugraha *et al.*, 2021)**.

It was reported that the area of seagrass ecosystems globall continues to decrease by 2.82% per year **(Stankovic *et al.*, 2021)**. One area that has the potential to have the highest seagrass ecosystem area in the world is the Indonesian waters **(Thorhaug *et al.*,**

2020). The area of seagrass ecosystems in several regions of Indonesia has decreased **(Unsworth *et al.*, 2018)**. The increase in Indonesia's seagrass ecosystem threat is caused by human activity around coastal waters. It impacts the sustainability of the surrounding ecosystems. These activities include developing coastal areas, mining, capturing fisheries, oil pollution and deforestation **(Unsworth *et al.*, 2018)**. Currently, seagrass ecosystems in Indonesia are in a moderate status, requiring management efforts to maintain their sustainability **(Hernawan *et al.*, 2021)**.

Several efforts have been exerted to address the decline in seagrass. Seagrass ecosystem restoration is part of a nature-based solution to the problem of the decreasing of seagrass ecosystem area **(Rifai *et al.*, 2022)**. One method of seagrass restoration that has been extensively studied and practised is the transplantation technique **(Orth *et al.*, 2006; van Katwijk *et al.*, 2009)**. The seagrass transplantation technique moves seagrass vegetative seedlings from donor areas to new habitat that have decreased in seagrass area **(Williams *et al.*, 2017)**. Remarkably, restoring seagrass requires a large number of seagrass vegetative seedlings; therefore, there are weaknesses in carrying out seagrass restoration using seagrass transplantation techniques including reducing the number of seagrass in the donor area. Hence, a recovery time is required for the donor area and reducing seagrass genetic diversity **(Williams & Orth, 1998; Orth *et al.*, 2006; van Katwijk *et al.*, 2016)**.

Another technique that can be done to restore seagrass ecosystem is via using a generative technique. This restoration method using generative (seedling) techniques will not reduce the number of seagrasses in the donor area **(Ambo-Rappe & Yasir, 2015; Escandell-Westcott *et al.*, 2023)**. This generative technique is carried out by utilizing the potential of seagrass seeds. Furthermore, a seedling process is needed, which aims to produce seeds that can be planted in the field to restore seagrass. One of the advantages of the seagrass ecosystem restoration method with the generative technique is less damaging for the seagrass donor site than taking adult plants **(Orth *et al*., 2006; Ambo-Rappe & Yasir, 2015)**.

In previous studies, efforts to use seagrass seeds in the restoration process began with the seedling process in the laboratory. After being old enough, seeds would be ready for planting in the field **(Ambo-Rappe *et al.*, 2019)**. The process of sowing seagrass seedling in the laboratory requires several equipment, treatments, media and periodic monitoring before the seeds can be planted properly in the field **(Ambo-Rappe & Yasir, 2015; Li *et al*., 2021; Nugraha *et al.*, 2022)**. Another research has been made by directly spreading seeds in the field, but a low survival rate was recorded upon using this method **(Yu *et al.*, 2019; Ambo-Rappe, 2022)**. Factors affecting the survival rate of seeds when planted directly in the field include: predation by biota, hydrodynamics process and buried sediment **(Campbell, 2016; Ambo-Rappe, 2022)**. With the aim of planting seeds directly in the field, yielding a reasonable survival rate, another effort is needed. In their study, **Unsworth *et al* (2019)** attempted to plant seagrass seeds directly in the field, where the seeds were put in a fiber hessian bag, and they succeeded in increasing the seed survival rate in the *Z.marina* restoration.

E. acoroides is a type of seagrass that belongs to the persistent seagrass group and is generally found in the Indonesian waters **(Kilminster *et al.*, 2015; Hernawan *et al.*, 2021)**. This research examined the performance of the survival rate and growth of *E. acoroides* seeds in the field by storing the seeds in bamboo boxes. It is worth noting that, the Indonesians have a history related to the use of bamboo since bamboo is easy to find and it grows in abundance in Indonesia **(Sujarwo, 2018)**.

MATERIALS AND METHODS

1. Study site

This research was conducted in the seagrass ecosystem located on Dompak Island, west of Bintan Island, Riau Islands. Two stations were selected for this study (Fig 1). The first is in the North of Dompak Island, facing Bintan Island. While, the 2nd is in the South of Dompak Island, facing the open ocean. The two stations in the current study are seagrass ecosystems consisting of several species of seagrass and the habitat of *E. acoroides.*

Fig. 1. Research location

Based on the characteristics of the substrate at each station, the first station has sandy mud substrate, and the second has gravel sandy substrate (Table 1). The water temperature during the observation was in the range of 26- 28^{o}C. The types of substrate found in both stations support seagrass life **(Nugraha *et al.*, 2022)**. Surface current conditions in the second station generally tend to be stronger compared to the 1st station.

The seagrass seeds used in this study were gathered from a seagrass fruit growing around the coast of Malang Rapat, Bintan Island. Ripe seagrass fruit was cut from the stalk, and then the seeds were removed from the fruit. Seeds were cleaned and taken to the research location to be planted.

2. Seedling process

Seagrass seeds samples cleaned were stored in bamboo boxes. The bamboo box used in the present study had dimensions of 25 x 25 x 15cm. In each bamboo box, 30 seagrass seeds were placed (Fig. 2). The substrates used in the bamboo box were those taken from each research location. There were two bamboo boxes at each study site, with a total of 60 seagrass seeds under observation. The observation process was carried out for 8 weeks. The parameters observed included the survival rate and growth of leaves and roots. The observation process was carried out every two weeks.

Fig. 2. Seed of *E. acoroides* in bamboo box

3. Data analysis

Comparison of the survival rate and growth of seagrass seeds in each research location was analyzed using the one-way ANOVA.

RESULTS

Measurements of the survival rate were carried out to find how far seagrass seeds can survive in their habitat. Based on 8- week observations, the survival rate of seagrass seeds of *E. acoroides* in each study location was decreased (Fig 3). The survival rate of seagrass seeds in the last week of observation in station 1 was 70%, and for station 2, it was 43%. One-way ANOVA test results showed no significant difference in the survival rates for the two study locations ($P > 0.05$)

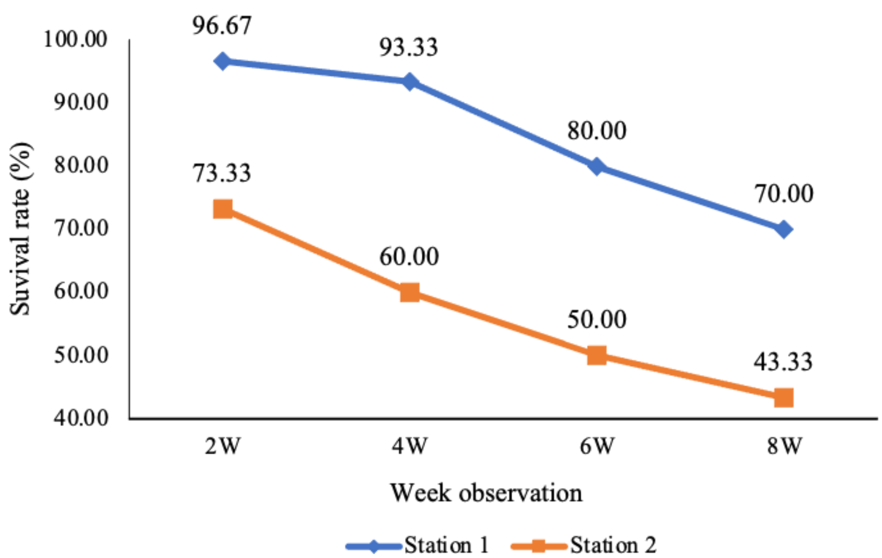


Fig. 3. Survival rate of seagrass seedling

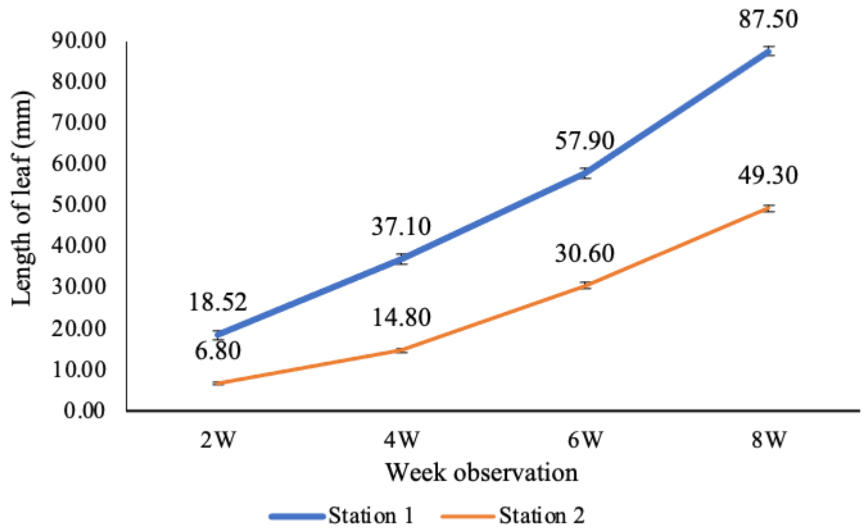


Fig. 4. Length of leaf seagrass seedling

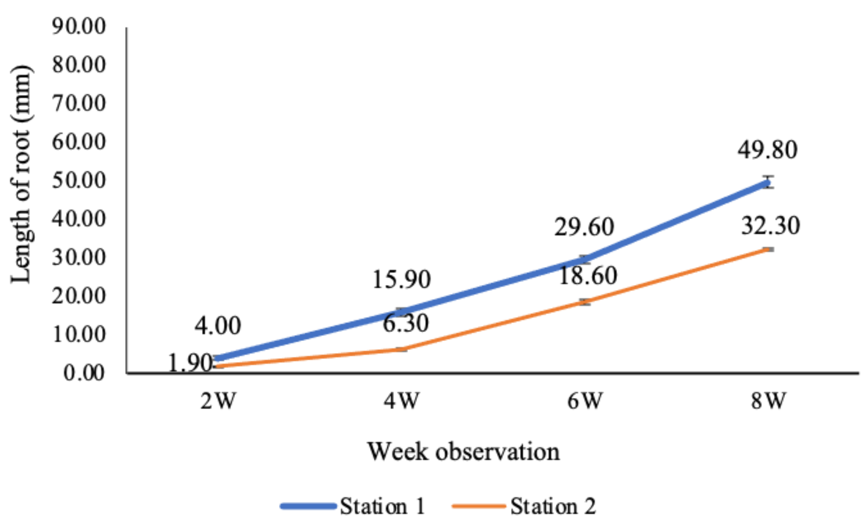
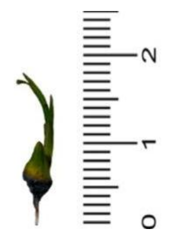
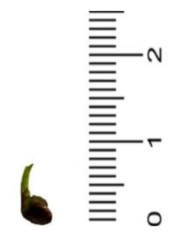
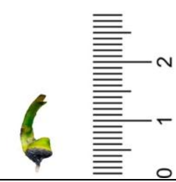
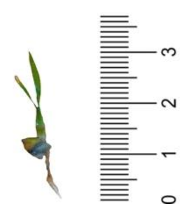
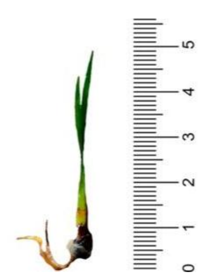


Fig. 5. Length of root seagrass seedling

The growth of leaves and roots of seagrass seeds continued to increase during the observation process (Figs. 4, 5). The highest growth of leaves and roots was observed in station 1. Based on the one-way ANOVA test, no significant difference was detected in the growth of leaves and roots in the two stations (*P*> 0.05). Visually, the condition of seagrass seeds during the observation process in each study location is presented in Table (2).

Table 2. Growth performance of *E. acoroides* seedling

Week observation	Station 1	Station 2
2nd		
4th		
6th		
8th		

DISCUSSION

All seagrass seeds in the field underwent a germination process. This indicates a good development of seagrass seeds. Several previous studies regarding seagrass restoration in subtropical areas using *Zostera marina* seeds showed a low percentage of seeds that were germinated **(Orth *et al*., 2008; Golden *et al*., 2010)**. The survival rate decreased during the observation process, and several factors had a role in this decrease, among which is the predation by marine biota. **Ambo-Rappe (2022)** stated that some marine invertebrates are predators of seagrass seeds. The high nutritional content and easy-to-digest properties of seagrass seeds make them much preferred by marine biota; moreover, several types of predators prey on seagrass seeds including the crustacean group **(Orth *et al.*, 2007)**. Beside predation, the substrate type affects seagrass seeds' survival. Substrate conditions are related to wave exposure conditions. On the other hand, water with fine substrate conditions has moderate wave exposure, providing a good support for the life and growth of seagrass seeds. In this study, the survival rate was high in locations with fine sand substrates. This finding is in line those of **Balestri *et al.* (2019)** and **Ambo-Rappe (2022)** who recorded the highest survival rate in seagrass seeds on fine substrate. Early life of seagrass seeds is a critical stage where seagrass seeds need a substrate to settle and grow roots **(Nugraha *et al*., 2022)**.

Seed survival rate in this study is higher compared to a previous study conducted by **Ambo-Rappe (2022)** who detected a survival rate with a value below 10% on coarse substrates and 70% on fine sand substrates on the 40th day of observation. **Ambo-Rappe and Yasir (2015)** noted a seed survival rate below 40% on the 11th day of observation. Both studies were carried out by spreading the seeds directly in the field. Planting *Z. marina* seeds directly in field using the Buoy Developed Seeding (BuDS) method cannot be determined to be successful since 99% of the seagrass seeds were lost **(Govers *et al*., 2022)**. Another study concerned with planting seagrass seeds using natural bio containers showed that the restoration process is effective with a high survival rate **(Balestri *et al*., 2019)**. The use of hessian bags has given good results in seagrass restoration using *Z. marina* seeds **(Unsworth *et al*., 2019)**. The use of bamboo boxes in this research is very useful in protecting seagrass seeds from hydrodynamic activity disturbances that occur.

The growth value of seagrass morphology continued to increase during the observation process (Figs. 4, 5). This is due to the condition of seagrass, which is in the early phase of life, where it is still in a fast growth phase. It is known that *E. acoroides* seagrass belongs to the group of persistent seagrass species that have a long life span **(Kilminster *et al.*, 2015)**. Environmental parameters influence the growth process of seagrass seeds **(Ambo-Rappe *et al.*, 2019)**. The highest growth in leaves and roots was found in station 1. Station 1 has a mixed mud substrate, which has a high organic matter content compared to other types of substrate. The organic matter content in the aquatic environment contributes to the growth process of seagrass seeds **(Statton *et al.*, 2013)**. The morphometric growth of seagrass seeds is faster in waters with a high organic matter content **(Fraser *et al*., 2016; Nugraha *et al*., 2022)**. Compared to the research of **Ambo-Rappe and Yasir (2015)**, the growth of seagrass seeds in this study has a smaller growth size. Furthermore, it was deduced that, the size of seagrass seeds is among the factors influencing growth **(Glasby *et al.*, 2015)**.

CONCLUSION

Overall, our study's results suggest that the bamboo box represents one effective measure to safeguard seagrass seeds from hydrodynamic forces, predation, and burial. According to our findings, utilizing bamboo boxes led to higher survival rates compared to past studies where seagrass seeds were dispersed directly in the field. The accessibility and simplicity of bamboo boxes render them a viable solution for implementing seagrass layering through generative techniques.

REFERENCES

Ambo-Rappe, R. (2022). The success of seagrass restoration using Enhalus acoroides seeds is correlated with substrate and hydrodynamic conditions. *Journal of Environmental Management.*, *310*:114692. https://doi.org/10.1016/j.jenvman.2022.114692

Ambo-Rappe, R.; La Nafie, Y. A.; Syafiuddin, Limbong, S. R.; Asriani, N.; Handayani, N. T. and Lisdayanti, E. K. A. (2019). Short communication: Restoration of seagrass enhalus acoroides using a combination of generative and vegetative techniques. *Biodiversitas.*, *20*(11): 3358–3363. https://doi.org/10.13057/biodiv/d201132

Ambo-Rappe, R. and Yasir, I. (2015). The effect of storage condition on viability of Enhalus acoroides seedlings. *Aquatic Botany.*, *127*: 57–61. https://doi.org/10.1016/j.aquabot.2015.07.004

Balestri, E.; Vallerini, F.; Seggiani, M. ;Cinelli, P. ;Menicagli, V.; Vannini, C. and Lardicci, C. (2019). Use of bio-containers from seagrass wrack with nursery planting to improve the eco-sustainability of coastal habitat restoration. *Journal of Environmental Management.*, *251*:109604. https://doi.org/10.1016/j.jenvman.2019.109604

Campbell, M. L. (2016). Burial Duration and Frequency Influences Resilience of Differing Propagule Types in a Subtidal Seagrass, Posidonia australis. *PLoS ONE.*, *11*(8): 1–11. https://doi.org/10.1371/journal.pone.0161309

Cullen-Unsworth, L. C.; Nordlund, L. M.; Paddock, J.; Baker, S.; McKenzie, L. J. and Unsworth, R. K. F. (2014). Seagrass meadows globally as a coupled social-ecological system: Implications for human wellbeing. *Marine Pollution Bulletin.*, *83*(2): 387–397. https://doi.org/10.1016/j.marpolbul.2013.06.001

Escandell-Westcott, A.; Riera, R. and Hernández-Muñoz, N. (2023). Posidonia oceanica restoration review: Factors affecting seedlings. *Journal of Sea Research.*, *191*:102337 https://doi.org/10.1016/j.seares.2023.102337

Fraser, M. W.; Statton, J.; Hovey, R. K.; Laverock, B. and Kendrick, G. A. (2016). Seagrass derived organic matter influences biogeochemistry, microbial communities, and seedling biomass partitioning in seagrass sediments. *Plant and Soil.*, *400*: 133–146. https://doi.org/10.1007/s11104-015-2721-0

Glasby, T. M.; Taylor, S. L. and Housefield, G. P. (2015). Factors influencing the growth of seagrass seedlings: A case study of Posidonia australis. *Aquatic Botany.*, *120*: 251–259. https://doi.org/10.1016/j.aquabot.2014.09.003

Golden, R. R.; Busch, K. E.; Karrh, L. P.; Parham, T. A.; Lewandowski, M. J. and Naylor, M. D. (2010). Large-scale Zostera marina (eelgrass) restoration in Chesapeake Bay, Maryland, USA. Part II: A comparison of restoration methods in the Patuxent and Potomac Rivers. *Restoration Ecology.*, *18*(4):501–513. https://doi.org/10.1111/j.1526-100X.2010.00691.x

Govers, L. L.; Heusinkveld, J. H. T.; Gräfnings, M. L. E.; Smeele, Q. and van der Heide, T. (2022). Adaptive intertidal seed-based seagrass restoration in the Dutch Wadden Sea. *PLoS ONE.*, *17*(2): e0262845. https://doi.org/10.1371/journal.pone.0262845

Hernawan, U. E.; Rahmawati, S.; Ambo-Rappe, R.; Sjafrie, N. D. M.; Hadiyanto, H.; Yusup, D. S.; Nugraha, A. H.; La Nafie, Y. A.; Adi, W.; Prayudha, B.; Irawan, A.; Rahayu, Y. P.; Ningsih, E.; Riniatsih, I.; Supriyadi, I. H. and McMahon, K. (2021). The first nation-wide assessment identifies valuable blue‑ carbon seagrass habitat in Indonesia is in moderate condition. *Science of the Total Environment.*, *782*:14618. https://doi.org/10.1016/j.scitotenv.2021.146818

Kawaroe, M.; Nugraha, A. H.; Juraij and Tasabaramo, I. A. (2016). Seagrass biodiversity at three marine ecoregions of Indonesia: Sunda shelf, sulawesi sea, and banda sea. *Biodiversitas.*, *17*(2): 585-591. https://doi.org/10.13057/biodiv/d170228

Kilminster, K.; McMahon, K.; Waycott, M.; Kendrick, G. A.; Scanes, P.; McKenzie, L.; O'Brien, K. R.; Lyons, M.; Ferguson, A.; Maxwell, P.; Glasby, T. and Udy, J. (2015). Unravelling complexity in seagrass systems for management: Australia as a microcosm. *Science of the Total Environment.*, *534*:97–109. https://doi.org/10.1016/j.scitotenv. 2015.04.061

Li, H., Liu, J. and Che, X. (2021). Establishing healthy seedlings of Enhalus acoroides for the tropical seagrass restoration. *Journal of Environmental Management.*, *286*:112200. https://doi.org/10.1016/j.jenvman.2021.112200

Nugraha, A.H.; Kawaroe, M.; Srimariana, E. S.; Jaya, I.; Apdillah, D. and Deswati, S. R. (2019). Carbon storage in seagrass meadow of Teluk Bakau - Bintan Island. *IOP Conference Series: Earth and Environmental Science.*, *278*(1). https://doi.org/10.1088/1755-1315/278/1/012051

Nugraha, A.H.; Almahdi, S.; Zahra, A. and Karlina, I. (2022). Morphometric Characteristic and Growth Responses of Enhalus acoroides Seedlings Under Different Substrate Composition Treatment. *Omni-Akuatika.*, *17*(2):112-117. https://doi.org/10.20884/1.oa.

2021.17.2.883

Nugraha, A.H.; Tasabaramo, I. A.; Hernawan, U. E.; Rahmawati, S.; Putra, R. D. and Darus, R. F. (2021). Diversity, coverage, distribution and ecosystem services of seagrass in three small islands of northern Papua, Indonesia: Liki island, Meossu island and Befondi island. *Biodiversitas., 22*(12): 5544–5549. https://doi.org/10.13057/biodiv/d221238

Orth, R. J.; Kendrick, G. A. and Marion, S. R. (2007). Posidonia australis seed predation in seagrass habitats of Two Peoples Bay, Western Australia. *Aquatic Botany*., *86*(1): 83–85. https://doi.org/10.1016/j.aquabot.2006.09.012

Orth, R. J.; Luckenbach, M. L.; Marion, S. R.; Moore, K. A. and Wilcox, D. J. (2006). Seagrass recovery in the Delmarva Coastal Bays, USA. *Aquatic Botany.*, *84*(1): 26–36. https://doi.org/10.1016/j.aquabot.2005.07.007

Orth, R. J.; Marion, S. R.; Granger, S. and Traber, M. (2008). Restoring Eelgrass (Zostera marina) from Seed : A Comparison of Planting Methods for Large-Scale Projects. *Submerged Aquatic Vegetation, March*.

Rifai, H.; Quevedo, J. M. D.; Lukman, K. M.; Sondak, C. F. A.; Risandi, J.; Hernawan, U. E.; Uchiyama, Y.; Ambo-Rappe, R. and Kohsaka, R. (2022). Potential of seagrass habitat restorations as nature-based solutions: Practical and scientific implications in Indonesia. *Ambio*., 52: 546-555. https://doi.org/10.1007/s13280-022-01811-2

Stankovic, M.; Ambo-Rappe, R.; Carly, F.; Dangan-Galon, F.; Fortes, M. D.; Hossain, M. S.; Kiswara, W.; Van Luong, C.; Minh-Thu, P.; Mishra, A. K.; Noiraksar, T.; Nurdin, N.; Panyawai, J.; Rattanachot, E.; Rozaimi, M.; Soe Htun, U. and Prathep, A. (2021). Quantification of blue carbon in seagrass ecosystems of Southeast Asia and their potential for climate change mitigation. *Science of the Total Environment.*, *783*:146858. https://doi.org/10.1016/j.scitotenv.2021.146858

Statton, J.; Cambridge, M. L.; Dixon, K. W. and Kendrick, G. A. (2013). Aquaculture of Posidonia australis Seedlings for Seagrass Restoration Programs: Effect of Sediment Type and Organic Enrichment on Growth. *Restoration Ecology.*, *21*(2):250–259. https://doi.org/10.1111/j.1526-100X.2012.00873.x

Thorhaug, A.; Gallagher, J. B.; Kiswara, W.; Prathep, A.,; Huang, X.; Yap, T. K.; Dorward, S. and Berlyn, G. (2020). Coastal and estuarine blue carbon stocks in the greater Southeast Asia region: Seagrasses and mangroves per nation and sum of total. *Marine Pollution Bulletin.*, *160*: 111168. https://doi.org/10.1016/j.marpolbul.2020.111168

Unsworth, R. K. F.; Ambo-Rappe, R.; Jones, B. L.; La Nafie, Y. A.; Irawan, A.; Hernawan, U. E.; Moore, A. M. and Cullen-Unsworth, L. C. (2018). Indonesia's globally significant seagrass meadows are under widespread threat. *Science of the Total Environment.*, *634*: 279–286. https://doi.org/10.1016/j.scitotenv.2018.03.315

Unsworth, R. K. F.; Bertelli, C. M.; Cullen-Unsworth, L. C.; Esteban, N.; Jones, B. L.;

Lilley, R.; Lowe, C.; Nuuttila, H. K. and Rees, S. C. (2019). Sowing the seeds of seagrass recovery using hessian bags. *Frontiers in Ecology and Evolution.*, *7*: 311. https://doi.org/10.3389/fevo.2019.00311

Unsworth, R. K. F., van Keulen, M., & Coles, R. G. (2014). Seagrass meadows in a globally changing environment. *Marine Pollution Bulletin*, *83*(2), 383–386. https://doi.org/10.1016/j.marpolbul.2014.02.026

van Katwijk, M. M.; Bos, A. R.; de Jonge, V. N.; Hanssen, L. S. A. M.; Hermus, D. C. R. and de Jong, D. J. (2009). Guidelines for seagrass restoration: Importance of habitat selection and donor population, spreading of risks, and ecosystem engineering effects. *Marine Pollution Bulletin.*, *58*(2): 179–188. https://doi.org/10.1016/j.marpolbul.2008.09.028

van Katwijk, Marieke M.; Thorhaug, A.; Marbà, N.; Orth, R. J.; Duarte, C. M.; Kendrick, G. A.; Althuizen, I. H. J.; Balestri, E.; Bernard, G.; Cambridge, M. L.; Cunha, A.; Durance, C.; Giesen, W.; Han, Q.; Hosokawa, S.; Kiswara, W.; Komatsu, T.; Lardicci, C.; Lee, K. S., … Verduin, J. J. (2016). Global analysis of seagrass restoration: The importance of large-scale planting. *Journal of Applied Ecology.*, *53*(2): 567–578. https://doi.org/10.1111/1365-2664.12562

Williams, S. L.; Ambo-Rappe, R.; Sur, C.; Abbott, J. M. and Limbong, S. R. (2017). Species richness accelerates marine ecosystem restoration in the Coral Triangle. *Proceedings of the National Academy of Sciences of the United States of America.*, *114*(45):11986–11991. https://doi.org/10.1073/pnas.1707962114

Williams, S. L. and Orth, R. J. (1998). Genetic diversity and structure of natural and transplanted eelgrass populations in the Chesapeake and Chincoteague bays. *Estuaries.*, *21*(1): 118–128. https://doi.org/10.2307/1352551