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Ex-situ propagation and transplantation of *Enhalus acoroides* in coastal waters of Maragondon, Cavite, Philippines

Glenn Bryan A. Creencia*, Ginno Esguerra
Cavite State University

ABSTRACT

The newly discovered seagrass meadow in Cavite was the initial site of rehabilitation efforts of Cavite State University. This study determined the physicochemical characteristics of potential sites for seagrass transplantation, donor site, and ex-situ setup; determined the germination rate of the seagrass *(Enhalus acoroides)* seeds planted in different substrates; determined the growth rate of seagrass seeds planted in different substrates; and monitored the survival rate of the transplanted cultured seagrass. The physicochemical parameters were assessed and monitored using a multiparameter water probe, while the substrate was analyzed using the hydrological soil group. The physicochemical parameters of the transplantation site were mimicked in the ex-situ setup, where the germination and growth rate of the seeds were observed for two months. Thereafter, the harvested seagrass seedlings from the ex-situ setup were transplanted in Patungan Cove, Maragondon, Cavite, and monitored once a month. The germination rate of seagrass grown in the substrate obtained from the transplantation site was 100% and 96.8% for the artificial substrate. The growth rate of seagrass planted in the substrate obtained from the transplantation site is 18.26%, while the seagrass planted in the artificial substrate was 16.94%. Overall, 80 seagrass seedlings were transplanted in Patungan Cove, Maragondon, Cavite, and the survival rate of seagrass after one month of transplantation resulted in 50 out of 80 (62.5%) seagrass seedlings survived. And after two months, 47 out of 80 seagrass (58.75%) of seagrass seedlings survived.

Keywords: rehabilitation, conservation, biodiversity

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1. Introduction

For decades, the main interest of marine scientists of Southeast Asia has focused almost solely on the corals, seaweed, animals, or fish that either live in the coastal habitats or are associated with them [1], thus, the seagrass ecosystem is considered least studied. Seagrass ecosystem services are difficult to value and rank, and in many areas, they believed that the loss of seagrass will not directly affect the local community [2]. In some other cases, alteration of seagrass leads to the downfall of economically important organisms [3]. Seagrass conservation, rehabilitation, and its persistent scientific research are now given a high priority in the coastal action agenda of governments in Southeast Asia [4].

Seagrass bed is a discrete community dominated by flowering plants with roots and rhizomes (underground stems), thriving in slightly reducing sediments, and normally exhibiting maximum biomass under conditions of complete submergence [3]. Seagrasses are home to many economically important marine organisms, including shrimps, sea urchins, clams, various fish species, and endangered animals like sea turtles and the enigmatic sea cow, some of whose diet is seagrasses [4]. Seagrass plays an important role in the functions of the ocean ecosystem and forms a critical marine biophysicochemical system [5]. Seagrass ecosystems could store as much as 19.9 Pg (Petagrams) of organic carbon and approximately the seagrass carbon pool, slander between 4.2 and 8.4 Pg carbon, reflecting the importance of seagrass ecosystems in mitigating the effects of global warming and climate change [6]. A seagrass meadow is a highly productive habitat that supplies essential services, including carbon and nutrient sequestration. Blue carbon is a known organic carbon in seagrass sediments; it is accumulated from in-situ production from the water column [7]. However, seagrass losses are expected to increase and continue, further worsened by climate change.

Moreover, the loss of seagrass meadows has a significant impact on the marine ecosystem as well as the local communities since it provides preservation and nursery for fish and other biotic diversity and an important source of livelihood for the fishermen and local communities in the coastal areas [8]. Although seagrass beds in Southeast Asia have experienced widespread deterioration due to changes in environmental qualities, many studies concerning seagrass transplantation have attempted to recolonize or restore the seagrass meadows in the area where seagrasses have existed and received disturbance from many anthropogenic activities in the coastal zone. Seagrass transplantation has been performed mostly in temperate regions with different species such as *Zostera marina, Z. noltii, Posidonia australis, P. coriacea, P. oceanica, P. sinuosa, Amphibolis griffithii, and Halophila ovalis,* whereas the studies on seagrass transplantation in tropical areas are rare [9], but in this study,

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*Corresponding author
Email address: gacreencia@cvsu.edu.ph

E. acoroides was used.

To rehabilitate the newly discovered seagrass meadows in Maragondon, Cavite, this study introduced *Enhalus acoroides* through the ex-situ propagation and transplantation to the selected coastal area of Maragondon, Cavite. This species can tolerate heavier siltation loads and thrive under diminished physical conditions [10]. It also shows no evidence of harm to other seagrass species when transplanted [11]. When transplanted in mixed meadows, it stabilizes sediments and boosts associated macrozoobenthos which indirectly aiding other seagrasses [12]. Its great tolerance to stressors such as temperature fluctuations reinforce meadow-wide resilience when co-transplanted [13,14]. Moreover, its seedlings can withstand prolonged exposure to elevated temperatures suggesting that exposure to temperatures expected under climate change will not affect seedling survival [13]. Furthermore, this could be a tool to promote societal awareness to the importance of seagrass ecosystems as the key factor for bold management and sound restoration and decision practices, which can be met with public support and involvement of the local communities and fishermen, achieving the conservation goals and sustainability of seagrass biodiversity [15].

Generally, this project aimed to determine the ex-situ propagation and transplantation of seagrass *(Enhalus acoroides)* to the coastal area of Maragondon, Cavite. Specifically, this study determined the physicochemical characteristics of a potential site for seagrass transplantation in terms of soil texture, pH, turbidity, temperature and salinity. Also, it determined the germination rate of the seagrass *(Enhalus acoroides)* seeds planted in different substrates: a) artificial sand, and b) soil substrate from the coastal area of Maragondon, Cavite. It also determined the growth rate of the seagrass *(Enhalus acoroides)* seeds planted in different substrates: a) artificial sand and b) substrate samples from the coastal area of Maragondon, Cavite, in terms of seedling size. Furthermore, it monitored the survival rate of the transplanted cultured seagrass *(Enhalus acoroides)* at the coastal area of Maragondon, Cavite, and formulated a strategic plan to be proposed as a recommendation for seagrass ex-situ propagation and transplantation to the coastal communities in Maragondon, Cavite.

2. Materials and methods

This section presents all the materials and methods that were used in gathering sufficient data for the study.

2.1. Study site

The selection of the site was based on the following criteria: (1) the transplantation site has an existing seagrass meadow; (2) there is an adequate area to conduct the study; and (3) the site where the seagrass seeds are collected and the transplantation site has the same physicochemical characteristics. The site where the seagrass seeds were collected is in Brgy. Gulod, Calatagan, Batangas (Figure 1), while the seagrass area for the transplantation site is in Patungan Cove, Maragondon, Cavite (Figure 2).

Figure 1. Satellite image of the site where the seagrass seeds *E. acoroides* are collected in Brgy. Gulod, Calatagan, Batangas (Source: Google Earth Pro, Maxar Technologies, Terra Metrics).

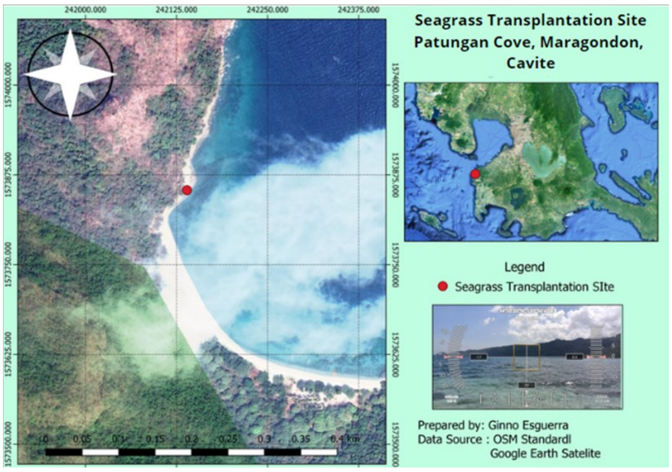

Figure 2. Transplantation site of seagrass *E. acoroides* at Patungan Cove, Maragondon, Cavite (Source: OSM Standard and Google Earth Satellite).

2.2. Physicochemical assessment of the study sites

Physicochemical characteristics of the site where seagrass seeds are collected, ex-situ setup, and transplantation site were assessed to determine their similarities and differences, which are vital in crafting the strategic plan.

2.2.1. Soil texture analysis

The hydrological soil group classified by the Natural Resource Conservation Service was used as a basis to determine the classification of the substrate, where A generally has the smallest runoff potential, and D has the greatest [16].

Group A: Sand, loamy sand, or sandy loam types of soil. It has low runoff potential and high infiltration rates even when thoroughly wet. They consist chiefly of deep, well-drained to excessively drained sand or gravels and have a high rate of water transmission.

Group B: Silt loam or loam. It has a moderate infiltration rate when thoroughly wet and consists chiefly of moderately deep to deep, moderately well to well-drained soils with moderately fine to moderately coarse textures.

Group C: Sandy clay loam. They have low infiltration rates when thoroughly wet and consist chiefly of soils with a layer that impedes downward movement of water and soils with moderately fine to fine structure.

Group D: Clay loam, silty clay loam, sandy clay, silty clay, or clay. This HSG has the highest runoff potential. They have very low infiltration rates when thoroughly wet and consist chiefly of clay soils with high swelling potential, soils with a permanent high-water table, soils with a claypan or clay layer at or near the surface, and shallow soils over nearly impervious material.

2.2.2. Water parameters testing

A multiparameter water probe was used to determine the physicochemical parameters of the water in terms of temperature, salinity, total dissolved solids (TDS), pH, and electroconductivity. This was done on the site where seagrass seeds were collected, an ex-situ setup, and the transplantation site.

2.2.3. Selection of seagrass species

The site where the seagrass seeds were collected was in Brgy. Gulod, Calatagan, Batangas. The selection of seagrass species was based on the following criteria: (1) the species *Enhalus acoroides* are not present in the newly discovered seagrass meadows in the transplantation site; (2) *E. acoroides* seeds was able to thrive in all conditions; and (3) *E. acoroides* is known as one of the largest species of seagrass and it will act as a barrier to other seagrasses in the transplantation site.

2.2.4. Seed collection

Brgy. Gulod in Calatagan, Batangas was the site for seagrass seeds collection. Matured fruits of seagrass were harvested and placed inside a container with seawater to avoid desiccation.

2.2.5. Ex-situ propagation of seagrass.

The seeds collected from the mature fruit of *E. acoroides* (Figure 3) were planted in the substrate from the transplantation site and artificial substrate in the ex-situ setup. A wave maker was also used to simulate waves and give circulation to the aquarium. Using artificial light was also considered to provide energy. Artificial salt was used to control the salinity of the water in the aquarium.

Figure 3. (A) *E. acoroides* matured fruits; and (B) *E. acoroides* seeds.

2.3. Monitoring of ex-situ propagated seagrass

2.3.1. Physicochemical parameters monitoring

Physicochemical parameters of the water in the ex-situ setup in terms of its temperature, salinity, total dissolved solids (TDS), pH, and electroconductivity were monitored weekly for two months.

2.3.2. Germination rate

Germination rate was determined by counting and recording the number of germinating seeds for two weeks. Germination percentage was recorded every other day during the two weeks. The rate of germination was estimated using modified Timpson's index of germination velocity. Mean germination time (MGT) was calculated to assess the rate of germination [17],

$$MGT = \frac{\Sigma D \cdot N}{n} \tag{1}$$

where N is the number of seeds which in D days grow, n is the total number of seeds grown, and D is the number of days from the date of germination, and the germination rate index will be obtained by reversing MGT at the end of the period. Then the final germination percentage was recorded.

2.3.3. Growth rate

Growth rate was determined by recording the initial and final length of the seedlings weekly for a 2-month observation. However, roots were not measured because they were fully planted in the substrate and uprooting may affect the growth rate of the samples. Using the modification of the growth rate formula to calculate the length and the leaf number growth rate.

$$G = \frac{L2 - L1}{T} \tag{2}$$

where G is the growth rate, $L2$ is the second time average leaf count / second time leaf, nodes, and stem size in cm, while $L1$ is the initial leaf count / initial leaf nodes and stem size in cm, and T is the time period. This study used percentage-based growth rates, which differ from the standard practice in seagrass biology where growth is typically expressed in units such as cm/day or through relative growth calculations. Some studies have also employed percentage-based growth rate to facilitate comparisons of proportional increases relative to initial size [18].

2.3.4. Transplantation of seagrass

The ex-situ propagated seagrass seedlings were transplanted; the method used was bare-root transplantation. Two-month-old seagrass seedlings were harvested and placed inside a container with seawater to avoid desiccation. The seedlings were transplanted to the sediment by hand. The transplantation site has a 1m x 1m square plot where there are 5 seedlings on each side of the plot with a total of 20 seedlings per plot and then planted with a 1-meter interval by plots (Figure 4). The total area used for seagrass transplantation is 2 m by 2 m, with a total of 80 seedlings planted.

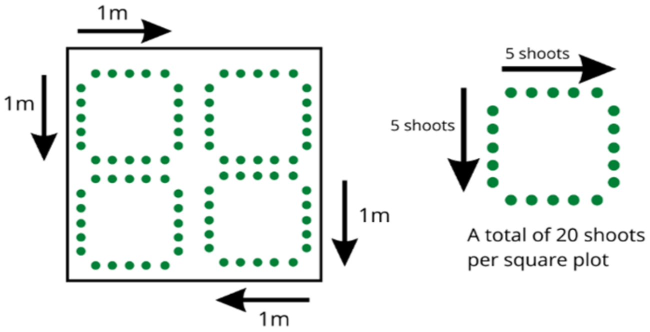

Figure 4. Configuration of seagrass transplantation.

2.3.5. Monitoring of transplanted seagrass

Monitoring of transplanted seagrass was done through on -site visits at least once a month. The population size of transplanted seagrass was measured. The population size was determined through actual counting of transplanted seagrass.

2.3.6. Survival rate

Survival rate was determined by monitoring the transplanted cultured seagrass once a month. The counting of the surviving cultured seagrass was compared to the initial number of transplanted cultured seagrass.

3. Results and discussion

This chapter presents and discusses the physicochemical characteristics of the site where the seagrass seeds are collected, the transplantation site, and the ex-situ propagation setup, as well as the germination rate and growth rate of seagrass *(Enhalus acoroides)* seeds in the ex-situ propagation setup and the survival rate of transplanted seagrass.

3.1. Water physicochemical analysis

Three sampling sites were selected for physicochemical testing of the water parameters from the site where the seagrass seeds are collected and the transplantation site. Three trials were done from each sampling site, and one trial was done in the ex-situ setup once a week for two months to monitor and maintain its stability.

Table 1 shows that the physicochemical parameters of the site where the seeds are collected, the ex-situ setup, and the transplantation site have differences. Still, the parameters of all the three mentioned sites were within the quality standards, as follows, pH: 7.0-8.5; TDS (g/l): 10-100; Temperature (°C): 26-30; Salinity (ppt): 30-35; Electroconductivity: 30-55 [19,20]. Since the parameter values remain within these acceptable ranges, but statistically different (significant at 0.05 level) to each other, the physicochemical factors do not inhibit the growth of *Enhalus acoroides* [21]. Monitoring of the water quality parameters is relevant in determining the survival and growth of the cultured and transplanted seagrass. It also plays a pivotal role in assessing the water environment, ecosystem, hydrochemistry, and ecology, and restoring water quality [22-24]. Making sure that the physicochemical parameters of both seed collection and transplantation sites, as well as the ex-situ setup, are the same is very important in supporting the germination, growth, and survival of the seagrass plants, especially in doing transplantation using ex-situ propagated seagrass.

Table 1. Physicochemical characteristics of the site where the seagrass seeds are collected, the transplantation site, and the ex-situ setup

Parameters	**Site where the seagrass seeds are collected**	**Transplan-tation site**	**Ex-situ setup**	**Standard deviation**	**ANOVA (*p-value*)**
pH	6.95	8.38	7.98	0.85	<0.001
TDS (g/l)	26.01	25.83	25.02	0.37	<0.001
Temperature (°C)	25.0	24.67	23.80	0.51	<0.001
Salinity (ppt)	33.30	34.18	34.90	0.71	<0.001
Electroconductivity	52.03	51.63	50.50	0.52	<0.001

3.2. Soil classification

Two substrates were used in the ex-situ propagation setup: the artificial sand and substrate from the transplantation site (Figure 5). The substrate from the transplantation site was used to ensure that the seagrass plants to be transplanted were already familiar with the type of substrate present in the transplantation site. The hydrological soil group classified by the Natural Resource Conservation Service is used as a basis to determine the classification of the substrate.

Figure 5. (a) Substrate from the transplantation site, and (b) artificial substrate.

Both substrates fall into the classification of Group A, where they have low runoff potential and high infiltration rates even when thoroughly wet. They consist chiefly of deep, well-drained to excessively drained sand or gravels and have a high rate of water transmission. The artificial substrate and the substrate obtained from the transplantation site are considered sandy.

3.3. Germination rate

Seed germination is a potential limiting stage in successful sexual reproduction for both terrestrial [25] and marine angiosperms [26-28] as well as for ex-situ propagation. Figure 6 shows that the seeds planted in the substrate from the transplantation site obtained a 100% germination rate in the span of 8 days, while the seeds planted in the artificial substrate obtained a 96.8% germination rate after 2 weeks. According to Juntaban et al. [29], *E. acoroides* can better tolerate the change of salinity level than other species. The level of different salinities, as well as Nitrogen (N) and Phosphorus (P) concentrations used in all treatments, did not affect the percentage of seed germination. In addition, germination of *E. acoroides* seeds was able to occur in all conditions, but it could germinate more greatly at low salinity levels. Furthermore, germination failure has predominantly been related to the characteristics of the surrounding microenvironment, which may lack the required signals to break seed dormancy [28].

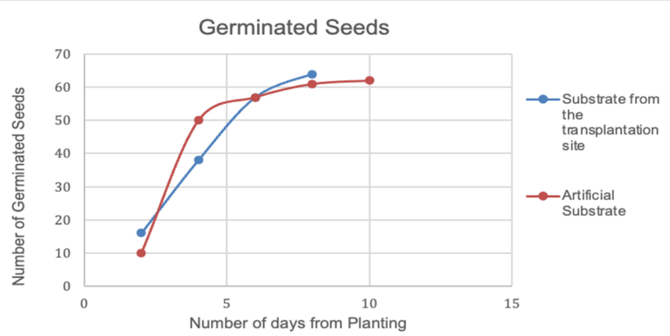

Figure 6. Germination rate of *Enhalus acoroides.*

Ex-situ conservation involves the maintenance and breeding of endangered plants and animals under partially or wholly controlled conditions in specific areas, including zoos, gardens, nurseries, etc. The stresses on living organisms due to competition for food, water, space, etc., can be avoided by ex-situ conservation, thereby providing conditions necessary for a secure life and breeding [30]. Based on the results, seagrass seeds coming from the mature fruit are viable for ex-situ propagation and suitable for use in transplantation projects.

3.4. Growth rate

The 128 seagrass seeds were planted in the two substrates. Table 2 shows the mean growth rates of seagrass planted in natural substrate. Seagrass seeds grown in substrates from the transplantation site exhibited a mean growth rate of 18.49%, while those grown in artificial substrates showed a mean growth rate of 16.26%. Nurul et al. [31] explained that seagrass seed culture needs proper places to grow faster. It is also important to monitor water parameters since some species are very sensitive. Growing them in laboratory conditions is more suitable in room conditions. Others advise artificial light, especially when indoors, and others use natural light. Based on this result, the ex-situ setup is favorable to the growth of seagrass seedlings, and this setup can be an option for seagrass propagation (Figure 7). Laboratory propagation of seagrass can be adopted by the local LGU since this technique is not place-specific.

Table 2. Growth rate of *Enhalus acoroides* in terms of the initial and final length of the seedlings

Substrate type	Growth rate
Artificial substrate	16.26%
Substrate from transplantation site	18.94%

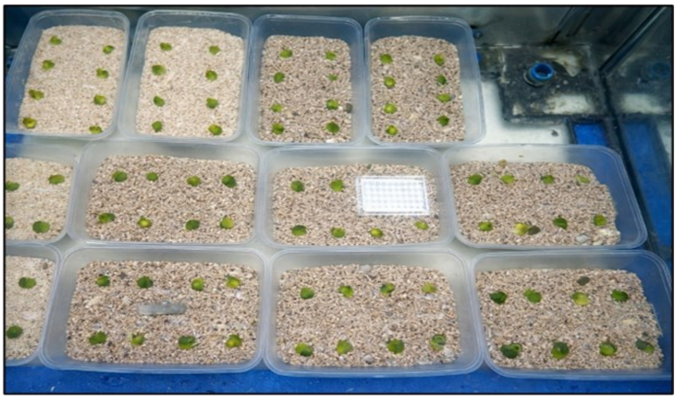

Figure 7. *Enhalus* acoroides seeds were planted in the ex-situ propagation setup.

3.5. Monitoring of the transplanted seagrass

A month after the transplantation (Figure 8), the transplanted seagrass resulted in a 62.5% survival rate, where 50 out of 80 seagrass plants survived. And after two months, a 58.75% survival rate where 47 out of 80 seagrass plants survived. The length of 47 surviving cultured seagrass after two months was measured (cm) to compare its average initial size before the transplantation. The growth rate of the transplanted cultured seagrass in terms of its length was 0.1365 or 13.65% with the length ranging from 12 cm to 27 cm.

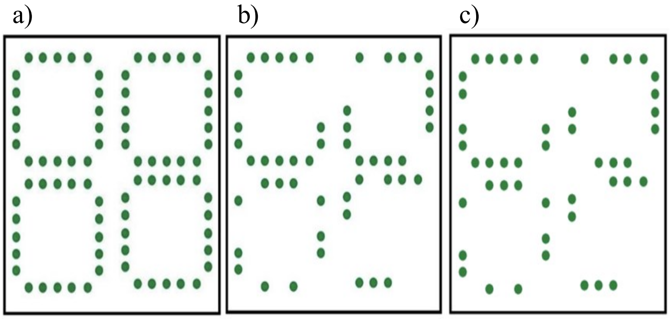

Figure 8. (a) Seagrass after transplantation (80 seedlings), (b) survived seagrass after one month of transplantation (50 seedlings), and (c) survived seagrass after two months of transplantation (47 seedlings).

Based on the field observation, anthropogenic, biological, and climatic factors may be the reasons to cause the transplanted seagrass mortality. There was little bad weather after the transplantation day, and there was a lot of solid waste in the area brought by the bad weather conditions. Aerial expansion of the other seagrass species in the transplantation site could be one of the possible causes since it overlapped with the transplanted seagrass plants.

Seagrass can adapt to its environment since it can withstand various degrees of salinity. They can also tolerate temperatures ranging from -6 to 40 °C. Their horizontal stems, called rhizomes, enable them to cope with the tugging of currents and waves. Roots grow down from the rhizome to anchor the plant to the seabed, while flexible blades grow straight up and can bend to the current without resistance. In mixed seagrass colonies, short-leaved, fast-growing grasses form a mat that traps sediment and stabilizes the seabed, allowing taller, slower-growing varieties to establish roots.

3.6. Strategic plan

Table 3 summarizes the strategic plan, highlighting project activities, objectives, stakeholders, implementing agencies, and expected outputs for seagrass ecosystem restoration in Maragondon, Cavite.

Table 3. Proposed strategic plan

Project activity	Objectives	Stakeholders	Implementing agency	Expected output
Seminars and trainings about the value, ex-situ propagation, and transplantation of seagrass	To educate the local coastal communities about the importance and value of seagrass To train local coastal communities about different ways and techniques in propagating and transplanting seagrass	Local Coastal Communities and volunteers from different private and public sectors	LGUs in partnership with MENRO	Local communities will be more aware and educated about the value and importance of seagrass ecosystem Local coastal communities will be more equipped with knowledge and skills in the ex-situ propagation and transplantation of seagrass
Seagrass ex-situ propagation projects	To propagate seagrass in ex-situ, either in the laboratory or in selected coastal area of Maragondon, Cavite	Local Coastal Communities and volunteers from different private and public sectors	LGUs in partnership with MENRO	Resilient coastal ecosystem A complete marine ecosystem Reintroduction of seagrass to the coastal areas in Maragondon, Cavite
Seagrass Transplantation Project	To transplant seagrass in the selected local coastal areas in Maragondon, Cavite	Local Coastal Communities and volunteers from different private and public sectors	LGUs in partnership with MENRO	Resilient coastal ecosystem A complete marine ecosystem Reintroduction of seagrass to the coastal areas in Maragondon, Cavite
Policy Making and ordinances for the protection of transplanted seagrass	To regulate possible man-made disturbances that hinders seagrass growth and survival	Local Coastal Communities and volunteers from different private and public sectors	LGUs in partnership with MENRO	Protection for seagrass ecosystem and diversify transplanted seagrass
Regular Monitoring	To have regular monitoring of the ex-situ propagated seagrass and the transplanted seagrass	Appointed individuals from LGUs and volunteers from different private and public sectors	LGUs in partnership with MENRO	Successful ex-situ propagation and transplantation of seagrass

4. Conclusions

In light of the results, the following conclusions were drawn. The water physicochemical parameters for seed collection site, ex-situ setup, and transplantation site have significant differences. The substrate classification for the seed collection and transplantation sites falls under Class A. These parameters became significant to ensure that there will be very minimal differences from the seed collection site, ex-situ setup, and transplantation site, which became significant in the germination, growth, and survival rates. The germination rate of seagrass seeds planted in the substrate obtained from the transplantation site is 100%, while the seagrass seeds planted in the artificial substrate is 96.8%. The growth rate of seagrass planted in the substrate obtained from the transplantation site is 18.26%, while the seagrass planted in the artificial substrate has 16.94%. The survival rate of transplanted seagrass after one month of transplantation resulted in 62.5% or 50 out of 80 seagrass seedlings surviving and 58.75% or 47 out of 80 seagrass surviving after two months. Science-based approach is recommended as the primary framework in doing seagrass ex-situ propagation and transplantation. Seagrass rehabilitation must involve various stakeholders. Different strategies in boosting and improving information and education, local community empowerment, and local government capability must be implemented to ensure the success of the rehabilitation project.

In line with the conclusions, the study suggested that a physicochemical assessment of the target transplantation site must be obtained to ensure the survival of the transplanted seagrass plants. Since the monitoring period for the transplanted seagrass lasted only 60 days, the observations are not sufficient to determine restoration success or long-term ecological performance hence it is suggested to employ a longer monitoring period to more accurately assess the sustainability and ecological outcomes of seagrass restoration efforts. Furthermore, the LGU and other stakeholders who are capable of doing ex-situ propagation of seagrass from seeds must be trained to equip them with the right knowledge and techniques to ensure that the project will yield a higher rate of success. The academe, DENR, local schools, and the LGU boost information and education campaigns about the presence of seagrass and its relevance as well as its rehabilitation through seagrass transplantation. It is also suggested that in the upland coastal areas, seagrass transplantation be promoted as their community service.

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