Transplantation of Tropical Seagrass *Enhalus acoroides* (L.) in Thai Coastal Water: Implication for Habitat Restoration

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***Abstract*— *Transplantation of tropical seagrass Enhalus acoroides (L.) was performed in Thai coastal water. The survival of transplants was related to environmental qualities at the initial period of transplantation time. Temperature and desiccation were accounted to be the major factors contributed to a success or failure of seagrass transplantation in the area. In addition, low survivorship of transplants was attributed to high sulfides in sediments. The transplanted populations exhibited survival rate of 26.15% whilst leaves growth rates were recorded as 1.29 cm d^{-1}. The critical period of transplants was within the first three months where plants needed to acclimatize to new environments. Winter was the most suitable period for transplanting seagrass in tropical area. The limitation of this seagrass meadows expansion was due to slow rhizome fragmentation characteristic of plant species. Although, the transplant has low survival rate but transplantation would potentially enhance seagrass meadows to support the coastal ecosystem functions.*

***Keywords*— About four key words or phrases in alphabetical order, separated by commas.**

1. INTRODUCTION

Seagrasses are highly productive and play many important ecological functions in coastal and marine ecosystems [1]. They are primary producer in the coastal food chain and provide habitat, nursing and spawning ground as well as food for numerous marine organisms. Seagrasses modify currents and trap fine-grained sediments from water column and retain organic matters produced by themselves and other photosynthetic organisms. Seagrass litter is also the carbon source for bacterial decomposition. However, declining of seagrass abundance has been reported worldwide due to deterioration of environmental qualities associated with the increase of human population [2]. Reduction of seagrass coverage has been linked to many activities in the coastal zone including anthropogenic nutrient input leading to eutrophication in the areas, loss of tidal marshes, mechanical damage from mining, dredge filling and scarring by boat propellers. Seagrass beds are also affected from many purposes of coastal development and land reclamation e.g. pier, hotel and resort constructions [3]. The increasing rate of seagrass loss has led to considerable concern about the health of coastal ecosystems by environmental agencies and regulatory authorities [2]. Recent environmental management programs have included some forms of seagrass rehabilitation and restoration in the projects. Restoration of seagrass habitats have been in focus for many decades [4]-[6] with the attempt for development and enhancement of vegetative cover gained for that lost in the area and increase in acreage where possible. Davis and Short [4] summarized three categories of seagrass transplanting methods using either plants with sediment intact, plants with bare root or seeds. From these categories, various techniques on seagrass transplantation have been applied in many parts of the world ranging from rapid and simple [5] to complicate mechanical used [7], [8] with varying degree of success. Advantages and disadvantages of these techniques have been reported in the literature; however, one common conclusion is that most methods are labor intensive and time consuming and many require either large numbers of plants, and/or an anchoring mechanism.

Seagrass transplantation has been performed mostly in temperate region with different species i.e. *Zostera marina* [4], [5], [9], *Z. noltii* [10], *Posidonia australis* [11], [12], *P. coriacea* [7], [8], *P. oceanica* [13], [14], *P. sinuosa*, [7], [8], *Amphibolis griffithii* [7], [8], *Halophila ovalis* [15] whereas the studies on seagrass transplantation in tropical areas are rare. The largest seagrass species *Enhalus acoroides* (L.) is commonly found and contributed to high ecological importance in SE Asian coastal waters [16], [17]. Although seagrass beds in SE Asia have been experienced widespread deterioration due to the change of environmental qualities. Many studies concerning seagrass transplantation have attempted to recolonisation or restoration of seagrass meadows in the area where seagrasses have existed and have received disturbance from many activities in the coastal zone. Chonburi province, located on the east coast of Thailand, has a long coastline of 157 km with a variety of coastal resources including seagrass beds. A fast developing of industrialization and urbanization has contributed to deterioration of coastal habitats along the Chonburi coastal area. To cope with the problem, the provincial government of Chonburi, in collaboration with

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Partnerships in Environmental Management for the Seas of East Asia (PEMSEA) organization, established the Integrated Coastal Management (ICM) program since 2001. Seagrass restoration has been considered as one of the possible ICM implementations at Sriracha Municipality coastal area. The major goal of our transplanting project was to find out the possibility to create seagrass beds in Sriracha coastal areas with emphasis on large and long-lived tropical seagrass *E. acoroides* and to elucidate the limitation of seagrass transplantation in the area.

2. MATERIALS AND METHODS

2.1 Donor plants sampling locations

Khung Krabaen Bay is a shallow semi-enclosed bay located in Chanthaburi province on the east coast of Thailand. The bay is an oval-shaped with 4.6 km long and 2.6 km wide. The mouth of the bay is 500 m width and maximum water depth is approximately 4.0-6.0 m. The bay is surrounded by mangrove forests which act as the barrier against intensive shrimp culture on the landward areas. The bay supplies water for shrimp culture activities inland via nine inlet canals. Due to the small opening of the bay, the influence of the bay water is minimal. Bottom sediment in the bay is characterized as muddy to sandy mud sediment. There are 6 species of seagrasses with *E. acoroides* and *Halodule uninervis* are dominant species and form dense meadows in the inner part of the bay.

2.2 Plant Collection

Seagrass (*E. acoroides*) was randomly retrieved from well developed seagrass beds. The collector followed the blades of the shoot to the substrate, uprooted approximately 15-20 cm of the rhizome by digging under the rhizome by hand, and snapped the rhizome to remove the plant. This technique allowed us to minimize disruption of root-rhizome layer. At the donor site, plants were temporarily stored in large coolers with a small amount of seawater to prevent exposure and desiccation and were later transferred back to Sriracha Fisheries Research Station. Collected seagrass remained viable for transplanting up to 3 d when stored in this manner.

2.3 Transplant locations

Sriracha Bay is opened and generally exposed to wave action. There is a small island with the area of 5500 m^2, namely Koh Loi, located 800 m form shore line. Koh Loi is a popular recreational location among local people and tourists because it has delightful scenery on the hilltop as well as a recreational park in the surrounding areas. For tourism and water transportation development purposes, the Sriracha Municipality has been constructed a road to connect between Koh Loi and the mainland. Although, the road has water gate underneath to allow water circulation continue through the bay; however, the constructed road has created small embayment in the north and south of the bay where current and wave action are reduced. Therefore, two sites; one in the north (St. N), another in the south (St. S) of Sriracha coastal areas were chosen for the transplant experiments. Both stations were located at 200 m from the shore line. Water depth at the transplanting sites varied between 2.5-4.0 m.

2.4 Transplanting procedure

Transplanting of *E. acoroides* was primarily performed in August 2005 at St. N and once again in November 2005 at St. S. Transplantation of *E. acoroides* at each site was done in triplicate to create three patch sizes. A plot size of 10 m $\times$ 10 m was designed with spacing of 0.5 m intervals between planting units within a row. Triple shoots per one planting unit were planted in twenty rows, yielding 1200 shoots in a plot. This design resulted in 3600 shoots being planted at each of two transplant locations. Seagrasses were transplanted at both stations during low tide no matter daytime or nighttime regardless of planting convenience. Sediment was removed to the depth of 20 cm by using spade, then three mature *E. acoroides* shoots were placed into the sediment by hand. Since sediment remains unconsolidated for some time when the finger was removed, the rhizome was pushed under a more compact area of the sediment which assisted in anchoring the plants. The final position of the plants is similar to what occurs naturally with the buried rhizome parallel to the sediment surface and the shoots erect into the water column.

Growth and survival rates of transplanted seagrasses were assessed monthly. Growth was measured using leaf marking technique where twenty four plants at each transplanting plot were marked on leaf sheath. After a period of 2-3 d, leaves were remarked again. Growth was then measured as the difference between the first mark and the initial baseline after the second mark has been done. The total number of remaining plants at each plot was counted to examine the survival rate.

2.3 Sediment and water qualities measurement

Sediment samples were collected from either donor sites or transplanted sites using two plexiglass hand corers. One was used to determined grain size distribution and another was used for physical and chemical analyses. To determine grain size distribution, sediment from each sites was oven-dried at 105 ºC for 24 h and grain size analysis performed by the dry sieving method using a set of British Standard Test sieves of mesh size in the range of 0.063-9.5 mm, all nested together on an automatic mechanical sieve shaker (Model Endecotts EFL2 MK3). Grain size classification was obtained from the weight data of the respective size fractions based on the Wentworth scale. Sediment samples in another core was sliced with cutting plate into 1 cm depth intervals from surface down to 5 cm and subsequently sediment at depth 9-10 cm was selected to represent sediment at depth. Sediment at each depth was kept in a zip-lock bag and was mixed well prior to the physical and chemical analyses. Water content was measured after drying of weighed sediment at 105 ºC for 24 h. Total organic matter content (TOM) in sediment was measured as ignition loss (IL) after drying at 550 ºC for 3 h. The acid volatile sulfide (AVS) concentrations in sediment were determined by acidifying sediment samples with sulfuric acid and collecting the discharged H_2S in a dosimeter tube with an H_2S-absorbent column (Gastec, Japan), which measures the amount of H_2S released [18]. Briefly, 1-2 g wet sediment was weighed into sulfide reactor column. One end of the detector tube was connected to sulfide reactor column via silicone tube and another end was connected to the suction pump. Then, add 2 ml of 18N H_2SO_4 into the reactor column where the solid phase sulfide in the sediments would convert to H_2S. The H_2S gas was pulled in through the detector tube by the vacuum pump with the sampling time of 2 min. The read value from the detector tube was then converted into sediment dry weight basis.

Field measurement of water qualities at the transplanting sites, including temperature, salinity, pH and dissolved oxygen (1 m depth from water surface) were examined using Multi-Parameter System (YSI 650 MDS) and transparency was measured using Secchi disk.

3. RESULTS

The chemical and physical characteristics of sediments from donor sites and selected sites for *E. acoroides* transplantation were determined and presented in Fig. 1.

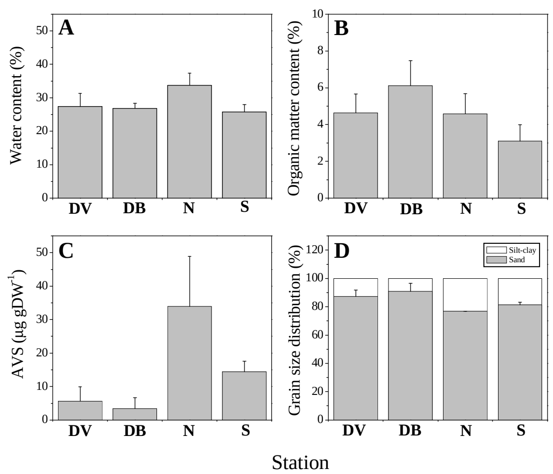


Fig. 1. Sediment properties including water content (A), organic matter content (B), AVS (C) and grain size distributions (D) from donor sites compared to selected sites for seagrass (*Enhalus acoroides*) transplantation. The values are expressed as mean±S.D. (n=3) and the abbreviations are denoted as DV; Vegetated donor site, DB; Bare donor site, N; Station North (St. N), S; Station South (St. S).

Sediment water contents were similar among sites (26-28%) with only exception for St. N where higher water content (34%) was recorded. Organic matter content in sediments were in the range between 3.11-6.11% with highest value was found in bare donor sites whereas organic matter content in sediments at the selected sites were closed to vegetated donor sites. When the comparison of acid volatile sulfide (AVS) in sediments was done, the difference between donor sites and transplanted sites was obviously shown. Two selected areas for transplants have 3-7 times higher AVS than those found in vegetated donor sites. Sediments for both vegetated and bare at donor sites were composed of slightly higher sand (87.2-90.9%) than two selected sites (76.7-81.4%) regardless of grain size distribution. The overall picture of sediment characteristics of the selected sites were similar to donor sites with only exception for significantly higher AVS were pronounced in transplanted sites. Seasonal variations of sediment characteristics at transplanted sites (St. S) including water content (A), organic matter content (B) and AVS (C) were monitored since *E. acoroides* had been transplanted in October 2005 (Fig. 2). Water content in sediment was uniformity reductions with depth with slightly lower values were found in the rainy season. Organic matter content was almost constant at ~2% in surface sediment through out the study period. It increased to reach the maximum at 3 cm depth before decreased with depth in the summer and rainy seasons. Sediment organic matter content was rather constant with depth with values between 1.43-2.02% in winter. Vertical depth profiles of AVS were similar among seasons where low AVS values (0.73-8.34 µg gDW^{-1}) were obtained in surface sediment then increased to reach the maxima at 3 cm depth before decreased downward. However, seasonal variation had significantly affected depth profiles of AVS in *E. acoroides* transplanted sediment (Fig. 2C). High AVS values were observed in summer whilst intermediate and low AVS were found in the winter and rainy seasons.

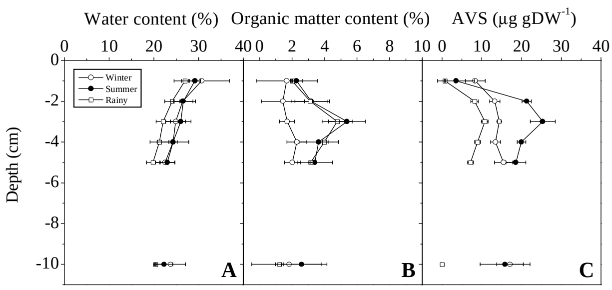
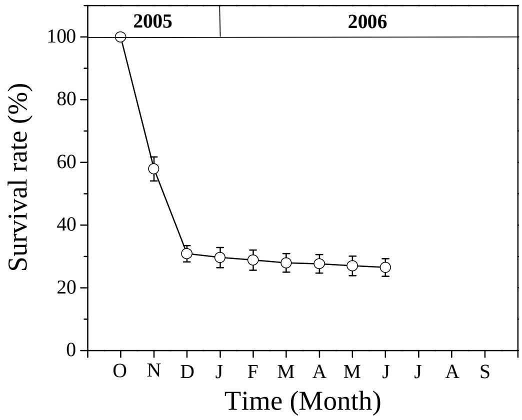


Fig. 2. Vertical depth profiles (mean±S.D., n=3) related to seasonal variation of water content (A), organic matter content (B) and AVS (C) from seagrass (*Enhalus acoroides*) transplanted sediments at St. S in Sriracha Bay.

Transplantation of *E. acoroides* at St. N occurred in August 2005 where low tide was presented during the daytime. Plants at all three transplanted plots did not survive after one week revisited. In addition to high AVS in sediment, desiccation of transplanted plants due to exposure to sunlight and high surface water temperature (≥40 ºC) could be the main reason for transplantation failure. Although, natural plants at the donor site were also treated at the same manner; however, revegetation was pronounced after tidal datum had changed following seasonal cycle.

Fig. 3. Survival rate of transplanted seagrass (*Enhalus acoroides*) at St. S in Sriracha Bay.



In contrast, transplantation success was recorded when site and transplanting period had changed to St. S in November 2005. In this period, low tide was recorded during the nighttime; therefore, the degree of plant exposure to sunlight and high water temperature were reduced. Individual shoots were successfully established with growth over the eight-month period. The survival rate of transplants reduced dramatically to 57.9 ± 3.83% within one month after transplantation. Two months survivorship was 30.9 ± 2.61% and it decreased to 28.8 ± 3.21% over the span of four months. The survival rate remained constant at about 27% after four months (Fig. 3). Leaf growth rate reduced from 1.44 cm d^{-1} to 1.28 cm d^{-1} within three months after the initial transplantation period before fluctuated close to this value and the overall average leaf growth rate of the transplants was recorded as 1.29 ± 0.08 cm d^{-1} (data not shown).

Water qualities from the transplanted sites at Sriracha Bay (St. S) were presented in Table 1. Water temperature varied between 26.88-31.67 ºC, pH fell within the range of 7.15-8.34, salinity fluctuated from 30.43 to 35.15 ppt, DO was in the range of 4.31 and 6.63 mg l^{-1} and secchi depth differed between 0.8-1.5 m, respectively. The examined water quality parameters in this study were within the range of those gennerally found in the Sriracha coastal water.

Table 1. Water qualities from the transplantation sites of seagrass (*Enhalus acoroides*) at Sriracha Bay (St. S).

Time	Temp (ºC)	pH	Salinity (ppt)	DO (mg l^{-1})	Secchi Depth (m)
Aug 05	30.17	8.34	30.43	4.50	1.0
Sep 05	30.10	7.84	31.80	4.31	0.8
Oct 05	29.67	7.73	32.99	4.55	1.8
Nov 05	29.71	7.15	33.70	5.14	1.5
Dec 05	28.45	7.70	34.65	6.35	1.5
Jan 06	26.88	7.54	35.15	6.63	1.0
Feb 06	30.70	7.75	31.10	5.53	1.5
Mar 06	30.64	7.73	31.27	6.21	1.5
Apr 06	31.67	7.79	30.60	6.70	0.3
May 06	30.80	7.88	28.80	5.43	0.1
Jun 06	31.50	7.89	28.85	4.41	0.5

4. DISCUSSIONS

In addition to reverse habitat degradation, to select transplantation habitats carefully, and to optimize the transplantation techniques, van Katwijk et al. [6] suggested that prior to launch the seagrass restoration program, it was necessary to consider on selection of an appropriate donor population, spreading of risks, and ecosystem engineering effects. Keeping this guideline in mind, we chose the similar physical properties between donor sites and transplanted sites. Exhibited sediment characteristic which could support seagrass growing during the initial transplanting period is regarding to be one of the key factors contributed to seagrass transplantation success. However, it is apparent that sediment physical properties cannot always predict the suitability of the areas for seagrass transplantation [6]. Although, sediment physical properties at the two selected sites were similar to the donor sites but transplantation success was gained only at St. S. The difference between donor sites as well as among the transplanting sites was the initial acid volatile sulfide (AVS) in sediments. High AVS values were observed in transplanting sediments where St. N had ~7 times higher whilst St. S obtained ~3 times higher than donor sites. AVS are shown to be the major fraction of reduced sulfur compounds in sediments [19] and are potential phytotoxins [20]. Although seagrasses appear to be well adapted for survival in anaerobic sediment environments by developing a lacunae system [21], [22]; however, periods of sediment hypoxia have been associated with the decline of seagrass beds [20], [23]. The increase of sediment sulfide levels have been related to depressed photosynthetic potential and resulted in loss of seagrass vegetation in stressed environments. It has been criticized that supplementary of oxygen from either above-ground plant parts or water column to below-ground tissues can reduce sulfides effects by oxidization of sulfides to elemental sulfur and accumulation in below-ground plant tissues [24], [25].

Availability of oxygen to support oxidation processes needs of below-ground tissues could enhance the survival of transplants. The unsuccessful transplantation at St. N could possibly be explained by high AVS content in sediments in accordance with plants inability to keep the balance between production and respiration in the below-ground tissues which made them vulnerable to sulfides effects. The transplantation process may have caused some kind of stress to the transplants due to uprooting and handling which made them more sensitive to the effects of adverse factors than non-transplanted plants. In addition, *E. acoroides* has large rhizome and subsequently larger wound on rhizome fraction in comparrison to other seagrass species; therefore, transplants may suffer from loss of important carbon reserve via exudation before the completion of wound healing [26]. Other environmental conditions have to be taken in to account for transplants survivorship as well as influencing sulfides effects on seagrasses [20], [24]. The rainy season in the area starts from May to October and tidal regime in this period following high tide during the nighttime and low tide during the daytime. Therefore, transplants at St. N in August will expose to sunlight and desiccation during the daytime period. Elevated water temperature can stimulate stress and exhibit a negative effect on health and survival of seagrass [10], [27] especially when this phenomenon is incorporated with high light irradiance and high organic nutrients concentrations [28], [29]. Increased turbidity in rainy season also affects underwater light quality and quantity and is related to photosynthetic processes which in turn influence survival of transplanted seagrass [9].

The success of transplants occurred at St. S in winter (November) although higher AVS was observed at the station than donor sites but it was only half of the amount found at St. N in August. The winter period stands between November and January where low tide occurs in nighttime while high tide pronounced in daytime. Less impact from desiccation and sunlight as well as the possibility of oxygen supply to overcome the sulfide effects in accordance with low temperature were thus influenced successful establishment and subsequent growth of *E. acoroides* in winter. Many studies indicated that seasonal variation involved on survival and success of transplanted seagrass with emphasis on temperature changes [4], [10], [27]; however, suitable season for seagrass transplantation varied among the regions. In high latitude temperate region, severe winter may cause scouring and rafting of sea ice on transplants [4] whilst winter seems to be the best season for transplantation of seagrasses in low latitude temperate region [10], [27]. Our results suggest that the success of seagrass transplantation in tropical areas varies seasonally and winter is the most suitable period to establish seagrass meadows via transplantation in the region. Physical and biological disturbances may play a significant role on transplantation success. For instance, Davis and Short [4] suggested that biological disturbance from crabs and worms could influence the survival of transplanted seagrass but these were not always important factors in other areas [5]. We did not see a significant impact from such biological disturbance although we observed the carbonaceous tube of polychaete on *E. acoroides* blade but this phenomenon was also present at the donor sites. A number of literatures reported success on transplantation of fast growing temperate seagrasses such as *Zostera marina* with meadows formation in many instances but it is difficult to draw conclusion and comparison to tropical seagrasses like *E. acoroides* which we were monitored the transplants up to eight months without any new erected shoot was observed. This is consistent with previous study on *E. acoroides* exhibited meadows which was characterized by long shoot, root and rhizome with slow rhizome branching frequency therefore exhibits a patchy distribution of clonal fragments [16], [30].

Monitoring of the transplants at St. S was performed in moderate periods with an attempt to clarify survival and growth of transplants and the properties of transplanted sediments. Both survival and growth of transplants reduced dramatically in the first three months before transplants exhibited the survival rate of 27% and average growth rate of 1.29 ± 0.08 cm d^{-1}. Growth of *E. acoroides* varied with season and location where leaf growth rate can be varied from less than 1 cm d^{-1} up to more than 2 cm d^{-1} [16], [31]; however, transplants grow with slower rate compared to plants at the donor sites [14] suggesting that *E. acoroides* transplants needed time to acclimatize to new environment. Exposure during the day and the resulting of desiccation of the seagrass leaves can cause catastrophic reductions in above-ground biomass and growth, whereas seagrasses continue to grow; although, the water is shallow and even they are exposed to the air during the night, when desiccation is unlikely [32]. Seasonal variations were present in both organic matter content and AVS in transplanted sediments. Low organic matter content (~2%) as well as low AVS (~13 µg gDW^{-1}) was pronounced at the initial transplantation period in winter (November to January) suggesting that less accumulation of organic matter in accordance with low temperature could reduce decomposition rate resulted in low AVS. High organic matter content in association with high AVS was pronounced in summer (February to April) suggesting that anaerobic decomposition under sulfate reducing conditions was dominated in sediments and was stimulated by higher temperature and organic matter [33]. The highest AVS concentrations of ~26 µg gDW^{-1} observed at 2-3 cm depth was closed to the value presented at St. N however, no sign of sulfide effects on transplants suggesting that transplants have settled and acclimatized to sulfide in sediments. It was observed, during the rainy season (May to October) with available data till June 2006 when this study was conducted, that the desiccation of surface sediments in combination with high temperature during the daytime were potentially influenced to low amount of AVS in sediments. An increased resuspension in consistent with a simultaneously observed large increase turbidity of surface water may be an additional factor from tidal flushing leading towards the lower AVS concentrations in the sediments.

5. CONCLUSION

Transplantation is a potential tool to restore and replace the ecological values lost due to both direct and indirect impacts from coastal disturbance on seagrass beds. In addition, transplantation of seagrass in suitable areas can also enhance the habitats and productivities in the coastal zones. However, the success of seagrass transplantation depends on a number of factors. Transplanting period in combination with site selection is the major factor that can contribute to the success or failure of seagrass transplantation. Seagrass (*E. acoroides*) transplantation success in tropical region such as in Thai coastal water depends on physical, biological and chemical factors including low temperature, low sulfide in sediments in accordance with less degree of desiccation and winter seems to be the best time to create transplantation in the region. Monitoring of transplanting *E. acoroides* over the span of eight months suggests that the most critical period to determine success of transplantation is within three months. After this period, exhibit plants can survive through the summer month although higher sulfide is pronounced in the sediments.

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