Transplantation of seagrass, *Halodule pinifolia (*MIKI) DEN HARTOG*:* survival between mangrove and urban areas in Makampom Bay, Rayong Province, Thailand

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ABSTRACT
The plug technique to transplant seagrass, *Halodule pinifolia* (Miki) den Hartog was employed to conduct a study of seagrass survival in Makampom Bay, Rayong Province for 17 months from November 2007- Mar 2009. The survival of seagrass was investigated and compared between mangrove and urban areas in which 3 different sizes of seagrass patches, 4, 6 and 8 inches in diameter, and coconut fiber pots were used as important factors in this study. The monthly results revealed that the percentage of seagrass growth transplanted with the seagrass patch having a diameter of 6 inches in the mangrove area presented the highest growth of 247.9% compared with the diameter of 4 and 8 inches. In addition, the statistical analysis showed that the growth of seagrass in the mangrove area was significant higher than the urban area ($p<0.05$). When considering the supporting material in the transplant process, the plug technique having coconut fiber pots provided an insignificance difference of the seagrass survival comparing with the plug technique without coconut fiber pots ($p>0.05$). Moreover, the shoot density evaluation indicated that the maximum shoot density of 12036.8 $\pm$ 5662.78 (Mean $\pm$ SD) in the urban area was higher than the maximum shoot density of 11713.1 $\pm$ 5308.81 in the mangrove area. However, the percentage of seagrass growth in this study showed a fluctuating manner depending on other environment factors such as soil sedimentation, inorganic materials, season and residual sewage. Therefore, to obtain the most benefit of the seagrass transplantation, the improvement of the marine environment and habitat should be considered carefully in relation to both natural and human factors.

KEYWORDS: survival, growth, transplantation, seagrass, plug technique, Thailand

INTRODUCTION

A seagrass bed is a habitat of small marine organisms and a source of energy transfer system in the sea (Satapoomin and Satapoomin, 2005). Unfortunately, nowadays, seagrass beds are damaged; consequently, marine resources will be depleted enormously because of environmental disaster and human activities. This anxious situation influences government agencies and local parties to look after and pay attention to rehabilitate injured seagrass beds. Makampom Bay was selected to be a study site because of the plentiful seagrasses and dugongs throughout the year in the past. However, the seagrass bed area has changed because of fisheries in the site, construction on the shore and urban intrusion. The study has been established in urban and mangrove areas close to and faraway from sewage areas, respectively. The plug technique to transplant the dominant species of seagrass, *Halodule pinifolia* (Miki) den Hartog (Phillips, 1990), was used to conduct the study of seagrass survival by investigating and comparing the environment factors (Calumpong and Fonseca, 2001) of the mangrove and urban areas, sizes of seagrass patch and with or without coconut fiber pot were factors as well.

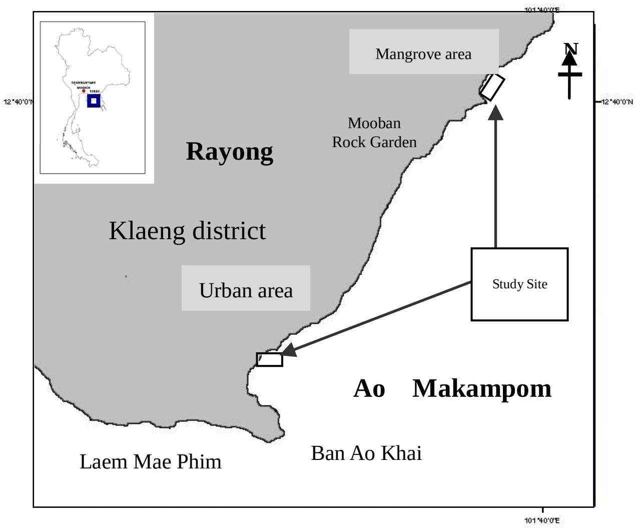


Fig. 1 Seagrass transplant experiment site, Rayong Province, Thailand.

MATERIAL AND METHODS

Study area and duration

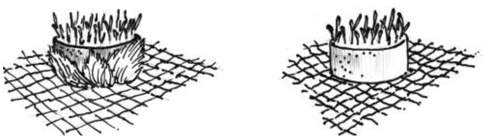
The H. *pinifolia* was chosen and employed to conduct the study of seagrass survival in Makampom bay on the west side of the Prasae River, Rayong province. The study was established at 2 locations (Figure 1). One was a mangrove area representing a clean area and another was an urban area representing a residential sewage area. The data was collected from the mangrove area at a depth range from 0 – 1.3m and a distance of 3-10 meter from donated seagrass bed, and from the urban area at a depth range from 0 – 1.1m. The study was performed during November 2007 – March 2009 (17 months).

Transplantation

The plug technique modified from the previous studies (Calumpong and Fonseca, 2001 and Phillips, 1990) was used to transplant the seagrass. PVC pipes having 3 different sizes of 4, 6 and 8 inches in diameter were used as a container. The study design comprised of 48 samples on 2 locations, there were 3 sizes of containers, 2 types of pots (with and without coconut fiber pots, which decompose slowly and reserve water well) and 4 replications. The working procedures are described as follows:

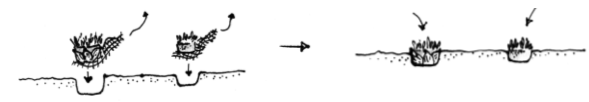
1) press PVC pipe into the primitive seagrass bed,
2) pull out the bulk of seagrass with the sediment intact with approximate 10cm height,

3) put bulk of seagrass on the net (with coconut fiber pot), or put on the net for use as a transport container (without coconut fiber pot),



4) prepare a seagrass coring hole having a 100 cm of inter-hole-gap and

5) plug the bulk of a seagrass with the sediment intact into the prepared hole having a coconut fiber pot after pulling the net off inside the hole (in case of using the pot as a container), or pull the net off (in case that no fiber pot was used).



Monitoring

The parameters necessary to be monitored in this study were the seagrass survival rate, the growth rate, the shoot density and rhizome elongation (RE) which measured the expansion of rhizome from the coring boundary by monthly. In addition, the sediment bottom characteristics of sediment grain size and organic matter values at 10cm depth from sea base were investigated monthly**.** Furthermore, the dissolved oxygen (DO) was also evaluated by in situ testing.

Analysis method

1) The seagrass survival rate was calculated from the number of remaining cored seagrass samples in each month by using the equation:

The percentage of seagrass survival (%)

$$= \frac{\text{(No.coring of SG at present)} * 100}{24}$$

2) The growth rate of seagrass was calculated from the different amount of shoots growing between the present time and the initial time in each month by using the equation:

The percentage of seagrass growth (%)

$$= \frac{\text{(No.shoots of SG at present - at initial)} * 100}{\text{No.shoots of SG at initial}}$$

3) The statistical analysis was investigated by using SPSS program with Mutivariate Analysis method and One-Way ANOVA method under a confidence level of 95%

4) The sediment analysis was evaluated by using wet sieve analysis modified from the study of Mudroch and colleagues (Mudroch *et al.*, 1997) and organic matter analysis by using a furnace at 500^{o}C for 3 hours adapted from the study of Dawes and colleagues (Dawes and Kenworthy, 1990).

RESULT

The seagrass survival rate

Table 1 shows the percentage of seagrass survival after transplantation. The results in the table indicate that the survival of seagrass started to decrease in March, 2008 at the mangrove area and in April, 2008 at the urban area. The minimum survival rate existed in November, 2008 until February, 2009 and in June, 2008, at the mangrove area and at the urban area, respectively.

The growth rate of seagrass and the shoot density

Table 2 shows the maximum percentage of seagrass growth was 238.1$\pm$142.78% in January, 2008 and the minimum growth was 6.3$\pm$17.81% in March, 2009 at the mangrove site. In case of the urban site, the maximum percentage of seagrass growth was 157.4$\pm$39.54 % in January, 2008 and the minimum growth was 0% (size 4”), all of the seagrasses were destroyed in July, 2008. To consider the shoot

Table 1 Mean values of growth, rhizome elongation, difference of rhizome elongation and leaf length by monthly at mangrove and urban areas

	Site	*Dec 08*	*Jan 08*	*Feb 08*	*Mar 08*	*Apr 08*	*May 08*	*Jun 08*	*Jul 08*	*Aug 08*	*Sep 08*	*Oct 08*	*Nov 08*	*Dec 08*	*Jan 09*	*Feb 09*	*Mar 09*
Survival	MG	100	100	100	71	75	67	58	50	42	33	29	25	25	25	25	29
(%)	UB	100	100	100	100	96	83	54	17	25	54	71	71	83	92	92	96
Growth	MG	*148*	*174*	*135*	*104*	*52*	*45*	*46*	*40*	*38*	*33*	*44*	*45*	*38*	*43*	*22*	*21*
(%)	UB	*107*	*144*	*119*	*120*	*39*	*17*	*11*	*2*	*4*	*12*	*21*	*46*	*59*	*56*	*32*	*42*
RE	MG	*11*	*34*	*55*	*58*	*70*	*63*	*78*	*87*	*97*	*117*	*123*	*127*	*141*	*150*	*165*	*127*
(cm.)	UB		*5*	*50*	*21*	*15*	*14*	*0*	*0*	*0*	*0*	*0*	*30*	*35*	*47*	*55*	*n.d*
ΔRE	MG		*23*	*21*	*3*	*12*	*-7*	*15*	*9*	*10*	*20*	*6*	*4*	*14*	*9*	*15*	*-38*
(cm)	UB			*45*	*-29*	*-6*	*-1*	*-14*	*0*	*0*	*0*	*0*	*30*	*5*	*12*	*8*	*n.d*
Leaf																	
length	MG				*9.2*	*7.4*	*9.6*	*7.8*	*8.8*	*9.4*	*11.8*	*14.4*	*17.8*	*20.8*	*19.3*	*17.2*	*12.3*
(cm)	UB				*6.5*	*2.2*	*2.3*	*5.1*	*2.1*	*2.3*	*3*	*2.8*	*3.9*	*3.8*	*7.2*	*5.4*	*4.9*

Notation: RE = Rhizome elongation, ΔRE =Difference of rhizome elongation by month

Table 2 Maximum and minimum (mean + standard deviation) of growth and the shoot density of transplanted seagrass between mangrove and urban areas

site	∅	growth (%)		Density (shoot/m^2)	
		Max.	Min.	Max.	Min.
Mangrove	4”	157.9+82.28	18.78+38.02	10480.2+4911.53	1202.1+2409.58
	6”	238.1+142.78	23.7+44.79	11713.1+5308.81	938.4+1803.56
	8”	130.4+26.62	6.3+17.81	10029.4+2590.62	1483.4+2073.02
Urban	4”	139.0+55.64	0	12036.8+5662.78	0
	6”	157.4+39.54	2.3+6.59	8891.0+2125.70	130.2+368.12
	8”	136.9+21.87	4.0+6.03	6496.1+1119.86	346.8+122.60

density of seagrass, we found that at the mangrove site, the maximum shoot density was 11713.1+5308.81 shoot/m^2 in January, 2008 and the minimum value was 938.4+1803.56 shoot/m^2 in March, 2009. In case of the urban site, the maximum value of shoot density was 12036.8+5662.78 shoot/m^2 in January, 2008 and the minimum value was 0 shoot/m^2, all of the seagrasses were destroyed in July, 2008.

The different relation of seagrass growth and the shoot density between the study sites, diameters of coring size and types of transplant were investigated by using Multivariate Analysis test. The results revealed that the percentage of seagrass growth and the shoot density between the study sites and diameters of coring size had a significant difference ($p>0.05$) but the type of transplant had insignificant difference ($p<0.05$).

Rhizome elongation and leaf length

Table 1 shows that the rhizome elongation had been increasing since conducting the seagrass transplantation in November, 2007 and deceasing in May, 2008 at the mangrove site having a maximum length of rhizome of 70 cm. The rhizome elongation started to decrease in March, 2008 with a maximum length of rhizome of 50 cm at the urban site. The highest elongation of rhizome was 165 cm and 55 cm at mangrove and urban sites, respectively in February, 2009. The largest different in rhizome elongations at the mangrove and urban sites were 23 cm observed in January, 2008 and 45 cm observed in February, 2008 respectively. The maximum length of leaf from the base was 20.8 cm observed in December, 2008 at the mangrove site and 7.2 cm observed in January, 2009 at the urban site.

Character of sediment

In case of organic matter in the transplanted seagrass bed area, Figure 2 and Table 3 show the maximum value of organic matter of 1.378% measured in March, 2008 and the minimum value of 0.294% observed in October, 2008 at the mangrove site whereas the maximum value of organic matter of 1.524% was measured in December, 2007 and the minimum value of 0.207% observed in January, 2009 at the urban site. In case of organic matter in the primitive seagrass bed area, the results showed the maximum value of organic matter of 1.719% measured in February, 2009 and the minimum value of 0.523% observed in April, 2008 at the mangrove site whereas the maximum value of organic matter of 1.590% was measured in January, 2008 and the minimum value of 0.229% observed in January, 2009 at the urban site. It can be noted that the quantity of organic matter was in the range of 0.294 – 1.378% at the transplanted seagrass bed area and in the range of 0.523 – 1.719% at the primitive seagrass bed in adjacent areas at the mangrove site whereas the quantity of organic matter was in the range of 0.203 – 1.524% at transplanted seagrass bed area and in the range of 0.229 – 1.590% at primitive seagrass bed in adjacent areas at the urban site.

In the case of the grain size factor, at the transplanted seagrass bed area, Figure 2 and Table 3 show a maximum grain size of sediment 0.114 mm. measured in August, 2008 and a minimum value of 0.047 mm. observed in February, 2008 at the mangrove site, while a maximum grain size of sediment of 0.070 mm. measured in September, 2008 and a minimum value of 0.047 mm. observed in April, 2008 at the urban site. For the grain size factor, at the primitive seagrass bed area, the results showed a maximum of grain size of 0.100 mm. measured in January, 2009 and a minimum value of 0.046 mm. observed in February, 2008 at the mangrove site, while a maximum grain size of 0.060 mm. measured in January, 2009 and a minimum value of 0.044 mm. observed in April, 2008 at the urban site. It can be concluded that the grain size of sediment in the transplanted seagrass bed area was in the range of 0.047 – 0.114 mm. and in the range of 0.046 – 0.100 mm. in the primitive seagrass bed in adjacent areas at the mangrove site, while the grain size of sediment was in the range of 0.047 – 0.070 mm. in the transplanted seagrass bed area and in the range of 0.044 – 0.060 mm. in the primitive seagrass bed in adjacent area at the urban site.

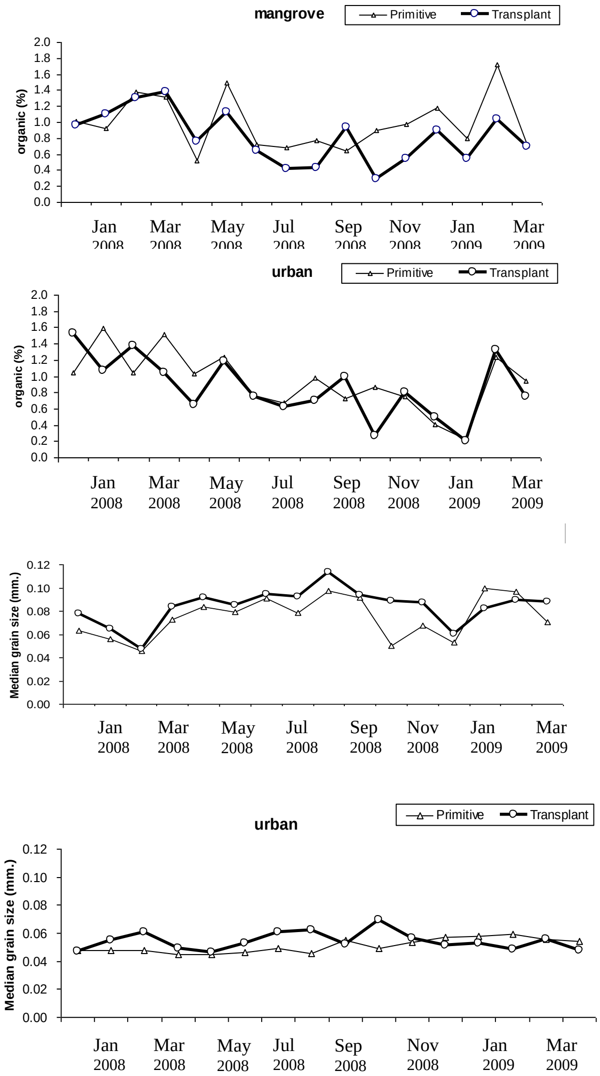


Fig. 2 Organic matter and median grain size of sediment comparing between transplant and primitive seagrass bed in adjacent area at mangrove and urban areas versus time (month)

Table 3 Percentage of organic matter and median grain size of sediment at mangrove and urban site between transplanted and primitive seagrass bed

		organic matter (%)	median grain size (mm.)
Transplanted	MG	0.294 – 1.378	0.047 – 0.114
seagrass bed	UB	0.207 – 1.524	0.047 – 0.070
Primitive	MG	0.523 – 1.719	0.046 – 0.100
seagrass bed	UB	0.229 – 1.590	0.044 – 0.060

DISCUSSION

The coring size of seagrass patch conducted in this study followed valuable suggestions of the previous studies (Keulen, 2003) and providing the highest growth of seagrass was 6 inch in diameter (∅6") under the plug technique transplantation method in both sites of study, the mangrove and urban areas as mentioned in Table 2. The result could reasonably described that the ∅6" coring might grip and provide a tighter sediment than other coring sizes when pulling them up from donor area. In addition, the growth comparison of seagrass between the study sites revealed that the mangrove area provided a percentage of seagrass growth higher than the urban area, in which the highest growth rate was 247.9% in January, 2008 (Figure 3).

The appearance of rhizome elongation throughout this study indicated that the seagrass grew enormously in the mangrove area compared to the urban area. In addition, in some periods of time such as during June to October, 2008, there was no rhizome elongation occurring in the urban area because the seagrass was destroyed (Table 1). Furthermore, the difference of rhizome elongation was incertain especially in the urban area and found that the extreme elongation of the seagrass was 30 cm. in November, 2008 after which the rhizome elongation disappearance occurred during July to October, 2008. However, the highest expansion of rhizome elongation of transplanted seagrass in both areas appeared in the same period of February, 2009.

The percentage of seagrass growth significantly decreased in April 2008 (Table 1) because of algae bloom. Therefore, the amount of floating algae provided a protection to a sunlight exposure which is necessary for seagrass photosynthesis. Consequently, during low tides in the ocean, seagrass leaves were wrapped with algae to reduce the dissolved oxygen (DO) and resulted in a growth inhibition of seagrass observed in the next month of results (in May, 2008) (Table 4). However, the damaged seagrass had an increase of organic matter in bottom sediment in the same period of May, 2008 (Figure 2). The percentage of seagrass growth decreased in the period of May to August, 2008 (Table 1 and Figure 3). The result complied with the study of Heidelbaugh and colleagues (Heidelbaugh ,2000) in that the phenomena of low water level occurred in June/July in shallow sites. This situation could be interpreted and described by a decrease in sea level at daytime, thereby receiving a long period of direct daylight that cause of seagrass broke which presented in a tide table at Laem Sing during May to August, 2008 in Figure 5. The lowest water level occurred in August 2008 and resulted in the largest decease of seagrass shoot density.

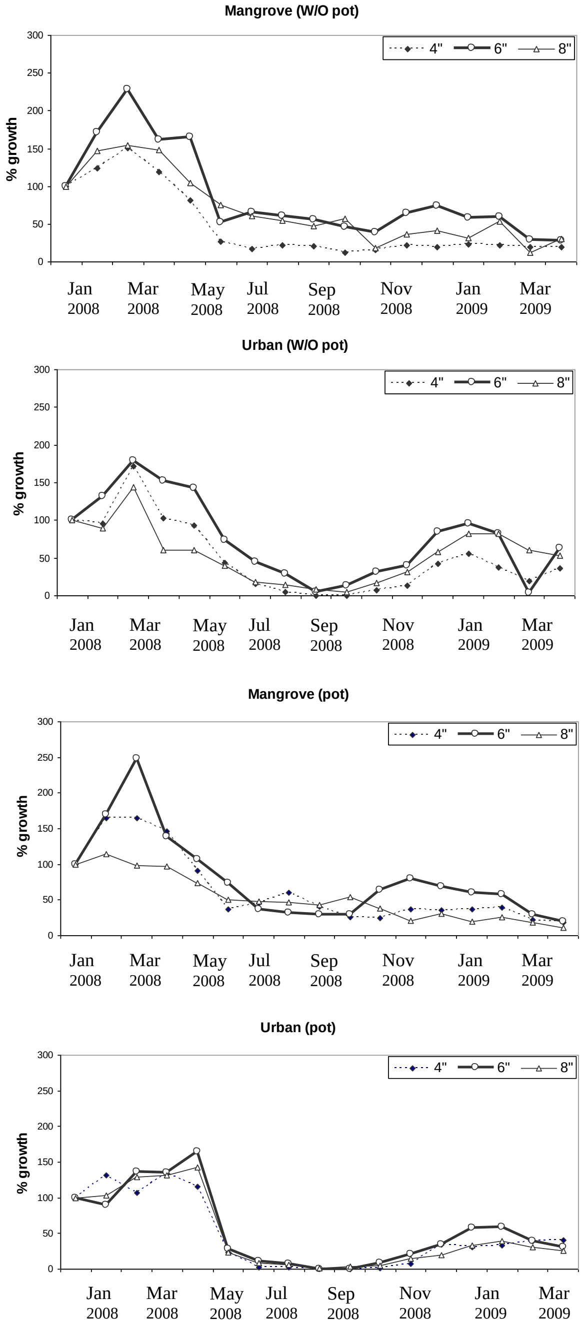
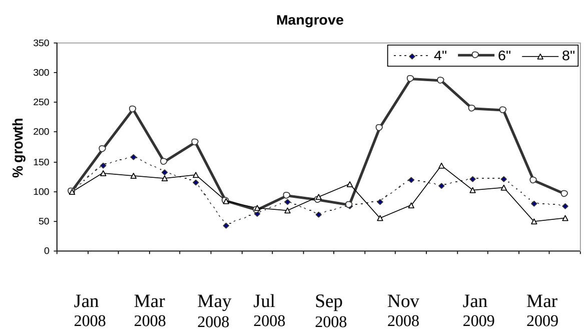


Fig. 3 The percentage of transplanted seagrass growth versus time (month) with and without coconut fiber pot in mangrove and urban areas



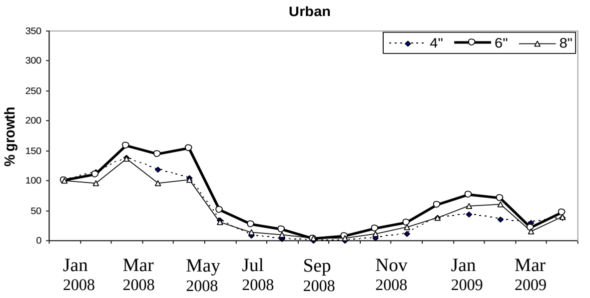


Fig. 4 The percentage of transplanted seagrass growth versus time (month) in different sizes of seagrass patches and do not concern the sediment transport areas

Table 4 Dissolved oxygen in water at mangrove and urban areas

	site	*Jan 08*	*Apr 08*	*Jul 08*	*Oct 08*	*Jan 09*
DO	MG	8.0	3.2	7.4	6.8	5.8
(mg/l)	UB	8.1	2.7	7.4	6.8	5.0

Table 5 Pearson Correlation analysis of growth & organic matter and growth & grain size at mangrove and urban site area

		r	Pearson Correlation Sig.(2-tailed)
mangrove	growth organic matter	0.556	0.025*
urban	growth organic matter	0.406	0.133
mangrove	growth grain size	- 0.599	0.014*
urban	growth grain size	- 0.305	0.251

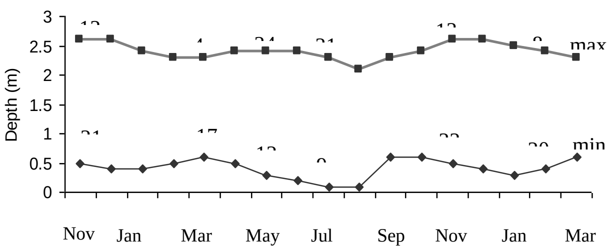


Fig. 5 Maximum and minimum depth range versus time at Laem Sing

In addition, we found that there were seagrasses covered with sand dunes in some areas at the mangrove area throughout the study period since March, 2008. However, the problem was solved in some seagrass areas in January 2009. Therefore, it was reasonable to describe that the seagrass survival in the mangrove area having seagrasses covered with sand dune was less than the urban area in the consecutive month. Nevertheless, in the ignorance of seagrass covered with sand dune area, the results showed that the growth rate of seagrass in the mangrove area was higher than in the urban as mentioned in Figure 4. The growth comparison between the horizontal direction and the vertical direction revealed that the seagrasses had been well developed in both directions of horizontal (rhizome elongation) and vertical (leave length) growths in the mangrove area and were better than in the urban area as presented in Table 1.

The Pearson Correlation statistical analysis comparing between organic matter and grain size of sediment, and the seagrass growth showed that all factors significantly related to each other ($p<0.05$) (Table 5). The results indicated that the growth presented a positive relation with organic matter and a negative relation with sediment grain size in the mangrove area, however, there was insignificant relation in the urban area ($p>0.05$). From these interpretations, it can be noted that organic matter and grain size were influential factors of the seagrass growth in the mangrove area while other environmental factors such as nutrient and suspended solid from sewage might be also involved to the seagrass growth in the urban area.

SUMMARY

1) The coring size for transplantation of *Halodule pinifolia* (MiKi) den Hartog providing the highest seagrass growth was 6 inches (~15cm) having a percentage of seagrass growth of 247.9% in January, 2008. The highest rhizome elongation (mean) during 15 months was 165 cm and 55 cm in the mangrove and urban areas, respectively.

2) The transplantation with and without coconut fiber pot had an insignificant difference ($p>0.05$).

3) The duration of October to March, after the rainy season to start of the summer season, was a suitable period for seagrass rehabilitation on these study sites.

4) The significant factors for seagrass rehabilitation at both sites of clean and sewage areas were depth of water level through the year, the time interval to protect against daylight causing seagrass damage, the character of topography, construction and human activities around the seagrass transplantation area that might cause sediment erosion or deposition to have affect on or to destroy the seagrass survival.

5) However, the plug transplantation method might not be the best method for seagrass rehabilitation because of an inappropriate procedure of translocation using a supportive patch. Therefore, this study might also be concerned with other

rehabilitation methods such as the tissue culture technique, seed culture or plant culture of the seagrass habitat in the future to avoid the negative factor from allocate seagrasses of donated seagrass bed.

ACKNOWLEGDEMENTS

I am thankful for the advice of Dr. Kanjana Adulyanukosol some suggestions of Mr. Supot Chanthapornsilp and appreciate editing of the manuscript by Wichit Nosoongnoen.

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