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QUALITATIVE ASSESSMENT OF FIVE ARTIFICIAL GROWTH MEDIA ON GROWTH AND SURVIVAL OF *THALASSIA TESTUDINUM* (HYDROCHARITACAE) SEEDLINGS

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Abstract

Growth and survival of turtle grass, *Thalassia testudinum* Banks *ex* Konig, seedlings from five Florida sites were monitored in four commercially available growth media (Jiffy-7® peat pellets, Rootcubes®, Horticubes® and Plant Plugs®) and a control medium of shell hash (aragonite). After six months in culture, survival was highest (40%) in Rootcubes and the control, but efficacy (growth x % survival) was highest for seedlings in Plant Plugs. Cost effectiveness (efficacy/cost per planting unit) was highest for Horticubes, followed by Rootcubes, Jiffy-7 peat pellets and Plant Plugs. A preliminary field transplanting experiment was performed using *Thalassia* seedlings collected during the summer of 1982, cultured in peat pellets in the laboratory over the winter of 1982/83, and transplanted during the spring and summer of 1983. Observations after seven months, indicated that planting the units in a *Halodule wrightii* bed, a "compressed successional" approach, resulted in higher survival than planting in bare sediments. In addition, survival of transplants that were anchored with steel staples was no greater than unanchored transplants. The variability of these results suggests that site, planting design and logistic considerations are all important in deciding what growth medium and anchoring system should be employed in a particular seagrass restoration project.

INTRODUCTION

Turtle grass, Thalassia testudinum Banks ex König, is the predominant seagrass species in coastal marine/estuarine ecosystems surrounding Florida (Thayer and Ustach, 1981; Zieman, 1982). Revegetation studies involving Thalassia have utilized a variety of planting units, ranging from sods to individual short-shoots and seedlings (see Knight et al., 1980; Phillips, 1980, 1982). Success of past transplanting efforts has been quite variable, but three basic principles, which were outlined by Phillips and Lewis (1983), have been fairly well established. First, planting units should be large enough to support the normal growth of plants after transplanting, but small enough for reasonable handling and transportation. Second, the harvesting of planting material should not cause damage to the donor site. Third, planting units should be planted close enough to coalesce within a reasonable time, but far enough apart to optimize costs. Fonseca et al. (1982) developed a cost-evaluation technique for seagrass restoration projects that will aid in spacing decisions.

Since the use of vegetative material is destructive to source beds, because Thalassia is slow to propagate into disturbed areas (Godcharles, 1971; Zieman, 1976), there is a growing interest in the use of Thalassia seedlings in restoration projects (Thorhaug, 1974; Durako and Moffler, 1981; Derrenbecker and Lewis, 1982; Phillips and Lewis, 1983). Several problems exist, however. Seed production in Thalassia appears to be variable spatially and temporally, ranging from rare (Phillips, 1960; Zieman, 1975; Grey and Moffler, 1978) to abundant (Lewis and Phillips, 1980; Williams and Adey, 1983). Fruiting beds also may not be close to the restoration sites, while seedlings may not be available in the field when the projects are being performed. Finally, seedlings exhibit distinctive morphogeographic growth patterns, a characteristic which may be important when selecting seed stock for revegetation projects (Durako and Moffler, 1981).

Recently, a technique was developed which may provide a non-destructive year-round source of seagrass planting units (Durako and Moffler, 1981). This technique utilized *Thalassia* seedlings grown in an artificial culture system using commercially available peat pellets. When the seedlings rooted in the pellets they were transplanted into the field. The present paper reviews several subsequent studies on the use of *Thalassia* seedlings grown in peat pellets as a source of transplanting material and presents preliminary growth data for seedlings from five sites in Florida grown in four commercially available growth media.

MATERIALS AND METHODS

Lab Culture/Field Transplant Study 1982/83

Seedlings of *Thalassia* were collected from Grassy Key in the Florida Keys (FK), Matheson Hammock in Biscayne Bay (BB) and along the Sunshine Skyway causeway in Tampa Bay (TB) during July 1982. The material was transported to the laboratory cooled in ice chests (see Durako and Moffler, 1981). Twenty seedlings per population were planted into Jiffy-7 peat pellets which were placed above a layer of aragonite shell hash in 5 x 5 x 12 cm plastic pots. The shell hash was utilized to raise the top surface of the peat pellets level with the top edge of the pots and to allow less restricted root growth. The seedlings were grown in 71 1 aquaria containing synthetic seawater (Instant Ocean®) at 32 ppt. Temperatures were maintained between 24-26 C. Illumination was provided by Duro-Test Vita Lites on a 14:10 L:D cycle. Additional material was planted as above, but grown in a large (2000 l) culture tank.

Leaf growth, as leaf area (Durako and Moffler, 1981), was measured monthly until the seedlings were transplanted into the field. Growth measurements were made only on seedlings in the aquaria. On 10 May 1983, seventeen TB seedlings from the large culture tank were transplanted to a site north of Bunces Pass in western Tampa Bay. Nine of the transplants were anchored with U-shaped metal staples (Derrenbecker and Lewis, 1982) and eight were transplanted without anchors (Fig. 1). Nine transplants (5 anchored and 4 unanchored) were planted within a small circular Halodule bed; the remaining eight (4 anchored and 4 unanchored) were planted in bare sand outside the bed. On 8 July 1983, thirty-six seedlings from the aquaria were transplanted into another Halodule bed approximately 10 m south of the above site (Fig. 1). Half of these transplants were anchored. Survival was monitored periodically and the production of new short-shoots was noted (all original transplants had one short-shoot).

Growth Media Study 1983/84

Thalassia seedlings from the Florida Keys and Biscayne Bay were collected during July, while those from Tampa Bay, Lake Worth (LW) and North Florida (St. Joseph Bay) (NF) were collected in August 1983. The experimental design for this study involved the five populations grown in five growth media: Rootcubes®, Horticubes®, Jiffy-7® peat pellets, Plant Plugs® and a control of shell hash. There were five replicates (one/aquarium) of each combination (Fig. 2). The low number of TB and NF seedlings collected in 1983 necessitated a reduction in the number of treatments for these two populations. Seedlings were planted into growth

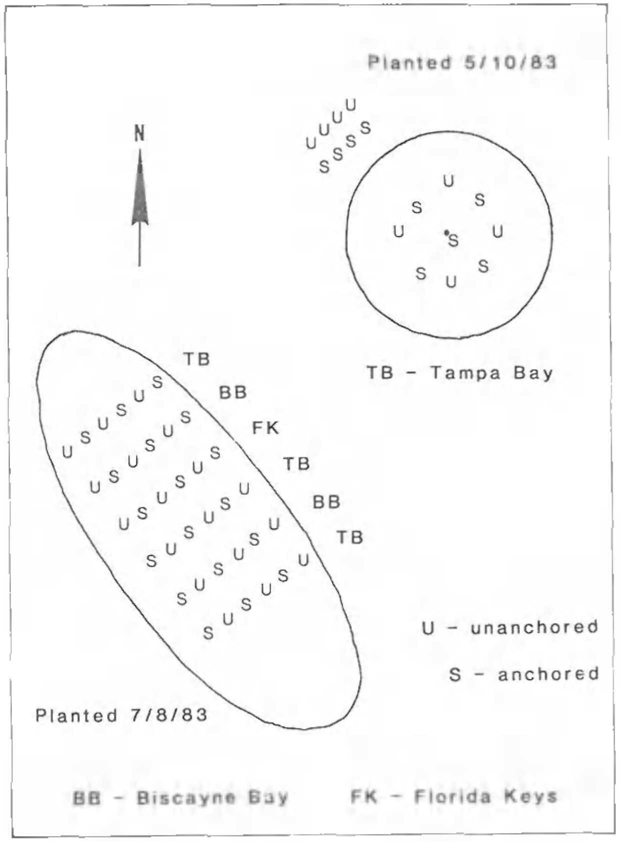

Figure 1. Schematic of field transplanting sites in western Tampa Bay.

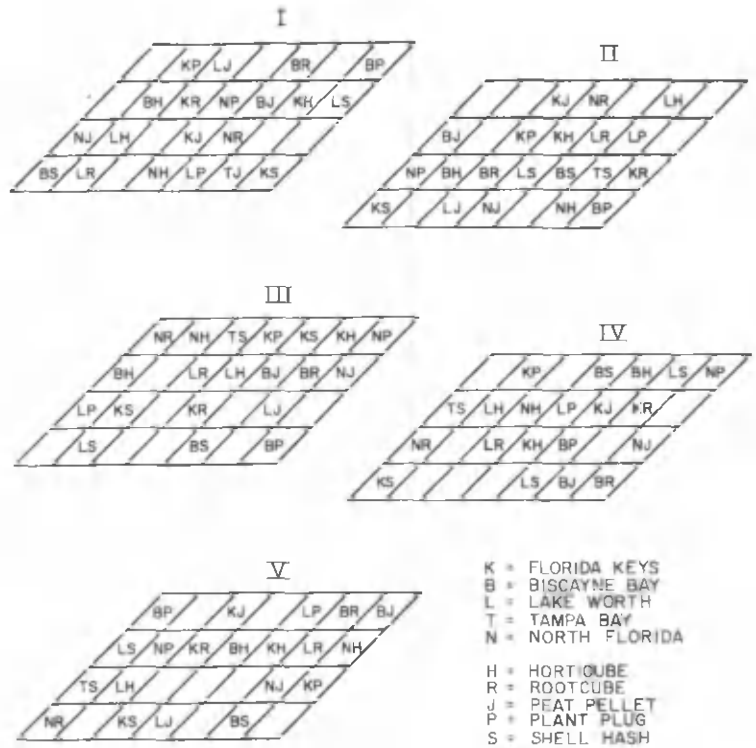

Figure 2. Experimental design of growth media/population study showing arrangement of treatment combinations in the five 71 l aquaria.

media in 5 x 5 x 12 cm plastic pots, as above. Within each aquarium, planting units were randomly arranged. Aquaria contained synthetic seawater at 30-32 ppt.; temperatures and photoperiod were as above.

Growth and survival of the seedlings were assessed after 3, 6 and 9 months in culture. Growth was measured as total leaf area. Due to the high mortality rates, efficacies and cost effectiveness of the growth media were evaluated without regard to population. Efficacy was used to assess the effectiveness or desirability of a growth medium considering both growth and survival. Cost effectiveness was based on the following prices: Horticubes-$50.35/3240 units or 1.6¢ each; Rootcubes-$49.40/1000 units or $4.9¢ each; Jiffy-7 peat pellets-$45.00/1000 units or 4.5¢ each; Plant Plugs-$24.00/100 units or 24.0¢ each. We did not consider space, handling or labor cost factors.

RESULTS

Lab Culture/Field Transplant Study

Leaf areas of the seedlings increased linearly for the first five months in culture (Fig. 3). FK seedlings had the highest rate of growth during this period. BB and TB seedlings had much lower growth rates; their leaf areas were only half (approximately 12 cm^2) that of the FK population (23.5 cm^2) after 5 months. Subsequent growth patterns were erratic. FK seedlings exhibited a decline in leaf area from five to ten months, while BB and TB seedlings continued to show an increase. Part of this pattern was due to variations in leaf width (Fig. 4). For the first five months, leaf widths followed the same trend as leaf areas. They increased the greatest in the FK seedlings with BB and TB seedlings having similar but lower rates of increase. Leaves of BB and TB

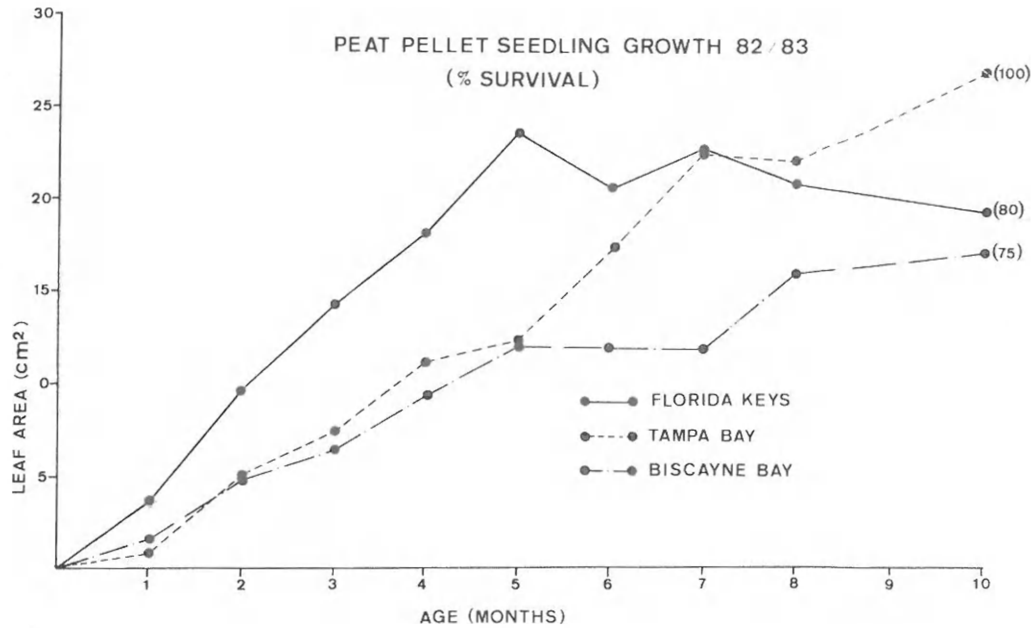

Figure 3. Leaf growth of three populations of Thalassia testudinum seedlings in laboratory peat pellet cultures. Numbers in parentheses are percent survival after ten months in culture.

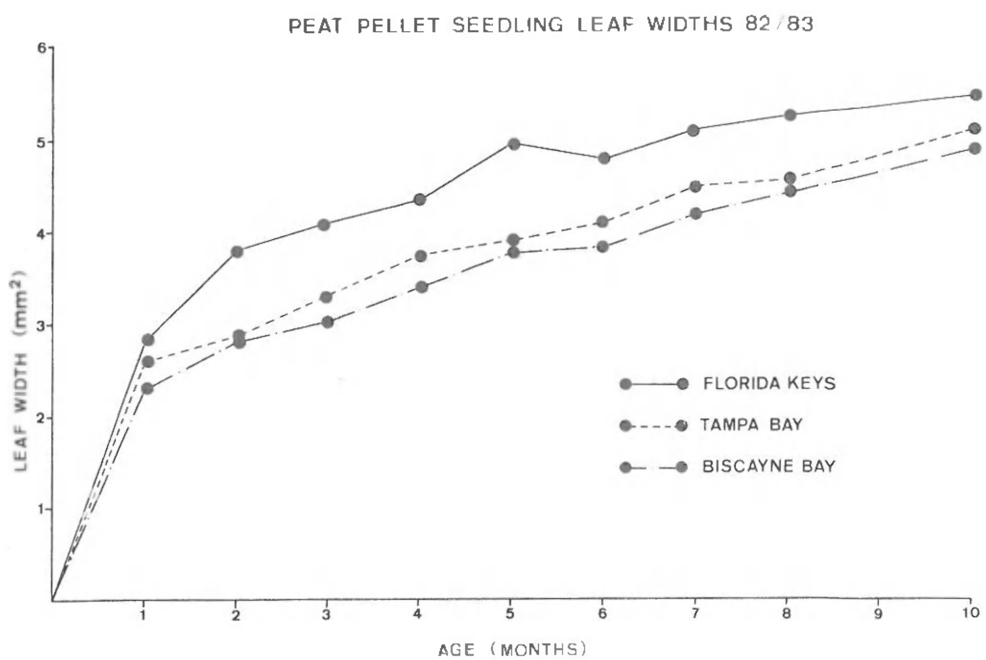

Figure 4. Leaf widths of three populations of Thalassia testudinum seedlings in laboratory peat pellet cultures.

seedlings continued to increase in width from five to ten months, while FK seedlings exhibited a lower rate of widening during this period. Survival after ten months in culture was 80, 75 and 100% for the FK, BB and TB seedlings, respectively. All mortality occurred during the first four months in culture.

Although survival of the seedlings transplanted to the field was periodically monitored, it was not accurately assessed until January 1984. The monitoring difficulty was mainly due to the lush growth of Halodule and high turbidities that were present during the summer and fall. Some of our early observations revealed that 3 of the 4 unanchored transplants in the bare substrate had disappeared two weeks after they had been transplanted. In addition, none of the transplants in the bare substrate were left by the time the second group of seedlings were transplanted. On 17 January 1984, we were able to obtain reliable counts of surviving transplants because of the coincidence of clear water and a leaf die-back in Halodule. Survival of Thalassia transplants in the small circular patch of Halodule after 252 days was approximately 44% (Table 1). One of the unanchored transplants had produced 3 additional short-shoots. Anchored transplants showed no more survival than unanchored transplants.

Survival of the second group of transplants, after 193 days was 61% overall (Table 1). The indigenous TB seedlings had a higher survival rate (73%) than the FK and BB seedlings (50%). One of the anchored TB seedlings had produced a second short-shoot. Once again, anchoring did not increase survival. In both groups of transplants some of the "mortality" seemed to be due to man-induced impacts rather than biological or environmental factors. We observed recent prop tracks

Table 1. Survival of Thalassia testudinum peat pellet transplants as of 17 January 1984.

Population	Planting Date	Units Planted A*	Units Planted U**	Units Planted T***	Units Surviving A*	Units Surviving U**	Units Surviving T***	% Survival A*	% Survival U**	% Survival T***
Tampa Bay	5/10/83	5	4	9	2	2	4	40	50	44
Florida Keys	7/8/83	3	3	6	2	1	3	67	33	50
Biscayne Bay	7/8/83	6	6	12	3	3	6	50	50	50
Tampa Bay	7/8/83	9	9	18	5	8	13	56	89	73
Total		18	18	36	10	12	22	56	67	61

*A-anchored **U-unanchored ***T-total

during several of our observation periods and found a number of peat pellets and staples that had been dislodged from the substrate along the tracks.

Growth Media Study

Growth and survival rates for the five populations of seedlings in this study were all lower than those of the previous year (Table 2). Since the density of the seedlings in the aquaria was the same (20/aquarium), the reduced growth and survival may have resulted from the mixing of different growth media (and their associated fertilizers) in the aquaria. Evidence for this was suggested by the continuous problems we experienced with algal blooms. These blooms resulted in highly turbid water and highly epiphytized Thalassia leaves. Interestingly, after nine months in culture, seedlings displayed a consistent morphogeographic pattern in leaf areas, which decreased as latitude of origin increased

Table 2. Growth and survival of Thalassia testudinum seedlings in five growth media after 3, 6 and 9 months in culture.

		Leaf Area (cm^2)					Population		
Population	Month	Horticube	Rootcube	Peat pellet	Plant plug	Shell hash	mean leaf area	n	% survival
Florida Keys	3	0.74	1.54	2.56	1.79	1.11	1.52	20	80
	6	0	0.80	1.65	1.95	0.63	1.05	6	24
	9	0	0	14.30	0	0	14.30	1	4
Biscayne Bay	3	0	2.50	0	0	0.50	1.83	6	24
	6	0	4.16	0	0	0	4.16	2	8
	9	0	10.80	0	0	0	10.80	1	4
Lake Worth	3	3.01	3.62	1.72	5.26	3.47	3.64	17	68
	6	4.76	2.70	2.85	6.44	5.16	4.46	13	52
	9	3.84	8.54	4.59	15.09	7.10	8.88	10	40
Tampa Bay	3	-	-	2.39	-	-	2.39	3	60
	5	-	-	5.88	-	-	5.88	1	20
	9	-	-	8.82	-	-	8.82	1	20
North Florida	3	1.35	0.99	0.48	2.12	-	1.27	19	95
	6	4.67	1.88	0	1.94	-	2.84	6	30
	9	7.26	1.00	0	2.00	-	3.90	5	25
Treatment									
mean leaf area	3	1.56	2.12	1.75	2.98	1.86			
	6	4.73	2.72	3.46	3.87	2.90			
	9	5.55	7.22	9.24	9.85	7.10			
n	3	12	16	13	13	11			
	6	4	8	3	7	6			
	9	4	4	3	5	2			
% survival	3	60	80	52	65	73			
	6	20	40	12	35	40			
	9	20	20	12	25	13			

(Fig. 5). This relationship may have been coincidental, however, and due to the very low survival rates (n=1 for FK, BB and TB populations. Because of low survival rates, the growth media had to be evaluated without regard to population effects.

Seedlings in Plant Plugs and peat pellets had higher growth rates than those in the shell hash control, and these had similar growth to seedlings in Rootcubes (Table 2). Seedlings in Horticubes had the lowest growth rates. Survival was higher than the control in all growth media except peat pellets. Efficacies at 9 months for all the commercial growth media were higher than the control, illustrating some enhancement of growth and/or survival (Table 3). High mortality of seedlings in peat

Table 3. Efficacy and cost effectiveness of growth media for _Thalassia testudinum_ seedlings after 3, 6 and 9 months in culture.

	Efficacy*			Cost Effectiveness**		
Treatment	3 months	6 months	9 months	3 months	6 months	9 month
Horticube	0.94	0.94	1.11	0.58	0.59	0.69
Rootcube	1.70	1.09	1.44	0.35	0.22	0.29
Peat pellet	0.91	0.42	1.11	0.20	0.09	0.25
Plant plug	1.94	1.35	2.46	0.08	0.06	0.10
Shell hash	1.36	1.16	0.94	-	-	-

*Efficacy = mean leaf area x % survival
**Cost Effectiveness = efficacy / cost per planting unit

pellets resulted in a low efficacy. Efficacy was also low for Horticubes, but in this medium it was mostly due to low growth rates rather than survival. The most expensive growth medium, Plant Plugs, had the highest growth rate and survival, hence the highest efficacy. Correspondingly, Rootcubes, which were the second most expensive growth medium, had the next highest efficacy. Cost effectiveness was the inverse of efficacy. The least expensive growth medium, Horticubes, was the most cost effective; Plant Plugs were least cost effective.

DISCUSSION

Lab Culture/Field Transplant Study

Clinal morphogeographic growth patterns of the *Thalassia* seedlings were evident for the first five months in culture. Growth patterns were similar to those previously reported for laboratory cultured seedlings (Durako and Moffler, 1981). However, actual growth rates of the 1982 seedlings in the present study were much higher than those of the 1980 seedlings. Both groups were cultured in peat pellets, although in the earlier study the pellets were planted in shallow (5 cm deep) plastic trays. This restricted root growth somewhat and may have resulted in less leaf growth. Most of the seedlings in the present study had roots which extended through the peat pellet, shell hash and out the bottom of the plastic pots (12 cm deep) well before the time they were transplanted into the field.

Leaf widths indicated a physiological and/or genetic similarity between BB and TB seedlings; both had narrower leaves than the FK seedlings. McMillan (1978) suggested that differing leaf widths in geographic variants of *Thalassia* were indicative of an ecotypic response. This response may be correlated with environmental stress over a latitudinal gradient (Phillips and Lewis, 1983). Leaf areas exhibited patterns similar to leaf widths for the first five months in culture.

The erratic nature of the patterns for all three populations from 5-10 months was due to differing leaf turnover rates, which altered mean leaf lengths and number of leaves/seedling.

Phillips and Lewis (1983) tested the hypothesis that leaf width is significant for evaluating transplant stock because the widest leaves may reflect the least environmental stress and constitute the most adaptable transplants. Their results indicated this was true for eelgrass (Zostera marina), but Thalassia exhibited narrow tolerances characteristic of genotypic rather than phenotypic responses. Our transplanting results, while preliminary, support their conclusions. TB seedlings had the highest survival rates, suggesting this population may be physiologically and/or genetically superior to the other two populations in its ability to adapt to Tampa Bay conditions (sensu McMillan, 1978; McMillan and Phillips, 1979). Further evidence for this is the observation that only TB transplants produced additional short-shoots. Coupled with possible problems of introducing non-indigenous pathogens when transplanting non-native seedlings in situ (Durako and Moffler, 1981), these data reinforce support for use of local seedling stock (when available) in transplanting projects.

Maintaining seedlings in a "nursery" may provide a year- round non-destructive source for Thalassia planting units. Low mortality of seedlings in the peat pellets after 10 months in culture indicates that they do provide a suitable growth medium for this species. This is in contrast to the results of Williams and Adey (1983) who reported 75% mortality of Thalassia seedlings after 8 months in a coral reef microcosm. The ability to maintain seedlings over winter may be beneficial in two respects. First, in Tampa Bay, low water temperatures coupled with extremely low tides result in a winter die-back of leaves (Phillips, 1960; Lewis et al., in press). Without the benefit of stored reserves in a rhizome, which are important in sustaining plants in winter (Dawes and Lawrence, 1980), transplants may experience high mortality. Consequently, spring may be the best time for transplanting seedlings in Tampa Bay, rather than August to November as previously suggested (Phillips, 1980). Second, our observations and those of Williams and Adey (1983) indicate mortality of seedlings is highest in the first several months of growth and those seedlings that survive seem to be more robust.

The peat pellet technique certainly conforms to the first two principles of seagrass transplanting outlined by Phillips and Lewis (1983). Peat pellets provide a suitable growth medium and readily fit into commercially available plastic trays which are easy to transport. In addition, the use of material that has washed ashore is non-destructive as this resource has already been lost to the donor beds. However, spread of the transplants was low, preventing evaluation of spacing requirements. Failure of all our transplants in bare sediments (this study, Durako and Moffler, 1981; and additional unpublished data), compared to the relatively good survival of seedlings planted within *Halodule* beds, indicates the "compressed succession" approach should be considered as a fourth technological principle for future transplanting efforts. This approach alleviates the need for anchoring devices for *Thalassia*/peat pellet planting units in low current areas. Significantly, Connel Associates (1983) reported that universal failure of *Thalassia* seedling transplants in an experimental project in Biscayne Bay was due to the lack of a suitable anchoring device. They noted that seedlings turned black where there was contact with metal anchors. We did not observe this reaction in our study. Metal staples were used to anchor the peat pellet with no direct contact between metal and plant. However, we agree that caution be used with metal anchoring devices because of the possibility of producing toxic metal sulfides in the reduced sediments characteristic of most transplant sites. In areas with relatively high current velocities, anchors may still be required, even with a "compressed succession" approach.

Growth Media Study

One of the initial reasons for using peat pellets as a growth medium in addition to ease of sowing, transport and handling (Durako and Moffler, 1981), was the provision of a uniform growth substrate. This was necessary to test effects of various biological and physical factors on seedling growth in order to optimize the "nursery" system and transplant procedure. Jiffy-7 peat pellets are designed as a medium for rooting cuttings and seeding vegetable and floral crops. Several characteristics of the pellets, when submerged, lead to a search for other growth media which might provide greater uniformity and possibly better growth. Imbibition was quite variable for peat pellets. This resulted in heterogeneity in the size and density of hydrated pellets. In addition, the peat becomes rather fluid, and care must be taken when pellets are moved to minimize resuspension of the peat. These characteristics may only present problems in research projects and may not be significant in applied transplanting programs.

Only one of the four commercial growth media tested in this study, Plant Plugs, was specifically designed for use with submerged plants. This specificity, with its comparatively small market, may be one reason why plant plugs were also the most expensive (and least cost effective) media. It seems, however, that "you get what you pay for"; growth and survival were highest for seedlings in Plant Plugs resulting in the highest efficacy. The soil matrix of Plant Plugs has a high degree of integrity; they can be removed from aquaria without harm and without clouding the water. Horticubes and Rootcubes are used primarily as growth media for floral crops. They differ from each other in size and density (Rootcubes are larger and denser). Rootcubes sink when placed in water. In contrast, Horticubes are initially quite buoyant, but they maintain their integrity after submergence better than Rootcubes. The low growth rates of seedlings in Horticubes may have been partially due to their small size (24.6 cc vs 55.7 cc for Rootcubes). One potential application for the use of Horticubes and Rootcubes may be in the production of "sods" of seagrasses. Both media come in sheets which reduces handling and space problems.

We feel the high mortality of seedlings in this study was partially a result of our experimental design. In an attempt to randomize treatments and populations, we may have created eutrophic conditions within the aquaria. Randomization was necessary to separate population and treatment effects from aquarium effects. However, each medium contains its own type of fertilizer, the combination of which may have been inhibitory to the seedlings while promoting prolonged algal blooms. This suggestion is reinforced by the fact that mortality was highest in the peat pellets, a medium we know is suitable for growth. Despite high mortality, a morphogeographic pattern in leaf area was present after 9 months and even applied to the additional LW and NF seedlings. These two populations should be better adapted to low water temperatures than the

BB and FK seedlings (McMillan, 1979; McMillan and Phillips, 1979), and may be more suitable as transplant stock for Tampa Bay.

Seedlings rooted in all the tested media and most of the media enhanced growth relative to the shell hash control. Future studies should segregate media into separate culture systems and evaluate success of field transplants in different media. Once an optimal medium is selected for a particular species or propagule, then development of efficient procedures for handling and transport can proceed. We have initiated work in this area using shellfish grow-out racks (Nestier) to hold trays of peat pellets and sheets of Horticubes or Rootcubes. The racks are rigid, stackable and reusable and hold promise as a seagrass nursery "Liner" system.

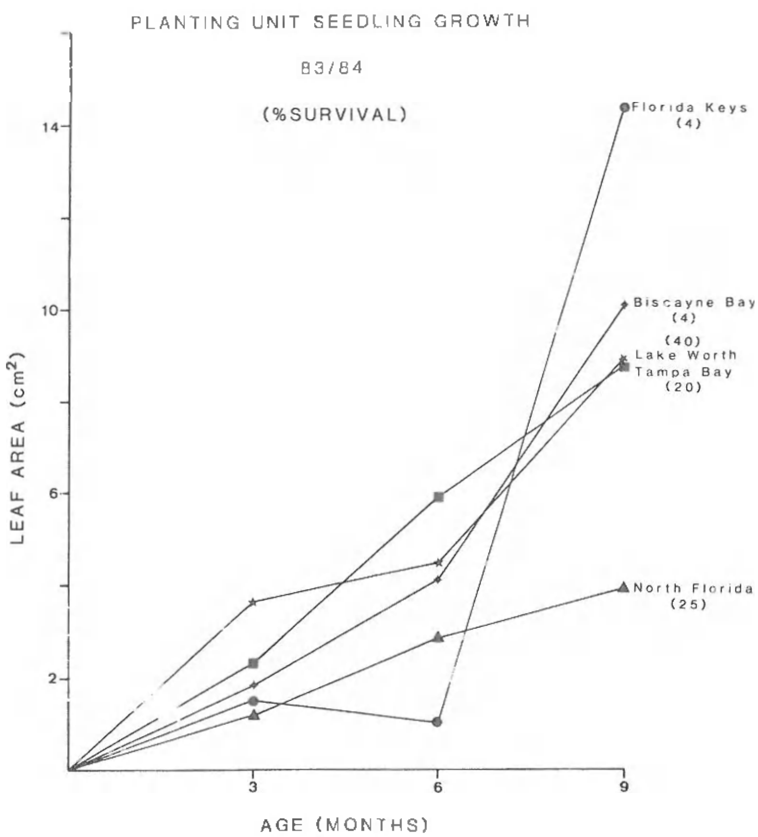

Figure 5. Leaf growth of five populations of Thalassia testudinum seedlings in laboratory cultures. Numbers in parentheses are percent survival after nine months in culture.

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