COMMON GARDEN OF SEAGRASSES *Halodule uninervis* (Forsskål) Aschershon AND *Halodule pinifolia* (Miki) den Hartog

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A simple culture system 'common garden' has been set up in Institute of Environmental and Marine Science (IEMS) Dumaguete City Philippines (N 09° 19' 52.8" and E 123° 18' 34.1") at the running seawater outdoor experiment tank aimed to monitor the variation of shoot density, leaf morphology and growth rates of marine plant genus Halodule. The mean shoot densities of *Halodule pinifolia* were varied and tend to decrease after the three month period of common garden. Using one-way ANOVA tests, significant differences were detected on the comparison among the months of common garden (F=6.400; p=0.005), mean leaf growth rate (mm/day) of *H. pinifolia* (F=13.510; p=0.000), and the mean leaf widths from Siquijor (F=9.274; p=0.001) among the three month period of common garden. Meanwhile, there were no significant differences detected on leaf width (mm) of *H. pinifolia* origin from Bantayan site, on mean leaf widths of *H. uninervis* from Bantayan, on mean leaf widths of *H. uninervis* from Banilad, and on mean leaf growth rate (mm) of *H. uninervis* among the three month period of common garden. The result suggests that the 'common garden' procedure could have possibly given a clearer pattern of genus Halodule leaf width, shoot density, and growth rate if the could be prolonged. It could also be suggested that an adequate number of shoot density on the initial procedure for 'common garden' should be properly determined. This is to avoid overcrowding of the samples later, when growth rate will tend to increase without compromising the leaf width and shoot density measurements.

Keywords: *common garden, seagrasses, shoot density, leaf width, growth rate, Halodule pinifolia, Halodule uninervis.*

Sistem kultur Common Garden sederhana telah dibangun di Institute of Environmental and Marine Science (IEMS) Dumaguete City Philippines (09° 19' 52,8" LU dan 123° 18' 34,1" BT) dalam tangki penelitian di luar ruangan untuk memantau variasi kepadatan tunas, morfologi daun dan laju pertumbuhan rumput laut genus Halodule. Kepadatan tunas rata-rata *Halodule pinifolia* bervariasi dan cenderung menurun setelah periode tiga bulan. Menggunakan uji ANOVA satu arah, perbedaan nyata terdeteksi pada pembandingan bulan (F = 6.400, p = 0,005), rata-rata laju pertumbuhan daun (mm/hari) *H. pinifolia* (F = 13,510, p = 0,000), dan rata-rata lebar daun dari Siquijor (F = 9,274, p = 0,001) antara ketiga bulan common garden. Sementara itu, tidak ada perbedaan nyata yang terdeteksi pada lebar daun (mm) *H. pinifolia* asal Bantayan, pada lebar daun rata-rata *H. uninervis* dari Bantayan, pada lebar daun rata-rata *H. uninervis* dari Banilad, dan laju pertumbuhan daun rata-rata (mm) dari *H. uninervis* dalam periode tiga bulan dari common garden. Hasil ini menunjukkan bahwa prosedur 'common garden' mungkin bisa memberikan pola yang lebih jelas tentang lebar daun, kepadatan tunas, dan laju pertumbuhan genus Halodule jika waktu bisa diperlama. Juga dapat disarankan bahwa kepadatan tunas pada prosedur awal common garden harus ditentukan dengan baik untuk menghindari kesesakan sampel di kemudian hari, karena laju pertumbuhan cenderung meningkat sehingga ukuran lebar daun dan kepadatan tunas tidak terpengaruhi.

Kata kunci: *common garden, lamun, kepadatan tunas, lebar daun, laju pertumbuhan, Halodule pinifolia, Halodule uninervis.*

INTRODUCTION

Seagrasses are dominant primary producers and play a central role in stability, nursery function, biogeochemical cycling, and trophodynamics in coastal ecosystems. In order to study the dynamic adaptation that allowed seagrass to survive in different ecological fluctuations, a common garden, an aquatic system of seagrass growing, has been developed to resemble natural environmental requirements in enclosed containers. A common garden experiment is an experiment where one or more organisms are moved from one environment to other environment. In a usual common garden experiment, two species of plants growing in their native environments would both be transplanted in a common environment (Molles, 2002). This common garden method will hopefully provides the expectation that it will represent a useful tool for studying seagrass growth and characteristics in responds to the environmental conditions.

A number of seagrass species has been successfully cultivated in outdoor tank with a plug growing in containers within different sizes (Mc. Millan 1980; Zimmerman *et al*., 1981, Pulich, 1982). Short (1985) was also able to successfully established a method for culture tropical seagrass into 1.5 m^2 culture tanks, whereas, Howard and Short (1986) had establish a simple cultures of the sub-tropical seagrass *Halodule wrightii* Aschers under the same conditions for 2 years to investigate the effects of epiphyte grazing on seagrass growth and biomass. Hughes *et al*. (2009) has conducted a common garden experiment to quantify variation within and between *Zostera* seagrass genotypes in morphology, shoot and biomass production, photosynthetic rate and nutrient uptake rate.

This research was to develop a common garden seagrass system to grow seagrass plant of Halodule under conditions most favorable to the plant. Hence, this present study represent a simple culture system 'common garden' which aims to monitor the variation of shoot density, leaf morphology and growth rates of marine plant genus Halodule.

MATERIAL AND METHOD

Common garden

Seagrasses of *H. uninervis* and *H. pinifolia* were harvested in Bantayan, Banilad and Siquijor Beach. Seagrass common garden were then set up in the Institute of Environmental and Marine Science (IEMS) Dumaguete City Philippines (N 09°19'88" and E 123°18'569") at the running seawater outdoor experiment tank using trays. The tank was 0.6x2x3 m in dimensions and was in cement vaults with an inflow pipe at one side and another outflow pipe installed at the other side. The incoming seawater was pumped from Bantayan Beach. The aerator was supplied by the main pipe which can be adjusted manually in order to maintained current flow, salinity ranges of 25-31‰ during the experiment (Figure 1).

The samples were collected by shovels in plugs (consist of seagrass plants with roots and rhizomes intact in the accompanying sediment) and then were placed in a labeled net bag. They were directly placed into 60 trays (21x21x6 cm) (figure 2 A, B and C) immediately to avoid desiccation, and planted into the designated tank. All the plugs chosen of the high shoot density since the preliminary experiment, all the low shoot density were not able to survive beyond two weeks cultivation. Each tray was randomly placed (random block design) and permanently fixed in the common garden for 3 month-period of cultivation in order to adapt the plants to culture condition. Prior to the common garden period, shoot density was measured and then 5 shots from each tray samples were taken for measuring the initial leaf width. After a month period of common garden cultivation, five young and healthy shoot of plants were chosen to be marked with a cotton string using a small needle for measuring the growth rate (mm/day) (Figure 3). At the end of the experiment, the number of shoots was determined for each plug at the final sampling as well as the final leaf width. Pictures were taken at all samples for leave width measurement. All the plants in the tray were harvested by the end of 3rd month cultivation and the measurements were recorded in the data log book and transferred immediately to Excel spreadsheet for subsequent analysis. Generated data were summarized using descriptive statistics such as mean and standard deviation.

Light and temperature measurement

Temperature and light intensity were measured in the tank using underwater sensor (a Hobo pendant logger) which record the data every 10 minutes. Hobo pendant logger was placed in the bottom center of tank Figure 2). The data were processed using a software HOBOware Lite 3.1 then was tabulated in excel program.

Data analysis

Statistical analysis using SPSS V.14.0 was used in an attempt to classify trends, or variances in the data. One-Way ANOVA's was used in other to detect significant differences of shot density leaf width and growth rate of the data sets among the sites. The Tukey's Test was used to compare means where ANOVA detected significant difference.

RESULT AND DISCUSSION

Shoot density, Leaf width, and growth rate

The mean shoot densities of *H. pinifolia* were varied and tend to decrease after the three month period of common garden. One-way ANOVA showed that there were a significant difference among the month of common garden (F=6.400; p=0.005). Tukey's Test showed that the mean shoot densities from Bantayan sample were higher in the first month than the second and the final month of common garden. Moreover, *H. pinifolia* from Banilad performed significantly different in each month period of common garden (F=6.75; p=0.006). Tukey's Test showed shoot densities were higher in the initial month of common garden compare to the second and the final month period. Similar result was found in samples origin from Siquijor. ANOVA showed that mean shoot densities were significantly different among the month period of common garden ((F=7.672; p=0.02). Furthermore, Tukey's-Test showed that mean shoot densities were higher at the initial month than the second and final month period of common garden.

One-way ANOVA revealed that leaf width (mm) of *H. pinifolia* origin from Bantayan site showed no significant difference (F=1.136; p=0.336) in all three month period of common garden. In contrast, leaf width of *H. pinifolia* origin from Banilad showed a high significant difference (F=21.143; p=0.000). Tukey'Test revealed that mean leaf width of *H. pinifolia* was high in the initial month, followed by the second month and the last month period of common garden. One-way ANOVA showed that mean leaf widths of *H. pinifolia* from Siquijor were significantly different among the three month period of common garden (F= 4.272; p=0.024). Moreover, Tukey's test mean of the leaf widths were higher in the first than the third month, however the mean leaf widths were similar in the second and the third month period of common garden.

The result of ANOVA performed on mean leaf growth rate (mm/day) comparison of Halodule pinifolia among the three month period of common garden showed highly significant difference (F=13.510; p=0.000). Furthermore, pos-hoc analysis (Tukey's test) showed that the mean leaf growth rate of *H. pinifolia* origin from the Banilad and Siquijor were higher than those from Bantayan. While the mean leaf growth rate of *H. pinifolia* origin from Banilad and Siquijor were not significantly different. The summary of average number of shoot, leaf width and growth rate of *H. pinifolia* can be seen on the Table 1.

The result of ANOVA performed on mean leaf shoot densities comparison of *H. uninervis* origin from Bantayan showed a high significant difference (F=17.803; p=0.000) among the three month period of common garden. This is revealed by Tukey's test that the mean of the shoot densities in the first month were higher than those from the second and the third month period of common garden (Table 5). Similar results of shoot densities were found in the sample which came from Banilad. One-way ANOVA showed that there were significant difference among the three months period of common garden (F=9.846; p=0.001). Tukey's test revealed that the shoot density was higher in the first and the third month than the second month period of common garden. One-way ANOVA showed that there was a significant difference on the shoot densities among the three months period of common garden found in the sample origin from Siquijor (F=9.274; p=0.001). Tukey's test showed that mean shoot densities were lower in the third (end) month than in the first and second month period of common garden.

In this study, the mean of leaf widths of *H. uninervis* sample origin from Bantayan were not significantly different in all three month period of common garden (F=0.046;p=0.956). Likewise, it was found that there were no signifycant difference among the mean of leaf widths of *H. uninervis* from Banilad F=0.2746; p= 0.82). However, the mean of the leaf widths from Siquijor showed significant difference among the three month period of common garden (F=9.274; p=0.001).

One-way ANOVA performed on the mean of leaf growth rate (mm) comparison of *H. uninervis* among the three month period of common garden showed that there is no signifycant difference (F=2.445; p=0.106) (Table 4).

Temperature and light intensity

The temperature in the common garden tank was varied with time and month. It reached a peaked at 31.64 ^{o}C ± 0.80 on November 2010 at 14:00–14:50 hours, 30.19 ^{o}C ± 0.85 on December 2010 at 15:00–15:50 hours and 30.44 ^{o}C

± 0.52 on January 2011 at 13:00–13:50 hours (Figure 6).

The Light intensity in the common garden tank was also different with months. Light intensity peaked at 25,718.68 ± 6,679.01 Lux on November 2010 at 13:00–13:50 hours, peak intensity was recorded at 16,366.73 ± 3,013.18 Lux on December 2010 at 13:00-13:50 hours. On January 2011 at the end of the common garden experiment, light intensity was recorded at 22,564.92 ± 3,214.19 Lux at 13:00–13:50 hours (Figure 7).

The result of 'common garden' of *H. uninervis* is different of that of *H. pinifolia. H. uninervis* shoot density showed no significant difference within the three months of 'common garden' with the samples that were taken from Banilad and Bantayan. On the other hand, the sample taken from Siquijor, showed that on the tertiary month there was a decline of shoot density. The leaf width showed significant differences among the samples within the 3 months treatment. The differences are slightly varied in the sense that samples taken from different sites showed different responses. All of the samples from Bantayan, Banilad, and Siquijor showed high shoot density during the primary month. On the second and third months, the leaf width of samples from Bantayan showed same result of species *H. pinifolia*. In contrast, results of samples from Siquijor showed only an increase during the first two months and declined on the third month. It is different with the sample taken from Banilad. The pattern showed that on the initial month, the shoot density is higher than that of the second month. From a low shoot density on the second month it increased on the third month. The growth rate is fluctuating with the samples from Banilad.

The 'common garden' procedure showed that although parameters, temperature and light intensity, were maintained within the growth range of *H. pinifolia*; there was a decline generally on its shoot density and leaf width. Its initial shoot density and leaf width on the primary month is greater as compared to its secondary and tertiary month. This observation could be attributed to the acclimatization of the samples in the procedure, hence, primary month showed a high shoot density and leaf width relative to the second and tertiary which showed a decline. It could also be that the shoot density and leaf width is too dense already on the initial month that it becomes too crowded within its tray that its shoot density and leaf width declined on the secondary and tertiary months. On the contrary, the growth rate of *H. pinifolia* in common garden is high. This is observed consistently with the different samples all throughout the three months 'common garden'.

CONCLUSION

The result suggests that the 'common garden' procedure could have possibly given a clearer pattern of the two species leaf width, shoot density, and growth rate if the treatment will be extended into six months the least. The extension may give a much more concrete pattern than the three-month span of the treatment. It is also highly suggested that an adequate number of shoot density on the initial procedure for 'common garden' should be properly determined. This is to avoid overcrowding of the samples later, when growth rate will tend to increase without compromising the leaf width and shoot density measurements.

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Table 1. Summary of average number of shoot, leaf width and growth rate of *H. pinifolia* after 3 month cultivation in a common garden. Data are presented as means of 10 replicates ± standard deviation.

Site origin	Shoot Density			Leaf Width (mm)		
	S_1	S_2	S_f	LW_1	L_2	LW_f
Bantayan	39	25	17	1.08	1.08	1.04
	±13	±7	±11	±0.09	±0.09	±0.04
Banilad	36	23	28	1.29	1.14	1.01
	±8	±9	±9	±0.11	±0.08	±0.07
Siquijor	40	27	28	1.12	1.06	1.02
	±11	±14	±15	±0.11	±0.06	±0.05

S_1= shoot first month, S_2=shoot second month, S_f =shoot third month culture, LW_1= leaf first month, LW_2=leaf second month, LW_f =leaf third month.

Table 2. Summary of average number of leaf growth rate of *H. pinifolia* after 3 month cultivated in a common garden. Data are presented as means of 10 replicates ± standard deviation

Site origin	Mean growth rate(mm)	Range
Bantayan	3.9±0.7	2.8-4.9
Banilad	5.2±0.9	4.1-6.4
Siquijor	5.9±1.0	4.7-7.3

Table 3. Summary of average number of shoot and leaf width of *H. uninervis* after 3 month cultivated in a common garden. Data are presented as means of 10 replicates ± standard deviation.

Site origin	Shoot Density			Leaf Width (mm)		
	S_i	S_{2nd}	S_f	LW_i	L_{2nd}	LW_f
	36	24	23	3.3	3.2	3.2
Bantayan	±6	±6	±3	±0.2	±0.2	±0.2
	33	22	28	3.3	3.3	3.1
Banilad	±8	±5	±3	±0.2	±0.2	±0.1
	38	29	27	3.4	3.4	3.1
Siquijor	±8	±7	±4	±0.2	±0.2	±0.2

S_1= shoot first month, S_2=shoot second month, S_f=shoot third month culture, LW_1= leaf first month, LW_2=leaf second month, LW_f =leaf third month.

Table 4. Summary of average number of leaf growth rate of *H. uninervis* after 3 month cultivated in a common garden. Data are presented as means of 10 replicates±standard deviation.

Site origin	Mean growth rate(mm)	Range
Bantayan	3.4±0.7	2.9-5.0
Banilad	4.0±0.7	2.4-4.9
Siquijor	3.5±0.6	2.3-4.4

Table 5. One-way ANOVA and compare means with Turkey's test.

Descriptives
Bantayan

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
1	10	37.5000	8.87255	2.80575	31.1530	43.8470	27.00	55.00
2	9	26.5556	7.53510	2.51170	20.7636	32.3475	16.00	35.00
3	11	26.0909	7.75183	2.33727	20.8832	31.2987	16.00	37.00
Total	30	30.0333	9.47040	1.72905	26.4970	33.5696	16.00	55.00

ANOVA
Bantayan

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	837.335	2	418.668	6.410	0.005
Within Groups	1763.631	27	65.320		
Total	2600.967	29			

Bantayan (Tukey HSD)

all sites	N	Subset for alpha = 0.05	
		1	2
3	11	26.0909	
2	9	26.5556	
1	10		37.5000
Sig.		0.9910	1.0000

Figure 1. Photograph the outdoor cultured tank in Silliman University Institute Environmental and Marine Science. Arrows showing the source of aerator pipe and the inlet-outlet pipes.

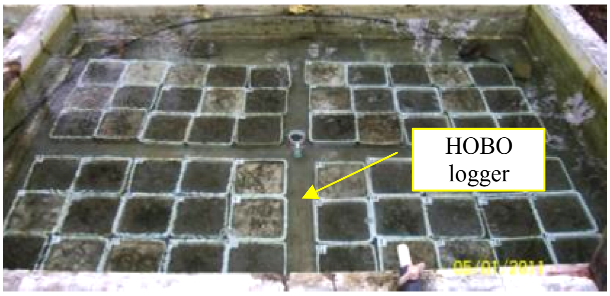

Figure 2. Photograph of common garden seagrass Halodule growing in an outdoor cultured tank (white arrow pointing the installation of Hobo pendant logger).

Figure 3. A tray of *Halodule pinifolia*

Figure 4. A tray of *Halodule uninervis*

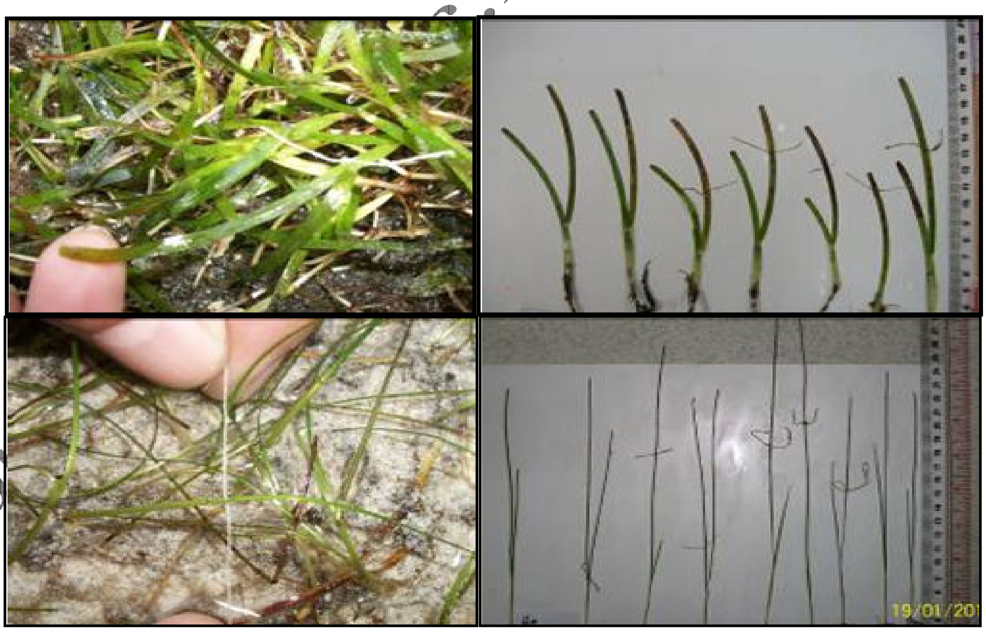

Figure 5 Photograph showed the initial marking and the final leaf growth result of *H. uninervis* and *H. pinifolia* using a white cotton string.

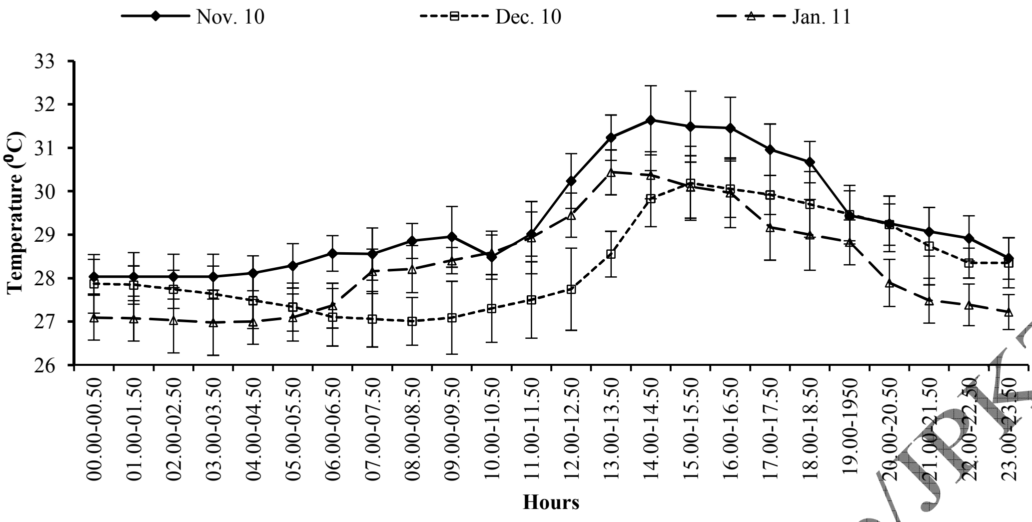

Figure 6. Daily temperature (°C) in common garden tank on November, December 2010, and January 2011. Data are presented as means ± standard deviation.

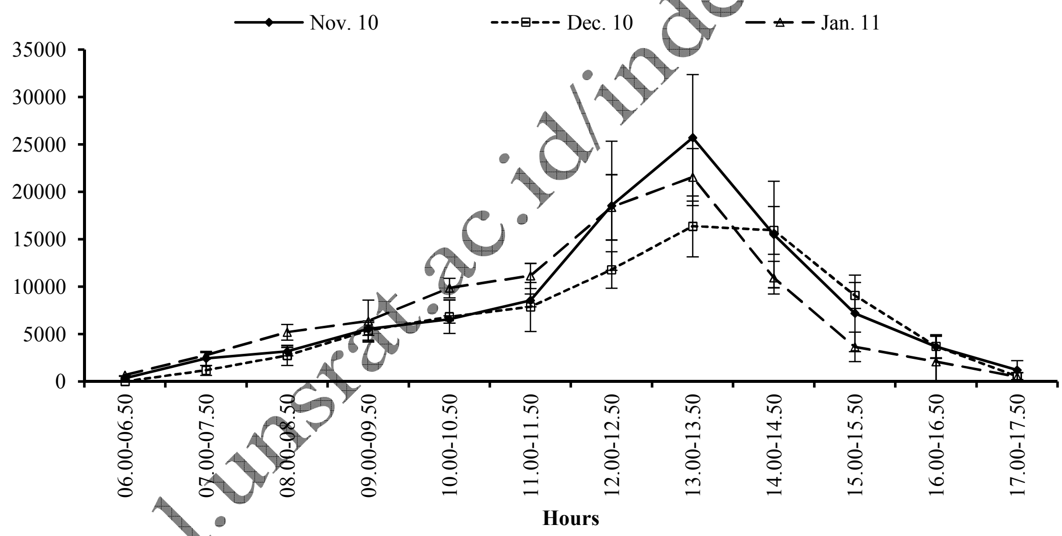

Figure 7. Daily light intensity (Lux) reading at common garden tank on November, December 2010, and January 2011. Data are presented as means of 6 replicates ± standard deviation.