Vol. 7 January 2016
CHED Accredited Research Journal, Category A-1
Print ISSN 2094-5019 • Online ISSN 2244-0461
doi: http://dx.doi.org/10.7828/ajob.v7i1.900

Asian Journal of Biodiversity
This Journal is in the Science Master Journal List of
Thomson Reuters (ISI) Zoological Record

Spatial Morphological Variability of the Seagrass *Halophila ovalis* (R.Br.) Hooker f in Minahasa Peninsula waters

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ABSTRACT

The study was conducted to explore the spatial morphological variability of *Halophila ovalis* (R.Br.) Hooker f in seven sites of Minahasa Peninsula waters. The sites were Tanjung Merah Beach, Likupang Beach, Mantehage Beach, Likupang

Beach, Tongkeina Beach, Manado Beach and Arakan Beach. The Image-J software was applied to measure the leaves (length, wide, and cross veins), rhizomes, and nodes of the samples. Simple descriptive statistics were used to characterize the populations. Hobo pendant tempt/light loggers (UA-002-08) were used to measure light intensity and temperature. This research indicated that most of the leaves were larger in the subtidal area than those in the intertidal area.

Keywords: Morphometric, segrass, variability, Image-J, Hobo Loggers, *Halophila ovalis*

INTRODUCTION

Seagrass (seagrass) are the only flowering plants found in the marine environment as well as the grass on the ground. They have leafy shoots, upright and creeping stalks effective to breed and have roots and an internal system for transporting gas and nutrient substances (Calumpong and Meňez 1997; Romimohtarto and Juwana 2001).

Seagrass is also a plant that has adapted to live buried in shallow waters. They have roots and rhizomes (rhizome) gripping the seabed so as to help the coastal defences of scouring waves and waves. Beds of seagrass vegetation can consist of a single type or a mixed type (Hemminga and Duarte 2000).

Seagrasses are angiosperms that are related to terrestrial flowering plants. They are living partially or entirely submerged in marine waters. They have erect leaves, buried root-like structure (rhizomes) that hold the plants in the sediments, and roots that take up nutrients from sediments for growth. As a result, seagrasses are not a taxonomically unified group but a 'biological' or 'ecological' group. The evolutionary adaptations required for survival in the marine environment have led to convergence in morphology (den Hartog 1970; den Hartog and Kuo 2006).

Seagrasses are interesting because they form long-lived, structurally-complex benthic communities. There are about 60 known seagrass species in the world, 12 of which have been recorded in Indonesian Waters. Because of this high diversity, there has recently been an expanding interest in evaluating various morphometric structural and dynamic parameters in seagrasses. Moreover, Seagrasses play an important role in the coastal ecosystem as one of the major primary producers, forming the base of the food chain. Seagrasses beds form an extremely complex ecosystem that function through detritus-based as well as herbivore food webs. The importance of seagrasses beds in contributing to fish abundance and diversity continue to be a major focus of marine biologists because of their ecological and economical role in many coastal ecosystems (Green and Short 2003; Kuriandewa *et al.* 2003).

Dealing with climate change (climate change), seagrass ecosystem is one of the most obvious affected. Seagrass beds could disappear, especially near the estuary of the river and in the intertidal waters. The main cause of this is rising temperatures, particularly in some places in the shallow water habitat. Increasing the temperature influence the distribution and reproduction process of seagrass. Besides the temperature, other factors that influence the increasing sedimentation and resuspension of sediment due to high rainfall and the frequency of floods from rivers that affect the intensity of light (Vermaat *et al.* 1997; Poejirahajoe 2013). Numerous researchers have related seagrass health to environmental stressors. Salinity, depth, light, nutrients, sediment characteristics and temperature are some of the variables identified as contributing to patterns in the measured seagrass response variable (Dennison 1987; Dawson and Denisson 1996).

OBJECTIVES OF THE STUDY

This study aimed to assess the morphometric variation of seagrass species *Halophila ovalis* (R.Br.) Hooker f on the dominant seagrass habitat in Minahasa Peninsula coastal waters.

MATERIALS AND METHODS

Sampling and Study Sites

Sampling was carried out from January to December 2014 at selected sites in the seagrass beds area of Arakan, Manado Bay, Tongkeina, Siladen, Mantehage, Likupang, and Tanjung Merah (Figure 1).

Sample plots were used for sampling measuring 50 cm x 50 cm which is divided again into 25 subplots with a size of 10 cm x 10 cm. Percentage cover of seagrass at some point is the average of the six replications.

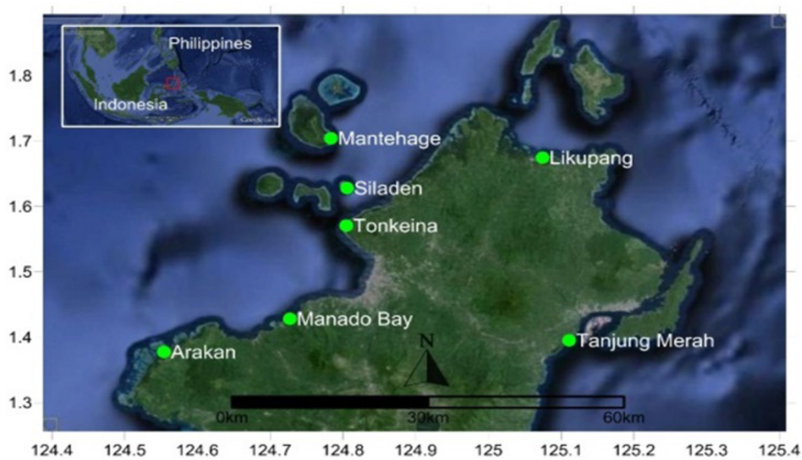


Figure 1. Showed the map of location of the sites
in Minahasa Peninsula Waters

Density Measurements

Percentage cover of each species was determined by counting the number of sub quadrats occupied. This was categorized into: 0 percentage cover (when frequency was 0), < 10 percentage cover, 10 - 50 percentage cover, and > 50 percentage cover (Tomasko *et al.* 1993; Short and Coles 2001).

In this present study, the shoot density has been broadly classified into two, low and high-density bed.

Morphometric Measurements and Analysis

A total of fifty samples (n = 50) of each species from the intertidal (with low *Halophila* density), intertidal (with high *Halophila* density), subtidal (with low *Halophila* density) and subtidal (with high *Halophila* density) were randomly collected from all the quadrats for measurements. Samples of *Halophila* were collected, rinsed free of sediment using seawater, then placed in labelled net bags. They were brought to Marine Laboratory Sam Ratulangi University and kept in a bucket for subsequent processing and analysis. The next day, shoots were carefully cleaned in filtered seawater, and epiphytes attached to the leaves were removed by gently scraping them with the hand. After cleaning, each sample was photographed with a ruler as a scale and the following were measured using Image-J software 1.42u;java 1,6; 32 bit (Rasband 2011). All photos were stored and saved in .jpg files according to its species name, site, tidal area or station and density, and the date of collection.

Measurements of the following morphological variables were taken: the length of rhizomes, length of upright shoot, the length of longest leaf (cm), the number of leaves, the number of nodes and the present of flower and fruit. The cross-veins in the leaves were counted. The blade width/length, petiole diameter/length and rhizome diameter/length ratios were computed. Simple descriptive statistics were used to characterize the populations.

Light and Temperature Measurements

Onset Hobo pendant loggers temp/light (UA-002-08) were used to measure temperature and light intensity. A pendant logger was deployed in each of the intertidal and subtidal zones for about a month at the time the seagrasses were collected. The loggers were programmed to store data of temperature and light intensity every fifteen minutes and were set up with actual date and time when installed. Each logger was covered with plastic tape and tied to an iron bar. The iron bars were stuck into the seafloor within the seagrass bed with the tip of the loggers facing the equator. This allowed each data logger to receive the maximum light intensity. The loggers were inspected every six days for any damage and were cleaned from all marine growths. Every two weeks, the loggers were taken out to upload the data. The data were exported and tabulated into Microsoft Excel 2010 program to get the mean of temperature and light intensity per day per month. Sampling and measurements were conducted during the southwest monsoon (August 2014). Generated data were summarized using descriptive statistics such as mean and standard deviation.

RESULTS AND DISCUSSION

The observation of morphometric variation of seagrass in seven (7) locations Minahasa Peninsula coast shows in detail the fluctuations in size according to the observation location. The images of this species based on the sites can be seen in Figure 2. Whereas the average number of cross veins, blade width (BW), length (BL), and BW/BL ratio of the second leaf, petiole diameter (PD), length (PL), and PD/PL ratio of the second petiole and rhizome diameter (RD), rhizome length (RL), and RD/RL ratio of the second rhizome can be seen in Table 1 below.

Figure 2. Variation in leaf morphology of *H. ovalis* collected from seven sites in Minahasa Peninsula, North Sulawesi, Indonesia.

Table 1. Average number of cross veins, blade width (BW), length (BL), and BW/BL ratio of the second leaf, petiole diameter (PD), length (PL), and PD/PL ratio of the second petiole and rhizome diameter (RD), rhizome length (RL), and RD/RL ratio of the second rhizome. Ranges are in parentheses. N = 50.

Site	No. x-veins	Ave BL (mm)	Ave BW (mm)	Ave 2BW/BL	Ave 2PL (mm)	Ave 2PD (mm)	Ave 2PD/2PL	Ave. RL	Ave RD	Ave 2RD/2RL
Arakan	15 ± 3	18.72 ± 2.67	9.67 ± 1.98	0.52 ± 0.06	17.34 ± 6.23	0.86 ± 0.12	0.06 ± 0.02	23.22 ± 7.45	0.06 ± 0.02	0.06 ± 0.02
(Range)	(10-26)	(14.04-27.07)	(5.21-14.5)	(0.32-0.65)	(6.69-33.21)	(0.63-1.29)	(0.02-0.1)	(12.01-43.32)	(0.91-2.04)	(0.04-0.11)
Manado Bay	13 ± 3	15.38 ± 2.15	7.75± 1.38	0.50 ± 0.05	15.40 ± 6.04	0.91 ± 0.17	0.07± 0.03	15.36 ± 5.58	1.20 ± 0.24	0.09 ± 0.04
(Range)	(9-18)	(9-19.4)	(4.29-10.68)	(0.39-0.65)	(6.41-28.89)	(0.052-1.42)	(0.02-0.17)	(5.22-32.48)	(0.79-1.56)	(0.03-0.20)
Mantehage	16± 3	16.05 ± 3.78	9.70 ± 2.21	0.61± 0.07	17.41± 7.56	0.9 ± 0.2	0.06± 0.03	20.91± 7.19	1.46 ± 0.33	0.07 ± 0.02
(Range)	(12-22)	(9.49-25.16)	(5.53-15.66)	(0.45-0.75)	(4.01-25.9)	(0.45-1.47)	(0.02-0.17)	(10.16-45.11)	(0.86-2.42)	(0.04-0.13)
Siladen	12 ± 2	12.0 ± 1.89	6.94 ± 1.17	0.58 ± 0.06	10.73 ± 5.76	0.68 ± 0.15	0.08± 0.04	20.30 ± 8.09	1.00 ± 0.024	0.05 ± 0.02
(Range)	(10-15)	(7.91-17.58)	(3.71-9.15)	(0.41-0.74)	(3.17-28.51)	(0.09-1.14)	(0.02-0.18)	(9.10-41.54)	(0.63 -1.44)	(0.03-0.11)
Tanjungmerah	16 ± 2	17.60 ± 2.72	10.47± 2.00	0.59 ± 0.06	17.67 ± 7.50	0.76 ± 0.15	0.06 ± 0.04	19.64 ± 6.32	0.92 ± 0.16	0.05 ± 0.02
(Range)	(12-24)	(11.07-22.58)	(5.61-13.51)	(0.50-0.71)	(4.12-34.02)	(0.46-1.43)	(0.01-0.19)	(6.69-34.01)	(0.60-1.42)	(0.02-0.15)
Likupang	14 ± 2	14.14± 2.68	7.39 ± 1.55	0.53 ± 0.10	15.28± 6.05	0.71± 0.33	0.05± 0.02	20.04± 8.94	1.07± 0.10.26	0.06± 0.03
(Range)	(10-20)	(8.15-22.31)	(3.74-16.58)	(0.39-1.47)	(4.35-35.69)	(0.35-1.10)	(0.02-0.19)	(5.06-34.13)	(0.62-1.44)	(0.03-0.19)

Morphology

Arakan – The plants collected from this site were relatively the largest among those collected from all sites and were found growing in 6 m to 12 m deep in rocky's substrate. The leaves are more round than ovate (Figure 2) with average blade to length ratio of 0.52 ± 0.06 (Table 1). The leaf pairs are also unevenly sized and shaped. Average leaf width for one of the second pair of leaves = 9.81 ± 2.02 mm; leaf length = 18.39 ± 2.58 mm or a ratio of 0.53 ± 0.07 while for the other leaf width was 9.60 ± 2.00 mm while leaf length was 19.15 ± 2.87 mm a ratio of 0.50 ± 0.06. Average petiole diameter= 0.87 ± 0.13 mm; petiole length = 16.46 ± 6.34 mm for the first the second pair of petioles or a ratio of 0.08 ± 0.04 while for the 2nd of the second pair of petioles, average petiole diameter = 0.84 ± 0.16 mm; petiole length of the second pair of petioles = 18.22 ± 6.41 mm; or a ratio of 0.07 ± 0.04; rhizome diameter = 1.34 ± 0.23 rhizome length of the second rhizome = 23.22 ± 7.45 or a ratio of 0.06 ± 0.02. Average number of cross veins is 15 ± 3 with a range of 10-26.

Likupang – The plants are small and were found growing in 0.5 m to 4 m deep in muddy mix with coral rubbles substrate. The leaves are more round than ovate (Figure 2) with average blade to length ratio of 0.53 ± 0.05 (Table 1). The leaf pairs are also unevenly sized and shaped. Average leaf width for one of the second pair of leaves was 7.49 ± 2.00 mm; leaf length was 13.82 ± 3.15 mm or a ratio of 0.56 ± 0.19 while for the other leaf width was 7.33 ± 1.51 mm while leaf length was 14.53 ± 2.65 mm or a ratio of 0.50 ± 0.05. Average number of cross veins was 15 ± 2 with a range of 10-20. Average petiole diameter was 0.78 ± 0.65 mm; petiole length was 14.95 ± 6.81 mm for the first the second pair of petioles or a ratio of 0.06 ± 0.03 while for the 2nd of the second pair of petioles, average petiole diameter was 0.66 ± 0.16 mm; petiole length of the second pair of petioles was 15.71 ± 5.84 mm; or a ratio of 0.05 ± 0.02; rhizome diameter was 1.07 ± 0.26 rhizome length of the second rhizome was 20.04 ± 8.94 or a ratio of 0.06 ± 0.03. Average number of cross veins was 15 ± 3 with a range of 12-18.

Manado Bay – The plants are small and were found growing in 6 m to 11 m deep in sandy substrate. The leaves are more round than ovate (Figure 2) with average blade to length ratio of 0.50 ± 0.05 (Table 2). The leaf pairs are also unevenly sized and shaped. Average leaf width for one of the second pair of leaves was 7.76 ± 1.42 mm; leaf length was 15.16 ± 2.18 mm or a ratio of 0.51 ± 0.06 while for the other leaf width was 7.92 ± 1.22 mm while leaf length was 15.93

± 2.13 mm or a ratio of 0.50 ± 0.06. Average petiole diameter was 0.93 ± 0.19 mm; petiole length was 14.86 ± 5.94 mm for the first the second pair of petioles or a ratio of 0.08 ± 0.04 while for the 2nd of the second pair of petioles, average petiole diameter was 0.91 ± 0.19 mm; petiole length of the second pair of petioles was 16.53 ± 6.51 mm; or a ratio of 0.07 ± 0.04; rhizome diameter was 1.20 ± 0.24 rhizome length of the second rhizome was 15.36 ± 5.58 or a ratio of 0.09 ± 0.04. Average number of cross veins is 13 ± 3 with a range of 9-18.

Mantehage – The plants are relatively big and were found growing in in 0.5 m to 4 m deep in sandy substrate. The leaves are more round than ovate (Figure 2) with average blade to length ratio of 0.50 ± 0.05 (Table 1). The leaf pairs are also unevenly sized and shaped. Average leaf width for one of the second pair of leaves was 9.77 ± 2.20 mm; leaf length was 15.83 ± 3.76 mm or a ratio of 0.62 ± 0.06 while for the other leaf width was 9.60 ± 2.30 mm while leaf length was 16.18 ± 3.90 mm or a ratio of 0.60 ± 0.08. Average number of cross veins is 16 ± 3 with a range of 12-22 and 17 ± 2 with a range of 14-20, respectively. Average petiole diameter was 0.90 ± 0.23 mm; petiole length was 16.46 ± 7.91 mm for the first the second pair of petioles or a ratio of 0.06 ± 0.03 while for the 2nd of the second pair of petioles, average petiole diameter was 0.90 ± 0.22 mm; petiole length of the second pair of petioles was 18.04 ± 7.29 mm; or a ratio of 0.06 ± 0.03; rhizome diameter was 1.46 ± 0.33 rhizome length of the second rhizome was 20.91 ± 7.19 or a ratio of 0.07 ± 0.02.

Siladen – The plants collected from this site were relatively the smallest among those collected from all sites and were found growing in in 0.6 m to 4.5 m deep in mud and sandy substrate. The leaves are more ovate than round (Figure 2) with an average blade to length ratio of 0.58 ± 0.06 (Table 1). The leaf pairs are also unevenly sized and shaped. Average leaf width for one of the second pair of leaves was 6.93 ± 1.15 mm; leaf length was 11.89 ± 1.98 mm or a ratio of 0.59 ± 0.07 while for the other leaf. Leaf width was 6.94 ± 1.24 mm while leaf length was 12.13 ± 1.97 mm a ratio of 0.58 ± 0.07. Average petiole diameter was 0.68 ± 0.20 mm; petiole length was 10.66 ± 5.75 mm for the first the second pair of petioles or a ratio of 0.08 ± 0.04 while for the 2nd of the second pair of petioles, average petiole diameter was 0.67 ± 0.16 mm; petiole length of the second pair of petioles was 10.80 ± 5.96 mm; or a ratio of 0.08 ± 0.04; rhizome diameter was 1.00 ± 0.24 rhizome length of the second rhizome was 20.30 ± 8.09 or a ratio of 0.05 0.02. Average number of cross veins is 12 ± 2 with a range of 9-15.

Tanjung merah – The plants are small and were found growing in xx m to xx m deep in xx substrate. The leaves are more ovate than round (Figure 2) with an average blade to length ratio of 0.59 ± 0.06 (Table 1). The leaf pairs are also unevenly sized and shaped. Average leaf width for one of the second pair of leaves was 10.49 ± 2.04 mm; leaf length was 17.26 ± 2.70 mm or a ratio of 0.6 ± 0.06. Average number of cross veins was 17 ± 3. For the other leaf, leaf width was 10.44 ± 2.04 mm while leaf length was 17.90 ± 2.90 mm a ratio of 0.58 ± 0.07. Average number of cross veins was 17 ± 2. Average petiole diameter was 0.75 ± 0.20 mm; petiole length was 16.94 ± 7.38 mm for the first the second pair of petioles or a ratio of 0.06 ± 0.04 while for the 2nd of the second pair of petioles, average petiole diameter was 0.77 ± 0.15 mm; petiole length of the second pair of petioles was 18.47 ± 7.89 mm; or a ratio of 0.05 ± 0.04; rhizome diameter was 0.92 ± 0.16 rhizome length of the second rhizome was 19.64 ± 6.32 or a ratio of 0.05 0.02. Average number of cross veins is 16 ± 2 with a range of 12-24.

Genetic Analysis for Species Confirmation

PCR Condition

Kit: 2X KAPA Taq ReadyMix with dye (Kapa Biosystem)

Primer

- 1F (5'-ATG TCA CCA CAA ACA GAA AC-3)
- 724R (5'-TCG CAT GTA CCT GCA GTA GC-3')

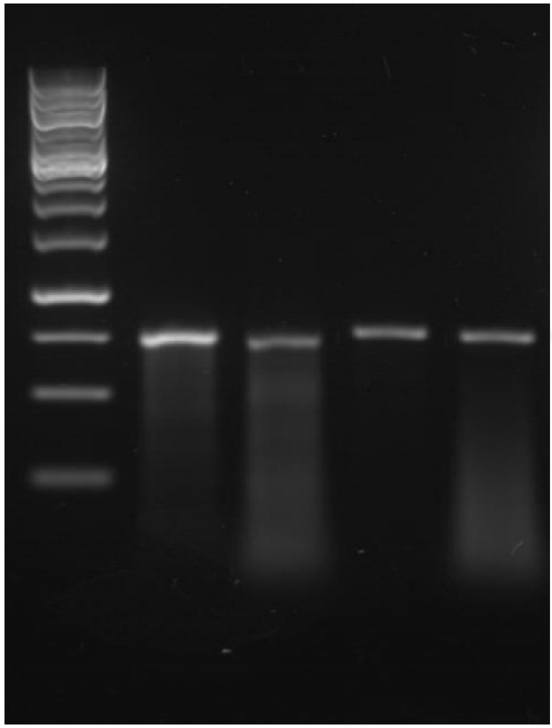
Setting PCR Machine

95°C 3 minutes

{ 95°C 30 second
50°C 30 second
72°C 40 second } 35 cycles

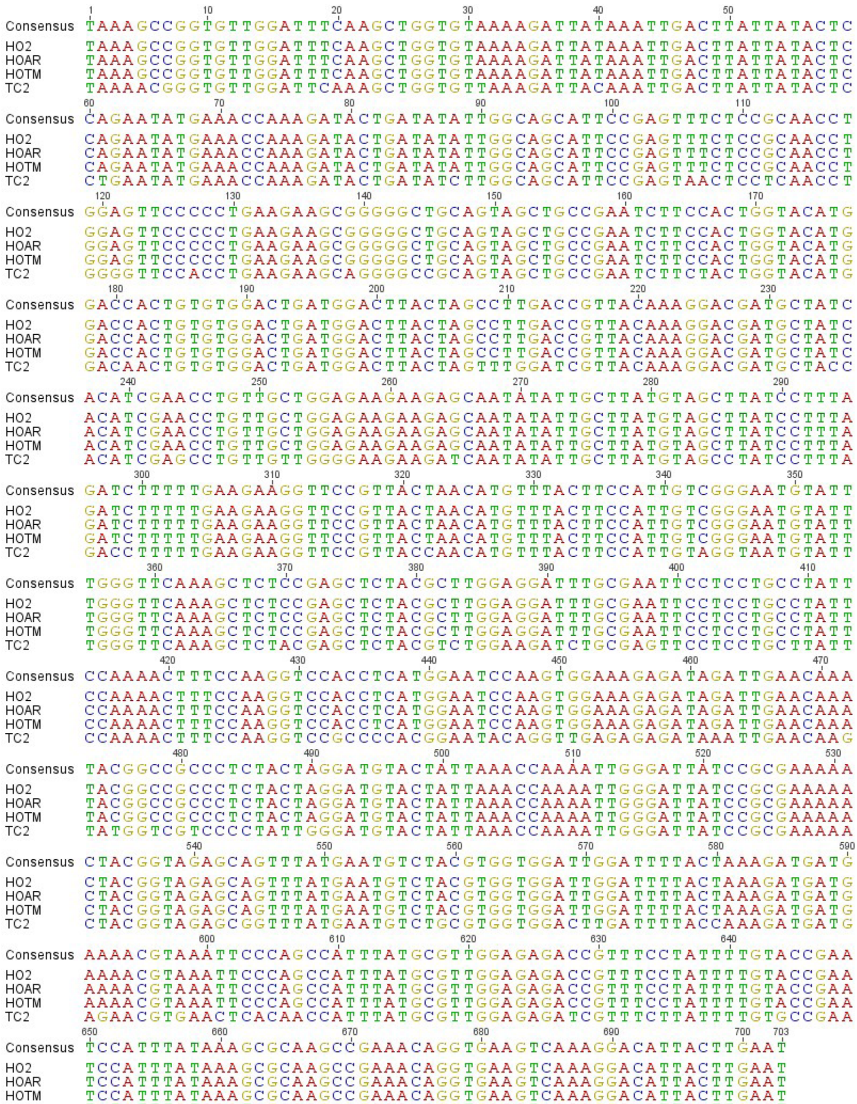
72°C 1 minute

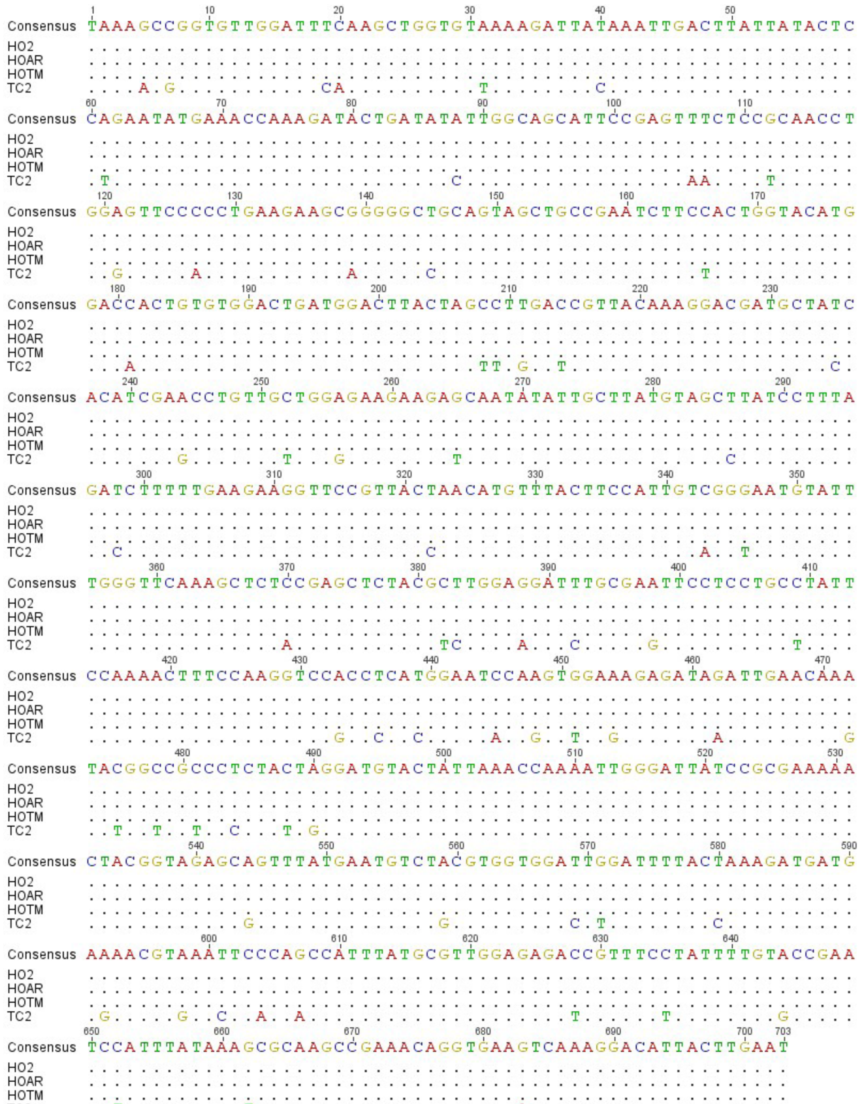
Gel Elektroforesis 1%



Ladder 1 kb (inbase pair): 250, 500, 750, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 5000, 6000, 8000, 10000

Alignment





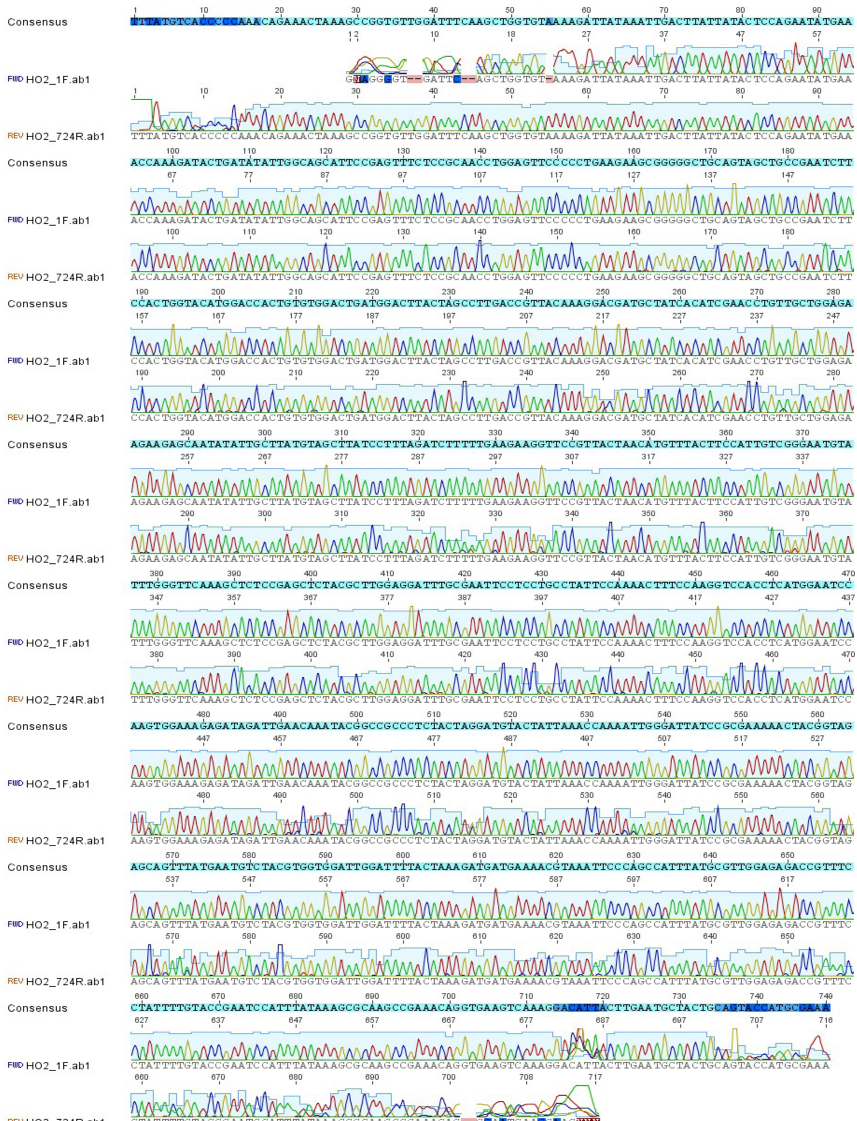
Distances

	HO2	HOAR	HOTM	TC2
HO2		100%	100%	90.2%
HOAR	100%		100%	90.2%
HOTM	100%	100%		90.2%
TC2	90.2%	90.2%	90.2%	

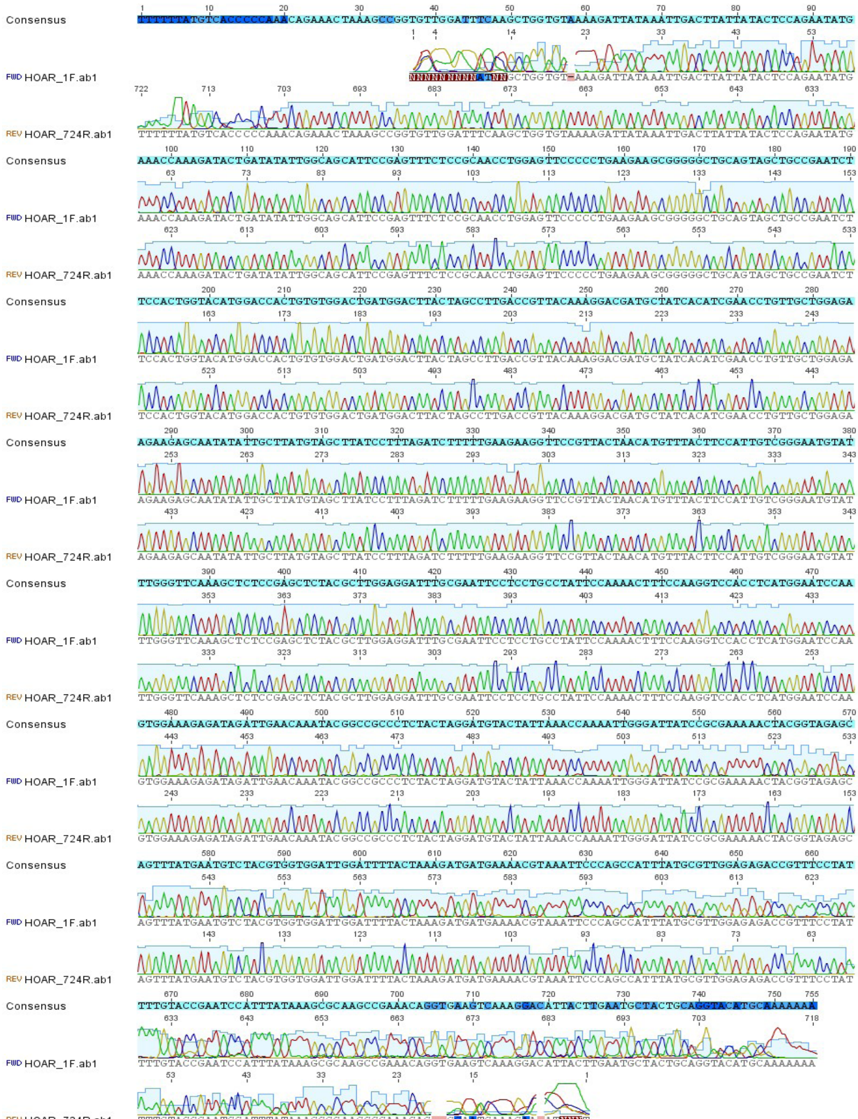
Sekuens

TAAAGCCGGTGTTGGATTTCAAGCTGGTGTAAAAGATTATAAATTGACTTATTATACT
CCAGAATATGAAACCAAAGATACTGATATATTGGCAGCATTCCGAGTTTCTCCGCAA
CCTGGAGTTCCCCCTGAAGAAGCGGGGGCTGCAGTAGCTGCCGAATCTTCCACTG
GTACATGGACCACTGTGTGGACTGATGGACTTACTAGCCTTGACCGTTACAAAGGA
CGATGCTATCACATCGAACCTGTTGCTGGAGAAGAAGAGCAATATATTGCTTATGTA
GCTTATCCTTTAGATCTTTTTGAAGAAGGTTCCGTTACTAACATGTTTACTTCCATTG
TCGGGAATGTATTTGGGTTCAAAGCTCTCCGAGCTCTACGCTTGGAGGATTTGCGA
ATTCCTCCTGCCTATTCCAAAACTTTCCAAGGTCCACCTCATGGAATCCAAGTGGAA
AGAGATAGATTGAACAAATACGGCCGCCCTCTACTAGGATGTACTATTAAACCAAAA
TTGGGATTATCCGCGAAAAACTACGGTAGAGCAGTTTATGAATGTCTACGTGGTGG
ATTGGATTTTACTAAAGATGATGAAAACGTAAATTCCCAGCCATTTATGCGTTGGAG
AGACCGTTTCCTATTTTGTACCGAATCCATTTATAAAGCGCAAGCCGAAACAGGTGA
AGTCAAAGGACATTACTTGAAT

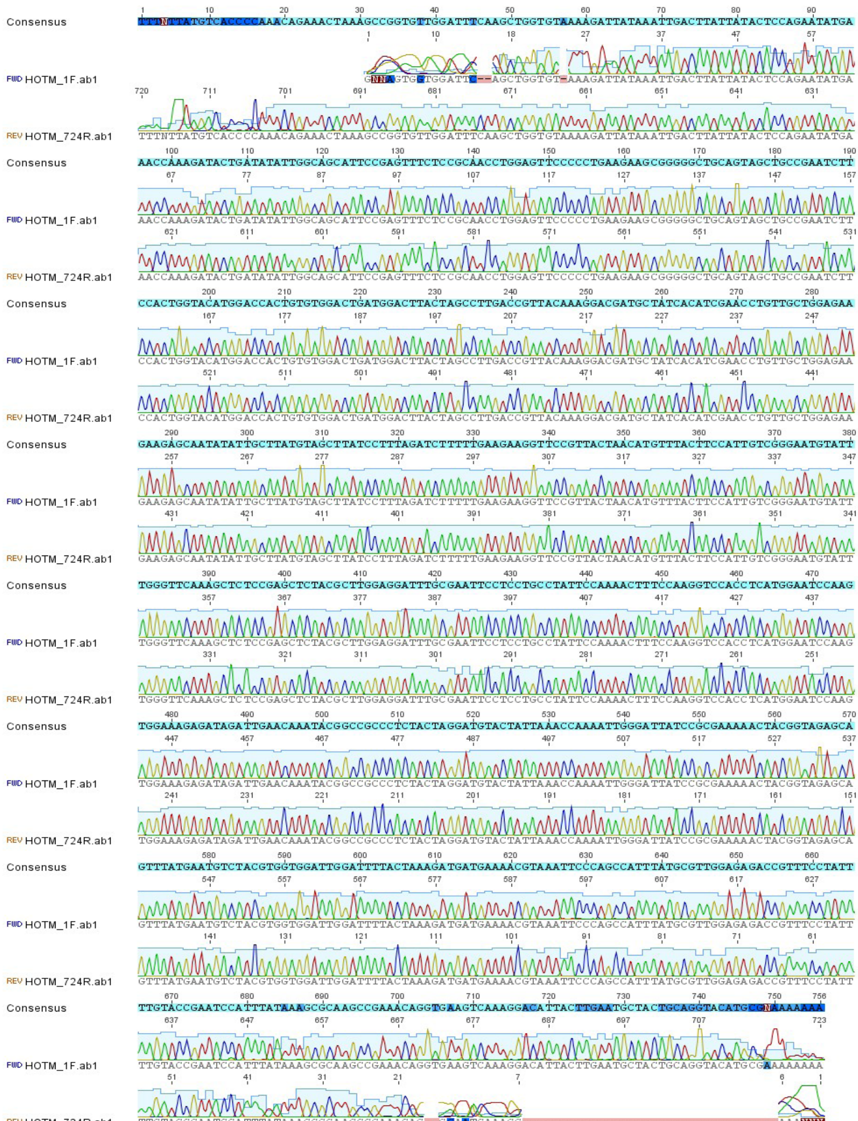
HO (Bali)



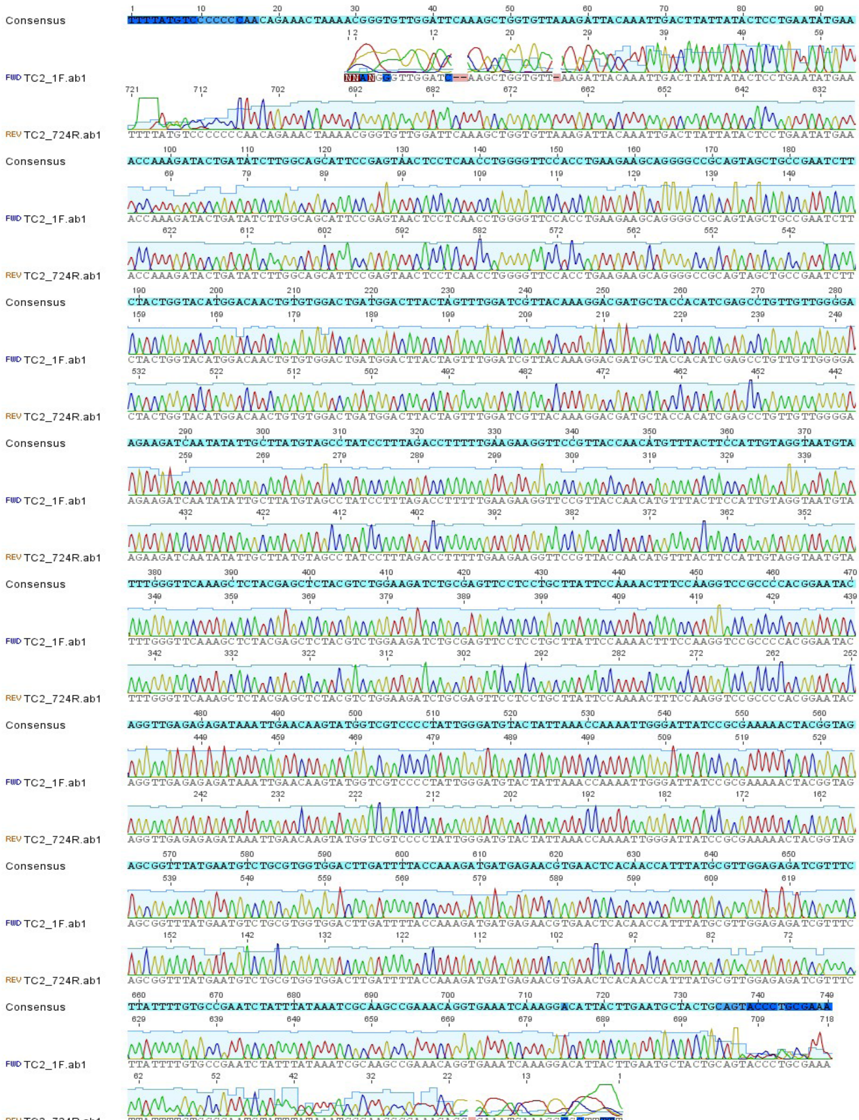
HOAR (Arakan)



HOTM (Tanjung Merah)



TC2 (Mantehage)



Environmental Factors

1. Temperature Reading

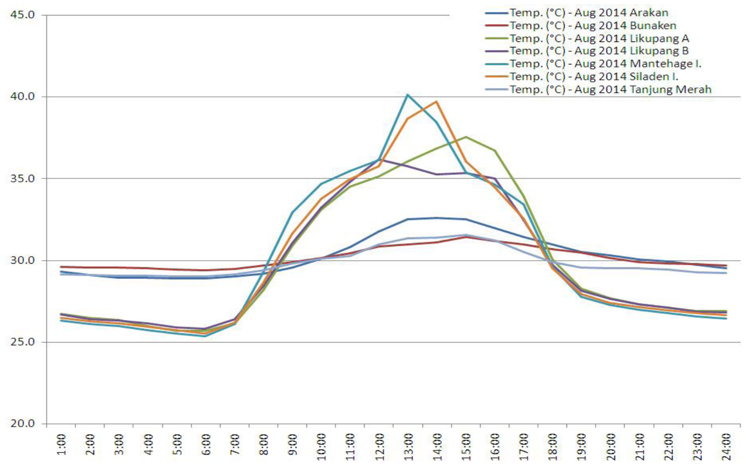


Figure 3. Mean Temperature reading on August 2014 in intertidal and subtidal areas (N=6).

2. Light intensity Reading

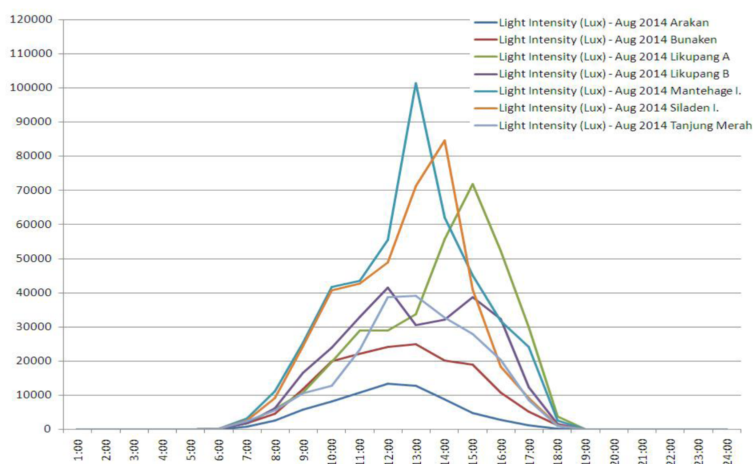


Figure 4. Mean light intensity reading on August 2014 in intertidal and subtidal areas (N=6).

The results showed that sites at Manado beach and Arakan beach have a percentage cover higher than the most other sites; 62% and 65% respectively. This is expected for such beaches that has the most extensive seagrass closure in this coastal region. Conditions quiet beach without much disruption of ecosystems, causing seagrass cover in this area appears to be in still quite good condition. Based the Decree of State Ministry of Environment No. 200/2004 on the status of seagrass beds by closing percentage above 60% indicated that the seagrass bed is considered as healthy ecosystem (Anonym 2004).

The low numbers of percentage cover found in the sites of Bunaken, Mantehage, and Siladen are 35%, 40%, and 37% respectively. Furthermore, the percentage cover of seagrass in Tanjung Merah only reaches below 25%, which indicates that this area is the poorest area among all sites. Allegedly for that the sites get crowded with tourists and high activities of fishing vessels in the region, making it difficult to avoid turbidity. As it is known that the turbidity inhibits photosynthesis. The reduced photosynthesis means reducing primary productivity of the growth of seagrass.

Overall, this research found only leaves that showed the variation of morphological difference compared with others. Moreover, despite the density it was found that most of the leaves were larger in the subtidal sites than those in the intertidal sites.

Environmental parameters like temperature reach the average of 29°C and showed a strong fluctuation ranging from 22°C to 40°C (see Figure 3). This research found that although the temperatures reach above 40°C, it can be assumed that the average temperature is still favourable for the seagrass growth for this range still within the range of dynamic seasonal fluctuation in the area of tropical waters (19.8-41°C) (McKenzie 1994; McKenzie and Campbell 2004; and Wagey 2015).

On the other hand, light intensity reaches maximum 105,734.1 Lux with the average of 3,444.9 Lux (Figure 4). Although the value of the maximum light intensity is exceeded more than expected, the average value of the light intensity obtained almost the similar with previous researches conducted on the other seagrass species (Zavodnik 1998; Rountos 2008; Wagey 2015). All the sequens of *Halophila* are identical (Clean without primer). Although this finding confirms that all the sequens are identical. It was shows that the rbcL primer which was using to observe in plant for chloroplast DNA cannot distinguish specifically the difference amongst the species. This research is recommended to be proceeded with a new marker such as Microsatellites to see the different among species found in different areas. Nonetheless this finding also projects that seagrass has a strong ability to survive in a wide range of environmental condition.

CONCLUSIONS

Among all the morphology of *Halophila ovalis* observed, only the leaves showed morphometric variations. Regardless the density, seagrass *Halophila ovalis* from intertidal had the lowest size range in leaf morphometric measurements, whereas those from subtidal had the highest. All the sequens are identical.

ACKNOWLEDGMENTS

The study was funded by Ministry of Research, Technology, and Higher Education of the Republic Indonesia; SIMLITABMAS in the skim research of International Collaboration and Publication. Appreciation given to Dr. Hilconida Calumpong as my co-researcher overseas, and to Dr. Janet Estacion as Director of Graduate Study, for the support and assistance. Thanks to IEMS graduate colleagues Ian Canlas, Julius, Diane, and my former undergraduate students; Webi Sake, Anas Sauyai and Enos Balaira for sampling and data collection. Finally, appreciation goes to LPPM ("Lembaga Penelitian dan Pengabdian") Sam Ratulangi University for administrative assistance research.

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