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Developing a methodology of bioindication of human-induced effects using seagrass morphological variation in Spermonde Archipelago, South Sulawesi, Indonesia

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ABSTRACT

Seagrass is particularly susceptible to environmental degradation. The objective of the study is to develop an effective bioindicator to assess human-induced effects using morphological variation and fluctuating asymmetry (FA) of seagrass. Samples were collected from eight islands situated at different distance from mainland with different human population density and therefore expected to experience different level of anthropogenic pressure. Cd, Pb, Cu, Zn, nitrate, and phosphate were measured. Metals were also measured in tissues of seagrass. Metal concentrations in sediment, water, and seagrass did not exceed the quality standards required for marine life. Heterogeneity of FA was found among sites suggesting that there are some factors changing developmental instability of seagrass which is not associated to particular toxicants. This baseline study indicates that the water condition is still natural and shows no signs of metal contamination, therefore it does not cause a detectable stress on morphological variation and FA of seagrass.

1. Introduction

Seagrass beds are common in shallow waters of the tropics, subtropics, and temperate regions (Green and Short, 2003). This habitat is used for shelter, foraging, spawning, and rearing place for marine organisms (Hemminga and Duarte, 2000). Seagrass beds are also positioned very close to the mainland, thereby, is influenced by anthropogenic effect.

Indonesia is a home to exceptional seagrass diversity and there is evidence that seagrass there is experiencing some decline due to a quick economical development with unavoidable increase of pollution (Kuriandewa et al., 2003), although relatively little is known about the ecology of its seagrass compared to other regions of the world (Waycott et al., 2009; Short et al., 2011). We characterized the seagrass in the Spermonde Archipelago, which is located in Makassar Strait, South Sulawesi, Indonesia contains more than a hundred small coral islands. Most of the islands in the archipelago are populated. Seagrass occupies most of the reef flat area in each island. Previous research in the Spermonde Archipelago concerned the distribution of seagrass was done by Verheij and Erftemeijer (1993). They found that seagrass occurred in five different habitat in this area, namely intertidal terrigenous mudflats, shallow terrigenous sandy bays, coastal reef flats, reef flat of patch reefs, and sandy reef bases. They also observed lower seagrass species diversity on islands close to the mainland due to heavy sedimentation and high nutrient load. Some previous research in this area also found differences in biotic and abiotic parameters based on distance of the island from the shore (Moll, 1983; Verheij, 1993). Moreover, de Voogd et al. (2006) found that species richness of sponges in Spermonde Archipelago declined with increased human settlement.

Population and industrial growth in coastal areas has placed severe pressure on marine organisms, including seagrassess. Organic matter and excess nutrients, sedimentation and heavy metal contamination, can result in loss of habitat quality or even loss of seagrass ecosystems in several locations. Ambo-Rappe (2010) found that heavy metal contamination in seagrass ecosystems did not reduce the productivity of seagrass itself because heavy metals accumulated in certain tissues of the plant that do not affect the metabolism (Ward, 1989). However, contaminants will be concentrated in the biota that utilizes seagrass ecosystems as a place to find food and passed into the higher trophic levels, including to humans. High content of heavy metals has been found in fish associated with seagrass beds (Batley, 1987). Therefore, the integration of both chemical and biological environmental assessment techniques will provide a significant contribution to managing pollution in the coastal environment.

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The objective of the research was to develop a methodology of bioindication of human-induced effects in the region using simple and inexpensive seagrass morphology measures integrated with chemical analysis of contaminants, in this case metals. In addition to standard leaf length and width measurements, we tested the application of fluctuating asymmetry as an indicator of seagrass condition. Fluctuating asymmetry (FA) is deviation from a symmetrical structure, which then is used to measure instability during development (Van Valen, 1962). Developmental instability can occur when environmental disruption, such as nutrient and heavy metal pollution in waters (Graham et al., 2010) exists during the development process resulted in inconsistently a genotype produce the same phenotype (Zakharov, 1992). Increases in FA were found in organisms associated with seagrass beds of a significantly contaminated with metals (Ambo-Rappe et al., 2008a); thus FA can be an important indicator of pollution.

2. Methods

2.1. Study site

Sampling of the seagrass was conducted in April–May 2010 in Spermonde Archipelago, South Sulawesi, Indonesia. Spermonde Archipelago is situated in the southern of Makassar Strait off the SW peninsula of Sulawesi. There were eight islands selected in this study based on the vicinity to the mainland of Makassar and the population density (Fig. 1).

Each of the location were described as follows: (1) Gusung Tallang is uninhabited island which is located approximately 1,5 km from west side of Makassar and directly opposite to Sukarno-Hatta Port, the biggest port in South Sulawesi, (2) Lae Lae Island is placed adjacent to Gusung Tallang, with an area of 3 ha and is inhabited by 1300 people, (3) Barrang Caddi Island is located northwest of Makassar with a distance approximately 11 km from Makassar, with an area of 4 ha and has a population of 1586 people, (4) Barrang Lompo Island is approximately 12 km from the mainland of Makassar, with an area of 14 ha and is occupied by 4452 people, (5) Bone Tambung Island is located in the northwest of Makassar with a distance of 17.2 km and the island's area is 2.5 ha, inhabited by 600 people, (6) Kodingareng Lompo Island is located to the west of Makassar with a distance approximately 15 km from the mainland with an area of 17 ha and is inhabited by 4700 people, (7) Bone Batang Island is an uninhabited island in the northwest of Makassar to the distance approximately 15 km from Makassar, (8) Kapopposang Island is approximately 70 km northwest of Makassar, the island's area is 10 ha and is inhabited by 484 people.

We sampled 40 stands of the largest dominant seagrass species (*Enhalus acoroides*) in each of three randomly selected 1m^2 quadrate placed at each station (island). Sampling of water for nitrate and phosphate analysis was also conducted in each quadrate. Nitrate content in the water were analyzed using Brucine method and phosphate with Ascorbique Acide method (APHA, 1989). Heavy metals (Zn, Cu, Cd and Pb) were analyzed in water, sediment and seagrass leaves from the same quadrate using AAS (Atomic Absorption Spectrophotometry) method (APHA, 1989). We also measured oceanographic parameters *in situ*. Turbidity, temperature, salinity, pH, and dissolved oxygen were measured using Water Quality Checker (WQC-22A), depth was measured using depth gauge, current speed was measured with current meter, and secchi disk to measure water clarity.

2.2. Measurement of seagrass morphometrics and fluctuating asymmetry

The length and width of leaves of seagrass were measured, then the measurement of fluctuating asymmetry was conducted according to Ambo-Rappe et al. (2007, 2008b, 2011) as follows: (a) twenty leaves from each quadrate were cleaned of attached epiphytes, (2) five points along the leaf were defined at a distance of 5 cm apart, (3) measurement of leaf width on the right and left

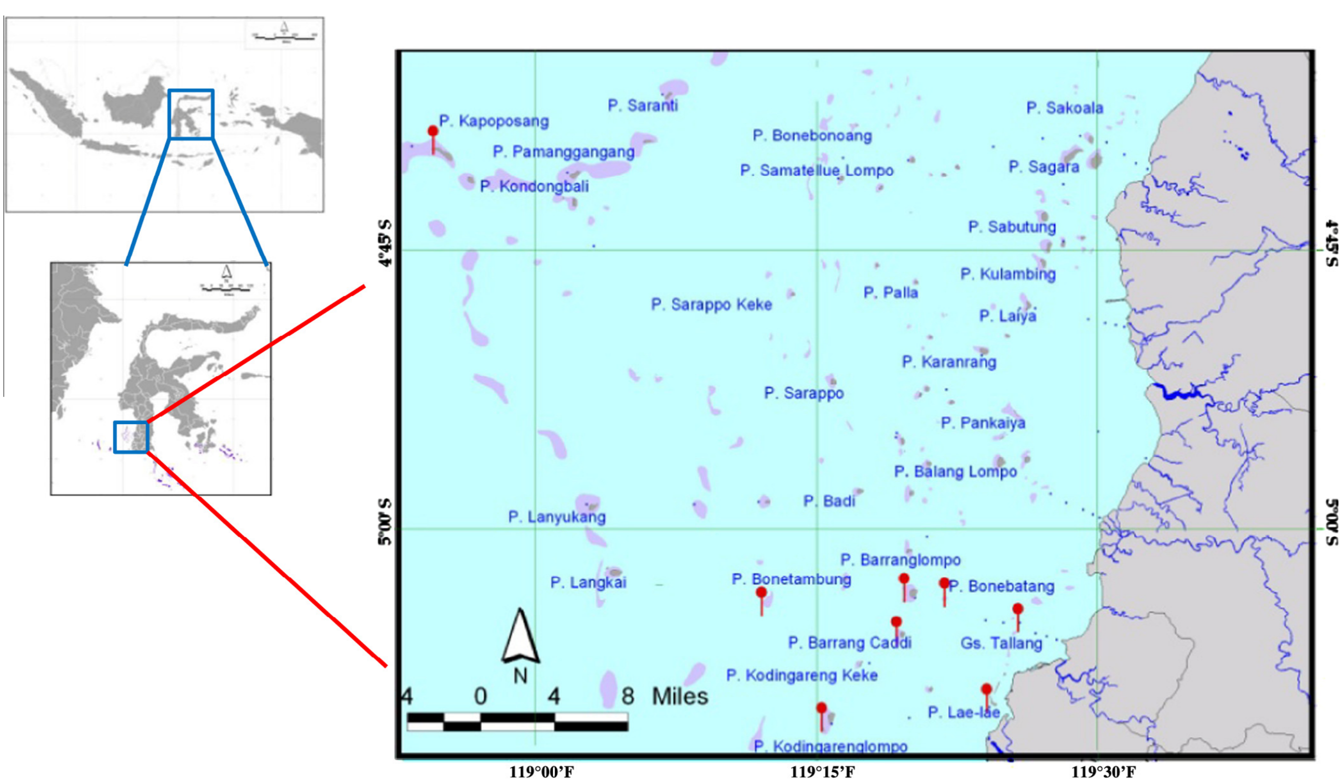

Fig. 1. Study site in Spermonde Archipelago, South Sulawesi, Indonesia.

of the center of the leaf was made at each point twice to control measurement error. The fluctuating asymmetry index for each of five points was calculated using the formula proposed by Palmer and Strobeck (1986), Palmer (1994), and Lajus et al. (2003) as follows:

$$\mathrm{FA} = \mathrm{Abs}(L - R)/((L + R)/2)$$

2.3. Data analysis

One-Way Analysis of Variance was used to analyse the difference in morphometric characters and fluctuating asymmetry (FA) of seagrass leaves among islands. Each of the environmental parameters measured in this study such as heavy metal (Zn, Cu, Cd, Pb) in the sediment, water, and seagrass leaves, and nitrate and phosphate content in the water were also analyzed with the One-Way ANOVA. We tested for normality of the data using Kolmogorov–Smirnov test. If data were not normally distributed, we performed a non-parametric Kruskal–Wallis test. When the result is significantly different between stations, a *post hoc test* was conducted (Sokal, 1981).

In order to analyze association between environmental variables and seagrass morphological variables, we used Principal Component Analysis to reduce number of environmental variables, and further on studied correlations between biological variables and PCs based on environmental variables.

3. Results

Data on oceanographic parameters are shown in Table 1. High water temperatures were found in Barrang Caddi, Bone Batang, and Gusung Tallang. Low salinity was observed in almost all stations due to recent rain. Dissolved oxygen, pH, clarity, and turbidity of water were relatively uniform at all locations. Current speeds measured at all sites was very slow and ranged from 0.013 to 0.046 m/s.

Phosphate was significantly higher in waters of Kapopposang compared to other locations ($F_{7,16} = 5.236$; $p < 0.01$; Fig. 2). Nitrate, on the other hand, did not differ between the locations.

Results of one-way ANOVA showed that there were significant differences in Zn-sediment concentration between locations ($F_{7,16} = 4.561$; $p < 0.01$), where Gusung Tallang, Lae-Lae, Barrang Lompo, and Bone Tambung had higher Zn content in sediment than other islands. Concentrations of copper in sediment varied among sites ($F_{7,16} = 10.188$; $p < 0.01$). Cu concentrations were significantly lower in Kapopposang than in other sites. The content of cadmium (Cd) in sediments also differed between locations ($F_{7,16} = 129.248$; $p < 0.01$) and Kapopposang contained higher Cd-sediment concentration than the other locations. The content of lead (Pb) in sediment also differed between sites ($F_{7,16} = 8.016$; $p < 0.01$), with Bone Tambung having higher Pb-sediment than the other sites.

The results of the analysis of non-parametric Kruskal–Wallis test showed that the concentrations of Zn, Cu, and Cd in water were not significantly different between locations. While Pb concentration was significantly different among locations ($p < 0.05$), in which Bone Tambung had higher content of Pb.

The results of the analysis of one-way ANOVA showed that there was no difference in Cu, Cd, and Pb in seagrass between locations. However, a highly significant difference was found in Zn-seagrass concentration among locations ($F_{7,24} = 5.704$; $p < 0.01$), where seagrass from Lae-Lae had higher Zn content than other locations.

Analysis of Spearman Correlation indicated a significant and strong positive correlation between concentration of Cu and Zn in sediment and seagrass ($r = 0.810$; $p < 0.05$). Mean values of all heavy metal studied in sediment, water, and seagrass are presented in Table 2.

Length and width of leaves of *E. acoroides* varied significantly among locations (leaf length, $F_{7,952} = 69.613$; leaf width, $F_{7,952} = 24.588$; $p < 0.01$). Seagrass leaves in Kapopposang Island were longer and wider than the leaves of the seagrass from different locations.

We did not observe directional asymmetry in our data; none of five t-test were significant ($p > 0.05$) while testing differences

Table 1
Data on oceanographic parameters.

Location	Temp (°C)	Salinity (ppt)	DO (ppm)	pH	Turbidity (NTU)	Depth (cm)	Clarity (%)	Current Speed (m/s)
Gusung Tallang	32.8	24	7.6	8.00	5	80.0	100	
	32.4	25	6.7	8.04	4	82.0	100	0.026
	32.4	25	6.9	8.03	5	84.9	100	
Lae-Lae	31.1	25	5.9	7.93	5	114.0	100	
	31.1	26	5.9	7.91	6	115.0	100	0.035
	31.1	25	6.0	7.94	7	115.5	100	
Barrang Caddi	33.0	29	8.7	8.19	7	35.0	100	
	33.0	28	8.4	8.19	5	42.0	100	0.040
	32.8	29	6.5	8.19	6	104.0	100	
Barrang Lompo	31.6	25	6.8	7.99	5	113.0	100	
	31.6	25	6.7	8.01	7	117.0	100	0.020
	31.3	25	6.3	7.95	7	119.1	100	
Bone Tambung	31.7	30	7.7	8.10	7	88.0	100	
	31.8	29	7.9	8.11	6	90.2	100	0.013
	31.8	29	8.3	8.12	7	93.0	100	
Kodingareng Lompo	29.7	25	6.0	7.90	7	96.0	100	
	29.9	25	6.5	7.97	8	91.3	100	0.046
	29.3	25	6.4	8.03	7	94.4	100	
Bone Batang	33.0	27	8.7	8.14	7	85.0	100	
	33.2	27	8.8	8.13	8	84.0	100	0.021
	32.5	29	10.2	8.25	7	85.9	100	
Kapopposang	30.9	33	6.9	7.48	7	290.0	100	
	30.8	31	5.8	7.61	8	280.0	100	0.024
	30.9	30	5.9	7.84	8	275.0	100	

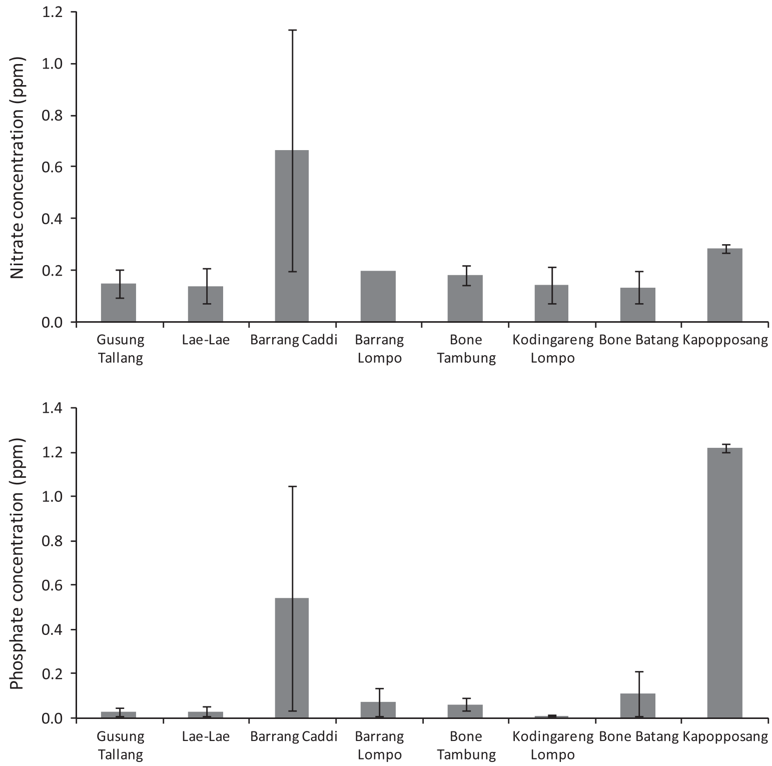

Fig. 2. Mean nitrate and phosphate concentration (mean ± SE, $n = 3$) in each location.

Table 2
Mean values of Zn, Cu, Cd, Pb concentration in sediment, water, and seagrass in Spermonde Archipelago.

Station/island	Heavy metal	Sediment (ppm)	Water (ppm)	Seagrass (ppm)
Gusung Tallang	Zn	3.053 ± 0.147	0.020 ± 0.010	3.163 ± 0.506
	Cu	11.230 ± 0.125	0.014 ± 0.000	3.160 ± 0.522
	Cd	0.983 ± 0.052	0.008 ± 0.002	0.935 ± 0.006
	Pb	9.463 ± 0.229	0.005 ± 0.000	2.433 ± 0.993
Lae-Lae	Zn	2.227 ± 0.760	0.010 ± 0.000	5.538 ± 0.313
	Cu	11.953 ± 1.771	0.014 ± 0.000	2.195 ± 0.195
	Cd	1.017 ± 0.038	0.012 ± 0.004	1.085 ± 0.006
	Pb	9.870 ± 0.531	0.005 ± 0.000	1.993 ± 0.206
Barrang Caddi	Zn	1.337 ± 0.367	0.010 ± 0.000	1.755 ± 0.438
	Cu	9.063 ± 0.127	0.014 ± 0.000	1.560 ± 0.178
	Cd	0.997 ± 0.048	0.005 ± 0.000	0.935 ± 0.006
	Pb	9.003 ± 0.213	0.005 ± 0.000	1.500 ± 0.352
Barrang Lompo	Zn	2.063 ± 0.124	0.010 ± 0.000	2.960 ± 0.340
	Cu	10.483 ± 0.207	0.014 ± 0.000	1.740 ± 0.127
	Cd	1.013 ± 0.058	0.007 ± 0.002	0.865 ± 0.006
	Pb	10.150 ± 0.176	0.005 ± 0.000	2.125 ± 0.241
Bone Tambung	Zn	1.860 ± 0.242	0.047 ± 0.037	3.688 ± 1.139
	Cu	13.217 ± 0.347	0.549 ± 0.535	2.090 ± 0.446
	Cd	1.257 ± 0.034	0.012 ± 0.004	0.885 ± 0.006
	Pb	11.747 ± 0.323	1.573 ± 1.568	1.300 ± 0.392
Kodingareng Lompo	Zn	0.863 ± 0.188	0.010 ± 0.000	1.438 ± 0.098
	Cu	9.873 ± 0.409	0.014 ± 0.000	1.873 ± 0.247
	Cd	1.007 ± 0.026	0.012 ± 0.004	0.945 ± 0.006
	Pb	9.493 ± 0.408	0.005 ± 0.000	1.028 ± 0.332
Bone Batang	Zn	1.203 ± 0.306	0.010 ± 0.000	1.620 ± 0.853
	Cu	11.023 ± 0.515	0.014 ± 0.000	1.578 ± 0.156
	Cd	1.150 ± 0.026	0.005 ± 0.000	0.905 ± 0.006
	Pb	10.607 ± 0.284	0.005 ± 0.000	1.223 ± 0.243
Kapopposang	Zn	1.067 ± 0.048	0.157 ± 0.132	1.948 ± 0.076
	Cu	5.887 ± 0.172	0.263 ± 0.249	0.180 ± 0.012
	Cd	2.433 ± 0.052	0.030 ± 0.006	0.808 ± 0.009
	Pb	9.047 ± 0.269	0.263 ± 0.112	2.073 ± 0.659

between left and right values pooled across islands. Measurement error as measured per point per station per side ($n = 240$) varied from 1.02% to 14.93% of magnitude of fluctuating asymmetry and in average comprised 6.44% of fluctuating asymmetry and we considered that it does not affect results of FA comparison.

Overall, fluctuating asymmetry based on five measurements of *E. acoroides* leaves showed statistically significant heterogeneity both among stations and islands (Kruskal–Wallis test, $p < 0.05$). Value of Kruskal–Wallis test was higher while comparing among stations, H (23, $N = 480$) = 72.4 than its value while comparing among islands, H (7, $N = 480$) = 22.6. This shows rather high heterogeneity of stations within islands, but still presence of differences between islands.

Magnitude of fluctuating asymmetry was the highest in the Kapopposang island, and the second highest was in the Lae-Lae island. Heterogeneity of stations within these islands was rather high. Pairwise comparison of islands with each other showed that overall heterogeneity of FA is mostly caused by high asymmetry in the Kapopposang island, which was significantly higher than in all other islands besides Lae-Lae ($p < 0.05$, Mann–Whitney test).

Principal Component Analysis showed that the PC1 describes 41.4% of total variance of environmental variables, and four first PCs describe 89.8% of total variance (Table 3). The PC1 shows high (absolute value higher than 0.8) positive correlations with Cu in sediments and seagrass, and high negative correlations with Cd in sediments and in water, Zn in water, depth and phosphorus in water.

Leaf length and width are highly correlated with each other ($r = 0.95$, $p < 0.01$) thus they show very similar patterns of correlation with environmental variables, in particularly, they show significant negative correlations with PC1 ($p < 0.05$) (Table 4). Seagrass with larger leaves tend to leave in deeper locations with higher concentrations of phosphorus, Cd (in water and sediments), Zn (in water) and lower concentrations of Cu (in seagrass and sediments). Fluctuating asymmetry show positive ($r = 0.50$), but not significant correlation with leaf length and width and, in general, shows quite similar patterns in terms of correlation with other environmental PCs although they are in most cases lower. This pattern is at least partly explained by the very distinctive patterns in Kapopposang island in terms of number of environmental variables and biological characteristics of leaves.

Table 3
Loadings of environmental variables on first four principal components and their contribution to total variance.

	PC1	PC2	PC3	PC4
Zn-sediment	0.559	−0.121	0.679	−0.449
Cu-sediment	0.836	0.412	0.293	0.187
Cd-sediment	−0.962	0.094	0.180	−0.098
Pb-sediment	0.327	0.852	0.264	0.253
Zn-water	−0.938	0.104	0.268	−0.133
Cu-water	−0.365	0.774	0.361	0.074
Cd-water	−0.901	−0.086	0.363	0.113
Pb-water	−0.092	0.841	0.330	0.118
Zn-seagrass	0.369	−0.038	0.774	0.030
Cu-seagrass	0.891	−0.061	0.294	−0.058
Cd-seagrass	0.633	−0.398	0.120	0.254
Pb-seagrass	−0.069	−0.449	0.643	−0.588
N-water	−0.185	−0.097	−0.575	−0.512
P-water	−0.913	−0.094	−0.125	−0.338
Salinity	0.354	0.323	−0.245	−0.803
Temperature	−0.747	0.504	−0.165	−0.241
pH	0.279	0.681	−0.512	−0.298
DO	0.759	0.399	−0.484	−0.158
Turbidity	−0.652	0.284	−0.338	0.552
Depth	−0.922	−0.135	0.323	−0.005
Current	0.097	−0.715	−0.454	0.317
Prp.total	0.414	0.201	0.169	0.113

Table 4
Pearson correlations of *Enhalus* characteristics with first four principal components based on environmental variables.

	PC1env	PC2env	PC3env	PC4env
Leaf length	−0.85	0.05	0.32	0.35
Leaf width	−0.83	0.06	0.18	0.47
FA	−0.76	−0.19	0.04	−0.44

4. Discussion

Relatively shallow depth may affect the high temperature measurement obtained for some locations. Low salinity was recorded due to dilution by heavy rain which fell before the observation. Dissolved oxygen, pH, clarity, and turbidity of water are within ranges typical for seagrass beds. Current speeds were very slow and possibly associated with the dampening effect of the large seagrass, *E. acoroides*, on currents (Hemminga and Duarte, 2000). Water depth and turbidity levels which were relatively high in Kapopposang (see Table 1) may contribute to the longer and wider leaves found in this location. In addition to the water depth and high turbidity levels of Kapopposang, there was the contribution of nutrients, particularly phosphate which was significantly higher in concentration, to the length and width of leaves of *E. acoroides* in this area.

Seagrass morphology is very sensitive to environmental conditions (McMillan and Phillips, 1979; Phillips, 1980; Durako, 1995; Hackney and Durako, 2004) including heavy metal concentrations in the sediment (Marin-Guirao et al., 2005). The morphology of *Enhalus*, however, was apparently unrelated to heavy metal concentrations. The wide and long leaves at Kapopposang were associated instead with depths and higher turbidity, which suggests that light attenuation was greater at this site. A similar phenomenon was obtained by Ambo-Rappe et al. (2007), where the variation in seagrass leaf morphology was more influenced by the water depth and turbidity than the heavy metal content in waters.

Enhalus tissue reflected the gradient in metal concentration in the environment, especially of the sediment across the different islands. Seagrasses have the ability to accumulate trace metals from the environment (Sanchiz et al., 2000; Marin-Guirao et al., 2005), and thus are increasingly employed to monitor heavy metals in aquatic systems due to their ability to accumulate heavy metals and to reflect the concentration of heavy metals present in the environment (Ward, 1989; Sanchiz et al., 2000; Campanella et al., 2001; Marin-Guirao et al., 2005; Ambo-Rappe et al., 2007; Govindasamy et al., 2011). The uptake of trace metals by seagrass varies between non-essential (e.g. Cd and Pb) and essential metals (e.g. Cu, Zn). Non-essential elements, as opposed to the essential ones, are predominantly incorporated into seagrass tissues via passive rather than active metabolic uptake across biological membranes (Whelan et al., 2005). This might explain the significant correlation between Cu and Zn concentration in seagrass and in the sediment, and no such significant correlation found for Cd and Pb.

Although the concentration of heavy metals was highest close to the mainland and decreased with increasing distance from the mainland, in general the average metal concentration found in sediment and water of Spermonde Archipelago was below the guideline for protection of aquatic biota (ANZECC, 2000). Heavy metal content in the leaves of seagrass *E. acoroides* found in Spermonde Archipelago was also below the background level reported elsewhere in Indonesia (Nienhuis, 1986).

As regards FA, the significant differences were observed suggesting that there are some factors changing developmental instability of seagrass. At the same time, it is quite difficult to link these changes to some specific environmental variables, in particular toxicants, because heterogeneity of FAs is mostly related to distinctiveness of Kapopposang island, and this distinctiveness is not only related to pollution characteristics but to most of other environmental variables. In this situation is not easy to separate effects of pollution from other effects.

In conclusion, our study shows that both environmental and biological variables are heterogeneous among islands of Spermonde Archipelago. For some of heavy metals we observe higher concentrations in locations adjacent to the source of pollution – Makassar city area. No biological variables, however, show similar trends, suggesting that their heterogeneity is not associated with gradient of pollution. This allows assuming that current level of pollution does not cause detectable effect on seagrass leaves length and width as well as on developmental instability. Therefore values of biological variables described in our work may be considered as a baseline characterizing comparatively undisturbed ecosystem. Information about such a baseline is necessary for effective monitoring of ecosystem status in situation when further quick economical development will unavoidably associated with increasing human-induced pressure and specifically with pollution.

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