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TAXONOMIC LEVEL NEEDED TO DETECT SIGNIFICANT CHANGES IN POLYCHAETE COMMUNITIES OVER DIFFERENT MACROPHYTE ASSEMBLAGES ON ROCKY INTERTIDAL SHORES

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ABSTRACT

Environmental degradation has more significant impacts on rocky intertidal communities after global changes increase progressively. Thus, ecological monitoring should be conducted properly to analyse potential drivers and their impacts. However, most of the ecological monitoring in rocky intertidal shores is more interested in macroalgae. Polychaetes associated with macrophyte assemblages should be also involved in the monitoring because they are important in determining coastal health and productivity. A successful ecological monitoring should consider three factors: taxonomic level, statistical power, and sample size. In this study, those factors were analysed in the relationships between polychaetes and macrophytes. Four taxonomic levels of polychaetes (*i.e.* order, family, genus, species) were tested based on 25 samples collected from rocky intertidal shores of Gunung Kidul, Yogyakarta, Indonesia. Relationships between each of taxonomic richness of polychaetes and each of macrophytes variables (*i.e.* species richness, biomass, species composition) were analysed using a Generalised Linear Models fitted by Poisson Distribution and log link. The statistical power of those relationships and the sample size needed to obtain a strong statistical power (>0.8) were also recorded. Relationships between each of taxonomic composition of polychaetes and each of macrophyte variables were analysed using a distance based Redundancy Analysis based on Bray Curtis dissimilarity on log(x+1) transformed abundance data with 999 permutations. Results showed that family based data analysis was sufficient to detect significant relationships between polychaetes and macrophytes. However, the statistical power of most relationships was relatively weak (< 0.8). Hence, the family-based data analysis should select a 44 sample size to gain significant relationships with a strong statistical power.

Keywords: family based data analysis, significant changes, polychaete communities, macrophyte assemblages, rocky intertidal shores

INTRODUCTION

Environmental degradation due to natural processes (*e.g.* seismic activities) and human activities (*e.g.* climate change and pollution) has impacted marine ecosystems. Loss in biodiversity and changes in community structures get faster after global changes increase progressively (Jones and Cheung, 2015). Compared to sublittoral and offshore ecosystems, rocky intertidal shores may be more vulnerable to global changes because they are influenced directly by both terrestrial and marine disturbances (Thompson *et al*., 2002). Total area of intertidal zones is also relatively smaller, so the entire communities living on the rocky intertidal zones, especially macrophytes (*i.e.* macroalgae and seagrass) and faunas associated with them are probably subjected to extinct by those disturbances.

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Many management plans with various approaches and actions have been proposed to reduce the effect of environmental degradation. The first step of any management plan is ecological monitoring that is purposed to detect potential drivers and their impacts (Elzinga *et al*., 2001; Hewitt *et al*., 2005; Kallimanis *et al*., 2012). Changes in community structure of rocky intertidal shores have been monitored using various methods that are focused on macrophytes (Ellis, 2003; Murray *et al*., 2016). It is reasonable because macrophytes are foundation group in the rocky intertidal shores that provide food and shelters for invertebrates, especially those attached on them (Gambi *et al*., 2000; Zamzow *et al*., 2010).

However, invertebrates associated with macrophytes should be also monitored because they have important roles in the rocky intertidal shores. They can be decomposer of organic materials as well as food sources for the higher taxa (*e.g.* fishes) (Duggins *et al*., 2016). Hence, the distribution of invertebrates on rocky intertidal shores determines coastal health and productivity. Polychaetes can be one of the targeted fauna in ecological monitoring on the rocky intertidal shores (Musco *et al*., 2009) because they show a high variability over different macrophyte assemblages (Abbiati *et al*., 1987; Giangrande, 1988; Giangrande *et al*., 2003; Pabis and Sicinski, 2010), and are generally diverse and dominant on rocky intertidal shores (Morais and Lee, 2014; Gusmao-Junior and Lana, 2015).

A successful ecological monitoring must consider three factors to gain statistically strong results: taxonomic level (Kallimanis *et al*., 2012), statistical power (Morrison, 2007), and sample size (Elzinga *et al*., 2001). In term of taxonomic level, most ecological studies in rocky intertidal shores have identified polychaetes up to the lowest taxonomic level (*i.e.* species level) (Abbiati *et al*., 1987; Giangrande, 1988; Giangrande *et al*., 2003; Casu *et al*., 2006; Pabis and Sicinski, 2010; Magalhaes and Bailey-Brock, 2014). Species based data analysis may provide strongest statistical results, but such analysis probably expends a lot of resources including time, cost, and taxonomist (Caughlan and Oakley, 2001; Kallimanis *et al*., 2012). Alternatively, many studies on marine invertebrates have shown that a higher taxonomic level (*e.g.* family and genus) can be applied in the ecological monitoring to reduce the expenditure for those resources (Olsgard *et al*., 1997; Defeo and Lercari, 2004; Jimenez *et al*., 2010). Unfortunately, those studies analysed non-associated invertebrates in the soft-bottom communities, so the results cannot be used to estimate the taxonomic level needed in the ecological monitoring of polychaetes associated with macrophyte assemblages on the rocky intertidal shores.

Secondly, statistical power generally tells the probability to reject type 2 error (the failing to reject the null hypothesis when it is false) (Bakus, 2007). In the ecological monitoring, type 2 error is worse than type 1 error (the failing to retain the null hypothesis when it is true) because it will not detect an environmental change that is actually occurring (Morrison, 2007). It is widely acknowledged that the statistical power should be >0.8 so that is strong enough to avoid falling into type 2 error. However, the statistical power is strongly influenced by data variation, by which the power increases as the data variation within population gets lower (Cohen, 1977). The statistical power of macrobenthic communities has been analysed but was limited to soft bottom communities (Charles *et al*., 2001; Franco *et al*., 2015).

Lastly, an increase in sample size will reduce the data variation, so it will increase the statistical power. Hence, there is a relationship among data variation, sample size, and statistical power. In general, the sample size needed in the ecological monitoring is 30 samples, called as the "n = 30 rule of thumb" (Elzinga *et al*., 2001). Over the 30 samples, the data will have generally a normal distribution, so the increasing of sample size may not increase the statistical benefits (Martnez–Abran, 2014). However, polychaetes show a spatial and temporal variability (Abbiati *et al*., 1987; Giangrande, 1988; Giangrande *et al*., 2003; Casu *et al*., 2006; Pabis and Sicinski, 2010; Magalhaes and Bailey-Brock, 2014). As a result, the data variation may differ between locations, and thus the "n = 30 rule of thumb" may not be always applicable (Martinez-Abran, 2014).

This study filled the gap of the current studies in the ecological monitoring of rocky intertidal shores, particularly those related to taxonomic level, statistical power, and sample size. More specifically, this study answered the following questions: (1) what is the highest taxonomic level that is selected to detect significant relationships between polychaetes and macrophytes? (2) how is the statistical power of those relationships? and (3) how many samples that are required to obtain a strong statistical power (0.8) in those relationships?

MATERIALS AND METHODS

Study Site

Rocky intertidal shores of Gunung Kidul, Yogyakarta, Indonesia, were selected as study sites because there are many variations in macrophyte assemblages. The shores are typically limestone system (Miftahussalam *et al*., 2004) with medium to high wave energy, ranging from 165 to 4036 J (Damayanti and Ayuningtyas, 2008). Macrophytes are foundation group on the shores, composed by 23 genera of macroalgae and 1 genus of seagrass. Echinoderms are also dominant, particularly *Ophiocoma*, *Ophiotrix*, *Macrophiotrix*, *Echinotrix*, and *Echinometra*, with the maximum density is usually in November (Jati, 1996).

The shores are utilised by various human activities. Tourisms, especially walking on the intertidal platforms, are the most common because the shores of Gunung Kidul are tourism destination for both local and international visitors. Extractive activities (*e.g.* local fisheries, sand mining) and macroalgae (*e.g. Ulva* sp.) harvesting are also found (Damayanti and Ayuningtyas, 2008). There is also an effort to conserve coral reefs in the Gunung Kidul but it is not well supported by local people (Harjiyatni, 2001).

Experimental Design

Polychaete samples were collected from five sites (*i.e.* Kukup, Sepanjang, Drini, Krakal, and Sundak) (Figure 1), in which each site had five transects. Then, the samples were analysed into four taxonomic levels: order, family, genus, and species.

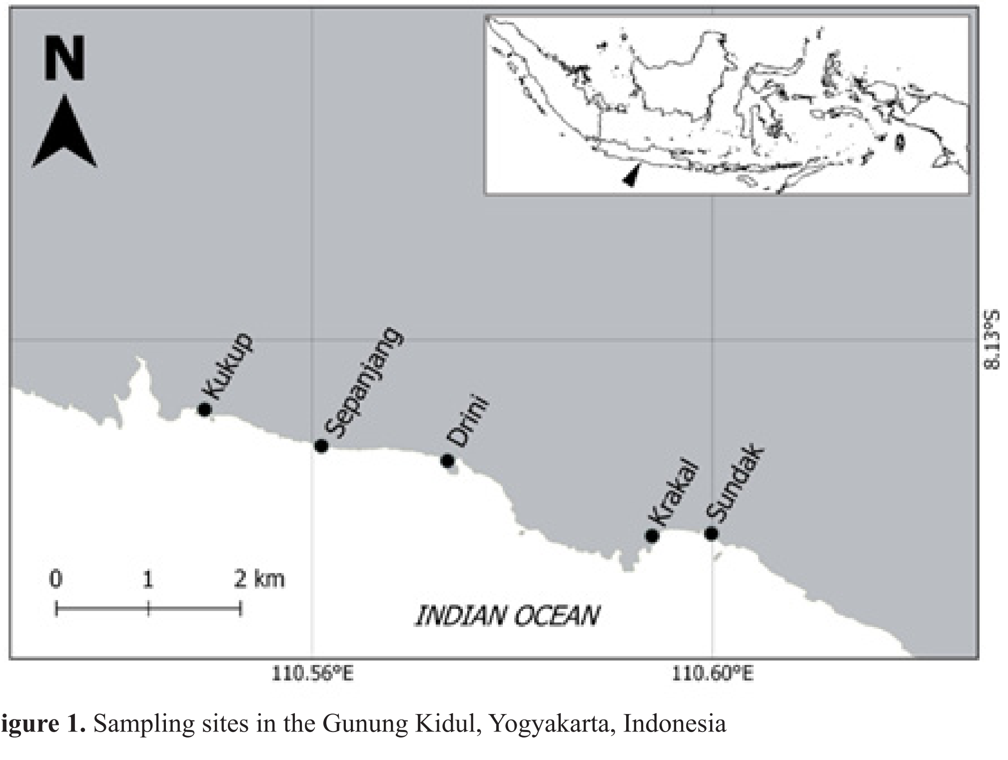

Figure 1. Sampling sites in the Gunung Kidul, Yogyakarta, Indonesia

Sampling Technique

Sampling was conducted in March 2012 mostly during low tide because most of the intertidal invertebrates are active during this time. At each of sampling sites, five line transects separated by 5 m were performed perpendicularly to shorelines. Then, at each of transects, there were three quadrats of 20x20 cm separated by 5 m. All of the transects were located at the middle of intertidal rock platforms with about 5 cm of depth to minimise the effect of desiccation during low tide.

Macrophytes inside the quadrats were scrapped totally and fixed using 4% formaldehyde-sea water solution. In the laboratory, the macrophyte samples were rinsed using freshwater and stirred gently to detach faunas. Then, the water was sieved through a 0.5 mm mesh to retain faunas.

The polychaete samples were identified up to four different taxonomic levels (*i.e.* order, genus, family, and species), counted individually, and preserved in 80% ethanol. The macrophyte samples were identified up to species level, counted as binomial data (*i.e.* absence (0) and presence (1)), and dried in the oven at 70-80°C for 3 days to obtain the constant biomass. The validity of taxonomic name of polychaetes and macrophytes was confirmed through a World Register of Marine Species website (www.marinespecies.org).

Data Analysis

Inferential statistics included relationships between each of polychaete variables (*i.e.* taxonomic richness and taxonomic composition) and each of macrophyte variables (*i.e.* species richness, biomass, and species composition). The taxonomic composition of polychaetes was visualised by Principle Coordinate Analysis (PCoA) based on Bray-Curtis dissimilarity on log (x+ 1) transformed abundance data. According to preliminary analyses, the variance of data was higher than the mean, so log (x + 1) transformation was needed as recommended by Bakus (2007). Then, the ordination of community structure of polychaetes was clustered based on the <25% Bray Curtis dissimilarity. Similarly, the species composition of macrophytes was also determined based the values of axis 1 of PCoA based on Bray Curtis dissimilarity but using absent-present data (Gower, 1966). Hence, the tests involved both univariate and multivariate analyses.

The univariate analyses included relationships between each of taxonomic richness of polychaetes (*i.e.* order, family, genus, species) and each of macrophyte variables. These were analysed using a Generalised Linear Models (GLMs) fitted by Poisson Distribution and log link. A Poisson Distribution was used because the data are count data with a lot of zero (Crawley, 2015). The statistical power of GLMs test and the sample size to obtain the statistical power of 0.8 was estimated based on the method of Signorini (1991).

The multivariate analyses included relationships between each of taxonomic compositions of polychaetes and each of macrophyte variables. These were analysed using a distance based Redundancy Analysis (dbRDA) based on Bray Curtis dissimilarity on log(x+1) transformed abundance data with 999 permutations (Legendre and Anderson, 1999).

All statistical tests and data visualisation were performed using various packages of R software (https://www.r-project.org). The package of vegan was used to perform PCoA and dbRDA (Oksanen *et al*., 2016), the package of powerMediation was used to analyse the statistical power of Poisson regression (Qiu, 2015), and the package of ggplot2 was used to visualise the data (Wickham, 2009).

RESULTS

Community Structure of Polychaetes and Macrophytes

In total, this study collected 1,280 individuals of polychaetes belonging to 30 species, 22 genera, 16 families, and 8 order (Table 1). In average, the species, genus, family, and order richness were 6.80 species/transect, 6.08 genus/transect, 4.96 families/transect, 3.40 order/transect, respectively. In term of community structure, PCoA plots visualised around 48.19% to 52.15%

Table 1. List of polychaetes collected in this study

Order	Family	Genus	Species
Amphinomida	Amphinomidae	*Eurythoe*	*Eurythoe complanata*
Capitellida	Capitellidae	*Notomastus*	*Notomastus* sp.
Capitellida	Capitellidae	*Pseudocapitella*	*Pseudocapitella* sp.
Capitellida	Maldanidae	*Maldane*	*Maldane* sp.
Eunicida	Eunicidae	*Lysidice*	*Lysidice* sp.
Eunicida	Oenonidae	*Oenone*	*Oenone* sp.1
Eunicida	Oenonidae	*Oenone*	*Oenone* sp.2
Opheliida	Opheliidae	*Polyophthalmus*	*Polyophthalmus pictus*
Phyllodocida	Glyceridae	*Glycera*	*Glycera brevicirris*
Phyllodocida	Nereididae	*Perinereis*	*Perinereis* af. *arabica*
Phyllodocida	Polynoidae	*Lepidonotus*	*Lepidonotus cristatus*
Phyllodocida	Polynoidae	*Lepidonotus*	*Lepidonotus tenuisetosus*
Phyllodocida	Sigalionidae	*Euthalenessa*	*Euthalenessa* sp.
Phyllodocida	Syllidae	*Syllis*	*Syllis* cf. *amica*
Phyllodocida	Syllidae	*Typosyllis*	*Typosyllis* af. *aciculata*
Phyllodocida	Syllidae	*Typosyllis*	*Typosyllis* cf. *ehlersioides*
Phyllodocida	Syllidae	*Typosyllis*	*Typosyllis* cf. *maculata*
Phyllodocida	Syllidae	*Trypanosyllis*	*Trypanosyllis gemmipara*
Sabellida	Sabellidae	*Manayunkia*	*Manayunkia* sp.
Spionida	Magelonidae	*Magelona*	*Magelona* sp.
Spionida	Spionidae	*Prionospio*	*Prionospio cirrifera*
Spionida	Spionidae	*Prionospio*	*Prionospio* sp.
Spionida	Spionidae	*Spio*	*Spio filicornis*
Terebellida	Cirratulidae	*Cirratulus*	*Cirratulus chrysoderma*
Terebellida	Cirratulidae	*Tharyx*	*Tharyx marioni*
Terebellida	Terebellidae	*Branchiomma*	*Branchiomma violacea*
Terebellida	Terebellidae	*Pista*	*Pista* af. *foliigera*
Terebellida	Terebellidae	*Pista*	*Pista* af. *macrolobata*
Terebellida	Terebellidae	*Pista*	*Pista* sp.1
Terebellida	Terebellidae	*Pista*	*Pista* sp.2

of total variation of polychaete communities depending on the taxonomic resolution. There were also around 11 to 20 clusters based on the 25% Bray-Curtis dissimilarity, in which the number of clusters decreased with the taxonomic resolution. The ordination of community data of polychaetes changed substantially from order to family level, and seemed to be more consistent from family to species level (Figure 2).

A total of 397.72 g of macrophytes was also collected in this study, grouped into 9 species

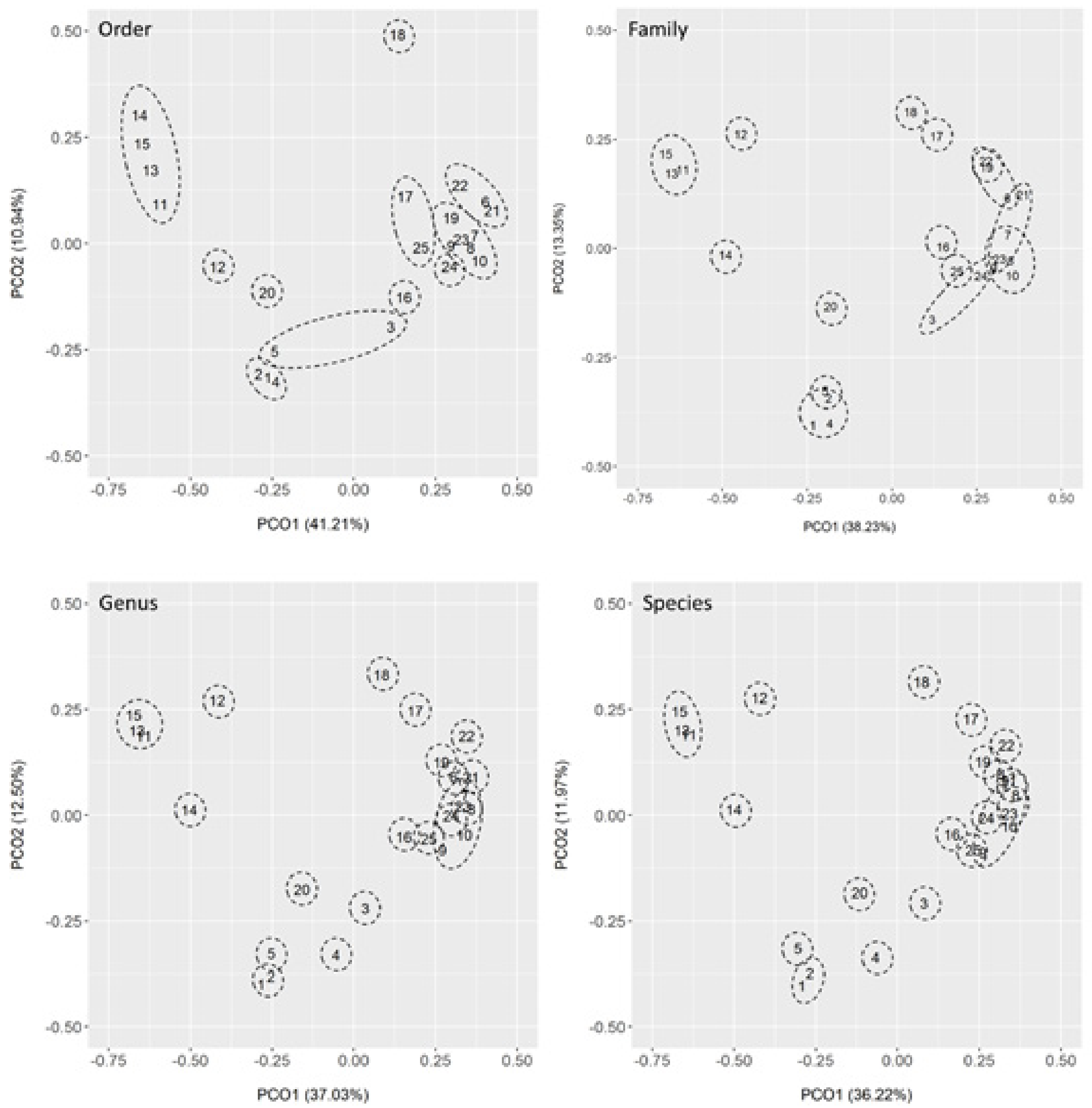

Figure 2. The ordination of polychaete communities at every taxonomic resolution

of macroalgae (*i.e. Gracilaria lichenoides*, *Gracilaria salicornia*, *Gracilaria* sp., *Ulva lactuca*, *Ulva* sp., *Chaetomorpha crassa*, *Acanthopora muscoides*, *Valonia aigagropilia*, and *Padina* sp.) and 1 species of seagrass (*i.e. Thalassia hemprichii*). The dry biomass of macrophytes was approximately 15.91 g per transect, while the species richness was about 4.52 per transect. In term of species composition, the assemblage structure of macrophytes along axis 1 of PCoA (25% of total variation) changed from 20.49 g per transect composed by 3 species (*i.e. Ulva lactuca*, *Chaetomorpha crassa*, and *Gracilaria* sp.) to 13.58 g per transect composed by 5 species (*i.e. Thalassia hemprichii*, *Gracilaria lichenoides*, *Ulva lactuca*, *Chaetomorpha crassa*, and *Acanthopora muscoides*) (Figure 3).

Univariate analyses on relationships between taxonomic richness of polychaetes and macrophyte assemblages

All taxonomic richness of polychaetes increased significantly with the species richness of macrophytes ($p<0.05$) (Table 2). Over the species richness of macrophytes (3-7 species per transect), the species richness of polychaetes increased more rapidly than other taxonomic levels (Figure 4). The statistical power increased gradually from 0.16 at order level to 0.62 at species level, but all of them were weak (<0.8). In order to obtain a strong statistical power (≥0.8) in these relationships, the sample size should be set minimally 70 samples at order level and decreased to 32 samples at species level (Table 2).

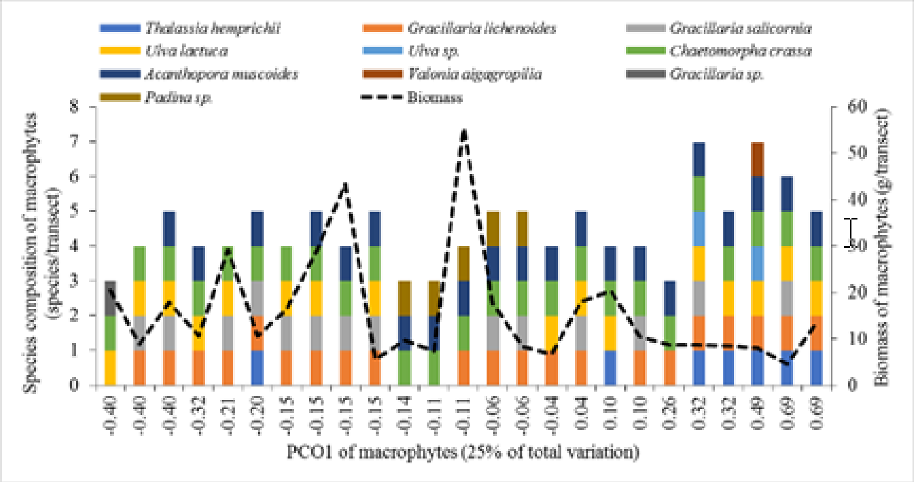

Figure 3. Change in species composition of macrophytes along axis 1 of PCoA (PCO1)

Table 2. p value, statistical power, and sample size (minimum size to obtain the statistical power of 0.8) of relationships between macrophyte variables (predictor) and taxonomic richness of polychaetes (response)

Predictor (Macrophyte Variables)	Response (Polychaete Variables)	p value	Statistical Power	Minimum Sample Size
Species richness	Order richness	0.00343	0.16	70
Species richness	Family richness	0.0000929	0.37	44
Species richness	Genus richness	0.000011	0.53	36
Species richness	Species richness	0.00000358	0.62	32
Biomass	Order richness	0.111	0.22	114
Biomass	Family richness	0.0134	0.45	53
Biomass	Genus richness	0.00297	0.60	38
Biomass	Species richness	0.00715	0.71	31
PCO1	Order richness	0.0242	0.74	30
PCO1	Family richness	0.00187	0.96	15
PCO1	Genus richness	0.000695	0.98	13
PCO1	Species richness	0.000299	0.99	11

Similarly, most of the taxonomic richness of polychaetes increased significantly with the biomass of macrophytes ($p<0.05$), except for the order richness ($p>0.05$) (Table 2). As the biomass of macrophytes increased from 4.607 g per transect to 55.536 g per transect, the species richness of polychaetes increased more quickly than other taxonomic levels (Figure 5). The statistical power also increased gradually between 0.22 at order level and 0.71 at species level, but all of them were weak (<0.8) as well. A strong statistical power (≥0.8) would be obtained in these relationships when the sample size was at least 114 samples at order level and declined to 31 samples at species level (Table 2).

In contrast, all the taxonomic level richness of polychaetes declined significantly along the axis 1 of PCoA of macrophytes ($p<0.05$) (Table 2). Within the changes in macrophyte composition, the species richness of polychaetes decreased more rapidly than other taxonomic levels (Figure 6). The statistical power increased from 0.74 at order level to 0.99 at species level, and most of them were strong (≥0.8), except that of order level. In these analyses, the minimum sample size required to obtain a strong statistical power (≥0.8) should be 30 samples at order level and decreased to 11 samples at species level (Table 2).

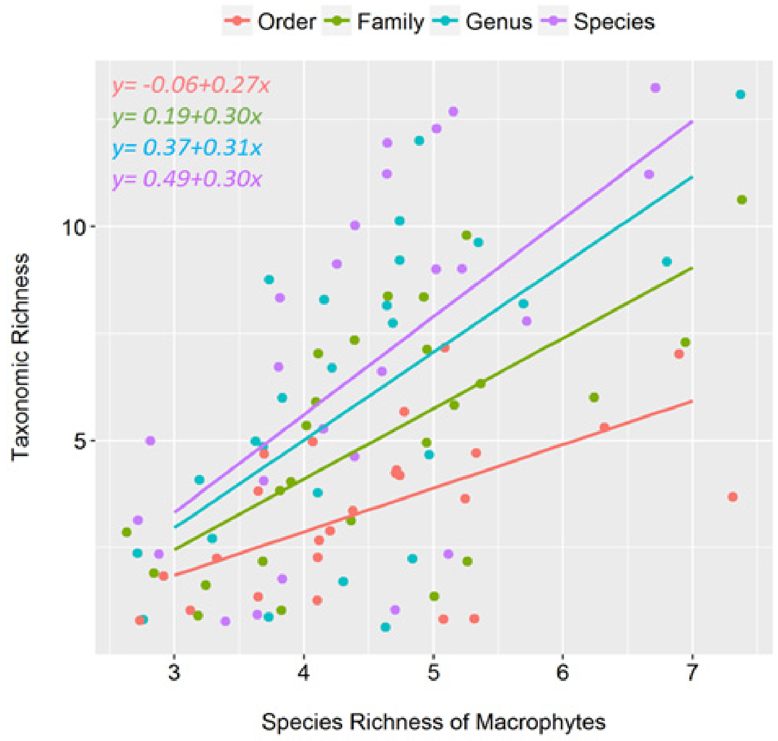

Figure 4. Linear relationships between each of taxonomic richness of polychaetes and species richness of macrophytes

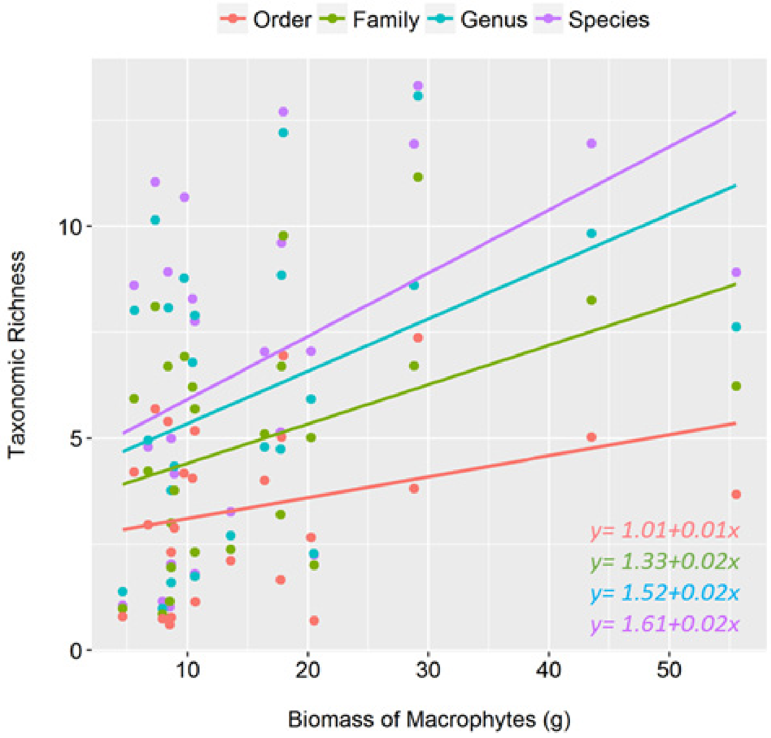

Figure 5. Linear relationships between each of taxonomic richness of polychaetes and biomass of macrophytes

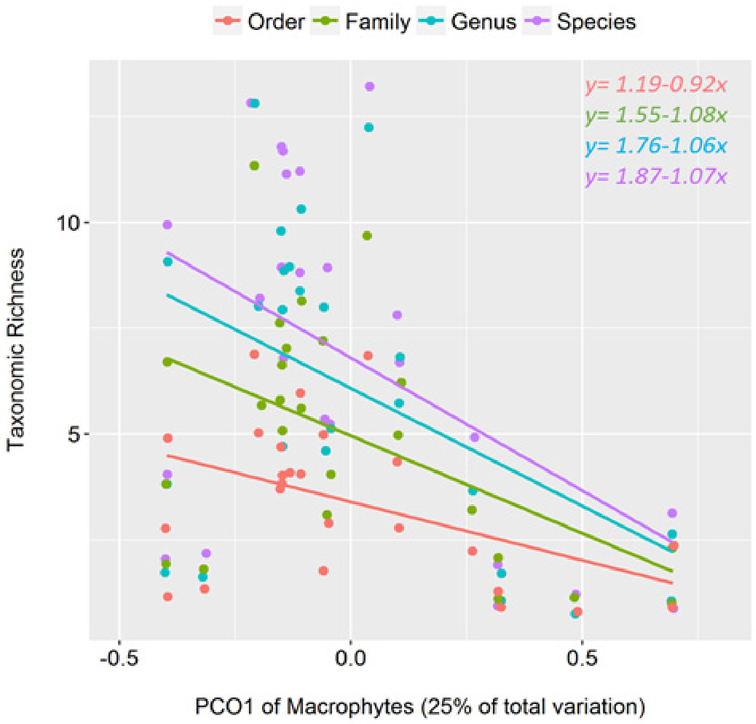

Figure 6. Linear relationships between each of taxonomic richness of polychaetes and species composition of macrophytes (PCO1)

Table 3. p value of relationships between macrophyte variables (predictor) and taxonomic composition of polychaetes (response)

Predictor (Macrophyte Variables)	Response (Polychaete Variables)	p value
Species richness	Order composition	0.013
Species richness	Family composition	0.012
Species richness	Genus composition	0.007
Species richness	Species composition	0.004
Biomass	Order composition	0.038
Biomass	Family composition	0.031
Biomass	Genus composition	0.044
Biomass	Species composition	0.034
PCO1	Order composition	0.003
PCO1	Family composition	0.001
PCO1	Genus composition	0.001
PCO1	Species composition	0.001

Multivariate analyses on relationships between taxonomic composition of polychaetes and macrophyte assemblages dbRDA tests showed that all taxonomic composition of polychaetes changed significantly with the species richness, the biomass, and the composition of macrophytes ($p<0.05$) (Table 3).

DISCUSSION

Prior to this study, changes in polychaete communities in respect to different macrophyte assemblages were mostly assessed based on species level data (Abbiati *et al*., 1987; Giangrande, 1988; Giangrande *et al*., 2003; Casu *et al*., 2006; Pabis and Sicinski, 2010; Magalhaes and Bailey-Brock, 2014). It may be true that such analysis has many advantages. For examples, it can detect biotic changes because each species may respond differently to environmental changes. Species identification also allows to monitor the introduction of alien species that may be useful for taxonomic studies and historical records (Bates *et al*., 2007). However, species based data analysis will need a lot of resources, including time, money, and availability of taxonomist (Caughlan and Oakley, 2001; Kallimanis *et al*., 2012). This study revealed that the effects of macrophytes on polychaete communities on rocky intertidal shores could be also detected significantly through data analysis based on some higher taxonomic levels (*e.g.* genus, family, and order). The statistical power of those analyses as well as the sample size recommended to obtain a strong statistical power were also discussed.

In general, both univariate and multivariate tests showed that most of the taxonomic levels of polychaete communities had significant relationships with macrophyte assemblages, although these relationships were stronger at species level. These suggest that the polychaete samples do not have to be identified until species level to detect a significant change of polychaete communities. Hence, this supports previous studies that a higher taxonomic resolution can be used to do a rapid assessment on the environmental changes (Olsgard *et al*., 1997; Defeo and Lercari, 2004; Jimenez *et al*., 2010). In this study, it is true that order data are sufficient to analyse relationships between polychaetes and macrophytes, except to detect a significant change in order richness. However, family data may be more appropriate because these had significant relationships with all macrophyte variables tested, including species richness, biomass, and species composition.

The use of family data in assessing polychaetes communities is likely related to the variation of taxonomic level of polychaetes within communities. Community structure data showed that taxonomic richness of polychaetes increased more rapidly from order to family level than from family to species level. It shows that the variation of polychaete communities on rocky intertidal shores are high within an order and low within a family. In fact, this study recorded that 9 of 16 families only had one species. Hence, the family data can be used as a proxy to estimate the species data of polychaetes on rocky intertidal shores. Indeed, there is a strong correlation between family richness and species richness of invertebrates on rocky intertidal shores, making the family richness is a good predictor to determine the species richness in an area (Musco *et al*., 2009).

In addition, the community structure of polychaetes based on the family data were visualised consistently with that on the species data. It is true that the total variations explained by two axes of PCoA were lower than the empirical rule-of-thumb (70%), so it may not describe the community structure well. However, the threshold of total variations in ecological studies is possibly difficult to set because it relies on the number of species and samples (Clarke and Warwick, 1994). Ordination plots of the community structure of polychaetes on the rocky shores of Mediterranean also visualise low total variations (<60%) (Abbiati *et al*., 1987; Giangrande, 1988).

Unfortunately, the sample size of this study resulted a weak statistical power, particularly for analyses of the effects of species richness and the biomass of macrophytes. The statistical power was getting lower with the taxonomic level, which implies that the probability to fall into type 2 error is getting bigger. To increase the statistical power up to 0.8, the sample size of polychaete samples for these analyses was around 31-114 samples, depending on how far the polychaete samples were identified. A species level data analysis only needs minimally 31 samples, but it possibly needs a taxonomist and will take longer for species identification. In contrast, a higher level data analysis, especially order and family levels, may not require a taxonomist and the polychaete samples can be identified faster, but more sample sizes are required to obtain the strong statistical power. Hence, the balance between the need of taxonomist and the sample size should be considered in the ecological monitoring of rocky intertidal shores.

However, the sample size of this study gained a strong statistical power in analysing the effects of species composition of macrophytes. In these analyses, the minimum sample size required to result a strong statistical power was much lower than that for analysing the effects of species richness and biomass of macrophytes. At the same taxonomic level, the slope of linear relationships was also steeper at the species composition of macrophytes than at the species richness and the biomass of macrophytes. These indicate that polychaete communities on rocky intertidal shores are more sensitive to changes in the species composition of macrophytes than to the species richness or biomass of macrophytes. It is likely related to biological interactions between polychaetes and different macrophyte assemblages. On the rocky intertidal shores, polychaetes use single layer and non-corticated macrophytes (*e.g. Cladophora* and *Ulva*) for food sources, and multilayer and corticated macrophytes (*e.g. Padina* and *Chondrus*) for shelters (Steneck and Dethier, 1994). Polychaetes need both types of macrophytes to survive in the rocky intertidal shores, and hence the complexity of macrophytes is always important for many intertidal invertebrates (Best *et al*., 2014; Torres *et al*., 2015; Martín *et al*., 2016). Indeed, this study showed that polychaetes were found more on the macrophytes composed by *Gracilaria salicornia*, *Chaetomorpha crassa*, and *Ulva lactuca*.

Compared to the taxonomic richness, the sample size of this study is sufficient to analyse the significant changes in the taxonomic composition of polychaetes. Hence, it can be suggested that the taxonomic composition is an efficient indicator to detect significant changes in polychaetes communities on rocky intertidal shores. It is likely because every taxon of polychaetes responds differently to the changes in macrophyte assemblages, and hence it does not need a lot of samples to detect those changes. Previous studies have also suggested that multivariate analysis (*e.g.* species composition) is more sensitive than univariate analysis (*e.g.* species richness and abundance) in detecting the significant effect of environmental changes because the relative abundance of each taxon is not compiled into a single dimension (Mueller *et al*., 2014).

Overall, this study provided some scenarios of taxonomic level and sample size required to detect significant changes in polychaete communities with a strong statistical power. Considering the significant level, the sample size, and the need of taxonomist, this study suggested that family-based data analysis is the best option in analysing significant relationships between polychaete communities and macrophyte assemblages on rocky intertidal shores. This agrees with many previous studies that family is a good descriptor to analyse the community structure of invertebrates (Olsgard *et al*., 1997; Thompson *et al*., 2003; Jimenez *et al*., 2010).

In term of sample size, it is true that the sample size of this study (*i.e.* 25 samples) is sufficient to detect significant changes in polychaete composition. However, 44 samples are probably a better size to gain a significant result and a strong statistical power at every analysis of polychaete variables. Hence, the recommended size is higher than the rule of thumb of sample size in the ecological monitoring (*i.e.* 30 samples) (Elzinga *et al*., 2001). It is likely because the data variation of this study may be higher than that of the common studies, so more samples are needed to reduce the variation within population. Hence, it supports Martnez–Abran (2014) that the analysis of data variation within population should be conducted before choosing a sample size or using the "n=30 rule of thumb". In fact, the rule of 30 samples is not appropriate for some analyses in this study due to low statistical power. Compared to world-wide studies, the recommended size is much higher than that of other studies which is around 10-19 samples (Abbiati *et al*., 1987; Giangrande, 1988; Pabis and Sicinski, 2010), but still lower enough than studies in Hawaii choosing 48 samples (Magalhaes and Bailey-

Brock, 2014) and Mediterranean region using 54 samples (Giangrande *et al*., 2003).

One limitation of this study is that the sampling was only conducted during a dry season, while Indonesia including the study area generally has three seasons: dry, transition, and wet season. The distribution of macrophytes on rocky intertidal shores shows a seasonal variation (Bellgrove *et al*., 2004; Masi *et al*., 2009; Marcías *et al*., 2017) so it may affect the diversity and abundance of polychaetes. Hence, future studies should compile polychaete and macrophyte data that are collected from whole seasons to obtain all those variations.

Regardless of the limitation, this study has provided a preliminary result on taxonomic level and sample size needed to detect significant changes in polychaete communities on rocky intertidal shores with a strong statistical power. Family level identification and 44 samples can be used sufficiently in the ecological monitoring of polychaetes on the rocky intertidal shores. Habitats on rocky intertidal shores changes rapidly due to ocean warming (Jueterbock *et al*., 2013), herbivory (Mayakun *et al*., 2010), pollution (Medina *et al*., 2005), and human activities (Bertocci *et al*., 2011). Hence, a rapid assessment with low investment and strong statistical result is needed, so the mitigation in respond to these changes can be proposed immediately.

CONCLUSION

Family-based data analysis is sufficient to detect significant changes in polychaete communities over different macrophyte assemblages. Unfortunately, most statistical powers of those analyses were relatively weak, although the strong one was detected in the effect of macrophyte composition. In order to gain significant results with a strong statistical power, family-based data analysis should select a 44 sample size.

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