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Research Article

## The Use of Multivariate and Graphical Methods in Assessing Environmental Disturbance: Temperate versus Tropical Regions

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**Abstract:** This study is aimed to assess the effectiveness of multivariate analyses and distributional/graphical techniques in assessing the response of macrobenthic assemblages to environmental disturbance caused by fish farming, comparing temperate and tropical regions. The biotic data were used from samples taken under southern blue-fin tuna farms in southern Spencer Gulf, South Australia, and under fish impoundment at coastal region of Demak District, Central Java, Indonesia. Principal Component Analysis (PCA) and Non Metric Multi-Dimensional Scaling (NMDS) were used for analysis the abiotic and biotic data, respectively. Abundance-Biomass Comparison (ABC) curves were employed to detect the level of disturbance. The results showed that the use of multivariate analyses and distributional/graphical techniques are effective to assess the severity of disturbance, owing to sensitive response of macrobenthic assemblages to environmental disturbance that made it possible to detect the effects of farming activities. The ordination of the MDS and ABC curves showed consistently with

*W* statistic and *H'* index values in assessing the status of the area in both tropical and temperate regions. They clearly separated the disturbed and undisturbed areas, whether in spatial and temporal, thus the methods may be applied for the data taken from temperate and tropical regions.

Keywords: macrobenthic assemblages, ABC curves, multivariate analyses, graphical method, environmental disturbance, fish farming

## INTRODUCTION

The environmental impact of aquaculture activities and their recovery process have been observed using macrobenthic assemblages by several authors<sup>1-5</sup> and used as environmental quality criteria for managing marine aquaculture in several countries, i.e. Japan<sup>6</sup>, Tasmania-Australia<sup>3</sup>, Norway<sup>7</sup>, and Hong Kong<sup>8</sup>. However, the results had been inconsistent and depending on the environmental variables and farming practices.

Marine benthic organisms have a variable sensitivity to disturbance. Therefore, an increasing level of disturbance may either decrease or increase diversity or it may even remain the same<sup>9</sup>, a condition that may not be easily detected by univariate statistical techniques. It has been suggested that univariate diversity measures are not consistent in response to environmental stress<sup>10</sup>. In order to assess the rates and degree of environmental disturbance, multivariate techniques are considered more sensitive in detecting community changes than univariate techniques<sup>11</sup>.

Graphical methods, such as ordination Non-metric Multi Dimensional Scaling (NMDS) and abundance biomass comparison (ABC), have been introduced and applied to assess the level of environmental disturbance<sup>11</sup>. The method has been illustrated initially in a graphical model by Ref.<sup>12</sup> in assessing the changes in numbers of species, abundance, and biomass (SAB) in response to organic enrichment applicable to all habitats where organic enrichment occurs. In theory, the macrobenthic assemblages under stable conditions or low level disturbance are competitively dominated in biomass but not in numbers by conservative species, which have a so-called "K-selected" life history characterised by large-body size and long life-span. In disturbed areas, macrobenthic assemblages are dominated in numbers but not in biomass by "r-selected" organisms or opportunistic species characterized by small-body size and short life-span, high reproductive potential and early maturation<sup>13</sup>. Depending on the level of disturbance, the biomass curve may lie above the abundance curve (for undisturbed areas) or under the abundance curve (for heavily/grossly disturbed areas), or they may be closely coincident for their entire length or may cross each other one or more times (for moderately disturbed areas)<sup>10</sup>.

Ordination is considered useful techniques for recognizing and summarizing the pattern present in community data of many species/taxa and many samples<sup>14</sup>. In particular, NMDS has been widely used as it is conceptually simple and sensitive technique for detecting differences in community structure that is generally applicable to a wide variety of data<sup>10</sup>.

## MATERIALS AND METHODS

**Sampling procedures:** Samples of macrobenthos were taken from sediments using HAPS corer under southern blue-fin tuna farms in southern Spencer Gulf, South Australia, representing a temperate region as Location I, and under fish impoundment at coastal region of Demak District, Central Java, Indonesia, representing tropical regions as Location II. Each location had a reference

site, which was at least 1 km away from farming site. Samples were taken from three stations for each location with four replicates. Physico-chemical parameters of water were measured *in situ*.

**Laboratory procedures:** Samples were then preserved with 4% formalin solution. Laboratory procedures include sieving, sorting, counting and identifying the fauna that has been preserved in 70% ethanol. Ash free dry weight (AFDW) analysis was carried out for biomass measurement. The biotic datas recorded for further analysis were abundance and biomass of macrobenthic animals.

**Data analyses:** The rates and degree of recovery were assessed and graphically illustrated by distributional technique using multivariate analysis using Non-metric Multi Dimensional Scaling (NMDS) plots. This provided more information on the time required for recovery, the level of environmental disturbance and patterns of benthic recovery in response to organic loading from fish farming, which are taken from both temperate and tropical regions. The changes in the dominance pattern of macrobenthic assemblages based on both abundance and biomass were assessed using the Abundance/Biomass Comparison (ABC) method<sup>15</sup>. The ABC method was used to determine a shift in the proportions of different phyla and in relative distributions of abundance and biomass among taxa between control sites and fish farmings sites, and over time. The '*W*' plotted in graphs is Clarke's *W* statistic describing the degree and direction of separation of the curves<sup>16-17</sup> and is calculated as shown in a formula (1):

$$W = \sum_{i=1}^S \frac{(B_i - A_i)}{[50(S-1)]} \quad (1)$$

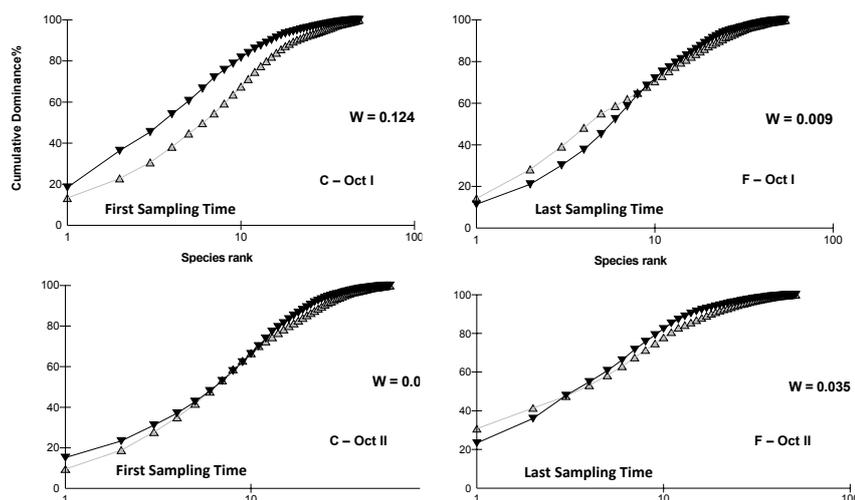
where *S* is number of species, *A<sub>i</sub>* is abundance of species *i*, and *B<sub>i</sub>* is biomass of species *i*. The value of *W* is in the range between -1 and +1, where *W* → +1 for even abundances across species but biomass is dominated by a single species (undisturbed), and *W* → -1 in the converse case (severely disturbed)<sup>10,16</sup>.

## RESULTS AND DISCUSSION

**Abundance-Biomass Comparison (ABC) curves:** At Location I, the results of the ABC analyses from control sites and fallowed sites of the temperate region for the first sampling time and the last sampling times in a year period are shown in **Fig 1**. At the beginning of the study, the biomass curve lies above the abundance curve for its entire length at the control sites, indicating an undisturbed area. Conversely, the abundance curve at the fallowed sites lies above the biomass curve from the starting point to the middle of the curves where they cross, indicating a moderately disturbed area<sup>18</sup>. At the reference site for beginning of the sampling time, the abundance curve lies below the biomass curve, indicating undisturbed areas. However, by the end of sampling time, the curve of biomass intercepts the abundance curve from the starting point to the middle where they cross to the end of the curve, indicating a moderate pollution. At the fallowed farm sites, the biomass curve intercept the abundance curve both from the beginning of the sampling time throughout the end of the study, indicating moderately disturbed areas by Ref<sup>18</sup> criterion. These results imply that a constant downstream flux of particulate organic matter produced by fish farming activities may be generated in such conditions.

The moderate level of disturbance at control sites by the end of the study time at Location I may be because of the extension of impacts of farming to the control sites, because the current velocity in the study site is relatively strong. Thus, hydrodynamic conditions with a relatively strong current velocity

may affect the dispersion of particulate matter, and cause an extended zone of impact. These results are in accordance with those of Ref.<sup>19</sup> in the Gulf of Castellammare, Mediterranean, where fish farm facilities generated an organic enrichment of the water column extended at least 1000 m downstream from the cages. The mean water current velocities throughout the year in the Gulf of Castellammare are about 10 - 12 cm s<sup>-1</sup>, which is similar to the 8 – 10 cm s<sup>-1</sup> currents in the southern Spencer Gulf. Conversely, the fallowed farm sites have been moderately polluted throughout the sampling period indicated by the abundance curve that intercept the biomass curve, both in the first and the end of sampling time.

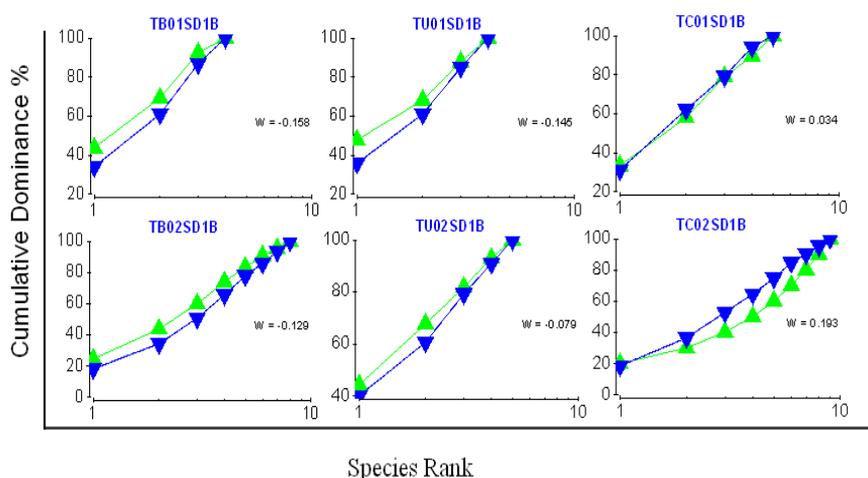


**Figure 1:** The ABC curves at control and fish farming sites plotted, comparing the first sampling period and the end of sampling time over a full year assessment at the temperate region. The curves were projected based on data transformation  $\log(X+1)$  of abundance ( $\blacktriangle$ ) and biomass ( $\blacktriangledown$ ) of macrobenthos.

Given that the environmental variables and benthic assemblages generally showed differences between the two sites, and diversity and evenness at control sites were considerably higher than at fallowed sites, the abundance of dominant taxa is more likely to be the main factor affecting the similar level of disturbance at both sites. The proportions of dominant taxa (which are all polychaetes) at both sites are the highest compared to the other macrobenthic taxa, suggesting that the contribution of these taxa to the level of disturbance, using ABC curves, was substantial. Most of them are considered as opportunistic species, which have small-body size, and grow rapidly in response to a disturbance, especially organic enrichment<sup>9,12,13</sup>. Ref.<sup>2</sup> found that, there was evidence of macrobenthic recovery fifteen months after fallowing. However, opportunistic species were still dominating, suggesting that the areas were moderately to slightly disturb at the end of the study. Response of opportunistic species may be due to the excess of organic matter in the sediment caused by fish farming. Most opportunistic taxa are categorized as sub-surface deposit feeders (SSDF), thus the more number of SSDF may indicate the higher level of disturbance<sup>20</sup>. Ref.<sup>21</sup> recorded decreased species richness under salmon farms, characterized by the dominance of small body sized macrobenthic fauna, with increasing proximity to the farm sites. Similar patterns of macrobenthic succession under three different fish farms were reported Ref.<sup>22</sup> In this study, statistical analyses showed that there were no significant differences in the abundance of the dominant taxa between

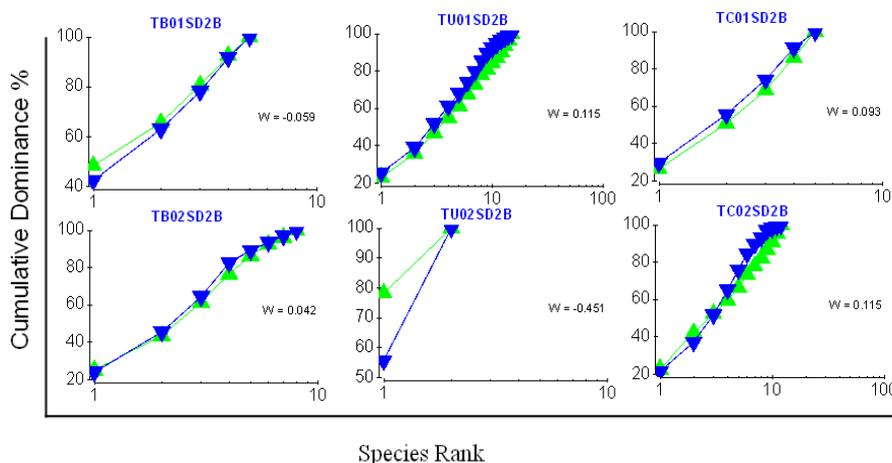
control and fallowed sites at the end of sampling period, leading to similar patterns of the ABC curves between control and fallowed farm sites.

At Location II, based on the criteria proposed by Ref<sup>10</sup>, all the sampling stations on the sampling time I (July 2009) is categorized as a disturbed/ polluted area, except station TC01SD1B (mixed ponds; inlet) and station TC02SD1B (mixed pond; outlet). These stations were used as mixtures to obtain the flow of water directly from the River Ronggolawe. The river is still affected directly by the tidal wave, so the quality of water used is still relatively good. While the input water for shrimp and milkfish ponds are from mixed pond, so that water quality has been affected by the activity of farming in the mixed pond. Unlike the sampling time I, the curve generated from the analysis of abundance and biomass of macrobenthos projected as ABC curve of the tropical region showed variability between stations (Fig. 2).



**Figure 2:** Abundance Biomass Curves Curve (ABC) is projected based on data transformation  $\log(X + 1)$  of abundance ( $\blacktriangle$ ) and biomass ( $\blacktriangledown$ ) macrobenthos in the first sampling time at the tropical region.

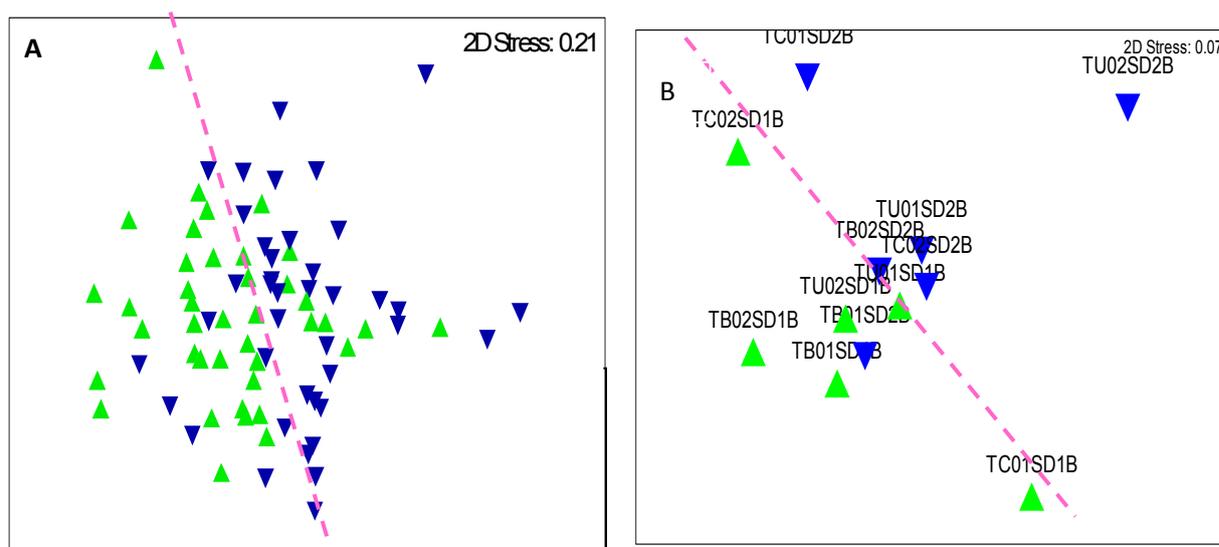
Each station on the sampling II (October 2009) of Location II is categorized as undisturbed areas (unpolluted), except station TB01SD2B (milkfish ponds; outlet) and station TU02SD2B (shrimp ponds; outlet) (Fig. 3).



**Figure 3:** Abundance Biomass Curves Curve (ABC) is projected based on data transformation  $\log(X + 1)$  of abundance ( $\blacktriangle$ ) and biomass ( $\blacktriangledown$ ) of macrobenthos in the second sampling time.

This indicates that the two stations above have been disturbed in October's samples. Based on the results of interviews with the owner/manager of shrimp farms (personal communication), the condition of shrimp pond a week before sampling II (early October 2009) has been environmentally disturbed, resulting in most of the stocked shrimp died (more than 70% of total population). It is not yet known the cause of the disturbance, but is expected related to the quality of feed and less aeration in the pond. Poor feed quality will reduce or even eliminate appetite of the animals. This can cause the accumulation of feed into body water and partially decomposed into sediment. The presence of high organic matter can trigger the growth of toxic microalgae and pathogenic bacteria in the waters, so it can result in lowered resistance of the cultured animals against the diseases.

**Multi-Dimensional Scaling (MDS) plots:** At the temperate region (Location I), ordination plots show separation between control and fish farming sites (Fig. 4A). This separation indicates a difference in assemblage composition between the two sites over the sampling period but with overlap. The sediments at fish farming sites had more silt and clay, and a lower proportion of coarse sands than control sites over the entire sampling period.



**Figure 4:** Ordination of *Non-Metric Multidimensional Scaling (NMDS)* projected based on  $\log(X + 1)$  transformed data of categorised according to: (A) Temperate region-sampling site :  $\blacktriangle$  reference sites;  $\blacktriangledown$  Fallowed farm sites; (B) Tropical region-sampling time:  $\blacktriangle$  Sampling I,  $\blacktriangledown$  Sampling II.

Based on data from tropical region (Location II), macrobenthic abundance varied between stations (spatially) and sampling time (temporally). Results from ordination (NMDS) showed no grouping if it was projected by fishpond types. On the contrary, the ordination based on the sampling time indicates grouping of sampling stations I and II sampling (**Fig. 4B**). These groupings may be due to differences in composition and abundance of species found between the two sampling time.

Overall, the ordination techniques showed the degree of variability between stations was spatially and temporally high. The shifts in direction through time shown in the MDS plots for certain sites were due to the changes of abundance and number of taxa throughout a year, with opportunistic taxa

(mostly surface and sub-surface deposit feeders) dominating the assemblages. These surface-dwelling organisms have an “r-selected” life history characterised by a small-body and a rapid life cycle, high reproductive potential and early maturation, and are tolerant of a wide range of environmental stressors, including hypoxia<sup>13</sup> The domination of deposit feeders, such as capitellids, spionids, and cirratulids, in moderate levels of organic enrichment has been reported<sup>8,23</sup>.

Shifts in the direction of the macrobenthic structure over the sampling period plotted on the MDS showed a major recovery or further disturbance, though this varied with site. In particular, a major recovery may occur at site P06 as the site shifted from the upper-right to the middle of the configuration, owing to similarity of taxa composition to those at control stations by the end of the study.

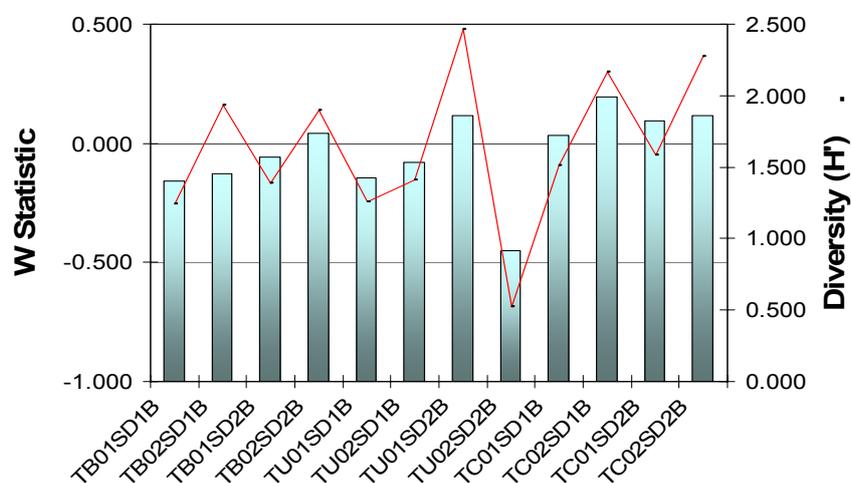
**Table-1:** The Selected Control and Fish Farm Sites Classified as Disturbed at Temperate Region (Location I).

No.	Site (Wstat) <sup>*)</sup>		Disturbance status
	Control	Fallowed	
1	-	P03 (0.119) P04 (0.075) P06 (0.031)	Moderately disturbed
2	RC1 (0.071) RC7 (0.085)	P01 (0.051) P02 (0.103)	Moderately disturbed
3	RC3 (0.045)	P01 (0.095)	Moderately disturbed
4	RC1 (0.075) RC3 (0.166) BC7 (0.094)	P01 (0.047) P04 (0.130) P08 (0.110)	Moderately disturbed
5	BC7 (0.163) RC3 (0.077) RC5 (0.119)	P01 (0.103) P04 (0.097) P08 (0.074)	Moderately disturbed

**Note:** <sup>\*)</sup> An index that describes the degree and direction of separation between abundance curve and biomass curve<sup>17</sup>.

Results from the Wstatistic and Shanon-Wiener diversity index ( $H'$ ) showed high variability for each sampling time (**Fig. 5**) at Location II. The values of diversity index exhibited higher variability compared to the Wstatistic index. Although variability occurs, both values tend to be recorded consistently for each sampling station. In particular, Station TU02SD2B (shrimp ponds; second sampling time), that formerly was categorised as disturbed area using ABC curve (see Fig. 3), is in

accordance with both  $W$  statistic and  $H'$  values, indicating that the diversity at this station is low and disturbed area



**Figure 5:** Values of  $W$  statistic and Shanon-Wiener diversity indices ( $H'$ ) projected for each sampling station at tropical region (Location II).

## CONCLUSIONS

The great variability between stations within each sampling time in this study resulted in high variability in rates and degree of recovery. Recovery from disturbance is likely to be dependent on where the samples were collected, owing to organic decomposition caused by farming activities. The variability between sites within each sampling time caused complex patterns of infaunal structure. At Location I, particularly site P06, shows recovery from disturbance from moderately disturbed to undisturbed, in which the benthic structure became similar to the control sites (the MDS plots, the ABC curves and  $W$  statistics). Conversely, a further disturbance is suggested for site P01, BC7 and RC3.

At Location II, although variability occurs, both values tend to be recorded consistently for each sampling station. In particular, Station TU02SD2B (shrimp ponds; second sampling time) is categorised as disturbed area using the ABC curve (see Fig. 3), also in accordance with both  $W$  statistic and  $H'$  values, indicating that the diversity at this station is low.

Seasonal fluctuations caused by natural variability, hydrodynamic conditions, sediment characteristics, and organic matter is likely to be responsible for the observed changes of the assemblages over the study period. The use of multivariate analyses and distributional/graphical techniques are effective to assess the severity of disturbance, owing to sensitive response of macrobenthic assemblages to environmental disturbance that made it possible to detect effects of farming activities. The ABC curve, in particular, showed consistently with  $W$  statistic and  $H'$  index values in assessing the status of the area. Furthermore, the ordination of MDS at both tropical and temperate regions clearly separated the disturbed and undisturbed areas, whether in spatial and temporal. This means that the similarity of the patterns of macrobenthic assemblages over sampling

station and times is effectively detected at both region, thus the methods may be applied at temperate and tropical regions.

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