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SPATIAL AND TEMPORAL PATTERNS OF THE MACROBENTHIC ASSEMBLAGES IN RELATION TO ENVIRONMENTAL VARIABLES

*Sapto P. Putro**

Biology Study Program, Faculty of Mathematics and Natural Science, ,Diponegoro University
Semarang 50275, Central Java, Indonesia

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ABSTRACT

This study focuses on the effect of fallowing of southern blue-fin tuna farms in southern Spencer Gulf, South Australia, on macrobenthic assemblage comparing spatial and temporal patterns of distribution and abundance at eight control sites and eight fallowed pontoon sites, during the period October 2002 to October 2003. Two stations at each site were sampled five times throughout the year with four replicates. Polychaetes were the most abundant organisms both at control sites (76.4%) and fallowed pontoon sites (80.5%). Five dominant taxa (Capitellidae, Cirratullidae, Lumbrineridae, Nephtyidae, and Spionidae), relatively tolerant to organic enrichment, were generally recorded in higher numbers at the fallowed sites than at control sites.

Assessed using univariate and multivariate analyses, a significant difference in abundance between the control and fallowed pontoon sites was found, which also showed a significant effect of time. A slight decreased in diversity, number of taxa, and evenness at fallowed pontoon sites compared to those at control sites was observed. Seasonal fluctuations caused by natural variability, especially hydrodynamic conditions and sediment characteristics, are likely to be responsible for the observed changes of the assemblages.

Keywords: Fallowed fish farms; macrobenthic assemblages; Polychaeta; opportunistic taxa.

***) Correspondence**: ; Phone: +62.24.70799494; Fax: +62.24.76480923; e-mail: saptoputro@yahoo.com

INTRODUCTION

The structure of macrobenthic assemblages vary at different spatio-temporal scales (Chapman, 2002; Ysebaert and Herman, 2002; Dauvin *et al.*, 2004), and thus need to be understood in order to identify the processes that govern ecosystems (Levin, 1992), and to measure the potential effects of environmental disturbance (Chapman, 2002; Hernandez-Arana *et al.*, 2003). An environmental disturbance, whether natural (Hernandez - Arana *et al.*, 2003; Aller, 1997) or anthropogenic (Karakassis *et al.*, 2000), may change the pattern of macrobenthic assemblages spatially and temporally in taxa richness, abundance, and biomass. The changes may be used as an indicator of "community" disturbance and provide an assessment of the levels of disturbance (Pearson and Rosenberg, 1978; Rosenberg, 2001). A shift in the proportions of different phyla, changes in relative distribution of species abundance and biomass with increasing levels of disturbance, reduction in trophic complexity, and increase in densities of opportunistic taxa are some of the benthic responses to a disturbance (Pearson and Rosenberg, 1978; Flemer *et al*., 1999; Warwick *et al*., 1990; Hernandez-Arana *et al.,* 2003; Norkko *et al*., 2006). Sediments organically enriched by fish farming, are dominated by small opportunistic species whose size decreased with proximity to the farm (Weston 1990).

In soft-sediment environments, spatial variability in the richness and abundance of macrobenthic fauna has been observed (Dauvin *et al.,* 2004), with a high degree of variability at the smallest temporal and spatial scales (Chapman, 2002). Temporal variability in the distribution and abundance of macrofauna caused by environmental disturbance has been recognized in both short-term (Livingston, 1987; Warwick and Uncles, 1980; Morrisey *et al.,* 1992) and long-term studies (Brooks *et al.,* 2004; Fraser *et al*., 2006; Macleod *et al*., 2004). Thus, understanding spatial and temporal distribution and abundance has become essential part of ecological research on benthic "communities" (Dauvin *et al*., 2004). Environmental variability is believed to play a major role in the change of structure expressed by variation in species richness, abundance, and biomass (Ysebaert and Herman, 2002). It has been suggested that spatial distribution of organisms in soft-bottom habitats is mainly controlled by both biotic factors (eg. life history, competition, predation) and abiotic factors (eg. food availability, sediment characteristics, tidal current) (Warwick and Uncles, 1980; Thrush, 1991; Rosenberg, 2001; Ysebaert *et al*., 2003; Cusson and Bourget, 2005).

The structure of macrobenthic assemblages has been used as an environmental quality criterion for managing marine aquaculture (Pearson and Black, 2001). However, their distributional patterns at different spatio-temporal scales are still neglected. The relationship between temporal and spatial variability of environmental variables and biological patterns are thus poorly understood (Ysebaert and Herman, 2002). Furthermore, most macrobenthic studies are from soft muddy sediments in different parts of the world (Kempf *et al*., 2002; Crawford *et al*., 2003; Mistri *et al.,* 2002; Rosenberg *et al.,* 2002; Constable, 1999), not really necessary comparable to the environments of the southern Spencer Gulf, South Australia, where southern bluefin tuna farms are located. As a consequence, only little information is available about spatial and temporal structure of macrobenthic assemblages in coarse sediments under fish farms. In this study, the changes in the dominance pattern of the abundance and biomass as an indicator of community disturbance are discussed. Abundance, diversity, evenness, and taxa richness between control and impacted sites are compared.

MATERIALS AND METHODS

The study sites

The sampling sites were located between 135^o 58.25' to 135^o 59.82' E and 34^o 35.41' to 34^o 42.43' S, in southern Spencer Gulf, South Australia, where farming of southern bluefin tuna (*Thunnus maccoyii*) takes place **(Fig 1).** The farms consist of a series of pontoons 40-50 m in diameter, with a 15 m deep net. Pontoons are stocked at rates of 1.5 – 2.5 kg m^{-3} (Cheshire *et al*., 2005). They are situated in areas with relatively strong microtidal (<2 m) currents with an average current velocity of 5-10 cm s^{-1} (Petrusevics, 1993; Bierman, 2005). The seawater temperatures fluctuate from 14^{o}C in winter to 25^{o}C in summer (Edyvane, 1997) The first sampling 2002 was sampled in October 2002 when farming season started. Fallowed sites were sampled after all fish and pontoons were removed and their coordinates were recorded.

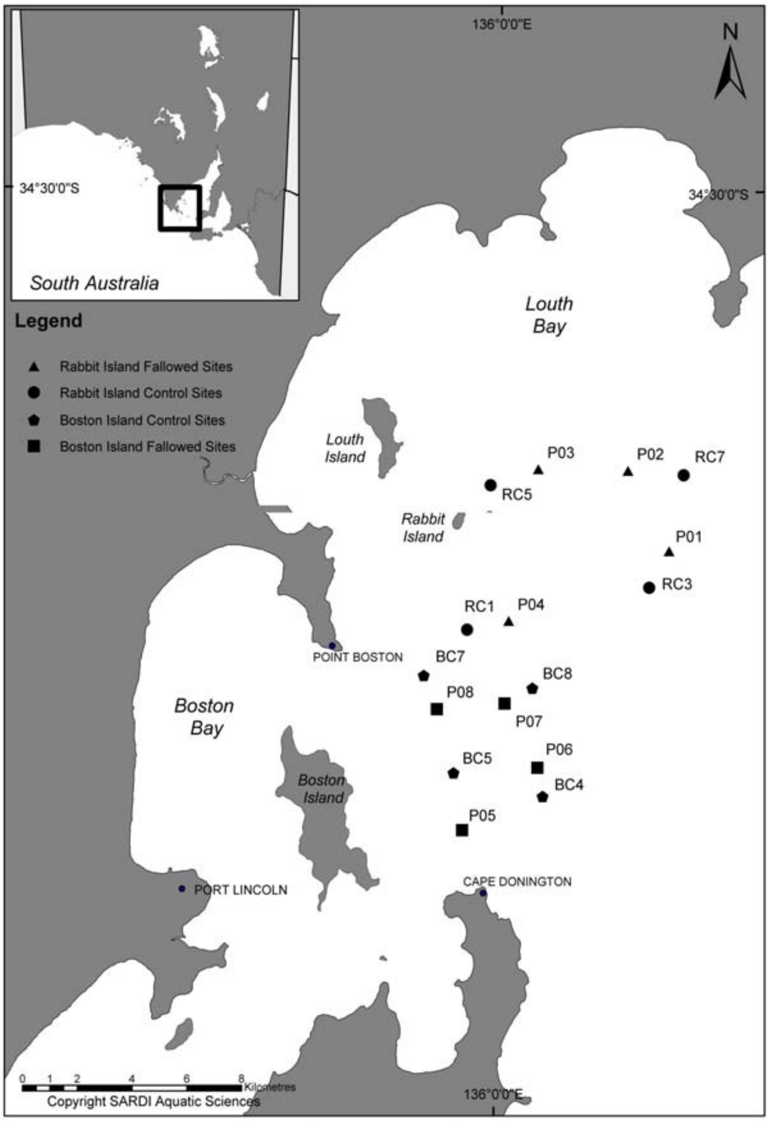

Fig. 1. Map of sampling stations representing eight control sites and eight fallowed pontoon sites adjacent to Rabbit Island and Boston Island. P= pontoon site; C= control site; B=Boston Island; R=Rabbit

Control sites were at least 1 km from any leased site.

Sampling procedures

Sediment samples were taken using a HAPS bottom corer equipped with a corer of 67 mm in diameter and 315 mm in length, operated from the research vessel R/V Ngerin. From the two zones of sampling located adjacent to Boston Island and Rabbit Island, 160 cores were collected for each sampling time for benthic animals. At each fallowed pontoon site, eight replicated cores were collected within a fallowed pontoon area. At each of two sampling stations, 4 replicate cores were collected (64 cores in total) **(Fig 2).** The same sampling strategy was used for each control site (64 cores in total). Sediment core was taken at each station for sediment composition. In total, thirty two cores were collected for each sampling time for sediment grain size analysis. Sediment organic matter was analysed only for the first set of samples.

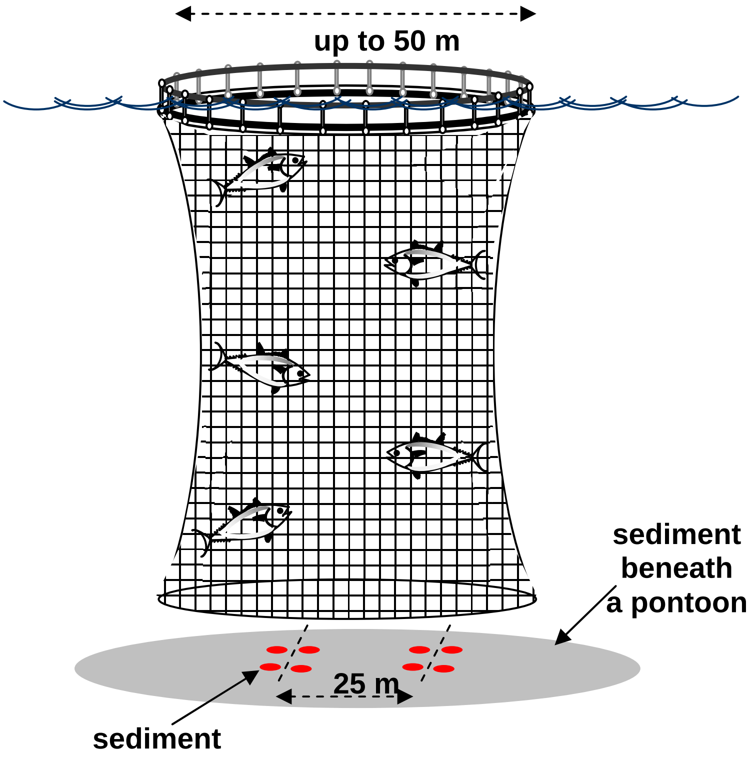

Fig. 2. Schematic design for sampling collection of sediment cores for benthic animals and sediment composition during the fallowing period. Fish and pontoons were removed and their coordinates were recorded before the start of the sampling program.

Samples were subsequently collected five times during the period from October 2002 to October 2003. The depth of sediment collected varied between 25 – 75 mm (mean = 40.9 mm) at control sites and between 22 – 85 mm (mean = 44.9 mm) at fallowed sites. Cores were collected at eight fallowed pontoon and control sites within the Rabbit Island aquaculture zone and the Boston Island aquaculture zone **(Fig 3).**

Laboratory procedures

Sediment samples taken in the HAPS corer were fixed in Bennett's solution and stored in 2 l plastic jars. The samples were then sieved through a 1.0 mm mesh.

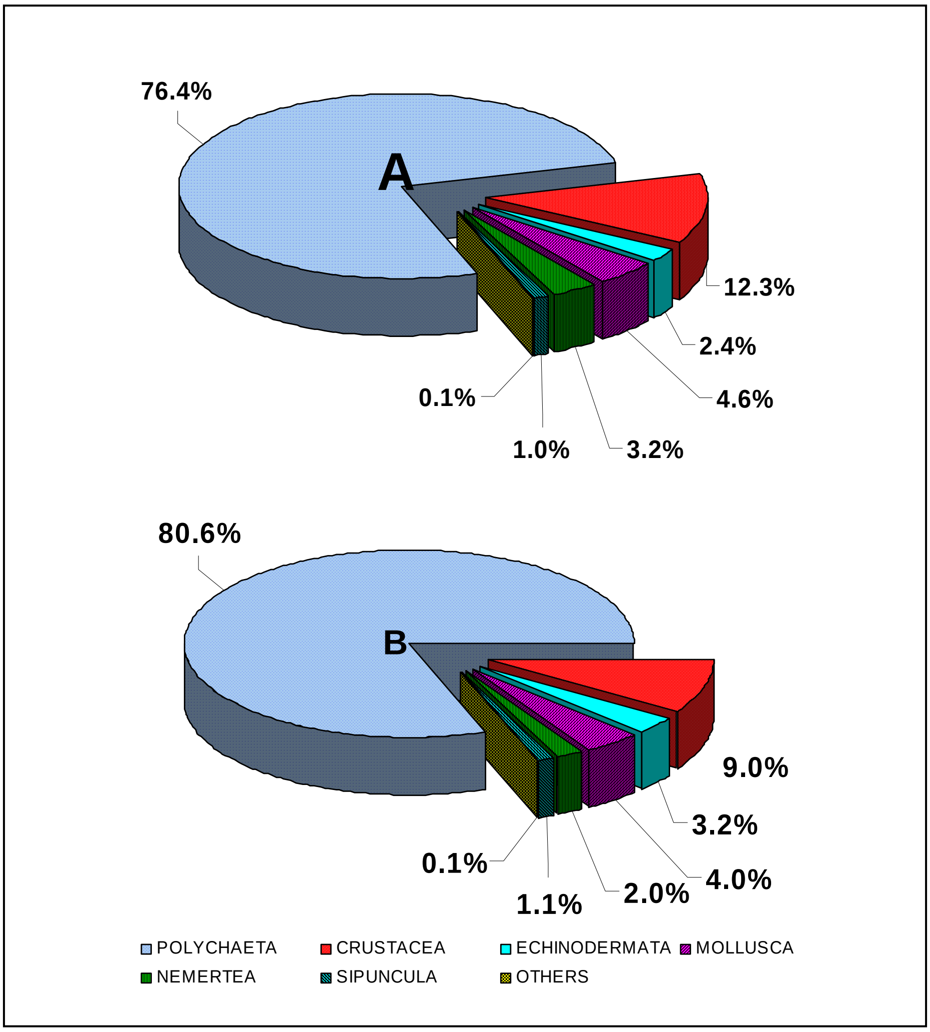

Fig. 3. The total proportion of major macrobenthic taxa at control sites (A) and fallowed pontoon sites (B) during the sampling period.

The macrobenthic animals from the sediment retained by the sieve were sorted under a binocular microscope. The sorted fauna was preserved in 70% ethanol for further analyses. Enumeration and identification of benthic animals were carried out at family level for polychaetes and bivalves. Other animals were identified to higher taxa.

Data analyses

Number of taxa (S) and Margalef's index were used to compare taxon richness between sites and times. The diversity of the macrobenthic assemblages was analysed using Shanon-Wiener index (H'). Pielou's evenness index was used to express equitability. All indices were presented graphically as plots of means and 95% confidence intervals within sampling times. One-way analysis of variance (ANOVA) was used to compare the differences in macrobenthic abundances between sites. As replicate samples from each sampling site exist, and normal distribution and constant variance across the groups are tested, two-way ANOVA was used to test the differences between sites and sampling time for all diversity indices (Clarke and Warwick, 2001). Kolmogorov-Smirnov's test and Levene's test were employed to test for normal distribution of data and homogeinity of variances. Further tests using Tukey's HSD post hoc for multiple comparisons were carried out if results revealed significant differences between treatments ($p < 0.05$).

A standard multiple regression was used to assess the environmental variables affecting the macrobenthic abundance based on the transformed data. First, all transformed environmental data were included in the procedure. Any independent variable strongly correlated with another independent variable (Pearson correlation value > 0.7) was excluded to avoid the violation of the multicollinearity assumption, while others were retained in the analysis (Tabachnick and Fidell, 2001). Outliers were checked by inspecting the values of Mahalanobis distances and scatterplot produced by the multiple regression program (Pallant, 2004). The coefficient of determination (r^2) was calculated to assess how much the level of the variance in the benthic abundance is explained by the regression model. The statistical significance of the result was then assessed using ANOVA at the 95% confidence level. The contribution of each environmental variable to explaining the dependent variable (benthic abundance) was compared using Beta values at the 95% confidence level.

RESULTS AND DISCUSSION

General trends of macrobenthic structure

The macrobenthic assemblages were dominated by polychaetes (28 families) at both control and fallowed sites (**Fig. 3)** The proportion of Polychaeta at the control and fallowed pontoon sites was 76.4% and 80.5%, respectively. Other major taxa in the assemblages were Crustacea, Echinodermata, Mollusca, and Sipuncula. control and fallowed sites (**Fig. 3)** The proportion of Polychaeta at the control and fallowed pontoon sites was 76.4% and 80.5%, respectively. Other major taxa in the assemblages were Crustacea, Echinodermata, Mollusca, and Sipuncula. The second most abundant group of animals was the Crustacea, which was relatively more abundant at control sites by 3.3% compared to the fallowed sites. Seven families of bivalve molluscs were recorded during sampling period. Other phyla were relatively rare and varied little between fallowed and control sites.

During the sampling period, the number of individuals increased at control sites, while they decreased at fallowed pontoon sites from October 2002 to May 2003 **(Fig 4).** From May 2003 and onwards, however, there was a slight decrease in the number of individuals for the control sites, while those at fallowed increased slightly. Both sites increased in their numbers after July 2003. Because the trends between the first six months and the last six months were different, a two-way analysis of variance was used to assess the effect of site and time for each six-month period. In the first six months, the abundance at fallowed sites decreased while at control sites increased. Although the effect was small (partial eta squared= 0.043), the two sites were significantly different during this period ($F_{(1,383)}$= 16.919, p<0.001). However, the interaction effect was significant ($F_{(1,383}$=14.691, p<0.001) with moderate effect (partial eta squared=0.072). In the last six months, there was no significant difference between the control and fallowed sites.No significant effect of interaction was observed.

These findings can be interpreted in two ways. First, the tendency of decreasing abundance at fallowed sites at the first six months may indicate the impact of farming activities on the sediment structure during this fallowing period, thus influence the structure of the fauna. The recovery process of the assemblages may occur at fallowed sites over the last six months after the disturbance. Secondly, the temporal variability in the assemblage patterns over the study period may be caused by the variability of deposited organic matter

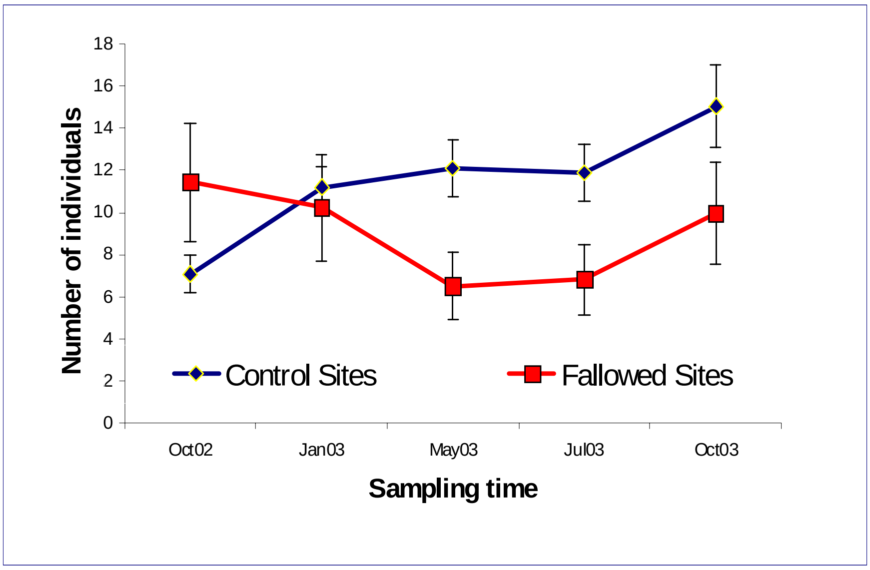

Fig. 4. Abundance of infauna per corer and 95% confidence intervals at control and fallowed pontoon sites over the sampling period.

availability in the forms of unconsumed pellets and faeces, because of whether variations and/or the difference in harvesting time of the farms, or organic dispersion by relatively strong water currents in this region.

The taxa richness, evenness, diversity, abundance of macrobenthic assemblages varied over time. Furthermore, there were differences in their patterns between the first six months and the last six months of sampling. In the spatial scales, the diversity indices were more variable at the fallowed sites than at the control sites owing to considerable differences in diversity and evenness between fallowed sites in the Boston- and Rabbit-Island zones. The results support the idea proposed by Warwick and Clarke (1993) that the increase variability is an indication of environmental stress/disturbance. It has been suggested that the increased variability might be due to an increase in the variability of abundances of the same set of species caused by disturbance and that due to changes in species identities (Warwick and Clarke, 1993}.

Taxon richness, evenness and diversity

Spatial variability

Fig 5 shows the spatial patterns of taxon richness, diversity and evenness of macrobenthic assemblages, representing eight control sites and eight fallowed sites in the Rabbit Island and Boston Island zones.

At the control sites, number of taxa between sites was not significantly different ($F_{(7, 40)} = 0.667$; p>0.05), and there was also no significant difference between zones (t = -0.44; p>0.05). However, at the fallowed sites, there were significant differences between sites ($F_{(7, 40)} = 4.425$; p = 0.002), with a large effect (partial eta squared = 0.492), explaining 49,2% of the variance. Post hoc comparisons using Tukey HSD test

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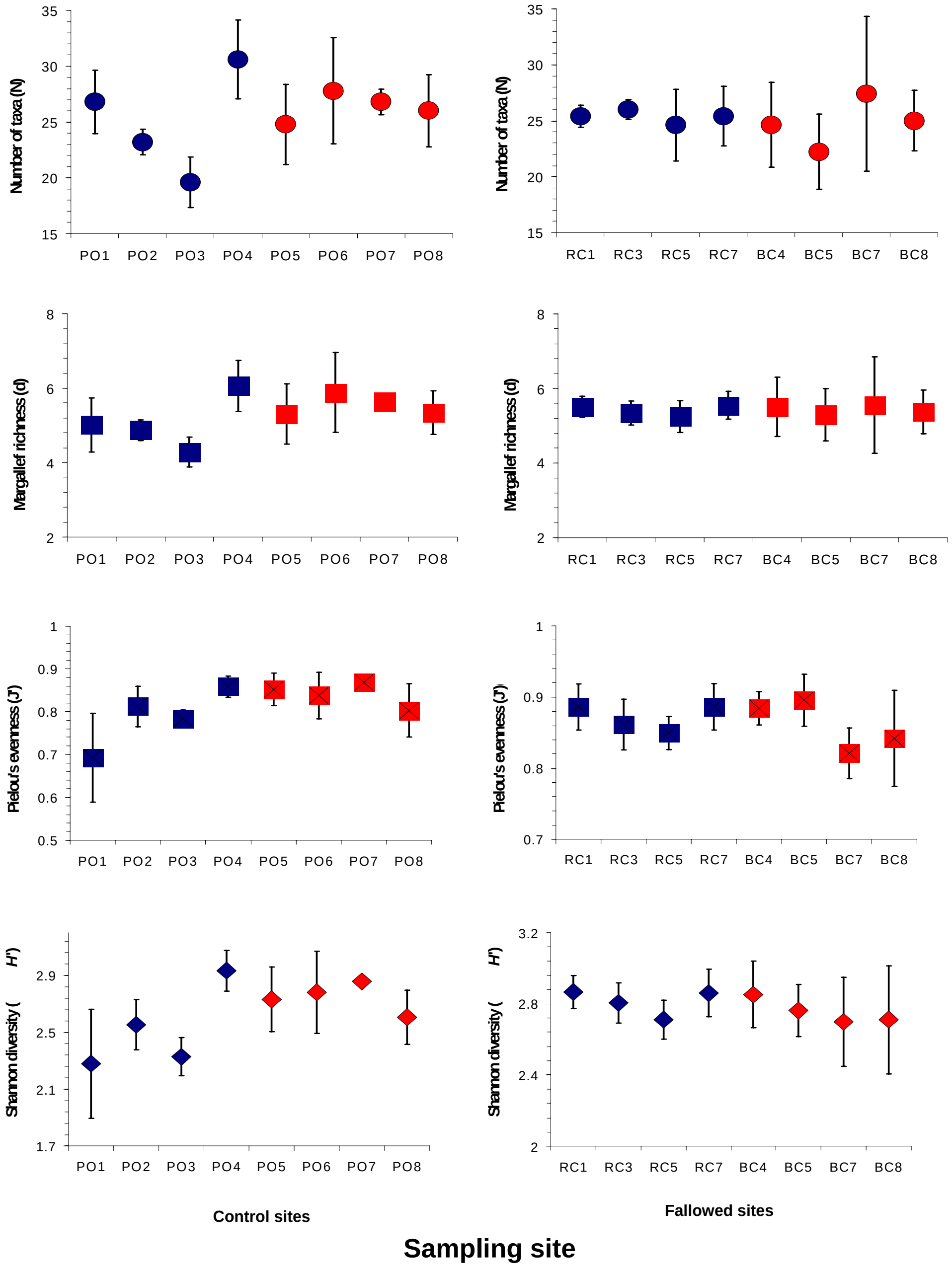

Fig. 5. Spatial patterns of taxon richness, diversity and evenness of macrobenthic assemblages averaged from five sampling times. Error bars displayed are 95% confidence intervals.

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P03 ($\overline{x}$ = 19.6, SD = 2.61); site P02 ($\overline{x}$ = 23.2, SD = 1.30) from site P04 ($\overline{x}$ = 30.6, SD = 4.04); site P03 from site P04, site P06 ($\overline{x}$ = 27.8, SD = 5.45), or site P07 ($\overline{x}$ = 26.8, SD = 1.30). However, no significant difference in the number of taxa was observed between zones (t = 0.927; p>0.05). Similarly results were obtained for other indices, i.e. Margalef richness, Pielou's evenness, and Shannon-Wiener diversity, with large magnitude differences.

Temporal variability

The diversity, evenness, and taxon richness at both control and fallowed sites differed at the five sampling times, but with high variability. At the last sampling time, higher values were observed for all indices at the control sites compared to the fallowed sites **(Fig 6).**

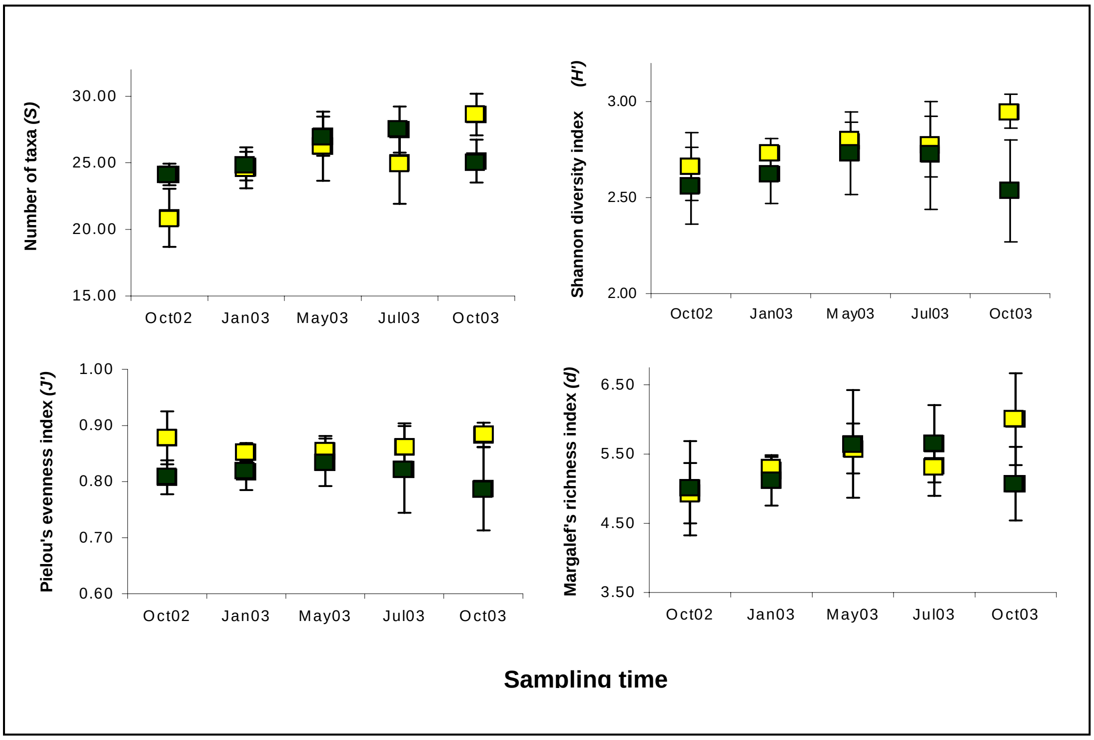

Fig. 6. Temporal patterns of taxon richness, diversity and evenness of macrobenthic assemblages generated from eight control sites (🟨) and eight fallowed sites (🟩). Error bars displayed are 95% confidence intervals.

In general, the number of taxa and Maragalef's richness index at both sites increased gradually throughout the investigated period. A two-way analysis of variance revealed no significant differences in Margalef's richness with site and time. However, the number of taxa varied significantly with sampling time ($F_{(4,\ 80)}$ = 3.594, p=0.01). Post hoc tests showed that the number of taxa varied significantly with the highest in May 2003 ($\overline{x}$ = 26.62, SD= 3.53) or October 2003 ($\overline{x}$ = 26.87, SD= 3.65) and lowest in October 2002 ($\overline{x}$ = 22.5, SD= 4.26). Shannon diversity was significantly higher at control sites than fallowed sites over the sampling period ($F_{(1,\ 80)}$= 6.129, p<0.05). However, no significant difference in diversity with time was observed. Likewise, Pielou’s evenness was significantly higher at the control sites than at the fallowed sites ($F_{(1, 80)}$= 12.640, p<0.001), but there were no significant differences between sampling periods.

The dominant taxa of the assemblages

Five polychaete families dominated the macrobenthic abundance during the study. They were the Capitellidae, Cirratulidae, Lumbrineridae, Nepthyidae, and Spionidae. The total number of individuals in each dominant family varied with time and site **(Fig7).**

The total abundances of the dominant families each sampling time were generally higher at fallowed sites than at control sites, except for the Nepthyidae that were slightly more abundant at control sites in January, May and October 2003. Capitellidae was the most abundant taxon at fallowed sites in October 2002 compared to the other sampling times, and was relatively more abundant at fallowed sites than at control sites over the sampling period. Cirratulidae was higher at fallowed sites than at control sites in all sampling times, except for the samples in July 2003. Lumbrineridae were more abundant at all sampling times with the highest values in October 2002.

Based on a two-way analysis of variance, the abundances of Capitellidae and Cirratulidae were not significantly different between sites and time of samples. The differences between Rabbit Island and Boston Island zones in both control and fallowed sites were also minor. However, the abundance of Nephtyidae varied significantly with sample time ($F_{(4, 160)}$ = 19.895, p < 0.001), with a large effect (Partial eta squared = 0.34). Post hoc tests showed that the number of taxa varied significantly with the highest in May 2003 ($\overline{x}$ = 26.62, SD= 3.53) or October 2003 ($\overline{x}$ = 26.87, SD= 3.65) and lowest in October 2002 ($\overline{x}$ = 22.5, SD= 4.26). Post hoc comparisons showed that Nephtyidae varied significantly with the highest abundance in January 2003 ($\overline{x}$ = 8.563, SD = 3.698) and the lowest abundance in October 2003 ($\overline{x}$ = 3.375, SD = 2.012). Although the effect size was moderate (Partial eta squared = 0.059), the difference between sites for Lumbrineridae was significant ($F_{(1, 160)}$ = 9.463, p = 0.002), and between zones ($F_{(3, 159)}$ = 4.499, p = 0.005). Spionidae showed significant different in time ($F_{(4, 160)}$ = 4.227, p = 0.003) with a moderate effect (Partial eta squared= 0.101), only between October 2003 ($\overline{x}$ = 16.219, SD = 23.165) and any of October 2002 ($\overline{x}$ = 4.563, SD = 4.983) or January 2003 ($\overline{x}$ = 5.813, SD = 5.619). Significant differences among zones were also observed ($F_{(3, 159)}$ = 6.378, p<0.001. Overall, no significant two-way interactions between sites and times for all dominant taxa were observed.

The five dominant taxa, which are the polychaete families: Capitellidae, Cirratulidae, Nephtyidae, Lumbrineridae, and Spionidae, were more abundant at the fallowed sites than at the control sites, suggesting more environmental disturbance occurred at fallowed sites. It is not surprising that the five dominant taxa at both sites are polychaete families, as some are known to be opportunistic species. Norkko (2006) observed that opportunistic species have life-history characteristics that allow them to respond quickly to disturbances. Their abundance during the early stages of succession is central to ecological models of benthic soft-sediment. The development of opportunistic responses can be influenced by the spatial scale or intensity of a disturbance.

Nonetheless, the composition of the dominant taxa in the sampled assemblage was spatially and temporally variable over October 2002 and decreased in numbers at the end of the study. Despite strong currents that may influence the availibity of organic matter over time, it is likely that this deposit-feeding family was efficient in decomposing organic matter generated

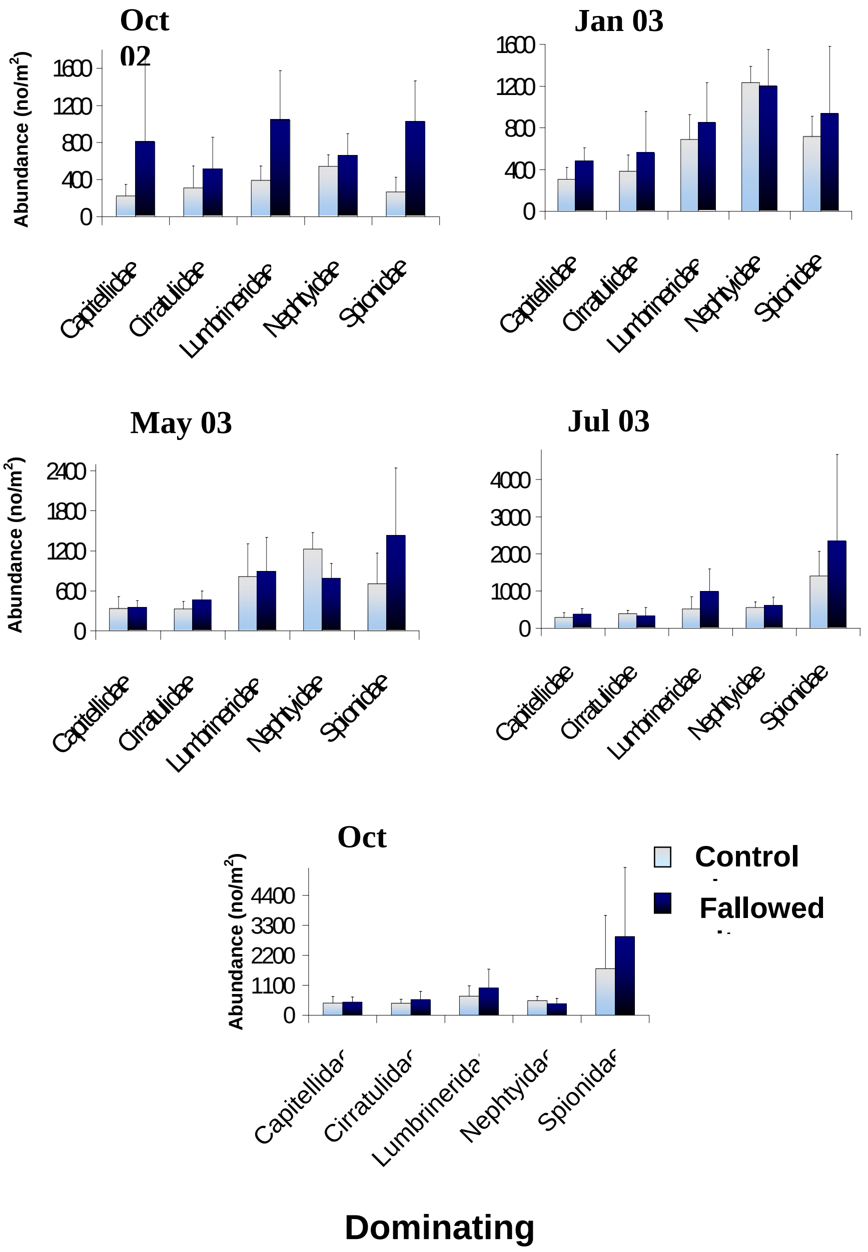

Fig. 7. Total number of individuals per m^2 per site with 95% CI of the dominating taxa for each sampling time, sampled during October 2002 to October 2003 at control and fallowed sites.

from fish farms as has been observed by Chareonpanich *et al.* (1994; 1994) using *Capitella sp.*, resulting the domination of this species at all observed sites/zones. As there is no clear trend found for nephtyids, it is suggested that they are an unresponsive to organic enrichment (Grall and Glemarec, 1997). This group is always present in relatively low densities with low variability over time. Therefore, it is reasonable that nepthyids were more evenly present at both sites than any other dominant taxa.

Although spatial variation may occur in any sedimentary habitat, most studies showed that polychaetes constituted the highest proportion of the benthic organisms in both muddy and sandy sediments, followed by molluscs and crustaceans. Cumaceans and amphipods dominate muddy sediments, whereas molluscs are likely to be restricted in their distribution. (Shin *et al.*, 2004; Quijon and Snelgrove, 2005; Dauvin *et al.,* 2004). Furthermore, Quijon and Snelgrove (2005) found that species associated with more than one type of sedimentary habitat were more broadly distributed than species associated with a single sediment type within a sub-artic fjord and across the region of Atlantic Canada.

Linking the assemblages to the environmental variables

The relationship between the biotic parameters (number of individuals, taxa richness, and diversity) and abiotic variables (hydrographic conditions, sediment chemistry and sediment grains size) was explored using a stepwise multiple regression. The best-fit models from the regression are shown in **Table 1.** Based on the biotic parameters, the spatial changes of the assemblages are explained mainly by changes in sediment grain size, particularly the proportion of clay and coarse sand, and the conductivity and turbidity of seawater. However, the variances in the three biotic parameters explained by the models were relatively small. Although all showed statistical significance (<0.0001), the models explain only 21.9%, 17.9%, and 21.7% of the variances in number of individuals, taxa richness, and diversity, respectively. Clay fraction is the strongest contribution to explaining the variance in diversity (β = -0.477) and taxa richness (β = -0.441), whereas coarse sand is best in explaining the variance in number of individuals (β = -0.369).

Environmental variability is believed to be the key to the changes in macrobenthic structure expressed by variation in taxa richness, abundance, and biomass (Ysebaert and Herman, 2002). It has been suggested that spatial distribution of organisms in soft-sediment habitats is mainly controlled by biotic (life history, competition, predation) and abiotic (food availability, sediment characteristics, tidal current) factors (Warwick and Uncles, 1980; Thrush, 1991; Rosenberg, 2001; Ysebaert *et al.*, 2003; Cusson and Bourget, 2005). Depending on habitat sampled, the main environmental factors affecting the distribution and structure of macrobenthic animals reported by most authors were food availability, particularly organic matter, salinity, current velocity, and sediment characteristics, especially mud or clay content, explained a significant part of macrobenthic spatial patterns (Pearson 1970; Pearson and Rosenberg, 1978; Rosenberg, 2001; Ysebaert *et al.*, 2003). Rosenberg (2001) reported that food availability influenced the structure of benthic assemblages characterized by number of species, abundance and biomass. Cusson (2005) observed that, in global patterns, the availability of food both in quality (assessed by C:N ratios) and quantity, temperature, depth gradients were the important variables affecting metabolism and growth of invertebrates.

The result of multiple regression showed that sediment characteristics, especially clay and coarse sand, are the most significant variables explaining the spatial variability of the assemblages. This may be due to high variability in food availability in sediments, thus affects the changes of macrobenthic structure.

The availability of organic matter varied, whether because of variations and/or the difference in harvesting time of the

Table 1. Best-fit models of the stepwise multiple regression analysis assessed from transformed number of individuals, taxa richness of Margalef index (*d*), and diversity of Shannon-Wiener index (*H*') of macrobenthic assemblages.

Predictor variables (*n* = 13)	Number of individuals		Taxa Richness (d)		Diversity (H')	
	Coefficient	***p*-value**	**Coefficient**	***p*-value**	**Coefficient**	***p*-value**
Constant	1.901	<0.0001	6.090	<0.0001	-49.885	0.042
Clay (%)	-0.284	0.003	-0.441	<0.0001	-0.477	<0.0001
Coarse sand (%)	-0.369	<0.0001	-0.405	<0.0001	-0.419	<0.0001
Conductivity					0.178	0.029
Turbidity	-0.255	0.002				
Regression		<0.0001		<0.0001		<0.0001
R-square	0.219		0.179		0.217	

farms, or organic dispersion by relatively strong water current velocity in this region. Furthermore, Blackburn *et al.* (1996) observed that the quality of organic matter (assessed by C:N ratio) was better in sandy habitats than in muddy sediments. However, the result from multiple regression showed that food availability (assessed as % organic carbon and total nitrogen) is not the main variable explaining the variability in the assemblages in this study, owing to a relatively small amount of organic matter recorded in the sediments at both control and fallowed sites. Although the excess of organic matter in sediments can decrease species richness, abundance and biomass, the impact of organic matter depends on its concentration. Total organic carbon in sediments has low impact on species richness only if its concentration less than 10 mg g^{-1}, but it has high impact at more than 35 mg g^{-1} (Hyland, 2005). In this study, the average concentration of organic carbon at control and fallowed sites were 0.3 – 0.7 % (3 – 7 mg g^{-1}) and 0.4 – 1.0 % (4 – 10 mg g^{-1}), respectively, in the levels that may have low impact on the assemblages. Thus, it is likely that sediment characteristics are more important in explaining the observed patterns rather than organic matter derived from fish farming.

CONCLUSIONS

a. Macrobenthic abundance was significantly different between the control and fallowed pontoon sites both spatially and temporally. The macrobenthic assemblages were dominated by polychaetes , followed by crustaceans, echinoderms, molluscs, and sipunculans.

b. Spatial and temporal variability of taxa richness, evenness, diversity, abundance of macrobenthic assemblages occurred. The diversity and evenness at the control sites were found to be slightly higher but less variable than those at fallowed pontoon sites, which is likely to be an indication of environmental disturbance. Sediment characteristics, especially clay and coarse sand, were the most significant variables explaining the spatial variability of the assemblages.

c. The tendency to decreasing abundance at fallowed sites during the first six months is more likely to be due to the direct impact of farming activities on the sediment characteristics. The recovery process of the assemblages may be started six months after the fallowing period. By the end of the study, however, the recovery process is still underway, as the areas are still moderately disturbed by the farming activities.

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