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Shift in Phytoplankton Community Structure in a Tropical Marine Reserve Before and After a Major oil Spill Event

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ABSTRACT: The present study reports the changes in the phytoplankton community structure in Taklong Island National Marine Reserve (TINMAR), Guimaras Island, Philippines. Quantification of PAH yielded undetectable results, whereas, primary productivity, phytoplankton density, and diversity values were higher as compared to samples before the oil spill and samples from the reference site. Sixty-nine genera representing 6 classes of phytoplankton were identified. Class distribution revealed that diatoms belonging to Coscinodiscophyceae, Bacillariophyceae and Fragilariophyceae were dominant in the area. Class Coscinodiscophyceae was the best represented class with 1,535 individuals/L seawater. The top-ranked diatom genera encountered were *Chaetoceros*, *Skeletonema*, *Thalassionema*, *Rhizosolenia*, and *Bacteriastrum*. The shifts in dominance of diatoms over dinoflagellates and fast-growing centric diatoms over pennate diatoms are indicative of a stressed phytoplankton community. Both the Simpsons (1/D') and Shannon-Weiner (H') values registered for the 2006 TINMAR samples (1/D':11.23; H':1.304) were higher than those obtained from pre-oil impacted samples (1/D':8.83; H':1.07) and samples from the reference site (1/D':8.798; H':1.039). The present findings provided information on the direct impact of a recent oil spill on phytoplankton community and demonstrate the suitability of using phytoplankton as bioindicators of environmental stress.

Key words: Phytoplankton, Oil spill, Marine reserve, Diversity, PAH

INTRODUCTION

In this present time and generation, oil spills remain to be a major threat to the stability of marine, freshwater, coastal, and estuarine ecosystems worldwide (Marshall and Edgar, 2003; Onwurah *et al*., 2007). Global inputs of petroleum hydrocarbons into marine ecosystems due to accidental oil spills and other events have been estimated to be in the order of 1,300,000 tonnes per year (NRC, 2003). Spill events are presumed to primarily affect aquatic invertebrates inducing both lethal and sublethal changes at the level of individuals that may often cascade towards population and community levels (Crowe *et al*., 2004; Otitoloju, 2010; Zahed *et al.*, 2010). However, apart from their very crucial role in the ecology of marine ecosystems, phytoplankton have also been demonstrated to quickly react to environmental stressors (El-Sheekh *et al*., 2000; Hjorth *et al*., 2007). Together with corals and macrophytic algae, phytoplankton provide the primary influx of energy to the coastal food web. In fact in some areas, these organisms can be responsible for over 50% of their primary production (MacIntyre *et al*., 1996). Perturbations to the phytoplankton community, therefore, could result in major havocs to marine ecosystems and have been of great interest (eg. Plante-Cuny *et al*., 1993; Suderman and Thistle, 2004). Understanding changes in planktonic photosynthetic activity, primary production, biomass accumulation and community assemblages could provide essential parameters to evaluate the hazard potential of aquatic pollution (Rakocevic-Nedovic & Hollert, 2005; Ekwu and Sikoki, 2006; Uttah *et al.*, 2008;). Previous studies (Batten *et al*., 1998; Edgar *et al*., 2003; Lee and Page, 1997; Suderman and Thistle, 2004; Varela *et al*. 2006) have identified three approaches that have been widely used for determining effects of oil in plankton communities. These include working with cultures of single species; applying mesocosms with natural phytoplankton assemblages; or using natural or *in situ* studies.

Taklong Island National Marine Reserve (TINMAR) is one of the most productive ecosystems

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in the Philippines. It is composed of Taklong and Tandog group of islands and surrounding islets covering an area of about 1,143.43 hectares and found within the municipality of Nueva Valencia, Guimaras Island (Fig. 1a). It was established primarily to protect the rich marine resources and to preserve the geological, scenic, scientific and educational features of the region. The island is home to white sand beaches, several marine sanctuaries, coral reefs and mangrove forests. However, the area became the focus of world-wide concern when a massive oil spill incident happened in August 2006 due to the sinking of M/T Solar carrying approximately 2 million liters of bunker fuel. Approximately two months after the oil spill incident, neither oil slicks nor oil sheens were noticeable in the area; however traces of adsorbed oil can be seen on rock surfaces and on prop roots of mangroves in the area.

The present study aimed to provide baseline information on the ecological and taxonomical aspects of the phytoplankton community in the Taklong Island National Marine Reserve (TINMAR), Nueva Valencia, Guimaras, Philippines before and after being impacted by the oil spill. Results from this study could serve as valuable inputs for the local government and various interested groups in their protection and conservation efforts of the marine reserve.

MATERIALS & METHODS

Water samples were collected from seven sampling stations, five of which have been previously determined following GPS plots of the 2001 Agriculture and Fisheries Modernization Act-Marine Fishery Reserves Program (AFMA– MFR) cruise around TINMAR while the other two stations which serve as reference sites were randomly selected 200 m off the coast of Brgy. Nagbuhi, Miag-ao, Iloilo (Fig. 1b). These sites have not been reported to be impacted by oil spill. Moreover, pre-oil spill samples of phytoplankton preserved in formalin dated September – October 2001 collected from AFMA-MFR cruise were made available and used as a reference sample (pre oil spill) for temporal comparisons of phytoplankton community only.

The study followed a descriptive research design aimed to assess the impact of the recent M/T Solar I oil spill in Taklong National Marine Reserve (TINMAR) Nueva Valencia, Guimaras, Philippines. The physical parameters measured include total nitrogen and phosphate content, polyaromatic hydrocarbon (PAH) content, dissolved oxygen and temperature while biological parameters measured include chlorophyll *a* concentration, primary productivity and phytoplankton community indices such as richness, diversity and evenness. Water samples were collected from seven sampling stations, five of which have been previously determined following GPS plots of the 2001 Agriculture and Fisheries Modernization Act- Marine Fishery Reserves Program (AFMA-MFR) cruise around TINMAR while the other two stations were randomly selected 200 m off the coast of Brgy. Nagbuhi, Miag-ao, Iloilo. The two Miag-ao stations served as the reference stations for spatial comparisons of phytoplankton community indices, chlorophyll *a* concentration, primary production and water quality analysis.

Seawater samples for physicochemical and PAH analyses were collected using improvised *Van Dorn* bottles lowered not more than 1 meter from the surface. For physicochemical analysis, constituted seawater samples were collected from the Miag-ao and TINMAR stations. They were all emptied and labeled separately into clean polyethylene bottles covered with aluminum foil, and kept refrigerated at a minimum temperature of 4ºC until analysis. The same sampling procedure was done for PAH analysis. However, two liters of seawater samples were taken from each station prior to constitutive sampling. Two liters subsamples were taken from each constituted samples of the Miag-ao and TINMAR stations. Subsamples for nutrient analysis were processed at the Chemical Oceanography Laboratory at the Marine Science Institute, University of the Philippines while subsamples for PAH analysis were treated at the Research and Analytical Services Laboratory at the National Science Research Institute, University of the Philippines.

Water samples for quantifying chlorophyll *a* concentration were collected using 250 milliliter improvised *Van Dorn* glass bottles entirely covered with aluminum foil. They were lowered into the water column not deeper than one meter from the surface and capped underwater. The samples were then stored in a cooler maintaining a temperature of at least 4ºC at transport and kept frozen until the next step. In filtration, the samples were poured into the Millipore Filtration equipment containing a 0.50 µm Gelman cellulose ester membrane filter. The samples were subjected under one-half atmospheric pressure vacuum to facilitate rapid filtration. Three to five drops of $MgCO_3$ solution were added for every sample being filtered to prevent acidity. The filter with the residue were then drained till dryness, removed and folded in half, backed with a piece of ordinary paper and fastened with a paper clip. The dried filter was immediately subjected to extraction and spectrophotometric (F2500 Fluorescence Spectrometer, Hitachi, Inc). For extraction, the filter was placed in fifteen milliliter centrifuge tubes with fifteen milliliters of 90% acetone.

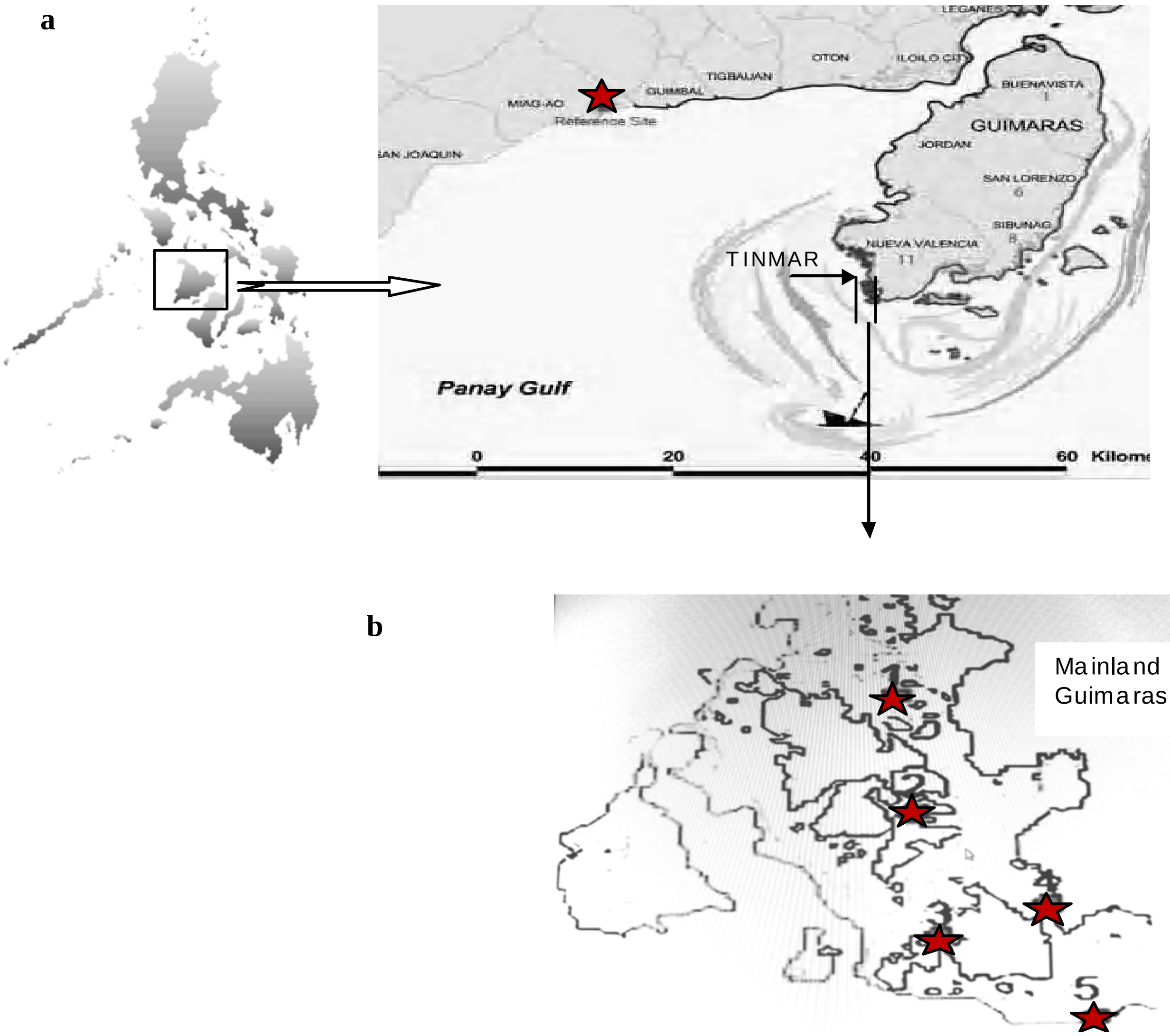

Fig. 1 (a) A map showing the location of Guimaras Island and Miag-ao, Iloilo, the reference site in the study and (b) A map of Taklong Island National Marine Reserve (TINMAR) showing the corresponding sampling stations used in the study

Then the tubes were shaken until the filter dissolved. The tubes were then allowed to stand overnight in a refrigerator for maximum chlorophyll extraction. In the analysis proper, the tubes were centrifuged at medium setting for 10 minutes and immediately decanted into 10-centimeter path length spectrophotometer cuvettes. Extinctions were then measured at wave lengths 750, 664, 647 and 630 nm. The extinction for a small turbidity blank was then corrected by subtracting 750 nm from the 664, 647 and 630 nm absorption readings. The amount of chlorophyll *a* in the original seawater was calculated. Pre-oil spill data were obtained from a previous study (Orbina, 2003).

For estimation of primary productivity, we used the light and dark bottle method developed by Adams (1990). Seawater was first filtered using a 200 μm sieve and temporarily contained in a clean pail. The light, dark and initial bottles each with a 250 milliliter capacity were then submerged in the filtered seawater, filled, and capped underwater. Primary productivity measures will then be estimated based on the measured DO of the three bottles using simple mathematical procedures. The Winkler-azide modification test reported DO in ppm O_2 (mg/L) and to be able to express these in values relevant to biomass and productivity, unit conversions were done. Pre-oil spill data were obtained from a previous study (Orbina, 2003).

Phytoplankton samples were collected not deeper than 1 m below the surface using a simulated pump technique. Fifty liters of seawater were passed through a 20 μm vertical plankton net with a 50 ml receiving container. Formaldehyde was added to the collected

50 ml phytoplankton samples emptied into amber bottles. The samples were stored in a cooler maintaining a temperature of at least 4ºC until microscopic observation. Phytoplankton cell counting was done in a Sedgwick-Rafter counting chamber. Two milliliters of the sample was dropped onto slide and three predetermined horizontal microtransect strips were followed. Microscopic observation was accomplished under phase contrast illumination at x200 and x400 magnifications of Leica™ microscope. Each organism encountered was photographed and documented until taxonomic identification. Individual cells represent the unit of phytoplankton density during microscopic examination. Phytoplankton occurring in colonies, filaments, and in pairs were counted as one cell as well as those undergoing cellular division. Other unique and distinguishable features such as theca or lorica that might suggest an organism's presence were considered when counting as long as they could be used to classify an organism down to its genus.

After counting and identification, different indices were used to interpret the data gathered. Species diversity (H') was calculated based on abundance and evenness of species after Shannon-Weiner and Simpson's (1/D'). Comparison of computed mean values was done on measurements of chlorophyll *a* concentrations and primary productivity of both study areas. Comparison of genera composition and distribution was accomplished using the Bray-Curtis Cluster Analysis. All statistical computations were done using Biodiversity Professional V.2 software.

RESULTS & DISCUSSION

Table 1 summarizes the physico-chemical parameters of 2006 TINMAR Stations and Miag-ao Stations. Pooled seawater samples of 2006 TINMAR stations revealed higher dissolved nitrite content (0.1041 µmol) and dissolved phosphate content (0.2063 µmol) than the pooled seawater samples from Miag-ao stations. On the other hand, dissolved nitrate (0.06 µmol) and ammonia (0.9826 µmol) for pooled seawater samples from Miag-ao stations were higher than that of 2006 TINMAR stations. Nevertheless, all values measured from both sampling sites were lower than the seawater standard. The PAH content of both water samples was less than the method detection limit set at 0.05 µg/L. No physicochemical data correspond to the 2001 TINMAR since we only obtained phytoplankton samples preserved in formalin. The negative detection for PAHs corroborated very well with ocular inspections we made. During the time of sampling (approximately two months after the oil spill), neither oil slicks nor oil sheens were observable on the sea surface. The only readily observable remnants of the oil spill were the adhered oil residues on rocks and mangrove prop roots. Similar result was reported in the 2002 Prestige oil spill in the coast of Galicia wherein despite the large release of fuel, relatively low concentrations were found in shelf waters in most of the area, even shortly after the wreckage (Gonzales *et al*., 2009). In both cases, the PAHs must have already undergone physicochemical transformations that may be due to the interplay of many processes including sedimentation of the heavy crude part, evaporation of the volatiles, rapid degradation of low molecular weight PAHs, and physical spreading via wind and water currents (Yamada *et al*., 2003; Garcia-Soto, 2004; Yumul and Cruz, 2006). Scientists from the Department of Science and Technology (DOST, Philippines) reported that the average oil slick displacement during August 11-13 was 50 km/day and 25 km/ day for August 14 – 18. During this time, the oil slick traveled north and affected the southern coastline of Guimaras including the study site. Oil slick displacement was further intensified by the southwest monsoon and by the two typhoons (Inday and Juan) which propelled the oil slick in an east to northeast direction away from the study site (Yumul and Cruz, 2006). PAHs were also reported to have high affinity to particulate matter and thereby readily transferred into the sediments after introduction to the marine environment (Den Besten *et al*., 2003). In the case of the MT/Solar oil spill, the nature of the spilled oil (No. 6 fuel oil) might have caused its rapid sedimentation (Suderman and Thistle, 2004). This type of oil was a residue after the lighter fractions of the oil have been removed by distillation or cracking. The University of Siliman Rapid Assessment Team reported that the type of spilled oil is not expected to be as acutely damaging to the water column as lighter oils (MLSU, 2006).

Table 1. Average values obtained from physicochemical analyses of pooled water samples from TINMAR and Miag-ao stations

Physicochemical Parameters	TINMAR	MIAG-AO
Temperature (°C)	31.2	30.0
Dissolved NO_3 (µmol)	0.0558	0.06
Dissolved NO_2 (µmol)	0.1041	0.0585
Dissolved NH_3 (µmol)	0.8839	0.9826
Dissolved PO_4 (µmol)	0.2063	0.1711
Polyaromatic Hydrocarbons (PAH)	ND	ND

*ND- not detected

A higher mean value for chlorophyll *a* concentration in TINMAR after the oil spill (0.857 mg Chl *a*/m^3) was obtained as compared to both the pre-oil spill data (0.774 mg Chl *a*/m^3) and to the reference site (0.684 mg Chl *a*/m^3) (Fig. 2). In a related microcosm study, Gonzalez *et al*. (2009) reported an initial decline in chlorophyll *a* concentration after oil contamination but a trend of higher chlorophyll *a* concentration towards the end in all set-ups when the PAH values have decreased. Similar trend was observed by Hjorth *et al.* (2007) concerning the chlorophyll *a* content in marine phytoplankton subjected to pyrene contamination. Furthermore, Fabregas *et al.* (1983) reported that at oil concentrations below 8 ppm, chlorophyll *a* content started to increase. These related works clearly support the result of our study wherein the measured PAH concentration after two months coincided with an increase in chlorophyll *a* concentrations in the water samples. Another worker, on the other hand, attributed the sudden increase in phytoplankton biomass, and subsequently chlorophyll *a* levels, to the decrease in zooplankton density which was greatly affected by the oil spill (Miller *et al*., 1978). Supplementing the trend in chlorophyll *a* values were the data on gross and net productivity. The TINMAR stations revealed higher productivity (GPP:71.82 mgC/m^3/hr, NPP: 61.06 mgC/m^3/hr) when compared to both the reference (Miag-ao) stations (GPP:29.4 mgC/m^3/hr, NPP:31.28 mgC/m^3/hr) and pre-oil spilled data (GPP:62.09 mgC/m^3/hr, NPP:57.35 mgC/m^3/hr) (Table 2). Other factors such as light intensity (ie. euphotic depth) (Khanna *et al*., 2009) and nutrient availability (Rakocevic-Nedovic and Hollert, 2005) may have also contributed to the outcome. Light was observed to penetrate well the water bottom in the TINMAR sampling as compared to the reference sampling site wherein light penetrated only part of the seawater surface. Dissolved nutrients such as nitrogen and phosphorous were also relatively higher in the pooled seawater samples from TINMAR stations compared to the reference samples.

Post-oil spill data also demonstrated higher mean productivity values than pre-oil spill data. The increase in primary productivity could be due to the stimulation of photosynthesis or to the decrease in zooplankton grazing (Barsdate *et al*., 1980; Gin *et al*., 2001; Varela *et al*., 2006). Though exposure to petroleum oil products has been previously linked to toxicity effects on photosynthesis (Wedding *et al*., 1952; Hsiao, 1978), the decline in the amount of PAHs after two months has subsequently gave rise to an increased trend in photosynthesis and productivity. Davenport *et al.* (1982) affirmed that oil spills are typically followed by rises in bacterial and yeast numbers, temporary falls in zooplankton densities and increases in phytoplankton

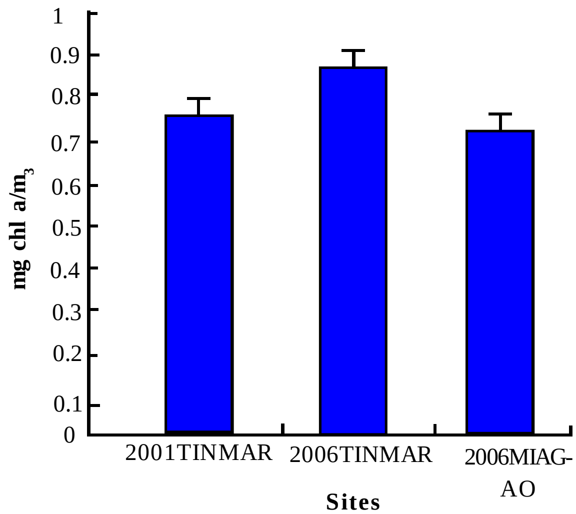

Fig. 2. Comparison of the computed mean values for chlorophyll *a* concentration for the post oil spill data (2006 TINMAR), pre oil spill data (2001 TINMAR) and reference site data (2006 Miag-ao)

Table 2. Calculated mean values for Gross Primary Productivity (GPP), and Net Primary Productivity (NPP) from pre-oil spill (TINMAR, Orbina, 2003), post-oil spill (TINMAR), and reference site (Miag-ao)

	Pre-oil Spill Data (TINMAR Stations)	Post-oil Spill Data (TINMAR Stations)	Reference Site (Miag-ao)
Mean GPP(mg C/m^3/hr)	62.09	71.82	29.40
Mean NPP(mg C/m^3/hr)	57.35	61.06	31.28

production. This was further confirmed by Miller *et al.* (1978) whose finding showed an initial decrease in primary production of phytoplankton after the oil spill but a proportional increase in primary production thereafter. Primary production and algal biomass were enhanced by increased contact with oil tars and a reduction in zooplankton density. Meanwhile, such observed increase in both the primary production and chlorophyll *a* content indicates that phytoplankton was not severely limited by nutrients during the spill event (Gonzales *et al*., 2009) while at the same time may suggest that certain level of stress is already operating in the phytoplankton community (Batten *et al*., 1998). Using a SeaWiFS satellite system to monitor impact of oil spill on primary production in the Galapagos Marine Reserve, Banks (2003) concluded that the stress induced by oil spill in terms of phytoplankton primary production was not significant in comparison to the normal high variation between years and within the El Nino/Souther Oscillation signal.

A total of 69 genera representing 6 classes of phytoplankton were identified in the 2006 sampling at TINMAR. Class Coscinodiscophyceae was best represented with 20 genera comprising of 1,535 individuals followed by Class Dinophyceae with 10 genera containing 625 individuals. Fragilariophyceae and Bacillariophyceae were moderately represented by 21 and 19 genera respectively, the latter with 376 individuals and the former with 518 individuals. Least represented were Class Ciliatea and Class Cyanophyceae as summarized in Fig. 3. The preserved samples from the 2001 AFMA-MFR cruise registered 75 genera. Class Dinophyceae was best represented, with all its 13 genera present, consisting of 451 individuals. Class Bacillario- and Coscinodiscophyceae followed with 16 and 15 representative genera respectively. Class Bacillariophyceae listed 322 individuals and Class Coscinodiscophyceae listed 242 individuals. Other less-represented classes were Fragilariaphyceae, Cyanophyceae, Ciliatea, Chrysophyceae and Chlorophyceae. *Cosmarium* of Class Chlorophyceae was only observed in this sample. The reference stations in Miag-ao only listed 5 taxonomic classes with Class Dinophyceae being well-represented with 473 individuals categorized in only 9 genera. Class Coscinodiscophyceae was moderately represented with 9 genera containing 104 individuals (Fig. 3).

The 2001 TINMAR samples showed dominance of four taxonomic classes with *Chaetoceros* and *Bacteriastrum* of Class Coscinodiscophyceae; *Nitzchia* of Class Fragilariophyceae; *Protoperidinium*, *Gonyolaux* and *Prorocentrum* of Class Dinophyceae and *Oscillatoria* of Class Cyanophyceae. The 2006 TINMAR samples were primarily characterized by the dominance of fast-growing centric diatoms of Class Coscinodiscophyceae like *Chaetoceros*, *Skeletonema*, *Rhizosolenia* and *Bacteriastrum*.

As stated earlier, diatoms represented by Classes Bacillariophyceae, Coscinodiscophyceae and Fragilariophyceae dominated the 2001 and 2006 TINMAR samples based on their abundance. The 2006 TINMAR samples, though, have the highest cumulative relative abundance of the diatom classes (75.764%) as compared to that of the 2001 TINMAR samples (65.514%). Mean phytoplankton density was also found to be highest in the 2006 TINMAR sampling with 21,374 individuals per liter seawater (Fig. 5). The adaptive capability of phytoplankton, especially the diatoms, to oil spills has already been demonstrated in a number of studies (*eg*. Desa, 1994; Varela *et al*., 2006). Their ability to use oil at low concentrations as carbon source (El-Sheekh *et al*., 2000), to reproduce, and to colonize rapidly after a perturbation might have been the cause of the observed increase in phytoplankton densities and biomass after the spill (Batten *et al*., 1998; Varela *et al*., 2006). Furthermore, decreased grazing due to hydrocarbon poisoning of zooplankton community was a common observation among oil spill assessment studies (Suderman and Thistle, 2004). Many authors consider this as a major contributing factor to the increase in population and diversification of marine

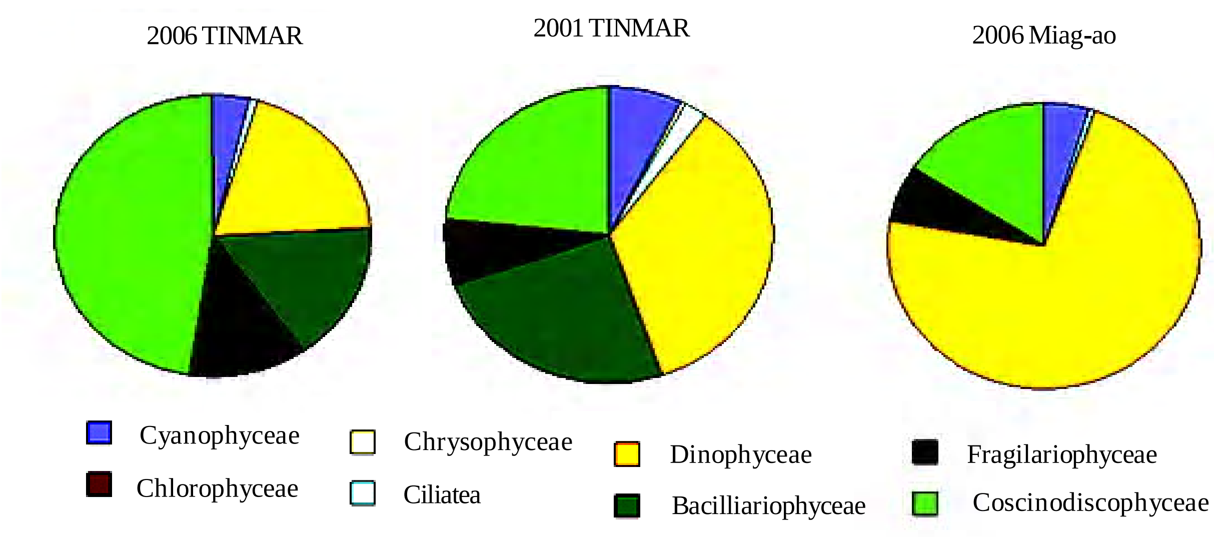

Fig. 3. Phytoplankton class distribution according to their relative abundance data after the oil spill (2006 TINMAR), before the oil spill (2001 TINMAR), and sample from a reference site (2001 Miag-ao)

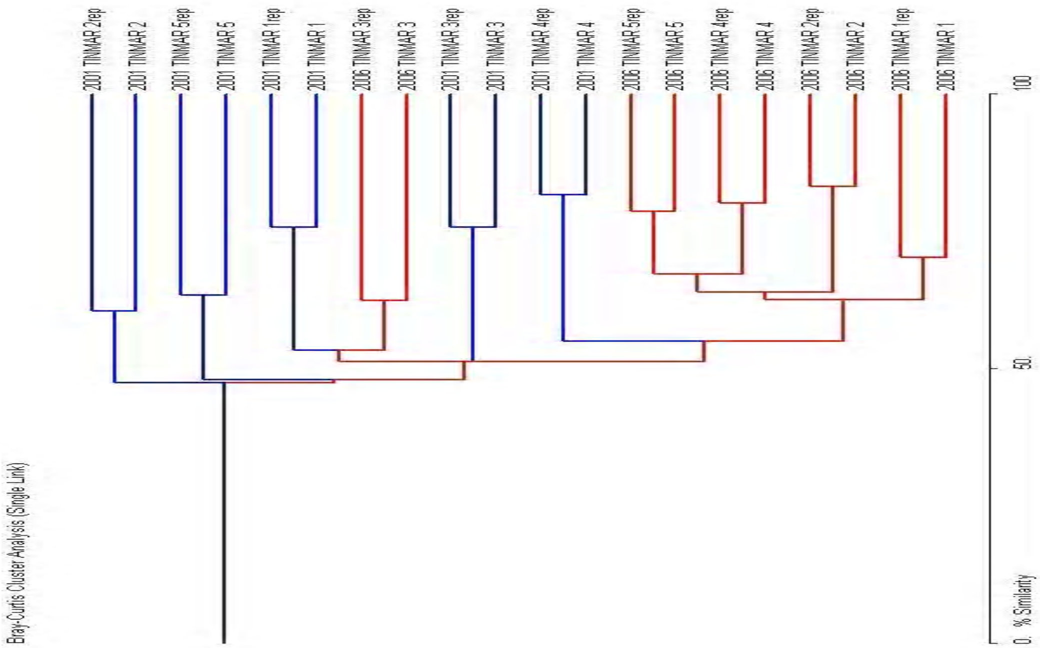

Fig. 4. Bray-Curtis Cluster Analysis showing similarities between the genera observed in 2001 and 2006 phytoplankton samples

phytoplankton following an oil spill event. This observed phenomenon was supported by studies noting the higher sensitivity of zooplankton than phytoplankton (Barsdate *et al*., 1980; Lee and Page, 1997; Marshall and Edgar, 2003; Hjorth *et al*., 2007). As a consequence, phytoplankton could take advantage of lower grazing rates and were able to reproduce more and diversify. Findings of Ikavalko (2005) further showed that the silica frustules of diatoms could serve as a protection of the cell from acute lethal effects of oil and had a higher chance of survival than uncovered cellular phytoplankton. This could explain the dominance of diatoms over other phytoplankton in the 2006 TINMAR samples and in several oil assessment studies. However, the present result was not in agreement with Harrison *et al*. (1986) who reported that diatoms were more affected by oil than other phytoplankton groups or by dinoflagellates (Adenkule *et al*., 2010). On the other hand, it cannot be ruled out also that the levels of PAHs in the present study were simply not sufficient to cause significant damage on diatom community.

Also, the dominance of fast-growing centric diatoms over pennates was also observed in 2006 TINMAR samples. A recent study reported that the dominance of fast-growing centric genera like *Chaetoceros*, *Rhizosolenia*, *Leptocylindrus*, *Bacteriastrum*, *Thalassiosira* and *Skeletonema* over pennates of Classes Bacillariophyceae and Fragilariophyceae could be an indication of a marine phytoplankton community being stressed by oil components (Verlecar *et al*., 2006). This might be due to the fact that pennates in general were more sensitive to pollutants like PAH than centrics (Hsiao, 1978: Siron *et al*., 1996; Jiang *et al*., 2002; Adenkule *et al*., 2010: Perez *et al*., 2010). The indicator species for oil-contaminated waters (i.e. centric diatoms) were actually observed to be the dominant genera in the 2006 TINMAR samples. It is therefore, highly probable that the phytoplankton community was still under stress from the M/T Solar I oil spill during the time of sampling.

The number of genera encountered in the 2001 and 2006 samples from TINMAR was higher than from the reference stations. This difference could be attributed to the topography of the sampling sites. The topography of Taklong Island which is characterized by shallow waters interspersed with mangrove forests, limestone cliffs, natural lagoons with coral gardens and seagrass beds could support more niches and more phytoplankton genera as compared to the beachfront, open waters of the reference site (Orbina, 2003). A change in genera composition was clearly manifested by the similarity values obtained through Bray-Curtis Cluster Analysis (Fig. 4). A change in the community assemblage of species might also be an indicator of stress in marine communities (Crowe *et al*., 2004). Furthermore, the Bray-Curtis Similarity Matrix (data not shown) revealed

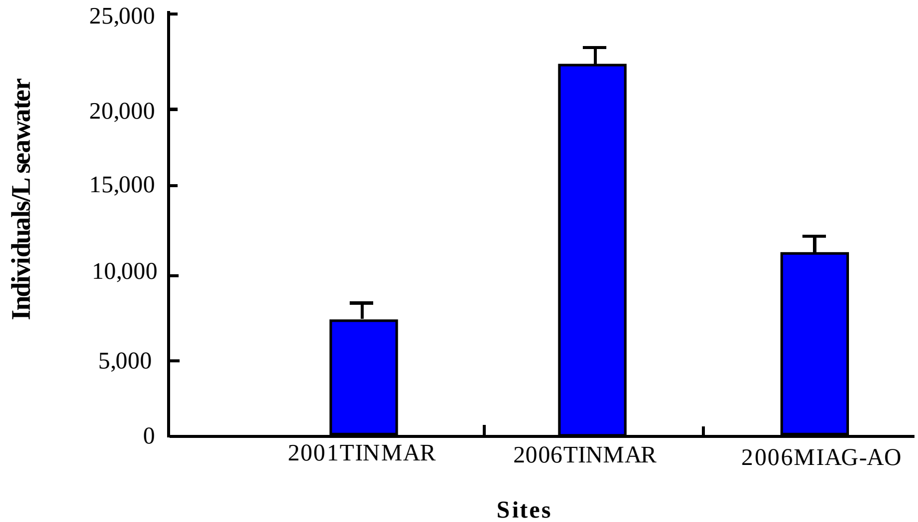

Fig. 5. Mean phytoplankton density values for the post oil spill data (2006 TINMAR), pre oil spill data (2001 TINMAR) and for the reference site data (2006 Miag-ao)

Table 3. Calculated average values for Simpsons and Shannon-Weiner's Indices for the TINMAR stations (pre and post oil spills) and for the Miag-ao reference stations

	TINMAR STATIONS (Pre- Oil Spill, 2001)*	TINMAR STATIONS (Post-Oil Spill, 2006)*	MIAG-AO STATIONS (Reference Site, 2006)**
Simpsons Diversity (1/D)	8.830	11.203	8.798
Shannon's Diversity (H´)	1.079	1.301	1.039

* Average values for the 5 stations
** Average values for the 2 stations

that all 2006 TINMAR Stations only resembled their 2001 counterparts by less than 50 %. Similarities of the 2006 TINMAR samples with their 2001 counterparts ranged from 17.90% similarity of TINMAR Station 5 to 47.02% similarity of TINMAR Station 4. However, no distinct cluster was observed to distinguish the 2006 TINMAR samples from the 2001 TINMAR samples.

The observed difference in genera composition of the TINMAR 2006 stations with their 2001 counterparts seem to reflect the effect of a disturbance causing stress to the phytoplankton community. However, change in species composition is fairly common to phytoplankton communities owing to their spatial and seasonal variation. Thus, community assemblage alone without data on hydrographic factors, long term seasonal fluctuations and phytoplankton patchiness makes determination of an effect of the oil spill difficult (Miller *et al*., 1978; Marshall and Edgar, 2003; Edgar *et al.*, 2003; Varela *et al.*, 2006).

Shannon-Weiner and Simpsons diversity values (Teble 3) were recorded highest in the 2006 TINMAR stations as compared to the spatial and temporal reference samples. The reason for this lies with the great number of genera and individuals encountered in the samples as compared to the Dinophyceae-dominated reference site samples and the low abundance observed in the 2001 AFMA samples. Evenness which is a measure of equity in species distribution was recorded lowest for 2006 TINMAR stations 4 and 5 where the bulk of the stations' population is heavily distributed in only three genera namely: *Chaetoceros*, *Rhizosolenia* and *Skelotonema*. Accordingly, these three genera were bioindicators of an oil-stressed phytoplankton community (Verlecar *et al.*, 2006).

CONCLUSION

Overall, the observed patterns in chlorophyll *a* concentration, primary productivity, phytoplankton abundance and diversity were consistently demonstrated in all investigated sites. The increase in primary productivity, increase in biomass, shift in phytoplankton community and the observed diversification of species all indicate that the phytoplankton community of the 2006 TINMAR post-oil spill site has undergone a certain level of stress. Furthermore, such shifts in phytoplankton community characteristics associated with oil pollution may cause sharp changes in the functioning of the entire aquatic systems, and represent an ecological risk. Results of this study could contribute to the growing body of knowledge regarding the effect of oil spill on phytoplankton communities.

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