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EFFECT OF THE MV SAINT THOMAS AQUINAS OIL SPILL ON ZOOPLANKTON COMPOSITION AND ABUNDANCE IN MACTAN ISLAND, CEBU, THE PHILIPPINES

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ABSTRACT – A survey of zooplankton composition and abundance was conducted on four nearshore waters last May 2014, 9 months after the oil spill by the passenger vessel "MV Saint Thomas Aquinas." Samples from three sites hypothesized to represent different degrees of oil contamination along the south coast of Mactan Island in Cebu, the Philippines were compared with those from the reference site in Dalaguete, Cebu. A total of six phyla with over 15 species and a density of 630 individuals/L of micro- and mesozooplankton were identified where ciliates specifically aloricates coming out as the most numerous. The average zooplankton density in the reference site (mean=143) differed significantly ($p=0.04$) from zooplankton density observed in S2 (mean=12.33), a moderately affected site. The aloricate ciliates, the copepods, and the zooplankton eggs were evident in all the sites. Although there was no significant spatial variation ($p<0.05$), the aloricates were more abundant in the unaffected and lesser affected sites. The copepods ($p<0.01$) and zooplankton eggs ($p=0.03$ and $p<0.01$) appeared to be good zooplankton predictors for the effects of the oil spill in this study as they have significantly higher abundance in the unaffected and least affected sites while controlling for other factors. Further investigation is recommended to monitor the temporal dynamics of the zooplankton population and abundance taking into consideration other intervening factors such as physicochemical factors, trophic relationships especially with bacteria, as well as the organisms' physiology, behavior, life stages, and sizes. The use of ratios of abundances or other indices can also be explored. Regular monitoring is strongly suggested especially in areas where the probability of oil spills is high and data is absent.

Keywords: ciliates, copepod, Mactan Island, mesozooplankton, oil spill

INTRODUCTION

Mactan Island in Central Philippines is characterized by shallow bays and channels, extensive intertidal flats, mangrove swamps, fish and seaweed ponds and salt pans, and coral reefs. It also serves as an important staging area for shorebirds in the Visayas (Asian Development Bank, 2014). These coastal habitats provide shelter, breeding, nursery and feeding grounds for fish, birds and other species. Due to increasing coastal development, these habitats have decreased and been degraded at an accelerating rate. The condition has been exacerbated by an oil spill in its southmost shallow nearshore section due to a

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collision between the passenger ship MV Saint Thomas Aquinas and the MV Sulpicio Express cargo on 16 August 2013 at the Lawis ledge portion of the island's fringing coral reef (Rubio et al., 2013).

In addition, the coastal habitats of Mactan Island are continually exposed to small-scale oil and grease contamination since the Mactan channel that separates mainland Cebu from Mactan Island is a navigational route that hosts heavy maritime activities (Picardal et al., 2012). Less than a kilometer away from the channel are Mactan Island's different coastal ecosystems such as the intertidal mangrove forests (Sotto et al., 2001; Sadaba and Niego, 2016); a large tract of the reef backwater in the area has also been reforested with mangrove saplings (INQUIRERPLUS, 2018; www.cordova.gov.ph., 2019), while the majority of the reef flat depressions are home to seagrass and seaweed beds (Montenegro et al., 2005; Tanduyan et al., 2013).

One of the areas affected by the oil spill in Mactan Island is Barangay Calawisan, Lapu-lapu City. Although considered as only moderately impacted based on the observed amount of oil slick on the water a week after the collision, the potential effect of the spilled oil on its coastal habitat is considered significant since majority of the remaining mangrove habitats of the island is found here including fishponds that are currently being used for the culture of several species (i.e. fish, shrimp and seaweed) (R. Tagaan, head of Lapu-Lapu City Environment and Natural Resources Office, personal communication, August 2013). The more impacted area in terms of the presence of the oil slick are four barangays in the Municipality of Cordova, with Day-as as the most severely affected. Considered less affected relative to Barangays Calawisan and Day-as is the coastal area of Barangay Alegria since this is farther away from where the oil spill started (Sadaba and Niego, 2016).

In order to monitor the long-term changes in the water quality following an oil spill, biological indicators found in the water column like zooplankton can be used as indices (Almeda et al., 2013; Buskey et al., 2016). The zooplankton is composed of microzooplankton (20 to 200 μm) such as ciliates, dinoflagellates and foraminiferans, as well as the mesozooplankton (200 μm to 20 mm) and the macrozooplankton (>20mm) where the suspension-feeding copepods, some gelatinous zooplankters and some first-feeding fish and invertebrate larvae belong. In marine systems, copepods dominate the mesozooplankton group (Calbet, 2008; Makabe et al., 2012; Alexandrov et al., 2014).

It is now widely recognized that aside from phytoplankton, microzooplankton represent food for mesozooplankton (Calbet and Saiz, 2005; Calbet, 2008; Almeda et al., 2014). In marine pelagic ecosystems, these two groups of zooplanktons play both direct and indirect role in transferring energy and materials from bacteria and phytoplankton to higher trophic levels (Calbet, 2008; Calbet and Landry, 2004). The zooplankton's unique role in the food web and its impact on the nutrient dynamics makes them a major player in the functioning and productivity of aquatic ecosystems (Sahu et al., 2012). Spatial distribution of these two groups are affected by a host of physico-chemical and biological factors while their temporal distribution has been attributed to monsoonal and seasonal changes (Elangovan et al., 2012). Hence, many zooplankton species can be used as biological indicators for water pollution, water quality and eutrophication (Sahu et al., 2012).

Knowledge about the zooplankton's distributions during episodic oil spill event or chronic oil contamination can provide insights as to possible changes that may also cascade up or down the trophic dynamics of the ecosystem (Abbriano et al., 2011; Buskey et al., 2016).

This study surveyed the effects of the "MV Saint Thomas Aquinas" oil spill on zooplankton composition and abundance in the southmost portion of Mactan Island, Cebu, 9 months after the oil spill, and compared this with that of a reference site in Dalaguete, Cebu.

MATERIALS AND METHODS

The study sites

Three sites along the south coast of Mactan Island were chosen to represent different degrees of oil contamination in relation to the distance from where the oil spill occurred (Fig. 1). The chosen sites identified to have potential low, mid and high oil spill impact status, respectively were: a) Alegria designated as S1 (123°58'8.105"E, 10°15'5.272"N), b) Calawisan or S2 (123°55'6.504"E, 10°16'9.688"N) and, c) Day-as or S3 (123°55'44.195"E, 10°15'13.495"N). The impact status was based on visual observations of the oil slick on the water and oil coating at the trunk base of the mangrove trees in the study sites a week after the oil spill occurrence. S3 (Day-as) had the highest oil height coating on the trunk base of mangrove trees, followed by those in S2 (Calawisan), with the least coating on S1 (Alegria) mangroves. A similar observation was reported by Sadaba and Niego (2016) in their study of the effect of the same oil spill incident on the mangroves of Cordova, Cebu. Since there was no pre-oil spill data on the zooplankton population in these affected areas, the shallow waters near the mangrove forest of Dalaguete was chosen as reference site (123°32'31.823"E 9°45'10.637"N) because it was not affected by the oil spill but this reference site has similar features to the affected sites in Mactan such as but not limited to presence of fringing mangroves and seagrass meadows. The reference site is about 97 km away from the oil spill affected areas in Mactan Island.

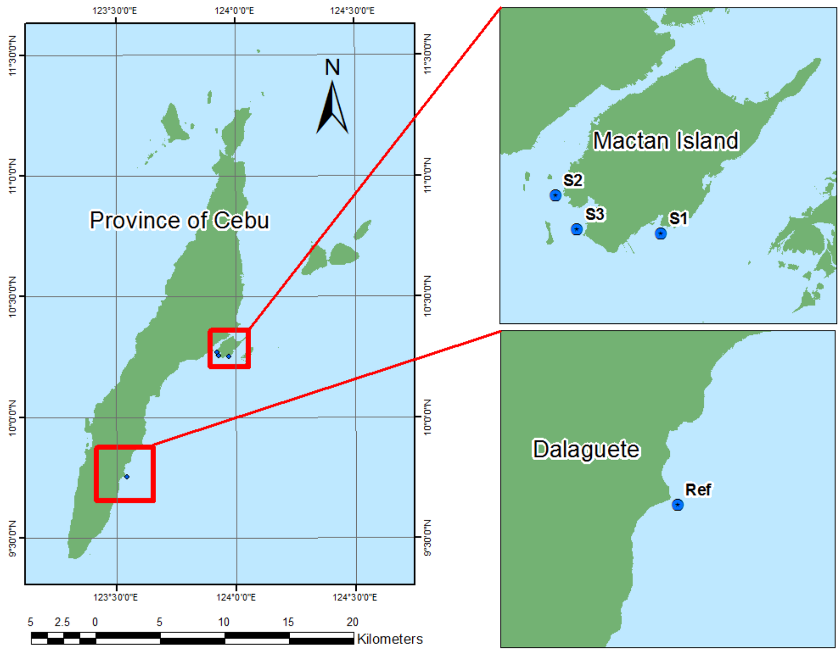

Figure 1. The sampling sites in Barangays Alegria (S1), Calawisan (S2), and Day-as (S3) in Mactan Island, Central Philippines, and the reference site in Dalaguete, South Cebu (Map Source: UP Cebu Center for Environmental Informatics).

The chosen sites in Mactan Island are about 500-900 m away from edge of the mangrove forest, which is a mixture of natural and reforested stands dominated by *Rhizophora*, with patches of *Sonneratia* and *Avicennia.* In the reference site (Dalaguete), the natural growth mangrove is composed primarily of *Sonneratia* and *Avicennia* sp. while the reforested mangrove is specifically planted to *Rhizophora mucronata*.

Zooplankton sample collection and identification

Protocols from Goswami (2004) were adopted for the collection and identification of zooplankton. Nearshore surface water was collected from the selected sampling sites using 250-mL polyethylene bottles. Care was taken so as not to disturb the water as much as possible during the collection to avoid flight reactions of the zooplankton. All samples were taken during high tide, around midday on May 18-20, 2014 to eliminate differences in abundance and diversity affected by the time of day and tidal variations. Water parameters like salinity and temperature were measured using a refractometer (Atago brand) and a field thermometer, respectively.

Immediately after collection, the samples were fixed with 10% Lugol's solution to avoid damage to the animal tissues by bacterial action and autolysis (Chagwa, 2013), then the samples were stored until further examination. The preserved samples were concentrated, settling to the final volume of 25 ml, 5 ml of which were used for the counting of the zooplankton. The equation to get the abundance/L of zooplankton in the sample, which was adopted from Godhantaraman (2001) is shown below:

$$\frac{\text{number of individuals}}{5\text{ mL}} \times 25\text{ mL} = \frac{\text{number of individuals}}{25\text{ mL}} \sim \frac{\text{number of individuals}}{\text{L}}$$

Data Analyses

Generalized linear model (GLM) was used to compare mean estimates of zooplankton abundance across sites. Student's t-test was used to test the significant difference of mean estimates of zooplankton abundance of each affected site from the mean estimates in the reference site at 95% and 99% confidence levels. Data processing and analysis were done using R version 3.5.1 (R Core Team, 2019).

RESULTS AND DISCUSSION

About six phyla with over 15 species and a total density of 630 individuals/L of micro- and mesozooplankton were observed from the three oil spill affected sites in Mactan Island (S1, S2, S3) and from the unaffected reference site in South Cebu, 9 months after the oil spill event that affected Mactan Island in Cebu. The microzooplankton ciliates (Ciliophora) had the highest density at 278 individuals/L (Fig. 2) or about 44% of the sample (Fig. 3). A laboratory study on the effects of crude oil and dispersant to marine microzooplankton reveals that these chemicals were highly toxic to microzooplankton, particularly to small ciliates (Almeda et al., 2014). Using ciliates in the assessment of water quality has become increasingly recognized. With short developmental times and delicate external membranes, they may react faster to environmental changes than many other eukaryotic organisms (Jiang et al., 2013a). However, these same characteristics may also explain why ciliate abundance in this study was relatively

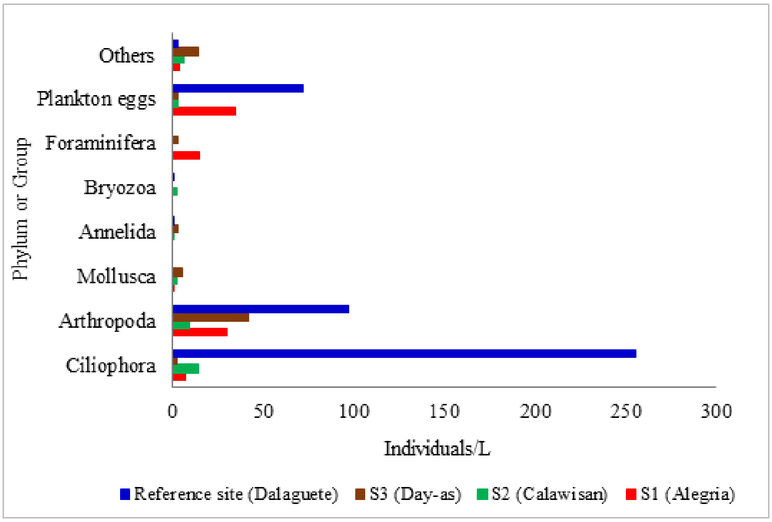

Figure 2. Zooplankton composition and abundance in the oil spill affected sites in Mactan Island (S1, Alegria; S2, Calawisan; S3, Day-as), Central Philippines and the reference site in Dalaguete, Cebu, May 18-20, 2014.

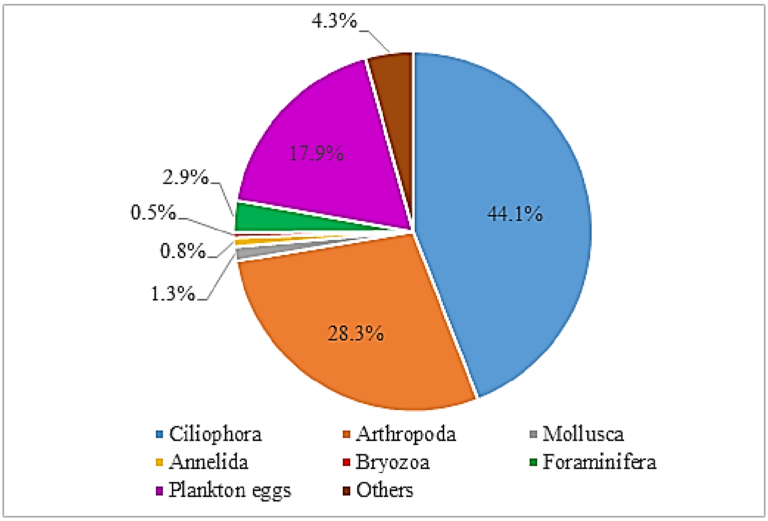

Figure 3. Zooplankton composition and relative abundance in the oil spill affected sites in Mactan Island (S1, Alegria; S2, Calawisan; S3, Day-as), Central Philippines and the reference site in Dalaguete, Cebu, May 18-20, 2014.

high 9 months after the oil spill. The shorter life cycles of ciliates allow the fast increase in biomass and the quick selection of resistant species or genotypes to cope with toxic prey, in this case, the oil droplets that are frequently in the food size spectra of many microzooplankton (Calbet, 2008). Furthermore, it is commonly observed for ciliates to dominate most marine and estuarine environments since they are a major component of the zooplanktons, and are increasingly regarded as important in nutrient cycling and trophic flux (Pierce and Turner, 1992; Sherr and Sherr, 2002; Calbet and Landry, 2004). Ciliate assemblages are responsible for most of the energy transfer from the lower trophic levels, occupied by the bacteria and phytoplankton, to higher trophic levels like copepods and fishes (Dolan and Coats, 1990; Stoecker and McDowell-Cappuzzo, 1990; Gifford, 1991; Sime-Ngando et al., 1995; Sherr and Sherr, 2002; Calbet and Saiz, 2005; Jiang et al., 2013b).

Following the ciliates in density were the arthropods, 99% of which were copepods, which form part of the mesozooplankton, with 178 individuals/L (about 28.3% of the sample). The smallest in density were the bryozoans (3 individuals/L, 0.5% of sample), annelids (5 individuals/L, 0.8% of sample), and mollusks (8 individuals/L, 1.3% of sample).

The average zooplankton density in the reference site differed significantly from zooplankton density observed in S2 (Fig. 4; Table 1, [GLM 1, p=0.04]). The difference in the means can be attributed to the high total abundance of zooplankton in the reference site compared with the oil spill affected sites. There were 429 individuals/L observed at the reference site compared to only 37 individuals in S2, a moderately affected site (Fig. 5). The results were not different from that of Bote (unpublished) that was conducted from December 2013 to February 2014, which was about 3-5 months earlier than this study where the abundance of zooplankton in the reference site (Olango Island, unaffected by oil spill) were significantly higher than that in the oil spill affected sites (Barangays Mactan and Day-as). Meanwhile, average zooplankton density in the reference site did not significantly differ from zooplankton density observed in S1 (Table 1, [GLM 1, p=0.07]) and S3 (Table 1, [GLM 1, p=0.06]).

Several studies have documented that abundance of zooplankton in oil spill affected sites generally decline shortly after the spill incident and rate of recovery varies depending on the species, type of oil, and other environmental factors (Guzmán del Próo et al., 1986; Suchanek, 1993). These studies cited lethal and sublethal effects of spilled oil on marine zooplankton could lead to mortality, narcosis and related diseases; and changes in feeding, development and reproduction (Samain et al., 1980; Elmgren et al., 1983; Abbriano et al., 2011; Almeda et al., 2013). However, most of these aforementioned studies were conducted in the laboratory or in controlled environments. Meanwhile, *in situ* studies have shown inconsistencies in the results depending on: the original components of the oil; products resulting from degradation of oil, the structure of planktonic communities, which can be species-specific or clone specific; natural environmental conditions (e.g. the inhibition of growth rate caused by oil is highly temperature-dependent); the relationships among organisms in the pelagic system, which may conceal the possible effects of contaminants; and natural variability in season, water movement, and/or distribution of planktonic organisms (National Research Council, 2003; Varela et al., 2006; Abbriano et al., 2011; Almeda et al., 2013). The natural variability of the ecosystem makes it difficult to accurately determine the effects of oil spills on planktonic organisms (Varela et al., 2006).

The aloricate ciliates, the copepods, and the zooplankton eggs were evident in all the sites (Fig. 6). In the reference site, the most numerous were the aloricate ciliates with 254 individuals/L followed by the copepods with 96 individuals/L. A similar trend was observed in S2 with 14 individuals/L of aloricates followed by 9 individuals/L of copepods. Meanwhile, zooplankton eggs dominated S1 with 35 individuals/L, followed by the copepods with 29 individuals/L. The copepods dominated S3 with 42

Table 1. Summary statistics of Generalized linear model (GLM) for the comparison of mean estimates of zooplankton abundance across sites. SE refers to standard error of the mean. Significant difference of mean estimates of zooplankton abundance of each affected site from the mean estimates in the reference site was determined using Student's t-test at 95%* and 99%** confidence levels.

Models	Terms	Estimates	SE	statistics	p value
GLM 1 Total Abundance ~ Sites	(Intercept)	143.00	38.54	3.71	0.01
	S1	-112.33	54.51	-2.06	0.07
	S2 *	-130.67	54.51	-2.40	0.04
	S3	-119.00	54.51	-2.18	0.06
GLM 2 Abundance of Aloricate ciliates ~ Sites	(Intercept)	84.67	35.08	2.41	0.04
	S1	-82.33	49.61	-1.66	0.14
	S2	-80.00	49.61	-1.61	0.15
	S3	-84.00	49.61	-1.69	0.13
GLM 3 Abundance of Loricate ciliates ~ Sites	(Intercept)	0.33	0.17	2.00	0.08
	S1	-0.33	0.24	-1.41	0.20
	S2	-0.33	0.24	-1.41	0.20
	S3	-0.33	0.24	-1.41	0.20
GLM 4 Abundance of Copepods ~ Sites	(Intercept)	32.00	2.88	11.12	0.00
	S1 **	-22.33	4.07	-5.49	<0.01
	S2 **	-29.00	4.07	-7.13	<0.01
	S3 **	-18.00	4.07	-4.42	<0.01
GLM 5 Abundance of Phyllopod ~ Sites	(Intercept)	0.33	0.24	1.41	0.20
	S1	0.00	0.33	0.00	1.00
	S2	-0.33	0.33	-1.00	0.35
	S3	-0.33	0.33	-1.00	0.35
GLM 6 Abundance of Bivalve veliger ~ Sites	(Intercept)	0.00	0.47	0.00	1.00
	S1	0.00	0.67	0.00	1.00
	S2	0.67	0.67	1.00	0.35
	S3	1.33	0.67	2.00	0.08
GLM 7 Abundance of Gastropod veliger ~ Sites	(Intercept)	0.00	0.24	0.00	1.00
	S1	0.33	0.33	1.00	0.35
	S2	0.00	0.33	0.00	1.00
	S3	0.33	0.33	1.00	0.35
GLM 8 Abundance of Polychaete trocophore larvae ~ Sites	(Intercept)	0.33	0.37	0.89	0.40
	S1	-0.33	0.53	-0.63	0.54
	S2	0.00	0.53	0.00	1.00
	S3	0.67	0.53	1.26	0.24
GLM 9 Abundance of Cyphonaute larvae ~ Sites	(Intercept)	0.33	0.37	0.89	0.40
	S1	-0.33	0.53	-0.63	0.54
	S2	0.33	0.53	0.63	0.54
	S3	-0.33	0.53	-0.63	0.54
GLM 10 Abundance of Foraminiferan ~ Sites	(Intercept)	0.00	1.83	0.00	1.00
	S1	5.00	2.58	1.94	0.09
	S2	0.00	2.58	0.00	1.00
	S3	1.00	2.58	0.39	0.71
GLM 11 Abundance of Zooplankton eggs ~ Sites	(Intercept)	24.00	3.26	7.37	0.00
	S1 *	-12.33	4.61	-2.68	0.03
	S2 **	-23.00	4.61	-4.99	<0.01
	S3 **	-23.00	4.61	-4.99	<0.01
GLM 12 Abundance of Other zooplankon ~ Sites	(Intercept)	1.00	1.62	0.62	0.56
	S1	0.33	2.30	0.15	0.89
	S2	1.00	2.30	0.44	0.67
	S3	3.67	2.30	1.60	0.15

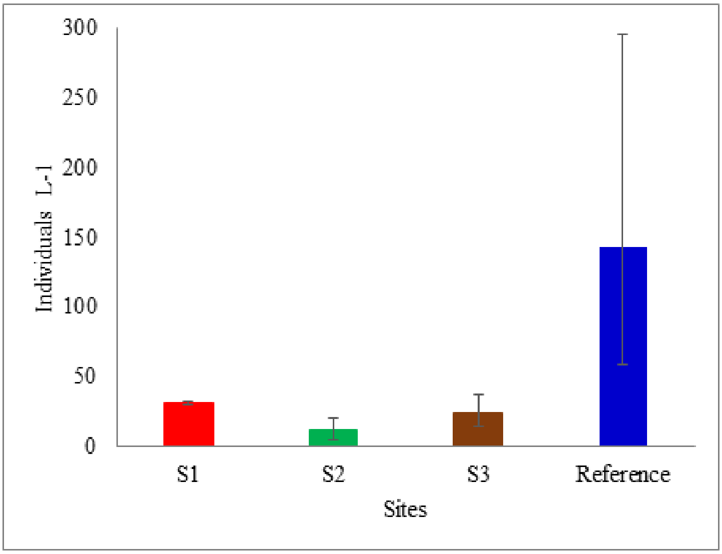

Figure 4. Average zooplankton density in the oil spill affected sites in Mactan Island (S1, Alegria; S2, Calawisan; S3, Day-as), and the reference site in Dalaguete, Cebu, May 18-20, 2014.

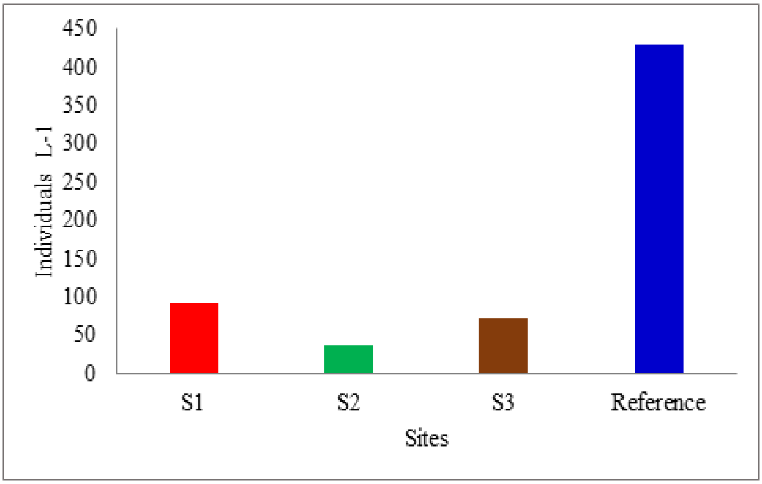

Figure 5. Total zooplankton abundance in the oil spill affected sites in Mactan Island (S1, Alegria; S2, Calawisan; S3, Day-as), and the reference site in Dalaguete, Cebu, May 18-20, 2014.

individuals/L. The results were again comparable to the study conducted by Bote (unpublished). Copepods dominated the zooplankton in the nearshore waters, while the aloricates dominated the offshore waters of the most affected site, Barangay Day-as (S3 in the current study). Meanwhile, the copepods, followed by the aloricates dominated both the nearshore and offshore waters of Barangay Mactan, another oil spill affected site in Mactan Island. Even the reference site, Olango Island had copepods dominating the samples followed by the aloricates.

Of the three dominant groups, only the copepods (Table 1 [GLM 4: all sites, $p<0.01$]) and zooplankton eggs (Table 1 [GLM 11: S1, $p=0.03$; S2 and S3, $p<0.01$]) showed significant to highly significant spatial variation. The copepod samples were predominantly in the nauplii larvae stage of development with only 10 adults present, six of which were found in the reference site. Adult copepods identified in the different study sites included *Euterpina* sp. (S3), *Clausocalanus* sp. (S3), *Cosmocalanus* sp. (S3), *Oncaea media* (reference site), *Oncaea* sp. (S3 and reference site), *Dioithona rigida* (reference site), and *Oithona* sp. (S1 and reference site).

Although there was no significant difference in abundance of aloricate ciliates between sites, they were generally more abundant in the reference site (Dalaguete) and in lesser affected sites, S1 (Alegria) and S2 (Calawisan). Aloricate ciliates, also known as "naked" ciliates, lack external skeletal structures, which render them more vulnerable to the effects of crude oil and other water contaminants. The presence of external skeletal structures in plankton may provide some protection against crude oil pollution (Dale, 1988). Almeda et al. (2014) validated these observations in a laboratory study where the growth rates of aloricate ciliates, specifically the oligotrichs, were significantly higher in the control set-ups than the experimental set-ups containing different crude oil concentrations, or dispersants, or combinations of both. The study of Asadullayeva and Alekperov (1999) showed that of ciliate specimens tested for sensitivity to oil pollution, the aloricate ciliate *Strombidium conicum* was the only specimen to exhibit considerable deviations from the control suggesting that *S. conicum* could be a new reliable sensitive species to oil pollution. The absence of significant differences in ciliate abundance between oil spill affected sites and unaffected sites is commonly observed among post oil spill studies on zooplankton; and this is due to several intervening extrinsic factors (i.e., temperature, trophic relationships especially with bacteria), as well as those intrinsic to the organisms like species, life stage, size (Straughan, 1972; Berdugo et al., 1977; Varela et al., 2006; Jiang et al., 2012; Sahu et al., 2012), which affect the sensitivity of microzooplankton, such as ciliates, to oil.

Copepods appeared to be tolerant of a wide range of fluctuations in different physico-chemical parameters and this could be the reason why copepods were the second most abundant group in this study. Previous studies confirm that copepods were the most versatile and abundant mesozooplankton in marine waters even in areas affected by oil spills (Longhurst, 1985; Sahu et al., 2012). The short generation times, high fecundity, and ability to sense and avoid oiled areas among copepods result to their less contact with spilled oil, less potential mortality, and/or rapid recovery for copepods (Seuront, 2010; Abbriano et al., 2011). The negative impact of oil spills on mesozooplankton such as copepods could also be reduced by crude oil-microbial food web interactions (Almeda et al., 2013). However, several post oil spill studies have reported the toxic, lethal and sublethal effects in copepods exposed to petroleum hydrocarbons (Suderman and Marcus, 2002; Barata et al., 2005; Bejarano et al., 2006; Calbet et al., 2007; Saiz et al., 2009; Seuront, 2011), which usually result to increases in their mortality with increasing oil concentration (Almeda et al., 2013). This could explain the significant differences in copepod abundance between sites, with more copepods observed in the unaffected reference site and the least affected site (S1).

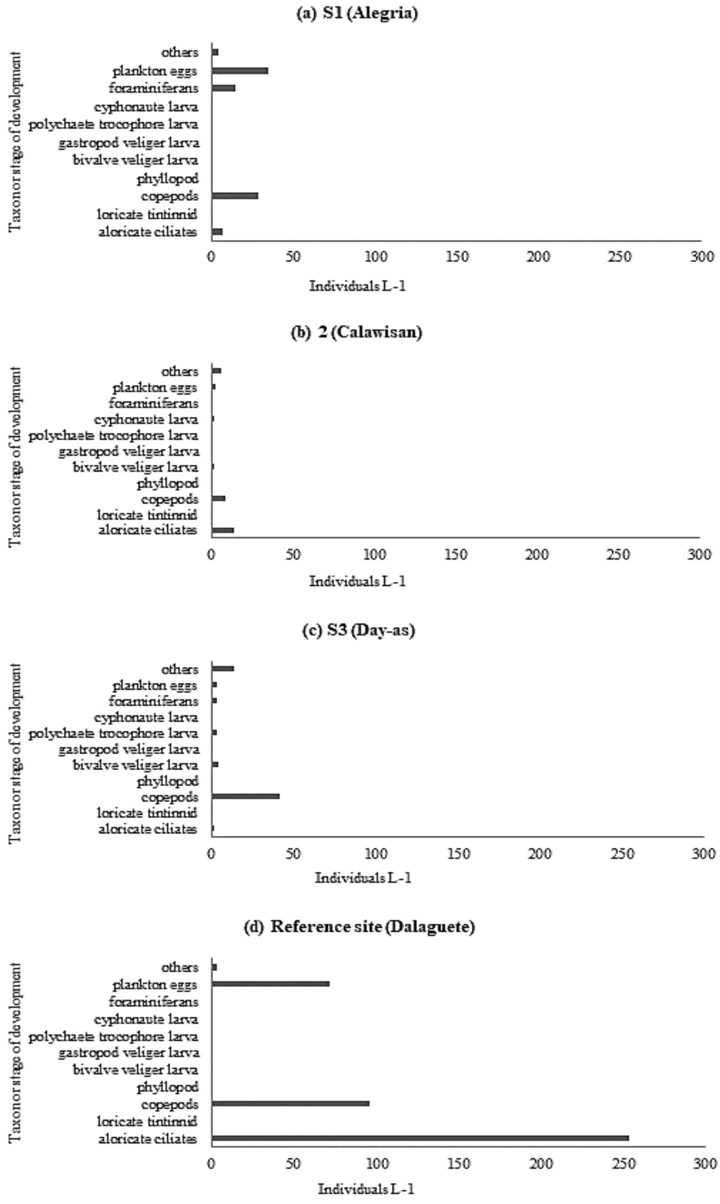

Figure 6. Zooplankton abundance per taxon or stage of development and per site: (a) S1 (Alegria), (b) S2 (Calawisan), (c) S3 (Day-as), and (d) Reference site (Dalaguete).

Zooplankton eggs, composed of eggs from fish, copepods, and other invertebrates (Verlecar et al., 2006; Sahu et al., 2012) can be negatively affected by petroleum hydrocarbons from oil spill incidents. Egg production rates of certain copepod species exposed to crude oil were reportedly lower than the non-exposed individuals (Hjorth and Nielsen, 2011; Almeda et al., 2013). The increase in mortality of larger zooplankton in the oil spill affected sites, and/or the migration of zooplankton away from the affected areas could have also led to decreased predation on the plankton eggs by zooplankton (Abbriano et al., 2011).

CONCLUSION AND RECOMMENDATIONS

Ciliates specifically aloricates were found to be in highest abundance in all sites compared to the other micro- and mesozooplankton samples collected and identified under six phyla with over 15 species and a total density of 630 individuals/L. The average density of zooplankton in the unaffected reference site was significantly higher compared with that in the oil spill affected sites. The identified dominant groups were the aloricate ciliates, the copepods, and the zooplankton eggs as they were evident in all the sites. Results of this study showed that copepods and zooplankton eggs appeared to be good zooplankton predictors for oil spill effects as these have significantly higher abundance in the unaffected and least affected sites of the oil spill compared to the other zooplankton groups.

Since the analyses in this study were based on data from a single sampling period that was done almost a year after the oil spill and the apparent lack of pre-oil spill information and information immediately after the spill, follow-up work is recommended to monitor if there are further changes in the zooplankton population composition and abundance in the study sites taking into consideration seasonal variability. Other intervening factors such as physicochemical factors, trophic relationships especially with bacteria, as well as the organisms' physiology, behaviour, life stages, and sizes need to be considered as well. The use of ratios of abundances or other indices can also be explored. Regular monitoring is strongly suggested especially in areas where the probability of occurrence of oil spills is high and information is lacking or absent.

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STATEMENT OF AUTHORSHIP

M.J.L. Flores drafted and finalized the manuscript (including the interpretation and analyses of data) with significant contribution from J.R. Silapan. B. Edullantes performed the statistical analysis of the field data. All authors identified the study sites, coordinated with the local government units, collected data, discussed the results, and commented on the manuscript.

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