

Summer phytoplankton community structure and distribution in a mariculture-affected coastal environment

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Many aspects of the ecological responses of phytoplankton communities toward environmental changes remain little understood. Here, the study investigated correlations of micro-phytoplankton communities across environmental gradients associated with mariculture activities during early (April) and late summer (May). Plankton and physico-chemical profiling of the upper surface waters were carried out in 16 stations from mariculture-affected areas inside the Guiguivanen channel in Bolinao to non-mariculture areas outside the channel. Community beta-diversity clustered the phytoplankton based on the environments where they were collected, with most communities correlating with high temperature and nutrients (phosphate). Phosphate loading in the area resulted in low total inorganic nitrogen and phosphorus ratio coupled with a shift from a diatom-dominated community (84.8%; non-mariculture areas) to a harmful dinoflagellate-dominated community (41.5%; mariculture-affected areas). In addition, zooplankton were generally negatively correlated with phytoplankton, which could indicate grazing. Increase in heavy rainfall towards late summer (late May) was accompanied by

increase in nutrients followed by a bloom of putative dinoflagellate *Takayama* spp. (99%), which coincided with a fish kill event inside the channel. This study shows that continuous intensive aquaculture activities could influence phytoplankton community structures, and in some cases, favor harmful algal bloom-forming species, thereby affecting ecosystem functioning.

KEYWORDS

Biology, Bolinao, eutrophication, harmful algal bloom, mariculture, phytoplankton

INTRODUCTION

Marine phytoplankton contribute significantly to primary productivity. They serve as foundation of marine food webs and are critical players in the biogeochemical cycles involving biological carbon pump and nutrient cycling (Pomeroy 1974; Huntley et al. 1991; Falkowski 1994). As photosynthetic organisms, they rely on the availability of dissolved inorganic carbon, light, and nutrients (Maddux and Jones 1964; Riebesell et al. 1993). Thus, factors affecting the availability of these resources would have significant implications for phytoplankton cellular growth, population abundance, and spatio-temporal

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distributions. Such factors could be natural (e.g., upwelling and wind-induced mixing) and/or anthropogenically induced (e.g., land-based pollution from coastal communities and nutrient loading from aquaculture) (Bach et al. 1998; Landsberg 2002; Sellner et al. 2003; Rabalais et al. 2009). In turn, phytoplankton community composition and structure reflect the conditions or environment where they proliferate. For example, low-nutrient environments tend to be dominated by small-cell taxa due to their lower metabolic requirements and surface area to volume ratio advantage (Irwin et al. 2006). In contrast, nutrient-rich environments are favorable to large-cell phytoplankton such as diatoms and dinoflagellates (Cloern and Dufford 2005). Moreover, phytoplankton community diversity may be regulated by their interactions with potential consumers or grazers such as zooplankton (Le Quéré et al. 2016). Information about zooplankton communities indicate prey limitation at low phytoplankton diversity and/or selective grazing at high phytoplankton diversity (Irigoien et al. 2004; Goldyn and Kowalczywska-Madura 2008). Thus, phytoplankton community composition could provide insights on water quality and the status of a particular aquatic environment (Alvarez-Gongora and Herrera-Silveira 2006; Casé et al. 2008).

Compared to the open ocean, phytoplankton tend to be more abundant along the coast. This is primarily due to increased availability of the nutrients nitrate (NO_3^-), phosphate (PO_4^{3-}), and silicate (SiO_4^{4-}) (Paerl 1988). Anthropogenic inputs from agriculture, industries, and aquaculture could significantly contribute to coastal eutrophication. In particular, mariculture or the cultivation of economically important organisms in the sea, has been reported to produce excess nutrients (San Diego-McGlone et al. 2008). The unconsumed feeds and waste products released in the water column and deposited to the sediments cause local eutrophication (Eng et al. 1989; Talbot and Hole 1994; Cao et al. 2007). This can subsequently lead to phytoplankton growth of one or a group of species, which can proceed to blooms (Cloern 2001; Anderson et al. 2002; San Diego-McGlone et al. 2008; Jiang et al. 2012). In some cases, blooms of toxic phytoplankton species, also known as harmful algal blooms (HABs), occur which then result in catastrophic events such as massive fish kills or toxin poisoning (Azanza et al. 2005; Azanza and Benico 2013). Although the link of eutrophication to HABs occurrence has been explored, its effects on other phytoplankton and components of the community remain little understood. The underlying factors that cause changes in the phytoplankton community in the context of regime shift from oligotrophic-mesotrophic to eutrophic conditions need further elucidation. Understanding responses of other components in the phytoplankton community could provide insights on ecosystem-level responses.

Global aquaculture production has been steadily increasing over the years primarily due to increased food demand. In the Philippines alone, marine products (including fish) constitute 56% of animal protein intake of most Filipinos (Espejo-Hermes 2004). Hence, aquaculture production in the Philippines is the fifth highest globally (2014), with milkfish (*Chanos chanos*) and tilapia (*Oreochromis niloticus*) as the most important cultured organisms (Food and Agriculture Organization 2016). The coastal towns of Bolinao and Anda in the province of Pangasinan, northwest Philippines have become large producers of milkfish locally since they started in 1998 (Verceles et al. 2000). Over time, water quality conditions have deteriorated due to the uncontrolled expansion of mariculture activities in these coastal towns (San Diego-McGlone et al. 2008). Conditions have become eutrophic and the area experienced several HAB events and major fish kills such as during the bloom of *Prorocentrum minimum* in 2002 and *Alexandrium* spp. in 2010 (Azanza and Benico 2013; Escobar et al. 2013). These occurrences show that phytoplankton are not only the

foundational component of ecosystems, but they could also be sentinels to detect significant changes in the environment.

Given the global increase in coastal eutrophication due to mariculture and its consequent negative impacts e.g. HABs (Paerl et al. 2014; Edwards 2015), there is a need to understand community level responses of phytoplankton to help gain insights for future scenarios especially in the wake of a changing climate. In this study, the composition of micro-phytoplankton and zooplankton communities was determined in Bolinao along a gradient of conditions associated with mariculture activities and investigated the drivers of phytoplankton structuring and distribution. The phytoplankton communities during no-fish kill (April) and fish kill (May) occurrences were also examined.

MATERIALS AND METHODS

Study site and sampling stations

Horizontal spatial variation in phytoplankton and zooplankton communities relative to gradients in physico-chemical factors associated with anthropogenic inputs were investigated during an early summer period (20–21 April 2018) inside (with fish cages) and outside the Guiguivanen channel in Bolinao, Pangasinan, Philippines. A total of 16 stations, situated between latitudes $16^{\circ}22'N$ and $16^{\circ}26'N$, and longitudes $119^{\circ}57'E$ and $119^{\circ}54'E$ were established in the area (Figure 1). Ten (10) stations (Guig1 to Guig10) were inside the channel or the mariculture-affected area. Six (6) stations were located outside the channel or the non-mariculture areas, specifically in a coral reef area (Corals), Lucero near the mouth leading to the channel (Lucero), seagrass reserve (SeaGr1 and SeaGr2), and Silaqui island beside a giant clam nursery (Sil1 and Sil2). These stations represented conditions during the no – fish kill period. For comparison, samples were collected in Guig4 during a fish kill event on 30 May 2018. However, due to logistical constraints during the abrupt occurrence of the fish kill, sampling was only done in Guig4 located at the middle of the channel, which was also surrounded by fish pens.

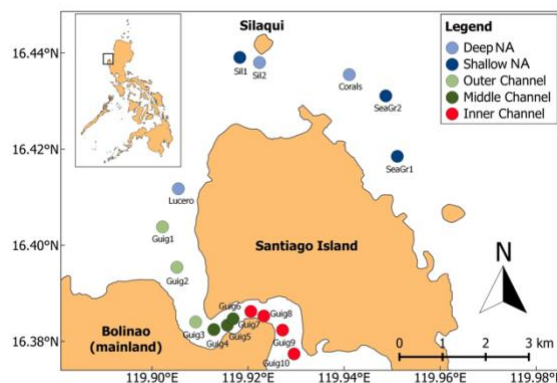


Figure 1: Map of 16 stations surveyed in Bolinao, Pangasinan in April 2018. Inset: Map of the Philippines with Bolinao enclosed in a box. Note: Each station was color-coded based on UPGMA clustering in Figure 2; NA = relatively non-affected waters.

Physico-chemical sampling and analysis

Due to the homogenous characteristic of the upper surface layer, which was indicative of a strong mixed layer, some physico-chemical parameters such as dissolved oxygen (DO), salinity, and temperature were measured using a calibrated HANA multi-parameter at 2 m below the surface. Samples for the other chemical parameters were collected at the same depth using a 5-L Niskin bottle.

Seawater samples for the dissolved inorganic nutrients were filtered through $0.45 \mu\text{m}$ cellulose acetate glass fiber syringe

membrane filters (Sartorius Minisart, Germany). For Chl *a*, a few drops of magnesium carbonate (MgCO₃) solution was added to water samples before being filtered through a 0.7 μm Whatman® GF/F using a 500 mL calibrated Nalgene® vacuum filter holder with a tygon tubing attached to a hand pump.

For analysis, samples were thawed and analyzed separately for NH₄⁺, NO₂⁻, PO₄³⁻, and SiO₄⁴⁻ concentrations following the colorimetric methods of Strickland and Parsons (1972) using a spectrophotometer (Shimadzu UV Mini 1240, Japan). NO₃⁻ concentrations were determined using a shaking technique modified from Jones (1984) to reduce NO₃⁻ to NO₂⁻ using cadmium granules, and subsequently detected using the nitrite colorimetric method. Concentrations obtained from this method were NO_x, which were NO₃⁻ and NO₂⁻. Subtracting the NO₂⁻ concentration from NO_x generated the NO₃⁻ concentration. Analysis of Chl *a* was done in a low light environment to avoid photodegradation. The samples in filter papers were soaked in 10 mL 90% acetone for 24 hours and then sonicated at 40KHz (Cole Palmer®) in a water bath for 10 minutes. After sonication, 5 mL of the solvent was treated with two drops of 1.2 M hydrochloric acid (HCl) for phaeophytin analysis. Both samples were analyzed using a Trilogy® laboratory fluorometer with a specialized built-in module that directly measures fluorescence absorbance for Chl *a* and phaeophytin (μg L⁻¹).

Biological sampling and analysis

Biological and physico-chemical data were collected at 16 stations during the no fish kill period (April) and in Guig 4 during a fish kill event (May). Phytoplankton and zooplankton samples were collected from the upper 2 m depth using a 20-μm mesh size plankton net with 30-cm diameter mouth opening that was towed vertically to filter approximately 141 L of water per deployment. Samples were concentrated to 25 mL, immediately transferred into 250-mL opaque Nalgene® bottles and fixed with concentrated Lugol's iodine solution (Yap et al. 2004).

Plankton samples were quantified using 1 mL aliquots transferred to a Sedgewick-rafter counting chamber and densities were calculated following Yap et al. (2004). Both phytoplankton and zooplankton counts were carried out using a compound light microscope with 100X magnification with replicate counts for each sample. For the phytoplankton, counting was limited to microphytoplankton including diatoms, dinoflagellates, and other microzooplankton such as ciliates and rhizaria. Smaller phytoplankton such as chlorophytes, cyanobacteria, and haptophytes, as well as resting and temporary cysts, were also noted but were not further identified. Identification was done at the lowest taxonomic level when possible following the classification guides of Omura et al. (2013), Yamaji (1986), and Matsuoka and Yasuwo (2000). Micrographs were taken using a compound inverted microscope (Motic AE 2000 Tri at 200X and 400X) and a confocal laser scanning microscope (Carl Zeiss LSM 710, Germany) following Onda et al. (2014). For the zooplankton, similar methods were used, categorized only at the phylum level (e.g., Annelida, Arthropoda, Chordata, Echinodermata, Foraminifera, Mollusca, and Nematoda) following Yamaji (1986). Various eggs and embryos were also noted, and their densities were computed based on Huber (2012) expressed as individuals L⁻¹.

Community structuring and ecological analyses

Stations were clustered using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on Bray-Curtis similarity derived from the measured physico-chemical parameters. On the other hand, phytoplankton community structuring based on Bray-Curtis dissimilarity was computed in PAST v3.0 and plotted in an ordinate system using Principal

Coordinate Analysis (PCoA). The abundance data was first log-transformed to normalize the data and to avoid negative values. The PC1 and PC2 coordinates were then regressed with the environmental variables to determine the possible drivers of community structuring. Correlations between phytoplankton, environmental variables, and zooplankton were determined using Spearman's rank correlation visualized as a heatmap generated in 'corrplot' package in R. Differences in physico-chemical parameters between and among sites were tested using analysis of variance (ANOVA) and student t-tests implemented in PAST v3.0.

RESULTS AND DISCUSSIONS

Environmental gradients in Bolinao stations

Phytoplankton are suggested to follow a ratio (e.g., 16 nitrogen : 1 phosphorus) in assimilating macronutrients, and limitations of either nitrogen (N) or phosphorus (P) could have profound implications (Smith 1984) on the structure and distribution of phytoplankton communities (Tilman et al. 1982). In this study, the 16 stations in Bolinao clustered into three main categories to represent a gradient of physico-chemical characteristics: (1) Guig5 to Guig10 as mariculture-affected waters with total inorganic nitrogen to total inorganic phosphorus (hereafter referred to as TIN:TIP) ratio of <4, (2) Guig1 to Guig4 as transitional waters (TIN:TIP = 4–11), and (3) Corals, Lucero, SeaGr1, SeaGr2, Sil1, and Sil2 as relatively non-affected waters (TIN:TIP>11; Figure 2). The gradient in TIN:TIP could be attributed to coastal eutrophication from the expansion of milkfish farming in the Guiguwanen channel (Vergeles et al. 2000; San Diego-McGlone et al. 2008). The low TIN:TIP suggests N as the limiting nutrient, which is a recurrent characteristic of polluted coastal waters with NO₃⁻ consumed first before PO₄³⁻, while the latter is enriched from the input of fish pellets (Downing et al. 1999; Souchu et al. 2010; Ferrera et al. 2016). The all-year-round fish farming in Bolinao with typically high stocking density has resulted in rapid degradation of water quality primarily due to excessive inputs of nutrients and organic matter from fish excreta and excess feeds (Vergeles et al. 2000; Tovar et al. 2000; Escobar et al. 2013; Ferrera et al. 2016). Unconsumed fish pellets along with fish feces release particulate nitrogen and phosphorus, that settle to the sediments, while metabolic wastes release most of the dissolved forms in the water column (Wu et al. 1994; Wu 1995; Lazzari and Baldisserotto 2008; Liang et al. 2010). Elevated nutrient levels have been observed over a 10-year period from 1995–2005, which led to eutrophic and hypoxic coastal waters (San Diego-McGlone et al. 2008). This was evident in the tenfold increase of nutrients from outside the channel going in (Table 1). Interestingly, lower overall surface NO₃⁻ and PO₄³⁻ concentrations were observed in April 2018 than previously reported (San-Diego McGlone et al. 2008; Ferrera et al. 2016).

In contrast to the TIN:TIP observed across stations, higher levels of SiO₄⁴⁻ (3.15 μM to 9.27 μM) were found in stations outside the channel compared to inside the channel (0.66 μM to 5.75 μM). The submarine groundwater discharge in the Santiago Reef Flat, near the stations outside the channel, may have contributed to these high SiO₄⁴⁻ levels (Senal et al. 2011). The SiO₄⁴⁻ values observed were within the range (4.3–19.1 μM from April to August 2009) reported by Senal et al. (2011).

Aside from nutrient gradients, surface water temperatures inside the channel (30.90 ± 0.49° C) were significantly higher (t-test, *p*<0.001) than temperatures outside (29.90 ± 0.51° C). This may be due to reduced water flow caused by the high density of fish pen structures, limiting flushing in the channel (San Diego-

Table 1: Summary of physico-chemical factors in 16 stations surveyed in mariculture-affected and non-affected areas in Bolinao, Pangasinan in April 2018, including chlorophyll a (Chl a), dissolved oxygen (DO), ammonia (NH₄⁺), nitrate (NO₃⁻), nitrite (NO₂⁻), phosphate (PO₄³⁻), silicate (SiO₄⁴⁻), and total inorganic nitrogen to total inorganic phosphorus ratio (TIN:TIP).

Type	Station	Latitude	Longitude	Chl a (mg L)	DO (ppm)	Nutrients						Phaeophytin (mg/L)	Salinity (psu)	Temp (°C)
						NH ₄ ⁺ (μM)	NO ₃ ⁻ (μM)	NO ₂ ⁻ (μM)	PO ₄ ³⁻ (μM)	SiO ₄ ⁴⁻ (μM)	TIN:TIP			
Transitional waters	Guig1	16.4038	119.9022	1.39	7.00	0.62	1.44	0.09	0.33	3.26	6.484173	0.26	33.97	30.64
	Guig2	16.39538	119.9052	1.34	7.14	0.70	1.97	0.07	0.26	5.19	10.71757	7.88	33.84	30.43
	Guig3	16.38401	119.9091	4.07	6.94	0.72	1.42	0.07	0.40	3.31	5.548683	2.62	33.62	30.53
	Guig4	16.38242	119.9129	5.26	6.34	1.30	1.47	0.09	0.57	6.02	4.982947	2.24	33.90	30.46
Mariculture-affected waters	Guig5	16.38333	119.9157	8.05	6.41	1.61	1.27	0.07	1.10	2.68	2.666534	0.38	33.84	30.80
	Guig6	16.38467	119.9169	20.92	6.46	1.24	1.31	0.09	0.84	2.84	3.143081	12.08	33.95	30.90
	Guig7	16.38619	119.9206	27.79	6.37	1.23	1.29	0.09	1.12	5.99	2.340445	22.88	34.01	30.98
	Guig8	16.38524	119.9233	18.77	7.25	0.74	1.27	0.09	1.04	4.78	2.029726	12.70	34.02	31.46
	Guig9	16.38231	119.9272	29.69	7.49	1.83	1.09	0.09	1.02	3.89	2.968921	22.01	33.96	31.38
	Guig10	16.37736	119.9296	32.17	8.15	1.21	2.16	0.08	2.15	0.86	1.601448 23.41677	18.75	34.25	31.89
	Corals	16.43549	119.9411	0.67	6.90	0.81	1.91	0.11	0.12	6.62		0.37	33.94	29.56
	SeaGr1	16.41848	119.9511	1.49	5.09	0.69	1.48	0.12	0.20	9.59	11.49615	0.13	34.77	30.44
	SeaGr2	16.43106	119.9487	0.40	6.05	0.62	1.35	0.10	0.13	9.08	15.45552	0.27	34.00	29.67
	Non-affected waters	Sil1	16.43906	119.9183	0.23	6.32	1.06	1.53	0.09	0.13	6.40	20.92552	0.09	33.95
Sil2		16.43943	119.9224	0.50	6.24	0.82	1.50	0.10	0.16	4.67	15.24002	0.44	34.05	29.90
Lucero		16.41173	119.9055	0.62	8.47	1.00	1.92	0.12	0.28	3.38	11.03299	0.38	34.06	30.90

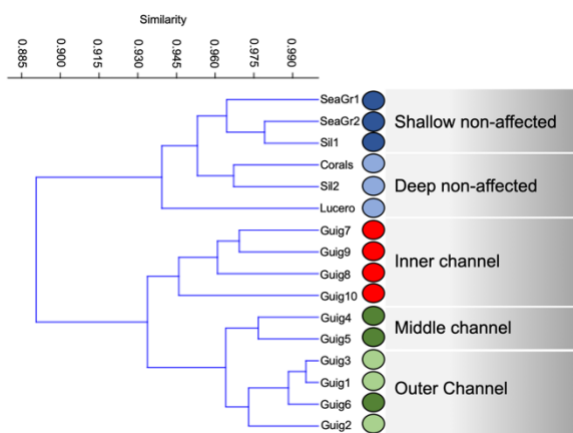


Figure 2: Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering of the stations based on Bray-Curtis similarity derived from physico-chemical factors (chlorophyll-a, chromophoric dissolved organic matter, dissolved oxygen, nitrate, nitrite, phaeophytin, phosphate, salinity, silicate, and temperature).

McGlone et al. 2008). The thermal difference, which creates a typically strong stratification in the channel (Hassenrück 2019), and the decreased flushing rate could potentially inhibit mixing and flow of nutrient-rich waters from inside to outside the channel. These could may in turn influenced the nutrient gradients across stations (San Diego-McGlone et al. 2008; Ferrera et al. 2016). In addition, the continuous supply of N and P from feeds and fish wastes could have sustained the high phytoplankton biomass in the mariculture-affected area based on Chl a during summer. These observations suggest that the strong gradients in physico-chemical factors significantly (see succeeding sections) influenced phytoplankton communities.

Microphytoplankton community structure and distribution

A total of 35 phytoplankton genera were identified from 16 stations in Bolinao, mainly composed of the taxa Bacillariophyceae (diatoms) and Dinophyceae (dinoflagellates) (Figures 3 and 4). Stations inside the channel, that had overall higher nutrient concentrations, were also characterized by higher phytoplankton density ($11,496 \pm 5,540$ cells mL⁻¹) but lower diversity ($H^2=1.79$). On the other hand, stations outside the channel had lower phytoplankton density ($3,533 \pm 1,950$ cells mL⁻¹) but higher diversity ($H^2=1.99$) except in SeaGr1. Such observations are typical of aquatic environments where richness (diversity) increases with decreasing evenness (relative abundance), and vice versa (Smayda and Reynolds 2003; Larson and Belovsky 2013). The high availability of nutrients inside the channel only favored a few species that are colonial, competitive, and/or fast-growing, leading to their dominance and exclusion of other taxa, and thus, lower diversity (Smayda and Reynolds 2003; Romo and Villena 2005). In contrast, low nutrient concentrations outside the channel may have allowed for the co-occurrences of more genera but in lower abundances (Vallina et al. 2014). Specifically, stations in the mid and inner channel (Guig4 to 10) were dominated by the diatom *Leptocylindrus* spp. ($5,592 \pm 3,752$ cells mL⁻¹), and dinoflagellates *Protoperidinium* spp. ($1,825 \pm 1,679$ cells mL⁻¹) and *Scrippsiella* spp. ($2,097 \pm 648$ cells mL⁻¹). The dominance of *Leptocylindrus* spp. may be explained by their consumption of previously high silicate concentrations, which at the time of sampling were already at lower concentrations compared to stations outside of the channel (Nashad et al. 2017). In addition, the dominance of dinoflagellates is thought to be associated with high nutrient concentrations and low turbulence, which were both present inside the channel due to presence of fish pen structures (Figueiras et al. 2006).

Meanwhile, stations outside the channel (Guig1 to Guig3) had higher dinoflagellate cyst densities, both temporary (781 ± 105 cysts mL⁻¹) and resting stages (112 ± 52 cysts mL⁻¹). Cyst formation has been suggested as an escape mechanism against unfavorable factors including nutrient limitation (e.g. Nehring 1996; Onda et al. 2014). Notably, increase in cyst density was accompanied by the drastic decrease in the availability of nutrients from the inner to the middle section of the channel (Table 1). In contrast, deeper (<2 m) stations in non-mariculture areas such as Corals, Lucero, and Sil2 had higher diversity and high abundances of diatoms such as *Chaetoceros* spp., *Cylindrotheca* spp., and *Licmophora* spp. While stations in the shallower non-mariculture areas (<2 m), especially in SeaGr1 and SeaGr2, were dominated by *Chaetoceros* spp. ($1,752 \pm 104$ cells mL⁻¹). Compared to dinoflagellates, diatoms generally have smaller cell sizes but higher surface area to volume ratios, making them more efficient in nutrient acquisition (Taguchi 1976; Irwin et al. 2006). Diatoms are also known to have lower half-saturation values for nitrogenous compounds, that allow them to thrive even in areas with low nitrogen availability (Thomas et al. 1978).

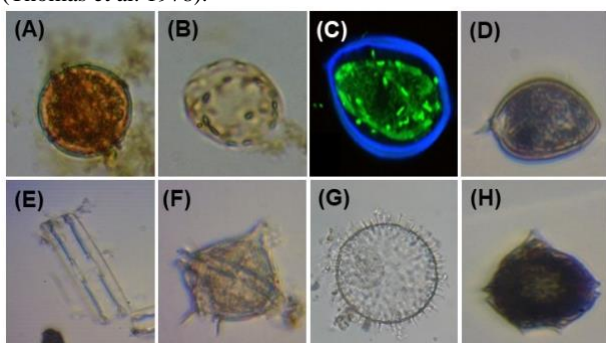


Figure 3: Micrographs of the phytoplankton: (a) *Alexandrium* sp. (400x), (b) *Coscinodiscus* sp. (400x), (c) *Prorocentrum micans* (using a confocal laser scanning microscope, 200x), (d) *P. minimum* (400x), (e) *Pinnularia* sp. (200x), (f) *Protoperidinium* sp. (400x), (g) Dinoflagellate resting cyst (400x), and (h) *Scrippsiella* sp. (400x).

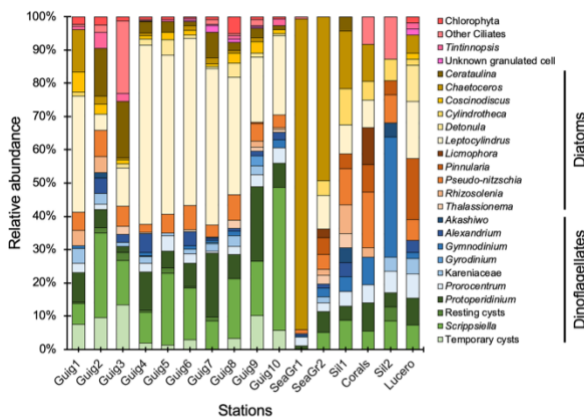


Figure 4: Relative abundance (%) of the dominant taxonomic groups found in the Guiguwanen channel (mariculture-affected area) and non-affected areas in April 2018.

At the community level, samples from different stations formed three distinct clusters based on phytoplankton composition and abundance (beta-diversity; Figure 5), which was also seen in the clustering of physico-chemical parameters (Figure 2). The first and second principal coordinates (Figure 5, PC1 and PC2) accounted for 67.84% of the variance observed. In particular, PC1 was negatively correlated with temperature ($r = -0.73$, $p < 0.001$) and PO₄₃₋ ($r = -0.61$, $p < 0.01$), with both being highest inside the channel, but positively correlated with SiO₄₄₋ ($r = 0.55$,

$p = 0.02$). This indicates that while phytoplankton proliferated better (seen as high Chl *a*) in waters with higher nutrients despite the low TIN:TIP, diversity on the other hand decreased significantly resulting in high similarity in phytoplankton community among stations inside the channel. These results were consistent with Rosenzweig's (1971) "paradox of enrichment", suggesting that increased supply of nutrients through anthropogenic eutrophication would disrupt and lower the diversity of phytoplankton communities by causing increased interspecific competitive interactions within the same trophic level (Tubay et al. 2013).

The composition of the community in specific conditions of resource availability could indicate the efficiency of species in utilizing limited resources (Ptcnik et al. 2008). The unique intra- and inter-specific variations of plankton physiology enable them to proliferate in different environmental conditions (Lagus et al. 2004). For example, most species present inside the channel, such as *Alexandrium* spp., *Leptocylindrus* spp., and *Scrippsiella* spp. have been reported to exhibit low half-saturation constant (K_N), possibly indicating that they are highly opportunistic and adapted to conditions with relatively high nutrient levels (Eppley et al. 1969). On the other hand, phytoplankton with high K_N can grow rapidly under low nutrient conditions. For example, *Chaetoceros* spp. has been reported to proliferate well in nutrient-limited environments (Timmermans et al. 2004), and incidentally the same taxa in the non-mariculture areas were found. However, species sorting may occur wherein species not able to tolerate changes in conditions are replaced by more adapted species. This may then lead to dominance of one or a group of species within the community (Litchman et al. 2012). For example, the dominance of dinoflagellates inside the channel which was characterized by high nutrient but low TIN:TIP and weak turbulence, could be associated with their different strategies to cope with varying nutrient regimes. Many dinoflagellates in particular are known to be mixotrophic, allowing them to combine different strategies to fill in their nutritional needs (Jeong et al. 2010), and to graze on some diatoms (Dagenais-Bellefeuille and Morse 2013; Yoo et al. 2009). Additionally, they undergo encystment (i.e., either resting or temporary) in order to adapt to shifting conditions, which was also observed in high densities in Guig1 to Guig3. They can also form symbiotic relationships with N-fixing and reducing bacteria that could aid them in (re)generation of the necessary inorganic nutrients, thus allowing dinoflagellates to fill in N limitation (Dagenais-Bellefeuille and Morse 2013; Onda et al. 2015). In contrast, diatoms remained constantly abundant in all stations due to their ability to tolerate high amounts of turbulence and oligotrophic conditions such as in stations outside the channel (Margalef 1978; Brzezinski 1985; Moncheva et al. 2001). Although diatoms also exhibited high abundance inside the channel, the tight coupling between their reproduction and mortality could have limited their abundance relative to dinoflagellates (Cloern and Dufford 2005, Ward et al. 2012).

In addition to environmental influences, biological interactions such as grazing by zooplankton may also drive the distribution of phytoplankton communities. In this study, abundances of some zooplankton taxa were negatively correlated with some of the phytoplankton, an indication of potential grazing (Figure 6). Grazing may be based on chemical properties and size, allowing grazers to be either specialist or generalist. For example, copepods utilize both chemical and visual cues to detect their preferred prey (Sommer et al. 2002). However, positive correlations of some zooplankton with certain phytoplankton genera were also observed. For example, Arthropoda was positively correlated with *Scrippsiella* spp., possibly suggesting low to no grazing activity. This was opposite to the findings of

Schnack (1983) where copepods had high feeding rates on *Scrippsiella* spp. over a diatom species due to the larger size of the dinoflagellate. However, unlike the latter experiment where the copepods had only two prey items, the present study involved zooplankters naturally exposed to a wide array of potential prey items, indicating potential prey selection and grazing preferences.

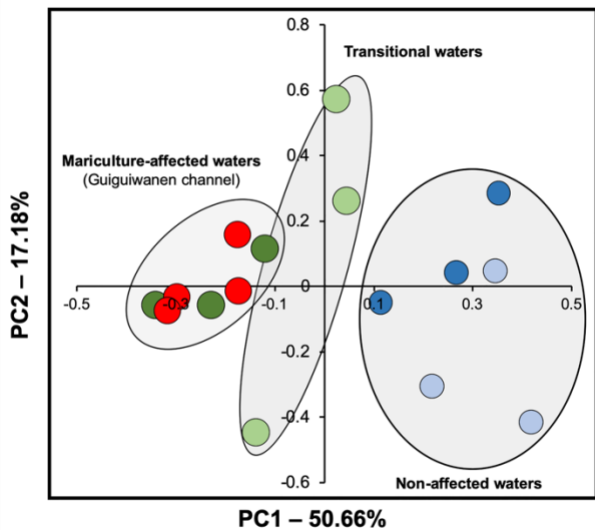


Figure 5: Principal coordinate analysis (PCoA) of the stations based on the dominant taxa (taxa present in at least three sites) with distinct clusters encircled showing three groups of phytoplankton composition based on species abundance and similarity: (1) left cluster – mariculture-affected waters, (2) middle cluster – transitional waters, and (3) right cluster – relatively non-affected waters.

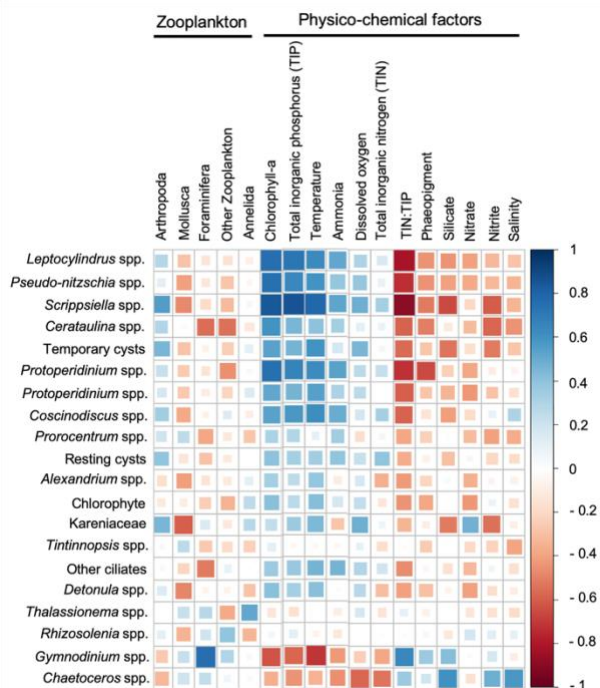


Figure 6: Heatmap showing Spearman's rank correlations of various phytoplankton taxa with zooplankton phyla and environmental variables with a color gradient indicating levels of correlation (bar).

Phytoplankton community structure and diversity are also influenced by biological interactions such as competition (Kremp et al. 2008) and predation (Lynch and Shapiro 1981; Irigoien et al. 2005), along with the prevailing environmental conditions such as nutrient concentration. The latter could also

lead to stable coexistence, several alternative stable states, or complete competitive exclusion by the well-adapted species (Passarge et al. 2006; Barton et al. 2010). Here, nutrient availability seemed to be a strong driver of community structuring and species sorting across environments with dinoflagellates dominating in the mariculture-affected area, while a more diverse diatom-dominated community was observed in the non-mariculture areas. The gradient in Bolinao waters simulates the transition from oligotrophic to eutrophic systems, allowing us to gain insights on possible future scenarios (Heisler et al. 2008).

Eutrophication and HABs

There are accumulating pieces of evidence linking nutrient enrichment or eutrophication to increased frequencies of HABs in coastal environments (e.g., Anderson et al. 2002; Heisler et al. 2008; Davidson et al. 2014). However, it is not just the individual concentrations of N and P that significantly affect HABs formation but the ratio of these two macronutrients (Ferber et al. 2004). For example, the relatively higher nutrient concentrations inside the channel allowed for the higher abundance of dinoflagellates such as HAB-forming species like *Prorocentrum* spp., *Protoperidinium* spp., and *Scrippsiella* spp. but at significantly lower densities than during bloom events (GA Benico, unpublished observations). However, the low TIN:TIP ratio or N-limitation during the summer period could have limited the bloom of toxic dinoflagellates in Guiguivanen channel. According to Ferrera et al. (2016), N inputs from watersheds during the rainy season could have triggered the blooms observed in June of 2002 and May of 2010.

Interestingly, a fish kill event due to hypoxia was reported in Bolinao and Anda waters in 30 May to 2 June 2018 with losses amounting to PhP 107M or USD 2M (Austria and Pasion 2018). According to the Municipal Agricultural Office of Bolinao, increased sea surface temperature and low DO likely caused the fish kill (GMA News 2018). Notably, the event was preceded by sustained average air temperature of 35 °C in Bolinao five days prior to the localized fish kill. Runoff due to heavy rainfall that occurred days prior to the fish kill may have brought in organic matter and nutrients to the area, leading to nutrient enrichment (i.e., N) that contributed to the overgrowth of select microalgae. Nutrients in the middle of the channel (from 2010 to 2014) had higher mean NO₃⁻ concentration during the wet season (0.79 ± 1.17 μM) than dry season (0.49 ± 0.37 μM; Ferrera et al. 2016). In this study, limited sampling at Guig 4 (Figure 7) during the fish kill event revealed that NO₃⁻ concentration increased from 1.46 μM in April to an average of 1.85 μM in the weeks of May 10 to May 20. It was followed by a drastic decline a few days prior to the appearance of dead fish, and then to 0.92 μM on 30th May, the day before the peak of the fish kill. PO₄³⁻ on the other hand, continuously increased prior to the fish kill event (Figure 7). The decrease in NO₃⁻ could indicate its utilization by the phytoplankton before the sampled days, indicating a slow buildup of the bloom since it further decreased to 0.89 μM during the appearance of the dead fish. The decrease in NO₃⁻, however, was accompanied by a sharp increase in NH₄⁺ from 8.30 μM in 30th May to 9.49 μM in 31st May, indicating possible release of N from the decaying fish carcasses (Figure 7).

Water sampling during the fish kill event in Bolinao revealed the high abundance (~99% cell dominance; GA Benico, personal communication; Figure 7) of *Takayama* spp. (Kareniaceae), a non-toxin-producing dinoflagellate that is related to the brevetoxin-producing *Karenia* spp. (Kareniaceae) (de Salas et al. 2005). Interestingly, blooms of *Takayama* spp. in Guiguivanen channel were also observed in 2016 when it co-occurred with the woloszynskiid dinoflagellate *Biecheleriopsis adriatica* (Benico et al. 2019). In our study, although several cells

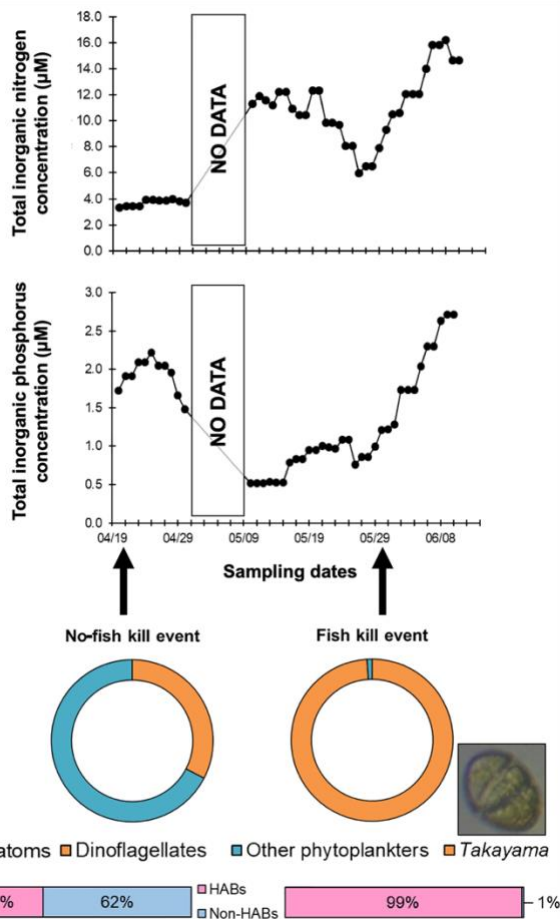


Figure 7: Summary of findings showing the shift from a diverse phytoplankton community during a no fish kill event (April 20-21, 2018) to a dominance of *Takayama* sp. (micrograph image) during a fish kill event (May 30, 2018) in Guig4, Bolinao, Philippines.

belonging to Kareniaceae were detected during the April sampling in the Guiguwanan channel, they were not the most abundant phytoplankton in the community (Figure 4). *Takayama* spp. has also been implicated in hypoxia and fish kill of other embayment and water bodies such as in New Zealand (de Salas et al. 2005), Singapore (Tang et al. 2012), and Florida (Florida Fish and Wildlife Conservation Commission 2018). Factors causing the sudden appearance of *Takayama* spp. in some bays in the Philippines remain unknown. However, warmer surface temperatures could be related to increased HABs occurrences in addition to nutrient loading or eutrophication. Alarming, HABs occurrences are expected to further increase with the changing climate (Wells et al. 2015). This then emphasizes the importance of monitoring and management efforts to mitigate and even prevent formation of HABs and their consequences.

CONCLUSION

Eutrophication in coastal areas has been a major problem across the globe. In the tropics, incidence of eutrophication usually results in algal blooms that can be potentially damaging (Anderson et al. 2002). This study was able to provide a snapshot of how micro-phytoplankton communities are structured in relation to biological interactions and physico-chemical gradients, particularly with nutrient loading. The phytoplankton community in stations inside the channel or in mariculture-affected area was significantly different from the stations outside the channel or in non-mariculture areas. The observed transition in community composition may be influenced by differences in

adaptation to maximize available resources, which could have an implication to ecosystem functioning.

The continuing intensification of mariculture inside the channel pose considerable threats, especially with occurrence of HABs, deterioration of the local ecosystem, and negative impact on economic activities in the area. HABs species are highly opportunistic and further P-enrichment due to fish feeding make the area more vulnerable to such events (Figure 7). The distinct micro-phytoplankton communities thriving under two opposite conditions (inside and outside the channel) provide valuable insights on how phytoplankton communities are structured in relation to varying levels of nutrients. This makes the waters in Bolinao a natural laboratory to test several fundamental microbial ecological questions, from which insights can be gained related to the effects of manmade changes such as eutrophication. Future studies should investigate the vertical distribution of phytoplankton communities in mariculture-affected and non-affected areas, the seasonal (dry and wet season) variability in terms of phytoplankton productivity and succession, and how climate change affects both the phytoplankton community structure and distribution.

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