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Phytoplankton Diversity and Macroinvertebrate Assemblage as Pollution Indicators in Sapangdaku River, Toledo City, Cebu, Philippines

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Abstract

The study assessed the phytoplankton diversity and macroinvertebrate assemblage in Sapangdaku River, Toledo City in Cebu, Philippines in relation to the habitat preference of the organisms. Three sampling stations were identified as downstream, midstream and upstream and 5 samples were collected in each site. Phytoplanktons were hauled vertically following the Bottle sampling method and were identified at the species and/or genus level using phytoplankton identification manuals. Macroinvertebrates were surveyed using the Kick Sampling method, and were identified up to the Order level using online taxonomic guides. Findings revealed that 16 phytoplankton genera belonging to five phyla, as well as eight macroinvertebrate orders, characterize the river. Genus Bacillariophyceae and Order Insecta are the most numerous in the freshwater sources. The downstream portion of the river, as indicated by the diversity of phytoplanktons and assemblage of macroinvertebrates, may reflect poor river water quality.

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Introduction

Biological indicators are species, which by their presence, provide information and indication of the status of their surrounding physical and/or chemical environment due to its rapid response to any biogeographic changes such as water pollution (Bellinger and Sigee, 2010; Parmar *et al.*, 2016). Biomonitoring has long been used at the onset of industrial revolution where habitat destructions started to emerge (Cairns and Pratt, 1993). This method has been primarily employed for water quality monitoring and assessment due to its advantages such as: (1) early state diagnosis; (2) easy enumeration due to pervasiveness and high abundance; (3) cost-efficient than standard measuring systems (Pourafrasyabi and Ramenzanpour, 2014; Parmar *et al.*, 2016), and (4) high ecological relevance (Bellinger and Sigee, 2010). In recent years, both phytoplanktons and macroinvertebrates are widely used for biomonitoring water quality in freshwater (Dacayana *et al.*, 2013; Sharma *et al.*, 2013; Jindal *et al.*, 2014; Veronica *et al.*, 2014).

Phytoplankton is among the primary producers of the aquatic food web in the aquatic ecosystem (Pourafrasyabi and Ramenzanpour, 2014; Parmar *et al.*, 2016; Barinova and Krupa, 2017). They photosynthesize by converting light and inorganic nutrients into organic material that freshwater and ocean life (Brahic, 2006; Valencar and Desai, 2004). Phytoplankton richness, distribution and diversity contribute to the productivity of water systems (Asis *et al.*, 2006), hence, these organisms are used as effective tool for monitoring water quality as bioindicators (Jindal *et al.*, 2014; Veronica *et al.*, 2014) since they are sensitive to environmental changes (Parmar *et al.*, 2016; Bellinger and Sigee, 2010). Phytoplanktons as tools for water quality assessment have been studied in freshwater areas such as river basins in Iran (Pourafrasyabi and Ramenzanpour, 2014) and lake in India (Jindal *et al.*, 2014). In the Philippines, several initiatives have been conducted to assess the abundance and distribution of phytoplanktons compliant to RA 927 or Philippine Clean Water Act. Local researchers have studied such abundance and distribution in different areas such as in Calamianes Islands in Palawan (Asis *et al.*, 2006), Boracay Island in Aklan (Limates *et al.*, 2016) and Lake Buhi in Camarines Sur (Baloloy *et al.*, 2016).

Along with phytoplanktons, benthic macroinvertebrates have long been used for monitoring several conditions in freshwater habitats. Researchers have characterized macroinvertebrates as slow moving, with longer life expectancy, and more sensitive or tolerant to water condition. These attributes made them ideal as indicator species in water quality monitoring (Supereles and Zafaralla, 2008; Sharma *et al.*, 2013; Dacayana *et al.*, 2013). W\ater conditions in freshwater habitats such as flow, light and nutrient availability create macroinvertebrate pattern (Dewson *et al.*, 2007; Dacayana *et al.*, 2013; Sharma *et al.*, 2013; Labajo-Villantes and Nuñeza, 2015). The common pattern of benthic macroinvertebrate assemblage is having a higher diversity index in the midstream compared to the upstream and downstream, following the humped-back model. However, this is not always true as indicated in the work of Flores and Zafaralla (2012). Edward and Ugwumba (2011) noted that diversity of habitat and its condition contributed to the difference in the assemblage of macroinvertebrates. The diversity of freshwater habitat may imply resource partitioning among macroinvertebrates, and its condition may result to varying diversity due to the difference in sensitivity of macro-invertebrates. Upstream species include pollution sensitive and moderately-sensitive species which indicate less polluted habitat while the downstream species include less-sensitive species (Flores and Zafaralla, 2012; Dacayana *et al.*, 2013). In Cebu Island, there are only few, yet distant past studies reported focusing on the biological and chemical investigations in aquatic systems. To mention some are the ecological zonation of gastropods in the Matutinao River (Bandel and Riedel, 1998), a study on the benthic organisms in the intertidal zone in Mactan (Faubel, 1984), the investigation of seawater bugs in Badian (Bendanillo *et al.*, 2016) and the study of zooplankton composition and abundance during the oil spill of MV Saint Thomas Aquinas in Mactan Island (Flores *et al.*, 2020). Lotic systems such as the river has weak water currents believed to always contain phytoplankton (Jindal *et al.*, 2014) and the Sapangdako River is one of the rivers in Cebu that requires water quality assessment due to reported sighting of the endemic species freshwater goby, *Sicyopus cebuensis* (Chen and Shao, 1998). This threatened species requires clean and pristine streams or rivers to survive. It was last seen in 1998 in Uling, City of Naga, Cebu and anecdotal reports from fisherfolks claimed that they have seen it recently. The intermittent sighting of this endemic fish is an indication that increased eutrophication in the river water may have caused the disappearance of the fish. It can be associated to the rich diversity of phytoplanktons present in the river as the *S. cebuensis* feeds on phytoplanktons. Aquatic biota in the river is oftentimes affected by current, erosion and sedimentation (Veronica *et al.*, 2014). The whole stretch of the river is manifested by various anthropogenic activities, as the Sapangdako River is inhabited with residents whose livelihood is heavily dependent on the river and the whole locale for mining and quarrying. Midstream and downstream portion of the river are also surrounded with residential structures in which anthropogenic activities contribute to the water quality condition. Hence, the number of phytoplanktons and macroinvertebrates varies as water travels down the downstream as the organisms are displaced by the current and the other factors such as the amount of nutrient content, DO, temperature, pH, salinity, among other. In this study, diversity of phytoplanktons and assemblage of benthic macroinvertebrates were assessed in relation to their habitat preference in different portions of the river such as upstream, midstream, and downstream.

Materials and methods

Research Site

This descriptive survey design identified the phytoplanktons and benthic macroinvertebrates present in Sapangdaku River as components in assessing the river water quality. Three sampling stations were selected up to 100 meters from the head of the river where three station points were established namely upstream (10°21'2.592" N, 123°41'34.368" E), midstream (10°21'32.364" N, 123°39'50.544" E), and downstream (10°23'28.932" N, 123°39'6.156" E). In each station, there were 5 substations (100 meter apart for each substation) chosen to obtain sample variations in the area.

Phytoplankton Survey

Phytoplanktons were hauled vertically from the river water following the Bottle sampling method (Valencar and Desai, 2004). For each sample, 100-mL sterile PET bottle was immersed in the middle surface of the water to collect the phytoplanktons. Immediately after collection, it was preserved with 1 mL of 1% Lugol's solution and covered. It was then transported to Cebu Normal University Biology Laboratory where the preserved sample was shaken for 30 seconds.

A drop of the sample was fixed in a hemacytomer and was observed for the presence of phytoplanktons under a compound microscope. Morphological structure used includes cell wall structure, frustule markings such as the presence of locomotive structures (i.e., flagella, cilia), colonies formed, colour, and shape. The images viewed in the microscope were compared to that in the identification guides of Bellinger and Sigge (2010) and the training manual by Gopinathan *et al.* (2007) at the species and/or genus level.

Macroinvertebrate Survey

Benthic macroinvertebrate survey was done using Kick Sampling Method (Hayslip, 2007). An identified area (approx. 61 cm x 61 cm) was kicked or disturbed by the kicker's foot for about 3 minutes. Dislodged invertebrates were collected downstream (approx. 1 meter away from the kicker) through a scooping motion using a D-frame net made of nylon with a mesh size of about 0.50-0.80 mm. The collected samples were washed with water and were placed in a white plastic container. Other macroinvertebrates present in the riverbanks (not caught in the Kick

Sampling) were also recorded. Morphological characteristics such as primary metal placement, head and dorsal sclerite patterns and others were used for identification of samples. Collected samples were identified up to the Order level only using online taxonomic guides.

Results and discussions

Diversity of Phytoplankton in Sapangdaku River

Results of the phytoplankton survey are presented in Table 1 below. Phytoplanktons comprised of 16 genera from five phyla were collected in three sampling river stations namely upstream, midstream, and downstream. The dominant phytoplankton was from family Bacillariophyceae followed in order by Cyanophyceae, Chlorophyceae, Chrysophyceae and Euglenophyceae. It is notable that in all points of the river, Bacillariophyceae has been consistently observed while the rest of the phytoplanktons' genera were mostly found in the downstream region. The observed presence could be attributed to the nutrient concentrations as well as other abiotic factors.

Table 1. Phytoplankton genus list for three stations in Sapangdaku River.

Sampling Station	Downstream					Midstream					Upstream				
Area	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Bacillariophyceae															
Thallassiosira	+	+	+	+			+				+	+			+
Synedra	+	+	+				+	+		+				+	
Licmophora				+		+									
Nitzchia		+		+											
Rhaphoneis		+													
Climacosphenia			+							+					
Cyanophyceae															
Trichodesmium															
Spirulina			+		+										
Oscillatoria	+														
Chlorophyceae															
Synechocystis															
Nannochloropsis			+					+	+						
Chlorella		+													
Spirogyra				+		+									
Chrysophyceae															
Chrysococcus	+	+	+		+			+			+				+
Oochromonas	+	+	+	+		+				+		+		+	
Euglenophyceae															
Euglena	+			+											

(+ = observed).

Planktonic species abundance is not consistent all throughout the three sampling sites which may be attributed to anthropogenic activities surrounding the river as well as various biotic and abiotic ecological components. Diatoms (members of Bacillariophyceae) were the highest number of phytoplanktons identified from the sampling stations which is similar to the findings in Lake Buhi, Camarines Sur (Baloloy *et al*., 2016). The abundance of pollution tolerant species could be attributed to the aquaculture activities present in the freshwater sources, which contributes to high nutrient availability.

In the upstream river, there were very few genera of phytoplanktons collected which is likely due to the strong river current and since phytoplankton are drifters, they are easily carried away by the water (Veronica *et al.*, 2014). Hence, they tend to be more concentrated in the downstream where current flow is low. Bellinger and Sigee (2010) reported that phytoplanktons build-up requires only low rate of flow and adequate amount of light to reproduce and this is evident in the diverse presence of Bacillophyceae in the river. The relatively high temperature in the area and light intensity that reaches the river water also favors the reproduction and photosynthetic activities of phytoplanktons (Yap-Dejeto and Batula, 2016).

Furthermore, the differences of phytoplanktons diversity among sampling sites, as purported by Baloloy *et al.* (2016), is caused by the variation in the physicochemical aspects of the water. In the present study, the downstream portion of the river is believed to have poor water quality as reflected by the presence of pollution-tolerant phytoplankton species (e.g. Bacillariophyceae, Chrysophyceae). Asis *et al.* (2006) accounted such abundance to the increase of nutrients transported from the upstream going down, while Parmar *et al.* (2016) attributed this to the high concentration of phosphorus and nitrogen indicating the increased rate of planktons reproduction. Conversely, Barinova and Krupa (2017) found out that the upper watershed of the river is the most polluted part as manifested by the high abundance of phytoplanktons due to the water source that is influenced by agricultural and industrial run-off. The diversity and richness of phytoplankton is dependent upon the water condition particularly the availability of nutrients N and P that are influenced by the anthropogenic inputs. Each species or group of phytoplankton has different reaction to nutrients causing their abundance could be different in different body of waters (Veronica *et al.*, 2014; Yap-Dejeto and Batula, 2016).

Diatoms or Bacillophyceae have been widely used in assessing the human impacts on the freshwater quality (Bellinger and Sigee, 2010). Two species of this phylum is of significant indication namely, Synedra ulna and Nitzchia palea, as these species are most tolerant to severe nutrient pollution. In this study, the habitat preference of the genera of phytoplanktons identified may reflect the water condition of the river. The bioindication implied that the downstream portion of the river is highly enriched with nutrients indicating eutrophication.

Assemblage of Macroinvertebrates in Sapangdaku River

The macroinvertebrates present in Sapangdaku River are shown in Table 2. Ten species of macroinvertebrates were observed during the survey in Sapangdaku River, Toledo City. These species were under Class Insecta, Class Arachnida, Class Malacostraca and Class Crustacea. Table 2 showed the different Orders in each Class of Phylum Arthropoda seen in the three sampling sites. It is notable that scuds, shrimps and crabs were only present in the downstream of the river. The rest of the observed species were present in all sampling sites.

The survey identified six species of macroinvertebrates that are useful as indicator of the water quality status of the area. These species includes crayfish, crabs, riffle beetles, water strider, dragonflies and freshwater shrimps. Crayfish (Voshell, 2002; Reynolds *et al.*, 2013), crabs (Sanders *et al.*, 1998), water strider (Pal *et al.*, 2012), freshwater shrimps (Voshell, 2002; Webb, 2011) and dragonflies (Sahlén and Ekestubbe, 2001) were somewhat tolerant species to pollution while riffle beetles (Elliott, 2008) is considered as intolerant species to pollution. The result of the survey conforms to this data except for the riffle beetles where it is present in all sampling sites.

Richardson (1925; 1929) and Gaufin (1958) identified crustaceans along with odonates as intolerant species to water pollution, where they could only be found in clean water (Cairns and Parts, 1993). Among the crustacean species, crayfish is considered as tolerant species to pollution. This species was present only in the downstream area of Sapangdako River. Alikhan *et al.* (1990) reported that crayfish absorb heavy metals such as copper, magnesium, zinc, nickel and cadmium in several parts of the body of the organisms such as the hepatopancreas, gills, exoskeleton and viscera with the highest concentrations in the gut, gills and hepatopancreas although the author indicated that the use of crayfish as an indicator species of heavy metal pollution is still debatable.

The study of David (2003) on bioaccumulation of heavy metals of macroinvertebrate species in Philippine streams particularly on caddisflies showed a positive result where caddisflies have high contaminants of copper in their body. The utilization of water resource of winged macroinvertebrates such as the dragonflies and other flies made them vulnerable to heavy metal accumulation. This could in turn be used as indication of the pollution of the rivers. Although this is outside of the scope of the present study but it is noteworthy of the possibility of using the species for future studies on heavy metal contamination.

Table 2. Orders of macroinvertebrates present in Sapangdaku River.

Order	Downstream	Midstream	Upstream
Arachnida			
Araneae (spiders)	+	+	+
Crustacea			
Decapoda (crayfish,crabs)	+		
Insecta			
Coleoptera (riffle beetles)	+	+	+
Diptera (flies)	+	+	+
Heteroptera (water striders)	+	+	+
Hymenoptera (ants)	+	+	+
Odonata (dragonflies)	+	+	+
Malacostraca			
Amphipoda (freshwater shrimp, scuds)	+		

(+ = observed).

Riffle beetle is the only observed species that is not tolerant to pollution. This species is usually found in running water. Elliot (2008) identified riffle beetles as sensitive to pollution and other conditions in the river such as water flow and temperature, which make it an excellent indicator of water pollution and climate change. However, in this survey, riffle beetles were found in upstream to downstream areas. Its presence in the downstream areas is probably due to drift movements in the water.

Conclusion and future directions

The diversity of phytoplanktons in Sapangdako River is characterized by sixteen genera that belong to the five phyla. The variation in the number of phytoplanktons observed may be attributed to various factors such as the water current, temperature, nutrient content among many others which may have been induced by the anthropogenic activities. The downstream portion of the river as indicated by the diversity of phytoplanktons may reflect poor river water quality. The water quality of Sapangdaku in Toledo City is somewhat polluted based on the presence of the following pollution tolerant macro invertebrates species - crayfish, crabs, scuds, water strider and dragonflies particularly on the downstream. The presence of riffle beetles all sampling sites which needs further verification as this species is sensitive to pollution.

This study provided only a one-time picture of Sapangdaku River. For future directions, constant monitoring of the freshwater indicator species is recommended for proper management of this important resource in Toledo City. It is also recommended to identify up to the species level of the different species recorded.

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