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RESEARCH PAPER

Particle selectivity based on pre-ingested phytoplankton composition in Charru Mussel *Mytella strigata* (Hanley, 1843)

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Abstract

Identification of phytoplankton trapped in the gills suggests initial selectivity and food preference in marine bivalve molluscs. In this study, pre-ingested phytoplankton in the gills of *Mytella strigata* were determined and compared to the existing phytoplankton in the waters of three sites in the Philippines (Pangasinan, Cavite, and Bataan). Pre-ingested phytoplankton of *M. strigata* varied with size ($p<0.05$) and site ($p<0.05$). *Coscinodiscus* spp. and *Skeletonema* spp. were most abundant across sizes (<20mm, 20–40mm, and >40mm) and sites. Particle selection observed in the gills suggests food preference for centric diatoms such as *Coscinodiscus* spp., *Skeletonema* spp., and *Navicula* spp. The ingestion of these diatoms ranged from 26% to 46% of the total phytoplankton composition in the waters of different sites. The results highlight particle selection during filtration and indicate a potential food preference of *M. strigata*.

Introduction

Bivalves filter food particles from the water column using their cilia-lined grooves sorting system, while deposit-feeders use their modified labial palps (Leal 2002). Their gills serve as sieve and the particle retention depends on the movement of the latero-frontal cilia, which varies among species (Dral 1967). When resting on a substrate, water is drawn into the bivalve through the inhalant opening or siphon and the gills. Particles trapped in the gills are considered to be pre-ingested particles (Widdows *et al*. 1979). Particles are then transferred along the ctenidium to the labial palps, which assists in particle selection. After selection, some particles are rejected as pseudo-feces while others are ingested through the mouth (Gosling 2003). Suspension feeders such as *Cerastoderma edule*, *Mya arenaria* and *Mytilus edulis* take their food out of the water column (Purchon 1968). In contrast, others take their food both from the surface of the sediment and out of the water column, like the tellinid clams *Macoma balthica*, *Scrobicularia plana* (Hummel 1985; Thompson and Nichols 1988), and *Placuna placenta* (Stella *et al*. 2010).

Bivalves feed on phytoplankton (Gosling 2003), including species that vary in size and other structural characteristics (Shumway et al. 1990), as well as on bacteria, zooplankton, and detritus organisms (Vakily 1989; Langdon and Newell 1990; and Lehane and Davenport 2002). Previous studies reported that bivalve grazing might influence phytoplankton abundance (Cloern 1982; Noren *et al*. 1999), eutrophication (Officer *et al.* 1982), trophic dynamics (Huang *et al.* 2008), light penetration (King and Mc Neal 2010), energy and nutrient flux of benthopelagic communities (Dame 1996) and hypoxia in estuaries (Konrad 2014). Several studies have reported that species in the family Mytilidae demonstrate selective feeding behavior towards phytoplankton with a higher preference for dinoflagellates (Soon and Ransangan 2014; Sivalingam 1977). Sidari (1998) revealed that *Mytillus galloprovincialis* seemed to feed on dinoflagellates rather than diatoms. *M. galloprovincialis* in the Adriatic Sea ingested Tintinnids (Jasprica 1997). Kreeger and Newell (2001) showed that the mussel *Geukensia demissa* preferred resuspended benthic microalgae over phytoplankton. Cowden *et al*. (1984) observed *Mytilus edulis* ingested larvae of polychaetes, asteroids, gastropods, and echinoids in laboratory settings. Mesozooplankton such as amphipods (size up to 6 mm), nematodes, polychates and *Carcinus maenas* zoea (2 mm) are being ingested by *M. edulis* larvae (Davenport *et al*. 2000) exhibiting particle selection (Kiorboe *et al*. 1980). Riisgard *et al*. (1980) reported that *M. edulis* larvae (5 and 13 days old with lengths 150 to 170 mm) cannot ingest particles with diameters smaller than 1 µm or larger than 9 µm. The same result was found by Sprung (1984) using 112–150 µm mussel larvae. In addition, Bass *et al*. (1990) found that *M. mercenaria* can filter cyanobacteria and picoplankton *Nannochloris atomus* 3 µm in diameter. The size of the mouth and esophagus may have contributed to the size selectivity of the veligers.

In early 2000, *Mytella strigata*, a non-native species, affected wild green mussel spats and other bivalves observed in the Philippines (Rice 2016). *M. strigata* is an epifaunal estuarine species colonizing rocky substrates in the Atlantic and Caribbean coasts of South America (Scarabino *et al*. 1975). Massive colonization of this species in different areas in the country has become a significant problem for the local farmers. This study was conducted to identify the pre-ingested phytoplankton in the gills of *M. strigata* as an indicator of its initial food particle selection. The influence of factors such as the size of *M. strigata* and the phytoplankton composition of sampling areas was also determined. The results of this study may explain the cause of the rapid proliferation of this organism in the waters of the Philippines and may reveal its invasive mechanism.

Materials and Methods

Sampling was conducted during November 2016 when *M. strigata* was abundant in (1) Abucay, Bataan (N 14043.057'; E 120033.862'), (2) Bacoor, Cavite (N14029.087'; E 120056.820') and (3) Lucao, Pangasinan (N 16001.282'; E120018.848) (Fig. 1). Sites were selected based on the abundance of charru mussel reported during a preliminary interview with the mussel farmers. Charru mussels were collected from bamboo, nets, and rubber tire substrate at 2 to 3 m depth. Pooled samples from different substrates were photographed with size reference, and 30 pieces were preserved in 10% buffered formalin and were further processed in the laboratory.

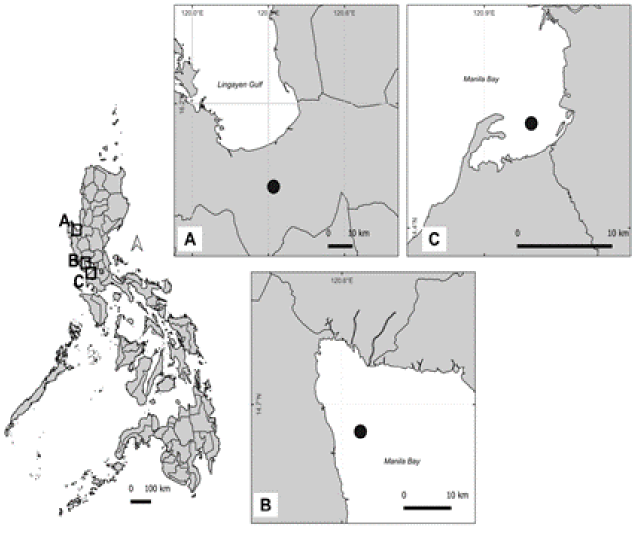

Figure 1. Study sites in (A) Pangasinan, (B) Bataan and (C) Cavite, Philippines.

Triplicate water samples were collected using a 50-L plastic container from approximately 0.5-meter sub-surface depth to determine the phytoplankton abundance and composition of each site. Pooled water samples were filtered in a 28-µm mesh phytoplankton net, preserved in Lugol's solution, and added with distilled water in a beaker to obtain 100 mL. One (1) ml of sub-sample was collected while constantly stirred for homogenization and transferred to Sedgewick rafter or counting under a compound microscope (Motic, USA). For replication, the same steps were followed 5 times. Photos of phytoplankton were captured using Motic software and identified using the guidebook of Isamu Yamaji (1966). All phytoplankton identified were pooled and averaged (n = 5). The diversity index was computed using Shannon and Simpson's diversity index (Table 1). To determine the pre-ingested phytoplankton, thirty (30) whole charru mussels from each size class (<20 mm, 20–40 mm, and >40 mm) were partially opened using a knife and were preserved in 10 % buffered formalin. In the laboratory, meat was separated from the shell. All gills were separated and scraped using a scalpel and transferred to a petri dish. Samples were homogenized and added to distilled water for a 10 mL volume. One mL of well-mixed samples was pipetted and moved into the Sedgewick rafter in triplicate. Phytoplankton was then counted and identified using a compound microscope. Photos were captured, and images of the samples were identified (Yamaji 1966). Pre-ingested phytoplankton abundance and composition were calculated using standard formula (Table 1).

Table 1. Summary of formula used in the study to determine different variables.

Variable (no. of samples)	**Formula**	**Description**	**References**
Phytoplankton Abundance	PA = (n/N) x 100%	PA = phytoplankton abundance n = total count per taxa N = total count of pooled taxa	Heip, *et al.* (1998)
Phytoplankton Composition	PC = (n/N) x 100%	PC = Phytoplankton composition n = total no. of species N = total phytoplankton species	
Phytoplankton Diversity Index (n = 30)	Shannon Diversity Index H’ = - Σ((n1/N) ln (n1/N)	H = Diversity Index n1 = number of samples N = total samples	
	Simpson's Diversity Index D = N (N-1) /Σ [n(n-1)]	D = diversity N =total no. of organisms n = no. of individual	
	Evenness E=H/S	E = Evenness H = Value derived from Shannon Index of Diversity S = No. of taxa identified	

Results

Phytoplankton composition in mussel areas

Results showed diverse phytoplankton species in water samples from Bataan, Cavite, and Pangasinan (Table 2). Out of 1406 total phytoplankton identified in Bataan, 66% were *Skeletonema* spp., 14.01% were *Ceratium* spp., 4.77% were *Tintinnopsis* spp., and 15.22 % of which were species with lower than 3% abundance including *Dytilum* spp., *Chaetoceros* spp., *Thalasionema* spp., *Gonyaulax* spp., *Rhizosolenia* spp., *Chaetoceros* spp., *Nitszchia* spp., *Hemialus* spp., *Biddulphia* spp., *Coscinodiscus* spp., *Hyalodiscus* spp., *Melosira* spp., *Proroceratium* spp., *Mougeotia* spp., *Eucampia* spp., and *Coscinosira* spp. (Fig. 2).

Table 2. Diversity profile of the phytoplankton community in three sampling sites (n = 30).

Diversity Index	**Bataan**	**Cavite**	**Pang**	**p-value (p<0.05)**	**Post Hoc (Tukey Test)**
Composition	18	11	14	0.110	(Bataan=Cavite=Pangasinan)
Abundance	469	69	114	0.000	(Bataan>Cavite=Pangasinan)
Shannon (H)	1.33	1.76	1.84	0.076	(Bataan=Cavite=Pangasinan)
Simpson's	2.22	3.92	4.47	0.016	(Bataan>Cavite>Pangasinan)
Evenness (e^H/S)	1.08	1.17	1.16	0.010	(Bataan<Cavite=Pangasinan)

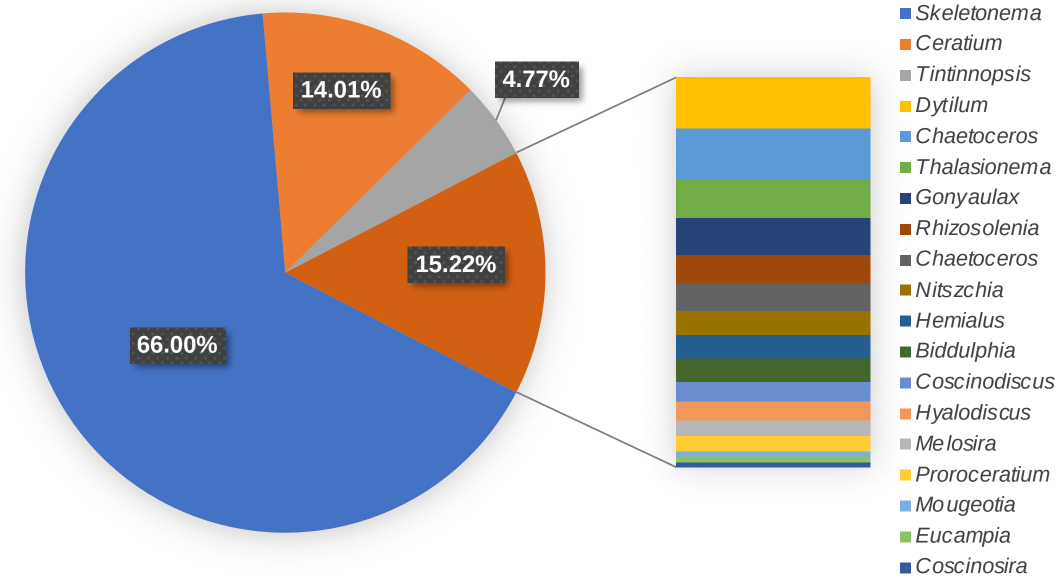

Figure. 2. Relative abundance of phytoplankton species in Abucay, Bataan. The dominant species are *Skeletonema* spp. (66%), *Ceratium* spp. (14.01%), and *Tintinnopsis* spp. (4.77%). The secondary bar graph reveals phytoplankton species with less than 3.0% abundance from a total of 15.22%.

Skeletonema spp. comprising 33.97%, was also found to be the most abundant phytoplankton in Bacoor, Cavite, followed by *Thalasiosera* spp. (19.14%), *Melosira* spp. (10.05%), *Gonyaulax* spp. (6.70%), *Coscinodiscus* spp. (5.74%), *Mougeotia* spp. (5.26%), *Dytilum* spp. (4.31%) and *Cyclotella* spp. (3.83%). Species with lower than 3.0% abundance include *Titinnopsis* spp., *Thalasionema* spp., *Pleurosigma* spp., *Hyalodiscus* spp., *Mougeotia* spp., *Proroceratium* spp., *Biddulphia* spp., *Triceratium* spp., *Peridinium* spp. A total of 17 phytoplankton species were found in the water samples collected (Fig. 3).

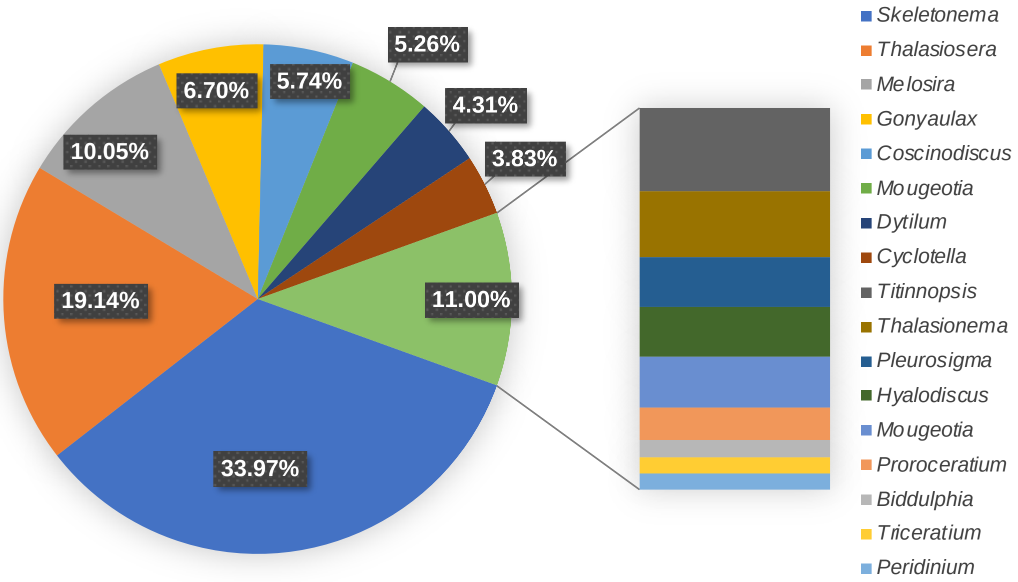

Figure 3. Relative abundance of phytoplankton species in Bacoor, Cavite. Phytoplankton with higher abundance includes *Skeletonema* spp. (33.97%), *Thalasiosera* spp. (19.14%), and *Melosira* spp. (10.05%). The secondary bar graph revealed phytoplankton species with less than 3.0% abundance from a total of 11.0%.

In Lucao, Pangasinan, 22 phytoplankton species were identified. Among them, *Actinoptychus* spp. had the highest percentage of 37.54%, followed by *Coscinodiscus* spp. (17.01%), *Thalasiosera* spp. (16.12%), *Bacteriastrum* spp. (5.87%), *Triceratium* spp. (5.28%) and *Tintinnopsis* spp. (4.11%). Species with lower than 3.0% percentage abundance include, *Pleurosigma* spp., *Leptocylindrus* spp., *Melosira* spp., *Gyrodinium* spp., *Skeletonema* spp., *Rhizosolenia* spp., *Chaetoceros* spp., *Asterolampra* spp., *Biddulphia* spp., *Ceratium* spp., *Dictyocha* spp., *Fragilaria* spp., *Protoperidinium* spp., *Thalasionema* spp., *Gymnodinium* spp., and *Gonyaulax* spp. (Fig. 4).

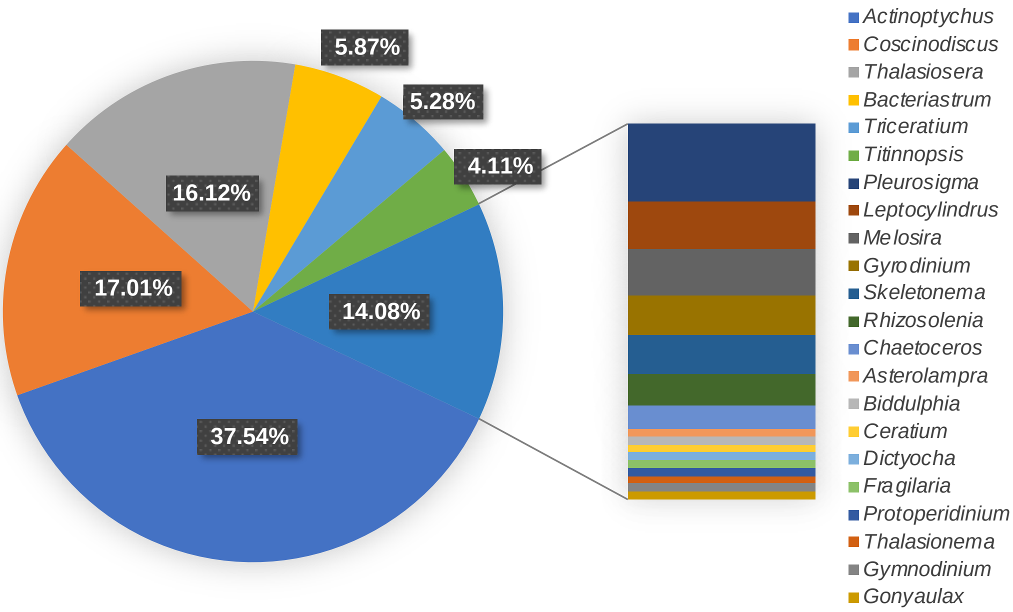

Figure 4. Relative abundance of phytoplankton in Lucao, Pangasinan. Phytoplankton with higher abundance includes *Actinoptychus* spp. (37.54%), *Coscinodiscus* spp. (17.01%), and *Thalasiosera* spp. (16.12%). The secondary bar graph revealed phytoplankton with less than 3.0% abuindance from a total of 14.0%.

The majority of phytoplankton identified were centric diatoms. Diatoms showed significantly higher ($p<0.05$) abundance in the different sites than dinoflagellates and chlorophytes (Fig. 5). Phytoplankton abundance was significantly higher ($p>0.05$) in Bataan (469) than in Pangasinan and Cavite (114). However, Shannon and Simpson's Diversity Index showed significantly lower diversity in Bataan (1.33 and 2.22) than in Pangasinan (1.84 and 4.47) and Cavite (1.76 and 3.92).

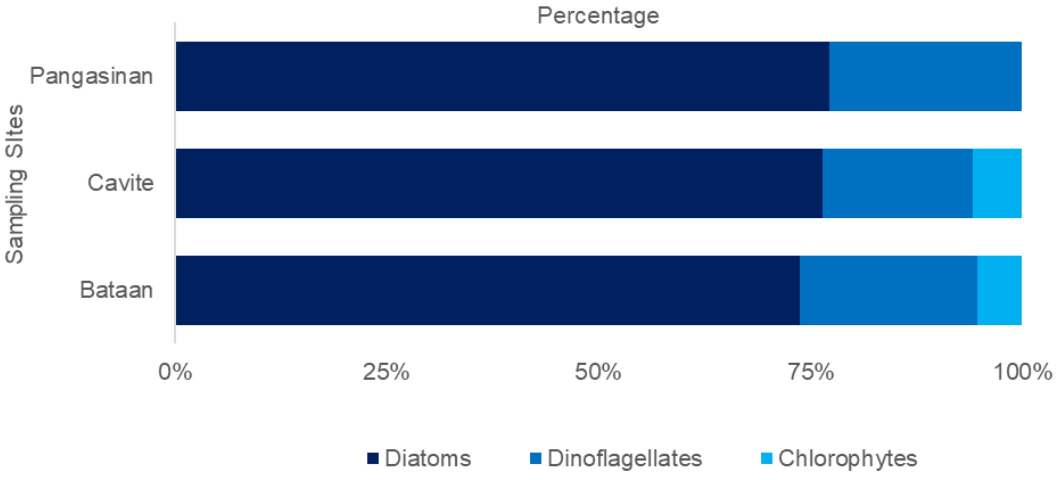

Figure 5. Phytoplankton abundance per group (Diatoms, Dinoflagellates, and Chlorophytes) in three sampling sites (Pangasinan, Cavite, and Bataan) presented as percentage.

Pre-ingested phytoplankton of charru mussel

Pre-ingested phytoplankton of *M. strigata* varied with size ($p<0.05$) and site ($p<0.05$). *Coscinodiscus* spp. and *Skeletonema* spp. had the highest abundance in different sizes and sites (Fig. 6). The same species were abundant in the water samples collected in Bataan and Cavite. *Navicula* spp. was also common but was not found in Pangasinan in size 20-40 mm. Phytoplankton composition was significantly higher in Pangasinan than in Bataan and Cavite ($p>0.05$, Tukey Test: Bataan=Cavite=Pangasinan) (Fig. 7). Size affected phytoplankton abundance in Bataan but not in Cavite and Pangasinan ($p>0.05$, Tukey Test: <20mm = 20–40mm < >40mm). The ingested material in the mussel samples ranged from 26% to 46% of the total phytoplankton composition in the waters of the different sites (Fig. 8).

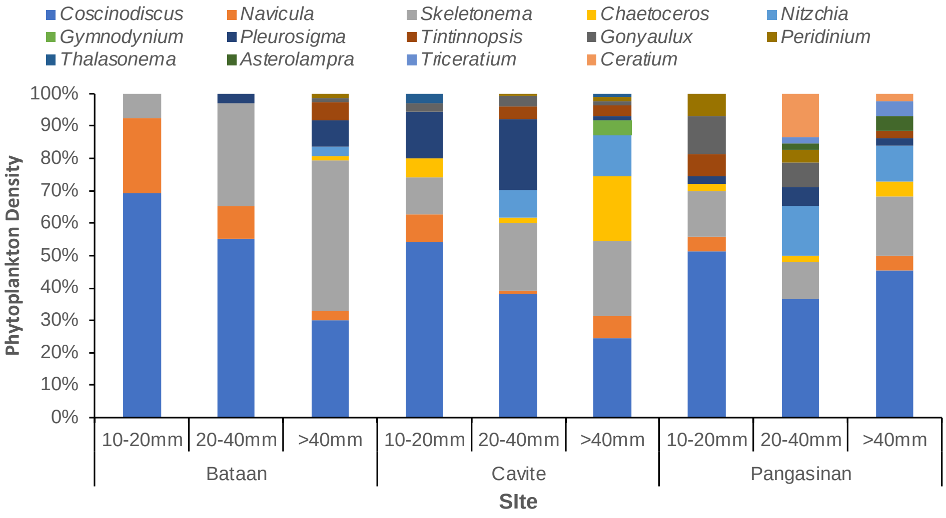

Figure 6. Pre-ingested phytoplankton of *M. strigata* in different sites and size class (n = 10).

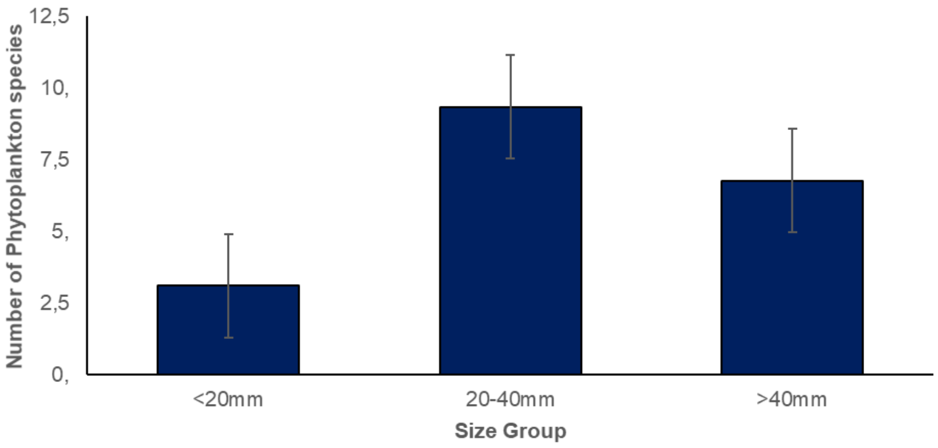

Figure 7. Phytoplankton composition in different size groups shows that 20 – 40mm has more species than the lower size classes (<20mm, >40mm).

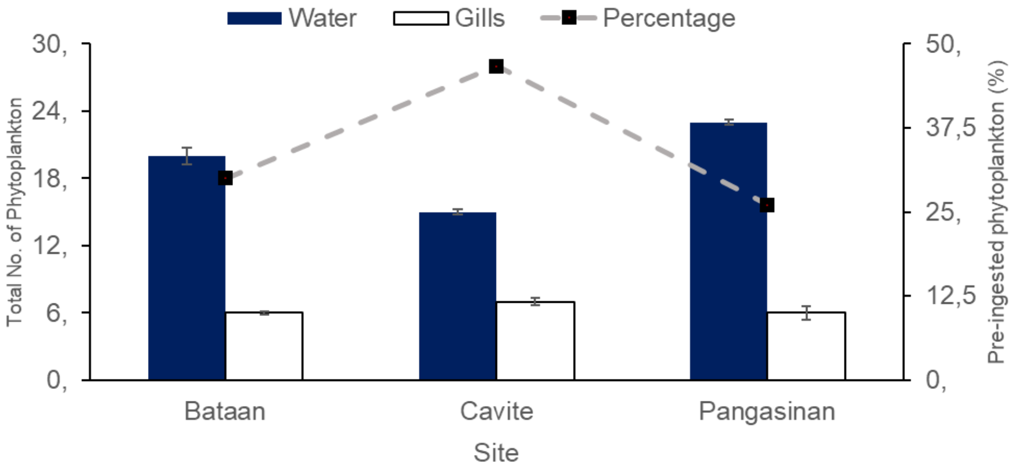

Figure 8. Total number of pre-ingested species in the gills compared to the total number of species in the water from different sites ranged from 25 % – 45 % (n = 30).

Discussion

The present study identified three pre-ingested phytoplankton genera in the gills of *M. strigata* that include *Coscinodiscus* spp., *Skeletonema* spp., and *Navicula* spp. Identified pre-ingested particles are phytoplankton, specifically centric diatoms, considered the most preferred food of bivalves (Gosling 2003), together with bacteria, zooplankton, and detritus organisms (Vakily 1989; Langdon and Newell 1990; and Lehane and Davenport 2002). However, several studies have reported that species in the family Mytilidea demonstrate selective feeding behavior towards phytoplankton with a higher preference for dinoflagellates (Soon and Ransangan 2014; Sivalingam 1977). Sidari (1998) revealed that *M. galloprovincialis* seemed to feed on dinoflagellates rather than diatoms. This study also revealed that during water filtration, there is already particle retention in the gills of *M. strigata* that can also be affected by the movement of latero-frontal cilia and gill structure and particle concentration (Dral 1967; Jorgensen 1996). Ward *et a*l. (1998) revealed that ctenidia are responsible for particle sorting in *Crassostrea virginica* and *Crassostrea gigas*, which can also be true for mytilid species. In addition, gills and labial palp have been used to determine the efficiency of particle selection for marine bivalves with no distinct visible gut (Owen 1966).

It was further revealed that body size significantly affects the potential food preference of *M. strigata.* According to Beecham (2008), particles from 1 to 7 µm were preferentially ingested, while particles > 50 µm were rejected because it does not fit in the ctenidium, though they can be ingested and processed. Rijsgard *et al*. (1980) revealed that five and 13-year-old *M. edulis* consume particles smaller than 1 µm or larger than 9µm. Berry and Schleyer (1983) also identified pre-injected particles (0.46 µm in diameter) in *Perna perna*. Further, digestible particles are preferred over inorganic particles. This study found higher phytoplankton compositions in larger shell sizes (>40 mm) of *M. strigata,* suggesting possible food and size selectivity. This linear relationship may be due to the increasing demand for food for mussels as it grows. Bass *et al*. (1990) found that the size selectivity of *M. mercenaria* veligers is linked to the size of the mouth and esophagus, which get larger (5-6 µm in diameter) as they grow. Factors such as chemotactic attractiveness, size, and shape are believed to affect the active selection of phytoplankton in bivalve larvae (Raby 1997).

The present study showed a wide range of phytoplankton species filtered in the gills of *M. strigata,* which varied with site depending on the availability of phytoplankton in the area. *Coscinodiscus* spp., *Skeletonema* spp., and *Navicula* spp. are the most dominant species found in the gills of *M. strigata,* which are also the dominant species in the waters of Bataan, Cavite, and Pangasinan. *Coscinodiscus* spp. found most prevalent is a free-living saucer- to petri-dish-shaped discoid with a valve mantle deeper on one side common in marine waters (Ehrenberg 1839). Five common species identified include *C. argus, C. centralis, C. lineatus, C. minor, and C. patina*. Lacuna *et al*. (2012) also considered *Coscinodiscus* spp. one of the four major phytoplankton identified in Panguil Bay, Philippines. In this study, *Coscinodiscus* sp. dominated in the month of November with 53.21% compared to in September and in October with only 11.51% and 13.05%, respectively. The abundance of *Coscinodiscus* sp. in this study is similar to the report of Lacuna *et al*. (2012), showing that this species is most abundant in November. In addition, *Skeletonema* spp. and *Navicula* spp. were also dominant in the different sites and are brackish water diatoms that have long been used as food for aquaculture species. A combination of this dominant species and some other culture microalgae can serve as the primary food for *M. strigata*, if the culture potential of this species is taken into account. There were also harmful algal cells such as *Gymnodynium* spp., *Skeletonema* spp., *Peridinium* spp., and *Navicula* spp. It shows that *M. strigata*, like any other bivalve, may still be able to ingest algal cells, resulting in diuretic or paralytic shellfish poisoning once eaten (Hermes and Villoso 1983; Bajarias *et al*. 2006). Aside from the phytoplankton identified, detritus forming matrix termed as dust fine detritus by Baker (1916) was also observed, which could not be estimated. Filter feeder sediment trapped in the gills may also serve as food for sessile bivalve species like *M. strigata*. The availability of food resources in the area is worth considering to understand further the bioinvasion ecology of *M. strigata* to control its likely further dispersal.

Conclusion

This study provided information on the particle selection of *Mytella strigata* based on the pre-ingested phytoplankton. A potential food preference for centric diatoms such as *Coscinodiscus* spp., *Skeletonema* spp., and *Navicula* spp. was observed. These phytoplankton species were dominant in the waters of Bataan, Cavite, and Pangasinan, where there was a rapid proliferation of *M. strigata*. Size influences the pre-ingestion, as shown in the higher species diversity in size 20– 40mm than in <20 mm and >40mm. Further, pre-ingested particles present in the gills can be used to determine the efficiency of particle selection and preference for bivalves with no distinct visible gut, such as that of *M. strigata*.

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Ethical approval

The authors declare that this study complies with research and publication ethics.

Informed Consent

Not Applicable

Conflict of interest

There is no conflict of interests for publishing this study.

Data availability statement

The authors declare that the data from this study are available upon request.

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Author contributions

DPMediodia - Conceptualization, Sampling, Analyses, Writing, Editing

NCAñasco - Conceptualization, Editing

CPBaylon - Conceptualization, Editing

SSSantander-de Leon - Conceptualization, Analyses, Writing, Editing

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