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Gut Content Analysis and Selective Feeding Behavior of the Asiatic Hard Clam *Meretrix meretrix* (Linnaeus, 1758) in Marudu Bay

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ABSTRACT

This study investigated the feeding behavior of the Asiatic hard clam, *Meretrix meretrix*, an important species in the artisanal fishery and commonly found in Sabah's coastal waters. The study aimed to identify the clam's primary diet and the environmental parameters that influence its feeding selection in its natural habitat. Sampling was monthly conducted for ten months, during which clam samples, environmental parameters, water samples, phytoplankton, and zooplankton were collected. Gut content analysis of 250 clams revealed that phytoplankton and zooplankton were the main food sources, representing 80.9% and 19.1% of the total food particles, respectively. Diatoms were the most common food particles ingested by the clams, while dinoflagellates only contributed a minor proportion of the total phytoplankton. The results of the dendrogram similarity analysis indicated significant differences in phytoplankton composition between gut and water samples. The one-way ANOSIM analysis indicated significant differences for all months, with an overall average R of 0.717 and $P<0.001$. Furthermore, the PERMANOVA following DistLM revealed that phytoplankton cell density, phytoplankton diversity, chlorophyll-a, and salinity significantly influenced the clam's particle selection process ($P<0.05$). In conclusion, this study offers a comprehensive understanding of feeding behavior and the dietary preference that can be utilized for the conservation of fishing grounds and enhancement of aquaculture production of the clam.

INTRODUCTION

Meretrix meretrix is a bivalve species commonly found in intertidal and sublittoral waters, inhabiting muddy-sand bottoms up to 20m deep (**Poutiers, 1998**). The clam, functioning as a shallow infaunal suspension filter feeder, feeds near the bottom within the benthic boundary layer, where it filters food particles from the water column (**Stanley, 1977; Gosling, 2015**). It is a valuable species in the artisanal fishery in Sabah.

It has the potential for commercial bivalve culture in Malaysia, but information on its diet and feeding behavior is limited.

Bivalves, including *M. meretrix*, primarily feed on phytoplankton such as diatoms and dinoflagellates. However, feeding preferences for specific phytoplankton species vary among bivalve species (**Hawkins *et al.*, 1998a; Gosling, 2003; Lehane & Davenport, 2006; Espinosa *et al.*, 2008**). Environmental factors and body size also influence the feeding rates of bivalves (**Grizzle *et al.*, 2001**). Food concentration is a key factor in the feeding physiology of bivalves (**Bayne *et al.*, 1987; Newell *et al.*, 2001; Zhuang & Wang, 2004**).

Gut content analysis is essential to understand the feeding patterns and food habits of bivalve populations and for fisheries management (**Amundsen & Sánchez-Hernández, 2019**). Comparing the phytoplankton composition in the gut content of bivalves to that in the water column of their surrounding habitat, provides insights into the bivalves' feeding preferences (**Alves *et al.*, 2014**). Detailed information on the natural food diet of bivalve populations, including phytoplankton composition, is crucial for understanding population growth and dynamics (**Beukema & Cadée, 1991**).

This study aimed to investigate the feeding preference and main diet of *M. meretrix* in its natural habitat by comparing the phytoplankton ingested by the clam to the phytoplankton community in their habitat. Temporal environmental variables were documented to investigate their relationship with clam feeding preference and selection. This study will aid in developing a suitable microalgae diet for the aquaculture of the clam, establish baseline data of the species, and develop a sustainable management plan for shellfishery in Sabah and bivalve aquaculture in general.

MATERIALS AND METHODS

1. Sampling site

The study was conducted from May 2019 to February 2020, lasting for ten months, in the inner region of Marudu Bay, which lies between 6°35’ to 7°N and 116°45’ to 117°E (Fig. 1). The sampling sites are well-known artisanal fishing grounds surrounded by dense mangrove forests. The grounds are covered with water during high tide and are exposed during low tide. The bay is subject to heavy rainfall from November to March every year during the northeast monsoon (NEM), but experiences limited rainfall from May to September during the southwest monsoon (SWM), as reported by the **Malaysian Meteorological Department (2021)**.

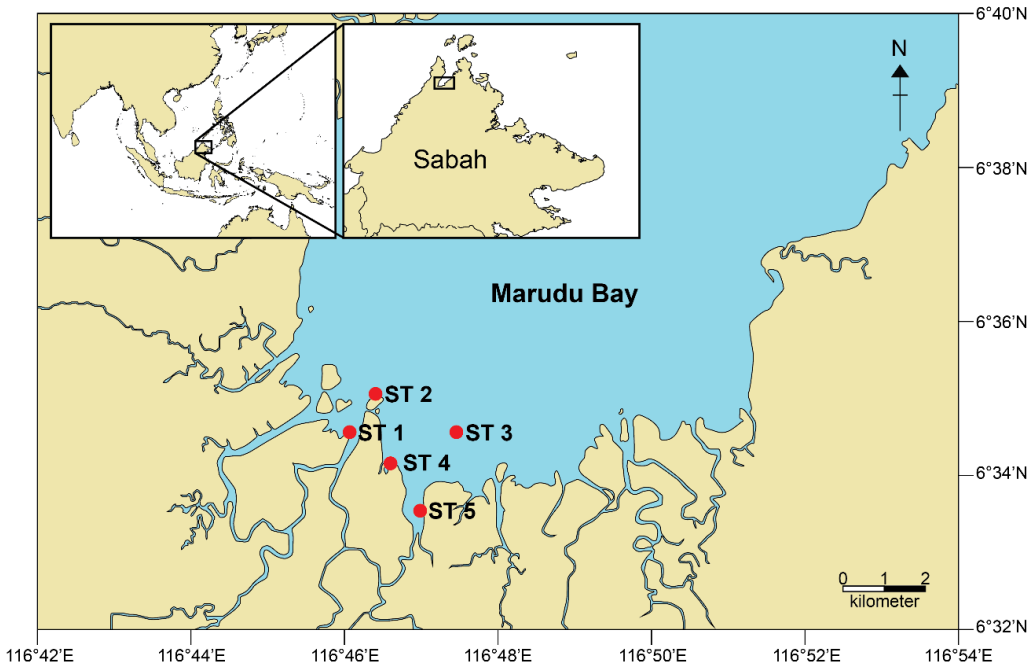

Fig. 1. Study sites in the inner part of Marudu Bay, Sabah, Malaysia

2. Clam collection and gut content analysis

A total of 250 *Meretrix meretrix* clams (n=25 clams/month) were collected using the 'kerek' method during low tide in mud-flat areas. The clams had a mean size of 40.54±7.12mm, ranging from 24.40 to 64.70mm. The 'kerek' is a handmade bivalve hand dredge typically used by artisanal fishermen for clam harvesting. The clams were immediately dissected in the field to collect their gut contents, and effectively stopping digestion. The valves were thoroughly cleaned and opened at the adductor's muscles, and the gut area was identified and dissected. The content was withdrawn using a Pasteur pipette and preserved in a glass vial. Gut contents were diluted with distilled water to 5ml and preserved with Lugol's solution (**Tan & Ransangan, 2017**).

In the laboratory, the gut contents were observed under an inverted microscope at 40x and 100x magnification, and food particles such as phytoplankton and zooplankton were counted using a Sedgewick Rafter Chamber. Phytoplankton cells in the gut contents were identified at the genus level using taxonomic keys recommended by **Hartley *et al.* (1996)** and **Tomas (1996)**. Zooplankton identification followed **Lenz (2000)** identification guide. The number of individuals of each food item found in each specimen was recorded and expressed as a percentage of the total number of food items analyzed in the sample (**Muñetón-Gómez *et al*., 2010**) as follows;

Percentage of food item i, %Ni: (Ni/Nt) x 100

Where,

Ni = number of particular food item

Nt = total number of food (gut content) items

3. Environmental parameters

Monthly *in-situ* measurements of physical parameters were conducted using a multi-function environmental probe (YSI; Loveland, Co, USA) positioned 0.5m above the sediment surface. The parameters measured were temperature (°C), salinity (ppt), pH, and dissolved oxygen (mg/L). Water transparency was measured using a Secchi disc, and the seawater depth (m) at the sampling site was determined by a depth sounder (Hondex, Japan). Monthly rainfall data from May 2019 to February 2020 were obtained from the Meteorology Department of Malaysia.

4. Total seston, chlorophyll- α and water nutrient analysis

Water samples were collected in triplicate, using a Van Dorn water sampler at 0.5m above the seafloor, to measure total seston (total particulate matter, TPM; particle organic matter, POM; particle inorganic matter, PIM, and organic content, OC), chlorophyll-α, and dissolved nutrients. A total of 2 liters (2L) of water samples were collected for the analysis. The determination of total seston followed the method described by **Wong and Cheung (1999)**.

The chlorophyll-α concentration was done using a spectrophotometer (UV-VIS Spectrophotometer, HACH DR3900) at 664, 647, and 630nm. The absorbance at each wavelength was recorded and used to calculate chlorophyll-α concentration following the procedure described by **Parson *et al.* (1984)**. The water filtrate collected for chlorophyll-α analysis was utilized for dissolved nutrient analysis, including total ammonia-nitrogen, total phosphate, nitrate, and nitrite, following the procedure outlined by **Parson *et al.* (1984)**.

5. Phytoplankton and zooplankton composition in water samples

Seawater samples were collected for phytoplankton quantitative analysis using a Van Don Water sampler. Three replicate one-liter (1L) samples were collected at a height of 0.5m above the seafloor. In the field, Lugol's solution was immediately added to the water samples to preserve the phytoplankton samples. The samples were concentrated in the laboratory for 24 hours into 50ml using the Utermöhl sedimentation method **(Utermöhl, 1958)**. Quantitative analysis of the phytoplankton in the water samples, including cell density and composition, was estimated using a Sedgwick Rafter chamber at 40x and 100x magnification. Cell density was calculated as cells/ml following the method described by **Aktan *et al.* (2005)**. While, phytoplankton was identified at the genus level using the taxonomic keys suggested by **Hartley *et al.* (1996)** and **Tomas (1996)**.

Meanwhile, phytoplankton species diversity and evenness were calculated and expressed using the Shannon-Wiener index (H') (**Shannon & Weaver, 1963**) and Pielou's evenness index (J') (**Pielou, 1966**), respectively, using the formula below;

Shannon-Wiener Index, H'

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

Where, pi is the composition of each phytoplankton taxa.

Pielou's Evenness Index, J'

$$J = \frac{H'}{\ln(S)}$$

Where,

H' = Shannon-Wiener index
S = Total number of phytoplankton taxa

Zooplankton samples were collected using a 50µm mesh-size plankton net with a calibrated flow meter at the center of the net opening. The flow meter measured the volume of water filtered, and the net was towed horizontally for about 3 minutes. The samples were immediately preserved in 70% ethanol. In the laboratory, the samples were sieved through a 50µm mesh and rinsed with running tap water to remove fine debris and dirt. The zooplankton samples were observed and quantified under the Carl Zeiss light microscope at 40x and 100x magnification in a Sedgewick Rafter Chamber (1ml). Identification was done following the identification guide suggested by **Lenz (2000)**.

6. Statistical analysis

Statistical comparisons between the gut content and surrounding water were based on the relative composition, which was calculated as a percentage of the cell number. The phytoplankton composition data were normalised using a square root transformation (**Clarke & Warwick, 1994**), while the environmental data were log(x+1) was transformed (**Clarke & Warwick, 1994**; **Rouillon *et al.*, 2005**). To test for significant differences between the phytoplankton assemblages in the gut content and its surrounding habitat, a one-way analysis of similarity (ANOSIM) was performed (**Clarke & Warwick, 1994; Rouillon *et al.*, 2005**).

Non-metric cluster analysis was conducted using the Bray-Curtis similarity index to compare the phytoplankton composition in each month. To determine the percentage contribution of each genus to the average dissimilarity between gut content and its surrounding water samples, similarity percentage analysis (SIMPER) was used (**Clarke, 1993**). Furthermore, to investigate the relationship between the selective feeding behavior of the clam and environmental variables, a PERMANOVA followed by a distance-based linear modelling (DistLM) routine was employed. All analyses were performed using the Primer V7 software (Plymouth Routines Multivariate Ecological Research) (**Tan & Ransangan, 2017**).

RESULTS

1. Gut content analysis of *Meretrix meretrix*

During the sampling period, 1475 food items were found in the gut of the clams, consisting of phytoplankton and zooplankton. Phytoplankton and zooplankton accounted for 80.9% and 19.1% of the total food items, respectively (Table 1). The gut content analysis identified 25 genera of diatoms and 7 genera of dinoflagellates in the clams. Diatoms were the most consumed phytoplankton type, representing 95.81% of the total phytoplankton ingested, while dinoflagellates only contributed 4.19%. *Nitzschia*, *Coscinodiscus*, *Cyclotella*, *Pleurosigma*, and *Navicula* were the top five dominant phytoplankton genera found in the gut of the clams during the study period, accounting for 24.71%, 23.38%, 13.70%, 12.68%, and 8.95%, respectively (Fig. 2).

Table 1. Number of phytoplankton and zooplankton found in the gut content of *M. meretrix* recorded from May 2019 to February 2020

Month	Phytoplankton	%	Zooplankton	%	Total food item
May 2019	92	93.9	6	6.1	98
June 2019	109	85.2	19	14.8	128
July 2019	79	78.2	22	21.8	101
August 2019	86	74.1	30	25.9	116
September 2019	108	81.8	24	18.2	132
October 2019	67	62.6	40	37.4	107
November 2019	283	80.2	70	19.8	353
December 2019	142	81.1	33	18.9	175
January 2020	69	77.5	20	22.5	89
February 2020	158	89.8	18	10.2	176
Total	1193	80.9	282	19.1	1475

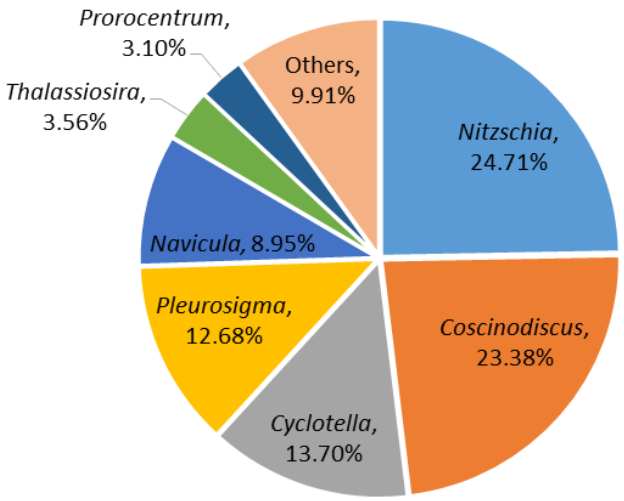

Fig. 2. Phytoplankton composition (%) in the gut of *M. meretrix* recorded from May 2019 to February 2020

2. Phytoplankton and zooplankton composition in the water column

The study identified a total of 50 phytoplankton genera from 37 families, consisting of 40 genera of diatoms and 10 genera of dinoflagellates. However, diatoms dominated the phytoplankton population with 95.31%, while dinoflagellates accounted for only about 4.69% of the total phytoplankton population. *Nitzschia* (16.24%), *Pleurosigma* (11.98%), *Chaetoceros* (10.84%), *Navicula* (8.34%), and *Odontella* (8.18%) were the five most dominant phytoplankton genera identified at the sampling sites throughout the study period (Fig. 3).

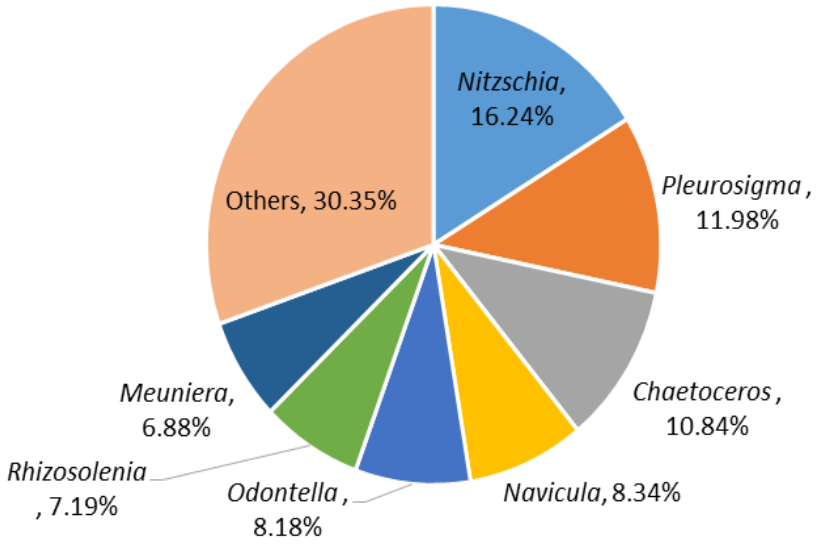

Fig. 3. Phytoplankton genus, composition and abundance (%) recorded in the sampling areas from May 2019 to February 2020

In September 2019, the highest cell density for phytoplankton was recorded, with 270±58.84 cells/ml. Its density was significantly higher (P<0.05) than the cell density

recorded in other sampling months (Fig. 4). The high cell density in September 2019 was primarily attributed to *Chaetoceros* and *Rhizosolenia*, which accounted for 44.31% and 44.91% of the total cell density in the phytoplankton communities that month. June 2019 recorded the lowest cell density with 13.05±2.33 cells/ml. The five most dominant zooplankton in the sampling sites throughout the study period were tintinnid (28.04%), calanoid (14.53%), cyclopoid (7.57%), nauplius (5.48%), and harpacticoid (3.66%).

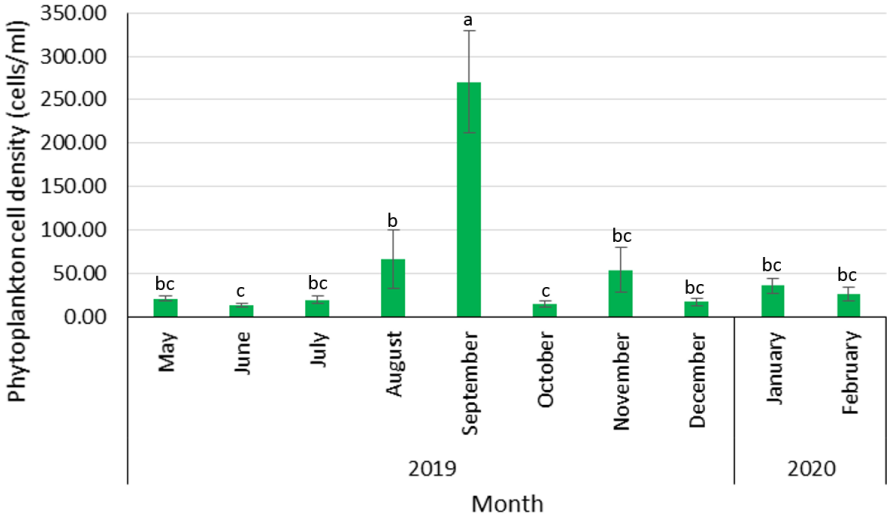

Fig. 4. Monthly phytoplankton cell density (cells/ml) recorded in the sampling areas from May 2019 to February 2020

3. Comparison of phytoplankton composition in water and the gut

Fig. (5) shows the dendrogram of similarity resulting from the non-metric cluster analysis of the gut and water samples, based on the similarity of phytoplankton composition.

The dendrogram analysis revealed that the phytoplankton composition in the water and gut content samples were significantly different, with an overall similarity of less than 50%. The analysis identified two clusters; Cluster 1 with a similarity of less than 40%, and Cluster 2 with a similarity between 40-50%. The one-way ANOSIM test indicated significant differences in phytoplankton composition between gut contents and water samples for all the months, as summarized in Table (2).

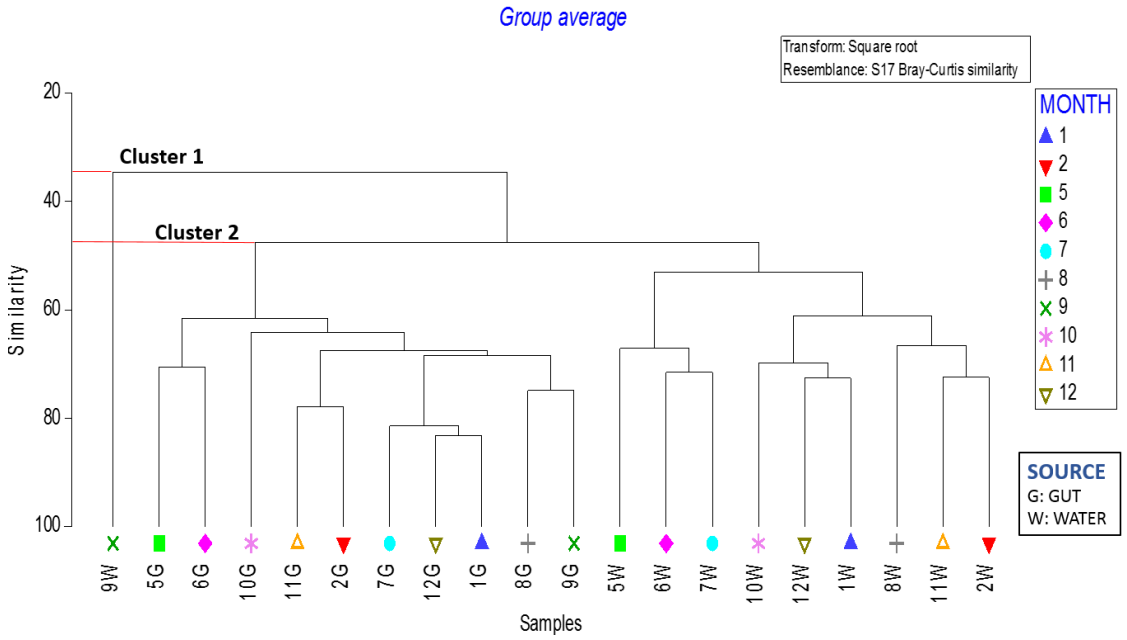

Fig. 5. Dendrogram of similarity of phytoplankton composition between surrounding water and the gut content of *M. meretrix* in the sampling areas

Table 2. Summary of analysis of similarity (ANOSIM) of phytoplankton in water samples and gut content of *M. meretrix*

Sample	**R**	**P**
Overall (May 2019 to February 2020)	0.717	< *0.001*
Cluster 1 (September 2019)	0.924	< *0.008*
Cluster 2 (All months except September 2019)	0.788	< *0.008*

Although *Chaetoceros* was the third most dominant phytoplankton genus in the water column, it was found in small numbers in the clam's gut during the study period. The highest number of *Chaetoceros* cells in the clam's gut was recorded in September 2019, when a major bloom of *Chaetoceros* and *Rhizosolenia* occurred in the water column. However, despite accounting for 44.31% of the total abundance of phytoplankton in September 2019, only 3.7% of *Chaetoceros* was found in the clam's gut. The phytoplankton composition in the clam's gut in September 2019 was dominated by the genera *Nitzschia* (29.6%), *Navicula* (15.74%), and *Coscinodiscus* (12.96%). In contrast, the relative abundance of these three genera in the water samples in September 2019 was much lower, with only 1.42% for *Nitzschia*, 1.80% for *Navicula*, and 0.33% for *Coscinodiscus* (Fig. 6).

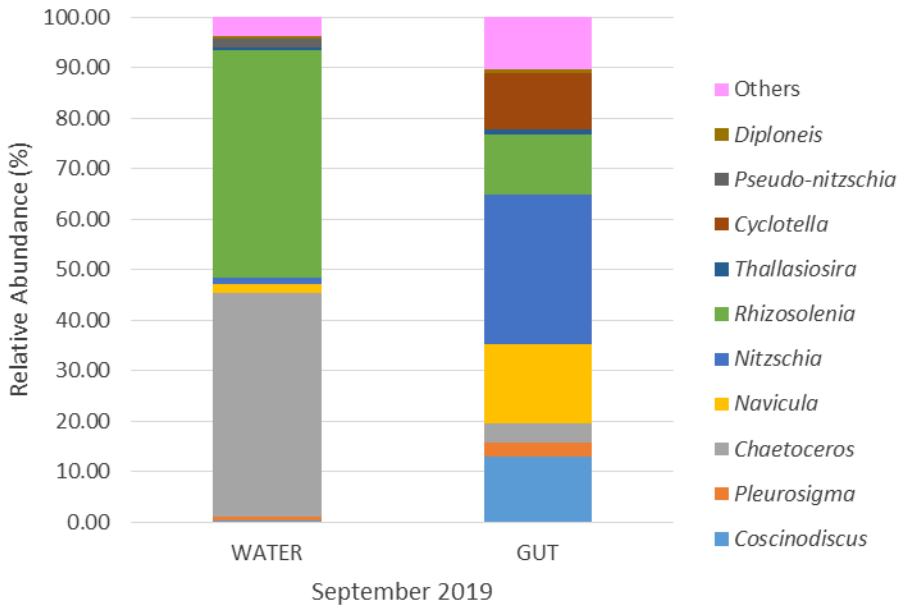

Fig. 6. Total phytoplankton composition in water and gut content of *M. meretrix* with the phytoplankton genera contribution to the overall dissimilarity recorded in September 2019

SIMPER analysis showed that the overall average dissimilarity between the gut content and water samples from May 2019 to February 2020 (Table 3) was 63.14%. Dissimilarity greater than 60% was recorded from July 2019 to November 2019, while other months recorded dissimilarity between 51-55%. In September 2019, the highest dissimilarity of phytoplankton composition between the gut content and surrounding water was recorded with 69.97%. The clam selectively ingested phytoplankton genera such as *Coscinodiscus* and *Cyclotella*, even though they were not the most abundant phytoplankton in the water column. Meanwhile, genera such as *Nitzschia*, *Pleurosigma*, and *Navicula* were the most abundant in the water column and were consistently ingested by the clams.

Table 3. Monthly average dissimilarity between groups (gut content and water sample)

Month	Average dissimilarity (%)
May 2019	51.89
June 2019	55.44
July 2019	63.47
August 2019	68.70
September 2019	69.67
October 2019	67.29
November 2019	63.95
December 2019	53.03
January 2020	54.62
February 2020	52.61

4. Selective feeding behavior of *Meretrix meretrix*

The distance-based linear modeling (DistLM) results revealed that several environmental factors significantly impacted the selective feeding behavior of the hard clam in Marudu Bay (Fig. 7). Specifically, phytoplankton productivity, including phytoplankton cell density, Shannon-Wiener Diversity Index (H'), and chlorophyll-α, as well as salinity, had a significant (P<0.001) effect. These four factors accounted for 19% of the total variance in the phytoplankton composition structure (R^2: 0.19, P<0.001).

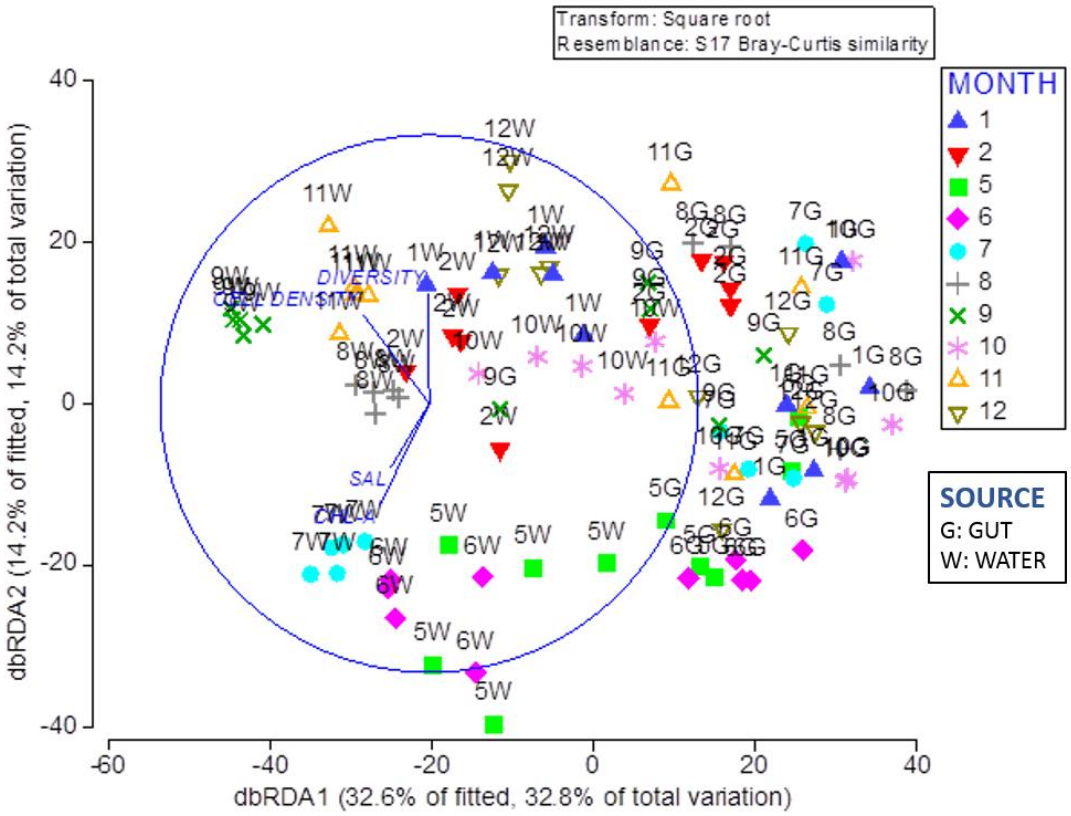

Fig. 7. Relationships of environmental parameters and phytoplankton composition in distance-based linear modelling (DistLM) of permutational multivariate analysis of variance (PERMANOVA).

DISCUSSION

The present study confirmed that phytoplankton is the main food source for *M. meretrix*, consistent with previous findings on other Meretrix species (**Khowhit *et al.*, 2014; Ramamoorthy *et al.*, 2020**). The clam preferred diatoms over dinoflagellates, with only a small number of dinoflagellates found in the gut compared to diatoms. The clam was found to avoid consuming dinoflagellates characterized by flagella, which enables them to avoid capture by bivalves despite their higher abundance in the water column (**Qiao *et al.*, 2021**). *M. meretrix* prefers diatoms because of their high percentage of

unsaturated and polyunsaturated fatty acids and oil reserves (**Pohl & Zurheide, 1979; Beukema & Cadée, 1991; Ahlgren *et al.*, 1992; Li *et al.*, 2002**).

While phytoplankton is the exclusive food source for *M. meretrix*, the gut content analysis revealed that a small percentage of zooplankton was also present, suggesting that the clam can ingest zooplankton as an alternative food source. Several studies have highlighted the importance of zooplankton as an additional or alternative food source for bivalves (**Lehane & Davenport, 2002**; **Prato *et al.*, 2010**). *M. meretrix* can ingest various types of zooplankton, including tintinnids, foraminifera, radiolarians, crustacean larvae, and others. *M. casta*, another species in the *Meretrix* genus, was also found to have zooplankton in its gut (**Ramamoorthy *et al.*, 2020**) although the percentage was lower than in the present study (19.1%).

The comparison of phytoplankton composition between bivalve gut content and the surrounding water column is commonly used to evaluate selective feeding behavior (**Lopes-Lima *et al.*, 2014**; **Tan & Ransangan, 2017**). This study examined the phytoplankton in the gut content of *M. meretrix* and compared it to the phytoplankton in the water column. The analysis revealed that the ingested phytoplankton composition differed significantly from the phytoplankton composition in the water column throughout the sampling period. Therefore, it can be inferred that *M. meretrix* exhibited selective feeding behavior.

Selective feeding behavior of *Meretrix meretrix* varied among the sampling months, with some months showing more intense selective feeding. The dissimilarity between phytoplankton in the gut and water column was at its highest in months with high phytoplankton cell density and chlorophyll-α and moderate total seston. September 2019 in Cluster 1 and August 2019 in Cluster 2 showed the highest dissimilarity between gut and water column phytoplankton, with *Chaetoceros*, *Rhizosolenia*, and *Meuniera* being selectively rejected by the clam in those months despite their high cell density in the water column.

Bivalve filtration and feeding processes depend on the gill structure and particle concentration in the water column (**Jørgensen, 1996**). *M. meretrix* has a lower filtration and ingestion rate than epifaunal species (**Hawkins *et al.*, 1998b; Rajesh *et al.*, 2001**), and thus secretes mucus to avoid oversaturation, which can damage its gills. The clam then sorts the particles caught in the mucus and ejects rejected particles as pseudofeces (**Jørgensen, 1981; Maire *et al.*, 2007; Arapov *et al.*, 2010**). The highest dissimilarity of phytoplankton composition was also recorded during months with higher salinity. Water salinity affects the feeding and filtration rate in bivalves (**Pourmozaffar *et al.*, 2019**). Moreover, *M. meretrix* achieves a greater ingestion rate within the salinity range of 27 to 30ppt (**Zhuang, 2006**). The optimal salinity for clam feeding recorded during the study months may have led to more efficient selective feeding activities.

The feeding behavior of clams is influenced by environmental factors, as well as the quality, quantity, and availability of food (**Tang *et al.*, 2006; Rosa *et al.*, 2015; Rosa *et al.*, 2018**). In the present study, benthic diatoms were the dominant phytoplankton genera in the gut of *M. meretrix*. The clam's infaunal behavior leads it to feed more on benthic diatoms, as feeding by infaunal suspension feeders like Meretrix occurs at the surface of the substrate within the benthic boundary layer (**Stanley, 1977**; **Gosling, 2015**). *Nitzschia* was the most dominant and consistent genus found in the gut of the clam and the water column throughout the study period. *Nitzschia* is known to contain high lipid content

(**Ben-Amotz *et al.*, 1985**) and is reported to have higher levels of protein, lipid, and eicosapentaenoic acid (EPA) than *Chaetoceros* sp. (**Rodríguez-Núñez & Toledo-Aquero, 2017**). Therefore, *Nitzschia* is suggested as a useful component of the microalgal diet for cultured marine organisms (**Renaud *et al.*, 1994; Rodríguez-Núñez & Toledo-Aquero, 2017**).

The clam selectively ingested several diatom genera, including *Coscinodiscus* and *Cyclotella*, even when they were not the most abundant genera found in the water column. The results of this study are consistent with those of **Khowhit *et al.* (2014)**, where *Coscinodiscus* and *Cyclotella* were the most dominant phytoplankton genera found in the gut of *Meretrix casta*. The organic molecules casing found on the external layer of *Coscinodiscus*, known as the "perifrustular envelope," serve as a cue for high-quality food items for bivalves (**Beninger & Decottignies, 2005**). In contrast, the clam selectively rejected *Chaetoceros*, which has poor nutritional content and siliceous spines structure that can entangle at the gills of bivalves (**Martínez-Fernández *et al.*, 2006**; **Ochieng *et al.*, 2015**). Reduction in food quality triggers a higher level of sorting efficiencies in bivalves (**Hawkins *et al.*, 1996; Pouvreau *et al.*, 2000**).

CONCLUSION

The results of this study suggested that phytoplankton is the primary diet and source of nutrition for the hard clam, *M. meretrix*, with a preference for diatoms over dinoflagellates. The clam was also observed to ingest a diverse range of zooplankton as alternative food sources. The gut content analysis revealed that the phytoplankton composition in the surrounding water did not closely resemble that in the clam's gut, confirming the clam's selective feeding behavior. This selectivity is influenced by environmental factors, including salinity, as well as the nutritional value, quality, and availability of phytoplankton in the water column. These findings highlight the significance of understanding the feeding behavior of clams for effective management of shellfishery, particularly in locations important for fishing grounds such as Marudu Bay, and the diet formulation for enhancing aquaculture production of the clam.

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