Gut content of mangrove gastropod, *Cerithidea obtusa* (Lamarck, 1822) from Kuala Selangor Nature Park, Selangor and Tanjung Piai National Park, Johor, Peninsular Malaysia

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Abstract. Commercially valuable gastropod *Cerithidea obtusa* is abundant in the Malaysian mangrove ecosystems, yet little research has been done on this species. The present study was conducted to examine the diet of *C. obtusa* from two sites, Kuala Selangor Nature Park, Selangor and Tanjung Piai National Park, Johor, Malacca Strait. A total of 90 individuals were randomly collected from both sites by hand and fixed in 10% buffered formalin. Data from the sample analysis revealed that the percentage frequency of occurrence (Fpi) of food items found in both sites representing six major groups, dominated by vegetal detritus (100%), leaf matter (84.34%), diatoms (71.08%), dinoflagellate (44.58%), porifera (38.55%) and foraminifera (19.28%). A total of 16 diatom genera were recorded from the gut content analysis. Percentage frequency of occurrence of diatoms (Fpi) indicated that three genera were frequently found in the guts from Kuala Selangor; *Cyclotella* (73.33%), *Navicula* (70%) and *Coscinodiscus* (40%) and in the guts from Tanjung Piai; *Cyclotella* (56.6%), *Navicula* (32.08%) and *Coscinodiscus* (26.42%). In addition, the diet of *C. obtusa* showed that this species is a non-selective bottom grazer and the composition might vary due to the different localities and environmental factors such as tidal level. Further study can be carried out to allocate the relationship between their feeding mechanism and food preferences for a better understanding of their ecological roles in the mangrove ecosystems.

1. Introduction
Gastropod is defined under the phylum Mollusca and it is the larger taxonomic class under this phylum [1]. They can be found in brackish and marine waterway. The study on the ecology and biological aspects of mangrove gastropod is essential to acknowledge the changes they contribute to the ecosystem. Previous studies have reported the study on mangrove snails including species from the genus of *Cerithidea* [2]. However, only few studies can be reached emphasizing the knowledge on *C. obtusa* in Malaysia. Most studies only focus on the diversity and abundance of *C. obtusa* in mangrove area associated with other species and on heavy metal accumulation in body parts of *C. obtusa* [3].
The notion to conduct a research on *C. obtusa* is crucial as this species is known as a commercial species sell in the market around Malaysia, yet limited study on this species has been documented. *Cerithidea* is known for its habit of living in the bottom of the waters and belong to benthic species. They are believed to have the capability of utilizing plankton, and organic materials in the form of detritus present in the mangrove ecosystem [4].

In the mangrove ecosystem, grazing gastropod is important in acknowledgement of its roles in tropic intermediaries between primary and secondary production [5]. One of the most dominant resources in a mangrove from the family Potamididae is *Cerithidea obtusa* [6]. Requirement of nutrients is a curial aspect of every living organism including snails [7]. Adequate nutrients will enhance the growth of snails and in providing enough nutrition needed by the snails. Thus, a study on stomach content is essential to indicate the sources of food of the invertebrate and for that, conservation efforts can be made to ensure the sustainability of the ecosystems for conserving their food sources.

Notably, several studies had been made on the feeding behaviour of snails in mangrove ecosystems by Fratini, Vannini and Cannicci [8], Christensen [9], Zaman and Jahan [4] and Pape [10]. In conjunction with that, this study is aimed to examine the gut and faecal content of *Cerithidea obtusa* in two different localities. This is to provide preliminary data on the food items ingest by *C. obtusa* from Malaysia mangrove ecosystems for conservation efforts of this valuable commercial species in future.

2. Materials and methods

2.1. Sampling sites and collections

Samples were collected from selected protected mangrove areas along the Malacca Strait, Peninsular Malaysia located in Kuala Selangor Nature Park, Selangor (KSNP) (03°20'06.10"N, 101°14'02.70"E) and Tanjung Piai National Park, Johor (TPNP) (01°15'58.00"N, 103°30'40.60"E). Sampling in Kuala Selangor Nature Park was conducted in August 2020 (KSNPA) while in Tanjung Piai National Park; the sampling was conducted in September (TPNPS) and December 2020 (TPNPD). According to the tidal chart of the nearest port, in KSNPA the sampling was conducted during low water at 2.8 meters of tidal height at 1500 hours until the next high tide at 1730 hours. In TPNPS sample collection was conducted during the beginning of low tide with the tidal height of 1.09 meters at 1530 hours while in TPNPD was during 0.24 meters of tidal height at 0744 hours. Samples were collected randomly by hand and preserved in 10% buffered formalin. The range size of collected snails was varied from 3 cm to 6 cm.

2.2. Gut content analysis

Total of 90 specimens from both sites were analysed for gut content. The shell had been washed using freshwater. After crushing the shell using a g-clamp, the soft body of the snail was dissected in a petri dish and the gut content was washed with distilled water in the petri dish. Gut contents were removed from the stomach using a micropipette and forceps. The gut contents were pipetted into a 1.5 mL Eppendorf tube and the suspended particles were mounted on a slide with a coverslip for light microscopic examination at 10x and 40x magnifications. Diatom identification was done by using [11] and algaebase.com was referred to for genus verification. Minor food items found in the gut which occurred in low percentage were quantified but not presented in the graphic. Diatoms with broken and unbroken frustules were noted only for the presence and absence.

2.3. Faecal content analysis

Faecal pellets were collected from the sample after collection of the snails were made during low tide when we let the snails excreted the faecal in the small container. By using a 1.5 mL Eppendorf tube, a total of 20 faecal pellets were preserved in 70% ethanol (1 mL) for analysis. In the laboratory, the samples were shaken thoroughly to break the bond of faecal pellets. Micropipette with a 0.1 mL scale was used to pipette the faecal solution for slide preparation. A total volume of 0.1 mL sample was
pipetted onto a microscope slide and covered with a coverslip. The content was then been observed under a compound microscope (Leica DM750) with 10x and 40x magnifications.

2.4. Data analysis
Gut content was calculated using the percentage frequency of occurrence (Fpi) and numerical importance index (%NIi) [12] [13]:

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Fpi = \frac{Ni}{N} \times 100
\]

Ni= Number of gut containing \( i \) food item
N = Total number of full gut

\[
%NIi = \frac{Ni}{N} \times 100
\]

Ni= Total number of food \( i \)
N = Total number of all food items

2.5. Statistical analysis
Statistical analysis was run using IBM SPSS 26. Independent t-test with the assumption of unequal variance was conducted to test the significance of gut content from two sampling sites with 95% confidence level.

3. Results
3.1. Gut content composition
A total of 90 specimens were analysed and seven were found to be emptied with no content after dissecting the gut. The gut contents found and quantified were mostly larger than 10µm. Table 1 is referring to the absence and presence of food items in the gut of *C. obtusa* from KSNP and TPNP. The gut content was divided into six major groups consists of vegetal detritus, leaf matter, diatoms, dinoflagellate, porifera and foraminifera. When refer to figure 1, results calculated from the data of gut content from both study sites revealed that vegetal detritus was the most dominant gut content with 91.05% composition and a total of 28983 particles were counted while leaf matter comes in second with 4.31% and the total number of counted leaf matter was 1371 found in the gut of *C. obtusa*. This followed by diatoms 1.85% with 590 individuals, dinoflagellate 0.41% with 132 individuals, porifera 2.29% with 729 individuals and foraminifera 0.08% with 26 individuals. For diatoms, the frustules were observed and two conditions were noted. First is the broken frustules and the second condition is unbroken frustules. From our finding, the broken and unbroken frustules were frequently found in both KSNP and TPNP with no intact of chloroplast. Only a small numbers of unbroken frustules were found in both locations were intact with chloroplast. Data analysis shows that only foraminifera (p<0.05) and dinoflagellate (p<0.05) were significantly different between both sites. Other food items were not significantly different (p>0.05) when tested using independent t-test.

**Table 1:** Presence of food items of *Cerithidea obtusa* found in the gut of all specimens from Kuala Selangor and Tanjung Piai.

| Food item          | GUT Kuala Selangor | Tanjung Piai |
|--------------------|-------------------|--------------|
| **Diatom**         | +                 | +            |
| **Pennate diatom** |                   |              |
| *Amphora*          | +                 | +            |
| *Cocconeis*        | +                 | +            |
| Diatom Species            | Percentage |
|--------------------------|------------|
| Diploneis                | +          |
| Eunotogramma             | +          |
| Gyrosigma                | +          |
| Navicula                 | +          |
| Nitzschia                | +          |
| Pinnularia               | +          |
| Pleurosigma              | +          |
| Surirella                | +          |
| Tabularia                | +          |
| Thalassionema            | +          |
| **Centric diatom**       |            |
| Coscinodiscus            | +          |
| Cyclotella               | +          |
| Paralia                  | +          |
| Thalassiosira            | +          |
| Other diatoms            | +          |
| **Dinoflagellate**       | +          |
| Foraminifera             | +          |
| **Porifera (siliceous spicules)** | + |
| Leaf matter              | +          |
| Vegetal detritus         | +          |
| Broken frustules         | +          |
| Unbroken frustules       | +          |

**Figure 1**: Percentage of food items composition in gut of *Cerithidea obtusa*
3.2. Percentage frequency of occurrence and numerical importance index of food items

The percentage of frequency occurrence (Fpi) and numerical importance index (%NIi) of food items found from these two sites was presented in table 2. Most dominated by vegetal detritus followed by leaf matter and diatoms these food items were frequently found in the gut of *C. obtusa* from both sites. Data from the sample analysis revealed that the Fpi of each food items is; vegetal detritus (100%), leaf matter (84.34%), diatoms (71.08%), dinoflagellate (44.58%), porifera (38.55%) and foraminifera (19.28%). A total of 16 genera of diatoms were recorded from the gut content analysis. The calculation on Fpi of diatoms from Kuala Selangor and Tanjung Piai respectively, using the same calculation method mentioned above indicated that three genera were frequently found in the guts from Kuala Selangor Nature Park in 295 individuals compared to their number found in Tanjung Piai National Park in September 183 individuals and in December 113 individuals. Figure 2 shows the items of the gut content found in *C. obtusa* from both localities.

As for dinoflagellate, it was recorded that the highest number of individual counted was in September in Tanjung Piai National Park with a total of 93 individuals while dinoflagellate from Tanjung Piai National Park in December was 25 in total followed by in Kuala Selangor Nature Park with 14 individuals and from their occurrence in gut, 44.58% was the Fpi for dinoflagellate found in both sampling sites. The mean percentage showed the average of percentage of the food items occurred in both gut and faecal.

**Table 2**: Percentage frequency occurrence; Fpi, Numerical importance index; % NIi, mean percentage of gut content and faecal pellets content of *Cerithidea obtusa* obtained from Kuala Selangor and Tanjung Piai.

| Food item   | Gut content Fpi | %NIi | Fecal content | Mean percentage |
|-------------|-----------------|------|---------------|-----------------|
| **Diatom**  |                 |      |               |                 |
| *Amphora*   | 21.31           | 0.08 | 0.02          | 10.69           |
| *Cocconeis* | 14.75           | 0.04 | 0.02          | 7.40            |
| *Diploneis* | 6.56            | 0.02 | 0.01          | 3.29            |
| *Eunotogramma* | 6.56    | 0.02 | 0.01          | 3.29            |
| *Gyrosigma* | 1.64            | 0.00 | 0.01          | 0.82            |
| *Navicula*  | 63.93           | 0.30 | 0.08          | 32.11           |
| *Nitzschia* | 11.48           | 0.03 | 0.01          | 5.75            |
| *Pinnularia*| 3.28            | 0.01 |               | 1.65            |
| *Pleurosigma* | 3.28  | 0.01 |               | 1.64            |
| *Surirella* | 1.64            | 0.01 |               | 0.82            |
| *Tabularia* | 3.28            | 0.02 | 0.01          | 1.65            |
| *Thalassionema* | 1.64 | 0.00 |               | 0.82            |
| **Pennate diatom** |       |      |               |                 |
| *Coscinodiscus* | 44.26 | 0.43 | 0.18          | 22.35           |
| *Cyclotella* | 85.25           | 0.55 | 0.17          | 42.90           |
| *Paralia*   | 19.67           | 0.05 | 0.02          | 9.86            |
| *Thalassiosira* | 9.84   | 0.03 | 0.01          | 4.93            |
| **Centric diatom** |      |      |               |                 |

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|                          |       |   |   |     |
|--------------------------|-------|---|---|-----|
| Other diatoms            | 59.02 | 0.27| 0.03| 29.64|
| Dinoflagellate           | 44.58 | 0.41| 0.15| 22.50|
| Foraminifera             | 19.28 | 0.08| 0.06| 9.68|
| Porifera (siliceous spicules) | 38.55 | 2.29| 4.99| 20.42|
| Leaf matter              | 84.43 | 4.31| 1.94| 44.37|
| Vegetal detritus         | 100   | 91.05| 92.28| 95.50|

**Figure 2:** Food items found in gut from Kuala Selangor and Tanjung Piai including diatoms and other organisms. (a) *Cosinodiscus* sp. found in gut from Kuala Selangor; (b) *Cyclotella* sp.; (c) *Eunototragmna* sp. from Tanjung Piai; (d) *Diploneis* sp. with chloroplast; (e) *Gyrosigma* sp.; (f) *Nitzschia* sp. the raphe located at the edge of frustules; (g) *Paralia* sp. (h) *Pinnularia* sp.; (i) *Surirella* sp.; (j) *Tabularia* sp.; (k) *Thalassionema* sp.; (l) *Thalassiosira* sp. from Tanjung Piai with fragmentation around the frustules; (m) Dinoflagellate with the present of plate; (n) Foraminifera; (o-p) Porifera (siliceous spicules). (Scale bar = 50μm)

3.3. *Faecal content*

There was a similarity in food item found in faecal pellets and gut content. Figure 3 is portraying several food items found in faecal pellets. Faecal content from both sites possessed vegetal detritus as the highest excreted item composition with 92.28% followed by leaf matter with 1.94%. Diatoms come in third with 0.59 % and dinoflagellate is 0.15% while foraminifera show the lowest percentage with 0.06% in total. Apart from that, other food items including porifera (siliceous spicules and
unidentified food items) was 4.99% surpassing the leaf matter composition. Table 3 shows the presence of each diatom genera in faecal pellets collected in KSNPA, TPNPS and TPNPD.

**Figure 3**: Excreted food items found in faecal pellets of *Cerithidea obtusa* from Kuala Selangor and Tanjung Piai. (a-b) *Coscinodiscus* spp. intact with sediment; (c) *Cyclotella* sp.; (d) *Thalassiosira* sp.; (e) *Thalassionema* sp.; (f) *Pleurosigma* sp. raphe in the middle; (g-h) *Diploneis* spp; (i) *Amphora* sp. organic matter inside the body; (j) *Navicula* sp.; (k) Porifera (siliceous spicule); (l) Fragment of microplastic from Kuala Selangor; (m) *Navicula* sp.; (n) Dinoflagellate; (o-p) Foraminiferans. (Scale bar = 50µm)

**Table 3**: Presence of diatoms in fecal pellets from Kuala Selangor Nature Park and Tanjung Piai National Park.

| Diatom          | KSNPA | TPNPS | TPNPD |
|-----------------|-------|-------|-------|
| Pennate diatom  |       |       |       |
| *Amphora*       | +     | +     | +     |
| *Cocconeis*     | +     | +     |       |
| *Diploneis*     | +     |       | +     |
| *Eunotogramma*  |       |       | +     |
4. Discussion
This study has shown that the gut content was mainly composed of vegetal detritus followed by leaf matter, diatoms, dinoflagellate, porifera and foraminiferans. Other food items for example microplastic and sand grain were also recorded which was not included in these six major groups of food items as only small numbers quantified. The vegetal detritus was indicated as the highest Fpi with 100% found in every gut with a total number of 28983 particles compared to the second dominated food item which is leaf matter with the Fpi of 84.43% and 1371 leaf particles found in the gut from both localities. The list followed by the Fpi of diatoms 71.08%, dinoflagellate 44.58%, porifera 38.55% and foraminifera 19.28%. With that, gastropod under family Potamididae was said to be a bottom grazer, non-selective detritus feeder as they inhabit mangrove area in large number. Although the mangrove tissue was found abundant in the gut, it was said to be accidentally ingested while the gastropod grazing (foraging) for microalgae including diatoms [5]. The statement has supported the results showing that the percentage of vegetal detritus ingested to the digestive system and excreted out in the form of faecal pellets were both high indicating the unnecessary importance of food item goes in and out from the digestive tract of the *C. obtusa*.

According to this study, it was demonstrated that the gut content of *C. obtusa* from Kuala Selangor Nature Park, Selangor shows a significantly higher percentage of food items when compared to those of Tanjung Piai National Park, Johor. Different timing during sampling was took place in this study. First, in Kuala Selangor, the snails were collected 2 hours before high tide happens while in Tanjung Piai, the sampling took place after the low tide at noon (TPNPS) and in the early morning (TPNPD). The time at which the snails are able to forage for food during low tide in Kuala Selangor awaiting for the high tide is believed to enable them to ingest more food when compared to the snail in Tanjung Piai. Similar activities were reported by a research from South Lumpung, Indonesia, elaborating on the activity of potamidid snails was related to the availability of tidal regime, the respiratory system and the moisture content within the ecosystem which triggered them to move horizontally and vertically on and off of the tree trunk [14]. The activity of potamidid snails in the South Lumpung, Indonesia was observed to be affected by the exposure to the low water in the sampling sites which the snails usually happened to feed during low water. *Cerithidia quadrata*, the accepted name is *Cerithidea quoyii* (Hombron & Jacquinot, 1848) as any other *Cerithidea* genus, was affected by the tidal changes for the snail is believed to respire in the air and their capability to respire in the water is limited [15] [16] [14]. These factors may explain the correlation between tidal level and feeding behaviour of the *C. obtusa* in conjunction with the different composition of food items found in both study sites. The scale of gut fullness might be an indication of this scenario as well. In Kuala Selangor, the gut
content in the stomach was observed to be from almost full (95%) to half full (50%) meanwhile the scale of the fullness of the gut from Tanjung Piai was seemed to be varied from almost full (95%) to empty stomach (0%).

Apart from that, in the major groups of food items, t-test analysis on the composition of dinoflagellate and foraminifera were tested to be significantly different (p<0.05) between these two items found in Kuala Selangor and Tanjung Piai. The presence of dinoflagellate in the gut collected from Tanjung Piai was an indication of the accumulation of organic materials in the water column coming from the nearest agricultural activities. The bloom of dinoflagellates was influenced by the increasing of nutrients in the water column affected by the agriculture industry and urbanization [17][18] which triggered the dinoflagellate to rapidly bloom in favourable conditions and dispersed in the water, reaching the mangrove bank and ingested by C. obtusa while grazing for food. Moruf and Lawal-Are in 2015 observed that favourable environmental conditions such as habitat and prey availability will enhance the diet intake of the snail in the surrounding ecosystems [19]. It has been demonstrated that the relative importance of different food items for a certain species might be varied depending on its location and that such difference in diet composition may be related to the relative availability of potential food sources in their habitat [20] [21]. The availability of foraminifers in mangroves was identified to be affected by pH, salinity and temperature of the environment [22]. They were possibly rasped together along with other food items [23]. Thus, the water conditions, locations and availability of potential food might be the factors contributing to the differences that occurred between dinoflagellate and foraminiferans composition in Kuala Selangor and Tanjung Piai. The changes occurred in the water properties altering to rapid growth of dinoflagellate in the study sites. While the availability of food contributed to the wide assimilation of food items in gut content as the snail is grazing on the mud which intact with various microorganisms.

The various food items found in the gut are believed to be ingested continuously at a time of foraging on mud. A component in the stomach of Cerithidea was known to aid in ingesting the food while grazing of food took place. A study on the physiology of the digestive system of Cerithidea has been made by Sreenivasan in 1995 revealed that there was an occurrence of style sack (crystal stalk) in the gut of Cerithidea [24]. The function of the style sack was discussed as a device to ensure the flow of the enzyme throughout the digestive tract is sufficient to help in breaking down the food materials in the stomach of the Cerithidea [6]. The mechanism of the style sack in accelerating the digestion process was happened by rotation of the crystal stalk for the digestion process to occur in slow continuously feeding snail [6] [24]. This shown that the function of style sack helps to break down the large and various food items that eaten by C. obtusa such as vegetal detritus, leaf matter and diatoms during feeding time on the mud in the mangrove areas.

Potentially, several studies on the Potamididae family suggested that the snails feed on microalgae including diatoms and phytoplankton [6]. Our study found that the occurrence of three diatom genera in the stomach can be traced known as Coscinodiscus, Cyclotella and Navicula. These results matched those observed from the earlier studies explaining the feeding of microalgae by potamidid snails. Cyclotella was found in large number both in the gut and faecal pellets. Coscinodiscus, Cyclotella and Navicula are known as the epipelic diatom in which they associated with the mud interfacing water and sediment and even on roots of mangrove trees [25].

Few studies had been focusing on benthic diatom and a study was executed in a neighbouring country, Indoneisa by Nitisuparjo and Hendarto in 2011 found that Navicula is inhabited benthic surrounding of three mangrove forest in Central Java, Indonesia [26]. The recognition of Navicula as a benthic diatom was documented in several regions across the countries such as in Malaysia [27] Australia [28] [29], Papua New Guineia [30] and some others [31]. This explained the behaviour of C. obtusa during low tide in which they were seen to be crawling down from the tree and grazing on the mud surface to search for food after resting on the tree trunk during high water occurrence. There are high possibilities of C. obtusa grazing for diatoms on the muddy sediment. The presence of both centric and pennate diatoms strengthen the idea of C. obtusa is a non-selective feeder in the mangrove ecosystems.
To date, the diatoms from the gut and faecal pellets were observed for their two conditions; broken and unbroken frustules. It was documented from this study that unbroken frustules were most commonly found in both gut and faecal pellets. Many of those unbroken frustules were seen to possess no organic matter (chloroplast) inside of their bodies in the gut and faecal pellet. Even though the unbroken frustules were dominated, it is not possible that the micro-scale rupture around the frustules might occur inside the digestive tract and leads to the release of organic materials from the diatoms as the energy sources for the snail to grow [13].

Interestingly, our finding was able to identify abundant siliceous spicules in both gut and faecal pellets. There were several types of poriferans had been observed ingested by C. obtusa. A study from Indonesia on sponges revealed that the porifera or marine sponges were found in the mangrove located near to the coastal area [32]. The sponges were driven by the water from the coastal area to the mangrove area and settle at the bottom. This revealed the reasons of the presence of spicules in the gut and faecal pellets of the C. obtusa. We believed that the spicules were also a forms of detritus which been accidentally grazed by the snail upon foraging for the food on the mangrove sediments during low tide as the presence of spicules both in gut and faecal pellets were fairly the same.

5. Conclusion
This study is set out to determine the diet of Cerithidea obtusa from two mangrove areas in Kuala Selangor Nature Park, Selangor and Tanjung Piai National Park, Johor. This study has shown that C. obtusa is like other potamidid snail is a bottom grazer specifically a non-selective feeder. The gut and faecal content were classified into six major groups concerning vegetal detritus as the highest food items that being consumed and excreted. Ingestion of vegetal detritus in the estuarine area was discussed to be an accidental food item while grazing for the microalgae. In both Kuala Selangor and Tanjung Piai, microalgae (in this study is diatom) possess three important genera of epipelic diatom which were frequently found in the gut; they are Cosinodisus, Cylotella and Navicula. On the other hand, the occurrence of dinoflagellate in the gut from Tanjung Piai was linked to the nearest anthropogenic activity; agricultural runoff which triggered the bloom of dinoflagellate. Few factors such as locations and the timing of sampling were observed to be the main factors for the various food intakes grazed by the snail. It is important to study the diet of the Potamididae which occur abundantly in the mangrove area. It is much needed to study their dietary composition to conserve the ecosystem and the sources of the food itself to maintain these species in our mangrove ecosystem from extinction. The study on Cerithidea obtusa for example can help in providing data on their habitat and food preferences which can be used as references for future studies. Further study can be carried out to allocate the relationship between their feeding mechanism and food preferences for a better understanding of their ecological roles in the mangrove ecosystems.

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