

## Research Article

# Food Preference, Fecundity, Proximate Analysis on Eggs and Meat of *Turbo crassus* (W. Wood 1828) and *Turbo setosus* (Gmelin 1791) in Sepanjang Beach, Gunungkidul, Special Region of Yogyakarta

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### ABSTRACT

Turbo snails have important roles in the ecosystem as prey for predators, and decomposition of dead objects, and it is starting to decrease. This is quite worrying so that conservation efforts must be carried out immediately. Turbo snail species found in Sepanjang Beach, Gunungkidul are *Turbo crassus* and *Turbo setosus*. The aims of this study were to determine food preferences, the relationship between body size and fecundity, analyze egg quality based on proximate levels of the two species, and meat proximate of *T. crassus* and *T. setosus*. Samples were taken from Sepanjang Beach, Gunungkidul. Food preference was observed using the sedgwick rafter, fecundity was counted using the gravimetric method. Moisture content, protein content, fat content, ash, and carbohydrate content of both species eggs and meat were determined with proximate analysis at the UGM Central Laboratory for Food and Nutrition Studies. The results showed that the dominant food for *T. setosus* was Phaeophyta, whereas the dominant food for *T. crassus* was *Microcoleus* sp. and Phaeophyta. The fecundity of both Turbo species is exponentially positively correlated with all body sizes. Proximate analysis showed that the levels of fat and protein in the eggs of both species were quite high, which meant that the eggs are in good quality. The conclusion of this study is both species have slightly different food preferences. The fecundity of both species will increase exponentially with increasing body size and the broodstock of *T. crassus* and *T. setosus* Sepanjang Beach has good quality.

**Keywords:** Fecundity, Food Preference, Proximate Analysis, *Turbo crassus*, *Turbo setosus*

### INTRODUCTION

Turbo snails have been sold as food since 1995 and have started to be in great demand since 1998 at Kukup Beach (Azizah 2019). Based on a preliminary study conducted through interviews with local residents, Turbo snails in Gunungkidul were abundant in certain seasons, especially the rainy season, around February to April. In this season, people hunt every size of Turbo snail. This activity causes decrease in Turbo snail's availability in nature. Thus, conservation efforts like breeding must be carried out immediately.

Breeding efforts of Turbo snails have been successfully carried out on *T. marmoratus* (Dwiono et al. 2001) in Ambon and *T. chrysostomus* (Setyono et al. 2013) in Lombok by taking wild sires from nature to be spawned in the laboratory, while their reproductive biology has not been studied extensively. Biological aspects that are important to study as breeding efforts include fecundity, food preferences, and egg quality. Robinson (1992) evaluated that, the fecundity of individual females *Turbo smaragdus* increases along with shell size. This pattern relates to the allocation of energy in organisms, younger snails need more energy to grow while the older can use more energy to reproduce. Egg quality is important to know because the early development of the embryo is strongly influenced by the quality of the eggs, which is related to the adequacy of energy reserves and raw materials for organogenesis. Larvae that hatch from eggs with the highest protein and fat content survive the longest without food. Proximate analysis of the gonads can be carried out to determine the quality of eggs based on their nutrient composition (Fukazawa et al. 2005).

Gastropods use the radula to scrape their food from the substrate. Turbo snails have a type of radula known as rhipidoglossan commonly possessed by Ordo Archaeogastropoda. Members of the Order Archaeogastropods eat filamentous algae and other algae that grow on rocks. Kikutani et al. (2002) studied that *Turbo marmoratus* has different food preferences in different habitats. Those found in the intertidal area prefer algae while those found in the tidal area prefer foraminifera, copepod, hydrozoa, nematode, bryozoa, and even snail juvenile for their food. Another research shows that *Megastraea undosa*'s diet consists of several macroalgae groups such as Rhodophyta, Chlorophyta, and even from Cnidaria groups such as *Dynamena* sp., Mollusca such as *Capulus* sp and *Petricola* sp., Arthropods such as *Idotea* sp and Amphipoda, and Chordata such as *Metandrocarpa* sp. (Mazariegos-villarreal et al. 2017).

Based on our preliminary study, there are two species of Turbo snails found in Sepanjang Beach, namely *Turbo crassus* and *Turbo setosus*. The aims of this study were to determine food preferences, the relationship between body size and fecundity, analyze egg quality based on proximate levels of the two species, and meat proximate of *Turbo crassus* and *Turbo setosus*.

## MATERIALS AND METHODS

### Materials

The materials used in this study were *T. crassus* and *T. setosus* snails; aquades, 4% formalin, and 70% alcohol, Gilson's solution (consisting of 100 mL 60% alcohol, 800 mL distilled water 15 mL 80% nitric acid, 18 mL glacial acetic acid, and 20 g mercuric chloride). The tools used in this study were analytical and semi-analytical scale, coolboxes and freezers, tweezers, pipettes, optilab and microscopes (Boeco, Germany), hand counters, calipers, millimeter blocks, and rulers, knives and scissors, flacons glass, microtubes, beaker glass, sedgewick rafter, and measuring cup.

### Methods

Data collection was divided into 4 steps, namely sample collection and morphometry data collection, fecundity counting, gut content analysis, and proximate analysis.

### Sample Collection

Sample collection was conducted at Sepanjang Beach, Gunungkidul, at night, 21 July, 15-16 August, and 26-27 September 2020 using the purposive random sampling method. The snail samples that have been caught were

immediately put in the freezer or icebox. The total weight of the snail, length, and width of the shell were measured in the laboratory, then the shell was carefully broken with a hammer so that the visceral mass was not damaged. The visceral mass was separated from the shell fragments and the operculum, weighed, then put in a container containing 4% formalin for 24 hours. Formalin was then replaced with alcohol 70%, sample can be stored until the time of observation. The sex of the samples was also observed based on the color of the gonads (female gonads are olive green and the males are yellowish cream). The gonad weight was measured using a semi-analytic scale.

### Gut Content Analysis

To determine the food preferences of both species, dissection was performed and the stomach contents were observed using the sedgewick rafter. The stomach content was diluted in 10 mL aquadest. The solution of the stomach contents is then taken using a 1 mL dropper and placed on top of the sedgewick rafter. The contents of the stomach were observed under a microscope, each type of food was counted using the sedgewick rafters and calculated as follows to evaluate the food preferences of *T. crassus* and *T. setosus*.

$$\text{Relative Frequency (RF)} = \frac{\text{Frequency of species X}}{\text{Total frequency}} \times 100\%$$

$$\text{Relative Abundance (RA)} = \frac{\text{Abundance of species X}}{\text{Total abundance}} \times 100\%$$

$$\text{Important Value Index (IVI)} = \text{RF} + \text{RA} \text{ (Islamiyah et al. 2013)}$$

Observations were made in 3 repetitions.

### Fecundity Counting

The number of eggs was count using the gravimetric method. Whole gonads were separated from other organs for weighing. A little piece of the whole gonad (sub-samples) was taken as much as 0.001-0.002 g from the posterior, middle, and anterior parts. Sub-samples of gonad were then placed in Gilson's solution for 48 hours or more, to separate the eggs from the connective tissue. Eggs were counted using a hand counter tool. The results of the sub-sample counting were used to obtain an estimate of the total number of eggs. Fecundity was estimated using the formula as follows:

$$\text{Fecundity} = \frac{\text{total gonad weight}}{\text{average of sub-samples weight}} \times \text{average of the number of eggs in sub - samples}$$

Note: The average of sub-samples (weight and the number of eggs) were from 3 parts of each gonad.

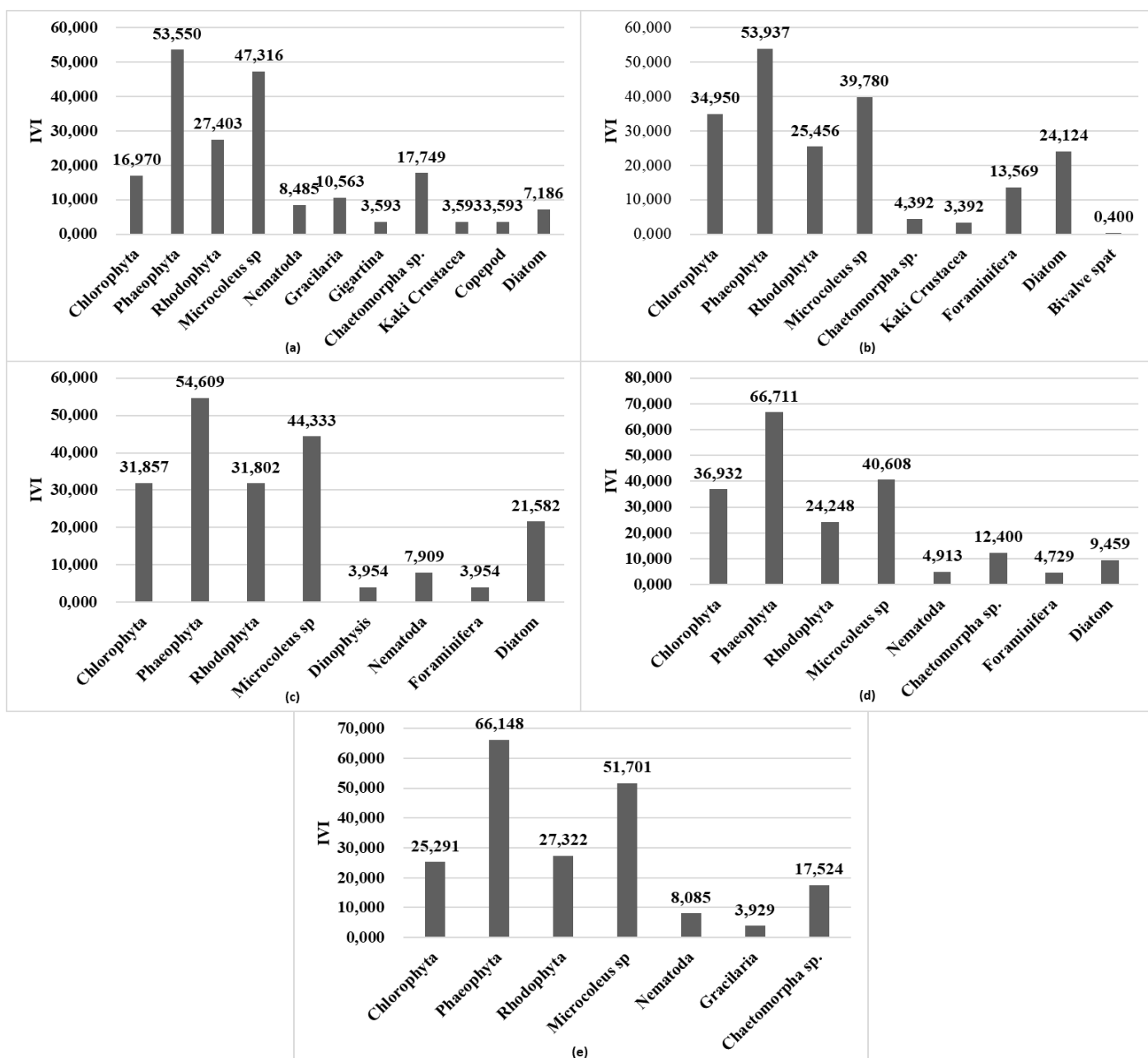
### Proximate Analysis

The samples of *T. crassus* eggs analyzed were 4.9 g from 11 samples, while the samples of *T. setosus* eggs analyzed were 6 g. Samples of meat or foot from *T. crassus* and *T. setosus* were taken as much as  $\pm 30$  g. The proximate analysis was done using the Kjeldahl method for protein content, Soxhlet for fat content, Thermogravimetry for moisture content, Muffle Furnace for ash content, and carbohydrate by difference for carbohydrate content (Ratna 2019).

## RESULTS AND DISCUSSION

### Gut Content Analysis

The stomach contents of *Turbo setosus* indicate a variety of natural food. *Turbo setosus* samples used in this observation were 24 individuals and divided

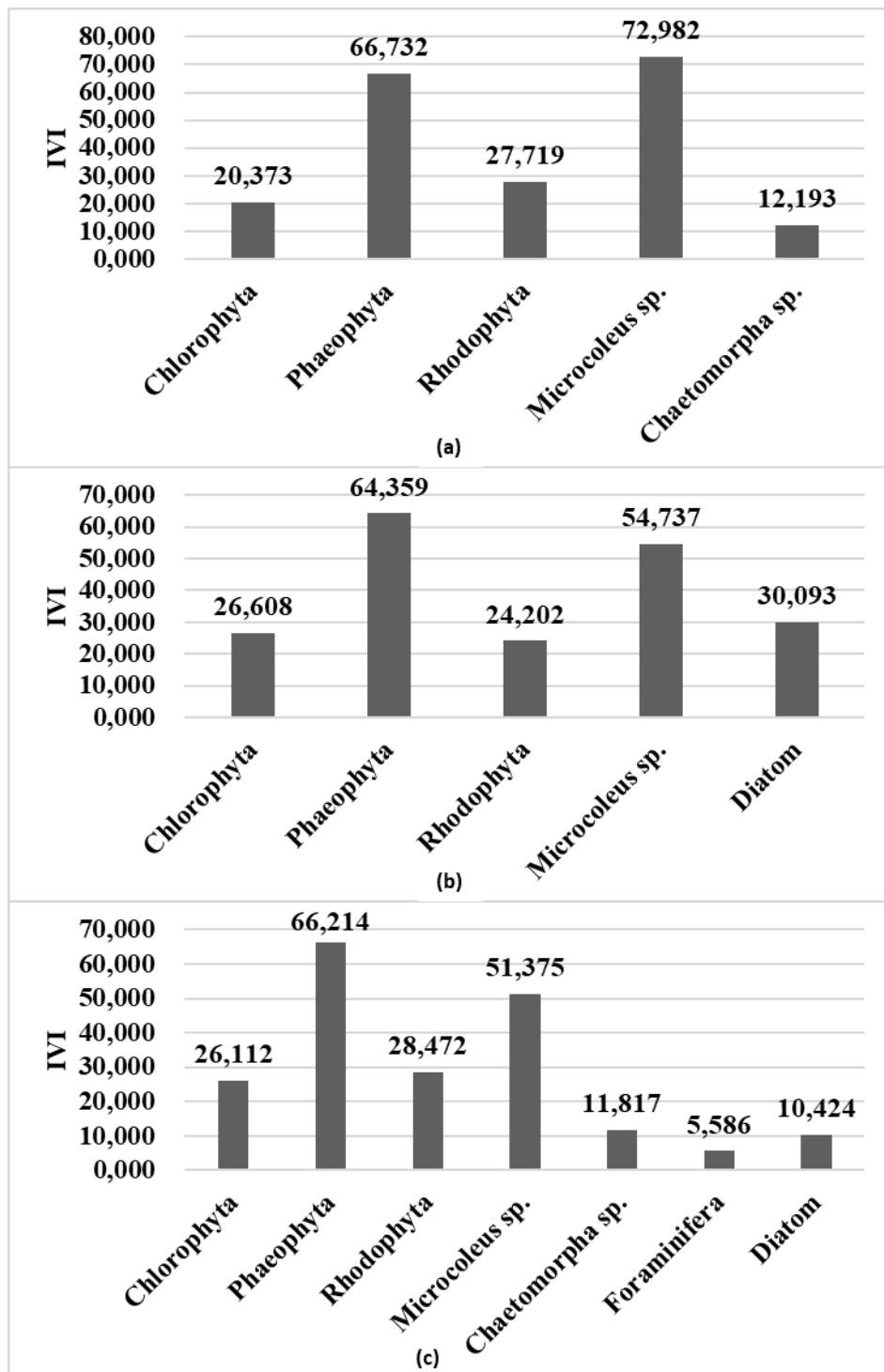


**Figure 1.** Importance value index (IVI) of *T.setosus* gut content (a) group 3,5-4 cm (b) group 4-4,5 cm (c) group 4,5-5 cm (d) group 5-5,5 cm (e) group 5,5-6 cm. n=1.

into 5 size groups based on shell length with each class having 5 samples. The first group consisted of 3.5-4 cm shell lengths. The second group consisted of 4-4.5 cm shell lengths. The third group consisted of 4.5-5 cm shell lengths. The fourth consisted of 5-5.5 cm shell lengths. The fifth group consisted of 5.5-6 cm shell lengths. Group size 5-5.5 cm in length has only 4 samples because of the inadequate sample quantity.

The IVI diagram in Figure 1 showed that Phaeophyta or brown algae have the highest Importance Value Index, followed by *Microcoleus* sp. and Rhodophyta or Chlorophyta in the third position. Importance Value Index (IVI) is used to analyze the dominance of a species in a particular community (Pamungkas & Zamzam 2017). The high IVI in Phaeophyta indicates the dominance of Phaeophyta in the stomach contents of *Turbo setosus* snails.

Based on the diagram in Figure 2, it is found that in the 3.5-4 cm size group *Microcoleus* sp. has the highest IVI, and Phaeophyta has the second-highest IVI. Phaeophyta has the highest IVI in class sizes 4-4.5 cm and 4.5-5 cm, and *Microcoleus* sp was in the second position.

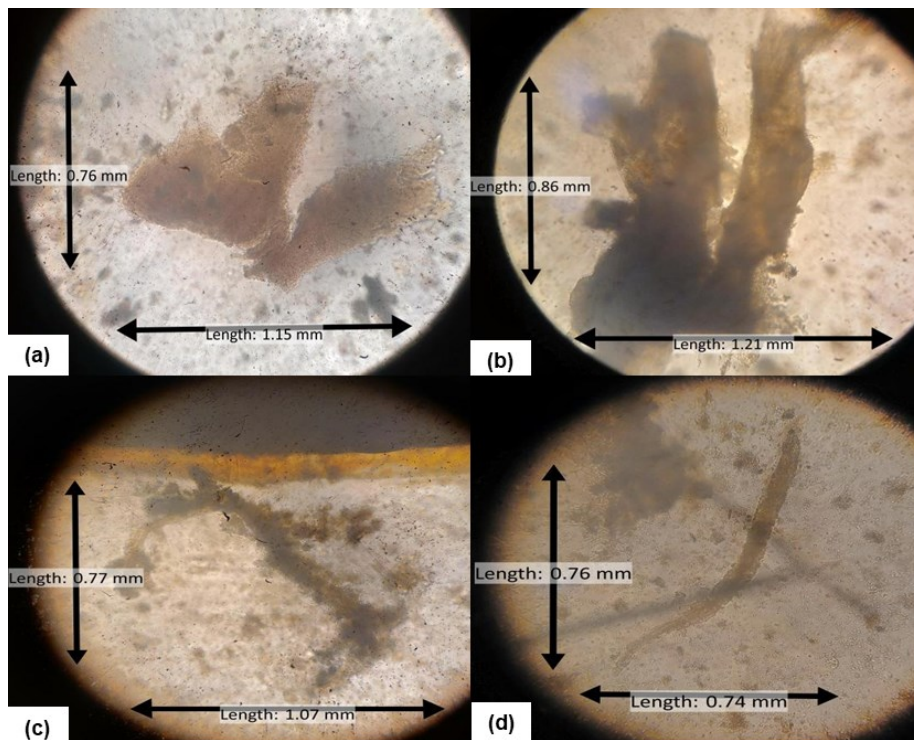


**Figure 2.** Importance value index of *T. crassus* gut content (a) group 3,5-4 cm (b) group 4-4,5 cm (c) group 4,5-5 cm. n=1.

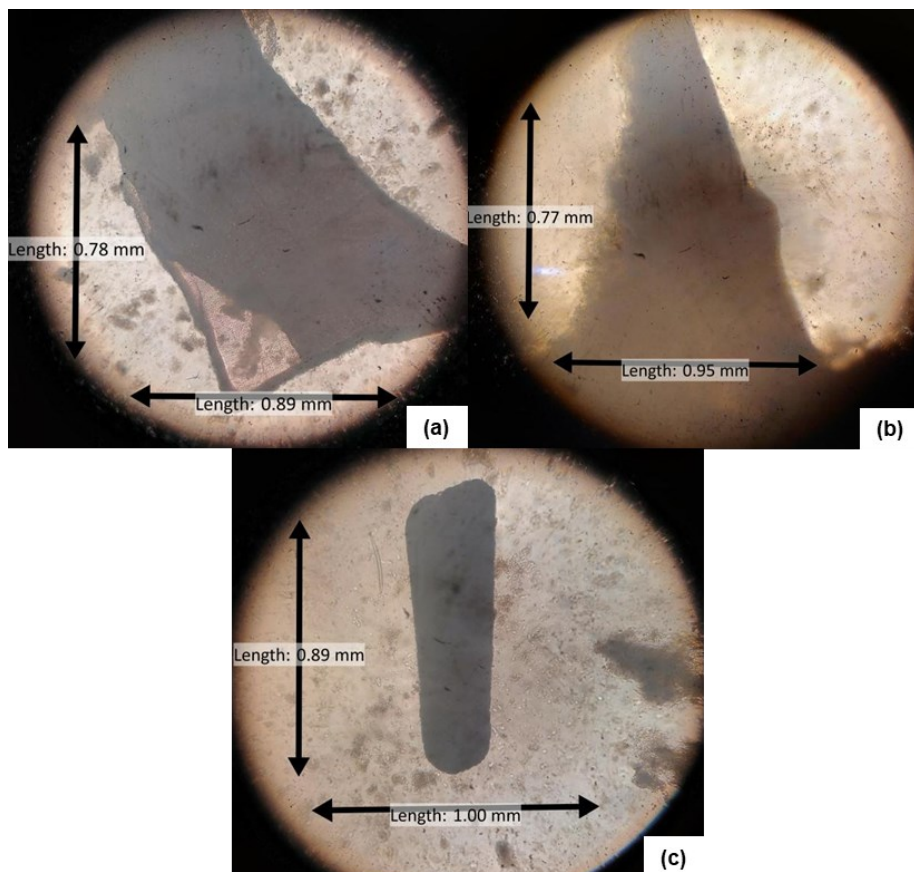
Figure 3 shows the conditions of Phaeophyta that can be found in the stomach of the *Turbo setosus* and *Turbo crassus*. Brown algae talus that can be found in the stomach indicates a tear that is thought to be due to the radula. The algae cell wall is also damaged due to the grinding process on the gizzard. The brown algae found in the stomach of *Turbo crassus* are shaped like filaments, while the Phaeophyta found in *Turbo setosus* is more sheet-like.

Rhodophyta in Figure 4 shows conditions that are not much different from Phaeophyta. Tearing is also estimated by the snail's radula, but it can be seen that the cell wall is still relatively better than the Phaeophyta found. The relatively better cell wall is estimated because the lag between taking and preserving the samples obtained and the time for the snails to feed is not too long so that the algae wall in the stomach is still relatively intact.



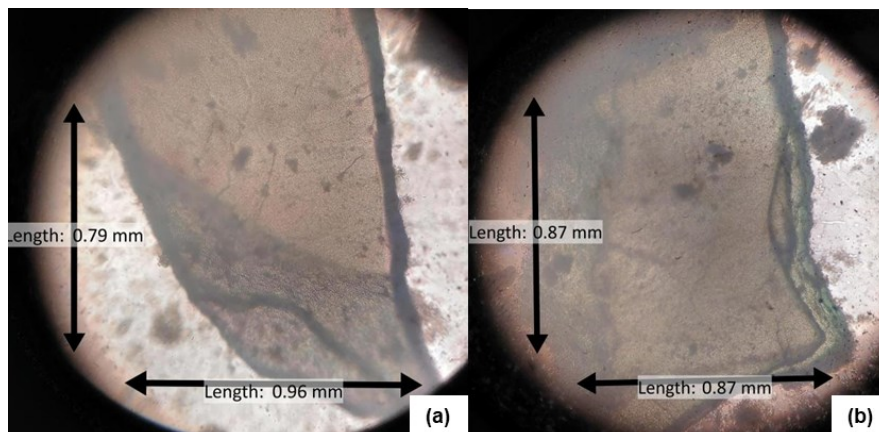


**Figure 3.** Phaeophyta found in *T. setosus*' stomach (a,b) and in *T. crassus*' stomach (c,d), magnification of  $\times 10$ .



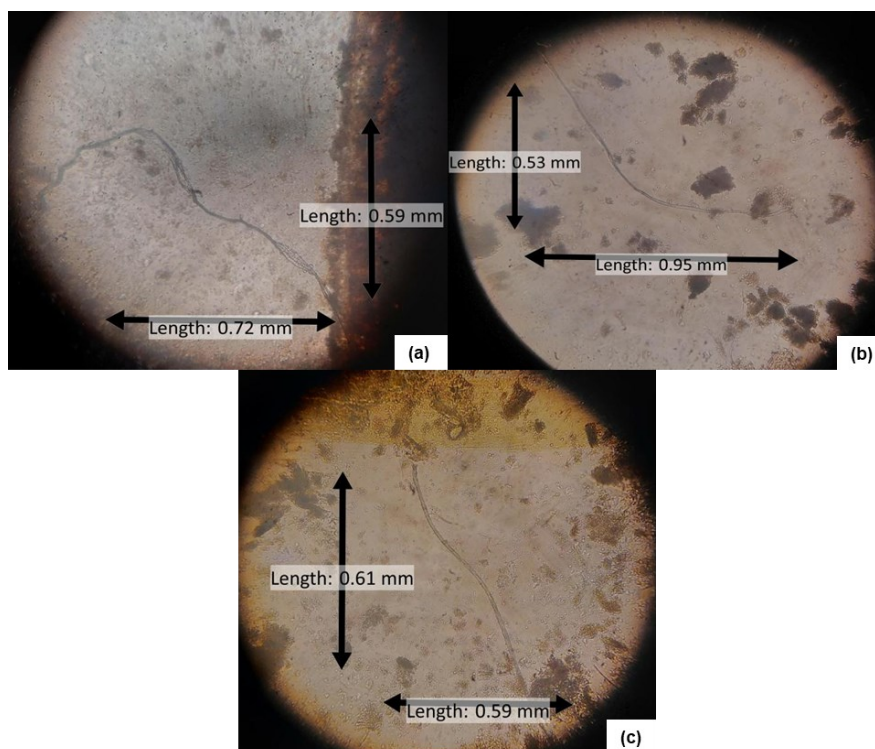
**Figure 4.** Rhodophyta found in *T. setosus*' stomach (a,b) and in *T. crassus*' stomach (c), magnification of  $\times 10$ .

Figure 5 showed the condition of Chlorophyta which has sheet-shaped talus and is overlapped by brown algae. The tearing of the talus is thought to be due to the snail's radula tearing the green algae talus. The cell wall is still in a relatively intact condition.



**Figure 5.** Chlorophyta found in *T. setosus* stomach (a,b) magnification of 10x10.

The condition of *Microcoleus* sp inside *Turbo crassus*' stomach is not much different from the condition of *Microcoleus* sp in *Turbo setosus*' stomach (Figure 6). The characteristic conditions seen in *Microcoleus* sp. are in the form of filaments, filaments that join together into a thin layer, arranged in parallel, and are colorless (Guiry 2020).



**Figure 6.** *Microcoleus* sp found in *T. setosus* stomach (a,b) and in *T. crassus*' stomach (c) magnification of 10x10.

In another study in the Tuamotu Islands, *Turbo setosus*' stomach contents could be found several types from various kinds of algae groups. The members of the Chlorophyta group that can be found were *Cladophora* sp. The members of the Phaeophyta group found were *Lobophora variegata* and *Sphacelaria* sp. The Rhodophyta group members found were *Jania* sp. and *Laurencia* sp. Members of the Cyanobacteria group that could be found were *Calothrix confervicola*, *Microcoleus lyngbyaceus*, *Schizothrix calciola*, and *Spirulina subsalsa* (Tsuda & Randall 1971).

Gastropods have chemoreceptor organs, one of which is osphradium. Osphradium is a primitive chemoreceptor organ that is connected to the visceral arch of the central nervous system (Kamardin et al. 2001). Studies have been taken to prove that Gastropods do have preferences on their food.

Oosphradium stimulation with L-aspartate in *Lymnaea stagnalis* causes an increase in oosphradial nerve activity (Kamardin et al. 2001). Rhinophores also have an active role in finding habitats for nudibranch larvae based on the presence of suitable food (Bornancin et al. 2017). Metamorphosis in *Aphysia californica* also occurs with several chemical signals from several algae such as *Rhodomyenia californica*, *Corallina officinalis*, *Dyctyopteris undulata*, *Pachydictyon coriaceum*, *Pterocladia capillacea*, *Centroceras clavulatum*, and *Chondria californica* (Bornancin et al. 2017).

The result from different shell length groups has only slight differences, all shell length sizes have Phaeophyta as their most dominant food, followed by *Microcoleus* sp. as the second most dominant food in their stomach. There is no different food preference based solely on shell length. This shows that their diet is roughly the same even among different sizes. Further research in captivity or cultivation may be needed for the confirmation of this result.

Based on the observations in this study, Turbo snails seem to have a preference for their food. Both Turbo species have shown some slight differences in their food preferences. *Turbo setosus* food preferences consist of 3 types of macroalgae, *Microcoleus* sp, 1 type of nematode, crustacean, and diatoms. *Turbo crassus* food preferences consist of 3 types of macroalgae, *Microcoleus* sp, foraminifera, and diatoms. The results showed that both species seem to be able to differentiate their food.

### Fecundity

Decapoda and Bivalvia's carapace or shell capacity is the limit of gamete productivity. A hard, closed-shell will limit the space available for the development of the gonads. In other words, carapace or shell size has a positive correlation with fecundity. The more space available, the more eggs that can be produced. The growth of the organism is also greatly influenced by the food available in its environment, if there is an increase in the amount of food, the rate of growth will increase and the egg production becomes more abundant. The energy possessed by living things will be allocated to growth and reproduction, and the distribution will adjust to the age or phase of their life. In some Bivalvia, there is massive growth in the early years of its life, meaning that the energy is focused on growth. The growth rate will begin to decline as gamete production increases. The increase in *Mytilus edulis* and *Yoldia notabilis* fecundity was more closely related to the body size of the broodstock than age (Llodra 2002). The fecundities of *T. crassus* and *T. setosus* per size group mean can be seen in Figure 7.

Based on Figure 7, of the 11 individual *T. crassus* species studied, the increase in shell length had a positive exponential correlation with the increase in egg number or fecundity. Likewise with the increase in shell width and total weight. The highest  $R^2$  value is 0.6683 in the ratio of the number of eggs to shell length (Figure 7a). The lowest  $R^2$  value is 0.3967 in the ratio of the number of eggs to shell width (Figure 7b).

Based on Figure 8, of the 18 individual *T. setosus* species studied, the increase in shell length had a positive exponential correlation with the increase in egg number or fecundity. Likewise with the increase in shell width and total weight. The highest  $R^2$  value is 0.5252 in the ratio of the number of eggs to shell width (Figure 8b). The lowest  $R^2$  value is 0.3964 in the ratio of the number of eggs to the total weight (Figure 8c).

The value of  $R^2$  is a marker of how much data can be explained by a regression formula. In this fecundity data, the exponential regression that has the best  $R^2$  value means that an increase in body size will increase the number of eggs exponentially. The body size parameter that describes fecundity well is the one with the highest  $R^2$  value. *T. crassus* had the highest



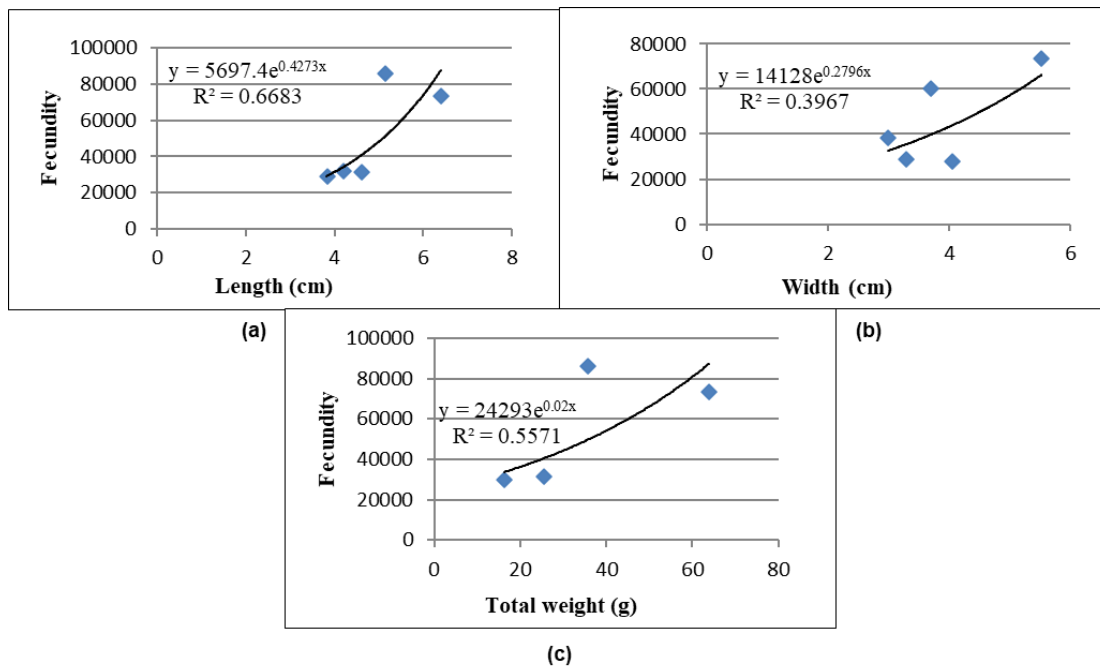


Figure 7. Comparison of the shell length (a), shell width (b), and total weight (c) of *T. crassus* to fecundity.

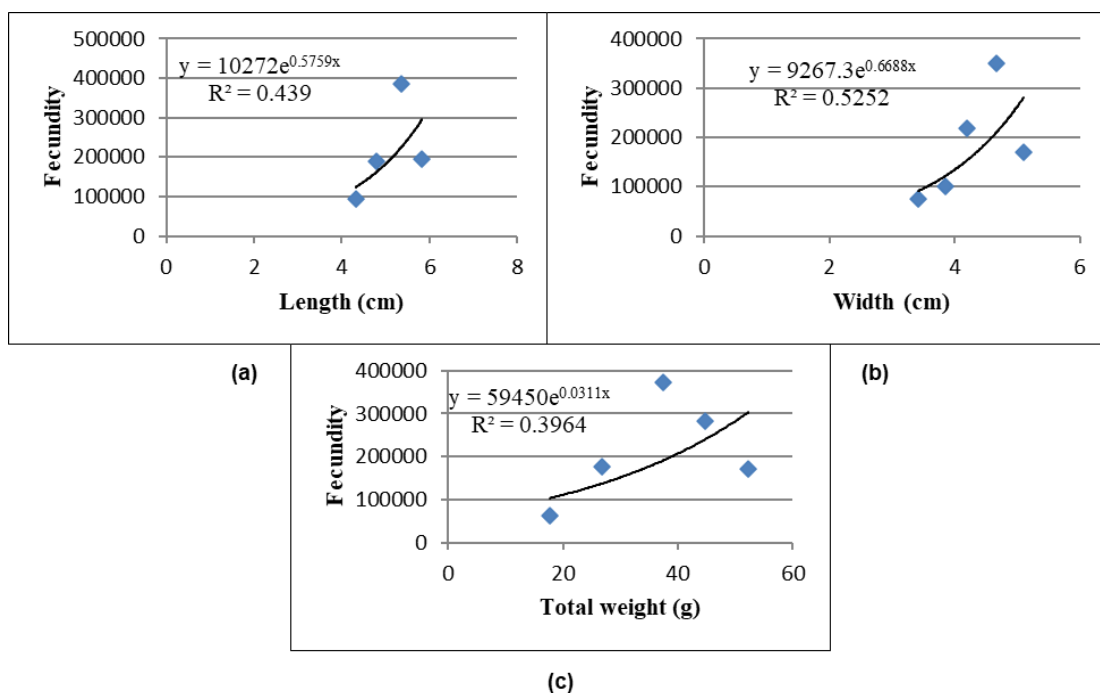


Figure 8. Comparison of shell length (a), shell width (b), and total weight (c) of *T. setosus* to fecundity.

$R^2$  value for shell length parameters, while *T. setosus* had the highest value for shell width. This difference can be attributed to the shell morphology of *T. crassus*, which has a longer spire than *T. setosus* (Poutiers 1998). The ratio between the length and width of the shells of the two species did not differ much, that is 1: 0.82 in *T. crassus* and 1: 0.85 in *T. setosus*.

Based on the description above, body size will determine the number of eggs that can be produced, in relation to the availability of space for gonad development in the shell. Larger snails have a higher number of eggs, so the comparison of the number of eggs to the length and width of the shell shows a positive correlation. The complete weight of the snail also correlates with its fecundity, as the number of eggs will also have an effect on its total body weight. The positive correlation between fecundity and body size also occurs in other gastropod members such as *Conus pennaceus* (Perron 1983); Limpet

species *Notoacmea petterdi*, *Patelloida alticostata*, and *P. latistrigata* (Creese 1980); Invasive gastropod *Crepidula fornicata* (Pechenik et al. 2017).

If a Turbo snail breeding efforts is carried out such as *T. chrysostomus* (Setyono et al. 2013) and *T. marmoratus* (Dwiono et al. 2001), the quality of broodstock must be considered so that the resulting spawns are also of good quality. One of the considerations to determine the quality of broodstock is their fecundity. The results of this study indicate that the first maturity period of *T. crassus* and *T. setosus* gonads were found in the shell length of 3-4.5 cm. The least gonads are produced at that size, so it is better to use broodstock at a shell length of at least 4-5 cm to increase the number of offspring. Turbo snails take a long time to grow. The government can create a program to release the seeds to help restore the population in the wild and create limits on their catch. The availability of nutrients also has an important effect, such as *Haliotis laevigata* and *H. cyclobates* experiencing the highest gonad growth when food is abundant (Robinson 1992).

In *T. smaragdus* (Robinson 1992) which has a certain period for spawning, fecundity observations were made in January where the gonads were in the immediate pre-spawn phase when the ovaries were filled with mature oocytes. In this study, there was no information regarding the reproductive cycle throughout the year. It is possible that *T. crassus* and *T. setosus* fecundities could reach higher numbers. The highest number of eggs in *T. crassus* individuals was counted is 85,876 eggs, while the least number of eggs was 8,881 items. The *T. setosus* individual counted at most 567,719 eggs, and at least 35,563 eggs. The number of *T. crassus* eggs that are less can occur due to different reproductive cycles, or indeed the reproductive ability is lower than that of *T. setosus*. This can be confirmed by observing fecundity every month, for a full year.

### Proximate Analysis

Proximate analysis is performed to determine the moisture, ash, fat, protein, and carbohydrate content in the sample being tested. In this study, testing was carried out on meat and egg samples of *T. crassus* and *T. setosus* at the Center for Food and Nutrition Studies Laboratory UGM. The results of 2 repetitions were averaged and shown as a percentage, it can be seen in Table 1.

Table 1. shows that overall, the highest composition of all samples is moisture content, where it is known that water is the main constituent of living bodies, and also because the samples analyzed are wet samples. In dry samples, the composition will be very different. The next highest level is protein, which is the building block for the structure and maintenance of body tissues. The highest water content measured in *T. crassus* meat was  $77.11 \pm 0.21\%$ , while the lowest was in *T. setosus* eggs of  $69.75 \pm 0.06\%$ .

As a comparison, previously researched proximate content of *T. setosus* meat in Ujung Genteng Waters, Sukabumi, wet sample had a water content of 74.8%, 0.8% ash, 0.02% fat, 16% protein, and 6.8% carbohydrates. The dry sample has a moisture content of  $10.15 \pm 0.69\%$ , ash  $6.87 \pm 0.12\%$ , fat  $2.20 \pm 0.01\%$ , protein  $70.34 \pm 0.13\%$ , and carbohydrates  $10.06 \pm 0.04$  (Merdekawati et al. 2017). The proximate analysis of *T. crassus* has not been studied, while the proximate of other Turbo species such as *T. militaris* has a water content of 73.1%, 2.1% ash, 5.6% fat, 16.2% protein, and 3% carbohydrates (Santhanam 2019). Ash content describes the mineral content in an organism's body, the regulation of which can be different for each organism (Merdekawati et al. 2017).

Egg fat content is much higher than meat fat content. Egg protein content is lower than meat, while egg carbohydrate content is higher. In Mollusca, most of the energy sources are stored in the leg muscles and

**Table 1.** Proximate analysis of *T. crassus* and *T. setosus*.

| Proximate content (%) | <i>T. Setosus</i> |            | <i>T. crassus</i> |            |
|-----------------------|-------------------|------------|-------------------|------------|
|                       | Eggs (n=2)        | Meat (n=2) | Eggs (n=2)        | Meat (n=2) |
| Moisture              | 69.75±0.06        | 75.89±0.55 | 75.60±0.19        | 77.11±0.21 |
| Ash                   | 1.02±0.01         | 0.825±0.05 | 1.04±0.24         | 0.925±0.13 |
| Lipid                 | 9.37±0.14         | 0.38±0.00  | 6.06±0.18         | 0.39±0.01  |
| Protein               | 15.27±0.22        | 20.04±0.13 | 14.23±0.66        | 18.59±0.17 |
| Carbohydrate          | 4.60±0.29         | 2.77±0.49  | 3.08±0.91         | 2.99±0.23  |

digestive glands (Najmudeen 2007), therefore the highest protein levels are in *T. crassus* and *T. setosus* measured in the foot. In gastropods, protein is an important component in egg yolk in the form of ferritin, which is derived from the hepatopancreas (Najmudeen 2007). The protein content of meat is influenced by growth and metabolic stages, as well as nutrient factors and environmental conditions (Najmudeen 2007). Research by Litaay & De Silva (2003) on the *Haliotis rubra* abalone stated that the main energy in reproduction is fat, as evidenced by the significant reduction in fat levels in the gonads after spawning. In a study on *Pecten maximus*, lipid reserves in the body will be used in gametogenesis and lipid levels will differ according to the reproductive cycle with higher levels in more mature gonads (Litaay & De Silva 2003). Lipids in eggs are very important because they are used in the formation of membranes and the main energy source for embryogenesis and early larval development (Litaay & De Silva 2003). The difference in fat content in the eggs of the two species in this study may be related to the phase of gametogenesis, wherein *T. setosus* the gonads are much thicker than *T. crassus* so that the fat content is also higher.

There are several instances where carbohydrates are converted into fat during gametogenesis (Najmudeen 2007). Carbohydrates in ordinary Mollusca tissue are in the form of glycogen and their levels can differ according to their utilization in the organism's body (Merdekawati 2017). The high carbohydrate content in *T. crassus* and *T. setosus* eggs may be related to the glycogen component in mature oocytes which becomes energy reserves for further development. Figures 3. and 4. showed that both species have a variety of natural food. This may result in a more balanced diet as compared to having only a single type of food. Variation in food types may lead to a better growth rate (Naidoo et al. 2006).

In marine invertebrates, the quality of female broodstock will affect the size and quality of eggs, which is related to the survival and growth of spawns (Fukazawa et al. 2005). Egg nutrient reserves are not only important in the process of embryogenesis, for example, abalone larvae and postlarva will also depend on nutrient reserves in eggs. Eggs with the highest levels of fat and protein will produce spawns that can last longer without food. Fat content is also directly proportional to protein content, both of which are important sources of energy for larval and postlarvae development. In *Haliotis discus hannai* which has multiple spawning types, it was found that protein and egg fat levels would be higher in eggs that were spawned at the last spawning period in one period (Fukazawa et al. 2005). From this study, it was found that the levels of fat and protein in the eggs of *T. crassus* and *T. setosus* were quite high, which meant that they had good quality and were expected to produce offspring with good growth and survival. In breeding or conservation efforts, the availability of suitable feed will be able to support the productivity of Turbo snails. If the nutritional needs of the broodstock are met, the quality of eggs or offspring will increase.

## CONCLUSION

From this study, we concluded that *Turbo crassus* and *Turbo setosus* have a slightly different diet. There is also a slightly different diet between different sizes. The most dominant food in *Turbo setosus* was Phaeophyta, *Microcoleus* sp, and Chlorophyta or Rhodophyta. The most dominant food in *Turbo crassus* is *Microcoleus* sp, Phaeophyta, and diatoms or Rhodophyta. The meat proximate of *T. setosus* has the highest protein content. The meat proximate of *T. crassus* showed higher water, fat, carbohydrate, and ash content than *T. setosus* meat. The fecundity of *T. crassus* and *T. setosus* will increase with increasing body size, where *T. crassus* has the highest R<sup>2</sup> value in the shell length parameter, while *T. setosus* on the shell width. The broodstock of *T. crassus* and *T. setosus* in Sepanjang Beach have good quality, because of the high levels of fat and protein in eggs and meat.

## AUTHORS CONTRIBUTION

All three authors conceived and designed the research. Trijoko was the supervisor for this research, provided critical revision, and also approved the final version to be published. Rijal R and Izzatul Auliya' both collected the samples for this research, analyzed the data and wrote the article.

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## CONFLICT OF INTEREST

There is no conflict interest regarding the research or the research funding.

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