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Plankton Community, Carbon-Nitrogen Ratios and Food Preference in Blind Feeding Phase of *Litopenaeus vannamei*

Maria Eva Kristiana[1]*, Suwarno Hadisusanto[1]* & Rustadi Rustadi[2]
[1]Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada, Sleman Regency, Special Region of Yogyakarta, Indonesia
[2]Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Sleman Regency, Special Region of Yogyakarta, Indonesia
*Corresponding Author, email: mariaeva97@mail.ugm.ac.id; suwarnohs@ugm.ac.id

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ABSTRACT *Litopenaeus vannamei* is one of the largest commodities having a major impact on the global economy. Shrimp larvae have drastic changes in morphology and physiology which require extra maintenance. Feed management in early rearing using a blind feeding system which plankton as the main food. The relation between plankton and C/N needs to be investigated to optimize the growth of natural feed. The aims of the study were to analyze plankton community structure; the relationship between plankton to C/N and the environmental parameters; and determine the shrimp preferences of plankton. Data were collected at 3 points in 3 different ponds (PA, PB, PC) on day 8 and day 15 using a stratified sampling method with criteria (intensive system and blind feeding phase). Plankton density and C/N relationship were analyzed using Linear Regression, plankton and environmental parameters using Canonical Correspondence Analysis (CCA). Shrimp food preferences were analyzed descriptively in percentage. The results showed that diatoms and dinoflagellates dominated phytoplankton, and rotifers dominate zooplankton. C/N showed a positive correlation to protozoa, but a negative correlation to pennate diatoms and copepods. The most influential environmental factors for both phytoplankton and zooplankton density were temperature, C-organic, salinity, pH, alkalinity, and dissolved oxygen.

Keywords: Blind feeding; C/N; density, intensive; *L. vannamei*; plankton; pond

INTRODUCTION

Aquaculture has developed rapidly over the last three decades and plays an important role in the global economy (Kumar et al., 2020). The stages of shrimp cultivation consist of several stages i.e., preparing, rearing, and harvesting. At the beginning of the rearing, shrimp seeds are in the post larvae phase (PL10 to PL12) (WWF, 2014). Larval is a critical phase because of the drastic changes in morphology, physiology, behavior, size, and weight that require extra maintenance (Brito et al., 2004; Wei et al., 2014). Feed management in an early stage is using blind feeding where larvae eat dissolved materials in the water column such as microbial flocs including plankton, organic detritus, protozoa, fungi, and small biota at the bottom of the water (Epa, 2017; Alfiansyah et al., 2022). Panjaitan et al. (2015) mention that shrimp in nauplius to post-larval stages eat natural food including phytoplankton and zooplankton. Plankton provides higher nutrients and is easily digested compared to commercial feed (Rihi, 2019) because of its body size. Plankton supply vitamins, amino acids and fatty acids that are needed by the shrimp such as diatoms that proved to increase the essential amino acids and unsaturated fatty acids in shrimp tissues which further increases shrimp growth (Brito et al., 2016; Supono, 2017; Llario et al., 2019).

In aquaculture, many carbon/nitrogen adjustments were investigated. C/N is the ratio between carbon and nitrogen present in a material and indicate the contribution of organic matter to water fertility (Putri, 2015; Purnomo *et al.*, 2017; Alfiansyah *et al.*, 2022). Avnimelech (1999) stated that organic carbon in aquaculture is needed to stimulate inorganic nitrogen controlling bacteria to decrease the toxic ammonia, in line with Alfiansyah *et al*. (2022). On the other hand, phytoplankton absorbs carbon e.g., carbohydrates as an energy source (Firdaus & Wijayanti, 2019). In addition, nitrogen is important for phytoplankton to construct amino acids in protein synthesis of cell division (Reynolds, 2006). Li *et al*. (2022) reported that C/N in the water column affects phytoplankton C/N cellular homeostasis.

A plankton community study in shrimp cultivation has been carried out by Arifin et al. (2018) acquire that Chlorophyta and Diatoms were dominant. Case et al. (2008) examined the same study with the dominance of Copepods, Protozoa, and Rotifers. However, the study on plankton in aquaculture and the relationship to C/N is very limited. This study is important to ensure the availability of natural food during the critical larval phase and its relationship to C/N ratio. The aims of this study were to analyze the structure of the plankton community and its relationship to C/N in the blind feeding phase, analyze the relationship between plankton density to the environmental parameters of pond water, and determine the shrimp preferences of plankton as a natural food.

MATERIALS AND METHODS

Materials

Plankton sampling used a bucket with a 5 L-capacity, 200-micron plankton net, 10 mL flacon bottle, pipette, microscope, Sedgewick rafter counting cell (SRCC), cover glass, and hand counter. Measurement of environmental factors used a thermometer, ATAGO S-28 refractometer, pH waterproof pen OHAUS SI-10, alkalinity kit, and dissolved oxygen kit. Total organic carbon (C) and total nitrogen (N) from the water were observed from surface water (30cm depth) and analyzed at the Chemix - Pratama laboratory.

Methods

Sampling stations

The study was carried out in May 2021 at the tropical pond of the Indian Ocean, precisely at Purworejo pond area, Central Java, Indonesia. Plankton and environmental parameters sampling were done at 3 shrimp ponds on the day-8 and day-15 of shrimp culture. The pond criterias that use in this study must be using intensive system, in the blind feeding phase, and shrimp stocked on the same day. During the blind feeding, the cultivation was not added any fertilizer, it only added commercial food. Sampling points for plankton and environmental parameters were determined by land and sea breezes shown in Figure 1. Environmental parameters that were observed directly were temperature, dissolved oxygen, alkalinity, salinity, and pH. Water samples were taken to the laboratory referring to APHA (1998).

The homogenized sample was placed into the SRCC 1 ml and covered by a cover glass. The number individual of plankton was counted using a microscope in 10x10 magnification and a hand counter with total stripe count and sub-sampling approach. Plankton identification referred to The Plankton of South Vietnam - Fresh Water and Marine Plankton from (Shirota, 1966) and algaeweb.net. Plankton density is calculated by the formula from Wetzel & Likens (2000):

$$N\,o\,/\,ml = \frac{(C)\,(1000mm^3)}{(L)\,(D)\,(W)\,(S)}$$

Notes: Total of the individual (C), SRCC length (L), SRCC width (W), SRCC depth (D), dan SRCC repetition (S).

Statistical analysis

The relationship between plankton and C/N was analyzed statistically using linear regression with a 90% confidence level. The relationship between plankton density and environmental parameters was analyzed used Canonical Correlation Analysis (CCA). Both statistical analyses using PAST4 software. Plankton in shrimp stomach was shown in percent (%) and analyzed descriptively.

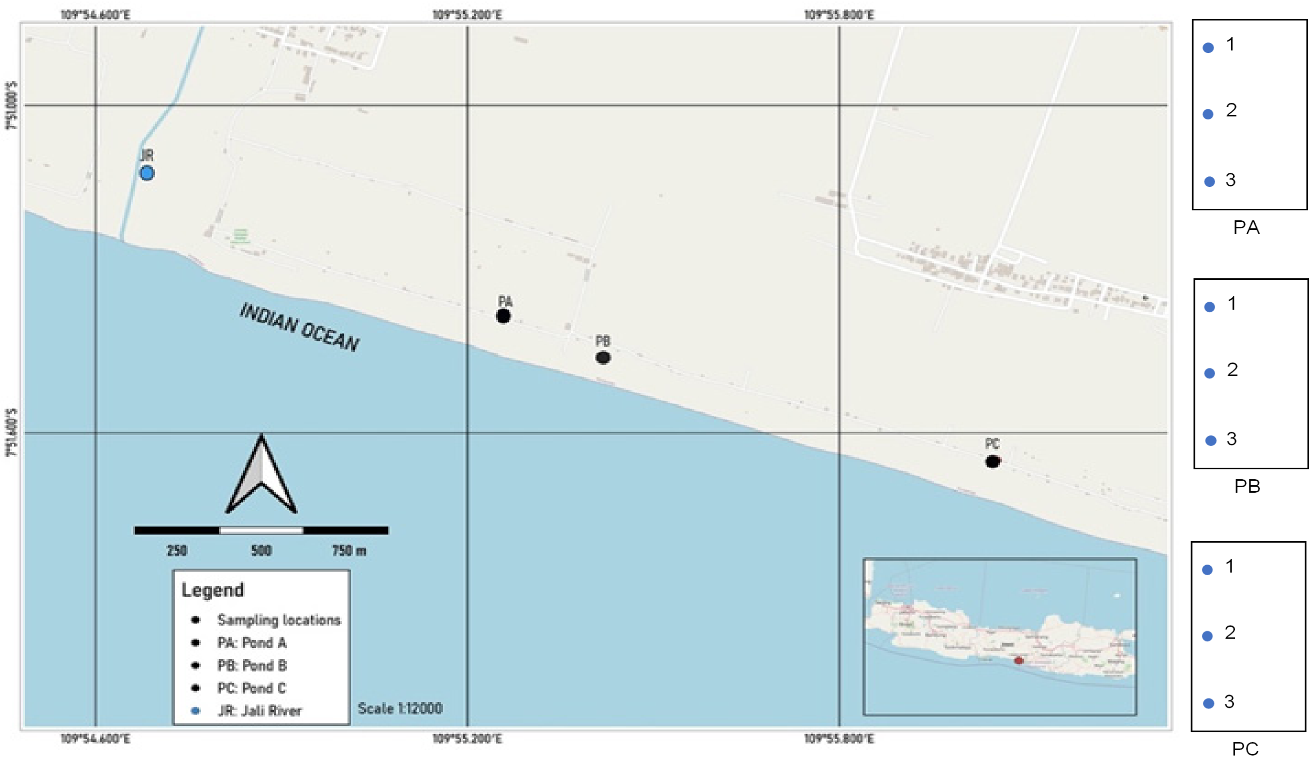

Figure 1. Location of sampling stations and sampling design. (PA : Pond APB : Pond B PC : Pond C)

Plankton sampling and gut analysis

Plankton samples were taken from each point at surface water in 10 liters using a bucket, filtered using a plankton net and placed in 10 ml falcon. Sampling were done with 3 repetitions. Three shrimp samples for each pond were collected and natural food for shrimp larvae were taken from the stomach and intestine, diluted with distilled water to 10 ml and stored in flacon. Plankton samples and gut contents were preserved with 4% formalin. The samples were stored at 4 °C for further observation in the laboratory.

RESULTS AND DISCUSSION

The phytoplankton consisted of 36 species divided into 6 functional groups, i.e., pennate diatoms, centric diatoms, unicellular algae, colony algae, filamentous algae, and dinoflagellates. Pennate diatom members were *Pleurosigma angulatum*, *Climacosphenia moniligera*, *Grammatophora angulosa*, *Synedra acus*, *Synedra cunningtoni*, *Synedra affinis* var. *fasciculata*, *Chaetoceros weissflogii* and *Bacteriastrum comosum*. Centric diatoms members were *Thalassiosira condensate*, *Melosira agussizi*, *Melosira distans* and *Hemiaulus membranaceus*. Unicellular algae were *Synechocystis aquatilis*, *Lyngbya contorta*, *Acanthosphaera acufera*, *Staurastrum megacanthum*, *Staurastrum rhychoceps*, *Staurastrum amithii*, *Staurastrum woltereckii*, and Closterium ehrenbergii. The colony algae were *Basicladia chelonum*, *Zygnemopsis americana*, *Merismopedia elegans*, *Treubaria crassispina*, *Trichodesmium* sp., *Trichodesmium lacustre*, *Anabaena circinalis*, *Globigerinella aequilateralis*, *Nostoc linckia*, *Selenastrum boryanum*, *Plectonema tomasinianum*, *Protococcus viridis*, and *Pediastrum boryanum*. The filamentous algae were *Spirulina major*, *Oedocladium protonema*, and *Oedogonium crispum*. The dinoflagellate member is *Gonyaulax apiculata*.

The zooplankton consisted of 17 species and divided into 5 functional groups, i.e., copepod, ostracod, rotifers, protozoa, and other plankton life stages. Copepod members were *Temora* sp. and nauplius. Ostracod member were *Heterocypris incongruens* and *Cypridina mediterranea*. Rotifer members were *Colurella adriatica*, *Brachionus plicatilis*, *Branchionus pala*, *Branchionus bakeri*, and *Kellicottia longispina*. Protozoa members were *Vorticella* sp., *Astramoeba radiosa*, *Euglena deses*, and *Euglena polymorpha*. Other plankton life stages were *Scyphistoma polyp*, rotifera egg, *Moina* sp. egg, and *Temora* sp. egg.

The density of plankton changed from day-8 to day-15. Dinoflagellates increased dramatically from 2,02 to 325,86 ind. L^{-1}. Diatom pennate increased from 43,59 to 222,13 ind. L^{-1}. Unisel algae increased dramatically from 1,43 to 200,11 ind. L^{-1}. Colony algae decreased from 4,09 to 2,45 ind. L^{-1}. Meanwhile, the density of centric diatoms and filamentous algae tends to be constant. The density of rotifers dropped drastically from 1799,52 to 446,14 ind. L^{-1}. Other life stages of plankton decreased from 246,04 to 107,28 ind. L^{-1}. Ostracod fell from 6,92 to 3,99 ind. L^{-1}. Protozoa increased dramatically from 1,04 to 74,76 ind. L^{-1}. Copepods increased slightly from 47,16 to 50,81 ind. L^{-1} (Figures 4 and 5).

Few plankton functional groups are associated with C/N. The average C/N ratio in pond A is 85,95, pond B is 123,81, and pond C is 24,87. Based on linear regression analysis, C/N had a significant negative correlation to copepods with p value <0,05. C/N has a negative relationship with diatom pennate with p <0,1. C/N had a positive relationship with protozoa with p <0,1. CCA analysis showed that the environmental factors that most influence phytoplankton were temperature and total organic carbon. While the factors that affect zooplankton were salinity and temperature. On the other hand, C/N and total N were less influential for both phytoplankton and zooplankton.

Shrimp gut analysis yielded 13 species of phytoplankton and 11 species of zooplankton. Plankton that found were transparent or in a piece form of plankton. Figure 7 shows the plankton preferences that selected by shrimp in the blind feeding phase. They were pennate diatoms as the most preferred, unicellular algae as the second choice, and other life stages as the third choice.

Plankton's number of species

The number of species of phytoplankton functional groups in each sampling point is shown in Figure 2. Algae colonies had the greatest number of species and were found in each sampling both day-8 and day-15. On the other hand, unicellular and filamentous algae were found with the least number of species. It is because of the competition between them and the colony algae. Colony algae are able to float and sink faster than unicellular and filamentous algae to find the optimal depth of nutrition and light intensity with a pattern of 'day-float' and 'night-sink' (Goldman & Horne, 1983; Xue *et al.*, 2022). The second great number of species was the pennate diatom, while the centric diatom was in the less number. Centric diatoms such as Melosira sp. had a heavy bodies because of their silica wall. The thick silica (SiO_2) wall makes the centric diatom difficult to float without water mixing (Goldman & Horne, 1983; Paidi *et al.*, 2022). It has become a disadvantage in sunlight competition for photosynthesis.

Based on Figure 3, all zooplankton functional groups occurred at each sampling location. According to Lu *et al*. (2021), zooplankton distribution is influenced by the nutrient concentration of the water body. High nutrient concentrations trigger the growth of phytoplankton and indirectly affect zooplankton distribution. Based on the

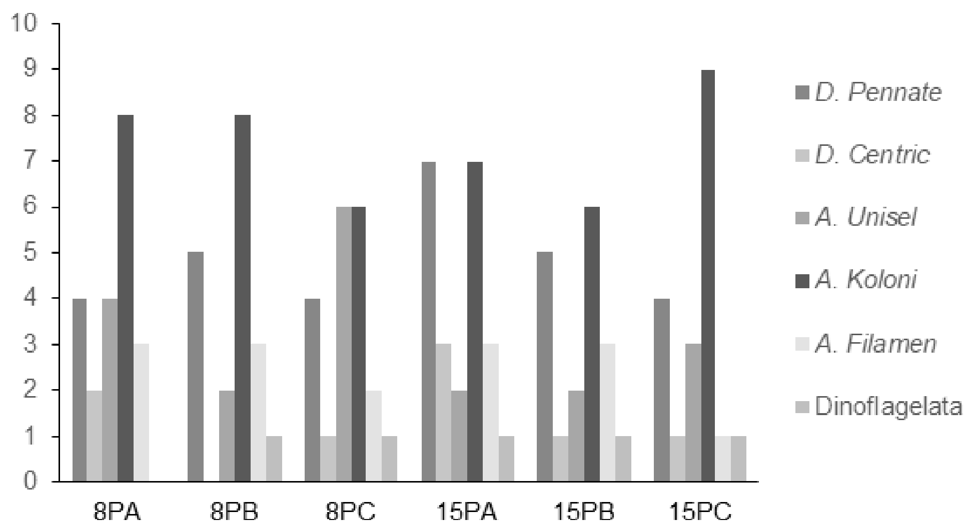

Figure 2. Number of species of phytoplankton functional groups.

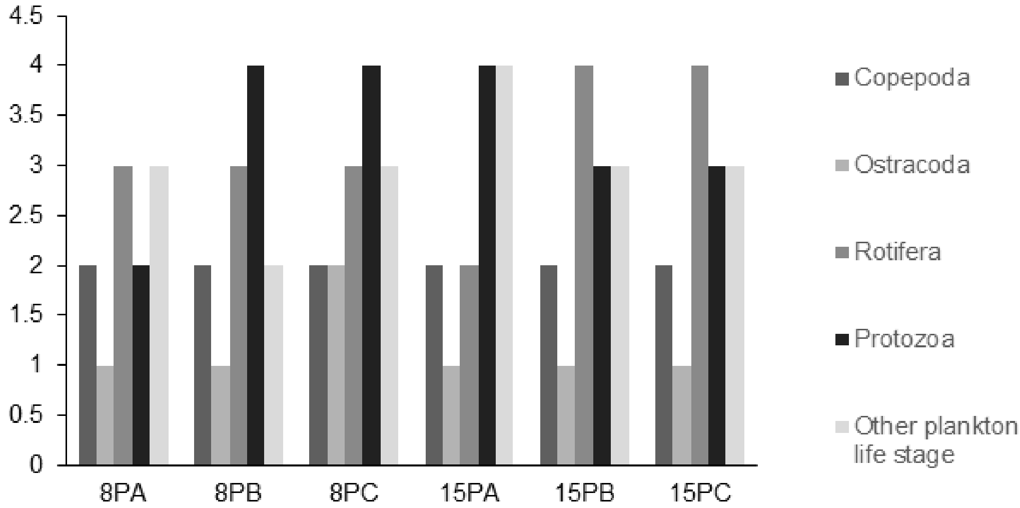

Figure 3. Number of species of zooplankton functional groups.

top-down interaction, phytoplankton is the food source that determines the abundance of zooplankton.

Overall, the abundance of rotifers, protozoa and other life stages of plankton tends to be even in this study. According to Roy & Chattopadhyay (2007), at non-equilibrium conditions two or more species can co-exist under the suitable conditions. Interspecies growth and dispersal rate induce spatial separation of the competing species. Then, spatial separation leads to species co-existence. In addition, competition encourages species to adapt through physiological and behavioral to be co-exist. Different species from different functional groups were also found in this study. According to Salcher (2014), different species can occupy the same location because of the niche partitioning. The ecological separation of niches from co-existing taxa is caused by limited resources (bottom-up control) and mortality (top-down control).

Many eggs of rotifers and copepods that grouped into other plankton stages were found in this study. Zooplankton eggs were found at all sampling points both on the day-8 and day-15. Zooplankton has a unique strategy to survive under adverse environmental conditions by producing resting/dormant stages. Zooplankton can produce a resting stage (diapause) by physiological and biochemical changes in order to survive. Examples are ephippium in Daphnia, resting egg in copepods, and resting state in Cladocera. Eggs also undergoes as a population dispersal mechanism for new habitats colonization (Goldman & Horne, 1983; Battauz *et al.*, 2017).

Ostracod has the fewest number of species. Ostracods are deposit-feeders and only a few species are in plankton form. Plankton ostracods are formed when their shells do not develop exoskeleton formation (Brandão *et al.*, 2019). Ostracod's diversity is influenced by environmental factors, especially the sediment type. Their diversity is low in coarse sediments and high in fine sediments (Aiello *et al.*, 2021).

Two copepod species, *Temora* sp. and nauplius found in all sampling points. Copepods are perennial crustaceans that always appear in various waters (Goldman & Horne, 1983). Copepods have several adaptation strategies, i.e., diapausing, resting eggs, high reproductive rate, ability to migrate vertically, and efficient use of energy (Kreibich *et al.*, 2008; Bandara *et al.*, 2021). These abilities make copepods consistent to present in pond during the blind feeding phase.

The dynamic of plankton density

Density is the number of individuals per unit volume or area (Krebs, 2014). The density of phytoplankton on day-8 and day-15 are shown in Figure 4. Dinoflagellates density on day-8 is 2,02 ind. L^{-1}. It was because of the early stages of shrimp cultivation, so there only a few dinoflagellates had been colonized. On the other hand, this group is not required to grow highly in aquaculture. On the day-15, dinoflagellates density increased drastically to 235,85 ind. L^{-1}. According to Sweeny & Hasting (1958) Gonyalux divide 85% within 24 hours especially at the end of the dark period and the beginning of the light. The trigger factor for rapid cell division is the high-level nutrient such as nitrate that accumulated from organism excretion and dead living things. Padmakumar *et al*. (2018) reported that nutrient enrichment was one of the factors causing Gonyalux bloom in the water column. The following are the advantages of dinoflagellates according to Goldman & Horne (1983). Dinoflagellates have flagellum to move towards the water column actively heading to the optimal light intensity and nutrients. They swim upward to the surface in the morning to photosynthesize and downward in the late afternoon. Their relatively large body makes them easy to swim quickly (several ms^{-1}) with phototaxis. About 60 genera of dinoflagellate members are dangerous because of their toxic substances, such as gonyautoxin in Gonyaluax (Nasution et al., 2021). Kumar *et al*. (2021) added that dinoflagellates are abundant in high anthropogenic activity areas.

Pennate diatoms had a high density of 43,59 ind. L^{-1} on day-8 and increased five times to 222,13 ind. L^{-1} on day-15 because *Chaetoceros weissflogii* occur in large numbers. Chaetoceros is one of the most diverse genera of the diatom group and is saline water cosmopolite species that is distributed in a largeareas in tropical and temperate areas (Niu *et al.*, 2022). According to Rahayu *et al*. (2022), *Synedra acus*, a member of pennate diatom has a higher tolerance for the environment than other functional groups. Bacillariophyceae class diatoms such as *Pleurosigma angulatum*, *Grammatophora angulosa*, *Synedra acus*, *Synedra cunningtoni* and *Synedra affinis* var. fasciculata are cosmopolitan and they can live in both fresh and saline water. Bacillariophyceae can tolerate high salinity conditions and easily adapt to the environment.Diatoms are able to compete with other phytoplankton groups because of their flat bodies to optimize the absorption of light and nutrients (Mawarni *et al.*, 2020).

Unicell algae had a low density of 1,43 ind. L^{-1} on day-8 and 200,11 ind. L^{-1} on day-15. The drastic increase was caused by an increase in *Closterium ehrenbergii* which spread in the water column. Closterium is Zygnematophyceae, they can grow optimally in slow currents on water bodies, with optimal temperature, sufficient brightness, and low turbidity (Hajong & Ramanujam, 2021). The availability of macro and micronutrients creates optimal conditions for phytoplankton to grow rapidly (Padmakumar *et al.*, 2018).

Colony algae had a density of 4,09 ind. L^{-1} on day-8 and decreased to 2,45 ind. L^{-1} on day-15. The decrease was caused by *vannamei* shrimp and zooplankton consumption. Colony algae such as Protococus and Treubaria are included in the Chlorophyceae. Chlorophyceae are expected to grow in vannamei shrimp culture as a natural food for shrimp and had a beneficial for shrimp (Katmoko *et al.*, 2021). This is supported by Gutiérrez *et al*. (2016) who reported that pennaeid shrimp in shallow water consume phytoplankton as a source of carbon and nitrogen for shrimp tissue formation. The most common colony algae were Merismopedia. Rahayu *et al*. (2022) stated that Merismopedia is a generalist organism that lives in both fresh and saline waters. Members of Cyanophyceae class e.g., *Merismopedia elegans*, *Anabaena circinalis*, *Nostoc linckia*, *Trichodesmium lacustre*, *Plectonema tomasinianum*, and *Pediastrum boryanum* are able to live in low nitrogen concentrations because of their ability to fix nitrogen. It makes them grow faster than other species.

Centric diatoms (0,10 – 0,26 ind. L^{-1}) and filamentous algae (0,62 – 0,79 ind. L^{-1}) had the lowest densities. Centric diatom eg., Melosira has a low growth rate (Goldman & Horne, 1983) lead this group tends to be constant with the low density both on the day-8 and day-15. In addition, centric diatom competed with pennate diatom but pennate had more advantages due to the body morphology as previously discussed. The low density of filamentous algae was because of the competition with unicellular and colony algae. Berger *et al*. (2003) added that filamentous algae is the main factor for eutrophication that indicated by increased turbidity, low light intensity in the water column and high sedimentation. In this study, filamentous algae were low because there was no blooming that indicated by low turbidity because of the Secchi disk visibility at the bottom of the pond.

Based on Figure 4, the highest density of zooplankton was owned by rotifers with 1799,52 ind. L^{-1}on day-8 and decreased drastically to 446,14 ind. L^{-1}. It is caused by the alkalinity change from 15.7 mg L^{-1} on the day-8 to 9.7 mg L^{-1} on day-15. According to Onadia *et al*. (2021), alkalinity is positively corellated to rotifers abundance. In line with Kumari (2022), Brachionus prefer a high alkalinity area and they build a higher population during high alkalinity period. The dominant species rotifers are *Brachionus plicatilis*, and *B. pala* is the second higher abundant. Patra (2022) stated when two or more species of a genus occur in a water body, only one species that more abundant. Brachionus is commonly found in tropical waters because Brachionus can tolerate various ecological conditions, both physical and chemical factors (Neelgund & Kadadevaru, 2021). Brachionus domination in rotifers community reflecting a wide tolerance limit towards environmental changes (Shah *et al.*, 2015). Rotifer's domination is supported by their ability to digest small organisms such as bacteria and organic detritus which are abundant in aquaculture (Neelgund & Kadadevaru, 2021).

The density of other plankton life stages decreased ½ times on the day 15 as well as rotifers as one of the egg producers of zooplankton. Rotifers have a short life cycle that makes them need to adapt to produce many generations in a year (multivoltine). Multivoltine zooplankton allocates their energy to producing eggs rather than growth. Rotifers reproduce rapidly when food is available (Goldman & Horne, 1983). Copepod eggs were also found in this study. *Temora* has many eggs that are

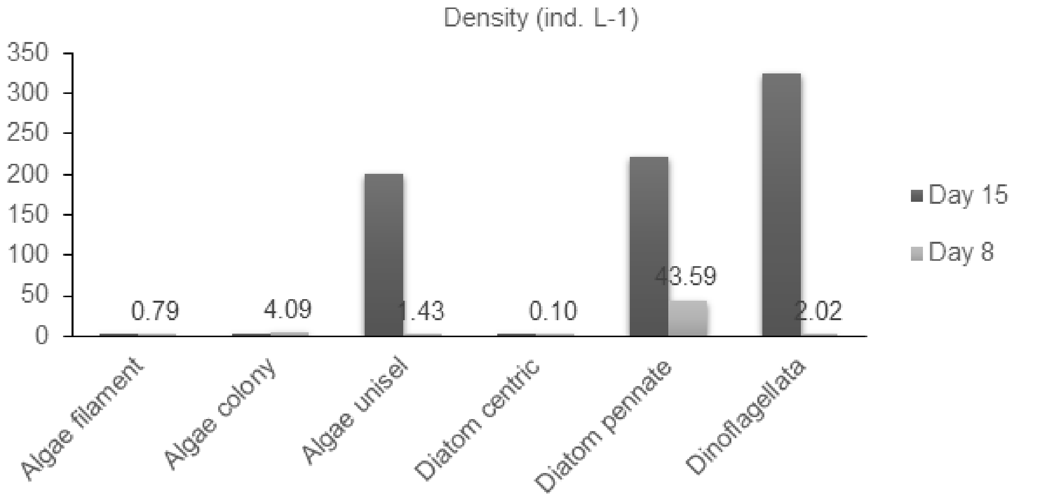

Figure 4. Zooplankton functional group density.

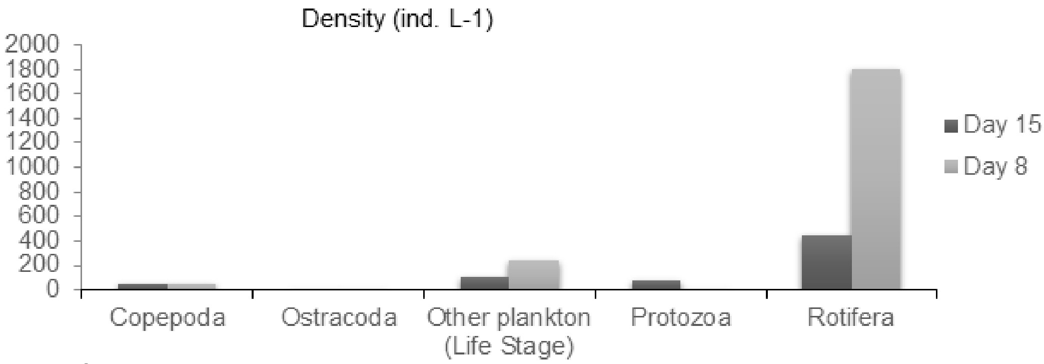

Figure 5. Phytoplankton functional group density.

shaped like a grape. Because the density of *Temora* tends to be constant, we assume that the decrease of other plankton life stage density were caused by the decrease of rotifers.

Protozoa increase rapidly up to 70 times from 1,04 ind. L^{-1} on day-8 to 74,75 ind. L^{-1} on day-15. They are filter feeders that feed on detritus, bacteria, fungi, yeast, algae, and other protozoa (Goldman & Horne, 1983). The dominant species of protozoa is Vorticella sp. Vorticella are solitary bell-shaped members of ciliate. Vorticella is an ectoparasite that lives on the swimming and walking legs, tail, and gills of shrimp. Ectoparasites population rise in poor water quality such as low dissolved oxygen (DO) (<3 ppm) and organic matter accumulation (Amalisa & Kismiyanti, 2021). Dissolved oxygen is normal in this study, so the organic matter in the cultivation such as carbon, nitrogen and phosphate may be triggers the protozoa growth. Amalisa & Kismiyati (2021) reported that there is a significant relationship between ectoparasites abundance and vibrio. The higher ectoparasites lead to the increased vibrio that triggers shrimp disease.

Copepod densities were constant during the study of 47,16 - 50,81 ind. L^{-1}. Copepods are crustaceans with exoskeletons from chitin. Copepod growth is slow due to several times molting before adults and being ready to reproduce (Goldman & Horne, 1983; Twombly & Burns, 1996). It makes copepod density stable and does not dominate like rotifers which have a fast generation time. Copepods swim upward at night to warmer surfaces and can migrate at about 0,85 cm s^{-1} speed (Goldman & Horne, 1983).

Carbon/Nitrogen and plankton relationship

Based on Figure 5, there were significant relationship between C/N and pennate diatom, protozoa and copepod. Ren *et al*. (2019) states that there are sensitivity

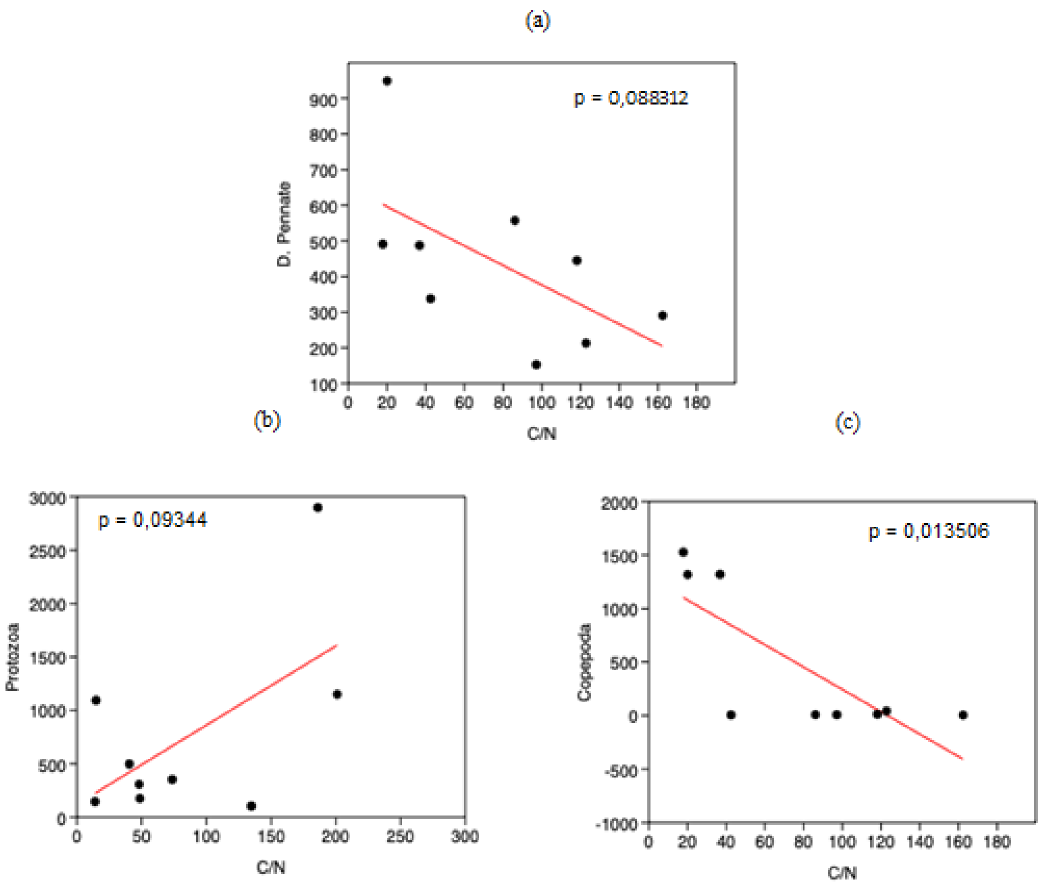

Figure 6. Regression curve of plankton and C/N relationship with α=0,1.

difference to pond C/N among each group member. The higher C/N waters, the lower density of diatom pennate (Figure 5a). It is related to the biofloc of the pond. The higher C/N, is in line with the suspended bacterial biofloc. The high suspension covers the surface water and reduces the light intensity in the water column, it makes the diatom not capable to photosynthesize optimally which leads to the lower density of diatoms. It is supported by Martins *et al*. (2016) that the presence of biofloc reduces light penetration and nutrient absorption by diatoms.

The higher C/N is in line with the protozoa's density (Figure 5b). The dominant protozoa is *Vorticella* sp. Singh *et al*. (2016) reported that protozoa are commonly appear in anthropogenic waters and play an important role in the wastewater process. As a ciliated protozoan, *Vorticella* sp. controll the growth of bacteria and organic particles in water. The higher C/N causes high levels of heterotrophic bacteria in the waters. Ballester *et al*. (2017) mention that bacteria will stimulate protozoa such as *Vorticella* sp. because they feed on the bacteria.

The higher C/N waters, the lower density of copepod (Figure 5c). The C/N was too high in this study and it exceeds the optimal limit for copepods that reduces their growth rate. Optimal C/N for copepods is 7-8 (van Nieuwerburgh *et al*., 2004). Jepsen *et al*. (2021) added that the adult copepod *Apocyplops royi* requires 4.7 of C/N to maximize egg production. *Apocyplops royi* and Temora are copepods in the class Hexanauplia. Belfiore appear in anthropogenic waters and play an important role in the wastewater process. As a ciliated protozoan, *Vorticella* sp. controll the growth of bacteria and organic particles in water. The higher C/N causes high levels of heterotrophic bacteria in the waters. Ballester *et al*. (2017) mention that bacteria will stimulate protozoa such as *Vorticella* sp. because they feed on the bacteria.

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The adjustmen on C/N affect the growth rate of different bacterial communities. Nitrifying bacteria such as Nitrosomonas and Nitrosococus grow at low C/N (Ren *et al*., 2019). C/N is associated with heterotrophic bacteria that will reduce toxic inorganic nitrogen such as ammonia. The application of probiotic bacteria in shrimp ponds with the right concentration will reduce the organic matter and maintain the nutrients from organic decomposition (Ren *et al*., 2019).

Environmental parameters and plankton relationship

Tabel 1. The average of water quality parameters.

	Temperature (°C)	DO (mgl-1)	pH	Alkalinity (mgl-1)	Salinity (‰)
Day-8	31,79	8,10	7,87	15,70	22,00
Day-15	30,76	9,77	8,13	9,70	22,00

Temperature, dissolved oxygen (DO), salinity and pH were in the normal range for rearing *L. vannamei* (Table 1). The temperature is relatively stable because of the shallow

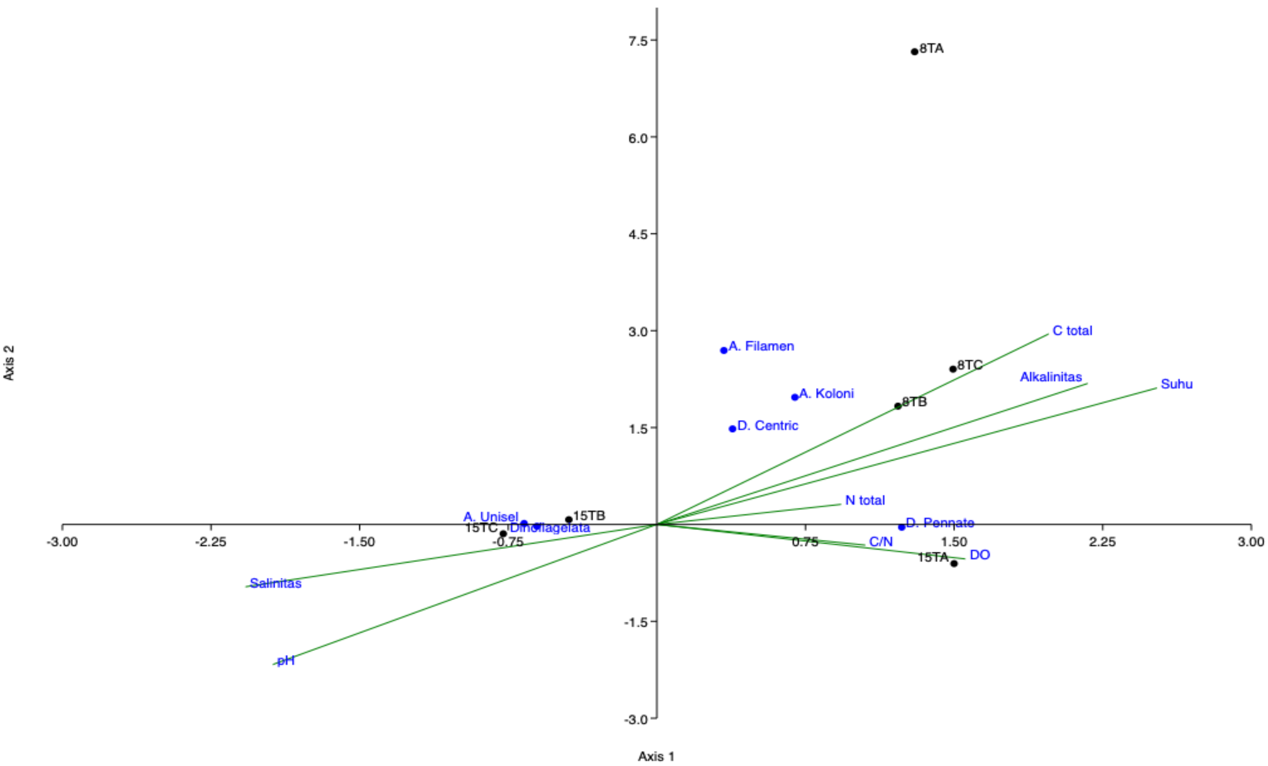

Figure 7. Canonical correlation analysis of plankton and C/N ratio relationship with α=0,1.

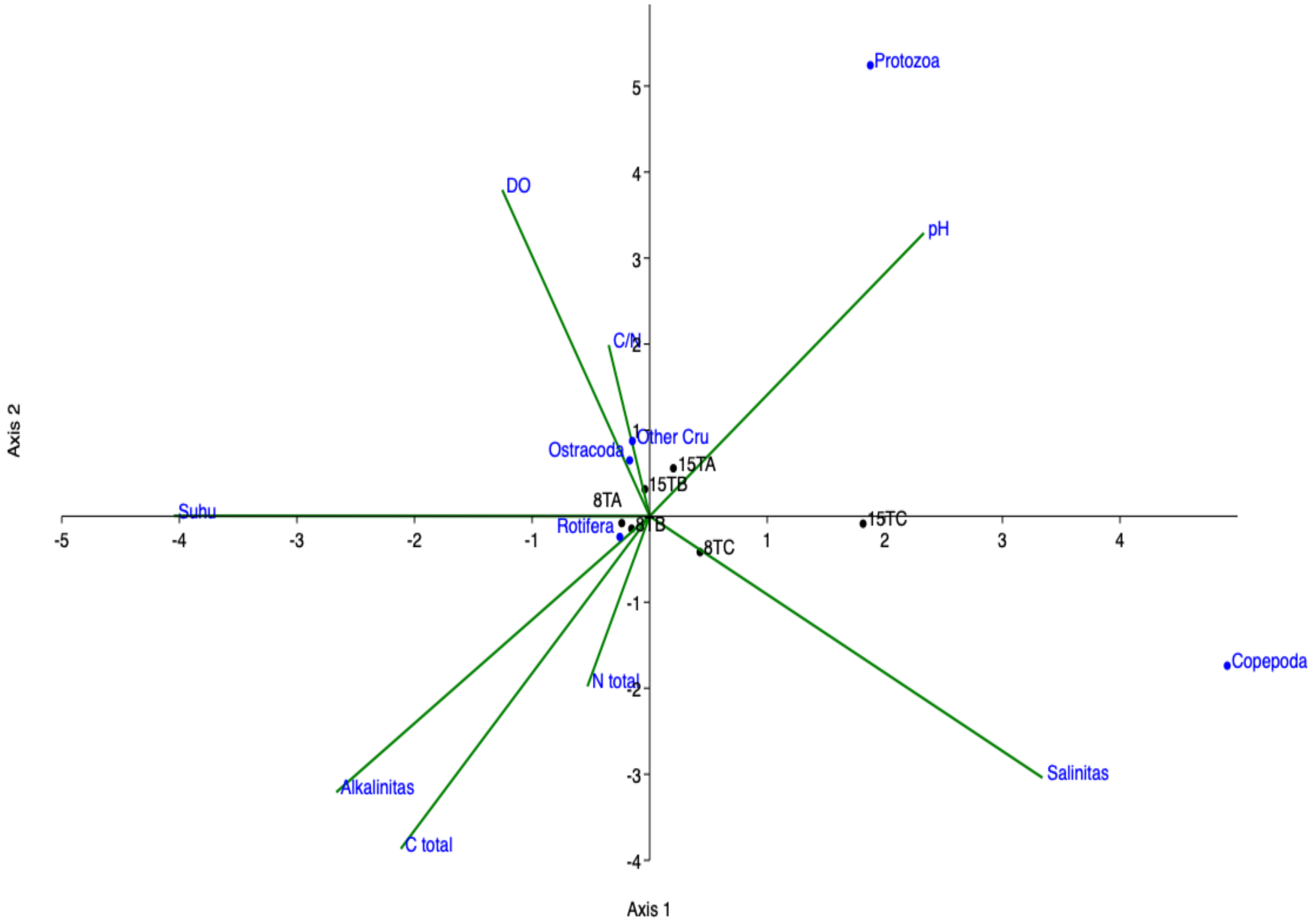

Figure 8. Canonical correlation analysis of plankton and C/N ratio relationship with α=0,1.

depth of the pond. The water is completely mixed to the bottom (well-mixing). In addition, the windmills help to mix the water which will prevent thermal stratification. The high temperatures accelerate diatom's growth rate because their metabolism runs faster (Montagnes & Franklin, 2001). DO tends to be stable. It indicates that oxygen that used by shrimp, plankton, bacteria, and other organisms in the pond compensated by photosynthesis and the paddle wheel aerator as the oxygen supply.

The pH was normal with slightly alkaline because of the photosynthesis. According to Wetzel (2001), the rapid run of phytoplankton photosynthesis will reduce dissolved inorganic carbon (DIC), increase pH, and shift the balance that ends up with low dissolved CO_2. Alkalinity is low compared to standard shrimp rearing (>75 mg l^{-1}) (McNevin *et al.*, 2004). Water's alkalinity is influenced by bicarbonate and carbonate. Dissolved organic anions that are derived from organic carbon can increase alkalinity (Wetzel & Likens, 2000), in addition, farmers usually use dolomite to increase it.

Salinity tends to be stable because of the closed pond during blind feeding. The process that affects salinity changes in this study are evaporation-precipitation. Sea water evaporates into the atmosphere. It forms salt particles and lead cloud condensation, then rain with high salinity drops in ponds area that are located near the sea (Wetzel & Likens, 2000). The study located at tropical region where the sun appears throughout the year makes high evaporation which supports the rain with high salinity. In addition, coastal sand containing salt particles blown by the wind and entering the pond can also increase salinity.

Canonical correlation analysis (CCA) was used to find out the environmental parameters and plankton density relationship. CCA is a multivariate analysis to explain the relationship between two sets of variables, particularly biological species and their environment. This method is used in various aquatic studies including plankton to determine variables that play an important role in determining the composition of the community (ter Braak & Verdonschot, 1995; El-Zeiny & Abd El-Hamid, 2022). Based on the CCA (Figure 7 & 8), the most influence environmental factors for phytoplankton density were water temperature, total organic-C, salinity, pH, alkalinity, and DO. Environmental factors that do not affect the density of phytoplankton were C/N and total N. C/N had less effect on phytoplankton in line with the linear regression in Figure 6 which only has a significant effect on the pennate diatoms, not on other phytoplankton groups. On the day-8, all the ponds had similarities distinguishing functional groups i.e., centric diatom, colony algae and filamentous algae. On the day-15, pond A had pennate diatoms, pond B had unicellular algae, and pond C dinoflagellates distinguishing functional groups.

Figure 6[b] shows that the most influential environmental factors for zooplankton were salinity, temperature, alkalinity, total organic-C, pH, and DO. Similar to phytoplankton, the functional group of zooplankton was not significantly affected by total N and C/N. On the day-8, ponds A and B had rotifers as a distinguishing functional groups, and pond C had copepods. On the day-15, pond A protozoa, pond B ostracoda and other plankton life stages, and pond C had protozoa.

Plankton preferences by shrimp as natural food

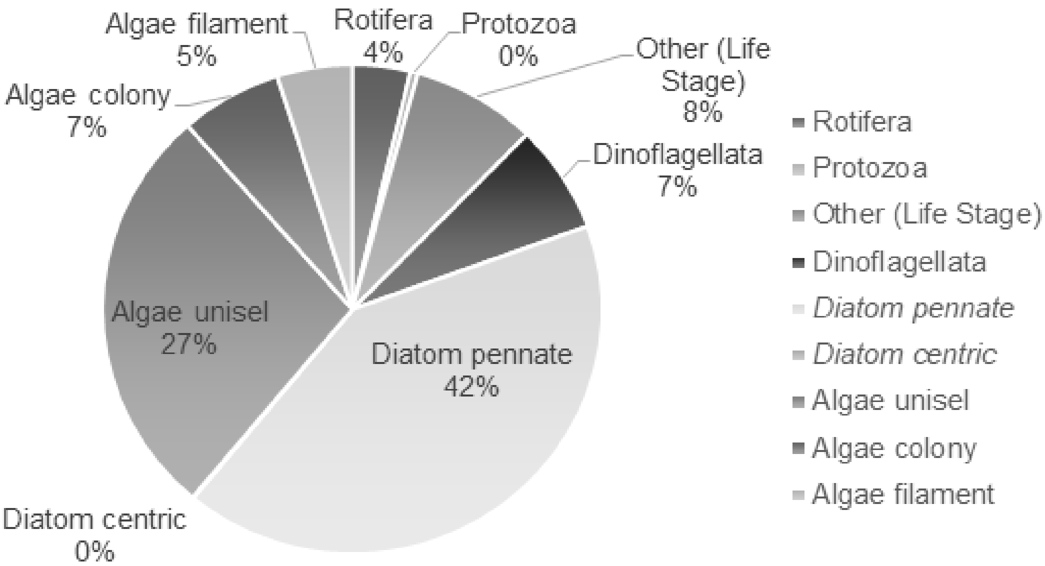

Figure 9. Plankton in the shrimp gut.

Diatom was the most disposed food because they contain nutrients that needed by vannamei shrimp. The most species found in shrimp stomachs was *Synedra acus*. Kent *et al*. (2011) reported that shrimp that feed on detritus and diatoms had a digestive efficiency up to 87% because they contained adhesive polysaccharides produced by algae and *L. vannamei* are able to digest diatoms with their teeth and stomach which break down the cell walls of silica, and enzyme to digest the contents of the cells. Unisel algae were the second choice. Unicellular algae such as Closterium with a body width of 44-170 μm can enter the mouth of juvenile shrimp which shrimp seta filter particles >10 μm (Kent *et al*., 2011). Nonwachai *et al*. (2010) stated that unicellular algae increased vibrio resistance for shrimp because it contains docosahexaenoic acid (DHA) and arachidonic acid (ARA). The increase of survival rate promotes cultivation success. Closterium is Chlorophyceae and diatom is Bacillariophyceae, they were expected to grow to support the aquaculture sustainability.

The other crustacean life stages commonly found were *Temora* sp. and *B. plicatilis* eggs. Eggs were still in shape because of their thick walls. The wall makes eggs pass through the digestive system without significant changes (Goldman & Horne, 1983). Because of it condition, we assume that eggs had less beneficial for shrimp because shrimp's stomachs and intestines cannot break the wall to absorb egg content.

Dinoflagellates were mostly found in shrimp stomachs. It is because of the inclusion of dinoflagellates when the shrimp filtered food considering the high density of dinoflagellates in the water column (Figure 4). The planktons that are expected to grow in culture are Chlorophyta and diatoms (50-90%), blue-green algae (10%), and dinoflagellates (<5%) (Widigdo, 2013; Supono, 2017).

CONCLUSION AND RECOMMENDATION

Conclusion

The phytoplankton species that dominated in the blind feeding phase were *Synedra acus* (diatom) on the day-8 and *Gonyalux apiculata* (dinoflagellate) on day-15. The dominant zooplankton species on the day-8 and day-15 was *Brachionus plicatilis* (rotifer). C/N affects protozoa group with a positive relationship, it means that the higher the C/N in line with protozoa density. C/N affects pennate diatoms and copepods with a negative relationship, it means that the higher the C/N, the lower the density of pennate diatoms and copepods. The most influential environmental factors for both phytoplankton and zooplankton density were temperature, C-organic, salinity, pH, alkalinity, and dissolved oxygen. Plankton as a natural food that selected by L. vannamei in the blind feeding phase were pennate diatoms, unicellular algae, and other life stages the third tendency.

Recommendation

Research with a longer period (temporal) needs to conduct to know the dynamics of plankton during shrimp rearing during blind feeding and rearing until the adult shrimp. Zooplankton growth needs to be controlled to minimize the competition to the shrimp eg; food competition. Dinoflagellate's growth needs to be controlled because of the toxic substances in the shrimp. *Vorticella* sp. growth needs to be controlled it is an ectoparasites that can affect the shrimp culture.

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AUTHORS' CONTRIBUTIONS

MEK initiate research topic, conduct research, and write manuscript; R initiate research topic, suggest research locations, suggest in manuscript writing and SH direct the research, suggest in making manuscript writing.

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