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Community structure of periphyton on bamboo substrate in whiteleg shrimp (*Litopenaeus vannamei* Boone, 1931) pond with semi-biofloc system

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Abstract. *Arfiati D, Zakiyah U, Anitasari S, Inayah ZN, Pratiwi RK. 2023. Community structure of periphyton on bamboo substrate in whiteleg shrimp* (Litopenaeus vannamei *Boone, 1931*) *pond with semi-biofloc system. Biodiversitas 24: 5080-5087.* Periphyton is one aquatic organism that attaches to the substrate and can be used as a bioindicator. This study analyzes the community structure and dynamics of periphyton on bamboo substrates in whiteleg shrimp ponds with a semi-biofloc system. The study was conducted from May to July 2022. The bamboo substrate was placed in the ponds, and the samples were taken every seven days to 8 weeks. Identification and calculation of periphyton using Lackey Drop Micro transect Counting Method. The periphyton was found on a bamboo substrate in whiteleg shrimp ponds with a semi-biofloc system consisting of phyto-periphyton and zoo-periphyton. The phyto-periphyton group contains 4 phylum, 21 species, including the Bacillariophyta, Chlorophyta, Cyanophyta, and Ocrophyta. The zoo-periphyton group has 4 phylum, 10 species consisting of Arthropoda, Ciliophora, Nematoda, and Rotifera. The abundance of periphyton found changes every week. The relative abundance of phyto-periphyton includes phylum Bacillariophyta (17-69%), Chlorophyta (1-22%), Cyanophyta (30-65%), and Ocrophyta (0-25%), while zoo-periphyton includes phylum Arthropoda (0-3%), Ciliophora (69-99%), Nematodes (0-2%) and Rotifers (0-28%). The total abundance of phyto-periphyton on bamboo substrate ranged from 223,806-989,398 cells/L, while zoo-periphyton ranged from 396-1,924 individual/L. The diversity, uniformity, and dominance index of phyto-periphyton and zoo-periphyton are moderate. Water quality parameters during the study were relatively good for aquatic organism growth, except for very high total organic matter levels in several weeks.

Keywords: Bamboo substrate, *Litopenaeus vannamei*, periphyton, pond, semi-biofloc, water quality

INTRODUCTION

Ponds are one of the artificial ecosystems for cultivation, especially whiteleg shrimp, which are currently widely cultivated (Yang et al. 2020). One organism that plays a role in the shrimp aquaculture ecosystem is the periphyton. Periphyton is a group of aquatic organisms that live attached to the surface of plants, wood, bamboo, stone, or other substrates in the water. Periphyton can be either animals or plants (Kumar et al. 2017). Animal-type periphyton generally consists of macroinvertebrates such as protozoa, rotifers, and cladocerans, while plant-type periphyton consists of microalgae (Abwao et al. 2014). In whiteleg shrimp cultivation, periphyton has many benefits, such as a natural food item. Natural food needs in shrimp culture, especially in the postlarva phase, there is a change in the predatory behavior of shrimp, which initially preyed on planktonic biota to become predators of benthic or periphytic biota. Appropriate feed nutrition is needed to support shrimp farming production (Effendi et al. 2016).

Another role of periphyton in shrimp ponds is as primary productivity that supports the life of aquatic organisms. The periphyton presence can contribute to increased dissolved oxygen for respiration. Periphyton can fix carbon, increase oxygen levels, and provide additional food for organisms in the water (Biswas et al. 2022). Periphyton can also be used as a very effective, efficient, and economical aquatic bioindicator, including ponds. The advantages of periphyton as a bioindicator include its wide distribution, short life cycle, and fast reproduction. Most of the periphyton species are sensitive or tolerant to pollution, both in the form of organic and inorganic pollution, so they can describe the health condition of the waters through their community structure (Chen et al. 2022). Using biological agents such as indicators of environmental health, such as periphyton, has various advantages, including describing the condition of the quality of environmental health as a whole and providing an accurate measurement of the effects of ecological change (Arsad et al. 2021).

Periphyton survival is related to the presence of the substrate in terms of the condition, type, and position. The substrate is important as a place to live for the periphyton, so that needs research using a natural substrate such as bamboo. Bamboo is easy to obtain and widely used in the cultivation environment, expected to reduce the need for cultivation activities. In addition, bamboo is utilized as a substrate for periphyton growth because of its ability to produce high-quality periphyton, availability in the tropics, ease of use, and durability (Amirtharaja et al. 2022).

Information on bamboo substrates as a place for periphyton communities to grow in whiteleg shrimp ponds using the semi-biofloc system needs studies. Currently, the cultivation system widely used in whiteleg shrimp farming is the semi-biofloc system. This research adds a small amount of *Bacillus* bacteria to form floc. The semi-biofloc system is a cultivation system with the addition of a small number of bacteria to form a floc. Biofloc can help maintain water quality (Schveitzer et al. 2013). Semi-floc technology utilizes the activity of microorganisms that form flocs as a food source for the shrimp that are reared. Semi-floc technology was initially applied to industrial wastewater treatment systems and is currently applied to aquaculture water treatment systems (Nisar et al. 2022). This technology is a combination of heterotrophic and autotrophic organisms. These heterotrophic organisms will control water quality mainly due to leftover feed, feces, plankton carcasses, and the remains of other organisms' activities, including the possibility of exfoliating the slime of organisms in the culture media water, thus forming environmental stability. At the same time, autotrophic organisms can be used as natural food for shrimp (Effendy et al. 2016). The basis for this research is the need for more information regarding the composition of periphyton on bamboo substrates. This study analyzed the community structure and dynamics of periphyton on bamboo substrates in whiteleg shrimp ponds with a semi-biofloc system.

MATERIALS AND METHODS

Research time and location

This research was conducted on three whiteleg shrimp ponds with a semi-biofloc system from May to July 2022. The pond belongs to Universitas Brawijaya and the Department of Marine and Fisheries of Probolinggo City, East Java, Indonesia. Table 1 lists the coordinates of the research ponds. The three ponds were used as a replicate in this research. The research location can be seen in Figure 1.

Research procedure

Placing of substrate

This research is experimental research conducted in the field. Bamboo substrate measuring 3 x 5 cm hanging using a rope and equipped with weights on the pipe frame. One pipe frame consists of 8 substrates. The frame is placed in the pond so the bamboo substrate is on the water column (25-50 cm below the water surface). Each pond consists of 3 frames as replicates (Figure 2).

Periphyton sampling method

The sample was carried out every seven days after placing the substrate for eight weeks. One substrate is taken from each frame in every sampling time. The bamboo was brushed gently on both sides using a toothbrush to separate the periphyton from the substrate. Then, the sample is put into a bottle containing aquades and reserved with 1% Lugol solution (Rodríguez and Pizarro 2015).

Table 1. Information of sampling side

Sampling site	Coordinates
Pond 1	7°44’32’’ S 113°13’58’’ E
Pond 2	7°44’36’’ S 113°13’48’’ E
Pond 3	7°44’37’’ S 113°13’48’’ E

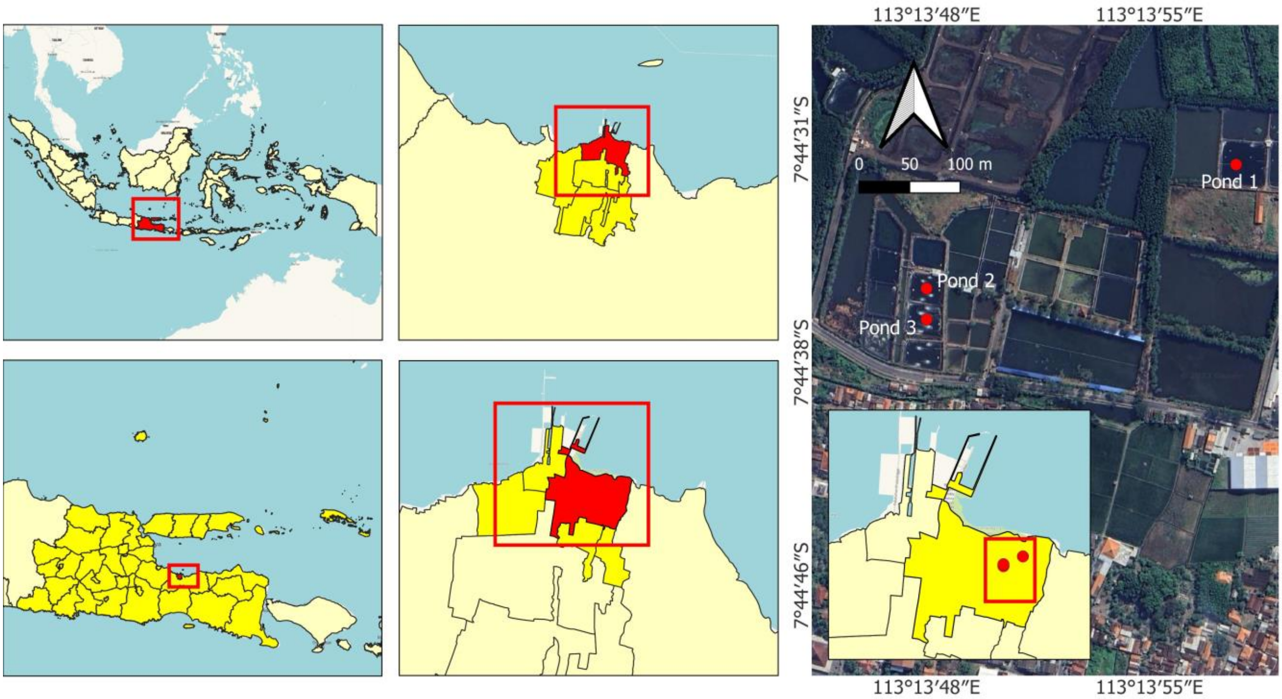

Figure 1. Research location in Probolinggo City, East Java, Indonesia

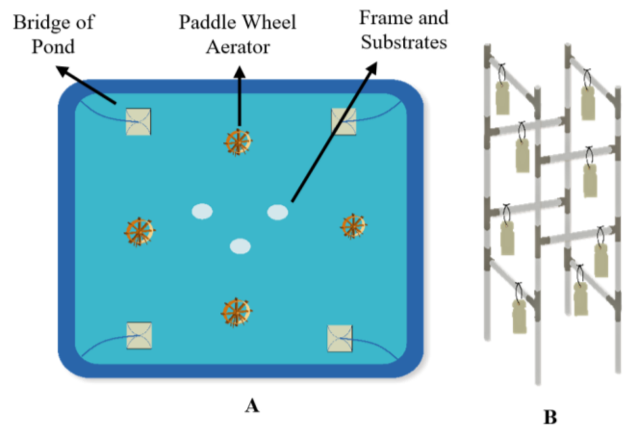

Figure 2. Placing of the substrate, A. Placing point; B. Frame and substrates

Periphyton sample observation

Observation of periphyton samples includes identification of the type and abundance of periphyton. This observation is according to Arsad et al. (2021) with the Lackey Drop Micro transect Counting Method using a binocular microscope at 400x magnification. Identification of the type of periphyton using the identification book such as Davis (1955), Conway et al. (2003), Reynolds et al. (2006), and Al-Yamani et al. (2011). Data analysis includes the calculation of abundance and relative abundance (Arsad et al. 2019), the Shannon-Weaver diversity index (Shannon and Weaver 1949; Odum 1971; Margalef 1978), the uniformity index (Odum 1971), and the Simpson dominance index (Simpson 1949; Magurran 1955; Odum 1971; Margalef 1978) with the following formula:

$$K = \frac{n \times At \times Vt}{Ac \times Vs \times As}$$

Where:

K : Abundance of periphyton (cell/cm^2 or ind/cm^2)
n : Number of periphyton found (cell or ind)
At : Cover glass area (mm^2)
Vt : Volume of samples (mL)
Ac : Wide from field of view (mm^2)
Vs : Observed sample volume (mL)
As : Scraped substrate area (mm^2)

$$KR = \frac{n_i}{N} \times 100\%$$

Where:

KR : Relative abundance
n_i : Number of individuals from species *i* in the sample
N : Total number of individuals from all species in the sample

$$H = -\sum_{i=1}^{S} pi \, log_2 \, pi \quad \text{Where:} \quad pi = \frac{n_i}{N}$$

Where:

H : Diversity Index
n_i : Number of individuals from species *i* in the sample
N : Total number of individuals from all species in the sample

The criteria for the diversity index are as follows:

$H < 1$: Low diversity
$1 < H < 3$: Moderate diversity
$H > 3$: High diversity

$$e = \frac{H}{H_{max}} \quad \text{Where:} \quad H_{max} = log_2 \, S$$

Where:

e : Uniformity Index
H : Diversity Index
S : Number of species

The uniformity index value (e) ranges from 0-1. The uniformity index value of 0-0.4 indicates low uniformity, 0.4-0.6 indicates moderate uniformity, and 0.6-1.0 indicates high uniformity.

$$C = \sum_{i=1}^{s} pi^2 \quad \text{Where:} \quad pi = \frac{n_i}{N}$$

Where:

C : Dominance Index
ni : Number of individuals from species *i* in the sample
N : Total number of individuals from all species in the sample

The value of the dominance index is between 0 and 1. A value of dominance index close to 0 means that there are no dominant species and vice versa, if closer to 1, it means that there is a dominant species. The greater of the dominance index value indicates that the diversity is low.

Measurements of water quality parameters

Water quality measurements based on in situ and ex-situ methods. Measurement water quality parameters as in situ include temperature and dissolved oxygen using a DO meter (Lutron PDO-520), brightness (Secchi disk, Indonesia), pH using pH paper (Ref 921 10, pH-Fix 0-14, Macherey-Nagel, Düren, Germany), and salinity using Salinometer (Smart Sensor Salinity Meter AR8012, China). Meanwhile, nitrate, phosphate, and ammonia using a spectrophotometer (Genesys 10S UV-Vis Spectrophotometer, Thermo Scientific, US) and total organic matter (titration method) carry ex-situ. The results of water quality measurements can be used to support the periphyton data obtained.

RESULTS AND DISCUSSION

Structure community of periphyton

This research was carried out in whiteleg shrimp ponds with a semi-bioflock system. The floc concentration in the ponds used for research was around 1 mL. Periphyton is found on a bamboo substrate in a whiteleg shrimp pond consisting of phyto-periphyton and zoo-periphyton (Figure 3). The phyto-periphyton found consisted of 4 phyla and 21 species. The phyto-periphyton phyla found consisted of the phyla Bacillariophyta, Chlorophyta, Cyanophyta, and Ochrophyta. Bacillariophyta is a phyto-periphyton phylum with the most species, namely 13 species consisting of

Achnanthes sp., *Amphiprora* sp., *Amphora* sp., *Campylodiscus* sp., *Chaetoceros* sp., *Cocconeis* sp., *Cyclotella* sp., *Cymbella* sp., *Gyrosigma* sp., *Melosira* sp., *Navicula* sp., *Nitzchia* sp., and *Pinnularia* sp. Phylum Chlorophyta consists of 4 species: *Chlorella* sp., *Dictyospaerium* sp., *Microspora* sp., and *Planktospaeria* sp. Phylum Cyanophyta consists of 3 species: *Aphanocapsa* sp., *Chroococcus* sp., and *Lyngby* sp. Phylum Ochrophyta only found *Nannochloropsis* sp. The zoo-periphyton found consisted of 4 phyla with 10 species. The five phyla consisted of Arthropoda, Ciliophora, Nematoda, and Rotifera. The phylum Arthropoda is *Microsetella* sp. and *Tanytarsus* sp. Phylum Ciliophora was the most abundant among other zoo-periphyton divisions and consisted of 5 species: *Halteria* sp., *Zoothamnium* sp., *Acineta* sp., *Thuricola* sp., and *Pyxicola* sp. Phylum Nematoda consists of only one species, namely *Mononchus* sp. Phylum Rotifera consists of 2 species, namely *Cephalodella* sp. and *Macrotrachela* sp.

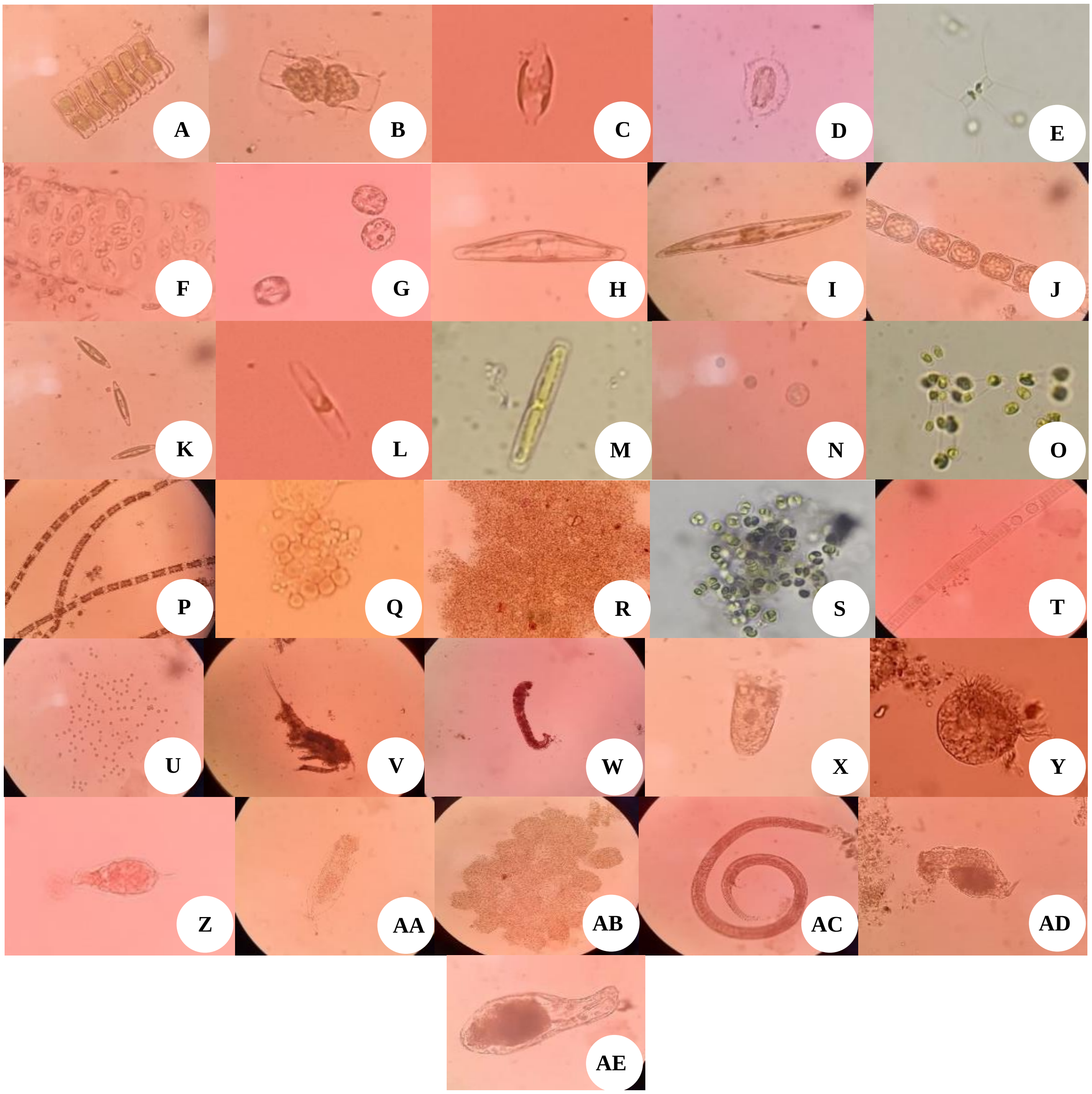

Figure 3. Periphyton was found on the bamboo substrate in whiteleg shrimp with a semi-biofloc pond system; A-U. Phyto-periphyton, V-AE. Zoo-periphyton. A. *Achnanthes* sp., B. *Amphiprora* sp., C. *Amphora* sp., D. *Campylodiscus* sp., E. *Chaetoceros* sp., F. *Cocconeis* sp., G. *Cyclotella* sp., H. *Cymbella* sp., I. *Gyrosigma* sp., J. *Melosira* sp., K. *Navicula* sp., L. *Nitzchia* sp., M. *Pinnularia* sp., N. *Chlorella* sp., O. *Dictyospaerium* sp., P. *Microspora* sp., Q. *Planktospaeria* sp., R. *Aphanocapsa* sp., S. *Chroococcus* sp., T. *Lyngby* sp. U. *Nannochloropsis* sp. V. *Microsetella* sp. W. *Tanytarsus* sp., X. *Halteria* sp., Y. *Zoothamnium* sp., Z. *Acineta* sp., AA. *Thuricola* sp., AB. *Pyxicola* sp. AC. *Mononchus* sp. AD. *Cephalodella* sp., AE. *Macrotrachela* sp.

The relative abundance of phyto-periphyton includes the phyla Bacillariophyta (17-69%), Chlorophyta (1-22%), Cyanophyta (30-65%), and Ocrophyta (0-25%) (Figure 4). The relative abundance of phyto-periphyton shows that the Bacillariophyta and Cyanophyta made up more phyto-periphytons than others. The phylum Cyanophyta abundance is high because this phylum can adapt to unfavorable conditions such as low CO_2, low or too high temperatures, and lack of light (Hood et al. 2016; Ciebiada et al. 2020; Briddon et al. 2022). Hu et al. (2022) and Samudra et al. (2022), stated that the Bacillariophyta phylum constituent of periphyton adapts to the environment, is cosmopolitan, and has a high tolerance. According to Ogi et al. (2021), most Bacillariophyta adapt to aquatic environmental conditions. This division can survive the worst environmental conditions and produce more mucus. In addition, most of the Bacillariophyta phylum secrete gelatinous stalk-like tools to attach to specific substrates.

The relative abundance of zoo-periphyton includes the phyla Arthropoda (0-3%), Ciliophora (69-99%), Nematodes (0-2%), and Rotifera (0-28%) (Figure 5). Class Ciliata (phylum Ciliophora) is a zoo-periphyton that is more abundant than other classes. Other phyla that compose zoo-periphyton consist of Rotifera, Nematoda, and class Crustacea (phylum Arthropoda). According to Gómez (2020), Ciliates (phylum Ciliophora) have stalks attached to the substrate equipped with an adhesive disk. In general, Ciliates live in benthic habitats.

The total abundance of phyto-periphyton on bamboo substrate ranged from 223,806-989,398 cells/L (Figure 6). The highest phyto-periphyton occurred in the 2nd week of 989,398 cells/L, but the lowest was in the 7th week of 22,806 cells/L. This abundance has decreased every week, presumably due to the declining quality of the environment. According to Ren et al. (2021) and Pacheco et al. (2022), the microalgae abundance on a substrate decreases every week due to reduced nutrients, light (water brightness) as well as utilization by the next level consumer (zoo-periphyton) or grazing.

The total abundance of zoo-periphyton ranged from 396-1924 individual/L. The zooplankton abundance tends to increase, the highest in the last week at 1,924 ind/L (Figure 7). The high zoo-periphyton is because of sufficient food (phyto-periphyton). The decrease of phyto-periphytons because consumed by zoo-periphytons, the zoo-periphyton abundance can be increased.

Diversity, uniformity and dominance index

The index of diversity, uniformity, and dominance of phyto-periphytons ranged from 1.988-2.880 (moderate), 0.460-0.666 (moderate), and 0.169-0.314 (moderate), while zoo-periphyton ranged from 0.931 to 2.262 (moderate), 0.280-0.681 (moderate) and 0.242-0.732 (moderate) (Table 1).

This index can be used as an indicator of water because its value can influence several factors that can cause pollution in the ecosystem. A high value of diversity indicates that the environment is very healthy, and a low value indicates the occurrence of pollution. Low diversity will be related to the value of dominance, which is an inverse relationship. If the diversity value is high, the dominance value will be small and indicate the absence of dominance (Setyono and Himawan 2018; Arsad et al. 2019).

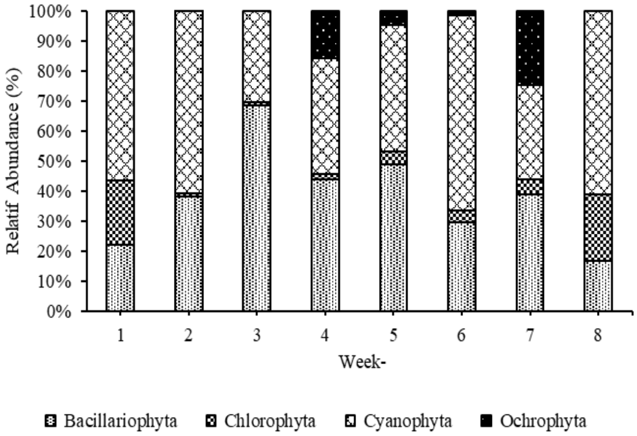

Figure 4. Mean relative abundance weekly of phyto-periphyton from Bamboo substrate in the whiteleg shrimp with semi-biofloc system

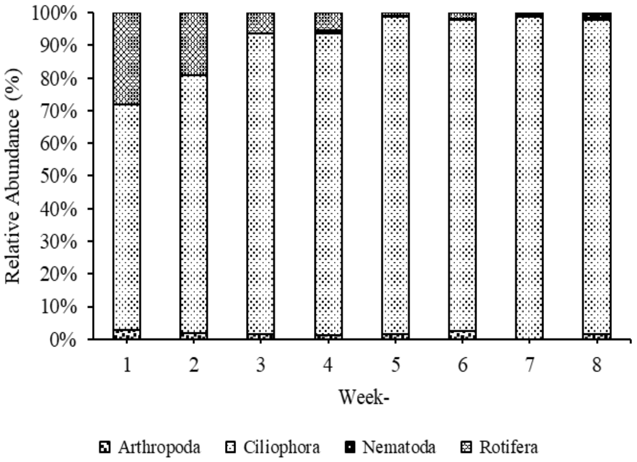

Figure 5. Mean relative abundance weekly of zooperiphyton from Bamboo substrate in the whiteleg shrimp with semi-biofloc system

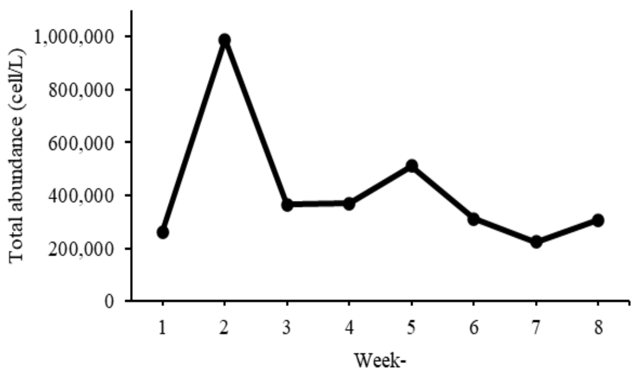

Figure 6. Mean total abundance weekly of phyto-periphyton from Bamboo substrate in the whiteleg shrimp with semi-biofloc system

Water quality parameters

The results of measuring water quality parameters consisting of temperature, brightness, acidity (pH), dissolved oxygen, carbon dioxide, nitrate, phosphate, ammonia, salinity, and total organic matter can be seen in Table 2. The average results temperature obtained during this study ranged from 27.2-32.6°C. Temperature changes in this study were not too significant every week because the measurement time was relatively the same. The high temperature is because of environmental conditions such as the intensity of sunlight, clouds, and rain. Water temperature serves as a regulator of physiological and metabolic roles for aquatic organisms. This temperature range is still quite good for periphyton life. The optimal temperature range for the growth of the diatom group (Bacillariophyta) is between 20-30°C, while for the Chlorophyta is between 30-35°C (Prelle et al. 2019; Bondar-Kunze 2021).

Whiteleg shrimp ponds have an average brightness value that is relatively decreased weekly. The lowest brightness value occurred in the 7th week, 35 cm, while the highest was 64.2 cm in the 3rd week. The low brightness value is presumably because of suspended particles in the waters, such as aquaculture organisms and organic materials (plankton, feed residues, and feces). Based on Katmoko et al. (2021), pond brightness is affected by plankton (phytoplankton and zooplankton) and dissolved materials. The low brightness results in low dissolved oxygen levels. Optimal brightness values in ponds generally range from 35-45 cm.

The average value of the degree of acidity (pH) obtained during this research is 8. This value can be a good category for periphyton growth. The optimal pH value for aquatic biota growth ranges from 7-8.5. If the pH value decreases below the optimal range, it can decrease biota production, especially periphyton. One factor that can affect water's pH value is the respiration process. The respiration process produces carbon dioxide. The higher carbon dioxide in the water showed lower pH values (Raven et al. 2020).

The average value of DO (Dissolved Oxygen) in ponds ranged from 6.7 to 10.6 mg/L. The high dissolved oxygen in the pond is due to the existence of a waterwheel to increase oxygen levels. The result of the dissolved oxygen level in this research is good for the growth of aquatic organisms. The dissolved oxygen level in the water is related to temperature factors and the concentration of various water ions. The optimum DO content for the growth of aquatic autotrophic organisms (phyto-periphyton and phytoplankton) is >4 mg/L (Anand et al. 2019).

The carbon dioxide (CO_2) results during this study showed a 0 (zero) or undetected value. Autotrophic organisms (microalgae include phytoplankton and phyto-periphyton) in ponds utilize it for photosynthesis. In addition, the measurements were carried out from morning until noon. Carbon dioxide is one of the main elements needed in photosynthesis for aquatic autotrophs. Free carbon dioxide (CO_2) in waters can decrease due to photosynthesis, evaporation, and water movement (Arsad et al. 2019).

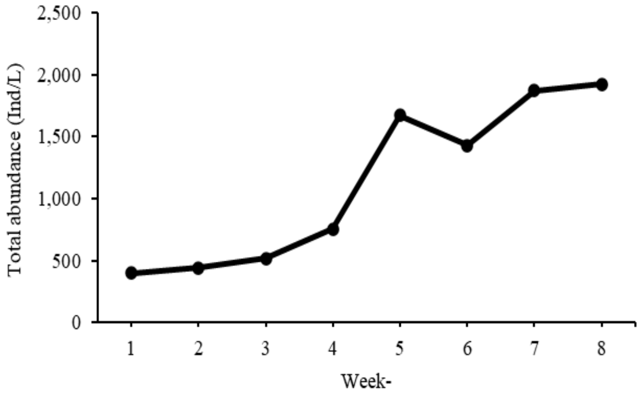

Figure 7. Mean total abundance weekly of zoo-periphyton from Bamboo substrate in the whiteleg shrimp with semi-biofloc system

Table 1. Value of diversity index, uniformity, and dominance of periphyton from Bamboo substrate in the whiteleg shrimp pond

Week-	Phyto-periphyton			Zoo-periphyton		
	H'	e	C	H'	e	C
1	2.181	0.505	0.269	2.112	0.636	0.242
2	1.988	0.460	0.280	2.213	0.666	0.253
3	2.031	0.470	0.314	2.019	0.608	0.310
4	2.547	0.589	0.192	2.262	0.681	0.271
5	2.773	0.642	0.174	1.647	0.496	0.410
6	2.630	0.609	0.198	1.366	0.411	0.527
7	2.880	0.666	0.169	0.931	0.280	0.732
8	2.832	0.655	0.177	1.439	0.433	0.551

Table 2. The average results from the measurement of water quality parameters in semi-biofloc shrimp pond systems

Water quality parameters	Unit	Week-							
		1	2	3	4	5	6	7	8
Temperature	°C	29.8	29.0	29.3	27.2	29.1	28.3	29.2	29.2
Brightness	Cm	56.7	55.8	64.2	54.2	49.2	45.0	35.0	37.5
pH		8	8	8	8	8	8	8	8
Dissolved oxygen	$mg.L^{-1}$	6.7	7.0	10.6	8.7	8.0	8.3	8.8	7.6
Carbon dioxide (CO_2)	$mg.L^{-1}$	0	0	0	0	0	0	0	0
Nitrate	$mg.L^{-1}$	0.039	0.042	0.123	0.061	0.050	0.032	0.121	0.164
Orthophosphate	$mg.L^{-1}$	0.026	0.013	0.033	0.008	0.102	0.012	0.029	0.014
Ammonia	$mg.L^{-1}$	0.013	0.030	0.036	0.023	0.087	0.063	0.059	0.134
Salinity	ppt	18.8	19.4	19.1	21.0	19.3	20.0	20.9	20.8
Total Organic Matter	$mg.L^{-1}$	31.60	16.85	23.17	23.17	15.53	97.96	53.72	63.20

The average value of nitrate, phosphate, and ammonia each week fluctuates. Nitrate values ranged from 0.032-0.164 mg/L, phosphate ranged from 0.008-0.102 mg/L, and ammonia ranged from 0.013-0.134 mg/L. The source of nitrate in the pond is the rest of the feed. The nitrate level was still in good condition for periphyton growth. Periphyton used nitrate as a growth nutrient. A good nitrate level for aquaculture is <0.2 mg/L (Nurhatijah et al. 2016). orthophosphate is a limiting factor for periphyton growth. An orthophosphate concentration of 0.01 mg/L supports algae growth, while concentrations from 0.03 to 0.1 mg/L or higher likely encourage blooming (Ballah et al. 2019).

The average value of salinity every week is relatively consistent. Whiteleg shrimp ponds have an average salinity value ranging from 18.8 to 21.0 ppt. The suitable salinity range for epiphytic microalgae growth is 10-40 ppt, with an optimum value of 35 ppt. The distribution of water salinity affects several factors, namely air circulation patterns, evaporation, rainfall, and river or freshwater input. Temperature also affects the evaporation process in the sea. The highest temperature causes an increase in salinity levels in the water (Mamun 2020).

The total organic matter (TOM) average in ponds showed a downward trend from the 1st week to the 5th week. The 5th week was the lowest TOM result in the pond, which was 15,533 mg/L. A high increase in TOM levels occurred at week 6, which was 97.960 mg/L. The high TOM levels are suspected from the accumulation of feed residues and the high abundance of periphyton. High levels of TOM will reduce dissolved oxygen and affect autotrophic organisms in photosynthesis (Arsad et al. 2021; McCabe et al. 2021).

In conclusion, periphyton found on a bamboo substrate in a semi-biofloc system whiteleg shrimp ponds consisted of phyto-periphyton (4 phyla and 21 species) and zoo-periphyton (4 phyla with 10 species). There has been a change in the abundance of periphyton, which indicates a change in water quality in whiteleg shrimp cultivation. Water quality parameters during the study were relatively good for aquatic organism growth, except for very high TOM levels in several weeks.

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