Wastewater Treatment Efficiency by a Freshwater Phylactolaemate Bryozoan and Experimental Feeding with Protozoa

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ABSTRACT

The wastewater treatment ponds of the King's Royally Initiated Laem Phak Bia Environmental Research and Development (LERD) Project in west-central Thailand provide habitats for freshwater bryozoans, which are colonial invertebrate animals. Bryozoans sieve food particles out of the water using a retractable lophophore and can play an important ecological role in wastewater treatment. In this unique environment, we: (1) investigated the efficiency of a phylactolaemate bryozoan (*Plumatella casmiana*) in wastewater treatment, measured by BOD_5, chlorophyll a and turbidity; and (2) determined the role of protozoans in the diet of the bryozoan *P. casmiana*. Comparison of growth rate and fecal pellet characteristics between protozoan-fed bryozoans and phytoplankton-fed bryozoans was investigated. At the end of our wastewater treatment experiment, water quality parameters were markedly improved in the treatment with bryozoans compared to the control (without bryozoans). The treatment efficiency levels for BOD_5, turbidity, and chlorophyll a were 24.04%, 59.21%, and 55.13%, respectively. The growth rates of bryozoans in the experimental treatment increased over time. Our study also revealed that this bryozoan can feed on a diet of protozoans under experimental conditions. However, the average daily growth rate of protozoan-fed bryozoans -20 zooids per day was lower than that of phytoplankton-fed bryozoans 19 zooids per day. This may have been due to incomplete digestion of protozoans or insufficient nutrition in the bryozoans. The results from this study provide better understanding of bryozoan ecology and their role in wastewater treatment systems.

1. INTRODUCTION

Bryozoans are moss-like invertebrates that coexist in colonies, where one colony consists of many zooids joined together (Schwaha et al., 2016). They are hermaphroditic and can reproduce both sexually and asexually (Schwaha et al., 2020). Most freshwater bryozoans can be propagated from encapsulated seed-like structures called statoblasts. These statoblasts are found in most bryozoans and are important in phylactolaemate systematics (Hirose et al., 2011). One species, *Plumatella casmiana* Oka, 1907 is capable of producing statoblasts that are dormant as well as statoblasts ("leptoblasts") that germinate immediately upon release from the colony (Wood, 2015). Bryozoans feed by filtering suspended solids such as microorganisms, algae, plankton and other debris from the surrounding water (Morris et al., 2002; Rutkauskaitė-Sucilienė and Šatkauskienė, 2016; Nimtim et al., 2020). They can also consume protozoans and rotifers (Wood, 2015). The food items are captured by an array of ciliated tentacles and then digested in a simple gut (Ryland, 2020). Undigested particles are packaged together and expelled in the form of fecal pellets (Wood, 2015). Wright (2014) and Yepes-Narváez (2020) reported that a single bryozoan zooid can filter up to 8.8 mL of water per day; thus, these animals have the potential to remove suspended particles and algae from water and contribute to nutrient cycling and regulation of the metabolism of freshwater ecosystems (Todini et al., 2018). Bryozoans also represent a food source for many aquatic species and provide microhabitats and refuge for smaller invertebrates (Gorgoglione et al., 2016).

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Most bryozoans (Phylum Bryozoa) are marine. However, some species can be found in freshwater habitats; these are members of Class Phylactolaemata and are referred to as phylactolaemate bryozoans (Massard and Geimer, 2007). Bryozoans grow on submerged objects such as glass, plastic and wood (Wood, 2009) in shallow water, and they can live in a wide variety of environments. They usually thrive in warm waters between 15ºC and 28ºC. However, some species can survive in cold (below 10ºC) and very warm waters (above 32ºC or more). A study by Shrivastava and Rao (1985) showed that *Plumatella emarginata* could survive in Bhopal Lake in India during the summer, when water temperatures rose to 34ºC.

Bryozoans can survive in both clean water and water that has been contaminated with pollutants such as wastewater treatment systems. Bryozoans are also tolerant to a wide range of pH, but thrive in slightly alkaline water bodies (Wood, 2015). Because bryozoans are sedentary organisms and have a certain degree of tolerance to water contamination, they are one of the most suitable organisms for use as bioindicators; they are capable of assessing water quality in a fixed location continuously and accurately (Seansupha, 2009). Bryozoans can also be problematic in the environment. They cause clogging of various pipes and drainage systems (Wood, 2005) via floatoblasts, which are characterized by their buoyancy and mass production. In marine environments, bryozoans spread easily by currents or by attaching to transport ships as well as in ballast water. Therefore, bryozoans are among the most invasive alien species and can greatly affect native species (Kocak et al., 2019).

In Thailand, a total of 18 species of freshwater bryozoans have been identified from 26 provinces (Wood et al., 2006). A member of the family Plumatellidae, *Plumatella casmiana* has a worldwide distribution (Wood et al., 2010). This paper deals primarily with *P. casmiana*, which is a phylactolaemate bryozoan. *P. casmiana* is the predominant bryozoan found in wastewater treatment systems of the King's Royally Initiated Laem Phak Bia Environmental Research and Development (LERD) Project in Thailand. In fact, the study by Nimtim (2020) identified three species of freshwater bryozoans living in these wastewater treatment ponds: *P. casmiana*, *P. vorstmani* Toriumi, 1952 and *P. vaihiriae* Hastings, 1929. Wastewater in the LERD Project is treated by a series of oxidation ponds. Notably, bryozoans can grow well in some of these wastewater treatment ponds; they tend to settle on the outfall of these ponds (Nimtim et al., 2020), where oxygen and food are plentiful and the colonies are beyond the reach of predatory fish. Thriving bryozoan colonies in this environment could be due to the combination of rich organic food materials coming from domestic wastewater as well as dense populations of highly nutritional phytoplankton. In particular, *Spirulina platensis* (Gomont) Geitler 1925 is dominant in oxidation ponds (Chaichana and Dumpin, 2016).

Bryozoans can consume a variety of foods such as plankton and organic particles suspended in the wastewater ponds of the LERD Project. Protozoans are also diverse, abundant and one of the main biological components in these man-made ecosystems (Madoni, 2011). Protozoans may be an important alternative food source for bryozoan nutrition. There is strong evidence that some species of freshwater bryozoans can feed not only on algae but also on protozoans (Wood, 2019). Nimtim et al. (2020) suggested that the role of ciliates in bryozoan nutrition at the LERD Project site should be further investigated. Nutrients derived from protozoa (as a food) may increase the growth rate of bryozoans and help maintain healthy communities of bryozoans in wastewater treatment ponds.

Research on the role of bryozoans in wastewater treatment is limited, especially in Thailand. Much of the research has focused on bryozoans in natural habitats. To gain more insight into the biology and ecology of freshwater bryozoans in the LERD Project (wastewater treatment ponds), this study aimed to explore the role of *P. casmiana* in wastewater treatment, particularly in terms of reduction of organic content (BOD_5), chlorophyll a, and turbidity. Since oxidation ponds are a rich source of food to bryozoans, knowledge of protozoa as a food source of bryozoans is important, but currently this topic is poorly understood. This study thus further examined the role of protozoans as a food source for bryozoans and characterized the fecal pellets after protozoan digestion. The results should add to our understanding of the efficiency of the *P. casmiana* digestive system and create wider interest in wastewater treatment and research in Thailand and the international research community.

2. METHODOLOGY

2.1 Study site

The wastewater treatment system at the LERD Project was established in 1991 with the aim of treating domestic wastewater from Phetchaburi municipality, Thailand. The municipal wastewater here is composed of organic matter, inorganic substances, oil and various flotation agents, solids, detergents, and nutrients. The LERD Project handles approximately 3,600 to 6,000 m^3/d. There is a total of five ponds, comprising one sedimentation pond, three oxidation ponds and one stabilization pond (Figure 1). The ponds range in surface area from 10,134-42,878 m^2, with a combined storage capacity of approximately 20,000 m^3/d. Wastewater flows by gravity from pond 1 to pond 5, and then is discharged to a nearby mangrove forest and then to the Gulf of Thailand. When the wastewater enters the system, the BOD_5 ranges from 1,000-1,600 mg/L. After treatment in all five ponds, the BOD_5 is reduced to only 10-15 mg/L.

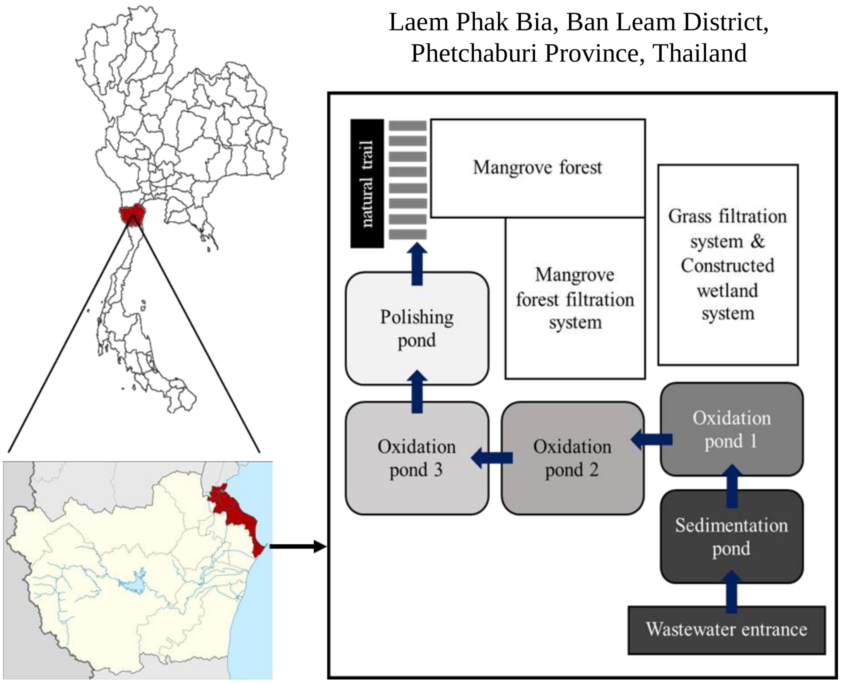

Figure 1. Map and flow diagram of the LERD Project's wastewater treatment system (13.0471°N, 100.0844° E)

2.2 Sampling of bryozoans and bryozoan culture

Portions of bryozoan colonies (*P. casmiana*) were collected by hand from the edge of the outflow weir of a pond where bryozoans were abundant (Figure 2). The color of living colonies of these bryozoans is light to dark brown and colonies are soft. Colonies are seen as tubular branches and look like tree roots. Portions of bryozoan colonies were then placed in a polyethylene bottle containing a small amount of water from the sampling point. The bryozoan samples were cultured and used for further experiments in the laboratory of the LERD Project.

Colonies of bryozoans were torn into small fragments about 0.5 cm in diameter, and then viewed under a stereo microscope (Nikon SMZ 445) to confirm that the bryozoans were alive. Then, 4-5 pieces of bryozoan colony were placed in plastic Petri dishes inside a plastic container filled with water from oxidation pond 2. We used water from oxidation pond 2 because bryozoans were most abundant there, and the environmental conditions were suitable for bryozoans. The Petri dishes were attached to the wall of the container using a pair of small magnets. The dishes were left at room temperature (30ºC) for a few days until each bryozoan colony had attached to the Petri dish. Then, the bryozoans were nurtured for another 3-5 days and supplied with constant aeration (Figure 3), with the water being renewed daily (3-4 L) to provide food for the bryozoan colonies.

Figure 2. Location of bryozoan colonies collected from oxidation pond 2 at the LERD Project indicated by red arrow (above); and living bryozoan colonies on hand (below left) and under microscope (below right).

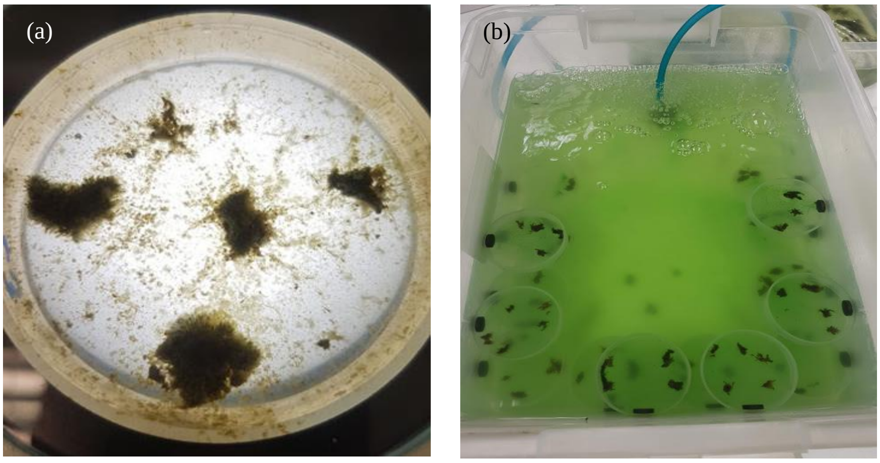

Figure 3. (a) Colonies of *P. casmiana* (Oka, 1907) fully established in a Petri dish; and (b) Petri dishes attached with paired magnets to the walls of a container

Water samples (n=3) from oxidation pond 2 were examined for several parameters. We measured temperature (ºC), pH, dissolved oxygen (mg/L) and conductivity (µs/cm) using a multimeter analyzer (WTW ProfiLine Cond 3310). Plant nutrients (mg/L) were also measured: soluble reactive phosphorus (SRP) by ascorbic acid method, ammonium nitrogen by phenol hypochlorite method, total nitrogen by the Kjeldahl method and total phosphorus by the vanadomolybdate method. Phytoplankton was collected by filtration of 10 L of water through a plankton net (20-micron mesh). Phytoplankton species were identified and counted for density (unit/L) using Sedgewick Rafter counting chamber. Analysis of water and biological samples was determined at Department of Environmental Technology and Management, Faculty of Environment, and Department of Soil Science, Faculty of Agriculture, Kasetsart University, Bangkok.

2.3 Efficiency of wastewater treatment by bryozoans

In this experiment, we explored the role of the freshwater bryozoan *P. casmiana* in wastewater treatment based on the method of Nimtim et al. (2020). The experiment included a control (without bryozoans) and an experimental treatment with bryozoans, containing around 100-150 zooids. Both the experimental and control treatments were done in triplicate. The water used in the experiment was taken from oxidation pond 2. Water was poured into 18 beakers of 1,000 mL (Figure 4). Each beaker was aerated at a constant rate to circulate food particles throughout water column and to maintain oxygen level, and was kept at room temperature (30-35°C) with light: dark (L:D) conditions of 12:12 h. We measured turbidity using a turbidity meter (WTW Turb® 430 IR) and measured chlorophyll a by an acetone extraction method, with wavelengths determined by spectrophotometer. Turbidity and chlorophyll a were measured at 0, 24, 48, and 72 h. BOD_5 measurement was done by Azide modification method at 0 and 72 h to compare the values between the beginning and end of the experiment. Wastewater treatment efficiency was calculated using the formula [(In-Out)/In] × 100, where In is the water quality before the experiment and Out is the water quality after the experiment. The growth of bryozoans was estimated by counting the number of zooids at 0, 24, 48, and 72 h, and then the average daily growth rate of bryozoans (ADG) was estimated using the formula (W2-W1)/time, where W1 is the number of zooids before the experiment and W2 is the number of zooids after the experiment. Water chemistry analysis was conducted in the laboratories of the LERD Project and of Department of Environmental Technology and Management, Faculty of Environment, Kasetsart University, Bangkok.

2.4 Feeding experiment of bryozoans using protozoans as diet

The bryozoans used in this study were cultured using the same procedure as described in section 2.2. For protozoan culture, water collected from oxidation pond 2 was poured into 50 mL Petri dishes and then three boiled mung bean seeds were added to each dish as a food source for the protozoans (Figure 5). The protozoans were grown for 3-5 days before they were used to feed the bryozoans. After 3-5 days, the water in the Petri dishes became milky white, indicating a dense population of protozoans (Figure 5). The density of cultured protozoans per 1 mL was calculated to obtain an estimate of the number of protozoa.

Figure 4. Experimental setup to investigate wastewater treatment efficiency by bryozoans

We divided the experiment into a control and experimental treatment, both performed in triplicate. The bryozoan colonies were placed in Petri dishes. In the control, three Petri dishes with colonies of bryozoans were attached to the wall of a 600 mL beaker and the bryozoans were fed daily by phytoplankton in water from oxidation pond 2 (600 mL of pond water).

In the experimental treatment, three Petri dishes with colonies of bryozoans were used and protozoans were provided as bryozoan diet. Prior to the experiment, pond water (500 mL) was taken from oxidation pond 2 and left for five days (we waited until phytoplankton in the water died and settled to the bottom of beaker). The resulting clarified water without phytoplankton was then mixed with protozoans obtained from our protozoan culture. On day 0, the bryozoans were fed with protozoans (500 mL of clarified pond water + 100 mL of protozoans). During days 1-5, 50 mL of protozoans was added to the beaker containing Petri dishes. The experiment was conducted for six days. The feeding of bryozoans on the protozoans was observed and photographed under a light microscope (Leica DM500).

We measured the growth rate of bryozoans by counting the number of zooids on days 0 to 5 under a stereo microscope, and then estimated the average daily growth rate. We also examined the fecal pellets produced by the bryozoans for both the control and experimental treatment. A pointed dropper was used to collect fecal pellets from the Petri dishes. The shape, appearance, color, and detail of fecal pellets were examined under a microscope at 400X magnification (Wood, 2021) and fecal pellets were photographed and compared.

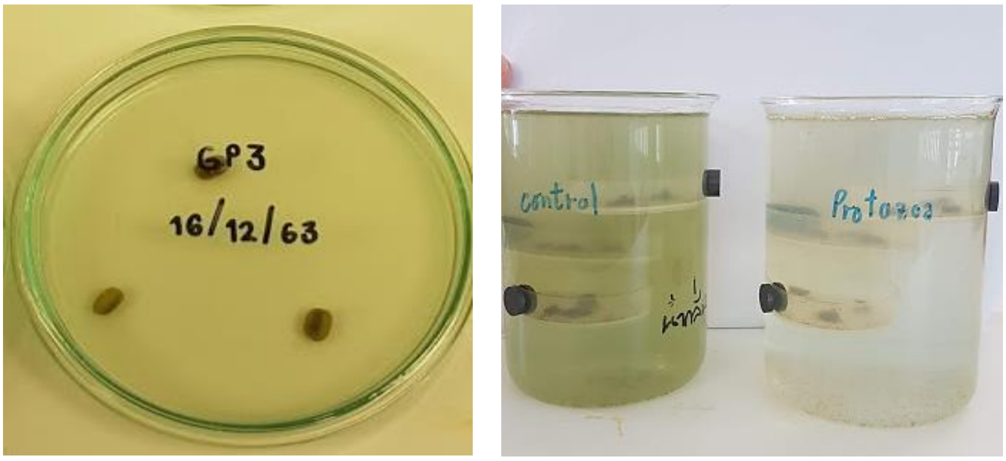

Figure 5. Culture of protozoans in Petri dish (left); and bryozoan feeding experiment using protozoans as main diet (right)

Data were analyzed using descriptive statistics (mean and standard deviation). Error bars are also presented in bar and line graphs. A statistical test was applied for comparing the control and the experimental treatment using the Student's t-test. Means with a significance value of less than 0.05 were considered significantly different. The statistical analysis was performed using the IBM SPSS Statistics software, authorized user version 22.

3. RESULTS

3.1 Efficiency of wastewater treatment by bryozoans

We gathered general information on water chemistry and phytoplankton communities in oxidation pond 2 since water from this pond was used in our experiments. Water chemistry results are presented in Table 1. Temperature, pH, conductivity, and dissolved oxygen were in suitable ranges for bryozoan survival and growth. Nutrient concentrations were high, especially nitrogen. Oxidation pond 2 was classified as hypereutrophic (TP>0.1 mg/L and TN>1.5 mg/L).

Phytoplankton were classified into two divisions (Table 2). Cyanobacteria represented the largest group in oxidation pond 2, with *Spirulina platensis* as the most abundant species followed by the genera *Oscillatoria* and *Microcystis*. High nutrient concentrations corresponded well with high densities of phytoplankton.

Table 1. Water quality measurements (mean±SD) in oxidation pond 2

Parameter	Value
Temperature (ºC)	30.69±0.71
pH	8.44±0.49
Dissolved oxygen (mg/L)	5.28±1.98
Conductivity (µs/cm)	496.63±16.49
NH_4^+-N (mg/L)	0.51±0.03
SRP (mg/L)	0.64±0.03
Total nitrogen (mg/L)	2.92±0.00
Total phosphorus (mg/L)	0.67±0.00

Table 2. Species and densities of phytoplankton in oxidation pond 2

Phytoplankton	Density (unit/L)
Division Cyanophyta	
Class Cyanophyceae (Blue-green algae)	
Merismopedia sp.	200
Microcystis sp.	15,050
Spirulina platensis (Nordstedt) Geitler	1,062,500
Oscillatoria sp.	85,500
Anabaena sp.	6,025
Division Chlorophyta	
Class Chlorophyceae (Green algae)	
Pandorina morum (Müller) Bory	150
Tetraedron gracile (Reinsch) Hansgirg	50
Tetraedron hastatum (Reinsch) Hansgirg	75
Tetraedron trigonum (Naegeli) Hansgirg	175
Radiococcus sp.	425
Actinastrum hantzschii Lagerheim	75
Coelastrum astroideum De Notaris	200
Coelastrum microporum Nägeli	75
Crucigenia sp.	800
Scenedesmus acuminatus (Lagerheim) Chodat	100

Table 2. Species and densities of phytoplankton in oxidation pond 2 (cont.)

Phytoplankton	Density (unit/L)
Scenedesmus quadricauda (Turpin) Brébisson	225
Scenedesmus sp.	125
Pediastrum duplex Meyen	100
Pediastrum simplex (Meyen) Lemmermann	50
Class Euglenophyceae (Euglenoids)	
Euglena sp.	1,275
Lepocinclis ovum (Ehrenberg) Lemmermann	25
Phacus longicauda (Ehrenberg) Dujardin	25
Trachelomonas hispida (Perty) Stein	25

The wastewater treatment efficiency of *P. casmiana* was determined by measuring the change in BOD_5, chlorophyll a concentration, and turbidity before and after the experiment. The results are shown in Table 3. In the experimental treatment (with bryozoans), after 72 h, the levels of BOD_5, chlorophyll a and turbidity were reduced. In the control (without bryozoans), chlorophyll a and turbidity values were reduced, while BOD_5 was elevated.

Statistical analysis revealed that after 72 h, the BOD_5 and turbidity levels between the control and treatment were significantly different at the 95% confidence level. In the control, treatment efficiency levels for BOD_5 and turbidity were -161.77±49.39% and 24.09±8.74%, respectively. In the experimental treatment, treatment efficiency levels for BOD_5 and turbidity were 24.04±18.13% and 59.21±15.40%, respectively. The efficiency of chlorophyll a removal in the control and experimental treatment was 36.89±5.30% and 55.13±14.34% (Figure 6).

Table 3. Water quality between control without bryozoans and experimental treatment with bryozoans

Variable	Prior to experiment	After experiment	
		Control	Treatment
BOD_5 (mg/L)	7.30±1.05		
- After 72 h		18.8±1.97	5.2±1.03
Chlorophyll a (mg/L)	0.27±0.01		
- After 24 h		0.37±0.01	0.37±0.02
- After 48 h		0.36±0.01	0.30±0.02
- After 72 h		0.17±0.01	0.11±0.04
Turbidity (NTU)	33.7±1.16		
- After 24 h		34.90±0.30	31.20±0.30
- After 48 h		31.63±0.49	22.50±3.06
- After 72 h		25.27±2.11	12.36±4.24

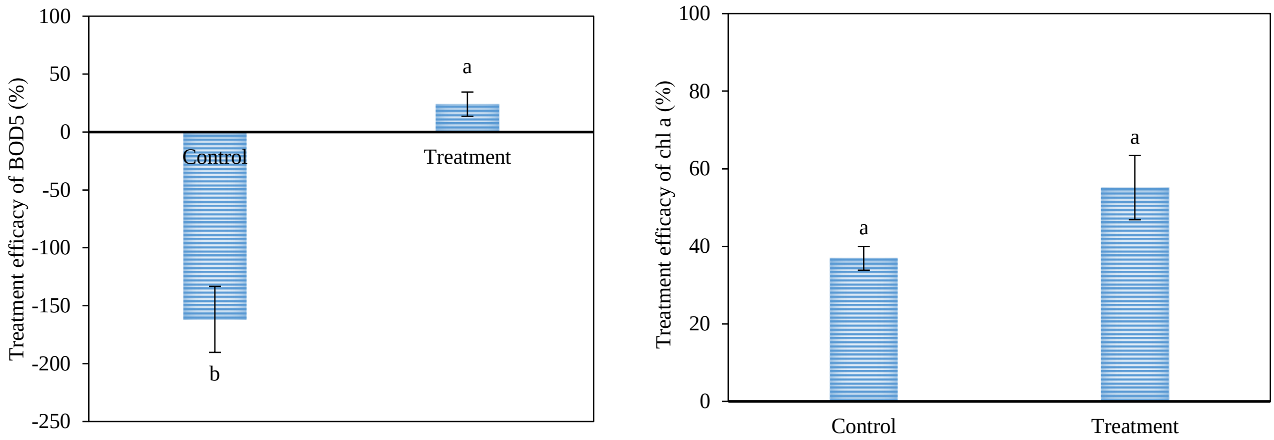

Figure 6. Comparison of wastewater treatment efficiency (BOD_5, turbidity and chlorophyll a) between control and experimental treatment (mean values with error bars). Different letters (a and b) above columns indicate significant differences between control and experimental treatment.

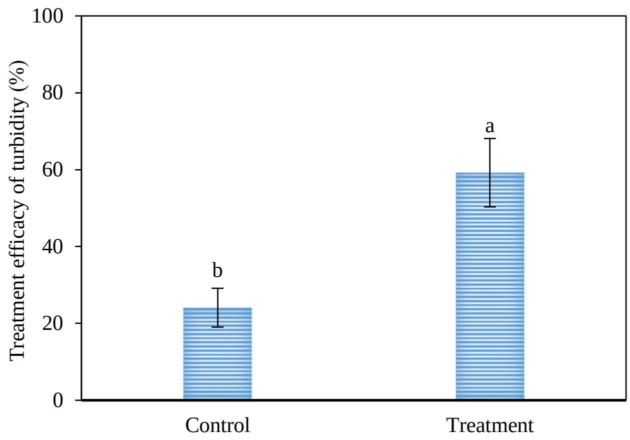

Figure 6. Comparison of wastewater treatment efficiency (BOD_5, turbidity and chlorophyll a) between control and experimental treatment (mean values with error bars). Different letters (a and b) above columns indicate significant differences between control and experimental treatment (cont.).

The results showed that during the experiment, the bryozoan growth rate increased over time (Figure 7). The average daily growth rate of bryozoans was 54±22 zooids per day.

3.2 Feeding studies of bryozoans using protozoa

In this experiment, protozoans were cultured as diet for bryozoans. The protozoans were identified as members of the Phylum Ciliophora (*Colpidium, Paramecium, Tetrahymena*). The density of protozoans was about 1,600 individuals/mL. The bryozoans were able to feed successfully on the protozoans. The lophophore of a bryozoan aids in waving protozoans into the mouth and further into the digestive system (Figure 8). The feeding rate of bryozoans was approximately 2 individual protozoans per minute during the experiment.

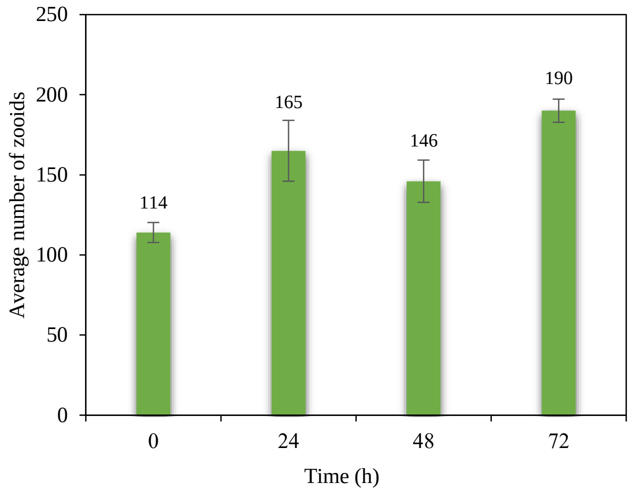

Figure 7. Growth rate of bryozoans (based on number of zooids) during 72 h of feeding experiment (mean values with error bars)

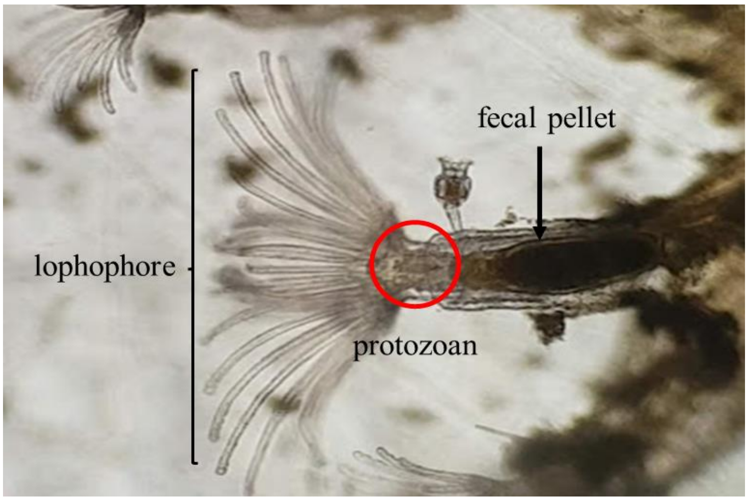

Figure 8. Protozoan (in red circle) entering the gastrointestinal tract of a bryozoan and presence of a fecal pellet (arrow) inside the bryozoan. Length of bryozoan is approximately 0.5 mm (Wood, 2021).

We examined the characteristics of fecal pellets produced by bryozoans feeding on protozoans and compared them with fecal pellets of phytoplankton-fed bryozoans in the control. The fecal pellets of protozoan-fed bryozoans were translucent and consisted of large fragments (Figure 9(a)), indicating incomplete digestion of the protozoans. In contrast, the fecal pellets of bryozoans in the control contained greenish particles from ingested phytoplankton. This was seen as a large mass of unidentified microscopic cells (Figure 9(b)).

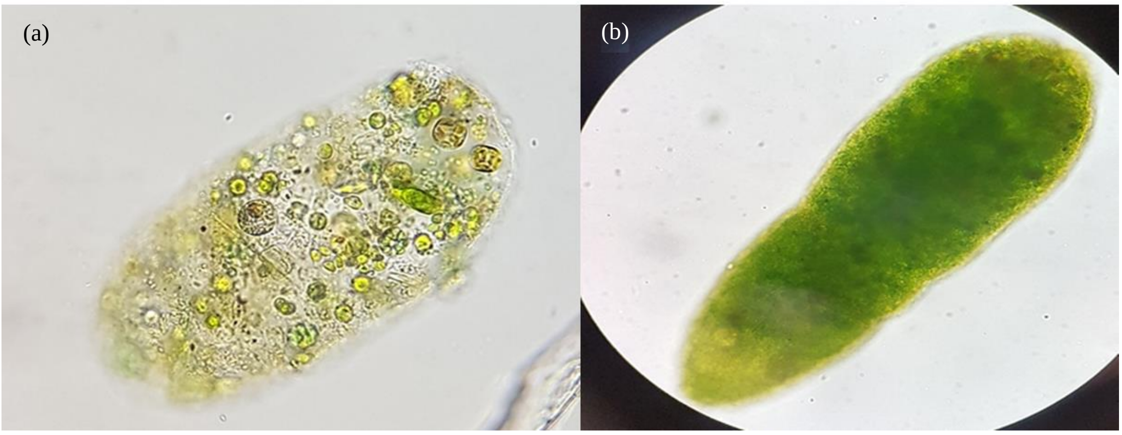

Figure 9. Fecal pellet of protozoan-fed bryozoan (a) and fecal pellet of phytoplankton-fed bryozoan in the control (b). Length of fecal pellet is approximately 100 µm (Wood, 2021).

Figure 10 shows the growth rates of protozona-fed bryozoans compared to bryozoans in the control that were fed with phytoplankton from oxidation pond 2. Based on the number of zooids, bryozoans in the control had relatively higher growth than protozoan-fed bryozoans, for which growth tended to decrease gradually throughout the experiment. The average daily growth rates of protozoan-fed bryozoans and phytoplankton-fed bryozoans were -20±6 and 19±34 zooids per day.

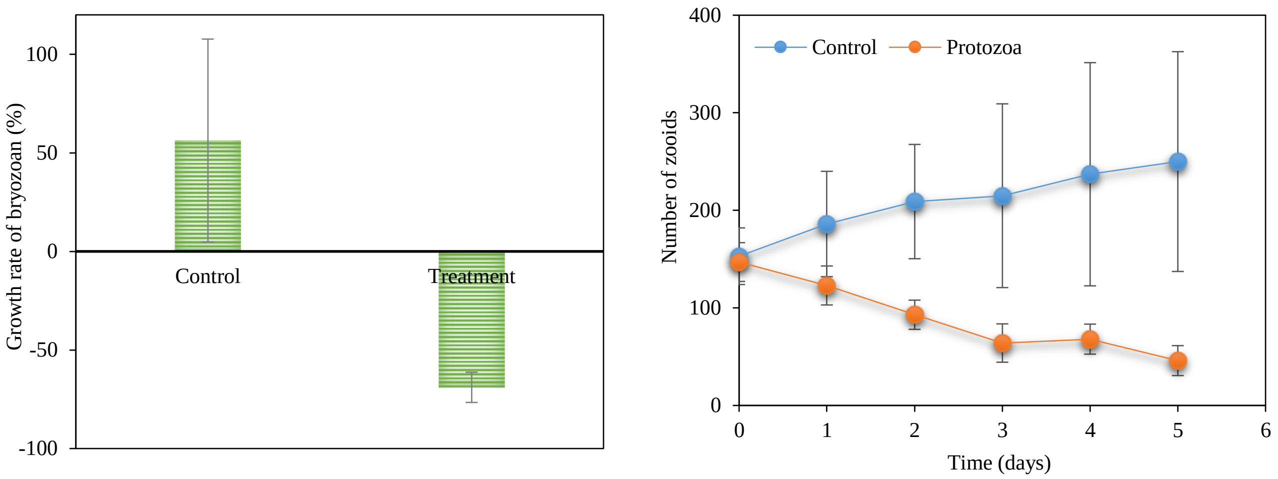

Figure 10. Comparison of growth rates of bryozoans between control (bryozoans fed with phytoplankton) and treatment (bryozoans fed with protozoans) (mean values with error bars)

4. DISCUSSION

Bryozoans are invertebrate animals that have the potential to be biological filters (Rutkauskaitė-Sucilienė and Šatkauskienė, 2016) and are part of the cycling of organic matter (Todini et al., 2018) in wastewater treatment ponds. The present study showed that in the laboratory, water quality (BOD_5 and turbidity in particular) in the LERD Project tended to improve in the presence of bryozoans. The increase of BOD_5 in the control may be linked to an increase in organic components from different sources such as phytoplankton, zooplankton and other microscopic organisms (Minnesota Pollution Control Agency, 2008). Further research should investigate the population dynamics and composition of microbial communities in wastewater ponds. The improvement in water quality in the experimental treatment could have resulted from the filter-feeding activity of the bryozoans, reducing the number of organic particles, small organisms and perhaps dissolved organic materials (Wood, 2021). The lophophores of bryozoans are used to carry these organic food materials into the mouth (Riisgård and Manríquez, 1997), with the ingested suspensions used for bryozoan growth and reproduction (Tamberg and Shunatova, 2016). Increasing growth rates of bryozoans toward the end of the experiment corresponded well with the continuous improvement in water quality. This was consistent with a previous study at the LERD Project, in which the filtration rates of suspended particles by bryozoans (*P. casmiana* and *P. vorstmani*) were as high as 74% (Nimtim et al., 2020). When food is digested, bryozoans excrete small oval fecal pellets that themselves are an important food source for aquatic life in natural systems, such as planktonic and benthic organisms (Orellana et al., 2019; Wood, 2019). Therefore, it is suggested that bryozoan communities in wastewater treatment ponds should be maintained for the benefit of water quality improvement. However, there are many factors that should be considered to support the colonization of bryozoans, such as control of water level and environmental conditions. In addition, bryozoan colonies in wastewater treatment ponds should be protected from fish predation, such as by putting fine screening over the colonies.

The food sources of bryozoans are diverse, such as plankton and organic matter suspended in water. Protozoans are also an alternative important diet for bryozoans (Wood, 2015), especially those that are smaller in size, such as *Paramecium*. In the present study, protozoans were offered as diet for the bryozoans, and we confirmed that *P. casmiana* can consume protozoans. This was consistent with a recent experimental feeding study by Wood (2021), revealing that several bryozoan species (*Fredericella indica* Annandale, 1909, *L. carteri* Hyatt, 1866) and *Plumatella emarginata* Allman, 1844) ingested protozoans. After digestion, the bryozoans released fecal pellets that were composed of digested and undigested protozoans. In contrast, bryozoans that were fed with phytoplankton released fecal pellets that had different characteristics in terms of color and internal composition. We found that the fecal pellets of phytoplankton-fed bryozoans contained densely packed, tiny phytoplankton cells. These tiny green particles in fecal pellets could be *Spirulina platensis* (Gomont) Geitler 1925, which is a cyanobacterium found predominantly in wastewater treatment ponds at the LERD Project (Nimtim, 2020). The fecal pellets of phytoplankton-fed bryozoans in the present study were similar to those in the study by Wood (2021), who described green fecal pellets of *Fredericella indica* after feeding on cultures of *Chlamydomonas* sp. Thus, the composition and characteristics of fecal pellets depend on the food types consumed by the bryozoans, and these can be distinguished under a microscope.

The growth rates of the protozoan-fed bryozoans were relatively slow, perhaps because the bryozoans could not break apart larger protozoans in the gut. Furthermore, the presence of some undigested particles in the fecal pellet may be the result of those items spending little time in the stomach. Therefore, the full complement of nutritional substances could not be absorbed and used for growth. The ability of bryozoans to capture food in water is another important factor in their growth. Because protozoans move quickly, bryozoans may have difficulty capturing them. Previous research also showed factors that influence the capture of particles by bryozoans, including characteristics of the food (such as type, size, and concentration) (Pratt, 2008; Todini et al., 2018). Okamura and Doolan (1993) suggested that large colonies of bryozoans ingested greater numbers of particles than small colonies, and that feeding of both large and small colonies depended on flow rate (feeding increased with flow). In contrast, the bryozoans that fed on phytoplankton tended to grow faster because the water from the pond offered a greater variety of food sources, especially phytoplankton (together with zooplankton, protozoans and other microscopic organisms) that are the staple foods of bryozoans (Amui-Vedel et al., 2007). In particular, dominant *S. platensis* can provide high nutritional value and may have produced the fast growth of bryozoans in this experiment, although the food eaten passes through the gut in less than an hour and looks apparently intact in fecal pellets (Cancino et al., 2019). However, the results from the present study differed from Wood (2021), who reported that phylactolaemate bryozoans (different species from this study) are carnivorous and feed on protozoans and other small zooplankton, such as rotifers, from which they get most of their nutrition and results in faster growth than those with any other diet. The results of this research on the diet of bryozoans have demonstrated that bryozoans are omnivorous and can be a link to the transfer of energy in ecosystems. In addition, bryozoans can regulate population dynamics not only of phytoplankton but also of protozoans and possibly other microscopic animals in the wastewater treatment system.

5. CONCLUSION

This study has highlighted the role of the freshwater bryozoan *P. casmiana* in wastewater treatment at the LERD Project. It was clearly demonstrated that bryozoans aid in improving the water quality, especially in terms of BOD_5 and turbidity. This is because suspended organic food particles in water are removed through filtration by bryozoans. Therefore, maintaining communities of bryozoans in wastewater treatment ponds can be beneficial to overall wastewater treatment efficiency. This study also has provided further insights into the biology and ecology of bryozoans in terms of their diet. It was demonstrated that protozoans can be a food source for bryozoans. However, protozoan-fed bryozoans had slower growth rates than those feeding on phytoplankton. This may be due to incomplete digestion of protozoans by bryozoans, as fecal pellets of protozoan-fed bryozoans contained some undigested particles. More research is still needed on the effects of wastewater contaminants (such as heavy metals, pharmaceuticals, and personal care products) on the growth, reproduction and survival of bryozoans in wastewater treatment systems.

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