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Research article

Role of freshwater bryozoans in wastewater treatment ponds at Laem Phak Bia Environmental Research and Development Project site, Phetchaburi province, Thailand

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Abstract

Roles of freshwater bryozoans in wastewater treatment ponds have been recognized. However, there are still limited studies on this issue. Established in 1991, the Laem Phak Bia Environmental Research and Development Project (LERD Project) in western central Thailand has provided natural oxidation of urban wastewater via treatment ponds. These oxidation ponds are suitable habitats for freshwater bryozoans. However, research on bryozoans in these wastewater treatment ponds has received little attention. Thus, this research: 1) studied the biodiversity of the bryozoans in five wastewater treatment ponds, 2) determined the impact of wastewater on the growth rates and survival of the bryozoans and 3) investigated the role of bryozoans as filter feeders. Field surveys and laboratory experiments were conducted in 2018–2019. The results identified three species of freshwater bryozoans (*Plumatella casmiana*, *P. vorstmani*, *P. vaihiriae*) in the ponds. The water quality (biological oxygen demand) in the oxidation pond affected the survival and growth rates of the bryozoans. Ponds 1 and 2 had high organic loadings leading to the death of most bryozoans. In contrast, ponds 3–5 supported colonies of bryozoans. The different water quality conditions reflected the different growth rates of the bryozoans. Furthermore, depth was not the key factor that impacted the growth rates of the bryozoans since there was no significant difference in the growth rates of the bryozoans between the shallow (exposure to light) and deep zones (no light). Lastly, the filtration rates of the bryozoans were notable, with *P. casmiana* and *P. vorstmani* removing up to 74% of the suspended particles.

Introduction

Freshwater bryozoans are sessile filter feeders that are widespread worldwide (Wood et al., 1998; Wood, 2010; Hirose and Mawatari, 2011; Hartikainen et al., 2009). They attach to submerged substrates and grow in both lentic and lotic habitats. Freshwater bryozoans are important and play vital roles in freshwater ecosystems. For example, bryozoans can be food sources of invertebrate and vertebrate animals (Wood, 2005a) and help in the flux of organic materials (Vohmann et al., 2009). In addition, several species of freshwater bryozoans can be used as bioindicators. A study by Hartikainen (2007) showed that growth rates of *Fredericella sultana* increased with increasing nutrient concentration. Being filter feeders, bryozoans can also remove suspended particles from the water (Rutkauskaitė and Šatkauskienė,

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2016). In Thailand, bryozoans are diverse and abundant due to suitable climatic and environmental conditions (such as warm temperatures, plentiful nutrients and diverse habitats). At least 18 freshwater bryozoan species (out of 88 species worldwide, Massard and Geimer [2007]) have been identified in Thailand (Wood et al., 2006; 2010). Most bryozoan studies have focused on the roles and diversity of the bryozoans in natural ecosystems. There is still a knowledge gap regarding bryozoans in wastewater treatment ponds.

Established in 1991, the Laem Phak Bia Environmental Research and Development Project (LERD Project) has the goal of treating wastewater from a municipality in Phetchaburi province using natural processes (Laem Phak Bia Environmental Research and Development Project, 2008). Wastewater is treated through a series of five oxidation ponds with the interaction of sunlight, wind, bacteria and algae (Chunkao et al., 2014). The connected ponds are large and shallow (Hoyt et al., 2012). The water surface area of each pond is in the range 10,134–42,878 m^2 with a storage capacity of approximately 20,000 m^3/d. These large ponds can be important habitats for freshwater bryozoans.

One bryozoan species, *Plumatella vaihiriae* Hastings (1929), has long been known to inhabit wastewater treatment facilities (Wood and Marsh, 1999). However, knowledge of its ecology, diversity and role in wastewater treatment ponds is limited. The LERD Project in Phetchaburi offers a good opportunity to examine freshwater bryozoans in this environment. Therefore, the objectives of this study were: 1) to investigate the biodiversity of freshwater bryozoans at the LERD project site, 2) to study the growth rates of bryozoan species when exposed to different levels of water quality and 3) to determine the functional roles of bryozoans as filter feeders in wastewater treatment ponds.

Materials and Methods

Study site

This study was conducted at the LERD Project site (UTM 1442240–1443480 N and 0619271–0619271 E) in Phetchaburi Province (Fig. 1), central Thailand. The LERD Project was initiated by the late King Rama 9 the Great to provide natural wastewater treatment for a municipality in Phetchaburi Province (Hoyt et al., 2012). The system consists of five connected treatment ponds where water flows by gravity from pond 1 to pond 5 and is then discharged to a mangrove forest along the coastal zone of Phetchaburi. Pond 1 functions primarily for sedimentation, ponds 2–4 are where most of the oxidation occurs and pond 5 is described as a stabilizing zone (Jitthaisong et al., 2012; Chunkao et al., 2014). Ponds 2–5 contain abundant animal life including water monitor lizards (*Varanus salvator* [Laurenti 1768]), several species of fish and abundant waterfowl. Macroinvertebrates are also present including shrimps, snails and aquatic insects. Fish in particular feed heavily on the bryozoans, and for this reason, the current experimental colonies were kept in protective cages.

Diversity of freshwater bryozoans

A qualitative survey of freshwater bryozoans was conducted in 2018–2019 based on Wood et al. (2006). Bryozoans were identified in the five ponds by looking at colonies attached on submerged material and plants, and on the cement outfalls of water gates. After colonies of bryozoans were spotted, samples were removed and placed in 1,000 mL plastic bottles filled with water for transportation to the nearby laboratory. In the laboratory, species were identified mostly on the basis of their statoblasts (Wood et al., 2010) using a compound microscope. Subsequently, colonies obtained from each species were used to culture additional colonies/statoblasts in the laboratory for further experimentation.

Growth rate of bryozoans and water quality

This study had two parts: part one compared the growth rate of bryozoans among the five ponds and part two determined the growth rates of two bryozoan species, *P. casmiana* (Oka, 1907) and *P. vorstmani* (Toriumi, 1952) between two depth zones. These two species were selected because they were the most common in the wastewater treatment ponds of the LERD Project. The bryozoans were cultured from parent colonies collected from ponds following standard methods for freshwater bryozoans (Wood, 2005b). Colonies were cultured by separating small branches from the parent colonies with forceps under a dissecting microscope. Then, three small branches were placed in a plastic Petri dish (diameter, 5 cm) and left for 3 d for the fragments to attach to the plastic Petri dishes. Subsequently, the Petri dishes with colonies were submerged in a 27 cm × 37 cm × 15 cm plastic container and were aerated with a submersible jetting air pump (AP-600, 220–240 V, frequency 50 Hz, 6 W power) for another 2 d to ensure that the colonies were attached firmly to the plates. Bryozoans were fed three times daily with fresh pond water.

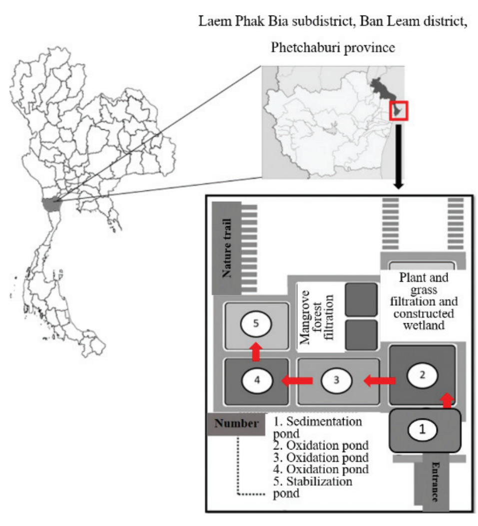

Fig. 1 Location of oxidation ponds in Laem Phak Bia subdistrict, Ban Laem district, Phetchaburi province, Thailand, where red arrows indicate flow direction of water in the ponds

Part 1 - Comparison of growth rates of two species in five ponds. The zooids of each colony on each Petri dish were counted prior to exposure. Three Petri dishes of *P. casmiana* and three Petri dishes of *P. vorstmani* were set out in a protected cage (16 cm ×16 cm × 4 cm) at the outfalls of ponds 1–5 with three replicates of each species (Fig. 2). It was intended that the cages would protect the bryozoan colonies from predation by fish and snails, which were abundant in the ponds. The protective cages were suspended by fishing line at a depth of approximately 30 cm below the water surface. The zooids on each Petri dish were counted daily for their growth rates and then returned to the ponds. The experiment lasted for 5 d.

The water quality in the five ponds was determined by measuring the pH, water temperature (°C), conductivity (μs/cm), and dissolved oxygen (mg/L) using a multi-parameter analyzer (Consort C933), with measurements taken daily at approximately 1000 hours. In addition, 3 L of water was sampled from each pond in plastic bottles for investigation in the laboratory, where the parameters measured were: turbidity (NTU), salinity (psu), soluble reactive phosphorus (μg/L), ammonium nitrogen (μg/L), total nitrogen (TN, mg/L), total phosphorus (TP, mg/L), biological oxygen demand (BOD, mg/L), alkalinity (mg/L), hardness (mg/L), chlorophyll a (μg/L) and total suspended solids (mg/L).

Part 2 - Experiment comparing the growth rates of two species (*P. casmiana* and *P. vorstmani*) at depths of 30 cm and 150 cm. It was hypothesized that the growth rate of the two species would be different in the two different depth zones (shallow zone, with light exposure and a deep zone, with no light exposure). Three Petri dishes with colonies of each species were put in a protective cage. Prior to the experiment, the zooids on each dish were counted before the protected cages were submerged in pond 3 for 5 d. The water quality of pond 3 was suitable to bryozoans compared with the other ponds. The growth rate was investigated daily. Water quality was compared between the two depth zones based on: temperature (°C), pH, DO (mg/L), conductivity (μs/cm), TDS (mg/L), and salinity (psu) using a multi-parameter analyzer (Consort C933). Total chlorophyll (μg/L) and blue green algae (μg/L) were measured using a YSI multi-parameter EXO.

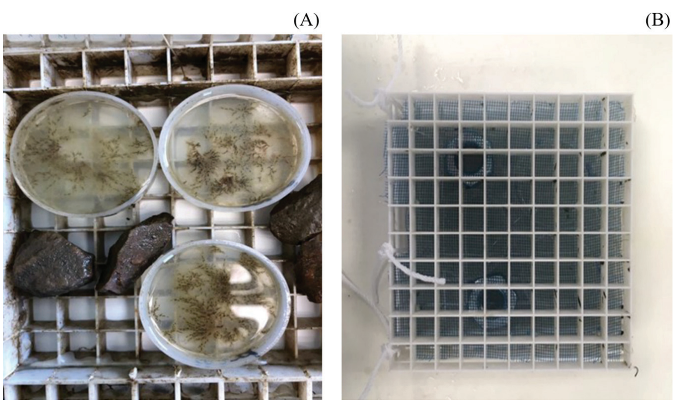

Fig. 2 Germination of freshwater bryozoans: (A) colonies established in Petri dishes; (B) colonies kept in protected cage covered inside with fine net prior to placing in ponds

Relative filtration rates of bryozoan species

This part of the study was conducted in the laboratory and compared the filtration rates between *P. casmiana* and *P. vorstmani*. Freshwater bryozoans are believed to feed on various suspended organic particles in the water (Wood, 2010). This experiment was adapted from a clearance method and measured the reduction in the number of particles as a function of time (Riisgard, 2001). Colonies of the two species were established on separate Petri dishes. The zooids of each species were counted prior to the experiment. A sample of 300 mL of water from pond 3 was collected and poured into a 400 mL beaker. Then, one Petri dish with each species was attached to the side of the beaker with magnets. The water was well mixed to obtain uniform turbidity, as confirmed by a turbidity meter (series TURB 430IR). Turbidity was measured at fixed time intervals of 0 hr, 1 hr, 2 hr, 4 hr, 8 hr, 16 hr and 32 hr in a beaker in which particles decreased during the experiment (Riisgard, 2001). Filtration rates between the two species and the control were calculated and compared. Studies were performed in three replicates with a control.

The filtration rate efficiency (%) was calculated using Equation 1:

$$\text{Filtration rate efficiency (\%)} = (\text{Turbidity}_0 - \text{Turbidity}_5) / 100 \quad (1)$$

where Turbidity_0 is the turbidity on day 0 and Turbidity_5 is the turbidity on day 5.

Data analysis

Descriptive statistics were used to present the data (mean ± SD and range). Statistical analysis involved a Student's t test to compare the growth rate (zooids) of the bryozoans and the water quality between the two depths. One-way analysis of variance was also used to determine whether or not there were any significant differences between the filtration rates of the two bryozoan species and the control using the SPSS 23 (trial version) software package. Significance was tested at $p < 0.05$. Primer seven (PRIMER-e version 7.0.13) was used to analyze for similarity of water quality data among the ponds (multidimensional scaling and cluster analysis). Data were log-transformed and standardized.

Results

Diversity of freshwater bryozoans

A survey of freshwater bryozoans in 2018 revealed that three species of bryozoans occurred in the ponds: *Plumatella casmiana, Plumatella vorstmani* and *Plumatella vaihiriae*., as shown in Fig. 3, whereas in 2019, only two species (*P. casmiana* and *P. vorstmani*) were identified, with *P. casmiana* being the more abundant and *P. vorstmani* less abundant. Colonies of bryozoans had established on pond outfalls. *P. casmiana* was found in ponds 3, 4 and 5, whereas *P. vorstmani* was found only in pond 2 and *P. vaihiriae* only in pond 1. *P. casmiana* produced two floatoblast types: leptoblasts and statoblasts. Fig. 3 shows the statoblasts of the three species.

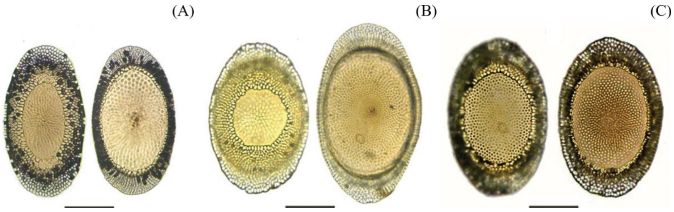

Fig. 3 Statoblast valves of three bryozoan species, with the dorsal valve of each pair always on the left: (A) *Plumtella casmiana*; (B) *P. vorstmani*; (C) *P. vaihiriae*, where scale bar = 100 μm (Photos by T.S. Wood)

Growth rate of bryozoans and water quality

Part I of this study showed that the growth rates of *P. casmiana* and *P. vorstmani* were different among the ponds (Fig. 4). Both species survived and grew well in ponds 3, 4 and 5 throughout the experiment (5 d). In contrast, both *P. casmiana* and *P. vorstmani* died in ponds 1 and 2. The relative growth rates of *P. casmiana* in ponds 3, 4 and 5 were 2.59, 2.90 and 2.49, respectively. The relative growth rates of *P. vorstmani* in ponds 3, 4 and 5 were 2.57, 2.38 and 1.00, respectively.

Water quality data are presented in Table 1. In general, conductivity, hardness, alkalinity and BOD all declined from pond 1 to pond 5, while dissolved oxygen, TSS, turbidity and pH all tended to increase. Nutrient concentrations were exceptionally high in pond 1 and decreased sharply towards pond 5. Levels of chlorophyll a and blue green algae increased. As expected, ponds 1 and 2 seemed to be more polluted (high organic loading) than the remaining ponds.

Cluster analysis and multidimensional scaling showed that each pond had distinct similarities in water quality (Fig. 5). In particular, ponds 3, 4 and 5 were similar but differed from ponds 1 and 2.

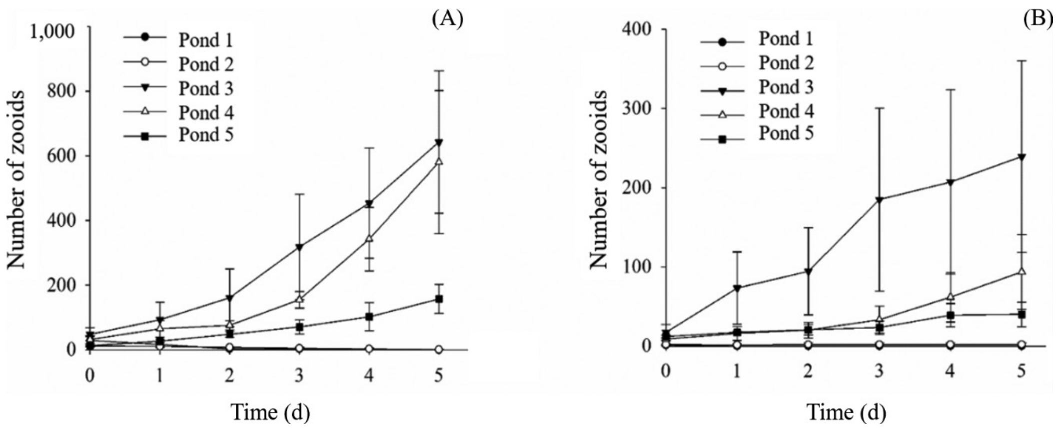

Fig. 4 Daily growth rate of bryozoans in ponds 1–5: (A) *P. casmiana*; (B) *P. vorstmani*, where error bars indicate ± SD

Table 1 Water quality (mean±SD) in ponds 1–5 during study period

Parameter	Pond				
	1	2	3	4	5
Temperature (°C)	32 ± 1	32 ± 1	31 ± 1	31 ± 1	31 ± 1
pH	7.6 ± 0.2	8.2 ± 0.1	8.5 ± 0.3	8.9 ± 0.2	9.1 ± 0.2
DO (mg/L)	4.9 ± 1.9	6.6 ± 0.3	7.6 ± 0.6	7.6 ± 0.4	8.8 ± 1.9
Conductivity (μs/cm)	204 ± 9	183 ± 3	155 ± 4	165 ± 3	181 ± 1
TDS (mg/L)	117 ± 6	105 ± 1	90 ± 3	96 ± 1	106 ± 1
Salinity (psu)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Turbidity (NTU)	31 ± 5	36 ± 5	70 ± 4	29 ± 2	61 ± 5
TSS (mg/L)	9.0 ± 1.2	18.3 ± 3.9	27.0 ± 4.5	31.7 ± 3.0	43.0 ± 1.9
BOD (mg/L)	45 ± 1	45 ± 1	25 ± 6	22 ± 3	17 ± 4
Alkalinity (mg/L)	195 ± 0	160 ± 0	110 ± 0	126 ± 0	128 ± 0
Hardness (mg/L)	128 ± 0	121 ± 0	96 ± 0	104 ± 0	103 ± 0
NH_4 (μg/L)	10,951.28 ± 4.44	4,960.26 ± 7.28	242.03 ± 6.67	16.46 ± 2.98	ND
SRP (μg/L)	1,379.72 ± 30.27	176.50 ± 31.07	874.61 ± 10.29	301.78 ± 4.70	102.06 ± 7.07
TP (mg/L)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.00 ± 0.00
TN (mg/L)	0.88 ± 0.00	0.88 ± 0.00	0.88 ± 0.00	0.88 ± 0.00	0.88 ± 0.00
Chlorophyll a (μg/L)	455.30 ± 1.10	608.70 ± 6.70	746.10 ± 5.10	578.50 ± 7.00	545.70 ± 2.00
Blue green algae (μg/L)	6.88 ± 0.76	21.76 ± 4.71	25.31 ± 1.58	20.98 ± 2.23	25.30 ± 2.31

DO = dissolved oxygen; TDS = total dissolved solid TSS = total suspended solid BOD = biological oxygen demand; SRP = soluble reactive phosphorus; TP = total phosphorus; TN = total nitrogen.

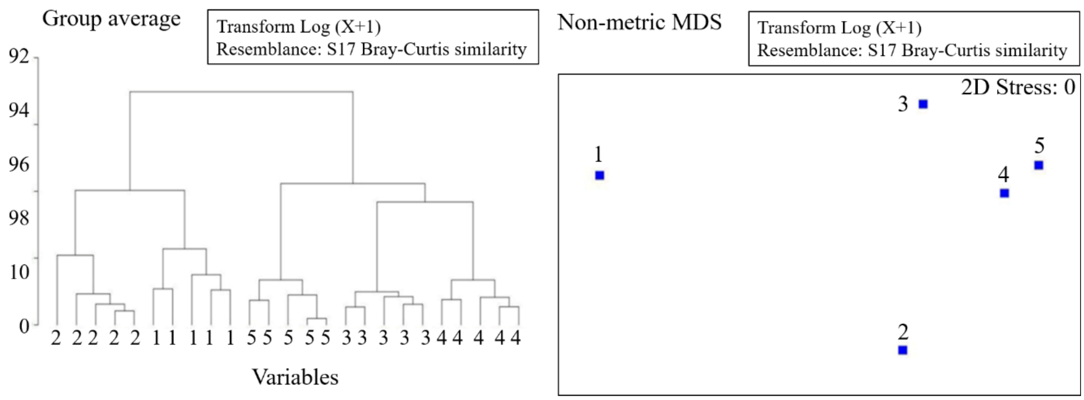

Fig. 5 Grouping of ponds based on similarity: (A) cluster analysis and (B) multidimensional scaling analysis, where MDS = Multidimensional scaling

Part II of this study examined the growth rates between *P. casmiana* and *P. vorstmani* in the two depth zones (Fig. 6). In the shallow zone (exposed to light), at 30 cm depth, the growth rates of *P. casmiana* and *P. vorstmani* were 91.85% and 92.80%, respectively. In the deep zone (no light exposure) at 150 cm depth, the growth rates of *P. casmiana* and *P. vorstmani* were 75.09% and 85.13%, respectively. In fact, there was no significant difference in the growth rates of *P. casmiana* and *P. vorstmani* between the two depths or between the two species. Similarly, statistical analysis of water quality values showed no significant difference between the two depth zones (Table 2).

Species filtration rate comparison

A comparison of the filter efficiencies showed that except for the control, *P. casmiana* and *P. vorstmani* removed suspended particles continuously from the beginning until the end of the experiment (Fig. 7). The filtration rates of *P. casmiana* and *P. vorstmani* were 73.90% and 53.14%, respectively. Statistical analysis showed that the filtration rates of *P. casmiana* were highly significantly ($p < 0.01$) different compared with the control (Fig. 6) but not for *P. vorstmani* and the control. Furthermore, there was no significant difference in the filtration rates between *P. casmiana* and *P. vorstmani*.

Discussion

The diversity of freshwater bryozoans at the LERD Project site was investigated for the first time since the establishment of the Project in 1991. *Plumatella casmiana, P. vorstmani* and *P. vaihiriae* have not previously been reported from Phetchaburi province, although they are widely distributed in other areas of Thailand (Wood et al., 2010). The only other species known in the area (in the Kaeng Krachan reservoir) has been *Asajirella gelatinosa* Oka (1891), where large lakes and reservoirs seem to be its preferred habitat (Wood et al., 2010).

Table 2 Comparison of water quality between shallow and deep zones (*n*=3)

Parameters	Zone	
	Shallow (30 cm)	Deep (150 cm)
Temperature (°C)	30 ± 1[a]	30 ± 1[a]
pH	8.9 ± 0.2[a]	8.9 ± 0.2[a]
Dissolved oxygen (mg/L)	12.3 ± 2.8[a]	10.3 ± 1.7[a]
Conductivity (μs/cm)	178 ± 2[a]	178 ± 2[a]
TDS (mg/L)	105 ± 1[a]	106 ± 0[a]
Salinity (psu)	0.1 ± 0.0[a]	0.08 ± 0.0[a]
Blue green algae (μg/L)	20.02 ± 2.27[a]	18.99 ± 1.17[a]

TDS = total dissolved solid values (mean ± SD) with the same lowercase superscript in a row indicate no significant ($p < 0.05$) difference between the two zones.

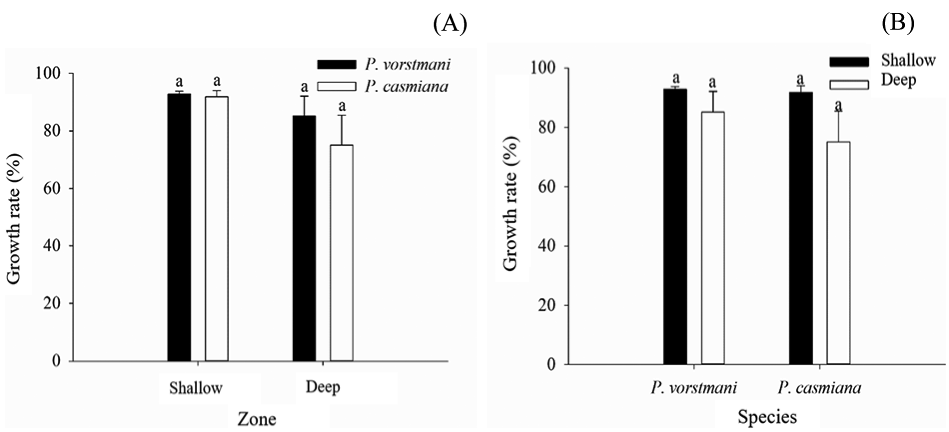

Fig. 6 Growth rates of *P. casmiana* and *P. vorstmani*: (A) between zones; (B) between species, where the same lowercase letter above a column indicates no significant ($p < 0.05$) difference and the error bars indicate ± SD

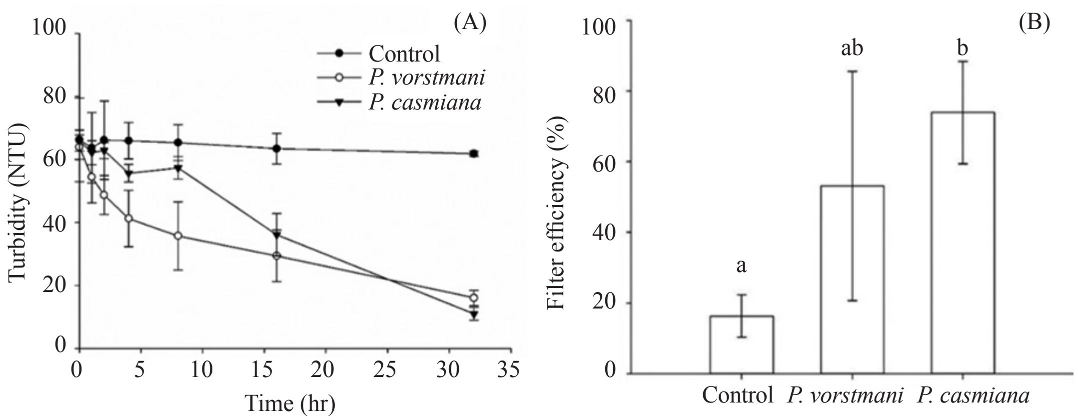

Fig. 7 Comparison between *P. casmiana* and *P. vorstmani*: (A) turbidity, showing a decrease over time; (B) filter efficiency compared to control (no bryozoan), where the same lowercase letter above a column indicates no significant ($p < 0.05$) difference and the error bars indicate ± SD.

At the LERD Project site, *P. casmiana* was the most abundant species, possibly because it produces two types of floatoblasts. Leptoblasts germinate and form a new colony immediately, allowing for rapid population growth (Wood et al., 2010), whereas capsuled statoblasts can remain dormant for weeks or months. *P. vorstmani* and *P. vaihiriae* were less abundant. It has been reported that *P. vaihiriae* usually thrives in highly eutrophic conditions (Wood et al., 2010), such as those at the LERD Project site. A study in the USA also reported large growths of the phylactolaemate bryozoan *Plumatella vaihiriae* in a domestic wastewater treatment facility (Wood and Marsh, 1999). The occurrence and abundance of each species may depend on multiple factors, such as biological characteristics (different food intake rates and growth rates), water quality thresholds, predation by animals and the regulation of the water level, which still need further investigation.

Freshwater bryozoans established their colonies well on outfalls where the water flowed by gravity from one pond to another (Fig. 8). It appeared that the overflowing water brought food and oxygen to the colonies. By growing at the outfalls, bryozoans avoided being grazed upon by fish and possibly other predators in the wastewater treatment ponds, thus resulting in higher growth rates at these locations than at other locations. On the other hand, bryozoans growing on outfalls can face other risks. First, if the water level is lowered, bryozoans could become exposed to drying conditions and die. Second, cleaning crews regularly removed algae and bryozoans from the oxidation pond outfalls, as observed during late November 2018. Although this cleaning procedure inevitably impacted the bryozoan colonies, the populations eventually recovered.

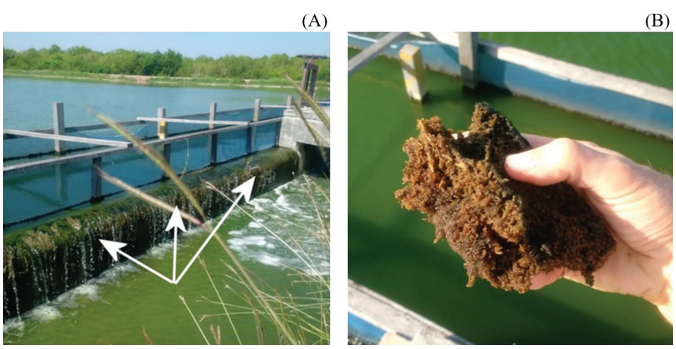

Fig. 8 Freshwater bryozoans: (A) location at outfall of pond 5, indicated by white arrows; (B) colony of bryozoans (photos by T.S. Wood)

The growth rates of freshwater bryozoans have been clearly related to the quality of water; therefore, bryozoans can be used as good bioindicators of environmental change (Amui-Vedel et al., 2007; Rutkauskaitė and Šatkauskienė, 2016). The water quality of ponds 1 and 2 was very poor, as indicated by the high BOD and other contaminants, which could explain the death of the experimental colonies in ponds 1 and 2. In contrast, *P. casmiana* and *P. vorstmani* had their highest growth rates in ponds 3, 4 and 5, where the wastewater was mostly treated by natural means (Dampin et al., 2012). Chlorophyll a was highest in pond 3, which may have been linked to the higher growth rates of *P. casmiana* and *P. vorstmani*. *Spirulina platensis* (high nutrition) was a likely food source (Orellana et al., 2019), as most ponds are dominated by such species (Chaichana and Dampin, 2016). This was consistent with the results of Hartikainen (2007), who reported that the abundance of bryozoans increased with increased planktonic productivity. Improvement in the water quality in consecutive ponds may also have supported the improved growth of bryozoans. In addition, a study in the USA showed that *Plumatella* exhibited rapid, aggressive growth and produced large quantities of statoblasts in domestic wastewater treatment plants (Wood and Marsh, 1999). The results from the cluster analysis and multidimensional scaling in the current study identified two distinct groups of ponds: 1) ponds 1 and 2 with poorer water quality and 2) ponds 3–5 with better water quality. In addition, the growth rate of *P. casmiana* was higher than for *P. vorstmani*, It seemed that *P. casmiana* ingested food particles more rapidly, although this observation has yet to be quantified.

No significant differences in water quality were found between the shallow and deep zones of the oxidation ponds, and this was reflected in the similar growth rates for the bryozoans at these two depths. Bryozoans can grow well in a completely dark environment, such as in pipeline as their survival depends on food availability and suitable environmental conditions other than the specific presence or absence of light (Wood, 2010. In the current case study, the wastewater treatment ponds were shallow (depth < 2 m), and phytoplankton in pond 3 were abundant. Therefore, food (such as phytoplankton) was available and plentiful throughout the water column. The dissolved oxygen content was apparently also sufficient to support bryozoans at the two depth zones. This result was in agreement with Wood (2005a; 2010), who stated that bryozoans could be found at almost any depth and could thrive in dark places wherever the supply of particulate food is continuous.

The current study revealed the roles of freshwater bryozoans in wastewater treatment for the first time. Bryozoans are biofilter feeders (Rutkauskaitė and Šatkauskienė, 2016) that feed on plankton, bacteria and tiny suspended particles in water (Todini et al., 2018). The current research demonstrated that both *P. casmiana* and *P. vorstmani* were efficient at filtering water from wastewater treatment ponds. In particular, *P. casmiana* tended to filter suspended particles faster than *P. vorstmani* at the beginning of the experiment. In general, *P. casmiana* tended to grow faster than *P. vorstmani* and thus had higher growth and filtration rates. Several studies have reported that the capture of food by bryozoans depends on food characteristics such as the type and size and on colony features, such as the size and number of zooids (Lisbjerg and Petersen, 2001; Okamura and Doolan, 1993; Pratt, 2008). Furthermore, toward the end of the experiment, the filtration rate of both species started to decline at a steady rate. This was possibly because there were fewer suspended particles to be filtered toward the end of the experiment. The role of bryozoans as filter feeders can partly improve water quality by reducing suspended particles and cells in the water. After ingestion of algal species as a food item, excretion of bryozoans via fecal pellets can also be an important food source for other animals (Orellana et al., 2019).

The current study provided a better understanding of the ecology and biology of freshwater bryozoans in wastewater treatment ponds. However, there are still some research aspects that can be addressed. For example, while it is known that freshwater bryozoans (phylactolaemates) feed mostly on algae, bacteria, and detritus, a recent study (Wood, 2019) provided strong evidence that there are some species that feed on not only algae but also on protozoans. Therefore, the role of ciliates as bryozoan nutrition could be a research topic that could be further investigated at the LERD Project site. Another research aspect could be the effect of seasonal variation (wet and dry periods) on freshwater bryozoans, since the water quality may differ between seasons and thus influence the growth and survival of bryozoans.

In conclusion, this study identified three species of freshwater bryozoans (*Plumatella casmiana, Plumatella vorstmani* and *Plumatella vaihiriae*) in wastewater treatment ponds, with *P. casmiana* being the most abundant species. The water quality in ponds 1 and 2 adversely affected the growth rates of all species, whereas these bryozoan species grew better in ponds 3-5. The growth rates of the bryozoans were not influenced by the water depth. Finally, freshwater bryozoans played a role in the wastewater treatment system through their filtration of particles suspended in the water, though the growth rates and filter rates varied among species. Additional investigations are now needed to understand other abiotic and biotic factors affecting the composition and abundance of freshwater bryozoans at the LERD Project site.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Amui-Vedel, A.M., Hayward, P.J., Porter, J.S. 2007. Zooid size and growth rate of the bryozoan *Cryptosula pallasiana* Moll in relation to temperature, in culture and in its natural environment. J. Exp. Mar. Biol. Ecol. 353: 1–12. doi.org/10.1016/j.jembe. 2007.02.020

Chaichana, R., Dampin, N. 2016. Unialgal blooms of cyanobacteria in oxidation ponds of the King's Royally Initiated Laem Phak Bia Environmental Research and Development project, Thailand. EnvironmentAsia. 9: 150–157. doi: 10.14456/ea.2016.19

Chunkao, K., Tarnchalanukit, W., Prabuddham, P., Phewnil, O., Bualert, S., Duangmal, K., Pattamapitoon, T., Nimpee, C. 2014. H.M. The King's Royally Initiated LERD Project on community wastewater treatment through small wetlands and oxidation pond in Phetchaburi, Thailand. Mod. Appl. Sci. 8: 233–246. doi: 10.5539/mas.v8n5

Dampin, N., Tarnchalanukit, W., Chunkao, K., Maleewong, M. 2012. Fish growth model for Nile tilapia (*Oreochromis niloticus*) in wastewater oxidation pond, Thailand. Procedia Environ. Sci. 13: 513–524. doi.org/10.1016/j.proenv.2012.01.042

Hartikainen, H.L. 2007. Freshwater bryozoan abundance, eutrophication and salmonid fish disease. Ph.D. dissertation, University of Reading. Reading, UK.

Hartikainen, H., Johnes, P., Moncrieff, C., Okamura, B. 2009. Bryozoan populations reflect
nutrient enrichment and productivity gradients in rivers. Freshw. Biol. 54: 2320–2334.
https://doi.org/10.1111/j.1365-2427.2009.02262.x

Hastings, A.B. 1929. Notes on some little-known *Phylactolaematous polyzoa* and description of a new species from Tahiti. Ann. Mag. Nat. Hist. 10: 300–311. doi.org/10.1080/ 00222932908672974

Hirose, M., Mawatari, S.F. 2011. Freshwater Bryozoa of Okinawa, Japan, with descriptions of
Rumarcanella gen. nov. (Phylactolaemata: Plumatellidae) and two new species. Zootaxa 2732: 1–19.

Hoyt, A.E., Loy, J.W., Ly, M., Zamora-Palacios, S.A. 2012. Assessment of Laem Phak Bia Environmental Research and Development Project's Outreach Program. Worcester Polytechnic Institute. Worcester, UK.

Jitthaisong, O., Dhanmanonda, P., Chunkao, K., Teejuntuk, S. 2012. Water quality from mangrove forest: The King's royally initiated Laem Phak Bia Environmental Research and Development Project, Phetchaburi province, Thailand. Mod. Appl. Sci. 6: 1-8. doi: 10.5539/mas.v6n8p1

Laurenti, J.N. 1768. Specimen Medicum, Exhibens Synopsin Reptilium Emendatam cum Experimentis circa Venena et Antidota Reptilium Austriacorum. Trattner. Vienna, Austria. [in Latin]

Laem Phak Bia Environmental Research and Development Project. 2008. Laem Phak Bia: The King's royally initiated Laem Phak Bia Environmental Research and Development Project. Petchpoom Printing. Bangkok, Thailand.

Lisbjerg, D., Petersen, J.K. 2001. Feeding activity, retention efficiency, and effects of temperature and particle concentration on clearance rate in the marine bryozoan *Electra crustulenta*. Mar. Ecol. Prog. Ser. 215: 133–141. doi: 10.3354/meps215133

Massard, J.A., Geimer, G. 2007. Global diversity of bryozoans (Bryozoa or Ectoprocta) in freshwater. Hydrobiologia 595: 93–99.

Oka, A. 1891. Observations on a fresh-water polyzoa (*Pectinatella gelatinosa* nov. sp.). Journ. Coll. Sci. Imp. Univ. Tokyo. 4: 89–150.

Okamura, B., Doolan, L.A. 1993. Patterns of suspension feeding in the freshwater bryozoan *Plumatella repens*. Biol. Bull. 184: 52–56. doi: 10.2307/1542379

Orellana, M.C., Cancino, J.M., Wood, T.S., Chaichana, R. 2019. Feeding and fecal pellet production in *Plumatella casmiana* Oka, at Laem Phak Bia, Thailand. 18th International Bryozoology Association Conference. Technical University of Liberec. Liberec, Czech Republic, p. 55.

Pratt, M.C. 2008. Living where the flow is right: How flow affects feeding in bryozoans. Integr. Comp. Biol. 48: 808–822. doi.org/10.1093/icb/icn052

Riisgard, H.U. 2001. On measurement of filtration rates in bivalves - the stony road to reliable data: review and interpretation. Mar. Ecol. Prog. Ser. 211: 275–291. doi: 10.3354/ meps211275

Rutkauskaitė, J., Šatkauskienė, I. 2016. Species composition of freshwater bryozoan (Bryozoa) in some regions of Lithuania. In: Proceedings of the 10th International Scientific Conference. Vytautas Magnus University. Kaunus, Lithuania, pp. 64.

Todini, C., Elia, A.C., Selvaggi, R., Scoparo, M., Taticchi, M.I. 2018. Food selection by *Plumatella geimermassardi* Wood and Okamura, 2004 (Phylactolaemata, Bryozoa). Knowl. Manag. Aquat. Ecosyst. 419: 26. doi.org/10.1051/kmae/2018008

Toriumi, M. 1952. Taxonomical study on fresh-water Bryozoa 4. On *Plumatella javanica* Kraepelin reported by Vorstman in 1928. Sci. Rep. Tôhoku Imp. Univ. Ser. 4. 19: 264–269.

Vohmann, A., Mutz, M., Arndt, H., Weitere, M. 2009. Grazing impact and phenology of the freshwater sponge *Ephydatia muelleri* and the bryozoans *Plumatella emarginata* and *Fredericella sultana* under experimental warming. Freshw. Biol. 54: 1078–1092. doi.org/10.1111/j.1365-2427.2008.02155.x

Wood, T.S. 2005a. The pipeline menace of freshwater bryozoans. Denesia 16: 203–208

Wood, T.S. 2005b. Study methods for freshwater bryozoans. Denesia 16: 103–110.

Wood, T.S. 2010. Chapter 13 - Bryozoans. In: Thorp, J.H., Covich, A.P. (Eds.). Ecology and Classification of North American Freshwater Invertebrates, 3th ed. Academic Press. San Diego, CA, USA, pp. 437–454.

Wood, T.S. 2019. Phylactolaemate bryozoans feed the zoobenthos. Australas. Palaeontol. Mem. 52: 163–167.

Wood, T.S., Anurakpongsatorn, P., Mahujchariyawong, J. 2006. Freshwater bryozoans of Thailand (Ectoprocta and Entoprocta). Nat. Hist. J. Chulalongkorn Univ. 6: 83–119.

Wood, T.S., Anurakpongsatorn, P., Mahujchariyawong, J. 2010. An Introduction to the Freshwater Bryozoans of Thailand. Kasetsart University Press. Bangkok, Thailand.

Wood, T.S., Marsh, 1999. Biofouling of wastewater treatment plants by the freshwater bryozoan, *Plumatella vaihiriae* (Hastings, 1929). Water Res. 33: 609–614. doi.org/ 10.1016/S0043-1354(98)00274-7

Wood, T.S., Wood, L.J., Geimer, G., Massard, J. 1998. Freshwater bryozoans of New Zealand: A preliminary survey. N. Z. J. Mar. Freshwater Res. 32: 639-648. https://doi.org/10.1080/00288330.1998.9516850