ORIGINAL ARTICLE

Influence of water quality and seasonal variations on freshwater macroinvertebrate diversity and community structure in wastewater treatment ponds, Phetchaburi Province, Thailand

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ABSTRACT

Wastewater originating from the Phetchaburi municipality undergoes treatment in a series of five distinct stages at the King's Royally Initiated Leam Phak Bia Environmental Research and Development Project (LERD) in Phetchaburi province, Thailand. These stages involve a sedimentation (pond 1), three oxidation ponds (ponds 2 to 4), and a final stabilization pond (pond 5). These ponds serve as habitats for macroinvertebrates; consequently, their diversity and composition might be influenced by fluctuations in water quality and seasonal variations. The primary aim of this research was to analyze the diversity and species composition of macroinvertebrate communities concerning varying levels of organic contamination across the five wastewater treatment ponds at LERD. This investigation spanned three seasons: cold season (December 2019), rainy season (July 2020), and hot season (April 2021). The findings revealed that the diversity and species composition of macroinvertebrate communities displayed distinct alterations across multiple environmental gradients, especially identifying the significant influence of organic loading levels observed in ponds 1 to 5. The macroinvertebrate communities exhibited two distinct groupings, with the Chironomidae and Candonidae or ostracods prevailing prominently in ponds 1 and 2 (heterogenous environments). This prevalence was attributed to the high levels of detrital food and the robust resilience of chironomid larvae and ostracods to organic pollution, thriving even in environments characterized by low dissolved oxygen levels. Conversely, the prevalence of snails from the Thiaridae family in ponds 3 to 5 (homogenous environments) indicated improved water quality conditions, notably lower organic matter levels, and a higher dissolved oxygen content. In addition, the study identified seasonal variations in macroinvertebrates, likely influenced by the differing organic loading and environmental conditions. Thus, this research provided insights into the factors shaping macroinvertebrate communities in a wastewater treatment system.

INTRODUCTION

Macroinvertebrates are prevalent organisms found within municipal wastewater treatment ponds, encompassing diverse groups, such as insect larvae, mollusks, and worms. These organisms are associated with varied habitats within the ponds, dwelling within the water column, pond substrates, and surfaces, and often associating with macrophytes along the edges of the wastewater treatment ponds. Several studies have documented a range of macroinvertebrate groups present in wastewater treatment ponds, including the Diptera, Coleoptera, Simuliidae, Oligochaeta, Hemiptera, Isopoda, Gyrinidae, Dytiscidae, Ephemeroptera, Plecoptera, Trichoptera and bryozoans (Loch *et al.,* 1996; Maranga *et al.,* 2016; Raburu *et al.,* 2017; Nimtim *et al.*, 2020). These findings have contributed to understanding the diverse and varied nature of the macroinvertebrates thriving within these unique environments, highlighting the complexity of the presence and roles of these organisms within wastewater treatment ecosystems.

Macroinvertebrates play a pivotal role within wastewater treatment ponds by actively contributing to the breakdown of organic materials into fine particles and by consuming organic matter through their feeding habits and biological activities (Nimtim *et al.,* 2020; Thongdang *et al.,* 2022). This vital role aids in the comprehensive treatment of wastewater, enhancing its overall quality. For example, Hirabayashi and Wotton (1998) documented the influence of chironomid larvae on organic matter recycling. Their findings revealed that a signifi-

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cant portion of the utilized organic matter was assimilated into tubes and fecal pellets, while the remainder resided within the larval gut, ultimately leading to its mineralization. This was in agreement with Kuntz and Tyler (2018), who highlighted the important role of tolerant benthic invertebrates (*Chironomus* and oligochaetes) that could consume algae and leaf litter and enhance organic matter mineralization in stormwater retention ponds.

Furthermore, macroinvertebrates serve as valuable indicators of water quality (Nzengy'a and Wishitemi, 2000), providing insights into the efficiency of the wastewater treatment process. The presence of specific species can serve as indicative environmental markers. For example, the prevalence of pollution-tolerant species, such as chironomid larvae, often signals high organic pollutant levels and a low dissolved oxygen content in the water. Conversely, the presence of less tolerant groups, such as the Ephemeroptera, Plecoptera, and Trichoptera (EPT), suggests improved environmental conditions within wastewater treatment systems (Raburu *et al.,* 2017). Okuku *et al.,* (2006) also developed a tolerance scale using macroinvertebrates in oxidation ponds, from the most pollution-tolerant species to the least tolerant species as: *Chironomus* sp., *Belistoma* sp., *Notonecta* sp., and *Corixa* sp., respectively. This differential presence of macroinvertebrates offers a practical tool for assessing and monitoring the effectiveness of wastewater treatment processes and the overall health of the aquatic ecosystem.

The King's Royally Initiated Leam Phak Bia Environmental Research and Development Project (LERD) was established in 1998 to treat wastewater from the Phetchaburi municipality in Phetchaburi Province, Thailand, utilizing five ponds (LERD, 2022). These ponds represent diverse habitats for a wide array of macroinvertebrates. Therefore, investigating the macroinvertebrate community across these ponds is of particular interest, as the environmental characteristics within wastewater treatment ponds can substantially influence the diversity and structure of these communities. Thus, the primary objective of this research was to explore the impact of the specific water quality in each treatment pond on macroinvertebrate diversity and assemblages. Additionally, it aimed to discern the variations in invertebrate communities across different seasons. The findings of this investigation are important as they should contribute to a better understanding of the environmental factors that shape macroinvertebrate communities within wastewater treatment ponds. The insights gained should also provide valuable information concerning the ecosystem's condition and the efficacy of the wastewater treatment process, contributing to a deeper understanding of the complex interplay between environmental factors and macroinvertebrate populations in these settings. Lastly, this study could provide a useful baseline for further studies into ecosystem of wastewater treatment ponds.

METHODS

Study site

The LERD Project comprises a series of five interconnected ponds (Fig. 1), collectively capable of treating up to 10,000 cubic meters per day. These ponds receive primarily wastewater originating from food processing units along with household usage (sugars, carbohydrates, and lipid production) and other sources within the Phetchaburi municipality. The wastewater is conveyed into the treatment system through a high-density polyethylene (HDPE) pipe with a diameter of 40 cm (LERD, 2022).

The ponds have varying water surface areas, spanning from 10,200 to 42,900 square meters, with an average depth ranging approximately between 1.42 and 2.43 meters. Each pond retains wastewater for a hydraulic retention period ranging from 5 to 17 days, totaling an overall retention time of 65 days (from pond 1 to pond 5). The treatment process commences in a sedimentation pond (pond 1), followed by sequential processing through oxidation ponds 2–4. Subsequently, the treated wastewater progresses to a stabilization pond (pond 5) for further refinement. Gravity serves as the mechanism for the flow of wastewater through these treatment stages. Upon completion of the treatment process, the treated effluent, which meets the standard of biochemical oxygen demand (BOD_5) levels of less than 20 mg L^{-1}, is discharged into the surrounding mangrove forest and the Gulf of Thailand (LERD, 2022).

Water sampling and analysis

This study assessed the water quality across the five ponds during three seasons: the cold season in December 2019, the rainy season in July 2020, and the hot season in April 2021. Phetchaburi Province is situated in the upper southern region near the sea. The weather patterns in each season (cold season from mid-October to mid-February, hot season from mid-February to mid-May, and rainy season from mid-May to mid-October) are generally similar, except during the rainy season, which experiences higher rainfall compared to the other dry seasons. Sampling was carried out across various years owing to COVID-related lockdowns. It is important to note that the weather conditions in each season may vary from year to year, which could potentially influence the impact of seasons on water quality and invertebrate communities. In fact, rainfall data from the Data Innovation and Governance Institute in Thailand, along with temperature data from Weather Spark, showed a similar pattern during the years 2019 to 2021 in Phetchaburi Province. Sampling locations were positioned along the edge of each pond, specifically focusing on areas adjacent to the outflow weir, chosen for their accessibility during the sampling process. Various water quality parameters were measured at three different stations: temperature (°C), dissolved oxygen (DO, mg L^{-1}), pH, salinity (psu), total dissolved solids (mg L^{-1}), and conductivity (μs cm^{-1}), measured using a Multimeter analyzer (WTW Profi-Line Cond 3310). Additionally, water samples were collected for further laboratory analysis.

The laboratory analysis focused on several variables: BOD_5 (mg L^{-1}) using the azide modification method; ammonium nitrogen (mg L^{-1}) using the phenol-hypochlorite method; soluble reactive phosphorus (mg L^{-1}) analyzed using the ascorbic acid method; total nitrogen (TN, mg L^{-1}) assessed using alkaline persulfate oxidation; total phosphorus (TP, mg L^{-1}) determined using the ascorbic acid method; and chlorophyll a (μg L^{-1}) measured using acetone extraction. The water samples were subsequently analyzed in the laboratory using Agilent Cary 60 UV-VIS Spectrophotometer. Each chemical parameter was analyzed using three separate samples, and then the average measurement was calculated.

Macroinvertebrate sampling and identification

Macroinvertebrate samples were collected across three seasons from the five ponds, with collections from three sampling points at each pond. Two methods were used for sample collection, with snail samples being manually collected for 30 sec on areas adjacent to the outflow weir, while other macroinvertebrates were captured using a hand net with a mesh size of 450 µm for the same one-minute duration. Following collection, invertebrate samples were preserved in 95% ethanol. The identification of macroinvertebrates was conducted at the family level, followed by counting. Additionally, measurements of length and weight were taken specifically for the snail specimens.

Data analysis

The data were presented as mean±SD values. We calculated the species richness (Margaleff, 1958) index, similarity index (Sorensen, 1948), evenness index (Pielou, 1984), and Shannon-Weaver biodiversity index (Shannon and Weaver, 1949). We performed an ANOVA using SPSS, v. 29.0.0.0 (241) as an authorized user to determine if there were any statistically significant differences in the taxonomic composition among ponds during each season. Additionally, we conducted further analyses on water quality and invertebrate data using multivariate methods including multidimensional scaling and Principal Component Analysis. The data were log-transformed and analyzed using PRIMER Version 7 with PERMANOVA+ (academic license, sn: Q781).

RESULTS

Water quality

There were variations in water quality across ponds and seasons, as indicated in Tab. 1. It became evident that ponds 1 and 2 had relatively poorer water quality compared to ponds 3, 4, and 5. Specifically, the BOD_5 levels were higher in ponds 1 and 2, gradually declining in ponds 3, 4, and 5, respectively. The trend for dissolved oxygen showed an incremental increase from pond

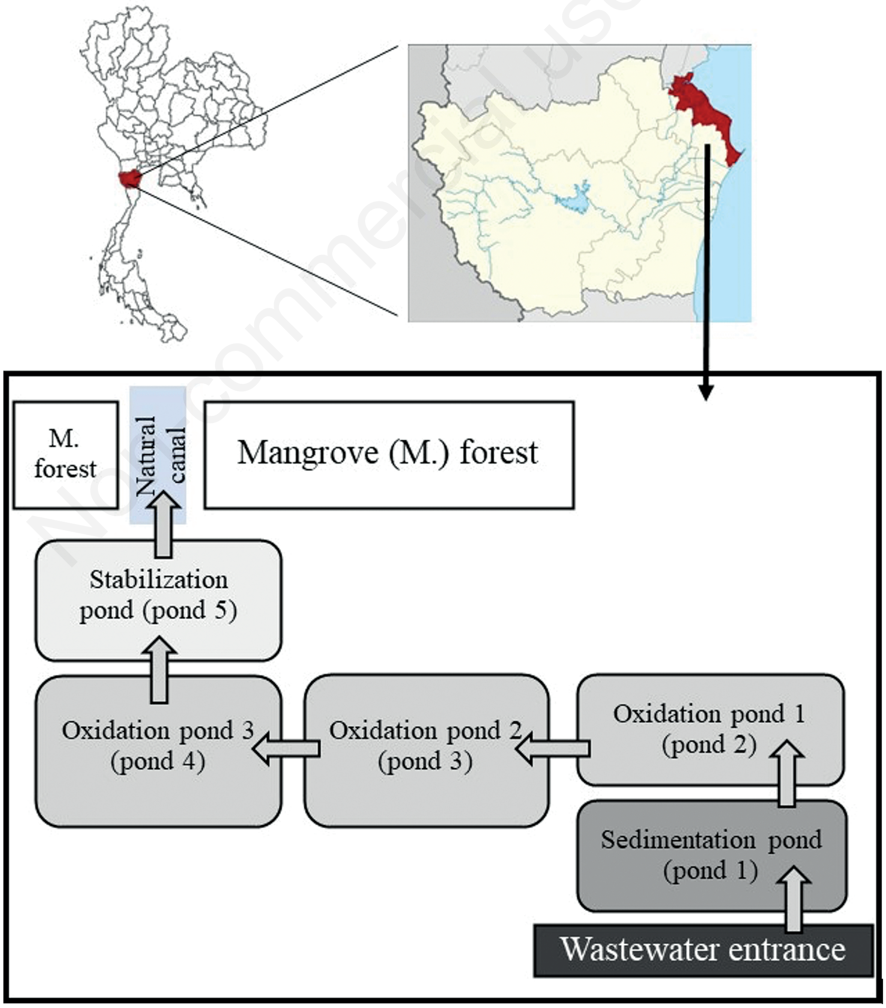


Fig. 1. Map of ponds at the King's Royally Initiated Leam Phak Bia Environmental Research and Development Project (13.047136373230497N, 100.08436544747366E).

1 to pond 5. Similarly, there was a decreasing trend in nutrient concentrations (nitrogen and phosphorus) from pond 1 to pond 5. Although the chlorophyll a concentrations varied among ponds, there was a tendency for it to decrease from pond 1 to pond 5.The evaluation of water quality across seasons indicated that there was comparatively lower water quality in the cold season than for the other seasons. For example, the BOD$_5$ levels in pond 1 during the cold season (26.00±1.93 mg L^{-1}) surpassed those observed in both the rainy season (11.20±2.11 mg L^{-1}) and the hot season (6.20±3.02 mg L^{-1}). Similarly, nutrient concentrations (nitrogen and phosphorus) peaked during the cold season and were lower during the rainy and hot seasons. These trends were consistent

Tab. 1. Water quality analysis conducted across ponds (P) 1 to 5 during various seasons (n=3).

Parameters			**Cold season**		
	P1	**P2**	**P3**	**P4**	**P5**
Temperature (°C)	30±1	30±1	29±1	30±1	30±1
pH	7.4±0.1	8.2±0.6	8.3±0.7	8.8±0.3	8.8±0.2
DO (mg L^{-1})	3.3±2.3	5.7±2.7	4.3±4.0	6.0±2.3	5.0±2.8
Conductivity (µs cm^{-1})	991±49	957±26	1,092±36	1,183±81	1,263±115
TDS (mg L^{-1})	597±29	577±6	657±22	719±48	788±71
Salinity (psu)	0.5±0.0	0.4±0.0	0.5±0.0	0.5±0.0	0.6±0.1
BOD5 (mg L^{-1})	26.00±1.93	29.23±5.45	17.50±0.46	12.20±1.42	13.40±0.46
NH4+ (mg L^{-1})	17.41±0.77	2.75±0.04	0.14±0.05	0.60±0.02	0.58±0.03
SRP (mg L^{-1})	2.55±0.05	1.47±0.11	0.88±0.04	0.42±0.03	0.11±0.00
TN (mg L^{-1})	19.30±0.00	10.50±0.00	5.25±0.00	3.50±0.00	1.75±0.00
TP (mg L^{-1})	2.15±0.00	1.34±0.00	0.35±0.00	ND	ND
Chl a (µg L^{-1})	223.92±61.32	224.69±50.22	236.79±8.45	89.14±10.67	129.80±28.82
Parameters			**Hot season**		
	P1	**P2**	**P3**	**P4**	**P5**
Temperature (°C)	30±1	31±1	30±1	30±1	30±1
pH	10.3±0.9	10.7±0.9	10.3±0.9	10.0±1.0	10.2±1.1
DO (mg L^{-1})	3.8±3.0	3.6±1.6	3.3±1.7	2.8±1.5	2.9±1.4
Conductivity (µs cm^{-1})	556±10	558±6	553±7	644±8	840±9
TDS (mg L^{-1})	328±6	326±3	327±2	381±1	499±3
Salinity (psu)	0.3±0.1	0.3±0.0	0.2±0.0	0.3±0.0	0.4±0.1
BOD5 (mg L^{-1})	6.20±3.02	15.60±0.3	14.40±1.97	8.40±0.79	8.20±0.87
NH4+ (mg L^{-1})	2.97±0.08	0.17±0.01	0.00±0.01	ND	0.01±0.01
SRP (mg L^{-1})	1.07±0.01	0.82±0.01	0.25±0.00	0.47±0.01	0.05±0.01
TN (mg L^{-1})	4.09±0.00	2.46±0.00	1.24±0.00	1.18±0.00	1.29±0.00
TP (mg L^{-1})	2.55±0.00	2.41±0.00	2.51±0.00	2.04±0.00	2.00±0.00
Chl a (µg L^{-1})	143.66±7.66	292.71±3.54	277.79±6.56	220.22±3.54	140.29±6.80
Parameters			**Rainy season**		
	P1	**P2**	**P3**	**P4**	**P5**
Temperature (°C)	32±1	32±1	31±1	31±1	31±1
pH	7.9±0.7	8.9±0.6	8.4±0.5	8.5±0.2	8.1±1.2
DO (mg L^{-1})	5.9±5.7	10.0±5.5	5.3±2.0	8.0±2.1	7.1±2.4
Conductivity (µs cm^{-1})	557±23	515±28	497±17	460±16	473±15
TDS (mg L^{-1})	319±16	299±15	289±11	270±10	277±11
Salinity (psu)	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
BOD5 (mg L^{-1})	11.20±2.11	5.80±2.27	11.40±0.60	12.20±0.35	11.30±2.21
NH4+ (mg L^{-1})	4.81±0.08	1.60±0.14	0.51±0.03	0.19±0.01	0.12±0.02
SRP (mg L^{-1})	0.99±0.02	1.10±0.04	0.64±0.03	0.41±0.08	0.26±0.03
TN (mg L^{-1})	2.92±0.00	2.92±0.00	2.92±0.00	5.83±0.00	5.83±0.00
TP (mg L^{-1})	1.37±0.00	1.46±0.00	0.67±0.00	0.69±0.00	0.93±0.00
Chl a (µg L^{-1})	214.83±3.06	483.49±36.80	195.14±16.84	123.68±2.50	144.39±44.10

DO, dissolved oxygen; TDS, total dissolved solids; SRP, soluble reactive phosphorus; BOD, biochemical oxygen demand; TN, total nitrogen; TP, total phosphorus; ND, not detected.

with the chlorophyll a levels, showing higher values during the cold season and lower values during the hot season. Fig. 2 displays the Principal Component Analysis illustrating variations in both physical and chemical characteristics, highlighting the positive and negative relationships among environmental variables, and visualizing the similarities and dissimilarities of ponds among seasons. *Supplementary Fig. S1* summarizes the relationships among the monitored environmental variables of the ponds, and highlights the similarities and dissimilarities of the ponds based on these variables during the cold, hot, and rainy seasons.

Macroinvertebrate diversity and assemblages

There were variations in the macroinvertebrates across ponds, with the highest diversity and abundance observed in pond 1, followed by a gradual decrease to pond 5 (Tab. 2). The average number of macroinvertebrate families recorded decreased from pond 1 to pond 5, with average values of 13±3, 12±3, 5±3, 3±1, and 3±0 families, respectively. Similarly, the average densities of macroinvertebrates followed a decreasing trend from pond 1 to pond 5, with average values of 625±152, 157±152, 250±51, 120±100, and 438±397 individuals, respectively. We conducted tests to assess differences in taxonomic composition (Tab. 3), and the results showed that during the cold season, Notonectidae ($p<0.001$), Candonidae ($p<0.001$), Palaemonidae ($p=0.024$), and Thiaridae ($p=0.04$) exhibited statistically significant differences among ponds. In the hot season, significant differences in taxonomic composition among ponds were observed in Naucoridae ($p=0.007$), Corixidae ($p=0.031$), Hydrometridae ($p=0.002$), Coenagrionidae ($p=0.034$), Elmidae ($p=0.034$), and Candonidae ($p=0.024$). Finally, during the rainy season, Pleidae ($p=0.006$), Naucoridae ($p<0.001$), Hydrometridae ($p<0.001$), Chironomidae ($p<0.001$), Candonidae ($p=0.031$), Palaemonidae ($p=0.001$),

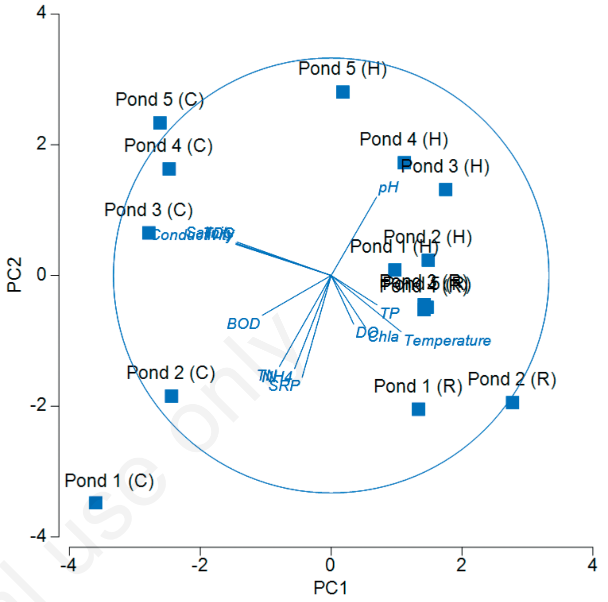


Fig. 2. Principal Component Analysis (PCA) of water quality among five ponds across three seasons (C, cold season; H, hot season; R, rainy season).

Tab. 2. Macroinvertebrate individuals (averaged) across ponds (P) 1 to 5 during various seasons.

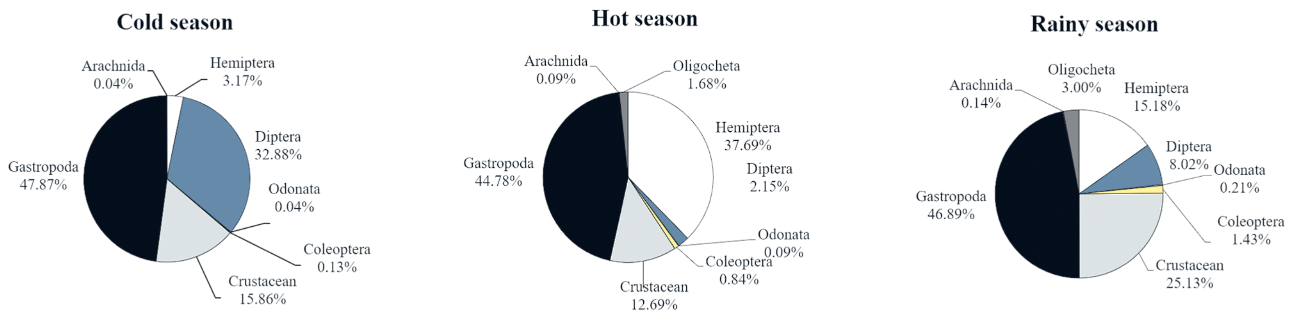
Invertebrate taxon	Cold season					Hot season					Rainy season				
	P1	P2	P3	P4	P5	P1	P2	P3	P4	P5	P1	P2	P3	P4	P5
Notonectidae	11	21	2	-	1	9	15	-	-	-	6	7	-	-	-
Mesoveliidae	2	3	-	-	-	18	1	-	-	-	5	2	1	-	-
Gerridae	-	1	-	-	-	-	-	-	-	-	-	4	-	-	-
Pleidae	6	-	-	-	-	2	-	-	-	-	107	4	-	-	-
Naucoridae	17	9	-	-	-	55	1	-	-	-	42	28	4	-	-
Corixidae	-	-	-	-	-	297	6	-	-	-	1	-	-	-	-
Hydrometridae	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Chironomidae	435	292	29	1	-	13	6	3	-	1	97	1	11	-	-
Statiomyidae	-	-	-	-	-	-	-	-	-	-	2	1	-	-	-
Coenagrionidae	1	-	-	-	-	1	-	-	-	-	-	2	1	-	-
Hydrophilidae	2	1	-	-	-	1	5	-	-	-	14	3	-	-	1
Elmidae	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-
Noteridae	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-
Candonidae	311	-	-	-	-	75	-	-	-	-	303	-	-	-	-
Palaemonidae	-	1	30	6	17	-	15	28	16	1	-	1	3	9	34
Sphaeromatidae	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-
Thiaridae	-	-	243	3	848	-	-	204	189	80	-	-	113	135	332
Lymnaeidae	1	4	3	-	-	-	1	-	-	-	1	1	65	-	-
Planorbidae	-	-	-	-	-	3	3	-	-	-	8	-	-	-	-
Cybaeidae	-	1	-	-	-	1	-	-	-	-	1	1	-	-	-
Naididae	-	-	-	-	-	8	10	-	-	-	15	17	10	-	-

Thiaridae (p=0.028), and Planorbidae (p=0.025) showed statistically significant differences among ponds.The predominant macroinvertebrate groups recorded in pond 1 were the Chironomidae and Candonidae. In pond 2, the Chironomidae were abundant during the cold season, whereas in ponds 3 to 5, the Thiaridae were numerically dominant. The variation in macroinvertebrate assemblages across seasons is shown in Fig. 3. The number of macroinvertebrate families peaked during the rainy season (20 families), followed by the hot season (18 families), and was at its lowest during the cold season (13 families). The Gastropoda constituted approximately 40% consistently throughout the year. The prevalence of the Diptera was highest during the cold season and decreased during the hot and rainy seasons. On the other hand, the Hemiptera were at their highest numbers during the hot season, followed by the rainy season and then the cold season. The result from multivariate analysis (multidimensional scaling) indicates two distinct groups within the invertebrate data: group I (ponds 1 to 2) and group II (ponds 3 to 5) (Fig. 4). This suggests that variations in the macroinvertebrate data were more closely related to the characteristics of the ponds. Notably, seasonal changes seemed to have different impacts on macroinvertebrate densities. The highest macroinvertebrate density was recorded during the cold season (460±358 individuals), followed by the rainy season (280±212 individuals), and the hot season (214±169

Tab. 3. Statistical analysis for assessing differences in taxonomic composition of macroinvertebrates among ponds during each season.

Taxonomic family	Statistical analysis					
	Cold season		Hot season		Rainy season	
	F-test	p-value	F-test	p-value	F-test	p-value
Notonectidae	13.842	<0.001*	1.480	0.279	1.285	0.339
Mesoveliidae	0.775	0.566	1.810	0.204	3.115	0.066
Gerridae	1.000	0.452			1.000	0.452
Pleidae	2.173	0.146	1.000	0.452	6.865	0.006*
Naucoridae	1.997	0.171	6.787	0.007*	14.605	<0.001*
Corixidae			4.142	0.031*	1.000	0.452
Hydrometridae	2.615	0.099	9.932	0.002*	14.207	<0.001*
Chironomidae	2.85	0.082	3.206	0.062	10.922	0.001*
Statiomyidae					3.200	0.062
Coenagrionidae	1.000	0.452	4.000	0.034*	1.875	0.191
Hydrophilidae					1.000	0.452
Elmidae			4.000	0.034*	3.000	0.072
Noteridae			0.850	0.525		
Candonidae	173.232	<0.001*	3.947	0.036*	4.147	0.031*
Palaemonidae	4.551	0.024*	1.092	0.412	11.868	<0.001*
Sphaeromatidae			1.000	0.452	3.000	0.072
Thiaridae	7.684	0.004*	3.176	0.063	4.274	0.028*
Lymnaeidae	2.765	0.088	1.000	0.452	1.102	0.407
Planorbidae			2.297	0.131	4.440	0.025*
Cybaeidae	1.000	0.452	1.000	0.452	0.750	0.580
Naididae			0.759	0.575		

*Statistically significant differences among ponds (p<0.05).



Cold season

Hot season

Rainy season

Fig. 3. The variations in macroinvertebrate composition across different seasons.

individuals), respectively. This study investigated the length, width, and weight of gastropods across five ponds in the cold, hot, and rainy seasons (Tab. 4). It was found that the Lymnaeidae and the Planorbidae in pond 2 had the maximum values for length, width, and weight. Additionally, the Thiaridae in pond 4 had the highest values for length, width, and weight. Seasonal variations were observed by the Lymnaeidae being greatest during the rainy season, while the Planorbidae had the highest values for length, width, and weight during the hot season. The Thiaridae had their peak values for length, width, and weight during the cold season. The findings from the biological indices are depicted in Tab. 5. They revealed that the highest values for the richness, evenness, and biodiversity indices were in pond 2 during the hot season. The similarity index delineated the macroinvertebrates into two distinct groups: group I, consisting of ponds 1 and 2; and group II, consisting of ponds 3 to 5. The highest value for the similarity index for ponds 1 and 2 (71.43%) was during the rainy season, while the highest values for the similarity index between ponds 3

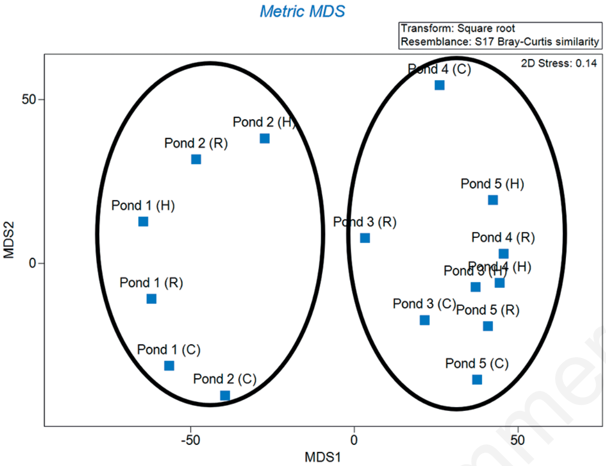


Fig. 4. Multidimensional scaling of macroinvertebrate taxa from five ponds across three seasons, revealing two distinct groups: group I (ponds 1 to 2) and group II (ponds 3 to 5) (C, cold season; H, hot season; R, rainy season).

Tab. 4. Measurements (mean ± SD) of length, width and weight of gastropods across ponds 1 to 5 during various seasons .

Pond	Family		Cold season	
		Length (cm)	Width (cm)	Weight (g)
1	Lymnaeidae	0.23±0.40	0.15±0.26	0.00±0.01
	Planorbidae	-	-	-
2	Lymnaeidae	0.61±0.12	0.39±0.06	0.01±0.01
	Planorbidae	-	-	-
3	Lymnaeidae	0.51±0.45	0.29±0.26	0.03±0.03
	Thiaridae	1.50±0.15	0.64±0.05	0.22±0.05
4	Thiaridae	1.73±0.24	0.77±0.12	0.33±0.12
5	Thiaridae	1.34±0.08	0.61±0.04	0.18±0.02
Pond	**Family**		**Hot season**	
		Length (cm)	**Width (cm)**	**Weight (g)**
1	Lymnaeidae	0.43±0.16	0.40±0.10	0.00±0.01
	Planorbidae	-	-	-
2	Lymnaeidae	0.50±0.00	0.31±0.00	0.00±0.00
	Planorbidae	0.57±0.10	0.37±0.08	0.01±0.00
3	Lymnaeidae	-	-	-
	Thiaridae	1.32±0.14	0.58±0.06	0.15±0.05
4	Thiaridae	1.30±0.09	0.59±0.04	0.24±0.14
5	Thiaridae	1.41±0.04	0.62±0.02	0.19±0.03
Pond	**Family**		**Rainy season**	
		Length (cm)	**Width (cm)**	**Weight (g)**
1	Lymnaeidae	0.55±0.49	0.39±0.31	0.05±0.07
	Planorbidae	0.01±0.00	0.01±0.00	0.01±0.00
2	Lymnaeidae	0.45±0.78	0.25±0.43	0.05±0.09
	Planorbidae	-	-	-
3	Lymnaeidae	0.42±0.37	0.26±0.22	0.02±0.02
	Thiaridae	1.09±0.59	0.49±0.27	0.15±0.14
4	Thiaridae	1.46±0.10	0.61±0.03	0.29±0.14
5	Thiaridae	1.32±0.04	0.56±0.02	0.18±0.08

Tab. 5. Comparison of biological indices (mean ± SD) across ponds 1 to 5 during various seasons.

Pond		Cold season			Hot season			Rainy season	
	Richness index	Evenness index	Biodiversity index	Richness index	Evenness index	Biodiversity index	Richness index	Evenness index	Biodiversity index
1	0.22±0.11	0.53±0.06	0.88±0.20	0.43±0.20	0.59±0.23	1.23±0.53	0.51±0.24	0.63±0.15	1.52±0.43
2	0.53±0.38	0.43±0.25	0.69±0.39	1.13±0.51	0.77±0.16	1.62±0.59	0.93±0.30	0.63±0.07	1.23±0.30
3	0.25±0.13	0.48±0.19	0.69±0.39	0.19±0.12	0.49±0.30	0.41±0.24	0.38±0.22	0.44±0.28	0.71±0.56
4	0.53±0.57	0.60±0.52	0.54±0.51	0.13±0.07	0.39±0.51	0.27±0.35	0.21±0.12	0.46±0.40	0.32±0.27
5	0.08±0.02	0.15±0.08	0.11±0.05	0.56±0.55	0.37±0.48	0.28±0.32	0.14±0.07	0.35±0.14	0.29±0.14
	Similarity index (%)	P1 x P2	66.67	Similarity index (%)	P1 x P2	59.26	Similarity index (%)	P1 x P2	71.43
		P1 x P3	42.86		P1 x P3	11.77		P1 x P3	45.46
		P1x P4	16.67		P1 x P4	0.00		P1 x P4	0.00
		P1x P5	16.67		P1 x P5	11.77		P1 x P5	11.77
		P2 x P3	57.14		P2 x P3	25.00		P2 x P3	63.64
		P2 x P4	33.33		P2 x P4	13.33		P2 x P4	12.50
		P2 x P5	33.33		P2 x P5	25.00		P2 x P5	23.53
		P3 x P4	75.00		P3 x P4	80.00		P3 x P4	40.00
		P3 x P5	75.00		P3 x P5	66.67		P3 x P5	36.36
		P4 x P5	66.67		P4 x P5	80.00		P4 x P5	80.00

P, pond.

and 4 (80.00%), as well as between ponds 4 and 5 (80.00%), were recorded during both the hot and rainy seasons. In addition, pond 2 during the hot season recorded the highest values for the richness, evenness, and biodiversity indices.

DISCUSSION

Water quality changes

Analysis of water quality across the ponds revealed notable variations. The DO levels varied among the ponds, with pond 1 having the lowest DO content. Sequentially, ponds 2 to 5 displayed an ascending trend in DO levels. Similarly, the BOD_5 values were highest in ponds 2 and 1, gradually declining in ponds 3, 5, and 4, respectively, similar to a previous study (Nimtim *et al*., 2020). This was likely because pond 1 was the initial recipient of wastewater from the Phetchaburi municipality, resulting in a relatively higher concentration of organic materials and waste compared to the other ponds. Despite this, some organic contamination might have been decomposed by anaerobic bacteria during the transportation of wastewater in a high-density polyethylene (HDPE) pipe before entering pond 1 (LERD, 2022). The decrease in BOD_5 suggested that the wastewater treatment across each pond collectively mitigated organic pollutants and waste. Dampin *et al.,* (2012) indicated that these wastewater treatment systems can achieve up to an 85% efficiency in BOD_5 treatment.

The nutrient composition within the wastewater treatment ponds is a result of the mineralization and biogeochemical function of organic nitrogen from the wastewater into nitrogenous inorganic compounds (LERD, 2022; Kuntz and Tyler, 2018; Saneha *et al.,* 2023). Consequently, pond 1 had the highest total ammonium nitrogen and nitrogen contents, with subsequent decreases observed in ponds 2 to 5. The soluble reactive phosphorus and TP levels followed a similar trend, with the highest concentrations recorded in pond 1 with a generally diminishing trend in ponds 2 to 5. Because pond 1 received the initial wastewater from Phetchaburi municipality into the treatment system, it may contain a considerable amount of washing and cleaning substances, as noted by Adesakin *et al.,* (2020). The nutrient quantities detected in the wastewater treatment ponds aligned consistently with the observed chlorophyll contents.

Macroinvertebrate diversity and assemblages

This study highlighted the diversity of macroinvertebrates and their variability across both wastewater treatment ponds and seasons. The predominant presence of the detrital feeding Chironomidae and Candonidae (ostracods) in ponds 1 and 2 was likely linked to the abundant organic content in these ponds, serving as their primary food sources (Montemezzani *et al.,* 2016). Additionally, the Chironomidae exhibit remarkable resistance to pollution, enabling their survival even in conditions characterized by low dissolved oxygen levels (Okuku *et al.,* 2006; Kuntz and Tyler, 2018). The Ostracods, recognized for their ability to endure broad variations in different environmental parameters (Kulkoyluoglu, 2007), have demonstrated a high tolerance to elevated organic pollution levels (Aiello *et al.,* 2020) and can persist in environments with low levels of dissolved oxygen (Kulkoyluoglu, 2004; Ruiz *et al.,* 2013; Parameswari *et al.,* 2020). However, in heavily organic-polluted waters near urban or industrial areas, the ostracods were notably scarce (Poquet *et al.,* 2008).

In addition, freshwater insects, notably belonging to the Corixidae and Pleidae, were abundant in ponds 1 and 2. This increased presence might have been linked to their association with the macrophytes located along the edges of the ponds, as well as a high organic food content. These findings were consistent with other studies that highlighted the factors influencing differences in macroinvertebrate community structures, such as vegetation composition and pollution levels (Jurado *et al.,* 2009). Snails from the Lymnaeidae and Planorbidae families were observed in ponds 1 to 3, although their numbers were lower compared to the prevalence of thiarid snails in the other ponds. Snails from the Lymnaeidae are amphibious and commonly inhabit pond sediment or aquatic plants (Eversham, 2013), often adopting periphytic behavior to feed on algae as a grazer, as well as feeding on detritus (Crichton, 2003; Pyron and Brown, 2015). Members of this family have lung-like organs, enabling them to survive in aquatic environments with reduced oxygen levels (Kuroda and Abe, 2020) and to exhibit resilience to high pollution levels (Pignata *et al.,* 2013).

Primarily, the macroinvertebrate composition within ponds 3 to 5 comprised thiarid snails (Thiaridae) and shrimps (Palaemonidae), largely attributed to their moderate resistance to pollution, aligning with the observed physical, and chemical water quality parameters (Tab. 1). The nutrient and organic matter levels in these ponds were notably lower compared to ponds 1 and 2. Additionally, the overflow of water at the edge of the outflow weir may continually transport organic material to these faunas. This condition proved particularly suitable for supporting the survival of freshwater gastropods and shrimps, as supported by Mejía-Ortíz *et al.,* (2019). A comparison of the lengths and body weights revealed that the thiarid snails in pond 4 had larger body sizes and weights compared to those in ponds 3 and 5. This disparity could be attributed to the fact that thiarid snails were relatively more abundant in ponds 3 and 5, leading to increased competition among snails for food and space.

Pond 1 had the highest biodiversity index value for macroinvertebrates. This elevated value could be attributed to the abundance in pond 1 of food sources and its heterogenous environmental context, including phytoplankton and various organic substances, fostering a richer diversity of life compared to the other treatment ponds. This was in agreement with Hill *et al.* (2018) and Viana *et al.* (2016), who reported that urbanization appeared to support highly heterogeneous macroinvertebrate communities in ponds within urban areas, possibly due to structured environmental variables. Organic matter breakdown releases particulate organic matter and dissolved organic substances into the water column and surface sediment. These serve as food sources for filter-feeding macroinvertebrates like larvae of insects and midges, enhancing their abundance and diversity (Jyväsjärvi *et al.,* 2013). The decomposition of organic matter can also lead to the formation of oxygen gradients, which acts as a significant driver (Dalu *et al.,* 2022), and this stratification can create distinct ecological habitats that support different macroinvertebrate communities (van der Lee *et al.,* 2017). In pond 4, both the species richness index and the evenness index had the highest values because the number of individuals was low. Conversely, in other ponds, the higher number of individuals led to a lower richness index. Furthermore, ponds 3 and 4, as well as ponds 3 and 5, had the highest values for the similarity index (75%), which reflected the similar water quality and enhanced environmental characteristics (homogenous environments – *Spirulina* was dominant) shared among ponds 3 to 5, resulting in comparable species composition within these ponds. Chaichana and Dampin (2016) and Thongdang *et al*. (2022) reported that *Spirulina platensis* made up more than 90% of all phytoplankton in ponds 3 to 5 and that this species may be beneficial to aquatic fauna because of its high nutritional value.

The results from this study raise several intriguing questions that warrant further investigation. Future studies should focus on a detailed examination of water quality and invertebrate communities on a monthly basis. It is also necessary to understand how environmental variables directly and indirectly influence each macroinvertebrate taxon, as well as to comprehend the impact of phytoplankton blooms in wastewater treatment ponds on the macroinvertebrate community. Additionally, exploring the cascade effects, such as the role of predator and prey, on biodiversity and the structure of the biotic component to unravel the complex interplay between different trophic levels and its implications for ecosystem functioning, would be valuable areas for follow-up studies.

CONCLUSIONS

In summary, the wastewater treatment ponds within the LERD Project serve as habitats for a diverse array of macroinvertebrates. Variations in water quality (e.g. DO levels and BOD_5 concentrations) and seasonal changes appear to greatly impact both the diversity and composition of these macroinvertebrates. Notably, macroinvertebrates from the Chironomidae and Candonidae families were abundant in ponds 1 and 2, where the water quality was relatively poorer. Conversely, in ponds 3 to 5, where there was improved water quality, the dominant family was the Thiaridae. This shift in dominant species among ponds with varying levels of water quality underscored the relationship between water quality and macroinvertebrate communities, offering valuable insights into this distinctive environmental context.

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Online supplementary material:

Fig. S1. Principal Component Analysis (PCA) of environmental variables among five ponds in each season.