Seasonal variation of zooplankton assemblages and their responses to water chemistry and microcystin content in shallow lakes in Thailand

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Abstract: This study examines zooplankton assemblage structure and density from five hypereutrophic urban shallow lakes between cool and hot periods in 2018-2019. We analyzed the variation of zooplankton and their relationship with environmental factors. Samples of zooplankton were collected from shallow lakes in different regions of Thailand. Four groups of zooplankton were identified, of which Rotifera was the most abundant group, followed by Copepoda, Protozoa, and Cladocera. Zooplankton assemblages were influenced by seasons, as indicated by multidimensional scaling analysis. The number of species and density of zooplankton were lower during the cool period than during the hot period. The increased density of zooplankton in the hot period may have been due to increased phytoplankton density as food sources. Pearson's correlation coefficient revealed that Rotifera and Copepoda positively correlated with the temperature and pH, and Rotifera was negatively correlated with total phosphorus; a negative correlation was also observed between Protozoa and dissolved oxygen. The microcystin content tended to have a negative impact on specific small species such as Protozoa (*Coleps* sp.). Information from this research is important for further study involving factors affecting the size structure of zooplankton communities, especially large-bodied species in tropical regions.

Keywords: hypereutrophic; lake; plankton; urban pond; water quality

Abbreviations: dissolved oxygen (DO); total nitrogen (TN); total phosphorus (TP); Nephelometric Turbidity Unit (NTU); enzyme-linked immunosorbent assay (ELISA); multidimensional scaling analysis (MDS)

INTRODUCTION

Zooplankton are heterotrophic plankton that are an important component in freshwater ecosystems. The diversity of zooplankton varies among geographical regions [1]. Protozoa, Rotifera, Copepoda, and Cladocera are common groups that can be found in freshwater habitats. In an ecosystem, zooplankton occupies trophic level two, links with producers, and transfers energy from primary producers through the food web [2]. They are a resource for consumers on higher trophic levels (invertebrate and vertebrate animals). Zooplankton also has potential value as bioindicators of the freshwater trophic state [3-4]; for example, *Brachionus angularis* and *Trichocerca cylindrical* are good bioindicators of eutrophic waterbodies [5-6].

Abiotic and biotic factors can influence zooplankton diversity, distribution, and abundance. Important abiotic factors include temperature, current, lake morphometry, and water chemistry [7-8]. Increased zooplankton assemblages, particularly rotifers, are associated with warmer summer temperatures [9]. A study in Brazil also revealed that rotifers (e.g., Brachionus calyciflorus and *Thermocyclops* sp.) were good indicators of high nutrient conditions. In contrast, Keratella tropica and Hexarthra mira were good indicators of high turbidity [10]. In eutrophic conditions, microcystin concentrations produced by cyanobacteria to deter zooplankton grazing can also affect zooplankton communities [11-12]. In Sweden, high microcystin concentrations were negatively correlated with Daphnia and calanoid copepods [13], and in China, the presence of microcystin



can contribute to increased dominance of small-bodied species [12]. For the biotic factor, predation by invertebrates (e.g., *Chaborus*) [14] and vertebrates (e.g., fish) [15] is attributed to shaping the size and structure of zooplankton in lakes. Thus, a negative correlation of zooplankton was found with the relative abundance of fish, meaning that the density of zooplankton is inversely proportional to fish density [16].

Zooplankton also plays a crucial role in the restoration of eutrophic waters. Large-body-sized classes such as cladocerans, particularly Daphnia, can feed on phytoplankton effectively and thus maintain a clear water state and mitigate the impact of eutrophication [17]. An experiment in Japan showed that biomanipulation using piscivore and the introduction of Daphnia improved lake clarity (from 2 m to more than 4 m) by reducing algal biomass [18]. In contrast, zooplankton with smaller body sizes, such as copepods and rotifers, have lower filtration capacity [19]. They tend to dominate in turbid water bodies characterized by the presence of fish and low densities of submerged macrophytes [19-20]. Information on zooplankton composition in a eutrophic lake is crucial and valuable for restoration planning and management. This can be done by increasing the composition of large-bodied zooplankton such as Daphnia to suppress phytoplankton biomass by removing zooplanktivorous fish through biomanipulation [21]. The result has been shown to be a successful method of improving water quality.

In Thailand, eutrophication is widespread and intense, especially during the hot season (February to May). Eutrophication is evident in urban shallow lakes nationwide due to rapid rural and industrial expansion and development. Most research in Thailand has focused on phytoplankton and the cyanotoxin potential since this can lead to a better understanding of some health concerns. The current study investigated seasonal variation in species and zooplankton abundance in hypereutrophic urban shallow lakes in Thailand. Eutrophication and environmental variables may influence and shape the ecological structure of zooplankton communities. Some cyanobacteria, such as Microcystis, can be toxic and harmful to zooplankton [22-24]. Zooplankton encountering toxic cells cease feeding, which can cause a reduction in the growth, reproduction, and survival of zooplankton [21]. This study aimed to investigate the zooplankton structure

and its seasonal variation during cool and hot periods in hypereutrophic urban shallow lakes in a tropical landscape. This study also determined the effect of environmental variables such as water quality values and microcystin content that are associated with species diversity and the density of zooplankton. The results of this research will lead to a better understanding of the ecology of zooplankton in urban shallow lakes and their response to water quality conditions.

MATERIALS AND METHODS

Study area

Zooplankton communities were investigated in five urban shallow lakes located in five provinces of Thailand. These shallow lakes were selected because they are highly hypereutrophic (dominated mainly by intense cyanobacterial blooms) and are examples of urban shallow lakes in each region of Thailand (north in Chiang Mai, northeast in Khon Kaen, east in Chanthaburi, and central regions in Bangkok and Pathum Thani, respectively) (Supplementary Fig. S1).

Water sampling and analysis

Water samples were collected in the cool period (December 2018-January 2019) and the hot period (March-May 2019). There were three sampling points in each shallow lake. Selection of the sampling point was determined by (i) the distribution of the sampling point around the shallow lake; (ii) the area where the plankton bloom was formed; (iii) accessibility to the sampling point. Physical and chemical variables were determined in situ and in the laboratory. Conductivity (µs·cm⁻¹), dissolved oxygen (DO, mg·L⁻¹), pH, and water temperature (°C) were measured in the field by a multiparameter analyzer (Consort 9116, Belgium). Two L of water samples were collected and kept in plastic bottles and then placed in a container at 4°C for further analysis in the laboratory. We analyzed the total nitrogen (TN, mg·L⁻¹) using the Kjeldahl method, total phosphorus (TP, mg·L⁻¹) using the vanadomolybdate method, and chlorophyll a $(\mu g \cdot L^{-1})$ using the acetone extraction method [25]. Turbidity (Nephelometric Turbidity Unit, NTU) was measured by a portable turbidity meter (WTW 430IR), and total suspended solid (TSS, mg·L⁻¹) was determined by measuring the dry weight of the suspended solid particles after filtration. All samples were analyzed in triplicate at the Department of Environmental Technology and Management, Faculty of Environment, Kasetsart University and Central Laboratory, Bangkok, Thailand.

The microcystin content was determined by the Microcystin-Adda enzyme-linked immunosorbent assay (ELISA) kit (Abraxis, Inc. USA). Water samples were collected and filtered using a glass Whatman microfiber filter, grade GF/C (diameter 47 mm). The toxin content in the wells of the test strips was analyzed by adding 50 µL of the standard solutions and then 50 µL of the antibody solutions. The wells were kept at room temperature for 90 min. The wells were then washed using a diluted Tris-based wash buffer. After washing, 100 µL of the enzyme conjugate solution (anti-sheep-HRP) was added to individual wells, and the wells were incubated for 30 min. The wells were rewashed using the diluted wash buffer, and subsequently, 100 μL of (color) solution (tetramethylbenzidine (TMB)) was added. The wells were incubated for another 30 min, and $50 \,\mu\text{L}$ of stop solution was added to the wells. The color reaction in the wells was stopped after 20-30 min, and the color was evaluated by measuring the absorbance at 450 nm wavelength using a microplate spectrophotometer. The concentrations of the samples were determined by interpolation using the standard curve constructed with each run.

Collection of zooplankton and identification

Zooplankton data were compared during cool and hot periods. Zooplankton samples were collected from the surface water (1 m below the water surface) at three sampling points (one sample per sampling point in a total of three samples per shallow lake) around each shallow lake. Twenty L of water was poured through a plankton net (64 μ m mesh size). Samples were preserved in 70% ethanol [26-27] and brought to the laboratory facilities of the Department of Environmental Technology and Management, Faculty of Environment, Kasetsart University, Bangkok, Thailand, for further investigation. One week after collection, 1 mL of sample was placed in a Sedgewick Rafter counting chamber. Zooplankton was counted in 1,000 grids of the chamber, and zooplankton identified up to species level using the identification key of [28]) under a compound microscope (Kruss MBL2000, Germany). Zooplankton density was calculated as individuals-L⁻¹ [29].

Data analysis

Values are expressed as the mean±standard deviation (n=3). In this study, we used PRIMER Version 7 with PERMANOVA+ (academic license (sn: Q781)) for multidimensional scaling (MDS) analysis. MDS was performed to visualize similarities or dissimilarities of zooplankton assemblages in two dimensions between the cool and hot periods. Biological data were log-transformed before analysis. We conducted Pearson's correlation coefficient (r) analysis using IBM SPSS Statistics software (authorized user version 22) to investigate the correlation between zooplankton group density and environmental variables and between individual zooplankton species and the microcystin content [30-32]. Statistical significance was tested at 0.05 and 0.01 levels.

RESULTS

Water quality

The results of water-quality analysis are given in Table 1. Conductivity values were in the normal ranges of freshwaters. Both total nitrogen and total phosphorus

Province	Temperature	pН	Turbidity (NTU)	Conductivity (µs·cm⁻¹)	TSS (mg·L ⁻¹)	TN (mg·L⁻¹)	TP (mg·L ⁻¹)	Chlorophyll a (µg·L ⁻¹)
Khon Kaen	28-34	8.1-8.6	17-23	620-714	28.9-32.0	8.75-13.13	0.10-0.25	46.00-119.70
Chiang Mai	27-35	7.2-9.9	101-111	170-243	59.3-81.3	4.38-8.75	1.19-34.80	187.22-4,022.09
Chanthaburi	30-33	6.7-7.4	38-44	282-837	34.4-51.1	2.92-5.83	0.18-26.69	45.68-301.20
Pathum Thani	31-34	8.8-9.4	16-309	411-450	36.4-181.8	2.92-4.38	1.33-17.35	72.59-1,288.25
Bangkok	29-35	8.3-8.7	32-40	715-762	70.4-98.7	1.46-2.92	1.43-26.69	134.08-576.51

Table 1. Water-quality values (range) among five shallow lakes.

Zooplankton group	Khon Kaen					Chant	haburi		Chiang Mai				
	Species		Der	Density		Species		Density		Species		Density	
	С	Н	С	Н	С	Н	С	Н	С	Н	С	Н	
Protozoa	3	2	12	589	1	3	4	125	3	1	600	314	
Rotifera	8	12	168	6,031	3	6	28	663	6	8	2,198	7,662	
Copepoda	1	1	11	618	1	1	124	664	1	1	68	936	
Cladocera	-	-	-	-	1	2	4	42	-	1	-	628	
Total	12	15	191	7,238	7	12	160	1,494	10	11	2,866	9,540	

Table 2. Zooplankton of five urban shallow lakes in the cool (C) and hot (H) periods.

7 1 1.		В	angkok	2	Pathum Thani				
Zooplankton	Species		Der	Spe	cies	Density			
group	С	Η	С	Н	C H		С	Н	
Protozoa	3	-	208	-	-	-	-	-	
Rotifera	5	5	177	13,658	10	14	1,442	8,126	
Copepoda	1	1	145	171	1	1	27	80,326	
Cladocera	1	-	630	-	2	2	426	1,172	
Total	10	6	1,160	13,829	13	17	1,595	89,624	

values were high in all studied shallow lakes, indicating eutrophic-hypereutrophic conditions. High chlorophyll a values were recorded in Chiang Mai and Pathum Thani and resulted in high values of turbidity and total suspended solid. Furthermore, all urban shallow lakes were classified as hypereutrophic, and nitrogen was a limiting nutrient in most shallow lakes (except Khon Kaen) [33].

Zooplankton composition and assemblages

The diversity of zooplankton varied among shallow lakes and with season. As can be seen in Supplementary Table S1, the highest number of zooplankton species (21) was recorded in Khon Kaen, whereas the lowest number of zooplankton species (11) was observed in Bangkok. In most shallow lakes, the maximum diversity of zooplankton was recorded during the hot period, and the minimum diversity was observed during the cool period. A species list and the number of zooplankton in each shallow lake are given in Supplementary Table S1.

Table 2 shows the densities of the zooplankton (Protozoa, Rotifera, Copepoda, and Cladocera) during the cool and hot periods. In the cool period, the highest zooplankton density (2,866 individuals·L⁻¹) was recorded in Chaing Mai, followed by Pathum Thani, Bangkok, Khon Kaen, and Chanthaburi. In the hot period, the highest zooplankton density (89,624 individuals·L⁻¹) was observed in Pathum Thani followed



Fig. 1. Species composition of zooplankton in five urban shallow lakes (a) Khon Kaen, (b) Chanthaburi, (c) Chiang Mai, (d) Bangkok, (e) Pathum Thani.

by Bangkok, Chiang Mai, Khon Kaen, and Chanthaburi. The population density trend of zooplankton showed a marked increase during the hot period.

Zooplankton composition exhibited similar patterns in hypereutrophic shallow lakes. In general, Rotifera dominated in most shallow lakes, followed by Copepoda, Protozoa, and Cladocera (Fig. 1). During the cool period, Rotifera was the most abundant component in Khon Kaen (88%), Chiang Mai (77%) and Pathum Thani (72%). *Brachionus angularis* (614 individuals·L⁻¹) was the most prominent species in Pathum Thani, whereas *B. caudatus* was abundant in Khon Kaen (68 individuals·L⁻¹). *Trichocerca* sp. (987 individuals·L⁻¹) was the dominant species in Chiang Mai. Chanthaburi's main zooplankton group was the cyclopoid copepod (124 individuals·L⁻¹). In Bangkok, Cladocera was dominant, with *Moina* sp. the most abundant genus (679 individuals·L⁻¹).



Fig. 2. Multidimensional scaling (MDS) plots with stress values of less than 0.2 in two dimensions showing two separated groups of zooplankton data between the cool and hot periods (**a**) Khon Kean, (**b**) Bangkok, (**c**) Pathum Thani, (**d**) Chiang Mai, (**e**) Chanthaburi. (1, 2, and 3 are replicates of 1, 2, and 3).

In the hot period, Rotifera was the main group in Khon Kaen (83%), Chiang Mai (80%), Bangkok (99%), and Pathum Thani (66%), respectively. *B. bidentatus* was the most dominant species in Khon Kaen (2,261 individuals·L⁻¹). In Chiang Mai, the predominant zoo-plankton species was *B. forficula* (4,528 individuals·L⁻¹), and in Bangkok, the dominant zooplankton species was *Brachionus angularis* (12,041 individuals·L⁻¹). Cyclopoid copepod was the key dominant group in Pathum Thani (80,326 individuals·L⁻¹). In Chanthaburi, cyclopoid copepod was dominant (664 individuals·L⁻¹).

MDS analysis

We performed multidimensional scaling (MDS) analysis to graphically analyze seasonal changes (between the cool and hot periods) in zooplankton assemblages using species diversity and density data. The results suggested that seasons influenced zooplankton communities. Fig. 2, derived from MDS, displayed an arrangement of sites in two dimensions. It can be partitioned into two groups of zooplankton assemblages in the cool and hot periods. The rank of proximity of points to one another reflects the similarity of the zooplankton community structure from the sites. Zooplankton data from Chiang Mai, Bangkok, and Pathum Thani displayed clearer results.

Pearson's analysis

The results of Pearson correlation coefficient (r) analysis showed some correlations between zooplankton density data and water quality variables (Table 3). Protozoa density was negatively correlated with DO ($r=-0.389^*$, P<0.05) and temperature ($r=-0.363^*$, P<0.05). The

density of Rotifera and Copepoda positively correlated with temperature ($r=0.717^*$, P<0.05 and $r=0.379^{***}$, P<0.01, respectively). Rotifera density was positively correlated with the pH ($r=0.554^{**}$, P<0.01) but negatively with the TP ($r=-0.501^{**}$, P<0.01).

Microcystin content

The microcystin content tended to have a negative effect on Protozoa density, as can be seen in Table 3. We tested the correlation between individual zooplankton species and the microcystin content. Only

Table 3. Correlation coefficients between zooplankton groups and environmental variables

Zooplankton	DO	Temperature	pН	Chlorophyll a	TN	ТР	Microcystin
Protozoa	-0.389*	-0.363*	-0.292	-0.092	0.201	-0.268	-0.345
Rotifera	0.153	0.717**	0.554**	0.262	-0.157	-0.501**	0.278
Copepoda	-0.065	0.379*	0.142	-0.316	-0.184	0.171	0.153
Cladocera	0.089	0.342	0.277	-0.224	-0.168	0.229	0.224

*Correlation significance at 0.05 level and **Correlation significance at 0.01 level

some zooplankton species were correlated with the microcystin content. *Coleps* sp. (Protozoa group) was negatively correlated with the microcystin content (r=-0.362*, P<0.05). *Brachionus calyciflorus* (r=0.435*, P<0.05) and *Ceriodaphnia cornuta* (r=0.429*, P<0.05) were positively correlated with the microcystin content. No correlation was found for other zooplankton species.

DISCUSSION

The diversity and density of zooplankton differed among shallow lakes and between the studied periods. Rotifera and Copepoda appeared to dominate in hypereutrophic urban shallow lakes. This is consistent with previous studies in which Rotifera Brachionus angularis and Trichocerca cylindrical were designated as bioindicators of eutrophy [5-6]. Protozoans tended to be more abundant in the cool period than in the hot period. For Cladocera, small-body-sized species such as Ceriodaphnia cornuta, Diaphanosoma sp., and Moina sp. were found in this study. In a tropical country like Thailand, there are fewer species of cladocerans, and these are generally smaller [34]. In contrast, largebody-sized cladocerans such as Daphnia magna and D. pulex are limited. In fact, large-bodied Daphnia have been reported in temperate regions [35-37]. There is very little information on Daphnia species found in the tropics, and this perhaps may be due to the lack of extensive and intensive field collections in the tropics [38]. Overall, the presence and absence of zooplankton species may be attributable to several factors, such as lake morphology, environmental and biotic factors [1].

Zooplankton composition among hypereutrophic urban shallow lakes showed interesting results. Copepoda dominated the shallow lake in Pathum Thani, contributing up to 88%. The dominance of Copepoda may have been associated with the intense plankton blooms in the hot period since some copepod species seem best adapted to utilizing large cyanobacteria [39]. In other shallow lakes, plankton blooms were not intense. This is in agreement with a previous study that showed that the blooming of cyanobacteria could contribute to the feeding and reproduction of copepods during summer and create a favorable growth environment for copepod communities [40]. The monthly succession of zooplankton groups should be further investigated.

In this study, we showed that seasons affected zooplankton assemblages. This could be linked to seasonal variation in abiotic and biotic factors that contribute to the shaping structure of zooplankton communities. In particular, abiotic factors (e.g., temperature and pH) strongly influence zooplankton succession and biomass in most zooplankton groups [8,32] except Protozoa. Crustacea and Rotifera showed positive correlations with water temperature [41], similar to data from a current study that zooplankton abundance with the highest density occurs during the hot period. This was probably because the hot season is a growing season for phytoplankton which can become a major food source for zooplankton. There are ample food supplies for successful offspring development at this time of year [30]. Similarly, changes in zooplankton diversity could be attributed to changes in spatial distribution and the type of phytoplankton [42].

In the current study, we observed a negative relationship between Rotifera and TP because TP promoted cyanobacteria blooms. Correlation analysis showed a strong positive relationship between toxic Microcystis and TP [43]. The cyanotoxin production by cyanobacteria may impact Rotifera abundance and Protozoa community structure (especially Coleps sp.) [44]. Microcystins, which are produced by Microcystis aeruginosa, are toxic to rotifers, causing changes in enzyme activity (superoxide dismutase (SOD)) and nutrient content [45]. Another explanation is that cyanobacteria are inadequate as a food source for zooplankton, whether due to their large size, low nutritious value, or due to feeding inhibitors [46]. Protozoa density was negatively correlated with DO. Maximum densities of planktonic ciliated Protozoa populations have been reported at depths where the oxygen concentration was low, $1 \text{ mg} \cdot L^{-1}$ or less [47]. Another study reported that abiotic factors, such as pH, phosphates, and nitrates, did not affect Rotifera communities [31].

The structure and assemblages of zooplankton may also be influenced by biotic factors such as predation by fish. The current study indicated that the main groups of zooplankton were those in small-body-sized classes. It was reported that zooplankton with smaller body sizes typically dominate in turbid waterbodies characterized by the presence of fish and low density of submerged macrophytes due to a lack of refuges [18-19]. Fish predation appears to be the main control factor of zooplankton size structure [48]. In the urban landscape, fish are stocked in public shallow lakes intentionally to attract visitors, and by mercy release, especially in Asian culture. This could result in the absence of large-body-sized zooplankton due to fish predation [49-50] and could lead to a turbid phase dominated by phytoplankton. Protozoa and Rotifera are small and thus have less impact on the grazing of phytoplankton communities [18].

CONCLUSIONS

Many urban shallow lakes have suffered from eutrophication. There is limited information on the species, densities, and seasonal variations of zooplankton in hypereutrophic shallow lakes in Thailand. This research revealed that small Rotifera and Copepoda dominated hypereutrophic shallow lakes, while large Cladocera, as well as Protozoa, were less abundant. Zooplankton diversity and density increased in the hot period compared to the cool period. Seasonal variation and abiotic factors (temperature, DO, pH, phosphate, microcystin content) appeared to impact zooplankton density and assemblages significantly. We suggest that further research should focus on restoring hypereutrophic shallow lakes in relation to adjusting zooplankton structure. Biomanipulation techniques, such as the removal of zooplanktivorous fish, and reintroducing macrophytes, refuges for zooplankton, should be tested and used. These techniques have proved effective and can restore turbid waters by promoting large-body-sized zooplankton communities.

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Phylum	Kho	n Kean	Chanthaburi		Chiang Mai		Bangkok		Pathum Thani	
Phylum	С	Н	С	Н	С	H	С	Н	С	Н
Phylum Protozoa										•
Arcella sp.	3									
Centropyxis aculeata				14						
<i>Centropyxis</i> sp.						314				
Coleps sp.		452			88					
Difflugia acuminata					19					
Difflugia lebes			4		493					
<i>Difflugia</i> sp.	4			14			19			
Didiunium sp.		137					160			
<i>Euglypha</i> sp	5									
Halteria sp.							28			
<i>Tintinnopsis</i> sp.				98						
Phylum Rotifera				·						
Anuraeopsis fissa	4									
Anuraeopsis sp.		821			493		9			
Ascomorpha sp									17	
Brachionus angularis	55	740	4	276					614	580
Brachionus bidentatus		2,261								
Brachionus caudatus	68	1,231		28						262
Brachionus calyciflorus		269			29				117	1,206
Brachionus diversicornis						2,042				
Brachionu falcatus					10					

SUPPLEMENTARY MATERIAL

Supplementary Table S1. Zooplankton species recorded in five urban shallow lakes (individuals:L-1).

Table S1 continued

Phylum	Khon	Kean	Chanthaburi		Chiang Mai		Bangkok		Pathum Thani	
Phylum	С	Н	С	Н	С	Н	С	H 1,457 161	С	Н
Brachionus forficula		603		165	68	4,528				171
Brachionus quadridentatus										334
Brachionus rotundiformis										84
Brachionus rubens							19			171
Brachionus sp.	9	64					47	1,457	356	334
Cephalodella sp.		137				156				
Epiphanes sp.	3									
Filina longiseta		904					9	161		
Keratella tropica		301	17						7	84
Keratella sp.				83						
<i>Mytilina</i> sp.				83		156			17	437
Philodina sp.	9									
Polyarthra	13	1,536	4		610		93		7	
<i>Synchaeta</i> sp.						156				
<i>Trichocera</i> sp.	51	151		28	987					4,469
Phylum Arthropoda										
Cyclopoid copepod	11	618	124	664	68	936	104	1716	27	80,326
Ceriodaphnia cornuta										666
Diaphanosoma sp.			4	14					169	
<i>Moina</i> sp.				28		628	670		313	262
C – cool period, H – hot period										

Image: displaying the image: disp

Supplementary Fig. S1. Five urban shallow lakes located in each region of Thailand (represents the sampling point). Source: Google Earth (modified from: www. maps.google.com).