

Diversity of aquatic insect families and their relationship to water quality in urban ponds in Phnom Penh, Cambodia

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សត្វល្អិតទឹកត្រូវបានគេប្រើប្រាស់ជាសូចនាករដើរសាស្ត្រគុណភាពទឹកទាំងនៅក្នុងប្រព័ន្ធទឹកធម្មជាតិ និងពាក់កណ្តាលធម្មជាតិ។ ទោះជាយ៉ាងនេះក្តី ការសិក្សាបែបនេះគឺនៅមានកម្រិតនៅឡើយនៅក្នុងបរិស្ថានទឹកក្រុងនៃព្រះរាជាណាចក្រកម្ពុជា។ យើងបានធ្វើការសិក្សាពីជីវិតសត្វល្អិតទឹក និងទំនាក់ទំនងរបស់ពួកវាទៅនឹងគុណភាពទឹកនៅក្នុងត្រពាំងទឹកចំនួន៥ ក្នុងបរិវេណសាកលវិទ្យាល័យភូមិន្ទភ្នំពេញក្នុងប្រទេសកម្ពុជា។ សត្វល្អិតទឹកសរុបចំនួន៧,៣៥០ក្បាលស្ថិតក្នុង ២៣អំបូរ និង៦លំដាប់ត្រូវបានប្រមូល និងកំណត់អត្តសញ្ញាណដល់កម្រិតអំបូរ។ លំដាប់ Hemiptera (ភាគច្រើនជាអំបូរ Micronectidae) គឺមានវត្តមានលើសលប់នៅក្នុងសំណាកទាំងអស់ ចំណែកអំបូរ Ephemeroptera និងLepidoptera គឺមានវត្តមានតិចតួចជាងគេ។ ចំនួនប្រភេទ ចំនួនឯកត្តៈ និងតម្លៃនានាភាព (Shannon-Wiener) មានភាពខុសគ្នាជាចំរាងត្រពាំងទឹកដែលបានសិក្សា។ ចំនួនប្រភេទ និងចំនួនឯកត្តៈរបស់សត្វល្អិតទឹកត្រូវបានរកឃើញច្រើនជាងគេនៅក្នុងត្រពាំងទឹក២ (P2 និងP4) ដែលមានទីតាំងនៅភាគអាគ្នេយ៍នៃសាកលវិទ្យាល័យភូមិន្ទភ្នំពេញ។ កត្តានេះអាចមកពីត្រពាំងទាំងពីរនោះមានគុណភាពទឹកល្អ និងមានរុក្ខជាតិទឹកច្រើន។ ត្រពាំងទឹក P5 ដែលមិនបានទ្រទ្រង់ជីវិតសត្វល្អិតច្រើននោះ មានរងនូវការបំពុលទឹក ព្រមទាំងសម្បូរទៅដោយពពួកសត្វល្អិតក្នុងអំបូរ Micronectidae និង Chironomidae ដែលជាពពួកសត្វល្អិតធំនឹងការបំពុល។ លទ្ធផលនៃការវិភាគបានបង្ហាញថា ចំនួនប្រភេទ និងចំនួនឯកត្តៈរបស់សត្វល្អិតទឹកគឺមានទំនាក់ទំនងជាវិជ្ជមានជាមួយនឹងកម្រិតអុកស៊ីសែនរលាយក្នុងទឹក ក៏ប៉ុន្តែវាមានទំនាក់ទំនងជាអវិជ្ជមានជាមួយនឹងកម្រិតកករនៅក្នុងទឹក។ ជាអនុសាសន៍ កិច្ចខិតខំប្រឹងប្រែងស្តារឡើងនូវគុណភាពទឹកនៃត្រពាំងដែលរងការបំពុលខ្លាំងនោះ គួរតែត្រូវបានធ្វើឡើង ដើម្បីធ្វើឲ្យជីវិតក្នុងទឹកត្រពាំងមានភាពសម្បូរបែបឡើងវិញ។

Abstract

Aquatic insects are commonly used as bioindicators of water quality in semi-natural and natural aquatic systems. However, such studies are still limited in urban environments in Cambodia. We investigated aquatic insect life and its relationship to water quality in five ponds located on the grounds of the Royal University of Phnom Penh in Cambodia. A total of 7,350 individuals of aquatic insects belonging to 23 families and six orders were collected and identified to family level. Hemiptera (mostly Micronectidae) were most abundant in our samples overall whereas Ephemeroptera and Lepidoptera were the least abundant. Taxonomic richness, abundance and Shannon-Wiener's diversity values

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differed significantly between our study ponds. Two ponds (P2 and P4) located in the southeast portion of the university were found to support the highest taxonomic richness and diversity, which was likely due to better water quality and greater aquatic vegetation. The least diverse study pond (P5) had rather polluted water and was dominated by members of the Micronectidae and Chironomidae which are more tolerant to pollution. Regression analysis showed that aquatic insect richness and abundance were positively associated with dissolved oxygen levels, but negatively associated with water turbidity. We recommend that restoration efforts be undertaken to improve the water quality of the most polluted of our study ponds to enhance their aquatic life.

Keywords Invertebrate diversity, Diptera, dissolved oxygen, Hemiptera, Ephemeroptera, water turbidity.

Introduction

Aquatic insects (animals without an internal skeleton) are an ecological and polyphyletic group of arthropods which live or spend part of their life cycle in water (Pennak, 1978; Arimoro & Ikomi, 2008). Some species are entirely aquatic whereas others are semi-aquatic, and they collectively comprise 12 orders and approximately 100,000 species in total (Dijkstra *et al.*, 2014). Aquatic insects are important in aquatic systems because they play major roles as consumers, detritivores, predators and/or pollinators (Balian *et al.*, 2008).

Aquatic insects are commonly used as indicators of water quality in lentic and lotic systems because changes in the physical and chemical properties of water can strongly influence their presence and abundance (Uherek & Pinto, 2014). As such, their differing levels of tolerance to the amount and type of pollution can indicate different water quality classes (Cairns & Pratt, 1993; Kamsia *et al.*, 2008). For example, the presence of most species in the orders Ephemeroptera, Plecoptera, and Trichoptera can indicate good water quality (Sor *et al.*, 2017), because most of these taxa are sensitive to poor water quality and thus often occur in pollution-free systems (Bonada *et al.*, 2006; Merritt & Cummins, 2008; Hamid & Rawi, 2011). On the other hand, some species belonging to the Odonata and Diptera orders can tolerate moderate to extremely polluted waters, respectively (Merritt & Cummins, 2008; Al-Shami *et al.*, 2010; Hepp *et al.*, 2013).

Anthropogenic activities such as releases of sewage, industrial and household water, coupled with run-off from agriculture and mining activities have reached a critical level in many aquatic systems in Asia (Prommi & Payakka, 2015; IPBES, 2018). As such, most waterbodies in the region are experiencing increasing pollution loads which are undoubtedly altering their physical-chemical properties e.g., temperature, dissolved oxygen, pH, conductivity, alkalinity, phosphates, nitrates, turbidity, and metal concentrations (Prommi & Payakka, 2015). Variations in these properties can greatly influence the distribution of aquatic insects because some taxa are

highly sensitive to pollution and environmental disturbance, whereas others are moderately to highly tolerant (Hepp *et al.*, 2013).

The diversity of aquatic insects in Cambodia and their relationships to water quality in urban aquatic systems is poorly studied to the best of our knowledge. We therefore aimed to investigate this in a well-known area of Phnom Penh, namely at the Royal University of Phnom Penh (RUPP). Specifically, we aimed to i) determine how diverse aquatic insects are in ponds at the RUPP, ii) identify which ponds are more or less vulnerable to pollution and consequently support a higher or lower aquatic insect diversity, and iii) quantify the relationship between aquatic insect diversity and specific water quality variables. We consequently provide baseline data regarding aquatic insect diversity and surface water quality at the Royal University of Phnom Penh.

Methods

Study area

Our study was carried out at five ponds located on the grounds of the RUPP which encompasses approximately 21 ha in Sangkat Teklark 1, Khan Toul Kork, Phnom Penh (between 11°34.0'N, 104°53.3'E and 11°34.3'N, 104°53.6'E) (Fig. 1). The ponds at the RUPP provide a water source for aquatic organisms and valuable cultural services to the public and whereas some ponds receive wastewaters, others do not. We assumed these differences would likely influence the aquatic communities present.

Our first study pond (P1, Fig. 1) occupied the southwest corner of the RUPP grounds. Its waters were moderately clear during our study and surrounded by trees. Our second study pond (P2) was located in the southeast portion of the RUPP, in front of the Institute of Foreign Languages. Aquatic vegetation was more abundant within the pond and its waters were rather clear and clean. Our third study pond (P3) was situated in the northeast corner of the RUPP and surrounded by

only a few trees. The pond had limited aquatic vegetation and its waters were dark-green, moderately turbid and pungent, with much plastic waste present. Our fourth study pond (P4) was located in front of the Cambodia-Japan Cooperation Centre in the southeast corner of the RUPP grounds. This appeared to be rich in aquatic vegetation, with clear waters. Our final study pond (P5) was located next to the Cambodia-Korea Cooperation Centre in the central-west portion of the RUPP. Aquatic and riparian vegetation were limited at the pond and its waters were turbid because the pond receives wastewater from the surrounding area.

Sampling design and data collection

Our sampling was undertaken between 18–20 January 2019. Four samples were obtained from different locations in each pond, providing a study total of 20 samples.

Aquatic insects were sampled at each location using an aquatic hand-net, which had an opening that measured 30 x 30 cm, a length (or depth) of 92 cm and a mesh size of 1 mm. This was used to collect aquatic insects near the shoreline for a total of 30 minutes at each pond. While this sampling method is biased towards natatonic and neustic organisms, it can also sample opportunistically benthic insects such as Chironomidae and was standardised at each pond.

Following collection, insects were placed on a white tray and rinsed with water for sorting and screening. These were then transferred with forceps into labelled

containers containing 75% ethanol. Large insects were sorted by naked eye, whereas smaller individuals were sorted using a dissecting microscope (Olympus SZ61). The sorted material was later identified to family level using keys provided by Dudgeon (1999), Yule & Yong Hoi (2004) and Burnhill (2006).

Several water quality variables were measured at each sampling location using a HI-7609829 Multiparameter Portable Water Quality Meter (Hanna Instruments Ltd., Bedfordshire, UK). These included pH, water temperature, dissolved oxygen, electrical conductivity, turbidity, and total dissolved solids. All measurements were made between 0.1–0.5 m in water depth, after sampling for aquatic insects.

Data analysis

We calculated taxonomic richness (=the number of taxa), abundance (=the number of individuals), and Shannon-Wiener's diversity index (H) values for each sampling location and pond (Hamid & Rawi, 2017; Sor *et al.*, 2017). We also quantified the total number of individuals per taxon. As a non-parametric distribution was assumed due to the small sample size for each pond, Kruskal-Wallis tests were employed to test for significant differences in the taxonomic richness, abundance and diversity values between the ponds.

To assess relationships, these community metrics were regressed against our water quality data, which were normalized using the zero minimum (Sor *et al.*,

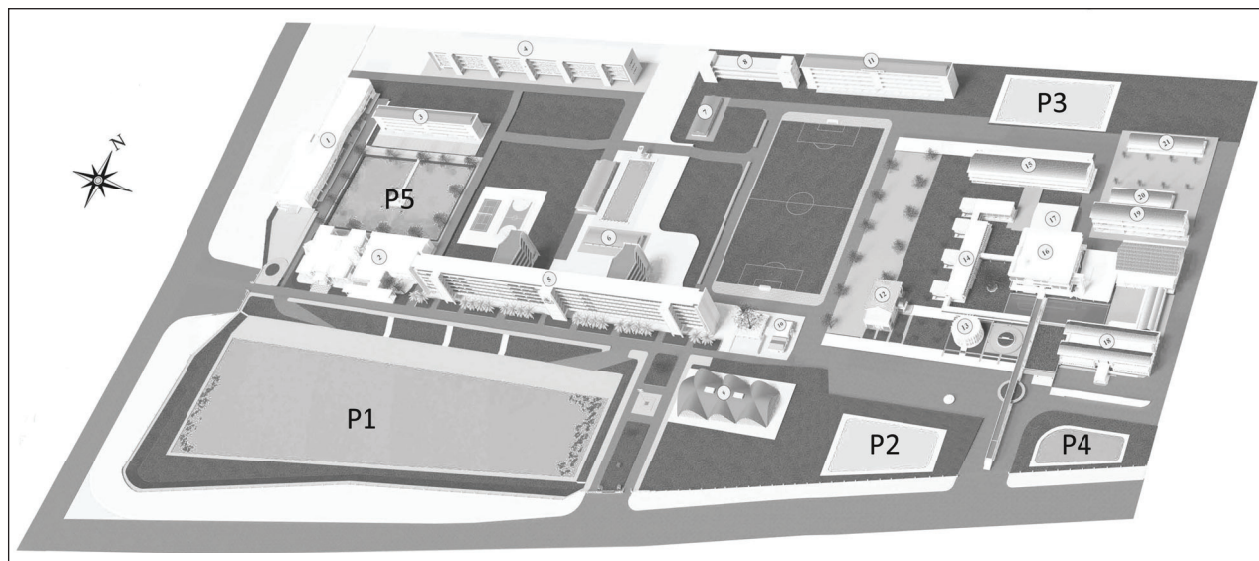


Fig. 1 Location of five study ponds (P1–P5) on the grounds of the Royal University of Phnom Penh (© RUPP Faculty of Engineering).

2017). An overall multiple linear regression was first employed to this end. All water quality variables that proved significant in these tests were then individually tested again against the community metrics using either simple or multiple regression. Values of $P < 0.05$ were considered significant in all tests and all analyses were performed in the R statistical environment (R Core Team, 2018).

Results

Overall diversity

We collected and identified a total of 7,350 individuals arranged in 23 families and six orders (Annex 1). The most diverse and abundant order in our study material was Hemiptera with eight families: Micronectidae (5,676 individuals), Notonectidae (216), Vellidae (208), Nepidae (132), Belostomatidae (108), Gerridae (52), Hydro-metridae (28), and Pleidae (1). This was followed by Odonata with five families: Coenagrionidae (98), Proto-neuridae (77), Libellulidae (73), Corduliidae (1), and Gomphidae (1). The order Coleoptera was represented by members of the Hydrophilidae (75), Hydroscaphidae (67), Dytiscidae (1), and Gyrinidae (1), whereas the order Diptera was represented by members of the Chironomidae (573), Culicidae (85), Stratiomyidae (39), and Tabanidae (3). Ephemeroptera and Lepidoptera were the least abundant orders and represented by members of the Baetidae (10) and Crambidae (5), respectively. The taxonomic richness, abundance and diversity of our study material is summarised along with water quality variables in Table 1.

Aquatic insect diversity between ponds

The aquatic insect communities of our study ponds differed significantly in taxonomic richness ($H=9.67$, $P=0.046$), abundance ($H=11.53$, $P=0.021$) and diversity ($H=11.54$, $P=0.021$) (Fig. 2).

Study ponds 2 and 4 had the greatest values for taxonomic richness (11.5 ± 1.7 and 10.8 ± 2.7 respectively), followed by pond 5 (8 ± 2.7), pond 1 (7.3 ± 3.8), and finally, pond 3 (4.8 ± 2.9). With respect to abundance, pond 5 had the greatest number of individuals ($1,600 \pm 2,087$), followed by pond 4 (80 ± 28), pond 2 (71 ± 27), pond 1 (52 ± 28), and finally, pond 3 (36 ± 41). In terms of diversity, study ponds 2 and 4 again showed the greatest values (with a mean H value of 1.94 ± 0.21 and 1.87 ± 0.47 respectively), followed by pond 1 (1.46 ± 0.44), pond 3 (0.88 ± 0.79), and finally, pond 5 (0.77 ± 0.55).

Relationship between aquatic insects and water quality

Our metrics for aquatic insects were significantly associated with just two water quality variables: turbidity and dissolved oxygen (Table 2). Taxonomic richness and abundance were negatively associated with turbidity, whereas taxonomic richness was significantly and positively associated with dissolved oxygen (Fig. 3, Table 2).

Discussion

Our study appears to be the first report on aquatic insects in urban areas in Cambodia. However, as we were only able to identify insect taxa to family level, the species diversity and composition of our study sites remains

Table 1 Summary of water quality values and community metrics recorded at five study ponds in the grounds of the Royal University of Phnom Penh.

Variable/Metric (unit)	Minimum	Maximum	Mean	Standard deviation
Temperature (°C)	28.4	31.2	29.7	0.9
Dissolved oxygen (%)	39.2	177.5	82.8	39.8
Electrical conductivity (mS/m)	78	526	292.9	161.5
Turbidity (FNU)	8.7	116	44.6	33.0
pH	7.5	9.3	8.2	0.6
Total dissolved solids (mg/L)	39	263	146.6	80.7
Taxonomic richness (taxa/sample)	1	14	8.5	3.6
Abundance (individuals/sample)	9	4684	367.5	1043.2
Shannon-Weiner diversity (H)	0	2.3	1.4	0.7

unknown. Although only 23 families arranged in six orders were documented, our study nonetheless contributes to knowledge of aquatic insects in urban areas in Cambodia. Relatively few studies have been conducted on aquatic insects in the country to date (e.g., Kosterin & Chartier, 2014; Kosterin, 2015a, 2015b; Zettel *et al.*, 2017; Freitag *et al.*, 2018) and fewer still have investigated the relationship between these and water quality and other environmental variables (e.g., Sor *et al.*, 2017, 2018).

Among the 12 orders of aquatic insects, Hemiptera and Diptera have been reported as most abundant in lentic (still water) systems, whereas Ephemeroptera, Plecoptera, and Trichoptera are the least abundant (Balian *et al.*, 2008). Our results are consistent with this finding as Hemiptera was the most commonly found order. This is likely because hemipterans can tolerate low concentrations of dissolved oxygen and high turbidity because various families in the order have different methods of replenishing air stores, including plastron respiration (Kurzatkowska, 2003; Chen *et al.*, 2015). This may explain why Micronectidae was the most abundant family in study pond 5, which had turbid and polluted waters. Similarly, the paucity of Ephemeroptera in our study likely reflects the fact that most taxa in the order prefer unpolluted waters and running waters (Collier & Lill, 2008; Sor, 2017; Sor *et al.*, 2017).

We found that study ponds 2 and 4 in the southeast portion of the RUPP grounds supported the highest taxonomic richness and diversity values for aquatic insects (Fig. 2). This may be because both ponds are characterised by diverse aquatic vegetation (including algae, water lilies, and water grass) and surrounding trees that provide good conditions for shelter and foraging and refuges from predators (Andersson, 2014). Many

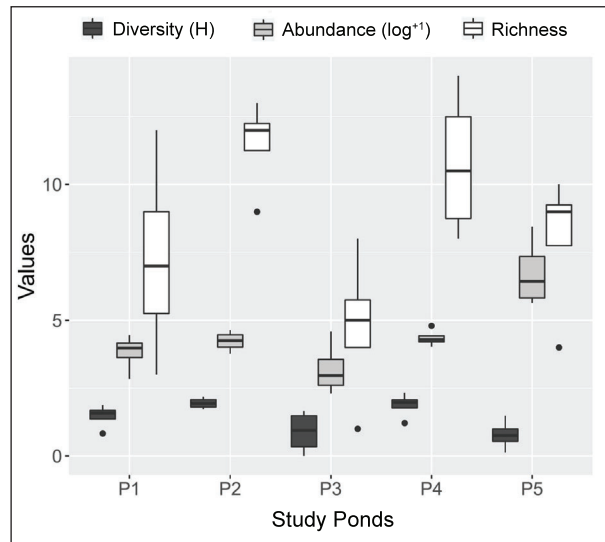


Fig. 2 Box and whisker plots of values for Shannon-Wiener’s diversity (H), abundance, and taxonomic richness of aquatic insects in five study ponds. Rectangles show the first and third quartiles, dark bars represent medians, whereas the lower and upper ends of vertical lines represent minimum and maximum values and dots represent outliers.

aquatic insect taxa thrive in undisturbed habitats (Mohd *et al.*, 2012) and changes in habitat quality (e.g., substrate composition, water quality, and physical conditions) can substantially influence their diversity. Study pond 1 supported comparatively lower diversity, which could be partly due to the reduced amount of aquatic vegetation present, whereas ponds 3 and 5 had the lowest diversity scores of all. The latter may be due to the fact that these ponds function as reservoirs which receive wastewater

Table 2 Linear regression models of community metrics and water quality variables recorded at the Royal University of Phnom Penh. Asterisks indicate that only variables that had significant relationships with community metrics (in overall multiple linear regression against all variables recorded) were employed.

Linear models	Co-efficient	Adjusted R ²	F statistic	P value
Simple*				
Taxonomic richness ~ turbidity	-7.22	0.355	11.46	0.003
Abundance ~ turbidity	0.13	-0.546	0.02	0.901
Abundance ~ dissolved oxygen	2.58	0.161	4.64	0.045
Multiple (abundance ~ turbidity + dissolved oxygen)*	-	0.49	10.17	0.001
~ Turbidity	-4.68	-	-	-
~ Dissolved oxygen	6.33	-	-	-

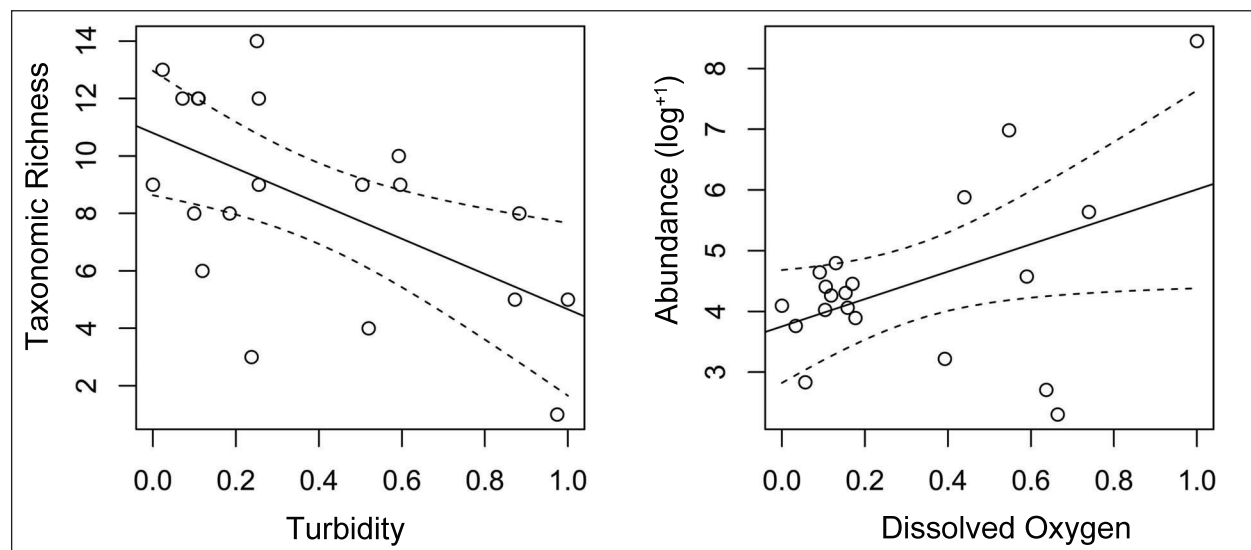


Fig. 3 Simple linear regression of the aquatic insect community metrics (taxonomic richness and abundance) and normalized water quality variables (turbidity and dissolved oxygen). Dashed-lines represent lower and upper 95% confidence intervals.

from the university and are thus more polluted. The lack of surrounding trees and aquatic vegetation at the two ponds may exacerbate this. However, our data suggest that the aquatic insect fauna of pond 3 is somewhat healthier than pond 5, which was highly dominated by members of the Micronectidae and Chironomidae, both of which are more tolerant of pollution (Slooff, 1983).

Among the water properties we tested, dissolved oxygen was the major variable which had a positive relationship with aquatic diversity in our study. This is consistent with the results of studies elsewhere, including the Mekong region in Asia (Sor *et al.*, 2017), New Zealand (Collier & Lill, 2008), and Europe (Królak & Korycińska, 2008). In contrast, water turbidity had a strong negative relationship with the taxonomic richness and abundance of aquatic insects. Limited richness and abundance of Ephemeroptera, Plecoptera and Trichoptera has been observed in highly turbid water bodies elsewhere for example (Hershey *et al.*, 2010) and it may be that water turbidity indirectly influences aquatic insects because high levels of turbidity can affect dissolved oxygen regimes (van Heest *et al.*, 2005). Factors which increase water turbidity include sediments induced by algae blooms, soil erosion and pollutants in industrial wastewater and sewage discharges (van Heest *et al.*, 2005; Ebenebe *et al.*, 2016). With respect to our study ponds, we recommend restoration efforts be undertaken to improve the water quality and surrounding environments of ponds 3 and 5.

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Annex 1 Aquatic insects and water quality variables recorded at the Royal University of Phnom Penh.

Water quality values represent mean (min–max). Asterisks indicate taxa that occurred in only one location and for which only one water quality measurement is therefore provided.

Order/Family	Abundance	Occurrence	pH	Dissolved oxygen (mg/L)	Electrical conductivity (m/L)	Total dissolved solids (mg/L)	Turbidity (FNU)	Temperature (°C)
Hemiptera								
Nepidae	132	14	8.1 (7.5-9.3)	71.4 (39.2-131.1)	260.6 (79.0-526.0)	130.4 (39.0-263.0)	36.1 (8.7-94.1)	29.7 (28.4-31.2)
Gerridae	52	10	8.0 (7.5-8.6)	63.5 (39.2-100.0)	255.7 (78.0-525.0)	127.9 (38.9-262.0)	30.7 (10.7-84.1)	29.6 (28.4-31.2)
Notonectidae	216	13	8.4 (7.7-9.3)	91.3 (39.2-177.5)	311.2 (78.0-526.0)	155.7 (39.0-263.0)	48.7 (10.7-94.1)	30.02 (28.4-31.2)
Belostomatidae	108	16	8.2 (7.5-9.3)	85.04 (39.2-177.5)	323.2 (79.0-526.0)	161.7 (39.0-262.0)	47.1 (10.7-116.0)	28.9 (27.9-31.0)
Vellidae	28	6	8.2 (7.5-9.3)	73.6 (53.8-120.8)	320.3 (155.0-408.0)	160.3 (78.0-204.0)	45.07 (18.0-84.1)	29.4 (28.3-30.4)
Micronectidae	5,676	8	8.3 (7.7-9.3)	109.4 (55.6-177.5)	406.9 (78.0-526.0)	203.5 (39.0-263.0)	63.33 (17.2-116.0)	29.8 (28.4-30.5)
Hydrometridae	28	4	7.6 (7.5-7.7)	55.8 (51.8-60.4)	287.5 (161.0-330.0)	144 (81.0-165.0)	25.5 (10.7-30.5)	29.1 (28.4-31.2)
Pleidae*	1	1	8.6	62.7	79.0	39.0	18.1	28.9
Diptera								
Chironomidae	573	13	8.2 (7.7-9.3)	87.3 (39.2-177.5)	299.4 (78.0-526.0)	149.8 (39.0-263.0)	44.9 (8.7-117.0)	29.0 (27.9-31.0)
Stratiomyidae	39	9	7.8 (7.7-8.4)	63.3 (39.2-114.9)	297.0 (155.0-526.0)	148.7 (78.0-263.0)	28.5 (8.7-59.6)	29.7 (28.4-31.2)
Tabanidae	3	3	7.8 (7.7-8.1)	57.3 (53.6-61.1)	245.7 (78.0-330.0)	123.0 (39.0-165.0)	25.9 (17.2-30.5)	28.8 (28.4-29.4)
Culicidae	85	2	8.1 (7.7-8.4)	138.8 (100.0-177.5)	518.5 (512.0-525.0)	259.0 (256.0-262.0)	55.6 (51.8-59.3)	30.4 (30.2-30.6)
Odonata								
Libellulidae	73	14	8.0 (7.5-8.6)	67.3 (39.2-177.5)	236.1 (78.0-526.0)	118.1 (39.0-263.0)	25.9 (8.7-59.6)	29.5 (28.4-31.1)
Corduliidae*	1	1	7.7	57.2	330.0	165.0	30.1	28.5
Gomphidae*	1	1	7.7	57.2	330.0	165.0	30.1	28.5
Protoneuridae	77	8	8.03 (7.7-8.6)	53.7 (39.2-63.7)	149.9 (78.0-329.0)	75.1 (38.9-165.0)	17.1 (8.7-30.5)	29.6 (28.3-31.1)
Coenagrionidae	98	11	7.9 (7.5-8.6)	71.5 (39.2-177.5)	249.3 (78.0-526.0)	124.7 (38.9-263.0)	26.5 (8.7-59.6)	29.4 (28.4-31.2)
Ephemeroptera								
Baetidae	10	5	8.1 (7.8-8.4)	75.6 (39.2-177.5)	212.8 (79.0-512.0)	106.6 (38.9-256.0)	22.4 (8.7-51.8)	30.4 (28.7-30.6)
Coleoptera								
Dytiscidae*	1	1	8.6	62.7	79.0	39.0	18.1	29.0
Hydrophilidae	75	13	8.0 (7.5-8.6)	80.2 (39.2-177.5)	341.0 (155.0-526.0)	170.6 (78.0-263.0)	41.4 (8.7-116.0)	29.7 (28.4-31.2)
Hydroscaphidae	67	10	8.1 (7.7-9.3)	63.3 (39.2-120.8)	247.9 (79.0-408.0)	124.1 (38.9-204.0)	32.3 (8.7-84.1)	29.7 (28.4-31.2)
Gyrinidae*	1	1	7.5	60.4	330.0	165.0	30.5	28.4
Lepidoptera								
Crambidae	5	5	8.02 (7.7-8.6)	65.1 (51.8-100.0)	250.0 (79.0-525.0)	125 (39.0-262.0)	27.24 (10.7-59.3)	29.8 (28.5-31.2)