



Article Species Composition of Aquatic (Nepomorpha) and Semiaquatic (Gerromorpha) Heteroptera (Insecta: Hemiptera) in Kaeng Krachan National Park, Phetchaburi Province, Thailand

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Abstract: The species composition of aquatic (Nepomorpha) and semiaquatic (Gerromorpha) Heteroptera were examined from protected and unprotected study sites in three streams associated with Kaeng Krachan National Park. At each stream, both quantitative and qualitative sampling methods were used during seven collecting events (November 2018 to June 2020). A total of 11 families, representing 33 genera and 60 species, were collected in this study, with more Nepomorpha families but higher species richness in Gerromorpha. The species richness of both protected and unprotected sampling sites were lowest during the fifth sampling event. Nevertheless, there was no significant difference in richness between protected and unprotected sampling sites for any sampling event based on a paired *t*-test analysis. Based on an nMDS analysis, the patterns of species composition of aquatic and semiaquatic heteropterans were unclear among protected and unprotected sampling sites. The use of aquatic and semiaquatic Heteroptera as bioindicators for habitat quality is still uncertain. Additional physiochemical characters of the water and physical characters of the stream may lead to a clearer picture of the relationship between aquatic and semiaquatic Heteroptera and stream habitat quality.

Keywords: Heteroptera; aquatic; species compositions; Thailand

1. Introduction

Kaeng Krachan National Park is the largest national park in Thailand, located in Phetchaburi and Prachuap Khiri Khan provinces, covering an area of approximately 2900 km² [1]. The area was announced as a national park in 1981 and as a World Heritage Site in 2021 [2]. The national park is apart of the Tenasserim Mountain Range where the Himalayan, Indochinese, and Sumatran faunae meet [3]. The national park is one of the best in Southeast Asia in terms of the preservation of wildlife habitats, with outstanding wildlife management and protection [4]. The national park fauna is highly diverse with many endemic species [5]. Therefore, numerous research projects have been conducted within the national park [5]. Nevertheless, the diversity on aquatic insects in this biologically rich national park remains unexplored. With a high biodiversity and distinguished conservation management, Kaeng Krachan National Park is a perfect study area to examine the differences in composition patterns of aquatic and semiaquatic Heteroptera between protected and unprotected sampling sites. Furthermore, studies within the park offer an opportunity to discover undescribed species of aquatic and semiaquatic Heteroptera within the faunistically complex area.

Semiaquatic Heteroptera species are in infraorder Gerromorpha [6,7]. Gerromorpha consists of eight families, 161 genera, and nearly 2120 species worldwide [8,9]. Aquatic Heteroptera are classified under infraorder Nepomorpha, with 11 families, 137 genera, and approximately 2006 species distributed worldwide [9,10]. Most Gerromorpha species live on the water surface, floating plants, and vegetation on the margins of freshwater habitats, whereas Nepomorpha live under water, including in both lentic and lotic habitats [7].



Citation: Attawanno, S.; Vitheepradit, A. Species Composition of Aquatic (Nepomorpha) and Semiaquatic (Gerromorpha) Heteroptera (Insecta: Hemiptera) in Kaeng Krachan National Park, Phetchaburi Province, Thailand. *Diversity* 2022, *14*, 462. https://doi.org/10.3390/d14060462

Academic Editors: Marina Vilenica, Zohar Yanai, Laurent Vuataz and Michael Wink

Received: 30 April 2022 Accepted: 6 June 2022 Published: 9 June 2022

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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). In the last several decades, the aquatic and semiaquatic Heteroptera in Thailand have received great attention [11–24]. Numerous new species have been discovered from this rich country [25–28]. Nevertheless, the diversity of aquatic and semiaquatic Heteroptera at Kaeng Krachan National Park remains unexplored.

Owing to their high diversity and habitat specialization, aquatic and semiaquatic Heteroptera are excellent organisms for studies in evolutionary biology, ecology, and conservation biology [29]. Most ecological studies on aquatic and semiaquatic Heteroptera investigate the relationship of these two infraorders with biotic factors (e.g., aquatic vegetation, and riparian vegetation) [30]. For example, riparian vegetation in lotic habitats is positively correlated with the nepomorphan and gerromorphan species richness [31]. Additionally, the communities of Nepomorpha and Gerromorpha in streams are influenced by abiotic factors, including stream velocity, electric conductivity, pH, organic matter, and water temperature [32,33]. Recently, ecological studies have shown effects of negative environmental changes on aquatic and semiaquatic Heteroptera communities [34–37]. Ephemeroptera, Plecoptera, Trichoptera, and Odonata were considered to be more sensitive to water quality and habitat degradation than aquatic and semiaquatic Heteroptera [38]. Nonetheless, aquatic and semiaquatic Heteroptera have been used as bioindicators in various aquatic systems [39,40]. For example, the species compositions of Nepomorpha communities may reflect ecological integrity, habitat diversity, and water quality, as well as stress by pollutants [41,42]. Furthermore, changes in the habitat structure can alter the physicochemical characters of water, which subsequently negatively effects the taxonomic diversity of Gerromorpha [35,36,43]. Therefore, gerromorphans are potential candidates as bioindicators to monitor environmental changes, especially those of anthropogenic source [30,44]. In this research, the compositions of aquatic and semiaquatic Heteroptera were compared between protected and unprotected sampling sites within three streams, each of which originates within Kaeng Krachan National Park. Aquatic and semiaquatic Heteroptera are suitable taxa for this study because (1) they are well-known taxonomically, and (2) the group has potential as indicators for habitat quality.

2. Materials and Methods

2.1. Study Area

Kaeng Krachan National Park covers the rain forests of the Tenasserim Mountain Range in the west of Thailand. The national park is located in the Mae Klong Watershed. The Phetchaburi and Pranburi rivers are two major rivers that originate from the uplands inside the national park and run through Phetchaburi and Prachuap Khiri Khan provinces, respectively.

The criteria for the paired study sites were: (1) the protected section was located in the national park, (2) the unprotected section was located at least 1 km outside the national park, and was noticeably impacted by human activities (e.g., agriculture, urbanization), and (3) sites must have been similar in size and stream morphology (e.g., the presence of riffles, stream width) for both sections (Figures 1 and 2). Three protected and unprotected sampling sites (six sites total) were chosen on three streams. The paired stream sites are (protected and unprotected sampling sites): (1) Mae Kra Dung La Waterfall (MWF) and Mae Kra Dung La Stream (MS); Ban Krang (BK) and Huai Sat Yai (HSY); and Pa La-U Waterfall (PWF) and Huai Palaw (HPL) (Figures 1 and 2).

2.2. Measurement of Physical Characters of Sampling Sites

Global Positioning System (GPS) technology was used to record latitude, longitude, and elevation (WGS84 datum). At each sampling site, basic physical characters were measured: stream width, stream depth, and riparian width (Table 1). Physical characters were measured at three randomly chosen spots and then averaged. The types of substrate and presence of marginal vegetation were also recorded at each sampling site (Table 1).



Figure 1. Map of Kaeng Krachan National Park showing three protected sampling sites located inside the national parks, including Mae Kra Dung La Waterfall (MWF); Ban Krang (BK); Pa La-U Waterfall (PWF), and three unprotected sampling sites located outside the national parks, including Mae Kra Dung La Stream (MS); Huai Sat Yai (HSY); Huai Palaw (HPL).

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Figure 2. Protected sampling sites located inside the national park: (**a**) Mae Kra Dung La Waterfall (MWF); (**b**) Ban Krang (BK); (**c**) Pa La-U Waterfall (PWF), and unprotected sampling sites located outside the national park: (**d**) Mae Kra Dung La Stream (MS); (**e**) Huai Sat Yai (HSY); (**f**) Huai Palaw (HPL).

Sampling Sites	GPS S Coordinates A	Stream Width (m) Average (Min-Max)	Depth (m) Average (Min-Max)	Riparian Width (m)	Marginal Vegetation	Substrate Types in Stream			
					warginar vegetation	Boulder	Cobble	Gravel	Sand
MWF *	13°11.175′ N 099°32.472′ E (335 m)	5.78 (2.90–11.50)	0.16 (0.09–0.31)	>30	\checkmark	\checkmark	\checkmark		
BK *	12°48.144' N 099°25.640' E (386 m)	3.12 (2.00–4.80)	0.19 (0.05–0.37)	>30	\checkmark		\checkmark		
PWF *	12°45.250' N 099°78.848' E (232 m)	7.36 (1.40–4.23)	0.20 (0.04–0.45)	>30	\checkmark	\checkmark	\checkmark		
MS	13°12.182′ N 099°32.500′ E (350 m)	3.16 (1.56–4.45)	0.26 (0.07–0.44)	5–30			\checkmark		
HSY	12°30.832′ N 099°34.151′ E (176 m)	7.80 (3.25–12.00)	0.33 (0.10–0.70)	1–5				\checkmark	\checkmark
HPL	12°32.017' N 099°29.940' E (199 m)	9.37 (1.72–12.26)	0.21 (0.10–0.46)	1–5			\checkmark		

Table 1. Physical characters of sampling sites in the study. * = protected sampling sites and $\sqrt{}$ = found.

2.3. Sampling and Identification of Aquatic and Semiaquatic Heteroptera

To determine the composition patterns of aquatic and semiaquatic Heteroptera, six sampling sites were sampled at each site (two in each stream) using both quantitative and qualitative sampling methods. Each site was sampled seven times between November 2018 and June 2020. Three mesohabitats (i.e., gravel, margin, water surface) were identified and sampled. At each sampling site, three samples of each mesohabitat (3×3) were collected using a quantitative method and one sample of each mesohabitat (1×3) was collected using a qualitative method. Therefore, 12 samples were taken at each sampling site during the sampling events. Samples were collected using an aquatic D-net, although the specific sampling techniques differed among mesohabitats. For quantitative sampling, gravel sampling was conducted over a 2 m swath by kicking the substrate while holding the net downstream. Marginal stream vegetation and roots were swept with the D-net back and forth three times. The net was swept over the water surface three times to collect surface-dwelling insects (e.g., Gerridae, Veliidae).

For qualitative sampling, one sample of each mesohabitat was collected using a similar technique as during quantitative sampling. However, sampling continued until no recognizably new morphospecies were collected in three consecutive samples. All samples were sorted in the field using soft forceps to remove specimens, which were placed into container with 80% ethyl alcohol and labeled. Specimens were identified to species level and counted under a stereo microscope using various taxonomic keys [24,45,46].

2.4. Data Analysis

Taxonomic richness was tested for a normal distribution using the Shapiro–Wilk test. A pairwise *t*-test was performed to compare protected and unprotected sampling sites, significance was set at alpha = 0.05. The normality test and paired *t*-test were performed by Jamovi 2.3.9 [47]. A non-metric multidimensional scaling algorithm (nMDS) was used to reveal community patterns of aquatic and semiaquatic Heteroptera between protected and unprotected sampling sites of the seven collecting events based on abundance data. nMDS was performed using PC–ORD 5.0 [48].

3. Results

Protected and unprotected sampling sites overlapped in physical characteristics (Table 1). Protected sampling sites are located 232 to 386 m above sea level with an average stream width from 3.12 to 7.36 m and an average stream depth from 0.16 to 0.20 m. Likewise, the unprotected sampling sites are located 176 to 350 m above sea level with an average stream width from 3.16 to 9.37 m and an average stream depth from 0.21 to 0.33 m. Conversely, the riparian width, substrate types, and the presence of marginal vege-

tation between protected and unprotected sampling sites were dissimilar. The protected sampling sites have a wider riparian width (>30 m) and marginal vegetation with larger substrates (boulder and cobble), whereas the unprotected sampling sites have a narrower riparian width (1–5 m) and lack of marginal vegetation with smaller substrates (cobble, gravel, and sand).

Approximately 2000 specimens were collected in this study. Specimens were identified to the species level when taxonomic knowledge was available, or were assigned to morphospecies otherwise. Nevertheless, at least three possible undescribed species of Heteroptera have been discovered. Two possible undescribed species of *Metrocoris* were collected from Ban Krang (BK) and Pa La-U Waterfall (PWF), and a single possible undescribed specimen of *Ranatra* was collected from root mats at Pa La-U Waterfall (PWF) (Table 2).

Table 2. Taxa collected from protected sampling sites located inside the national park and uprotected sampling sites located outside the national park. * = protected sampling sites.

	Sites						
Species	MWF *	BK *	PWF *	MS	HSY	HPL	
Nepomorpha							
Aphelocheiridae							
Aphelocheirus (M.) asiaticus (Hoberlandt & Stys)					х	х	
Aphelocheirus (A.) grik Polhemus & Polhemus					х	х	
Helotrephidae							
Helotrephes otoeis Nieser & Chen				х		х	
Hydrotrephes jani Zettel	х					х	
Tiphotrephes indicus (Distant)	х				х	х	
Micronectidae							
Micronecta quadristrigata Breddin			х			х	
Naucoridae							
Gestroiella limnocoroides Montandon					х	х	
Gestroiella siamensis Polhemus, Polhemus & Sites					х	х	
Heleolaccocoris ovatus (Montandon)			х		х	х	
Heleolaccocoris strabus (Montandon)			х				
Naucoris scutellaris (Stål)	х						
Nepidae							
<i>Cercotmetus asiaticus</i> Amyot & Serville		х	x			х	
Ranatra thai Lansbury			х	х			
Ranatra sp. A			х				
Notonectidae							
Anisops nigrolineatus (Lundblad)	х			x			
Aphelonecta gavini (Lunsbury)	х			х			
Enithares ciliata (Fabricius)	х						
Nychia sappho Kirkaldy	х			х			
Gerromorpha							
Gerridae							
Amemboa armata Polhemus & Andersen	х		х	х		х	
Amemboa cristata Polhemus & Andersen	х	х	х			х	
Limnogonus nitidus (Mayr)	х		х	х		х	
Limnometra matsudai (Miyamoto)		х	х				
Metrocoris acutus Chen & Nieser			х				
Metrocoris borneensis Polhemus		х					
Metrocoris malayensis Chen & Nieser			х				
Metrocoris nigrofasciatus Distant			х				
Metrocoris nigrofascioides (Chen & Nieser)		х	х				
Metrocoris sp. A		х	х				
Metrocoris sp. B			х				
Onychotrechus esakii Andersen			х				
Pleciogonus wongsirii Chen, Nieser &							
Wattanachaivingchareon			х	х			
Ptilomera jariyae Vitheepradit & Sites	х	х	х				
Ptilomera tigrina Uhler	x	x	x	х	х	х	
Rhagadotarsus kraepelini Breddin		50				x	
Rheumatogonus intermedius Hungerford					х	х	
Rheumatagonus vietnamensis Zettel & Chen					х	x	
0							

Table 2. Cont.

Caracian	Sites					
Species	MWF *	BK *	PWF *	MS	HSY	HPL
Ventidius modulatus Lundblad					х	х
Ventidius pulai Cheng					х	х
Hebridae						
Hebrus longisetosus Zettel	х		х			
Hyrcanus saxatilis Andersen	х					
Hydrometridae						
Hydrometra annamana Hungerford & Evans	х	х		х		
Hydrometra greeni Kirkaldy			х	х		
Hydrometra kelantan Polhemus & Polhemus		х				
Hydrometra longicapitis Torre-Bueno			x	х	х	
Hydrometra orientalis Lundblad			х			
Mesoveliidae						
Mesovelia horvathi Lundblad	х		х	х	х	х
Mesovelia vittigera (Horváth)			х	х	х	х
Veliidae						
Microvelia douglasi Scott	х	х	х	х	x	x
Microvelia genitalis Lundblad			х	х	х	x
Microvelia leveillei (Lethierry)	х					х
<i>Microvelia</i> sp. A		х	х	х		
<i>Microvelia</i> sp. B			х	х		
Neoalardus typicus (Distant)	х					
Perittopus asiaticus Zettel	х					
Rhagovelia femorata Dover	х	х	х		х	х
Rhagovelia inexpectata Zettel	х					
<i>Rhagovelia sondaica</i> Polhemus & Polhemus	х	х				
<i>Rhagovelia sumatrensis</i> Lundblad			х			
Strongylovelia setosa (Zettel & Tran)			х	х	х	
Strongylovelia sp. A	х			Х		
Species of Nepomorpha	7	1	6	5	6	10
Species of Gerromorpha	17	13	27	15	12	15
Total species	24	14	33	20	18	25

Species richness was not significantly different between protected and unprotected sampling sites (Table 3). Species richness varied from season to season, appearing the highest at the beginning of the year (January 2019 and March 2020) and falling after that (November 2019 and June 2020). Species richness was nearly always lower at unprotected sampling sites (Figure 3). However, based on the results of the paired *t*-test, there was no significant difference between protected and unprotected sampling sites during each sampling event (Table 3, Figure 3).

Table 3. Paired *t*-test of species richness between three protected and three unprotected sites (n = 3 for each). Mean = average species richness of the three study sites.

		Mea	Student t-Test		
Months	Ν	Protected Sampling Sites	Unprotected Sampling Sites	(p) *	
November 2018	3	38.70 ± 4.04	28.00 ± 3.61	0.239	
January 2019	3	43.30 ± 9.29	38.30 ± 4.51	0.402	
April 2019	3	28.70 ± 4.16	26.70 ± 7.09	0.197	
July 2019	3	24.30 ± 3.79	17.00 ± 6.56	0.19	
November 2019	3	14.00 ± 6.24	14.30 ± 4.51	0.944	
March 2020	3	52.30 ± 4.93	45.70 ± 8.96	0.109	
June 2020	3	37.30 ± 5.69	29.39 ± 4.73	0.134	

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* p > 0.05.

The results of ordination using an nMDS analysis based on abundance data showed no clear community patterns between protected and unprotected sampling sites (Figure 4). In the protected group, the community at Mae Kra Dung La Waterfall (MWF) from June 2020 was clearly separated from other sampling sites from any other sampling event because of the positive correlation with *Enithares ciliata*. Additionally, communities at Ban Krang (BK) from every sampling event were aligned together with a positive correlation with *C. asiaticus*, *H. kelantan*, *R. inexpectata*, *Ranatra* sp. A, and *R. thai*. In the unprotected group, communities at Mae Kra Dung La Stream (MS) from April 2019 to March 2020 were separated from other communities.



Figure 3. Species richness of protected and unprotected sampling sites during seven collecting events.



Figure 4. nMDS ordination plot samples by taxa dissimilarity (abundance data) between protected and unprotected sampling sites in the seven collecting events (stress = 14.80). Green refers to protected sampling sites, red refers to unprotected sampling sites, and blue refers to species.

In general, conservation management is a vital tool to promote aquatic insect diversity, especially in preserved areas [49–51]. In protected areas, the presence of a riparian zone and marginal vegetation within aquatic ecosystems provides a wide variety of suitable microhabitats for aquatic insects [52,53]. Anthropogenic activities (e.g., agriculture, deforestation, urbanization) clearly impact the diversities of aquatic insects throughout the world [54–58]. Given the inferred large number of undescribed species and higher richness of insects, the paucity of aquatic surveys, and continuing habitat destruction in tropical areas, the aquatic insect fauna of these regions are more threatened than those of temperate regions [59]. This study focused on comparisons of species composition of aquatic and semiaquatic Heteroptera between protected and unprotected sampling sites that are located inside and outside of a national park, which reflect conservation management and the influence of human disturbance, respectively.

Season affects the insect communities of both aquatic and terrestrial ecosystems [60]. The richness and composition of aquatic insects in temperate regions fluctuates throughout the year, partially due to temperature changes among the seasons [61]. Similarly, the aquatic insect diversity in the tropical zones is strongly controlled by the seasons [61–63]. Richness and abundance of tropical aquatic insects are generally positively correlated with amount of rainfall [64,65]; however, an excessive amount of rainfall can significantly cause the decline of aquatic insects in streams [66].

The lowest richness was observed during November 2019 (Figure 3), which occurred during a drought during 2019 [67,68]. Most of the streams dried up and consisted of only stagnant pools at the sampling sites. The change in water level and flow clearly effected benthic nepomorphans, especially Aphelocheiridae and Naucoridae, since most of them are adapted to living in running waters [69,70]. Pools became the only available refuges for aquatic insects during these unfavorable periods [71–73]. These aquatic true Heteroptera have been reported to colonize new suitable habitat by short flights, and some are known to estivate during unfavorable conditions [74,75]. In this study, numerous species of semiaquatic Heteroptera were found at high densities in pools during the drought period. Gerromorpha is known to temporarily colonize these mesohabitats until lotic habitats revert to their normal stage [76–78].

Although the richness of aquatic and semiaquatic Heteroptera was not significantly different, this study was similar to previous studies that found aquatic true Heteroptera were more commonly found in altered areas [79]. Two species within each family, Aphelocheiridae and Naucoridae, were associated with gravel and sandy substrates in unprotected sampling sites (Table 2) [69,70,79], and they were not abundantly present in protected sampling sites within the national park (Table 1). Although most species of Gerridae and Veliidae are not strongly associated with specific mesohabitats [11,80], some members are found only in specific mesohabitats within aquatic systems [76]. For example, species of *Metrocoris* and *Perritopus* occur abundantly at margins and rock pools of forested streams in the highlands [25,76,81,82], whereas species of *Ventidius* are commonly found in open streams in lowlands [83]. These habitat preferences were observed in this study: species of *Metrocoris* and *Perritopus* were present only from protected sampling sites, and species of *Ventidius* were found only in unprotected sampling sites.

Based on the species abundance of aquatic and semiaquatic Heteroptera, the results of nMDS showed an unclear pattern. Nevertheless, there are several interesting arrangements of sampling sites in both the protected and unprotected group. In the protected group, the Mae Kra Dung La Waterfall in March 2020 (MWF6) was separated from other protected sampling sites because of *Enithares ciliata*. In Thailand, *E.ciliata* has only been reported from forested streams [84], which describes MWF6. *E.ciliata* was not collected at any other sampling sites. Various sampling events at Ban Krang (BK) were grouped together because of the presence of *C. asiaticus*, *H. kelantan*, *Ranatra* sp. A, and *R. thai*. Ban Krang (BK) is located deep in the national park and had a higher species richness than other sampling sites (Table 2). The cluster of Ban Krang (BK) in the nMDS is probably due to location

because the diversity of aquatic insects is generally higher in remote streams in forests which are less affected by humans [85,86]. Furthermore, vegetation and root mats were abundant observed at the stream margins of Ban Krang, which provide suitable habitat for *C. asiaticus*, *H. kelantan*, *Ranatra* sp. A, and *Ranatra thai* [87–89]. In the unprotected sampling sites, several sampling events at Mae Kra Dung La Stream (MS) were separated from other sampling sites in the group. Although Mae Kra Dung La Stream (MS) is an unprotected sampling site located outside the conservation management, the physical characters of this sampling site are similar to those protected sampling sites because it is well-shaded with numerous large trees in the riparian zone and contains large emergent rocks in the stream (Table 1).

Previously, research on aquatic insect diversity and structure adjacent to different land uses (i.e., agriculture, forest, urban) indicated that both richness and abundance of streams located in forested areas are higher than those of streams associated with other land uses [90–94]. Based on this study, the use of aquatic and semiaquatic Heteroptera as bioindicators of stream habitat quality is still unclear. Additional physiochemical characteristics of water and additional physical characteristics of streams with different species richness and composition may allow for a better understanding of the relationship between aquatic and semiaquatic heteropterans and land use adjacent to stream systems [95,96]. Including additional aquatic insect orders (e.g., Ephemeroptera, Plecoptera, Trichoptera, Odonata) in assessments may assist the study as bioindicators for the quality of stream physical structure [97].

Although the aquatic and semiaquatic Heteroptera are not directly influenced by forest types or vegetative zones (they are predacious), numerous species are restricted to forests, especially gerromorphans [36,76]. The specific reason for their restricted habitat is unknown. Nonetheless, preserved forests protect habitat integrity of streams, which provide preferred or suitable habitats for aquatic and semiaquatic Heteroptera [6,30]. Therefore, conservation management is vital to protect the diversity of aquatic insects from human disturbance [98,99].

5. Summary

In total, 60 species, representing 33 genera, and 11 families of aquatic and semiaquatic Heteroptera were collected during this study. Species richness and composition did not differ significantly between protected and unprotected sampling sites. However, unprotected sampling sites tended to have lower absolute species richness than protected sampling sites. Conservation management and quality of riparian zones play a major role in shaping the composition of not only herbivorous, but also predaceous, aquatic insects. The ability to use aquatic and semiaquatic Heteroptera as indicators for habitat quality remains unclear, but this may be useful after further study.

Author Contributions: Conceptualization, A.V. and S.A.; methodology, S.A. and A.V.; software, A.V.; validation, S.A. and A.V.; investigation, S.A.; resources, A.V. and S.A.; specimen curation, A.V. and S.A.; data curation, S.A. and A.V.; writing—original draft preparation, S.A.; writing—review and editing, A.V.; funding acquisition, A.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by financial support for research provided by the Kasetsart University Research and Development Institute, KURDI (Grant No. P-2.2(D) 198.61, and FF(KU) 14.64) and the Graduate School, Kasetsart University.

Institutional Review Board Statement: This research was approved by the Institutional Animal Care and Use Committee, Faculty of Agriculture, Kasetsart University, Thailand under Project number ACKU59-AGR-008.

Data Availability Statement: Data is available from the corresponding author upon request.

Acknowledgments: We are grateful to La-au Nakthong (Kasetsart University) and Prabseuk Sritipsak (Kasetsart University) for their assistance in the field. We are grateful to Boonsatien Boonsoong (Kasetsart University) and Ratchadawan Ngoen-klan for their analysis assistance. We express our gratitude to Kaeng Krachan National Park and the Department of National Park, Wildlife and Plant Conservation for permission and logistic support to conduct the fieldwork. We thank the Department of Entomology, the Faculty of Agriculture, Kasetsart University for facility support. Lastly, we thank Michael L. Ferro (Clemson University) and reviewers for their critical comments.

Conflicts of Interest: The authors declare no conflict of interest.

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