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Aquatic Insects and Anurans in Pristine and Altered Streams in Bundu Tuhan, Sabah, for Freshwater Quality Monitoring

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Abstract Biological indicators are important components for freshwater biomonitoring to assess freshwater water quality. Aquatic insects are well-established biological indicators, but there is scarce information on anurans as potential biological indicators in Sabah. This study was conducted to (i) investigate aquatic insects in Bundu Tuhan, (ii) provide assessment for stream water quality, and (iii) understand anurans as potential biological indicators. Both aquatic insects and anurans were collected from pristine and altered streams, together with water quality data. Overall, 43 families of aquatic insects and 20 species of anurans were collected. Water quality information derived from Interim National Water Quality Standard, and biotic indices revealed that overall stream water quality of the four streams sampled were in good quality. Pearson's correlation analysis showed no association between aquatic insect and anuran taxa richness. Conversely, anuran occurrence between pristine and altered streams showed that few species have potential as biological indicators for stream.

Keywords Aquatic Insects, Anurans, Biological Indicator, Pristine and Altered Streams, Bundu Tuhan, Borneo

1. Introduction

Biological indicators are the biological processes, species or communities which assess environmental conditions and their changes over time[1]. As a part of aquatic biota, aquatic insects are one of the indicator groups that are well studied and widely implemented worldwide. Various approaches were developed to improve the efficiency to assess water quality of aquatic ecosystem. These included the earliest saprobic system once used in Europe, diversity indices, biotic indices, multimetric approaches, and multivariate approaches[2, 3, 4].

Anurans are generally considered as good biological indicators due to their semi-permeable skin and high sensitivity to environmental changes in both aquatic and terrestrial habitats[5]. Several studies investigated the potential of anurans as biological indicators in wetlands[5, 6]; streams and rivers[7, 8]; agricultural land uses[9]; and other habitats[10]. Although there has been an increasing understanding on the relationship between amphibian abundance and environmental degradation, detailed studies and empirical data to suggest amphibians as good indicators are still lacking[11].

In addition, occurrence relationship between aquatic insects and anurans in Sabah is still unknown. Therefore, the objectives of this study were to (i) understand the potentials of anurans as bioindicators by determining the occurrence relationship between anurans and aquatic insects; (ii) investigate unexplored area (Bundu Tuhan, Sabah, Malaysia) for aquatic insect diversity, and (iii) documenting the stream water quality at Bundu Tuhan based on aquatic insect data.

2. Materials and Methods

2.1. Study Site

Bundu Tuhan (N 05° 58'60, E 116° 31'60) is located 92 km from Kota Kinabalu in the Ranau District. Bundu Tuhan is considered as a montane forest, with high relative humidity and low temperature. Bundu Tuhan has three main villages: Sokid, Siba, and Kinasaraban. Total population of Bundu Tuhan is estimated to be over 3,200 people[12]. Main income of the local community is from agriculture; with 59% of the villagers are farmers[12].

Samplings for aquatic insects and anurans were carried out in July and December 2010 for five consecutive days (aquatic insect samplings) and nights (anuran samplings) for each sampling. Samples were collected from four streams in Bundu Tuhan (Figure 1). Two of the streams are in pristine area (Sungai Sinunsuyanan and Sungai Kepungit); away from human settlement. Samples for altered area were collected from streams that are affected by human activities (Sungai Geludu and Sungai Mayampak).

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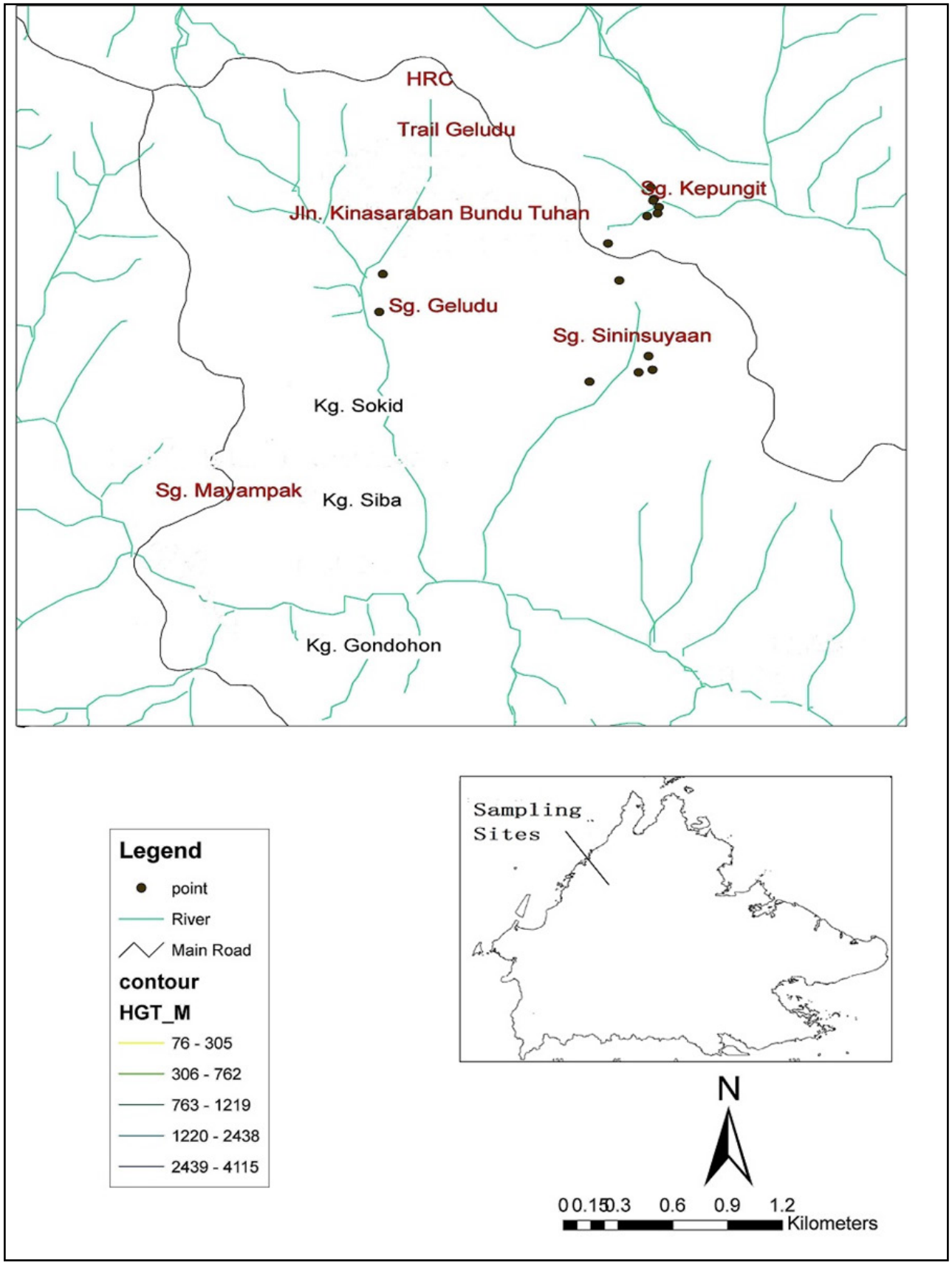

Figure 1. Map showing the four sampling sites in Bundu Tuhan: Sg. Geludu, Sg. Mayampak, Sg. Sinunsuyanan, and Sg. Kepungit

2.2. Aquatic Insect Sampling

Samplings for aquatic insects were done during the day using kick net at riffle and run habitat. The kick net was held at the bottom of the stream perpendicular to the current flow. Area of $1m^2$ was sampled for each of these habitats. Three samples were collected from each habitat. Aquatic insects from microhabitats (leaf litter and stone substrates) were collected manually. Stone substrates were selected randomly from each stream. Five packs of leaf litters were collected randomly from every stream and placed in separate plastic bags. These leaf packs were sorted upon returning from sampling. The specimens were preserved and stored in 70% ethanol. Specimens collected were identified to the family level with McCafferty keys[13]. Water quality parameters were measured with HANNA Multi-parameter Meter (HI 9828). The parameters taken were the water temperature, salinity, pH, conductivity, dissolved oxygen (DO), and dissolved oxygen percentage (DO %).

2.3. Anuran Sampling

Anuran samplings were done nightly from 1930h until 2230h. The sampling method used is opportunistic examination, which is a thorough search of all possible habitats along a sampling path for a fixed period of time[14]. Specimens were placed in a separate, clean, and transparent plastic bag. Air temperature and relative humidity were measured using a Thermo-hygrometer.

Table 1. Aquatic insect composition and distribution between pristine and altered streams

Order	Family	Sg.kepungit	Sg. Sinunsuyanan	Sg. Geludu	Sg. Mayampak	Total
Ephemeroptera	Baetidae	14	3	8	6	31
	Ephemerellidae			1		1
	Heptageniidae	111	86	57	20	274
	Siphlonuridae	4	2	4	6	16
	Tricorythidae		3	11	3	17
Plecoptera	Capniidae		2			2
	Chloroperlidae		5	5		10
	Leuctridae	1				1
	Nemouridae	4	4	13	5	26
	Peltoperlidae	12	61	25		98
	Perlidae	3	23			26
	Perlodidae		6	1		7
	Taeniopterygidae	1		1		2
Trichoptera	Brachycentridae	18	13	68	17	116
	Glossosomatidae	1	1	2	3	7
	Hydropryschidae	59	50	21	37	167
	Hydroptilidae				1	1
	Lepidostomatidae	91	49	137	23	300
	Philopotamidae	2	2		2	6
	Rhyacophilidae	3		1	4	8
	Phryganeidae	1	3		1	5
	Polycentropodidae	2	1		3	6
	Calamoceratidae		7	3		10
Diptera	Blephariceridae	1		1		2
	Chironomidae	2		2		4
	Dixidae	1	2	3		6
	Empididae				1	1
	Simuliidae	5	9	5	7	26
	Tabanidae			3		3
	Tipulidae	3	1		1	5
Coleoptera	Amphizoidae		1	1		2
	Elmidae	2	14		4	20
	Haliplidae				1	1
	Hydraenidae		1		1	2
	Hydrophilidae	1	3		4	8
	Ptilodoctylidae	1			1	2
	Psephenidae		1	1	1	3
	Limnichidae				1	1
	Lampyridae				1	1
Hemiptera	Pleidae	1	2		1	4
Lepidoptera	Pyralidae	1		1		2
Odonata	Calopterygidae			1	2	3
	Corduliidae			1		1
Total		345	355	377	157	1234

Table 2. Anuran composition and distribution between pristine and altered streams

Species	Pristine		Altered	
	Sg. kepungit	Sg. Sinunsuyanan	Sg. Geludu	Sg. Mayampak
Ansonia hanitschi	4	3	3	
Ansonia longidigita	3	3	5	5
Leptolalax dringi	1	2	1	
Leptolalax pictus			1	4
Leptobrachium abbotti				1
Megophrys nasuta		1		
Chaperina fusca				1
Limnonectes kuhlii	3		5	
*Limnonectes finchi**		1		
*Limnonectes palavanensis**		1		
Staurois tuberilinguis	5	2		
Meristogenys orphnocnemis			1	
*Occidozyga baluensis**			1	
Rana raniceps			1	
*Fejervarya limnocharis**				2
*Philautus aurantium**	1			
*Philautus bunitus**	2	2		
*Philautus mjobergi**	1			
*Philautus petersi**	4	1	1	
Rhacophorus angulirostris		2		
Total	24	18	19	13
Overall	74			

* Stream independent anurans, neither stream sitters nor stream breeders.

Anurans collected were identified using the keys in Inger and Stuebing, and Malkmus et al.[15, 16]. The anurans were euthanized in saturated Chlorobutanol solution. Anuran setting was done immediately after the specimens were euthanized. The specimens were sprayed with 10% formalin, and kept overnight in a covered specimen flat container for fixing. On the following day, the specimens were transferred into 10% formalin. Specimens were then rinsed with tap water upon coming back from the fieldwork. These specimens were stored in 70% ethanol in BORNEENSIS, the Institute for Tropical Biology and Conservation for long-term preservation.

2.4. Data Analyses

Commonly used biodiversity indices: Shannon-Weiner Index (H') and Eveness Index (J') were calculated for aquatic insects and anurans. Four indices were used for measuring the biotic index of aquatic insects in Bundu Tuhan. These indices were EPT richness[17], Family Biotic Index (FBI)[18], Biological Monitoring Work Party (BMWP)[19], and Average Score Per Taxa (ASPT)[17].

The association between aquatic insects and anurans was tested with parametric Pearson's correlation coefficient using taxa richness between anurans and aquatic insects. Anuran species that were not related to streams were excluded in this analysis. The statistical analyses were calculated using the IBM SPSS 17.0 Statistics software.

3. Results and Discussion

3.1. Composition of Aquatic Insects and Anurans

Accumulatively, a total of 43 families and 1234 individuals of aquatic insects were collected from Bundu Tuhan (Table 1). Trichoptera (50.7%) was the most abundant and the largest order (10 families) of the overall aquatic insects collected. The aquatic insects collected was dominated by family Lepidostomatidae (24.3%), followed by family Heptageniidae (22.2%).

For anurans, 20 species with 74 individuals were collectedfrom the four sampling sites (Table 2). *Ansonia longidigita* dominated 21.6% of the whole anuran collection, and followed by *Ansonia hanitschi* (13.5%).

Compared with previous studies of anurans in Bundu

Tuhan[20, 21], there was a lack of 15 species in this study. This was reasonable as the sampling areas in the previous studies covered larger terrestrial area than streams.

3.2. Aquatic Insects and Anurans Comparison between Pristine and Altered Streams

Comparing between the four sampling sites, aquatic insects showed higher abundance (700 individuals) in pristine streams. More taxa were found in altered streams (39 taxa) as compared to pristine streams with 34 taxa. There were four families found exclusively in pristine streams, namely Capniidae, Leuctidae, Perlidae, and Phrygeneidae. As for altered streams, 10 families (Ephemerellidae, Hydroptilidae, Empididae, Tabanidae, Haliplidae, Lampyridae, Ptilodoctylidae, Limnichidae, Calopterygidae, Corduliidae) were collected exclusively.

The abundance and species richness of anurans were higher in pristine streams (42 individuals; 13 species) as compared to altered streams (32 individuals; 12 species). In pristine streams, eight species, namely *Limnonectes finchi*, *Limnonectes palavanensis*, *Philautus aurantium*, *Megophrys nasuta*, *Philautus bunitus*, *Philautus mjobergi*, *Staurois tuberilinguis*, and *Rhacophorus angulirostri* were found exclusively. There were seven species only found in altered streams, which were *Occidozyga baluensis*, *Fejervarya limnocharis*, *Leptolalax pictus*, *Leptobrachium abbotti*, *Chaperina fusca, Meristogenys orphnocnemis*, and *Rana raniceps*.

3.3. Diversity of Aquatic Insects and Anurans

Both diversity indices calculated showed that Sg. Mayampak had the highest diversity (H'=2.60; J'=0.79) of aquatic insects (Table 3). The lowest values of both diversity indices (H'=2.60; J'=0.79) were for Sg. Kepungit.

The diversity of anurans was different as compared to the aquatic insect diversity. For the Shannon-Weiner diversity index, the highest diversity was for Sg. Kepungit (H'=2.05), while the lowest in Sg. Mayampak (H'=1.42). The evenness index showed that anurans were distributed most evenly in Sg. Sinunsuyanan (J'=0.87) as compared to the other three streams.

Table 3. Diversity indices of aquatic insects and anurans between the four sampling sites

Sampling sites	Aquatic insects		Anurans	
	Shannon (H')	Evenness (J')	Shannon (H')	Evenness (J')
Sg. Kepungit	2.00	0.61	2.05	0.93
Sg. Sinunsuyanan	2.38	0.72	2.21	0.96
Sg. Geludu	2.10	0.64	1.81	0.87
Sg. Mayampak	2.60	0.79	1.42	0.88

Both the Shannon-Weiner diversity and Evenness indices calculated for anurans showed higher diversity in pristine streams as compared to altered streams. Diversity indices values for aquatic insects had varied patterns between pristine and altered streams as compared to anurans. Generally, it is assumed that high diversity occurs in undisturbed environment. In this study, diversity indices performed well to reflect the disturbance that occurred in altered streams. Conversely, diversity indices of aquatic insects were consist- ent with the criticism of the usefulness of diversity indices for stream water quality assessment[4].

Table 4. Stream classification based on the Malaysia Interim National Water Quality Standard (INWQS)

Parameter	Streams	Range	INWQS	Classification
Temperature (°C)	Sg. Kepungit	19.06	Normal	-
	Sg. Sinunsuyanan	18.52-18.63	Normal	-
	Sg. Geludu	17.92-18.17	Normal	-
	Sg. Mayampak	20.73	Normal	-
pH	Sg. Kepungit	8.61-8.76	6.00-9.00	IIA
	Sg. Sinunsuyanan	8.00-8.15	6.50-8.50	I
	Sg. Geludu	7.78-8.22	6.50-8.50	I
	Sg. Mayampak	7.92-8.26	6.50-8.50	I
Conductivity (μS/cm)	Sg. Kepungit	64-133	1000	I
	Sg. Sinunsuyanan	43-46	1000	I
	Sg. Geludu	31-113	1000	I
	Sg. Mayampak	47-55	1000	I
Dissolved Oxygen (mg/l)	Sg. Kepungit	4.60-6.00	5.00-7.00	IIA
	Sg. Sinunsuyanan	4.70-5.85	5.00-7.00	IIA
	Sg. Geludu	5.31-6.75	5.00-7.00	IIA
	Sg. Mayampak	4.82-5.54	5.00-7.00	IIA

Table 5. Biotic indices values between the four sampling sites

Streams	EPT	FBI	BMWP	ASPT
Sg. Kepungit	16 (non impacted)	2.87 excellent	145 high water quality	7.25 rather clean
Sg. Sinunsuyanan	18 (non impacted)	2.57 excellent	148 high water quality	7.40 rather clean
Sg. Geludu	16 (non impacted)	2.05 excellent	126 high water quality	7.88 rather clean
Sg. Mayampak	14 (non impacted)	3.16 excellent	132 high water quality	6.95 rather clean

3.4. Water Quality of the Four Sampling Sites

Water quality data derived from the water quality parameters measured and biotic indices. The parameters measured were used to classify the streams based on the Interim National Water Quality Standards of Malaysia (Table 4). The table shows that most streams were classified into Class I and IIA with little variation.

All of the biotic indices calculated ranked the four streams as good and clean (Table 5). In general, altered streams shows lower values as compared to pristine streams. For instance, Sg. Mayampak had the lower values as compared to pristine streams except for FBI, which in contrast have the highest value for Sg. Mayampak.

3.5. Correlation Analysis

The Pearson's correlation analysis in Table 6 shows that there was no correlation between the taxa richness of aquatic insects and aquatic anuran species ($r = -0.397$; $p = 0.331$) of the four streams sampled. Therefore, the occurrence of stream anurans showed no association with aquatic insect occurrence that was highly influenced by stream water quality.

Table 6. Correlation analysis between aquatic insect taxa and stream anuran species

		anuran	insects
anuran	Pearson correlation	1	-0.397
	Sig. (2-tailed)		0.331
	N	8	8
insects	Pearson correlation	-0.397	1
	Sig. (2-tailed)	0.331	
	N	8	8

The insignificant results were possibly due to various reasons. In comparison to aquatic insects that live in streams, most anurans in this study were caught on the banks or on bank vegetation. The variation of time anuran species spent between aquatic and terrestrial habitats[11] might contribute to this result. In addition, tadpoles that are directly associated with stream were not sampled in this study due to the difficulty in Borneon tadpole identification[16].

3.6. Anurans as Potential Biological Indicator for Stream Habitat

Despite the insignificant correlation, aquatic anuran occurrence revealed their potential as biological indicators. Among the aquatic anurans sampled, it could be grouped into three groups: species which occurred only in pristine streams (*Megophrys nasuta*, *Staurois tuberilinguis*); species that occurred only in altered streams (*Leptolalax pictus*, *Leptobrachium abbotti*, *Chaperina fusca*, *Meristogenys orphnocnemis*, *Rana raniceps*); and species which occurred at both types of streams (*Ansonia hanitschi*, *Ansonia longidigita*, *Leptolalax dringi*, *Limnonectes kuhlii*). Anuran species from group one and two showed their potential to indicate stream habitat alteration, as their occurrence in pristine and altered streams was consistent with their ecological habitat description in literature[15].

4. Conclusions

This study managed to provide baseline information on aquatic insect diversity and stream water quality in Bundu Tuhan. The potential of anurans as biological indicators was determined based on their occurrence in pristine and altered streams. This is important for providing additional approaches for stream biological assessment in the future.

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