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The use of aquatic insects as bio-indicator to monitor freshwater stream health of Liwagu River, Sabah, Malaysia

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Abstract
A study was conducted to investigate the water quality in the Liwagu River, Sabah using aquatic insects as biological indicators. Sampling was carried out at different land use of the Liwagu River (forest, agricultural and urban settlement). Aquatic insects were sampled using a Surber net from August to October 2015. YSI Multiparameter was utilized to measure *in situ* parameters including dissolved oxygen (DO), salinity, conductivity, temperature and pH. *Ex situ* measurements were undertaken for Total Suspended Solid (TSS), ammonia nitrogen, phosphate and nitrate. A total of 44 genera, 34 families, eight orders and 3,126 individuals of aquatic insects were collected. Kruskal-Wallis test showed that most of the physico-chemical parameters have significant difference between the land uses of the river ($p<0.05$). Interim National Water Quality Standard for Malaysia (INWQS) showed that physico-chemical parameters in three types of land use were categorized as Class I – Class V. Biotic indices showed similar results where both forest and agricultural areas have good water quality while the human settlement area had scored moderate water quality. Pearson's correlation showed that aquatic insect abundance and taxa richness had significant relationships ($P<0.05$ or 0.01) with all physico-chemical water quality parameters except for pH and phosphate.

Keywords: Aquatic insects, Biological indicator, Water quality, Tropical stream.

1. Introduction
The effects of anthropogenic water use activities and land use management in the environment can now be easily detected. In general, rivers mirror the landscape, providing insights on their catchments [1]. Thus, ecological water management is important to enhance, the ecological integrity of aquatic systems. Such management requires a profound understanding on how the ecosystem functions and how communities are associated with their environment [2]. Vincent and March-Anre [3] had defined ecological integrity as the concept that seeks to incorporate the biotic and abiotic components of an ecosystem in regards to how they relate to their functions, goods and service output as well as their regeneration rates.

Studies by using aquatic insects as bio-indicators of anthropogenic impacts on aquatic ecosystem have shown a general decrease in aquatic insect population and the reduction in species diversity and richness. They possess a higher ability to tolerate pollution-induced environmental stress than fish and plankton [4]. Aquatic insect is a useful bio-indicator that provides a more accurate understanding of the changing water body or river system than chemical data [5]. The response of the aquatic insect to pollution gives an early warning to possible harm of the water resources because the aquatic insect spends nearly its entire life in a water body and they show the effects of physical habitat alteration, point and nonpoint contaminants over their life cycles. Aquatic insect assemblages are sensitive to disturbance and water pollution. Therefore, they are the most frequently used biological parameters in monitoring water quality [6].

Liwagu River has been subjected to agricultural and human activities that had lead to the degradation of the river water quality. Thus, this study was carried out to assess the water quality of the Liwagu River by using aquatic insect communities as a biological indicator. In addition, the study produces baseline information on the structure, diversity and group composition of aquatic insects in Liwagu River, Sabah, Malaysia.

2. Materials and Methods

2.1 Study site

The study was carried out at Liwagu River, Ranau, northeast of the state of Sabah, Malaysia (Figure 1). The river basin is located between latitudes 5° 43’ N and 5° 05’ N and longitudes 116° 51’ E and 116° 85’ E. Samplings were carried out in three types of land use consisting of the forest (Kinabalu Park), agricultural (Kundasang) and human settlement area (Ranau). Kinabalu Park covers six vegetation zones from lowland rainforest through to alpine scrub headquarters. Kundasang has a temperate climate which allows intensive vegetable production and dairy farms. Ranau is surrounded by residential area and road network.

2.2 Methodologies

The study was conducted from August to October 2015. Aquatic insects were collected from three types of land use (forest, agricultural and human settlement) using a Surber net (mesh size 125 µm, 900 cm^2area). Three replicates of the six important microhabitat/ habitats (riffle, run, pool, aquatic vegetation, leaf litter, and stone substrate) were sampled. The specimens were placed in white trays for sorting and screening. Sorted specimens were preserved in 95 % ethanol and identified with identification keys available [7-8]. At each land use, measurements of physical and chemical parameters such as dissolved oxygen (DO), temperature, salinity, conductivity and pH were made *in situ* using YSI multiparameter water quality. To randomly collect three replicates of water samples, HDPA bottles were used along the river. By using DR900 Colorimeter and gravimetric process, ammonia nitrogen, nitrate, phosphate and total suspended solid (TSS) were measured from the collected samples [9].

2.3 Data analysis

The Species Diversity and Richness software version 2 was employed to calculate the Shannon diversity index (H’) and Simpson index (1/D). Three biotic indices such as Biological Monitoring Work Party (BMWP), Average Score per Taxa (ASPT) and the richness of Ephemeroptera, Plecoptera and Trichoptera (EPT) were used to measure the biological quality of the Liwagu River Basin, Sabah, Malaysia. Kruskal-Wallis test ($p<0.05$) was used to compare the diversity indices, biotic indices, physical and chemical water quality parameters between the land use by using SPSS software version 20. Pearson’s correlation analysis was used to determine the relationships between aquatic insect communities and water quality parameters.

3. Results and Discussions

3.1 Physical and chemical parameters

Generally, Liwagu River is characterized by good water quality (Table 1). The pH of water samples varied between 7.47±0.11 in the forest area to 7.88±0.04 in the human settlement area and according to the Interim National Water Quality of Malaysia (INWQS), these areas can be categorized into Class I. In general, pH values recorded were almost at a neutral level, indicating that land use did not affect the water pH.

Table 1: Mean±SE and Kruskal-Wallis results of physico-chemical parameters along the Liwagu River, Sabah

Parameters	Liwagu River		
	Forest	Agricultural	Human settlement
Temperature (°C)*	16.92±0.12	20.01±0.31	22.62±0.47
DO (mg/L)*	7.85±0.02	7.41±0.15	7.22±0.06
pH	7.47±0.11	7.35±0.17	7.88±0.04
Salinity (%)*	0.01±0.00	0.02±0.01	0.09±0.01
Conductivity (µs/cm)*	22.07±4.08	60.8±8.18	177.33±22.05
TSS (mg/L)*	4.39±0.32	42.97±7.15	359.25±83.21
Ammonia-nitrogen (mg/L)*	0.00±0.00	0.02±0.00	0.24±0.07
Nitrate (mg/L)*	0.40±0.02	1.17±0.04	2.07±0.05
Phosphate (mg/L)*	0.12±0.01	0.99±0.19	2.02±0.25

Note: * Kruskal-Wallis test significant at $p<0.05$

Dissolved oxygen varied between 7.85±0.02 mg/L to 7.22±0.06 mg/L at every sampling area and according to the INWQS of Malaysia, these amounts categorized these sampling areas into Class I. In this study, the concentration of dissolved oxygen was highest in the upstream (forest) compared to downstream (human settlement). This could be due to the effect of temperature variation from upstream (16.92±0.12 °C) to downstream (22.62±0.47 °C). Increase in temperature will result in the decrease of dissolved oxygen [10]. Therefore, determining dissolved oxygen is important in biological assessment since it influences biological and chemical processes, which is vital for aquatic insect communities [11].

In accordance to INWQS of Malaysia, phosphorus values categorized the water quality along the Liwagu River as Class I. The significant difference in phosphorus levels between forest and human settlement area might be due to the fact that these study sites have minimum and maximum concentrations of water oxygen. Low dissolved oxygen levels are known to favor phosphorus release to the freshwater ecosystem [12]. Thus, the impacts of phosphorus on water quality were mainly found in human settlement areas due to its low oxygen levels caused by organic pollution. The level of ammonia nitrogen recorded had varying concentrations from 0.00±0.00 to 0.24±0.07 mg/L. According to INWQS, the amount of ammonia nitrogen found in the Liwagu River categorized the river as Class II. Nitrate was also detected, but in low concentrations from 0.40±0.02 to 2.07±0.05 mg/L. The low levels of nutrients may be the result of continual use of soap and fertilizer in agricultural and human settlement areas contributing to the nutrient input into the river.

Total suspended solid was found to be highest in human settlement area (359.25±83.21 mg/L) that fall under Class V. This could be due to the construction works at this area. Exposed soil at construction sites has often resulted in large sediment inputs to the streams through runoff events. In addition, the extent of fine substrate particles is generally greater in streams around the residential area [13].

3.2 Diversity of Aquatic Insects

A total of 3,126 individuals belonging to 44 genera and eight orders were recorded in the Liwagu River (Table 2). Order

Ephemeroptera was the most abundant in Liwagu River with 42.28% of the total samples. Megaloptera represented by family Corydalidae had the least abundance that was comprised of 0.48% of the total composition. For taxa richness, Coleoptera had the highest with 20.45% of the overall genera identified. Megaloptera had 2.27% of the total general, showed the lowest for generic richness.

Table 2: Mean population of aquatic insects along the Liwagu River, Sabah

Orders	Families	Genera	Liwagu River		
			Forest	Agricultural	Human settlement
Trichoptera	Hydropsychidae	*Hydropsyche* sp.	99	151	51
		Cheumatopsyche sp.	23	25	4
	Lepidostomatidae	*Lepidostoma* sp.	161	46	0
	Glossosomatidae	*Glossosoma* sp.	43	0	0
	Limnocentropodidae	*Limnocentropus* sp.	7	0	0
	Philopotamidae	*Warmaldia* sp.	39	2	0
Plecoptera	Perlidae	*Tetropina* sp.	24	0	0
		Neoperla sp.	24	0	0
	Peltoperlidae	*Peltoperlopsis* sp.	55	0	0
	Nemouridae	*Amphinemura* sp.	32	0	0
Ephemeroptera	Heptageniidae	*Epeorus* sp.	83	4	0
		Heptagenia sp.	125	19	0
		Rhithrogena sp.	2	8	0
	Baetidae	*Baetis* sp.	60	234	14
		Pseudocloeon sp.	101	398	0
	Potamanthidae	*Potamanthus* sp.	53	0	0
	Tricorythidae	*Teloganella* sp.	21	195	11
	Leptopheliibidae	*Habrophlebiodes* sp.	2	81	1
Coleoptera	Elmidae	*Grouvellinus* sp.	64	26	3
		Stenelmis sp.	123	28	8
	Psephenidae	*Odontanax* sp.	48	0	0
		Macroeubria sp.	4	0	0
	Scirtidae	*Cyphon* sp.	45	0	0
	Lampyridae	Unknown	3	0	0
	Hydrophilidae	*Berosus* sp.	0	0	0
	Gyrinidae	*Gyrinus* sp.	7	0	0
	Eulichadidae	*Stenocolus* sp.	2	0	0
Hemiptera	Gerridae	*Metrocoris* sp.	64	1	3
		Ptilomera sp.	0	0	1
	Aphelocheiridae	*Aphelocheirus* sp.	11	0	0
	Vellidae	*Rhagovelia sp.*	77	0	1
Diptera	Chironomidae	*Chironomus* sp.	8	0	108
	Simuliidae	*Simulium* sp.	122	12	0
	Blephariceridae	*Philorus* sp.	47	16	0
	Athericidae	*Atrichops* sp.	11	0	1
	Tipulidae	*Tipula* sp.	3	1	1
		Hexatoma sp.	23	0	0
	Ceratopogonidae	*Bezzia* sp.	2	16	2
Odonata	Coenagrionidae	*Ceriagrion* sp.	0	0	0
		Pseudagrion sp.	2	0	0
	Corduliidae	*Cordulia* sp.	5	9	0
	Macromiidae	*Macromia* sp.	3	0	0
	Calopterygidae	*Hetaerina* sp.	0	2	0
Megaloptera	Corydalidae	*Protohermes* sp.	15	0	0
Total			1643	1274	209

The upstream (forest area) of Liwagu River recorded the highest taxa richness (44 genera) and abundance of aquatic insects (1643 individuals) due to the diverse habitats found in this area. Mountain stream creates a heterogeneous landscape with unique and diverse habitats that support rare or endemic species [14-16]. Meanwhile, the decrease in aquatic insect diversity in the downstream (human settlement) confirmed that the intensive modification of natural habitat surrounding the river bank influence the biodiversity of aquatic invertebrates [17-23].

Table 3 showed the Shannon-Weiner index (H') and Simpson index (1/D). Aquatic insect diversity showed significant differences (P<0.05) between forest (H'=3.19; 1/D=19.89), agricultural (H'=2.08; 1/D=5.69) and human settlement area (H'=1.52; 1/D=3.00). Pearson's correlations (Table 4) showed that aquatic insect abundance and taxa richness had significant relationships (P<0.05 or 0.01) with all water quality parameters except pH and phosphate that had no significant relationship with total abundance and taxa richness. This showed that temperature, salinity, dissolved oxygen, conductivity, ammonia nitrogen, nitrate and TSS were influential factors on aquatic insect communities in Liwagu River, Sabah.

Table 3: Diversity index along the Liwagu River, Sabah

Liwagu River	Shannon (H')*	Simpson (1/D)*
Forest	3.19	19.89
Agricultural	2.08	5.69
Human settlement	1.52	3.00

Note: *Kruskal-Wallis test significance at $P<0.05$

Table 4: Pearson's correlation analysis of aquatic insect communities and water quality parameters of Liwagu River, Sabah

Parameters	Total abundance	Taxa richness
Temperature	-0.788*	-0.934**
pH	-0.744^{ns}	-0.252^{ns}
Salinity	-0.859**	-0.871**
Conductivity	-0.745*	-0.903**
DO	0.821**	0.876**
Ammonia-nitrogen	-0.748*	-0.721*
Nitrate	-0.774*	-0.942**
Phosphate	-0.664^{ns}	-0.912^{ns}
TSS	-0.817**	-0.824**

Notes: * $P<0.05$; ** $P<0.01$; ns $P>0.01$, ns=not significant

3.3 Biotic Indices
The assessment of the impacts of land use on river catchment is an important issue that many resource managers face in the modern world. Aquatic insect assemblages are widely used in the biological assessment of freshwater ecosystems due to their diverse taxa that exhibit a range of responses to river pollution levels [18]. In this study, the water quality assessment of the Liwagu River showed significant differences ($P<0.05$) between sampling sites.

The highest biotic index value at forest area corresponded with good water quality, while the lowest value in the human settlement area indicated moderate water quality (Table 5). Moderate water quality is synonymous with polluted rivers. Biotic index is an appropriate approach for this study because it takes into account the relative abundance of aquatic taxa, even reflecting the really minor changes in both abundances and community structures in the river ecosystem. It is also a flexible approach and gives the researcher freedom to select any pollution-sensitive score system based on family, generic characteristics and specific levels. Biotic index is universally applicable and usually affected by the geographical location of the river [24, 25].

Table 5: Biotic index along the Liwagu River, Sabah

Biotic Index	Liwagu River		
	Forest	Agricultural	Human settlement
EPT*	18 (Non impacted)	11 (Non impacted)	5 (Moderate impacted)
BMWP*	165 (Very high water quality	109 (High water quality)	50 (good water quality)
ASPT*	6.60 (Rather clean)	6.40 (Rather clean)	4.60 (Average)

Note: *Kruskal-Wallis test significance at $P<0.05$

Biotic index is a good approach for effective monitoring the aquatic ecosystem's health in selected rivers because it is an effective indicator of environmental pollution [26]. This method was applied in this particular study due to its simplicity, as well as being cost effective and less time consuming.

4. Conclusion
In general, the aquatic insect communities had high diversity in Liwagu River, Sabah, Malaysia. This study demonstrated the usage of biotic indices for water quality assessment in a freshwater ecosystem. Interim National Water Quality Standard for Malaysia (INWQS) showed that physico-chemical parameters in all sampling sites were categorized as Class I - Class V. Biotic indices showed similar results where both forest and agricultural areas have good water quality except for urban settlement area that had scored moderate water quality. In addition, only the human settlement area had a lack of pollutant sensitive taxa: Ephemeroptera, Plecoptera and Trichoptera. The results from this study revealed a greater diversity of aquatic insect communities and suggest for a stringent and effective biological water quality monitoring program in Liwagu River, Sabah. Further research could be conducted to determine the interaction between physical habitat quality characteristics, biological and ecological components in the tropical freshwater ecosystem.

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