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Benthic Macroinvertebrates and Trichoptera Adults for Bioassessment Approach in Streams and Wadeable Rivers in Lao People's Democratic Republic

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ABSTRACT. – Rapid bioassessment for water quality has not been established in Loa PDR. This study provides baseline information on the composition and distribution of benthic macroinvertebrate diversity and Trichoptera adults in two streams and three wadeable rivers for future rapid bioassessment in Lao PDR. Benthic macroinvertebrates were collected at each site in 20 sweeps using a D-Frame net (450 μm mesh size) in April 2017, 2018, and November 2017. Adults of Trichoptera were collected using an ultraviolet light trap. A total of 4,539 benthic macroinvertebrates belonging to 176 taxa, 84 families, 15 orders, and 3 phyla were found. Ephemeroptera and Trichoptera were the two orders with the most taxa richness and abundance. A total of 160 species in 16 families of Trichoptera adults were found. Leptoceridae and Hydropsychidae were diverse and abundant family. Agricultural activities had altered water to high electrical conductivity, high total dissolved solids and increased nutrient input even though almost all sampling sites were classified by the National Water Quality Standard as good water quality. Total taxa richness of benthic macroinvertebrates decreased with increasing disturbance. In contrast, high electrical conductivity and total dissolved solids at Nam Hinboun, a preserved area with no human disturbance exhibited the natural characteristics of a karst river. Canonical Correspondence Analysis revealed that physical variables, especially water velocity and water depth, determined benthic macroinvertebrate assemblages rather than chemical variables. The composition and distribution of Trichoptera larvae were determined by their species-specific ecological requirements. Benthic macroinvertebrate assemblages could respond to physico-chemical variables of streams better than the EPT taxa or Trichoptera taxa (both larvae and adults) and using macroinvertebrate assemblages to detect change in streams is promising.

KEYWORDS: EPT group, karst river, water quality, biomonitoring

INTRODUCTION

Freshwater ecosystems have been severely damaged worldwide by human activities. As a result of these impacts, species diversity in stream ecosystems is seriously threatened (Santos and Ferreira, 2020; Pander and Geist, 2013). Physico-chemical measurements are basic methods for monitoring the health of water; biological monitoring is an essential supplement to these measurements in many countries (López-López and Sedeño-Díaz, 2015). Aquatic organisms such as plants, plankton, animals, and microbes are natural biological indicators used to monitor changes in ecosystem health. They are also an essential tool for detecting changes in the environment and the impacts of human activities (Parmar et al., 2016). Biological indicators are important in the management and conservation of freshwater. The ecological knowledge they yield can be applied to assess the state of rivers and streams (Friberg, 2014). One popular biological indicator in monitoring the quality of lotic water is benthic macroinvertebrates. They are reliable indicators because they are ubiquitous, species-rich, abundant, and with long life cycles; their taxonomy, sampling methods, and data analyses are well established (Barbour et al., 1999). Benthic macroinvertebrates can exhibit a response to environmental changes and pollutants or other disturbance effects in water (Rosenberg and Resh, 1993). The tolerance of contamination ranges from sensitive groups to those that are highly pollution resistant, so the structure of benthic macroinvertebrate assemblages changes when environmental disturbances occur in predictable ways (López-López and Sedeño-Díaz, 2015).

The Lao People's Democratic Republic (Lao PDR), a land in Indo-Burma is mountainous and one of the richest biodiversity hotspots in Asia (Ministry of Natural Resources and Environment, 2019). The freshwater ecosystem is diverse, providing food and livelihood security for fisheries; more than 481 fish species, 37 amphibian species, 7 crab species, and 10 shrimp species have been reported (UNDP, 2021). The quality of surface water in rivers is good and subsistence fisheries are important to the Lao population, but an increasing population, expanding agricultural areas (including rubber plantations, sugarcane, and other cash crops), hydropower development, mining, infrastructure, and urban expansion may cause pollution problems in the future.

Some freshwater fish and aquatic species have been well studied. Among benthic macroinvertebrates, there was a study of aquatic insects at Vang Vieng, northern Laos (Jung et al., 2012). Benthic macroinvertebrates, particularly aquatic insects, have received interest for monitoring the health of freshwater ecosystems in many Asian countries (Mustow, 2002; Sharma et al., 2008; Varnosfaderany et al., 2010; Xu et al., 2014; Tan and Beh, 2015; Rattanachan et al., 2016; Patang et al., 2018). This study provides background information on benthic macroinvertebrates and environmental factors and correlations between them in streams and wadeable rivers in Lao PDR., compares different fauna groups collected from the same location at the same time, and considers physico-chemical variables of the streams to determine whether one fauna group was more informative than others for detecting changes in water quality or habitat among the sampling sites.

MATERIALS AND METHODS

Study sites

The Lao People's Democratic Republic is in the heart of the Indomalayan biogeographical zone and is one of the most biologically diverse countries in the world due to its relatively wide ranges of latitude and altitude and its abundance of forest and water resources. Eighty percent of the land is mountains and hills. The country is surrounded by China, Myanmar, Thailand, Cambodia, and Vietnam. The Mekong River is the major waterway of the country; it runs through the country and drains water from Lao PDR.'s land area, and it forms the border between Lao PDR, Myanmar, and Thailand. The climatic conditions of the country are a tropical-monsoon climate type, with a rainy season from May to October (MoNRE and IUCN, 2016). The study was conducted during hot (April) seasons of 2017, 2018 and cool (November) season of 2017. We selected the following five study sites in Lao PDR (Fig. 1): Nam Khan (NK, 19º43'55.58''N, 102º9'24.31''E, altitude 332 m a.s.l.) is situated in Luangprabang Province and flows through agricultural areas (with cultivation of mainly rice and millet) with the edges of the water channel having intensive erosion. Nam Thang is a tributary of Nam Song (NT, 19º6'15.12''N, 102º29'54.98''E, altitude 324 m a.s.l.). Nam Song (NS, 19º6'13.07''N, 102º30'3.16''E, altitude 315 m a.s.l.) is situated in Vientiane Province and surrounded by forested areas and small agriculture patches. Nam Hinboun (NH, 17º57'19.47''N, 104º45'28.73''E, altitude 164 m a.s.l.) is situated in Phou Hin Poun National Biodiversity Conservation Area, in Khammouan Province, flowing through limestone mountains; upstream of the sampling site is a limestone cave (Kong Lor cave) allocated for recreation. Xe Namnoy (NN, 15º13'37.99''N, 106º44'45.30''E, altitude 132 m a.s.l.) is situated in Xekong Province, which is a wadeable river with few agriculture areas among dense riparian vegetation. All study sites are perennial water bodies flowing throughout the year, with substrate types of most sampling sites including boulder, cobble, pebble, gravel, sand, and detritus; however, Nam Khan exhibited erosion from agricultural activities, and substrates consisted of mostly cobble and muck-mud in some areas.

Physico-chemical parameters of water

Three replicates of water samples at each site were randomly collected. Fifteen physico-chemical parameters were measured. They consisted of air temperature (ºC) with a thermometer, water temperature (ºC) with a Dissolved Oxygen meter (YSI model 550A), water channel width (m) with a tape measure, water depth (cm) with a steel rod measure, water velocity (m/s) with a Teledyne Gurley model 625 Pygmy Water Current meter, dissolved oxygen (DO, mg/L) with a Dissolved Oxygen meter (YSI model 550A). pH, electrical conductivity (EC, µS/cm), and total dissolved solids (TDS, mg/L) were measured with a pH/EC/TDS meter (Hanna model HI 98129). Nitrate nitrogen (NO_3-N, mg/L, cadmium reduction method), orthophosphate (PO_4^{3-}, mg/L, ascorbic acid method), and suspended solids (SS, mg/L, photometric method) were measured using spectrophotometer (Hach model DR 1900). Turbidity (NTU) was measured with a turbidimeter (Hach model 2100N). Biochemical oxygen demand (BOD_5, mg/L) was determined as the difference between initial and 5-day oxygen concentrations in dark bottles after incubation at 20ºC and measured with a Dissolved Oxygen meter (YSI model 550A), chlorophyll a (µg/L) with an extracted methanol method (APHA AWWA WPCF, 1998).

Benthic macroinvertebrates

A D-frame aquatic dip net (30 cm width, 450 µm mesh size) was used for benthic macroinvertebrate sampling. A total of 20 kicks were taken from all habitats (i.e., boulders, cobbles, pebbles, gravels, macrophytes and wood debris) over an approximately 100-m-long reach. The 20 kicked samples were then pooled, representing a single sample for each sampling collection, and immediately preserved in the field with 95% ethanol and taken to the laboratory for further processing and identification. The benthic macroinvertebrate samples from each sampling collection were sorted and subsampled in a standard tray for a total count of 300±20% individuals (Boonsoong et al., 2009). The samples of benthic macroinvertebrates were identified to the lowest

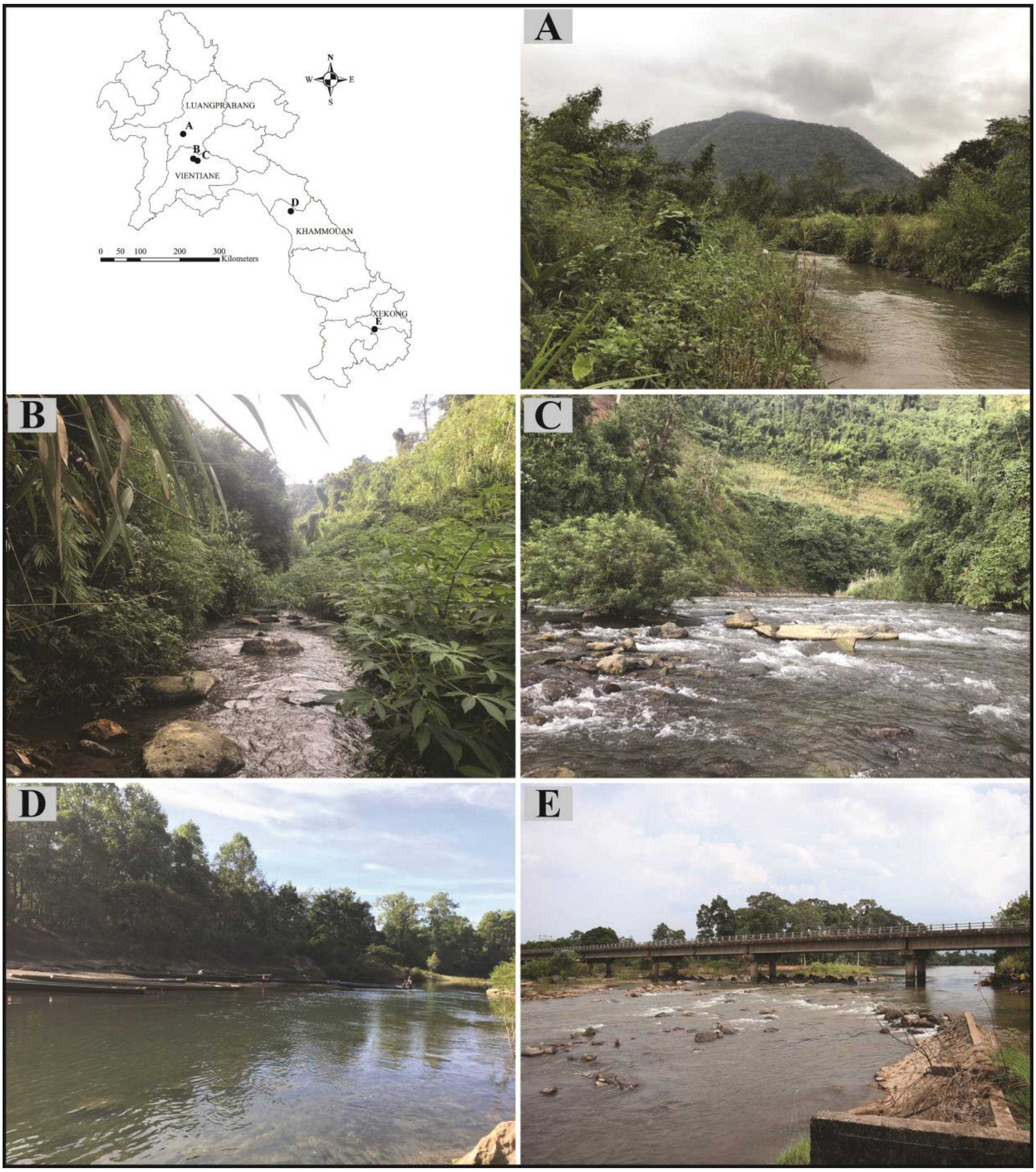

FIGURE 1. Map showing the location of study sites (●) in Lao PDR. Photographs of study sites during this study. A. Nam Khan, B. Nam Thang, C. Nam Song, D. Nam Hinboun, E. Xe Namnoy.

feasible taxon, usually family or genus. Identifications were based on Morse et al. (1994), Sangpradub and Boonsoong (2006).

Trichoptera adults

Adult aquatic insect samples were collected by using an ultraviolet light tube (8-W fluorescent, powered by an 18-volt DC battery) suspended over a pan of 95% ethanol. The ultraviolet light trap was placed at the edge of the water body adjacent to the study site, it remained lit from 5:00 p.m. to 7:00 a.m. and was always installed at the same point. Samples of the adult aquatic insects were collected the following morning and poured into a plastic container containing 95% ethanol for later identification. The adult aquatic insects were sorted and adult Trichoptera were separated from other adult insects and preserved in 95% ethanol. The male sex was used to identify species. An apex of abdominal segment of adult male genitalia was dissected, and tissue was cleared with warm 10% potassium hydroxide (KOH) for 30 min–1h. Male genitalia was identified under a stereo microscope (Nikon model C-LEDS, China) mainly following the atlas by Malicky (2010). The voucher specimens of benthic macroinvertebrates and adult Trichoptera were deposited in the Freshwater Biology Laboratory, Department of Biology, Faculty of Science, Khon Kaen University (KKU), Thailand.

Data analysis

The mean and standard deviation (Mean±SD) of 15 physico-chemical variables of the streams were presented for all study sites. The difference of each parameter between study sites was statistically tested using One-way ANOVA or Kruskal Wallis depending on the distribution of data. A Post-hoc or Pairwise comparison test was calculated to describe which study sites were significantly different from one another in each variable. Statistical testing of physico-chemical variables was performed in IBM SPSS Statistics version 23 (IBM Corp., 2015). Cluster analysis was used to group sampling sites based on physico-chemical variables. Sorensen (Bray-Curtis) similarity distance among sampling sites was calculated and the flexible beta method was used for group linkage. In addition, clusters of sampling sites based on data of benthic macroinvertebrates, Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa, Trichoptera larvae, and Trichoptera adults were performed, clusters of physico-chemical variables and clusters based on faunal data were compared to find potential faunal assemblage correspondences to physico-chemical variables. Canonical Correspondence Analysis (CCA) was used to find the relationships among physico-chemical variables and potential faunal assemblages. A Monte Carlo permutation test with 998 runs was used to verify whether physico-chemical variables were correlated with faunal distribution. Multivariate analyses were performed in PC-ORD version 5 (McCune and Mefford, 2006).

RESULTS

Physico-chemical parameters

The mean±SD of environmental variables at each site are presented in Table 1. Air temperature and water temperature ranged between 25.50–30.50ºC and 24.44–28.58ºC, respectively; Water channel width and water depth ranged 3.23–26.11 m and 16.17–42.22 cm, respectively; DO and BOD_5 ranged between 5.59–6.66 mg/L and 0.30–1.17 mg/L, respectively, EC and TDS range was 72.67–439.11 µS/cm and 37.78–226.56 mg/L, respectively; NO_3-N, PO_4^{3-}, and chlorophyll a ranged 0.22–0.33 mg/L, 0.13–0.32 mg/L and 0.80–1.51 µg/L, respectively. The results of Kruskal Wallis test revealed that DO, BOD_5, NO_3-N, and chlorophyll a were not significantly different among sites. The overall results of Pairwise tests showed that Nam Khan and Nam Thang were narrow and shallow streams, Xe Namnoy was a deep and wide water channel, Nam Song and Nam Hinboun had values of channel width and the water depth between those extremes. Moreover, chemical variables (EC, NO_3-N, PO_4^{3-}) showed that Xe Namnoy had low pollutant or nutrient input. This implied that the water quality of Xe Namnoy was better than those of Nam Thang and Nam Song, Nam Hinboun, and Nam Khan, respectively.

TABLE 1. Mean±SD and Kruskal-Wallis test of physico-chemical parameters among study sites in Lao PDR.

Physico-chemical parameter	Study sites					*p-value*
	Nam Khan	Nam Thang	Nam Song	Nam Hinboun	Xe Namnoy	
Air temperature (ºC)	25.50±4.52^{ab}	30.50±0.55^{c}	29.75±6.30^{abc}	26.17±1.64^{a}	30.00±0.87^{bc}	0.001*
Water temperature (ºC)	24.44±2.51^{a}	25.75±0.82^{ab}	26.70±1.86^{ab}	24.54±0.89^{a}	28.58±1.24^{b}	0.000*
Water channel width (m)	3.23±1.50^{a}	3.55±0.80^{a}	17.50±4.18^{ab}	19.58±3.87^{b}	26.11±7.41^{b}	0.000*
Water depth (cm)	19.22±8.24^{a}	16.17±1.33^{a}	19.67±5.50^{ab}	40.11±26.28^{ab}	42.22±13.41^{b}	0.001*
Water velocity (m/s)	0.40±0.15^{a}	0.62±0.12^{b}	0.63±0.13^{b}	0.43±0.10^{a}	0.58±0.24^{ab}	0.020*
Dissolved oxygen (mg/L)	5.59±1.42	5.88±0.50	6.37±0.81	6.52±0.72	6.66±0.77	0.248
pH	7.67±0.45^{ab}	7.74±0.25^{ab}	8.11±0.03^{b}	7.63±0.39^{a}	7.86±0.58^{ab}	0.031*
Electrical conductivity (µS/cm)	439.11±20.27^{c}	199.33±20.46^{ab}	290.00±42.75^{ab}	426.44±30.07^{bc}	72.67±10.78^{a}	0.000*
Total dissolved solids (mg/L)	226.56±12.31^{b}	104.83±11.16^{a}	153.00±22.65^{ab}	221.22±14.58^{b}	37.78±5.65^{a}	0.000*
Nitrate nitrogen (mg/L)	0.29±0.08	0.32±0.13	0.22±0.04	0.33±0.07	0.26±0.07	0.060
Orthophosphate (mg/L)	0.31±0.13^{b}	0.27±0.08^{ab}	0.32±0.12^{ab}	0.16±0.09^{ab}	0.13±0.12^{a}	0.003*
Suspended solid (mg/L)	11.78±7.36^{b}	6.67±1.03^{ab}	5.67±4.27^{ab}	3.33±1.22^{a}	8.11±3.98^{ab}	0.006*
Turbidity (NTU)	2.52±2.28^{b}	1.07±0.50^{ab}	1.17±0.73^{ab}	0.38±0.20^{a}	3.83±2.32^{b}	0.001*
Biochemical oxygen demand (mg/L)	0.99±1.17	0.71±0.47	0.30±0.19	1.03±0.95	1.17±0.30	0.065
Chlorophyll a (µg/L)	1.51±0.89	0.94±0.37	0.80±0.28	0.94±1.22	0.92±0.34	0.183

***Significant difference at p<0.05**

TABLE 2. Composition of benthic macroinvertebrate collected during the study period from sampling sites of Lao PDR.

Phylum	Benthic macroinvertebrate Order	Number of taxa	Percentage %	Number of individuals	Percentage %
Annilida	Oligochaeta	1	0.57	3	0.07
Arthropoda	Decapoda	4	2.27	31	0.68
	Isopoda	1	0.57	4	0.09
	Coleoptera	26	14.77	313	6.90
	Diptera	16	9.09	552	12.16
	Ephemeroptera	34	19.32	1614	35.56
	Hemiptera	19	10.80	294	6.48
	Lepidoptera	4	2.27	53	1.17
	Megaloptera	1	0.57	14	0.31
	Odonata	27	15.34	302	6.65
	Orthoptera	1	0.57	1	0.02
	Plecoptera	4	2.27	75	1.65
	Trichoptera	29	16.48	1203	26.50
Mollusca	Mesogastropoda	8	4.55	67	1.48
	Veneroida	1	0.57	13	0.29
Total		176		4539	

Benthic macroinvertebrate, EPT, and Trichoptera assemblages

A total of 4,539 benthic macroinvertebrates belonging to 176 taxa in 84 families, 15 orders and three phyla were found from five studied sites (Table 2). Phylum Arthropoda was the most diverse (166 taxa) and abundant group (98.16% of total individuals), followed by Phylum Mollusca and Phylum Annelida. Among Arthropoda, 96.98% of taxa (161 taxa) and 99.21% of relative abundance were insects, followed by decapods and isopod, respectively. The most diverse order was Ephemeroptera (34 taxa), followed by Trichoptera (29 taxa), and Odonata (27 taxa). Moreover, Ephemeroptera (35.56%) and Trichoptera (26.50%) were the most abundant of all benthic macroinvertebrates, while Diptera (12.16%) was the third most abundant order. Among the EPT, *Choroterpes (Dilatognathus)* was the most abundant Ephemeroptera genus, followed by *Caenis* and *Baetis*, respectively. Plecoptera consisted of 4 genera; *Neoperla* (Perlidae) was predominant, with 88% of total plecopteran individuals. The genera *Cheumatopsyche* (Hydropsychidae), *Chimarra* (Philopotamidae), and *Potamyia* (Hydropsychidae) were the three most abundant Trichoptera larvae.

A total of 40,557 individuals in 160 species, 16 families of Trichoptera adults were collected from light trapping (Table 3). Leptoceridae (54 species) was the most diverse family, followed by Hydropsychidae (32 species), and Hydroptilidae and Psychomyiidae (each with 14 species). Hydropsychidae represented 44.97% of all individuals followed by Leptoceridae (27.83%) and Philopotamidae (18.83%). In addition, thirteen families of Trichoptera were found in both adult and larval stages. Members of three families Ecnomidae, Helicopsychidae and Lepidostomatidae were found only in the adult stage.

Table 4 summarizes mean taxa richness of total benthic macroinvertebrates, EPT taxa, Ephemeroptera taxa, and Trichoptera taxa (larvae and adults) found in each study site. The results showed that Nam Thang had the highest values of total taxa, EPT taxa, and Ephemeroptera taxa and these mentioned taxa were at their lowest values at Nam Khan. The mean taxa richness of Trichoptera in larval or adult stages was highest at Xe Namnoy, but lowest at Nam Hinboun.

Multivariate analyses

Clusters were based on physico-chemical variables; site groups were associated with a gradient of chemical variables, particularly EC and TDS, rather than physical variables. Nam Khan, Nam Hinboun, Nam Song, and Nam Thang had higher values of EC and TDS than Xe Namnoy. Xe Namnoy was classified as one cluster (group 2) and the other sites were grouped to another cluster (group 1) (Fig. 2A). Clusters based on benthic macroinvertebrates, EPT taxa, and Trichoptera larvae were associated with both physical and chemical parameters of sites. In clusters based on benthic macroinvertebrates, cluster-group 1 consisted of Nam Khan, Nam Song, and Nam Thang, which were shallower sites than those of Nam Hinboun and Xe Namnoy, which were grouped as cluster group 2.

TABLE 3. Composition of adult Trichoptera collected during the study period from sampling sites of Lao PDR.

Family	Number of species	Percentage %	Number of individuals	Percentage %
Calamoceratidae	4	2.50	13	0.03
Dipseudopsidae	3	1.88	35	0.09
Ecnomidae	10	6.25	400	0.99
Glossomatidae	1	0.63	10	0.02
Goeridae	2	1.25	5	0.01
Helicopsychidae	1	0.63	6	0.01
Hydropsychidae	32	20.00	18238	44.97
Hydroptilidae	14	8.75	270	0.67
Lepidotomatidae	1	0.63	1	0
Leptoceridae	54	33.75	11288	27.83
Odontoceridae	4	2.50	291	0.72
Philopotamidae	6	3.75	7638	18.83
Polycentropodidae	9	5.63	145	0.36
Psychomyiidae	14	8.75	2203	5.43
Stenopsychidae	4	2.50	13	0.03
Xiphocentronidae	1	0.63	1	0
Total	160		40557	

TABLE 4. Mean±SD total taxa, EPT taxa, Ephemeroptera taxa, Trichoptera taxa (larvae) and Trichoptera taxa (adults) in the study sites.

Study sites	Total taxa	EPT taxa	Ephemeroptera larvae taxa	Trichoptera larvae taxa	Trichoptera adult taxa
Nam Khan	45.3±4.0	17.3±1.1	9.6±1.5	6.6±2.0	23.3±11.5
Nam Thang	68.5±13.4	27.0±7.0	14.5±0.7	10.0±5.6	36.5±3.5
Nam Song	54.0±5.6	22.5±4.9	11.5±2.1	11.0±2.8	22.0±2.8
Nam Hinboun	42.0±10.1	19.3±2.5	13.6±3.5	4.6±1.1	17.3±12.4
Xe Namnoy	48.3±5.0	27.0±2.6	13.3±2.5	12.3±0.5	48.3±19.0

Subgroups among each cluster were discriminated by the gradients of EC and TDS (Fig. 2B). For clusters based on EPT taxa and Trichoptera larvae, sampling sites were primarily grouped according to water velocity; cluster group 1 consisted of Nam Khan, Nam Song, Nam Thang, and Xe Namnoy, which had a greater water current than Nam Hinboun (cluster group 2). Subgroups in cluster group 1 were grouped by gradients of EC and TDS (Fig. 2C, D). Clusters based on Trichoptera adults differed from the four previous clusters (Fig. 2E) and did not associate with the given physico-chemical variables in this study. From the cluster analysis based on five datasets, the results showed that grouping of sampling sites based on physico-chemical variables was similar to grouping based on benthic macroinvertebrate assemblages because sampling collections of Nam Khan, Nam Thang, and Nam Song were always arranged in the same group, but sampling collections of Xe Namnoy was grouped into another one, so the data concerning benthic macroinvertebrate assemblages were used for ordination.

The ordination result revealed that cumulative percentage variance of species-environment relation for the first two axes explained 31.1% of the variance, while the eigenvalues of axes 1 and 2 were 0.358 and 0.302, respectively. The correlation coefficient values between the species-environment of both axes were all equal to 1.0 which suggests they are highly related, and a Monte Carlo test also supported a significant relationship between the two ($p<0.05$). The Monte Carlo test also revealed the significance of the eigenvalue for axis 1 ($p<0.05$). Axis 1 of CCA described the gradient of physical variables (water velocity and water depth) that correlated with changes in benthic macroinvertebrate taxa composition. Axis 2 described the gradient of chemical variables. Results of ordination showed that TDS, EC, water depth, water channel width, water temperature, water velocity, turbidity, and PO_4^{3-} were detected as the most

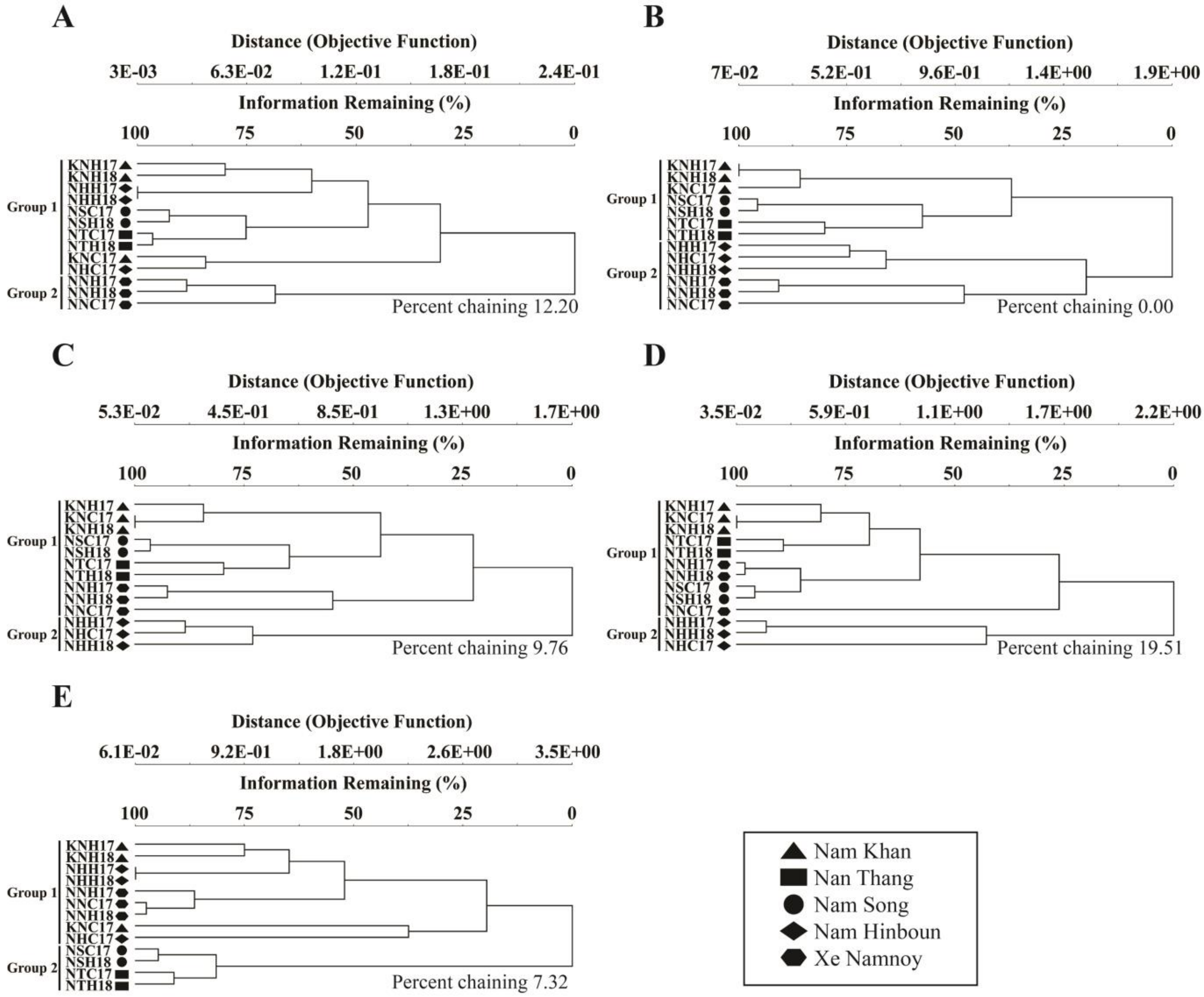

FIGURE 2. Dendrogram of cluster analysis of 13 sampling collections based on A. environmental variables, B. benthic macroinvertebrates, C. EPT taxa, D. larval of Trichoptera, E. adult of Trichoptera.

important variables explaining the distribution of benthic macroinvertebrates amongst the sampling sites (Table 5, Fig. 3). Nam Thang had relatively high water velocity and was a shallow stream while Nam Hinboun had relatively slow current and a deeper wadeable river. The highest and the lowest mean total taxa of benthic macroinvertebrates were found at Nam Thang (68.5 taxa) and Nam Hinboun (42 taxa), respectively. Axis 2 described the gradient of chemical variables. EC, TDS, and PO_4^{3-} were higher at Nam Khan, Nam Hinboun, Nam Song, and Nam Thang than at Xe Namnoy. Larvae of Trichoptera genera *Chimara* and *Potamyia*, nymphs of Ephemeroptera genera *Choroterpes (Dilatognathus)*, *Afronurus*, and *Potamanthus*, and nymphs of Plecoptera genus *Neoperla* were predominant at Xe Namnoy, which had less nutrient contamination and high current speed. In contrast, nymphs of Ephemeroptera genera *Baetis*, *Caenis*, *Ephemera*, and *Choroterpes (Euthraulus)*, larvae of Trichoptera genus *Marilia*, and larvae of Diptera genus *Hexatoma* were abundant at Nam Hinboun, which had high EC and TDS but low current speed. Larvae of Trichoptera genera *Cheumatopsyche*, *Hydropsyche*, and *Stenopsyche*, nymphs of Ephemeroptera genera *Acentrella* and *Nigrobaetis*, nymphs of Odonata genus *Melliogomphus*, larvae of Coleoptera genus *Stenelmis*, larvae of Diptera genera *Antocha* and *Simulium*, and nymphs of Hemiptera genus *Naucoris* were found together at Nam Khan, Nam Song, and Nam Thang, where high nutrient concentrations were recorded.

DISCUSSION

Physico-chemical characteristics of sampling sites

Univariate statistical analyses (Table 1) and clustering based on environmental variables of sampling sites (Fig. 2A) revealed that Xe Namnoy differed from the other sampling sites by being a deep and wide water channel with high suspension of solids and turbidity but lower nutrient input. Xe Namnoy had markedly low EC and TDS and low NO_3-N and PO_4^{3-} concentration compared to other sampling sites. Based on the results of statistical analyses on physico-chemical measurements, the order of water quality status, best to worst, is as follows: Xe Namnoy, Nam Thang and Nam Song, Nam Hinboun, and Nam Khan. High values of EC and TDS at Nam Hinboun, due to being a natural limestone mountain (or karst) wadeable river, are consistent with the study by Taylar and Ferreira (2012) at Retiro cave, where high pH and EC were found to be related to the solubility of limestone. Nam Hinboun is in a forest protected area and the study site was downstream of a recreation zone, with no agricultural activities or human disturbance occurring at the study site. In contrast, the agricultural activities at Nam Khan, Nam Song and Nam Thang were related to high values of PO_4^{3-}, EC and TDS (Boonsoong et al., 2009; Getwongsa et al., 2010; Rattanachan et al., 2016). According to the surface water quality standards of the Pollution Control Department, Lao PDR (Department of Pollution Control, 2017), water qualities of almost all sampling sites in the present study were classified as good conditions. This means that the studied sites were undisturbed or less disturbed by humans. These water resources can be used for consumption after sterilization, for aquatic animal nursery, fisheries, and water sports. Considering cluster analyses based on data of different faunal assemblages, the results showed that sampling sites were gathered into groups regarding according to both physical and chemical variables. Physical variables, particularly water depth and water velocity, and gradients of chemical variables, especially EC and TDS, effected composition and distribution of the fauna.

Relationships between environmental parameters and faunal composition and distribution of benthic fauna

Results of Canonical Correspondence Analysis (Fig. 3, Table 5) showed the same trend and strongly supported the relationship between physico-chemical variables and benthic macroinvertebrate assemblages. Water depth, water velocity, water channel width, water temperature, turbidity, PO_4^{3-}, EC, and TDS all affected the composition and distribution of benthic macroinvertebrates in our study sites. This finding concurs with the previous studies for EC and TDS in Africa (Edia et al., 2016), China (Li et al., 2016), Thailand (Mustow, 2002; Getwongsa et al., 2010; Rattanachan et al., 2016), Vietnam (Nguyen et al., 2014), and Indonesia (Patang et al., 2018). Turbidity (Sirisinthuwanitch et al., 2017), and water velocity (Mustow, 2002) also affected benthic macroinvertebrate assemblages. Moreover, water temperature, SS, nutrients (such as NO_3-N, NH_3-N and PO_4^{3-}), DO, BOD_5, chorophyll a, and pH also affected benthic macroinvertebrate assemblages (Nguyen et al., 2014; Edia et al., 2016; Li et al., 2016; Rattanachan et al., 2016; Sirisinthuwanitch et al., 2017).

TABLE 5. Results of canonical correspondence analysis (CCA) based on physico-chemical variables and benthic macroinvertebrates.

Variable	Axis 1	Axis 2	*p-value*
Eigenvalue	0.358	0.302	
Variance in species data percentage of variance explained	16.9	14.2	
Percentage of cumulative variance explained	16.9	31.1	
Pearson correlation, species-environment	1.000	1.000	
Monte Carlo test			
Test significance of eigenvalue of axis 1			0.001
Test significance of species-environment correlations of axis 1			0.011
Total dissolved solids	0.241	0.799	
Electrical conductivity	0.252	0.794	
Water depth	0.708	-0.442	
Water channel width	0.452	-0.677	
Water temperature	-0.066	-0.589	
Water velocity	-0.562	-0.400	
Turbidity	0.035	-0.483	
Orthophosphate	-0.460	0.438	

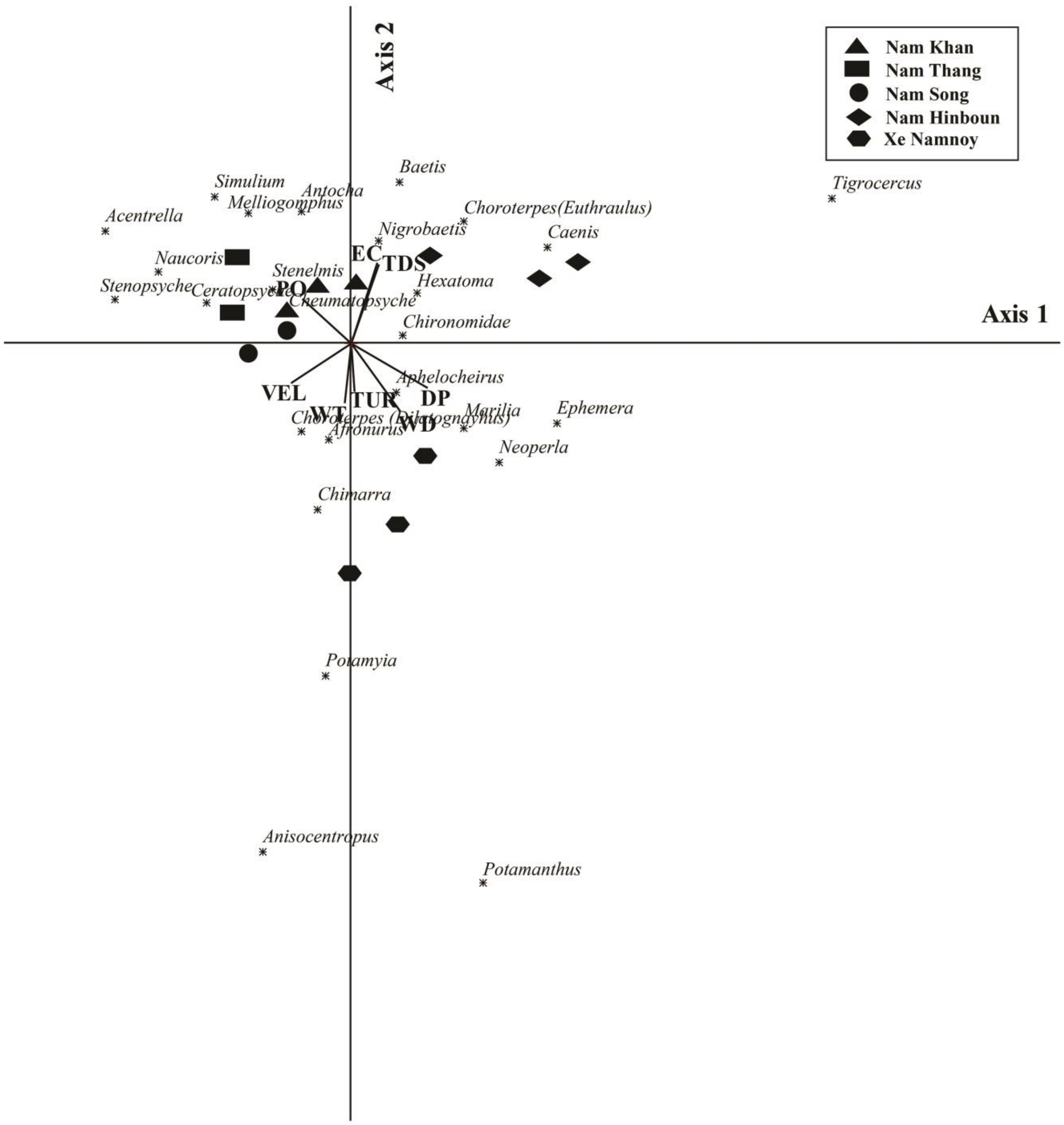

FIGURE 3. Canonical correspondence analysis (CCA) ordination of 13 sampling collections based on physico-chemical variables and 26 abundant taxa of benthic macroinvertebrates. **Abbreviations:** EC, electrical conductivity; TDS, total dissolved solids; DP, water depth; WD, water channel width; TUR, turbidity; WT, water temperature; VEL, water velocity; PO, PO43-.

Ephemeroptera (34 taxa, relative abundance 35.56%) and Trichoptera (29 taxa, relative abundance 26.50%) were diverse and dominant among benthic macroinvertebrate orders in this study (Table 2), and this trend is in support of other work such as studies in tributaries of the Mekong River (Getwongsa et al., 2010), Song and Suswa Rivers (Samweel and Nazir, 2013), and Sangi Stream (Ertaş et al., 2020). Ephemeroptera were reported to be the most diverse and abundant aquatic insect in rivers of the Vang Vieng area, northern Laos (Jung et al., 2012). Ephemeroptera and Trichoptera are diverse aquatic insect orders with world-wide distributions, their immature stages inhabit a variety of freshwater habitats (Dudgeon, 1999). Unlike the Song and Suswa Rivers in India, where Plecoptera were the second highest in genus diversity among aquatic insects (Samweel and Nazir, 2013), four genera of Plecoptera were found in the present study, whereas seven genera of Plecoptera were recorded from the Vang Vieng area, northern Laos (Jung et al., 2012). The genus and species diversity of this order is low in tropical Asian streams (Covich, 1988).

Nymphs of the ephemeropteran genera *Baetis*, *Caenis*, *Choroterpes (Euthraulus)*, and *Ephemera* were predominant at Nam Hinboun. These ephemeropteran larvae are usually found in depositional zones of streams and rivers (Waltz and Burian, 2008). Moreover, larvae of *Marilia* and *Setodes* trichopteran genera inhabit sandy areas with low flow velocity (Merrill and Wiggins, 1971; Reynaga and Rueda-Martín, 2014). Among Hydropsychidae, larvae of *Cheumatopsyche* are usually found in slower water velocities than those of other hydropsychid larvae that spin silk to make shelter retreats and catch food in fast-flowing water (Wiggins, 1996; Morse and Holzenthal, 2008). The occurrence of *Cheumatopsyche* and *Marilia* larvae, and the four previously mentioned ephemeropterans at Nam Hinboun implied that the majority of habitats at this study site were lotic-depositional zones which corresponded with the results of the physical variables measurement (slow water current average 0.43 m/s and average 40.11 cm water depth). This finding could explain the cluster for Nam Hinboun apart from other clusters in the dendrograms based on data of EPT taxa and data of Trichoptera larvae. *Acentrella*, *Antocha*, *Cheumatopsyche*, *Hydropsyche*, *Melliogomphus*, *Naucoris*, *Simulium*, and *Stenelmis* were abundant at Nam Khan, Nam Song, and Nam Thang. *Acentrella*, *Antocha*, *Baetis*, *Caenodes*, *Cheumatopsyche*, *Hydropsyche*, *Choroterpes*, *Simulium*, and Gomphidae were found in higher numbers in agricultural areas than in forest areas (Getwongsa et al., 2010). *Cheumatopsyche*, a tolerant trichopteran of family Hydropsychidae which can exploit disturbed areas (Dudgeon, 1999) or agricultural areas (Getwongsa et al., 2010), was a dominant taxon at Nam Khan, consisting of 20% of total individuals of benthic macroinvertebrates. *Chimarra*, *Choroterpes (Dilatognathus)*, *Afronurus*, *Potamanthus*, *Potamyia*, and *Neoperla* were abundant at Xe Namnoy. *Chimarra* and *Neoperla* were more abundant in forest areas (Feio et al., 2005; Getwongsa et al., 2010). Shredder trichopterans *Anisocentropus* and *Ganonema* (family Calamoceritidae) were found only at Xe Namnoy, which implied that input of allochthonous coarse particulate organic matter is high at this site. High numbers of *Chimarra* and *Leptocerus* caddisfly larvae also indicated availability of allochthonous energy sources at Xe Namnoy. Larvae of *Chimarra* usually fasten their nets on leaves litter, while *Leptocerus* construct portable cases with wood debris in the stream (Wiggins, 1996).

Benthic fauna and Trichoptera adults for bioassessment approach in Lao PDR

Benthic macroinvertebrates and especially EPT taxa are widely used in biomonitoring programs (Morse et al., 2007; Boonsoong et al., 2009; Xu et al., 2014; Rattanachan et al., 2016; Wimbaningrum et al., 2016). EPT taxa are considered generally sensitive or intolerant to pollution. Richness of benthic macroinvertebrates or EPT taxa decrease in reaction to increasing levels of anthropogenic disturbance (Rothrock et al., 1998; Houghton, 2011; Edia et al., 2016; Ertaş et al., 2020). Apart from the relatively undisturbed sampling site Nam Hinboun, our study showed the same trend, that values of mean total macroinvertebrates taxa, and mean EPT taxa, were lower in more human-disturbed site (Nam Khan) than those of less disturbed sites (Nam Thang, Nam Song, and Xe Namnoy). However, almost all sampling sites had good water quality based on the values of physico-chemical variables of water.

The diversity and composition of benthic macroinvertebrates, particularly the aquatic insect assemblages, are complex. At Nam Hinboun, the average of Ephermeroptera taxa was high, but Trichoptera taxa was the lowest taxa (Table 4). Low diversity of total taxa of benthic macroinvertebrates, EPT taxa, Ephemeroptera taxa, and Trichoptera taxa at Nam Khan were correlated with anthropogenic influences. Intolerant taxa decrease with increasing human environmental impact. In contrast, it is possible that total taxa of benthic macroinvertebrates and EPT taxa at Nam Hinboun may be naturally low, caused by physical features of the stream, and the lowest number of Trichoptera taxa due to their species-specific ecological requirements (Wiggins, 1996; Altermatt et al., 2013). Most Trichoptera genera found in this study usually inhabited lotic-erosional habitats that did not match the depositional zones which comprised the major habitat characteristics at Nam Hinboun. The diversity and composition of aquatic insects were affected by network centrality, catchment area, elevation, and their interactions. The drainage basin provides the available regional species pool, but dependence on local and spatial factors are different across orders of aquatic insects, which indicate diversity and species richness patterns (Altermatt et al., 2013). Many studies have shown that water velocity variability and its related physico-chemical factors were more important to the change of benthic macroinvertebrate assemblages than the chemicals used in agricultural activities (Crosa et al., 2001; Ricart et al., 2010; Brix et al., 2012). Our findings supported the correlation of those physical factors, particularly finding that water depth, and water current velocity influence the substrate characteristics at the site by affecting substrate particle size, food source availability, exerting a physical force on aquatic organisms to meet their physiological requirements, and consequently determining the inhabiting fauna (Allan, 1995; Edington and Hildrew, 1995).

In the present study, 13 and 16 families of larvae and adults of Trichoptera were found, respectively. Larvae were identified to genera while adults were identified to species. The dendrogram of Trichoptera adults was different from other dendrograms, possibly because of the different levels of identification, with more families in adult stage. Houghton et al. (2011) also found more genera of caddisfly adults than those of larvae at the same sampling sites. Many studies mentioned that most Ephemeroptera, Plecoptera, and Trichoptera adults dispersed for 10–20 m from emergent streams, but some species could fly for over 100 meters to several kilometers from inhabited streams, and several EPT species were found to disperse to adjacent streams (Crichton and Fisher, 1982; Griffith et al., 1998; Briers and Gee, 2004; Petersen et al., 2004; Macneale et al., 2005; Brakel et al., 2015). Long distance dispersal in aquatic insects may be highly beneficial in exchanging genetic materials between populations (Schmidt et al., 1995). It is possible that some of the adult caddisflies in the light traps came from freshwater sources other than the streams beside which the traps were placed, perhaps explaining why a greater species diversity and the additional three families were captured with light traps. Houghton (2004) mentioned that hydropsychids *Cheumatopsyche campyla*, *Hydropsyche simulans*, and *Potamyia flava* (Family Hydropsychidae) could be used as indicators to show organic enrichment in small and medium streams in Minnesota, but they failed to indicate organic pollution in large rivers (Houghton, 2006). Small and medium streams naturally contain coarse particulate organic matter, especially allochthonous inputs, and these smaller streams then export fine particulate organic matter to the large rivers downstream, resulting in different relative abundances of invertebrate functional feeding groups along the river (Vannote et al., 1980). Increases of fine particle organic matters and large proportions of filter-feeders are naturally observed in large rivers. This could explain why responses of the same species differed in different habitats (small streams vs large rivers). A component of ecological diversity was not captured using only assemblages of EPT, and trichopteran as monitors because the physical features of streams had important roles indicating that species inhabited them to meet their physiological and behavioral requirements.

In conclusion, benthic macroinvertebrate assemblages can respond to physico-chemical variables of sampling sites better than using just one or some selected aquatic insect groups for monitoring, and the results showed that using benthic macroinvertebrates to detect changes in streams and wadeable rivers in Lao PDR. is a promising approach. More research for appropriate methods is required to associate various aspects of the diversity and distribution of benthic macroinvertebrates and their relationships among them, physico-chemical parameters, and different land uses in Lao PDR.

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