# The response of aquatic insect assemblages to diverse land-use types and environmental factors in Mae Ram River basin, Thailand.

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**ABSTRACT**: We assessed the response of aquatic assemblages to 3 types of land uses (horticulture, forest, and rural/urban areas) and the relationship between physicochemical and environmental parameters of the Mae Ram River basin. The physicochemical and biological variables were significantly varied among the types of land uses. Diversity significantly declined in both horticultural and rural/urban areas. Traits were satisfactorily explained by environmental variables across land-use gradients. The main drivers of trait variations were divided into 2 groups: ammonia and nitrate at upstream horticultural sites and total dissolved solids, orthophosphate, and conductivity in downstream rural/urban areas which also influenced Caenidae distribution. Functional feeding groups (FFGs) did not conform completely to the river continuum concept. This could be related to the degradation of the basin resulting from human activities from upstream to downstream. Our results indicate that land-use type can help predict aquatic ecosystem health and anthropogenic support of a diverse community of aquatic insects in the Mae Ram River basin.

KEYWORDS: aquatic insect, intensive agriculture, land use, water quality

## INTRODUCTION

Mae Ram River basin is in Northern Thailand, Chiang Mai province, with a drainage area of 53.72 km<sup>2</sup> and is one of the headstreams of Ping River, a major river in Northern Thailand. This river runs from the top of the mountain, passing through a wide range of human activities. It is divided into 3 agro-ecological zones where the upper zone is dominated by Hmong people, the middle zone by the forest and small Karen villages, and the lower stream by rural urbanization of local Thais [1,2]. Mae Ram River has been facing an ongoing process of land use and suffered from intensive agricultural activities on the upstream, while the downstream has been impacted by increasing urbanization in river catchment areas [2]. The agricultural intensification in Mae Ram River basin can be distinguished by the use of fertilizers, pesticides, agricultural machinery, and intensive labor [3]. Human activities along the river often lead to anthropogenic pollutants in aquatic ecosystems. Especially, agriculture and urbanization can lead to soil erosion and runoff as well as nutrient loading. As a consequence of this, the anthropogenic disturbance in stream habitats can cause dramatic ecological transformations, including changes in ecosystem processes and community structure of aquatic organisms. Alterations of aquatic insect communities have been the most extensively studied ecological responses to agricultural and human impacts in a freshwater ecosystem. Several studies

over the last decade have shown that changes in land use are one of the primary causes of local and regional biodiversity loss [4]. These changes to the structure of aquatic insect communities have been documented with the conversion of natural landscapes for both agricultural uses and urban development [5, 6].

The examination of aquatic insect communities is useful for biomonitoring to assess the environmental constraints in a lotic ecosystem that can provide great insights into ecosystem health based on the diversity of life histories and stressor tolerance exhibited by different species. Also, the functional feeding group (FFG) approach can be used to classify aquatic ecosystem health by considering aquatic insect assemblages which are well-known to respond rapidly to pollutants. Shredders and scrapers are said to be more sensitive to environmental changes, while filterers and gatherers are more tolerant to pollutions [7]. Hence, FFGs of aquatic insects can be used to reflect the anthropogenic impacts on aquatic ecosystems and the environment.

Although many publications paid attention to aquatic insects and their distribution, abundance, and diversity in Thailand and Southeast Asia [8–10], none of them have directly connected the structure of insect community to land use or land cover in the river catchment area. In addition, few studies that describe invertebrate dynamics of tropical streams over several years are available.

Therefore, this is the first report on the Mae Ram River basin that provides information on the impacts of



Fig. 1 Location of study sites at Mae Ram River basin.

different land uses on aquatic insect assemblages from upstream to downstream. Understanding freshwater degradation and community patterns across the landuse gradient is necessary to investigate specific factors within agricultural or developed areas that are affecting stream ecosystems in developing countries like Thailand. This study will contribute to the knowledge of how aquatic insects are distributed under various human activities as well as the environmental factors and land-use characteristics for their abundance that reflects on functional feeding group and will identify key areas for protection efforts concerning the assemblages of aquatic taxa which are crucial for ensuring long-term aquatic insect biodiversity in intensive agricultural areas. Moreover, the knowledge of the characteristics of land-use types will help illuminate the distribution characteristics of the organisms and aid local conservation planning and future actions, helping to predict conditions under which loss of freshwater biodiversity occurs.

## MATERIALS AND METHODS

## Study sites

We aimed to identify suitable sampling locations along the Mae Ram River basin, focusing on 3 types including forest, agricultural, and residential areas. The 9 sampling sites and their catchment are all situated in the Mae Ram River basin. Its centroid geographic coordinate is between  $8^{\circ}54'59''$  N to  $18^{\circ}58'43''$  N latitude and  $98^{\circ}46'31''$  E to  $98^{\circ}55'56''$  E longitude. It has a total area of  $53.72 \text{ km}^2$ . Nine sampling sites were selected along the Mae Ram River with different landuse and land-cover patterns: MR1, MR2, and MR3 located upstream (agricultural area: horticulture); MR4, MR5, and MR6 located midstream (forest area); and MR7, MR8 and, MR9 located downstream (residential area: rural/urban area) (Fig. 1).

# Land use

To evaluate the potential of Landsat imagery to assess land use and land cover in the Mae Ram River basin, the primary data and secondary data need to be obtained. The primary data have been collected by field surveys and a topography map. In terms of the secondary data, the satellite data from Landsat8 required preprocessing which was performed using geometric correction. The geometric correction method is essential to preprocess the satellite imagery data and eliminate the geometric distortion by Pan sharpening [11]. The land use and land cover were identified using the open-source Quantum GIS (QGIS) software with supervised classification by the maximum likelihood method. Then, the data on land use and land cover were validated in the field before being used.

#### **Environmental sampling**

Physicochemical parameters were assessed in laboratory analysis including conductivity (mg/l), total dissolved solids (mg/l), pH, velocity (m/s), dissolved oxygen (mg/l), biochemical oxygen demand (mg/l), air temperature, water temperature, and nutrients (ammonia, nitrate, and orthophosphate). All parameters were analyzed according to the procedures described in Standard Methods for the Examination of Water and Wastewater [12].

#### Aquatic insect sampling

Each site was sampled on 2 occasions in June and October 2019 with 3 replicated sampling of aquatic insects from random locations with riffle zone using a D frame net (250  $\mu$ m mesh size). Aquatic insects were sampled using the multi-microhabitat sampling technique. The samples were preserved in 80% ethanol prior to laboratory analysis. In the laboratory, aquatic insects were sorted and identified under stereomicroscope and preserved in 95% ethanol. The taxonomical identification was conducted to the family level using taxonomic references by Dudgeon [13] and Mekong River Commission [14]. All taxa were categorized into FFGs based on available information [15].

# Data analysis

To assess the response of aquatic insect communities to land use and environmental variables, Richness, Shannon-Wiener index (H'), and Pielou evenness index (J) were applied to describe the characteristics of the aquatic insect communities using the diversity function in the R package vegan [16]. Before performing comparison analyses, data normality was checked using Shapiro-Wilk test. As the physicochemical and biological data follow normal distribution (p > 0.05), the parametric test one-way ANOVA was performed to compare data variability between sampling sites. When significant differences were detected, post hoc pair-wise comparisons would be performed using Tukey's test. If the data were not normally distributed, non-parametric Kruskal-Wallis test would be used, followed by Dunn's post hoc pairwise comparison [16]. The associations between the significant differences among environmental factors were tested using SpearmanâĂŹs rank correlations. Aquatic insects that appeared in less than 5% (rare species) of all sampling sites were removed before multivariate analysis was performed to avoid skewness of the ordination plots. Detrended correspondence analysis (DCA) was performed to determine gradient lengths to select the appropriate response model. If it was less than 3 standard deviations, Redundancy Analysis (RDA) would be used. RDA is a constrainedordination technique defined by selecting the linear combination of environmental variables that best explain the variation of the dependent matrix [17]. Before RDA test, the aquatic insect abundance data were Hellinger-transformed to standardize absolute values. The proportion of variance explained by each set of the explanatory variables was described by  $R^2$  adjusted, and significance levels were calculated using Monte Carlo unrestricted 999 permutation tests [18]. All data analyses for diversity and multivariate analyses were created using the statistical software RStudio version 1.2.5033 [19].

# RESULTS

# Environmental factors and land use

The environmental factors in different land-use types of the Mae Ram River basin were shown in box plots and tests. There was a significant association between land-use type and biochemical oxygen demand (F =5.355, p < 0.05), total dissolved solids (F = 31.395, p < 0.01), and conductivity (F = 19.520, p < 0.01). It showed that the values of those parameters for the rural/urban areas tended to be higher than those for forest and horticulture areas (Fig. 2(a,c,d)), while nitrate (F = 5.416, p < 0.05) and ammonia (F = 15.521, p < 0.01) values tended to be low in rural/urban areas but high in horticultural areas (Fig. 2(e,g)). In addition, we found that the ammonia level upstream was greater than 0.5 mg/l, which is over the limit of Surface Water Quality Standards of Thailand. However, there were no significant differences (p > 0.05)for dissolved oxygen and orthophosphate among the types of land use (Fig. 2(b,f)). Mean values  $(\pm SD)$ of environmental variables at each sampling site in different land uses are provided in the supplementary document (Tables S1 and S2).

#### The relationship among environmental variables

Considering the correlation among 12 environmental variables, the overall physical and chemical components showed that the total dissolved solid had a strong positive correlation to conductivity (r = 0.99, p < 0.001) and orthophosphate (r = 0.77, p < 0.01) but had a negative correlation to ammonia (r = -0.84, p < 0.01), nitrate (r = -0.79, p < 0.01), and elevation (r = -0.74, p < 0.01) (Fig. S1).

#### Aquatic insect community structure

A total of 20,538 individual aquatic insects were sampled across all sampling sites, representing 62 families. Aquatic insects in the order Ephemeroptera were the most abundant throughout the sampling periods at 43.4%, followed by order Diptera, order Trichoptera, order Odonata, order Coleoptera, order Hemiptera, and others (order Lepidoptera and Plecoptera) at 31.9%, 16.7%, 4.8%, 1.7%, 1.3%, and 0.2%, respectively. The overall organism distribution showed that Baetidae in the order Ephemeroptera was the most common family, followed by Chironomidae in the order Diptera, Caenidae in the order Trichoptera, and Simuliidae in the order Diptera, respectively.

Aquatic insect abundance showed greater differences between the types of land use, (Tukey test, p < 0.05). The highest abundance of aquatic insects was recorded in the horticultural areas, followed by rural/urban areas and forest areas, respectively. Moreover, the Shannon diversity and Pielou index showed significant differences among the types of land use (Tukey test, p < 0.05). Surprisingly, the highest value



**Fig. 2** Differences in environmental variables and land-use types of Mae Ram River; (a) Biochemical oxygen demand (BOD), (b) Bissolved oxygen (DO), (c) Total dissolved solid (TDS), (d) Conductivity, (e) Nitrate, (f) Orthophosphate, (g) Ammonia, and (h) Elevation. Letters indicate significant differences according to Tukey's test (except Elevation; Dunn's test). Bold horizontal lines represent medians, boxes represent interquartile ranges (25th–75th percentiles), and range bars show maximum and minimum values.



**Fig. 3** Box plots of different land-use types and macroinvertebrate communities. (a) Abundance, (b) Family richness, (c) Shannon diversity, and (d) Pielou evenness. Letters indicate significant differences according to Tukey's test. Bold horizontal lines represent medians, boxes represent interquartile ranges (25th–75th percentiles), and range bars show maximum and minimum values.

of Shannon diversity index and Pielou index were found in forest and rural/urban areas. There was no significant difference in family richness among land-use types (Tukey, p < 0.05) (Fig. 3).

#### Functional feeding groups and the environment

Overall, the most present FFG was gathering collectors which made up 48.71%, followed by predators (33.14%), filtering collectors (11.05%), scrappers (3.61%), and shredders (3.49%) (Fig. S2a). There were no significant variations in terms of the distribution of the FFGs along sampling sites upstream (MR1-MR3), midstream (MR4-MR6), and downstream (MR7–MR9) (p < 0.05). However, the proportion of FFGs was slightly different from up- to downstream. Upstream where horticultural areas are located, the FFGs with the highest proportion were predators, followed by gathering collectors. Midstream where forest areas are located, the FFGs with the highest proportion were gathering collectors, followed by predators. Finally, downstream, gathering collectors were the dominant group in rural/urban areas (Fig. 2b).

## Ordination analysis

The RDA was performed to explore the effects of selected environmental parameters and topographical influence on aquatic insect distribution. The results revealed that 5 parameters including ammonia, conductivity, orthophosphate, total dissolved solids (TDS), and nitrate were the most significant influences on aquatic insect assemblages. The RDA model based on those selected environmental parameters was found significant (F = 2.9963, p = 0.007). The numerical output showed that the first 2 axes explain together 68.34% of the response data, the first axis alone explaining 58.97%. The  $R^2$  adjusted of 0.555 showed that the first 2 axes have a 45.57% variance. It can be said that the 5 selected environmental parameters have been well modeled in this analysis. The triplot showed 2 groups of sampling sites correlated with explanatory variables and aquatic insects. The first group of Caenidae is strongly related with high orthophosphate, conductivity, and total dissolved solid level in rural/urban areas at MR7, MR8, and MR9, while Hydroptilidae (Hydrot) is related to high ammonia and nitrate in horticultural areas at MR2, MR3 and forest area at MR4 (Fig. 4).

## DISCUSSION

The 9 sampling sites exhibited significantly varied anthropogenic alterations for different land-use types. High-elevation upstream sites (MR1–MR3) were located in horticultural areas that use intensive chemical fertilizers. High ammonia levels were registered on these sites. Indeed, the results from this study



**Fig. 4** Ordination triplot of Hellinger-redundancy analysis (RDA) for the aquatic insect data constrained by selected environmental variables at Mae Ram River basin. Points represent taxa. Arrows show significant (p < 0.05) main effects or interactions of treatments following a permutation test. Species significant explanatory variables aligned with the direction of arrows show positive associations with those treatments.

showed high correlation between elevation, ammonia, and nitrate. Agricultural water quality has been well-documented to be a major environmental issue [20,21], particularly nutrients such as nitrate and ammonia that most likely result from high loads from agricultural area. This may explain why aquatic insect assemblages are highly abundant upstream but low in richness, diversity, and evenness. It could be said that habitats at MR1-MR3 are not appropriate and not varied enough for aquatic insects [22, 23]. In addition, the anthropogenic pollutants from agricultural discharge often lead to a decline of aquatic insect groups that are sensitive to chemicals. Moreover, it has been reported that nitrate and ammonia could affect larval stage of aquatic insects [24], while MR4-MR6 which are characterized by forest land use showed high levels of dissolved oxygen which did not differ between sampling sites or land-use types. However, interestingly, aquatic assemblage showed low abundance but high diversity and evenness. It indicated that forest land may be suitable for aquatic insect communities. Likewise, Vennote [25] reported that species richness should peak at the midstream of the river where high environmental heterogeneity enables the co-occurrence of species with widely differing niches to occur. The downstream MR7-MR8 sites were characterized as rural and urban areas. Not surprisingly, the values of conductivity, TDS, BOD, and orthophosphate were higher than those from upstream and midstream. In addition, our findings revealed that TDS, conductivity, and orthophosphate are strongly

correlated in downstream sites. This finding supports the fact that human activities affect water quality. Few studies have reported that, among the various human activities, urban areas produce the most consistent and ubiquitous effects on surface water quality [26, 27].

The FFGs of aquatic insects at Mae Ram River revealed that the dominant group was gathering collectors at 48.71% and the lowest abundance was the shredder group at 3.49%. Both gathering and filtering collectors tended to increase from upstream to downstream. At the downstream sites, collectors were favored by high turbidity and conductivity in open-canopy agricultural streams. There have been reports that the collectors are commonly abundant in streams and have highly positive correlation between increased abundance and stream size [25, 28]. On the contrary, shredders are commonly abundant in temperate headwaters but are less abundant and diverse in tropical and regulated temperate streams [29, 30]. However, this study showed a higher proportion at the upstream stream and a slightly decreased one towards the downstream with the total of 3.49%. This result strongly agrees with previous studies [29, 31]. The low number of shredders upstream may result from a higher rate of microbial activity in the warmer water temperatures of these streams [28].

RDA ordination clearly divided the response of aquatic insects based on land-use types and environmental factors into 2 groups. The first group was all the upstream sampling sites where the horticulture areas were significantly influenced by nitrate and ammonia. Those nutrients were widely used in horticulture areas as the main fertilizers for promoting crop plants such as cauliflower, cabbage, and lettuce [2]. Therefore, it is clearly shown that agricultural activities lead to anthropogenic chemical pollutants in surface water [32]. However, the results showed that the response of aquatic insect communities to nitrate and ammonia is not clear, except for Hydroptilidae. Unfortunately, there are taxonomic limitations relating to the nutrient stressor knowledge of this fauna. In contrast, the downstream river sites, MR7-MR9 with rural and urban area land use, showed that water quality is significantly influenced by household anthropogenic pollutants including total dissolved solids, conductivity, and orthophosphate. Mayflies in family Caenidae were abundant in this area. Our report supports previous research claiming that Caenidae nymphs were dominant and clearly in higher numbers downstream. Caenidae have been recognized to tolerate some household pollutants known as organic pollution [33].

# CONCLUSION

Our study revealed various existing stressors in the Mae Ram River basin which are mostly due to intensive human activities such as agriculture and direct loading of wastewater from household effluents. Increasing use of chemical fertilizers in upstream sampling sites and detergent in downstream sampling sites are likely to impact aquatic insect assemblages. As seen in this work, different land-use types lead to differing anthropogenic pollutions and impacts on organisms. These results provide valuable insights into how nutrient enrichment may alter aquatic assemblages in tropical streams in Thailand. Our findings have important implications regarding the management of land use and good practice on chemical use in agriculture.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874. 2023.013.

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# Appendix A. Supplementary data



**Fig. S1** Spearman's correlation between physicochemical parameters among sampling sites in Mae Ram River basin. \*p < 0.05, \*\*p < 0.01, and \*\*\*p < 0.001.



**Fig. S2** (a) Proportion of aquatic insect FFGs in Mae Ram River basin, (b) The proportion of aquatic insect FFGs in different land-use types.

Site	Picture sampling site	Latitude	Longitude	Elevation (m a.s.l.)	Land used/ land cover
MR1	A STATE	18°57′40.7″ N	98°48′30.2″ E	883	Horticulture
MR 2		18°57′28 2″ N	08°48′57 4″ F	866	Horticulture
		10 5/ 20.2 1	2010 07.1		horiculture
MR3	<b>对第</b> 4条公	18°57′26.3″ N	98°49′17.5″ E	838	Horticulture
MD 4		10°57/01.0// N	00°51/00 5// 5	551	Conort
MK4 		10 5/ 31.9 N	98 51 02.5 E	551	Forest
MR5		18°57′22.4″ N	98°51′42.3″ E	484	Forest
MR6		18°57′06.8″ N	98°52′03 3″ E	467	Forest
MR7		18°56′26.9″ N	98°53′22.1″ E	361	Urban/rural area
MDC			0005 4/00 0// 5	2/2	
MR8		18°55′56.3″ N	98°54′00.9″ E	368	Urban/rural area
MR9		18°55′45.8″ №	98°54′41 9″ F	355	Urban/rural area
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 Table S1 Geographical, land-cover, and land-use characteristics and physicochemical parameters.

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Site	pH	DO (mg/l)	BOD (mg/l)	TDS (mg/l)	Conductivity ( $\mu s/cm$ )	$NO_3^-$ (mg/l)	PO <sub>4</sub> <sup>3-</sup> (mg/l)	NH <sub>3</sub> (mg/l)
MR1	7.79±0.24	6.49±1.49	1.67±0.84	115.13±4.78	170.59±14.39	0.87±0.19	0.48±0.15	0.67±0.35
MR2	$7.30 \pm 0.32$	$5.97 \pm 1.22$	$1.56 \pm 0.91$	105.86±3.13	$157.35 \pm 1.88$	$0.80 \pm 0.45$	$0.33 \pm 0.06$	$0.67 \pm 0.55$
MR3	7.56±0.24	6.31±1.45	$0.80 \pm 0.91$	$116.28 \pm 0.78$	172.71±7.59	0.81±0.34	$0.52 \pm 0.11$	$0.62 \pm 0.41$
MR4	7.89±0.09	6.75±1.39	$1.80 \pm 0.97$	98.55±7.31	$146.56 \pm 16.7$	0.86±0.47	$0.51 \pm 0.07$	$0.60 \pm 0.60$
MR5	7.63±0.36	6.47±1.27	1.83±1.98	$116.30 \pm 4.52$	$172.36 \pm 11.4$	$0.57 \pm 0.32$	$0.65 \pm 0.32$	0.44±0.34
MR6	8.13±0.18	6.51±1.13	1.43±0.89	$117.60 \pm 1.08$	174.94±7.70	0.41±0.29	$0.60 \pm 0.36$	$0.30 \pm 0.15$
MR7	7.74±0.09	6.26±1.09	$1.93 \pm 0.87$	152.77±25.93	$210.99 \pm 7.17$	$0.44 \pm 0.18$	$0.59 \pm 0.08$	$0.26 \pm 0.04$
MR8	$7.70 \pm 0.25$	$5.85 \pm 1.00$	$2.56 \pm 0.83$	$150.52 \pm 5.07$	$222.32 \pm 1.99$	0.54±0.39	$0.70 \pm 0.16$	$0.22 \pm 0.11$
MR9	$7.70 \pm 0.21$	$6.00 \pm 1.05$	$3.16 \pm 0.84$	161.45±1.55	240.89±9.49	$0.30 \pm 0.16$	$0.92 \pm 0.06$	$0.26 \pm 0.17$

 Table S2
 The physicochemical parameters of water quality at 9 sampling sites.