

## Use of Aquatic Insects as Bioindicators of Water Quality of Mae Kham Watershed, Chiang Rai Province การใช้แมลงน้ำเป็นตัวบ่งชี้คุณภาพน้ำในลุ่มน้ำแม่คำ จังหวัดเชียงราย

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### Abstract

This study measured the aquatic insect diversity and its application as a bioindicator to monitor water quality in Mae Kham Watershed, Chiang Rai Province. Shannon–Wiener index, BMWP<sup>Thai</sup> score with ASPT, EPT ratio and HBI were used to assess water quality. Physical, chemical and biological parameters were also measured to compare with the surface water quality standard of Thailand. 241 aquatic insect morphotaxa from 86 families in 10 orders were identified. The most abundant family found during the year was Baetidae in the Ephemeroptera order. Using aquatic insects as bioindicators, it can be concluded that all sampling sites were shown to have good to polluted water quality, depending on land use and human activities. From the PCA, biological indices were related to some physico–chemical properties of water quality. ASPT, EPT ratio and diversity index were related to some parameters such as velocity, DO, alkalinity and conductivity. From the results, ASPT (based on BMWP<sup>Thai</sup>) is suitable for use in the Mae Kham Watershed while the Shannon–Wiener index is appropriate for use in watershed areas for quantitative study. The EPT ratio is suitable for use along upstream areas while the HBI index is not appropriate for use in small streams.

### บทคัดย่อ

การศึกษานี้เป็นการศึกษาความหลากหลายของแมลงน้ำและการประยุกต์ใช้เพื่อเป็นตัวบ่งชี้ทางชีวภาพในการติดตามตรวจสอบคุณภาพน้ำในลุ่มน้ำแม่คำ จังหวัดเชียงราย โดยใช้ดัชนีความหลากหลายทางชีวภาพของแซนนอนวีเนอร์ (Shannon–Wiener index) ดัชนีบีเอ็มดับเบิลยูพีไทย (Biomonitoring Working Party Score: BMWP<sup>Thai</sup>) ร่วมกับค่าเอเอสพีที (Average Score Per Taxon: ASPT) ดัชนีอีพีที (Ephemeroptera Plecoptera Trichoptera Index: EPT) และดัชนีเอชบีไอ (Hilsenhoff Biotic Index: HBI) ในการประเมินคุณภาพน้ำ นอกจากนี้ยังมีการวัดค่าทางเคมีและกายภาพของแหล่งน้ำและเปรียบเทียบกับมาตรฐานคุณภาพน้ำผิวดินของประเทศไทย พบแมลงน้ำทั้งสิ้น 241 มอร์โฟทักซา จาก 86 วงศ์ ใน 10 อันดับ แมลงที่พบมากที่สุดคือแมลงชีปะขาวในวงศ์ Baetidae อันดับ Ephemeroptera จากการใช้แมลงน้ำเป็นตัวบ่งชี้ทางชีวภาพ สามารถสรุปได้ว่า คุณภาพน้ำของจุดเก็บตัวอย่างทั้งหมดตลอดทั้งปี อยู่ในช่วง ดี ถึงสกปรก โดยขึ้นอยู่กับการใช้ประโยชน์ที่ดินและกิจกรรมของมนุษย์ จากการวิเคราะห์ด้วย PCA พบว่าการใช้ดัชนีชีวภาพมีความสัมพันธ์กับคุณภาพน้ำทางเคมีและกายภาพ นอกจากนี้ค่าดัชนีเอเอสพีที ดัชนีอีพีที และดัชนีความหลากหลายทางชีวภาพมีความสัมพันธ์กับค่าความเร็วกระแสน้ำ ค่าออกซิเจนละลายน้ำ ค่าความเป็นด่าง และค่าการนำไฟฟ้า ผลการศึกษาสรุปได้ว่า ดัชนีเอเอสพีที มีความเหมาะสมที่จะนำมาใช้กับลุ่มน้ำแม่คำ ขณะที่ดัชนีอีพีทีเหมาะสมที่จะใช้กับพื้นที่ต้นน้ำ ส่วนดัชนีเอชบีไอไม่เหมาะสมที่จะนำมาใช้กับลำธารขนาดเล็ก

**Keywords:** aquatic insect, BMWP<sup>Thai</sup>, Shannon–Wiener index, EPT ratio, bioindicator

**คำสำคัญ:** แมลงน้ำ บีเอ็มดับเบิลยูพีไทย ดัชนีแซนนอนวีเนอร์ ดัชนีอีพีที ตัวบ่งชี้ทางชีวภาพ

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## Introduction

Urbanization and agricultural development have been encroaching on highlands in the north of Thailand for decades. This impacts forests and the sources of streams and causes a lack of fresh water for consumption throughout the year. Moreover, a high volume of wastewater is produced from communities, agriculture and livestock. Chiang Rai is located in the mountains and is near the origin of many rivers such as Kok, Ing, Mae Lao, Mae Sai and Mae Kham. Thus, it is a suitable place for plantations such as rice, corn, tobacco, tea, coffee, and vegetable growing and recently orange plantations have been initiated in large numbers. This research studied the Mae Kham Watershed in Mae Chan and Mae Fah Luang Districts, Chiang Rai Province. Wastewater is discharged into the streams in this area from many plantations. Concerns about water pollution in Mae Kham Watershed are considered alongside water conservation in order to protect the water resources of the local people and their villages. Conservation begins by studying characteristics and water quality to help in planning, water use management and conservation for the future.

The study was to assess and compare water quality in each study site where different water was used. In this research aquatic insects were used as bioindicators and this method was compared to some physico-chemical properties. The diversity of aquatic insects in the watershed was also studied. The results were used to assess the water resource management of the local people.

### Objectives:

1. To assess water quality of the Mae Kham River using aquatic insects as bioindicators.
2. To monitor water quality of Mae Kham River and its tributaries, Chiang Rai Province, using

physical, chemical and biological parameters compared with the surface water quality standard of Thailand.

## Material and Methods

### *Sampling site descriptions*

Eight sampling sites with different land use were assigned on the Mae Kham River and tributaries in Mae Chan and Mae Fah Luang Districts (Figure 1). The land use had different impact on water quality and aquatic insects.

#### **Site 1: Mae Pern Stream**

The Mae Pern Stream is located in Sri Kham Village, Mae Chan District at approximately 20°12' 298" N 99°49' 719" E at an altitude of 396 meters above sea level. This stream is downstream of the Mae Pern Pha Mieng (sampling site 8) and passes through many communities including agricultural areas and areas which possess livestock. The stream width is 5 meters wide in the rainy season and approximately 1.5 meters in the dry season with 10–40 cm depth. The substrates of the stream are silt and sand. The water current is very slow.

#### **Site 2: Mae Salong Stream**

Mae Salong Stream is located in Sri Kham Village, Mae Chan District at approximately 20°13' 258" N 99°48' 926" E with an altitude of 385 meters above sea level. This stream is downstream of the Huai Moh Khang (sampling site 4). The majority of the activities beside the stream are agricultural, including rice fields and corn plantations. The stream width is 4 meters with 30–60 cm depth. The profile of the stream changes in each season and the stream bottom is covered with sand and gravel. The water current is very high with soil erosion and so the sampling site has high turbidity.

**Site 3: Mae Salap 1**

Mae Salap 1 is located in Samakkeemai Village, Mae Salong Nai Sub – District in Mae Fah Luang District at approximately 20°14' 464" N 99° 45' 951" E with an altitude of 433 meters above sea level. The sampling point is near to the junction of Mae Salap Stream and the Mae Kham River. The stream width is 4 – 5 meters with 30 – 50 cm depth. Both sides of the stream are covered with grass and plants. The substrates are boulder, gravel and sand but in the rainy season they change to silt and clay with high speed water current.

**Site 4: Huai Moh Khang Stream**

Huai Moh Khang Stream is located in Ban Sansuk Village, Pha Sang Sub-District in Mae Chan District at approximately 20°14' 743" N 99°46'273" E with an altitude of 454 meters above sea level. The stream width is 2.5 meters with 20 – 30 cm depth. The sampling site is covered by many aquatic plants, with small plants at the riverbanks and some bushes. The substrates are boulder, gravel and sand.

**Site 5: Mae Kham River**

Mae Kham River is located in Samakkeemai Village, Mae Salong Nai Sub – District in Mae Fah Luang District at approximately 20°14' 262" N 99° 45' 028" E with an altitude of 433 meters above sea level. The stream width is 15 meters with 1 – 4 meters depth. The stream bottom is sand, boulder and has some rock. There is very high speed water current.

**Site 6: Huai Jai Stream**

Huai Jai Stream is located in Ban Sansuk Village, Pha Sang Sub – District in Mae Chan District at approximately 20°14' 496" N 99° 45' 978" E with an altitude of 461 meters above sea level. The stream width is 20 – 40 cm with 10 – 20 cm

depth. It is an origin of the Huai Jai Reservoir. This is the character of the head of the stream. The sampling point is in a deciduous forest with high percent canopy cover of trees and there are many plants beside the stream. The substrate contains sand, gravel, boulder and some rock. There is low speed water current.

**Site 7: Mae Salap 2**

Mae Salap 2 is located in Ban Sanjaimai Village, Pha Sang Sub – District in Mae Chan District at approximately 20°12' 924" N 99°45' 025" E with an altitude of 466 meters above sea level. The stream width is 4 – 5 meters with 20 – 60 cm depth. The area around this sampling point includes rice fields and livestock areas but it does not pass any large communities.

**Site 8: Mae Pern Pha Mieng Stream**

Mae Pern Pha Mieng is located in Mae Pern Pha Mieng Village, Pha Tung Sub – District in Mae Chan District at approximately 20°09' 192" N 99°46' 805" E with an altitude of 448 meters above sea level. The stream width is 4 meters with 20 – 50 cm depth. It is located in the forest with a very high percent of canopy cover and is between mountains with low light penetration. There is leaf litter on the stream sides and bottom. The substrate varies with leaf litter, silt, soil sediment, sand, gravel and boulder to bed rock. Some parts of the stream are like a small waterfall.

The study was conducted from October 2003 until August 2004. The samples were collected twice in each season.

**Biological properties****Aquatic insects****Method of aquatic insect sampling**

The pond net, kicking sample and pick method were used for collecting aquatic insects from several

habitats. There were two replications for each sampling method. Aquatic insects were collected by pond net at the stream side. Aquatic plants were collected. A basin was used for sediment and leaf litter. A pond net was used for 5 minutes per replication. The kick sample method was also used to collect aquatic insects from the stream substrates. The kicking net had a one meter sieve bag. The insects which live in gravel and sand drifted into the sieve bag. This sampling method was done for 10 meters per replication. Lastly, the pick method was used to collect insects from boulders. In this sampling method boulders were picked up and washed by brushing. All insect samples were preserved in 4% formalin before sorting and identifying by the keys of McCafferty (1983), Merritt and Cummins (1984), Stehr (1991), Dudgeon (1992) and Wiggins (1996) in the laboratory. Aquatic insects were identified to the family level and the numbers of individuals in each family was counted. Identification was done using biological indices as follows.

#### Diversity Index

The results of aquatic insect communities were calculated for the diversity index by using Shannon - Wiener Index (H) (Table 1).

#### BMWP<sup>Thai</sup> score and ASPT

In the Biomonitoring Working Party Score (BMWP score), each family is assigned a score according to its tolerance to pollution and the score varies from 1 to 10. This study used the score following Mustow (2002). The least tolerant to pollution species groups are assigned a high score and the highly tolerant groups are assigned low scores. The total BMWP score is divided by the number of corresponding scoring families to calculate the ASPT. Then, the ASPT was compared with the water

quality standard which was modified from the water quality standard of Thailand by Lotic Insects Ecology Research Unit.

#### EPT Ratio

An EPT Index was calculated for the Arlington County sites. This is another commonly used water quality assessment index that is based on the abundance of three pollution sensitive insect orders (Ephemeroptera, Plecoptera, and Trichoptera) present in the stream. The %EPT equals the number of EPT taxa found at the monitoring site divided by the total number of aquatic insects found (Table 2).

#### HBI Index

The Hilsenhoff Biotic Index is based on family level by using the tolerance of organisms to pollution. Tolerance values range from 0 for organisms very sensitive to pollution to 10 for organisms very tolerant of pollution. Then the results are compared with the table to assess water quality.

$$\text{HBI index} = \frac{\sum [(X_i)(t_i)]}{n_i}$$

$X_i$  = the number of individuals in the species

$t_i$  = tolerance value for each species

$n_i$  = the total number of individuals in the collection

#### Physical properties

**Velocity** was measured by using flow probe velocity meter model FP 101 serial no. 17884 of Global Water. Velocity values were measured at 5 cm under surface water at three points and then calculated to the average in units of meters per second.

**Turbidity** was determined by DR 2000 HACH spectrophotometer in Formazin Turbidity Units (FTU).

### **Chemical properties**

**Conductivity and pH** were measured at a depth of 10 cm by multi-parameter analyzer consort C533 version 2.2.

**Alkalinity** was examined by Phenolphthalein methyl orange indicator (APHA, 1998). Water samples were titrated with 0.02 N  $\text{H}_2\text{SO}_4$  until the pH at the end point was 4.3–4.5.

**Dissolved oxygen (DO):** Water samples were collected from each sampling site very carefully in 300 ml BOD bottles and the Azide Modification method (APHA, 1998) followed.

**Biochemical Oxygen Demand ( $\text{BOD}_5$ ):** Water samples were collected from sampling sites very carefully in 300 ml BOD bottles and the Azide Modification method (APHA, 1998) followed.

#### **Nutrients:**

– Ammonia–nitrogen ( $\text{NH}_3\text{-N}$ ) was determined by the Nesslerization technique, using the DR 2400 HACH spectrophotometer.

– Nitrate–nitrogen ( $\text{NO}_3^-\text{-N}$ ) was determined employing the cadmium reduction method using Nitra Ver 5 Nitrate reagent by DR 2400 HACH spectrophotometer.

– Orthophosphate ( $\text{O-PO}_4^{3-}$ ): was measured by the Ascorbic Acid Method, using Phos Ver 3 powder and a DR 2400 HACH spectrophotometer.

The results of physico–chemical and biological parameters were analyzed by the Multivariate Statistical Package (MVSP). Principal Component Analysis (PCA) was used to study the effect of the relative proportion of insect orders on water quality and of each physico–chemical parameter on biological indices. Cluster analysis was used to group the sampling periods from the similarity of physico–chemical properties and aquatic insects.

Therefore, Statistical Program for Social Science (SPSS) version 10 was used to compare the means of the results in each sampling site and seasons and the differences between biological indices.

### **Results and Discussion**

After collecting samples from all sites throughout the duration of the research, the raw data were used to calculate the 4 diversity indices as shown in Table 3.

The results of all indices were related to physical and chemical properties. There were differences between sampling sites ( $p < 0.05$ ) at all indices and velocity, conductivity, ammonia–nitrogen, alkalinity and turbidity. From the results, it can be concluded that the water quality in each sampling site was different because of the impacts from several types of land use along the streams.

The Multivariate Statistical Package (MVSP) program was used to analyze the data in this research. The classification method cluster analysis (UGPMA) was used to segregate the sampling site. By using physico – chemical properties, the UPGMA showed 4 groups (Figure 2). The first group was at sampling site 5 Mae Kham River in the hot dry season; the second group at sampling site 1 Mae Pern in the rainy season; the third group at sampling site 5 Mae Kham River in the rainy season; and the last group at the other sampling sites and all seasons at site 2 Mae Salong, site 3 Mae Salap 1, site 4 Huai Moh Khang, site 6 Huai Jai, site 7 Mae Salap 2 and site 8 Mae Pern Pha Mieng and at site 1 in the cool dry and hot dry seasons and at site 5 in the cool dry season. In addition, they could be classified by the diversity of aquatic insects into 4 groups (Figure 3). The first group was at site 2 in

the hot dry season; the second group was at site 4 in the hot dry season; the third group was at site 3 and site 7 in the hot dry and cool dry seasons and site 2 and site 4 in the cool dry season; and the last group was at sites 1, 5, 6 and 8 throughout the year and sites 2, 3, 4 and 7 in the rainy season.

Nevertheless, there was no obvious difference between groups from both the segregation by physico-chemical properties and the diversity of aquatic insects. Principal Component Analysis (PCA) was used to analyze the correlation of all sampling times. Only site 2 in the hot dry season was clearly different from other sampling periods (Figure 4). As a result, the water quality in each sampling site did not show obvious differences, because the downstream sites had an impact from humans and nature. The upstream sites also had human impact, but from different sources. Therefore, clear difference between sites was not seen. Moreover, the chosen sampling sites for this study were not of specific character.

The PCA analysis revealed the correlation between aquatic insect orders and water quality (Figure 5). Insects in orders Coleoptera, Diptera, Odonata, Megaloptera, Lepidoptera and Hemiptera were related to the concentration of nitrate nitrogen. Insects in order Ephemeroptera, Plecoptera, Trichoptera and Collembola had relationships with conductivity and phosphates. Conductivity was an important property to indicate water quality. Therefore, insects in sensitive orders, such as Ephemeroptera, Plecoptera and Trichoptera were affected.

Correlation between biological indices and water quality in some physico-chemical properties were evaluated (Figure 6). The values of ASPT, EPT ratio and diversity index were clearly related to

velocity, DO, alkalinity and conductivity. From this correlation it can be concluded that the 3 indices are appropriate to be used to indicate water quality in the Mae Kham Watershed. They are related to physico-chemical parameters which are significant in specifying the quality of running water. However, the HBI index was not related to any parameters.

## Conclusions

The conclusions of this study are as follows:

1. Ten orders, 86 families and 241 morphotaxa of aquatic insects were identified. The most abundant family during the year was Baetidae (Ephemeroptera). Site 6 was upstream with less contamination by human activities. Numerous aquatic insect families, particularly insects in the Trichoptera Order were discovered there.

2. Using physico-chemical parameters to compare the water quality to the water quality standard of Thailand, the streams were classified into 3 groups. Site 1 was classified in class 3 with medium clean water resources. Sampling site 2, site 3, site 4 and site 5 were classified in class 2 with very clean water resources and sampling site 6, site 7 and site 8 were classified as being extra clean water resources. Water quality depended on land use and human activities beside each stream. The results were related to biological indices.

3. Biological indices were used. The HBI index was not appropriate to be used in small streams in which almost all macro-invertebrates are insects. However, from the results, the diversity index is appropriate for use in the watershed area but the quantitative index should be considered. The high diversity levels might have been from order Diptera which occurs in polluted water. The EPT ratio is

suitable for use and should be applied in Thailand. ASPT (based on  $BMWP^{Thai}$ ) is more suitable for use at the Mae Kham Watershed than other indices. From those indices, sampling site 6, site 7 and site 8 were indicated as having good water quality, while sampling site 2, site 3, site 4 and site 5 had moderate water quality and sampling site 1 had quite polluted water.

4. Using cluster analysis, the water quality at all sampling times can be classified into 4 groups using the physico-chemical properties and can also be classified into 4 groups using the diversity of aquatic insects. Nevertheless, the water quality at each sampling site is not obviously different. PCA analysis can expose the correlation between aquatic insect orders and water quality with conductivity, nitrates and phosphates. The biological indices were related to water quality in some physico-chemical properties. ASPT, EPT ratio and diversity index are related to some parameters such as velocity, DO, alkalinity and conductivity.

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**Table 1.** Shannon–Wiener index for each category of water quality.

	Good	Moderate Pollution	Polluted Stream
Shannon–Wiener Index	>3	1–3	<1

**Table 2.** EPT index for each category of water quality.

	Good	Acceptable	Marginal	Poor
EPT to total ratio	0.75–1.00	0.5–0.75	0.25–0.50	0–0.25

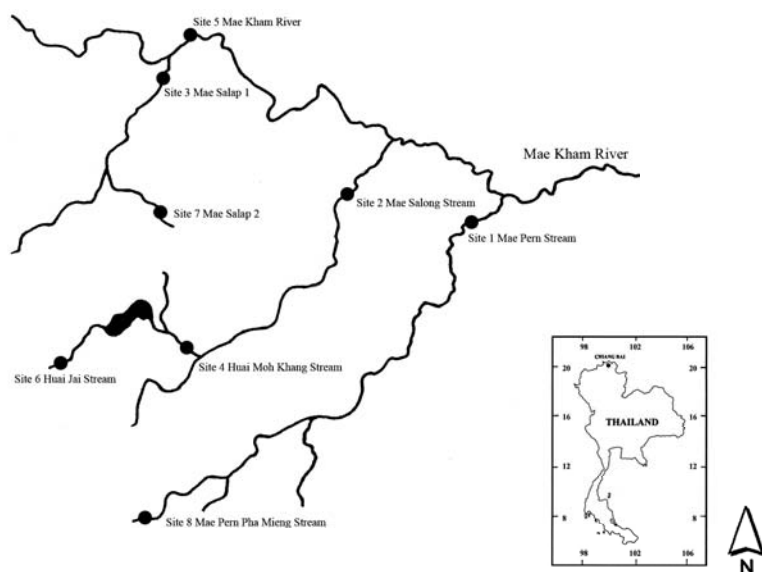
**Table 3.** Comparison of biological indices at each sampling time for the Mae Kham Watershed from October 2003 to August 2004.

Site	Month	Diversity	ASPT	EPT ratio	HBI
1	Oct.	2.7	5.8	0.56	5.16
	Dec.	2.1	5.8	0.25	5.29
	Mar.	2.5	5.2	0.17	5.63
	May	2.4	4.7	0.09	5.39
	Jul.	1.8	5.4	0.18	5.40
	Aug.	1.9	5.0	0.53	5.46
2	Oct.	1.7	6.4	0.85	4.94
	Dec.	1.9	6.2	0.77	5.02
	Mar.	2.2	6.2	0.50	5.24
	May	2.3	6.1	0.73	5.24
	Jul.	2.0	6.2	0.81	4.93
	Aug.	2.3	5.5	0.61	4.73
3	Oct.	2.0	6.4	0.74	5.13
	Dec.	2.7	6.7	0.60	5.28
	Mar.	3.1	6.7	0.67	4.63
	May	3.3	7.0	0.58	4.20
	Jul.	2.7	6.8	0.79	4.29
	Aug.	2.4	6.2	0.66	4.57

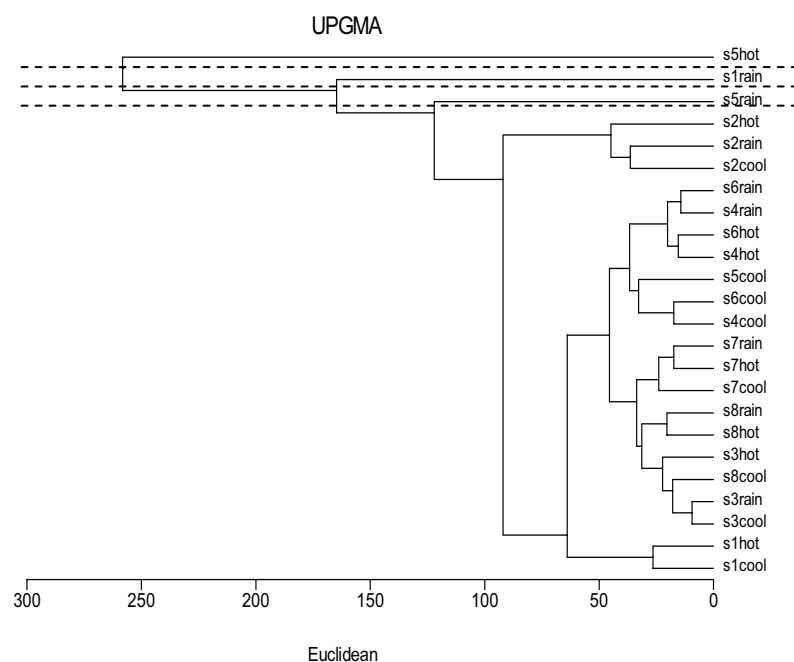


**Table 3.** Comparison of biological indices for each sampling time for the Mae Kham Watershed from October 2003 to August 2004. (Cont.)

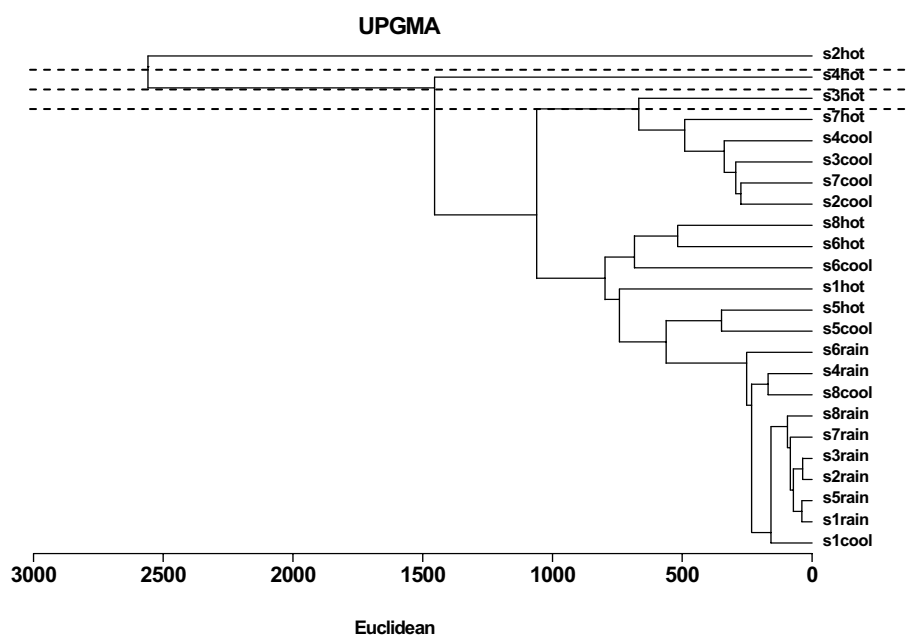
Site	Month	Diversity	ASPT	EPT ratio	HBI
4	Oct.	2.4	5.9	0.65	5.39
	Dec.	2.3	6.3	0.71	5.05
	Mar.	2.5	5.9	0.20	6.11
	May	3.3	6.3	0.59	5.61
	Jul.	2.7	6.2	0.68	4.86
	Aug.	2.9	6.1	0.60	5.08
5	Oct.	1.8	6.3	0.70	5.30
	Dec.	2.5	7.2	0.56	5.29
	Mar.	2.6	7.0	0.58	4.77
	May	1.5	5.0	0.24	3.86
	Jul.	1.9	7.4	0.55	3.63
	Aug.	3.0	7.1	0.59	3.76
6	Oct.	2.1	6.8	0.81	4.86
	Dec.	2.9	6.6	0.61	4.94
	Mar.	3.1	6.7	0.57	5.14
	May	3.4	6.8	0.81	3.37
	Jul.	3.6	6.7	0.65	3.79
	Aug.	3.2	6.5	0.62	4.38
7	Oct.	2.2	6.8	0.73	5.05
	Dec.	2.3	6.7	0.82	4.89
	Mar.	3.0	7.0	0.72	4.45
	May	1.9	6.6	0.86	4.63
	Jul.	3.6	6.8	0.61	3.99
	Aug.	3.3	6.6	0.42	4.27
8	Oct.	2.8	6.8	0.65	4.77
	Dec.	3.3	6.8	0.71	4.64
	Mar.	3.3	6.7	0.60	5.07
	May	3.4	6.9	0.74	3.88
	Jul.	–	–	–	–
	Aug.	3.3	6.6	0.72	4.28



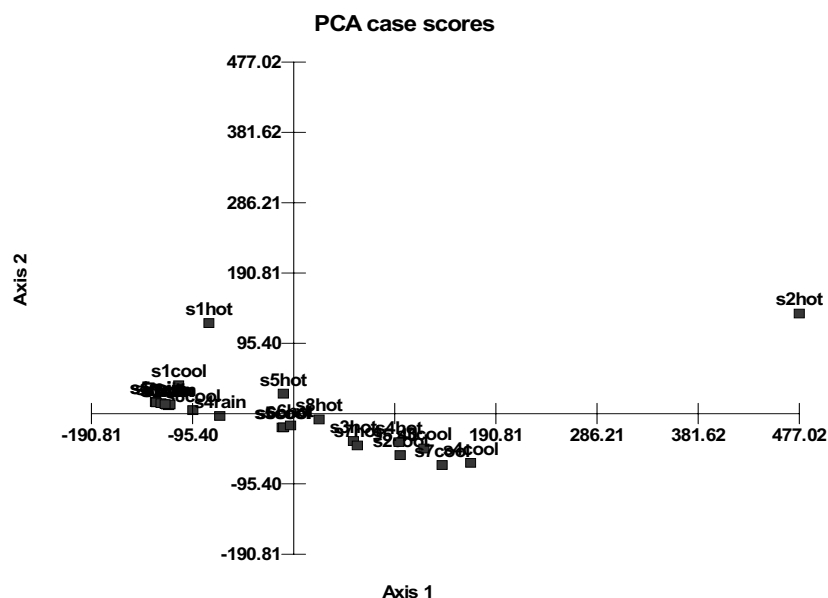
**Figure 1.** Map of the Mae Kham Watershed (showing 8 sampling sites), Mae Chan and Mae Fah Luang District, Chiang Rai Province.



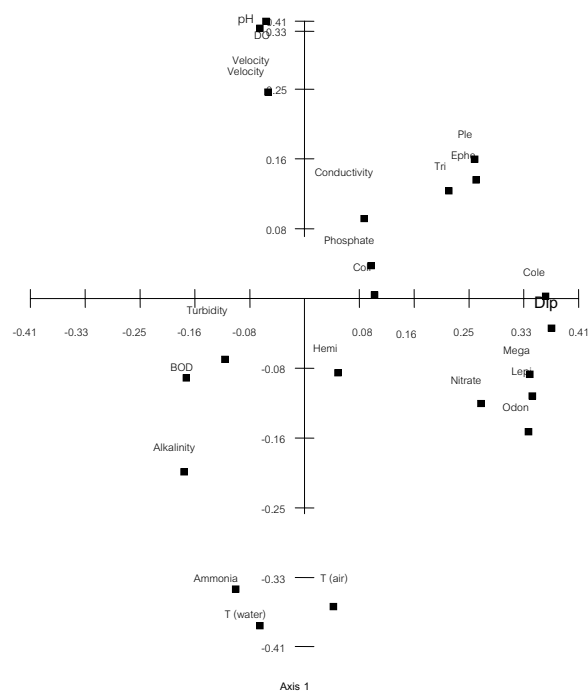
**Figure 2.** Cluster analysis of sampling period using physico-chemical properties for the Mae Kham Watershed from October 2003 to August 2004.



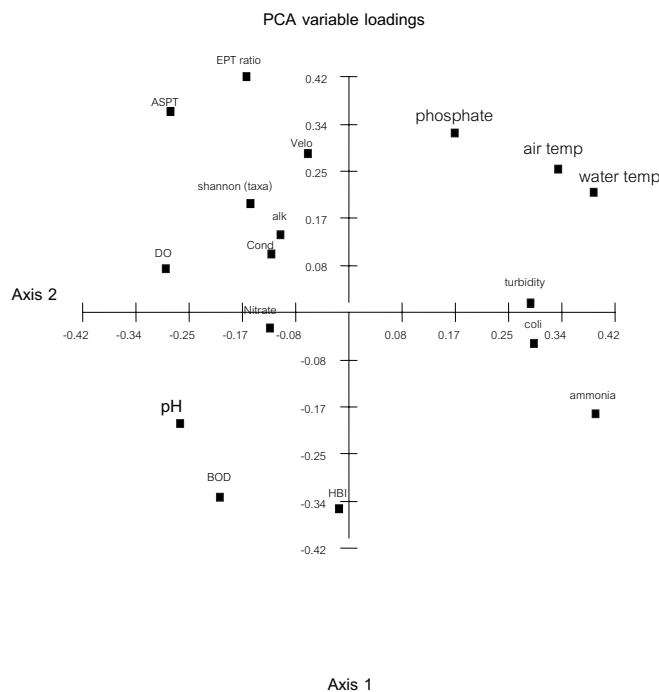
**Figure 3.** Cluster analysis of sampling period using aquatic insects diversity for the Mae Kham Watershed from October 2003 to August 2004.



**Figure 4.** Correlation of sampling periods using aquatic insect diversity for the Mae Kham Watershed from October 2003 to August 2004.



**Figure 5.** Correlation between aquatic insect orders and water quality for the Mae Kham watershed from October 2003 to August 2004.



**Figure 6.** Correlation between biotic indices and water quality in some physico-chemical properties for the Mae Kham Watershed from October 2003 to August 2004.