CHAPTER 3

MATERIALS AND METHODS

1. Equipment

Field equipment

1. Polyethylene bottles (2 liter-size)
2. BOD bottles (300 ml size)
3. Pond net
4. Kick net
5. 4 % formalin concentration
6. Plastic bowls
7. Plastic bags (8x12 inch size)
8. Rubber band
9. Medical flat bottles (150 ml Size)
10. Multi-parameter analyzer Consort C533 version 2.2
11. Velocity meter
12. DO kit

Laboratory equipment and chemicals

1. Stereomicroscope
2. Glass sorting dishes
3. Dropper
4. Ethanol 70%
5. Distilled water
6. Forceps
7. Vials
8. Needle
9. Label papers and pencils
10. Equipment to analyze turbidity

11. Equipment to analyze alkalinity
12. Equipment to analyze nutrient
13. Equipment to analyze total coliform bacteria

2. The sampling site descriptions

The Mae Kham River and tributaries surveyed in Mae Chan and Mae Fah Laung Districts (Figure 3-1) that could assign to 8 sampling sites with different land used. Thus, there were different impacts to 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 $20^{O}12^{'}298^{"}$ N $99^{O}49^{'}719^{"}$ E at an altitude of 396 meters above sea level. This stream is downstream of the Mae Pern Pha Mieng (sampling site 8) by passing through many communities including agricultural areas and areas which possessed livestock. The stream width was 5 meters wide in the rainy season and approximately 1.5 meters in the dry season with 10-40 cm. depth. Some parts of the sampling area were under a concrete bridge and some were covered by small plants. There was road construction near the site and it had a land slide problem. The substrates of the stream were silt and sand. The water current was very slow and it changed to stand still during some parts of the year (Figure 3-2 (A)).

Site 2; Mae Salong Stream

Mae Salong Stream is located in Sri Kham Village, Mae Chan District at approximately $20^{O}13^{'}258^{"}$ N $99^{O}48^{'}926^{"}$ E with an altitude of 385 meters above sea level. This stream is downstream of the Huai Moh Khang (sampling site 4). The majorities of the activities beside the stream were agricultural, or included rice fields corn plantations. The stream width was 4 meters with 30-60 cm. depth. The sampling site was at the open area and it changed the character of stream, in the rainy season it had high sediment causing the stream to be shallower. The riparian zones of the stream were covered by high grasses and reeds and there was soil erosion in some parts. The profile of the stream always changed in each season and the stream bottom

was covered with sand and gravel. The water current was very high with soil erosion then the sampling site had high turbidity (Figure 3-2 (B)).

Site 3; Mae Salap 1

Mae Salap 1 is located in Samakkeemai Village, Mae Salong Nai Sub-District in Mae Fah Laung District at approximately $20^{O}14^{'}464^{"}$ N $99^{O}45^{'}951^{"}$ E with an altitude of 433 meters above sea level. The sampling point was near to the junction of Mae Salap Stream and the Mae Kham River. The stream width was 4-5 meters with 30-50 cm. depth. The area around each sampling site was grass fields for livestock, cows, buffaloes and goats. Both sides of the stream were covered by grasses and plants. The substrates were boulder, gravel and sand but in the rainy season it changed to silt and clay with high speed water current (Figure 3-2 (C)).

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 $20^{O}14^{'}743^{"}$ N $99^{O}46^{'}273^{"}$ E with an altitude of 454 meters above sea level. The stream width was 2.5 meters with 20-30 cm. depth. Water is from Huai Jai Reservoir and wastewater from Sansuk Village. The sampling site was covered by many aquatic plants, small plants at the shore and some bushes. The substrates were boulder, gravel and sand and it did not have high speed water current (Figure 3-2 (D)).

Site 5; Mae Kham River

Mae Kham River is located in Samakkeemai Village, Mae Salong Nai Sub-District in Mae Fah Laung District at approximately $20^{O}14^{'}262^{"}$ N $99^{O}45^{'}028^{"}$ E with an altitude of 433 meters above sea level. The stream width was 15 meters with 1-4 meters depth. It is a big river with a basin of many small streams and the sampling point just received water from Mae Salap Stream under the bridge with a concrete shoreline, without any plant cover, it was very deep and had high water quantity. The stream bottom was sand, boulder and some rock. There was very high speed water current (Figure 3-3 (A)).

Site 6; Huai Jai Stream

Huai Jai Stream is located in Ban Sansuk Village, Pha Sang Sub-District in Mae Chan District at approximately $20^{O}14'496''$ N $99^{O}45'978''$ E with an altitude of 461 meters above sea level. The stream width was 20-40 cm with 10-20 cm depth. It was a very small stream and as an origin of the Huai Jai Reservoir. This was a character of the head of the stream, a small stream which is shallow but there was low water level all year long. The sampling point was in a deciduous forest with high percent canopy cover of trees and there were many plants beside the stream. There were cattle that had been raised near the area. The substrate characters were sand, gravel, boulder and some rock. There was low speed water current (Figure 3-3 (B)).

Site 7; Mae Salap 2

Mae Salap 2 is located in Ban Sanjaimai Village, Pha Sang Sub-District in Mae Chan District at approximately $20^{O}12'924''$ N $99^{O}45'025''$ E with an altitude of 466 meters above sea level. The stream width was 4-5 meters with 20-60 cm. depth. The area around this sampling point included rice fields and livestock area but did not pass any large communities. Beside the stream there was a density of several plants. At some part of the sampling was a point where local people used vehicles to cross the stream so there was clay and sand on the stream bottom. But for other parts there was gravel and rock. And it had a basin about 1 meter deep. It did not have high water current (Figure 3-3 (C)).

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 $20^{O}09'192''$ N $99^{O}46'805''$ E with an altitude of 448 meters above sea level. The stream width was 4 meters with 20-50 cm depth. It was located in the forest with a very high percent canopy cover and was between mountains. Beside the stream there was cover by many big trees so there was less light able to penetrate through to the stream. There was leaf litter on the stream sides and bottom. The substrates were varied as leaf litter, silt, soil sediment, sand, gravel and boulder to bed rock. And some part of the stream was like a small water fall. It did not have high water current (Figure 3-3 (D)).

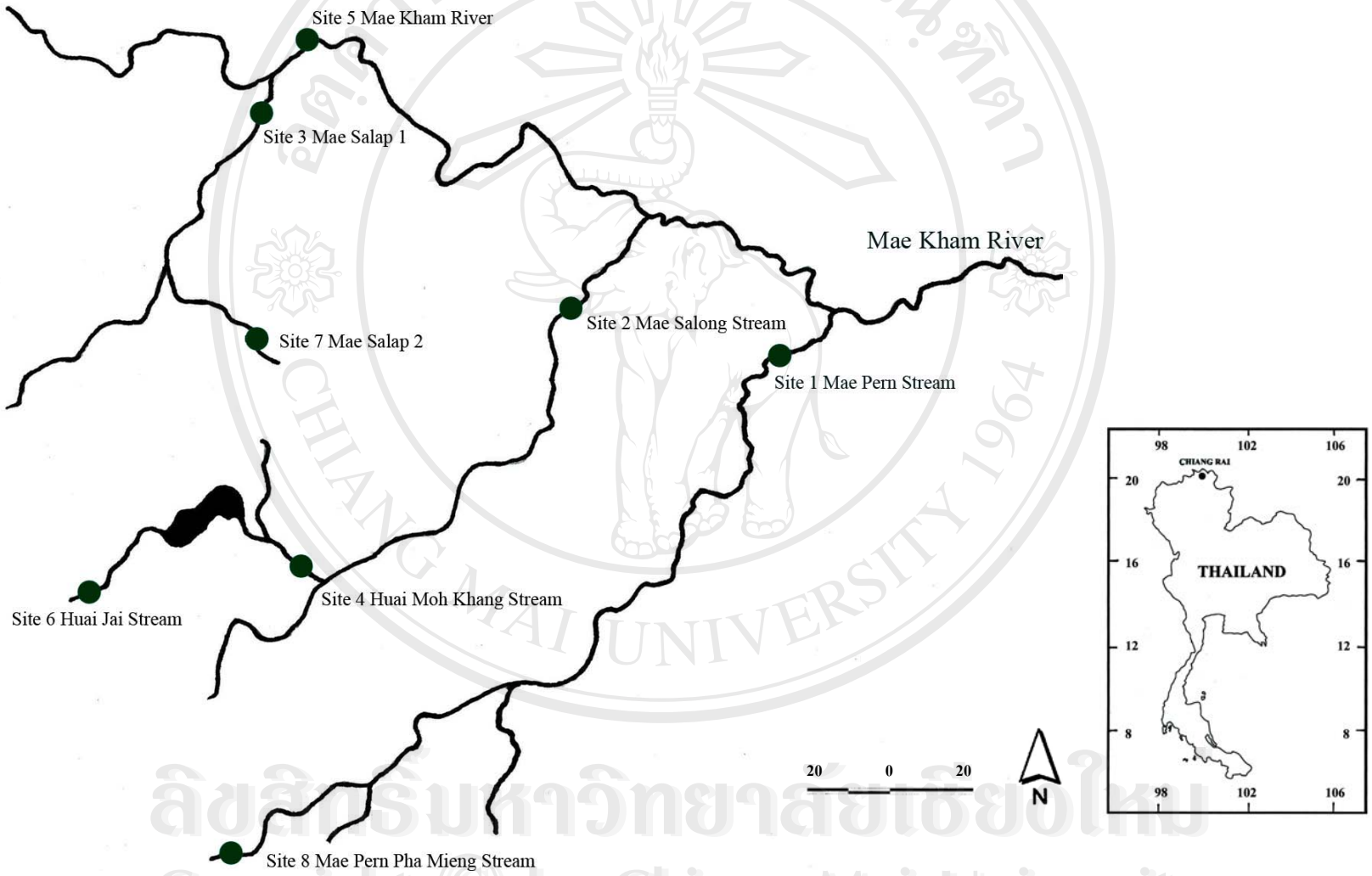

Figure 3-1 Map of the Mae Kham Watershed (showing 8 sampling sites), Mae Chan and Mae Fah Laung District, Chiang Rai Province

Figure 3-2 Study sites 1-4: (A) Site 1 Mae Pern Stream; (B) Site 2 Mae Salong Stream; (C) Site 3 Mae Salap 1; (D) Site 4 Huai Moh Khang Stream

Figure 3-3 Study sites 5-8: (A) Site 5 Mae Kham River; (B) Site 6 Huai Jai Stream; (C) Site 7 Mae Salap 2; (D) Site 8 Mae Pern Pha Mieng Stream

3. Biological properties

3.1 Aquatic insects

3.1.1 Method of aquatic insect sampling

The pond net, kicking sample and pick method had been used for collecting aquatic insects from several habitats. There were two replications in each sampling method. Aquatic insects were collected by pond net (Figure 3-4 (A)) at the stream side, aquatic plants, basin with sediment and leaf litter, a pond net was used for 5 minutes for a replication. Kick sample method was also used to collect aquatic insects from the stream substrates. The kicking net had a one meter sieve bag (Figure 3-4 (B)). The sampler was put in to the bottom and across the stream then the area in front of the sampler was disturbed by foot. The insects which live in gravel and sand drifted into the sieve bag. This sampling method was done for 10 meters for one replication. Lastly, the pick method was used to collect insects from boulders (Figure 3-4 (C)). This sampling method was used to pick up boulders and wash them by brush. The insects which hold to boulders were collected. All sampling method did not disturb the aquatic insects by sampling from downstream to upstream. All samples had been preserved in 4% formalin before being sorted in the laboratory.

3.1.2 Sorting and identification

All sorting and identification was done in the Lotic Insect Ecology Research Unit. Before examining the samples, the formalin was away with water while retaining the sample in a 0.5 mm sieve net. All insect samples had been preserved in 70 % ethanol.

Aquatic insects were identified up to the family level and the numbers of individuals in each family were counted. Identification was done by using, McCafferty (1983), Merritt and Cummins (1984), Stehr (1991), Dudgeon (1992) and Wiggins (1996).

Figure 3-4 Sampling methods

A. Pond net

B. Kicking sample

C. Pick method

3.2 Biological Indices

3.2.1 Diversity Index

The results of aquatic insect communities were calculated as to the diversity index by using the formula of Shannon-Wiener Index (H′)

$$H' = -\Sigma(P_i)(\ln P_i)$$

H′ = Diversity Index

P_i = Proportion of individuals of the total sample belonging to the *i*th species (or order)

The diversity index can be used to assess water quality by comparing with the table below.

	Good	Moderate Pollution	Polluted Stream
Shannon-Weiner Index	>3	1-3	<1

3.2.2 BMWP^{Thai} score and ASPT

In the Biomonitoring Working Party Score (BMWP score), each family was assigned a score according to its tolerance to pollution and the score varies from 1 to 10. This study gave the score following Mustow (2002). The least tolerant species to pollution groups are assigned a high score and the highly tolerant groups are assigned low scores (Appendix A).The total score of BMWP score was divided by the number of corresponding scoring families to calculate the ASPT. Then, to compare the ASPT with the water quality standard which was applied from the water quality standard of Thailand by Lotic Insects Ecology Research Unit.

$$ASPT = \frac{\text{BMWP score}}{\text{Number of families with score}}$$

3.2.3 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 orders of macroinvertebrates (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 (http://www.vsmp.org).

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

3.2.4 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 intolerant of pollution to 10 for organisms very tolerant of pollution. Then the results are compared with the table to assess water quality (Appendix B).

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

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

3.3 Total coliform bacteria

Total coliform bacteria were determined by using Multiple Tube Method (Sontichai, 1994) (Thai version) in Appendix C.

4. Physico-chemical properties

4.1 Physical properties

4.1.1 Temperature: Air and water temperatures were measured by thermometer. Water temperature was measured 10 cm. under the water surface.

4.1.2 Velocity: 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 meters per second unit.

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

4.1.4 Substrate components: To separate the stream to four parts by stream line (1 meter distant) and also divide by crosswise to four parts. Then, the relative proportion of the substrate particle types that were presents at each sampling sites were visually estimated.

4.2 Chemical properties

4.2.1 Conductivity: Conductivity was measured at a depth of 10 cm. by Multi-parameter analyzer consort C533 version 2.2 with µ/cm units.

4.2.2 pH: The pH was determined at a depth of 10 cm. by Multi-parameter analyzer consort C533 version 2.2.

4.2.3 Alkalinity: Alkalinity was examined by Phenolphthalein methyl orange indicator (APHA, 1998). Water samples were titrated with 0.02 N H_2SO_4 until the pH at the end point was 4.3-4.5.

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

4.2.5 Biochemical Oxygen Demand (BOD_5): Water samples were collected from sampling sites very carefully in 300 ml BOD bottles and following the Azide Modification method (APHA, 1998) for determining the dissolved oxygen value (DO_0). Final dissolved oxygen (DO_5) was kept in the incubator at 20°C for 5 days and the final DO value was taken. BOD_5 was then calculated using the formula:

$$BOD_5 = DO_0 - DO_5$$

4.2.6 Nutrients:

- Ammonia-nitrogen (NH_3-N): Ammonia was determined by the Nesslerization technique, using Nessler reagent, mineral stabilizer and polyvinyl alcohol and measured by the DR 2400 HACH spectrophotometer.

- Nitrate-nitrogen (NO_3^--N): Nitrate was determined employing the cadmium reduction method using Nitra Ver 5 Nitrate reagent by DR 2400 HACH spectrophotometer.

- Orthophosphate (O-PO_4^{3-}): The Ascorbic Acid Method, using Phos Ver 3 powder and a DR 2400 HACH spectrophotometer, determined phosphate.

5. Research duration

The duration for collecting samples was from October 2003 until August 2004. The samples were collected twice in each season.

6. Statistical analysis

The results of physico-chemical and biological parameters were analyzed by Multivariate Statistical Package (MVSP), Principal Component Analysis (PCA) had been used to study the relative proportion of insect orders to water quality and in each the physico-chemical parameters to 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.