CHAPTER 3

MATERIALS AND METHODS

3.1 Study sites

Phu Hin Rongkla National Park is located between $16^0 53^/$ to $17^0 07^/$N latitude and $100^0 56^/$ to $101^0 06^/$E longitude. Four sites; Romglao-Paradorn Waterfall, Waterwheel Waterfall, Kha Mun Noi Stream and Man Dang Noi Stream, were chosen (Figure 2-5). Substrate and land use characteristics of each sampling site are shown in Table 1. Map of study sites in Phu Hin Rongkla National Park are shown in Figure 6.

3.2 Methodology for Trichoptera larvae collection

Chemicals and Equipment

1. Pond net

2. Sorting tray

3. Vials

4. Ethanol 70%

5. Labels

6. Glass sorting dishes

7. Forceps and needles

8. Stereomicroscope and compound microscope

Figure 2 Romglao-Paradorn Waterfall
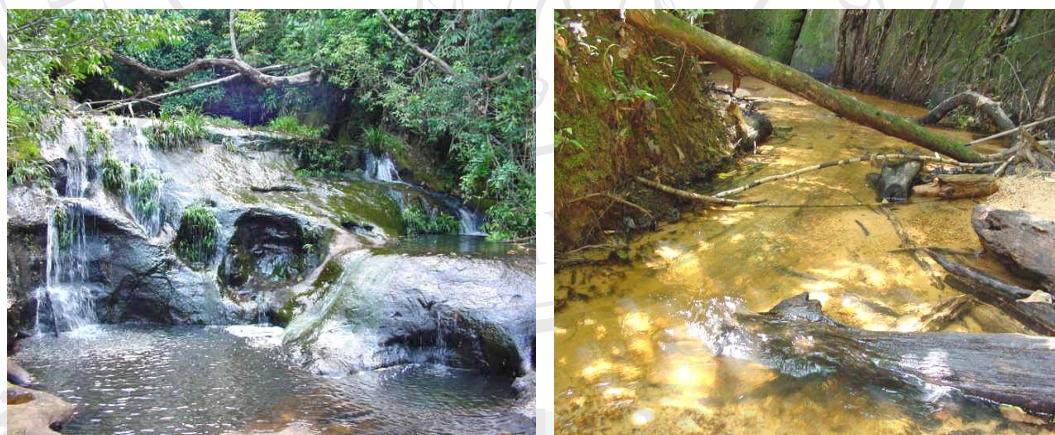
Figure 3 Waterwheel Waterfall

Figure 4 Kha Mun Noi Stream

Figure 5 Man Dang Noi Stream

Table 1 The characteristics of four stream sites in Phu Hin Rongkla National Park (Abbreviation: RPW, Romglao Paradorn Waterfall; WWW, Waterwheel Waterfall; KMS, Kha Mun Noi Stream; MDS, Man Dang Noi Stream)

Stream site Categories	WWW	RPW	KMS	MDS
coordination	$(16^059^/N101^000^/E)$	$(16^059^/N101^000^/E)$	$(16^059^/N101^000^/E)$	$(16^057^/N101^003^/E)$
Altitude	1,280 m asl.	1, 190 m asl.	1,220 m asl.	1, 600 m asl.
*Canopy	60-70% canopy	60-70% canopy	70-80% canopy	Closed canopy
Land use	Usually visited by tourists from October to April	Usually visited by tourists from October to April	sometimes visited by tourists	Forest area
Streambed Character	Bedrock predominant at above, sand with little gravel and leaf accumulation below	Lower than 20 meter accessible reach with bedrock and boulders predominant, few sand, debris pools leaf accumulation, in between bedrock and boulders	Bedrock predominant at above, large deep pool in the middle, bedrock with few debris pools in bedrock bellow	Bedrock predominant at above, leaf accumulation, debris pool below

Note: *Canopy (Source: Forest Land Resources Division, 2002)
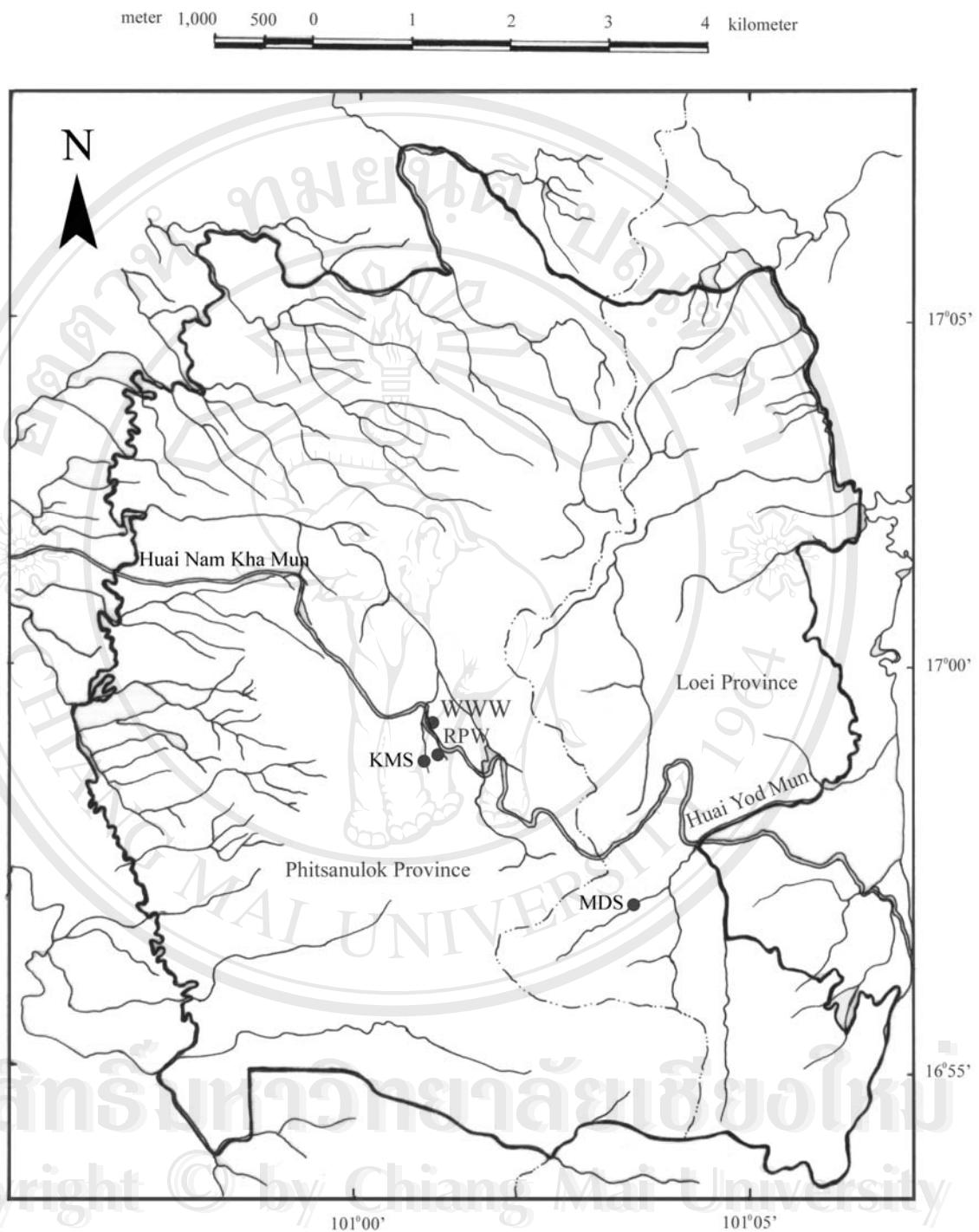
Figure 6 Map of Phu Hin Rongkla National Park, with four study sites of Romglao-

Paradorn Waterfall, Waterwheel Waterfall, Kha Mun Noi Stream, Man Dang

Noi Stream (Source: Forest Land Resources Division, 2002)

Method for Trichoptera larvae collections

Trichoptera larvae were collected from four study sites once a month for 15 months (May, 2002-July, 2002). Larvae were collected by kick sampling method for 3-5 minutes from each of 6 types of microhabitats: bedrock, sand, leaf pack, stream bank, debris pool and moss patch. Data from different microhabitats were pooled to be analyzed. Larvae were sorted in sorting trays, labeled and preserved in 70% ethanol. Then, they were brought to the laboratory of Environmental Monitoring: Aquatic Insects Research Unit (AIRU), Department of Biology, Chiang Mai University, for identification.

Trichoptera specimens were sorted and identified under a stereomicroscope. Keys of Dudgeon, (1999) and Wiggins (1996) were used to identify caddisfly to genera.

3.3 Methodology for Trichoptera adult collection

Chemical and Equipment

1. Portable black light, 10 W 12 Volt DC

2. Malaise Trap

3. Pond net

4. Sorting tray

5. Vials

6. Ethanol 70%

7. Labels

8. Petri dishes

9. Forceps and needles

10. Hot plate

11. Stereo microscope and compound microscope

12. 10% sodium hydroxide solution (NaOH)

13. Detergent

Method of adult Trichoptera collections

Light traps (portable black light, 10 W 12 Volt DC) (Figure 7A) and malaise trap (Figure 7B) were used to collect adult Trichoptera. The sampling period was 15 months from May, 2002-July, 2003. Black light trap was operated once a month and placed overnight near the stream bank. The malaise trap was set over the stream at Man Dang Noi Stream for 15 months. Specimens from Malaise trap were collected one a month. Trichoptera specimens were preserved in 70% ethanol and then were brought to the AIRU laboratory.

A   B

Figure 7 A) Black light trap, and B) Malaise trap

Identification of adult Trichoptera

Trichoptera were identified to species level by using male genitalia, terminal segments of males were cut and cleared in 10% NaOH. To identify specimens, the key of Malicky (1997c) was mainly used. Many publications were also used, such as Malicky (1987; 1989a, b; 1994; 1995; 1997a, b; 1998a, b; 1999a, b; 2002, 2005), Chantaramongkol and Malicky (1989; 1995a, b), Malicky and Chantaramongkol (1989a, b; 1990; 1991,a b; 1992a, b; 1993a, b; 1995; 1996; 1997; 1999; 2000; 2003), Luadee and Malicky (1999) and Malicky *et al.* (2000a,b; 2001; 2002; 2004; 2005)

3.4 Physical and chemical water quality study

Material for sampling water quality parameters

1. DR/2000 HACH spectrophotometer

2. pH meter

3. Thermometer

4. Velocity meter

5. Conductivity meter

6. BOD bottle (300 ml)

Methods

Thirteen parameters of water quality (i.e. Discharge, Dissolved oxygen, Air temperature, Water temperature, pH, Turbidity, Alkalinity, Nitrate, Orthophosphate, Sulfate, Ammonia Nitrogen, Conductivity and Total Dissolve Solids) were measured monthly and analyzed for 15 months. Dissolved oxygen was sampled by 300 ml BOD bottles. To determine nutrients and turbidity, water samples were collected in

1,000 ml polyethylene bottles and taken to the laboratory for analysis. Environmental variables and their analysis methodology included:

1) Air Temperature

Air temperatures were measured in four stream sites every month using a thermometer.

2) Water Temperature

Water temperatures were measured in four stream sites every month using a thermometer.

3) pH

pH was measured using the microprocessor pH-meter at all sites.

4) Conductivity

Conductivity was measured by using conductivity meter.

5) Total Dissolved Solids

Total Dissolved Solids (TDS) were measured by using conductivity meter.

6) Dissolve Oxygen (DO)

Water samples from all sites were collected in BOD bottles (300 ml) and analyzed using the Azide Modification method (APHA, 1992)

7) Alkalinity

Alkalinity was analyzed by the Phenolpthalein methyl orange indicator method (APHA, 1992).

8) Nitrate-Nitrogen

Nitrate-nitrogen (NO_3-N) was analyzed by the Cadmium Reduction Method using a HACH DR/2000 spectrophotometer with Nitra Ver5 Nitrate reagent.

9) Orthophosphate

Orthophosphate (PO_4^{3-}) was tested by the ascorbic acid methods using Phos Ver3 reagent and a HACH DR/2000 spectrophotometer.

10) Ammonia Nitrogen

Ammonium-nitrogen (NH_3-N) was determined by the Nessler method, combined with mineral stabilizer, Nessler reagent and polyvinyl alcohol, and measured for colorimeteically using a HACH DR/2000 spectrophotometer.

11) Sulfate

Sulfate (SO_4^{2-}) was determined by a HACH DR/2000 spectrophotometer with SulfaVer4 reagent.

12) Turbidity

Turbidity was determined by a HACH DR/2000 spectrophotometer.

13) Discharge

To calculate the discharge, velocity and cross section area were recorded and divided by cross section to reach small cells, measured for width and depth in each cell, measured for velocity in a representative run area, for which discharge was calculated as

$$Q = \overset{i=1}{\underset{N}{\sum}} (A_i . v_i)$$

When   Q = discharge

A = area in each cell (width x depth)

v = velocity in each cell

N = number of cell

3.5 Data Analysis

The data of larvae and adults were determined for the diversity and distribution. Water quality data were analyzed by Statistical Package for Social Science Program (SPSS/PC).