CHAPTER 3

MATERIALS AND METHODS

3.1 Thai Trichoptera Database

The database has been constructed using secondary data; mainly from the 43 published studies on caddisflies in Thailand (Malicky, 1987; Chantaramongkol and Malicky, 1989; Malicky and Chantaramongkol, 1989a; Malicky, 1989a, Malicky, 1989b; Malicky and Chantaramongkol, 1989b; Malicky and Chantaramongkol, 1990; Malicky and Chantaramongkol, 1991a; Malicky and Chantaramongkol, 1991b; Malicky and Chantaramongkol, 1992a; Malicky and Chantaramongkol, 1992b; Malicky and Chantaramongkol, 1993a; Malicky and Chantaramongkol, 1993b; Malicky, 1994; Malicky and Chantaramongkol, 1994; Chantaramongkol and Malicky, 1995a, Malicky and Chantaramongkol, 1995; Chantaramongkol and Malicky, 1995b; Malicky, 1995; Malicky and Chantaramongkol, 1996; Malicky and Chantaramongkol, 1997; Malicky, 1997; Malicky, 1998a; Malicky, 1998b, Malicky, 1999a, Malicky and Chantaramongkol, 1999; Luadee and Malicky, 1999; Malicky, 1999b; Malicky and Chantaramongkol, 2000; Malicky *et al.*, 2000a; Malicky *et al.*, 2000b; Malicky *et al.*, 2001; Malicky *et al.*, 2002; Malicky, 2002; Malicky and Chantaramongkol, 2003; Malicky *et al.*, 2004; Malicky *et al.*, 2005; Malicky, 2005; Malicky and Chantaramongkol, 2006; Malicky, 2006; Malicky *et al.*, 2006; Malicky and Prommi, 2006; Malicky and Chantaramongkol, 2007), and also from other published papers (i.e. Malicky, 2009b; Malicky, 2010b; Malicky, 2011). The publications were sorted for information such as:

■ the taxonomic information (Holotype and/or Paratype) of each species

■ the location of specimen collection (province, national park, site and coordinates)

■ the date of specimen collection (month and year)

■ the number of male individuals found

■ other existed information i.e. number of female and place of specimen deposition

The Thai Trichoptera Database included secondary data from previous post-graduate studies (Chaibu, 2002; Chaiyapa, 2001; Cheapudee, 2006; Cheunbarn, 2002; Klaytong, 2000; Luadee and Prommi, 2011; Nawvong, 2005; Nuangchalerm, 2001; Nuntakwang, 2006; Prommi, 1999; Prommi 2011; Thapanya, 2004 and Thamsenanupap, 2005 - see table 3.1). Additionally, the recent study involved field work and specimen collections from Mae Hong Son, Chiang Mai, Lampang, Phrae and Nan (table 3.2).

Table 3.1 Secondary data sources which were added in Thai Trichoptera Database.

Data source	**National Park**	**Province**	**Sampling site(s)**
Chaibu, 2002		Chiang Mai	12 sampling sites along Ping river
Chaiyapa, 2001	Nam Nao	Phetchabun	Yakruea and Phromlaeng streams
Changthong, 2005	Phu Hin Rong Kla	Phitsanulok	Romglao-paradorn WF, Water Wheel WF, Kha Meun Noi WF, and Man Dang Noi WF

Table 3.1 continued

Data source	National Park	Province	Sampling site(s)
Cheapudee, 2006		Chiang Mai	Huay Thung Tao Reservoir, Mae Jok Luang Reservoir, Nong Huay Yuak Reservoir, Mae Yuak Noi Reservoir, Ang Kaew Reservoir, Lotus Fields and Paddy Fields
Cheunbarn, 2002		Chiang Mai	Along Ping river from A. Chiang Dao, Chiang Mai province to Lampun province
Klaytong, 2000	Nam Nao	Phetchabun	Phromlaeng stream
Luadee and Prommi, 2011		Surat Thani	along Tapee River
Nuangchalerm, 2001	Nam Nao	Phetchabun	Yakruae and Phromlaeng streams
Nawvong, 2005	Wieng Ko Sai	Phrae	Pan Chen WF and Mae Koeng Yai WF
Nuntakwang, 2006		Lampun, Chiang Rai, Chiang Mai, KampaengPhet and Phayao	Koh Luang WF, Pu Kaeng WF, Sri Sang Wan WF, Klong Lan WF, Champathong WF, and Chiang Dao Wildlife Research Center
Prommi, 1999	Doi Suthep-Pui	Chiang Mai	Huay Kaew, Huay Palad, Huay Koo Kaw
Prommi, 2011		Southern Thailand	24 sampling sites in Southern part of Thailand
Thapanya, 2004	Doi Inthanon and Doi Suthep-Pui	Chiang Mai	3 sampling sites in Doi Inthanon and 3 sampling sites in Doi Suthep-Pui
Thamsenanupap, 2005	Doi Inthanon and Doi Suthep-Pui	Chiang Mai	Siribhum WF, Mae Klang at Ban Sop Aep, Huai Sai Lueng WF, Mae Pan Noi stream, Sai Yoi WF and Monthatarn WF

Table 3.2 Location, habitat type and altitude of light trap collecting in current study during 2009-2010.

Province/Amphoe Sampling site	Coordination	Habitat type	Altitude (m asl)
Mae Hong Son/Salawin			
Huay Mae Kaa1 (Ban Mae Samlab)	18° 9' 30.5"N, 97° 52' 45.7"E	stream, (cobble, boulder)	420
Huay Mae Kaa2 (Ban Mae Samlab)	18° 9' 42.4"N, 97° 53' 12"E	stream (cobble, boulder)	350
Huay Tok	18° 18' 26.9"N, 97° 51' 4.9"E	stream (gravel and sand)	397
Pai River	19° 22' 38.6"N, 98° 26' 24.1" E	wide stream (gravel)	517
Chiang Mai/Mueng			
***Doi Suthep-Pui National Park**			
Pa Ngerb	18° 48' 47" N 98° 56' 18.4" E	stream (cobble, gravel)	461
Montatarn lower	18° 49' 0.4" N 98° 55' 24.7" E	waterfall + stream (bedrock, sand)	693
Montatarn upper	18° 49' 2.7" N 98° 55' 24.5" E	waterfall + stream (bedrock, cobble)	746
Pa Lad	18° 47' 2.7" N 98° 55' 24.5" E	waterfall + stream (bedrock, gravel)	686
Pu Ping water supply	18° 48' 19.6" N 98° 54' 28.5" E	waterfall + stream (bedrock, sand)	1,171
Chiang Mai/Jomthong			
***Doi Inthanon National Park**			
Mae Klang Sob Ab	18° 31' 47.68"N, 98° 36' 34.45" E	stream (boulder, cobble, sand)	630
Wachiratarn	18° 32' 30.45" N 98° 35' 57.07" E	waterfall + stream (bedrock, boulder)	750
Siritarn	18° 32' 36.74" N 98° 34' 47.33" E	waterfall + stream (bedrock)	890

Table 3.2 continued

Province/Amphoe Sampling site	Coordination	Habitat type	Altitude (m asl)
Siribhum	18° 33' 11.36" N 98° 34' 33.50" E	waterfall + stream (cobble, boulder, bedrock)	1,380
Kaew Mae Pan lower	18° 32' 47.55" N 98° 30' 58.35" E	stream (boulder, cobble, organic materials)	2,051
Kaew Mae Pan upper	18° 32' 37.93" N 98° 28' 34.93" E	stream (boulder, gravel, organic materials)	2,074
Chiang Mai/Chiang Dao			
Huay Mae Ka 1	19° 21'41.49"N, 98° 55'6.3"E	stream (boulder, gravel)	540
Huay Mae Ka 2	19° 21'42.01"N, 98° 55'13.01"E	stream (boulder, gravel)	520
Huay Mae Ka 3	19° 21'41.73"N, 98° 55'20.13"E	stream (boulder, gravel)	518
Chiang Mai/Om Koi			
Mae Tuen River	17° 28'14.1"N, 98° 26'50.2"E	river (gravel, sand)	481
Lampang			
Jae Sorn WF	18°46' N, 99°28' E	waterfall + stream (bedrock, boulder)	500
Phrae/Mueang			
Yom River 1	18° 10'52.9"N, 100° 8'19.2"E	river (gravel, sand)	147
Yom River 2	18° 10'52.9"N, 100° 8'19.2"E	river (gravel, sand)	147
Paddy field at Ban Tung Hong Tai	18° 9'56.4"N, 100° 8'31.6"E	temporary wetland	127
Nan/Tung Chang			
Huay Satang	-	stream	-

Note * indicated monthly collecting sites during November 2009 - November 2010

3.2 Trichoptera in Doi Suthep-Pui and Doi Inthanon NPs

Sampling sites

Doi Suthep-Pui National Park The total area of Doi Suthep and Doi Pui National Park is 261 km^2 (declared as National Park on 1981). The study sites are situated 350 - 1,610 m asl (Doi Suthep) and 1,685 (Doi Pui). The vegetation zone comprises of evergreen forest (in areas above 800 m) and deciduous forest (below 800 m). During 1952-1997, annual average precipitation at foothill was 1,067.8 mm , 1,670.1 mm at the National Park headquarters, and 2,095 mm at Pu Ping village, and the wet season occurred during May - October (August - September had the highest rainfall and December-January had the lowest rainfall). Annual average temperature were 21.1^o C for the cool season (December-January) and 29^o C for the hot season (in April - May). Threats to natural areas within Doi Suthep-Pui National Park include tourism, forest fires, and agriculture (Maxwell and Elliott, 2001).

Study sites in Doi Suthep-Pui National Park (see figure 3.1 and 3.2)

Pa Ngerb (PNG) – A medium stream, with an average width of 3 - 5 m, situated at 461 m asl, and with an instream substrate comprised of cobble (64 - 225 mm diameter) and gravel (2 - 64 mm diameter). A small concrete dam is located upstream of the sampling site. Trash, such as plastic bags and glass bottles, were observed at the study site, indicating that the area is frequented by tourists, or that trash is arriving to the site from upstream locations.

Montatarn lower (Mon_lower) - a popular location for tourists, situated at 693 m asl. The instream substrate was comprised dominantly of bedrock and was sandy in the downstream waterfall. One stream bank and riparian zone remained natural, while others have been modified as recreation areas. The average stream width was about 3-

6 meters. Trash was not a major problem at this site, and overall the site appeared in good-keeping.

Montatarn upper (Mon_upper) - The upper section of Montatarn water fall (2nd level) is situated approximately 500 m from the lower section of Montatarn water fall (746 m asl). The sampling site was located at a waterfall approximately 10-12 m high , with an instream substrate consisting predominantly of bedrock and cobble. In order to create a swimming area for recreationists, instream substrate has been moved, thus changing channel flow. Riparian forest remained in good conditions.

Pa Lad (PL) - Situated at 686 m asl, and located near the main road, this small waterfall had a predominate substrate of bedrock and gravel. The stream width was 1 - 3 meters. Riparian forest remained in good conditions. Some trash was observed instream and along the banks. The habitat was not stable. Moreover, in April, the channel dried out for few days (during light trap sampling).

Pu Ping water supply (PP) - This sampling site a water supply for Pu Ping Palace. , and a permanent dam has been built upstream of the waterfall. The riparian forest remained in good conditions. Sediment accumulation and bank instability was observed in front of dam. The predominant substrates were bedrock and sand.

Doi Inthanon National Park - Doi Inthanon was established as a national park in 1978 to preserve the unique ecosystems and organisms that are located within its particularly high elevation range. The national park covers an area approximately 482 km^2, and is situated from 400 - 2,565 m asl. This wide altitudinal range relates roughly to three climate zones . The areas below 1,000m, between 1,000m – 2,000m, and above 2,000m represent a sub-tropical climate, tropical climate, and temperate climate, respectively. Cloud forest exists in the high elevations and moist areas (e.g. at

Angka). Temperatures are relatively low whole year round, and particularly low during December and January (frost occurs occasionally). Annual average rainfall is relatively high (2,000 - 2,100 mm), and the wet season occurs between June and November) (Source: Department of National Parks, Wildlife and Plant Conservation :http://web3.dnp.go.th/parkreserve/nature.asp?lg=2 [27 Nov 2011]).

Study sites in Doi Inthanon National Park (see figure 3.1 and 3.3)

Mae Klang Sob Ab (MK) - sampling site was situated at 630 m asl. Stream width was between 12 - 14m, and average depths of 0.5 - 2.0 m. Predominant substrates were boulder (> 256 mm diameter), cobble and sand. Bank stability was low. Riparian forest remained in good natural conditions.

Wachiratarn (WRCT) - sampling site was located at 750m asl. The waterfall was large and turbulent, with a stream width between 8 - 10 m, and with depths greater than 0.5 m. This area is a popular tourist attraction. One stream bank has been modified for tourist parking and as a scenic area, whereas another bank remained in its natural conditions. The instream habitat was not disturbed. The predominant substrate was bedrock and boulder. Trash was observed along the stream bank.

Siritarn (SRT) - sampling site was situated at 890 m asl. The waterfall was large, turbulent, and about 40 m in height. Stream width was between 8 and 12 m, and depth greater than 0.5 m. The dominant substrate was bedrock. The sampling site was difficult to access due to its steep slope and, as a result, there was less observed impacts of tourism. The riparian forest remained in good natural conditions.

Siribhum (SRB) - sampling site was located at 1,380 m asl. The area contained a turbulent waterfall and stream which was between 3-4 m wide and less than 0.5 meter deep. Boulder and cobble was the predominant substrate. Areas adjacent to stream

banks have been heavily modified for tourism purposes, with gardens present. Relatively little trash was observed at the site. Channel and instream modification was not observed, and stream banks were stable.

Kaew Mae Pan lower (KMP_lower) - sampling site was located at 2,051 m asl, hidden in forest about 200 m from the Kaew Mae Pan tourist station. Streams were small, with widths between 2 – 4m and with depths of less than 0.3 m. Riparian forest covered the area densely. Low average annual temperatures, along with high relative humidity, allows for presence of mosses and ferns at the site, often growing on tree trunks and instream rocks. Predominant substrates were boulder, gravel and organic materials.

Kaew Mae Pan upper (KMP_upper)- sampling site located at 2,074 m asl. Stream width was between 2-4 m and depth was less than 0.5 m. Predominant substrates were boulder (covered with mosses), gravel and organic materials. Dense forest covered the stream channel. Average annual temperatures are low and relative humidity is high, which has allowed the formation of cloud forest at this site.

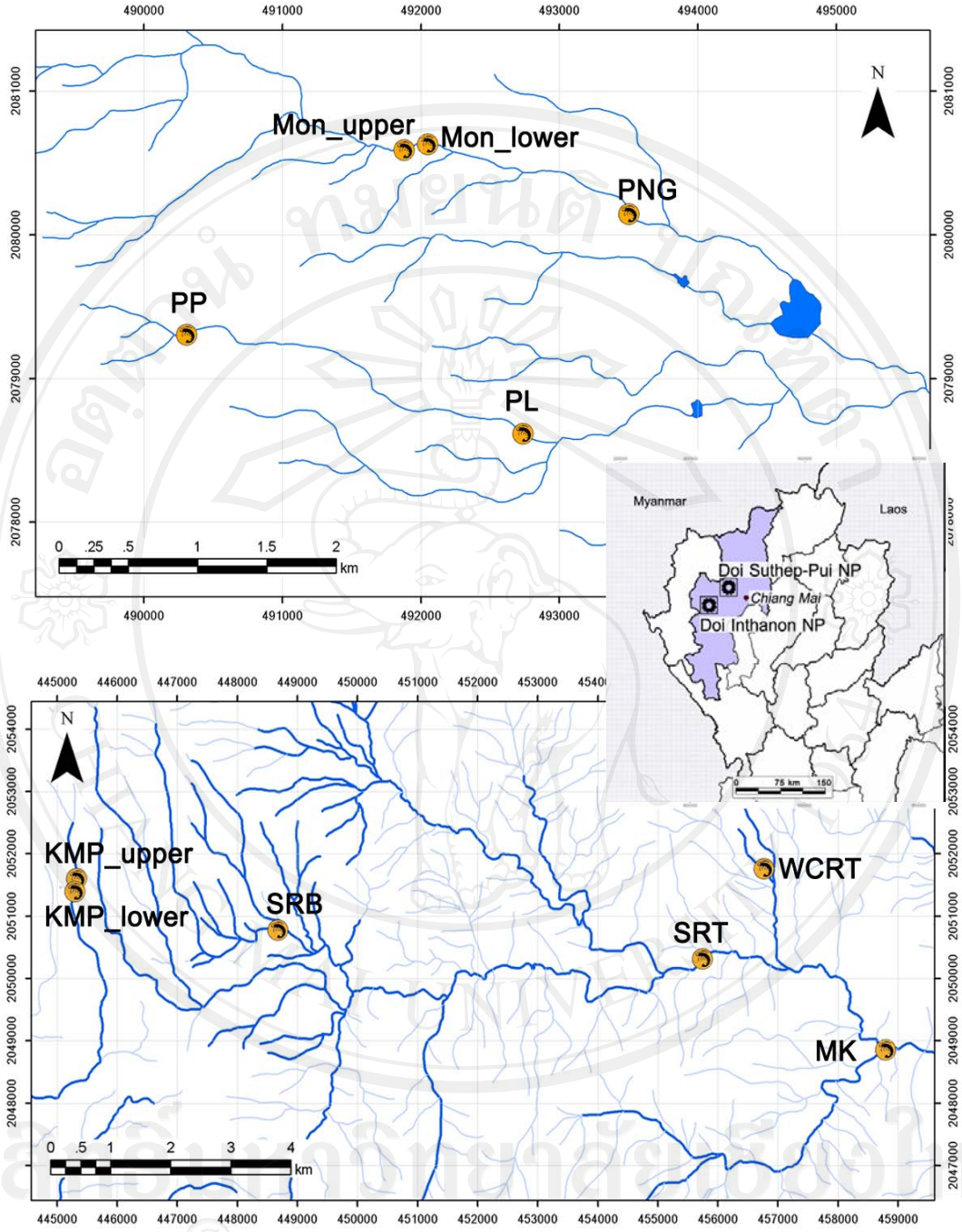

Figure 3.1 Sampling site location in Doi Suthep-Pui (upper) National Park (PNG= Pa Ngerb; Mon_lower=Montatarn lower; Mon_upper=Montatarn upper; PL=Pa Lad and PP=Pu Ping water supply) and Doi Inthanon National Park (lower: MK=Mae Klang Sob Ab; WCRT=Wachiratarn; SRT=Siritarn; SRB=Siribhum; KMP_lower=Kaew Mae Pan lower and KMP_upper=Kaew Mae Pan upper)

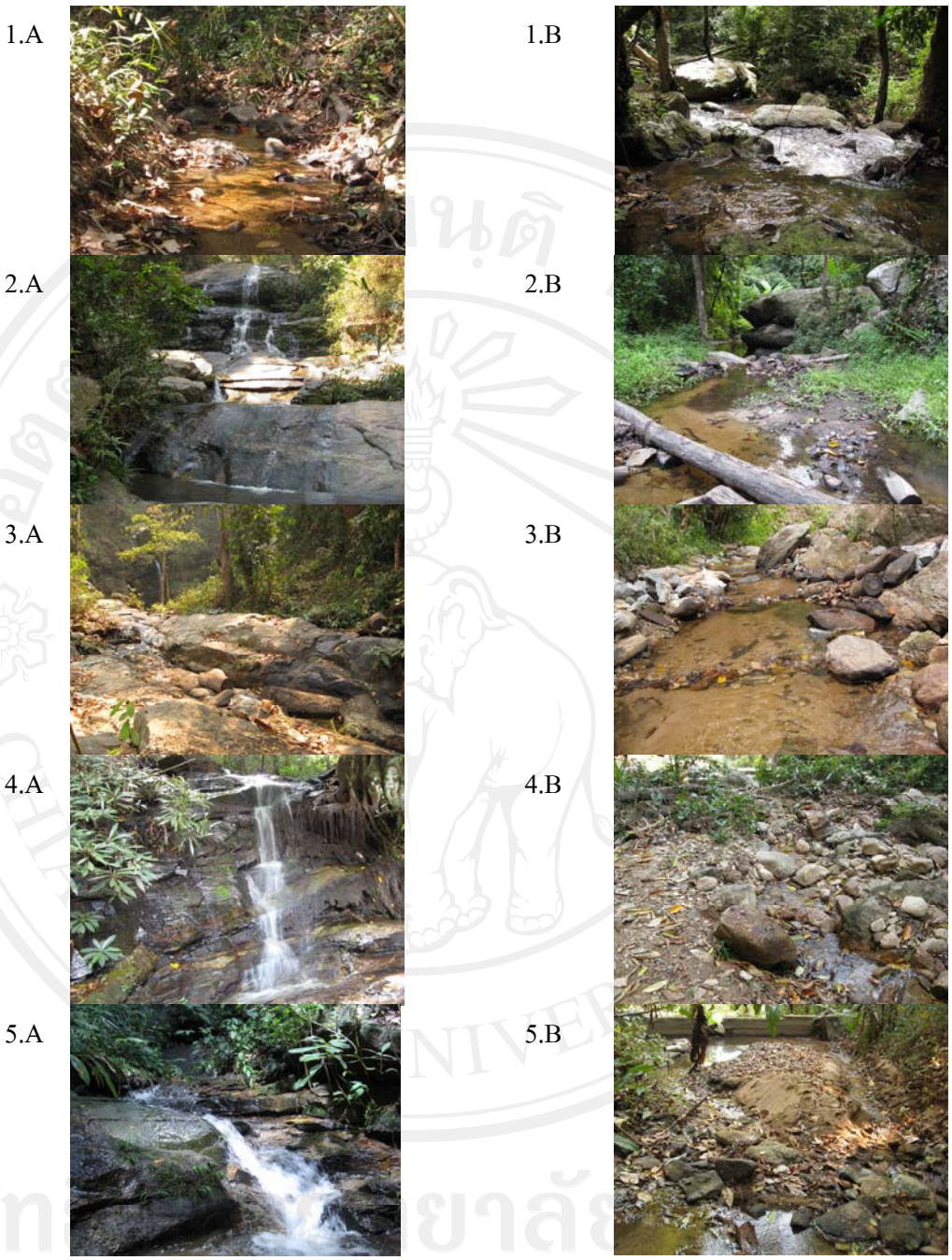

Figure 3.2 Sampling sites in Doi Suthep-Pui NP (A=upstream, B=downstream, 1=Pa Ngerb; 2=Montatarn lower; 3=Montatarn upper; 4=Pa Lad; and 5=Pu Ping water supply).

Figure 3.3 Sampling sites in Doi Inthanon NP (A=upstream, B=downstream; 1=Mae Klang Sob Ab; 2=Wachiratarn; 3=Siritarn; 4=Siribhum; 5=Kaew Mae Pan lower; and 6=Kaew Mae Pan upper).

Water quality sampling and measurement

Chemical reagents and glass wares

- 1 liter polyethylene bottles
- glass wares e.g. 250 ml flask, 25 ml beakers, pipettes, burettes
- $MnSO_4$
- conc. H_2SO_4
- 300 ml DO and BOD bottles
- 0.025 N $Na_2S_2O_3$
- thermometers
- CONSORT C533 multiparameter analyzer
- tape measure
- Nessler reagent
- ice box
- Mineral stabilizer
- Starch reagent
- Polyvinyl Alcohol Dispersing agent
- Global Water FP101 velocity meter
- HACH, NitraVer 5 Nitrate reagent powder pillow for 25 ml sample
- HACH DR/2000 spectrophotometer
- AIA (Alkali Iodide Azide)
- 80 % Ethyl alcohol
- HACH, PhosVer 3 Phosphate reagent powder pillow for 25 ml sample

Measurement procedure

During November 2009 - November 2010, adult trichoptera was trapped using light (pan) traps. Water quality was measured using measurements of air temperature, water temperature, width, depth, velocity, discharge, pH, conductivity, total dissolved solid (TDS), dissolved oxygen (DO), biochemical oxygen demand (BOD), nitrate nitrogen, ammonia nitrogen, ortho-phosphate, turbidity and habitat assessments. The analysis methods and instruments used are provided in Table 3.3.

Habitat assessment is based on 1) Epifaunal substrate available, 2) Embeddedness, 3) Velocity/Depth Regime, 4) Sediment deposition, 5) Channel flow status, 6) Channel alteration, 7) Frequency of riffles (or bends), 8) Band stability (score each bank), 9) Vegetation protection (score each bank), and 10) Riparian vegetation zone (score each bank). The score were given in each parameters from 0 to 20 at the sampling sites and then average score was calculates. There are 4 ranges of condition categories i.e. poor (0 -5), marginal (6-10), suboptimal (11-15), and optimal (16-20) (see the category criteria based on Barbour *et al.*, 1999 in appendix A).

Figure 3.4 Light (pan) trap in operation and adult trichoptera which were caught.

Adult trichoptera sampling procedure - light (pan) trap

Adult trichoptera were sampled monthly from 11 sites using light (pan) traps. Light traps were made up of rechargeable 12N11 batteries, a black light trap, pan and detergent (dish washing reagent).

The pan was filled one third with water and added with one tea spoon of detergent, then bubbled. A light bulb and ballast was attached on one side of the tripod and positioned over the pan. Batteries were located relatively far from the

tripod (figure 3.4). The light trap was set on the stream bank before sunset and left overnight. In the morning insects were collected from the pan and were preserved with 70% ethyl alcohol. Adult trichoptera was attracted by black light and fell into the pan, and the detergent was used to break surface tension.

In the laboratory

Adult trichoptera was sorting and identified under a microscope. Only male trichoptera are identifiable down to species level (by using genitalia characters). The procedure for identification followed that of Malicky (2010a). Male trichoptera was classified into family level using ocelli presence and absence, spur formula, number of segment of maxilary palp. For some genera wing pattern was also useful. Male genitalia was observed by cutting at segment 5 of the abdomen, placed in a glass block and filled NaOH or KOH 10% w/v over the piece, then covered with glass plate. The glass block was then put on a hot plate (with warm heat) for between 5 minutes - 1 hour, depending on the size of the abdominal piece. The abdominal piece was moved into another glass block which was filled with water, and with a drop of detergent. Residue of the soft tissue was removed from the harden sclerite carefully using fine forceps under stereomicroscope. The piece was then removed and placed in 80% ethyl alcohol with the remaining body parts (1 specimen per vial). This procedure is called maceration. Genitalia characteristics were compared using the '*Atlas of Southeast Asian Trichoptera'* (Malicky, 2010a). For further verification, at least three views (dorsal, ventral and lateral) of genitalia were sketched under a stereoscope, using a drawing tube.

Table 3.3 Physico-chemical parameters and their analysis method and unit.

Parameters	Unit	instrument /analysis method
Physical parameters		
Temperature	^{o}C	Thermometer
Stream width and depth	meter	Tape measure
Velocity	mph	Global Water FP101 velocity meter
Total dissolved solid (TDS)	mg/l	CONSORT C533 multiparameter analyzer
Conductivity	µS/cm	CONSORT C533 multiparameter analyzer
Turbidity	FTU	HACH DR/2000 spectrophotometer
Chemical parameters		
pH		CONSORT C533 multiparameter analyzer
Dissolved oxygen (DO)	mg/l	Azide Modification Method
Biological Oxygen Demand (BOD)	mg/l	Azide Modification Method
Nutrients		
• ammonia nitrogen	mg/l	Nessler Method and HACH DR/2000 spectrophotometer
• nitrate nitrogen	mg/l	Cadmium Reduction Method and HACH DR/2000 spectrophotometer
• ortho-Phosphate	mg/l	Ascorbic Acid Method and HACH DR/2000 spectrophotometer

Data analysis

Thai Trichoptera Database

Biotope, occurrence, and monthly distribution and abundance were summarized for each species.

- biotope type was classified into 7 groups based on altitude distribution and habitat type, as following:

Biotope type	altitude distribution	habitat type
I	> 1,700 m asl	stream
II	1,200 - 1,700 m asl	stream
III	500 - 1,200 m asl	stream
IV	< 500 m asl	stream
V	< 500 m asl	river
VI	< 500 m asl	permanent standing water
VII	< 500 m asl	temporary standing water

The frequency of biotope types was recorded for each species, respectively.

- occurrence was summation.

- monthly distribution was revealed for the number of individuals of each species, based on monthly recordings

- abundance was revealed as a summation of all existing records. There were some reports didn't show the number of individuals, and sometimes also for present/absent data and abundance rank data, respectively. Present data was showed with "p".

Trichoptera in Doi Suthep-Pui and Doi Inthanon NPs

The presence of species found in sampling sites was shown, and occurrence and abundance was summarized. The data were compared with Prommi (1999) and Thamsenanupap (2005).

Temporal distribution was classified based on the seasonal pattern via monsoonal influences (from Thapanya, 2004 and Thamsenanupap, 2005), such as:

non seasonal - existed all year

hot season - only existed in March - May

wet season - only existed in June - October or three months in the period

dry season - only existed in November - February

In the case of scattered existence, that species would be labeled with the month in abbreviation.

Abundance status was categorized (adopt from Wells and Cartwright, 1992) as

rare (r) a total of 1-2 male trichoptera from all collecting.

common (c) a total of 3-100 male trichoptera from all collecting.

abundant (a) a total of > 100 male trichoptera from all collecting.

EstimateS statistic program was used to express species richness in both national parks.

SPSS was used to reveal differences among physico-chemical parameters in all sampling sites (ANOVA).

PC-ORD statistical program was used for site clustering and for identifying indicator species (using TWINSPAN).