CHAPTER 3

Material and methods

3.1 Sampling area; Mae Ngat Somboonchol dam

Mae Ngat Somboonchol dam, the part of Sri Lanna National Park, was built by His Majesty the King Rama IX's order in 1977 for purposes of irrigation and prevention of downstream flooding, by blocking the Mae Ngat River, Mae Tang district, Chiang Mai province. This earthen fill dam is 1,950 meters long and 95 meters high, the reservoir has an area of 300 square kilometers, maximum water storage capacity up to 256 million cubic meters. Addition to purpose of irrigation, two hydropower plants were constructed with 9,000 kilowatts electric capacity, they can produce electrical energy up to 19 million kilowatt hours per year.

The dam has two outlets for draining water. The first outlet from hydropower plant was a large narrow cement-block and sloping channel. The second outlet from bottom of spillway, which widened and be wetland formed, at the end both joined to tributary of Mae Ngat River, see in Figure 3.1 and Figure 3.2.

3.2 Description of sampling sites

Study area in Mae Ngat Somboonchol dam consist of seven sampling sites covered area of Sri Lanna National Park, Royal Irrigation Department; Regional Irrigation Office 1, and Electricity Generating Authority of Thailand; Hydroelectric generator of Mae Ngat Somboonchol dam. The sampling route was beginning from tributary stream of Mae Wa stream along through the Ping river in Mae Tang District, approximately 6.48 km. long and elevations ranging over 344 to 521 m asl. All sampling sites were divided into two groups by two upstream sites which following by upstream sampling site 1 (SU1) and upstream sampling site 2 (SU2), five downstream sampling sites which following by downstream sampling site 3 (SD3), downstream

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

sampling site 4 (SD4), downstream sampling site 5 (SD5), downstream sampling site 6 (SD6) and downstream sampling site 7 (SD7) (Figure 3.2)

Upstream sampling site (SU1) – The reference site, there was natural tributary stream from Mae Wa stream in mixed deciduous forest close to the Sri Lanna National Park, situated at 345 m asl., coordinate 19°11'19.76"N, 99°12'16.98"E. The channel shape was narrow with average width of 3 – 4 m and covered with vegetation plant along the channel. There were many riffles zone along the channel. The average depth (from bank to bank) was 0.1 – 5 m and the dominant substrate sediment was gravel, cobble and some bed rock. The average velocities were 0.2 – 0.7 m/s. The stream structure was remaining the same along year period except the depth and velocities which were high in monsoon season and finally flood (in November to January). There was a crossing local road across the stream at the end of the sampling site, which acted like a check dam in this stream. The channel was continuing to the second sampling site. There was none disturbance or activities of human activities close to the stream. (Figure 3.3)

Site 2 Upstream (SU2) – A natural stream, flow channel was continuous from upstream sampling site 1 approximately 1 km long, more width, depth and velocities, of 3 – 5m, 0.2 – 1.4 m and 0.2 – 9.4 m/s, respectively. This site was opened area, no big trees. The area was surrounded with *Mimosa pigra* and Cyeraceae plants. There were mix with many substrates such as rock, gravel, pebble, sand, mud and clay. At the end point of the sampling site are close to the reservoir, this spot was flood in monsoon season or when the reservoir capacity was full. Flashflood situation were taking for a few months and become a lentic ecosystem (Figure 3.4). The road was blocked by the water level, so researcher had to use a boat to collecting the materials. Additions, on this situation the benthic macroinvertebrate in the stream were hardly to found. After the flood, there were some actives by villager such as cattle.

Site 3 Downstream (SD3) – the first sampling site below the reservoir, located next to the hydro electrical generator, the water was release to the channel through the village, situated at 333 m asl. The water channel was 25 m width and average depth of 0.05 – 2 m, cement covered along the both side of the channel. The water was daily released every evening. Water velocities was depending on release time from the gate,

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

when the gate was close the averages speed was 0-0.2 m and up to 27 m/s when the gate was open. Normally, this area was shallow and clearly sees the substrate, mostly boulder (> 256 diameter) and some cobble covered with algae. (Figure 3.5)

Site 4 Downstream (SD4) – This wetland closed to the spillway. This wetland, situated at 333 asl., located on junction of stream channel from the hydro electrical generator and the channel size become larger to be a river form, flows along through Ping river. The wetland still has water seepage entire year, most of area are covered with parrot's feather (*Myriophyllum aquaticum*). The water discharges are come from the hydroelectric generator channel and spill way. Mostly substrate material was sand and clay. (Figure 3.6)

Site 5 Downstream (SD5) – This sampling site was the river, range of width was 20- 100 m and the depth was depending on released from the hydroelectric generator, up to 5 m. On the begins of the survey, this area was s river which has width of 30 – 50 m and depth of 3-4 m, but there were degraded the canal to increase the size, continues during research period. In daytime, before 5 pm, the water level was low and we took the sampling on the time and after 5 pm the water gate will release the water from the generator that increase to width and depth, not suitable for sampling. The substrate types were sand and clay. (Figure 3.7)

Site 6 Downstream (SD6) – This area was braided channel with longitudinal and transverse, far from the bridge for 1 km, the width of 3 – 20 m and has an erosion bank and cement in opposite side. This flow channel was flow through village and supplied for town activities. There had some rain tree (*Samanea saman*) for shade along the channel. The substrate of the stream was coarse gravel to very coarse sand. (Figure 3.8)

Site 7 Downstream (SD7) – the braided channel with longitudinal flows to the Ping river, far from site 6 average 800 m. situated 343 m asl. Approximately width and depth was 10-25 m and 0.3 – 0.8 m, respectively. There was an opened area without a big tree, one side was a concrete and another side was an erosion bank and riparian plants, mostly *Mimosa pigra*. This area was the last channel point from this river before the flows go through the Ping River. (Figure 3.9)

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Figure 3.1 All 7 sampling sites sorting by upstream to downstream. (moderated from google earth, 2014)

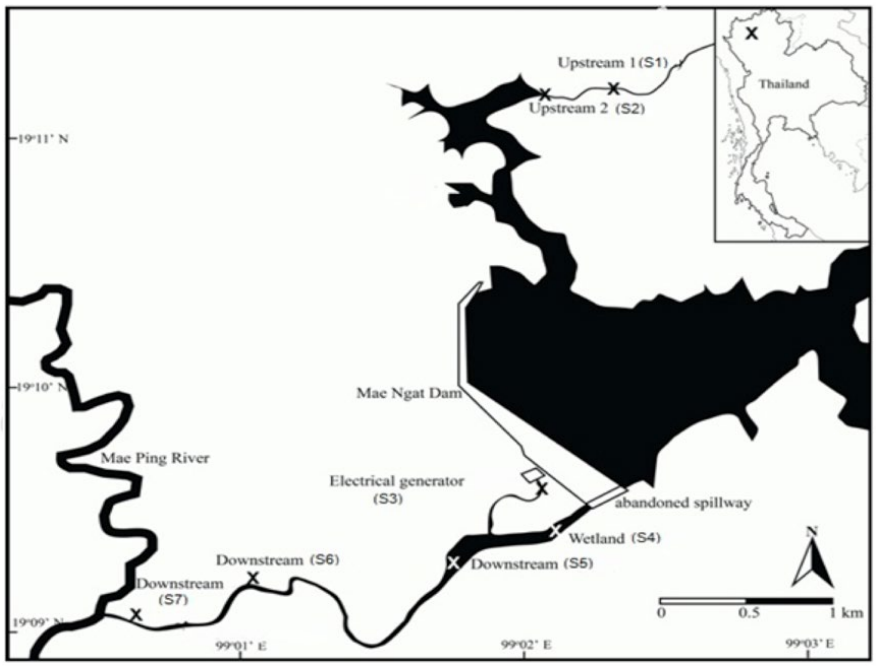

Figure 3.2 Drawing map showed all sampling sites on Mae Ngat Somboonchol dam. (edited from Thapanya *et al*, 2015)

Sampling sites

a) b)

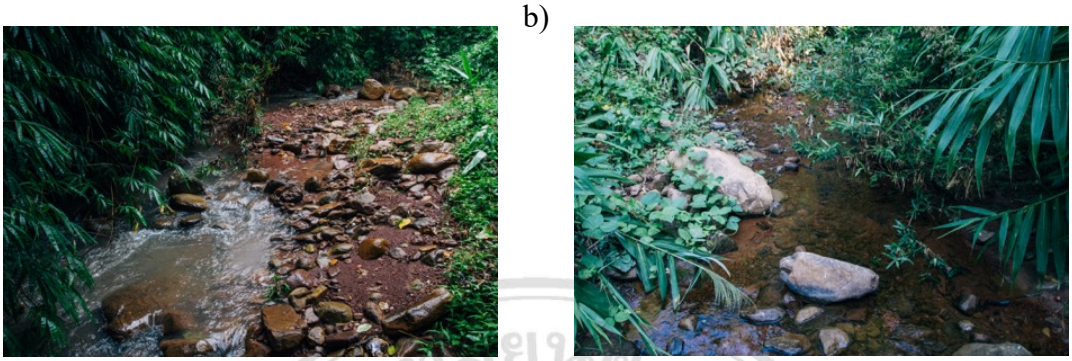

Figure 3.3 Upstream sampling site 1 (SU1) on a) July 2012. b) August 2013.

a) b)

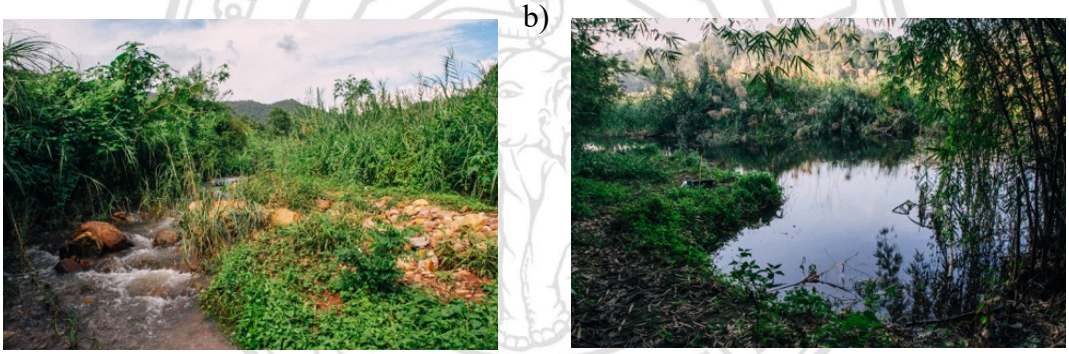

Figure 3.4 Upstream sampling site 2 (SU2) on a) August 2012 b) January 2013.

a) b)

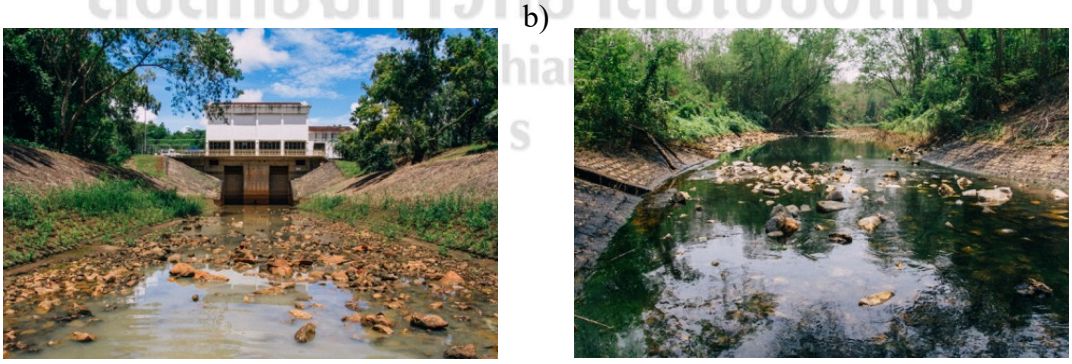

Figure 3.5 Downstream sampling site (SD3) on a) July 2012. b) September 2012.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

a) b)

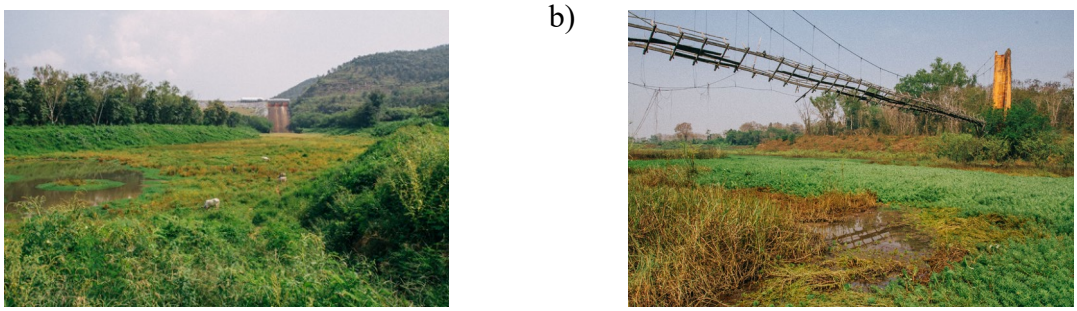

Figure 3.6 Downstream sampling site 4 (SD4) on a) October 2012. b) March 2013.

a) b)

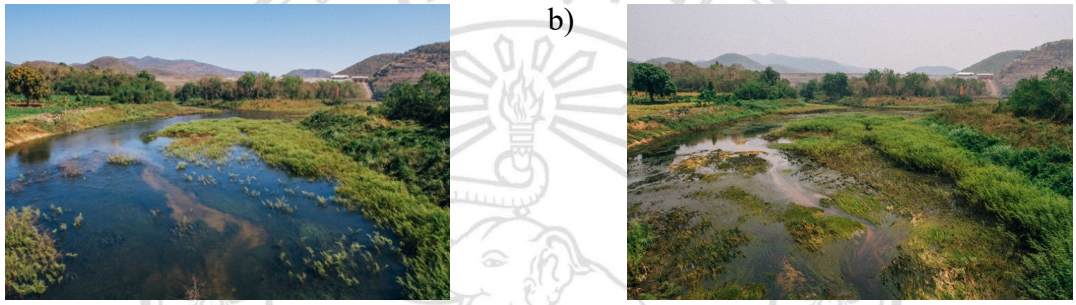

Figure 3.7 Downstream sampling site 5 (SD5) on a) September 2012. b) April 2013.

a) b)

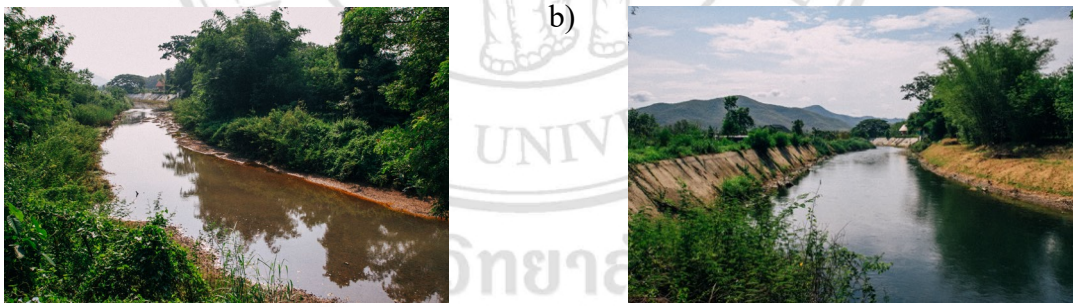

Figure 3.8 Downstream sampling site 6 (SD6) on a) October 2012. b) May 2013.

Copyright© by Chiang Mai University
All rights reserved

a) b)

Figure 3.9 Downstream sampling site 7 (SD7) on a) August 2012. b) May 2013.

3.3 Physicochemical water sampling

Water quality and measure equipment in field

- Thermometer
- Multiparameters analyzer (CONSORT C533)
- Measuring tape
- Velocity meter (Global Water FP101)
- Polyethylene bottles (1 litter)

Water quality and measure equipment in laboratory

- Glass wares
    - 250 ml flasks
    - 25 ml beakers
    - Pipettes
    - Burettes
- Spectrophotometer (HACH DR/2000)
- AIA (Alkali Iodide Azide) solution
- $MnSO_4$ solution
- Conc. $H_2S_2O_3$
- Starch reagent
- Nessler reagent
- Mineral stabilizer
- HACH, NitraVer 5 Nitrate reagent powder pillow for 25 ml sample.
- HACH, PhosVer 3 5 Phosphate reagent powder pillow for 25 ml sample.
- 80% ethyl alcohol

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Water quality measurement procedure

Generally, from the physical habitat quality assessment, the water quality measurement should have completed before sampling larvae, for not disturbed the streambed.

The air-water temperature, width, depth, velocity, discharge, pH, conductivity, total dissolved solid (TDS) were measure directly on sites by using a multiparameters analyzer CONSORT C533. The water sampling was collected in a glass bottle for

analyzed the dissolved oxygen (DO), biochemical oxygen demand (BOD) and collected in the polyethylene bottles for analyzed the nitrate nitrogen, ammonia nitrogen, ortho-phosphate with Azide Modification Method, those were analyzed in the laboratory (APHA AWWA WPCE, 1992).

Ammonia nitrogen (NH_3-N) was analyzed by using the Nessler reagent technique. Nitrate nitrogen (NO_3-N) was analyzed by cadmium Reduction method by using Nitra Ver5 Nitrate chemical pillows. Orthophosphate (PO_4-P) was analyzed by the Ascorbic Acid method using Phos Ver3 powder. All three methods were recorded in milligrams per liter (mg/l) and using a HACH DR/2000 for analyzed, see in Table 3.1.

Table 3.1 Physicochemical of water parameters method of sampling and procedure for analysis.

List	Water parameter	Unit	Method	procedure
1.	Air temperature	°C	Mercury thermometer	On site
2.	Water temperature	°C	Mercury thermometer	On site
3.	pH		pH meter	On site
4.	Conductivity	µS/cm	multiparameters analyzer	On site
5.	TDS		multiparameters analyzer	On site
6.	DO	mg/l	Azide modification	Laboratory
7.	BOD_5	mg/l	Azide modification	Laboratory
8.	Ammonia nitrogen	mg/l	Nessler reagent	Laboratory
9.	Nitrate	mg/l	NitraVer 5 Nitrate pillow	Laboratory
10.	Orthophosphate	mg/l	PhosVer 3 pillow	Laboratory
11.	Depth	cm	Measuring tape	On site
12.	Width	m	Measuring tape	On site
13.	Velocity	m/s	Velocity meter	On site

Copyright© by Chiang Mai University

3.4 Trichoptera sampling and identification

Larvae Trichoptera sampling procedure

Semiquantitative methods were used for collecting the larvae of Trichoptera, including kick sampling, hand picking, sweeping on riparian zone. D-frame net (mesh size 0.35 x 0.21 cm^2) was used to collecting the larvae. Larvae of Trichoptera were collected from many types of microhabitat such as, stream bed and river such as, cobble, gravel, sand, clay, leaf pack, organic debris, stream bank, riparian zone and water surface. Different of velocities areas were selected for collected as well.

After collecting a group of sample, pieces of debris was removed from the Trichoptera's sample (such as gravel sand rock, stick and leaves) and put those sample into the tray for sorting in a field. After sorting, each sample were preserved in 70% ethanol in plastic bag which labeled a data of sampling site name, sample number, sampling method, collector's name and date of sampling. All samples were taken to the lab, AIRU; Aquatic Insects Research Unit, Department of Biology, Faculty of Science, Chiang Mai University. Sorting of larvae were done in water with trays and after sorting the larvae were kept in vials glass filled with 80% ethanol, and labeled.

All larvae of Trichoptera taxa were identified to family level by used stereoscopic microscope and taxonomic keys were used following Dudgeon, (1999), McCafferty, (1981) and Wiggins, (1996). There were no a complete guide taxonomic keys of larvae of Trichoptera in Thailand, mostly of taxonomic keys were published from others region of the world (China and North America), so used of those key should be realized and prevent in confusing taxa, because our sample were collected in Oriental region.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Adults Trichoptera sampling procedure

The adults of Trichoptera were collected from all 7 sites overnight each month during year, from June 2012 to May 2013. Using light for catch is the most effective method for sampling adult Trichoptera, light pan trap (with 12v battery and black light fluorescent lamp) were used (Figure 3.11). The container was filled liquid that mixed by detergent for decreasing a water surface tension, prevent adult Trichoptera to escape

from the trap. The traps were set up close to the stream or river on the sites, operated from dusk to next dawn. In the next morning, all adult samples were taken to the lab for sorting and identification.

Only male Trichoptera specimens were identified and identified to species level with the pictorial key, Atlas of Southeast Asian Trichoptera (Malicky, 2010). The important Trichoptera's character for use to identify first step were 1) the spur formula 2) the presence or absence of ocelli and 3) the number of segments in the maxillary palp of the males (mostly female had 5 segments of maxillary palp), all three characters could guide for identified to genus level. Some specimens could be clearly to identified to species level by theirs characteristic in first step but some specimens were not possible, there should be make a preparation of genitalia to look for others details in genitalia structure.

Preparation and taxonomic work of adult Trichoptera specimens in laboratory

The preparation of Trichoptera specimens was similar to Lepidoptera's preparations. Posterior half of abdomen were cut with dissecting needles or tiny scissors, 10% solution of KOH or NaOH was used to soak the whole specimens overnight, 6 hours for tiny specimens. Clean the debris by horse brush for 3 times submerge into the 50%, 70% and 80% ethyl alcohol respectively. Identification of the specimens must process under the microscope, OLYMPUS SZH10 stereo with OLYMPUS DF PLANAPO IX were used in research. Genitalia specimens and abdomen part were in 80% ethyl alcohol into the vials. Labeling the data by printed form and put into the vials and label again on the tip of vials. Male genitalia morphological were showed in example, see Figure 3.10.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

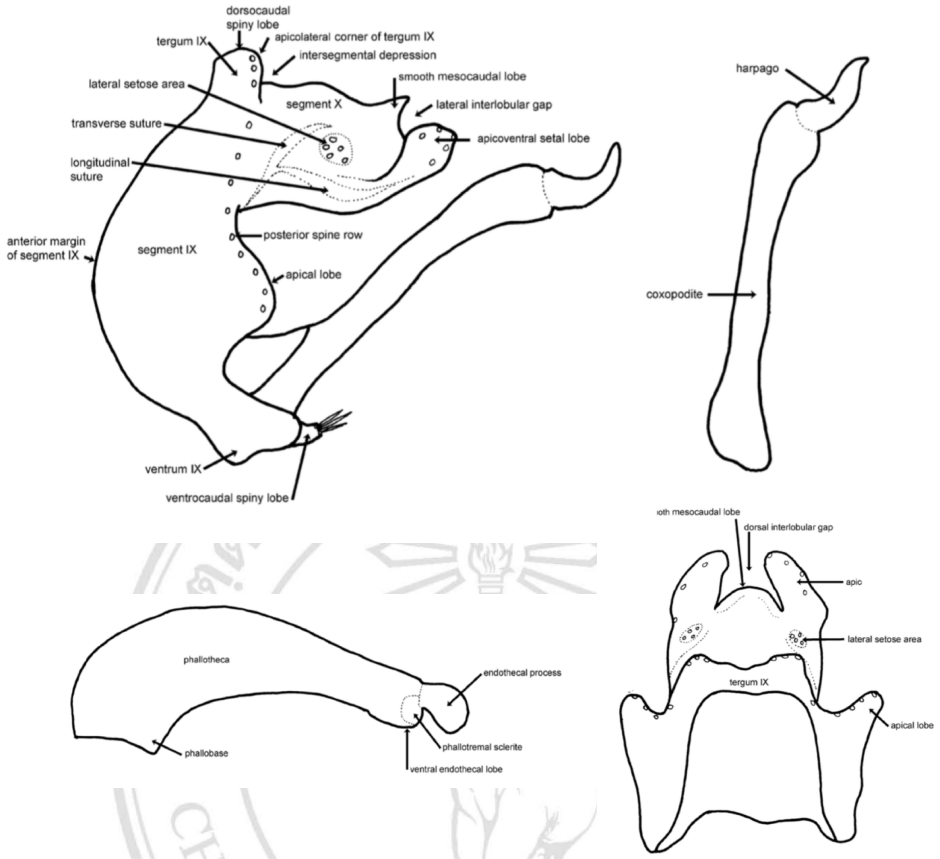

Figure 3.10 Genitalia of a hypothetical *Cheumatopsyche* species on genital structures (from Olah *et al,* 2008)

Figure 3.11 Light pan trap for adult Trichoptera on sampling site.

3.5 Data analysis

Water quality parameters were compared between upstream and downstream sampling sites and analyzed by using correlation. Cluster analysis was determined for grouping water parameters as well.

Larvae of Trichoptera diversity and richness index were compared by Shannon and Weiner index and analyzed by One-way ANOVA and Cluster analysis. Adult male Trichoptera diversity and richness among year were recorded and analyzed by Shannon and Weiner index.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved