

BIODIVERSITY OF SOME AQUATIC INSECTS FROM CHIANG
DAO WATERSHED, CHIANG MAI PROVINCE FOR
ENVIRONMENTAL BIOASSESSMENT

PONGSAK LUADDEE

DOCTOR OF PHILOSOPHY
IN BIOLOGY

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**A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
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ชื่อเรื่องวิทยานิพนธ์ ความหลากหลายทางชีวภาพของแมลงน้ำบางกลุ่มจากลุ่มน้ำเชิงดาว
จังหวัดเชียงใหม่เพื่อการประเมินทางชีวภาพของสิ่งแวดล้อม

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บทคัดย่อ

จากการศึกษาความหลากหลายทางชีวภาพของแมลงน้ำกลุ่มแมลงชีปะขาว แมลงเกาะหิน และแมลงหนอนปลอกน้ำ และการศึกษาผลกระทบของการใช้สารเคมีทางการเกษตรต่อการทำงานของเอนไซม์คลอโรตินเอสเตอเรส ผลกระทบต่อผิวหนังและวงจรชีวิตของแมลงหนอนปลอกน้ำชนิด *Stenopsyche siamensis* ในพื้นที่ลุ่มน้ำคอยเชิงดาว จังหวัดเชียงใหม่ เพื่อใช้เป็นดัชนีชี้วัดคุณภาพสิ่งแวดล้อม พบแมลงน้ำในกลุ่มแมลงชีปะขาวจำนวน 1,315 ตัว แมลงเกาะหิน 88 ตัวและแมลงหนอนปลอกน้ำเพศผู้ 4,460 ตัว ผลจากการจัดจำแนกแมลงน้ำพบแมลงน้ำในกลุ่มแมลงชีปะขาว 7 วงศ์ แมลงน้ำในกลุ่มแมลงเกาะหิน 2 วงศ์ 5 สกุลและ 10 ชนิด ส่วนแมลงหนอนปลอกน้ำพบ 17 วงศ์ 38 สกุลและ 127 ชนิด ในแมลงน้ำกลุ่มชีปะขาว พบแมลงในวงศ์ Heptageniidae, Baetidae และ Ephemeridae มีจำนวนมากที่สุดคือ 370 ตัว (28.1%), 350 ตัว (26.6%), และ 292 ตัว (22.2%) ตามลำดับ สำหรับแมลงน้ำกลุ่มแมลงเกาะหินพบ 2 วงศ์คือ วงศ์ Perlidae และ Peltoperlidae ส่วนแมลงหนอนปลอกน้ำวงศ์ Hydropsychidae, Philopotamidae และ Psychomyiidae เป็นวงศ์ที่มีจำนวนชนิดมากที่สุดคือ 39 ชนิด (30.7 %), 19 ชนิด (15%), และ 11 ชนิด (8.7%) ตามลำดับ

ผลจากการวิเคราะห์หลายตัวแปร (Multivariate analysis) โดยโปรแกรม PATN- (TWINSPAN และ Semi-Strong Hybrid Multidimensional Scaling (ssh mds)) สามารถจัดกลุ่มจุดศึกษาออกเป็น 4 กลุ่มคือ กลุ่มที่ 1 กลุ่มที่ได้รับผลกระทบจากการใช้พื้นที่ทางการเกษตรในระดับปานกลาง กลุ่มที่ 2 เป็นกลุ่มควบคุม กลุ่มที่ 3 และ 4 เป็นกลุ่มที่ได้รับผลกระทบจากการใช้พื้นที่ทางการเกษตรในระดับสูง การวิเคราะห์สหสัมพันธ์ระหว่างคุณภาพน้ำและชนิดของแมลงหนอนปลอก

น้ำพบ มีคุณภาพน้ำจำนวน 10 ปังจัยมีความแตกต่างระหว่างกลุ่มจุดศึกษา อันประกอบด้วย ค่าความเป็นกรดเป็นด่าง ค่าความนำไฟฟ้า ปริมาณของแข็งละลายน้ำ ในเตรท-ไนโตรเจน ซัลเฟต อุณหภูมิ น้ำ ความไวกระแสน้ำ ปริมาณออกซิเจนที่ละลายน้ำ ปริมาณออกซิเจนที่จุลินทรีย์ใช้ในการย่อยสลายสารอินทรีย์ และ ค่าเปอร์เซ็นต์การยับยั้งการทำงานของของเอนไซม์โคลีนเอสเตอเรส (Cholinesterase enzyme) จากตะกอน และ 28 ชนิดของแมลงหนอนปลอกน้ำมีความสัมพันธ์กับการวิเคราะห์ห่อดินชั้น

จากความสัมพันธ์ในแนวแกนระหว่างชนิดแมลงหนอนปลอกน้ำ ปังจัยทางสิ่งแวดล้อม และกลุ่มจุดศึกษาสามารถแบ่งแมลงหนอนปลอกน้ำได้ 2 กลุ่มคือ

1. Sensitive caddisfly species ประกอบด้วย *Psychomyia barata*, *Hydropsyche arcturus*, *H. cerva*, *Hydromanicus truncatus*, *Cheumatopsyche joliviti*, *C. cocles* และ *Ugandatrichia maliwan*
2. Tolerant caddisfly species ประกอบด้วย *Marilia sumatrana*, *Macrostemum floridum*, *Hydropsyche clitumnus*, *Cheumatopsyche globosa*, *C. cognita*, *C. charites*, *C. chrysothemis*, *Potamyia flavata*, *P. baenzigeri*, *Oecetis tripunctata*, *Maesaipsyche prichapanyai*, *Ecnomus volovicus*, *E. puro*, *Agapetus halong*, *Psychomyia mithila*, *Setodes argentiguttatus*, *Goera uniformis*, และ *Goera redsat*

ผลการศึกษาทางสัณฐานวิทยาของแมลงหนอนปลอกน้ำชนิด *Stenopsyche siamensis* จากจุดศึกษาในสองลำธารที่มีการใช้พื้นที่ต่างกันด้วยเทคนิคทางกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด พบว่าตัวอย่างของแมลงจากลำธารที่ไหลผ่านพื้นที่เกษตร ผิวน้ำบริเวณปล้องอกและท้องมีลักษณะผิดปกติ

ผลการศึกษาผลกระทบยาฆ่าแมลงในกลุ่มออร์กาโนฟอสเฟต (organophosphate insecticide) และ คาร์บาเมท (carbamate insecticide) ต่อระดับการทำงานของเอนไซม์โคลีนเอสเตอเรสในแมลงน้ำชนิด *Stenopsyche siamensis* พบว่าระดับการทำงานของเอนไซม์โคลีนเอสเตอเรส ในแมลงจากลำธารที่ไหลผ่านพื้นที่เกษตรต่ำกว่าระดับการทำงานของเอนไซม์โคลีนเอสเตอเรสในแมลงจากลำธารที่ไหลผ่านป่า โดยค่าที่ตรวจวัดได้คือ 2.39 ± 0.56 (ฤดูร้อน), 1.26 ± 0.19 (ฤดูฝน), 0.99 ± 0.21 (ฤดูหนาว) $\mu\text{mole/min/mg}$ ในจุดควบคุมและ 0.63 ± 0.36 (ฤดูร้อน), 0.57 ± 0.22 (ฤดูฝน), 0.85 ± 0.38 (ฤดูหนาว) $\mu\text{mole/min/mg}$ ในจุดศึกษาที่เป็นพื้นที่เกษตรกรรม การศึกษาวงจรชีวิตของแมลงน้ำชนิด *Stenopsyche siamensis* พบว่าตัวอ่อนของแมลงมี 5 ระยะ มีวงจรชีวิตแบบ univoltine ตัวอ่อนแมลงมีพฤติกรรมการกินเป็นแบบ filtering collectors โดยอาหารที่พบในส่วนของทางเดินอาหารคือ ซากสารอินทรีย์ขนาดเล็ก benthic algae จำพวก diatom ชนิด *Aulacoseira granulata* เป็นหลัก

Thesis Title Biodiversity of Some Aquatic Insects from Chiang Dao
Watershed, Chiang Mai Province for Environmental
Bioassessment

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Ph.D. Biology

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ABSTRACT

A study of biodiversity of some aquatic insects (Ephemeroptera, Plecoptera, and Trichoptera) and its application for use as indicator species to assess aquatic environmental quality and study of the impact of agricultural chemicals on life cycle, cholinesterase enzyme (ChE) activity, and tegumental surface of *Stenopsyche siamensis* (Trichoptera) were studied in Doi Chiang Dao watershed, Chiang Mai Province. The total of 1,315 mayflies, 88 stoneflies and 4,460 male caddisflies were trapped. Seven families of mayflies, two families, 5 genera, and 10 species of stoneflies and seventeen families, 38 genera, and 127 species of caddisflies were identified. The families Heptageniidae, Baetidae, and Ephemeridae yielded the most individuals: 370 (28.1%), 350 (26.6%), and 292 (22.2%) respectively in Ephemeroptera order. The families of Plecoptera that found in this study were Perlidae and

Peltoperlidae. In caddisflies, the families Hydropsychidae, Philopotamidae, and Psychomyiidae were the most abundant, yielding 39 species (30.7 %), 19 species (15%), and 11 species (8.7%), respectively.

The assemblages of male caddisfly adults and physico-chemical parameters were clustered using two-way indicator species analysis TWINSpan and ordinated using semi-strong hybrid multidimensional scaling (ssh mds) by multivariate techniques supported in the ecological pattern analysis package PATN. TWINSpan organized the study sites into 4 recognizable groups at the second level division. Group I represents moderately impacted sites. Group II represents forested control sites. Group III and IV represent impacted sites. ANOVA showed 10 environmental parameters which differed between the TWINSpan groups, including pH, conductivity, TDS, nitrate-nitrogen, sulfate, water temperature, velocity, dissolved oxygen, BOD₅, and % inhibition of ChE activity in sediment and there were 28 caddisfly species significantly correlated with the ordination. Consideration of the orientation of vectors of various caddisflies species, environmental parameters, and the study sites within the ordination suggests *Psychomyia barata*, *Hydropsyche arcturus*, *H. cerva*, *Hydromanicus truncatus*, *Cheumatopsyche joliviti*, *C. cocles* and *Ugandatrichia maliwan* were the most sensitive caddisflies to pesticides from agricultural and organic pollution. Conversely, *Marilia sumatrana*, *Macrostemum floridum*, *Hydropsyche clitumnus*, *Cheumatopsyche globosa*, *C. cognita*, *C. charites*, *C. chrysothemis*, *Potamyia flavata*, *P. baenzigeri*, *Oecetis tripunctata*, *Maesaipsyche prichapanyai*, *Ecnomus volovicus*, *E. puro*, *Agapetus halong*, *Psychomyia mithila*, *Setodes argentiguttatus*, *Goera uniformis*, and *Goera redsat* were relatively tolerant caddisfly species.

A study of the morphology and a comparison of *Stenopsyche siamensis* larvae from two streams (control and impacted sites) by using SEM showed the chronic effects of agricultural chemicals to the tegumental surface of this

insect. SEM micrographs of the larvae stage in agricultural stream site showed deformities of the tegumental surface of the thorax and abdomen.

A study of the effects of organophosphate and carbamate insecticides on Stenopsychid ChE activity showed significant differences between the impacted and the control stream sites. The results showed a ChE activity of 2.39 ± 0.56 (hot season), 1.26 ± 0.19 (rainy season) and 0.99 ± 0.21 (cool season) $\mu\text{mole/min/mg}$ at the control site, and 0.63 ± 0.36 (hot season), 0.57 ± 0.22 (rainy season) and 0.85 ± 0.38 (cool season) $\mu\text{mole/min/mg}$ at the impacted site. This study indicates that chronic effects of insecticides used in agriculture on aquatic fauna were evident.

A study of the life cycle of *Stenopsyche siamensis* showed that the insect have 5 larval instars and exhibited a non-seasonal univoltine life cycle. The gut content analysis showed that the stenopsychid larvae are filtering collectors that consume fine particulate organic matter and benthic algae predominately the diatom, *Aulacoseira granulata*, which is a common species in northern Thailand.

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CHAPTER I

INTRODUCTION

1.1 Introduction and the concept of using organisms for environmental assessment

The modification of catchment and headwater areas for plant cultivation is one of the main causes that has adversely affected on stream and river ecosystems in northern Thailand (Mustow 1999). Impacts of agricultural practices on stream ecosystems include soil erosion that causes high turbidity, pesticide runoff, nutrient enrichment, destruction of riparian animals and vegetation in and along stream margins, and water irrigation. Recent studies of the impact of land use on water quality and aquatic insect communities in Thailand reveals significant inputs of agricultural chemicals, heavy metals, and domestic waste (Mustow, 1999; Chaibu and Chantaramongkol, 1999).

In freshwater ecosystems, aquatic insects are dominant components of stream food webs. They are almost all primary consumers that feed on primary producers (algae, phytoplankton, macrophytes) and/or decomposers (bacteria or fungi) and transfer energy to higher trophic levels (fish and aquatic birds). In recent years, aquatic insects have often been used as indicators of ecological conditions and environmental quality (Watson *et al.*, 1981; Lemly, 1982; Resh, 1992; Usseglio-Polatera *et al.*, 2000; Ometo *et al.*, 2000). Aquatic insects have been used in attempts to detect, measure, and interpret environmental disturbance resulting from pollution by fertilizers, pesticides, nutrient enrichment, sewage, and chemical waste disposal (Lemly, 1982; Metcalfe, 1989; Hardersen and Wratten, 1998; van der Geest *et al.*, 1999; Cuppen *et al.*, 2000; Ometo *et al.*, 2000; Tessier *et al.*, 2000). These insects are particularly

useful because they are ubiquitous, diverse, and have long life cycles. Several species have been used as test animals in studies concerning the effects of chemicals on living organisms (McCahon *et al.*, 1989; Day and Scott, 1990; Hardersen and Wratten 2000; Meier *et al.*, 2000).

Nowadays, the concept of using organisms for environmental assessment is popular worldwide, especially macroinvertebrates and aquatic insects (Hellawell, 1986). One of them is the bio-indicator concept which uses the responses of living organisms to environmental changes to determine environmental conditions. There are many ways in which biological studies are used to assess environmental quality. The terms biological indicator or bio-indicator or bio-indicator species can be understood in two main ways, *viz.* using individuals and/or populations of biota as indicators and using the community structure of the organisms as an indicator.

The use of individuals and populations of aquatic insects as indicators includes:

i). pollution indicator species whose presence or absence indicates environmental water quality conditions are within the tolerance limits of those species (Lemly, 1982; Hellawell, 1986; Metcalfe, 1989; Ometo *et al.*, 2000).

ii). bio-marker analysis of tissues or/and body fluids for chemicals, enzymes, and other biochemical substances to document the interaction of chemicals with biological systems of biota for evaluating the impact of xenobiotic chemicals (Day and Scott, 1990; World Health Organization, 1993; Guedes *et al.*, 1997).

iii). Bio-magnification and bio-accumulation studies assess exposure of organisms to pollutants and the likely effects along the food chain (Spehar *et al.*, 1978; Memmert, 1987; Oliver, 1987; Norris and Norris, 1995).

iv). bio-sensors involve studies on the effects of pollutants on physiological changes, reproduction, and growth which are associated with

changes in morphology and behavior (Hellawell, 1986; Heimbach *et al.*, 1998; McCahon *et al.*, 1989; van der Geest *et al.*, 1999; Meier *et al.*, 2000; Tessier *et al.*, 2000; Tukker, 2000).

Another way is using the community structure of organisms as an indicator of the changes in community composition and biomass (Dance and Hynes, 1980; Lemly, 1982; Metcalfe, 1989; Greenwood *et al.*, 2001).

According to the above concepts of indicator organisms, the present study evaluated the impact of land-use patterns (forested control site, rice field, tea plantation, orchard, and urbanization) on aquatic environmental quality by using the diversity of some aquatic insects (Ephemeroptera, Plecoptera, and Trichoptera) in Chiang Dao District, Chiang Mai Province. Evidence of the chronic effects of pollutants on aquatic insects was investigated by studies on the life cycle, cholinesterase enzyme (ChE) activity, and integumental surface deformities of *Stenopsyche siamensis* (Trichoptera) from a polluted stream (impacted site) and a non-polluted stream (control site).

1.2 Aims of the present study

- To survey the biodiversity of some aquatic insect orders (Ephemeroptera, Plecoptera, and Trichoptera) in Chiang Dao District, Chiang Mai Province.
- To investigate the impact of various land-use patterns on nearby aquatic insect communities.
- To identify and recommend some species of aquatic insects as bioindicators for environmental assessment.
- To study the impact of pollution on the life cycle, cholinesterase enzyme activity, and integumental deformities of *Stenopsyche siamensis* (Trichoptera).

1.3 Education Application Advantages

- Improve the knowledge of the biodiversity of aquatic insects in Chiang Dao District, Chiang Mai Province, and contribute to a preliminary list of aquatic insects in Thailand.
- To demonstrate the impact of land use activities on aquatic insect communities and aquatic ecosystems.
- To demonstrate the utility of aquatic insect species as bioindicators for environmental assessment.
- To understand the effects of pollution on the life cycle, cholinesterase enzyme activity, and integumental deformities of *Stenopsyche siamensis* (Trichoptera).

CHAPTER II

LITERATURE REVIEWS

Streams are the products of their catchments and exhibit features resulting from their role as channels for the transport of excess water derived from precipitation which terrestrial environments release. Streams are places where rapid flow of shallow water produces a shearing stress on the streambed and generates a wide range of habitats for aquatic organisms (Wetzel, 1975). There are many aspects to the understanding of streams including the study of geological processes, physical processes, chemical processes and biological processes. Moreover, recent trends in stream research include an increasing emphasis on the study of the effects of land-use management on stream ecosystems and/or the aquatic environment through their effects on the aquatic fauna.

2.1 A history of biological monitoring using aquatic insects

The history of using aquatic insects to assess surface water quality began in 1908 in Germany by Kolk-witz and Marsson who developed the idea of saprobity in rivers as a measure of the extent of contamination by sewage (Rosenberg and Resh, 1996). The study of freshwater quality using the responses of aquatic insects to prevailing environmental conditions is becoming increasingly common (Lemly, 1982; Ometo *et al.*, 2000) and many indices have been developed. Many studies have produced lists of potential indicator organisms and recommended methods to evaluate stream quality. For example, chironomid midges (Diptera) were considered to be pollution tolerant, whereas caddisflies and stoneflies were considered to be sensitive to

pollution (Metcalf, 1989). Today, the notion of biological assessment has been extended to many other sciences such as toxicology, biochemistry, chemistry, physiology and molecular biology.

2.2 Using aquatic insects for assessing quality of the aquatic environment

Many groups of organisms have been used as indicators of water quality or environmental changes in fresh water bodies. These include bacteria, algae, macrophytes, protozoa, invertebrates and fish, but aquatic invertebrates have been used most extensively, especially in flowing water (Pinder and Morley, 1995). Aquatic insects have been used for this purpose in two main ways: trawling individuals and/or populations, or alternatively community structure as environmental indicators (Hellawell, 1986). The reasons that aquatic insects are useful for environmental assessment are (Rosenberg and Resh, 1993):

- They are ubiquitous and can be affected by environmental perturbations in many different types of aquatic systems and in habitats within those waters.
- The large number of species involved offers a spectrum of responses to environmental stresses.
- Their basically sedentary nature allows effective spatial analyses of pollutant or disturbance effects.
- They have long life cycles compared to other groups, which allows elucidation of temporal changes caused by perturbations.

2.3 Aquatic insects as bioindicators

The definition of indicator species or bioindicator is a species or species assemblage that indicates the environmental condition by changes in presence/absence, numbers, morphology, physiology, or behavior (Spellerberg, 1994; Johnson *et al.*, 1993). The term of bioindicator was recently discussed in detail by Lindenmayer *et al.* (2000)

In aquatic environmental quality assessment, insects have been used in many ways as bio-indicators including:

2.3.1 Pollution indicator species- The use of pollution indicator species for monitoring water quality has been described for many years such as the presence of chironomids e.g. *Camptochironomus tentans*, *Chironomus plumosus* and *Chironomus thummi* indicating the existence of organic pollution. In contrast, the presence of Plecoptera such as *Amphinemeura sulcicollis*, *Leuctra nigra*, and *Perla bipunctata*; Ephemeroptera such as *Baetis alpinus*, *Ecdynurus venosus*, and *Rhithrogena semicolorata*; and Trichoptera such as *Agapetus fuscipes*, *Apatania fimbriata*, *Hydropsyche siltali*, and *Rhyacophila philopotamoides* separated non-polluted waters from those with organic pollution in some European countris (Hellawell, 1986).

2.3.2 Biomarkers or biochemical indicators- Responses to environmental stress originate at the biochemical level in aquatic insects. The use of biochemical indicators in aquatic insects consists of energy metabolism, enzyme activities, as well as RNA, DNA, aminoacid, and protein content (Johnson *et al.*, 1993). Examples of studies concerned with the effects of pollution on biochemical levels in aquatic insects by Day and Scott (1990) showed the effects of organophosphate insecticides (azinphosmethyl,

chlorpyrifos, fenitrothion) at sublethal concentrations during field spraying activities on acetylcholinesterase activity in *Ephemerella* sp. nymphs (mayfly), *Hydropsyche slossonae*, *Hydropsyche betteni* larvae (caddisfly). In addition, Sturm and Hansen (1999) investigated the potential use of cholinesterase and biotransformation enzyme activities in toxicity tests with *Chironomus riparius*. The results suggest that enzyme activity may be a useful variable in toxicity tests with aquatic insects.

2.3.3 Bioaccumulation and biomagnification- There is much interest in the accumulation of polluting chemicals (heavy metals, DDT etc.) in the bodies of aquatic insects and their transfer along the food chain. Bioaccumulation of heavy metals and pesticides and their effects on freshwater aquatic insects are well documented. Spehar *et al.* (1978) studied the bioaccumulation of cadmium and lead in *Ephemerella* sp. (mayfly), *Pteronarcys dorsata* (stonefly), and *Hydropsyche betteni* (caddisfly). Their results showed increases of cadmium and lead concentrations in the insects up to 30,000 and 9,000 times higher respectively, than the metal concentrations in the water.

2.3.4 Biosensors- Xenobiotic stresses on physiological changes, reproduction, and growth rate are associated with changes in the morphology and behavior of aquatic insects (Hellowell, 1986). The effects of pollutants on structural deformities and behavioral changes have been demonstrated with several aquatic insect species and are used as a bioindicator of the chronic effect of pollutants in running waters. Tessier *et al.* (2000) showed that nets spun by *Hydropsyche slossonae* larvae were characterized by two distinct anomalies after chronic exposure to sublethal cadmium concentrations. Net anomalies were also observed for malathion (organophosphate insecticide) (Tessier *et al.*, 2000) and near a zinc mining operation (Balch *et al.*, 1999)(refer to Tessier *et al.*, 2000). Simpson (1980) studied abnormalities in the tracheal

gills of the aquatic insect *Phasganophora capitata* (Plecoptera) and net-spinning caddisfly larvae (Trichoptera;Hydropsychidae) collected from streams receiving chlorinated or crude oil wastes. De Bisthoven *et al.*, (1998) indicated that morphologically deformed *Chironomus thummi* resulted from heavy metals and domestic sewage.

2.3.5 Community composition as an indicator- This method considers population, community structure, and biomass of aquatic insects for the assessment water or aquatic environment quality. The study of community structures of insects for assessing environmental condition has been developed. Many indices have been used to summarize water quality such as the Trent Biotic Index, Chandler Biotic Score, Average Score Per Taxon (ASPT) Index and Biological Monitoring Working Party (BMWP) Score in Europe, Rapid Bioassessment Procedure in North America, and Stream Invertebrate Grade Number-Average Level (SIGNAL) index in Australia (Norris & Norris 1995).

2.4 General biology of aquatic insects

Aquatic insects are those which live in moist places or partially or completely in freshwater habitats, or have some part of their life cycle living in water. There are thirteen orders with at least some aquatic species, including Collembola, Ephemeroptera, Odonata, Hemiptera, Orthoptera, Plecoptera, Coleoptera, Diptera, Hymenoptera, Lepidoptera, Megaloptera, Neuroptera, and Trichoptera (McCafferty, 1989). In some fresh water biotopes, aquatic insects may comprise over 95% of the total individuals or species of macroinvertebrates (Mackay and Kersey, 1985; Ward, 1992).

2.4.1 Ephemeroptera or mayflies (Figure 1) are a small order of aquatic insects with about 23 families, 200 genera and 2,500 species recorded around the world (Zborowski and Storey, 1995; Williams and Feltmate, 1992). In Thailand, Sangpradub *et al.* (1999) reported 45 species of mayflies found in northeast Thailand.

Mayflies are exopterygotes, medium-sized (average wingspan is about 15 mm), most with two pair of membranous wings with the hind wings very much smaller than the forewings and absent in a few. The head has mandibulate mouthparts in the aquatic nymphs, very large eyes, three ocelli, and very short filiform antennae. The thorax consists of three segments, each with one or two pair of appendages. The abdomen usually has 10 segments, with gills usually located entirely on the abdomen in larvae stage. Most species have three caudal filaments, composed of terminal filament and two cerci (Edmunds, 1996; Zborowski and Storey, 1995).

Mayflies occur in an extremely wide variety of standing and running fresh water habitat, ranging from lakes, streams, rivers, headwaters, ponds, and swamps. The major part of the life cycle of mayflies is spent in an aquatic environment, while their short terrestrial life is primarily concerned with reproduction (Brittain, 1990). The adults of most mayflies live near water, but some species may fly several kilometers from their place of emergence to headwaters for mating and spawning. Mating occurs in flight and eggs are mainly laid on the water surface and then sink. Several hundred to several thousand eggs are laid per female which hatch two to three weeks later. The nymphs live on the bottom where they undergo 20-50 molts. Emergence to sub-adult (sub-imago) and adult (imago) is synchronized in many species to allow the short-lived adults maximum time to mate and lay eggs (Zborowski and Storey, 1995). Ephemeroptera display a wide range of life cycle types. Univoltine or an annual life cycle is most common in this order, but multivoltine life cycles are found in warm temperate and tropical areas (Brittain, 1990).



Figure 1 Adult Ephemeroptera

Family Leptophlebiidae (male), Doi Chiang Dao, Thailand.

2.4.2 Plecoptera or stoneflies (Figure 2) are a small order of aquatic insects, with about 15 families and 2,000 species recorded around the world (Zborowski and Storey, 1995; Williams and Feltmate, 1992). Twenty-four Oriental Plecoptera species are reported, while 10 new species were recently found in Thailand (Stark, 1983; 1987; 1989; Stark and Sivec, 1991).

Larval and adult stoneflies fit the general morphological pattern of a primitive insect, small to large (average wingspan is about 10-110 mm), body elongate, flattened and soft (Zborowski and Storey, 1995). The head has a pair of well-developed compound eyes, ocelli, and long thread like antennae consisting of many segments covered with short setae. The mouthparts are of the primitive mandibular type. The forewings and hindwings are membranous, held flat or almost curved around the body at rest and with the hind wings broader than the slightly longer forewings (Stewart and Harper, 1996). The abdomen of both the adult and nymphal stonefly is typically soft, cylindrical or slightly flattened dorso-ventrally, and consists of 10 segments. The 10th segment bears a pair of long cerci (Williams and Feltmate, 1992).

Stoneflies are always found in clean cool stream. In most species, embryonic development proceeds directly and is complete within 3-4 weeks, hatchlings then undergo gradual development requiring 12-24 months depending on the species, sex, and environment conditions. The nymphs live on sand, gravel, or cobble substrates where they feed on fine particles of organic matter, debris, precipitated organic matter from the water column, algae aquatic fungi and bacteria. Stonefly adults are short-lived, most living for several days and even weeks. Adults mate on or near the ground, under stones or in vegetation, and female lay 100-2,000 eggs (Williams and Feltmate, 1992).



A



B

Figure 2 Adult Plecoptera,
Family Perlidae, *Etrocorema* spp., Doi Chiang Dao, Thailand.

2.4.3 Trichoptera or caddisflies (Figure 3) is one of the largest groups of aquatic insects, with about 43 families and 7000 species recorded around the world (Zborowski and Storey, 1995). More than 494 caddisfly species are reported from Thailand in 18 families (Malicky and Chantaramongkol (1999).

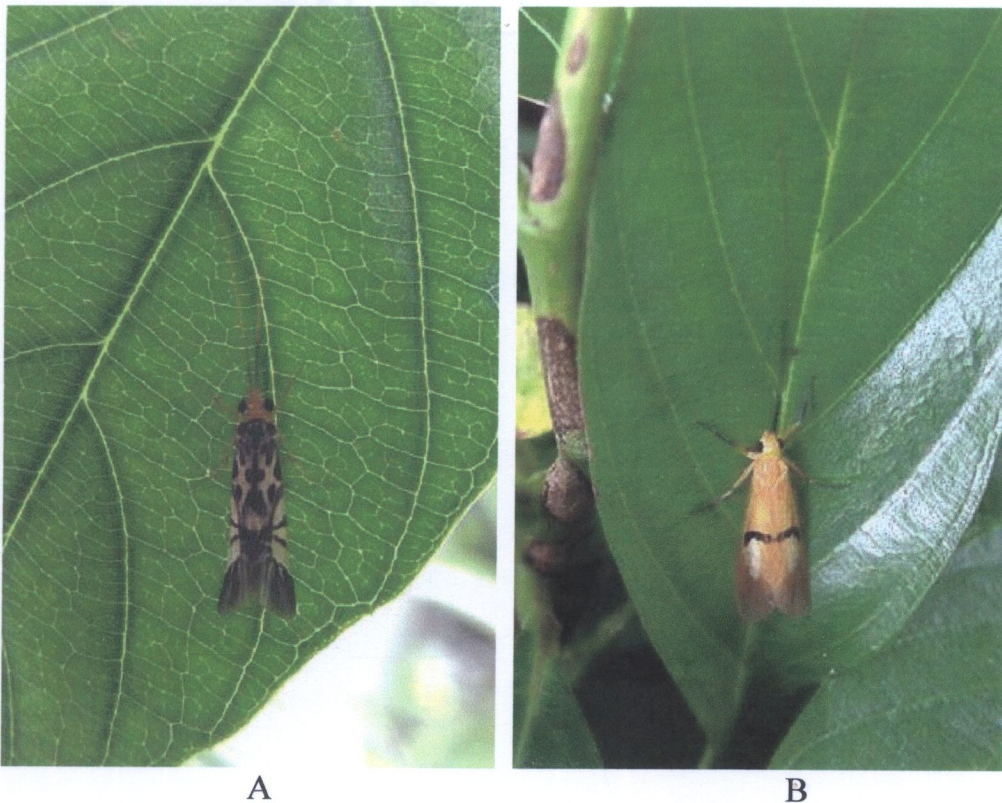
Caddisflies are holometabolous neopteran insects, small to moderate-sized (average 1.5-40 mm in body length) (Zborowski and Storey, 1995). In adults, the compound eyes are well developed and there may be up to three ocelli. The mouthparts are weak and capable only of ingesting liquids. All three thoracic segments are distinct. The wings are covered with setae. When at rest, the wings are held in an erect roof-like fashion over the body. The abdomen consists of 10 segments. Caddisfly larvae are quite similar to Lepidoptera caterpillars, but have only a single pair of prolegs on the terminal segment. A pair of eyes on the head capsule and with two very small antennae. The larval thorax is well developed. In some families, there is a membranous horn on the prosternum. There are nine abdominal segments that in some families are filamentous. At the end of abdominal segment, has anal prolegs and are each equipped with an apical anal claw (Williams and Feltmate, 1992).

Trichoptera occur on all continents except Antarctica, and are found in fresh and brackish waters and the sea. Most occur in inland waters where they have adapted to condition in a variety of permanent and temporary running and standing water biotopes (Ward, 1992). Most caddisflies mate at dusk or in the early evening. The eggs are small, more or less spherical, and are laid in strings or in a mass. Many species crawl beneath the water to attach their eggs directly on to substrate or deposit their eggs on objects above the water or on the water surface (Williams and Feltmate, 1992). Most caddisflies have five larval instars and a wide diversity of larval forms.

Wiggins (1996) divided the case construction of caddisfly larva into 5 groups (Figure 4):

- Free-living forms (Rhyacophilidae)
- Saddle-case makers (Glossosomatidae)
- Purse-case makers (Hydroptilidae)
- Net-spinners or retreat-makers (Hydropsychidae)
- Tube-case makers (Lepidostomatidae, Calamostomatida)

The larvae of caddisflies consume a wide range of foods such as fine organic particles, dead vascular plants, riparian leaves, bacteria, algae, diatoms, and aquatic insect prey.



A

B

Figure 3 Adult Trichoptera

A). *Macrostemum floridum* (male) B). *Macrostemum midas* (female),

Doi Chiang Dao, Thailand.

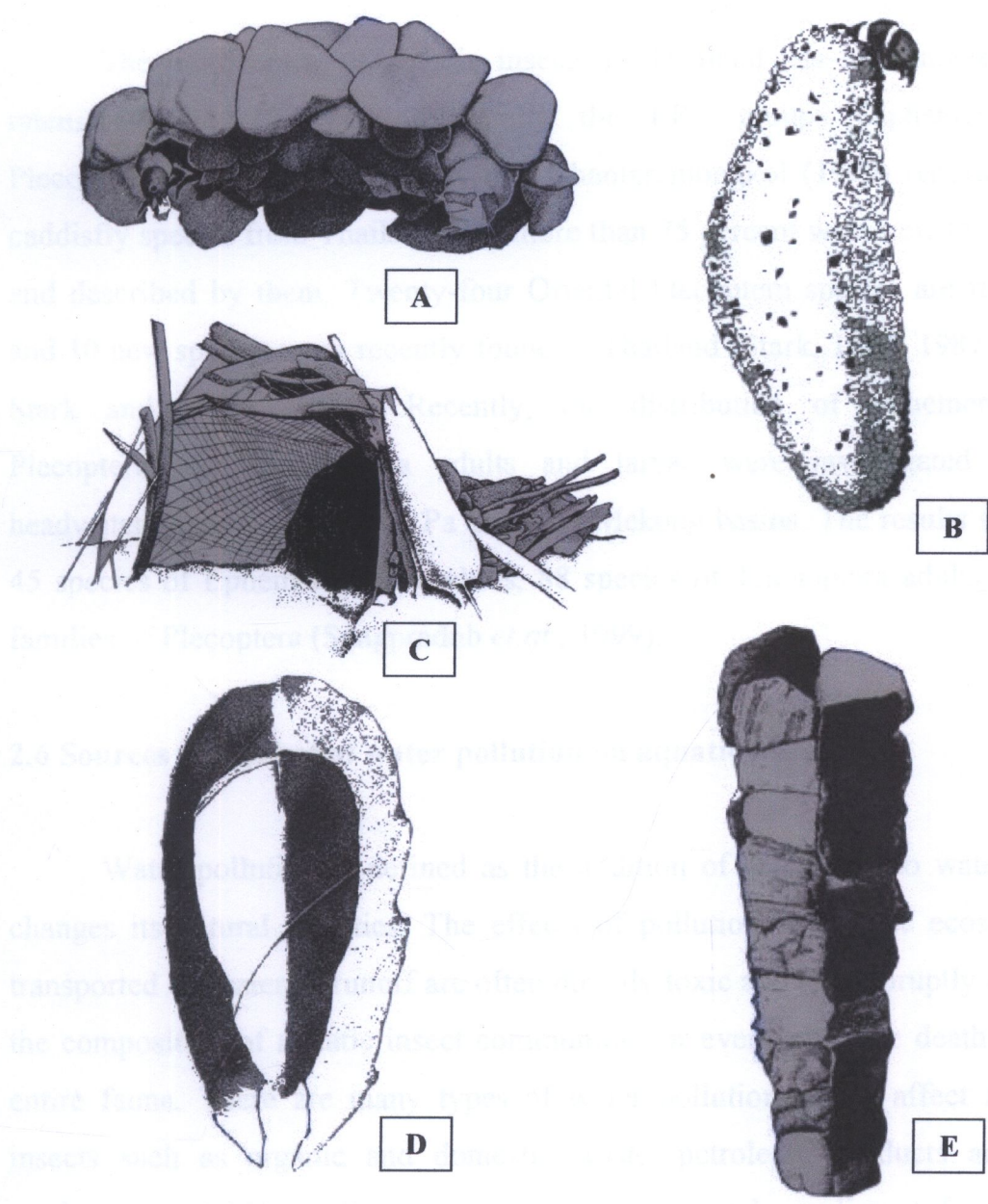


Figure 4. Case types of caddisflies.

- A). Glossosomatidae
- B). Hydroptilidae
- C). Hydropsycidae
- D). Calamoceratidae
- E). Lepidostomatidae (from Wiggan, 1996)

2.5 Biodiversity of aquatic insects in Thailand

The biodiversity of aquatic insects in Thailand has been investigated intensively for 10 years, especially the EPT groups (Ephemeroptera, Plecoptera, Trichoptera). Malicky and Chantaramongkol (1999) reported 494 caddisfly species from Thailand, and more than 75 percent were new to science and described by them. Twenty-four Oriental Plecoptera species are reported and 10 new species were recently found in Thailand (Stark, 1983; 1987; 1989; Stark and Sivec, 1991). Recently, the distribution of Ephemeroptera, Plecoptera, and Trichoptera adults and larvae, were investigated in 21 headwater streams of the Chi, Pa Sak, and Mekong basins. The results showed 45 species of Ephemeropteran adults, 88 species of Trichoptera adults, and 4 families of Plecoptera (Sangpradub *et al.*, 1999).

2.6 Sources and effect of water pollution on aquatic insects

Water pollution is defined as the addition of something to water with changes its natural qualities. The effects of pollution in aquatic ecosystems transported by water or runoff are often directly toxic and may abruptly change the composition of aquatic insect communities or even cause the death of the entire fauna. There are many types of water pollution which affect aquatic insects such as organic and domestic waste, petroleum products and by-products, pesticides, radioactive wastes, heavy metals, sediment from road construction sites, dams and regulated rivers.

2.6.1 Organic wastes- The major sources of organic pollution are sewage, agricultural runoff from inadequately stored animal wastes and silage, food processing and manufacture, and industries processing natural materials such as textile and paper manufacture (Abel, 1989). Most organic wastewater contains a high proportion of suspended matter. The most important

consequences of organic pollution affect the dissolved oxygen concentration in the water and sediment. Pearson and Penridge (1987) (refer to Heliövaara and Väisänen, 1993) showed that the discharge of organic effluent from a sugar mill into a stream had effects on the macroinvertebrates fauna by increasing pollution and led to decreased diversity. In addition, Lemly (1982) monitored the responses of the benthic insect community of a southern Appalachian trout stream to inorganic sedimentation and nutrient enrichment. The zone receiving nutrient runoff from livestock pastures exhibited elevated levels of nitrate and phosphate. Species richness, diversity, and total biomass of filter feeding Trichoptera and Diptera, predaceous Plecoptera and certain Ephemeroptera were significantly reduced in the polluted zones.

2.6.2 Domestic waste- Jones and Clark (1987) compared the benthic insects of several watersheds from un-urbanized to highly urbanized areas in Virginia (USA). Urbanization in the watershed caused marked changes in the composition of stream insect communities. Tolerant taxa, such as some chironomids, increased in abundance, while non-tolerant taxa decreased or were eliminated. Highly urbanized streams were strongly dominated by Diptera, with Trichoptera being co-dominant in some cases. Less urbanized streams were characterized by the presence of a large number of genera belonging to Diptera, Trichoptera, Ephemeroptera, Coleoptera, Plecoptera, and Megaloptera. The genera *Cricotopus* and *Orthocladius* were most common in urbanized streams, while several genera, such as *Constempellina*, *Hydropsyche*, *Stenelmis*, and *Stenonema*, were virtually absent from all moderately to highly urbanized streams.

2.6.3 Petroleum products and by-products- The widespread use of petroleum and its products has resulted in the discharge of oil to the aquatic environment. The toxic effects of these contaminants to aquatic insects fall in

two general categories. The first category includes the effects associated with coating or smothering of organisms with oil. The second toxic effect involves disruption of an organism's metabolism due to the ingestion of oil and the incorporation of hydrocarbons into lipids or other tissues in sufficient concentrations to upset the normal functioning of the organism (Laws, 1993). The damage of stream fauna from oiling has often been reported. Lytle and Peckarsky (2001) concluded that diesel fuel spills significantly reduced the density of invertebrates by 90% and taxonomic richness by 50% at least 5.0 km downstream. Moreover, crude oil wastes are the suspected cause of abnormalities in the tracheal gills of some aquatic insects e.g. *Phasganophora capitata* (Perlidae: Plecoptera) and *Cheumatopsyche* sp. (Hydropsychidae: Trichoptera) collected from streams receiving crude oil wastes (Simpson, 1980). In addition, water soluble fractions of crude oil impair feeding, damage the reproductive, defense mechanisms, and limit oxygen transport through the gill epithelium in aquatic invertebrates (Parker *et al*, 1976) (refer to Simpson, 1980).

2.6.4 Pesticides- There are many effects on aquatic ecosystems caused by pesticides that originate from terrestrial spraying operations directed against pests, control of harmful aquatic insects, drainage, runoff, and leaching from agricultural or forested land. Maund *et al.* (1992) studied the acute and chronic toxicities of lindane to *Chironomus riparius*, *Chaoborus flavicans*, and *Sigara striata* in mesocosm compartments of an experimental pond. They reported that the median lethal concentrations (LC₅₀s) of lindane were 240-h LC₅₀ of 20 µg/l for the second instar, 72-h LC₅₀ of 6.5µg/l for fourth instar *C. riparius*, and 96-h LC₅₀ of 4.0 and 3.9µg/l for the fourth instar of *C. flavicans*, and fourth and fifth instars for *S. striata*, respectively. Lindane significantly reduced the growth during 10 days of the second instar of *C. riparius* at concentrations where larvae survived (1.0 to 7.0 µg/l).

Hatakeyama *et al.* (1990) used a drift net to investigate the effects of aerial spraying of fenitrothion in a Japanese mountain stream. The insecticide concentration in the river water increased to 20 µg/l 3-h after the spraying and decreased rapidly to half the peak value after 2 hours. The drift of a large number of aquatic insect species started immediately after the spraying and was almost coincident with the peak of the insecticide concentration in the water. The number of species (e.g. *Baetis*, heptageniid, and chironomid species) which drifted during the 24 hours period following the spraying increased from 17 on the previous day to 43.

The effects of pesticides at sublethal levels has been studied in aquatic insects by Anderson (1982) who showed *Brachycentrus americanus* (Trichoptera) larvae left their cases with at 0.064 µg/l of Permethrin. Hardersen and Wratten (2000) investigated the impact of azinphos-methyl and carbaryl insecticides on the hatching rate of *Xanthocnemis zealandica* (Zygoptera: Odonata). At 600-ppb of carbaryl concentration, there was a reduced hatching rate.

2.6.5 Heavy metals- Heavy metals is an imprecise term that is generally taken to include the metallic elements with an atomic weight greater than 40, but excluding the alkaline earth metals, alkali metals, lanthanides and actinides. The most important heavy metals from the point of view of water pollution are zinc, copper, lead, cadmium, mercury, nickel and chromium (Abel, 1989). Oladimeji and Offem (1989) (refer to Heliövaara and Väisänen, 1993) investigated the accumulation, depuration, and toxicity of lead to *Chironomus tentans* larvae. The mortality data indicated that *Chironomus* larvae were the most tolerant to lead poisoning. The maximum “safe” concentration of lead for Chironomid larva is 0.27 mg/l, and concentrations higher than 0.33mg/l will damage both the fish population and the organisms that make up their food. McCahon *et al.* (1989) studied the acute toxicity of cadmium to the first, third,

and fourth larval instars of the caddisfly *Agapetus fuscipes*. First instar larvae were more sensitive than older instars, and for all age classes, cased animals were more resistant than uncased ones. Wentsel *et al.* (1978) (refer to Heliövaara and Väisänen, 1993) reared midge larvae of *Chironomus tentans* for 17 days in metal-contaminated sediment collected from Palesline Lake, Indiana (USA). They reported a significant decrease in midge growth compared to that in the control sediment.

2.6.6 Radioactive waste- Radioactive wastes are considered highly toxic substance which include irradiated reactor fuels and liquid or solid wastes resulting from the reprocessing of reactor fuel to recover fissionable isotopes (Laws, 1993). The four possible sources of radioactive water pollution are from radioisotopes used in hospitals, universities and industry; mining and uranium production; nuclear reactor operation; and fuel reprocessing plants (Davidson *et al.*, 1970). The levels of radioactive wastes on aquatic systems known to be lethal to aquatic organisms lie in the 2-550 Gy range for invertebrates and adult fish but there are not many reports noting the effects of chronic low-level radiation exposure on aquatic organisms. However, Warwick (1987) (refer to Heliövaara and Väisänen, 1993) reported that high frequencies of structural deformities in chironomid larvae may be associated with high levels of chemicals and radioactive wastes. Chironomid larvae and adults collected from several freshwater habitats around the Chernobyl disaster site in Russia from 1990 to 1994, 4-8 years after a nuclear accident, showed deformities in larvae of nine of the 13 chironomid species. Deformities were most obviously found in headcapsule structures, particularly the antennae, mentum, mandibles, and epipharyngeal pectens (Williams *et al.*, 2001).

2.6.7 Dams and regulated rivers- Some effects of dams on river ecosystems and their biota are obvious (Walker, 1985; Kingsford, 2000). The

impacts of dams on aquatic ecosystems due to biota migration blockage, altered thermal flows, nutrients, sediment loading, and flow regimes have been extensively documented (Walker, 1985). Bishop (1979) (refer to Walker, 1985) described the macroinvertebrate fauna of the Tallowa Dam (Australia) which changed from benthic to nektonic species after being dammed. More than 70 aquatic insect species (caddisflies, mayflies, and stoneflies) were eliminated or became rare. Kigsford (2000) reviewed the ecological impacts of dams, water diversions, and river management on floodplain wetlands in Australia. The research showed that Australian floodplain wetlands with high biodiversity that depend on natural flows river flow have been reduced flooding to these wetlands, altering their ecology, and causing the death or poor health of aquatic biota by dams, diversions and river management.

CHAPTER III

MATERIALS AND METHODS

3.1 Description of the study area

3.1.1 Location

The streams sampled during this study are located in the Doi Chiang Dao watershed of the Pee Pan Nam Mountain Ranges, Chiang Dao District, Chiang Mai Province. The watershed covers the areas between 19° 15' to 19° 22'N latitude and 98° 50' to 98° 59'E longitude. The area has an elevation range between 350 to 1,300 meters above average mean sea level. The 20 study sites are located in 5 tributaries *viz.* Huai Mae Thalai, Huai Tad, Huai Kaeng Pan Tao, Huai Khun Mae Mae, Huai Mae Sai, and Huai Mae Mae Ngae Sai of the Ping River, which is the main river in the Chiang Mai basin (Figures 6-7).

3.1.2 Climate

Data from Doi Chiang Dao Watershed Research Station shows that the upper Ping River (study area) has a tropical monsoon climate with three seasons (hot, rainy, and cool). The rainy season lasts from May through October when the area is under the influence of the local climate, and depressions from the South China Sea and the Indian Ocean. Precipitation peaks in August and September. The temperature in the rainy season averages between 21.0-35.6 °C. The cool season is from November to February with an average temperature between 12.5-33.5 °C. This season has cool dry weather due to the influence of the northeast monsoon from the southern part of China and Siberia. The hot-dry season is from March to April with has average

temperatures between 15.6-38.4 °C. The average precipitation in this season is 12.8 mm per Inch². The annual mean temperature and precipitation volume in this area were 26.3 °C and 103.8 mm per Inch², respectively. (Table 1).

3.1.3 Geology and soil

Doi Chiang Dao watershed is elongated in a north-south direction. Many types of rocks, quartzite, granodiorite, Kaeng Krachan formation, Ratburi formation, sandstone, shale, and limestone are in the watershed. The major groups of soil are reddish brown lateritic soils and red yellow podzolic soils.

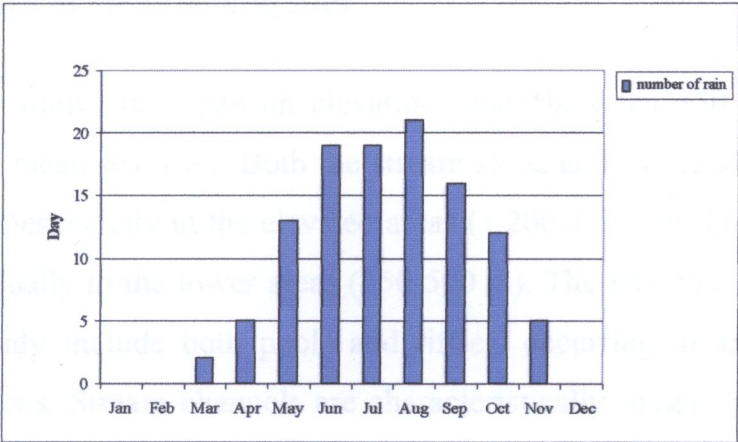
3.1.4 Land use and the terrestrial environmental

The ecosystems of Doi Chiang Dao Watershed can be divided into 8 types, including predominantly evergreen forest, predominantly deciduous/bamboo forests, predominantly dry dipterocarp forest, predominantly pine/Fagaceae forests, mixed forests, incomplete classification area, plantation area, and open area. Smitinand (1966) first reported 109 families and 570 species of vegetation at Doi Chiang Dao Mountain, and divided the forest types into 5 categories *viz.* Mixed deciduous forest below 500 m elevation, Dry evergreen forest at 500-600 m, Teak (*Tectona grandis* L.F.) forest at 600-700 m, Hill evergreen forest at 700-1900 m, and Open hill evergreen forest from 1900m-summit (Maxwell, 1992). Maxwell (1992) reported on the lowland vegetation between 450-800 m of the Doi Chiang Dao Wildlife Sanctuary. He noted 4 forest types including mixed evergreen-deciduous forest, deciduous dipterocarp-oak (savanna) forest, intermediate areas, and secondary growth.

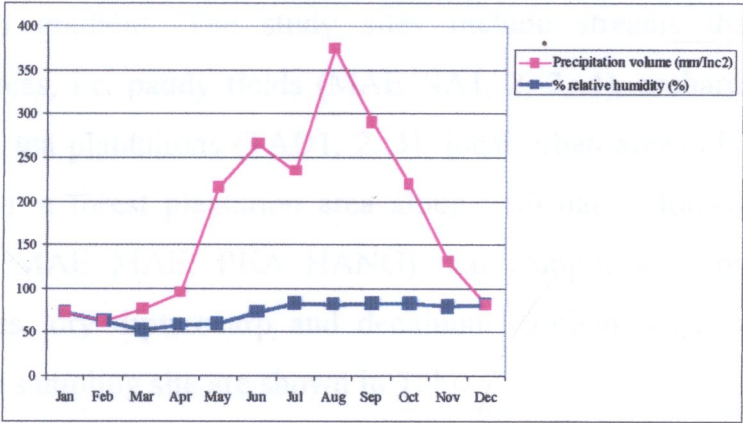
The study area is mainly used for agriculture. Large parts are planted to paddy fields (rice is the main crop of Thailand), tea plantations (main crop of high lands), orchards (*Litchi chinensis* Sonn., *Dimocarpus longan* Lour. and *Citrus maxima* Merr.). The minor of land use is village.

Table. 1 Average monthly rainfall, temperature, and % relative humidity at Doi Chiang Dao Watershed Research Station, Chiang Dao District, Chiang Mai Province, 1987-1997 (from Doi Chiang Dao Watershed Research Station, 1998).

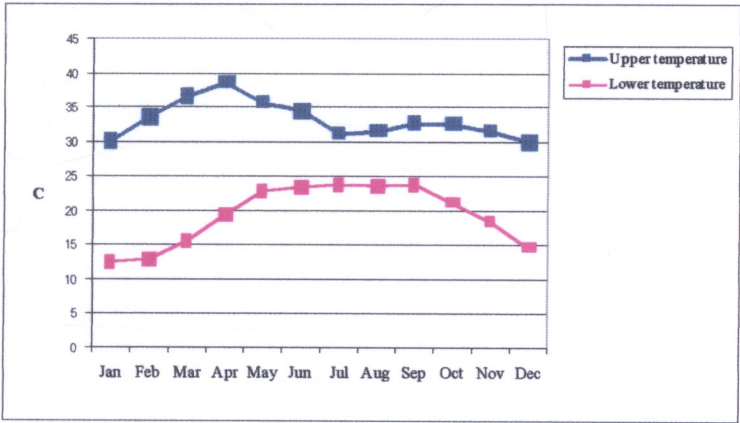
Month	Air Temperature C°		% relative humidity	Precipitation volume	number of rainy
	Upper temp.	Lower temp.			
			(%)	(mm/Inc ²)	(days)
Jan	30.1	12.5	72.9	0	0
Feb	33.5	12.9	62.7	0	0
Mar	36.6	15.6	53.4	22.5	2
Apr	38.4	19.5	57.6	37.3	5
May	35.6	22.9	60.9	154.1	13
Jun	34.5	23.4	72.9	192.4	19
Jul	31.0	23.7	81.9	152.2	19
Aug	31.4	23.5	81.8	291.8	21
Sep	32.6	23.8	82.5	208.4	16
Oct	32.5	21.0	82.7	136.0	12
Nov	31.4	18.4	79.6	51.2	5
Dec	29.9	14.5	81.2	0	0
Total				1245.9	112
Mean	33.1	19.3	72.5	103.83	9.33



(A)



(B)



(C)

Figure 5. Average monthly rainfall (A), temperature (B), and % relative humidity (C) at Doi Chiang Dao Watershed Research Station, Chiang Dao District, Chiang Mai Province, 1987-1997 (from Doi Chiang Dao Watershed Research Station, 1998).

3.2 Descriptions of the sampling sites

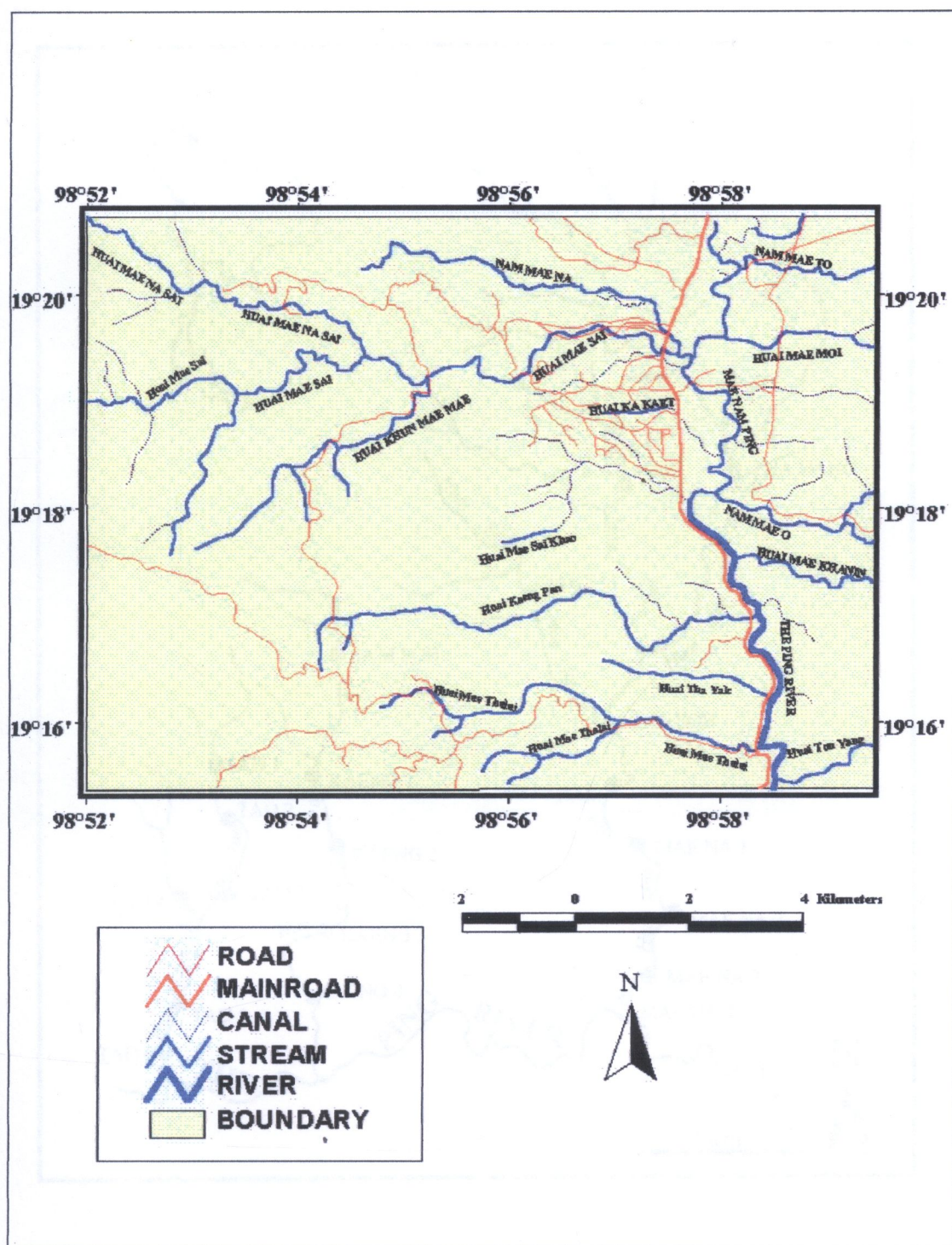
The 20 study sites span an elevation range between 350 to 1,300 m. above average mean sea level. Both the stream slope and overland slope are at a steep incline, especially in the elevated areas (1,200-1,300 m) but this incline tapers off gradually in the lower areas (350-500 m). The five streams observed during this study include both pools and riffles, occurring in approximately equal proportions. Stream channels are characteristically rough, dominated by substrates consisting of boulders, cobbles, gravel and sand in proportions that correspond to gradient. The study sites include streams that crisscross agricultural areas, i.e. paddy fields (MAE NA1, 2, 3, 4), orchards (TAD5, 6, KAENG3, 4), tea plantations (TAD1, 2, 3), local urban areas (TAD6, TAD1, MAE NA3, 4), a forest plantation area along with native forest (MAE SAI, SONG SOB, MAE MAE, PHA HANG) that comprises of predominantly evergreen trees, dry dipterocarp and deciduous/bamboo (figures 8-27). The details of each sampling site are shown in Table 2.

Table 2. Description of sampling sites in Doi Chiang Dao Watershed, Chiang Dao District, Chiang Mai Province.

Site	Elevation (m AMSL)	Coordination	Substrates Characters	% Canopy cover
TAD 1	840	19°16'N 98°55'E	mainly sand with some boulders and cobbles	50-60
TAD 2	820	19°16'N 98°55'E	mainly boulders with some sand	60-70
TAD 3	700	19°16'N 98°57'E	mainly cobbles with some boulders and sand	15
TAD 4	500	19°15'N 98°56'E	mainly sand with some cobbles and boulder	70
TAD 5	460	19°16'N 98°57'E	mainly boulders with some cobbles and sand	30
TAD 6	440	19°16'N 98°58'E	mainly boulders with some cobbles and sand	10
SAM SOB	830	19°17'N 98°55'E	mainly sand with some boulders and cobbles	20
KAENG 1	560	19°17'N 98°55'E	mainly boulders with some sand	40
KAENK 2	500	19°17'N 98°56'E	mainly bed rock and sand	40
KAENG 3	400	19°17'N 98°57'E	mainly sand and riparian vegetation	10
KAENG 4	360	19°17'N 98°57'E	mainly boulders with some cobbles and sand	15
MAE MAE	740	19°19'N 98°53'E	mainly boulders with some cobbles	20

Table 2 continued.

Site	Elevation (m AMSL)	Coordination	Substrates Characters	% Canopy
PHA HANG	840	19°20'N 98°53'E	mainly boulders and sand	60
MAE SAI	690	19°19'N 98°54'E	mainly boulders with some cobbles and sand	30
SONG SOB	540	19°19'N 98°55'E	mainly cobbles and boulders with some sand	30
DAM	460	19°19'N 98°56'E	mainly boulders and cobbles	1-2
MAE NA 1	380	19°20'N 98°56'E	mainly boulders and cobbles with sand	5
MAE NA 2	360	19°20'N 98°57'E	mainly sand with some cobbles and clay	2-5
MAE NA 3	360	19°20'N 98°57'E	mainly boulders with some cobbles	10
MAE NA 4	350	19°20'N 98°57'E	mainly cobbles with sand	2



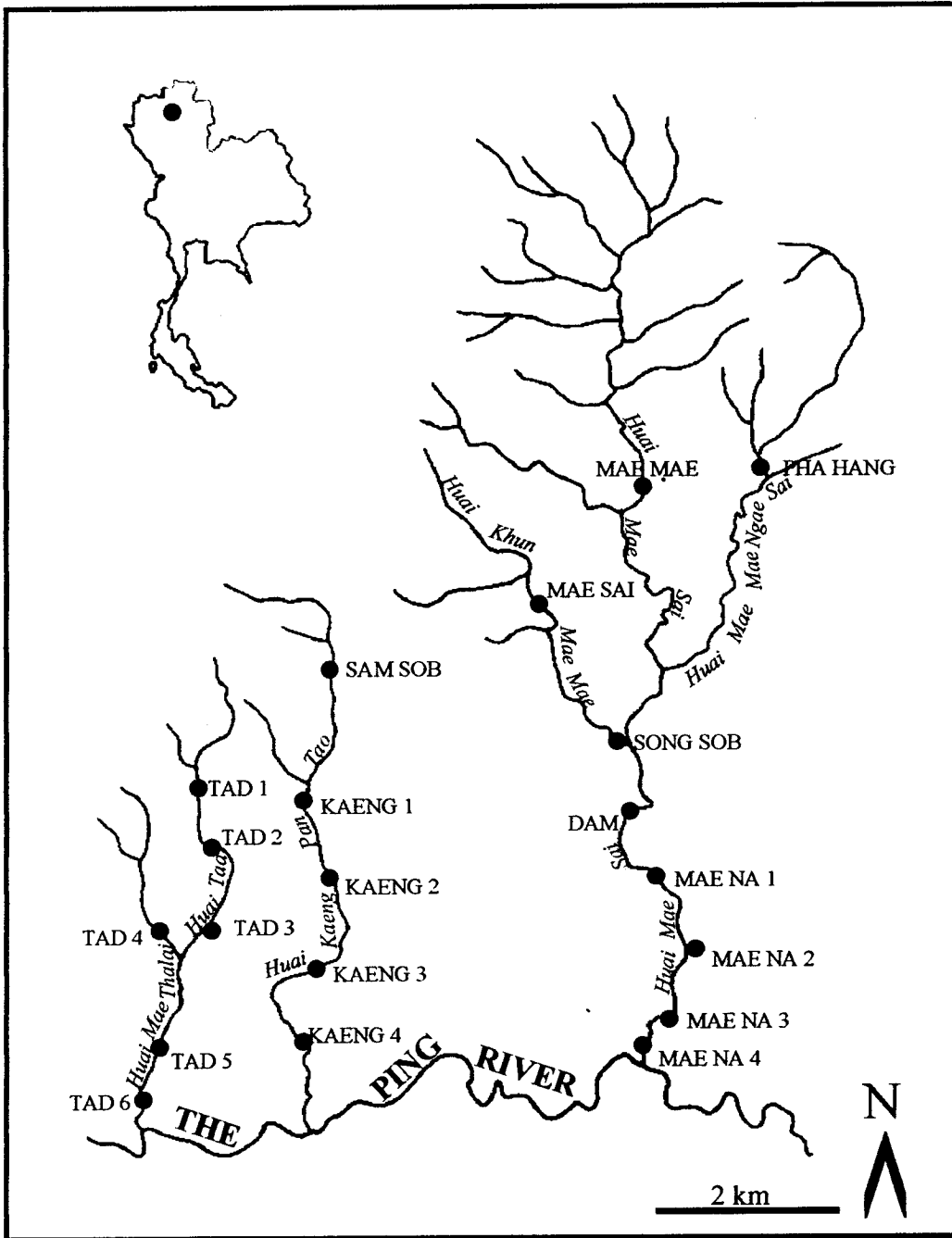


Figure 7. Map of study sites showing the locations of sampling sites along stream tributaries of the Ping River.



Figure 8 Study site, TAD 1, 30 January 2000.

Activities in this site: Mainly tea plantation, hill evergreen forest and village.



Figure 9 Study site, TAD 2, 30 January 2000.

Activities in this site: Mainly tea plantation and hill evergreen forest.



Figure 10 Study site, TAD 3, 30 January 2000.

Activities in this site: Mainly forest, orchard (*Citrus maxima* Merr.)



Figure 11 Study site, TAD 4, 30 January 2000.

Activities in this site: Mainly forest (mixed deciduous forest)

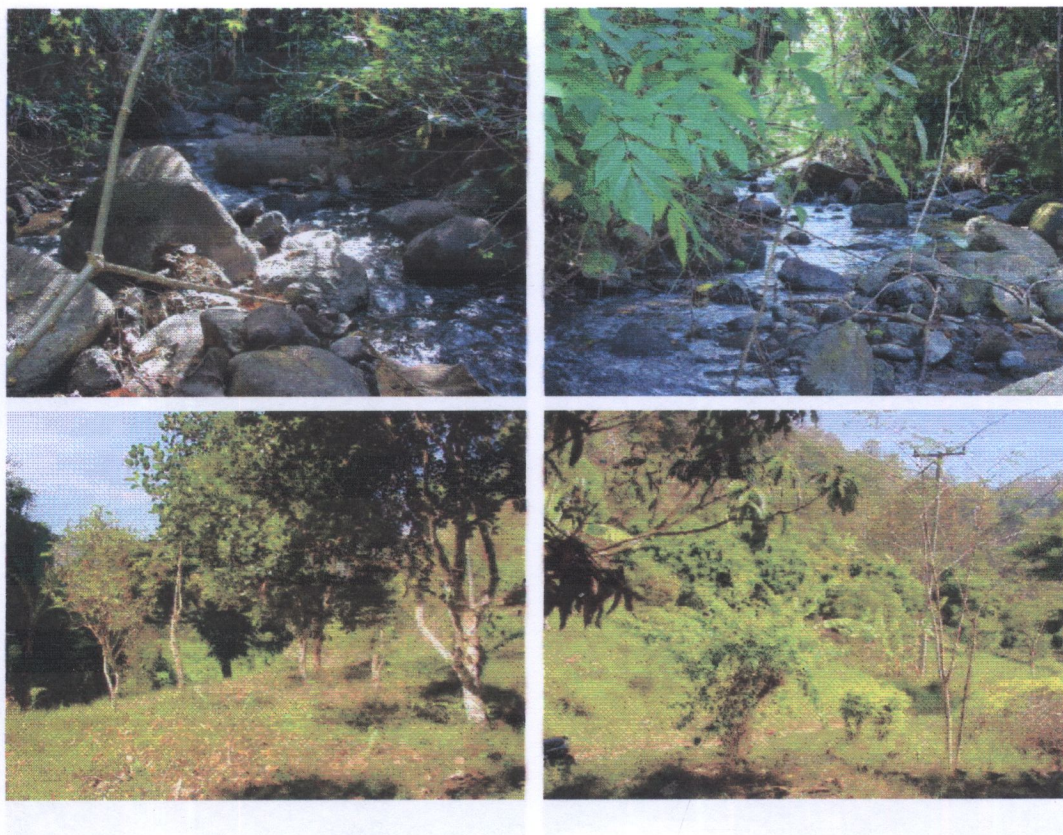


Figure 12 Study site, TAD 5, 30 January 2000.

Activities in this site: Mainly Orchards (*Litchi chinensis* Sonn.
and *Artocarpus heterophyllus* Lank.)

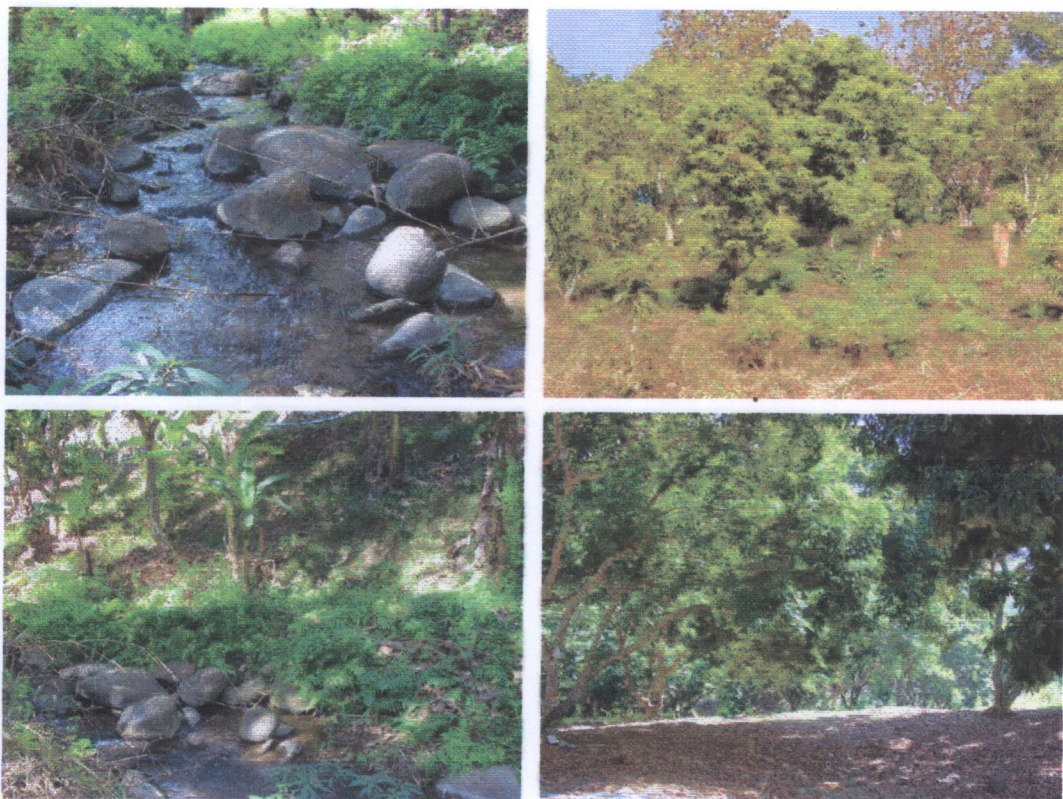


Figure 13 Study site, TAD 6, 30 January 2001.

Activities in this site: Mainly Orchards (*Litchi chinensis* Sonn.
and *Dimocarpus longan* Lour.)



Figure 14 Study site, SAMSOB, 30 January 2000.
Activities in this site: Mainly evergreen forest and small village.



Figure 15 Study site, KAENG 1, 30 January 2000.
Activities in this site: Mainly mixed deciduous forest.

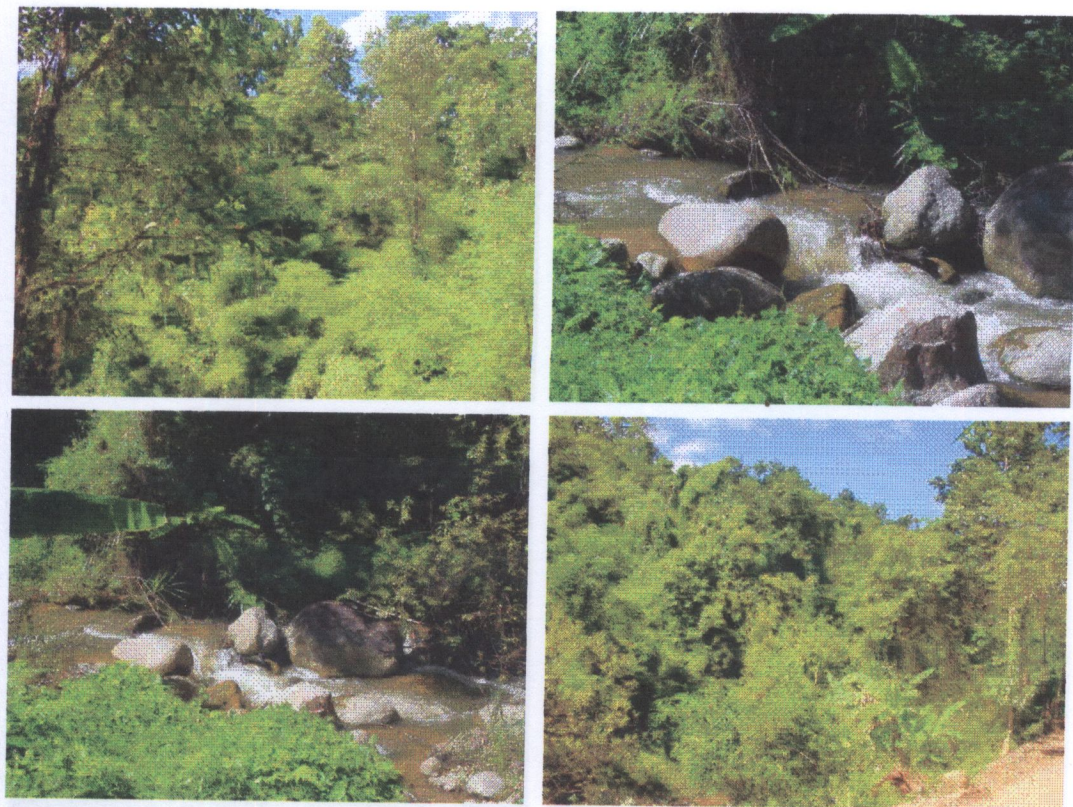


Figure 16 Study site, KAENG 2, 30 January 2000.

Activities in this site: Mainly mixed deciduous forest.



Figure 17 Study site, KAENG 3, 30 January 2000.

Activities in this site: Mainly orchards (*Litchi chinensis* Sonn. and *Dimocarpus longan* Lour.) and cultivation of lowland crop (corn and soybean)



Figure 18 Study site, KAENG 4, 30 January 2000.

Activities in this site: Mainly orchards (*Litchi chinensis* Sonn.
and *Dimocarpus longan* Lour.) and village.



Figure 19 Study site, MAE MAE, 30 January 2000.
Activities in this site: Mainly hill evergreen forest and village.

Figure 20 Study site, PHA HANG, 30 January 2000.
Activities in this site: Mainly forest and a small village.

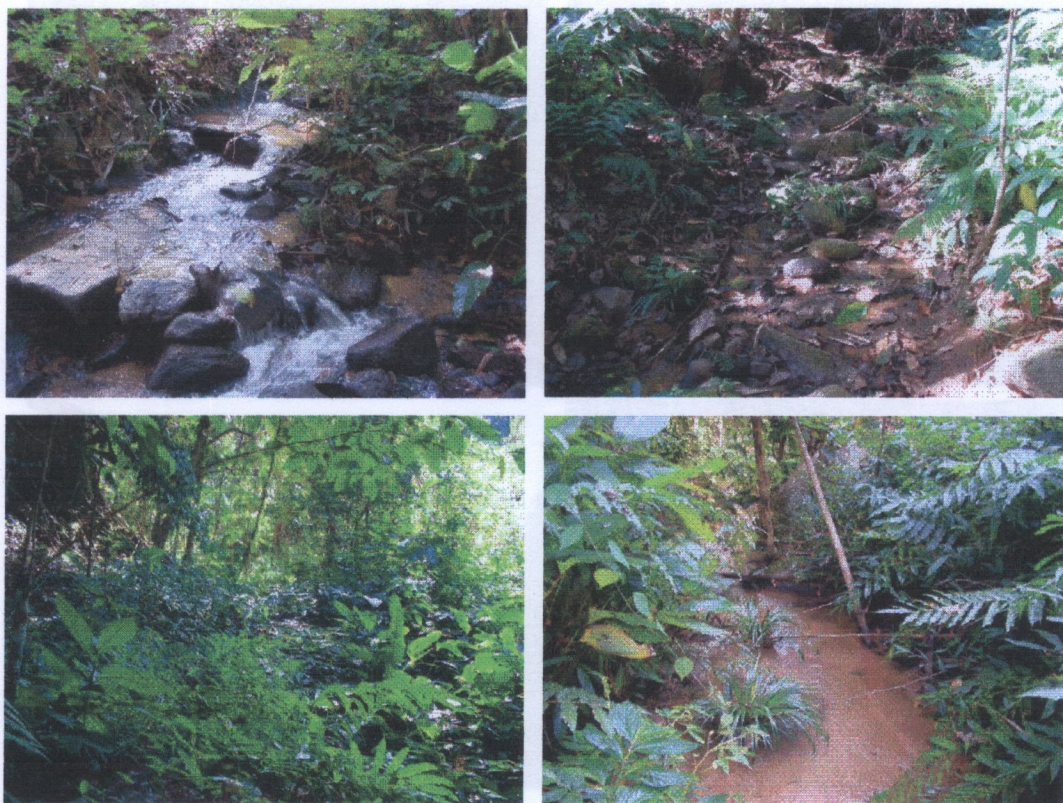


Figure 20 Study site, PHA HANG, 30 January 2000.
Activities in this site: Mainly forest and a small village

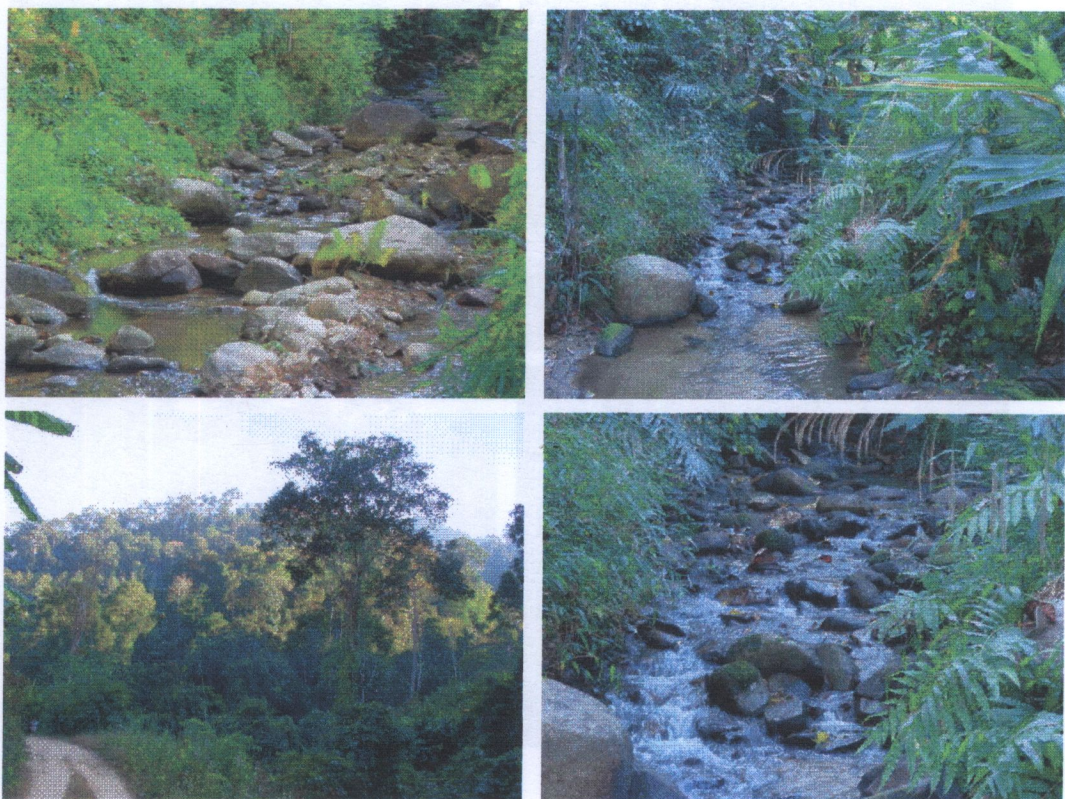


Figure 21 Study site, MAE SAI, 30 January 2000

Activities in this site: Mainly evergreen forest



Figure 22 Study site, SONG SOB, 30 January 2000

Activities in this site: Mainly mixed deciduous forest



Figure 23 Study site, DAM, 30 January 2000

Activities in this site: Weir, sand extraction and orchard (*Litchi chinesis* Sonn.)

Activities in this site: paddy field, poultry live stock and kak plantation



Figure 24 Study site, MAE NA 1

Activities in this site: paddy field, poultry live stock and teak plantation.



Figure 25 Study site, MAE NA 2

Activities in this site: paddy field, crop cultivation and small village.



Figure 26 Study site, MAE NA 3
Activities in this site: community area

3.3 Descriptions of physico-chemical parameters measurement

3.3.1 Equipment for physico-chemical measurement

1. Conductivity/TDS meter



Figure 27 Study site, MAE NA 4

- 8. 300 ml BOD bottles
- 9. 1-liter pol. ethylene bottles

3.3.3 Measurement of physico-chemical parameters and site aspects

Figure 27 Study site, MAE NA 4
Activities in this site: community area and paddy field

- 2. Alkalinity by phenolphthalein methyl orange indicator titration.
- 3. Dissolved oxygen by azide modification method.

3.3 Descriptions of physico-chemical parameters measurement

3.3.1 Equipment for physico-chemical measurement

1. Conductivity/TDS meter
2. pH meter
3. Velocity meter
4. Tape measure and ruler
5. DR/2000 Spectrophotometer HACH

3.3.2 Materials for physico-chemical investigation

1. PhosVer3 Phosphate reagent powder pillows
2. NitraVer5 Nitrate reagent powder pillows
3. SulfaVer4 Sulfate reagent powder pillows
4. Nessler reagent
5. Mineral stabilizer
6. Polyvinyl alcohol
7. Distilled and deionized water
8. 300 ml BOD bottles
9. 1-liter polyethylene bottles

3.3.3 Measurement of physio-chemical parameters and site aspects

1. Organophosphate and carbarmate residues from soil and sediment by Thoophom *et al.* (1998)
2. Alkalinity by phenophthalein methyl orange indicator titration.
3. Dissolved oxygen by azide modification method.

4. Biochemical oxygen demand (BOD_5) by azide modification method.
5. Nitrate-Nitrogen using a Spectrophotometer (HACH model DR/2000) by cadmium reduction method.
6. Ammonia-nitrogen using a Spectrophotometer (HACH model DR/2000) by Nessler method.
7. Phosphate-Phosphorus using a spectrophotometer (HACH model DR/2000) by ascorbic acid method.
8. Nitrate-Nitrogen using spectrophotometer (HACH model DR/2000) by the cadmium reduction method.
9. Sulfate using spectrophotometer (HACH model DR/2000) by Sulfaver 4 method.
10. Air and water temperatures using hand mercury thermometer.
11. pH using a pH meter.
12. Conductivity and total dissolved solid using a conductivity /TDS meter.
13. Velocity using a velocity meter.
14. Stream width and depth using a plummet and ruler.
15. Discharge (D) calculated from a function of velocity (V) and cross section (A), $D=AV$
16. Turbidity using a spectrophotometer (HACH model DR/2000) by absorptometric method.
17. Recording stream substrates by observation.

3.4 Description of Aquatic insects data

3.4.1 Equipment and Materials for collecting and sorting

1. 20 portable light-traps, 10 watt (Figure 28)
2. Sieve
3. Plastic bottles
4. Scintillation vial
4. Ethanol 70%
5. Label papers and pencils
6. Plastic bowls
7. Glass sorting dishes
8. Hot plate
9. Stereomicroscope
10. 10% potassium hydroxide (KOH) solution
11. Detergent
12. Staining block

3.4.2 Methodology for aquatic insects sampling

To collect adults of aquatic insects, 20 portable light traps (10 watt, 12 volt DC) with water and detergent liquid was placed along near the water edge at each study site. The traps were operated overnight, every season (hot-March 2000, rainy-June 2000, and cool-October 2000). The specimens were later separated into order groups (Ephemeroptera, Plecoptera, and Trichoptera), labeled and preserved in 70 % alcohol.

3.4.3 Aquatic Insects identification

1. Mayflies were identified to family level using McCafferty, 1981.
2. Stonefly specimens were identified by Prof. Dr. Ignac Sivec.
3. Caddisflies specimens were further separated into distinct groups under a stereo microscope. The two last abdominal segments of males caddisflies were cut and macerated in 10% potassium hydroxide (KOH) solution for 30 minutes–2 hours. The preparations of genital segments were studied under a stereo-microscope. Male genitalia morphologies were correlated with the pictorial key by Malicky (1997). Others references are: Chantaramongkol and Malicky, 1989; 1995; Malicky, 1987; 1989; 1994; 1998a; 1998b; Malicky and Chantaramongkol, 1989a; 1989b; 1991a; 1991b; 1991c; 1992a; 1992b; 1993; 1994; 1996; 1999, and Malicky *et al.*, 2000a; Malicky *et al.*, 2000b.

3.5 Data analysis

The assemblages of male caddisfly adults and physico-chemical parameters were clustered using two-way indicator species analysis TWINSpan and ordinated using semi-strong hybrid multidimensional scaling (ssh mds) by multivariate techniques supported in the ecological pattern analysis package PATN (Belbin, 1995). The Bray-Curtis measure of similarity was used to summarize relationships between all pairs of sites and the association matrix was used as input to the multivariate analysis. Analysis of Variance was used to test for differences in the mean values of environmental variables between groups of sites identified by the TWINSpan analysis. Kruskal-Wallis One Way Analysis of Variance was used if the data violated the

parametric assumptions. Contrasts were explored using the Student-Newman-Keuls Method. Product-moment correlations were determined for each variable within the ordination space using the PCC option in PATN. These were tested for significance using 100 Monte Carlo randomizations (MCAO option in PATN). To determine the relative of significant parameters and caddisfly species, the using of correlation were performed by SPSS.

3.6 Studying the effects of pollution on cholinesterase (ChE) activity

3.6.1 Equipment and Materials

3.6.1.1 Apparatus

1. Eppendorf safe-lock tubes
2. Ice box
3. Glass dropper
4. Pyrex test tube
5. pH meter
6. Thermometer
7. Shaking incubator
8. Automatic pipette
8. Homoginizer
9. Centrifuge
10. Vortex shaker
11. Spectrophotometer, Cecil model CE292, England
12. Refrigerator
13. ELISA plate reader, CERES UV900 Hdi, Bio-tek Instrument, INC.
14. Microwell plates (96 wells, flatted bottom), MaxiSorp Surface
15. Microwell plates shaker, Cooke Laboratory Products, England

3.6.1.2 Chemical reagents

1. Homogenizing reagent, tris-HCl 0.1 M pH 7.4
2. DTNB (5,5' dithiobis-2-nitrobenzoic acid) 0.5 mM in buffer
3. Acetylthiocholine iodide 156 mM (substrate), Sigma, USA
4. Eserine 12 mM, Sigma, USA
5. Bovine serum albumin (BSA) standard, Bio-Rad
6. Bio-Rad Protein dye.
7. Whatman filter paper No. 7
8. Pesticide solutions, 1250, 625, 156.2, 78.2, 19.5, and 9.8 μ M of carbaryl (Dr. Ehrenstorfer, Augsburg, Germany) and 625, 156.2, 39.1, 9.8, 2.4, and 0.6 μ M of mevinphos (Dr. Ehrenstorfer, Augsburg, Germany).

3.6.2 Measurement of cholinesterase (ChE) activity

Twelve final instar larvae of *Stenopsyche siamensis* (Trichoptera) were collected from 2 different sites (Song Sob-control, Mae Na-impacted sites) in the hot (April 2000), rainy (August 2000) and cool (October 2000) seasons. The samples were kept in an icebox with ice. After that the samples were transported to the Toxicology Laboratory, Research Institute for Health Sciences, Chiang Mai University and stored in the freezer. Frozen samples were thawed in tap water. ChE was extracted from the head of each insect with 0.5 ml of 1M tris-HCl in a motor-homogenizer. Homogenates of each larva were centrifuged at 10,000 rpm for 10 minutes. Supernatants of each larva were analyzed using the colorimetric reaction between the hydrolyzed choline analogue, acetylthiocholine iodide, and DTNB (5,5' dithiobis-2-nitrobenzoic acid) according to Ellman *et al.* (1961) (Figure 29). The

remnant supernatants were assayed for protein using Biorad reagent (Figure 30) to standardize the ChE activity results for differences in stenopsychid mass. ChE activity was calculated as unit of activity per mg ($\mu\text{mole}/\text{min}/\text{mg}$ protein) of protein as equation 3.1. The data were compared using a student *t* test.



Figure 28. Portable light-trap, 10 watts

Reagent/Solution	Blank	Test (2 tubes)
DTNB	3.0 ml	3.0 ml
Acetylthiocholine iodide	0.01 ml	0.01 ml
Eserine	0.05 ml	-
Mixed and incubated at 25 ⁰ C for 10 minutes		
Stenopsychid supernatant	0.1 ml	0.1 ml
Mixed and incubated at 25 ⁰ C for 15 minutes		
Eserine	-	0.05 ml



Mixed them with vortex and measure absorbance at 415 nm



Calculated ΔA using
test absorbance - bank absorbance
for ChE activity calculation with eq 3.1

Figure 29. Flow chart showing the processes to detected stenopsychid cholinesterase (ChE) activity.

$$R = \frac{\Delta A}{136,000} \times \frac{3.16}{0.1} \times \frac{1}{C_p} \times \frac{1}{15} \quad \text{Eq 3.1}$$

Where: R = ChE activity ($\mu\text{mole}/\text{min}/\text{mg}$ protein), ΔA = change in absorbency in 15 minutes, C_p = stenopsychid protein concentration (mg/ml)

3.6.3 Inhibition test

In vitro assay was conducted to check *in vivo* ChE inhibition as a direct result of pesticides exposure. 12 stenopsychid larvae were collected from the 2 different sites in the rainy season (8 July, 2000). ChE was extracted from each insect head with 0.5 ml of 1M tris-HCl in a motor-homogenizer. Homogenates of each single larva were pooled into 1 test tube and mixed in vortex shaker. 12 tubes of 0.1 ml of stenopsychid supernatant from the control and impacted sites were incubated with 3 ml of DTNB at 25°C. Pesticides solutions of 1250, 625, 156.2, 78.2, 19.5, and 9.8 μM of carbaryl, 625, 156.2, 39.1, 9.8, 2.4, and 0.6 μM of mevinphos were mixed with 100 μl in test tubes. The samples were analyzed using a colorimetric reaction according to Ellman *et al.* (1996) (Figure 31). The remnant supernatants were assayed for protein using Biorad reagent to standardize the ChE activity results for differences in stenopsychid mass. ChE activity was calculated as unit of activity per mg of protein as equation 3.2. The data were plotted and compared at 50% ChE inhibition.

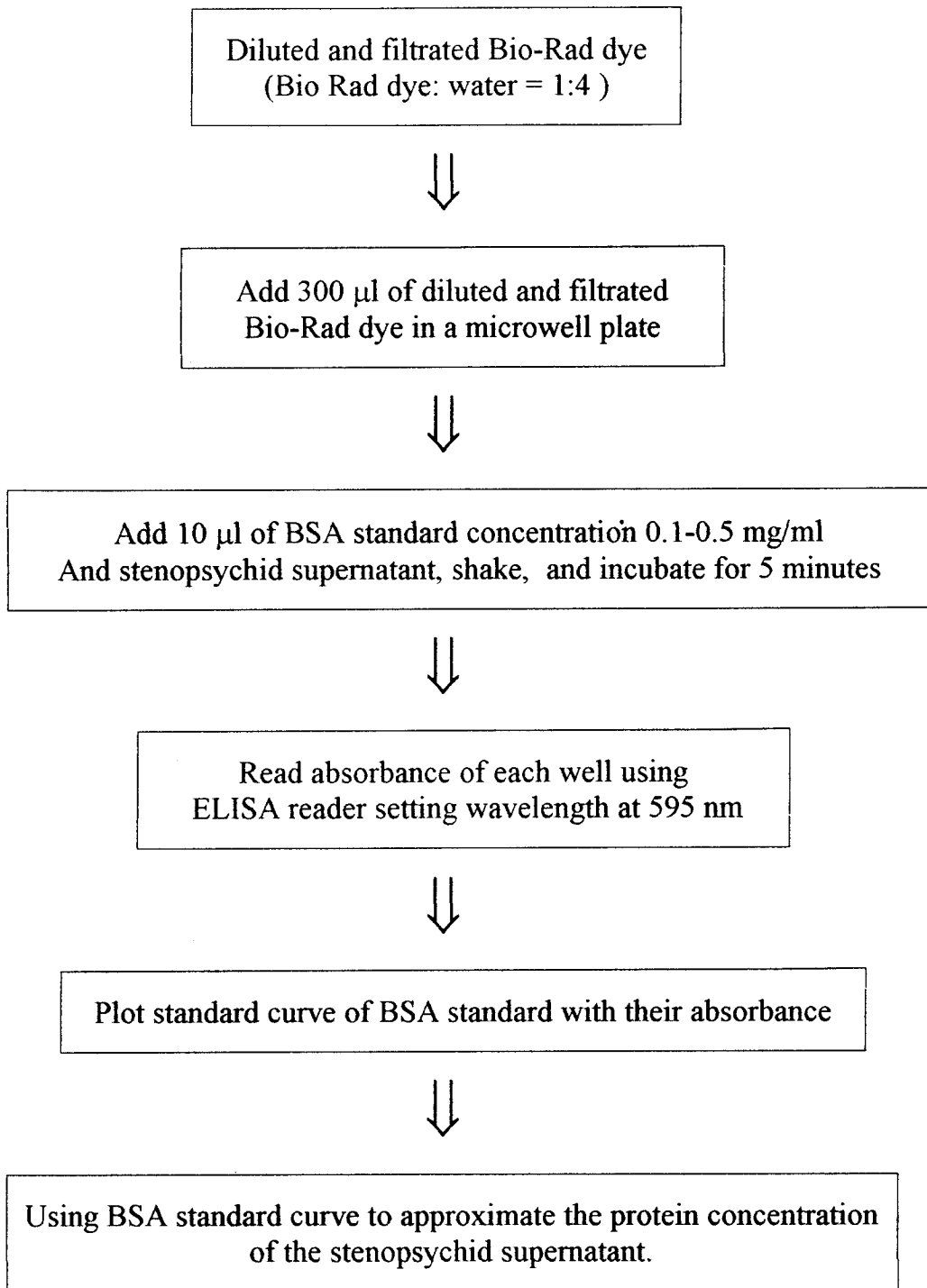


Figure 30 Flow chart showing the processes of stenopsychid protein determination

Reagent/Solution	Blank	Test (2 tubes)
DTNB	3.0 ml	3.0 ml
Acetylthiocholine iodide	0.01 ml	0.01 ml
Eserine	0.05 ml	-
Cabaryl or Mevinphos solutions	-	0.1 ml
Mixed and incubated at 25 ⁰ C for 10 minutes		
Stenopsychid supernatant	0.1 ml	0.1 ml
Mixed and incubated at 25 ⁰ C for 15 minutes		
Eserine	-	0.05 ml



Mixed them with vortex and measure absorbance at 415 nm



Calculated ΔA using
test absorbance - blank absorbance
for ChE activity calculation with eq. 3.2

Figure 31. Flow chart showing the processing of *in vitro* stenopsychid cholinesterase (ChE) activity inhibition test.

$$R = \frac{\Delta A}{136,000} \times \frac{3.27}{0.1} \times \frac{1}{C_p} \times \frac{1}{15} \quad \text{Eq 3.2}$$

Where: R = ChE activity ($\mu\text{mole}/\text{min}/\text{mg}$ protein), ΔA = Change in absorbency in 15 minutes, C_p = Stenopsychid protein concentration (mg/ml)

3.7 Studying the effects of pollution on integumental surfaces

3.7.1 Equipment and Materials

1. Scanning Electron microscope (SEM) model JEOL-JSM 840A
2. Sputtering coater
3. Critical point dryer
4. Stub and copper sticky tape
5. 2.5% glutaraldehyde
6. 1% osmium tetroxide
7. 0.1 M phosphate buffer pH 7.4
8. 0.85 normal saline
9. Ethyl alcohol serial solutions (%): 10, 20, 30, 40, 50, 70, 85, 95 and absolute
10. Acetone
11. 0.2 M sodium phosphate monobasic buffer
12. 0.2 M sodium phosphate dibasic buffer
13. Transitional fluid; liquid CO_2

3.7.2 Studying integumental surfaces by scanning electron microscopy

30 *Stenopsyche siamensis* larvae were collected from each stream (control and impacted sites). Specimens were killed and cleaned in cold water, fixed in 2.5% glutaraldehyde in phosphate buffer, pH 7.4, and 1% osmium tetroxide for 24 and 2 hours, respectively. Fixed specimens were dehydrated with a graded series of ethanol (%) (10, 20, 30, 50, 70, 85, 95, and absolute) and acetone, and finally dried in a critical point dryer. The dried samples were mounted on stubs, coated with gold, and examined with SEM JEOL-JSM840A (Figure 32). SEM micrographs were compared between different study sites to determine the impact of pollution on integument morphology.

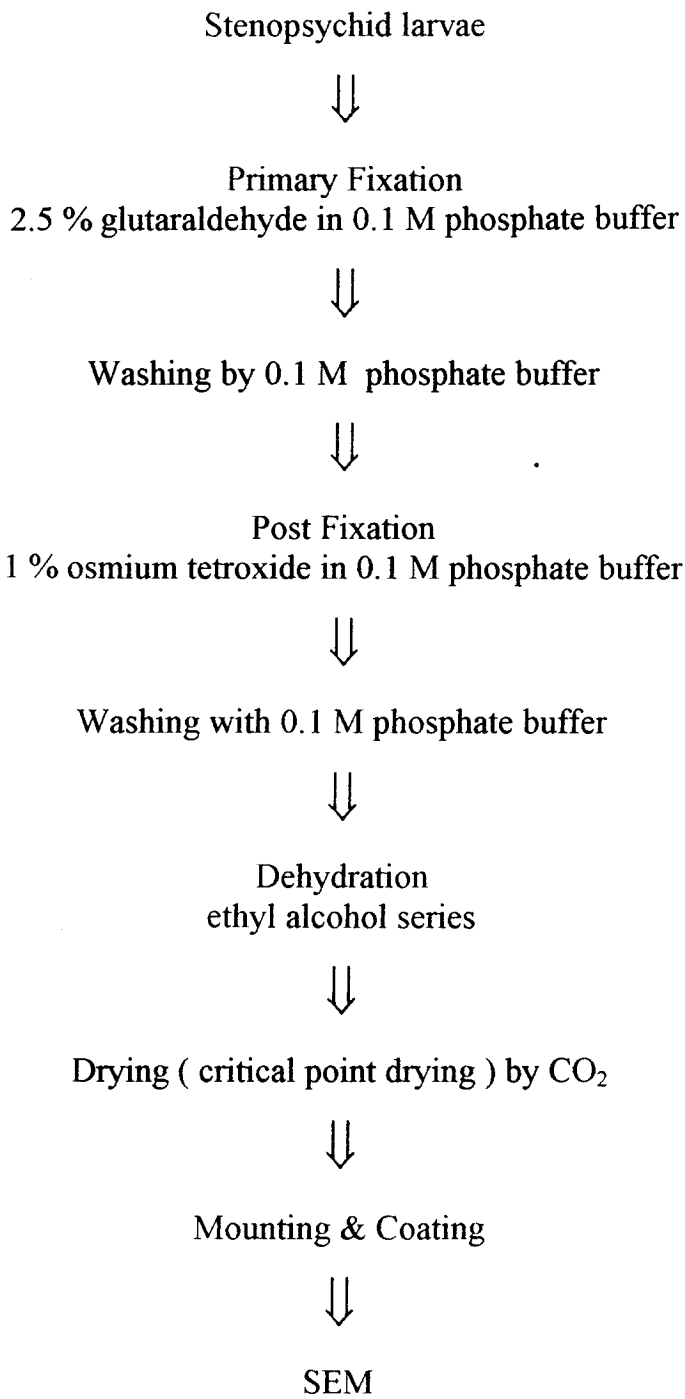


Figure 32 Flow chart showing the processes of specimen preparation for SEM.

3.8 Studying the impact of pollution on the life cycle of *Stenopsyche siamensis*

3.8.1 Equipment and Materials

1. Compound microscope with ocular micrometer
2. Tray
3. Soft forceps
4. Enclosure (Figure 32)
5. Refrigerated incubator
6. Plastic boxes
7. Air pump
8. Glass slide
9. Needle

3.8.2 Life cycle studies

Larvae of *Stenopsyche siamensis* were collected monthly by hand picking from the 2 different stream sites (control and impacted site) during March 1999–February 2000. The head capsule widths were measured with an ocular micrometer. The distribution of head capsule width was plotted to determine the instar larvae of the insect.

To determine the impact of pollution on the life cycle of this aquatic insect, first and second instar larvae were collected in 2 different stream sites (control and impacted site) and cultured in their sites in an enclosure (one enclosure has 8 sections). The head capsule widths and motility rate of the larvae were observed every

week until the larvae developed to the fourth. To complete the study of the life cycle, the forth instars and adults were reared in a 20⁰ C refrigerated incubator in the Environmental Monitoring: Aquatic Insects Research Unit to known the developing time of the fifth instars.

To study the feeding behavior of this insect, fifth instar larvae were collected from the 2 study sites. The heads were pulled off by forceps. Each fore gut, which should remain intact and attached to the head, was dissected and examined under a compound microscope. The food in each gut was identified and taken the photos.

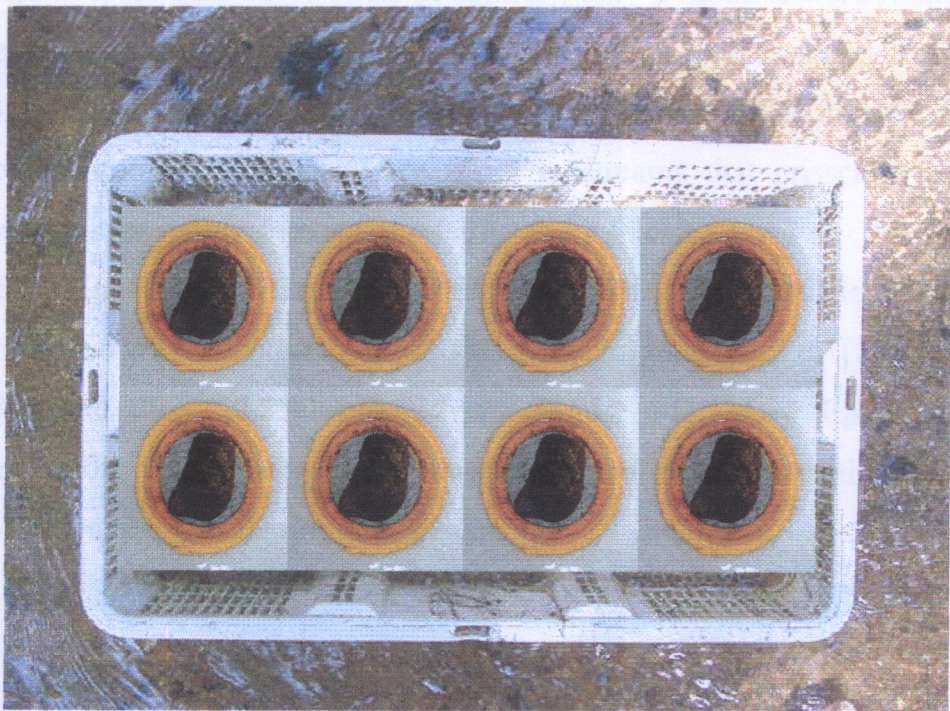


Figure 33. Enclosure

CHAPTER IV

RESULTS

4.1 Biodiversity of aquatic insects in Doi Chiang Dao watershed

4.1.1 Biodiversity of Ephemeroptera

A total of 1,315 Ephemeroptera were trapped at the 20 sites over all the following seasons: hot season-February 2000, rainy season- July 2000 and cool season October 2000. Seven families of Ephemeroptera were identified: Baetidae, Caenidae, Ephemeridae, Heptageniidae, Leptophlebiidae, Polymitarcyidae and Siphonuridae (Appendix 1). The most widespread families were Heptageniidae, Baetidae, Ephemeridae, and Caenidae, which occurred in more than 13-18 sites. In contrast, only 2 families were found in 4-6 sites including Siphonuridae, usually found in forest stream sites, and Leptophlebiidae in organic polluted streams (village site). The highest number of Ephemeroptera specimens were taken in streams that crisscross through paddy fields or village areas or weirs, and typically were in the families Baetidae Ephemeridae and Heptageniidae. The families Heptageniidae, Baetidae, and Ephemeridae yielded the most individuals: 370 (28.1%), 350 (26.6%), and 292 (22.2%) respectively (Figure 34). More Ephemeroptera emerged in the hot and rainy season than in the cool season, in both numbers of adults and numbers of families (Appendix 1).

4.1.2 Diversity of Plecoptera

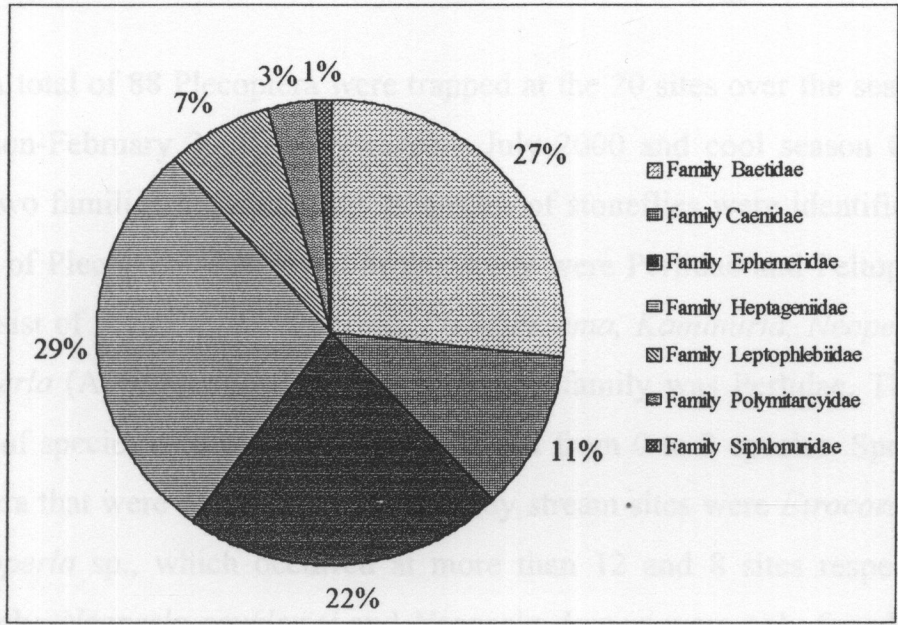


Figure 34. Percentage composition of Ephemeroptera families in Doi Chiang Dao watershed, Chiang Mai Province, Thailand

4.1.2 Biodiversity of Plecoptera

A total of 88 Plecoptera were trapped at the 20 sites over the seasons of hot season-February 2000, rainy season- July 2000 and cool season October 2000. Two families, 5 genera, and 8 species of stoneflies were identified. The families of Plecoptera that found in this study were Perlidae and Peltoperlidae that consist of 5 genera, *Etrocorema*, *Phanoperema*, *Kaminuria*, *Neopera*, and *Cryptoperla* (Appendix 2). The most abundant family was Perlidae. The total number of species recorded at the sites ranged from 0 to 3 species. Species of Plecoptera that were often found in the study stream sites were *Etrocorema* sp. and *Neoperla* sp., which occurred at more than 12 and 8 sites respectively. Conversely, *Neoperla cavaleriei* and *Neoperla desperi* were only found at one site. More Plecoptera emerged in the hot season than in the rainy and cool seasons, in both of numbers of adults and numbers of families (Appendix 2).

4.1.3 Biodiversity of Trichoptera

A total of 4,460 male caddisflies were trapped at the 20 sites from February to October 2000. Seventeen families, 38 genera, and 127 species of caddisflies were identified in the samples. Species richness and numbers of specimens caught in the different sites varied moderately. The highest species richness was found in the forested sites, whereas the lowest numbers of species were trapped in agriculture and household areas. The highest numbers of caddisflies specimens were taken in village sites, while the lowest numbers that were found in agricultural sites. Only 8 species of caddisflies were found at more than 12 sites: *Agapetus halong*, *Kissaura consagia*, *Psychomyia kaiya*, *Cheumatopsyche cocles*, *Hydropsyche atropos*, *H. camillus*, *H. dolosa*, *Potamyia phaidra*, and *Goera doligung*. However, forty-five species were highly localized and restricted to one or two sites including *Rhyacophila tosagan*, *A. atuus*, *Glossosoma malayana*, *G. voccus*, *Ugandatrichia maliwan*, *Chimarra aneca*, *C. htinorum*, *C. khamuorum*, *C. litussa*, *C. monorum*, *C. ravanna*, *C. yaorum*, *Gunungiella segsafiazga*, *Nyctiophylax amaltheia*, *Polyplectropus admin*, *P. sampati*, *P. barata*, *P. monto*, *P. semarangensis*, *Drepanocentron curmisagius*, *Ecnomus cincibilus*, *E. jojachin*, *E. mammus*, *E. pseudotenellus*, *E. volovicus*, *E. uttu*, *Amphipsyche gratiosa*, *C. carmentis*, *C. carna*, *C. gaia*, *H. scotosius*, *H. sealthiel*, *H. askalaphos*, *P. alleni*, *P. baenzigeri*, *Moropsyche huaisailianga*, *Dinarthrum matius*, *Leptocerus Chiangmaiensis*, *L. lanzenbergeri*, *L. promkutkaewi*, *L. posticoideis*, *Adinathrum brevipennis*, *A. diana*, *Inthanopsyche trimeresuri*, and *Marilia mogtiana* (Appendix 3).

Hydropsychidae, Philopotamidae, and Psychomyiidae were the most abundant families, with 39 species (30.7 %), 19 species (15%), and 11 species (8.7%), respectively (Figure 35). More caddisflies emerged in the hot season

than in the rainy and cool seasons, in both of numbers of adults and numbers of species (Figure 36). The relative abundance of caddisfly families in each site is shown in figure 37.

Flight periods of the caddisflies also varied considerably. Forty-two species (33.1%) were found in all seasons, 49 species (38.6%) were active in a single season and 36 species (28.4%) were active in two seasons. In the hot season, 2,985 male caddisflies were trapped in the study sites, and these comprised 16 families, 36 genera, and 103 species (Appendix 4). The relative abundance of caddisfly families in each site is shown in figure 38. The families Hydropsychidae, Philopotamidae, and Psychomyiidae were the most abundant, yielding 34 species, 16 species, and 8 species respectively. In the rainy season, 1,099 male caddisflies were trapped in the study sites. Fifteen families, 30 genera, and 81 species of caddisflies were identified in these samples (Appendix 5). The relative abundance of caddisfly families in each site is shown in figure 39. The families Hydropsychidae, Philopotamidae, Ecnomidae, and Leptoceridae were the most abundant, yielding 31 species, 9 species, 7 species and 7 species respectively. In the cool season, 376 male caddisflies were trapped in the study sites. Thirteen families, 30 genera, and 63 species of caddisflies were identified in these samples (Appendix 6). The relative abundance of caddisfly families in each site is shown in figure 40. The families Hydropsychidae, Philopotamidae, and Psychomyiidae were the most abundant, yielding 23 species, 9 species, and 6 species respectively.

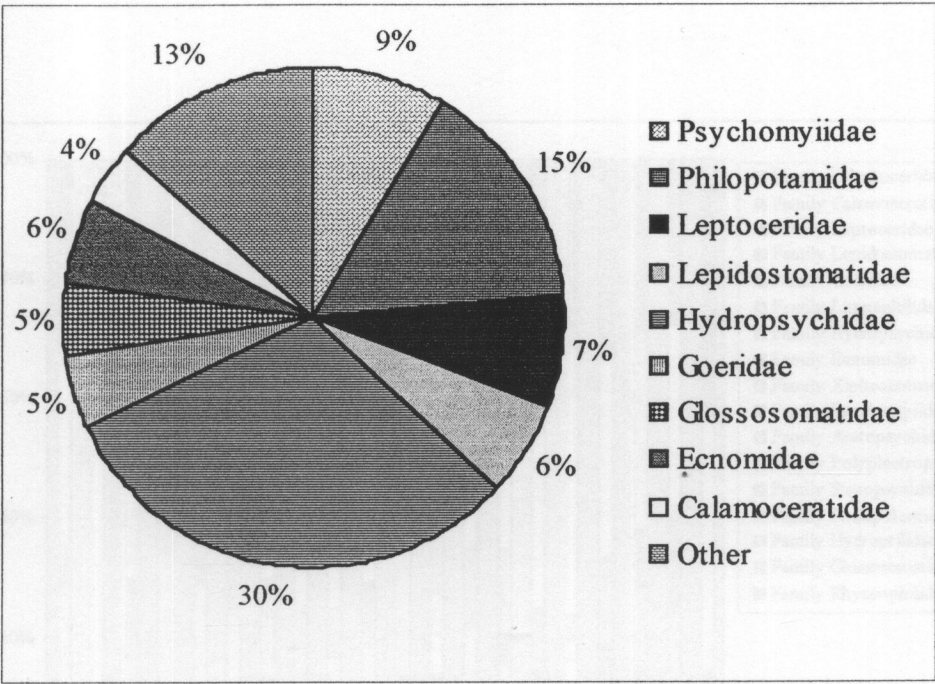


Figure 35. Percentage composition of caddisflies families in Doi Chiang Dao watershed, Chiang Mai Province, Thailand.

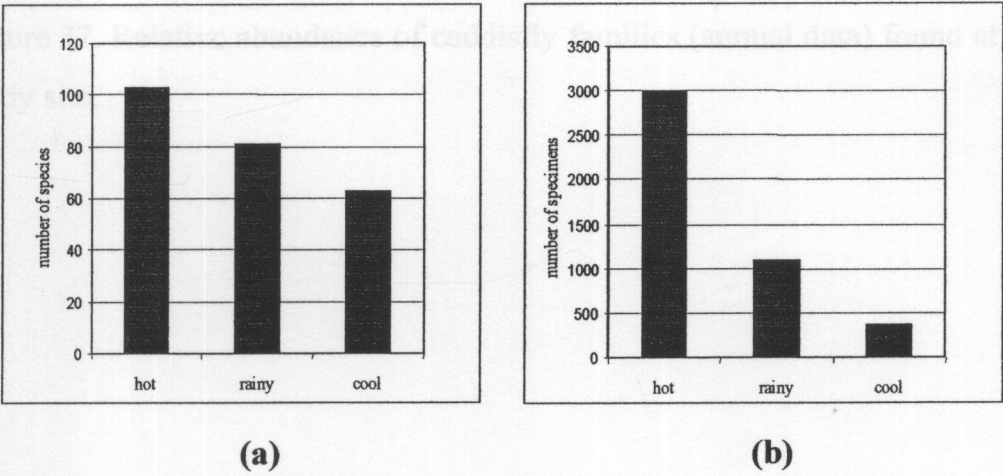


Figure 36. Seasonal occurrence of numbers of caddisflies species (a) and numbers of caddisflies specimens (b) in Doi Chiang Dao watershed, Chiang Mai Province, Thailand.

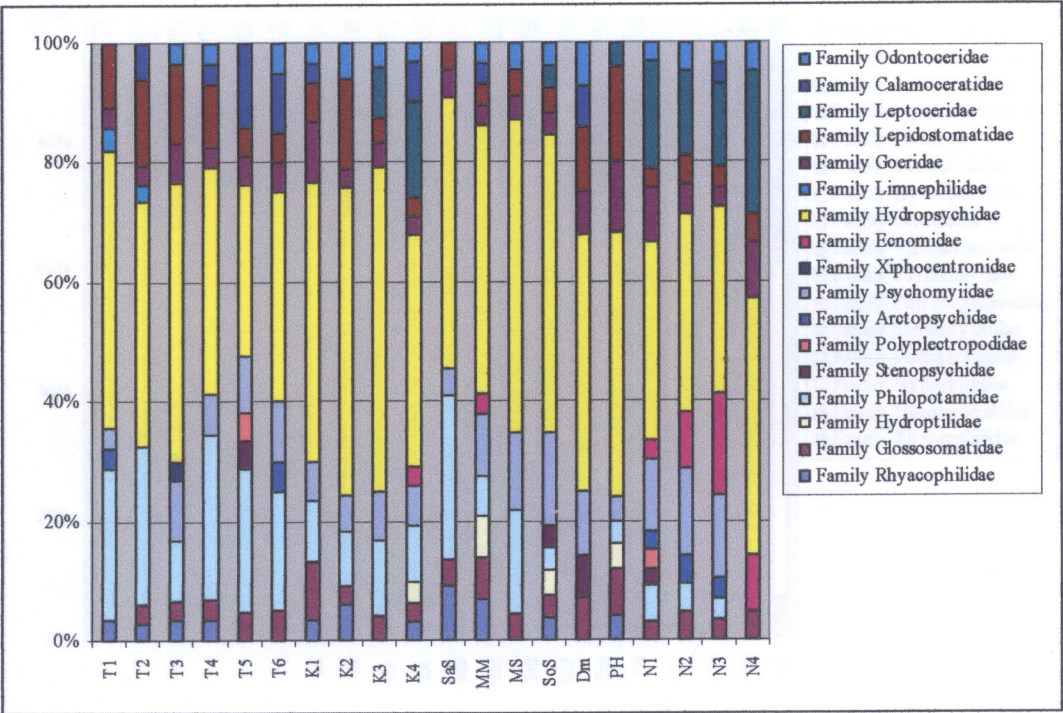


Figure 37. Relative abundance of caddisfly families (annual data) found at each study site.

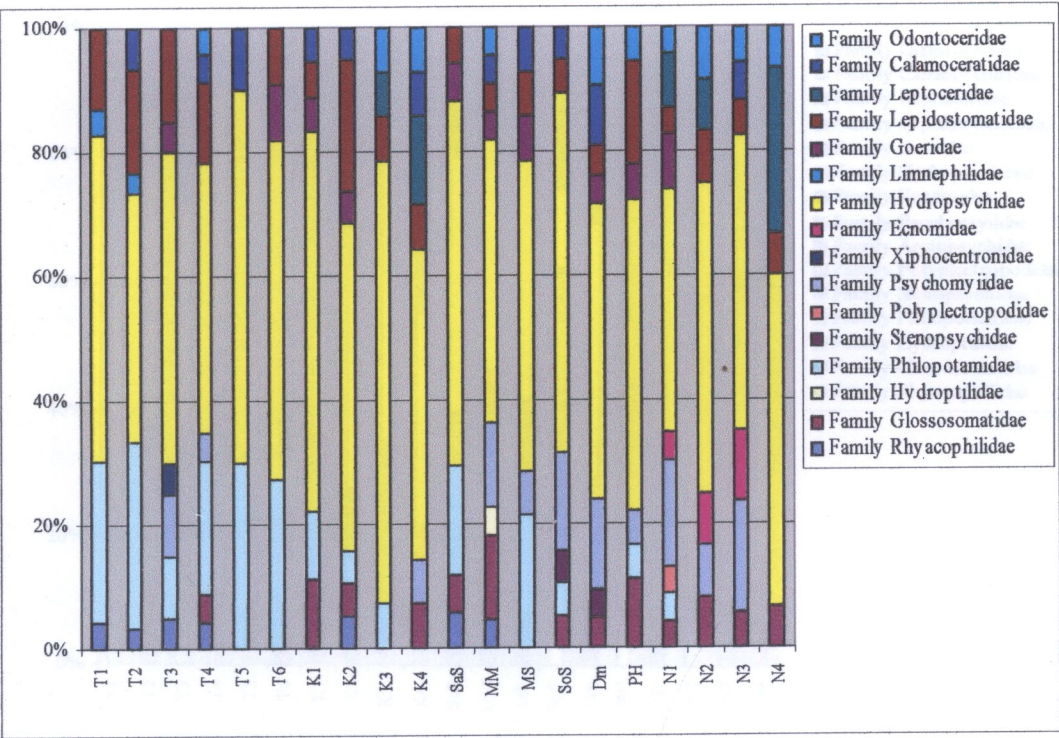


Figure 38. Relative abundance of caddisfly families found in the hot season at each study site.

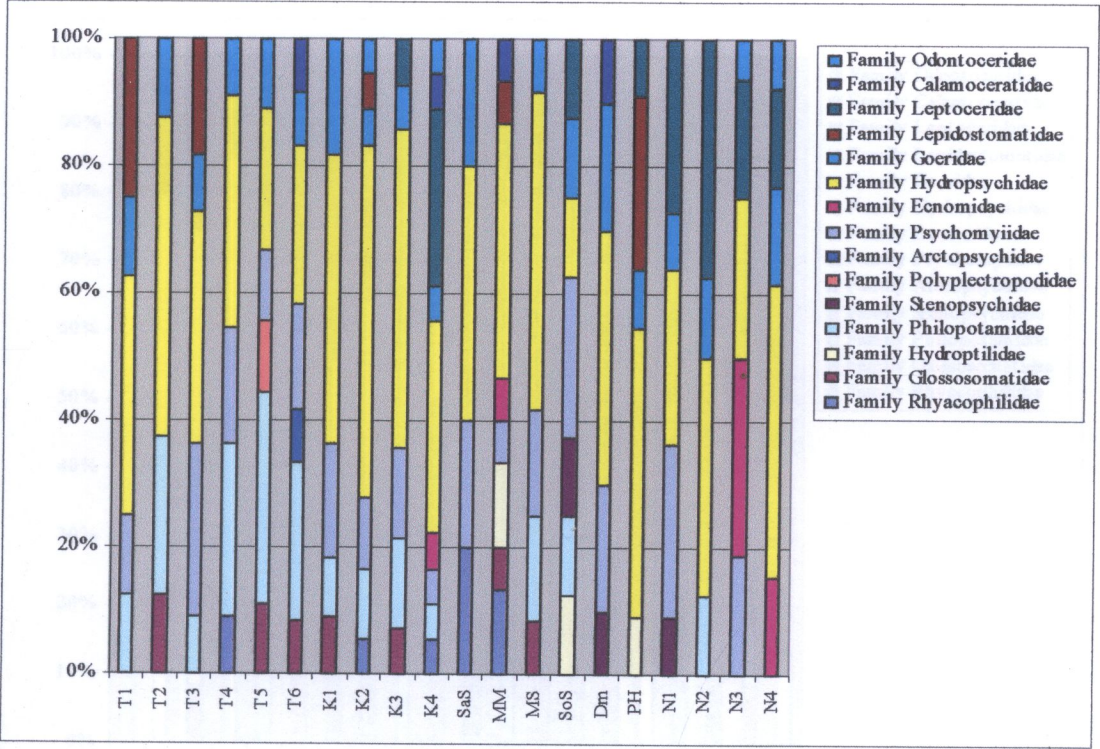


Figure 39. Relative abundance of caddisfly families which occurred in the rainy season in each of the study sites.

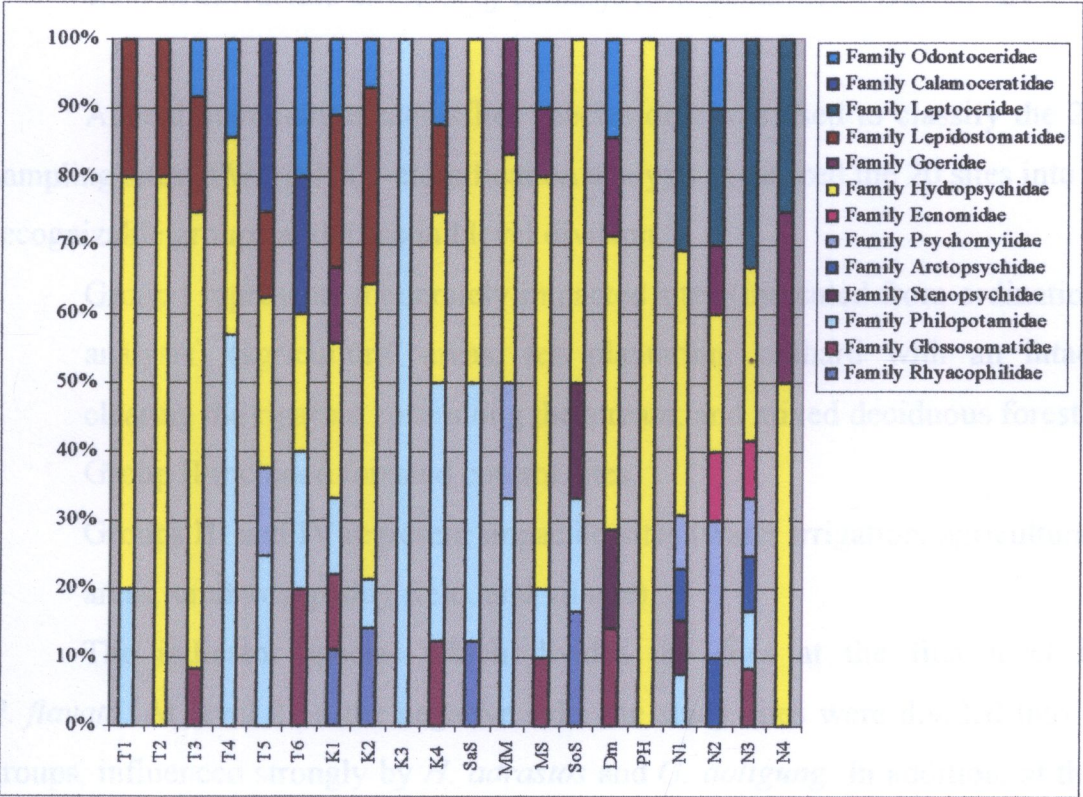


Figure 40. Relative abundance of caddisfly families which occurred in the cool season at each study site.

4.2 Biodiversity of caddisflies as bio-indicators of aquatic ecosystem health in Doi Chiang Dao watershed, Chiang Mai, Thailand

4.2.1 Multivariate analysis of caddisflies community: TWINSpan

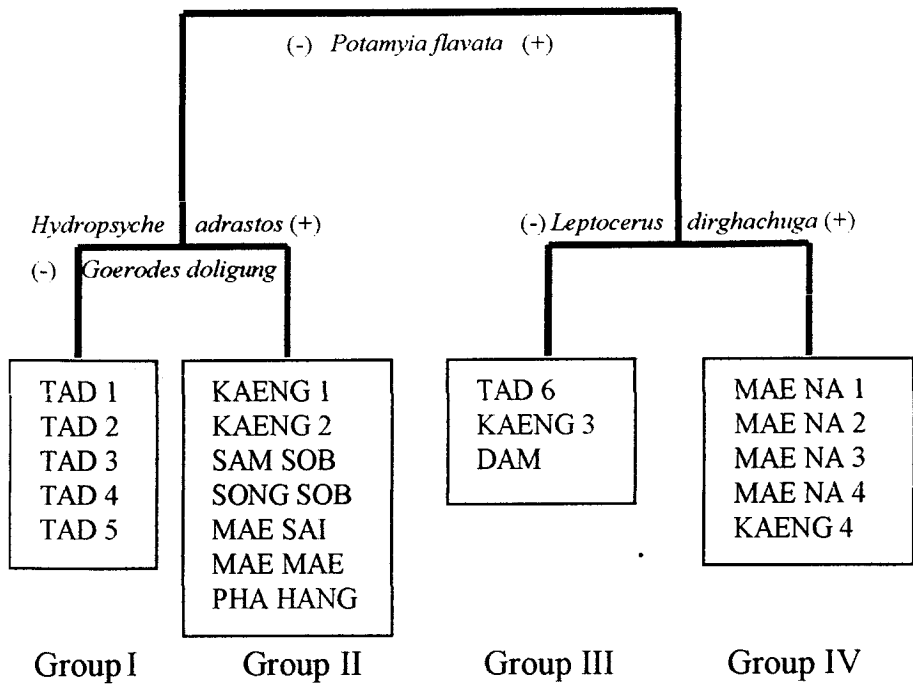
Annual quantitative caddisflies species data was used to classify the 20 sampling sites. TWINSpan-classification analysis organized the 20 sites into 4 recognizable groups at the second level division.

Group I represents moderately impacted sites (indicated from ordination analysis) (agricultural areas, tea plantation, orchard with an intact clearing the riparian zone along the stream, and mixed deciduous forest). Group II included forested control sites.

Groups III and IV represent impacted sites (water irrigation, agricultural areas, orchards, paddy field, and village).

The indicator species which divides the sites at the first level is *P. flavata*. At level 2 on the negative side, the study sites were divided into 2 groups, influenced strongly by *H. adraetos* and *G. doligung*. In addition, at the positive side, 8 study sites were separated into 2 groups by *L. dirghachuga* (Figure 41).

The average seasonal environmental parameters (Appendices 7-9) of each TWINSpan group were compared using analysis of variance. Ten parameters were shown to differ significantly between the groups (Figures 42-53). Group I sites differed from the others in their high conductivity, high TDS and nitrate-nitrogen, and low sulfate concentration in the hot season. Group II sites were significant for their high average stream velocity in the rainy season. Group III sites had significantly higher BOD, and Group IV sites had significantly lower pH, sulfate, dissolved oxygen, and higher % Inhibition of ChE from sediment (Table 3).



Trichoptera Family	Group I	Group II	Group III	Group IV
Rhyacophilidae	1±0.707 ^{ab}	2.143±2.143 ^a	0±0 ^b	0.2±0.447 ^b
Philopotamidae	2.94±19.113 ^a	6.857±6.857 ^b	7±8.888 ^b	5.8±10.756 ^b
Leptoceridae	0±0 ^a	0.286±0.286 ^a	0.667±1.154 ^a	5.2±53.474 ^b
Odontocerida	0.8±1.304 ^a	1.143±1.143 ^a	4±3 ^a	13.4±9.289 ^b

Figure 41. TWINSpan dendrogram of study sites based upon species of caddisflies and Mean ± SD of number of Trichoptera in family level of each TWINSpan group with differences at the P<0.05 level indicated from ANOVA analysis in Doi Chiang Dao watershed, Chiang Mai Province, Thailand.

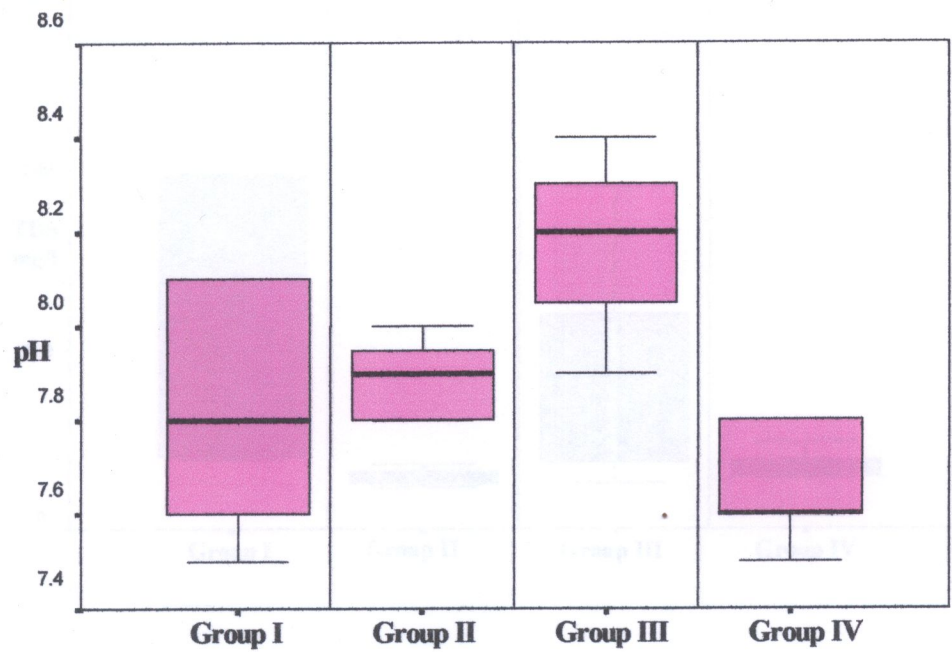


Figure 42. Boxplot of pH values in the hot season of each TWINSpan group.

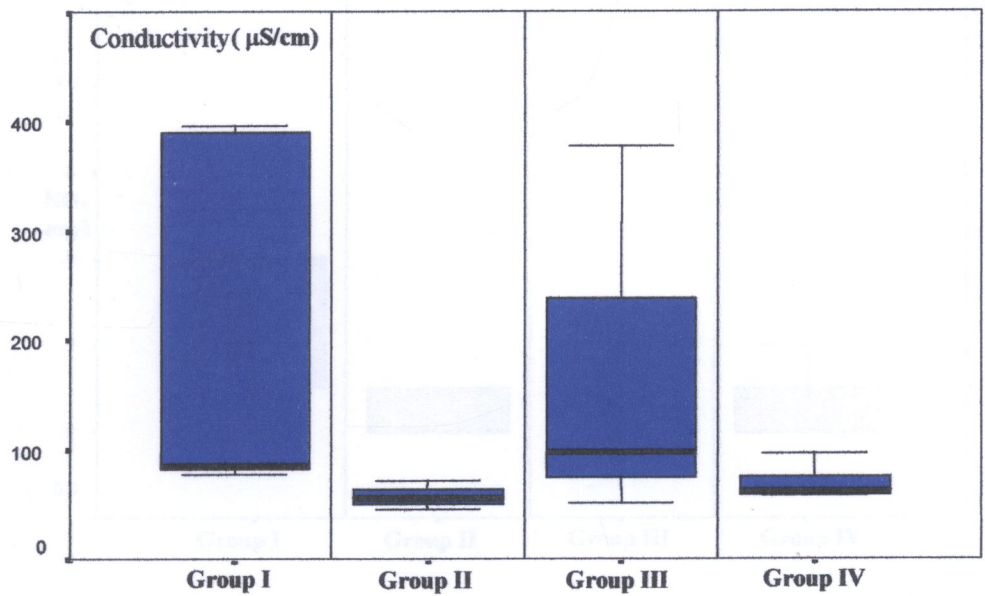


Figure 43. Boxplot of conductivity values in the hot season of each TWINSpan group.

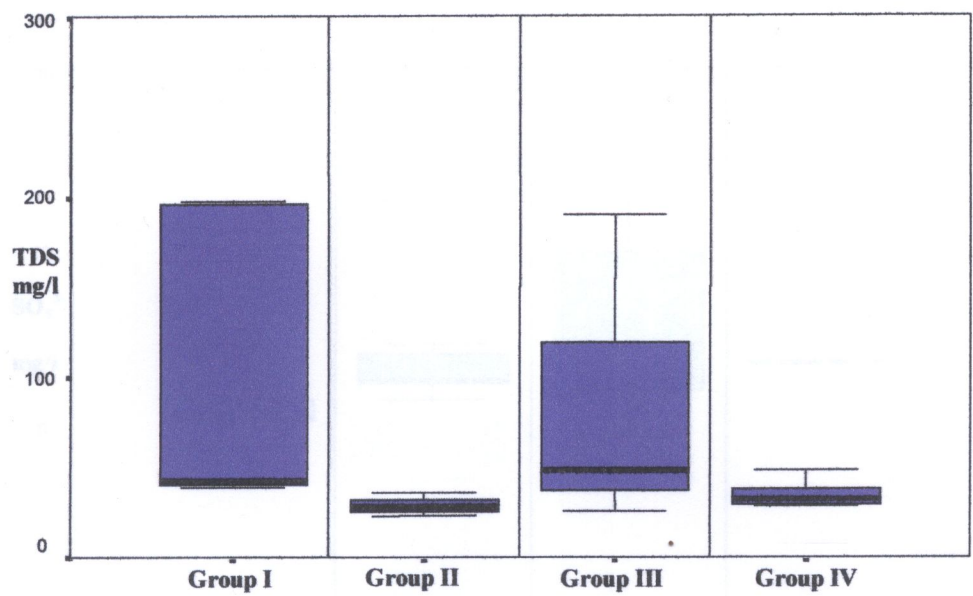


Figure 44. Boxplot of TDS values in the hot season for each TWINSpan group.

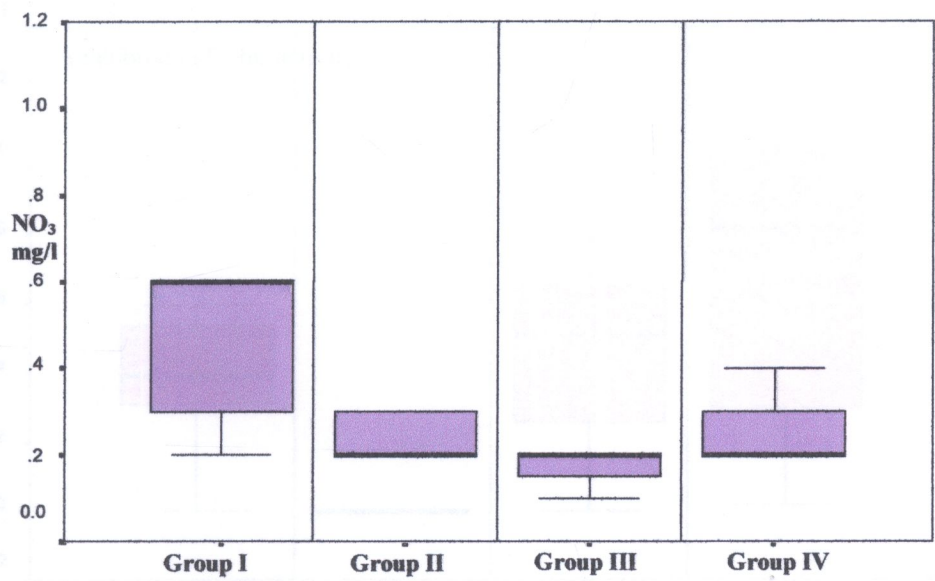


Figure 45. Boxplot of nitrate-nitrogen values in the hot season of each TWINSpan groups.

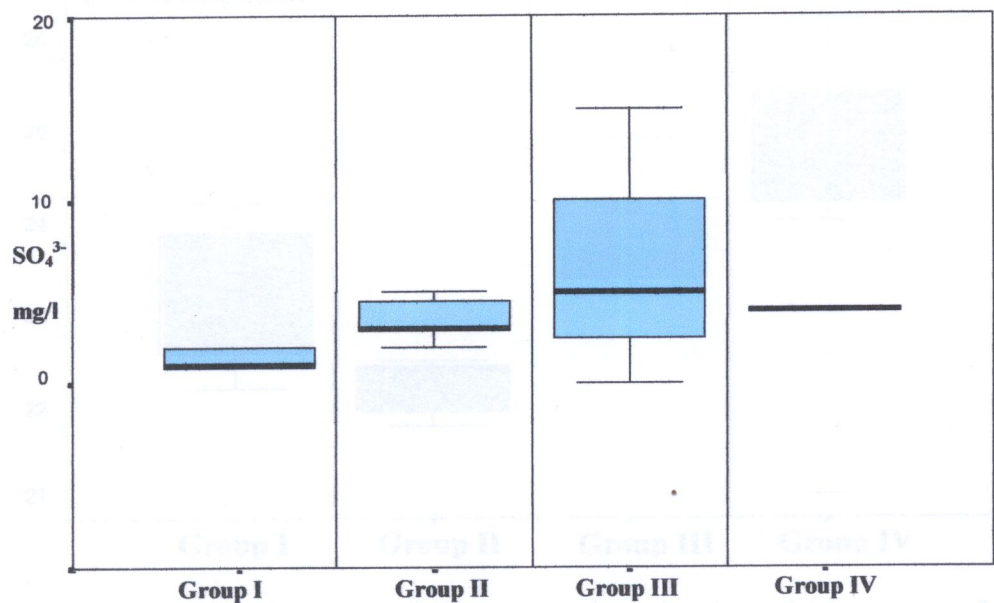


Figure 46. Boxplot of sulfate values in the hot season for each TWINSPAN group.

Figure 46. Boxplot of sulfate values in the hot season for each TWINSPAN group.

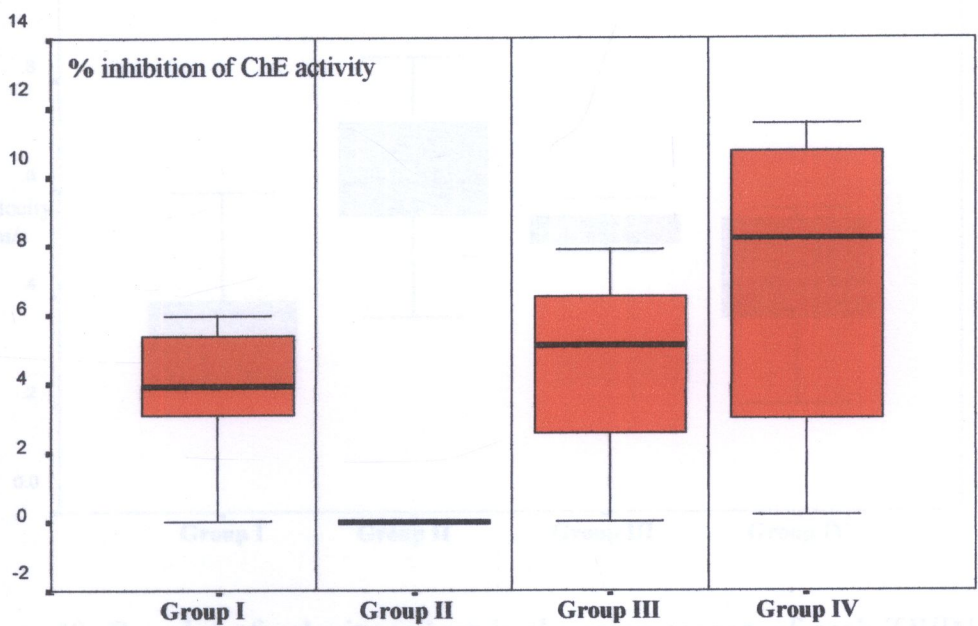


Figure 47. Boxplot of % inhibition of ChE activity from soil in the hot season of each TWINSPAN group.

Figure 47. Boxplot of % inhibition of ChE activity from soil in the hot season of each TWINSPAN group.

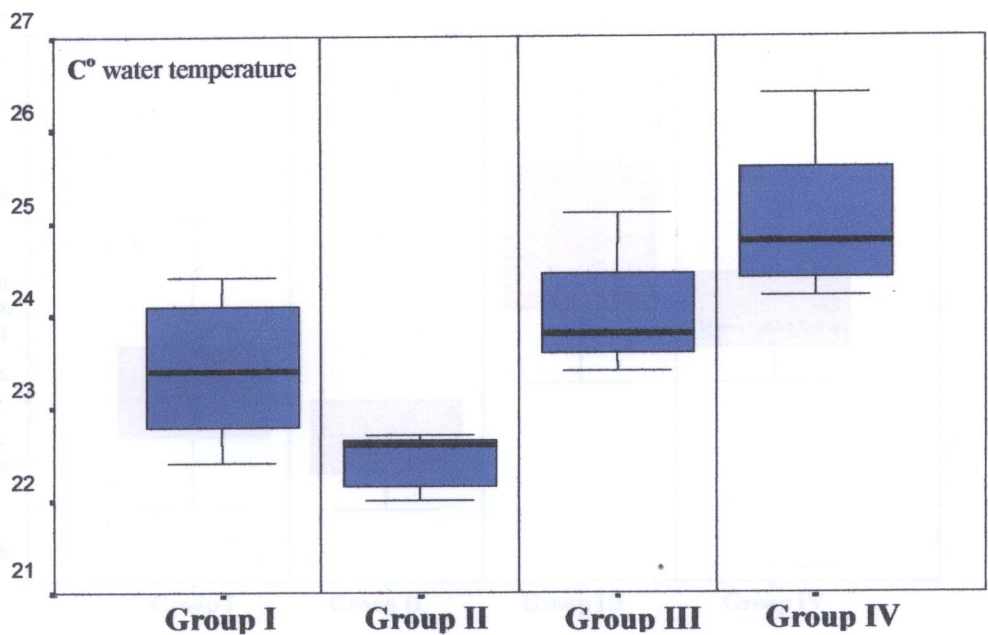


Figure 48. Boxplot of water temperature values in the rainy season of each TWINSpan group.

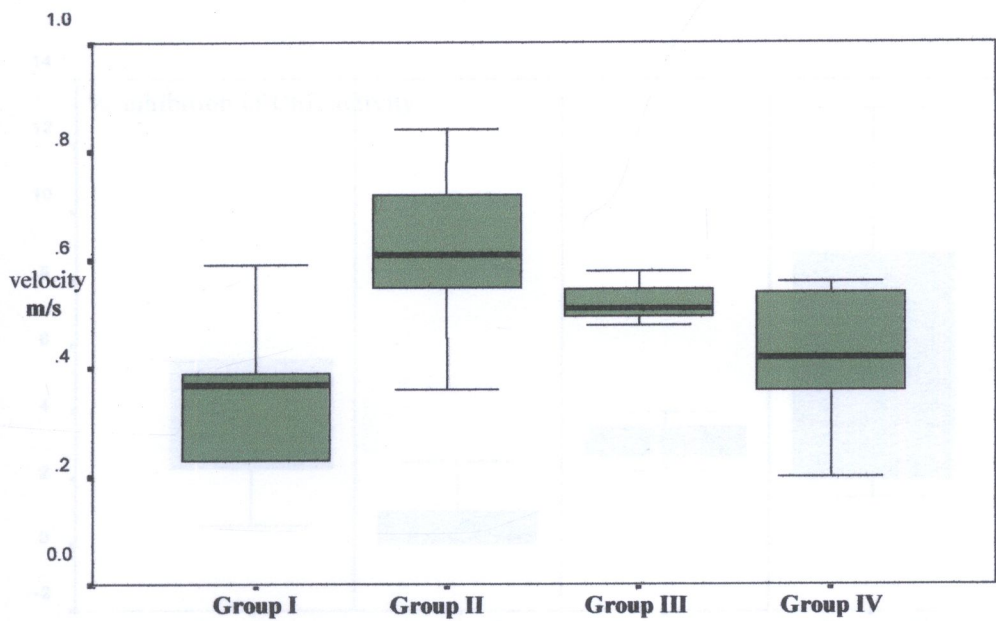


Figure 49. Boxplot of velocity values in the rainy season of each TWINSpan group.

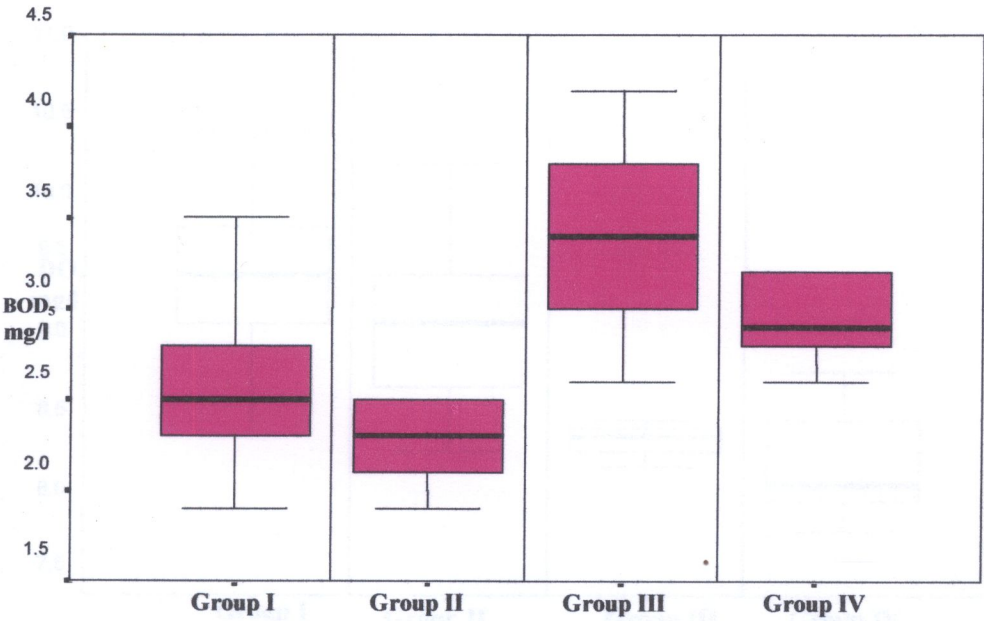


Figure 50. Boxplot of BOD₅ values in the rainy season of each TWINSPAN group.

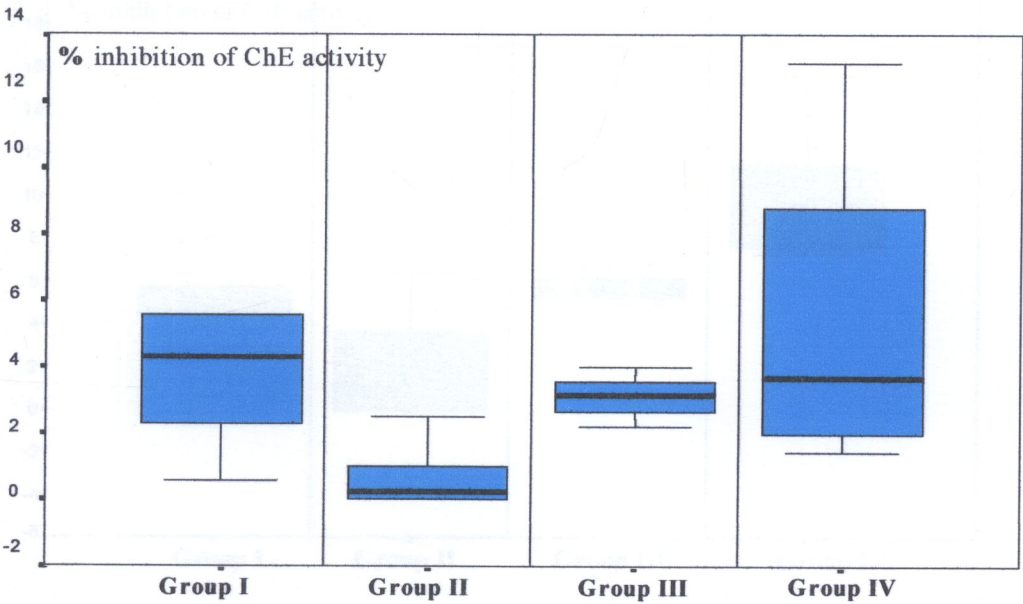


Figure 51. Boxplot of % inhibition of ChE activity from soil in the rainy season of each TWINSPAN group.

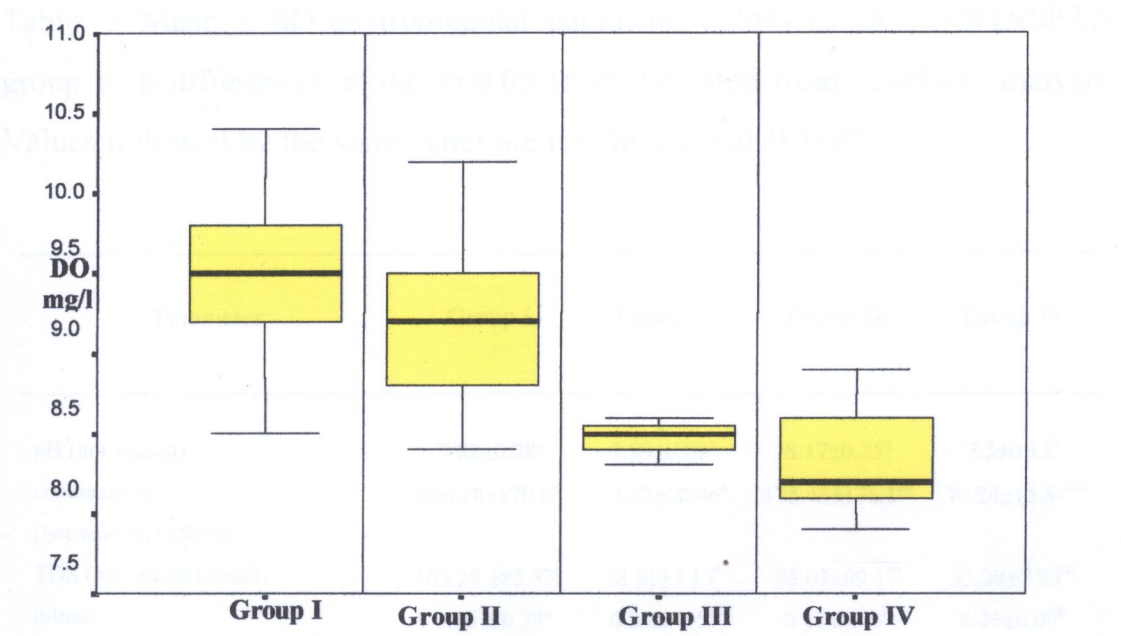


Figure 52. Boxplot of dissolved oxygen in the cool season of each TWINSpan group.

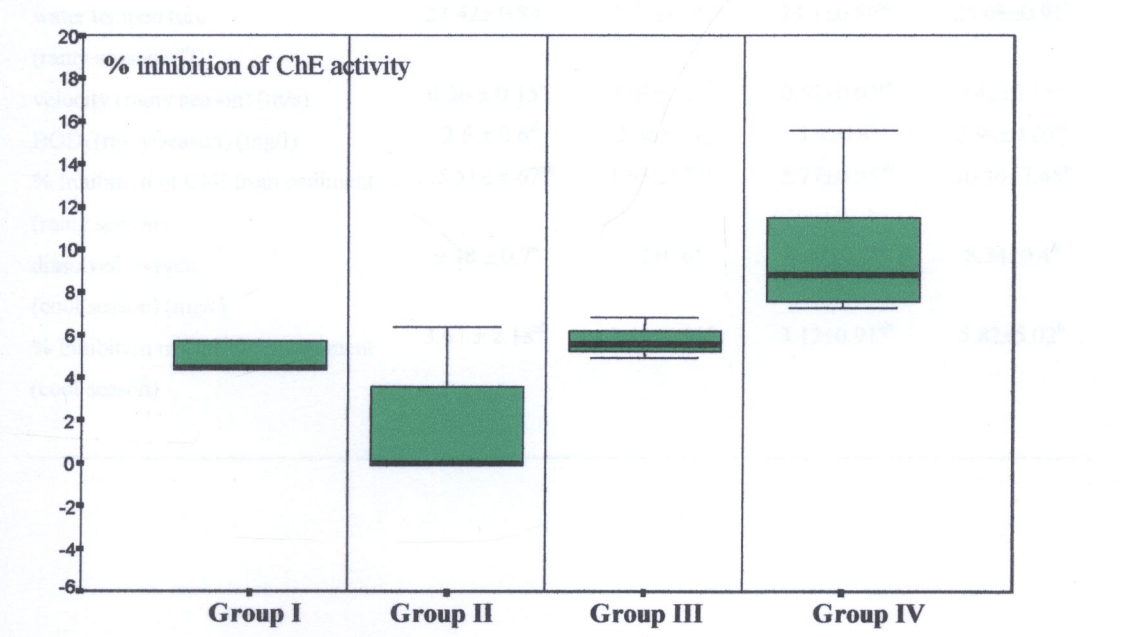


Figure 53. Boxplot of % Inhibition of ChE activity from soil in the cool season of each TWINSpan group.

Table. 3 Mean ± SD environmental parameter values of each TWINSpan group with differences at the $P<0.05$ level indicated from ANOVA analysis. Values followed by the same letter are not different at $P<0.05$

Parameter	Group I	Group II	Group III	Group IV
pH (hot season)	7.82±0.28 ^a	7.89±0.09 ^a	8.17±0.25 ^a	7.5±0.13 ^b
conductivity (hot season) (µS/cm)	206.18±170.6 ^a	57.67±10.46 ^b	175.43±176.1 ^{ab}	70.24±15.81 ^{ab}
TDS (hot season) (mg/l)	103.28 ±85.57 ^a	28.81±5.13 ^b	88.03±89.1 ^{ab}	35.28±7.95 ^{ab}
nitrate (NH ₃ -N, hot season)(mg/l)	0.56 ± 0.35 ^a	0.24±0.05 ^b	0.17±0.06 ^b	0.26±0.09 ^b
sulfate (SO ₄ , hot season)(mg/l)	1.4 ± 0.55 ^a	4±2 ^{ab}	6.67±7.64 ^{ab}	5.8±4.02 ^b
% Inhibition of ChE from sediment (hot season)	3.68 ±2.35 ^{ab}	0.85±2.26 ^a	4.34±4 ^{ab}	6.74±4.95 ^b
water temperature (rainy season) (°C)	23.42± 0.84 ^a	22.51±0.49 ^{ac}	24.1±0.89 ^{ab}	25.08±0.91 ^b
velocity (rainy season) (m/s)	0.36 ± 0.15 ^a	7.98±0.16 ^b	0.52±0.05 ^{ab}	0.42±0.15 ^{ab}
BOD ₅ (rainy season) (mg/l)	2.6 ± 0.6 ^{ab}	2.36±0.4 ^a	3.4±0.8 ^b	2.94±0.26 ^{ab}
% Inhibition of ChE from sediment (rainy season)	5.51± 4.67 ^{ab}	1.93±2.75 ^a	5.77±0.95 ^{ab}	10.16±3.45 ^b
dissolved oxygen (cool season) (mg/l)	9.48 ± 0.7 ^a	9.2±0.6 ^a	8.47±0.2 ^{ab}	8.34±0.4 ^b
% Inhibition of ChE from sediment (cool season)	3.67 ± 2.18 ^{ab}	0.69±0.93 ^a	3.12±0.91 ^{ab}	5.82±5.02 ^b

4.2.2 Multivariate analysis of caddisflies community: HMDS-Ordination

HMDS ordination was used to ordinate the sites using the annual quantitative caddisfly data. The three dimensional solution was selected to arrange the sampling sites and caddisfly associations. The 20 study sites were plotted in ordination space on axes II and III (which gave the best visual separation of sites), and grouped into 4 sets as determined by the TWINSpan analysis. A three dimensional solution gave a satisfactorily low level of stress (0.138). There were 28 caddisfly species significantly correlated with the ordination as plotted in the same ordination space. Ten of these were typical of the control and moderately impacted sites including *P. barata* (Psybar), *H. arcturus* (Hydarc), *H. truncatus* (Hydtru), *H. serubabel* (Hydser), *H. cerva* (Hydcer), *C. joliviti* (Chejol), *C. cocles* (Checoc), *C. chryseis* (Chechr), *U. maliwan* (Ugamal) and *C. pipake* (Chipip). Eighteen significant species were associated with the impacted sites including *M. sumatrana* (Marsum), *M. floridum* (Marflo), *O. tripunctata* (Oectri), *P. mithila* (Psymit), *H. clitumnus* (Hydcli), *C. globosa* (Cheglo), *C. charites* (Checha), *C. chrysothemis* (Checho), *C. cognita* (Checog), *P. flavata* (Potfla), *P. baenzigeri* (Potbae), *M. prichapanyai* (Maepri), *E. volovicus* (Ecnvol), *G. uniformis* (Goeuni), *A. halong* (Agahal), *E. puro* (Ecnpur), *S. argentiguttatus* (Setarg), and *G. redsat* (Goered) (Figure 54).

The ten significant parameters from the ANOVA analysis were plotted in the ordination space. The result showed the vectors that related strongly to Groups I and III were BOD₅, sulfate, pH and water temperature; to Group II were dissolved oxygen and velocity; and to Group IV were water temperature, nitrate-nitrogen, % inhibition of ChE activity in sediment, conductivity, TDS and dissolved oxygen (Figure 55)

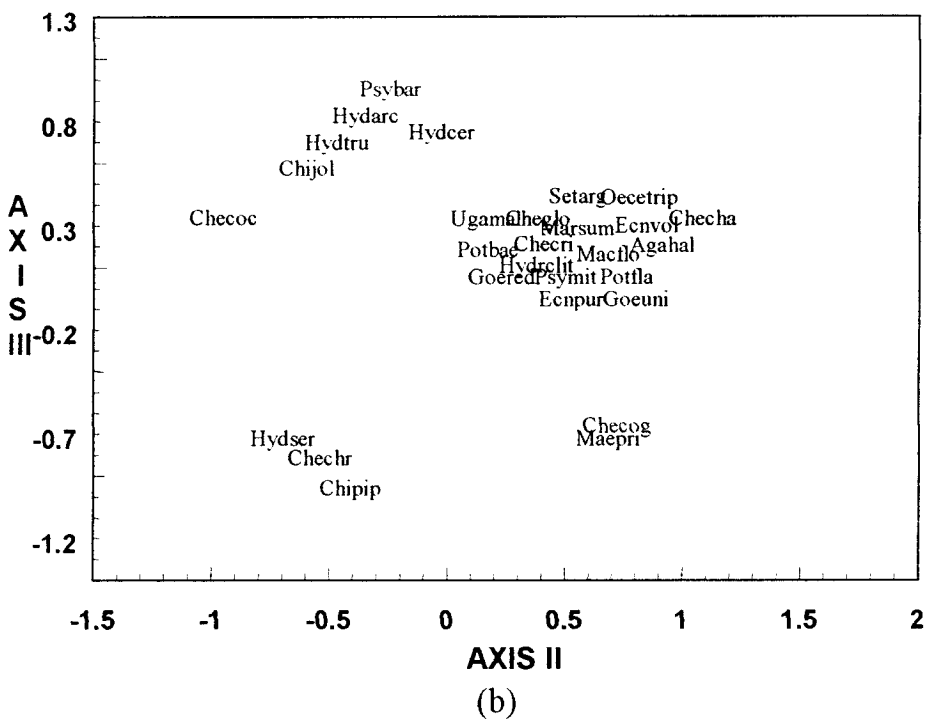
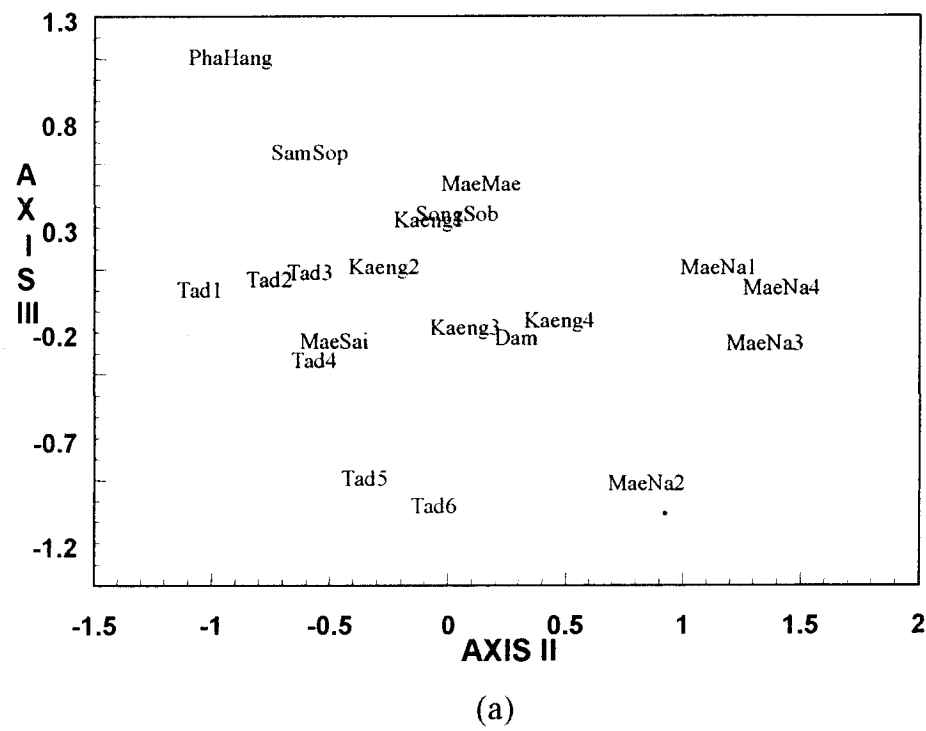


Figure 54. Ordination of study sites based on caddisflies species data (a). Caddisflies species significantly correlated with the ordination were plotted as vectors in the same ordination space (b). Stress = 0.138.

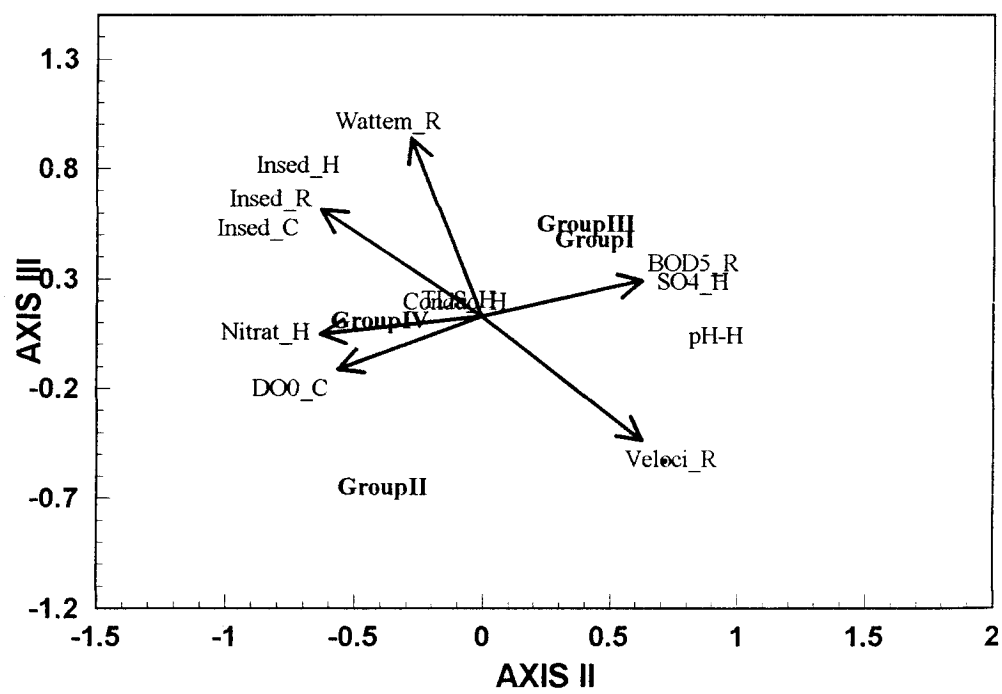


Figure 55. Relationships between the TWINSpan groups and their significantly correlated environmental parameters. Stress = 0.002

4.2.3 Relationship of environmental parameters to land management

Consideration of the orientation of vectors of various caddisflies species, environmental parameters, and the study sites within the ordination suggests relationships between certain taxa and impacted sites. *P. barata*, *H. arcturus*, *H. truncatus*, *H. cerva*, *C. joliviti*, *C. cocles* and *U. maliwan* were positioned opposite to the vector of % inhibition of ChE activity from sediment in all seasons and nitrate-nitrogen in the hot season, indicating these taxa are the most sensitive caddisflies to pesticides from agricultural and organic pollution. Conversely, *M. sumatrana*, *M. floridum*, *O. tripunctata*, *P. mithila*, *C. globosa*, *P. flavata*, *M. prichapanyai*, *E. volovicus*, *C. charites*, *G. uniformis*, *A. halong*, *C. cognita*, *E. puro*, *C. chrysothemis*, *H. clitumnus*, *S. argentiguttatus*, *P. baenzigeri*, and *G. redsat* were positioned in the direction of the vector of % inhibition of ChE activity in sediment in all seasons and nitrate-nitrogen, indicating they are relatively tolerant species (Figures 54&55).

In addition, Pearson product moment correlations between the significant caddisflies species counts and values of environmental parameters at the same site were performed. *C. chrysothemis* was positively correlated with pH, whereas *C. globosa* and *M. floridum* were negatively correlated with pH. *C. pipake*, *C. chrysothemis* and *H. serubabel* were positively correlated with conductivity and TDS. *C. cocles* was positively correlated with nitrate nitrogen and *H. serubabel* was negatively correlated with sulfate.

A. halong, *C. charites*, *O. tripunctata*, *M. prichapanyai*, *E. puro*, *C. cognita*, *M. floridum* were positively correlated with % inhibition of ChE from sediment. *E. puro*, *C. globosa*, *M. floridum*, *P. flavata*, *M. sumatrana* were negatively correlated with dissolved oxygen. Inversely, *H. cerva* and *C. joliviti* had negative correlation to % inhibition of ChE from sediment (Table 4).

Table 4. Pearson correlation values and significance levels between caddisflies species and environmental parameters. Only significant values are reported. * P<0.05, ** P<0.01

	pH (hot season)	Conductivity (hot season)	TDS (hot season)	Nitrate (hot season)	SO ₄ (hot season)
<i>A. halong</i>					
<i>C. joliviti</i>					
<i>C. pipake</i>		0.936**	0.936**		
<i>M. prichapanyai</i>					
<i>E. puro</i>					
<i>E. volovicus</i>					
<i>C. charites</i>					
<i>C. chrysothemis</i>	0.542*	0.931**	0.931**		
<i>C. cocles</i>				0.658*	
<i>C. cognita</i>					
<i>C. globosa</i>	-0.474*				
<i>H. serubabel</i>		0.739**	0.793**		-0.480*
<i>H. truncatus</i>					
<i>H. arcturus</i>					
<i>H. cerva</i>					
<i>M. floridum</i>	-0.471*				
<i>P. flavata</i>					
<i>G. uniformis</i>					
<i>O. tripunctata</i>					
<i>S. argentiguttatus</i>					
<i>M. sumatrana</i>					

Table 4 (continued)

	Insed (hot)	Water temperature (rainy season)	Insed (rainy season)	DO (cool season)	Insed (cool season)
<i>A. halong</i>	0.579**				
<i>C. joliviti</i>		-0.487*	-0.456*		
<i>C. pipake</i>					
<i>M. prichapanyai</i>		0.539*	0.563**		
<i>E. puro</i>		0.459*	0.469*	-0.483*	
<i>E. volovicus</i>		0.584**			
<i>C. charites</i>	0.504*	0.608**			
<i>C. chrysothemis</i>					
<i>C. cocles</i>		-0.486*			
<i>C. cognita</i>		0.473*	0.638**		
<i>C. globosa</i>		0.658**		-0.486*	
<i>H. serubabel</i>					
<i>H. truncatus</i>		-0.479*			
<i>H. arcturus</i>		-0.485*			
<i>H. cerva</i>	-0.494*	-0.564**			
<i>M. floridum</i>		-0.638**	0.570**	-0.547*	
<i>P. flavata</i>		0.643**		-0.495*	
<i>G. uniformis</i>		0.644**			
<i>O. tripunctata</i>	0.695**	0.516*			0.594**
<i>S. argentiguttatus</i>		0.583**			
<i>M. sumatrana</i>		0.659**		-0.597*	

Abbreviations: Insed= % inhibition ChE from sediment, DO=Dissolved oxygen.

4.3 Observations on the morphology and abnormalities in the tegumental surface of *Stenopsyche siamensis* larvae using scanning electron microscopy

A study of the morphology and a comparison of *Stenopsyche siamensis* larvae from two streams (control and impacted sites) by using SEM was conducted. The fine structure of the insect exoskeleton and the chronic effects of agricultural chemicals to the tegument of this insect were investigated.

4.3.1 General morphology of *Stenopsyche siamensis* larvae

The larvae of stenopsychid are large and slender. The head is black and the abdomen is dark brown. The head capsule is elongated and tapers slightly anteriorly (Figure 56). The head capsule is covered with a scale-like cuticle (Figure 57). The frontoclypeus is about two-thirds the length of the head capsule (Figure 58). The membranous anteclypeus has four lobes. The sclerotised labrum has two pairs of a bristles and a dense row of comb-like bristles along the antero-ventral margin (Figures 59-61). The sclerotised labrum is covered with ridge-liked cuticle (Figure 62). The oblong mentum has labial palps with sensilla and a silk gland opening at the anterior. Between the labial palps, there is a tuft of bristles and under the silk gland opening, a couple of hairs is found. The palpiger is wide at the base and the maxillary palps have four segments. At the end of the fourth-maxillary palps, sensilla were found (Figures 63-64). The prothorax is shorter and smaller than the mesothorax and metathorax, and covered entirely by pronotal sclerite with a single row of bristles along the anterior margin (Figure 56). The mesothorax and metathorax are membranous, covered with undulate-like cuticle (Figures 65-66). The forelegs are slightly longer than each of the other two legs. The forecoxae possess two stout processes on the anterior margin and the apical process is

longer than the basal process (Figures 67-68). The distal end of the fore, middle, and hind femora have a ventral row of fringed setae. The fore tarsal claw has basal setae with hairs; the middle and hind tarsal claw bear basal setae without hairs (Figures 69-71). The leg cuticle is rugose-like (Figure 72). The abdominal segments are membranous and covered with a ridge-like cuticle (Figures 73-74). The anal proleg is short and its outer surface is covered by sclerite. The anal claw is bent and sharply pointed at the apex (Figure 75). There are five anal papillae protruding from the anal papillae pore (Figure 76). The end of the anal proleg has a pore situated near the base of each anal claw (Figure 77).

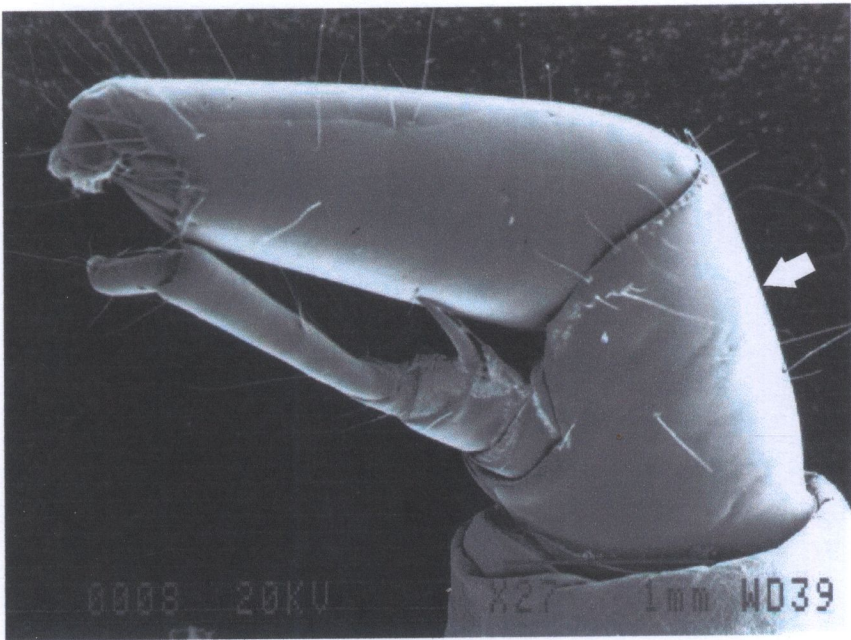


Figure 56. Head capsule and prothorax (⇨) of *Stenopsyche siamensis*

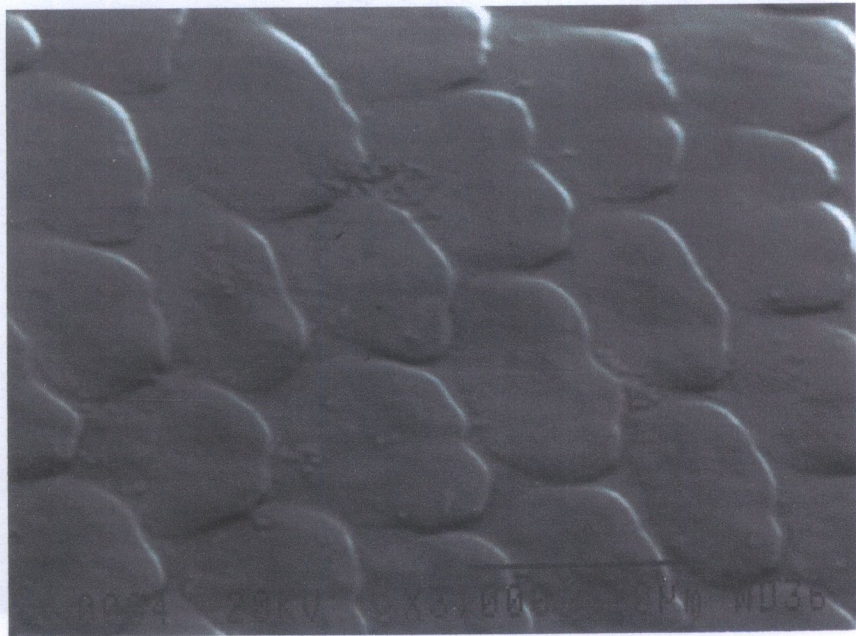


Figure 57. Scale-like cuticle of *Stenopsyche siamensis* (©)

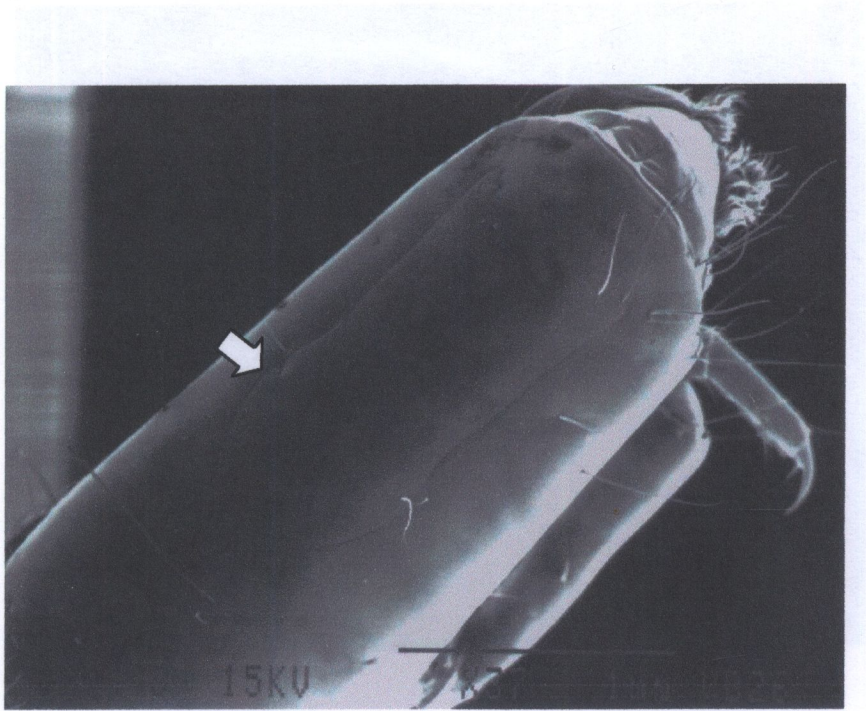
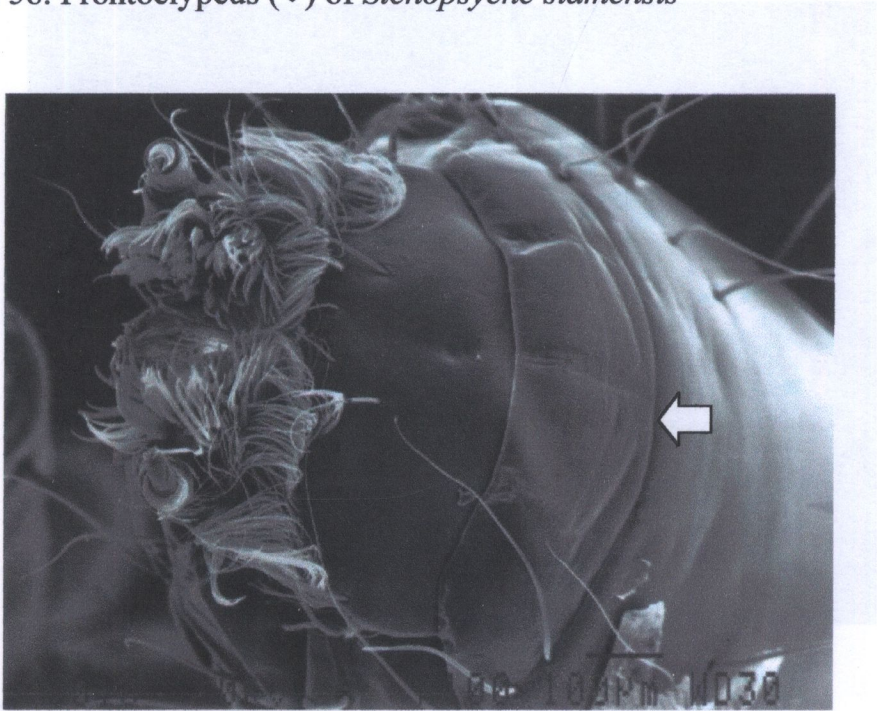


Figure 60. Sclerotised labrum (⇨) and dense row of comb-like bristles (⇦).

Figure 58. Frontoclypeus (⇨) of *Stenopsyche siamensis*



Figure

Figure 59. Membranous anteclypeus (⇨)

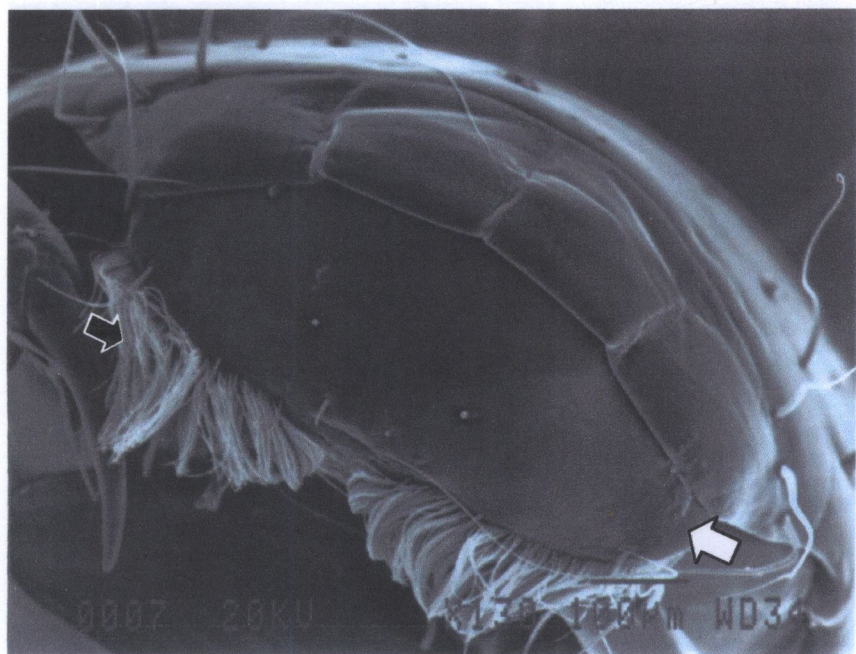


Figure 60. Sclerotised labrum (⇨) and dense row of comb-like bristles (⇨).

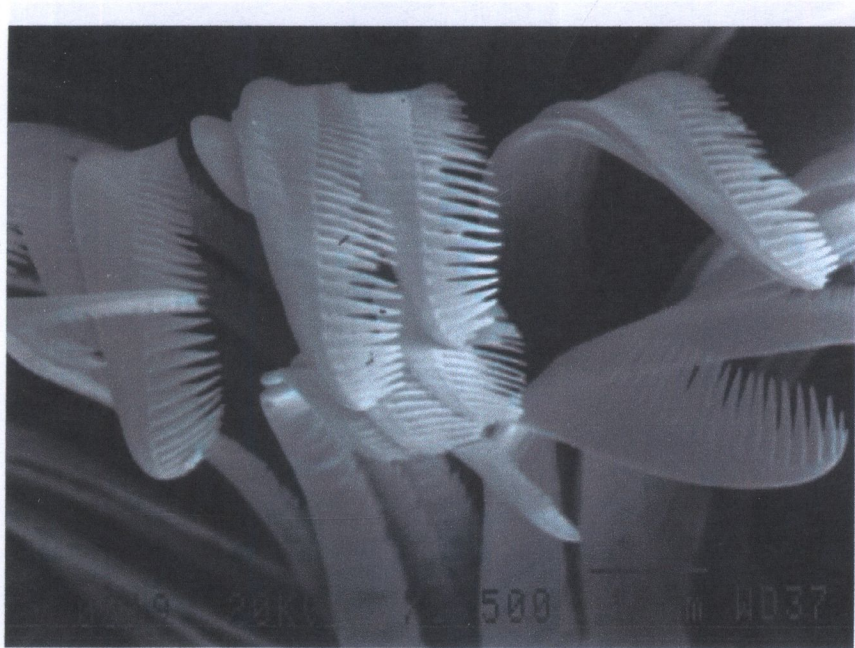


Figure 61. Comb-like bristles (Δ), labial palps with sensilla (⇨), a silk gland opening (•), and a tuft of bristles (Δ).

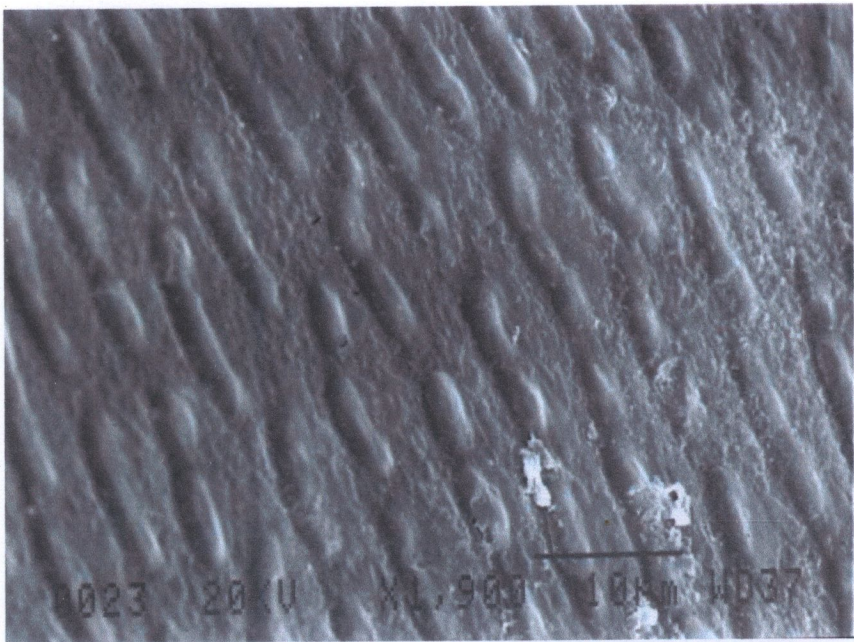


Figure 62. Ridge-liked cuticle



Figure 63. Oblong mentum (▲), labial palps with sensilla (⇔), a silk gland opening (➡), and a tuft of bristles (Δ).



Figure 64. Palpiger (Δ), maxillary palps with sensilla (⇨)

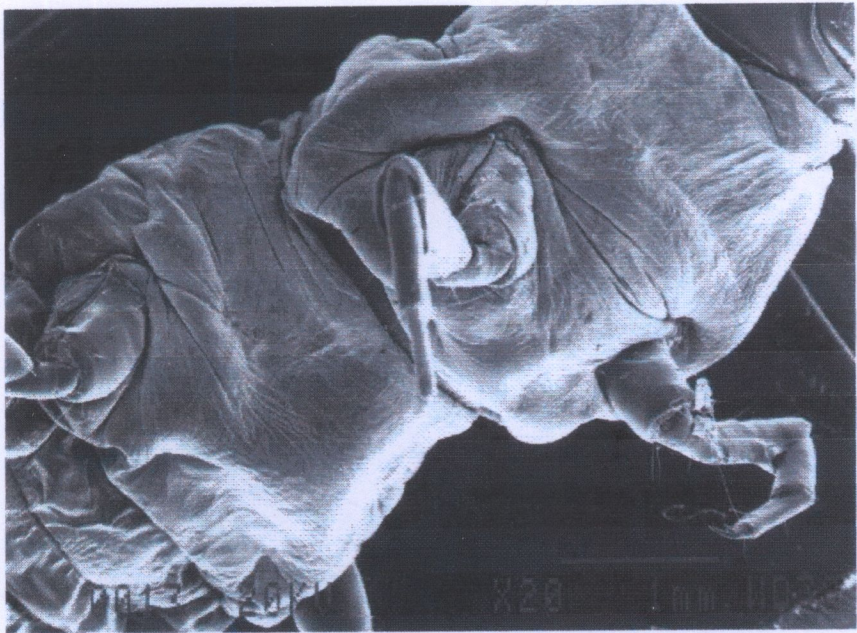


Figure 65. Mesothorax and metathorax

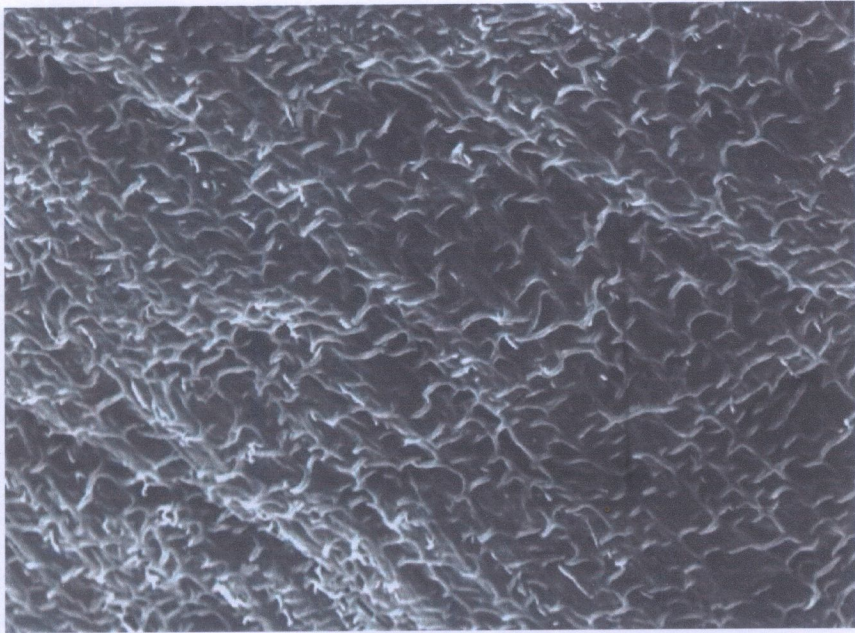


Figure 66. Pericoxae possess typical process (A) and basal process (A')

Figure 66. Undulate-like cuticle

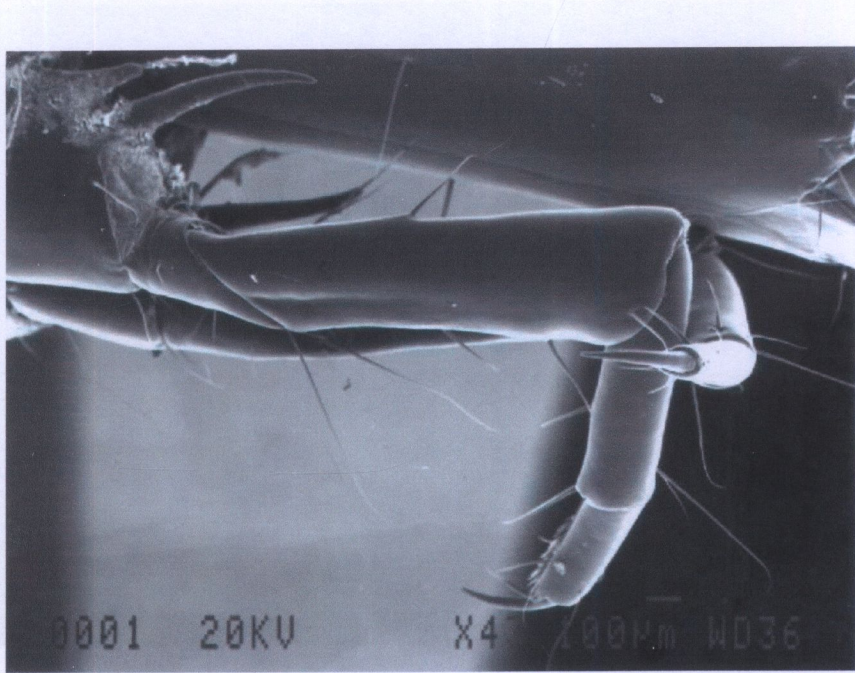


Figure 67. Ventral view of fringed setae

Figure 67. Forelegs

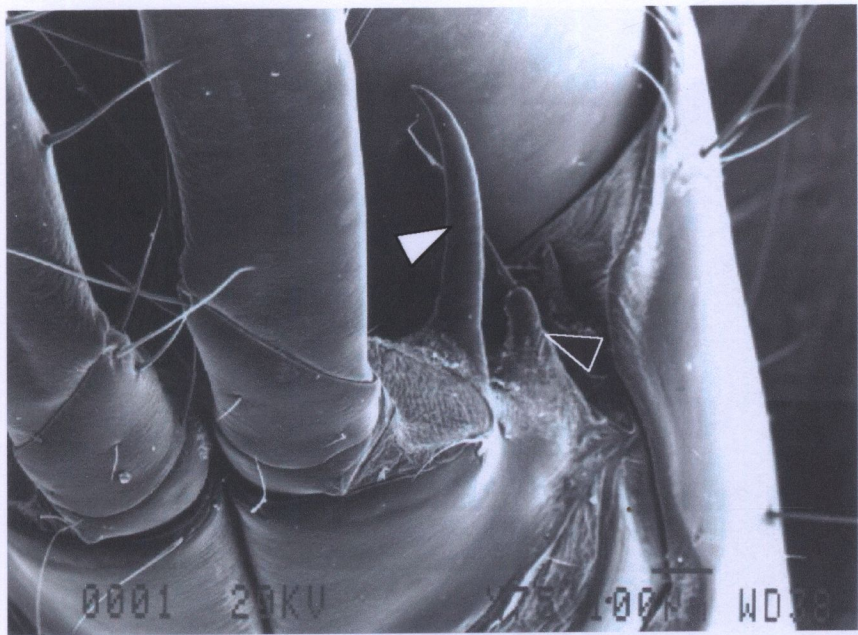


Figure 68. Forecoxae possess (apical process (Δ) and basal process (▲))

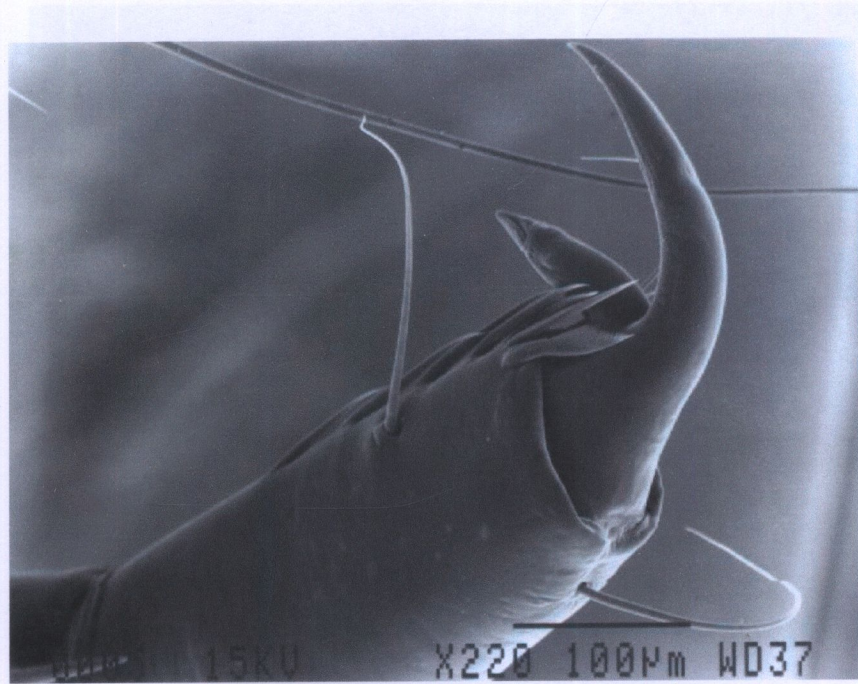


Figure 71. Middle and hind ventral claws have basal setae without hair

Figure 69: Ventral row of fringed setae.

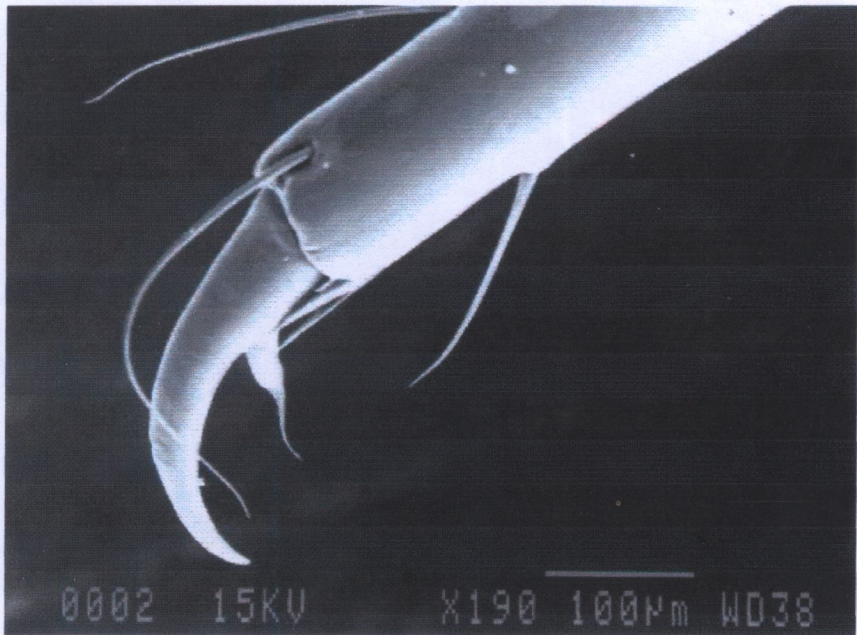


Figure 72. Fore tarsal claw

Figure 70. Fore tarsal claw with basal setae and bristles

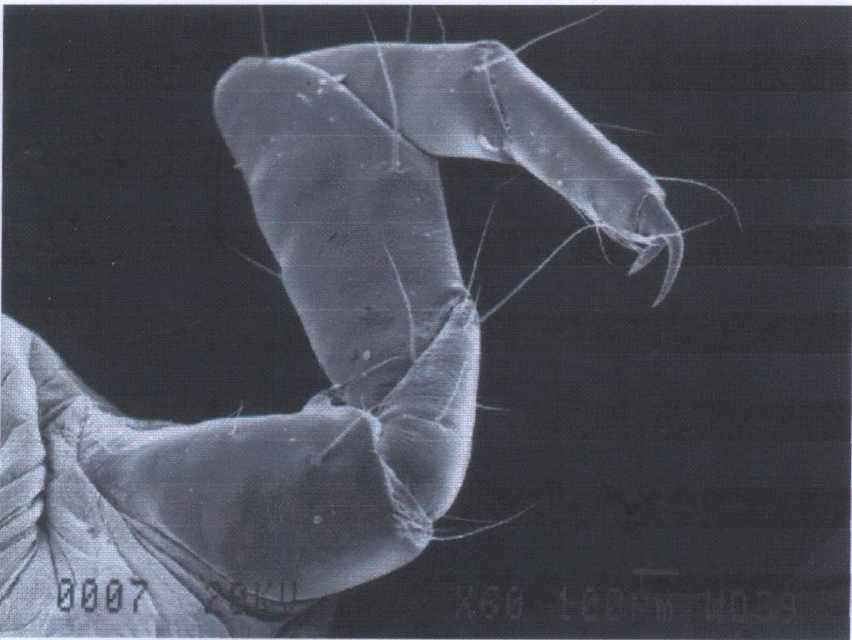


Figure 71. Middle and hind tarsal claws have basal setae without hair

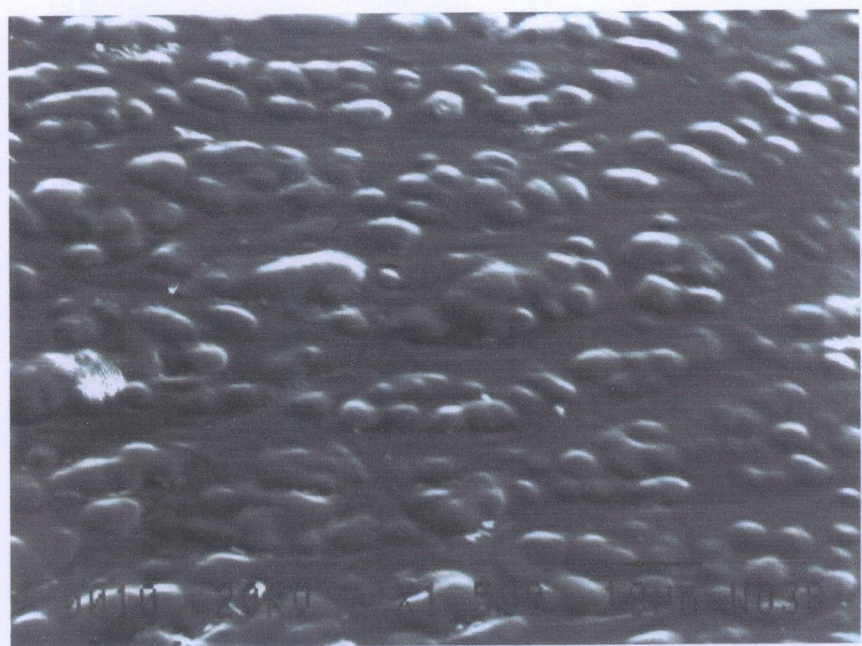


Figure 72. Rugose-like cuticle

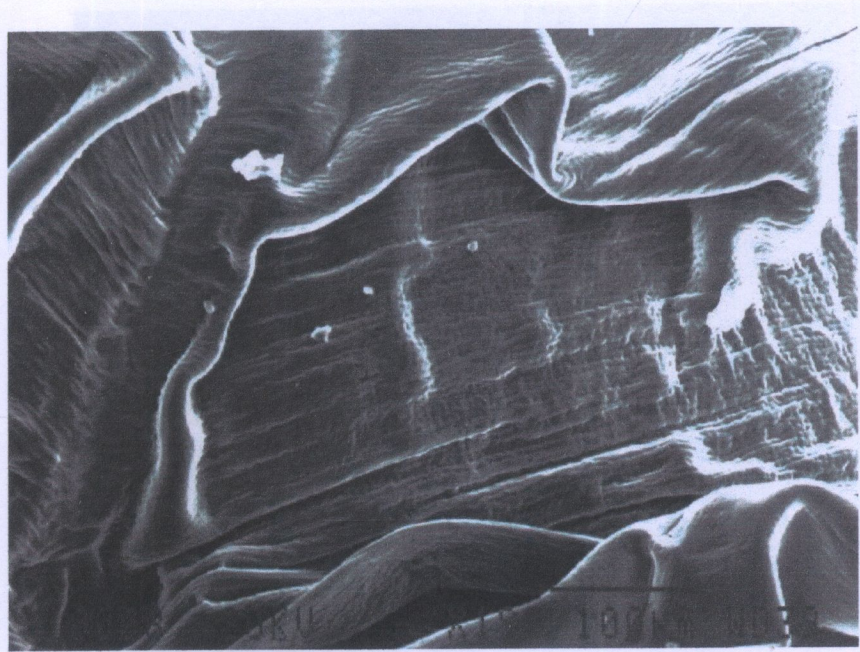


Figure 73. Membranous abdominal segments

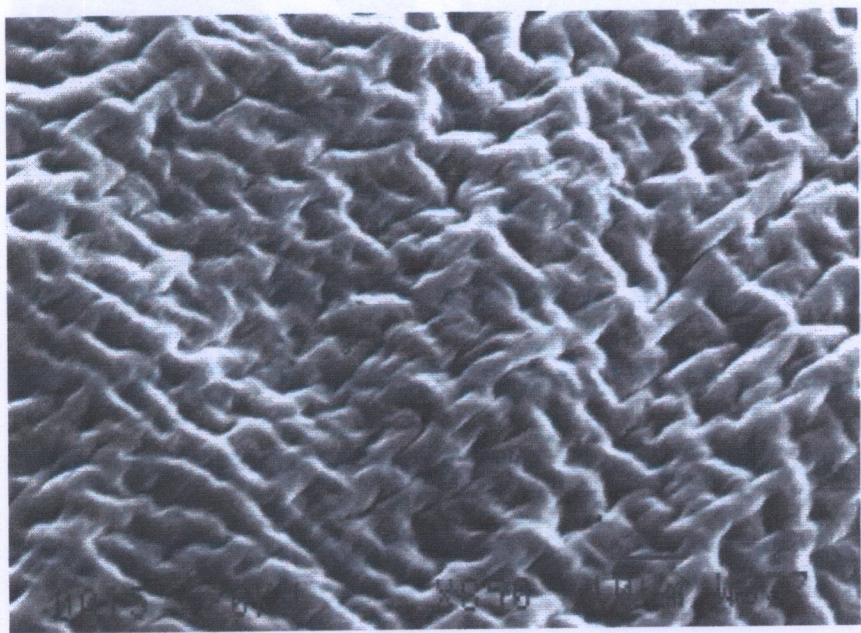


Figure 74. Ridge-like cuticle

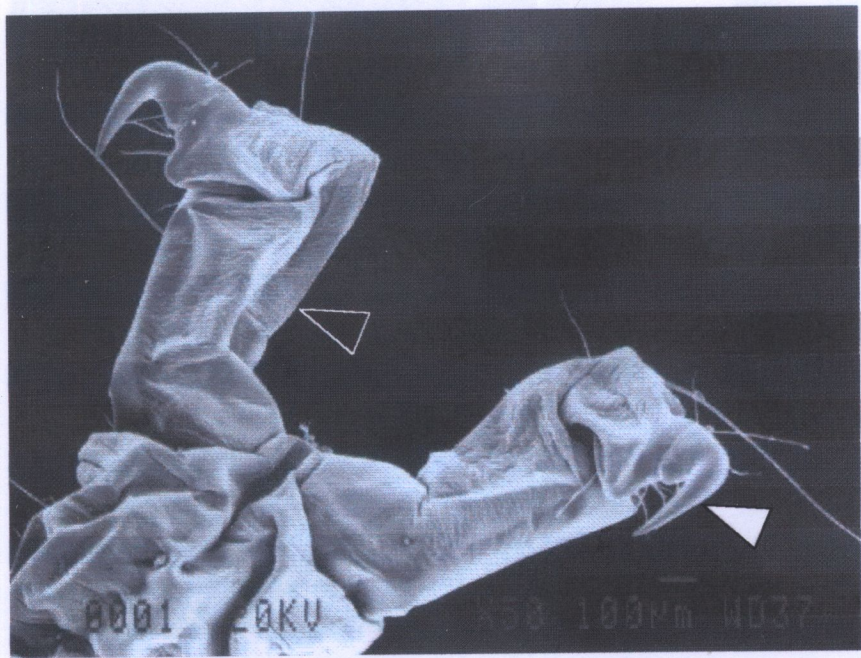


Figure 75. Anal proleg (▲) and anal claw (△)

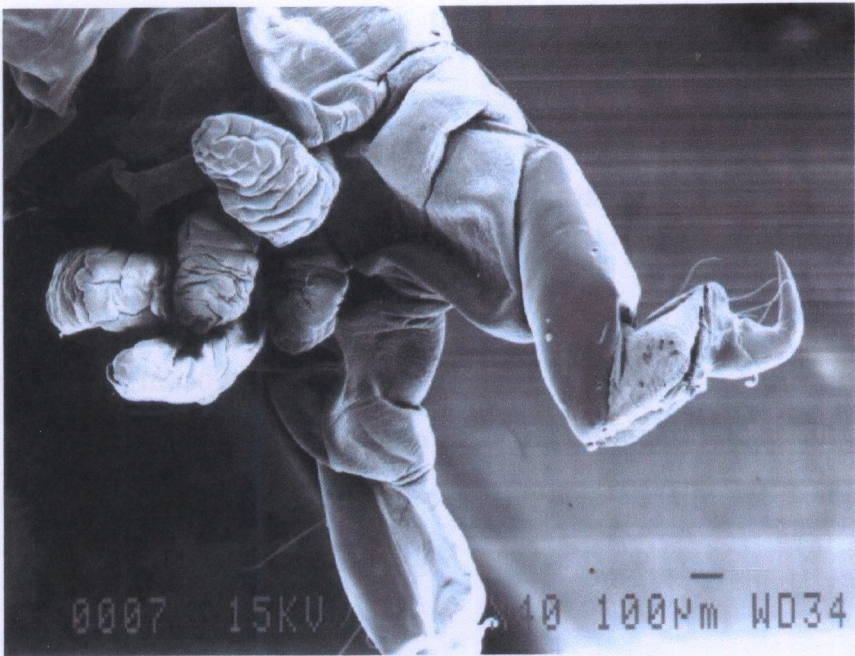


Figure 76. Anal papillae

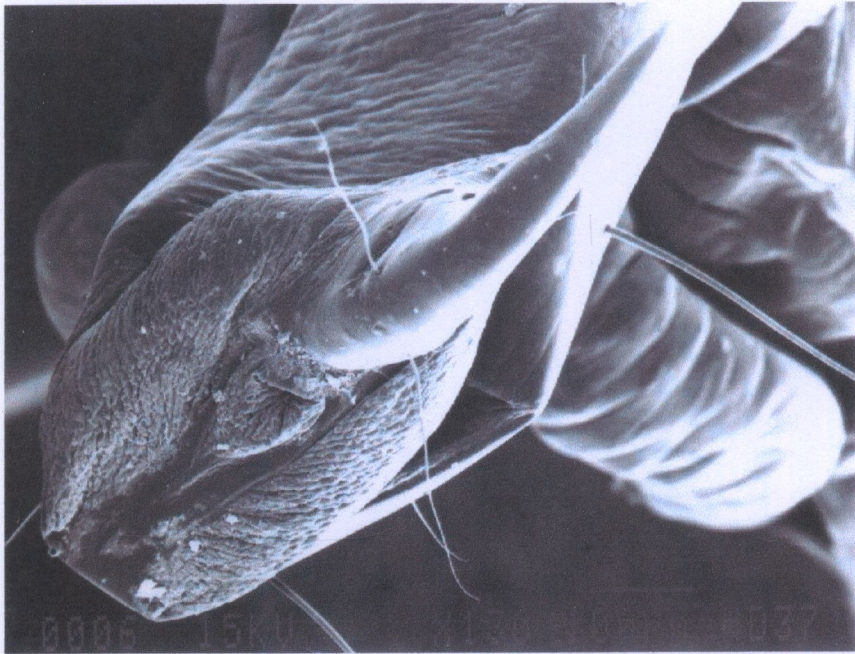
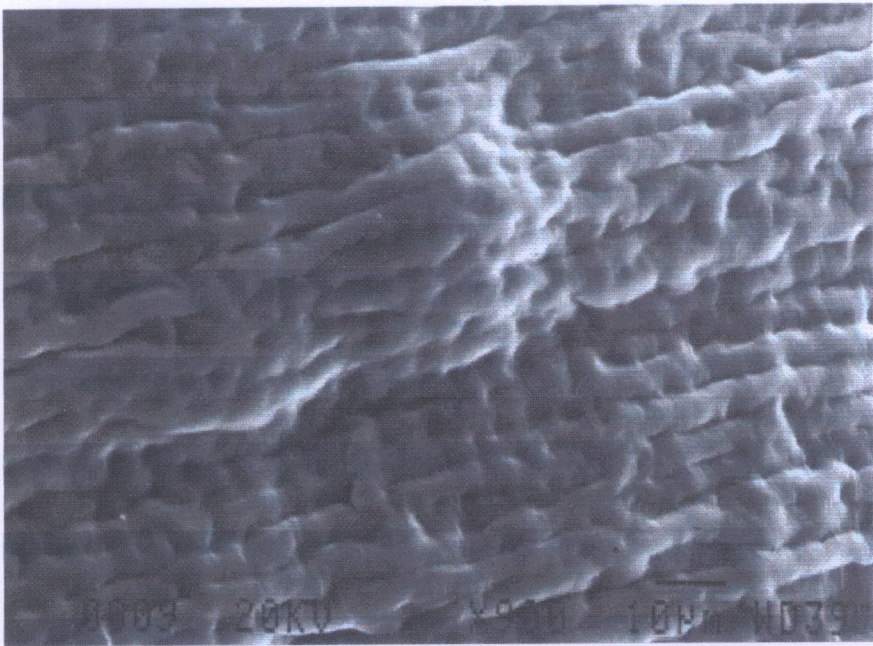


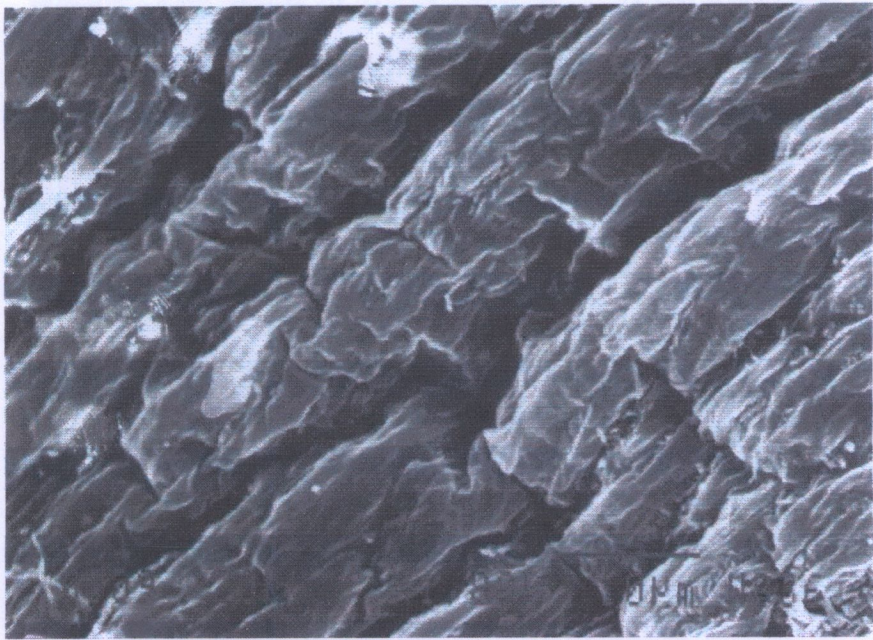
Figure 77. Anal pore

4.3.2 Abnormalities in the tegumental surface of *Stenopsyche siamensis* Larvae

A comparison of SEM micrographs of larval tegument in two different stream sites showed that 60% (n=30) deformities of the tegumental surface of the thorax and abdomen were present in the impacted site. In the impacted site the membranous mesothorax and metathorax teguments were frayed and swollen (Figures 78&79). In addition, the abdominal integument of larvae which lived in the impacted stream site was fragmented (Figures 80&81).

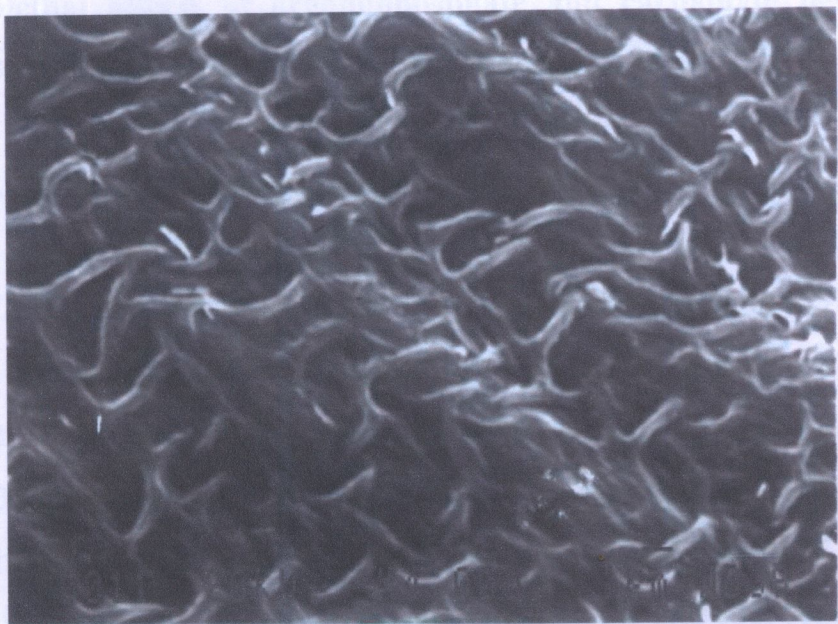


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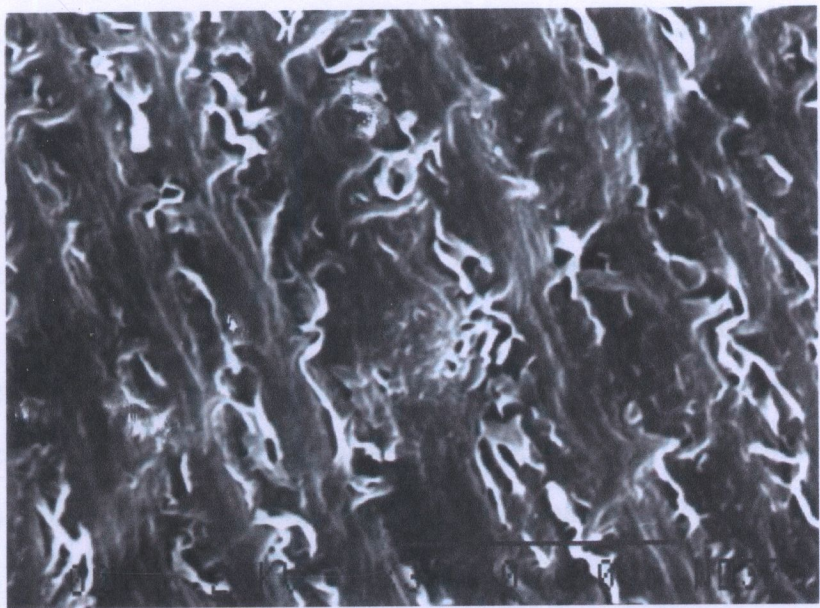


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Figures 78&79. Thoracic tegument of *Stenopsyche siamensis* larvae (78- from the control site, 79- from the impacted site)



80



81

Figures 80&81. Abdominal tegument of *Stenopsyche siamensis* larvae (80- from the control site, 81- from the impacted site)

4.4 Sublethal effects of organophosphate and carbamate pesticides on cholinesterase activity of *Stenopsyche siamensis*

4.4.1 *In vivo stenopsychid ChE activity test*

The *in vivo* test results showed a mean ChE activity of 2.39 ± 0.56 (hot season), 1.26 ± 0.19 (rainy season), and 0.99 ± 0.21 (cool season) $\mu\text{M}/\text{min}/\text{mg}$ in the control site and 0.63 ± 0.36 (hot season), 0.57 ± 0.22 (rainy season), and 0.85 ± 0.38 (cool season) $\mu\text{M}/\text{min}/\text{mg}$ in the impacted site. Mean differences of ChE activity between both sites were significant (*t-test*, $P < 0.01$). Mean ChE activity in the hot season was higher than in the rainy and cool seasons at both sites (Tables 5-7).

4.4.2 *In vitro stenopsychid ChE activity inhibition test*

In vitro stenopsychid ChE inhibition tests were done to confirm the inhibition of ChE activity resulting from organophosphorous and carbamate pesticides. This was also compared to 50% ChE activity inhibition from the control and impacted sites. The results showed that the activity of ChE decreases gradually with increased insecticide concentrations. The concentration at 50% ChE inhibition (IC_{50}) is $9.83 \mu\text{M}$ for carbaryl and $3.32 \mu\text{M}$ for mevinphos in the control site. At the impacted site, 50% ChE inhibition (IC_{50}) is $4.53 \mu\text{M}$ for carbaryl and $0.26 \mu\text{M}$ for the mevinphos. At 50% ChE inhibition, the concentration of mevinphos is lower than carbaryl (Figures 82-85).

Table 5. Cholinesterase activity (μ mole/min/mg of protein) of stenopsychid larva from the control and impacted sites in the hot season.

Larvae Sample	hot season	
	Control site	Impacted site
1	2.24	.1.09
2	2.58	0.65
3	3.33	1.17
4	2.66	0.04
5	1.78	0.15
6	1.89	0.84
7	1.80	1.01
8	2.01	0.74
9	1.97	0.48
10	2.96	0.48
11	2.20	0.34
12	3.23	0.57
mean	2.39	0.63
SD	0.56	0.36

Table 6. Cholinesterase activity ($\mu\text{mole/min/mg}$ of protein) of stenopsychid larva from the control and impacted sites in the rainy season.

Larvae Sample	rainy season	
	control site	impacted site
1	1.38	0.46
2	1.17	0.57
3	1.62	0.80
4	1.55	0.71
5	1.20	0.84
6	1.30	0.71
7	1.41	0.38
8	0.84	0.29
9	1.36	0.40
10	1.11	0.94
11	1.18	0.36
12	1.40	0.40
mean	1.26	0.57
SD	0.19	0.22

Table 7. Cholinesterase activity ($\mu\text{mole/min/mg}$ of protein) of stenopsychid larva from the control and impacted sites in the cool season.

Larvae Sample	cool season	
	Control site	Impacted site
1	1.09	1.13
2	0.92	1.74
3	0.80	0.92
4	0.82	0.90
5	0.98	0.71
6	1.05	1.28
7	0.89	0.52
8	0.76	0.78
9	0.99	0.54
10	0.80	0.42
11	1.45	0.65
12	1.3	0.61
mean	0.99	0.85
SD	0.21	0.38

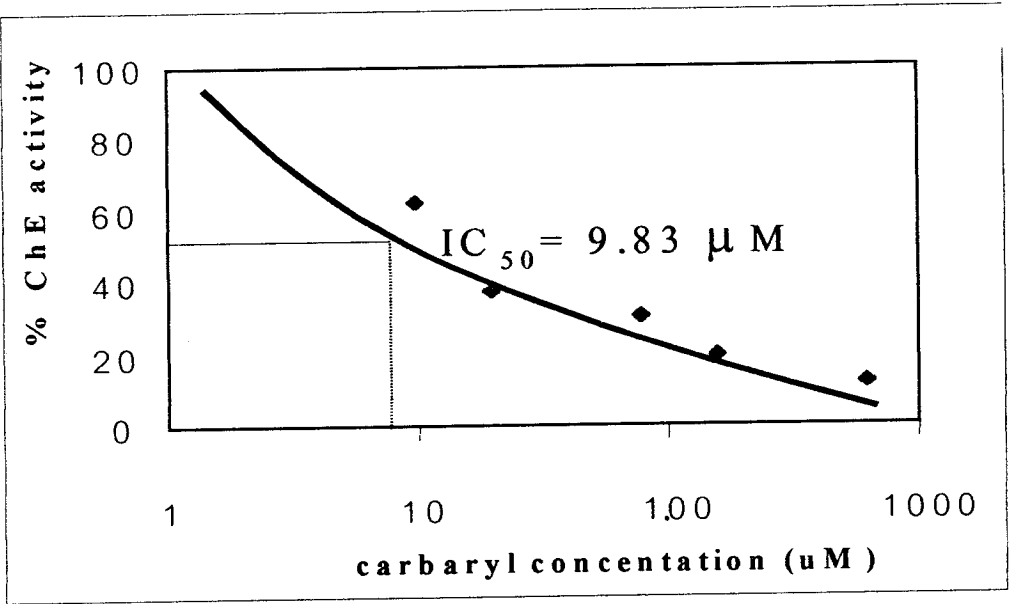


Figure 82. Inhibition test of carbaryl insecticide on stenopsychid ChE activity at the control site.

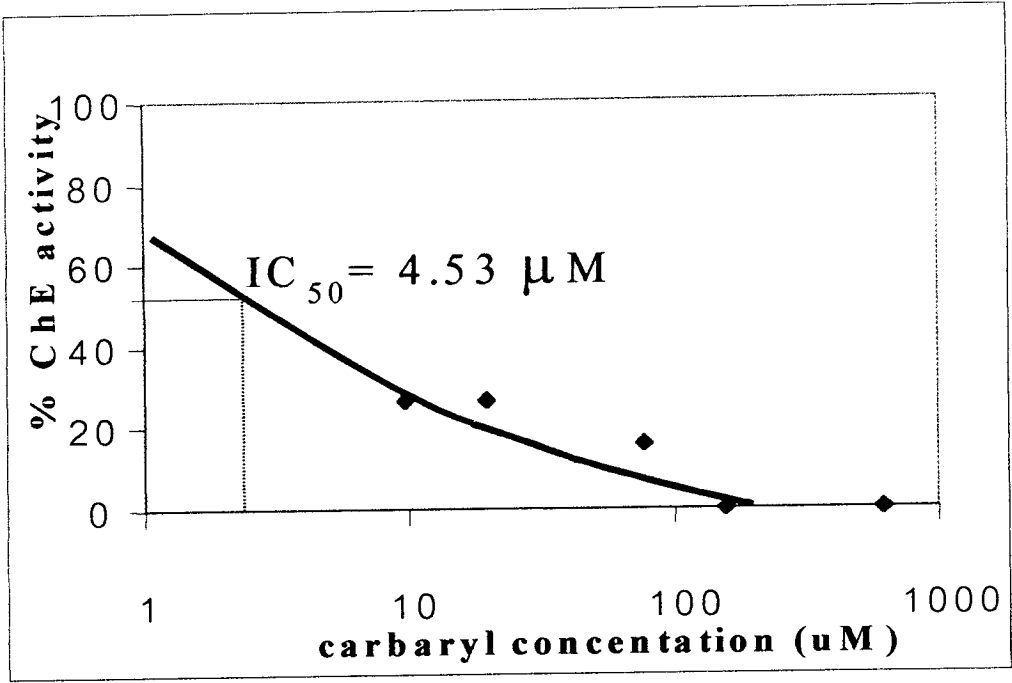


Figure 83. Inhibition test of carbaryl insecticide on stenopsychid ChE activity at the impacted site.

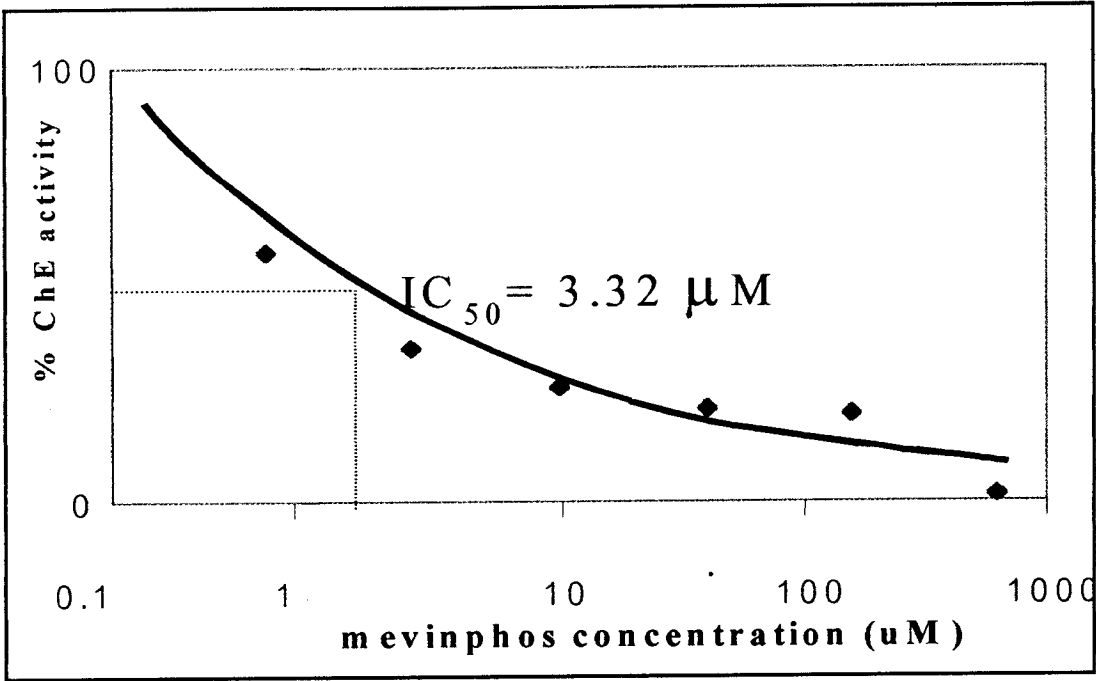


Figure 84. Inhibition test of mevinphos insecticide on stenopsychid ChE activity at the control site.

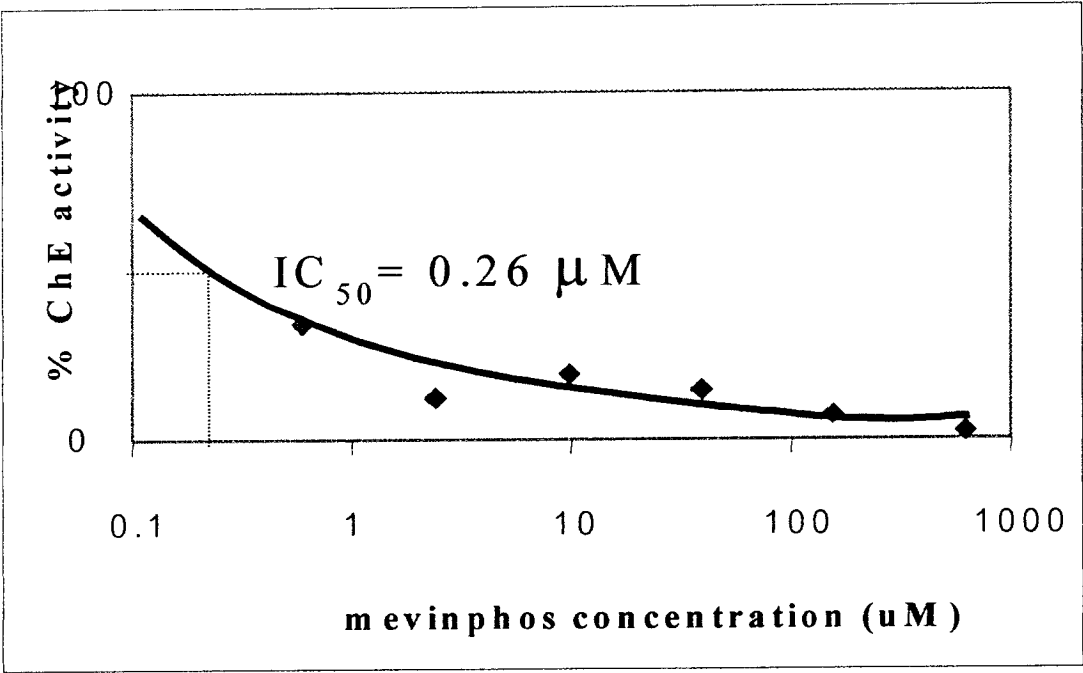


Figure 85. Inhibition test of mevinphos insecticide on stenopsychid ChE activity at the impacted site.

4.5 Life history strategies and impact of land-use patterns on the life cycle of *Stenopsyche siamensis*

4.5.1 Life cycle of *Stenopsyche siamensis*

The results of seasonal light trap collecting (hot season-March, 2000; rainy season-July, 2000; cool season-October, 2000) of adult of *Stenopsyche siamensis* showed a non-seasonal flight period pattern. Adults of *S. siamensis* were found all seasons, peaked in the hot season, and decreased in the cool season (Appendices 4-6).

From monthly samples, a total of 431 stenopsychid larvae were gathered. The head capsule widths were discontinuously distributed from 0.300-1.450 mm (Figure 86). Monthly distribution ratio of each instar is showed in figure 87. Frequency analysis of the larval head capsule width revealed the presence of five instars. The ranges and mean \pm SD of head capsule width of each instar are:

Instar I: HCW = 0.300-0.325 mm, 0.306 ± 0.012 mm (n=4)

Instar II: HCW = 0.375-0.450 mm, 0.412 ± 0.018 mm (n=16)

Instar III: HCW = 0.600-0.725 mm, 0.664 ± 0.032 mm (n=79)

Instar IV: HCW = 0.950-1.100 mm, 1.009 ± 0.033 mm (n=140)

Instar V: HCW = 1.200-1.450 mm, 1.327 ± 0.057 mm (n=192)

The size distribution of head capsule width showed an increasing pattern in a regular geometric progression by a factor of 1.48.

Larvae rearing experiments at the study sites and in the laboratory showed a period of development time of 2 weeks in instar I, 3 weeks in instar II, 3-5 weeks in instar III, 7-8 weeks in instar IV, and 22 - 26 weeks in instar V. The time for pupation was 4-5 weeks and the adults of *S. siamensis* lived for only 1-2 weeks after emerging (Figure 88).

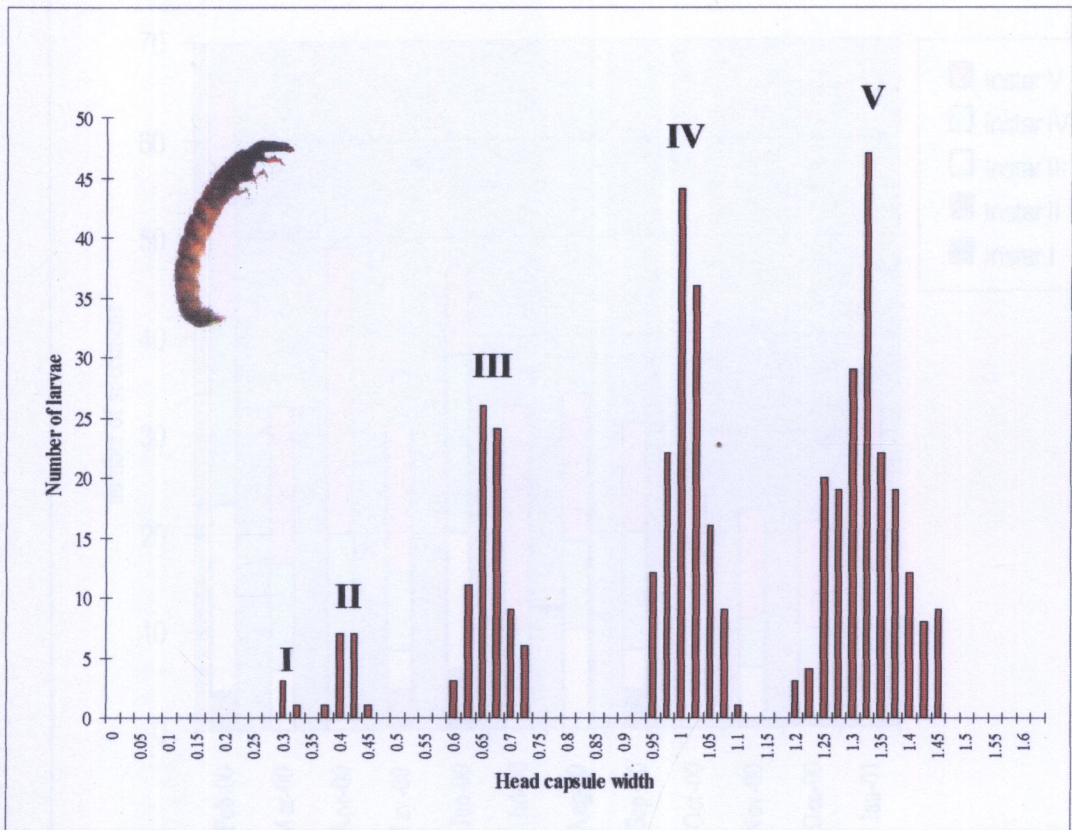


Figure 86. Distribution of head capsule width (mm) of larvae of *Stenopsyche siamensis*.

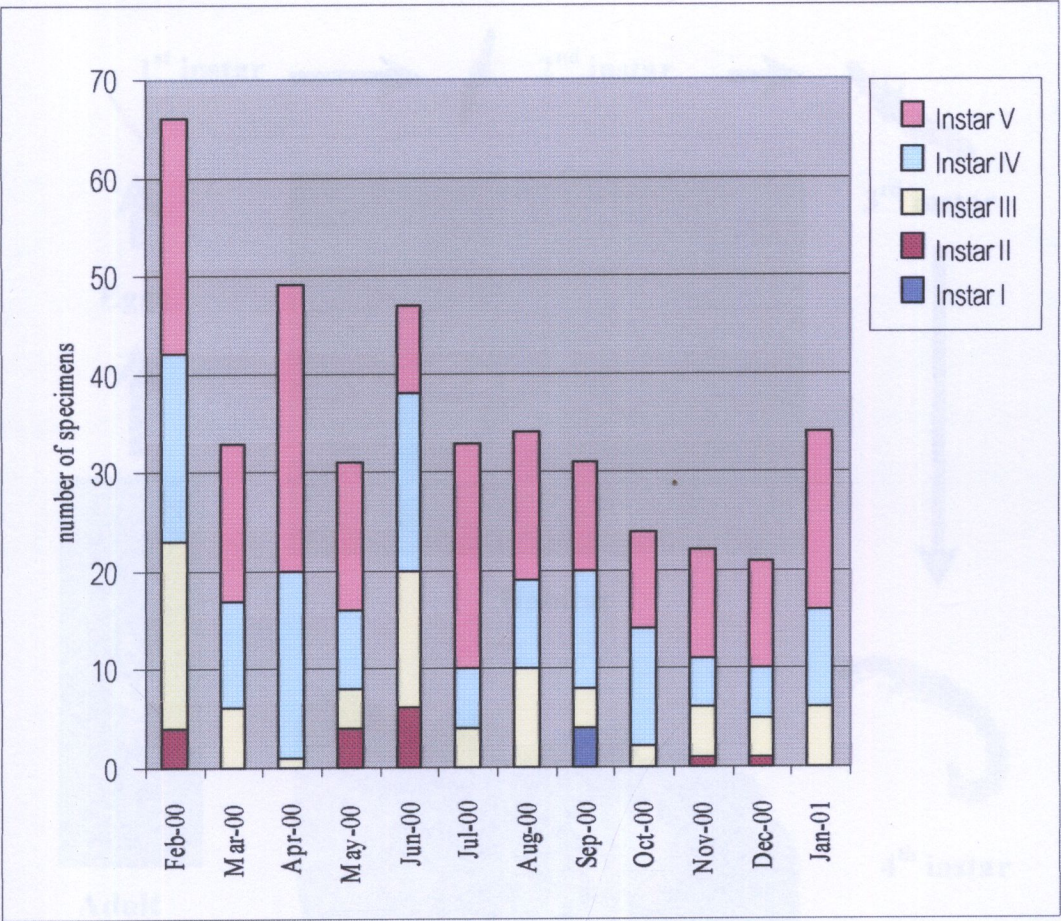


Figure 87. Monthly distribution Ratio of each instar of *Stenopsyche siamensis*

Figure 88. Life cycle of *Stenopsyche siamensis*

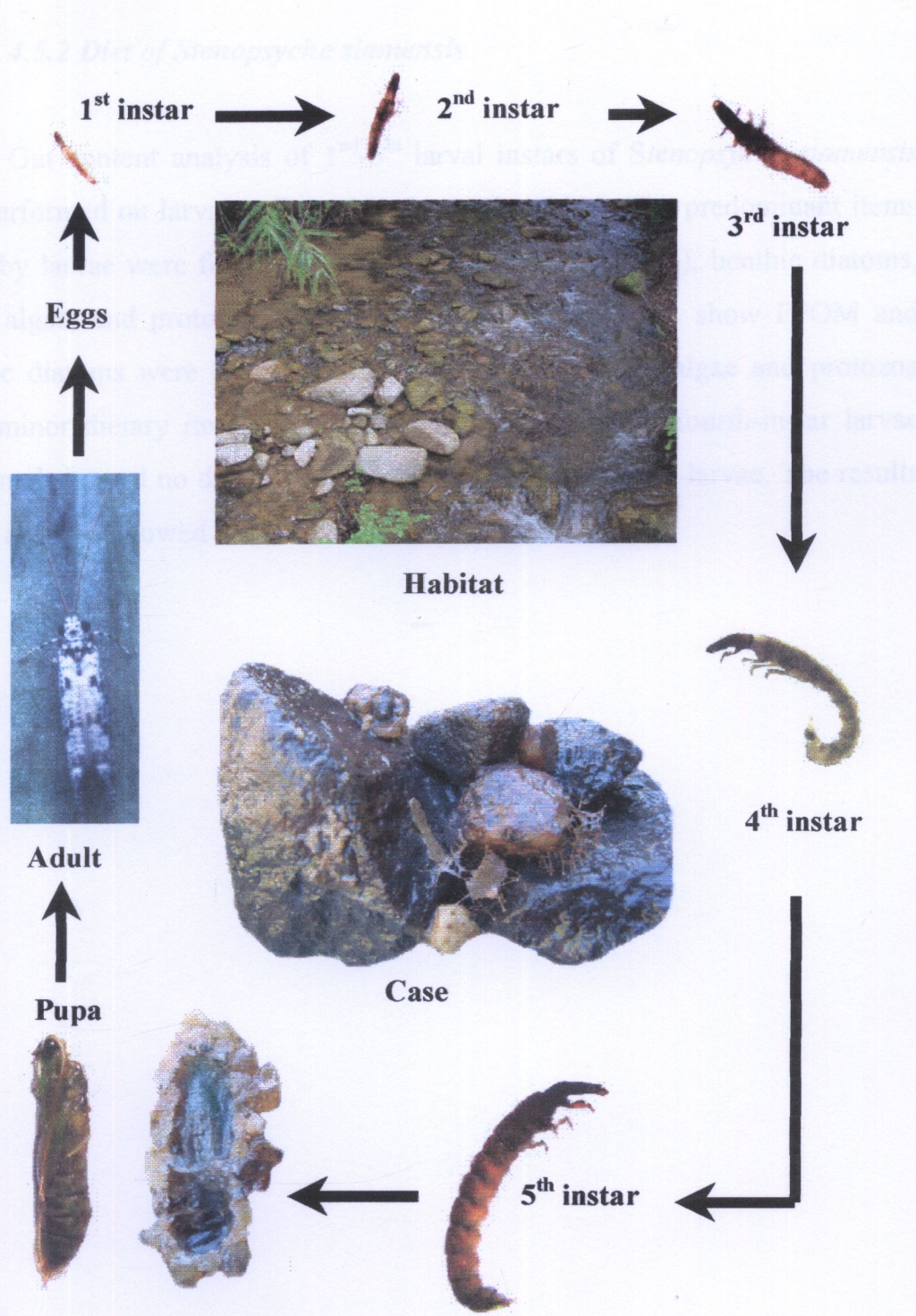


Figure 88. Life cycle of *Stenopsyche siamensis*

4.5.2 Diet of *Stenopsyche siamensis*

Gut content analysis of 1nd-5th larval instars of *Stenopsyche siamensis* was performed on larvae collected in September 2001. The predominant items eaten by larvae were fine particulate organic mater (FPOM), benthic diatoms, green algae, and protozoa (Figures 89&90). These results show FPOM and benthic diatoms were the major dietary items and green algae and protozoa were minor dietary items. The diet of the second, third, fourth-instar larvae examined showed no difference from that of the final-instar larvae. The results of gut analysis showed the larvae are filter feeders.

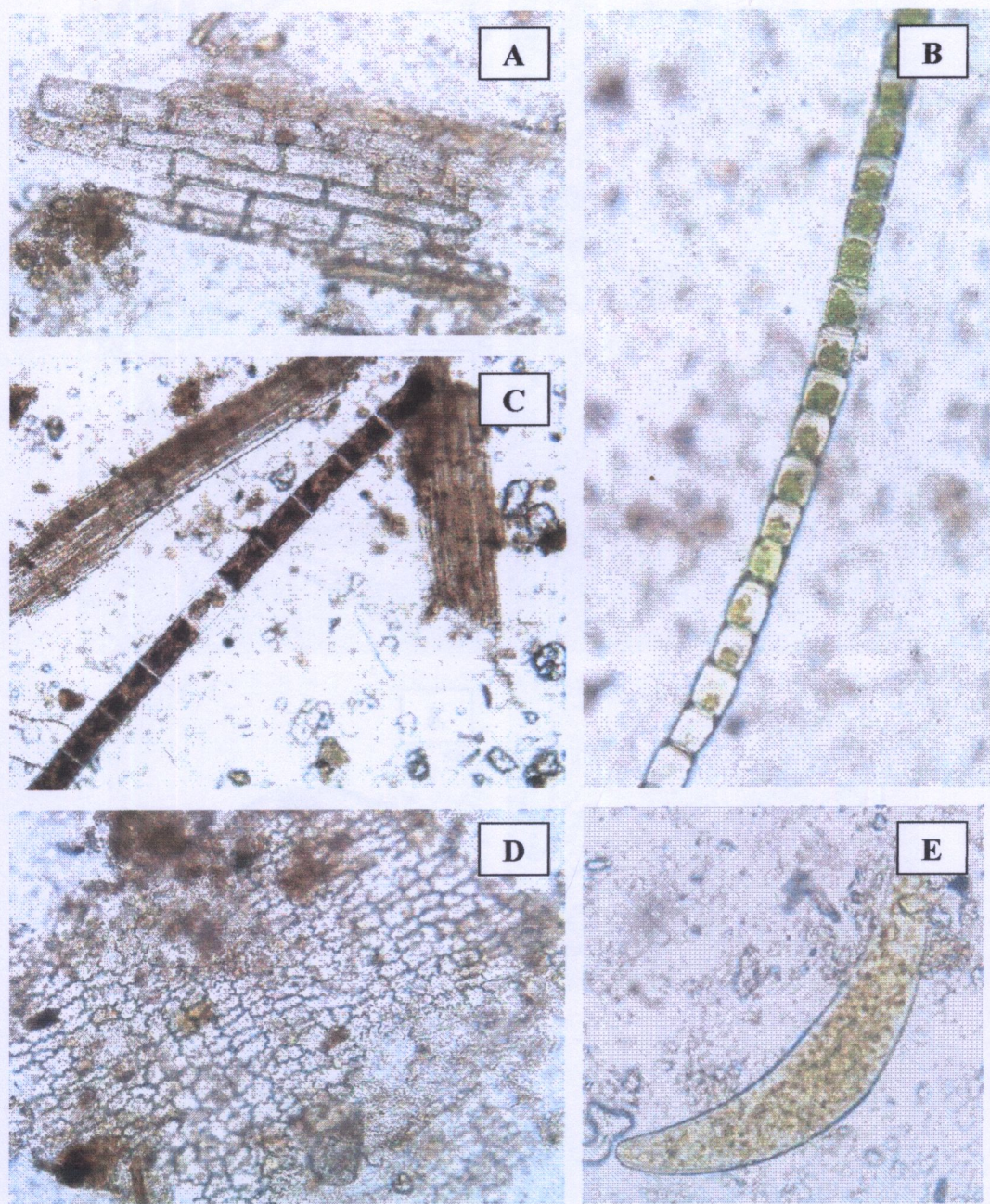


Figure 89 Gut contents of *Stenopsyche siamensis*

A-D-plant tissues, B- *Oedogonium* sp., C- *Microspora* sp., E- *Closterium* sp.

A- *Cymbella* sp., B- *Aulacoseira granulata*, C- *Gomphonema* sp. 1, D- *Pinnularia* sp., E- *Cymbella nana*, F- *Fragilaria* sp., G- *Achnanthes* sp., H- *Gomphonema* sp. 2

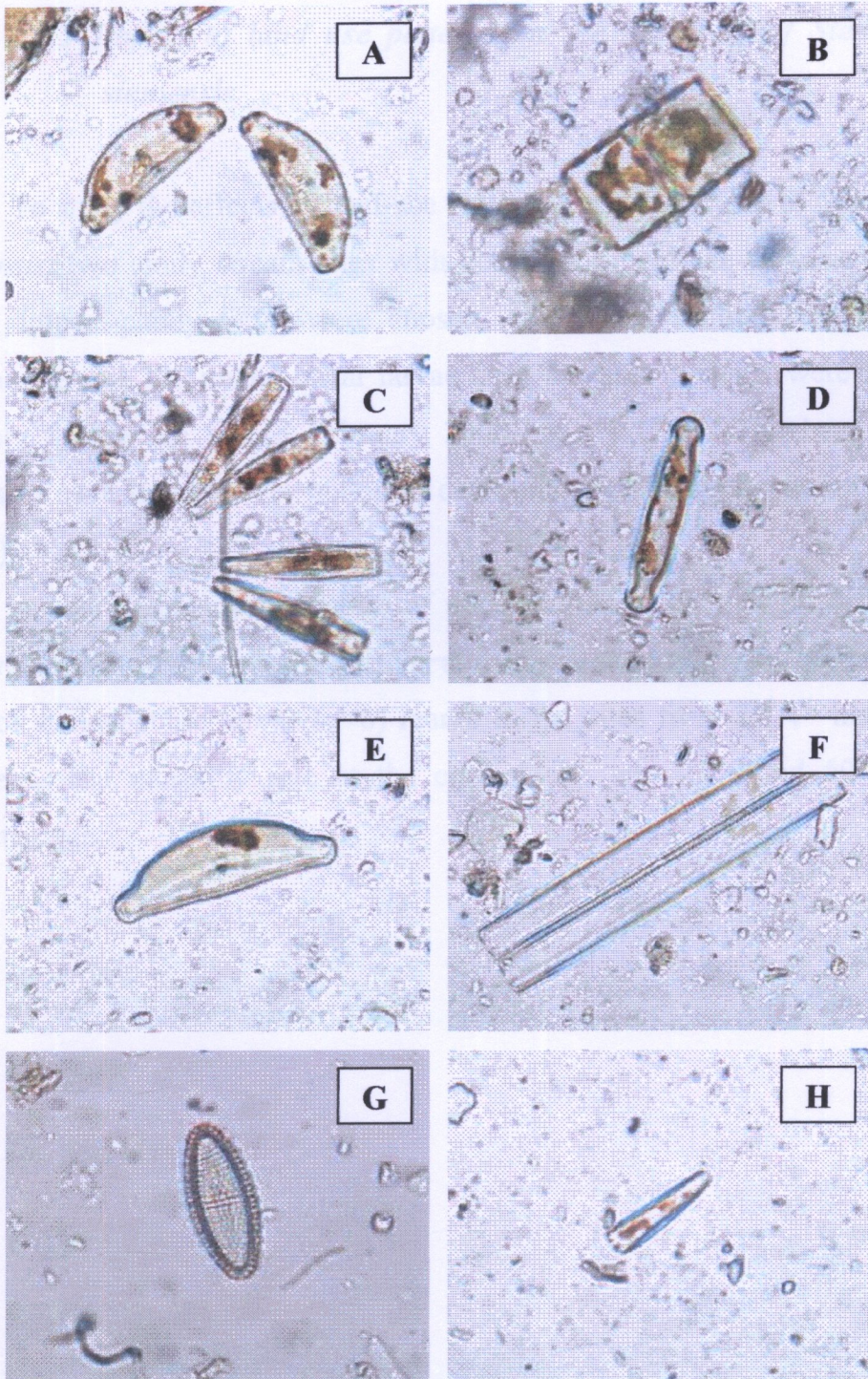


Figure 90. Gut contents of *Stenopsyche siamensis*

A- *Cymbella* sp., B- *Aulacoseira granulata*, C- *Gomphonema* sp. 1, D- *Pinnularia* sp. E- *Cymbella tumida*, F- *Fragilaria* sp., G- *Achnanthes* sp., H- *Gomphonema* sp. 2

*4.5.3 Impact of land use patterns on the life cycle of *Stenopsyche siamensis**

To study the effects of land-use pattern on the life cycle of *Stenopsyche siamensis*, two study stream sites with contrasting land-use patterns (control-forest, impact-paddy field) were chosen. 152 mixed instar larvae from the control site and 289 mixed instar larvae from the impacted site were collected monthly during the period February 2000-January 2001. The head capsule widths of each sample were potted to determine the life cycle pattern (Figures 91-94).

A size-frequency histogram of the populations showed no different in life cycle pattern between populations at different land-use sites. The mortality rates of stenopsychid larvae (n=8) reared in the sites from 2nd-4th instar were different. The mortality rate at the control site was 25% and 50% at the impacted site.

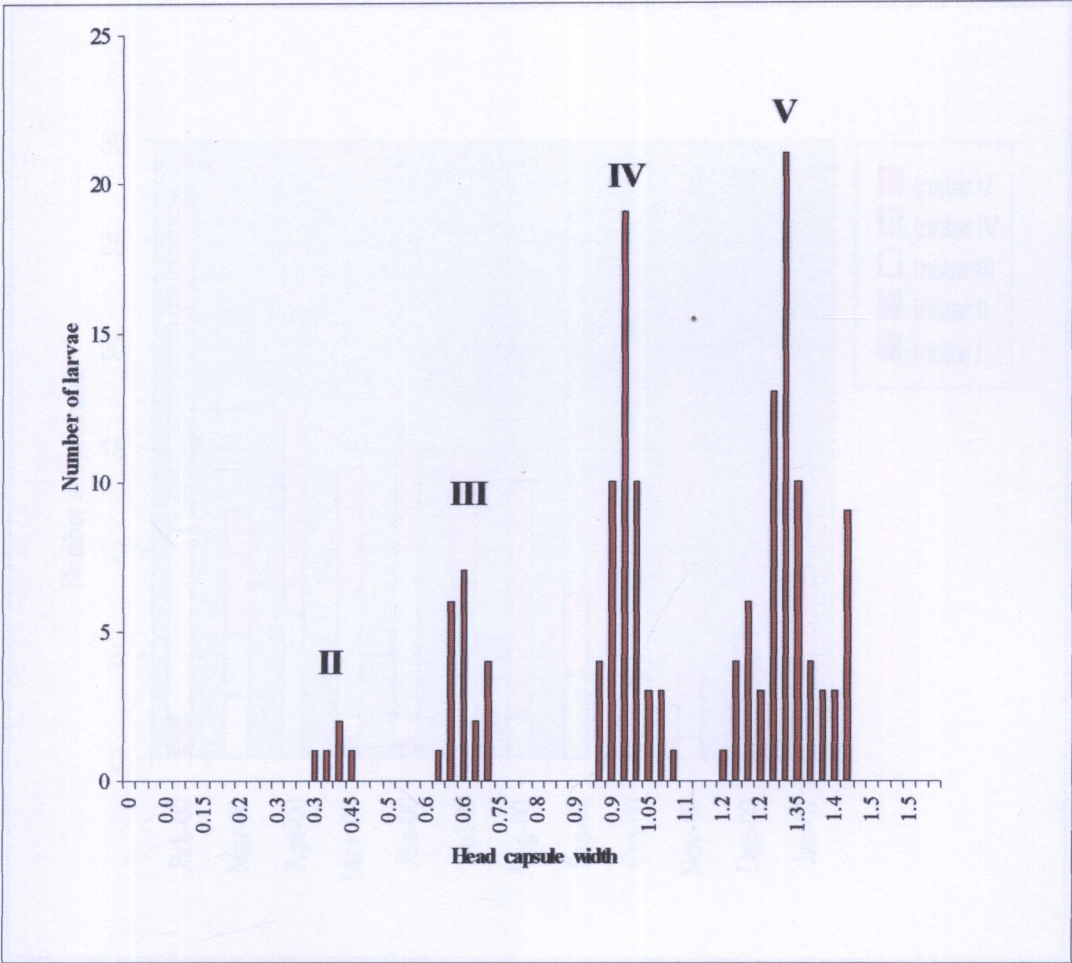


Figure 91. Size distribution of head capsule width (mm) of *Stenopsyche siamensis* from forest stream site showing larval instars I-V.

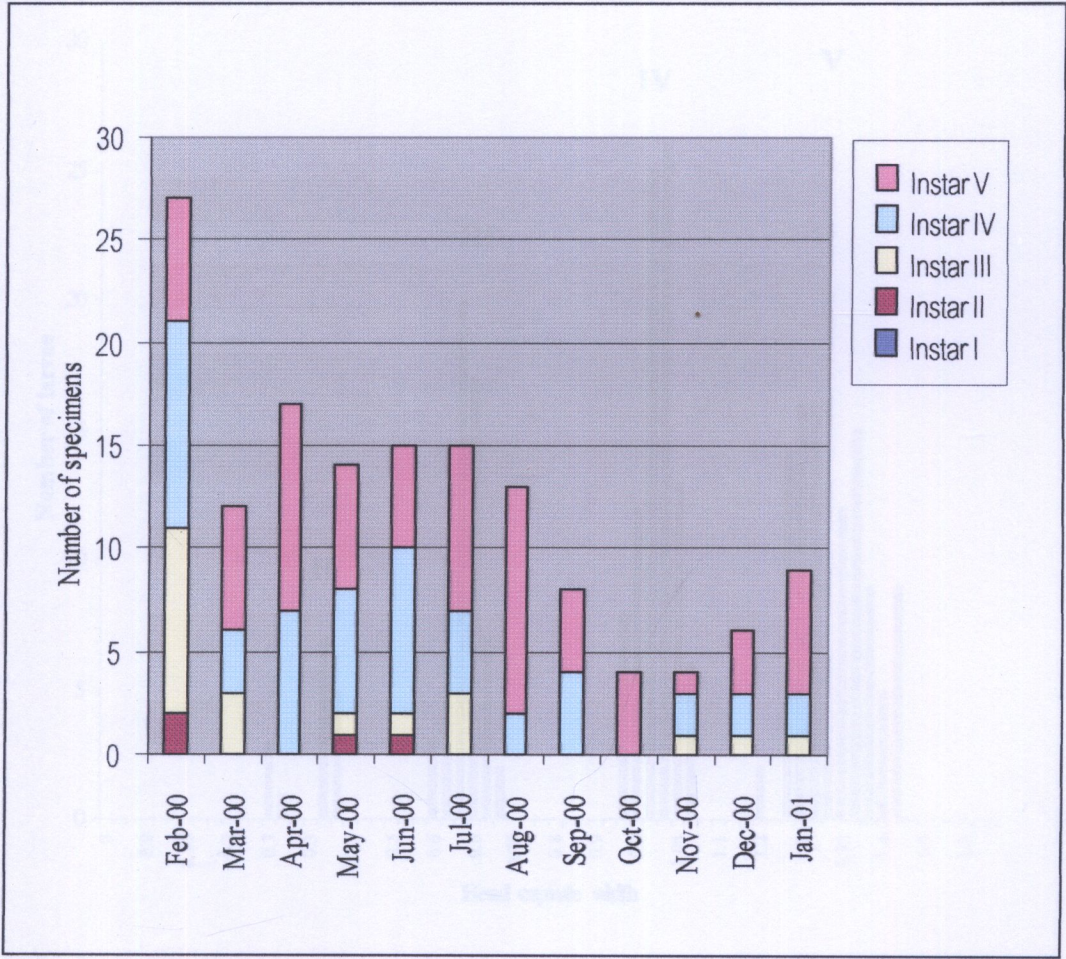


Figure 92. Monthly distribution ratio (control site) of each instar of *Stenopsyche siamensis*

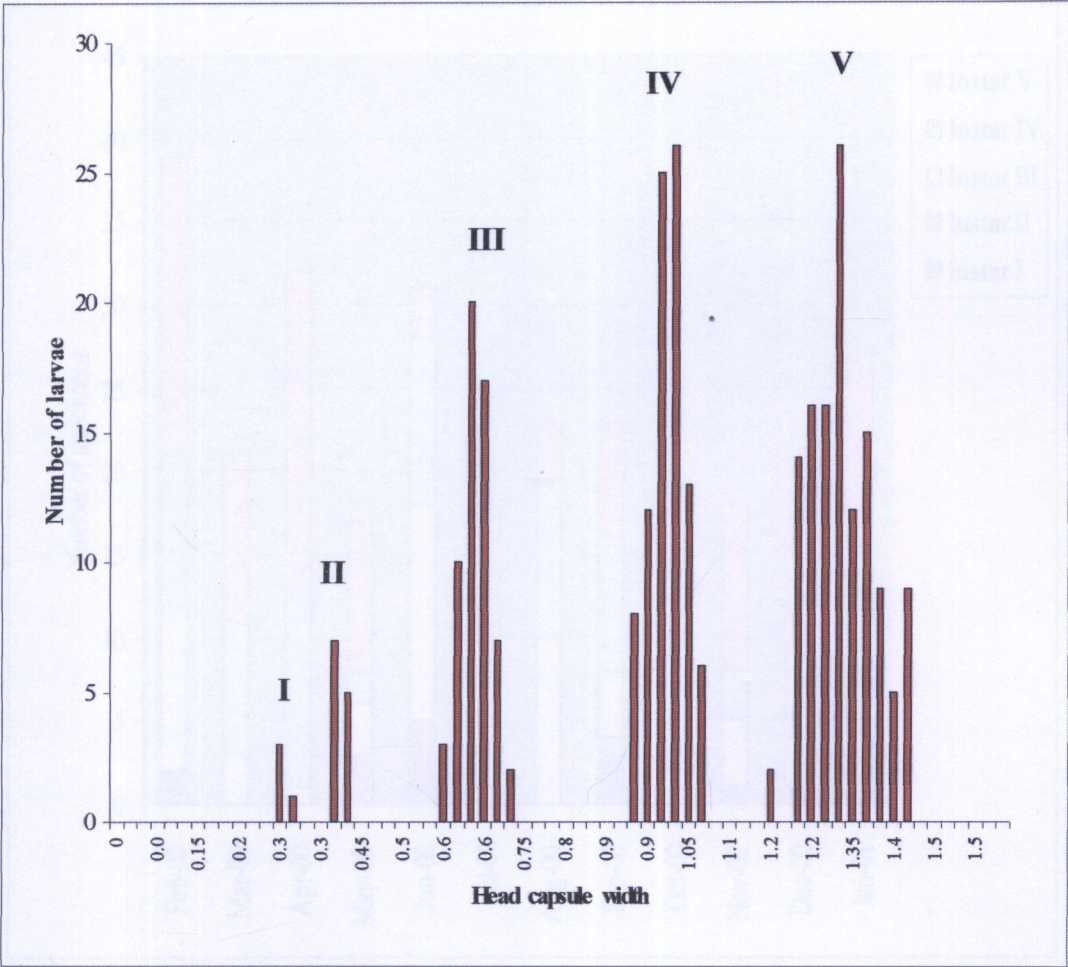


Figure 93. Size distribution of head capsule width (mm) of *Stenopsyche siamensis* from a paddy field stream site showing larval instars I-V.

CHAPTER V

DISCUSSION

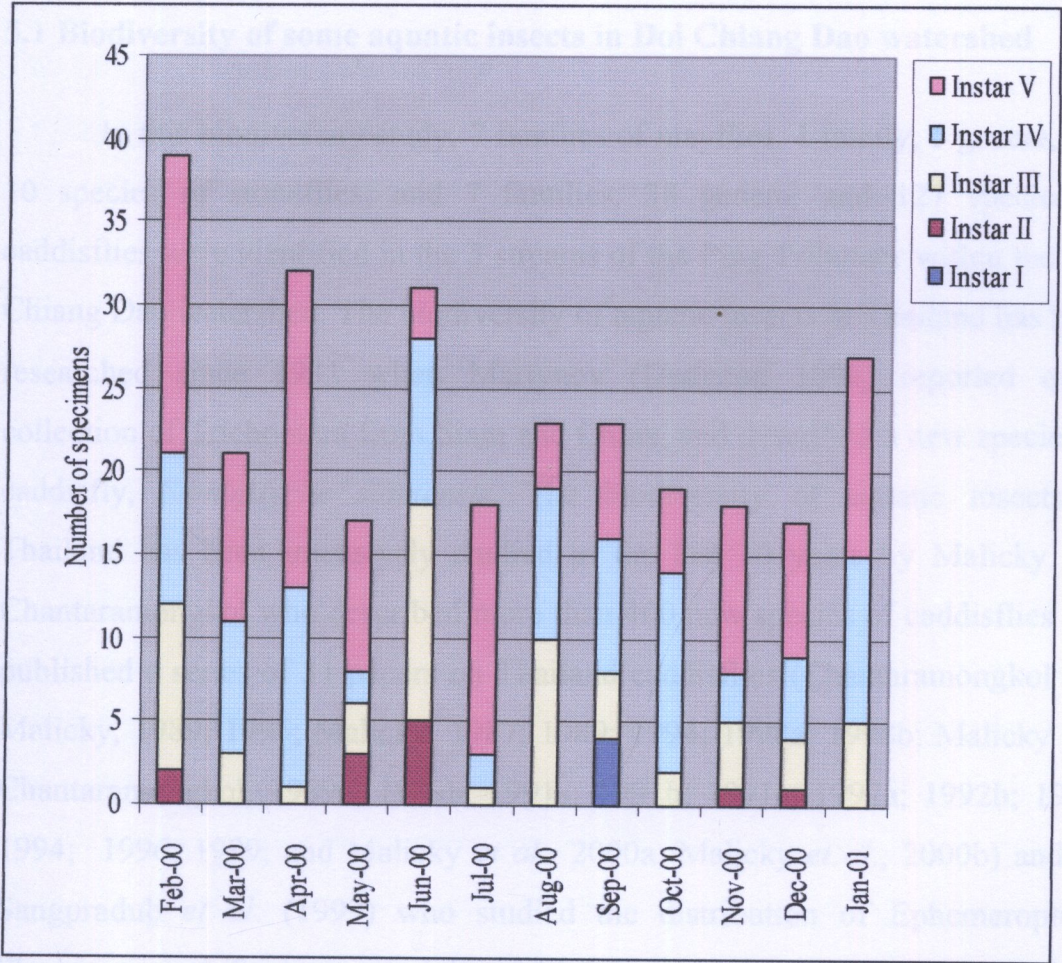


Figure 94. Monthly distribution ratio (impacted site) of each instar of *Stenopsyche siamensis*.

CHAPTER V

DISCUSSION

5.1 Biodiversity of some aquatic insects in Doi Chiang Dao watershed

In this biodiversity study, 7 families of mayflies, 1 family, 5 genera, and 10 species of stoneflies; and 7 families, 38 genera, and 127 species of caddisflies were identified in the 5 streams of the Ping Tributary within the Doi Chiang Dao watershed. The biodiversity of aquatic insects in Thailand has been researched since 1931 when Martynov (Dudgeon 1999) reported on a collection of Trichoptera from Siam and China, and described a new species of caddisfly, *Stenopsyche siamensis*. The biodiversity of aquatic insects in Thailand has been intensively studied in the last 10 years by Malicky and Chantaramongkol who described more than 400 new species of caddisflies and published a series of 31 papers on Thailand caddisflies (Chantaramongkol and Malicky, 1989; 1995; Malicky, 1987; 1989; 1994; 1998a; 1998b; Malicky and Chantaramongkol, 1989a; 1989b; 1991a; 1991b; 1991c; 1992a; 1992b; 1993; 1994; 1996; 1999, and Malicky *et al.*, 2000a; Malicky *et al.*, 2000b) and by Sangpradub *et al.* (1999) who studied the distribution of Ephemeroptera, Plecoptera, and Trichoptera adults and larvae in 21 head water streams of the Chi, Pa Sak, and Mekong basins. Their results showed 45 species of Ephemeropteran adults, 88 species of Trichopteran adults, and 4 families of Plecopteran. In addition, twenty-four Oriental Plecoptera species are reported, and 10 new species were recently found in Thailand (Stark, 1983; 1987; 1989; Stark and Sivec, 1991).

The most abundant family of stonefly in this study was Perlidae, which is also the dominant family of Oriental stonefly (Stark and Sivec, 1991) and the dominant family of caddisfly in Doi Chiang Dao was Hydropsychidae. A

preliminary checklist of caddisflies showed that Hydropsychidae is the most diverse family of caddisfly in Thailand (Malicky and Chantaramongkol, 1999). The seasonality of Doi Chiang Dao watershed caddisfly varies between species. Many species have a flight period all year and some species have a seasonal flight pattern. Analysis of the flight period of aquatic insects in the Doi Chiang Dao watershed showed the greatest numbers of individuals and species occurred in the hot season. Dudgeon (1996) described the factors which cause aquatic insects in tropical streams to emerge in the hot season as i) the survival of small larvae is higher than in the wet season which has spate induced mortality; ii) a physiological response to rising temperature and/or day length.

5.2 Biodiversity of caddisflies as bio-indicators of aquatic ecosystem health

Thailand is one of the few tropical countries where resolution of the Trichoptera fauna to species level is possible and therefore the group is a potentially powerful tool for monitoring change in freshwater environments. The assumption that most adult caddisflies do not wander far from their natal stream is reasonable and rare adults are generally more easily sampled than rare larvae. In this study, responsiveness of caddisflies to prevailing environmental conditions was apparent in the associations of species and changes in their abundance. Certain environmental parameters such as pH, conductivity, TDS, nitrate-nitrogen, sulfate, water temperature, velocity, BOD5, dissolved oxygen, and pesticide residues in stream sediment were associated with changes in the population and community composition of aquatic caddisflies. In addition, many species of caddisflies showed distribution and community patterns that related to particular environmental factors. Many taxa were sensitive to environmental quality but others were identified as being relatively tolerant to agricultural chemicals and organic pollution. Thus, the analysis of the

environmental parameters and their insects at 20 different study sites showed that the use of adult Trichoptera as bioindicators for environmental impacts in running water is useful in Thailand.

The main pollutants that probably affected the insects in this study were pesticides present in the sediment and nutrient enrichment from agricultural areas. Pesticides commonly contaminate streams by runoff from the adjacent agricultural areas in Thailand. Reice and Wohlenberg (1993) noted that stream or river sediments are repositories of accumulated toxins that may cause environmental deterioration in freshwater systems especially in aquatic insects which live on and in the sediment. Moreover, the study of the impact of pesticides applications on aquatic insects in irrigated rice field showed the total biomass and the numbers of Odonata, Ephemeroptera, Nematocera (Diptera) and Hydrocoridae (Heteroptera) were severely reduced after treatment (Heliovaara and Vaisanen, 1993) and Lemly (1982) reported the species richness, diversity, and total biomass of filter feeding Trichoptera was significantly reduced in the polluted streams that received inorganic sediment and nutrient enrichment.

The multivariate analysis identified the most tolerant caddisflies to be certain Hydropsychidae in the genera *Hydromanicus*, *Hydropsyche*, and *Cheumatopsyche*, as well as certain Leptoceridae and Odontoceridae. However, many of the most sensitive caddisflies were also Hydropsychidae-species in the genera *Hydromanicus*, *Hydropsyche*, and *Cheumatopsyche*. Thus the limitation of using only family or genus level resolution of aquatic insects in the evaluation of environmental quality is evident. The use of species level identifications for assessing environmental condition is likely to be more satisfactory than family level resolution because the concept of species as the basic biological unit is widely accepted and this level is assumed to have the greatest information content as a result of studies on individual populations (Resh and McElravy, 1993).

Chaibu (2000) indicated that Trichoptera species could be used as indicators to assess anthropogenic pollution in lowland rivers in tropical regions and reported some species of genus *Cheumatopsyche*, *Macrostemum* (Hydropsychidae) were pollution tolerant species. Likewise, the result of this study showed some species of genus *Cheumatopsyche*, *Macrostemum* were tolerant species including *C. globosa* and *M. floridum*.

The sampling of caddisfly adults showed many species in Thailand have a seasonal life cycle and that the flight periods of various species depends on the season. Therefore the comprehensive use of adult caddisflies for assessing environmental quality requires that insects should be sampled in every season. In addition, multivariate approaches are successful for broadly analyzing the impacts of land used on aquatic ecosystems in northern Thailand and for identifying candidate taxa that have particular use as indicators of such impacts.

5.3 Observation of morphology and abnormalities in the tegumental surface of *Stenopsyche siamensis* Martynov 1931 Larvae Using Scanning Electron Microscopy

Results of the study showed that deformities of the membranous thorax and abdomen were evident. These tegumental deformities might be caused by agricultural chemicals which are well documented in the literature to cause structural deformities in chironomid larvae exposed to organochlorine compounds and other pollutants (Warwick, 1989). In addition, the investigation of organophosphate and carbamate pesticides residue from sediment of the streambed showed significant differences between the control and impacted stream sites.

Study of the chronic effects of pollutants (petrol oils, pesticides, chlorine etc.) has reported abnormalities and physiological changes in aquatic insects.

Simpson (1980) showed that abnormalities in tracheal gills of *Phasganophora capitata* (stoneflies) and net spinning caddisfly larvae (Trichoptera: Hydropsychidae) were due to chlorinated or crude oil wastes. Tessier (2000) showed that chronic exposure to malathion (an organophosphate insecticide) at concentrations as low as $0.1 \mu\text{g/l}^{-1}$ can induce significant abnormalities in the capture nets of *Hydropsyche slosonae* larvae (Trichoptera: Hydropsychidae).

Biological water-quality evaluations often are based on community structure. This study has indicated that the physiological condition of resident organisms can also be useful for evaluating some effects of pollutants on the aquatic environment.

5.4 Sublethal effects of Organophosphate and Carbamate insecticides on Cholinesterase activity of *Stenopsyche siamensis* Martynov 1931

Effects of agricultural pesticides on stenopsychid ChE activity was significant in both *in vivo* and *in vitro* studies. Sublethal doses of insecticides can lead to the death of the individual's natural environment, changes in behavior, lead to drifting, and/or might facilitate predation (Kuhn and Streit, 1994; Kreutzweiser and Sibley, 1991). Field observation indicated that the study stream site was contaminated from carbaryl carbamate insecticide by drainage, run-off, and leaching from paddy field and orchard areas (figure 95).

From the inhibition ChE tests, we found a concentration of carbaryl at 50% inhibition was lower than the concentration of mevinphos at the same inhibition. This means that mevinphos is more toxic to these animals than carbaryl. This result is consistent with the WHO pesticides classification by hazard that classify mevinphos in Class Ia- extremely hazardous (oral LD_{50} 4 mg/kg (rat)) and carbaryl in Class II Moderately hazardous (oral LD_{50} 300 mg/kg (rat))(ARSAP/CIRAD, 1991). From the results of this study, we conclude that it is possible to use *Stenopsyche* ChE activity tests to evaluate

the impact of pesticides used in aquatic catchments. Trichoptera (caddisflies) can be used as a new bioindicator to assess toxic aquatic environments in Thailand.



Figure 95 Insecticides that were used in the study area

5.5 Life history strategies and impact of land used pattern on life cycle of *Stenopsyche siamensis* Martynov 1931

The family Stenopsychidae is divided into three genera: *Stenopsychodes* (nine species) from Australia, *Pseudostenopsyche* (two species) from Chile, and *Stenopsyche* (about 78 species) found mainly in the Oriental and southeastern Palaearctic (Dudgeon, 1999). Stenopsychid larvae are a distinctive element in the fauna of streams in Asia. Malicky and Chantaramongkol (1999) reported six species of stenopsychid in Thailand. From their research, *Stenopsyche siamensis* is known to occur in all parts, i.e. northern, northeast, southern and western Thailand. In Asia, the life cycle of stenopsychids has been reported from Japan and Hong Kong. The number of larval stages of the Japanese and Hong Kong stenopsychid, *Stenopsyche angustata*, is similar to

the Thai stenopsychid, *Stenopsyche siamensis* with has five larval instars. *Stenopsyche siamensis* from the Doi Chiang Dao watershed exhibited non-seasonal univoltine life cycle which is also similar to the Hong Kong stenopsychid, *Stenopsyche angustata* (Dudgeon, 1996). In Japan, Gose (1970) (refer to Dudgeon, 1996) reported that *Stenopsyche griseipennis* that lives in cooler streams or in up-stream situations, has a univoltine life cycle in contrast those that live in warm streams or down-stream, which have a bivoltine life cycle. However, Williams *et al.*, (1995) has concluded that the life history plasticity of many aquatic insects results from extrinsic factors such as stream temperature and intrinsic factors such as genetic differentiation.

The gut content analysis showed that the stenopsychid larvae consume fine particulate organic matter and benthic algae predominately the diatom, *Aulacosira granulata*. Nishimura (1966) (refer to Dudgeon, 1999) and Ismail (1996) reported that stenopsychid larvae feed on detritus (mainly) and algae (predominately diatoms).

The comparison of details of the life cycle of *Stenopsyche siamensis* under different land-use patterns (forest stream site and agricultural stream site) showed no evidence of the effects of agricultural chemicals on the life cycle. However, the mortality rate of larvae reared in the agricultural stream site, which has pesticide contamination higher than the control site, was higher than the mortality rate at the control site.

CHAPTER VI

CONCLUSIONS

1. A total of 4,460 male caddisflies, 1,315 mayflies and 88 stoneflies were trapped at 20 different land-use sites in three streams within the Doi Chiang Dao watershed during the period April – November 2000. Seven families of mayflies, one family, 5 genera, and 10 species of stoneflies and seventeen families, 38 genera, and 127 species of caddisflies were identified in the samples. In caddisflies, the families Hydropsychidae, Philopotamidae, and Psychomyiidae were the most abundant, yielding 39 species (30.7 %), 19 species (15%), and 11 species (8.7%) respectively.

2. More caddisflies emerged in the hot season than in the rainy and cool seasons, in both of numbers of adults and numbers of species. Flight period of the caddisflies also varied considerably. Forty-two species (33.1%) were found in all seasons, 49 species (38.6%) were active in a single season and 36 species (28.4%) were active in two seasons.

3. Using the database comprising 127 caddisflies species, TWINSpan organized the sites into 4 recognizable groups at the second level division. Group I represents moderately impacted sites. Group II represents forested control sites. Group III and IV represent impacted sites.

4. ANOVA showed 10 environmental parameters which differed between the TWINSpan groups, including pH, conductivity, TDS, nitrate-nitrogen, sulfate, water temperature, velocity, dissolved oxygen, BOD₅, and % inhibition of ChE activity in sediment and there were 28 caddisfly species significantly correlated with the ordination.

5. Consideration of the orientation of vectors of various caddisflies species, environmental parameters, and the study sites within the ordination suggests *P. barata*, *H. arcturus*, *H. truncatus*, *H. cerva*, *C. joliviti*, *C. coulees* and *U. maliwan* were the most sensitive caddisflies to pesticides from agricultural and organic pollution. Conversely, *M. sumatrana*, *M. floridum*, *O. tripunctata*, *P. mithila*, *C. globosa*, *P. flavata*, *M. prichapanyai*, *E. volovicus*, *C. charites*, *G. uniformis*, *A. halong*, *C. cognita*, *E. puro*, *C. chrysothemis*, *H. clitumnus*, *S. argentiguttatus*, *P. baenzigeri*, and *G. redsat* were relatively tolerant caddisfly species.

6. A study of the morphology and a comparison of *Stenopsyche siamensis* larvae from two streams (control and impacted sites) by using SEM showed the chronic effects of agricultural chemicals to the tegumental surface of this insect. SEM micrographs of the larvae stage in agricultural stream site showed deformities of the tegumental surface of the thorax and abdomen.

7. A study of the effects of organophosphate and carbamate insecticides on Stenopsychid ChE activity showed significant differences between the impacted and the control stream site. The results showed a ChE activity of 2.39 ± 0.56 (hot season), 1.26 ± 0.19 (rainy season) and 0.99 ± 0.21 (cool season) $\mu\text{mole}/\text{min}/\text{mg}$ at the control site, and 0.63 ± 0.36 (hot season), 0.57 ± 0.22 (rainy season) and 0.85 ± 0.38 (cool season) $\mu\text{mole}/\text{min}/\text{mg}$ at the impacted site. This study indicates that chronic effects of insecticides used in agriculture on aquatic fauna were evident.

8. A study of the life cycle of *Stenopsyche siamensis* showed that the insect have 5 larval instars and exhibited a non-seasonal univoltine life cycle. The gut content analysis showed that the stenopsychid larvae are filtering collectors that consume fine particulate organic matter and benthic algae predominately the diatom, *Aulacoseira granulata*, which is a common species in northern Thailand.

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Appendix 1. Check list of Ephemeroptera were found in Chiang Dao Watershed, Chiang Dao Distric, Chiang Mai Province, Thailand.

Study Sites	T1	T2	T3	T4	T5	T6	SaS	K1	K2	K3	K4	MM	MS	SoS	Dm	PH	N1	N2	N3	N4
Hot Season																				
Family Baetidae		1					5	36	5	5	4	8	5	1		5	4	1	60	
Caenidae				54	3	2				1				1	26					
Ephemeridae		1								1	7				1	2	20	10	65	18
Heptageniidae	1	5	1			1	3	1	2	12	1	2	2	1	5	3	20	1	34	3
Leptophlebiidae																	3			
Polymitarcyidae			1								3							1		
Siphonuridae								1				2								
Rainy Season																				
Family Baetidae			9	5	28	30	5	1	2	1	45		1				17	7	50	5
Caenidae	4		24	7					1	12						3			4	
Ephemeridae				53	10	2	2		3		76	1	1	1	3		1	2	3	4
Heptageniidae	1		3	5	2		8		1	2	37	36	14	5	43		22	7	21	
Leptophlebiidae															17		42	10	6	
Polymitarcyidae					1		1		1		2	2					3	3		
Siphonuridae										1		4		1		1				
Cool Season																				
Family Baetidae					1		1							1				1		
Caenidae								2				2			2					
Ephemeridae				2		1													1	1
Heptageniidae			2			2					2			2	10		43	2	2	
Leptophlebiidae															4		11	3		
Polymitarcyidae											24				1		1			
Siphonuridae							1	1		1		1				1				
Total	6	7	40	126	45	38	26	42	15	36	201	58	23	13	112	15	187	48	246	31

Appendix 2. Check list of Plecoptera were found in Chiang Dao Watershed, Chiang Dao Distric, Chiang Mai Province, Thailand.

Study Sites	T1	T2	T3	T4	T5	T6	SaS	K1	K2	K3	K4	MM	MS	SoS	Dm	PH	N1	N2	N3	N4
Hot Season																				
Family Peridae																				
<i>Etrocorema nigrogeniculatum</i>																				
<i>Etrocorema</i> sp.		1	2	3	5					1					10	1		2		
<i>Kaminuria</i> sp.		1											2	3		2				
<i>Neoperla cavaleriei</i>										1										
<i>Neoperla desperi</i>																				
<i>Neoperla</i> sp.				1														4	2	5
<i>Phanoperla</i> sp.																				
Family Peltoperlidae																				
<i>Cryptoperla</i> sp.																				
Rainy Season																				
Family Peridae																				
<i>Etrocorema nigrogeniculatum</i>												1								
<i>Etrocorema</i> sp.				3					2	1					1	3				
<i>Kaminuria</i> sp.																				
<i>Neoperla cavaleriei</i>																				
<i>Neoperla desperi</i>															1					
<i>Neoperla</i> sp.			1		1				1											
<i>Phanoperla</i> sp.				1	3															
Family Peltoperlidae																				
<i>Cryptoperla</i> sp.																				
Cool Season																				
Family Peridae																				
<i>Etrocorema nigrogeniculatum</i>														3			1			
<i>Etrocorema</i> sp.	1			2	2	2										5				
<i>Kaminuria</i> sp.																				
<i>Neoperla cavaleriei</i>																				
<i>Neoperla desperi</i>																				
<i>Neoperla</i> sp.					1											1		1		
<i>Phanoperla</i> sp.																				
Family Peltoperlidae																				
<i>Cryptoperla</i> sp.			1									1					2			
Total	1	2	4	10	12	2	0	0	3	3	1	1	5	5	19	3	3	7	2	5

Appendix 3. Check list of Trichoptera were found in Chiang Dao Watershed, Chiang Dao Distric, Chiang Mai Province, Thailand.

[illegible]

Study Sites	T1	T2	T3	T4	T5	T6	K1	K2	K3	K4	SaS	MM	MS	SoS	Dam	PH	N1	N2	N3	N4	
Family Hydropsychidae																					
Amphipsyche gratiosa									2	2											
Cheumatopsyche admetos	3	1		7														1			
Cheumatopsyche carmentis																					
Cheumatopsyche carna				4																	
Cheumatopsyche charites									1	1				1			2		2	13	
Cheumatopsyche chryseis					61	28	34		1	8				4							
Cheumatopsyche chrysothemis									2	1							1	2			
Cheumatopsyche cocles	16	21	21					2	2		3	1	10			15					
Cheumatopsyche cognita										1					2		2	17	3	6	
Cheumatopsyche criseyde												11		1		1					
Cheumatopsyche dhanikari																			8		
Cheumatopsyche gaia															1						
Cheumatopsyche globosa				1		1			2	70							29	36	505	323	
Hydratomanicus adonis	1	1	1																		
Hydratomanicus klanklini			2						2					1	1						
Hydratomanicus scotosius	1																				
Hydromanicus inferior			2				18	22	2			3	4	23	44		1				
Hydromanicus sealthiel																1					
Hydromanicus serubabel	4	12	10	14	26	9	2	2			*		1								
Hydromanicus truncatus	13	8	3				1				6						31				
Hydropsyche adraetos				9			2	3			9	28	6	5			10				
Hydropsyche angkangensis												1	8				3				
Hydropsyche arcturus	3	3	3				3	3			12						31				
Hydropsyche askalaphos								1													
Hydropsyche atropos	2	4	10			1	7	4	3	8	4	1	3	5	7						
Hydropsyche briareus	3	5	5	22	3		4	2	2	1				8	27						
Hydropsyche camillus	1	3	15	38	2	1	12	50	42	2	2	3	1	13	23		6	2	7	2	
Hydropsyche cerva		1									1	2		1	1	3					
Hydropsyche clitumnus							1					19	1								
Hydropsyche dolosa	7	16	78	72	9		14	21	35	13	19		5	34	43		1	3	3	4	
Hydropsyche pallipenne			4	2	3	6							1	1							
Macrostemum fastosum	2	3					3	1				1	1	1		1					
Macrostemum floridum									1	2	1						3	3	7	3	
Macrostemum midas									1			2			1						
Potamyia alleni																				2	
Potamyia baenzigeri												2									
Potamyia flavata						2				2					2		2	1	23	11	
Potamyia phaidra			1	8	3		9	23	73	49				4	4	1	8		1	5	
Trichomacronema paniae	1	2	1				1	8			2		2			2					
Family Limnephilidae																					
Moropsyche huaysailanga	1	2																			
Family Goeridae																					
Goera ilo				6			7	2			3						1				
Goera matuilla					2	4	1		1	3					15		2			3	
Goera redsat													7								
Goera redsamar	1	1	6				1							30	1	39	1	1			
Goera solicur																	1				
Goera uniformis																		1	4	13	
Family Lepidostomatidae																					
Adinarthrum moulmina	3	11	10	6					11								1				
Dinarthrum brueckmanni														1	2	1					
Dinarthrum daidalion	10	22	19	1			19	13			1	2					11				
Dinarthrum februaryi																	1				
Dinarthrum matius							1	3													
Dinarthrum pratetiaensis	2	5	2					2									3				
Dinarthrum tungyawensis			2					1													
Goerodes doligug		1	1	1	1	2				3	11					6		1	2	6	3
Family Leptoceridae																					
Leptocerus Chiangmaiensis																		12		1	
Leptocerus dirghachuga										16							1	4	2	2	
Leptocerus lanzenbergeri																				1	
Leptocerus promkutaewi																	1				
Leptocerus posticoides											2										
Oecetis empusa											1						3	5	22		

Study Sites	T1	T2	T3	T4	T5	T6	K1	K2	K3	K4	SaS	MM	MS	SoS	Dm	PH	N1	N2	N3	N4
Family Leptoceridae																				
<i>Oecetis tripunctata</i>									1	4				1			6		2	4
<i>Setodes argentiguttatus</i>									1	3							2	5	15	138
<i>Setodes endymion</i>																	9			
Family Calamoceratidae																				
<i>Anisocentropus brevipennis</i>					1														3	
<i>Anisocentropus janus</i>		1					1			2		3			1					
<i>Anisocentropus diana</i>		1																		
<i>Anisocentropus pan</i>				6	1	1														
<i>Ganonema fuscipenne</i>					1	1				1					2					
Family Odontoceridae																				
<i>Inthanonopsycha trimeresuri</i>													1							
<i>Lunnapsyche chantaramongkolae</i>			1	3		1	1	3						1						
<i>Marilia mogiana</i>																				
<i>Marilia sumatrana</i>								2	7	19					2					
Total	122	183	227	285	96	87	139	211	207	250	77	131	110	149	300	134	346	96	690	620

Appendix 4. Check list of Trichoptera were found in Chiang Dao Watershed in hot season, March, 25, 2000.

Study Sites	T1	T2	T3	T4	T5	T6	K1	K2	K3	K4	SaS	MM	MS	SoS	Dm	PH	N1	N2	N3	N4
Family Rhyacophilidae																				
<i>Himalopsyche acharai</i>	1										1	1								
<i>Rhyacophila inaequalis</i>				1																
<i>Rhyacophila petersorum</i>								1								1				
<i>Rhyacophila suthepensis</i>			1																	
<i>Rhyacophila tosgani</i>		1																		
Family Glossosomatidae																				
<i>Agapetus atuius</i>												1								
<i>Agapetus dangorum</i>							1													
<i>Agapetus halong</i>							1			1		2		2	1		172	1	16	65
<i>Glossosoma elvisso</i>				1				2			1					1				
<i>Glossosoma malayana</i>																1				
<i>Glossosoma voccus</i>												1								
Family Hydroptilidae																				
<i>Ugandatrichia maliwan</i>													4							
Family Philopotamidae																				
<i>Chimarra akkaorum</i>					2	5											1			
<i>Chimarra aneca</i>	1	1																		
<i>Chimarra atara</i>	3	1		6																
<i>Chimarra hinorum</i>			1																	
<i>Chimarra jolivetii</i>	1			1			1				2					1				
<i>Chimarra khamuorum</i>									1											
<i>Chimarra lavuaorum</i>	1	1																		
<i>Chimarra litussa</i>		1																		
<i>Chimarra monorum</i>													2							
<i>Chimarra pipake</i>				2	1	1														
<i>Chimarra ravanna</i>		1																		
<i>Chimarra suthepensis</i>	21	13					1				1		1							
<i>Chimarra yaorum</i>		6																		
<i>Gunungiella segsafiaza</i>		1																		
<i>Kisaura consagia</i>		26	7	4	3	5		3						1	1					
<i>Kisaura sura</i>	14			2							1									
Family Stenopsychidae																				
<i>Stenopsyche siamensis</i>														4	32		2			
Family Polyplectropodidae																				
<i>Polyplectropus admin</i>																	1			
Family Psychomyiidae																				
<i>Lype athia</i>			1	2																
<i>Paduniella maeclangensis</i>												1	14	1						
<i>Paduniella sampati</i>																	1		4	
<i>Psychomyia barata</i>			1																	
<i>Psychomyia kaiya</i>												1		1	7		13		1	
<i>Psychomyia kiskinda</i>																		1		
<i>Psychomyia lak</i>										1					10		20		1	
<i>Psychomyia mithila</i>												1		1	8		11			
Family Niphocentronidae																				
<i>Drepanocentron curmisagius</i>			1																	
Family Ecnomidae																				
<i>Ecnomus cincibilus</i>																			4	
<i>Ecnomus joachin</i>																		3		3
<i>Ecnomus mammus</i>																	1			
Family Hydropsychidae																				
<i>Amphipsyche gratioa</i>									1	2										
<i>Cheumatopsyche admetos</i>	3	1		6																
<i>Cheumatopsyche charites</i>														1			1		2	13
<i>Cheumatopsyche chryseis</i>				16	11	22		1	3					4						
<i>Cheumatopsyche chrysothemis</i>									1								1	2		
<i>Cheumatopsyche cocles</i>	12	19	12				2				2	1	4			15				
<i>Cheumatopsyche cognita</i>									1						2		1	2		3
<i>Cheumatopsyche criseyde</i>												9		1		1				
<i>Cheumatopsyche dhanikari</i>																			8	
<i>Cheumatopsyche gaia</i>															1					
<i>Cheumatopsyche globosa</i>				1		1				32							27	28	372	257
<i>Hydratomanicus adonis</i>	1		1																	
<i>Hydratomanicus klanklini</i>															1					

Study Sites	T1	T2	T3	T4	T5	T6	K1	K2	K3	K4	SaS	MM	MS	SoS	Dm	PH	N1	N2	N3	N4
Family Hydropsychidae																				
<i>Hydratomanicus scotosius</i>	1																			
<i>Hydromanicus inferior</i>			1				16	17	2			3		16	19		1			
<i>Hydromanicus serubabel</i>	3	8	8	6	4	3	1													
<i>Hydromanicus truncatus</i>	12	5									3					26				
<i>Hydropsyche adraetos</i>				9							7	15	1	3		4				
<i>Hydropsyche angkangensis</i>												1				1				
<i>Hydropsyche archurus</i>	2	2	1				3	2			4					9				
<i>Hydropsyche atropas</i>	2	4	10			1	7	3	3		4	1	3	5	6					
<i>Hydropsyche briareus</i>	3	5	5	22	3		4	2	2	1				8	21					
<i>Hydropsyche camillus</i>	1	3	14	38	2	1	12	40	42	2	2	3	1	13	23		6	2	3	
<i>Hydropsyche cerva</i>		1									1	1		1	1	3				
<i>Hydropsyche clitumnus</i>												1								
<i>Hydropsyche dolosa</i>	7	16	78	72	9		14	16	35	13	19		5	34	43			3	3	1
<i>Hydropsyche pallipenne</i>				2	1	6														
<i>Macrostemum fastosum</i>		2					2						1							
<i>Macrostemum floridum</i>											1						1		6	2
<i>Macrostemum midas</i>								1				2								
<i>Potamyia alleni</i>																				2
<i>Potamyia flavata</i>																	2	1	5	6
<i>Potamyia phaidra</i>		1	8	3			9	14	72	49				4	4	1	8		1	5
<i>Trichomacronema paniae</i>	1	1					1	6			1		1			1				
Family Limnephilidae																				
<i>Moropsyche huaysailianga</i>	1	2																		
Family Goeridae																				
<i>Goera ilo</i>			6				2	1			2						1			
<i>Goera matuilla</i>						1										7	2			
<i>Goera redsat</i>												6								
<i>Goera redsomar</i>													6							
<i>Goera solicur</i>																1				
<i>Goera uniformis</i>																	1		1	6
Family Lepidostomatidae																				
<i>Adinathrum moulmina</i>	3	11	10	6				9												
<i>Dinarthrum brueckmanni</i>													1	2	1					
<i>Dinarthrum daidaloin</i>	7	17	14	1			15	9			1	1				7				
<i>Dinarthrum februaris</i>																1				
<i>Dinarthrum pratetaiensis</i>	1	5						1								2				
<i>Dinarthrum tungyavensis</i>		2						1												
<i>Goerodes doligung</i>	1	1	1			2			3	2					6		1	1	6	3
Family Leptoceridae																				
<i>Leptocerus dirghachuga</i>										2							1	1		2
<i>Leptocerus lanzenbergeri</i>																				1
<i>Oecetis tripunctata</i>									1	1							3		1	4
<i>Setodes argentiguttatus</i>																				13
Family Calamoceratidae																				
<i>Anisocentropus brevipennis</i>					1														3	
<i>Anisocentropus janus</i>		1					1					1			1					
<i>Anisocentropus diana</i>		1																		
<i>Anisocentropus panm</i>				6																
<i>Ganonema fuscipenne</i>										1					1					
Family Odontoceridae																				
<i>Inthanonopsyche trimeresuri</i>												1								
<i>Lannapsyche chantaramongkolae</i>				1																
<i>Marilia mogiana</i>															2					
<i>Marilia sumatrana</i>									7	17					1		5	1	22	16
taxa richness	102	160	181	209	37	48	93	129	174	124	53	58	41	102	198	86	283	46	462	399

[illegible]

Study Sites	T1	T2	T3	T4	T5	T6	K1	K2	K3	K4	SaS	MM	MS	SoS	Dm	PH	N1	N2	N3	N4
Family Hydropsychidae																				
<i>Hydropsyche cerva</i>								1					1							
<i>Hydropsyche clitumnus</i>													18	1						
<i>Hydropsyche dolosa</i>								5									1			3
<i>Hydropsyche pallipenne</i>			1																	
<i>Macrostemum fastosum</i>	2	1					1	1					1		1					
<i>Macrostemum floridum</i>									1	1							1	2	1	1
<i>Potamyia baenzigeri</i>													2							
<i>Potamyia flavata</i>						2				2					2				17	5
<i>Potamyia phaidra</i>								9	1											
<i>Trichomacronema paniae</i>		1	1									1				1				
Family Goeridae																				
<i>Goera ilo</i>							5	1				1								
<i>Goera matuilla</i>				2	2	3	1		1	3						8				3
<i>Goera redsoma</i>	1	1	6										23	1	38	1	1			
<i>Goera uniformis</i>																		3	1	2
Family Lepidostomatidae																				
<i>Adinarthrum moulimina</i>																1				
<i>Dinarthrum daidalon</i>	2		1					1				1				4				
<i>Dinarthrum pratetianensis</i>	1		1													1				
Family Leptoceridae																				
<i>Leptocerus Chiangmaiensis</i>																	10			1
<i>Leptocerus dirghachuga</i>										14								2	1	
<i>Leptocerus promkutkaewi</i>																1				
<i>Leptocerus posticoides</i>										2										
<i>Oecetis tripunctata</i>										3				1						
<i>Oecetis empusa</i>										1							2	3	21	
<i>Setodes argentiguttatus</i>									1	3							2	5	14	124
Family Calamoceratidae																				
<i>Anisocentropus janus</i>										2			2							
<i>Ganonema fuscipenne</i>						1									1					
Family Odontoceridae																				
<i>Marilia sumatrana</i>								2		1									1	2
taxa richness	15	10	19	58	41	32	27	52	32	88	6	64	48	33	79	24	33	25	202	211

Appendix 6. Check list of Trichoptera were found in Chiang Dao Watershed in cool season, November, 3, 2000.

Study Sites	T1	T2	T3	T4	T5	T6	K1	K2	K3	K4	SaS	MM	MS	SoS	Dm	PH	N1	N2	N3	N4
Family Rhyacophilidae																				
Himalopsyche acharai							1	1			2			1						
Rhyacophila petersorum								2												
Family Glossosomatidae																				
Agapetus halong			1			1	7			2									1	
Glossosoma elvisso											1		4		1					
Family Philopotamidae																				
Chimarra akkaorum										13									1	
Chimarra atara				1								1								
Chimarra chiangmaiensis					1				1	9							1			
Chimarra pipake				1																
Chimarra spinifera				2						2										
Chimarra sultheopsis	1								4											
Chimarra yaorum											1									
Gunungieella segsafiazga											1									
Kisaura consagia				1	5	2	2			*	1	1	2	2						
Family Stenopsychidae																				
Stenopsyche siamensis													1		8		7			
Family Arctopsychidae																				
Moesaipsyche prichapanyai																	1	1	1	
Family Psychomyiidae																				
Lype athia					1															
Psychomyia amphiarao																		1		
Psychomyia barata																		1		
Psychomyia kaiya																	1			
Psychomyia mithila												3							1	
Psychomyia semarangensis																				
Family Ecnomidae																				
Ecnomus jojachin																			1	
Ecnomus puro																		1		
Family Hydropsychidae																				
Cheumatopsyche carmentis																		1		
Cheumatopsyche charites																		1		
Cheumatopsyche chryseis				9	6															
Cheumatopsyche coeles	1		7					2			1		1							
Cheumatopsyche cognita																	1	14	3	1
Cheumatopsyche globosa																	1		13	3
Hydratomanicus adonis		1																		
Hydratomanicus klanklini			2											1						
Hydromanicus inferior			1					2					3	7	7					
Hydromanicus sealthiel																1				
Hydromanicus serubabel		4	1	2		2		1												
Hydromanicus truncatus	1	2	3								1									
Hydropsyche adraatos							1	3			2	2	3	2						
Hydropsyche angkangensis													4				1			
Hydropsyche arcturus	1	1	2					1			8						22			
Hydropsyche briareus															4					
Hydropsyche camillus			1																	
Hydropsyche citumnus							1													
Hydropsyche pallipenne			3		2							1	1	1				1	1	
Macrostemum floridum										1										
Macrostemum midas										1					1					
Potamyia flavata																			1	
Trichomacronema paniae								2					1							
Family Goeridae																				
Goera redsat												1								
Goera redsamar							1						1		1					
Goera uniformis																		1		5
Family Lepidostomatidae																				
Adinarthrum moulimina								2												
Dinarthrum daidalion	1	5	4				4	3												
Dinarthrum matius							1	3												
Dinarthrum pratetaiensis			1					1												
Goerodes doligung					1					9								1		

Study Sites	T1	T2	T3	T4	T5	T6	K1	K3	K2	K4	SaS	MM	MS	SoS	Dm	PH	N1	N2	N3	N4
Family Leptoceridae																				
<i>Leptocerus dirghachuga</i>																	2	1	1	
<i>Oecetis tripunctata</i>																	3		1	
<i>Oecetis empusa</i>																	1	2	1	
<i>Setodes argentiguttatus</i>																			1	1
<i>Setodes endymion</i>																	9			
Family Calamoceratidae																				
<i>Anisocentropus pan</i>					1	1														
<i>Ganonema fuscipenne</i>					1															
Family Odontoceridae																				
<i>Lannapsyche chantaramongkolae</i>			1	2		1	1	3					1							
<i>Marilia sumatrana</i>										1					1			1		
taxa richness	5	13	27	18	18	7	19	30	1	38	18	9	21	14	23	24	30	25	26	10

Appendix 7. Values of environmental variables in hot season 2000 at the study sites, Doi Chiang Dao Watershed, Chiang Mai.

Parameters\Sites	T1	T2	T3	T4	T5	T6	K1	K2	K3	K4
Altitude (m)	840	820	700	500	460	440	560	500	400	360
pH	7.8	7.6	7.5	8.1	8.1	8.2	8	7.9	8.4	7.8
Conductivity (uS/cm)	77.8	84.9	82.2	396	390	377	56.1	57.6	97.6	95.8
TDS (mg/l)	38.8	42.7	40.9	198	196	190	28.2	29	48.9	48.1
Air temperature (C)	23.8	19	22	25	27.2	27	18	18	18	20.5
Water temperature (C)	18	18	18.5	30	22	22	16	16	16	19
Velocity (m/s)	0.37	0.2	0.21	0.32	0.23	0.4	0.35	0.25	0.33	0.31
Alkalinity (mg/l)	30.5	28.5	28.5	173	177	174	28	24	32.5	29.5
Dissolved oxygen (mg/l)	11	11.2	12.2	11.2	13	11.6	12.4	12.2	12	10.2
Phosphorus (mg/l)	0.22	0.27	0.29	0.42	0.39	0.66	0.29	0.28	0.21	0.31
Nitrate-nitrogen (mg/l)	0.3	1.1	0.6	0.6	0.2	0.2	0.3	0.2	0.2	0.2
Sulphate (mg/l)	2	1	2	1	1	0	3	3	15	13
Turbidity (FTU)	11	9	12	13	7	8	21	14	17	26
Ammonia-nitrogen (mg/l)	0.2	0.08	0.09	0.02	0.04	0.03	0.1	0.08	0.07	0.07
BOD5 (mg/l)	2.9	2.2	1.8	3.1	2.8	2	2.7	2.5	2.2	4
% Inhibition-soil	0	2.16	2.29	0	10.1	1.8	0	0	11.7	26.6
% Inhibition-sed	3.12	5.38	5.97	0	3.95	5.14	0	0	7.88	8.23

Appendix 7. Continued.

Parameters\Sites	SaS	MM	MS	SoS	Dm	PH	N1	N2	N3	N4
Altitude (m)	830	740	690	540	460	840	380	360	360	350
pH	7.8	7.9	7.8	8	7.9	7.8	7.8	7.6	7.5	7.6
Conductivity (uS/cm)	45.7	46.6	71	71.9	51.7	54.8	58.3	59.4	62.4	75.3
TDS (mg/l)	23	23.4	35.1	36	25.2	27	28.7	29.8	32.2	37.6
Air temperature (C)	24	25.5	24	23	30	18	20	21	18	14
Water temperature (C)	16	18	18	19.5	21	16	18	18	17	17
Velocity (m/s)	0.35	0.63	0.46	0.54	0.58	0.27	0.21	0.77	0.26	0.21
Alkalinity (mg/l)	21	24	32	36	22	26	25	28	26	31
Dissolved oxygen (mg/l)	10.8	10.4	10.2	10.8	11.2	11.2	12	10.4	9.8	9.8
Phosphorus (mg/l)	0.48	0.64	0.48	0.49	0.74	1.03	0.78	0.41	0.8	0.71
Nitrate-nitrogen (mg/l)	0.2	0.2	0.3	0.2	0.1	0.3	0.2	0.2	0.4	0.3
Sulphate (mg/l)	3	5	4	2	5	8	4	4	4	4
Turbidity (FTU)	10	44	18	9	20	33	11	12	12	13
Ammonia-nitrogen (mg/l)	0.12	0.12	0.09	0.07	0.13	0.21	0.13	0.13	0.14	0.09
BOD5 (mg/l)	0.4	1.6	0.8	0.6	2.7	1.1	1.6	2.4	1.2	1.2
% Inhibition-soil	0	0	25.4	2.84	11	8.71	10	20.6	2.84	10
% Inhibition-sed	0	0	5.97	0	0	0	11.6	3	0.2	10.7

Appendix 8. Results of environmental variables in rainy season at the study sites, Doi Chiang Dao Watershed, Chiang Mai.

Parameters\Sites	T1	T2	T3	T4	T5	T6	K1	K2	K3	K4
pH	8.2	8.1	8.2	8.2	8.2	8.3	8.3	8.2	8.1	8.2
Conductivity (uS/cm)	56.3	72.1	73.3	314	248	244	48.2	44.6	45.9	68.1
TDS (mg/l)	27.9	35.8	36.6	156	123	123	24.2	22.3	23	34.1
Air temperature (C)	28	26	28	28	27	28	26	25	25	27
Water temperature (C)	22.4	22.8	23.4	24.1	24.4	25.1	22.7	22.6	23.4	24.4
Velocity (m/s)	0.23	0.59	0.23	0.39	0.37	0.51	0.72	0.72	0.58	0.42
Alkalinity (mg/l)	21	28	26	232	200	195	18	16	17	25
Dissolved oxygen (mg/l)	8.2	8.4	7.8	7.5	9.8	10	6.8	7.1	6.8	7.2
Phosphorus (mg/l)	0.45	0.49	0.54	0.67	0.6	0.46	0.37	0.53	0.88	0.48
Nitrate-nitrogen (mg/l)	0.6	1.6	0.4	1	1	0.7	0.8	1	0.6	1.6
Sulphate (mg/l)	0	0	3	0	0	0	0	2	0	4
Turbidity (FTU)	490	320	147	24	86	78	2813	2737	2425	240
Ammonia-nitrogen (mg/l)	0.47	0.46	0.23	0.01	0.01	0.03	2.34	2.15	1.89	0.14
BOD5 (mg/l)	3.5	2.8	1.9	2.5	2.3	4.2	2.1	2.5	3.4	3.2
% Inhibition-soil	0	0	0	0	2.9	1.68	0	0	4.24	1.79
% Inhibition-sed	4.46	12.9	5.72	0	4.46	6.81	0	0	5.56	7.28

Appendix 8. Continued.

Parameters\Sites	SaS	MM	MS	SoS	Dm	PH	N1	N2	N3	N4
pH	8.3	8.1	8.3	8.3	8.3	8.2	8.2	7.1	7.3	7.3
Conductivity (uS/cm)	43.1	51.7	65.5	62.4	56	58.5	55.1	65.2	75.6	78.6
TDS (mg/l)	20.3	25.8	32.4	31.3	28.7	29.4	27.4	32.7	37.9	39.3
Air temperature (C)	28	26	26	26	26	26	27	28	28	28
Water temperature (C)	22	22.3	22.6	23.4	23.8	22	24.2	24.8	25.6	26.4
Velocity (m/s)	0.84	0.61	0.52	0.58	0.48	0.36	0.36	0.2	0.56	0.54
Alkalinity (mg/l)	18	21	24	23	21	22	20	28	28	27
Dissolved oxygen (mg/l)	7.4	7.8	7.8	7.4	7	8.4	6.8	7.2	7.6	7
Phosphorus (mg/l)	0.19	0.77	0.53	0.73	0.71	0.16	0.71	0.62	1.63	0.73
Nitrate-nitrogen (mg/l)	0.7	0.05	0.8	0.6	1	0.1	0.5	4	0.9	0.9
Sulphate (mg/l)	0	0	1	1	2	0	2	9	0	1
Turbidity (FTU)	2150	46	89	147	194	57	214	167	55	63
Ammonia-nitrogen (mg/l)	1.26	0.19	0.28	0.28	1.31	0.23	0.56	0.69	0.64	0.47
BOD5 (mg/l)	2.5	2.1	2.3	3.1	2.6	1.9	2.8	2.6	2.9	3.2
% Inhibition-soil	0	0	1.52	0.13	2.32	2.68	1.56	5.34	0.35	2.35
% Inhibition-sed	0	0	6.34	1.9	4.94	5.25	7.59	15.6	11.5	8.84

Appendix 9. Results of environmental variables in cool season at the study sites, Doi Chiang Dao Watershed, Chiang Mai.

Parameters\Sites	T1	T2	T3	T4	T5	T6	K1	K2	K3	K4
pH	7.1	7	7.9	7.5	7.5	7.8	8.2	8.3	8.4	8.3
Conductivity (uS/cm)	61.4	69.1	80.7	355	296	290	61.3	58.2	76.1	80.6
TDS (mg/l)	30.7	34.7	40.9	178	148	146	30.2	29	38.2	40.2
Air temperature (C)	21	22	23	26	27	25	26	27	26	25
Water temperature (C)	20.5	20.5	20.3	22.9	23.6	23.3	25.1	25.3	21.4	21.7
Velocity (m/s)	0.42	0.47	0.41	0.24	1.07	1.02	0.62	0.51	47	0.51
Alkalinity (mg/l)	25	24.5	29	163	138	136	24.2	23	26.5	28
Dissolved oxygen (mg/l)	9.8	9.2	8.5	10.4	9.5	8.3	10.2	9.2	8.5	8.1
Phosphorus (mg/l)	0.27	0.23	0.23	0.31	0.33	0.47	0.22	0.22	0.23	0.24
Nitrate-nitrogen (mg/l)	1.5	1.6	2	1.4	1.3	1.8	1.3	1.2	1.4	1.2
Sulphate (mg/l)	0	0	1	0	0	0	0	0	2	3
Turbidity (FTU)	27	26	22	15	20	20	14	16	21	18
Ammonia-nitrogen (mg/l)	0.09	0.1	0.04	0.01	0.02	0.04	0.01	0.04	0.06	0.04
BOD5 (mg/l)	2.3	1.8	0.6	0.8	2.1	1.8	0.7	0.6	0.8	1.2
% Inhibition-soil	2.8	0	0	0	4.22	0	0	0	3.28	3.1
% Inhibition-sed	5.57	2.31	4.31	0.57	5.57	3.15	0	0.26	4.02	13.2

Appendix 9. Continued.

Parameters\Sites	SaS	MM	MS	SoS	Dm	PH	N1	N2	N3	N4
pH	7.3	7	7.4	7.3	7.2	7.8	8.5	8.4	8.3	7.3
Conductivity (uS/cm)	54	48.5	70	70.6	54	73.6	54	54.1	55.4	56.9
TDS (mg/l)	27	24.1	35.2	35.3	27	36.8	27.1	27.1	28	28
Air temperature (C)	22	21	22	22	22	26	30	26	25	25
Water temperature (C)	20.6	19.1	20	20.5	20.6	19.5	22	25	21.1	21.8
Velocity (m/s)	0.94	0.75	0.3	0.35	0.94	0.32	0.54	0.66	0.62	0.42
Alkalinity (mg/l)	21	23	32	31	25	25	23	25	24	23
Dissolved oxygen (mg/l)	8.6	9	9.3	8.4	8.6	9.7	8.9	8.6	7.9	8.2
Phosphorus (mg/l)	0.77	0.62	0.77	0.54	0.51	0.61	0.56	1.27	0.52	0.6
Nitrate-nitrogen (mg/l)	1.1	1.4	1.5	1.8	1.4	1.2	0.8	1.3	1.1	1.6
Sulphate (mg/l)	0	0	0	1	0	0	0	0	0	0
Turbidity (FTU)	20	20	7	15	20	46	14	14	14	15
Ammonia-nitrogen (mg/l)	0.01	0.04	0.01	0.02	0.11	0.09	0.09	0.07	0.09	0.11
BOD5 (mg/l)	0.9	1.7	1.6	0.6	1.6	2.1	1.5	1.4	1.9	1.8
% Inhibition-soil	0	0	0.7	0	0.9	0	2.8	2.3	0	2.8
% Inhibition-sed	1.02	0	1.04	0	2.2	2.52	8.8	3.7	1.46	1.99

Appendix 10. ChE activity ($\mu\text{mole}/\text{min}/\text{mg}$) of stenopsychids sampled from control sites in hot season 2000.

No.	Blank	R1	R2	Average	delta	Protein(mg/ml)	ChE Activity $\mu\text{mole}/\text{min}/\text{mg}$
1	0.635	0.692	0.695	0.6935	0.0585	3.61	2.2367
2	0.538	0.604	0.607	0.6055	0.0675	4.44	2.5808
3	0.68	0.765	0.769	0.767	0.087	4.79	3.3264
4	0.571	0.643	0.638	0.6405	0.0695	5.78	2.6573
5	0.528	0.577	0.572	0.5745	0.0465	4.01	1.7779
6	0.519	0.565	0.572	0.5685	0.0495	2.95	1.8926
7	0.585	0.627	0.637	0.632	0.047	3.01	1.797
8	0.652	0.701	0.708	0.7045	0.0525	4.92	2.0073
9	0.638	0.687	0.692	0.6895	0.0515	4.26	1.9691
10	0.654	0.739	0.724	0.7315	0.0775	3.46	2.9632
11	0.698	0.756	0.755	0.7555	0.0575	3.49	2.1985
12	0.586	0.666	0.675	0.6705	0.0845	3.44	3.2308

Appendix 11. ChE activity ($\mu\text{mole}/\text{min}/\text{mg}$) of stenopsychids sampled from impact sites in hot season.

No.	Blank	R1	R2	Average	delta	Protein(mg/ml)	ChE Activity $\mu\text{mole}/\text{min}/\text{mg}$
1	0.555	0.588	0.579	0.5835	0.0285	4.02	1.0897
2	0.542	0.555	0.563	0.559	0.017	3.67	0.65
3	0.531	0.56	0.563	0.5615	0.0305	3.91	1.1661
4	0.632	0.644	0.622	0.633	0.001	3.08	0.0382
5	0.639	0.653	0.633	0.643	0.004	3.66	0.1529
6	0.626	0.646	0.65	0.648	0.022	4.12	0.8411
7	0.597	0.622	0.625	0.6235	0.0265	3.08	1.0132
8	0.609	0.625	0.632	0.6285	0.0195	4.33	0.7455
9	0.584	0.595	0.598	0.5965	0.0125	4.48	0.4779
10	0.546	0.555	0.562	0.5585	0.0125	3.01	0.4779
11	0.628	0.635	0.639	0.637	0.009	3.17	0.3441
12	0.647	0.661	0.663	0.662	0.015	3.87	0.5735

Appendix 12. ChE activity ($\mu\text{mole}/\text{min}/\text{mg}$) of stenopsychids sampled from control sites in rainy season.

No.	Blank	R1	R2	Average	delta	Protein (mg/ml)	ChE Activity $\mu\text{mole}/\text{min}/\text{mg}$
1	0.498	0.532	0.536	0.534	0.036	1.025	1.3764
2	0.482	0.509	0.516	0.5125	0.0305	1.827	1.1661
3	0.49	0.528	0.537	0.5325	0.0425	1.691	1.625
4	0.486	0.525	0.528	0.5265	0.0405	2.507	1.5485
5	0.519	0.555	0.546	0.5505	0.0315	1.796	1.2044
6	0.488	0.518	0.526	0.522	0.034	2.008	1.3
7	0.473	0.508	0.512	0.51	0.037	1.056	1.4147
8	0.476	0.498	0.498	0.498	0.022	1.403	0.8411
9	0.465	0.502	0.499	0.5005	0.0355	1.675	1.3573
10	0.495	0.526	0.522	0.524	0.029	1.615	1.1088
11	0.475	0.508	0.504	0.506	0.031	3.082	1.1852
12	0.47	0.505	0.508	0.5065	0.0365	2.341	1.3955

Appendix 13. ChE activity ($\mu\text{mole}/\text{min}/\text{mg}$) of stenopsychids sampled from impact sites in rainy season.

No.	Blank	R1	R2	Average	delta	Protein(mg/ml)	ChE Activity $\mu\text{mole}/\text{min}/\text{mg}$
1	0.53	0.538	0.546	0.542	0.012	2.507	0.4588
2	0.537	0.556	0.548	0.552	0.015	2.719	0.5735
3	0.538	0.561	0.557	0.559	0.021	3.505	0.8029
4	0.512	0.525	0.536	0.5305	0.0185	3.581	0.7073
5	0.539	0.564	0.558	0.561	0.022	3.082	0.8411
6	0.501	0.515	0.524	0.5195	0.0185	2.341	0.7073
7	0.491	0.491	0.511	0.501	0.01	1.842	0.3823
8	0.493	0.493	0.508	0.5005	0.0075	3.717	0.2867
9	0.498	0.498	0.519	0.5085	0.0105	2.492	0.4014
10	0.501	0.527	0.524	0.5255	0.0245	2.688	0.9367
11	0.519	0.519	0.538	0.5285	0.0095	3.324	0.3632
12	0.482	0.482	0.503	0.4925	0.0105	1.963	0.4014

Appendix 14. ChE activity ($\mu\text{mole}/\text{min}/\text{mg}$) of stenopsychids sampled from control sites in cool season.

No.	Blank	R1	R2	Average	delta	Protein(mg/ml)	ChE Activity $\mu\text{mole}/\text{min}/\text{mg}$
1	0.349	0.367	0.388	0.3775	0.0285	0.69	1.0897
2	0.348	0.369	0.375	0.372	0.024	1.318	0.9176
3	0.383	0.4	0.408	0.404	0.021	1.431	0.8029
4	0.374	0.395	0.396	0.3955	0.0215	2.929	0.822
5	0.398	0.423	0.424	0.4235	0.0255	0.872	0.975
6	0.395	0.423	0.422	0.4225	0.0275	2.793	1.0514
7	0.346	0.366	0.372	0.369	0.023	0.962	0.8794
8	0.346	0.36	0.372	0.366	0.02	1.265	0.7647
9	0.386	0.416	0.408	0.412	0.026	1.401	0.9941
10	0.378	0.396	0.402	0.399	0.021	2.369	0.8029
11	0.402	0.436	0.444	0.44	0.038	1.068	1.4529
12	0.398	0.428	0.436	0.432	0.034	3.94	1.3

Appendix 15. ChE activity ($\mu\text{mole}/\text{min}/\text{mg}$) of stenopsychids sampled from impact sites in cool season.

No.	Blank	R1	R2	Average	delta	Protein(mg/ml)	ChE Activity $\mu\text{mole}/\text{min}/\text{mg}$
1	0.355	0.396	0.373	0.3845	0.0295	0.796	1.1279
2	0.352	0.378	0.417	0.3975	0.0455	1.431	1.7397
3	0.349	0.372	0.374	0.373	0.024	1.537	0.9176
4	0.377	0.402	0.399	0.4005	0.0235	3.02	0.8985
5	0.31	0.325	0.332	0.3285	0.0185	0.977	0.7073
6	0.374	0.406	0.409	0.4075	0.0335	2.974	1.2808
7	0.361	0.386	0.363	0.3745	0.0135	1.098	0.5161
8	0.358	0.374	0.383	0.3785	0.0205	1.386	0.7838
9	0.351	0.362	0.368	0.365	0.014	1.507	0.5352
10	0.386	0.396	0.398	0.397	0.011	2.49	0.4205
11	0.32	0.336	0.338	0.337	0.017	1.174	0.65
12	0.379	0.388	0.402	0.395	0.016	4.109	0.6117

Appendix 16. ChE activity ($\mu\text{mole}/\text{min}/\text{mg}$) at each concentration of carbaryl of *Stenopyschids* sampled from study sites in rainy season 2000.

site	carbaryl conc(μM)	Blank	R1	R2	R3	Average	delta	Protein(mg)	% ChE Activity
control	1250	1.132	1.13	1.128	1.136	1.1313	-7E-04	1.1462	0
control	625	1.132	1.132	1.138	1.142	1.1373	0.0053	1.1467	12.1212
control	156.25	1.132	1.14	1.144	1.138	1.1407	0.0087	1.1467	19.6969
control	78.2	1.132	1.146	1.148	1.142	1.1453	0.0133	1.1467	30.303
control	19.5	1.132	1.146	1.148	1.152	1.1487	0.0167	1.1467	37.8787
control	9.8	1.132	1.158	1.166	1.154	1.1593	0.0273	1.1467	62.1212
control	0	1.132	1.178	1.172	1.178	1.176	0.044	1.1467	100
impact	1250	1.134	1.128	1.124	1.124	1.1253	-0.009	1.3584	0
impact	625	1.134	1.132	1.13	1.128	1.13	-0.004	1.3584	0
impact	156.25	1.134	1.132	1.13	1.138	1.1333	-7E-04	1.3584	0
impact	78.2	1.134	1.142	1.136	1.144	1.1407	0.0067	1.3584	15.625
impact	19.5	1.134	1.148	1.144	1.144	1.1453	0.0113	1.3584	26.5625
impact	9.8	1.134	1.148	1.146	1.142	1.1453	0.0113	1.3584	26.5625
impact	0	1.134	1.174	1.178	1.178	1.1767	0.0427	1.3584	100

Appendix 17. ChE activity ($\mu\text{mole}/\text{min}/\text{mg}$) at each concentration of mevinphos of *Stenopyschids* sampled from study sites in rainy season.

site	Mevinphos conc(μM)	Blank	R1	R2	R3	Average	delta	Protein(mg)	% ChE Activity
control	625	0.52	0.518	0.52	0.523	0.5203	0.0003	1.1467	1.5503
control	156.25	0.52	0.523	0.523	0.527	0.5243	0.0043	1.1467	20.155
control	39.1	0.52	0.527	0.523	0.524	0.5247	0.0047	1.1467	21.7054
control	9.8	0.52	0.526	0.524	0.527	0.5257	0.0057	1.1467	26.3565
control	2.4	0.52	0.527	0.527	0.529	0.5277	0.0077	1.1467	35.6589
control	0.6	0.52	0.533	0.529	0.535	0.5323	0.0123	1.1467	57.3643
control	0	0.52	0.54	0.543	0.5415	0.5415	0.0215	1.1467	100
impact	625	0.503	0.501	0.506	0.503	0.5033	0.0003	1.3584	2.3809
impact	156.25	0.503	0.501	0.504	0.507	0.504	0.001	1.3584	7.1428
impact	39.1	0.503	0.504	0.507	0.504	0.505	0.002	1.3584	14.2857
impact	9.8	0.503	0.503	0.506	0.508	0.5057	0.0027	1.3584	19.0476
impact	2.4	0.503	0.506	0.503	0.505	0.5047	0.0017	1.3584	11.9047
impact	0.6	0.503	0.507	0.506	0.51	0.5077	0.0047	1.3584	33.3333
impact	0	0.503	0.521	0.513	0.517	0.517	0.014	1.3584	100

Appendix 18 Monthly collection of *Stenopsyche siamensis* larvae data during February, 2000-January, 2001 from study sites.

HCW mm	Feb-00 Impact	Feb-00 Control	Mar-00 Impact	Mar-00 Control	Apr-00 Impact	Apr-00 Control	May-00 Impact	May-00 Control	Jun-00 Impact	Jun-00 Control	Jul-00 Impact	Jul-00 Control
0.275												
0.3												
0.325												
0.35												
0.375										1		
0.4	1								4			
0.425	1	1					3	1	1			
0.45		1										
0.475												
0.5												
0.525												
0.55												
0.575												
0.6												
0.625									2		1	
0.65	3	1		1					5	1		2
0.675	6	3	1	1			2	1	6			1
0.7	1	1	2	1	1							
0.725		4					1					
0.75												
0.775												
0.8												
0.825												
0.85												
0.875												
0.9												
0.925												
0.95	3	2								2	1	
0.975	1	1	2	1	1	3	1		3	4		
1	3	6	2		5	3	1	1	3	2		3
1.025		1	3	1	5			2	4			1
1.05				1	1			1			1	
1.075	2		1					2				
1.1						1						
1.125												
1.15												
1.175												
1.2	1	1			1							
1.225										1		2
1.25			1	1	3		6		1	1	1	
1.275			1		1	1	1		3	1	6	
1.3			1	1	2						1	3
1.325		3	3	1	4	4	1	1		1	5	3
1.35		1	2		3		1	3		1	1	
1.375	8			2	2	1						
1.4	5		1		1			2				
1.425			1	1	1	2						
1.45	4	1			1	2					1	
1.475												
1.5												
Total pupae	39 p	27 p	21 p	12 p	32 p	17 p	17 p	14 p	32 p	15 p	18 p	15 p

Appendix 18 (continued)

HCW mm	Aug-00 Impact	Aug-00 Control	Sep-00 Impact	Sep-00 Control	Oct-00 Impact	Oct-00 Control	Nov-00 Impact	Nov-00 Control	Dec-00 Impact	Dec-00 Control	Jan-01 Impact	Jan-01 Control
0.275												
0.3			3									
0.325			1									
0.35												
0.375												
0.4							1		1			
0.425												
0.45												
0.475												
0.5												
0.525												
0.55												
0.575												
0.6	1		2									
0.625	1				1		2		2	1	1	
0.65	5		1		1		2	1	1		2	
0.675	1										1	1
0.7	1		1								1	
0.725	1											
0.75												
0.775												
0.8												
0.825												
0.85												
0.875												
0.9												
0.925												
0.95					1						3	
0.975			2	1					1		1	
1	4		2	3	3						2	1
1.025	2	1	2		4		1	2	3	2	2	
1.05	2		2		4		2		1			1
1.075	1	1									2	
1.1												
1.125												
1.15												
1.175												
1.2												
1.225		1										
1.25				2	1					1	1	1
1.275			1			1	1		1		1	
1.3	3	4	1	1	1	3	4		3			1
1.325		4	1		1		4		2	2	5	2
1.35	1	1	1	1		1	1		1		1	2
1.375		1	3		1						1	
1.4					1			1			1	
1.425									1		2	
1.45												
1.475												
1.5												
Total pupar	23 p	13 p	23 p	8 p	19 p	5 p	18 p	4 p	17 p	6 p	27 p	9 p

Remark p=present

Appendix 19. Male genitalia figures of some unidentified Trichoptera

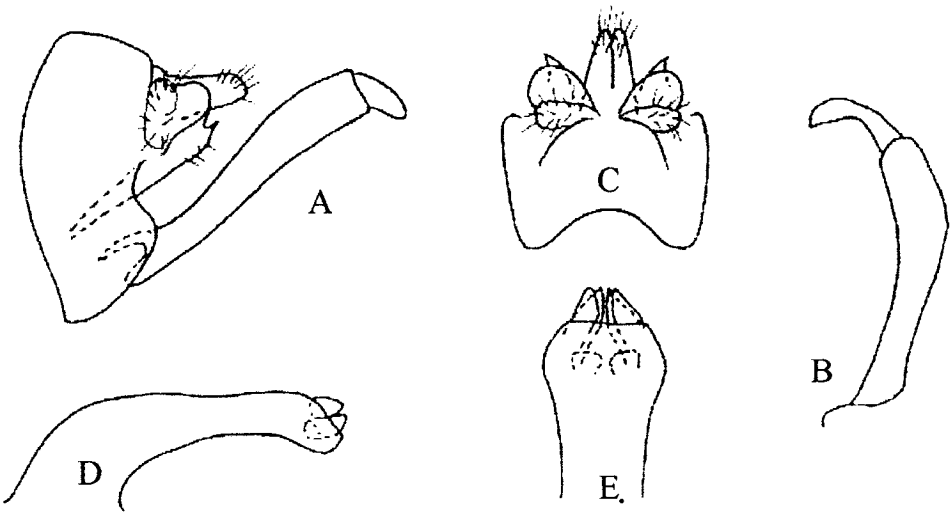


Figure 94. Male genitalia drawing of *Diploma* sp. T10
A) lateral view B) ventral view C) dorsal view D) and E) aedeagus

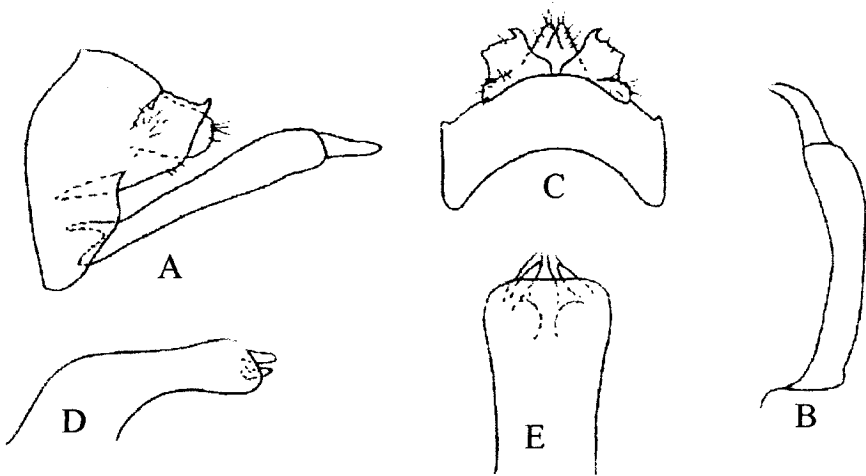


Figure 95. Male genitalia drawing of *Diploma* sp. T7
A) lateral view B) ventral view C) dorsal view D) and E) aedeagus

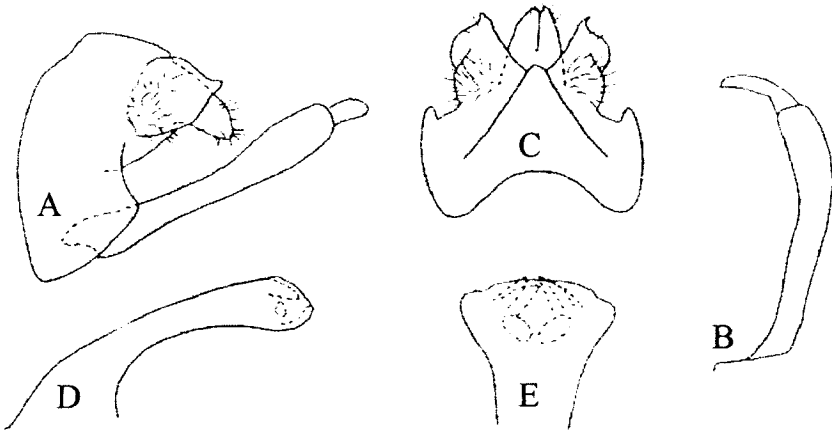


Figure 96. Male genitalia drawing of *Diploma* sp. T 6
A) lateral view B) ventral view C) dorsal view D) and E) aedeagus

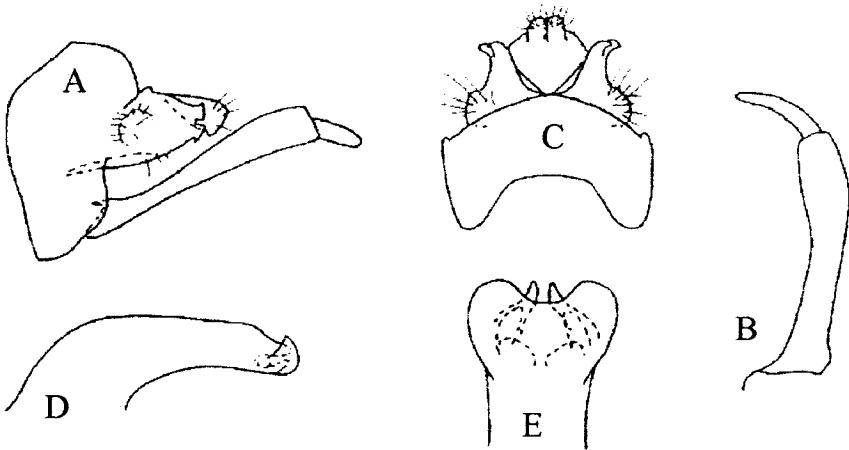


Figure 97. Male genitalia drawing of *Diploma* sp. T4
A) lateral view B) ventral view C) dorsal view D) and E) aedeagus

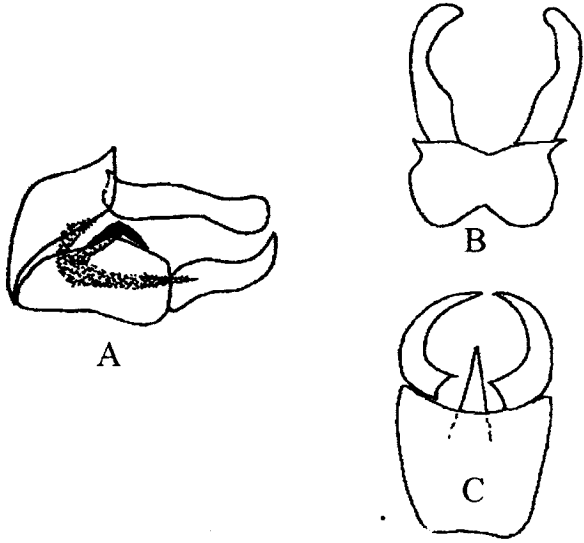


Figure 98. Male genitalia drawing of *Ecnomus* sp.
A) lateral view B) ventral view C) dorsal view

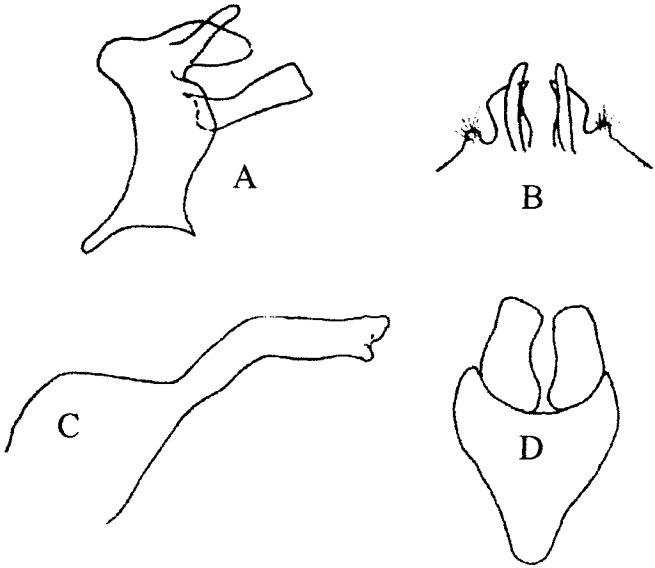


Figure 99. Male genitalia drawing of *Chimarra* sp.
A) lateral view B) ventral view C) dorsal view

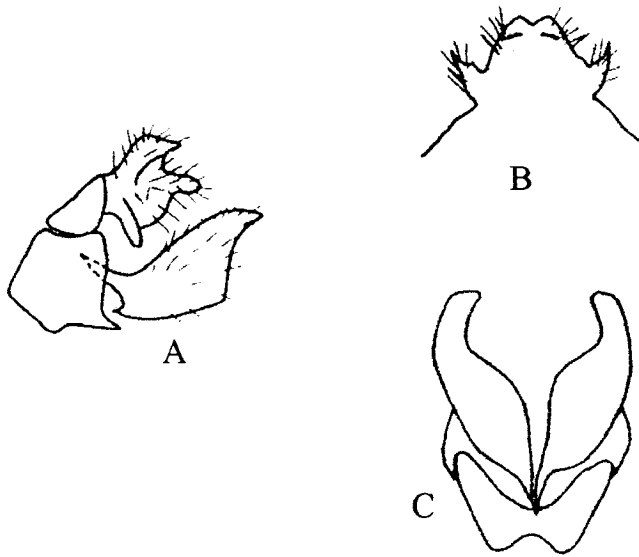


Figure 100. Male genitalia drawing of unknown species
A) lateral view B) ventral view C) dorsal view

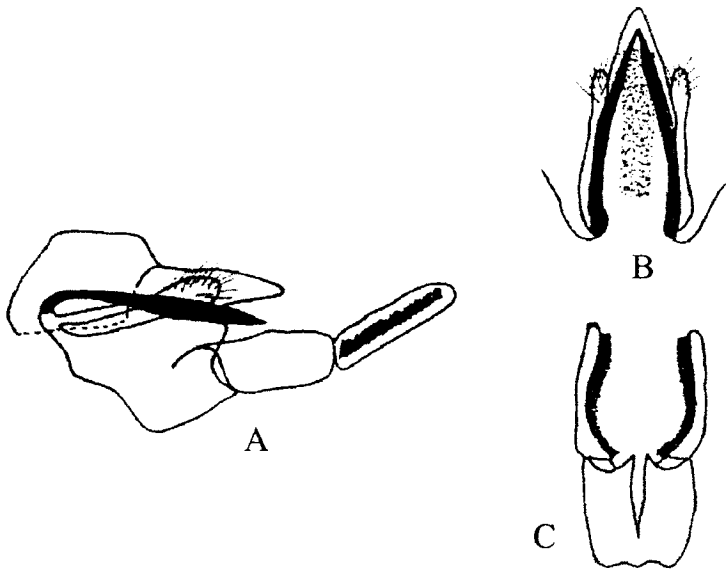


Figure 101. Male genitalia drawing of *Kissura* sp.
A) lateral view B) ventral view C) dorsal view

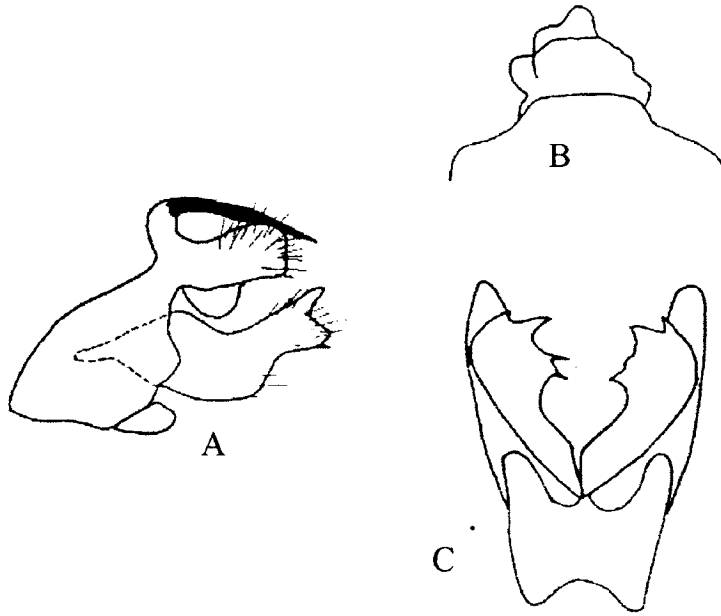


Figure 102. Male genitalia drawing of *Polycentropus* sp.

A) lateral view B) ventral view C) dorsal view

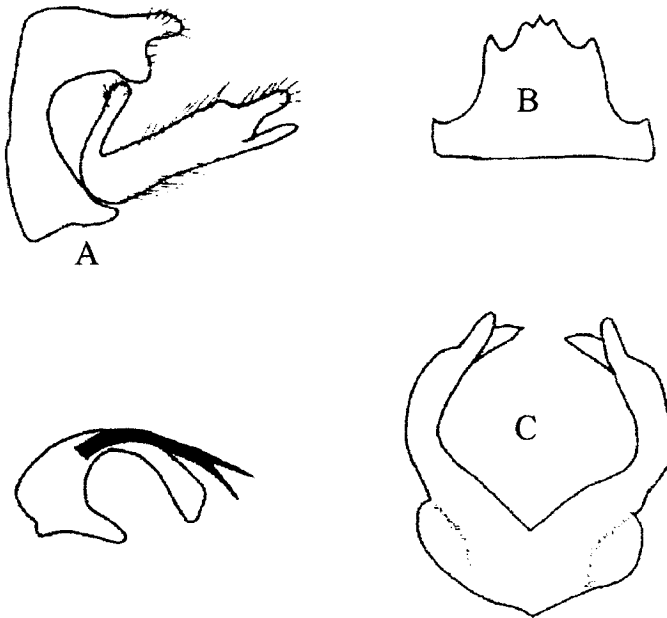


Figure 103. Male genitalia drawing of *Dinarthum* sp.

A) lateral view B) ventral view C) dorsal view

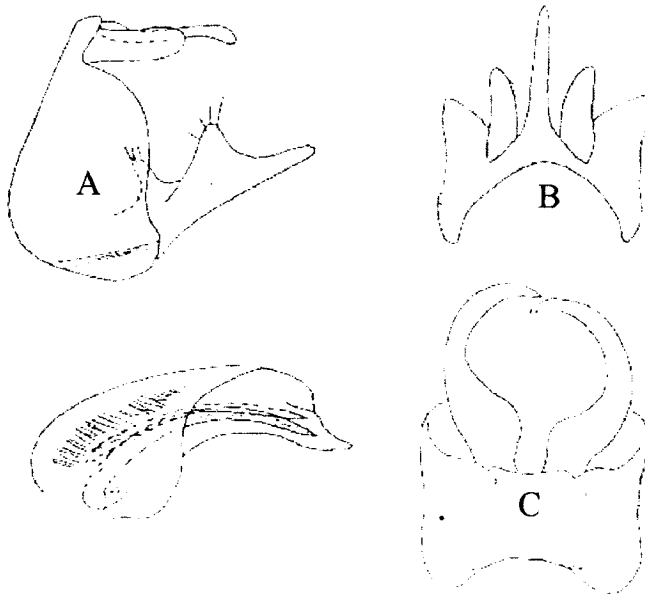


Figure 104. Male genitalia drawing of *Oecetis* sp 1.

A) lateral view B) ventral view C) dorsal view

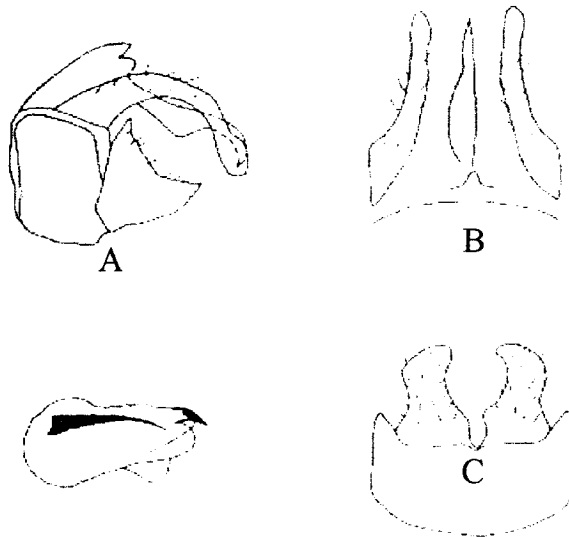


Figure 105. Male genitalia drawing of *Oecetis* sp 2.

A) lateral view B) ventral view C) dorsal view

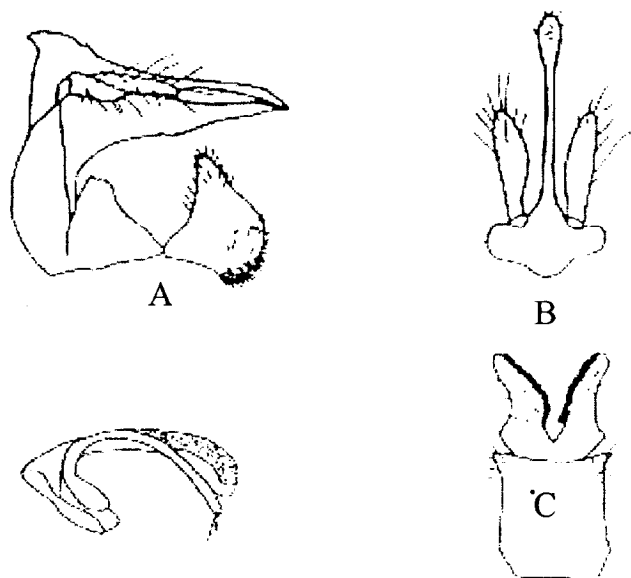


Figure 106. Male genitalia drawing of *Oecetis* sp 3.
A) lateral view B) ventral view C) dorsal view

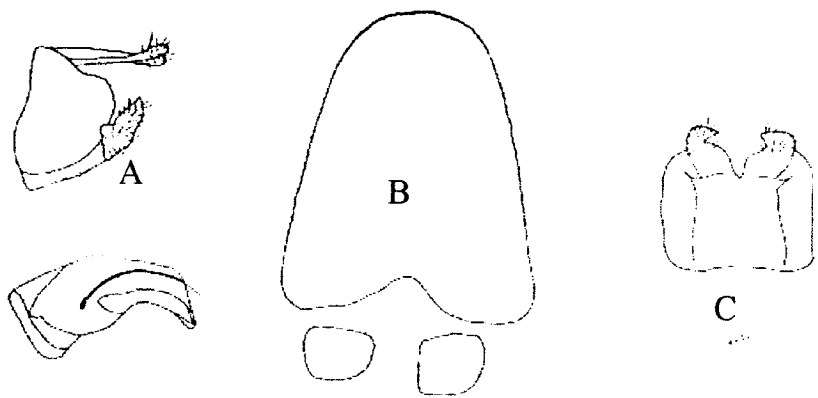


Figure 107. Male genitalia drawing of *Oecetis* sp 4.
A) lateral view B) ventral view C) dorsal view

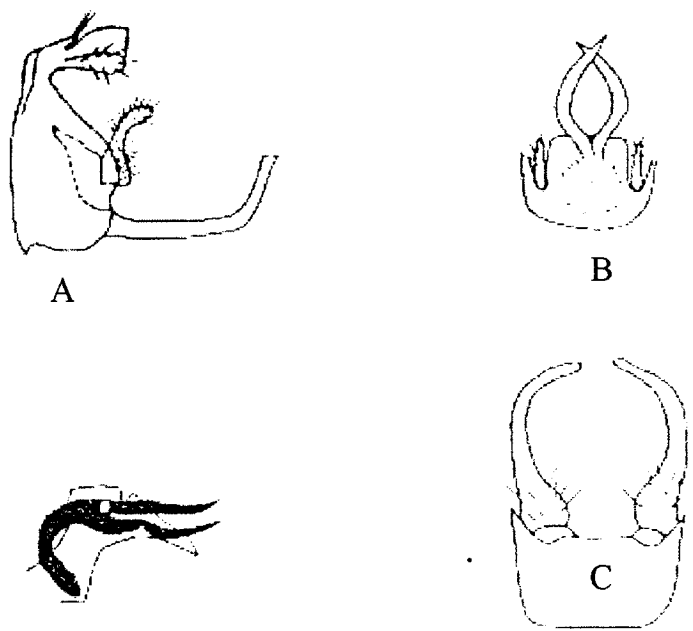


Figure 108. Male genitalia drawing of *Oecetis* sp 5.
A) lateral view B) ventral view C) dorsal view

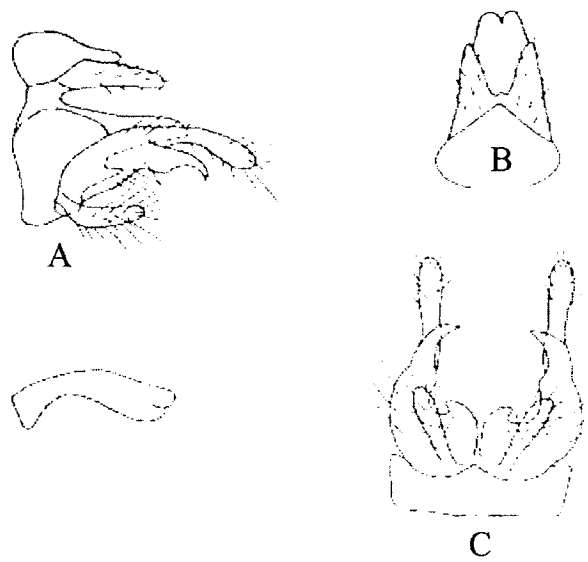


Figure 109. Male genitalia drawing of *Triplectides* sp 1.
A) lateral view B) ventral view C) dorsal view

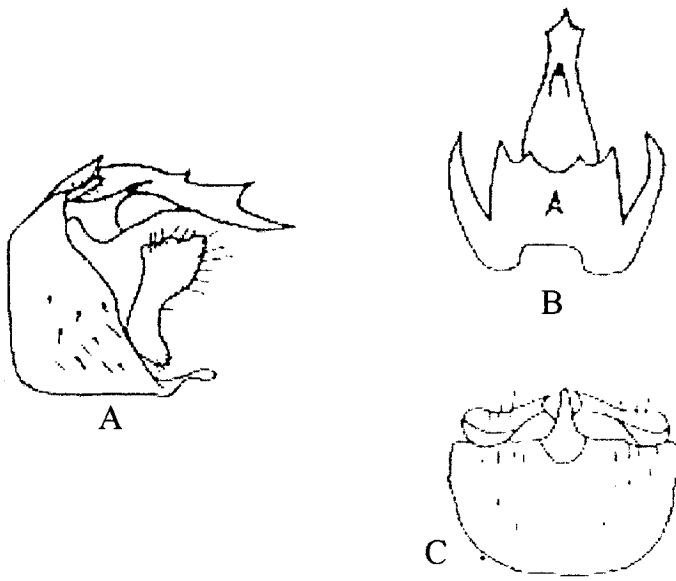


Figure 110. Male genitalia drawing of *Ceraclea or Athripsodes* sp 1.

A) lateral view B) ventral view C) dorsal view

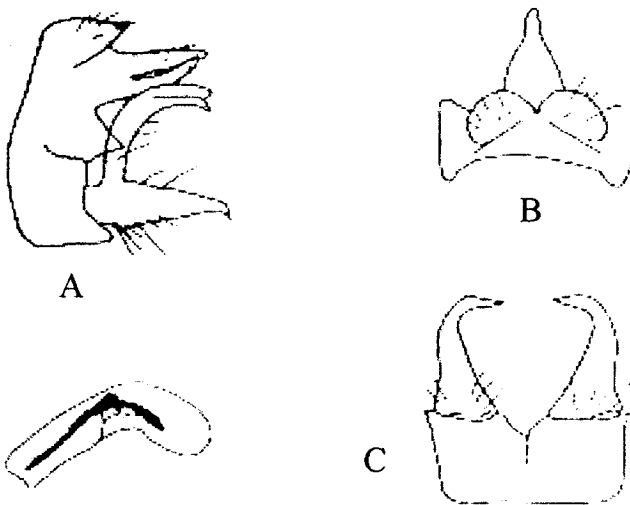


Figure 111. Male genitalia drawing of *Ceraclea or Athripsodes* sp 2.

A) lateral view B) ventral view C) dorsal view

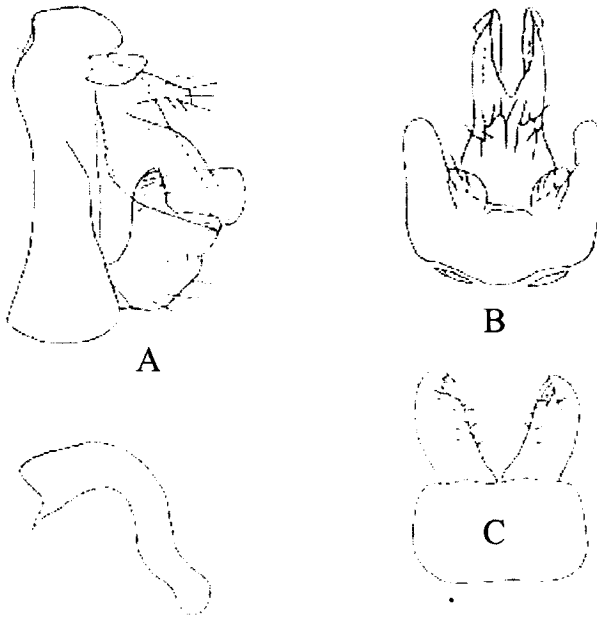


Figure 112. Male genitalia drawing of *Adicella* sp.

A) lateral view B) ventral view C) dorsal view

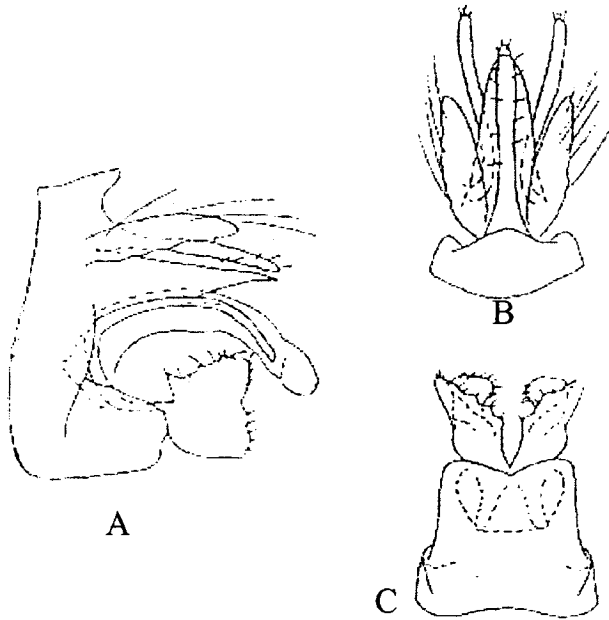


Figure 113. Male genitalia drawing of *Triaenodes* sp.

A) lateral view B) ventral view C) dorsal view

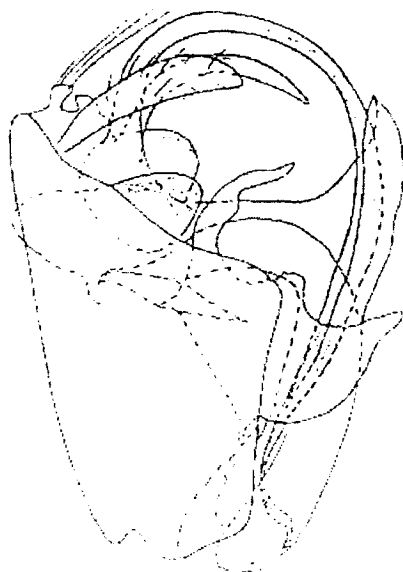


Figure 114. Male genitalia drawing of *Setodes* sp 1.

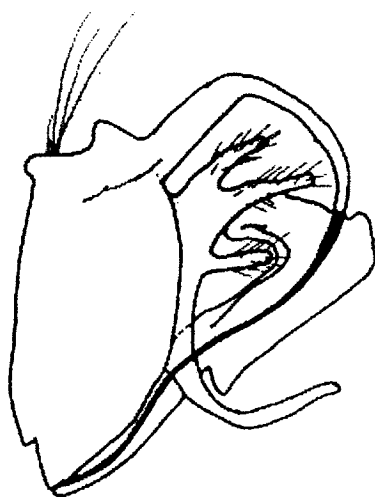


Figure 115. Male genitalia drawing of *Setodes* sp 2.

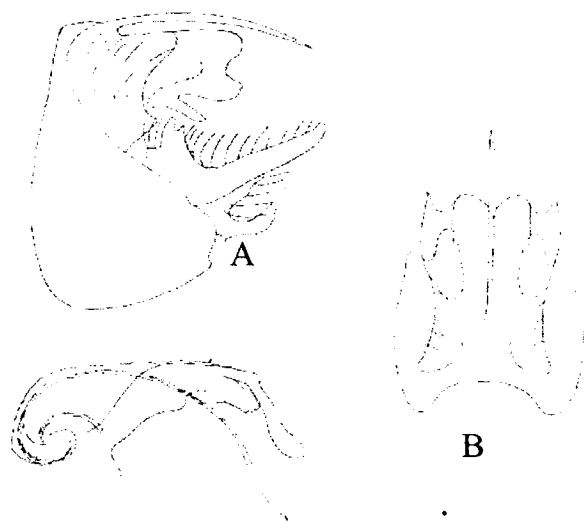


Figure 116. Male genitalia drawing of *Setodes* sp 3.
A) lateral view B) ventral view

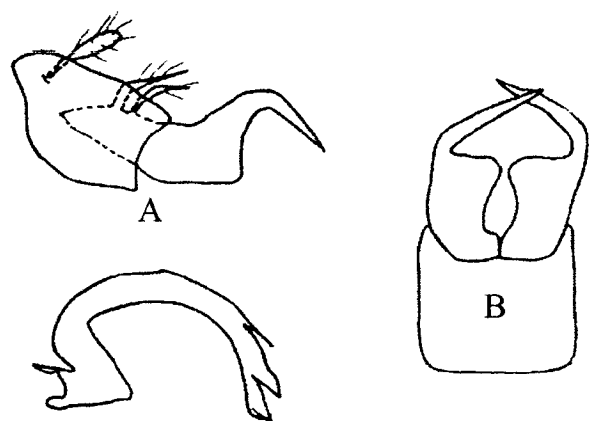


Figure 117. Male genitalia drawing of *Leptoceridae* sp 1.
A) lateral view B) ventral view

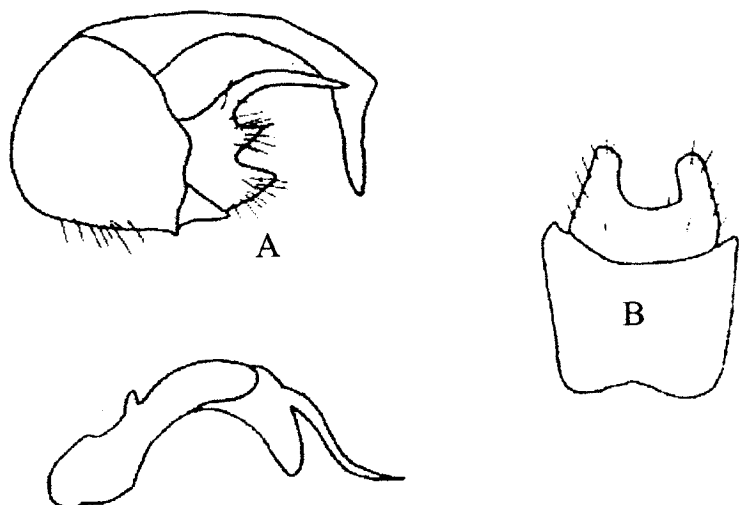


Figure 118. Male genitalia drawing of Leptoceridae sp 2.
A) lateral view B) ventral view

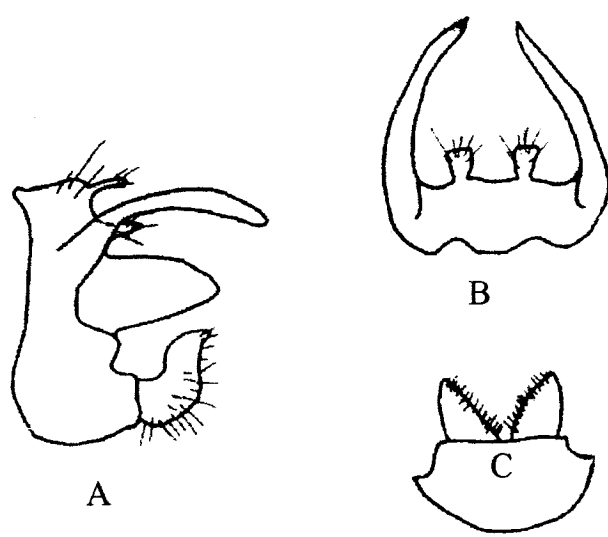


Figure 119. Male genitalia drawing of Leptoceridae sp 3.
A) lateral view B) ventral view C) dorsal view

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Publications:

- Luadee P.,** C. Wongsawad, S. Naboonmee, B. Kantalue and P. Vanittanakom. 1997. Scanning Electron Microscopic Observation of *Heterakis galinarum* Schrank, 1788 (Nematoda:Heterakidae). Journal of Electron Microscopy Society of Thailand, 11 (1): 63-64.
- Luadee P.,** I. Thani and P. Chantaramongkol. 1999. Diel flight activity of caddisflies (Insecta:Trichoptera) in northern Thailand. J. Sci. Technol. 21 (3):293-299.
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