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# Influence of water quality parameters on larval stages of *Pseudoleptonema quinquefasciatum* Martynov 1935 (Trichoptera: Hydropsychidae) in streams of western Thailand

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# Abstract

Aspects of the life cycle of *Pseudoleptonema quinquefasciatum* Martynov 1935 (Trichoptera: Hydropsychidae) were investigated including the possible influence of water quality parameters on larval stages. Larvae were sampled by hand picking at five sites in three streams in western Thailand in December 2014 and April 2015. In total, 2,139 larvae were collected and, by measurement of head capsules, the number of larval instars present was confirmed to be five. Analyses were conducted to determine if the number of larval instars present at any time was influenced by physicochemical water quality parameters. The number of first instar larvae was found to be positively correlated with electrical conductivity, whereas number of second instar larvae was correlated with quantity of dissolved solids and water turbidity, orthophosphate, pH, air and water temperature. A positive correlation was found also between third to fifth instar larvae and levels of dissolved solids, water turbidity, orthophosphate, pH, air and water turbidity, may be major factors in determining the Trichoptera assemblages present in the streams of western Thailand.

Keywords: Life cycle, Pseudoleptonema quinquefasciatum, water variables, larval diet

# 1. Introduction

Aquatic insects are significant elements in lentic and lotic trophic webs, participating in energy flow and nutrient cycling (Whiles and Wallace, 1997). They are also important food resources for fish (Wallace and Webster, 1996) and some insectivorous birds (Ward et al., 1995). The distribution and abundance of insects in freshwater systems is the result of complex interactions between their ecological roles and the physico-chemical conditions that characterize the habitat, and food availability (Merritt and Cummins, 1996). Thus, the community structure depends on a number of factors, such as water quality, type of substrate, particle size of sediment, water flow, sediment organic matter availability, oxygen concentration as well as environmental conditions surrounding the watercourse (Ward et al., 1995; Buss et al., 2004). Because they reflect environmental changes, aquatic insects are often used as indicators of the effects of human activity on water systems, providing information on habitat and water quality (Woodcock and Huryn, 2007). Amongst the aquatic insects, order Trichoptera (or caddisflies) are the most widely distributed; their larvae are common in running water (8-13% of total abundance) (Roback, 1962; Ward, 1992) and they are one of the relatively well-studied orders of aquatic insects in South East Asia (Malicky,

2010; Morse, 2017). The larvae of many species coexist in running waters and are known to have specific habitat and environmental requirements (de Moor, 2007).

Pseudoleptonema Mosely 1933 is a small genus of eight species in the subfamily Macronematinae, family Hydropsychidae. All eight species are described from adults, and larvae have been described for one of the Thai species, P. quinquefasciatum (Prommi et al., 2006). The life cycles of a number of Hydropsychidae species have been described (Edington and Hildrew, 2005), but the life cycle of P. quinquefasciatum in Thailand is not well known. Data are available on adult emergence periods, which may vary across Thailand and Laos (Hoang et al., 2011). This paper explores aspects of the life cycle of this species, and is the first study on a caddisfly life cycle in streams in the Mae Klong watershed of western Thailand, investigating the relationship between environmental factors and its life history. Also, the study of feeding habits and trophic guilds in aquatic insects is important to understand their functional role and relevance in the conservation of the Asian Tropical streams.

# 2. Materials and methods

# 2.1. Study Area

This study was conducted in streams of the Mae Klong Watershed in western Thailand (Fig. 1), the most

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important watershed in western Thailand. Upstream in the watershed area are two main rivers: the Khwae Noi and the Khwae Yai. These rivers run into the Khao Laem and Srinagarind Dams located in the upper region of the Mae Klong Watershed. Downstream in the Kanchanaburi Province, the rivers flow through Ratchaburi Province and enter the Gulf of Thailand in the Samut Songkhram Province. Five sampling sites in three streams were chosen for this study. The streams were in the upstream section of Khwae Noi River before it flows into the Khao Laem Dam. These three streams are the Huai Pakkok, PK1 and PK2; the Huai Kayeng, KY1 and KY2; and the Huai Lijia, LJ (Fig. 1). Details of latitude and longitude of the stations and stream characteristics are shown in Table 1. The streams pass through areas of forest and cultivation, and three of the collecting sites are bordered by villages.

Site	Latitude/ Longitude	Description of stream
PK1	13°32.135´ N, 099°17.842´ E	Cobble predominant, forest and cultivation on both sides
PK2	13°32.077´ N, 099°15.495´ E	Bedrock and cobble predominant, pool in the middle, gravel and sand. Village and cultivation on both sides
KY1	13°27.658´N, 099°15.348´E	Man-made concrete upstream, gravel, woody debris and other stable substrates. Forest and highland, village both sides
KY2	13°30.241´N, 099°15.883´E	Cobble predominant, pool in the middle, gravel and sand. Village and cultivation on both sides



Figure 1. Map of Thailand showing locations of the five sampling sites, PK1, PK2, KY1, KY2 and LJ, and photographs of the streams at the sites.

## 2.2. Environmental Variables

The physicochemical water quality variables were measured simultaneously with collection of samples. Three replicates of selected physicochemical parameters were recorded directly at the sampling sites: pH (using a portable pH meter); water temperature (WT, °C) and dissolved oxygen (DO, mg/L) (using an oxygen meter — EcoScan DO 110); total dissolved solid (TDS, mg/L) and electrical conductivity (EC,  $\mu$ S/cm) (using an EC meter — CyberScan CON 11). Water samples from each site and sampling period were collected in polyethylene bottles (500 mL) and held at 4°C for study in the laboratory. Ammonia-nitrogen (NH<sub>3</sub>-N, mg/L) was measured by the Nessler method, using a spectrophotometer (DR/2010 model 49300-00; Hach, USA); nitrate-nitrogen (NO<sub>3</sub>-N, mg/L) by the cadmium reduction method, using a

spectrophotometer (DR/2010 model 49300-00; Hach, USA); orthophosphate; (PO<sub>4</sub><sup>3–</sup>, mg/L) by the ascorbic acid method, using a spectrophotometer (DR/2010 model 49300-00; Hach, USA); and turbidity (TUB, NTU) using a spectrophotometer (DR/2010 model 49300-00; Hach, USA). Alkalinity (ALK, mg/L) was measured by the bromocresol green-methyl red indicator titration in accordance with the standard method procedures (APHA *et al.*, 1992).

#### 2.3. Sampling Procedure

The larvae and pupae of *Pseudoleptonema quinquefasciatum* (Fig. 2A, B) were collected at five sampling sites in the streams of western Thailand (Figure 1). At each sampling site, a stretch of approximately 50 m was chosen for collection of samples. The samples were collected in December 2014 and April 2015, by hand

picking from the surface of boulders that were lifted from water around steps and rapids (Fig. 2C). The sampling time at each site was 2h. Collected *P. quinquefasciatum* larvae and pupae were preserved in 80% ethanol. Larval

characteristics were described and illustrated by Prommi *et al.* (2006). Larvae of *P. quinquefasciatum* construct an open-ended chamber retreat of fine sand, silk and plant debris, and fastened to a rock and capture nets (Fig. 2C).



Figure 2. *Pseudoleptonema quinquefasciatum* larvae (A), head, dorsal view (B) and larval case (red arrows) constructed from sand and detritus attached on stony substrate (C).

# 2.4. Life Cycle Analysis

A total of 2,139 individual of larvae samples were sampled in this study. In the laboratory, all larvae were sorted to instar using head width, following the method described by MacKay (1978). Head capsule widths were measured using an ocular micrometer. The number of larval instars was determined by analyzing the size frequency distributions of head capsule widths, following which the width ranges for instars were determined for each site. Size frequency distribution diagrams were constructed for each site based on the relative percent of the total number of individuals at each instar collected each month. Once larvae were assigned to instars, the variability in size at each instar was assessed for each site. Voucher specimens were deposited in the Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom Province, Thailand.

## 2.5. Gut Content Analysis

For a qualitative determination of the food items of *P. quinquefasciatum*, 10 larvae from each sampling site and sampling occasion were dissected under a stereomicroscope (Olympus SZ51). The whole digestive tracts were removed to a glass slide with water, shredded, and examined under a compound microscope (Olympus CX31). Contents were separated into plant tissue, algae, plankton, arthropod fragments, and amorphous tissue.

### 2.6. Data Analysis

The relationship between all larval stages and recorded environmental variables was determined for all sampling dates together. A Principle Component Analysis (PCA) was performed using the PC-ORD 5.1 software.

# 3. Results

## 3.1. Environmental Variables

The means and standard deviations of measured physicochemical water quality parameters at the sampling sites taken during the two sampling periods were summarized in Table 2. The water temperature, dissolved oxygen, turbidity, ammonia-nitrogen, orthophosphate and nitrate-nitrogen did not vary significantly over the sampling period (P>0.05), whereas total dissolved solids, electrical conductivity, pH and alkalinity varied significantly during the period (P < 0.05). The temperature varied from 26.38 (KY2) to 29.96 C° (PK2). The lowest mean value for dissolved oxygen (3.58 mg/L) was taken in Huai Lijia (LJ), and the highest values in Huai Kayeng (KY2) (5.76 mg/L). All sampling sites were slightly alkaline with pH showing little variation of (8.05 (LJ) -8.51 (KY1)); the highest mean value for alkalinity was recorded for KY2 (8.43 mg/L) and lowest was registered for PK1 (12.80 mg/L). The highest mean value of total dissolved solids and electrical conductivity (129.52 mg/L, 258.28 µS/cm) were observed at KY2 and the lowest at PK1 (40.25 mg/L, 74.08 µS/cm). The mean turbidity was highest in LJ (18.66 NTU) and lowest at PK1 (6.00 NTU). The mean dissolved nutrients, ammonia-nitrogen, orthophosphate, nitrate-nitrogen concentrations varied from 0.19 (PK2) to 0.36 mg/L (PK1), 0.40 (PK1) to 0.64 mg/L (LJ), and 0.95 (PK2) to 1.70 mg/L (PK1), respectively.

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Parameters/sites	PK1	PK2	KY1	KY2	LJ
WT (°C)	27.33±1.97 <sup>a</sup>	29.96±3.91 <sup>a</sup>	$28.48 \pm 5.43^{a}$	26.38±1.34 <sup>a</sup>	27.58±1.71 <sup>a</sup>
DO (mg/L)	$4.59{\pm}2.89^{a}$	$4.59 \pm 4.89^{a}$	$5.10{\pm}2.78^{a}$	$5.76 \pm 1.80^{a}$	$3.58 \pm 4.12^{a}$
TDS (mg/L)	$40.25 \pm 997^{a}$	$63.48{\pm}21.37^{a}$	127.3±53.31 <sup>b</sup>	$129.52 \pm 70.92^{b}$	114.34±25.92 <sup>ab</sup>
ES (µS/cm)	$74.08 \pm 28.63^{a}$	132.17±35.58 <sup>ab</sup>	272.22±82.18 <sup>b</sup>	258.28±142.43 <sup>b</sup>	228.16±51.14 <sup>b</sup>
pH	8.46±0.09 <sup>a</sup>	8.36±0.19 <sup>ab</sup>	$8.51 \pm 0.12^{ab}$	$8.43{\pm}0.00^{ab}$	$8.05{\pm}0.07^{b}$
ALK (mg/L)	$12.80{\pm}0.28^{a}$	$16.00 \pm 0.56^{a}$	33.3±10.32 <sup>ab</sup>	37.50±12.02 <sup>b</sup>	29.50±7.21 <sup>a</sup>
TUB (NTU)	$6.00{\pm}4.24^{a}$	$10.00 \pm 0.46^{a}$	16.00±11.31 <sup>a</sup>	7.50±5.33ª	$18.66 \pm 7.54^{a}$
NH <sub>3</sub> -N (mg/L)	$0.36{\pm}0.07^{a}$	$0.19{\pm}0.09^{a}$	$0.22 \pm 0.03^{a}$	0.23±0.01 <sup>a</sup>	$0.20\pm0.24^{a}$
$PO_4^{3-}$ (mg/L)	$0.40{\pm}0.11^{a}$	$0.59{\pm}0.09^{a}$	$0.47{\pm}0.12^{a}$	$0.48{\pm}0.01^{a}$	$0.64{\pm}0.53^{a}$
NO <sub>3</sub> -N (mg/L)	1.70±0.07 <sup>a</sup>	0.95±0.21 <sup>a</sup>	$0.96{\pm}0.04^{a}$	1.58±0.63 <sup>a</sup>	1.15±0.67 <sup>a</sup>

Table 2. Physico-chemical variables recorded at all the sampling sites (Mean ±SD).

Remark: a, b = the relationship of environmental factors is similar in the sampling sites, ab = the relationship of environmental factors is different in the sampling sites.

# 3.2. Larvae Instars of Pseudoleptonema Quinquefasciatum

Five instars were observed in *Pseudoleptonema* quinquefasciatum using 2,139 specimens. Head widths of first instars ranged from 0.20 to 0.29 mm (n = 22); of second instars from 0.30 to 0.39 mm (n = 143); of thread range of head capeula widths (mm) for larged

instars from 0.40 to 0.59 mm (n = 491); of fourth instars from 0.60 to 0.79 mm (n = 1,404); and of fifth instars from 0.80-1.15 mm (n = 73) (Fig. 3–4, Table 3). Accordingly, larvae determined as first to fifth instar were collected from four of the sampling sites; but no first and second instar larvae were found at site KY2 (Table 3).

Table 3. Median and range of head of	psule widths (mm) for larval instars of Pseudo	leptonema quinquefasciatum.
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Instar	Number of measurements			Total	Median head width (mm)	Range of head width (mm)		
	PK1	PK2	KY1	KY2	LJ	-		
Ι	1	1	4	-	22	28	0.245	0.20-0.29
II	1	26	20	-	96	143	0.345	0.30-0.39
III	9	197	89	3	193	491	0.495	0.40-0.59
IV	41	296	316	26	725	1404	0.695	0.60-0.79
V	5	17	31	3	17	73	0.975	0.80-1.15



Figure 3. The frequency distribution of larval instars of *Pseudoleptonema quinquefasciatum* Martynov 1935 at all sampling sites, based on head capsule width (n = 2,139) in December 2014 and April 2015.



Figure 4. Proportion of larval instars of *Pseudoleptonema quinquefasciatum* Martynov 1935 at all sampling sites, assigned on basis of the distribution of head capsule width at each sampling site.

# 3.3. Gut Content Of Pseudoleptonema Quinquefasciatum Larvae At Five Sampling Sites

Larval gut contents were assessed qualitatively. Gut contents of 50 larvae were plant tissue, algae, plankton, arthropod fragments, and amorphous detritus. The first three instars appeared to select plankton, algae and plant tissue, whereas guts of larvae of the final two instars contained amorphous detritus and arthropod fragments. Thus, the gut content analysis indicated that larvae are omnivorous filterers (Fig., Table 4).



**Figure 5.** Gut contents from *Pseudoleptonema quinquefasciatum* Martynov 1935 larvae. A = plant tissue, B = algae, C = plankton, D = arthropod fragment, E = amorphous detritus.

**Table 4.** Frequency of food items found in gut contents of eachlarval instar *Pseudoleptonema quinquefasciatum*. + = low, ++ =medium, +++ = high

Larval instar/type of food	1	2	3	4	5
Plant tissue	+	+	++	++	++
Algae	++	++	++	+++	+++
Plankton	+++	+++	+++	+++	+++
Amorphous detritus	+	+	+	++	++
Animal tissue	+	+	+	++	++

3.4. Larval instars of Pseudoleptonema

quinquefasciatum and environmental variables

According to the PCA ordination (Fig. 6), the upper portion of the ordination indicates that the factors with greatest influence on larvae were dissolved oxygen (DO), ammonia-nitrogen (NH<sub>3</sub>-N), nitrate-nitrogen (NO<sub>3</sub>-N) and alkalinity (AKL) in the 3<sup>rd</sup> to 5<sup>th</sup> instar larva at site KY2. The electrical conductivity (EC) appeared to be correlated positively with presence of the 1<sup>st</sup> instar larva in PK1. In contrast, as shown by the lower portion of the ordination, at sites KY1, PK2 and LJ, number of 2<sup>nd</sup> instar larvae appear to be correlated with turbidity (TUB), total dissolved solids (TDS), orthophosphate (PO<sub>4</sub><sup>3-</sup>), pH, air temperature (AT) and water temperature (WT).



Figure 6. PCA ordination plot based on larval instars of *Pseudoleptonema quinquefasciatum* Martynov 1935 and physico-chemical variables and sampling date.

# 4. Discussion

#### 4.1. Environmental Variables

In freshwater ecosystems, variables such as water temperature and water temperature fluctuations or dissolved oxygen can influence life histories through increased growth rates and number of generations produced annually (Sweeney, 1984). Principle Component Analysis (PCA) showed that dissolved oxygen, ammonianitrogen, nitrate-nitrogen and alkalinity had an influence on the 3<sup>rd</sup> to 5<sup>th</sup> instar larvae in KY2. The electrical conductivity was correlated positively with number of 1<sup>st</sup> instar larva in PK1, whereas the turbidity, total dissolved solids, orthophosphate, pH, and water temperature influenced presence 2<sup>nd</sup> instar larva in the KY1, PK2 and LJ.

Water temperature varied from 26.38 (KY2) to 29.96 C° (PK2). Water temperatures were relatively lower during the wet season than during the dry season. The minimum (25.0°C) and maximum temperatures (35.5°C) were within the range usually observed for tropical waters. At the upstream site, the riparian areas were covered by vegetation that shaded the water and may have kept water temperatures slightly lower — Hauer and Hill (1996) reported that shading from riparian trees beside a smaller watershed stream helped to maintain a lower water temperature.

The lowest mean value of dissolved oxygen (3.58 mg/L) was found in the Huai Lijia (LJ), and the highest values were observed in Huai Kayeng (KY2) (5.76 mg/L). Sources of dissolved oxygen in aquatic environments include the atmosphere and photosynthesis, the concentration being dependent on solubility (which decreases with increasing water temperature). Dissolved oxygen is also reduced by respiration of submerged plants, animals and aerobic bacteria, including their metabolic activity in decomposing dead organic matter (Gupta and Gupta, 2006). Boulton and Brock (1999) pointed out that strong water currents result in turbulent flow which tends to increase or replenish dissolved oxygen.

The pH of the water at all sampling sites was slightly alkaline and showed very little variation (pH 8.05 (LJ) – 8.51 (KY1). The accumulation of free carbon dioxide due

to reduced photosynthesis by phytoplankton and rooted macrophytes can result in lower pH values in the water while intense photosynthesis reduces free carbon dioxide content and results in higher pH values (Egborge, 1994; Gupta and Gupta, 2006). The pH showed a decrease in the rainy season. This may be attributed to the increased organic matter washed into the stream by surface runoff during the wet season. This would tend to reduce dissolved oxygen through organic decomposition, thus lowering pH.

The highest mean value of total dissolved solids and electrical conductivity (129.52 mg/L, 258.28  $\mu\text{S/cm})$  was observed at KY2 and lowest was at PK1 (40.25 mg/L, 74.08 µS/cm). The general trend in this study was that electrical conductivity tended to increase in the dry season compared with the wet season. Increases in electrical conductivity could result from low precipitation, higher atmospheric temperature resulting in higher evapotranspiration rates, higher total ion concentration, and saline intrusions from underground sources. It could also be due to a high rate of decomposition and mineralization by microbes and by nutrient replenishment from bottom sediments (Egborge, 1994). Increased electrical conductivity and total dissolved solids could also be due to contamination of water from agricultural activities and industrial effluent, degrading water quality (Lenat and Crawford, 1994).

The mean turbidity was highest at LJ (18.66 NTU); the lowest was recorded at PK1 (6.00 NTU). The higher turbidity was recorded during the wet season and may have been due to heavy rainfall, the increase in suspended solids impeding light and thereby increasing turbidity. The adverse effects of turbidity on freshwater include decreased penetration of light which then reduces primary and secondary production, increased adsorption of nutrient molecules to suspended materials, making the nutrients unavailable for plankton production, and decreased oxygen concentration. The particulate matter can clog the filterfeeding apparatus and digestive organs of planktonic organisms, which in turn may adversely affect the development of larvae (Gupta and Gupta, 2006).

The highest mean of alkalinity was recorded in KY2 (8.43 mg/L) and lowest was registered in PK1 (12.80 mg/L). Water bodies in the tropics usually show wide fluctuations in total alkalinity, the values depending on the location, season, plankton populations and nature of bottom depositions. Highly productive waters tend to have alkalinity values above 100 mg/L and for freshwater aquaculture the values optimally should be between 40-200 mg/L. Alkalinity values above 300 mg/L have been reported to affect the spawning and hatching of freshwater fish adversely (Gupta and Gupta, 2006).

The mean dissolved nutrients, ammonia-nitrogen, orthophosphate, nitrate-nitrogen concentrations varied from 0.19 (PK2) to 0.36 mg/L (PK1), 0.40 (PK1) to 0.64 mg/L (LJ), and 0.95 (PK2) to 1.70 mg/L (PK1), respectively. Nitrates are the most oxidized forms of nitrogen and the end product of aerobic decomposition of organic nitrogenous matter. Natural waters in their unpolluted state contain only minute quantities of nitrates. The highest nitrate values occurring during the monsoon/post monsoon season may be primarily due to organic materials entering from the catchment area during periods of high rainfall (Das *et al.*, 1997). The increasing levels of nitrates are likely to be due to freshwater inflow,

litter fall decomposition and terrestrial run-off during the monsoon/post monsoon season (Karuppasamy and Perumal, 2000). Another possible source of nitrate recruitment is through oxidation of ammonia from nitrogen to nitrites (Rajasegar, 2003). The low values during the summer/pre-monsoon period may have been due to utilization of nitrates by phytoplankton activity. In addition, nitrates may be obtained from natural and human activities, both from household wastes and from fertilizers used in agriculture, consistent with observations by Omernik (1977) who indicated that the levels of nutrients in streams were positively correlated with the percentage of land in agriculture. Furthermore, although orthophosphate is usually a minor compound in nature (Jarvie et al., 2002), its higher values in stream water at our sampling sites was probably from agricultural fertilizers which are leached into water when it rains.

4.2. Life Cycle of P. Quinquefasciatum

The adults of *P. quinquefasciatum* occur all year round (Prommi, 2007; 2015). In our study, pre-pupae and pupae of *P. quinquefasciatum* were recorded throughout the study period. Hydropsychidae undergo five larval instars (Edington and Hildrew, 1995; Waringer and Graf, 1997). In our samples, five larval instars of *P. quinquefasciatum* were recognized at all sampling sites except for the KY2, where no first and second instar larvae were recorded (Table 2). This is due to different collecting efforts but the discrepancy between the lists partly results from the often important adult flight activity.

Trichoptera play an important role in community biomass and secondary production in the stream in Mae Klong watershed. As with many tropical aquatic invertebrates, P. quinquefasciatum seems to have a multivoltine life cycle, the year-round favorable environmental conditions resulting in continuous growth and development (Humantinco and Nessimian, 2000). Our study, the first such report for Thailand, showed that larval P. quinquefasciatum in the streams from the Mae Klong watershed are mainly omnivorous filterers (Maneechan et al., 2018), moving through several trophic guilds as development from instar to instar takes place. Analyses of gut contents of larvae indicated that the first three instars appear to select plankton, algae and plant tissue, whereas the later instars had consumed amorphous detritus and arthropod fragments.

### 5. Conclusion

The development of *P. quinquefasciatum* was asynchronous, with many overlapping cohorts coexisting in the rivers. It appears that physico-chemical factors such as electrical conductivity, total dissolved solids, water turbidity, orthophosphate, pH, air and water temperature, dissolved oxygen, ammonia-nitrogen, nitratenitrogen, and alkalinity are significant in influencing the life stages and thus the biomass of this hydropsychid caddisfly species in streams of western Thailand. Hydropsychidae are richly diverse in Thai streams and other species can be expected to be affected in the same way.

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