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Assessment of Water Quality in the Stream Flows into the Krasiow Dam based on Hydropsychid Larvae (Insecta, Trichoptera)

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Abstract

The use of altered gill morphology in hydropsychid larvae (Hydropsychidae: Trichoptera) as water quality biomonitoring was conducted in streams flows into Krasiow Dam, Dan Chang district, Suphan Buri Province, Thailand. Samples were collected over a period of one year January to December 2019. In total, 5,583 individual hydropsychid larvae belonging to four genera (*Amphipsyche meridiana, Macrostemum indistinctum, Cheumatopsyche* spp. and *Potamyia* spp.) were identified in this study. The percentage of individuals with at least some abnormality (HAI) and hydropsychid gill abnormality indice (HYI) was the highest in February and lowest in October. Canonical Correspondence Analysis (CCA) indicated the species *Amphipsyche meridiana* were positively correlated with water temperature, pH, and ammonia-nitrogen. Total dissolved solids were negatively associated with the *Cheumatopsyche* spp. and *Macrostemum indistinctum*. The outcomes of this study is expected that analyses of morphological deformations in tracheal gills could become a support for traditional physicochemical analyses of water quality or traditional biomonitoring.

Introduction

The net-spinning Hydropsychidae are one of the largest families of caddisflies (Trichoptera), with about 1,900 described adult species worldwide (Morse, 2017). Larvae of hydropsychids live in running waters and are generally collectors-filterers, although some species are predators of larval black flies (Merritt & Cummins, 1996). They usually construct a silken filter net at the entrance to their fixed tubular retreat (Wiggins, 1996). Larvae present a high ecological diversity and display a wide range of tolerance to different levels of pollution, which makes them very useful organisms in biological water quality monitoring programs (Resh, 1995). Due to their high abundance, wide distribution and important role in the ecology of the lotic ecosystem, the hydropsychid caddisflies have been increasingly utilized in the biomonitoring and impact assessment of the river (Vuori, 1995). Hydropsychid larvae have been used as sentinel organisms when measuring the environmental concentration levels of contaminants (Cain et al., 1992; Vuori, 1994; Clements & Kiffney, 1994) and also as monitoring and toxicity test organisms both in the laboratory and in the field (Vuori, 1995). In these studies, the individual, population and guild responses of the hydropsychid larvae have been found to be sensitive indicators of

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river pollution.

The assessment of the impact of pollution using individuals and populations of benthic invertebrates has a number of advantages over the traditional biomonitoring methods utilizing community structure of benthic invertebrates (Rosenberg & Resh, 1993). Although importance in describing the general state of the river ecosystem, the community derived parameters are robust, inconclusive and often insensitive to gradual environmental changes. Pollution, as a selective factor, mainly affects the mortality, growth and reproduction of individuals and populations. Hence, individual and population parameters are likely to be more sensitive and accurate indicators of increasing stress than parameters utilizing whole communities. Furthermore, while the objective criteria for the determination of healthy ecosystems and communities is lacking, the occurrence of unhealthy individuals is often easily distinguishable and can be linked to the effects at the population level (Forbes & Forbes, 1994). However, studies dealing only individual and population responses are unable to detect changes in such community level phenomena as species interactions and diversity. These changes may become apparent if a group of ecologically related species, is studied (Petersen, 1986).

Upon exposure to pollutants, some changes at the biochemical, cellular or tissue levels must precede changes at the higher levels of biological organization (Giesy & Graney, 1989). Morphological abnormalities in the tracheal gills and ion regulation organs, and anal papillae of hydropsychid larvae indicate cellular and tissue which leads to the impairment of the individual's respiratory or osmoregulatatory functions (Camargo, 1991; Vuori, 1994). Hence, the simultaneous study of the morphology, life cycle and species composition of the hydropsychid larvae may provide information on the impact of pollution at a cellular, individual and population level and even offer clues to possible mechanisms at the community level (Vuori, 1995). The aim of this study was to analyze the usefulness of hydropsychid larvae in evaluation of water quality in stream that surrounding by agricultural activities. Our intention was to find a relationship between gill morphological changes and level of water contamination in the studied streams. The outcome of this study is expected that analyses of morphological deformations in tracheal gills could become a support for traditional physicochemical analyses of water quality or traditional biomonitoring.

Materials and methods

1. Study area

The samples were conducted in the stream flows into the Kasiow Dam, Dan Chang district, Suphan Buri Province (14°56.859′ N, 099°38.118′ E, 81 m asl.) (Fig. 1). Kasiow stream, the stream that flows through Kasiow Dam, is a main branch of Thachin River, approximately 140 kilometers. The upstream is located between Khao La and Khao Yai in the north part of Ban Rai district, Uthai Thani Province. This stream flows pass the area in Dan Chang district, which the sugarcane plantation and sugar factories are located near the stream. In addition, fish cage farming are reared in the area of Kasiow Dam.

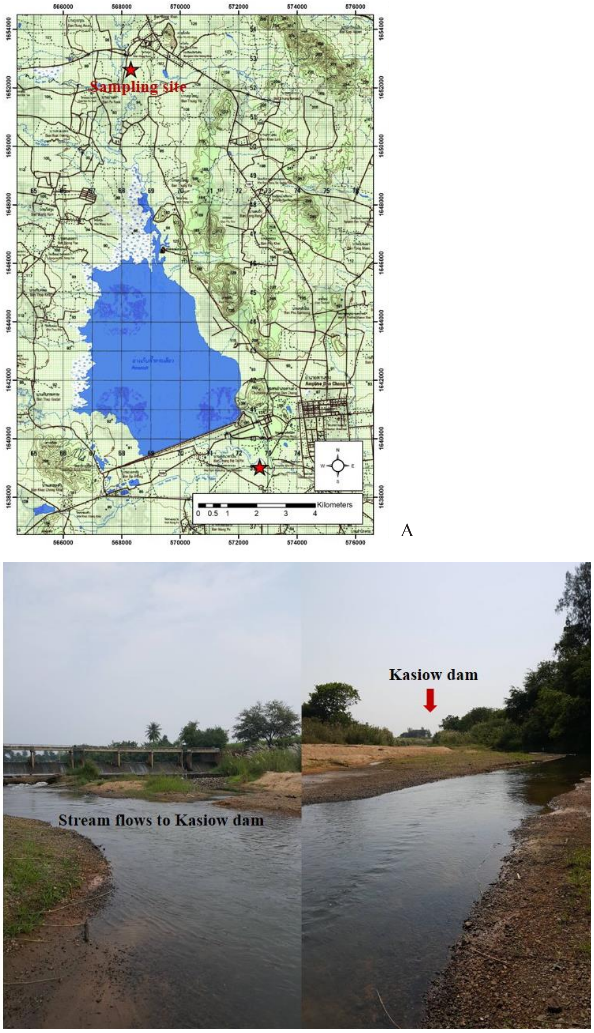

Fig. 1 Map showing the sampling site (A) of the stream flows (B) into Kasiow Dam in Dan Chang district, Suphan Buri Province, Thailand

2. Environmental parameters

In order to observe the relationship of caddisflies species occurrence to environmental parameters the following data were recorded for water body. Three replicates of selected physicochemical water quality parameters were recorded directly at the sampling site. Water temperature (°C) and dissolved oxygen (DO, mg/L) were measured in situ with HQ40D Portable Dissolved Oxygen and Conductivity/TDS Meter. The pH was measured by a pH-meter Waterproof Model Testr30. Water samples from each collecting period were stored in polyethylene bottles (500 mL). Nitrate-nitrogen (mg/L NO_3-N, ascorbic acid method), orthophosphate (mg/L PO_4^{3-}, cadmium reduction method) and ammonia-nitrogen (mg/L NH_3-N, the Nessler method) were measured by using the Hach DR/2000 spectrophotometer (APHA, 1992).

3. Sample collection and identification

The caddisfly larvae were collected from stream flows into Kasiow Dam during January to December 2019, except the month of April, May and June. In April, when the stream water was particularly warm, the caddisfly larvae were not found. In May and June during the storm season the stream water was very turbid. At each sampling period, semi-quantitative samples of caddisfly larvae from the different microhabitats (riffles, depositional zones and different types of vegetation) were collected. A D-frame (Merritt & Cummins, 1996) aquatic hand net (mesh sieve 250 µm) was used. The samples of the aquatic hand net were poured into white trays. Living caddisflies were sorted and transferred into properly-labelled plastic containers, preserved in 80% ethanol and brought to the laboratory for analysis.

In the laboratory, caddisfly larvae were sorted on a petri dish and were identified to the genus and species level using taxonomic keys by several authors (Wiggins, 1996; Dudgeon, 1999; Yule & Yong, 2004; Prommi, 2007). All the sorted samples were kept in properly-labelled vials containing 80% ethanol.

4. Data analysis

Structural changes in the hydropsychid gills were studied under a stereomicroscope and quantified using an ocular micrometer. Small and light pigmentation spots were considered as part of natural variation and so were not categorized as a morphologically abnormal. Two biomarkers were evaluated: (1) Hydropsychid abnormality incidence (HAI), referring to the proportion of individuals with at least some abnormalities, and (2) Hydropsychid gill abnormality indice (HYI), referring to the average number of abnormal gill tufts for all individuals (Vuori, 1994; Vuori & Kukkonen, 1996). One way ANOVA was used to determine the difference in physicochemical characteristics among each month. Canonical correspondence analysis (CCA) was applied to test the influence of environmental variables on the hydropsychid assemblages. Biological data were log (X+1) transformed prior to canonical correspondence analysis. A Monte Carlo permutation test with 998 random permutations was used to examine a statistical significance of the model determination. Multivariate analyses were performed by using PC-ORD ver.5 (McCune & Mefford, 2006).

Results and discussion

1. Physicochemical parameters of water quality

One-way ANOVA showed significant difference of all parameters such as water temperature, total dissolved solids (TDS), dissolved oxygen (DO), pH, orthophosphate (PO_4^{2-}), ammonia-nitrogen (NH_3-N) and nitrate-nitrogen (NO_3-N) in all sampling occasions ($p<0.05$) (Table 1). The annual variations in environmental factors in the stream flows into Kasiow Dam for one years are shown in Table 1. The water temperature showed very wide range of fluctuation, (22.37±0.21 −39.5±1.15ºC). Also, the total dissolved solids showed very wide range of fluctuation, (95.93±1.05−385.50±0.35 mg/L). The higher values of TDS were the indicators of higher ionic concentrations, probably due to the high anthropogenic activities in the region and geological weathering conditions acquiring high concentrations of the dissolved minerals (Costello et al., 1984).

The local anthropogenic activities could be the discharges from intensive and prolonged agricultural activities and discharges from industrial and domestic wastes. Agricultural activities introduce ions and metals from fertilizers and other agrochemicals (Hamid et al., 2020). The DO showed very narrow range of fluctuation, (6.13±0.16−8.63±0.50 mg/L). The range of DO of the stream water flows into Kasiow Dam was found within the normal range (>2 to 6 mg/L) in the stream water and considered very good for most stream biotas (PCD, 2014). The pH of water was found slightly alkaline (7.47±0.15 −9.30±0.44). The pH range from 5.0 to 9.0 is permissible as per PCD (2014). Although pH usually has no direct impact to consumers, it is one of the most important operational water quality parameters.

Table 1 Mean ±SD water quality parameters of the stream flows into Kasiow Dam during January to December 2019.

Factor/ month	WT	DO	pH	TDS	NH_3-N	NO_3-N	PO_4^{3-}
Jan	27.67 ± 0.31^{bc}	8.21 ± 0.13^{c}	8.20 ± 0.10^{bc}	228.67 ± 0.58^{f}	0.08 ± 0.04^{a}	4.13 ± 0.06^{abd}	1.18 ± 0.08^{d}
Feb	30.13 ± 1.18^{de}	7.21 ± 0.13^{b}	7.97 ± 0.40^{abc}	111.67 ± 11.50^{b}	0.69 ± 0.34^{c}	7.20 ± 0.17^{d}	2.25 ± 0.15^{ef}
Mar	31.30 ± 0.53^{e}	8.63 ± 0.50^{c}	8.53 ± 0.06^{c}	134.10 ± 0.75^{c}	0.23 ± 0.01^{ab}	4.10 ± 0.17^{ab}	0.84 ± 0.04^{cd}
Apr	39.5 ± 1.15^{f}	6.88 ± 0.65^{c}	7.87 ± 0.1^{2ab}	95.93 ± 1.05^{a}	0.72 ± 0.04^{c}	-	0.76 ± 0.25^{bcd}
May	-	-	-	-	-	-	-
Jun	-	-	-	-	-	-	-
Jul	29.13 ± 0.06^{cd}	6.13 ± 0.16^{a}	8.03 ± 0.06^{abc}	136.70 ± 3.91^{c}	0.22 ± 0.13^{ab}	1.77 ± 0.15^{abc}	0.26 ± 0.27^{a}
Aug	29.87 ± 0.06^{de}	6.65 ± 0.07^{ab}	8.17 ± 0.06^{bc}	162.57 ± 0.41^{d}	0.20 ± 0.03^{ab}	1.57 ± 0.46^{ab}	0.17 ± 0.07^{a}
Sep	29.10 ± 0.53^{cd}	7.07 ± 0.03^{c}	7.47 ± 0.15^{a}	175.00 ± 0.26^{e}	0.48 ± 0.30^{bc}	5.63 ± 4.21^{cd}	0.40 ± 0.19^{abc}
Oct	31.60 ± 1.08^{e}	6.89 ± 0.07^{ab}	8.30 ± 0.00^{bc}	245.67 ± 2.52^{g}	0.26 ± 0.01^{ab}	2.93 ± 0.06^{abc}	0.67 ± 0.10^{abcd}
Nov	22.37 ± 0.21^{a}	6.99 ± 0.02^{b}	9.30 ± 0.44^{d}	385.50 ± 0.35^{i}	0.15 ± 0.03^{a}	3.70 ± 0.00^{abcd}	1.82 ± 0.39^{e}
Dec	26.80 ± 0.17^{b}	7.06 ± 0.04^{c}	8.43 ± 0.06^{bc}	359.00 ± 1.00^{h}	0.24 ± 0.02^{ab}	4.40 ± 0.62^{bcd}	2.43 ± 0.06^{f}

Remark: Data shown with different letters are statisti cally significant at the $P < 0.05$ level

The orthophosphate concentration was highest (2.43±0.06 mg/L) in December and lowest in August (0.17±0.07 mg/L). The high concentration of phosphate in the stream may be due to land use management practices. Land use operations such as fertilizer application to promote agricultural products results in increased P release (Cummins & Farrell, 2003), and may result in increasing of P concentration in receiving water bodies (Lawniczak et al., 2016). Other important sources of phosphorus to freshwater are atmospheric precipitation, geochemical condition, and ground water. Concentration of nitrate-nitrogen and ammonia-nitrogen ranged from 1.57±0.46 to 7.20±0.17 mg/L and 0.08±0.04 to 0.72±0.04 mg/L, respectively. In natural aerobic water, most nitrogen occurs as nitrates in varying amount depending upon the nature of water shed, seasons, degree of pollution and the abundance of plankton (Maitland, 1978).

2. Biodiversity of hydropsychids larvae

In total 5583 individual hydropsychid larvae belonged to four genera. There are consists of the *Amphipsyche meridiana* which contains 2,442 individual (Fig. 2), followed by *Macrostemum indistinctum* (1438 individual, *Cheumatopsyche* spp. (1423 individual) and *Potamyia* spp. (280 individual) (Table 2). All four hydropsychid genera were found in each month, except in September. Only the *Potamyia* spp. were found, because of the stream water was slightly turbid and high water level during sampling.

Table 2 Number of normal and abnormal hydropsychid larvae collected in stream flows into Kasiow Dam during January to December 2019

Month	Taxa	Total	Total number	
			Normal	Abnormal
January	*Amphipsyche meridiana*	6	3	3
	Cheumatopsyche sp.	104	77	27
	Macrostemum indistinctum	33	24	9
	Potamyia sp.	217	183	34
February	*Amphipsyche meridiana*	89	24	65
	Cheumatopsyche sp.	33	14	19
	Macrostemum indistinctum	3	0	1
	Potamyia sp.	2	1	1
March	*Amphipsyche meridiana*	170	102	68
	Cheumatopsyche sp.	401	401	0
	Macrostemum indistinctum	130	113	17
July	*Amphipsyche meridiana*	670	489	181
	Cheumatopsyche sp.	11	4	6
	Macrostemum indistinctum	303	252	51
	Potamyia sp.	3	1	0
August	*Amphipsyche meridiana*	650	357	293
	Cheumatopsyche sp.	24	22	2
	Macrostemum indistinctum	270	180	90
	Potamyia sp.	4	2	2
September	*Potamyia* sp.	8	8	0
October	*Amphipsyche meridiana*	278	268	10
	Cheumatopsyche sp.	133	133	0
	Macrostemum indistinctum	39	36	3
	Potamyia sp.	33	33	0
November	*Amphipsyche meridiana*	156	22	134
	Cheumatopsyche sp.	191	151	13
	Macrostemum indistinctum	421	276	37
	Potamyia sp.	6	6	0
December	*Amphipsyche meridiana*	423	397	67
	Cheumatopsyche sp.	526	476	50
	Macrostemum indistinctum	239	215	34
	Potamyia sp.	7	7	0

3. Morphological abnormalities

Normal, undamaged gills are whitish and branching. According to Ratia et al. (2012), a gill tuft can be considered damaged if it is totally reduced or its basal or distal parts darkened, or if the gill tuft has dark spots on > 50 % of its branches. However, some studies consider only totally reduced gill tufts as damaged (Vuori, 1994; Vuori & Kukkonen 1996). Also darkening of the anal papillae of hydropsychid larvae has been observed as a response to metal exposure (Vuori, 1994).

The morphological abnormalities observed included the darkening and reduction of the tracheal gills (Fig. 2B) and the malformation of the whole gill tuffs (Fig. 2C) in all hydropsychids larvae collected. The wound-like malformations were also discovered in the abdominal segments of the larvae (Fig. 2D-F).

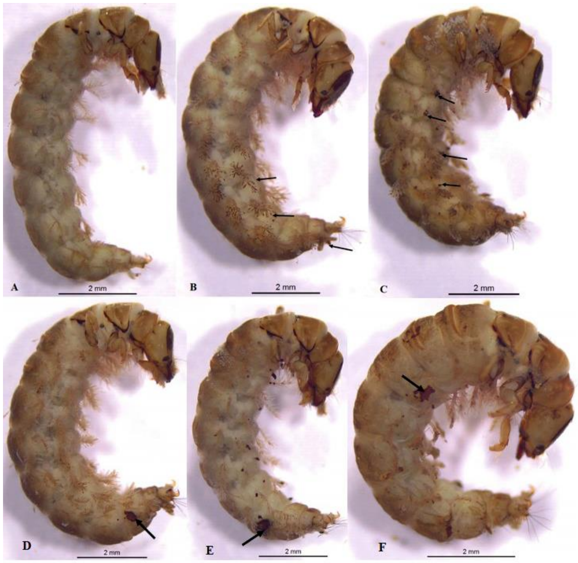

Fig. 2 Example of Hydropsychidae species, *Amphipsyche meridiana* that are found the most individual from this study. Gill tufts are the transparent branches on the ventral side (A), slightly damaged (B), seriously damaged (C), and wound-like in the body surface (D-F). Arrows indicate malformation symptoms. Bar represent 2 mm.

The percentage of individuals with at least some abnormality (HAI) (Fig. 3) and hydropsychid gill abnormality indice (HYI) (Fig. 4) were the highest in February and lowest in October. A remarked increase of HYI values with increasing concentration of pollutants concentration reflected a clear abnormality-contaminant relation, whereas the mere dicotomic classification of larvae as normal or abnormal (HAI) was less informative. High values of both HAI and HYI were associated with high concentration of contaminant. The HAI indicated deleterious effects, but failed to quantify the severity of degradation. The application of individual gill tufts, as response units in deriving HYI, revealed a simple solution to the quantification problem (Vuori, 1994).

3. Influence of physicochemical parameters on caddisfly larvae communities.

Canonical correspondence analysis is a multivariate method to calculate the relationships between biological

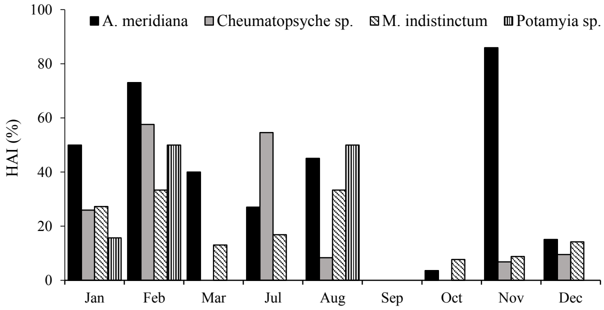

Fig. 3 Hydropsychid abnormality incidence (HAI) during January to December 2019

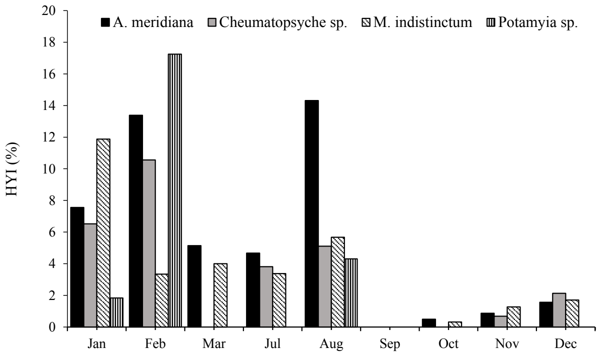

Fig. 4 Hydropsychid gill abnormality indice (HYI) during January to December 2019

assemblages of species and their environment (Ter Braak & Verdonschot, 1995). CCA revealed association between aquatic insect species and environmental variables at different months. Eigenvalue associated with each axis equal the correlation coefficient between species scores and environmental variables scores (Gauch, 1982).Thus an eigenvalue close to one will represent a high degree of correspondence between species and environmental variables, and an Eigenvalue close to zero will indicate very little correspondence (Palmer, 1993). In the present study, total eigenvalue 0.87 indicated a high degree of correspondences between species and environmental variables (Table 3).

The axis 2 was found to be strongly positive associated with ammonia-nitrogen (NH_3-N), water temperature (WT) and pH, but total dissolved solids (TDS) was found to be inversely associated with each other (Fig. 5). The species; *Amphipsyche meridiana* was positively correlated with all the measured variables, except with the TDS in February and October. Negative association of *Cheumatopsyche* spp. and *Macrostemum indistinctum* with TDS in November and December. In the stream water, ammonia-nitrogen concentration was high ranging from 0.08±0.04 (January) to 0.72±0.04 mg/L (April). According to (PCD, 2014) ammonia-nitrogen concentrations over 0.5 mg/L will certainly have impact on a river. The local anthropogenic activities could be the discharges from intensive and prolonged agricultural activities and discharges from industrial and domestic wastes. Agricultural activities may introduce ions and metals from fertilizers and other agrochemicals (Hamid et al., 2020). The higher incidence of gill abnormalities in hydropsychid larvae may reflect the cumulative impact of contaminant exposure via food and water during larval development. As the increasing incidence of morphological abnormalities in the larvae imply an increased concentration of pollutants in the tissue (Vuori & Kukkonen, 1996). Prommi (2011) studied the altered gills morphology in *Amphipsyche meridiana* from the Pasak Jolasit Dam Outlet, central Thailand. The results showed that the tracheal gills were mostly altered followed by body surface and anal papillae. Factors such as conductivity, and inputs of orthophosphate, sulfate, turbidity, other contaminants and/or stream profiles may effect to the abnormalities, corresponding with the work by Prommi & Thamsenanupap (2013) and Ninon & Prommi (2019).

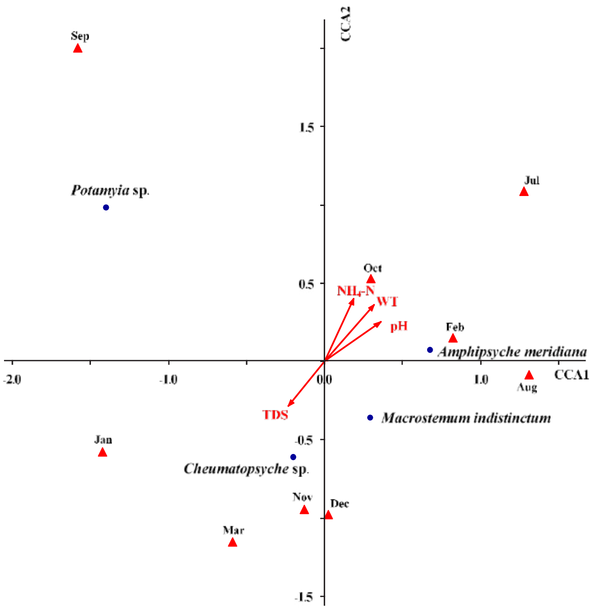

Fig. 5 CCA showing the correlation between caddisflies larvae taxa and physicochemical variables

Table 3 Summary of CCA for the caddisfly larvae and water quality variables. Axis 1 and Axis 2 significantly followed the monte-carlo permutation procedure ($P<0.05$)

	Axis 1	**Axis 2**	**Axis 3**
Eigenvalue	0.705	0.165	0.128
Variance in species data			
% of variance explained	65.2	15.3	11.8
Cumulative % explained	65.2	80.4	92.3
Pearson Correlation, species–environments	0.963	0.934	0.992
Water temperature	0.094	**0.439**	0.462
Dissolved oxygen	-0.005	0.147	0.483
pH	0.181	**0.374**	0.359
Total dissolved solids	-0.058	**-0.346**	-0.518
Ammonia-nitrogen (NH_3-N)	-0.035	**0.410**	0.509
Nitrate-nitrogen (NO_3-N)	-0.210	0.085	0.574
Orthophosphate (PO_4^{3-})	0.205	-0.190	0.525

Conclusion

The altered gill morphology in hydropsychids larvae related to multiple environmental variables. We can conclude that the malformation in tracheal gills were highly correlated with water temperature, pH, ammonia-nitrogen, and total dissolved solids. Our data indicate that the malformation in tracheal gills could be used as an early warning in aquatic ecosystems combine with physical and chemical factor.

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