

Chironomid Mouthpart Deformity as an Early Signal to Assess the Impact of Dyeing Process Waste in an Aquatic Environment

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Received: October 1, 2021; Revised: November 3, 2021; Accepted: January 12, 2022

Abstract

Weaving and dyeing, as both a household industry and in small factories, are very popular in Chonnabot District, Khon Kaen Province, Thailand. The dyes used are inorganic and organic substances that can cause high electrical conductivity (EC) and increase biochemical oxygen demand (BOD), affecting the aquatic food chain as well as human health. The mouthpart deformities of chironomid larvae, a member of benthic macroinvertebrates, occur when the developmental homeostasis is insufficient to compensate for environmental stress and has been proposed as a bioindicator of stress in aquatic environment. Studies on the effects of waste from the dyeing processes contaminating freshwater environments were conducted in August 2018. Water quality variables were measured, and benthic macroinvertebrates were investigated in five sampling sites in Kong Kaew Pond. The chironomid larvae were analyzed. The results demonstrate that sites which received dyeing process waste (K02, K03 and K05 sites) directly had higher EC and BOD, lower diversity index, lower evenness index, higher relative abundance of tolerate taxa (Chironomidae and air-breathing freshwater snails) and a higher percentage of chironomid mouthpart deformity than those of less polluted sites (K01 and K04 sites). Dissolved oxygen (DO) concentrations were high in all sampling sites. A complete wastewater treatment system is recommended to treat dyeing process wastes before they are discharged and contaminate natural freshwater and to avoid hazards to human health. The combination of benthic macroinvertebrate diversity measurement and the incidence of mouthpart deformity in chironomid larvae may be useful as an early warning to indicate the ecological state of freshwater habitats.

Keywords: Wetland; Textile dyeing process; Macroinvertebrates; Non-biting midges; Deformity

1. Introduction

Wastewater from textile dyeing processes may have an effect on various organisms and lead to an alteration of the habitats of aquatic communities (Muñoz and Prat, 1996). Dyes are considered as major pollutants, and heavy metal contamination in the effluent which is non-biodegradable with high toxicity can cause long-term health effects (Berradi *et al.*, 2019; Lellis *et al.*, 2019). In Thailand, Mud Mee silk is a very famous product of Chonnabot District, Khon Kaen Province. As silk

personal consumption increased and high market demand led to the use of chemical dyes and inorganic materials, replacing traditional methods (Ngern-Tong, 2016). Wastewater from the domestic textile dyeing process has flowed into Kong Kaew Pond for a long time. Currently, people near the community do not eat aquatic fauna caught from this pond, due to the fear of contamination with textile dyeing chemicals. Benthic macroinvertebrates play an important role in the cycling of organic matter and are an essential food

resource in the aquatic food web. They dwell in almost all aquatic habitats and analysis of the species present can be used to assess the health of their habitats (Resh and Jackson, 1993). Larvae of chironomid, a member of the macroinvertebrates, can tolerate low oxygen and accumulate heavy metals into their tissues (Roosa *et al.*, 2016). Chironomid larvae have been largely used as a bioindicator of freshwater quality, because they are abundant even in highly polluted water, where they often present phenotypic and development defects that can be easily detected (Armitage *et al.*, 1995; Clarke, 1993; Nicacio *et al.*, 2015). As pollution levels rise, more chironomid larvae will be exposed to stressors that cause abnormalities (Janssens de Bisthoven *et al.*, 1992), particularly deformities in mouthpart structures such as the mentum and mandibles (Martinez *et al.*, 2001). Deformities of chironomid larvae have been widely reported in freshwater sediment contaminated with heavy metals, pesticides and organic pollution (Servia *et al.*, 2004; Di Veroli *et al.*, 2012; Weeraprapan *et al.*, 2018). Most studies of chironomid deformities have been involved with mine operations, industries, urban areas and

pesticides. However, no studies in water contaminated with waste from textile dyeing process. The present study, we investigated benthic macroinvertebrate community structures and the incidence of deformity in the mouthparts of chironomid larvae in order to be used as the biological tool to indicate contamination in Kong Kaew Pond, a wetland contaminated by waste from the dyeing process.

2. Materials and Methods

2.1 Study area and sampling sites

The study was carried out in Kong Kaew Pond, a natural wetland with a surface area of 6.6 km². Five sampling sites (K01-K05) were selected as representative of the large lake in the Reference Condition Approach of Bailey *et al.* (2004). They consisted of the control site (K01), a site close to a sewage disposal outlet (K02), downstream of a sewage disposal outlet (K03 and K04), and a small waterway connected to Kong Kaew Pond (K05). Details of the sampling sites are listed in Table 1 and a map shown in Figure 1.

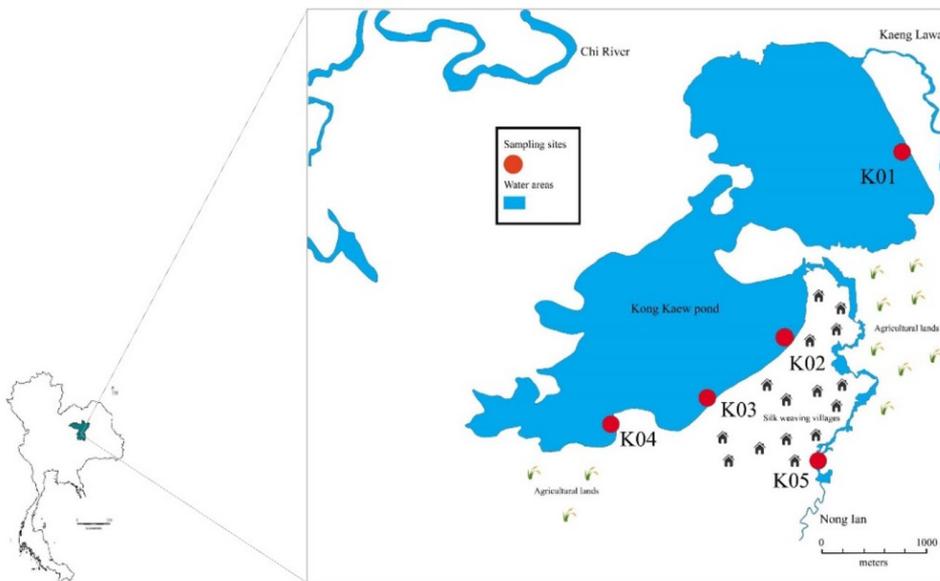


Figure 1. Map showing location of sampling sites (K01-K05), Kong Kaew Pond, Khon Kaen Province

2.2 Environmental variables

Three replicates of water samples were taken from each sampling site for water quality analysis in August 2018. Physico-chemical parameters of water quality were measured as follows: air temperature (°C) by thermometer, pH, electrical conductivity (EC, µS/cm) and total dissolved solid (TDS, mg/L) by a pH/EC/TDS meter Hanna model HI 98129. Water temperature (°C), dissolved oxygen (DO, mg/L) and biochemical oxygen demand (BOD, mg/L) were measured using a dissolved oxygen meter YSI model 550A, while chlorophyll a (µg/L) was measured by the extracted methanol method (APHA AWWA WPCF, 1998).

2.3 Collecting benthic macroinvertebrate samples and identification

At each sampling site, a D-frame net (0.3 m wide, 450 µm mesh) was used to collect benthic macroinvertebrates by twenty sweeps. They were then composited into a plastic bottle containing 95% ethyl alcohol.

In the laboratory, collected specimens were washed through a 500 µm mesh sieve. Benthic macroinvertebrates were sorted and identified to the lowest taxon possible based on Morse et al. (1994). Most individuals were identified to the genus level, but some could be identified to family or higher level, because the immature stages of most benthic invertebrates cannot be identified to the species level.

2.4 Mouthpart deformities of chironomid larvae

Permanent slides of chironomid larvae were processed, which was modified from Epler (2001) to identify the species level using taxonomic keys based on Cranston (2019) and Epler (2001). If the numbers of chironomid larvae from each site were more than 100 individuals, only 100 individuals were randomly selected. On the other hand, when the number of specimens was less than 100 individuals, the total number of specimens was examined for deformity mouthpart incidence by observation of mentum. Mentum were classified as deformed if they exhibited

Table 1. Latitude, longitude, and details of habitats of sampling sites

Site code	Habitat	Latitude	Longitude
K01	Site is located behind the Chi River barrier flap. The site had no obvious source of pollution and is far away from the residential area, and it is approximately 5 km, which is sufficiently distant, from K02.	16°06'53.3"N	102°37'55.6"E
K02	Site is close to a sewage disposal outlet, which releases waste from the textile dyeing process. The district municipality has created an embankment for treating wastewater using water hyacinth (<i>Eichornia crassipes</i>).	16°5'58.45"N	102°37'21.07"E
K03	Site is approximately 1 km downstream of the embankment. The site receives water that could seep through the embankment.	16°5'40.38"N	102°36'57.28"E
K04	Site is located approximately 1 km downstream of K03. Local people have renovated this area into a public park for recreation.	16°5'31.43"N	102°36'31.23"E
K05	Site is located on the east side of the village where there is a small waterway connecting to Kong Kaew Pond. The waterway receives wastewater from the fresh market and silk weaving village.	16°5'27.92"N	102°37'26.83"E

extra teeth, missing teeth including gaps, very round teeth, bifid teeth or severe asymmetry or abnormality in shape (Bird, 1994; Di Veroli et al., 2012). The frequency of deformities was expressed as a percentage of the total of analyzed larvae.

2.5 Statistical analysis

One-way analysis of variance (ANOVA) in the SPSS program version 26.0 (SPSS Inc., Chicago, IL, USA) was used to test the significant difference of physico-chemical parameters of water quality, and non-normal distribution of data was analyzed with the Kruskal-Wallis nonparametric tests. Shannon-Wiener species diversity index ($H' = -\sum_{i=1}^s (p_i) \ln(p_i)$) and evenness index ($E' = H' / \ln(s)$) were calculated to measure the diversity of benthic macroinvertebrates, where s = Number of species, $p_i = n_i/N$ (where n_i = Number of individuals in each species and N = Total number of all species) (Krebs, 1999). Relationships between the benthic macroinvertebrate community structure and the environmental variables were analyzed using Canonical Correspondence Analysis (CCA) ordination, and Monte Carlo permutation tests with 998 iterations, which were obtained by the PC-ORD program for Window version 5.10 (McCune and Mefford, 2006).

3. Results and Discussion

3.1 Environmental variables

The ranges of water temperatures of sampling sites were 34°C and 37°C. Chlorophyll a of phytoplankton was highest in K05 (13.48 µg/L), followed by K02 (1.95 µg/L) and K03 (0.87 µg/L), in order. The mean ± standard deviation (SD) of physico-chemical parameters of water quality at the five sampling sites are shown in Table 2. One-way ANOVA revealed that average values of DO (7.51 - 9.27 mg/L) and BOD (3.01 - 6.3 mg/L) were significantly different ($p < 0.05$), but pH had non-significant difference ($p > 0.05$) among sampling sites.

High DO during the daytime may result from photosynthesis by algae and aquatic

plants. Algae and aquatic plants need nutrients to grow, and human activity can cause rising nutrient levels.

Dead algae and aquatic vegetation in Kong Kaew Pond provided some sources of organic matter for microorganisms, which used up DO in the water and consequently caused high BOD. Higher values of BOD indicate that the site is contaminated by organic pollutants (Custodio et al., 2018).

Electrical conductivity and TDS ranged from 524.33 - 750.33 µS/cm and 247.33-398.33 mg/L, respectively. The Kruskal-Wallis non-parametric test showed that EC and TDS were significantly different ($p < 0.05$) among sampling sites. K01 and K04 sites had lower values of EC and TDS than those variables from K02, K03 and K05. In general, water in the same pond tends to have a relatively constant range of EC, while significant increases to relative EC could indicate contamination of pollutants in the water body (De Sousa et al., 2014; US EPA, 2021). The average EC at K01 and K04 sites were not different ($p > 0.05$), but they were significantly lower than K02, K03 and K05 sites ($p < 0.05$). These findings imply that K01 and K04 sites had less contamination from pollutants than those of the three latter sites.

Multiple comparison test after ANOVA and pairwise comparison test after Kruskal-Wallis revealed that sites K02, K03 and K05 were grouped together as the higher contamination sites than those of the other group (K01 and K04) sites. Based on the standard of surface water quality in Thailand (NNEB, 1994), the BOD in the sampling sites were between 3.01 - 6.3 mg/L which can be categorized as water quality class 3 and class 4.

3.2 Benthic macroinvertebrate community and relationship with environmental parameters

A total of 4,968 individuals representing 65 taxa, 43 families, 11 orders and 3 phyla was identified. The majority of benthic macroinvertebrates was classified as the family Chironomidae (2,919 individuals), which

was the most abundant group, followed by Mollusca (851 individuals), ephemeropterans (180 individuals) and hemipterans (180 individuals). The top three abundant benthic macroinvertebrates from each sampling site were the following: K01 consisted of *Cloeon* (Order Ephemeroptera), Chironomidae (Order Diptera) and Coenagrionidae (Order Odonata), in order. Site K02 consisted of Chironomidae, amphipod (Order Amphipoda) and *Pomacea* (Order Mesogastropoda), in order. Site K03 consisted of Chironomidae, isopod (Order Isopoda) and *Cloeon*, in order. Site K04 consisted of Hydrophilidae (Order Coleoptera), Chironomidae and Culicidae (Order Diptera), in order. Site K05 consisted of Chironomidae, *Indoplanorbis exustus* and *Bithynia* (Order Mesogastropoda), in order. It was found that K03 and K05 sites had the highest taxa richness (38 taxa), followed by K04 (31 taxa), K02 (26 taxa) and K01 (16 taxa) sites. Site K04 had the highest Shannon-Wiener diversity index (2.88), followed by K03 (2.33), K01 (2.29), K05 (2.06) and K02 (0.80) sites. Site K04 also had the highest evenness index (0.84), followed by K01 (0.83), K03 (0.64), K05 (0.57) and K02 (0.24) sites (Table 3).

K01 and K04 sites had high diversity index and high evenness index compared to those of K02, K03 and K05 sites. High evenness at K01 and K04 sites indicate community structure of benthic macroinvertebrates at these sites were more stable than those of K02, K03 and K05 sites. Many studies show that the diversity of benthic macroinvertebrates decreases

in higher polluted habitats (Phaphong and Sangradub, 2012; Rattanachan et al., 2016), and chironomid genus *Chironomus* was more tolerant than that of *Polypedilum* (Tang et al., 2009; Sriariyanuwath et al., 2015). Chironomids and air-breathing freshwater snails (such as Planorbidae and Lymnaeidae) were representative of high tolerance benthic macroinvertebrates in freshwater habitats, which could survive in polluted water (Hawkes, 1997). Chironomids and air-breathing snails (*Indoplanorbis exustus*, *Physa acuta* and *Pomacea*) were found in large numbers at K02, K03 and K05 sites. *Chironomus* were more relatively abundant at K02, K03 and K05 sites, but *Polypedilum* were more abundant at K01 and K04 sites. These results suggest that K02, K03 and K05 sites contain more pollutants than K01 and K04 sites, and this result correlates with the results of physico-chemical parameters of water quality at these sites.

The relationships between the environmental parameters and benthic macroinvertebrate community structures were investigated using CCA. The first two CCA axes explained 54.0% of the variance (the first axis 29.8% and the second axis 24.2%). The results of Ordination by CCA (Figure 2) showed that sampling sites were clearly discriminated by EC, DO and BOD. Sites K01 and K04 are located closely on ordination space, which indicates their similar composition. Sites K01 and K04 had lower DO, BOD and EC, whereas K02 and K03 sites had higher BOD, and K03 and K05 sites had

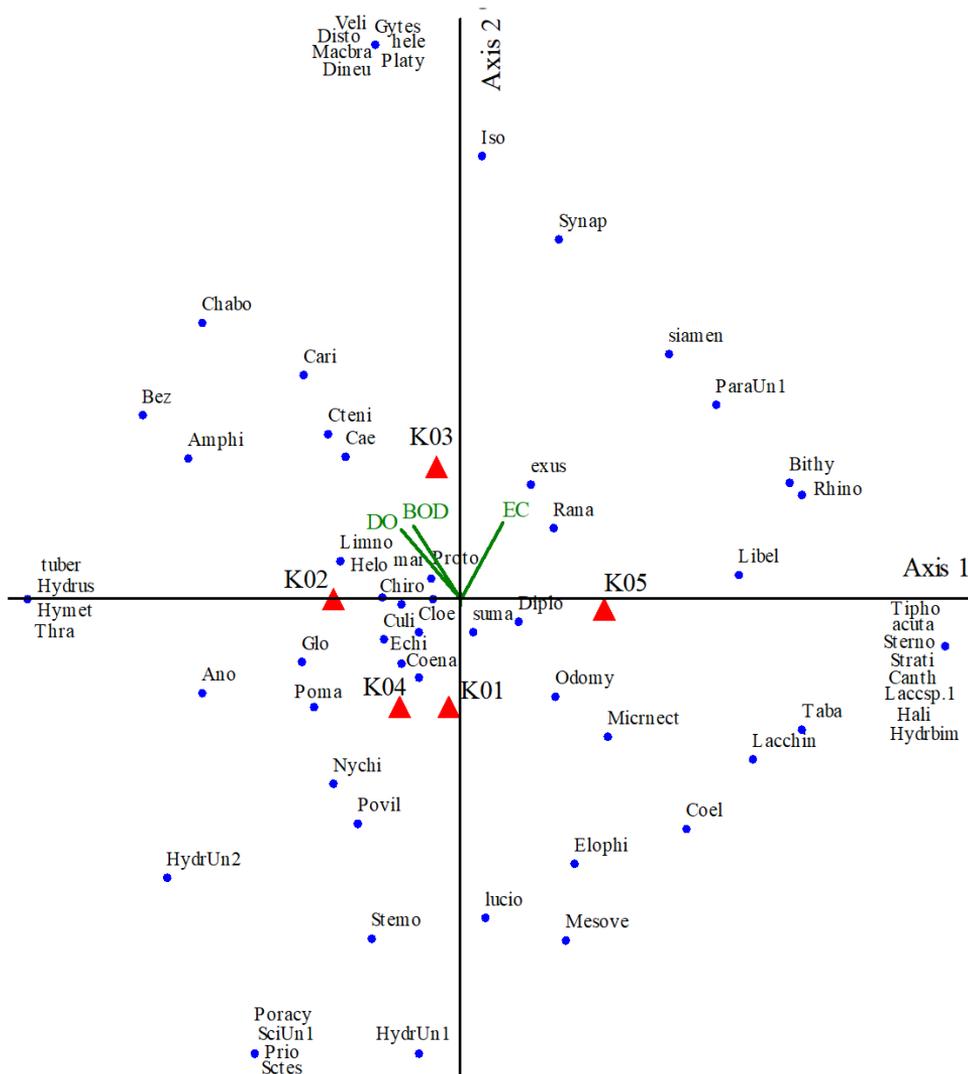
Table 2. Mean ± SD of physico-chemical parameters of water quality from Kong Kaew Pond

Physico-chemical parameters	K01	K02	K03	K04	K05	p-Value
pH	7.13 ± 0.12	7.07 ± 0.22	7.08 ± 0.10	7.17 ± 0.12	7.55 ± 0.49	0.105 ^{ns}
Electrical conductivity (µS/cm)	552 ± 1.73 ^a	642 ± 8.96 ^b	750.33 ± 2.65 ^d	524.33 ± 1.15 ^a	714 ± 1.00 ^c	0.009 [*]
Total dissolved solids (mg/L)	282.667 ± 17.90 ^a	340 ± 2.89 ^a	398.33 ± 1.00 ^c	247.33 ± 6.35 ^a	378.666 ± 1.15 ^b	0.011 [*]
Dissolved oxygen (mg/L)	7.51 ± 0.11 ^a	9.16 ± 0.48 ^b	9.27 ± 0.52 ^b	7.68 ± 0.27 ^a	7.59 ± 0.42 ^a	< 0.001 [*]
Biochemical oxygen demand (mg/L)	3.25 ± 0.99 ^a	4.42 ± 0.40 ^a	6.3 ± 0.51 ^b	3.76 ± 1.30 ^a	3.01 ± 1.40 ^a	0.016 [*]
Chlorophyll a (µg/L)	0.52	1.95	0.87	0.63	13.48	na

(* = significant difference at $p < 0.05$, ns = non-significant difference at $p > 0.05$, na = not analyzed)

higher EC values. Monte Carlo permutation test showed neither eigenvalue nor species-environment correlations were significantly different ($p > 0.05$), which indicates no relationship between species distribution and physico-chemical parameters. This may be due to declining water quality in all samplings sites at Kong Kaew Pond.

Freshwater snails and Micronecta (Order Hemiptera) which primarily feed on algae (Chen *et al.*, 2005) were more abundant at the K05 site, coinciding with high chlorophyll a at this site. Sampling site K05 received sewage from the fresh market and community, which promotes the growth of algae and aquatic plants.



(Red triangle = sampling sites, Blue dot = macroinvertebrates taxa).

Figure 2. Biplot between axis 1 and axis 2 from all sampling sites ordination by Canonical Correspondence Analysis (CCA) using physico-chemical parameters and benthic macroinvertebrates abundance data

Table 3. Summary results of water quality variables, diversity index, evenness index, % tolerant groups (Chironomidae and air-breathing snails), % Mollusca and % mouthpart deformity in Chironomidae at 5 sampling sites

Site	K01	K02	K03	K04	K05
EC ($\mu\text{S}/\text{cm}$)	552	642	750	524	714
BOD (mg/L)	3.25	4.42	6.30	3.76	3.01
Diversity index	2.29	0.8	2.33	2.88	2.06
Evenness index	0.83	0.25	0.64	0.84	0.57
% Chironomidae	17.22	83.85	39.44	13.04	39.09
% Air breathing snails	0	5.75	2.91	2.17	32.05
% Mollusca	16.56	7.80	9.34	6.09	40.17
% Deformed mouthpart in Chironomidae	3.70	10.00	9.00	6.67	12.00

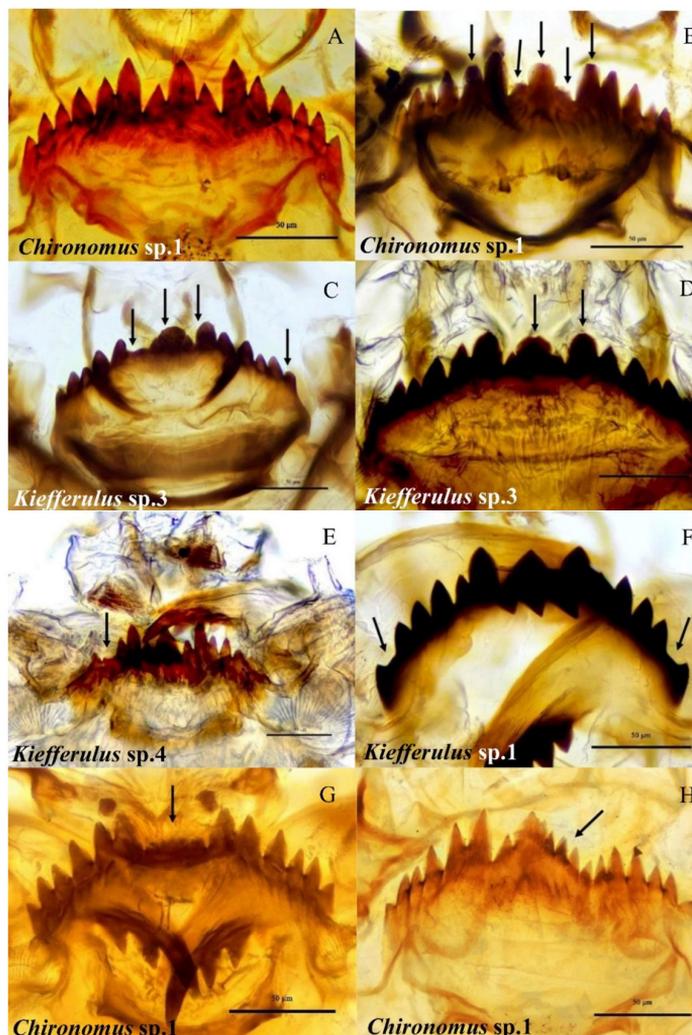


Figure 3. Deformities of mentum in chironomid larvae consist of *Chironomus sp.1* (A, B, G and H), *Kiefferulus sp.3* (C and D), *Kiefferulus sp.4* (E), *Kiefferulus sp.1* (F) collected from Kong Kaew Pond: (A) normal mentum, (B - H) deformed menta. Scale = 50 μm (\rightarrow indicates deformed points)

3.3 Mouthpart deformities of chironomid larvae

A total of 2,919 chironomid larvae were collected from Kong Kaew Pond. The highest numbers of chironomid larvae were found from K02 site (n = 1,999), followed by K05 (n = 539), K03 (n = 325), K04 (n = 30) and K01 (n = 26) sites, in order. The top three abundant generic levels were *Chironomus* (mostly found in K02, K03 and K05 sites), *Kiefferulus* (mostly found in K05 site) and *Polypedilum* (mostly found in K01 and K04 sites). Deformities were found in 7 out of 15 chironomid species collected. *Chironomus* sp.1 showed the highest incidence of mouthpart deformities. The highest to the lowest percentage of mouthpart deformities were as follows: K05 site had the highest incidence of deformities (12%) followed by K02 (10%), K03 (9%), K04 (6.67%) and K01 (3.70%) sites. A summary of the study including main water quality variables, diversity measurement, percentage of tolerant groups, and percentage of mouthpart deformities of chironomids is presented in Table 3. Deformities of mentum in chironomid larvae are shown in Figure 3.

In the present study, the percentages of mentum deformities ranged from 3.7 to 12; K01 site may be categorized as a slightly to moderately polluted site, while K02, K03, K04 and K05 sites may be moderate to severely polluted sites based on Bird (1994). In this study, we did not measure heavy metals in the water or sediment at Kong Kaew Pond, Mahachai *et al.* (2010) analyzed wastewater of dyeing water before treatment from households at Chonnabot and Mancha Kiri Districts, Khon Kaen Province, and found that concentrations of Pb (1.494 mg/L), Cd (0.124 mg/L) and Hg (0.010 mg/L) exceeded the effluent standard for industry of Thailand (NMNRE, 2016). As this waste cannot be purified by the process of natural biological degradation, such heavy metals are likely accumulated in sediment and water in Kong Kaew Pond. Sublethal concentrations of Pb, Cd, Zn and As can induce deformities in chironomid mouthparts in the laboratory (Vermeulen *et al.*, 2000; Martinez *et al.*, 2001).

Sites K01 did not receive waste water from dyeing process. The high percentage of mouthpart deformation in the other four sampling sites could be attributed to heavy metal accumulated in the pond. Higher frequencies of deformities were associated with the level of pollution at the site and the ranking of the sites. The results coincide with water quality based on physico-chemical variables and macroinvertebrate communities at each site. In comparison with other studies in Southeast Asia, mentum deformities of chironomids were reported in three polluted rivers in the Juru River System, Malaysia ranging from 44.05 - 59.6% (Al-Shami *et al.*, 2010), while in Thailand, a stream was contaminated with acid mine drainage from a gold mine (17%) in Loei Province (Sangpradub, 2017), ditches (8.83 - 22.85%) in Kasetsart University, Nakhon Pathom Province (Thanee and Prommi, 2017), and a stream contaminated with Cd (0.20 - 5.5%) in Tak Province (Weeraprapan *et al.*, 2018). The percentages of deformities are much different, probably due to the different deformation screening resolution in each study, since very subtle deformities may be included or excluded from the count. The results from this study, together with other studies, suggest that mentum deformities in chironomid larvae are sensitive to heavy metal and organic pollutants and could potentially be a biological tool for early warning in freshwater habitats. Proper treatment of dye processing wastewater before it is discharged to natural waters is essential to avoid adverse effects on the freshwater environment and human health.

4. Conclusion

The benthic macroinvertebrates diversity index and evenness index declined, but the percentage of mouthpart deformity of chironomid larvae increased in contaminated sites, as individual responses occur before community responses. The benthic macroinvertebrate diversity measurement and mouthpart deformity of chironomid larvae could provide an early warning of wastewater from dyeing process contamination.

Acknowledgements

The authors would like to thank Dr. Rungnapa Somnak and Miss Sirikamol Phai-ngam for helping in the field sampling.

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