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Mentum Deformities in Chironomidae (Diptera, Insecta) as Indicator of Environmental Perturbation in Freshwater Habitats

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Abstract

Deformities in the mouthparts of Chironomidae larvae, particularly of the teeth on the mentum, have been proposed as a bioindicator of sediment quality and environmental stress. The Chironomidae larvae were collected in the six-ditch canalization in Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom Province over a period of six months between October 2016 to March 2017. Three replicates of sampling by aquatic D-frame dip net were used at sampling sites. A total of 1,868 Chironomidae larvae, representing 2 subfamilies, Chironominae and Tanypodinae, were screened for mentum deformities. The observation under the stereomicroscope showed the typical mentum teeth deformity in Chironomidae larvae with various degrees of worn, such as median teeth with moderately worn, substantial worn, median and lateral teeth folked. The percentage of deformities was higher in the ditch canalization received wastewater from dormitory and household than other sites. Data of water quality and chironomids larvae were analyzed with Principal Component Analysis (PCA). Strong correlations were found between Chironomidae larvae and the water quality parameters of orthophosphate (PO_4^{3-}), ammonia-nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N), dissolved oxygen (DO), pH and water temperature.

Key words*:* Chironomidae, Mentum deformities, Ditch canalization**,** Water quality.

1. Introduction

Freshwater bodies in developing countries are subjected to degradation, mainly due to domestic as well as industrial wastes. This will in turn have a serious impact on aquatic organisms. One way of monitoring the health of aquatic ecosystems is through critical observation of benthic macroinvertebrates, especially the chironomids (Cranston, 2007). Because of their ubiquitous distribution, sensitivity to various pollutants and relatively short life cycle, chironomids are an ideal candidate for ecotoxicological studies. The head capsule of chironomid larva is one of the most impacted structures in the body of the organism when the environment, in which it lives, is altered, and it is an indication of stress (Bisthoven and Gerhardt, 2003). Deformities of the head capsule in larval Chironomidae indicate sub-lethal effects of exposure to contaminants and are considered an early warning signal for environmental water quality deterioration (Bisthoven and Gerhardt, 2003). Several studies, especially in the temperate region, have examined the use of head capsules of different species of chironomid larvae in response to a variety of contaminants (Bisthoven and Gerhardt, 2003), including an increase in morphological deformities of antennae and other parts of chironomid larvae as a result of heavy metals elevation in surface water and sediments. Although Chironomidae larval deformities have been successfully used as a biomonitoring tool in other parts of the world, its potential as an indicator of pollution stress in Thai freshwater system is yet to be explored.

Effluent from the domestic and agricultural area consists of a complex mixture of chemicals, varying in composition over time. In the production processes, these plants generate both inorganic and organic wastes (major ions, organic solvents, nutrients etc.) mixed with water, which change the concentration of suspended solids, biological oxygen demand (BOD), conductivity, temperature, color and odor of the receiving water-bodies (Al-Shami *et al*., 2010a). The aim of the present study is to examine the head capsules of Chironomidae larvae for determine the health of freshwater habitats in Kasetsart University, Kamphaeng Campus which are received the effluent-impacted river from domestic and agricultural area.

2. Materials and Methods

2.1. Study Area

Six freshwater ditches (KU_KPS1, KU_KPS2, KU_KPS3, KU_KPS4, KU_KPS5, and KU_KPS6)

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(Figure 1) were selected to sample water quality parameters and Chironomidae larvae.

2.2. Sampling

Sampling was carried out monthly over a period of 6 months during October 2016 to March 2017. Three replicates of sampling by aquatic D-frame dip net were used at sampling sites (Figure 1). Sample collected in the field were preserved in 95%ethanol. On each sampling occasion, measurements of physicochemical parameters in the field, such as pH, Water Temperature (WT), Dissolved Oxygen (DO), Total Dissolved Oxygen (TDS) and Electrical Conductivity (EC) were made in situ at three randomly selected locations at each sampling site. To analyze selected chemical parameters of water, three separate samples of water from each site were randomly collected using 500-ml plastic bottles. Each appropriately labeled bottle was thoroughly rinsed out with the river water immediately before collecting a sample. All water samples were transported to the laboratory in an ice chest and kept at 4 °C until analyzed. The ammonium-nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N), orthophosphate (PO_4^{3-}) and turbidity (TUB) were measured at appropriate wavelength using a spectrophotometer HACH 2000. Alkalinity was titration (APHA, 1992).

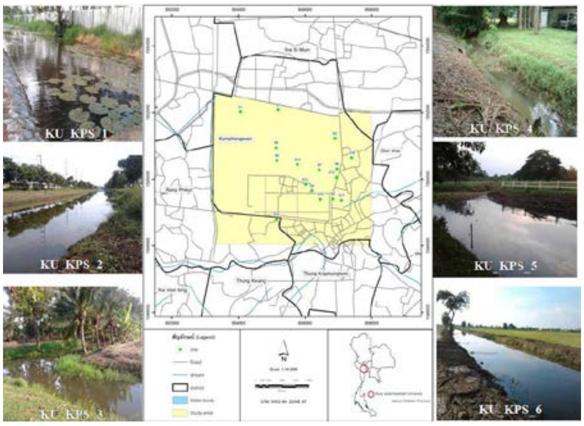

Figure 1. Six sampling sites at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom Province

2.3. Chironomid Larval Sampling

Concurrent with physicochemical sampling, Chironomidae larvae were sampled monthly over a period of 6 months from October 2016 to March 2017 at the six sampling sites. A D-frame aquatic dip net was used for collecting the larval samples. Sampling was time-limited. Three minutes total sampling time for each ditch was split equally between different habitat types. Chironomidae larvae were preserved in 95% ethanol and transported to the laboratory for sorting, identification, abundance counts and deformity screening.

2.4. Chironomid Deformities Investigation

The Chironomidae larvae were investigated under light microscope (40X to 400X) for checking normal and deformed head capsule. Head capsules of each deformed Chironomidae larvae were removed from the six sampling sites. All chironomid head capsules were mounted for light microscopical identification following the methods described by Al-Shami *et al*. (2010b) and Gerhardt and Bisthoven (1995). The head capsules were mounted ventral side up and squeezed under a cover slip until maximum visibility of mentum was achieved. The mentum structure was systematically screened for morphological deformities (Dickman *et al*., 1992).

2.5. Data Analysis

One-way ANOVA in combination with Tukey's (HSD) *post hoc* test was used to test for physicochemical parameters among various sampling occasions and among the sampling sites using SPSS Version 20.0. The Principal Component Analysis (PCA) was used to evaluate relationships between chironomid deformities and environmental variables with PC-ORD version 5.10. Cluster analysis and non-metric multidimensional scaling (NMDS) were used to classify the sampling sites based on the chironomid deformities using Ward's linkage method with Euclidean distance measure using PC-ORD software.

3. Results and Discussion

3.1. Environmental Variables

The mean and standard errors of the environmental variables at each sampling site are indicated in Table 1. Mean water temperature was significantly lower at site KU_KPS1 compared with sites KU_KPS2 and KU_KPS4 with significantly higher values ($p<0.05$). Mean concentrations of DO and pH were significantly higher at sites KU_KPS2, KU_KPS4 and KU_KPS6 than at sites KU_KPS1, KU_KPS3 and KU_KPS5 ($p<0.05$). Mean TDS and EC were higher at sites KU_KPS3 and KU_KPS5 than other sites ($p<0.05$). Mean water turbidity was lower at the site KU_KPS6 than other sites ($p<0.05$). Mean alkalinity was higher at the site KU_KPS3 than other sites ($p<0.05$). Mean nitrate-nitrogen and orthophosphate were higher at the site KU_KPS1 than other sites, whereas mean ammonia-nitrogen was higher at the site KU_KPS6 than other sites ($p<0.05$). The result suggests that the sites KU_KPS2, KU_KPS4 and KU_KPS6 were relatively free of either dormitory and household wastes compared with the sites KU_KPS1, KU_KPS3 and KU_KPS5.

Table 1. Environmental factors measured at six sampling sites of ditch canalization (October 2016 to March 2017)

Sites/parameter	KU_KPS1	KU_KPS2	KU_KPS3	KU_KPS4	KU_KPS5	KU_KPS6
WT (°C)	26.22±1.24[a]	31.36±2.40[c]	27.99±2.13[ab]	30.74±1.95[c]	27.56±1.84[ab]	29.82±1.84[bc]
DO (mg/L)	1.99±1.49[a]	7.95±0.67[b]	2.23±1.36[a]	7.75±0.34[b]	3.10±2.22[a]	8.69±0.49[b]
pH	7.44±0.24[a]	8.88±0.16[b]	7.58±0.07[a]	8.75±0.19[b]	7.53±0.23[a]	8.91±0.43[b]
TDS (mg/L)	121.70±23.16[a]	98.04±3.82[a]	630.67±102.35[c]	98.58±8.17[a]	264.25±75.22[b]	99.52±9.29[a]
EC (µS/cm)	243.79±45.93[a]	195.76±7.97[a]	1263.08±204.90[c]	199.25±14.24[a]	527.17±148.53[b]	198.72±18.82[a]
TUB (NTU)	36.33±3.23[c]	27.92±8.43[bc]	20.08±9.56[b]	20.75±9.96[b]	29.67±4.75[c]	11.25±3.62[a]
ALK (mg/L)	80.67±8.24[ab]	79.33±5.61[a]	271.33±53.82[c]	78.00±8.78[a]	108.17±13.76[b]	78.92±9.28[a]
NO_3-N (mg/L)	2.05±1.05[b]	1.10±0.39[a]	1.05±0.43[a]	1.25±0.43[a]	1.18±0.42[a]	1.43±0.67[ab]
PO_4^{3-} (mg/L)	0.65±0.20[b]	0.26±0.12[a]	0.49±0.32[ab]	0.37±0.10[ab]	0.52±0.25[ab]	0.35±0.39[a]
NH_3-N (mg/L)	0.57±0.27[ab]	0.38±0.07[a]	0.44±0.12[a]	0.46±0.20[a]	0.43±0.11[a]	0.72±0.26[b]

3.2. Deformity in Chironomidae Larvae

A total of 1868 chironomid larvae belonging to the subfamily Chironominae and Tanypodinae were analyzed for incidence of deformity. The presence of relatively high levels of organic materials at station KU_KPS1 gives credence to the fact that the sediments as well as the water chemistry were severally altered by the effluent. The domestic effluent-impacted site recorded a high percentage (22.85%) of deformed Chironomidae larvae when compared to the other five sites (Table 2). Various types of deformities were encountered in the present study ranging from normal median teeth, normal–moderately worn, worn and folked (Figure 2).

Table 2. The number of normal and deformed mouth parts of Chironominae and Tanypodinae head capsules at each site

Sites	Chironominae					Tanypodinae					Total individual				
	Normal	Abnormal	Total	% Normal	% Abnormal	Normal	Abnormal	Total	% Normal	% Abnormal	Normal	Abnormal	Total	% Normal	% Abnormal
KU_KPS1	204	60	264	77.27	22.73	2	1	3	66.67	33.33	206	61	267	77.15	22.85
KU_KPS2	155	19	174	89.08	10.92	45	14	59	76.27	23.73	200	33	233	85.84	14.16
KU_KPS3	98	26	124	79.03	20.97	9	1	10	90.00	10.00	107	27	134	79.85	20.15
KU_KPS4	183	15	198	92.42	7.58	92	6	98	93.88	6.12	275	21	296	92.91	7.09
KU_KPS5	141	10	151	93.38	6.62	5	1	6	83.33	16.67	146	11	157	92.99	7.01
KU_KPS6	656	65	721	90.98	9.02	56	4	60	93.33	6.67	712	69	781	91.17	8.83
Total	1482	195	163			209	27	236			1646	222	1868		

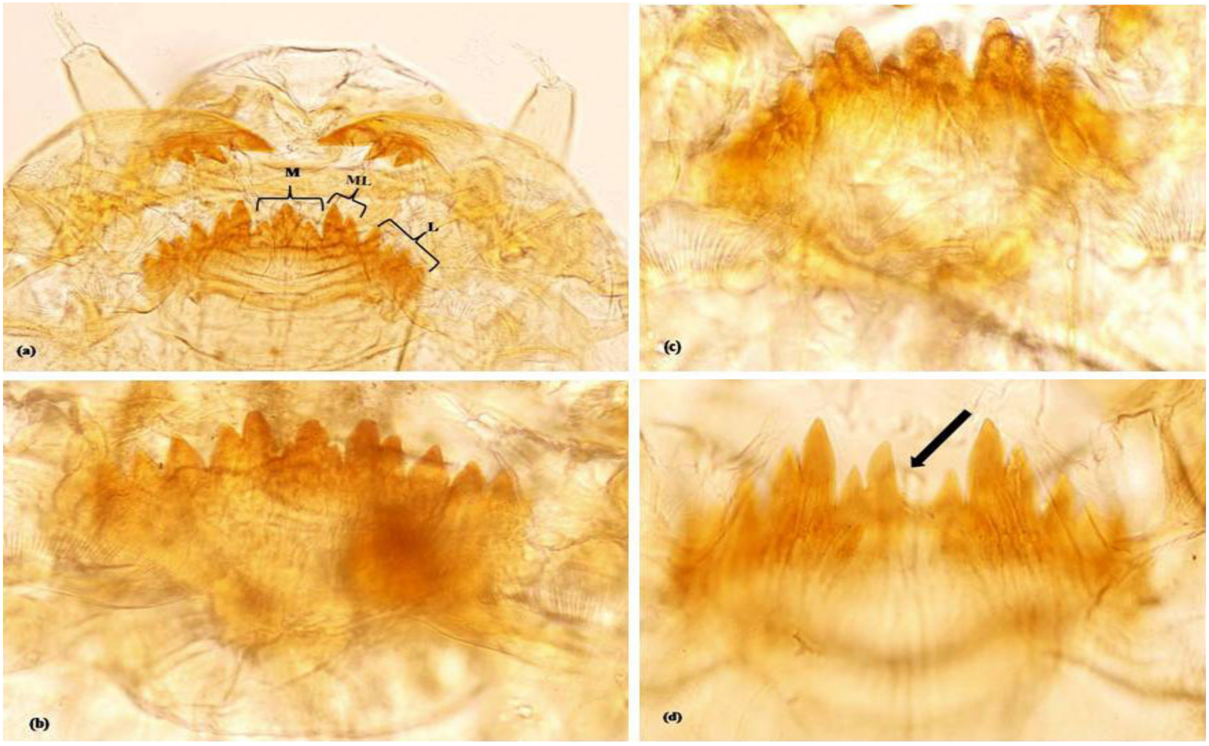

Figure 2. Some forms of deformities observed in chironomids larvae in the present study (a) normal median teeth, (b) normal – moderately worn, (c) worn, and (D) folked. (M=median teeth, ML=median lateral, L=lateral)

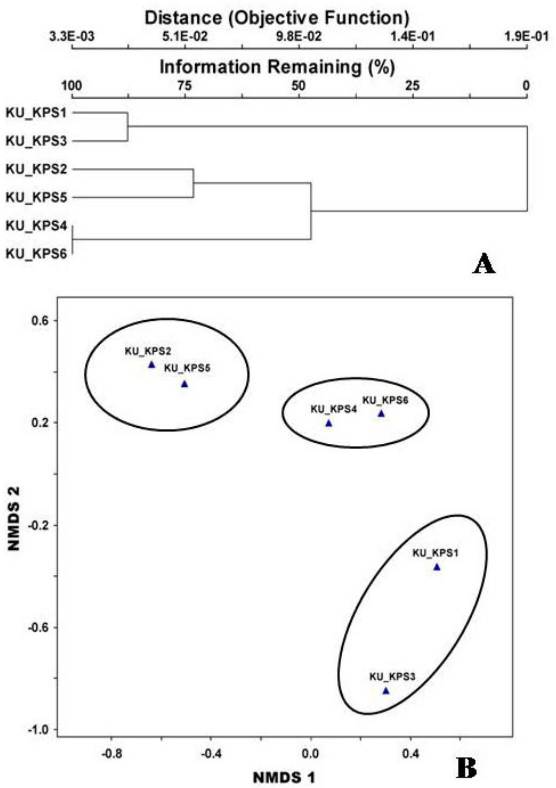

Figure 3. Cluster analysis (A) and Non-metric Multidimensional (NMDS) Scaling (B) of sampling sites based on deformed Chironomidae larvae

Cluster analysis based on Bray-Curtis similarity values showed that sites KU_KPS4 and KU_KPS6 showing high similarity followed by sites KU_KP1 and KU_KPS3, sites KU_KPS2 and KU-KPS6, respectively (Figure 3).

In order to determine the relationship between the physicochemical parameters with the percentage chironomid deformity, a principal component analysis (PCA) was performed. PCA first and second axes were significant and accounted for 75 and 24.9% of the total variance explained, respectively. According to PCA ordination (Figure 4), percentage chironomid deformity was positively correlated with the NO_3-N, PO_4^{3-} and water turbidity. Major nutrient concentration (i.e., PO_4^{3-} and NO_3-N) in an ambient aquatic environment was the primary factor explaining variation in benthic assemblages (Ponader *et al*., 2007). In the present study, PCA showed that proximate determinants, such as water turbidity, PO_4^{3-} and NO_3-N, were vital in explaining the variation in chironomid deformity. Considerable morphological abnormalities were revealed in the mouthparts with various degrees of worn such as, median teeth with moderately worn, substantial worn, median and lateral teeth folked. The deformities reported here are similar to some other chironomid species in polluted waters (Bisthoven and Gerhardt, 2003). These abnormalities represent sub-lethal effects and can be considered as early warning signals of environmental degradation by chemical contaminants (Warwick, 1990). Similarly, Al-Shami *et al.* (2010b) reported high percentage of chironomid deformity in rivers contaminated with industrial discharges from garment and rubber factories. Head capsule deformities can be used as biomarkers for pollution stress.

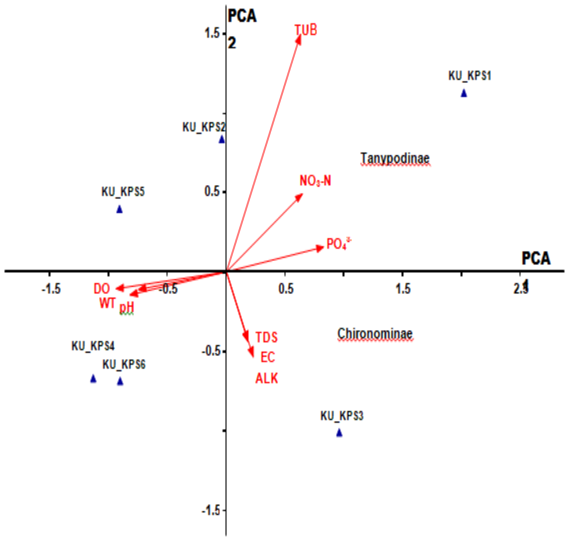

Figure 4. PCA ordination plot based on deformed Chironomidae larvae and environmental variables and sampling sites

4. Conclusions

The present study provides baseline data on some physicochemical conditions prevailing in the investigated pond with respect to the site receiving effluent from the domestic and agricultural activities, and could serve as a reference for future investigations. In addition, there was positive influence of domestic effluent discharge in the deformity of Chironomidae larvae inhabiting ponds. Therefore, chironomidae larvae appear to be useful taxa in deformity screening method owing to its ubiquitous distribution and relative abundance in tropical freshwater bodies. Their inclusion in monitoring, especially in Tropical Asian freshwater habitats, will add inferring power to assess ecosystem health and contaminated sediments.

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