Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2010, 22(11) 1718–1727
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Distribution of Chironomidae (Insecta: Diptera) in polluted rivers of the Juru River Basin, Penang, Malaysia

Salman A. Al-Shami[1,*], Che Salmah Md Rawi[1], Abu HassanAhmad[1], Siti Azizah Mohd Nor[1,2]

1. School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia. E-mail: alshami200@gmail.com
2. Centre for Marine and Coastal Studies, Universiti Sains Malaysia, 11800 Penang, Malaysia

Received 10 November 2009; revised 23 March 2010; accepted 08 April 2010

Abstract

The influence of physical and chemical parameters on the abundance and diversity of chironomids was studied in six rivers with moderate to highly polluted water in the Juru River Basin. The rivers: Ceruk Tok Kun (CTKR) as reference site, and polluted rivers of Pasir (PR), Juru (JR), Permatang Rawa (PRR), Ara (AR) and Kilang Ubi (KUR) were sampled over a period of five months (November 2007–March 2008). Nine chirnomid species: *Chironimus kiiensis*, *C. javanus*, *Polypedilum trigonus*, *Microchironomus* sp., *Dicrotendipes* sp., *Tanytarsus formosanus*, *Clinotanypus* sp., *Tanypus punctipennis* and *Fittkauimyia* sp. were identified. Assessment of their relationships with several environmental parameters was performed using the canonical correspondence analysis (CCA). *Tanytarsus formosanus* was the most dominant in the relatively clean CTKR and moderately polluted JR with mean densities of 19.66 and 25.32 m^{-2}, respectively while *C. kiiensis* was abundant in more polluted rivers. *Tanytarsus formosanus*, *Dicrotendipes* sp. and *Microchironomus* sp. were grouped under moderate to high water temperature, total organic matter (TOM), total suspended solids (TSS), velocity, pH, phosphates and sulphates. However, *Tanypus punctipennis*, *Fittkauimyia* sp., and *Clinotanypus* sp. were associated with high contents of river sediment such as TOM, Zn and Mn and water ammonium-N and nitrate-N and they were associated with higher dissolved oxygen (DO) content in the water. *Chironomus kiiensis*, *C. javanus* and *P. trigonus* showed positive relationships with TOM, ammonium-N and nitrate-N as well as trace metals of Zn, Cu and Mn. These three species could be considered as tolerant species since they have the ability to survive in extreme environmental conditions with low DO and high concentrations of pollutants. Based on the water parameter scores in all rivers, the highest diversity of chironomid larvae was reported in CTKR. With higher concentrations of organic and/or inorganic pollutants as reported in PPR, KUR and AR, the chironomid larval diversity decreased, and the abundance of tolerant species, mainly *Chironomus* spp., increased.

Key words: chironomid larvae; Juru River; physicochemical parameters; canonical correspondence analysis

DOI: 10.1016/S1001-0742(09)60311-9

Introduction

Biological indicators are becoming useful in assessing the overall effect of environmental contaminations by virtue of their role in aquatic ecosystems (MacDonald and Taylor, 2006; Wang et al., 2007; Ghedira et al., 2010; Meng et al., 2010). The larvae of non-biting aquatic midges (Chironomidae: Diptera) are dominant in many aquatic ecosystems especially those with moderately to highly polluted environments (Al-Shami et al., 2010a). This group of insects is considered as ideal organisms for bioassays in the laboratory because they are relatively easy to culture, (Warwick, 1985). They are suitable for ecotoxicological assessment because the larvae of some species are sensitive to specific forms of pollution, whereas others are quite tolerant when continuously exposed to stress or pollution. Late instar larvae of some chironomid species frequently develop deformities especially in the mouthparts which make them ideal bioindicators for aquatic ecosystem's disturbance (Doherty et al., 1999; Bhattacharyay et al., 2005; MacDonald and Taylor, 2006; Al-Shami et al., 2010a). In Malaysia, three subfamilies of Chironomidae have been reported; Chironominae, Tanypodinae, and Orthocladiinae. The Chironominae subfamily are represented by two tribes: Chironomini and Tanytarsini (Cranston, 2004).

In lentic and lotic environments, the analysis of benthic fauna, particularly Chironomidae, has played a dominant role (Saether, 1979). The benthic fauna are exposed to variations in their environment both in the nutrient cycle and in the oxygen level (Saether, 1979). However, the use of communities of Chironomidae in describing and monitoring lotic ecosystems still lacks inferring power, due to a lack of knowledge on their ecological niches and their species distribution (de Bisthoven and Gerhardt, 2003).

In industrial and urbanized areas, streams are subject to physical (canalization, modification of banks, cleansing, regulation, etc.) and chemical (industrial or municipal sewage, etc) stress, which strongly modifies the water

* Corresponding author. E-mail: alshami200@gmail.com

quality (Grumiaux et al., 1998). This stress is reflected in the composition of benthic macroinvertebrate communities that respond quantitatively, not only to the availability of trophic resources, but also to variations in their physical habitat as well as physical and chemical variations in water and sediments (Grumiaux et al., 1998; Wang et al., 2007).

The pollution of aquatic ecosystems in Malaysia has emerged as a major ecological problem coinciding with rapid industrialization and urbanization (Ho, 1996). In 1994, the Malaysian Department of Environment (DOE) classified the Juru River Basin in the northwestern peninsular Malaysia as “very polluted” based on the water quality index (WQI) categorization (DOE, 1994). According to the study of Lim and Kiu in 1995, the Juru River is one the most polluted rivers in Malaysia, with sediments highly contaminated with non-residual heavy metals, such as Cd, Cu, Pb, and Zn. These contaminants were likely introduced by the discharges from the light and heavy industries in the Prai Industrial Estate that was established in the early 1970s (Mat and Maah, 1994).

Among macroinvertebrate taxa, Chironomidae is one of the richest groups having a lot of species inhabiting lotic and lentic habitats (Cranston, 2004). Due to their ubiquity and different species habitat preference, chironomids are well known as indicators of organic and inorganic pollution including heavy metal contamination (Marziali et al., 2010). The chironomids are widely reported from many moderately to highly polluted Malaysian rivers including Juru River (Siregar et al., 1999; Azrina et al., 2006; Al-Shami et al., 2010a).

Considerable efforts have been made in the past two decades to analyze chemical pollution in several Malaysian rivers (including the Juru River Basin) (Mat and Maah, 1994; Lim and Kiu, 1995). However, relatively much less attention has been paid to utilize aquatic organisms for purposes of environmental bioassessments (Tan and Yap, 2006; Morse et al., 2007). The available studies concerning the effects of contaminants on aquatic invertebrates in Malaysia at present primarily focus on the diversity and abundance of benthic macroinvertebrates inhabiting contaminated rivers (Azrina et al., 2006). The utility of chironomid for bioindication purposes seems not to be popular or widespread in the Asian ecoregions (Gopal, 2005), although this technique provides empirical biomonitoring tool and is widely used in the Northern American and European ecoregions (Groenendijk et al., 1998; Faria et al., 2008; Marziali et al., 2010). Al-Shami et al. (2010a) investigated the induction at organismal level of the organic and inorganic pollutants in Juru River Basin, in which the deformities among *Chironomus* spp. larvae collected from Juru River were correlated with different industrial, anthropogenic and agricultural inputs. However, no attempt to investigate the effect of the different inputs into the Juru River system on the chironomid species and distribution was made.

The present study was undertaken to investigate the influence of physicochemical parameters and environmental stressors (agricultural, industrial and anthropogenic) on the distribution and abundance of chironomid larvae in selected rivers receiving different contaminants in the Juru River Basin, Malaysia. It is hoped that chironomids as a bioindicator could be applied in water quality biomonitoring program in Southeast Asia, specifically Malaysia.

1 Materials and methods

1.1 Study area and sampling sites

The Juru River system originates from Mertajam Hill (Bukit Mertajam), located at approximately 05°22′N and 100°28′E in the northwestern part of the Malaysian Peninsula (Fig. 1). It drains a basin of about 75 km^2 with several small tributaries that flow through urbanized areas, highly polluted with industrial, agricultural, and domestic wastes. For the present study, six rivers in the Juru River Basin (Ceruk Tok Kun River (CTKR), Pasir River (PR), Juru River (JR), Permatang Rawa River (PRR), Ara River (AR) and Kilang Ubi River (KUR)) were selected as study areas. In each river, one permanent site for collecting chironomid larvae, water and sediment samples was established (Fig. 1). This river basin starts from pristine springs in the Ceruk Tok Kun recreational forest. CTKR was considered as a reference river because it is located nearest to its origin, at the foot of the Bukit Mertajam hill and was the least disturbed. It flows through a recreational park that is maintained and protected by the state government. The park has high density of vegetation with canopies dominated by bamboo trees fully covering the water surface of the river. The river sediment comprises mainly of gravel and small pebbles with some small rocks. The second river of PRR flows through a large area of paddy fields, consequently receives some discharges that may contain organic and inorganic contaminants. The river sediment is primarily sand-mud mixture and scattered grasses and shrubs grow along the river margins. KUR and JR are outlets of industrial discharges from surrounding garment and rubber factories. The bottom sediment in KUR consists of small pebbles and the water surface is slightly shaded. JR is a large, open river with sediments made of mainly clay. PR and AR are polluted primarily by anthropogenic activities from residential areas situated along the course of these rivers. The river bed in PR consists of small pebbles and its surface is slightly shaded. In AR, the river sediment consists of small pebbles and sand. This river passes through a village with a lot of vegetation. The water surface is largely covered by vegetative canopies.

1.2 Chironomid larval sampling

Chironomid larvae were randomly collected every month from November 2007 to March 2008 by sampling benthos across an approximately 100 m stretch in each river. The larval sampling area was generally in the vicinity of the water and sediment sampling sites in each river. A D-shaped aquatic net (20 cm in radius, 40 cm in width, 300 μm in pore size) equipped with a 1.2 m long handle was used for collecting the larval samples by dragging the aquatic net above 1 m of the sediment-water interface. The number of benthic samples required to yield

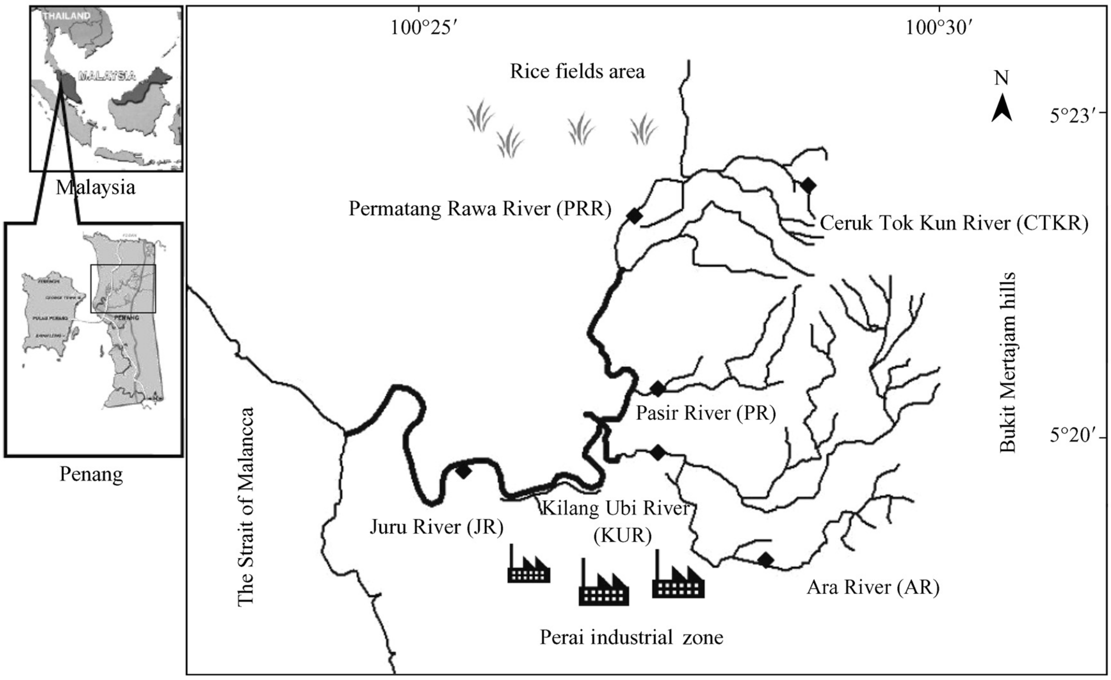

Fig. 1 Map of the Juru River system showing approximate locations of sampling sites in the Juru River Basin, Penang, Malaysia.

a representative estimation of chironomid population were calculated following Elliot (1971). Ten benthic samples were collected monthly from each river except in KUR (30 samples). All benthic materials collected during each sampling were transported to the laboratory on ice. In the laboratory, each sample was washed with tap water through a sieve (300 μm pore). The residue on the sieve was transferred into a white plastic pan and sufficient tap water was added to examine midge larvae. The larvae were collected with a dropper and transferred to small vials. They were preserved in 80% ethanol for subsequent taxonomic identification.

1.3 Chironomid identification

Permanent slide mounts of chironomid larvae (3–4th instar) were prepared to identify chironomid larvae. The preserved larvae were transferred to a Petri dish containing 10% KOH solution and left for 24–48 hr to eliminate the larval muscles. Thereafter, the permanent slide mounts of the larvae were prepared following the method of Epler (2001). The slide-mounted larvae were identified to generic level using appropriate taxonomic keys (Kikuchi et al., 1985; Hasegawa and Sasa, 1987; Morse et al., 1994; Merritt and Cummins, 1996; Epler, 2001; Cranston, 2004). The larvae of *C. kiiensis*, *C. javanus*, *Tanytarsus formosanus* and *Tanypus punctipennis* were compared with other verified records in our laboratory.

1.4 Physicochemical parameters of water

Measurements of physicochemical parameters in the field, such as water depth, river width, water pH, water temperature, and dissolved oxygen (DO) were made *in situ* at three randomly selected locations at each sampling site.

To analyze selected chemical parameters, three separate samples from each river were randomly collected. All water samples were transported to the laboratory in an ice chest and kept at 4°C until analyzed. The total suspended solids (TSS) were estimated using the method of Tomar (1999). The ammonium-N, nitrate-N and phosphate-P, chlorides, sulphates, and aluminum contents in water were measured at appropriate wavelengths using a YSI 9100 photometer test kit (YSI Inc., USA).

1.5 Sediment samples

At each river, three sediment samples were randomly collected using grab sampler and these samples were air-dried (25–30°C) in the laboratory. Total organic matter (TOM) content was analyzed following the method of Faithful (2002). The non-residual metals, Zn, Mn, Cu, and Ni, in the sediment samples were analyzed according to the method of Chester and Voutsinou (1981). Five grams of a dried sediment samples were placed in a 100 mL wide neck glass flask to which 75 mL of 0.5 mol/L HCl was added and the flasks shaken mechanically for approximately 16 hr. The solutions were filtered through 0.45 μm filter paper. The filtrates were sprayed directly into a flame atomic absorption spectrophotometer Model A Analyst 100 (PerkinElmer, USA) using hollow cathode lamps at wavelengths of 213.9 nm (Zn), 279.5 nm (Mn), 324.8 nm (Cu) and 232 nm (Ni) to determine the concentrations in mg/kg of the dry weight (Robinson, 1966).

1.6 Statistical analysis

The one-way ANOVA ($P < 0.05$) was used to test the difference in means of chironomid larval abundance among various sampling occasions and among the sampling locations. The nonparametric Kendall's tau-b correlation was used to assess the influence of physicochemical variables on the abundance of the Chironomidae using the SPSS (Statistical Package for Social Science), Version 13.

Canonical correspondence analysis (CCA) of CANOCO program (ter Braak and Prentice, 1988; ter Braak, 1989) investigate the contribution of the environmental stressors on the distribution and abundance of transformed Chironomidae species data (log(X+1)). The Monte-Carlo test was applied to test the significance of the produced canonical axes with 499 permutations at P <0.05. The biplot ordination diagram was produced using CanoDraw for Windows 4.1.

2 Results

2.1 Chironomid taxa abundance and distribution

Six species of subfamily Chironominae; *C. kiiensis*, *C. javanus*, *Polypedilum trigonus*, *Microchironomus* sp., *Dicrotendipes* sp. and *Tanytarsus formosanus* and three species of subfamily Tanypodinae; *Clinotanypus* sp., *Tanypus punctipennis* and *Fittkauimyia* sp. were identified from the six rivers studied. As shown in Table 1, the abundance of chironomid taxa recorded from Juru River basin did not show any significant difference among the sampling dates (ANOVA, P > 0.05). However, *C. kiiensis*, *C. javanus* and *Microchironomus* sp. abundances differed significantly (P <0.05) among the investigated rivers (ANOVA, F = 2.81, 11.41, and 2.95, respectively).

As shown in Fig. 2, two rivers: PRR and CTKR supported a high diversity of chironomid species. However, in other rivers *C. kiiensis* was the most dominant with very few other genera present. In CTKR, six species of Chironomidae were recorded. *Polypedilum trigonus* was reported in March with a low density (< 1 m^{-2}). The abundance of *Tanytarsus formosanus* was obvious. It was found in the samples of all months except in November. The highest density was reported in December (68.31 m^{-2}). All the three species of Tanypodinae; *Clinotanypus* sp., *Tanypus punctipennis* and *Fittkauimyia* sp. were found in December with densities of 3.33, 3.33 and 6.66 m^{-2}, respectively. They were also collected in February with densities of 0.83, 4.17 and 2.5 m^{-2}, respectively (Fig. 2a). *Chironomus kiiensis* was recorded in December (5 m^{-2}), February (4.17 m^{-2}) and March (5.83 m^{-2}) (Fig. 3).

In PRR, six species were recorded (Fig. 2b) with a dominance of *C. kiiensis* (Fig. 3). In AR, three species were collected. *Chironomus kiiensis* was found during all sampling occasions reaching its highest density in February 2008 with 52.48 m^{-2}, and the lowest density in March 2008 (14.16 m^{-2}). *Polypedilum trigonus* and *Clinotanypus* sp. were both reported in December with a density of 3.33 and 1.67 m^{-2}, respectively. In KUR, PRR, AR and PR, *C. kiiensis* larvae were the most dominant species with a total mean density (5 months) of 34.32 and 1509.06, 23.99 and 73.97 m^{-2}, respectively.

2.2 Physical and chemical parameters of water and sediment

Various water and sediments physicochemical parameters are summarized in Table 2. The mean water depths of 11.77, 12.80, 20.43, 25.87, and 19.07 cm respectively at CTKR, AR, KUR, PR, and PRR were relatively shallow. JR (71.27 cm) was rather deep. The widths of the rivers varied quite significantly with AR, PRR, PR, KUR and

Table 1 Results of the one-way ANOVA on monthly variation of the chironomid larval abundance from November 2007 to March 2008 in six rivers in Juru River Basin, Penang, Malaysia

Parameter	ANOVA (factor month, df = 4)		ANOVA (factor site, df = 5)	
	F	Significance	F	Significance
C. kiiensis	0.75	0.569	2.81*	0.039
C. javanus	0.25	0.909	11.41*	0.000
Polypedilum trigonus	1.32	0.289	1.69	0.176
Microchironomus sp.	0.88	0.488	2.95*	0.032
Dicrotendipes sp.	0.76	0.561	2.58	0.053
Tanytarsus formosanus	0.52	0.723	1.53	0.218
Clinotanypus sp.	0.91	0.472	0.80	0.559
Tanypus punctipennis	0.98	0.436	1.57	0.206
Fittkauimyia sp.	1.47	0.240	1.81	0.149

* P < 0.05.

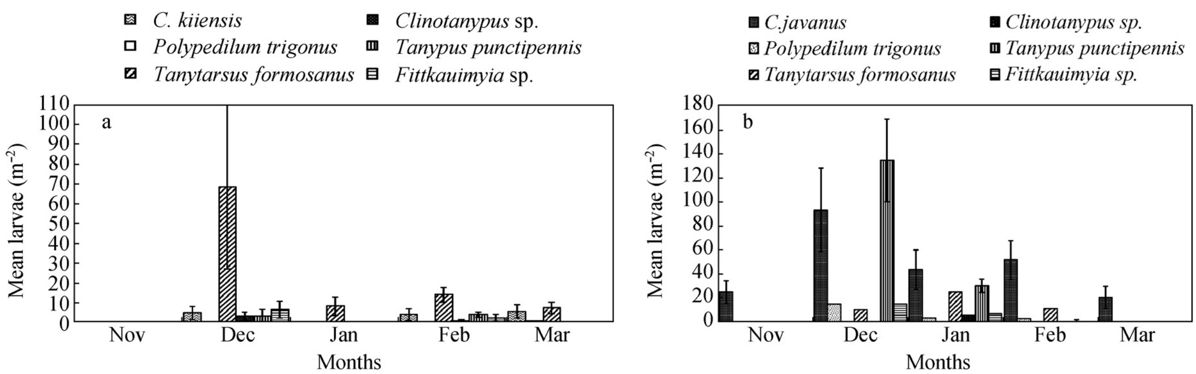

Fig. 2 Larval diversity of chironomid species sampled monthly (November 2007–March 2008) from Ceruk Tok Kun River (CTKR) (a) and Permatang Rawa River (PRR) (b), Penang, Malaysia.

Table 2 Selected physicochemical parameters of water and sediments measured monthly from November 2007 to March 2008 at permanent sampling sites in Juru River Basin, Penang, Malaysia

Water parameter	CTKR	PR	KUR	PRR	AR	JR
pH	6.42 ± 0.05	6.90 ± 0.01	6.97 ± 0.03	7.10 ± 0.03	6.58 ± 0.04	7.83 ± 0.22
DO (mg/L)	6.99 ± 0.17	2.75 ± 0.20	1.14 ± 0.29	3.30 ± 0.36	1.09 ± 0.13	9.80 ± 1.26
Temperature (°C)	25.34 ± 0.14	27.01 ± 0.15	27.86 ± 0.16	30.15 ± 0.26	27.15 ± 0.07	31.61 ± 0.49
Velocity (m/sec)	0.32 ± 0.05	0.61 ± 0.22	0.48 ± 0.133	0.30 ± 0.05	0.43 ± 0.08	0.04 ± 0.03
Depth (cm)	11.77 ± 1.31	25.87 ± 1.83	20.43 ± 1.45	19.07 ± 2.03	12.80 ± 1.20	71.27 ± 11.56
Width (m)	2.88 ± 0.18	4.65 ± 0.18	3.42 ± 0.09	3.36 ± 0.08	3.55 ± 0.16	9.13 ± 0.44
TOM (%)	2.11 ± 0.18	6.21 ± 0.75	3.29 ± 0.82	8.57 ± 1.38	4.66 ± 0.77	9.17 ± 0.61
TSS (mg/L)	12.41 ± 1.69	22.57 ± 2.53	68.57 ± 1.36	81.07 ± 1.18	68.75 ± 3.60	60.75 ± 3.87
Phosphate (mg/L)	1.23 ± 0.44	2.67 ± 0.35	3.97 ± 0.41	2.15 ± 0.09	2.19 ± 0.15	2.74 ± 0.42
Ammonia (mg/L)	0.34 ± 0.14	3.30 ± 0.22	5.62 ± 0.50	3. 83 ± 0.31	2.19 ± 0.22	2.78 ± 0.25
Nitrate (mg/L)	0.74 ± 0.14	0.85 ± 0.14	0.96 ± 0.11	1.50 ± 0.30	0.94 ± 0.174	1.17 ± 0.27
Sulphates (mg/L)	7.20 ± 2.32	6.38 ± 0.44	27.69 ± 3.16	13.29 ± 2.03	10.20 ± 0.901	47.53 ± 4.05
Chloride (mg/L)	2.17 ± 0.19	3.25 ± 0.21	4.24 ± 0.20	5.45 ± 0. 84	7.28 ± 0.28	16.07 ± 1.47
Aluminium (mg/L)	0.12 ± 0.03	0.08 ± 0.01	0.17 ± 0.03	0.22 ± 0.04	0.30 ± 0.08	0.14 ± 0.03
Sediment Zn (mg/kg)	4.57 ± 0.18	24.81 ± 1.11	44.72 ± 4.17	38.83 ± 4.76	20.72 ± 0.99	101.08 ± 12.16
Sediment Mn (mg/kg)	35.47 ± 2.6	16.78 ± 3.12	17.91 ± 1.22	50.54 ± 6.19	10.59 ± 2.20	33.02 ± 0.34
Sediment Cu (mg/kg)	0.39 ± 0.09	1.98 ± 0.91	1.64 ± 0.57	3.13 ± 0.70	1.054 ± 0.06	6.07 ± 0.191
Sediment Ni (mg/kg)	3.73 ± 4.50	3.01 ± 2.04	nd	2.92 ± 2.23	2.73 ± 5.47	2.64 ± 3.64

nd: not detected.
Data are presented as mean ± SE.

CTKR were below 5 m (3.55, 3.36, 4.65, 3.42, and 2.88 m respectively), JR (9.13 m) was the widest. The mean value of water pH ranged from 6.42 (CTKR) to 7.83 (JR). Except for CTKR (6.99) and JR (9.80), the DO content in the water was rather low in the other four habitats, with AR showing the lowest mean value of 1.09 mg/L. Water temperature generally remained 2–3°C higher in the JR and PRR (mean 31.61 and 30.15°C, respectively),

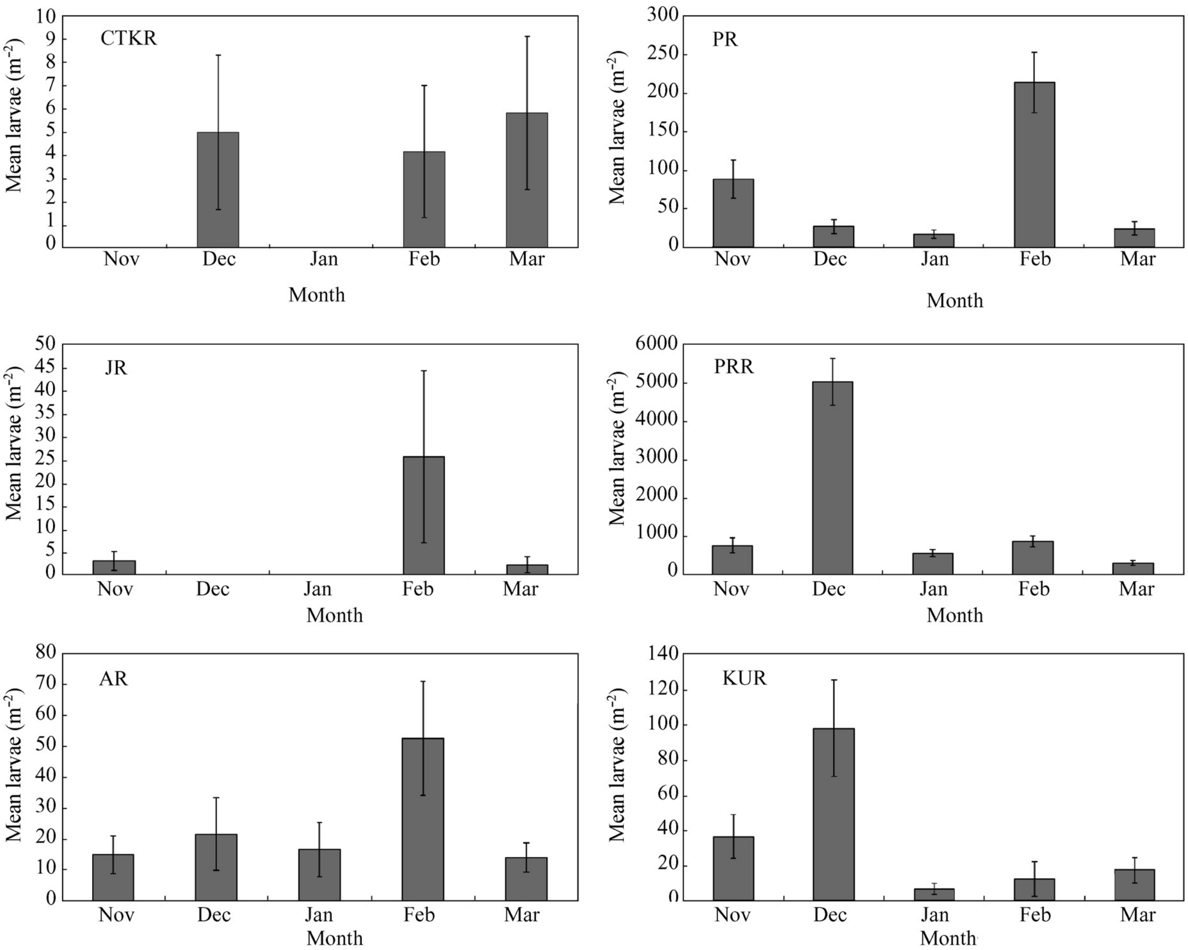

Fig. 3 Larval density (mean ± standard error (SE)) of *C. kiiensis* sampled monthly (November 2007–March 2008) from Ceruk Tok Kun River (CTKR), Pasir River (PR), Juru River (JR), Permatang Rawa River (PRR), Ara River (AR) and Kilang Ubi River (KUR), Penang, Malaysia.

compared to the other four habitats. Mean TOM content of 9.17% in JR was 3–4 folds higher than in CTKR (2.11%); in PR, PRR, AR and KUR, mean values of TOM were 6.21%, 8.57%, 4.66%, and 3.29%, respectively. The TSS content in PRR (81.07 mg/L) was the highest, followed by KUR (68.57 mg/L), AR (68.75 mg/L), and JR (60.75 mg/L). In CTKR and PR, the TSS contents were comparatively lower (12.41 and 22.57 mg/L, respectively). The phosphate content of water in the six rivers ranged from 1.23 mg/L (CTKR) to 3.97 mg/L (KUR); in PR, AR, PRR, and JR the mean values amounted to 2.67, 2.19, 2.15, and 2.74 mg/L. CTKR had the lowest ammonium-N content in water (0.34 mg/L). In PR, AR and JR, their mean values ranged from 2.19 to 3.30 mg/L. The highest content (5.62 mg/L) of this parameter was recorded in KUR followed by PRR (3.83 mg/L). Surrounded by rice fields, PRR recorded the highest content of nitrate-N (1.50 mg/L) followed by JR with 1.17 mg/L. In other rivers the nitrate-N contents were much lower ranging from 0.74 to 0.96 mg/L The sulphate content in the six rivers showed a large variation, with the lowest mean value of 6.38 mg/L recorded in PR and the highest of 47.53 mg/L in JR. The highest concentration of chloride was also reported from JR (16.07 mg/L) and its lowest content was observed in CTKR (2.17 mg/L). The aluminum concentration in the water from the six rivers ranged from 0.08 to 0.3 mg/L, with the highest concentration occurring in AR and the lowest in PR. The concentration of this metal was nearly three fold higher in AR compared to PR.

In the least contaminated CTKR, sediments concentrations of non-residual metals, Zn, Mn, Cu, and Ni were 4.57, 35.47, 0.39 and 3.73 mg/kg, respectively. In other rivers, their sediments were highly contaminated with Zn compared to other metals. The highest Zn contamination was recorded in JR (101.08 mg/kg) followed by KUR (44.72 mg/kg), PRR (38.83 mg/kg), PR (24.81 mg/kg) and AR (20.72 mg/kg). Mn concentrations in river sediments were relatively high with 33.02 mg/kg in JR, 50.54 mg/kg in PRR, 16.78 mg/kg in PR, 17.91 mg/kg and 10.59 in KUR and AR respectively. Contaminations of two other metals were comparatively low with Cu ranging from 1.054 mg/kg in AR to 6.07 mg/kg in JR and Ni from undetected in KUR to 3.73 mg/kg in CTKR.

2.3 Influence of environmental stressors on the chironomid distribution

Table 3 shows the correlation coefficients (non-parametric Kendall's tau-b correlation test at $P < 0.05$) between the physicochemical parameters and chironomid taxa abundance. *Chironomus kiiensis*, *C. javanus* ($P < 0.01$) and *P. trigonus* ($P < 0.5$) showed positive correlation with pH, indicating their preference for alkaline habitat. *Chironomus javanus*, *P. trigonus*, *Tanypus punctipennis* and *Fittkauimyia* sp. abundances were positively influenced by DO ($P < 0.01$) in the water. The abundance of *Chironomus* species were positively influenced by water temperature ($P < 0.01$). *Chironomus javanus*, *Tanypus punctipennis* and *Fittkauimyia* sp. preferred lower water velocity. Only *C. kiiensis* and *C. javanus* preferred deeper and wider rivers as their abundances correlated positively with river depth and width ($P < 0.01$). The tolerant species, *C. kiiensis*, *C. javanus* and *P. trigonus* preferred high organic matters in the river sediments. They also tolerated increasing sulphate contents in the water as they were positively correlated with sulphates content. However, *C. kiiensis* and *C. javanus* were tolerant to chloride contents in the water as well as Zn and Cu contents in the sediments, hence their abundances were associated with high concentrations of these pollutants.

CCA was utilized to investigate the effect of the environmental parameters on the distribution of chironomid larvae. The CCA biplot is shown in Fig. 4. The first axis explained 78% and the second axis 11.4% of the variances and the Monte Carlo permutation test (499 permutations) was significant at $P < 0.05$. The CCA output indicated that *T. formosanus*, *Dicrotendipes* sp. and *Microchironomus* sp. preferred environmental conditions characterized as having relatively, moderate to high water temperature, TOM, TSS, velocity, pH, phosphates and sulphates. The chironomid group of Tanypodinae, including *Tanypus punctipennis*, *Fittkauimyia* sp., *Clinotanypus* sp. and other species of *P. trigonus*, *C. kiiensis*, and *C. javanus* were associated with higher DO, depth and other organic pollutants such as TOM, ammonia and nitrate.

3 Discussion

The success in the economic growth and industrialization in Malaysia has lead to environmental problems with ever-increasing land, air and water pollution (Tan and Yap, 2006). Consequently, many rivers in the country including Juru River Basin, were listed as highly polluted water bodies. Out of 189 rivers managed by the Department of Drainage and Irrigation Malaysia, only 75 of them

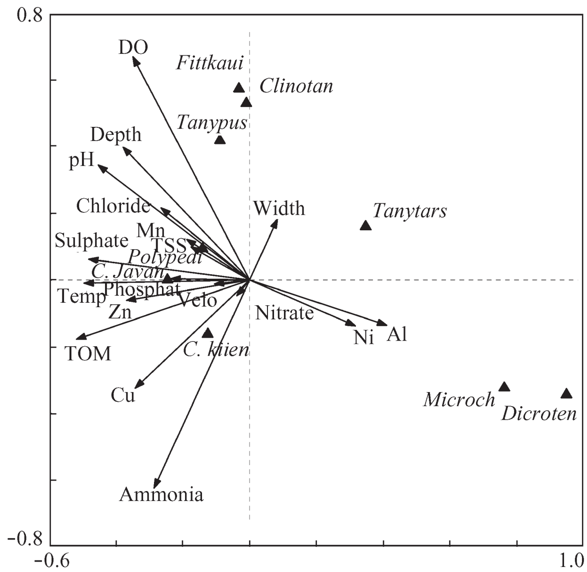

Fig. 4 First two axes of canonical correspondence analysis (CCA) showing the relationship between selected (forward selection) environmental parameters and distribution of chironomid larvae (log(X+1)) collected from Juru River tributaries, Penang, Malaysia.

Table 3 Non-parametric correlation (correlation coefficient values) between chironomid species larvae and water and sediment parameters

	C. kiiensis	*C. javanus*	*Polypedilum trigonus*	*Microchironomus* sp.	*Dicrotendipes* sp.	*Tanytarsus formosanus*	*Clinotanypus* sp.	*Tanypus punctipennis*	*Fittkauimyia* sp.
pH	0.460**	0.417**	0.356*	–0.120	–0.204	–0.180	0.018	0.168	0.060
DO (mg/L)	0.118	0.451**	0.391**	–0.211	–0.197	0.232	0.293	0.428**	0.437**
Temperature (°C)	0.570**	0.478**	0.216	–0.051	–0.057	–0.175	0.009	0.096	–0.009
Velocity (m/sec)	–0.203	–0.317*	–0.19	0.106	0.073	–0.218	–0.121	–0.388*	–0.393*
Depth (cm)	0.436**	0.453**	0.278	–0.148	–0.141	–0.149	0.023	0.088	0.102
Width (m)	0.256*	0.425**	0.202	–0.134	–0.121	–0.032	–0.165	0.034	–0.037
TOM (%)	0.371**	0.458**	0.333*	0.014	–0.095	–0.046	–0.055	0.145	0.032
TSS (mg/L)	0.177	0.050	0.073	0.150	0.116	–0.073	–0.009	0.016	–0.079
Phosphate (mg/L)	0.283	0.210	0.216	0.028	–0.025	–0.08	0.041	0.050	0.133
Ammonia (mg/L)	0.167	–0.050	0.022	–0.023	–0.006	–0.300	–0.188	–0.157	–0.295
Nitrate (mg/L)	0.149	0.125	–0.143	0.267	0.240	0.038	–0.103	0.051	–0.056
Sulphates (mg/L)	0.428**	0.531**	0.314*	0.037	0.038	0.058	0.018	0.285	0.153
Chloride (mg/L)	0.341**	0.504**	0.154	0.198	0.184	0.117	–0.165	0.179	0.069
Aluminium (mg/L)	–0.040	0.016	–0.217	0.231	0.209	–0.059	–0.197	–0.110	–0.033
Sediment									
Zn (mg/kg)	0.51**	0.42**	0.082	0.087	0.019	–0.126	–0.174	0.023	–0.170
Mn (mg/kg)	0.074	0.175	0.125	–0.280	–0.260	0.120	0.128	0.145	0.087
Cu (mg/kg)	0.286*	0.32*	0.16	–0.010	–0.027	–0.219	–0.083	–0.174	–0.039
Ni (mg/kg)	0.013	0.037	0.010	nd	0.151	0.048	–0.006	–0.101	–0.026

$N = 30$, nd: not detected.
*$P < 0.05$; **$P < 0.01$.

Table 4 Tolerance of the chironomid species collected from six rivers in the Juru Basin based on their abundance

	CTKR	KUR	PRR	AR	JR	PR	Previous records
Chironomus spp.	+	++	++++	+	+	+++	Very tolerant (Saether, 1979; Mousavi et al., 2003)
Polypedilum trigonus	+	+	+	+	–	+	Tolerant (Mousavi et al., 2003)
Microchironomus sp.	–	–	–	–	+	+	Not available
Dicrotendipes sp.	–	–	–	–	+	–	Tolerant (Saether, 1979)
Tanytarsus formosanus	++	+	+	–	++	+	Sensitive (Mousavi et al., 2003; Helson et al., 2006; Carew et al., 2007)
Clinotanypus sp.	+	–	+	+	–	–	Tolerant (Dermott, 1991)
Tanypus punctipennis	+	–	++	–	–	+	Tolerant (Saether, 1979; Dermott, 1991)
Fittkauimyia sp.	+	–	+	–	–	+	Not available

–: Absent; +: present; ++: abudant; +++: moderately abundant; ++++: very abundant.

have clean water, and 56 rivers (including Juru River) are categorized as polluted while 58 rivers are slightly polluted (Ho, 1996; Tan and Yap, 2006).

Application of living organisms in bio-monitoring of aquatic ecosystems has not been commonly practiced in Malaysia due to a lack sufficient data of the aquatic invertebrate's ecology and taxonomy (Morse et al., 2007). There are very few reported studies of aquatic organism utilisation in biomonitoring programs of Malaysian rivers. Maznah and Mansor (2002) assessed the aquatic pollution in one of the most polluted rivers, Pinang River, based on attached diatom communities. Our study is the first report on application of chironomid species as an indicator for water and sediment quality in polluted Malaysian river.

In this study, chironomid community composition was shown to differ between an unpolluted river and polluted rivers irrespective of the pollution sources (Table 4). A number of chironomid species collected from these rivers such as *T. formosanus*, *C. kiiensis*, *C. javanus* and *P. trigonus* proved to be good bioindicators for the pollution stress in Juru River system with tolerance characteristics comparable to other findings in the temperate rivers (Table 4). Agricultural activities, through the use of fertilizers and organic manures, often lead to the nutrient enrichment of water and sediment, as does the input of both treated and untreated domestic wastes (Harding et al., 1999). Consequently, PRR, PR and AR that pass such areas, were dominated by pollution-tolerant species, such as *C. kiiensis* and *C. javanus*.

Other than CTKR, none of the rivers in the Juru River Basin investigated in this study is perceived to be pristine or even clean because of their location amid areas of high nutrient loading from various, agricultural, and anthropogenic sources. PRR was highly contaminated, presumably due to agricultural chemicals applied periodically to the surrounding paddy fields. The highest density of *Chironomus* spp. (> 1000 m^{-2}) was observed in this river. Paddy fields are known to be a suitable habitat for *C. kiiensis* (Steven, 2006; Al-Shami et al., 2010b). Therefore, a nearby river would be equally inhabited by a similar species when their females lay eggs there. Furthermore, larvae from these rice fields could continually enter the river when excess water is drained out of the paddy plots. Consequently, the larval population peaked at a very high density in the PRR as compared to other rivers. Other than *C. kiiensis*, *C. javanus* and *Tanypus punctipennis* were also abundant in this river, which is in agreement with the study of Winberg (1978) who predicted that Tanypodinae could dominate polluted sites such as paddy fields.

KUR and JR receive industrial discharges from garment and rubber factories. Industrially polluted sites are typically dominated by chironomid larvae (Wright and Burgin, 2009). Some chironomid genera such as *Procladius*, *Chironomus* and *Cricotopus* are increasingly dominant in more polluted sites (Dermott, 1991; Marziali et al., 2010). Similar findings were previously reported from other Malaysian rivers such as Langat River (Azrina et al., 2006) and Linggi River (Ahmad et al., 2002). *Chironomus* hemoglobin appears to fulfill physiological roles in transporting and storing oxygen in the larvae that burrow in polluted and hypoxic mud (Osmulski and Leyko, 1986). Moreover, adaptation to pollutants can involve a wide range of mechanisms including detoxification, transport, essential element regulation and behaviour (Newman, 1995). Therefore, *Chironomus* spp. larvae have the ability to survive in organically and inorganically polluted rivers with low concentration of dissolved oxygen and high concentrations of pollutants including heavy metals such as Zn (Mousavi et al., 2003; Wright and Burgin, 2009; Marziali et al., 2010).

The trace metals contamination of the sediment was evident in some rivers such as KUR, JR and PRR. Considerably high content of Zn was found in the sediment of KUR that received discharges from garment and rubber factories. The highest concentrations of Zn (101.08 mg/kg) and Cu (6.07 mg/kg) were recorded from JR due to some industrial and anthropogenic discharges. The highest concentration of Mn (50.54 mg/kg) was detected in PRR. Some pesticides that contain Zn and Cu are commonly applied to the paddy fields, which are likely the case in PRR. *Chironomus* spp. was the most dominant species in PRR. It is well known that *Chironomus* spp. is highly tolerant and adaptable to extremely polluted conditions and show deformities as a response (Bhattacharyay et al., 2005). The deformities of *Chironomus* spp. larvae collected from polluted Juru River Basin has been reported and found to be strongly related to heavy metal contamination (Al-Shami et al., 2010a).

The lowest abundance of chironomid was recorded in PR and AR. Both rivers were polluted primarily with domestic wastes resulting from anthropogenic activities in the surrounding residential areas. The balance of river ecosystem is often disturbed after receiving excessive amounts of either organic matter or inorganic nutrient salts, nitrates and phosphates (Hawkes, 1979). A poor quality effluent containing high concentrations of ammonia (Mason, 1981), phosphorus, nitrogen, and carbon (Dudgeon, 2008) influence the structures of residence macroinvertebrate communities. Rivers with such contaminations are characterized by a dominance of *Chironomus* (Azrina et al., 2006). In a low dissolved oxygen environment such as a rice field, chironomid *Tanypus punctipennis* larval abundance is much regulated by variations in oxygen concentrations in the water (Al-Shami et al., 2010b). Similarly, our results revealed a positive relationship between the abundance of *C. javanus*, *P. trigonus*, *T. punctipennis* and the concentration of oxygen in the river water.

It is well established that multivariate analysis methods such as CCA have the potential to explain the complex interaction between chironomid community and environmental stressors (Mousavi et al., 2003; Marziali et al., 2010). In the present study, the CCA facilitated identification of the influence of various physicochemical environmental variables on the distribution of chironomid larvae in the Juru River Basin. *Chironomus kiiensis*, *C. javanus* and *P. trigonus*, showed a positive relationship with most of the organic pollutants such as TOM, ammonium, nitrate as well as trace metals of Zn, Cu and Mn. From observations in this study, these three species are considered as tolerant species because they have the ability to survive in extreme environmental conditions with low amounts of dissolved oxygen and high concentrations of pollutants. The finding of Mousavi et al. (2003) that *Chironomus* spp. and *Polypedilum* sp. were very tolerant to heavy metal contamination in subarctic watercourse further supported this categorization.

As displayed in the CCA output, the three Tanypodinae species favored habitats with high contents of sediment TOM, Zn and Mn and water ammonium and nitrate. They were also associated with higher levels of DO content. In this case, Tanypodinae larvae might be able to tolerate some organic and inorganic contaminants, but they could not survive at low oxygen concentrations. *Tanytarsus formosanus* preferred environmental conditions with relatively moderate to high water temperatures, DO, velocity and pH. However, unlike the Tanypodinae species, some organic (ammonium and nitrate) and inorganic pollutants (Zn and Mn) were unfavorable to this species. Similar findings were also reported by Mousavi et al. (2003). In their study, *Tanytarsus* spp. appeared to be more sensitive to pollution and in consequence was commonly found at the least polluted sites of Pasvik Lake in Norway-Russia border.

Utilization of indicator organisms such as Chironomidae (in this study) has several advantages over traditional chemical analyses for water quality assessment because aquatic organisms live almost continuously in the water and respond to all environmental stressors, including synergistic combinations of pollutants (Morse et al., 2007). According to Goodnight (1973), the presence of the aquatic organisms often provides the best indication of the condition of a water body. Other than chironomids (Wright and Burgin, 2009), many groups of aquatic organisms have also been suggested as valuable indicator species such as algae and diatoms (Padisak et al., 2006), mollusks (Amin et al., 2009), Oligochaeta (Lafont et al., 1992), fish (Lyons et al., 1995) and other macroinvertebrates (Hellawell, 1986).

4 Conclusions

The present study demonstrates the possible influence of industrial and anthropogenic contaminants on the distribution and diversity of chironomid larvae inhabiting rivers of the Juru River Basin. In general, severe levels of pollution appeared to have marked impacts on the chironomid community. *Tanytarsus formosanus* appeared to be the most reliable bioindicators of the least polluted sites (such as CTKR and JR). With higher concentrations of organic pollutants as reported in PPR, KUR and AR, the abundance of tolerant species *Chironomus* spp. increased.

This study provides baseline data for some physicochemical conditions prevailing in the investigated rivers for future reference to develop the biomonitoring programs using macroinvertebrates, specifically Chironomidae. Our results also provide further support for using chironomid larvae as bioindicators for water pollution in other Malaysian rivers. *Tanytarsus formosanus* was evidently sensitive to deterioration of water and sediment quality in Juru River Basin. However, *C. kiiensis*, *C. javanus*, and *P. trigonus* were highly tolerant to organic and heavy metal contamination in all tributaries of Juru River Basin. Application of ordination techniques like the CCA on chironomid community is useful for differentiation of sites with varying degrees of contamination.

Acknowledgments

This study was partially funded by the Research University Grant 1001/BIOLOGI/ 630166, awarded to the second and the third authors. We are indebted to Mohd Shukri, Kalimuthu, Shahbudin, and Siti Khatijah for their assistance in the field and in the laboratory. We thank the School of Biological Sciences, Universiti Sains Malaysia for providing laboratory and field facilities to undertake this study. Thanks to two anonymous reviewers for helpful revisions and critical comments to earlier drafts of this manuscript.

References

Ahmad A, Maimon A, Othman M S, Mohd Pauzi A, 2002. The potential of local benthic macroinvertbrates as a biological monitoring tool for river quality assessment. In: Proceedings of the Regional Symposium on Environment and Natural Resources. 10–11 April, Kuala Lumpur, Malaysia. 464–471.

Al-Shami S, Rawi C S M, Nor S A M, Ahmad A H, Ali A, 2010a. Morphological deformities in *Chironomus* spp. (Diptera: Chironomidae) larvae as a tool for impact assessment of anthropogenic and environmental stresses on three rivers in the Juru River system, Penang, Malaysia. *Environmental Entomology*, 39: 210–222.

Al-Shami S A, Salmah M R C, Abu Hassan A, Azizah M N S, 2010b. Temporal distribution of larval Chironomidae (Diptera) in experimental rice fields in Penang, Malaysia. *Journal of Asia-Pacific Entomology*, 13: 17–22.

Amin B, Ismail A, Arshad A, Yap C K, Kamarudin M S, 2009. Gastropod assemblages as indicators of sediment metal contamination in mangroves of Dumai, Sumatra, Indonesia. *Water Air and Soil Pollution*, 201: 9–1.

Azrina M Z, Yap C K, Rahim Ismail A, Tan S G, 2006. Anthropogenic impacts on the distribution and biodiversity of benthic macroinvertebrates and water quality of the Langat River, Peninsular Malaysia. *Ecotoxicology and Environmental Safety*, 64: 337–347.

Bhattacharyay G, Sadhu A K, Mazumdar A, Chaudhuri P K, 2005. Antennal deformities of chironomid larvae and their use in biomonitoring of heavy metal pollutants in the River Damodar of West Bengal, India. *Environmental Monitoring and Assessment*, 108: 67–84.

Carew M E, Pettigrove V, Cox R L, Hoffmann A A, 2007. The response of Chironomidae to sediment pollution and other environmental characteristics in urban wetlands. *Freshwater Biology*, 52: 2444–2462.

Chester R, Voutsinou F G, 1981. The initial assessment of trace metal pollution in coastal sediment. *Marine Pollution Bulletin*, 12(3): 84–91.

Cranston P S, 2004. Chironomidae. In: The freshwater invertebrates of Malaysia and Singapore (Yule C M, Yong H S, eds.). Academy of Sciences, Malaysia. 711–735.

de Bisthoven L J, Gerhardt A, 2003. Chironomidae (Diptera, Nematocera) fauna in three small streams of Skania, Sweden. *Environmental Monitoring and Assessment*, 83: 89–102.

Dermott R M, 1991. Deformities in larval *Proclaius* sp. and dominant Chironomini from the St. Clair River. *Hydrobiologia*, 219: 171–185.

DOE (Department of Environment Malaysia), 1994. Classification of Malaysian Rivers, Juru River. Department of Environment, Ministry of Science, Technology and the Environment Malaysia.

Doherty M S E, Hudson P L, Ciborowski J J H, Schloesser D W, 1999. Morphological deformities in larval Chironomidae (Diptera) from the Western Basin of Lake Erie: A historical comparison. In: Proceedings of the 25th Annual Aquatic Toxicity Workshop. 18–21 October, Quebec.

Dudgeon D, 2008. Tropical Streams Ecology. Elsevier, USA.

Elliot J M, 1971. Some Methods for the Statistical Analysis of Samples of Benthic Invertebrates. Scientific Publication, Freshwater Biology Association, UK.

Epler J H, 2001. Identification manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida. Special Publication SJ2001-SP13. North Carolina Department of Environmental and Natural Resources, Raleigh, NC.

Faithful N T, 2002. Methods in Agricultural Chemistry Analysis. CABI Publishing, UK.

Faria M S, Lopes R J, Malcato J, Nogueira J A, Soares A M V M, 2008. *In situ* bioassay with *Chironomus riparius* larvae to biomonitor metal pollution in rivers and to evaluate the efficiency of restoration measures in mine areas. *Environmental Pollution*, 151(1): 213–221.

Ghedira J, Jebali J, Bouraoui Z, Banni M, Chouba L, Boussetta H, 2009. Acute effects of chlorpyryphos-ethyl and secondary treated effluents on acetylcholinesterase and butyrylcholinesterase activities in *Carcinus maenas*. *Journal of Environmental Sciences*, 21(10): 1467–1472.

Goodnight C J, 1973. The use of aquatic macroinvertebrates as indicators of streams pollution. *Transactions of the American Microscopical Society*, 92(1): 1–13.

Gopal B, 2005. Does inland aquatic biodiversity have a future in Asian developing countries? *Hydrobiologia*, 542: 69–75.

Groenendijk D, Zeinstra L W M, Postma J F, 1998. Fluctuating asymmetry and mentum gaps in populations of the midge *Chironomus riparius* (Diptera: Chironomidae) from a metal contaminated river. *Environmental Toxicology and Chemistry*, 17: 1999–2005.

Grumiaux F, Lepretre A, Dhainaut-Courtois N, 1998. Effect of sediment quality on benthic macroinvertebrate communities in streams in the north of France. *Hydrobiologia*, 385: 33–46.

Harding J S, Young R G, Hayes J W, Shearer K A, Stark J, 1999. Changes in agricultural intensity and river health along a river continuum. *Freshwater Biology*, 42: 345–357.

Hasegawa H, Sasa M, 1987. Taxonomical notes on the chironomid midges of the tribe Chironomini collected from Ryukyu

Islands, Japan, with description of their immature stages. *Japan Journal of Sanitary Zoology*, 38(4): 275–295.

Hawkes H A, 1979. Invertebrates as indicators of river water quality. In: Biological Indicators of Water Quality (James A, Evison L, eds.). John Wiley and Sons Ltd., UK.

Hellawell J M, 1986. Biological Indicators of Freshwater Pollution and Environmental Management. Elsevier Applied Science, London.

Helson J E, Williams D D, Turner D, 2006. Larval chironomid community organization in four tropical rivers: human impacts and longitudinal zonation. *Hydrobiologia*, 559: 413–431.

Ho S C, 1996. Vision 2020: Towards an environmental sound and sustainable development of freshwater resources in Malaysia. *GeoJournal*, 40(1-2): 73–84.

Kikuchi M, Kikuchi T, Okubo S, Sasa M, 1985. Observations on the seasonal prevalence of chironomid midges and mosquitoes by light traps set in a rice paddy area in Tokushima. *Japan Journal of Sanitary Zoology*, 36(4): 333–342.

Lafont M, Durbec A, Ille C, 1992. Oligochaete worms as biological describers of the interactions between surface and groundwaters: a first synthesis. *Regulated Rivers: Research and Management*, 7(1): 65–73.

Lim P E, Kiu M Y, 1995. Determination and speciation of heavy metals in sediment of the Juru River, Penang, Malaysia. *Environmental Monitoring and Assessment*, 35: 85–95.

Lyons J, Navarro-Perez S, Cochran P A, Santana C E, Guzman-arroyo M, 1995. Index of biological integrity based on fish assemblages for the conservation of streams and rivers in west central Mexico. *Conservation Biology*, 9: 569–584.

MacDonald E E, Taylor B R, 2006. Incidence of mentum deformities in midge larvae (Diptera: Chironomidae) from North Nova Scotia, Canada. *Hydrobiologia*, 563: 277–287.

Marziali L, Armanini D G, Cazzola M, Erba S, Toppi E, Buffagni A, Rossaro B, 2010. Responses of chironomid larvae (Insecta, Diptera) to ecological quality in Mediterranean river mesohabitats (South Italy). *River Research Applications*. 26: 1036–1051.

Mason C F, 1981. Biology of Freshwater Pollution. Longman Inc., New York.

Mat I, Maah M J, 1994. Sediment trace metal concentrations from the mudflats of Kuala Juru and Kuala Muda of Malaysia. *Bulletin of Environmental Contamination and Toxicology*, 53: 740–746.

Maznah W O W, Mansor M, 2002. Aquatic pollution assessment based on attached diatom communities in the Pinang River Basin, Malaysia. *Hydrobiologia*, 487: 229–241.

Meng W, Zhang N, Zhang Y, Zheng B H, 2009. Integrated assessment of river health based on water quality, aquatic life and physical habitat. *Journal of Environmental Sciences*, 21(8): 1017–1027.

Merritt R W, Cummins K W, 1996. An Introduction to the Aquatic Insects of North America (3rd ed.) Kenndall/ Hunt Publication, Dubuque, IA.

Morse J C, Yang L, Tian L, 1994. Aquatic Insects of China Useful for Monitoring Water Quality. Hohai University Press, China.

Morse J C, Bae Y J, Munkhjargal G, SangpraDub N, Tanida K, Vshivkova T S et al., 2007. Freshwater biomonitoring with macroinvertebrates in East Asia. *Frontiers in Ecology and the Environment*, 5(1): 33–42.

Mousavi S K, Primcerio R, Amundsen P, 2003. Diversity and structure of Chironomidae (Diptera) communities along a gradient of heavy metal contamination in a subarctic watercourse. *Science of the Total Environment*, 307: 93–110.

Newman M C, 1995. Quantitative Methods in Aquatic Ecotoxicology. Lewis Publishers, USA.

Osmulski P A, Leyko W, 1986. Structure, function and physiological role of *Chironomus* haemoglobin. *Comparative Biochemistry and Physiology–Part B*, 85: 701–722.

Padisak J, Borics G, Grigorszky I, Soroczki-Pinter E, 2006. Use of phytoplankton assemblages for monitoring ecological status of lakes within the Water Framework Directive: the assemblage index. *Hydrobiologia*, 553(1): 1–14.

Robinson J W, 1966. Atomic Absorption Spectroscopy. Dekker Inc., New York.

Saether O A, 1979. Chironomid communities as water quality indicators. *Holarctic Ecology*, 2: 65–74.

Siregar A Z , Che Salmah M R, Abu Hassan A, 1999. Distribution of aquatic insect and its implication on water quality in the Kerian River Basin, Kedah-Perak, Malaysia. In: Proceedings of River 99, University Science Malaysia. 329–334.

Stevens M M, Helliwell S, Cranston P S, 2006. Larval chironomid communities (Diptera: Chironomidae) associated with establishing rice crops in southern New South Wales, Australia. *Hydrobiologia*, 556: 317–325.

Tan S G, Yap C K, 2006. Biochemical and molecular indicators in aquatic ecosystems: current status and further applications in Malaysia. *Aquatic Ecosystem Health and Management*, 9(2): 227–236.

ter Braak C J F, 1989. CANOCO – an extension of DECORA-NA to analyze species-environment relationships. *Hydrobiologia*, 181: 169–170.

ter Braak C J F, Prentice I C, 1988. A theory of gradient analysis. *Advances in Ecological Research*, 18: 271–317.

Tomar M, 1999. Quality Assessment of Water and Waste Water. Lewis Publishers, New York.

Warwick W F, 1985. Morphological abnormalities in Chironomidae (Diptera) larvae as measures of toxic stress in freshwater ecosystem: indicating antennal deformities in *Chironomus* Meigen. *Canadian Journal of Fisheries and Aquatic Sciences*, 42: 1881–1914.

Wang X L, Lu Y L, Han J Y, He G Z, Wang T Y, 2007. Identification of anthropogenic influences on water quality of rivers in Taihu watershed. *Journal of Environmental Sciences*, 19(4): 475–481.

Winberg G G, 1978. Experimental application of various systems of biological indication of water pollution, In: Proceeding of first and second USA-USSR Symposium on effect of pollutants upon aquatic ecosystems (Mount D I, ed.). vol. I. Environ Res Lab, US EPA, Duluth, MN. 14–149.

Wright I A, Burgin S, 2009. Effects of organic and heavy metal pollution on chironomids within a pristine upland catchment. *Hydrobiologia*, 635: 15–25.