



Macroinvertebrate assemblage patterns as indicators of water quality in the Xindian watershed, Taiwan



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ABSTRACT

In the present study, we assessed the water quality along a stretch of the watershed with considerable economic importance at the Xindian in Taiwan, using macroinvertebrate assemblages, along with environmental variables. The research was carried out at the seven sampling sites (abbreviated as XD1–XD7) where human impacts varied in intensity from upstream tributaries to the downstream of the Xindian watershed from December 2010 to December 2011. All variables except for the hardness, pH, dissolved oxygen, conductivity, turbidity, phosphate, ammonia, and alkalinity were significantly different ($P < 0.05$) between the sampling sites. A total of seventy seven taxa belonging to forty five families within eight insect orders, along with three non-insect invertebrate taxa were recorded, with most representative orders being Ephemeroptera and Diptera. Mean values of the density, abundance of macroinvertebrates, Shannon index, Simpson index, and Pielou's evenness were much higher in the reference sites, XD2, XD3, and XD4 compared with impacted sites, XD5, XD6, and XD7. Most of the benthic metrics were greatest in the reference site compared to the impacted site. Only the composition measures, percentages of Chironomidae and Oligochaeta which are more tolerant to pollution were dominant in the impacted site, XD7. As the results of assessment by different benthic metrics, water quality of Xindian watershed became gradually worse from upstream to downstream. Generally, our results suggest that macroinvertebrate assemblages can be used for assessment of water quality.

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Introduction

Human activities are a major force affecting the ecosystems of the earth nowadays (Vitousek et al., 1997). Aquatic ecosystems most affected by the inadequate land use which influence the overall quality of the ecosystems by directly altering habitat, channel structure, and water quality posing severe threats to aquatic biodiversity (Lenat and Crawford, 1994; Allan, 2004; Azrina et al., 2006; Dudgeon et al., 2006; Smith and Lamp, 2008; Carlson et al., 2013). Riparian canopy removal in agriculture and urban development are common types of land use which influences river ecosystem by changing hydrological regimes and creating impervious areas, and increases the input of sediments, nutrient loads, and other pollutants (Allan et al., 1997; Nessimian et al., 2008). Even slight changes can affect the diversity, apparently causing alteration in community measures, resulting in the reduced intolerant taxa and even resulting in the local extinction of native species (Lenat and Crawford, 1994; Roy et al., 2003; Helms et al., 2009).

Use of macroinvertebrates is a widely used method for assessments of river water quality, especially for organic contamination (Johnson et al., 1993). Most aquatic macroinvertebrates reside in the benthic habitat for at least part of their life, relatively immobile, and very sensitive, therefore any disturbances in the aquatic environment may cause them to disappear or reduce diversity (Hilsenhoff, 1988; Zamora-Muñoz et al., 1995; Morse et al., 2007). By now, multimetric approaches have been the most widely used approach for biomonitoring in the world because the individual metrics that respond to different types of stressors are scored against the assumptions of human disturbances (Barbour and Yoder, 2000). Community indices, diversity indices, and functional feeding group measures were the most effective measures that can be used in water quality assessment, and have a response across a range of human influence (Rosenberg and Resh, 1993; Karr and Chu, 1999).

Disturbances in aquatic ecosystems are intense in the municipal area of Taiwan, reflecting the environmental degradation associated with its large population. The Environmental Protection Administration (EPA) of Taiwan developed the river pollution index (RPI) which measures the dissolved oxygen, biochemical oxygen demand, ammonia nitrogen, and suspended solids in water to assess river quality (EPA, 2010). Among the 126 rivers monitored in Taiwan, 50% of them rated as

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heavily polluted by industrial and municipal wastewater discharges along with diffuse runoff of fertilizers from agricultural areas. One of the eleven heavily polluted rivers of Taiwan is the Danshui River Basin, the largest river in the northern area which accumulates its catchment from the tributary rivers and which passes through the urban and agricultural area (ROC, 2012).

Previous studies reported the effect of pollution on community structure and functional feeding groups of macroinvertebrate and their relations to environmental factors in northern and central Taiwan (Shieh and Yang, 1999, 2000; Hsu and Yang, 2005; Shieh et al., 2007). Hsu and Yang (1997) modified the family level biotic index for northern rivers of Taiwan which initially developed by Hilsenhoff (1988). The family level biotic index was a reliable method for assessing water quality of Keelung River, northern Taiwan. However, a limited number of studies are carried out in a northern river ecosystem which flows through the high population area of Taiwan, use of macroinvertebrate assemblages for biomonitoring seems not to be widespread in Taiwan.

The Xindian River is one of the main tributaries of the Danshui River which runs through densely-populated metropolitan Taipei (with a population of 6,900,273) (ROC, 2011). According to the Taiwan Water Department, more than 4 million people obtain 97% of their drinking water supplies from this river and the regional economy based on the agricultural activities and urban development developed along the river. Totally 22 bridges and dams are constructed on the Xindian River (ROC, 2011). The Xindian watershed is one of the main drinking water resources of Taipei city, essential to develop appropriate bioassessment approaches for maintaining water quality. The aim of this study was to investigate changes in macroinvertebrate assemblage patterns and environmental variables along the Xindian watershed, and to assess the status of macroinvertebrate assemblages in response to human impacts in the Xindian watershed.

Materials and methods

Site description

The Xindian River basin is located in the northern part of Taiwan, and one of the major tributaries of the Danshui River which flows through New Taipei and Taipei city (Huang et al., 2012). The Xindian River is 82 km long, with a drainage area of 910 km². Its main tributaries are Nanshi River and Beishi River basins being confluence at the Guishan town to form the Xindian River. The Beishi River is about 50 km long, and the catchment area is 310 km². It originates from Sanfonsun and Yingtzuling mountains. The Nanshi River flows in the deep valley towards the north for 45 km which originates from Chilan mountain (elevation is 2130 m) (Hu et al., 2007).

Seven study sites (abbreviated as XD1 to XD7) were selected from upstream tributaries to the downstream corresponding to the different land uses in the Xindian watershed. Three sites (site XD2 located in Beishi River, sites XD3 and XD4 located in Nanshi) were selected in

the upstream reaches, two (XD1-located in Beishi River and XD5 in Xindian River) in the middle, and two (XD6 and XD7 in Xindian River) in the downstream reach. The sites were chosen based on the absence of anthropogenic disturbances, presence of natural riparian forest, and a variety of habitats on the stream bed. XD1 and XD5 are located in the midstream reach near the downtown, it is impacted by urban activities and agriculture. The riparian area is dominated by natural forest with discontinuous distribution of cultivated land by local villagers. The reference sites XD3 and XD4 are relatively undisturbed by human activities because of they are located within the National Forest Protected Area. XD6 and XD7 are located in areas that are heavily impacted by industrial effluent, urban sewage, and agricultural discharges (Table 1). The distribution of the sampling sites is represented in Fig. 1.

Macroinvertebrate sampling

Field sampling was carried out once every month from December 2010 to December 2011. Three replicates of macroinvertebrate samples were collected from the riffle and run habitats of each study site using a Surber net (with an area of 0.5 × 0.5 m, and a mesh size of 250 μm). All samples from the randomly chosen sampling points in the reaches were preserved in 95% ethanol in the field. Samples are rinsed on a sieve (500 μm mesh size) to remove silt and detritus, sorted in a Petri dish, and processed for identification in the laboratory. Specimens preserved in 80% ethanol were identified using the dissecting microscope.

Ephemeroptera, Plecoptera, and Trichoptera (EPT) larvae were identified to the lowest taxonomic level possible, mostly to the genus level. Odonata, Hemiptera, and Diptera were identified to family level and the non-insect taxa to the order level. Functional feeding groups (FFGs) were assigned according to Merritt et al. (2008). Identification of macroinvertebrates also followed the key of Merritt et al. (2008), Kawai (1985), Kang (1993), and other local references available.

Environmental variables

Water variables, temperature (°C), dissolved oxygen (DO, mg/L), electric conductivity (EC, μS/cm), and pH were measured in the field using portable meters, such as temperature and DO (CyberScan, model DO 100), EC (HANNA, model HI 9635 microprocessor conductivity/TDS meter), and pH (CyberScan, model pH310). In the laboratory, ammonia (HANNA, model HI93700), phosphate (HANNA, model HI93713), and turbidity (HANNA, model HI93703) microprocessor meters, hardness (HANNA, model HI3812) and alkalinity (HANNA, model HI 3811) test kits are used to measure ammonium (NH₄⁺, mg/L), phosphate (PO₄³⁻, mg/L), turbidity (mg/L), hardness (mg/L), and alkalinity (mg/L), respectively.

Current velocity (m/s) and water depth (m) were measured in several equal transects with a flow meter (Water Globe, model FP101) at sites XD1–XD5. However, downstream sites XD6 and XD7 were difficult to wade therefore; only the velocity of sampling reaches was used for

Table 1
Descriptions of the seven sampling sites in Xindian watershed.

Site	1	2	3	4	5	6	7
Code	XD1	XD2	XD3	XD4	XD5	XD6	XD7
Stream name	Beishi	Beishi	Tonghou	Nanshi	Nanshi	Xindian	Xindian
Location	Midstream	Upstream	Upstream	Upstream	Midstream	Midstream	Downstream
Latitude	24°56'02.06"	24°54'48.56"	24°50'36.93"	24°46'30.93"	24°53'26.81"	24°55'40.84"	25°00'42.00"
Longitude	121°42'34.09"	121°42'24.09"	121°37'48.69"	121°30'18.18"	121°33'02.22"	121°31'52.41"	121°31'36.28"
Altitude (m)	187	211	395	400	90	39	1
Distance from source (km)	21.92	13.83	8	12.65	18.77	49.6	64.32
Width (m)	29–45.3	25.6–42.4	9.1–26.6	14.1–21	15.1–31.9	– ^a	– ^a
Canopy (%)	5.01	2.67	6.58	27.26	2	0.86	0.64
Riparian zone impacts	Rural, local farm	Local farm	Undisturbed, natural forest	Undisturbed, natural forest	Rural, hot springs	Local farm, Urbanization, dam	Local farm, urbanization

^a Not measured.

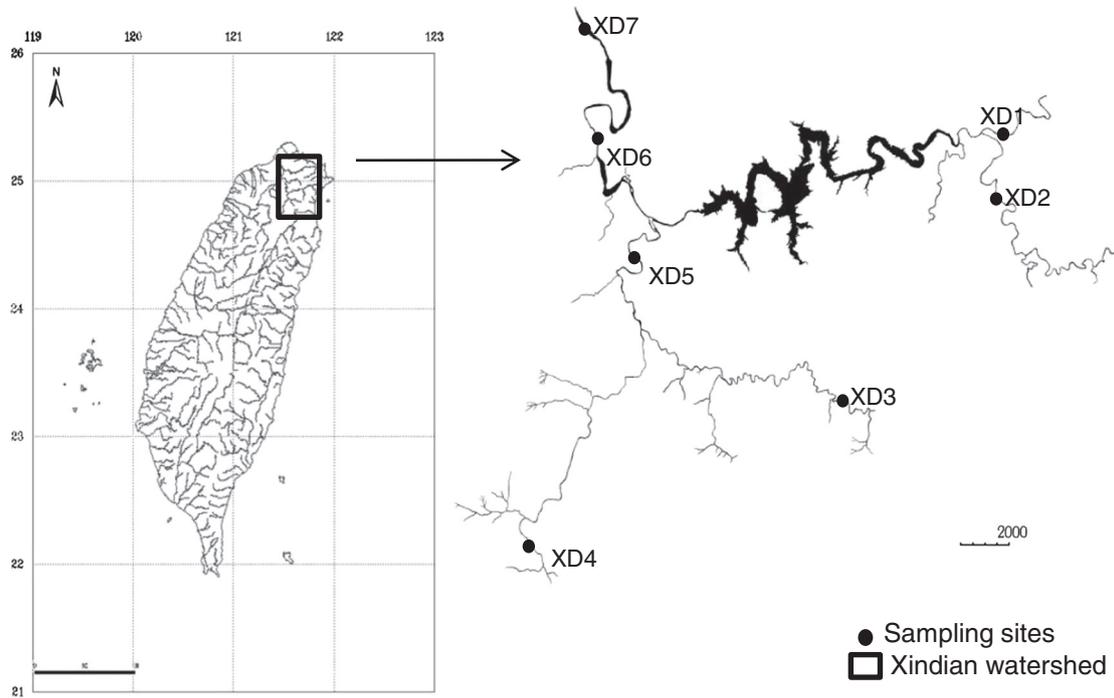


Fig. 1. Locations of the sampling sites in the Xindian watershed.

the measurement. Simple approximations of percentages of river covered by the tree canopy were estimated using densitometer model-C. Monthly total precipitation was represented by the Taipei station which is near the sampling sites.

Data analysis

A multimetric approach has been proposed to assess the water quality based on benthic macroinvertebrate assemblages, where different structural and functional attributes of the assemblage are characterized as “benthic metrics”. Benthic metrics were classified into the richness measures, composition measures, tolerance measures, and trophic measures (Barbour et al., 1995). Two community indices, biotic indices at genera (HBI) (Lenat, 1993) and family levels (FBI) (Hilsenhoff, 1988) were used to evaluate the water quality. Taxon richness, abundance, density, diversity indices, including Pielou's (J') (Pielou, 1966), Shannon–Wiener's (H') (Shannon and Weaver, 1949), and Simpson's (D) (Simpson, 1949) indices were calculated using PRIMER version 6.0 (Clarke and Gorley, 2006). Univariate analysis was used to describe the patterns of macroinvertebrate assemblages on a temporal and a spatial scale. One-way analysis of variance (ANOVA) with Tukey's multiple-comparisons was used to test any significant differences in environmental and community variables using JMP 9.0 (SAS, 2010).

We used multivariate techniques to examine the relationships of the macroinvertebrate assemblages with environmental variables. Variables were first examined to see if they were normally distributed. The water temperature, conductivity, turbidity, and concentration of phosphate and ammonia were log-transformed to improve the normality. An arcsine-square-root transformation was used for the canopy. Abundances of aquatic macroinvertebrates were $\log(x + 1)$ -transformed (Zar, 1998). The proportion of each species was calculated and only taxa constituting more than 1% of the total abundances were included in the multivariate analyses to reduce their influence on the ordinations (Braak and Verdonschot, 1995). A total of 15 dominant taxa was used in the analyses (Appendix A1). A prior detrended correspondence analysis (DCA) was separately conducted to assess the gradient length of the first DCA axis. The lengths of the gradients were 2.25, which suggested that the linear model

(e.g., the redundancy analysis, RDA) was more appropriate in the analysis (Smilauer and ter Braak, 2002). We ran the analysis using the automatic forward-selection mode, and only variables explaining a significant proportion of the remaining variation were included (based on a Monte Carlo test with 999 permutations at $p < 0.1$). Ordination analyses were performed using CANOCO for Windows version 4.5 (Smilauer and ter Braak, 2002).

Results

Environmental variables

During the period of the studies, average annual air temperature of the Xindian watershed was observed to fluctuate from a minimum of 13.7 °C in January 2011 to a maximum of 29.7 °C in the month of July 2011. Higher precipitation occurred in June and July 2011 with 284.61 mm and 264.22 mm respectively. Lowest precipitation was 27.4 mm in Apr 2011.

The mean values and SE of the physicochemical variables are summarized in Table 2. All sites were well-oxygenated (>91% saturation) and within the neutral pH (6.8–8.1) for all of sampling sites. Mean values of temperature (18.27 °C), turbidity (0.14 mg/L), ammonia (0.06 mg/L), alkalinity (0.28 mg/L), and hardness (0.28 mg/L) were lower at XD3 while conductivity (176.12 mg/L), temperature (21.23 °C), phosphate (0.49 mg/L), ammonia (0.81 mg/L), and alkalinity (0.46 mg/L) were higher at XD7. However, some variables such as DO (9.61 mg/L), turbidity (48.88 mg/L), and canopy cover (27.26%) were the highest in XD4 than in the other sites. The mean discharge was higher at XD1 (7.47 m³/s) while lower at sites XD6 and XD7 with 0.55 m³/s.

pH, DO, and turbidity were significantly different ($P < 0.05$) among the sampling sites during the four seasons (Table 3). However, phosphate and alkalinity were not significant during autumn, spring, and summer seasons. Moreover, hardness did not differ significantly during all the seasons. The seasonal trends can be observed with regard to DO, temperature and nutrient levels. DO increased during winter and spring while the temperature increased in summer and autumn. Nutrients increased during the spring and autumn.

Table 2
Mean values and SE of environmental variables at the seven sampling sites in the Xindian watershed. Means \pm SE in a row followed by the same letter show no significance difference at $p < 0.05$, by Tukey's range test. * $P < 0.05$; ** $P < 0.005$; *** $P < 0.0001$; NS, not significant.

	XD1	XD2	XD3	XD4	XD5	XD6	XD7	F ratio
pH	7.4 \pm 0.1 ^b	8.11 \pm 0.14 ^a	7.33 \pm 0.05 ^b	7.52 \pm 0.04 ^b	8.01 \pm 0.10 ^a	7.72 \pm 0.15 ^{ab}	6.89 \pm 0.05 ^c	8.96***
Dissolved oxygen (mg/L)	9.27 \pm 0.12 ^a	9.23 \pm 0.14 ^a	9.46 \pm 0.09 ^a	9.61 \pm 0.10 ^a	9.29 \pm 0.08 ^a	9.6 \pm 0.14 ^a	6.42 \pm 0.27 ^b	2.82*
Conductivity (μ S/cm)	82.07 \pm 3.46 ^{bc}	91.32 \pm 3.42 ^{bc}	73.96 \pm 1.73 ^c	123.59 \pm 2.33 ^b	127.62 \pm 4.5 ^a	118.87 \pm 28.47 ^{bc}	176.12 \pm 7.31 ^a	12.85***
Temperature ($^{\circ}$ C)	20.49 \pm 0.81 ^{ab}	20.74 \pm 0.91 ^{ab}	18.78 \pm 0.55 ^{ab}	18.27 \pm 0.53 ^b	21.05 \pm 0.73 ^{ab}	19.58 \pm 0.55 ^{ab}	21.23 \pm 0.67 ^a	44.61***
Turbidity (FTU)	1.23 \pm 0.29 ^b	0.53 \pm 0.26 ^b	0.14 \pm 0.05 ^b	48.88 \pm 11.54 ^a	17.04 \pm 3.4 ^b	19.82 \pm 4.59 ^b	7.3 \pm 0.98 ^b	53.33***
Phosphate (mg/L)	0.12 \pm 0.04 ^b	0.06 \pm 0.02 ^b	0.15 \pm 0.09 ^b	0.09 \pm 0.02 ^b	0.15 \pm 0.03 ^b	0.15 \pm 0.04 ^b	0.49 \pm 0.11 ^a	5.306***
Ammonia (mg/L)	0.09 \pm 0.03 ^b	0.16 \pm 0.07 ^b	0.06 \pm 0.02 ^b	0.1 \pm 0.05 ^b	0.09 \pm 0.02 ^b	0.16 \pm 0.08 ^b	0.81 \pm 0.28 ^a	5.105**
Alkalinity (mg/L)	0.32 \pm 0.03 ^{ab}	0.32 \pm 0.03 ^{ab}	0.28 \pm 0.028 ^b	0.41 \pm 0.03 ^{ab}	0.39 \pm 0.02 ^{ab}	0.33 \pm 0.02 ^{ab}	0.44 \pm 0.04 ^a	2.93*
Hardness (mg/L)	0.36 \pm 0.06 ^a	0.32 \pm 0.05 ^a	0.29 \pm 0.06 ^a	0.39 \pm 0.05 ^a	0.34 \pm 0.04 ^a	0.39 \pm 0.05 ^a	0.37 \pm 0.04 ^a	0.43 ^{NS}
Width (m)	37.2 \pm 13.91 ^a	31.8 \pm 5.63 ^b	18.6 \pm 7.34 ^d	16.2 \pm 9.62 ^d	24.6 \pm 8.78 ^c	NS	NS	80.18***
Discharge (m ³ /s)	6.8 \pm 1.7 ^a	3.83 ^{bc}	2.73 \pm 0.23 ^{cd}	6.69 \pm 0.45 ^{ab}	2.06 \pm 0.13 ^d	NS	NS	11.23***
Depth (cm)	37.01 \pm 1.42 ^a	26.13 \pm 1.16 ^{cd}	28.67 \pm 1.2 ^{bcd}	33.6 \pm 1.5 ^{ab}	30.9 \pm 1.73 ^{bc}	34.3 \pm 1.42 ^{ab}	24.8 \pm 1.04 ^d	9.62***
Velocity (cm/s)	0.33 \pm 0.06 ^a	0.35 \pm 0.04 ^a	0.82 \pm 0.05 ^a	1.06 \pm 0.53 ^a	0.33 \pm 0.03 ^a	0.35 \pm 0.03 ^a	0.48 \pm 0.03 ^a	2.56*
Canopy (%)	5.35 \pm 0.89 ^b	2.6 \pm 0.64 ^{bc}	5.98 \pm 1.18 ^b	25.7 \pm 2.3 ^a	2.19 \pm 0.64 ^{bc}	0.74 \pm 0.34 ^c	0.79 \pm 0.26 ^c	65.68***
Substrate size (mm)	95.13 \pm 5.04 ^b	41.77 \pm 1.25 ^c	34.18 \pm 0.83 ^c	47.7 \pm 3.91 ^c	121 \pm 4 ^a	108 \pm 5.56 ^{ab}	101 \pm 3.2 ^b	89.42***

Macroinvertebrate communities

A total of seventy seven macroinvertebrate taxa belonging to 45 families of 8 insect orders Ephemeroptera, Plecoptera, Trichoptera, Diptera, Odonata, Hemiptera, Coleoptera, and Megaloptera along with 3 classes of Annelida (Oligochaeta, Polychaeta, and Hirudinea) was collected from the 7 sites during this study period (Appendix A1). Family Prosoptistomatidae (Ephemeroptera) was first recorded in Taiwan which was collected from sampling site XD3. However, it was not included in the analysis because of lower abundance.

In general, macroinvertebrate communities were dominated by the orders Ephemeroptera (51.54% of total abundance) and Diptera (29.51%) along with Plecoptera (5.98%), Trichoptera (5.52%), Hirudinea (3.04%), Coleoptera (2.13%), and Oligochaeta (1.03%). Ephemeroptera was the most diverse and abundant order which possessed 20 taxa and comprised about a half percentage of the total abundance in the Xindian watershed. However, Trichoptera showed higher taxa (20 taxa) and less abundance than Ephemeroptera. The other key order Diptera possessed 13 taxa. Among them, *Baetis* (Ephemeroptera) was the most abundant genus possessing 23.15% and abundant at XD1, XD3, and XD5. *Choroterpes* (Ephemeroptera; Leptoplebiidae) was dominant at XD2, Tipulidae (Diptera) at XD4, and Chironomidae (Diptera) at XD6 and XD7, respectively. Oligochaeta, Polychaeta, and Hirudinea were only dominant in site XD7, which possessed above 90% of the total taxa. Total taxon richness was higher in spring (sites XD3 and XD4) while it was lower in the summer season (Fig. 2(a)). The Shannon diversity index was higher during winter, at XD4. However, both total abundance of macroinvertebrates and EPT abundances were higher during autumn (Fig. 2(b)).

Comparison of different community variables of macroinvertebrates at each sampling site is shown in Table 4. According to the ANOVA, all of the variables, taxon richness, abundance, EPT abundance, Shannon, Simpson's indices, Pielou's evenness, and density were significantly different ($P < 0.05$) among the seven sampling sites. The highest total and

EPT abundances occurred at site XD2 than at the others (Fig. 2(b)) which has a more efficient habitat for macroinvertebrates. Moreover, site XD2 showed the highest density with a value of 1260 individuals per 0.25 m² while site XD4 showed lower density during the study. The Shannon index and Simpson index were higher in the reference sites, XD3 and XD4 compared with the lower sites, XD6 and XD7. Pielou's evenness was higher in site XD4 than in XD1 and XD2.

Macroinvertebrate metrics

Table 5 shows the mean values and SE of benthic metrics at the 7 sampling sites. Twenty one metrics were classified into four basic categories: richness, composition, tolerance, and trophies. Except the ratio of scraper and collector-filterer, most metrics showed significant differences ($p < 0.05$) between the sites. The ratio of scraper and collector-filterer was not statistically different among the sites.

The six richness measures, such as; richness-family, richness-genus, Plecoptera taxa, Trichoptera taxa, EPT taxa, and total numbers of taxa were the greatest at site XD3 while the lowest at XD7. For the trophic and composition measures, the percentages of predator and EPT, and ratio of composition of EPT/Chironomidae were the greatest at site XD3 and the lowest at XD7. However, the taxa and the percentage of Ephemeroptera, the ratio of scraper and collector-filterer, percentage of gathering collector were the highest at site XD2. The percentages of the shredder and scraper were higher at sites XD4 and XD6, respectively. For the percentages of dominant species, collector-filterer, Chironomidae, and Oligochaeta were higher at XD7. The tolerance measures, HBI and FBI values were the smallest at XD3 and the highest at the XD7. According to the Hilsenhoff's water quality assessment by biotic indices at the genus and family level, the findings indicated "excellent" or "very good" water quality for the non-impacted sites, XD3 and XD4. For XD6, it is classified into "good" and "fair" category with biotic index scores of 4.79 and 4.84 for FBI and HBI respectively. There is some organic pollution probable at XD6. Both of the biotic index scores

Table 3
Water quality variables among the seven sampling sites during the 4 seasons in Xindian watershed. * $P < 0.05$; ** $P < 0.005$; *** $P < 0.0001$; NS, not significant.

	Autumn		Spring		Summer		Winter	
	F ratio	P value						
pH	5.20	**	8.29	***	4.81	**	3.42	*
DO (mg/L)	37.75	***	35.96	***	17.50	***	5.63	***
Conductivity (μ S/cm)	33.11	***	32.76	***	17.51	***	1.05	NS
Temperature ($^{\circ}$ C)	1.16	NS	2.56	*	5.07	**	2.46	*
Turbidity (FTU)	2.93	*	5.07	**	4.53	**	8.89	***
Phosphate (mg/L)	1.82	NS	2.02	NS	1.47	NS	5.24	**
Ammonia (mg/L)	0.71	NS	2.79	*	1.94	NS	12.88	***
Alkalinity (mg/L)	1.38	NS	0.40	NS	0.52	NS	2.88	*
Hardness (mg/L)	0.47	NS	1.21	NS	0.62	NS	0.10	NS

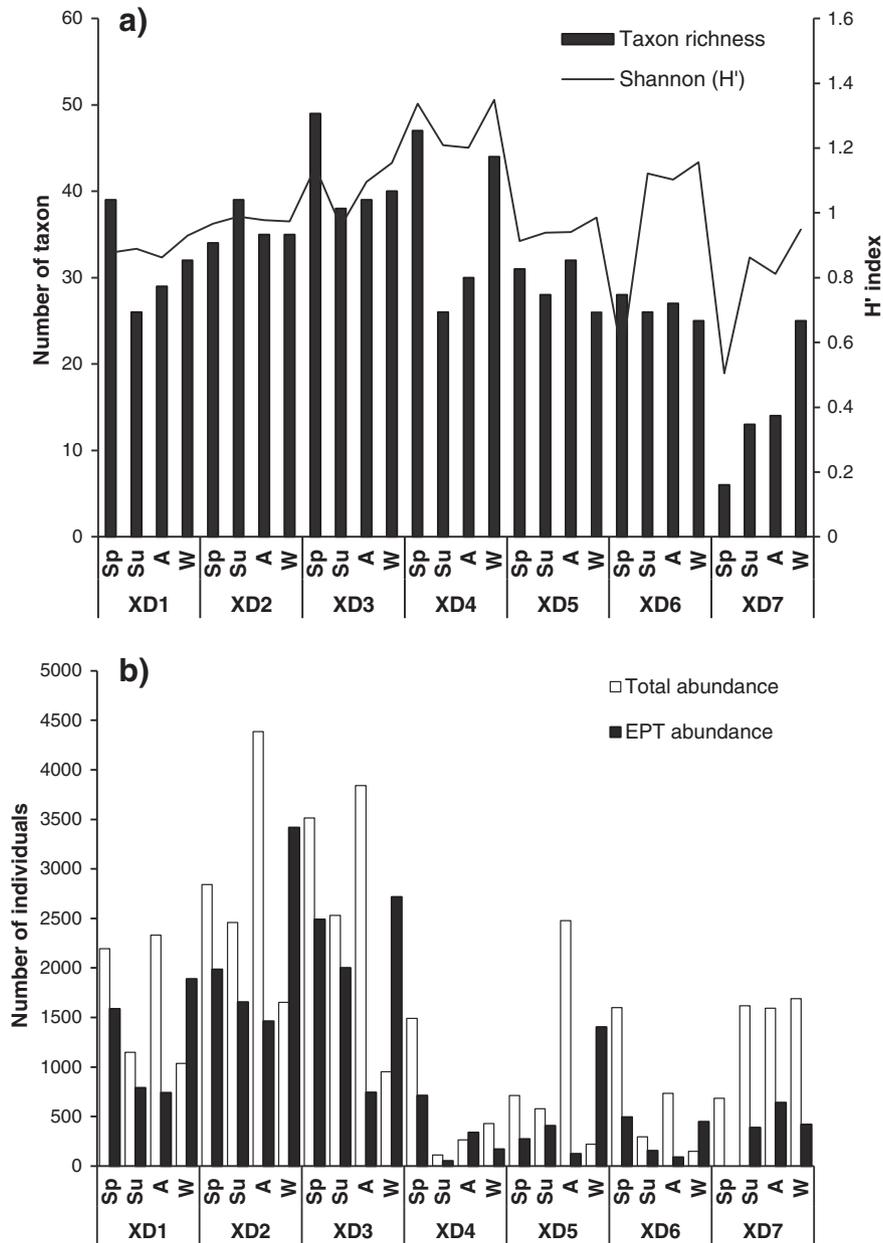


Fig. 2. Taxon richness and Shannon index (a), and total abundance and EPT abundance (b) during the different seasons in Xindian watershed. Abbreviations of four seasons: Sp, spring; Su, summer; A, autumn; W, winter.

Table 4

Mean values and SE of total taxon richness, abundance, density, and diversity indices of macroinvertebrates at the sampling sites of the Xindian watershed. ***P* < 0.005; ****P* < 0.0001.

	XD1	XD2	XD3	XD4	XD5	XD6	XD7	<i>F</i> ratio	<i>P</i> value
	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)		
Taxon richness	12.25 (0.7)	15.47 (0.7)	18.91 (0.8)	12.83 (1.61)	9.77 (0.8)	7.5 (1.01)	6.52 (0.6)	21.13	***
Total abundance	186.33 (26.5)	315.08 (42.09)	301.05 (42.8)	63.83 (14.3)	110.91 (35.7)	77.27 (22.7)	155.16 (18.5)	10.86	***
EPT abundance	139.16 (20.3)	230.19 (29.2)	220.94 (28.3)	35.44 (7.5)	60.75 (18.4)	33.08 (11.6)	40.25 (9.6)	20.09	***
Density	747 (106.8)	1263.22 (168.2)	1209.44 (171.1)	258.11 (57.6)	443.89 (142.7)	313.78 (91.1)	634.11 (73.5)	10.88	***
Evenness	0.66 (0.02)	0.65 (0.02)	0.69 (0.01)	0.88 (0.02)	0.74 (0.02)	0.75 (0.04)	0.69 (0.03)	10.13	***
Shannon	0.7 (0.03)	0.76 (0.03)	0.87 (0.02)	0.81 (0.06)	0.69 (0.03)	0.52 (0.06)	0.51 (0.04)	11.40	**
Simpson	0.69 (0.03)	0.72 (0.02)	0.78 (0.02)	0.88 (0.02)	0.73 (0.03)	0.66 (0.05)	0.58 (0.04)	9.67	***

Table 5
Mean values and SE of community metrics at the sampling sites in Xindian watershed. EPT/Chir, EPT abundance/Chironomidae abundance; Sc/Cf, scraper/collector-filterer; Dom%, dominant species %; Fil. collector, filtering collector; Gat. collector, gathering collector. ** $P < 0.005$; *** $P < 0.0001$; NS, not significant.

	XD1		XD2		XD3		XD4		XD5		XD6		XD7		F ratio	P value
	Mean	SE														
<i>Richness measures</i>																
Richness-Family	9.53	0.58	11.67	0.52	14.58	0.50	9.78	1.12	7.75	0.64	6.42	0.81	3.61	0.44	26.42	***
Richness-Genus	12.56	0.73	15.97	0.75	19.36	0.83	12.86	1.63	9.81	0.87	7.75	1.00	5.19	0.55	25.22	***
Ephemeroptera taxa	4.83	0.24	6.42	0.29	6.03	0.27	3.97	0.43	3.53	0.31	2.75	0.44	1.86	0.29	24.90	***
Plecoptera taxa	0.56	0.09	1.08	0.08	1.89	0.15	1.56	0.24	0.72	0.10	0.17	0.07	0.03	0.03	29.52	***
Trichoptera taxa	1.58	0.30	2.44	0.28	3.36	0.35	2.69	0.50	1.56	0.33	1.67	0.34	0.53	0.12	7.77	***
EPT taxa	6.97	0.48	9.94	0.46	11.28	0.57	8.22	1.06	5.81	0.60	4.58	0.76	2.42	0.41	22.09	***
<i>Tolerance measures</i>																
HBI value	4.19	0.08	3.63	0.13	3.06	0.10	3.35	0.23	4.40	0.13	4.84	0.24	6.52	0.15	51.87	***
FBI value	4.20	0.08	3.64	0.13	3.28	0.09	3.71	0.19	4.42	0.12	4.79	0.25	6.52	0.15	49.31	***
Dominant sp. %	45.83	2.99	43.20	2.30	35.63	2.16	34.08	3.58	42.00	2.90	50.04	4.51	52.77	3.49	4.61	**
Sc/Cf	3.32	0.98	6.47	5.14	2.87	1.00	0.43	0.12	0.39	0.10	0.73	0.22	0.41	0.23	1.29	NS
<i>Trophic measures</i>																
Shredder	0.84	0.32	0.78	0.22	3.15	0.92	6.00	1.75	4.47	1.81	7.10	2.96	0.02	0.02	3.34	**
Fil. Collector	7.17	1.36	8.42	1.85	9.46	1.52	18.32	3.36	11.71	2.27	15.02	3.66	25.64	4.74	5.02	***
Gat. Collector	68.37	2.55	74.06	1.98	53.42	2.86	51.33	4.16	63.55	3.25	48.32	5.41	68.26	4.75	7.08	***
Scraper	13.98	2.42	6.11	1.15	7.59	0.98	7.06	1.75	7.97	1.65	16.26	3.73	3.52	0.77	5.03	***
Predator	9.63	1.14	10.38	1.22	25.98	2.69	16.62	3.28	12.20	2.22	9.06	1.85	2.56	0.93	12.54	***
<i>Composition measures</i>																
%EPT	72.32	2.44	75.62	2.49	75.64	1.84	60.14	4.07	59.22	3.62	40.79	5.49	17.28	3.50	37.53	***
%Ephemeroptera	69.07	2.69	69.58	2.67	47.04	3.26	33.90	3.43	52.44	3.80	28.52	4.69	15.03	3.01	36.22	***
%Chironomidae	21.27	2.10	19.42	2.44	13.36	1.76	20.29	3.56	27.27	2.80	39.77	5.13	43.62	4.12	11.54	***
%EPT/Chironomidae	6.48	1.89	7.44	1.29	11.94	2.54	4.97	1.84	4.94	1.25	2.13	0.57	0.49	0.12	5.94	***
%Oligochaeta	0.40	0.30	0.03	0.02	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	13.70	3.69	13.48	***

were higher in XD7 with biotic index scores of 6.52 for both FBI and HBI, respectively, and which suggested a “poor” and “fairly poor” water quality with substantial pollution probable at XD7.

Relationships between macroinvertebrate assemblages and environmental variables

The RDA was performed on the seasonal data on relative abundance of macroinvertebrates with environmental variables (Figs. 3(a) and (b)). The result shows that six environmental variables including pH, temperature, conductivity, turbidity, hardness, and canopy cover were detected as significant factors explaining the macroinvertebrate assemblages.

The results of the RDA ordination for 15 taxa and 6 environmental variables showed statistically significant results ($F = 4.98$, $P = 0.001$). A total of 80% of the variance in taxa abundance accounted for by the first two axes. A strong species–environment correlation (0.933 and 0.832 respectively) was found for both of the two first axes (Table 6). There was a strong positive correlation of the first axis with conductivity, turbidity, and hardness, and negative correlation with pH and canopy cover. The second axis had the strongest correlation with temperature. The variables temperature, conductivity, and turbidity were mainly responsible during spring, winter, and autumn seasons for XD7, pH was for spring and winter seasons for XD2, turbidity and hardness were for summer and winter seasons for XD6, and the canopy was related with sites XD1 and XD3 during the spring and winter seasons. Characteristic taxa for temperature are *Caenis sp.*, Hirudinea for conductivity, and Chironomini for hardness. Taxa like *Neoperla sp.*, *Baetiella sp.*, *Stenopsyche sp.*, *Antocha sp.*, and *Rithrogena sp.* have been found to occur most dominant with relating pH and canopy cover.

Discussion

Patterns of macroinvertebrate assemblages reflected the human impacts along the Xindian watershed. Higher taxon richness, abundance, dominance of intolerant taxa (EPT), and diversity indices were found at the non-impacted sites (XD2, XD3, and XD4) which are located

upstream part of the watershed with higher DO and canopy cover. In contrast, the lower taxon richness and diversity indices, and higher abundance of tolerant taxa (Diptera and Oligochaeta) occurred at the lower sites (XD6 and XD7) of the Xindian watershed, where higher values of temperature, conductivity, ammonia, and phosphate were measured. Conductivity, temperature, and DO are always related each other and discriminate between these three variables because temperature affects conductivity and DO (Kefford, 1998). Furthermore, conductivity is usually caused by a change of ion concentrations by affecting land use (Hsu and Yang, 2005). Ammonia and phosphate increase by the use of fertilizer in agricultural activities (Shaviv and Mikkelsen, 1993). It is clearly indicated that indiscriminate agricultural activity directly influenced the macroinvertebrate assemblages by reducing the diversity and abundance. Moreover, habitats with higher forest canopy cover are characterized by higher DO and lower temperature which are important conditions that support diverse aquatic organisms. However, sites located in agricultural or urban area had lower DO but had higher temperature, nutrients, and fine particular sediments that are associated with removal of riparian vegetation and effluence of pollutants (Meyer et al., 2005). Land use, pollution, and frequent habitat disturbance impact the water quality and habitats and eliminate intolerant species, and therefore reduce diversity in the river (Arheimer et al., 2004; Dudgeon et al., 2006).

Ordination analysis also can be used to indicate changes of macroinvertebrate assemblages in relating environmental factors and predict the changes likely to occur as a result of human impacts in aquatic ecosystems. In the RDA diagram, most of the intolerant taxa were distributed in the non-impacted sites with relating canopy cover while the dominant taxa of EPT were replaced by pollution tolerant taxa (such as Chironomini, Hirudinea, and Oligochaeta) in the downstream (XD5, XD6 and XD7) associated with higher conductivity, temperature, and hardness. The consistent results from univariate and multivariate analyses confirmed that macroinvertebrate assemblages reflected the human impacts along the Xindian watershed.

Moreover, benthic metrics have been used for biomonitoring all around the world based on important indicator attributes to assess

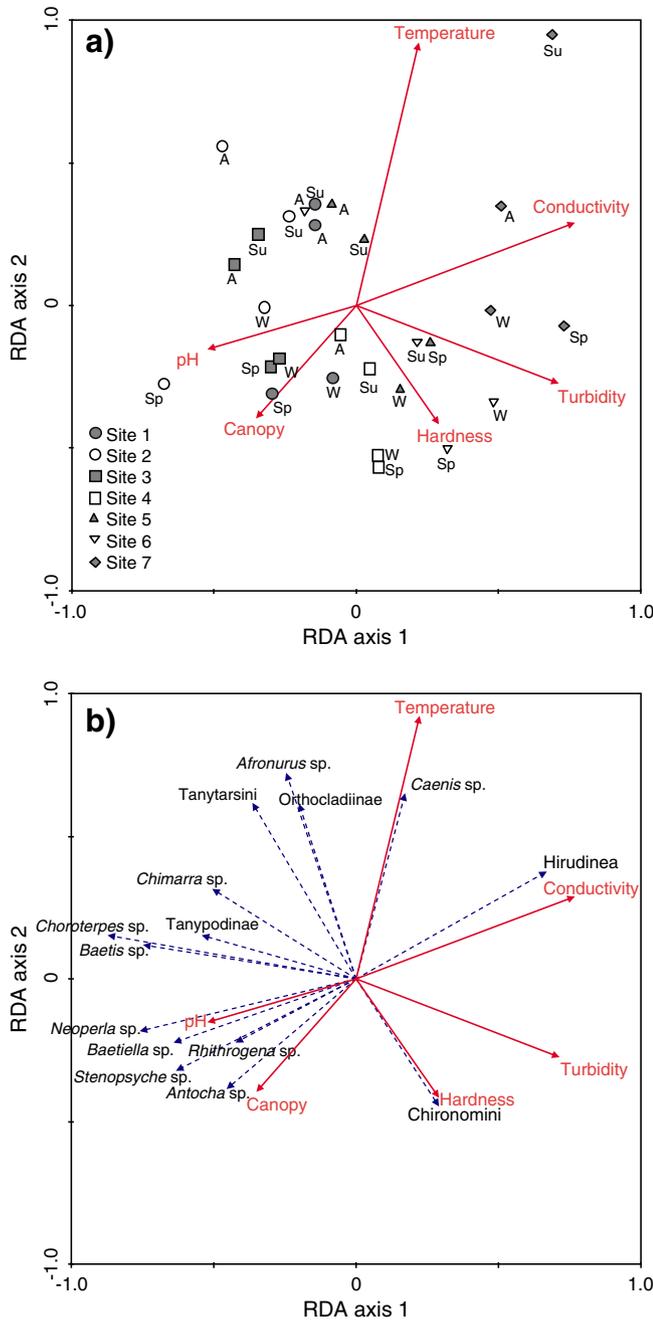


Fig. 3. RDA ordination diagrams of sampling sites (a) and macroinvertebrate taxa (dashed arrows) (b) in the Xindian watershed with environmental variables (solid arrows). Correlations between RDA axes and environmental variables are in Table 6. List of the selected taxa placed in Appendix A1. Each symbol was labeled with the season of collection. Abbreviations of four seasons: Sp, spring; Su, summer; A, autumn; W, winter.

the status of communities in response to disturbances (Johnson et al., 1993; Barbour et al., 1999). The overall results of benthic metrics showed that the richness-family, richness-genus, EPT taxa, the number of Plecoptera taxa and Trichoptera taxa, compositions of EPT, and EPT/Chironomidae indices were greater in the reference sites compared to the impacted sites. The EPT taxa are sensitive to disturbances and their presence is often considered as an indicator of good quality of the ecosystems (Rosenberg and Resh, 1993). Higher values of EPT index were found at the reference site (XD3) while much lower EPT and total taxa were found at the impacted sites, XD6 and XD7. In contrast, the percentage of Chironomidae index was higher in the impacted sites which increases with impairment (Barbour et al., 1999). For the trophic measure, percentage of predator was higher at site XD3 while lower at XD7. Collector-filterer was higher at site XD7 than at the other sites. Specialized feeders, such as predator, shredders and scrapers, are presumed to be more sensitive to disturbance, while generalists, such as gatherers and filterers, are more tolerant to pollution that might alter the availability of certain food (Barbour et al., 1999).

Water quality was evaluated using Hilsenhoff's biotic indices at the genus and family levels. The higher biotic index's score indicates that a stream may have been subjected to organic pollution while lower score indicates less environmental impact. The biotic index score was higher in XD7 with biotic index score of 6.52 for both FBI and HBI, which suggested a poor and fairly poor water quality. Reference sites were classified as excellent categories. According to the water quality evaluation based on the biotic indices, there was substantial pollution probable at XD7 and some organic pollution probable at XD6. Previous studies of water quality monitoring provided evidence that a severe pollution in water quality occurred at the lower sites in the Xindian River due to the inputs of urban and industrial sewages from illegal industry (EPA, 2010). According to the result, DO was lower with 6.05 mg/L and RPI score was 2.7 which indicate light pollution occurred at Zhongzheng station, Taipei (close to our sampling site XD7). Therefore, pollution and urbanization were most likely the determinant factors affecting macroinvertebrate assemblages in the Xindian watershed.

Conclusion

The impacts of human activities on the water quality and macroinvertebrate assemblages of Xindian watershed were determined clearly. Our results showed that the taxon richness, density, abundance, diversity indices of the macroinvertebrates, and benthic measures were higher in upstream reaches which are referred as the reference sites compared with downstream sites or impacted sites. The results of RDA suggest that conductivity, temperature, canopy cover, and pH were highly correlated factors for macroinvertebrate assemblages. Changes of environmental variables are mainly caused by municipal and agricultural activities along the watershed. Thus, macroinvertebrate assemblages can be used as a biomonitoring tool to monitor water quality in this river systems and better management and protection in the metropolitan area.

Table 6

Axes eigenvalues and weighted intraset correlation between the RDA axes and environmental variables related to macroinvertebrate assemblages in Xindian watershed. Significance of the axes by Monte Carlo test is given.

	Ordination axis				Total variance	Full model	
	1	2	3	4		F	p
Eigenvalue	0.316	0.168	0.044	0.041	1	4.984	0.001
Species-env. ^a correlations	0.933	0.832	0.729	0.655			
Cumulative % variance explained							
of species data	31.6	48.4	52.7	56.9			
of species-env. relation	53.8	82.3	89.8	96.8			
Sum of all canonical eigenvalues				0.587			

^a env., environment.

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Appendix A1. Relative abundances of macroinvertebrates in the seven sampling sites in the Xindian watershed

Taxa	XD1	XD2	XD3	XD4	XD5	XD6	XD7
<i>Ephemeroptera</i>							
<i>Afronurus</i> spp.*	10.89	4.85	1.39	0.39	6.24	7.42	3.84
<i>Electrogena</i> sp.	0.35	1.59	0.49	0.13	0.00	0.18	0.00
<i>Epeorus erratus</i>	0.00	0.01	0.05	2.39	0.00	0.00	0.00
<i>Rhithrogena</i> spp.*	0.04	0.05	5.33	5.75	0.54	0.00	0.00
<i>Nixe</i> spp.	0.15	0.41	0.02	0.00	0.18	0.72	0.02
<i>Choroterpes</i> spp.*	15.00	27.06	8.90	5.22	1.97	2.37	0.21
<i>Choroterpides nigella</i>	0.04	0.20	0.00	0.00	0.00	0.04	0.00
<i>Habrophlebiodes</i>	0.01	0.00	0.00	0.00	0.00	0.00	0.00
<i>Thraulius</i> spp.	0.07	0.00	0.11	0.22	0.05	0.18	0.00
<i>Ephemeriformosana</i>	0.04	0.54	0.13	0.22	0.05	0.07	0.00
<i>Torleya</i> spp.	0.41	0.64	0.56	0.78	0.90	1.15	0.00
<i>Kangella</i>	0.65	0.66	1.15	1.04	0.61	1.15	0.00
<i>Kangella</i> spp.	0.05	1.99	0.34	0.57	0.00	0.00	0.00
<i>Cincticostella</i> spp.	0.00	0.03	0.39	0.00	0.00	0.00	0.00
<i>Caenis</i> spp.*	1.89	0.41	0.59	0.13	2.23	2.69	15.56
<i>Baetis</i> spp.*	39.45	23.07	24.68	7.97	31.84	17.50	2.19
<i>Baetiella bispinosa</i> *	3.85	3.78	3.23	7.31	0.64	0.47	0.04
<i>Pseudocloeon</i> sp.	0.01	0.00	0.00	0.00	0.03	0.00	0.04
<i>Procloeon</i> sp.	0.12	0.12	0.05	0.00	0.00	0.22	0.00
<i>Potamanthus</i>	0.00	1.16	0.20	0.00	0.00	0.00	0.00
<i>Plecoptera</i>							
<i>Neoperla</i> spp.*	0.74	1.97	17.64	4.61	0.74	0.11	0.00
<i>Neoperla</i> sp.	0.00	0.00	0.03	0.00	0.00	0.00	0.00
<i>Agnetina</i> sp.	0.00	0.00	0.00	0.22	0.00	0.07	0.00
<i>Kamimuria</i> sp.	0.00	0.02	0.26	0.09	0.00	0.00	0.00
<i>Paragnetina</i> sp.	0.00	0.02	0.05	0.57	0.00	0.00	0.00
<i>Nemoura</i> sp.	0.00	0.00	0.00	0.09	0.03	0.00	0.00
<i>Amphinemura</i> sp.	0.04	0.00	1.08	1.70	0.08	0.00	0.00
<i>Protonemura</i> sp.	0.01	0.00	0.06	0.44	0.00	0.00	0.00
Leuctridae	0.07	0.14	0.42	0.04	0.05	0.04	0.02
<i>Cerconychia</i> spp.	0.00	0.00	0.06	0.04	0.00	0.00	0.00
<i>Cryptoperla</i>	0.00	0.00	0.00	0.04	0.00	0.00	0.00
<i>Trichoptera</i>							
<i>Stenopsyche</i> spp.*	0.62	1.31	2.85	2.39	0.49	5.09	0.00
<i>Chimarra</i> sp.*	0.99	1.52	0.42	0.30	3.45	0.00	0.00
<i>Philopotamidae</i> sp.	0.19	0.28	0.73	2.70	0.15	1.04	0.00
<i>Cheumatopsyche</i> sp.	0.08	0.41	0.61	1.70	0.41	0.57	0.02
<i>Hydropsyche</i> spp.	0.19	0.04	0.74	3.70	2.61	0.43	0.07
<i>Arctopsyche</i> sp.	0.00	0.08	0.01	0.00	0.13	0.04	0.00
<i>Potamyia</i> sp.	0.00	0.00	0.21	1.26	0.38	0.04	0.00
<i>Macrostemum</i> sp.	0.00	0.01	0.00	0.00	0.00	0.00	0.00
<i>Rhyacophila</i> sp.	0.16	0.20	0.46	0.96	0.10	0.04	0.00
<i>Micrasema</i> sp.	0.00	0.00	0.00	0.00	0.00	0.07	0.00
<i>Ceraclaea</i> sp.	0.32	0.34	0.08	0.13	0.03	0.14	0.00
<i>Mystacides</i> sp.	0.00	0.01	0.01	0.09	0.00	0.00	0.00
<i>Oecetis</i> sp.	0.00	0.00	0.01	0.48	0.03	0.00	0.00
<i>Leptocerus</i> sp.	0.00	0.00	0.00	0.04	0.00	0.00	0.00
<i>Hydroptila</i> sp.	0.00	0.00	0.01	0.04	0.00	0.00	0.00
<i>Goera</i> sp.	0.03	0.01	0.02	0.00	0.00	0.00	0.00
<i>Glossosoma</i> sp.	0.00	0.00	0.00	1.26	0.00	0.00	0.00
<i>Psychomyia</i> sp.	0.00	0.01	0.03	0.00	0.20	0.75	3.35
<i>Polycentropus</i> sp.	0.03	0.00	0.00	0.00	0.00	0.11	0.00
<i>Lepidostoma</i> sp.	0.00	0.01	0.04	0.52	0.00	0.00	0.00
<i>Odonata</i>							
<i>Euphaea formosa</i>	0.59	0.49	0.13	0.09	1.74	0.68	0.05
Gomphidae	0.08	0.11	0.20	0.22	0.26	0.04	0.05

Appendix A1. (continued)

Taxa	XD1	XD2	XD3	XD4	XD5	XD6	XD7
<i>Megaloptera</i>							
<i>Prothormes</i> sp.	0.03	0.04	0.40	0.44	0.15	0.11	0.00
<i>Coleoptera</i>							
<i>Psephenoides</i> sp.	1.35	0.71	0.61	0.30	0.82	2.69	0.05
<i>Stenelmis</i> sp. (adult)	0.03	0.03	0.66	0.74	0.00	0.04	0.02
<i>Stenelmis</i> sp. (larvae)	0.11	0.16	1.89	1.44	0.15	0.11	0.07
Hydrophilidae (adult)	0.03	0.11	0.04	0.09	0.00	0.00	0.00
Hydrophilidae (larvae)	0.15	0.92	0.46	0.13	0.10	0.25	0.00
Hydraenidae	0.01	0.01	0.06	0.00	0.00	0.04	0.00
<i>Diptera</i>							
Chironomini*	0.28	3.34	0.59	7.88	3.27	35.15	9.11
Orthoclaadiinae*	6.56	3.81	7.68	2.92	8.54	7.10	14.54
Tanytarsini*	6.18	10.19	7.63	3.83	19.39	4.95	16.39
Tanypodinae*	4.54	5.31	1.52	2.05	6.68	3.84	0.26
<i>Atherix</i> sp.	0.58	0.00	0.17	0.52	0.00	0.00	0.00
<i>Hemerodromia</i> sp.	0.01	0.00	0.00	0.00	0.00	0.14	0.00
<i>Simulium</i> sp.	1.03	0.34	0.92	6.49	0.15	0.00	0.00
<i>Tipula</i> sp.	0.08	0.17	0.18	2.00	1.43	0.72	0.00
<i>Hexatoma</i> sp.	0.22	0.06	0.67	0.35	0.08	0.04	0.00
<i>Antocha</i> sp.*	1.23	0.94	2.66	12.71	3.02	1.08	0.02
<i>Psychoda</i> sp.	0.01	0.00	0.00	0.00	0.00	0.00	0.04
<i>Blepharocera</i> sp.	0.11	0.00	0.01	1.61	0.03	0.00	0.00
Ceratophronidae	0.00	0.00	0.07	0.52	0.03	0.29	0.00
<i>Hemiptera</i>							
<i>Gerris</i> sp.	0.00	0.33	0.01	0.00	0.00	0.07	0.00
<i>Other invertebrates</i>							
Oligochaeta	0.01	0.00	0.01	0.00	0.00	0.00	8.02
Polychaeta	0.27	0.00	0.00	0.00	0.03	0.00	2.46
Hirudinea*	0.00	0.00	0.02	0.13	0.00	0.00	23.58

Taxon indicated "*" was included in the RDA analysis.

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