# Examining the Relationship between Aquatic Insect Assemblages and Water Variables by Ordination Techniques

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#### ABSTRACT

Aquatic insects were sampled quarterly at five sites along the upper Keelung River, which runs through the Taipei metropolitan area and receives various forms of pollution and anthropogenic disturbances, between August 1996 and April 1997 to examine the distribution patterns of aquatic insects and their relationships with the water variables using univariate and multivariate analyses. Upstream sites 1 to 3 significantly differed from the downstream sites 4 and 5 in the number of taxa, density, Shannon diversity index, and the proportion of dominant taxon in the univariate analyses. Baetis spp. was the dominant taxon (14.22%) and Chironomidae was the dominant family (21.78%) in the entire study reach. The results of canonical correspondence analysis (CCA), a eigenanalysis-based ordination, were compared with the results of non-metric multidimensional scaling (MDS), a distance-based ordination. Differences in aquatic insect assemblages at the first three sites were only found by the multivariate approaches, not by the univariate approach. High number of taxa and abundances at the three upstream sites contributing to variances in the aquatic insect assemblages were perceived on the CCA ordination diagrams. However, the common taxa at the three sites also increased the community similarities and formed an upstream group of samples separate from the downstream group in the MDS plots. Differences in the results between the univariate and multivariate analyses at the three upstream sites were mainly caused by the limited distributions of some taxa, such as Epeorus erratus, Amphinemura sp., Protonemura sp., and Rhyacophila spp. at site 1. In the CCA, conductivity and biochemical oxygen demand (BOD) on axis 1 and water temperature and pH on axis 2 were the water variables that best explained about 42.9% of the variance in aquatic insect assemblages on the first two axes. In the MDS, chemical oxygen demand (COD) and BOD showed the highest correlation with aquatic insect assemblages in the upper Keelung River.

#### Key words: community structure, Keelung River, canonical correspondence analysis, multidimensional scaling

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# Introduction

Species surveillance, as a part of biological monitoring to examine stream conditions, always forms the basis of related ecological studies (Kedwards *et al.*, 1999). In recent decades, studies on aquatic insects have revealed their ability to reflect water pollution (Winner *et al.*, 1980; Metcalfe, 1989; Hannaford and Resh, 1995) and its impact on ecological integrity (Kerans and Karr, 1994; Fore and Karr, 1996). Understanding the distribution patterns of aquatic insects is of value in assessing environmental changes for further studies on stream ecology.

difficulties in studying However. biotic communities arise from the need to deal with a large amount of data. The complexity of the data makes it difficult to explicitly elucidate spatial and temporal variations of species assemblages and the relationships between environmental variables and assemblage structures. Conventional univariate statistics, such as analysis of variance and regression, are well documented and easy to interpret (Washington, 1984; Lenat and Barbour, 1994). But they only use some single biotic parameters derived from large data sets. Ordination techniques, a collective term for multivariate techniques (ter Braak, 1995), provide useful tools to assess changes in biological communities and has been adopted bv manv researchers in their studies of aquatic ecology (e.g. Ormerod and Edwards, 1987; Boulton and Lloyd, 1991; Shieh and Yang, 2000).

Urban land uses usually have adverse effects, such as physical habitat modifications and water quality problems, on adjacent river systems. Stream channel networks in urban areas always need to be modified to accommodate the greater surface runoff (Whitlow and Gregory, 1989). Urban runoff and waste discharges are the major pollution problems in urban areas (Duda et al., 1982). Both of changes these cause in benthic invertebrate assemblages (Pedersen and Perkins, 1986; Beavan et al., 2001; Walsh et al., 2001). The Keelung River is an urban stream running through the Taipei metropolitan area. It is heavily polluted by municipal and industrial wastewater (Lo and Chen, 1991). A study on the biotic indices indicated a downstream trend of degradation in water quality (Hsu and Yang, 1997). Furthermore, unexpected floods always cause severe damage to human lives and properties in recent vears. and а levee system has continuously been built along the river. However, the effects of urbanization on the aquatic insect assemblages in the Keelung River are poorly known, and little attention has been paid to the threaten to aquatic environment posed by the common engineering practices. A lack of data on the aquatic fauna has resulted in most major development projects proceeding without an assessment of the potential impacts on the riverine biota. Therefore, this study presents the distribution patterns of aquatic insects along the upper reaches of the Keelung River by ordination techniques. Our objectives were 1) to understand the compositions and distribution patterns of aquatic insects along the upper reaches of the river, 2) to examine variations among sampling sites by mapping the samples in ordination diagrams, and 3) to identify the relative importances of water variables that might shape the distribution pattern of aquatic insects.

# Materials and Methods

## Study area and sampling sites

The Keelung River, located in northeasttern Taiwan, is one of the three main tributaries of the Tanshui River. It originates in Mt. Ching-Tung in Taipei County and has a total length of 87 km (or 81 km after straightening of the river

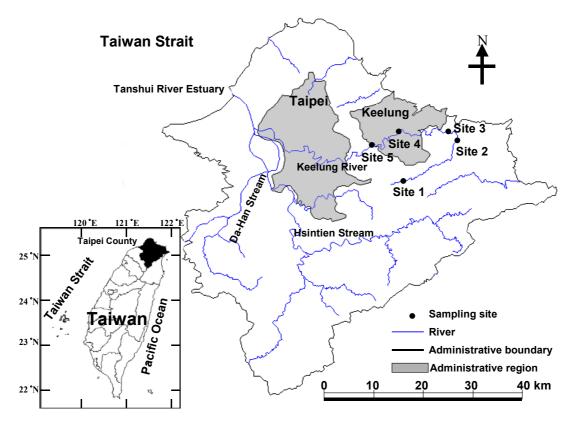


Fig. 1. Map of the study area and sampling sites on the Keelung River.

channel in 1994) before connecting with the Tanshui River at Guandu. The catchment area is 501 km<sup>2</sup>, accounting for about 32.1% of the Tanshui River drainage area. The study area is affected by the northeastern monsoonal climate; the annual precipitation ranged from 2000 to 5000 mm from 1991-1993 (Chen *et al.*, 1997). Seventy to 80% of rainfall occurs between September and March (Chen *et al.*, 1997).

Five sampling sites were selected in the study area (Fig. 1). Site 1 is located on a confluence of two first-order tributaries, about 0.27 km downstream from the headwaters. There is low-density housing in this stream reach. The surrounding area at this site is predominately abandoned and agricultural fields. Site 2 is about 2 km downstream from a small town, Hou-Tung, which was once prosperous with mining activities. The major impact at site 2 is a recreational riverside park. Sites 3 and 4 are located in an urban area and are mainly affected by domestic sewage. Site 5 is located in an industrial area. The substrate types are primarily bedrock and boulders at site 1, boulders and cobbles at site 2, cobbles, gravel, and sand at site 3, bedrock with potholes and sand at site 4, and gravel and sand at site 5. Except for site 1, all sampling sites have open canopies.

#### Aquatic insect sampling

Samples were taken from a 50 m riffle section at each of the five sampling

sites quarterly for 1 year (August and November in 1996 and January and April in 1997) by a modified Surber net (with an area of  $0.5 \times 0.5$  m, and a mesh size of 250 µm). Riffle sections were chosen because these habitats typically have the highest species richness and of benthic invertebrates abundance (Hynes, 1970). Three samples at each site were taken and combined together as a sample unit. All aquatic insects were picked from the samples and preserved in 70% ethanol in the field. Most organisms were identified to species and genus when possible in the laboratory and were assigned to functional feeding groups according to Merritt and Cummins (1996). The identification of aquatic insects was based on Kawai (1985), Chang (1992), Kang (1993), Kang and Yang (1994a, b, c), Morse et al. (1994), Ishida (1996), Merritt and Cummins (1996) and Wiggins (1996).

#### Water variables

Eight water variables, including water temperature, pH, dissolved oxygen (DO), conductivity, turbidity, and the concentrations of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) (Table 1), at the 5 sites were provided by Chen *et al.* (1997). The methods used to produce these variables are described in Lin *et al.* (1998).

## Data analysis

Five community parameters, including the number of taxa, density, Shannon-Weaver diversity index, Pielou's evenness index (Ludwig and Reynolds, 1988), and the proportion of the dominant taxon, for each sample were calculated.

One-factor repeated-measures designs and Duncan's multiple-range tests were conducted to test for differences in the aquatic insect community parameters and water variables among sites. Two types of logarithmic transformation,  $\log_{10}(x+1)$  and  $\log_{10}x$ , were used. The former was applied to density data of aquatic insects and the latter to the concentration data of water chemistry. The arcsine square root transformation  $(\arcsin (p^{1/2}))$  was applied to proportional data prior to the statistical analyses (Zar, 1996). Univariate analysis was conducted using the SAS program (Der and Everitt, 2001).

Ordination techniques were used to describe variations in aquatic insect assemblages among samples and to identify possible relationships between their distributions and water variables. An initial analysis using the detrended correspondence analysis (DCA) showed that the aquatic insect data had a long gradient length (> 2 standard deviations),and so ordination with the unimodal model was a better approach than the linear model (McGarigal et al., 2000; Shiu and Lee, 2003). The CCA is a constrained ordination, and the water variables are directly projected on taxon and site coordinates. In the CCA, taxa constituting more than 0.1% of the total abundance were selected, and in totals, 40 taxa were used in the analyses. Since seasonal factors are considered nominal variables, they are best represented by a series of "dummy" variables and presented as centroids in the CCA diagrams as suggested by Kovach (1999). The CCA was conducted using the software MVSP plus v.3.1, and the abundance data were logarithmic-transformed,  $\log_{10} (x+1)$ , before the analyses (Kovach, 1999).

Unlike the CCA. non-metric multidimensional scaling (MDS) belongs to another group of ordination techniques based on sample dissimilarity matrices instead of eigenanalyses. The major strength of MDS is that it uses rank order information to identify similarities in a data set and makes no assumption of linearity. In this analysis, spatial and temporal variations in the aquatic insect assemblages were examined by the analysis of similarity test (ANOSIM), a randomized permutation approach (Clarke

Table 1.	Community parameters of aquatic insects and mean values of water variables at five sites on the Keelung
	River during the sampling period (August 1996-April 1997)

	Site 1	Site 2	Site 3	Site 4	Site 5
Community parameters					
Number of taxa	$23.8 \pm \  \  3.7a^{\rm 1)}$	$19.3 \pm 4.9a$	$20.0~\pm~~2.7a$	$6.5 \pm 3.3b$	$4.8~\pm~~2.4b$
Density	$184.7~\pm~78.0a$	$250.0\pm188.4a$	$429.7~\pm~314.9a$	$13.0~\pm~5.4b$	$18.0~\pm~12.4b$
Evenness	$0.80 \pm 0.09a$	$0.85~\pm  0.05a$	$0.78~\pm 0.03a$	$0.89 \pm  0.12 a$	$0.81 \pm 0.11a$
Shannon diversity	$2.53~\pm~~0.35a$	$2.49~\pm 0.23a$	$2.34~\pm 0.14a$	$1.59~\pm~0.75b$	$1.19~\pm~0.41b$
Dominant taxon (%)	$25.6~\pm~13.4b$	$19.1~\pm 3.5b$	$22.9~\pm 4.2b$	$41.4~\pm~26.2ab$	$54.7 \pm 14.1a$
Water variables					
Water temperature (°C)	$19.38~\pm 4.06a$	$20.38~\pm 5.11a$	$21.13~\pm 4.93a$	$21.90~\pm~~5.03a$	$22.28 \pm  5.37a$
pH	$7.00 \pm 0.46 a$	$7.28~\pm 0.12a$	$7.05 \pm 0.28 a$	$6.90~\pm~0.35a$	$6.96 \pm  0.41 a$
Dissolved oxygen (DO) (ppm)	$8.28 \pm  1.18 a$	$7.20 \pm 1.59a$	7.70 ± 2.33a	$7.10~\pm~1.01a$	$7.06 \pm 0.77a$
Conductivity (µs/cm)	$148.9~\pm6.7c$	$223.8~\pm66.5b$	$204.5 \pm  32.8 bc$	$240.3 \pm 41.1b$	$310.3 \pm 39.7 a$
Turbidity (NTU)	$3.34 \pm  3.24 a$	6.78 ± 7.01a	$3.71~\pm 2.96a$	$8.56 \pm  7.18 a$	$6.25 \pm  2.01 a$
Biochemical oxygen demand	$0.48~\pm 0.31b$	$0.67~\pm 0.25b$	$1.28~\pm1.46b$	$6.25~\pm~4.02a$	$2.75~\pm~1.93b$
(BOD) (mg/L)					
Chemical oxygen demand	$1.65 \pm 1.37c$	$4.15~\pm 2.86bc$	$2.15~\pm  0.84 bc$	$15.13\pm10.17a$	$10.20~\pm4.11ab$
(COD) (mg/L)					
Ammonia (mg/L)	$1.97 \pm  3.22 a$	$0.18~\pm 0.23a$	$2.40~\pm 4.13a$	$2.02 \pm 2.18a$	$1.50~\pm~1.32a$

<sup>1)</sup> Means  $\pm$  S.D. in a row followed by the same letter do not significantly differ at p < 0.05, by Duncan's multiple-range test (n = 4).

and Warwick, 1994). R-statistic values and significance levels were calculated for (global) and pairwise both overall comparisons of aquatic insect assemblages among sites. In order to produce sample dendrograms, the hierarchical agglomerative clustering with groupaverage linking and Bray-Curtis species similarity were used. The SIMPER (similarity percentage) procedure was used to determine the contribution of each species to the overall change in assemblages among samples (Clarke and Warwick, 1994). The BIOENV procedure provided by the PRIMER program to relate  $\mathbf{the}$ biotic and environment data matrices was used to link the observed changes in the community structure to environmental variables by searching for the best combination of environmental variables, which was most highly correlated to the similarity matrix of sites based on the biotic data (Clarke and Warwick, 1994). MDS was performed on all taxa using the PRIMER computer software (Clarke and

Warwick, 1994; Clarke and Gorley, 2001). Abundance data were log-transformed before analysis.

#### Results

#### Univariate analyses

According to the water variables measured at the sampling sites, only conductivity (F = 7.78, p < 0.01) and the concentrations of biochemical oxygen demand (BOD) (F = 5.16, p < 0.01) and chemical oxygen demand (COD) (F = 5.19, p < 0.01) showed significant differences among sites in the repeated-measures designs. Conductivity increased downstream and was the highest at site 5, but the concentrations of COD and BOD were the highest at site 4 (Table 1).

In total, 51 aquatic insect taxa were collected during the sampling period (Table 2). Mayfly taxa were the most diverse and comprised 25% of the total taxa collected. The other major orders were the Diptera and Coleoptera, which

Taxon	Abbr.	Site 1	Site 2	Site 3	Site 4	Site 5	Total
Ephemeroptera							
Afronurus sp.* <sup>1)</sup>	afro	2.34	5.87	1.86	15.38	1.85	3.28
Epeorus erratus*	epeo	0.72	0	0	0	0	0.15
Choroterpes sp.*	chtp	0	0.93	0.23	0	0	0.37
Choroterpides nigella	chtn	0	0	0.16	0	0	7
Habrophlebiodes tenella*	habr	3.79	0.93	0	0	0	1.04
Thraulus sp.*	thra	0	1.33	0	0	0	0.37
Ephemera formosana*	epfo	2.17	0.13	0	0	0	0.48
Torleya sp.*	torl	0	0	0.23	2.56	0	0.15
Kangella sp.*	kage	0	0.27	0.78	0	0	0.45
Caenis spp.*	caen	1.62	11.87	8.38	10.26	0	7.82
Baetis spp.*	baet	19.49	14.93	12.41	5.13	0	14.22
Baetiella bispinosa*	babi	1.81	4.4	2.95	0	0	3.02
Pseudocloeon sp.*	pseu	3.07	9.07	3.10	0	1.85	4.77
Plecoptera	1						
Neoperla sp.*	neop	0.36	0.27	0.31	0	0	0.30
Nemoura sp.	nemo	0	0.13	0	ů 0	ů 0	0.04
Amphinemura sp.*	amph	1.26	0.13	0	0	0	0.30
Protonemura sp.*	prne	0.54	0	0	0	0	0.11
Trichoptera	1 .						
Stenopsyche sp.*	sten	0	2.13	0.39	2.56	0	0.82
Chimarra sp.*	chim	5.78	3.33	6.21	0	14.81	5.40
Cheumatopsyche sp.*	chps	9.21	1.47	7.29	10.26	7.41	6.11
Hydropsyche spp.*	hyps	8.12	4.53	9.39	5.13	1.85	7.56
Rhyacophila spp.*	rhya	0.54	0	0	0	0	0.11
Ceraclea sp.*	cera	0	0.4	0	0 0	0	0.11
Megaloptera	ooru	Ũ	011	Ũ	Ū	Ū	0111
Protohermes costalis*	prco	0.72	0	0.16	0	1.85	0.26
Neochauliodes sinensis*	nesi	1.44	0	0.08	2.56	0	0.37
Odonata	11001		Ū	0.000	2.00	Ū	0.01
Euphaea formosa*	eufo	7.04	4.4	4.34	0	1.85	4.80
Matrona basilaris subsp.	maba	0.18	0	0	ů 0	0	0.04
Heliogomphus retroflexus	here	0.18	0	0	2.56	0	0.07
Onychogomphus formosanus*	onfo	0.36	0	0.62	5.13	0	0.45
Sieboldius deflexus	side	0.18	0	0.02	0	0	0.40
Stylogomphus shirozui*	stsh	0.10	0	0	0	0	0.04
Zygonx takasago*	zyta	1.08	0	0.23	0	1.85	0.37
Coleoptera	290a	1.00	0	0.20	0	1.00	0.01
Psephenoides sp.*	psep	2.53	2.27	1.09	2.56	0	1.71
Eubrianax sp.	eubr	0.18	0	0	2.50	0	0.04
Mataeopsephus sp.	mata	0.10	0.13	0	0	0	0.04
Ectopria hsui	echs	0.18	0.15	0	0	0	0.04
Stenelmis formosana*	stfo	3.07	0.13	15.05	15.38	1.85	10.76
Stenelmis vongi	stwo	0.18	0.13	15.05	10.56	0	0.04
Grouvellinus sp.*		0.18	0.27	0.39	0	0	0.04
Zaitzevia sp.	grou zait		0.27	0.39			0.30
-		$0.36 \\ 0.18$	0	0	0 0	0 0	0.07
Elmomorphus sp.	elmo	0.18	0	0	0	0	0.04
Diptera	-1. :	0.17	C 90	19.10	F 19	95.09	0.97
Chironomini species*	chir	2.17	6.80	13.19	5.13	25.93	9.27
Orthocladiinae species*	orth	4.51	8.00	6.44	7.69	31.48	7.00
Tanytarsini species*	tata	3.79	0.93	0.78	0	0	1.41
Tanypodinae species*	tapo	6.68	4.27	3.03	2.56	1.85	4.10
Atherix sp.*	athe	0.54	0	0	0	0	0.11
Simulium sp.*	simu	1.99	0.4	0	0	0	0.52
Hexatoma sp.*	hexa	0.54	0.13	0.70	0	0	0.48
Antocha sp.*	anto	0	0.53	0.23	0	0	0.26
Psychoda sp.*	psyc	0	0	0	0	5.56	0.11
Ceratopogonidae species*	ceto	0.36	0.27	0	0	0	0.15

Table 2. Name list and the relative composition (%) of aquatic insects collected at sampling sites of the Keelung River during the sampling period (August 1996-April 1997)

<sup>1)</sup> Taxon denoted "\*" was included in the canonical correspondence analysis.

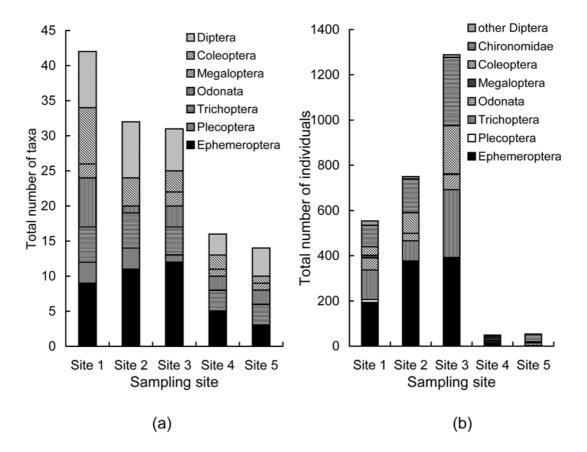


Fig. 2. Total number of (a) taxa, and (b) individuals of aquatic insects collected at five sites along the Keelung River during the sampling period (August 1996-April 1997).

possessed 10 and nine taxa, respectively. Baetis spp. was the most-abundant taxa, accounting for 14.22% of the total abundance, but the Chironomidae was the most-abundant family, accounting for 21.78% of the total abundance (Table 2). For each sampling site, the dominant taxon varied a little from upstream to downstream. Baetis spp. showed the highest relative abundances at sites 1 and 2. **Stenelmis** formosana and Chironomini at site 3, Afronurus sp., and S. formosana at site 4, and Orhtocladiinae and Chironomini at site 5 (Table 2). Some taxa exhibited local distribution patterns. *Epeorus* erratus, Protonemura sp., Rhyacophila spp. and Atherix sp. appeared

at site 1 with relative proportions exceeding 0.5%. *Nemoura* sp., *Ceraclea* sp., and *Mataeopsephus* sp. were only recorded at site 2 but with low relative proportions (0.13-0.4%). Except the *Psychoda* sp. at site 5, no other taxon showed a limited distribution at downstream sites (Table 2).

The total number of taxa decreased downstream (Fig. 2), and the mean number of taxa per sample at each site ranged from 4.8 to 23.8 taxa. The mostdiverse taxon was the Ephemeroptera at sites 1-4 and Diptera at site 5. The total number of individuals collected was highest at site 3 (Fig. 2), while those at sites 4 and 5 were fewer than 100 individuals, and accounted for only 3.8% of

total abundance. The Shannon the diversity index was highest at site 1 (mean, 2.53) and decreased downstream (Table 1). In the one-factor repeatedmeasures designs, significant differences in the number of taxa (F = 21.11, p <0.001), density (F = 20.35, p < 0.001), diversity index (F = 6.98, p < 0.05), and the proportion of dominant taxon (F = 3.46, p < 0.05) were observed among sampling sites (Table 1). The number of taxa, density, and diversity index were significantly higher at the 3 upstream sites (p < 0.05). Pielou's evenness index was highest at site 4 (mean, 0.89) and did not significantly differ among sites  $(p \rightarrow p)$ 0.05) (Table 1). The proportion of the dominant taxon was the highest at site 5 (54.7%) and significantly different from those of sites 1 (25.6%), 2 (19.1%), and 3 (22.9%) (p < 0.05) (Table 1).

## Multivariate analyses

Forty taxa from five sites were selected in the CCA (Table 2). The ordination results of the CCA are given in Table 3. The eigenvalues were low, and the first four axes accounted for 40.2% of the variance in the aquatic insect assemblages, of which, 66.3% was explained by the gradient extracted in the CCA (Table 3). The results indicated that the water variables extracted were not sufficient to predict the main variance in aquatic insect data set, but the high correlation coefficients (multiple correlation coefficients. 0.792 - 0.936)indicated that they did predict a substantial part of the remainning variance (Table 3). Canonical coefficients indicated that the first CCA axis contained the conductivity and BOD gradient and the second axis consisted of a combined gradient of temperature and pH (Table 4). In Fig. 3, the direction and length of each arrow indicate the direction and rate of maximum changes in each variable. Except for some samples from sites 1 and 5, most of the samples were

centered in the CCA ordination diagrams. The results implied most samples were less affected by these water variables, and sites 1 and 5 were on the opposite ends of the gradient changes of water variables depicted in Table 4 (Fig. 3a). In the joint plot of taxon and water variables, Epeorus erratus, Amphinemura sp., Protonemura sp., and *Rhyacophila* spp., which belong to Ephemeroptera, Plecoptera, the and Trichoptera, showed high abundance at site 1, were negatively correlated with conductivity, and were positively correlated with dissolved oxygen (DO). Taxa, such as the Psychodidae, which attained the highest value at site 5, corresponded with increases in conductivity and the concentrations of BOD and COD (Fig. 3b).

Seasonal variations in the aquatic insect assemblages and water variables were evident in summer and spring (Table 4). Temperatures increased and pH values decreased in summer. Conductivity and the concentrations of COD and BOD decreased, and DO and the concentration of ammonia increased in winter (Fig. 3a). Most of the taxa centered in the diagram showed little seasonal changes in our data, but Torleya sp., Zygonx takasago, Psephenoides sp., and Protohermis costalis appeared to be abundant in summer, while Epeorus erratus, Protonemura sp., Amphinemura, Stylogomphus shirozui, and Habrophlebiodes tenella revealed increases in abundance in winter (Fig. 3b). As to the patterns of seasonal changes of aquatic insects at each site, the upstream sites 1-3 gave clearer seasonal patterns than did downstream sites 4 and 5.

In the MDS plot (Fig. 4), the value of stress represents the degree of distortion when the MDS plot is constructed using the similarity rankings (Clarke and Warwick, 1994). A stress value of < 0.2 means that the plot provides a potentially useful two-dimensional picture.

The results of the MDS identified two site groups: samples from upstream Table 3. Eigenvalues, cumulative percentage variance explained by aquatic insect data, cumulative percentage variance explained by the taxon-water relationship, and taxon-water correlation coefficients for the first four axes of the CCA

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalue	0.187	0.133	0.101	0.074
Cumulative % variance explained by the	15.176	26.005	34.197	40.240
taxon-water relationship				
Cumulative constrained % variance explained by	25.008	42.851	56.350	66.308
the taxon-water relationship				
Taxon-water correlation coefficients	0.899	0.936	0.936	0.792

Table 4. Canonical coefficients and intra-set correlation coefficients of water variables and seasonal factors represented by dummy variables for the first two axes of the CCA

	Canonical	coefficients	Correlation coefficients	
Variable	Axis 1	Axis 2	Axis 1	Axis 2
Water variables				
Temperature	0.373	0.696	0.415	0.743
pH	-0.196	-0.741	-0.219	-0.792
Dissolved oxygen	-0.211	-0.083	-0.235	-0.088
Conductivity	0.639	0.199	0.711	0.212
Turbidity	0.223	-0.364	0.248	-0.389
Biochemical oxygen demand	0.443	-0.256	0.493	-0.273
Chemical oxygen demand	0.348	-0.094	0.387	-0.100
Ammonia	-0.374	0.096	-0.416	0.103
Season				
Spring	0.135	-0.544	0.150	-0.581
Summer	0.050	0.754	0.056	0.806
Autumn	0.169	0.243	0.188	0.260
Winter	-0.349	-0.313	-0.388	-0.334

sites 1-3 were close to each other, and those from downstream sites 4 and 5 tended to be further separated (Fig. 4a). The results indicated that assemblages of aquatic insects at sites 1, 2, and 3 were similar throughout the sampling period, but not to sites 4 and 5. In the ANOSIM test, *R*-statistics are proposed to measure the differences of the aquatic insect assemblages among sites on the MDS plot (Clarke and Warwick, 1994). There were significant differences in the aquatic insect assemblages among sites (R =0.490, p < 0.01). The further pairwise tests indicated that, except for sites 1 and 2 (p = 0.086), sites 2 and 3 (p = 0.514), and sites 4 and 5 (p = 0.143), there was significant difference between each pair of sites (p < 0.05) (Table 5).

In the cluster analysis, the dendrogram showed the observed distribution pattern on the MDS plot. Seven sample groups were marked with  $G_1$  to  $G_7$  at the 40% similarity level and were superimposed on the MDS ordination diagram (Fig. 4b). The largest group,  $G_1$ , included all samples from upstream sites 1 to 3 at about a 50% similarity level. Within this group, the strongest similarity occurred between sites 2 and 3 in January and

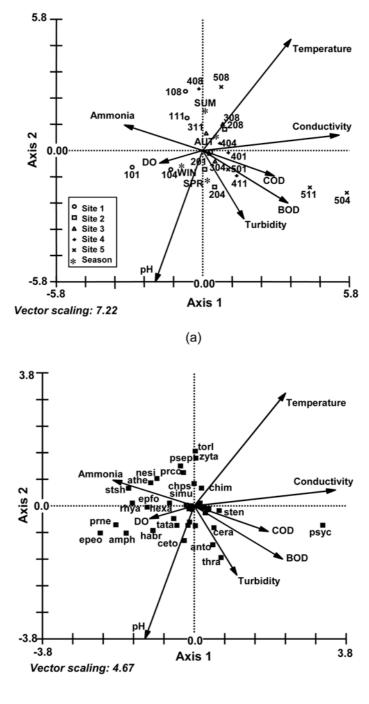
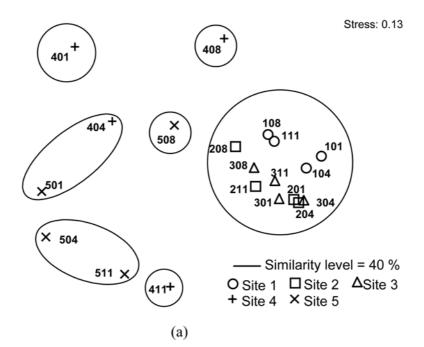




Fig. 3. Biplots of the CCA ordination diagrams of (a) samples and (b) taxa, with water variables represented by arrows and seasons represented by asterisks. Samples are labeled with numbers. The first digit indicates the sampling site, and the second and third digits indicate the month. Abbreviations of aquatic insect taxa are shown in Table 2.



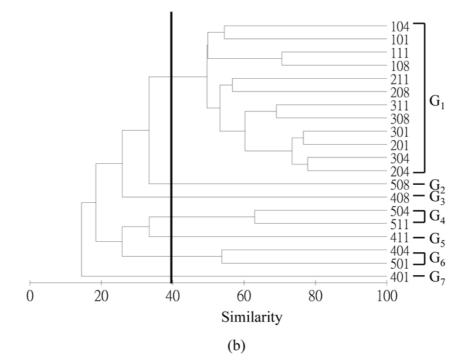


Fig. 4. (a) MDS plot, and (b) cluster dendrogram of aquatic insect samples at the species/genus level. Samples are labeled with numbers. The first digit indicates the sampling site, and the second and third digits indicate the month.

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Table 5. *R*-statistics for pairwise tests of aquatic insect assemblages among sites by the ANOSIM procedure<sup>1)</sup>

	Site 1	Site 2	Site 3	Site 4
Site 2	$0.344 \text{ n.s.}^{2)}$			
Site 3	0.510 *	-0.010 n.s.		
Site 4	0.688 *	0.573 *	0.667 *	
Site 5	0.802 *	0.854 *	0.813 *	0.271 n.s

<sup>1)</sup> Overall R = 0.490, significance level = 0.001.

<sup>2)</sup> Significance level: n.s., not significant; \*, p < 0.05.

Table 6. Optimal BIOENV solutions for the combinations of the environmental variables matched with the aquatic insect assemblages for site groups in the Keelung River, evaluated by the weighted Spearman rank correlation ( $\rho_w$ ) of both similarity matrices

No. of variables in combination	Best variable combination	$\rho_{\mathbf{w}}$
1	COD	0.623
2	BOD, COD	0.694
3	Conductivity, BOD, COD	0.630
4	Conductivity, BOD, COD, ammonia	0.592
5	pH, conductivity, BOD, COD, ammonia	0.570

April 1997 (with similarities of 76.6% and 77.8%, respectively). Groups  $G_2$  to  $G_7$ included samples from downstream sites 4 and 5, and four groups  $(G_2, G_3, G_5, and$ G<sub>7</sub>) contained only one sample. The importances of taxa responsible for these observed differences among sample groups were identified by the SIMPER procedure. Using group  $G_{2-7}$  to represent groups  $G_2$  to  $G_7$  as a single group, an average 80% dissimilarity was found between groups  $G_1$  and  $G_{2-7}$ . Nineteen taxa were responsible for more than 80% of the dissimilarity between  $G_1$  and  $G_{2-7}$ . All of these taxa were more abundant in  $G_1$  than in  $G_{2.7}$ , and five of them were absent from G<sub>2-7</sub>. The most-frequently found taxa were Baetis spp., Stenelmis formosana, Chironomini, Caenis spp., and *Hydropsyche* spp. in the  $G_1$  group and Chironomini and Orthocladiinae in the G<sub>2-7</sub> group.

By using the BIOENV procedure in the MDS, the best single environmental variable which constructed a similar MDS plot with the aquatic insects was COD with a  $\rho_w$  value of 0.623 (Table 6). The best combination contained BOD and COD with a  $\rho_w$  of 0.694, and the value of  $\rho_w$  in the second-best combination dropped a little to 6.30 when conductivity was added. An increase in the number of variables did not reflect a comparable increase in the correlation coefficient (Table 6). The MDS plot constructed by the best combination of BOD and COD was similar to the one of aquatic insect assemblages (Fig. 5).

## Discussion

Along the Keelung River, it was noted that the reduction in the number of taxa at sites 4 and 5 (Fig. 2a) was accompanied by a reduction in the number of individuals (Fig. 2b). Both of these reductions, particularly in the number of taxa, affected changes in the community diversity of aquatic insects. Diversity is composed of two distinct components: total number of species and evenness (Ludwig and Reynolds, 1988).

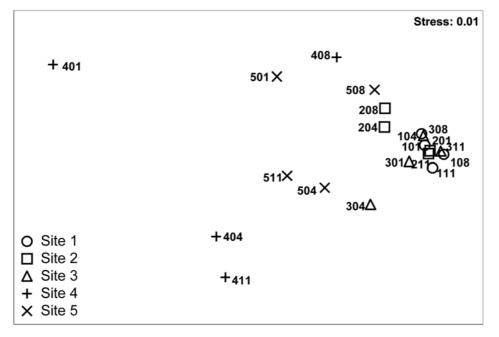


Fig. 5. MDS plot of water variables, including BOD and COD, selected by the BIOENV procedure for the sampling sites on the Keelung River. Samples are labeled with numbers. The first digit indicates the sampling site, and the second and third digits indicate the month. The correlation coefficient with the MDS plot of aquatic insect samples was 0.694.

Because the evenness values showed no significant difference among sites, differences in diversity values were mainly related to the total number of species. This result suggested that the number of taxa was the most-important determinant of changes in diversity values in the study reach.

The distribution pattern of aquatic insects more or less reflected the pollution conditions along the upper Keelung River. Lin et al. (1998) indicated a severe degradation in water quality at site 4 due to the inputs of municipal and industrial wastewater. The polluted condition also explained the dominance of pollution-intolerant taxa at upstream sites (Lenat, 1988; Lenat and Barbour, 1994). In this study, we found that Ephemeroptera dominanted at sites 1-3 and Diptera at sites 4 and 5. Furthermore, the number of taxa (Lenat,

1988; Plafkin et al., 1989; Lenat and Barbour, 1994; Barbour et al., 1996; Cao et al., 1996; Sadin and Johnson, 2000; Whiles et al., 2000) and the proportion of the dominant taxon (Plafkin et al., 1989; Lenat and Barbour, 1994; Barbour et al., 1996) are often related to river pollution conditions and are used as biotic indices. Although some researchers did not agree that the diversity measure is a useful method for describing the pollution level of water bodies (Washington, 1984; Cao et al., 1996). It is evident that diversity and biotic indices may be influenced by any stresses, including pollution (Sandin and Johnson, 2000; Ravera, 2001).

Two methods of ordinations, CCA and MDS, were used here. They extracted different aspects of the aquatic insect assemblages. The CCA is based on the eigenanalysis and extracts the maximum variance of the community data set

(McGarigal et al., 2000). In the MDS ordination diagram, distances between samples attempt to correlate corresponding dissimilarities in community structures (Clarke and Warwick, 1994). In our study, the ordination diagrams revealed different information from the aquatic insect data by these two methods. Samples from sites 1-3 with diverse and abundant aquatic insects and large amounts of variance were separately distributed on the CCA diagrams, especially on the CCA axis 1. In the ordination diagram of the MDS, samples from upstream sites had more in common and had taxa higher similarities, thus they were closely distributed on the diagram.

The ordination diagrams mostlv corresponded to the distribution patterns observed in the univariate analyses. In the CCA diagram, most of the taxa were distributed with an evident association with samples at sites 1-3. Although the dominant taxa of the Ephemeroptera in the upstream reaches were replaced by pollutant-tolerant taxa downstream, such as the Chironomidae and Psychodidae, most chironomids were still collected at sites 1-3. This explains why only the Psychodidae was superimposed on samples of site 5 in the CCA plots (Fig. 3). ANOSIM also indicated that there were significant differences between the upstream sites 1-3 and the downstream sites 4 and 5. The SIMPER procedure identified two site groups of samples, G<sub>1</sub> G<sub>2-7</sub>, by cluster analysis. The and difference between the two groups was attributed to the reduction in average abundances of Baetis spp., Stenelmis formosana, Euphaea formosa, Chimarra spp., and *Caenis* spp. from  $G_1$  group, containing samples at sites 1-3, to the G<sub>2-7</sub> group, containing samples at sites 4 and 5. The sharp declines in abundances of several taxa appeared to occur promptly between sites 3 and 4, rather than as gradual changes from upstream to downstream.

The results inconsistent from univariate and multivariate analyses implied their abilities to detect subtle differences among sites with similar aquatic insect assemblages. The univariate approach indicated a significant change in aquatic insect assemblages between sites 3 and 4, but differences in the number of taxa, densities and diversity among sites 1-3 were not explicit. However, the multivariate approach revealed significant differences in $\operatorname{the}$ aquatic insect assemblages between sites 1 and 3 (Figs. 3, 4, Table 5). Examining the relative proportions of taxa (Table 2), the limited distribution of several taxa made the assemblages at the each of the upstream sites quite different from each other. These taxa exhibiting local distributions might have been caused by the low sampling intensity or their naturelly low abundance, which was reflected in the low relative (Table 2). However, proportions the distribution patterns might have contributed little to the differences between sites in the univariate approaches, but an increase in differences in the aquatic insect assemblages was only perceived by the multivariate approach.

Centroids of the nominal variables, such as seasonal factors, in a CCA diagram will lie at the centers of these samples from groups of variables (Kovach, 1999). Results of the CCA indicated that the upstream sites 1-3 showed evident seasonal changes in aquatic insect assemblages. elevations The in concentrations of ammonia in winter were probably due to incidental pollution, and they increased the deviations of concentrations of ammonia at each of the five sites (Table 1). The CCA plot implied that site 1 appeared to be the possible source of pollution, which did not appear to have substantially adverse effects on the pollution-intolerant taxa, such as Rhyacophila spp., E. erratus, Protonemura sp., and Amphinemura sp. Samples at sites 4 and 5, especially in winter and

autumn, were far away from the centroids, which implied that the water variables were more influential on the distributions of aquatic insects than were the seasonal factors.

Environmental variables affecting the community structure of aquatic insects have been broadly studied but the conclusions are diverse. Both substrate and water variables were found to be influential on the distribution of aquatic insects; however, only water variables were included in our study, which might have been the reason a large portion of variance remained unexplained here.

In the study reach, the water variables that best explained the patterns of aquatic insect assemblages illustrated probable constraints arising from geology and pollution. The CCA had similar results that conductivity and BOD on axis 1 and water temperature and pH on axis 2 showed the strongest relationship with the variance in aquatic insect assemblages. But the short distribution gradients of taxa and sites on the CCA axis 2 implied that water temperature and pH were less important in shaping the aquatic insect assemblages. In the BIOENV procedure, COD was selected as the best single variable for correlating the configuration of aquatic insect assemblages. Among sites 1-3, there was no significant difference in COD values (Table 1) and therefore, the similarities among the three sites increased, which corresponded with the pattern of aquatic assemblages. and insect BOD conductivity were also found to be important in the BIOENV procedure. Some studies have demonstrated that water chemistry is controlled by catchment geology or land uses. Conductivity is thought to be one of the geological characters of catchments, and increases in the concentrations of BOD and COD usually reflect degradation of water quality. Some factors, like urbanization (Walsh et al., 2001), might cause changes

in conductivity and BOD. Shieh et al. (1999) and Kay et al. (1999) also reported significant relationship between ล conductivity and the distribution patterns of macroinvertebrates. Hsu and Lien (1988) constructed a water quality model simulating the BOD and dissolved oxygen (DO) patterns of change and applied it to the Keelung River. Their results indicated that the degradation in water quality caused by low DO and high BOD values was serious in the river. Conductivity is primarily affected by the geology of the area through which the river runs and also by the landuses that change the concentrations of ions in the runoff. The increases in conductivity proceeding downstream along the upper Keelung River might reflect natural changes in geological patterns, although this was not well documented in Lin et al. (1998). However, their results corresponded to the changes in aquatic insect assemblages from this study, showing that pollution and urbanization were more likely the determinant factors influencing the aquatic insect assemblages in the Keelung River.

# Conclusions

Our results revealed that the number of taxa and abundance of aquatic insects were dominantly distributed in the upper reaches of the Keelung River, with sharp declines between sites 3 and 4. In a comparison of the diagrams from the CCA and MDS, variations in aquatic insect assemblages explained the wide distribution of samples at sites 1-3 in the CCA plot, while samples from those sites sharing more-common taxa and having higher similarities formed closer relationships in the MDS plot. The results obtained by the CCA and MDS suggest that conductivity, BOD, and COD are highly correlated with the aquatic insects assemblages. The gradient changes in water chemistry are mainly caused by

of inputs municipal and industrial wastewater along the study reaches. Without the substrate variables, there was a large proportion of variance of aquatic insect data which remained unexplained. The multivariate approach appeared to be a good tool for detecting the slight differences among the assemblages and for relating biotic data and environmental data by ordination plots. To get a better understanding of relationships between aquatic insect assemblages and environmental variables, further study is needed to increase the sampling frequencies and periods and to examine substrate variables in analyses.

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# 以排序技術分析河川水棲昆蟲群聚及水質變數之關係

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## 摘 要

本研究自1996年8月至1997年4月間,在基隆河上游五個樣站進行每季採樣, 以瞭解水棲昆蟲的分佈型式及其與水質變數之關係。由單變質分析方法顯示,分類群 數量、密度、Shannon 歧異度指標與優勢分類群比例,在上游3個樣站與下游2樣 站間具有顯著之差異。全河域以 Baetis spp.為優勢分類群 (佔 14.22%),搖蚊科 (Chironomidae) 則爲最優勢的科 (21.78%)。多變質分析以典型對應分析 (canonical correspondence analysis, CCA) 及多維空間尺度 (multidimensional scaling, MDS) 二種方法比較,結果顯示在上游3個樣站間水棲昆蟲組成的差異僅能以多變質 分析方法區辦。高物種數及豐度增大了上游樣站中水棲昆蟲群聚的變異,而反應於 CCA 的排序圖中。而上游樣站共同物種的存在,亦增加樣站間群聚的相似度,在 MDS 的排序圖上形成上游與下游兩個不同群組的分佈形態。由於部份物種呈現侷限分布, 如 Epeorus erratus、Amphinemura sp.、Protonemura sp.及 Rhyacophila spp.僅 在第一樣站記錄,為主要造成上游三個樣站中單變質與多變質分析結果差異的原因。 CCA 指出在第一軸上的電導度與生化需氧量 (biochemical oxygen demand, BOD) 與第二軸上之水溫及酸鹼值爲解釋此河段水棲昆蟲群聚變異最佳的水質變數,解釋的 變異量約為 42.9%。MDS 顯示化學需氧量 (chemical oxygen demand, COD) 及 BOD,與水棲昆蟲群聚所構成之排序圖具有最高之相似性。

關鍵詞:群聚結構、基隆河、典型對應分析、多維空間尺度。

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