Knowledge and Management of Aquatic Ecosystems (2015) 416, 17
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DOI: 10.1051/kmae/2015013

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Knowledge &
Management of
Aquatic
Ecosystems

Journal fully supported by Onema

Spatial variation of macroinvertebrate community structure and associated environmental conditions in a subtropical river system of southeastern China

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Received December 20, 2014
Revised April 5, 2015
Accepted May 18, 2015

ABSTRACT

Key-words:
Biomonitoring, macroinvertebrate communities, spatial differentiation, Dongjiang River basin, indicators

Knowledge of macroinvertebrate distributions and associated environmental drivers in subtropical Asian rivers is relatively scarce. To fill this knowledge gap, we examined the spatial variation of macroinvertebrate community structure and associated environmental conditions in a subtropical river system, the Dongjiang River Basin, in southeastern China. A total of 70 families and 9 classes of macroinvertebrates were identified from 74 sites sampled in January 2013. Our study has the following findings: (1) a distinct spatial differentiation of macroinvertebrate communities was present in the Dongjiang River Basin indicated by non-metric multidimensional scaling (NMDS), which corresponded to the northern region (NR), middle region (MR), and southern region (SR) gradient; (2) ANOVAs showed that diversity indices (total taxa, Margalef index and the Shannon diversity index), biotic indices (richness of EPT, percentage of EPT, and family biotic index) and most of the studied environmental conditions (elevation, slope, steam order, water temperature, electrical conductivity, dissolved oxygen, pH, substrates, ammoniacal nitrogen, total phosphorus, percentage of urban land, percentage of rural land, and percentage of forest land) differed significantly among the three regions and a degradation gradient was observed in the NR–MR–SR direction; (3) Canonical correspondence analysis (CCA) revealed that NR sites were characterized by steep slope and coarse substrate, MR sites were characterized by high water temperatures and shallow slopes, and SR sites were primarily characterized by high total phosphorus and ammoniacal nitrogen concentrations; and (4) the Indicator Species Analysis, in conjunction with CCA analysis indicated that the most representative indicator taxon is Tipulidae for NR, *Semisulcospira* sp. for MR, and *Branchiura* sp. for SR.

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RÉSUMÉ

La variation spatiale de la structure de la communauté des macroinvertébrés et les conditions environnementales associées dans un système fluvial subtropical du sud-est de la Chine

Mots-clés : *biosurveillance, communautés de macro-invertébrés, différenciation spatiale, bassin de la rivière Dongjiang, Indicateurs*

La connaissance des distributions de macroinvertébrés et des facteurs environnementaux associés dans les rivières asiatiques subtropicales est relativement limitée. Pour combler cette lacune, nous avons examiné la variation spatiale de la structure de la communauté de macroinvertébrés et les conditions environnementales associées dans un système fluvial subtropical, le bassin de la rivière Dongjiang, dans le sud-est de la Chine. Au total 70 familles et 9 classes de macroinvertébrés ont été identifiées à partir de 74 sites échantillonnés en janvier 2013. Notre étude a tiré les conclusions suivantes : (1) une différenciation spatiale nette des communautés de macroinvertébrés dans le bassin de la rivière Dongjiang mise en évidence par « non-metric multidimensional scaling » (NMDS), ce qui correspond à un gradient de la région du nord (NR), à la zone médiane (MR), puis la région sud (SR) ; (2) des analyses de variance ont montré que les indices de diversité (total des taxons, indice de Margalef, de diversité de Shannon), les indices biotiques (richesse de l'EPT, pourcentage en EPT, et indice biotique), et la plupart des conditions environnementales étudiées (altitude, pente, ordre de Strahler, température de l'eau, conductivité, oxygène dissous, pH, substrat, azote ammoniacal, phosphore total, pourcentage de terres urbaines, pourcentage de terres rurales, et pourcentage de terres forestières) différaient sensiblement entre les trois régions et un gradient de dégradation était observé dans le sens NR-MR-SR ; (3) l'analyse canonique des correspondances (ACC) a révélé que les sites NR ont été caractérisés par la pente raide et un substrat grossier, les sites MR ont été caractérisés par des températures élevées de l'eau et des pentes faibles, et les sites de RS ont été principalement caractérisés par le phosphore total élevé et les concentrations d'azote ammoniacal ; et (4) l'analyse des indicateurs spécifiques, en conjonction avec l'analyse CCA, a indiqué que le taxon indicateur le plus représentatif est Tipulidae pour NR, *Semisulcospira sp.* MR et *Branchiura sp*. pour SR.

INTRODUCTION

Biomonitoring is one of the most valuable tools for making management policies and practices for aquatic ecosystems (Barbour *et al*., 1996; Friberg *et al*., 2011). Macroinvertebrate assemblage measures are considered as effective and reliable indicators for environmental degradation and overall river health (*e.g.* Thorne and Williams, 1997; Smith *et al*., 1999; Bieger *et al*., 2010).

Relative to European and American river systems, subtropical Asian rivers support rich but poorly known macroinvertebrate communities (Morse *et al*., 2007). In addition, research on linkages between macroinvertebrates and their environment are lacking at the watershed scale in this region, resulting in poor river management practices, especially in China (Jiang *et al*., 2010). In many rivers, water is severely polluted as a result of untreated agricultural, urban, and industrial discharges with high concentrations of nutrients, organic materials, toxicants, and sediments (Pan *et al*., 2013). Several studies have suggested that nutrient pollutants (*e.g.* nitrogen and phosphorus) were dominant factors in determining macroinvertebrate assemblages compared with natural factors (*e.g.* water velocity and substrate size) in human-disturbed rivers (Chen *et al*., 2012; Liu *et al*., 2003; Pan *et al*., 2013). As a result, anthropogenic pollutants modify aquatic community composition from natural to tolerant dominated assemblages (Xu *et al*., 2013). Previous studies also showed that macroinvertebrate assemblages were affected by elevation (Chessman, 2006; Loayza-Muro *et al.*, 2013), water temperature (Brown and May, 2000), conductivity (Miserendino *et al.*, 2001; Stenert *et al.*, 2008), fow velocity (Boyero and Bailey, 2001; Beauger *et al.*, 2006), dissolved oxygen (Gabriels *et al.*, 2007; Kaller and Kelso, 2007), substrates (Buss *et al.*, 2004; Beauger *et al.*, 2006), and

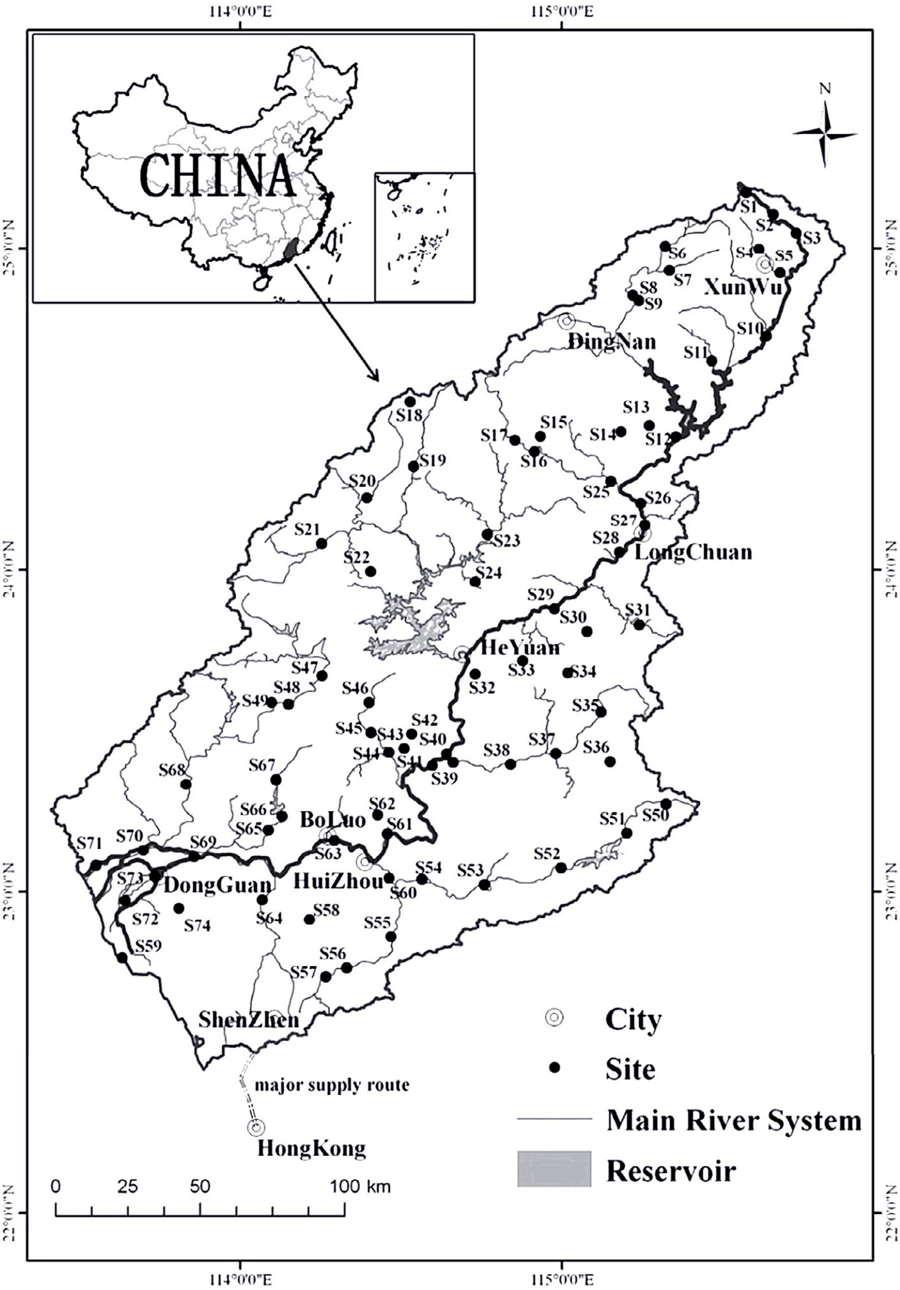

Figure 1
Map of the Dongjiang River Basin, showing the location of the study area and sampling sites (74 sites in total).

slope (Miserendino and Pizzolon, 2004; Roy *et al.*, 2003). Therefore, a better understanding of macroinvertebrate communities in relation to environments is of great interest to river management (Bucker *et al*., 2010; Neff and Jackson, 2011).

The Dongjiang River is a major tributary of the Pearl River (the second largest river by discharge in China). It lies predominantly within the east-central part of Guangdong Province of southeast China (Zhang *et al*., 2008) (Figure 1). The lower Dongjiang River belongs to the Pearl River Delta region, where has been undergoing rapid economic development since the 1980s. Over the last few decades, this region has been transformed from predominantly fishing villages into urban centres. Land use has changed dramatically due to a variety of human activities, including agriculture, logging, sand extraction, industrial development, urban sewage discharges, and flow modification (Zhang *et al*., 2010). Rapid land development has increased the amount of anthropogenic disturbance to the river ecosystem and has damaged river health, including physical habitat alteration (Jiang *et al*., 2012; Zhou *et al*., 2012).

In the 1980s, a preliminary investigation of macroinvertebrates in Dongjiang River was conducted (Lai, 1988). More recently, some studies have explored the relationships between macroinvertebrates and substrate characteristics (Wang *et al*., 2007), land use (Zhang *et al*., 2010) and water quality (Li *et al*., 2013; Wang *et al*., 2011). However, knowledge in macroinvertebrate distribution and its associated governing factors at a basin-wide scale is still lacking due to the large area and the complexity of spatial variation. The studies mentioned above in this region are mainly focused at small scales (*e.g.*, river main stem) or are based on a small number of samples, and do not reveal the broad-scale spatial differentiation and regulation of community structures across the entire basin. In addition, information on macroinvertebrate distributions and their governing environmental conditions is rarely reported at the level of the entire basin.

The objectives of this study were to (1) identify the large spatial differentiation in macroinvertebrate community structure across the entire Dongjiang River Basin, (2) examine how the distributions of macroinvertebrates are influenced by natural and anthropogenic factors, and (3) identify sensitive indicators that respond to spatial differentiation for future bioassessment.

MATERIALS AND METHODS

> *STUDY AREA*

The Dongjiang River Basin (22°21′N–25°12′N, 113°04′E–115°50′E) encompasses an area of 35 340 km^2. It is the primary source of drinking water for Hong Kong and other regions of the Pearl River Delta in China (Ho *et al*., 2003). The main river channel is 562 km long, originating in the northeast of Jiangxi Province and extending to southwest of Guangdong Province with a decline in altitude, eventually discharging into the Pearl River estuary. The Dongjiang River basin is located in a subtropical climate region with a mean annual temperature of approximately 21°C and a mean annual precipitation of approximately 1900 mm. Front- and typhoon-type rainfalls are predominant in the basin (Liu *et al*., 2010). The average annual discharge is 32.4 billion m^3, of which 80% occurs during the wet season from April to October and 20% occurs between November and March (Lee *et al*., 2007). Forests cover the headwater mountain areas, and intensive cultivation dominates the other areas of hills and plains (Jiang *et al*., 2012). Large cities (*e.g.*, Shenzhen, Dongguan, Huizhou, and Heyuan) with rapid development and growing economies are primarily located in the southern downstream areas of the basin (Figure 1).

> *MACROINVERTEBRATE SAMPLING AND LABORATORY PROCEDURES*

Fieldwork was carried out during the dry season in January 2013 during stable flow conditions. A total of 74 sites were sampled to represent all major habitat types across the entire basin (Figure 1). Quantitative macroinvertebrate samples were taken using multi-habitat sampling procedures (Lenat, 1988). At each sampling site (50−100 m reach, along the riverside), a single sample of macroinvertebrates was taken from one or more microhabitats: silt/sand, gravel/cobble, boulder, macroalgae/macrophyte, and marginal plant with three replicates. The number of habitat types sampled varied depending on their presence at each site. Silt/sand, macroalgae/macrophyte, and marginal plant habitats were sampled using D-frame nets (0.3 m, mesh size 500 μm) for a total channel length of approximately 3−5 m at water depth about 50 cm Gravel/cobble and boulder habitats were sampled using a Surber net sampler (30 cm × 30 cm, mesh size: 500 μm) at water depth approximately 20 cm. For all habitats of a site, the total composite sampling area was approximately 3 m^2. The sampling gears were washed thoroughly between sites. The sampled materials were sieved through a 500-mm mesh sieve in the field for approximately 30−60 min. The specimens were manually separated from the sediment on a porcelain plate and preserved in 100-ml plastic bottles containing 10% formaldehyde solution.

Where possible, macroinvertebrates were identified to the family (Crustacea, Insecta, Arachnida, Polychaeta, and Turbellaria) and genus (Gastropoda, Oligochaeta, Hirudinea, and Bivalvia) in the laboratory using identification keys (Liu *et al*., 1979; Morse *et al*. 1994; Thorp and Covich, 2001; Wang, 2002). Taxon identification to the family level has been reported adequate for providing a representative and persistent differentiation of macroinvertebrates distribution in response to pollution, land-use changes, and spatial differentiation (Bowman and Bailey, 1997; Chessman *et al*., 2007; Olsgard *et al*., 1998).

> ENVIRONMENTAL CONDITIONS

At each site, water samples were collected below the water surface prior to macroinvertebrate sampling for measuring the physical and chemical conditions. For nutrient analyses, water samples were transported to the laboratory at 4 °C. The water temperature (WT, °C), electrical conductivity (EC, $mS \cdot cm^{-1}$), dissolved oxygen (DO, $mg \cdot L^{-1}$), and pH were measured using a portable YSI probe (YSI 6600) at the sites. The water velocity ($m \cdot s^{-1}$) was measured near the river bed for 60 seconds using hydrometric propeller (LS1206B, Nanjing, China). Total nitrogen (TN, $mg \cdot L^{-1}$), ammoniacal nitrogen (NH_3-N, $mg \cdot L^{-1}$), nitrate nitrogen (NO_3-N, $mg \cdot L^{-1}$), and total phosphorus (TP, $mg \cdot L^{-1}$) were analysed using a UV spectrophotometer immediately upon the arrival of the samples at the laboratory.

The percentages of substrate types of each site were estimated based on the improved Wentworth scale. These types included sand/silt (diameter 0.1–2 mm), gravel (2–16 mm), pebble (16–64 mm), cobble (64–256 mm), and boulder (>256 mm). Using the substrate data, the average substrate score (MSUBST), a quantization indicator of substrate constitution, was calculated (WFD-UKTAG 2008) as follows:

$$\text{MSUBST} = \frac{-7.75 \times \text{BOLDCOBB} - 3.25 \times \text{PEBBGRAV} + 2 \times \text{SAND} + 8 \times \text{SILTCLAY}}{\text{TOTSUB}} \quad (1)$$

$$\text{TOTSUB} = \text{BOLDCOBB} + \text{PEBBGRAV} + \text{SAND} + \text{SILTCLAY}$$

where BOLDCOBB, PEBBGRAV, SAND, and SILTCLAY are percentage of bolder/cobble, pebble/gravel, sand, and silt/clay, respectively. A high score of MSUBST indicates a high proportion of sand and silt; conversely, a lower score indicates a high proportion of large rocks and cobble.

Percentages of forest agriculture, and urban land covers were calculated using Landsat 5 thematic mapper (TM) images taken in December 2009 at a spatial resolution of 30 m. Land cover types were measured at a 1-km radius of the sampling site using ArcGIS 10 (Kong *et al*., 2013). The elevation (m) of the sampling site was recorded with portable GPS (GARMIN ETR eTrex Venture). Channel slopes were determined using a 90-m-resolution digital altitude model (DEM; Shuttle Radar Topography Mission, SRTM). The stream Strahler order (Strahler, 1957) of a sampling site was determined using a river system map layer at a scale of 1:250 000 in ArcGIS 10.

> DATA ANALYSIS

Macroinvertebrate diversity indices, including total taxa (S), Margalef index (*d*; Margalef, 1957) and the Shannon diversity index (*H′*; Shannon, 1948), were calculated as follow:

$$d = (S - 1) / \ln N \quad (2)$$

$$H' = \sum_{s}^{i=1} p_i \ln p_i \quad (3)$$

where N represents the total number of individuals at a site; and p_i represents the proportion of individuals found in the ith taxon, with values summed across all taxa (S).

Number of Ephemeroptera, Plecoptera, and Trichoptera taxa (EPT-S) and percentage of Ephemeroptera, Plecoptera and Trichoptera individuals (%EPT) for each site were calculated. Family biotic index (*FBI*; Hilsenhoff, 1988) was calculated as follow:

$$FBI = \sum_{i}^{F} n_i t_i / N \tag{4}$$

in which F is the family number, n_i is the number of individuals in the ith family, t_i is the tolerance value of the ith family, and N is the sum of individuals. The tolerance values of macroinvertebrate families were from Hilsenhoff (1988) and Duan (2010).

To identify the spatial differentiation of macroinvertebrate assemblages, non-metric multidimensional scaling (NMDS) was performed with log (x + 1) transformed abundance data (ind·m^{-2}). We used the Bray-Curtis similarity coefficient as the distance measure and computed the data with 250 maximum iterations, 40 real runs, and 50 randomized runs using the PC-ORD computer package (version 5.0; MjM Software, Gleneden Beach, Oregon, USA).

Bray-Curtis similarities analysis (ANOSIM; Clarke, 1993) was used to evaluate the degree of separation among the NMDS groups. Rank abundance data and presence/absence data were used in the analyses. High positive R values (up to 1) indicated dissimilarity between groups. The number of Monte Carlo permutations was set to 9999. ANOSIM analyses were conducted using the PRIMER 5.0 software (Clarke and Warwick, 2001).

The Indicator Value method (IndVal; Dufrêne and Legendre, 1997) was used to identify the most representative macroinvertebrate taxa of each sampling site group using PC-ORD 5.0 program. IndVal is based on the relative frequency of taxa in the samples of one group and the mean abundance of taxa in the samples of that group relative to all groups. The indicator value (IV) varies from 0 to 100, attaining its maximum value when all individuals belonged to one taxon for a group at all sites (Dufrêne and Legendre, 1997). The significance of the indicator values for each taxon was tested using Monte Carlo permutations (4999 permutations).

One-way analysis of variance (ANOVA) and non-parametric rank-based one-way ANOVA (Kruskal-Wallis) analyses were used to test the significance of differences in the diversity indices, biotic indices, and environmental conditions among the site groups. The mean values of normally distributed datasets were analysed by one-way ANOVA. In cases when variance homogeneity (Levene's tests) was not achieved, the data were log (x + 1) transformed. The non-parametric Kruskal-Wallis test was applied if normality was not achieved after transformation. If significant differences were observed by ANOVA, *post hoc* pair-wise comparisons were performed using Tukey's test. All analyses were conducted using SPSS 19.0.

To assess the relationships between environmental conditions and the macroinvertebrate abundance (ind·m^{-2}), constrained ordination was used. Detrended correspondence analysis (DCA) was used to determine the appropriate type of model for direct gradient analysis. DCA indicated that the unimodal model (gradient lengths $>$ 4 standard units) would best fit the data, and hence Canonical Correspondence Analysis (CCA) was used. Prior to the analysis, macroinvertebrate data were transformed ($\log_{10}(x+1)$). Taxa that occurred less than three sites and the relative abundance was less than 1% in a site were removed from the analysis to reduce the influence of rare taxa. Environmental variables were logarithmically transformed (except pH) to approximate normality. CCA forward selection procedure was performed to select key environmental variables ($p < 0.05$, Monte Carlo randomization test with 999 permutations). All analyses were conducted using CANOCO version 4.5 (ter Braak and Šmilauer, 2002). Exponential regression analyses were used to confirm the relationships between the sensitive taxa (abundance data) in each site group and their key influential variables.

RESULTS

> *MACROINVERTEBRATES COMPOSITION AND SPATIAL DIFFERENTIATION*

A total of 79 taxa of 9 classes were identified from 74 sampling sites (Appendix I), including Insecta (50 taxa), Gastropoda (13 taxa), Oligochaeta (4 taxa), Hirudinea (4 taxa), Lamellibranchia

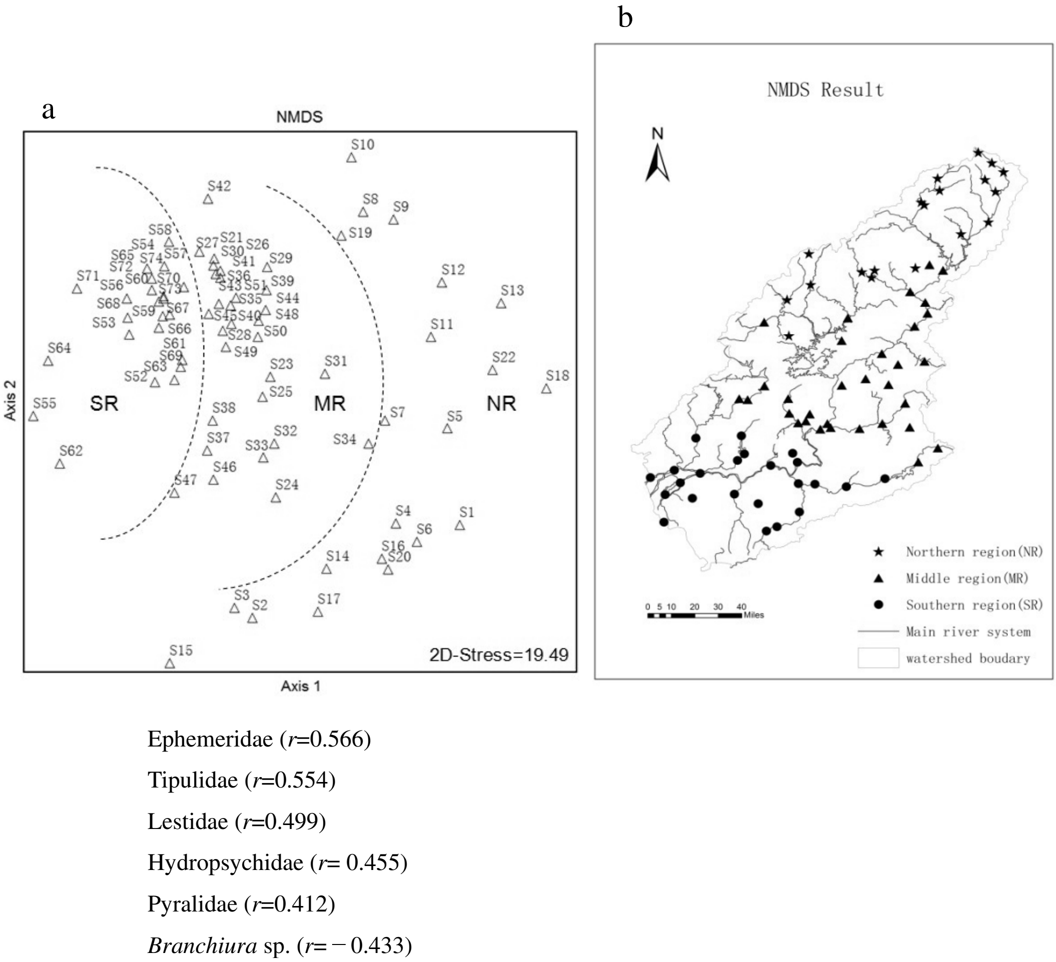

Figure 2

(a) The ordination plot of macroinvertebrate communities in the Dongjiang River Basin obtained based on the NMDS method. The sites were divided into three groups corresponding to the southern region (SR), the middle region (MR), and the northern region (NR). The stress value is 19.49, suggesting that the ordination is reliable but not an excellent interpretation. Taxa that are highly correlated ($r > 0.4$) with the first axis are listed below the plot; (b) Map showing the three regions identified by NMDS. Sites represented by asterisks belong to the northern region (NR), sites represented by triangles belong to the middle region (MR), and sites represented by circles belong to the southern region (SR).

(3 taxa), Crustacea (2 taxa), Arachnoidea (1 taxon), Turbellaria (1 taxon), and Polychaeta (1 taxon). Insecta accounted for 63.3%, Gastropoda accounted for 16.5%, and the remaining 7 classes accounted for 20.3% of the macroinvertebrate taxa.

With a final stress of 19.49 and instability of <0.0001, the two-dimensional solution in the NMDS ordination revealed spatial differentiation in macroinvertebrate assemblage structure. Two axes explained 79.1% of the variation (Axis 1 = 41.1%; Axis 2 = 38.0%). Three groups were clearly distinguished along the first axis gradient (Figure 2a). Considering their geographical locations, we found that the spatial distribution of macroinvertebrate communities exhibited strong regional differentiation (Figure 2b). The first group consisting of 21 sampling sites was located in the northwestern region (NR, sites represented by asterisks); the 2nd group consisting of 30 sampling sites was located in the middle region (MR, sites represented by triangles); and the third group consisted of 23 sampling sites was located in the southern region (SR, sites represented by circles). Accordingly, NMDS ordination revealed strong gradients associated with particular taxa. The first NMDS axis was highly correlated with the Ephemeridae ($r = 0.566$), Tipulidae ($r = 0.554$), Lestidae ($r = 0.499$), Hydropsychidae ($r = 0.455$) and Pyralidae ($r = 0.412$). These taxa were associated with mountain streams with

Table I
Results of ANOSIM global and pair-wise comparisons among northern region (NR), middle region (MR), and southern region (SR) sites using rank abundance data and presence/absence data.

Data type	Global	Pair-wise comparisons		
		NR & MR	MR & SR	NR & SR
	Global *R*	*R*	*R*	*R*
Abundance	0.107**	0.084*	0.091**	0.176***
Pres/Abs	0.11***	0.085*	0.094**	0.193***

Significant *R* values: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

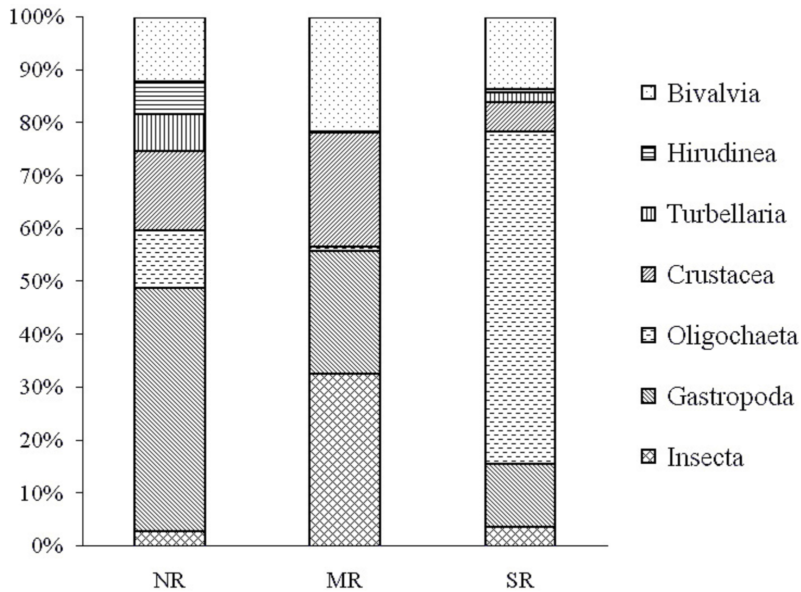

Figure 3
Stacked bar plots showing the percentage contributions of the macroinvertebrate compositions (abundance) for the northern region (NR), the middle region (MR), and the southern region (SR).

high dissolved oxygen concentrations, coarse substrates, and clean water. In contrast, the taxon *Branchiura* sp. was strongly negatively correlated with the first axis ($r = 0.433$) and was associated with habitats with low dissolved oxygen concentrations, fine substrates, and organic pollution. The second NMDS axis did not show clear associations between taxa and specific environmental variables.

The ANOSIM test showed significant differences in assemblage composition among the three site groups using both abundance data (Global $R = 0.107$, $P < 0.05$) and presence/absence (Global $R = 0.11$, $P < 0.001$) data (Table I). Pair-wise tests showed significant differences between regions ($p < 0.05$). The *R*-value obtained from the abundance data revealed that the difference between NR and SR ($R = 0.176$, $p < 0.001$) was greater than that between MR and SR ($R = 0.091$, $p < 0.01$) and that between NR and MR ($R = 0.084$, $p < 0.05$). The same conclusion was drawn using the presence/absence data (Table I). These results indicate that the community similarity decreased with increasing geographic distance.

The NMDS and ANOSIM analyses characterized the macroinvertebrate assemblage composition of each site group. The total family-level taxa for NR, MR and SR were 56, 52 and 29; and the average abundances were 740, 362 and 556 individuals·m^{-2}, respectively. The percentage contributions of different macroinvertebrate compositions (abundance data) of the three groups are shown in Figure 3. Gastropoda (46%), Crustacea (15%), and Bivalvia (12%) had the highest percentage contributions to the assemblage composition in NR; and Insecta (33%) and Gastropoda (23%) made the greatest contributions to the assemblage composition in MR. Oligochaeta (63%) was predominant phylum in SR, accounting for more than

Table II
Indicator taxa and indicator values (IV) identified using IndVal analysis for northern region (NR), middle region (MR), and southern region (SR). Only taxa with $p < 0.05$ were listed, which were identified using Monte Carlo permutation test (4999 runs).

NR		MR		SR	
Indicator taxa	IV	Indicator taxa	IV	Indicator taxa	IV
***Radix* sp.**	34.8	*Semisulcospira* sp.	23.8	*Branchiura* sp.	29.1
Libellulidae	33.3	Haliplidae	14.3	*Angulyagra* sp.	23.7
Tanypodinae	32.1	Dytiscidae	13.3	Ampullariidae	22.8
Tipulidae	30.3			*Tubifex* sp.	22.8
***Cipangopaludina* sp.**	28.6				
Lestidae	28.1				
***Helobdella* sp.**	27.9				
Calopterygidae	22.8				
***Hippeutis* sp.**	21.8				
Ephemeridae	21.8				
***Glossiphonia* sp.**	21.2				
Pyralidae	19.0				
Leptophlebiidae	14.3				

the sum of the remaining six phyla, and was much higher than in NR (11%) and in MR (1%). Arachnoidea and Polychaeta were excluded from the analysis because their low abundance (<1%). The IndVal analysis identified 12 significant representative taxa for NR, 3 for MR, and 4 for SR (Table II). Two EPT taxa (Ephemeridae and Leptophlebiidae) were identified in the NR as clean water quality indicators, and 2 taxa of Tubificidae (*Tubifex* sp. and *Branchiura* sp.) were identified in the SR as organic pollution indicators.

> DIFFERENCES IN INDICES AND ENVIRONMENTAL CONDITIONS AMONG REGIONS

Macroinvertebrate diversities and biotic indices differed significantly ($p < 0.05$) among the three NMDS groups, and the mean values of the indices consistently ranked NR > MR > SR in water quality (Table III). *Post hoc* comparisons revealed that the mean value of *S* (*Kruskal-Wallis*, $H = 18.939$, $p < 0.001$), H' (*Kruskal-Wallis*, $H = 14.176$, $p < 0.01$), EPT-S (*Kruskal-Wallis*, $H = 10.178$, $p < 0.01$), and EPT% (*Kruskal-Wallis*, $H = 8.071$, $p < 0.05$) were the highest in NR, which were remarkably different from MR and SR. The *d* showed significant differences (*Kruskal-Wallis*, $H = 16.531$, $p < 0.001$) among the three groups and mean value of each group decreased from NR to MR and to SR. The FBI score showed significant differences ($F_{2,71} = 3.323$, $p < 0.01$) among the three groups and mean value of each group increased from NR to MR and to SR. In general, a degradation gradient of macroinvertebrate communities was observed in the NR-MR-SR direction.

All of the geographical and land-use conditions, as well as the majority of the water physicochemical conditions (WT, EC, DO, TP, NH_3-N, pH and MSUBST), differed significantly ($p < 0.05$) among the three groups (Table III); however, TN (*Kruskal-Wallis*, $H = 3.006$, $p > 0.05$), NO_3-N ($F_{2,71} = 0.722$, $p > 0.05$) and velocity ($F_{2,71} = 0.685$, $p > 0.05$) showed no evidence of spatial differentiation. *Post hoc* comparisons showed that NR had a significantly higher percentage of forest land (*Kruskal-Wallis*, $H = 35.602$, $p < 0.001$), elevation ($F_{2,71} = 94.131$, $p < 0.001$), slope ($F_{2,71} = 25.559$, $p < 0.001$) and DO ($F_{2,71} = 5.725$, $p < 0.005$) than the other regions, and had a significantly lower values of rural land ($F_{2,71} = 5.284$, $p < 0.01$), urban land (*Kruskal-Wallis*, $H = 39.762$, $p < 0.001$), MSUBST ($F_{2,71} = 5.253$, $p < 0.05$), and WT ($F_{2,71} = 16.252$, $p < 0.001$. In contrast, SR had a significantly higher values of urban land (*Kruskal-Wallis*, $H = 39.762$, $p < 0.001$), stream order ($F_{2,71} = 4.128$, $p < 0.05$), EC ($F_{2,71} = 3.614$, $p < 0.05$), TP (*Kruskal-Wallis*, $H = 9.104$, $p < 0.05$), and NH_3-N ($F_{2,71} = 4.229$, $p < 0.05$) than the other regions, but a significantly lower values of forest land (*Kruskal-Wallis*, $H = 35.602$, $p < 0.001$), slope ($F_{2,71} = 25.559$, $p < 0.001$), and pH ($F_{2,71} = 8.459$, $p < 0.01$).

Table III
Means ± SE for diversity indices, biotic indices, and environmental variables of each region. n = number of sampling sites per region. An asterisk indicates p $<$ 0.05*. The results of post hoc tests among the three regions are indicated by superscripts, and significant differences (p* $<$ 0.05*) are indicated by different letters (a, b, c).*

Variables	NR (n = 21)	MR (n = 30)	SR (n = 23)	ANOVA		Kruskal-Wallis	
				F	p	H'	p
Diversity and biotic indices							
S	12 ± 1^a	8 ± 1^b	5 ± 1^b			18.939	**0.000***
d	2.46 ± 0.20^a	1.65 ± 0.24^b	1.15 ± 0.12^c			16.531	**0.000***
H′	2.29 ± 0.15^a	1.58 ± 0.19^b	1.28 ± 0.14^b			14.176	**0.001***
EPT-S	1.4 ± 0.4^a	0.8 ± 0.4^b	0.1 ± 0.1^b			10.178	**0.006***
EPT %	10 ± 2.9^a	5 ± 2.1^b	2 ± 1.2^b			8.071	**0.018***
FBI	4.2 ± 0.2^a	5.6 ± 0.2^b	6.4 ± 0.2^c	3.323	**0.002***		
Environmental variables							
Urban (%)	0.7 ± 0.3^a	2.8 ± 0.8^b	24.8 ± 4.2^c			39.762	**0.000***
Rural (%)	11.1 ± 2.4^a	25.5 ± 2.6^b	25.8 ± 3.7^b	5.284	**0.007***		
Forest (%)	84.3 ± 2.6^a	66.1 ± 4.5^b	31.3 ± 5.2^c			35.602	**0.000***
Stream order	3 ± 0.2^a	4 ± 0.3^{ab}	5 ± 0.4^b	4.128	**0.020***		
Elevation (m)	243 ± 13^a	78 ± 9^b	17 ± 4^c	94.131	**0.000***		
Slope (°)	5.75 ± 0.54^a	3.77 ± 0.49^b	1.51 ± 0.18^c	25.559	**0.000***		
WT (°C)	14.0 ± 0.44^a	16.6 ± 0.35^b	17.1 ± 0.39^b	16.252	**0.000***		
DO (mg·L^{-1})	9.0 ± 0.34^a	7.9 ± 0.30^b	7.1 ± 0.48^b	5.725	**0.005***		
TP (mg·L^{-1})	0.12 ± 0.03^{ab}	0.09 ± 0.02^a	0.23 ± 0.07^b			9.104	**0.011***
NH_3-N (mg·L^{-1})	1.59 ± 0.79^a	0.84 ± 0.21^a	4.54 ± 1.72^b	4.229	**0.018***		
EC (mS·cm^{-1})	0.07 ± 0.01^a	0.08 ± 0.01^a	0.17 ± 0.36^b	3.614	**0.032***		
pH	7.28 ± 0.05^a	7.23 ± 0.09^a	6.87 ± 0.06^b	8.459	**0.001***		
MSUBST	1.52 ± 0.82^a	3.07 ± 0.64^b	3.74 ± 0.72^b	5.253	**0.034***		
TN (mg·L^{-1})	2.64 ± 0.43	2.07 ± 0.28	4.57 ± 0.07			3.006	0.222
NO_3-N (mg·L^{-1})	1.33 ± 0.22	1.02 ± 0.13	2.37 ± 0.77	0.722	0.489		
Velocity (m·s^{-1})	0.26 ± 0.03	0.22 ± 0.02	0.20 ± 0.05	0.685	0.513		

> RELATIONSHIP BETWEEN MACROINVERTEBRATE COMMUNITIES AND ENVIRONMENTAL CONDITIONS

The first two CCA axes explained 57.6% of the variation in macroinvertebrate abundance. The forward selection procedure with the Monte-Carlo permutation test ($p < 0.05$) retained eight explanatory variables (NH_3-N, TP, NO_3-N, pH, WT, substrate, urban-cover percentage, and slope) (Table IV). The first axis (eigenvalue 0.545) accounted for 34.5% of the species-environment association. NH_3-N (r = 0.70) and TP (r = 0.66) were the most important environmental variables related to water quality from the first axis. The second axis (eigenvalue 0.234), mainly contributed by slope (r = 0.49) and WT (r = −0.46), accounted for 23.1% of the macroinvertebrate-environment correlation, reflecting the basin's topographical features and climate conditions (Table IV).

CCA biplot of samples versus environmental factors (Figure 4a) showed three distinct site groups: (1) almost all NR sites were clustered within the second quadrant, characterized by steeper slopes and coarser substrates; (2) the MR sites were situated on the lower half of the plot, characterized by relatively high temperatures and gradual slopes; and (3) the SR sites were located in the right side of the plot, distinguished by high organic pollution indicators (TP, NH_3-N, NO_3-N) and high percentages of urban land. The CCA site grouping is consistent with the NMDS results.

The associations among sensitive macroinvertebrate taxa and key environmental factors (TP, NH_3-N, slope, and WT) were apparent in the CCA analysis (Figure 4b). The abundance of *Tubifex* sp., *Limnodrilus* sp., *Branchiura* sp., and Coenagrionidae that had the highest scores on the first axis were significantly, positively correlated with TP (R^2 = 0.64; $P < 0.001$) and

Table IV
Summary statistics for the canonical correspondences analysis (CCA) relating macroinvertebrate abundance to environmental variables. The important variables in Axis 1 and Axis 2 are shown in bold.

Variables	Correlations with canonical axes		Forward selection of variables	
	Axis 1	Axis 2	*F*-ratio	*p*-value
TP	**0.66**	0.26	2.063	0.012
NH_3-N	**0.70**	0.20	3.792	0.002
Slope	–0.43	**0.49**	2.724	0.002
Water temperature	0.28	**–0.46**	1.957	0.006
NO_3-N	0.58	0.21	1.977	0.002
pH	–0.49	0.24	2.098	0.010
Substrates	0.18	–0.40	1.538	0.024
percentage of urban cover	0.59	–0.11	2.111	0.004
Eigenvalues	0.352	0.234		
Species-environment correlations	0.848	0.792		
Cumulative percentage variance of species-environment relation	34.5	57.6		

NH_3-N ($R^2 = 0.63$; $P < 0.001$) (Figures 5a and 5b). The abundance of *Semisulcospira* sp., *Limnoperna* sp., *Nais* sp., and *Angulyagra* sp. showed a positive correlation with water temperature ($R^2 = 0.52$; $P < 0.01$) (Figure 5c). The abundance of the Rhyacophilidae, Odontoceridae, Ephemeridae, Tipulidae, Leptophlebiidae, and Elmidae from NR were positively associated with slope ($R^2 = 0.28$; $P < 0.001$) (Figure 5d).

DISCUSSION

> *SPATIAL DIFFERENCES AND REGIONAL CHARACTERISTICS OF MACROINVERTEBRATE COMMUNITIES*

The characteristics of the macroinvertebrate communities were closely associated with geographical conditions, land cover, and anthropogenic disturbance of the three identified regions. NR is a mountain forest dominated region that has relatively low levels of anthropogenic disturbance and has retained its original natural habitat. These conditions likely have maintained the least disturbed macroinvertebrate communities, which is reflected by that the EPT taxa, such as members of the Ephemeridae, Hydropsychidae and Baetidae, were more abundant in this region than in the other regions. The majority sites in NR were predominated by cobble and boulder substrates, and taxon occurrence of macroinvertebrates was influenced primarily by substrate type (Beisel *et al*., 1998). Usually, aquatic insect larva, especially the taxa preferring sticking onto the stones (*e.g.* Ephemeroptera and Trichoptera), were abundant in coarse substrate (Buss *et al*., 2004) Furthermore, although the second NMDS axis did not show clear associations between taxa and specific environmental variables, we found an evident gradient change of MSUBST in the sites of NR along this axis (Figure 2a).

MR is a hill-dominated, transitional region where sand extraction for construction is widespread (Zhang *et al*., 2010). The high percentage of Insecta species recorded in MR (Figure 3) was due largely to the high abundance of Chironomidae, which reached a maximum of 2290 individuals$\cdot$m^{-2}. We speculate that sand extraction might increase the growth of Chironomidae because most Chironomidae species prefer sand-bottom substrates (Michael and David, 1984; John, 2004). Moreover, Chironomidae larvae can recolonize quickly to dredging due to their high dispersal ability and fecundity (Vermonden *et al*., 2011).

SR is a plain-urbanized region, where human impacts have greatly altered aquatic ecosystems. The agriculture and urban land-use practices degraded water quality by exporting fine sediments, nutrients, and pesticides; changing river channel physical properties; and reducing riparian vegetation. All of these could impact macroinvertebrate community composition

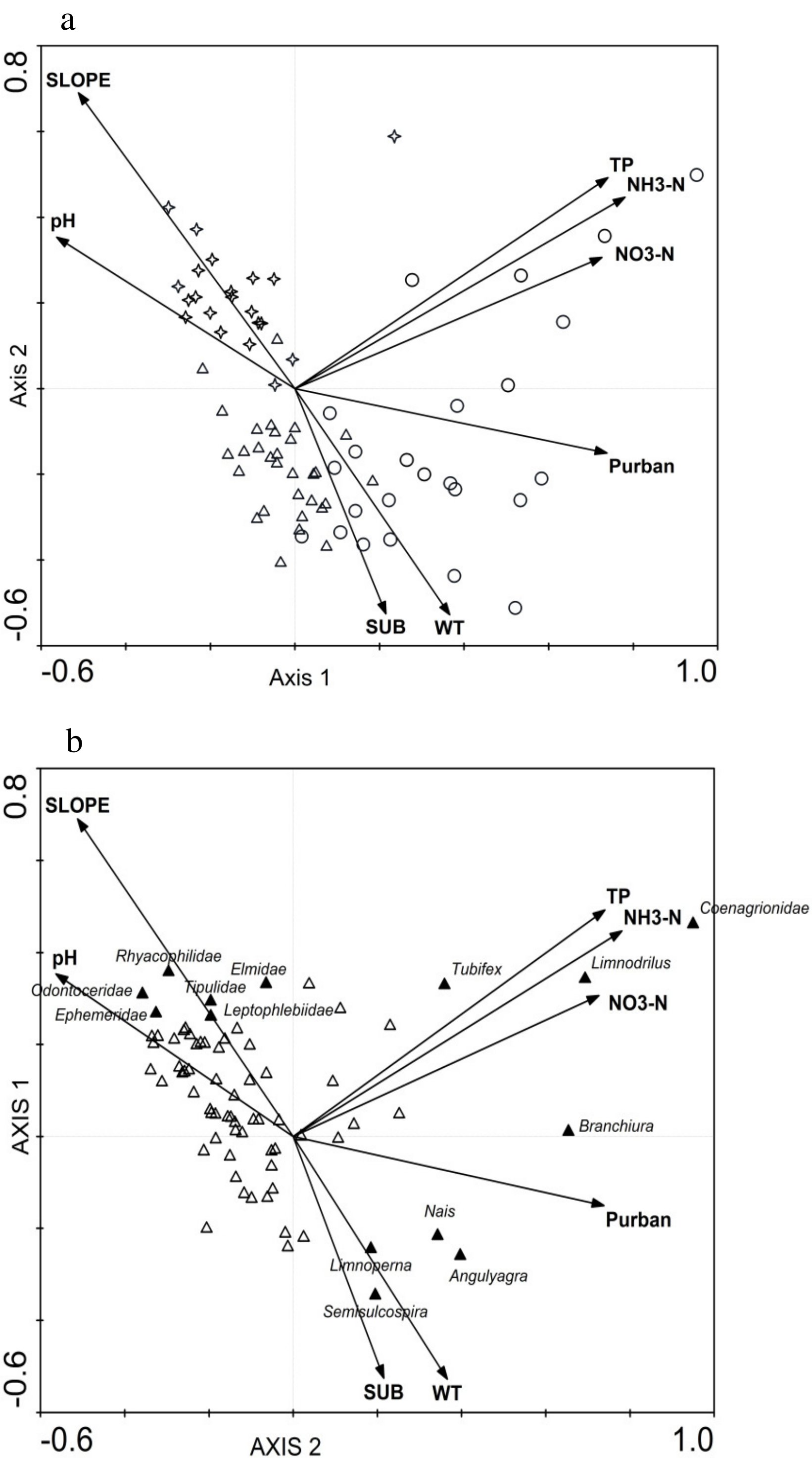

Figure 4
(a) Biplot of the CCA results of the sampling sites and environmental variables of the study area. The northern region (NR), the middle region (MR), and the southern region (SR) sites are represented by asterisks, triangles, and circles, respectively. (b) Biplot of the CCA results for relations among macroinvertebrate taxa (abundance, triangles) and environmental variables (arrows). Taxa strongly associated with the environmental variables are indicated by solid triangles. Purban = percentage of urban; SUB = average substrate score; WT = water temperature (°C).

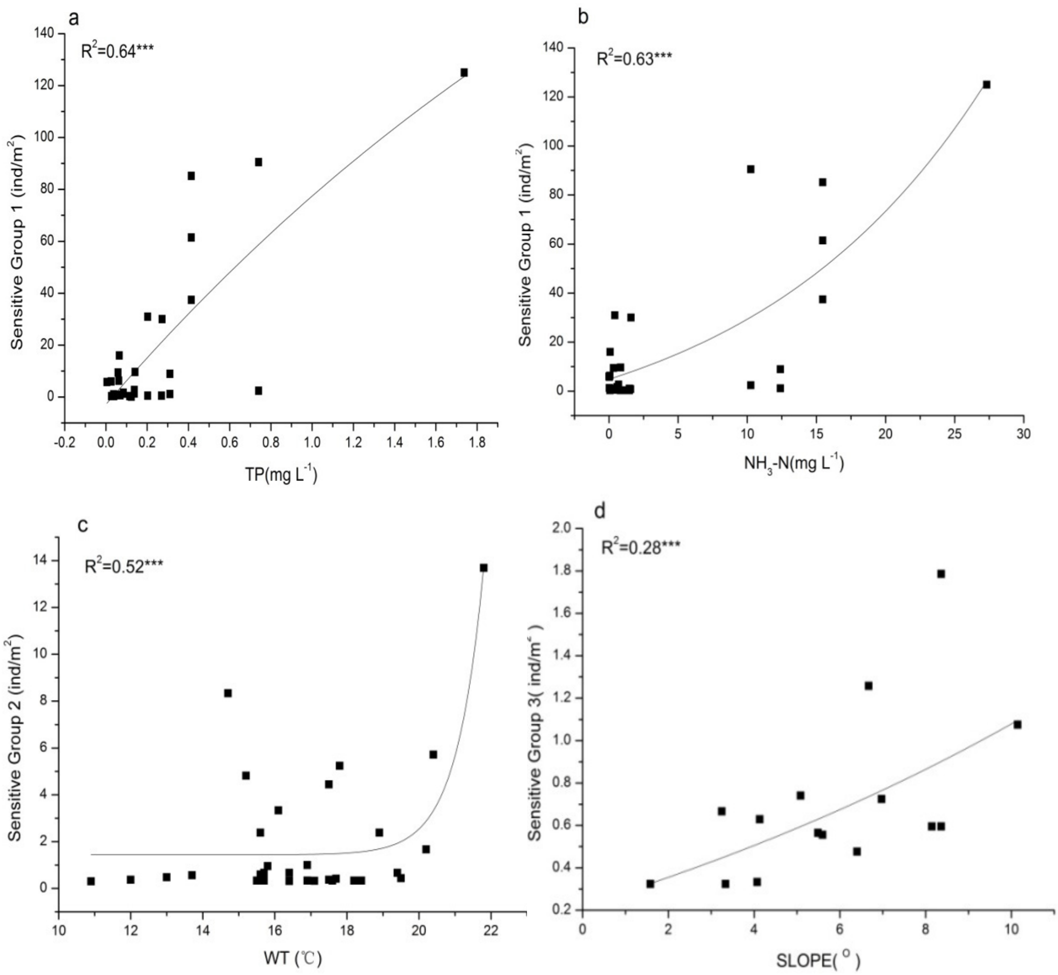

Figure 5

*Relationships between the abundance of select taxa and key environmental variables. (a) Relationship between abundance of sensitive taxon group 1 (Tubifex sp., Limnodrilus sp., Branchiura sp., and Coenagrionidae) and concentration of TP; (b) Relationship between abundance of sensitive taxon group 1 and concentration of NH_3-N; (c) Relationship between abundance of sensitive taxon group 2 (Semisulcospira sp., Limnoperna sp., Nais sp., and Angulyagra sp.) and water temperature; (d) Relationship between abundance of sensitive taxon group 3 (Rhyacophilidae, Odontoceridae, Ephemeridae, Tipulidae, Leptophlebiidae, and Elmidae) and slope. Statistical significance: ***p* < 0.001.

(Paul and Meyer, 2001; Genito *et al*., 2002; Allan, 2004). The most abundant taxa were *Tubifex* sp. and *Branchiura* sp., and only 2 EPT taxa (Neoephemeridae and Baetidae) occurred in SR. These results suggest that the habitat and water quality conditions are much poorer in SR than in the other two regions. A survey by Gallacher (2001) indicated that domestic sewage was the most commonly listed problem facing this region, followed by agriculturally derived pollutions. The large amount of nutrient and sediment loads and other pollutants from such land uses jointly impact stream biological integrity by lowering water quality, degrading habitat, and interrupting ecosystem processes (Sponseller *et al*., 2001).

Using various analyses, we identified spatial distribution and regional differentiation of macroinvertebrate communities based on taxon data, diversity indices, biotic indices and their relations with environmental conditions. Our results are similar to those of Zhang *et al.* (2010) who showed that the macroinvertebrate community composition are distinctly different among the upper (4 sites), middle (4 sites) and lower (3 sites) sections of the Dongjiang River Basin. Similar distribution differentiation was also observed for the Lijiang River in southwestern China (Chen *et al*., 2012) and for the Taizi River in northeastern China (Qu *et al*., 2013).

> EFFECTS OF NITROGEN AND PHOSPHORUS ON MACROINVERTEBRATES

Our CCA results indicated that chemical conditions (NH_3-N and TP) and, to a lesser extent, physical properties (slope and WT) were the main environmental factors influencing macroinvertebrate distributions in the Dongjiang River Basin. These results suggest that nitrogen and phosphorus pollutants from human activity have greater effects on macroinvertebrate communities than natural factors. Nitrogen and phosphorus have been previously shown to be important in determining macroinvertebrates distributions and have been used to indicate the level of organic pollution of rivers (Maul *et al*., 2004; McCormick *et al*., 2004; Meng *et al*., 2009). Some studies in Asia, such as in Tamiraparani River in south India (Martin *et al*., 2000) and Juru River Basin in Malaysia (Al-Shami *et al*., 2011) reported that chemical parameters (*e.g.* NH_3-N and TP) were main factors controlling the spatial heterogeneity of macroinvertebrate communities.

Inputs of phosphorus and nitrogen to freshwater systems not only compromise water quality but also deteriorate the diversity of aquatic fauna (Chambers *et al*., 2012). In the present study, as TP and NH_3-N increased, the abundances of *Tubifex* sp., *Limnodrilus* sp., *Branchiura* sp. and Coenagrionidae increased pronouncedly (Figures 5a and 5b). This nutrient-macroinvertebrate association is likely resulted from that the four taxa are tolerant species (with tolerance scores up to 9; Xu *et al*., 2013), have low dissolved oxygen requirements, and prefer organic-polluted habitats. The sites with high nitrogen and phosphorus concentrations were predominantly located within SR and associated with high percentages of urban land use (Figure 4a). With the local human population increase and economic growth, sewage output from industrial and residential sectors has rapidly increased, overwhelming municipal sewage disposal systems. Therefore, the harmful substances in sewage have directly or indirectly been released into the river system, causing eutrophication and pollution of the aquatic system.

In the present study, the concentration of NH_3-N in many SR sites was above 1.00 $mg{\cdot}L^{-1}$, at times exceeded 10.00 $mg{\cdot}L^{-1}$ (Figure 5b). These concentrations exceed the freshwater aquatic life criteria for ammonia (0.07–3.92 $mg{\cdot}L^{-1}$) in China (Yan *et al*., 2011). The sites (*e.g.*, S55, S56, S64, S65) with the highest NH_3-N concentrations ($>$10.00 $mg{\cdot}L^{-1}$) are primarily located in industrial district cities (*e.g.*, Shenzhen and Dongguan), and sewage outlets are the primary pollution sources. The macroinvertebrate diversity in these sites was very low, limited to several extremely tolerant taxa (*e.g. Branchiura* sp.). Hence, there is an urgent need to reduce the input of nitrogenous pollutants into freshwater system, especially in the southern urban area of the river basin.

> POTENTIAL INDICATORS IN DIFFERENT REGIONS

Relative to community-level statistical indices, such as total macroinvertebrate density, biomass, and richness, taxa-specific indicators generally provide a higher-resolution indication of the effects of anthropogenic disturbances (Johnson *et al*., 1992). Taxa-specific indicators of a region typically yield patterns with agglomerative distributions and are often associated with particular environmental conditions, especially conditions that closely reflect water quality and degree of pollution of the ecosystems.

Our IndVal method extracted “indicator taxa” for each region based on abundance and frequency (Table II) and those taxa represent the environmental condition where they occur (Figures 4b and 5). Our results suggest that the Tipulidae, Ephemeridae, and Leptophlebiidae are suitable indicator taxa for NR, representing natural habitats with minimal human disturbance and clean water; *Semisulcospira* sp. are suitable indicator taxa for MR, preferring sandy substrates and relatively high water temperatures within a suitable range; and *Branchiura* sp. are useful indicator taxa for SR, indicating organic pollution and extensive habitat degradation.

Our results are consistent with those of Xu *et al*. (2013) who studied taxa-specific macroinvertebrate indicators from several sites in 14 rivers in China (including the Guanlan River in the

Dongjiang River Basin) with varying pollution levels. They found that members of the Tipulidae, Ephemeridae, and Leptophlebiidae were indicators of good or very good water quality, *Semisulcospira* sp. were indicators of moderate or poor water quality, and *Branchiura* sp. were indicators of extremely poor water quality. Their results are consistent with the regional indicators of water quality identified for the three regions in our study.

ACKNOWLEDGEMENTS

The authors thank Professor Dayong Wu for his help with the specimen identifications. We are grateful to Bo Wang, Chenglong Tang, Kai Wu, and Tinghai Li for their help with the fieldwork. We also thank Prof. Han Y. H. Chen for reviewing this manuscript. This work was supported by the National Natural Science Foundation of China (41271104) and the National Science and Technology Major Project: Water Pollution Control and Management Technology of China (No. 2012ZX07501002-04).

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Cite this article as: L. Fu, Y. Jiang, J. Ding, Q. Liu, Q.Z. Peng, M.Y. Kang and L.Z. Wang, 2015. Spatial variation of macroinvertebrate community structure and associated environmental conditions in a subtropical river system of southeastern China. *Knowl. Manag. Aquat. Ecosyst.*, 416, 17.

***Appendix I**. List of macroinvertebrates in the study sites.*

Phylum	Class	Order	Family	Genus
Annelida	Polychaeta	Phyllodocida	Nereididae	
	Oligochaeta	Tubificida	Tubificidae	*Tubifex*
				Limnodrilus
				Branchiura
			Naididae	*Nais*
	Hirudinea	Rhynchobdellida	Glossiphonidae	*Helobdella*
				Glossiphonia
				Hemiclepsis
		Herpobdellida	Erpobdellidae	*Erpobdella*
Mollusca	Gastropoda	Mesogastropoda	Viviparidae	*Cipangopaludina*
				Bellamya
				Angulyagra
			Hydrobiidae	*Oncomelania*
				Tricula
				Bithynia
			Ampullariidae	
			Melaniidae	*Semisulcospira*
		Basommatophora	Physidae	*Physa*
			Planorbidae	*Hippeutis*
			Lymnaeidae	*Radix*
				Gabla
			Assimineidae	*Assiminea*
	Lamellibranchia	Unionoida	Unionidae	*Anodonta*
		Mytiloida	Mytilidae	*Limnoperna*
		Eulamellibranchia	Corbiculidae	*Corbicula*
Arthropoda	Crustacea	Decapoda	Atyidae	
			Palaemonidae	
	Arachnoida	Acariformes	Lebertiidae	
	Insecta	Hemiptera	Corixidae	
			Naucoridae	
			Notonectidae	
			Nepidae	
			Gerridae	
		Ephemeroptera	Ephemeridae	
			Baetidae	
			Siphlonuridae	
			Ephemerellidae	
			Neoephemeridae	
			Caenidae	
			Heptageniidae	
			Leptophlebiidae	
		Plecoptera	Perlidae	
		Coleoptera	Pyralidae	
			Tortricidae	
		Trichoptera	Hydropsychidae	
			Leptoceridae	
			Lepidostomatidae	
			Rhyacophilidae	
			Limnephilidae	
			Calamoceratidae	
			Hydroptilidae	
			Odontoceridae	

***Appendix I**. Continued.*

Phylum	Class	Order	Family	Genus
		Megaloptera	Corydalidae	
		Coleoptera	Elmidae	
			Haliplidae	
			Staphylinidae	
			Gyrinidae	
			Dytiscidae	
			Hydrophilidae	
			Hydraenidae	
		Odonata	Gomphidae	
			Macromiidae	
			Corduliidae	
			Libellulidae	
			Calopterygidae	
			Coenagrionidae	
			Aeshnidae	
			Lestidae	
			Cordulegastridae	
			Tanypodinae	
			Orthocladiinae	
		Diptera	Chironominae	
			Simuliidae	
			Dolichopodidae	
			Stratiomyidae	
			Tipulidae	
			Psychodidae	
			Tabanidae	
Platyhelminthes	Turbellaria	Tricladida	Dugesiidae	