



Fine-scale environmental gradients formed by local pollutants largely impact zooplankton communities in running water ecosystems

Heng Peng^{1,2}, Wei Xiong¹, Aibin Zhan^{1,2,*}

¹Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, Haidian District, Beijing 100085, PR China

²University of Chinese Academy of Sciences, Chinese Academy of Sciences, 19 Yuquan Road, Shijingshan District, Beijing 100049, PR China

ABSTRACT: Many freshwater ecosystems suffer from multiple environmental stressors derived from anthropogenic activities. It is therefore necessary to investigate how environmental changes influence composition and functioning of biological communities such as zooplankton. At fine geographical scales, a well-known view on meta-community dynamics suggests that high dispersal can strongly homogenize community structure along water flows, largely erasing signals left by species sorting. However, a recent study by Xiong et al. (2017; *Mol Ecol* 26:4351–4360) challenges this view, showing that species sorting derived from an environmental gradient overrode the process of dispersal to determine the zooplankton community structure in running river ecosystems at fine geographical scales (the fine-scale species sorting hypothesis). Here we chose zooplankton communities from Fuyang River in north China to test the newly proposed hypothesis and identified the environmental factors contributing to meta-community dynamics in running water ecosystems. Multiple analyses based on high-throughput sequencing showed significantly varied zooplankton community composition and geographical distribution determined by an environmental gradient. Our study clearly shows that local chemical pollution, such as metal pollutants Cu and Mg, largely contributes to the observed patterns. Our study successfully identified local pollutants that influenced meta-community dynamics. Thus, we support the fine-scale species sorting hypothesis, indicating that a strong environmental gradient at fine geographical scales can strengthen the process of species sorting. As many rivers suffer from anthropogenic environmental stressors, an urgent need exists to integrate both environmental and community information when investigating how environmental changes influence community composition and functioning.

KEY WORDS: Community · Dispersal · Environmental pollution · High-throughput sequencing · Metal pollution · River · Species sorting · Zooplankton

INTRODUCTION

Freshwater ecosystems are suffering increasingly from multiple interacting environmental stressors derived from intensive anthropogenic activities, leading to catastrophic impacts on biodiversity and ecosystem functioning globally (Sandin & Solimini 2009,

Woodward 2009, Xiong et al. 2018). Vulnerable freshwater ecosystems, particularly rivers flowing through cities, gradually lose their ability to provide ecosystem goods and services, which substantially influences the future of life on earth (Sala et al. 2000, Woodward et al. 2012). Although significant negative influence of environmental stressors on river ecosys-

*Corresponding author: zhanaibin@hotmail.com or azhan@rcees.ac.cn

© The authors 2018. Open Access under Creative Commons by Attribution Licence. Use, distribution and reproduction are unrestricted. Authors and original publication must be credited.

tems has been well documented in the published literature, we still have limited knowledge on the responsible mechanisms, such as how environmental stressors derived from intensive anthropogenic activities alter ecosystems, particularly chemical pollution in river ecosystems (Xiong et al. 2016a, 2017).

Based on several well-known models (see details in Leibold et al. 2004, Heino et al. 2012, Winegardner et al. 2012), species sorting and dispersal are widely recognized as 2 fundamentally competing processes that determine zooplankton meta-community structure and geographical distribution. Species sorting, based on niche theory and environmental filtering, needs sufficient dispersal for species to track environmental heterogeneity among sites. Following sufficient dispersal, local factors, including abiotic and biotic interactions with existing species, largely filter the species based on habitat suitability to determine community composition, leading to varied community structure in different habitats (Duggan et al. 2002). Dispersal, which is based on a neutral and random process (Hubbell 2001), homogenizes the geographical distribution of species irrespective of environmental conditions, thus obscuring the function of species sorting.

Numerous studies have shown that these 2 processes (dispersal and species sorting) are highly scale-dependent when determining community composition and geographical distribution in running freshwater ecosystems (Heino et al. 2015a, Xiong et al. 2016a). At relatively large and medium geographical scales, such as across a basin or across rivers within a basin, studies have generally reached a common conclusion that species sorting is usually dominant, mainly owing to dispersal limitation (Landeiro et al. 2012, Grönroos et al. 2013). At fine geographical scales, such as within single streams or rivers, a high level of dispersal can largely erase signals left by species sorting, as communities can be largely homogenized by high dispersal (Cottenie 2005, Heino et al. 2015a). Indeed, an environmental gradient rarely exists at fine geographical scales, leading to weak signals of species sorting in communities, particularly those highly influenced by dispersal, such as zooplankton (Duggan et al. 2002, Datry et al. 2016). However, a recent study by Xiong et al. (2017) has challenged this view of meta-community dynamics: they found significant signals of species sorting in zooplankton communities in a running water ecosystem at fine geographical scales (i.e. ~200 km at a single river level), and such a pattern was mainly derived from an environmental gradient caused by local pollution from various intensive anthropogenic

activities along the river. As a result, the authors proposed the 'fine-scale species sorting hypothesis', where they emphasized that species sorting derived from various local environmental conditions could largely outcompete the strong influence of high dispersal at fine geographical scales, resulting in significantly diverse community structure along an environmental gradient (Xiong et al. 2017).

In order to test the fine-scale species sorting hypothesis and validate whether the presence of an environmental gradient can eliminate the significant influence caused by dispersal, we selected zooplankton communities from Fuyang River for this study. Fuyang River, belonging to the Haihe River Basin located in north China, is one of 2 branches of the Ziya River. It originates from Fengfeng County of Handan City, Hebei Province, and flows through 5 major cities, including Handan, Xingtai, Shijiazhuang, Hengshui, and Cangzhou. The average annual river flow is 12 million m³ (Yang et al. 2013). We chose zooplankton in this study mainly as the taxa are easily dispersed through ambient water currents (Liu et al. 2013). As a result, zooplankton represents a good model to test the dispersal versus species sorting hypotheses (Xiong et al. 2016a, 2017). In addition, zooplankton play a crucial role in aquatic food webs through their consumption and processing of phytoplankton and other food sources, as a resource for consumers at higher trophic levels, and/or as a conduit for packaging organic material in the biological pump (Battuello et al. 2016). Moreover, widely accepted evidence among ecologists suggests that species assemblages in zooplankton communities can rapidly respond to environmental changes such as eutrophication and metal pollution (Zhou et al. 2008, Gagnetten & Paggi 2009, Xiong et al. 2017).

In this study, we combined information on species composition and environmental variables to analyze the structure and geographical distribution of a zooplankton community. Specifically, we aimed to (1) test the fine-scale species sorting hypothesis and (2) investigate what environmental factors contribute to the observed patterns at fine geographical scales in running water ecosystems.

MATERIALS AND METHODS

Site selection and sampling

Fuyang River is 402 km long and has an area of 22814 km² with a northern temperate continental monsoon climate and annual precipitation of 550 mm

and an average temperature of 13.4°C. In addition to its main river course, dendritic tributaries are distributed in a palm-like network, which contains the Niuwei River, Xiao River, Wangyang Ditch, and Shaocun Canal, etc. (Fig. 1). We sampled 26 sites, 8 in Handan and South Xingtai (Region I), 12 in North Xingtai and Shijiazhuang (Region II), and 6 in Hengshui and Cangzhou (Region III), at a relatively fine geographical scale (~300 km) along the environmental gradient (see 'Results'; Table S1 in the Supplement at www.int-res.com/articles/suppl/b027p043_supp.pdf). The 26 sites covered all 5 cities that Fuyang River flows through, namely Handan, Xingtai, Shijiazhuang, Hengshui, and Cangzhou (Fig. 1).

Zooplankton and water samples were collected from June 12–20, 2014 using the methods of Xiong et al. (2016a). At each sampling site, we took three 10 l water samples, with each collected from the bottom to water surface, and the 3 samples from each site were combined and filtered through a 20 µm mesh net. All collected zooplankton samples were immediately preserved in anhydrous alcohol with a final volume of 100 ml. At the same time, 500 ml water samples were collected for physicochemical analyses in the laboratory. All collected samples were stored at 4°C and shipped to the laboratory. Geographical coordinates were recorded by GPS (Garmin Legend) in the field.

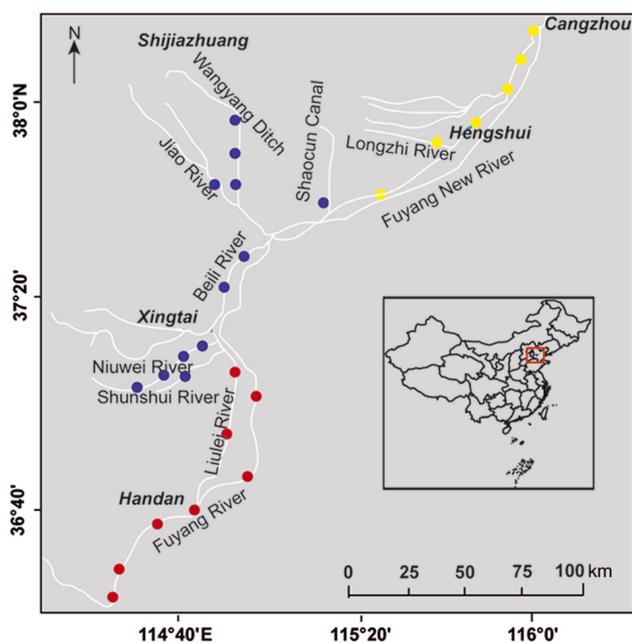


Fig. 1. Sampling sites in Fuyang River. Dots in red, blue, and yellow stand for sampling sites in Regions I, II, and III, respectively

Physicochemical analysis

Water temperature (T), pH, electrical conductivity (EC), total dissolved solids, and oxidation-reduction potential were measured *in situ* with a multi-parameter water quality sonde (MYRON Company). Total nitrogen, nitrate nitrogen, ammonia nitrogen, total phosphorus, and soluble reactive phosphorus were measured using the methods of Xiong et al. (2016a). Concentrations of heavy metals (Cd, Cr, Cu, Ni, Zn, Pb, and As) were determined by inductively coupled plasma-mass spectrometry (ICP-MS; 7500a, Plasma Quad 3). Concentrations of K, Ca, Na, Mg, and dissolved total phosphorus were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES; OPTIMA 2000, PerkinElmer).

Spatial variable analysis

In order to model the spatial structure of zooplankton communities, we used principal coordinates of neighbor matrices (PCNM) analysis to create spatial predictors (Borcard & Legendre 2002, Dray et al. 2006, Blanchet et al. 2011). Based on longitude and latitude of the sampling locations, we used PCNM to truncate a geographical distance matrix to generate a set of explanatory spatial variables (i.e. PCNM variables, V1–V6). Positive eigenvalues with positive spatial correlation were retained as spatial explanatory variables for subsequent redundancy analysis (RDA) and variance partitioning. The PCNM analysis was performed in the R language environment using the *pcnm* function from the PCNM package (Oksanen et al. 2013, R Core Team 2015).

DNA extraction, PCR amplification, and high-throughput sequencing

For DNA extraction, bottles containing zooplankton were vigorously shaken and all the samples were transferred into Eppendorf tubes. Tubes containing preserved zooplankton were centrifuged at $13\,500 \times g$ for 3 min to remove anhydrous alcohol. The total genomic DNA was extracted from collected zooplankton using the DNeasy Blood and Tissue Kit (Qiagen). The quality and quantity of each sample was measured by a UV spectrophotometer (NanoDrop, Thermo Scientific).

We used the primer pair Uni18S and Uni18SR, which was specifically designed for zooplankton based on the V4 region of nuclear small subunit ribo-

somal DNA (Zhan et al. 2013), to amplify all zooplankton samples. Eight nucleotides (also known as tags) were added to the 5' ends of both the forward and reverse primers to identify pooled PCR products (Parameswaran et al. 2007). PCR reactions were assembled in a 25 μ l mix, including approximately 100 ng of genomic DNA, 1 \times PCR buffer, 2 mM of Mg^{2+} , 0.2 mM of dNTP, 0.4 μ M of each primer, and 2 U of *Taq* DNA polymerase (LA *Taq*, Takara). The amplification program was: 95°C for 5 min, 25 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 90 s, and a final extension at 72°C for 10 min. To reduce biased amplification, each sample was replicated 5 times during PCR amplification (Zhan et al. 2013, 2014b, Xiong et al. 2016b). Meanwhile, a negative control without DNA template was included in all experiments. Finally, all generated amplicons were purified using the QIAquick PCR Purification Kit (Qiagen). In order to pool all tested communities together, an equal quantity of PCR product derived from each community was used to construct a sequencing library. Sequencing was performed on an Illumina MiSeq platform using a paired-end 300 bp sequence read run.

Data analysis

To determine the community structure of zooplankton, we used the UPARSE pipeline for high accuracy in biological sequence recovery and richness estimates on zooplankton communities (Edgar 2013). Sequence reads without errors in both primers and tags were obtained using Python scripts provided by UPARSE (Majaneva et al. 2015). Additionally, sequence reads were denoised, trimmed, and filtered to eliminate errors and artifacts by using the UPARSE pipeline in USEARCH (Edgar 2013). In general, we discarded sequences with Phred quality (Q) scores <30, undetermined nucleotides (N's), and expected error >0.5. Furthermore, sequence length was trimmed to 225 bp for clustering alignment and sequences <225 bp were discarded (Xiong et al. 2017). The filtered reads were de-replicated and then clustered into similarity-based operational taxonomic units (OTUs) at a similarity cut-off value of 97% (Zhan et al. 2014a) using the UPARSE-OTU algorithm. After clustering, taxonomic assignment was performed against the nucleotide database of GenBank using BLASTn implemented in the pipeline Seed (Větrovský & Baldrian 2013) with the parameters of E value <10⁻⁸⁰, minimum query coverage >80%, and similarity >85% (Zhan et al. 2014a, Xiong et al. 2017).

Non-metric multidimensional scaling ordination (NMDS) was used to identify the inter-regional difference of species composition based on Bray-Curtis distance. ANOSIM based on Bray-Curtis similarity was used to compare the variance of zooplankton community composition in the 3 regions. Furthermore, the major OTUs responsible for the contribution of community variation were identified using SIMPER analysis at both the intra- and inter-region levels. We also used NMDS to group sampling sites based on the Euclidean distance of the environmental variables. Prior to statistical analyses, the abundance of OTUs and physiochemical variables were $\log_{10}(x + 1)$ transformed, with the exception of pH, to improve normality and homoscedasticity. All tests of ANOSIM, SIMPER, and NMDS were conducted in PRIMER 5.0 (Clarke 1993, Clarke & Warwick 1994, Clarke & Gorley 2001).

Canonical ordination such as RDA is an invaluable tool to assess the relationship between community structure and environmental variables (McArdle & Anderson 2001, Legendre et al. 2011, Xiong et al. 2018). The RDA was chosen based on a preliminary detrended correspondence analysis (DCA) on zooplankton communities, which indicated that the longest length of the gradient (1.904) was shorter than 4, suggesting that the majority of taxa exhibited a linear response to environmental variables, including spatial and environmental factors (Lepš & Šmilauer 2003). In order to prevent a Type I error and a consequent overestimation of explained variation, variables which were highly collinear with others were removed in variance partitioning according to Blanchet et al. (2008). Then, 2 separate forward selection procedures were performed to select a set of environmental and spatial variables significantly contributing to zooplankton community variation ($p < 0.05$ after 999 permutations). Finally, the selected environmental and spatial variables were combined to build a parsimonious model of RDA (Gauch 2003).

To test the relative roles of species sorting versus dispersal in structuring the zooplankton community, a variance partitioning approach was used to quantify the effects of environmental factors and spatial factors (Borcard et al. 1992). The relative roles of species sorting versus dispersal were estimated through calculating the proportion of the explained variation of zooplankton community composition by non-collinear environmental variables and spatial variables of PCNM. The total variation was partitioned into 4 parts: explained by pure environmental variables independent of spatial variables [E/S], pure spatial variables independent of environmental vari-

ables [S/E], shared explained by environmental and spatial variables [$E \cap S$], and unexplained variation ($1 - [E/S] - [S/E] - [E \cap S]$). For all analyses, the species data was log transformed and a Monte Carlo permutation test (999 permutations under reduced model) was used to compute the significance for all these different parts. Variance partitioning was performed using the CANOCO 4.5 package (Lepš & Šmilauer 2003).

RESULTS

Environmental gradient

We detected a significant environmental gradient along the direction of water flow, and the whole river was divided into 3 regions (I–III) based on NMDS analysis of environmental factors (Stress = 0.12; Fig. 2). ANOSIM revealed significant variation of environmental conditions in the 3 regions ($R = 0.655$; $p = 0.001$), which was confirmed by pairwise analysis of regions ($p < 0.01$; Fig. 2). The physicochemical environmental variables largely varied among the 3 regions, particularly the heavy metal Cu. Its concentration was the lowest in Region I ($4.64\text{--}16.38 \mu\text{g l}^{-1}$, mean: $11.94 \mu\text{g l}^{-1}$; Table 1), and gradually increased along the river course, reaching the highest values in Region III ($75.87\text{--}171.80 \mu\text{g l}^{-1}$, mean: $120.05 \mu\text{g l}^{-1}$). Similarly, the concentration of Mg was relatively lower in Regions I (mean: 31.48mg l^{-1}) and II (mean: 31.56mg l^{-1}), and was highest in Region III (mean: 81.60mg l^{-1}). All analyses clearly showed a distinct environmental gradient along the river.

Composition of zooplankton communities

A total of 1833257 sequences were generated for the pooled 26 zooplankton samples (NCBI SRA accession no. SRP114914). After all processes of filtering, trimming, removal of sequences annotated

to non-zooplankton taxa, and OTU grouping, a total of 530 OTUs were recovered across all 26 zooplankton communities. The number of OTUs per sampling site varied from 122 (Sites ZYN49 and ZYN66 in Region II and Site ZYN22 in Region III, see Table S1 in the Supplement) to 242 (Site ZYN23 in Region III; Table S1), and the average number of OTUs per sampling site was 169, 159, and 176 in Regions I, II, and III, respectively. The estimated species-accumulation curves indicated that taxa in zooplankton communities were relatively well recovered based on our sequencing effort (Fig. 3).

After BLASTn, 179 and 351 OTUs belonged to metazoans and protozoans, respectively, across all 26 sampling sites (Fig. S1). Among metazoans, Arthropoda was the most abundant (68 OTUs; 38.0%), including 57, 8, and 3 OTUs of Crustacea, Hexapoda, and Chelicerata, respectively, followed by Rotifera (63 OTUs; 35.2%), including 54 OTUs for Monogononta and 9 OTUs for Bdelloidea. Among the protozoans, Ciliophora was the largest group (286 OTUs; 81.5%), including Oligohymenophorea (96 OTUs; 27.4%), Spirotrichea (34 OTUs; 9.7%), and Litostomatea (30 OTUs; 8.5%), and followed by Cercozoa (29 OTUs; 8.3%) and Amoebozoa (11 OTUs; 3.1%).

Geographical distribution of zooplankton communities

Zooplankton communities largely varied along the environmental gradient (global $R = 0.269$; $p = 0.003$; Fig. 2). Further, a pairwise global test and SIMPER analysis showed that zooplankton communities were significantly different between regions (Table S2). Dissimilarity between Regions I and II, between Regions I and III, and between Regions II and III was 47.22 (global $R = 0.290$, $p = 0.003$), 44.85 (global $R = 0.152$, $p = 0.091$), and 49.50 (global $R = 0.317$, $p = 0.015$), respectively. When SIMPER analysis was performed at the intra-regional level, we also detected a high level of dissimilarity (Table S3): the similarity

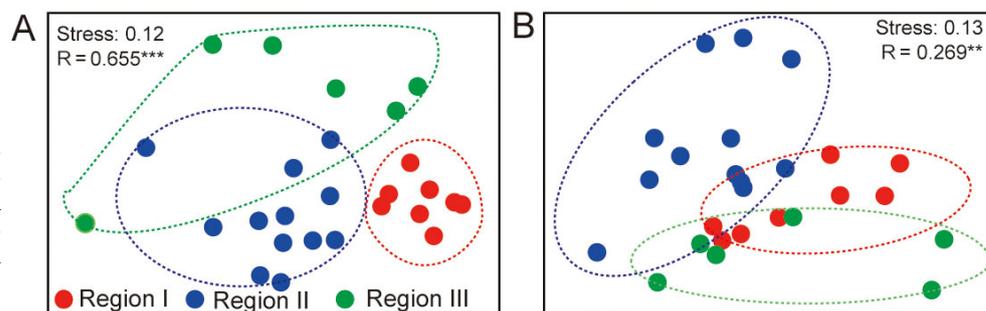


Fig. 2. Non-metric multi-dimensional scaling ordination of (A) environmental variables and (B) zooplankton communities at each sampling site. ** $p < 0.01$, *** $p < 0.001$

Table 1. Environmental variables measured in the 3 regions in Fuyang River

Environmental variable	Region I		Region II		Region III	
	Mean	SD	Mean	SD	Mean	SD
pH	8.80	0.38	7.96	0.32	8.50	0.50
Water temperature (°C)	29.44	2.85	25.57	1.94	24.20	0.91
Electrical conductivity ($\mu\text{S cm}^{-1}$)	1173.94	298.27	2943.17	2002.68	4292.33	1941.10
Oxidation-reduction potential (mV)	7.13	27.96	-11.42	51.64	-55.50	52.61
Total dissolved solids (mg l^{-1})	814.41	213.64	2228.45	1656.16	2117.78	1079.19
Total N (mg l^{-1})	6.49	4.15	53.76	50.17	20.38	22.88
$\text{NH}_4\text{-N}$ (mg l^{-1})	0.82	1.16	39.53	43.04	11.25	18.26
$\text{NO}_3\text{-N}$ (mg l^{-1})	3.46	3.74	5.47	5.65	0.82	1.09
Total P (mg l^{-1})	1.68	0.84	6.63	6.16	5.28	5.02
Soluble reactive P (mg l^{-1})	0.05	0.08	0.45	0.67	0.64	0.82
Dissolved total P (mg l^{-1})	0.33	0.20	1.27	1.19	1.56	1.83
Cr ($\mu\text{g l}^{-1}$)	3.52	1.92	10.91	6.74	16.45	7.86
Ni ($\mu\text{g l}^{-1}$)	6.39	2.33	18.30	9.16	10.67	8.45
Cu ($\mu\text{g l}^{-1}$)	11.94	4.13	40.62	65.10	120.05	34.94
Zn ($\mu\text{g l}^{-1}$)	8.86	3.37	16.26	7.72	359.83	841.27
As ($\mu\text{g l}^{-1}$)	4.96	1.69	4.04	1.70	21.61	22.40
Cd ($\mu\text{g l}^{-1}$)	0.10	0.05	0.06	0.04	0.23	0.22
Pb ($\mu\text{g l}^{-1}$)	1.48	0.80	1.62	0.45	4.01	5.76
K (mg l^{-1})	5.61	2.69	16.12	8.18	10.02	5.80
Na (mg l^{-1})	45.63	17.34	171.75	144.91	308.79	169.86
Mg (mg l^{-1})	31.48	4.78	31.56	6.95	81.60	36.69
Ca (mg l^{-1})	45.26	13.50	54.25	7.18	42.95	14.12

values were 58.13, 56.11, and 53.29 in Regions I, II, and III, respectively. Meanwhile, the taxa responsible for the intra-regional similarity were largely different, even for the high-abundance species. For example, OTU7 (Rotifera), which had a wide geographical distribution and varied abundance in each region, largely contributed to the dissimilarity between regions, especially between Regions I and III (Tables S2 & S3). OTU3 (*Thermocyclops* sp.), whose abundance generally decreased along the environmental gradient in the 3 regions, contributed only 2.69%, 2.41%, and 2.2% to the similarity within Regions I, III, and II, respectively.

Variables responsible for the observed community structure

Among the 22 measured environmental variables, 12 environmental factors were retained as non-collinear variables. Therein, T, EC, Mg, and Cu were selected as significant variables responsible for the variation of zooplankton community structure (Table S4, Fig. 4). For spatial factors, 4 (V1, V2, V4, and V6) out of 7 spatial variables were selected as significant ones for further analyses.

To assess the relationship between zooplankton communities and explanatory variables, 4 environmental (Fig. 5) and 4 spatial variables were combined together to establish a parsimonious RDA model (Trace = 0.542; $F = 2.518$, $p = 0.001$ for all canonical axes; Fig. 4). The results showed that the metal Mg was the largest contributor to the variation of zooplankton communities among environmental variables, followed by T, EC, and Cu. The strong influence of Mg was also detected in the results of sample ordination in RDA, as the communities collected from the 3 regions were grouped into 3 clusters, corresponding to the gradient of Mg among regions. In addition, the gradients of EC, Cu, and T also separated communities among different regions.

For spatial variables, V1 and V4 were the largest contributors to the geographical variation of zooplankton communities. V1 and V4 modeled a broad spatial scale to form a trend along the environmental gradient, while V2 and V6 altogether modeled a fine spatial scale but the trend was not so obvious as with V1 and V4 (Fig. 4).

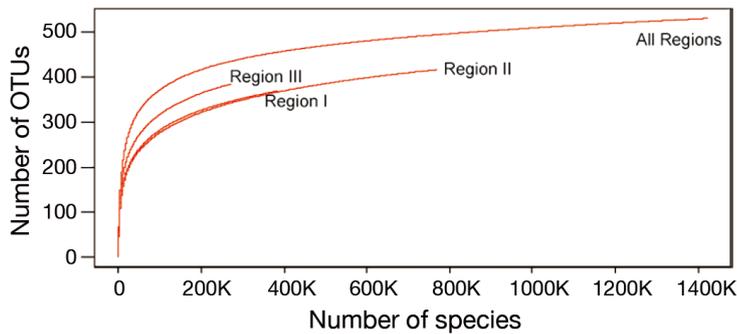


Fig. 3. Rarefaction curves of similarity-based operational taxonomic units (OTUs) for each region and all 3 regions

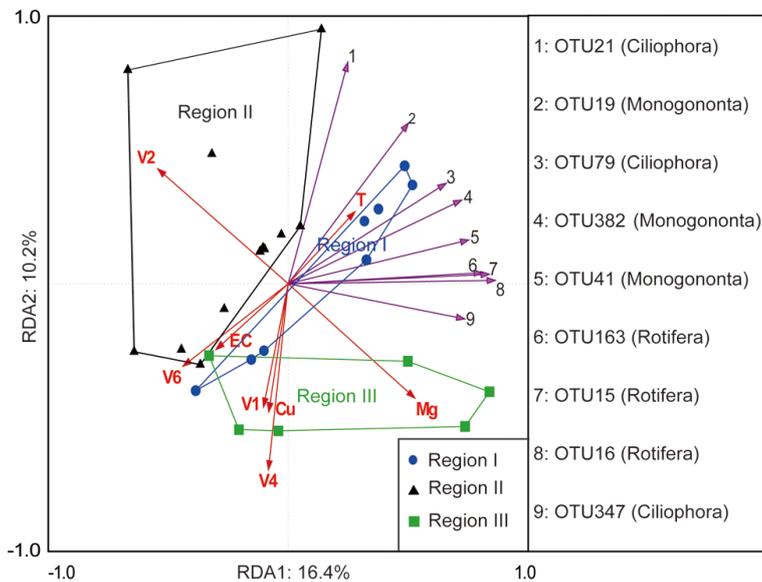


Fig. 4. Ordination plot based on redundancy analysis (RDA) of zooplankton communities. Plots are enclosed by a coloured line for each region. Scores of operational taxonomic units (OTUs) and environmental variables are scaled to fit the sample ordination. Species weakly associated with the first 2 axes (i.e. with fitness <55%) are omitted from the ordination for clarity. Arrows in purple and red represent OTUs and measured physicochemical variables, respectively. EC: electrical conductivity; T: water temperature; V1, V2, V4, and V6: spatial variables (i.e. PCNM variables)

Considering the relationship between taxonomic groups and environmental factors, we found that the explanatory variables had varied influence on different taxa. For example, Rotifera and Ciliophora were generally positively affected by Mg, while they negatively responded to the gradient of Cu (Fig. 4).

Determinative role of species sorting versus dispersal

To understand the relative role of species sorting versus dispersal in structuring zooplankton communities, 12 non-collinear environmental and spatial

variables were used for the variance partitioning analysis. All factors together explained 86.6% of the variation of zooplankton communities, and only 13.4% of variation could not be explained by the measured environmental or spatial variables. The environmental component could explain 60.1% ([E]: $p = 0.002$) of the total variation. After excluding the spatial effect, the environmental effect was still significant and explained the largest total variation ([E/S]: 43.3%). In contrast, spatial factors could explain far lower variation when compared to environmental factors ([S]: 43.3% versus [S/E]: 26.5%). When calculating the conjunct part, environmental and spatial factors could explain 16.8% ($[E \cap S]$) of the total variation.

DISCUSSION

Relative role of dispersal versus species sorting

Multiple analyses including variance partitioning and RDA clearly showed that species sorting prevailed to determine zooplankton community structure and geographical distribution at fine scales in Fuyang River, thus supporting the fine-scale species sorting hypothesis proposed by Xiong et al. (2017). In contrast, dispersal has often been considered to be more vital at fine geographical scales (Grönroos et al. 2013, Heino et al. 2015a), and studies have confirmed that local communities were homogenized to some degree by dispersal (Heino et al. 2015b, Datry et al. 2016). Nevertheless, Fuyang River, which has been highly disturbed by anthropogenic activities (Palmer & Yan 2013), demonstrated a strong environmental gradient along the river. The presence of a significant environmental gradient derived from local pollution in Fuyang River largely wiped out the signals left by dispersal (Fig. 2, Table S2), leading to varied community composition and geographical distribution largely determined by local environmental factors.

The presence of a significant environmental gradient is largely responsible for the different conclusions between our study and many other studies on less disturbed/polluted rivers. Similar to Xiong et al. (2017), we propose that the presence of a significant environmental gradient is indispensable to reject the dispersal

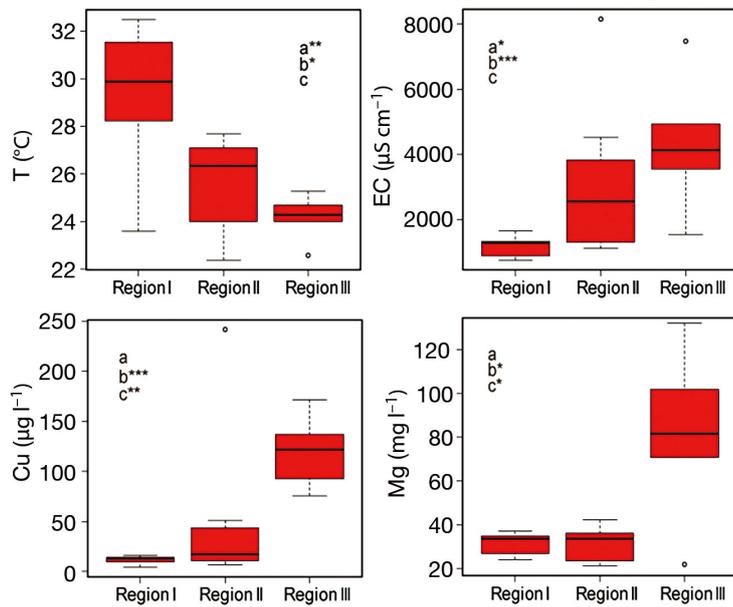


Fig. 5. Boxplots of 4 environmental variables retained by forward selection: water temperature (T), electrical conductivity (EC), Cu, and Mg. Boxes represent the interquartile range, central bars represent the median, whiskers represent the range of the data, and outliers are circles lying outside $1.5 \times$ the interquartile range. Mann-Whitney *U*-test: a: between Regions I and II, b: between Regions I and III, and c: between Regions II and III. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

hypothesis in running water ecosystems. Indeed, in natural river ecosystems with less disturbance by anthropogenic activities, high dispersal largely homogenizes communities along a river. Usually a significant environmental gradient cannot be easily observed at a fine geographical scale. The significant environmental gradient strengthened the process of species sorting in this study, accelerating the divergence of local communities along the Fuyang River at a fine geographical scale. Therefore, the fine-scale species sorting hypothesis should be considered as the null hypothesis when strong environmental gradients exist at both large and fine geographical scales.

Geographical distribution patterns of zooplankton communities

In this study, the dispersal hypothesis was rejected, mainly owing to strong species sorting caused by an environmental gradient within the 3 regions of the Fuyang River (Fig. 2, Table S2). Species sorting resulted in strong local community structure (Table S2) rather than a homogenized community composition determined by dispersal. However, the composition of communities within each region was more similar than that among regions, suggesting that dispersal is

still functional when the environmental gradient is not strong enough to override the influence of dispersal (Table 1, Fig. 2). Collectively, although species sorting is more competitive than dispersal in determining the geographical distribution patterns of zooplankton communities when environmental gradients exist, we cannot ignore the effect of uniformity caused by dispersal, particularly when environmental gradients are not strong enough at fine geographical scales.

Various local human activities determine the observed environmental gradient. Fuyang River receives industrial and agricultural wastewater and domestic sewage mainly from the 5 cities it flows through, and such intensive human activities are largely responsible for the exacerbation of its water quality (Tang et al. 2016). In terms of non-point source pollution, the agricultural areas along Fuyang River have been greatly transformed by intensified agricultural cultivation such as the use of a large amount of chemical fertilizers and pesticides, yielding a high concentration of P and N (Table 1). Further, under the circumstances of a relatively low level of precipitation and overuse of water resources, point source of pollution has largely affected the water quality along Fuyang River. Indeed, various toxic materials (e.g. Cd, Cu, Zn, Pb) have been released into Fuyang River from local industries such as mining and smelting (Audry et al. 2004, Tang et al. 2016). The geographical distribution of such industries along the river leads to an environmental gradient (Fig. 2), and the resulting gradient largely determines the zooplankton community composition and geographical distribution. Therefore, in order to reduce threats to the environment and health of river ecosystems, it is crucial to find, trace, and ecologically manage possible sources of pollutants and further reduce their potential influence in local river ecosystems.

Contribution of environmental variables to community structure

Local physicochemical variables and biotic constraints have been documented to affect zooplankton communities (Ritterhoff & Zauke 1997, Wang et al. 2010, Datry et al. 2016, Yang et al. 2018). A large number of studies have addressed the toxicity of metals in aquatic systems (Rainbow 2002, Förstner &

Wittmann 2012); however, mechanisms at the community level have not been well studied. In the present study, the results of parsimonious modeling showed that metal pollution was a major driver in discriminating the divergence of community composition in a running water ecosystem. At a low metal concentration, many zooplankton species can accumulate and metabolize many kinds of metals, which makes zooplankton a good model for biomonitoring of water quality (Attayde & Bozelli 1998, Budka et al. 2010, Battuello et al. 2016). However, at a high concentration, the metals may be transformed into hyper-toxic materials, causing an acute decrease of biodiversity in zooplankton communities (Baudouin & Scoppa 1974, Förstner & Wittmann 2012). Similarly, the varied concentration of metals along the environmental gradient in this study largely contributed to the variation of zooplankton communities (Figs. 4 & 5), and the high concentration of metals has had negative effects on biodiversity (Fig. 4).

In this study, we identified the environmental gradient formed by metal pollution, particularly Cu and Mg, as being largely responsible for the observed patterns in zooplankton communities. Toxic effects of Cu on aquatic biota have been abundantly documented in the literature (e.g. Zhou et al. 2008); however, reports of toxic effects of Mg are rare. As Mg is an essential element involved in multiple biological processes of metabolism, it may play an important role in food webs. For the environmental gradient formed by Mg, it is possible that Mg may be released with other pollutants derived from mining and smelting industries along the river. Thus, biological communities may be influenced by Mg alone or by synergistic interactions with other pollutants. As there may exist a large number of unknown pollutants, further investigations are needed to clarify the role of Mg in affecting biological communities in polluted water bodies.

Results obtained in this study imply that in addition to significant environmental factors, other environmental variables also had impacts on community composition. Based on national quality standards for surface waters (China GB3838-2002), the minimum metal concentration for Zn, As, Cd, and Pb in each sampling site (Table 1) exceeded the Class V standard, which is considered to be severe water pollution (Huang et al. 2010). Despite that significant environmental variables had a significant contribution in explaining the variation of community structure (Fig. 4), the strong selection cannot be ignored by non-significant environmental variables in the Fuyang River. For example, excess Cd is an environmental stressor with negative consequences for

human health and maintenance of biodiversity in affected ecosystems and can be bioaccumulated in organisms and magnified in the food chain, further threatening the health of both zooplankton and human beings (Zhou et al. 2008).

As rivers can be affected by various human activities, it is essential to identify key environmental factors that already or potentially have negative impacts on aquatic communities. In the Chaobai River, Xiong et al. (2017) demonstrated distinct patterns of zooplankton communities along the environmental gradient, and chemical pollutant-related factors such as total P and chl *a* were identified as the major drivers for the observed patterns. The present study verifies that metal pollution largely determined zooplankton community structure at fine geographical scales in Fuyang River. Consequently, multiple interdisciplinary investigations, including biomonitoring studies, such as pollutant bioaccumulation and biochemical alterations, and ecological studies, such as species, interactions and biological responses to pollution, should be conducted to understand the causes and consequences of environmental pollution, as well as to assist in planning comprehensive ecological remediation for polluted rivers.

CONCLUSIONS

In this study, we found that the geographical distribution of zooplankton community structure was mainly controlled by a high level of local selection pressure formed by anthropogenic activity-mediated pollutant release. The environmental gradient formed by metal pollution, particularly Cu and Mg, was largely responsible for the observed patterns in zooplankton communities at fine geographical scales. Multiple layers of evidence obtained in this study support the fine-scale species sorting hypothesis in determining the community structure of zooplankton, and we also confirm that the prerequisite for the fine-scale species sorting hypothesis is the presence of a significant environmental gradient. When integrating both environmental factors and community information, it is crucial to understand anthropogenic activity-mediated environmental changes and to identify the key environmental factors responsible for such changes. Understanding these interactions will help us to develop biomonitoring programs for early warning of potentially negative effects and to establish ecological bioremediation plans to restore water quality and biological integrity of communities in highly polluted rivers.

Acknowledgements. This work was supported by the National Natural Science Foundation of China (grant no. 31572228), the Innovation in Cross-functional Team Program of the Chinese Academy of Sciences (grant no. 2015), Chinese Academy of Science (grant no. ZDRW-ZS-2016-5-6), the State Key Joint Laboratory of Environment Simulation and Pollution Control (RCEES, Chinese Academy of Sciences; grant no. 15K01ESPCR), and the Water Pollution Control and Treatment Special Project (grant no. 2015ZX 07201-008-09) to A.Z.

LITERATURE CITED

- Attayde JL, Bozelli RL (1998) Assessing the indicator properties of zooplankton assemblages to disturbance gradients by canonical correspondence analysis. *Can J Fish Aquat Sci* 55:1789–1797
- Audry S, Schafer J, Blanc G, Jouanneau JM (2004) Fifty-year sedimentary record of heavy metal pollution (Cd, Zn, Cu, Pb) in the Lot River reservoirs (France). *Environ Pollut* 132:413–426
- Battuello M, Brizio P, Mussat Sartor R, Nurra N, Pessani D, Abete MC, Squadrone S (2016) Zooplankton from a North Western Mediterranean area as a model of metal transfer in a marine environment. *Ecol Indic* 66:440–451
- Baudouin MF, Scoppa P (1974) Acute toxicity of various metals to freshwater zooplankton. *Bull Environ Contam Toxicol* 12:745–751
- Blanchet FG, Legendre P, Borcard D (2008) Forward selection of explanatory variables. *Ecology* 89:2623–2632
- Blanchet FG, Legendre P, Maranger R, Monti D, Pepin P (2011) Modelling the effect of directional spatial ecological processes at different scales. *Oecologia* 166:357–368
- Borcard D, Legendre P (2002) All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecol Modell* 153:51–68
- Borcard D, Legendre P, Drapeau P (1992) Partialling out the spatial component of ecological variation. *Ecology* 73:1045–1055
- Budka M, Gabrys B, Ravagnan E (2010) Robust predictive modelling of water pollution using biomarker data. *Water Res* 44:3294–3308
- Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. *Aust J Ecol* 18:117–143
- Clarke KR, Gorley RN (2001) PRIMER v5: user manual/tutorial. PRIMER-E, Plymouth
- Clarke KR, Warwick RM (1994) Similarity-based testing for community pattern: the two-way layout with no replication. *Mar Biol* 118:167–176
- Cottenie K (2005) Integrating environmental and spatial processes in ecological community dynamics. *Ecol Lett* 8:1175–1182
- Datry T, Melo AS, Moya N, Zubieta J, De la Barra E, Oberdorff T (2016) Metacommunity patterns across three Neotropical catchments with varying environmental harshness. *Freshw Biol* 61:277–292
- Dray S, Legendre P, Peres-Neto PR (2006) Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). *Ecol Modell* 196:483–493
- Duggan IC, Green JD, Shiel RJ (2002) Distribution of rotifer assemblages in North Island, New Zealand, lakes: relationships to environmental and historical factors. *Freshw Biol* 47:195–206
- Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 10:996–998
- Förstner U, Wittmann GT (2012) Metal pollution in the aquatic environment. Springer Science & Business Media, Berlin
- Gagneten AM, Paggi JC (2009) Effects of heavy metal contamination (Cr, Cu, Pb, Cd) and eutrophication on zooplankton in the lower basin of the Salado River (Argentina). *Water Air Soil Pollut* 198:317–334
- Gauch HG (2003) Scientific method in practice. Cambridge University Press, Cambridge
- Grönroos M, Heino J, Siqueira T, Landeiro VL, Kotanen J, Bini LM (2013) Metacommunity structuring in stream networks: roles of dispersal mode, distance type, and regional environmental context. *Ecol Evol* 3:4473–4487
- Heino J, Grönroos M, Soininen J, Virtanen R, Muotka T (2012) Context dependency and metacommunity structuring in boreal headwater streams. *Oikos* 121:537–544
- Heino J, Melo AS, Siqueira T, Soininen J, Valanko S, Bini LM (2015a) Metacommunity organisation, spatial extent and dispersal in aquatic systems: patterns, processes and prospects. *Freshw Biol* 60:845–869
- Heino J, Soininen J, Alahuhta J, Lappalainen J, Virtanen R (2015b) A comparative analysis of metacommunity types in the freshwater realm. *Ecol Evol* 5:1525–1537
- Huang F, Wang X, Lou L, Zhou Z, Wu J (2010) Spatial variation and source apportionment of water pollution in Qiantang River (China) using statistical techniques. *Water Res* 44:1562–1572
- Hubbell SP (2001) The unified neutral theory of biodiversity and biogeography (MPB-32). Princeton University Press, Princeton, NJ
- Landeiro VL, Bini LM, Melo AS, Pes AMO, Magnusson WE (2012) The roles of dispersal limitation and environmental conditions in controlling caddisfly (Trichoptera) assemblages. *Freshw Biol* 57:1554–1564
- Legendre P, Oksanen J, ter Braak CJF (2011) Testing the significance of canonical axes in redundancy analysis. *Methods Ecol Evol* 2:269–277
- Leibold MA, Holyoak M, Mouquet N, Amarasekare P and others (2004) The metacommunity concept: a framework for multi-scale community ecology. *Ecol Lett* 7:601–613
- Lepš J, Šmilauer P (2003) Multivariate analysis of ecological data using CANOCO. Cambridge University Press, Cambridge
- Liu J, Soininen J, Han BP, Declerck SAJ, Whittaker R (2013) Effects of connectivity, dispersal directionality and functional traits on the metacommunity structure of river benthic diatoms. *J Biogeogr* 40:2238–2248
- Majaneva M, Hyytiäinen K, Varvio SL, Nagai S, Blomster J (2015) Bioinformatic amplicon read processing strategies strongly affect eukaryotic diversity and the taxonomic composition of communities. *PLOS ONE* 10:e0130035
- McArdle BH, Anderson MJ (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* 82:290–297
- Oksanen J, Blanchet FG, Kindt R, Legendre P and others (2013) Package ‘vegan’ Community ecology package, version 2(9). <https://cran.r-project.org/package=vegan>
- Palmer ME, Yan ND (2013) Decadal-scale regional changes in Canadian freshwater zooplankton: the likely consequence of complex interactions among multiple anthropogenic stressors. *Freshw Biol* 58:1366–1378
- Parameswaran P, Jalili R, Tao L, Shokralla S, Gharizadeh B, Ronaghi M, Fire AZ (2007) A pyrosequencing-tailored

- nucleotide barcode design unveils opportunities for large-scale sample multiplexing. *Nucleic Acids Res* 35: e130
- ✦ Rainbow PS (2002) Trace metal concentrations in aquatic invertebrates: why and so what? *Environ Pollut* 120: 497–507
- R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. www.r-project.org
- ✦ Ritterhoff J, Zauke GP (1997) Trace metals in field samples of zooplankton from the Fram Strait and the Greenland Sea. *Sci Total Environ* 199:255–270
- ✦ Sala OE, Chapin FS, Armesto JJ, Berlow E and others (2000) Global biodiversity scenarios for the year 2100. *Science* 287:1770–1774
- ✦ Sandin L, Solimini AG (2009) Freshwater ecosystem structure-function relationships: from theory to application. *Freshw Biol* 54:2017–2024
- ✦ Tang W, Duan S, Shan B, Zhang H, Zhang W, Zhao Y, Zhang C (2016) Concentrations, diffusive fluxes and toxicity of heavy metals in pore water of the Fuyang River, Haihe Basin. *Ecotoxicol Environ Saf* 127:80–86
- ✦ Větrovský T, Baldrian P (2013) Analysis of soil fungal communities by amplicon pyrosequencing: current approaches to data analysis and the introduction of the pipeline SEED. *Biol Fertil Soils* 49:1027–1037
- ✦ Wang S, Xie P, Geng H (2010) The relative importance of physicochemical factors and crustacean zooplankton as determinants of rotifer density and species distribution in lakes adjacent to the Yangtze River, China. *Limnologica* 40:1–7
- ✦ Winegardner AK, Jones BK, Ng IS, Siqueira T, Cottenie K (2012) The terminology of metacommunity ecology. *Trends Ecol Evol* 27:253–254
- ✦ Woodward G (2009) Biodiversity, ecosystem functioning and food webs in fresh waters: assembling the jigsaw puzzle. *Freshw Biol* 54:2171–2187
- ✦ Woodward G, Gessner MO, Giller PS, Gulis V and others (2012) Continental-scale effects of nutrient pollution on stream ecosystem functioning. *Science* 336:1438–1440
- ✦ Xiong W, Li J, Chen Y, Shan B, Wang W, Zhan A (2016a) Determinants of community structure of zooplankton in heavily polluted river ecosystems. *Sci Rep* 6:22043
- ✦ Xiong W, Li H, Zhan A (2016b) Early detection of invasive species in marine ecosystems using high-throughput sequencing: technical challenges and possible solutions. *Mar Biol* 163:139
- ✦ Xiong W, Ni P, Chen Y, Gao Y, Shan B, Zhan A (2017) Zooplankton community structure along a pollution gradient at fine geographical scales in river ecosystems: the importance of species sorting over dispersal. *Mol Ecol* 26:4351–4360
- ✦ Xiong W, Yang Y, Zhan A (2018) Reliability of simplifying strategies for rapid biodiversity assessment in studying community-environment interactions. *Ecol Indic* 85: 861–868
- ✦ Yang T, Liu J, Chen Q, Zhang J, Yang Y (2013) Estimation of environmental flow requirements for the river ecosystem in the Haihe River Basin, China. *Water Sci Technol* 67: 699–707
- ✦ Yang Y, Ni P, Gao Y, Xiong W, Zhao Y, Zhan A (2018) Geographical distribution of zooplankton biodiversity in highly polluted running water ecosystems: validation of fine-scale species sorting hypothesis. *Ecol Evol*, doi: 10.1002/ece3.4037
- ✦ Zhan A, Hulák M, Sylvester F, Huang X and others (2013) High sensitivity of 454 pyrosequencing for detection of rare species in aquatic communities. *Methods Ecol Evol* 4:558–565
- ✦ Zhan A, Bailey SA, Heath DD, Macisaac HJ (2014a) Performance comparison of genetic markers for high-throughput sequencing-based biodiversity assessment in complex communities. *Mol Ecol Resour* 14:1049–1059
- ✦ Zhan A, He S, Brown EA, Chain FJJ and others (2014b) Reproducibility of pyrosequencing data for biodiversity assessment in complex communities. *Methods Ecol Evol* 5:881–890
- ✦ Zhou Q, Zhang J, Fu J, Shi J, Jiang G (2008) Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. *Anal Chim Acta* 606:135–150

*Editorial responsibility: J. Rudi Strickler,
Milwaukee, Wisconsin, USA*

*Submitted: November 6, 2017; Accepted: March 16, 2018
Proofs received from author(s): April 24, 2018*