

# RELATIONSHIPS BETWEEN MACROINVERTEBRATE COMMUNITIES AND ENVIRONMENTAL PARAMETERS OF THE HU-LAN ESTUARY NATURAL WETLAND RESERVE AND ITS SURROUNDING WATERS, NORTHEAST CHINA

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**Abstract.** This study analyzed the relationships between environmental parameters and benthic macroinvertebrate faunas. Invertebrate richness, Shannon-Wiener diversity, functional-group and CCA were used to evaluate conditions of waterbodies of the Hu-lan Estuary Natural Wetland Reserve and its hinterlands. We measured 10 physicochemical variables and collected 165 species belonging to 6 functional feeding groups (FFGs) during spring, summer and autumn. Some of the physicochemical factors changed with seasons. Nitrate nitrogen, total nitrogen and total phosphate significantly varied with seasons ( $p < 0.05$ ), unlike water temperature, depth, pH, dissolved oxygen, chemical oxygen demand, Ammonia nitrogen and electronic conductivity ( $p > 0.05$ ). The taxa richness of benthic macroinvertebrates did not vary significantly within the sites ( $p = 0.508$ ); while the Shannon-Wiener diversity showed significantly difference ( $p < 0.01$ ) within the sites over these three seasons. Gathering-collectors were the most abundant macroinvertebrate FFG assemblages all over the sites, though there's no significant difference in the total abundance of 6 functional feeding groups identified among the sites during spring, summer and autumn ( $p = 0.677$ ,  $p = 0.681$ ,  $p = 0.302$ , respectively). Our CCA results indicated positive correlations of macroinvertebrate community compositions with environmental variables in the Hu-lan Estuary Natural Wetland Reserve and its surrounding waters.

**Keywords:** taxa richness, Shannon-Wiener diversity, functional feeding groups, water parameters, canonical correspondence analysis

## Introduction

Wetlands suffer from anthropogenic pressure and therefore are altered with respect to their physicochemical composition and species diversity (Rivera-Usme et al., 2015). They are essential for the maintenance of biodiversity that depends on the wetlands for their water supply. These aquatic systems are fragile because of high population densities subsequent to accelerated urban development that seriously threatens most of its wetlands (Rivera-Usme et al., 2015). Anthropogenic activities have severely affected the condition of wetlands worldwide (Wilkins et al., 2015; Tan and Beh, 2016). Physical alteration, habitat loss, water withdrawal, pollution (Cao et al., 1997; Morse et al., 2007), overexploitation and the introduction of exotic species all contribute to the decline in freshwater species and water quality as well (Tan and Beh, 2016). The stream

community structure is a result of both long-term environmental factors and short-term critical conditions of short duration (Morse et al., 2007); and an experienced stream biologist with good knowledge of normal and stressed stream community structures often can evaluate the water quality of a stream with considerable accuracy just after a few minutes' examination of its fauna (Hilsenhoff, 1977).

Benthic macroinvertebrates are an important component of the aquatic ecosystem and reflect a multitude of physical, chemical, and biological stream features allowing them to be excellent indicators of stream health (Hilsenhoff, 1977; Allan, 2004); i.e, the impacts of stressors such as organic pollution, toxins, and physical habitat alterations or foodweb links at higher trophic levels such as fish and birds (Cummins, 1992). Among members of the aquatic environments, they are probably best suited because they are numerous in almost every stream, and they are readily collected and identified, and they are not very mobile, and generally have life cycles of a year or more (Hilsenhoff, 1977). Monitoring wetland ecosystems using aquatic macroinvertebrates has been an effective tool for documenting changes in community health (Lenat, 1987; Plafkin et al., 1989). Some macroinvertebrate indices of stream health have been identified as most useful and efficacious. These include the EPT taxa richness-Ephemeroptera, Plecoptera, Trichoptera- (Lenat and Penrose, 1996), and total benthic macroinvertebrate taxa richness (Plafkin et al., 1989). The effective use of EPT or taxa richness measures for monitoring water quality in China requires richness rating systems to be developed by biologists (Lenat, 1994). However, physicochemical monitoring tools are usually expensive and can only be used at limited site number and they are, thus, unable to achieve computation of distribution patterns (Swaminathan, 2003). Hence, biological monitoring is considered one of the alternatives which is a useful as a rapid assessment instrument to check up the status of water quality (Tan and Beh, 2016). Changes in benthic macroinvertebrate fauna with water pollution (Morse et al., 2007), have been documented and measured in several instances using various aspects including biomass, density and composition (Cao, 1997).

Benthic macroinvertebrate functional feeding-group analyses were developed initially for streams (Cummins, 1973, 1974) as key components of the river continuum concept (Vannote et al., 1980). Various ratios of the functional groups have been successfully used as surrogates for ecosystem attributes to assess the ecological condition of flowing water environments (Masese et al., 2014; Fu et al., 2016; Shabani et al., 2019). The evaluation is based on easily observed morphological and behavioral attributes associated with feeding (functional-feeding groups, FFGs) and modes of attachment, concealment, and locomotion (functional-habit groups, FHGs), together with life history patterns and drift propensity (Merritt et al., 2002).

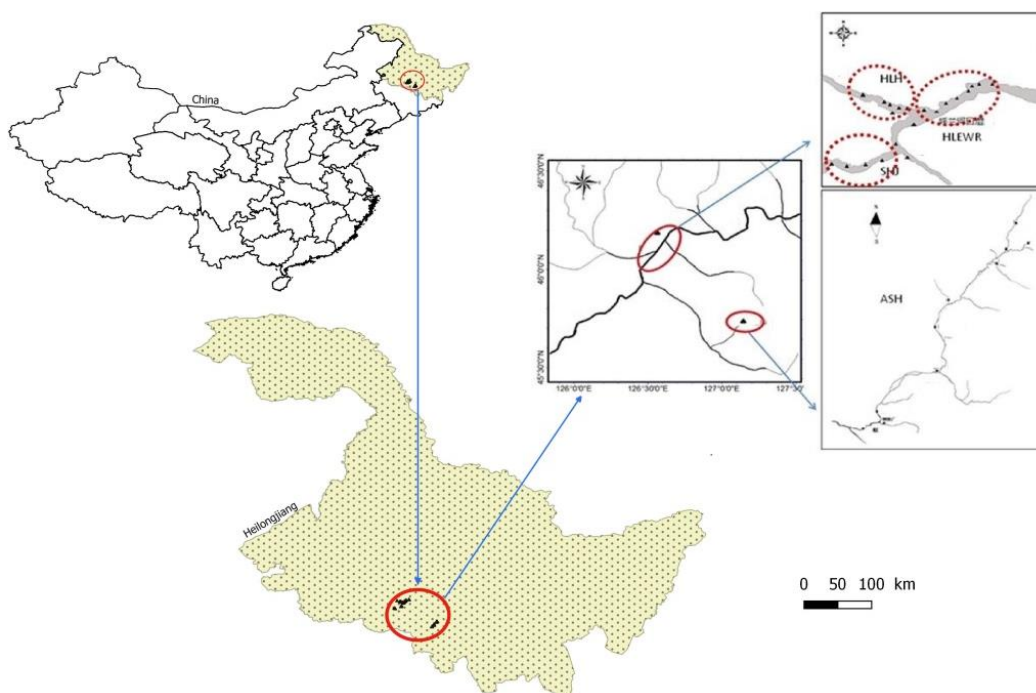
In this paper, we determined the functional group composition of benthic macroinvertebrate fauna and modeled relationships between benthic macroinvertebrate metrics and environmental factors in the Hu-lan Estuary Natural Wetland Reserve and its surrounding waters to evaluate the effects of environmental characteristics on benthic macroinvertebrate assemblages.

## Materials and methods

### *Study area*

The Hu-lan Estuary Natural Wetland Reserve is located in the southern part of Hu lan district, Harbin city in Heilongjiang Province, Northeast China (*Fig. 1*); and extends

along of the north bank of Songhua River from East to West band. The Hu-lan River enters the estuary of Songhua River and reaches the Dadingshan dam. The natural reserve extends between  $45^{\circ}53'44''$  and  $46^{\circ}54'04''$  N latitude and between  $126^{\circ}41'00''$  and  $127^{\circ}15'00''$  E longitude. The length of the reserve from East to West is 63.5 km, with a width of 21.3 km from North to South (Liu, 2012). It covers around  $192.62 \text{ km}^2$ . The annual temperature average is  $3.3^{\circ}\text{C}$ , with mean annual precipitations of 500.4 mm (Liu, 2012). The Hu lan Estuary Natural Wetland Reserve was created in 2008 and mainly aims to protect wetland ecosystems and endangered waterfowls. The wetland is rich in biological resources with more than 465 species of plants and 348 species of vertebrates, including fishes, amphibians, reptiles, birds and mammals (Liu, 2012). According to the Classification Criteria of Nature Reserve Types and Grades of the People's Republic of China (GB/T15629-93), the Hu-lan Estuary Natural Wetland Nature Reserve belongs to the inland wetland type and natural ecosystem category. Ashihe River is an important tributary of Harbin section of Songhua River in Hu-lan Estuary. The upper reaches of Ashihe River are alpine forest streams and the middle reaches of the river pass through a large area of farmland to reach the Harbin city and join the Songhua River (Liu, 2012).



**Figure 1.** Map of study site with the sampling locations in China

### ***Sampling of physical and chemical parameters***

Four sampling locations were selected, including the Songhua River along Harbin (SHJ:  $N45^{\circ}45'29.2''$  and  $E126^{\circ}45'10.4''$ , sites 1-3, 13-14), the Ashihe River upstream (ASH:  $N45^{\circ}16'38.7''$  and  $E127^{\circ}39'23.1''$ , sites 4-12), the Hu-lan river downstream (HLE:  $N45^{\circ}55'33.1''$  and  $E126^{\circ}46'35.8''$ , sites 15-22) and the Hu-lan estuary wetland reserve (HLEWR:  $N45^{\circ}55'31.3''$  and  $E126^{\circ}55'53.3''$ , sites 23-30). Physicochemical water conditions were determined in spring, summer and autumn in 2009. Water temperature

(WT, °C), pH, electronic conductivity (EC, mS/m) and dissolved oxygen (DO, mg/l) were measured in situ with a multiparameter probe. Water depth (m) was measured using Secchi disk and longline method when biological samples were collected. 500 ml of water were sampled at each site per season and put in labelled plastic containers, and then taken to the Laboratory of Hydrobiology, where nitrate nitrogen ( $\text{NO}_3^-$ -N, mg/l), Ammonia nitrogen ( $\text{NH}_3$ -N, mg/l), total phosphate (TP, mg/l), total nitrogen (TN, mg/l) and chemical oxygen demand with permanganate index ( $\text{COD}_{\text{Mn}}$ , mg/l) were determined following the Chinese national water quality standards method GB3838-2002.

### ***Benthic macroinvertebrate sampling***

Several qualitative and quantitative sampling tools are designed to sample benthic macroinvertebrates (Barbour et al., 1999; Merritt et al., 2008). In this study, we used qualitative (D-frame net and handle net) and quantitative (Surber sampler: 0.09 m<sup>2</sup> and Petersen grab sampler: 1/16 m<sup>2</sup>) sampling tools to collect the benthic macroinvertebrates from each of the four sampling locations. We used 500- $\mu\text{m}$  mesh D-net and handle-net type devices at each site, the nets were dragged against the water flow over an area of 1 m<sup>2</sup> while large substrates were gently rubbed or kicked for 3-5 minutes with hands or feet, respectively, to dislodge free-living and burrowing macroinvertebrates from benthic substrates. Surber type sampling devices of 30 cm wide  $\times$  30 cm long with 0.09 m<sup>2</sup> catching area and a net of 500  $\mu\text{m}$  in mesh size were placed along the river substrate to catch any organisms dislodged initially and carried downstream at low velocities; and the animals were subsequently swept by the water and caught in the net. We also used the Petersen sampler in wetland sites to collect macroscopic macroinvertebrates in sand, gravel, marl, or clay.

All samples were transferred into labelled plastic bags and preserved in 95% ethanol. Taxonomic determinations were conducted in the Laboratory of Hydrobiology using identification keys of Thorp and Covich (1991), Morse et al. (1994), Merritt et al. (1996), Tong (1996), and Dudgeon (1999). Each taxon was assigned to a general functional group according to their trophic specializations, as categorized by Cummins and Klug (1979), Morse et al. (1994), Vannote et al. (1980), Merritt et al. (1996), and Barbour et al. (1999). The FFGs identified were scrapers (SC), that are herbivores and feed on periphyton; shredders (SH), that feed on coarse particulate organic matter (CPOM); gatherers/collectors (GC), that consume fine particulate organic matter (FPOM); filterers/collector (FC), which filter FPOM in the water column; omnivores (OM), or those which simply do not fit neatly into the other categories; and predators (PR) that feed on other living organisms (Rivera-Usme et al., 2015).

### ***Data analysis***

The benthic macroinvertebrate taxa richness and Shannon-Wiener diversity were computed for each site and sampling season using PAST software (Hammer et al., 2001; Hammer and Ryan, 2008). Differences in benthic macroinvertebrate taxa richness and Shannon-Wiener diversity among sampling sites during spring, summer and autumn were tested using the ANOVA or Kruskal-Wallis tests in R software, and tests were considered significant at the  $p < 0.05$  level. Canonical correspondence analysis was performed using CANOCO 4.5 software and Pearson correlation test (SPSS, version 16.0) to model on the correlation between benthic macroinvertebrate metrics and environmental factors. For data which fitted to the normal distribution, one-way

ANOVA and Tukey's honestly significant difference (HSD) tests, followed by Levene's test of homogeneity of variance were performed for multiple comparisons. On the other hand, data which did not fit to the normal distribution were submitted to the non-parametric Kruskal-Wallis test to evaluate the relationships between water quality variables and macroinvertebrate community metrics among sites over three seasons.

## Results and discussion

### *Environmental variables*

Table 1 shows the mean values of ten water parameters measured in the Hu-lan estuary wetland sites and its surrounding waters. Some of the physicochemical factors changed among seasons. Nitrate nitrogen ( $\text{NO}_3^-$ -N), total nitrogen (TN) and total phosphate (TP) significantly varied with seasons (Kruskal-Wallis test,  $p < 0.05$ ); unlike water temperature (WT), depth, pH, dissolved oxygen (DO), chemical oxygen demand with permanganate index ( $\text{COD}_{\text{Mn}}$ ), Ammonia nitrogen ( $\text{NH}_3$ -N) and electronic conductivity (EC) which did not vary significantly (Kruskal-Wallis test,  $p > 0.05$ ). Shabani et al. (2019) also found  $\text{NO}_3^-$ -N, TN and TP concentrations significantly varied with seasons in the waterbodies of Sanjiang plain wetlands. Maloney and Feminella (2006) also recorded the highest mean value of water temperature in summer in the Southeastern plains ecoregion of central western Georgia, USA. Ortiz and Puig (2007) also found the highest mean value of water temperature in summer at the downstream reach in a Mediterranean stream. Shabani et al. (2019) also reported the highest mean value of water temperature in summer in the Sanjiang plain wetlands, obviously as the warmest season.

**Table 1.** Seasonal dynamics of environmental parameters (Mean±SE). WT = Water temperature, DO = dissolved oxygen,  $\text{COD}_{\text{Mn}}$  = chemical oxygen demand,  $\text{NH}_3$ -N = Ammonia nitrogen,  $\text{NO}_3^-$ -N = nitrate nitrogen, EC = electronic conductivity, TN = total nitrogen, TP = total phosphate

Environmental parameters	Spring	Summer	Autumn
WT (°C)	12.95±0.70 <sup>a</sup>	21.20±0.78 <sup>c</sup>	16.31±0.96 <sup>b</sup>
Depth (m)	6.24±1.02 <sup>a</sup>	6.93±1.09 <sup>a</sup>	5.05±0.85 <sup>a</sup>
pH	7.39±0.10 <sup>ab</sup>	7.31±0.06 <sup>a</sup>	7.62±0.08 <sup>b</sup>
DO (mg/l)	7.67±0.39 <sup>a</sup>	7.38±0.31 <sup>a</sup>	6.29±0.45 <sup>a</sup>
$\text{COD}_{\text{Mn}}$ (mg/l)	3.32±0.44 <sup>a</sup>	8.74±0.83 <sup>c</sup>	5.31±0.28 <sup>b</sup>
$\text{NH}_3$ -N (mg/l)	0.54±0.15 <sup>a</sup>	0.41±0.15 <sup>a</sup>	0.57±0.27 <sup>a</sup>
$\text{NO}_3^-$ -N (mg/l)	0.68±0.14 <sup>a</sup>	1.24±0.12 <sup>a</sup>	0.75±0.07 <sup>b</sup>
EC (mS/m)	15.73±1.67 <sup>a</sup>	17.12±1.95 <sup>a</sup>	15.39±1.94 <sup>a</sup>
TN (mg/l)	3.41±0.27 <sup>b</sup>	1.80±0.16 <sup>a</sup>	1.51±0.42 <sup>a</sup>
TP (mg/l)	0.11±0.03 <sup>a</sup>	0.05±0.002 <sup>a</sup>	0.30±0.06 <sup>b</sup>

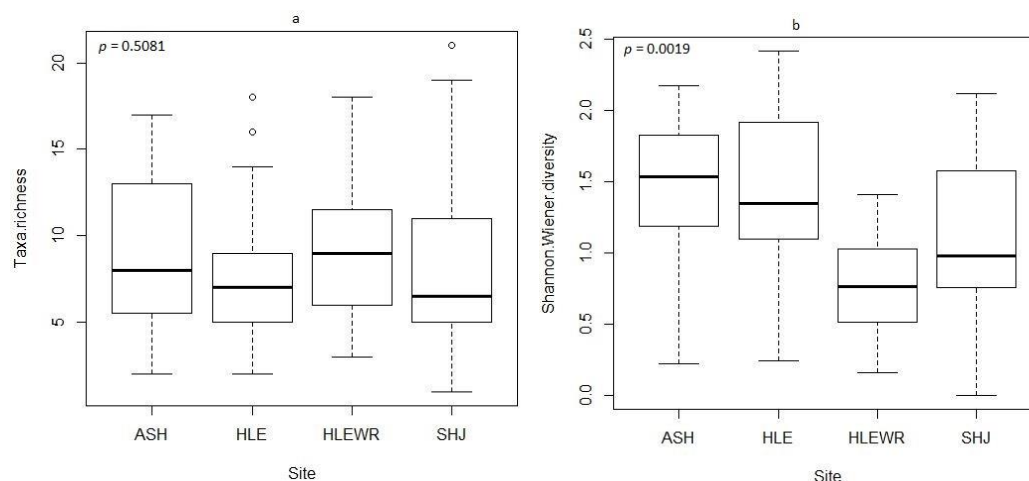
### *Benthic macroinvertebrate communities*

A total of 165 benthic macroinvertebrate species belonging to 16 orders were recorded during the study period (Appendix 1, Table 2). Insects were the most species-rich group, with 8 orders (Trichoptera, Ephemeroptera, Plecoptera, Diptera, Heteroptera, Odonata, Coleoptera and Neuroptera), and 41.8% of the species belonged to order of Dipterans (Table 2). *Limnodrilus hoffmeisteri* (Tubificida) was considerably abundant in the Hu-lan estuary wetland and the surrounding Waters. 78 species were

collected in the Hu-lan river downstream (HLE) followed by the Ashihe River upstream (ASH) with 71 species, the Songhua River along Harbin (SHJ) with 59 species and the Hu-lan estuary wetland reserves (HLEWR) with 52 species (*Appendix 1*). The benthic macroinvertebrate taxa richness of in these four regions did not vary significantly during the three seasons (ANOVA results,  $p = 0.5081$ ; *Fig. 2a*). While the Shannon-Wiener diversity showed high significant difference (Kruskal-Wallis test,  $p = 0.0019$ , *Fig. 2b*) within the sampling zones during these three seasons.

**Table 2.** Orders and number of families and species of benthic macroinvertebrates

Order	Family number	%	Species number	%
Arhynchobdellida	1	2.63	2	1.21
Coleoptera	1	2.63	2	1.21
Decapoda	1	2.63	2	1.21
Diptera	5	13.2	69	41.80
Ephemeroptera	6	15.80	13	7.88
Heteroptera	2	5.26	6	3.64
Heterostropha	1	2.63	2	1.21
Hygrophila	2	5.26	10	6.06
Neuroptera	1	2.63	1	0.61
Odonata	2	5.26	2	1.21
Plecoptera	3	7.89	6	3.64
Rhynchobdellida	1	2.63	3	1.82
Trichoptera	8	21.10	20	12.10
Tubificida	2	5.26	22	13.30
Unionoida	1	2.63	3	1.82
Veneroida	1	2.63	2	1.21
Total	38	100.00	165	100.00



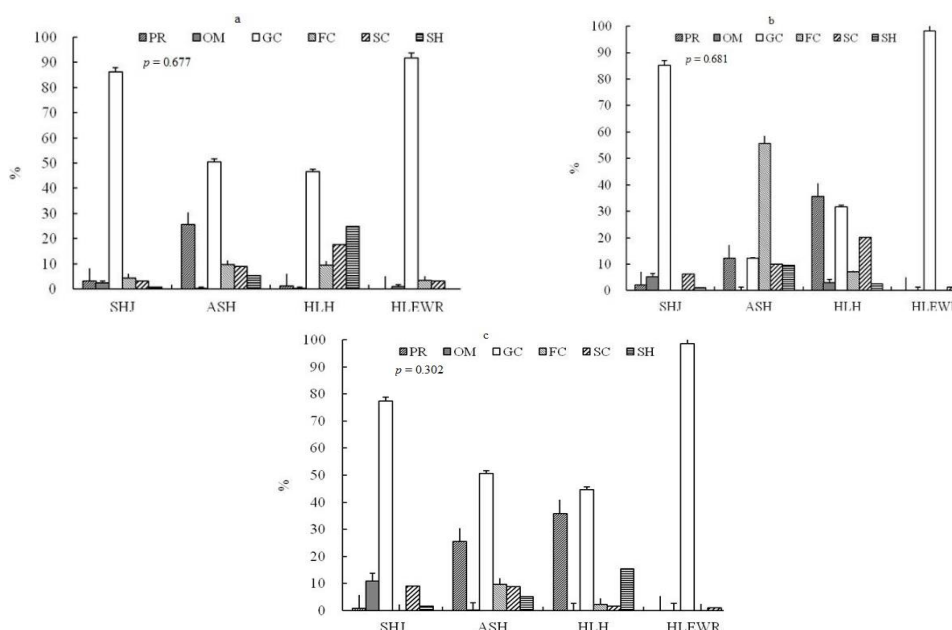
**Figure 2.** Comparison of mean values of macroinvertebrate taxa richness (a) and Shannon-Wiener diversity (b) at sampling sites. ASH = Ashihe River upstream, HLE = Hu-lan river downstream, HLEWR = Hu-lan estuary wetland reserve, SHJ = Songhua River along Harbin

Our results contracted with those of Šporka et al. (2006) in the stream of the Carpathian Mountains of central Europe; Wang et al. (2007) in Taihu lake watershed, Changzhou area; Zhao et al. (2012) in the bed sediment of the Yellow River; Pan et al.

(2013) in the Upper Yellow and Yangtze Rivers; Zhang et al. (2014) in streams and rivers of lake Taihu Basin; Pham (2017) in Saigon river and its tributaries, Vietnam; Rosser and Pearson (2018) in tropical streams; Shabani et al. (2019) in the wetlands of the Sanjiang plain, who reported that aquatic insects were the most species-rich group and occurred in almost all of the sites. Their dominance may depend on the availability of allochthonous organic detritus from riparian structure (Vannote et al., 1980).

### ***Benthic macroinvertebrate functional feeding groups***

Figure 3a,b,c report the percentage contributions of the various benthic macroinvertebrate FFGs at the sites during the three seasons. Gathering-collectors (GC) were the most dominant benthic macroinvertebrate FFG assemblages within all sites in spring and autumn. They also dominated the benthic communities in the HLEWR and SHJ in summer. Filtering-collectors (FC) and predators were the most abundant in the ASH and HLE, respectively in summer. It appeared that there's no significant difference in the total abundance of these six functional feeding groups among the sites during spring, summer and autumn (Kruskal-Wallis rank sum test,  $p = 0.677$ ,  $p = 0.681$ ,  $p = 0.302$ , respectively). Pan et al. (2013) also found that gathering-collectors were the predominant macroinvertebrate FFGs in the source region of the Yangtze River. Fogelman et al. (2018) also reported that the gathering-collectors were the most dominant in the macroinvertebrate collection from the Susquehanna River, USA.



**Figure 3.** Percentages of macroinvertebrate FFGs in spring (a), summer (b) and autumn (c), SH = shredders, SC = scrapers, FC = filtering-collectors, GC = gathering-collectors, PR = predators, SHJ = Songhua River along Harbin, ASH = Ashihe River upstream, HLE = Hu-lan river downstream, HLEWR = Hu-lan estuary wetland reserve

### ***Relationships between physicochemical variables and benthic macroinvertebrates***

Figure 4 shows the CCA ordinations during the three seasons. During the spring period, the first two axes respectively explained 18.90 and 36.60% of benthic

macroinvertebrate variances, with eigenvalues of 0.964 and 0.907. Biotic data as *Chironomus pallidivittatus*, *C. okinawanus*, *C. plumosus*, *Limnodrilus hoffmeisteri*, *Branchiura sowerbyi*, *Ephemera shengmi* and *Polypylis hemisphaerula* exhibited high positive relationships with water temperature ( $r = 0.93$ ) and  $\text{COD}_{\text{Mn}}$  ( $r = 0.94$ ) at sites 1, 2, 3, 21, 23, 25, 26, 27, 28, 29 and 30 on the first axis. CCA indicated that *Nipoptipula* spp., *Ameletus* spp., *Iron* spp., *Heptagenia* spp., *Ephemera sachalinensis*, *Serratella* spp., *Ephemerella fusongensis*, *Leptophlebia* spp., *Paraleptophlebia* spp., *Corixa substriata*, *Hesperocorixa distanti*, *Gyraulus convexiuculus*, *Galba truncatula*, *Galba pervia*, *Radix auricularia*, *Valvata piscinalis*, *Stenopsyche marmorata*, *Hydropsyche nakaharai*, *Hydropsyche* spp., *Polycentropus* spp. and *Alloperla sapporoensis* were positively correlated with TN and DO at sites 5, 7, 8, 10 and 11 on the second axis (Fig. 4a,b).

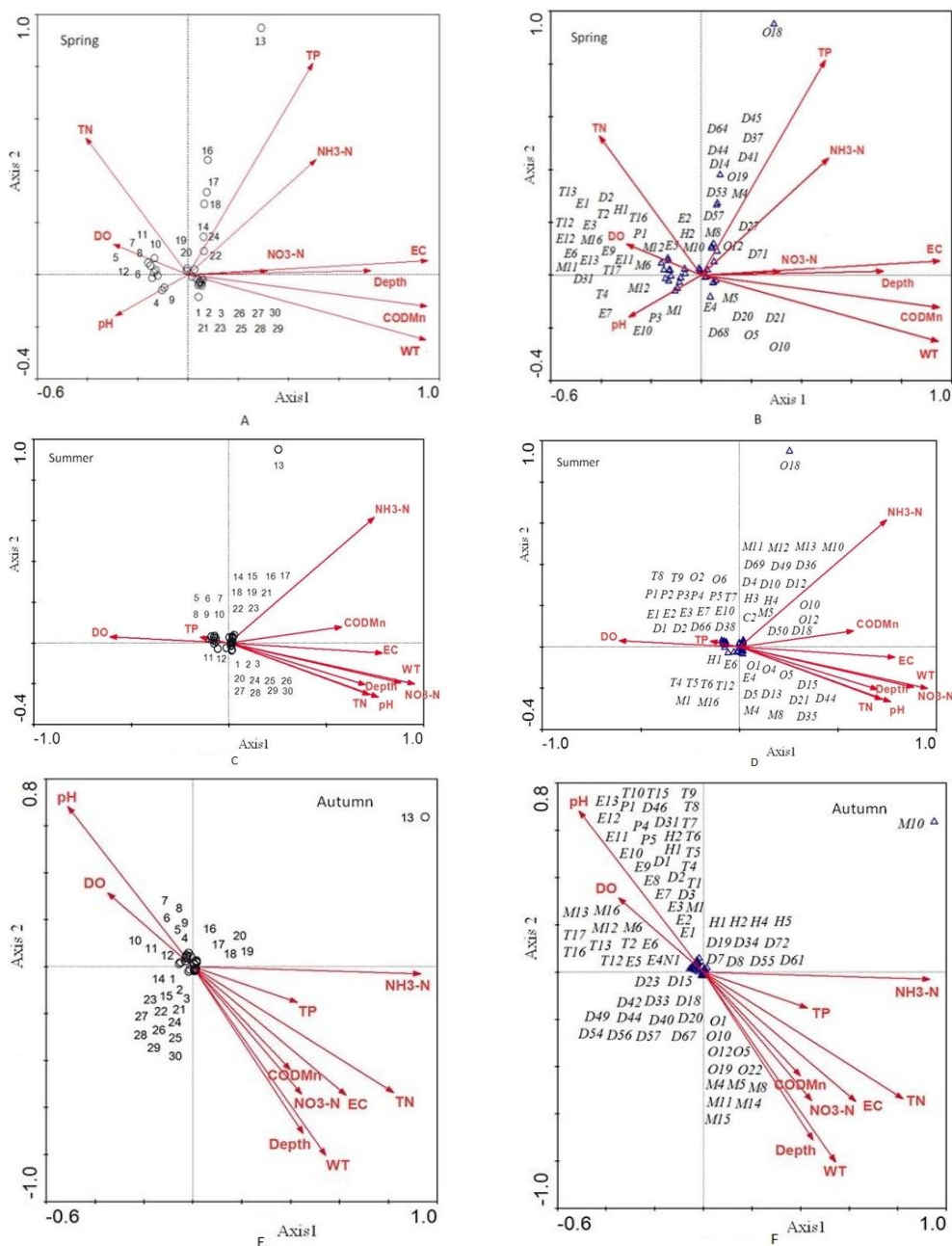
In summer season, the first two axes explained 55.40% of total variances, with eigenvalues of 0.915 and 0.958, respectively. There were positive significant correlations of *Tipulidae* spp., *Cryptochironomus defectus*, *Stictochironomus maculipennis*, *Chironomus okinawanus*, *Pentaneurella katterjokki*, *Glyptotendipes pallens*, *Ephemera shengmi*, *Unio douglasiae*, *Viviparus chui*, *Limnodrilus helveticus*, *Teneridrilus mastix*, *Limnodrilus hoffmeisteri* with electronic conductivity ( $r = 0.78$ ), depth ( $r = 0.69$ ), water temperature ( $r = 0.94$ ), TN ( $r = 0.71$ ),  $\text{NO}_3^-$ -N ( $r = 0.87$ ) and pH ( $r = 0.76$ ) at sites 1, 2, 3, 20, 24, 25, 26, 27, 28, 29, and 30 on the first axis. While *Tabanus* spp., *Nipoptipula* spp., *Cricotopus sylvestris*, *Eukiefferiella gracei*, *Ameletus* spp., *Iron* spp., *Heptagenia* spp., *Baetis* spp., *Ephemerella* spp., *Nais* spp., *Limnodrilus claparedeianus*, *Alloperla sapporoensis*, *Alloperla nikkoensis*, *Alloperla* spp., *Haploperla* spp., *Stenophylax ondakesis*, *Stenophylax koizumii* and *Astenophylax grammicus* were associated with DO and TP at sites 5, 6, 7, 8, 9 and 10 on the second axis (Fig. 4c,d).

During autumn, the first two CCA axes respectively explained 20.60 and 41.00% of total variances, with eigenvalues of 0.958 and 0.954. Axis 1 showed positive correlations of water temperature, TP, TN, depth,  $\text{NH}_3$ -N, electronic conductivity,  $\text{COD}_{\text{Mn}}$  and  $\text{NH}_3$ -N on *Unio douglasiae*, *Polypylis hemisphaerula*, *Viviparus chui*, *Galba pervia*, *Radix swinhoei*, *Cipangopaludina chinensis*, *Limnodrilus helveticus*, *Limnodrilus hoffmeisteri*, *Branchiura sowerbyi*, *Tubifex tubifex*, *Nais communis*, *Dero* spp. While the second axis revealed that pH and DO were the most important variables to impact the distribution of *Tabanus* spp., *Nipoptipula* spp., *Holorusia* spp., *Procladius choreus*, *Stictochironomus akizukii*, *Ameletus* spp., *Iron* spp., *Heptagenia* spp., *Baetis* spp., *Baetis* spp., *Ephemera sachalinensis*, *Serratella* spp., *Ephemerella* spp., *Ephemerella fusongensis*, *Leptophlebia* spp., *Paraleptophlebia* spp., *Ephemera nigroptera*, *Corixa substriata*, *Hesperocorixa distanti*, *Sphaerium lacustre*, *Gyraulus convexiuculus*, *Radix auricularia*, *Radix ovata*, *Valvata piscinalis*, *Protohermes grandis*, *Parastenopsyche* spp., *Stenopsyche marmorata*, *Goera ramosa*, *Goera japonica*, *Goera kyotonis*, *Stenophylax ondakesis*, *Stenophylax koizumii*, *Astenophylax grammicus*, *Glyptotendipes admorsus*, *Hydropsyche nakaharai*, *Hydropsyche* spp., *Oecetis morii*, *Ganonema* spp. and *Polycentropus* spp. at sites 4, 5, 6, 7, 8, 9, 10, 11 and 12 (Fig. 4e,f).

Understanding the relative importance of environmental conditions is critical for understanding how to preserve generate benefits to aquatic macroinvertebrate communities (Wilkins et al., 2015). Our modeling demonstrated that benthic macroinvertebrate species richness values were strongly associated with physical and



chemical conditions in the Hu-lan Estuary Natural Wetland Reserve and its surrounding waters. Environmental parameters as measured were very influential on macroinvertebrate diversity in freshwater habitats of this reserve. Moreover, several studies have demonstrated that differences in physical and chemical characteristics are detected anytime and with strong influence on aquatic community composition (Bertaso et al., 2015). Habitat quality was second important factor, implying that macroinvertebrate diversity also benefited from improved environmental conditions (Wilkins et al., 2015). Palmer et al. (1997), and Harper et al. (1998) argued that habitat heterogeneity can promote biotic recovery and biodiversity in aquatic environment and is often a goal of environment restoration.



**Figure 4.** CCA plots relating the benthic macroinvertebrates associated with physicochemical variables in spring (A, B), summer (C, D) and autumn (E, F)

In our study, we recorded pollution-sensitive taxa, including Ephemeroptera, Plecoptera, Trichoptera and Odonata (EPTO) and benthic communities characterized by pollution-tolerant taxa, such as Diptera, Heterostropha, Hygrophila and Tubificida. Luo et al. (2017) also collected sensitive species (e.g., EPT taxa) and less diverse communities with dominance of tolerant species (e.g., Tubificids, Chironomids, and Physids) in the Liangjiang New Area. Although some taxa (e.g. EPTO) are sensitive to aquatic environment conditions (Barbour et al., 1992), many species in these orders are of conservation interest (Wilkins et al., 2015).

## Conclusion

Our findings demonstrated the positive of benthic macroinvertebrate community compositions with environmental parameters. We found that Gathering-collectors were the most dominant benthic macroinvertebrate FFG assemblages, and recorded more sensitive and tolerant species in the Hu-lan Estuary Natural Wetland Reserve and its surrounding waters. In order to reach the conservation status assessment of species, we suggest that local faunas should be inventoried and described (many species remain unknown). Tolerance values for macroinvertebrates should be determined. More research teams using benthic macroinvertebrates to assess water quality should operate in this reserve. Wetland conservation efforts, such as planting aquatic vegetation in a watershed, can also help to improve water quality and restore natural flow regimes, and then may structure macroinvertebrate populations.

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## APPENDIX

**Appendix 1.** Benthic macroinvertebrate taxa list in the Hu-lan Estuary Natural Wetland Reserve and its surrounding waters. FFG= functional feeding groups, ASH = Ashihe River upstream, SHJ = Songhua River along Harbin, HLE = Hu-lan river downstream, HLEWR = Hu-lan estuary wetland reserve, SH = shredders, SC = scrapers, FC = filtering-collectors, GC = gathering-collectors, PR = predators

Taxon	Code	FFG	ASH	HLE	HLEWR	SHJ
<b>ARHYNCHOBEDELLIDA</b>						
<i>Whitmania</i> spp.	H3	PR	1			1
<i>Erpobdella octoculata</i>	H5	PR				
<b>COLEOPTERA</b>						
<i>Chlaenius</i> spp.	C1	PR	1			
<i>Sialis rotunda</i>	C2	PR	1	1		
<b>DECAPODA</b>						
<i>Leander modestus</i>	N3	OM		1		
<i>Palaemon sinensis</i>	N4	OM		1	1	1
<b>DIPTERA</b>						
<i>Tabanus</i> spp.	D1	PR	1	1		
<i>Nippotipula</i> spp.	D2	SH	1	1		
<i>Holorusia</i> spp.	D3	SH	1			
<i>Antocha</i> spp.	D4	GC	1	1		
<i>Tipulidae</i> spp.	D5	SH		1		1
<i>Pilaria</i> spp.	D6	PR	1			
<i>Cryptochironomus digitatus</i>	D7	PR				1
<i>Limonia</i> spp.	D8	SH	1			
<i>Chaoborus</i> spp.	D10	PR	1	1		
<i>Chironomus lugubris</i>	D11	GC				1
<i>Harnischia fuscimana</i>	D12	GC		1		1
<i>Cryptochironomus defectus</i>	D13	PR		1		1
<i>Polypedilum albicorne</i>	D14	SH		1		
<i>Stictochironomus maculipennis</i>	D15	OM				1
<i>Tanypus punctipennis</i>	D16	OM			1	
<i>Stictochironomus</i> spp.	D17	OM				1
<i>Chironomus dorsalis</i>	D18	GC		1	1	1
<i>Polypedilum pedestre</i>	D19	SH		1		
<i>Chironomus pallidivittatus</i>	D20	GC		1	1	1
<i>Chironomus okinawanus</i>	D21	GC		1	1	1
<i>Dicrotendipes pelochloris</i>	D23	GC			1	
<i>Polypedilum cultellatum</i>	D24	SH				1
<i>Glyptotendipes tokunagai</i>	D25	FC			1	1
<i>Glyptotendipes gripekoveni</i>	D26	FC			1	
<i>Cladotanytarsus vanderwulpi</i>	D27	GC		1		
<i>Acricotopus lucens</i>	D28	GC				1
<i>Cryptochironomus fulvus</i>	D29	PR				1
<i>Smittia aterrima</i>	D30	GC	1	1	1	
<i>Procladius choreus</i>	D31	PR	1		1	
<i>Polypedilum flavum</i>	D32	SH		1		
<i>Chironomus flaviplumus</i>	D33	GC		1		1
<i>Tanytarsus mendex</i>	D34	FC		1		
<i>Pentaneurella katterjokki</i>	D35	–	1			1
<i>Cricotopus sylvestris</i>	D36	SH		1	1	1

Taxon	Code	FFG	ASH	HLE	HLEWR	SHJ
<i>Hydrobaenus lugubris</i>	D37	SC		1		
<i>Cricotopus annulator</i>	D38	SH	1			
<i>Tanypus villipennis</i>	D39	PR		1		
<i>Chironomus anthracinus</i>	D40	GC			1	1
<i>Polypedilum sordens</i>	D41	SH		1		
<i>Demicryptochironomus</i> spp.	D42	GC		1		
<i>Procladius</i> spp.	D43	PR				1
<i>Glyptotendipes pallens</i>	D44	FC		1	1	1
<i>Polypedilum asakawanense</i>	D45	SH		1		
<i>Stictochironomus akizukii</i>	D46	OM	1		1	
<i>Demicryptochironomus vulneratus</i>	D47	GC		1		
<i>Chironomus circumdatus</i>	D48	GC		1	1	
<i>Lipiniella sekunada</i>	D49	GC		1	1	
<i>Cricotopus trifasciatus</i>	D50	SH		1		
<i>Dicrotendipes tritonus</i>	D51	GC		1		
<i>Endochironomus tendens</i>	D52	SH		1		
<i>Diplocladius</i> spp.	D53	GC		1		1
<i>Hexatoma bicolor</i>	D54	PR		1		
<i>Dicrotendipes tamaviridis</i>	D55	GC		1		
<i>Polypedilum scalaenum</i>	D56	SH			1	1
<i>Orthocladius thienemanni</i>	D57	GC		1		1
<i>Orthocladius vaillanti</i>	D58	GC		1		
<i>Cryptotendipes</i> spp.	D59	GC				1
<i>Parachironomus arcuatus</i>	D60	PR		1		
<i>Cryptochironomus ussouriensis</i>	D61	PR		1	1	
<i>Chironomus riparius</i>	D62	OM				1
<i>Chironomus salinarius</i>	D63	OM			1	
<i>Polypedilum surugense</i>	D64	SH		1		1
<i>Chironomus attenuatus</i>	D65	OM			1	
<i>Eukiefferiella gracei</i>	D66	GC	1			
<i>Polypedilum nubeculosum</i>	D67	SH		1	1	1
<i>Chironomus plumosus</i>	D68	OM				1
<i>Macropelopia nebulosa</i>	D69	PR		1	1	1
<i>Paracladopelma undine</i>	D70	GC	1			
<i>Acricotopus longipalpus</i>	D71	GC		1		
<b>EPHEMEROPTERA</b>						
<i>Ameletus</i> spp.	E1	GC	1	1		
<i>Iron</i> spp.	E2	FC	1	1	1	
<i>Heptagenia</i> spp.	E3	SC	1			
<i>Ephemera shengmi</i>	E4	GC	1	1	1	1
<i>Ephemera nigroptera</i>	E5	GC	1			
<i>Ephemera sachalinensis</i>	E6	GC	1			1
<i>Baetis</i> spp.	E7	GC	1			
<i>Baetiell</i> spp.	E8	GC	1			
<i>Serratella</i> spp.	E9	GC	1			
<i>Ephemerella</i> spp.	E10	GC	1			
<i>Ephemerella fusongensis</i>	E11	GC	1			
<i>Leptophlebia</i> spp.	E12	GC	1			
<i>Paraleptophlebia</i> spp.	E13	GC	1			
<b>HETEROPTERA</b>						
<i>Corixa substriata</i>	H1	PR	1	1		
<i>Hesperocorixa distanti</i>	H2	PR		1		
<i>Hesperocorixa kirkaldy</i>	H3	PR		1		
<i>Sigra distanti</i>	H4	PR		1		
<i>Ranatra chinensis</i>	H5	PR	1			
<i>Hesperocorixa vulgaris</i>	H6	PR		1		
<b>HETEROSTROPHA</b>						
<i>Cipangopaludina chinensis</i>	M15	SC			1	

Taxon	Code	FFG	ASH	HLE	HLEWR	SHJ
<i>Valvata piscinalis</i>	M16	SC	1		1	1
<b>HYGROPHILA</b>						
<i>Polypylis hemisphaerula</i>	M5	SC		1	1	
<i>Gyraulus convexus</i>	M6	SC	1		1	
<i>Hippeutis cantori</i>	M7	SC		1		
<i>Viviparus chui</i>	M8	SC		1	1	1
<i>Semisulcospira amurensis</i>	M9	SC	1		1	1
<i>Galba truncatula</i>	M10	SC		1		1
<i>Galba perversa</i>	M11	SC		1	1	1
<i>Radix auricularia</i>	M12	SC	1	1	1	1
<i>Radix ovata</i>	M13	SC	1	1	1	1
<i>Radix swinhoi</i>	M14	SC	1	1	1	
<b>NEUROPTERA</b>						
<i>Protohermes grandis</i>	N1	PR		1		
<b>ODONATA</b>						
<i>Ictinogomphus</i> spp.	Od1	PR		1		1
<i>Macromidae</i> spp.	Od2	PR	1			
<b>PLECOPTERA</b>						
<i>Alloperla sapporoensis</i>	P1	PR	1	1		
<i>Alloperla nikkoensis</i>	P2	PR	1			
<i>Alloperla</i> spp.	P3	PR	1			
<i>Haploperla</i> spp.	P5	PR	1			
<i>Cyamia</i> spp.	P6	PR	1			
<i>Doddia iaponica</i>	P7	SH	1			
<b>RHYNCHOBDELLIDA</b>						
<i>Parabdella quadrioculata</i>	H1	PR	1			
<i>Glossiphonia lata</i>	H2	PR	1	1		1
<i>Helobdella stagnalis</i>	H4	PR		1		
<b>TRICHOPTERA</b>						
<i>Parastenopsyche</i> spp.	T1	GC	1			
<i>Stenopsyche marmorata</i>	T2	PR	1			
<i>Parastenopsyche sauteri</i>	T3	GC	1			
<i>Goera ramosa</i>	T4	SC	1			
<i>Goera japonica</i>	T5	SC	1			
<i>Goera kyotonis</i>	T6	SC	1			
<i>Stenophylax ondakesis</i>	T7	SH	1			
<i>Stenophylax koizumii</i>	T8	SH	1			
<i>Astenophylax grammicus</i>	T9	SH	1			
<i>Glyphotaelius</i> spp.	T10	SH	1			
<i>Apatania</i> spp.	T11	SC	1			
<i>Hydropsyche nakaharai</i>	T12	FC	1	1		
<i>Hydropsyche</i> spp.	T13	FC	1			
<i>Ptilocolepus</i> spp.	T14	GC	1			
<i>Oecetis morii</i>	T15	GC	1			
<i>Ganonema</i> spp.	T16	SH	1			
<i>Polycentropus</i> spp.	T17	FC	1			
<i>Hydroptila</i> spp.	T18	SC	1			
<i>Goera</i> spp.	T19	SC	1			
<i>Astenophylax</i> spp.	T20	SH	1			
<b>TUBIFICIDA</b>						
<i>Limnodrilus helveticus</i>	O1	GC		1		1
<i>Nais</i> spp.	O2	GC			1	1
<i>Limnodrilus udekemianus</i>	O3	GC		1	1	1
<i>Teneridrilus mastix</i>	O4	GC				1
<i>Limnodrilus hoffmeisteri</i>	O5	GC	1	1	1	1
<i>Limnodrilus claparedeianus</i>	O6	GC	1			
<i>Spirosperma nikolskyi</i>	O7	GC				1
<i>Aulodrilus pigueti</i>	O8	GC		1	1	

<b>Taxon</b>	<b>Code</b>	<b>FFG</b>	<b>ASH</b>	<b>HLE</b>	<b>HLEWR</b>	<b>SHJ</b>
<i>Aulodrilus japonicus</i>	O9	GC		1	1	
<i>Branchiura sowerbyi</i>	O10	GC		1	1	1
<i>Chaetogaster diaphanus</i>	O11	GC		1	1	1
<i>Tubifex tubifex</i>	O12	GC		1	1	1
<i>Nais bretscheri</i>	O13	GC			1	1
<i>Nais pseudobtusa</i>	O14	GC				1
<i>Paranais frici</i>	O15	GC			1	1
<i>Pristinella acuminata</i>	O16	GC			1	
<i>Nais simplex</i>	O17	GC			1	
<i>Slavina</i> spp.	O18	GC			1	1
<i>Nais communis</i>	O19	GC		1	1	1
<i>Uncinaiis uncinata</i>	O20	GC		1		
<i>Dero</i> spp.	O22	GC			1	
<i>Dero digitata</i>	O23	GC				1
<b>UNIONOIDA</b>						
<i>Corbicula nittens</i>	M2	FC			1	
<i>Lanceolaria grayana</i>	M3	FC				1
<i>Unio douglasiae</i>	M4	FC		1	1	1
<b>VENEROIDA</b>						
<i>Sphaerium lacustre</i>	M1	FC	1			
<i>Sphaerium</i> spp.	N2	FC	1	1	1	
Total			71	78	52	59

<sup>1</sup> = present