A freshwater ecoregion delineation approach based on freshwater
macroinvertebrates community features and spatial environmental data in
Taizi River Basin, northeastern China

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Abstract

The ecoregion is currently widely used as the basic geospatial unit in freshwater biodiversity conservation. The popularly used delineation is usually based on the assumption that attributes of aquatic ecosystems are influenced by landscape-scale environmental variables. However, few ecoregion delineations attempt to establish the local validity of this assumption prior to delineation, and few studies check the correspondence of the derived ecoregion boundaries with the distributions of attributes of aquatic biota. In this study, we established an approach to overcome these shortcomings. The notable features of the approach are: 1) the delineation variables were filtered through a series of analytical steps to select those that best represented the aquatic community traits, and which avoided redundancy in the data; 2) the method was quantitative and repeatable; and 3) the derived ecoregion boundaries were checked for consistency with the spatial attributes of aquatic biota.

The approach was applied in the Taizi River basin, northeast China. The procedure proposed here filtered out altitude and annual precipitation as the best variables to include in the freshwater ecoregion delineation. Then, using the quantitative ISODATA classification method, the basin was classified into three ecoregions. A test of accuracy indicated that freshwater ecoregions matched well with the spatial distribution pattern of macroinvertebrate community attributes. Statistical analysis showed that natural geographical attributes and river attributes were different in the three ecoregions, and indices representing macroinvertebrate community attributes are significantly different as a whole among the 3 ecoregions. The case study proved this

23 approach effective on ecoregion delineation.

24 **Keywords** Freshwater ecoregion delineation; Environmental variables; Aquatic biota;

25 Variables filtration; Spatial cluster; Taizi river basin

26 **Introduction**

27 Ecoregions are geospatial units that are distinguished from others with respect to
28 biological, chemical and/or physical criteria, usually at the landscape scale. Ecoregion
29 delineation is undertaken for the purposes of understanding, auditing and managing
30 natural systems within a framework based on macro-scale spatial units. The
31 distribution of freshwater ecosystem processes partly reflects landscape-scale
32 bio-physical patterns, so ecoregions defined on this basis have application in
33 freshwater biodiversity conservation management frameworks (Omernik 1987;
34 Maxwell et al. 1995; Groves et al. 2002; Higgins et al. 2005; Abell et al. 2008;
35 Kennard et al. 2010; Soranno et al. 2010).

36 Ecoregion delineation initially focused on terrestrial ecosystems, but Omernik
37 (1987) expanded the concept to include aquatic ecosystems. Since then, many aquatic
38 ecoregion delineation systems have been defined for the purposes of conservation and
39 management of water resources (Kennard et al. 2010), aquatic species (Maxwell et al.
40 1995; Abell et al. 2008), aquatic ecosystems and habitats (Munne and Prat 2004;
41 Higgins et al. 2005), and also for the purpose of river health assessment (Binckley et
42 al. 2010). Ecoregion delineation usually aims to define natural ecoregions, but human
43 alteration could give rise to a distinct pattern of modified ecoregions.

44 Freshwater ecoregion delineation approaches fall into two main categories. One
45 approach is to define ecoregions directly using freshwater taxon data (Abell et al.
46 2008), and the other is to use, as surrogates, landscape-scale environmental attributes

that are thought to influence the distribution of freshwater ecosystem processes (Maxwell et al. 1995; Munne and Prat 2004; Higgins et al. 2005; Soranno et al. 2010). Stream biological data can be highly variable through time, and costly to collect; also, in areas that are highly stressed by human activities, the data would not necessarily define the natural ecoregions. Thus, the use of landscape-scale environmental variables to represent the pattern of biological attributes and processes is the more common approach.

The use of non-aquatic environmental attributes to define freshwater ecoregions is based on the assumption that freshwater ecosystem processes are systematically influenced by environmental processes operating at the landscape-scale (Minshall et al. 1985; Omernik 1987; Maxwell et al. 1995; Soranno et al. 2010). Environmental factors influence freshwater ecosystem processes over a range of spatial scales. For example, life history and behavioral attributes of aquatic biota are best related to reach-scale physical features of streams (Richards et al. 1997), while community traits are mostly related to large-scale factors such as altitude (Poff et al. 1997). However, biological traits that are linked directly to local environmental factors can also be indirectly associated with large-scale factors through their controlling influence over the distribution of habitat characteristics (Richards et al. 1997; Poff 1997; Munne and Prat 2004). Environmental variables popularly used in ecoregion delineation are climate, vegetation, soil, topography, altitude, land use, hydrology and geology (Omernik 1987; Maxwell et al. 1995; Higgins et al. 2005; Soranno et al. 2010). The spatial distribution of these variables at the meso- and macro-scales has been linked to

the spatial distribution of freshwater species traits (Quinn et al. 1997; Labbe and Fausch 2000; Vinson and Hawkins 2003), community structure (Allan and Johnson 1997; Jacobson et al. 1997; Poff et al. 2001; Vinson and Hawkins 2003; Santoul et al. 2004) and ecosystem function (Poff 1997; Jones et al. 2003; Binckley et al. 2010).

Many previous attempts at defining ecoregions used all available data concerning environmental variables in the delineation process, rather than assessing the significance of the contribution of each variable in influencing freshwater biological traits (Maxwell et al. 1995; Higgins et al. 2005; Soranno et al. 2000). It should not be assumed that all environmental variables are statistically important in determining the spatial pattern of ecosystem traits and processes (Soranno et al. 2010). For example, Wright et al. (1998) found that vegetation patterns did not correspond well with ecoregions, with most vegetation types having a small proportion of their total area in a given ecoregion, and with no dominance by one or more vegetation types in any ecoregion. This indicated that the pattern of vegetation types, which is a popular variable in freshwater ecoregion delineation, had little connection with the freshwater ecoregion boundary. This issue could also be relevant to other environmental variables. Another problem is the use of the mean value of environmental attributes, when the characteristics of the ecosystems, and the delineation of ecosystem boundaries, might be more sensitive to the variability of the environmental attributes (Allan and Johnson 1997; Palmer et al. 1997).

In this paper we hypothesize that selected biological characteristics of freshwater ecosystems are influenced by certain environmental factors at the meso- and

macro-scale, and that these relationships can be used to define freshwater ecoregions. The most appropriate environmental variables for the ecoregion delineation were selected using a series of quantitative steps that took into account the variability of the data. The approach is illustrated through its application to the Taizi River basin, China.

Materials and methods

Study area

The Taizi River basin is located in Liaoning Province, northeast of China, between 122°25'-124°55'E and 40°28'-41°38'N. The basin drains an area of 13,900 km², and the Taizi River main stem has a length of 413 km (Fig. 1). The climate is temperate terrestrial monsoon, with the highest temperatures in July and August, and the lowest temperatures in January and February. The pattern of precipitation is almost synchronous with that of temperature, being highest in July and August. The basin includes mountain, hill and plain landform zones. The main land cover types are forest, distributed mainly in the mountain and hill zones, and farmland, distributed mainly in the plain zone. The population of the Taizi River basin in 2008 was 6.9 million, with the majority of people living in the 4 main cities of Benxi, Liaoyang, Anshan and Haicheng (Fig. 1). Being rich in iron and coal, mineral extraction is the main industry in this region, with Anshan city the biggest source of iron in China. Liaoyang is the main base for the chemical industry of Liaoning province (mainly chemical fiber textile). Rapid expansion of economic activity since the 1980s has led to decline of the health of the Taizi River system, and biodiversity

conservation is currently a major management concern in the basin.

Outline of the delineation approach

The framework of the approach taken here to ecoregion delineation included the following steps: 1) Data on landscape-scale environmental variables were collated and then analyzed using statistical variation analysis, spatial visualization, geostatistical analysis and expert judgment to find those with distinct patterns of heterogeneity within the basin; 2) these potentially suitable environmental variables were then filtered by finding those most strongly linked to field-collected data concerning freshwater biological traits using correlation analysis, ordination analysis, and other statistical methods; 3) unsupervised clustering was undertaken using the ISODATA clustering algorithm to establish tentative ecoregions; 4) the ecoregion boundaries were then rectified to fit sub-catchment boundaries, and these ecoregions were then checked for their consistency with the spatial pattern of field-measured biological attributes.

The main strengths of the approach taken to definition of freshwater ecoregions are that: 1) it makes use of quantitative and repeatable methods of data collection, environmental variable selection and boundary definition, and 2) environmental variables are selected on the basis of a demonstrated association with freshwater biological traits.

Environmental data collection and preprocessing

Data were collated on the environmental variables altitude, slope, annual average

temperature, annual precipitation, annual evaporation, terrestrial vegetation type, soil type and geomorphological type (Table 1). Data were in the form of both continuous raster and categorical vector data (Table 1). All datasets were projected into Albers conical equal area projection.

Climate data were supplied from the monitoring network of the Chinese National Meteorological Bureau. Daily data over the period 1961 to 2001 were obtained from 11 stations located within and adjacent to the Taizi River basin. Annual rainfall and evaporation was calculated for each station as the average of the calendar year totals of 41 years of data (using calendar year). Annual temperature of each station was calculated as the average of 41 years of calendar year annual average temperature. A kriging interpolation method was used convert the spatial climate data into a grid format with resolution of 100 m (Li et al. 2007). Terrestrial vegetation, soil and geomorphology data were mapped at scales of 1:4,000,000, 1:1,000,000 and 1:5,000,000 respectively (Data source: National Geomatics Center of China). Altitude was from the SRTM DEM produced by NASA and the USGS National Imagery Mapping Agency (NIMA) with 90 m resolution (<http://srtm.csi.cgiar.org>). Slope was calculated from the SRTM DEM in ArcGIS with spatial analysis module, and its resolution is 90 m (Kong et al. 2010).

All environmental variables were analyzed in ArcGIS using spatial visualization, basic statistics and geostatistical methods to examine the landscape-scale distributions of the data. For each selected environmental variable, a characteristic value was derived for each biological field sampling point by averaging the values of the

variable within a radius of 1.5 km of the survey point.

Macroinvertebrate data acquisition and analysis

Macroinvertebrates assemblages, which are known to be sensitive to environmental factors and which are an important component of the freshwater ecosystem food web, were selected to represent the freshwater biology. Macroinvertebrate species diversity and assemblage composition are both important indicators of ecosystem integrity and health. Macroinvertebrates were collected in May 2009 at 69 randomly selected sampling sites using a Surber net (30 cm ×30 cm, 500 µm mesh). At wadeable sampling sites, three replicates were obtained from two riffles and one shallow pool (<40 cm deep). For the sampling sites that could not be waded, the Surber net was used at the shallow water (<40 cm deep) with three replicates along the riverside, and the forth replicate was added by using a D-frame net to scrape the surface of substrates in the deep water areas (>40 cm deep). Each sample was separately put into a 500 mL jar and preserved with 70% alcohol. In the laboratory, the samples were rinsed through a sieve (500 µm mesh). The retained macroinvertebrates were sorted and preserved in 70% alcohol. The macroinvertebrates were identified to the lowest taxonomic classification, mainly to species and genus level, with a few to subfamily level and one to class level due to difficulty in identification, using the Japanese freshwater insect manual (Kawai and Tanida 2005), and the number of individuals (abundance) of each taxon was counted. At the time of sampling, river physical and water chemical features were also measured, including river width, substrate particle size, dissolved oxygen

concentration (DO), pH, suspended solids concentration (SS), total dissolved solids concentration (TDS) and electrical conductivity (EC).

The Shannon-Wiener index was calculated to represent macroinvertebrate diversity using the equation:

$$H' = -\sum_{i=1}^n p_i \log p_i \quad (\text{Huston 1994})$$

H' is the value of the Shannon-Wiener diversity index; p_i is the proportion of individual numbers of i th taxon on total individual numbers in the site; for log we used the logarithm to base 10; and n is the number of species at the site.

The spatial distribution pattern of the macroinvertebrate community was determined using cluster analysis with individuals of taxa. The sampling sites were classified based on the taxonomic composition similarity using Ward's linkage method with Euclidean distance measure (PC-ORD software; McCune and Mefford 1999). Differences among clusters were tested by the multi-response permutation procedure (MRPP), which is a nonparametric method for testing multivariate differences among pre-defined groups (Zimmerman et al. 1985). The Sørensen coefficient was used as the distance measure in the MRPP. The null hypothesis that there was no statistical difference among groups was tested using a Monte Carlo randomization procedure with 1,000 permutations using PC-ORD software. The indicator value analysis (Dufrêne and Legendre 1997) was used to determine indicator species for different clusters. The indicator value for each taxon in different clusters is the product of its relative individual abundance and relative frequency (100), and

ranges from 0 (no indication) to 100 (perfect indication) (Peterson and Keister 2003). A perfect indicator of a particular group should be faithful and exclusive to that group, never occurring in another group. The significance of the indicator value for each species was tested with a Monte Carlo randomization procedure with 1,000 permutations. The indicator value analysis was also carried out with the PC-ORD software (McCune and Mefford 1999).

Macroinvertebrate taxon individuals, taxon numbers, and diversity measures were correlated with environmental variables as a way of filtering the environmental attributes most suitable for the ecoregion delineation. The clustering procedure should use uncorrelated variables (Hargrove and Hoffman 2005), so correlation analysis was undertaken to remove redundant environmental variables (with those least conceptually related to the distribution of freshwater biology attributes being excluded from groups of correlated variables). Correlation analysis was done in SPSS, testing to a significance level of $\alpha = 0.05$. All work was done in ArcGIS with spatial analysis module and SPSS.

Delineation approach and results assessment

Layers of filtered environmental variables were stacked to form a grid layer database. ISODATA, a spatial clustering method (ERDAS Inc. 1999), was used for the delineation. The number of classes was based on the spatial pattern of the selected environmental attributes. This clustering work was done in ERDAS.

The boundary of each ecoregion was rectified to fit the boundaries of sub-catchments (the drainage areas of individual stream lengths). Although the spatial

pattern of sub-catchments is unsuitable as an input to the ecoregion delineation process (Omernik 1987), water resources management is usually based on the catchment geospatial unit, so there is a practical advantage in adjusting statistically determined ecoregion boundaries to follow sub-catchment boundaries. Catchment boundaries were extracted from DEM data in ArcGIS hydrologic module. The ecoregions were then checked for consistency with the delineation based on macroinvertebrate data.

The match degree value was used to quantitatively assess the degree of consistency between ecoregions based on delineation of environmental variables and the delineation based on cluster analysis of macroinvertebrate data. The match degree (MD) in each ecoregion was calculated using the following equation:

$$MD\% = \frac{\text{site numbers belonging to the spatial cluster group}}{\text{total sites in the ecoregion corresponding to the cluster group}} \times 100$$

Results

First-stage filtering of environmental variables based on spatial patterns

All environmental variables except terrestrial vegetation type exhibited a pattern of upstream to downstream zonation through the basin (Fig. 2). The patterns suggested three main zones for most variables, and two zones for geomorphology type and soil type. The dominant geomorphological types were undulating mountain in the upper and middle region and flat plain in the lower region (Fig. 2). The geomorphological types were defined on the basis of a combination of altitude and slope characteristics, except for karst landforms (which occupied a relatively small area of the basin), so it

was considered a redundant environmental attribute. The main soil types were brown soil in the upper and middle regions of the basin, and meadow soil dominating the lower region and following drainage lines into the middle and upper regions (Fig. 2). Soil types tended to be distributed according to geomorphological zones (Fig. 2). As soil type was relatively consistent along the drainage lines, it was deemed insensitive for the purpose of distinguishing a gradient of aquatic biology attributes. Oak shrub and oak forest dominated in the upper and middle region, and cultivated land dominated the lower region (Fig. 2). Terrestrial vegetation was distributed as relatively small isolated patches in the upper and middle regions (Fig. 2), which deemed it unsuitable for use in defining macro-scale ecoregions.

Altitude ranged from -149 m (an artificial elevation caused by mineral extraction) to 1,287 m, with a CV (coefficient of variation) of 164%. Slope ranged from 0 to 72.9 degrees, with a CV of 169%. Annual average temperature ranged from 2.27°C in the upper region to 9.99°C in the lower region, with a CV of 125%. Annual precipitation ranged from over 900 mm in the upper and middle region to 655 mm in the lower region, with a CV of 121%. Annual evaporation ranged from just over 1,000 mm in the upper region to a low of 734 mm in the lower region, with a CV of 121% (Fig. 2). The high values of CV for altitude, slope, temperature, precipitation and evaporation indicated that these environmental attributes had high spatial variability. Semivariance analysis showed that altitude, temperature, precipitation and evaporation were spatially autocorrelated, and the minor ranges were 100.3 km, 92.3 km, 116 km and 171 km respectively, while slope was not spatially auto correlated. The Taizi River

basin is more than 200 km long, suggesting that the spatial variation of environmental variables can be reflected in the delineation.

Thus, the first coarse filtering step identified the environmental variables altitude, temperature, precipitation as potentially suitable for the purpose of ecoregion delineation, while geomorphology type, soil type and terrestrial vegetation were less promising.

Macroinvertebrate assemblage traits and spatial pattern

Over the 69 sampled sites, 131 taxa of macroinvertebrates were identified in the Taizi River. At most sites, the numbers of individuals collected ranged from 500 to 4000, and the number of species ranged from 10 to 40. The diversity index (H') of all samples ranged from 0.14 to 3.63, with high values occurring mainly in the upper and middle part of Taizi River and tributaries. The highest value occurred in the Taizi River north tributary, which is the main water source area of the basin. Low values occurred mainly in the lower region, with the lowest value in the tributary Yangliu River, which is near the end of Taizi River. The dominant taxa were Orthocladinae spp., Oligochaeta spp., *Baetis thermicus*, *Serratella rufa* and Chironominae spp. with relative abundance of 40.9%, 20.3%, 8.9%, 4.7% and 4.1% over all sites.

Cluster analysis based on community similarity showed that in the Taizi River basin, macroinvertebrates can be classified into 4 groups distributed spatially along a general upland to lowland gradient (Fig. 3). The indicator species were different among the 4 groups (Table 2). Cluster 1 comprised 24 points distributed mainly in the

upper reaches of the Taizi River and some tributaries, and had more indicator species than the other 3 clusters (Table 2); taxa with high indicator value were *Serratella rufa*, *Epeorus latifolium* and *Hydropsyche orientalis*. Cluster 2 included 21 points distributed mainly in the middle reaches of the Taizi River. Indicator species in cluster 2 were *Gammarus*, *Nephelopsis* and *Nematoda*. Cluster 3 included 16 points distributed mainly in the lower part of Taizi River. Indicator species in cluster 3 were the mollusks *Bithynic fuchsiana* and *Unio douglasiae*. Cluster 4 comprised 8 points located mainly in the lower part of the Taizi River together with Cluster 3. Indicator species in cluster 4 were the mollusk *Branchiura sowerbyi*, *Oligochaeta* species and *Elophila*. Dominance by *Oligochaeta* is indicative of serious organic pollution and would not be expected naturally. Clusters 3 and 4 occurred in the same area of the basin, and if the cluster distance were increased the 2 clusters will merge into one group (Fig. 3).

Second-stage filtering of environmental variables based on correlation analysis

Macroinvertebrate species number and diversity were highly significantly correlated to altitude, slope, annual precipitation, annual average temperature and annual evaporation (Table 3). Altitude, slope and annual precipitation were positively correlated, while annual temperature and annual evaporation negatively correlated (Table 3). Macroinvertebrate abundance was not significantly correlated with altitude, slope or annual average temperature, but was significantly correlated with annual precipitation and annual evaporation (Table 3). Thus, annual precipitation and annual evaporation were correlated with both abundance and diversity.

The five environmental variables were highly inter-correlated ($p < 0.01$; Table 4). Of these, altitude and slope are landform attributes, and precipitation, evaporation and temperature are climate attributes. Slope and temperature are physically linked to altitude. It is normal for precipitation and evaporation to be inversely correlated. Of these, precipitation more directly influences river flows, so it was regarded as more closely linked to freshwater biological processes and traits than evaporation.

The correlation analysis suggested that ecoregion delineation based on just altitude and annual precipitation would be ecologically meaningful and free of redundant information.

Freshwater ecoregion delineation and evaluation

The distribution of environmental variables suggested that the basin had three natural geographical zones (Fig. 2). Thus, the spatial cluster analysis classified the basin into three areas on the basis of the altitude and precipitation attributes (Fig. 4). This produced a division between the upper, middle and lower catchment, but the boundaries were complex because of the dissected nature of the drainage pattern (Fig. 4).

This basin was classified into 105 sub-catchments (Fig. 4). Combined with the spatial cluster results, the freshwater ecoregion boundaries were then established using the principle of maintaining the boundary integrity of sub-catchment (Fig. 4). The three freshwater ecoregions of Taizi River basin were then named highland, midland and lowland, according to the dominant environmental factors. Average

altitudes of the highland, midland and lowland ecoregions were 511 m, 282 m and 65 m (Table 5). ANOVA showed that altitude was highly significantly different between the three ecoregions ($p < 0.01$; Table 5).

The freshwater ecoregions matched well with the macroinvertebrate cluster results (Fig. 3 and Fig. 4). The match degree was 85.7%, 65% and 75% for the highland, midland and lowland ecoregions. The total match degree was 75.4%.

Features of freshwater ecoregions in the Taizi River basin

The data suggested that the three defined ecoregions were significantly different in landscape-scale natural geographical attributes, river physical and water chemistry attributes, and macroinvertebrate attributes (Table 5). The natural geographical attributes were all significantly different among the three ecoregions, with a strong gradient from the highland to the midland and to the lowland ecoregions (Table 5).

River physical and water chemistry attributes of the lowland ecoregion were significantly different to those of the upland and midland ecoregions for most parameters. These attributes were not different between the upland and midland ecoregions, except for substrate index, suspended solid, total dissolved solid and total nitrogen (Table 5). Attributes of macroinvertebrate community were also different among the three freshwater ecoregions (Table 5). Differences among upland, midland and lowland freshwater ecoregions suggested that the delineation was efficient in reflecting the broad-scale spatial differences in environmental attributes and freshwater biology traits across the basin.

Discussion

This study demonstrated that in the Taizi River basin the spatial distributions of environmental variables were correlated with the spatial distributions of attributes of the macroinvertebrate communities. The results support our hypothesis that carefully selected environmental variables could be used to define distinct freshwater ecoregions. The ecoregion delineation approach was quantitative and repeatable, and the results were consistent with the observed spatial pattern of the macroinvertebrate communities.

The Taizi River basin lacks historical survey data concerning macroinvertebrates, fish and other biota, so this study relied on data from a recent snapshot field survey. According to the River Continuum Concept (Vannote et al. 1980), freshwater community structure changes from headwater to mouth. Macroinvertebrate species in this region obeys the model and the assemblage species showed their adaption to natural environmental changes from upper to the lower region along the river. Among the 24 sites in the lowland ecoregion, indicator species of 8 sites is pollution tolerant, while all species of the 24 sites are adaptable for the lowland habitat. Although the freshwater ecosystem of the Taizi River basin is stressed by the impacts of anthropogenic activities, especially in the lowland ecoregion, on water quality, the distributions of macroinvertebrate attributes still broadly followed the gradients of the river physical habitat attributes and landscape-scale natural geographical attributes.

Our findings support the contention of Gallant et al. (1989) that where suitable

spatial data are available, quantitative methods are superior to qualitative methods for addressing regional delineation problems. The delineation approach suggested here can be applied to any freshwater biology component (e.g. fish, or aquatic plants), as the environmental variables selected for use in the delineation are specific to the biological data. Paavola et al. (2006) found that the degree to which patterns in ecological community structure across a set of sites are similar between different taxonomic groups (i.e. community concordance), in this case, among macroinvertebrate, fish and bryophytes, was strong across multiple drainage systems and ecoregions. This suggests that the freshwater ecoregions defined on the basis of one taxonomic group are likely to be relevant to the management of whole ecosystems.

Freshwater ecoregions have application in river health assessment and freshwater ecosystem conservation management (Hawkins et al. 2000). Esselman and Allan (2010) considered that the sub-catchment or larger scale (i.e. ecoregion scale) is a biologically relevant scale at which to conduct fish conservation planning to protect the integrity of local habitat conditions and fish communities. As the natural physical, chemical and biological characteristics of streams in the Taizi River basin showed a level of consistency within the highland, midland and lowland ecoregions, it follows that different river health assessment standards should be applied in these ecoregions. Also, it might be appropriate to establish different freshwater ecosystem conservation targets in these three ecoregions.

The Taizi River lies near latitude 40°N, which is known as one of the most naturally

diverse zones for invertebrates in the world (Vinson and Hawkins 2003). In the Taizi River basin, total phosphorus, COD_{cr} and EC were characteristically higher in the lowland ecoregion compared to the highland and midland ecoregions. Also, the data suggested that the macroinvertebrate communities in lowland showed degradation tendency compared to the midland and highland ecoregions (Table 5). The dominant macroinvertebrate species in the lowland ecoregion was pollution tolerant, indicating an impact from mining, chemical industries and other anthropogenic activities. This finding suggests that in the Taizi River basin, the lowland ecoregion is the most important region for stream rehabilitation, while ecosystem protection is the appropriate strategy for the midland, and particularly the highland ecoregion.

Wright et al. (1998) found that vegetation type, which is commonly used in ecoregion delineation (Omernik 1987), was almost completely incongruent with ecoregion boundary. There may be a temptation to include in ecoregion delineation all variables for which data are available, especially potentially manageable variables that might influence river health (e.g. terrestrial vegetation). However, this study demonstrated that not all environmental variables are well correlated with the pattern of freshwater biological attributes, so their inclusion could lead to inappropriate representation of the main target of management (i.e. stream ecological health). Thus, filtering of environmental variables is an important step in ecoregion delineation.

This study found that the selected landscape-scale environmental variables were significantly correlated with macroinvertebrate taxon number and diversity, while precipitation and evaporation, which are related to availability of water in streams,

were also correlated with taxon abundance. The results from the Taizi River basin support the many studies from around the world that have found altitude is strongly related to the distribution of the characteristics of freshwater ecosystems at global and regional scales (Vinson and Hawkins 2003; Santoul et al. 2004). In general, a diverse gravel-cobble substrate favors high macroinvertebrate diversity. This habitat type is typical of streams in high altitude mountain zones, while lowland streams tend to have finer-grained substrates (sand to mud) and lower macroinvertebrate species richness (Allan and Castillo 2007). The Taizi River basin includes mountain and plain geomorphological zones, and the bed material size and macroinvertebrate diversity generally followed the expected pattern. The indicator species of the three ecoregions of the Taizi River basin highlighted the differences in macroinvertebrate community structure. The favored habitat for the indicator species of the highland ecoregion is torrent and heterogeneous substrate; the indicator species of the midland ecoregion prefer relatively slow flowing habitats; while the indicator species of the lowland ecoregion prefer sandy or muddy substrates (Kawai and Tanida 2005). For freshwater ecosystems, precipitation is quite important as it largely determines the pattern of runoff in streams (Poff et al. 2001). In the Taizi River basin, while macroinvertebrate abundance was not correlated with altitude, it was correlated with precipitation, suggesting that there are a number of mechanisms by which environmental variables can influence freshwater community attributes.

It is widely recognized that freshwater ecosystem features and functions are organized hierarchically, and the influence of environmental variables on aquatic

ecosystem attributes is also hierarchical (Frissel 1986; Tonn 1990; Maxwell et al. 1995; Soranno 2010). The results of this study support the contention of Maxwell et al. (1995) that attributes of biological communities correspond with meso-scale environmental variables. A study of a basin with an area of 45,750 km² in Belize, Mesoamerica (Esselman and Allan 2010), and one of a basin with an area of 57,000 km² in France (Santoul et al. 2004), also found that landscape-scale factors had a stronger influence on the distribution of fish assemblages than did environmental conditions at the reach scale.

Conclusion

The results of this study supported the hypothesis that freshwater biology traits were influenced meso- and macro-scale environmental variables. A series of analytical steps can be followed to obtain a parsimonious group of ecologically meaningful environmental variables suitable for classifying ecoregions. Although the macroinvertebrate communities of the Taizi River basin are impacted by poor water quality, the approach suggested here produced ecoregions that would be expected of the natural system. Concordance among different biological groups suggests that ecoregions that are relevant to macroinvertebrate attributes can also represent other components of the aquatic ecosystem. Ecoregions that are aligned with the distribution patterns of biological attributes of streams can be a useful tool to assist conservation management of freshwater ecosystems.

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575 Fig. 1 Location of Taizi River basin and the field sampling points

576 Fig. 2 Spatial distribution of environmental variables in Taizi River basin

577 Fig. 3 Cluster analysis for macroinvertebrates in the Taizi River basin based on

578 community taxon individual similarity

579 Fig. 4 Spatial clustering and freshwater ecoregion delineation results in Taizi River

580 basin

581

Fig. 1

Fig. 2

Fig. 3

Macroinvertebrate cluster

Fig. 4

Table 1. Information of environmental data

Parameters	Data form	Data type	Resolution /Scale	Data source
Annual average temperature	Raster	Continuous	1km	China Meteorological Data Sharing Service System
Annual precipitation	Raster	Continuous	1km	As above
Annual evaporation	Raster	Continuous	1km	As above
altitude	Raster	Continuous	90m	SRTM, download from NASA web
Slope	Raster	Continuous	90m	Calculated from DEM in ArcGIS
Soil	Vector	Categorical	1:4000000	National Geomatics Center of China
Terrestrial vegetation	Vector	Categorical	1:4000000	As above
Geomorphology	vector	Categorical	1:5000000	As above

Table 2. Indicator species analysis in the clusters in Taizi river basin

Taxa	Cluster	% Occurrence frequency	% Relative abundance	Indicator values	p
<i>Serratella rufa</i>	1	50.0	4.44	80.0	0.001
<i>Epeorus latifolium</i>	1	38.6	0.71	74.2	0.001
<i>Hydropsyche orientalis</i>	1	28.6	0.20	74.1	0.001
<i>Acentrella sibirica</i>	1	30.0	0.60	69.4	0.001
Ceratopogoniidae	1	51.4	0.32	69.3	0.001
<i>Baetiella japonica</i>	1	30.0	2.98	60.4	0.001
<i>Beatis thermicus</i>	1	64.3	8.44	56.4	0.001
<i>Rhyacophila igrocephala</i>	1	18.6	0.06	54.2	0.001
Tanypodiinae	1	75.7	3.01	46.7	0.001
<i>Antocha</i>	1	38.6	0.10	46.2	0.001
<i>Beatis</i> sp.	1	10.0	0.13	44.6	0.001
<i>Cheumatopsyche</i>	1	35.7	1.35	42.5	0.002
Chironominae	1	91.4	3.88	37.8	0.002
<i>Simulium yonagoense</i>	1	46	82	37.4	0.003
Orthocladinae	1	92.9	37.2	35.9	0.001
<i>Paraleptophlebia estoni</i>	1	14.3	0.39	34.8	0.002
<i>Ephemera strigata</i>	1	17.1	0.13	34.3	0.005
<i>Setodes turbatus</i>	1	20.0	0.08	34.1	0.011
Diamesinae	1	25.7	0.71	33.7	0.008

<i>Gyraulus convexiusculus</i>	1	28.6	0.14	32.5	0.017
<i>Ecdyonurus kibunensis</i>	1	12.9	0.31	31.4	0.005
<i>Ecdyonurus viridis</i>	1	14.3	0.13	30.4	0.009
<i>Gammarus</i>	2	32.9	3.59	30.9	0.031
<i>Nephelopsis</i>	2	27.1	0.11	20.7	0.118
Nematoda	2	17.1	0.03	12.2	0.357
<i>Bithynic fuchsiana</i>	3	8.57	0.01	16.7	0.052
<i>Unio douglasiae</i>	3	7.14	0.005	12.7	0.202
Oligochaeta	4	94.3	19.33	36.2	0.001
<i>Branchiura sowerbyi</i>	4	15.7	0.09	26.9	0.014
<i>Elophila</i>	4	5.71	0.01	16.7	0.057

Table 3. Correlation between freshwater community structure and environmental variables

Community features	Altitude	Slope	AT	AP	AE
Taxon	0.21	0.19	-0.19	0.25*	-0.28*
Abundance					
Number of taxa	0.52**	0.43**	-0.5**	0.54**	-0.54**
Shannon Wiener	0.54**	0.47**	-0.56**	0.52**	-0.51**
diversity index					

AT: Annual average temperature; AP: Annual precipitation; AE: Annual average evaporation

*significance $p < 0.05$; ** significance $p < 0.01$

Table 4. Correlation analysis among environmental variables

	Slope	AT	AP	AE
Altitude	0.69**	-0.69**	0.86**	-0.84**
Slope		-0.49	0.59**	-0.58**
AT			-0.80**	0.79**
AP				-0.98**
*significance $p < 0.05$; ** significance $p < 0.01$				

Table 5. Freshwater ecoregion features among upper, middle and lower ecoregion.

(Abbreviations: A: altitude, AAT: annual average temperature, AP: annual precipitation, S: slope, SI: substrate index, SS: suspended solid, EC: electrical conductivity, TN: total nitrogen, TP: total phosphorus, CODcr: chemical oxygen demand, MI: microinvertebrate individual numbers, MS: microinvertebrate specie numbers, MB: microinvertebrate biodiversity. ANOVA was conducted among features and value in bracket is the average value for parameters. Statistical significant level: $p=0.05$. a, b, c represents the difference among the 3 ecoregions based on site data)

Ecoregions		Highland	Midland	Lowland
landscape-scale natural attributes	Area (km ²)	4557	3896	4749
	A (m)	153-1181(511a)	45-910(282b)	0-520(65c)
	AAT (°C)	2.28-7.75(5.86a)	4.38-9.22(7.07b)	4.9-10(8.74c)
	AP(mm)	786-954(870a)	717-868(786b)	653-775(709c)
	AS(°)	0-60.5(18.7a)	0-51.7 (16.1b)	0-44.7(4.1c)
River attributes	Width (m)	0.5-100 (14.8 a)	0.3-300 (44.4b)	1-400m (84bc)
	SI	1-4.8 (3.6 a)	1-4.4 (3.4 a)	1-4.2 (2.1 b)
	pH	8.08-9.30(8.35a)	7.8-9.12(8.30a)	6.82-8.6(7.78b)
	SS (mg/L)	3.5-347 (33.7 a)	5.5-81.5 (30.3 a)	3.5-109 (32.3 a)
	TDS (mg/L)	84.5-598.5(223.1a)	130.5-287.5(229.6a)	60-795(274.9a)
	DO (mg/L)	4.3-15.6 (10.8 a)	3.5-14.6 (9.4 a)	0.4-13 (6.4 b)
	EC (μ s/cm)	11.7-826 (183 a)	57-566 (336.8 a)	177-1126(515 b)
	TN(mg/L)	1.5-17.8 (4.7a)	0.8-15.5 (4.6a)	0.7-16 (6.1a)
	TP(mg/L)	0.01-0.73(0.09a)	0.01-0.4(0.1a)	0-3.03 (0.57b)

	CODcr(mg/L)	12.1-38.8 (22.75a)	0-53.4 (21.08a)	0-110.5 (34.93b)
Ecosystem attributes	MI	6-3045(1095)ab	26-2577(1221)a	75-2679(756)b
	MS	2-41(23)a	4-33(15)b	4-24(11)b
	MB	0.65-3.63(2.5)a	0.14-2.8(1.7)b	0.16-3.18(1.4)b

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