



# Distribution and Trophic Pattern of Non-Native Fish Species Across the Liao River Basin in China

# Kangshun Zhao <sup>1,3</sup>, Chao Li<sup>2</sup>, Tao Wang<sup>2</sup>, Bowen Hu<sup>2</sup>, Min Zhang<sup>2,\*</sup> and Jun Xu<sup>1,\*</sup>

- <sup>1</sup> Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China; zhaokangshun@ihb.ac.cn
- <sup>2</sup> College of Fisheries, Freshwater Aquaculture Collaborative Innovation Center of Hubei Province, Hubei Provincial Engineering Laboratory for Pond Aquaculture, Huazhong Agricultural University, Wuhan 430070, China; chaoli0032@163.com (C.L.); waaqgr@163.com (T.W.); hbw21@webmail.hzau.edu.cn (B.H.)
- <sup>3</sup> University of Chinese Academy of Sciences, Beijing 100049, China
- \* Correspondence: zhm7875@mail.hzau.edu.cn (M.Z.); xujun@ihb.ac.cn (J.X.)

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**Abstract:** Controlling the invasion of non-native fish species necessitates a complete understanding of the distribution of these species and the key factors that influence such distribution. In order to research the situation of non-native fish and the relevant influencing factors in the Liao River Basin, we investigated and analyzed the characteristics of the distribution and the trophic levels of non-native fish species, as well their response to different types of factors in the basin. Nine non-native fish species were found during the investigation, and the trophic levels of these species ranged between 2.00 and 3.84. The results of generalized linear models indicate that the distribution of non-native fish species in the basin is mainly related to anthropogenic activities, socioeconomic development, and climate. The southeastern part of the Liao River Basin is conducive to the distribution of non-native fish species species was not correlated with anthropogenic activities and socioeconomic development in the Liao River Basin. By providing evidence for the links between non-native fishes and different types of factors, our study contributes to increasing the relevant references for and experiences in the early detection and management of non-native fishes on a basin scale.

**Keywords:** non-native fish; distribution; trophic level; Liao River Basin; environmental factors; socioeconomic factors; anthropogenic activities

# 1. Introduction

Invasive non-native species have become a serious environmental issue worldwide. Invasive species are considered threats to the environment and the economy [1], and are known to cause major economic losses in agriculture, forestry, and other sectors of the world economy [2,3]. For example, the economic costs inflicted by invasive non-native species are considerable, i.e., amount yearly to hundreds of billions of dollars worldwide [4]. Along with other drivers of ecosystem degradation, such as climate change, pollution, and habitat change, biological invasion is one of the major causes of biodiversity decline that reduces ecosystem services around the world [5].

Freshwater ecosystems are subject to a wide range of anthropogenic threats, and the introduction of non-native species is one of the major threats [6]. Non-native species can cause impacts in the ecosystems to which they are introduced [7]. In some cases, these impacts are dramatic and may result in the extinction of native species or radical changes in ecosystem functioning [7–10].

Biological invasion follows a certain process whereby after an introduction event, non-native species may become established, increase in number, and spread [11,12]. The impacts of non-native



species generally increase if the species establish themselves and spread in their new environment [13–15]. Once non-native species become established and a nuisance, it will be almost impossible to eradicate them [16]. Therefore, the mechanisms of biological invasion should be understood to ensure the efficient management and conservation of freshwater ecosystems [17]. Early detection, monitoring, and prediction are critical for mitigating the threats posed by non-native species [18].

Among vertebrate groups, freshwater fish have been widely introduced around the world [19]. Non-native fish species have successfully invaded ecosystems primarily through aquaculture (51%), ornamental fishery (21%), recreational fishing (12%), fish transportation (7%), and other approaches (9%) [20]. These situations have exerted serious impacts on local fish populations and have even led to the extinction of some local species [21].

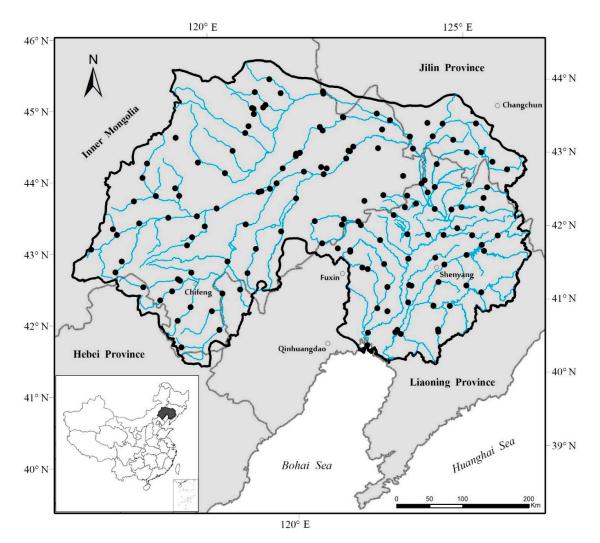
In the Liao River Basin in Northeast China, the climate ranges from arid and semi-arid to humid and semi-humid monsoon [22]. Over the years, studies on non-native fish species in the freshwater ecosystems of semi-humid and semi-arid areas on the basin scale remain rare. Therefore, we systematically investigated the current situation and trophic level characteristic of non-native fish species, as well as their response to the environmental changes in the freshwater ecosystem of the Liao River Basin. China has achieved rapid urban socioeconomic development and urban spatial expansion in recent decades, which have led to the loss and fragmentation of habitats [23,24]. However, the disturbance of ecological environments caused by human activities constitutes an important reason for non-native fish invasion [25]. Therefore, we hypothesize that (1) the distribution of non-native fish species in the Liao River Basin is considerably positively correlated with anthropogenic activities and socioeconomic development. The sustained increase in consumer income experienced by China over recent decades has resulted in people changing towards a more diversified diet, demanding greater quality in food [26,27]. Freshwater fish is an important and growing source of protein for China [27], and the traditional inland fishery species (e.g., carps and tilapia) usually have lower trophic levels than the newly developing species (e.g., Chinese perch, prawn, crab, and soft-shelled turtle). Therefore, we also hypothesize that (2) higher trophic level non-native fish species will likely prevail with the growth of socioeconomic development and anthropogenic activity intensity on a spatial scale.

## 2. Materials and Methods

## 2.1. Study Area

The Liao River Basin (116°40′ E to 125°35′ E, 40°28′ N to 45°12′ N), located in Northeastern China, covers the Liaoning, Jilin, and Hebei Provinces and the Inner Mongolia Autonomous Region (Figure 1). Its total drainage area covers 229,000 km<sup>2</sup>. The Liao River Basin belongs to a temperate semi-humid and semi-arid continental monsoon climate. The mean annual precipitation (MAP) in the area ranges from 350 mm to 900 mm, and the mean annual temperature (MAT) ranges from 4 °C to 10 °C. Land use in the Liao River Basin is dominated by row crop agriculture (38.29%), followed by grassland (23.65%), forestry (22.78%), bare land (6.45%), urban–rural residential land (4.43%), and water body (4.4%) [28]. The upper and middle reaches of the river basin are mainly covered by meadows and deserts, and the lower reach is mainly characterized by intensive human activity and urbanization.





**Figure 1.** Location of the study sites in the Liao River Basin in Northeast China. (black line: boundary of the Liao River Basin; filled circle: sampling sites; blue line: main streams; gray line: boundary of provinces).

# 2.2. Field Sampling and Identification

For a detailed investigation of the non-native fish species in the basin, we distributed the sampling sites in the basin as evenly as possible. A total of 153 sampling sites, which mainly include rivers, alongside lesser numbers of reservoirs and lakes, were surveyed from August to October 2016 and July to August 2017 (Figure 1). At each sampling site, fish specimens were captured using different sizes of fishing gears: three fixed nets (meshes from 10 mm to 50 mm, and 15 m to 30 m in length) and three ground net cages (lengths ranging from 20 m to 30 m) for 5 to 6 h between 9 am and 5 pm in the visual range (100 m). Furthermore, we investigated fishermen's catches to identify the non-native fishes near the sampling sites. The local fish markets near the sampling sites were also investigated. Fish were counted, identified, and recorded immediately after collection. All fish were then released, with the exception of those that could not be identified in the field. Unidentified fish were stored in a 10% formalin solution and brought back to the laboratory for identification [29–32].

## 2.3. Water Quality Parameters

In situ measurements included water temperature (TEM,  $^{\circ}$ C), pH, dissolved oxygen (DO, mg/L), electrical conductivity (EC, µs/cm) (YSI-80; YSI Inc., Yellow Springs, OH, USA) and current velocity (measured using a flotation method at two transects, 10 m apart from each other). Water samples

were collected in plastic bottles (200 mL) from 50 cm below the water surface. The water samples were transferred to polyethylene bottles and acidified by adding sulfuric acid to adjust the pH to below 2. The bottles were stored at 4 °C until they were ready for laboratory analysis. The water quality chemical parameters were analyzed in a laboratory. The permanganate index (COD<sub>Mn</sub>, mg/L) was determined using the potassium permanganate method. Total phosphorus (TP, mg/L), ammonia nitrogen (NH<sub>4</sub>–N, mg/L), soluble reactive phosphorus (SRP, mg/L), and total nitrogen (TN, mg/L) contents were measured with an ultraviolet spectrophotometer (PhotoLab S12, WTW Company, Munich, Germany). Nitrate nitrogen (NO<sub>3</sub>–N, mg/L) was detected using the digestion–ultraviolet (UV) spectrophotometric method (UV2800, UNICO Company, South Brunswick, NJ, USA). COD<sub>Mn</sub>, TP, and TN were measured using unfiltered samples, whereas NH<sub>4</sub>–N, SRP, and NO<sub>3</sub>–N were measured using filtered samples. Storage, preservation, and chemical analysis in the laboratory followed national standard methods of examining water and wastewater, which were provided by the Ministry of Environment Protection, China [33].

#### 2.4. Physical Habitat Factors and Climate Factors

The quality of physical habitats within the river was determined using the habitat evaluation index system which includes the following habitat factors: substrate, habitat complexity, velocity–depth combination, bank stability, channelization, channel flow status, water quality conditions, bank vegetative diversity, intensity of human disturbance, riverside land use, and aquatic plant abundance (Table A1). The habitat assessment index was derived from [34,35] and from the indices originally used in the U.S. Rapid Bioassessment Protocols [36]. Each parameter has four scoring categories: optimal (score of 16–20), suboptimal (score of 11–15), marginal (score of 6–10), and poor (score of 0–5). The score for each parameter at each sampling site was determined by expert visual evaluation in the field. Furthermore, the factors of the current climatic conditions, including mean annual precipitation (MAP) and mean annual temperature (MAT), were collected from national standard meteorological stations and rain gauging stations located within the Liao River Basin.

## 2.5. Data Analysis

The trophic level estimates of the non-native fish species were obtained from [37] and FishBase [29] according to their diet composition. The mean trophic level (MTL) of non-native fish species at each sampling site was calculated using the following formula:

$$MTL = \frac{Y_1 + Y_2 + \dots + Y_i}{N_i} \tag{1}$$

where  $Y_i$  is the trophic level of each non-native fish species i, and  $N_i$  is the number of non-native fish species at each sampling site.

ArcGIS 10.2 (ESRI Company, Redlands, CA, USA) was used to obtain the acreage of various types of land use areas (grassland, farmland, forest, construction, water, wetland, and others) within a radius of 5 km from the sampling sites. Furthermore, distribution maps of non-native fish species in the Liao River Basin were produced using the kriging interpolation tool in ArcGIS 10.2 (ESRI Company, Redlands, CA, USA). All explanatory variables are listed in Table 1.

Variables	Factors	Unit
Habitat factors	Substrate	/
	- Habitat complexity	/
	Velocity-depth combination	/
	Bank stability	/
	Channelization	/
	Channel flow status	/
	Water quality conditions	/
	Bank vegetative diversity	/
	Intensity of human disturbance	/
	Riverside landuse	/
	Aquatic plant abundance	/
Water quality	Water temperature (TEM)	°C
1 5	- pH	/
	Dissolved oxygen (DO)	mg/L
	Electrical conductivity (EC)	μs/cm
	Permanganate index (COD <sub>Mn)</sub>	mg/L
	Total phosphorus (TP)	mg/L
	Ammonia nitrogen (NH <sub>4</sub> –N)	mg/L
	Soluble reactive phosphorus (SRP)	mg/L
	Total nitrogen (TN)	mg/L
	Nitrate nitrogen (NO <sub>3</sub> –N)	mg/L
Land use	Farmland area	km <sup>2</sup>
	Water area	km <sup>2</sup>
	Construction area	km <sup>2</sup>
	Grassland area	km <sup>2</sup>
	Forest area	km <sup>2</sup>
	Wetland	km <sup>2</sup>
	Other	km <sup>2</sup>
Climate	Mean annual precipitation (MAP)	mm
	Mean annual temperature (MAT)	°C

Table 1. List of all explanatory variables.

The relationship between habitat factors, water quality parameters, land use, and climate factors and the presence, number, and MTL of non-native fish species was assessed using generalized linear models (GLMs) in the package stats [38]. The multicollinearity among the explanatory variables was measured by variance inflation factors (VIF). Despite there being no formal rule, it is generally accepted that a VIF value greater than 10 is often thought to indicate harmful collinearity [39]. Then, these explanatory variables causing multicollinearity were dropped from the model. The data of the explanatory variables were root transformed to improve their normality, and the data were scaled before performing the analysis. The GLMs were fitted to a binomial distribution (link = "logit"), Gaussian distribution (link = "identity") and Poisson distribution (link = "logi"), according to the response variable form. The data of the response variable MTL was the square root transformed to Gaussian distribution. Model selection was performed by ranking the candidate models based on their Akaike information criterion (AIC) and selecting the model that minimizes AIC as the best supported model [40,41]. All the statistical analyses were performed in R for Windows (R Foundation for Statistical Computing, Vienna, Austria) [38].

# 3. Results

#### 3.1. Information and Distribution of Non-Native Fish Species

Nine non-native fish species in the Liao River Basin were found during the investigation (Table 2). Non-native fish species found in the basin include the following: (1) fish native to China that

had been translocated outside their natural range (*Mylopharyngodon piceus*, *Ctenopharyngodon idellus*, *Hypophthalmichthys molitrix*, *Aristichthys nobilis*, *Carassius auratus* var. Pengze, and *Megalobrama amblycephala*); and (2) non-native species from other countries or regions that had been introduced to China (*Micropterus salmoides*, *Tinca tinca*, and *Cyprinus carpio* var. *specularis*). These species are listed in Table 2. The trophic levels of the non-native fish species ranged between 2.00 and 3.84 (Table 2).

Latin Name	Trophic Level	Origin	
Mylopharyngodon piceus	3.00	Lower reaches of Heilong River, Wusuli River, Songhua River Basin, Haihe Plain, middle and lower reaches of Yellow River, Huaihe Plain, Yangtze River Basin, Qiantang River Plain, Pearl River Plain (China)	
Ctenopharyngodon idellus	2.00	Heilong River Basin, Haihe Plain, middle and lower reaches of Yellow River, Huaihe Plain, Yangtze River Basin, Qiantang River Plain, Pearl River Plain, Pearl River (China)	
Hypophthalmichthys molitrix	2.30	Heilong River Basin, Wusuli River, Songhua River, lower reaches of Liao River, Haihe River Plain, middle and lower reaches of Yellow River, Huaihe River Plain, Yangtze River, Qiantang River Plain, Pearl River Plain (China)	
Aristichthys nobilis	2.40	Haihe River Plain, Yellow River Plain, Yangtze River Plain, Qiantang River Plain, Pearl River Plain, Pearl River (China)	
<i>Carassius auratus</i> var. Pengze	2.50 <sup>1</sup>	Pengze County, Jiangxi Province (China)	
Megalobrama amblycephala	2.00	Middle and lower reaches of Yangtze River (China)	
Micropterus salmoides	3.84	North America	
Tinca tinca	3.69	Europe	
Cyprinus carpio var. specularis	3.06 <sup>2</sup>	Europe	

Table 2. List of non-native fish species in the Liao River Basin.

<sup>1</sup> The trophic levels of *Carassius auratus* var. Pengze and *Carassius auratus* estimated by Zhang, Wu et al. (2013) were considered the same. <sup>2</sup> The trophic level of *Cyprinus carpio* var. *specularis* was considered the same as *Cyprinus carpio* in FishBase.

The distribution map of non-native fish species in the Liao River Basin shows that more cities in the southeast have non-native fish species than other regions in the basin and non-native fish species are mostly concentrated around cities (Figure 2). The southeastern part of the basin has the highest number of non-native fish species. By contrast, the rainless areas in the middle–northern part of the basin have the lowest number of fish species (Figure 2).

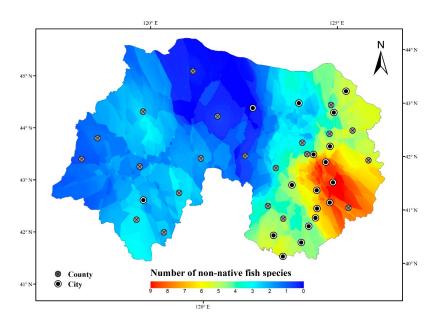


Figure 2. Distribution of non-native fish species in the Liao River Basin.

## 3.2. Associations Between Environmental Variables and the Distribution of Non-Native Fish Species

There was a significant relationship between the presence of non-native fish species and the habitat factors and land use in the Liao River Basin (Table 3). Channelization was negatively correlated with the presence of non-native fish species (Table 3). Channelization was scored on the spot. The higher the score, the less channelization; the lower the score, the wider the channelization, an embankment or bridge prop appears on two sides, and the habitat is completely changed (Table A1). Water area was positively correlated with the presence of non-native fish species. It was showed that non-native fish species were also more likely to occur where there was greater water area (Table 3). There was no significant correlation between water quality parameters, climate factors, and the presence of non-native fish species.

Variables	Coefficient	SE	<i>p</i> Value
Habitat factors			
Channelization Water quality	-2.9993	1.1520	<0.01
- Land use	-	-	-
Water area Climate	1.7646	0.6502	<0.01
-	-	-	-

Table 3. Associations between environmental variables and the presence of non-native fish species.

The number of non-native fish species was correlated with habitat factors, water quality, land use, and climate factors within the Liao River Basin (Table 4). Changes in channelization exert a statistically negative relationship with the number of non-native fish species. By contrast, channel flow status, dissolved oxygen, water area, construction area, and MAP were positively correlated with the number of non-native fish species (Table 4).

Variables	Coefficient	SE	p Value
Habitat factors			
Channelization	-1.1272	0.2821	< 0.01
Channel flow status <b>Water quality</b>	0.8538	0.4055	0.035
DO Land use	2.0883	0.6739	<0.01
Water area	0.7327	0.2295	< 0.01
Construction area <b>Climate</b>	0.5239	0.2376	0.027
MAP	2.7251	1.0483	< 0.01

Table 4. Associations between environmental variables and the number of non-native fish species.

## 3.3. Associations Between Environmental Variables and the MTL of Non-Native Fish Species

The MTL of non-native fish species in the sampling sites was only correlated with habitat factors within the Liao River Basin (Table 5). The substrate and riverside land use had a statistically positive relationship with the MTL of non-native fish species, which was negatively correlated with habitat complexity and bank stability (Table 5). Water quality parameters, land use, and climate factors were not correlated with the MTL of non-native fish species (Table 5).

**Table 5.** Associations Between Environmental Variables and the mean trophic level (MTL) of Non-Native Fish Species.

Variables	Coefficient	SE	p Value
Habitat factors			
Substrate	0.0848	0.0260 0.0219	<0.01 0.018
Habitat complexity Bank stability	-0.0531 -0.0735	0.0219	0.018
Riverside land use <b>Water quality</b>	0.0633	0.0297	0.037
- Land use	-	-	-
- Climate	-	-	-
-	-	-	-

# 4. Discussion

To our knowledge, this study is the first to comprehensibly investigate the relationship between different types of influencing factors and non-native fish species on a basin scale in China. Our results show that the factors which exert a significant relationship with the distribution of non-native fish species in the Liao River Basin can be divided into four categories: (1) habitat factors, including channelization and channel flow status; (2) water quality parameters, including dissolved oxygen; (3) land use, including water and construction areas; (4) climate factors, including MAP. Interestingly, the MTL of non-native fish species at the sampling sites was only correlated significantly with habitat factors.

Our initial expectations are partially supported by the results of the GLMs, which indicate that the distribution of non-native fish species is positively correlated to the channelization extent and construction area, two factors which represent anthropogenic activities and socioeconomic development

(Tables 1 and A1). China's inland fisheries have developed rapidly in recent decades [42]. The nine non-native fish species investigated in the current study are all aquaculture species introduced from the 1950s to the 1990s in order to increase yield and economic benefits [31]. In addition, population size exhibits a direct relationship with the development of inland fisheries because population growth is always correlated with an increase in aquatic food consumption and aquaculture production [43,44]. To meet the increase in demand, the frequent transportation and aquaculture of a large number of aquatic products have enabled many non-native fish species to enter the region. The anthropogenic activity indicators of the world's river basins were positively related to the number of established non-native fish species [45]. Thus, more non-native fish species are present in basin areas with a higher intensity of anthropogenic activities and socioeconomic development. However, our second hypothesis, namely that the mean trophic level of non-native fish species will increase with the growth of socioeconomic development and anthropogenic activity intensity, is not supported by the results. The results indicate that only habitat factors (substrate, riverside land use, habitat complexity, and bank stability) were correlated with the MTL of sampling sites, and these factors have almost no direct connection with human activities and socioeconomic development (Tables 5 and A1). The results suggest that the mean trophic level of non-native fish species does not increases with the intensity of anthropogenic activities and socioeconomic development on a spatial scale within the Liao River Basin. This finding is very likely to be related to the size of the basin, which is not very large. Thus, the close interaction between socioeconomic activities and the convenience of transportation have probably resulted in no differences in fish trophic levels across the Liao River Basin.

In general, successful non-native species tend to adapt to a wide range of environmental factors and exhibit high tolerance to the environment conditions [46]. We have found that the presence of non-native fish species is not correlated with water quality, and that the abundance of species is only related to dissolved oxygen. Species with wide niche breadths can effectively use various resources to establish wild populations and wide distributions [47]. For example, *Procambarus clarkii*, which is native to North America, has been introduced to many parts of the world as an important economic species for fisheries [48,49]. Given its extensive habitat adaptability, *Procambarus clarkii* can survive in water bodies with poor water quality, establish wild populations rapidly, and considerably impact local habitats [49–52]. Our results show that water quality exerts almost no significant relationship with the distribution of non-native fish species in the Liao River Basin. This finding suggests that non-native fish species are probably adaptable to environmental conditions, and the water quality conditions in the basin are not constraints to their survival or even to their reproduction. Thus, these non-native fish species may establish wild populations rapidly and then affect local freshwater ecosystems.

Water area, channel flow status and MAP are positively correlated with the distribution of non-native fish species, thereby suggesting an increase in the possibility of the appearance and an increase in numbers of non-native fish species in areas where abundant rainfall occurs. In general, areas with more water are more suitable for the survival of aquatic organisms than arid regions, and thus, increased precipitation in arid and semiarid areas allows the invasion of non-native species [53]. The southeastern part of the basin receives the most annual precipitation within the basin. This condition suggests that the southeastern part of the Liao River Basin is more likely to be abundant in non-native fish species. Furthermore, our results also indicate that the water area in the southeastern part of the basin where invasive species may potentially exist is larger.

Nowadays, the rapid development of inland fisheries can exacerbate pressures on already threatened inland water bodies [54,55]. The introduction of non-native fish species may cause serious biodiversity problems in the future, such as species invasion, extinction, genetic pollution, and economic loss [56]. Our study allows the quantification of the threats of non-native fish species by simultaneously considering different sources of factors within the basin. In view of our findings, the prioritization of prevention and management must be strengthened in the southeastern region, and the arid freshwater ecosystems in the central and western regions should also receive increased attention. Besides, ecologically sustainable development needs to seek a balance between the benefits

and costs (environmental, economic, social) of an activity in relation to the introduction of new non-native fish species [57].

# 5. Conclusions

We provide the evidence for the links between non-native fish species and different types of factors on a basin scale. Our results demonstrate that the distribution of non-native fish species is positively correlated with anthropogenic activities and socioeconomic development, but the mean trophic level of non-native fish species is not correlated with socioeconomic development and anthropogenic activities on a spatial scale across the Liao River Basin. The southeastern part of the Liao River Basin is conducive to the distribution of non-native fish species. Such evidence is highly important for early detection and managing freshwater ecosystems to prevent non-native fish invasion on a basin scale.

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Conflicts of Interest: The authors declare no conflict of interest.

# Appendix A

Habitat Factors	Optimal	Suboptimal	Marginal	Poor
Substrate	More than 75% are gravel, pebbles and boulders	50%~75% are gravel, pebbles and boulders	25%~50% are gravel, pebbles and boulders	Less than 25% are gravel, pebbles and boulders
Habitat complexity	Composition of aquatic vegetation, dead branches and leaves, fallen wood, sunken dikes, boulders and other microhabitats	Composition of aquatic vegetation, dead leaves, dikes and other small habitats	Domination by two or three kinds of microhabitat	One kind of microhabitat
Velocity-depth combination	Four types of velocity-depth habitat (slow-deep, slow-shallow, fast-deep, fast-shallow) (slow <0.3 m/s, deep >0.5 m)	Three types of velocity-depth habitat	Two types of velocity-depth habitat	Only one type of velocity–depth habitat
Bank stability	Less than 5% of the riverbanks have eroded areas in the visual range (100 m)	5%~30% of the riverbanks have eroded areas in the visual range (100 m)	30%~60% of the riverbanks have eroded areas in the visual range (100 m)	More than 60% of the riverbanks have eroded areas in the visual range (100 m)

**Table A1.** Habitat assessment scoring descriptions in Liao River Basin.

Habitat Factors	Optimal	Suboptimal	Marginal	Poor
Channelization	No channelization	Channelization is rare, usually around the piers	Channelization is normal, embankment or bridge prop appear on two sides	Channelization is wide, embankment or bridge prop appear on two sides and habitat completely changed
Channel flow status	Large water volume and channel sediment is not exposure	Relatively large water volume and less than 25% channel sediment exposure	25%~75% of channel sediment exposure	Small water volume and dry river course
Water quality conditions	Very clear, no smell, no sediment at static condition	Relatively clear, with a small amount of odor, a small amount of sediment at static condition	Turbid, odor, has sediment at static condition	Very turbid and foul smelling at static condition
Bank vegetative diversity	More than 50% coverage of vegetation	25%~50% coverage of vegetation	Less than 25% coverage of vegetation	Hardly any vegetation coverage
Intensity of human disturbance	No human activities	Minimal human disturbance by few walkers or bikes	Less human disturbance by vehicles	Serious human disturbance by vehicles or ships
Riverside land use	No cultivated soil on both riversides	No cultivated soil on the one riverside and cultivated soil on the other riverside	Cultivated soil on both riversides	Weathered soil after fallow conditions present on both riversides
Aquatic plant abundance	75%~100% aquatic plant coverage	50%~75% aquatic plant coverage	25%~50% aquatic plant coverage	Less than 25% aquatic plant coverage
Score	16–20	11–15	6–10	0–5

## Table A1. Cont.

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