EVALUATION OF WATER ECOLOGICAL STATUS OF THE BILIU RIVER BASIN BASED ON FISH INDEX OF BIOLOGICAL INTEGRITY IN NORTHEAST CHINA

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Abstract. A total fish of 31 species, 20 genera 9 families were found in the Biliu River Basin in northeast China, including 1384 fish in the autumn of 2015, 1148 fish in the spring and 1801 fish in the summer of 2016. Through interference response, index distribution, discriminant ability analysis, normal distribution test and correlation analysis of 31 candidate indicators, and fish index of biological integrity in the Biliu River was established. Moreover, by using the ration scoring method and the step method to unify the standardized index, the indices were added to get the Fish Index of Biological Integrity (F-IBI) value. The results showed that the overall situation of the Biliu River downstream was bad while the situation of its upstream was good. The results indicated that the specimen near the source point (sampling site 12 and 13) and Ge Li River (sampling site 14, 15 and 16) was healthy or sub-healthy. The other sampling points were sub-healthy or generally well. The results of the two evaluation methods were consistent, indicating that the F-IBI evaluation results were more reliable and could be used as an effective evaluation method to promote the application.
Keywords: *F-IBI, ecosystem health, assessment, freshwater, river*

Introduction

Ecosystem healthy rivers with normal function can meet the basic needs of the ecosystem (Boulton, 2010; Norris and Thoms, 2010). As a developing country, Australia was notoriously short of funds for sustained long-term surveys of their water courses (Fairweather, 1999). Historically, the assessment of river ecosystem always focused on water quality. According to physical and chemical factors, water was divided into several levels (Silveira et al., 2005; Lu et al., 2019). Although this is convenient, especially today with advanced instruments, it is not very comprehensive and it only reflects partially (John et al., 2021; Li et al., 2022; Lu et al., 2022). However, assemblages of freshwater organisms could be used to assess the biotic condition of rivers, lakes, and wetlands because the integrity of these assemblages provides a direct measure of ecological conditions of these freshwater resources such as fish, macroinvertebrates and plankton (Worthington, 1954; Jones et al., 2016; Lu et al., 2020). Fishes have been regarded as effective biological indicators of environmental quality and anthropogenic stress in aquatic ecosystems (Hu et al., 2006; Luque and Poulin, 2010; Freedman et al., 2012). Fish

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responses to environmental disturbances are different in spatiotemporal distribution, which always have been selected as an indicator to assess the environmental health (Harrison and Whitfield, 2010; Williams et al., 2010; Wolter et al., 2015; Sarrazin et al., 2021).

Rivers are providing food, industry, agriculture, and domestic water, also they are main channels for material circulation, energy flow and information exchange between terrestrial ecosystems and aquatic ecosystems (Wu et al., 2005; Zhu et al., 2014; Zhang et al., 2015). A healthy aquatic ecosystem can maintain its own organizational structure and has a certain degree of resilience and self-regulation (Bain et al., 2000; Li et al., 2021a). In recent years, human interference and damage to nature have become increasingly intense (Li et al., 2013). Urban sewage, industrial wastewater and agricultural waste water are continuously discharged into rivers, causing great harm to river ecological environment (Lu et al., 2021; Schweizer et al., 2022). Even causing river flow interruption, wetland loss, regional ecological environment degradation, biodiversity reduction and other problems in serious cases (Huang, 2010; Chatterjee et al., 2015). Maintaining the health of rivers is very important to the earth's ecosystem and human beings, which is becoming the most dynamic frontier field in ecology today (An and Choi, 2003; Al-Janabi et al., 2016; Zhu et al., 2021; Bacigalupi et al., 2021).

The index of biological integrity (IBI) was first proposed and defined by Karr and Dudley (1981). It was initially established with fish as the research object and applied to streams and rivers in the Midwest of the United States. The fish index of biological integrity (F-IBI) was originally applied to streams and rivers in the Midwest of the United States (Karr, 1981). Fish is used as an indicator species because it is widely distributed and is a highly trophic organism in the aquatic ecosystem (Moyle and Randall, 1998; Lydy et al., 2000; Mebane et al., 2003). Following the application of F-IBI in the United States, scholars were also evaluated the health of rivers in various countries through improved indicators and other methods in other countries (Oberdorff and Hughes, 1992; Klemm et al., 2003). In China, Zhu and Chang (2004) studied the spatio-temporal changes in the biological integrity of shallow lakes in the middle reaches of the Yangtze River for the first time. Besides, Song et al. (2010) was selected 5 metrics from 23 candidate metrics and used stepped scoring metrics to evaluate the 61 sites of the Taizi River Basin in northeast of China.

Biliu river is the largest river in Dalian City of Liaoning Province in China, which is the largest source of drinking water in Dalian. While the research on the evaluation of water ecosystem in Biliu River Basin has not been reported. This study screened the biological integrity index and constructed the F-IBI system through the annual survey of fish composition and quantity (spring, summer, and autumn) in the Biliu River Basin. By evaluating the health status of water ecosystem in the Biliu River Basin, this study aims to provide reference for the direction and methods of environmental protection and pollution control, which could establish an index of biological integrity evaluation system suitable for the characteristics of rivers in China.

Materials and methods

Study area

The source of Biliu River basin is Miaogou which is located in the vicinity of Chishan scenic area of Gaizhou city, the mountains near Miaogou belong to the Changbai Mountains, dominated by Karst landform and dense vegetation in the mountains. It has a

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relatively primitive ecological environment, there are no roads and cars cannot pass. The Biliu River drains about 2814 km^2 and flows over 156 km. The Biliu River flows into the Yellow Sea at the estuary of Jianshan village, Pulandian city in northeast China.

According to the merging of the tributaries and the extent of human interference, the sampling sites were selected at intervals of 10-15 km in the Biliu River basin (*Fig. 1*), every sampling site was marked with a Global Positioning System code (*Table 1*). Between October 2015 and July 2016, all 16 sites were sampled seasonally (autumn: October; spring: March; summer: July; winter: Not sampled because of ice). The longitude and latitude, elevation, environment (including the presence or absence of a village, factory, bridge, highway nearby, also vegetation coverage rate, and any anthropogenic stress), width, depth, transparency of every site were measured and recorded.

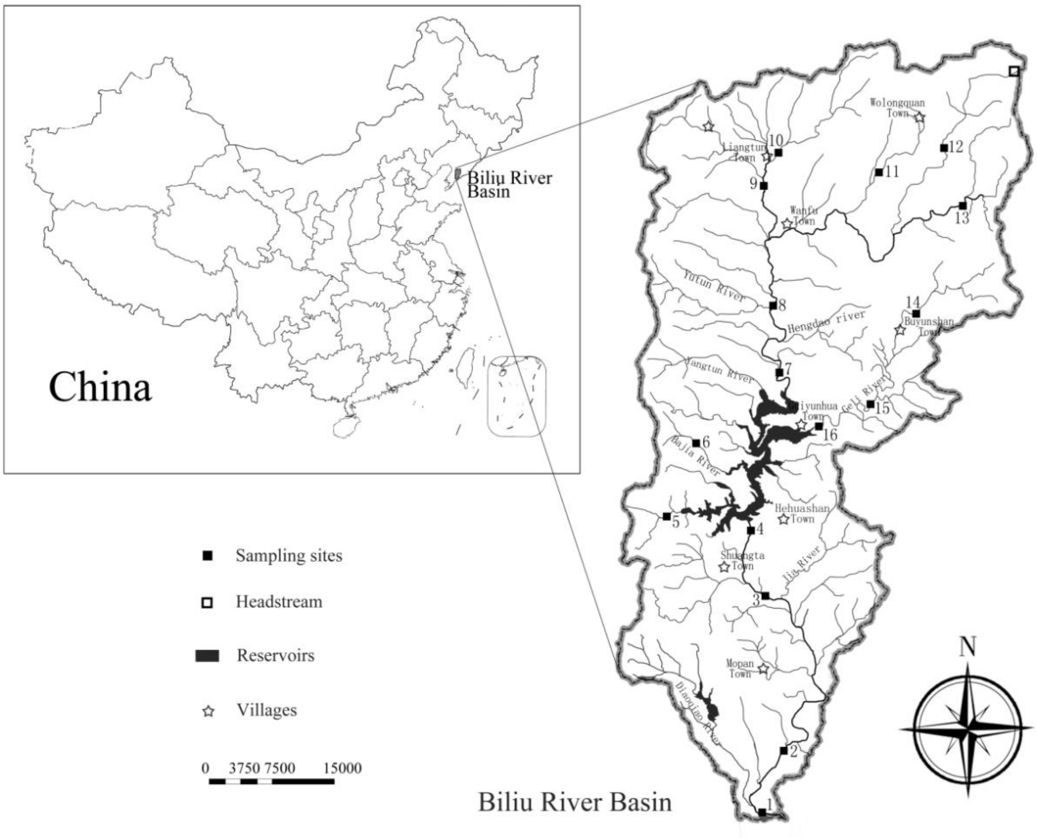

Figure 1. *Study area*

Field sampling and laboratory analysis

Fish assembles were collected along 500 m line transects for 15 min following the under 1.5 m depth contour using standard electric fishing protocols (350 V, 3-4 A). At a depth of more than 1.5 m according to the local condition of the river, sampling was carried out with a three-drift net (length of 200 m, net size of 1.5-3 cm) for 30 min. The samples were identified at once, scientific name, quantity, length, weight, spawn were recorded and then replaced back into the river. Unknown fish were fixed with methanol and taken back to the laboratory for further identification, according to the "Fauna of Liaoning Fish" (Liu and Qin, 1987), and "Fishes in Northeast Region of China" (Xie, 2007).

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***Table 1.** Information of sampling sites*

Sampling sites	Name	Longitude	Latitude	Elevation (m)	Property
1	Entrance to the sea	122°34'04" E	39°28'32" N	25	Impaired
2	Biliu River Bridge	122°32'42" E	39°34'03" N	36	Impaired
3	Badger cave	122°30'43" E	39°44'16" N	52	Impaired
4	Downstream of Biliu River reservoir	122°29'49" E	39°48'12" N	62	Impaired
5	Jindian Bridge	122°22'56" E	39°49'28" N	43	Impaired
6	Xiongcheng line	122°25'59" E	39°53'32" N	52	Impaired
7	Upstream of Biliu River reservoir	122°32'19" E	39°58'43" N	50	Impaired
8	Xiaojialu Bridge	122°31'40" E	40°02'58" N	60	Impaired
9	Under Zhuang-Gai Expressway Bridge	122°30'49" E	40°11'11" N	49	Impaired
10	Liang village	122°32'09" E	40°13'03" N	38	Reference
11	Fangshen village	122°40'24" E	40°11'44" N	32	Impaired
12	Birthplace downstream	122°46'17" E	40°13'04" N	34	Reference
13	Taiping village	122°47'55" E	40°09'53" N	20	Impaired
14	Buyunshan township	122°43'42" E	40°02'37" N	25	Impaired
15	Sandao River Bridge	122°39'47" E	39°56'24" N	45	Impaired
16	Guiyunhua township	122°35'02" E	39°55'03" N	63	Impaired

Data analysis

Reference site selection

The reference site is the sample point in the original state or undisturbed state, and the damaged point is the sample point that has been obviously disturbed by human activities. For aquatic ecosystem health assessment, the selection of reference points is a key factor (Karr, 1981). The principle of selecting reference site generally needs to refer to historical research as the basis and select the original state or the river section without human interference as the reference point. However, with the increasing intensity of human activities in today's society, there are few river sections that have been disturbed by no one. And so far, there is no unified standard for the selection of reference points (Blocksom et al., 2002; Morley and Karr, 2002), and no historical data on the environment of the Biliu River Basin has been published. In this study, combined with the pre-investigation and field sampling, sampling site 10 and 12 met the standards of reference sites, and the other 14 points were impaired site (*Table 1*), which were the least affected by human interference, and there were no farmlands, roads and villages around.

F-IBI candidate indicators

The formulation of candidate indicators refers to the relatively mature indicators in the application of F-IBI to evaluate river health at home and abroad, combined with the research content and actual investigation of this survey. In this study, a total of 31 candidate indicators (*Table 2*) are applied to reflect the impact of environmental changes on the number, structure, and function of fish (individuals, populations, communities) as much as possible, so as to effectively evaluate the health status of the river.

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Table 2. *List of candidate indicators*

Indicator types	No.	Indicators	Indicators description	Response to interference
Species composition and richness	F1	Total fish species	Number of species collected at each sampling site	Decrease
	F2	Total catch	Total number of fish collected at each sampling site	Decrease
	F3	Shannon-Wiener diversity index (*H'*)	Fish species diversity index at each sampling site	Decrease
Proportion of individual number of species	F4	%Petromyzonidae	*Lampetra mori*	Decrease
	F5	%Leuciscinae	*Zacco platypus*, *Phoxinus lagowskii*, *Phoxinus oxycephalus*	Decrease
	F6	%Acheilognathinae	*Rhodeus ocellatus*, *Rhodeus sinensis*, *Rhodeus sericeus*	Decrease
	F7	%Gobioninae	*Pseudorasbora parva*, *Abbottina rivularis*, *Abbottina liaoningensis*, *Pseudogobio vaillanti*, *Huigobio chinssuensis*	Increase
	F8	%Cyprininae	*Carassius auratus*	Increase
	F9	%Cobitidae	*Nemachilus nudus*, *Lefua costata*, *Barbatula barbatula nuda*, *Cobitis granoei*, *Misgurnus anguillicaudatus*	Decrease
	F10	%Bagridae	*Pelteobagrus fulvidraco*, *Pelteobaggrus nitidus*	Decrease
	F11	%Siluridae	*Silurus asotus*	Decrease
	F12	%Oryziidae	*Oryzias latipes*	Decrease
	F13	%Eleotridae	*Perccottus glehni*, *Hypseleotris swinhonis*	Decrease
	F14	%Gobiidae	*Tridentiger obscurus*, *Tridentiger trigonocephalus*, *Ctenogobius pflaumi*, *Ctenogobius giurinus*, *Ctenogobius brunneus*, *Ctenogobius cliffordpopei*	Decrease
	F15	%Channidae	*Ophiocephalus argus*	Decrease
	F16	%Endemic fish species	*Perccottus glehni*	-
	F17	%Economic fish species	*Carassius auratus*, *Misgurnus anguillicaudatus*, *Silurus asotus*, *Perccottus glehni*, *Ophiocephalus argus*	Increase
Tolerance	F18	%Sensitive fish species	*Cobitis granoei*, *Perccottus glehni*	Decrease
	F19	%Tolerant fish species	*Misgurnus anguillicaudatus*, *Carassius auratus*	Increase
Nutritional structure	F20	%Number of carnivorous fish	*Pelteobagrus fulvidraco*, *Pelteobaggrus nitidus*, *Silurus asotus*, *Oryzias latipes*, *Ophiocephalus argus*	Decrease
	F21	%Number of omnivorous fish	*Lampetra mori*, *Zacco platypus*, *Phoxinus lagowskii*, *Phoxinus oxycephalus*, *Pseudorasbora parva*, *Abbottina rivularis*, *Abbottina liaoningensis*, *Pseudogobio vaillanti*, *Huigobio chinssuensis*, *Carassius auratus*, *Nemachilus nudus*, *Lefua costata*, *Nemachilus nudus*, *Misgurnus anguillicaudatus*, *Perccottus glehni*, *Hypseleotris swinhonis*, *Tridentiger obscurus*, *Tridentiger trigonocephalus*, *Ctenogobius pflaumi*, *Ctenogobius giurinus*, *Ctenogobius brunneus*	Increase

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Indicator types	No.	Indicators	Indicators description	Response to interference
	F22	%Number of phytophagous fish	*Rhodeus ocellatus*, *Rhodeus sinensis*, *Rhodeus sericeus*, *Cobitis granoei*, *Ctenogobius cliffordpopei*	Decrease
Individual condition	F23	%Abnormal individuals	Deformities, lesions, tumors, fin erosion	Increase
	F24	%Number of brood fish	Number of brooding fish / total fish	Decrease
Habitat quality	F25	%Pelagic fish	*Phoxinus lagowskii*, *Phoxinus oxycephalus*	Decrease
	F26	%Upper-middle layer fish	*Zacco platypus*, *Rhodeus ocellatus*, *Rhodeus sinensis*, *Rhodeus sericeus*, *Oryzias latipes*	Decrease
	F27	%Mid-lower layer fish	*Pseudorasbora parva*, *Abbottina rivularis*, *Abbottina liaoningensis*, *Pseudogobio vaillanti*, *Huigobio chinssuensis*, *Nemachilus nudus*, *Lefua costata*, *Carassius auratus*, *Perccottus glehni*, *Tridentiger obscurus*, *Tridentiger trigonocephalus*, *Ctenogobius brunneus*	Decrease
	F28	%Demersal fish	*Lampetra mori*, *Barbatula barbatula nuda*, *Cobitis granoei*, *Misgurnus anguillicaudatus*, *Pelteobagrus fulvidraco*, *Pelteobaggrus nitidus*, *Silurus asotus*, *Hypseleotris swinhonis*, *Ctenogobius pflaumi*, *Ctenogobius brunneus*, *Ctenogobius cliffordpopei*, *Ophiocephalus argus*	Increase
	F29	%Cold water fish	*Phoxinus lagowskii*, *Phoxinus oxycephalus*, *Nemachilus nudus*, *Perccottus glehni*	-
	F30	Number of fish with egg protection behavior	*Pseudorasbora parva*	Increase
Environmental quality	F31	Number of widely distributed species	*Phoxinus lagowskii*, *Phoxinus czekanowskii*	Decrease

F-IBI determination

All indicators proposed in *Table 2* were screened as follows, and the indicators that met the conditions were retained, and the indicators that did not meet the conditions were deleted. Finally, the retained indicators were determined as the F-IBI evaluation index system of the Biliu River Basin.

Response of candidate indicators to interference was determined as follows: the index value of each candidate indicator was calculated according to the survey data of reference points and damaged points and the response of candidate indicators to human interference were analyzed. The indicators that increase or decrease unidirectionally with the increase of human interference intensity were selected and those that do not change unidirectionally or not change with human interference were deleted.

Indicator distribution range was determined as follows: the indicators filtered in the previous step were further filtered, and the indicators with more than 90% of the sampling points being 0 were deleted, and the indicators with the difference between the maximum and minimum values of all sampling points being less than 10% were deleted.

Discriminant ability analysis of biological parameters was carried out as follows: the overlap of reference points and damaged points of each index in the range of 25%-75% quantiles were compared with the box graph method and different values were assigned to each index. As shown in *Figure 2*, A is no overlap, IQ=3,; B is partial overlap, but their median values are outside the range of the other box, IQ=2; C and D are partial overlaps, and there is only one median within the range of the other box, IQ=1,; E refers to most overlaps, and their median values are within the range of each other's box, IQ=0. Indicators with IQ less than 2 were deleted. Only indicators with IQ≥2 (*Fig. 2A and B*) can be further analyzed.

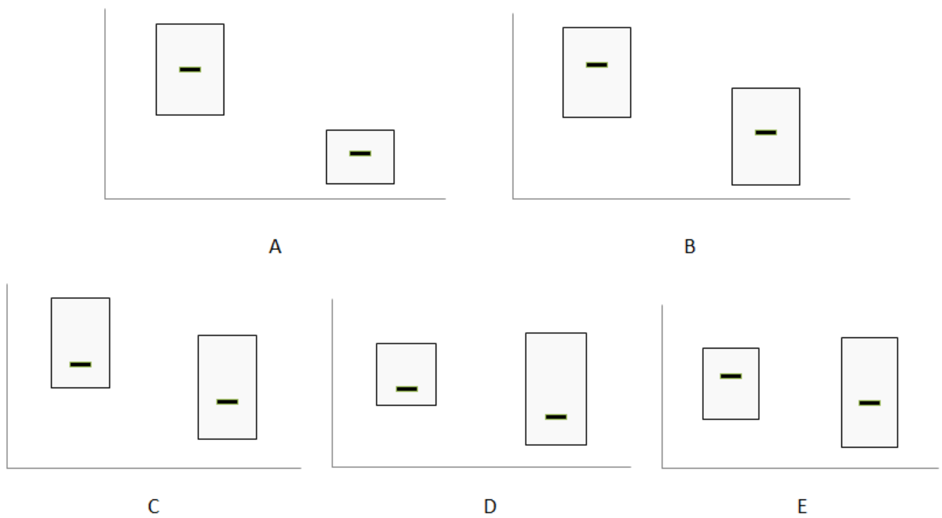

Figure 2. *Discriminatory power analysis of index*

In the correlation analysis the normal distribution of the remaining indicators were tested. For the indicators that conform to the normal distribution, the Pearson correlation coefficient was calculated, While the indicators that did not conform to the normal distribution, the Sperman correlation coefficient was calculated, and the degree of information overlap between biological indicators was determined according to the significant level of correlation. If the two indicators correlated significantly (|R| > 0.9), indicating that most of the information reflected by the two overlaps, the more reflective indicator was chosen.

After the above screening, the remaining indicators constituted the F-IBI index system for evaluating the water ecological health of the Biliu River Basin.

F-IBI calculation method and evaluation standard

The purpose of scoring the candidate indicators is to unify the evaluation dimension. In this study, the general ratio method and 1,3,5 assignment method (three scoring method) are used to evaluate the Biliu River Basin F-IBI, respectively.

(1) Ratio method

For the index that value decreases with the increase of interference, the standardized index formula is:

$$P_{ij} = O_{ij}/S_{i95} \quad \text{(Eq.1)}$$

where, P_{ij} is the standardized index of the ith index at the jth sampling site, O_{ij} is the original observation value of the ith index at the jth sampling site, S_{i95} is the 95% quantile value of the ith index at the jth sampling site.

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For the index that value increases with the increase of interference, the standardized index formula is:

$$P_{ij} = (\max O_{ij} - O_{ij})/(maxO_{ij} - S_{i5}) \quad \text{(Eq.2)}$$

where, $\max O_{ij}$ is the maximum value of the ith index at the jth sampling site, S_{i5} is the 5% quantile value of the ith index at the jth sampling site.

The final score of F-IBI at each sampling site is the sum of the standardized index values.

(2) 1,3,5 assignment method (three scoring method)

This method is the most widely used method at present, and its parameter standardization method is: the actual values measured for each index are divided into three equal parts in the range from the lowest to the highest, and are divided into three areas. The best grade area is recorded as 5, the middle is recorded as 3, and the worst is recorded as 1. The final F-IBI score of each sampling point is the sum of the standardized values of each index.

Evaluation standard: The health standard is determined according to the 75% quantile of the final score of F-IBI at all sampling sites. If the F-IBI score is bigger than 75%, it means that the point is health. The data smaller than 75% quantile of all sampling sites are quartered to represent different health levels in turn. According to the above methods, the evaluation criteria of F-IBI system in the Biliu River Basin were determined as healthy, sub-healthy, general, poor, and bad.

Results

Fish community structure characteristics in the Biliu River Basin

A total of 4333 fish belonging to 9 families, 20 genera and 31 species (*Appendix*) were collected in this survey, 1384 in autumn, 1148 in spring and 1801 in summer. Among the fish species, Cyprinidae owned the largest number of species, accounting for 38.71%, followed by Gobiidae (accounting for 19.35%), while Siluridae and Channidae were both the least, accounting for only 3.23% (*Fig. 3*). In terms of quantity, Cyprinidae accounted for 75.55% of the total fish collected, followed by Cobitidae accounting for 17.35%, and Cyprinidae accounting for 4.02% (*Fig. 4*).

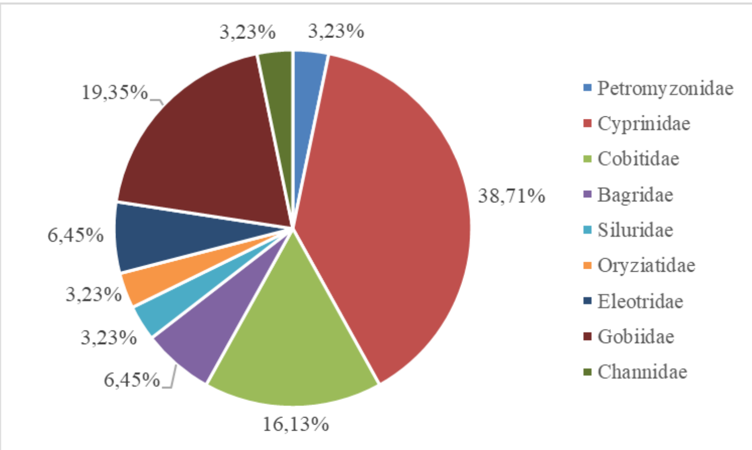

Figure 3. *Fish assemblage structure*

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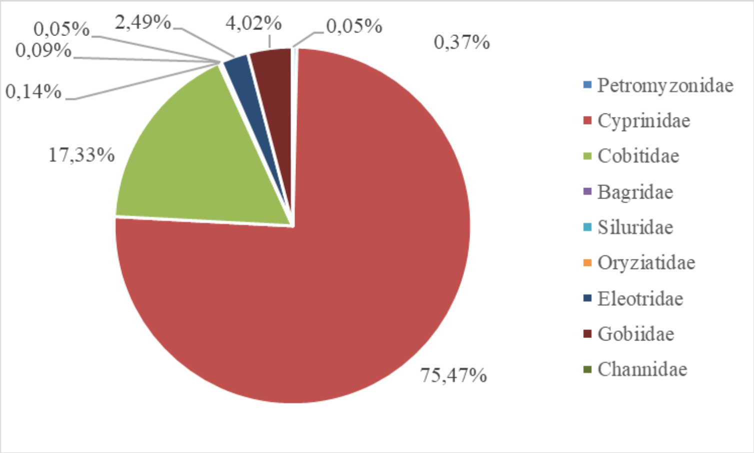

Figure 4. *Fish quantitative distribution*

Pale chub (*Zacco platypus*) and Granoc's spined loach (*Cobitis granoei*) are widely distributed species in the Biliu River Basin, and their distribution accounts for 87.50%. Besides, weatherfish (*Misgurnus anguillicaudatus*), Amur minnow (*Phoxinus lagowskii*), Rounded gudgeon (*Abbottina rivularis*), Northern stone loach (*Nemachilus nudus*), Sharp-head minnow (*Phoxinus czekanowskii*) and topmouth minnow (*Pseudorasbora parva*) are also widely distributed at various points. Among them, sampling site 1 is located at the estuary of the downstream of the Biliu River. The current is turbulent and restricted by objective conditions, so it is impossible to collect fish. Rare protected fish include mori's Lamprey (*Lampetra mori*), Chinese gudgeo (*Huigobio chinssuensis*), and swinhon's Sleeper (*Hypseleotris swinhonis*).

Establishment of F-IBI in the Biliu River Basin

This study requires annual sampling. Due to the long sampling period and the interference of seasonal changes and human factors, the environment in the Biliu River Basin changes greatly in each season. Therefore, when determining the F-IBI index system, first the index system of the three quarters (autumn, spring, and summer) was selected according to the time unit, and then the three quarters were integrated to select the F-IBI for evaluating the annual environment of the Biliu River Basin.

Response of candidate indicators to interference

After screening the response of 31 candidate indicators to interference, the results showed that, %Endemic fish species (F16) and %Cold water fish (F29) did not change unidirectionally with the enhancement of human interference intensity. Therefore, the above two indicators are not suitable to participate in the construction of F-IBI indicator system in the Biliu River Basin.

Index distribution range

The insufficient data indicators of %Siluridae (F11), %Oryziidae (F12), %Channidae (F15), %Abnormal individuals (F23), and %Number of brood fish (F24), which were cannot be carried out in the next screening. Therefore, the above five indicators are not suitable to participate in the construction of F-IBI indicator system in Biliu River Basin.

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Discriminant analysis

For the remaining 24 indicators, the box plot method was used to analyze the discrimination ability, and the overlap of reference points and damaged points in the quantile range of 25%-75% was compared. The indicators of autumn, spring, summer, and the anniversary were discriminated and screened in chronological order.

In autumn (*Fig. 5*), the total catch (F2), %Leuciscinae (F5), %Gobioninae (F7), %Cyprininae (F8), %Eleotridae (F13), %Economic fish species (F17), %Sensitive fish species (F18) and %Demersal fish (F28) can be further analyzed, if IQ values ≥2.

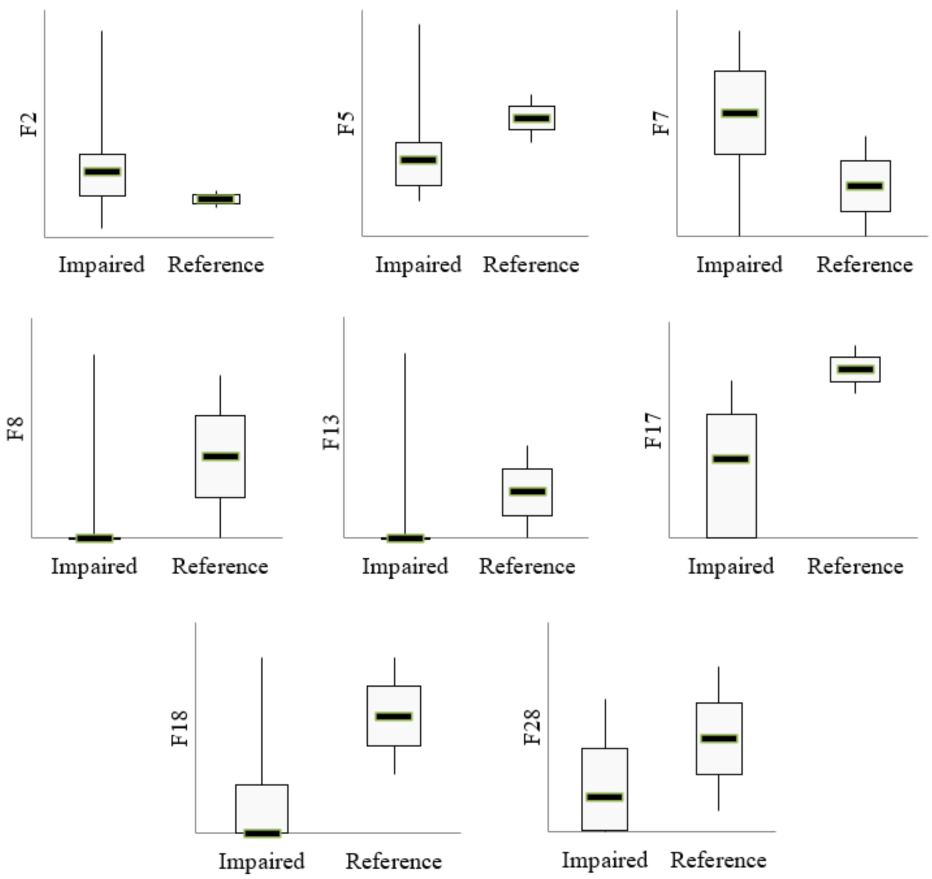

Figure 5. *Discriminatory power of selected metrics for reference and impaired sites using box and whisker plots in autumn. F2: Total catch, F5: %Leuciscinae, F7: %Gobioninae, F8: %Cyprininae, F13: %Eleotridae, F17: %Economic fish species, F18: %Sensitive fish species, F28: %Demersal fish*

In spring (*Fig. 6*), the Total fish species (F1), Shannon-Wiener diversity index *(H')* (F3), %Petromyzonidae (F4), %Leuciscinae (F5), %Cobitidae (F9), %Sensitive fish species (F18), %Number of omnivorous fish (F21), %Number of phytophagous fish (F22) and %Demersal fish (F28) can be further analyzed, if IQ values ≥2.

In summer (*Fig. 7*), the Total catch (F2), Shannon-Wiener diversity index (H') (F3), %Petromyzonidae (F4), %Cyprininae (F8), %Gobiidae (F14), %Economic fish species (F17) and Number of widely distributed species (F31) can be further analyzed, if IQ values ≥2.

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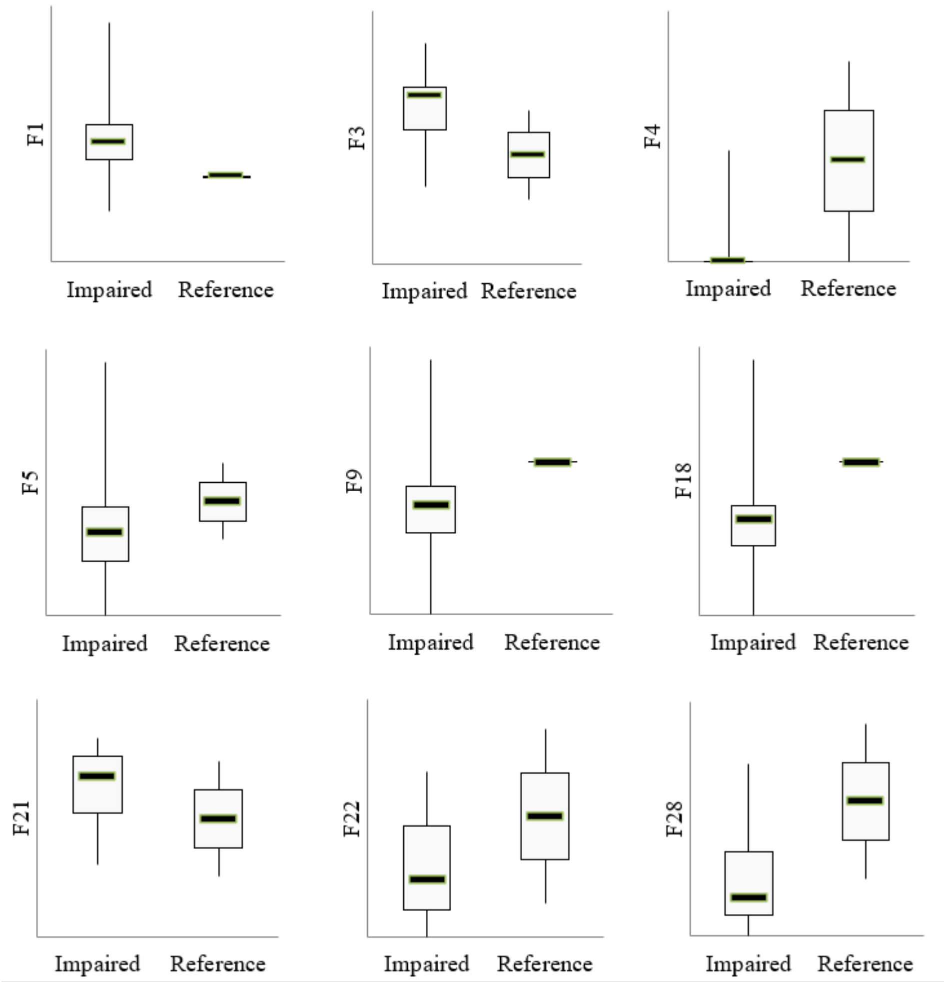

Figure 6. *Discriminatory power of selected metrics for reference and impaired sites using box and whisker plots in spring. F1: Total fish species, F3: Shannon-Wiener diversity index (H'), F4: %Petromyzonidae, F5: %Leuciscinae, F9: %Cobitidae, F18: %Sensitive fish species, F21: %Number of omnivorous fish, F22: %Number of phytophagous fish, F28: %Demersal fish*

In anniversary (*Fig. 8*), the %Petromyzonidae (F4), %Leuciscinae (F5), %Gobiidae (F14), %Sensitive fish species (F18) and %Demersal fish (F28) can be further analyzed, if IQ values ≥2.

Correlation analysis

In autumn, all indicators were not conform to the normal distribution, except %Gobioninae (F7). The correlation analysis results show that the correlation between the indicators is not high, so the eight indicators can be used to evaluate the autumn health status of the Biliu River Basin (*Table 3*).

In spring, the Total fish species (F1), Shannon-Wiener diversity index (H') (F3), %Leuciscinae (F5), %Cobitidae (F9) and %Demersal fish (F28) were conform to the normal distribution. While %Petromyzonidae (F4), %Sensitive fish species (F18), %Number of omnivorous fish (F21) and %Number of phytophagous fish (F22) were not conform to the normal distribution. The results of correlation analysis show that %Number of omnivorous fish (F21) and %Number of phytophagous fish (F22)

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significantly correlated, and the other indicators did not highly correlated. Due to %Number of omnivorous fish (F21) containing more information than %Number of phytophagous fish (F22) this latter was deleted. So, the eight indicators can be used to evaluate the spring health status of the Biliu River Basin (*Table 4*).

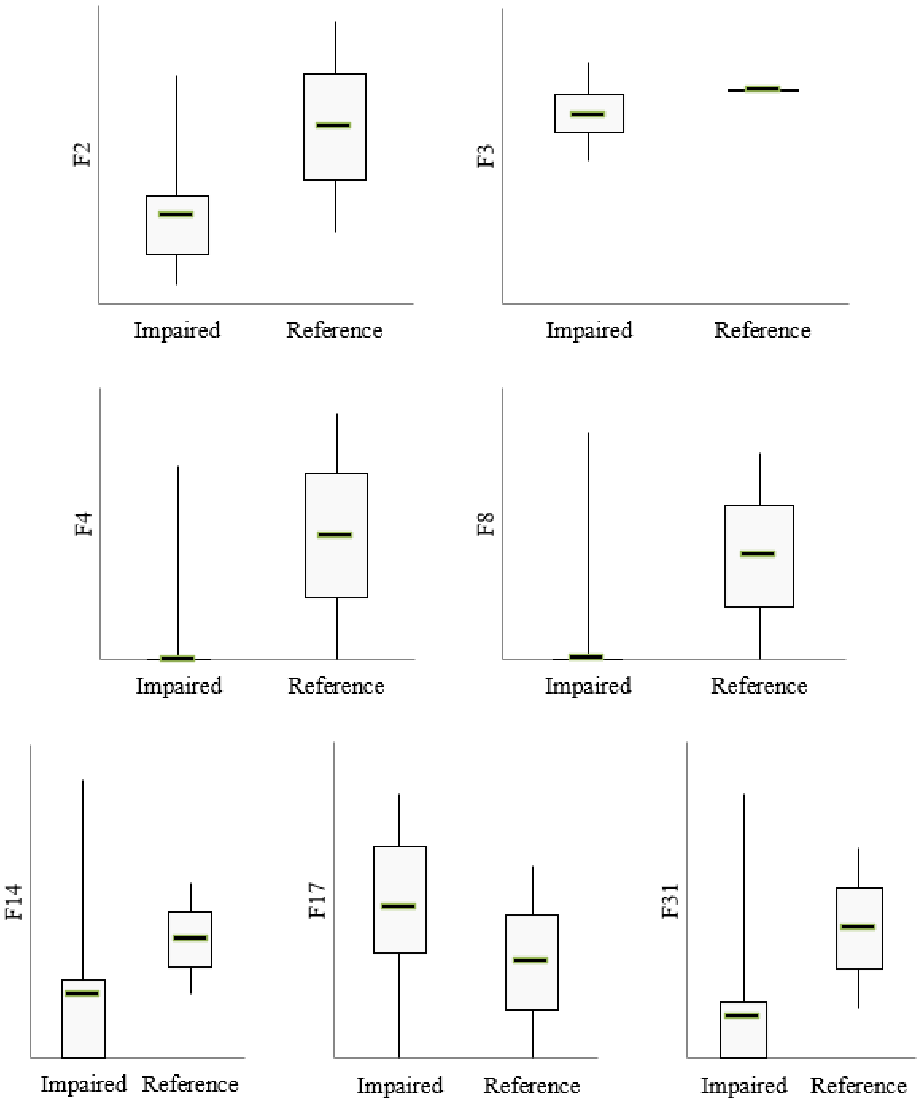

Figure 7. *Discriminatory power of selected metrics for reference and impaired sites using box and whisker plots in summer. F2: Total catch, F3: Shannon-Wiener diversity index (H'), F4: %Petromyzonidae, F8: %Cyprininae, F14: %Gobiidae, F17: %Economic fish species, F31: Number of widely distributed species*

In summer, all seven indicators were not conform to the normal distribution, except Shannon-Wiener diversity index (*H'*) (F3). The correlation analysis results show that the correlation between the indicators is high, so the seven indicators can be used to evaluate the summer health status of the Biliu River Basin (*Table 5*).

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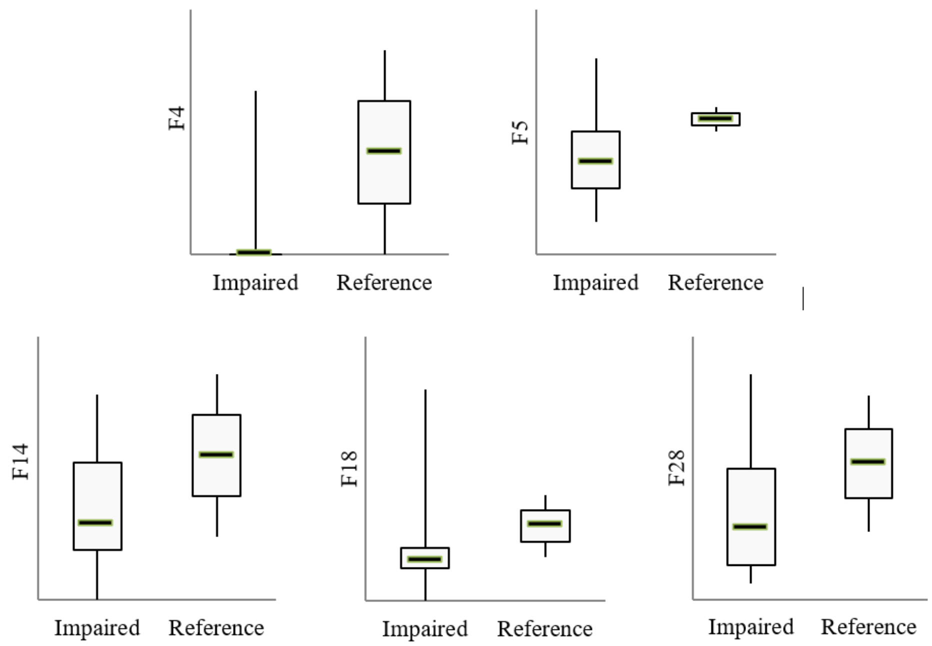

Figure 8. *Discriminatory power of selected metrics for reference and impaired sites using box and whisker plots in anniversary. F4: %Petromyzonidae, F5: %Leuciscinae, F14: %Gobiidae, F18: %Sensitive fish species, F28: %Demersal fish*

Table 3. *Candidate index Spearman correlation coefficient in autumn*

	F2	F5	F8	F13	F17	F18	F28
F2	1						
F5	-0.187	1					
F8	0.000	-0.377	1				
F13	-0.353	-0.090	-0.124	1			
F17	-0.028	-0.436	0.628	0.114	1		
F18	-0.527	0.231	-0.102	0.025	0.201	1	
F28	-0.120	-0.126	0.039	0.317	0.275	0.408	1

F2: Total catch, F5: %Leuciscinae, F8: %Cyprininae, F13: %Eleotridae, F17: %Economic fish species, F18: %Sensitive fish species, F28: %Demersal fish

Table 4. *Candidate index correlation coefficient in spring*

Pearson	F1	F3	F5	F9	F28
F1	1				
F3	0.786	1			
F5	-0.452	-0.516	1		
F9	-0.566	-0.555	0.549	1	
F28	-0.073	-0.272	-0.254	0.351	1
Spearman	**F4**	**F18**	**F21**	**F22**	
F4	1				
F18	0.092	1			
F21	-0.465	-0.213	1		
F22	0.507	0.249	**-0.979**	1	

F1: Total fish species, F3: Shannon-Wiener diversity index (H’), F4: %Petromyzonidae, F5: %Leuciscinae, F9: %Cobitidae, F18: %Sensitive fish species, F21: %Number of omnivorous fish, F22: %Number of phytophagous fish, F28: %Demersal fish

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Table 5. *Candidate index Spearman correlation coefficient in summer*

	F2	F4	F8	F14	F17	F31
F2	1					
F4	-0.136	1				
F8	-0.003	0.171	1			
F14	-0.053	0.210	-0.201	1		
F17	-0.329	-0.073	0.516	-0.608	1	
F31	0.459	-0.079	-0.274	-0.087	-0.117	1

F2: Total catch, F4: %Petromyzonidae, F8: %Cyprininae, F14: %Gobiidae, F17: %Economic fish species, F31: Number of widely distributed species

In anniversary, the %Leuciscinae (F5), %Gobiidae (F14) and %Demersal fish (F28) were conform to the normal distribution, while %Petromyzonidae (F4) and %Sensitive fish species (F18) were not conform to the normal distribution. The correlation analysis results show that the correlation between the indicators is high, so the five indicators can be used to evaluate the anniversary health status of the Biliu River Basin (*Table 6*).

Table 6. *Candidate index correlation coefficient in anniversary*

Spearman	F4	F18
F4	1	
F18	-0.207	1

Pearson	F5	F14	F28
F5	1		
F14	-0.198	1	
F28	-0.409	-0.054	1

F4: %Petromyzonidae, F5: %Leuciscinae, F14: %Gobiidae, F18: %Sensitive fish species, F28: %Demersal fish

Ecosystem health assessment in the Biliu River Basin

According to the screening of candidate indicators in the above process, the construction of F-IBI indicator system in the Biliu River Basin is finally determined as shown in *Table 7* below.

Table 7. *The index system of F-IBI*

Seasons	F-IBI system
Autumn	Total catch (F2), %Leuciscinae (F5), %Gobioninae (F7), %Cyprininae (F8), %Eleotridae (F13), %Economic fish species (F17), %Sensitive fish species (F18), %Demersal fish (F28)
Spring	Total fish species (F1), Shannon-Wiener diversity index (F3), %Petromyzonidae (F4), %Leuciscinae (F5), %Cobitidae (F9), %Sensitive fish species (F18), %Number of omnivorous fish (F21), %Demersal fish (F28)
Summer	Total catch (F2), Shannon-Wiener diversity index (F3), %Petromyzonidae (F4), %Cyprininae (F8), %Gobiidae (F14), %Economic fish species (F17), Number of widely distributed species (F31)
Anniversary	%Petromyzonidae (F4), %Leuciscinae (F5), %Gobiidae (F14), %Sensitive fish species (F18), %Demersal fish (F28)

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Ratio method result

Firstly, the ratio method was used to score the ecosystem health status of the Biliu River Basin (*Table 8*). The results showed that sampling sites 2~4 were seriously polluted in the downstream, and the scores of each season were at a low level. In upstream, sampling sites 5~9 were at the medium level, and the evaluation results were general. While sampling sites 10~16 were in good health status, and most of them were sub-healthy or healthy.

Table 8. *F-IBI result for each sampling sites by ratio method score*

Sampling site	Autumn		Spring		Summer		Anniversary	
	Score	**Health level**	**Score**	**Health level**	**Score**	**Health level**	**Score**	**Health level**
1	0	-	0	-	0	-	0	-
2	1.63	Bad	2.44	Bad	3.95	Sub-health	1.86	General
3	4.20	Sub-health	3.65	Sub-health	1.65	Bad	0.88	Bad
4	2.90	Poor	3.91	Sub-health	1.97	Bad	1.06	Bad
5	3.34	General	3.44	General	4.84	Health	2.16	General
6	3.18	General	3.79	Sub-health	2.35	Poor	2.30	Sub-health
7	3.18	General	4.44	Health	2.57	Poor	2.13	General
8	2.86	Poor	3.20	General	3.40	General	1.69	Poor
9	1.81	Bad	4.35	Health	1.55	Bad	1.91	General
10	1.70	Bad	4.36	Health	3.04	General	2.33	Sub-health
11	3.70	General	4.38	Health	3.97	Sub-health	2.01	General
12	5.45	Health	3.93	Sub-health	5.56	Health	3.15	Health
13	4.18	Sub-health	3.57	Sub-health	3.07	General	2.68	Health
14	4.97	Sub-health	3.21	General	2.06	Bad	3.06	Health
15	4.05	Sub-health	4.37	Health	3.12	General	2.41	Sub-health
16	3.08	General	3.60	Sub-health	4.68	Health	2.70	Health

In terms of seasons, the health status of the Biliu River Basin in autumn was poor, and most sampling sites were at a general or even worse level. The health status in spring was good, which 11 sampling sites reaching the level above sub-health. Compared with spring, the health status was decreased in summer. Only 5 sampling sites reached the level of sub-healthy or above, and 4 sampling sites were bad. In anniversary, there were 12 sampling sites reaching the general level or above.

Three scoring method result

Secondly, the three-scoring method was used to score the ecosystem health status of the Biliu River Basin (*Table 9*). The results showed that sampling sites 2~4, 6,7, 11 and 13 were seriously polluted, and the results of health status in each season were general, poor or bad. Sampling sites 8,14~16 were at the medium level, and sampling sites 5,9 and 10 were in good health status, which were sub-healthy and healthy.

In terms of seasons, there are 8 sample sites that reached the general and above health level in autumn, 12 sites in spring and 9 sites in summer. Meanwhile, sites with the worst status were the most in autumn, and the river was in good health in spring. In anniversary, sampling sites 2,4 and 7 were the most seriously polluted, and seven sites reached the general level or above.

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Table 9. *F-IBI result for each sampling sites by three scoring method*

Sampling site	Autumn		Spring		Summer		Anniversary	
	Score	**Health level**	**Score**	**Health level**	**Score**	**Health level**	**Score**	**Health level**
1	0	-	0	-	0	-	0	-
2	18	General	16	Bad	11	Bad	7	Bad
3	16	Poor	24	Health	19	Sub-health	11	General
4	20	Sub-health	16	Bad	21	Health	7	Bad
5	20	Sub-health	22	Sub-health	19	Sub-health	9	Poor
6	22	Sub-health	20	General	13	Poor	9	Poor
7	12	Bad	20	General	9	Bad	7	Bad
8	12	Bad	24	Health	15	General	9	Poor
9	24	Health	26	Health	17	Sub-health	13	Sub-health
10	26	Health	20	General	23	Health	15	Health
11	14	Bad	26	Health	13	Poor	9	Poor
12	22	Sub-health	20	General	21	Health	15	Health
13	12	Bad	22	Sub-health	11	Bad	11	General
14	16	Poor	20	General	11	Bad	13	Sub-health
15	16	Poor	20	General	15	General	9	Poor
16	22	Sub-health	18	Poor	19	Sub-health	15	Health

Combining the above two scoring methods to evaluate the health status of the Biliu River Basin, although the results were not exactly the same, the overall trend was consistent (*Table 10*). Overall, sampling sites 9~12 were in good health status, and sites 5,8,13~ 15 were at medium level, while sites 2~4,6 and 7 were not good.

Table 10. *The health statistics between two evaluation methods in different season*

	Ratio method					Three scoring method				
	Health	**Sub-health**	**General**	**Poor**	**Bad**	**Health**	**Sub-health**	**General**	**Poor**	**Bad**
Autumn	1	4	5	2	3	2	5	1	3	4
Spring	5	6	3	0	1	4	2	6	1	2
Summer	3	2	4	2	4	3	4	2	2	4
Anniversary	4	3	5	1	2	3	2	2	5	3

Discussion

Fish composition in the Biliu River Basin

A total of 31 species of fish were collected in this survey. Compared with the survey results of Shi et al. (1960), more species of fish were collected this time, covering most species collected by predecessors. While there are still some that have not been collected, such as Sweetfish (*Plecoglossus altivelis*) and Common seaperch (*Lateolabrax maculatus*). Before the completion of the Biliu River reservoir, there were 65 fish species, and the number of fish species decreased significantly in the past 33 years (Mu et al., 1982). Indigenous freshwater fish decreased by 10 species, and migratory fish Sweetfish (*Plecoglossus altivelis*) and Japonese eel (*Anguilla japonica*) were not found in this survey. It can be seen that the completion of the Biliuhe reservoir has a great impact on the aquatic ecosystem of the Biliu River Basin. In the past 30 years, China's economy has advanced by leaps and bounds, and the rapid economic development has also accelerated

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the deterioration of the natural environment (Hou et al., 2015; Li et al., 2021b). The construction of roads and bridges (Kvitko et al., 2018), the construction of farms (Oh et al., 2015), and the large discharge of domestic sewage and agricultural sewage (Wang et al., 2022) will all have an impact on the river ecosystem, which is also closely related to the substantial reduction of fish species (Yanesroca, 2001).

The fish composition of the Taizi River Basin was similar to that of the Biliu River Basin (Song et al., 2010). Cyprinidae, Cobitidae and Gobiidae accounted for the top three percentages of the total number of species. Amur minnow (*Phoxinus lagowskii*) was the dominant species, and Mori's Lamprey (*Lampetra mori*) and Chinese gudgeon (*Huigobio chinssuensis*) distributed in both basins. The basin area of the Taizi River is five times that of the Biliu River, and also longer than the Biliu River Basin. Meanwhile, there are abundant tributaries and more diverse water ecological environment, so the total number of species is more than that of the Biliu River Basin. In the Hun River Basin, 33 fish species were found, which were similar to the number of species in this study (Zhang et al., 2015). Freshwater aquaculture was popularized earlier in the Hun River Basin, so economic fish account for a large proportion in the river. The Hun River basin covers a wide area, with a large number of sampling points and a wide range, so the tail number of fish collected is more than that of the Biliu river. What the Hun River and the Biliu River have in common is that Amur minnow (*Phoxinus lagowskii*) was the dominant species, and there are a large number of Pale chub (Zacco platypus). Cyprinidae fish account for a large proportion of the collected fish. Compared with other rivers in northern China, the Liao River (Zhang et al., 2015) and its tributaries (Pei et al., 2010), the Xiliao River Basin (Zhang et al., 2015) and the Biliu River Basin were dominated by Cyprinids. Most pollution tolerant species are fish, and sensitive species cannot survive. The reason is that the river is located in the old industrial base in Northeast China, and the pollution is relatively serious.

In terms of the number of fish captured, the most fish were caught in summer, followed by autumn and the least in spring. Song et al. (2010) collected fish samples from 64 sampling points in the Taizi River Basin. The results showed that the number of fish species collected in the two samples was similar, and the catch in autumn was more than that in spring, which was consistent with this study. Due to the large area of the Taizi River Basin and more sampling sites, the total catch was more than that of this study. In addition, winter sampling was not carried out in this survey because the temperature was low in winter, the water flow was small, the river gradient was slow, and continuous bottom freezing often occurred. There was no river water merging and flowing between tributaries, and even the river water depletion occurred in smaller tributaries. Therefore, the collected organisms were not representative of the ecological environment, so winter sampling and health evaluation were not carried out.

F-IBI system

At present, it is common to use fish index of biological integrity to evaluate river health. Liu et al. (2010) conducted F-IBI evaluation on the mainstream and affiliated lakes in the middle and upper reaches of the Yangtze River. By monitoring the changes of fish species and quantity, they used a unified index system to directly assign scores to the points, which was consistent with the monitoring method of this study, but the index system was different. Cai et al. (2014) used a unified index system to conduct a total of 11 seasonal surveys of the Tai Lake ecosystem. Due to the large water area of the Yangtze River and Tai Lake, which have formed a very stable aquatic ecosystem, and are located

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in the middle of China, the annual temperature difference is small, so it is more reasonable to adopt the same index system in different years. The drainage area and runoff of the Biliu River are much smaller than that of the Yangtze River and Tai Lake. The annual temperature difference is large, which is greatly affected by seasonal changes. Therefore, different index systems should be used to evaluate river health in different seasons.

Because of higher nutritional level and niche, fish is more sensitive to changes in the aquatic ecosystem and can reflect the health of the aquatic ecosystem more intuitively and accurately (Weis et al., 2000). It can not only evaluate quarterly, but also summarize the quarterly sampling results for annual evaluation (Ruprecht et al., 2022). Meanwhile, fish assembles are the most suitable for water ecological health assessment, indicator species and flagship species can be selected to further improve the water ecological health evaluation model (Noh et al., 2015). Plankton can also reflect the changing trend of water environment to a certain extent, but the index system is not perfect (Carpenter et al., 2006; Kane et al., 2009; Wu et al., 2012). Therefore, it is necessary to further study the evaluation index system, and the comprehensive evaluation of biological indicators can better evaluate the river health status.

The selection and setting of reference sites are crucial in IBI evaluation, which determines the credibility of the final evaluation results (Wang et al., 2005, 2009; Wu et al., 2012). For the selection principle of reference sites, historical data should be referred to and rivers without human interference should be selected. However, there is still no unified standard (Blocksom et al., 2002). During the sampling period of this study, we found that there was no historical data for reference in the Biliu River Basin, and the river have been subject to varying degrees of human interference, and there are no rivers in the original state. Therefore, through the combination of pre investigation and the surrounding environment, the area with the least human interference is selected as the reference point. Similar situations can be followed at home and abroad, adding different parameters or using different analysis methods to select reference sites (Pei et al., 2010).

F-IBI index system construction in the Biliu River Basin

The areas with low scores and extremely poor and poor evaluation results in the Biliu River Basin were mainly concentrated in the downstream and upstream points close to villages. Sampling sites 2, 3 and 4 were located at the junction of Pulandian City and Zhuanghe City at the downstream of the reservoir. Particularly, there was a factory near site 2, which was close to the sewage outlet, and the river was muddy. There were farmlands near site 3 and 4, which often discharged agricultural wastewater. The villages around site 7 were dense, and the domestic sewage of residents was often discharged into the river, which was muddy and stinking. Sites 8 and 13 were located next to the sand pit and tunnel respectively. The river channel was seriously damaged, the surrounding vegetation coverage was very low, and seriously disturbed by human beings, and the water ecological environment was greatly damaged. At these sampling sites, a large amount of wastewater and organic pollutants were discharged into the river, causing water pollution and deterioration of the water environment. The low F-IBI score is related to the discharge of organic matter, industrial wastewater, and domestic sewage, which has been reported in foreign river health research (Hughes and Gammon, 1987; Ganasan et al., 1998).

In general, indicators such as Total catch and Shannon-Wiener diversity index are used in other river health assessment studies (Pei et al., 2010; Zhang et al., 2015). In addition, the construction of the reservoir also has a great impact on the composition of fish in the

Biliu River Basin (Mu et al., 1982). Before the completion of the reservoir, the composition of fish in the upstream and downstream were similar. After completion, due to the interception of the reservoir, the spawning conditions of spawning fish in the downstream estuary have been destroyed, affecting the reproduction of fish. Meanwhile, fish in the upper reaches of the reservoir cannot migrate downstream through the reservoir, thus affecting the F-IBI score. In the future research, we can add more parameters such as the species richness of surrounding vegetation, population density, village distance, vegetation coverage, etc. While collecting data and conducting pre investigation, so as to obtain more accurate data can improve the selection criteria of reference points, and also accuracy.

F-IBI evaluation method in the Biliu River Basin

In this study, the ratio method and 1,3, 5 assignment method were used to assign the indices, respectively. Both methods are widely used in the evaluation of river health by biological integrity index (Dolah et al., 1999; Qadir and Malik, 2009; Maulood et al., 2011; Baek et al., 2014; Al-Janabi et al., 2016). There were some differences in the evaluation results between the two methods. The overall evaluation results of the ratio method were higher, but the results of the two evaluation methods were the same in the proportion of above general and poor, and the overall trend was the same. Pei et al. (2010) applied F-IBI for evaluating the health status of the Liao River Basin, which results showed that only six of the 32 sampling sites had different grades. Although the results obtained by the two methods were not exactly the same, the overall trend was the same. The above studies were consistent with the results of this study, so it can be seen that the ratio method and the 1,3,5 assignment method can reflect the river health status to a certain extent. There is no contradiction between the two, thus further studies are required to decide which method has higher accuracy.

In addition, reference sites selection is also one of the important factors affecting the evaluation results, which affect the final evaluation credibility directly (Detenbeck and Cincotta, 2008). When selecting the reference sites, the historical data should be the first reference, and the river reach without human interference should be selected (Lacouture et al., 2006). However, according to the survey results during the pre-survey period of this study, there is no historical data for reference in the Biliu River Basin, and the upstream to downstream are subject to varying degrees of human interference. Therefore, no part of the river is in its original state, so the selection standard of the reference site is the area with the least human interference. There are some subjective factors in the reference points selected according to this method. Hence, in the future research, further exploration is needed to formulate a unified standard for selecting reference points to improve the accuracy of evaluation results.

According to the evaluation method of this study, the point with the final score of F-IBI above the 75% quantile is the healthy sampling sites. Then, the health level of the quartile with a score below the 75% quantile will decrease gradually with the decrease of the score. It can be seen that the "health" and "bad" in the evaluation results are relative, and both are related to the overall situation of the surveyed basin (Xu, 2014; Ma, 2015; Zhang et al., 2015). In the study of river health evaluation, most evaluation methods divide the health grade standard by 75% quantile of the score, so the evaluation results contain health sampling sites and bad sampling sites (Song et al., 2010; Chen et al., 2011; Wu et al., 2012; Baek et al., 2014; Al-Janabi et al., 2016; Zhu et al., 2021). However, there are great differences in environmental conditions and temperatures in different

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countries and regions, resulting in different species composition and distribution (Baptista et al., 2021; Haulsee et al., 2022). There is no clear regulation on this when selecting the reference point, and the selection is the key factor to determine the final evaluation result. Therefore, there are still some differences in the evaluation results in different regions, and there is still some subjectivity in the concept of water ecological environment health.

Conclusions

Through fish collection and identification at 16 sampling sites in the Biliu River Basin, the index system suitable for different seasons in the Biliu River basin was finally determined after screening. The ratio method and 1,3,5 assignment method was used to evaluate the health of the Biliu River basin. By contrast, it is found that the ratio method is more consistent, while the 1, 3 and 5 scoring methods are more intermittent. Although the evaluation results of the two methods are slightly different, they have the same trend and can reflect river health status to a certain extent.

Overall, F-IBI is significantly affected by seasonal variations. The health status of the Biliu River Basin decreased with seasonal changes. The health status of each sampling site was the best in spring, followed by autumn and the worst was in summer. The health status of the sampling site 1 nearing the estuary was the worst, the health status of the 3 sampling site nearing the origin of the upstream and the sampling site 3 in the clam river was healthy or sub-healthy, and the health status of the rest of the upstream points was average. The results of F-IBI showed that the health status of the downstream sites was lower than that of the upstream sites, and the health status of the upstream sites near the villages was worse.

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APPENDIX

Table A1. *Composition and distribution of fish individuals in Biliu River Basin (Sampling sites 1~16)*

Family	Species	Autumn	Spring	Summer	Total	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Petromyzonidae	*Lampetra mori*	0	9	7	16									+	+						+
Cyprinidae	*Zacco platypus*	288	49	304	641		+	+	+	+	+	+	+	+	+	+	+	+		+	+
	Phoxinus lagowskii	268	401	491	1160					+	+	+	+	+	+	+	+	+	+	+	+
	Phoxinus czekanowskii	113	219	267	599					+	+	+	+	+	+	+	+	+	+	+	
	Rhodeus ocellatus	2	0	1	3		+				+										+
	Rhodeus sinensis	2	25	56	83		+	+	+		+	+	+	+						+	+
	Rhodeus sericeus	153	42	45	240		+	+	+		+	+	+	+							+
	Pseudorasbora parva	114	26	16	156		+		+	+	+	+	+	+	+	+		+		+	
	Abbottina rivularis	135	49	100	284		+	+	+	+	+	+	+	+	+	+	+				+
	Abbottina liaoningensis	5	1	2	8							+	+								+
	Pseudogobio vaillanti	2	0	0	2								+							+	
	Huigobio chinssuensis	26	1	0	27		+		+		+		+	+	+						+
	Carassius auratus	55	7	5	67		+	+	+		+		+	+	+						
Cobitidae	*Nemachilus nudus*	31	57	61	149			+	+	+	+	+	+	+	+	+	+	+		+	
	Lefua costata	1	2	4	7		+	+		+	+										
	Barbatula barbatula	56	36	15	107				+	+		+				+					
	Cobitis granoei	39	142	226	407			+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Misgurnus anguillicaudatus	24	9	48	81			+	+	+	+	+	+	+	+	+		+	+	+	+
Bagridae	*Pelteobagrus fulvidraco*	0	1	1	2								+	+							
	Pelteobaggrus nitidus	0	0	4	4													+		+	
Siluridae	*Silurus asotus*	0	0	2	2							+									
Oryziatidae	*Oryzias latipes*	0	4	0	4				+												
Eleotridae	*Perccottus glehni*	2	0	7	9												+		+	+	
	Hypseleotris swinhonis	36	12	51	99			+	+					+							
Gobiidae	*Tridentiger obscurus*	3	1	3	7						+							+		+	+

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Family	**Species**	**Autumn**	**Spring**	**Summer**	**Total**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
	Tridentiger trigonocephalus	0	1	20	21												+				+
	Ctenogobius pflaumi	0	4	0	4								+								
	Ctenogobius giurinus	16	24	17	57		+			+	+	+	+		+		+	+			+
	Ctenogobius brunneus	11	22	48	81		+	+		+	+		+				+	+			+
	Ctenogobius cliffordpopei	0	4	0	4						+										
Channidae	*Ophiocephalus argus*	2	0	0	2		+														
Total		1384	1148	1801	4333	0	10	11	13	12	18	13	19	15	12	9	10	11	5	12	15

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