

# Development and evaluation of a Planktonic Integrity Index (PII) for Jingpo Lake, China

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## ABSTRACT

A Planktonic Integrity Index (PII) for the China's largest alpine barrier lake (Jingpo Lake) was developed to assess the water quality of Jingpo Lake by using phytoplankton and zooplankton metrics. Phytoplankton and zooplankton assemblages were sampled at 26 sites in Jingpo Lake. A total of 140 species of phytoplankton and 92 species of zooplankton were obtained in the investigations. We used a stepwise process to evaluate properties of candidate metrics and selected five for the PII: Algal cell abundance, Species richness of algae, Trophic diatom index, Zooplankton Shannon index, and Zooplankton Margalef index. Evaluation of the PII showed that it discriminated well between reference and impaired sites and the discriminatory biocriteria of the PII were suitable for the assessment of the water quality of Jingpo Lake. The further scoring results from the 26 sites showed that the water quality of Jingpo Lake was fair to good. The results of analyses between PII and major environmental factors indicated that water temperature (WT), transparency (SD), dissolved oxygen (DO), potassium permanganate ( $\text{COD}_{\text{Mn}}$ ) and total nitrogen (TN) were the main factors influencing on the composition and distribution of phytoplankton and zooplankton. Additionally, more metrics belonging to habitat, hydrology, physics and chemistry should be considered for the PII, so as to establish comprehensive assessment system which can reflect the community structure of aquatic organisms, physical and chemical characteristics of water environment, human activities, *etc.*

## INTRODUCTION

As primary producers at the beginning of the food chain in aquatic ecosystem, phytoplankton have the characteristics of short life cycle are sensitive to pollution, and their community structure can transform along with the changes in water chemistry. Phytoplankton are also amongst the most widely used indicators of biological integrity and physicochemical conditions in aquatic ecosystems (Hill *et al.*, 2003; Miller *et al.*, 2006; Zalack *et al.*, 2010; Wu *et al.*, 2012b). Zooplankton, as the second trophic level of the food webs in aquatic ecosystem, play a vital role in ecological processes of material transformation and energy flow. Further their community

structure, abundance, dominant taxa, and pollution indicator species are widely used to reflect the water status (Swadling *et al.*, 2000; Echaniz *et al.*, 2006; Jiang *et al.*, 2012). Since 1990s, the water quality bioassessment by using single biotic metrics began to be displaced by the integrated water quality bioassessment based on multiple metric (Kerans and Karr, 1994; Blocksom *et al.*, 2002; Lugoli *et al.*, 2012). However, there are several problems in the actual operation and assessment as followed: i) the growth of aquatic organisms is influenced by not only water quality but also parameters of physics, chemistry, climate, hydrology and so on, increasing the random errors of sampling; ii) the precision of identification of specimens may affect the accuracy of bioassessment; iii) the bio-metrics based on one assemblage can only represent one aspect of the communities and functions in ecosystem, or respond to limited stressors, which may also affect the accuracy of bioassessment for water quality (Wang *et al.*, 2015c). Therefore, the use of at least two assemblages has been suggested for more robust biological assessment of condition, as each assemblage may respond differently to potential stressors (Yoder and Rankin, 1995). Multi-biotic indicators and metrics for fish, phytoplankton, zooplankton, or macroinvertebrate assemblage condition have been developed for streams (Angermeier *et al.*, 2000; McCormick *et al.*, 2001; Klemm *et al.*, 2003; Whittier *et al.*, 2007; Chon *et al.*, 2013) and lakes (O'Connor *et al.*, 2000; Wilcox *et al.*, 2002; Kane *et al.*, 2009; Wang *et al.*, 2015c). However, there are still some inadequacies in the present multi-assemblage assessment. For example, the subjectivity of

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the choice of metrics, and the lack of rigorous screening based on discrimination and redundancy of the chosen metrics, leading to some alternative metrics being left off during the development of the multi-metric index. So far, multi-assemblage assessment has just been used for Chinese lakes, that is why we need to establish a multi-metric index based on multi-assemblages to assess the water quality of the lakes in China. Such an index should be able to reflect to a variety of potential stressors. Jingpo Lake is the largest alpine barrier lake of China, which located at N43°30'-44°20' and E128°07'-129°06' in Ningan country of Heilongjiang province. As a nature reservoir belonging to Mudanjiang River, Jingpo Lake contains the storage capacity of 1.6 billion m<sup>3</sup>, the annual average impoundage of 1.1 billion m<sup>3</sup>, the basin area of 11,820 km<sup>2</sup>, the scenic spot area of 1,726 km<sup>2</sup>, the nature reserve area of 1260 km<sup>2</sup>, and the average lake area of 79.3 km<sup>2</sup> (Chen *et al.*, 1994). Jingpo Lake plays an important role in freshwater aquaculture, tourism, electricity, transportation, drinking water sources. However, in the past few years, environment pollutions of the Jingpo Lake have become more and more serious because of development of agriculture and fisheries, land use changes, industrial pollutants etc. These pollutants have caused the gradual reduction of species, abundance, and diversity of phytoplankton and zooplankton (Yu *et al.*, 2008b; Song and Yu, 2009; Liu *et al.*, 2012; Wang *et al.*, 2015a, 2015b). In this study, we developed and tested the Planktonic Integrity Index (PII) using a training data set and a testing data set of phytoplankton and zooplankton, respectively, from Jingpo Lake. Our specific objectives were to: i) develop a PII based on phytoplankton and zooplankton metrics, so as to create an offshore water quality monitoring tool that reflected Beneficial Use Impairments to Jingpo Lake and would be generally applicable to other lakes in China; ii) deduce the water quality of the study area by implementing the developed PII; iii) test the accuracy of the PII index by its relationship with local physical-chemical parameters.

## METHODS

### Study area

Jingpo Lake has a maximum length of 45 km (N-S), width of 6 km (E-W), is shallow at its southern and deep at the northern part. It has an average depth of 13.8 m and a maximum depth of 70 m. The annual average influx of water is 90.8 m<sup>3</sup> per second and runoff of 2.87 billion m<sup>3</sup>. The study area lies in a temperature zone with annual average temperature of 4.3°C its climate belongs to monsoon climate zone with long cold winters and short cool summers, with annual average relative humidity of 71.5%, precipitation of 619.8mm (Li,

2013). The study area consists of 26 sample sites located throughout Jingpo Lake (Fig. 1). In detail, S1 represents the water quality of inflowing water from Jilin Province; S2 to S6 represents the water quality of Xidapao, Dongdapao, Big-Little Jiaji River and Songyi River, respectively; S7 to S11 represents the water quality of upstream of Jingpo Lake; S12 and S13 represents the water quality of the entrance from Erzhan River to Jingpo Lake and inflowing water from upstream of Erzhan River, respectively; S14 to S22 represents the water quality of lake region; S23 and S24 represents the water quality of Jingpo mount and inflowing water from Baoyue Bay; S25 represents the water quality of out-water of Jingpo Lake; S26 represents the water quality of inflowing water from Ziling Lake.

### Sampling and processing of phytoplankton and zooplankton

Phytoplankton was sampled and concentrated by a 25-micron mesh through the entire water column (from the bottom to the top) in September and October of 2013. Collected organisms were stored in 5‰ non-acetic Lugol's iodine solution. Non-diatom phytoplankton was

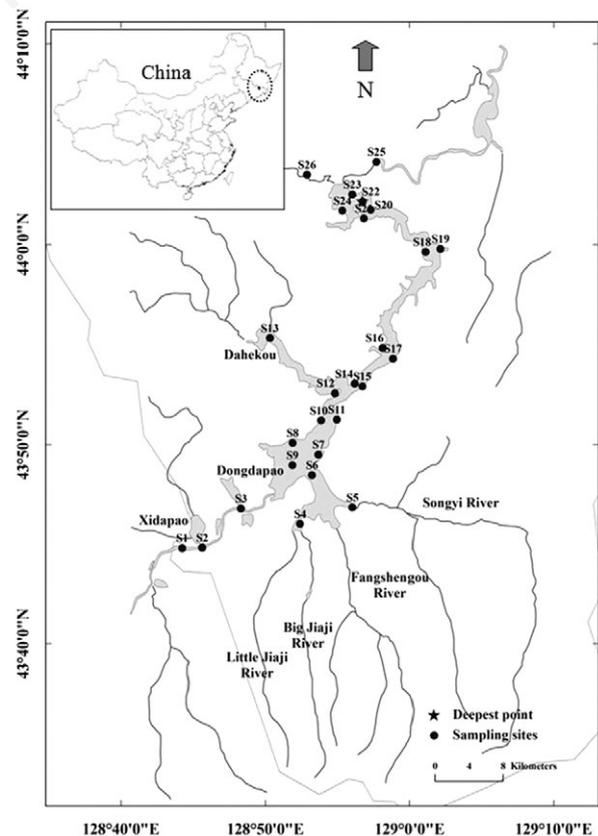


Fig. 1. Sampling sites of Jingpo Lake in 2013.

analyzed using a 0.1 mL counting chamber at a magnification of 400× (Zeiss Axioskop microscope). Permanent diatom samples were cleaned using a strong acid solution ( $\text{HNO}_3 + \text{H}_2\text{SO}_4$ ; 2:1). From each sample, about 500 valves were counted for each sample using a Zeiss Axioskop microscope at 1000× under oil immersion. Phytoplankton was identified to the lowest taxonomic level possible (mainly species level) and its densities were expressed as cell  $\text{L}^{-1}$  (Wu *et al.*, 2012a).

Zooplankton at each site was sampled with a Wisconsin net (mesh size: 50  $\mu\text{m}$ ; mouth diameter: 25 cm) that was towed through the water column, during the same sampling event as phytoplankton. Each catch was preserved in 4% formaldehyde and kept refrigerated until further work. Zooplankton was identified to the lowest taxon level possible, usually to species level. Copepod nauplii could not be identified as calanoid or cyclopoid and were pooled. If the number of individuals in a sample was less than 1500 the entire catch was sorted, otherwise it was split so that between 1000 and 1500 organisms were counted. The number of zooplankton cells in each species or genus was calculated by determining the cells collected at each site and converted to cell  $\text{L}^{-1}$  (Swadling *et al.*, 2000).

### Water quality measures and characterization of reference and impaired sites

Water quality samples were collected and composited at the same time and locations as phytoplankton and zooplankton samples. For each sampling site, *in situ* measurements included conductivity (EC), pH, dissolved oxygen (DO), and water temperature (WT), whose values

were averaged from two depths (0.5 m below the surface and 0.5m above the bottom), and the transparency (SD) was measured by Secchi disk at each site. The measurements of total phosphorus (TP), total nitrogen (TN), ammonia nitrogen ( $\text{NH}_3\text{-N}$ ), potassium permanganate ( $\text{COD}_{\text{Mn}}$ ) and fluoride (F) were carried out with reference to Chinese EPA methods for the monitoring and analysis of water and wastewater (Chinese EPA, 2002).

A Chinese surface water quality standard (Tab.1) was applied to help defining bioregional reference and impairment criteria for each site (Tab. 2).

### Metric selection and calibration

Seventeen phytoplankton metrics and seventeen zooplankton metrics were selected for evaluation of the multi-metric index (Tabs. 3 and 4). Statistical analyses for screening candidate metrics were performed with SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). The metrics were calculated based on the sampling results of September. A stepwise process based on US EPA technical guidance (US EPA, 1998) for establishing biocriteria for lakes and reservoirs was used to evaluate the metrics for use in a multi-metric index. Three characteristics were evaluated for each metric: 1) discriminatory power, 2) redundancy, and 3) scoring of metrics.

### Discriminatory power

We defined discriminatory power of a metric as the ability of that metric to distinguish between reference and impaired sites and evaluated metrics by examining their

**Tab. 1.** Five classes of surface water bodies (I-V) of water environmental quality standards (GB3838-2002) in China.

Indicators(mg/L)		Classes				
		I	II	III	IV	V
TN	≤	0.2	0.5	1.0	1.5	2.0
TP (river)	≤	0.02	0.1	0.2	0.3	0.4
TP (lake) and reservoirs)	≤	0.01	0.025	0.05	0.1	0.2
$\text{NH}_3\text{-N}$	≤	0.15	0.5	1.0	1.5	2.0
DO	≥	90% or 7.5	6	5	3	2
COD	≤	15	15	20	30	40
$\text{BOD}_5$	≤	3	3	4	6	10
$\text{COD}_{\text{Mn}}$	≤	2	4	6	10	15

**Tab. 2.** Discriminatory factors for reference and impaired water.

	$\rho(\text{DO})$	$\rho(\text{COD}_{\text{Mn}})$	$\rho(\text{TN})$ mg/L	$\rho(\text{TP})$	$\rho(\text{NH}_3\text{-N})$
Reference	≥ 8.0	≤6.0	≤1.0	≤0.05	≤0.50
Impaired	<8.0	>6.0	>1.0	>0.05	>0.50

$\rho$ , indicator concentration.

distributions by using box-and-whisker plots. The degree of overlap between interquartile (IQ) ranges (the box) of reference and impaired sites was considered a signal of the discriminatory capability of the metric. Using the system developed by Barbour *et al.* (1996), metrics scoring 2 or 3 were retained for further analysis.

### Redundancy

We evaluated redundancy among metrics to ensure that each metric in the final index provides new information. Using the remaining metrics, Pearson correlation coefficients was used to identify highly correlated metrics. A simple correlation alone is not

**Tab. 3.** Candidate biotic-metrics of algae.

Category	Metrics and serial number	Response	Reference
Richness and abundance	P <sub>1</sub> : Relative abundance of diatoms (RAD)	Decrease	Griffith <i>et al.</i> , 2005
	P <sub>2</sub> : Algal cell abundance (CA)	Decrease	
	P <sub>3</sub> : Margalef diversity index (Margalef)	Decrease	Margalef, 1958
	P <sub>4</sub> : Shannon-Wiener diversity index (Shannon)	Decrease	Shannon, 1949
	P <sub>5</sub> : Simpson diversity index (Simpson)	Decrease	Simpson, 1949
	P <sub>6</sub> : Pieloud evenness index (Pieloud)	Decrease	Yang <i>et al.</i> , 2011
	P <sub>7</sub> : Species richness (SpR)	Decrease	Wu <i>et al.</i> , 2012b
	P <sub>8</sub> : Chlorophyll a (Chla)	Increase	
Taxonomic composition	P <sub>9</sub> : Percent motile diatoms (PMD)	Increase	Bellinger <i>et al.</i> , 2006
	P <sub>10</sub> : Generic diatom index (GI)	Decrease	Wu and Kow, 2002
	P <sub>11</sub> : Diatom quotient (DQ)	Increase	Kane <i>et al.</i> , 2009
	P <sub>12</sub> : % Abundance of Microcystis, Anabaena, Aphanizomenon (MAA)	Increase	
	P <sub>13</sub> : % <i>Cymbella</i> sp. ( <i>Cym.</i> )	Decrease	Wang <i>et al.</i> , 2005
P <sub>14</sub> : % <i>Navicula</i> sp. ( <i>Nav.</i> )	Increase		
Tolerance and intolerance index	P <sub>15</sub> : Pollution tolerance index for diatoms (PTI)	Decrease	Muscio, 2002
	P <sub>16</sub> : Percent sensitive diatoms (PSD)	Decrease	Barbour <i>et al.</i> , 1999
	P <sub>17</sub> : Trophic diatom index (TDI)	Increase	Kelly and Whitton, 1995

*P<sub>p</sub>* algae index.

**Tab. 4.** Candidate biotic-metrics of zooplankton.

Category	Metrics and serial number	Response	Reference
Richness and abundance	Z <sub>1</sub> : Total zooplankton abundance (TZA)	Decrease	Carpenter <i>et al.</i> , 2006
	Z <sub>2</sub> : Biomass of zooplankton/biomass of phytoplankton (Z/P)	Decrease	Kane <i>et al.</i> , 2009
	Z <sub>3</sub> : Taxa richness (TAR)	Decrease	O'Connor <i>et al.</i> , 2000
	Z <sub>4</sub> : Calanodia abundance ( <i>Cal.</i> )	Decrease	Carpenter <i>et al.</i> , 2006 Kane <i>et al.</i> , 2009
	Z <sub>5</sub> : Rotifer abundance ( <i>Rot.</i> )	Decrease	
	Z <sub>6</sub> : Cladocera abundance ( <i>Cl.</i> )	Decrease	Carpenter <i>et al.</i> , 2006
	Z <sub>7</sub> : Copepoda abundance ( <i>Cop.</i> )	Decrease	
	Z <sub>8</sub> : Copepod nauplii abundance ( <i>Cop. n.</i> )	Decrease	
	Z <sub>9</sub> : Cyclopoida abundance ( <i>Cyc.</i> )	Increase	
	Z <sub>10</sub> : Harpacticoida abundance ( <i>Har.</i> )	Decrease	
	Z <sub>11</sub> : Zooplankton ratio (ZR)	Decrease	Kane <i>et al.</i> , 2009
	Z <sub>12</sub> : Biomass of crustacean zooplankton (CZ)	Increase	
Diversity	Z <sub>13</sub> : Margalef diversity index (Margalef)	Decrease	Margalef, 1958
	Z <sub>14</sub> : Shannon-Wiener diversity index (Shannon)	Decrease	Shannon, 1949
	Z <sub>15</sub> : Simpson diversity index (Simpson)	Decrease	Simpson, 1949
	Z <sub>16</sub> : Pieloud evenness index (Pieloud)	Decrease	Yang <i>et al.</i> , 2011
Tolerance	Z <sub>17</sub> : Wetland zooplankton index (WZI)	Decrease	Lougheed and Chow-Fraser, 2002

*Z<sub>i</sub>*: Zooplankton index.

considered sufficient to regard two metrics as redundant (US EPA, 1998). It is suggested that usually a tight correlation ( $r > 0.75$ ) and a linear relationship is necessary to consider two metrics redundant. Pairs of metrics with lower correlation coefficients usually showed enough scatter or nonlinearity to indicate that each metric provided some new information. We selected one metric from each group of redundant metrics. We retained the one that had a tight correlation ( $r < 0.75$ ) for further analysis (Maxted *et al.*, 2000).

### Scoring of metrics

We used the 95<sup>th</sup> or 5<sup>th</sup> percentile value because this method avoids using anomalously high or low outliers as the best expected value (US EPA, 1999). The frequency was distributed at the reference sites of metrics 95<sup>th</sup> percentile and the maximum value. Calculated metric values were converted (normalized) to metric scores of 5, 3 or 1 depending on their proximity to the optimal values. For the metrics whose values decreased with the increase of stress (positive metrics), metric values above the 50<sup>th</sup> percentile were scored as 5, metric values between and including the 5<sup>th</sup> and 50<sup>th</sup> percentiles were scored as 3, and all metric values below the 5<sup>th</sup> percentile were scored as 1. For the metrics whose values increased with the increase of stress (negative metrics), metric values below the 50<sup>th</sup> percentile were scored as 5, metric values between and including the 50<sup>th</sup> and 95<sup>th</sup> percentiles were scored as 3, and metric values above the 95<sup>th</sup> percentile were scored as 1.

A final multi-metric index of biotic integrity was created by summing selected metrics of phytoplankton and zooplankton to establish the Planktonic Integrity Index (PII).

### Power and accuracy analysis

To assess the ability of the PII to distinguish sites or conditions, we also used box-and-whisker plots to define discriminatory power of the PII to distinguish between reference and impaired sites. The degree of overlap between interquartile (IQ) ranges (the box) of reference and impaired sites was considered a signal of the discriminatory capability of the PII, which scoring 2 or 3. We chose the data of September and October in 2013 to verify the accuracy of the PII.

### Relationship with stressors

The PII index was also tested for significant relationships with potential stressors. The potential stressors included limnological variables, such as WT, pH, SD, EC, DO, TP, TN, NH<sub>3</sub>-N, COD<sub>Mn</sub>, and fluoride. Principal component analysis (PCA) with SPSS version 13.0 was used to choose the major potential stressors (value of PC > 0.7) and Pearson correlation analysis was

used to identify important stressor-metric relationships (Wang *et al.*, 2012).

## RESULTS

### Species composition of phytoplankton and zooplankton

A total of 140 species of phytoplankton was obtained through sampling from 26 sites of Jingpo Lake: 58 species of Chlorophyta, 49 species of Bacillariophyta, 17 species of Cyanophyta, 8 species of Euglenophyta, 2 species of Pyrrophyta, Cryptophyta, Chrysophyta and Xanthophyta, respectively, accounting for 41.5%, 35.0%, 12.05%, 5.7%, 1.4%, 1.4%, 1.4% and 1.4% of the total number of collected species, respectively.

A total of 92 species of zooplankton were obtained through sampling from 26 sites of Jingpo Lake: 46 species of Rotifer, 20 species of Protozoa, 16 species of Copepoda and 10 species of Cladocera, accounting for 50.0%, 21.7%, 17.4% and 10.9% of the total number of collected species, respectively.

### Evaluation of metrics

Following the water quality criteria (Type II and III) derived from Chinese surface water quality standard, 10 sites (S2, S4, S6, S7, S8, S9, S10, S12, S13 and S26) were determined as reference sites, the remained 16 sites were impaired sites. The information of water quality measures and characterization was shown in Tab. 5.

### Discriminatory power

On the basis of this classification, of 17 phytoplankton metrics and 17 zooplankton metrics evaluated, 7 phytoplankton and 5 zooplankton metrics scored a 2 or 3 in discriminatory power between reference and impaired sections (Fig. 2). The 7 phytoplankton metrics were P<sub>2</sub>, P<sub>3</sub>, P<sub>4</sub>, P<sub>5</sub>, P<sub>6</sub>, P<sub>7</sub> and P<sub>17</sub>, and the 5 zooplankton metrics were Z<sub>1</sub>, Z<sub>3</sub>, Z<sub>5</sub>, Z<sub>13</sub> and Z<sub>14</sub>.

### Redundancy

Among the 7 phytoplankton metrics, several pairs or groups were highly correlated and considered redundant (Tab. 6), including P<sub>3</sub> with P<sub>4</sub>, P<sub>5</sub> and P<sub>7</sub> (all  $r > 0.75$ ,  $P < 0.01$ ), P<sub>4</sub> with P<sub>5</sub>, P<sub>6</sub> and P<sub>7</sub> (all  $r > 0.75$ ,  $P < 0.01$ ), P<sub>5</sub> with P<sub>6</sub> ( $r = 0.954$ ,  $P < 0.01$ ). Finally we selected P<sub>7</sub> from the three groups of redundant metrics, because it was simpler to calculate than the other metrics. The two other metrics (P<sub>2</sub> and P<sub>17</sub>) that were not redundant with any other metrics were also candidates for final selection, leaving a total of three algae metrics for establishing the PII. Among the 5 zooplankton metrics, several pairs or groups were highly correlated and considered redundant (Tab. 7), including Z<sub>1</sub> with Z<sub>3</sub> and Z<sub>5</sub>

(all  $r > 0.75$ ,  $P < 0.01$ ),  $Z_3$  with  $Z_{13}$  ( $r = 0.824$ ,  $P < 0.01$ ). Finally we selected  $Z_{13}$  from the two groups of redundant metrics.  $Z_{14}$ , which was not redundant with any other metrics, was also a candidate for final selection, leaving a total of 2 zooplankton metrics for establishing the PII. Final metrics we selected were: Algal cell abundance, Species richness of algae, Trophic diatom index, Zooplankton Shannon index and Zooplankton Margalef index.

**Scoring of metrics**

Frequency distribution statistics and scoring criteria allowed us to select 5 metrics for our index (Tab. 8). Based on the scoring of each metric, a multi-metric on a scale ranging from 1 to 25 for bioassessment was developed for each site by addition of the 5 metrics. Four levels of discriminatory biocriteria for water quality were eventually obtained by quartation: 5-10, very poor; 11-15, poor; 16-20, fair; 21-25, good.

**Power and accuracy analysis**

As the results show that the PII can effectively distinguish between reference and impaired sites with no overlap between interquartile (IQ) ranges (the box) in September or October (Fig. 3), which indicated that the available power and accuracy of the PII.

**Relationship to stressors**

The results of principal component analysis showed that WT, pH, EC, SD, DO,  $COD_{Mn}$ , TN, TP and  $NH_3-N$  were the main factors influencing the water quality of Jingpo Lake (Tab. 9).

The results of correlation between PII and main factors indicated that the PII scores were influenced more significantly in October ( $R = -0.527$ ,  $P < 0.01$ ) than in September ( $R = -0.420$ ,  $P < 0.05$ ) by WT, more significantly in September ( $R = -0.534$ ,  $P < 0.01$ ) than in October

**Tab. 5.** Water quality of sampling sites in Jingpo Lake.

Sites	T °C	pH	SD (m)	$\rho(EC)$ (ms/m)	$\rho(DO)$	$\rho(COD_{Mn})$	$\rho(TN)$	$\rho(TP)$	$\rho(NH_3-N)$	$\rho(F)$
					(mg/L)					
S1	14.20±7.70	7.82±0.45	0.38±0.24	0.70±0.41	10.56±0.57	5.60±1.04	1.00±0.03	0.08±0.00	0.18±0.06	0.14±0.01
S2	<b>13.97±6.79</b>	<b>7.83±0.47</b>	<b>0.23±0.06</b>	<b>9.90±0.96</b>	<b>12.51±0.81</b>	<b>5.47±0.67</b>	<b>0.80±0.09</b>	<b>0.05±0.00</b>	<b>0.19±0.08</b>	<b>0.16±0.01</b>
S3	15.40±7.43	7.70±0.35	0.22±0.08	12.57±3.11	10.34±1.47	6.00±1.08	1.05±0.33	0.12±0.01	0.20±0.11	0.21±0.05
S4	<b>12.07±6.45</b>	<b>8.10±0.62</b>	<b>0.33±0.15</b>	<b>11.87±1.31</b>	<b>13.31±0.37</b>	<b>5.70±0.56</b>	<b>0.85±0.23</b>	<b>0.06±0.01</b>	<b>0.17±0.07</b>	<b>0.16±0.01</b>
S5	9.90±3.93	7.43±0.12	0.17±0.12	3.47±5.31	10.29±2.00	5.77±0.71	0.88±0.28	0.07±0.01	0.21±0.09	0.18±0.04
S6	<b>14.90±3.94</b>	<b>8.20±1.08</b>	<b>0.41±0.29</b>	<b>6.50±5.28</b>	<b>12.17±2.98</b>	<b>5.80±0.10</b>	<b>0.77±0.11</b>	<b>0.05±0.00</b>	<b>0.20±0.08</b>	<b>0.14±0.03</b>
S7	<b>13.27±3.90</b>	<b>7.77±0.32</b>	<b>0.37±0.15</b>	<b>5.13±4.10</b>	<b>12.05±2.18</b>	<b>5.47±0.76</b>	<b>0.76±0.30</b>	<b>0.05±0.01</b>	<b>0.21±0.08</b>	<b>0.14±0.01</b>
S8	<b>14.30±4.59</b>	<b>7.87±0.45</b>	<b>0.27±0.06</b>	<b>5.10±3.52</b>	<b>11.02±2.14</b>	<b>5.33±0.64</b>	<b>0.85±0.14</b>	<b>0.05±0.00</b>	<b>0.25±0.07</b>	<b>0.13±0.01</b>
S9	<b>14.83±3.77</b>	<b>7.90±0.50</b>	<b>0.40±0.10</b>	<b>5.47±3.78</b>	<b>10.61±1.85</b>	<b>5.20±1.22</b>	<b>0.93±0.01</b>	<b>0.05±0.01</b>	<b>0.24±0.04</b>	<b>0.18±0.02</b>
S10	<b>15.47±2.60</b>	<b>7.90±0.56</b>	<b>0.35±0.23</b>	<b>5.23±4.37</b>	<b>10.40±0.00</b>	<b>5.47±0.58</b>	<b>0.92±0.08</b>	<b>0.05±0.02</b>	<b>0.21±0.09</b>	<b>0.15±0.01</b>
S11	16.70±4.44	7.63±0.29	0.33±0.12	8.37±0.32	8.90±0.84	6.17±1.37	0.94±0.01	0.07±0.02	0.24±0.09	0.16±0.02
S12	<b>16.37±2.56</b>	<b>7.77±0.40</b>	<b>0.32±0.16</b>	<b>7.67±0.90</b>	<b>10.44±3.03</b>	<b>5.30±1.13</b>	<b>0.68±0.28</b>	<b>0.05±0.00</b>	<b>0.22±0.13</b>	<b>0.16±0.02</b>
S13	<b>16.90±3.22</b>	<b>7.87±0.51</b>	<b>0.47±0.06</b>	<b>5.93±4.71</b>	<b>11.44±2.06</b>	<b>5.07±1.36</b>	<b>0.92±0.08</b>	<b>0.05±0.01</b>	<b>0.22±0.14</b>	<b>0.14±0.01</b>
S14	16.30±2.30	7.67±0.47	0.47±0.06	8.23±1.15	8.10±0.71	5.73±1.27	1.12±0.34	0.06±0.00	0.22±0.14	0.17±0.02
S15	16.57±2.76	7.70±0.36	0.47±0.06	8.30±0.60	8.65±0.78	5.93±1.51	1.16±0.09	0.06±0.00	0.25±0.07	0.18±0.02
S16	16.73±2.06	7.77±0.45	0.47±0.12	5.70±3.82	9.00±1.44	6.17±1.31	1.01±0.06	0.07±0.02	0.21±0.08	0.16±0.01
S17	16.57±2.20	7.87±0.49	0.43±0.06	8.20±0.46	8.23±1.09	5.90±1.23	1.11±0.09	0.07±0.02	0.26±0.07	0.14±0.02
S18	16.67±2.21	7.77±0.35	0.43±0.06	8.23±0.55	7.86±0.57	6.37±1.55	0.90±0.04	0.06±0.01	0.28±0.06	0.15±0.02
S19	16.80±2.10	7.80±0.40	0.62±0.18	8.00±0.46	8.65±0.07	6.50±1.66	0.98±0.11	0.05±0.00	0.23±0.13	0.19±0.03
S20	16.97±1.88	7.77±0.32	0.67±0.13	6.80±2.61	8.23±0.89	5.93±1.07	0.96±0.09	0.05±0.00	0.27±0.09	0.18±0.02
S21	16.90±1.78	7.93±0.50	0.50±0.00	7.90±0.35	8.43±1.17	6.20±1.61	0.98±0.07	0.06±0.00	0.33±0.06	0.16±0.01
S22	16.63±2.22	7.80±0.50	0.87±0.12	7.87±0.40	7.93±0.88	6.17±1.46	1.01±0.04	0.08±0.00	0.41±0.08	0.18±0.03
S23	16.60±2.10	7.90±0.56	0.72±0.03	8.77±0.86	7.86±1.47	6.20±1.39	1.11±0.08	0.07±0.01	0.29±0.09	0.14±0.01
S24	16.50±2.25	7.97±0.59	0.57±0.06	9.30±1.45	8.13±0.01	6.07±1.63	1.16±0.06	0.06±0.00	0.25±0.06	0.15±0.01
S25	15.37±2.99	7.97±0.59	0.5±0.10	7.10±1.22	9.56±2.23	6.33±1.59	1.03±0.04	0.07±0.01	0.21±0.07	0.17±0.01
S26	<b>13.90±7.20</b>	<b>7.90±0.53</b>	<b>0.57±0.06</b>	<b>8.80±1.42</b>	<b>13.33±3.01</b>	<b>5.33±1.07</b>	<b>0.60±0.02</b>	<b>0.05±0.00</b>	<b>0.19±0.10</b>	<b>0.22±0.02</b>

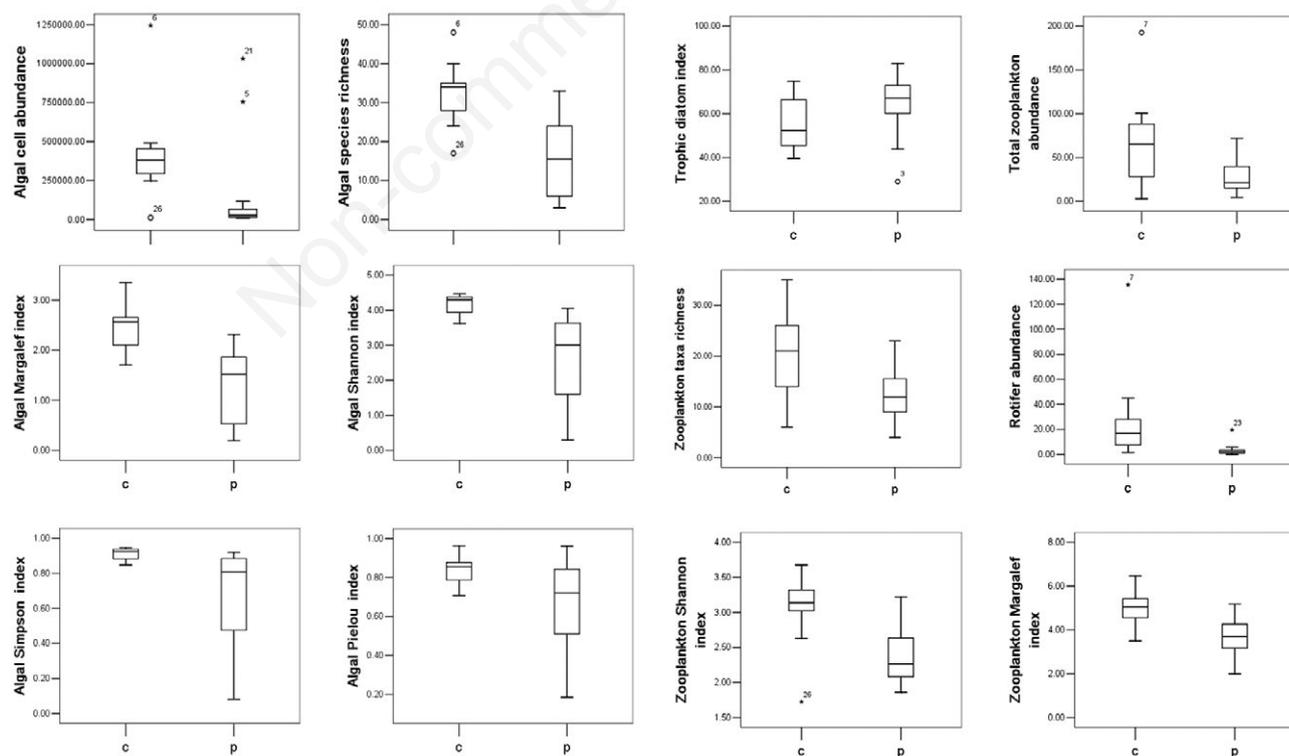
T, temperature; SD, transparency; EC, electrical conductivity; DO, dissolved oxygen; TP, total phosphorus; TN, total nitrogen;  $COD_{Mn}$ , potassium permanganate;  $NH_3-N$ , ammonia nitrogen; F, fluoride;  $\rho$ , indicator concentration.

**Tab. 6.** Pearson correlation analysis of 7 algae candidate metrics.

	P <sub>2</sub>	P <sub>3</sub>	P <sub>4</sub>	P <sub>5</sub>	P <sub>6</sub>	P <sub>7</sub>	P <sub>17</sub>
P <sub>2</sub>	1						
P <sub>3</sub>	0.633**	1					
P <sub>4</sub>	0.413*	0.868**	1				
P <sub>5</sub>	0.343	0.792**	0.973**	1			
P <sub>6</sub>	0.142	0.610**	0.908**	0.954**	1		
P <sub>7</sub>	0.746**	0.982**	0.805**	0.712**	0.510	1	
P <sub>17</sub>	0.021	-0.385	-0.479	-0.524	-0.471	-0.332	1

\* $P < 0.05$ ; \*\* $P < 0.01$ .**Tab. 7.** Pearson correlation analysis of 5 zooplankton candidate metrics.

	Z <sub>1</sub>	Z <sub>3</sub>	Z <sub>5</sub>	Z <sub>13</sub>	Z <sub>14</sub>
Z <sub>1</sub>	1				
Z <sub>3</sub>	0.910**	1			
Z <sub>5</sub>	0.846**	0.747**	1		
Z <sub>13</sub>	0.632	0.824**	0.618**	1	
Z <sub>14</sub>	0.539**	0.730**	0.442*	0.710**	1

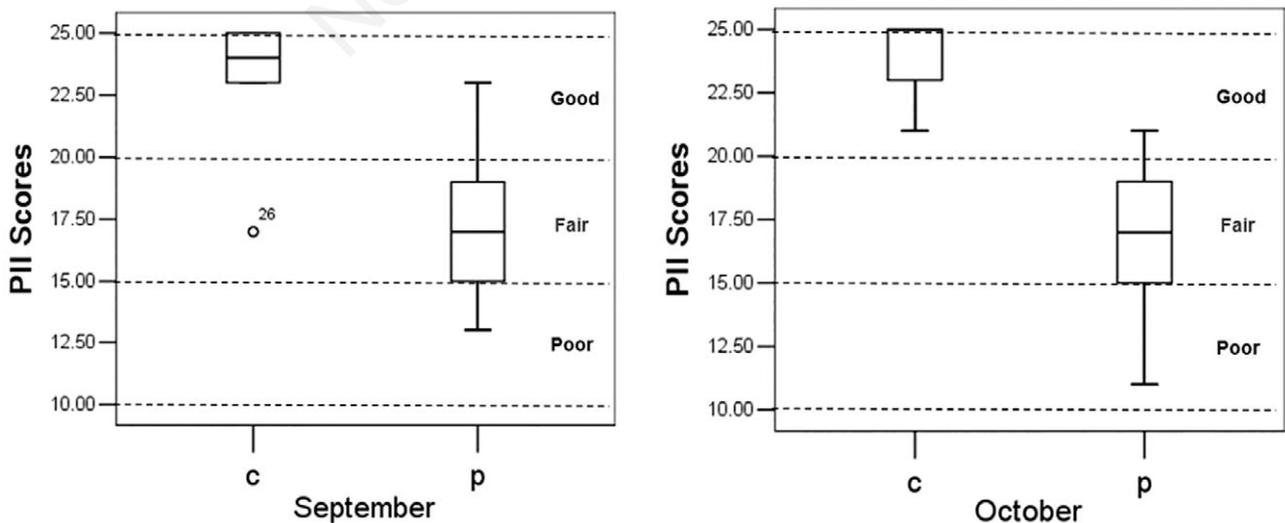
\* $P < 0.05$ ; \*\* $P < 0.01$ .**Fig. 2.** Discriminatory of algae and zooplankton metrics by box-and-whisker plots. The degree of overlap between interquartile (IQ) ranges (the box) of reference and impaired sites was considered a signal of the discriminatory capability of the PII, which scoring 2 or 3, in this study, the metrics with  $IQ \geq 2$  could go to the next screening steps. c, reference sites; p: impaired sites.

**Tab. 8.** Frequency distribution statistics of the final metrics and associated scoring criteria.

Metrics	Frequency distribution					Score		
	Min	5 <sup>th</sup> percentile	50 <sup>th</sup> percentile	95 <sup>th</sup> percentile	Max	5	3	1
Algal cell abundance ( $\times 10^4$ )	0.75	0.87	6.53	117.04	124.5	>6.53	0.87~6.53	<0.87
Species richness of algae	3	3	23	45.2	48	>23	3~23	<3
Trophic diatom index	28.97	32.68	63.47	80.17	82.95	<63.47	63.47~80.17	>80.17
Zooplankton Shannon index	1.72	1.77	2.61	3.59	3.68	>2.61	1.77~2.61	<1.77
Zooplankton Margalef index	1.99	2.13	4.26	6.15	6.46	>4.26	2.13~4.26	<2.13

**Tab. 9.** Rotated component matrix.

Factors	September				October		
	PC1	PC2	PC3	PC4	PC1	PC2	PC3
WT	<b>0.71</b>	0.55	-0.18	0.04	<b>0.91</b>	0.29	-0.03
DO	<b>0.80</b>	0.36	-0.28	-0.25	<b>0.88</b>	0.13	-0.12
pH	-0.00	-0.12	-0.00	<b>-0.97</b>	0.08	0.03	<b>0.92</b>
EC	-0.10	-0.36	<b>0.82</b>	-0.22	-0.47	-0.18	<b>0.73</b>
SD	<b>0.82</b>	-0.31	0.13	0.19	<b>0.85</b>	0.04	-0.08
COD <sub>Mn</sub>	<b>0.74</b>	0.13	-0.28	0.11	<b>0.82</b>	-0.10	-0.06
TN	0.18	<b>0.91</b>	0.03	-0.16	<b>0.71</b>	-0.13	0.29
TP	-0.12	0.38	<b>0.83</b>	0.24	-0.18	<b>-0.80</b>	-0.15
NH <sub>3</sub> -N	<b>0.85</b>	0.23	0.03	-0.19	-0.10	<b>0.74</b>	-0.23
Characteristic value	3.13	1.70	1.58	1.22	3.73	1.35	1.31
Variance %	34.8	18.9	17.5	13.5	41.4	15.0	14.6
Cumulative variance %	34.8	53.7	71.2	84.7	41.4	56.4	71.0



**Fig. 3.** Distinguish efficiency of PII in September and October. c, clean sites; p: impaired sites.

( $R=-0.461$ ,  $P<0.05$ ) by SD, significantly in September ( $R=0.588$ ,  $P<0.01$ ) and October ( $R=0.559$ ,  $P<0.01$ ) by DO, significantly in September ( $R=-0.581$ ,  $P<0.01$ ) and October ( $R=-0.587$ ,  $P<0.01$ ) by  $\text{COD}_{\text{Mn}}$ , significantly in October ( $R=-0.432$ ,  $P<0.05$ ) but not significantly in September ( $R=-0.300$ ,  $P>0.05$ ) by TN, and the other factors (pH, EC, TP and  $\text{NH}_3\text{-N}$ ) had no significant correlations with the PII scores (Fig. 4).

## DISCUSSION

### Selection of metrics and reference sites

The reference condition approach (RCA) has recently emerged as a broadly applicable protocol to monitor quality of streams, rivers, and lakes at regional level (Tall *et al.*, 2008). Usually, it is difficult to define the actual clean site (or reference site) in a homogeneous ecological

region owing to frequent occurrence of pollution in freshwater bodies of China. So in this study, the classification of clean and impaired sites was only a relative division. Generally, there are two methods including Shannon-Wiener species diversity index method (Huang *et al.*, 1982; Wang and Yang, 2003), and physico-chemical index method (Stribling *et al.*, 1998), through which the reference and impaired sites can be differentiated. The physicochemical index method is undoubtedly more powerful, but it needs too many physicochemical indices for classification, and further leads to time consuming calculation and analysis. Only simple calculation is needed when Shannon-Wiener species diversity index method is employed; however, it has a low accuracy or some misjudgments because of limitation of sampling area (small area of the grab). Finally, we chose physicochemical index method for classification of clean and impaired sites.

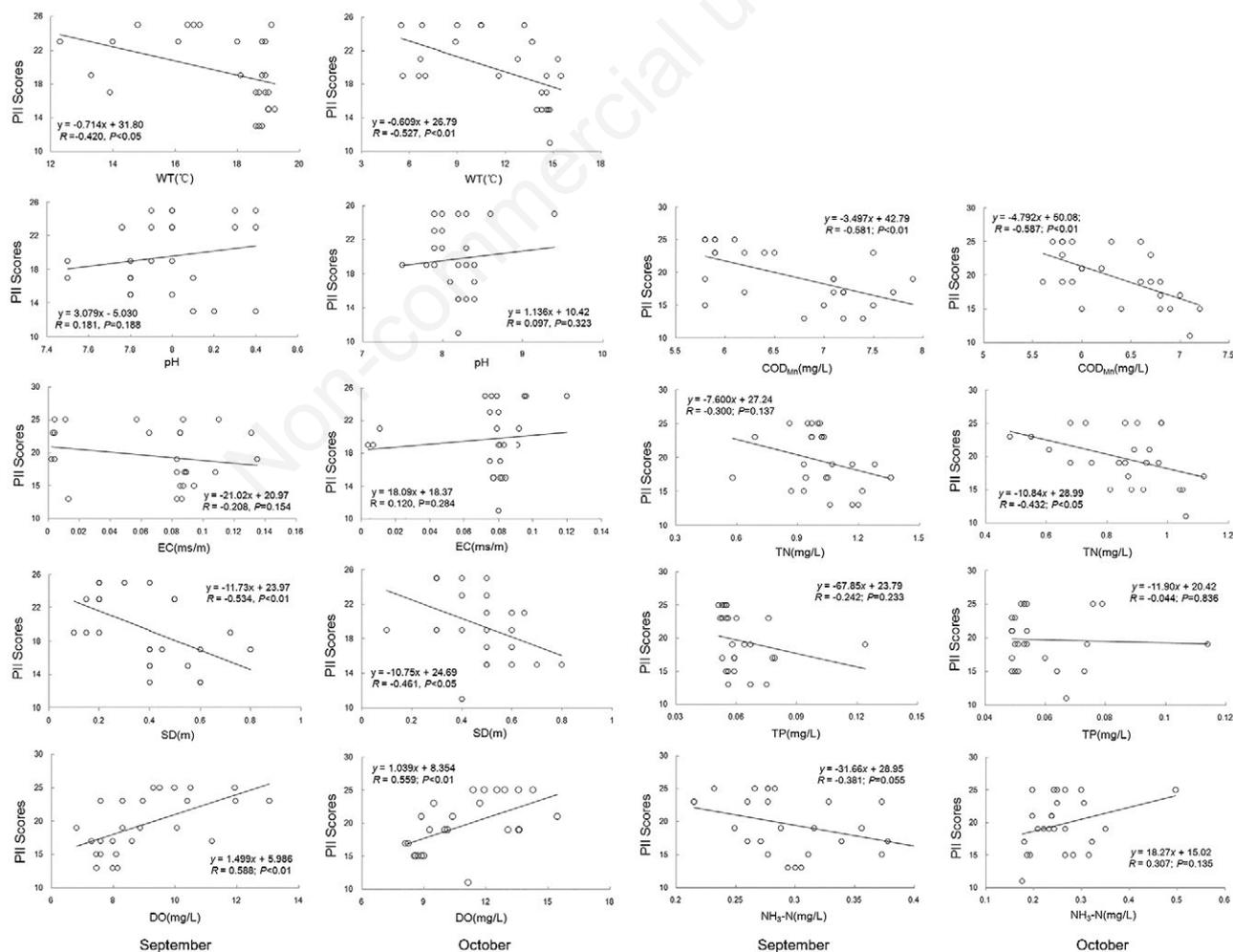


Fig. 4. The relationship between PII Scores and major environment factors.

### Power and accuracy of the PII

Discriminatory power of a metric is defined as the ability of that metric to distinguish between reference and impaired sites and the metric needs to be evaluated by examining its distribution by using box-and-whisker plots. In this study, the reference and impaired sites could be effectively distinguished by the PII with no overlap between IQ ranges (the box) in September or October (Fig. 3), which indicated the available power and accuracy of the PII. Additionally, the bioassessment results of Jingpo Lake by PII indicated that the water quality of upstream region was better than central and outlet of Jingpo Lake, which was completely consistent with previous studies assessed by Algal cell abundance, Species richness of algae, Phytoplankton Shannon index, Phytoplankton Margalef index, Species richness of zooplankton, Zooplankton Shannon index and Zooplankton Margalef index, respectively (Yu *et al.*, 2008a; Song and Yu, 2009; Liu *et al.*, 2012; Wang *et al.*, 2015a, 2015b).

However, the factors of habitat, hydrology, physics and chemistry, human activities, for example, flow velocity, climate, land use, man-made dams, cultivation, deforestation, which can influence the power and accuracy of the assessment results were not yet considered into the PII. So the PII with comprehensive assessment systems needs to be established, so as to improve the power and accuracy of the assessment metric established in our present study.

### Relationship between PII and environment factors

WT (Mageed and Heikal, 2006; Jiang *et al.*, 2014), SD (Arhonditsis *et al.*, 2004; Dejen *et al.*, 2004; Wang *et al.*, 2015b), pH (Wu *et al.*, 2011), COD<sub>Mn</sub>, TN, NH<sub>3</sub>-N (Yu *et al.*, 2008a; Yang *et al.*, 2012; Wang *et al.*, 2015a, 2015b), DO, TP, Chl *a* (Wu *et al.*, 2011; Wang *et al.*, 2012; Jiang *et al.*, 2014), chloride, orthophosphate and silica (Swadling *et al.*, 2000; Jiang *et al.*, 2014) can all become the major factors influencing the community composition of phytoplankton and zooplankton. As our results show, WT, SD, DO, COD<sub>Mn</sub> and TN were the major factors influencing PII, indicating that these five environmental parameters were also the limiting factors influencing the community distribution of phytoplankton and zooplankton in Jingpo Lake. As a cold lake in the northeastern of China, the water temperature showed significantly differences in day and night and different seasons in Jingpo Lake, which may affect the composition of phytoplankton and zooplankton (Liu and Xu, 1996; Yu *et al.*, 2008a; Wang *et al.*, 2015a, 2015b). Phytoplankton need light for photosynthesis and oxygen for respiration, which indicated that SD is one key factors for phytoplankton growth (Wang *et al.*, 2015b).

Likewise, the growth of zooplankton needs not only requisite ingestion of algae, but also oxygen (DO) for respiration, which is rather a response parameter to photosynthesis (indicator, response parameter) and secondary production, than a major (affective) parameter for primary production (Wang *et al.*, 2015b). COD<sub>Mn</sub> and TN are usually used for monitoring of the point and nonpoint pollution. As shown in some studies, the external contaminations of Jingpo Lake mainly came from the industrial and domestic wastewater of Dunhua City, located upstream of Jingpo Lake. Agricultural fertilizer entered via runoff into Jingpo Lake and domestic wastewater and landfill leachate also entered Jingpo Lake. Thus COD<sub>Mn</sub> was mainly affected by industrial wastewater, whereas TN was mainly affected by domestic wastewater and agricultural fertilizer (Jin *et al.*, 2009; Li, 2013). The results of this study also indicated that point and nonpoint pollution may be the direct factors influencing on composition and distribution of phytoplankton and zooplankton in Jingpo Lake.

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### CONCLUSIONS

In the present study, a Planktonic Integrity Index (PII) for the China's largest alpine barrier lake (Jingpo Lake) was developed to assess the water quality of Jingpo Lake by using phytoplankton and zooplankton metrics. A total of 140 species of phytoplankton and 92 species of zooplankton were obtained in the investigations. Algal cell abundance, Species richness of algae, Trophic diatom index, Zooplankton Shannon index, and Zooplankton Margalef index were selected for the PII. Evaluation of the PII showed that it discriminated well between reference and impaired sites and the discriminatory biocriteria of the PII were suitable for the assessment of the water quality of Jingpo Lake. The further scoring results from the 26 sites showed that the water quality of Jingpo Lake was fair to good. Additionally, more metrics belonging to habitat, hydrology, physics and chemistry should also be considered into the PII, so as to establish comprehensive assessment system which can reflect the community structure of aquatic organisms, physical and chemical characteristics of water environment and, human activities.

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