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Water quality assessment of benthic diatom communities for water quality in the subalpine karstic lakes of Jiuzhaigou, a world heritage site in China

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Abstract: Jiuzhaigou, characterized by its magnificent waterscapes and subalpine karstic features, is both a World Heritage Site and a World Biosphere Reserve in southwestern China. In recent years, this unique ecosystem has shown signs of stress due to increasing tourism activities within the reserve. The various routine methods, which monitor physical and chemical properties, do not fully reflect water quality in the subalpine and alpine lakes, while the indicators using aquatic organisms to evaluate the water quality or status of the subalpine lakes are poorly reported. Thus, in this study, benthic diatoms from multiple habitats in Jiuzhaigou were sampled and assessed for water quality monitoring. Canonical Correspondence Analysis (CCA) showed that the canonical coefficients for elevation, water temperature and total nitrogen on the first Canonical Correspondence Analysis axis were -0.84, 0.78 and -0.53, respectively, environmental variables associated with the distribution patterns of benthic diatoms. The dominance of diatom taxa indicative of nutrient enrichment indicates a clear trend toward eutrophication in the Pearl Shoal and Colorful Lake, two of the sites mostly visited by tourists. It was observed that the effect of the type of substratum on diatom community composition is not significant in subalpine lakes. The most dominant species in Jiuzhaigou lakes are the genera *Achnanthes*, *Fragilaria*, *Cymbella*, *Cocconeis*, *Diatoma* and *Denticula*. In combination with dominant and sensitive species in the benthic diatom communities, CCA and CA methods can be used to evaluate the impact of human activities on subalpine karstic lakes. The dominance of diatom taxa is indicative of nutrient enrichment and the results of CCA and CA indicate a clear trend toward eutrophication in the Pearl Shoal and Colorful Lake, two of the sites mostly visited by tourists.

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Introduction

A vast number of lakes have suffered varying degrees of pollution across the world, and to monitor lake water quality, physical and chemical proxies have been commonly used (Akkoyunlu and Akiner 2012; Wang et al. 2014). However, whilst these physical and chemical methods definitely indicate the water quality status at the sampling points at a particular time, usually they cannot comprehensively reflect the overall situation regarding changes in water quality (Brendan et al. 2013; Catherine et al. 2013).

Benthic algae are primary producers in freshwater lake ecosystems. They are at the bottom of the food chain in ecosystems with stable habitats, and are often used as biological indicators of water quality due to their sensitivity to pollutants (Winter and Duthie 2000; Wang et al. 2013; Smucker and Vis 2013). There is a long history of using diatoms to evaluate the environmental status of lakes, rivers and streams worldwide (Descy and Coste 1991; Kelly and Whitton 1995; Potapova and Charles 2007; Bere and Tundisi 2012; Gudmundsdottira et al. 2013), and the diverse assessment methods include various indicators and predictive models (Rimet 2012). However, some researchers have suggested that these indicators and models, typically, are only applicable to certain regions, particularly in contaminated lakes and streams (Rimet 2012). Reports on evaluating the water quality of alpine and subalpine lakes using benthic diatoms are fewer than those of plain rivers (Kilroy et al. 2006; Rott et al. 2006; Moser et al. 2010; Blanco et al. 2013). Furthermore, existing studies favor using benthic algae growing on rocks to monitor water quality because such substrata are widely available and relatively easy to access (Kelly et al. 1998; Moravcova et al. 2013). However, there are also benthic diatoms that are epiphytes on macrophytes and macroalgae. Studies of the results of the impact of these different types of substratum on benthic diatom communities have sparked major controversies. Some researchers argue that the type of substratum affects the distribution and composition of the attached diatom communities (Potapova and Charles 2005; Kroepfl et al. 2006; Bere and Tundisi 2010; Majewska et al. 2012; Sweat and Johnson 2013), while other researchers hold an opposing view (Millie and Lowe 1983).

Benthic diatom assemblages may be ideal indicators of water quality in the lakes of Jiuzhaigou National Nature Reserve (hereafter referred to as Jiuzhaigou), in southwestern China. Liu et al. (2007) reported that 47 species of algae can be used as indicators in Jiuzhaigou, and 39 of the 47 species are diatoms. The distribution of algae in the Jiuzhaigou lakes is characterized by the sparse planktonic assemblage and biomass due to ultra-oligotrophic conditions (Bao et al. 1986). Considering that most of lakes in Jiuzhaigou are shallow and clear, light can reach the bottom of the lakes, which is favorable for growth of benthic diatoms, and as a result benthic organisms often dominate the food chain. Therefore, we used benthic diatoms to evaluate the water quality in the Jiuzhaigou lakes. Unlike other subalpine lakes, Jiuzhaigou is unique due to its fragile karstic nature coupled with heavy tourism, and there is an urgent need for more scientifically sound management solutions.

In this paper, we investigated the species composition of the benthic diatoms in 8 lakes and wetlands in Jiuzhaigou National Nature Reserve and their relationships with environmental factors. The specific objectives include (1) characterizing the patterns of distribution of benthic diatoms in the Jiuzhaigou lakes and (2) assessing the water quality of subalpine karstic lakes using the environmental significance of the dominant species and communities of benthic diatoms.

1 Materials and Methods

1.1 Study area

Jiuzhaigou National Nature Reserve is situated in Aba, Sichuan Province, in southwestern China (103°46'-104°05' E longitude and 32°53'-33°20' N latitude). This subalpine region (elevation: 1996-4789 m) includes three valleys: the Rize Valley, the Shuzheng Valley and the Zechawa Valley. The reserve is protected by a single tourist entrance and exit located on the northernmost side of the reserve.

The total area of the Jiuzhaigou waters is approximately 2.85 km^2, and the surface water flows from south to north. There are a total of 118 lakes, 17 waterfall groups, 47 springs and 11 streams/rivers in the reserve. The reserve is well known for its emerald water, and the overall water quality complies with national standards for first class surface water quality (Zhou et al. 2009). Since Jiuzhaigou was officially opened to the public in 1984, the number of visitors has increased sharply each year, from just 27,500 in 1984 to 2,521,800 in 2007. The number of tourists reached 3,639,000 in 2012, and most tourists were concentrated in a 55 km^2 area (Li et al. 2014). The more tourists, the more rubbish discarded. In addition, some antisocial tourism behavior, such as washing feet in the lakes, feeding the fish and so on, also has had a bad influence on the environment. As a result, tourism activities have resulted in a series of environmental problems in several lakes, such as degraded travertine and water quality. Thus the water quality of Jiuzhaigou has attracted considerable attention (Li et al. 2014).

Benthic diatom samples were collected from 26 sampling sites in 8 lakes in the Rize Valley: Grass Lake, Swan Lake, the wetland of Arrow Bamboo Lake, Arrow Bamboo Lake, Panda Lake, Colorful Lake, Pearl Shoal and Mirror Lake (Figure 1) (Jiuzhaigou Administrative Bureau issued permits for each location, and the field studies did not threaten endangered or protected species. The location of our study was specifically constrained between 103.86°-103.89°E longitude and 33.08°-33.16° N latitude), and we sampled in July and September, respectively. In addition, diatom samples of different substrate types were collected to compare the effect of different substrate types on the diatom growth (Table 1). These substrates were dead tree branches, *Hippuris vulgaris*, *Equisetum ramosissimum*, *Potamogeton ectinatus*, moss, rock and *Spirogyra*. A total of 47 diatom samples were collected in July, and 30 were collected in September.

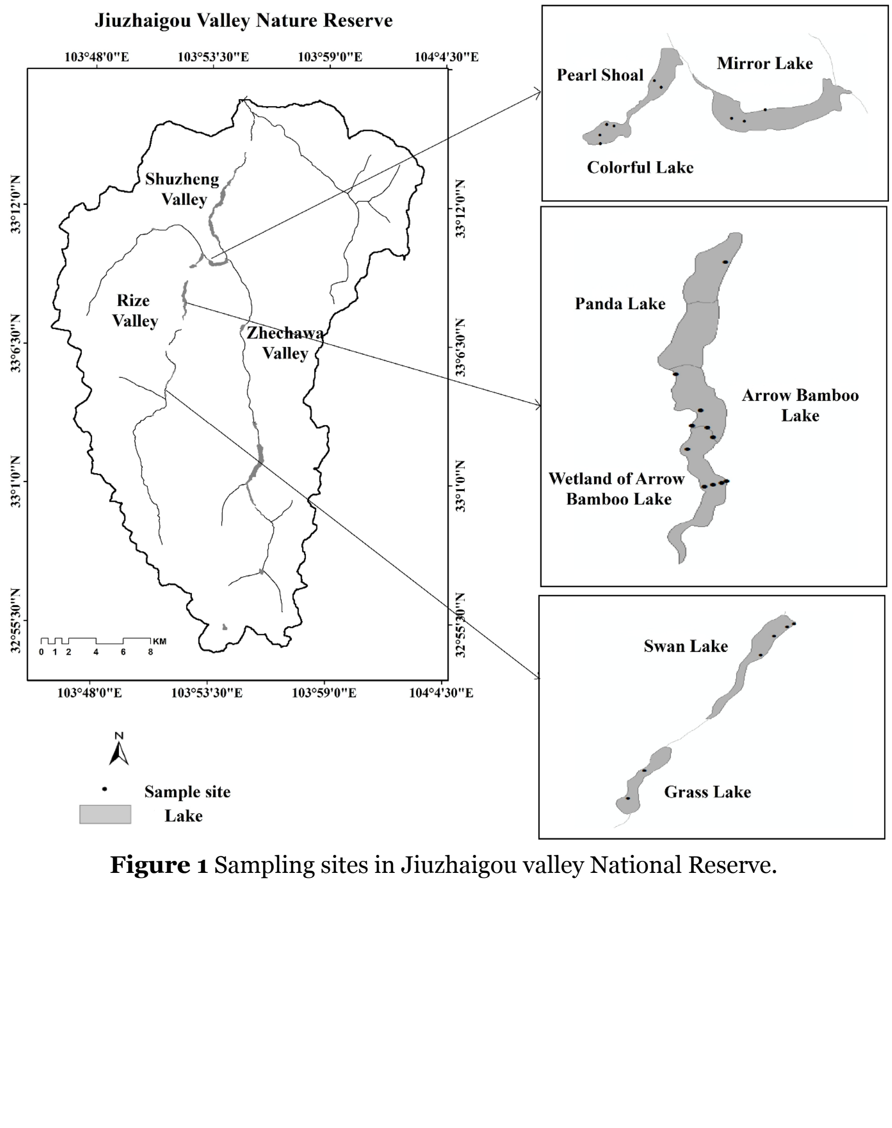

Figure 1 Sampling sites in Jiuzhaigou valley National Reserve.

1.2 Sample collection

We first identified the major diatom habitats in the littoral zone and then collected the natural matrix of the benthic diatoms and sealed them in zip lock bags and took them back to the lab. In the lab, benthic diatoms attached to some substrates, such as stones and branches, were scraped with a toothbrush and preserved with 35%~40% formaldehyde. Other substrates were squeezed or shaken to dislodge the epiphyton and rinsed thoroughly with distilled water. Diatom samples were digested with concentrated hydrochloric acid, concentrated sulfuric acid and concentrated nitric acid and repeatedly rinsed with deionized water until the pH was approximately neutral (Kelly et al. 1998). Clean diatom valves were mounted on slides

Table 1 Environmental data of the sampling sites in Jiuzhaigou in July, 2012

Sampling lake	Sampling site	Elevation (m)	Substratum type
Grass Lake	S1 S2 S3	2910	dead tree branches moss *Hippuris vulgaris*
Mirror Lake	S4 S5 S6 S7 S8 S9 S10	2390	dead tree branches *Equisetum ramosissimum* *Hippuris vulgaris* *Hippuris vulgaris* *Equisetum ramosissimum* *Hippuris vulgaris* *Potamogeton pectinatus*
Wetland of Arrow Bamboo Lake	S11 S12 S13 S14 S15 S16 S17	2618	Hippuris vulgaris dead tree branches *Hippuris vulgaris* *Hippuris vulgaris* dead tree branches *Potamogeton pectinatus* dead tree branches
Arrow Bamboo Lake	S18 S19 S20 S21 S22 S23 S24 S25 S26 S27	2618	*Hippuris vulgaris* dead tree branches *Equisetum ramosissimum* *Potamogeton pectinatus* *Hippuris vulgaris* *Hippuris vulgaris* *Hippuris vulgaris* dead tree branches rock *Hippuris vulgaris*
Pearl Shoal	S28 S29 S30 S31	2443	moss moss rock rock
Swan Lake	S32 S33 S34 S35 S36 S37 S38 S39	2905	rock moss dead tree branches moss moss dead tree branches *Hippuris vulgaris* Rock
Colorful Lake	S40 S41 S42 S43 S44 S45	2472	*Equisetum ramosissimum* moss dead tree branches Spirogyra *Equisetum ramosissimum* moss
Panda Lake	S46 S47	2587	moss dead tree branches

using gum (Kelly et al. 1998; You 2006).

Water temperature, pH and conductivity were analyzed in situ using a portable conductivity meter (Hanna Instrument Company, USA). A 250 ml water sample was collected for the measurement of total nitrogen and total phosphorus because the growth of algae is greatly affected by nitrogen and phosphorus. Total nitrogen was analyzed using a TOC (Total organic carbon) analyzer (Analytik Jena Company, Germany), and total phosphorus was analyzed using the ammonium molybdate spectrophotometry method (Ministry of Environmental Protection of the People's Republic of China 2002).

1.3 Diatom analysis method

Using an optical biological microscope (OLYMPUS CX31, Olympus, Japanese) at 1000×

magnification, slides were scanned along transects until at least 500 diatom valves were enumerated and identified to the species level, as much as possible. Taxonomic identification followed Krammer et al. (1988), Zhu et al. (2000) and Hu et al. (2006).

1.4 Data analysis method

Correspondence analysis (CA) is a method for multivariate statistical analysis. It can reveal the mutual relationships between variables and categories in qualitative data (Kargıoğlu et al. 2012; Andrus et al. 2013; Michelutti et al. 2013). We used CA to analyze the differences between sites with different types of diatom community compositions.

Canonical correspondence analysis (CCA) is a method that can elucidate the relationships between biological communities and their environment (McGarigal et al. 2000; Pappas 2010). We used CCA to identify the environmental variables that have the greatest impact on the benthic diatom communities. Water temperature, pH, conductivity, total nitrogen, total phosphorus and elevation were used as the environmental variables in this study. CCA represents the similarity at sampling sites or dissimilarity in a simplified and condensed form from which patterns of variation, can be identified and interpreted by extracting the most important dimensions (Lavoie et al. 2006; Luis et al. 2013). The environmental variables are represented as arrows in the CCA plot, and the longer the arrow, the greater the correlation between the environmental variables and the distribution of the diatom communities. The wider the angle between the arrow and the axis is, the greater the correlation between the environmental variables and the axis (Zhang 2011). Sampling sites are shown in the CCA plot, and the position of the site on the axis varies according to the composition of the benthic diatom community. Thus the relationship between benthic diatom community composition and environmental variables might be interpreted from the trend of each site along the gradient of maximum variance, inferred from its position on the first axis.

According to the similarities revealed by sorting the sampling sites on the CCA and CA axes, CA was used to evaluate the condition of the benthic diatom habitats.

2 Results

2.1 Environmental parameters of each sampling site in July 2012

The result of ANOVA analysis revealed that the environmental conditions varied substantially among the 8 lakes/wetlands (Table 2). Total phosphorus concentration in the Pearl Shoal was 5 times higher than that in Mirror Lake, but it is interesting that Pearl Shoal had the lowest concentration of total nitrogen. In other lakes/wetlands, the total phosphorus concentration was also high when the corresponding total nitrogen concentration was low, and total phosphorus concentration was low when the corresponding total nitrogen concentration was high. In addition, conductivity values in the 8 lakes/wetlands were not significantly different; pH

Table 2 pH, conductivity, water temperature, content of total nitrogen and total phosphorus in water collected from eight lakes in Jiuzhaigou

	Water temperature (°C)	pH	Conductivity (μs/cm)	Total N (μg/L)	Total P (μg/L)
Grass Lake	6.30±0.87^{a}	8.47±0.28^{b}	263.00±0.00^{a}	450^{d}	30±10^{abc}
Swan Lake	5.13±0.67^{a}	7.36±0.55^{a}	327.25±72.25^{ab}	390±10^{bcd}	30±20^{abc}
Wetland of Arrow Bamboo Lake	6.66±1.16^{a}	8.44±0.35^{b}	330.29±8.52^{ab}	380±20^{abc}	20±10^{ab}
Arrow Bamboo Lake	6.40±0.69^{a}	8.56±0.22^{b}	335.60±13.09^{b}	410±30^{cd}	20±10^{ab}
Panda Lake	9.70±0.00^{b}	8.75±0.00^{b}	316.00±0.00^{ab}	340^{ab}	30^{abc}
Colorful Lake	12.25±2.75^{c}	8.29±0.15^{b}	377.67±96.85^{b}	340±60^{ab}	50±60^{bc}
Pearl Shoal	9.70±0.23^{b}	8.45±0.12^{b}	347.50±4.04^{b}	330±10^{a}	60±20^{c}
Mirror Lake	11.56±0.95^{c}	9.72±0.42^{c}	349.00±20.15^{b}	400±80^{cd}	10^{a}

Note: Different superscript letters show that significant difference exists between the items ($P<0.05$). Total N means Total Nitrogen; Total P means Total Phosphorus.

values were higher than 7 and lower than 10.

2.2 Composition of benthic diatom communities

A total of 158 taxa were found in the sampled lakes and wetlands, and 152 of those taxa were found in July 2012. There were 91, 83, 78, 77, 75, 66, 53 and 51 taxa in Swan Lake, Arrow Bamboo Lake, the wetland of Arrow Bamboo Lake, Colorful Lake, Mirror Lake, Grass Lake, Pearl Shoal and Panda Lake, respectively. In September 2012, we again investigated the lakes and wetlands, except for Panda Lake, and found 118 diatom taxa. There were 76, 72, 56, 55, 54, 53 and 40 taxa in the wetland of Arrow Bamboo Lake, Swan Lake, Grass Lake, Arrow Bamboo Lake, Mirror Lake, Colorful Lake and Pearl Shoal, respectively.

We identified the dominant taxa when its relative abundance was more than 10% (Weilhoefer and Pan 2008). The numbers of the dominant taxa found in July and September 2012 were 12 and 8, respectively. In September, *Achnanthes gracillima*, *Cymbella delicatula, C. Microcephala* and *Denticula elegans* disappeared from all sites. *Cocconeis placentula* disappeared in Grass Lake, Swan Lake, the wetland of Arrow Bamboo Lake, Arrow Bamboo Lake, Panda Lake and Pearl Shoal. *Diatoma tenue* disappeared in Grass Lake, Swan Lake, the wetland of Arrow Bamboo Lake, Arrow Bamboo Lake, Panda Lake, Pearl Shoal and Mirror Lake. Two new dominant species, *A. linearis* appeared in thelakes, except Pearl Shoal, and *C. placentula* var. *Euglypta* appeared in all lakes, except Colorful Lake. In July, the most dominant species in each lake were *A. microcephala* and *A. minutissima*, followed by *Fragilaria pinnata* and *C. delicatula*. In September, the most dominant species in each lake was *A. minutissima* followed by *A. microcephala* and *C. placentula* var. *Euglypta*.

2.3 Significance of dominant species for lake water quality

The dominant species found at each sampling site have significant implications for assessing water quality, because diatoms are sensitive to numerous environmental variables and are considered as a powerful indicator for environmental changes (Shubert 1984; Dam et al. 1994; Shepard et al. 2000; Wang and Zhang 2003; Stevenson et al. 2008). The dominant diatom species sampled in July indicate that there was little difference in the environmental conditions of Grass Lake, Swan Lake, Panda Lake and Mirror Lake. The three dominant species in Pearl Shoal are indicators of an α-mesosaprobous zone, a β-mesosaprobous zone or eutrophic conditions. All of the diatom samples from Pearl Shoal were collected along the same plank road accessed by tourists.

The three dominant species of Colorful Lake indicate very different environmental conditions. *C. cessatii* indicates the best environmental conditions, and the sampling sites with this dominant species are located on large, dead tree branches at the center of the lake and at the water inlet. These sampling sites are both far from the plank road and have abundant macrophytes such as *Equisetum ramosissimum* and benthic diatoms. They may have absorbed ambient nutrient concentrations, which would have resulted in low nutrient concentrations in the water column at the time of sampling (Lavoie et al. 2006). The environmental conditions indicated by *Fragilaria delicatissima* and *Fragilaria pinnata* are relatively poor (van Dam et al. 1994), and the sampling sites with these dominant species are distributed in the *Equisetum ramosissimum* along the right side of the observation deck, the littoral zone of observation deck and around the dead tree branches at the center of the lake, respectively.

In summary, when comparing sample sites with high tourist activity, such as plank roads, with sampling sites where there are fewer tourists are not concentrated, such as high observation decks and lake centers far from the plank roads, the water quality of the former is inferior to the latter as indicated by the dominant species.

Compared to the results from July, the water quality, as indicated by the dominant species of most of the lakes, was in decline in September, and the reason for this change may be that many macrophytes in the lakes begin to rot, thereby releasing nutrients into the water and affecting quality.

2.4 Relationship between diatom community composition and environmental variables

For samples collected in July 2012, for example, the canonical coefficients of the environmental variables on the CCA axis show that elevation is most closely associated with the first axis on the CCA graph (the canonical coefficient is -0.84 followed by water temperature and total nitrogen (TN), with canonical coefficients of 0.78 and -0.53, respectively). The canonical coefficient represents the dependency between the environmental variables and the CCA axis. The higher the absolute value of the canonical coefficients, the more significant is the relationship between the environmental variables and the CCA axis. Temperature is positively correlated with the first axis, and elevation and total nitrogen are negatively correlated with the first axis. The result indicates that these three environmental variables seriously influence the diatom communities. Comparing the positions of the 47 sampling sites on the CCA graph (Figure 2) shows the sampling sites S28-S31 in Pearl Shoal and S40-S45 in Colorful Lake are clearly separated from the other sampling sites along the first CCA axis. We suggest that this separation is primarily caused by the variability in elevation, water temperature and total nitrogen.

2.5 Significance of benthic diatom community composition for lake water quality

In addition to the environmental significance of the dominant species, changes in the composition of the diatom community can also indicate water quality. The results of the CCA reflect the relationship between benthic diatom community composition and environmental variables, however the significance of CCA for water quality is different from that of dominant species. We hoped to evaluate environmental conditions based on changes in the composition of the benthic diatom communities, independent of the environmental variables. So, we used CA where the position of the sites along the maximum variance gradient (first axis) is strictly determined by the diatom community structure and is therefore independent of the analyzed environmental variables. The CCA of 47 sites sampled in July 2012 revealed that the diatom communities were strongly influenced by elevation, water temperature and total nitrogen. Based solely on the CA of the July 2012 diatom data, the ordination of the samples showed patterns similar to the CCA ordinations (Figures 2 and 3), and the partial sampling sites (i.e., the Arrow Bamboo Lake upstream wetland, Arrow Bamboo Lake and Pearl Shoal) were significantly separated from the other sampling sites. Therefore, we evaluated environmental conditions for each sampling site according to the CA graph. The larger the difference between the scores for the sampling sites along the first CA axis, the larger the difference in environmental conditions.

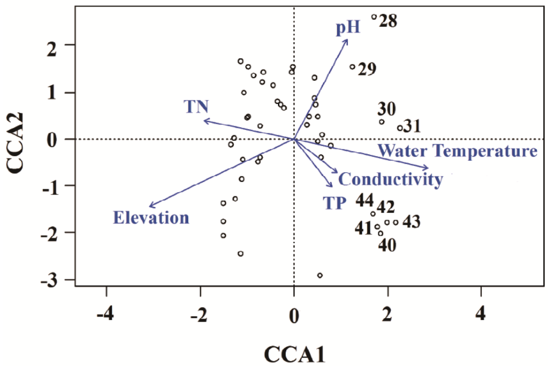

Figure 2 Canonical correspondence analysis biplots of the 47 sampling sites.(The number of sampling sites is shown in Table 1, with ○ represents the sampling site).

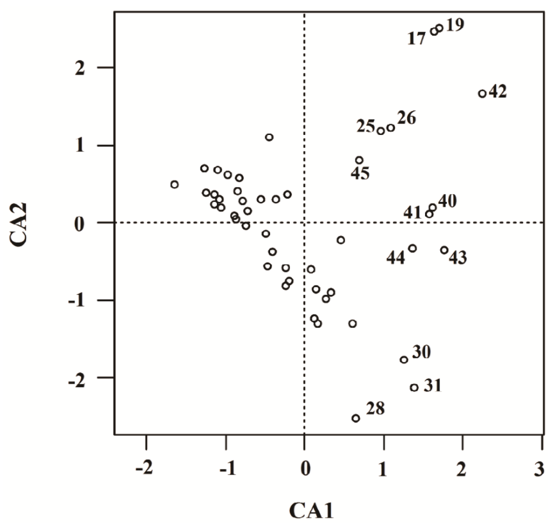

Figure 3 Correspondence analysis biplots of the 47 sampling sites in July, 2012 (○ represents the sampling site).

The scores for sample S17 in the Arrow Bamboo Lake wetland, samples S19, S25, and S26 in Arrow Bamboo Lake, samples S28, S30, and S31 in Pearl Shoal and samples S40-S45 in Colorful Lake differed significantly from the other samples on the first axis of the CA, which indicates that water quality is different in different sites. Furthermore, correlation analysis between the scores for each sampling site on the first axis of the CA and its corresponding TN and TP values was conducted. The results demonstrated that the CA scores are significantly and negatively correlated with total nitrogen and, to a certain extent, are positively correlated with total phosphorus, but not significantly (Table 3), indicating that the differences between the diatom samples can be specifically treated as the differences in total nitrogen. The scores for partial samples in the Arrow Bamboo Lake upstream wetland, Arrow Bamboo Lake and Pearl Shoal as well as all of the sampling points for Colorful Lake on the first CA axis were higher than those of the other samples, which indicate lower total nitrogen. The reason for this will be discussed in the next section.

Table 3 The Pearson correlation coefficients of Total nitrogen, Total phosphorus and CA scores

	Total N	Total P	CA scores
Total N	1	-0.042	-0.358*
Total P		1	0.160
CA scores			1

Notes: *Correlation is significant at the 0.05 level (2-tailed); CA: correspondence analysis; Total N means Total Nitrogen; Total P means Total Phosphorus.

Furthermore, it should be noted that a total of 7 and 10 samples were collected from the Arrow Bamboo Lake upstream wetland and Arrow Bamboo Lake, respectively, while in the CA graph, only sample S17 from the Arrow Bamboo Lake upstream wetland and 3 samples (S19, S25 and S26) from Arrow Bamboo Lake were separated from the other samples. This merely accounts for 1/7 and 3/10, respectively, of the total number of samples collected from each lake (Table 1). Thus, we suggest that these samples cannot represent the overall environmental conditions of the Arrow Bamboo Lake upstream wetland and Arrow Bamboo Lake. The CA of samples from July 2012 can only separate the environmental conditions of Pearl Shoal and Colorful Lake from the conditions of the other lakes (Figure 3).

In the CA graph of samples collected in September 2012, the positions of the sampling sites along the first axis were roughly the same as those for July. The sampling sites and their ranks are shown in Table 4. The results of the CA (Figure 4) show that Arrow Bamboo Lake sample S16, Pearl Shoal sample S19 and all of the samples (S27-S30) from Colorful lake differ significantly from the other samples on the first axis, and the results are similar to those for July. As previously noted, the Arrow Bamboo Lake sample S16 cannot represent the overall condition of Arrow Bamboo Lake, and the CA graph can only reveal that the environmental conditions in Pearl Shoal and Colorful Lake is different from that of the other lakes and wetlands (Figure 4). In addition, we compile a global CA with both the data of September and July (Figure 5). The red circles (S40-S45) and light red circles (S72-S75) indicate the samples from Colorful Lake in July and September, respectively. The blue circles (S28-S31) and light blue circles (S64, S65) indicate the

Table 4 Sampling sites in Jiuzhaigou National Nature Reserve in September, 2012

Sampling lake	Sampling site
Grass Lake	S1~S3
Mirror Lake	S4~S7
Wetland of Arrow Bamboo Lake	S8~S13
Arrow Bamboo Lake	S14~S18
Pearl Shoal	S19~S20
Swan Lake	S21~S26
Colorful Lake	S27~S30

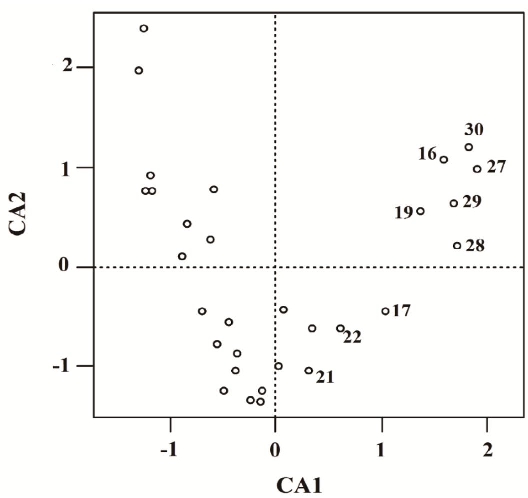

Figure 4 Correspondence analysis biplots of the 30 sampling sites in September, 2012 (○ represents the sampling site).

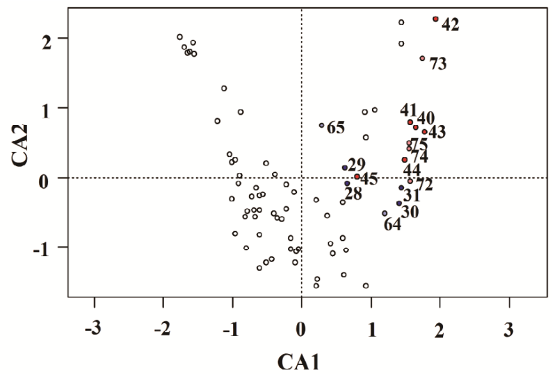

Figure 5 Correspondence analysis biplots of the all sampling sites in July and September, 2012 (○ represents the sampling site).

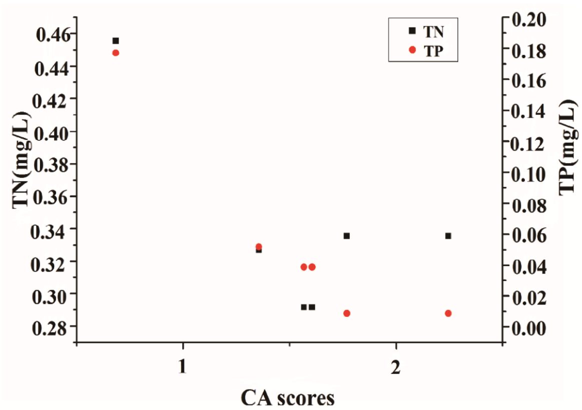

Figure 6 Change of TN and TP along with CA scores of each sampling site in Colorful Lake.

samples from Pearl Lake in July and September, respectively. As is shown in the figure, the positions of diatom samples in September along the first CA axis almost moved left compared with those of July.

Pearl Shoal and Colorful Lake have low concentrations of total nitrogen (Table 2), but most of the dominant species in the diatom communities of these two lakes are classified between the mesotrophic and eutrophic levels, so the total nitrogen concentration should be high (Florida Lakewatch 2004). Therefore, we should explain this contradiction.

In July 2012, TN concentration at the sampling sites in Colorful Lake ranged from 290-460μg/L, TP concentration ranged from 10-180μg/L, and total phosphorus concentration at different sampling sites in the same lake also had a large range. The relationship between TN, TP and the score on the first CA axis for each sampling site is shown in Figure 6. The corresponding R^2 values for these two correlation curves are 0.92 and 0.99, respectively, indicating that TP is more closely related to the CA value. We conducted correlation analyses for the scores, TN and TP, for the sampling sites in Colorful Lake. The results showed that the CA scores are significantly and negatively correlated with TP, and the correlation coefficient is -0.921. The CA scores are negatively correlated with TN, but the correlation is insignificant (P=-0.676). The obvious travertine deposition in the lakes of the Jiuzhaigou valley may accelerate the deposition of dissolved phosphorus (Anderson and Ring 1999; Kufel et al. 2013). Therefore, the concentration of phosphorus in the Jiuzhaigou lakes is generally low. Furthermore, the results from a previous study showed that phosphorus is the limiting factor to algae growth when the nitrogen to phosphorus ratio is 10 (Horne and Goldman 1994). The average annual TN/TP of Jiuzhaigou is 29.91, far greater than 10. So, we suggest that total phosphorus is the main nutrient limiting eutrophication in the Jiuzhaigou lakes. This would explain why the algae bloomed in some of the sampling sites of Colorful Lake and the water quality was poor. There are also research results that show a trend of deteriorating water quality in the littoral zone of Colorful Lake in which the water has been lightly polluted (Zhu 2007), but our results show that the TN concentration in Colorful Lake is low. The reason is that Colorful Lake is phosphorus limited, and the total phosphorus concentration in Colorful Lake is higher than in the other lakes (Table 2). The biomass of algae increased with the increase in phosphorus, and the nitrogen was absorbed and converted into algal biomass. The increase in the relative abundance of *D. kuetzingii*, which has the ability to fix nitrogen, in Colorful Lake also supports our view.

The situations in Pearl Shoal and Colorful Lake are similar. Tourists always gather along the plank roads of Pearl Shoal, with the filamentous green algae and benthic diatoms growing in massive amounts, and the dominant species of the diatom communities indicate poor water quality. A previous study also showed that the water quality of Pearl Shoal has a downward trend(Zhu 2007). However, our results show that the TN concentration in Pearl Shoal is low. The reason is that Pearl Shoal is also phosphorus limited, and compared with the other lakes sampled at the same time, the total phosphorus concentration of Pearl Shoal showed an increasing trend (Table 2). The algal biomass increased with the increase in phosphorus, and the ambient nitrogen was absorbed and converted into algal biomass.

In summary, CA should be combined with an assessment of the dominant diatom species to evaluate the water quality.

3 Discussion

3.1 Influence of substratum type and season on benthic diatom communities

We took samples from Arrow Bamboo Lake as an example; the dominant diatom species from the two different sampling sites were *C. delicatula* and *C. microcephala*. The environmental conditions indicated by the two species were different, but their substrates were both dead tree branches. Some diatom samples on different types of substrates have the same dominant species, as has been the case in the other lakes. Therefore, we suggest that the influence of the substrate type on the benthic diatom communities may not be of great significance. In addition, we added confidence ellipses on the CA results to show the effects of sampling months and substrate types (Figures 7 and 8). Both the season and substrate type had effects on benthic diatom community compositions but the effects were not statistically significant.

Although it is proposed that epiphytic diatom assemblages are significantly influenced by type of substrates reported from the waters at low elevation (Sweat and Johnson 2013; Delgado and Pardo 2015), our results show that the effect of substrate type on diatom community composition is less important. This contradiction may be attributed to the impact of elevation on diatom community that is far greater than that of substrates at the high elevation of Jiuzhaigou.

3.2 Comparison of water quality indicated by dominant species and benthic diatom communities

As mentioned in the section on the significance of the dominant species for lake water quality, we compared the differences in water quality as indicated by the dominant species at each sampling site. The results from July 2012 can be divided into three categories. The first is the presence of dominant species that indicate poor water quality, most of which appeared in Arrow Bamboo Lake and Pearl Shoal followed by Colorful Lake. The second is the presence of dominant species that indicate the best water quality; they also appeared in the Arrow Bamboo Lake upstream wetland, Arrow Bamboo Lake and Colorful Lake but in small proportions. The third is the dominant species in Grass Lake, Swan Lake, Panda Lake and Mirror Lake, most of which are species with broad environmental tolerance and indicate a level of water quality between the best and the worst. The

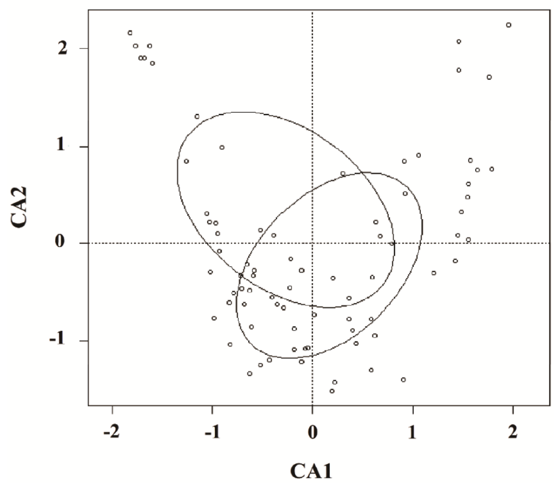

Figure 7 Correspondence analysis and confidences ellipses biplots of the all sampling sites in July and September, 2012 (Ellipses represent different months).

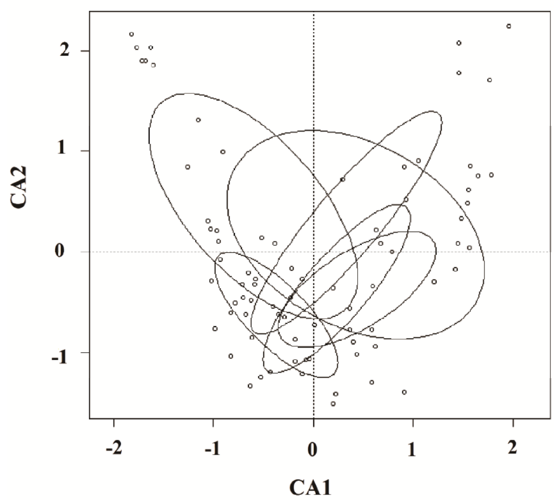

Figure 8 Correspondence analysis and confidences ellipses biplots of the all sampling sites in July and September, 2012 (ellipses represent different types of substrate).

results from September are roughly the same as July. The diatom species that indicate poor water quality appeared in the Arrow Bamboo Lake upstream wetland, Pearl Shoal, Colorful Lake and Grass Lake. Overall, the results show that most of the dominant species in Pearl Shoal and Colorful Lake indicate poor water quality.

The composition of the benthic diatom community varies with the position of the sampling sites on the CA axis, and the difference in the composition of the diatom community indicates the change of environmental conditions. Thus, the CA of samples from July 2012 separated the environmental conditions of Pearl Shoal and Colorful Lake from the conditions of the other lakes (Figure 3). The CCA also showed that elevation, water temperature and total nitrogen are the important environmental variables affecting community composition of the benthic diatoms in Jiuzhaigou. The CA for July 2012 revealed that the diatom community composition in samples from Pearl Shoal and Colorful Lake was different from that at other sampling sites, which indicates that the total nitrogen of Pearl Shoal and Colorful Lake was less than that at other sampling sites. The reason may be that the characteristics of the karst at Jiuzhaigou cause phosphorus to become a factor restricting eutrophication. As the phosphorus concentration at some of the sampling sites, such as Colorful Lake and Pearl Shoal, increases, the diatom biomass increases accordingly and absorbs the ambient nitrogen, so the total nitrogen concentration decreases. At other sampling sites, the total concentration of phosphorus was relatively low, which inhibited the growth of the diatoms, so the ambient nitrogen accumulated without being used. The September results also revealed that the water quality of Pearl Shoal and Colorful Lake was different from the other lakes, which was consistent with the results from July.

Water temperature, pH, conductivity, total nitrogen, total phosphorus and elevation were used as the environmental variables in the CCA of this study. In the Jiuzhaigou valley, water temperature, elevation, pH and conductivity might be the proxies of natural variation. The nutrient concentration in the lake water may easily be affected by the tourist activities in Jiuzhaigou, so total nitrogen and total phosphorus may be proxies of anthropogenic influences. According to the result of CCA, elevation, water temperature and total nitrogen are the important environmental variables affecting community composition of the benthic diatoms in Jiuzhaigou, which means that human activities may affect the benthic diatoms. Thus, the community composition of benthic diatoms may be used to assess the influence of human activities on the lake water of Jiuzhaigou.

Furthermore, it is important to note that there are also diatom species which are tolerant to pollution and indicate the presence of a polysaprobic zone; these include *Nitzschia palea*, *Synedra acus*, *N. frustulum* and *Gomphonema parvulum*. These species indicate poor water quality when they become dominant (Shubert 1984; Wang and Zhang 2003), but the relative abundance of these species at each sampling site is low and does not necessarily indicate pollution.

In a word, the water quality of Pearl Shoal and Colorful Lake is inferior to the other lakes and may be due to human interference. It is unreasonable to assess the water quality of the two lakes solely employing TN concentration. Tourists always gather along the Pearl Shoal and Colorful Lake, where the plank roads are near the water, and dabble in the water and throw food to the fish. All of these activities can pollute the water. Therefore, we should pay more attention to the human impact to enhance the management of the Jiuzhaigou Valley Nature Reserve.

4 Conclusions

In this research, a total of 158 taxa of benthic diatoms were observed in the lakes sampled at Jiuzhaigou. The most dominant species belong to the genera *Achnanthes*, *Fragilaria*, *Cymbella*, *Cocconeis*, *Diatoma and Denticula*.

The CCA revealed that the composition of the diatom communities of the subalpine karstic lakes in Jiuzhaigou is mainly influenced by elevation, water temperature and total nitrogen. The influence of the type of substratum on diatom community composition is not significant in subalpine lakes. The results of the CCA and CA illustrate that the compositions of the diatom communities of Pearl Shoal and Colorful Lake are different from those of the other lakes and are due to changes in elevation, water temperature and total nitrogen. In addition, compared with the sampling sites of other lakes, the dominant species of the benthic diatom communities in Pearl Shoal and Colorful Lake indicated poor water quality. Therefore, we combined the environmental significance of the dominant species with the CCA and CA to evaluate the water quality. The results show that the possibilities for eutrophication in Pearl Shoal and Colorful Lake are greater than in the other lakes because of tourist activities.

In summary, to evaluate the impact of human activities on subalpine karstic lakes, such as the lakes of Jiuzhaigou Valley, CCA and CA methods should be combined with the environmental significance of the dominant and sensitive species in the benthic diatom communities.

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