EFFECTS OF ENVIRONMENTAL FACTORS ON THE BIOMASS OF BENTHIC DIATOM IN RIVERS

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Abstract

This paper aims to disclose the quantitative relationship between benthic diatom biomass in natural rivers and environmental factors. For this purpose, the author selected seven natural rivers in the basin of Minjiang River as the objects. First, the samples of benthic diatoms and the environmental factors were collected from 14 different sampling sites of five rivers and the diatom density and Chlorophyll a (Chl a) content were selected to characterize the diatom biomass. Then, the number of diatom cells of the collected samples was counted and the Chl a content was measured in the lab. After that, the correlations between diatom biomass (diatom density and Chl a content) and environmental factors were analysed in details, revealing that turbidity, water depth, chemical oxygen demand (COD) and flow velocity are primary influencing factors of diatom biomass. On this basis, two mathematical models were established through multivariate linear regression analysis to calculate the correlations between diatom density and Chl a content, respectively, with the primary influencing factors. The models were validated through a case study on the remaining two rivers. The results show that the calculated diatom density and Chl a content of most sampling site could mirror the actual impacts of COD, turbidity, water depth and flow velocity on diatom biomass in the study area. The research findings provide an indirect way to estimate the biomass of benthic diatoms and shed new light on the evaluation of the health of rivers.

Key words: diatom density, Chlorophyll a (Chl a) content, environmental factors, correlation analysis, empirical prediction models

1. Introduction

The growth of algae is directly related to the regulation of the ecosystem in lotic environments. The algae plants, serving as food providers and nutrient maintainers (Stevenson et al., 1996; Velichkova et al., 2018), are essential to the energy cycle of river ecosystems. As important parts of algae, benthic algae exist widely in many aquatic ecosystems. Benthic algae both functions as primary producers and chemical regulators and provide important habitats for other organisms (Bunn et al., 2003). Generally, diatoms (Bacillariophyta) are the dominant taxonomic group in freshwater algae, comprising of more than 80% of the total abundance and biovolume (Stevenson et al., 1996). With the accumulation of water pollutants, the biomass of benthic diatoms in river systems is severely impacted.

Thanks to their rapid cell cycle, sensitivity to disturbances and wide distribution, benthic diatoms have widely been considered as indicators of water quality, such as the specific polluosensitivity index (IPS) and index of European Economic Community (CEE) (Noga et al., 2014; Rimet et al., 2015; Tan et al., 2014). Stevenson and Pan (1999) proposed to evaluate the river environment against the biomass, species composition, morphology, metabolism, and other features of benthic diatoms, owing to its quick response to various physical, chemical and biological changes in the river ecosystem. Sabater et al. (2010)
took the diatom community as the biomarker for water quality and river pollution monitoring. Ndiritu et al. (2003) discovered the good indicative function of the water quality among certain diatom species or assemblage in Kenya. Stratification of water had an effect on fluid flow and heat transfer characteristics (Sarojamma et al., 2018). Kingsley et al. (2006) explored the relationships among the biomass, species composition and environmental factors of benthic diatoms in Salmonid River, British Columbia, Canada, revealing that the content of dissolved phosphorus and velocity gradient have major impacts on the structural change of diatom communities.

Many scholars have investigated the influence mechanism of environmental factors on the biomass of benthic diatoms, aiming to evaluate water quality of rivers or lakes using these algae. For instance, Lohman et al. (1992) revealed the significant effects of hydraulic disturbance and flow on the biomass of benthic diatoms. Wang et al. (2017) examined the correspondence between diatom biomass and hydrodynamic conditions in natural rivers through 1D and 2D numerical simulations, concluding that the velocity of 0.85-1m/s and the shear stress of 24-28N/m2 are suitable for 1D simulation and that the velocity of 0.9-1.1m/s and the water depth of 0.40-0.48m are suitable for 2D simulation. Dodds et al. (2002) found that the chlorophyll level of benthic algae has a significant positive correlation with the total phosphorus (TP) content and total nitrogen (TN) content in water, and a slightly weaker but still very positive correlation with high temperature and low altitude. Winslow et al. (2014) discovered that the light, positively correlated with the chlorophyll level at low latitudes, determines the limit of nutrient level and maximum depth of growth of benthic diatoms, indicating that the growth and distribution of benthic diatoms in streams are affected by light availability. Dalu et al. (2017) suggested that species richness varies with dissolved oxygen (DO), turbidity, temperature, channel width, pH and water column nitrate contents. Teittinen et al. (2016) argued that the richness of benthic diatoms also depends on electrical conductivity (Cond), rock size and pH.

In summary, the biomass of benthic diatoms in natural rivers is influenced by such factors as water depth, velocity, nutrient content, temperature, turbidity, Cond, pH, light availability, DO. The existing studies concentrate on using benthic diatoms as water quality indicators and the relationships among diatom community structure, richness variation and regional environmental factors.

There are few reports on the quantitative relationship between benthic diatom biomass and the aforementioned environmental parameters factors in rivers. To manage the eutrophication of freshwater lakes and reservoirs, the algal biomass has been successfully linked with water column nutrients via empirical regression models (Cooke et al., 1993; Smith, 1998; Smith et al., 1999). By contrast, the attempts to derive similar relationships for nutrients and periphyton biomass in streams are less successful (Biggs, 2000). Dodds et al. (2002) created a generic model to predict the benthic chlorophyll content in streams, and constructed a large database for various temperate zone streams. In this way, the statistical relationships between the chlorophyll content and TN and TP were identified through multiple regression analyses, yet the empirical prediction model fails to cover other environmental factors.

Considering the above, this paper aims to quantify the relationship between benthic diatoms biomass and various environmental factors in natural rivers. For this purpose, the rivers upstream Zipingpu Reservoir, Dujiangyan, Sichuan, China were selected as the study area to sample benthic diatoms and measure environmental factors and hydraulic conditions.

The dominant benthic diatoms in the samples were identified and counted to further analyse the correlation between diatom biomass and environmental factors and hydraulic parameters. Then, empirical prediction models were established based on the main influencing factors of diatom biomass in the study area.

The innovation of our research mainly lies in the quantification of the biomass and influencing factors of benthic diatoms and the creation of a water quality management framework based on the quantitative relationships. The research findings can be extended to predict the biomass of benthic diatoms and evaluate the health of rivers.

2. Methodology

2.1. Study area and sampling

The study area lies between Yuzixi Hydropower Station, located in the middle and upper reaches of Minjiang River, and Dujiangyan (Fig. 1). The following rivers flow through the study area: Minjiang River, Shouxi River, Baisha River, Puyang River, Zouma River, Baitiao River and Jiang’an River. The last four rivers crisscross the downtown of Dujiangyan. Due to the proximity to residential areas, field surveys showed that the water quality of the four rivers was severely affected by the domestic waste water discharged. According to the hydrological features, a total of 20 sampling sites were selected across the study area to reflect the complex structure of the seven rivers. The first sampling was carried out on Minjiang River, Shouxi River, Baisha River, Puyang River and Zouma River in April 2017 for constructing the model.

Since the sampled rivers were rehabilitated, the second sampling of the model was implemented to verify the model on Baitiao River and Jiang’an River, on April 8, 2018. The location of sampling sites is given in Fig. 1. Targeting the benthic diatoms in natural rivers, measurement methods based on unit area mass (eg dry weight and ash dry weight) are relatively economical methods for assessing algal biomass (Fielding et al., 1988), but their accuracy is lower than cell counting (Stevenson et al., 1996).
For reliability, Chl a and cell density-based methods (Prestipino et al., 2018; Stevenson et al., 1996) were selected to characterize the benthic diatom biomass. According to the sampling method recommended by the US Environmental Protection Agency (EPA, 1998), three 20cm diameter pebbles were randomly selected at each sampling site. Then, algae were collected through the following steps from the specific surface area of the pebble: cover the algae on the specific area with a circular lid (radius: 2.7cm), brush away the algae surrounding the lid, and then relocate the algae covered by the lid to 200mL distilled water. After mixing these fully, the mixture of algae and water was filled into two 100mL sampling bottles, and brought back to the lab for the qualitative and quantitative analyses on benthic diatoms. Next, 1% Lugol’s iodine solution was added to each sample to fix the algae. The sample with the fixing solution was used to identify the species and density of diatoms, while other samples were used to measure the Chl a content.

2.2. Measurement of environmental factors

Some environmental indicators are recorded on sampling sites by portable instruments. Portable thermometer, pH meter, hand-held dissolved oxygen apparatus, turbidimeter, water depth ruler, velocity meter and portable conductivity meter respectively were used to measure the water temperature (T), pH, DO, turbidity, water depth, flow velocity and electric conductivity (Cond) in the study area. The water samples were collected against the flow direction. The sampling bottles were washed by the sample water for three times and each bottle was filled up with water. These bottles were brought back to the lab to determine the Chlorophyll a (Chl a) content, TP, TN and chemical oxygen demand (COD) of benthic diatoms.

2.3. Sample processing and data analysis

According to the Water and Wastewater Monitoring and Analysis Methods released by the State Environmental Protection Administration of China (2002), during density measurement, the samples for benthic diatoms analysis were stored to supernate for 24 hours in the dark, and then the sedimeted benthic diatoms was transferred into a smaller bottle. Benthic diatoms were identified and counted directly in cell counting chambers using a light microscope. Three–five lines should be counted in each sample, or the entire slide glass should be counted if there are too few diatoms. For each sample, two slide glasses should be counted at the least. If the number counted on the two slide glasses deviates from the mean value by over 15%, a third slide glass should be counted. After counting, the number of diatoms in each sample was calculated. The diatom density per unit area of pebble can be obtained as (Eq. 1):

$$ N_i = \frac{C_i \cdot L \cdot n_i}{C_j \cdot R \cdot h \cdot S} $$

(1)

where: $N_i$ is the number of diatoms per unit area $i$ (cells/cm²);
$C_i$ and $C_j$ are the measured volume and actual volume of sample water, respectively (mL);
$L$ is the side length of the counting chamber (µm);
$R$ is the number of lines; $h$ is the distance of parallel lines in the view field (µm); $n_i$ is the counted number of diatoms $i$; $S$ is the total substrate scraping area (cm$^2$).

Chl a content was an important indicator for characterizing biomass of benthic diatoms since there was a positive correlation between chlorophyll a content and diatom density. Bowman et al. (2005) used Chl a content to predict the abundance of epiphytic algae and studied the relationship between this abundance and anthropogenic changes in phosphorus bioavailability and limitation in mountain rivers. Rolland et al. (1997) calculated the correlation between Chl a content and total diatoms density (TDD), and found that Chl a content was positively correlated with TDD ($r=+0.69$; $p<0.05$). Therefore, the Chl a of the whole algae community was taken as the research object in this paper. According to the Water and Wastewater Monitoring and Analysis Methods released by the State Environmental Protection Administration of China (2002), 90% acetone was added to extract the Chl a. Then, the absorbance was measured at such four wavelengths as 750nm, 665nm, 645nm and 630nm. The Chl a content can be calculated as follows (Eq. 2):

$$\text{Chl a} = \frac{[11.64(D_{750}-D_{645})-2.16(D_{750}-D_{630})+0.10(D_{750}-D_{645})]}{V \cdot \delta}$$  

where: $V$ is the volume of water sample (L); $D$ is the absorbance; $V_1$ is the measured volume of the extracted liquid (mL); $\delta$ is the optical path of colour dish (cm).

Next, the impacts of environmental factors on benthic diatom biomass were subjected to correlation analysis by Spearman’s model. After calculating the correlation and significance analysis between environmental factors separately, and those with relatively high correlations (with a significance level of 0.05) were selected as the primary influencing factors for model construction. Based on these factors, a mathematic model was set up to describe relationships between the primary influencing factors through multivariate linear regression analysis: $f(x)=a_0+a_1x_1+a_2x_2+...+a_nx_n$, where $f(x)$ is the biomass and $x_1...x_n$ are environmental factors like water depth, velocity and turbidity.

3 Results

3.1. Variation of environmental factors

The environmental factors of all sampling sites were obtained by taking the average values of the sampling results in three consecutive days (Table 1). The trophic states were evaluated based on the trophic level index (TLI) (Burns et al., 2005; Li et al., 2015; Wang et al., 2019).

The results show that TP varied from 0.11 to 0.14mg/L at most sites, except M-2 (Yingxiu Town) (0.23mg/L), and that according to the classification of eutrophication levels by Evaluation Methods and Classification Technical Regulations for Eutrophication Assessment of Lakes (China National Environmental Monitoring Center, 2001), all sites were severely eutrophic, except M-1 (meso-eutrophic).

3.2. Biomass distribution

Ten genera of diatoms were identified from the samples, including Navicula, Diatoma, Pinnularia, Cymbella, Gomphonema, Achnanthes, Eunotia, Synedra, Cyclotella and Nitzschia (Fig. 2, Fig. 3). Specifically, Navicula dominated most sampling sites with a maximum relative abundance of 94.8% (Fig. 4).

<table>
<thead>
<tr>
<th>Sampling sites</th>
<th>TP /mg·L$^{-1}$</th>
<th>TN /mg·L$^{-1}$</th>
<th>Velocity /m·s$^{-1}$</th>
<th>Water depth /m</th>
<th>Turbidity /NTU</th>
<th>pH</th>
<th>DO /mg·L$^{-1}$</th>
<th>COD /mg·L$^{-1}$</th>
<th>TLI</th>
<th>Trophic state</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-1</td>
<td>0.13</td>
<td>0.52</td>
<td>0.8</td>
<td>0.2</td>
<td>105</td>
<td>8.12</td>
<td>9.3</td>
<td>22.79</td>
<td>64.9</td>
<td>Middle</td>
</tr>
<tr>
<td>M-2</td>
<td>0.23</td>
<td>2.62</td>
<td>0.2</td>
<td>0.35</td>
<td>121</td>
<td>8.05</td>
<td>9.2</td>
<td>23.63</td>
<td>73.0</td>
<td>Hyper</td>
</tr>
<tr>
<td>M-3</td>
<td>0.12</td>
<td>0.08</td>
<td>0.1</td>
<td>0.3</td>
<td>20.5</td>
<td>8.03</td>
<td>9.5</td>
<td>22.79</td>
<td>74.2</td>
<td>Hyper</td>
</tr>
<tr>
<td>M-4</td>
<td>0.12</td>
<td>1.22</td>
<td>0.3</td>
<td>0.1</td>
<td>17.8</td>
<td>8.03</td>
<td>9.6</td>
<td>19.41</td>
<td>72.0</td>
<td>Hyper</td>
</tr>
<tr>
<td>M-5</td>
<td>0.11</td>
<td>0.52</td>
<td>0.1</td>
<td>0.2</td>
<td>9.58</td>
<td>8.24</td>
<td>9.3</td>
<td>16.88</td>
<td>91.6</td>
<td>Hyper</td>
</tr>
<tr>
<td>M-6</td>
<td>0.11</td>
<td>0.91</td>
<td>0.45</td>
<td>0.35</td>
<td>6.12</td>
<td>7.99</td>
<td>9.1</td>
<td>18.57</td>
<td>100.0</td>
<td>Hyper</td>
</tr>
<tr>
<td>M-7</td>
<td>0.12</td>
<td>1.24</td>
<td>0.3</td>
<td>0.37</td>
<td>10.2</td>
<td>8.2</td>
<td>9.4</td>
<td>23.63</td>
<td>83.7</td>
<td>Hyper</td>
</tr>
<tr>
<td>P-1</td>
<td>0.14</td>
<td>0.13</td>
<td>0.7</td>
<td>0.23</td>
<td>10.1</td>
<td>8.09</td>
<td>9.8</td>
<td>20.25</td>
<td>87.2</td>
<td>Hyper</td>
</tr>
<tr>
<td>P-2</td>
<td>0.13</td>
<td>0.44</td>
<td>0.5</td>
<td>0.45</td>
<td>9.07</td>
<td>7.95</td>
<td>9.6</td>
<td>18.99</td>
<td>98.7</td>
<td>Hyper</td>
</tr>
<tr>
<td>P-3</td>
<td>0.13</td>
<td>0.38</td>
<td>0.4</td>
<td>0.4</td>
<td>9.56</td>
<td>8.22</td>
<td>10.5</td>
<td>22.79</td>
<td>96.0</td>
<td>Hyper</td>
</tr>
<tr>
<td>P-4</td>
<td>0.12</td>
<td>0.43</td>
<td>0.3</td>
<td>0.3</td>
<td>11.1</td>
<td>8.18</td>
<td>9.2</td>
<td>23.21</td>
<td>70.1</td>
<td>Hyper</td>
</tr>
<tr>
<td>Z-1</td>
<td>0.12</td>
<td>2.12</td>
<td>0.5</td>
<td>0.42</td>
<td>11</td>
<td>8.15</td>
<td>9.6</td>
<td>19.41</td>
<td>80.5</td>
<td>Hyper</td>
</tr>
<tr>
<td>Z-2</td>
<td>0.11</td>
<td>3.29</td>
<td>0.4</td>
<td>0.4</td>
<td>17.8</td>
<td>8.12</td>
<td>10.2</td>
<td>20.25</td>
<td>80.2</td>
<td>Hyper</td>
</tr>
<tr>
<td>Z-3</td>
<td>0.12</td>
<td>2.93</td>
<td>0.5</td>
<td>0.35</td>
<td>7.69</td>
<td>8.31</td>
<td>8.6</td>
<td>17.72</td>
<td>94.5</td>
<td>Hyper</td>
</tr>
</tbody>
</table>

Note: ‘+’ stands for medium eutrophication; ‘-’ stands for severe eutrophication; the temperatures of the sites range between 11.4 and 12.4°C; transparency is negligible due to the shallow depth of the rivers.
Effects of environmental factors on the biomass of benthic diatom in rivers

Fig. 2. Dominant benthic diatoms (a) *Navicula*; (b) *Diatoma*; (c) *Pinnularia*; (d) *Achnanthes*; (e) *Gomphonema*;

Fig. 3. Dominant benthic diatoms (a) *Eunotia*; (b) *Achnanthes* or *Eunotia*; (c) *Synedra*; (d) *Nitzschia*; (e) *Cyclotella*

Fig. 4. Relative abundance of benthic diatom
Fig. 5 presents the variations of diatom density and Chl a content. The diatom biomass differed from site to site, owing to the difference in environmental factors. In general, the density of benthic diatoms varied from 80 to 109,215 cells/cm², while the Chl a content ranged between 0.29 and 39.98 μg/m². The positive correlation \( r = +0.71; \ p < 0.01 \) can be observed between the variation in diatom density and that of Chl a content (Fig. 6). The results agree well with the research of Rolland et al. (1997). This relationship helps to characterize the biomass of benthic diatoms and facilitates the subsequent analysis.

3.3. Data analysis results and model building

Spearman’s correlation was analyzed between the data on environmental factors and those on diatom biomass at the 14 sampling sites. The resulting correlation coefficient matrix is shown in Table 2. The results reveal that the biomass of benthic diatoms is positively correlated with TN, water temperature, flow velocity, water depth, pH and Cond, negatively with TP, turbidity, DO and COD. The correlations with turbidity, water depth, COD and flow velocity are particularly significant, indicating that these parameters are primary influencing factors of the diatom biomass. The Spearman’s correlation was calculated between the data on environmental factors and those on diatom biomass at the 14 sampling sites. The resulting correlation coefficient matrix is shown in Table 2. The results reveal that the diatom biomass with turbidity \( (p < 0.01, \ r = -0.935) \), water depth \( (p < 0.01, \ r = +0.662) \), COD \( (p < 0.01, \ r = -0.638) \) has high correlations, indicating that these parameters are primary influencing factors of the diatom biomass. According to literature review (Besemer et al., 2007; Gundersen and Jorgensen, 1990; Odum, 1956; Wang et al., 2017) and correlation analysis, flow velocity also has a nonnegligible impact on the diatom biomass. Thus, this factor was also taken as a primary influencing factor in model building, aiming to enhance the model accuracy.
Table 2. Correlation coefficient matrix

<table>
<thead>
<tr>
<th>Biomass Density</th>
<th>Chl a</th>
<th>TP</th>
<th>TN</th>
<th>T</th>
<th>Velocity</th>
<th>Water depth</th>
<th>Turbidity</th>
<th>pH</th>
<th>DO</th>
<th>COD</th>
<th>Cond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chl a 0.785**</td>
<td></td>
<td>-0.189</td>
<td>-0.215</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP 0.253</td>
<td>-0.007</td>
<td>-0.321</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TN 0.102</td>
<td>0.144</td>
<td>0.145</td>
<td>-0.024</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T Velocity 0.442</td>
<td>0.224</td>
<td>0.314</td>
<td>0.018</td>
<td>-0.050</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water depth 0.662**</td>
<td>0.439</td>
<td>0.037</td>
<td>0.275</td>
<td>-0.165</td>
<td>0.213</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turbidity -0.816**</td>
<td>-0.935**</td>
<td>0.253</td>
<td>0.062</td>
<td>0.080</td>
<td>-0.297</td>
<td>-0.327</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 0.119</td>
<td>0.033</td>
<td>-0.174</td>
<td>0.214</td>
<td>-0.215</td>
<td>-0.062</td>
<td>-0.006</td>
<td>-0.183</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO -0.022</td>
<td>0.029</td>
<td>0.185</td>
<td>-0.243</td>
<td>-0.486</td>
<td>0.135</td>
<td>0.284</td>
<td>0.091</td>
<td>-0.119</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COD -0.505</td>
<td>-0.638*</td>
<td>0.471</td>
<td>-0.157</td>
<td>0.184</td>
<td>-0.239</td>
<td>0.037</td>
<td>0.636</td>
<td>-0.002</td>
<td>0.099</td>
<td>1</td>
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</tr>
<tr>
<td>Cond 0.318</td>
<td>0.291</td>
<td>0.146</td>
<td>-0.214</td>
<td>-0.405</td>
<td>0.183</td>
<td>0.278</td>
<td>-0.366</td>
<td>0.392</td>
<td>0.377</td>
<td>-0.210</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: The unit of T is °C and that of Cond is μs/cm; ** means significant correlation at 0.01 level (both sides); * means significant correlation at 0.05 level (both sides).

Table 3. Mean values and standard deviations of the primary influencing factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number</th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Average value</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turbidity (NTU)</td>
<td>14</td>
<td>6.12</td>
<td>121</td>
<td>26.18</td>
<td>37.15</td>
</tr>
<tr>
<td>Water temperature (℃)</td>
<td>14</td>
<td>11</td>
<td>13.6</td>
<td>11.71</td>
<td>0.71</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>14</td>
<td>16.88</td>
<td>23.63</td>
<td>20.74</td>
<td>2.34</td>
</tr>
<tr>
<td>Cond (μs/cm)</td>
<td>14</td>
<td>209</td>
<td>333</td>
<td>306.29</td>
<td>33.86</td>
</tr>
<tr>
<td>Chl a content (μg/m2)</td>
<td>14</td>
<td>0.29</td>
<td>39.98</td>
<td>10.29</td>
<td>12.96</td>
</tr>
<tr>
<td>Diatom density (cells/cm2)</td>
<td>14</td>
<td>80</td>
<td>100478</td>
<td>22102</td>
<td>33357</td>
</tr>
</tbody>
</table>

As shown in Table 3, the mean values and standard deviations of turbidity, water depth, COD, flow velocity, Chl a content and cell density were 26.18 and 37.15, 0.32 and 0.1, 20.74 and 2.34, 0.3 and 0.2, 10.29 and 12.96, 22,102 and 33,357, respectively.

Then, the diatom density was subjected to log transform with \( Y_1 \) being the number of diatoms and \( X_1 - X_4 \) being the turbidity, water depth, COD and flow velocity, respectively. The multivariate linear regression equation was established as \( Y_1 = 4.558 - 0.1X_1 + 6.095X_2 - 0.15X_3 + 1.251X_4 \) (\( F=11.1, P=0.002, R^2=0.832 \)). It can be seen that the log value of diatom density is negatively correlated with turbidity and COD, and positively with water depth and flow velocity. Therefore, the mathematical model for the response of diatom density to the environmental factors can be written as (Eq. 3):

\[
\log(D) = 4.558 - 0.1T + 6.095H - 0.15C + 1.251V
\]  

(3)

where: D is the diatom density (cells/cm2); Tb is turbidity (NTU); H is the water depth (m); Co is the COD (mg/L); V is the flow velocity (m/s).

Similarly, the Chl a content was also subjected to log transform with \( Y_2 \) being the log value of Chl a and \( X_1 - X_4 \) being the turbidity, water depth, COD and flow velocity, respectively. The multivariate linear regression equation was established as \( Y_2 = 2.94 - 0.008T + 3.02H - 0.19C + 0.13V \) (\( F=4.704, P=0.025, R^2=0.676 \)). It can be seen that the log value of Chl a content is negatively correlated with turbidity and COD, and positively with water depth and flow velocity. Therefore, the mathematical model for the response of Chl a content to the environmental factors can be written as (Eq. 4):

\[
\log(Chl\ a) = 2.94 - 0.008T + 3.02H - 0.19C + 0.13V
\]  

(4)

3.4. Model verification

Since the sampled rivers were rehabilitated, the mathematical models for the response of benthic diatoms to the environmental factors were verified through sample analysis on Baitiao River and Jiang’an River in the same basin. The samples of benthic diatom were collected from three sites of each river on April 8, 2018, and the environmental factors were measured at the same time.

The diatom biomasses and environmental factors were listed in Table 4. The turbidity, water depth, COD and flow velocity measured at the six sampling sites were imported into our models to calculate the diatom biomass of each site (Fig. 7).

The comparison shows that the calculation accuracy of diatom density and Chl a content fell in the range of 71~89% and 63~89%, respectively. Site J-2 had the smallest biomass among all sampling sites. For this site, the accuracies of the calculated diatom density and calculated Chl a content were relatively low, which are 71% and 63%, respectively.

4. Discussions

In our research, the responses of diatom density and Chl a were modelled to the primary four...
influencing factors, i.e. turbidity, COD, water depth and flow velocity. Our models demonstrate that log (D) and log (Chl a) are negatively correlated with turbidity and COD while positively with water depth and flow velocity. The results agree well with the research of Wang et al. (2017). Targeting Baitiao River downstream Zipingpu Hydropower Station, Wang et al. (2017) determined the suitable ranges of flow velocity and water depth for benthic diatoms as 0.9-1.1m/s and 0.4-0.48 m, separately.

Table 4. Diatom biomasses and environmental factors

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>B-1</th>
<th>B-2</th>
<th>B-3</th>
<th>J-1</th>
<th>J-2</th>
<th>J-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turbidity (NTU)</td>
<td>8.71</td>
<td>10.9</td>
<td>8.11</td>
<td>7.82</td>
<td>9.43</td>
<td>6.86</td>
</tr>
<tr>
<td>Velocity (m/s)</td>
<td>0.2</td>
<td>0.7</td>
<td>0.5</td>
<td>0.3</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>17.75</td>
<td>18.19</td>
<td>16.71</td>
<td>20.14</td>
<td>21.62</td>
<td>17.68</td>
</tr>
<tr>
<td>Water depth (m)</td>
<td>0.2</td>
<td>0.35</td>
<td>0.2</td>
<td>0.13</td>
<td>0.33</td>
<td>0.17</td>
</tr>
<tr>
<td>Diatom density (cells/cm²)</td>
<td>56865</td>
<td>21625</td>
<td>109215</td>
<td>33493</td>
<td>4485</td>
<td>107759</td>
</tr>
<tr>
<td>Chl a concentration (μg/m²)</td>
<td>22.4</td>
<td>8.98</td>
<td>40.87</td>
<td>11.17</td>
<td>2.05</td>
<td>44.51</td>
</tr>
</tbody>
</table>

Fig. 7. Comparison between calculated and sampled biomass values: (a) Calculated and sampled diatom densities; (b) Calculated and sampled Chl a contents
In this paper, the flow velocity and water depth varied in 0.2-0.5m/s and 0.25-0.4 m, respectively, failing to reach the suitable ranges determined by Wang et al. (2017). The negative correlation between COD and diatom biomass may be due to the negative impact of COD on diatom abundance. The correlation \((r=-0.45; p<0.01)\) can be observed between the variation in COD and diatom species richness (Wu et al., 2014). In addition, Vogt et al. (2010) proved that species richness was positively associated with total diatom biovolume at the level of the entire diatom community. The following studies have tackled the influence mechanisms of flow velocity and water depth on diatom density. Besemer et al. (2007) explored the impact of flow velocity on the growth of benthic diatoms, pointing out that high flow velocity contributes to physiological processes like nutrient absorption and photosynthesis, and that the nutrient acquisition speed and efficiency may be boosted or suppressed by affecting resource distribution, biological localization and resource collection ability. Gundersen and Jorgensen (1990) proved that the increase of flow velocity can elevate the exchange rate of nutrients between aquatic organism and water bodies. Reference states that shallow natural streams are featured by small flow velocities, which hinders the elimination of by-product accumulation and nutrient supply (Odum, 1956), and subjected to severe external interference. Wang et al. (2017) found that diatom biomass begins to drop once the water depth exceeds 0.55m, owing to the variations in transparency and light availability with the water depths (Sellers et al., 2003).

In previous studies, we found that nutrients have a positive correlation with the biomass of benthic diatoms (Dodds et al., 2002). However, the data correlation analysis in this research shows that the biomass of benthic diatoms was inversely proportional to the TP concentration and that the correlation between benthic diatom density and Chl a concnet and TN in rivers was not significant. We analyze the reasons as follows: (1) this result is related to the concentration of nutrients at the sampling sites. According to Table 1, except for M-1, which is medium eutrophic, the other sampling points have reached a state of severe eutrophication. The nutrient concentration is no longer the main factor limiting the biomass of benthic diatoms since the sampling sites provide sufficient nutrients for the growth and reproduction of benthic diatoms, thus causing the insignificant correlation between benthic diatom biomass and TP and TN in rivers. (2) the excess nutrients were beneficial to the growth of Chl a on the surface of water (Nazeer et al., 2017), which will affect the photosynthesis of benthic diatoms, thus affecting the biomass of diatoms. It can be seen from the correlation analysis results of various indicators in Table 1 that the TP concentration has a strong positive correlation with turbidity, while turbidity was affected by the biomass of phytoplankton in the water (Oviatt et al., 2017), which can also explain that the negative correlation between the benthic diatom biomass and TP concentration may be related to the propagation of phytoplankton. Through studying the correlation between the steady-state transition mechanism and the threshold determination, Schindler (2006) found that the increasing of nutrient concentration (especially P) could result in the transformation of lake system from the submerged plant steady state to the Chl a steady state. The Chl a in shallow lakes mainly respond to changes in P, while the primary productivity of Chl a was the key factor that causes the death of aquatic plants by shading, so the concentration of P in water was an early warning indicator for the changes of ecological homeostasis (Schindler et al., 2008). Therefore, interspecific competition caused by nutrients exceeding a certain critical concentration may be the main reason for the negative correlation trend, which is one of the focuses of our future research. However, the purpose of this study was to establish a mathematical model between benthic diatom biomass and various environmental factors, providing an easy way to predict the trend of benthic diatom biomass in regional rivers. Through the above analysis, the model is applicable to the area of nutrients.

After the model was established, two rivers in the same basin were select for simultaneous sampling in order to model verification. The model verification results showed that both mathematical models had good predictability. The minimum calculation accuracy of the model appeared at the J-2 sampling site. Since the diatom density and the Chl a content were small at the sampling site, the error had a great influence on the accuracy. In summary, the above two models can initially evaluate the the biomass of benthic diatoms in the river with changes of environmental factors and the results of the study can provide a reference for the evaluation of river health.

The existing studies agree that the diatom biomass is deeply influenced by various environmental factors through a complex mechanism. Thus, the proposed models are particularly suitable for the fish breeding season in mild spring when the nutrients in the river are dense enough to stimulate the growth of benthic diatoms and the changes of pH value, DO and Cond are extremely small. In other words, the quantitative relationships between the diatom biomass and environmental factors only apply to specific area and period, provided that other environmental factors remain basically the same. Despite the great ecological significance, our research only tackles fish spawning period, failing to consider the fattening period, wintering period or different flow periods. In the neglected periods, the primary influencing factors may vary, calling for revisions to the mathematical models and the inclusion of the grey prediction and linear programming models (Hue et al., 2018; Jian et al., 2018; Rehar et al., 2017; Shannon, 2017; Song, 2018). The local meteorological factors are also worth considering during model construction (Evola et al., 2018; Magrini et al., 2018).
5. Conclusions

In this paper, 10 diatom genera were identified from the samples collected from 14 sites between Yuzixi Hydropower Station, which lies in the middle and upper reaches of Minjiang River, and Dujiangyan. Then, the environmental factors were measured to determine the differences in nutrients (TP and TN), water temperature, hydraulic conditions (velocity and water depth), turbidity, pH, DO, COD, Cond, etc. Through data analysis, the author acquired the correlation between each environmental factor and diatom biomass, and established the mathematical models for the responses of diatom density and Chl a to the four primary influencing factors (i.e. turbidity, COD, water depth and flow velocity).

Our models demonstrate that log (D) and log (Chl a) are negatively correlated with turbidity and COD, and positively with water depth and flow velocity. These results reflect the responses of benthic diatom biomass to environmental factors in fish breeding and spawning periods in the downstream of Zipingpu Reservoir, offering a valuable reference for the nutritional demand of fish in the spawning period. The future research will probe deeper into the responses of benthic diatom biomass to environmental factors in other periods.

Acknowledgments

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References

Bunn S.E., Davies P.M., Winning M., (2003), Sources of organic carbon supporting the food web of an arid zone floodplain river, Freshwater Biology, 48, 619-635.
Burns N., McIntosh J., Scholes P., (2005), Strategies for managing the lakes of the Rotoura District, New Zealand, Lake and Reservoir Management, 21, 61-72.
Lohman K., Jones J.R., Perkins B.D., (1992), Effects of nutrient enrichment and flood frequency on periphyton biomass in Northern Ozark streams, Canadian Journal of Fisheries and Aquatic Sciences, 49, 1198-1205.
Odum H.T., (1956), Primary production in flowing waters, Limnology and Oceanography, 1, 102-117.
Oviatt C., Smith L., Krumholz J., (2017), Managed nutrient reduction impacts on nutrient concentrations, water clarity, primary production, and hypoxia in a north temperate estuary, Estuarine Coastal & Shelf Science, 199, 25-34.
Effects of environmental factors on the biomass of benthic diatom in rivers


Rimet F., Bouchez A., Montuelle B., (2015), Benthic diatoms and phytoplankton to assess nutrients in a large lake: complementarity of their use in Lake Geneva (France-Switzerland), Ecological Indicators, 53, 231-239.


Smith V.H., Tilman G.D., Nekola J.C., (1999), Eutrophication: effects of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems, Environmental Pollution, 100, 179-196.


Teittinen A., Kallajoki L., Meier S., Stigzelius T., Soininen J., (2016), The roles of elevation and local environmental factors as drivers of diatom diversity in subarctic streams, Freshwater Biology, 61, 1509-1521.


