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Biodiversity and distribution of microzooplankton in *Spirulina* (*Arthrospira*) *platensis* mass cultures throughout China

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ABSTRACT

Spirulina (*Arthrospira*) *platensis* is the most commonly produced microalgae for commercial applications, such as nutraceuticals and feed. While crop productivity of commercial *Spirulina* farms is often compromised by grazers and contaminating microzooplanktons, the biodiversity and identity of the most harmful microzooplanktons in *Spirulina* farms have not been extensively studied. As China is the number one producer of *Spirulina* in the world, comprehensive information on the biodiversity and identity of microzooplanktons in *Spirulina* farms is essential for the long-term commercial viability of these farms. Therefore, we determined the biodiversity and identity of the major microzooplanktons that are present in eight commercial *Spirulina* cultivation sites throughout China. Furthermore, we identified the major grazers that appear to directly affect the productivity of *Spirulina* cultures. Among twenty-three species that include 2 flagellates, 2 amoebae, 15 ciliates, and 4 rotifers, *Brachionus plicatilis*, *Frontonia* sp. and one unknown Heterolobosean amoeba appeared to be the most harmful to *Spirulina* due to their high density and ability to graze *Spirulina*. The similarity of the biodiversity and abundance of the microzooplankton was > 80% among two out of eight mass cultivation sites (C and D), while the remaining cultivation sites exhibited their own unique microzooplankton biodiversity characteristics. Redundancy analysis (RDA) showed that there was a positive relationship between harmful species of *Brachionus plicatilis* and salinity, while the other two harmful species of Heterolobosean amoeba and *Frontonia* sp. had a positive relationship with oxidation-reduction potential (ORP). As this is the first report to identify the major harmful microzooplankton species in commercial *Spirulina* farms, our study not only provides a theoretical basis for the relationship between environmental factors and biodiversity of harmful grazers but also lays a scientific foundation for developing effective monitoring and management strategies for commercial *Spirulina* farms.

1. Introduction

Among the various microalgal species that are currently used for commercial applications, *Spirulina* is produced in the greatest quantity for the production of nutraceuticals, aquaculture feed, food, and cosmetics [1–3]. China annually produces over 10,000 tons of *Spirulina* biomass, which represents > 70% of the global supply. *Spirulina* has been successfully cultivated for many decades at commercial scale throughout the world, as it can be cultivated in extreme conditions, such as high alkalinity, salinity, and temperature. This robustness allows *Spirulina* to outcompete other contaminating microalgae or grazers [4,5]. Additionally, its large and spiral morphology helps *Spirulina* to resist grazing by various microzooplanktons. However, *Spirulina* cultures often crash based on personal communication with algal producers, and the productivity of *Spirulina* is mainly affected by the invasion of various grazers [6].

Algivore zooplankton are most harmful to microalgae cultivation because they can rapidly reduce algal biomass through direct grazing, causing serious economic losses [7]. The grazers and unwanted algae present major challenges in mass cultivation of microalgae and complicate the management of industrial algal farms [6]. Wang et al. found that the algivore rotifer *Brachionus plicatilis* was the main contaminating

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Table 1
Geographical positions and descriptions of *Spirulina* cultivation systems from different companies in China.

Sites	Geographical position	Size of ponds	Medium depth	Sampling time
A	N19°59′6.52″ E110°27′48.92″	550–560 m^2	15–20 cm	Sept. 21
B	N25°43′46.54″ E119°26′17.20″	1200 m^2	32–33 cm	Sept. 20
C	N25°03′44.57″ E102°39′57.78″	800 m^2	18–20 cm	Sept. 26
D	N25°03′33.18″ E102°39′39.69″	800 m^2	18–20 cm	Sept. 26
E	N27°15′29.72″ E114°42′20.48″	900–1800 m^2	30 cm	Jul. 30
F	N33°14′38.80″ E120°44′3.29″	710–776 m^2	17–25 cm	Aug. 3
G	N37°29′19.72″ E118°37′47.09″	300–640 m^2	30 cm	Aug. 6
H	N39°05′58.42″ E107°59′18.87″	300–640 m^2	15–20 cm	Aug. 19[a]

[a] The samples of H site were collected from *Spirulina* cultures in 2014, while the rest of the samples were collected in 2015.

species and cause of major losses of *Spirulina* biomass productivity [8]. Microalgal contaminants, protozoa and fungi negatively affect the overall productivity of open outdoor cultures of *Spirulina platensis* [5,9]. However, there are few publications on the comprehensive biodiversity of major grazers or on how to manage the grazing of microalgal 'crops' by predators [10].

As there is limited information on microzooplankton species in mass cultivation ponds of *Spirulina* [9,11], the aim of this research was to determine the biodiversity of microzooplankton and to identify the major harmful grazers in commercial *Spirulina* farms throughout China. We further attempted to establish relationships between various environmental factors and the occurrence of harmful grazers and the biodiversity of microzooplanktons in these large-scale *Spirulina* culture ponds.

2. Materials and methods

2.1. Cultivation of Spirulina platensis and sample collection

The microalga *Spirulina platensis* was semi-continuously cultivated with modified Zarrouk medium [12] by eight companies (Table 1; Fig. 1) located in seven provinces that represent the main regions of *Spirulina* mass cultivation throughout China. All culture systems were open raceway ponds of various sizes that ranged from 300 to 1800 m^2 of water surface, and the ponds from four sites (C, D, F, and H) were covered (Fig. 2).

Sampling was conducted from July 30th to September 16th, 2015 for sites A-G and on August 19th, 2014 for site H (Table 1). Six ponds were randomly selected in each sampling site, and samples consisted of mixtures from three points in each pond. Samples of the original culture including algae and contaminating organisms were collected in two 50 mL centrifuge tubes. One tube with 50 mL culture was fixed with Lugol's solution to a final concentration of 1.0% for quantitative analysis [13]. The remainder was used for morphological analysis and molecular identification of microzooplanktons. Additional samples (250 mL) were collected and subjected to various analyses of abiotic factors.

2.2. Analyses of pond abiotic factors

During this study, water temperature (WT), salinity, pH, conductivity, dissolved oxygen (DO) and Oxidation-Reduction Potential (ORP) were measured on site with a YSI Professional Plus meter (Yellow Springs, USA). Samples (250 mL) were collected under the water surface for nutrient analysis. The samples were filtered through 0.45-μm millipore filters immediately after collection and stored at − 20 °C for future analyses. The total nitrogen (TN) and phosphorus (TP) were determined by standard methods [14], and dissolved organic carbon (DOC) was quantitated with a TOC-L analyzer (Shimadzu Corporation).

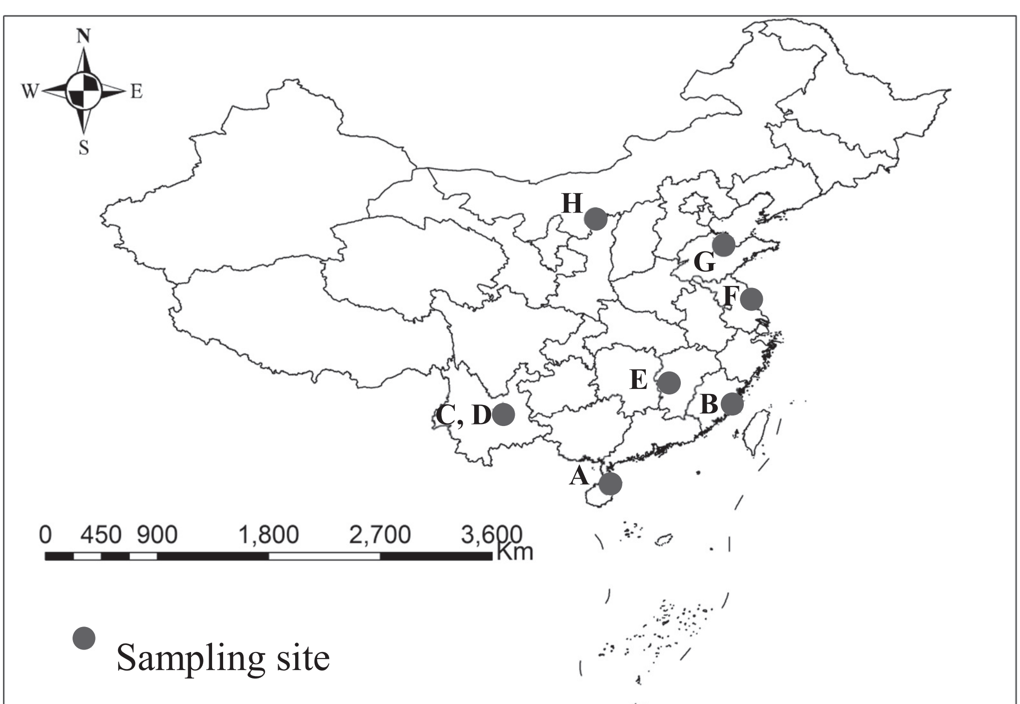

Fig. 1. *Spirulina* culture sampling sites throughout China. A, in Hainan province; B, in Fujian province; C and D, in Yunnan province; E, in Jiangxi province; F, in Jiangsu province; G, in Shandong province; H, in Inner Mongolia.