Spatial variations in the predation of nano-protozoa on bacteria in Lake Taihu, China

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ABSTRACT
We investigated the predation of nano-protozoa (size fraction < 16 \( \mu \)m) on bacteria in four regions of Lake Taihu, China using the dilution method. The results showed that the growth rate of bacteria in the Meiliang Bay, Gonghu Bay, Dapu River mouth, and Lake Center was 0.613 ± 0.024 \( d^{-1} \), 1.015 ± 0.074 \( d^{-1} \), 1.324 ± 0.052 \( d^{-1} \), and 0.924 ± 0.034 \( d^{-1} \), respectively. The predation rate of nano-protozoa on bacteria at these sites was 0.733 ± 0.061 \( d^{-1} \), 1.245 ± 0.096 \( d^{-1} \), 1.930 ± 0.039 \( d^{-1} \), and 1.040 ± 0.025 \( d^{-1} \), respectively. The growth rate at the Dapu River mouth was significantly higher than that at Lake Center and Meiliang Bay, and that at Gonghu Bay was significantly higher than that at Meiliang Bay. The predation rate was significantly higher at the Dapu river mouth than in the other three regions, and the predation rate at Gonghu Bay was significantly higher than in Meiliang Bay. Wind-induced waves, nutrient levels and algal blooms may explain these variations of the nano-protozoa predation rate in Lake Taihu. Our results also indicated that the mean predation rate in Lake Taihu was higher than those of other freshwater, or marine water environments.

KEYWORDS
Lake Taihu; spatial variations; nano-protozoa; predation; bacteria

Introduction
In many environments, protozoan predation of bacteria is of great biological and ecological importance (deLeo and Baveye 1997). In freshwater ecosystems, metazoan and protozoan predation regulates bacterial biomass and community structure (Langenheder and Jürgens 2001). In an oligotrophic system, predation pressure affected bacterial abundance, diversity, and size–structure distribution (Corno et al. 2008). Furthermore, microcosm experiments have shown that metazoan and protozoan grazers impact the distribution of bacterial biomass (Jürgens and Arndt 1997). Although zooplankton grazing on bacteria has received increasing attention, such studies have focused on lakes and experimental microcosms, rather than the spatial variations of predation within lakes that differ in trophic status and humic content.

Lake Taihu, a large shallow eutrophic lake in China, has four distinct regions characterized by significant differences in their environmental conditions (Qin et al. 2007). The Dapu River mouth in the west is adjacent to land with vegetation cover, and intensive tourism and urban development, which increase anthropogenic inputs. Gonghu Bay in the northeast is the macrophyte-dominated region, characterized by submersed vegetation, clear water, and diverse communities of fishes and invertebrates. The Lake Center is less enriched with nitrogen and phosphorus than the other regions,
but exposed to frequent wind mixing. Meiliang Bay in the north is in the algae-dominated region, characterized by the dominance of phytoplankton, high water turbidity, and strong algal blooms.

A single-location study of protozoan predation on bacteria in Lake Taihu was conducted in Meiliang Bay by Chen et al. in 2007. They reported that 90% of the predation on bacteria was by nano-sized protozoa (less than 16 μm), dominated by heterotrophic nanoflagellates (HNFs). However, there is no further data about how differences between the distinct lake regions are reflected in differences in the predation of nano-protozoa on bacteria of this aquatic ecosystem. In the present study, our objective was to investigate the spatial differences in the predation of nano-protozoa on bacteria in Lake Taihu using the dilution method.

**Methods**

Lake Taihu is a large (2238 km²), shallow (maximum water depth < 3.3 m, mean depth = 1.9 m), eutrophic, freshwater lake in China (Qin et al. 2007). Within the lake, we sampled the four different regions described above (Meiliang Bay, Gonghu Bay, Dapu River mouth and Lake Center), with three sampling sites within each region (Figure 1). Samples were collected on 12 June 2015. We measured seven water quality parameters at each site. Water temperature (WT), pH, and dissolved oxygen (DO) were measured *in situ* using an YSI 6600 V2 multiparameter water quality sonde (Yellow Spring Instruments, USA). Total nitrogen (TN), total phosphorus (TP), and total suspended solids (TSS) were measured according to standard methods described in Jin and Tu (1990). Chlorophyll a

Figure 1. Map showing three sampling sites in each of four regions (Meiliang Bay, Gonghu Bay, Dapu River mouth and Lake Center) of Lake Taihu, China. Samples were collected on 12 June 2015.
(Chl-a) concentrations were analyzed by spectrophotometric measurements after extraction in hot 90% ethanol (85 °C) (Papista et al. 2002).

At each site, about 5 L of water was collected from the water column by using a 5 L Schindler sampler. Water samples were filtered through a 16-μm sieve to remove large plankton, and stored in a portable refrigerator (about 4 °C), before being transported to the laboratory for subsequent grazing experiments. Nano-protozoa grazing was determined using the dilution method (Landry and Hassett 1982). The filtered lake water was combined with sterile water, in five volume ratios, 10:0, 7:3, 5:5, 3:7, and 1:9, to make up 500 mL. Three replicate sterilized glass reagent bottles of 1 L were filled with each dilution mixture. All bottles were sealed without air bubbles and then incubated in situ for 3 h at ambient light levels and subsurface water temperatures, to determine the number of bacteria and HNFs. The apparent bacterial growth rates were calculated from the following equation used in the dilution method of Landry and Hassett (1982):

\[
Pt = P_0 \times e^{(k-g)\cdot t}
\]

\[
r = \ln(P_t/P_0)/t
\]

where \(g\) is the predation rate of nano-protozoa on bacteria, \(k\) is the growth rate of bacteria, \(P_0\) and \(P_t\) are initial and final cell densities of bacteria, \(r\) is the rate of population growth (day\(^{-1}\)) and \(t\) is the duration of incubation. Instantaneous coefficients of bacterial growth (\(k\)) and nano-protozoa grazing (\(g\)) were determined from least-squares and linear regression analysis of the relationship between the rate of change of bacteria and nano-protozoa.

Samples for enumeration of bacteria and HNFs were preserved in formalin (final concentration 2%) and stored at 4 °C until further processing (usually within the next 24 h). A 1 mL subsample was filtered on black polycarbonate filters (25 mm, pore size 0.2 mm, Millipore) and stained with 4,6-diamidino-2-phenylindol (DAPI, final concentration 100 mg/mL) (Porter and Feig 1980). The filters were cut into halves, one of which was immediately mounted on a slide and used to count bacteria and HNFs under an epifluorescence microscope (Axiophot II; Zeiss).

The significance of differences in the predation rate of nano-protozoa on bacteria in different lake regions were determined using Wilcoxon signed-rank nonparametric test, using SPSS (Statistical Program for Social Sciences) 13.0 software. Significance levels were set at \(p < 0.05\) as significant and \(p < 0.01\) as very significant.

**Results and discussion**

The environmental conditions varied between the four regions of the lake, as shown by TN, TP, Chl-a, SS, DO and pH (Figure S1 (Online Supplemental data)). There were regional differences in the growth rate of bacteria within Lake Taihu as follows: Meiliang Bay 0.613 ± 0.024 d\(^{-1}\), Gonghu Bay 1.015 ± 0.074 d\(^{-1}\), Dapu River mouth 1.324 ± 0.052 d\(^{-1}\), Lake Center 0.924 ± 0.034 d\(^{-1}\) (Figure 2). The growth rate of bacteria at the Dapu River mouth was significantly higher than that at Lake Center and Meiliang Bay (\(p < 0.05\)), and that at Gonghu Bay was significantly higher than that at Meiliang Bay (\(p < 0.05\)) (Table 1). There were regional differences in the predation rate of nano-protozoa on bacteria within Lake Taihu as follows: Meiliang Bay 0.733 ± 0.061 d\(^{-1}\), Gonghu Bay 1.245 ± 0.096 d\(^{-1}\), Dapu River mouth 1.930 ± 0.039 d\(^{-1}\), and Lake Center 1.040 ± 0.025 d\(^{-1}\) (Figure 3). The predation rate was significantly higher at the Dapu river mouth than in the other three regions (\(p < 0.05\)), and the predation rate at Gonghu Bay was significantly higher than in Meiliang Bay (\(p < 0.05\)) (Table 1).

We suggest that three key environmental characteristics within the different regions of Lake Taihu account for the differences, namely wind-induced waves, nutrient, and algal blooms. We suggest that wind-induced waves are a main cause of the site-specific variation in the predation of nano-protozoa on bacteria among lake regions. Waves are frequent in Lake Taihu due to its shallow
waters and special shape (Qin et al. 2007). The wind-induced waves frequently disturb the water-sediment interface, leading to intensive exchange of nutrients and organic matter particles between the water column and surface sediments. Due to its large size, however, the exchange frequency of different sites within Lake Taihu may vary (Wu et al. 2016). Thus, significant environmental gradients in the water column were observed between different sampling sites within a lake region, which could lead to the site-specific variations in the predation of nano-protozoa on bacteria within the same region.

The predation of nano-protozoa on bacteria was highest in the Dapu River mouth, the region with the highest nutrient concentrations (TN, TP and SS), and extensive anthropogenic inputs due to tourism and urban development. The nutrient concentrations may therefore be related to the sediment contaminant status, with higher nutrient concentrations normally being found in seriously polluted areas. Increasing nutrients promote high-density bacterial community, which in turn provides abundant resources for protozoa, eventually leading to increased predation rate (Güde 1979). In addition, phytoplankton enrichment in this region is often accompanied by an increase in particulate organic carbon, which also provides abundant food resources for protozoa (Cai 1998).

Finally, the predation rate of nano-protozoa on bacteria in Meiliang Bay is very low, which may be related to cyanobacterial blooms. In the northern part of Lake Taihu, especially in the most eutrophicated Meiliang Bay, cyanobacterial blooms (principally of Microcystis) occurred from March to November, with the highest biomass from June to September. We suggest that dense phytoplankton inhibit the movement of nano-protozoa, and thereby reducing the probability of their predation on bacteria. Concurrently, the increase of phytoplankton caused the formation of combination of bacteria and algae, and these structures provide shelter for the bacteria, thereby reducing their vulnerability to predation. The low predation by nano-protozoa on bacteria in Gonghu Bay and Lake Center may be related to the relatively low nutrient levels and low phytoplankton abundance in these

Table 1. Differences in the bacteria growth rate and nano-protozoa predation rate between four regions of Lake Taihu: Dapu River mouth (D), Gonghu Bay (G), Lake Center (L), and Meiliang Bay (M). Significant differences between the pairs of regions were determined by a t-test.

|                      | D-G  | D-L  | D-M  | G-L  | G-M  | L-M  |
|----------------------|------|------|------|------|------|------|
| Bacterial growth rate| -1.347 | -1.943 * | -2.048 * | -1.002 | -1.802 * | -1.024 |
| Nano-protozoa predation rate | -1.923 * | -2.033 * | -2.148 * | -1.045 | -1.876 * | -1.096 |

The significant levels are represented as significant, $p < 0.05$ (*), and very significant, $p < 0.01$ (**).
regions. The east part of Gonghu Bay, dominated by floating macrophytes, is generally the clearest region of Lake Taihu, and Lake Center is less enriched with nitrogen and phosphorus and phytoplankton abundance is also less. Furthermore, the diversion of water from Yangtze River to Lake Taihu may contribute to the low predation rate in Gonghu Bay, because of the reduction of nutrient level.

The mean grazing rate of HNFs in Lake Taihu (1.237 ± 0.055 d⁻¹) was markedly higher than that in other environments, such as Laguna Bufeos Lake, Bolivia (tropical floodplain lake), and the east coast of New Zealand (subtropical ocean) (James and Hall 1998; Rejas et al. 2005). Two reasons may account for this phenomenon. One reason is that abundant bacteria and organic debris in the eutrophic water of Lake Taihu provide a rich source of food for HNFs, allowing abundance and biomass to greatly increase. The second reason is that predation of nano-protozoa is the most important influence on bacterial biomass (Lee et al. 2001a, 2001b); in Lake Taihu, nano-protozoa, the most efficient group that grazes on bacteria, are dominant (Burns and Schallenberg 2001; Mayer et al. 1997). The bacterial biomass in Lake Taihu is maintained in a relatively stable level, due to the efficient grazing of the nano-protozoa on bacteria.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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References

Burns CW, Schallenberg M. 2001. Short-term impacts of nutrients, *Daphnia*, and copepods on microbial food-webs of an oligotrophic and eutrophic lake. New Zeal J Mar Fresh. 35:695–710.

Cai HJ. 1998. [The response of ciliated protozoans to eutrophication in Meiliang Bay of Taihu Lake]. J Lake Sci. 10(3):43–48. Chinese.

Chen M, Gao G, Zhu LP, Feng SH. 2007. [Predation of microprotozoa on bacteria in Taihu Lake]. Chinese J Appl Ecol. 18(10):2384–2388. Chinese.

Corno G, Caravati E, Callieri C, Bertoni R. 2008. Effects of predation pressure on bacterial abundance, diversity, and size-structure distribution in an oligotrophic system. J Limnol. 67(2):107–119.

DeLeo PC, Baveye P. 1997. Factors affecting protozoan predation of bacteria clogging laboratory aquifer microcosms. Geomicrobiol J. 14(2):127–149.

Güde H. 1979. Grazing by protozoa as selection factor for activated sludge bacteria. Microb Ecol. 5:225–237.

James MR, Hall JA. 1998. Microzooplankton grazing in different water masses associated with the subtropical convergence round the South Island, New Zealand. Deep-Sea Res Pt I. 45:1689–1707.

Jin XC, Tu QY. 1990. Survey specificity for lake eutrophication. Beijing: China Environmental Science Press. Chinese.

Jürgens K, Arndt ZH. 1997. Impact of metazoan and protozoan grazers on bacterial biomass distribution in mesocosm experiments. Aquat Microb Ecol. 12(2):131–138.

Landry MR, Hassett RP. 1982. Estimating the grazing impact of marine microzooplankton. Mar Biol. 67(3):283–288.

Langenheder S, Jürgens K. 2001. Regulation of bacterial biomass and community structure by metazoan and protozoan predation. Limnol Oceanogr. 46:121–134.

Lee CW, Kudo I, Morishita H, Yanada M, Maita Y. 2001a. Examining the relationship between bacteria and heterotrophic nanoflagellates in Funka Bay (Japan) using the size fractionation method. Aquat Ecol. 35:1–8.

Lee CW, Kudo I, Yanada M, Maita Y. 2001b. Bacterial abundance and production and heterotrophic nanoflagellate abundance in subarctic coastal waters (Western North Pacific Ocean). Aquat Microb Ecol. 23:263–271.

Mayer J, Dokulilm T, Salbrechter MB, Posch T, Pfister G, Kirschner AKT, Velimirov B, Steitz A, Ulbricht T. 1997. Seasonal successions and trophic relations between phytoplankton, zooplankton, ciliate and bacteria in a hypertrophic shallow lake in Vienna, Austria. Hydrobiology. 342/343:165–174.

Papista E, Acs E, Boddi B. 2002. Chlorophyll-alpha determination with ethanol–a critical test. Hydrobiology. 485:191–198.

Porter KG, Feig YS. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr. 25(5):943–948.

Qin BQ, Xu PZ, Wu QL, Luo LC, Zhang YL. 2007. Environmental issues of Lake Taihu, China. Hydrobiology. 581:3–14.

Rejas D, Muyaert K, Meester LD. 2005. Trophic interactions with in the microbial food web in a tropical floodplain lake (Laguna Buefos, Bolivia). Rev Biol Trop. 53(1):85–96.

Wu TF, Timo H, Qin BQ, Zhu GW, Janne R, Yan WM. 2016. In-situ erosion of cohesive sediment in a large shallow lake experiencing long-term decline in wind speed. J Hydrol. 539:254–264.