



Identification and feeding characteristics of the mixotrophic flagellate *Poterioochromonas malhamensis*, a microalgal predator isolated from planting water of *Pontederia cordata*

Hao Yan^{1,2} · Qin Li¹ · Bo Chen¹ · Mei Shi¹ · Tingting Zhang¹

Received: 4 May 2021 / Accepted: 7 January 2022 / Published online: 27 January 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract

The microorganisms and allelochemicals in *Pontederia cordata* planting water may have a synergistic inhibitory effect on algae. To study this synergy, an algae-inhibiting organism was isolated and identified, and its growth and feeding characteristics were studied. The organism was identified as *Poterioochromonas malhamensis* yzs924 based on both its morphology and molecular barcoding employing 18S rDNA gene sequences.

The growth and feeding of *P. malhamensis* were affected by environmental factors and the state of its prey. (1) *P. malhamensis* is a mixotrophic flagellate. Its heterotrophic growth was the fastest in a wheat grain medium, and its growth rate in this study reached 2.5 day^{-1} . (2) Within a short period of time (2 days), *P. malhamensis* growth was slower under continuous dark conditions than under alternating light and dark conditions, but it fed on *Microcystis aeruginosa* more rapidly under dark conditions. (3) High pH was disadvantageous to the growth and grazing of *P. malhamensis*. When the pH was kept stable at 9, *P. malhamensis* could not grow continuously. (4) When the initial density of *M. aeruginosa* was 5×10^7 cells/mL or is in a period of decline, *P. malhamensis* could not remove all *M. aeruginosa*. The combined use of *P. malhamensis* and allelochemicals may represent a method of *M. aeruginosa* control, but this approach requires further research.

Keywords *Poterioochromonas malhamensis* · *Microcystis aeruginosa* · *Pontederia cordata* · Algae inhibition · Allelochemicals

Introduction

The proliferation of *Microcystis aeruginosa* leads to bloom and adversely affects the environment. Plants can control the excessive reproduction of *M. aeruginosa* by releasing allelochemicals (Xiang et al. 2015; Wang et al. 2017a, b, Qian et al. 2018). When evaluating the allelopathic potential of *Pontederia cordata* to *M. aeruginosa*, we found that small amounts of *P. cordata* planting water that had not been filtered to remove microorganisms could quickly kill

M. aeruginosa, indicating the possible presence of algae-killing organisms in the planting water.

Algae-inhibiting organisms include mainly viruses, bacteria, microfungi, amoebae, ciliates, eukaryotic microalgae, and protozoa (Van Wichelen et al. 2016). Among them, there have been fewer studies on fungi and viruses, and most studies are on algae-inhibiting bacteria. Most of these bacteria inhibit the growth of algae by releasing chemicals, and a high density of bacteria is often required to noticeably inhibit algal growth (Guo et al. 2015; Lin et al. 2016). Myxobacteria can lyse algae cells directly when they come into contact, but the living conditions of myxobacteria are relatively harsh (Shilo 1970; Daft et al. 2010). Phagocytosis of cyanobacteria by protozoan ciliates and amoebae has also been reported (Van Wichelen et al. 2016), while phagocytosis by flagellates, mainly mixotrophic flagellates of the Chrysoophyta and Cryptophyta, has been reported even more often (Zhang and Watanabe 2001; Touloupakis et al. 2016; Princiotta et al. 2019).

Responsible Editor: Vitor Vasconcelos

✉ Tingting Zhang
zttahnu@163.com

¹ College of Life Sciences, Anhui Normal University, 241000 Wuhu, China

² Anhui Provincial Key Laboratory of Molecular Enzymology and Mechanism of Major Diseases, Anhui Normal University, Wuhu 241000, China

Mixotrophic flagellates have strong environmental adaptability, grow rapidly, can feed a variety of algae, can degrade Microcystins, and favor more diverse phytoplankton community (Baek et al. 2009; Wilken et al. 2010; Ma et al. 2018a, b; Zhang et al. 2020). Flagellates can prey on algae cells, resulting in a rapid decrease in their biomass, while bacteria tend to inhibit the growth of algae cells. Flagellates are also important predators of bacteria (Isaksson et al. 1999, Boenigk and Arndt 2002). In the presence of a large number of flagellates, bacteria are unlikely to reach a density that can inhibit *M. aeruginosa*.

In nature, plants and microorganisms always coexist and influence each other. Plants affect the community structure of the surrounding microorganisms by releasing organic carbon and allelochemicals. Studies have found that more algae-lysing bacteria can be isolated from water bodies with aquatic plants than from water bodies without aquatic plants, indicating that plants may stimulate the growth of algae-lysing bacteria (Kojima et al. 2016; Sakami et al. 2017). The presence of benthic fauna also stimulates plants to produce more allelochemicals, thus enhancing their allelopathic and algae-inhibiting ability (Zuo et al. 2015, 2016). Therefore, the main purpose of this article is to isolate and identify algae-inhibiting microorganisms and to study their growth and feeding characteristics. This will lay the foundation for further research on the synergistic inhibition of flagellates and allelochemicals and the evaluation of the effects of plants on the growth of flagellates.

Methods and materials

Experimental materials

The toxin-producing cyanobacteria *M. aeruginosa* (FACHB-915) used in the experiment were provided by Freshwater Algae Culture Collection at the Institute of Hydrobiology, China.

Obtaining of *P.cordata* planting water *P.cordata* was collected from the water body of the Sculpture Park in Wuhu City (31.34°N, 118.41°E). The *P.cordata* collected in the field was washed, and three stalks (wet weight 118.41 ± 30 g) were planted in 1 L beakers with water culture baskets. They were respectively added to 500 mL BG11 medium and cultured in light incubators for 20 days. The planting water was merged and filtered to remove physical particles. Use 100 mL of 10 times concentrated BG11 medium to supplement nutrition, and then use ultrapure water to make the volume to 1.5 L for use.

Comparison of algae inhibition by allelochemicals and microorganisms in planting water

A sterile 0.22 μm cellulose acetate membrane filter was used to filter the planting water. The filtrate containing only allelochemicals made up the allelochemicals treatment (group A), and the organisms remaining on the filter membrane made up the biological treatment (group B). The unfiltered planting water was used as a mixed treatment (group A + B) that contained both allelochemicals and organisms. Twenty milliliters of planting water from the different groups was added to sterilized Erlenmeyer flask of 50 mL. Additionally, 20 mL BG11 medium was added to an Erlenmeyer flask as the control group (CK). *M. aeruginosa* suspension (5 mL) with a density of 1×10^6 cells/mL was added to each group above. Three replicates were set up for each group and cultured in a light incubator with a light intensity of 2000 Lux and a temperature of 25 ± 1 °C. The cell density of *M. aeruginosa* was detected every 24 h, and the inhibition rate was calculated.

M. aeruginosa contains phycocyanin, which has its largest fluorescence peak at 650 nm. Fluorescence is not influenced by bacteria or *P. malhamensis*. The fluorescence intensity(y) has a good linear relationship with the density of *M. aeruginosa* ($y = 0.554x - 0.858$ $R^2 = 0.998$) and can be used to quickly characterize the biomass of *M. aeruginosa* (Bastien et al. 2011). The fluorescence intensity was detected by excitation (EX) light 610 and emission (EM) light 650 with a microplate reader (Spark 10 M, Tecan, Switzerland).

The inhibition rate was calculated as follows: $IR_t (\%) = (N_0 - N_t) / N_0 \times 100$, where t is time, IR_t is inhibition rate at day t , N_t is the mean value of EM 650 of *M. aeruginosa* in the treatment group at day t , and N_0 is the mean value of EM 650 of *M. aeruginosa* in the control group at day t (Qian et al. 2019).

Isolation and identification of predatory flagellate based on cell morphology and phylogenetic analysis

After the algae inhibition experiment, the liquid was concentrated by centrifugation, and a large number of swimming flagellates were observed. Using the gradient dilution separation method, the flagellates were diluted to one cell per drop with *M. aeruginosa* in the logarithmic phase (3×10^5 cells/mL), cultured in a 96-well plate under illumination, and observed and shaken gently every day. After a period of co-cultivation, if the liquid in the well became transparent, it indicated that *M. aeruginosa* had been preyed upon and that was a monoclonal algicidal microorganism in the well. In contrast, if *M. aeruginosa*

grew normally, the liquid in the well remained green. The purified flagellates were subjected to the above process, but *M. aeruginosa* was replaced with autoclaved wheat grain medium. Finally, the purified flagellates were extracted and preserved in wheat grain medium for later experiments.

The purified flagellate was observed and photographed under the light microscope (Leica DMi8) and transmission electron microscope (TEM). The TEM methods were essentially the same as those described in (Gong et al. 2015).

Purified flagellates were sent to be sequenced (Sangon, China). DNA was extracted and amplified using universal forward primer SSU-F (5'-ACCTGGTTGATCCTGCCA GT-3') and reverse primer SSU-R (5'-TCACCTACGGAA ACCTTGT-3') (Wang et al. 2006). For the temperature cycling, pre-denaturation at 94 °C for 4 min was followed by 30 cycles of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min, and finally an elongation at 72 °C for 10 min. Products of polymerase chain reaction were purified ligated into a pMD®18-T vector transformed into competent cell ligation product transformation, blue-white screening, plasmid extraction, and sequencing. The sequences were submitted to GeneBank (Accession No. MN022220.1). Alignment and arrangement were conducted by MEGA 7.0; the phylogenetic tree was constructed with 26 sequences of representative chrysophytes by the neighbor-joining method.

Growth of *P. malhamensis* in different media

P. malhamensis growth was studied in the following mediums. (1) *M. aeruginosa* was initially cultivated on BG11 medium. (2) The logarithmic phase of *M. aeruginosa*: *M. aeruginosa* was cultivated for 6 days, starting with an initial density of 1.5×10^6 cells/mL (3) The decline phase of *M. aeruginosa*: *M. aeruginosa* was cultivated for 30 days and then diluted to 1.5×10^6 cells/mL. (4) Culture solution of *M. aeruginosa*: *M. aeruginosa* cultures in the logarithmic growth phase were collected by centrifugation at 4000 rpm for 5 min. The supernatant was collected and filtered with a 0.22 µm filter membrane for later use. (5) Wheat grain medium: One hundred wheat seeds were added to 1 L ddH₂O, autoclaved and saved for later use.

P. malhamensis was inoculated onto the above mentioned growth media at 50 mL, and the initial density reached 1×10^5 cells/ml. Three replicates were set up for each group and cultured in a light incubator with a light intensity of 2000 Lux (light: dark = 14:10) and a temperature of 25 ± 1 °C.

One milliliter of the culture liquid was sampled every 24 h and fixed with Lugol's solution. The density of *P. malhamensis* cells was counted by a hemocytometer, and the specific growth rate was calculated as follows: $\mu = \ln(M_t/M_0)/t$, where M_0 and M_t represent the cell density (cells/

mL) of *P. malhamensis* at the beginning and at time t , respectively (Ma et al. 2018a, b).

Effects of environmental factors on the growth and feeding behavior of *P. malhamensis*

Environmental factors including light intensity, temperature, and the pH values of the growth medium were tested as follows:

- (1) Light intensity experiment: Three different light intensities were tested: 0 Lux, 2000 Lux, and 10,000 Lux, with a light period of 14 h:10 h (light: dark). The experiment was conducted in three light incubators (PRX-350B, Saifu Apparatus, China). During the cultivation, the temperature was 25 ± 1 °C, and the medium pH was not adjusted artificially.
- (2) Temperature experiment: three different temperatures of 20 ± 1 °C, 25 ± 1 °C, and 30 ± 1 °C were tested. The experiment was conducted in three temperature-controlled incubators. The light intensity was 2000 Lux and a photoperiod of 14 h:10 h (light: dark). Medium pH was not artificially adjusted during the cultivation.
- (3) Medium pH experiment: four different medium pH values of 6.0 ± 0.2 , 7.0 ± 0.2 , 8.0 ± 0.2 , and 9.0 ± 0.2 were tested. Medium pH was measured and adjusted every day with 1 mM HCl or 1 mM NaOH, as appropriate, using a pH meter (PHS-3C, Leici, China). The light intensity was 2000 Lux and a photoperiod of 14 h:10 h (light: dark). The temperature was 25 ± 1 °C.

For each treatment, the co-culture was incubated in triplicate 100 mL flasks with 50 mL *M. aeruginosa* in the logarithmic growth phase. Initial inoculation concentrations of *P. malhamensis* and *M. aeruginosa* were 1×10^4 cells/mL and $1-3 \times 10^6$ cells/mL. The cell densities of *M. aeruginosa* and *P. malhamensis* were detected every 24 h, with reference to 2.2 and 2.4.

Effects of *M. aeruginosa* growth phase and density on *P. malhamensis* growth and grazing

P. malhamensis feeding on *M. aeruginosa* in different growth phase

M. aeruginosa cultures employed were brought to exponential phase in continuous cultures. *M. aeruginosa* cultures in the decline phase were cultured for 1 month. During the experiment, the pH of the exponential phase culture was measured to be 9. In order to eliminate the influence of pH on grazing, the initial pH of the culture in the decline phase was adjusted to 9 and 7 with 1 M hydrochloric acid. *P. malhamensis* was inoculated into the above three

algal suspensions (50 mL) at a density of 1×10^4 cells/mL. The experiments were carried out in a light incubator as described in “Comparison of algae inhibition by allelochemicals and microorganisms in planting water.” Three replicates were set up for each group, and the biomass of *M. aeruginosa* was measured every day.

Effects of *M. aeruginosa* density on the growth and grazing of *P. malhamensis*

M. aeruginosa in the logarithmic phase was centrifuged at 4000 rpm, and the precipitated cells were resuspended in BG11 to an initial algal density of 5×10^7 cells/mL. Then, *M. aeruginosa* suspensions were prepared at densities of 5000, 2500, 1250, 625, 312, 156, and 78×10^4 cells/mL by twofold gradient dilution. The 50 mL *M. aeruginosa* suspensions mentioned above were combined with *P. malhamensis* at a density of 2×10^4 cells/mL. The initial density ratios of *M. aeruginosa* to *P. malhamensis* were 2500:1, 1250:1600:1300:1150:1, 70:1, and 35:1. The densities of *M. aeruginosa* and *P. malhamensis* were detected every 2 days after co-culturing for 8 days. The culture conditions are the same as those described in “Comparison of algae inhibition by allelochemicals and microorganisms in planting water.”

Statistics

The significance test was carried out by one-way ANOVA by IBM SPSS Inc.22.

Results

Comparison of inhibitory effects of allelochemicals and microorganisms in planting water

Figure 1 shows that the allelochemicals and microorganisms in the planting water had a good inhibitory effect on algae. The mixture of allelochemicals and microorganisms inhibited algae faster than allelochemicals or microorganisms alone. With the extension of the culture time, the inhibitory effect of microorganisms on algae became stronger than that of allelochemicals. After 3 days of culture, the inhibition rate of microorganisms was 86.35%, the inhibition rate of allelochemicals was only 48.25%, and that of the mixed group was 97.47%, indicating that there may be a synergistic algae inhibition effect between allelochemicals and microorganisms.

Identification of an unknown predatory flagellate

A monoclonal flagellate was successfully isolated and purified. Most flagellates are spherical or oval, and their cells are deformable. Most flagellates are 10–12 μm in width,

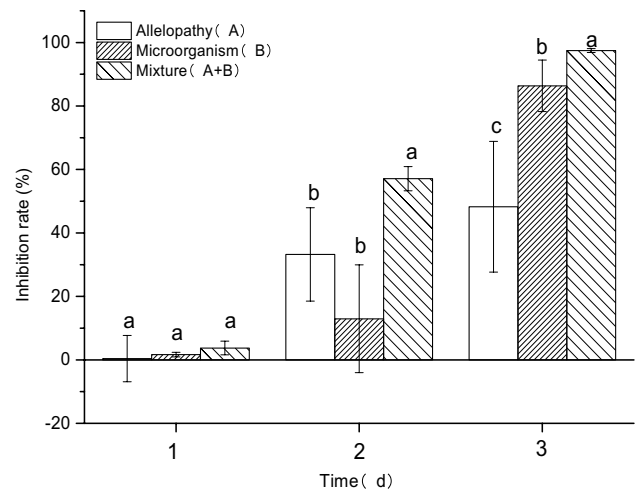


Fig. 1 Inhibition effect of allelochemicals and microorganisms on algae in planting water. Significant differences are indicated by different lowercase letters (one-way ANOVA, $P < 0.05$). Error bars represent the standard errors of the mean, $n = 3$. The following is the same

but some of the newly divided offspring are narrower. After phagocytosis of *M. aeruginosa*, the cells were irregularly spherical and could be enlarged. The flagellate isolated in this study had two flagella of different lengths, and the long flagellum was approximately 1–1.5 times the length of the body. Most flagellate cells contained two peripheral flaky yellowish pigmented components. In the grazing experiments, we did not observe the formation of *M. aeruginosa* colonies, but we often observed the formation of flagellate colonies. In addition, round objects without cellular structures were often observed, and they tended to cluster together (Fig. 2).

Predation and digestion Under the microscope, we observed flagellates swimming around the *M. aeruginosa* cells and touching them with a flagellum. The flagellates moved the water by vibrating and rotating their flagella, and the cells of *M. aeruginosa* followed the currents alongside the flagellate cells. Then, the *M. aeruginosa* cells rotated rapidly between the two flagella and were brought into the flagellates by phagocytic vacuoles. The cell wall and cell membrane of the ingested *M. aeruginosa* cells were separated, the cell contents were gradually absorbed by the flagellates, and the cell wall shrank into small black particles (Fig. 3).

To further confirm the morphological results, the predatory flagellate was also characterized by the gene sequence of its 18S rDNA. The 18 s sequence length is 1661 bp, NCBI accession number: MN022220.1. Blast results showed that it was 99.46% identical to that of *Poterioochromonas malhamensis* SAG933.1c (Gene Bank No., EF165114). The coverage is 100% (Fig. 4). So the isolated flagellate can be

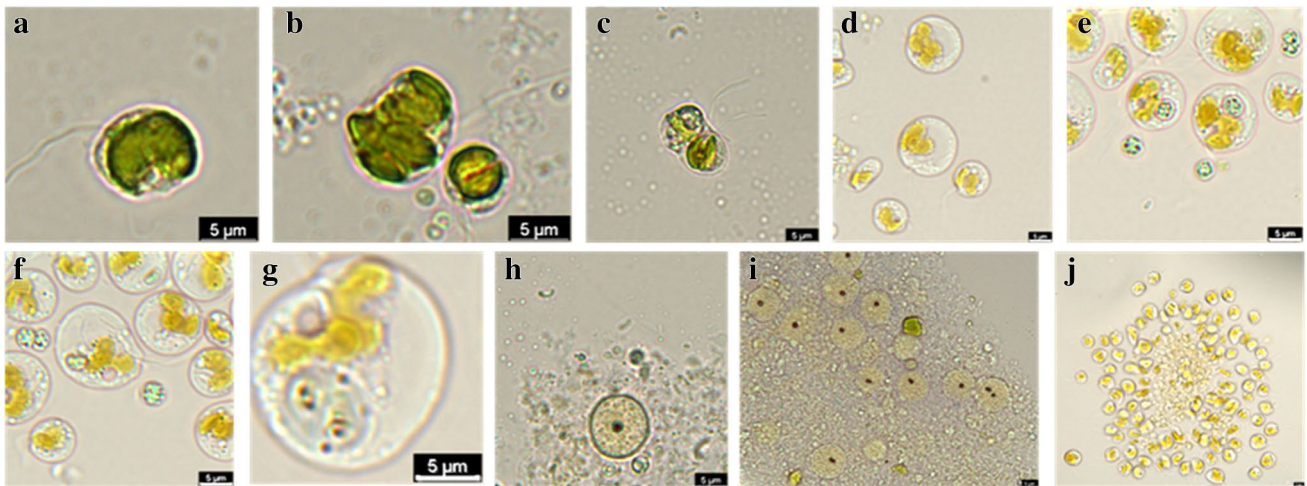


Fig. 2 a–c Cells fixed with Lugol's solution in order to more easily photograph the flagella; c shows a dividing cell. d Living cells of different shapes and sizes. e–g Phagocytosis and digestion of *M. aeruginosa*. h, i Round objects with no cell structure. j Flagellate clusters. Bar = 5 μm

identified as Chrysophyceae, Ochromonadales, and Ochromonadaceae *Poterioochromonas malhamensis* yzs924.

The growth of *P. malhamensis* in different media

Fig. 5 shows that *P. malhamensis* rapidly proliferated in the wheat grain medium. The density increased from the initial 1×10^5 cells/mL to 1.33×10^6 cells/mL in 1 day. The generation time was 5–6 h, and the growth rate reached 2.56 day^{-1} . After 4 days of inoculation, the growth rate and generation time gradually decreased, and the growth rate was 0.71 day^{-1} at 5 days.

The growth rates of *P. malhamensis* in the logarithmic phase (LP) and the decline phase (DP) were not significantly different, indicating that *M. aeruginosa* in different growth states had no effect on the growth of *P. malhamensis*. *P. malhamensis* could also grow in *M. aeruginosa* culture (cell-free) and reached a density of $2\text{--}4 \times 10^5$ cells/mL. The growth rate was $0.26/\text{day}$, close to that in the DP and LP groups.

In the first 2 days after inoculation into BG11 medium, the *P. malhamensis* cell density decreased and then gradually increased. After 1 month, the cell density had reached 6×10^5 cells/mL, and the specific growth rate was 0.06 d^{-1} . *P. malhamensis* was stored in sterilized wheat grain medium

Fig. 3 a–c The ultrastructure of the unknown flagellate and the digestion process of *M. aeruginosa*. d–f The digestion process. e is a local magnification of d in which the cell wall and contents of *M. aeruginosa* began to separate (arrow). f The cell wall and protoplast of *M. aeruginosa* in the digestive vesicle had become seriously separated, and the protoplast shrank into a mass, indicating that digestive enzymes had hydrolyzed and digested the cell contents through the cell wall. FV (food vacuole), CP (chloroplast), MT (mitochondria), N (nucleus), M (*Microcystis aeruginosa*), Scale bars, a, d, f = 1 μm ; b, c, e = 500 nm

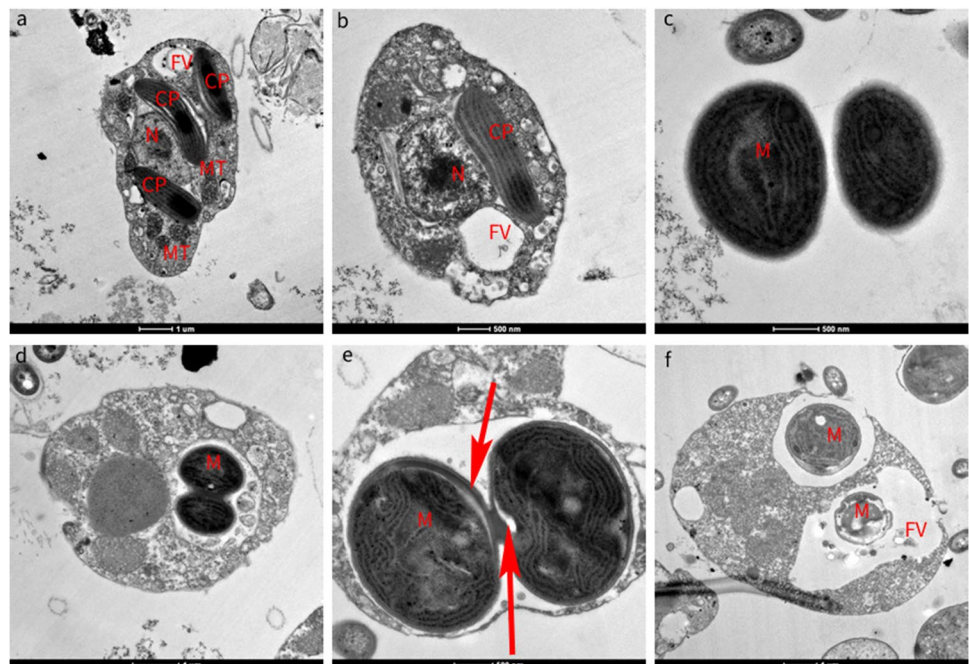
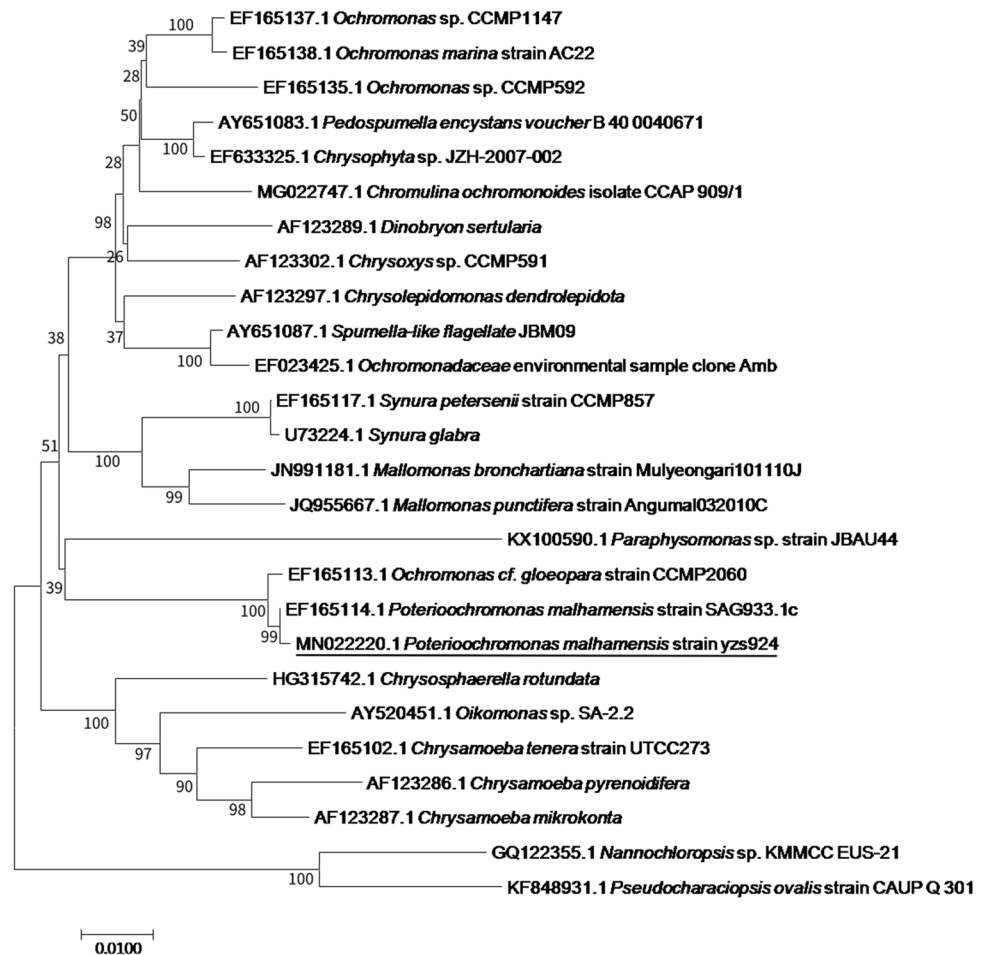


Fig. 4 The 18S rDNA sequence phylogenetic tree constructed using the neighbor-joining method indicates the evolutionary status of this flagellate (*Poterioochromonas malhamensis*), the number on the branch point indicates bootstrap support values, and the scale indicates the genetic distance



and grew in heterotrophic mode; in contrast, BG11 medium lacks organic nutrients, which caused part of the *P. malhamensis* to die. After 2 days, *P. malhamensis* gradually changed from organic heterotrophic mode to autotrophic mode, at which point the cell density began to increase again.

Effects of light, temperature, and pH on the growth and grazing of *P. malhamensis*

As shown in Fig. 6a, *P. malhamensis* grazed on *M. aeruginosa* under both light and dark conditions. Under dark conditions, the removal of *M. aeruginosa* was faster than that under light conditions in the first 2 days but did not continue after the second day. Under light conditions (2000 and 10,000 Lux), the removal of *M. aeruginosa* was slower in the first 2 days but continued after the second day. After 4 days, the biomass of *M. aeruginosa* had been reduced to a very low level, and *M. aeruginosa* cells could hardly be found under the microscope. Figure 6b shows that there

were no significant differences in the biomass of *P. malhamensis* under 2000 and 10,000 Lux illumination. However, the biomass of *P. malhamensis* under light conditions was significantly higher than that under dark conditions. In summary, different light intensities had no significant effect on the growth or grazing of *P. malhamensis*, but continuous dark conditions were not conducive to the growth and grazing of *P. malhamensis*.

As shown in Fig. 6c, the removal of *M. aeruginosa* at different temperatures was similar at 1 day and 4 days, but the removal of *M. aeruginosa* was the lowest at 20 °C at 2 days and 3 days. As shown in Fig. 7d, the biomass of *P. malhamensis* at 30 °C was higher than that at 20 °C and 25 °C. Therefore, 30 °C was the temperature that was the most beneficial to *P. malhamensis* and growth and grazing.

Figure 6e shows that when the pH was maintained at 9, the biomass of *M. aeruginosa* gradually increased and could not be reduced, indicating that *P. malhamensis* may not be able to graze or digest *M. aeruginosa* at pH 9. When the pH was controlled at 6, 7, or 8, *P. malhamensis* grazed

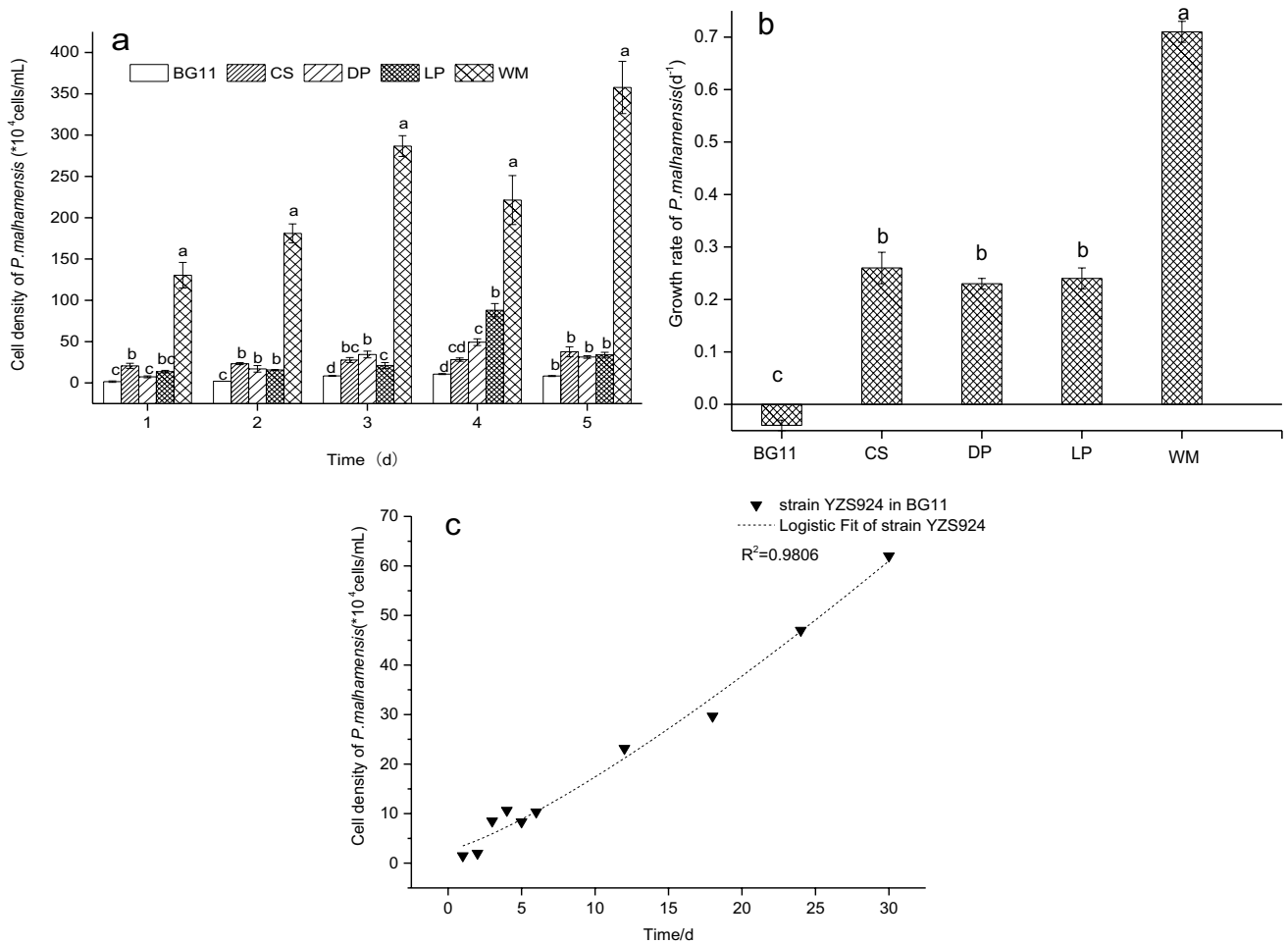


Fig. 5 Growth rate of *P. malhamensis* in different growth media. BG11 BG11 medium, CS culture solution in the logarithmic growth phase of *M. aeruginosa*, DP the decline phase of *M. aeruginosa*, LP the log phase of *M. aeruginosa*, WM wheat grain medium

M. aeruginosa, and the grazing speed decreased with increasing pH value. As shown in Fig. 6f, the density of *P. malhamensis* decreased with increasing pH on the 2nd day, but the differences in *P. malhamensis* density were not significant among pH values of 6, 7, and 8 after the 3rd day, possibly because of food restrictions. When the pH was maintained at 9, *P. malhamensis* was almost undetectable under the microscope.

As shown in Fig. 7, when the pH was controlled at 6, the daily measured pH value increased to approximately 7, and when the pH was controlled at 9, the measured pH was reduced to approximately 8, indicating that the pH tended to adjust to reach similar values (7 and 8). In summary, a low pH is more beneficial to the growth and grazing of *P. malhamensis*. When the pH is continuously controlled at 9, *P. malhamensis* cannot survive.

Effects of *M. aeruginosa* growth phase and density on *P. malhamensis* growth and grazing

As shown in Fig. 8, *M. aeruginosa* in the logarithmic growth phase could be grazed, and its biomass was reduced to an extremely low value, but *M. aeruginosa* in the decline growth phase was not easily grazed by *P. malhamensis*. The biomass of *M. aeruginosa* in the decline phase decreased in the first 3 days but no longer decreased after three days.

Though *M. aeruginosa* in the decline phase could not be completely grazed by *P. malhamensis*, the initial pH value affected the final density of *M. aeruginosa*. Compared with that in the initial pH 9 group, the *M. aeruginosa* biomass in the initial pH 7 group decreased faster and eventually remained at a lower level; this may have occurred because low pH is conducive to the growth and grazing of *P. malhamensis* (Fig. 6e). The results in Fig. 6f show that *P.*

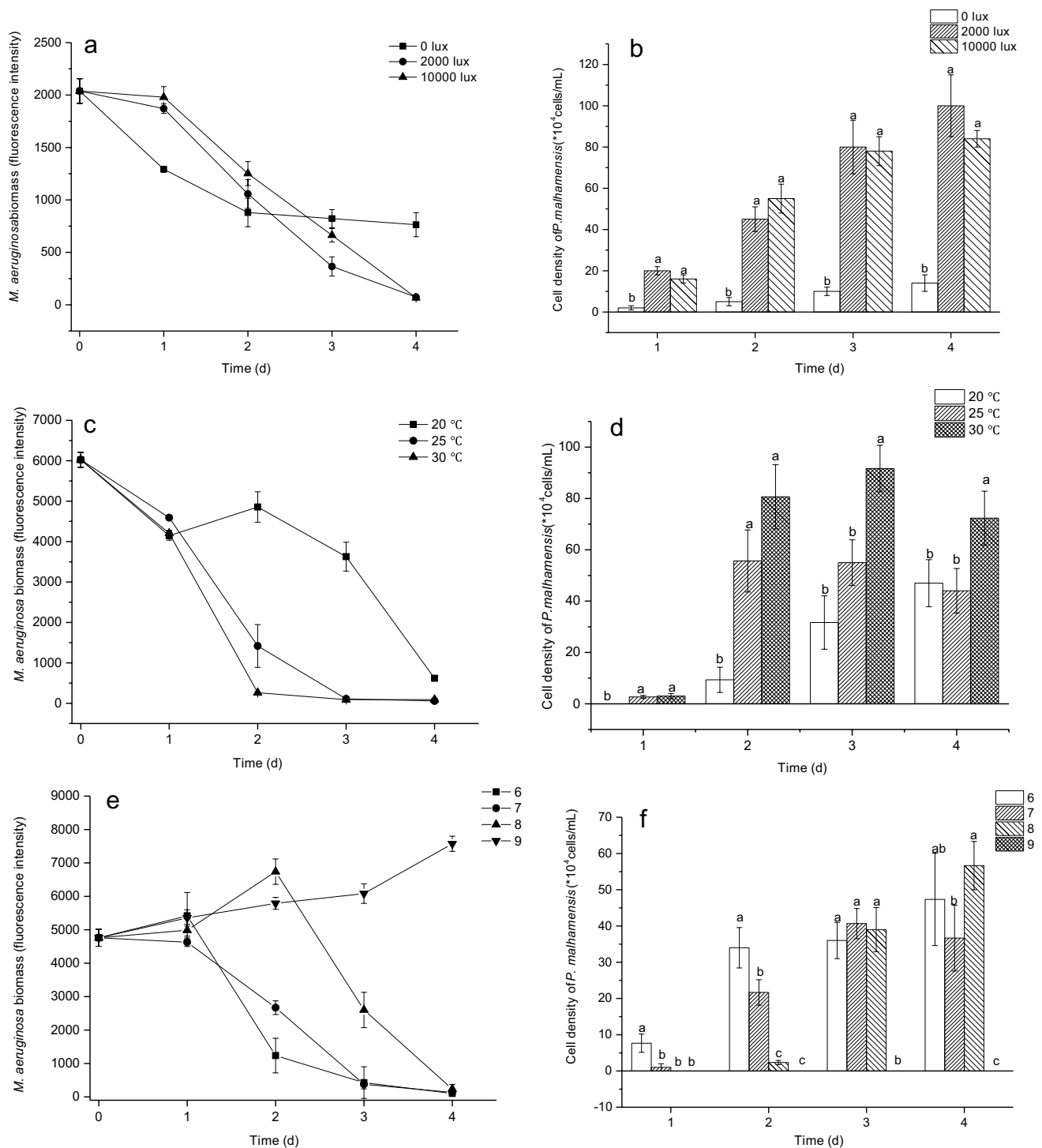


Fig. 6 Effect of light intensity (a, b), temperature (c, d), and pH (e, f) on the growth and grazing of *P. malhamensis*

malhamensis could not grow at pH 9, but the pH had to be continuously artificially maintained at 9. As Fig. 7 shows, the pH of the culture decreased to approximately 8 when the initial pH was 9. Therefore, adjusting the initial pH to 9 does not inhibit *P. malhamensis* feeding on *M. aeruginosa*.

As shown in Fig. 9a, when the initial density of *M. aeruginosa* was lower than 2.5×10^7 cells/mL, the density of *M. aeruginosa* gradually decreased. The *M. aeruginosa* density was reduced to extremely low values after 7 days in all experimental treatments except the S0 group.

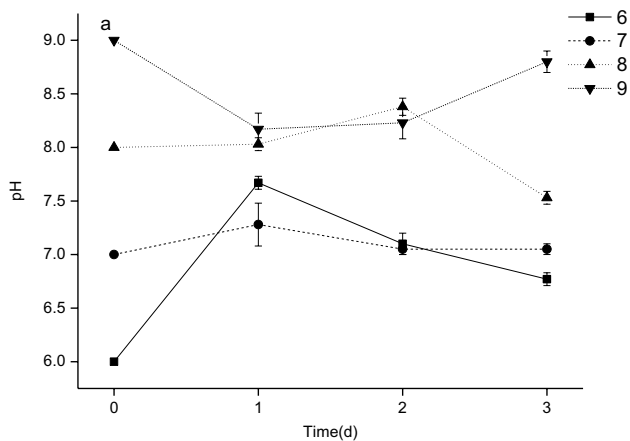


Fig. 7 Measured pH values in the different groups on each day

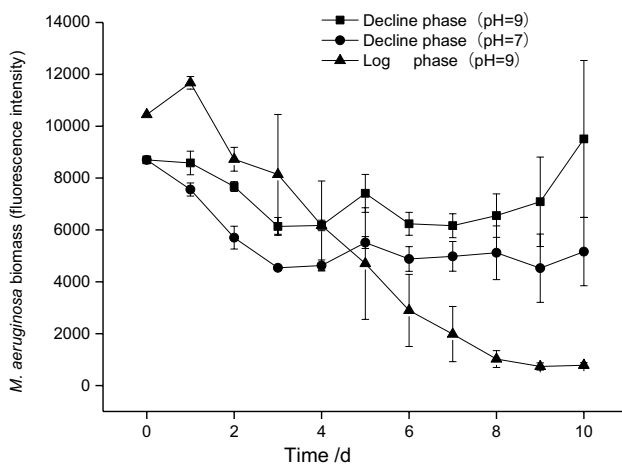


Fig. 8 Effect of *M. aeruginosa* growth phase on the grazing of *P. malhamensis*

The initial density of *M. aeruginosa* was 5×10^7 cells/mL; then, it was diluted by 50%. However, the density of *P. malhamensis* obviously did not decrease by 50% (Fig. 9b).

In the S0 group, the density of *P. malhamensis* first increased and then decreased after the 4th day. Initially, the high-density *M. aeruginosa* provided ample food for *P. malhamensis*, causing the flagellate population to grow rapidly. After 4 days, the density of the flagellate in this group gradually decreased. One possible reason for this decrease is that the pH (Fig. 6) or growth phase (Fig. 8) of *M. aeruginosa* changed, which caused grazing resistance in *P. malhamensis*. As a result, the density of *M. aeruginosa* during this period showed almost no reduction (Fig. 9a).

Comparing the density of *P. malhamensis* among the different groups revealed that the differences between S2, S3, S4, S5, and S6 were larger than the differences between S3, S4, S5, and S6. In the S6 group, the initial

density of *M. aeruginosa* was only 7.5×10^5 cells/mL, but the density of *P. malhamensis* still reached 2.05×10^6 cells/mL. This result shows that the density of *P. malhamensis* is less affected by *M. aeruginosa* densities within the ranges in the S3–S6 groups.

During the experimental period, the density of *P. malhamensis* in the S1 group increased gradually. In addition, the density of *P. malhamensis* in the S1 group was much higher than that in the S2–S6 groups because the S1 group had sufficient food, and *M. aeruginosa* exhibited no resistance to grazing by *P. malhamensis*.

Discussion

Caron et al. (1993) studied the effect of light on the uptake rate of *Dinobryon cylindricum*. The results showed that the uptake rate of algae at light intensities $> 150 \mu\text{Em}^{-2} \text{s}^{-1}$ was approximately $5\text{--}10$ bacteria $\text{alga}^{-1} \text{h}^{-1}$, while that of *D. cylindricum* cultured under $30 \mu\text{Em}^{-2} \text{s}^{-1}$ continuous light was consistently low (1.8 bacteria $\text{alga}^{-1} \text{h}^{-1}$). *D. cylindricum* cultured in the dark quickly stopped ingesting bacteria. In contrast, the results of this paper (Fig. 6a) show that in the first 2 days of the experiment, *P. malhamensis* ingested *M. aeruginosa* faster in the dark than in the light; that is, light had a negative effect on the feeding rate. Holen (1999) found that in 120 min after adding bacteria, light had a negative effect on the feeding rate of *Poteroiochromonas malhamensis* on bacteria. After incubation for 15 min under high-intensity light, 29% of flagellates ingested FLB (fluorescently labeled bacteria), while under low light, 39% of flagellates ingested FLB; 53% of flagellates ingested FLB in the dark.

P. malhamensis could not continue to graze on *M. aeruginosa* under dark conditions in the last two days of this experiment, while *P. malhamensis* did continue to graze on *M. aeruginosa* under light conditions. These results indicate that the feeding and digestion processes of *P. malhamensis* on *M. aeruginosa* may depend on light or on photosynthetic products. Zhang and Watanabe (2001) found that phagotrophic populations of *P. malhamensis* were incapable of growth in continuous darkness for longer than 5 days and were light-dependent for phagotrophy. Li et al. (2000) found that phagocytosis by the dinoflagellate *Gyrodinium galatheanum* (Braarud) Taylor might be light-dependent and that photosynthesis-derived metabolites were needed to support its growth. Strom (2001) found that relative to complete darkness, bright light ($900 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) resulted in a 40-fold increase in food vacuole loss rates (a proxy for digestion) in the heterotrophic dinoflagellate *Noctiluca scintillans* when fed phytoplankton prey. However, light had no effect on the vacuole loss rate when *N. scintillans* was fed heterotrophic (nonpigmented) prey. One possible mechanism

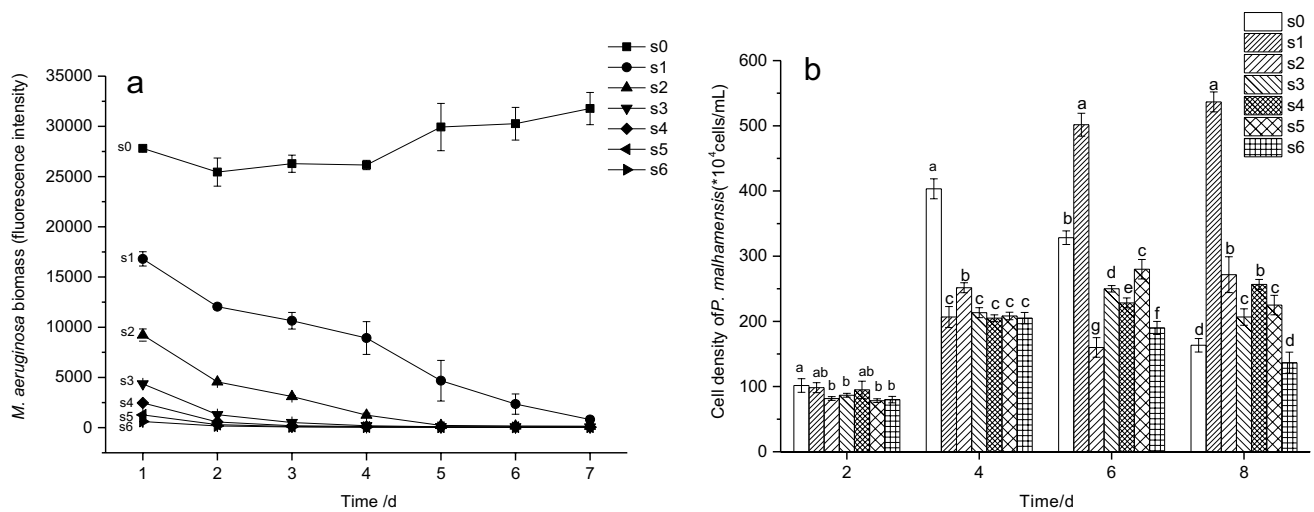


Fig. 9 Population dynamics of *M. aeruginosa* (a) and *P. malhamensis* (b) in different inoculation proportions (*M. aeruginosa*: *P. malhamensis*) with the same concentration of *P. malhamensis* (1×10^4 cells/mL). The *M. aeruginosa* densities were as follows: **S0**: 5×10^7 cells/

mL; **S1**: 2.5×10^7 cells/mL; **S2**: 1.25×10^7 cells/mL; **S3**: 6.25×10^6 cells/mL; **S4**: 3×10^6 cells/mL; **S5**: 1.5×10^6 cells/mL; **S6**: 7.5×10^5 cells/mL

to explain this difference is the photo oxidative breakdown of ingested organic matter in these nearly transparent grazers. Klein et al. (1986) measured algal pigments during grazing experiments with the protozoan *Oxyrrhis marina* Dujardin on *Rhodomonas* sp. and found that the rates of chlorophyll and carotene degradation were the highest in the light.

Can predatory flagellates live without light? Holen (1999) found that *Poterioochromonas malhamensis* could grow in DYIV + glucose medium without light, and the growth rate was similar to that under light, indicating that light was not necessary for osmotic nutrition. However, when predatory flagellates survive through prey consumption, light may also be necessary. Andersson et al. (1989) studied *Ochromonas* sp. that were grown on *E. coli* and transferred to an inorganic salt medium. *Ochromonas* sp. survived without additional bacteria as food in the light but died quickly under dark conditions, and only 1% of the cells remained intact at the end of the experiment. In our research (Fig. 6a), the density of *P. malhamensis* under dark conditions was lower than that under light conditions after 4 days of culture, but *P. malhamensis* did not die in large numbers, which may have been due to the secretion of dissolved organic carbon by *M. aeruginosa*. In the first 2 days of the experiment, the phagocytosis of *M. aeruginosa* by *P. malhamensis* and the growth of *P. malhamensis* may have depended on the dissolved organic carbon secreted by *M. aeruginosa*. In the continuously dark environment, *M. aeruginosa* could not continue to secrete dissolved organic carbon, so *P. malhamensis* could not remove the remaining *M. aeruginosa* cells and could not continue to divide and proliferate.

Mixotrophic Chrysophyta can often adapt to extreme environments and exhibit a wide range of adaptations to pH due to their variety of nutrient acquisition methods. Some flagellates of the genus *Mallomonas* can even reach high abundances in environments with pH values of 4 (Roijackers 1988). Moser and Weisse (2011) found that there are flagellates in acidic lakes that are the main algae in acidic lakes. This study shows that *P. malhamensis* can live at pH values between 6 and 9, which is a suitable pH environment for the growth of most aquatic organisms. Guo (2008) found that the phagocytic rate of *Poterioochromonas* sp. decreased with increasing pH within a pH range of 6–10 and that there was a strong negative correlation between the phagocytic rate and pH. The growth performance of *Poterioochromonas* sp. was the best in the pH range of 7–8, and its algal phagocytosis rate was also high within this range. Touloupakis et al. (2016) found that flagellates could not grow at a pH of 11. Moreover, *P. malhamensis* was very sensitive to pH shock, resulting in a rapid loss of motility followed by cell lysis within a few hours when the pH was suddenly increased to 11. The tolerance range of *P. malhamensis* is usually between 6 and 10, but an alkaline environment is not conducive to its growth. This may be because the digestive enzymes in *Ochromonas* cells, such as amylase, show higher activity under weakly acidic conditions (Tarayre et al. 2014). Many plants, especially floating plants and ecological floating beds, can reduce the pH value in water (Hu et al. 2010). Higher temperature and pH are beneficial to cyanobacteria (Yang et al. 2018), and the reproduction of cyanobacteria promotes an increase in the water pH, which inhibits the

growth of *P. malhamensis*. Therefore, in the competition between predatory flagellates and *M. aeruginosa*, the presence of plants benefits *P. malhamensis* by adjusting the environmental pH.

P. malhamensis has three nutrition modes: photo autotrophy, phagocytosis, and osmosis. Of these, osmosis results in the fastest growth. In the wheat grain medium, the growth rate of *P. malhamensis* on the first day was $2.56 \pm 0.12 \text{ day}^{-1}$, and the generation time was approximately 5–6 h. Röderer (1986) reported that *Poterioochromonas malhamensis* grows rapidly under heterotrophic conditions (Bacto-tryptone, yeast extract, and d-glucose, each 0.1% (wt/vol) in double-distilled water); the generation time was approximately 5–6 h. Zhang et al. (2016) reported that the growth rate of *Ochromonas* sp. reached $2.66 \pm 0.36/\text{day}$ when 150 mg/L glucose was added to the BG11 medium. These studies suggest that a growth rate of 2.6 day^{-1} may be the maximum growth rate of *Ochromonas* and *Poterioochromonas*. However, when bacteria or algae were used as food for phagocytosis, the growth rate could not reach this value (Zhang and Watanabe 2001; Zhang et al. 2009; Ma et al. 2018a, b). This may be because phagocytosis consumes energy not only to take in prey and form food vacuoles but also to digest prey. Osmotic nutrients can be absorbed and utilized directly, which is more efficient. At the same time, flagellates can achieve higher cell density when acquiring nutrients through osmosis. In this study, the density of *P. malhamensis* reached more than 10^7 cells/mL in the wheat grain medium, but *P. malhamensis* could not reach this density when *M. aeruginosa* was provided as prey, even when the density of *M. aeruginosa* reached 5×10^7 cells/mL (Fig. 9a). When *Chlorella* was used as prey, even if the density of *Chlorella* reached 4×10^8 cells/ml, the maximum density of *P. malhamensis* was only approximately 4×10^6 cells/ml, regardless of whether the experiment was performed in a 250-mL flask or at the larger scale of greenhouse (Ma et al. 2018a, b). Guo (2008) found that when *Poterioochromonas* sp. DO2004 was fed on *M. aeruginosa*, the cell density of the flagellate could not reach 10^7 cells/ml. Zhang et al. (2016) reported that the growth rate and maximum cell density of the flagellate *Ochromonas* sp. could be increased by adding glucose alone to an inorganic nutrient medium.

In this paper, it was found that when the initial density of *M. aeruginosa* was 5×10^7 cells/mL (*M. aeruginosa*: *P. malhamensis* = 2500:1), *P. malhamensis* could not remove all of the *M. aeruginosa*, and *M. aeruginosa* maintained a stable density. There are three possible explanations for this process. First, the high density of *M. aeruginosa* leads to a higher pH value in the environment, which inhibits the grazing of *P. malhamensis* on *M. aeruginosa*. Second, *M. aeruginosa* may change under these conditions to resist *P. malhamensis* grazing, and many studies have reported the phenomenon of resistance in bacteria and algae to

grazing by flagellates (Jousset 2012; Ma et al. 2019). It has been reported in the literature that *M. aeruginosa* can form colonies under grazing pressure (Wang et al. 2010) but that *Poterioochromonas malhamensis* can still feed on smaller *M. aeruginosa* colonies (Kim and Han 2007). However, we did not observe that *M. aeruginosa* formed colonies during these experiments, indicating that *M. aeruginosa* may resist grazing by other means. Third explanation may be that *M. aeruginosa* produces a certain substance that inhibits feeding by flagellates. *Microcystis aeruginosa* can produce allelochemicals and inhibit phytoplankton such as cyanobacteria (*Microcystis wesenbergii*), green algae (*Scenedesmus quadricauda*, *Chlorella pyrenoidosa*), and diatom (*Cyclotella meneghiniana*) (Yang et al. 2014; Wang et al. 2017a, b). Studies have shown that micro-plastics and green algae can inhibit flagellate feeding (Zhang et al. 2018; Kong et al. 2021). *Chlorella* could still be grazed even if its density reached 4×10^8 cells/ml (Ma et al. 2018a, b), which is an unfortunate result for the high-density cultivation of *Chlorella*. Exploring the reasons why high-density *M. aeruginosa* resists grazing may be beneficial for controlling the influence of flagellates when cultivating *Chlorella* at high density.

In summary, environmental factors and the density and growth of prey affect the growth and grazing of *P. malhamensis*.

Acknowledgements Thanks to Dr. Ling Lin for his help in species identification.

Author contribution All authors contributed to the study conception and design. Tingting Zhang provided support for experimental design, manuscript revision, and funding. Material preparation, data collection, and analysis were performed by Hao Yan, Qin Li, Bo Chen, and Mei Shi. The first draft of the manuscript was written by Hao Yan, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding The research was funded by the National Natural Science Foundation of China through Grant 31170443.

Availability of data and materials Not applicable.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication The manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

Competing interests The authors declare no competing interests.

References

- Andersson A, Falk S, Samuelsson G et al (1989) Nutritional characteristics of a mixotrophic nanoflagellate, *Ochromonas* sp. *Microb Ecol* 17(3):251–262. <https://doi.org/10.1007/BF02012838>
- Baek SH, Hong S-S, Song S-Y et al (2009) Grazing effects on toxic and non-toxic microcystis aeruginosa by the mixotrophic flagellate *Ochromonas* sp. *J Freshw Ecol* 24(3):367–373. <https://doi.org/10.1080/02705060.2009.9664308>
- Bastien C, Cardin R, Veilleux E et al (2011) Performance evaluation of phycocyanin probes for the monitoring of cyanobacteria. *J Environ Monit* 13(1):110–118. <https://doi.org/10.1039/c0em00366b>
- Boenigk J, Arndt H (2002) Bacterivory by heterotrophic flagellates: community structure and feeding strategies. *Antonie Van Leeuwenhoek* 81(1–4):465–480. <https://doi.org/10.1023/a:1020509305868>
- Caron DA, Sanders RW, Lim EL et al (1993) Light-dependent phagotrophy in the fresh-water mixotrophic chrysophyte *Dinobryon-Cylindricum*. *Microb Ecol* 25(1):93–111. <https://doi.org/10.1007/BF00182132>
- Daft MJ, Mccord SB, Stewart WDP (2010) Ecological studies on algal-lysing bacteria in freshwater. *Freshw Biol* 5(6):577–596. <https://doi.org/10.1111/j.1365-2427.1975.tb00157.x>
- Gong Y, Patterson DJ, Li Y et al (2015) *Vernalophrys algivore* gen. nov., sp. nov. (Rhizaria: Cercozoa: Vampyrellida), a new algal predator isolated from outdoor mass culture of *Scenedesmus dimorphus*. *Appl Environ Microbiol* 81(12):3900–3913. <https://doi.org/10.1128/AEM.00160-15>
- Guo X, Liu X, Pan J et al (2015) Synergistic algicidal effect and mechanism of two diketopiperazines produced by *Chryseobacterium* sp strain GLY-1106 on the harmful bloom-forming *Microcystis aeruginosa*. *Sci Rep* 5. <https://doi.org/10.1038/srep14720>
- Guo S (2008) The Physiological Characteristics of *Poterioochromonas* sp. DO-2004 and its potential role in Cyanobacteria bloom control Ph.D, Chinese Academy of Science
- Holen DA (1999) Effects of prey abundance and light intensity on the mixotrophic chrysophyte *Poterioochromonas malhamensis* from a mesotrophic lake. *Freshw Biol* 42(3):445–455. <https://doi.org/10.1046/j.1365-2427.1999.00476.x>
- Hu M-H, Yuan J-H, Yang X-E et al (2010) Effects of temperature on purification of eutrophic water by floating eco-island system. *Acta Ecol Sin* 30(6):310–318. <https://doi.org/10.1016/j.chnaes.2010.06.009>
- Isaksson A, Bergstrom AK, Blomqvist P et al (1999) Bacterial grazing by phagotrophic phytoflagellates in a deep humic lake in northern Sweden. *J Plankton Res* 21(2):247–268. <https://doi.org/10.1093/plankt/21.2.247>
- Jousset A (2012) Ecological and evolutive implications of bacterial defences against predators. *Environ Microbiol* 14(8):1830–1843. <https://doi.org/10.1111/j.1462-2920.2011.02627.x>
- Kim BR, Han MS (2007) Growth and grazing of the mixotrophic flagellate *Poterioochromonas malhamensis* on the cyanobacterium *Microcystis aeruginosa*. *Korean J Nat Conserv* 5(3):183–194. <https://doi.org/10.30960/kjnc.2007.5.3.183>
- Klein B, Gieskes WWC, Krray GG (1986) Digestion of chlorophylls and carotenoids by the marine protozoan *Oxyrrhis marina* studied by h.p.l.c. analysis of algal pigments. *J Plankton Res* 8(5):827–836. <https://doi.org/10.1093/plankt/8.5.827>
- Kojima S, Miyashita Y, Hagiwara T et al (2016) Possible control strategy of cyanobacterial blooms by bacteria from reed (*Phragmites australis*) communities in lakes of Ohnuma Quasi-National Park, Hokkaido. *Bull Fish Sci Hokkaido Univ* 66:19–28
- Kong Q, Li Y, Xu X et al (2021) Microplastics interfere with mixotrophic *Ochromonas* eliminating toxic *Microcystis*. *Chemosphere* 265:129030. <https://doi.org/10.1016/j.chemosphere.2020.129030>
- Li A, Stoecker DK, Coats DW (2000) Mixotrophy in *gyrodinium galatheanum* (DINOPHYCEAE): grazing responses to light intensity and inorganic nutrients*. *J Phycol* 36(1):33–45. <https://doi.org/10.1046/j.1529-8817.2000.98076.x>
- Lin S, Geng M, Liu X et al (2016) On the control of *Microcystis aeruginosa* and *Synechococcus* species using an algicidal bacterium, *Stenotrophomonas* F6, and its algicidal compounds cyclo-(Gly-Pro) and hydroquinone. *J Appl Phycol* 28(1):345–355. <https://doi.org/10.1007/s10811-015-0549-x>
- Ma M, Gong Y, Hu Q (2018a) Identification and feeding characteristics of the mixotrophic flagellate *Poterioochromonas malhamensis*, a microalgal predator isolated from outdoor massive *Chlorella* culture. *Algal Res* 29:142–153. <https://doi.org/10.1016/j.algal.2017.11.024>
- Ma MY, Gong YC, Hu Q (2018b) Identification and feeding characteristics of the mixotrophic flagellate *Poterioochromonas malhamensis*, a microalgal predator isolated from outdoor massive *Chlorella* culture. *Algal Res Biomass Biofuels Bioprod* 29:142–153. <https://doi.org/10.1016/j.algal.2017.11.024>
- Ma M, Wei C, Wang H et al (2019) Isolation and evaluation of a novel strain of *Chlorella sorokiniana* that resists grazing by the predator *Poterioochromonas malhamensis*. *Algal Res* 38:101429. <https://doi.org/10.1016/j.algal.2019.101429>
- Moser M, Weisse T (2011) Combined stress effect of pH and temperature narrows the niche width of flagellates in acid mining lakes. *J Plankton Res* 33(7):1023–1032. <https://doi.org/10.1093/plankt/fbr014>
- Princiotta SD, Hendricks SP, White DS (2019) Production of cyanotoxins by *Microcystis aeruginosa* mediates interactions with the mixotrophic flagellate *Cryptomonas*. *Toxins (Basel)* 20(4). <https://doi.org/10.3390/toxins11040223>
- Qian Y-P, Li X-T, Tian R-N (2019) Effects of aqueous extracts from the rhizome of *Pontederia cordata* on the growth and interspecific competition of two algal species. *Ecotoxicol Environ Saf* 168:401–407. <https://doi.org/10.1016/j.ecoenv.2018.10.086>
- Qian Y, Xu N, Liu J et al (2018) Inhibitory effects of *Pontederia cordata* on the growth of *Microcystis aeruginosa*. *Water Sci Technol*:99–107. <https://doi.org/10.2166/wst.2018.090>
- Röderer G (1986) *Poterioochromonas malhamensis*—a unicellular alga as test system in ecotoxicology, toxicology, and pharmacology. *Environ Toxicol* 1(1):123–138. <https://doi.org/10.1002/tox.2540010110>
- Roijackers RMM (1988) External morphology as taxonomic characteristic in planktonic scale-bearing Chrysophyceae and scaled Heliozoa. *Hydrobiol Bull* 22(1):69–73. <https://doi.org/10.1007/BF02256785>
- Sakami T, Sakamoto S, Takagi S et al (2017) Distribution of three algicidal *Alteromonas* sp. strains in seagrass beds and surrounding areas in the Seto Inland Sea, Japan. *Fish Sci* 83(1):113–121. <https://doi.org/10.1007/s12562-016-1048-y>
- Shilo M (1970) Lysis of blue-green algae by myxobacter. *J Bacteriol* 104(1):453. <https://doi.org/10.1128/JB.104.1.453-461.1970>
- Strom SL (2001) Light-aided digestion, grazing and growth in herbivorous protists. *Aquat Microb Ecol* 23(3):253–261. <https://doi.org/10.3354/ame023253>
- Tarayre C, Bauwens J, Brasseur C et al (2014) Isolation of an amylolytic chrysophyte, *Poterioochromonas* sp. from the digestive tract of the termite *R. santonensis*. *Biotechnol Agron Soc Environ* 18(1). <https://doi.org/10.1007/BF02524171>
- Touloupakis E, Cicchi B, Silva Benavides AM et al (2016) Effect of high pH on growth of *Synechocystis* sp PCC 6803 cultures and their contamination by golden algae (*Poterioochromonas* sp.). *Appl Microbiol Biotechnol* 100(3):1333–1341. <https://doi.org/10.1007/s00253-015-7024-0>
- Van Wichelen J, Vanormelingen P, Codd GA et al (2016) The common bloom-forming cyanobacterium *Microcystis* is prone to a wide

- array of microbial antagonists. *Harmful Algae* 55:97–111. <https://doi.org/10.1016/j.hal.2016.02.009>
- Wang B, Tie-Zhu MI, Song-Hui L et al (2006) Cloning and sequences analysis of 18S rRNA gene of five prorocentrum species/strains. *Oceanol Limnol Sinica* 37(5):450–456. <https://doi.org/10.1007/s11676-006-0017-1>
- Wang W, Liu Y, Yang Z (2010) Combined effects of nitrogen content in media and *Ochromonas* sp grazing on colony formation of cultured *Microcystis aeruginosa*. *J Limnol* 69(2):193–198. <https://doi.org/10.4081/jlimnol.2010.193>
- Wang H, Liu F, Luo P et al (2017a) Allelopathic effects of *Myriophyllum aquaticum* on two cyanobacteria of *Anabaena flos-aquae* and *Microcystis aeruginosa*. *Bull Environ Contam Toxicol* 98(4):556–561. <https://doi.org/10.1007/s00128-017-2034-5>
- Wang L, Zi J, Xu R et al (2017b) Allelopathic effects of *Microcystis aeruginosa* on green algae and a diatom: Evidence from exudates addition and co-culturing. *Harmful Algae* 61:56–62. <https://doi.org/10.1016/j.hal.2016.11.010>
- Wilken S, Wiezer S, Huisman J et al (2010) Microcystins do not provide anti-herbivore defence against mixotrophic flagellates. *Aquat Microb Ecol* 59:207–216. <https://doi.org/10.3354/ame01395>
- Xiang W, Hao W, Jinyun Y et al (2015) Study on the release routes of allelochemicals from *Pistia stratiotes* Linn., and its anti-cyanobacteria mechanisms on *Microcystis aeruginosa*. *Environ Sci Pollut Res* 22(23):18994–19001. <https://doi.org/10.1007/s11356-015-5104-4>
- Yang J, Deng X, Xian Q et al (2014) Allelopathic effect of *Microcystis aeruginosa* on *Microcystis wesenbergii*: microcystin-LR as a potential allelochemical. *Hydrobiologia* 727(1):65–73. <https://doi.org/10.1007/s10750-013-1787-z>
- Yang J, Tang H, Zhang X et al (2018) High temperature and pH favor *Microcystis aeruginosa* to outcompete *Scenedesmus obliquus*. *Environ Sci Pollut Res* 25(5):4794–4802. <https://doi.org/10.1007/s11356-017-0887-0>
- Zhang XM, Watanabe MM (2001) Grazing and growth of the mixotrophic chryomonad *Poterioochromonas malhamensis* (Chrysochyceae) feeding on algae. *J Phycol* 37(5):738–743. <https://doi.org/10.1046/j.1529-8817.2001.00127.x>
- Zhang X, Hu H-Y, Men Y-J et al (2009) Feeding characteristics of a golden alga (*Poterioochromonas* sp.) grazing on toxic cyanobacterium *Microcystis aeruginosa*. *Water Res* 43(12):2953–2960. <https://doi.org/10.1016/j.watres.2009.04.003>
- Zhang L, Li B, Wu Z et al (2016) Changes in growth and photosynthesis of mixotrophic *Ochromonas* sp in response to different concentrations of glucose. *J Appl Phycol* 28(5):2671–2678. <https://doi.org/10.1007/s10811-016-0832-5>
- Zhang L, Gu L, Hou X et al (2018) Chlorophytes prolong mixotrophic *Ochromonas* eliminating *Microcystis*: Temperature-dependent effect. *Sci Total Environ* 639:705–713. <https://doi.org/10.1016/j.scitotenv.2018.05.196>
- Zhang L, Wang Z, Wang N et al (2020) Mixotrophic *Ochromonas* addition improves the harmful microcystis-dominated phytoplankton community in in situ microcosms. *Environ Sci Technol*. <https://doi.org/10.1021/acs.est.9b06438>
- Zhang XM, Watanabe MM (2001) Grazing and growth of the mixotrophic chryomonad *Poterioochromonas malhamensis* (Chrysochyceae) feeding on algae. *J Phycol* 37(5):738–743. <https://doi.org/10.1046/j.1529-8817.2001.00127.x>
- Zuo S, Wan K, Ma S (2015) Combined effect of predatory zooplankton and allelopathic aquatic macrophytes on algal suppression. *Environ Technol* 36(1):54–59. <https://doi.org/10.1080/09593330.2014.936520>
- Zuo S, Fang Z, Zhou S et al (2016) Benthic fauna promote algicidal effect of allelopathic macrophytes on *Microcystis aeruginosa*. *J Plant Growth Regul* 35(3):646–654. <https://doi.org/10.1007/s00344-015-9566-x>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.