Journal of Hazardous Materials 385 (2020) 121625

Contents lists available at ScienceDirect

Journal of Hazardous Materials

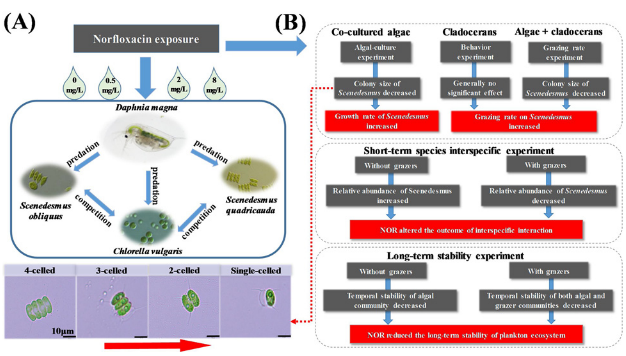
journal homepage: www.elsevier.com/locate/jhazmat

Norfloxacin pollution alters species composition and stability of plankton communities

Ying Pan[a,b,c], Jinyan Dong[a,b], Lingling Wan[a,b], Shucun Sun[c], Hugh J. MacIsaac[d], Ken G. Drouillard[d], Xuexiu Chang[a,d,*]

[a] School of Ecology and Environmental Science, Yunnan University, Kunming, 650091, Yunnan, China
[b] Yunnan Key Laboratory for Plateau Mountain Ecology and Restoration of Degraded Environments, Yunnan University, Kunming, 650091, Yunnan, China
[c] Department of Ecology, School of Life Sciences, Nanjing University, Nanjing, China
[d] Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON, N9 B 3P4, Canada

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: R. Debora

Keywords:
Antibiotic
Plankton ecosystem
Inducible defense
Interspecific interaction
Community composition
Temporal stability

ABSTRACT

Despite recent advances in assessing lethal effects of antibiotics on freshwater organisms, little is known about their potential consequences on community composition and function, which are essential for assessing the ecological risk of these pollutants. Here, we investigated the impact of norfloxacin (NOR) on the short-term (≤ 6 days) dynamics of co-cultured *Scenedesmusquadricauda-Chlorella vulgaris* and *Scenedesmusobliquus-C. vulgaris*, and the long-term (≤ 70 days) dynamics of co-cultured *S.obliquus-C. vulgaris* in experiments with or without grazer *Daphnia magna* at sublethal antibiotic concentrations (0, 0.5, 2 and 8 mg L^{-1}). NOR increased the relative abundance of *Scenedesmus* species in the absence of grazers but exerted opposite effects when *Daphnia* was present in both short- and long-term experiments due to reduced colony size. Meanwhile, increasing NOR concentrations led to quickly increased total algal density in the initial stage, followed by a sharp decline in the long-term experiment in the absence of grazers; when *Daphnia* was present, population fluctuations were even larger for both prey and predator species (e.g., grazer extinction at the highest concentration). Thus, NOR affected the outcome of species interactions and decreased temporal stability of plankton ecosystems, suggesting that antibiotics have more extensive impacts than presently recognized.

* Corresponding author.
E-mail address: changxx@ynu.edu.cn (X. Chang).

https://doi.org/10.1016/j.jhazmat.2019.121625
Received 12 July 2019; Received in revised form 18 October 2019; Accepted 5 November 2019
Available online 06 November 2019
0304-3894/

1. Introduction

Since their widespread advent in the 1940s, antibiotics have been used worldwide to treat bacterial, fungal, and parasitic infections in humans, and to promote growth in livestock and poultry industries, particularly in developing countries including China (Kümmerer, 2009; Zhao et al., 2010). Annual antibiotic usage reached 162,000 tonnes in China in 2013, which was nine times more than in the USA and 150 times more than in the UK (Zhang et al., 2015; Tang et al., 2016). Owing to their low absorbability and incomplete metabolism, a large proportion of these compounds passes through the bodies of those ingesting them and is released into the environment (Alcock et al., 1999; Chen et al., 2017). For example, Zhang et al. (2015) studied the consumption and emission of 36 frequently detected antibiotics in China and found that about 58 % of the total used entered adjacent soil and water, resulting in an accumulation of residual antibiotics in these zones. Antibiotic pollution has thus become a serious environmental problem in China (Qiao et al., 2018).

Considering the ecological consequences of antibiotics, an overwhelming majority of research has focused on the potential impacts of bacterial resistance, and thus public health (Marti et al., 2014; Rodriguez-Mozaz et al., 2014; Grenni et al., 2018). Relatively little is known about antibiotic effects on natural communities (Costanzo et al., 2005; Pan et al., 2017a). Freshwater aquatic communities are particularly vulnerable to antibiotic pollution since their residuals are directed to aquatic environments via rainfall wash-off and water infiltration processes (Zhang et al., 2015). Although antibiotic residuals have no direct effect on growth and survival of large animals (e.g., fishes and invertebrates (Robinson et al., 2005; Park and Choi, 2008), they could exert direct detrimental influences on smaller organisms in freshwater ecosystems. For example, acute toxicity effects of antibiotics were observed in phytoplankton species including *Chlorella vulgaris* (Eguch et al., 2004), *Pseudokirchneriella subcapitata* (Yang et al., 2008), *Scenedesmus obliquus* (Nie et al., 2009), and *Microcystis aeruginosa* (Robinson et al., 2005; Guo and Chen, 2012), and in the zooplankton species *Artemia salina* (Macrì et al., 1988), *Acartia tonsa* (Lanzky and Halling-Sørensen, 1997), and *Daphnia magna* (Wollenberger et al., 2000; Zalewski et al., 2011). Additionally, antibiotics at sub-lethal concentrations have impacts on the fitness of these organisms, affecting both physiological and morphological characteristics of phytoplankton species (Zhu et al., 2014; Teixeira and Granek, 2017) and behavior of zooplankton (Pan et al., 2017a).

To date, most studies have been carried out at the individual level using relatively high toxicant concentrations, while community-level consequences (e.g., composition and function) at environmentally relevant concentrations remain poorly studied. Understanding these community consequence is particularly important to assess the total threat of antibiotics, given that detectable concentrations of antibiotic residuals in the environment are usually far below known lethal doses but may result in species loss and ecosystem disfunction (Park and Choi, 2008; Pan et al., 2017a; Grenni et al., 2018). Specifically, pollutants at relatively low concentrations may induce species-specific fitness changes owing to differences in species' tolerance thresholds. If interspecific differences in response are large, species interactions including competition, predation, and facilitation may be affected (Selck et al., 2002; Peng et al., 2017), and community composition will subsequently be altered in consequence. Additionally, altered species interactions may induce large population fluctuations and further disrupt temporal persistence and stability of the ecosystems, as evidenced by studies addressing plankton systems (Davis et al., 2010; Pan et al., 2014). However, there is no data to support these conjectures because it is difficult to establish a relationship between degree of antibiotic pollution and alterations of species interactions, let alone assess its consequences at the community level.

This study attempts for the first time to experimentally examine the influence of antibiotics on the composition and function of plankton communities. Norfloxacin (NOR), a synthetic antibacterial agent belonging to the fluoroquinolone family, is one of the most widely used antibiotics globally and is frequently detected in surface and ground waters (Nie et al., 2009; Zhang et al., 2012; Zhang et al., 2015). Our previous studies determined that NOR decreased the formation of colonies (four- and eight-celled colonies) in *Scenedesmusquadricauda* (Pan et al., 2017a, b). Colony formation is an effective defense by *Scenedesmus* species against zooplankton grazers, thus the shift to fewer colonies makes them more vulnerable to grazing by zooplankton, such as the cladoceran *Daphnia magna*. However, this phenotypic plasticity comes at a cost, whereby colonies exhibit lower resource (e.g., nutrients and light) absorption ability due to a decrease in the surface area to volume ratio. Thus, in the absence of zooplankton grazers, colonial bodies of *Scenedesmus* could have reduced competitive outcomes for environmental resources relative to other algal species (Zhu et al., 2015). In contrast, *Chlorella vulgaris* exists only as single cells and does not exhibit phenotypic plasticity in forming colonies (Pan et al., 2017a). Therefore, we hypothesize that when co-cultured with *C. vulgaris*, NOR will increase the abundance of *Scenedesmus* species relative to that of *C. vulgaris* when no grazers are present. Alternatively, in the presence of a grazer, we hypothesize that NOR will increase the relative abundance of *C. vulgaris* owing to a reduction in colony formation ability of *Scenedesmus* species. Meanwhile, given that reducing *Scenedesmus* anti-grazer ability will lead to rapid exploitation by grazer species (Taghavi et al., 2013; Davis et al., 2010), the temporal stability of the above apparent competition system (i.e., the system involves indirect competition between two prey species that share a common predator; (Holt, 1997)) may be affected by NOR as well.

To test these hypotheses, we conducted a series of microcosm experiments to examine the potential effects of NOR on: 1) short-term interactions between *Scenedesmus* species (*S.quadricauda* or *S.obliquus*) with the unicellular green alga *C. vulgaris*, and on 2) the long-term temporal stability of co-cultures *S.obliquus* and *C. vulgaris* in the absence and presence of *D. magna*.

2. Method

2.1. Experimental organisms and culture conditions

We used three green algae, *S.quadricauda*, *S. obliquus*, and *C. vulgaris*, as well as *D. magna* as test species. These species have been widely used in toxicological research and are known for their sensitivity to antibiotics (Nie et al., 2009; Zalewski et al., 2011; Pan et al., 2017a). The two *Scenedesmus* species are reported to coexist with *C. vulgaris* in many freshwater ecosystems worldwide, and all three algal species can be efficiently consumed by co-occurring *D. magna* (Gutseit et al., 2007; Roy et al., 2016; Pan et al., 2018).

Algal species *S. quadricauda* (FACHB-507), *S.obliquus* (FACHB-416), and *C. vulgaris* (FACHB-32) were obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences. The cell size of each algal species is listed in the supporting information (Table S1). Axenic samples of each alga were batch-cultured in 500-mL Erlenmeyer flasks with 200 mL liquid COMBO medium, a widely used culture medium for algae and zooplankton (Kilham et al., 1998). Cultures were placed in oscillation incubators (TS-2102GZ, Shanghai Anjing laboratory equipment Co. Ltd., Shanghai, China) at 25 °C under a light: dark cycle of 14 h: 10 h with a light intensity of 120 μmol photons m^{-2} s^{-1}. Cultures in the incubators were mechanically stirred at 80 rpm for 5 min every hour to facilitate gas exchange and keep algae in suspension. All culture experiments were conducted under the same conditions. Only algae in the exponential growth phase were used for the experiments.

The grazer *D. magna* was a laboratory clone maintained on the above three algal species alternately every other day at a rate of 10^5 cells mL^{-1} day^{-1} for about one month. Prior to experiments, neonates (about one day old) of *D. magna* were transferred to clean COMBO medium and starved for 12 h to clean their guts and to increase their motivation to forage.

2.2. Experimental design

Four NOR concentrations (0, 0.5, 2, and 8 mg L^{-1}) were used across all experiments. Previous studies have found that the lethal dose (96 h-LC_{50}) of NOR on *D. magna* was 107.6 mg L^{-1} (Pan et al., 2017b), while the effective dose (96 h-EC_{50}) on the growth of *Scenedesmus* (*S.obliquus*) and *Chlorella* (*C. pyrenoidosa*) species was 38.5 and 30.8 mg L^{-1}, respectively (Nie et al., 2007, 2009), which are all much higher than the concentrations used in the present study. Meanwhile, the concentrations used conform with those in natural aquatic environments ranging from micrograms to a few milligrams per liter (Robinson et al., 2005; Nie et al., 2009; Zhang et al., 2012; Zhang et al., 2015; Liu et al., 2018), and are markedly lower than the exposure concentrations of 15–60 (Nie et al., 2009), 25–100 (Pan et al., 2017b), and 20–140 mg L^{-1} (González-Pleiter et al., 2013) used in recent studies investigating the ecological consequences of NOR.

The test mainly included two parts: First, we conducted a "short-term species interaction experiment" to examine variation in relative abundance of two *Scenedesmus* species (*S.quadricauda* or *S.obliquus*) with *C. vulgaris*, respectively, using four NOR concentrations with a constant density of grazers or without grazers. This experiment contained a total of 16 treatments (2 co-culture systems × 4 NOR concentrations in the absence and presence of grazers). Additionally, we set up 12 treatments that included each single-algal culture at each NOR concentration, such that the species-specific growth-sensitivity of these algae to NOR could be estimated. All short-term trials had six replicates per treatment and the trial lasted for 6 days.

Meanwhile, we conducted additional separate experiments in the short-term trials to examine NOR's effects on the grazing rate (i.e., the grazing rate on each of the three algae species that contained three algal systems and four NOR concentrations in the absence and presence of grazers) and on individual behavior (i.e., the behavior experiment that contained four NOR concentrations in the absence of algal cells) of *D. magna*, respectively. Each treatment had six replicates and the trials lasted for 8 h. Results of these two experiments could be used to deduce the impacts of grazers on the relative abundance of co-cultured algal species.

Secondly, we conducted a "long-term stability experiment" to investigate the effects of NOR on the temporal stability of co-cultured *S.obliquus* and *C. vulgaris* in the absence and presence of grazers. This experiment included eight treatments (i.e., contained four NOR concentrations in the absence and presence of grazers), with five and six replicates in treatments without and with grazers, respectively, that lasted for 70 days.

All laboratory experiments were conducted at Yunnan University, Kunming, China, starting on October 20, 2016. NOR was purchased from Dalian Meilun Biology Technology Company, Ltd. (Liaoning, China). All reagents used in the present work were of analytical-reagent grade. Ultrapure water (resistivity = 18.25 MΩ · cm at 25 °C) from Water Purifier (Shanghai Liding Co. Ltd., Shanghai, China) was used throughout the experiments. Prior to the experiment, algal cells were transferred into 500 mL beakers containing 200 mL of COMBO medium and the tested NOR. Beakers were gently aerated with sterile air (filtered through a sterilized Sartorius filter) to homogenize dissolved oxygen (DO), followed by introduction of *D. magna* individuals for treatments containing grazers.

Initial grazer density was 50 individuals L^{-1} in all treatments containing grazers, while the initial density of each algal species in both separate and co-culture treatments was 2×10^5 cells mL^{-1} regardless of the presence or absence of grazers, similar to that in many other studies that explored grazer activity and phytoplankton-zooplankton interactions (Olmstead and LeBlanc, 2003; Pan et al., 2014). Beakers used in the short-term species interaction, grazing rate, and long-term stability experiments were capped with breathable polyethylene and then transferred to the incubators mentioned above, while those used in the behavioral experiment were placed on an observation platform.

2.2.1. Short-term species interaction experiment

Throughout this experiment, the number of grazers was fixed in each beaker to preclude effects caused by variable grazer density fluctuations on interactions between phytoplankton species. Microcosms were sampled on days 2, 4, and 6 at 8:00 AM. For each sampling, 2 mL of the solution contained within each beaker was transferred to a 10 mL tube containing 0.1 mL Lugol's preservative to examine each algal species using a microscope at 400× magnification (Olympus BX43 with a DP27 imaging system, Olympus, Tokyo, Japan). For both *Scenedesmus* species, the number of cells in single-, two-, three-, or four-celled morphs were recorded (colonies of more than four cells were rarely detected in the present study), and total algal density as well as the average proportion of cells in each morph were determined from these counts. For *C. vulgaris*, no colony was observed in any beakers; therefore, only cell number was counted. Relative growth rate (RGR) was calculated as $100 \times (\ln(L_2) - \ln(L_1)) / (t_2 - t_1)$, where L_1 is the initial algal density, L_2 the algal density at harvest time t_2, and $(t_2 - t_1)$ the experimental time. Algal density ratio (ADR) was used to indicate the abundance of *Scenedesmus* species relative to *C. vulgaris*, which was defined as the cell density ratio of these species. This variable to some extent reflects the competitive interaction between co-cultured species, regardless of the presence or absence of grazers (Pan et al., 2018). Throughout the study, algae were sampled, counted and calculated following these methods.

No added grazers were found dead during this experiment. The number of grazers was fixed by removing neonates immediately after birth. However, the number of neonates was recorded to calculate grazer birth rate under different NOR concentrations. Birth rate (*No/Ni*, %) was estimated as the ratio of the number of offspring (*No*) to the total number of individuals added to the chamber at the beginning of the experiment (*Ni*). All grazers were picked using an inverted 5 mL serological pipette to examine body size and clutch size (embryos per female) using the same light-microscope at 40× magnification. Body size was determined as the length from the tip of the head to that of the abdomen. After measurement, grazers were placed back into their respective beakers.

2.2.2. Grazing rate experiment

This experiment was designed to examine the vulnerability of each algae species to grazing pressure by *D. magna* and the species-specific responses to NOR exposure in order to reveal the mechanisms by which the algae and grazers interact. Zooplankton grazing rate (*GR*, mL animal $individual^{-1}$ h^{-1}) was determined after 2, 4, and 8 h exposure to NOR following the procedure described in the supporting information (see Text S1).

2.2.3. Behavior experiment

Grazer behaviors (hopping frequency and heart-beat rate) were examined after 2, 4, and 8 h exposure to NOR without adding algal foods, so that the separate effects of NOR on grazer individuals could be estimated. Detailed information about the measurement of these two variables can be found in the supporting information (see Text S2).

2.2.4. Long-term stability experiment

Two plankton systems were used in this experiment, including co-cultured *S.obliquus* and *C. vulgaris* in the absence and presence of *D. magna*. Algae were sampled on days 2, 4, 6, 8, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 45, 50, 55, 60, 65, and 70 at 8:00 AM. During sampling, three beakers were randomly chosen from each treatment to measure the concentration of DO using a Hach HQ40d oxygen probe (Hach, Loveland, Co, USA) as soon as the beakers were taken out of the incubator. Throughout the experiment, DO concentration was generally

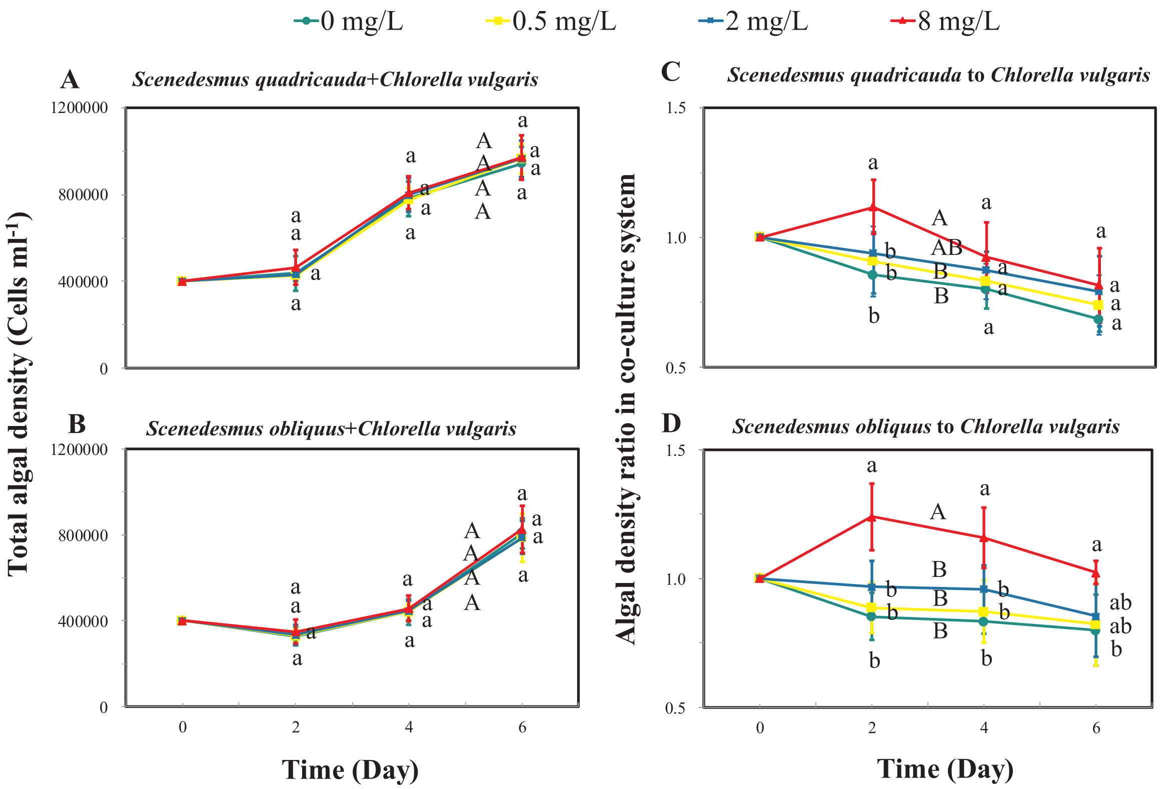


Fig. 1. Variation (means ± SD, $n = 6$) in total algal density of *Scenedesmusquadricauda* + *Chlorella vulgaris* (A) and *Scenedesmusobliquus* + *C. vulgaris* (B), and density ratio of *S.quadricauda* to *C. vulgaris* (C) and *S.obliquus* to *C. vulgaris* (D) after exposure to norfloxacin in corresponding co-culture experiment without adding grazers. Different lowercase letters indicate significant differences among treatments on each observation day through one-way ANOVA at the 0.05 significance level. Different uppercase letters indicate significant differences among treatments through one-way RM-ANOVA at the 0.05 significance level.

unaff ;ected by NOR regardless of experimental time or the absence or presence of grazers, and was always higher than 6.0 mg L^{-1} (see Table S2). Five percent of the volume (10 mL) was removed from each beaker, of which 2 mL was transferred into a 10 mL tube containing Lugol's preservative for the measurement of phytoplankton species. Finally, 10 mL of fresh medium (containing a corresponding amount of NOR) was added to the beaker to replenish nutrients and prevent metabolic waste build-up.

Throughout the experiment, NOR was added (with a similar dose as in the preceding treatment) in each beaker every 12 days to maintain continuous, long-term control of NOR concentration according to Struyf et al. (2007). An additional experiment with the same sampling procedure as mentioned above was carried out to evaluate NOR degradation rate in two of our experimental systems (i.e., the control system without plankton organisms, and the mixed system with co-cultured *S.obliquus*, *C. vulgaris*, and *D. magna*) by using three NOR concentrations (0.5, 2, and 8 mg L^{-1}). Results showed that NOR degradation rates were less than 22.0.% and 23.5.% after 6 days of exposure, and less than 44.5.% and 51.5.% after 12 days of exposure in the control and mixed systems, respectively (see Table S3), indicating that the NOR gradient among different treatments should be effective throughout the observation period of the long-term experiment. As predicted, by the end of the long-term experiment, NOR concentration in the treatment with 0.5, 2, and 8 mg L^{-1} of NOR and in the presence of grazers was 0.8, 3.6, and 12.4 mg L^{-1}, respectively.

The temporal stability of the algal or grazer populations was calculated as the inverse coefficient of the variation in community biomass, i.e., the ratio of mean biomass (here referring to the mean density of algal or grazer species in each beaker across time) to its standard deviation (Haddad et al., 2011).

2.3. Data analysis

For each observation time, a one-way analysis of variance (ANOVA) followed by the Tukey test was performed to determine the effects of NOR on algae species (density, RGR, and proportion of cells in each morph), grazer individuals (hopping frequency, heart-beat rate, grazing rate, birth rate, and clutch size), and species interactions (ADR) in short-term experiments, as well as on population dynamics (density, ADR, and temporal stability) in long-term experiments.

When considering the whole experiment, we conducted one- and two-way repeated measures analysis of variance (RM-ANOVA) followed by a Bonferroni post-test to test the eff ;ect of NOR concentration and/or plankton ecosystem on algae species, grazer species, and species interactions in short-term experiments, as well as algal and grazer populations in long-term trials. Sphericity was evaluated with Mauchly's test, and, if violated, the Greenhouse-Geisser correction was applied to recalculate the *F*-value.

We used linear regression analyses to determine the relationships between proportion of four-celled colonies of *Scenedesmus* species and ADR in both short-term and long-term experiments, and between four-celled colonies of *S.obliquus* and grazer density in long-term experiments.

All data were tested for normality and homogeneity of variance prior to analyses. Data were $\log_{10}$-transformed to reach normality when needed. All analyses were carried out in SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

3. Result

3.1. Short-term interactions

3.1.1. Algal responses in single culture experiment

In single-culture systems, the algal density ($P > 0.05$; Figure S1A-C), RGR ($P > 0.05$; Figure S1D-F) and colony size (Figure S2A-F) were generally unaffected by NOR. The exception was a gradual increase in the proportion of single cells of *Scenedesmus* species with increasing NOR concentrations ($P < 0.05$; Table S4), which exhibited significant differences between treatments of 0 and 8 mg L^{-1} NOR for *S.quadricauda* on days 4 ($F = 3.3$, $P = 0.042$; Figure S2B) and 6 ($F = 5.7$, $P = 0.005$; Figure S2C). Moreover, all these parameters exhibited significant differences among the species ($P < 0.001$; Table S4). For example, algal density and RGR were higher in *C. vulgaris* than those in either *Scenedesmus* species.

3.1.2. Species interactions in the absence of grazers

In mixed culture systems without grazers, ADR and proportion of single-, two- and four-celled colonies were significantly affected by NOR ($P < 0.05$; Table S4), while total algal density ($P > 0.05$; Fig. 1A and B) and proportion of three-celled colonies ($P > 0.05$; Fig. 2B, C, E, and F) were generally not ($P > 0.05$; Table S4). First, after an early adaptive phase (from 0 to 2 days), algal density of both co-cultured species increased with time in treatments with 0 mg L^{-1} of NOR; however, the growth rate was higher in *C. vulgaris* than in *Scenedesmus* species (Figure S3), resulting in consistently decreased ADR (Fig. 1C and D). The presence of NOR significantly promoted the growth of *Scenedesmus* species compared with *C. vulgaris* ($F > 4.8$, $P < 0.05$; Figure S3). As a result, ADR consistently increased with increasing NOR concentrations at each observation time, reaching significance for the whole experiment for both co-cultured systems ($P < 0.05$; Fig. 1C and D).

Regarding the colony size of *Scenedesmus* species, the proportion of single cells increased while the proportion of four-celled colonies decreased with increasing NOR concentrations (Table S4). In particular, the single cells had significant differences between treatments of 0 and 8 mg L^{-1} of NOR regardless of time and system ($P < 0.05$; Fig. 2A–F). Meanwhile, we observed significant negative relationships between the proportion of four-celled colonies and ADR for the whole experiment, regardless of experimental system ($P < 0.001$; Figure S4).

3.1.3. Species interactions in the presence of grazers

Apart from the proportion of three-celled colonies, all other characteristics of phytoplankton species were markedly affected by NOR in mixed culture systems in the presence of grazers ($P < 0.001$; Table S4). Specifically, algal density of two co-cultured algal species generally increased with increasing NOR concentrations for the whole experiment ($P < 0.05$; Figure S5). However, this effect was more prominent for *C. vulgaris* than for *Scenedesmus* species. As a result, total algal density increased, while ADR decreased with increasing NOR concentrations at each observation time and for the whole experiment ($P < 0.05$; Fig. 3A–D). At the end of the experiment, total algal density increased by 85.7.% ($F = 15.9$, $P < 0.001$; Fig. 3A) and 149.7.% ($F = 11.9$, $P < 0.001$; Fig. 3B), while ADR decreased by 46.5.% ($F = 14.6$, $P < 0.001$; Fig. 3C) and 51.6.% ($F = 12.6$, $P < 0.001$; Fig. 3D) from 0 to 8 mg L^{-1} of NOR for the *S.quadricauda* - *C. vulgaris* and *S.obliquus* - *C. vulgaris* systems, respectively. Moreover, the presence of grazers caused significantly decreased total algal density in both experimental systems compared to co-cultured ecosystems without grazers ($P < 0.001$; Table S5).

The proportion of four-celled colonies consistently decreased with increasing NOR concentration, regardless of time and system ($P < 0.05$; Fig. 4A–F). At the end of the experiment, the proportion of four-celled colonies decreased by 12.0.% ($F = 4.4$, $P < 0.05$; Fig. 4C) and 24.7.% ($F = 6.2$, $P < 0.01$; Fig. 4F) from 0 to 8 mg L^{-1} of NOR for *S.quadricauda* and *S.obliquus*, respectively. However, the presence of grazers increased the proportion of four-celled colonies ($P < 0.001$; Table S5). Significantly positive relationships were observed between the proportion of four-celled colonies and ADR regardless of time and system ($P < 0.01$; Figure S6A-H).

Both the birth rate and clutch size of *D. magna* were significantly affected by NOR concentration ($P < 0.001$), while the effects of NOR on body size were dependent on time, with a significant time × NOR interaction ($P < 0.001$; Table S4). Specifically, body size initially decreased on day 2 ($P < 0.05$), then stabilized on day 4 ($P > 0.05$) and increased on day 6 ($P < 0.05$) with increasing NOR concentrations, regardless of plankton system (Figure S7A and B). *D. magna* started to produce offspring (neonates or eggs) after 6 days of culture and exhibited significant differences among NOR treatments, i.e., birth rate increased ($P < 0.01$; Figure S7C), while clutch size decreased ($P < 0.05$; Figure S7D) with increasing NOR concentrations, regardless of plankton system.

3.2. Grazing rate and behavior of D. magna

The grazing rate of *D. magna* varied significantly among algal food species ($P < 0.001$), and the effects of NOR were dependent on species as well, with significant species × NOR interactions ($P < 0.001$; Table S6). Considering the whole experiment, grazing efficiency of *D. magna* was higher on *C. vulgaris* than on either *Scenedesmus* species ($P < 0.001$). Moreover, the grazing rate of *D. magna* on *C. vulgaris* decreased ($P < 0.05$; Figure S8A), while it increased on both *Scenedesmus* species with increasing NOR concentrations over the whole experiment ($P < 0.05$; Figure S8B and C).

At each exposure time and in the absence of grazers, the proportion of four-celled colonies was unaffected by NOR in both plankton ecosystems ($P > 0.05$; Figure S9A and C). However, in the presence of grazers, the proportion of four-celled colonies consistently decreased with increasing NOR concentrations, particularly for the whole experiment ($P < 0.05$; Figure S9B and D).

Both hopping frequency and heart-beat rate of *D. magna* exhibited decreasing trends with increasing NOR concentrations (Figure S10), but only heart-beat rate was significantly different by the end of the experiment ($F = 3.5$, $P = 0.034$; Figure S10B).

3.3. Long-term stability experiment

3.3.1. System dynamics in the absence of grazers

Except for total density, algal characteristics were affected significantly by NOR in the absence of grazers ($P < 0.01$; Table S7). Considering the whole experiment, NOR exerted contrasting effects on population density between the two algal species, as algal density decreased for *C. vulgaris* but increased for *S.obliquus* ($P < 0.01$; Figure S11), and thereby ADR increased with increasing NOR concentrations ($P < 0.001$; Fig. 5B). As in the short-term experiments, the proportion of four-celled colonies of *S.obliquus* decreased with increasing NOR concentrations ($P < 0.001$; Fig. 5A; Table S7).

More importantly, the stability of the experimental system was affected by NOR. Generally, under higher concentrations of NOR, total algal density increased more quickly to a maximum after the beginning of the experiment (using 8 days under 0.5, 2, and 8 mg L^{-1}, while taking 10 days under 0 mg L^{-1} of NOR) than those at low concentrations, and peak values were generally higher, followed by a sharper decline during the next few days (10–19th day). These cycles were repeated two more times throughout the experiment (Fig. 5C). As a result, the temporal stability of *C. vulgaris* and of the whole algal community decreased significantly with increasing NOR concentrations, and that of *S.obliquus* was lowest under the highest NOR concentration ($P < 0.05$; Fig. 6).

3.3.2. System dynamics in the presence of grazers

In the presence of grazers, algal population characteristics were

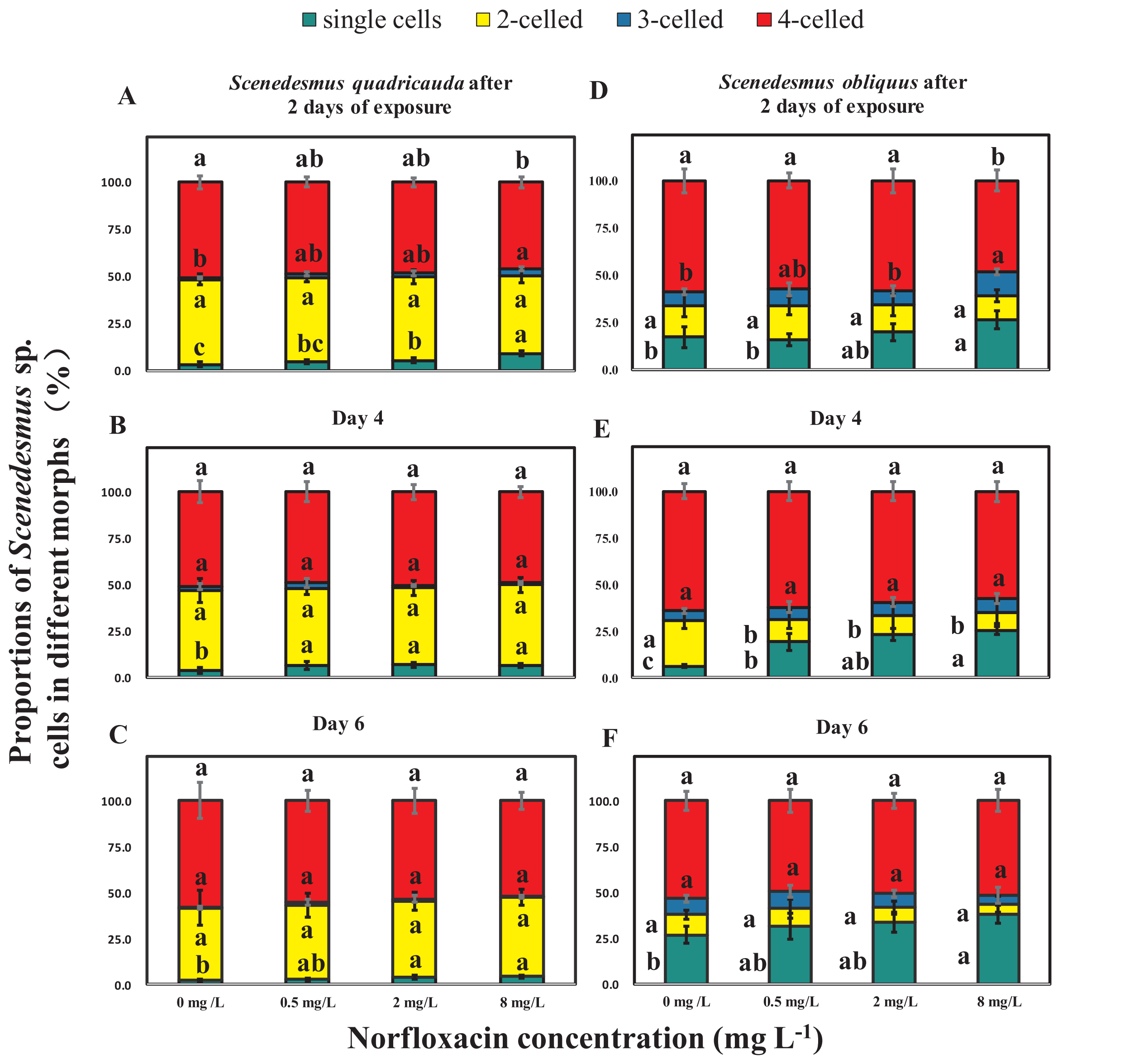


Fig. 2. Proportions ($n = 6$) of single cell, two-, three-, and four-celled colonies of *Scenedesmusquadricauda* (A, B, C) and *Scenedesmusobliquus* (D, E, F) after 2 (A, D), 4 (B, E), and 6 (C, F) days of exposure to norfloxacin in corresponding short-term mixed culture experiment without adding grazers. Different letters indicate significant differences among treatments. Multiple comparisons of means were performed using Tukey test at the 0.05 significance level.

significantly affected by NOR exposure ($P < 0.05$; Table S7). However, species dynamics were completely different from those in the absence of grazers. Considering the whole experiment, density of *C. vulgaris* increased ($P < 0.05$; Figure S12B), while that of *S.obliquus* decreased ($P < 0.001$; Figure S12A), resulting in decreased ADR with increasing NOR concentrations ($P < 0.001$; Fig. 7B). Furthermore, the proportion of four-celled colonies decreased with increasing NOR concentrations for the whole experiment ($P < 0.001$; Fig. 7A).

The stability of the apparent competition system also decreased with increasing NOR concentrations. Total algal density decreased significantly with increasing NOR concentrations throughout the experiment ($P < 0.001$; Fig. 7C). *Daphnia* density at higher NOR concentrations quickly increased to a maximum (on day 13 under 8 mg L^{-1} of NOR, versus on day 16 for the other three treatments), and peak values were generally higher (Fig. 7D) than those with lower NOR concentrations. Subsequently, grazer density declined more rapidly and remained at a relatively lower level throughout the experiment when compared with treatments with lower NOR concentrations. As a result, the temporal stabilities of *C. vulgaris*, *S.obliquus*, the whole algal community, and the grazer population all decreased with increasing NOR concentrations ($P < 0.05$; Fig. 8). Additionally, *Daphnia* almost disappeared at the highest NOR concentration (disappeared entirely in three chambers) at the end of the experiment, suggesting that the persistence of the apparent competition system was reduced by the presence of NOR.

We observed significant negative relationships between the proportion of four-celled colonies and ADR in the absence of grazers ($P < 0.001$; Figure S13A). By contrast, four-celled colonies were significantly and positively related to ADR ($P < 0.001$; Figure S13B), and to grazer density ($P < 0.05$; Figure S14C), in the presence of grazers.

4. Discussion

Understanding ecological effects of environmental pollutants requires knowledge of individual, population and community responses; among them, a community response is direct evidence that a natural ecosystem is disturbed by the pollutant (Peeters et al., 2010). The results of this study demonstrate that sub-lethal concentrations of NOR

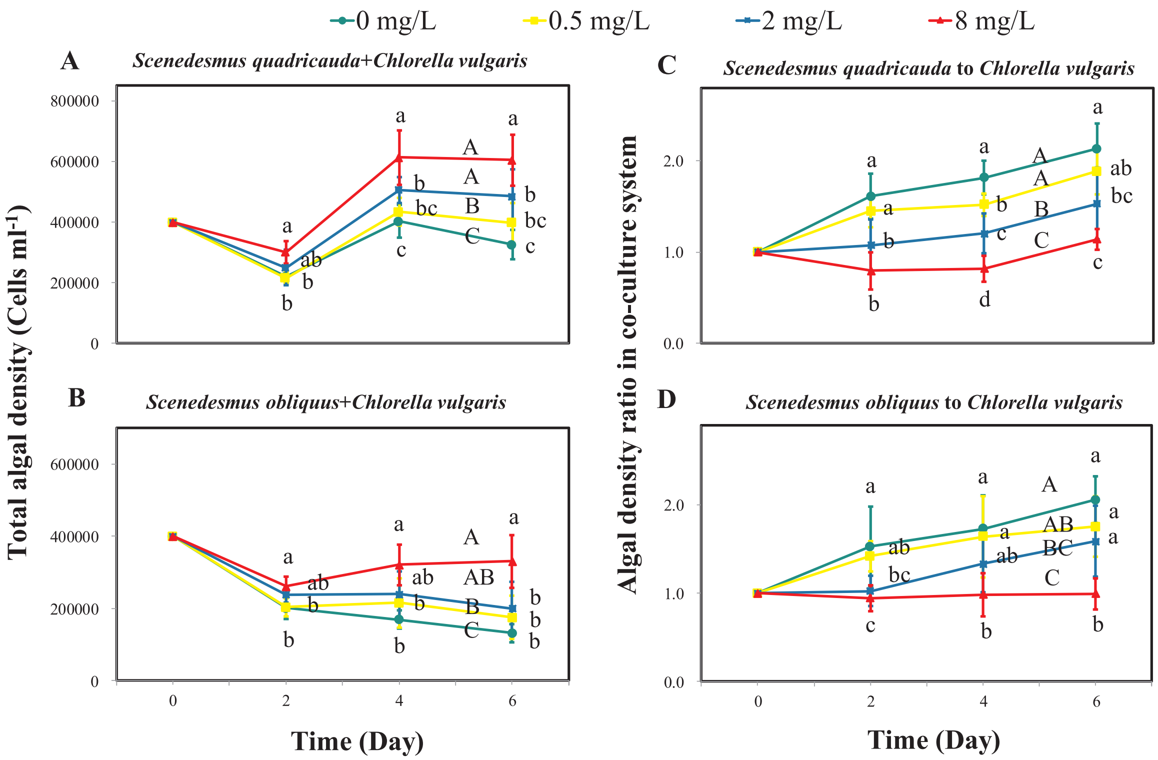


Fig. 3. Variation (means ± SD, *n* = 6) in total algal density of *Scenedesmusquadricauda* + *Chlorella vulgaris* (A) and *Scenedesmusobliquus* + *C. vulgaris* (B), and density ratio of *S.quadricauda* to *C. vulgaris* (C) and *S.obliquus* to *C. vulgaris* (D) after exposure to norfloxacin in corresponding co-culture experiment adding grazers. Different lowercase letters indicate significant differences among treatments on each observation day through one-way ANOVA at the 0.05 significance level. Different uppercase letters indicate significant differences among treatments through one-way RM-ANOVA at the 0.05 significance level.

residuals influenced the dynamics of interspecific interactions between co-cultured phytoplankton species and decreased the temporal stability of the tested ecosystems, regardless of grazer presence or absence. To our knowledge, this is the first study to offer quantitative insight into how antibiotics influence plankton communities.

4.1. Interspecific interactions in the absence of NOR

In this study, growth rates of three algal species were higher in the single-culture experiment (Figure S1) than those in the co-cultured system without grazers (Figure S3), indicating that competition would occur between co-cultured species since they require almost the same resources (e.g., spaces, nutrients, and light). In co-cultured systems without grazers, density of all algal species increased with time, but this increase was more prominent for *C. vulgaris* than for *Scenedesmus* species in treatments with 0 mg L^{-1} of NOR (Figure S3A-D), resulting in increased relative abundance of *C. vulgaris* (Fig. 1C and D). In this study, differences in DO (Table S2), temperature, light conditions (light intensity and period), and NOR degradation (Table S3) had been excluded. Therefore, the dominance of *C. vulgaris* in these treatments resulted both from its higher growth rate (see Figure S1) and greater competitive ability (see also Tilman (1977), Lürling et al. (2013)).

4.2. Effect of NOR on species interactions

NOR altered the balance of populations in co-cultured systems regardless of the presence (i.e., apparent competition system) or absence (i.e., algal competition system) of *D. magna* grazers. Due to the relatively low NOR concentrations used in this study, NOR did not significantly influence the growth of any of the three species in single cultures (Figure S1), consistent with previous studies (Nie et al., 2007, 2009). Thus, the action mechanism of NOR regarding interspecific interactions in mixed cultures may be associated with variation in species fitness, i.e., colony size.

Under both natural and experimental conditions, large colonies can be an effective defense of phytoplankton species against grazers owing to a size mismatch and inhibited algal ingestion, and members of the family Scenedesmaceae are the best studied phytoplankton group that can form large colonies (van Donk et al., 2011; Zhu et al., 2014, 2015). Our grazing rate experiments demonstrated that the presence of grazers promoted the formation of large (four-celled) colonies (Table S6; Figure S9) in *Scenedesmus* species. However, elevated NOR concentrations inhibited their ability to form large colonies, which may be related to variation in polysaccharide content which, in turn, affects cell aggregation (Pan et al., 2017a; Duan et al., 2018). Consequently, more algal cells were consumed by grazers with increasing NOR concentrations despite grazers' feeding behavior being inhibited (Figure S8 and S10), suggesting that colony formation is vital for *Scenedesmus* species to defend against grazers.

By affecting grazing efficiency, colony size further affected the relative abundance of this species over others. Predictably, phytoplankton species with the ability to form large colonies are more likely to achieve dominance in systems containing grazers (Li et al., 2013; Duan et al., 2018). In agreement, the formation of large colonies (Table S5; Fig. 4) increased the dominance of *Scenedesmus* species in the apparent competition systems without adding NOR (Figure 3CD) due to the higher grazing rate of *D. magna* on unicellular *C. vulgaris* than on *Scenedesmus* species (Figure S8). For the same reason, the decreases in *Scenedesmus* dominance with increasing NOR concentrations in the apparent competition systems could be attributed to the disaggregation of large colonies. These deductions are based on the significant positive relationships between the proportion of four-celled *Scenedesmus* colonies and ADR in apparent competition systems, regardless of experimental system and time (Figure S6).

By contrast, *Scenedesmus* dominance in the algal competition systems consistently increased with increasing NOR concentrations,

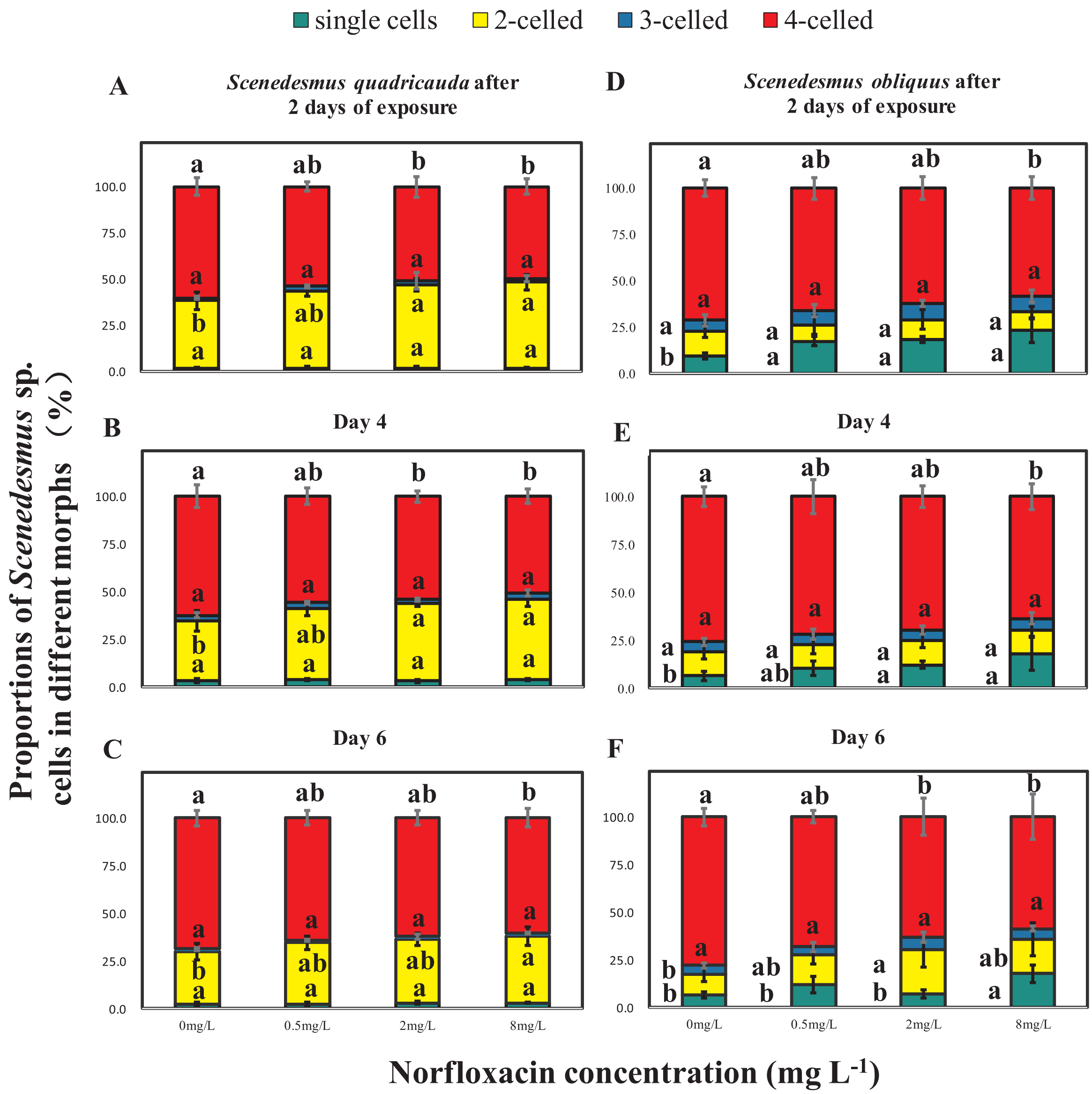


Fig. 4. Proportions (n = 6) of single cell, two-, three-, and four-celled colonies of *Scenedesmusquadricauda* (A, B, C) and *Scenedesmusobliquus* (D, E, F) after 2 (A, D), 4 (B, E) and 6 (C, F) days of exposure to norfloxacin in corresponding co-culture experiment adding grazers. Different letters indicate significant differences among treatments. Multiple comparisons of means were performed using Tukey's test at the 0.05 significance level.

regardless of experimental system (Fig. 1C and D). Colony size gradually decreased (Fig. 2), and significant negative relationships were observed between the proportion of four-celled *Scenedesmus* colonies and ADR in these systems (Fig. S4). Zhu et al. (2015) also found that the formation of large colonies exerted negative effects on *Scenedesmus* dominance in the absence of grazers because being in a large colony decreases a cell's ability to acquire nutrients, owing to the reduced surface area to volume ratio. Considering this competitive disadvantage, it is increasingly recognized that *Scenedesmus* species only activate the resistance mechanism when predation risk is sensed (Zhu et al., 2014; Pan et al., 2017a), particularly from *D. magna* (van Donk, 2007; Zhu et al., 2014; Zhu et al. (2015)). Therefore, a tradeoff exists between forming large colonies to reduce grazing risk and reducing colony size to maintain high growth. In our algal competition systems, colony size gradually declined as NOR increased because the cost of large colonies exceeded the benefit of improving nutrient absorption ability to obtain a growth advantage.

In conclusion, in the present study, NOR affected the outcome of interspecific interactions (i.e., relative abundance and thus competitive ability to a certain extent) between phytoplankton species by affecting colony size, regardless of the presence or absence of grazers.

4.3. Effects of NOR on system stability

In long-term experiments, the relative dominance of *S. obliquus* over *C. vulgaris* consistently increased in algal competition systems (Fig. 5B), but decreased in apparent competition systems with increasing NOR (Fig. 7B) due to the disaggregation of large colonies (see Figure S13 and S14). These results demonstrate the importance of NOR in determining interspecific interactions of plankton ecosystems, regardless of whether grazer density remained constant.

More importantly, system stability was greatly affected by NOR, i.e., significantly decreased when exposure to NOR, regardless the absence of presence of grazers (Figs. 6 and 8). In algal competition systems, increasing NOR concentrations led to quickly increased total algal density in the initial stage due to the accelerated the growth of *S. obliquus* (Figure S11A), followed by a sharper decline as a result of reducing system resources (e.g., nutrients, space and light; Pan et al. (2018)) within each density fluctuation. The negative effect of NOR on system stability was exaggerated when the grazer was present.

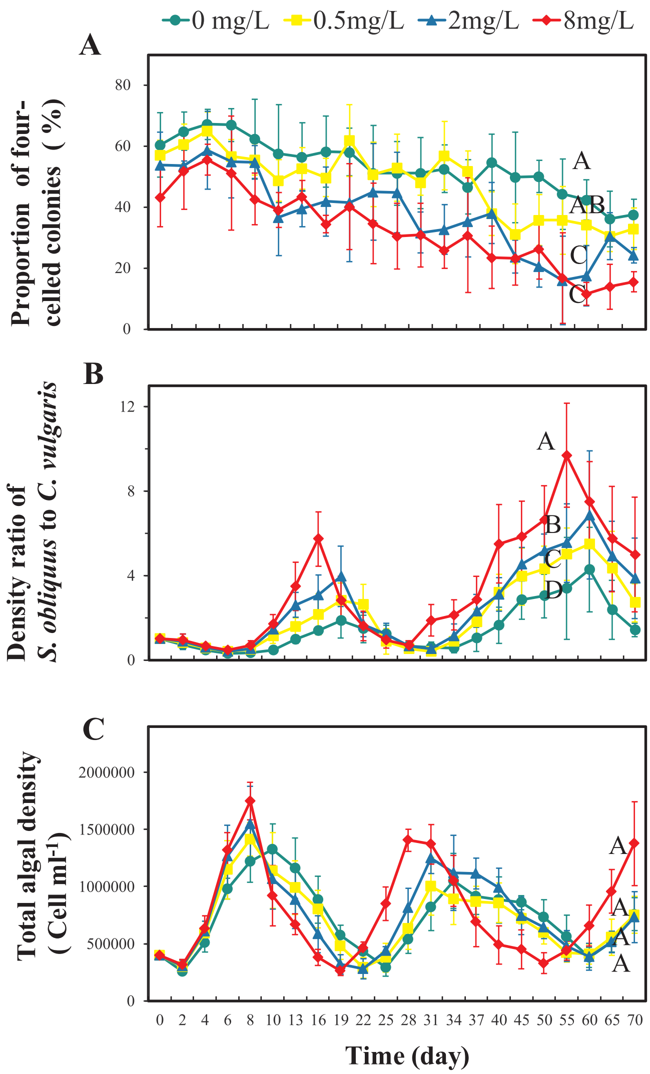


Fig. 5. Variation (means ± SD, *n* = 5) in proportion of four-celled colonies of *Scenedesmusobliquus* (A), density ratio of *S.obliquus* to *Chlorella vulgaris* (B), and total algal density of *S.obliquus* and *Chlorella vulgaris* (C), after exposure to norfloxacin in long-term mixed culture experiment without adding grazers. Different uppercase letters indicate significant differences among treatments through one-way RM-ANOVA at the 0.05 significance level.

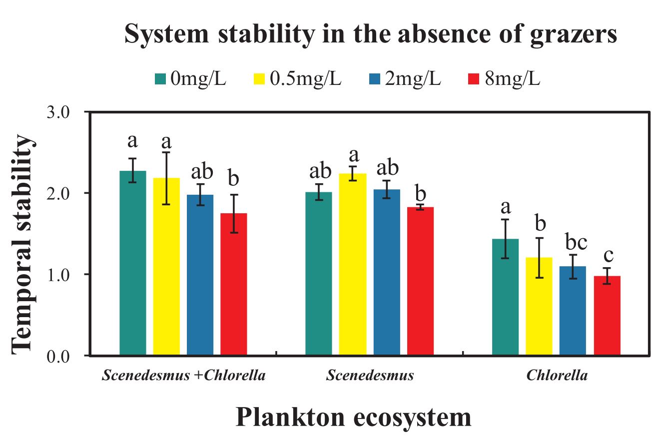


Fig. 6. Temporal stability of *Scenedesmusobliquus*, *Chlorella vulgaris*, and the whole algal community (*S.obliquus* + *C. vulgaris*) after exposure to norfloxacin in long-term mixed culture experiment without adding grazers. Different lowercase letters indicate significant differences among treatments through one-way ANOVA at the 0.05 significance level.

Zooplankton population dynamics are a key factor determining the stability of phytoplankton-zooplankton ecosystems (Peeters et al., 2010; Pan et al., 2018). In short-term apparent competition systems, we found that body size and birth rate increased with increasing NOR concentrations (Figure S7). This could be explained by the inhibition of the formation of large algal colonies, and thus increasing the filtrating efficiency of *D. magna* on *Scenedesmus* cells, which is known to promote the growth and reproduction of grazer species (Taghavi et al., 2013; Pan et al., 2018). Therefore, it is not surprising that in the long-term experiment, grazer density under high NOR concentrations increased faster and their peak values were generally higher than under low concentrations (Fig. 7D, Figure S14). Because of the close coupling between phytoplankton and zooplankton (Davis et al., 2010; Pan et al., 2014), total algal cells were almost depleted at the early experimental stage (Fig. 7C, Figure S12), resulting in large population (density) fluctuations of both prey and predator species in treatments with higher NOR levels, or even grazer extinction in the treatment with 8 mg L^{-1} of NOR.

Collectively, alteration of *S. obliquus* colony size by NOR further affected the stability and persistence of plankton ecosystems, verifying previous assumptions about the effects of colony size in maintaining system function (Davis et al., 2010; Taghavi et al., 2013). Notably, repeated addition of NOR should have resulted in a higher final NOR at the highest exposure level (12.43 mg L^{-1} on day 70 in the system containing grazer) than the environmentally-relevant concentrations (Robinson et al., 2005; Nie et al., 2009; Zhang et al., 2012; Zhang et al., 2015; Liu et al., 2018). However, this does not alter our conclusions, because system stability had been reduced in treatments with the second highest exposure level, where the exposure concentration remained within the range throughout the experiment (in view of the NOR degradation rate and NOR concentration of 3.60 mg L^{-1} on day 70 in the treatment containing grazers).

4.4. Summary

This study provides insights into how NOR at environmentally-relevant concentrations affects the composition and function of plankton systems. It is clear that the negative effects of environmental pollutants on aquatic ecosystems cannot be fully evaluated by their assessing lethal or sublethal effects on individual organisms alone. Destabilization of individual functions such as affected colony formation in *Scenedesmus* under mild pollution conditions can be transmitted through ecological networks and endangers the structure and function of aquatic ecosystems. Therefore, the ecological risks of antibiotic residuals are more pervasive than previously recognized. Additionally, our study did not investigate the uptake and accumulation of antibiotics in plankton organisms, which must be addressed in future studies in order to reveal the underlying mechanism of these processes (see Kováčik et al., 2014; Kováčik et al., 2018).

Author contributions

Y. P. and X.X.C designed the experiments. Y. P. wrote the manuscript. L. L.W. and J.Y.D. performed all experiments. Y.P analyzed the data and all authors contributed comments.

Additional information

This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. All authors have read and approved the manuscript.

Declaration of Competing Interest

The authors declare no competing financial interests.

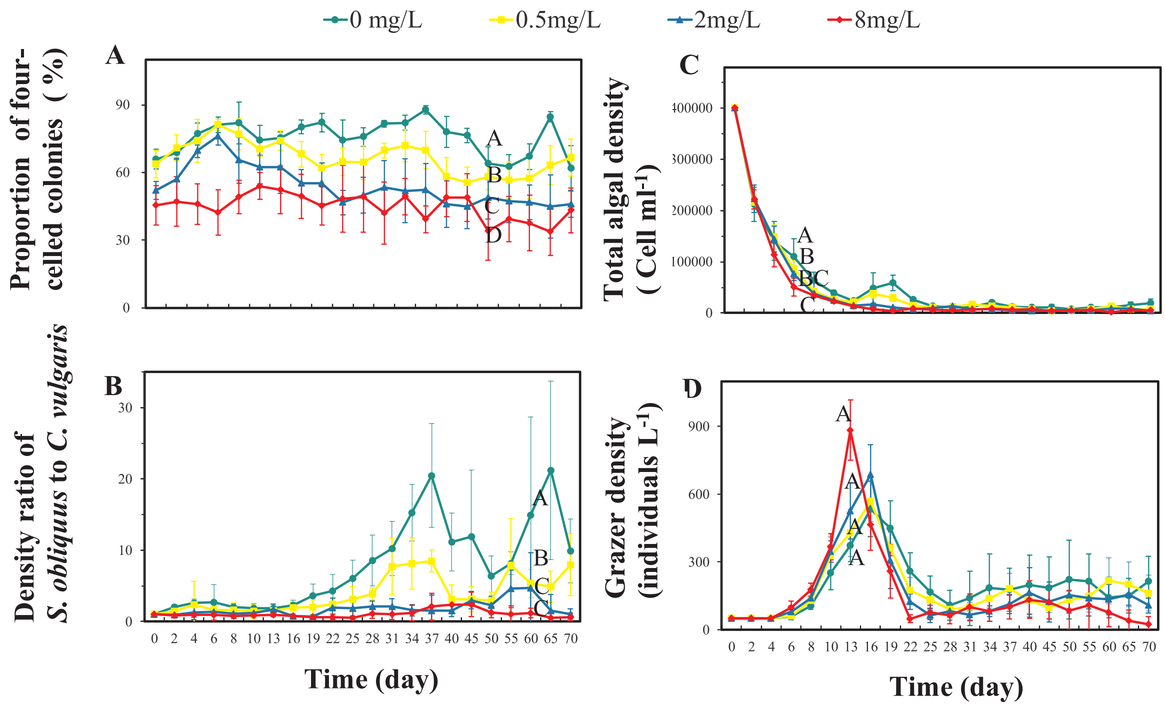


Fig. 7. Variation (means ± SD, $n = 6$) in proportion of four-celled colonies of *Scenedesmusobliquus* (A), density ratio of *S.obliquus* to *Chlorella vulgaris* (B), total algal density of *S.obliquus* and *Chlorella vulgaris* (C), and grazer density (D) after exposure to norfloxacin in long-term mixed culture experiment adding grazers. Different uppercase letters indicate significant differences among treatments through one-way RM-ANOVA at the 0.05 significance level.

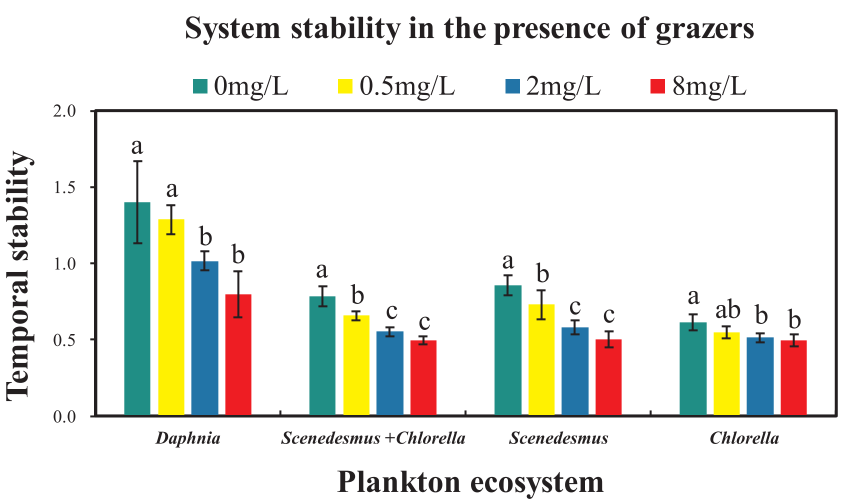


Fig. 8. Temporal stability of *Scenedesmusobliquus*, *Chlorella vulgaris*, the whole algal community (*S.obliquus* + *C. vulgaris*), as well as *Daphnia magna* population after exposure to norfloxacin in long-term mixed culture experiment in the presence of grazers. Different lowercase letters indicate significant differences among treatments through one-way ANOVA at the 0.05 significance level.

Acknowledgements

We thank Shiwei Yan, Guangdi Yi, Dan Liu, Jun Luo, Yuhe Wu, Deqin Gao, Han Yang, Guanwen Chen, Jie-xin Tan, and Zi-yong Cao for their help with data collection. This research was financially supported by the National Natural Science Foundation of China (Grant numbers 31670440), Yunnan Province Key Research Program for Science and Technology (2018BC002; S2017BC041), and the Undergraduate Innovation and Entrepreneurship Training Program of China (S201910673095).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2019.121625.

References

Alcock, R.E., Sweetman, A., Jones, K.C., 1999. Assessment of organic contaminant fate in wastewater treatment plants I: selected compounds and physiochemical properties. Chemosphere 38, 2247–2262. https://doi.org/10.1016/S0045-6535(98)00444-5.

Chen, Y., Zhou, J.L., Cheng, L., Zheng, Y.Y., Xu, J., 2017. Sediment and salinity effects on the bioaccumulation of sulfamethoxazole in zebrafish (*Danio rerio*). Chemosphere 180, 467–475. https://doi.org/10.1016/j.chemosphere.2017.04.055.

Costanzo, S.D., Murby, J., Bates, J., 2005. Ecosystem response to antibiotics entering the aquatic environment. Mar. Pollut. Bull. 51, 218–223. https://doi.org/10.1016/j.marpolbul.2004.10.038.

Davis, J.M., Rosemond, A.D., Eggert, S.L., Crossa, W.F., Wallace, J.B., 2010. Long-term nutrient enrichment decouples predator and prey production. Proc. Natl. Acad. Sci. U. S. A. 107, 121–126. https://doi.org/10.1073/pnas.0908497107.

Duan, Z.P., Tan, X., Parajuli, K., Upadhyay, S., Zhang, D.F., Shu, X.Q., Liu, Q.Q., 2018. Colony formation in two *Microcystis*morphotypes: Effects of temperature and nutrient availability. Harmful Algae 72, 14–24. https://doi.org/10.1016/j.hal.2017.12.006.

Eguch, K., Nagase, H., Ozawa, M., Endoh, Y.S., Goto, K., Hirat, K., Miyamoto, K., Yoshimura, H., 2004. Evaluation of antimicrobial agents for veterinary use in the ecotoxicity test using microalgae. Chemosphere 57, 1731–1738. https://doi.org/10.1016/j.chemosphere.2004.07.017.

González-Pleiter, M., Gonzalo, S., Rodea-Palomares, I., Leganés, F., Rosal, R., Boltes, K., Marco, E., Fernández-Piñas, F., 2013. Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: implications for environmental risk assessment. Water Res. 47, 2050–2064. https://doi.org/10.1016/j.watres.2013.01.020.

Grenni, P., Ancona, V., Caracciolo, A.B., 2018. Ecological effects of antibiotics on natural ecosystems: a review. Microchem. J. 136, 25–39. https://doi.org/10.1016/j.microc.2017.02.006.

Guo, R.X., Chen, J.Q., 2012. Phytoplankton toxicity of the antibiotic chlortetracycline and its UV light degradation products. Chemosphere 87, 1254–1259. https://doi.org/10.1016/j.chemosphere.2012.01.031.

Gutseit, K., Berglund, O., Granéli, W., 2007. Food quality for *Daphnia* in humic and clear water lakes. Freshw. Rev. 52, 344–356. https://doi.org/10.1111/j.1365-2427.2006.01697.x.

Haddad, N.M., Crutsinger, G.M., Gross, K., Haarstad, J., Tilmanet, D., 2011. Plant diversity and the stability of foodwebs. Ecol. Lett. 14, 42–46. https://doi.org/10.1111/j.1461-0248.2010.01548.x.

Holt, R.D., 1997. Predation, apparent competition, and the structure of prey communities. Theor. Popul. Biol. 12, 197–229. https://doi.org/10.1016/0040-5809(77)90042-9.

Kilham, S.S., Kreeger, D.A., Lynn, S.G., Goulden, C.E., Herrera, L., 1998. COMBO: A defined freshwater culture medium for algae and zooplankton. Hydrobiologia 377, 147–159. https://doi.org/10.1023/a:1003231628456.

Kováčik, J., Klejdus, B., Babula, P., 2014. Oxidative stress, uptake and bioconversion of 5-fluorouracil in algae. Chemosphere 100, 116–123. https://doi.org/10.1016/j.chemosphere.2013.11.074.

Kováčik, J., Antoš, V., Micalizzi, G., Dresler, S., Hrabák, P., Mondello, L., 2018. Accumulation and toxicity of organochlorines in green microalgae. J. Hazard. Mater. 347, 168–175. https://doi.org/10.1016/j.jhazmat.2017.12.056.

Kümmerer, K., 2009. Antibiotics in the aquatic environment-a review-part II. Chemosphere 75, 435–441. https://doi.org/10.1016/j.chemosphere.2008.11.086.

Lanzky, P.F., Halling-Sørensen, B., 1997. The toxic effect of the antibiotic metronidazole on aquatic organisms. Chemosphere 35, 2553–2561. https://doi.org/10.1016/S0045-6535(97)00324-X.

Li, M., Zhu, W., Gao, L., Lu, L., 2013. Changes in extracellular polysaccharide content and morphology of Microcystis aeruginosa at different specific growth rates. J. Appl. Phycol. 25, 1023–1030. https://doi.org/10.1016/10.1007/s10811-012-9937-7.

Liu, X.H., Lu, S.Y., Guo, Y., Xi, B.D., Wang, W.L., 2018. Antibiotics in the aquatic environments: a review of lakes, China. Sci. Total Environ. 627, 1195–1208. https://doi.org/10.1016/j.scitotenv.2018.01.271.

Lürling, M., Eshetu, F., Faassen, E.J., Kosten, S., Huszar, V.L.M., 2013. Comparison of cyanobacterial and green algal growth rates at different temperatures. Freshw. Rev. 58, 552–559. https://doi.org/10.1111/j.1365-2427.2012.02866.x.

Macrì, A., Stazi, A., Dojmi Di Delupis, G., 1988. Acute toxicity of furazolidone on *Artemia salina*, *Daphnia magna* and *Culex pipiens molestus* larvae. Ecotoxicol. Environ. Safe. 16, 90–94. https://doi.org/10.1016/0147-6513(88)90021-8.

Marti, E., Variatza, E., Balcázar, J.L., 2014. The role of aquatic ecosystems as reservoirs of antibiotic resistance. Trends Microbiol. 22, 36–41. https://doi.org/10.1016/j.tim.2013.11.001.

Nie, X.P., Gu, J.G., Lu, J.Y., Pan, W.B., Yang, Y.F., 2009. Effects of norfloxacin and butylated hydroxyanisole on the freshwater microalga Scenedesmus obliquus. Ecotoxicology 18, 677–684. https://doi.org/10.1007/s10646-009-0334-1.

Nie, X.P., Lu, J.Y., Li, X., Yang, Y.F., 2007. Toxic effects of Norfloxacin to the growth and the activity of antioxidase of Chlorella pyrenoidosa. Asian J. Ecotox. 2, 327–332. https://doi.org/10.3969/j.issn.1673-5897.2007.03.013. (In Chinese with an English abstract).

Olmstead, A.W., LeBlanc, G.A., 2003. Insecticidal juvenile hormone analogs stimulate the production of male offspring in the Crustacean Daphnia magna. Environ. Health Persp. 111, 919–924. https://doi.org/10.1289/ehp.5982.

Pan, Y., Zhang, Y.S., Sun, S.C., 2014. Phytoplankton–zooplankton dynamics vary with nutrients: A microcosm study with the cyanobacterium*Coleofasciculus chthonoplastes* and cladoceran *Moina micrura*. J. Plankton Res. 36, 1323–1332. https://doi.org/10.1093/plankt/fbu057.

Pan, Y., Zhang, Y.S., Sun, S.C., 2018. Habitat orientation alters the outcome of interspecific competition: a microcosm study with zooplankton grazers. Ecol. Evol. 1–16. https://doi.org/10.1002/ece3.3909.

Pan, Y., Liu, C.E., Li, F., Zhou, C.Q., Yan, S.Y., Dong, J.Y., Li, T.R., Duan, C.Q., 2017a. Norfloxacin disrupts *Daphnia magna*-induced colony formation in *Scenedesmus quadricauda* and facilitates grazing. Ecol. Eng. 102, 255–261. https://doi.org/10.1016/j.ecoleng.2017.02.037.

Pan, Y., Yan, S.Y., Li, R.Z., Hu, Y.W., Chang, X.X., 2017b. Lethal/sublethal responses of *Daphnia magna* to acute norfloxacin contamination and changes in phytoplankton-zooplankton interactions induced by this antibiotic. Sci. Rep. 7, 40385. https://doi.org/10.1038/srep40385.

Park, S., Choi, K., 2008. Hazard assessment of commonly used agricultural antibiotics on aquatic ecosystems. Ecotoxicology 17, 526–538. https://doi.org/10.1007/s10646-008-0209-x.

Peeters, F., Li, J., Straile, D., Rothhaupt, K.O., Vijverberg, J., 2010. Influence of low and decreasing food levels on *Daphnia*-algal interactions: numerical experiments with a new dynamic energy budget model. Ecol. Model. 221, 2642–2655. https://doi.org/10.1016/j.ecolmodel.2010.08.006.

Peng, C., Chen, Y., Pu, Z.C., Zhao, Q., Tong, X., Chen, Y.S., Jiang, L., 2017. CeO_2 nanoparticles alter the outcome of species interactions. Nanotoxicology 11, 1–38. https://doi.org/10.1080/17435390.2017.1340527.

Qiao, M., Ying, G.G., Singer, A.C., Zhu, Y.G., 2018. Review of antibiotic resistance in China and its environment. Environ. Int. 110, 160–172. https://doi.org/10.1016/j.enint.2017.10.106.

Robinson, A.A., Belden, J.B., Lydy, M.J., 2005. Toxicity of fluoroquinolone antibiotics to aquatic organisms. Environ. Toxicol. Chem. 24, 423–430. https://doi.org/10.1897/04-210r.1.

Rodriguez-Mozaz, S., Chamorro, S., Marti, E., Huerta, B., Gros, M., Sànchez-Melsió, A., Borrego, C.M., Barceló, D., Luis, J., 2014. Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water Res. 69, 234–242. https://doi.org/10.1016/j.watres.2014.11.021.

Roy, R., Parashar, A., Bhuvaneshwari, M., Chandrasekaran, N., Mukherjee, A., 2016. Differential effects of P25 TiO_2 nanoparticles on freshwater green microalgae: *chlorella* and *Scenedesmus* species. Aquat. Toxicol. 176, 161–171. https://doi.org/10.1016/j.aquatox.2016.04.021.

Selck, H., Riemann, B., Forbes, V.E., Christoffersen, K., Gustavson, K., Hansen, B.W., Jacobsen, J.A., Kusk, O.K., Petersen, S., 2002. Comparing sensitivity of ecotoxicological effect endpoints between laboratory and field. Ecotoxicol. Environ. Saf. 52, 97–112. https://doi.org/10.1006/eesa.2002.2172.

Struyf, E., van Damme, S., Gribsholt, B., Bal, K., Beauchard, O., Middelburg, J.J., Meire, P., 2007. *Phragmites australis* and silica cycling in tidal wetlands. Aquat. Bot. 87, 134–140. https://doi.org/10.1016/j.aquabot.2007.05.002.

Taghavi, D., Farhadian, O., Soofiani, N.M., Keivany, Y., 2013. Effects of different light/dark regimes and algal food on growth, fecundity, ephippial induction and molting of freshwater cladoceran, Ceriodaphnia quadrangular. Aquaculture 410–411, 190–196. https://doi.org/10.1016/j.aquaculture.2013.06.026.

Tang, Q., Song, P., Li, J., Kong, F., Sun, L., Xu, L., 2016. Control of antibiotic resistance in China must not be delayed: the current state of resistance and policy suggestions for the government, medical facilities, and patients. Biosci. Trends 10, 1–6. https://doi.org/10.5582/bst.2016.01034.

Teixeira, J.R., Granek, E.F., 2017. Effects of environmentally-relevant antibiotic mixtures on marine microalgal growth. Sci. Total Environ. 580, 43–49. https://doi.org/10.1016/j.scitotenv.2016.11.207.

Tilman, D., 1977. Resource competition between planktonic algae: an experimental and theoretical approach. Ecology 58, 338–348. https://doi.org/10.2307/1935608.

van Donk, E., 2007. Chemical information transfer in freshwater plankton. Ecol. Inform. 2, 112–120. https://doi.org/10.1016/j.ecoinf.2007.03.002.

van Donk, E., Ianora, A., Vos, M., 2011. Induced defences in marine and freshwater phytoplankton: a review. Hydrobiologia 668, 3–19. https://doi.org/10.1007/s10750-010-0395-4.

Wollenberger, L., Halling-Sùrensenb, B., Kuska, K.O., 2000. Acute and chronic toxicity of veterinary antibiotics to Daphnia magna. Chemosphere 40, 723–730. https://doi.org/10.1016/S0045-6535(99)00443-9.

Yang, L.H., Ying, G.G., Su, H.C., Stauber, J.L., Adams, M.S., Binet, M.T., 2008. Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga Pseudokirchneriella subcapitata. Environ. Toxicol. Chem. 27, 1201–1208. https://doi.org/10.1897/07-471.1.

Zalewski, A., Wagner, N.D., Frost, P.C., 2011. Antibiotics aff ;ect the growth responses of *Daphnia magna* to poor food quality. Aquat. Microb. Ecol. 45, 493–504. https://doi.org/10.1007/s10452-011-9370-z.

Zhang, Q.Q., Ying, G.G., Pan, C.G., Liu, Y.S., Zhao, J.L., 2015. Comprehensive evaluation of antibiotics emission and fate in the river basins of China: source analysis, multimedia modelling, and linkage to bacterial resistance. Environ. Sci. Technol. 49, 6772–6782. https://doi.org/10.1021/acs.est.5b00729.

Zhang, J.W., Fu, D.F., Wu, J.L., 2012. Photodegradation of Norfloxacin in aqueous solution containing algae. J. Environ. Sci. 24, 743–749. https://doi.org/10.1016/S1001-0742(11)60814-0.

Zhao, L., Dong, Y.H., Wang, H., 2010. Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. Sci. Total Environ. 408, 1069–1075. https://doi.org/10.1016/j.scitotenv.2009.11.014.

Zhu, X.X., Nan, H.H., Zhang, L., Zhu, C., Liu, J.X.Y., Yang, Z., 2014. Does microcystin disrupt the induced effect of *Daphnia* kairomone on colony formation in *Scenedesmus*? Biochem. Syst. Ecol. 57, 169–174. https://doi.org/10.1016/j.bse.2014.08.001.

Zhu, X.X., Wang, J., Lu, Y.C., Chen, Q.W., Yang, Z., 2015. Grazer-induced morphological defense in *Scenedesmus obliquus* is aff ;ected by competition against *Microcystis aeruginosa*. Sci. Rep. 5, 12743. https://doi.org/10.1038/srep12743.