Combined Effects of the Pesticide Spinetoram and the Cyanobacterium *Microcystis* on the Water Flea *Daphnia Pulex*

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Abstract

Spinetoram is one of the most worldwidely used pesticides for its high insecticidal efficacy and low human toxicity Following the large usage of spinetoram, the ecotoxicity and environmental risks to aquatic ecosystems have call for urgent study In the present study, we investigated the combined effects of spinetoram and the harmful alga *Microcystis aeruginosa* in freshwater, on survival and reproduction of *Daphnia pulex* Acute toxicity test of spinetoram resulted in negative effects on survival, with a 48h-LC_{50} value of 3771 μg L^{-1} Under the long-time exposure to environmentally relevant concentrations (018 and 035 μg L^{-1}) of spinetoram and a low composition of *Microcystis* (30%) in the diet, *D pulex* showed both shorter longevity and lower fecundity, the time to first brood was also increased At population level, carrying capacity was highly decreased by spinetoram and *Microcystis*, whereas a significant decrease of intrinsic growth rate was observed at 035 μg L^{-1} spinetoram with 30% *Microcystis* as food The present study highlighted that pesticide spinetoram had highly toxic effects on *D pulex* and could reduce the tolerance of *D pulex* to *M aeruginosa*, causing great effects on *D pulex* population in natural waterbodies

1 Introduction

Spinosyns are a series of macrocyclic natural bio-insecticides derived from the soil actinomycete *Saccharopolyspora spinosa*, which have been registered in more than 100 countries to control above 200 species of crops since the launch in 1996, because of their high efficiency, low unit area usage, and short residue period (Huang et al 2009; Bacci et al 2016; Sparks et al 2020) Spinosyns act mainly on the nicotinic acetylcholine receptors (nAChRs) and γ-aminobutyric acid receptors (GABARs) in the nervous system of arthropods, through depolarizing nerve cells and cause extensive hyperactivation of central nervous system Spinetoram, the next-generation product of spinosyn insecticides launched in 2007, has large global market for its high insecticidal efficacy and low human toxicity (Galm and Sparks, 2016) Following the worldwide adoption of spinetoram, the ecotoxicity and environmental risks have call for urgent study In particular, risk assessments of spinetoram on beneficial arthropods like natural enemies are crucial because potential side effects on these non-target arthropods can greatly affect their natural populations and ecological functions (Biondi et al 2012)

Aquatic environments receive direct and indirect pesticides inputs, the enormous number of pesticides induce a series of aquatic environmental problems A great deal of research have documented the ecotoxicological risks of pesticides on the aquatic organisms and ecosystems eg (i) physiological and behavioral sublethal effects (Bownik et al 2019), (ii) long-term suppression in population levels (Dalkvist et al 2009), (iii) alternation of aquatic community composition (Vonesh and Kraus 2009) Due to the large inputs of pesticides, aquatic organisms at different trophic levels are exposed to the threat of spinetoram For example, the development (ie body length and heartbeat rate) and immune system of zebrafish embryos were negatively affected under the exposure to an effective concentration of 025 g L^{-1} spinetoram (Cheng et al 2020) Spinosyn-based products, especially spinetoram, has great efficacy and selectivity to target pests (Sparks et al 2008) Nonetheless, the non-target impact of spinetoram on

beneficial arthropods requires systematic risk assessments, for these including natural enemies and pollinators, play crucial roles in ecosystems (Biondi et al 2012) Recently, spinetoram as well as other spinosyn-based products are also used to control aquatic insects, such as mosquitoes at developmental stages (Mohamed et al 2021), and control ectoparasitic infestations in aquaculture raised fish (Dick et al 2013) Therefore, although aquatic arthropods are also exposed to spinetoram threat, very few studies investigated the potential effect of spinetoram on those organisms

The freshwater cladocerans of the genus *Daphnia* are keystone pelagic filter feeders in the food webs of temperate ponds and lakes, and are served as an important model for ecological, ecotoxicological, biogeographical and evolutionary researches (Eads et al 2008; Seda and Petrusek, 2011; Perhar and Arhonditsis, 2015) The ability of *Daphnia* to suppress phytoplankton biomass, especially during the bloom of toxic cyanobacteria, indicates that the *Daphnia* seems to be critically important to the biomanipulation efforts in eutrophicate lakes (Chislock et al 2013) However, artificial pollutants in aquatic environments would have a significant impact on the ecological function of *Daphnia* (Pestana et al 2010; Liu et al 2019) The major pollutants like heavy metals and pesticides have been reported in previous studies not only affect the life-history traits but also inhibit the feeding rates of *Daphnia* (Hartgers et al 1999; Lessard and Frost, 2012) Whereas, artificial pollutants could suppress the grazing control of *Daphnia* on *Microcystis*, due to direct adverse effects on *Daphnia* or indirect interference on species interactions (Cerbin et al 2010; Yang et al 2011)

Besides artificial pollutants, intensive human activities also increase the nutrients flux in natural waterbodies, and as a result, cyanobacterial blooms occur frequently in freshwaters In freshwater aquaculture ponds, cyanobacterial blooms due to eutrophication is now becoming a major issue during pond system development (Bi et al 2019) *Microcystis aeruginosa* is one of the most common bloom-forming cyanobacteria in freshwater ecosystems worldwide, and it is assumed that, global warming together with eutrophication will promote *Microcystis* blooms (O'Neil et al 2012) In natural freshwaters, *Daphnia* spp play a central role in controlling *Microcystis* blooms (Triest et al 2016) Nonetheless, compared with green algae, *Microcystis* is poor in nutrient quality (Hairston et al 2001), and toxic strains cause negative effects on the fitness of zooplankton (Lei et al 2020) Additionally, study on the effects of non-toxic *Microcystis* on *Daphnia* grazing and life-history traits demonstrated that, *Microcystis* must contain some substances other than microcystins that have adverse effects on *Daphnia* (Lürling 2003)

It has been well documented that, spinosyn-based products have high toxicity risk on *Daphnia* (Duchet et al 2010) With the increasing global market of spinetoram, *Daphnia* is supposed to be under higher threat and to experience combined stress of spinetoram and *Microcystis* In this study, we hypothesized that the exposure to spinetoram would reduce the tolerance of *Daphnia* to *Microcystis* For this purpose, three separate experiments were conducted An acute experiment was run to assess the toxic effect of spinetoram on *D pulex* Whereas chronic exposure to selected concentrations of spinetoram, with or without *Microcystis* as food, were run to analyze the combined effects of spinetoram and *Microcystis*

2 Materials And Methods

21 Test organisms and culture conditions

A clone of *D pulex*, which was collected from the pond in the campus of Hohai University, Nanjing, China, and has been maintained in our laboratory since 2017, was used in this study Daphniids were cultured with a density of less than 100 individuals in 1-L beakers containing 800 mL dechlorinated tap water The suspensions of *Chlorella pyrenoidosa* at the stage of exponential growth were harvested by centrifugation and supplied as the food of *D pulex* with a density of 5×10^5 cells mL^{-1} *C pyrenoidosa* and a non-toxic strain of *Microcystis aeruginosa* (FACHB-469) were mono-cultured separately in 3 L Erlenmeyer flasks axenically using BG-11 medium and aerated with 022-μm filtrated air Erlenmeyer flasks were shaken manually 3 times a day The algae were collected by centrifugation (500 g for 10 min) and stored at 4 °C The cultivation of daphniids and algae and all the experiments were conducted in a constant temperature chamber at 25 °C, under fluorescent light at 40 μmol photons $m^{-2}s^{-1}$, with a light–dark period of 14:10 h

22 Chemical and test solution

Spinetoram was purchased from Dow AgroSciences (USA) and stored in a dark and dry place To prepare the stock solution, spinetoram was ultrasonic crushed for ten minutes and then dissolved in acetone (C995% analyticalgrade purity) The stock solution was immediately prepared prior to each experiment In all experiments, the maximum percentage of acetone in the cultural medium was below 001% (v/v) Prior to the experiments, the toxic effects of the acetone solution at a concentration of 001% (v/v) on *D pulex* was tested and showed no toxic effects (data not shown) Determination of the actual concentration of spinetoram in media was conducted by LC-MS/MS method, using Agilent 1290 HPLC-6495B The column was an Ascentis Express 90A C18 (30 × 150 mm, 27 μm) operated at 35°C, flow rate of 450 μL/min and the injection volume was 5 μL A prior enrichment of media was conducted using Cleanert PestiCarb/NH2 (Agela Tech China) before the concentration measurement

23 Acute exposure to spinetoram

To start a clonal culture, one ovigerous female was isolated from the *D pulex* population and cultured in a 100 mL beaker with 80 mL cultural medium *C pyrenoidosa* was supplied as food of *D pulex* with a final density of 5×10^5 cells mL^{-1} every day and renewed the cultural media every three days A continuous range of spinetoram concentrations was set: 0, 05, 1, 25, 5, 10, 20, 30, 40, 50, 75, 100, 200, 500 μg L^{-1} The actual concentrations of spinetoram were 0, 044, 088, 219, 428, 875, 1752, 2628, 3504, 4380, 6569, 8758, 17518 and 43795 μg L^{-1}, respectively For each concentration, 5 replicates with 10 individuals per replicate were taken Daphniids were selected from the clonal culture randomly and cultured in 50 mL beakers with 40 mL media, and no food was offered during the acute exposure to spinetoram Mortality was recorded at 12 h, 24 h, and 48 h The death of *D pulex* was determined as the time when the heart stopped beating under an inverted microscope Additionally, the phenomena of rapid movement and immobilization of *D pulex* were recorded Dose-response curves were adopted to fit the mortality rate and immobilization rate against time, respectively A special dose-response curve was adopted to fit the

mortality rate against time at 48 h, $M = M_0 + \frac{(M_m - M_0)}{1+10^{(lgC-C)\times\kappa}}$ In the equation, *M* represented the mortality rate, M_m and M_0 represented the maximal and minimum mortality rates, respectively *C* means concentration of spinetoram and *k* represented the slope of this curve The value of LC_{50} at 48 h was calculated according to the fitted curve

24 Chronic effects of spinetoram and Microcystis at individual level

Neonates of *D pulex* within 24 hours of birth were selected randomly and individually placed in 50 mL beakers containing 40 mL tested media A chronic 21-d toxicity experiment was carried out using a full factorial design composed of three concentrations of spinetoram (actual concentrations tested by LC-MS/MS: 0, 018 and 035 μg L^{-1}) and two diet compositions (*Ch*: 100% *C pyrenoidosa*; *Ch+Ma*: 70% *C pyrenoidosa* + 30% *M aeruginosa*) with a final algal biomass of 35 mg C L^{-1} To maintain constant concentration of spinetoram, chemical test solutions were replaced by fresh ones every day Parameters of individual level, including survival days and reproduction were recorded The death of *D pulex* was determined as the time when the heart stopped beating under an inverted microscope

25 Chronic effects of spinetoram and Microcystis at population level

To investigate the long-term effects of spinetoram and *Microcystis* on *D pulex* population, a third experiment was conducted Similarly to the previous experiment set-up of the second one, we started the third experimental with a clonal culture Neonates of *D pulex* within 24 hours of birth in this clonal culture were raised in good diet conditions (100% *C pyrenoidosa*) for 3 days Then a chronic population experiment was conducted using a full factorial design similar to the second experiment, three concentrations of spinetoram (0, 018 and 035 μg L^{-1}) and three diet compositions (*Ch*: 100% *C pyrenoidosa*; *Ch+Ma*: 70% *C pyrenoidosa* + 30% *M aeruginosa*; *Ma*: 100% *M aeruginosa*) with a final algal biomass of 35 mg C L^{-1} This experiment was established at an initial density of 5 neonates in each tested medium at 500 mL The population abundance was recorded every day, and the population growth curves were fitted by the formula, $D = \frac{K}{1+e^{a-rt}}$ In the equation, *r* represented the intrinsic rate of population growth, *K* represented the carrying capacity and *a* was a constant

26 Statistical analysis

The results were presented as means±1 standard error To test the effects of spinetoram and *Microcystis* on survival and reproduction parameters, two-way ANOVAs were performed The population growth was fitted according to logistic regression, and the significance of fitted logistic regression curves with observations were assessed through analysis of variances Two-way ANOVAs were performed to analyze the significant effects of spinetoram and *Microcystis* on carrying capacity and population growth rate All analyses were performed with SigmaPlot 140 Statistical significance was established at $\alpha < 005$

3 Results

31 Acute toxic effect of spinetoram on D pulex

The exposure to spinetoram stimulated rapid movement of *D pulex*, second antennae flapped quicker than in control medium The response of *D pulex* movement to spinetoram presented both concentration effect and temporal effect As shown in Table 1, at 12 h, most of individuals showed quick and nondirectional swimming trajectory in the treatments with spinetoram concentration above 1752 μg L^{-1} After 24 hours, observation of rapid movement decreased to 088 μg L^{-1}, and at 48 h, the concentration which could induce rapid movement was as low as 044 μg L^{-1} However, enhancement of movement was followed by loss of motion ability, which indicated the paralytic effect of spinetoram on *D pulex* At 12 h, immobilization of *D pulex* was observed at the spinetoram concentration of 2628 μg L^{-1}, and the concentration decreased to 428 and 088 μg L^{-1} at 24 h and 48 h, respectively (Table 1)

Table 1
The concentrations at which the phenomena of quick mobilization and immobilization of *D pulex* were observed at 12 h, 24 h, and 48 h, respectively

	Observation of rapid movement	***Observation of immobilization***
12 h	1752 μg L^{-1}	2628 μg L^{-1}
24 h	088 μg L^{-1}	428 μg L^{-1}
48 h	044 μg L^{-1}	088 μg L^{-1}

Increasing trends were determined in mortality rate and immobilization rate with both the increasing concentration of spinetoram and time (Fig. 1) During the first 24 hours, mortality rate was below 50% under all concentrations of spinetoram (Fig. 1A) According to the fitted dose-response curve, the value of 48h-LC_{50} equals to 3771 μg L^{-1} The value of EC_{50} of immobilization rate was also calculated according to dose-response curves and the EC_{50} values at 24 h and 48 h were 1976 and 319 μg L^{-1}, respectively (Fig. 1C, D)

32 Chronic effects of spinetoram and Microcystis at individual level

During the 21-day chronic experiment, the survivorship of *D pulex* was affected by both spinetoram and *Microcystis* (Fig. 2) Generally, individuals fed on *Chlorella* as the only food survived for longer time than these fed on a mixed diet containing 30% *Microcystis* at all concentrations of spinetoram In the *Chlorella* groups, 90% individuals cultured in medium without spinetoram survived until the end of experiment, whereas mortality occurred on 5th day and 2nd day when exposed to 018 and 035 μg L^{-1} spinetoram (Fig. 2A) However, 30% *Microcystis* in diet caused a negative effect on survival, the survival rate of individuals cultured with no spinetoram decreased to 80% at the end of experiment Mortality was

observed on 3rd day and 2nd day and 21-day mortality rate decreased to 40% and 30% at 018 and 035 μg L^{-1} spinetoram, respectively (Fig. 2B)

Besides survivorship, the reproduction was also affected significantly by spinetoram and *Microcystis* In both diet groups, exposure to spinetoram increased the time to first brood, especially at higher spinetoram concentration (035 μg L^{-1}) (Fig. 3A), and the spinetoram concentration had a significantly effect on the time to first brood (Table 2) Fewer offspring was produced by *D pulex* at first brood (Fig. 3B) and per brood (Fig. 3C) The result of Two-way ANOVA showed a significant effect of spinetoram concentration on offspring number of the first brood, and the number of offspring per brood was affected significantly by both spinetoram concentration and food composition (Table 2) Fewer broods were reproduced by *D pulex* under the exposure to spinetoram and *Microcystis* as compared to the control group (Fig. 3D), but no statistical significance was found As for the average number of total offspring produced by one female, significant decrease was found at 035 μg L^{-1} spinetoram treatments of both food groups (Fig. 3E), and both spinetoram concentration and food showed significant effects The interval of molting showed different trend in two food groups (Fig. 3F), but the concentration of spinetoram had no significant effect (Table 2)

Table 2

The results of two-way ANOVAs on the difference of the time to first brood, offspring of first brood, number of broods, offspring per brood, total offspring per female and number of molting of *D pulex* fed with different food under the exposure to spinetoram at different concentrations during the 21-day experiment of individual level; and carrying capacity and intrinsic growth rate of *D pulex* populations fed with different food under the exposure to spinetoram at different concentrations In the last column, * denotes $p < 005$, ** denotes $p < 0001$

Traits	*Factors*	*SS*	*DF*	*MS*	*F (DFn, DFd)*	*p*
Individual level						
Time to first brood	Interaction	168	2	08402	F (2, 28) = 02450	07843
	Spinetoram concentration	7206	2	3603	F (2, 28) = 1051	00004 **
	Food composition	384	1	384	F (1, 28) = 1120	0299
Offspring number of first brood	Interaction	577	2	2885	F (2, 28) = 06815	05141
	Spinetoram concentration	3425	2	1713	F (2, 28) = 4046	00286 *
	Food composition	1344	1	1344	F (1, 28) = 3176	00856
Offspring per brood	Interaction	2408	2	1204	F (2, 28) = 09108	0965
	Spinetoram concentration	4104	2	2052	F (2, 28) = 1552	00577
	Food composition	1173	1	1173	F (1, 28) = 8869	00831
Number of brood	Interaction	009821	2	00491	F (2, 28) = 003564	04138
	Spinetoram concentration	8717	2	4358	F (2, 28) = 3164	< 00001 **
	Food composition	4449	1	4449	F (1, 28) = 3229	00059 **
Total offspring per female	Interaction	8388	2	4194	F (2, 28) = 02649	07692
	Spinetoram concentration	1965	2	9824	F (2, 28) = 6205	00059 **
	Food composition	9963	1	9963	F (1, 28) = 6293	00182 *

Traits	*Factors*	*SS*	*DF*	*MS*	*F (DFn, DFd)*	*p*
Interval of molting (d)	Interaction	02666	2	01333	F (2, 52) = 09713	03853
	Spinetoram concentration	01246	2	006229	F (2, 52) = 04539	06376
	Food composition	07782	1	07782	F (1, 52) = 5671	00209 *
Population level						
Carrying capacity	Interaction	392993	4	98248	F (4, 18) = 1653	< 00001**
	Spinetoram concentration	666427	2	333213	F (2, 18) = 5606	< 00001**
	Food composition	1467725	2	733862	F (2, 18) = 1235	< 00001**
Population growth rate	Interaction	0004114	4	0001028	F (4, 18) = 7864	00008**
	Spinetoram concentration	003612	2	001806	F (2, 18) = 1381	< 00001**
	Food composition	03056	2	01528	F (2, 18) = 1168	< 00001**

33 Chronic effects of spinetoram and Microcystis at population level

During the first days of the experiment, *D pulex* population were composed by immature individuals and, afterwards, following the first reproduction, population kept growing exponentially, except in the 100% *Microcystis* treatment (Fig. 4) Generally, at three spinetoram concentrations (0, 018, 035 μg L^{-1}), the populations of *D pulex* fed on mixed food (70% *Chlorella* + 30% *Microcystis*) grew slower than those fed on 100% *Chlorella* However, feeding on 100% *Microcystis* could not support the population establishment of *D pulex* (Fig. 4) The population growth was fitted following the logistic growth curve in both food groups at three spinetoram concentrations

To compare the growth dynamics of different treatments, carrying capacity and intrinsic growth rate were analyzed Carrying capacity was affected significantly by both food and spinetoram concentration (Table 2) It significantly decreased with the increasing spinetoram concentrations, and showed significant decreases in mixed food groups at all three spinetoram concentrations (Fig. 5A) The carry capacity was higher than 800 inds L^{-1} when the population of *D pulex* was raised in medium with no spinetoram and fed on 100% *Chlorella* However, the carry capacity decreased to about 110 inds L^{-1} under the exposure of 035 μg L^{-1} spinetoram and fed on 30% *Microcystis* As for the population of *D pulex* feeding on 100% *Microcystis*, the carry capacity was much lower than other groups, and there was no

significant difference of carry capacity among the three spinetoram concentrations (Fig. 5A) Besides, a significant interaction between spinetoram concentration and food composition was also found (Table 2) The population growth rate was significantly affected by the concentration of spinetoram and food composition (Table 2), especially in the mixed food group (Fig. 5B) In the groups fed on 100% *Chlorella*, the growth rate in two spinetoram concentrations were significantly lower than that in no spinetoram treatment Under mixed food and total *Microcystis*, growth rate decreased with increasing spinetoram concentration (Fig. 5B) In the groups fed on 100% *Microcystis*, the growth rate was below zero when exposed to spinetoram (Fig. 5B), indicating a negative growth of *D pulex* population

4 Discussion

Spinetoram, one of the most worldwidely used pesticides, not only caused toxic effects on *D pulex* at both individual and population levels, but also affected the tolerance to *Microcystis*, in our study Increasing mortality of *D pulex* was caused by the increasing concentration of spinetoram Whereas the negative effect on longevity, as well as reproduction of *D pulex* was aggravated when fed on mixed food (70% *Chlorella* + 30% *Microcystis*) under long-time exposure Results of this present study demonstrated an interactive effect of spinetoram and *Microcystis* on the population of *D pulex*

In the present study, the adverse influence on the survival of *D pulex* caused by spinetoram was assessed During the acute experiment of direct exposure to spinetoram, under a wide concentration range, the mortality rate was lower than 50% during the first 24 hours (Fig. 1A) Nonetheless, mortality rate showed a quick increase at 48 h, especially the individuals exposed to spinetoram at concentrations higher than 875 μg L^{-1} (Fig. 1B) For evaluating both the efficacy on target insects and the potential ecological risk impact on beneficial arthropods of spinetoram, acute toxic studies have been reported previously It could be found in previous studies that the 48 h-LC_{50} value to diamondback moth, *Plutella xylostella*, was ranged from 0131 to 1001 mg L^{-1} (Li et al 2015), the values of LC_{50} of spinetoram to the third instar larvae of *Lampides boeticus* at 24, 48, and 72 h was 671, 223 and 128 mg L^{-1} (Sanjeevikumar and Muthukrishnan, 2017) Our experiment figured out the value of 48h-LC_{50} was approximately 4306 μg L^{-1} According to the toxicity categories based on LC_{50}, spinetoram could be classified as “highly toxic pesticide” to *Daphnia* Besides, the study of cytotoxicity on human liver cells demonstrated that spinetoram could inhibit the proliferation of human liver HepG2 cells and induce the oxidative DNA damage (Zhang et al 2019) Therefore, because spinetoram occupies a great market share, the ecological risk assessment should be taken into serious consideration

For the complex and varied environmental factors faced by *Daphnia* in natural waterbodies, our present study assumed a mixed threat to *D pulex*, spinetoram and *Microcystis*, that is real phenomena in freshwater Results turned out that, over a longer time-scale, both spinetoram and *Microcystis* had adverse effect on the survival and reproduction of *D pulex*, and these two factors had interactive effects Fecundity of *D pulex* was inhibited by both spinetoram and *Microcystis*, that less offspring were produced under the effects of single and mixed factors Previous studies demonstrated the adverse effect of *Microcystis* on

fecundity of *Daphnia* mostly reflected in the smaller size of each brood and smaller body size of offspring (Lürling 2003; Huang et al 2020; Li et al 2020) As for spinetoram, the main target sites in arthropods are nAChRs and GABARs in the nervous system, most studies focused on the acute toxic effect of spinetoram on insects (Dissanayaka et al 2020; Sammani et al 2020) However, widely application of spinetoram in agriculture along with the surface run-off, the environmental concentration of spinetoram was stable at relatively low concentrations Our study focused on the long-term exposure to spinetoram, and figured out reproduction of *D pulex* was also sensitive to spinetoram even at very low concentrations

The impact of spinetoram and *Microcystis* on the reproduction of *D pulex* in our study was not only in the fecundity but also in the time to brood Time to first brood was delayed with the increasing spinetoram concentration (Fig. 3A) Exposure to pollutions often caused increased time to maturity along with the decreased fecundity of *Daphnia*, which might be due to the delayed development and shortage in energy storage (Reynaldi et al 2006; Saebelfeld et al 2017) Usually, *Daphnia* has high phenotypic plasticity in life history under complex environments (Otte et al 2019; Gu et al 2020) Slight delay in first brood was also observed in *D pulex* fed on *Microcystis* (Fig. 3A), which was similar to previous studies (Wang et al 2019; Lei et al 2020; Zhou et al 2020) Resources and energy might be reallocated from somatic growth to reproduction so that *Daphnia* reaches maturity earlier under the influence of spinetoram and *Microcystis* in the present study

Because of the adverse effects caused by both spinetoram and *Microcystis*, population dynamics of *D pulex* showed significant changes (Fig. 5A) Generally, carrying capacity of *D pulex* population was highly decreased by both spinetoram and *Microcystis*, and these two factors had significant interactive effect The potential explanation of the reduced carrying capacity in *D pulex* population might be due to the energy allocation between development and the elimination of toxic effects of spinetoram and bad food conditions caused by *Microcystis* Lower intrinsic growth rate of *D pulex* population was observed at the higher spinetoram with 30% *Microcystis* as food (Fig. 5B), but other treatments had no significant difference Decline in carrying capacity of *D magna* population has also been observed in the condition of microplastics-induced food limitation (Bosker et al 2019) Research on the effects of cadmium showed lower population growth rate and population biomass of *D manga* (Connon et al 2008) Researches show the profound influence of artificial pollutants on *Daphnia* populations at a long term, which might also disrupt the ecological functions of *Daphnia* such as consuming *Microcystis* blooms Extensive application of pesticides is now gradually influencing the structure and biodiversity of both terrestrial and aquatic ecosystems For spinetoram, the application disrupts the structure and the abundance of surface-active arthropod fauna causing considerable changes in the ground dwelling biodiversity of this ecosystem (Lefkaditis et al 2017) As for aquatic ecosystems, very few studies focused on the risk assessment of spinetoram The findings of our study indicated that spinetoram not only affected the fitness of *D pulex* but also reduced the tolerance of *D pulex* to *Microcystis*

With the worldwide use of spinetoram, resistance has been frequently demonstrated in various species of pests such as *Plutella xylostella* (Lima Neto et al 2016), environmental concentration of spinetoram in

waterbodies is assumed increasing in the future Results of our study showed the interactive effects of spinetoram and *Microcystis* on *D pulex*, suggesting the consideration of complexed factors in the environmental risk assessment of pesticides Given the importance of *Daphnia* in freshwater food webs, the interactive effects caused by spinetoram and *Microcystis* can potentially impact the ecological function of *D pulex*, and cause great influence on aquatic ecosystems

5 Conclusions

In the present study, we exposed *D pulex* to the pesticide spinetoram, and tested the fitness-related traits Survival and reproduction of *D pulex* showed significant reduction under the acute and chronic exposure to spinetoram Simultaneously, we demonstrated the interactive effects of spinetoram and the main bloom-forming phytoplankton, *M aeruginosa*, on the individual fitness and population dynamics of *D pulex* This highlighted that spinetoram had severe toxic effects on *D pulex* and could reduce the tolerance of *D pulex* to *M aeruginosa*, that would cause great effects on *D pulex* population in natural waterbodies

Declarations

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Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare no competing interests

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Conceptualization, data curation, and writing: QS, XJ and YZ; review and editing: XZ and TG; methodology and formal analysis: BL; funding acquisition: QS and TG All authors have read and agreed to the published version of the manuscript

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Figures

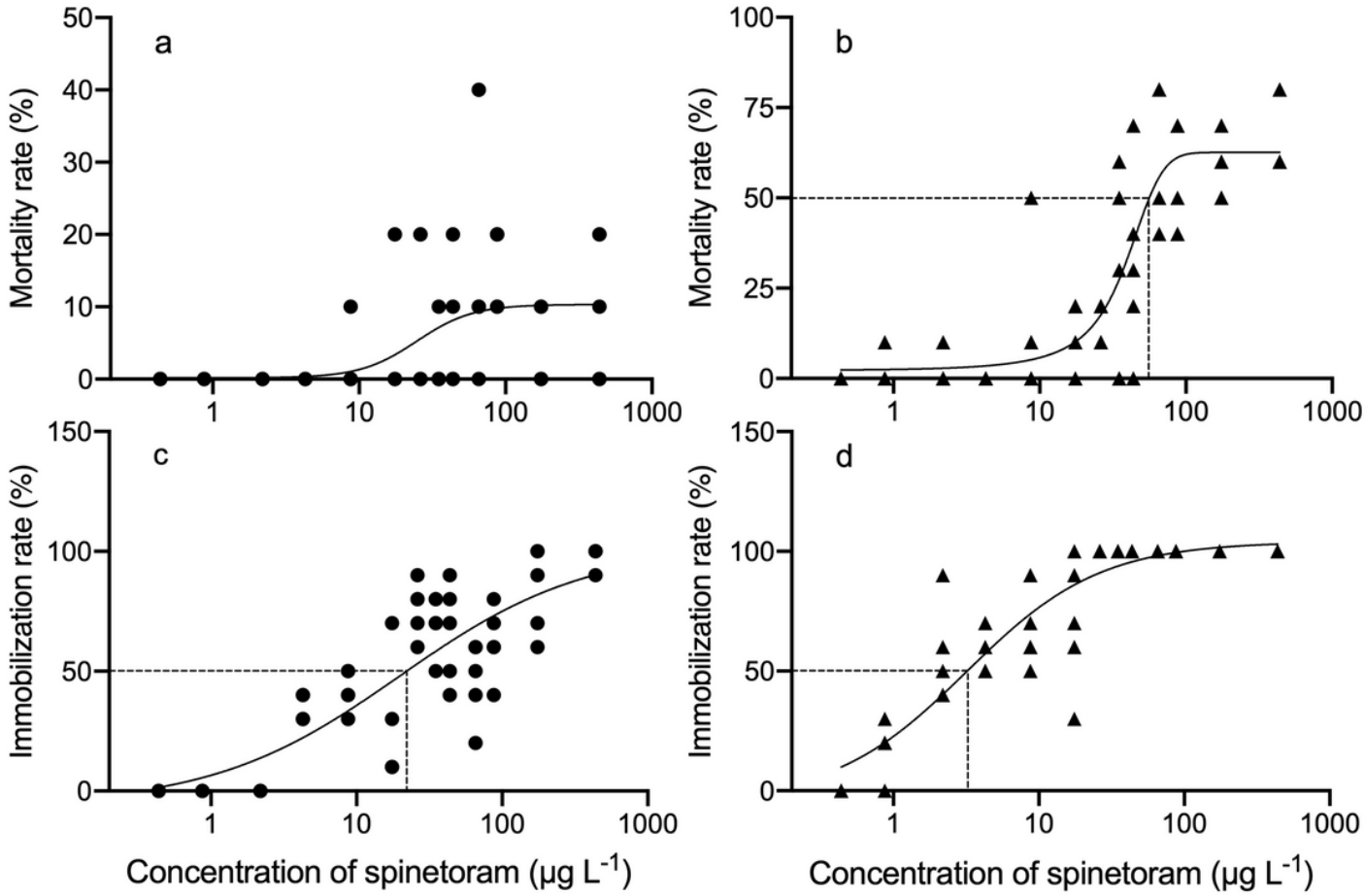

Figure 1

Mortality rate (A: 24 h, B: 48 h) and immobilization rate (C: 24 h, D: 48 h) of D pulex under the acute exposure to spinetoram at different concentrations Dose-response curves were adopted to fit the mortality rate and immobilization rate against time, respectively The dotted lines indicated the value of LC50 at 48 h (B), and the value of EC50 of immobilization rate at 24 h (C) and 48 h (D), respectively

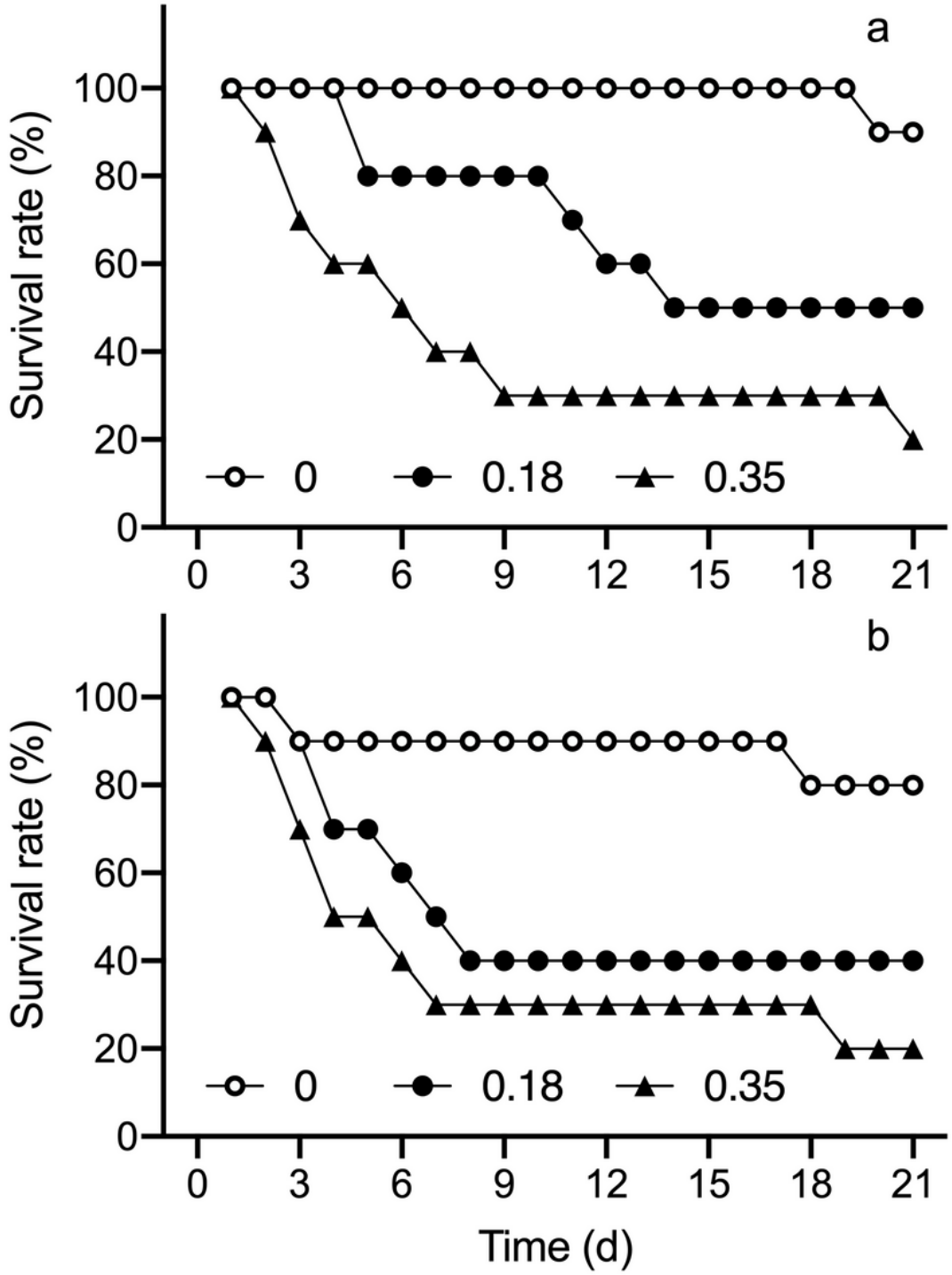

Figure 2

The survival of D pulex fed on different food (A: Chlorella, B: Chlorella + Microcystis) under the chronic exposure to spinetoram during the 21-day experiment

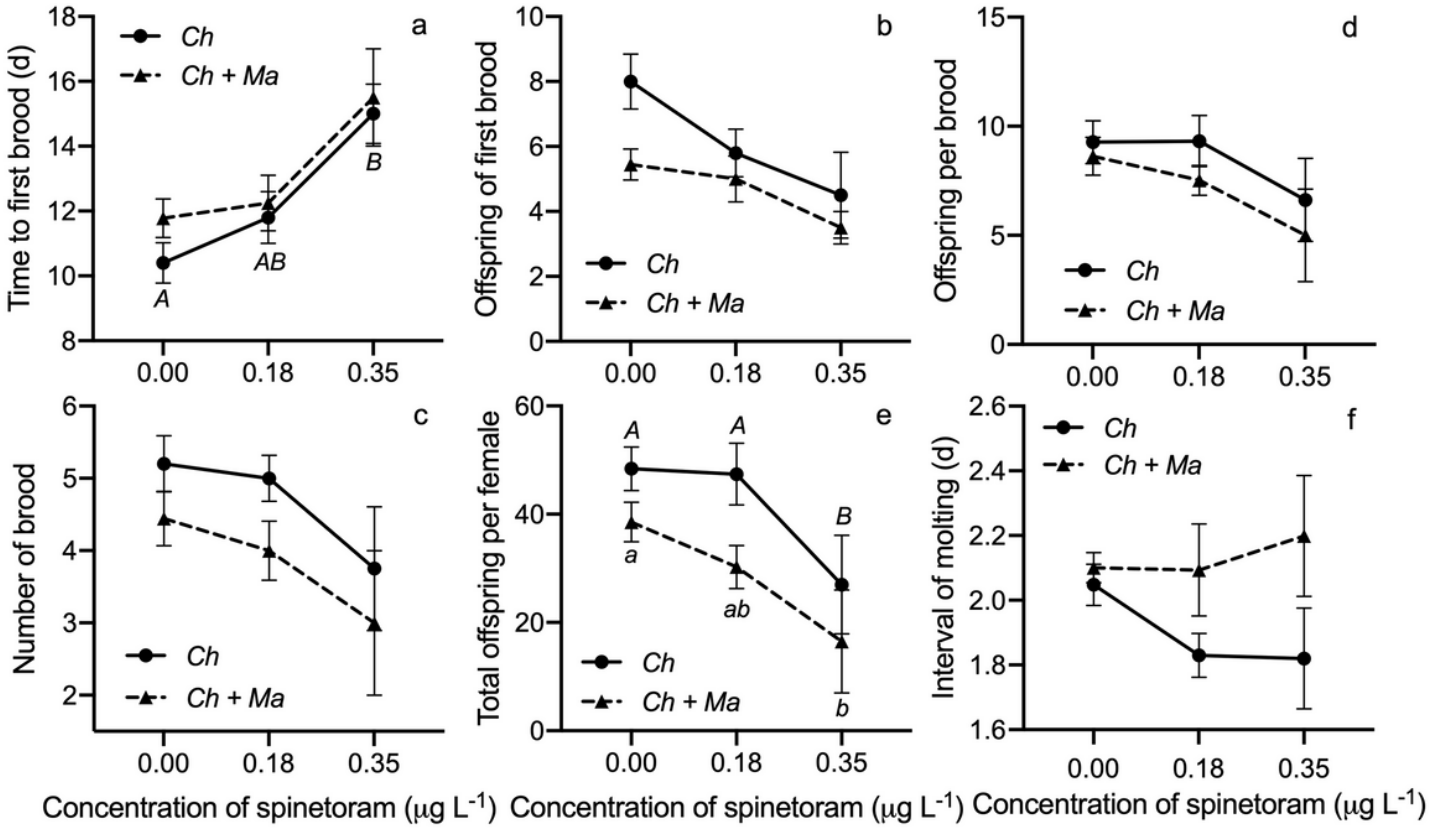

Figure 3

The time to first brood (A), offspring of first brood (B), number of brood (C), offspring per brood (D), total offspring per female (E) and number of molting (F) of D pulex fed on different food (solid lines: Chlorella, short dashed lines: Chlorella + Microcystis) under the exposure to spinetoram at different concentrations during the 21-day experiment Error bars indicate one SE, and different letters denotes significant difference at p < 005 among the three spinetoram concentration treatments at certain food treatment

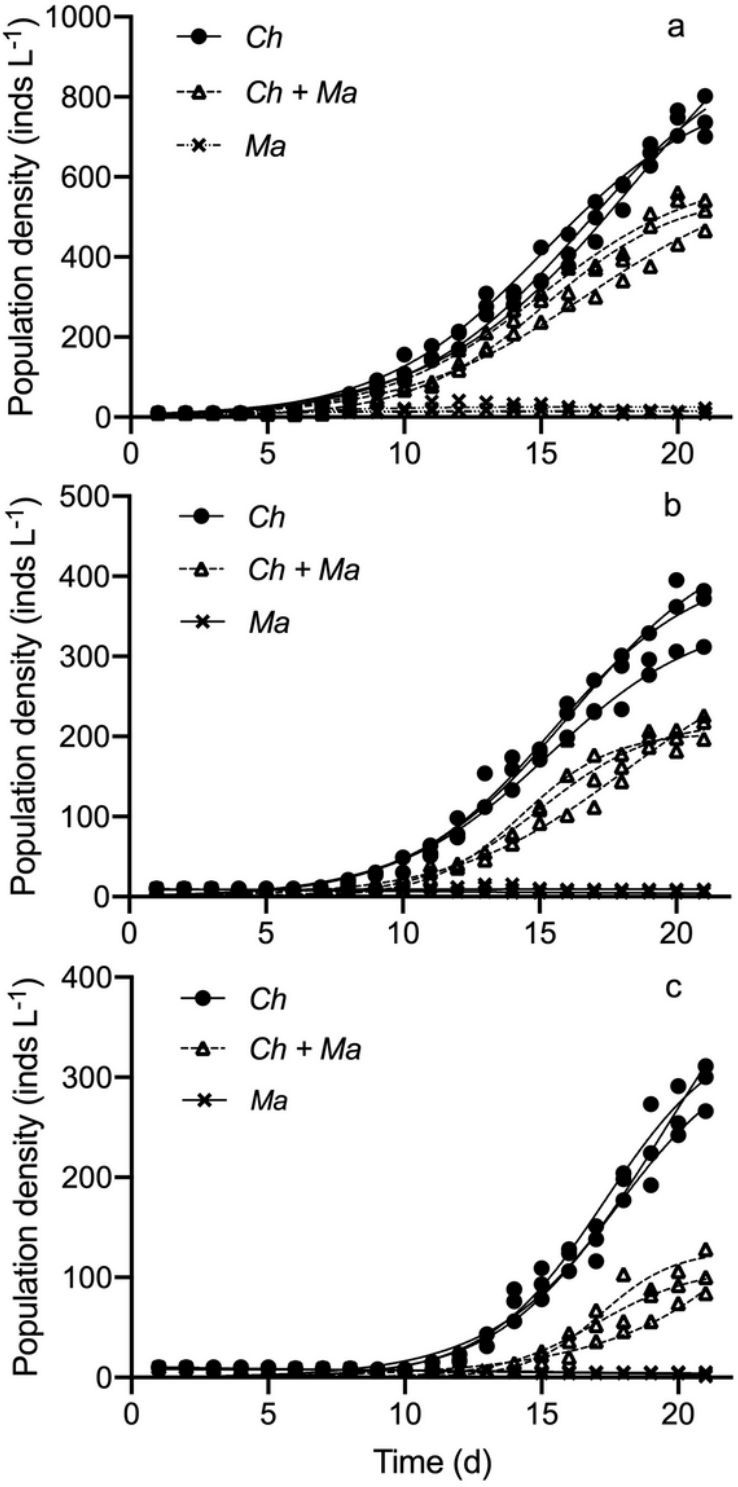

Figure 4

The population dynamics of D pulex fed on different food (black dots: Chlorella, empty triangles: Chlorella + Microcystis) under the exposure to spinetoram at different concentrations (A: 0 µg L−1, B: 018 µg L−1, C: 035 µg L−1) Logistic population growth curves were adopted to fit the population dynamics against time (solid lines: Chlorella, short dashed lines: Chlorella + Microcystis), respectively

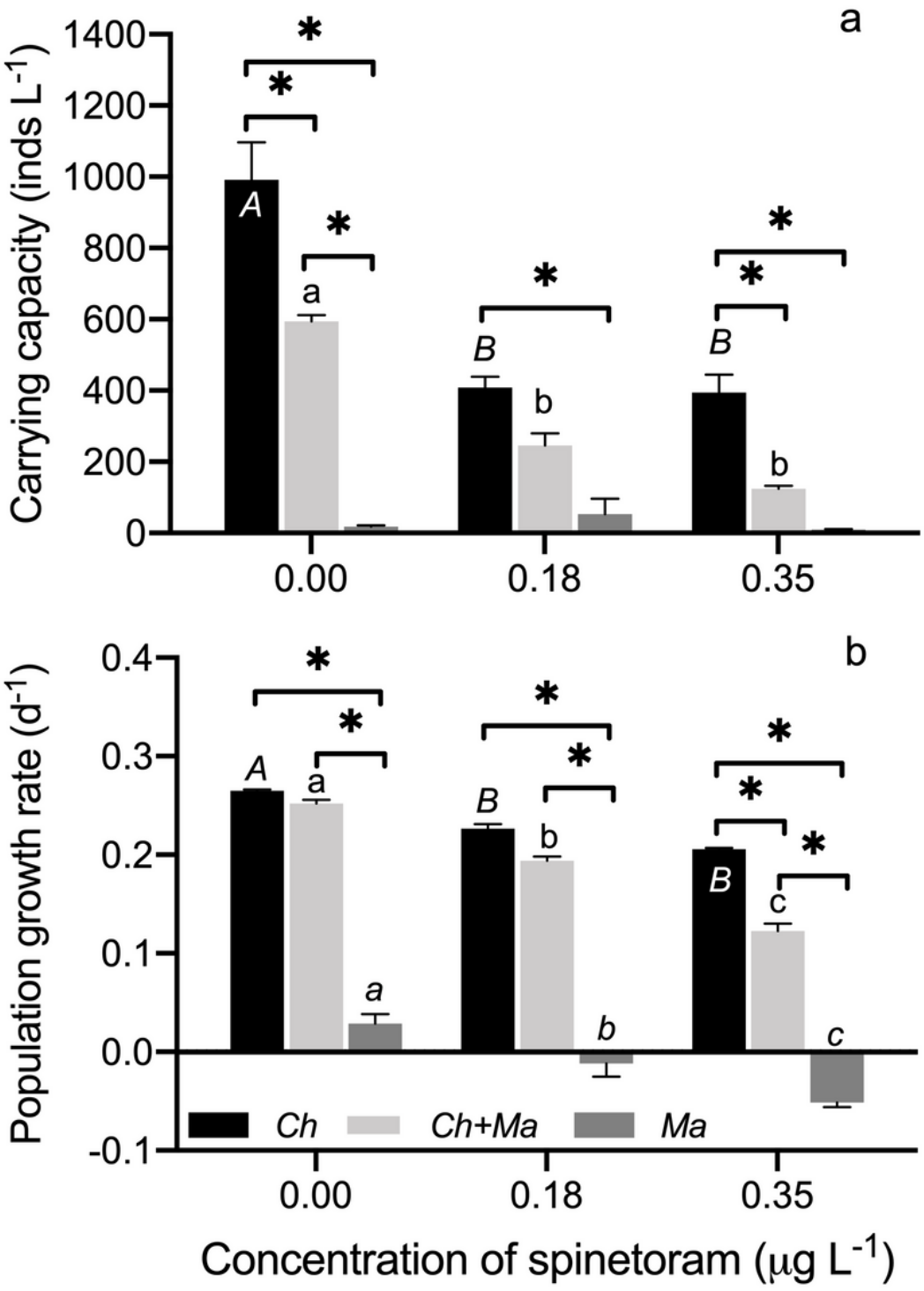

Figure 5

Carrying capacity (A) and population growth rate (B) of D pulex populaitons fed with different food (black bars: Chlorella, grey bars: Chlorella + Microcystis) under the exposure to spinetoram at different concentrations Error bars indicate one SE, and different words denotes significant difference among the three spinetoram concentration treatments at certain food treatment ($p < 005$) The asterisk above short

line denotes significant difference between two food treatments at certain spinetoram concentration (p < 005)