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Changes in the life history traits of *Daphnia magna* are associated with the gut microbiota composition shaped by diet and antibiotics

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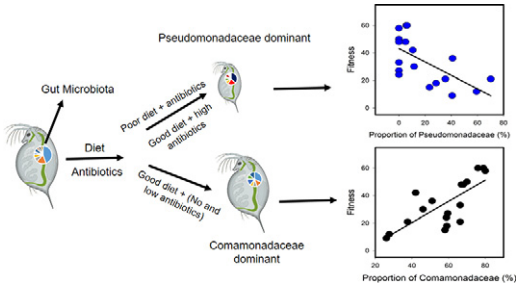
HIGHLIGHTS

- Environmental stressors can alter host-microbiota relationship.
- Diet and antibiotics shaped *Daphnia* gut microbiota.
- Life history traits were correlated with changes in bacterial abundance.
- Changes in phenotypes are important to understand host-microbiota interactions.

GRAPHICAL ABSTRACT



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ABSTRACT

The gut microbiota has a crucial role in host physiology and fitness. Host-microbiota relationships can be disrupted by environmental stressors, which further affect host growth and survival. However, the link between host performance and the gut microbiota composition shaped by increasing antibiotic pollution under different food conditions is not clearly understood. In the present study, we used *Daphnia magna* as a model organism to investigate the interactive effects of diets (*Chlorella* with or without *Microcystis*) and antibiotics on its life history traits, gut microbiota alterations, and their relationship. The results showed that poor diet consumption by *D. magna* at low and high antibiotic concentrations reduced reproduction and survival. Under good diet conditions, the fitness was reduced only at a high antibiotic concentration. Under good diet conditions, high concentration of antibiotics reduced the abundance of Comamonadaceae and increased the abundance of Pseudomonadaceae, whereas under poor diet conditions, both low and high concentrations of antibiotics increased the abundance of Pseudomonadaceae. Performances of life history traits were positively correlated with an increased abundance of Comamonadaceae but were negatively correlated with increased Pseudomonadaceae abundance. The results of this study revealed the interactive effects of diet and antibiotics on *D. magna* fitness and correlations between bacterial abundance and life history traits, which has important implications for understanding the effects of pollutants on host-microbiota interactions through changes in phenotypes.

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1. Introduction

The host microbiota is increasingly recognized as an integral part of animals due to its cooperation with the host in many biological processes. Recent advances in sequencing technology and other molecular

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techniques have revealed that the microbiota plays an essential role in host fitness, adaptation and evolution (Foster et al., 2017; King et al., 2013; Qi et al., 2009). The gut microbiota benefits its host in several ways, such as promoting digestion, providing essential nutrients, detoxifying harmful compounds, and competing with pathogens (Blaser et al., 2013; Koskella et al., 2017; Nicholson et al., 2012). The compositions of the gut microbiota associated with hosts are central to the overall health and performance of the host. Variations in gut microbiota influence hosts on different scales, ranging from life history traits to adaptation and ecological processes (Bang et al., 2018; Macke et al., 2017b). These variations are multifarious and dependent on genetic and environmental factors that influence host-microbiota interactions (Mitri and Richard Foster, 2013; Sison-Mangus et al., 2018). It will be difficult to investigate host genetic factors that shape gut microbiome without understanding the effects of environmental stressors. To understand these complex host-microbiomes interactions and how different microbiomes carry out ecosystem functions in cooperation with their hosts, we need simple model organisms for use in investigations.

Daphnia is a central food web component in freshwater habitats and has been used as model organism for many toxicological and ecological studies (Lyu et al., 2014; Lyu et al., 2013; Miner Brooks et al., 2012; Yang et al., 2011). Recently, the *Daphnia* gut microbiota has been shown to influence its fitness and survival (Peerakietkhajorn et al., 2016; Peerakietkhajorn et al., 2015; Sison-Mangus et al., 2015). In addition, tolerance of *Daphnia* to toxic *Microcystis* is linked with its gut microbiota (Macke et al., 2017a). The susceptibility of a *Daphnia* isolate susceptible to toxic *Microcystis* was significantly reduced after being transplanted with the gut microbiota from genotype of *Daphnia* exhibiting tolerance to *Microcystis* (Macke et al., 2017a). Moreover, *Daphnia* lacking gut bacterial communities are more sensitive to chemicals compared to *Daphnia* associated with bacteria (Manakul et al., 2017). In *Daphnia*, increasing temperature (≥26 °C) in the absence of bacteria led to a reduction in hatching success and severe morphological abnormalities compared to *Daphnia* grown with bacteria (Mushegian et al., 2016). However, these beneficial effects can be affected by environmental factors due to the disruption of *Daphnia*-microbiota relationships (Deng et al., 2016; Gorokhova et al., 2015).

Increases in specific environmental stressors in aquatic environments, such as toxic diets, antibiotics, and nanoparticles can disturb the gut microbiome, i.e. loss of beneficial or increase of harmful microbes, a phenomenon typically termed as "dysbiosis" (Chen et al., 2018; Cox et al., 2014; Jin et al., 2019; Langdon et al., 2016). The gut microbiota of mice was altered by microplastics and induced bile acids metabolism disorder (Jin et al., 2019). In addition, the gut microbiota of collembolans is regulated by the diet and changes in microbiota are linked to collembolan growth (Xiang et al., 2019). Among these environmental factors, diet is an important determinant affecting *Daphnia* growth, reproduction, and survival (Bovyn et al., 2018; Callens et al., 2015; Xiang et al., 2019). Recent studies have shown that the beneficial effects of a good diet in daphnids are increased by the gut microbiota (Callens et al., 2015; Mushegian and Ebert, 2017). A poor diet is one that either lacks essential nutrients necessary for the host or contains toxins that the host is unable to metabolize (Demott and Müller-Navarra, 2003). The gut microbiota assist hosts during these adverse conditions. Recently, toxic *Microcystis* had dominated many freshwater bodies and can reduce *Daphnia* growth and survival due to its toxicity and low nutritional value (Ger et al., 2016; Lyu et al., 2015; Yang et al., 2011). Previous studies showed that some daphnids can tolerate *Microcystis* toxicity through induced tolerance and maternal effects (Akbar et al., 2017b; Lyu et al., 2019; Lyu et al., 2016a; Lyu et al., 2016b), which are affected by environmental factors (Lyu et al., 2017) and nutrients availability (Akbar et al., 2017a). Later, it was observed that tolerance to *Microcystis* is due to gut microbiota (Macke et al., 2017a). However, multiple stressors exist in natural environments, and the presence of additional stressors can affect these beneficial effects by disruption of the gut microbiota. This disruption may be due to direct negative effects on host physiology and/or indirect effects from alteration of the gut microbiota (Evariste et al., 2019; Sun et al., 2019). To better understand the mechanisms of host-microbiota interactions, it is important to investigate the combined impact of environmental factors. In this way, we can link changes in microbiota composition with host fitness, which could promote understanding of these interactions under multiple stressors.

As outbreaks of cyanobacterial blooms frequently occur and antibiotic contamination is increasing worldwide, most microbiome studies have focused on the individual effects of diet regimes and antibiotics in shaping and disrupting microbiota and its influence on host fecundity and survival (Gorokhova et al., 2015; Mushegian and Ebert, 2017; Zhu et al., 2018). *Microcystis* species are toxic to *Daphnia*, which can be alleviated by the gut microbiota, but this relationship can be disrupted by antibiotics (Dalla Bona et al., 2015; Gorokhova et al., 2015). However, to date, the interactive effects of diets and antibiotics on the relationship between the gut microbiota and fitness of daphnids remain poorly understood. In this study, we hypothesized the following: (1) the combined effects of diet and antibiotics shape gut microbiota of *D. magna*; (2) poor diet and antibiotics negatively affect key life history traits of *D. magna*; and (3) changes in the life history traits of *D. magna* are associated with the composition of the gut microbiota shaped by diet and antibiotics. To test these hypotheses, we exposed *D. magna* to different combinations of diets and antibiotics and recorded the key life history traits and the composition of the gut microbiota.

2. Materials and methods

2.1. Experimental organisms and cultivation

The freshwater crustacean *Daphnia magna* Straus, maintained in the laboratory for over 20 years, was cultured in COMBO medium, which was refreshed three times a week. *D. magna* were fed the green alga *Chlorella pyrenoidosa* (1.5 mg C L^{-1}) daily in 500-mL glass beakers containing 400 mL of COMBO medium at 25 °C and 40 μmol photons m^{-2} s^{-1} with a 14:10 h light:dark cycle.

C. pyrenoidosa and toxic *Microcystis aeruginosa* (PCC 7806) were mass cultured separately in 1-L Erlenmeyer flasks axenically using BG-11 medium under continuous aeration at 25 °C, with a light intensity of 40 μmol photons m^{-2} s^{-1} using a fluorescent light and a light:dark period of 14:10 h. The cultures were manually shaken three times daily. The algae, with single or two-cell morphotypes, were harvested at the exponential growth phase by centrifugation at 4500 rpm for 15 min and then stored at 4 °C.

2.2. Experimental design

Tetracycline hydrochloride (Sigma Aldrich) is a broad spectrum antibiotic against both gram-positive and gram-negative bacteria that inhibits bacterial protein synthesis by preventing the association of aminoacyl-tRNA with the bacterial ribosome. A stock solution was first made by dissolving tetracycline in 1 M NaOH to yield a 1 mg L^{-1} solution, which was then diluted in sterile COMBO medium to obtain the desired concentrations.

Third-brood *D. magna* were cultured in 200 mL of COMBO medium in 250-mL beakers in triplicate (eight daphnids per beaker) and were fed *C. pyrenoidosa* (1.5 mg C L^{-1}). The incubation parameters (temperature, light intensity, light-dark ratio) were the same as those used for the algae culture conditions. On the 8th day, neonates produced by mothers were used for further experiments, while the mothers were used to analyze the gut microbiota, as neonates too small for analyses of gut microbiota. The microbiota of these mothers were used as a second control for comparisons with the gut microbiota of control daphnid treatments after two weeks. The microbiota of the bulk media was also analyzed in triplicate. The neonates were transferred to fresh COMBO media and grown for 24 h at 100% *C. pyrenoidosa*, as previous studies

have shown that the gut microbiota in *Daphnia* neonates can be established within 1–2 days (Macke et al., 2017a). After 24 h, neonates were transferred to their respective treatments. Five *D. magna* were transferred into each 150-mL sterile flask with 100 mL of COMBO media (15 individuals per replicate per treatment). The neonates were cultured under good diet (100% *C. pyrenoidosa*) or poor diet (80% *C. pyrenoidosa*: 20% *Microcystis*) conditions with low (1 μg L^{-1}) or high (10 μg L^{-1}) antibiotic concentrations. The control did not contain antibiotics (0 μg L^{-1}). In addition, ten *D. magna* were used for survival analysis, as preliminary experiments showed a high mortality of *D. magna* in the poor diet and antibiotics treatments after 15 days, and not enough daphnids could be obtained for gut microbiota analysis if experiments lasted for three weeks. Furthermore, because a previous study showed differences in gut microbiota between live and dead daphnids (Preiswerk et al., 2018), the use of dead daphnids for microbiota analysis may not represent the actual symbionts. Life history traits are common fitness indicators of *Daphnia* adaptation under adverse conditions (Olijnyk and Nelson, 2013). The time to first brood, the number of neonates produced at first brood, body length at maturation (measured from the head to the base of the spine), and total number of neonates produced were determined for two weeks. The survival of *D. magna* (10 daphnids) under different diet and antibiotic concentration treatments was investigated for 21 days.

2.3. Bacterial composition analysis

After two weeks, *D. magna* cultured in different conditions were placed in fresh sterile COMBO medium for 24 h, to remove food particles and non-symbiotic bacteria from their guts. Subsequently, *D. magna* were gently washed twice with sterile water to remove the external bacterial communities and then dissected under microscope in a laminar flow hood to isolate their guts. Microbial DNA was extracted using an E.Z.N.A.® soil DNA kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer's protocols (for more details see Supporting information S1 (Materials and methods)). Sequence data is deposited in the Sequence Read Database (SRA) and is available online under BioProject PRJNA561047.

2.4. Statistical analysis

Life history parameters, such as the time to first brood, the number of neonates produced at first brood, body length at maturation and the total number of neonates were determined by two-way ANOVA and presented as the means and standard deviation. Statistical analyses were performed using SigmaPlot v.14.0. Post hoc analysis between various treatments and multiple comparisons were determined by Tukey's HSD, with P value < 0.05 considered significant. The data were analyzed for normality, and Levene's test was performed to assess the equality of variance. Differences in survival rates between treatments were tested with the Kaplan-Meier log-rank test, which estimates the probability of an event that is death (SigmaPlot v.14.0).

Differences in the abundances of six dominant bacterial genera between different treatments that had three replicates were assessed by analysis of variance (ANOVA) with post-hoc Scheffe test. Treatments with $P < 0.05$ were considered significantly different. To avoid false discovery rate (FDR) in significant results, an FDR adjusted P-values were used. To observe the overall diversity of microbiota between different treatments, nonmetric multidimensional scaling (NMDS) with Bray-Curtis (Vegan 2.3-5) and weighted and unweighted UniFrac distances were calculated using Phyloseq. The individual and interactive effects of diet and antibiotics were determined using PERMANONA ANOSIM. Treatments with $P < 0.05$ were considered significantly different.

Spearman's correlation analysis between two variables (bacterial abundance in treatment and life history traits) was performed by bivariate analysis in SigmaPlot v.14.0, where a slope that was significantly different from zero ($\alpha = 0.05$) indicated that the bacterial abundance had a significant effect on a given life-history trait.

3. Results

3.1. Life history traits

Daphnia adopt different fitness strategies, with variables such as the time to first brood, the number of neonates produced at first brood, body length and total number of neonates varying for local adaptation during limited resources and environmental stressors. The time to first brood was reduced in *D. magna* grown in good and poor diet at a low tetracycline concentration (1 μg L^{-1}) compared to the high antibiotic concentration (10 μg L^{-1}) and no antibiotic treatments (two-way ANOVA; $P < 0.05$; Fig. 1a). *D. magna* grown under good diet and high antibiotic conditions produced fewer neonates at the first brood compared to those treated without or with a low concentration of antibiotic (two-way ANOVA; $P < 0.05$; Fig. 1c). Neonates produced at the first brood were not significantly different (two-way ANOVA; $P = 0.87$; Fig. 1c) between good diet with high antibiotic concentrations and poor diet without antibiotics. Under good diet conditions, the *D. magna* body length at maturation was reduced under high antibiotic concentrations compared to that observed in *D. magna* treated without or with a low antibiotic concentration (two-way ANOVA; $P < 0.05$; Fig. 1b). In contrast, under poor diet conditions, *D. magna* body length at maturation was reduced both at the low and high antibiotic concentration treatments compared to the treatment without antibiotics (two-way ANOVA; $P < 0.05$; Fig. 1b). The total number of neonates produced by *D. magna* was significantly reduced by the high antibiotic concentration treatment under good diet conditions compared to that observed in *D. magna* treated without or with a low antibiotic concentration (two-way ANOVA; $P < 0.05$; Fig. 1d), whereas poor diet conditions in the presence of antibiotics produced the lowest number of *D. magna* neonates (two-way ANOVA; $P < 0.001$; Fig. 1d).

3.2. Survival

Under good diet conditions, the survival of *D. magna* was significantly reduced by antibiotics compared to conditions without antibiotics (log rank test, $P < 0.035$; Fig. 2a), but a significant difference in survival was not observed between the low and high antibiotic concentration treatments (log rank test, $P = 0.063$; Fig. 2a). Under poor diet conditions, *D. magna* survival was reduced in all the treatments after 15 days, whereas the survival of *D. magna* grown in the high antibiotic concentration was significantly reduced compared to the low and without antibiotics treatments (log rank test; $P < 0.003$; Fig. 2b). A significant difference was not observed in the survival of *D. magna* grown under poor diet conditions without antibiotics and treated with a low concentration of antibiotic (log rank test; $P = 0.574$; Fig. 2b).

3.3. D. magna gut microbiota

The V3-V4 hypervariable regions of the bacteria 16S rRNA gene amplified with primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) produced a total number of 2139 reads. After trimming the raw sequences, the optimized sequences were clustered at 97% sequence identity, resulting in a total number of 186 operational taxonomic units (OTUs). The gut microbiota of *D. magna* comprised of few dominant OTUs accounting for the majority of the reads, with Proteobacteria (10.5–92%), Bacteroidetes (5–49%) and Actinobacteria (0.8–9.2%) as the dominant phyla (Figs. S1, S2). At the family level, Comamonadaceae and Pseudomonadaceae dominated most of the treatments, followed by Sphingobacteriaceae. However, the composition of these taxa varied among the different treatments (Fig. 3). In the good diet treatments without antibiotics or a low antibiotic concentration, the *D. magna* microbiota was dominated by

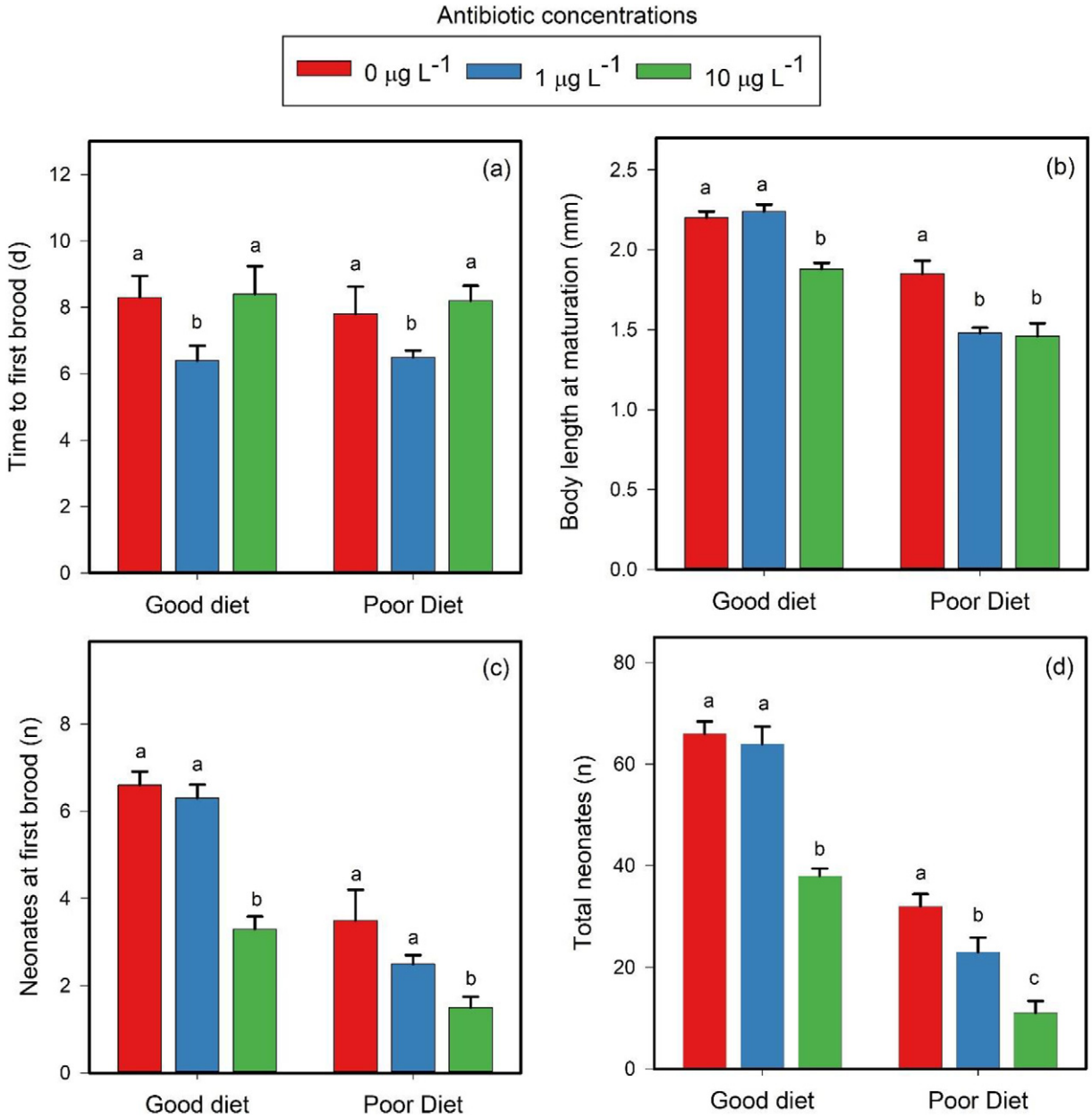

Fig. 1. Effects of diet and antibiotic concentrations on *D. magna* life history parameters: time to first brood (a); body length at maturation (b); number of neonates produced at first brood (c); total number of neonates (d); error bars are the standard deviation, and different letters represent significant differences between treatments (two-way ANOVA; $P < 0.05$).

Comamonadaceae (70 ± 10.8%) and was not significantly different from that of the mothers (one-way ANOVA; $P < 0.05$; Figs. 3, S3). The abundance of Comamonadaceae was significantly decreased by 50 ± 4.5% in the high antibiotic concentration treatment under good diet conditions, with Pseudomonadaceae and Sphingobacteriaceae becoming more abundant compared to *D. magna* cultured under good diet conditions without antibiotics and treated with a low antibiotic concentration. The abundance of bacteria in *D. magna* fed a poor diet with different antibiotics varied from that observed in *D. magna* from the good diet treatment. For *D. magna* under poor diet conditions, treatment with a high antibiotic concentration reduced the abundance of Comamonadaceae (28 ± 5.4%) and increased that of Pseudomonadaceae (60 ± 6.2%), whereas in the low antibiotic concentration treatment, the abundance of Comamonadaceae was not reduced compared to the treatment without antibiotics, while that of Pseudomonadaceae was increased by 23 ± 2.5% (Figs. 3, S3). Furthermore, the low and high antibiotic concentration treatments also reduced the abundances of Sphingobacteriaceae and *Sphingobacteriales* compared to the treatment without antibiotics (Figs. 3, S3).

3.4. Specific bacterial abundance

3.4.1. Ideonella

The genus *Ideonella* of the family Comamonadaceae was the most dominant in all the treatments, although its proportion varied among the different treatments (one-way ANOVA; $P < 0.001$; Table S1). The abundance of *Ideonella* was 60 and 65% in *D. magna* treated with 1 μg L^{-1} of antibiotic under both good and poor diet conditions respectively, and was significantly different from that observed in the other treatments (one-way ANOVA; $P < 0.001$; Table S1). However, the abundance of *Ideonella* significantly decreased under poor diet in the low or high antibiotic concentration treatments compared to all other treatments (one-way ANOVA; $P < 0.001$; Table S1).

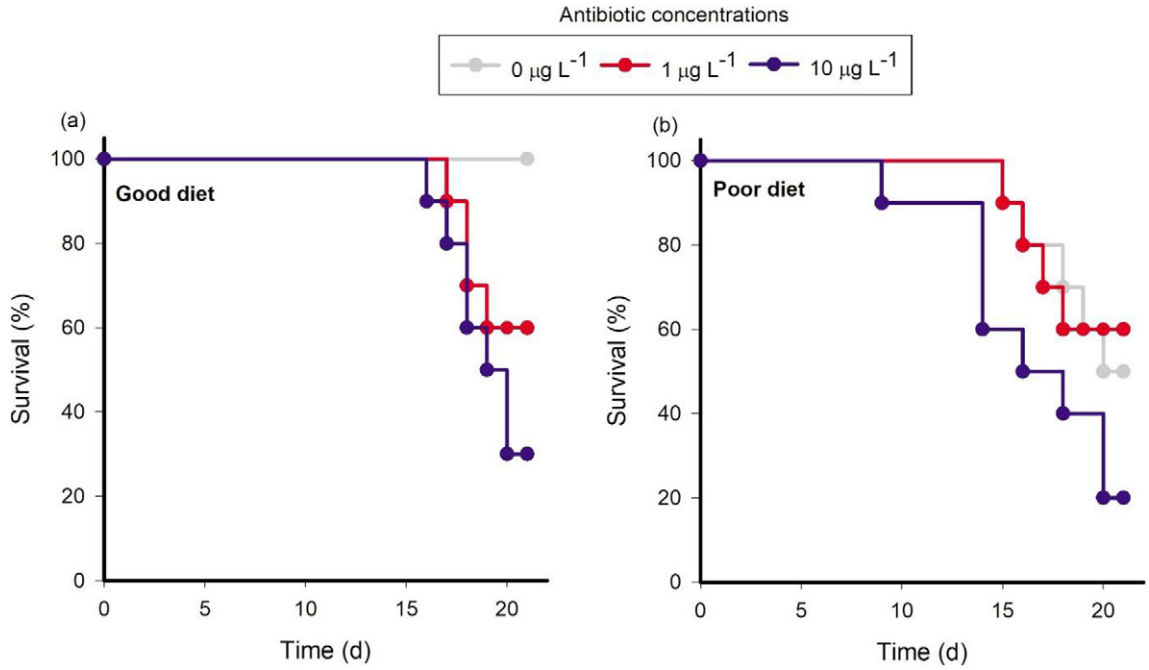

Fig. 2. Survival (%) of *D. magna* under different diets: good diet (a), poor diet (b), and antibiotic concentrations for three weeks.

3.4.2. Limnohabitans

Another dominant Comamonadaceae member present in the *D. magna* gut microbiota was the genus *Limnohabitans*, the abundance of which varied among the different treatments (one-way ANOVA; $P < 0.001$; Fig. 4; Table S1). Under poor diet conditions in the low or high antibiotic concentrations, the abundance of *Limnohabitans* was significantly decreased compared to that observed under poor diet conditions without antibiotics (one-way ANOVA; $P < 0.035$; Table S1).

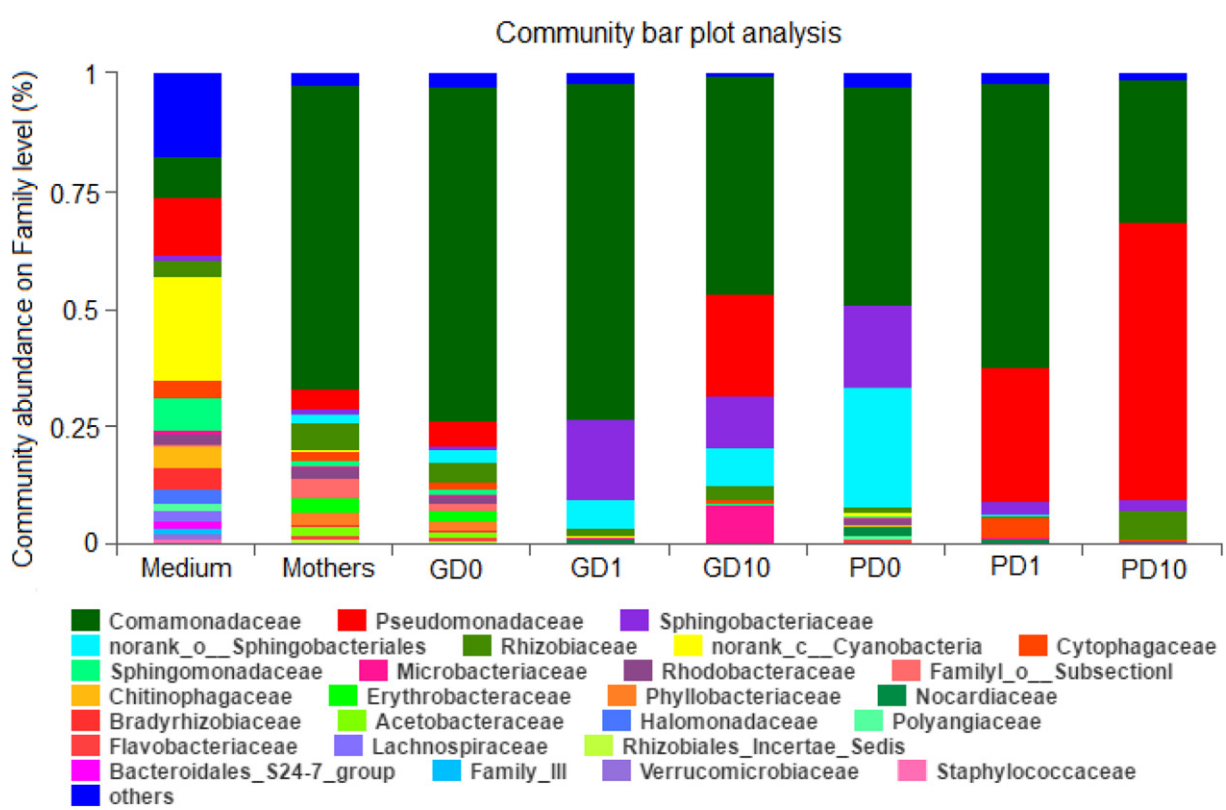

Fig. 3. Relative abundance of the *D. magna* gut microbiota at the family level under different diets and antibiotic concentration conditions. GD0 (good diet without antibiotics), GD1 (good diet with a low antibiotic concentration, 1 μg L^{-1}), GD10 (good diet with a high antibiotic concentration, 10 μg L^{-1}), PD0 (poor diet without antibiotics), PD1 (poor diet with a low antibiotic concentration, 1 μg L^{-1}), PD10 (poor diet with a high antibiotic concentration, 10 μg L^{-1}). Each value is an average of triplicate measurements.

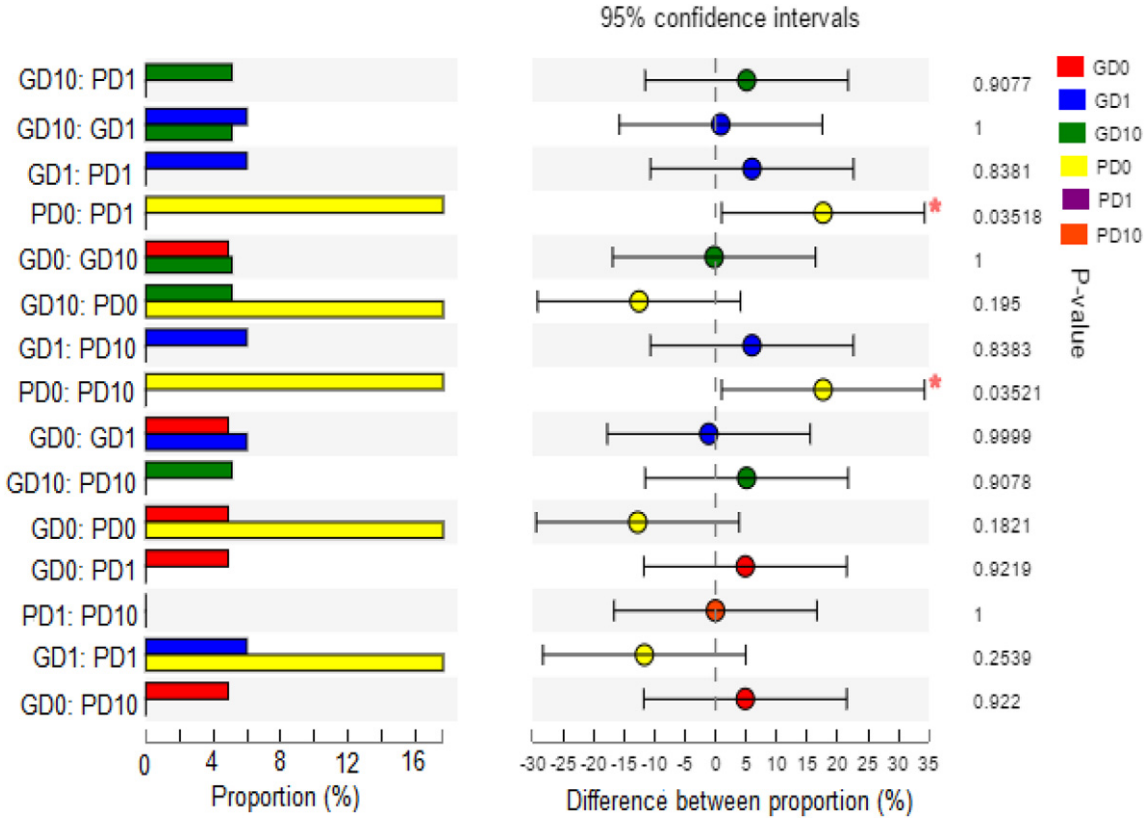

Fig. 4. Percent proportion of *Limnohabitans* in the different treatments. *P*-values are given between different treatments (one-way ANOVA), and treatments with significant difference are marked with asterisk. GD0 (good diet without antibiotics), GD1 (good diet with a low antibiotic concentration, 1 μg L^{-1}), GD10 (good diet with a high antibiotic concentration, 10 μg L^{-1}), PD0 (poor diet without antibiotics), PD1 (poor diet with a low antibiotic concentration, 1 μg L^{-1}), PD10 (poor diet with a high antibiotic concentration, 10 μg L^{-1}).

However, a significant difference was not observed between treatments under good diet conditions with or without antibiotics (one-way ANOVA; $P = 0.99$; Table S1).

3.4.3. Pseudomonas

Members of the genus *Pseudomonas* (*Pseudomonas brenneri* & unclassified *Pseudomonas* sp.) were significantly abundant in the

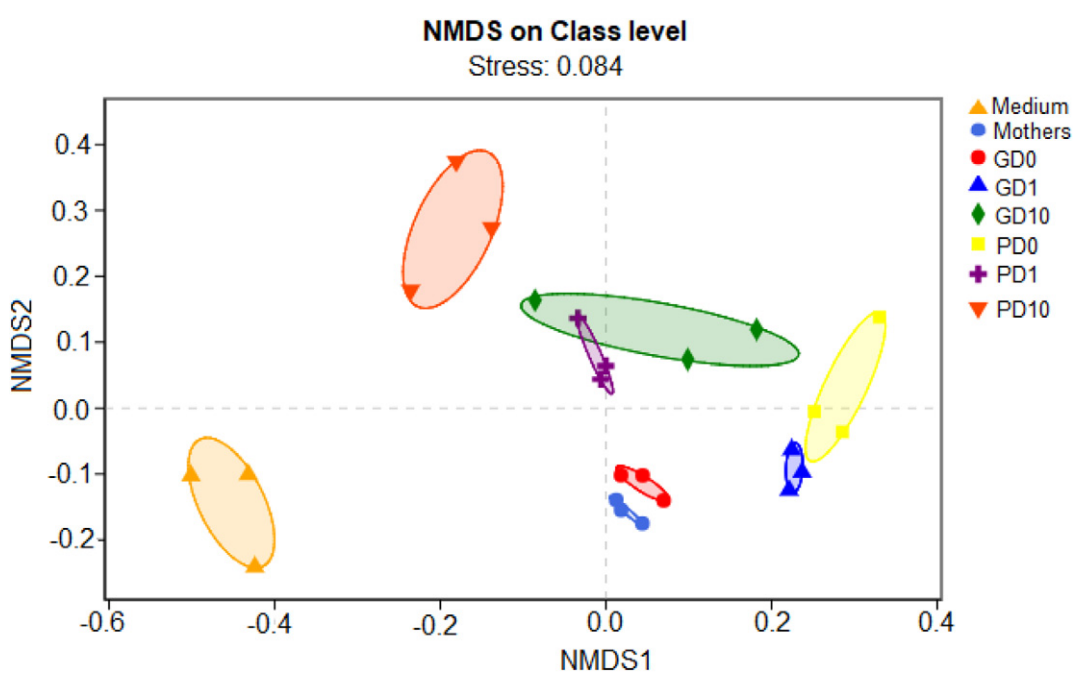

Fig. 5. Nonmetric multidimensional scaling (NMDS) with Bray-Curtis. GD0 (good diet without antibiotics), GD1 (good diet with a low antibiotic concentration, 1 μg L^{-1}), GD10 (good diet with a high antibiotic concentration, 10 μg L^{-1}), PD0 (poor diet without antibiotics), PD1 (poor diet with a low antibiotic concentration, 1 μg L^{-1}), PD10 (poor diet with a high antibiotic concentration, 10 μg L^{-1}).

D. magna gut microbiota under poor diet conditions in the high antibiotic concentration treatment, followed by the poor diet and low antibiotic concentration treatment (one-way ANOVA; $P < 0.0001$; Table S1). A significant difference in the abundance of *Pseudomonas* in *D. magna* was not observed between the low and high antibiotic concentration treatments under good diet conditions (one-way ANOVA; $P = 0.12$; Table S1), and between the good and poor diet conditions without antibiotics (one-way ANOVA; $P = 0.983$; Table S1).

Other abundant genera included *Pedobacter*, *Sphingobacteriales* and *Rhizobium*. In addition, some rare species detected in some treatments included *Rhodobacter*, *Rhodococus*, *Actinobacteria* and *Ensifer* (for details, see Supporting information (Results); Table S1).

3.5. NMDS analysis

The NMDS analysis for Bray-Curtis and weighted and unweighted UniFrac distances between treatments showed that the *D. magna* gut microbiota was different from that of the culture medium (Fig. 5). The PERMANOVA results showed that there was no significant difference in the microbiota at the diet level (Fig. 5; Table 1). The interactive effects of diet and antibiotics significantly altered the microbiota composition. Specifically, the interactive effects of poor diet and antibiotics significantly altered the microbiota composition more than good diet or antibiotics alone (Fig. 5; Table 1).

3.6. Correlation between bacteria and life history traits

Pearson's correlation analysis results showed that life history traits were significantly correlated with the relative abundance at the family level (Figs. 6, S4–6). The total number of neonates ($R^2 = 0.55$; $P < 0.004$; Table S2; Fig. 6c), body length at maturation ($R^2 = 0.493$; $P < 0.001$; Table S2; Fig. S6) and number of neonates produced at first brood ($R^2 = 0.463$; $P < 0.001$; Table S2; Fig. S5) were positively correlated with the relative abundance of Comamonadaceae. In contrast, the total number of neonates ($R^2 = 0.41$; $P < 0.0042$; Fig. 6a; Table S2), body length ($R^2 = 0.41$; $P < 0.004$; Table S2; Fig. S6) and number of neonates produced at first brood ($R^2 = 0.245$; $P < 0.036$; Table S2; Fig. S5) were negatively correlated with the abundance of Pseudomonadaceae. The abundance of Sphingobacteriaceae did not have a significant correlation with any life history trait ($R^2 = 0.04$; $P = 0.41$; Table S2; Fig. 6b), and a negative correlation between the abundances of Comamonadaceae and Pseudomonadaceae was observed ($R^2 = 0.51$; $P < 0.008$; Fig. 6d).

4. Discussion

In this study, the gut microbiota and the abundances of dominant bacteria associated with *D. magna* reproductive output were examined under good and poor diet conditions for the different antibiotic concentration treatments. As we hypothesized, poor diet and the presence of an antibiotic altered the gut microbiota and reduced growth and survival of *D. magna*, exhibiting synergistic interactions. The correlation analysis results showed that host life history traits, such as the number of neonates produced at first brood, body size at maturation, and total number of neonates were positively affected by the increased abundance of Comamonadaceae but negatively correlated with an increased abundance of Pseudomonadaceae. *Ideonella* and *Limnohabitans* were the most abundant Comamonadaceae. A poor performance of *D. magna* was due to the interactive effects of poor diet and antibiotics, which was associated with a reduction in the abundance of *Limnohabitans* and an increased abundance of *Pseudomonas* sp. Such results have an important implication for understanding *Daphnia*-microbiota interactions during the consumption of *Microcystis* under an additional stressor.

Table 1
PERMANOVA outcomes of the effects of diet and antibiotics (Ab) treatments individually and their effects on Bray-Curtis, weighted and unweight UniFrac distances.

Factor	df	Bray-Curtis			Weighted UniFrac			Unweighted UniFrac		
		R^2	*F*	*P*	R^2	*F*	*P*	R^2	*F*	*P*
Diet	1	0.8	1.83	0.1	0.24	2.48	0.094	0.445	3.2	0.1
Antibiotics	2	0.85	14.7	**0.001**	0.76	8.75	**0.002**	0.33	1.33	0.162
Diet × Ab	5	0.32	3.57	**0.024**	0.248	2.48	0.094	0.136	1.18	0.267
Good diet × Ab	2	0.7	8.5	**0.006**	0.64	5.53	**0.005**	0.38	1.89	**0.042**
Poor diet × Ab	2	0.90	30.2	**0.003**	0.90	27.1	**0.005**	0.34	1.56	0.065

4.1. D. magna core microbiota

The results of our study showed that *D. magna* has a unique gut microbiota dominated by Proteobacteria and Bacteroidetes. In agreement with previous reports, the most dominant family was Comamonadaceae (Freese and Schink, 2011; Kasalický et al., 2010; Qi et al., 2009). However, we observed that *Ideonella* was the most abundant Comamonadaceae rather than *Limnohabitans* and *Aeromonas*, as was described in previous studies (Freese and Schink, 2011; Peerakietkhajorn et al., 2016). In addition, in our study, the *Limnohabitans* species was different from previously identified *Limnohabitans* species in *Daphnia* (Peerakietkhajorn et al., 2016), demonstrating the generality of *Daphnia-Limnohabitans* symbiosis. The second most abundant group at the family level was Pseudomonadaceae, followed by Sphingobacteriaceae. *Pseudomonas brenneri* was detected in *D. magna*, the abundance of which varied in the different treatments. However, unclassified *Pseudomonas* sp. was more abundant in *D. magna* under good diet conditions treated with high antibiotic concentrations and under poor diet conditions treated with low and high antibiotic concentrations, resulting in reduced reproduction and survival. This unclassified strain may be more harmful to daphnids compared to *Pseudomonas brenneri*. In addition to *Ideonella*, *Limnohabitans* and *Pseudomonas* sp., other taxa detected in the gut of daphnids grown under different diet and antibiotic conditions included *Pedobacter*, *Sphingobacteriales*, *Ensifer* and *Rhizobium*. Moreover, other rare taxa, such as *Flectobacillus*, *Sphingomonas*, *Actinobacteria*, *Rhodococus*, *Hydrogenophaga* and *Pelomonas* were only present in some specific treatments. Detangling the individual effects of symbionts on *Daphnia* is difficult due to limitations in the ability to culture the associated microbes, and correlation studies of symbionts could further improve investigations of these interactions.

4.2. Interactive effects of diet and antibiotics on D. magna microbiota

Diet quality and quantity are two of the most important factors that influence host-microbiota interactions. In a previous study, the positive impacts of the gut microbiota were obvious for the good quality diet compared to a poor nutritional diet (Callens et al., 2018), where *Scenedesmus* was used as a food source at concentrations. In the current study, we used *Chlorella* mixed with *Microcystis*, which is more similar to natural conditions, as *Microcystis* species are frequently present in freshwater ecosystems and threaten daphnid growth and survival. In previous studies, the positive effects of diet and the negative effects of antibiotics on host gut microbiota have been studied separately (Callens et al., 2018; Gorokhova et al., 2015; Mushegian and Ebert, 2017). Diet and antibiotics may increase competition and/or cooperation between microbes. In natural environments, most of these interactions are competitive, as can be observed by culturing microbes (Foster et al., 2017). In contrast, microbial communities associated with hosts are predicted to exhibit cooperative behavior, such as in the human gut microbiota (Mitri and Richard Foster, 2013).

We observed that the interactive effects of diet and antibiotics caused significant changes in bacterial communities, and variations in

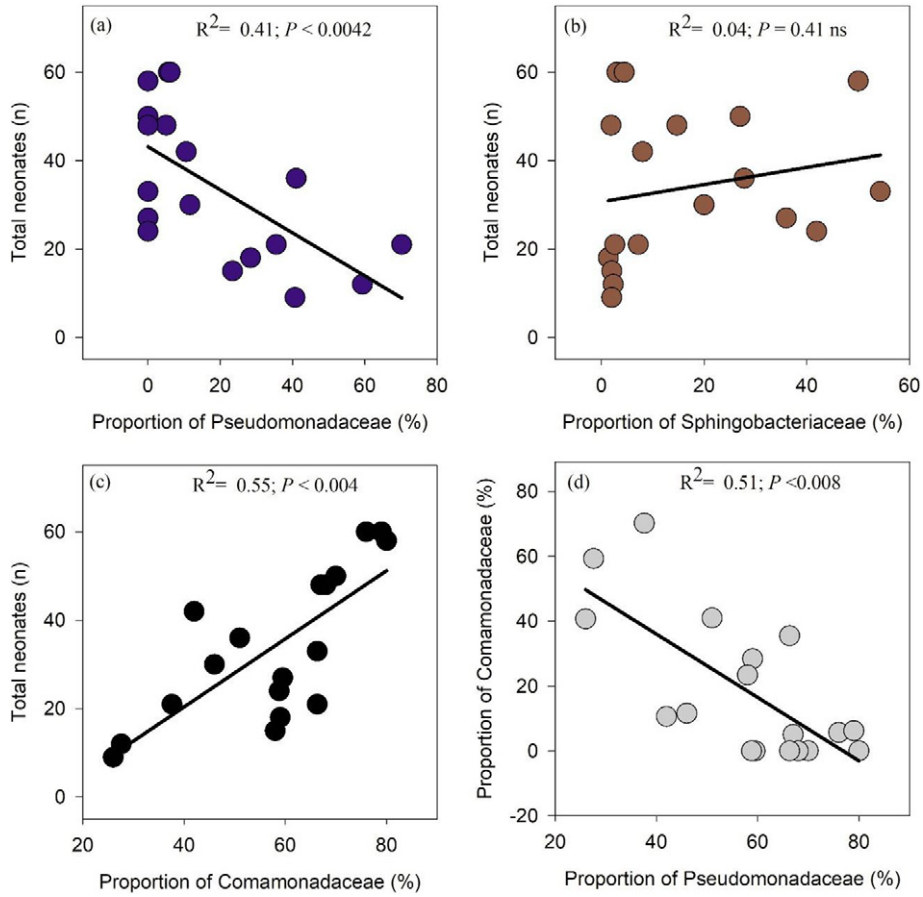

Fig. 6. Scatterplot of the total number of neonates and bacterial group proportion (family level) fitted by linear regression model. Pseudomonadaceae was significantly negatively correlated with the total number of neonates (a), while Sphingobacteriaceae has no significant correlation with the total number of neonates (b); Comamonadaceae abundance was significantly positively correlated (c); the abundances of two important bacterial groups (Comamonadaceae and Pseudomonadaceae) were negatively correlated (d). R^2 and *P* values are shown in each figure.

some specific bacterial strains affected host performance. Prior work has shown that single bacterial species identified from whole microbiota (*Acetobacter* or *Lactobacillus*), when inoculated into *Drosophila*, recover development and metabolism defects (Newell and Douglas, 2014). *Daphnia* grown under aseptic conditions exhibit reduced fecundity and survival compared to those supplemented with *Limnohabitans* and *Aeromonas* (Peerakietkhajorn et al., 2016; Sison-Mangus et al., 2015). In the current study, *Ideonella* was the dominant genus in most of the treatments. The abundance of *Ideonella* was significantly reduced by high antibiotic concentrations both at good and poor diet conditions. However, the *D. magna* performance was not linked with the abundance of *Ideonella*, as it was significantly reduced under poor diet without antibiotics compared to poor diet with low antibiotics concentrations. In contrast, *Limnohabitans* and *Sphingobacteriales* were less abundant compared to *Ideonella* but their reduction in daphnids grown under poor diet conditions with both low and high antibiotic concentrations negatively affected *D. magna* performance. This indicates that *Limnohabitans* and *Sphingobacteriales* may protect daphnids from *Microcystis* toxicity. Previous studies showed that planktonic bacteria can degrade algal organic matter and slowly utilize acetate, an important short chain fatty acid that is essential for animal hosts (Pérez and Sommaruga, 2006; Pester and Brune, 2007). Our study lacks controls for antibiotics effects on the food, as antibiotics affected *Microcystis* and *Chlorella* nutritional value and photosynthetic activity (Jin et al., 2012; Shang et al., 2015). However, the lowest antibiotics concentrations used in previous studies which negatively affected *Microcystis* and *Chlorella* nutritional value was higher than the high antibiotic concentration used in current study. Therefore, we expect that the indirect effects of tetracycline might be negligible. However, further studies are encouraged to decipher these indirect effects. Many free living and cyanobacterial bloom-associated bacteria have been demonstrated to degrade *Microcystis* and microcystins (Bourne et al., 1996; Li et al., 2016; Saito et al., 2003), but these interactions are not clearly understood in the *Daphnia* gut. Because *Microcystis* species lack essential nutrients, *Limnohabitans* and *Sphingobacteriales* may provide these nutrients to *Daphnia* or degrade *Microcystis* and microcystins, but the additional antibiotic stressor either kills these species and/or negatively affects *Daphnia* physiology to prevent symbiosis. *Pseudomonas* and *Pedobacter* were detected in *Daphnia* gut but their abundance varied in different treatments. The abundance of *Pseudomonas* and *Pedobacter* in different treatments showed their opportunistic nature. For instance, *Pedobacter* was abundant in good diet conditions both at low and high antibiotic concentrations compared to no antibiotic treatment, whereas, under poor diet conditions, it was only abundant in no antibiotic treatment. However, there were no obvious negative effects of *Pedobacter* on *D. magna* performance. *Pseudomonas* became abundant in daphnids consuming poor diets at both low and high antibiotic concentrations compared to no antibiotic treatment and was negatively linked with fitness. Similarly, under good diet conditions, *Pseudomonas* became abundant under high antibiotic concentration compared to low and no antibiotic treatments and also negatively affected *D. magna* performance. Prior work showed that *Daphnia* are dominated by opportunistic bacteria, including *Pseudomonas*, before death (Preiswerk et al., 2018), a phenomenon called the sit-and-wait (SAW) strategy, where opportunists wait and proliferate during an inactive or weak immune stage of the host (Lennon and Jones, 2011). However, it is unclear if *Pseudomonas* uses

this strategy or is recruited by daphnids to cope with antibiotics, as some *Pseudomonas* species are tetracycline resistant (Pang et al., 2019). Similarly, the tetracycline resistance gene tet (A) was detected in both rare and abundant species in the microbiome of *Daphnia obtusa* when grazing on pelagic aquatic bacterial communities (Eckert et al., 2016). However, the *Pseudomonas* species detected in this study was different from those previously reported resistant species and needs to be further investigated.

The gut microbiota transplantation from a *Microcystis*-resistant *Daphnia* genotype to a sensitive one increases the *Microcystis* tolerance of the sensitive genotype (Macke et al., 2017a). Our results showed a linkage between gut microbiota and life history traits, such as the total number of neonates, which is considered to be a strong trait for *Daphnia* success in stressful conditions. The abundance of Comamonadaceae was positively associated with the total number of neonates, whereas Pseudomonadaceae abundance was negatively correlated. This linkage can be further investigated if some specific trait, such as the time to first brood, body length, and total number of neonates is transferrable by microbiota or a specific bacterial species. The existence of a relationship between a life history trait and the microbiota may also be helpful in understanding small daphnid-microbiota interactions.

4.3. Gut microbiota and D. magna life history traits

Despite variations in the composition of microbiota at the species level in the different treatments, life history traits were positively correlated with the abundance of Comamonadaceae and negatively correlated with that of Pseudomonadaceae. Under good and poor diet conditions, either with no antibiotics or in the high antibiotic treatment, the time to first brood of *D. magna* was delayed compared to that observed under the poor and good diet conditions in the low antibiotic concentration. One possible reason for this result is that the high abundance of the genus *Ideonella* compared to that observed in the other treatments. The abundance of *Ideonella* was even higher in *D. magna* grown under good diet conditions without antibiotics. Similarly, the time to first reproduction decreased due to the interactive effects of a cocktail of antibiotics and food quality compared to that observed in daphnids grown without antibiotics (Zalewski et al., 2011). The time to first brood represents an early preparatory stage that is crucial for *Daphnia* growth and survival later on. *Daphnia* either prolong or shorten the time to first brood based on the nature of the stressful conditions (Ger et al., 2016). The physiology of the time to first brood remains poorly understood but is still an important trait used for assessing *Daphnia* fitness (Macke et al., 2017b). The lag phase of bacteria has been extensively studied and is an important factor for bacterial adaptation to new environment but is still poorly understood (Rolfe et al., 2012). How the bacterial lag phase and the time to first brood of *Daphnia* are interconnected, and may be an important issue to explore to assess *Daphnia*-microbiota interactions.

Daphnia performance in terms of large body size, the number of neonates produced at first brood, and the total number of neonates produced was linked with the abundance of specific bacterial groups in different treatments. Total neonate production is an important trait for reproductive output and organismal survival. In the current study, the relationship between the total number of neonates was strongly correlated with the abundance of Comamonadaceae and negatively correlated with that of Pseudomonadaceae. The body size of apo-symbiotic *D. magna* was shown to be increased after being inoculated with *Aeromonas* sp. (Sison-Mangus et al., 2015). In addition, an acute antibiotic concentration was shown to reduce daphnid gut microbiota abundance along with reductions in growth and body size (Gorokhova et al., 2015). *Daphnia* gut microbial communities exposed to the high antibiotic concentration were dominated by *Polynucleobacter* sp., *Xanthobacteriaceae* sp., and *Hansschlegelia* sp., which can increase body size when inoculated into *Daphnia*. In contrast, *Daphnia* treated with the low antibiotic concentration and without antibiotics were small and colonized by *Neisseriaceae* sp. Moreover, the OTUs richness of host associated microbiota shows positive correlations with normalized body size (Callens et al., 2018). In contrast, the body size of *D. magna* in our study was reduced in response to the high antibiotic concentration. Moreover, the microbiota in large-sized daphnids were dominated by Comamonadaceae, whereas Pseudomonadaceae was abundant in small-sized daphnids. The antibiotics used and bacterial communities identified in this study were different from those in a previous study (Callens et al., 2018). Variations in the identified bacterial communities may be due to the resistance potential of bacteria to different types of antibiotics, variations in *Daphnia* genotypes and their resistance to antibiotics. It is unclear whether a large body size is due to the abundance of Comamonadaceae and/or nutrients produced by Comamonadaceae or any other bacteria, as previous studies showed nitric oxide produced by bacteria increased longevity in *Caenorhabditis elegans* (Gusarov et al., 2013; Macke et al., 2017b).

Due to the high mortality rate observed after 15 days in our experiment, we could not correlate *Daphnia* survival and bacterial abundance, as not enough *Daphnia* survived after 21 days to evaluate the gut microbiota. However, the results showed trade-offs between reproductive output and survival in some treatments. Trade-offs between growth, reproduction, and survival with respect to changes in life history traits represent survival strategies used under adverse conditions (Olijnyk and Nelson, 2013). Our results showed that under good diet conditions, the performance of *Daphnia* under a low antibiotic concentration and without antibiotics was not significantly different with respect to life history traits except the time to first brood, and *Daphnia* treated with a low antibiotic concentration reproduced earlier. However, the survival of these daphnids was reduced compared to those fed a good diet without antibiotics (Figs. 1, 2a). We speculated that there is trade-off between early reproduction and survival. Another possible explanation is that a low antibiotic concentration reduces the abundance of Pseudomonadaceae and increased the proportion of *Sphingobacteriales* compared to *D. magna* provided a good diet without antibiotics. A previous study showed that doubling the abundance of *Commensalibacter* species increases the life span of butterfly *Speyeria mormonia* by 0.5 days, while *Rhodococcus* species abundance decreased by 0.7 days (Ravenscraft et al., 2019). A low antibiotic concentration may also be detrimental in the long term and requires further study to determine the possible effects and their correlations.

In summary, the results of this study demonstrated that the composition of the gut microbiota of *D. magna* was significantly shaped by the interactive effects of diet and antibiotics. Antibiotics can exert strong effects on the *Daphnia* gut microbiota under poor diet conditions, as observed by the reduction in the abundance of Comamonadaceae and the increased abundance of Pseudomonadaceae. Moreover, analysis of microbial community revealed that under good diet conditions without antibiotic and low antibiotic concentration, members of the gut microbiota were clearly distinguishable from that of high antibiotics concentration. Life history traits, such as the total number of neonates, body size at maturation, and neonates produced at the first brood were positively correlated with the increased abundance of Comamonadaceae and negatively correlated with a high abundance of Pseudomonadaceae. Under good diet conditions, only the high antibiotic concentration treatment reduced the reproductive output of *D. magna*, while survival was reduced by treatment with both low and high antibiotic concentrations compared to the no antibiotic treatment. Linking life history traits and changes in microbiota may enable us to better predict host-microbiota interactions under multiple stressors and requires further analysis to better understand these relationships.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.135827.

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