To flee or not to flee: detection, avoidance of and attraction to food resources by *Daphnia magna* studied with an olfactometer

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ABSTRACT

The cladoceran herbivore Daphnia magna *is a major consumer of phytoplankton in lakes. Therefore, this organism may control the phytoplankton community and the proliferation of some algae or cyanobacteria. Cladoceran behaviour and migration in relation to temperature, light or presence of planktivorous fishes have been well studied. In particular, it is known that the detection of kairomones produced by predators may induce avoidance. Avoidance could also occur with other semiochemicals such as cyanotoxins. In order to explore this hypothesis, we used an olfactometer to observe and measure the exploratory behaviour of* D. magna *individuals based on the motivation for food. Daphnids were allowed to choose between different compounds: water, a pure cyanotoxin,* i.e. *the microcystin-RR [(MC)-RR], extracts of one MC-producing strain (PMC 75.02) and one MC-free strain (PMC 87.02) of* Planktothrix agardhii, *or a green algae* Scenedesmus obliquus. *With this experimental design, we observed that i) cladocerans are able to detect resources with different qualities, ii) they can explore before exhibiting preferences, and iii) daphnids are able to avoid compounds that are potentially toxic (*e.g., *microcystins). First, daphnids explored the environment, subsequently (after about 1.5 h), they showed a significant tendency to stay where there is a profitable resource such as* S. obliquus. *These results also suggest that specimens of* D. magna *cannot detect MC compounds from* P. agardhii, *but they respond to it as a food resource. The study of zooplankton ability to explore the environment when exposed to semiochemicals needs further investigation.*

Key words: Daphnia magna, Planktothrix agardhii, *olfactometer, migration, secondary metabolites, cyanotoxins.*

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INTRODUCTION

Relationships between cyanobacteria and zooplankton are complex and affect aquatic ecosystems acting as positive or negative controls in microorganism communities (Ghadouani *et al.*, 2006; Oberhaus *et al.*, 2007). Cyanobacteria are considered as inadequate food for zooplankton such as daphnids (Wilson *et al.*, 2006). Indeed, i) cyanobacteria may be deficient in essential nutrients for zooplankton (De Bernardi and Giussani, 1990; Müller-Navarra *et al.*, 2000; Von Elert and Wolfram, 2001), and ii) some cyanobacteria are known to produce toxic metabolites (*e.g.,* cyanotoxins) that may harm or kill not only animals and humans (Carmichael, 1992; Hitzfield *et al.*, 2000; Codd *et al.*, 2005; Dittmann and Wiegand, 2006; Purdie *et al.*, 2009) but also daphnids and other zooplankton (Rohrlack *et al.*, 1999). Cyanotoxins may have various effects on zooplankton: decrease of swimming activity of freshwater cladocerans (Ferrao-Filho *et al.*, 2007), inhibition of digestive enzymes (Arnold, 1971; Nogueira *et al.*, 2006), inhibition of feeding rate, growth

and reproduction (Arnold, 1971; Hawkins and Lampert, 1989; DeMott, 1999), decrease of weight (Gliwicz and Lampert, 1990) or even lethally toxic effect (Nogueira *et al.*, 2004). These results, however, vary with experimental conditions (Lampert, 1987; Tillmanns *et al.*, 2008). Among the many parameters which may influence these impacts we found: i) density of the food provided to *Daphnia* (Kurmayer, 2001), ii) temperature and viscosity of the water (Abrusán, 2004), iii) size of the zooplankton (Hawkins and Lampert, 1989; Kirk and Gilbert, 1992), iv) species of the grazer (Kirk and Gilbert, 1992), v) species and strains of cyanobacteria (Nogueira *et al.*, 2006; Wilson *et al.*, 2006; Tillmanns *et al.*, 2008), or vi) type of metabolites provided (Nogueira *et al.*, 2006).

Among toxic secondary metabolites, cyanotoxins microcystins (MC) are the most abundant ones. Still, several studies have shown that other compounds produced by cyanobacteria can have a negative impact on zooplankton as well (Kurmayer, 2001; Reinikainen *et al.*, 2002; Lürling, 2003a; Rohrlack *et al.*, 2004; Wilson and Hay, 2007; Hulot

et al., 2012). Although the biological role of these compounds is debated (Schatz *et al.*, 2007), one hypothesis is their negative effect on planktonic herbivores (Lürling, 2003b). Indeed, through its predatory activity, herbivorous zooplankton, and particularly *Daphnia* sp., has a direct impact on phytoplankton (Vanni, 1987; Vanni and Findlay, 1990; Hansson and Carpenter, 1993; Brett and Goldman, 1996; Oberhaus *et al.*, 2007). *Daphnia* sp. is a major predator of algae (Kerfoot and Sih, 1987) and, in the absence of fish, zooplankton increases and phytoplankton decreases as a result of cascading trophic interactions (Christoffersen *et al.*, 1993). Therefore, the cyanobacterial dominance may be partly due to a low degree of control from large generalist zooplankton, as suggested by the negative relationship between cyanobacteria and cladoceran (Ghadouani *et al.*, 2006; Catherine *et al.*, 2008). Cyanobacteria secondary metabolites production may help to explain food selection and avoidance in freshwater ecosystems (Jüttner, 2005). Thus, studying *Daphnia* sp. behaviour facing different nutrition choices may contribute to explain why some phytoplankton species are favoured and may dominate the phytoplankton community.

Diel vertical migration of zooplankton is a daily pattern of movement in relation to temperature (Lampert and Grey, 2003), light (Stearns, 1975) and predator-prey interactions (De Meester *et al.*, 1995). Usually, the pattern is an evening ascent to surface waters and a morning descent to deep waters. The diel vertical migration of daphnids is associated with considerable metabolic cost (Stich and Lampert, 1984), but plays a key role in predator avoidance. This migration may be motivated by fish semiochemicals (Dodson, 1988; Loose, 1993; Lampert, 1993; De Meester *et al.*, 1995). Thus, daphnids are able to detect kairomones released by fishes (Beklioglu *et al.*, 2006). Other compounds may also modify the behaviour of zooplankton. For instance, organic compounds produced by algae and cyanobacteria can play an attractive and defensive role in aquatic ecosystems (Lass and Bittner, 2002; Fink *et al.*, 2006). Jüttner (2005) showed that diatoms and other phytoplanktonic species damaged by grazers may produce compounds that are toxic and repellent to herbivorous zooplankton (Jüttner, 2005). In a more recent article, Jüttner *et al.* (2010) showed that the cell disruption of the cyanobacterium *Microcystis* produces a defense signal, the b-Cyclocitral, that induces an increase in swimming velocity of *D. magna*. As a consequence, the signal induces the escape of the daphnids. Thus, the presence of fish or algae and the production of organic compounds such as kairomones influence Daphnia migration (Beklioglu *et al.*, 2008; Rinke and Petzoldt, 2008; Jüttner *et al.*, 2010; Slusarczyk and Pinel-Alloul, 2010).

The relationships between cyanobacteria and daphnids reflect the typical complexity of prey-predator interactions and much is still unknown. In particular, to our knowledge, the influence of the prey on the spatial displacement of the predator has been poorly studied. Since Daphnia's predators may elicit its migration, one might ask whether its prey (cyanobacteria) may also induce migration of the grazer (daphnids). To address this question, Van Gool and Ringelberg (1996) were the first to use an Y-tube olfactometer with an inflows of water. Their results showed that Daphnia may discriminate between clean water and water that previously contained either *Scenedesmus acuminatus* or *Planktothrix limnetica* but not *Planktothrix agardhii* producing MC. Olfactometers were rarely used in studies on aquatic invertebrates, but such devices were validated by numerous behavioural studies of semiochemical recognition in terrestrial species (Janssen *et al.*, 1990; Scutareanu *et al.*, 1996).

Based on studies on the effects of chemical compounds on terrestrial insects displacements (Dornhaus and Chittka, 2001; Meinwald and Eisner, 2008), of chemical defences against predation (Lass and Spaak, 2003), and on migration of zooplankton stimulated by the presence of kairomones (Rinke and Petzoldt, 2008; Beklioglu *et al.*, 2008; Slusarczyk and Pinel-Alloul, 2010), we explored – with olfactometers adapted to aquatic species – the migration of starved daphnids when exposed to different signals. Unlike other studies (Van Gool and Ringelberg, 1996; Roozen and Lürling, 2001), our design without inflow allowed movement of daphnids in the absence of rheotaxis influence. Thus, the main aim of our study was to observe not only daphnids' first choices, but also daphnids' behaviour over a long time, and to test assumptions on their exploratory ability. The second aim was to explore daphnids' behaviour facing different resources extracted from green algae or cyanobacteria that are potentially attractive or repulsive with a particular attention to MC.

METHODS

Experimental design

Origin and culture of organisms

We used two monoclonal and non-axenic strains of *Planktothrix (P.) agardhii* isolated from the *Base Nautique de Viry*, France (Yéprémian *et al.*, 2007) and maintained in the Paris museum collection (PMC). The *P. agardhii* strain PMC 75.02 produces mainly three variants of microcystins (*m/z* 981.6 [D-Asp³]MC-LR; *m/z* 1024.8 [D-Asp³]MC-RR; and *m/z* 1045.6 [D-Asp³]MC-HtyR). The *P. agardhii* strain PMC 87.02 is a MC-free strain (Yéprémian *et al.*, 2007). We refer to these strains in the following as the MC-strain and the MC-free strain respectively. However, both strains are able to produce other secondary metabolites that may affect *Daphnia magna* population dynamics (Hulot *et al.*, 2012). Thus, these two strains will allow a comparison among the effects of cyanobacterial compounds. Each strain was cul-

tured in modified 2 L Duran bottles containing 1 L of Z8 medium (Kotai, 1972) and inoculated with 100 mL of an exponential pre-culture. The bottles were placed in growth chambers at 20±1°C, and illuminated with cool white fluorescent tubes (L18W/21-840, Osram lumilux Plus Eco; OSRAM GmbH, Munich, Germany) with a 16:8 light:dark cycle, under 10±2 µmol photons m^{-2} s^{-1} (Yéprémian *et al.*, 2007). Growth of the cyanobacteria cultures was monitored by measuring optical density at 436 and 750 nm every two days with a spectrophotometer (Foy and Smith, 1980). The cultures were stopped at the plateau phase, and the MC-strain and MC-free strain were kept at -80°C for conservation, and thawed 24 h before use as a treatment. Microcystin-RR (Alexis® Biochemicals, San Diego, CA, USA) was selected as pure toxin whose toxicity mimics an exposure to a less toxic MC such as the demethyl forms (*e.g.,* LD$_{50}$ of [D-Asp^3]MC-LR ranged from 160 to 300 on mouse) (Sivonen and Jones, 1999).

Daphnia magna has a big size and is easily cultured in laboratory. Individuals were isolated from a pond situated at the *École Normale Supérieure* (Paris, France), and kept in an aquarium with Volvic water (Societé des Eaux de Volvic, Volvic, France) and fed on *Scenedesmus obliquus* (CCAP 276/6A). This alga, which is known to be a good resource for Daphnia (Lampert, 1987), was cultured at 22±1°C and under 14:10 h light-dark cycle in COMBO medium (Kilham *et al.*, 1998).

Our experiments in olfactometers were based on feeding stimulation, so daphnids were starved prior to the experiment. To do so, we isolated adults *D. magna* from stock culture 24 h prior to the experiments in vials containing pure Volvic (Societé des Eaux de Volvic) water.

Experimental system

The olfactometer consists of three glass jars connected *via* a Y-junction and corridors made of silicone tubes (Fig. 1). In one jar, *i.e.* the introduction jar, daphnids are introduced at the beginning of the experiment and they may eventually return in it. The two other jars are the migration jars containing the treatments and the individuals which can migrate into it. We used a Y-junction to have no asymmetry in our olfactometer. All corridors have the same diameter (5 mm) and the same length (50 mm), and the jars have a diameter of 50 mm and a volume of 100 mL. After filling the whole system with 100 mL of water, tubes were closed with clips adjoining the Y-junction to
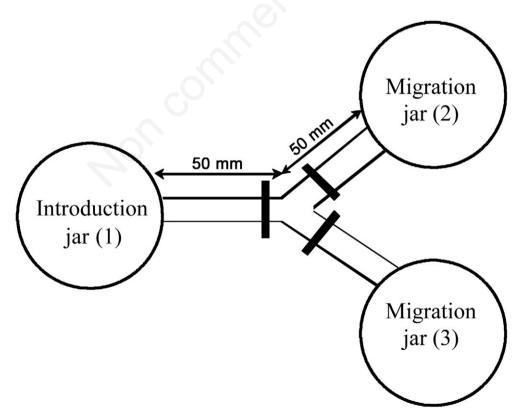

Fig. 1. Olfactometer. Individuals are introduced in an introduction jar (1) and may migrate to the migration jars (2 and 3) that contained the treatment. Clips are represented by black bars at the end of each Y-junction.

isolate the three parts of the olfactometer (Fig. 1). After removing equivalent volumes of water, we added the corresponding treatments in migration jars. One single starved daphnid was added to the introduction jar, the clips were removed and the movements of the individual were recorded during 1.5 h. This device thus allowed individual daphnids leaving the jar of introduction to choose between the migration jars depending on different treatments. Two 36 watt fluorescent tubes were suspended above the olfactometers and the light intensity arriving on experimental systems was 1500-2000 lux»21-28 µmol photons m^{-2} s^{-1} (PAR). This ensured homogeneous light for each olfactometer. Eight olfactometers were used at the same time to replicate all the experiments. To avoid any bias due to the relative position of the systems, olfactometers rotated at each repetition.

Experiments

Preliminary experiment

Our goal was to observe the behaviour of zooplankton in response to olfactory *stimuli*: we planned to measure the first choice as well as the choice made after some time, allowing exploration. To assess whether the observation lasted long enough (movements of daphnids became rare after 1.5 h) to give reliable data, and to ensure that there was no bias in the movements of daphnids due to the device, a preliminary test was conducted. In this test, the migration jars contained pure Volvic water (Societé des Eaux de Volvic) and one adult daphnid starved for 24 h was introduced in the jar of insertion. Seventeen repetitions with eight systems at the same time were conducted. For this preliminary experiment, we obtained 136 observations of different daphnids during 17 sessions of 90 min each.

Main experiment

We recorded daphnids' behaviour when they had the choice between i) MC-strain *vs* water, ii) MC-free strain *vs* water, iii) pure MC-RR *vs* water, iv) *S. obliquus vs* water, v) MC-strain *vs S. obliquus*, vi) MC-free strain *vs S. obliquus* vii) MC-free strain *vs* MC-strain, and viii) water *vs* water as a control. These eight treatments were tested in parallel with eight olfactometers and the tests were repeated 100 times strictly in the same conditions. Thus, we obtained 100 observations of different starved daphnids for each of the eight oppositions of treatments (Fig. 2) and our dataset was composed of 800 observations of displacements during 1.5 h. The water treatment contained pure Volvic (*Societé des Eaux de Volvic*) water. The density of resource in the treatments with MC-strain, MC-free strain and *S. obliquus* was calculated so that the final density of phytoplankton was 10 times Daphnia daily carbon requirement (0.2 mg.day^{-1}) (Sim *et al.*, 1994; Lampert, 1987). Treatments were kept at -80°C and thawed 24

hours before use, which disrupted cells. Unlike previous studies (Van Gool and Ringelberg, 1996; Roozen and Lürling, 2001), the present design devoid of filtering step, allowed to have the whole *S. obliquus* and *P. agardhii* cellular contents directly available to grazers.

The MC-RR treatment volume was calculated to get the same microcystin concentration as in the MC-strain treatment. The MC concentration in MC-strain treatment was 9.6 µg eq. MC-RR.L^{-1} assessed with a PP2A phosphatase assay (Rivasseau, 1999; Yepremian *et al.*, 2007; Müller *et al.*, in preparation). In the pure MC-RR treatment, we used purified MC-RR (Alexis® Biochemicals) solubilised with MeOH diluted at 1‰ and conserved at -20°C.

We analysed the first choice recorded for all individuals regardless of the migration time (between 0 and 1.5 h) and the position at the end of the observation period (after 1.5 h). We refer thereafter to these choices as the *first choice* and the *last choice* respectively. For the first choice, daphnids could choose between both treatments. For the last choice, daphnids could either rest in the first choice jar, move to the second treatment jar or come back to the introduction jar. These observations allowed us to study *Daphnia*'s behaviour (exploration, detection, choice) according to the presence or absence of compounds, as well as to test the attractive, repellent or neutral effects of compounds studied.

Statistical analysis

Two types of analysis were done: i) comparisons of the distribution of daphnids between the treatments for each choice, and ii) comparisons of the distributions of daphnids between the first and the last choices. We used Fisher's exact test with R software. Fisher's exact test is used in the analysis of contingency tables when sample sizes are small. For the first and last choices, we tested the significance of the deviation from a null hypothesis of equal distribution. For the comparison between the first and last choices, we tested the significance of the deviation of the last choice from the first choice. We set significant differences at a threshold of α=5% and marginal significance at a threshold of α=10%.

RESULTS

The results of the preliminary experiment performed with 135 replicates show that there is no bias in the experimental design (Tab. 1). One replicate was lost because of daphnid's death during the experiment. Tests on the first and last choices did not show significant difference between the number of daphnids present in the two migration jars (P=0.57). In addition, we did not observe any difference between the distribution of first and last choices (after 1.5 h). We observed that 71.8% of the individuals remained in the introduction jar; 7.4% of the individuals that left introduction jar returned to it; only 20.7% of the

individuals migrated definitely to either of the two treatment jars (Tab. 1).

In the main experiment, according to the treatments, between 66 and 79% of the individuals stayed in the introduction jar (Tab. 2). Between 0 to 6% of the individuals left this jar and returned to it later (Tab. 2). Again, there were no significant differences in the treatment water *vs* water at the first (P=0.885) and last choices (P=0.88) (Fig. 2a, Tab. 3) confirming the absence of bias from the device. Similarly, there are no significant differences when daphnids had to choose between MC-RR and water (Fig. 2b, Tab. 3), MC-strain and water (Fig. 2c, Tab. 3), MC-free strain and *S. obliquus* (Fig. 2e, Tab. 3), and MC-free strain and MC-strain (Fig. 2h, Tab. 3).

When submitted to the treatments MC-strain and *S. obliquus*, at the last choice, there were more daphnids in the jar with *S. obliquus* than in the jar with MC-strain (P=0.0007) (Fig. 2f, Tab. 3). The change between the first and last daphnids choices is significant (P=0.002). Indeed, daphnids seem to prefer *S. obliquus* after an exploratory phase. A marginal difference is revealed in the treatment *S. obliquus vs* water at the first choice (Fig. 2g, Tab. 3) with more daphnids in the jar containing *S. obliquus* than in the jar with water (P=0.09). The difference in daphnids choice for *S. obliquus vs* water is significant at the last choice (P=0.02). In the treatment where *D. magna* have the choice between MC-free strain and water, we also observed a significant effect of the treatment, with a prefer-

Tab. 1. Preliminary experiment. Daphnids' choice in olfactometers with pure Volvic water (*Societé des Eaux de Volvic*, Volvic, France).

		No. of daphnids			
	Motionless°	Jar 2[#]	Jar 3[§]	Return[^]	Total
First choice	97	21	17	-	135
Choice after 1.5 h	97	14	14	10	135

°*Number of individuals that stayed in introduction jar during the experiment;* [#]*number of individuals that migrated to the jar of migration 2;* [§]*number of individuals that migrated to the jar of migration 3;* [^]*number of individuals that returned to jar 1 after leaving it.*

Tab. 2. Main experiment. Distribution of individual daphnids after 1.5 h.

Treatments (%)	Water *vs* water	MC-RR *vs* water	MC-Pa *vs* water	MC-free Pa *vs* water	MC-free Pa *vs* Sc	MC-Pa *vs* Sc	Sc *vs* water	MC-Pa *vs* MC-free Pa
Motionless°	71	66	76	73	68	79	73	66
With motion[#]	29	28	23	25	31	19	24	30
Return[§]	0	6	1	2	1	2	3	4

MC-RR, treatment with pure microcystin-RR; MC-Pa, treatment with Planktothrix agardhii *microcystin-strain (PMC 75.02); MC-free Pa, treatment with* Planktothrix agardhii *microcystin-free strain (PMC 87.02); Sc, treatment with* Scenedesmus obliquus *extracts.*
°*Daphnids that did not move;* [#]*daphnids that moved;* [§]*daphnids that returned into the introduction jar.*
One hundred replicates were realised.

Tab. 3. P values of the Fisher's exact test on the distribution of daphnids among the treatments for each choice, and on the distributions of daphnids between the first and the last choice.

Treatment	Water *vs* water	MC-RR *vs* water	MC-Pa *vs* water	MC-free Pa *vs* water	MC-free Pa *vs* Sc	MC-Pa *vs* Sc	Sc *vs* water	MC-Pa *vs* MC-free Pa
Distribution for first choice	0.88	0.77	0.32	0.02	0.77	0.88	0.09	0.77
Distribution after 1.5 h	0.88	0.39	0.39	0.06	0.88	0.0007	0.02	1
Distribution for first choice *vs* distribution after 1.5 h	1	0.2	1	<0.0001	1	0.002	0.65	0.77

*MC-RR, treatment with pure microcystin-*RR*; MC-Pa, treatment with* Planktothrix agardhii *microcystin-strain (PMC 75.02); MC-free Pa, treatment with* Planktothrix agardhii *microcystin-free strain (PMC 87.02); Sc, treatment with* Scenedesmus obliquus *extracts.*

ence for water at the first choice (P=0.02) and a marginal effect with a preference for the MC-free strain at the last choice (P=0.06) (Fig. 2d, Tab. 3). Interestingly, we observed a highly significant inversion between the first and last choices (P<0.0001): daphnids migrated preferably at the beginning of the experiment to the jar containing water and finally in the jar with the MC-free strain treatment (Fig. 2d, Tab. 3).

DISCUSSION

Inspired by olfactometers used with terrestrial and aerial species and by previous studies with aquatic species, we used an aquatic Y-tube olfactometer to study the daphnids' behaviour towards semiochemicals. Preliminary tests of our device showed the absence of bias in our experimental system. Therefore, the differences in distribution observed in the main experiment when daphnids were subjected to different treatments are only due to their choices. Our results showed that *D. magna* did not exhibit preferences in four situations: MC-RR *vs* water, MC-strain *vs* water, MC-free strain *vs* S. obliquus, and MC-strain *vs* MC-free strain. Yet, *D. magna* showed preferences for *S. obliquus* and MC-free strain as an alternative from water, and *S. obliquus* as an alternative to MC-strain.

These results lead to several conclusions. First, the pure cyanotoxin MC-RR has no positive or repellent effects on daphnids. Therefore, the preference for *S. obliquus* as an alternative for the MC-strain cannot be attributed solely to the production of MC by the *P. agardhii* strain. Second, thanks to our design without inflows, we showed that *D. magna* is able to explore its environment and can modify its choice as a result. At their *last choice*, daphnids chose the treatment with *S. obliquus* when they had the choice between this green alga and the MC-strain, and they tended to choose the treatment with *S. obliquus* extracts when they had the choice between this alga and water. Daphnids being starved, this result shows that, after an exploration phase, they migrated to a nutritious source whatever the species. Indeed, when daphnids had the choice between MC-strain and *S. obliquus*, the analysis of the movements shows first a homogeneous distribution in both jars of migration but, after 1.5 h, they significantly moved to the jar containing *S. obliquus*, the resource they were fed on before the experiments. When daphnids had the choice between MC-free strain and water, *D. magna* preference changed between first and last choice, from water to the MC-free strain. These results show that these organisms might express displacement tropism as suggested by other studies (Van Gool and Ringelberg, 1996; Roozen and Lürling, 2001) and highlight the ability of *D. magna* individuals to explore their environment before making choices.

Van Gool and Ringelberg (1996) concluded from their

experiments that *D. galeata* x *hyalina* may be attracted by odours associated with edible algae and not by non-edible algae. In contrast, Roozen and Lürling (2001) did not observe preference of *D. galeata* x *hyalina*, nor of *D. pulex* or *D. magna* for the edible alga *S. obliquus*. However, they showed that the swimming speed of *D. magna* decreased with increased algal concentration. In the present
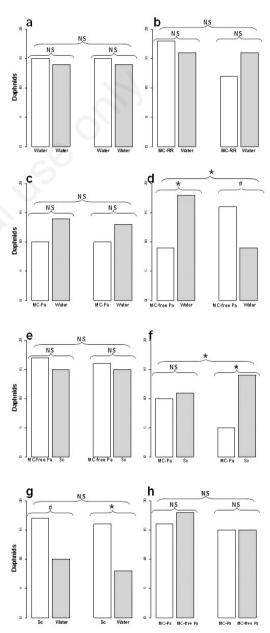

Fig. 2. Distribution of daphnids in the different treatments. In each panel, the first two bars give the first choice and last two bars the last choice. Legends as in Tab. 2. Results of statistical analyses are given as NS when not-significant, * when significant (α=5%) and # when marginally significant (α=10%).

study, we confirmed attraction by nutritious sources, but we also showed that the choice strategy of daphnids might be more complex than those measured in a *first-choice* test, as they might explore their environment and adapt their response accordingly.

Daphnids do not discriminate between the MC-strain and MC-free strain of *P. agardhii*. During blooms, the resource selectivity of major herbivorous zooplankton has an important control effect on the dynamic development of cyanobacteria (Catherine *et al.*, 2008). The daphnids' capacity to metabolise MC is of great importance. Thostrup and Christoffersen (1999) showed that microcystin can accumulate in daphnids. This observation and the absence of effects of MC on *D. magna* migration or discrimination between cyanobacterial strains that produce or not MC suggest that daphnids may play a role of vectors for the transfer of microcystins to higher trophic levels in the aquatic food web.

Besides the results obtained in this study, the experimental design presented here opens a way for further experiments. Indeed, daphnids migrate vertically in the water column according to light intensity (Stearns, 1975), temperature and food concentration (Lampert, 2003; Kessler and Lampert, 2004), presence of predators (De Meester *et al.*, 1995), but they may be confronted to variation of these factors at different depths. Their ability to detect kairomones raises the question of their behaviour in presence of multiple semiochemicals. More specifically, in our main experiment we used disrupted cells. With this procedure, *S. obliquus* and *P. agardhii* intracellular contents were directly available to grazers. We explicitly manipulated only one compound, MC-RR, but many other compounds in different treatments can influence daphnids. Thus, for a better understanding of daphnids' behaviour and of the effects of cyanobacterial secondary products on the relationship between cyanobacteria and herbivorous zooplankton, ideally we should study the metabolome of both *P. agardhii* strains and test daphnids' preference for cyanobacterial compounds or mixture of compounds with these new aquatic olfactometers.

Pawlik-Skowrońska *et al.* (2008) showed that the vertical distribution of *P. agardhii* was almost homogeneous in a shallow lake, whereas the MC distribution was not. In this way, potential signals for zooplankton were heterogeneously distributed in water. Horizontal gradients of abiotic conditions, such as light, temperature, dissolved oxygen and pH, are weaker in shallow lakes relative to vertical gradients in deep lakes and are less likely to influence horizontal migration (Burks *et al.*, 2002) than semiochemicals (Kvam and Kleiven, 1995). Heterogeneity in signals emerges also from the presence of predators and compounds produced by macrophytes and this heterogeneity can elicit different responses of zooplankton (Trochine *et al.*, 2009). However, during our experiments,

a big number of daphnids did not move and stayed in the introduction jar. This might be due to the absence of water inflows in our experimental device or because daphnids lost their rheotaxis with starvation as suggested by Roozen and Lürling (2001). Our experiments were based on feeding motivation: daphnids were starved with the aim of forcing them to look for food. This stimulation proved to be not enough. Further experiments could manipulate light that attracts daphnids in the absence of predators (Ringelberg, 1987).

CONCLUSIONS

Our results show that the olfactometer is a promising experimental device for understanding the relationship between phytoplankton and zooplankton and, more generally, the signals that could modify the spatial distribution of zooplankton in aquatic ecosystems. Moreover, our results indicate that daphnids are able to explore their environment in a first phase and express preference for profitable resources in a second phase.

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