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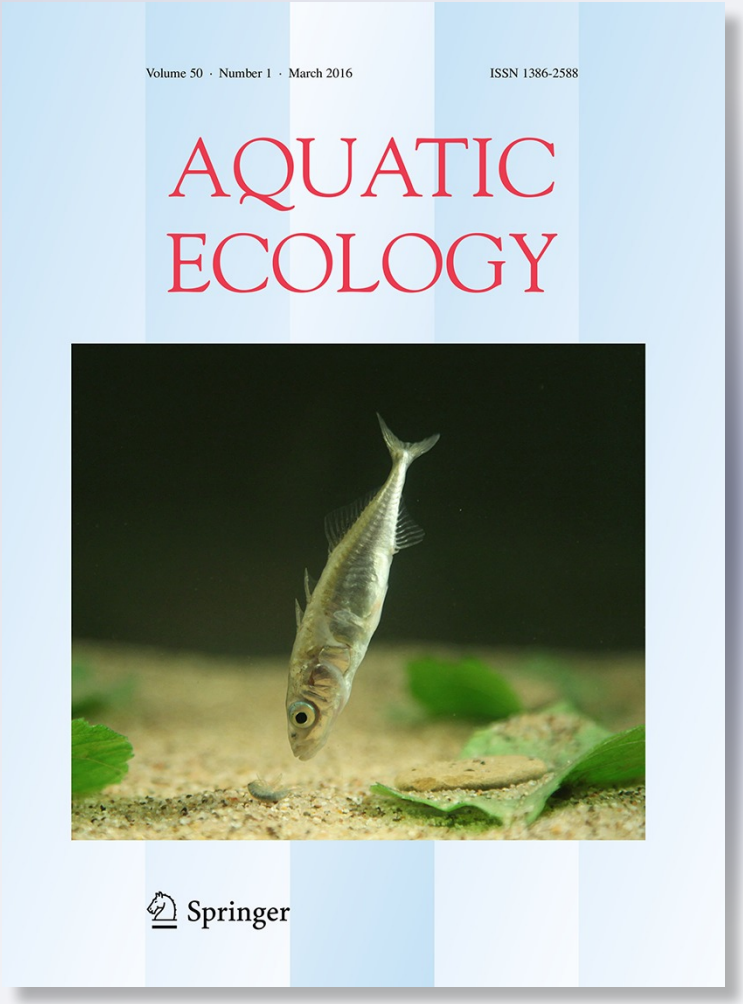

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Influence of macrophyte integrity on zooplankton habitat preference, emphasizing the released phenolic compounds and chromophoric dissolved organic matter

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Abstract Macrophyte health status can influence the composition of their exudates causing different effects on zooplankton behavior and distribution in nature. We hypothesize that: (1) the release of phenolic compounds and chromophoric dissolved organic matter (CDOM) depends on macrophyte species and its health status (broken macrophytes: BM, or healthy macrophytes: HM); (2) the repellency effect depends on zooplankton species, macrophyte species and its health status; and (3) higher concentrations of phenolic compounds and CDOM produce a stronger repellency effect. Phenolic compounds and CDOM were analyzed in exudates of BM and HM of *Salvinia* sp., *Eichhornia crassipes*, *Pistia stratiotes*, *Azolla* sp. and *Ludwigia peploides*. Through a flow-through experiment, the repellency produced by these exudates was assessed in two copepods (*Notodiaptomus conifer* and *Argyrodiaptomus falcifer*) and one cladoceran (*Ceriodaphnia dubia*). Our hypotheses were partially validated. The quantity of exudated phenolic compounds and CDOM depended on macrophyte species and, to a lesser extent, on the plant health status. The repellency effect was affected by macrophyte and zooplankton species but not by the health status of plants. Only *C. dubia* and *A. falcifer* increased their evasion behavior when phenolic compound and CDOM concentrations increased. In brief, the structuring effect of repellent substances depends on different factors. Under a certain threshold concentration, zooplankton behavior might depend on the information associated with the plant odor (e.g., predation risk, structural complexity) more than on the quantity of the released chemical compounds. Above this threshold, evasion would be the only possible option to avoid damaging effects.

Keywords Aquatic plants · Chemical ecology · Evasion behavior · Microcrustaceans

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Introduction

Aquatic vegetation has been considered one of the main factors promoting high biodiversity in wetlands (Burks et al. 2006; Dudgeon et al. 2006). It can perform different functions such as increasing habitat heterogeneity (Dibble and Thomaz 2009), providing food and shelter for many organisms (Harrison et al. 2005; González Sagrario and Balseiro 2010), and creating patches with particular physicochemical

conditions through the retention of suspended sediments and the release of substances into the surrounding water (Madsen et al. 2001; Thomaz and Ribeiro da Cunha 2010).

Macrophyte exudates include secondary metabolites (mainly phenolic compounds) that are frequently used as repellents, as defense against herbivores (Bolser et al. 1998; Choi et al. 2002), as competitive strategy against other macrophytes or algae (Gross et al. 2007; Dandelot et al. 2008), or as protection against tissue infection by fungi or bacteria (Mithraja et al. 2011). However, it has been recognized that such substances may have negative secondary effects on nontarget organisms. They can seriously affect vertebrates such as amphibians (Maerz et al. 2005) and invertebrates such as cladocerans (Gutierrez and Paggi 2013) as well as interfere with the natural chemical communication between organisms (Steinberg et al. 2008).

There are many factors that may influence the production and release of the above-mentioned exudates as well as their deleterious effects on the aquatic biota. For example, the concentration of phenolic compounds is usually much higher in free-floating or emergent macrophytes than in submerged ones, being highly dependent on the species (Smolders et al. 2000). Disturbances and ruptures of roots, leaves and stems by natural or anthropogenic causes can also determine the quantity and quality of the released phenolic compounds, chromophoric dissolved organic matter (CDOM) and other potential allelochemicals (Mann and Wetzel 1996; Mangas-Ramíreza and Elías-Gutiérrez 2004). In this regard, human intervention on macrophytes for weeding purposes, grazing and trampling by commercial livestock or other mammals (Steinman et al. 2003; Sabattini and Lallana 2007) and grazing by herbivorous insects play an important role in the release of chemical compounds. The analysis of this phenomenon would provide valuable knowledge about the dynamics of littoral areas, as well as the structuring effects of aquatic macrophytes upon other communities. Moreover, given the increasing invasion of aquatic macrophytes from warm environments in temperate zones (Michelan et al. 2010), the analysis of how much their exudates can affect other aquatic communities would be especially useful for managers or decision makers of affected regions.

Zooplankton is a key component of freshwater systems that frequently use macrophytes as a refuge from vertebrate predators or as a substrate where to stay and feed (Jeppesen et al. 1998; Wojtal et al. 2003). Even though of offering such advantages, macrophytes through their exudates can repel or negatively affect the inhabiting zooplankton organisms (Trochine et al. 2009; Gutierrez and Paggi 2013). To date, there is little information on how different the effect is when these exudates are naturally released by healthy plants or when they are released after a structural break. Furthermore, the specific sensitivity of zooplankton species with different life habits and/or belonging to different taxonomic groups is still largely unknown.

The aims of this work are to: (1) quantify the phenolic compounds and CDOM in exudates of five different species of macrophytes under two different conditions: healthy (healthy macrophytes, HM) and broken (broken macrophytes, BM); (2) assess the repellency produced by the exudates of each macrophyte on different zooplankton species (two copepods and one cladoceran); and (3) test the possible relationship between the observed repellency of zooplankton and quantity of phenolic compounds and CDOM released by macrophytes. We hypothesize that: (1) the release of phenolic compounds and CDOM depends on the macrophyte species and its health status (BM vs. HM); (2) the repellency produced by the exudates of each macrophyte is different depending on the zooplankton species, macrophyte species and its health status; and (3) exudates with higher concentrations of phenolic compounds and CDOM produce stronger repellency of zooplankton.

Materials and methods

Macrophyte collecting and experimental setup to obtain the "exudates"

The macrophytes used in this study were *Salvinia* sp., *Eichhornia crassipes*, *Pistia stratiotes*, *Azolla* sp. (free floating) and *Ludwigia peploides* (emergent), which were selected because they are common in littoral environments of subtropical water bodies, they often cause problems due to their rapid growth and also because their allelopathic potentiality was registered in previous studies (Sabattini and Lallana 2007; Dandelot et al. 2008; Mithraja et al. 2011; Dethe et al. 2014). Moreover, *E. crassipes* and *L. peploides* are of particular interest because they are invasive in

many places of the world. Plants were collected from a shallow lake belonging to the Middle Paraná River floodplain (31°38′20″S; 60°40′18″W), transported to the laboratory and carefully washed with tap water to eliminate invertebrates and biofilm from their roots and leaves.

Prior to the experiments, plants were placed inside plastic containers with dechlorinated tap water (characteristics are shown in Table 1) during 2 days for acclimation to the laboratory conditions: photoperiod: 16-h light: 8-h darkness, temperature: 22 ± 1 °C.

After the acclimation period, the plants of each species were weighed and carefully separated in six groups inside new containers with 3 L of dechlorinated tap water, ensuring that each group has similar wet weigh (~80 g). Three groups were left intact, hereafter "healthy macrophytes" (HM), and the other three groups were manually broken in approximately seven parts, including leaves, roots and stems (broken macrophytes, BM). *Azolla* sp. and *L. peploides* were exceptions because the former one was used only in the HM assays and *L. peploides* only in the BM assays. *Azolla* sp. was used only in its healthy state because since it is a very small plant, it was difficult to keep uniformity in the breaking technique between replicates and according the other plant species. *L. peploides* could not be used in this experiment under healthy condition because it is a rooted plant and requires an accurate substrate to be appropriately maintained in the laboratory. As a result, eight treatments (four for HM assays and four for BM assays) plus the controls (dechlorinated tap water without macrophytes) were carried out.

Table 1 Average values of chemical variables measured in the water used at the start of the experiment

Variable	Average (SD)
Conductivity (μS cm^{-1})	240 (41)
pH	7.3 (0.5)
Dissolved oxygen (mg L^{-1})	8.6 (0.9)
Phenolics compounds (μg L^{-1})	2.8 (3.9)
a440 (m^{-1})	0.0 (0.13)
CDOM spectral slope (250–350 nm)	0.0196 (6.9×10^{-4})

The determinations are made by triplicate. Standard deviations (SDs) are shown in parentheses. a_{440}: absorption coefficient of chromophoric dissolved organic matter (CDOM) at 440 nm

Measurement of chemical variables, phenolic compounds and CDOM

After 5 days, conductivity, pH and dissolved oxygen were measured in each treatment and control using Hanna portable probes. For the analysis of CDOM and phenolic compounds, water samples were collected in glass bottles and filtered through membrane filters (pore size: 0.45 μm). Immediately, UV–visible absorption spectra (250–700 nm) were measured using 1-cm quartz cuvettes and a HACH DR5000 ultraviolet–visible spectrophotometer with 1-nm resolution. Since the CDOM absorbance is assumed to be equal to zero above 700 nm, the absorbance at this wavelength was subtracted from each spectrum to correct for offsets. Absorption coefficients at each wavelength were calculated according to Kirk (1994):

$$a_\lambda = 2.303 A_\lambda / l$$

where a_λ is the CDOM absorption coefficient (m^{-1}) at wavelength λ, A_λ is the absorbance at wavelength λ and l is the cuvette path length (m). The absorption coefficient at 440 nm (a_{440}) was used as a measure of CDOM concentration (Kirk op. cit.). In addition, CDOM spectral slopes in the region 250–450 nm were calculated using linear regression of Ln-transformed absorption coefficients. This region was selected because r values were higher than 0.99, while that including values above 450 nm the low absorbance of the samples produced a poor fit to the straight line. CDOM spectral slopes of controls and water with which the assays were initiated were calculated for the range 250–350 nm because the absorbance was lower than in treatments, and therefore, the region with a good fit to the straight line was shorter. Spectral slopes in different ranges of wavelength are widely used to characterize CDOM because they are inversely related to CDOM molecular weight (Stedmon and Markager 2001).

Phenolic compound concentrations were determined using Folin–Ciocalteu reagent (Box 1983). Control average value for each variable was subtracted to values observed in each treatment. Thus, values were under zero for variables that were lower in the treatment groups than in the control group and above zero when it was the other way round. The wet weight of the plants was used to normalize the chemical condition differences between controls and each treatment in relation to the mass of vegetal material.

Repellency experiments with zooplankton

The zooplankton species used in the experiments were the copepods *Notodiaptomus conifer* (Sars 1901) and *Argyrodiaptomus falcifer* (Daday 1905), and the cladoceran *Ceriodaphnia dubia* (Richard 1894). They were chosen because of their wide distribution and because despite being limnetic, they usually are associated with littoral macrophytes, depending on the sampling site. Besides, previous behavioral experiments demonstrated that they are appropriate organisms for being cultured under laboratory conditions and used in experimental assessments (Gutierrez et al. 2011, 2013). The animals were collected from the same system where plants were taken and identified using specific keys (Lopretto and Tell 1995; Dussart and Frutos 1986; Paggi 2006). The collecting was performed 1 week before the experiments, and the selected organisms were acclimated and maintained at a photoperiod of 16-h light: 8-h darkness and 22 ± 1 °C in aerated and dechlorinated tap water. The organisms were fed every 2 days ad libitum with a *Chlorella vulgaris* concentrate (algal density: 2.8×10^5 cells mL^{-1}).

To test the response of the zooplankton organisms to macrophyte exudates, a similar wet weight (~ 80 g) of macrophytes was used in the eight treatments (exudates of both HM and BM for *P. stratiotes*, *Salvinia* sp. and *E. crassipes*; exudates of HM for *Azolla* sp.; and exudates of BM for *L. peploides*). These treatments were prepared following the same procedures as described above for measuring chemical variables, phenolic compounds and CDOM.

A "flow-through experiment" was carried out in order to assess the repellency/attraction response of zooplankton species to the presence of exudates of HM and BM. This consisted of a plastic channel (75 cm length × 3 cm deep × 4 cm width) with a small hole in each extreme, both connected to a glass buret by plastic hoses. Each buret was carefully regulated in order to provide a low flow of water running in the channel (~ 2.2 drops per second). There were also two small holes in the middle of the channel (in the upper part of each wall) sealed with a plankton mesh (50 μm) to allow the output of the excess of water and to maintain a constant water level. One of the two burets contained aerated and dechlorinated tap water (control), and the other contained tap water with macrophyte exudate prepared as mentioned above. Each exudate was tested individually. During the development of the experiment, burets with control water and macrophyte exudates were alternated in order to discard external effects (e.g., light) on the responses of the animals.

Inside the channel, the opposing flows allowed the development of a gradient in the macrophyte exudates that was previously tested with colored water. In addition, we corroborated the absence of a trawling effect, by action of the water flow, through several preliminary experiments using styrofoam spheres and living animals.

Several preliminary experiments were also carried out to determine the suitable exposure time for the animals, and a 10-min time was finally chosen. The experiments with each treatment were replicated three times with ten animals of the same species each, so 30 healthy and new individuals were used in each case (240 individuals in total).

The experimental mechanism consisted in placing the animals in the middle of the channel and registering their position after 10-min exposure. The channel was divided into three sections (tap water section, middle section and exudate section). The animals that moved to the section with tap water were considered as repelled by the exudate, the animals that moved to the section with exudate were considered as attracted, and the animals that remained in the middle were considered non-reactive.

Statistical analyses

To test for effects of plant species and their health status (HM, BM) on chemical exudates in the water (e.g., phenolic compounds, CDOM, etc., $n = 6$, see Table 1), two sets of analyses were run. Firstly, differences in the measured chemical variables were analyzed in exudates from three macrophytes available in both health statuses by two-way ANOVAs (Table 2). Dependent variable: individual chemical variable; Factor 1: plant species ($n = 3$; only *Salvinia* sp., *E. crassipes* and *P. stratiotes* were considered because they were used in both health statuses); Factor 2: health status of the plant (HM or BM; $n = 2$). Tukey post hoc tests served to analyze the pair-wise differences within Factor 1 (plant species). Secondly, six additional one-way ANOVAs (again, one for each of the six chemical variables) were performed to test for significant differences among altogether eight

Table 2 Results of the two-way ANOVA test to assess differences in chemical variables measured in water that contained the studied macrophytes according to the plant species (*Salvinia* sp. *Eichhornia crassipes* and *Pistia stratiotes*), the plant health status (healthy and broken macrophytes) and their interaction

Two-way ANOVA test	*df*	Mean square	F	*p*	Tukey test	*p*
Phenolic compounds						
Plant species	2	8.931	20.364	**<0.001**	*Sal–Pis*	**<0.001**
Health status	1	1.184	2.699	0.126	*Sal–Eich*	**0.001**
Interaction	2	2.734	6.233	**0.014**	*Eich–Pis*	0.883
a_{440}						
Plant species	2	0.001	57.53	**<0.001**	*Sal–Pis*	**<0.001**
Health status	1	1.217×10^{-6}	0.1379	0.7169	*Sal–Eich*	**0.006**
Interaction	2	2.29×10^{-6}	0.2595	0.7757	*Eich–Pis*	**<0.001**
CDOM spectral slope (250–450 nm)						
Plant species	2	1.2×10^{-8}	43.35	**0.006**	*Sal–Pis*	**<0.001**
Health status	1	1.1×10^{-8}	40.68	**0.001**	*Sal–Eich*	**0.003**
Interaction	2	1.7×10^{-9}	6.37	**0.013**	*Eich–Pis*	**0.03**
Dissolved oxygen						
Plant species	2	0.005	158.964	**<0.001**	*Sal–Pis*	**<0.001**
Health status	1	0.002	62.591	**<0.001**	*Sal–Eich*	0.100
Interaction	2	0.001	16.616	**<0.001**	*Eich–Pis*	**<0.001**
pH						
Plant species	2	8.733×10^{-5}	135.058	**<0.001**	*Sal–Pis*	0.810
Health status	1	6.825×10^{-6}	10.554	**0.002**	*Sal–Eich*	**0.003**
Interaction	2	2.204×10^{-5}	34.084	**<0.001**	*Eich–Pis*	**0.009**
Conductivity						
Plant species	2	0.167	7.297	**0.008**	*Sal–Pis*	0.214
Health status	1	0.192	8.392	**0.013**	*Sal–Eich*	0.148
Interaction	2	0.165	7.202	**0.009**	*Eich–Pis*	**0.006**

Tukey test comparisons between *Salvinia* sp. (*Sal*), *Eichhornia crassipes* (*Eich*) and *Pistia stratiotes* (*Pis*) are shown. Bold letters indicate statistical differences ($p < 0.05$). a_{440}: absorption coefficient of chromophoric dissolved organic matter (CDOM) at 440 nm

different types of water (incubated with HM and BM of *Salvinia* sp., *E. crassipes* and *P. stratiotes,* HM of *Azolla* sp. and BM of *L. peploides*). Thus, dependent variable: individual chemical variable; independent variable: treatment ($n = 8$) (Table 3).

In order to test for differences in the behavioral responses of the animals according to the macrophyte species and/or its health status (HM or BM), another set of two-way ANOVAs was performed. In this case, the percentage of repelled animals was considered as dependent variable, plant species as Factor 1 ($n = 3$) and health status of the plant (HM or BM) as a second factor ($n = 2$). As for the chemical analysis, only *Salvinia* sp., *E. crassipes* and *P. stratiotes* were considered in the two-way ANOVAs because they were used in both health statuses (HM and BM). Two-way ANOVAs were run separately for each of the three zooplankton species (Table 4). Tukey post hoc tests were used to analyze the pair-wise differences among plant species. In addition, the difference between the percentage of repelled animals and the percentage of attracted ones in response to exudates of each macrophyte species was tested for individual zooplankton species using one-way ANOVAs (Table 5). Since the previous two-way ANOVAs demonstrated that the health status of the plants did not affect the zooplankton behavior (Table 4), the analyses were based on the pooled data of zooplankton responses to individual plant species ($n = 5$) independent on their health status. Thus, the percentage of animals was considered as dependent variable and the animal response (attracted/repelled) as the independent factor. For this analysis (and the later ones), no additional experiment was conducted, but data from the previous analyses were reused. The number of tested zooplankton individuals per plant species was then 60 for macrophytes available in both health statuses (HM and BM) (*Salvinia* sp., *E. crassipes* and *P. stratiotes*) and 30 for macrophytes used only in one health status (HM or BM; *Azolla* sp. and *L. peploides*, respectively). Altogether 15 one-way ANOVAs were run to test for differences in behavioral responses of

Table 3 Results of the one-way ANOVA to assess differences in chemical variables measured in water that contained the studied macrophytes

Phenolic compounds								
ANOVA test Differences between groups: F = 75.95; $p < 0.001$								
Tukey test	H-*Sal*	H-*Eich*	H-*Pis*	B-*Sal*	B-*Eich*	B-*Pis*	H-*Azo*	B-*Lud*
H-*Sal*		0.9072	0.0868	1	**0.0158**	0.2089	**0.0005**	**0.0002**
H-*Eich*	1.756		0.5568	0.8226	0.1557	0.8422	**0.0044**	**0.0002**
H-*Pis*	4.473	2.717		0.0595	0.9811	0.9994	0.1609	**0.0002**
B-*Sal*	0.292	2.048	4.765		**0.0106**	0.1489	**0.0004**	**0.0002**
B-*Eich*	5.754	3.998	1.281	6.046		0.8351	0.5684	**0.0002**
B-*Pis*	3.744	1.988	0.729	4.035	2.01		0.0649	**0.0002**
H-*Azo*	8.443	6.687	3.97	8.735	2.689	4.7		**0.0002**
B-*Lud*	26.62	24.870	22.15	26.92	20.87	22.88	18.18	
Absorption coefficient of chromophoric dissolved organic matter (CDOM) at 440 nm (a_{440})								
ANOVA test Differences between groups: F = 124.9; $p < 0.001$								
Tukey test	H-*Sal*	H-*Eich*	H-*Pis*	B-*Sal*	B-*Eich*	B-*Pis*	H-*Azo*	B-*Lud*
H-*Sal*		0.4203	**0.0002**	0.9870	0.4457	**0.0002**	**0.0002**	**0.0002**
H-*Eich*	3.058		**0.0015**	0.1137	1	**0.0011**	**0.0002**	**0.0002**
H-*Pis*	10.57	7.515		**0.0002**	**0.0014**	1	0.0965	**0.0050**
B-*Sal*	1.199	4.257	11.77		0.1234	**0.0002**	**0.0002**	**0.0002**
B-*Eich*	2.991	0.06658	7.582	4.191		**0.0010**	**0.0002**	**0.0002**
B-*Pis*	10.86	7.801	0.2861	12.06	7.868		0.1373	**0.0073**
H-*Azo*	14.96	11.9	4.389	16.16	11.97	4.103		0.7648
B-*Lud*	17.17	14.11	6.598	18.37	14.18	6.312	2.209	
CDOM spectral slope (region 250–450 nm)								
ANOVA test Differences between groups: F = 8.272; $p = 0.0002516$								
Tukey test	H-*Sal*	H-*Eich*	H-*Pis*	B-*Sal*	B-*Eich*	B-*Pis*	H-*Azo*	B-*Lud*
H-*Sal*		**0.04289**	**0.0047**	0.6514	**0.0002**	**0.0002**	**0.0088**	**0.0002**
H-*Eich*	5.013		0.9350	0.6388	**0.0463**	**0.0002**	0.9889	**0.0007**
H-*Pis*	6.64	1.626		0.1300	0.3245	**0.0002**	1	**0.0054**
B-*Sal*	2.492	2.522	4.148		**0.0016**	**0.0002**	0.2222	**0.0002**
B-*Eich*	9.97	4.956	3.33	7.478		**0.0024**	0.1983	0.3666
B-*Pis*	17.14	12.12	10.5	14.64	7.166		**0.0002**	0.1627
H-*Azo*	6.18	1.167	0.4595	3.689	3.79	10.96		**0.0030**
B-*Lud*	13.17	8.162	6.535	10.68	3.205	3.961	6.995	
Dissolved oxygen								
ANOVA test Differences between groups: F = 53.95; $p < 0.001$								
Tukey test	H-*Sal*	H-*Eich*	H-*Pis*	B-*Sal*	B-*Eich*	B-*Pis*	H-*Azo*	B-*Lud*
H-*Sal*		0.9155	**0.0007**	1	**0.0471**	**0.0002**	**0.0002**	0.9073
H-*Eich*	1.72		**0.0002**	0.9379	**0.0046**	**0.0002**	**0.0002**	0.2797
H-*Pis*	8.191	9.911		**0.0006**	0.3516	**0.0012**	**0.0004**	**0.0062**
B-*Sal*	0.1085	1.611	8.299		**0.0408**	**0.0002**	**0.0002**	0.8792

Table 3 continued

Dissolved oxygen								
ANOVA test Differences between groups: F = 53.95; $p < 0.001$								
Tukey test	H-*Sal*	H-*Eich*	H-*Pis*	B-*Sal*	B-*Eich*	B-*Pis*	H-*Azo*	B-*Lud*
B-*Eich*	4.942	6.662	3.249	5.051		**0.0002**	**0.0002**	0.3731
B-*Pis*	15.88	17.6	7.69	15.99	10.94		0.9843	**0.0002**
H-*Azo*	17.12	18.84	8.93	17.23	12.18	1.24		**0.0002**
B-*Lud*	1.755	3.475	6.436	1.864	3.187	14.13	15.37	
pH								
ANOVA test Differences between groups: F = 99.19; $p < 0.001$								
Tukey test	H-*Sal*	H-*Eich*	H-*Pis*	B-*Sal*	B-*Eich*	B-*Pis*	H-*Azo*	B-*Lud*
H-*Sal*		0.9614	**0.0161**	**0.0002**	**0.0002**	**0.0086**	**0.0002**	**0.0002**
H-*Eich*	1.465		0.1118	**0.0003**	**0.0002**	0.0620	**0.0002**	**0.0002**
H-*Pis*	5.736	4.271		**0.0431**	**0.0002**	1	**0.0002**	**0.0002**
B-*Sal*	10.75	9.28	5.009		**0.0047**	0.0790	**0.0002**	**0.0002**
B-*Eich*	17.39	15.92	11.65	6.643		**0.0002**	0.1026	**0.0002**
B-*Pis*	6.199	4.734	0.4629	4.546	11.19		**0.0002**	**0.0002**
H-*Azo*	21.73	20.26	15.99	10.98	4.34	15.53		**0.0127**
B-*Lud*	27.64	26.17	21.9	16.89	10.25	21.44	5.909	
Conductivity								
ANOVA test Differences between groups: F = 2.52; $p = 0.05959$								
Tukey test	H-*Sal*	H-*Eich*	H-*Pis*	B-*Sal*	B-*Eich*	B-*Pis*	H-*Azo*	B-*Lud*
H-*Sal*		0.7319	0.9908	0.9983	0.9984	0.7339	0.8043	1
H-*Eich*	2.294		0.9890	0.9653	0.9644	0.0754	0.0958	0.8685
H-*Pis*	1.129	1.165		1	1	0.2969	0.3582	0.9994
B-*Sal*	0.8587	1.435	0.2701		1	0.3871	0.4582	1
B-*Eich*	0.8517	1.442	0.2771	0.007029		0.3897	0.4611	1
B-*Pis*	2.289	4.583	3.418	3.148	3.14		1	0.5719
H-*Azo*	2.101	4.395	3.229	2.959	2.952	0.1882		0.6509
B-*Lud*	0.3922	1.902	0.7366	0.4665	0.4595	2.681	2.493	

Tukey test comparisons between all eight treatments (considering exudates of each macrophyte species and health status individually) are shown: the statistic Q is given in the lower left triangle of the array, and the p values are shown in the upper right. Bold letters indicate statistical differences ($p < 0.05$). Sal: *Salvinia* sp., Eich: *Eichhornia crassipes* (*Eich*), Pis: *Pistia stratiotes*, Azo: *Azolla* sp., Lud: *Ludwigia peploides*, H: healthy macrophytes, B: broken macrophytes

the three zooplankton species to exudates from the five different plant species (Table 5).

Finally, simple linear regressions were performed to assess whether the zooplankton repellency (dependent variable) was significantly intensified or decreased in response to phenolic compounds, CDOM concentration or CDOM molecular weight (independent variables). Behavioral responses of animals to chemicals in plant exudates were used from altogether eight treatments (three macrophyte species in both health statuses and two species in only one). The percentage of repelled animals and the change in chemical variables in a given treatment were used as input. Four linear regressions were run for each of the three chemical variables of interest (Table 6). Prior to all the analyses, data were tested for normality and homogeneity of

Table 4 Results of the two-way ANOVA test to assess differences in the behavioral response (% repelled individuals) of studied zooplankton species (*Ceriodaphnia dubia*, *Notodiaptomus conifer* and *Argyrodiaptomus falcifer*) according to the plant species (*Salvinia* sp., *Eichhornia crassipes* and *Pistia stratiotes*), the plant health status (healthy and broken macrophytes) and their interaction

Two-way ANOVA test	*df*	Mean square	F	*p*	Tukey Test	*p*
C. dubia						
Plant species	2	346.898	2.572	0.118	*Sal–Pis*	0.322
Health status	1	50.045	0.401	0.539	*Sal–Eich*	0.108
Interaction	2	112.779	0.836	0.457	*Eich–Pis*	0.760
N. conifer						
Plant species	2	1352.55	6.779	**0.011**	*Sal–Pis*	0.942
Health status	1	42.936	0.215	0.651	*Sal–Eich*	**0.015**
Interaction	2	650.521	3.260	0.074	*Eich–Pis*	**0.027**
A. falcifer						
Plant species	2	682.505	4.455	**0.036**	*Sal–Pis*	0.603
Health status	1	325.125	2.122	0.171	*Sal–Eich*	**0.031**
Interaction	2	38.452	0.251	0.782	*Eich–Pis*	0.167

Tukey test comparisons between *Salvinia* sp. (*Sal*), *Eichhornia crassipes* (*Eich*) and *Pistia stratiotes* (*Pis*) are shown. Bold letters indicate statistical differences ($p < 0.05$)

Table 5 Results of one-way ANOVA tests to assess differences between the percentage of repelled animals and the percentage of attracted ones for each zooplankton species exposed to exudates from different macrophyte species

	Ceriodaphnia dubia			*Notodiaptomus conifer*			*Argyrodiaptomus falcifer*		
	df	F	*p*	df	F	*p*	df	F	*p*
Salvinia sp.	1	57.555	**<0.001**	1	57.808	**0.0370**	1	0.4802	0.5040
E. crassipes	1	13.0799	**0.0047**	1	0.0078	0.9310	1	0.1572	0.7000
P. stratiotes	1	0.0205	0.8889	1	70.348	**0.0242**	1	24.9426	**<0.001**
Azolla sp.	1	0.5941	0.4838	1	0.0509	0.8324	1	0.2480	0.6446
L. peploides	1	73.111	**0.0438**	1	7.9737	**0.0476**	1	91.8025	**<0.001**

The experiments are replicated three times with ten animals of the same species each. So, the quantity of tested zooplankton individuals per plant species is 60 for macrophytes used in both health statuses (HM and BM; *Salvinia* sp., *E. crassipes* and *P. stratiotes*) and 30 for macrophytes used only in one health status (HM or BM; *Azolla* sp. and *L. peploides*). Bold letters indicate statistical differences ($p < 0.05$)

variances using the Shapiro–Wilk test and Levene test, respectively. When data did not meet the normality assumption, they were log-transformed.

Results

Chemical variables, phenolic compounds and CDOM

Reference values for the chemical variables of interest are shown in Table 1. This table summarizes the averages of variables measured at the start of the experiment, when no plant material was in the water. These values were used as baselines to determine the changes after 5 days of incubation in the eight treatments (Fig. 1). For all variables, a change was observed. The degree of change varied among chemical variables, plant species and health statuses, but no consistent patterns emerged dependent on their healthy or broken status (Fig. 1).

Focusing on exudates from three plant species which were available in both health statuses (six treatments), the species effect on measured chemical

variables explained most variation in the data (two-way ANOVA, $p < 0.05$, Table 2). Although the health status (HM or BM) of plants showed also an effect, this was only significant for four of the six chemicals. Regarding the phenolic compounds and CDOM (a_{440}), there was no difference between healthy or broken plants. Interestingly, in all but one chemical measurement (a_{440}), the interaction of the two main factors (health status x plant species) was also significant, indicating that the effect of exudates from individual plant species in healthy or broken conditions was species specific (Table 2, Fig. 1). Thus, the quantity of released phenolic compounds and CDOM (a_{440}) showed higher differences among macrophyte species than comparing the two health statuses only. In this regard, the quantity of released CDOM depended on the plant species ($p < 0.01$) but not on their health status, while the quantity of released phenolic compounds depended on both plant species ($p < 0.01$) and its interaction with plant health status ($p < 0.05$) (Table 2). When macrophytes were broken, only *E. crassipes* increased the exudation of phenolic compounds (Fig. 1; see Table 3 for statistical differences among all eight treatments, one-way ANOVA). Plants that released high quantities of phenolic compounds also exuded high quantities of CDOM (Fig. 1). Accordingly, the following order of macrophytes can be established: *Ludwigia peploides* > *Azolla* sp. > *P. stratiotes* > *E. crassipes* > *Salvinia* sp. (Figure 1). In particular, *L. peploides* which was only used in the broken status was characterized by its high exudation of phenolic compounds (between 4 and 15 times higher than the other plants) and CDOM (Fig. 1), differing significantly from the other macrophytes (Table 3; $p < 0.01$, one-way ANOVA). This result suggests that the species effect may severely outweigh the health status effect.

The molecular weight of released CDOM presented a clear increase in BM (lower CDOM spectral slope) compared to HM (Fig. 1; Table 2; $p < 0.01$, two-way ANOVA). The molecular weight of released CDOM also varied among macrophyte species (Table 2; $p < 0.01$, two-way ANOVA). CDOM in exudates of *Salvinia* sp. had the lowest molecular weight in comparison with the other exudates analyzed, differing significantly from *P. stratiotes* and *E. crassipes* (Table 2; $p < 0.01$, Tukey test). The molecular weight of CDOM in the culture water of *P. stratiotes* increased more than in the culture water of the other species (Fig. 1), especially regarding BM (Fig. 1; Table 3; $p < 0.01$, one-way ANOVA).

In addition, broken macrophytes (BM) showed a larger decrease in dissolved oxygen (DO) and pH ($p < 0.01$), as well as a larger increase in conductivity ($p < 0.05$) (Fig. 1; Table 2: two-way ANOVA). Macrophyte species also showed a significant effect on these variables (Table 2; $p < 0.01$, two-way ANOVA). Considering healthy macrophytes (HM), the culture water of *Azolla* sp. showed the highest decrease in pH and DO ($p < 0.01$) and the highest increase in conductivity (without statistical significance) regarding controls (Fig. 1; Table 3: one-way ANOVA). Among BM, the culture water of *L. peploides* showed the highest decrease in pH ($p < 0.01$), whereas the culture water of *P. stratiotes* showed the highest decrease in DO ($p < 0.01$) and the highest increase in conductivity (without statistical significance) (Fig. 1; Table 3: one-way ANOVA).

Table 6 Linear regression analysis testing the effects of phenolic compound concentration, absorption coefficient of chromophoric dissolved organic matter (CDOM) at 440 nm (a_{440}) and CDOM spectral slope between 250 and 450 nm on the repellency behavior of all the animals considered together and of each particular zooplankton species

	Slope	Intercept	*R*	*p*
Phenolic compounds				
C. dubia	4.9277	−0.0666	0.44888	**0.02778**
N. conifer	4.9937	−0.0301	0.12622	0.57566
A. falcifer	5.2307	−0.1419	0.69834	**0.00014**
All species	5.2187	−0.1206	0.75043	**0.00002**
a_{440}				
C. dubia	4.8971	−14.738	0.26463	0.21144
N. conifer	5.3067	−29.632	0.39547	0.068498
A. falcifer	5.4735	−51.563	0.67609	**0.000287**
All species	5.3616	−39.67	0.69595	**0.000159**
CDOM spectral slope (250–450 nm)				
C. dubia	4.6881	252.7	−0.01794	0.93369
N. conifer	5.4455	9003.6	−0.52369	**0.01237**
A. falcifer	5.4718	12269	−0.63566	**0.00084**
All species	5.3204	8816.4	−0.57774	**0.00311**

Bold letters indicate statistical differences ($p < 0.05$)

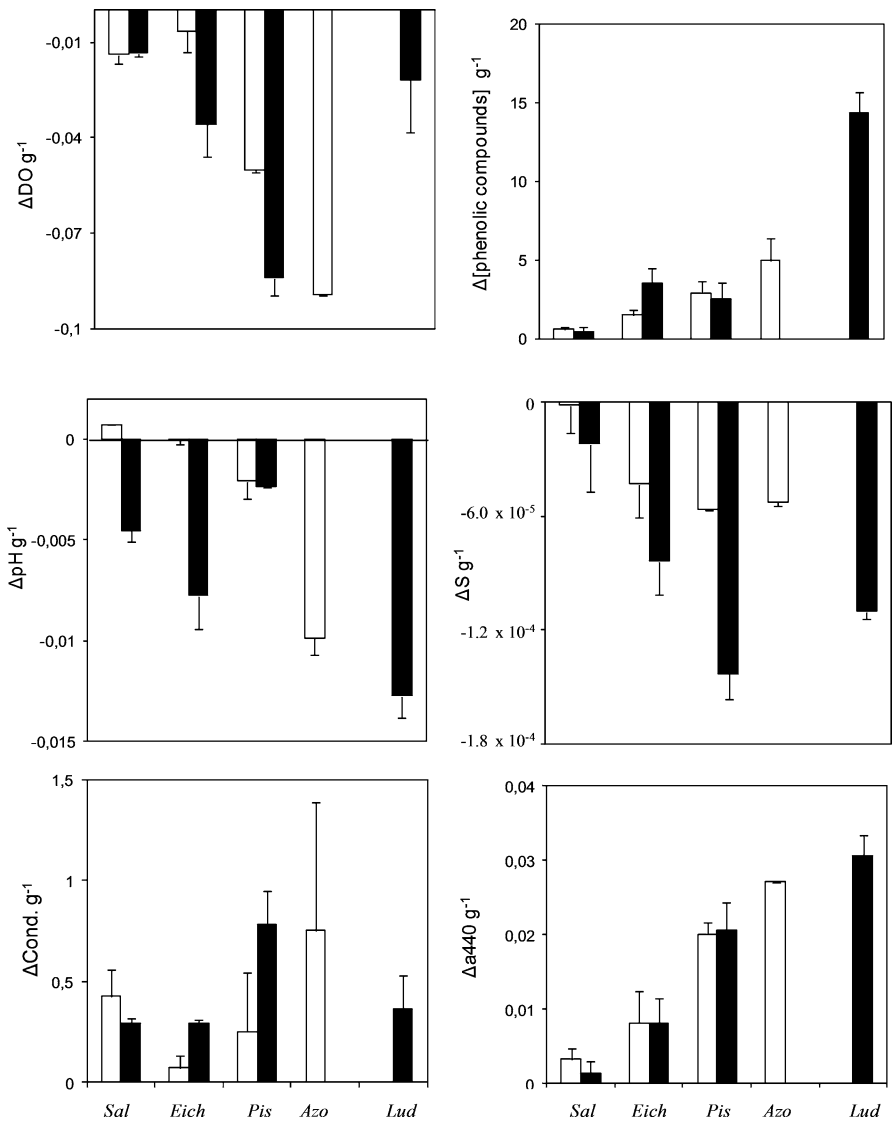

Fig. 1 Chemical variables measured in water that contained the studied macrophytes during 5 days (treatments). For each variable, the average value of controls (water that remained 5 days under the same conditions but without macrophytes) is subtracted to values observed in each treatment: positive values indicate that the variable is higher in the treatment than in the control, and negative values indicate the opposite. The wet weight of the plants is used to normalize the chemical condition differences between controls and each treatment. The *p* values of the one-way ANOVA test, comparing all treatments (considering individually exudates of each species and health status), are shown in Table 3. White columns: healthy macrophytes; black columns: broken macrophytes. Sal: *Salvinia* sp., Eich: *Eichhornia crassipes*, Pis: *Pistia stratiotes*, Azo: *Azolla* sp., Lud: *Ludwigia peploides*; DO: dissolved oxygen, Cond.: conductivity, S: CDOM spectral slope (250–450 nm), a_{440}: absorption coefficient of CDOM at 440 nm. *Vertical bars* are standard deviations based on the averaged three replicates

Repellency experiments with zooplankton

The copepods *N. conifer* and *A. falcifer* responded differently depending on the plant species ($p < 0.05$) but not regarding their health status (HM vs. BM) or the interaction among plant species and health status ($p > 0.05$) (Fig. 2; Table 4: two-way ANOVA). For both copepods, *E. crassipes* repelled a higher percentage of individuals than *Salvinia* sp. ($p < 0.05$), but a lower percentage of individuals than *P. stratiotes* (statistical significance only for *N. conifer:* $p < 0.05$) (Fig. 3; Table 4: Tukey test).

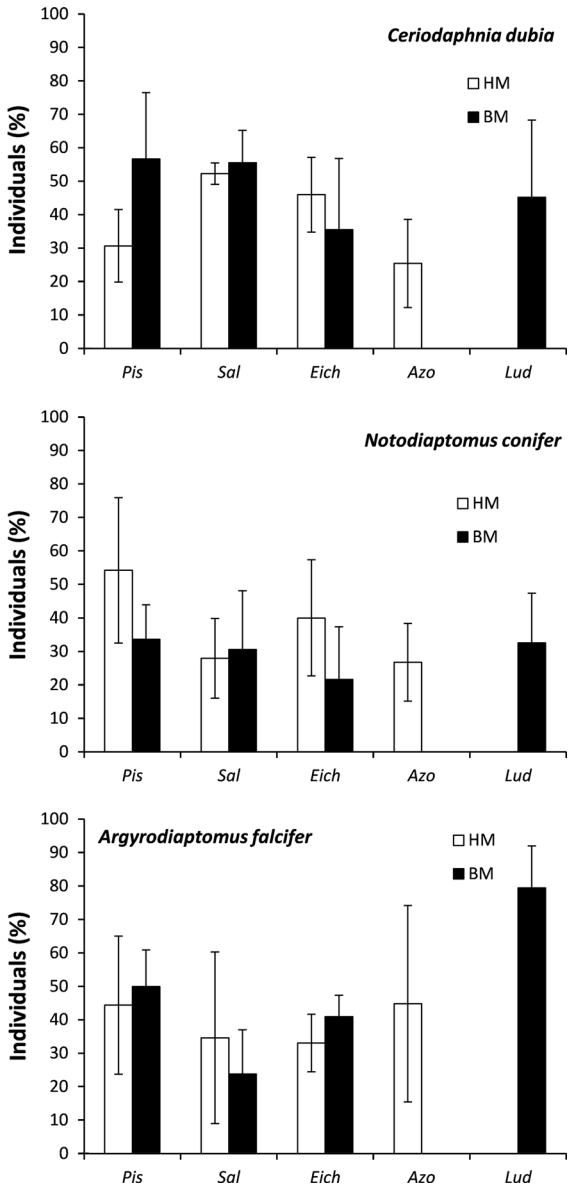

Fig. 2 Evasive response (measured as % of repelled individuals) of *Ceriodaphnia dubia*, *Notodiaptomus conifer* and *Argyrodiaptomus falcifer* to exudates of each plant species and health status. *White bars* healthy macrophytes (HM), *black bars* broken macrophytes (BM). Only the first three plant species (*P. stratiotes, Salvinia* sp. and *E. crassipes*) are tested in both health statuses. *Error bars* represent the standard deviations based on the averaged replicates

Since the health status of the plants did not affect the zooplankton behavior, we analyzed the effect of exudates pooling repellency and attraction data according to macrophyte species (with HM and BM together for *Salvinia* sp., *E. crassipes* and *P. stratiotes;* BM for *L. peploides*; HM for *Azolla* sp.). As a result of this analysis, the following patterns were manifested: the cladoceran *C. dubia* showed a higher percentage of repelled animals than attracted ones for all the macrophyte species ($p < 0.01$ for *Salvinia* sp. and *E. crassipes*, $p < 0.05$ for *L. peploides*), while the copepods were significantly repelled by *P. stratiotes* and *L. peploides* (*N. conifer*: $p < 0.05$; *A. falcifer*: $p < 0.01$) and attracted by *Salvinia* sp. (statistical significance only for *N. conifer*: $p < 0.05$) (Fig. 3; Table 5: one-way ANOVA). In sum, *L. peploides* and *Salvinia* sp. showed the clearest effects on zooplankton responses as the former one significantly repelled all the animals, while the latter repelled only *C. dubia* and attracted both copepods. Even though *Azolla* sp. did not show any statistically significant attraction nor repellency effects on the zooplankton species under study, there was a trend to repel *C. dubia* and *A. falcifer*.

The simple linear regression analysis showed that a higher concentration of phenolic compounds caused a stronger repellency effect of all the animals considered together and in particular of *C. dubia* and *A. falcifer* (Table 6). The same relationships emerged for higher concentration of CDOM (a_{440}) and higher CDOM molecular weight (lower CDOM spectral slope) which also produced a higher repellency of all the animals considered together and in particular of the copepods (Table 6).

Discussion

This work confirms that the chemical composition of exudates belonging to different macrophyte species effectively influences the evasion behavior of zooplankton and hence their possible habitat selection. A detailed analysis of the data allowed us partially accepting the three proposed hypotheses.

First, as expected, macrophyte species and their health status influenced the release of phenolic compounds and chromophoric dissolved organic matter, CDOM (measured as a_{440}). However, when macrophytes were broken, only *E. crassipes* increased the exudation of phenolic compounds. This latter difference may mirror the high potential in defensive and competitive abilities of *E. crassipes* to the predation pressure from the herbivores to which it is usually exposed in the field (Choi et al. 2002). In turn, the rupture of macrophytes produced a significant increase in the molecular weight of CDOM, particularly for *P.*

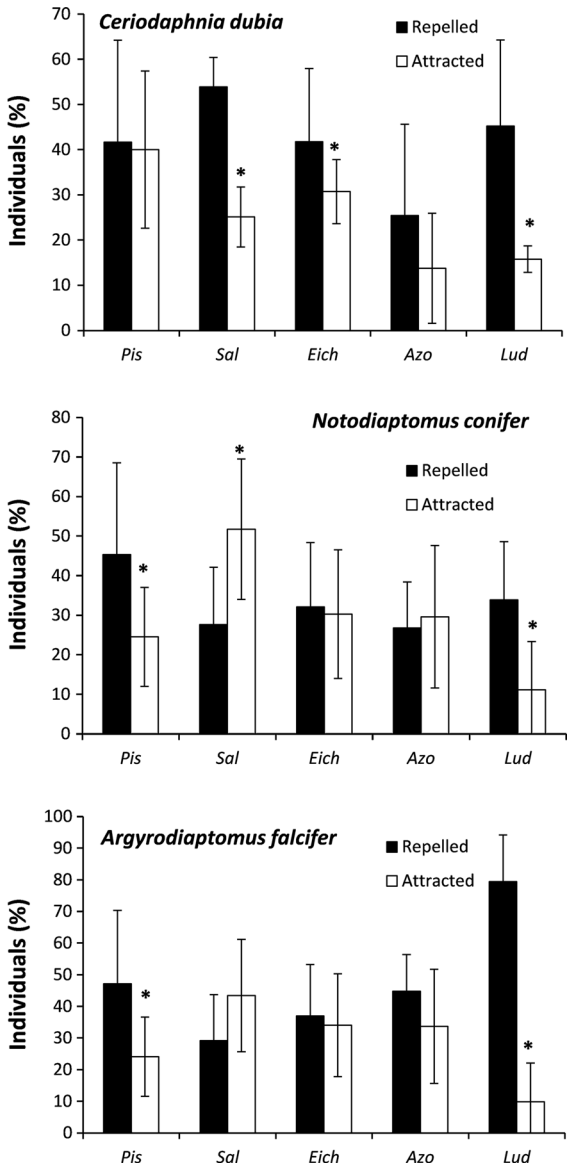

Fig. 3 Zooplankton responses to exudates of each macrophyte species, including both health statuses (healthy and broken macrophytes) for the first three plant species (*P. stratiotes, Salvinia* sp., *E. crassipes*) and only one health status for the fourth and fifth one (*Azolla* sp.: healthy; *L. peploides*: broken). Because some individuals showed an intermediate behavior (neither attraction nor repellence), the percentage of repelled and attracted animals does not sum up to 100 % per plant species. *Asterisks* indicate significant differences between the percentage of attracted individuals and repelled ones (one-way ANOVA test, *p* values are in Table 5). *Error bars* represent the standard deviations based on the averaged replicates

stratiotes, which is in line with expectations since large molecules are not able to passively pass through intact cell membranes (Gross et al. 2012).

Independently of their health status, there were also differences in the measured chemical variables among all the plant species. Those macrophytes releasing high concentrations of phenolic compounds also released high concentrations of CDOM. It is well known that many phenolic compounds are precursors of CDOM (Steinberg 2003) and the techniques used for determining them react positively to some compounds that comprise CDOM (APHA 2005). This may explain why the release of CDOM and that of phenolic compounds had a similar trend in this study.

Regarding the second hypothesis, it was confirmed that zooplankton repellency was different depending on the plant species. However, the health status (healthy macrophytes HM vs. broken macrophytes BM) did not influence the responses. On the other hand, animal responses also were different according to zooplankton species, being these differences mainly observed between both copepods and the cladoceran. Only *L. peploides* exudates repelled all the studied animals. *L. peploides* was the macrophyte species with the highest exudation of phenolic compounds and CDOM. Unfortunately, so far there are no reliable studies on the lethal or repellent effects of *L. peploides* exudates on zooplankton. However, this genus has been included within the 200 most aggressive ones worldwide (Cronk and Fuller 1995), with a great capacity to colonize new habitats and invade environments through the displacement of native species, which is attributed to its high production of allelochemicals (Dandelot et al. 2008; Sakpere et al. 2010). Therefore, the similar response in all zooplankton species in this study with respect to *L. peploides* exudates could be associated with the fact that the concentration of phenolic compound exceeded their tolerance range and caused the need for avoiding the toxic effect in a similar way. These results are in agreement with previous findings in the field, in which *L. peploides* registered much higher phenolic content in their leaves than other floating or emergent macrophytes from the Middle Paraná River (Mayora pers. obs.) and with other similar studies with *L. octovalvis* leaves (Yakob et al. 2012).

In addition to releasing a high concentration of phenolic compounds and CDOM, *L. peploides* also produced an important change in the water by decreasing the pH, which probably caused a negative effect on the zooplankton species as demonstrated in previous publications (Havas and Rosseland 1995;

Gagneten and Vila 2001; Nurminen and Horppila 2002). In our experiments, the combined effect of these factors could have been physiologically detected by the studied animals as a potential risk, which stimulated the escape from the exudate section of the tube.

The small free-floating fern, *Azolla* sp., followed *L. peploides* in the amount of phenolic compounds and CDOM released, which also confirmed previous measurements in plants collected in the field (Mayora pers. obs.). However, despite a clear trend on *C. dubia* and *A. falcifer* to be repelled by *Azolla* sp., its exudates did not have any statistically significant attraction or repellency effect in the studied animals, which may be related to a high variability in the zooplankton behavior manifested toward this plant. On the other hand, although it is possible to expect that high phenolic concentrations produce repulsive effects on the zooplankton, it is also reasonable to think that due to the structural and morphological characteristics of this particular plant, the animals do not evade its presence if the phenolic compounds do not exceed the toxicity threshold. This reasoning resides in the fact that *Azolla* sp. has a very simple and small root structure which is not appropriate for hosting predators, which would imply a real risk for zooplankton animals in nature. This suggests that despite the allelopathic substances of macrophytes, their ecological role (mostly determined by its structure) may be probably chemically recognized by the signature cue of the plant itself, in the same way as the specific odor of a predator can be recognized by its prey (Lass and Spaak 2003; Schoeppner and Relyea 2009; Ferrari et al. 2010).

Salvinia sp. released the least concentration of phenolic compounds and CDOM. However, the *C. dubia* cladoceran was significantly repelled by its exudates. Even though we did not perform a detailed analysis of the chemical composition of the exudates, they comprise a very high diversity of aromatic compounds such as tannins, lignin, saponins, flavonoids and gallic acid (Chantiratikul et al. 2009; Abraham and Aeri 2012; Oyedeji et al. 2014). Probably, the chemical recognition of each plant by zooplankton may be more related to the specific combination of each compound than to the total concentration of allelochemicals in the released exudate.

Exudates of *P. stratiotes* and *E. crassipes* registered intermediate values of phenolic compounds and CDOM, and produced the most complex behavioral responses in the studied animals. Exudates of *P. stratiotes* repelled all the animals although this effect was statistically significant only for copepods. This result shows that *P. stratiotes* was the most repelled plant after *L. peploides*. Although it did not release a high quantity of phenolic compounds and CDOM, the latter had the highest molecular weight in comparison with CDOM from the other plant species in both analyses (with BM and HM). This may cause a negative effect on the microcrustaceans, as suggested by the regression analysis that showed that a higher CDOM molecular weight caused a stronger repellency effect on all the animals considered together and in particular on both copepods. On the other hand, *E. crassipes* did not cause a significant repulsive effect on copepods, but it was true on *C. dubia*. Although some studies have demonstrated the repulsive effects of this macrophyte on different zooplankton species (Meerhoff et al. 2006; Gutierrez and Paggi 2013), our results suggest that the effect of this plant is species specific. This may be probably associated with the complex physical structure of its roots that may be chemically recognized by some zooplankton organisms. The complexity of the responses observed in the organisms studied in this work suggests the need for further analysis both under experimental conditions and in the field, where many environmental factors may contribute to determine the interactions between zooplankton and aquatic macrophytes.

Finally, a higher quantity of phenolic compounds and CDOM (a_{440}) generally increased the evasion of the exposed animals as predicted. Nevertheless, the analysis of each particular microcrustacean species revealed that only *C. dubia* and *A. falcifer* responded in this line to the phenolic compounds gradient and only *A. falcifer* responded according the CDOM increasing gradient. Therefore, our third hypothesis which claims that an increased concentration of phenolic compounds and CDOM produces an increase in the repellency of zooplankton organisms can be accepted but only partially.

The effects of CDOM on zooplankton have been poorly evaluated in laboratory assays. However, field surveys have found beneficial as well as deleterious effects on the animals. In the former case, since CDOM increases the availability of food, it protects against UV radiation and reduces visual predation by fishes. The deleterious effects may be the reduction in

pH and dissolved oxygen (Keskitalo and Eloranta 1999; Cooke et al. 2006). From our study, we are not able to determine the mechanism by which CDOM repelled the animals, but it is possible to suggest that CDOM released by macrophytes can stimulate zooplankton evasion, similarly to phenolic compounds, probably by providing ecological information on the predation risk, competence and chemical composition of the microenvironment associated with each specific plant. Consistently, the increase in CDOM molecular weight was associated with increased repellency, suggesting that CDOM quality can also be recognized by zooplankton.

In brief, habitat selection of the studied animals depended more on the plant species than on their health status. At the same time, each zooplankton species in particular manifested a different response, probably according to their particular sensitivity to the changes on the surrounding environmental conditions. In a natural system, it is probable to find a very different situation from the one found in this study, especially when the structural architecture of each plant is considered in addition to its chemical aspects, and due to the fact that vegetated patches are rarely monospecific. However, from this experimental research, we are able to state that chemical compounds released by each plant can alter the zooplankton habitat preference as well as provide good ecological information on the benefit or risk that different plants may have for each particular zooplankton species.

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References

Abraham G, Aeri V (2012) A preliminary examination of the phytochemical profile of *Azolla microphylla* with respect to seasons. Asian Pac J Trop Biomed 2:S1392–S1395

APHA (2005) Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC

Bolser RC, Hay ME, Lindquist N, Fenical W, Wilson D (1998) Chemical defenses of freshwater macrophytes against crayfish herbivory. J Chem Ecol 24:1639–1658

Box JD (1983) Investigation of the Folin–Ciocalteau phenol reagent for the determination of polyphenolic substances in natural waters. Water Res 17(511):525

Burks RL, Mulderij G, Gross E, Jones I, Jacobsen L, Jeppesen E, van Donk E (2006) Center stage: the crucial role of macrophytes in regulating trophic interactions in shallow lake wetlands. In: Robbink R, Beltman B, Verhoeven JTA, Whigham DF (eds) Wetlands: functioning, biodiversity conservation, and restoration. Springer-Verlag, Heidelberg, pp 37–59

Chantiratikul P, Meechai P, Nakbanpotecc W (2009) Antioxidant activities and phenolic contents of extracts from *Salvinia molesta* and *Eichornia crassipes*. Res J Biol Sci 4:1113–1117

Choi C, Bareiss C, Walenciak O, Gross EM (2002) Impact of polyphenols on growth of the aquatic herbivore *Acentria ephemerella*. J Chem Ecol 28:2245–2256

Cooke SL, Williamson CE, Hargreaves BR, Morris DP (2006) Beneficial and detrimental interactive effects of dissolved organic matter and ultraviolet radiation on zooplankton in a transparent lake. Hydrobiologia 568:15–28

Cronk QCB, Fuller JL (1995) Plant invaders: the threat to natural ecosystems. Chapman & Hall, London

Dandelot S, Robles C, Pech N, Cazaubon A, Verlaque R (2008) Allelopathic potential of two invasive alien *Ludwigia* spp. Aquat Bot 88:311–316

Dethe UL, Joshi SS, Desai SS, Aparadh VT (2014) Screening of bioactive compounds of *Sesbania grandiflora* and *Pistia stratiotes*. Indian J Adv Plant Res 1:27–30

Dibble ED, Thomaz SM (2009) Use of fractal dimension to assess habitat complexity and its influence on dominant invertebrates inhabiting tropical and temperate macrophytes. J Freshw Ecol 24:93–102

Dudgeon D, Arthington AH, Gessner MO, Kawabata Z, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard AH, Soto D, Stiassny ML, Sullivan CA (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. Biol Rev Camb Philos Soc 81:163–182

Dussart BH, Frutos SM (1986) Sur quelques Copépodes d'Argentine. Revue d'Hydrobiolgie Tropicale 19(3–4): 241–262

Ferrari MCO, Wisenden BD, Chivers DP (2010) Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus. Can J Zool 88:698–724

Gagneten AM, Vila I (2001) Effects of Cu^{+2} and pH on the fitness of *Ceriodaphnia dubia* (Richard 1894) (Crustacea, Cladocera) in microcosm experiments. Environ Toxicol 16:428–438

González Sagrario MA, Balseiro E (2010) The role of macroinvertebrates and fish in regulating the provision by macrophytes of refugia for zooplankton in a warm temperate shallow lake. Freshw Biol 55:2153–2166

Gross EM, Hilt S, Lombardo P, Mulderij G (2007) Searching for allelopathic effects of submerged macrophytes on phytoplankton-state of the art and open questions. Hydrobiologia 584:77–88

Gross EM, Legrand C, Rengefors K, Tillmann U (2012) Allelochemical interactions among aquatic primary producers. In: Brönmark C, Hansson L-A (eds) Chemical ecology in aquatic systems. Oxford University Press, Oxford, pp 196–209

Gutierrez MF, Paggi JC (2013) Chemical repellency and adverse effects of free-floating macrophytes on the cladoceran *Ceriodaphnia dubia* under two temperature levels. Limnology 15:37–45

Gutierrez MF, Gagneten AM, Paggi JC (2011) Behavioural responses of two cladocerans and two copepods exposed to fish kairomones. Mar Freshw Behav Physiol 44:289–303

Harrison SS, Bradley DC, Harris IT (2005) Uncoupling strong predator–prey interactions in streams: the role of macrophytes. Oikos 108:433–448

Havas M, Rosseland BO (1995) Response of zooplankton, benthos, and fish to acidification: an overview. Water Air Soil Pollut 85:51–62

Jeppesen E, Sondergaard M, Jensen JP, Mortensen E, Hansen AM, Jorgensen T (1998) Cascading trophic interactions from fish to bacteria and nutrients alter reduced sewage loading: an 18-year study of a shallow hypertrophic lake. Ecosystems 1:250–267

Keskitalo J, Eloranta P (1999) Limnology of humic waters. Backhuys Publishers, Liden

Kirk JTO (1994) Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge

Lass S, Spaak P (2003) Chemically induced anti-predator defenses in plankton: a review. Hydrobiology 491:221–239

Lopretto EC, Tell G (1995) Ecosistemas de aguas continentales. Metodologías para su estudio 3. Hemisferio Sur, La Plata:1401 pp

Madsen JD, Chambers PA, James WF, Koch EW, Westlake DF (2001) The interaction between water movement, sediment dynamics and submersed macrophytes. Hydrobiologia 444:71–84

Maerz JC, Brown CJ, Chapin CT, Blossey B (2005) Can secondary compounds of an invasive plant affect larval amphibians? Funct Ecol 19:970–975

Mangas-Ramíreza E, Elías-Gutiérrez M (2004) Effect of mechanical removal of water hyacinth (*Eichhornia crassipes*) on the water quality and biological communities in a Mexican reservoir. Aquat Ecosyst Health Manag 7:161–168

Mann CJ, Wetzel RG (1996) Loading and utilization of dissolved organic carbon from emergent macrophytes. Aquat Bot 53:61–72

Meerhoff M, Fosalva C, Bruzzone C, Mazzeo N, Noordoven W, Jeppesen E (2006) An experimental study of habitat choice by Daphnia: plant signal danger more than refuge in subtropical lakes. Freshw Biol 51:1320–1330

Michelan TS, Thomaz S, Mormul RP, Carvalho P (2010) Effects of an exotic invasive macrophyte (tropical signalgrass) on native plant community composition, species richness and functional diversity. Freshw Biol 55:1315–1326

Mithraja MJ, Antonisamy JM, Mahesh M, Paul ZM, Jeeva S (2011) Phytochemical studies on *Azolla pinnata* R. Br., *Marsilea minuta* L. and *Salvinia molesta* Mitch. Asian Pac J Trop Biomed 1:S26–S29

Nurminen LKL, Horppila JA (2002) A diurnal study on the distribution of filter feeding zooplankton: effect of emergent macrophytes, pH and lake trophy. Aquat Sci 64:198–206

Oyedeji O, Taiwo FO, Ayinde FO, Ajayi OS, Oziegbe M, Kelani MT, Adewole AH (2014) In vitro antimicrobial and antioxidant analysis of gallic acid from the leaves of *Ludwigia abyssinica* A Rich. European J Med Plants 4:1098–1112

Paggi JC (2006) Redescription and re-evaluation of the taxonomic status of the Neotropical copepod *Diaptomus falcifer* Daday, 1905 (Calanoida: Diaptomidae). Stud Neotrop Fauna Environ 41:67–78

Sabattini RA, Lallana VH (2007) Aquatic macrophytes. In: Iriondo MH, Paggi JC, Parma MJ (eds) The Middle Paraná River: limnology of a subtropical wetland. Springer-Verlag, Berlin Heidelberg, pp 205–226

Sakpere AM, Oziegbe M, Bilesanmi IA (2010) Allelopathic effects of *Ludwigia decurrens* and *L. adscendens* subsp. *diffusa* on germination, seedling growth and yield of *Corchorus olitorious* L. Not Sci Biol 2:75–80

Schoeppner NM, Relyea RA (2009) Interpreting the smells of predation: how alarm cues and kairomones induce different prey defenses. Funct Ecol 23:1114–1121

Smolders AJP, Vergeer LHT, van der Velde G, Roelofs JGM (2000) Phenolic contents of submerged, emergent and floating leaves of aquatic and semiaquatic macrophyte species: Why do they differ? Oikos 91:307–310

Stedmon CA, Markager S (2001) The optics of chromophoric dissolved organic matter (CDOM) in the Greenland Sea: an algorithm for differentiation between marine and terrestrially derived organic matter. Limnol Oceanogr 46: 2087–2093

Steinberg CEW (2003) Ecology of humic substances in freshwaters: from whole-lake geochemistry to ecological niche determination. Springer, New York, p 442

Steinberg CEW, Meinelt T, Timofeyev MA, Bittner M, Menzel R (2008) Humic substances (review series). Part 2: Interactions with organisms. Environ Sci Pollut Res 15: 128–135

Steinman AD, Conklin J, Bohlen PJ, Uzarski DG (2003) Influence of cattle grazing and pasture land use on macroinvertebrate communities in freshwater wetlands. Wetlands 23:877–889

Thomaz SM, Ribeiro da Cunha E (2010) The role of macrophytes in habitat structuring in aquatic ecosystems: methods of measurement, causes and consequences on animal assemblages' composition and biodiversity. Acta Limnol Bras. doi:10.4322/actalb.02202011

Trochine C, Modenutti BE, Balseiro EG (2009) Chemical signals and habitat selection by three zooplankters in Andean Patagonian ponds. Freshw Biol 54:480–494

Wojtal A, Frankiewicz P, Izydorczyk K, Zalewski M (2003) Horizontal migration of zooplankton in a littoral zone of the lowland Sulejow Reservoir (Central Poland). Hydrobiologia 506–509:339–346

Yakob HK, Sulaiman SF, Uyub AM (2012) Antioxidant and antibacterial activity of *Ludwigia octovalvis* on *Escherichia coli* O157:H7 and some pathogenic bacteria. World Appl Sci J 16:22–29