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1 **Decline of freshwater gastropods exposed to recurrent interacting stressors implying cyanobacterial**  
2 **proliferations and droughts**

3

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12

13 **Abstract**

14 Freshwater biota increasingly undergo multiple stressors but we poorly understand to what extent they influence  
15 dynamics of community structure. Here, we study the impact of combined stressor exposure on gastropods at 9-  
16 year interval, through a monthly one-year (2013) monitoring, also providing data on the occurrence of other  
17 macroinvertebrate taxa. Previous study in 2004 showed the occurrence of cyanobacteria proliferations, drought,  
18 trematode parasites and invasive non-native pulmonate *Physa acuta*.

19 During 2013, we always detected cyanobacterial microcystins (MCs) in gastropods, from 59 to 4149 ng.g<sup>-1</sup> fresh  
20 mass (vs 0-246 ng.g<sup>-1</sup> in 2004), suggesting a continuous and increased MC-intoxication. Environmental  
21 intracellular MC concentrations were high (8-41 µg.L<sup>-1</sup>) from August to October 2013, whereas only detected in  
22 August 2004 (17 µg.L<sup>-1</sup>). In 2013, we recorded no trematodes among the 2490 sampled gastropods, and *P. acuta*  
23 represented 94% of gastropods (vs 58% in 2004). After August 2013, nearly all gastropods disappeared as most  
24 other macroinvertebrates (except Chironomidae, Ephemeroptera and Trichoptera). The whole decline of  
25 gastropods and other macroinvertebrates, and the absence of trematodes strongly suggest adverse conditions in  
26 the study-site. Despite acute stressful conditions suggested above, gastropod abundance was 13-fold higher in  
27 June 2013 (vs 2004), reflecting successful recolonization and efficient breeding. Most gastropods exposed to  
28 drought and toxic bloom were young vulnerable stages. Thus, we supposed alternation of local gastropod  
29 extinctions vs recolonization that could induce on a long term a loss of diversity to the detriment of the most  
30 sensitive species.

31

32 **Keywords**

33 Gastropoda; co-occurring multiple stressors; toxic blooms; water withdrawal; invasive species; trematodes.

34

## 35 **Introduction**

36 Deterioration of freshwaters is increasing worldwide due to anthropogenic activities and global change  
37 (Relyea and Hoverman 2006; Sumpter 2009). Therefore, freshwater biota increasingly suffer from exposure to  
38 multiple abiotic and biotic stressors that threaten biodiversity (e.g., Vinebrooke et al. 2004; Dewson et al. 2007;  
39 Gérard et al. 2008; Strayer 2010; Nunes et al. 2015; Botana 2016). Stressors can be punctual, recurrent or  
40 continuous; consequently, organisms usually undergo combined mixtures of interacting stressors, which effects  
41 may be synergistic, but are often unknown or difficult to predict (Holmstrup et al. 2010; Lemm and Feld 2017).  
42 Because of the health hazard for biota induced by exposure to multiple stressors, there is a growing interest on  
43 this research topic (Fischer et al. 2013; Lemm and Feld 2017). Unfortunately, we still poorly understand to what  
44 extent interactive effects of multiple recurrent and concomitant stressors (and related stresses) influence the  
45 dynamics of community structure, potentially inducing resistance, resilience or decline of the populations  
46 depending on their sensitivity (Connell and Sousa 1983).

47 Freshwater gastropods are efficient bioindicators of ecosystem health (Tallarico 2016). They constitute  
48 a substantial part of the freshwater biomass and, as primary consumers, an important link between primary  
49 producers (e.g., potentially toxic cyanobacteria) and higher consumers (leeches, crayfish, insects, fish,  
50 waterfowl, and mammals) (Habdija et al. 1995; Dillon 2000). They can represent an intoxication route to  
51 predators in food web as demonstrated for microcystins (MCs) (Lance et al. 2014), well-known hepatotoxins  
52 distributed worldwide and produced by some strains of cyanobacteria (Chorus and Bartram 1999). Freshwater  
53 gastropods can be intoxicated via direct ingestion of toxic cyanobacteria as grazers, and via oral and/or  
54 transtegumental absorption of cyanotoxins dissolved in water and/or adsorbed on particles (Zurawell et al. 1999;  
55 Zhang et al. 2007). Recurrent proliferations of toxic cyanobacteria, typically occurring during summer and  
56 autumn in temperate regions, may lead to a strong decrease in gastropod abundance and species richness (Gérard  
57 et al. 2008; Lance et al. 2010). Freshwater gastropods, typically inhabiting shallow littoral zones, also frequently  
58 experience drought with consequent water withdrawal and habitat loss, potentially representing a severe  
59 environmental stressor (Schwarz and Jenkins 2000; Strzelec and Michalik-Kucharz 2003; Dewson et al. 2007).  
60 The increase of predator pressure and interspecific competition exerted by various taxa of macroinvertebrates  
61 and vertebrates due to the restricted habitat area lead to the elimination of most gastropods, but new generations  
62 typically appear after the water level rises again (Dudgeon 1983; Gérard 2001a; Gérard et al. 2008). Larval  
63 trematodes frequently parasitize freshwater gastropods and constitute a biotic stressor that decreases host fitness  
64 (Esch and Fernandez 1994; Combes 1995 for reviews). Parasites interact with natural and anthropogenic

65 stressors to increase mortality and reduce animal health in myriad ways in a wide spectrum of host and parasite  
66 taxa (Morley 2010; Marcogliese and Pietroock 2011 for reviews). The combined effects of parasites and other  
67 stressors can reduce either resistance or tolerance to infection. However, most ecotoxicological studies  
68 completely neglected trematodes (and other parasites) despite their potential confounding factor in the responses  
69 of gastropods exposed to stresses (Morley 2006; Marcogliese and Pietroock 2011; Martiguez et al. 2012), and  
70 despite trematodes are indicators of environmental changes such as decrease of free-living biodiversity in aquatic  
71 ecosystems over time (e.g., Keas and Blankespoor 1997; Morley and Lewis 2007).

72         Here, we conducted a monthly one-year monitoring (2013) of a gastropod community submitted to  
73 multiple interacting stressors in a French eutrophic dam reservoir. In this site, gastropods, including the invasive  
74 North American *Physa acuta*, experienced recurrent blooms since at least 1997 and punctuated droughts (as in  
75 2004) (Gérard et al. 2009), and some of them harbored larval trematodes (Gérard, pers. obs.). We examined the  
76 temporal fluctuations of two major abiotic parameters: water temperature, which is crucial for ectotherms such  
77 as gastropods and which can correlate with other physicochemical parameters or with cyanobacterial blooms,  
78 and depth, which reflects the availability of habitat/space resources. We investigated the cyanobacterial  
79 occurrence in water and the concentration of intracellular MCs in cyanobacteria. We measured the intoxication  
80 of gastropods by toxic cyanobacteria through the accumulation of MCs in their tissues. We also examined the  
81 monthly occurrence of parasitic trematodes in gastropods in the reservoir. Finally, we recorded the monthly  
82 occurrence of macroinvertebrate taxa other than gastropods because of their potential impact on gastropods as  
83 predators or competitors, and their own response to environmental stressors. We compared data collected during  
84 the year 2013 with data obtained during some months of 2004 (Gérard et al. 2009; Gérard, pers. com.). We  
85 aimed to check long-term changes at 9-year interval in the composition of gastropods, under the influence of  
86 exposure to the following interacting stressors: cyanobacteria proliferations, droughts, trematode parasites, and  
87 invasive non-native *P. acuta*.

88

## 89 **Material and methods**

### 90 *Study-site*

91 The Frémur River (western France, 48°32' N, 2°04' W), representative of many small coastal catchments in  
92 Western Europe, is scattered with many dam obstructions that reduce the average velocity and increase the water  
93 depth. As a result, the total extent of the catchment is 5 ha of running water (streams) and 75 ha of still waters  
94 (ponds and reservoirs). The most important dam (14 m high) creates a 3 x 10<sup>6</sup> m<sup>3</sup> reservoir for drinking water.

95 Winters are generally mild and quite rainy. During summer, cyanobacterial scum regularly occurred on the water  
96 surface since at least the year 1997, and measurements made in September 2003 revealed a density of 221 000  
97 cells.mL<sup>-1</sup>, dominated by *Microcystis aeruginosa* (Acou et al. 2008).

98 We conducted monitoring at the station “Les Rues”, a eutrophic reservoir below dam obstructions. According to  
99 Gérard et al. (2009), high densities of cyanobacteria occurred in this station from May to October 2004 including  
100 90% of genera potentially producing toxins among them MCs [i.e., *Anabaena*, *Aphanizomenon*, *Microcystis*,  
101 *Planktothrix*, *Trichodesmium* (Skulberg et al. 1993)]. Moreover, in August 2004, Gérard et al. (2009) detected  
102 MCs in water samples with intracellular MCs of 17.05 µg L<sup>-1</sup> MC-LR equivalent (MC-LReq).

103

#### 104 *Samplings and measurements*

105 We made monthly samplings from January to December 2013. On each occasion, we measured water  
106 temperature (°C) and maximum water depth (cm) in a littoral area of 20 m length by 2 m width, and we sampled  
107 500 mL of water in the uppermost part of the water column with a bailer to measure intracellular MC  
108 concentrations. We also visually qualified the intensity of cyanobacterial proliferations in four classes: 0 (no  
109 visible cyanobacteria), 1 (some cyanobacteria visible), 2 (some patches of cyanobacteria accumulation at the  
110 surface and/or on the shore line), 3 (continuous layer of cyanobacteria at the surface, ‘green’ water). Then, we  
111 sampled gastropods (and other macroinvertebrates) with a pond-net (nylon mesh: 1 mm, square aperture: 0.5 ×  
112 0.5 m) trawled from the surface sediment through the water column for three minutes along the 20 m long × 2 m  
113 wide area, as in Gérard et al. (2009).

114 In the laboratory, we harvested phytoplankton cells by filtration (1 µm Cyclopore track etched membrane,  
115 Whatman, Maidstone, UK) of the water sample and we froze the filter (-80°C) before MC analysis. We carefully  
116 examined the pond-net contents in tap water, and we counted and identified all the gastropods following Glöer  
117 and Meier-Brook (1994). The descriptors used to characterize the gastropod community were species richness  
118 (S), abundance (A), frequency (F, relative abundance of a species in the community), and occurrence frequency  
119 (FO, percentage of months a species was collected in the 12 months sampled). We also noted the general  
120 occurrence of other macroinvertebrate taxa in the pond-net contents according to Tachet et al. (2006), but  
121 without specific identification.

122 We measured the shell size (shell height for conic shells and diameter for discoid shells) of each gastropod to the  
123 nearest 0.1 mm to establish the age structure of populations and to discriminate juveniles and adults according to  
124 Glöer and Meier-Brook (1994). Then, we removed each gastropod from its shell under stereoscopic microscope

125 to detect parasitic infection by larval trematodes (sporocysts or rediae, and cercariae). If present, we observed  
126 parasites alive under light glass coverslip pressure using bright-field and phase-contrast microscopy. We used  
127 trematode prevalence as parasitic descriptor [i.e., number of hosts infected with a particular parasite species /  
128 number of examined hosts (Bush et al. 1997)]. Finally, we froze and weighted gastropod tissues (-80°C) before  
129 MC analysis. For each species, we quantified MCs in gastropods using one or several individuals depending on  
130 body fresh mass (FM). In total, we realized 36 MC-analyses corresponding to 268 gastropods belonging to seven  
131 species.

132 To compare data at a 9-year interval in the same study-site, we used data published in Gérard et al. (2009) and  
133 we provided unpublished data obtained in June 2004 and October 2004: water temperature and maximal depth,  
134 mean shell size of gastropods, trematode prevalence, and MC concentrations in healthy versus parasitized  
135 gastropods.

136

### 137 *MC analysis*

138 We used the High Pressure Liquid Chromatography (HPLC) to measure the MC concentration (in the  
139 cyanobacterial biomass) of the water samples. The intracellular MCs contained in the filtered phytoplanktonic  
140 biomass were extracted with 2 mL of 100% methanol, placed in an ultrasound water bath for 15 min at 35 kHz,  
141 and then subjected to probe sonication (Hielscher Ultrasonics GmbH, Germany) with a cycle of 0.5 s at 50% of  
142 amplitude during 1 min. The supernatant was analysed by HPLC as described in Lance et al. (2006). The MC  
143 detection limit (DL) in cyanobacterial samples was 0.04 µg.L<sup>-1</sup>. Below this level, the identification process can  
144 be uncertain due to the poor quality of spectra. We expressed the monthly intracellular MC concentration in  
145 water in µg.L<sup>-1</sup> MC-LReq using three standards (MC-RR, MC-YR and MC-LR).

146 We performed MC analysis on frozen tissues of gastropods by immuno-assay method with ELISA Microcystin  
147 Plate Kit (Envirologix INC) which detects free MCs from a 0.05 µg.L<sup>-1</sup>-threshold to the nearest 0.01 µg.L<sup>-1</sup>  
148 (Codd et al. 1997; Gilroy et al. 2000). After an elution with 1mL of 80 % MeOH, samples were placed in an  
149 ultrasound water bath for 15 min at 35 kHz, and next subjected to three cycles of centrifugation-probe sonication  
150 (Hielscher Ultrasonics GmbH, Germany) with a probe sonication cycle of 0.5 s at 70% of amplitude during 1  
151 min. After the last centrifugation, the supernatant was kept and diluted to ensure the presence of less than 10%  
152 methanol in wells of the ELISA tests, according to the manufacturer's protocols (Abraxis LLC, Warminster, PA,  
153 USA). Fluorescence was measured at 450 nm using an absorbance microplate reader (Tecan, Männedorf,  
154 Switerland). We expressed MC concentration in gastropods (MCCG) in MC-LReq ng.g<sup>-1</sup> FM.

155

156 *Data analyses*

157 We performed analyses using R software (R Core Team 2014). Differences were regarded as significant when  $P$   
158  $\leq 0.05$ . The data were log-transformed to meet the assumptions of normality and homogeneity of variance. We  
159 used two-way analysis of variance (ANOVA) to test for differences in MCCG according to the sampling date,  
160 the gastropod species, the developmental stage (juvenile vs adult), and the presence of larval trematodes. We fit  
161 linear regression to examine the relationship between MCCG and snail size.  
162 We reported mean values followed by 95% confidence interval (CI). For percentages (i.e., F, FO, and  
163 prevalence), we calculated CI using the score method (Newcombe 1998).

164

165 **Results**

166 **1) Temporal fluctuations in environmental conditions in 2013: water temperature, maximal water depth,**  
167 **cyanobacteria occurrence and intracellular MC concentration**

168 During the year 2013, the water temperature fluctuated from 0.5°C in March, corresponding to a snowy  
169 episode with ice of 3 mm thick covering the littoral area, to 22.0°C in July (Table 1, mean monthly temperature  
170 of  $13 \pm 4^\circ\text{C}$ ).

171 The maximal water depth in the sampling area fluctuated from 20 cm in August 2013 to 65 cm in  
172 January 2013 (Table 1, mean monthly maximal depth of  $48 \pm 9$  cm). Because of the summer drought, the depth  
173 decreased by half between July and August, and remained  $\leq 39$  cm until the end of the year (Table 1). Due to the  
174 consequent water withdrawal, the littoral area became dry and the distance between the initial shore and the  
175 water edge was comprised between 8 and 14 m from August to December 2013; therefore, neither vegetation nor  
176 organic debris were available as habitat/refuge and food for the snails.

177 We observed the occurrence of cyanobacteria (class 1 in abundance) for the first time in June 2013, and  
178 then increasing proliferations (classes 2 and 3) occurred from August to October 2013 (Table 1). During this  
179 period (August-October), we detected high concentrations of intracellular MCs, i.e., from 7.8 to  $41.3 \mu\text{g.L}^{-1}$ ,  
180 compared to the low values ( $< 0.3 \mu\text{g.L}^{-1}$  or  $< \text{DL}$ ) recorded at the other months of the year (Table 1, mean  
181 monthly intracellular MC concentration of  $5.4 \pm 7.7 \mu\text{g.L}^{-1}$ ).

182

183 **2) Monitoring of the gastropod community in 2013**



184 In total, we sampled 2490 gastropods over the year 2013 belonging to seven species among five  
185 families (in decreasing abundance: Physidae, Planorbidae, Lymnaeidae, Bithyniidae, and Ancyliidae) (Table 2).  
186 The exotic pulmonate *Physa acuta* was the highly dominant species and the most occurrent species of the  
187 gastropod community, each other species representing less than 3% in abundance frequency (Table 2).  
188 The monthly abundance of gastropods greatly varied depending on the sampling date, from 0 in September,  
189 October and December to 1258 individuals in July (Table 1). The mean monthly abundance and species richness  
190 were respectively  $207.50 \pm 275.69$  gastropods and  $2.33 \pm 1.32$  species. We collected 91.41% of the gastropods  
191 during June and July, whereas only 0.08% from August to December 2013. The disappearance of gastropods  
192 coincided with the increase of intracellular MCs in the water samples (Table 1).  
193 Concerning *P. acuta*, based on its relative size-frequency distributions (Fig. 1), two or even three generations  
194 were observed in most months with two periods of recruitment in May and July, corresponding to a decrease in  
195 the mean shell size, respectively  $4.0 \pm 0.4$  and  $3.0 \pm 0.1$  mm (Table 3).  
196 According to the mean shell size of each species per month (Table 3), the gastropod community was mainly  
197 composed of juveniles in June and July, when cyanobacteria began to proliferate and water depth decreased  
198 (Table 1).  
199 No gastropods infected by larval trematodes occurred in 2013 among the 2490 gastropods sampled (prevalence =  
200 0.00%, CI = 0.00-0.15%).

201

### 202 **3) MC accumulation in gastropods in 2013**

203 The MC-analyses made in 2013 revealed the presence of MCs in all gastropod tissues whatever the  
204 gastropod species and the sampling date (Table 4). MCCG values were extremely variable and ranged from 59 to  
205 4149 MC-LReq  $\text{ng.g}^{-1}$  FM (both values corresponding to *P. acuta* tissues) with a mean of  $1085 \pm 411$   $\text{ng.g}^{-1}$  FM.  
206 Accumulation of MCs significantly differed depending on the sampling date ( $F = 3.419$ ;  $P = 0.027$ ). The greatest  
207 values of MCCG occurred in February ( $3109 \pm 1075$   $\text{ng.g}^{-1}$  FM) and the lowest in spring and summer (May-  
208 August,  $385 \pm 168$   $\text{ng.g}^{-1}$  FM) (Table 4). MCCG was not significantly different according to the gastropod  
209 species ( $F = 0.094$ ;  $P = 0.996$ ), the shell size ( $F = 2.725$ ;  $P = 0.108$ ) and the developmental stage ( $F = 2.606$ ;  $P =$   
210 0.121).

211

### 212 **4) Temporal fluctuations of the occurrence of macroinvertebrates other than gastropods in 2013**

213 In addition to Gastropoda, we recorded the occurrence of 21 taxa of macroinvertebrates during the year  
214 2013 with a mean of  $8 \pm 2$  taxa per month, including mostly Arthropoda, and in a lesser extent Annelida, and  
215 then Bivalvia (Table 5).

216 Depending on taxa considered, the frequency of occurrence greatly varied from 8.33% (CI = 1.49-35.39%) to  
217 100% (CI = 75.75-100%) (Table 5). Chironomidae, Ephemeroptera, and Trichoptera occurred all along the year  
218 whatever environmental conditions ( $FO \geq 75\%$ ), whereas Limnadiidae, Asellidae and Corixidae were frequent  
219 only before the stressful period (August-December 2013), and then completely disappeared after August 2013.  
220 The taxa diversity doubled from April to May (13 taxa) and reached a peak in July (17 taxa), and then, strongly  
221 decreased from August (seven taxa) to the end of the study (three taxa in December) (Table 5).

222

### 223 **5) Comparison at a 9-year interval with data collected in June and October 2004**

224 Water temperatures in June and October 2004, respectively 18.5°C and 15°C, were on the same order  
225 than in June and October 2013 (Table 1).

226 The maximum water depths in June and October 2004, respectively 55 and 35 cm, were similar to those  
227 in 2013 (Table 1), as well as the water withdrawal recorded in October 2004 due to summer drought, i.e., 15 m  
228 between the initial shore and the water edge.

229 In June 2004, we sampled the same five species of gastropods than in June 2013, mainly at a juvenile  
230 stage based on their mean shell size (Tables 1, 3, 6). However, the total gastropod abundance was 13-fold higher  
231 in June 2013 than in June 2004. In particular, the dominant non-native species, *P. acuta*, was 20-fold more  
232 abundant in June 2013 than in June 2004, representing 93.52% of the gastropod community (vs 63.16% in June  
233 2004). The native pulmonates *Radix ovata* and *Planorbis planorbis* were respectively thrice and twice more  
234 abundant in June 2013 (vs 2004) but represented a lower part of the community, respectively 1.18% (vs 5.26% in  
235 2004) and 4.62% (vs 25.00%). No gastropods infected by larval trematodes occurred in June 2004 among the 76  
236 gastropods sampled (prevalence = 0.00%, CI = 0.00-4.81%).

237 In October 2004, we collected seven individuals of *P. acuta* and eleven individuals of *R. auricularia* at  
238 an adult stage, whereas we found no gastropods in October 2013 (Tables 1, 6). Three snails among 18 were  
239 infected by rediae and patent cercariae of a species of Echinostomatidae (prevalence = 16.67%, CI = 5.84-  
240 39.22%). These infected snails belonged to *R. auricularia* and were adults with shell size of 16.7, 18.4 and 21.7  
241 mm (mean size of  $18.9 \pm 4.7$  mm), thus greater than that of uninfected *R. auricularia* varying from 9.2 to 18.0  
242 mm (mean size of  $13.4 \pm 2.5$  mm).

243 The comparison with MCCG values recorded previously by Gérard et al. (2009) showed that MCCG  
244 was highly superior in 2013 than in 2004 ( $F = 57.502$ ;  $P = 1.404e^{-9}$ ) (Tables 4, 7). Moreover, MC accumulation  
245 was significantly higher in October 2004 than in June 2004 ( $F = 11.240$ ;  $P = 0.008$ ) (Table 7). When larval  
246 trematodes occurred in October 2004, MCCG was not different between infected and healthy snails ( $F = 0.154$ ;  
247  $P = 0.715$ ) (Table 7).

248

## 249 **Discussion**

250 Disentangle intricate effects of multiple interacting stressors, and related stresses, on freshwater biota  
251 over time in the field is challenging and of growing interest for scientists (Fischer et al. 2013; Lemm and Feld  
252 2017). Our field survey contributes to study responses of gastropods to two frequent combined stressors  
253 recurrent in stagnant waters, i.e., toxic cyanobacteria proliferations and droughts, typically occurring during  
254 summer in most temperate regions (Chorus and Bartram 1999; Lake 2000; Gérard et al. 2008). In addition, we  
255 take into account concomitantly the potential stress induced by i) the often-neglected parasitism by larval  
256 trematodes, ii) the non-native invasive gastropod *Physa acuta* originated to North America, and iii) the  
257 macroinvertebrates commonly acting as predators or competitors of gastropods (Dillon 2000). The disappearance  
258 of nearly all freshwater gastropods (and most other macroinvertebrate taxa) from August to December 2013  
259 reveals stressful conditions in our study-site, probably resulting from negative impact of interacting multiple  
260 stressors and their various related stresses.

261

### 262 **1) Impact of the main stressors and their related stresses on gastropods**

#### 263 *Cyanobacteria proliferations*

264 In addition to toxin production by some strains leading to health hazards, proliferations of cyanobacteria  
265 result in related stresses such as disequilibria in phytoplankton representing food resources for freshwater  
266 gastropods, but also ammonia release and hypoxia / anoxia (Chorus and Bartram 1999; Zurawell et al. 2005;  
267 Paerl and Otten 2013 for reviews). Ammonia (unionized  $\text{NH}_3$ ) is toxic for most aquatic biota with different  
268 tolerance degree between species (Richardson 1997; Alonso and Camargo 2003), and abundance of gastropods  
269 decreases with increasing ammonia concentrations due to toxicity (Cabuk et al. 2004). Depletion of dissolved  
270 oxygen may also be detrimental for gastropods using exclusive gill breathing such as *Bithynia tentaculata*  
271 (recorded in our study), and in a lesser extent for pulmonates potentially aerial breathing with differential

272 sensitivity (Aldridge 1983; McMahon 1983 for reviews). For instance, *P. acuta* is among the most tolerant  
273 macroinvertebrates to hypoxic-anoxic conditions (Pardo and García 2016).

274         Concerning the cyanotoxins, at least some strains of cyanobacteria produced MCs during our study in  
275 2013, in particular from August to October (from 8 to 41  $\mu\text{g.L}^{-1}$  vs  $< 0.3 \mu\text{g.L}^{-1}$  for the other months), and with  
276 higher concentrations than in 2004. Indeed, from May to October 2004, intracellular MCs were detected only in  
277 August (17  $\mu\text{g.L}^{-1}$ ) in water column samples (Gérard et al. 2009). In other waterbodies submitted to toxic  
278 blooms, the highest intracellular MC concentration recorded in phytoplankton was lower or on the same order (7  
279  $\mu\text{g.L}^{-1}$  and 9  $\mu\text{g.L}^{-1}$  in French lakes, 11  $\mu\text{g.L}^{-1}$  in Canadian lakes), even if surface scum samples could contain  
280 considerably higher MC concentrations (400  $\mu\text{g.L}^{-1}$  at the peak of the bloom) (Kotak and Zurawell 2007; Gérard  
281 et al. 2009; Lance et al. 2010). Experiments in controlled conditions showed that realistic MC intoxications via  
282 ingestion of toxic cyanobacteria or exposure to dissolved MCs (from 3 to 50  $\mu\text{g.L}^{-1}$ ) negatively affect gastropod  
283 performance, e.g., fecundity decrease (Gérard et al. 2005; Gérard and Poullain 2005; Lance et al. 2007, 2008).  
284 Such fecundity decrease can negatively influence the dynamic of gastropod populations (Lance et al. 2011). In  
285 the field, recurrent toxic proliferations of cyanobacteria may result in profound changes and even decline of  
286 gastropod communities (Gérard et al. 2008 2009; Lance et al. 2010), decline also observed during our one-year  
287 monitoring in 2013.

288         MC accumulation occurred in all gastropod tissues whatever species and sampling date in 2013,  
289 implying exposure to toxic cyanobacteria throughout the year and/or partial detoxification of MCs, not degraded,  
290 or metabolized at a slower rate than intoxicated. The concentration of MCs in gastropod tissues is correlated with  
291 toxin in the phytoplankton, likely resulting of grazing herbivory (Zurawell et al. 1999), and is assessed to be  
292 higher in gastropods from highly vs lowly MC-contaminated waters (Gérard et al. 2009; Lance et al. 2010).  
293 Therefore, higher values of gastropod MC accumulation in 2013 (vs 2004) reflect higher contamination of the  
294 study-site over time.

295         During 2013, mean monthly MC accumulation in gastropods greatly fluctuated depending on sampling date, and  
296 highest values occurred in February when we recorded no cyanobacteria and no intracellular MCs in the water. It  
297 may be surprising but: i) lowest values recorded in spring and summer 2013 corresponded mostly to neonates  
298 and juveniles, much less exposed to MCs during their short life than adults, and ii) intracellular MC  
299 concentrations in the water were lower in spring and beginning of summer compared to late summer and  
300 autumn. Highest values of mean monthly MC accumulation in winter may suggest MC intoxication of  
301 gastropods by overwintering vegetative filaments of potentially toxic cyanobacteria such as *Aphanizomenon flos*

302 *aquae* and *Planktothrix agardhii* observed in temperate lakes (Head et al. 1999; Briand et al. 2002; Mankiewicz-  
303 Boczek et al. 2011) and dominant in our study-site (Gérard et al. 2009).

304 According to some studies, extremely high inter- and intraspecific variations in the MC contents are commonly  
305 observed in molluscs, and MC accumulation is on average four times greater in pulmonates than in prosobranchs  
306 and bivalves (Zurawell et al. 1999; Gérard et al. 2009; Lance et al. 2010). Surprisingly, in our study, no  
307 statistically significant differences appeared in MC accumulation between gastropod species. This result may be  
308 due to insufficient and biased sampling effort (36 MC-analyzes among them 19 for *P. acuta* at different  
309 developmental stages and 17 for the six other species). However, the maximal values of MC accumulation were  
310 always reached in the non-native invasive *P. acuta* among various co-occurring gastropod species as shown in  
311 other studies (up to 24268 ng.g<sup>-1</sup> FM for *P. acuta* vs 3523 ng.g<sup>-1</sup> FM for other gastropods) (Gérard et al. 2009;  
312 Lance et al. 2010), suggesting inter-species differences in MC metabolism capacities and detoxification  
313 pathways.

314 Further investigations are needed on the possible relationships between inter-species differences of MC  
315 accumulation and physiological stress sensitivity to MCs (and other cyanotoxins), and the consequences on the  
316 dynamics of gastropod community structure exposed to toxic blooms.

317

### 318 *Droughts*

319 As the water level decreased in our study-site, the littoral area formerly inhabited by freshwater  
320 gastropods dried out as observed both in 2004 and 2013. It resulted in a restricted habitat area, depleted in  
321 vegetation and organic debris potentially used as habitat/refuge and food (i.e., decrease of resource availability).  
322 Reduction and fragmentation of habitat space due to drought periods generally result in increased biotic  
323 interactions such as predation and competition, deterioration of water quality, increased sedimentation, high  
324 water temperatures, and hypoxia (< 4 mg O<sub>2</sub>/l) or even anoxia (Schwarz and Jenkins 2000; Lake 2000; Dewson et  
325 al. 2007; Pardo and García 2016 for reviews). Most freshwater gastropods exposed to drought disappear (e.g.,  
326 Dudgeon 1983; Gérard 2001a) as in our study, despite adaptive strategies such as aestivation and desiccation-  
327 resistant eggs to survive drying conditions (Aldridge 1983; MacMahon 1983 for reviews). Nevertheless,  
328 gastropod communities are resilient to drought stressor since recolonization occurs with gradual restoration of  
329 aquatic habitat (Dudgeon 1983; Gérard 2001a).

330

### 331 **2) Larval trematodes acting as biotic stressors and/or stress bioindicators**

332 Helminth parasites such as trematodes provide information on host populations, and more generally, on  
333 free-living biodiversity and changes in ecosystem structure and functioning; a healthy ecosystem being one that  
334 is rich in parasite species (Keas and Blankespoor 1997; Hudson et al. 2006; Morley and Lewis 2007; Hechinger  
335 et al. 2007; Marcogliese 2005, 2016). The heteroxenous life cycle of most trematodes comprises three obligate  
336 hosts including a vertebrate as definitive host and a mollusc as first intermediate host (harboring rediae or  
337 sporocysts producing cercariae), the second intermediate host (with metacercariae) generally being an  
338 invertebrate (Esch and Fernandez 1994 for review). Larval trematodes frequently infect freshwater gastropods as  
339 first intermediate hosts, with detrimental effects on their life-traits, e.g., survival decrease, partial or total  
340 parasitic castration (Combes 1995 for review), and thus may be considered as a biotic stressor. Prevalence may  
341 vary from 0 to 100% in a same site, depending on gastropod species and sampling date, with a mean monthly  
342 prevalence around 6% for the whole gastropod community as for instance in the Combours Lake (Gérard et al.  
343 2008).

344 In our study-site, none of the 2490 snails sampled in 2013, among them 156 belonging to six native  
345 species, were parasitized by larval trematodes. This total absence of trematodes in 2013 may be indicative of a  
346 loss of free-living diversity (including intermediate and definitive hosts) and of a stressed ecosystem where  
347 vulnerable/sensitive stages of trematodes (e.g., free larval miracidia and cercariae) exposed to various  
348 environmental stressors probably died. Indeed, survival of larval trematodes often decreases following stress  
349 exposure (Mackenzie et al. 1995; Morley et al. 2003 for reviews), and trematode prevalence correlates inversely  
350 with perturbation degree (Huspeni and Lafferty 2004; King et al. 2007; Merlo and Etchegoin 2010). Moreover,  
351 local biomasses of all trematode life stages correlate positively with those of their hosts (Laguerre and Poulin  
352 2016), and both diversity and frequency of trematode species also correlate positively with diversity of gastropod  
353 species acting as first intermediate hosts (Gérard 2001b; Gordy et al. 2016). In the eutrophic Combours Lake, 15  
354 of the 17 species of gastropods present at the beginning of the long-term study played the role of first  
355 intermediate host for eleven morphotypes of cercariae (Gérard 2001b; Gérard et al. 2008). The recurrent  
356 exposure to toxic blooms and summer droughts induced the total disappearance of larval trematodes in this lake  
357 due to the decline of the gastropod community, and possibly, due to their own stress sensitivity (Gérard et al.  
358 2008). The single trematode species found only in 2004 in the study-site was an echinostomatid at the redial and  
359 cercarial stages infecting the native lymneid *R. auricularia*. No other gastropod species was infected [even by  
360 metacercariae generally characterized by low host-specificity (Esch and Fernandez 1994; Żbikowski and  
361 Żbikowska 2009)]. The cercarial morphotype was previously recorded in the Combours Lake and named

362 “echinostome 1” in absence of specific identification (Gérard et al. 2008). In this lake, redial and cercarial stages  
363 of “echinostome 1” mainly infected *R. auricularia*, but also *Radix peregra*, *Planorbis planorbis* and *Gyraulus*  
364 *albus* (Gérard et al. 2008). In general, members of Echinostomatidae are allogenic, i.e., temporary resident with  
365 the life cycle not entirely within the lake, with molluscs as first and second intermediate hosts, and a waterfowl  
366 as definitive host (Esch and Fernandez 1994; Gérard et al. 2008). Thus, recolonization of the water body by  
367 “echinostome 1” we recorded in 2004 may occur via passive dispersion of its molluscan hosts and frequentation  
368 of the site by waterfowl definitive hosts, but its maintenance may fail due to sensitivity to stressful  
369 environmental conditions and/or absence of one host species in the life cycle.

370 Finally, larval trematodes did not constitute a biotic stressor for gastropods in our study-site, and their  
371 absence may be indicative of a poor environmental quality and a lack of free-living biodiversity.

372

### 373 **3) The invasive non-native *Physa acuta* as a biotic stressor exposed to multiple combined stressors**

374 The freshwater snail, *P. acuta*, is an exceptionally efficient invader native to North America, introduced  
375 mainly via aquarium trade in Europe during the 19th century, and then established in many regions of Africa,  
376 Asia, Australia, New Zealand, and South America (Albrecht et al. 2009; Bousset et al. 2014 for reviews). In  
377 North American waters, *P. acuta* occupied every habitat from highly ephemeral pools to deep permanent lakes,  
378 and its exceptional habitat breadth stands in contrast to distributional studies of other freshwater taxa (Turner and  
379 Montgomery 2009). In contrast with other hermaphroditic invasive species, worldwide invasion of *P. acuta* is  
380 not associated with a genetic variation loss nor a change in outcrossing mating system towards more selfing  
381 (Bousset et al. 2014). The expansion of its invaded range is partly attributed to efficient passive dispersal using  
382 animal vectors (waterbirds and large mammals) and off-road vehicles (Kappes and Haase 2012; Van Leeuwen et  
383 al. 2013; Banha et al. 2014). Successful invasion of *P. acuta* is also explained by high reproductive output and  
384 high tolerance to water quality decrease (e.g., sewage, pollutants such as heavy metals, cyanobacterial  
385 proliferations, hypoxia/anoxia) and changing environmental conditions (e.g., depth fluctuations, temperature  
386 increase) (Brackenbury and Appleton 1993; Blakely and Harding 2005; Gérard et al. 2008; Albrecht et al. 2009;  
387 Turner and Montgomery 2009; Zukowski and Walker 2009; Lance et al. 2010; Pardo and García 2016).  
388 Moreover, in contrast to native species, *P. acuta* is not or rarely parasitized in introduced areas such as Europe,  
389 Africa and Australia (Brackenbury and Appleton 1993; Gérard et al. 2008; Mitchell and Leung 2016), in  
390 accordance with enemy release hypothesis (Torchin et al. 2003). For all these reasons, *P. acuta* is often the  
391 dominant species in stressed ecosystems (Brackenbury and Appleton 1993; Blakely and Harding 2005; Gérard et

392 al. 2008; Albrecht et al. 2009; Turner and Montgomery 2009; Lance et al. 2010), as shown in our study. In its  
393 introduced area, some authors demonstrated that the generalist and tolerant *P. acuta* may outcompete native  
394 gastropod species, and thus constitutes a biotic stressor for these latter with consequent changes in community  
395 structure and potential decline of some native species (Zukowski and Walker 2009; Höckendorff et al. 2015).  
396 However, stress exposure may also lead to the disappearance of *P. acuta* as in Lithuanian inland waters (Europe)  
397 where the locally established snail was not recorded since 1950s, possibly due to long term application of  
398 herbicides and consequent depletion of oxygen and/or food resources (Buktus et al. 2014).

399 In our study, the predominance of *P. acuta* in the gastropod community clearly increased in 2013  
400 compared to 2004 despite stressful environmental conditions and increased MC contamination. As for other  
401 gastropods, *P. acuta* disappeared from August onwards, probably due to acute combined stress exposure (mainly  
402 high MC contamination of water and drought). Nevertheless, we can suppose successful recolonization of the  
403 study-site by immigration mainly due to efficient passive dispersal that can also benefit to other gastropod  
404 species (Kappes and Haase 2012; Van Leeuwen et al. 2013; Banha et al. 2014). Despite high MC level in its  
405 tissues (as mentioned previously), *P. acuta* survived and reproduced (two recruitment periods in May and July)  
406 in the study-site as also shown in other studies (Gérard et al. 2009; Lance et al. 2010). It may suggest an adaptive  
407 strategy of limited investment in energy-consuming detoxification, allowing saving energy allocated to  
408 maintenance and life traits, and consequently leading to better competitive ability compared to native gastropod  
409 species. We need further investigations in controlled conditions to verify this hypothesis by determining if MC-  
410 toxicity threshold is higher for *P. acuta* than for native gastropod species, and exploring relations between  
411 gastropod ability to cope to multiple stressors and to detoxify MCs.

412

#### 413 **4) Macroinvertebrates acting as predators or competitors for gastropods (biotic stressors) and/or stress** 414 **bioindicators**

415 Many taxa of freshwater macroinvertebrates recorded in our study-site may prey on gastropods  
416 (Astacidae, Notonectidae, Nepidae, Gerridae, Naucoridae, Pleidae, Anisoptera and Zygoptera) or compete with  
417 them for food and space (Enchytraeidae, Sphaeridae, Limnadiidae, Asellidae, Gammaridae and Corixidae)  
418 (Dillon 2000). Based on our one-year monitoring study, predatory and competitive pressures potentially exerted  
419 by macroinvertebrates on gastropods differ among taxa according to their occurrence frequency. Further  
420 investigations including specific identification and monthly abundances are needed to determine which  
421 macroinvertebrate species can be considered as biotic stressors for gastropods in our study-site. Nevertheless, the



422 impact of predation and competition by macroinvertebrates appears limited over time since nearly all  
423 macroinvertebrates disappear after August 2013. Exposure to combined toxic blooms and droughts and their  
424 related stresses probably explain the disappearance of most macroinvertebrates, consequently which may be  
425 rather considered as stress bioindicators (vs gastropod biotic stressors). Indeed, drought commonly induces  
426 decrease of macroinvertebrate richness due to decrease of habitat diversity (Gérard 2000; Dewson et al. 2007;  
427 Pardo and García 2016). Increases in cyanobacterial toxicity generally coincide with decreases in total  
428 abundance and richness in macroinvertebrate communities (White et al. 2005). Intensive blooms lead to the  
429 dying out of most benthic macroinvertebrates, except some taxa less sensitive to cyanotoxin exposure and  
430 becoming more abundant (Krzyzanek et al. 1993; Oberholster et al. 2009). Moreover, ammonia exposure and  
431 hypoxia/anoxia related to both toxic blooms and droughts may have adverse effects on macroinvertebrates  
432 (Richardson 1997; Pardo and García 2016).

433           Among the macroinvertebrate taxa recorded in our study-site, three taxa, i.e., Chironomidae,  
434 Ephemeroptera and Trichoptera, maintain themselves throughout the year despite stressful conditions. Future  
435 studies are necessary to identify the species belonging to the three persistent taxa, and to test their stress  
436 tolerance in order to understand how they cope to combined toxic blooms and droughts and their related stresses.

437

#### 438 **Conclusion**

439           According to our results and previous studies on stress effects, the main stressors acting on gastropods  
440 in our study-site are almost certainly toxic blooms and droughts. The combination of both stressors and their  
441 related stresses result in extremely stressful conditions for native freshwater gastropods, i.e., decrease of space  
442 and food availability, intoxication by ammonia, MCs and probably other cyanotoxins, increase of predation and  
443 competition pressures, hypoxia/anoxia, warming. These interacting stresses which effects may be synergistic  
444 seem to induce profound and long-lasting effects on taxonomic richness and abundance of gastropods, leading to  
445 the elimination of gastropods, as also shown through a long-term study on 10 year-interval in a eutrophic lake  
446 submitted to recurrent toxic blooms and droughts (Gérard et al. 2008).

447           On the contrary, the biotic stressors studied here: parasitic trematodes, non-native invasive *P. acuta*, predatory  
448 and competitive macroinvertebrates, have probably no or minor impact on gastropods, because they are  
449 themselves affected by the above-mentioned combined stressors and almost all disappear following stress  
450 exposure, indicating adverse environmental conditions.

451           Despite the fact that gastropods tend to disappear at the end of summer due to acute stressful conditions,  
452 they showed extremely high reproductive effort in late spring-early summer 2013. Highly efficient breeding may  
453 be an adaptive response to environmental fluctuations and recurrent exposure to multiple interacting stressors  
454 and related stresses (Sulmon et al. 2015). Both high breeding capacities of gastropods and successful  
455 recolonization of the stressed ecosystem, probably by efficient passive dispersion from healthier surrounding  
456 waterbodies (Kappes and Hause 2012), suppose a relative resilience of gastropods with alternation of local  
457 extinctions vs recolonizations. On a long term, recurrent and increasing stresses could induce a loss of diversity  
458 to the detriment of the most sensitive species and to the benefit of the most resistant or tolerant. This hypothesis  
459 is supported by the increasing dominance at 9-year interval of the invasive non-native *P. acuta*, a species  
460 commonly dominant in stressed ecosystems and which can outcompete native gastropods, in relation with its  
461 better ability to cope with co-occurring stresses and its higher capacity of recolonization compared to native  
462 species (see numerous references detailed previously). Further long-term investigations on population dynamics  
463 are needed to understand how recurrent and stressful environmental conditions may influence interspecific  
464 competitive interactions.

465           In the future, we also need to include parasites in ecotoxicological studies as already suggested by  
466 several authors (Morley 2010; Marcogliese and Pietroock 2011), and to obtain more data on parasites and their  
467 complex interactions with hosts and other stressors, as well as on responses of parasites to multiple interacting  
468 stressors. In our study, the absence of trematode parasites due to acute stressful conditions and decline of  
469 gastropod host species, but also decline of macroinvertebrates potentially acting as second intermediate hosts,  
470 confirms their potential use as indicators of poor free-living biodiversity and stressed ecosystem (e.g., Keas and  
471 Blankespoor 1997; Morley and Lewis 2007). One question asks about potential differences in toxin  
472 accumulation between infected and uninfected snails, with what consequences on toxin transfer through the food  
473 web since trematodes generally favor predation of parasitized molluscs by vertebrate definitive hosts (Combes  
474 1995). Some authors demonstrated changes in pollutant (xenobiotics and cadmium) accumulation in freshwater  
475 and marine bivalves due to larval trematode parasitism (Heinonen et al. 1999; Baudrimont and De Montaudouin  
476 2007), but studies on this subject are scarce and do not investigate trematodes-gastropods associations.

477

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479

480 **Conflict of interest**

481 All of the authors read and approved the paper that has not been published previously nor is it being considered  
482 by any other peer-reviewed journal. The authors declare that there are no conflicts of interest.

483

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686

687

**Figure caption**

688

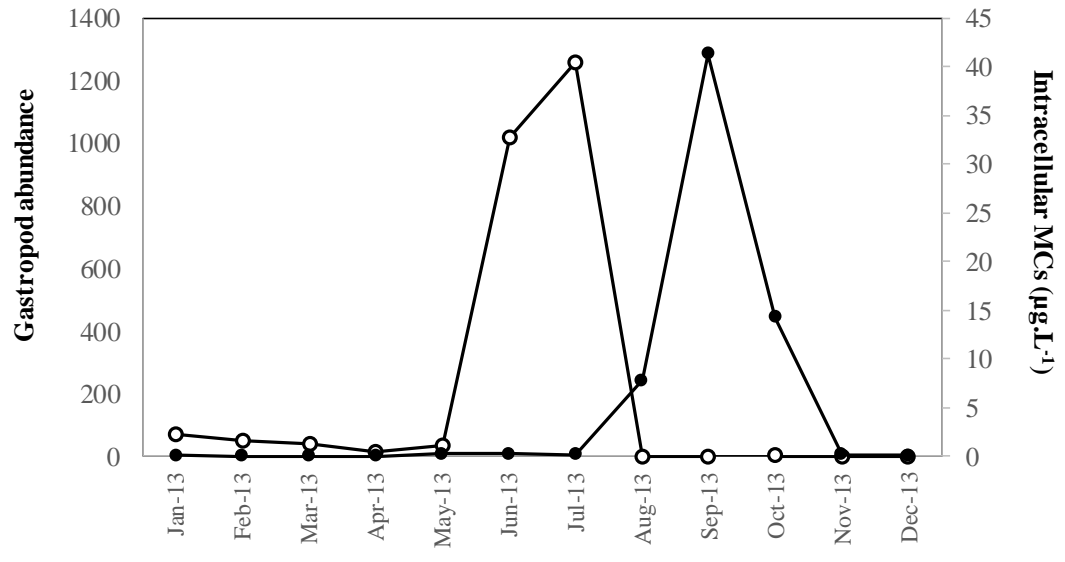
689 **Fig. 1** Relative size-frequency distributions and mean shell size ( $\pm$  CI) of the invasive gastropod *Physa acuta*  
690 each month from January to August 2013 in the station “Les Rues” of the Frémur River (mean size and size  
691 classes in mm, relative abundance from 0 to 30% with 10%-step graduation). We sampled no *P. acuta* from  
692 September to December 2013. Sample sizes are indicated in Table 1.

693

694 Figure 1

695

696



697

698 **Table 1. Environmental conditions, i.e., water temperature (°C), maximal water depth (cm), intensity of cyanobacteria occurrence (class from 0 to 3) and**  
 699 **intracellular MCs ( $\mu\text{g.L}^{-1}$ ), and abundance and species richness of gastropods sampled in the Frémur during 2013. DL = detection limit ( $0.04 \mu\text{g.L}^{-1}$ ).**

700

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Water temperature (°C)	5	3	0.5	11	15	16	22	21	17	17	9.5	7	
Max water depth (cm)	65	65	60	60	60	55	40	20	38	38	39	37	
Cyanobacteria occurrence	0	0	0	0	0	1	0	2	3	3	0	0	
Intracellular MCs ( $\mu\text{g.L}^{-1}$ )	≤ DL	≤ DL	≤ DL	≤ DL	0.260	0.274	0.192	7.819	41.320	14.249	0.171	0.073	
Caenogastropoda													
<i>Bithynia tentaculata</i>	1				2	2	6						11
Heterobranchia													
<i>Physa acuta</i>	34	44	33	14	30	952	1226	1					2334
<i>Planorbis planorbis</i>	15	3	5		2	47							72
<i>Hippeutis complanatus</i>	21	4	3			5	12						45
<i>Radix ovata</i>					1	12	11						24
<i>Radix auricularia</i>							2				1		3
<i>Ancylus fluviatilis</i>							1						1
Total abundance	71	51	41	14	35	1018	1258	1	0	0	1	0	2490
Species richness	4	3	3	1	4	5	6	1	0	0	1	0	7

701

702

703 **Table 2. Structure of the gastropod community in the Frémur from January to December 2013. A: abundance, F: frequency ± CI (%), FO: mean frequency of**  
 704 **occurrence ± CI among the 12 sampled months (%), CI: confidence interval. Abbreviations of gastropod species in parentheses.**

	A	F (CI)	FO (CI)
Caenogastropoda			
<i>Bithynia tentaculata (Bte)</i>	11	0.44 (0.25-0.79)	33.33 (13.81-60.94)
Heterobranchia			
<i>Physa acuta (Pac)</i>	2334	93.73 (92.71-94.62)	66.67 (39.06-86.19)
<i>Planorbis planorbis (Ppl)</i>	72	2.89 (2.30-3.63)	41.67 (19.33-68.05)
<i>Hippeutis complanatus (Hcom)</i>	45	1.81 (1.35-2.41)	41.67 (19.33-68.05)
<i>Radix ovata (Rov)</i>	24	0.96 (0.65-1.43)	25.00 (8.89-53.23)
<i>Radix auricularia (Rau)</i>	3	0.12 (0.04-0.35)	16.67 (4.70-44.80)
<i>Ancylus fluviatilis (Afl)</i>	1	0.04 (0.01-0.23)	8.33 (1.49-35.39)

705

706

707 **Table 3. Mean shell size  $\pm$  confidence interval (CI) of each gastropod species from January to December 2013 in the Frémur. We sampled no gastropods in**  
 708 **September, October and December.**

709

	January	February	March	April	May	June	July	August	November
<i>Bithynia tentaculata</i>	8.5				8.15 $\pm$ 0.45	8.45 $\pm$ 0.65	2.78 $\pm$ 0.98		
<i>Physa acuta</i>	5.32 $\pm$ 0.32	5.59 $\pm$ 0.28	5.58 $\pm$ 0.32	5.64 $\pm$ 0.40	3.98 $\pm$ 0.42	4.05 $\pm$ 0.12	2.98 $\pm$ 0.07	6.0	
<i>Planorbis planorbis</i>	4.73 $\pm$ 0.35	3.73 $\pm$ 0.99	5.60 $\pm$ 0.33		5.60 $\pm$ 0.40	2.86 $\pm$ 0.26			
<i>Hippeutis complanatus</i>	2.63 $\pm$ 0.08	2.73 $\pm$ 0.09	2.77 $\pm$ 0.15			3.94 $\pm$ 0.65	2.40 $\pm$ 0.19		
<i>Radix ovata</i>					3.5	4.85 $\pm$ 1.99	5.96 $\pm$ 1.55		
<i>Radix auricularia</i>							13.00 $\pm$ 0.43		14.3
<i>Ancylus fluviatilis</i>							4.3		

710

711



712 **Table 4. Abundance (A), frequency (F) ± CI (%) and mean shell size ± CI of gastropods in June and October 2004 in the Frémur (CI: confidence interval).**

713 Gérard et al. (2009) observed cyanobacterial occurrence from May to October 2004 with a peak in July, but they did not detect intracellular MCs excepted in August 2004

714 (17.05 µg.L<sup>-1</sup>).

	June 2004			October 2004		
	A	F (CI)	Size ± CI	A	F (CI)	Size ± CI
<i>Bithynia tentaculata</i>	3	3.95 (1.35-10.97)	4.08 ± 5.83			
<i>Physa acuta</i>	48	63.16 (51.92-73.12)	4.78 ± 0.66	7	38.89 (20.30-61.38)	8.66 ± 1.32
<i>Planorbis planorbis</i>	19	25.00 (16.63-35.78)	4.09 ± 0.71			
<i>Hippeutis complanatus</i>	2	2.63 (0.72-9.10)	2.25 ± 0.00			
<i>Radix ovata</i>	4	5.26 (2.07-12.77)	4.50 ± 2.44			
<i>Radix auricularia</i>				11	61.11 (38.62-79.70)	14.93 ± 2.54

715

716 **Table 5. Accumulation of microcystins (ng.g<sup>-1</sup> FW) for each gastropod species and mean value (± CI) per month during the year 2013 in the Frémur (total of 36 MC-**  
717 **analyses using the tissues of one or several individuals depending on their body fresh weight, number of individuals per sample in parentheses; juveniles in italics,**  
718 **adults in bold). See abbreviations of gastropod species in Table 2.**

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Nov
<i>Bte</i>	<b>1237.26 (1)</b>				<b>262.82 (2)</b>	<b>313.59 (2)</b>			
<i>Pac</i>	1325.45 (16)	4148.95 (8)	<i>3860.08 (9)</i>	<i>319.84 (5)</i>	<i>1332.00 (4)</i>	<i>436.56 (18)</i>	<b>143.15 (17)</b>	58.83 (1)	
	<i>528.37 (16)</i>	3973.87 (3)	<b>84.48 (12)</b>		211.04 (4)	<b>259.48 (10)</b>	69.89 (39)		
		<b>1606.98 (6)</b>	<b>201.97 (6)</b>		<b>342.78 (1)</b>	<b>496.16 (3)</b>			
		<b>2127.65 (4)</b>							
<i>Ppl</i>	582.30 (6)	3274.39 (3)	1664.86 (2)		937.78 (2)	548.33 (17)			
	<i>1087.17 (9)</i>								
<i>Hco</i>	<b>1237.26 (17)</b>	<b>3522.92 (4)</b>					807.22 (6)		
<i>Rov</i>						206.44 (6)	253.11 (5)		
<i>Rau</i>							<b>84.49 (2)</b>		<b>1351.53 (1)</b>
<i>Afl</i>							<b>163.36 (1)</b>		
Mean	999.64 ± 352.63	3109.13 ± 1023.39	1452.85 ± 2440.78	319.84	617.28 ± 568.99	376.76 ± 136.99	253.54 ± 278.71	58.83	1351.53

719

720

721 **Table 6. Accumulation of microcystins (ng.g<sup>-1</sup> FW) for each gastropod species in June and October 2004 and mean value (± CI) per month in the Frémur (total of 11**  
 722 **MC-analyses using the tissues of one or several individuals, number of individuals per sample in parentheses; juveniles in italics, adults in bold; '+' indicated**  
 723 **parasitized snails).**

724

	June 2004	October 2004
<i>Bithynia tentaculata</i>	0.00 (1)	
<i>Physa acuta</i>	<b>12.75 (6)</b>	<b>246.29 (7)</b>
<i>Planorbis planorbis</i>	10.79 (8)	
<i>Hippeutis complanatus</i>	0.00 (2)	
<i>Radix ovata</i>	12.66 (3)	
<i>Radix auricularia</i>		<b>18.98 (3)</b>
		<b>46.87 (5)</b>
		<b>37.95 (1+)</b>
		<b>103.83 (1+)</b>
		<b>19.65 (1+)</b>
Mean value ± CI	7.24 ± 7.65	78.93 ± 87.60

725

726

727 **Table 7. Monthly occurrence and frequency of occurrence (FO%, confidence interval CI) of macroinvertebrates other than gastropods in the Frémur during 2013.**

728 **Abundance is indicated in parentheses when only some individuals were recorded.**

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	FO% (CI)
<b>Annelida</b>													
Enchytraeidae					x	x	x				x		33.33 (13.81-60.94)
Piscicolidae					x	x	x						25.00 (8.89-53.23)
Erpobdellidae							x						8.33 (1.49-35.39)
<b>Bivalvia</b>													
Sphaeriidae					x (1)		x (1)						16.67 (4.70-44.80)
<b>Crustacea</b>													
Limnadiidae			x	x	x	x	x	x					50.00 (25.38-74.62)
Asellidae	x	x	x	x	x	x	x	x					66.67 (39.06-86.19)
Gammaridae	x	x (1)											16.67 (4.70-44.80)
Astacidae							x (1)	x (4)		x (1)			25.00 (8.89-53.23)
<b>Hexapoda</b>													
Chironomidae	x	x	x	x	x	x	x	x	x	x	x	x (1)	100.00 (75.75-100.00)
Corixidae	x	x	x		x	x	x	x					58.33 (31.95-80.67)
Notonectidae		x				x	x						25.00 (8.89-53.23)
Nepidae					x		x						16.67 (4.70-44.80)
Gerridae					x								8.33 (1.49-35.39)
Naucoridae							x						8.33 (1.49-35.39)
Pleidae									x (1)				8.33 (1.49-35.39)
Trichoptera	x	x	x	x	x			x	x	x	x		75.00 (46.77-91.11)
Anisoptera						x	x						16.67 (4.70-44.80)
Zygoptera	x	x				x	x						33.33 (13.81-60.94)
Ephemeroptera	x	x	x	x	x	x	x	x	x	x	x	x (4)	100.00 (75.75-100.00)
Coleoptera					x	x	x		x (1)			x (1)	41.67 (19.33-68.05)
<b>Arachnida</b>													
Hydracarina		x (1)	x (10)	x	x	x	x				x		58.33 (31.95-80.67)