Decline of freshwater gastropods exposed to recurrent interacting stressors implying cyanobacteria proliferations and droughts

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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^{-1} fresh

20   mass (vs 0-246 ng.g^{-1} in 2004), suggesting a continuous and increased MC-intoxication. Environmental

21   intracellular MC concentrations were high (8-41 µg.L^{-1}) from August to October 2013, whereas only detected in

22   August 2004 (17 µg.L^{-1}). 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

34

Introduction

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

Freshwater gastropods are efficient bioindicators of ecosystem health (Tallarico 2016). They constitute a substantial part of the freshwater biomass and, as primary consumers, an important link between primary producers (e.g., potentially toxic cyanobacteria) and higher consumers (leeches, crayfish, insects, fish, waterfowl, and mammals) (Habdija et al. 1995; Dillon 2000). They can represent an intoxication route to predators in food web as demonstrated for microcystins (MCs) (Lance et al. 2014), well-known hepatotoxins distributed worldwide and produced by some strains of cyanobacteria (Chorus and Bartram 1999). Freshwater gastropods can be intoxicated via direct ingestion of toxic cyanobacteria as grazers, and via oral and/or transtegumental absorption of cyanotoxins dissolved in water and/or adsorbed on particles (Zurawell et al. 1999; Zhang et al. 2007). Recurrent proliferations of toxic cyanobacteria, typically occurring during summer and autumn in temperate regions, may lead to a strong decrease in gastropod abundance and species richness (Gérard et al. 2008; Lance et al. 2010). Freshwater gastropods, typically inhabiting shallow littoral zones, also frequently experience drought with consequent water withdrawal and habitat loss, potentially representing a severe environmental stressor (Schwarz and Jenkins 2000; Strzelec and Michalik-Kucharz 2003; Dewson et al. 2007). The increase of predator pressure and interspecific competition exerted by various taxa of macroinvertebrates and vertebrates due to the restricted habitat area lead to the elimination of most gastropods, but new generations typically appear after the water level rises again (Dudgeon 1983; Gérard 2001a; Gérard et al. 2008). Larval trematodes frequently parasitize freshwater gastropods and constitute a biotic stressor that decreases host fitness (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 Pietrock 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 Pietrock 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 3×10^6 m^3 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^{-1}, 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^{-1} 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^{-1}. 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^{-1} 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^{-1}-threshold to the nearest 0.01 μg.L^{-1}

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, Warminister, 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^{-1} FM.

155

156   *Data analyses*

157   We performed analyses using R software (R Core Team 2014). Differences were regarded as significant when P

158   ≤ 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 ± 4°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 ± 9 cm). Because of the summer drought, the depth

173   decreased by half between July and August, and remained ≤ 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 µg.L^{-1},

180   compared to the low values (< 0.3 µg.L^{-1} or $<$ DL) recorded at the other months of the year (Table 1, mean

181   monthly intracellular MC concentration of 5.4 ± 7.7 µg.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 Ancylidae) (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 ± 275.69 gastropods and 2.33 ± 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 ± 0.4 and 3.0 ± 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 ng.g^{-1} FM (both values corresponding to *P. acuta* tissues) with a mean of 1085 ± 411 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 ± 1075 ng.g^{-1} FM) and the lowest in spring and summer (May-

208   August, 385 ± 168 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 ± 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 ≥ 75%), whereas Limnadidae, 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 ± 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 ± 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 NH3) 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 μg.L^{-1} vs < 0.3 μg.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 μg.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   μg.L^{-1} and 9 μg.L^{-1} in French lakes, 11 μg.L^{-1} in Canadian lakes), even if surface scum samples could contain

280   considerably higher MC concentrations (400 μg.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 μg.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^{-1} FM for *P. acuta* vs 3523 ng.g^{-1} FM for other gastropods) (Gérard et al. 2009;

312 Lance et al. 2010), suggesting inter-species differences in MC metabolization 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 0_2/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 Combourg 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 (Lagrue 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 Combourg 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 Combourg 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, Limnadidae, 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 Pietrock 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

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

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688

689   **Fig. 1** Relative size-frequency distributions and mean shell size (± 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
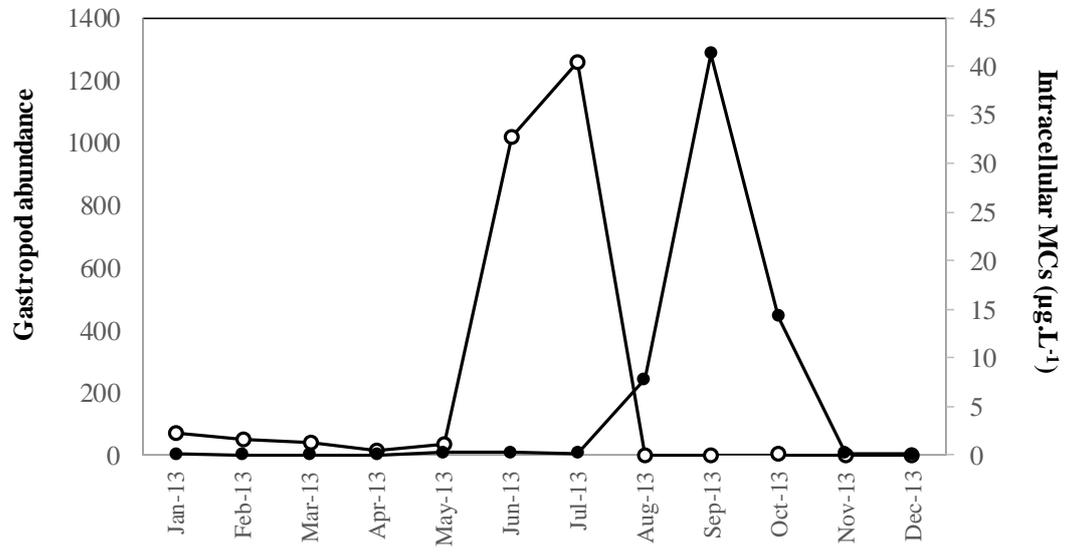
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700

	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 (µg.L^{-1})	≤ DL	≤ DL	≤ DL	≤ DL	0.260	0.274	0.192	7.819	41.320	14.249	0.171	0.073	
Caenogastropoda													
Bithynia tentaculata	1				2	2	6						11
Heterobranchia													
Physa acuta	34	44	33	14	30	952	1226	1					2334
Planorbis planorbis	15	3	5		2	47							72
Hippeutis complanatus	21	4	3			5	12						45
Radix ovata					1	12	11						24
Radix auricularia							2				1		3
Ancylus fluviatilis							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			
Bithynia tentaculata (Bte)	11	0.44 (0.25-0.79)	33.33 (13.81-60.94)
Heterobranchia			
Physa acuta (Pac)	2334	93.73 (92.71-94.62)	66.67 (39.06-86.19)
Planorbis planorbis (Ppl)	72	2.89 (2.30-3.63)	41.67 (19.33-68.05)
Hippeutis complanatus (Hcom)	45	1.81 (1.35-2.41)	41.67 (19.33-68.05)
Radix ovata (Rov)	24	0.96 (0.65-1.43)	25.00 (8.89-53.23)
Radix auricularia (Rau)	3	0.12 (0.04-0.35)	16.67 (4.70-44.80)
Ancylus fluviatilis (Afl)	1	0.04 (0.01-0.23)	8.33 (1.49-35.39)

705

706

707   **Table 3. Mean shell size ± 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
Bithynia tentaculata	8.5				8.15 ± 0.45	8.45 ± 0.65	2.78 ± 0.98		
Physa acuta	5.32 ± 0.32	5.59 ± 0.28	5.58 ± 0.32	5.64 ± 0.40	3.98 ± 0.42	4.05 ± 0,12	2.98 ± 0,07	6.0	
Planorbis planorbis	4.73 ± 0.35	3.73 ± 0.99	5.60 ± 0.33		5.60 ± 0.40	2.86 ± 0.26			
Hippeutis complanatus	2.63 ± 0.08	2.73 ± 0.09	2.77 ± 0.15			3.94 ± 0.65	2.40 ± 0.19		
Radix ovata					3.5	4.85 ± 1,99	5.96 ± 1,55		
Radix auricularia							13.00 ± 0.43		14.3
Ancylus fluviatilis							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^{-1}).

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

715

716 **Table 5. Accumulation of microcystins (ng.g^{-1} 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
Bte	**1237.26 (1)**				**262.82 (2)**	**313.59 (2)**			
Pac	1325.45 (16)	4148.95 (8)	*3860.08 (9)*	*319.84 (5)*	*1332.00 (4)*	*436.56 (18)*	**143.15 (17)**	58.83 (1)	
	528.37 (16)	3973.87 (3)	**84.48 (12)**		211.04 (4)	**259.48 (10)**	69.89 (39)		
		1606.98 (6)	**201.97 (6)**		**342.78 (1)**	**496.16 (3)**			
		2127.65 (4)							
Ppl	582.30 (6)	*3274.39 (3)*	1664.86 (2)		937.78 (2)	548.33 (17)			
	1087.17 (9)								
Hco	**1237.26 (17)**	**3522.92 (4)**					807.22 (6)		
Rov						206.44 (6)	253.11 (5)		
Rau							**84.49 (2)**		**1351.53 (1)**
Afl							**163.36 (1)**		
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^{-1} 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
Bithynia tentaculata	*0.00 (1)*	
Physa acuta	**12.75 (6)**	**246.29 (7)**
Planorbis planorbis	*10.79 (8)*	
Hippeutis complanatus	*0.00 (2)*	
Radix ovata	*12.66 (3)*	
Radix auricularia		**18.98 (3)**
		46.87 (5)
		37.95 (1+)
		103.83 (1+)
		19.65 (1+)
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)
Annelida													
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)
Bivalvia													
Sphaeriidae					x (1)		x (1)						16.67 (4.70-44.80)
Crustacea													
Limnadidae			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)
Hexapoda													
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)
Arachnida													
Hydracarina		x (1)	x (10)	x	x	x	x				x		58.33 (31.95-80.67)

729