Effect of water warming on the structure of biofilm-dwelling communities

Nabil Majdi, Jana Uthoff, Walter Traunspurger, Pascal Laffaille, Anthony Maire

Effect of water warming on the structure of biofilm-dwelling communities

Nabil MAJDI [a,b *], Jana UTHOFF [a], Walter TRAUNSPURGER [a], Pascal LAFFAILLE [b] and Anthony MAIRE [c]

a) *Animal Ecology, Bielefeld University, Bielefeld, Germany.*

b) *Laboratoire Ecologie Fonctionnelle et Environnement, EcoLab, Université de Toulouse, CNRS, INPT, UPS, Toulouse, France.*

c) *EDF R&D LNHE - Laboratoire National d'Hydraulique et Environnement, Chatou, France*

* Corresponding author: N. MAJDI, Email: nabil.majdi@uni-bielefeld.de

Abstract

Water warming, either resulting from global or local drivers, is of growing concern for aquatic environments. Changes in thermal regimes may alter the functioning and the trophic dynamics of river ecosystems since most aquatic biota like micro-algae, invertebrates, or fish are poikilotherms. Since biofilms integrate basal and intermediate components of aquatic food webs, examination of their responses to warming should contribute to a better understanding of the effects of ongoing climate change on river ecosystems. In this study, we considered sites that were or were not exposed directly to thermal effluents from a nuclear power plant as "in situ laboratories" of the effect of climate change and water warming. To compare the structure of algal and invertebrate communities dwelling in epilithic biofilms subjected to different thermal regimes in the Garonne River, sets of concrete tiles were placed in the river and the biofilm growing on these standard substrates was sampled after two months of colonisation. The site located within the thermal plume directly downstream of the thermal effluents showed warmer average water temperature (mean ΔT of about 1.5°C over the study period) and greater daily temperature fluctuations in comparison to the control site. Algal and invertebrate biomass did not differ significantly, but the structure of communities differed markedly between sites. At the heated site, the proportion of cyanobacteria was higher, while there were fewer micro-crustaceans and larvae of insects and bivalves. However, the population of chironomids showed a greater proportion of relatively large individuals. Interestingly, nematodes were more abundant, and their communities comprised more species at the heated site. Their feeding-types were also more diverse showing a greater proportion of algivores, large-sized omnivorous and predatory species. Changes in the abundance and age structure further showed that some bacterivore species, such as *Eumonhystera vulgaris*, thrived especially well at the heated site, whereas another bacterivore, *Monhystrella paramacrura*, thrived well at the control site. In the context of

climate change, our results highlight that warmer and more fluctuating water temperatures have the potential to alter the body-size spectrum, species composition, distribution of feeding specialisations and age structure within biofilm-dwelling communities.

Keywords

Meiofauna, Warming, Freshwater, Community ecology, Species distribution

1. Introduction

The question of how organisms and ecosystems respond to a rise in temperatures has attracted growing attention from the scientific community because of our need to understand and foresee the effects of the ongoing climate change on biological diversity and ecosystem functionality. In aquatic environments, spatial variability in water temperature can be natural (e.g. geothermal activity, presence of a water source) or result from direct local (e.g. deforestation, industrial activities) or indirect global (i.e. climate change) anthropogenic disturbances (Caissie, 2006; Van Vliet et al., 2011). Alteration of thermal regimes can be a major determinant of changes in the diversity and resilience of aquatic biota (Steel et al., 2017; Reid et al., 2019). For example, Mouthon and Daufresne (2006) recorded a dramatic reduction in density and diversity of mollusc communities in the Saône River (France) during the exceptional European heat wave in the summer of 2003. The recovery of the mollusc communities studied was not fully observed even eight years after the heat wave, and profound changes in community structure have persisted over the long term (Mouthon and Daufresne, 2015). Streams and rivers that are naturally or artificially heated can be considered relevant and realistic "*in situ* laboratories" to study the effects of climate change and water warming on aquatic organisms from the individual to the ecosystem level (Woodward et al., 2010). Several examples of such experiments have contributed to a better understanding of the effects of global warming on aquatic biodiversity and ecosystem functioning. In their review, O'Gorman et al. (2012) summarised a decade of research conducted in an active geothermal region (Hengill, Iceland). The underground geothermal heating of streams in this region allowed for comparative studies between naturally heated and non-heated systems. These works have revealed strong influences of water temperature on the structure of macroinvertebrate and algal assemblages, and on ecosystem functioning. Sandblom et al. (2016), Svensson et al. (2017), and Huss et al. (2019) have documented some

of the most remarkable results obtained from the "Biotest Enclosure" experiment carried out in the Baltic Sea (Sweden). For research purposes, heated cooling water from a nuclear power plant was diverted into an isolated atoll in the late 1970s. Since then, a temperature difference of 5–10°C has been maintained between the inside and outside of the enclosure. This made possible the study of the physiological responses of the European perch (*Perca fluviatilis*) to water warming, highlighting the fact that global warming could outpace the adaptive capacity of ectotherms (Sandblom et al., 2016). Lastly, Boulêtreau et al. (2014) compared biofilms collected inside or outside the thermal plume of a nuclear power plant located on the Garonne River (France) and demonstrated that water warming affected the denitrification activity of the biofilms through changes in the composition of their bacterial community. Despite the key insights brought by these studies into the responses of aquatic ecosystems to climate change, we still lack a comprehensive understanding of the warming-induced ecological effects that can be expected in the future on more complex and realistic communities made up of different interacting biological compartments.

Biofilms are colonies of microscopic organisms that become large enough to create visible, macroscopic structures. In aquatic ecosystems, biofilms are hotspots of biodiversity supporting pivotal ecosystem functions such as primary production and nutrient cycling. Thereby, biofilms fix and transform drifting particles and dissolved inorganic nutrients into attached biomass that can then be used by a variety of consumers (Battin et al., 2016; Lyautey et al., 2013; Risse-Buhl et al., 2012; Romaní et al., 2004; Teissier et al., 2007; Weitere et al., 2018). Ecological successions in river biofilms are characterised by an accretion phase followed by a detachment phase as the thickness and porosity of mature biofilms increase their sensitivity to shear stress and grazers (Biggs, 1996). Nevertheless, biofilms can also self-detach in summer under warmer temperatures and low flow because of bacterial decomposition of senescent basal algal layers (Boulêtreau et al., 2006). The density and

biomass of biofilm-dwelling grazers correlate well with the total biomass and chlorophyll *a* content of the biofilm because biofilms represent both a source of food and a habitat for micro-grazers like nematodes (Majdi et al., 2011, 2012a; Peters and Traunspurger, 2005; Schroeder et al., 2012; Vidakovic et al., 2011). Overall, most ecosystem services provided by aquatic biofilms could be strongly affected by global warming (Romaní et al., 2016). Experimental studies of the response of biofilm communities to water warming have shown faster accretion phases (especially higher bacterial growth rates), greater heterotrophic use of organic compounds (Norf et al., 2007; Villanueva et al., 2011), and enhanced filtration rates of peritrich ciliates and rotifers under warmer conditions (Kathol et al., 2009). In a 21-days *in situ* warming experiment (on average +2.5°C), warmed-up biofilms showed faster colonisation and maturation, and greater respiration and denitrification rates due to changes in the structure of bacterial communities at the expense of phototrophic biomass (Boulêtreau et al., 2014).

The responses of species to water warming essentially depends on their physiological ability to cope with warmer temperatures, or fluctuations thereof, and their capacity to cope with changes in the abundance of the species with which they interact (i.e. their predators, competitors, and prey) (O'Gorman et al., 2012; Woodward et al., 2010). Our aim with this study was to compare heated and non-heated natural river biofilms, with their complex assemblage of prey, micro-grazers, and predators to examine whether their community structure showed coherent responses to different thermal conditions. We exposed biofilms to heated effluents from the nuclear power plant of Golfech (Garonne River, France). At the chosen experimental site, the warming averages +2.5°C (Boulêtreau et al., 2014), which lies in the range of most Earth surface warming scenarios expected for the end of the 21st century (IPCC, 2014). Moreover, the amplitude of daily fluctuating temperatures is increased because of the passage of discharged water in natural draft wet-cooling towers. We monitored the

algal and invertebrate communities in biofilms, with particular emphasis on the taxonomic and functional structure of nematodes (numerically dominant metazoans). The biofilms grew during a low-flow period during the summer-fall, right after a substantial heat wave had hit Europe (29 July – 7 August 2018). Our hypotheses regarding the responses of biofilm-dwelling communities to warmer water temperature conditions were: (1) lower algal biomass and changes in the structure of algal communities; (2) changes in the structure of invertebrate communities, especially through the loss of thermosensitive taxa; (3) shift in the size-spectrum of insect larvae due to changes in cohort maturation rates; (4) greater taxonomic and trophic diversity of biofilm-dwelling nematodes, as previously observed in the summer in the Garonne River (Majdi et al., 2011).

2. Material and methods

2.1. Study site

The experiment was conducted from August to October 2018 downstream of the nuclear power plant of Golfech on the Garonne River (SW France, 44°07'04.3"N, 0°50'02.7"E). This nuclear power plant is operated in an open loop with recirculation and is equipped with natural draft wet-cooling towers to reduce the temperature of the blowdown water. The water is withdrawn from the river for its cooling system and the blowdown water is then discharged heated at a series of outlet pipes located directly in the middle of the riverbed over a width of about 100 m (Fig. 1). The resulting thermal plume covers a cone of a few hundred meters long by a hundred meters wide at its base. At this site, the Garonne River is 200 m wide with a relatively shallow (from 0.5 to 1 m depth) riverbed consisting of clayey bedrocks with large stone slabs, cobble bars, or sand/pebbles banks depending on local hydrodynamics, with substantial coverage of several hydrophyte species (e.g., *Potamogeton* spp., *Myriophyllum spicatum* L.).

157

158 *2.2. Experimental design*

159 Forty concrete tiles (60 x 40 x 4 cm, 21.5 Kg each) were used as standard substrates for the
160 growth of biofilm and were immerged on 7 August 2018. They were arranged in two blocks
161 of 20 tiles each (5 rows of 4 tiles) facing the flow direction at a depth of 50 cm. One block
162 (heated site) was positioned within the thermal plume, 20 m downstream from the outlet
163 pipes of the nuclear power plant and about 40 m from the right bank of the river (Fig. 1). The
164 other block (control site) was positioned outside the thermal plume on the same transect
165 about 120 m from the right bank of the river (Fig. 1). We observed that each tile in both
166 blocks showed homogeneous and similar depths and bottom flow velocities (depth = 50 ± 2
167 cm; flow velocity = 30 ± 5 cm s^{-1}). A temperature data-logger (measurement frequency = 5
168 min) linked to a buoy was positioned 4 cm above the riverbed and fixed to a steel rod
169 hammered in the riverbed at the centre of each block. Oxygen concentration, conductivity,
170 and pH were recorded monthly using a portable probe (U50, HORIBA) (Table S1 in
171 Appendix). When sampling biofilms, water samples were also collected at each site and
172 analysed after filtration on glass fibre filters (GF/C, Whatman) to measure the concentration
173 of dissolved organic carbon using the combustion catalytic oxidation method (TOC-L,
174 Shimadzu), and of anions and cations using high-performance ionic chromatography (ISC-
175 5000+ and DX-120, Dionex).

176

177 *2.3. Sampling of biofilm*

178 All tiles were sampled on 2 October 2018 (i.e. for a total of 40 samples: one sample per tile
179 with 20 tiles for each of the two sites) after 56 days of immersion; a period deemed long
180 enough to allow for the settlement of mature biofilm communities (Majdi et al., 2012b; Norf
181 et al., 2009). We collected 3.14 cm^2 patches of biofilm directly underwater using a brush-

sampler (Peters et al., 2005). For invertebrates, four patches were collected at random locations on each tile, poured on 20 µm meshes, and pooled in a 50 ml centrifuge tube. Invertebrate samples were preserved in a buffered formaldehyde solution (final concentration 4%) and stained with a few drops Rose Bengal. For algal pigments, two patches were collected and poured in a 50 ml centrifuge tube. Samples were returned to the laboratory in an icebox. The river discharge was very stable (low-flow conditions) during the biofilm colonisation period (i.e. from 7 August to 2 October 2018), but a major flood occurred two weeks after collecting the samples (16 October 2018), which prevented us from continuing the experiment.

2.4. Invertebrate identification and data pretreatment

Preserved invertebrates were counted, categorised into size classes (based on maximal length and width), and identified under a dissecting microscope at 40x magnification. While counting, 50 nematode individuals were picked out randomly from each sample, progressively transferred to glycerol, and mounted on slides following the method of Seinhorst (1959). Nematodes were identified at the species level and classified into age and sex categories. Nematode individuals were also classified according to their feeding type based on the morphology of their buccal cavity, after the classification of Traunspurger (1997): deposit-feeders (mainly bacterivorous given the small size of their unarmed buccal cavity), epistrate-feeders (potent algivores given the presence of small teeth in their buccal cavity allowing them to crack diatom frustules), chewers (omnivores or strict predators given their wide mouth armed with large teeth), and suction-feeders (omnivorous or fungal/plant-feeders given that their hollow stylet allows them to perform asymmetrical predation on other animals and hyphae). Thirty individuals of chironomids were also randomly sorted out from each sample during the counting, photographed under the microscope, and measured to the

207 nearest µm using the ImageJ image analysis software. Invertebrate biomass was estimated
208 from allometric relationships found in the literature (see Table S4 in Supplementary
209 Information) and expressed as dry mass per area of biofilm (µgDM $10cm^{-2}$).
210
211 *2.5. Pigment analysis*
212 In the laboratory, biofilm samples were pelletized by centrifugation (3000 g, 20 min), freeze-
213 dried, and stored at –80°C until analysis. The pellets were then homogenised and 200 mg
214 subsamples were extracted in 5 mL 98% cold-buffered methanol (with 2% of 1 M ammonium
215 acetate) in a 4°C sonication tub followed by a 15 min extraction in the dark at –20°C. The
216 solution was centrifugated (3000 g, 20 min), the supernatant was kept aside at –20°C, and the
217 pellet was extracted again following the same steps as described above. Thereby, for each
218 sample, a total of 10 mL pigment solution was obtained from which 2 mL was subsampled
219 and filtered through a 0.2 µm polytetrafluoroethylene syringe filter. The pigment solution
220 was analysed using a high-performance liquid chromatograph consisting of a 100 mL loop
221 autosampler and a quaternary solvent delivery system coupled to a diode array
222 spectrophotometer (LC1200 series; Agilent). The mobile phase was set after the protocol
223 described by Barlow et al. (1997). Algal pigments were identified and quantified by
224 comparing their retention time and absorption spectra with those of pure standards (DHI LAB
225 products; see Majdi et al., 2011 for further details). We compared the concentration of
226 biomarker pigments typical of specific algal taxonomic groups to estimate potential changes
227 in the composition of the algal community (Jeffrey et al., 1997).
228
229 *2.6. Statistical analyses*
230 All statistical analyses were performed using the 'vegan' (Oksanen et al., 2013) and
231 'indicspecies' (Cáceres and Legendre, 2009) packages of the R software (R Development

232 Core Team, 2018). Changes in the pigment composition of the biofilm and the structure of
233 biofilm-dwelling invertebrate and nematode communities between heated and control sites
234 were assessed using permutational analyses of variance based on the Bray-Curtis distance
235 matrices (PERMANOVA, 9999 permutations) calculated from Hellinger-transformed
236 abundance data. Multivariate homogeneity of group dispersion was tested using Anderson's
237 PERMDISP2 procedure which is a multivariate analog of Levene's test and which was met in
238 all cases. We used non-metric multidimensional scaling (nMDS) based on Hellinger-
239 transformed abundance data and Bray-Curtis similarity to ordinate the species in a bi-
240 dimensional space. Sample scores were then plotted as an illustrative variable in this 2D-
241 space to visually assess how different the communities were between the heated and control
242 sites. We used multilevel pattern analysis (9999 permutations) to test the significance of
243 species-site group associations and derive candidate indicator species for each site (Cáceres
244 and Legendre, 2009).
245 We calculated a set of diversity indices based on the untransformed abundances of
246 each taxon in the nematode community. First, the Index of Trophic Diversity (ITD) was
247 computed as ITD = $\Sigma\theta^2$ with θ being the relative contribution of each of the four functional
248 feeding guilds identified (Heip et al., 1985). This index ranges from 0.25 to 1, which
249 corresponds to the highest (each trophic group contributes 25%) and lowest (only one trophic
250 group present) feeding type diversity, respectively. Species richness (S), Shannon's entropy
251 (H, ln-based), Simpson's dominance (D), and Pielou's evenness (J) were calculated.
252 We used either one-way ANOVA when normality assumptions were met (tested using
253 Shapiro-Wilk and Bartlett test), or non-parametric Kruskal-Wallis rank sum test on
254 untransformed data to test for differences in total abundance and biomass, pigment
255 concentration, diversity indices, the proportion of nematode feeding types, and age ratios
256 between the heated and control sites. Regarding the temperature data and the body mass of

chironomid individuals, we compared cumulative density functions between sites using the Kolmogorov-Smirnov test.

3. Results

3.1. Abiotic background

Oxygen, pH, streambed flow velocity, and water depth showed very little variations between sites and along the biofilm colonisation period (Table S1). The most noticeable difference in water chemistry between sites, but minor, was the slightly higher concentrations of major ions like Mg^{2+}, Na^{+}, Ca^{2+}, Cl^{-}, and SO_4^{2-}, which increased water conductivity by 16.3% on average at the heated site (Tables S1 and S2). This difference was probably due to the partial evaporation of the water as it passed through the natural draft wet-cooling towers, thus concentrating the ions in the water discharged upstream of the heated site. Nevertheless, the relative concentration of ions were very similar across sites (Table S2).

At the beginning of the colonisation period (7 August 2018), the water temperature was very high (> 28°C) at both sites (Fig 2A). Water temperature then progressively diminished and the temperature contrast between the control and heated sites became more pronounced in September (Fig. 2C). Overall, the temperature regime differed across the two sites over the 56 days exposure period (total: 1344 h). The control site showed an average temperature of 23.81°C (min: 19.85°C; max: 28.85°C). The heated site showed an average temperature of 25.32°C (min: 19.85°C; max: 30.86°C). Thus, the heated site was, on average, 1.5°C warmer than the control site during the study period (min measured $\Delta T = –2.69$°C; max $\Delta T = +4.38$°C; Fig. 2C). When comparing the cumulative distribution of temperature functions, there was a significant difference in the local thermal regime between the control and heated sites (Kolmogorov-Smirnov test, $D^{+} = 0.47$, $P < 0.001$, Fig. S1). The average daily fluctuation of water temperature (i.e., the range between the minimum and maximum water

282 temperature recorded each day) was also significantly higher at the heated site (on average
283 2.69°C) than at the control site (0.72°C) (Kruskal-Wallis test, $\chi^2 = 90$, $P < 0.001$) (Fig. 2B).
284
285 *3.2. Biofilm pigment composition*
286 Biofilm pigment composition was significantly different between the heated and control sites
287 (PERMANOVA, $F_{1,38} = 15.8$, $R^2 = 0.29$, $P < 0.001$) (Fig. 3). More specifically, the biofilms
288 showed significantly higher concentrations of chlorophyllide *a*, zeaxanthin, β-carotene,
289 myxoxanthophyll, and echinenone at the heated site in comparison to the control site, while
290 concentrations of chlorophyll *b*, violaxanthin, and lutein were significantly lower at the
291 heated site (Table 1). The concentrations of the most abundant pigments, chlorophyll *a* and
292 fucoxanthin, were not significantly different across sites.
293
294 *3.3. Invertebrate community*
295 The total abundance of invertebrates was not significantly different across sites (Table 1)
296 with 335.2 ± 38.3 (mean ± SD) and 295.9 ± 17.3 ind $10cm^{-2}$ at the control and heated sites,
297 respectively. Nematodes were numerically dominant in the invertebrate community,
298 representing 27.2% and 47.3% of all individuals found at the control and heated sites,
299 respectively (Table 1). The structure of the invertebrate community differed significantly
300 across sites (PERMANOVA, $F_{1,38} = 13.9$, $R^2 = 0.26$, $P < 0.001$) (Fig. 4). Nematodes and
301 oligochaetes were significantly more abundant at the heated site, while larvae of chironomids,
302 *Corbicula*, and Ephemeroptera, as well as harpacticoid copepods and their nauplii larvae,
303 were significantly more abundant at the control site (Table 1). No significant differences in
304 the abundances of rotifers, ostracods, and cladocers were found across sites (Table 1).
305 The estimated total biomass of invertebrates was not significantly different across
306 sites, with 385.8 ± 51.4 (mean ± SD) and 284.5 ± 58.1 μgDM $10cm^{-2}$ at the control and

heated sites, respectively (Table 1). Chironomids were strongly dominant in terms of biomass and represented 59.1% and 86.9% of the total invertebrate biomass at the control and heated sites, respectively. The biomasses of Ephemeroptera larvae, *Corbicula* larvae, harpacticoid copepods, ostracods, and nauplii were significantly dampened at the heated site, whereas nematodes showed significantly higher biomass (Table 1). Full test-statistics are provided in Table S3.

The individual body-mass of chironomid larvae was on average 5.37 and 9.11 µgDM ind^{-1} at the control and heated sites, respectively (Fig. 5A). Most individuals showed a body-mass < 1 µgDM (Fig. 5A). However, the cumulative distribution function of the chironomid population at the control site was significantly above that of the heated site (Kolmogorov-Smirnov test, D+ = 0.09, P = 0.045) (Fig. 5B), showing a greater proportion of relatively large chironomid individuals with a body-mass > 15 µgDM at the heated site (Fig. 5B).

3.4. Nematode community

Of the 2,000 nematode individuals that have been identified, a total of 21 species have been found, with 14 and 17 species at the control and heated sites, respectively (Table 2). *Monhystrella paramacrura* (Meyl, 1954), *Eumonhystera vulgaris* (de Man, 1880), and *Chromadorina bioculata* (Schultze *in* Carus, 1857) were the three dominant species at both sites, accounting cumulatively for approximately 92 and 80% of the total community at the control and heated sites, respectively (Table 2). However, the structure of the nematode community differed significantly across sites (PERMANOVA, $F_{1,38} = 24.8$, $R^2 = 0.39$, $P < 0.001$) (Fig. 6). The multilevel pattern analysis highlighted three species that were significantly associated with the heated site (test statistics > 0.56, P <0.008): the two large predatory-omnivorous *Mylonchulus lacustris* (Cobb, 1915) and *Mesodorylaimus bastiani* (Bütschli, 1873), as well as the bacterivorous *Eumonhystera dispar* (Bastian, 1865).

Mylonchulus lacustris was absent at the control site while representing approximatively 6.3% of the total number of individuals in the community at the heated site. *Mesodorylaimus bastiani* was absent at the control site while having a relative abundance of about 5.1% at the heated site. Finally, *E. dispar* was > 10 times more abundant at the heated site (1.71 ind $10cm^{-2}$) in comparison to the control site (0.13 ind $10cm^{-2}$) (Table 2).

Nematode species richness was significantly higher at the heated site (on average 6.95 species found in each sample) than at the control site (on average 4.40 species) (Table 1). Shannon's entropy (H) was also significantly higher at the heated site, while Simpson's dominance (D) was lower (Table 1). Pielou's evenness (J) did not differ significantly across sites (Table 1). The index of trophic diversity (ITD) was significantly lower at the heated site, indicating a more homogeneous distribution between the different feeding types (Table 1). Nematode communities were strongly dominated by deposit-feeders at the control site (average relative abundance of 84.2%), while they represented on average only 54.2% of the total abundance at the heated site. In contrast, the relative abundance of epistrate-feeders, chewers, and suction-feeders was significantly higher at the heated site than at the control site (Table 1, Fig. 7).

With a substantial number of individuals sampled, we could compare age ratios in the two dominant bacterivorous species, *Monhystrella paramacrura* and *Eumonhystera vulgaris,* across sites. *M. paramacrura* showed a ratio of juveniles:adults significantly lower at the heated site (0.75 and 1.20 at the heated and control sites, respectively; ANOVA, $F_{1,38} = 5.42$, $P = 0.024$). *E. vulgaris* showed a ratio gravid:non-gravid females almost 10x higher at the heated site (0.65 and 0.07 at the heated and control sites, respectively; Kruskal-Wallis, $\chi^2 = 5.3$, $P = 0.021$). Other ratios did not differ significantly across sites.

4. Discussion

356 *4.1. A realistic in situ warming experiment?*

357 Several studies have used naturally or artificially heated streams or river as "*in situ*
358 laboratories" to study the effects of water warming and climate change on aquatic organisms
359 and ecosystem functioning (e.g. Woodward et al., 2010, O'Gorman et al., 2012, Boulêtreau et
360 al., 2014, Worthington et al., 2015, Sandblom et al., 2016). In this study, we carried out an
361 experiment within the thermal plume downstream of heated effluents from a nuclear power
362 plant because we expected that all environmental factors that could influence the structure of
363 the biofilm community (e.g., light, nutrients, hydrodynamics), other than water temperature,
364 would be similar at the two experimental sites (inside or outside the thermal plume). The
365 results on the abiotic environment showed that, given our caution regarding local
366 hydrodynamics, this hypothesis was verified, and thus water temperature appeared to be the
367 major environmental determinant that differed between the heated and control sites. During
368 the experiment, the average difference in water temperature between the control and heated
369 sites was about 1.5°C, which is within the range of optimistic projections of temperature
370 increases by the end of the century (IPCC, 2014). The passage through natural draft wet-
371 cooling towers implies a partial evaporation of the cooling water, thus concentrating
372 dissolved substances in the released effluent. We observed this effect also for dissolved
373 nutrients like nitrate and phosphate. However, when comparing their relative concentrations,
374 we found minor differences between sites, suggesting that the nutrient balance was similar for
375 biofilm-dwelling organisms at both sites. For example, the nitrate:phosphate ratio was 42.5
376 and 41.9 at the control and heated sites, respectively. The passage of the water through
377 natural draft wet-cooling towers, whose efficiency primarily varies with air humidity, has
378 also resulted in greater daily fluctuations in water temperature. Those secondary effects
379 probably enhanced the environmental contrast found across sites. Nevertheless, they also are
380 compatible with future climate change scenarios as the frequency of heat waves are expected

to increase in line with the rise in temperature and evaporation (Michel et al., 2019; Morrill et al., 2005; Webb, 1996). It is worth noting that the experimental heated site was located within the thermal plume only 20 m downstream from the outlet pipes of the nuclear power plant. This area does not correspond to the regulatory downstream area (which is located a few kilometres further downstream, after complete mixing between the river and the cooling water released) where ecological differences potentially induced by the operation of the nuclear power plant are under regulatory control, in accordance with local legislation and the requirements of the Water Framework Directive.

4.2. Effects of water warming on the microphytobenthic community of biofilms

At both sites, we found relatively high concentrations of fucoxanthin and diadinoxanthin, which are two biomarker pigments for diatoms. This result is consistent with previous studies that have highlighted that epilithic biofilms of the Garonne River were strongly dominated by diatoms in terms of biomass (Boulêtreau et al., 2014; Majdi et al., 2011, 2012a; Tekwani et al., 2013). However, the absence of significant differences in fucoxanthin and diadinoxanthin concentrations across sites showed that diatom biomass was not affected by the temperature contrast studied. Similarly, chlorophyll *a* concentration (as a proxy of phototrophic biomass) did not vary significantly across sites. However we found that the concentrations of chlorophyllide *a*, myxoxanthophyll, and echinenone were more than twice as high at the heated site in comparison to the control site. These three latter pigments are reported biomarkers for cyanobacteria (Hertzberg et al., 1971; Hodgson et al., 2004; Takaichi and Mochimaru, 2007). Several studies showed that warming can selectively promote cyanobacterial growth in rivers (Butterwick et al., 2005; Watkinson et al., 2005). Majdi et al. (2011, 2012a) and Tekwani et al. (2013) also reported an increase of the relative biomass of cyanobacteria in the plankton and in biofilms of the Garonne River during summer low-flow

406 periods. Further studies should focus on identifying which species of cyanobacteria are
407 favoured under warmer conditions and how such changes in the structure of the
408 microphytobenthic community could affect biofilm grazers and the ecosystem-level functions
409 supported by biofilm communities.
410
411 *4.3. Effects of water warming on invertebrate communities*
412 We found that the total abundance of invertebrates did not differ across sites. However, the
413 structure of the community was different, with a relatively diverse community at the control
414 site whereas, at the heated site, it was crowded with nematodes and oligochaetes but lacked
415 micro-crustaceans, larvae of *Corbicula,* and Ephemeroptera. Given the standard procedures
416 used to sample biofilm and the relatively straightforward changes observed at coarse
417 taxonomic-level, our results point out a potential to indicate water warming effects based on
418 changes in the taxonomic structure of biofilm-dwelling invertebrates. It is possible that the
419 warmer temperature and/or the greater daily fluctuation of temperature could have negatively
420 affected thermosensitive taxa such as Ephemeroptera, which need time to acclimatise to
421 critically high temperatures (Houghton and Shoup, 2014). For *Corbicula* larvae, Weitere et
422 al. (2009) showed that a 3°C increase in water temperature could reduce body-mass,
423 reproduction rate, and increase mortality. Such a rise in temperature occurred almost daily at
424 the heated site during the experimental period. In contrast, biofilm-dwelling nematodes and,
425 in a lesser extent, oligochaetes thrived well at the heated site. In addition to warmer average
426 temperatures, greater thermal fluctuations may also represent a stress for aquatic biota
427 (Coulter et al., 2016; Thome et al., 2016), which could lead to the selection of species able to
428 cope with temperature extremes and large temperature fluctuations.
429 In our study, chironomids were the major contributors to invertebrate biomass.
430 However, the reduction of their abundance observed at the heated site did not result in a

reduction in total invertebrate biomass. We further showed that this was due to the presence of chironomid individuals that were, on average, twice as large at the heated site in comparison to the control site. Ectotherms can develop adaptive responses to warming such as increased investment in reproduction, decreased body-size at sexual maturity, or higher heat tolerance limits (Daufresne et al., 2009; Loisel et al., 2019; Majdi et al., 2019; Nelson et al., 2017; Ohlberger, 2013; Sandblom et al., 2016; Yvon-Durocher et al., 2011). We did not observe such a reduction of the size spectrum for chironomids. The potential rationales to this result are probably complex and must take into account the fact that (1) we have no information at the species-level for chironomids, therefore we cannot rule out the hypothesis that different species assemblages at the heated site may account for the observed body-size differences. (2) Chironomids complete only part of their life cycle in the aquatic environment. For larvae destined to metamorphose and leave the aquatic environment, warmer temperatures have the potential to boost larval growth and reduce the duration of the larval stage, thereby hastening cohort succession (Cheney et al., 2019). The increase in the size of chironomids observed in our study could therefore also result from the hastening of early summer cohorts. Temperature-boosted larvae could also emerge earlier, with potential far-ranging consequences for species' ecology and biogeography. Nevertheless, this is likely context- and time-dependent. Further large-scale studies examining chironomid communities at a finer taxonomic-level are needed before envisaging the development of a bio-indicator of river warming based on the shape of the size spectrum of chironomids (but see Walker et al., 1991 for bio-indication of past climate based on fossil chironomid species assemblages preserved in lake sediments).

4.4. Changes in the structure of nematode communities

We observed that the structure of nematode communities differed across sites, both in terms of species composition and in terms of feeding-type composition. There is evidence that the taxonomic and feeding-type structure of freshwater nematode assemblages can shift in response to broad-scale mechanisms like ecosystem productivity and disturbance regime (Barbuto and Zullini, 2005; Kazemi-Dinan et al., 2014; Majdi et al., 2011; Michiels and Traunspurger, 2005; Ristau et al., 2013; Höss et al., 2011). Here we observed that nematode diversity was greater at the heated site, and we identified a few species that were clearly associated with heated biofilms: *Mylonchulus lacustris*, *Mesodorylaimus bastiani* and *Eumonhystera dispar*. The latter is a rather small opportunistic species, thus it made sense to find it at the heated site. However, the former two species are relatively large and it was surprising that they thrived well at the heated site. Whether those species can be considered indicators of thermal effects in river biofilms needs further studies. However, it has to be noted that several freshwater nematode species have been already classified as sensitive or tolerant to toxic pollution in freshwater sediments leading to the development of a "nematode species at risk" index (NEMAspear; Höss et al., 2011). Among the most common species dwelling river sediments, Höss et al. (2011) showed that *Eumonhystera dispar* was also one of the most resistant to metal and organic pollutions.

Warming can affect biomass production and food web structure with mixed effects, from body size-reductions and shortening of food chains in laboratory microbial food webs (Petchey et al., 1999), to body-size increases and elongation of food chains in natural geothermal streams (Woodward et al., 2016). For nematodes, we observed that the distribution between the different feeding-types was more even at the heated site than at the control site. The community was also made up of a greater number of large-sized omnivorous or predatory species at the heated site. In addition, *Chromadorina bioculata,* an algivorous species typical of epilithic biofilms of the Garonne River (Majdi et al. 2011), was about five

480 times more abundant at the heated site than at the control site. These results suggest that
481 warming fostered the establishment of an assemblage of nematodes with more diverse
482 feeding-types, which would presumably play a more important role in trophic transfers in
483 comparison with the control site. Previous studies examining the biofilm ecological
484 succession in the Garonne River have shown that warmer temperatures during summer low-
485 flow periods can trigger profound change in the structure of the algal and bacterial
486 communities (Leflaive et al., 2008; Lyautey et al., 2005; Tekwani et al., 2013) with
487 consequences on nutrient cycling and standing stocks available for grazers (Boulêtreau et al.,
488 2014; Graba et al., 2014; Teissier et al., 2007). Free-living nematodes show complex trophic
489 behaviours including feeding-selectivity and attraction to food patches (Derycke et al., 2016;
490 Estifanos et al., 2013; Höckelmann et al., 2004; Moens et al., 2013; Schroeder et al., 2010).
491 Although more studies are needed to establish robust causalities, we may presume that the
492 presence of a more functionally diverse assemblage of nematode species could be explained
493 by at least two mechanisms: (1) A diversification of food resources that nematodes could
494 exploit in the biofilm, hence agreeing with studies showing the importance of bottom-up
495 effects at structuring nematode communities (Michiels and Traunspurger, 2005; Ristau et al.,
496 2013). (2) Predation and/ or competition for resources as several studies have shown that
497 many organisms can prey on or compete with freshwater nematodes (Majdi and
498 Traunspurger, 2015). A decrease in competition for biofilm basal resources (due e.g. to the
499 reduction of Ephemeroptera and *Corbicula* larvae) may have increased the number of trophic
500 niches available for algivorous nematodes. Furthermore, the decrease in intraguild
501 competition between omnivores and predators (as the numbers of micro-crustaceans and
502 insect larvae were dampened at the heated site) may have favoured the establishment of
503 relatively large omnivorous and predatory nematode species instead. Nematodes are
504 extremely abundant in biofilms and their feeding type does not require specific taxonomic

505 expertise to be recognized under the microscope. Previous studies have shown that the
506 distribution of feeding types within freshwater nematode communities can be influenced by
507 key environmental drivers such as ecosystem productivity (see Traunspurger et al., 2020, and
508 references therein). Whether nematode feeding types may be envisaged as a straightforward
509 indicator of disturbance needs further confirmation, but this is promising as we showed a
510 more even distribution of feeding types at the heated site.
511 Not all nematode species thrived well in warmed-up biofilms. The case of
512 *Monhystrella paramacrura* was especially striking. Although this species was commonly
513 found at both sites, the abundance of this species was half as low at the heated site as at the
514 control site. This difference was accompanied by a much lower number of juveniles in
515 comparison to the control site. This result suggests that populations of *M. paramacrura* were
516 seemingly less successful under a warmer and more fluctuating temperature regime. This
517 response may be physiological as free-living nematodes show species-specific thermal niches
518 with associated patterns of population growth rate, age structure, egg production, or body-
519 size at maturity (Majdi et al., 2019). An average temperature difference of 1.5°C across
520 experimental sites did not seem huge at first. However, Majdi et al. (2019) have evidenced
521 the existence of temperature thresholds between 25 and 30°C above which survival and
522 population growth rate of several freshwater nematode species could be severely reduced.
523 Such upper temperature tolerance thresholds were more likely to be exceeded at the heated
524 site due to an overall higher temperature and a greater amplitude of daily temperature
525 fluctuations. Over the whole 1344 h of study, water temperatures were >25°C for 229 h (i.e.
526 17% of time) and 814 h (i.e. 61%) at the control and heated sites, respectively. This likely
527 explains the reduced success of *M. paramacrura*, at the heated site. Furthermore, nematodes
528 can associate a certain temperature with conditions of food availability and thereby exhibit
529 active attraction or repulsion to a given temperature where appropriate (Mohri et al., 2005).

530 *M. paramacrura* is a small species with a very slender body and a minute mouth cavity
531 without teeth. This may constrain the diet of *M. paramacrura.* On the other hand,
532 *Eumonhystera vulgaris* belongs to the same feeding guild as *M. paramacrura*, but unlike *M.*
533 *paramacrura*, *E. vulgaris* was very successful at the heated site showing higher abundances
534 and a much greater number of gravid females. In comparison, to *M. paramacrura*, *E. vulgaris*
535 is a bit larger showing a larger mouth cavity. Thereby, its diet could have been less limited by
536 prey size, which might be advantageous in warmed-up biofilms. Indeed, at this stage, it is
537 premature to suggest what precisely causes such a species turnover, laboratory studies
538 examining the effects of different conditions of food and temperature on single species and
539 species mixtures could help identify underlying mechanisms more accurately.
540

541 ## 5. Conclusion

542 The studied differences in thermal regime did not affect neither the biomass of biofilm-
543 dwelling phototrophs nor the total abundance and biomass of invertebrates. However, the
544 community structure was different with a higher proportion of cyanobacteria and nematodes
545 and lower abundances of crustaceans and insects under warmer conditions. The response of
546 the dominant invertebrate groups in terms of biomass (chironomids) and abundance
547 (nematodes) was further examined, and the results suggested that the body-size structure of
548 chironomids and the taxonomic and functional structure of nematode assemblages could be
549 good tools to indicate the impact of warming on river biofilm communities. The highlighted
550 differences in body-size and community structure may be explained by direct modification of
551 the metabolism and different specific tolerances to temperatures among biofilm-dwelling
552 organisms. The shifted biofilm landscape may, in return, create conditions that are beneficial
553 (or not) for the further settlement of colonists. In summary, our results suggest that water
554 warming coupled with larger daily temperature fluctuations have the potential to profoundly

555 affect biofilm communities. Further studies are recommended to correlate those community
556 shifts to potential changes in important ecosystem functions supported by river biofilms.
557

558 ## 6. Aknowledgements

559 We are grateful to Sylvain Lamothe, Louna Riem, Stephanie Boulêtreau, and Arthur Compin
560 for their help in the field and Stefanie Gehner for mounting the nematodes prior to their
561 identification. We also thank the PAPC platform (UMR EcoLab) and Frederic Julien and
562 Didier Lambrigot for their help with water analyses and HPLC. We thank Électricité de
563 France for their financial support and for providing part of the data. We thank two
564 anonymous reviewers for their helpful comments on a previous version of the manuscript.
565 We thank Helena Adão and our meiobenthologist colleagues for invigorating discussions and
566 hosting the 17th International Meiofauna Conference in Evora in July 2019, which has
567 resulted in the preparation of this special issue "Meiofauna in a changing world".
568

569 ## 7. References

570 Barbuto, M., Zullini, A., 2005. The nematode community of two Italian rivers (Taro and
571 Ticino). Nematology 7, 667–675.
572 Barlow, R.G., Cummings, D.G., Gibb, S.W., 1997. Improved resolution of mono-and divinyl
573 chlorophylls a and b and zeaxanthin and lutein in phytoplankton extracts using
574 reverse phase C-8 HPLC. Marine Ecology Progress Series 161, 303–307.
575 Battin, T.J., Besemer, K., Bengtsson, M.M., 2016. The ecology and biogeochemistry of
576 stream biofilms. Nature Reviews Microbiology 14, 251.
577 Biggs, B.J.F., 1996. Patterns in benthic algae of streams, in: Stevenson R. J, Bothwell M. L,
578 Lowe R. L (Eds.), Algal Ecology: Freshwater Benthic Ecosystems. Academic Press,
579 San Diego, CA, USA, pp. 31–56.

580 Boulêtreau, S., Garabetian, F., Sauvage, S., Sanchez-Perez, J.M., 2006. Assessing the
581 importance of a self-generated detachment process in river biofilm models.
582 Freshwater Biology 51, 901–912.
583 Boulêtreau, S., Lyautey, E., Dubois, S., Compin, A., Delattre, C., Touron-Bodilis, A.,
584 Mastrorillo, S., Garabetian, F., 2014. Warming-induced changes in denitrifier
585 community structure modulate the ability of phototrophic river biofilms to denitrify.
586 Science of the Total Environment 466, 856–863.
587 Butterwick, C., Heaney, S.I., Talling, J.F., 2005. Diversity in the influence of temperature on
588 the growth rates of freshwater algae, and its ecological relevance. Freshwater
589 Biology 50, 291–300.
590 Cáceres, M.D., Legendre, P., 2009. Associations between species and groups of sites: indices
591 and statistical inference. Ecology 90, 3566–3574.
592 Caissie, D., 2006. The thermal regime of rivers: a review. Freshwater Biology 51, 1389–
593 1406.
594 Cheney, K.N., Roy, A.H., Smith, R.F., Dewalt, R.E., 2019. Effects of Stream Temperature
595 and Substrate Type on Emergence Patterns of Plecoptera and Trichoptera From
596 Northeastern United States Headwater Streams. Environmental Entomology 48,
597 1349–1359.
598 Coulter, D.P., Sepúlveda, M.S., Troy, C.D., Höök, T.O., 2016. Species‐specific effects of
599 subdaily temperature fluctuations on consumption, growth and stress responses in
600 two physiologically similar fish species. Ecology of Freshwater fish 25, 465–475.
601 Daufresne, M., Lengfellner, K., Sommer, U., 2009. Global warming benefits the small in
602 aquatic ecosystems. Proceedings of the National Academy of Sciences 106, 12788–
603 12793.
604 Derycke, S., De Meester, N., Rigaux, A., Creer, S., Bik, H., Thomas, W.K., Moens, T., 2016.

605 Coexisting cryptic species of the Litoditis marina complex (Nematoda) show
606 differential resource use and have distinct microbiomes with high intraspecific
607 variability. Molecular Ecology 25, 2093–2110.
608 Estifanos, T.K., Traunspurger, W., Peters, L., 2013. Selective feeding in nematodes: a stable
609 isotope analysis of bacteria and algae as food sources for free-living nematodes.
610 Nematology 15, 1–13.
611 Graba, M., Sauvage, S., Majdi, N., Mialet, B., Moulin, F.Y., Urrea, G., Buffan-Dubau, E.,
612 Tackx, M., Sabater, S., Sanchez-Pérez, J.-M., 2014. Modelling epilithic biofilms
613 combining hydrodynamics, invertebrate grazing and algal traits. Freshwater Biology
614 59, 1213–1228.
615 Heip, C., Vincx, M., Vranken, G., 1985. The ecology of marine nematodes. Aberdeen
616 University Press.
617 Hertzberg, S., Liaaen-Jensen, S. t, Siegelman, H.W., 1971. The carotenoids of blue-green
618 algae. Phytochemistry 10, 3121–3127.
619 Höckelmann, C., Moens, T., Jüttner, F., 2004. Odor compounds from cyanobacterial biofilms
620 acting as attractants and repellents for free‐living nematodes. Limnology and
621 Oceanography 49, 1809–1819.
622 Hodgson, D.A., Vyverman, W., Verleyen, E., Sabbe, K., Leavitt, P.R., Taton, A., Squier,
623 A.H., Keely, B.J., 2004. Environmental factors influencing the pigment composition
624 of in situ benthic microbial communities in east Antarctic lakes. Aquatic Microbial
625 Ecology 37, 247–263.
626 Houghton, D.C., Shoup, L., 2014. Seasonal changes in the critical thermal maxima of four
627 species of aquatic insects (Ephemeroptera, Trichoptera). Environmental Entomology
628 43, 1059–1066.
629 Höss, S., Claus, E., Von der Ohe, P.C., Brinke, M., Güde, H., Heininger, P., Traunspurger,

W., 2011. Nematode species at risk — A metric to assess pollution in soft sediments of freshwaters. Environment International 37, 940–949.

Huss, M., Lindmark, M., Jacobson, P., van Dorst, R.M., Gårdmark, A., 2019. Experimental evidence of gradual size‐dependent shifts in body size and growth of fish in response to warming. Global change biology.

IPCC, 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC.

Jeffrey, S.W., Mantoura, R.F.C., Wright, S.W., 1997. Phytoplankton pigments in oceanography: guidelines to modern methods. UNESCO publishing, Paris, France.

Kathol, M., Norf, H., Arndt, H., Weitere, M., 2009. Effects of temperature increase on the grazing of planktonic bacteria by biofilm-dwelling consumers. Aquatic Microbial Ecology 55, 65–79.

Kazemi-Dinan, A., Schroeder, F., Peters, L., Majdi, N., Traunspurger, W., 2014. The effect of trophic state and depth on periphytic nematode communities in lakes. Limnologica 44, 49–57.

Leflaive, J., Boulêtreau, S., Buffan-Dubau, E., Ten-Hage, L., 2008. Temporal patterns in epilithic biofilm-relation with a putative allelopathic activity. Fundamental and Applied Limnology 173, 121–134.

Loisel, A., Isla, A., Daufresne, M., 2019. Variation of thermal plasticity in growth and reproduction patterns: Importance of ancestral and developmental temperatures. Journal of Thermal Biology 84, 460–468.

Lyautey, E., Hallin, S., Teissier, S., Iribar, A., Compin, A., Philippot, L., Garabetian, F., 2013. Abundance, activity and structure of denitrifier communities in phototrophic river biofilms (River Garonne, France). Hydrobiologia 716, 177–187.

655 Lyautey, E., Jackson, C.R., Cayrou, J., Rols, J.-L., Garabétian, F., 2005. Bacterial
656 Community Succession in Natural River Biofilm Assemblages. Microbial Ecology
657 50, 589–601.
658 Majdi, N., Mialet, B., Boyer, S., Tackx, M., Leflaive, J., Boulêtreau, S., Ten-Hage, L., Julien,
659 F., Fernandez, R., Buffan-Dubau, E., 2012a. The relationship between epilithic
660 biofilm stability and its associated meiofauna under two patterns of flood
661 disturbance. Freshwater Science 31, 38–50.
662 Majdi, N., Tackx, M., Buffan-Dubau, E., 2012b. Trophic positionning and microphytobenthic
663 carbon uptake of biofilm-dwelling meiofauna in a temperate river. Freshwater
664 Biology 57, 1180–1190.
665 Majdi, N., Traunspurger, W., 2015. Free-living nematodes in the freshwater food web: a
666 review. Journal of Nematology 47, 28–44.
667 Majdi, N., Traunspurger, W., Boyer, S., Mialet, B., Tackx, M., Fernandez, R., Gehner, S.,
668 Ten-Hage, L., Buffan-Dubau, E., 2011. Response of biofilm-dwelling nematodes to
669 habitat changes in the Garonne River, France: influence of hydrodynamics and
670 microalgal availability. Hydrobiologia 673, 229–244.
671 Majdi, N., Traunspurger, W., Fueser, H., Gansfort, B., Laffaille, P., Maire, A., 2019. Effects
672 of a broad range of experimental temperatures on the population growth and body-
673 size of five species of free-living nematodes. Journal of Thermal Biology 80, 21–36.
674 Michel, A., Brauchli, T., Lehning, M., Schaefli, B., Huwald, H., 2019. Stream temperature
675 evolution in Switzerland over the last 50 years. Hydrology and Earth System
676 Sciences.
677 Michiels, I.C., Traunspurger, W., 2005. Impact of resource availability on species
678 composition and diversity in freshwater nematodes. Oecologia 142, 98–103.
679 Moens, T., Vafeiadou, A.-M., De Geyter, E., Vanormelingen, P., Sabbe, K., De Troch, M.,

2013. Diatom feeding across trophic guilds in tidal flat nematodes, and the importance of diatom cell size. Journal of Sea Research 92, 125–133.

Mohri, A., Kodama, E., Kimura, K.D., Koike, M., Mizuno, T., Mori, I., 2005. Genetic control of temperature preference in the nematode Caenorhabditis elegans. Genetics 169, 1437–1450.

Morrill, J.C., Bales, R.C., Conklin, M.H., 2005. Estimating stream temperature from air temperature: implications for future water quality. Journal of Environmental Engineering 131, 139–146.

Mouthon, J., Daufresne, M., 2015. Resilience of mollusc communities of the River Saone (eastern France) and its two main tributaries after the 2003 heatwave. Freshwater Biology 60, 2571–2583.

Mouthon, J., Daufresne, M., 2006. Effects of the 2003 heatwave and climatic warming on mollusc communities of the Saône: a large lowland river and of its two main tributaries (France). Global Change Biology 12, 441–449.

Nelson, D., Benstead, J.P., Huryn, A.D., Cross, W.F., Hood, J.M., Johnson, P.W., Junker, J.R., Gíslason, G.M., Ólafsson, J.S., 2017. Shifts in community size structure drive temperature invariance of secondary production in a stream‐warming experiment. Ecology 98, 1797–1806.

Norf, H., Arndt, H., Weitere, M., 2009. Responses of biofilm-dwelling ciliate communities to planktonic and benthic resource enrichment. Microbial Ecology 57, 687–700.

Norf, H., Arndt, H., Weitere, M., 2007. Impact of local temperature increase on the early development of biofilm-associated ciliate communities. Oecologia 151, 341–350.

O'Gorman, E.J., Pichler, D.E., Adams, G., Benstead, J.P., Cohen, H., Craig, N., Cross, W.F., Demars, B.O., Friberg, N., Gislason, G.M., 2012. Impacts of warming on the structure and functioning of aquatic communities: individual-to ecosystem-level

responses, in: Advances in Ecological Research. Elsevier, pp. 81–176.

Ohlberger, J., 2013. Climate warming and ectotherm body size–from individual physiology to community ecology. Functional Ecology 27, 991–1001.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. Package 'vegan.' Community ecology package, version 2.

Petchey, O.L., McPhearson, P.T., Casey, T.M., Morin, P.J., 1999. Environmental warming alters food-web structure and ecosystem function. Nature 402, 69.

Peters, L., Scheifhacken, N., Kahlert, M., Rothhaupt, K.O., 2005. Note: An efficient in situ method for sampling periphyton in lakes and streams. Archiv für Hydrobiologie 163, 133–141.

Peters, L., Traunspurger, W., 2005. Species distribution of free-living nematodes and other meiofauna in littoral periphyton communities of lakes. Nematology 7, 267–280.

R Development Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Retrieved from http://R-project.org, Vienna, Austria.

Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., Kidd, K.A., MacCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., Taylor, W.W., Tockner, K., Vermaire, J.C., Dudgeon, D., Cooke, S.J., 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biological Reviews 94, 849–873.

Risse-Buhl, U., Trefzger, N., Seifert, A.-G., Schönborn, W., Gleixner, G., Küsel, K., 2012. Tracking the autochthonous carbon transfer in stream biofilm food webs. FEMS Microbiology Ecology 79, 118–131.

Ristau, K., Faupel, M., Traunspurger, W., 2013. Effects of nutrient enrichment on the trophic structure and species composition of freshwater nematodes-a microcosm study.

730 Freshwater Science 32, 155–168.
731 Romaní, A.M., Boulêtreau, S., Diaz Villanueva, V., Garabetian, F., Marxen, J., Norf, H.,
732 Weitere, M., 2016. Microbes in aquatic biofilms under the effect of changing
733 climate, in: Climate Change and Microbial Ecology: Current Research and Future
734 Trends. Caister Academic Press, Norfolk, UK, pp. 83–96.
735 Romaní, A.M., Guasch, H., Munoz, I., Ruana, J., Vilalta, E., Schwartz, T., Emtiazi, F.,
736 Sabater, S., 2004. Biofilm structure and function and possible implications for
737 riverine DOC dynamics. Microbial Ecology 47, 316–328.
738 Sandblom, E., Clark, T.D., Gräns, A., Ekström, A., Brijs, J., Sundström, L.F., Odelström, A.,
739 Adill, A., Aho, T., Jutfelt, F., 2016. Physiological constraints to climate warming in
740 fish follow principles of plastic floors and concrete ceilings. Nature Communications
741 7, 11447.
742 Schroeder, F., Muschiol, D., Traunspurger, W., 2010. Fluctuating food availability may
743 permit coexistence in bacterivorous nematodes. Fundamental and Applied
744 Limnology 178, 59–66.
745 Schroeder, F., Traunspurger, W., Pettersson, K., Peters, L., 2012. Temporal changes in
746 periphytic meiofauna in lakes of different trophic states. Journal of Limnology 71,
747 216–227.
748 Seinhorst, J.W., 1959. A rapid method for the transfer of nematodes from fixative to
749 anhydrous glycerin. Nematologica 4, 67–69.
750 Steel, E.A., Beechie, T.J., Torgersen, C.E., Fullerton, A.H., 2017. Envisioning, quantifying,
751 and managing thermal regimes on river networks. BioScience 67, 506–522.
752 Svensson, F., Karlsson, E., Gårdmark, A., Olsson, J., Adill, A., Zie, J., Snoeijs, P., Eklöf, J.S.,
753 2017. In situ warming strengthens trophic cascades in a coastal food web. Oikos
754 126, 1150–1161.

755 Takaichi, S., Mochimaru, M., 2007. Carotenoids and carotenogenesis in cyanobacteria:
756 unique ketocarotenoids and carotenoid glycosides. Cellular and Molecular Life
757 Sciences 64, 2607.
758 Teissier, S., Torre, M., Delmas, F., Garabetian, F., 2007. Detailing biogeochemical N budgets
759 in riverine epilithic biofilms. Journal of the North American Benthological Society
760 26, 178–190.
761 Tekwani, N., Majdi N, Mialet B, Tornés E, Urrea-Clos G, Buffan-Dubau E, Sabater S, Tackx
762 M, 2013. Contribution of epilithic diatoms to benthic-pelagic coupling in a
763 temperate river. Aquatic Microbial Ecology 69, 47–57.
764 Thome, C., Mitz, C., Somers, C.M., Manzon, R.G., Boreham, D.R., Wilson, J.Y., 2016.
765 Incubation of lake whitefish (Coregonus clupeaformis) embryos in cooling water
766 discharge and the impacts of fluctuating thermal regimes on development. Canadian
767 journal of fisheries and aquatic sciences 73, 1213–1221.
768 Traunspurger, W., 1997. Bathymetric, seasonal and vertical distribution of feeding-types of
769 nematodes in an oligotrophic lake. Vie et Milieu 47, 1–7.
770 Traunspurger, W., Wilden, B., Majdi, N., 2020. An overview of meiofaunal and nematode
771 distribution patterns in lake ecosystems differing in their trophic state.
772 Hydrobiologia, in press; doi: 10.1007/s10750-019-04092-1.
773 Van Vliet, M.T.H., Ludwig, F., Zwolsman, J.J.G., Weedon, G.P., Kabat, P., 2011. Global
774 river temperatures and sensitivity to atmospheric warming and changes in river flow.
775 Water Resources Research 47.
776 Vidakovic, J., Palijan, G., Cerba, D., 2011. Relationship between nematode community and
777 biomass and composition of periphyton developing on artificial substrates in
778 floodplain lake. Polish Journal of Ecology 59, 577–588.
779 Villanueva, V.D., Font, J., Schwartz, T., Romaní, A.M., 2011. Biofilm formation at warming

780 temperature: acceleration of microbial colonization and microbial interactive effects.
781 Biofouling 27, 59–71.
782 Walker, I.R., Mott, R.J., Smol, J.P., 1996. Allerød-Younger Dryas lake temperatures from
783 midge fossils in Atlantic Canada. Science 253, 1010–1012.
784 Watkinson, A.J., O'Neil, J.M., Dennison, W.C., 2005. Ecophysiology of the marine
785 cyanobacterium, Lyngbya majuscula (Oscillatoriaceae) in Moreton Bay, Australia.
786 Harmful Algae 4, 697–715.
787 Webb, B.W., 1996. Trends in stream and river temperature. Hydrological processes 10, 205–
788 226.
789 Weitere, M., Erken, M., Majdi, N., Arndt, H., Norf, H., Reinshagen, M., Traunspurger, W.,
790 Walterscheid, A., Wey, J.K., 2018. The food web perspective on aquatic biofilms.
791 Ecological Monographs 88, 543–559.
792 Weitere, M., Vohmann, A., Schulz, N., Linn, C., Dietrich, D., Arndt, H., 2009. Linking
793 environmental warming to the fitness of the invasive clam Corbicula fluminea.
794 Global Change Biology 15, 2838–2851.
795 Woodward, G., Bonada, N., Brown, L.E., Death, R.G., Durance, I., Gray, C., Hladyz, S.,
796 Ledger, M.E., Milner, A.M., Ormerod, S.J., 2016. The effects of climatic
797 fluctuations and extreme events on running water ecosystems. Philosophical
798 Transactions of the Royal Society B 371, 20150274.
799 Woodward, G., Perkins, D.M., Brown, L.E., 2010. Climate change and freshwater
800 ecosystems: impacts across multiple levels of organization. Philosophical
801 Transactions of the Royal Society of London B: Biological Sciences 365, 2093–
802 2106.
803 Worthington, T.A., Shaw, P.J., Daffern, J.R., Langford, T.E.L., 2015. The effects of a
804 thermal discharge on the macroinvertebrate community of a large British river:

implications for climate change. Hydrobiologia 753, 81–95.

Yvon-Durocher, G., Montoya, J.M., Trimmer, M., Woodward, G., 2011. Warming alters the size spectrum and shifts the distribution of biomass in freshwater ecosystems. Global Change Biology 17, 1681–1694.

Tables

Table 1. Mean, standard deviation (SD), and minimum (Min) and maximum (Max) values of the variables measured or indices computed at each site (N = 20 samples for each site). Differences were tested using one-way ANOVA or Kruskal-Wallis rank sum test depending on whether the data met the linear assumptions or not, respectively. The level of significance (p-values: * = 0.01–0.05; ** = 0.001–0.01; *** < 0.001) is given. Full test statistics are provided in Table S3. Mean values highlighted in bold show significantly higher values at a given site.

Variables	Control site				Heated site				Test signif.
	Mean	SD	Min	Max	Mean	SD	Min	Max	
Pigment concentrations (ng.mgBiofilmDM^{-1})									
Chlorophyll *a*	254.49	42.41	21.51	713.53	240.71	23.21	66.59	418.75	
Fucoxanthin	83.72	13.10	4.49	221.34	71.59	8.95	21.78	154.10	
Chlorophyll *b*	**25.15**	5.51	1.77	119.52	7.36	1.11	1.11	18.40	***
Diadinoxanthin	13.05	2.31	1.31	39.67	10.19	1.46	2.35	24.22	
Chlorophyllide *a*	10.48	7.58	0.00	156.57	**46.15**	14.45	0.00	190.65	**
Hexanoyloxy fucoxanthin	8.44	1.45	0.00	18.65	10.99	1.28	0.29	21.91	
Zeaxanthin	7.66	1.48	0.46	25.06	**13.48**	1.48	3.06	24.42	*
Violaxanthin	**5.78**	1.24	0.45	26.40	2.27	0.20	1.01	4.61	*
Lutein	**5.67**	1.03	0.00	19.23	1.98	0.26	0.00	4.35	**
Pheophytin *a*	4.79	0.79	0.00	13.81	6.26	0.64	0.00	13.09	
Alloxanthin	1.82	0.38	0.00	5.38	2.05	0.36	0.00	5.36	
β Carotene	0.99	0.14	0.00	2.57	**1.64**	0.18	0.57	3.14	**
Myxoxanthophyll	0.81	0.58	0.00	10.99	**1.80**	0.32	0.00	4.49	***
Echinenone	0.00	0.00	0.00	0.00	**0.89**	0.25	0.00	3.80	***
Abundance (ind.10cm^{-2})									
Total invertebrates	335.22	38.30	157.17	839.54	295.87	17.34	145.23	467.52	
Nematodes	91.12	8.66	37.80	194.96	**139.98**	11.80	27.85	228.79	**
Rotifers	85.05	6.09	27.85	137.27	94.30	7.86	41.78	185.02	
Chironomids	**60.38**	6.31	1.99	123.35	39.81	4.92	6.37	97.48	*
Corbicula	**55.80**	16.82	0.00	326.27	3.76	1.16	0.00	17.90	***
Harpacticoids	**19.70**	6.09	0.00	109.42	3.68	1.00	0.00	17.90	**
Nauplii	**8.06**	2.14	0.00	33.82	2.19	0.54	0.00	9.95	*
Oligochaetes	7.56	2.36	0.00	33.82	**9.67**	1.34	0.00	21.88	*

Ephemeroptera	**5.67**	1.04	0.00	17.90	1.39	0.45	0.00	7.96	***
Ostracods	1.59	0.41	0.00	5.97	0.90	0.36	0.00	5.97	
Cladocers	0.30	0.16	0.00	1.99	0.20	0.13	0.00	1.99	
Biomass (µgDM.10cm^{-2})									
Total invertebrates	385.75	51.43	43.17	996.37	284.54	58.11	12.01	1080.08	
Chironomids	227.82	31.61	0.28	472.95	247.14	56.17	3.14	1041.08	
Ephemeroptera	**79.59**	16.98	0.00	294.06	16.97	5.67	0.00	92.53	***
Harpacticoids	**48.19**	17.02	0.00	345.32	5.69	1.94	0.00	33.99	***
Corbicula	**13.57**	4.09	0.00	79.36	0.91	0.28	0.00	4.35	***
Oligochaets	6.83	2.65	0.00	40.73	6.27	1.07	0.00	21.46	
Ostracods	**5.23**	1.36	0.00	19.62	2.94	1.18	0.00	19.62	*
Nauplii	**3.06**	0.82	0.00	12.67	0.82	0.20	0.00	3.73	*
Nematodes	0.94	0.11	0.21	2.20	**3.28**	0.38	0.36	7.16	***
Rotifers	0.41	0.03	0.13	0.63	0.45	0.04	0.19	0.85	
Cladocers	0.10	0.05	0.00	0.64	0.06	0.04	0.00	0.64	
Nematode feeding types (%)									
Deposit-feeders	**84.23**	2.35	54.55	100.00	54.19	2.49	28.21	75.00	***
Epistrate-feeders	12.81	2.50	0.00	45.45	**33.12**	2.78	10.00	61.54	***
Chewers	1.91	0.83	0.00	15.38	**6.79**	1.14	0.00	18.75	***
Suction-feeders	0.92	0.43	0.00	6.25	**5.90**	0.97	0.00	12.50	***
Nematode diversity indices									
Species richness (S)	4.40	0.24	3.00	7.00	**6.95**	0.32	5.00	10.00	***
Shannon's entropy (H)	1.09	0.04	0.73	1.45	**1.48**	0.05	0.94	1.90	***
Simpson's dominance (D)	**0.40**	0.02	0.27	0.61	0.30	0.02	0.19	0.54	***
Pielou's eveness (J)	0.76	0.03	0.53	0.96	0.77	0.02	0.59	0.92	
Trophic diversity (ITD)	0.75	0.03	0.50	1.00	**0.44**	0.02	0.30	0.59	***

Table 2. Mean (ind 10cm^{-2}) and relative (%) abundance of biofilm-dwelling nematode species. Abbreviations for nematode species names are provided in Table S5. Suffixes refer to species feeding types (DF = deposit-feeders; EF = epistrate-feeders; C = chewers; SF = suction-feeders).

Control site			Heated site		
Species	Mean (ind.10cm^{-2})	%	Species	Mean (ind.10cm^{-2})	%
MonParaDF	38.06	41.77	EuVulgDF	46.89	33.50
EuVulgDF	37.90	41.59	ChroBiocEF	45.86	32.76
ChroBiocEF	7.84	8.61	MonParaDF	18.95	13.54
MonPaluDF	1.92	2.10	MyloLacC	8.80	6.29
EuSimpDF	1.66	1.82	DoryBasSF	7.20	5.14
TobStefC	1.10	1.21	EuSimpDF	2.49	1.78
DoryStagSF	1.03	1.13	RhabAquaDF	2.00	1.43
IroTenuC	0.42	0.46	EuDispDF	1.71	1.22
EuPseuDF	0.38	0.42	EuPseuDF	1.52	1.08
EthmoPratEF	0.27	0.30	RhabTerrDF	1.25	0.90
TripGloC	0.15	0.16	TobStefC	1.16	0.83
EuDispDF	0.13	0.15	DoryStagSF	0.68	0.49
Mermi-Par	0.13	0.15	MonPaluDF	0.54	0.39
PlecAquaDF	0.13	0.14	PlecAquaDF	0.36	0.26
			EuLongDF	0.24	0.17
			ChroVirEF	0.20	0.15
			DoryAgiSF	0.14	0.10

Figure caption

Fig. 1. Aerial view of the study site (source: IGN 2020) and photographs of the experimental set-up at the heated and control sites.

Fig. 2. A. Hourly water temperature recorded at the control (green line) and heated (orange line) sites over the entire experimental period (1344 h, starting on 7 August 2018 at 1 PM and ending when the samples were retrieved on 2 October 2018 at 1 PM). **B.** Boxplots of the daily fluctuation of water temperature (i.e., the range between the minimum and maximum water temperature for each day) at each site over the entire period. **C.** Hourly ΔT (difference in water temperature between heated and control sites).

Fig. 3. Non-metric multidimensional scaling (nMDS) of the Bray–Curtis similarities of pigment composition across samples (N = 40 concrete tiles). Pigment concentrations are ordinated on the biplot. 'spider web' extremities indicate the position of the samples in the 2D-space. The labels in black (control or heated) indicate the centroid of the site they belong to, and the labels in red indicate the centroid of each pigment concentration (pigment abbreviations can be found in Table S5).

Fig. 4. Non-metric multidimensional scaling (nMDS) of the Bray–Curtis similarities of invertebrate group abundances across samples. Group abundances are ordinated on the biplot. 'spider web' extremities indicate the position of the samples in the 2D-space. The label in black (control or heated) indicate the centroid of the site they belong and the labels in red indicate the centroid of each invertebrate group (group abbreviations can be found in Table S5).

Fig. 5. A. Chironomid body-mass distribution at each site (heated site: dark orange, control site: green). Vertical dotted lines indicate the mean body-mass value for each site. **B.** Cumulative density functions for chironomid individual body-mass.

Fig. 6. Non-metric multidimensional scaling (nMDS) of the Bray–Curtis similarities of nematode species abundances across samples. Species abundances are ordinated on the biplot. 'spider web' extremities indicate the position of the samples in the 2D-space. The label in black (control or heated) indicate the centroid of the site they belong and the labels in red indicate the centroid of each species (species abbreviations can be found in Table S5; suffixes refer to species feeding type, see Table 2).

Fig. 7. Relative abundance of nematode feeding types across sites. Values are medians, boxes and whiskers show interquartile range.

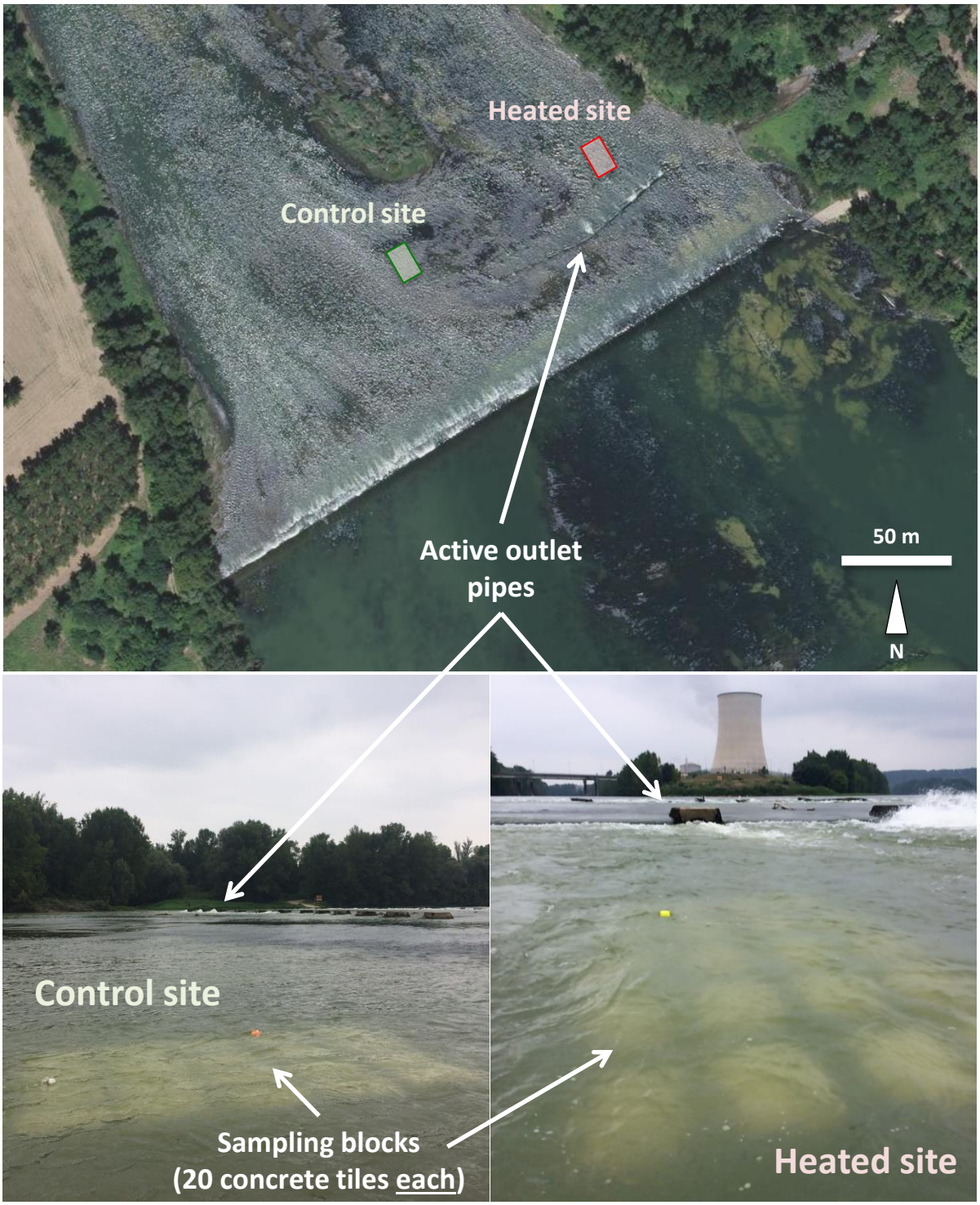

Heated site
Control site
Active outlet pipes
50 m
N
Control site
Sampling blocks
(20 concrete tiles each)
Heated site

Fig. 1

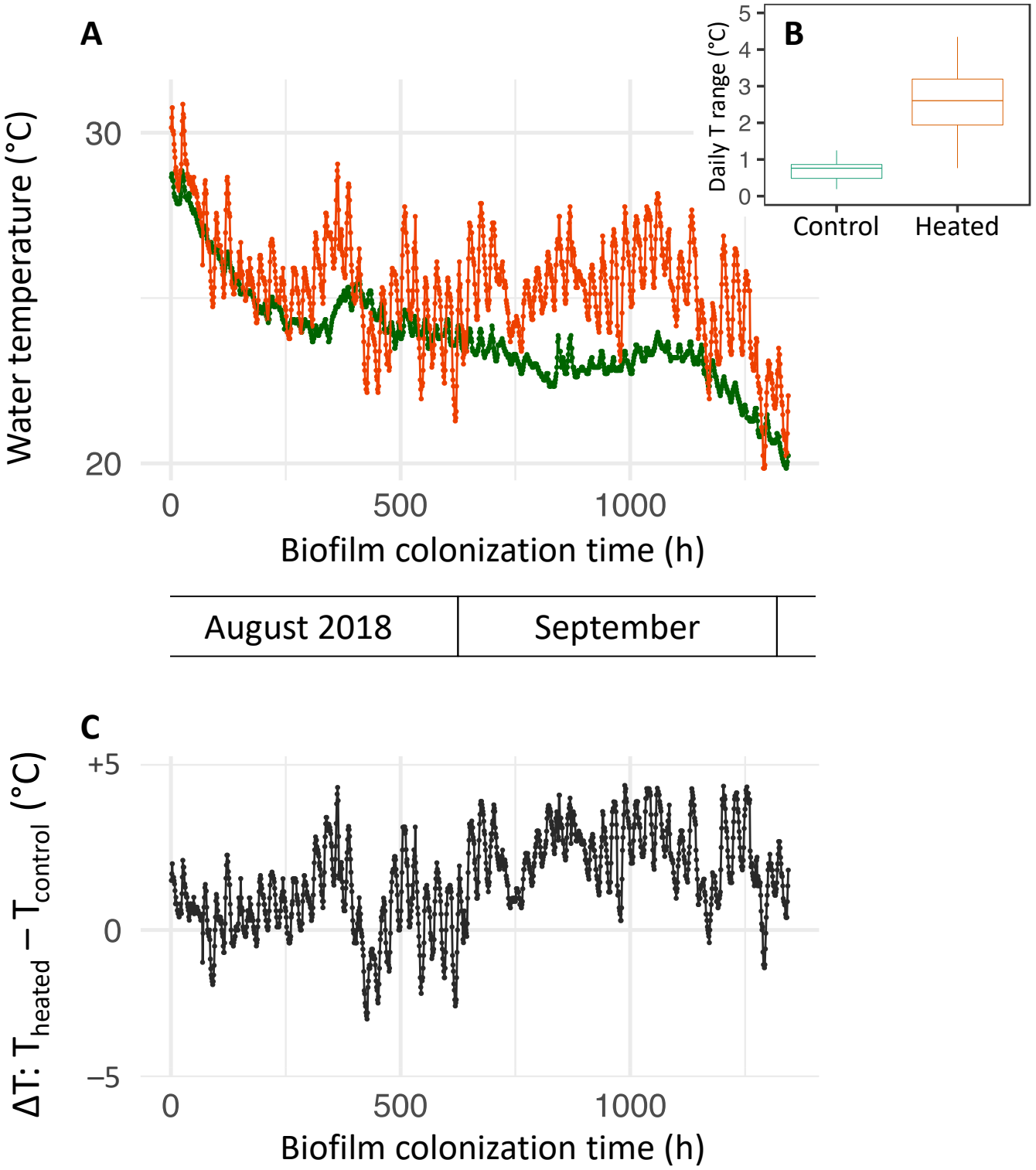

A
B
Water temperature (°C)
30
20
0
500
1000
Biofilm colonization time (h)
Daily T range (°C)
5
4
3
2
1
0
Control
Heated
August 2018
September
C
ΔT: T_heated − T_control (°C)
+5
0
−5
0
500
1000
Biofilm colonization time (h)

Fig. 2

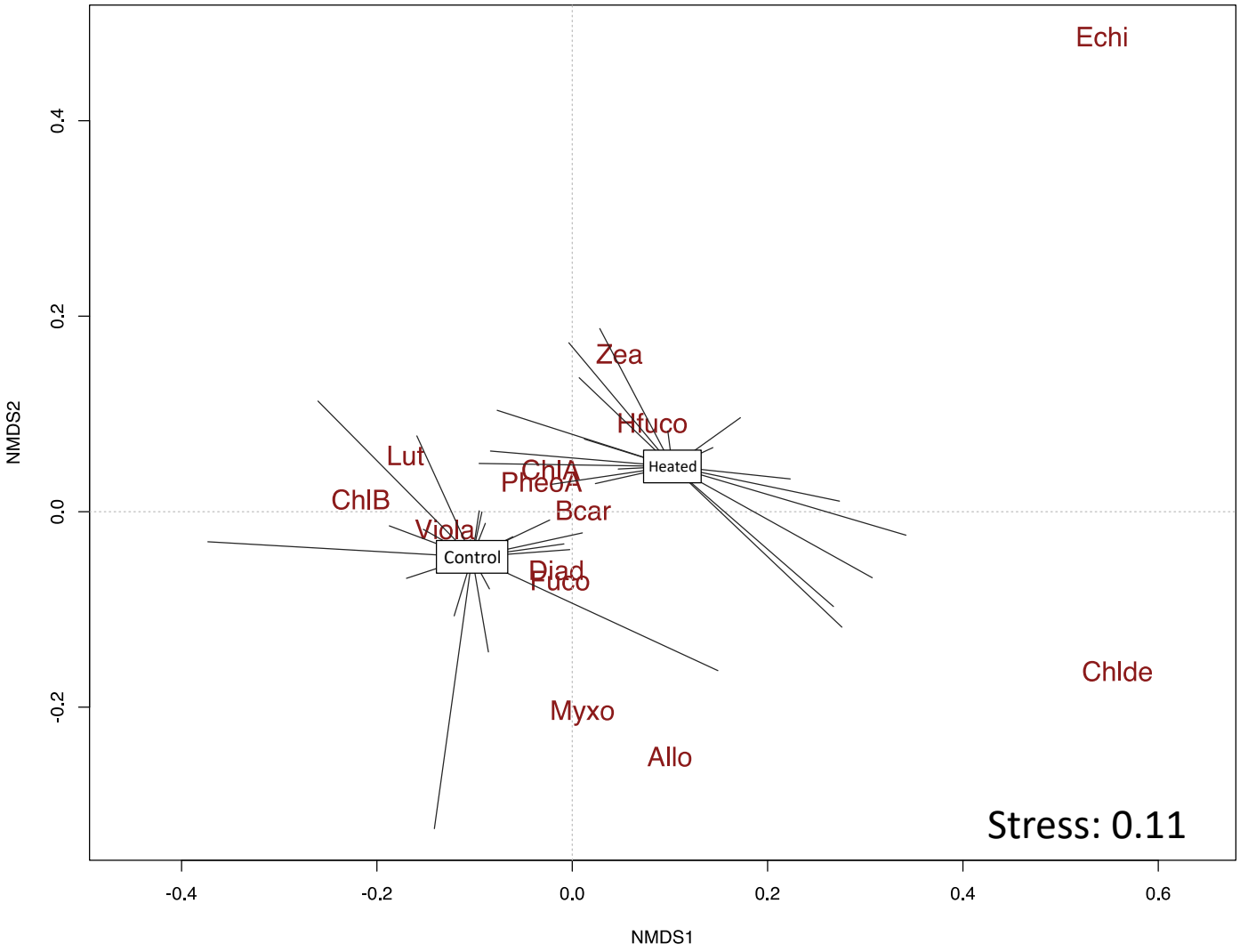

Echi
Zea
Hfuco
Lut
ChlA
PheoA
Heated
ChlB
Bcar
Viola
Control
Diad
Fuco
Chlde
Myxo
Allo
Stress: 0.11
NMDS2
NMDS1
0.4
0.2
0.0
-0.2
-0.4
-0.2
0.0
0.2
0.4
0.6

Fig. 3

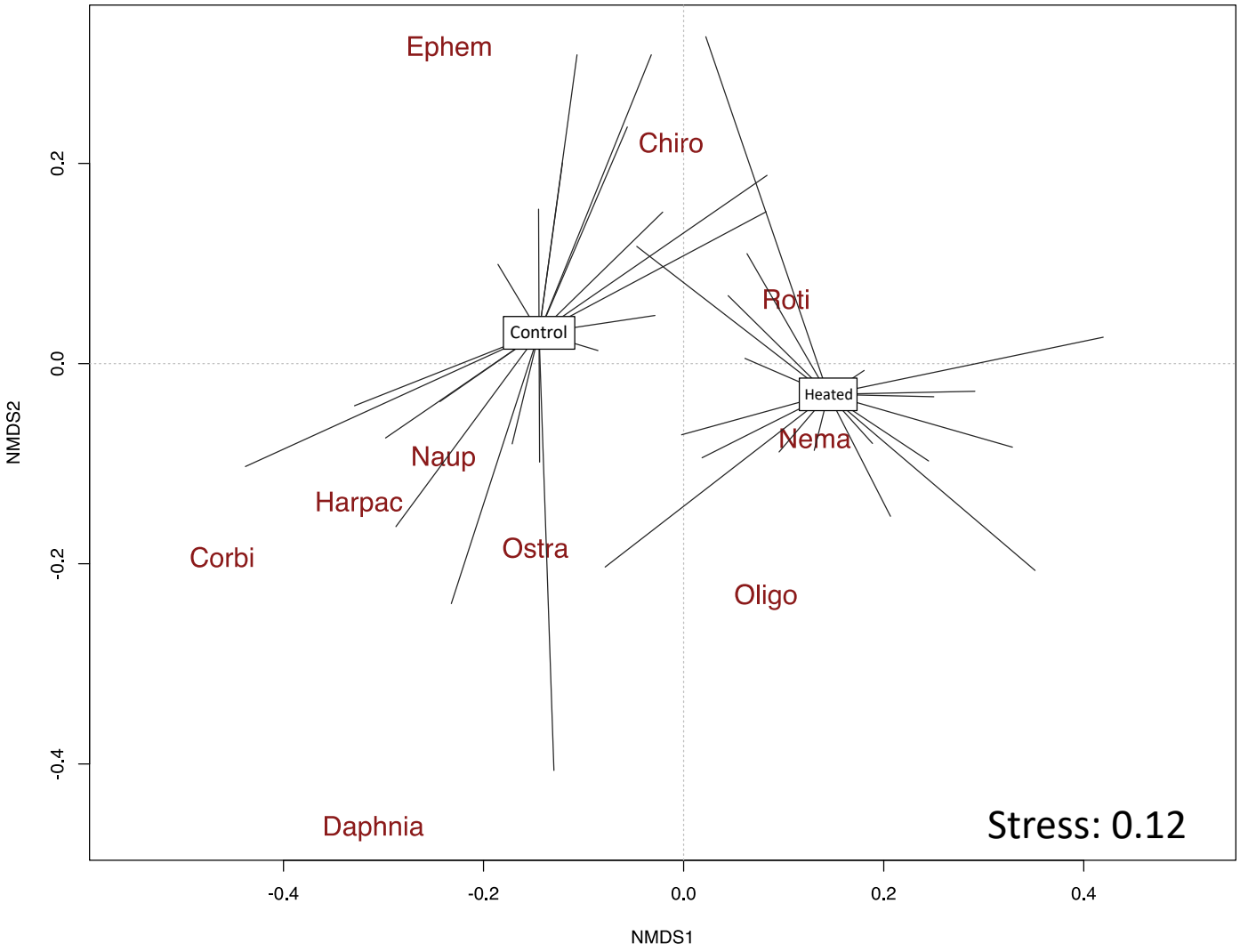
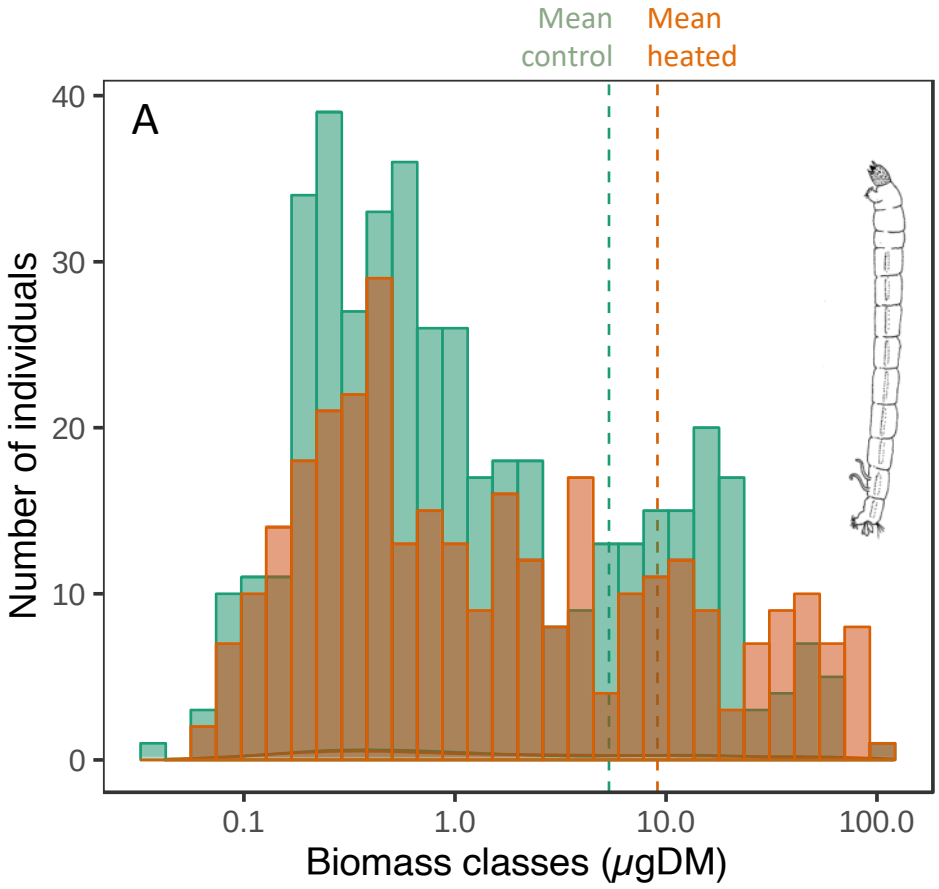

Ephem
Chiro
Roti
Control
Heated
Nema
Naup
Harpac
Corbi
Ostra
Oligo
Daphnia
Stress: 0.12
NMDS1
NMDS2
-0.4
-0.2
0.0
0.2
0.4

Fig. 4



Cumulative body-mass distribution of chironomids

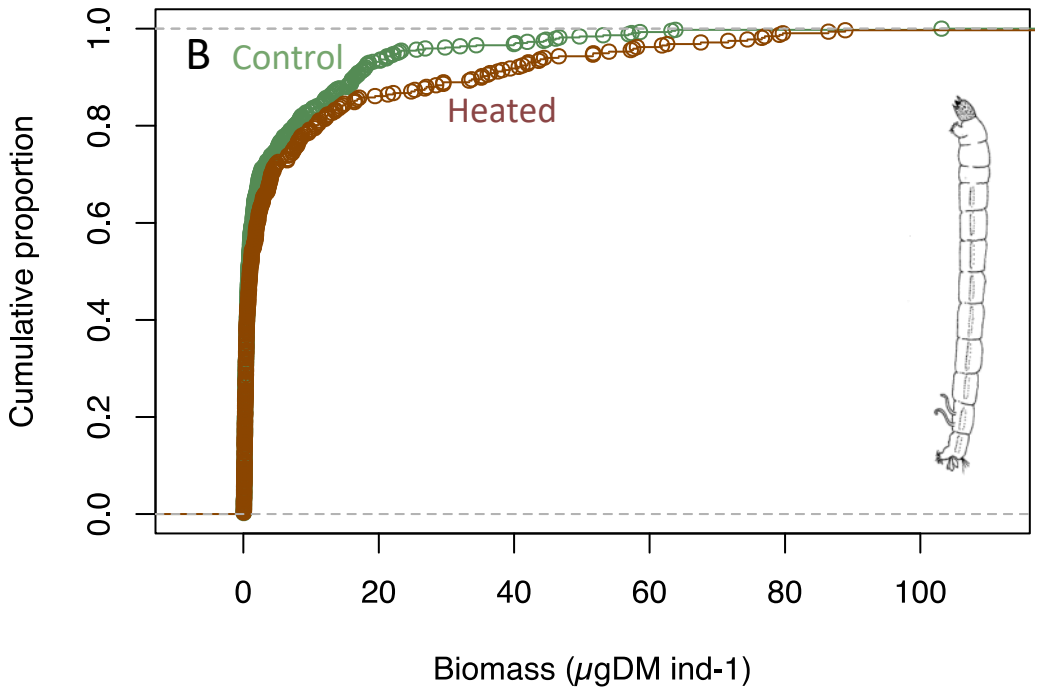


Fig. 5

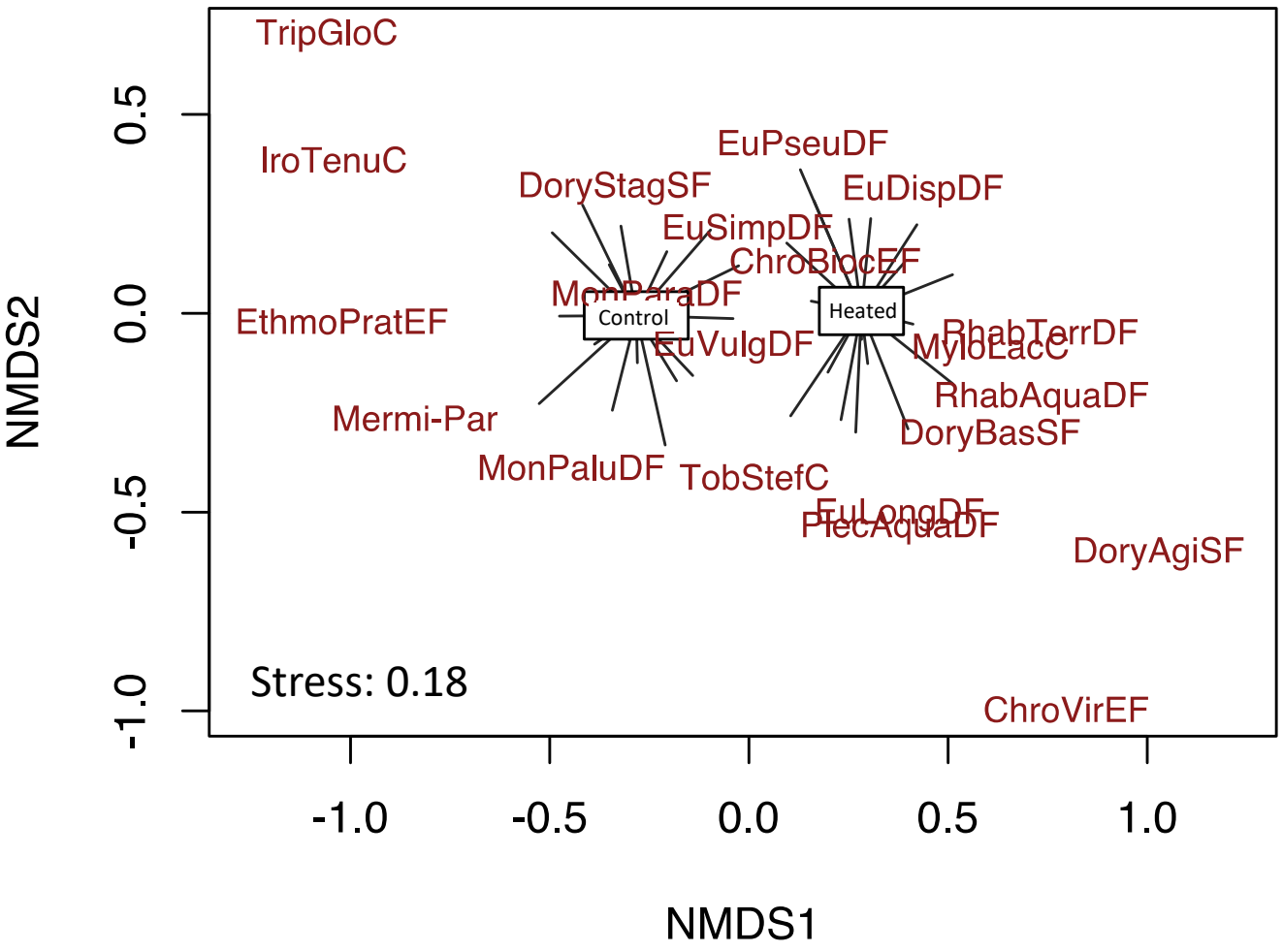

TripGloC
IroTenuC
EthmoPratEF
Mermi-Par
MonPaluDF
TobStefC
EuLongDF
PlecAquaDF
DoryAgiSF
ChroVirEF
DoryBasSF
RhabAquaDF
RhabTerrDF
MyloLacC
EuVulgDF
MonParaDF
ChroBiocEF
EuSimpDF
DoryStagSF
EuPseuDF
EuDispDF
Control
Heated
Stress: 0.18
NMDS1
NMDS2
-1.0
-0.5
0.0
0.5
1.0

Fig. 6

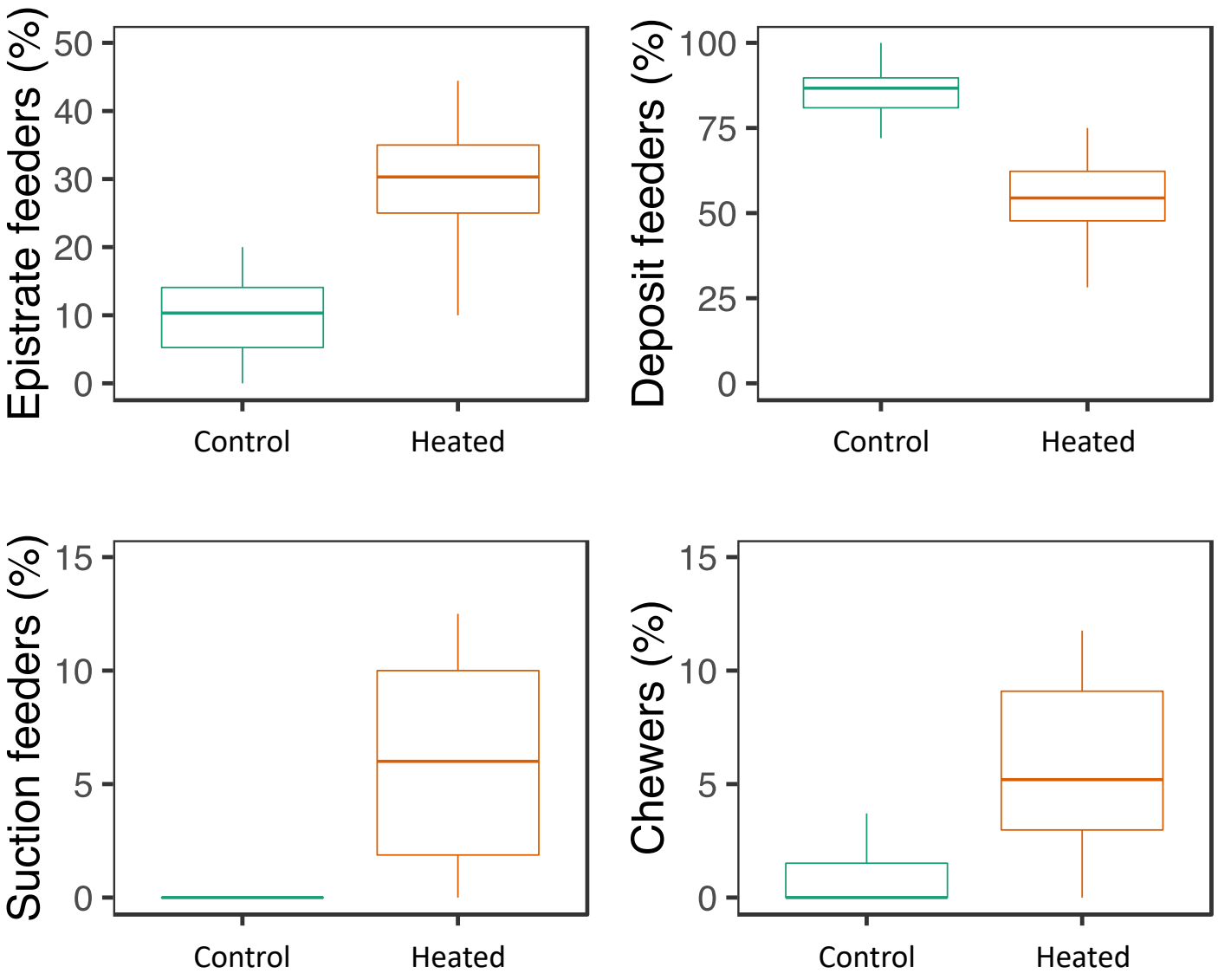
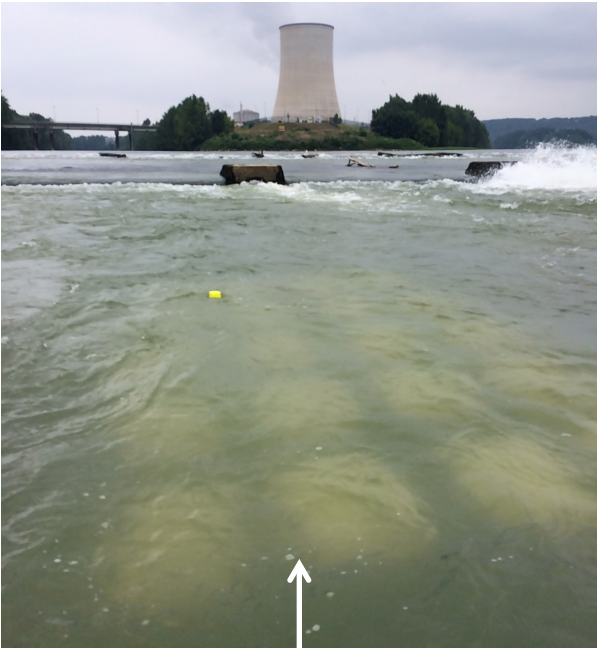

Epistrate feeders (%)
50
40
30
20
10
0
Control
Heated
Deposit feeders (%)
100
75
50
25
0
Control
Heated
Suction feeders (%)
15
10
5
0
Control
Heated
Chewers (%)
15
10
5
0
Control
Heated

Fig. 7

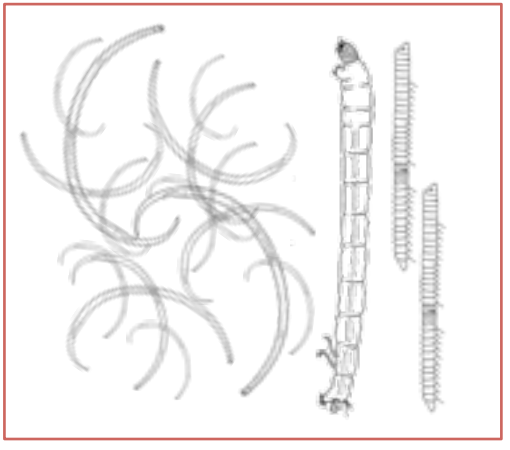
In situ **warming experiment** in the Garonne River (France)

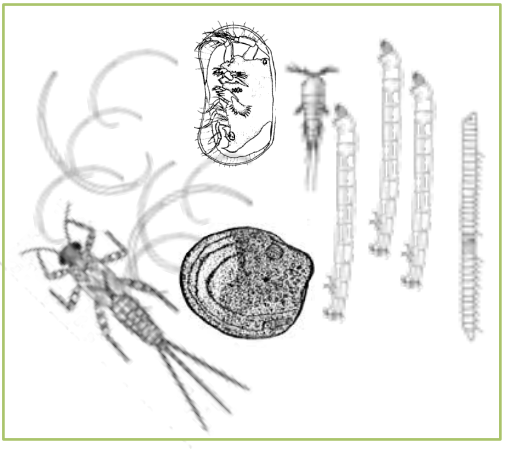
Heated site



Control site

Biofilm-dwelling communities





Comparable standing stocks
Different assemblages