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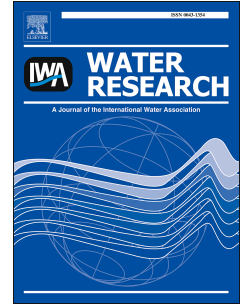
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Accepted Manuscript

The phantom midge menace: Migratory *Chaoborus* larvae maintain poor ecosystem state in eutrophic inland waters

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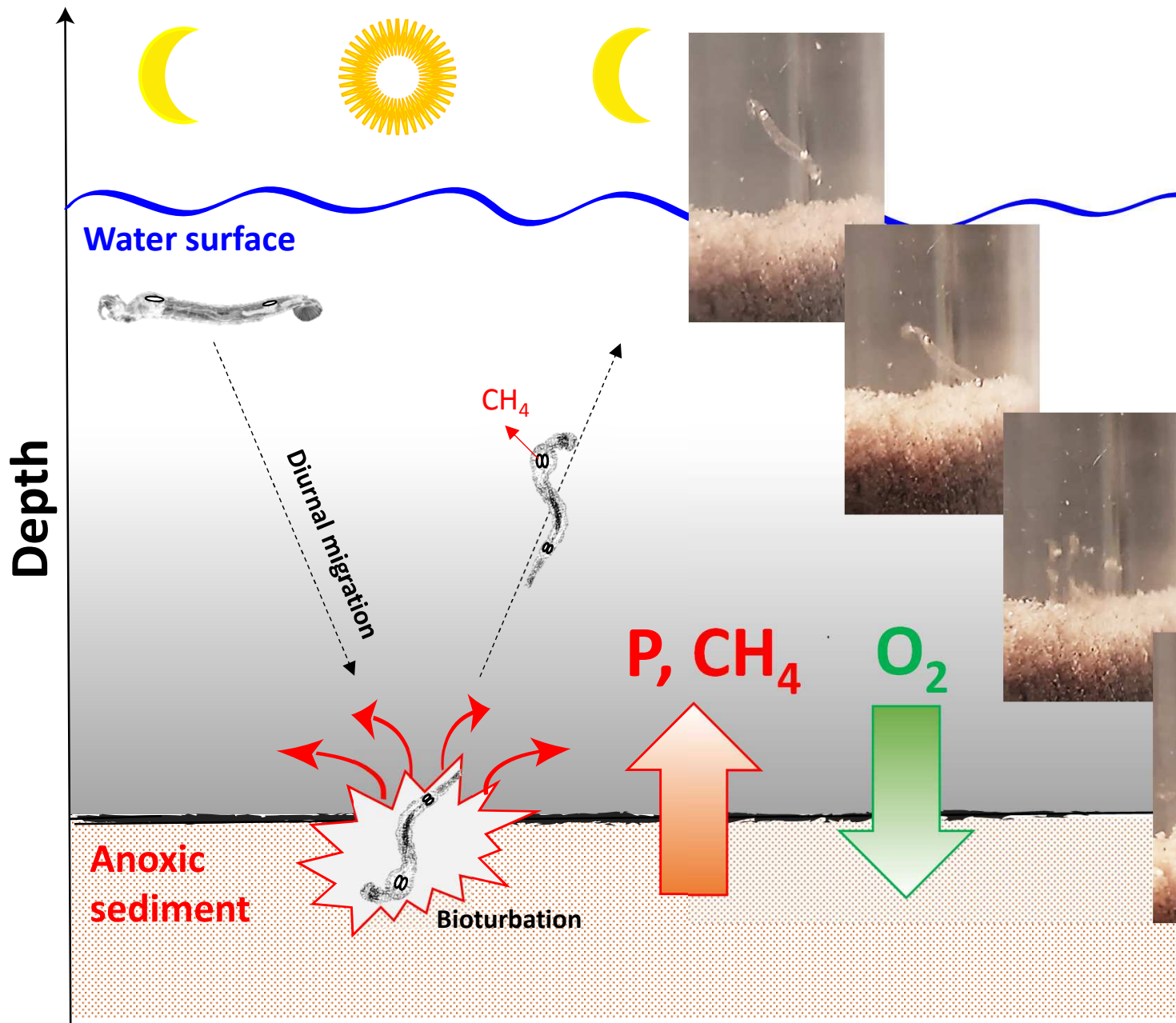
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3 eutrophic inland waters

4

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27 **Abstract**

28 *Chaoborus* spp. (phantom midge) are prevalent in eutrophic inland waters. In Lake Soppen,
29 Switzerland, *C. flavicans* larvae diurnally migrate between the methane-rich, oxygen-
30 depleted hypolimnion and sediments, and the methane-poor, oxygen-rich epilimnion. Using
31 a combination of experiments and system modelling, this study demonstrated that the
32 larvae's burrowing activities in and out of the sediment perturbed the sediment and re-
33 introduced sequestered phosphorus into the overlying water at a rate of $0.022 \mu\text{g P ind}^{-1} \text{d}^{-1}$,
34 thereby exacerbating internal nutrient loading in the water column. Fluxes of sediment
35 methane and other reduced solutes enhanced by the larval bioturbation would consume
36 oxygen and sustain the hypoxic/anoxic condition below the thermocline. In addition to
37 increasing diffusive fluxes, migrating larvae also directly transported methane in their gas
38 vesicles from the deep water and release it in the surface water at a rate of 0.99 nmol CH_4
39 $\text{ind}^{-1} \text{d}^{-1}$, potentially contributing to methane emission to air. As nutrient pollution and
40 climate warming persist or worsen in the coming decades, proliferation of *Chaoborus* could
41 intensify this positive feedback loop and delay lake recovery.

42

43 **Graphic abstract**

- 46 • Eutrophication promotes proliferation of *Chaoborus* larvae in inland waters.
- 47 • The larvae's bioturbation activities release phosphorus and methane from sediment.
- 48 • These in turn exacerbate eutrophication and deoxygenation of the water column.
- 49 • The larvae thereby maintain a poor ecosystem state despite restoration efforts.

50

51 **Keywords**

52 *Chaoborus*, eutrophication, methane, positive feedback, nutrient internal loading

55 production and deoxygenation of the hypolimnion, is one of the major man-made
56 environmental problems (Smith et al., 2006). Aggressive management practices such as
57 nutrient reduction and artificial aeration often yield very limited success, and the eutrophic
58 conditions can persist for decades or longer (Gächter and Wehrli, 1998; McCrackin et al.,
59 2017). These failures continue to puzzle and frustrate scientists and resource managers
60 (Ibelings et al., 2016). We hypothesize that the successful colonization of inland waters (i.e.
61 lakes) by the phantom midge larvae (*Chaoborus* spp.) drives internal processes that
62 counteract external mitigation efforts and maintains the status quo.

63 The prevalence of *Chaoborus* larvae in eutrophic inland waters—with population
64 densities up to 130,000 ind m⁻² (Gosselin and Hare, 2003; Sweetman and Smol, 2006)—may
65 be attributed to the larvae's tolerance of low oxygen environments and even toxic hydrogen
66 sulfide that characterize the hypolimnion and sediment in eutrophic lakes. Many *Chaoborus*
67 spp. larvae perform diurnal vertical migration where they reside at depth (i.e. sediment or
68 anoxic deep-water) to avoid planktivorous fish, and ascend to the epilimnion at night to
69 prey on other zooplankton (Dawidowicz et al., 1990; Bezerra-Neto et al., 2012). In tropical
70 lakes, this process may divert energy from planktivorous fish and suppress the production of
71 the latter (Hecky, 1984; Lewis, 1996). Accordingly, much research has been done on the
72 roles of *Chaoborus* spp. larvae in food web dynamics in the water column (e.g. Vanni et al.,
73 1997; Cole et al., 2006).

74 The instars of *Chaoborus* larvae can exchange gases, including CH₄ (McGinnis et al.,
75 2017; Carey et al., 2018) between their gas vesicles and ambient water, thereby adjusting

78 sediments, the gas typically abundant for this purpose (high partial pressure, low solubility)
79 is methane (CH₄) (McGinnis et al., 2017).

80 Paleolimnological studies have shown that the appearance of *Chaoborus* remains (e.g.
81 mandibles) in sediments often coincided with a major shift in the lake's trophic state
82 (Sweetman and Smol, 2006; Quinlan and Smol, 2010), but the direct link and feedback
83 between *Chaoborus* colonization and lake eutrophication history remain unclear. To our
84 knowledge, Gosselin and Hare (2003) were the first to observe in the laboratory the
85 bioturbation effects of individual *Chaoborus* larvae as they burrow in and out of the
86 sediment. Based on subsequent experimental and modelling studies, it is hypothesized that
87 this bioturbation activity would release sequestered nutrients and CH₄ into the overlying
88 water at an enhanced rate, thereby pushing the system over an ecological tipping point
89 where the eutrophic state may self-sustain indefinitely (McGinnis et al., 2017; Tang et al.,
90 2017). This hypothesis has not been empirically tested, but would have important
91 implications for lake ecology and management. This study therefore aimed at
92 demonstrating the population-level effect that migratory *Chaoborus* larvae have on nutrient
93 and methane dynamics in lakes.

94 Lake Soppen (47°5'25"N, 8°4'51"E) is a small eutrophic kettle lake (area 0.26 km², max
95 depth 27 m, mean depth 12 m) in the Canton of Lucerne, Switzerland. Paleolimnological
96 evidence suggests a drastic increase in total phosphorus level in the last decades (Lotter,
97 2001). Strong bioturbation by *C. flavicans* has caused the destruction of calcareous
98 laminations in the last century in Lake Soppen sediments despite the high calcite deposition

101 nutrient loading in Lake Soppen. In addition, by taking up CH₄ in the porewater and
102 releasing it in the water column, migrating *Chaoborus* larvae are expected to accelerate the
103 upward CH₄ flux over passive diffusion and affect the ambient CH₄-carbon isotope
104 composition in the epilimnion. We therefore also investigated how *C. flavicans* larvae affect
105 CH₄ transport and isotope composition in Lake Soppen. Taken together, this study provides
106 novel insights into how migratory *Chaoborus* larvae could drive a positive feedback loop
107 between eutrophication, methane transport and deoxygenation in inland waters.

108

109 **2. Material and Methods**

110 *2.1. Sediment incubation experiment*

111 Sediment was collected from Lake Soppen by a gravity corer. On shore, the sediment
112 was thoroughly mixed with an electric drill-mixer to standardize the initial conditions among
113 all replicates. Incubations were performed in 12 plexiglass cylinders (diameter 5.6 cm,
114 length 29 cm) with bottom caps. The caps were used to measure out ca. 73 ml of the mixed
115 sediment (ca. 4 cm thick); the cylinders were then pushed into the sediment-containing
116 caps. Surface lake water was gently siphoned into each cylinder, letting the water overflow
117 to replace ca. 3 times its volume to standardize the initial water condition among the
118 replicates. The final volume of the overlying water was ca. 0.5 l. *C. flavicans* larvae (instar 3–
119 4) were collected from ca. 20 m depth, rinsed with lake water and sorted into cups in
120 different numbers (0, 30, 60, 90, 120 and 180, in duplicates), equivalent to an in situ areal
121 density of 0 – 73,000 ind m⁻². Each cup was randomly assigned to a cylinder and the larvae

124 cylinders remained open and exposed to the natural day light (Fig. S1).

125 The incubation lasted from 12th June night until 22nd June 2017 morning (9.5 d total). On
126 the last day, the cylinders were removed one at a time in random order for processing. First,
127 we measured the water temperature and dissolved oxygen (HACH® Portable Multi Meter,
128 model HQ40D); the color appearance of the water was recorded. Afterward, 50 ml of the
129 water was taken from the top with a syringe, and stored refrigerated in an opaque plastic
130 bottle for total phosphorus (TP) (see below). 300–400 ml of the remaining water was gently
131 siphoned, without disturbing the sediment, into a glass bottle to measure CO₂ and CH₄
132 concentrations and $\delta^{13}\text{C-CH}_4$ (see below).

133

134 *2.2. Water column measurements, total phosphorus and dissolved gases*

135 Water column profiles were measured with a Seabird SBE 19plus V2 SeaCAT Profiler
136 CTD (Sea-Bird Scientific, Bellevue, Washington, USA) at a sampling frequency of 4Hz. The
137 profiler was lowered in the water at $\sim 10 \text{ cm s}^{-1}$ and recorded temperature, pressure,
138 conductivity and dissolved oxygen with about a 3 cm resolution.

139 Lake water was sampled with a Niskin bottle (5 l) at the deepest point on 22nd May for
140 total phosphorus (TP) and on 13th June 2017 for water column dissolved gases. For TP, water
141 samples were preserved in the cold until measurements. TP was measured spectrophoto-
142 metrically after potassium persulfate (K₂S₂O₈) digestion (45 minutes at 130°C). The same
143 method was used to measure TP in the sediment incubation experiment.

146 (Donis et al. 2017). About half of the water was replaced by atmospheric air. The bottle was
147 immediately capped to create a headspace, then shaken vigorously for at least 2 minutes to
148 equilibrate the dissolved gases with the headspace. Afterward, the headspace was extracted
149 through a top valve into a gas-sample bag (SupelTM Inert Multi-Layer Foil Gas Sampling Bags)
150 by slowly injecting lake water into the bottom of the bottle through a rubber tubing. CO₂
151 and CH₄ concentrations and $\delta^{13}\text{C-CH}_4$ of the gas bag content were measured within one day
152 on a Cavity Ringdown Spectrometer (Picarro G2201-i). Initial concentrations of CO₂ and CH₄
153 in the sampled water was calculated accounting for initial headspace CH₄ and CO₂
154 concentrations (before equilibrium assuming atmospheric concentration of 2 and 400 ppmv
155 for CH₄ and CO₂, respectively), volume ratio (i.e. headspace:water), air and water
156 temperatures, in situ barometric pressure and lake water total alkalinity. The same method
157 was used to measure dissolved gases in the sediment incubation experiment.

158

159 2.3. Day-night sampling of *Chaoborus*

160 *Chaoborus flavicans* larvae were sampled with an open-close net (0.3 m diameter; 200
161 μm mesh) through discrete vertical strata: 0–5, 5–10, 10–15, 15–20, 20–25 m (max. depth
162 ca. 27 m). Upon retrieval of the net, the cod-end content was washed into a container. *C.*
163 *flavicans* larvae were counted on shore immediately afterward. The mesh size was not
164 suitable for capturing the small instars 1–2; hence, only instars 3–4 were counted. Between
165 13th and 14th June, 2017, sampling was done at sunset (ca. 21:00 h local time), sunrise (ca.

169 2.4. Bottle incubation experiments

170 Surface lake water was aerated for several hours using an aquarium-type air pump to
171 equilibrate its background CH₄ with ambient air. The aerated water was used for washing
172 and for the blank. *C. flavicans* larvae were collected from depths where they were most
173 abundant at the time of the experiment (15–20 m in day; 0–5 m at night). The experiments
174 were done three times (13th June at 16:30, 22nd June at 02:40, 22nd June at 14:30). For the
175 first experiment, the larvae were brought back to shore, rinsed with the aerated lake water,
176 and unknown numbers were added to 120-ml serum bottles. For the latter experiments, the
177 larvae were immediately concentrated on a 1-mm mesh on the boat, briefly rinsed with the
178 aerated lake water, and immediately added to the serum bottles. All serum bottles were
179 topped off with the aerated lake water and crimp sealed. Bottles with only aerated lake
180 water were used as the blank.

181 The sealed bottles were left overnight to allow the release of CH₄ from *C. flavicans* gas
182 vesicles. Afterward, Synthetic Air (Carbagas: 80% N₂, 20% O₂; ± 1%) was injected to create
183 headspace (ca. 50 ml). The bottles were shaken vigorously for ca. 2 min. to equilibrate
184 headspace and dissolved gases. The headspace gas was then displaced into a gas-tight
185 syringe by slowly injecting lake water into the bottle. The gas was then injected immediately
186 into the Picarro spectrometer to measure CH₄ concentration and carbon isotopic signature
187 of CH₄. Total CH₄ was calculated by accounting for dissolved CH₄ in the displaced headspace
188 water. Afterward, the numbers of larvae in the serum bottles were counted.

$$\delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \cdot 1000$$

191 with R_{sample} as the ratio of heavy to light C isotope of the sample and R_{standard} the isotope
 192 ratio of the Vienna Pee Dee Belemnite standard.

193

194 2.5. System modelling of methane and oxygen dynamics

195 In the sediment incubation experiment, *C. flavicans* bioturbation would increase
 196 sediment-water exchange of not only nutrients but also dissolved gases including CH_4 . To
 197 examine the effect of bioturbation on CH_4 dynamics in the experiment, we apply a system
 198 modelling approach by accounting for input (bioturbation, F_{sed} plus gas vesicle transport,
 199 R_{chab}) and output (oxidation, R_{ox} plus emission to air, F_{sur}) of CH_4 . The mass balance is
 200 expressed as

$$201 \quad \frac{\partial C_{\text{CH}_4}}{\partial t} V = F_{\text{sed}} A - F_{\text{sur}} A + R_{\text{chab}} N_{\text{chab}} - R_{\text{ox}} C_{\text{CH}_4} V \quad \text{Eqn. 1}$$

202 where C_{CH_4} is the dissolved methane concentration, V is volume of water in the cylinder, t is
 203 time, A is the cross-sectional area of the cylinder. Measurements of CH_4 oxidation rate and
 204 Fickian diffusion coefficient (k_{600}) are explained in the supplementary materials. Average CH_4
 205 transport by *C. flavicans* gas vesicles was taken from the bottle incubation experiments
 206 (section 2.4).

207 Assuming the system was at steady state at the end of the experiment (i.e. $\frac{\partial C_{\text{CH}_4}}{\partial t} = 0$;
 208 input = output), we then derive the relative CH_4 increase against the control due to *C.*

211 *C. flavicans* gas vesicles transport and air-water gas exchange (Suppl. materials).

212

213 **3. Results**

214 *3.1. Water column chemistry*

215 The study was conducted on 12th–22nd June, 2017 when the lake was thermally
216 stratified at 5–9 m (Fig. 1A) with a strong oxycline at 5–6 m separating the oxygen-rich
217 epilimnion and the nearly anoxic water below 6 m (Fig. 1B). Dissolved CH₄ was low (ca. 1
218 μmol l⁻¹) at the surface and increased with depth in the hypolimnion to a maximum of 700
219 μmol l⁻¹ (Fig. 1C). The average δ¹³C-CH₄ was -51.0‰ in 0–5 m, and was more negative below
220 the thermocline reaching -65.1‰ within 10–25 m (Fig. 1D). Phosphorus concentration
221 increased by an order of magnitude between the surface (14.9 μg l⁻¹) and the bottom (289.6
222 μg l⁻¹) (Fig. 1E).

223

224 *3.2. Chaoborus effects on sediment-water exchange*

225 To test the hypothesis that migrating *C. flavicans* larvae enhance sediment-water
226 exchange of dissolved substances (i.e. phosphorus and CH₄), we conducted an experiment
227 where homogenized lake sediment was incubated in Plexiglas cylinders with surface lake
228 water and different number of larvae (instars 3–4) added. Because the cylinders were
229 uncapped during the experiment, some water (8.96 ± 4.95 %) was lost to evaporation. At
230 the end of the experiment, the water was increasingly more turbid and green with

233 Total phosphorus in the overlying water increased linearly by a factor of 5 with increasing
234 number of larvae (Fig. 2). The amount of phosphorus added to the overlying water by the
235 larvae (slope of regression line) was $0.21 \mu\text{g P ind}^{-1}$ over the course of the experiment (9.5
236 d), or $0.022 \mu\text{g P ind}^{-1} \text{d}^{-1}$.

237 Because one cylinder was approaching hypoxia, its gas dynamics (e.g. respiration, CH_4
238 oxidation) were not comparable with the others, and was excluded from further data
239 analysis. Final dissolved CH_4 concentrations increased linearly by a factor of ca. 5 with
240 increasing number of larvae; however, $\delta^{13}\text{C-CH}_4$ was quite stable at an average of -49.9‰
241 (Fig. 3A & B). Concurrently, dissolved pCO_2 decreased exponentially (Fig. 3C).

242

243 3.3. Diurnal migration of *Chaoborus*

244 On 13th–14th June, net sampling showed the integrated density of *C. flavicans* larvae
245 (instars 3–4) was quite consistent throughout the sampling cycle (mean \pm sd; 9229 ± 1393
246 ind m^{-2}), but their vertical distribution showed marked diurnal changes (Fig. 4). At 21:00 h,
247 most of them were at 5–15 m (87.5% of the total); at 05:00 h, almost all were concentrated
248 at 5–10 m. As the day progressed, the majority descended to 10–15 m (Fig. 4). The second
249 sampling cycle (21st–22nd June) was conducted at a higher frequency to better capture the
250 nighttime ascent of the larvae. Before sunset, most of the larvae were at 10–20 m. They
251 ascended at sunset (ca. 21:00 h) and occupied the 0–5 m layer at night (between 00:00 h
252 and 02:15 h). The larvae descended again in the morning and reached 15–20 m by mid-day

256 *3.4. Methane release from Chaoborus gas vesicles*

257 Bottle incubation experiments to measure the release of CH₄ from the larvae's gas
258 vesicles (instars 3–4) were performed three times using larvae collected from the deep (15–
259 20 m) and shallow layers (0–5 m). The total CH₄ released was linearly correlated with
260 number of larvae; i.e. the amount of CH₄ released per individual was quite constant within
261 each trial (Fig. 5A). The amounts of CH₄ released per individual were almost identical
262 between the two trials with deep-water larvae: 0.97 and 1.02 nmol ind⁻¹. Because the
263 shallow-water larvae would have already lost some of their CH₄ to the surrounding water
264 before capture, as expected, the amount of CH₄ was much lower for them, at 0.19 nmol ind⁻¹.
265 Both trials with deep-water larvae gave similar δ¹³C-CH₄ of -65.5 to -62.9 ‰, which is very
266 close to that of the bottom water (-65.4 ‰; Fig. 1D). Shallow-water larvae showed a wider
267 range of values, from -66.15 ‰ up to -58 ‰ (Fig. 5B).

268

269 *3.5. System dynamics of methane and oxygen*

270 The CH₄ oxidation rate was measured to be 0.03 d⁻¹ (Table S2). Emission to air was
271 calculated using a measured Fickian diffusion coefficient (k_{600}) of 0.23 m d⁻¹ (Suppl.
272 materials) and the final dissolved CH₄ concentrations. Average CH₄ transport by *C. flavicans*
273 gas vesicles was 0.99 nmol ind⁻¹ d⁻¹ (Fig. 5). Under a steady-state condition, our model
274 predicts a linear relative increase in CH₄ as a function of *C. flavicans* abundance (Fig. 6).

277 increase due to bioturbation (regression slope = 0.035 ind^{-1}) closely matches the observed
278 relative increase in TP (regression slope = 0.032 ind^{-1}); i.e. *C. flavicans* bioturbation
279 increased the effective diffusion of TP and CH_4 by nearly equal magnitude (Fig. 6). The
280 model performance was not sensitive to CH_4 oxidation rate. For example, decreasing and
281 increasing the specific oxidation rate by 10-fold (to 0.003 and 0.3 d^{-1} , respectively) will
282 change the regression slope by $\leq 6\%$. The percent contribution by gas vesicle transport of
283 sediment CH_4 to the overlying water increased with the number of larvae and asymptotically
284 approached 18% of the total CH_4 input (Fig. 6). In other words, the percent contributions of
285 CH_4 by both bioturbation and vesicle transport would approach constant as *C. flavicans*
286 abundance increases, though both rates would presumably continue to increase.

287

288 **4. Discussion**

289 *4.1. Bioturbation and nutrient internal loading*

290 In the sediment incubation experiment, final total phosphorus (TP) concentrations in
291 the control cylinders (without added *C. flavicans*) averaged $15.4 \mu\text{g l}^{-1}$, almost identical to
292 the in situ surface water value ($14.9 \mu\text{g l}^{-1}$; Fig. 1). In contrast, TP increased at a rate of 0.022
293 $\mu\text{g P ind}^{-1} \text{ d}^{-1}$ in the cylinders with added *C. flavicans* larvae. Because we measured TP
294 (particulate + dissolved), the observed increase in TP cannot be attributed to nutrient
295 recycling within the water (e.g. via excretion or remineralization). While we did not count
296 the final number of larvae, we did not observe any dead (floating) larvae in the experiment,

299 From the data we can infer that bioturbation by *C. flavicans* increased the effective
300 sediment diffusivity and re-introduced sequestered phosphorus to the overlying water, as
301 has been previously hypothesized (Gosselin and Hare, 2003), and is further supported by
302 our CH₄ data and system modelling. As expected, this extra nutrient stimulated primary
303 production and CO₂ drawdown in the experiment, as indicated by the observed pCO₂ values
304 (Fig. 3C) and the color appearance of the water in the cylinders (Table S1). Extrapolating the
305 experimental results to the observed in situ population density (ca. 9,300 ind m⁻²), *C.*
306 *flavicans* bioturbation would add ca. 205 µg P m⁻² d⁻¹ to the water column. This extra
307 phosphorus is likely to first accumulate in the hypolimnion and become available for
308 primary production during spring turnover. Lake remediation strategies often focus on
309 curbing external phosphorus input (Schindler, 2006). Our results, however, suggest that *C.*
310 *flavicans* bioturbation is a powerful mechanism to release nutrients from within the
311 sediments, and may explain in some cases the ineffectiveness of external nutrient
312 management for improving water clarity (McCrackin et al., 2017).

313

314 4.2. Breaking the diffusive barrier

315 Oxygen loss from the water column to the sediment is generally limited by diffusion
316 across the sediment-water interface (Bryant et al., 2010). However, hypolimnetic oxygen
317 demand would increase due to increased CH₄ oxidation as a result of enhanced introduction
318 of sediment CH₄ to the overlying water via bioturbation. In the present study, the in situ *C.*
319 *flavicans* larvae population (9,300 ind m⁻²) was equivalent to 23 larvae added to the

322 averaged $445 \mu\text{mol l}^{-1}$. Assuming a steady state condition, *C. flavicans* bioturbation would
323 increase hypolimnetic CH_4 concentration by $352 \mu\text{mol l}^{-1}$, and an extra O_2 demand of ca. 703
324 $\mu\text{mol l}^{-1}$ (or 22 mg l^{-1} ; assuming 1 mol CH_4 : 2 mol O_2 for complete oxidation), more than
325 sufficient to deplete all hypolimnetic O_2 (100% saturation at 12.8 mg l^{-1} at 5°C). The
326 *Chaoborus*-driven O_2 demand would be even stronger when we consider other reduced
327 solutes and organic carbon re-introduced by bioturbation, plus CH_4 release and respiration
328 by the larvae themselves (Tang et al., 2017). Taken together, *C. flavicans* can greatly
329 increase the hypolimnetic O_2 demand and maintain the hypoxic/anoxic condition below the
330 thermocline, as well as enhance phosphorus loading, which may explain why artificial
331 aeration often fails to restore lake quality (Gächter and Wehrli, 1998).

332

333 4.3. A positive feedback loop on climate warming

334 It is estimated that globally lakes account for 6–16% of natural CH_4 emissions ($7\text{--}11,300$
335 $\text{mmol m}^{-2} \text{ yr}^{-1}$), driven mainly by physical processes such as ebullition and diffusion, whereas
336 the roles of biota, besides a small contribution from rooted vegetation, are largely ignored
337 (Bastviken et al. 2004). In light of our findings, it would be interesting to consider how
338 *Chaoborus* may contribute to CH_4 emission. The enhanced CH_4 flux across the sediment-
339 water interface by bioturbation certainly has immediate implications for hypolimnetic
340 oxygen demand. The added hypolimnetic CH_4 due to *Chaoborus* bioturbation would not be
341 captured by conventional flux measurements at the air-water interface, and the eventual
342 emission of this CH_4 to the atmosphere depends on how fast the lake would overturn, the

345 (Bezerra-Neto et al., 2012), which is expected to be the strongest when the larvae perturb
346 the sediment during burrowing (at dawn and at dusk). Likewise, the amount of methane
347 directly transported and released by their gas vesicles to the surface water is expected to
348 be the highest during nighttime upward migration. Both of these processes are unlikely to
349 be resolved by conventional daytime flux measurements.

350 The observed *C. flavicans* in situ population density (instars 3–4) was ca. 9,300 ind m⁻².
351 Based on the bottle incubation experiments with deep-water larvae (averaged 0.99 nmol
352 CH₄ ind⁻¹), we estimate that the amount of deep-water CH₄ transported by gas vesicles
353 would be ca. 9.2 μmol m⁻² d⁻¹. The actual amount would likely vary in time and in space as
354 the *Chaoborus* population density and activity change. For example, Tang et al. (2017)
355 measured a higher population density of 34,000 ind m⁻² for Lake Soppen in an earlier year.
356 Likewise, McGinnis et al. (2017) estimated that in some lakes, migrating *Chaoborus* larvae
357 may transport up to 2,000 mmol CH₄ m⁻² yr⁻¹ from the sediment to the overlying water. It is
358 likely that some of this methane would be lost to oxidation within the water column, and
359 only a small fraction would contribute to emission to air. In a recent study, Carey et al.
360 (2018) estimated that direct transport by migratory *Chaoborus* spp. (mainly *C. punctipennis*
361 in their study) accounted for <1 % of the diffusive CH₄ flux to air, although it should be noted
362 that they observed an order of magnitude lower population density than ours.

363 Eutrophication of inland waters is expected to persist or worsen due to growing human
364 populations and increasing agricultural activities (Tilman et al., 2001; Smith, 2003;
365 McCrackin et al., 2017). Compounding the problem, global warming will exacerbate

368 deoxygenation of the bottom layer (Jeppesen et al., 2009; Moss et al., 2011). These
369 conditions are conducive to sediment methanogenesis and favor range expansion and
370 proliferation of *Chaoborus* (Teraguchi, 1975; Taylor et al., 2016). As shown in the present
371 study, bioturbation by *Chaoborus* larvae would significantly increase diffusivity at the
372 sediment-water interface and re-introduce sequestered nutrients, organic carbon and CH₄
373 to the overlying water. Concurrently, the migrating larvae enhance the direct transport of
374 sediment CH₄ to the surface water for water-air gaseous exchange. By linking the nutrient
375 and CH₄ dynamics in eutrophic lakes, *Chaoborus* can thereby play a unique role in driving a
376 positive feedback loop between lake eutrophication, CH₄ transport and climate warming in
377 the coming decades.

378

379 4.4. *The Chaoborus curse in lake restoration?*

380 The long history of eutrophication has led to a host of public health concerns such as
381 nuisance algal blooms, water quality deterioration and wildlife die-off, causing substantial
382 economic damages (Pretty et al., 2003; Dodds et al., 2008). Studies in eutrophic coastal
383 systems have shown that nutrient reduction often fails to revert the systems to the pre-
384 eutrophication state (Duarte et al., 2009), at least in the short term. Similarly, aggressive
385 lake management and restoration strategies have a checkered record of success (Gächter
386 and Wehrli, 1998; McCrackin et al., 2017). These observations suggest that the systems may
387 have passed the ecological tipping point such that internal forcing tends to maintain the
388 new status quo despite external interventions such as reducing nutrient runoff and artificial

391 positive feedback loop to sustain or even intensify eutrophication and deoxygenation,
392 hindering the system's recovery. We therefore argue that remediation measures will remain
393 limited until sediment CH₄ concentrations drop, and *Chaoborus* abundance is greatly
394 reduced. Understanding this internal *Chaoborus* feedback mechanism may allow resource
395 managers to devise more effective lake restoration strategies.

396

397 **5. Conclusion**

398 The phantom midge larvae (*Chaoborus* spp.) are prevalent in eutrophic lakes. By virtue of
399 their diurnal migration in and out of the sediment, they significantly perturb the sediment
400 and reintroduce sequestered nutrients and pollutants to the overlying water, essentially
401 maintaining eutrophic lakes in the status quo despite external efforts to curb nutrient inputs
402 and re-oxygenate the water column. Understanding this *Chaoborus*-driven positive feedback
403 mechanism may prove critical in effective lake restoration and in predicting lake ecosystem
404 response to intensifying eutrophication and climate change.

405

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505 (C) Dissolved CH₄; (D) δ¹³C of CH₄; all measured on 13th June, 2017; (E) Total phosphorus,
506 measured on 22nd May, 2017.

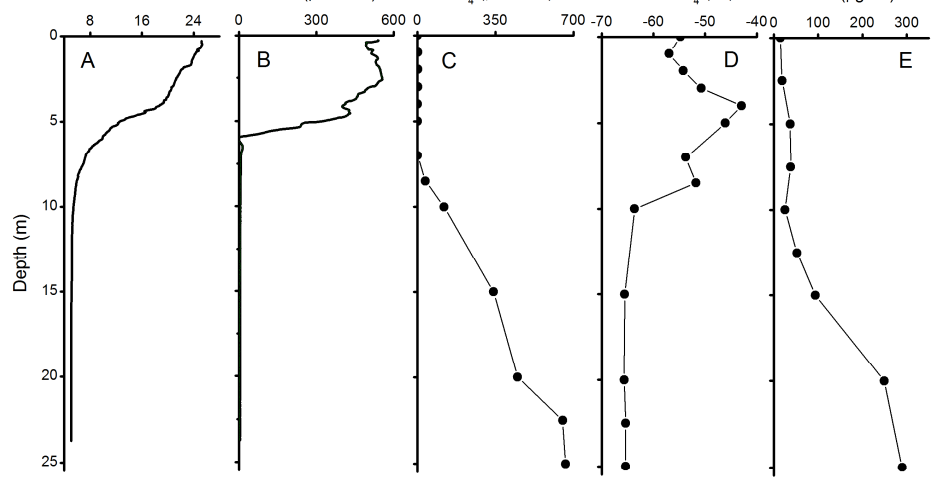
507 **Fig. 2.** Final total phosphorus in the water as a function of number of *C. flavicans* larvae in
508 the sediment incubation experiment. The lower x-axis shows the equivalent areal density of
509 the larvae in situ.

510 **Fig. 3.** Water chemistry as a function of number of *C. flavicans* larvae in the sediment
511 incubation experiment. (A) dissolved CH₄; (B) δ¹³C-CH₄; (C) dissolved pCO₂. Outliers
512 indicated by arrow and open square were excluded from regression analysis.

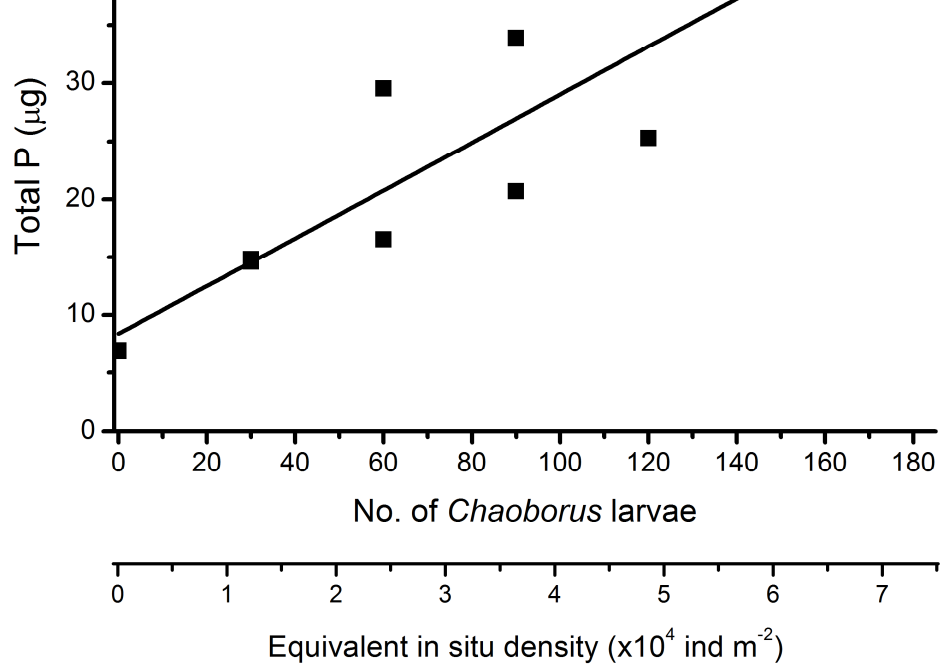
513 **Fig. 4.** Percent distribution of *Chaoborus flavicans* larvae (instars 3–4) in the different strata
514 in Lake Soppen at different times on 13th–14th June and 21st–22nd June, 2017.

515 **Fig. 5.** CH₄ released from gas vesicles of deep- and shallow-water *C. flavicans* larvae (instars
516 3–4). (A) Total CH₄ as a function of number of *C. flavicans* larvae for deep-water (13th June
517 and 22nd June) and shallow-water (22nd June) samples. (B) δ¹³C-CH₄ of the corresponding
518 samples.

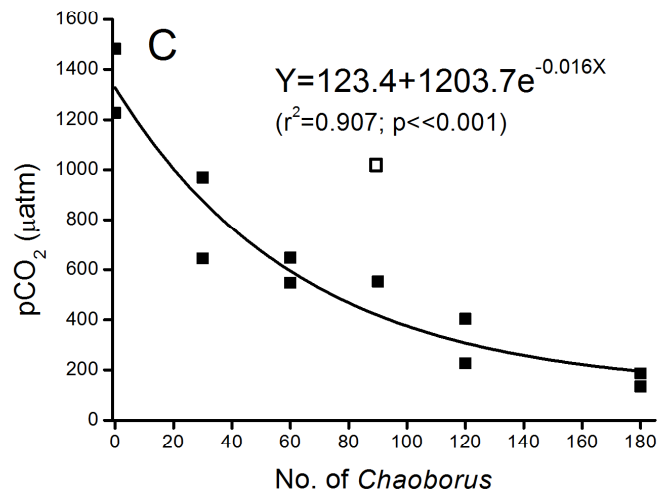
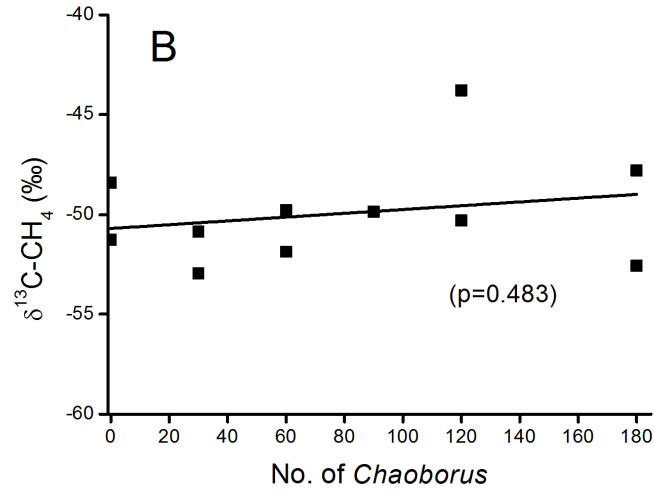
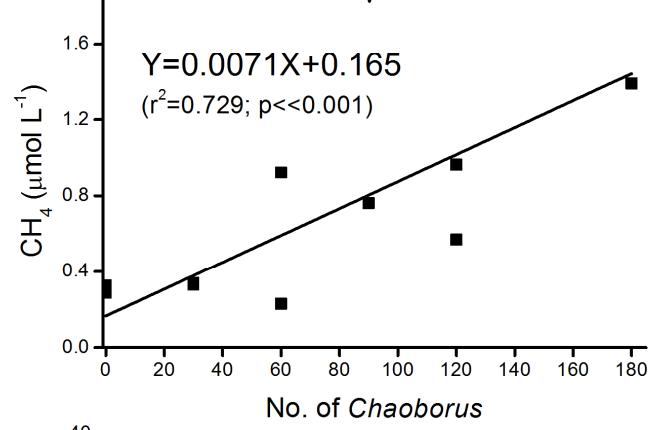
519 **Fig. 6.** System modelling results expressed as relative increase against the control, as a
520 function of number of *C. flavicans* larvae. ● = observed TP increase due to bioturbation; ▲ =
521 observed CH₄ increase; ■ = predicted CH₄ increase due to bioturbation; ◆ = predicted total
522 CH₄ increase (bioturbation + gas vesicle transport). Error analysis of the model is explained
523 in supplementary materials. Insert: Fraction of total CH₄ contributed by *C. flavicans* gas
524 vesicles as a function of number of larvae.

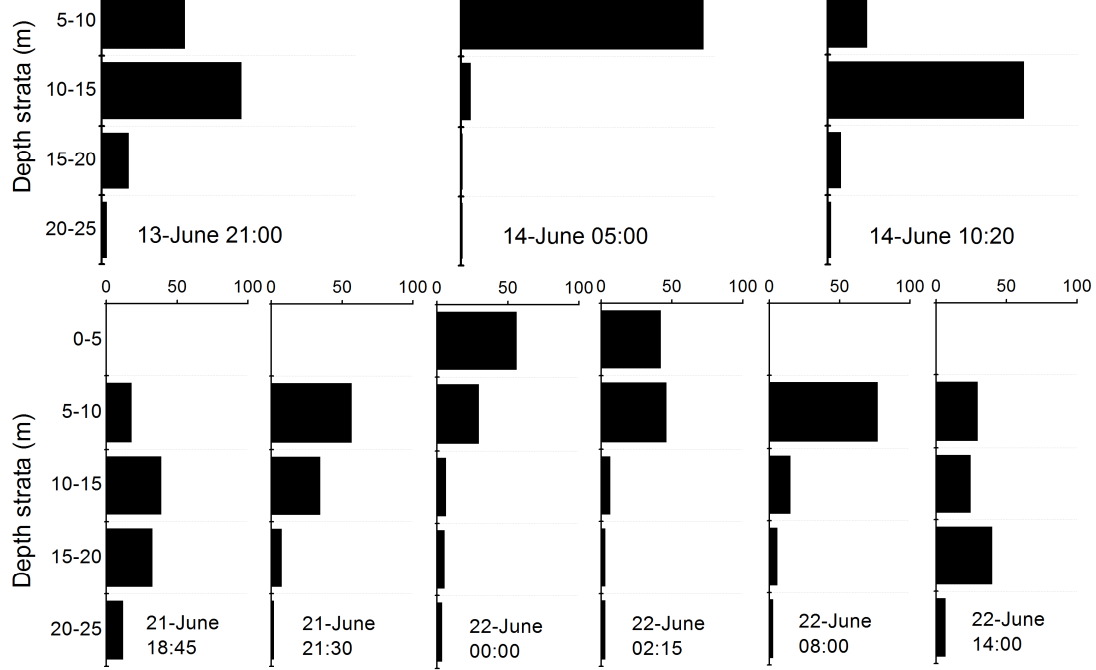


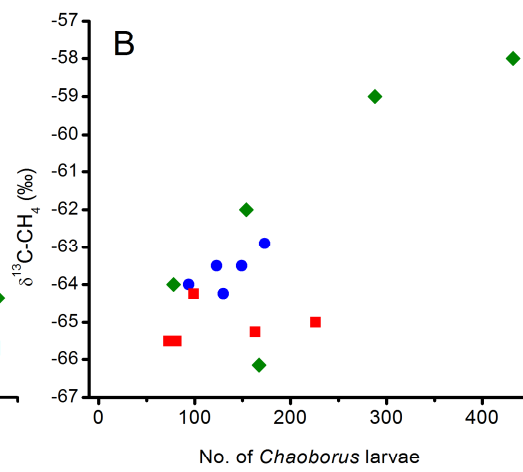
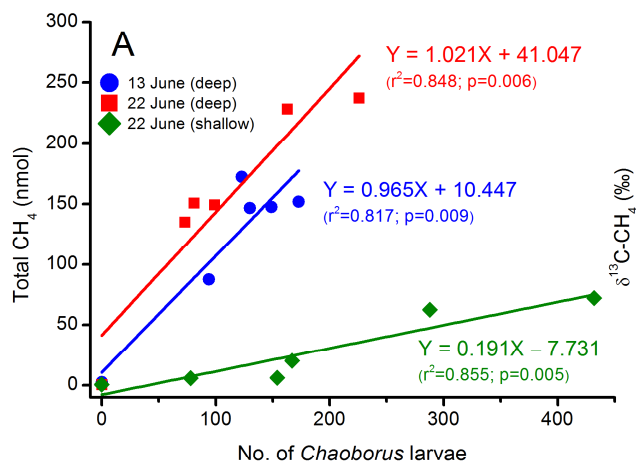
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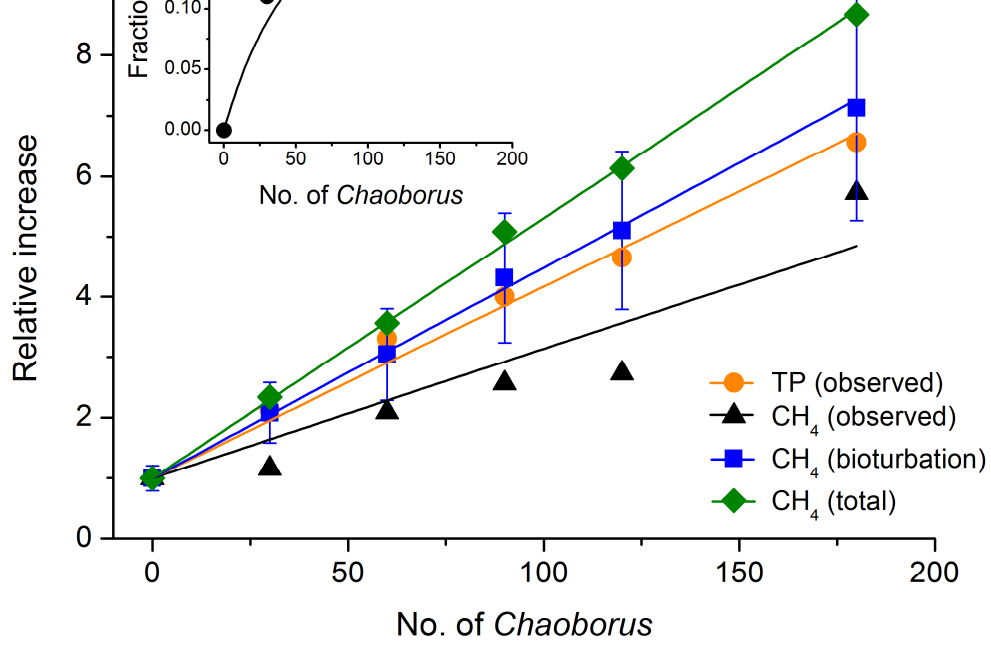
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