Swansea University
Prifysgol Abertawe

Cronfa
Setting Research Free

Cronfa - Swansea University Open Access Repository

_____

This is an author produced version of a paper published in:
Water Research

_____

Cronfa URL for this paper:
http://cronfa.swan.ac.uk/Record/cronfa39194

_____

Paper:

_____

http://www.swansea.ac.uk/library/researchsupport/ris-support/

Accepted Manuscript

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

Kam W. Tang, Sabine Flury, Dominic Vachon, César Ordóñez, Daniel F. McGinnis
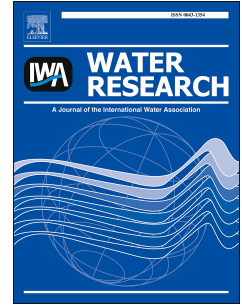
Please cite this article as: Tang, K.W., Flury, S., Vachon, D., Ordóñez, Cé., McGinnis, D.F., The phantom midge menace: Migratory *Chaoborus* larvae maintain poor ecosystem state in eutrophic inland waters, *Water Research* (2018), doi: 10.1016/j.watres.2018.03.060.

Depth

Water surface

Diurnal migration

CH$_4$

P, CH$_4$ O$_2$

Anoxic
sediment

Bioturbation
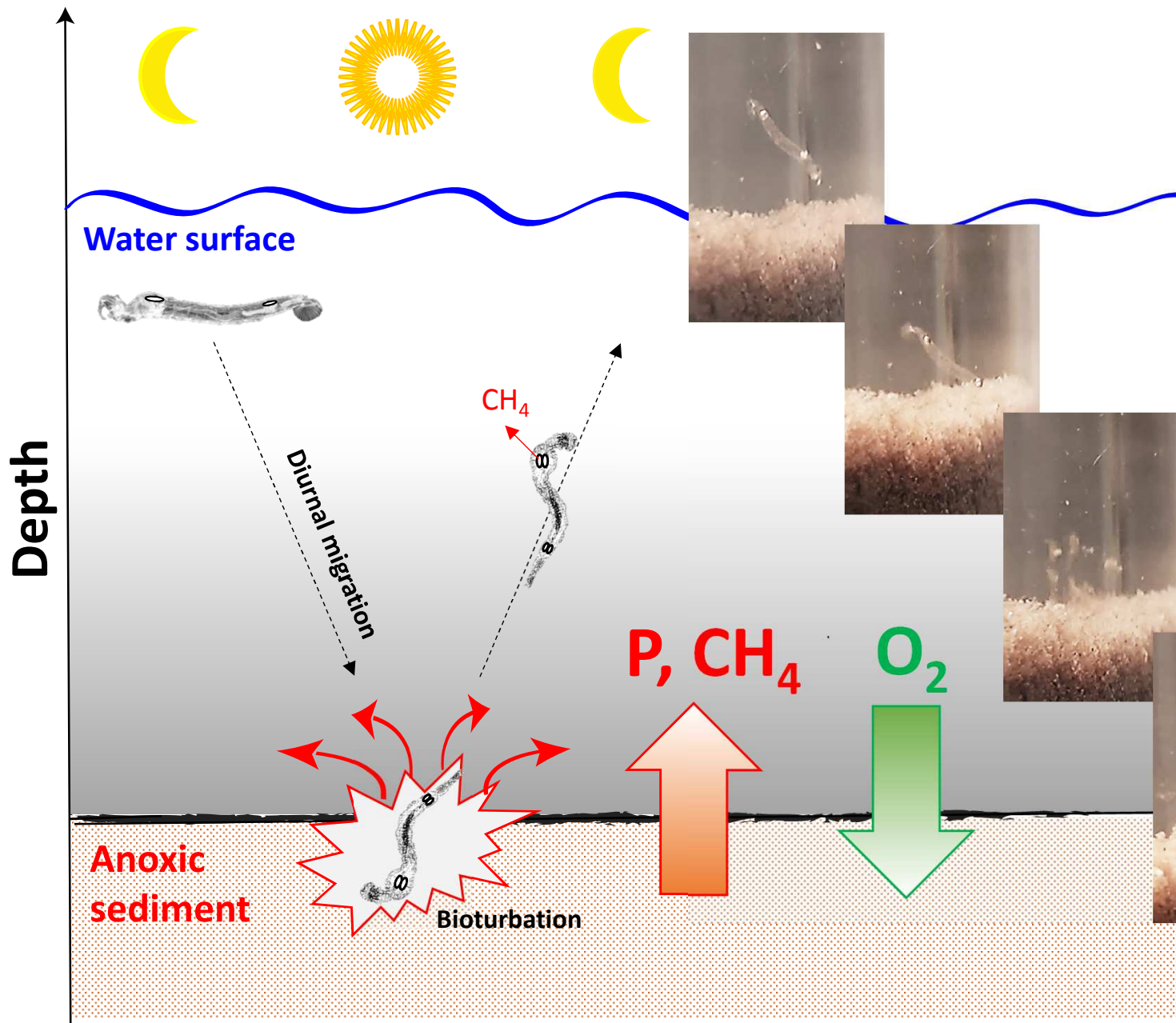
3   eutrophic inland waters

4

5   **Authors**

6   Kam W. Tang[a,*], Sabine Flury[b], Dominic Vachon[c], César Ordóñez[c], Daniel F. McGinnis[c,*]

7

8   **Author Affiliation**

9   a. Department of Biosciences, Swansea University, Swansea, SA2 8PP, United Kingdom

10   b. Stream Biofilm and Ecosystem Research Laboratory, Institute of Environmental

11   Engineering, School of Architecture, Civil and Environmental Engineering, Ecole

12   Polytechnique Fédérale de Lausanne, Station 2, 1015 Lausanne, Switzerland.

13   c. Aquatic Physics Group, Department F.-A. Forel for Environmental and Aquatic

14   Sciences (DEFSE), Faculty of Science, University of Geneva, Uni Carl Vogt, 66

15   boulevard Carl-Vogt, 1211 Geneva, Switzerland

16

17   ***Corresponding Authors**

18   Kam W. Tang

19   Department of Biosciences, Swansea University, Swansea, SA2 8PP, United Kingdom

20   Email: k.w.tang@swansea.ac.uk

21   Daniel F. McGinnis

24   Switzerland

25   Email: daniel.mcginnis@unige.ch

26

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 μg P ind^{-1} 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   ind^{-1} 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**

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 planktivorus 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 CH_4 (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 (CH_4) (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 CH_4 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 km^2, 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 CH_4 in the porewater and

102 releasing it in the water column, migrating *Chaoborus* larvae are expected to accelerate the

103 upward CH_4 flux over passive diffusion and affect the ambient CH_4-carbon isotope

104 composition in the epilimnion. We therefore also investigated how *C. flavicans* larvae affect

105 CH_4 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 CO_2 and CH_4

132　concentrations and $\delta^{13}C$-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 ~10 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 ($K_2S_2O_8$) 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 (Supel[TM] Inert Multi-Layer Foil Gas Sampling Bags)

150   by slowly injecting lake water into the bottom of the bottle through a rubber tubing. CO_2

151   and CH_4 concentrations and $\delta^{13}C$-CH_4 of the gas bag content were measured within one day

152   on a Cavity Ringdown Spectrometer (Piccaro G2201-i). Initial concentrations of CO_2 and CH_4

153   in the sampled water was calculated accounting for initial headspace CH_4 and CO_2

154   concentrations (before equilibrium assuming atmospheric concentration of 2 and 400 ppmv

155   for CH_4 and CO_2, 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.

168

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 CH_4 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 CH_4 from *C. flavicans* gas

182   vesicles. Afterward, Synthetic Air (Carbagas: 80% N_2, 20% O_2; ± 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 CH_4 concentration and carbon isotopic signature

187   of CH_4. Total CH_4 was calculated by accounting for dissolved CH_4 in the displaced headspace

188   water. Afterward, the numbers of larvae in the serum bottles were counted.

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

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 $$\frac{\partial C_{CH4}}{\partial t}V = F_{sed}A - F_{sur}A + R_{chab}N_{chab} - R_{Ox}C_{CH4}V \qquad \text{Eqn. 1}$$

202 where C_{CH4} 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. $\frac{\partial C_{CH4}}{\partial t} = 0$;

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 CH_4 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 δ^{13}C-CH_4 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 CH_4), 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

11

233   Total phosphorus in the overlaying 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 µg P ind^{-1} over the course of the experiment (9.5

236   d), or 0.022 µg P ind^{-1} 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, δ^{13}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 ± 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

255

256   *3.4. Methane release from Chaoborus gas vesicles*

257   Bottle incubation experiments to measure the release of CH_4 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 CH_4 released was linearly correlated with

260   number of larvae; i.e. the amount of CH_4 released per individual was quite constant within

261   each trial (Fig. 5A). The amounts of CH_4 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 CH_4 to the surrounding water

264   before capture, as expected, the amount of CH_4 was much lower for them, at 0.19 nmol ind^{-1}

265   ^{1}. Both trials with deep-water larvae gave similar $\delta^{13}C$-CH_4 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 CH_4 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 CH_4 concentrations. Average CH_4 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 CH_4 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 ≤ 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 µg l^{-1}, almost identical to

292   the in situ surface water value (14.9 µg l^{-1}; Fig. 1). In contrast, TP increased at a rate of 0.022

293   µg P ind^{-1} 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,

14

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 CH_4 data and system modelling. As expected, this extra nutrient stimulated primary

303   production and CO_2 drawdown in the experiment, as indicated by the observed pCO_2 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 CH_4 oxidation as a result of enhanced introduction

318   of sediment CH_4 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

15

322 averaged 445 μmol l⁻¹. Assuming a steady state condition, *C. flavicans* bioturbation would

323 increase hypolimnetic CH_4 concentration by 352 μmol l⁻¹, and an extra O_2 demand of ca. 703

324 μmol 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 (7–11,300

335 mmol m⁻² 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 hypolimnic 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

16

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 CH_4 ind^{-1}), we estimate that the amount of deep-water CH_4 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 CH_4 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 CH_4 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 inducive 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 CH_4

373 to the overlying water. Concurrently, the migrating larvae enhance the direct transport of

374 sediment CH_4 to the surface water for water-air gaseous exchange. By linking the nutrient

375 and CH_4 dynamics in eutrophic lakes, *Chaoborus* can thereby play a unique role in driving a

376 positive feedback loop between lake eutrophication, CH_4 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

18

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 CH_4 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

414 KWT, SF and DFM conceived the idea; KWT, SF, DV, CO and DFM performed the study; KWT

415 and DFM analyzed the data; KWT and DFM wrote the manuscript with input from

416 coauthors.

417

420   Dependence of lake characteristics, two regional assessments, and a global

421   estimate. Global Biogeochem. Cycles 18 (4), GB4009.

422 Bezerra-Neto, J.F., Brighenti, L.S., Teixeira de Mello, N.A.S., Pinto-Coelho, R.M., 2012.

423   Hydroacoustic assessment of fish and *Chaoborus* (Diptera-Chaoboridae) distribution in

424   three Neotrophical lakes. Acta Limnol. Brasiliensia 24 (1), 18–28.

425 Bryant, L.D., Lorrai, C., McGinnis, D.F., Brand, A., Little, J.C., 2010. Variable sediment oxygen

426   uptake in response to dynamic forcing. Limnol. Oceanogr. 55 (2), 950–964.

427 Carey, C.C., McClure, R.P., Doubek, J.P., Lofton, M.E., Ward, N.K., Scott, D., 2018. *Chaoborus*

428   spp. transport CH_4 from the sediments to the surface waters of a eutrophic reservoir,

429   but their contribution to water column CH4 concentrations and diffusive efflux is minor.

430   Environ. Sci. Technol. 52 (3), 1165–1173.

431 Cole, J. J., Carpenter, S. R., Pace, M. L., Van de Bogert, M. C., Kitchell, J. L., Hodgson, J. R.,

432   2006. Differential support of lake food webs by three types of terrestrial organic

433   carbon. Ecol. Lett. 9 (5), 558–568.

434 Dawidowicz, P., Pijanowska, J., Ciechomski, K., 1990. Vertical migration of *Chaoborus* larvae

435   is induced by the presence of fish. Limnol. Oceanogr. 35 (7), 1631–1637.

436 Dodds, W.K., Bouska, W.W., Eitzmann, J.L., Pilger, T.J., Pitts, K.L., Riley, A.J., Schloesser, J.T.,

437   Thornbrugh, D.J., 2008. Eutrophication of US freshwaters: analysis of potential

438   economic damages. Environ. Sci. Technol. 43 (1), 12–19.

439 Donis, D., Flury, S., Stöckli, A., Spangenberg, J.W., Vachon, D., McGinnis, D.F., 2017. Full-

440   scale evaluation of methane production under oxic conditions in a mesotrophic lake.

441   Nat Comm. 8 (1), 1661.

444   29–36.

445   Gächter, R., Wehrli, B., 1998. Ten years of artificial mixing and oxygenation: No effect on

446   internal phosphorus loading of two eutrophic lakes. Environ. Sci. Technol. 32 (23),

447   3659–3665.

448   Gosselin, A., Hare, L., 2003. Burrowing behavior of *Chaoborus flavicans* larvae and its

449   ecological significance. J. North Amer. Benthol. Soc. 22 (4), 575–581.

450   Hajdas-Skowronek, I., 1993. Extension of the radiocarbon calibration curve by AMS dating of

451   laminated sediments of Lake Soppensee and Lake Holzmaar. PhD thesis, Swiss Federal

452   Institute of Technology Zurich.

453   Hecky, R.E., 1984. African lakes and their trophic efficiencies: A temporal perspective. In:

454   Meyers, Strickler, J.R. (Eds.) Trophic interactions within aquatic ecosystem. AAAS,

455   Rome, pp. 405–448.

456   Ibelings, B.W., Fastner, J., Bormans, M., Visser, P.M., 2016. Cyanobacterial blooms. Ecology,

457   prevention, mitigation and control. Aquat. Ecol. 50 (3), 327–331.

458   Jeppesen, E., Kronvang, B., Meerhoff, M., Søndergaard, M., Hansen, K.M., Andersen, H.E.,

459   Lauridsen, T.L., Liboriussen, L., Beklioglu, M., Özen, A., Olesen, J.E., 2009. Climate

460   change effects on runoff, catchment phosphorus loading and lake ecological state, and

461   potential adaptations. J. Environ. Quality 38 (5), 1930–1941.

462   Lewis, W.M.,Jr., 1996. Tropical lakes: how latitude makes a difference. In: Schiemer, F.,

463   Boland, K.T. (Eds.) Perspectives in tropical limnology. SPB Academic Publishing,

464   Amsterdam, pp. 43–64.

467   McCrackin, M.L., Jones, H.P., Jones, P.C., Moreno-Mateos, D., 2017. Recovery of lakes and

468   coastal marine ecosystems from eutrophication: A global meta-analysis. Limnol.

469   Oceanogr. 62 (2), 507–518.

470   McGinnis, D.F., Flury, S., Tang, K.W., Grossart, H.-P., 2017. Porewater methane transport

471   within the gas vesicles of diurnally migrating *Chaoborus spp.*: An energetic advantage.

472   Scientific Reports 7, 44478.

473   Moss, B., Kosten, S., Meerhoff, M., Battarbee, R.W., Jeppesen, E., Mazzeo, N., Havens, K.,

474   Lacerot, G., Liu, Z., de Meester, L., Paerl, H., Scheffer, M., 2011. Allied attack: climate

475   change and eutrophication. Inland Waters 1 (2), 101–105.

476   Pretty, J.N., Mason, C.F., Nedwell, D.B., Hine, R.E., Leaf, S., Dils, R., 2003. Environmental

477   costs of freshwater eutrophication in England and Wales. Environ. Sci. Technol. 37 (2),

478   201–208.

479   Quinlan, R., Smol, J.P., 2010. Use of subfossil *Chaoborus* mandibles in models for inferring

480   past hypolimnetic oxygen. J. Paleolimnol. 44 (1), 43–50.

481   Schindler, D.W., 2006. Recent advances in the understanding and management of

482   eutrophication. Limnol. Oceanogr. 51 (1), 356–363.

483   Smith, V.H., 2003. Eutrophication of freshwater and coastal marine ecosystems a global

484   problem. Environ. Sci. Pollution Res. 10 (2), 126–139.

485   Smith, V.H., Joye, S.B., Howarth, R.W., 2006. Eutrophication of freshwater and marine

486   ecosystems. Limnol. Oceanogr. 51 (1), 351–355.

487   Sweetman, J.N., Smol, J.P., 2006. Reconstructing fish populations using *Chaoborus* (Diptera:

488   Chaoboridae) remains- a review. Quat. Sci. Rev. 25 (15), 2013–2023.

491   loading in lakes. Water Res. 122, 36–41.

492   Taylor, D. J., Ballinger, M. J., Medeiros, A. S., Kotov, A. A., 2016. Climate-associated tundra

493   thaw pond formation and range expansion of boreal zooplankton predators. Ecography

494   39 (1), 43–53.

495   Teraguchi, S., 1975. Correction of negative buoyancy in phantom larva. *Chaoborus*

496   *americanus*. J. Insect Physiol. 21 (10), 1659–1670.

497   Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D.,

498   Schlesinger, W.H., Simberloff, D., Swackhamer, D., 2001. Forecasting agriculturally

499   driven global environmental change. Science 292 (5515): 281–284.

500   Vanni, M. J., Layne, C. D., Arnott, S. E., 1997. "Top-down" trophic interactions in lakes:

501   effects of fish on nutrient dynamics. Ecology, 78 (1), 1-20.

502

505 (C) Dissolved CH_4; (D) $\delta^{13}C$ of CH_4; all measured on 13th June, 2017; (E) Total phosphorus,
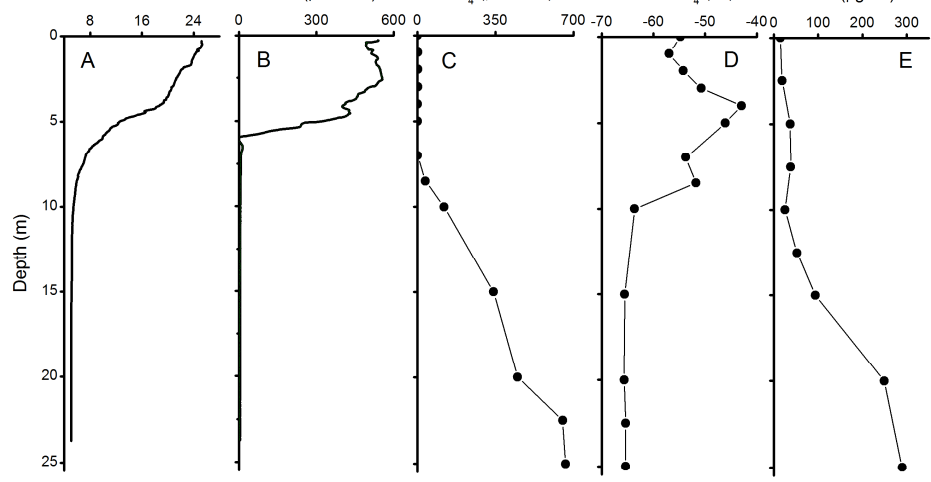
506 measured on 22nd May, 2017.

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 CH_4; (B) $\delta^{13}C$-CH_4; (C) dissolved pCO_2. Outliers

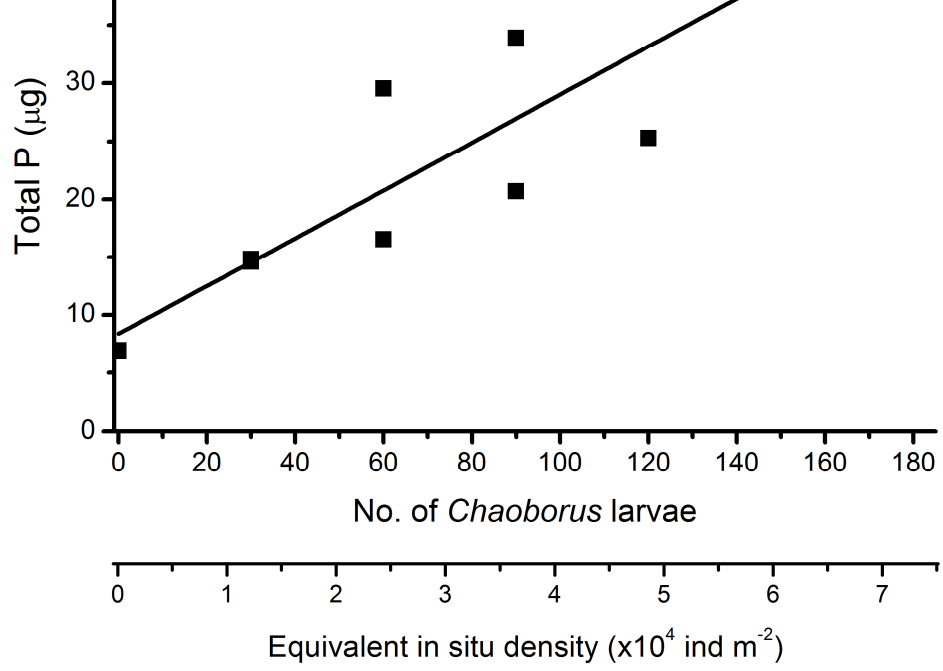512 indicated by arrow and open square were excluded from regression analysis.

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.** CH_4 released from gas vesicles of deep- and shallow-water *C. flavicans* larvae (instars

516 3–4). (A) Total CH_4 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) $\delta^{13}C$-CH_4 of the corresponding
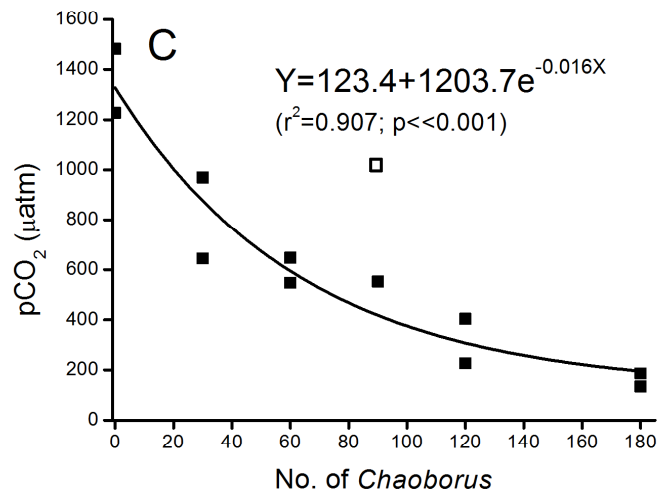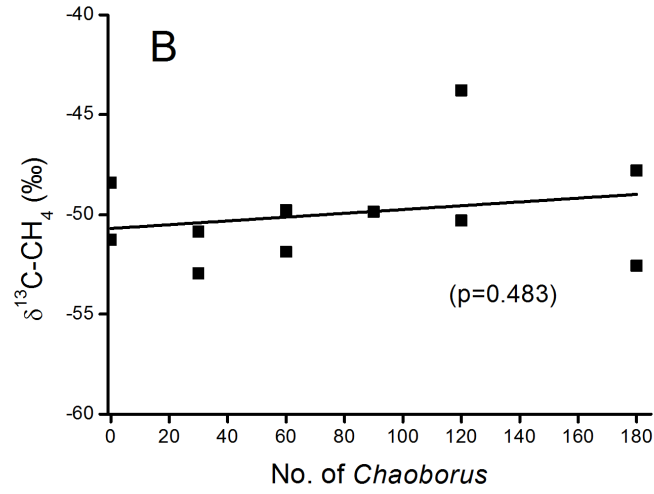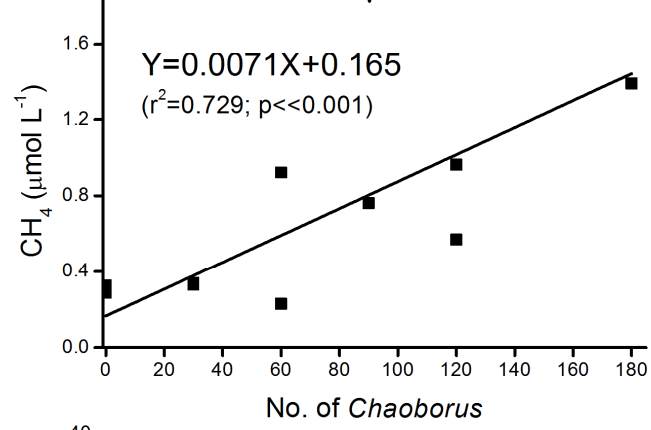
518 samples.

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 CH_4 increase; ■ = predicted CH_4 increase due to bioturbation; ♦ = predicted total

522 CH_4 increase (bioturbation + gas vesicle transport). Error analysis of the model is explained

523 in supplementary materials. Insert: Fraction of total CH_4 contributed by *C. flavicans* gas

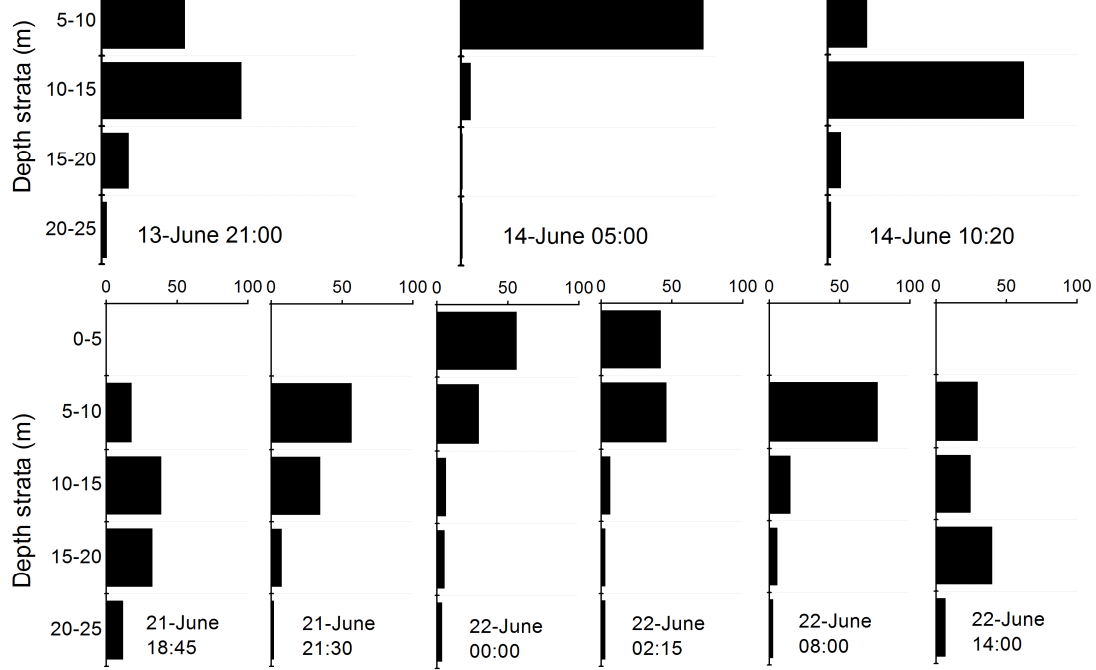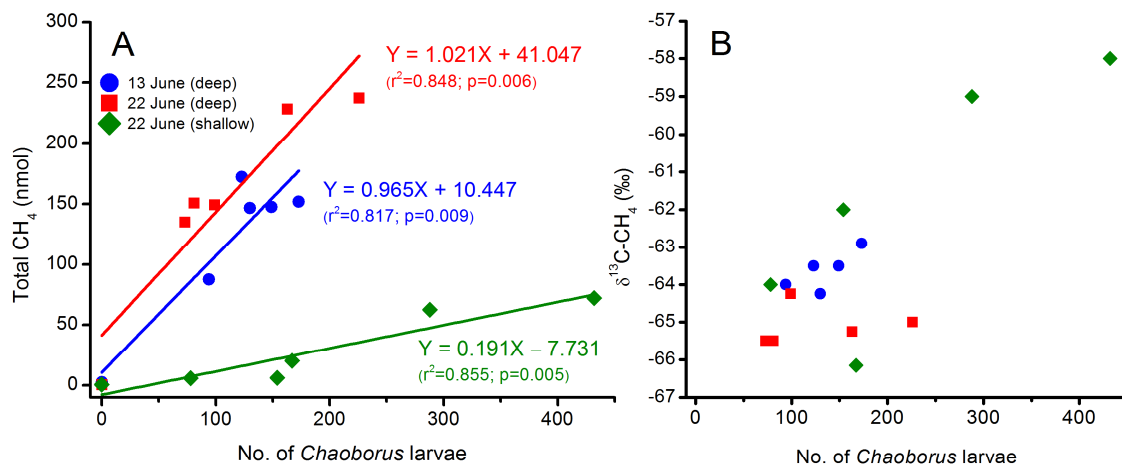524 vesicles as a function of number of larvae.

Total P (µg)

No. of *Chaoborus* larvae

Equivalent in situ density (x10^4 ind m^{-2})

$Y=0.0071X+0.165$
$(r^2=0.729; p<<0.001)$

CH$_4$ (μmol L^{-1})

No. of *Chaoborus*

B

δ^{13}C-CH$_4$ (‰)

$(p=0.483)$

No. of *Chaoborus*

C

$Y=123.4+1203.7e^{-0.016X}$
$(r^2=0.907; p<<0.001)$

pCO$_2$ (μatm)

No. of *Chaoborus*

A

13 June (deep)
22 June (deep)
22 June (shallow)

$Y = 1.021X + 41.047$
($r^2=0.848$; $p=0.006$)

$Y = 0.965X + 10.447$
($r^2=0.817$; $p=0.009$)

$Y = 0.191X - 7.731$
($r^2=0.855$; $p=0.005$)

Total CH_4 (nmol)

No. of *Chaoborus* larvae

B

$\delta^{13}C\text{-}CH_4$ (‰)

No. of *Chaoborus* larvae