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

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

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Abstract

Chaoborus spp. (phantom midge) are prevalent in eutrophic inland waters. In Lake Soppen, Switzerland, C. flavicans larvae diurnally migrate between the methane-rich, oxygen-depleted hypolimnion and sediments, and the methane-poor, oxygen-rich epilimnion. Using a combination of experiments and system modelling, this study demonstrated that the larvae’s burrowing activities in and out of the sediment perturbed the sediment and re-introduced sequestered phosphorus into the overlying water at a rate of 0.022 µg P ind$^{-1}$ d$^{-1}$, thereby exacerbating internal nutrient loading in the water column. Fluxes of sediment methane and other reduced solutes enhanced by the larval bioturbation would consume oxygen and sustain the hypoxic/anoxic condition below the thermocline. In addition to increasing diffusive fluxes, migrating larvae also directly transported methane in their gas vesicles from the deep water and release it in the surface water at a rate of 0.99 nmol CH$_4$ ind$^{-1}$ d$^{-1}$, potentially contributing to methane emission to air. As nutrient pollution and climate warming persist or worsen in the coming decades, proliferation of Chaoborus could intensify this positive feedback loop and delay lake recovery.

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

**Keywords**

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

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

The instars of *Chaoborus* larvae can exchange gases, including CH$_4$ (McGinnis et al., 2017; Carey et al., 2018) between their gas vesicles and ambient water, thereby adjusting...
the gas vesicle volume (Teraguchi, 1975) and gaining considerable energetic advantage through buoyancy for vertical migration (McGinnis et al., 2017). In eutrophic waters and sediments, the gas typically abundant for this purpose (high partial pressure, low solubility) is methane (CH$_4$) (McGinnis et al., 2017).

Paleolimnological studies have shown that the appearance of Chaoborus remains (e.g. mandibles) in sediments often coincided with a major shift in the lake’s trophic state (Sweetman and Smol, 2006; Quinlan and Smol, 2010), but the direct link and feedback between Chaoborus colonization and lake eutrophication history remain unclear. To our knowledge, Gosselin and Hare (2003) were the first to observe in the laboratory the bioturbation effects of individual Chaoborus larvae as they burrow in and out of the sediment. Based on subsequent experimental and modelling studies, it is hypothesized that this bioturbation activity would release sequestered nutrients and CH$_4$ into the overlying water at an enhanced rate, thereby pushing the system over an ecological tipping point where the eutrophic state may self-sustain indefinitely (McGinnis et al., 2017; Tang et al., 2017). This hypothesis has not been empirically tested, but would have important implications for lake ecology and management. This study therefore aimed at demonstrating the population-level effect that migratory Chaoborus larvae have on nutrient and methane dynamics in lakes.

Lake Soppen (47°5′25″N, 8°4′51″E) is a small eutrophic kettle lake (area 0.26 km$^2$, max depth 27 m, mean depth 12 m) in the Canton of Lucerne, Switzerland. Paleolimnological evidence suggests a drastic increase in total phosphorus level in the last decades (Lotter, 2001). Strong bioturbation by C. flavicans has caused the destruction of calcareous laminations in the last century in Lake Soppen sediments despite the high calcite deposition
nutrient loading in Lake Soppen. In addition, by taking up CH$_4$ in the porewater and releasing it in the water column, migrating *Chaoborus* larvae are expected to accelerate the upward CH$_4$ flux over passive diffusion and affect the ambient CH$_4$-carbon isotope composition in the epilimnion. We therefore also investigated how *C. flavicans* larvae affect CH$_4$ transport and isotope composition in Lake Soppen. Taken together, this study provides novel insights into how migratory *Chaoborus* larvae could drive a positive feedback loop between eutrophication, methane transport and deoxygenation in inland waters.

2. Material and Methods

2.1. Sediment incubation experiment

Sediment was collected from Lake Soppen by a gravity corer. On shore, the sediment was thoroughly mixed with an electric drill-mixer to standardize the initial conditions among all replicates. Incubations were performed in 12 plexiglass cylinders (diameter 5.6 cm, length 29 cm) with bottom caps. The caps were used to measure out ca. 73 ml of the mixed sediment (ca. 4 cm thick); the cylinders were then pushed into the sediment-containing caps. Surface lake water was gently siphoned into each cylinder, letting the water overflow to replace ca. 3 times its volume to standardize the initial water condition among the replicates. The final volume of the overlying water was ca. 0.5 l. *C. flavicans* larvae (instar 3–4) were collected from ca. 20 m depth, rinsed with lake water and sorted into cups in different numbers (0, 30, 60, 90, 120 and 180, in duplicates), equivalent to an in situ areal density of 0 – 73,000 ind m$^{-2}$. Each cup was randomly assigned to a cylinder and the larvae
cylinders remained open and exposed to the natural day light (Fig. S1).

The incubation lasted from 12th June night until 22nd June 2017 morning (9.5 d total). On the last day, the cylinders were removed one at a time in random order for processing. First, we measured the water temperature and dissolved oxygen (HACH® Portable Multi Meter, model HQ40D); the color appearance of the water was recorded. Afterward, 50 ml of the water was taken from the top with a syringe, and stored refrigerated in an opaque plastic bottle for total phosphorus (TP) (see below). 300–400 ml of the remaining water was gently siphoned, without disturbing the sediment, into a glass bottle to measure CO₂ and CH₄ concentrations and δ¹³C-CH₄ (see below).

2.2. Water column measurements, total phosphorus and dissolved gases

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

Lake water was sampled with a Niskin bottle (5 l) at the deepest point on 22nd May for total phosphorus (TP) and on 13th June 2017 for water column dissolved gases. For TP, water samples were preserved in the cold until measurements. TP was measured spectrophotometrically after potassium persulfate (K₂S₂O₈) digestion (45 minutes at 130°C). The same method was used to measure TP in the sediment incubation experiment.
(Donis et al. 2017). About half of the water was replaced by atmospheric air. The bottle was immediately capped to create a headspace, then shaken vigorously for at least 2 minutes to equilibrate the dissolved gases with the headspace. Afterward, the headspace was extracted through a top valve into a gas-sample bag (Supel™ Inert Multi-Layer Foil Gas Sampling Bags) by slowly injecting lake water into the bottom of the bottle through a rubber tubing. CO$_2$ and CH$_4$ concentrations and $\delta^{13}$C-CH$_4$ of the gas bag content were measured within one day on a Cavity Ringdown Spectrometer (Piccaro G2201-i). Initial concentrations of CO$_2$ and CH$_4$ in the sampled water was calculated accounting for initial headspace CH$_4$ and CO$_2$ concentrations (before equilibrium assuming atmospheric concentration of 2 and 400 ppmv for CH$_4$ and CO$_2$, respectively), volume ratio (i.e. headspace:water), air and water temperatures, in situ barometric pressure and lake water total alkalinity. The same method was used to measure dissolved gases in the sediment incubation experiment.

2.3. Day-night sampling of Chaoborus

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

Surface lake water was aerated for several hours using an aquarium-type air pump to equilibrate its background CH$_4$ with ambient air. The aerated water was used for washing and for the blank. *C. flavicans* larvae were collected from depths where they were most abundant at the time of the experiment (15–20 m in day; 0–5 m at night). The experiments were done three times (13$^{th}$ June at 16:30, 22$^{nd}$ June at 02:40, 22$^{nd}$ June at 14:30). For the first experiment, the larvae were brought back to shore, rinsed with the aerated lake water, and unknown numbers were added to 120-ml serum bottles. For the latter experiments, the larvae were immediately concentrated on a 1-mm mesh on the boat, briefly rinsed with the aerated lake water, and immediately added to the serum bottles. All serum bottles were topped off with the aerated lake water and crimp sealed. Bottles with only aerated lake water were used as the blank.

The sealed bottles were left overnight to allow the release of CH$_4$ from *C. flavicans* gas vesicles. Afterward, Synthetic Air (Carbagas: 80% N$_2$, 20% O$_2$; ± 1%) was injected to create headspace (ca. 50 ml). The bottles were shaken vigorously for ca. 2 min. to equilibrate headspace and dissolved gases. The headspace gas was then displaced into a gas-tight syringe by slowly injecting lake water into the bottle. The gas was then injected immediately into the Picarro spectrometer to measure CH$_4$ concentration and carbon isotopic signature of CH$_4$. Total CH$_4$ was calculated by accounting for dissolved CH$_4$ in the displaced headspace water. Afterward, the numbers of larvae in the serum bottles were counted.
Stable carbon isotope data are reported in delta notation (δ ‰) relative to VPDB (Vienna Pee Dee Belemnite) following the equation:

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

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

2.5. System modelling of methane and oxygen dynamics

In the sediment incubation experiment, \( C. \ flavicans \) bioturbation would increase sediment-water exchange of not only nutrients but also dissolved gases including CH4. To examine the effect of bioturbation on CH4 dynamics in the experiment, we apply a system modelling approach by accounting for input (bioturbation, \( F_{sed} \) plus gas vesicle transport, \( R_{chab} \)) and output (oxidation, \( R_{ox} \) plus emission to air, \( F_{sur} \)) of CH4. The mass balance is expressed as

\[
\frac{\partial C_{CH4}}{\partial t} V = F_{sed} A - F_{sur} A + R_{chab} N_{chab} - R_{ox} C_{CH4} V \quad \text{Eqn. 1}
\]

where \( C_{CH4} \) is the dissolved methane concentration, \( V \) is volume of water in the cylinder, \( t \) is time, \( A \) is the cross-sectional area of the cylinder. Measurements of CH4 oxidation rate and Fickian diffusion coefficient (\( k_{600} \)) are explained in the supplementary materials. Average CH4 transport by \( C. \ flavicans \) gas vesicles was taken from the bottle incubation experiments (section 2.4).

Assuming the system was at steady state at the end of the experiment (i.e. \( \frac{\partial C_{CH4}}{\partial t} = 0; \text{ input = output} \)), we then derive the relative CH4 increase against the control due to \( C. \)
3. Results

3.1. Water column chemistry

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

3.2. Chaoborus effects on sediment-water exchange

To test the hypothesis that migrating C. flavicans larvae enhance sediment-water exchange of dissolved substances (i.e. phosphorus and CH₄), we conducted an experiment where homogenized lake sediment was incubated in Plexiglas cylinders with surface lake water and different number of larvae (instars 3–4) added. Because the cylinders were uncapped during the experiment, some water (8.96 ± 4.95 %) was lost to evaporation. At the end of the experiment, the water was increasingly more turbid and green with...
Total phosphorus in the overlying water increased linearly by a factor of 5 with increasing number of larvae (Fig. 2). The amount of phosphorus added to the overlying water by the larvae (slope of regression line) was 0.21 µg P ind$^{-1}$ over the course of the experiment (9.5 d), or 0.022 µg P ind$^{-1}$ d$^{-1}$.

Because one cylinder was approaching hypoxia, its gas dynamics (e.g. respiration, CH$_4$ oxidation) were not comparable with the others, and was excluded from further data analysis. Final dissolved CH$_4$ concentrations increased linearly by a factor of ca. 5 with increasing number of larvae; however, δ$^{13}$C-CH$_4$ was quite stable at an average of -49.9‰ (Fig. 3A & B). Concurrently, dissolved pCO$_2$ decreased exponentially (Fig. 3C).

### 3.3. Diurnal migration of Chaoborus

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

Bottle incubation experiments to measure the release of CH$_4$ from the larvae’s gas vesicles (instars 3–4) were performed three times using larvae collected from the deep (15–20 m) and shallow layers (0–5 m). The total CH$_4$ released was linearly correlated with number of larvae; i.e. the amount of CH$_4$ released per individual was quite constant within each trial (Fig. 5A). The amounts of CH$_4$ released per individual were almost identical between the two trials with deep-water larvae: 0.97 and 1.02 nmol ind$^{-1}$. Because the shallow-water larvae would have already lost some of their CH$_4$ to the surrounding water before capture, as expected, the amount of CH$_4$ was much lower for them, at 0.19 nmol ind$^{-1}$. Both trials with deep-water larvae gave similar $\delta^{13}$C-CH$_4$ of -65.5 to -62.9 ‰, which is very close to that of the bottom water (-65.4 ‰; Fig. 1D). Shallow-water larvae showed a wider range of values, from -66.15 ‰ up to -58 ‰ (Fig. 5B).

3.5. System dynamics of methane and oxygen

The CH$_4$ oxidation rate was measured to be 0.03 d$^{-1}$ (Table S2). Emission to air was calculated using a measured Fickian diffusion coefficient ($k_{600}$) of 0.23 m d$^{-1}$ (Suppl. materials) and the final dissolved CH$_4$ concentrations. Average CH$_4$ transport by *C. flavicans* gas vesicles was 0.99 nmol ind$^{-1}$ d$^{-1}$ (Fig. 5). Under a steady-state condition, our model predicts a linear relative increase in CH$_4$ as a function of *C. flavicans* abundance (Fig. 6).
The accuracy of our model can be checked by using the phosphorus data as reference for bioturbation influence on diffusivity. In our case, the model outcome for the relative CH$_4$ increase due to bioturbation (regression slope = 0.035 ind$^{-1}$) closely matches the observed relative increase in TP (regression slope = 0.032 ind$^{-1}$); i.e. C. flavicans bioturbation increased the effective diffusion of TP and CH$_4$ by nearly equal magnitude (Fig. 6). The model performance was not sensitive to CH$_4$ oxidation rate. For example, decreasing and increasing the specific oxidation rate by 10-fold (to 0.003 and 0.3 d$^{-1}$, respectively) will change the regression slope by ≤ 6%. The percent contribution by gas vesicle transport of sediment CH$_4$ to the overlying water increased with the number of larvae and asymptotically approached 18% of the total CH$_4$ input (Fig. 6). In other words, the percent contributions of CH$_4$ by both bioturbation and vesicle transport would approach constant as C. flavicans abundance increases, though both rates would presumably continue to increase.

4. Discussion

4.1. Bioturbation and nutrient internal loading

In the sediment incubation experiment, final total phosphorus (TP) concentrations in the control cylinders (without added C. flavicans) averaged 15.4 µg l$^{-1}$, almost identical to the in situ surface water value (14.9 µg l$^{-1}$; Fig. 1). In contrast, TP increased at a rate of 0.022 µg P ind$^{-1}$ d$^{-1}$ in the cylinders with added C. flavicans larvae. Because we measured TP (particulate + dissolved), the observed increase in TP cannot be attributed to nutrient recycling within the water (e.g. via excretion or remineralization). While we did not count the final number of larvae, we did not observe any dead (floating) larvae in the experiment,
From the data we can infer that bioturbation by *C. flavicans* increased the effective sediment diffusivity and re-introduced sequestered phosphorus to the overlying water, as has been previously hypothesized (Gosselin and Hare, 2003), and is further supported by our CH$_4$ data and system modelling. As expected, this extra nutrient stimulated primary production and CO$_2$ drawdown in the experiment, as indicated by the observed pCO$_2$ values (Fig. 3C) and the color appearance of the water in the cylinders (Table S1). Extrapolating the experimental results to the observed in situ population density (ca. 9,300 ind m$^{-2}$), *C. flavicans* bioturbation would add ca. 205 µg P m$^{-2}$ d$^{-1}$ to the water column. This extra phosphorus is likely to first accumulate in the hypolimnion and become available for primary production during spring turnover. Lake remediation strategies often focus on curbing external phosphorus input (Schindler, 2006). Our results, however, suggest that *C. flavicans* bioturbation is a powerful mechanism to release nutrients from within the sediments, and may explain in some cases the ineffectiveness of external nutrient management for improving water clarity (McCrackin et al., 2017).

### 4.2. Breaking the diffusive barrier

Oxygen loss from the water column to the sediment is generally limited by diffusion across the sediment-water interface (Bryant et al., 2010). However, hypolimnetic oxygen demand would increase due to increased CH$_4$ oxidation as a result of enhanced introduction of sediment CH$_4$ to the overlying water via bioturbation. In the present study, the in situ *C. flavicans* larvae population (9,300 ind m$^{-2}$) was equivalent to 23 larvae added to the
incubation cylinder, which according to our model would increase water column CH$_4$ by 79\% over the baseline value via bioturbation alone. The in situ hypolimnetic CH$_4$ concentrations averaged 445 µmol l$^{-1}$. Assuming a steady state condition, $C. flavicans$ bioturbation would increase hypolimnetic CH$_4$ concentration by 352 µmol l$^{-1}$, and an extra O$_2$ demand of ca. 703 µmol l$^{-1}$ (or 22 mg l$^{-1}$; assuming 1 mol CH$_4$: 2 mol O$_2$ for complete oxidation), more than sufficient to deplete all hypolimnetic O$_2$ (100\% saturation at 12.8 mg l$^{-1}$ at 5°C). The $Chaoborus$-driven O$_2$ demand would be even stronger when we consider other reduced solutes and organic carbon re-introduced by bioturbation, plus CH$_4$ release and respiration by the larvae themselves (Tang et al., 2017). Taken together, $C. flavicans$ can greatly increase the hypolimnetic O$_2$ demand and maintain the hypoxic/anoxic condition below the thermocline, as well as enhance phosphorus loading, which may explain why artificial aeration often fails to restore lake quality (Gächter and Wehrli, 1998).

4.3. A positive feedback loop on climate warming

It is estimated that globally lakes account for 6–16\% of natural CH$_4$ emissions (7–11,300 mmol m$^{-2}$ yr$^{-1}$), driven mainly by physical processes such as ebullition and diffusion, whereas the roles of biota, besides a small contribution from rooted vegetation, are largely ignored (Bastviken et al. 2004). In light of our findings, it would be interesting to consider how $Chaoborus$ may contribute to CH$_4$ emission. The enhanced CH$_4$ flux across the sediment-water interface by bioturbation certainly has immediate implications for hypolimnetic oxygen demand. The added hypolimnic CH$_4$ due to $Chaoborus$ bioturbation would not be captured by conventional flux measurements at the air-water interface, and the eventual emission of this CH$_4$ to the atmosphere depends on how fast the lake would overturn, the
Lake bathymetry (surface area to volume ratio) and oxidation rates. An additional route by which *C. flavicans* can mediate CH$_4$ flux is by enhancing bubble release through bioturbation (Bezerra-Neto et al., 2012), which is expected to be the strongest when the larvae perturb the sediment during burrowing (at dawn and at dusk). Likewise, the amount of methane directly transported and released by their gas vesicles to the surface water is expected to be the highest during nighttime upward migration. Both of these processes are unlikely to be resolved by conventional daytime flux measurements.

The observed *C. flavicans* in situ population density (instars 3–4) was ca. 9,300 ind m$^{-2}$. Based on the bottle incubation experiments with deep-water larvae (averaged 0.99 nmol CH$_4$ ind$^{-1}$), we estimate that the amount of deep-water CH$_4$ transported by gas vesicles would be ca. 9.2 µmol m$^{-2}$ d$^{-1}$. The actual amount would likely vary in time and in space as the *Chaoborus* population density and activity change. For example, Tang et al. (2017) measured a higher population density of 34,000 ind m$^{-2}$ for Lake Soppen in an earlier year. Likewise, McGinnis et al. (2017) estimated that in some lakes, migrating *Chaoborus* larvae may transport up to 2,000 mmol CH$_4$ m$^{-2}$ yr$^{-1}$ from the sediment to the overlying water. It is likely that some of this methane would be lost to oxidation within the water column, and only a small fraction would contribute to emission to air. In a recent study, Carey et al. (2018) estimated that direct transport by migratory *Chaoborus* spp. (mainly *C. punctipennis* in their study) accounted for <1 % of the diffusive CH$_4$ flux to air, although it should be noted that they observed an order of magnitude lower population density than ours.

Eutrophication of inland waters is expected to persist or worsen due to growing human populations and increasing agricultural activities (Tilman et al., 2001; Smith, 2003; McCrackin et al., 2017). Compounding the problem, global warming will exacerbate
deoxygenation of the bottom layer (Jeppesen et al., 2009; Moss et al., 2011). These conditions are inducive to sediment methanogenesis and favor range expansion and proliferation of Chaoborus (Teraguchi, 1975; Taylor et al., 2016). As shown in the present study, bioturbation by Chaoborus larvae would significantly increase diffusivity at the sediment-water interface and re-introduce sequestered nutrients, organic carbon and CH\textsubscript{4} to the overlying water. Concurrently, the migrating larvae enhance the direct transport of sediment CH\textsubscript{4} to the surface water for water-air gaseous exchange. By linking the nutrient and CH\textsubscript{4} dynamics in eutrophic lakes, Chaoborus can thereby play a unique role in driving a positive feedback loop between lake eutrophication, CH\textsubscript{4} transport and climate warming in the coming decades.

4.4. The Chaoborus curse in lake restoration?

The long history of eutrophication has led to a host of public health concerns such as nuisance algal blooms, water quality deterioration and wildlife die-off, causing substantial economic damages (Pretty et al., 2003; Dodds et al., 2008). Studies in eutrophic coastal systems have shown that nutrient reduction often fails to revert the systems to the pre-eutrophication state (Duarte et al., 2009), at least in the short term. Similarly, aggressive lake management and restoration strategies have a checkered record of success (Gächter and Wehrli, 1998; McCrackin et al., 2017). These observations suggest that the systems may have passed the ecological tipping point such that internal forcing tends to maintain the new status quo despite external interventions such as reducing nutrient runoff and artificial
As shown in this and earlier studies (McGinnis et al., 2017; Tang et al., 2017), Chaoborus larvae that have successfully colonized eutrophic inland water bodies drive a positive feedback loop to sustain or even intensify eutrophication and deoxygenation, hindering the system’s recovery. We therefore argue that remediation measures will remain limited until sediment CH$_4$ concentrations drop, and Chaoborus abundance is greatly reduced. Understanding this internal Chaoborus feedback mechanism may allow resource managers to devise more effective lake restoration strategies.

5. Conclusion

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

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KWT, SF and DFM conceived the idea; KWT, SF, DV, CO and DFM performed the study; KWT and DFM analyzed the data; KWT and DFM wrote the manuscript with input from coauthors.


Fig. 1. Water column profiles in Lake Soppen. (A) Water temperature; (B) Dissolved oxygen; (C) Dissolved CH$_4$; (D) $\delta^{13}$C of CH$_4$; all measured on 13$^{th}$ June, 2017; (E) Total phosphorus, measured on 22$^{nd}$ May, 2017.

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

Fig. 3. Water chemistry as a function of number of C. flavicans larvae in the sediment incubation experiment. (A) dissolved CH$_4$; (B) $\delta^{13}$C-CH$_4$; (C) dissolved pCO$_2$. Outliers indicated by arrow and open square were excluded from regression analysis.

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

Fig. 5. CH$_4$ released from gas vesicles of deep- and shallow-water C. flavicans larvae (instars 3–4). (A) Total CH$_4$ as a function of number of C. flavicans larvae for deep-water (13$^{th}$ June and 22$^{nd}$ June) and shallow-water (22$^{nd}$ June) samples. (B) $\delta^{13}$C-CH$_4$ of the corresponding samples.

Fig. 6. System modelling results expressed as relative increase against the control, as a function of number of C. flavicans larvae. ● = observed TP increase due to bioturbation; ▲ = observed CH$_4$ increase; ■ = predicted CH$_4$ increase due to bioturbation; ♦ = predicted total CH$_4$ increase (bioturbation + gas vesicle transport). Error analysis of the model is explained in supplementary materials. Insert: Fraction of total CH$_4$ contributed by C. flavicans gas vesicles as a function of number of larvae.
\[ Y = 0.0071X + 0.165 \]
\[ r^2 = 0.729; p < 0.001 \]
Figure A: Relationship between the total CH$_4$ (nmol) and the number of Chaoborus larvae.

- **13 June (deep)**: $Y = 1.021X + 41.047$ ($r^2=0.848; p=0.006$)
- **22 June (deep)**: $Y = 0.965X + 10.447$ ($r^2=0.817; p=0.009$)
- **22 June (shallow)**: $Y = 0.191X + 7.731$ ($r^2=0.855; p=0.005$)

Figure B: Scatter plot of $\delta^{13}$C-CH$_4$ (%) vs. the number of Chaoborus larvae.