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Chaoborus spp. Transport CH_4 from the Sediments to the Surface Waters of a Eutrophic Reservoir, But Their Contribution to Water Column CH_4 Concentrations and Diffusive Efflux Is Minor

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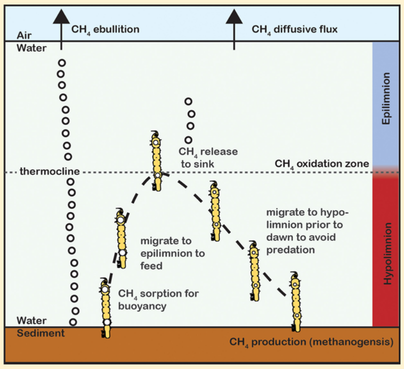
Supporting Information

ABSTRACT: *Chaoborus* spp. (midge larvae) live in the anoxic sediments and hypolimnia of freshwater lakes and reservoirs during the day and migrate to the surface waters at night to feed on plankton. It has recently been proposed that *Chaoborus* take up methane (CH_4) from the sediments in their tracheal gas sacs, use this acquired buoyancy to ascend into the surface waters, and then release the CH_4, thereby serving as a CH_4 "pump" to the atmosphere. We tested this hypothesis using diel surveys and seasonal monitoring, as well as incubations of *Chaoborus* to measure CH_4 transport in their gas sacs at different depths and times in a eutrophic reservoir. We found that *Chaoborus* transported CH_4 from the hypolimnion to the lower epilimnion at dusk, but the overall rate of CH_4 transport was minor, and incubations revealed substantial variability in CH_4 transport over space and time. We calculated that *Chaoborus* transport ~0.1 mmol CH_4 m^{-2} yr^{-1} to the epilimnion in our study reservoir, a very low proportion (<1%) of total CH_4 diffusive flux during the summer stratified period. Our data further indicate that CH_4 transport by *Chaoborus* is sensitive to water column mixing, *Chaoborus* density, and *Chaoborus* species identity.



INTRODUCTION

Lakes and reservoirs are substantial sources of methane (CH_4) to the atmosphere via diffusive flux across the water−air interface.[1,2] Annually, these waterbodies contribute as much as 9.9 Tg CH_4 yr^{-1} to the atmosphere via diffusive fluxes from their surface waters.[1] The magnitude of CH_4 diffusive flux is dependent on the concentrations of CH_4 in the surface waters (epilimnion) and the atmosphere, as well as the physical exchange rate of CH_4 across the air−water interface.[3] Because epilimnetic CH_4 concentrations can be extremely variable both over space and time,[4,5] determining the factors that increase CH_4 in the epilimnion over diel and seasonal time scales is critically important for estimating CH_4 diffusive fluxes.

It has recently been proposed that *Chaoborus* spp. (midge larvae; order Diptera) may increase CH_4 concentrations in the epilimnion.[10] *Chaoborus* may transport CH_4 from the sediments to the epilimnia of lakes and reservoirs by taking up CH_4 in their tracheal gas sacs, using this buoyancy to reach surface waters, and then releasing the CH_4 near the surface (Figure 1).[10] Gas sac inflation may allow *Chaoborus* to control their position in the water column,[10,11] and may be initiated by a decrease in light intensity.[11] This translocation of CH_4 may occur daily as part of *Chaoborus*' diel vertical migration (DVM), in which *Chaoborus* remain in the lower hypolimnion and sediments during the day to avoid visual predation from fish and ascend to the surface waters in the evening to feed on migrating zooplankton prey. *Chaoborus* DVM has been documented in lakes and reservoirs around the world.[11−14]

Chaoborus transport of CH_4 has multiple implications for CH_4 dynamics in freshwaters (Figure 1). Most importantly, if *Chaoborus* serve as a CH_4 "pump" from anoxic sediments and the hypolimnion to the surface waters, while also decreasing the exposure of CH_4 to oxidation, they may substantially increase dissolved CH_4 concentrations in the epilimnion. Moreover, increasing epilimnetic CH_4 concentrations could in turn increase the diffusive flux of CH_4 to the atmosphere.

Direct observations are needed to quantify the contribution of the *Chaoborus* CH_4 pump to lake and reservoir carbon (C) cycling and efflux. While an earlier laboratory study provides an important proof of concept of *Chaoborus*' ability to absorb and release CH_4,[10] additional measurements of CH_4 release using *Chaoborus* collected from the natural environment are needed to determine the magnitude of their CH_4 transport. Further studies incorporating both diel variation and depth through the water column will provide valuable insight beyond the single time and depth sampling by McGinnis et al.,[10] as many *Chaoborus* only migrate to the metalimnion, not epilimnion,

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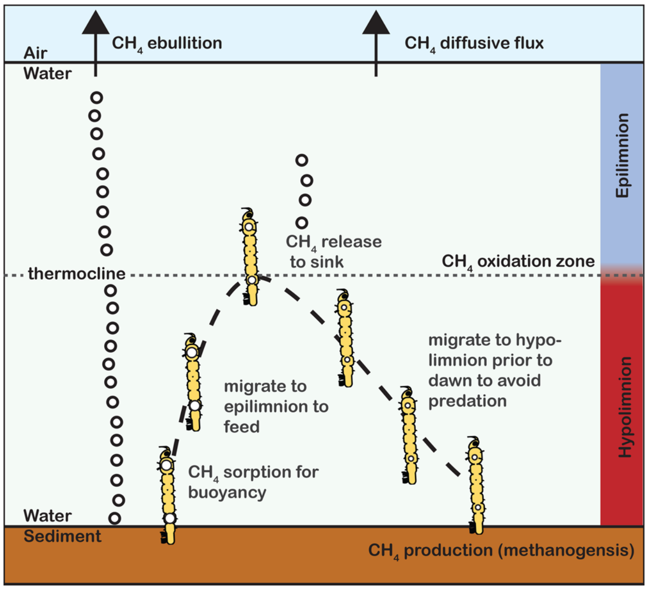

Figure 1. Schematic of *Chaoborus* diel vertical migration (DVM) and potential transport of methane (CH_4) from the hypolimnion and sediments into the epilimnion, where CH_4 increases could result in higher diffusive flux rates to the atmosphere.

and the timing of their DVM may vary throughout a diel period.[15−17]

The goal of our study was to quantify *Chaoborus* transport of CH_4 from the hypolimnion and sediments to the epilimnion and atmosphere over time using in situ observations. We conducted diel sampling of a reservoir where *Chaoborus* are commonly found in the summer. We collected 1 m-resolution depth profiles of *Chaoborus* density and CH_4 concentrations throughout two 24 h periods, measured CH_4 diffusive efflux, and isolated *Chaoborus* individuals to measure how much CH_4 they were transporting at different depths and times. These diel data were complemented by fortnightly surveys of *Chaoborus* and CH_4 throughout the summer stratified period to provide a reference for the magnitude of diel *Chaoborus* CH_4 transport. We used these data to answer three research questions: (1) Do *Chaoborus* transport CH_4 from the hypolimnion and sediments to the epilimnion?; (2) How does the concentration of CH_4 transported by *Chaoborus* vary by depth and time of day?; and (3) How does *Chaoborus*-mediated CH_4 transport affect epilimnetic CH_4 concentrations and CH_4 diffusive efflux? We predicted that if *Chaoborus* did serve as a CH_4 pump, concentrations of CH_4 within *Chaoborus* would be greatest at dusk and early evening, when *Chaoborus* begin migrating upward, with increases in CH_4 epilimnetic concentrations and diffusive efflux occurring throughout the evening.

■ MATERIALS AND METHODS

Diel CH_4 and *Chaoborus* Sampling. Beaverdam Reservoir (BVR) is a dimictic reservoir located in Vinton, Virginia (37.31°N, 79.81°W; Supporting Information (SI) Figures SI1 and SI2).[18] We sampled BVR throughout the diel periods of 3−4 August and 16−17 September 2016, collecting depth profiles of *Chaoborus*, CH_4, dissolved oxygen (DO), and temperature at noon, dusk, midnight, 2 a.m., dawn, and then noon the next day. Sunset and sunrise times for 3−4 August were approximately 20:30 and 6:00, respectively, and 20:00 and 6:30 for 16−17 September. On the second sampling event, in addition to the aforementioned sampling times, we collected additional profiles at 1 h before and after dusk to more finely resolve *Chaoborus* and CH_4 dynamics around dusk.

At every sampling time, our team simultaneously collected depth profiles of CH_4 and *Chaoborus* at the deepest site of BVR (SI Figure SI1). CH_4 samples were collected with a Van Dorn sampler every meter in the water column from subsurface (0.1 m) to just above the sediments at 11 m. Water samples were transferred from the Van Dorn into two replicate 20 mL serum vials, capped without headspace, and kept on ice until analysis within 24 h. Profiles of *Chaoborus* and their zooplankton prey were collected every meter from the surface to 10 m using a 30 L Schindler trap; 11 m was not sampled to prevent submerging the Schindler trap in the sediments. All Schindler trap samples were collected in <30 s, the duration from when the trap was closed with a messenger at depth to when the trap was raised to the water's surface and poured into an opaque sample bottle. All *Chaoborus* collected at night were kept in near-complete darkness from the time of collection to the beginning of the incubations to limit any light effects. Zooplankton samples were collected to compare the depth of maximum *Chaoborus* density with the depth of maximum crustacean zooplankton density in the water column and preserved with 70% ethanol.

We collected ~0.1 m-resolution depth profiles of temperature and DO concentrations at every sampling time using a 4-Hz SBE 19plus CTD profiler with an SBE 43 DO sensor (SeaBird Electronics, Bellevue, WA).

***Chaoborus* Incubations.** At every sampling time, we measured the CH_4 released from *Chaoborus* with methods adapted from McGinnis et al.[10] In brief, immediately after collection, the *Chaoborus* were transported to a mobile lab set up onshore. We immediately separated *Chaoborus* into counting trays, discarded any individuals not in their third or fourth instar stage, and then rinsed, counted, and gently added *Chaoborus* to 125 mL Erlenmeyer flasks. The *Chaoborus* were thoroughly rinsed with distilled water pre-equilibrated to atmospheric CH_4 concentrations to prevent high-CH_4 water from contaminating the flasks.[10] The flasks had a magnetic stir bar and were filled with 100 mL of equilibrated distilled water. Each flask was tightly capped with a rubber septum stopper and placed on a stir plate, where the *Chaoborus* were spun at ~60 rpm for 60 min to release any gas in their tracheal sacs and equilibrate CH_4 concentrations in the water with the headspace. The total duration of time from *Chaoborus* collection in the reservoir to the beginning of the incubations was approximately 10−30 min, and much care was taken to limit any unnecessary *Chaoborus* handling. After 60 min, 30 mL of gas were removed from the flask headspace using a syringe and injected into a serum vial that was kept on ice until analysis within 24 h. All flasks were thoroughly cleaned with atmosphere-equilibrated distilled water between incubations.

The goal of the incubations was to examine how the concentration of CH_4 in the flask headspace varied by the depth and time of *Chaoborus* collection, and by the number of *Chaoborus* per flask. Our experimental design during the August diel sampling aimed to incubate at least 25 *Chaoborus* per flask collected from 0.1, 5, and 10 m at every sampling time (noon, dusk, midnight, 2 AM, dawn, and noon the next day). Every sampling time included at least one distilled water flask without *Chaoborus* as a control. At some sampling times and depths, *Chaoborus* were not present or very rare (e.g., *Chaoborus* density at 0.1 m at noon was consistently zero). In these cases, we incubated fewer *Chaoborus* from those depths if any were

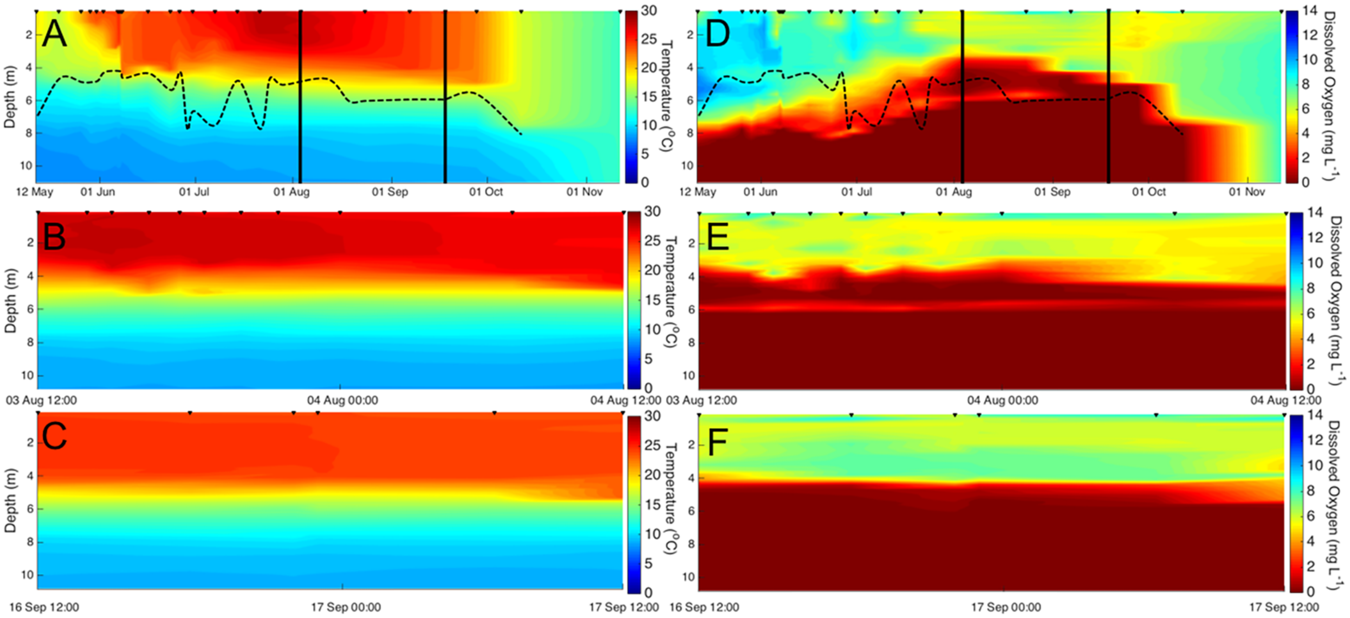

Figure 2. Depth profiles from the surface to the sediments of water temperature (A) and dissolved oxygen concentrations (D) in BVR from May to November 2016. The vertical black lines denote the noon to noon diel sampling events on 3−4 August (B,E) and 16−17 September (C,F). The inverted triangles on the top of the plots denote sampling times; the intervening data were interpolated.

collected (n ranging from 10 to 24 individuals). In September, in addition to replicating the methods from August, we incubated *Chaoborus* collected from additional depths near the thermocline (3, 6, and 8 m) at each sampling time, again aiming for 25 *Chaoborus* per flask but occasionally incubating fewer individuals when *Chaoborus* were rare or absent. CH_4 concentrations per individual *Chaoborus* were calculated for each flask as

$$\begin{aligned} &CH_4\textit{chaoborus}\text{ concentration} \\ &\quad = (\text{change in}[CH_4]\text{in the headspace during incubation} \\ &\qquad \times \text{headspace volume})/n \qquad (1) \end{aligned}$$

where n = number of *Chaoborus* in the flask.

Seasonal *Chaoborus* and CH_4 Surveys. From 6 April to 11 November 2016, we monitored BVR fortnightly during the daytime to compare the seasonal pattern of *Chaoborus* and CH_4 throughout the summer stratified period with the diel sampling. On each sampling date, we collected temperature and DO profiles with the CTD and quantified the daytime density of *Chaoborus* in the water column with 74-μm mesh vertical net tows from 0.5 m above the sediments to the surface. Samples were preserved with 70% ethanol. Finally, on every sample day, we measured depth profiles of dissolved CH_4 concentrations in the water column at five depths (0.1, 3, 6, 9, and 11 m), following the methods described above.

Laboratory Methods. We used the CTD temperature profiles to calculate thermocline depth and Schmidt stability, a metric of thermal stratification, using rLakeAnalyzer,[19] a lake physics package in R.[20]

Chaoborus densities were calculated for each Schindler trap and vertical net tow sample following Downing and Rigler.[21] For all Schindler trap samples, we also calculated the density of crustacean zooplankton (prey of *Chaoborus*).

We used standard methods for determining dissolved water column and flask CH_4 concentrations.[22] At the time of analysis, a 2 mL helium headspace in the reservoir samples was created and equilibrated by shaking the vials for 15 min. We injected 1 mL of the headspace gas into a gas chromatograph (GC; SRI model 8010, SRI Instruments, Torrance, CA) with a flame ionization detector (FID). We then back-calculated dissolved CH_4 concentrations in the water from headspace concentrations measured on the GC-FID using Henry's law. *Chaoborus* gas samples were also analyzed using GC-FID. The CH_4 method detection limit was 5.7×10^{-5} μM.

CH_4 Diffusive Flux. We calculated the diffusive flux of CH_4 from the surface of BVR into the atmosphere each time we sampled dissolved CH_4 at 0.1 m depth during the diel and seasonal monitoring following eq 2 (from ref 3):

$$\text{diffusive flux} = k \times [CH_{4(\text{surface})} - CH_{4(\text{air})}] \qquad (2)$$

where k is the piston velocity (m d^{-1}), or the depth of the water column that equilibrates with the atmosphere,[23] and $CH_{4(\text{surface})}$ and $CH_{4(\text{air})}$ are the concentrations of dissolved CH_4 at 0.1 m and above the water's surface, respectively. We calculated k using the LakeMetabolizer package in R using the Vachon model[24,25] with *U10*-corrected wind speed measured at a meteorological station located ~2.2 km from BVR.

■ RESULTS

From May to November 2016, BVR's water column was strongly thermally stratified (Figure 2A). Hypolimnetic anoxia (DO < 0.5 mg L^{-1}) developed immediately after thermal stratification set up in early spring and lasted until fall turnover (Figure 2A,D). During the two diel samplings, the hypolimnion was anoxic from the sediments to the thermocline (5.8 m depth in August and 6.7 m in September; Figure 2B,C,E,F). The August sampling coincided with a storm that persisted throughout the noon to noon sampling. During the storm, winds gusted up to 4.3 m s^{-1}, with a mean wind speed of 1.4 ± 0.76 m s^{-1} (1 SD) and total precipitation of 11.7 mm (SI Figure SI3). In comparison, the weather during the September sampling was calm, with mean winds of 1.2 ± 0.61 m s^{-1} and no precipitation. Because of the storm, Schmidt stability

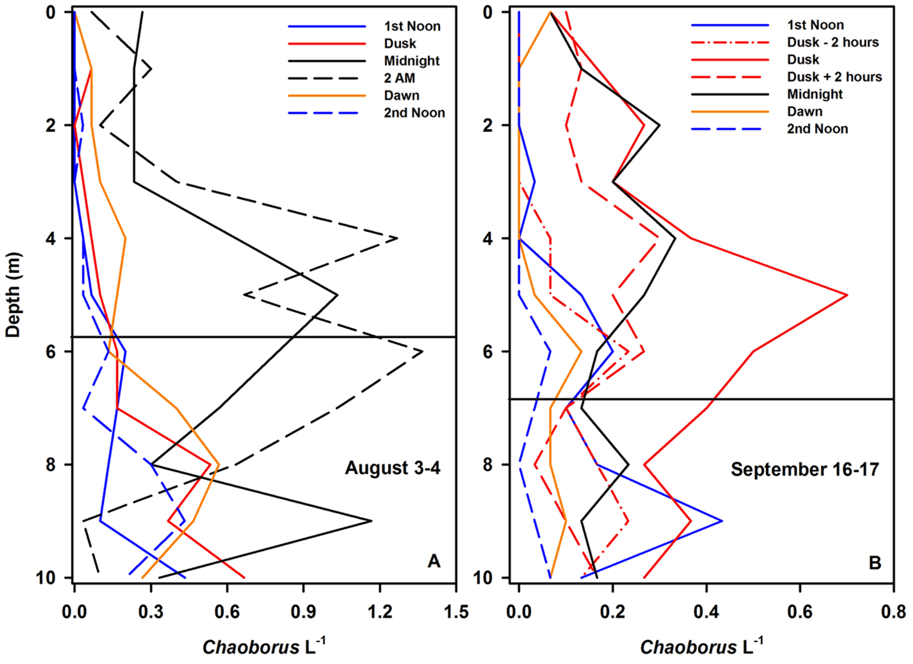

Figure 3. Depth profiles of *Chaoborus* measured every 1 m from the surface to 10 m depth during the 3−4 August (A) and 16−17 September (B) diel sampling events. The profiles measured over time show that *Chaoborus* densities above the thermocline (the horizontal black lines) were highest in the nighttime.

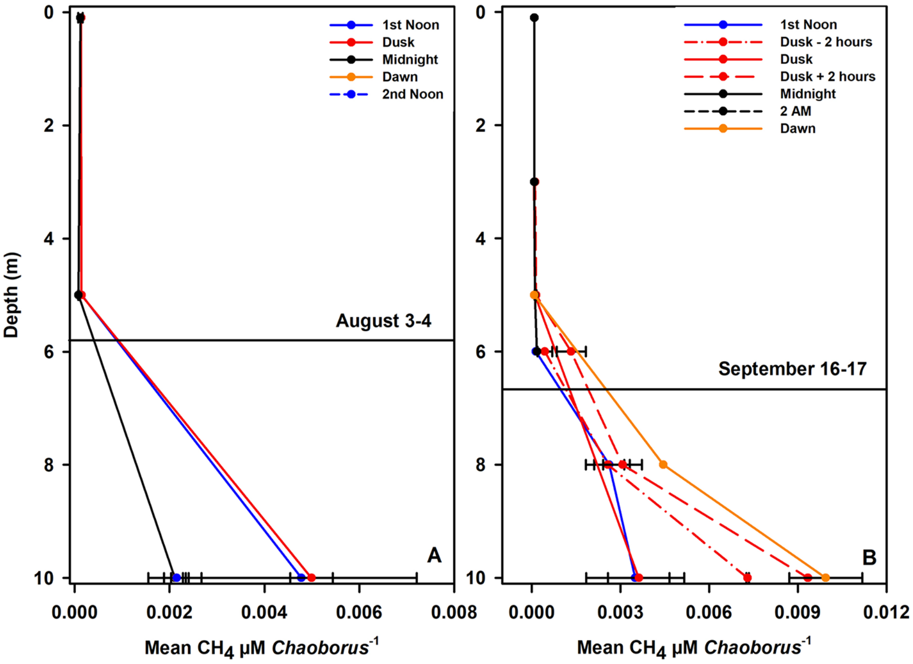

Figure 4. Mean ± standard error concentration of CH_4 within *Chaoborus* individuals collected at different times and depths from the 3−4 August (A) and 16−17 September (B) diel sampling events, calculated from the flask incubations. CH_4 concentrations within *Chaoborus* consistently decreased as they migrated upward from the hypolimnion to lower epilimnion at dusk; the thermocline is represented by the horizontal lines.

decreased by 20.5 J m^{-2} from the beginning to end of the August diel sampling (SI Figure SI3).

On both diel samplings, *Chaoborus* exhibited DVM (Figure 3), reaching a maximum of 6000 *Chaoborus* m^{-2} in the water column in August and 3600 m^{-2} in September. At noon in both August and September, *Chaoborus* density in the epilimnion was consistently <0.2 *Chaoborus* L^{-1}; hypolimnetic densities were slightly higher, up to 0.4 *Chaoborus* L^{-1}. In August, *Chaoborus* began increasing in the epilimnion after dusk, with maximum observed densities in the surface waters at midnight and 2 AM (~1.2 *Chaoborus* L^{-1}; Figure 3A). In September, *Chaoborus* began increasing above the thermocline at dusk, when they also exhibited their maximum density in the epilimnion (0.7 *Chaoborus* L^{-1}; Figure 3B). In both August and September, the peak epilimnetic *Chaoborus* density was consistently observed at 1−2 m above the thermocline; densities at the surface never exceeded 0.3 *Chaoborus* L^{-1}, even at midnight. The depth of peak *Chaoborus* density

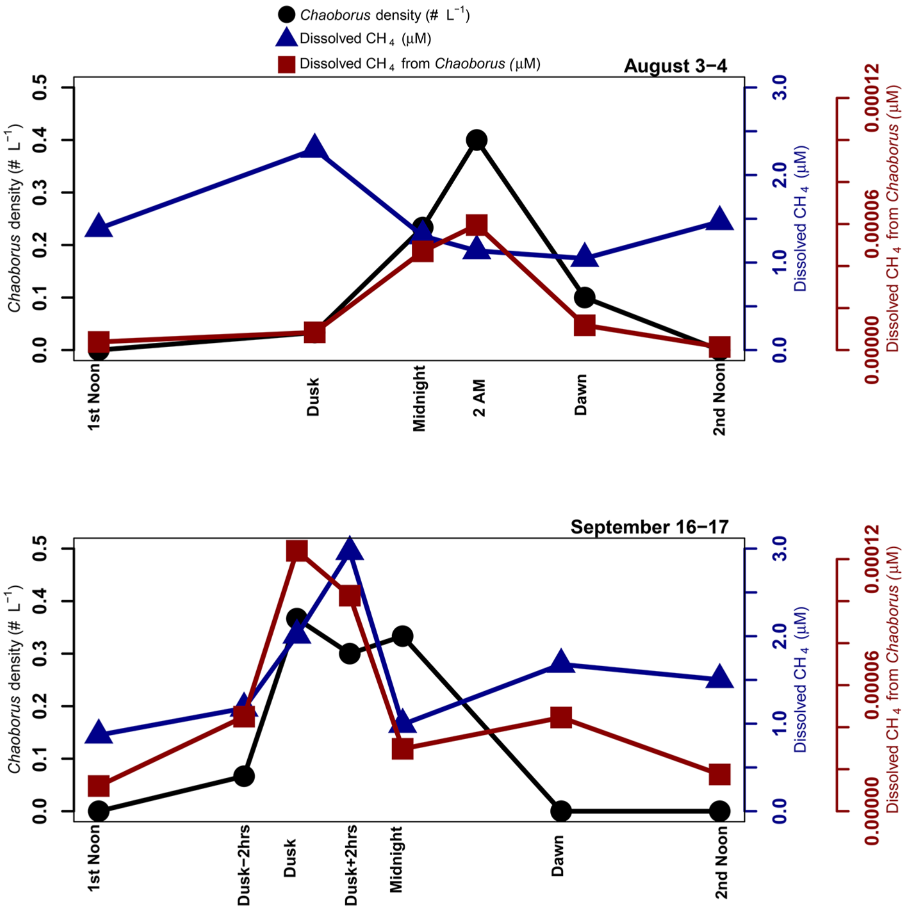

Figure 5. Volume-weighted *Chaoborus* densities (black lines) in the 3 m-layer above the thermocline (the lower epilimnion) during the 3−4 August (top) and 16−17 September (bottom) diel sampling events (left *y*-axis), in comparison to the volume-weighted ambient dissolved CH$_4$ concentrations (blue lines) and CH$_4$ concentrations within *Chaoborus* in the same layer (red lines; shown on the right *y*-axes). The amount of CH$_4$ transported within *Chaoborus* was four to five orders of magnitude below the ambient dissolved CH$_4$ concentrations in the water.

coincided with the depth of peak density of their zooplankton prey, which also ascended just above the thermocline after dusk (SI Figure SI4).

In both diel sampling events, all observed *Chaoborus* were *C. punctipennis*, ranging from 2.4−8.5 mm in size (mean 5.8 ± 1.3 mm). The *Chaoborus* diel data were representative of the seasonal monitoring, which indicated that *C. punctipennis* were present in the water column at low densities during the daytime from June to early October, peaking in late August (SI Figure SI5).

The magnitude of CH$_4$ in *Chaoborus* varied substantially over both depth and time (Figure 4, SI Figure SI6). The amount of CH$_4$ released by *Chaoborus* during the flask incubations ranged from below detection to 0.016 μM *Chaoborus*$^{-1}$ (median = 0.001 ± 0.0036 μM *Chaoborus*$^{-1}$) across both sampling events. Increasing *Chaoborus* density in the incubations generally resulted in higher CH$_4$ concentrations in flask headspace, but the result was strongly dependent on the depth from which the *Chaoborus* were collected (Figure 4, SI Figure SI6). *Chaoborus* collected from the water's surface never exhibited any detectable CH$_4$ release in the flask incubations, whereas *Chaoborus* collected near the sediments consistently released the highest CH$_4$ concentrations, especially during the nighttime (Figure 4). Incubations of *Chaoborus* collected above the thermocline (at 5 m) in August at dusk resulted in headspace CH$_4$ concentrations above detection, but values were only slightly greater than controls, and concentrations decreased again by midnight (Figure 4A). In comparison to August, flasks with *Chaoborus* collected above the thermocline (at 6 m) in September exhibited an order of magnitude higher CH$_4$ increases at dusk −2 h and dusk +2 h (Figure 4B). Similar to August, the CH$_4$ concentrations released from *Chaoborus* collected at 6 m in September had decreased to daytime levels by midnight.

While *Chaoborus* densities and CH$_4$ concentrations within *Chaoborus* in the lower epilimnion peaked during the nighttime, the CH$_4$ contribution from *Chaoborus* to the water column was likely minimal (Figure 5). At dusk, we observed an increase in the volume-weighted ambient dissolved CH$_4$ concentrations in the 3 m-thick layer above the thermocline that coincided with (September) or just preceded (August) an increase in *Chaoborus* densities for the same lower epilimnetic layer (Figure 5, SI Figure SI7). When the amount of CH$_4$ within the *Chaoborus* (calculated from the incubations) was multiplied by the *Chaoborus* density in that layer, a noted increase in *Chaoborus*-derived CH$_4$ was evident at early nighttime (Figure 5). However, the concentrations of CH$_4$ potentially attributable to *Chaoborus* were 4−5 orders of magnitude below the ambient water dissolved CH$_4$ concentrations in August and September (Figure 5). By dawn in both August and September, both volume-weighted dissolved CH$_4$ concentrations potentially attributable to *Chaoborus* and *Chaoborus* densities had declined to daytime levels.

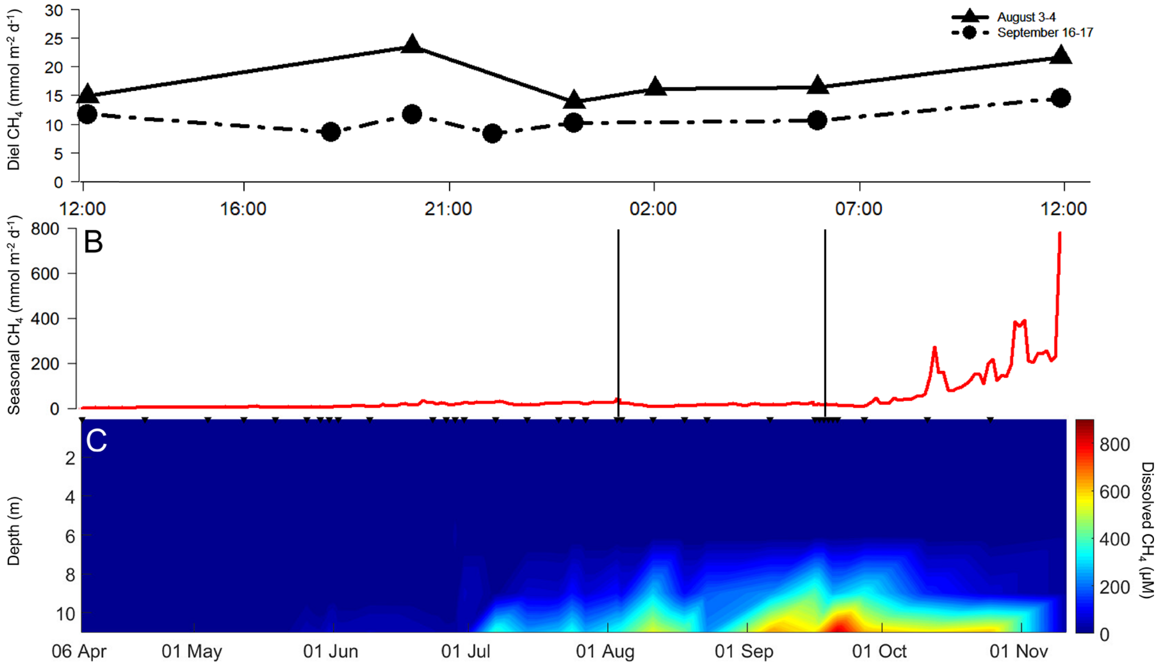

Figure 6. (A) Diffusive efflux of CH$_4$ peaked at dusk on 3−4 August (solid line) but did not exhibit any diel changes on 16−17 September (dashed line). (B) Diffusive efflux of CH$_4$ calculated from daytime CH$_4$ profiles collected throughout the summer stratified period was much higher than any diel increase potentially attributable to *Chaoborus*, with seasonal efflux rates peaking at fall turnover in November. The vertical black lines denote the noon to noon diel sampling events on 3−4 August and 16−17 September. (C) CH$_4$ concentrations in BVR were consistently much higher in the hypolimnion than epilimnion, reaching 842 μM on 20 September. The inverted triangles on the top of the panel denote sampling times for the fortnightly monitoring; the intervening data were interpolated.

Although CH$_4$ increased in the lower epilimnion at dusk in both August and September, there was a corresponding increase in evening CH$_4$ diffusive efflux only in August, not September (Figure 6A), and any CH$_4$ contributed by *Chaoborus* was a very small proportion of total seasonal CH$_4$ diffusive efflux (Figure 6B). In August, peak diffusive efflux was observed at dusk (24 mmol m^{-2} d^{-1}), a ~ 10 mmol m^{-2} d^{-1} increase above efflux rates observed in the daytime or at midnight. In September, however, efflux was relatively consistent throughout the diel period (~10 mmol m^{-2} d^{-1}), without any peak at dusk (Figure 6A). By comparison, throughout the summer stratified period (12 May to 11 November), the mean observed CH$_4$ diffusive efflux calculated from daytime monitoring was 37 ± 82 mmol m^{-2} d^{-1}, ranging from 2.5 to 780 mmol m^{-2} d^{-1} at fall turnover (Figure 6B). Thus, any diel diffusive flux of CH$_4$ potentially contributed by *Chaoborus* at dusk was much lower than the total diffusive flux calculated from daytime sampling throughout the summer stratified period.

Finally, we note that while dissolved CH$_4$ concentrations in the 3 m-layer above the thermocline were highest at early nighttime in August and September, they were still several orders of magnitude lower than dissolved CH$_4$ concentrations in the hypolimnion (Figure 6C). Throughout the stratified period, the epilimnion exhibited slightly supersaturated CH$_4$ concentrations (mean 1.9 ± 4.3 μM), whereas CH$_4$ concentrations in the hypolimnion peaked at 842 μM in September (Figure 6C).

■ DISCUSSION

Our data support an earlier investigation that *Chaoborus* are able to transport CH$_4$ from the hypolimnion and sediments to the lower epilimnion;[10] however, our study also reveals substantial variability in the magnitude of CH$_4$ transport by *Chaoborus*. Overall, the maximum amount of CH$_4$ diffusive efflux potentially attributable to *Chaoborus* is very small relative to the total seasonal diffusive flux in BVR. Summed throughout the summer stratified period, the total amount of CH$_4$ emitted via daytime diffusive flux was 6700 mmol m^{-2}. By comparison, the maximum amount of CH$_4$ diffusive flux potentially contributed by *Chaoborus* in the evening (up to 10 mmol m^{-2} d^{-1} at the August sampling) multiplied by the number of days when both *Chaoborus* densities in the water column (SI Figure S15) and wind speeds were equal to or greater than those observed on 3−4 August, would only result in ~40 mmol m^{-2} throughout the total stratified period. This value is very likely an overestimate because we cannot definitively attribute the increase in diffusive efflux at dusk in August to *Chaoborus*: below, we use the concentrations of CH$_4$ transported within *Chaoborus* to calculate a more realistic and conservative estimate. Regardless of calculation method, any diffusive CH$_4$ flux attributable to *Chaoborus* is a very small fraction (much less than 1%) of total diffusive CH$_4$ flux in BVR during the summer.

Our results exhibit multiple differences from the earlier findings of McGinnis et al.[10] First, the amount of CH$_4$ released by *Chaoborus* collected from BVR into flasks was higher than the amount of CH$_4$ released into flasks by *Chaoborus* from Lake Dagow, Germany. In that study, *Chaoborus* that had been incubated in 1.5 mM CH$_4$-saturated solution for 12 h released 0.255 ppm of CH$_4$ *Chaoborus*$^{-1}$ (0.000011 μM CH$_4$ *Chaoborus*$^{-1}$) into the headspace after being transferred to flasks filled with ambient water.[10] In contrast, we found that BVR *Chaoborus* that had been collected from hypolimnetic depths with lower dissolved CH$_4$ concentrations ($\leq$0.8 mM) released a median concentration of 0.001 μM CH$_4$ *Chaoborus*$^{-1}$

into the headspace of flasks filled with ambient water. Both studies' incubations had the same duration (60 min).

The varying results between the two studies may be due to differing methods and/or the *Chaoborus* used: Dagow was dominated by *C. flavicans* and BVR was dominated by *C. punctipennis*, and physiological differences between populations (e.g., varying gas sac volume and uptake rate) could result in different CH_4 transport capacity.[26] The preincubation in CH_4-saturated water may have also resulted in lower release rates from the Dagow *Chaoborus*, especially if the 12+ hours of handling time prior to the ambient water incubation stressed the animals. It is also possible that our hypolimnetic measurements may not reflect the actual CH_4 environment experienced by *Chaoborus* during their gas uptake period if they were burrowing into the anoxic sediments,[13] where porewater CH_4 concentrations would likely be much higher than in the water column. We note that it is possible that some CH_4 may have been released from the BVR *Chaoborus* as a result of the light and pressure changes that occurred during their collection with the Schindler trap, but we think that any CH_4 loss was likely minimal due to the short duration of time from when the *Chaoborus* were collected at depth and raised to the surface (<30 s) and the beginning of the flask incubations (10−30 min).

Despite the higher CH_4 release rate in *Chaoborus* measured in this study, the flux of CH_4 from the hypolimnion and sediments to the surface waters attributable to *Chaoborus* in BVR was lower than what was estimated by McGinnis et al.[10] The earlier study estimated that in a waterbody with 2000−130 000 *Chaoborus* m^{-2}, *Chaoborus* could transport 10−2000 mmol CH_4 m^{-2} yr^{-1} from the sediments to the surface waters.[10] Those calculations were based on a fixed gas sac volume (12 μL) within each individual *Chaoborus*, not measurements of CH_4 release from Dagow *Chaoborus*, and assumed that all CH_4 was emitted from the *Chaoborus*' gas sacs at the surface, with no CH_4 lost during the ascent. In BVR, which had a maximum of 3600−6000 *Chaoborus* m^{-2} (within the range observed by [13]), we calculated that the flux of CH_4 from the hypolimnion into the epilimnion attributable to *Chaoborus* was 0.093 mmol CH_4 m^{-2} yr^{-1} in August and 0.11 mmol CH_4 m^{-2} yr^{-1} in September. These rates are 2−7 orders of magnitude lower than the previous estimates and use the measured amount of CH_4 in *Chaoborus* collected at the thermocline, which was 1 to 2 orders of magnitude lower than CH_4 concentrations in *Chaoborus* collected near the sediments. If we used the highest measured CH_4 concentration released from *Chaoborus* collected just above the sediments (0.016 μM), the flux estimates would increase to 10−17 mmol m^{-2} yr^{-1}. However, we note that these higher rates are unlikely to be representative of BVR conditions because the 3,600−6,000 *Chaoborus* m^{-2} density is a maximum estimate and only 60−97% (mean 85 ± 14%) of the BVR *Chaoborus* population migrated above the thermocline at nighttime. Moreover, the flask incubations demonstrate that *Chaoborus* consistently released most of their CH_4 before they reached the thermocline while migrating upward (Figure 4). Consequently, we are confident that our estimate of ~0.1 mmol CH_4 m^{-2} yr^{-1} is realistic for BVR, and much lower than the potential maximum contribution of ~40 mmol m^{-2} calculated from diffusive flux estimates above.

As a result of using *Chaoborus* collected from multiple depths and times, our study reveals substantial variability in CH_4 transport by *Chaoborus* that was masked in the earlier study,[10] in which *Chaoborus* were collected from only one depth (1−2 m) and time (midnight). Both Lake Dagow and BVR are similar in morphometry, trophic state, mixing regime, and summer hypolimnetic oxygen depletion,[18,27] suggesting that the differences between studies are likely due to physiological differences between *Chaoborus* populations and study methods.

Differences in BVR *Chaoborus* and CH_4 dynamics between August and September are likely due to both seasonal changes as well as the August storm. First, *Chaoborus* densities were twice as high in August, yet the amount of CH_4 transported by *Chaoborus* was twice as high in September, likely due to the higher dissolved CH_4 concentrations in the hypolimnion. The net result of these two factors is that the amount of CH_4 transported by *Chaoborus* was similar (~0.1 mmol CH_4 m^{-2} yr^{-1}) between sampling events. This finding suggests that *Chaoborus* density and hypolimnetic CH_4 concentration are both important drivers of the magnitude of CH_4 *Chaoborus* transport to surface waters. Second, CH_4 diffusive flux in August exhibited diel fluctuations, with a peak at dusk and efflux rates that were twice as high as in September, when no detectable diel changes were observed. The higher efflux rates in August may be potentially due to increased turbulence in the water column from the storm, which could transport CH_4 from the lower epilimnion to the surface (SI Figure SI3). Following this hypothesis, depth profiles indicate that increases in CH_4 in the epilimnion extended higher above the thermocline in August than September SI Figure SI7. Regardless of the storm, the thermocline was ~1 m deeper in September than August, which also could have reduced any effect of *Chaoborus*-derived CH_4 on efflux rates because most *Chaoborus* did not migrate more than 3 m above the thermocline (Figure 2). The *Chaoborus*' migration to the lower epilimnion (not surface) is likely because their zooplankton prey were primarily located just above the thermocline at nighttime (SI Figure SI4), similar to other studies (e.g., ref 15). Altogether, these observations suggest that physical mixing and other lake characteristics influence the contribution of *Chaoborus*-mediated CH_4 transport to epilimnetic CH_4 concentrations and diffusive efflux.

We cannot attribute the increases in CH_4 in the lower epilimnion at dusk solely to *Chaoborus* transport because other processes, such as entrainment of CH_4 across the thermocline, likely also contributed. In addition, our study only investigated the effects of *Chaoborus* on the vertical distribution of CH_4 in the water column and diffusive flux, not ebullition: a past study found that *Chaoborus* bioturbation could substantially increase ebullitive CH_4 flux from the sediments.[29] Thus, we recommend that additional surveys of *Chaoborus* and CH_4 dynamics be conducted in a range of waterbodies with different mixing patterns and *Chaoborus* densities and species, and incorporate ebullition to quantify the importance of these invertebrates to lake and reservoir CH_4 budgets.

This study addressed three questions: Q1) Do *Chaoborus* transport CH_4 from the hypolimnion and sediments to the epilimnion?; Q2) How does the concentration of CH_4 in *Chaoborus* vary by depth and time of day?; and Q3) How does *Chaoborus*-mediated CH_4 transport affect epilimnetic CH_4 concentrations and CH_4 efflux? For Q1, we observed that *Chaoborus* collected from different depths in BVR transported CH_4 from hypolimnion to the lower epilimnion, but the overall rate of CH_4 transport was much lower than estimated in an earlier study.[10] For Q2, the flask incubations revealed substantial temporal variability in CH_4 transport by *Chaoborus*, with peak epilimnetic concentrations of CH_4 in *Chaoborus*

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Environ. Sci. Technol. 2018, 52, 1165−1173

observed just above the thermocline at dusk, though hypolimnetic *Chaoborus* contained overall much higher CH_4. For Q3, it appears that *Chaoborus* may contribute a small mass of CH_4 to the epilimnion, which could potentially result in slightly higher CH_4 efflux in the nighttime. However, the magnitude of CH_4 transport was very low relative to seasonal fluctuations in CH_4, suggesting that if CH_4 transport by *Chaoborus* does occur, it is highly sensitive to water column mixing, *Chaoborus* density and vertical distribution during DVM, and other lake processes. In summary, our study builds on McGinnis et al.[10] by providing a more detailed in situ data set highlighting the variability of *Chaoborus*-mediated CH_4 transport both over depth and time. While our work indicates that *Chaoborus* may potentially increase epilimnetic CH_4 concentrations, it is likely not a major pump of CH_4 from the hypolimnion to the surface waters in lakes and reservoirs.[6−9,28]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b04384.

> Bathymetric map of Beaverdam Reservoir (SI1); site description of Beaverdam Reservoir (SI2); comparison of weather and thermal stratification between the August and September diel sampling events (SI3); diel profiles of zooplankton prey (SI4); daytime *Chaoborus* density in the water column during the seasonal fortnightly sampling of Beaverdam Reservoir (SI5); the amount of CH_4 released into flask headspace during the incubations (SI6); and dissolved CH_4 concentrations in the water column during both diel sampling events (SI7) (PDF).

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Author Contributions

C.C.C. conceived the original idea for the manuscript. All authors collected field data; C.C.C. led the *Chaoborus* incubations; RPM led GHG analyses; J.P.D. conducted the zooplankton microscopy; and all authors contributed to data analysis and interpretation. C.C.C. led the writing of the manuscript, and all authors provided feedback and approved the final version.

Notes

The authors declare no competing financial interest.

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