1	<i>Chaoborus</i> spp. transport CH ₄ from the sediments to the surface waters of a eutrophic reservoir,
2	but their contribution to water column CH4 concentrations and diffusive efflux is minor
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19 Abstract:

20 *Chaoborus* spp. (midge larvae) live in the anoxic sediments and hypolimnia of freshwater 21 lakes and reservoirs during the day and migrate to the surface waters at night to feed on plankton. 22 It has recently been proposed that *Chaoborus* take up methane (CH₄) from the sediments in their 23 tracheal gas sacs, use this acquired buoyancy to ascend into the surface waters, and then release 24 the CH₄, thereby serving as a CH₄ "pump" to the atmosphere. We tested this hypothesis using 25 diel surveys and seasonal monitoring, as well as incubations of Chaoborus to measure CH4 26 transport in their gas sacs at different depths and times in a eutrophic reservoir. We found that 27 Chaoborus transported CH4 from the hypolimnion to the lower epilimnion at dusk, but the 28 overall rate of CH₄ transport was minor, and incubations revealed substantial variability in CH₄ transport over space and time. We calculated that *Chaoborus* transport ~0.1 mmol CH₄ m⁻² yr⁻¹ to 29 30 the epilimnion in our study reservoir, a very low proportion (<1%) of total CH₄ diffusive flux 31 during the summer stratified period. Our data further indicate that CH₄ transport by *Chaoborus* is 32 sensitive to water column mixing, Chaoborus density, and Chaoborus species identity. 33

34 Keywords: Diffusive emissions, Freshwaters, Greenhouse gases, Methane, Reservoirs

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37 Introduction

Lakes and reservoirs are substantial sources of methane (CH₄) to the atmosphere via 38 diffusive flux across the water-air interface.^{1,2} Annually, waterbodies contribute as much as 9.9 39 Tg CH₄ yr⁻¹ to the atmosphere via diffusive fluxes from their surface waters.¹ The magnitude of 40 CH₄ diffusive flux is dependent on the concentrations of CH₄ in the surface waters (epilimnion) 41 42 and the atmosphere, as well as the physical exchange rate of CH₄ across the air-water interface.³ Because epilimnetic CH₄ concentrations can be extremely variable both over space and time.^{4,5} 43 determining the factors that increase CH₄ in the epilimnion over diel and seasonal timescales is 44 45 critically important for estimating CH₄ diffusive fluxes.

46 It has recently been proposed that *Chaoborus* spp. (midge larvae; order Diptera) may increase CH₄ concentrations in the epilimnion.¹⁰ Chaoborus may transport CH₄ from the 47 48 sediments to the epilimnia of lakes and reservoirs by taking up CH₄ in their tracheal gas sacs, 49 using this buoyancy to reach surface waters, and then releasing the CH₄ near the surface (Figure 1).¹⁰ Gas sac inflation may allow *Chaoborus* to control their position in the water column.^{10,11} 50 51 and may be initiated by a decrease in light intensity.¹¹ This translocation of CH₄ may occur daily as part of *Chaoborus*' diel vertical migration (DVM), in which *Chaoborus* remain in the lower 52 53 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 54 documented in lakes and reservoirs around the world.¹¹⁻¹⁴ 55

56 *Chaoborus* transport of CH₄ has multiple implications for CH₄ dynamics in freshwaters 57 (Figure 1). Most importantly, if *Chaoborus* serve as a CH₄ "pump" from anoxic sediments and 58 the hypolimnia to the surface waters, while also decreasing exposure of CH₄ to oxidation, they 59 may substantially increase dissolved CH₄ concentrations in the epilimnion. Moreover, increasing epilimnetic CH₄ concentrations could in turn increase the diffusive flux of CH₄ to the
atmosphere.

Direct observations are needed to quantify the contribution of the *Chaoborus* CH₄ pump 62 63 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 CH₄,¹⁰ additional 64 65 measurements of CH₄ release using *Chaoborus* collected from the natural environment are needed to determine the magnitude of their CH₄ transport. Further studies incorporating both diel 66 variation and depth through the water column will provide valuable insight beyond the single 67 time and depth sampling by McGinnis et al.,¹⁰ as many *Chaoborus* only migrate to the 68 69 metalimnion, not epilimnion, and the timing of their DVM may vary throughout a diel period.¹⁵⁻ 17 70

71 The goal of our study was to quantify *Chaoborus* transport of CH₄ from the hypolimnion 72 and sediments to the epilimnion and atmosphere over time using *in situ* observations. We 73 conducted diel sampling of a reservoir where Chaoborus are commonly found in the summer. 74 We collected 1 m-resolution depth profiles of *Chaoborus* density and CH₄ concentrations throughout two 24-hr periods, measured CH₄ diffusive efflux, and isolated *Chaoborus* 75 76 individuals to measure how much CH₄ they were transporting at different depths and times. 77 These diel data were complemented by fortnightly surveys of *Chaoborus* and CH₄ throughout 78 the summer stratified period to provide a reference for the magnitude of diel *Chaoborus* CH₄ 79 transport. We used these data to answer three research questions: 1) Do Chaoborus transport 80 CH_4 from the hypolimnion and sediments to the epilimnion?; 2) How does the concentration of 81 CH₄ transported by *Chaoborus* vary by depth and time of day?; and 3) How does *Chaoborus*-82 mediated CH₄ transport affect epilimnetic CH₄ concentrations and CH₄ diffusive efflux? We

predicted that if *Chaoborus* did serve as a CH₄ pump, concentrations of CH₄ within *Chaoborus*would be greatest at dusk and early evening, when *Chaoborus* begin migrating upwards, with
increases in CH₄ epilimnetic concentrations and diffusive efflux occurring throughout the
evening.

87

88 Materials and Methods

89 *Diel CH*⁴ and Chaoborus sampling

Beaverdam Reservoir (BVR) is a dimictic reservoir located in Vinton, Virginia, USA 90 (37.31°N, 79.81°W; SI1, SI2).¹⁸ We sampled BVR throughout the diel periods of 3-4 August and 91 92 16-17 September 2016, collecting depth profiles of *Chaoborus*, CH₄, dissolved oxygen (DO), 93 and temperature at noon, dusk, midnight, 2 AM, dawn, and then noon the next day. Sunset and 94 sunrise times for 3-4 August were approximately 20:30 and 6:00, respectively, and 20:00 and 95 6:30 for 16-17 September. On the second sampling event, in addition to the aforementioned sampling times, we collected additional profiles at one hour before and after dusk to more finely 96 97 resolve Chaoborus and CH₄ dynamics around dusk.

98 At every sampling time, our team simultaneously collected depth profiles of CH₄ and 99 *Chaoborus* at the deepest site of BVR (SI1). CH₄ samples were collected with a Van Dorn 100 sampler every meter in the water column from subsurface (0.1 m) to just above the sediments at 101 11 m. Water samples were transferred from the Van Dorn into two replicate 20-mL serum vials, 102 capped without headspace, and kept on ice until analysis within 24 h. Profiles of Chaoborus and 103 their zooplankton prey were collected every meter from the surface to 10 m using a 30-L 104 Schindler trap; 11 m was not sampled to prevent submerging the Schindler trap in the sediments. 105 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 depths of maximum *Chaoborus* density
coincided with the depth of maximum crustacean zooplankton density in the water column
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, Washington, USA).

115

116 Chaoborus *incubations*

117 At every sampling time, we measured the CH₄ released from *Chaoborus* with methods adapted from ¹⁰. In brief, immediately after collection, the *Chaoborus* were transported to a 118 119 mobile lab set up onshore. We immediately separated *Chaoborus* into counting trays, discarded any individuals not in their 3rd or 4th instar stage, and then rinsed, counted, and gently added 120 121 Chaoborus to 125 mL Erlenmeyer flasks. The Chaoborus were thoroughly rinsed with distilled 122 water pre-equilibrated to atmospheric CH₄ concentrations to prevent high-CH₄ water from 123 contaminating the flasks.¹⁰ The flasks had a magnetic stir bar and were filled with 100 mL of 124 equilibrated distilled water. Each flask was tightly capped with a rubber septum stopper and 125 placed on a stir plate, where the *Chaoborus* were spun at ~60 rpm for 60 minutes to release any 126 gas in their tracheal sacs and equilibrate CH_4 concentrations in the water with the headspace. The 127 total duration of time from *Chaoborus* collection in the reservoir to the beginning of the 128 incubations was approximately 10-30 minutes, and much care was taken to limit any unnecessary

Chaoborus handling. After 60 minutes, 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.

133 The goal of the incubations was to examine how the amount of CH₄ in the flask 134 headspace varied by the depth and time of *Chaoborus* collection, and by the number of 135 *Chaoborus* per flask. Our experimental design during the August diel sampling aimed to 136 incubate at least 25 Chaoborus per flask collected from 0.1 m, 5 m, and 10 m at every sampling 137 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 138 139 depths, *Chaoborus* were not present or very rare (e.g., *Chaoborus* density at 0.1 m at noon was 140 consistently zero). In these cases, we incubated fewer *Chaoborus* from those depths if any were 141 collected (*n* ranging from 10 to 24 individuals). In September, in addition to replicating the 142 methods from August, we incubated *Chaoborus* collected from additional depths near the 143 thermocline (3, 6, and 8 m) at each sampling time, again aiming for 25 Chaoborus per flask but 144 occasionally incubating fewer individuals when *Chaoborus* were rare or absent. CH₄ 145 concentrations per individual Chaoborus were calculated for each flask as: 146 CH_4 Chaoborus concentration = (Change in [CH₄] in the headspace during incubation × 147 headspace volume)/n(eqn. 1) 148 where n = number of *Chaoborus* in the flask. 149

150 Seasonal Chaoborus and CH₄ surveys

151 From 6 April to 11 November 2016, we monitored BVR fortnightly during the daytime to

152 compare the seasonal pattern of *Chaoborus* and CH₄ throughout the summer stratified period 153 with the diel sampling. On each sampling date, we collected temperature and DO profiles with 154 the CTD and quantified the daytime density of *Chaoborus* in the water column with 74-um mesh 155 vertical net tows from 0.5 m above the sediments to the surface. Samples were preserved with 156 70% ethanol. Finally, on every sample day, we measured depth profiles of dissolved CH₄ concentrations in the water column at five depths (0.1, 3, 6, 9, and 11 m), following the methods 157 described above. 158 159 160 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 ²¹. For all Schindler trap samples, we also calculated the density of crustacean
zooplankton (prey of *Chaoborus*).

166 We used standard methods for determining dissolved water column and flask CH₄ concentrations.²² At the time of analysis, a 2-mL helium headspace in the reservoir samples was 167 168 created and equilibrated by shaking the vials for 15 minutes. We injected 1 mL of the headspace 169 gas into a gas chromatograph (GC; SRI model 8010, SRI Instruments, Torrance, California, 170 USA) with a flame ionization detector (FID). We then back-calculated dissolved CH₄ 171 concentrations in the water from headspace concentrations measured on the GC-FID using 172 Henry's law. *Chaoborus* gas samples were also analyzed using GC-FID. The CH₄ method detection limit was $5.7 \times 10^{-5} \mu$ M. 173

175 CH_4 diffusive flux

We calculated the diffusive flux of CH₄ from the surface of BVR into the atmosphere
each time we sampled dissolved CH₄ at 0.1 m depth during the diel and seasonal monitoring
following:

179 Diffusive flux =
$$k \times [CH_{4(surface)} - CH_{4(air)}]$$
 (eqn. 2)³

180 where *k* is the piston velocity (m d⁻¹), or the depth of the water column that equilibrates with the 181 atmosphere,²³ and $CH_{4(surface)}$ and $CH_{4(air)}$ are the concentrations of dissolved CH₄ at 0.1 m and 182 above the water's surface, respectively. We calculated *k* using the LakeMetabolizer package in R 183 using the Cole model^{24,25} with *U10*-corrected wind speed measured at a meteorological station 184 located ~2.2 km from BVR.

185

186 Results

From May to November 2016, BVR's water column was strongly thermally stratified 187 (Figure 2A). Hypolimnetic anoxia (DO <0.5 mg L⁻¹) developed immediately after thermal 188 189 stratification set up in early spring and lasted until fall turnover (Figure 2A,D). During the two 190 diel samplings, the hypolimnion was anoxic from the sediments to the thermocline (5.8 m depth 191 in August and 6.7 m in September; Figure 2B,C,E,F). The August sampling coincided with a 192 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 S.D.) and total precipitation of 11.7 mm 193 194 (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 decreased by 20.5 J 195 m^{-2} from the beginning to end of the August diel sampling (SI3). 196

197 On both diel samplings, *Chaoborus* exhibited DVM (Figure 3), reaching a maximum of

198	6,000 Chaoborus m ⁻² in the water column in August and 3,600 m ⁻² in September. At noon in
199	both August and September, Chaoborus density in the epilimnion was consistently <0.2
200	Chaoborus L ⁻¹ ; hypolimnetic densities were slightly higher, up to 0.4 Chaoborus L ⁻¹ . In August,
201	Chaoborus began increasing in the epilimnion after dusk, with maximum observed densities in
202	the surface waters at midnight and 2 AM (~1.2 Chaoborus L-1; Figure 3A). In September,
203	Chaoborus began increasing above the thermocline at dusk, when they also exhibited their
204	maximum density in the epilimnion (0.7 Chaoborus L ⁻¹ ; Figure 3B). In both August and
205	September, the peak epilimnetic Chaoborus density was consistently observed at 1-2 m above
206	the thermocline; densities at the surface never exceeded 0.3 Chaoborus L ⁻¹ , even at midnight.
207	The depth of peak Chaoborus density coincided with the depth of peak density of their
208	zooplankton prey, which also ascended just above the thermocline after dusk (SI4).
209	In both diel sampling events, all observed Chaoborus were C. punctipennis, ranging from
210	2.4 - 8.5 mm in size (mean 5.8 ± 1.3 mm). The <i>Chaoborus</i> diel data were representative of the
211	seasonal monitoring, which indicated that C. punctipennis were present in the water column at
212	low densities during the daytime from June to early October, peaking in late August (SI5).
213	The magnitude of CH4 in <i>Chaoborus</i> varied substantially over both depth and time
214	(Figure 4, SI6). The amount of CH ₄ released by <i>Chaoborus</i> during the flask incubations ranged
215	from below detection to 0.016 μ M <i>Chaoborus</i> ⁻¹ (median = 0.001 ± 0.0036 μ M <i>Chaoborus</i> ⁻¹)
216	across both sampling events. Increasing Chaoborus density in the incubations generally resulted
217	in higher CH ₄ concentrations in flask headspace, but the result was strongly dependent on the
218	depth from which the Chaoborus were collected (Figure 4, SI6). Chaoborus collected from the
219	water's surface never exhibited any detectable CH4 release in the flask incubations, whereas
220	Chaoborus collected near the sediments consistently released the highest CH4 concentrations,

especially during the nighttime (Figure 4). Incubations of *Chaoborus* collected above the
thermocline (5 m) in August at dusk resulted in headspace CH₄ 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 (6 m) in September exhibited an order of magnitude higher CH₄ increases at dusk - 2
hours and dusk + 2 hours (Figure 4B). Similar to August, the CH₄ concentrations released from *Chaoborus* collected at 6 m in September had decreased to daytime levels by midnight.

228 While Chaoborus densities and CH₄ concentrations within Chaoborus in the lower 229 epilimnion peaked during the nighttime, the CH₄ contribution from *Chaoborus* to the water 230 column was likely minimal (Figure 5). At dusk, we observed an increase in the volume-weighted 231 dissolved CH₄ concentrations in the 3 m-thick layer above the thermocline that coincided with 232 (September) or just preceded (August) an increase in *Chaoborus* densities for the same lower 233 epilimnetic layer (Figure 5, SI7). When the amount of CH₄ within the Chaoborus (calculated 234 from the incubations) was multiplied by the *Chaoborus* density in that layer, a noted increase in 235 *Chaoborus*-derived CH₄ was evident at early nighttime (Figure 5). However, the concentrations 236 of CH₄ potentially attributable to *Chaoborus* were four to five orders of magnitude below the 237 ambient water CH₄ concentrations in August and September (Figure 5). By dawn in both August 238 and September, both volume-weighted dissolved CH₄ concentrations and *Chaoborus* density had 239 declined to daytime concentrations.

Although CH₄ increased in the lower epilimnion at dusk in both August and September,
there was a corresponding increase in evening CH₄ diffusive efflux only in August, not
September (Figure 6A), and any CH₄ contributed by *Chaoborus* was a very small proportion of
total seasonal CH₄ diffusive efflux (Figure 6B). In August, peak diffusive efflux was observed at

244	dusk (24 mmol m ⁻² d ⁻¹), a \sim 10 mmol m ⁻² d ⁻¹ increase above efflux rates observed in the daytime
245	or at midnight. In September, however, efflux was relatively consistent throughout the diel
246	period (~10 mmol m ⁻² d ⁻¹), without any peak at dusk (Figure 6A). By comparison, throughout the
247	summer stratified period (12 May to 11 November), the mean observed CH ₄ diffusive efflux
248	calculated from daytime monitoring was $37 \pm 82 \text{ mmol m}^{-2} \text{ d}^{-1}$, ranging from 2.5 to 780 mmol m
249	2 d ⁻¹ at fall turnover (Figure 6B). Thus, any diel diffusive flux of CH ₄ potentially contributed by
250	Chaoborus at dusk was much lower than the total diffusive flux calculated from daytime
251	sampling throughout the summer stratified period.
252	Finally, we note that while dissolved CH ₄ concentrations in the 3 m-layer above the
253	thermocline were highest at early nighttime in August and September, they were still several
254	orders of magnitude lower than dissolved CH ₄ concentrations in the hypolimnion (Figure 6C).
255	Throughout the stratified period, the epilimnion exhibited slightly supersaturated CH4
256	concentrations (mean $1.9 \pm 4.3 \ \mu$ M), whereas CH ₄ concentrations in the hypolimnion peaked at
257	842 μM in September (Figure 6C).
258	

259 **Discussion**

Our data support an earlier investigation that *Chaoborus* are able to transport CH₄ from the hypolimnion and sediments to the lower epilimnion;¹⁰ however, our study also reveals substantial variability in the magnitude of CH₄ transport by *Chaoborus*. Overall, the maximum amount of CH₄ 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 CH₄ emitted via daytime diffusive flux was 6,700 mmol m⁻². By comparison, the maximum amount of CH₄ diffusive flux potentially contributed by *Chaoborus* in the evening (up

267	to 10 mmol m ⁻² d ⁻¹ at the August sampling) multiplied by the number of days when both
268	Chaoborus densities in the water column (SI5) and wind speeds were equal to or greater than
269	those observed on 3-4 August, would only result in ~40 mmol m ⁻² throughout the total stratified
270	period. This value is very likely an overestimate because we cannot definitively attribute the
271	increase in diffusive efflux at dusk in August to Chaoborus: below, we use the concentrations of
272	CH ₄ transported within <i>Chaoborus</i> to calculate a more realistic and conservative estimate.
273	Regardless of calculation method, any diffusive CH4 flux attributable to Chaoborus is a very
274	small fraction (much less than 1%) of total diffusive CH4 flux in BVR during the summer.
275	Our results exhibit multiple differences from the earlier findings of McGinnis et al. ¹⁰
276	First, the amount of CH ₄ released by <i>Chaoborus</i> collected from BVR into flasks was higher than
277	the amount of CH4 released into flasks by Chaoborus from Lake Dagow, Germany. In that study,
278	Chaoborus that had been incubated in 1.5 mM CH ₄ -saturated solution for 12 hours released
279	$0.255 \text{ ppm CH}_4 \text{ Chaoborus}^{-1} (0.000011 \ \mu\text{M CH}_4 \text{ Chaoborus}^{-1})$ into the headspace after being
280	transferred to flasks filled with ambient water. ¹⁰ In contrast, we found that BVR Chaoborus that
281	had been collected from hypolimnetic depths with lower dissolved CH ₄ concentrations (≤ 0.8
282	mM) released a median concentration of 0.001 μ M CH ₄ Chaoborus ⁻¹ into the headspace of flasks
283	filled with ambient water. Both studies' incubations had the same duration (60 minutes).
284	The varying results between the two studies may be due to differing methods and/or the
285	Chaoborus used: Dagow was dominated by C. flavicans and BVR was dominated by C.
286	punctipennis, and physiological differences between populations (e.g., varying gas sac volume)
287	could result in different CH ₄ transport capacity. ²⁶ The pre-incubation in CH ₄ -saturated water may
288	have also resulted in lower release rates from the Dagow Chaoborus, especially if the 12+ hours
289	of handling time prior to the ambient water incubation stressed the animals. It is also possible

290 that our hypolimnetic measurements may not reflect the actual CH₄ environment experienced by *Chaoborus* during gas uptake if they were burrowing into the anoxic sediments,¹³ where 291 292 porewater CH₄ concentrations would likely be much higher than in the water column. We note 293 that it is possible that some CH₄ may have been released from the BVR *Chaoborus* as a result of 294 the light and pressure changes that occurred during their collection with the Schindler trap, but 295 we think that any CH₄ loss was likely minimal due to the short duration of time from when the 296 Chaoborus were collected at depth and raised to the surface (<30 seconds) and the beginning of 297 the flask incubations (10-30 minutes).

298 Despite the higher CH₄ release rate in *Chaoborus* measured in this study, the flux of CH₄ 299 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 300 waterbody with 2,000 to 130,000 Chaoborus m⁻², Chaoborus could transport 10 to 2,000 mmol 301 CH₄ m⁻² yr⁻¹ from the sediments to the surface waters.¹⁰ Those calculations were based on a fixed 302 303 gas sac volume (12 µL) within each individual Chaoborus, not measurements of CH4 release 304 from Dagow Chaoborus, and assumed that all CH₄ was emitted from the Chaoborus' gas sacs at 305 the surface, with no CH₄ lost during the ascent. In BVR, which had a maximum of 3,600 - 6,000*Chaoborus* m^{-2} (within the range observed by ¹³), we calculated that the flux of CH₄ from the 306 hypolimnion into the epilimnion attributable to Chaoborus was 0.093 mmol CH₄ m⁻² yr⁻¹ in 307 August and 0.11 mmol CH₄ m⁻² yr⁻¹ in September. These rates are two to seven orders of 308 309 magnitude lower than the previous estimates and use the measured amount of CH₄ in *Chaoborus* 310 collected at the thermocline, which was one to two orders of magnitude lower than CH₄ 311 concentrations in *Chaoborus* collected near the sediments. If we used the highest measured CH₄ 312 concentration released from *Chaoborus* collected just above the sediments (0.016 μ M), the flux

313	estimates would increase to 10-17 mmol m ⁻² yr ⁻¹ . However, we note that these higher rates are
314	unlikely to be representative of BVR conditions because the $3,600 - 6,000$ Chaoborus m ⁻²
315	density is a maximum estimate and only 60-97% (mean $85 \pm 14\%$) of the BVR <i>Chaoborus</i>
316	population migrated above the thermocline at nighttime. Moreover, the flask incubations
317	demonstrate that Chaoborus consistently released most of their CH4 before they reached the
318	thermocline while migrating upwards (Figure 4). Consequently, we are confident that our
319	estimate of ~0.1 mmol CH ₄ m ⁻² yr ⁻¹ is realistic for BVR, and much lower than the potential
320	maximum contribution of \sim 40 mmol m ⁻² calculated from diffusive flux estimates above.
321	As a result of using Chaoborus collected from multiple depths and times, our study
322	reveals substantial variability in CH4 transport by Chaoborus that was masked in the earlier
323	study ¹⁰ , in which <i>Chaoborus</i> were collected from only one depth (1-2 m) and time (midnight).
324	Both Lake Dagow and BVR are similar in morphometry, trophic state, mixing regime, and
325	summer hypolimnetic oxygen depletion, ^{18,27} suggesting that the differences between studies are
326	likely due to physiological differences between Chaoborus populations and study methods.
327	Differences in BVR Chaoborus and CH4 dynamics between August and September are
328	likely due to both seasonal changes as well as the August storm. First, Chaoborus densities were
329	twice as high in August, yet the amount of CH4 transported by Chaoborus was twice as high in
330	September, likely due to the higher dissolved CH4 concentrations in the hypolimnion. The net
331	result of these two factors is that the amount of CH ₄ transported by <i>Chaoborus</i> was similar (~0.1
332	mmol CH ₄ m ⁻² yr ⁻¹) between sampling events. This finding suggests that <i>Chaoborus</i> density and
333	hypolimnetic CH ₄ concentration are both important drivers of the magnitude of CH ₄ Chaoborus
334	transport to surface waters. Second, CH4 diffusive flux in August exhibited diel fluctuations,
335	with a peak at dusk and efflux rates that were twice as high as in September, when no detectable

336 diel changes were observed. The higher efflux rates in August may be potentially due to 337 increased turbulence in water column from the storm, which could transport CH₄ from the lower 338 epilimnion to the surface (SI3). Following this hypothesis, depth profiles indicate that increases 339 in CH₄ in the epilimnion extended higher above the thermocline in August than September (SI7). 340 Regardless of the storm, the thermocline was ~ 1 m deeper in September than August, which also 341 could have reduced any effect of *Chaoborus*-derived CH₄ on efflux rates because most 342 Chaoborus did not migrate more than 3 m above the thermocline (Figure 2). The Chaoborus' 343 migration to the lower epilimnion (not surface) is likely because their zooplankton prey were primarily located just above the thermocline at nighttime (SI4), similar to other studies (e.g., ¹⁵). 344 345 Altogether, these observations suggest that physical mixing and other lake characteristics 346 influence the contribution of *Chaoborus*-mediated CH₄ transport to epilimnetic CH₄ 347 concentrations and diffusive efflux.

348 We cannot attribute the increases in CH₄ in the lower epilimnion at dusk solely to 349 Chaoborus transport because other processes, such as entrainment of CH4 across the 350 thermocline, likely also contributed. In addition, our study only investigated the effects of 351 *Chaoborus* on the vertical distribution of CH₄ in the water column and diffusive flux, not 352 ebullition: a past study found that *Chaoborus* bioturbation could substantially increase ebullitive CH₄ flux from the sediments.²⁹ Thus, we recommend that additional surveys of *Chaoborus* and 353 354 CH₄ dynamics be conducted in a range of waterbodies with different mixing patterns and 355 Chaoborus densities and species, and incorporate ebullition to quantify the importance of these 356 invertebrates to lake and reservoir CH₄ budgets.

This study addressed three questions: Q1) Do *Chaoborus* transport CH₄ from the hypolimnion and sediments to the epilimnion?; Q2) How does the concentration of CH₄ in

359 Chaoborus vary by depth and time of day?; and Q3) How does Chaoborus-mediated CH₄ 360 transport affect epilimnetic CH₄ concentrations and CH₄ efflux? For Q1, we observed that 361 Chaoborus collected from different depths in BVR transported CH₄ from hypolimnion to the 362 lower epilimnion, but the overall rate of CH₄ transport was much lower than estimated in an 363 earlier study.¹⁰ For Q2, the flask incubations revealed substantial temporal variability in CH₄ 364 transport by *Chaoborus*, with peak epilimnetic concentrations of CH₄ in *Chaoborus* observed 365 just above the thermocline at dusk, though hypolimnetic *Chaoborus* contained overall much 366 higher CH₄. For Q3, it appears that *Chaoborus* may contribute a small mass of CH₄ to the 367 epilimnion, which could potentially result in slightly higher CH₄ efflux in the nighttime. However, the magnitude of CH₄ transport was very low relative to seasonal fluctuations in CH₄, 368 369 suggesting that if CH₄ transport by *Chaoborus* does occur, it is highly sensitive to water column 370 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* dataset 371 372 highlighting the variability of *Chaoborus*-mediated CH₄ transport both over depth and time. 373 While our work indicates that *Chaoborus* may potentially increase epilimnetic CH₄ 374 concentrations, it is likely not a major pump of CH₄ from the hypolimnion to the surface waters 375 in lakes and reservoirs.

376

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386

Supporting Information. The Supporting Information includes a 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 CH₄ released
into flask headspace during the incubations (SI6); and dissolved CH₄ concentrations in the water
column during both diel sampling events.

394

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470 Figure Captions

471 Figure 1. Schematic of *Chaoborus* diel vertical migration (DVM) and potential transport of

472 methane (CH₄) from the hypolimnion and sediments into the epilimnion, where CH₄ increases

473 could result in higher diffusive flux rates.

474

475 Figure 2. Depth profiles from the surface to the sediments of water temperature (A) and

476 dissolved oxygen concentrations (D) in BVR from May to November 2016. The vertical black

477 lines denote the noon to noon diel sampling events on 3-4 August (B,E) and 16-17 September

478 (C,F). The inverted triangles on the top of the plots denote sampling times; the intervening data479 were interpolated.

480

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.

485

Figure 4. The mean ± standard error concentration of CH₄ 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. CH₄ concentrations within *Chaoborus*consistently decreased as they migrated upwards from the hypolimnion to lower epilimnion at
dusk; the thermocline is represented by the horizontal lines.

491

492 Figure 5. Volume-weighted *Chaoborus* density in the 3 m-layer above the thermocline (the lower

493	epilimnion) during the 3-4 August (top) and 16-17 September (bottom) diel sampling events (left
494	y-axis), in comparison to the volume-weighted dissolved CH ₄ concentrations and CH ₄
495	concentrations within <i>Chaoborus</i> in the same layer (right y-axis). The amount of CH ₄ transported
496	within Chaoborus was four to five orders of magnitude below the ambient dissolved CH4
497	concentrations in the water.
498	
499	Figure 6. (A) Diffusive efflux of CH ₄ peaked at dusk on 3-4 August (solid line) but did not
500	exhibit any diel changes on 16-17 September (dashed line). (B) Diffusive efflux of CH ₄
501	calculated from daytime CH ₄ profiles collected throughout the summer stratified period was
502	much higher than any diel increase potentially attributable to Chaoborus, with seasonal efflux
503	rates peaking at fall turnover in November. The vertical black lines denote the noon to noon diel
504	sampling events on 3-4 August and 16-17 September. (C) CH ₄ concentrations in BVR were
505	consistently much higher in the hypolimnion than epilimnion, reaching 842 μ M on 20
506	September. The inverted triangles on the top of the panel denote sampling times for the
507	fortnightly monitoring; the intervening data were interpolated.