UNIVERSITÉ DU QUÉBEC À MONTRÉAL

COMPRENDRE L'INFLUENCE DE LA MIGRATION DE *CHAOBORUS* SP. SUR LA DYNAMIQUE DU MÉTHANE DANS LES LACS À L'AIDE DE MÉSOCOSMES EXPÉRIMENTAUX

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MARTIN DEMERS

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CH_4	Méthane
CO_2	Dioxyde de carbone
O_2	Oxygène
UQAM	Université du Québec à Montréal
Chl*a*	Chlorophylle *a*
pH	Potentiel hydrogène
H	Hypothèse
Zoo	Zooplanctons
Chao	*Chaoborus*
13C	Carbone 13

DVM	Diurnal vertical migration
DOC	Dissolved organic carbon
DIC	Dissolved inorganic carbon
DO	Dissolved oxygen
TP	Total phosphorus
TN	Total nitrogen
SD	Standard deviation
SE	Standard error

LISTE DES SYMBOLES ET DES UNITÉS

cm	Centimètre
mm	Millimètre
µm	Micromètre
µl	Microlitre
mL	Millilitre
l	Litre
m	Mètre
ha	Hectare
m^2	Mètre carré
m^3	Mètre cube
h	Heure
mmol	Milimole
µmol	Micromol
d	Day
y	Year
p	p-value
%	Pour cent
‰	Pour mille
Tg	Téragramme
mg/L	Milligramme par litre
±	Plus ou moins
<	Plus petit
>	Plus grand
=	Égal
~	Environ
khz	Kilohertz
nm	Nanomètre
ind	Individus

RÉSUMÉ

Les lacs du nord sont potentiellement d'importants émetteurs de méthane (CH_4), un puissant gaz à effet de serre. Cependant, la dynamique du CH_4 est complexe et la régulation des flux lacustres n'est toujours pas bien comprise. Le CH_4 est principalement produit dans les sédiments, où il s'accumule et est relâché par ébullition ou par diffusion dans la colonne d'eau. Les larves de *Chaoborus* (Diptera-Chaoboridae) sont des agents de bioturbation importants en raison de leur migration journalière des sédiments vers la colonne d'eau, ce qui augmente le transport de CH_4 vers la colonne d'eau. De plus, ces larves utilisent le CH_4 des sédiments pour gonfler leurs sacs trachéaux, acquérant de la flottabilité pour faciliter la migration verticale, puis libèrent le CH_4 à la surface lors du retour dans les sédiments. Malgré leur rôle potentiel en tant que transporteurs passifs et actifs de CH_4, il existe très peu d'informations sur la contribution des activités de *Chaoborus* aux émissions globales de CH_4. Nous avons utilisé des mésocosmes expérimentaux dans deux lacs du Québec pour quantifier l'effet de différentes densités larvaires sur la dynamique du CH_4 en mesurant sa concentration et sa signature isotopique à différentes profondeurs au cours de la migration. Nos résultats montrent que la présence de *Chaoborus* n'a pas affecté les concentrations épilimnétiques de CH_4 et les flux dans l'atmosphère. Cependant, notre étude démontre que les larves augmentent considérablement les concentrations de CH_4 hypolimnétique au cours de la migration en raison de la bioturbation. Une partie de ce CH_4 est transportée dans la colonne d'eau alors que la majeure partie est oxydée, mais il y a une accumulation de ce nouvel apport dans l'hypolimnion anoxique pendant la saison estivale. Cette accumulation peut être importante pour les flux de CH_4 lacustre lors du renouvellement à l'automne. Cette étude met en évidence le rôle potentiel de l'activité biologique dans la régulation de la dynamique du CH_4 dans les lacs.

MOTS CLÉS : Transport de méthane, larves, migration, mésocosmes, bioturbation

ABSTRACT

Northern lakes are potentially important methane (CH_4) emitters, but CH_4 dynamics are complex and the regulation of their fluxes in lakes is still not well understood. CH_4 is mostly produced in sediments, where it accumulates, and is released by ebullition or by diffusion to the water column. The sediment release pathways play a critical role in shaping ecosystem methane fluxes. *Chaoborus* larvae (Diptera-Chaoboridae) are important bioturbation agents due to their daily migration from sediments to the water column, which increase CH_4 transport to the water column. Moreover, these larvae use CH_4 from sediments to inflate their tracheal sacs, gaining buoyancy to facilitate vertical migration, and then releasing the CH_4 at the surface when returning to the sediments. Despite their potential role as passive and active CH_4 transporters, very little information exists on the contribution of *Chaoborus* activity to overall CH_4 emissions from lakes. We used experimental mesocosms in two Québec lakes to quantify these effects of *Chaoborus*. We evaluated the effect of different larval densities on water column CH_4 dynamics by measuring CH_4 concentration and its isotopic signature at different depths during diel vertical migration. Our results show that the presence of *Chaoborus* did not affect the epilimnetic CH_4 concentrations and fluxes to the atmosphere. However, *Chaoborus* larvae significantly increased hypolimnetic CH_4 concentrations during migration due to their bioturbation behavior. We show that some of this CH_4 is transported through the water column while most of it is oxidised when the water is still oxic. However, there is still an accumulation of this new CH_4 import from bioturbation in the anoxic hypolimnion over the summer season. This accumulation may be important for CH_4 lake fluxes during the turnover in autumn. This study highlights the potential role of biological activity in the regulation of CH_4 dynamics in lakes.

KEY WORDS: Methane transport, larvae, migration, mesocosms, bioturbation

INTRODUCTION

Le méthane (CH_4) est un important gaz à effet de serre qui contribue à environ 20% de l'effet de serre global et il est 23 fois plus puissant que le CO_2 (Bastviken 2009). Les lacs sont des sources naturelles de CH_4, émettant de 8 à 73 Tg de CH_4 par année dans l'atmosphère, ce qui représenterait 2 à 21% des émissions naturelles globales (Saunois et al. 2016b). Ces estimations restent cependant encore incertaines et controversées. De même, certains des facteurs contrôlant les mécanismes de transport du méthane dans les écosystèmes lacustres sont encore incompris. Le CH_4 est principalement produit dans les lacs à la suite de la décomposition de la matière organique par les microorganismes en milieu anoxique. Il peut ensuite être libéré dans l'atmosphère via trois voies principales (Bastviken 2009). Premièrement, le CH_4 dissout dans l'eau peut être émis dans l'atmosphère par diffusion à travers la colonne d'eau. Deuxièmement, le CH_4 peut être émis sous forme des bulles voyageant directement des sédiments anoxiques à la surface de l'eau, un phénomène appelé ébullition. Finalement, le CH_4 peut être transporté des sédiments à l'atmosphère à travers les canaux racinaires des plantes émergentes (Bastviken 2009).

La faune benthique peut avoir une forte influence sur le cycle du CH_4 dans les écosystèmes lacustres en augmentant les échanges entre les sédiments et la colonne d'eau (White et Miller 2008). De récentes études suggèrent que les émissions de CH_4 peuvent être altérées par les larves de *Chaoborus* (Diptera-Chaoboridae), vivant dans les sédiments et dans la colonne d'eau (Gosselin and Hare 2003, Bezerra-Neto et al. 2012, McGinnis et al. 2017). La migration journalière verticale de *Chaoborus*, des sédiments vers la colonne d'eau, ainsi que leurs mouvements dans les sédiments peuvent augmenter les émissions de CH_4 par diffusion et ébullition dans les lacs. McGinnis et al. (2017) ont démontré que les larves de *Chaoborus* emplissent leurs sacs trachéaux du méthane dissout dans l'eau, ce qui leur permet de migrer vers la surface par flottaison. Les chercheurs suggèrent donc que la migration des larves de *Chaoborus* facilite le transport de CH_4 accumulé dans les sédiments vers l'atmosphère par diffusion. De même, ces larves tendent à s'enfouir dans les sédiments pendant la journée pour échapper aux prédateurs (Roth 1968, LaRow 1969), créant de la bioturbation et stimulant ainsi l'émission de méthane via diffusion et ébullition (Gosselin and Hare 2003). Bezerra-Neto et al. (2012) ont par ailleurs pu observer un grand volume de bulles émanant des sédiments pendant l'émersion de *Chaoborus*.

La migration de *Chaoborus* est étudiée par les chercheurs depuis près de 60 ans (Wood 1956; Northcote 1964; Roth 1968; Fedorenko et Swift 1972; Voss et Mumm 1999) ainsi que les facteurs qui l'influencent (LaRow 1968; Chaston 1969; LaRow 1970; Elser et al., 1987; Dawidowicz et al., 1990; Haney et al. 1990; Tjossem, 1990). Son rôle écologique majeur au sein des réseaux trophiques ainsi que ces relations proie-prédateur font de *Chaoborus* un sujet récurrent de la littérature (Dermott et al. 1977, Elser et al. 1987, Mackay et al. 1990, Moore et al. 1994). Bien que le potentiel qu'ont les larves de *Chaoborus* à influencer les émissions de CH_4 ait été clairement démontré, cet effet ne reste néanmoins que très rarement quantifié, et son ampleur est méconnue. De ce fait, l'effet des larves de *Chaoborus* n'est pas pris en considération dans les budgets de CH_4 des lacs. Étant un phénomène très ponctuel dans le temps, au lever et coucher du soleil, il n'est pas détecté par les mesures communément prises durant le jour. Ceci rajoute à l'incertitude déjà présente dans les estimations des émissions de CH_4 des lacs. *Chaoborus* est déjà très commun dans les lacs et les étangs (Eaton 1983) et sa distribution va augmenter avec les changements climatiques (Taylor et al. 2016). Sa contribution à l'effet de serre risque d'augmenter considérablement. En effet, Taylor et al. (2016) ont récemment souligné l'expansion de l'habitat de *Chaoborus* à travers les étangs nouvellement créés dans la toundra par la fonte du pergélisol. Ces larves subissent donc une expansion de territoire, formant ainsi rapidement de grandes populations stables dans la toundra sur une échelle de centaines de kilomètres (Taylor et al. 2016). La quantité de CH_4 libérée par ces macroinvertébrés pourrait donc s'accroitre dans le futur, d'où l'importance de comprendre les effets que ces organismes peuvent avoir sur la dynamique du CH_4 dans les écosystèmes lacustres.

0.1 ÉTAT DES CONNAISSANCES

0.1.1 Le méthane

Le méthane (CH_4) est un important gaz à effet de serre ayant un potentiel de réchauffement 23 fois plus puissant que le dioxyde de carbone (CO_2) et contribuant à environ 20% de l'effet de serre global (Bastviken 2009). Les lacs sont reconnus comme étant une source naturelle importante de méthane atmosphérique, émettant de 8 à 73 Tg de CH_4 par année dans l'atmosphère. Ceci représenterait 2 à 21% des émissions naturelles globales (Saunois et al. 2016a). Cependant, ces chiffres sont encore très incertains en raison des mesures limitées sur le terrain et des grands inconnus dans la variabilité spatio-temporelle des émissions (Wik et al. 2013). Les estimations des études actuelles sont présentement débattues principalement à cause de la prédominance d'études à court terme et du faible nombre d'études qui s'intéressent spécifiquement sur les émissions de CH_4 dans les lacs (Bastviken 2009).

Le méthane est produit suite à la décomposition de la matière organique par les archéobactéries méthanogènes, essentiellement en milieu anoxique comme dans les sédiments lacustres. Il peut ensuite être libéré dans l'atmosphère via trois voies principales (Bastviken 2009). Premièrement, le CH_4 dissout à la surface de l'eau peut être émis vers l'atmosphère par diffusion à travers la colonne d'eau, ce qu'on appelle aussi le flux diffusif. Dans les lacs stratifiés, le CH_4 produit dans les sédiments peut s'accumuler au fil du temps dans l'hypolimnion et être relâché vers la surface et vers l'atmosphère pendant les périodes de brassages saisonniers des eaux, provoquant ainsi des pics de flux diffusif de CH_4 (Bastviken et al. 2004). Deuxièmement, le CH_4 accumulé dans les sédiments anoxiques peut être émis sous forme de bulles migrant rapidement à travers la colonne d'eau jusqu'à la surface, un phénomène appelé ébullition (Bastviken 2009). Ce processus est facilité par la faible solubilité du CH_4 dans l'eau, de sorte que l'environnement est rapidement sursaturé en CH_4 et crée facilement des bulles (Casper et al. 2000). Le taux d'ébullition est étroitement lié au taux de production de CH_4 dans les sédiments ainsi qu'à la pression hydrostatique (Bastviken 2009). Les bulles sont ainsi relâchées lorsque la somme des pressions partielles du CH_4 dissous dans les sédiments dépasse la pression hydrostatique (Chanton et al. 1989). Finalement, le CH_4 peut être transporté des sédiments vers l'atmosphère à travers les canaux racinaires des plantes émergentes. Plusieurs plantes aquatiques transportent de l'air aux racines pour les approvisionner avec de l'oxygène. Un tel système de transport transfère aussi du CH_4 à partir des racines jusqu'aux feuilles et à l'atmosphère (Bastviken, 2009). La morphologie du lac, la productivité et la quantité de carbone organique qui arrive dans le lac sont des facteurs importants dans la dynamique du CH_4 en milieu lacustre (Bastviken et al. 2004). De plus, le taux d'ébullition montre une forte variabilité saisonnière, soit des valeurs plus faibles pendant l'hiver et des flux plus élevés pendant l'été. Cette relation reflète la dépendance de la production de CH_4 à la température de l'eau (Maeck et al. 2013). Ceci explique pourquoi les estimations des émissions de CH_4 des lacs varient largement. Des études plus approfondies sur certains de ses mécanismes derrière ces voies d'émissions nous permettront de mieux réduire l'incertitude de ces estimations des émissions.

0.1.2 Les larves de *Chaoborus*

0.1.2.1 Caractéristiques générales de Chaoborus

La larve de *Chaoborus* est aussi connue sous le nom de "larve fantôme" ou encore "vers de cristal". Son nom commun lui vient de sa couleur transparente ainsi que de sa forme longue et mince (Von Ende 1982). Elle fait partie de la famille des *Chaoboridea* et il existe plusieurs espèces de *Chaoborus*. Ces larves sont très communes dans les lacs et les étangs des écosystèmes tempérés et tropicaux et les principales espèces qu'on retrouve dans les lacs des régions tempérées sont *C. punctipenis*, *C. flavicans*, *C. trivittatus* et *C. americanus* (Eaton 1983, Moore et al. 1994, Taylor et al. 2016). *Chaoborus* passe la majorité de sa vie sous forme larvaire (Fedorenko and Swift 1972; Von Ende 1982). Il existe quatre stades larvaires. Les premiers et les seconds stades sont toujours limnétiques, c'est-à-dire qu'ils vivent dans la zone supérieure d'un lac (épi-métalimnion), et sont attirés par la lumière. Les troisièmes et quatrièmes stades sont principalement limnétiques, mais ils peuvent aussi être benthiques, c'est-à-dire la zone inférieure d'un lac (hypolimnion et sédiments) et ils fuient la lumière. Les larves se développent jusqu'au stade final en quatre à six semaines dans les régions tempérés (Wood 1956, Von Ende 1982). Les stades nymphe et adulte sont très brefs, ils durent de deux à quatre jours et moins de six jours respectivement. *Chaoborus* est donc principalement aquatique, mais il est aussi considéré comme un animal terrestre (Wood 1956; Diomande et al. 2010). Son cycle de vie est généralement univoltin, c'est-à-dire que son cycle de vie dure une saison, donc une génération par année. Cependant, en fonction des conditions de l'habitat, du climat et de l'espèce, il est possible d'y avoir deux à trois générations de *Chaoborus* par année dans certains lacs (Stahl 1966). Certaines espèces comme *C. punctinpenis* et C. *trivitatus* sont considérées comme ayant deux générations par an, soit une génération d'hiver (se développant pendant l'été, passant l'hiver sous la glace et émergeant au printemps) et une génération d'été (se développant durant l'été et émergeant au début de l'automne) (Fedorenko and Swift 1972, Eaton 1983). Au sein d'un même lac, une à trois espèces de *Chaoborus* sont généralement présentes (Roth 1968, Fedorenko and Swift 1972).

0.1.2.2 Écologie trophique de Chaoborus

Les larves de *Chaoborus* sont considérées comme des prédateurs féroces et voraces. Elles peuvent se nourrir d'une grande variété d'invertébrés, dont principalement de copépodes, de rotifères et de cladocères, tout dépendant du stade larvaire et de la taille de l'individu (Stahl 1966; Swift and Fedorenko 1975). Elles peuvent aussi se nourrir de larves de chironomides, d'oligochètes ainsi que d'autres larves de diptères (Stahl 1966). Les larves de *Chaoborus* sont opportunistes dans leur alimentation. N'ayant pas un comportement de chasseur, ces larves sélectionnent toute proie qui se déplace près d'elles et qui peut entrer entre leurs mandibules, pièces buccales qu'elles utilisent principalement pour se nourrir (Swift and Fedorenko 1975). Malgré le fait que les larves de *Chaoborus* sont considérées comme de grands prédateurs, le zooplancton n'est pas leur seule source de nourriture, elles sont omnivores. Même si les proies principales sont abondantes, elles se nourrissent fréquemment d'une espèce de phytoplanctons, des phytoflagellés qui sont détectables par les larves à cause de leur mobilité (Moore et al. 1994, Liljendahl-Nurminen et al. 2002). Tout au long du développement des larves et des saisons, il y a aussi des changements dans leurs préférences de proies (Moore et al. 1994). Ces changements dans leur régime alimentaire dépendent surtout de la grosseur de la larve, plus spécifiquement de la grosseur de ses mandibules (Stahl 1966, Swift and Fedorenko 1975). Les larves de *Chaoborus* se nourrissent uniquement pendant la nuit dans la colonne d'eau, à proximité de la surface (Nilssen 1974). Elles utilisent des récepteurs sensibles aux vibrations pour capturer leurs proies, ce qui leur permet donc de se nourrir dans la noirceur totale (Swift and Fedorenko 1975). La consommation de quantité importante de zooplanctons ainsi que de phytoflagellés par les larves *de Chaoborus* peut avoir d'importantes répercussions dans le transfert de carbone organique de la chaine alimentaire microbienne jusqu'au niveau trophique le plus élevé dans les lacs (Moore et al. 1994). De plus, ce prédateur est aussi la proie principale de plusieurs espèces de poissons planctivores (Dermott et al. 1977, Elser et al. 1987, Tjossem 1990). *Chaoborus* occupe donc une position centrale dans plusieurs réseaux trophiques lacustres. Un changement dans la composition et/ou dans la densité de *Chaoborus* peut complètement modifier le réseau trophique d'un lac et avoir un effet indirect sur les communautés planctoniques et microbiennes (Dermott et al. 1977; Elser et al. 1987).

0.1.2.3 Comportement migratoire

La plupart des larves de *Chaoborus* effectuent une migration verticale, c'est-à-dire qu'ils se déplacent vers la surface de l'eau la nuit pour se nourrir et retournent vers le

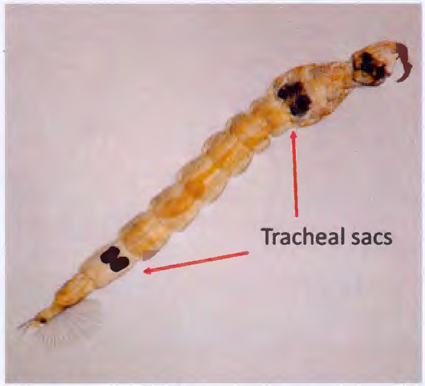


Figure 0.1 : Larve de *Chaoborus punctipennis* avec mise en évidence des sacs trachéaux

fond de l'eau, ou dans les sédiments, le jour pour se protéger de ses prédateurs (Northcote 1964; Roth 1968; LaRow 1969). Pour ce faire, les larves de *Chaoborus* régulent leur position dans la colonne d'eau en augmentant ou en diminuant le volume de gaz dans leurs deux pairs de sacs d'air (Teraguchi 1975a). Ces derniers sont en fait des organes hydrostatiques, faits à partir d'extension de tubes trachéaux (Figure 0.1). Ces sacs leur permettent de contrôler leur flottabilité, ce qui leur permet de demeurer stables dans l'eau, mais aussi de se déplacer au travers des zones stratifiées d'un lac (Teraguchi 1975a, 1975b). Les parois de ces sacs sont semi-rigides et elles peuvent ajuster leur volume à +/- 20 - 25%, qui est pour une larve moyenne de 0,12µL (Teraguchi 1975a). L'expulsion de gaz (contraction du sac) permet la descente de l'organisme, tandis que l'injection de gaz (expansion du sac) va entrainer l'organisme à s'élever dans la colonne d'eau. L'expansion initiale des sacs est causée par une force d'extension qui peut être générée par des muscles ou par la pression des gaz environnants (Teraguchi 1975b). Ensuite, l'augmentation ou la réduction de la quantité de gaz retenus par les sacs est due au revêtement cuticulaire des sacs qui joue un rôle actif dans le processus d'ajustement (Teraguchi 1975b, 1975a). Les gaz retenus dans les sacs reflètent la composition en gaz dissous de l'eau environnante, car la paroi des sacs est perméable aux gaz dissous (Teraguchi 1975b). Lors de leur migration, il est possible de distinguer trois phases évidentes. Au coucher du soleil, les larves de *Chaoborus* entament la montée (Rising), une fois près de la surface elles y restent un moment pour se nourrie et ensuite commencent à descendre lentement dans la colonne d'eau (Night sinking). Finalement, au lever du soleil, elle commence à descendre plus rapidement vers les sédiments (Dawn descent) (Teraguchi and Northcote 1966).

La cause principale du comportement migratoire des larves de *Chaoborus* est d'éviter les prédateurs (Swift 1976). L'amplitude de la migration verticale est majoritairement liée à l'intensité de la prédation exercée par les poissons planctivores (Dawidowicz et al. 1990), dont les larves détectent les signaux chimiques (kairomones) qui leur sont associés (Northcote 1964, Tjossem 1990). Bien que secondaire, la luminosité reste un facteur crucial dans la régulation de la migration, plus particulièrement dans la phase d'initiation (Teraguchi and Northcote 1966). Un seuil d'intensité lumineuse est nécessaire à *Chaoborus* pour commencer leur migration dans la colonne d'eau. À la sortie des sédiments, les larves testent, en quelque sorte, la condition de lumière à l'interface eau-sédiment avant d'entamer leur migration (Chaston 1969, LaRow 1969). Si l'intensité de la lumière est au-dessus du seuil, les larves s'enfouissent à nouveau dans les sédiments ou restent simplement à la surface de ceux-ci (Chaston 1969, LaRow 1969). Le mécanisme régulant leur mouvement dans les sédiments est continuellement réinitialisé pour se synchroniser avec les longueurs des journées qui changent durant l'année (Teraguchi and Northcote 1966, LaRow 1969).

Les larves peuvent facilement s'adapter à un changement brusque dans leur environnement en modifiant leur alimentation et leur comportement de migration (Dermott et al. 1977). La distribution verticale des larves de *Chaoborus* dans la colonne d'eau est différente entre les stades larvaires (Teraguchi and Northcote 1966). Plusieurs chercheurs ont démontré que les jeunes larves tendent à être continuellement pélagiques tandis que les larves de stade 3 et 4 tendent à être benthiques durant le jour et effectuent une migration verticale la nuit (Wood 1956, Stahl 1966, Sardella and Carter 1983, Voss and Mumm 1999). L'observation de l'absence de migration verticale chez les plus jeunes stades larvaires dans les lacs avec présence de poisson suggère donc qu'elles sont moins vulnérables aux prédateurs planctivores. Ceci est expliqué principalement par leur petite taille ainsi que par leur transparence (Teraguchi and Northcote 1966). Il est possible que certaines larves de stades larvaires 1 et 2 migrent, mais elles ne s'enfouissent pas, comparées aux stades 3 et 4 (LaRow 1969).

L'amplitude de la migration augmente donc avec le stade larvaire (Voss and Mumm 1999). La distribution des différentes espèces de larves dans la colonne d'eau peut varier, mais aucune d'elle n'est retrouvée à moins d'un mètre de la surface (Roth 1968). Lorsqu'il y a plusieurs espèces, leurs phases de migration (montée et descente) sont intercalées dans le temps, ce qui réduit la compétition pour la nourriture, permettant ainsi une telle coexistence entre les espèces (Roth 1968, Fedorenko and Swift 1972). Une plus grande densité et diversité de larves sont généralement retrouvées dans les zones profondes, typiquement au centre du lac, qui procure un meilleur abri contre les prédateurs et des sédiments peu compact dans lequel les larves peuvent s'enfouir plus facilement (Parma 1971, Rabette and Lair 1999, Voss and Mumm 1999, Liljendahl-Nurminen et al. 2002, White and Miller 2008).

L'amplitude de migration des larves change aussi au cours de la saison. Dans les régions tempérées, la période des grandes migrations est de juin à août, ce qui correspond aussi à la période de forte prédation. Les journées durant cette période sont plus longues et la lumière du jour pénètre davantage en profondeur dans la colonne d'eau (Wood 1956, Voss and Mumm 1999). On observe une diminution de la migration vers la fin de l'été, car les larves plus matures, ayant atteint leur croissance maximale, ont moins besoin d'aller se nourrir (Tjossem 1990). À l'automne, la plupart des larves cessent la migration et restent au fond de l'eau sans nécessairement s'enfouir dans les sédiments comme en été (Voss and Mumm 1999, Liljendahl-Nurminen et al. 2002). En hiver, les larves qui n'ont pas émergé s'accumulent dans les zones plus profondes et s'enfouissent de nouveau dans les sédiments, cette fois plus profondément, pour y rester tout l'hiver (Rabette and Lair 1999, Liljendahl-Nurminen et al. 2002). L'augmentation de la température dans les sédiments ainsi que de la luminosité au printemps pourrait être la cause de la réactivation de l'activité métabolique de larves et contribuer à leur retour dans la colonne d'eau (Parma 1971, Rabette and Lair 1999). Outre la migration verticale, les larves effectuent aussi une migration horizontale pendant la saison. En effet, la pupation se produit généralement dans les zones moins profondes du lac. Les larves migrent vers les zones littorales peu de temps avant leur émergence, où la température est plus élevée, ce qui favorise le développement et accélère le processus de pupation (Wood 1956, Roth 1968, Liljendahl-Nurminen et al. 2002).

0.1.2.4 Comportement d'enfouissement

Le facteur principal contrôlant l'enfouissement des larves dans les sédiments est la concentration en oxygène (O_2) dans l'hypolimnion. Plus la concentration en O_2 est élevée dans l'hypolimnion, plus la densité de larves augmente dans les sédiments pour se protéger des possibles prédateurs qui peuvent s'y retrouver (Rabette and Lair 1999). Tandis que plusieurs zooplanctons ont besoin d'oxygène pour respirer, les larves de *Chaoborus* utilisent un cycle de malate anaérobique pour leur fournir en énergie quand l'oxygène est manquant dans la zone hypolimnique ce qui leur permet de résister à une hypoxie prolongée (LaRow 1970, Wissel et al. 2003). Lorsqu'une grande partie de l'hypolimnion est anoxique, ce dernier devient donc un refuge contre la prédation, car les poissons ont besoin d'une plus grande concentration en oxygène que *Chaoborus* pour survivre. L'enfouissement des larves n'est donc pas nécessaire et elles restent à la surface des sédiments (LaRow 1970, Voss and Mumm 1999, Wissel et al. 2003).

0.1.3 Le lien entre le méthane et *Chaoborus*

La faune benthique peut avoir une forte influence sur le cycle du méthane dans les écosystèmes lacustres en augmentant les échanges entre les sédiments et la colonne d'eau (White et Miller 2008). Cependant, le rôle de ces macroinvertébrés comme vecteurs dans les émissions de CH_4 demeure peu étudié. De récentes études suggèrent que les émissions de CH_4 peuvent être augmentées par les larves de *Chaoborus* (Diptera-Chaoboridae), vivant dans les sédiments et dans la colonne d'eau des lacs (Gosselin and Hare 2003, Bezerra-Neto et al. 2012, McGinnis et al. 2017). Les chercheurs suggèrent que le comportement migratoire des larves de *Chaoborus* facilite le transport de CH_4 accumulé dans les sédiments vers l'atmosphère par diffusion et ébullition, augmentant ainsi les émissions de CH_4 des lacs (Figure 2).

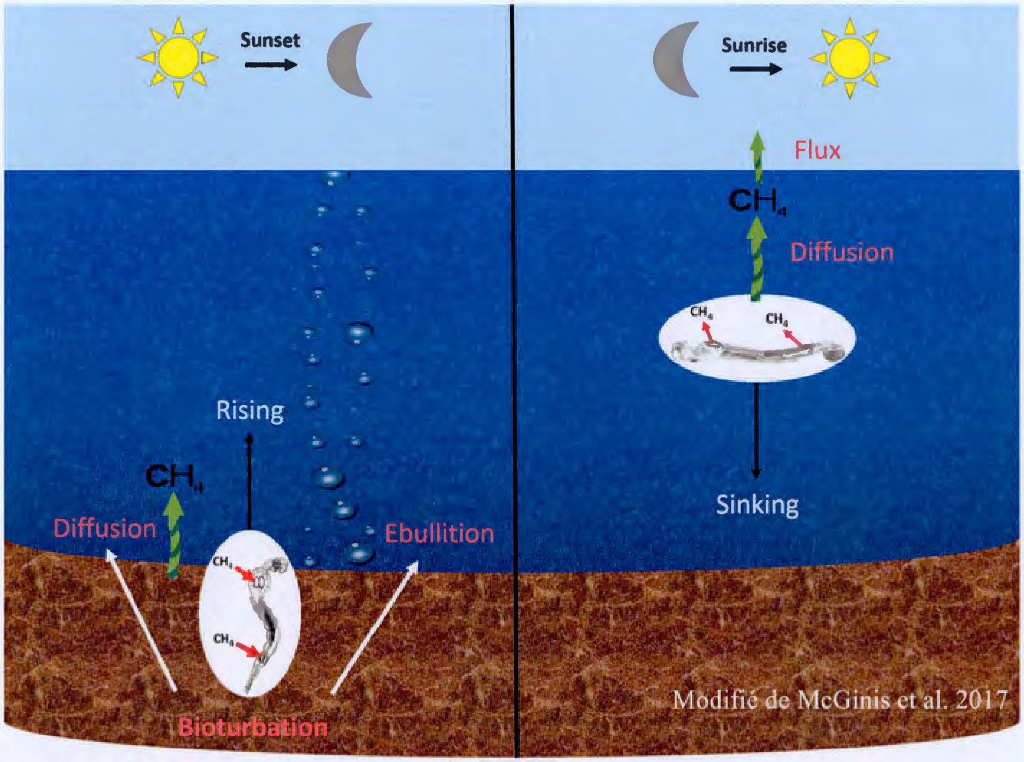


Figure 0.2 : Schéma du lien entre la migration de *Chaoborus* et des émissions de CH_4 dans les lacs

0.1.3.1 Relâchement du CH_4 pendant la migration

Selon une étude de McGinnis et al. (2017), dans les lacs ayant une forte densité de larves de *Chaoborus* (jusqu'à 130 000 ind. m^{-2}), ces larves émettraient un flux de CH_4 comparable au flux typique causé par le phénomène d'ébullition. McGinnis et al. (2017) ont pu démontrer que les larves utilisent la haute pression partielle du CH_4 dissout pour gonfler leurs sacs trachéaux. Le CH_4 dissout, étant généralement sursaturé dans les sédiments, fournit une pression partielle assez élevée pour gonfler les sacs trachéaux, comparé au dioxyde de carbone (CO_2) qui est aussi présent dans les sédiments, mais 27 fois plus soluble, fournissant une pression partielle insuffisante pour l'expansion des sacs (Sander 2015, McGinnis et al. 2017). Ce mécanisme fournit un avantage énergétique significatif qui permet aux larves de migrer par flottaison passive plutôt que par la nage qui peut être très coûteuse en énergie (McGinnis et al. 2017). Cette migration verticale journalière de *Chaoborus* peut donc faciliter le transport de CH_4 accumulé dans les sédiments vers l'atmosphère. En effet, lors de la descente des larves dans la colonne d'eau, elles libèrent à la surface, le gaz accumulé dans leurs sacs trachéaux contenant du CH_4 pouvant ainsi perturber le flux diffusif du CH_4 dans l'atmosphère (McGinnis et al. 2017).

0.1.3.2 Relâchement du CH_4 par la bioturbation

La bioturbation consiste à la perturbation des sédiments par un organisme vivant, soit par pénétration à la surface des sédiments ou par mouvement interne (White and Miller 2008). L'enfouissement des macroinvertébrés peut augmenter l'aire de surface de l'interface eau-sédiments ainsi que les échanges entre les sédiments et la colonne d'eau. Cela peut affecter entre autres le cycle du CH_4 dans les écosystèmes lacustres (Charbonneau and Hare 1998, Gosselin and Hare 2003, White and Miller 2008). De par leur migration journalière, les larves de *Chaoborus* sont de grands agents de bioturbation (Gosselin and Hare 2003). La perturbation des sédiments par *Chaoborus* peut augmenter significativement le flux de CH_4 provenant des sédiments dans la colonne d'eau par diffusion. De plus, la bioturbation effectuée par *Chaoborus* est susceptible de promouvoir le relâchement de CH_4 par ébullition. Des chercheurs ont remarqué, lors d'une émersion massive de larves de *Chaoborus*, un relâchement d'un grand volume de bulles à partir des sédiments (Bezerra-Neto et al. 2012). Juste avant de pénétrer dans les sédiments, les larves orientent leur corps perpendiculairement à l'interface eau-sédiments, avec la partie postérieure vers le haut. À partir de cette position, les larves retournent leur corps très rapidement afin de pousser leur extrémité postérieure dans les sédiments en premier. Une fois la partie postérieure entrée dans les sédiments, les larves conduisent leurs corps plus profondément dans les sédiments par une succession de mouvements latéraux très rapides (Gosselin and Hare 2003). Dans cette couche, les larves adoptent une position verticale, la partie antérieure vers le haut, en forme de S, sans connexion avec l'eau où elles pourraient pomper de l'eau oxygénée comme plusieurs autres zooplanctons le font (Gosselin and Hare 2003). Au moment de quitter les sédiments, les larves utilisent les mêmes mouvements de va-et-vient que lors de l’entrée (Gosselin and Hare 2003). À chaque mouvement latéral, le côté du corps larvaire entre en contact avec la surface des sédiments, ce qui provoque la levée de sédiments en un nuage autour de la larve (Gosselin and Hare 2003). Comparées à d’autres zooplanctons benthiques, les larves de *Chaoborus* ne se nourrissent pas dans les sédiments, ce qui limiterait la période de bioturbation seulement aux entrées et sorties des sédiments (Rabette and Lair 1999).

0.2 L'ENJEU

Bien que le potentiel qu'ont les larves de *Chaoborus* à influencer les émissions de CH_4 ait été clairement démontré, ces effets ne restent néanmoins que très rarement quantifiés, et leur ampleur est méconnue. Il serait donc important de comprendre et quantifier les effets que ces organismes peuvent avoir sur la dynamique du CH_4 dans les écosystèmes lacustres. De ce fait, la libération de CH_4 causée par les larves de *Chaoborus* n'est généralement pas prise en considération dans les budgets de CH_4 des lacs. Étant un phénomène très ponctuel dans le temps, au lever et coucher du soleil, il n'est pas détecté par les mesures communément prises durant le jour. Ceci s'ajoute à l'incertitude déjà présente dans les estimations des émissions de CH_4 des lacs. Ces effets directs ont été observés avec de grandes densités de larves seulement. Nous ne connaissons donc pas encore s'il y a des effets directs ou indirects lorsqu'il y a seulement une faible densité de *Chaoborus*. Cette étude, étant une des premières sur le sujet, permettra d'améliorer notre compréhension encore très préliminaire de cet aspect sur la dynamique lacustre du CH_4. De plus, il a récemment été démontré que *Chaoborus* étend son habitat à travers des étangs nouvellement créés dans la toundra par la fonte du pergélisol causé par le réchauffement climatique (Taylor et al. 2016). En effet, ces larves subissent une expansion de territoire, formant ainsi des populations stables dans la toundra sur une échelle de centaines de kilomètres. Ces nouveaux habitats sont très propices au développement des larves de *Chaoborus*, entre autres grâce au substrat boueux qui facilite l'hivernation ainsi qu'au manque de prédateurs. Ceci aide grandement à la colonisation de grandes populations rapidement (Taylor et al. 2016). Les lacs constituent une composante importante du cycle du CH_4 global et leur contribution risque de changer en réponse aux futurs changements climatiques. De grandes émissions de CH_4 sont déjà attendues des lacs créés par la fonte du pergélisol (Tranvik et al. 2009). De plus, la quantité de CH_4 libérée par ces macroinvertébrés pourrait s'accroitre dans le futur (Taylor et al. 2016). Il devient donc important de comprendre et de connaitre davantage les effets que ces organismes peuvent avoir sur la dynamique du CH_4 dans les écosystèmes lacustres.

0.3 HYPOTHÈSES

Le but de cette étude est d'évaluer et de quantifier les effets de *Chaoborus* sur les émissions de méthane dans un lac. Plus particulièrement, nous voulons suivre le mouvement des larves de *Chaoborus* et associer ces mouvements à la dynamique du CH_4 dans la colonne d'eau en utilisant des mésocosmes expérimentaux et des observations en milieu naturel (*in situ*) dans deux lacs du Québec. Nous voulons donc mesurer un changement dans la concentration du CH_4 à différents moments qui pourrait être causé par la migration des larves et la bioturbation créée par ces dernières. Le tout sera évalué lors des pics d'activité des larves, c'est-à-dire au lever et au coucher du soleil. Selon un gradient de densité de larves, deux hypothèses principales peuvent être émises :

> H1 : Il y aura une augmentation du taux de relâchement de CH_4 et de la concentration de CH_4 dans l'hypolimnion lors de la sortie des larves de *Chaoborus* des sédiments.
>
> H1.1. : Le taux de relâchement de CH_4 ainsi que la concentration de CH_4 dans l'hypolimnion seront positivement corrélés à la densité de larves de *Chaoborus*.

À la sortie (coucher du soleil) des larves des sédiments, ces dernières perturbent les sédiments par bioturbation et créeraient des échanges à l'interface eau-sédiments ce qui augmenterait les apports en CH_4 par ébullition et diffusion.

> H2 : Il y aura une augmentation de la concentration de CH_4 dans l'épilimnion ainsi que du flux de CH_4 dans l'atmosphère lors de la descente des larves vers les sédiments.
>
> H2.1 : La concentration de CH_4 dans l'épilimnion ainsi que le flux de CH_4 dans l'atmosphère sera positivement corrélée à la densité de larves de *Chaoborus*.

Au lever du soleil, lors de leur migration vers les sédiments, les larves expulsent le méthane dont elles avaient préalablement empli dans leurs sacs d'air pour ajuster leur position dans la colonne d'eau.

CHAPITRE I

UNDERSTANDING THE INFLUENCE OF *CHAOBORUS* MIGRATION ON LAKE METHANE DYNAMICS USING EXPEMRIMENTAL MESOCOSMS

This chapter is written in the form of a scientific paper and will be submitted to a peer-reviewed journal in January 2020

1.1 ABSTRACT

Northern lakes are potentially important methane (CH_4) emitters, but CH_4 dynamics are complex and the regulation of their fluxes in lakes is still not well understood. CH_4 is mostly produced in sediments, where it accumulates, and is released by ebullition or by diffusion to the water column. The sediment release pathways play a critical role in shaping ecosystem methane fluxes. *Chaoborus* larvae (Diptera-Chaoboridae) are important bioturbation agents due to their daily migration from sediments to the water column, which increase CH_4 transport to the water column. Moreover, these larvae use CH_4 from sediments to inflate their tracheal sacs, gaining buoyancy to facilitate vertical migration, and then releasing the CH_4 at the surface when returning to the sediments. Despite their potential role as passive and active CH_4 transporters, very little information exists on the contribution of *Chaoborus* activity to overall CH_4 emissions from lakes. We used experimental mesocosms in two Québec lakes to quantify these effects of *Chaoborus*. We evaluated the effect of different larval densities on water column CH_4 dynamics by measuring CH_4 concentration and its isotopic signature at different depths during diel vertical migration. Our results show that the presence of *Chaoborus* did not affect the epilimnetic CH_4 concentrations and fluxes to the atmosphere. However, *Chaoborus* larvae significantly increased hypolimnetic CH_4 concentrations during migration due to their bioturbation behavior. We show that some of this CH_4 is transported through the water column while most of it is oxidised when the water is still oxic. However, there is still an accumulation of this new CH_4 import from bioturbation in the anoxic hypolimnion over the summer season. This accumulation may be important for CH_4 lake fluxes during the turnover in autumn. This study highlights the potential role of biological activity in the regulation of CH_4 dynamics in lakes.

KEY WORDS: Methane transport, larvae, migration, mesocosms, bioturbation

1.2 INTRODUCTION

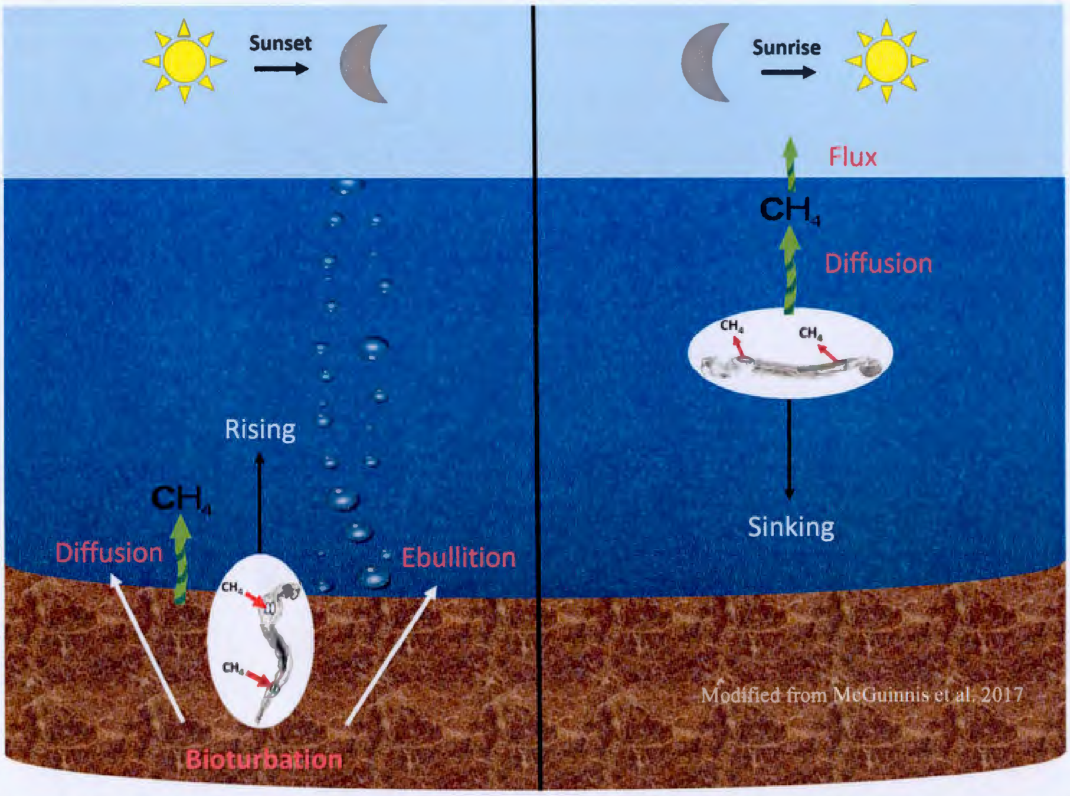
Methane (CH_4) is an important greenhouse gas that contributes about 20% to the global greenhouse effect and is 23 times more powerful than carbon monoxide (CO_2) (Bastviken 2009). Lakes are natural sources of CH_4, emitting 8 to 73 Tg of CH_4 per year into the atmosphere, representing 2 to 21% of global natural emissions (Saunois et al. 2016a). This estimate remains uncertain, in part due to a lack of knowledge of some mechanisms controlling CH_4 transport in lakes. Methane, in lakes, is mainly produced in anoxic environments following the decomposition of organic matter by methanogenic archaea. It can then travel and be released from the sediments to the atmosphere via three main routes: dissolved CH_4 can diffuse through the water column to the water surface, it can be emitted as bubbles travelling directly from anoxic sediments to the surface (ebullition) or, it can be transported through the vascular canals of emergent plants (Bastviken 2009).

Benthic fauna can have a strong influence on the CH_4 cycle in lake ecosystems by increasing the opportunity for exchange between sediments and the water column (White et Miller 2008). Recent studies suggest that CH_4 emissions may be altered by *Chaoborus* (Diptera-Chaoboridae) larvae; their diurnal vertical migration (DVM) from sediments to the water column and their movements within sediments have been shown to increase CH_4 emissions by diffusion and ebullition in lakes (Gosselin and Hare 2003, Bezerra-Neto et al. 2012, McGinnis et al. 2017). McGinnis et al. (2017) demonstrated that *Chaoborus* larvae fill their tracheal sacs with dissolved CH_4, allowing them to migrate to the surface by flotation, and further suggested that *Chaoborus* larvae migration facilitates the transport of CH_4 accumulated in sediments to the atmosphere by releasing the CH_4 accumulated in their tracheal sacs into the surface water, thus increasing CH_4 diffusion to the atmosphere. Also, *Chaoborus* larvae tend to burrow into sediments during the day to escape from predators (Roth 1968, LaRow 1969), creating bioturbation and thus stimulating CH_4 release from the sediment and subsequent transport to surface waters and emission via diffusion and ebullition (Gosselin and Hare 2003).

Although the potential for *Chaoborus* larvae to influence CH_4 emissions has been clearly demonstrated (Gosselin and Hare 2003, Bezerra-Neto et al. 2012, McGinnis et al. 2017, Carey et al. 2018, Tang et al. 2018), this effect remains only very rarely quantified, and its magnitude is unknown under densities typically found in northern lakes. *Chaoborus* generally plays a major ecological role in lake food webs via their predatory relationship with other plankton (Dermott et al. 1977, Elser et al. 1987, Mackay et al. 1990, Moore et al. 1994) and because they are very commonly present in lakes and ponds (Eaton 1983), *Chaoborus* migration has been studied by researchers for nearly 60 years (Wood 1956; Northcote 1964; Roth 1968; Fedorenko et Swift 1972; Voss et Mumm 1999), including the factors that influence the diurnal dynamics (LaRow 1968; Chaston 1969; LaRow 1970; Elser et al., 1987; Dawidowicz et al., 1990; Haney et al. 1990; Tjossem, 1990). Furthermore, the limits of *Chaoborus* distribution have been predicted to increase with climate change, including through newly established ponds in the tundra through melting permafrost (Taylor et al. 2016), and thus its contribution to the greenhouse effect via methane is likely to increase considerably. This range expansion, fuelled by climate change itself, is resulting in the appearance of new and stable populations of *Chaoborus* in water bodies across the tundra, where they were never recorded before (Taylor et al. 2016). Lakes, independently, are an important component of the global CH_4 cycle and their contribution is also likely to change in response to future climate change. Large CH_4 emissions are already expected from karst lakes created by melting permafrost (Tranvik et al. 2009). The amount of CH_4 released by their associated and range-expanding macroinvertebrates could therefore increase in the future; hence the importance of understanding the effects that these organisms may have on CH_4 dynamics in lake ecosystems.

1.2.1 CH_4 releasing during DVM

Previous work has demonstrated that in lakes with extremely high *Chaoborus* densities, the CH_4 flux generated by *Chaoborus* emptying their tracheal sacs in surface waters is comparable to that typically associated to ebullition in these same lakes (McGinnis et al. 2017). Utilization of tracheal sacs provides a significant energy benefit, allowing larvae to migrate diurnally by passive flotation rather than by swimming, which can be very energetically costly (McGinnis et al. 2017). Dissolved CH_4, being generally oversaturated in sediments, provides a relatively high partial pressure to inflate these tracheal sacs. Methane is thus preferred over CO_2, also present in sediments, but the former is 27 times more soluble and as a result, provides lower partial pressure for tracheal sac expansion (Sander 2015, McGinnis et al. 2017). *Chaoborus* DVM can therefore facilitate the transport of CH_4 accumulated in sediments to the atmosphere. At sunrise, *Chaoborus* release CH_4 accumulated in their tracheal sacs at the surface water enabling the descent of the larvae. This synchronized release of CH_4 at the surface has been hypothesized to potentially enhance diffusive flux of CH_4 in the atmosphere, provided that the density of the animals is sufficiently high (up to 130 000 ind. m^{-2}) (Figure 1.1).



Modified from McGuinnis et al. 2017

Figure 1.1: Schematic of the link between *Chaoborus* migration and CH_4 emissions in lakes.

1.2.2 CH_4 diffusion and ebullition by bioturbation

Burial activity by macroinvertebrates may increase the surface area of the water-sediment interface as well as the exchanges between the sediments and the water column. This can affect, among other things, the CH_4 cycle in lake ecosystems (Charbonneau and Hare 1998, Gosselin and Hare 2003, White and Miller 2008). Owing to their DVM, *Chaoborus* larvae are important bioturbation agents (Gosselin and Hare 2003). The disruption of sediments by *Chaoborus* can significantly increase CH_4 flux from sediments in the water column by diffusion. In addition, *Chaoborus* bioturbation is likely to promote the release of CH_4 by ebullition (Figure 1.1) as Bezerra-Neto et al. (2012) observed a large volume of bubbles emanating from sediments during *Chaoborus* rising. To enter, the larvae orient their bodies perpendicular to the water-sediment interface, very quickly pushing their posterior end into the sediment first and, using a succession of very fast lateral movements, bury themselves deeper into the sediments (Gosselin and Hare 2003). With each lateral movement, the larval body side comes into contact with the sediment surface, causing sediment to emerge in a cloud around the larva (Gosselin and Hare 2003). Unlike many other benthic zooplankton, *Chaoborus* larvae do not feed in the sediments, limiting the bioturbation period only to DVM-associated entrances and exits (Rabette and Lair 1999).

1.2.4 The current study

The purpose of this study was to evaluate and quantify the effects of *Chaoborus* on CH_4 emissions in lakes. Previous studies have focused on lakes with extremely high *Chaoborus* densities, but the extent to which the observed effects persist under more average densities is unknown. Here we tested *Chaoborus* effects under a range of densities that are more representative of those typically found in oligo- to mesotrophic temperate lakes, and simultaneously assessed several potential effects: an ability to influence CH_4 release from sediments through bioturbation, a role in transporting CH_4 to the surface, and also a role in influencing CH_4 processing within the water column of lakes. We followed the DVM movement of *Chaoborus* larvae and associated their activity to the observed CH_4 dynamics in the water column in experimental lake mesocosms, where we manipulated the density of *Chaoborus,* as well as in the surrounding waters of the two temperate Québec lakes in which the experiments occurred. We measured CH_4 concentration change at different times of the day and of the summer season, associated with larval migration and their bioturbation activity.

1.3 MATERIALS AND METHODS

1.3.1 Study site

The experiment took place in two small lakes typical of the Canadian Shield, located at the Station de Biologie des Laurentides (Saint-Hippolyte, Quebec, Canada). Lake Croche (45°59'34" N, 73°00'34" W) has an area of 17.9 ha, a maximum and mean depth of 11.4 m and 4.7 m respectively, and a volume of 836 000 m^3. The lake is oligo-mesotrophic and *Chaoborus punctipennis* is the dominant chaoborid species, with *Chaoborus flavicans* also rarely observed. Lake Cromwell (45°59'21" N, 73°59'55" W) has a smaller area (10.2 ha), a maximum and mean depth of 9.8 m and 3.5 m respectively, and a volume of 361 000 m^3. The chaoborid community of this mesotrophic lake is composed mainly of *Chaoborus trivittatus*. Both lakes are dimictic and their hypolimnia usually become anoxic towards mid to end of summer. The experiments and observations took place from July to August 2017.

1.3.2 Experimental design

We used mesocosms inoculated with two densities (High and Low) of *Chaoborus* larvae, each replicated twice in each lake, to evaluate the effects of theses densities on CH_4 dynamics associated to their migration. Comparison of simultaneous measurements taken in the lake and the mesocosms allowed a determination of the CH_4 released by the bioturbation of the *Chaoborus* larvae during their entry and exit from the sediments.

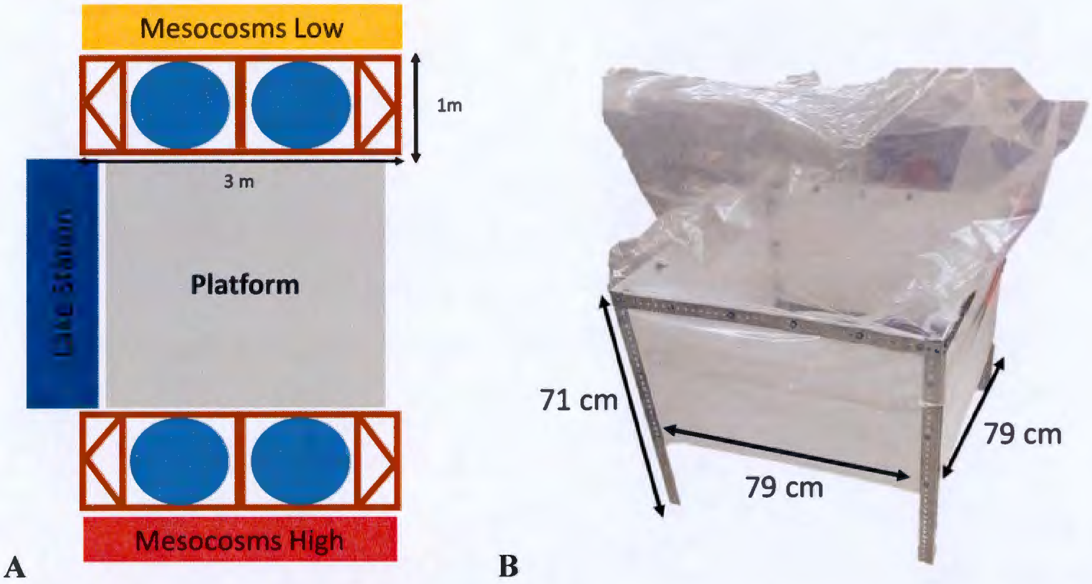


Figure 1.2: Schematic of the platform holding mesocosms, view from above (A) and a base prototype with a bag attached to it (B).

The mesocosms consisted of large, tubular plastic bags, 1m in diameter and 7m deep, suspended as replicate treatment pairs on a floating wooden structure (Figure 1.2A). The plastic liner was previously shown to be impermeable to CH_4 (Bogard et al. 2014) and therefore appropriate for this experimental set up. The top of the mesocosms was open to the air to allow gas exchange with the atmosphere. The bottom of the bags was attached to a base (79 cm x 79 cm x 71 cm) made of white plastic sheeting (5mm) and metal rods (Figure 1.2B), which was embedded in the sediments and maintained there with weights. The mesocosms thus isolated a column of water but had the same thermal and light properties as the surrounding lake. These floating wooden supports were attached along the sides of an anchored floating platform (Figure 1.2A), with one such platform in each lake.

The mesocosms were installed in each lake in May 2017 by lowering the empty bags with the aid of divers to the bottom of the lake, where the base was pushed into the sediments. At the time of deployment, some lake water entered the bags, but not enough to fill the bags completely. Filling of the bags was accomplished by pumping lake water directly from the epilimnion (1-2 m), leading to initial concentrations of zooplankton and *Chaoborus* similar to ambient values found in the surface waters of the lakes in May. A one-week acclimation period then allowed for the re-establishment of disturbed sediments and sediment anoxia so that CH_4 production was initiated prior to the introduction of additional experimental densities of zooplankton and *Chaoborus* larvae. A plastic tube (0.5 cm diameter) was mounted in each mesocosm, extending down to 1m above the sediment surface (6m in Croche and 5m in Cromwell), and was used to sample the hypolimnion of the mesocosms and the lakes from the platform without disturbing the water column and the sediments.

Zooplankton densities stocked in each mesocosms were concentrated (relative to the lake) along with low or high *Chaoborus* densities prior to the start of each observation period. At the beginning of every experiment (each month), crustacean zooplankton were added on days 1 and 2 (Table 1.1) to create densities 2-3 times higher in the Low and High mesocosms over those normally found in each lake. This was to ensure that there were sufficient prey items available to the *Chaoborus* in the mesocosms during the 5-day observation period. To concentrate zooplankton, we used repeated vertical hauls of a plankton net (54µm mesh, 0.5m diameter) through the lake water column from the deepest area of the lake. This collection was done during the day to minimize the capture of *Chaoborus* larvae; any *Chaoborus* were removed after visual inspection of the inocula. To achieve augmented *Chaoborus* densities in the High *Chaoborus* treatments, larvae were collected in the water column at night using the same zooplankton net (54µm mesh, 0.5m diameter), separately in each lake. *Chaoborus* larvae were isolated manually with a plastic pipette before addition to each mesocosm to avoid the addition of zooplankton. Following the introduction of *Chaoborus* larvae on days 1 and 2, we allowed 24 hours before any sampling or measurement were carried out (Days 2-3; Table 1.1).

1.3.3 Sampling

We performed two series of experiments, and a suite of biotic and abiotic variables were measured during the sampling period (5 days) of each month (July and August) in the mesocosms and in the surrounding waters of each lake (Table 1.1). Sampling always occurred first in lake Croche followed by Cromwell immediately after.

Table 1.1: The sampling schedule for the experimental mesocosms with the type of data collected on each day.

Day 1	Day 2	Day 3	Day 4	Day 5
Initial profiles (epi, meta and hypo) of all samples below Zoo + Chao additions (night)	Zoo + Chao additions (day)	First part of 24h sampling (Hypo – 16:00 to 00:00)	Second part of 24h sampling (Epi – 00:00 to 16:00)	Final profiles (epi, meta and hypo) of all samples below
		Samples		
CH_4 concentration + isotope TP, TN, DOC, DIC +chl*a*		CH_4 concentration + isotope CH_4 ebullition Echosounding Zoo+Chao density	CH_4 concentration + isotope CH_4 flux Echosounding	CH_4 concentration + isotope TP, TN, DOC, DIC + chl*a*

At the beginning and at the end of each experimental series (on days 1 and 5), water samples were taken from each of the layers (epilimnion [1m], metalimnion [3.5m Croche and 2.5 Cromwell], hypolimnion [6m Croche and 5m Cromwell]) of each mesocosm and lake, using a 7-meter long plastic tube (0.5 cm diameter) attached to a temperature sensor lowered at the desired depth. The samples were first put into 2L brown plastic bottles and then transferred partly into acid-washed 80-mL glass tubes for total phosphorus (TP) and total nitrogen (TN) concentrations and partly filtered through a 0.45-μm filter and collected in acid-washed 40-mL glass vials for dissolved organic and inorganic carbon (DOC and DIC). Surface water samples (30 cm depth) were also collected for chlorophyll *a* (chl*a*) analysis. All the samples were stored in the fridge at 4°C until lab analyses. To obtain CH_4 concentration and isotopic ^{13}C-CH_4 signature profiles, we used 60 mL of water from the thermal layers taken with the same plastic tube and collected into two 60-mL gas tight plastic syringes equipped with two-way Luer-lock valve. Half of the water in each syringe was replaced by ultrahigh purity zero air (Praxair, Canada) to create a headspace, then was shaken vigorously for at least 2 min to equilibrate the dissolved gases with the headspace. The equilibrated air was transferred using a needle (23G1; Becton Dickinson & Co., USA) attached to the Luer-lock valve to gas-tight 12-mL pre-evacuated exetainer vials (Labco Ltd., UK), for later concentration and isotopic analysis in the laboratory. A YSI multiprobe was used to obtain depth profiles (0.5m resolution) of dissolved oxygen levels, pH and temperature of the mesocosms and lake water column on days 1 and 5 (Table 1.1). The O_2 probe was calibrated with water vapor-saturated air at ambient pressure and surface water temperature before each profile measurement and the pH probe was calibrated everyday.

During the 24h sampling (days 3-4 of each experimental series), measurements were made at 3-hour intervals during the day, and at 1-hour intervals for around two hours prior and following sunrise and sunset. These later periods are associated with the peak activity of *Chaoborus* larvae: (i) sediment bioturbation by *Chaoborus* during exit around sunset and (ii) descent from surface waters around sunrise via tracheal sac emptying. During the first part of the 24h monitoring (16:00 to 0:00), 60 mL water samples were collected in syringes from the plastic tubes mounted 1m above the sediment surface to obtain CH_4 concentrations and CH_4 isotopic signatures with the headspace method described above (Day 3; Figure 1.3). A bubble trap was also deployed each time over the entire period (16:00 to 0:00) to measure CH_4 ebullition rates, corresponding to the total volume of CH_4 bubbles released during this period.

The bubble trap consisted of an inverted plastic funnel (63.5 cm diameter, 0.32 m^2 surface area). The neck of the funnel was fitted with a threaded plastic tube, which allowed the attachment of a submerged graduated 500 mL glass bottle. The funnel was kept afloat at 0.5m depth by attaching it to a cylindrical foam float of the same diameter as the funnel itself, with the collector bottle just below the water surface. At the time of deployment, the sample bottle was filled with lake water and attached to the neck of the funnel. No bubbles were detected during migration by any of the bubble traps deployed, or not enough gas was captured to effectively measure the volume, so this component will not be further discussed.

A net haul (54µm mesh, 0.05m diameter) was collected at midnight, through the water column of each mesocosm and lake (0-5m) to determine the densities of *Chaoborus* larvae and zooplankton. Zooplankton and *Chaoborus* were anesthetized with club soda and then preserved in 70% ethanol (final concentration). During the second part of the 24h monitoring (0:00 to 16:00), samples for CH_4 concentration and CH_4 isotopic signature were collected with 60 mL plastic syringes filled with water samples from the epilimnion (1m) using a 7-m long plastic tube (0.5 cm diameter) attached to a temperature sensor; following the headspace method described before (Day 4; Table 1.1). CH_4 fluxes at the air-water interface were measured at the surface of each mesocosm and the lake using a round floating chamber (0.9 m diameter, 16 L total volume) covered with aluminum foil to reduce solar heating and equipped with an internal thermometer to monitor temperature changes that may affect the exchange rates during the measurements (Vachon et al. 2010). The chamber was connected to an ultraportable greenhouse gas analyzer (UGGA, ABB-Los Gatos Research, USA). Measurements were conducted until a 5-min linear change in gas concentration was observed. The rates of change in pCH_4 in the chamber were used to estimate CH_4 fluxes (f_{CH4}, µmol m^{-2} d^{-1}) with the following equation:

$$f_{CH4} = \left(\frac{s \times V}{V_m \times S}\right) \times t \qquad (1)$$

where (s) is the rate of change of the gas in the chamber (μatm min^{-1}), (V) is the volume of the chamber (L), (S) is the surface area of the chamber (m^2), (V_m) is the molar volume (L mol^{-1}) adjusted for in situ temperature, and (t) is a conversion factor from minutes to a day and from mol to μmol. We further calculated the gas transfer coefficients (k, m d^{-1}) based on CH_4, using the measured pCH_4, CH_4 flux, and the temperature-dependent solubility of the gas with the following equation:

$$\mathrm{k} = \frac{f}{k_h \times \Delta gas} \tag{2}$$

where (f) is flux of CH_4 (mmol m^{-2} d^{-1}), (K_h) is the temperature-corrected Henry's constant (Sander 2015), and (Δgas) is the difference in partial pressures between the air and the water phases for CH_4.

We used an echosounder (HydroBox HD Dual 33/210khz, SyQwest) to estimate the position and migration of *Chaoborus* larvae through the water column (Northcote 1964). The echosounder was installed in the lake or in the High mesocosms and was used to continuously monitor *Chaoborus* migration and to capture the release of CH_4 bubbles from sediments during larval exit. The distinction between bubbles and larvae is possible because gas bubbles appear as oblique lines owing to their rapid rise through the water column whereas *Chaoborus* larvae appear as small dots (Ostrovsky et al. 2008). No bubbles were detected during migration by the echosounder, so this component will not be further discussed. Using the echosounder also allowed us to identify depths at which water samples should be taken to coincide with those at which the larvae were actually stationed, as migration patterns shifted as a function of day length over the summer.

1.3.4 Laboratory analyses

Dissolved CH_4 concentration and the isotopic signature of the carbon in CH_4 in the 12-mL exetainer vials were obtained using a Cavity Ring Down Spectrometer (CRDS) equipped with Small Sample Isotopic Module (SSIM, Picarro G2201-i, Picarro Inc., CA, USA). Initial concentrations of CH_4 (μmol L^{-1}) in the sampled water were calculated accounting for headspace $p$$CH_4$ (before and after the equilibrium, volume ratio headspace:water of 1:1]), air and water temperatures (°C), solubility of CH_4 at *in situ* temperature and at sampling temperature, and *in situ* barometric pressure (kPa). Isotopic data are reported in the standard delta (δ) notation expressed in ‰ relative to the standard Vienna Pee Dee Belemnite (Whiticar 1999). The calibration of $\delta^{13}C$-CH_4 values was performed using three certified isotopic standards (Liso1: δ13C-CH_4 = -66.5 ± 0.2 ‰; Tiso1: δ13C-CH_4 = -38.3 ± 0.2 ‰; Hiso1: δ13C-CH_4 = -23.9 ± 0.2 ‰; Isometric Instruments, Canada). The carbon signature measurements of CH_4 are used to determine if there is a change in the processing of the CH_4 in the water column, in terms of both CH_4 source and the degree of CH_4 oxidation across different treatments. A more negative value of isotopic signature of carbon in CH_4 indicates that it is less oxidised by methanotrophic bacteria while a less negative value indicates more oxidised CH_4.

Samples for total phosphorus (TP) concentrations were oxidised via a potassium persulfate digestion, analysed using a standard protocol (Wetzel and Likens 2000) and then quantified using colorimetric detection with a spectrophotometer at 890 nm (Ultrospec 2100pro spectrophotometer). Samples for total nitrogen (TN) concentrations were analyzed with a continuous flow analyzer (ALPKEM Flow Solution IV ©) using an alkaline persulfate digestion method, coupled with a cadmium reactor, following a standard protocol (Patton and Kryskalla 2003) and then detected using an in-line spectrophotometer at 540 nm. Dissolved organic and inorganic carbon (DOC and DIC) were assessed using an Aurora 1030W TOC Analyzer and a persulfate oxidation method. For chlorophyll α concentrations, water samples were immediately filtered through GF/F (Whatman, UK) filters and kept frozen until spectrophotometric analysis after hot ethanol (90%) extraction with an acidification step to correct for phaeophytin (665 and 750 nm, Ultrospec 2100 pro, Thermo Fisher Scientific Inc., USA). Finally, entire samples, but sometimes 5 mL subsamples, of zooplankton and *Chaoborus* were counted and identified to genus level using a dissecting microscope.

1.3.5 Statistical analyses and calculations

Values of response variables measured in the lakes or in the High treatments were used primarily to directly assess the effect of different densities of *Chaoborus* larvae on CH_4 concentrations and isotopic signatures and compared to the values in the Low treatments, where there was a lower *Chaoborus* density. The analyses addressed the following main questions:

1) Does *Chaoborus* larval migration alter hypolimnetic and epilimnetic CH_4 concentration?
2) Do different *Chaoborus* larval densities alter CH_4 concentrations differently across the water column and at different times of the day?
3) Are there significant changes in CH_4 dynamics in the different treatments, based on the patterns of CH_4 isotopic signatures?

We performed various analyses of variance for each combination of lakes and months as different experiments (2-way ANOVA; main factor: treatments [Lake, High or Low mesocosms], crossed with either thermal layers [Epilimnion or Hypolimnion] and migration times [16:00, 19:00, 20:00, 21:00, 22:00, 01:00, 03:00, 04:00, 05:00, 07:00 or 10:00]) to compare the effect of treatments on mean CH_4 concentrations, CH_4 isotopic signatures and mean CH_4 fluxes in each thermal layer, at specific times and differences between different times. A Tukey post hoc test was used to test simple effects where significant interactions were detected. We did not compare between lakes and months as these were not primary research questions. A t-test was used to compare mean metalimnetic and hypolimnetic CH_4 concentrations by *Chaoborus* treatment before and after the addition of *Chaoborus*. We also examined linear correlations between dissolved oxygen concentrations and CH_4 isotopic signatures. Statistical analyses were carried out using R software (R version 3.5.1).

We estimated the maximum amount of CH_4 (in µmol m^{-2} d^{-1}) that the *Chaoborus* larvae in each treatment could theoretically transport from the sediments using their tracheal sacs based on the maximum density of *Chaoborus* during each sampling period and an average reference volume of *Chaoborus* tracheal sacs of 0.12 µl (Teraguchi 1975b). These results are reported in Table 1.5. We also estimated CH_4 fluxes from sediments to the hypolimnion (µmol m^{-2} d^{-1}) that are due to *Chaoborus* migration, based on the differences between initial hypolimnetic CH_4 concentration (before migration) and maximum hypolimnetic CH_4 concentration measured during migration as described in the following equation:

$$f_{\text{sed}} = \frac{\left(\text{CH}_{4_{\text{peak}}} \times \text{Volume}_{\text{hypo}}\right) - \left(\text{CH}_{4_{\text{ini}}} \times \text{volume}_{\text{hypo}}\right)}{\text{Surface sediment}} \times \text{Time}^{-1} \tag{3}$$

where ($f\text{Sed}$) is CH_4 flux from sediment (µmol m^{-2} d^{-1}), ($CH_{4\text{peak}}$) is CH_4 maximum concentration during the migration (µmol L^{-1}), ($\text{Volume}_{\text{hypo}}$) is the volume of the water column from the sampling point to the sediments (L), ($CH_{4\text{ini}}$) is CH_4 minimum concentration before the migration (µmol L^{-1}), (Surface sediment) is the surface area of the sediments over which bioturbation is occurring (m^2) and (Time) is considered one day as this process is occurring once per day. These results are reported in Table 1.6. We further estimated the average increase in hypolimnetic CH_4 concentration (in µmol L^{-1}) (4-7m for Croche and 3-6m for Cromwell) that would result from the redistribution of all the CH_4 released from a diurnal bioturbation event under anoxic conditions (in the absence of oxidation), as described in the following equation:

$$\text{Delta}_{CH4} = \frac{f_{\text{sed}} \times \text{Surface}_{\text{hypo}}}{\text{Vol}_{\text{hypo}}} \tag{4}$$

where ($\text{Delta}_{\text{CH4}}$) is CH_4 concentration in the hypolimnion after redistribution (µmol L^{-1}), (f_{sed}) is CH_4 flux from sediment (in µmol m^{-2} d^{-1}), ($\text{Surface}_{\text{hypo}}$) is the surface area of the hypolimnion layer (m^2) and (Vol_{hypo}) is the volume of the hypolimnion layer (L). The results of this calculation are reported in Table 1.4. We also estimated the extent of oxidation of CH_4 by using a isotopic mass-balance approach based on stable isotopic discrimination during microbial CH_4 consumption (Thottathil et al. 2018), as described in the following equation:

$$\ln(1 - f_{ox}) = \frac{\left[\ln(\delta^{13}C_{source} + 1000) - \ln\left(\delta^{13}C_{depth} + 1000\right)\right]}{[\alpha - 1]} \tag{5}$$

where (f_{ox}) is the fraction of CH_4 oxidised, ($\delta^{13}C_{source}$) is the $\delta^{13}C$-CH_4 signature in the sediment, ($\delta^{13}C_{depth}$) is $\delta^{13}C$-CH_4 signature at the depth we sampled in the hypolimnion and (α) is the isotopic fractionation factor. We used $\delta^{13}C_{source}$ value of -73.98‰, corresponding to the most negative value measured in lake Cromwell under complete anoxia, and we used an α value of 1.027 as in Thottathil et al. (2018). Results are reported in Table 1.7. Finally, we estimated the long-term accumulation of CH_4 (μmol L^{-1}) that is strictly due to *Chaoborus* bioturbation in the anoxic hypolimnia (4-7m for Croche and 3-6m for Cromwell) between July and August, using the values obtained from equation 4, as described in the following equation:

$$\text{Accumulated}_{CH4} = \text{Conc}_{ini} + (\text{Delta}_{CH4} \times d) \tag{6}$$

where (Accumulated_{CH4}) is the estimated CH_4 concentration in August in the hypolimnion (μmol L^{-1}) that would be due to *Chaoborus* bioturbation, (Conc_{ini}) is the observed average CH_4 concentration in the hypolimnion at the end July (μmol L^{-1}), (Delta_{CH4}) is average CH_4 concentration increase in the hypolimnion after redistribution of a daily *Chaoborus* bioturbation event (μmol L^{-1}) and (d) is the number of days between sampling of July and August. Results are reported in Table 1.8.

1.4 RESULTS

1.4.1 Water column characteristics: mesocosms and lakes

The temperature profile was similar between the lakes and their respective mesocosms (Appendix A and B). The oxygen profiles amongst the mesocosms were similar, but different from that of the lake, the latter always having higher O_2 depletion in the hypolimnion for any given date. For example, Lake Croche was oxic at 6 m (2.15 mg/L) in July and sub-oxic (1.23 mg/L) in August, yet the mesocosms were oxic to the bottom (~5 mg/L in July, ~4 mg/L in August) (Appendix A, Figures A.1 and A.2). Lake Cromwell was anoxic starting at 3.5 m in July and 3.0 m in August, but the mesocosms were anoxic only near the bottom (4.0 m for High and 4.5 m for Low in July and 4.0 m for High and Low in August) (Appendix B, Figures B.1 and B.2).

Ambient *Chaoborus* densities in both lakes were close to the lower range of the global densities observed (2 000 to 130 000 ind. m^{-2}) (Gosselin and Hare 2003). The High treatment mesocosms always had greater (8-23 fold) *Chaoborus* densities than the Low treatment mesocosms and had 3-4.5 fold higher densities than the ambient lake densities (Table 1.2). The High and Low mesocosms had similar zooplankton densities (with the exception of lake Cromwell in August where an exceptionally high number was observed; Table 1.2).

Table 1.2: Mean ± SE zooplankton and *Chaoborus* densities in each treatment in July and August taken at 01:00 during the experimental period on day 4.

Lake	Time	Treatments	Mean zooplankton density (ind. m^{-2})	Mean *Chaoborus* density (ind. m^{-2})
Croche	July	Lake	**122 194** ± 29 909	**2 350** ± 277
		High	**301 066** ± 28 891	**7 503** ± 938
		Low	**349 837** ± 53 460	**938** ± 690
	August	Lake	**158 617** ± 36 002	**2 350** ± 692
		High	**405 642** ± 54 867	**10 786** ± 1 407
		Low	**305 756** ± 47 833	**469** ± 469
Cromwell	July	Lake	**162 132** ± 51 147	**3 954** ± 1 282
		High	**534 300** ± 48 737	**11 733** ± 4 513
		Low	**494 588** ± 28 881	**1 354** ± 451
	August	Lake	**237 283** ± 34 557	**3 116** ± 1 014
		High	**749 716** ± 32 243	**11 109** ± 3 522
		Low	**542 169** ± 19 508	**858** ± 927

1.4.2 Migration patterns of *Chaoborus* larvae

The echosounder approach was able to capture the spatial dynamics of the vertically migrating *Chaoborus* larvae in both the lake and in the mesocosms (Figure 1.3). The light regime during the experiment was between 13,5 to 15,0 hours of light (July: sunrise at 05:30 and sunset at 20:30, August: sunrise at 06:10 and sunset at 19:40). Observations in July showed very low larval densities close to the sediments at around 21:30 hrs, suggesting that most of the animals had migrated from the sediments at that point. During the night, however, there were still some *Chaoborus* observable in the bottom layer (Figure 1.3 A). The migration pattern was similar between the two lakes and between the lakes and the High mesocosms (Figure 1.3 B); we did not assess the migration patterns in the Low mesocosms owing to their low *Chaoborus* densities. In all cases, the larvae left the sediments around 20:00-21:00, reaching their shallowest depths in the surface waters around 22:00-23:00 (1-2 m), then beginning to descend again toward 03:00. Most larvae had disappeared into the sediments by 05:00-06:00 (Figure 1.3 B). In August, the upward migration from the sediments began earlier, corresponding to the earlier sunset (between 30 minutes to 1 hour earlier), but the return to the sediments occurred roughly at the same time as observed in July (Figure 1.4).

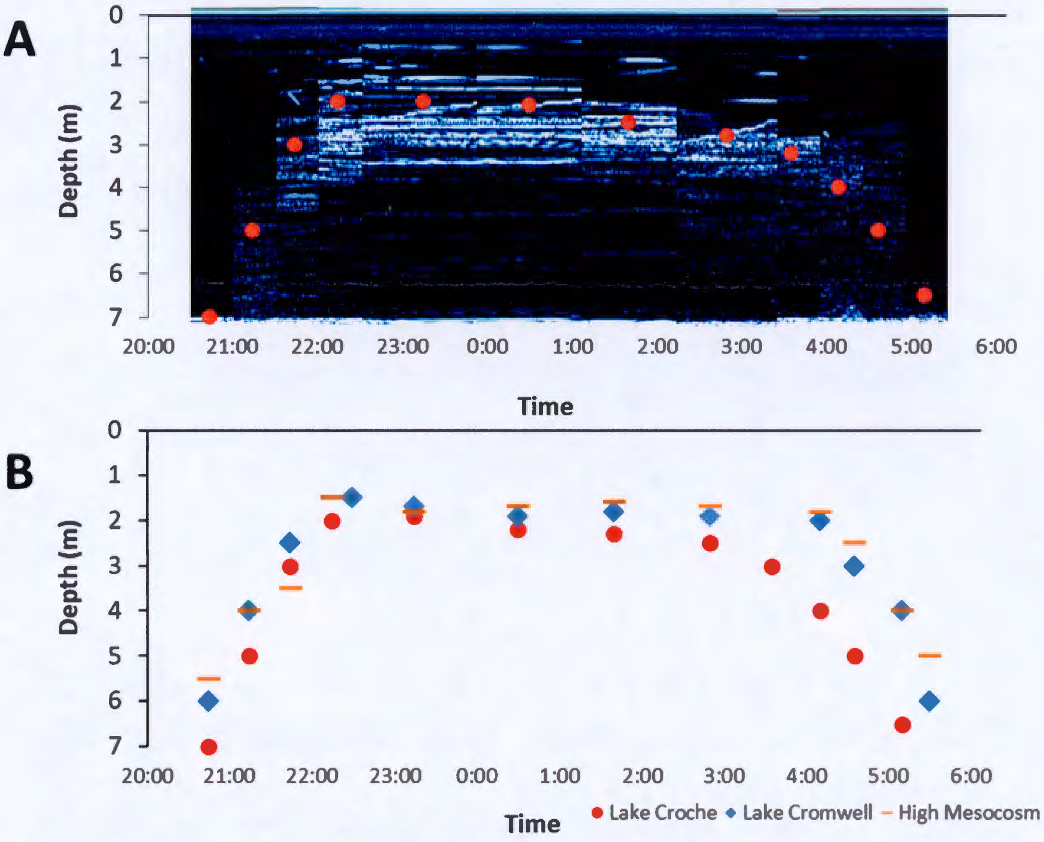


Figure 1.3: Example of echogram at different times through the migration time in lake Croche in July (A) and mean larvae position with time in the lakes and High mesocosms in July (B) *Chaoborus* larvae at different times in the water column are represented by the blue lines, consisting of small blue dots (A). Lighter dots indicated greater densities. Mean positions at each time point are represented by the red circles, blue diamonds and orange lines (A, B).

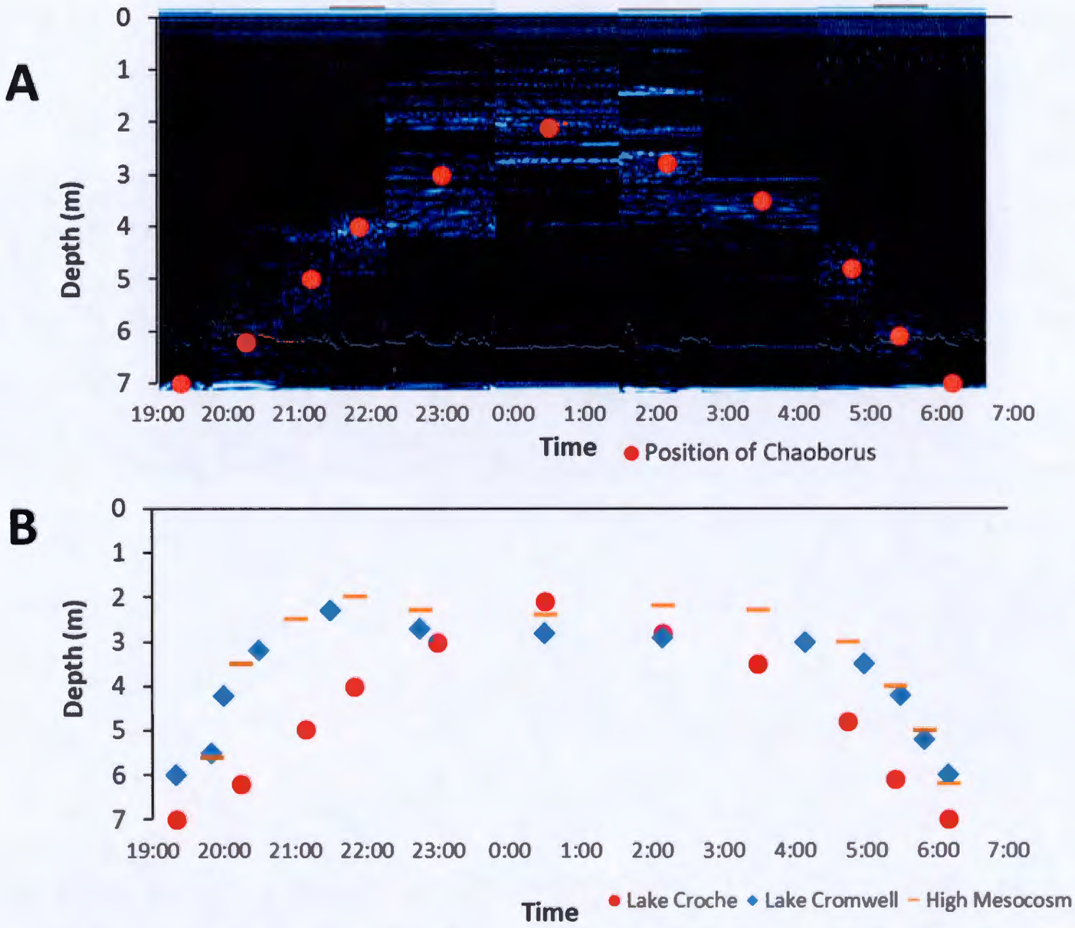


Figure 1.4: Example of echogram at different times through the migration time in lake Croche in August (A) and mean larvae position with time in the lakes and High mesocosms in August (B) *Chaoborus* larvae at different times in the water column are represented by the blue lines, consisting of small blue dots (A). Lighter dots indicated greater densities. Mean positions at each time point are represented by the red circles, blue diamonds and orange lines (A, B).

1.4.3 Comparison of mean response by treatment

Mean CH_4 concentrations were consistently higher in the lake waters compared to the mesocosms (2-way ANOVA, $p < 0.001$), except in the epilimnion of Cromwell where there were no differences between all the treatments in July and August (simple effect, $p > 0.95$) (Figure 1.5). There were no statistically significant differences in July between mesocosm treatments (High and Low) for the epilimnetic and hypolimnetic CH_4 concentrations in either lake (2-way ANOVA, $p > 0.92$). However, CH_4

concentrations in High mesocosms were marginally higher than Low mesocosms in Cromwell in August (simple effect, $p = 0.073$) (Figure 1.5).

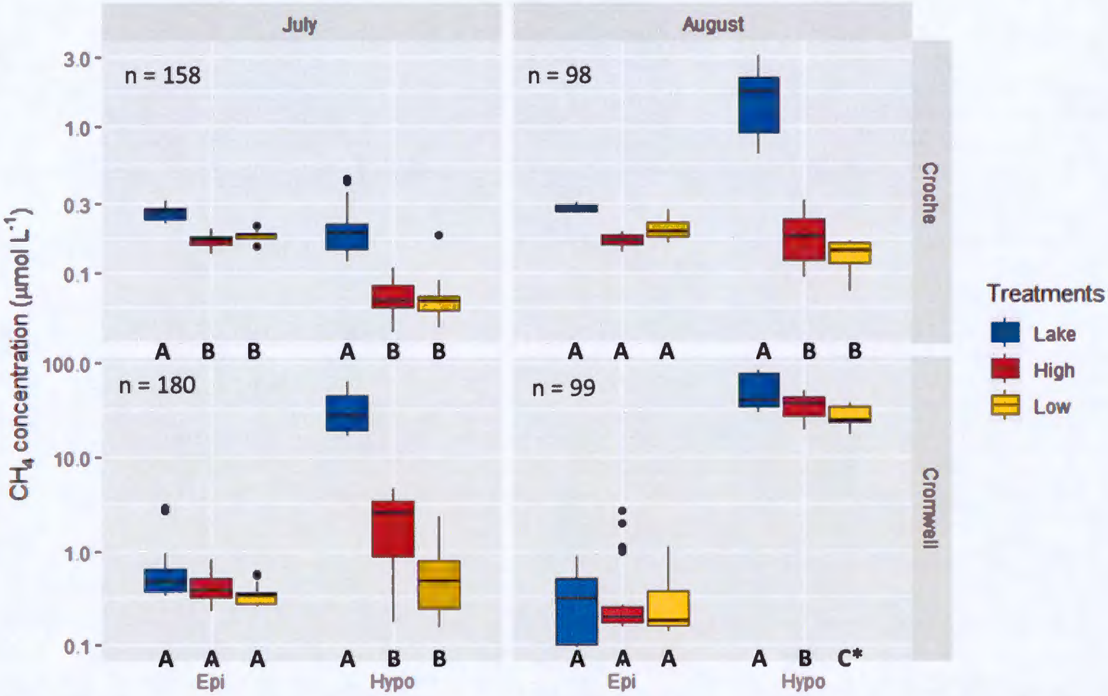


Figure 1.5: Box plot distribution of CH_4 concentrations in each thermal layer for the lake and *Chaoborus* treatments in July and August for lakes Croche and Cromwell. For each lake, all time points across the whole experiment were averaged by treatment (Lake, High, Low) and by depth layer: epilimnion (1m) and hypolimnion (6m Croche and 5m Cromwell). Different letters indicate significant differences between treatments for every combinations of layer in a lake in a month. The asterisk after a letter means that the difference is marginally [$p = 0.073$] significant.

Mean CH_4 isotopic values were similar between all treatments and layers in Croche in July and August (2-way ANOVA, $p > 0.2$) except in the hypolimnion in August when means were less negative in the mesocosms than in the lake (simple effect, $p = 0.003$, Figure 1.6). Isotopic signatures in lake Cromwell were always significantly less negative in the epilimnion and more negative in the hypolimnion, compared to the High and Low mesocosms (2-way ANOVA, $p < 0.001$). Also, in lake Cromwell, there was never any statistically significant difference between isotopic values in the High and Low mesocosms (simple effect, $p > 0.5$, Figure 1.6). Finally, hypolimnetic isotopic signatures in lake Croche and the mesocosms of lake Cromwell became more negative through the season (from July to August, Figure 1.6).

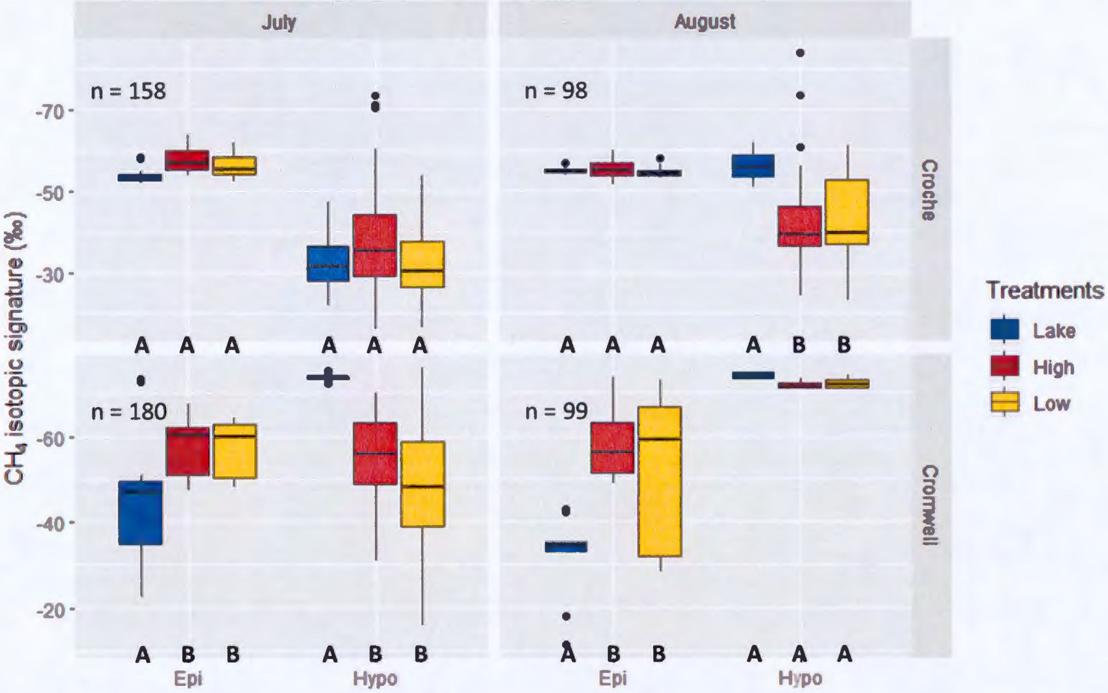


Figure 1.6: Box plot distribution of CH_4 isotopic signatures in each thermal layer for the lake and *Chaoborus* treatments in July and August for lakes Croche and Cromwell. For each lake, all time points across the whole experiment were averaged by treatment (Lake, High, Low) and by depth layer: epilimnion (1m) and hypolimnion (6m Croche and 5m Cromwell). Different letters indicate significant differences between treatments for every combinations of layer in a lake in a month.

1.4.4 Temporal changes in CH_4 concentrations and isotopic signatures

Each combination of lake, months and layers were analysed separately here to assess the effect of the *Chaoborus* treatments, the time of the day, and the interaction between those two factors on CH_4 concentration and CH_4 isotopic signature (Figure 1.7 to 1.10). Epilimnetic CH_4 concentrations in Croche were mostly stable through time in each treatment in July (Figures 1.7 A) and August (Figure 1.7 C) and there was no effect of time on CH_4 concentrations (2-way ANOVA, $p > 0.1$). The treatments had a significant effect on the epilimnetic CH_4 concentrations (2-way ANOVA, $p < 0.008$), with lake Croche waters having higher values than the mesocosms in both months. There were no significant differences between High and Low mesocosms for July and August (simple effects, $p > 0.05$; Figure 1.7 A, C). Similarly, CH_4 isotopic signatures remained stable in the epilimnion in July and August (2-way ANOVA, $p > 0.1$; Figure 1.7 B, D).

Results were very different in the hypolimnion of Croche where both treatment and time had a significant effect on CH_4 concentrations (2-way ANOVA, $p < 0.001$). Increase of CH_4 concentrations were observed in lake Croche in both July and August (simple effect, $p < 0.001$) and High treatments in August only (simple effect, $p = 0.04$) following the onset of *Chaoborus* migration (Figures 1.8 A, C). In the lake, CH_4 peaks of 0.29 $\mu mol\ L^{-1}$ in July and 2.57 $\mu mol\ L^{-1}$ in August were observed around 20:00-21:00, when almost all the larvae had exited the sediments (Figures 1.3 and 1.4). CH_4 was lower, but still elevated in the High mesocosms following the onset of migration around 19:00-20:00 (0.10 $\mu mol\ L^{-1}$ in July and 0.30 $\mu mol\ L^{-1}$ in August). In all cases, CH_4 significantly decreased again to close to pre-migration values during the remainder of the night, between 21:00 to 1:00 (Simple effect, $p < 0.01$) (Figure 1.8 A, C). In the Low mesocosms, hypolimnetic CH_4 concentrations remained stable and low ($0.06 \pm 0.006\ \mu mol\ L^{-1}$ in July and $0.13 \pm 0.01\ \mu mol\ L^{-1}$ in August) during these same migration times (simple effect, $p > 0.1$). In July, only CH_4 concentration in the lake was significantly different from the other treatments, but in August, all three treatments were significantly different from each other (Figure 1.8 A, C, simple effect, $p < 0.001$). Only time had an effect on CH_4 isotopic signatures in Croche in July and August (2-way ANOVA, $p \leq 0.05$), as there were generally no significant differences between the treatments (2-way ANOVA, $p > 0.05$). The hypolimnetic CH_4 isotopic signature in High and Low treatments showed comparable diurnal trends in July and August, when the mesocosms were still oxic, becoming significantly more negative around 19:00 from ~ -35‰ to ~ -60‰ (simple effect, $p \leq 0.03$), then shifting back to less negative during the night returning to close to -35‰ (Figure 1.8 B, D), but relative to the mesocosms, values in the lake were stable thought time, as well as less negative in July, and more negative in August (Figure 1.8 B, D).

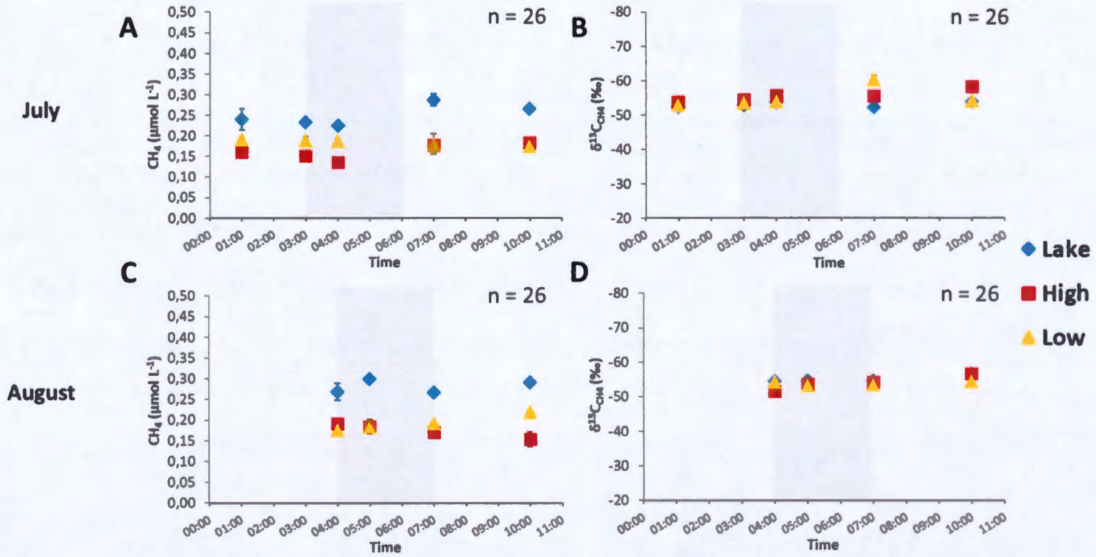


Figure 1.7: Mean ± SE epilimnetic (1m) CH_4 concentrations (A, C) and CH_4 isotopic signatures (B, D) in lake Croche in July (A, B) and August (C, D) through the diurnal migration period. Gray areas represent the time during which the larvae were descending to the sediments.

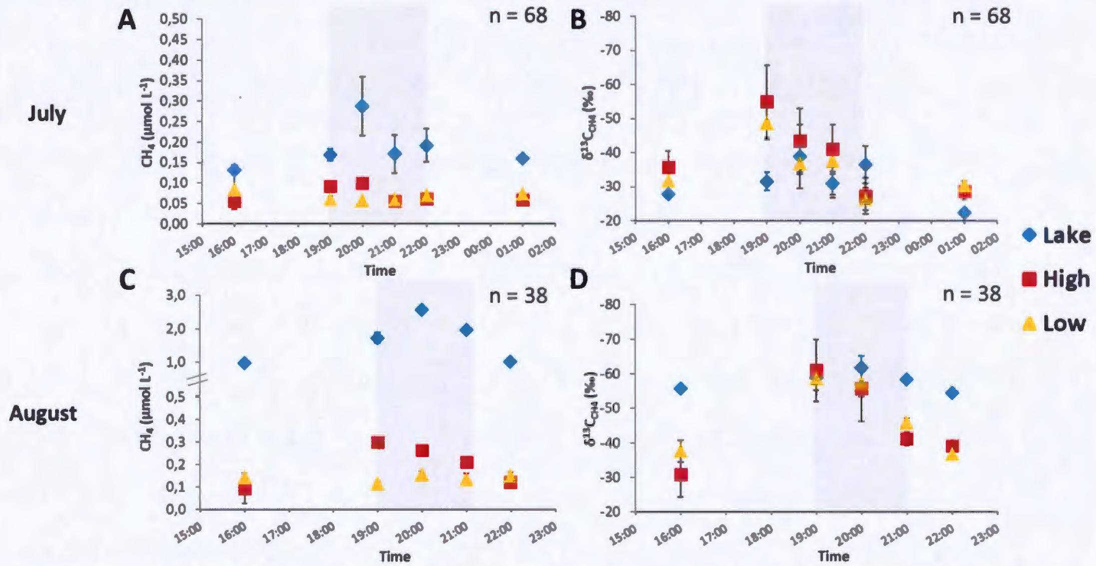


Figure 1.8: Mean ± SE hypolimnetic (6m) CH_4 concentrations (A, C) and CH_4 isotopic signatures (B, D) in lake Croche in July (A, B) and August (C, D) through the diurnal migration period. Gray areas represent the times during which the larvae were leaving the sediments.

In Cromwell, epilimnetic CH_4 concentrations in July (Figure 1.9 A) were mostly stable through time in each treatment and there was no effect of time or treatments on CH_4 concentrations (2-way ANOVA, $p > 0.06$). However, in August (Figure 1.9 C), both time and treatments had an effect on CH_4 concentrations (2-way ANOVA, $p < 0.001$). In August, Low and High treatments were not significantly different from each other and between time points during migration time (simple effect, $p > 0.1$, Figure 1.9 C) but there was a significant increase in the lake between 04:00 and 06:00, from 0.22 µmol L^{-1} to 0.76 µmol L^{-1} (simple effect, $p = 0.006$, Figure 1.9 C). CH_4 isotopic signatures in the surface waters of Cromwell did not vary in the same way as concentrations (Figures 1.9 B, D). There were effects of both treatments and time in the epilimnetic isotopic signatures in July only (2-way ANOVA, $p < 0.001$) where we noted significant differences between lake and mesocosms (simple effect, $p < 0.004$) with a trend towards less negative values during the night-time migration period, then becoming significantly lower (more negative) again by the morning (simple effect, $p < 0.05$).

In Cromwell, both treatments and time had an effect on CH_4 hypolimnetic concentrations in July and August (2-way ANOVA, $p < 0.05$, Figures 1.10 A, C). There was a significant increase in the lake and the High treatments during *Chaoborus* migration in both months, and these increases were more pronounced than those observed in Croche. In the lake hypolimnion, there was a significant peak of CH_4 (47.96 µmol L^{-1} in July and 81.50 µmol L^{-1} in August) around 19:00-20:00 (simple effects, $p < 0.002$), when almost all the larvae had exited the sediments. There was also a significant peak of CH_4 in the High treatments, but this time lower (3.95 µmol L^{-1} in July and 45.41 µmol L^{-1} in August) around 19:00-20:00 (simple effects, $p < 0.05$). In all cases, there was a subsequent decrease during the night, from 21:00 to 01:00, and the concentrations were significantly lower (simple effect, $p < 0.02$). CH_4 concentrations in the hypolimnion of the Low mesocosms remained stable and relatively low during migration time (0.76 ± 0.28 µmol L^{-1} in July and 19.23 ± 0.67

µmol L^{-1} in August), and there were no significant increases in CH_4 concentrations during the period of migration (simple effects, $p > 0.1$, Figure 1.10 A, C). Treatments and time had an effect on hypolimnetic CH_4 isotopic signature (2-way ANOVA, $p < 0.001$). The values in lake Cromwell, which was anoxic for the entire period, remained stable in July, around -70‰ (simple effect, $p < 0.05$, Figure 1.10 B). In contrast, in the High and Low mesocosms in July, when there was still oxygen in the hypolimnion, CH_4 isotopic signatures became significantly higher (less negative) during the night, from ~ -60‰ at 19:00 to ~ -40‰ at 01:00 (simple effects, $p < 0.05$, Figure 1.10 B). In August, neither treatment nor time had an effect on CH_4 isotopic signature (2-way ANOVA, $p > 0.1$). The CH_4 isotopic signatures in all treatments stayed stable through migration time at around -70‰ (Figure 1.10 D) and there were no significant differences between them.

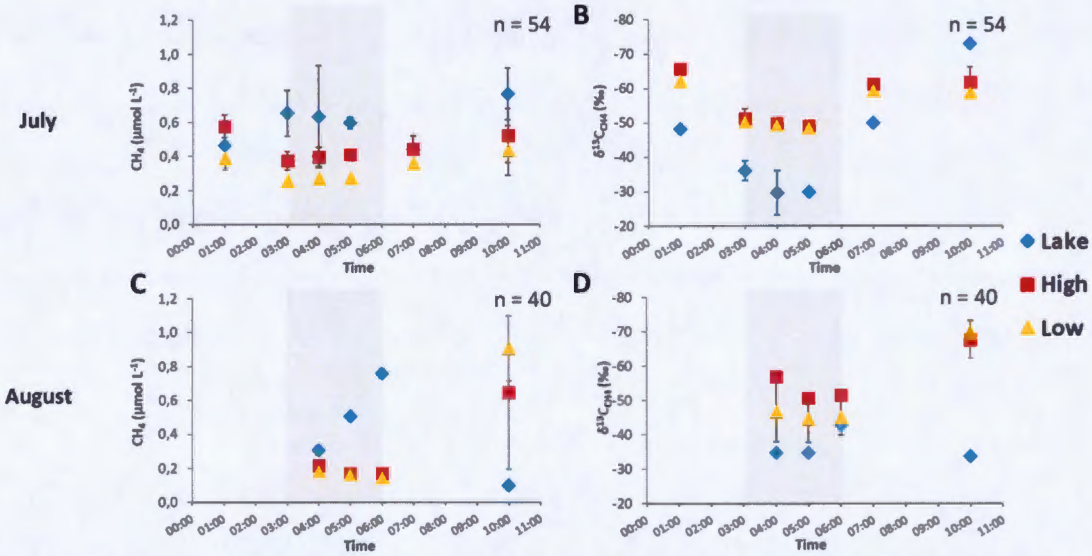


Figure 1.9: Mean ± SE epilimnetic (1m) CH_4 concentrations (A, C) and CH_4 isotopic signatures (B, D) in lake Cromwell in July (A, B) and August (C, D) through the diurnal migration period. Gray areas represent the time during which the larvae were descending to the sediments.

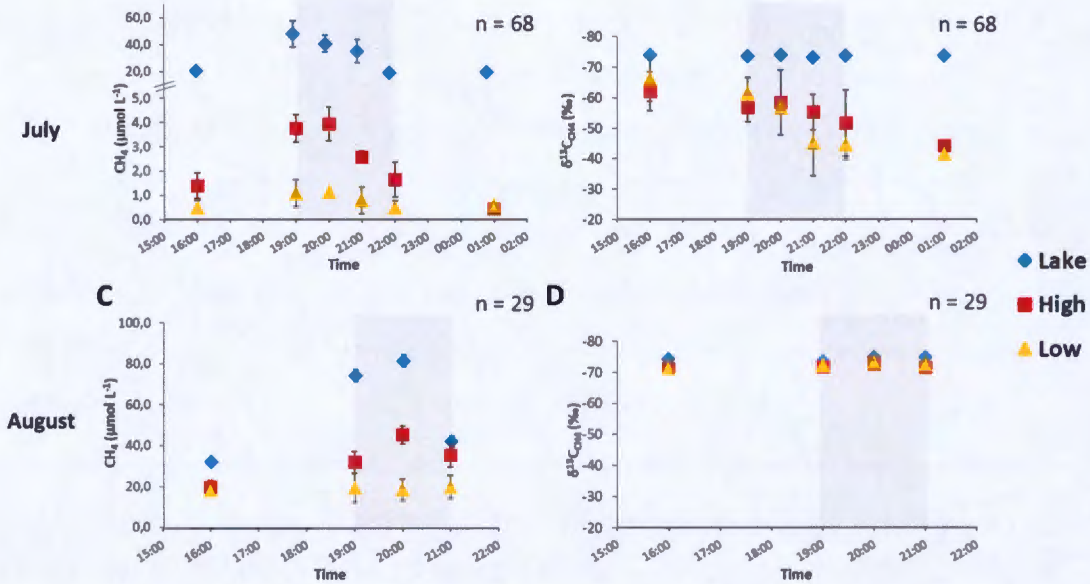


Figure 1.10: Mean ± SE hypolimnetic (5m) CH_4 concentrations (A, C) and CH_4 isotopic signatures (B, D) in lake Cromwell in July (A, B) and August (C, D) through the diurnal migration period. Gray areas represent the time the larvae are going out of the sediments.

As we had observed in the epilimnetic CH_4 concentration, there was no observable pattern attributable to migration time in CH_4 fluxes to the atmosphere in July in either of the lakes (Appendix E, Figure E.1 A, C). In August, CH_4 fluxes were similar in all the mesocosms treatments and for all times and there was no interaction between the time and the treatments (2-way ANOVA, $p > 0.1$; Appendix E, Figure E.1 B). The fluxes in the lakes were on average significantly higher compared to the mesocosms (simple effect, $p < 0.048$). Unfortunately, we were unable to collect data for August in lake Cromwell. There is the same pattern in the gas transfer coefficients values (Appendix E, Figure E2), suggesting that there was no wind effect on those fluxes.

1.4.5 CH_4 concentrations decrease after migration time in the hypolimnion

1.4.5.1 Patterns in ambient CO_2 and the oxidation hypothesis

We measured dissolved CO_2 concentrations to detect potential changes that would be due to CO_2 production associated to CH_4 oxidation. First, mean epilimnetic and hypolimnetic CO_2 concentrations were always higher in both lakes compared to the concentration in their respective mesocosms (2-way ANOVA, $p < 0.001$) except in the epilimnion of lake Croche (simple effect, $p > 0.7$) (Appendix C). Also, CO_2 concentrations between the treatments were never significantly different in each lake through the whole experiment (simple effects, $p > 0.99$). The CO_2 concentrations in the hypolimnion followed a pattern similar to that of CH_4 during the periods of migration (Appendix D). There was a significant increase of CO_2 concentrations during migration time (16:00 to 20:00) in July in the hypolimnion of lake Croche (peak at 253 μmol L^{-1}), (Simple effect, $p = 0.008$) (Appendix D, Figure D.1). There was also a significant decrease in CO_2 concentration during the night following the onset of migration (20:00 to 22:00) in the hypolimnion of the lake (simple effect, $p = 0.009$). In Cromwell, there was a small increase in CO_2 concentrations in the lake and High mesocosms during migration time in July and August, but this was not significant (Simple effects, $p > 0.1$) (Appendix D, Figure D.2). The CO_2 isotopic signatures in both lakes in July and August were stable in all treatments and there were no significant differences between them (2-way ANOVA, $p < 0.1$) (Appendix D). We did observe that the isotopic signature of CO_2 in the hypolimnion was on average more negative (around -20‰ and -25‰ in Croche and around -30‰ in Cromwell) compared to in the epilimnion (-around -10‰ and -15‰ in Croche and around -20‰ in Cromwell). This could suggest an input of highly depleted CO_2 derived from CH_4 in the hypolimnion but also an input from sediments.

Patterns of mean hypolimnetic CH_4 isotopic signatures showed that less negative values correspond to more oxygenated waters (oxidised CH_4 in presence of oxygen) and more negative values correspond to anoxic conditions (Figure 1.6). Indeed, Table 1.3 shows that there was a strong relationship between CH_4 isotopic signature in the hypolimnion and dissolved oxygen concentration. In particular, when the water column was still oxic, the hypolimnetic CH_4 isotopic signatures sharply declined as the larvae were exiting the sediments, suggesting a large input of fresh CH_4, and then consistently shifted back to less negative values during the night, suggesting that oxidation of this new CH_4 was taking place (Figure 1.8 B, D). In contrast, when the water column was anoxic, the hypolimnetic CH_4 isotopic signatures were consistently very negative and stable at onset of migration and throughout the night (Figure 1.8 B, D), suggesting that there was no oxidation occurring. This implies that other process(es) would be responsible for the decline in CH_4 concentration for this particular case in lake Cromwell.

Table 1.3: Correlation and linear regression between values of dissolved oxygen and CH_4 isotopic signature.

Correlation	**Coef**	**t**	**df**	***p*-value**
All layers	0.368	0.205	31	0.839
Epi	-0.349	-1.179	10	0.266
Meta	0.206	0.556	7	0.596
Hypo	**0.636**	**2.607**	**10**	**0.026**

Regression	**Slope + intercept**	**F**	**R^2**	***p*-value**
All layers	0.689 -55.311	0.828	0.005	0.370
Epi	-3.592 -27.483	0.316	0.066	0.586
Meta	1,905 -62.156	2.998	0.200	0.127
Hypo	**6,409** **-61.645**	**17.670**	**0.602**	**0.002**

1.4.5.2 CH_4 transport and re-distribution hypothesis

Another hypothesis to explain the decrease in CH_4 concentrations following the increases associated with diel migration (Figures 1.8 A, C and 1.10 A, C) could be that some CH_4 is diffusing or being redistributed by advection across the water column vertically and horizontally or even by the turbulence generated by the animals themselves. The vertical redistribution can be quantified by comparing the CH_4 concentrations at the beginning of the experiment (before the addition of zooplankton and *Chaoborus*) to those at the end (2-3 nights later) in the hypolimnion and also the metalimnion (Figures 1.12 and 1.13). There was no significant increase of CH_4

concentrations in the metalimnion or in the hypolimnion for Croche in July and August for any of the treatments (Paired t-test, $p > 0.2$) (Figure 1.12 A, B). Our results also indicate no significant net increase in the hypolimnion in July and August in lake Cromwell (Paired t-test, $p > 0.3$; Figure 1.13 A, C), but that there was a marginally significant increase of metalimnetic CH_4 concentrations in the lake and High mesocosms in July and August ($p < 0.09$), suggesting that some CH_4 accumulated in the hypolimnion could have diffused upward (Figure 1.13 A, B, C).

We further calculated the actual shift in mean hypolimnetic CH_4 concentration that would have resulted from re-distributing in the whole hypolimnion the net CH_4 released from the sediments to the bottom waters as a result of *Chaoborus* bioturbation events (Table 1.4). Our calculations suggest that the CH_4 re-distribution in the hypolimnion of these bioturbation-mediated CH_4 releases could amount to an increase of 0.05 μmol L^{-1} and 0.01 μmol L^{-1} in July, and of 0.50 μmol L^{-1} and 0.07 μmol L^{-1} in August in lake Croche and in the Croche High treatments respectively. In lake Cromwell and the lake Cromwell High treatments, these releases could amount to an increase of 2.60 μmol L^{-1} and 0.85 μmol L^{-1} respectively in July and an increase of 4.60 μmol L^{-1} and 8.40 μmol L^{-1} in August. Compared to the initial CH_4 concentration (μmol L^{-1}) in the hypolimnion before the increase, these calculations imply increases that amount to 13 to 61% of the amount of CH_4 already present in the hypolimnion, suggesting that a part of the decline in bottom water CH_4 observed during the night could be explained by the redistribution of this CH_4 throughout the hypolimnion layer.

Table 1.4: The initial CH_4 concentration (μmol L^{-1}) in the hypolimnion (6m for Croche and 5m for Cromwell) compared to the estimated increase in CH_4 concentration (μmol L^{-1}) in the hypolimnion layer (4-7m for Croche and 3-6m for Cromwell) as a result of re-distributing to the entire hypolimnetic volume of the CH_4 released from sediments to bottom waters through bioturbation.

	Initial concentration (μmol L^{-1})				Estimated increase (μmol L^{-1})			
	Croche		Cromwell		Croche		Cromwell	
	July	August	July	August	July	August	July	August
Lake	0.13	0.98	20.3	32.0	0.05	0.50	2.60	4.60
High mesocosm	0.06	0.09	1.4	20.2	0.01	0.05	0.85	6.40

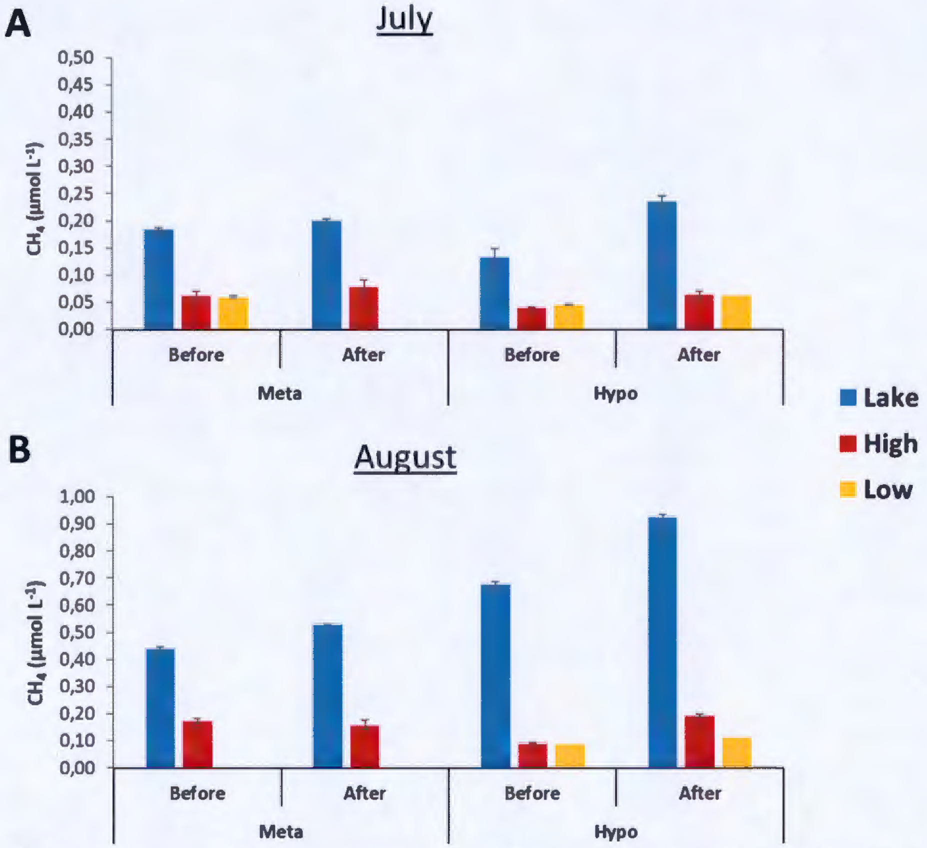


Figure 1.12 Mean ± SE metalimnetic (3.5m) and hypolimnetic (6m) CH_4 concentrations before and after the addition of zooplankton and *Chaoborus* to the mesocosms as measured in lake Croche, High and low mesocosms across July (A) and August (B).

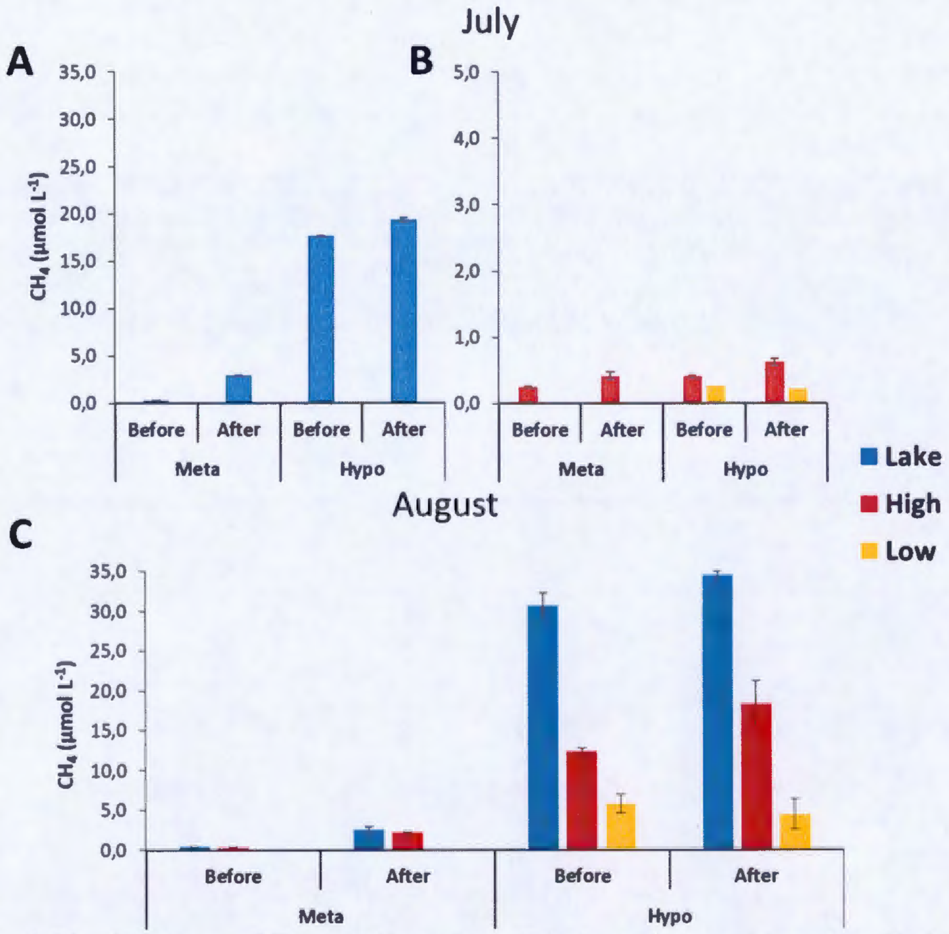


Figure 1.13: Mean ± SE metalimnetic (2.5m) and hypolimnetic (5m) CH_4 concentrations before and after the addition of zooplankton and *Chaoborus* to the mesocosms as measured in lake Cromwell across July (A), in High and Low mesocosms across July (B) and lake Cromwell, High and Low mesocosms across August (C).

1.5 DISCUSSION

1.5.1 General experimental conditions

It is very important to point out in the context of these experiments that the oxygen dynamics differed greatly between the two lake settings, also between the lake and its respective mesocosms, and these O_2 dynamics have a strong bearing on the patterns in CH_4 that we observed. The hypolimnion layer of lake Croche and its respective mesocosms (4-7 m) were constantly oxic, except the bottom-most layer (6.5-7 m) of the lake in August. In contrast, the hypolimnion of lake Cromwell (3-6 m) was anoxic throughout the experimental period, yet its respective mesocosms turned anoxic only in August and this only in the bottom most layer, so there was a range of hypolimnetic oxic/anoxic scenarios between lakes and mesocosms.

The lakes as well as the mesocosms were systematically supersaturated in CH_4 in all layers at all sampling times, although the lake had consistently higher CH_4 concentrations than did the mesocosms. Recent research has demonstrated that CH_4 supersaturation in the pelagic zone as Croche and Cromwell is mainly derived by transport from littoral sources (DelSontro et al. 2018). Mesocosm walls block such continuous lateral inputs and without this source of CH_4, concentrations should be lower than in the lakes. It is interesting to note, however, that despite their isolation from any lateral sources of CH_4, and the fact that they were strongly vertically stratified and therefore also physically isolated from benthic sources, the mesocosms were systematically supersaturated in CH_4 throughout the summer. This would suggest the existence of a pelagic source of CH_4 in this oxic surface waters, which has been previously demonstrated for Lake Cromwell in particular (Bogard et al. 2014).

1.5.2 *Chaoborus* responses to the mesocosms

Our observations confirmed that *Chaoborus* displayed a migratory behaviour in both lakes, and that they also did so in our mesocosms. Echosounder readings confirmed that *Chaoborus* were in the sediments during the day, even in the mesocosms (High treatment: Figures 1.3 and 1.4), exiting the sediments and beginning their daily migration towards the surface waters in early evening, reaching minimum depths around 20:00- 21:00 and returning to depth by early morning. At night, most larvae remained at water depths shallower than 4.5 m in Croche (DO ~ 9 mg L^{-1}) and 3 m in Cromwell (DO ~ 4 mg L^{-1}), but we observed some signals near the sediments, suggesting that some larvae likely remained there (Figures 1.4 A and 1.5 A). The same diurnal migration pattern has been reported in other studies (Bezerra-Neto et al. 2012, Weisser et al. 2018). It appears that DO levels through the water column may influence migration patterns across *Chaoborus* larval development stages. Weisser et al. (2018) demonstrated that during nightime, no larvae remained at water depths where DO was lower than 3.5 mg L^{-1} and when DO exceeded that amount across the water column, all the larvae were distributed over its entirety. Our results show that *Chaoborus* were leaving the sediments somewhat earlier in the High mesocosms compared to the lakes and that the amplitude of migration was larger (Figures 1.4 B and 1.5 B). This could be due to the fact that the oxygen profiles in the High mesocosms differed from those in the lake and that there were no fish in the mesocosms. There was always more oxygen at the bottom of the mesocosms compared to the lakes. Furthermore, the vertical distribution of light intensity can affect migration patterns. Older instars are developing eyes that make them more sensitive to light and this may explain why some individuals do not migrate to the surface (Weisser et al. 2018). Several studies have shown that young larvae tend to be permanently pelagic while older instars larvae tend to be benthic during the day and migrate vertically at night. (Wood 1956, Stahl 1966, Sardella and Carter 1983, Voss and Mumm 1999, Weisser et al. 2018). This can explain why our results show that not all individuals perform a complete migration at night, and that some larvae can remain in the water column during the day, never actually penetrating sediments (LaRow 1970, Voss and Mumm 1999, Wissel et al. 2003).

1.5.3 *Chaoborus* effects on CH_4 dynamics

We were able to introduce a maximum larval density of 10 786 ± 1 407 ind. m^{-2} in the High mesocosms in Croche and 11 733 ± 4 513 ind. m^{-2} in the High mesocosms in Cromwell (Table 1.2). These values are in the lower range of reported average densities of 2 300 to 130 000 individuals m^{-2} across lakes worldwide (Gosselin and Hare 2003), but within the same order of magnitude of recent research that showed links between CH_4 and *Chaoborus* larvae (6 000 ind. m^{-2} [Carey et al. 2018] and 9 300 ind. m^{-2} [Tang et al. 2018]). Our results suggest that at these ambient densities there are measurable effects on some aspects of lake CH_4 dynamics but not on others.

1.5.3.1 CH_4 transport from sediments to epilimnion by tracheal sacs

Our estimates of amount of CH_4 that *Chaoborus* could potentially transport from sediments by their tracheal sacs, based on maximum observed densities and mean tracheal sac volume of 0.12 µl (Teraguchi 1975b), ranges from 22 to 102 µmol m^{-2} d^{-1} (Table 1.5). These are likely overestimates, as not all larvae were observed to migrate to surface waters, and the calculation does not consider that some larvae may have been smaller than others (with smaller tracheal sac volumes). Based on previous work, only about 60-97% of *Chaoborus* population migrate above the thermocline each night and release CH_4 in the epilimnion; some also release CH_4 during the uprising migration (Carey et al. 2018). Our chamber-based measurements of water/air CH_4 flux during the period of migration in the lakes and in the High mesocosms ranged from 50 to 240 µmol m^{-2} d^{-1} (Appendix E, Figure E1). The maximum amounts of CH_4 that *Chaoborus* larvae could theoretically transport from sediments to surface water by their tracheal sacs (22–102 µmol m^{-2} d^{-1}, Table 1.5) could potentially account for 32 to 47 % of the measured water/air CH_4 fluxes that we measured during the night (Table 1.5). This potential contribution is a direct function of the animal density and peaked in the High treatment (102 µmol m^{-2} d^{-1} with 16 246 ind. m^{-2} in Cromwell in July, Table 1.5), but it was on average much lower in the ambient lake waters (42 µmol m^{-2} d^{-1}, Table 1.5). Although this potential contribution of direct *Chaoborus* transport to surface fluxes is not negligible, we observed stable epilimnetic CH_4 concentrations and isotopic signatures throughout most of *Chaoborus* migration periods and thus no discernible effect on the surface water CH_4 pools, suggesting that *Chaoburous*-mediated transport was insufficient to produce a spike in CH_4 concentrations and therefore of fluxes, over the background levels. This in turns suggests that our estimates of transport may greatly overestimate the actual contribution of this pathway.

In this regard, it is clear that the amount of CH_4 released in the epilimnion and its potential contribution to total diffusive CH_4 fluxes to the atmosphere is a function of the density of *Chaoborus* larvae. Carey et al. (2018) showed only minor effects of *Chaoborus* migration on epilimnetic CH_4 concentrations and fluxes to the atmosphere in a temperate reservoir at larval densities of 6 000 ind. m^{-2}. These authors concluded that the maximum amount of CH_4 diffusive efflux potentially attributable to *Chaoborus* would be less than 1% relative to the total seasonal diffusive flux (Carey et al. 2018). In contrast, Tang et al. (2018) suggested a very significant epilimnetic CH_4 release by *Chaoborus* and that this CH_4 has the same isotopic signature as that in the deeper waters, in an eutrophic temperate lake with higher *Chaoborus* densities (up to 34 000 ind. m^{-2}). Estimates of migration-driven methane imports from the sediment to the water column by *Chaoborus* tracheal sacs range widely, from 27 to 5495 µmol m^{-2} d^{-1} for 2000 to 130 000 ind. m^{-2} (McGinnis et al. 2017), 27 to 47 µmol m^{-2} d^{-1} for 6000 ind. m^{-2} (Carey et al. 2018) and 9.2 µmol m^{-2} d^{-1} for 9300 ind. m^{-2} (Tang et al. 2018). Together, studies converge to point out that CH_4 transport is highly dependent on *Chaoborus* larvae density and vertical distribution, the whole water column CH_4 concentration and water column mixing (McGinnis et al. 2017, Tang et al. 2018, Carey et al. 2018, our study). We conclude that at the larval densities found in our lakes, the effect of *Chaoborus* migration and the associated direct CH_4 transport on epilimnetic CH_4 concentrations, isotopic composition and fluxes to the atmosphere is modest and barely discernible from the background CH_4 dynamics in these surface waters; this potential contribution is nevertheless not entirely negligible. Previous studies have demonstrated that there is a large diurnal variability in CH_4 concentrations and isotopic signatures in the surface waters of lake Croche (Thottathil et al. 2018), but our results suggest that this does not appear to be related to *Chaoborus* migration. Higher CH_4 concentrations and more negative isotopic signature occur during the day relative to night and results in part from CH_4 oxidation inhibition by light during the day (Thottathil et al. 2018).

Table 1.5: Comparison of the range of measured water/air CH_4 fluxes in lake and High mesocosms during migration period, compared to estimated maximum amounts of CH_4 that the *Chaoborus* larvae could have theoretically transported from the sediments to the epilimnion by their tracheal sacs, based on maximum observed *Chaoborus* density in lake and High mesocosms for each sampling period multiplied by the reference mean volume of a *Chaoborus* tracheal sacs of 0.12 µl (Teraguchi 1975b).

		CH_4 fluxes measured ($\mu mol\ m^{-2}\ d^{-1}$)	CH_4 transport estimated ($\mu mol\ m^{-2}\ d^{-1}$)
Croche	**July**	49 - 181	22 - 57
	August	56 - 175	26 - 82
Cromwell	**July**	103 - 240	42 - 102
	August	-	32 - 91

1.5.3.2 CH_4 release from sediment by bioturbation

We observed significant increases in hypolimnetic CH_4 concentrations in the waters overlying the sediment in both the lakes and High mesocosms at the onset of migration (Figures 1.8 and 1.10). These peaks occurred right after the onset of the nightly migration, and in addition, the CH_4 concentrations in bottom hypolimnetic waters remained stable during the same period in the Low mesocosms, further supporting the notion that bioturbation by *Chaoborus* is responsible for these patterns. In addition, we observed a significant effect of migration on CH_4 isotopic signatures in the bottom layers of the hypolimnia of both lakes, where under oxic conditions there was a systematic shift from less negative values to more negative signature upon the onset of migration, consistent with the input of fresh sedimentary CH_4. These results are in agreement with a previous study by Tang et al. (2018), who reported an increase of

79% in CH_4 concentration in the overlying water due to *Chaoborus* bioturbation at densities similar to ours.

We calculated maximum *Chaoborus*-mediated CH_4 fluxes from sediments in the hypolimnion from the difference in concentrations in the overlying water during the migration period (Table 1.6). It should be noted out that these values are net fluxes, because in the case of Croche in July and in all mesocosms, the bottom waters were oxic and the isotopic signatures suggest extensive oxidation of the CH_4 released (see section below). This explains in part the large differences in the apparent rates obtained between lakes and treatments (Table 1.6). The values we observed in lake Croche (200 - 1600 µmol m^{-2} d^{-1}) are comparable to those of another study that measured sediment fluxes derived from sediment cores in this same lake (800 ± 200 µmol m^{-2} d^{-1}; Thottathil, 2018). On the other hand, the estimated bioturbation-mediated fluxes for lake Cromwell (27 500 - 49 500 µmol m^{-2} d^{-1}) were 12 to 23 times higher than the sediment fluxes also measured by Thottathil (2018) for this lake (2 200 ± 800 µmol m^{-2} d^{-1}). The differences in apparent release rates between the two lakes are unlikely driven by differences in ambient *Chaoborus* density, which were roughly similar (Table 1.2), but rather may be related to sediment properties, and more importantly, by the extent of anoxia in the bottom waters, which was much more extensive in Cromwell, and therefore the influence of oxidation on the apparent CH_4 accumulation in these waters overlying the sediments.

Collectively, these observations would suggest that the amount of CH_4 that *Chaoborus* may release via bioturbation may be comparable or in fact greatly exceed the purely diffusive sediment/water CH_4 fluxes in these temperate lakes, and this even at the relatively low densities found in these lakes. Furthermore, these bioturbation-mediated sediment/water CH_4 fluxes are much higher, by at least one to three orders of magnitude, than the direct transport of CH_4 to the epilimnion by larvae in their tracheal sacs.

Table 1.6: Calculated *Chaoborus*-mediated flux from sediments to the hypolimnion via bioturbation. CH_4 and CO_2 fluxes from sediments in the hypolimnion were calculated with the difference between initial CH_4 and CO_2 concentration before the migration and maximum CH_4 and CO_2 concentrations measured during migration divided by the time between the two measurements. These represent net sediment release fluxes expressed as µmol m^{-2} d^{-1}.

		July		August	
		CH_4 (µmol m^{-2} d^{-1})	CO_2 (µmol m^{-2} d^{-1})	CH_4 (µmol m^{-2} d^{-1})	CO_2 (µmol m^{-2} d^{-1})
Croche	**Lake**	200	70 000	1 600	80 200
	High	50	30 600	200	57 400
Cromwell	**Lake**	27 700	30 400	49 500	37 700
	High	2 600	42 000	25 300	61 600

Another direct consequence of bioturbation is the release of CH_4 bubbles from the disturbed sediments, but it should be noted that we did not capture any bubbles during bioturbation events, neither with the echosounder nor the bubble trap. Increase in ebullitive fluxes was recorded during *Chaoborus* exiting the sediments in a tropical system (Bezerra-Neto et al. 2012). At the latitude of our study however, temperature and hydrostatic pressure (depth) play a much larger role in the release of CH_4 bubbles. In temperate lakes, bubbles are rarely observed in deeper pelagic areas and at the water column depths of our mesocosms (6-7 m maximum depth) (DelSontro et al. 2016). According to DelSontro et al. (2016), in lakes Croche and Cromwell, ebullition only occurred in waters shallower than 3 meters. Also, a regular daily physical disturbance of the sediment by bioturbation may prevent the build-up of CH_4 gas bubbles in the sediment, therefore reducing the actual overall ebullitive flux (Leal et al. 2007). For example, bioturbation induced by feeding benthivorous fish can strongly reduce CH_4 ebullition (67% lower) and reduce total CH_4 diffusive emissions to the atmosphere (by 33%) (Oliveira Junior et al. 2018).

It is important to note that we have not considered bioturbation associated to the return of *Chaoborus* to the sediments, which is likely to result in a second peak in CH_4 release, although our own results would suggest that this return is more gradual than the actual onset and therefore does not generate such a clear signal in either CH_4 concentration or isotopic signature.

1.5.4 Fate of the new CH_4 import to hypolimnetic waters

The sharp increases in CH_4 concentration in bottom layers of the hypolimnion associated to the onset of the nightly upwards migration of *Chaoborus* were systematically followed by a marked decline in CH_4 concentrations during the night, back to almost initial concentrations before migration. There are three processes that could explain these nightly patterns in CH_4 decline: 1) CH_4 oxidation in oxic bottom waters, 2) re-distribution of CH_4 within the hypolimnion, and 3) vertical transport of CH_4 to upper layers. As we discuss below, we find strong empirical support for the first two.

1.5.4.1 Oxidation of CH_4 released from the sediments via bioturbation, and links to hypolimnetic CO_2 dynamics

The oxidation hypothesis to explain CH_4 decrease over time can only potentially function in lake Croche and its mesocosms, and in the Cromwell mesocosms in the month of July, when bottom hypolimnetic waters were still oxic. The ambient CH_4 isotopic signatures suggest that the hypolimnetic CH_4 was on average relatively oxidised in those sites, as evidenced by the high fraction of oxidised CH_4 in the hypolimnion layer (39 to 81%, Table 1.7). However, there was a diurnal pattern related to the inputs of new CH_4 from sediments, with a shift to more negative values of CH_4 upon the onset of migration which resulted in a decrease in the fraction of oxidised CH_4 (0 to 61%, Table 1.7), followed by a slow return to less negative values during the night, clearly associated with the declining concentrations of CH_4 and therefore to the oxidation of this newly released CH_4. In this regard, Table 1.7 clearly shows the difference between the treatments that were oxic or anoxic at the time. Our results suggest that as long as there were traces of O_2 in the hypolimnion, most of CH_4 released by the *Chaoborus*-induced bioturbation was quickly oxidised within the hypolimnion itself. However, upon the onset of anoxia, (in lake Cromwell in July and August, lake Cromwell mesocosms in August) the CH_4 isotopic signature remained essentially constant at values that resemble a fresh source (around -70 to -75‰), yet there was still a decrease in CH_4 concentrations along the night, following the post-migration peak. This suggests that there is re-distribution and perhaps vertical diffusion of this accumulated CH_4 (see section below).

Table 1.7: Extent of CH_4 oxidation (f_{ox}) in the hypolimnion layers (6m for Croche and 5m for Cromwell) before the migration (f_{ox} initial), during the peak (f_{ox} peak) and after the migration (f_{ox} final).

Lake	Month	Treatment	f_{ox} initial	f_{ox} peak	f_{ox} final
Croche	July	Lake	0.81	0.61	0.82
		High	0.74	0.45	0.80
		Low	0.77	0.57	0.79
	August	Lake	0.43	0.28	0.46
		High	0.78	0.30	0.70
		Low	0.72	0.36	0.73
Cromwell	July	Lake	0.03	0.03	0.04
		High	0.40	0.48	0.50
		Low	0.39	0.41	0.59
	August	Lake	0.02	0.00	0.00
		High	0.13	0.08	0.12
		Low	0.13	0.06	0.08

As we discussed above, the net *Chaoborus*-mediated sediment flux is closely related to the degree of CH_4 oxidation in the hypolimnion, since oxidation appears to quickly dispose of much of the CH_4 that is released. Given the fact that *Chaoborus* density was not that different between the lakes, the differences we observed may be a function of oxidation rates. This is further evidenced by the relationship that exists between the net rate of sediment CH_4 release as a function of the fraction of CH_4 oxidised in the bottom hypolimnion waters where this CH_4 accumulates (Figure 1.14). This pattern would suggest that the actual gross rates of *Chaoborus*-mediated release may not be substantially different at comparable animal densities between the two lakes, once oxidation is accounted for. The gross sediment release rates that can be extrapolated from Figure 1.14, which coincide with those measured under anoxic conditions in lake Cromwell, are extremely high, and further emphasizes the key role of oxidation in disposing of this large mass of CH_4 that might otherwise find its way to the atmosphere.

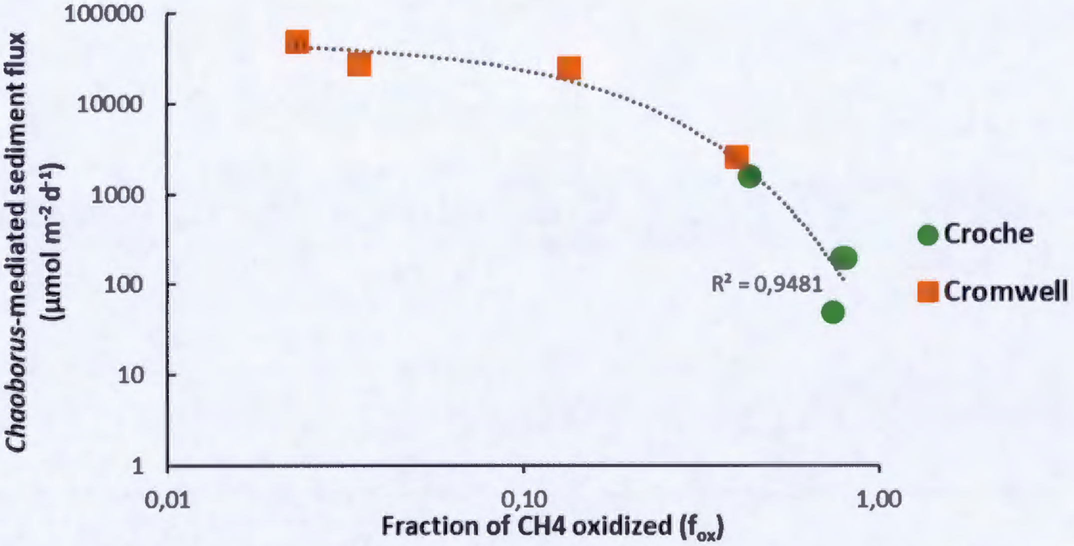


Figure 1.14: Relationship between the extent of CH_4 oxidation (f_{ox}) and the net *Chaoborus*-mediated sediment flux (µmol m^{-2} d^{-1}) in lake Croche and Cromwell. The dash line represents the exponential tendency between the two variables.

Significant CH_4 oxidation in hypolimnetic bottom waters should be mirrored by a pattern of local increase in CO_2 concentration and a lowering of the ^{13}C signature of the ambient CO_2. We followed the CO_2 concentrations in the bottom waters of the hypolimnion during the migration period, and at all times, there was a pattern of increasing hypolimnetic CO_2 concentration in both lakes and mesocosms (Supplementary Figures D1 and D2), leading to often extremely high rates of *Chaoborus*-mediated CO_2 release from sediments (Table 1.6). During oxic situations, the CO_2 also become more depleted in ^{13}C during the migration period, consistent with the oxidation of a ^{13}C-depleted substrate such as CH_4. It is difficult to apportion the contribution of CH_4 oxidation to ambient CO_2 dynamics in bottom waters, because the hypolimnetic CO_2 pool is so large relative to the relatively small changes in concentration and isotopic composition that results from the oxidation of CH_4. The fact that we observed equally large CO_2 fluxes in anoxic Cromwell hypolimnetic waters would suggest that not all this CO_2 is derived from the oxidation of the ambient CH_4, but rather that *Chaoborus* bioturbation also induces significant release of CO_2 from sediments. A previous study had concluded that bioturbation created by fish strongly increased CO_2 emissions from the sediments (Oliveira Junior et al. 2018), and Tang et al. (2017) had further suggested that bioturbation created by *Chaoborus* larvae contributed the same order of magnitude of CH_4 and CO_2 from exchange between the water-sediment interface. Our CO_2 results show that *Chaoborus* can also influence CO_2 dynamics in lakes by adding CO_2 during bioturbation events.

1.5.4.2 The re-distribution within the hypolimnion and diffusion

Vertical diffusive transport can occur from the hypolimnion to metalimnion and could reduce hypolimnetic concentrations over time diurnally, although this should be of minor importance in strongly thermally stratified systems such as the ones we were studying. We only recorded a marginally significant increase in CH_4 metalimnetic concentrations in lake Cromwell in August that could be associated to the transient increase in hypolimnetic CH_4 due to bioturbation. During the summer season, the hypolimnion layer is becoming enriched in CH_4, and this implies higher diffusive exchange with the overlying metalimnion (Thottathil et al. 2018). The oxidation rates are higher at the oxic/anoxic interface and this zone was always including at the top of the hypolimnion layer in our experiments, so we think that CH_4 from the bottom part of hypolimnion could be transported to the less enriched-CH_4 part of the hypolimnion, where oxidation at the oxic-anoxic interface was occurring. This pathway, however, would also imply a change in CH_4 isotopic signature, something that we did not observe in Cromwell, where the isotopic signature within the hypolimnion remained remarkably constant at close to source values once anoxia was attained. Since the *Chaoborus* larva are moving en masse, it is possible that they generate enough turbulence as they ascend the water column to significantly increase exchanges between hypolimnion and metalimnion waters beyond what would be expected from molecular diffusion alone. However, we did not observe any changes in CH_4 in the metalimnion that could be attributable to this phenomenon. We conclude that these two CH_4 transport processes would be only a small part of the CH_4 decrease and those hypotheses are not sufficient to explain CH_4 decline in bottom waters during the nightly cycle under anoxic conditions.

According to our calculations, however, a significant portion of the decrease of CH_4 concentration in bottom waters during the night could be explained by its re-distribution within the entire hypolimnetic layer. The dilution calculation (Table 1.4) shows that re-distribution of the CH_4 released by bioturbation, and that has escaped immediate oxidation to the whole hypolimnion, can account for the observed nightly decline that oxidation cannot explain. When the hypolimnion is completely anoxic, as in lake Cromwell, essentially all of the decrease can be explained by redistribution. The resulting daily step increase in overall hypolimnetic CH_4 concentration is small, even under anoxic conditions, because of the relatively large volume of hypolimnion, but this nevertheless becomes significant over the summer season, as we describe below.

1.5.4.3 The long-term impact of Chaoborus bioturbation on hypolimnetic CH_4 accumulation

As we discussed above, the diurnal migration of *Chaoborus* generates a comparatively large CH_4 release events in the bottom layers from sediments. We have further shown that whereas most of this CH_4 is rapidly consumed in the overlying hypolimnetic waters under oxic conditions, upon the onset of anoxia, this CH_4 released tends to accumulate in the bottom waters. We estimated the potential contribution of *Chaoborus*-mediated CH_4 release to the long-term CH_4 hypolimnetic build up for both lake Croche and Cromwell. The average hypolimnetic CH_4 concentrations varied from July to August from 0.13 μmol L^{-1} to 0.98 μmol L^{-1} in lake Croche and from 20.3 μmol L^{-1} to 32.04 μmol L^{-1} in lake Cromwell. We observed the same trend of accumulation in the High mesocosms, as the average hypolimnetic CH_4 concentrations varied from July to August from 0.06 µmol L^{-1} to 0.09 µmol L^{-1} in Croche and from 1.4 µmol L^{-1} to 20.15 µmol L^{-1} in Cromwell. The difference between Croche and Cromwell is striking, and this is due mainly by the fact that there was much less oxidation in Cromwell, or not at all.

The predicted hypolimnetic concentration in August that would result only from the accumulation of the bioturbation-mediated released CH_4 is shown in Table 1.8. Our calculations suggest that the accumulation over mid-summer of these bioturbation-mediated CH_4 releases could amount to 1.59 µmol L^{-1} and 0.45 µmol L^{-1} over a period of 30 days between July and August in the hypolimnion of lake Croche and in the Croche High treatments, and of 92.44 and 25.20 µmol L^{-1} for the hypolimnion of lake Cromwell and the lake Cromwell High treatment, respectively. This implies that this pathway could potentially account for between 125 and 500% of the net increase in mean hypolimnetic CH_4 concentration that was actually observed in these waters during the period. These predicted values are systematically higher than the observed concentrations in both ambient lake waters and in the mesocosms, but they are nevertheless in the same order of magnitude. This would suggest that 1) bioturbation may be an important source of CH_4 leading to hypolimnetic CH_4 buildup over the stratified period, and 2) there is further loss of this CH_4 released, even under the generalized anoxic conditions prevailing in the hypolimnia of lake Cromwell and its mesocosms. This could be oxidation at the oxic/anoxic interface in upper part of hypolimnion, which has been shown to be a site of major CH_4 loss in these lakes (Thottathil et al. 2018). It could also be the turbulence generated by the animals themselves that enhances vertical mixing, as we discussed previously. Finally, other processes, such as anaerobia CH_4 oxidation, which we did not measure in this study, may explain a part of this mismatch between the predicted and measured values in Table 1.8. We note that the values are closer in the scenarios when there was still oxygen in the hypolimnion (every experiment except in lake Cromwell), reflecting that under oxic conditions, our predicted rates of accumulation more realistically capture the ambient dynamics of *Chaoborus*-mediated flux. These calculations are admittedly simplistic, since they assume a fixed accumulation rate throughout this 30-day period and no further losses of CH_4, but nevertheless they do allow us to place the *Chaoborus*-mediated process within the context of the observed seasonal CH_4 dynamics in the hypolimnion.

Table 1.8: Predicted and measured concentrations of CH_4 at the beginning of August (μmol L^{-1}) in the hypolimnion (4-7m for Croche and 3-6m for Cromwell). Predicted concentrations (from equation 6) are based on the accumulation over 30 days using the values from Table 1.4.

	Croche		Cromwell	
	Predicted	**Measured**	**Predicted**	**Measured**
Lake	1.6	1.0	92.4	32.0
High mesocoms	0.5	0.1	25.2	20.2

It is interesting to note that we observed a similar pattern of long-term accumulation between the Low and High Cromwell mesocosms from July (0.50 μmol L^{-1}) to August (18.65 μmol L^{-1}), despite the Low mesocosms having much lower *Chaoborus* densities (around 400 to 1400 ind. L^{-1}) and the fact that we never observed any evidence of a *Chaoborus*-mediated diurnal pattern of sediment CH_4 release. This implies that the CH_4 release by sediments may occur regardless of the presence of *Chaoborus*, and it is difficult to separate the *Chaoborus* effect from other process, at least across the range of densities that we assessed. Alternatively, the fact that there is a convergence in the long-term CH_4 accumulation between High and Low mesocosms despite massive differences in the rates of sediment release may point to the existence of processes that remove hypolimnetic CH_4 that we did not observe, but which should be addressed in future studies.

We conclude that even under lower densities than studied to date, bioturbation by *Chaoborus* larvae contributes to CH_4 release to the hypolimnion. Under oxic conditions this biological activity supports extremely high rates of CH_4 oxidation in the overlying bottom waters. The CH_4 that escapes this initial oxidation is then redistributed to the entire hypolimnion, where it will undergo further oxidation, with a small fraction eventually accumulating. This accumulation of CH_4 originating from bioturbation in the hypolimnion of lakes may represent a CH_4 flux during the turnover in autumn. In this way, in lakes such as the ones we studied, *Chaoborus* may have a delayed effect on CH_4 fluxes to the atmosphere (during turnover), which can have more significance than the diurnal effects on the water column that we measured in this study.

1.6 CONCLUSION

This study has focused mainly on the potential effects of the direct transport of CH_4 by *Chaoborus* in tracheal sacs from sediments to surface waters in lakes, and also the effect of bioturbation created by *Chaoborus* larvae when they migrate out on hypolimnetic CH_4 dynamics. Our results suggest that epilimnetic CH_4 concentrations stay stable through most of *Chaoborus* migration periods and there is no quantifiable effect on the fluxes to the atmosphere indicating that transport of CH_4 by *Chaoborus* from sediments to the surface waters is not large enough to discern a direct change at the densities we observed. However, our calculation of possible CH_4 transport from sediments to epilimnetic waters by their tracheal sacs is on the same order of magnitude of the water/air fluxes we measured at night, suggesting that *Chaoborus* can nevertheless contribute significantly to those fluxes. Overall, our study demonstrates that *Chaoborus* larvae significantly increase hypolimnetic CH_4 concentrations though bioturbation, in both ambient lake waters and in the High mesocosm treatments as a result of diel migration. In lake Croche and in the experimental mesocosms, which remained oxic longer during the summer, most of this CH_4 was oxidised in the water layer immediately overlying the sediments, with the remainder redistributed within the hypolimnion. Under anoxic conditions, and in the absence of oxidation, the redistribution of the CH_4 released from the sediments led to larger shifts in the overall hypolimnetic CH_4 concentrations. The accumulation of this *Chaoborus*-associated CH_4 explained much of the increase in CH_4 concentrations in the hypolimnion of the two lakes over the summer, and this accumulation is likely important for CH_4 lake fluxes during the turnover in autumn. This implies that *Chaoborus* may have a delayed effect on CH_4 fluxes to the atmosphere, but one that is likely larger than potential diurnal effects on surface fluxes.

CONCLUSION GÉNÉRALE

2.1 CONCLUSION DU PREMIER CHAPITRE

Le CH_4 est principalement produit et accumulé dans les sédiments. La faune benthique peut avoir une forte influence sur le cycle du CH_4 dans les écosystèmes lacustres en augmentant les possibilités d'échange entre les sédiments et la colonne d'eau. Les larves de *Chaoborus* (*Diptera-Chaoboridae*) sont des agents de bioturbation importants de par leur migration journalière des sédiments vers la colonne d'eau. L'enfouissement de ses larves dans les sédiments peut augmenter la surface d'interface eau-sédiment ainsi que les échanges entre les sédiments et la colonne d'eau. Cela peut considérablement augmenter le flux de CH_4 provenant des sédiments dans la colonne d'eau par diffusion. En outre, la bioturbation de *Chaoborus* est susceptible de favoriser la libération de CH_4 par ébullition. De plus, ces larves utilisent le méthane dans les sédiments pour gonfler leurs sacs trachéaux, leur permettant de migrer par flottaison, puis elles le relâchent à la surface pour revenir dans les sédiments. Le CH_4 dissous, étant généralement sursaturé dans les sédiments, fournit une pression partielle suffisante pour gonfler les sacs trachéaux. Au lever du soleil, *Chaoborus* libère du CH_4 accumulé dans ses sacs trachéaux à la surface des eaux, permettant ainsi la descente des larves. Ces migrations facilitent donc le transport du CH_4 accumulé dans les sédiments dans la colonne d'eau et possiblement dans l'atmosphère.

Malgré leur rôle en tant que transporteur passif et actif du méthane, très peu d'information existe sur la contribution du *Chaoborus* aux émissions de méthane vers l'atmosphère. Le but de cette étude est d'évaluer et de quantifier les effets de *Chaoborus* sur les émissions de CH_4 et la dynamique de CH_4 dans les lacs. Ainsi, pour compléter les travaux antérieurs (axés sur des densités de *Chaoborus* extrêmement élevées), nous avons testé différentes densités représentatives de lacs tempérés typiques afin de déterminer s'il existe des effets sur les concentrations de méthane, améliorant ainsi notre compréhension encore très préliminaire de la dynamique du CH_4 dans les lacs. Étant difficile de contrôler plusieurs facteurs (densité de *Chaoborus* et de zooplanctons, concentration de CH_4, tout apport organique et non organique) en milieux naturels, nous avons utilisé des mésocosmes expérimentaux dans deux lacs naturels du Québec. Ces mésocosmes, faits de grands sacs en plastique tubulaires, étaient suspendus en paires de traitements identiques sur une structure en bois flottante. Le sommet des mésocosmes était ouvert à l'air pour permettre les échanges de gaz avec l'atmosphère et le fond des sacs était fixé à une base, constituée de feuilles de plastique et de tiges de métal, qui était enfoncée dans les sédiments et maintenue par des poids. Les mésocosmes étaient ainsi isolés du lac, mais possédaient les mêmes propriétés thermiques. Finalement, ces mésocosmes étaient inoculés de différentes densités de larves de *Chaoborus*, soit de très grande (High) et très faible (Low). Cela nous a permis de suivre le mouvement des larves de *Chaoborus* pendant leur migration et avons associé leurs activités à la dynamique du CH_4 dans la colonne d'eau. Des comparaisons simultanées avec les lacs naturels ont pu être faites. Nous avons principalement mesuré l'évolution de la concentration de CH_4 ainsi que sa signature isotopique à différents moments de la journée et de la saison estivale, associée à la migration larvaire et à leur activité de bioturbation. Des données sur la densité de *Chaoborus* et de zooplanctons ont aussi été prélevées.

Les résultats obtenus démontrent que les larves de *Chaoborus* ont le même comportement dans les mésocosmes que dans les lacs. Le suivi par échosondeur a permis de connaitre les heures d'activité susceptible d'affecter la dynamique du CH_4 dans la colonne d'eau, soit la bioturbation autour du coucher du soleil (20h00-22h00) et le relâchement du CH_4 par les sacs trachéaux autour du lever du soleil (03h00-05h00). Nous avons observé des concentrations épilimnétiques de CH_4 stables pendant la plupart des périodes de migration de *Chaoborus* et aucun effet sur les flux dans l'atmosphère. Cependant, nous avons observé une augmentation exceptionnelle dans la concentration du CH_4 épilimnétique en août dans le lac Cromwell. En revanche, pour un certain nombre de raisons, nous concluons que cette augmentation ne peut être attribuée seulement aux larves de *Chaoborus*. La quantité maximale de CH_4 que les *Chaoborus* pourraient potentiellement transporter des sédiments par les sacs trachéaux à ce moment-là, sur la base des densités maximales observées et du volume moyen des sacs trachéaux de 0,12µl, représente seulement le quart de l'augmentation mesurée. Cette augmentation serait davantage liée au cycle naturel journalier du CH_4. D'autres études sont aussi arrivées à la conclusion que la quantité maximale de flux diffusif de CH_4 potentiellement attribuable à *Chaoborus* est très faible par rapport au flux diffusif saisonnier total. Il faut une très grande densité de larves pour avoir un effet significatif sur la concentration de CH_4 à la surface et sur les émissions dans l'atmosphère. De plus, les flux dans l'air ne correspondraient pas toujours à la quantité de CH_4 libérée par les larves, car une partie de ce CH_4 est perdue en raison de l'oxydation dans la colonne d'eau, de sorte que seulement une petite partie contribuerait aux émissions atmosphériques.

Nous avons observé des augmentations significatives des concentrations hypolimnétiques de CH_4 dans les lacs et les mésocosmes à densité de *Chaoborus* élevés (High) au cours de la migration. Les augmentations correspondent aux moments de sortie des larves pendant que les concentrations de CH_4 sont restées stables au cours de la même période dans les mésocosmes à faible densité de larves (Low). Les résultats obtenus confirment donc l'hypothèse de la bioturbation. Ainsi, bien que nos densités maximales soient proches des valeurs globales minimales, nous avons néanmoins constaté un effet significatif dans l'hypolimnion. La bioturbation des *Chaoborus* peut affecter fortement la biogéochimie des sédiments en accélérant les flux chimiques des sédiments vers la colonne d'eau. En outre, si nous comparons ces augmentations à la quantité possible que les larves peuvent transporter par leurs sacs trachéaux, la bioturbation a un effet beaucoup plus important sur la dynamique du CH_4.

Les résultats obtenus dans cette recherche démontrent aussi qu'après la plupart des augmentations de CH_4 mesuré dans l'hypolimnion associé à la bioturbation, les concentrations de CH_4 ont de nouveau diminué pendant la nuit, revenant presque aux concentrations initiales avant la migration. La diminution dans le temps des concentrations hypolimnétiques de CH_4 pourrait résulter de l'oxydation de ce CH_4 en présence d'oxygène. Les signatures isotopiques du CH_4 deviennent plus positives au cours de la nuit, associées à l'oxydation du CH_4 hypolimnétique tandis qu'en absence d'oxygène, les signatures isotopiques restent stables et plus négatives. Les tests de corrélation et de régression ont montré qu'il existait effectivement une relation entre la signature isotopique et la concentration en oxygène dissous dans les eaux hypolimnétiques. Des valeurs de signature isotopiques plus positives correspondent à des eaux plus oxygénées (CH_4 oxydé en présence d'oxygène) et des valeurs négatives à des eaux moins oxygénées (CH_4 non oxydé sous anoxie). Il est également possible que le transport vertical par diffusion de CH_4 dans la colonne d’eau ait pu réduire les concentrations hypolimnétiques au cours de la nuit. Le transport vertical peut se produire de l’hypolimnion au métalimnion, bien qu’il soit d’une importance mineure en raison de la barrière physique due à la stratification. Les résultats ont montré seulement une légère augmentation marginale des concentrations de CH_4 métalimnétique et seulement dans le lac Cromwell en août. Il pourrait s’agir d’une diffusion de l’hypolimnion, mais les résultats ne montrent aucun autre changement significatif. Au cours de la saison estivale, la zone oxydée est réduite dans le lac Cromwell, ce qui implique un échange diffus plus important de CH_4 accumulé de l'hypolimnion en métalimnion afin de soutenir l'oxydation dans le métalimnion. Ce phénomène peut expliquer la diminution de l'hypolimnion si l'oxydation à l'interface oxique-anoxique dans le métalimnion aspire le CH_4 de la partie inférieure de l'hypolimnion vers la partie moins enrichie en CH_4 de l'hypolimnion. Bien que nous ayons observé une diminution du CH_4 hypolimnétique après les augmentations au coucher du soleil, les valeurs ne sont pas revenues complètement aux concentrations initiales. De plus, nous avons observé une augmentation des concentrations hypolimnétiques globales de juillet à août dans les deux lacs. Il semble donc y avoir une accumulation lente dans la partie anoxique pendant l’été. Nous nous attendons à ce qu'une accumulation de CH_4 provenant de la bioturbation dans l'hypolimnion des lacs puisse être importante pour les flux de CH_4 lors du mélange des eaux à l'automne.

En résumé, l'étude portait principalement sur l'effet de la bioturbation créée par les larves de *Chaoborus* sur la dynamique du CH_4 et sur l'effet du relargage de CH_4 de leurs sacs trachéaux dans la colonne d'eau lorsqu'ils retournent dans les sédiments. Notre étude indique que les concentrations épilimnétiques de CH_4 restent stables pendant les périodes de migration de *Chaoborus* et n'affectent donc pas les émissions dans l'atmosphère. Notre étude démontre que les larves de *Chaoborus* augmentent considérablement les concentrations de CH_4 hypolimnétique dans les lacs et les traitements de densité de larves élevée (High) pendant la migration en raison de leur comportement de bioturbation. Une partie de ce CH_4 est transportée à travers la colonne d'eau tandis que la majeure partie est oxydée. Cependant, il y a toujours une accumulation de cette nouvelle importation de CH_4 provenant de la bioturbation dans l'hypolimnion anoxique pendant la saison estivale. Cette accumulation peut être importante pour les flux de CH_4 du lac lors du mélange des eaux en automne. Les mesures de flux classiques à l'interface air-eau ne tiennent pas encore compte du CH_4 hypolimnétique ajouté en raison de la bioturbation de *Chaoborus*. Ainsi, dans des lacs tels que ceux que nous avons étudiés, *Chaoborus* pourrait avoir un effet direct retardé sur les flux de CH_4 dans l'atmosphère, ce qui est plus important que les effets instantanés que nous avons mesurés dans cette étude.

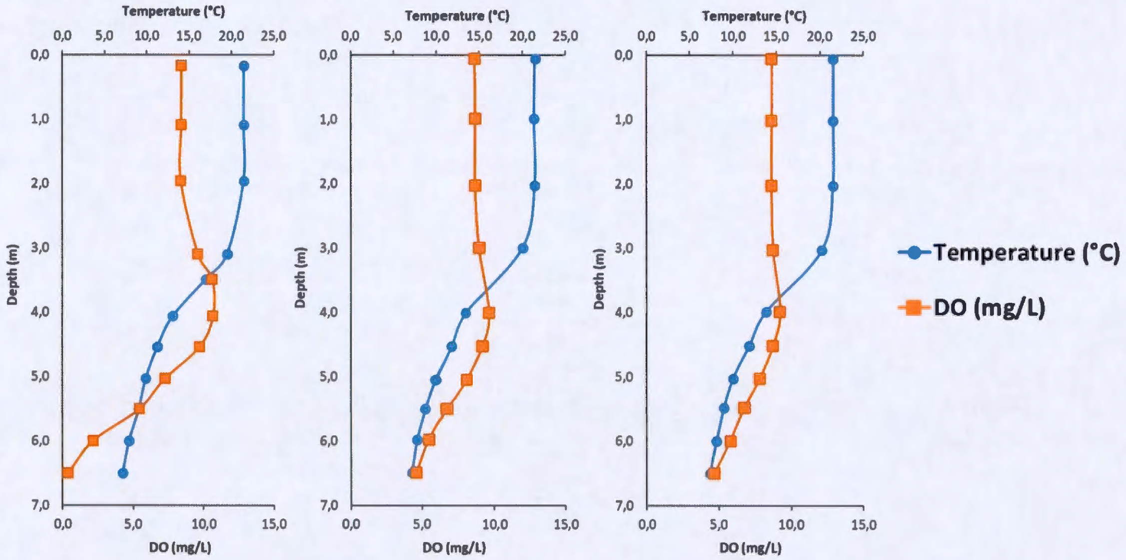
2.2 RECOMMANDATIONS

Cette étude en mésocosmes était une des premières à quantifier l'effet de différente densité de larve de *Chaoborus* sur la dynamique de CH_4 à travers la colonne d'eau ainsi que la première à observer des augmentations significatives des concentrations hypolimnétiques dues à la bioturbation. Ce résultat démontre l'importance du rôle potentiel des activités biologiques dans la régulation de la dynamique du CH_4 dans les lacs. Si nous avions à faire de nouveau l'expérience, il serait important de tester les sédiments au fond des mésocosmes avant et après l'expérience pour s'assurer l'homogénéité entre les mésocosmes et tester si les *Chaoborus* peuvent avoir un effet sur la composition des sédiments. Ces éléments peuvent avoir un rôle important dans la production de CH_4 dans les sédiments. De plus, il faudrait davantage de mésocosmes pour avoir davantage de réplicats et de traitements de densité larvaire. L'effet des larves sur la dynamique de CH_4 aurait été mieux capté si nous avions plusieurs sondes automatiques prenant des données de profile de concentration de CH_4 en continu, ainsi nous aurions pu mesurer l'étendue de l'impact des augmentations de CH_4 plus précisément dans la colonne d'eau.

Il faut une très grande densité de larves de *Chaoborus* pour avoir un effet direct et significatif sur le transport du CH_4. Cependant, à plus faible densité, elles pourraient quand même avoir un effet indirect via les interactions trophiques. Il serait intéressant pour de futures recherches de pousser plus loin et de tester l'effet de différente densité de larves sur la communauté de zooplanctons et de bactéries. Les larves de *Chaoborus* sont de très grandes consommatrices de zooplanctons et elles peuvent donc contrôler la biomasse des communautés de zooplancton ce qui pourrait avoir un effet sur la prédation par les zooplanctons sur la communauté bactérienne, par exemple sur les bactéries oxydatives de méthane (méthanotrophes). Ces changements dans la biomasse de la communauté de méthanotrophes pourraient ainsi modifier la dynamique d'oxydation du CH_4 et ainsi la concentration dans la colonne d'eau et les émissions dans l'atmosphère. Les larves de *Chaoborus* peuvent donc aussi avoir un effet indirect, à l'aide de l'effet des zooplanctons sur les bactéries méthanotrophes, sur l'oxydation du CH_4 dans la colonne d'eau. La compréhension des effets des interactions de la chaîne alimentaire sur le flux de carbone entre les lacs et l'atmosphère est particulièrement importante. L'importance quantitative des zooplanctons, qui peuvent modifier les fonctions ainsi que les cycles biogéochimiques de l'écosystème, comme l'activité méthanotrophique et les flux de CH_4, a besoin d'être étudiée plus en profondeur dans des conditions de milieu naturel.

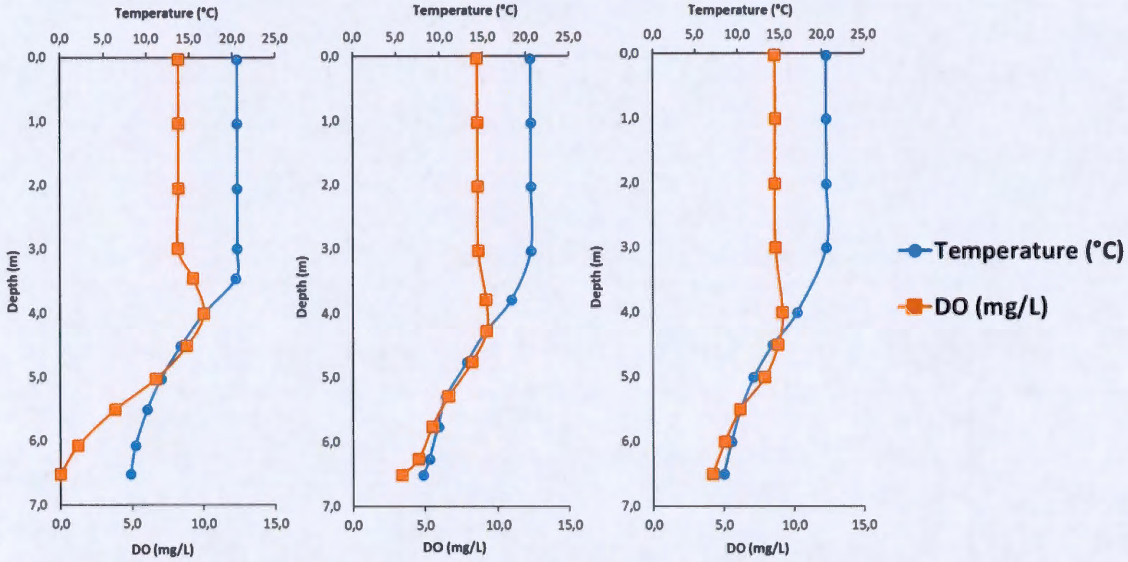
APPENDIX A

TEMPERATURE AND OXYGEN PROFILE OF LAKE CROCHE AND THE MESOCOSMS



Lake High Low

Figure A.1: Temperature and oxygen profile of lake Croche and the mesocosm in July

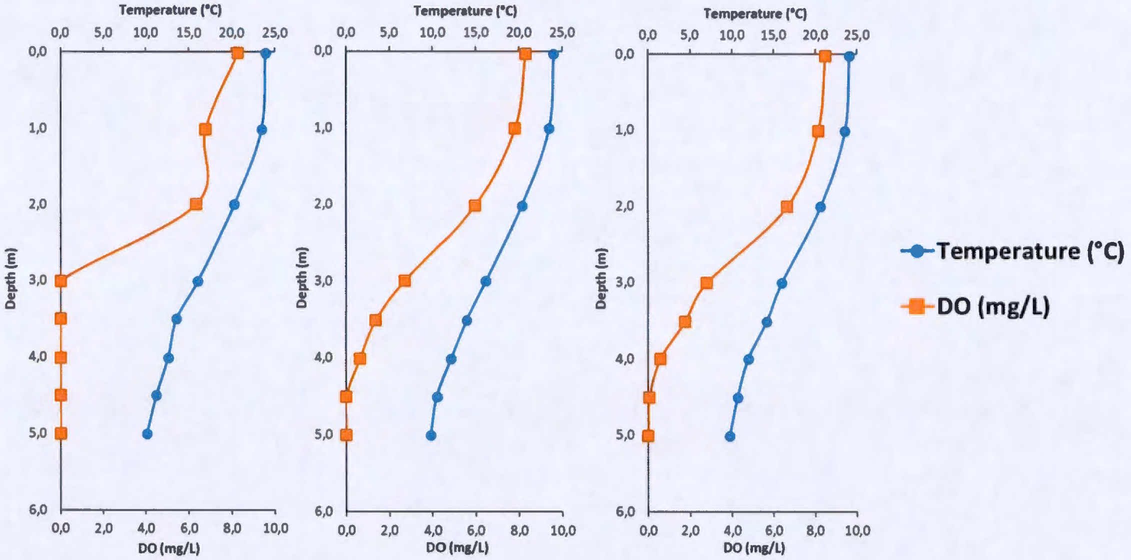


Lake High Low

Figure A.2: Temperature and oxygen profile of lake Croche and the mesocosms in August

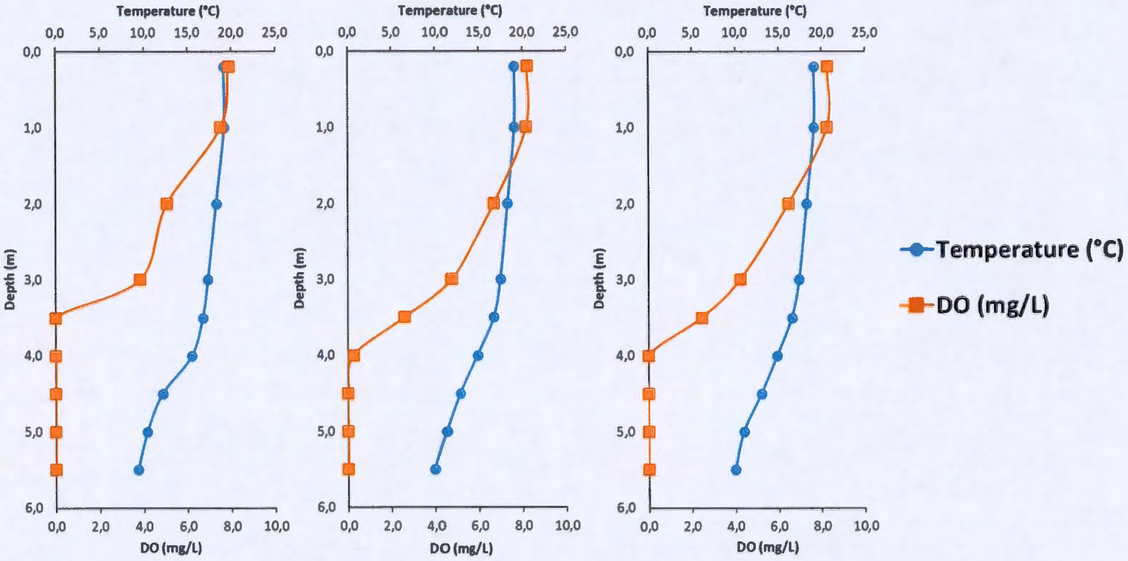
APPENDIX B

TEMPERATURE AND OXYGEN PROFILE OF LAKE CROMWELL AND THE MESOCOSMS



Lake High Low

Figure B.1: Temperature and oxygen profile of lake Cromwell and the mesocosms in July



Lake High Low

Figure B.2: Temperature and oxygen profile of lake Cromwell and the mesocosms in August

APPENDIX C

MEAN CO_2 CONCENTRATIONS AND CO_2 ISOTOPIC SIGNATURES DURING THE WHOLE EXPERIMENT

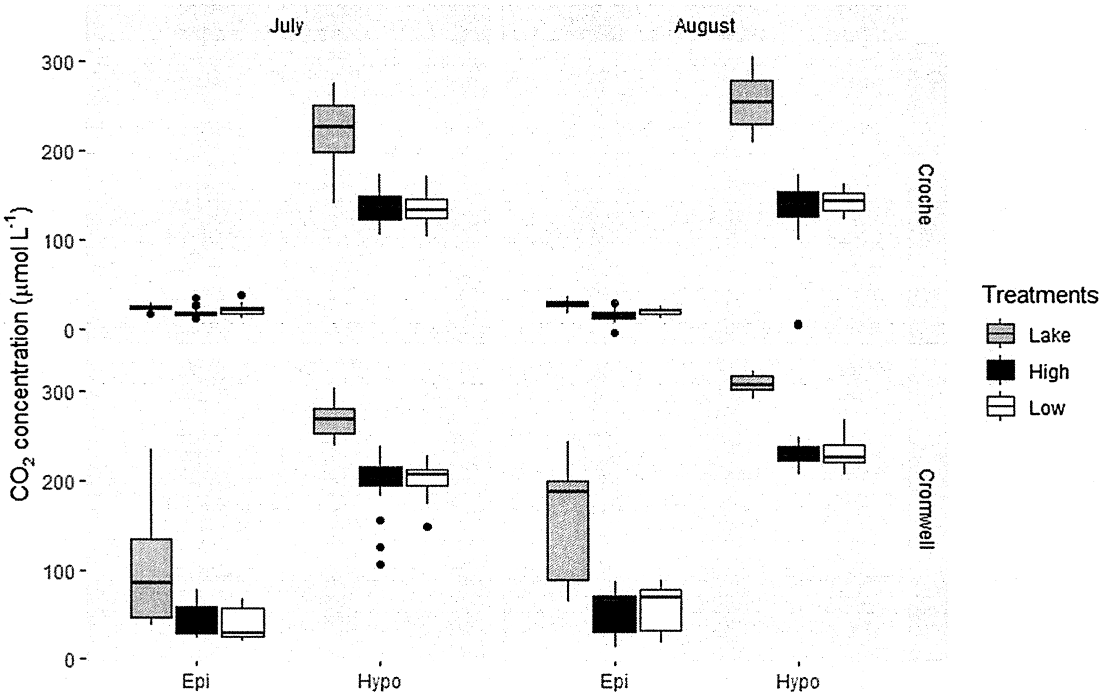


Figure C.1: Box plot distribution of CO_2 concentrations in each thermal layer for the lake and *Chaoborus* treatments in July and August for lakes Croche and Cromwell. For each lake, all time points during the whole experiment were averaged across treatment (Lake, High, Low) and by depth layer: epilimnion (1m) and hypolimnion (6m Croche and 5m Cromwell).

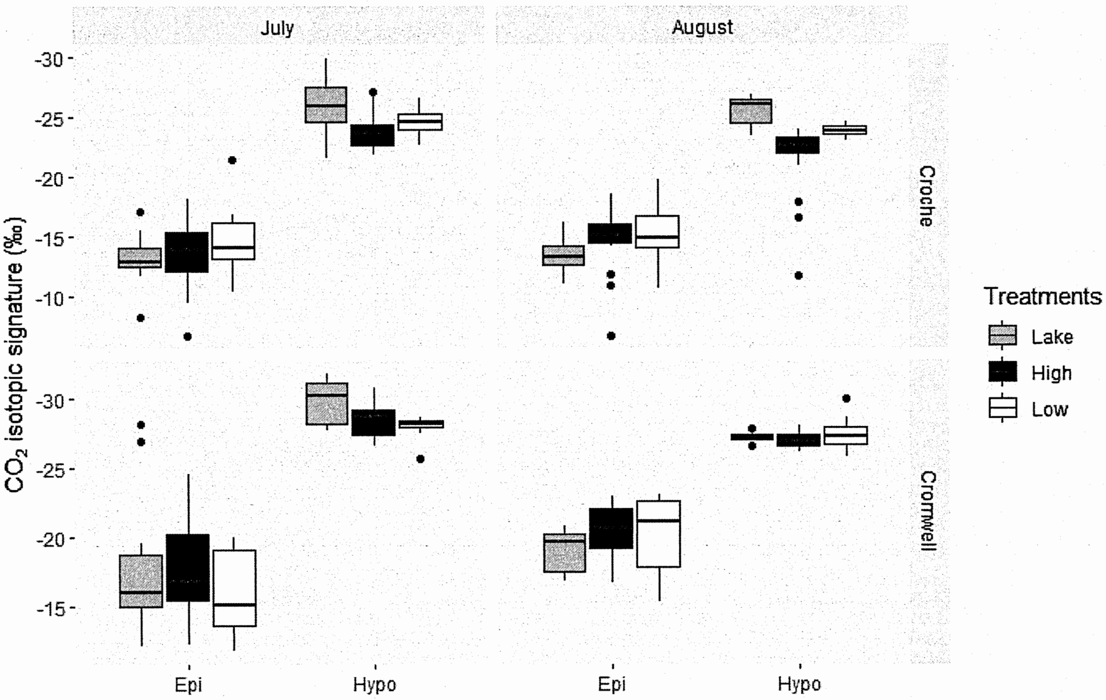


Figure C.2: Box plot distribution of CO_2 isotopic signatures in each thermal layer for the lake and *Chaoborus* treatments in July and August for lakes Croche and Cromwell. For each lake, all time points during the whole experiment were averaged across treatment (Lake, High, Low) and by depth layer: epilimnion (1m) and hypolimnion (6m Croche and 5m Cromwell).

APPENDIX D

MEAN CO2 CONCENTRATIONS AND CO2 ISOTOPIC DURING SPECIFIC MIGRATION TIME

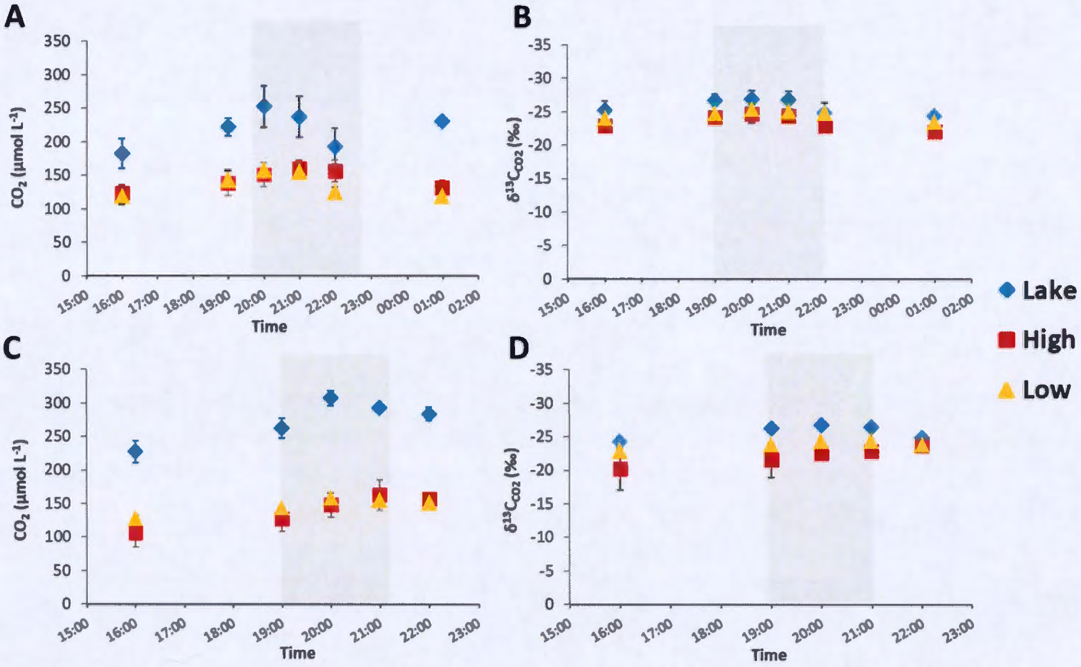


Figure D.1: Mean ± SE hypolimnetic (6m) CO_2 concentrations (A, C) and CO_2 isotopic signatures (B, D) in lake Croche in July (A, B) and August (C, D) through the diurnal migration period. Gray areas represent the times during which the larvae were leaving the sediments.

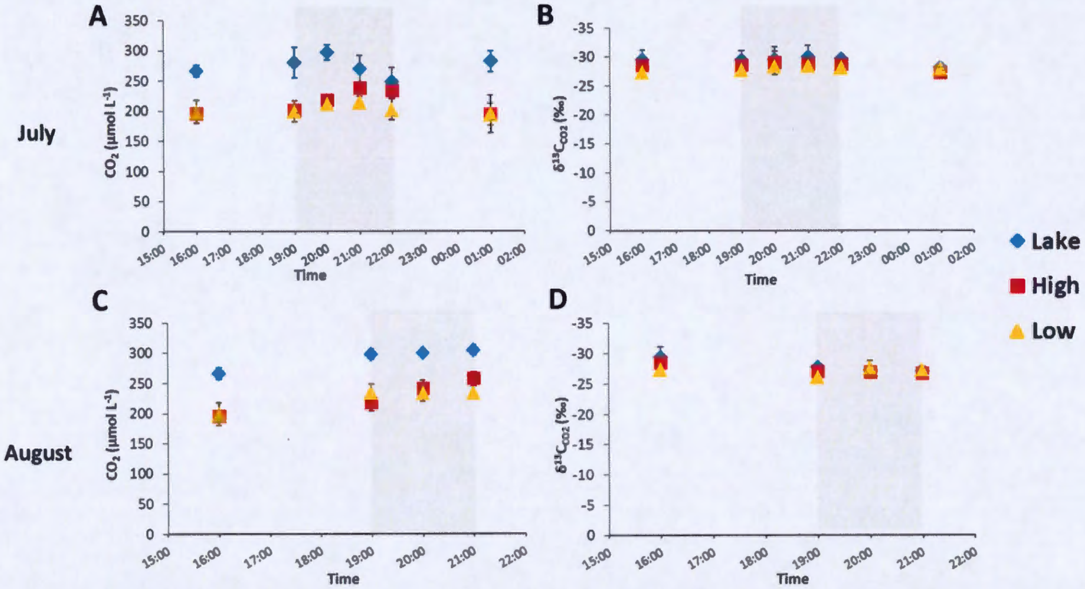


Figure D.2: Mean ± SE hypolimnetic (5m) CO_2 concentrations (A, C) and CO_2 isotopic signatures (B, D) in lake Cromwell in July (A, B) and August (C, D) through the diurnal migration period. Gray areas represent the time the larvae are going out of the sediments.

APPENDIX E

CH_4 FLUXES TO THE ATMOSPHERE AND VALUES OF K DURING MIGRATION TIME

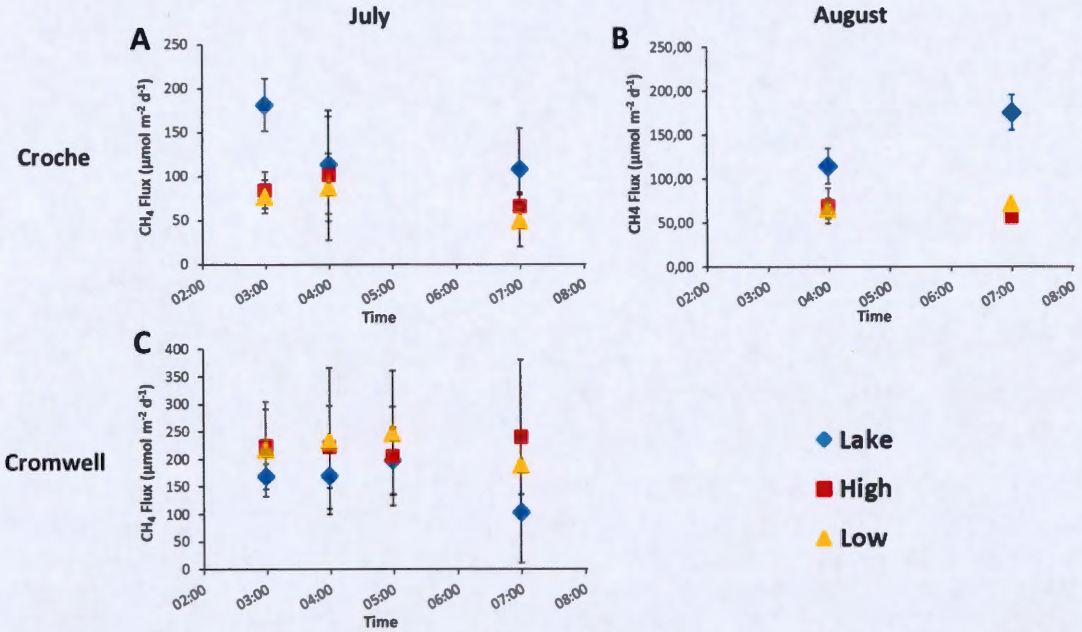


Figure E.1: Mean ± SE CH_4 flux to the atmosphere in July (A, C) and August (B) in lakes Croche, Cromwell and their respective mesocosms. Unfortunately, we were unable to collect data for August in lake Cromwell.

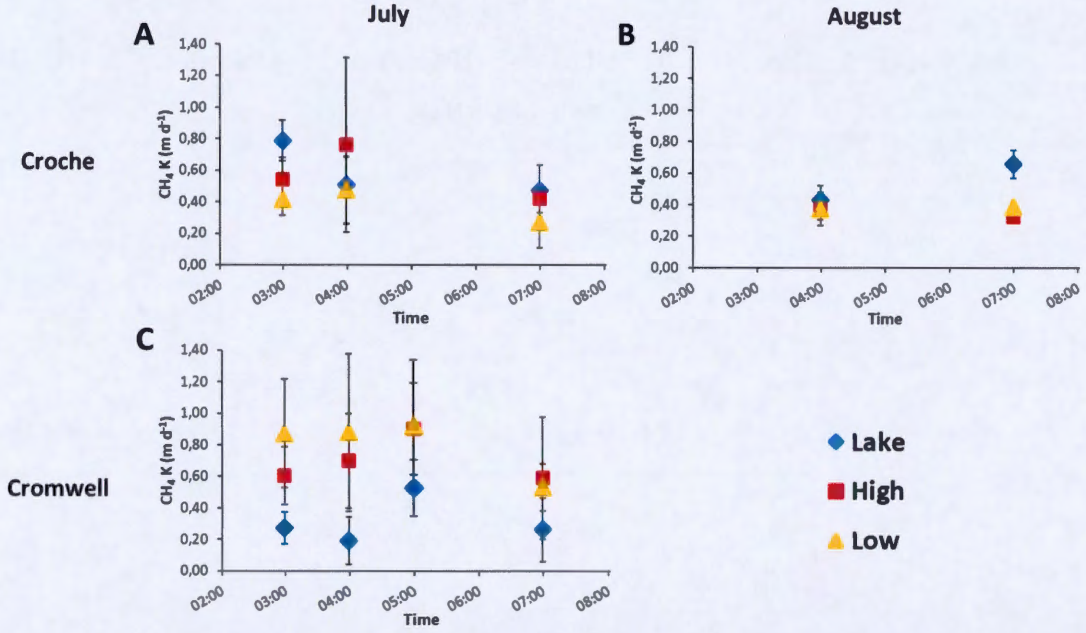


Figure E.2: Mean ± SE CH$_4$ K values of CH$_4$ fluxes in figure E.1 in July (A, C) and August (B) in lakes Croche, Cromwell and their respective mesocosms. Unfortunately, we were unable to collect data for August in lake Cromwell.

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