



Winter-active plankton community in a boreal lake
A high-resolution study of seasonal and vertical abundance patterns

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Résumé

Un couvert de glace se forme sur plus de 85 % des lacs de la planète pendant l'hiver, induisant une réduction de la pénétration lumineuse et de la diffusion d'oxygène dans la colonne d'eau, avec des implications en cascade sur le réseau trophique planctonique. J'ai étudié la communauté planctonique pélagique entre le retournement automnal en novembre 2020 et le retournement printanier en avril 2021, englobant une période de 5 mois durant laquelle la surface du lac était gelée, afin d'analyser comment le plancton est affecté et influencé par les modifications de la biogéochimie et de l'hydrodynamique sous la glace. Le lac boréal Simoncouche a été échantillonné pour la chlorophylle-*a*, le zooplancton (rotifères, cladocères, copépodes) et le microplancton (bactérioplancton, picoplancton, nanoflagellés) à cinq profondeurs lors de six dates d'échantillonnage. La température, l'oxygène, la conductivité et la lumière ont été mesurés par profilage à haute fréquence et par des capteurs ancrés. Une grande diversité de zooplancton a été observée, avec une richesse taxonomique sous la glace variant entre 18 espèces (en décembre) et 22 (en février et avril). La plupart des espèces privilégiaient les couches plus profondes caractérisées par une température de l'eau plus élevée, tandis que la biomasse de zooplancton la plus faible était la plupart du temps en surface. Plusieurs espèces de rotifères et de cladocères ont présenté une production d'œufs considérable (jusqu'à 20 % de la population portant des œufs), laquelle a perduré jusqu'au milieu de l'hiver en février. La reproduction hivernale des copépodes était restreinte au printemps, lorsque l'activité biologique globale augmentait dans la colonne d'eau avant le dégel. L'hétérotrophie représentait la principale voie énergétique chez les nanoflagellés, et la biomasse des nanoflagellés hétérotrophes augmentait de manière significative au début du printemps sous la glace, tandis que les nanoflagellés pigmentés étaient abondants durant les saisons intermédiaires. Cette étude met en lumière une hétérogénéité saisonnière et verticale au sein de la communauté planctonique active en hiver, en correspondance avec l'évolution de la stratification de la colonne d'eau, la disponibilité des ressources et des adaptations évolutives, soulignant ainsi l'activité biologique substantielle de la saison hivernale et ses conséquences potentiellement significatives sur la saison suivante en eau libre.

Abstract

Ice covers > 85% of the world's lakes during winter, preventing light penetration and oxygen diffusion to the water column, with cascading effects on the planktonic food web. I studied pelagic plankton community between the fall overturn in November 2020 and spring overturn in April 2021, including a 5-month ice-covered season, focusing on how plankton is enabled and driven by changing biogeochemistry and hydrodynamics under ice. Boreal Lake Simoncouche was sampled for chlorophyll-*a*, zooplankton (rotifers, cladocerans, copepods) and microplankton (bacterioplankton, picoplankton, nanoflagellates) at five depths on six sampling dates. Temperature, oxygen, conductivity, and light were measured by high frequency profiling and by moored sensors. A large diversity of zooplankton was encountered, with an under-ice taxa richness that varied between 18 (December) and 22 species (February and April). Most species preferred deeper strata that were characterized by warmer water, while the lowest zooplankton biomass was near the surface at most times. Several species of rotifers and cladocerans sustained considerable egg production (up to 20% of population egg-bearing) that ceased only in mid-winter in February. Copepod winter reproduction was restricted to spring when the overall biological activity increased in the water column before ice-off. Heterotrophy was the main energy pathway among the nanoflagellates and the heterotrophic nanoflagellate biomass increased considerably in early spring under the ice, while pigmented nanoflagellates were abundant during the shoulder seasons. This study demonstrates that there is seasonal and vertical heterogeneity in the winter-active plankton community, following the shifting water column stratification, resource availability, and evolutive adaptations, and emphasises that winter is a biologically active season with likely important repercussions to the following open water season.

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chl- <i>a</i>	Chlorophyll- <i>a</i>
DAPI	4',6-diamidino-2-phenylindole
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DW	Dry weight
HNF	Heterotrophic nanoflagellates
OM	Organic matter
PAR	Photosynthetically active radiations
PNF	Pigmented nanoflagellates
PUFA	Polyunsaturated fatty acid
TN	Total nitrogen
TP	Total phosphorus
Z _{max}	Deepest point of the lake

Acknowledgements

Most of the world's lakes freeze during winter. However, limnologists often chose to conduct their fieldwork during summer and used the winter as a writing season. The logistical challenges and the physical labour involved in the gathering of limnological data through a layer of ice is a frontier that might have slowed down the science in the past, but with a devoted group of curiosity-driven, frost-loving scientists, the boundaries of knowledge may be pushed to newer horizons. This is the kind of motivation that has driven my team all along this study, in the face of the cold winds and during the late nights spent filtering water in the lab. The conviction that winter is not merely a season of underwater inactivity, that understudied processes allow lakes to remain "alive" in a different way during the cold months, and that the seasonality of lakes cannot be fully understood without consideration for winter.

This *memoire* represents a portion of the collaborative work of a team of winter scientists that studied Lake Simoncouche in winter 2020-2021, each of whom deserve a sincere acknowledgement. Thanks to my supervisor, professor Milla Rautio, for the inspiration about winter ecology, zooplankton, the Arctic, scientific communication, and for introducing me to an exceptional team. Thanks also to my co-supervisors, professor Beatrix Beisner and Henriikka Kivilä, who were always available for answering questions about the science, the writing, and about the general worries of a graduate student. Thanks to Catherine Girard and Paschale Noel Bégin, for the corrections and comments in the *memoire*. Thanks to GRIL, for the financial support and for the networking opportunities. Thanks to Crysta and Balla, our lab technicians who helped us tremendously to prepare for the fieldwork and to process the samples. Thanks to the FERS staff, Pierre-Luc and Patrick, who were piloting the airboat, doing occasional emergency mechanical repairs and at times rescued us from challenging conditions on lake. Thanks to every student in the team, Paola, Vilmantas, Henriikka, Mukund, Noémie, Pénélope and Thanuri, for being there for each other, for listening to practice presentations and helping to improve them, for the scientific insights coming from different backgrounds, and for the fun weekend activities. Thanks to my colleagues in the program, to Véronique, Anthony and Laurie, for the solidarity and reciprocal interest in each other's projects. Thanks to my friends outside university, who supported me and were interested in what I was doing during my studies. Thanks to my mother, my father, my brother, and stepfamilies, who would encourage me and support me through anything. And a final and warm thank you to Thanuri, a friend who became family, thank you for being here with me, sharing all things in life that are shared.

Introduction

Studying winter limnology

Most of the world's lakes are located between 60°N and 66°N, in a region where seasonal temperature fluctuations cause surface water to freeze during winter (Grosse *et al.* 2013). Globally, around 86% of all lakes are seasonally part of the cryosphere and are covered by ice and snow for a period of 1 to 12 months, annually (Korver *et al.* 2024). Around 62% of earth's 1.4 million lakes (>10 ha) are located in Canada, and all of them are subjected to freezing temperatures annually (Messenger *et al.* 2016). The water column of a lake can be affected to a great extent by the presence of an ice layer, which forms a physical barrier and seals the body of water from its catchment and from the atmosphere (Cavaliere *et al.* 2021). The ice cover prevents gas exchanges and light penetration in the water column and modulates other physical, chemical, and biological parameters. Phases of ice accumulation, white ice formation and ice melting, along with changes in light and thermal regimes, punctuate the progression of winter into distinct periods, with influence on the biological dynamics that may reach the following open water season (Hébert *et al.* 2021; Jansen *et al.* 2021). Furthermore, the effects of climate change are disproportionately affecting the cryosphere and the duration of ice cover is decreasing at a rate of 17 ± 6.5 days per century, with events of intermittent ice cover and rain becoming more frequent (Magnuson *et al.* 2000; Sharma *et al.* 2019; Sharma *et al.* 2021). Such changes can be expected to have profound impacts on lake ecology during the winter and into the following open water period (Hébert *et al.* 2021; Hrycik *et al.* 2022; Socha *et al.* 2023). Nonetheless, winter has often been conventionally viewed as a rather homogeneous and biologically inactive season (Powers and Hampton 2016) and until recently, it was only about 2% of scientific publications in limnology that considered processes associated with winter (Hampton *et al.* 2015). This has created a knowledge gap in aquatic ecology and little is known about the ecosystem dynamics under ice (Sutton *et al.* 2021), despite more than 150 years of limnological studies. Considering the profound ecological effects of surface ice phenology on planktonic organisms, and that most of the world's lakes are exposed to negative hiemal temperatures and ice cover formation, it becomes clear that studying the winter ecology of the freshwater planktonic community is crucial to construct adequate

models of the seasonality of lakes and to better predict and anticipate the effects of climate change on frozen lentic ecosystems.

Physical and chemical effects of an ice-cover

In fall, the solar zenith angle decreases, the air temperature drops and the typically occurring summer thermal stratification of the water column breaks. As surface water becomes colder, it also becomes denser and sinks. This process is referred to as the fall overturn and continues until the entire water column reaches a homogeneous temperature close to 3.98°C, which represents the maximum density for freshwater. Further cooling of the surface water translates to lower density, allowing it to float and to reach colder temperatures until ice forms and seals the lake. Under the ice, inverse thermal stratification takes place, wherein colder water rests on top of warmer strata. During winter, the temperature directly below the ice layer is often close to 0°C, while deep water can exceed 4°C (Figure 1). A steep thermocline can be present below ice, where temperatures and density increase rapidly with depth, strongly separating the upper and lower parts of the water column (Bengtsson and Svensson 1996). This contrasts with the summer thermal stratification, where surface water is warmer than lower strata (Figure 1).

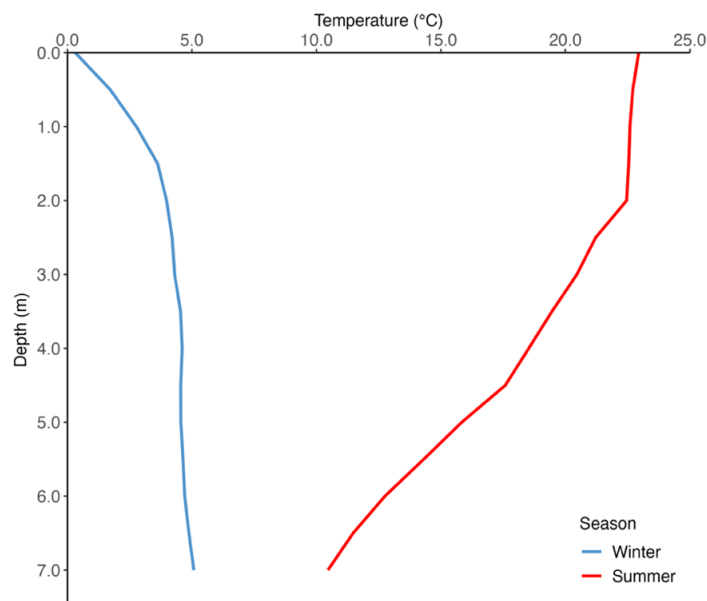


Figure 1: Temperature profiles showing thermal stratification in summer (July) and inverse stratification in winter (February) in lake Simoncouche. Data were measured using a RBR Concerto multi sensor.

As winter progresses, more clear ice accumulates from below the cover while white ice may accumulate from the top, with consequences on the light availability in the water column and hence the availability of solar energy for primary producers. The packing of the snow, events of rain on ice, snowmelt or resurgence of water over the ice cover, may form a slushy layer that becomes white ice when freezing. Clear, black ice and white ice have different physical and optical properties, including major differences in their opacity. Light transmits easily through clear, crystalline ice, which presents similar optical properties than those of liquid water (Bolsenga and Vanderploeg 1992). White ice on the other hand has a granular structure, causing greater reflection, absorption, and scattering and increasing opacity. Light availability in the water column can be greatly reduced by a cover of ice, particularly when white ice and snow are present. Most incoming radiations from the visual spectrum can be blocked by ice and snow, making the water column a rather dark environment in which photosynthesis is heavily restricted (Hrycik and Stockwell 2021; Jansen *et al.* 2021; Kivilä *et al.* 2023). A solid surface also prevents gas exchanges and blocks oxygen from diffusing from the atmosphere to the lake. Coupled with respiration from winter-active heterotrophic organisms, this can lead to hypoxia in the water column and anoxia at the deepest depths, especially in mid-winter when the ice and snow covers are thickest and until the melting season, when thinner frozen layers allow enough light to reach the water column and sustain photosynthesis (Kirillin *et al.* 2012). An oxycline can form near the bottom of the water column, where O₂ saturation decreases rapidly. Low oxygen near the sediments can lead to the production of reduced forms of phosphorus, which may accumulate below the oxycline and create a chemocline, where specific conductivity increases sharply with the higher concentration of solutes (Joung *et al.* 2017). These inorganic molecules usually become available as nutrients to primary producers during the melting season and the spring overturn, when the light returns, and the convective currents homogenize the water column.

The progression of winter gives place to distinct stages, or key periods, during the season (Kirillin *et al.* 2012; Jansen *et al.* 2021). In early winter, with the thin ice and snow layers, light can still reach the water column. The oxygen concentration in early winter is less than during the fall because ice blocks oxygen diffusion from the air and limits light availability for primary production but is still higher than in mid or late winter, when strong oxygen gradients and hypoxia may occur (Cavaliere *et*

al. 2021; Huang *et al.* 2021). The mid-winter water column contains less oxygen because there is close no light to support photosynthesis and at the same time, respiration of heterotrophs consumes the remaining oxygen (Hampton *et al.* 2017; Kivilä *et al.* 2023). The effects are more pronounced in late winter when the ice cover is thickest, and resources are more depleted. In the melting season, light passes more easily through the thinning ice and oxygenated water rushes from the inlets to the lake as laterally flowing currents, increasing the biological activity of both primary producers and heterotrophic organisms (Stefanovic and Stefan 2002; Cortés and MacIntyre 2020; Yang *et al.* 2020).

Effects of winter on the biology of boreal lakes

The density stratification under the ice creates a template for the development of hiemal ecological niches. Distinct oxygenated and oxygen-depleted water strata affect the type of energy used by micro-organisms, as well as the community structure and habitat of plankton. Boreal lakes are home to many planktonic groups playing important ecological roles in the pelagic food web (Figure 2). Photo-autotrophic organisms are primary producers that use sunlight as a source of metabolic energy, by producing pigments that enable photosynthetic reactions. This group includes various types of algae, including picoplankton and nanoflagellates, that are a part of phytoplankton and constitute the base of the classical pelagic food web by transferring energy towards herbivores and eventually predators. Photo-autotrophic organisms are strongly limited by the availability of light from the visible spectrum, or photosynthetically active radiation (PAR; 400-700 nm), as they use photosynthetic pigments sensitive to these wavelengths (Wetzel 2001). When an ice cover is present, primary production can be greatly reduced, depending on the properties and thickness of the ice and snow (Jewson and Granin 2015). The reduced light penetration restricts photosynthesis to the upper part of the water column, close to the ice. Non-motile primary producers such as diatoms sink to the bottom in winter, while motile cells can migrate between the irradiated epilimnion and the deeper chemocline, where they reach a zone of higher nutrient availability and warmer temperatures (Pettersson 1985). Alongside the classical food web, a considerably important portion of matter and energy is cycled in the microbial loop (Figure 2). Dissolved organic matter (DOM) from the excretion and death of other organisms (autochthonous DOM), as well as from the catchment (allochthonous DOM) can be degraded and used as a source of carbon for bacteria production, with different groups

of bacteria favouring DOM from different sources (Kivilä *et al.* 2023). Bacteria can metabolize DOM that is inaccessible to other heterotrophs, thus putting energy back in circulation and providing resources to heterotrophic microzooplankton and mixotrophic nanoflagellates (Butler *et al.* 2019). Mixotrophic organisms can use autotrophy and/or heterotrophy to remain active in low light environments by ceasing pigment production and by consuming bacteria as a source of energy (Anderson *et al.* 2017). Mixotrophic metabolism among nanoflagellates have been shown to be favored during the harsh winter months (Rautio *et al.* 2011) and their study can provide important information on the energy transfers in the lake, considering their dual role in the classical pelagic food web as autotrophs (Figure 2A, phytoplankton), as well as in the microbial loop as heterotrophs (Figure 2B, mixotrophic algae).

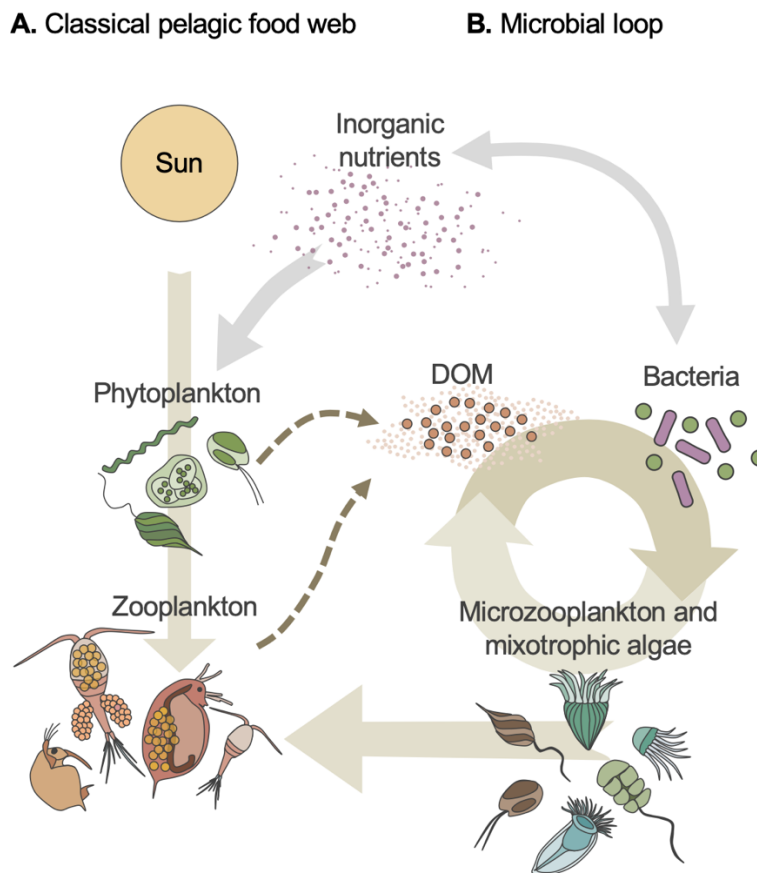


Figure 2: Planktonic food web in lakes. Classical pelagic food web (A) is driven by sunlight and inorganic nutrients, whereas the microbial loop (B) is fueled by organic carbon. Modified from Jansen *et al.* (2021)

Under the ice, especially in mid-winter, cold temperatures and oxygen depletion in the water column can limit the aerobic metabolism of winter-active organisms, while the low irradiance strongly limits primary production and reduces the contribution of phytoplankton to the food web, both as food for grazers and as sources of DOM for the microbial loop. Additionally, the ice cover and the frozen soil around the lake also affect the sources of DOM, as the inputs of fresh terrestrial organic matter (allochthonous DOM) are substantially scaled down. While autochthonous carbon is limited by the low primary production and allochthonous carbon is limited by the sealing effects of the ice cover, excretions and senescence of winter-active organisms and accumulated stocks of allochthonous organic matter become the main sources of carbon for the microbial loop (Bertilsson *et al.* 2013). Previous studies reported that the water column during winter contains relatively high concentrations of allochthonous carbon, which is preferentially used by bacterioplankton and transferred to higher trophic levels by zooplankton groups that feed under the ice, such as species of rotifers and cladocerans (Rautio *et al.* 2011). Despite the reduced activity of aerobic metabolism under the ice, the microbial loop can become the main energy pathway to winter-active food web, following the steep decline in primary production (Stockner and Porter 1988; Charvet *et al.* 2014).

Both the primary producers and the microbial loop provide resources for zooplankton, composed of rotifers, cladocerans, and copepods, all of which are known to be present in boreal lakes fauna during winter. Rotifers are small (50-200 μm), ubiquitous animals that are often present in the zooplankton communities of lentic ecosystems. This group is very diverse: some species are grazers while others are predators, some are pelagic when others are benthic and some are solitary while others, sometimes similar species, are colonial. Notable differences in feeding strategies can be observed within a rotifer population. Generalist species like *Kellicottia longispina* and *Keratella* spp. are filter feeders that may consume protists and other small organisms like ciliates and bacteria. Other species are herbivore, like *Gastropus stylifer* and *Filinia terminalis*, while others like *Ploesoma* spp. preys on other rotifers (Pourriot 1977; Sanders *et al.* 1989). Rotifers play an important role in the pelagic food web dynamics, and the community composition of a rotifer population is likely to undergo significant variations when environmental and ecological conditions change, most notably responding to temperature, oxygen saturation and preferred-food availability (Walz 1995). Few studies report the

seasonality of rotifers in boreal lakes, but some species, such as *K. longispina* are known to be winter-active while others are dormant (Virro *et al.* 2009). The winter ecology of rotifers remains elusive, but their different traits suggest that the changing physical and chemical properties of the water column may induce modifications in the community composition.

Large (adults generally > 200 µm) herbivorous crustacean zooplankton include cladocerans like *Daphnia* sp. and *Bosmina* sp.. Cladocerans need a constant supply of food and they rely on primary producers, on which they exercise top-down control, and are thus indirectly limited by light availability (Bertilsson *et al.* 2013). Winter can be a harsh season for herbivores, but different adaptations allow cladocerans to avoid winter altogether or to stay active. Some species produce ephippia, which are parthenogenetic resting eggs resistant to environmental conditions. Ephippia sink to the bottom of the water column and are covered in sediments. When conditions become favourable again, usually during spring overturn, these eggs hatch and allow for a new generation (Larsson and Wathne 2006; Lampert *et al.* 2010; Mariash *et al.* 2016). Some species are also known to delay their growth by slowing the metabolic rate, or to change their diet from algae to bacteria or small eukaryotes like nanoflagellates when algal diet resources are scarce (Sommer *et al.* 2012). Lipid reserves accumulated during the previous open water period may also help cladocerans to stay active under the ice (Mariash *et al.* 2016).

Copepods are largest among freshwater crustacean zooplankton; adults measure 1000-2000 µm while individuals in the larval stage (nauplii) measure 200 µm or less. Their hunting and grazing ability allows them to prey on a wide range of planktonic organisms. Rotifers are often the preferred prey for predatory copepods, but they also consume protists and small cladocerans, and can be herbivorous or omnivorous (Berggren *et al.* 2014). Some species of copepods are absent from the water column during winter and lay in the sediments until the environmental conditions meet their metabolic needs (Grosbois *et al.* 2020). Several calanoids, such as *Leptodiatomus minutus*, stay active and even reproduce under the ice in late winter (Grosbois *et al.* 2017). *L. minutus* is the most abundant zooplankton species in Lake Simoncouche, and it accumulates large amounts of lipids in the fall to be used in winter while fasting, when food availability is low (Schneider *et al.* 2017). Cyclopoids may also stay active under the ice, with similar strategies as the calanoids but they

typically only reproduce during the open water period (Kers *et al.* 2024). *Cyclops scutifer* is a common species of omnivorous cyclopoid that may feed on rotifers and nauplii, as well as algae.

The physical and chemical environment in winter, characterized by overall cold temperatures, low oxygen saturations and reduced luminosity, can be expected to have profound ecological impacts on the plankton community. In addition, the inverse thermal stratification and strong chemical gradients in the water column imply changing habitats for plankton communities. Given the central role of zooplankton in pelagic food webs where they both improve water quality (Talling 2003) and provide support for fish production (Bunnell *et al.* 2003), it is important to understand how warming winters change water quality and the health of lake ecosystems. This, however, cannot be done without fundamental understanding of plankton ecology in winter.

Objectives and hypotheses

This study, articulated around three objectives, aims to provide knowledge on plankton community dynamics, reproductive patterns, and energy transfers during the winter ice-covered period and the shoulder seasons of a boreal lake, and is following the key winter periods structure as described in Jansen *et al.* (2021). The first objective is to analyze the temporal and vertical community structure of winter-active zooplankton by quantifying the biomass of rotifers, copepods, and cladocerans during different stages of winter and at different depths. We expect an overall decrease in the biomass of all three zooplankton groups (rotifers, copepods, and cladocerans) during winter (H1), correlating with limitations in temperature, light, and oxygen (Sommer *et al.* 2012). Additionally, we hypothesize a negative correlation between cladoceran biomass and both depth and time (H2), reflecting the diminishing availability of algal diet resources over the winter period. Since cladocerans depend on a constant supply of algae for their diet, their position would be expected to be limited to the irradiated upper water column and their abundances to drastically decrease during mid and late winter, when most light is reflected and absorbed by snow and ice (Hampton *et al.* 2017). Further, vertical and temporal heterogeneity in the zooplankton community structure is expected (H3). This variation is anticipated to align with shifts in thermal stratification, changes in abiotic conditions of the water column, the temporal winter gradient (early winter, mid-winter, late winter and melting season), and the diverse adaptations and life history strategies of zooplankton species (Jansen *et al.* 2021). The second objective focuses on the reproductive patterns of winter-active zooplankton species by examining the presence of eggs and larval stages. The first hypothesis for this second objective suggests an earlier increase in copepod nauplii abundance under the ice towards the end of winter, driven by the under-ice reproduction of *L. minutus* (H4), as reported by Grosbois and Rautio (2017). Additionally, we posit that rotifer species will engage in reproductive activity under the ice, reflective of the overall robustness of this taxonomic group in adapting to winter conditions (H5). The third objective involves studying energy transfers at the base of the food web, using the biomass of pigmented and heterotrophic nanoflagellates as proxies for the classical food web and microbial loop, respectively. We anticipate a decrease in photo-autotrophic cell biomass with progressing winter and with depth, due to limitations in light availability (H6). Moreover, we hypothesize an increasingly higher

heterotrophy/autotrophy ratio during winter, peaking just before the ice melts and indicating a surge of biological activity within the water column (H7). Together, these objectives and hypotheses provide a comprehensive framework for studying the ecological dynamics of plankton communities in winter, addressing urgent need for baseline data in plankton research.

Material and methods

Study site

This study was carried out during winter 2020-2021 at lake Simoncouche (48° 13' N, 71° 14' W; Figure 3). This boreal lake is in the Simoncouche Teaching and Research Forest of Université du Québec à Chicoutimi (UQAC) in the region of Saguenay, Québec, Canada. The lake is dimictic and mesotrophic, with a mean depth of 2.2 m and a maximum depth of 8 m. Lake Simoncouche is annually covered in ice for five months, usually between mid-November and early May. Species of calanoid and cyclopoid copepods, cladocerans, and rotifers constitute the zooplankton community of this lake and previous studies of lake Simoncouche demonstrated the ability of *L. minutus* to reproduce under ice (Grosbois *et al.* 2017; Grosbois and Rautio 2017; Schneider *et al.* 2017).

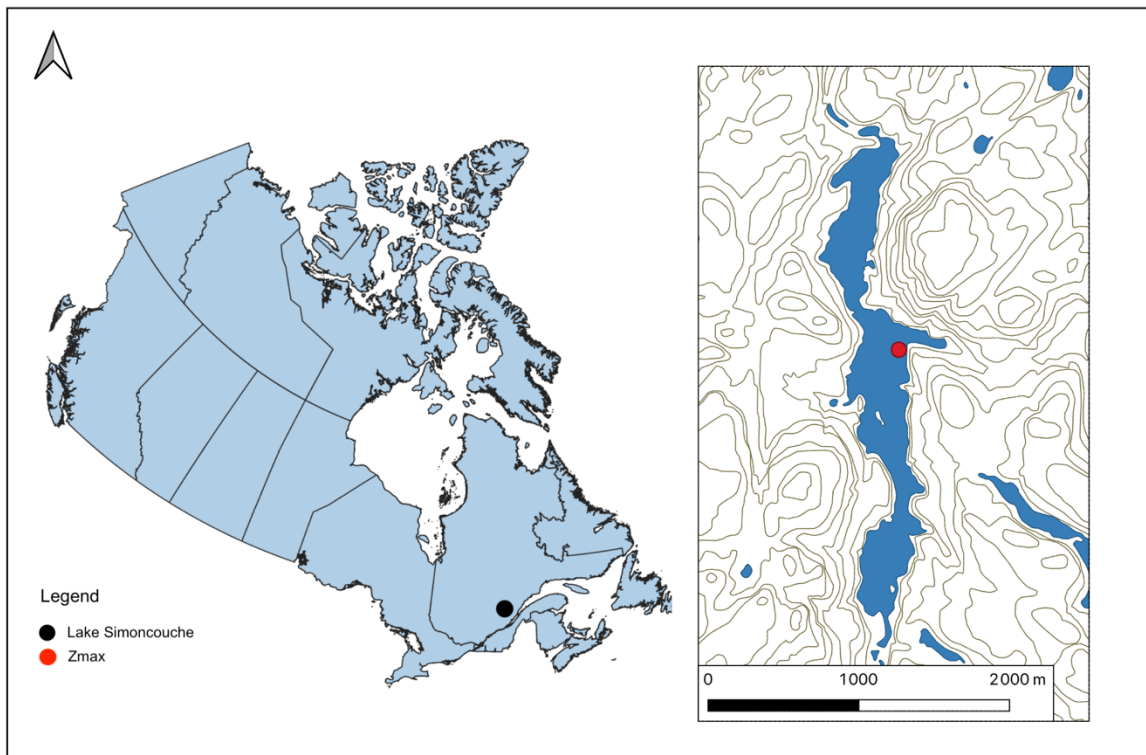


Figure 3: Study site: Lake Simoncouche, Saguenay, Québec, Canada (48° 13' N, 71° 14' W). All samples were collected at the deepest point in the lake (Zmax).

Sampling design

The sampling campaign, as represented in figure 4, was conducted four times during the ice-covered season, from 23 November 2020 to 13 April 2021, and at five fixed depths. In addition, the lake was sampled during the fall overturn 21 days before the ice-on (13 November) and during the spring overturn 7 days after the ice-off (19 April). Sampling dates corresponding to when the lake was frozen were chosen to overlap with key winter time periods, sensu Jansen *et al.* (2021): early winter (14 December), mid-winter (23 February), late winter (23 March) and melting season (12 April). As for the depths of sampling, five depths were chosen to represent the whole water column. Samples were taken at 0 m, immediately below ice, and from 1.5 m, 3 m, 4.5 m, and 7 m, measured from the top of the ice and excluding snow. All other samples were taken at the same relative depths from the surface, except during the overturns when integrated samples of the whole water column were taken given its vertical homogeneity in temperature, oxygen, and conductivity, as measured with the RBR probe (see below). In the broader context of the Simoncouche 2020-2021 winter campaign, two long-term RBR moorings (1.5 m and 6 m depths) were set to record temperature, PAR, O₂ saturation and specific conductivity, at 30 minutes intervals throughout winter 2020-2021 and at Z_{max}.

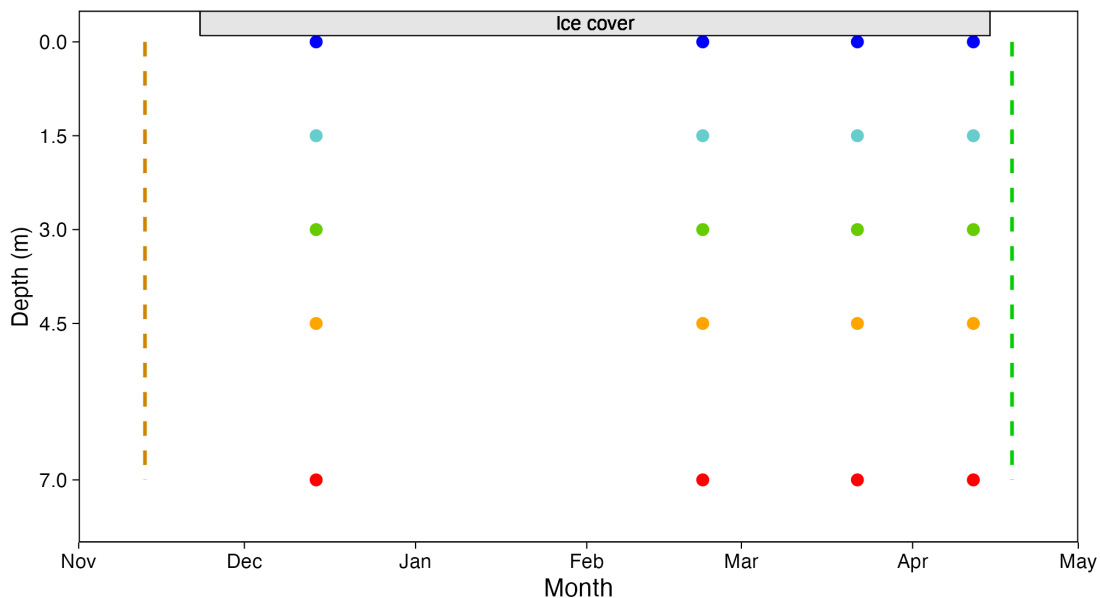


Figure 4: Diagram of the sampling design, showing the time and depths of sample collection. Integrated water column samples were taken during both overturns (coloured dashed lines) and punctual samples at 5 fixed depths when the lake was ice-covered and thermally stratified (coloured dots). The depth-specific colour coding shown here is used in following figures.

Sample analyses

Water was collected with a 2 L Limnos sampler (Limnos Ltd., Poland) and transferred into 4 L opaque Nalgene bottles (three replicates per depth) for chlorophyll-*a* (chl-*a*), bacteria, picoplankton and nanoflagellates. Zooplankton samples were produced from a volume of 20 liters taken at each sampling depth with the Limnos sampler, concentrated to a smaller volume with a 50 µm zooplankton net and transferred to a 250 mL Nalgene bottle. A few drops of formaldehyde were added in the field to euthanize the organisms, and more (4% final concentration) in the laboratory. Two zooplankton sample replicates were collected for each depth. Physical water column properties (temperature, dissolved oxygen saturation, specific conductivity) were profiled with a Ruskin RBR Concerto multi-channel logger (RBR Ltd., Canada) at each sampling date. All samples and data were collected from the deepest point of the lake (Z_{\max} ; Figure 3).

Sample preparation in the laboratory was done on the same day as the sampling on the lake to maximize the accuracy of the data. For chl-*a* analyses, 200–350 mL of lake water was filtered through a 25 mm GF/F filter (Whatman, UK; nominal pore size 0.7 µm) and stored frozen (-80°C) until extracted in ethanol and analyzed with a Cary Eclipse spectrofluorometer (Agilent Technologies, USA) according to Nusch (1980). The extracts were scanned before and after acidification to subtract phaeopigments.

The microbial samples were prepared immediately after arriving to the laboratory; a 10 mL aliquot of water from each sample bottle was preserved with 2 mL of 0.2 µm filtered formaldehyde solution (2% final concentration) for bacteria enumeration and subsequent biomass analyses. Another aliquot of 50 mL was preserved with 5 mL filtered glutaraldehyde solution (1% final concentration) for a combined sample of nanoflagellates and picoplankton. The preserved samples were stored in 25 mL Nalgene bottles at 4°C and slides were prepared within 48h. Bacteria slides were made from a volume of 1 mL of lake water and were stained with nucleic acid-staining fluorochrome 4',6-diamidino-2-phenylindole (DAPI) prior to filtration through a 0.2 µm Nucleopore membrane (Whatman, UK) and permanent mounting on microscope slides with immersion oil, as in Rautio *et al.* (2011). Combined nanoflagellates and picoplankton slides were prepared in a similar

fashion, except that 25 mL of lake water was taken, and the filtration was through a 0.6 μm black polycarbonate membrane (Whatman, UK). Samples were stored at -20°C until examination.

Enumeration of the microbial organisms was done at 100X magnification using an Axio Observer A1 (Zeiss, Germany) microscope with ultraviolet (UV) illumination. DAPI strongly binds to AT-rich regions in DNA and is fluorescent under UV light. DAPI-stained cells therefore appear bright in UV illuminated microscopy, allowing cell enumeration and abundance estimates. Counting was done using a graduated grid in the eyepiece of the microscope, both as a field of enumeration and to assess the size category of each nanoflagellate. Bacterioplankton was enumerated on their own slides, and at least 400 individuals were enumerated per slide and the total number of fields required to count the bacteria was noted. At 100X magnification, the 10X10 grid in the eyepiece covered an area of 0.01 mm^2 on the filter, which had an effective area of 346.185 mm^2 . For the nanoflagellate, a transect method was used; all NF were counted in a field then the slide was moved to the side, at the edge of the previous field, to continue the counting. The microscope was equipped with a millimetric scale, used to measure the required length of the transect for the enumeration of at least 400 NF individuals. The length of the transect was multiplied with the height of the grid at 100X magnification (0.1 mm) to measure the area of enumeration. A combination of UV excitation and light filters was used to visually distinguish between pigmented (PNF) and heterotrophic (HNF) nanoflagellates. The fluorescence from DAPI is detected in both PNF and HNF when excited with UV light, and all the stained cells are clearly defined and emit bright light. When a green light filter is used, the fluorescence from DAPI is blocked and HNF are no longer clearly visible. The photosynthetic pigments produced by the PNF have fluorescent properties when exposed to UV light, with emission wavelengths that allow the bright image of the pigmented cells to appear through the green filter. This method of combined UV illumination and green light filters allows to visually assess whether single cell organisms are producing pigments or not and in the context of this study, whether nanoflagellates were heterotrophic (HNF) or pigmented and likely photo-autotrophic (PNF). Photo-autotrophic picoplankton was counted in parallel with the nanoflagellates using the UV light and green filter method. Their robust cell wall prevents the DAPI from reaching the DNA, but their photosynthetic pigments are fluorescent under UV light, and their smaller size ($< 2\ \mu\text{m}$) differentiates them from the

NF. Bacterial carbon biomass was calculated using an average measured cell volume of $0.1 \mu\text{m}^3$ and a volume–biomass conversion factor of $0.308 \text{ pg C } \mu\text{m}^3$ (Fry 1988). Nanoflagellates were enumerated in three different size categories (diameter 2–5, 5–10, and $> 10 \mu\text{m}$) to allow volume estimation and subsequent carbon content for biomass conversion, using a conversion factor of $0.22 \text{ pg C } \mu\text{m}^3$ (Børsheim *et al.* 1990). Picoautotroph carbon biomass was calculated using an average cell volume of $4.2 \mu\text{m}^3$ and the same carbon conversion factor as for nanoflagellates.

Zooplankton samples were size-fractionated, using a $200 \mu\text{m}$ sieve, to two parts before microscopy so that the smaller size fraction containing mainly rotifers and nauplii was counted separately from the larger fraction dominated by adult copepods and cladocerans. Samples were divided as needed using a Folsom’s plankton divider into half, quarter or eighth of the original sample, so that at least 400 individuals could be enumerated and identified. Zooplankton were counted after 24 hours in Utermöhl sedimentation chambers and using an inverted microscope in white light illumination (Axio Observer, A1, Zeiss, Germany, 10–100X). Species identification was done at the lowest possible taxonomic level, and was based on taxonomy guides from Edmondson (1959) and Czaika (1982), and an online image-based taxonomic key (Haney *et al.* 2013). The number of organisms per species and per developmental stage was recorded, and according to the original sample volume (20 L) and the portion of the sample that was counted (whole, half, quarter or eighth), measures of abundance (10^3 ind. m^{-3}) for each species per date and per depth could be made, as an average (\pm standard error) of the two replicates. Mean dry weight (DW) was estimated using taxa specific length–DW regressions (Bottrell *et al.* 1976; Lawrence *et al.* 1978; McCauley 1984; Culver *et al.* 1985). For every species and development stages, ten individuals were measured using an optical camera (AxioCam ERC 5S, Zeiss, Germany) and microscope software (AxioVision, Zeiss, Germany). The mean of the ten length measures per species was used in the biomass (DW) conversion to estimate the mean DW of each specie’s individual. The conversion of abundance into biomass (mg DW m^{-3}) was done by multiplying the abundance results with the taxa-associated DW constants.

Data analyses

To evaluate the effects of time (4 levels, fixed) and depth (5 levels, fixed) on zooplankton biomass and HNF:PNF mass ratio, permutational analyses of variance (PER-ANOVA) with Euclidian distance matrices were conducted, following methods from Anderson *et al.* (2008). Zooplankton biomass and HNF:PNF mass ratio were Log + 1 transformed prior to analyses. Similarly, the same design was used to observe the community structure (using dispersion weighing and log-transformed biomass data) with a permutational multivariate analysis of variance (PERMANOVA) (Anderson 2014) using a Bray-Curtis dissimilarity matrix. Post-hoc permutation t-tests were performed when at least one of the main factors was significant and interaction between factors was also tested. Changes in zooplankton community was visualized with a non-metric multidimensional scaling (nMDS). SIMPER analysis was conducted on the zooplankton community to determine which taxa contributed the most to the separation of the groups (*i.e.* times and depths). The software JMP (version 14.3) was used for all univariate tests while PRIMER + PERMANOVA (version 7.0.1) was used for the multivariate analyses, with a significance level of $\alpha = 0.05$ for all analyses. Data visualisations were produced with R statistical software (R Core Team 2023) and ggplot2 package (Wickham 2016), except the nMDS that was made using PRIMER.

Results

Thermal stratification and water column properties

Lake Simoncouche presented thermal gradients throughout the water column, with temperatures colder at the surface and warmer at the bottom (Figure 5A, Figure 6A). Surface water was coldest in early winter at 1.28°C and increased to 4.75°C in the melting season. The bottom water remained stable throughout winter, with temperatures ranging between 4°C and 5°C. During the overturns, temperature was homogeneous in the water column (5.8°C in fall and 6.6°C in spring) as thermal mixing occurred. The oxygen saturation was generally higher in the upper strata (Figure 5B, figure 6C), and O₂ saturation gradually decreased at the surface as winter progressed but never decreased below 85%. The bottom stratum reached a minimum of O₂ saturation in mid-winter with 2.5%, then increased to 45% in late winter until declining again to 18% in the melting season. The fall overturn displayed a homogeneous 98% O₂ saturation while the spring overturn was saturated at 83%. Chlorophyll-*a* concentrations were highest during the overturns at around 3.7 µg L⁻¹ (Figure 5C). During the ice-covered period, the highest average water column concentration of chlorophyll-*a* was in early winter (2.3 µg L⁻¹ at 0 m), then decreased in mid-winter (0.35 µg L⁻¹ at 0 m) before increasing again towards late winter and the melting season. In early and mid-winter, the surface layer contained greater chlorophyll-*a* concentrations than other depths, but in late winter and in the melting season, the maximum concentration was at 1.5 m. The light availability in the water column decreased rapidly after the onset of the ice cover, and close to no PAR was recorded by the long term RBR moorings until late winter at the surface sensor (Figure 6B). The irradiation of the water column continued to increase during the melting period and was highest then the lake became free of ice. The specific conductivity recorded by the sensor at 1.5 m remained stable throughout winter and was equal between the two sensors during both the fall and spring overturns (Figure 6D). Values increased threefold at the 6 m sensor in early winter, then almost doubled again in late winter before decreasing abruptly during the spring overturn. During the melting of the ice cover, minimal values of conductivity were recorded by the top sensor.

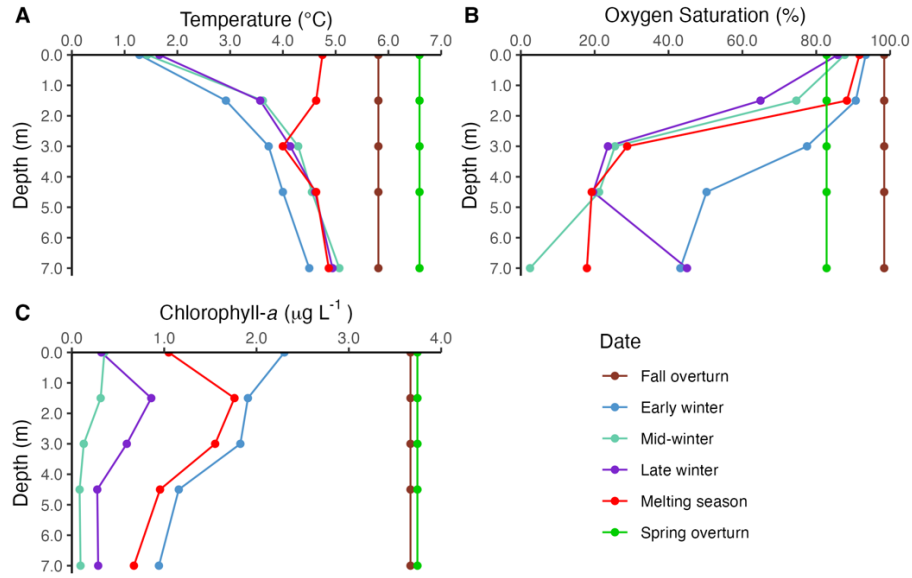


Figure 5: Water column profiling of temperature (A), oxygen saturation (B) and chlorophyll-a concentration (C) at the six samplings (fall overturn, early, mid, and late winter, melting season, spring overturn) during winter 2020-2021 in Lake Simoncouche. Temperature and oxygen measurements were taken using a RBR Concerto multi sensor profiler, while chlorophyll-a concentrations are means of three replicates determined by extraction from water samples at five fixed sampling depths.

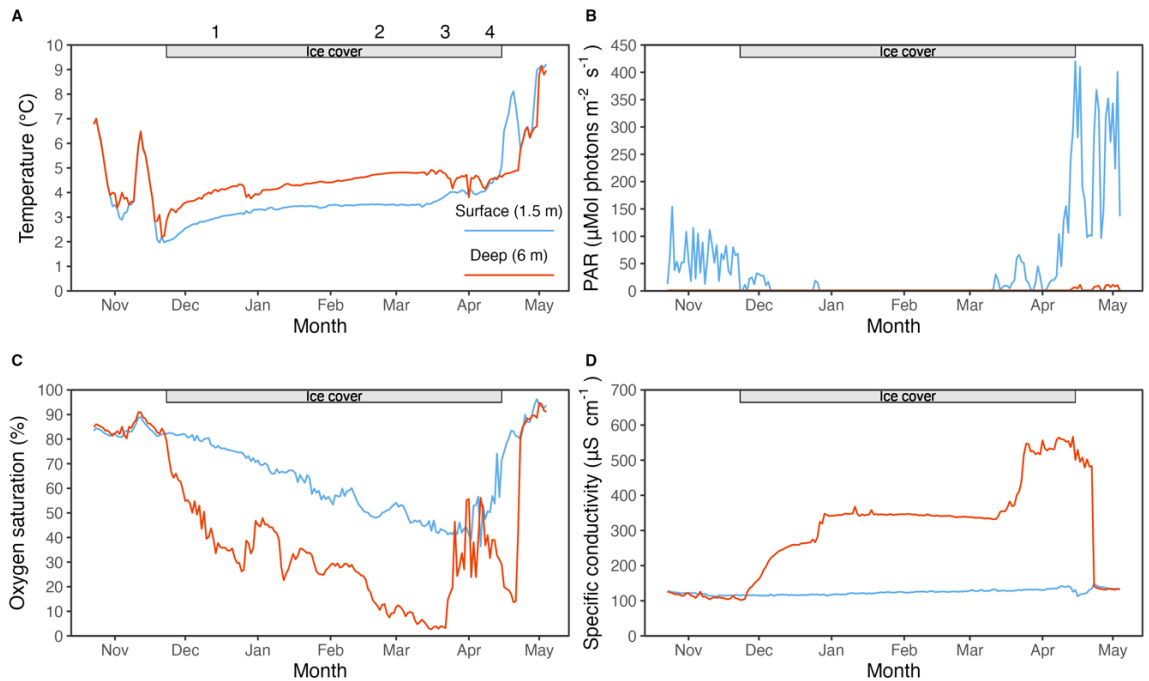


Figure 6: Daily mid-day (12:00 pm) measures of temperature (A), photosynthetically active radiation (B), oxygen saturation (C) and specific conductivity (D) collected from two long term RBR Concerto multisensors moorings placed near the surface (1.5 m) and bottom (6 m) of the water column in lake Simoncouche during winter 2020-2021. Key winter periods considered in this project are marked in panel A: early winter (1), mid-winter (2), later winter (3) and melting season (4).

Zooplankton community under the ice

A total of 28 zooplankton taxa were found in the lake between the fall and the spring overturns. Rotifers were the most species-rich with 20 taxa, followed by copepods that had 6 species and cladocerans that had 2 genus (Table 1). With 14 to 22 taxa per sampling date (Table 2), there was no difference over time in total zooplankton species richness between the samples collected from under the ice and during the overturns (PER-ANOVA pseudo- $F_{5,38} = 2.2$, $P_{mc} = 0.07550$), (Table 3).

Table 1 : Zooplankton taxa encountered under the ice and during the shoulder seasons in winter 2020-2021 in Lake Simoncouche. The species are listed in taxonomical order.

Rotifera	Copepoda	Cladocera
<i>Hexarthra mira</i>	<i>Epischura lacustris</i>	<i>Bosmina</i> sp.
<i>Pompholyx sulcata</i>	<i>Leptodiptomus minutus</i>	<i>Daphnia</i> sp.
<i>Testudinella</i> sp.	<i>Tropocyclops prasinus</i>	
<i>Conochilloides</i> sp.	<i>Cyclops scutifer</i>	
<i>Conochilus unicornis</i>	<i>Eucyclops speratus</i>	
<i>Filinia terminalis</i>	<i>Mesocyclops edax</i>	
<i>Filinia longiseta</i>		
<i>Gastropus stylifer</i>		
<i>Polyarthra</i> sp.		
<i>Ploesoma</i> sp.		
<i>Synchaeta</i> sp.		
<i>Ascomorpha</i> sp.		
<i>Lepadella</i> sp.		
<i>Kellicottia longispina</i>		
<i>Kellicottia bostoniensis</i>		
<i>Notholca squamula</i>		
<i>Keratella serrulata</i>		
<i>Keratella hiemalis</i>		
<i>Keratella cochlearis</i>		
<i>Lecane</i> sp.		

Table 2: Species richness of the three main zooplankton groups at each sampling date during winter 2020-2021 in Lake Simoncouche.

Sampling date	Total taxa	Rotifera	Copepoda	Cladocera
Fall overturn	20	12	6	2
Early winter	18	11	5	2
Mid-winter	22	15	5	2
Late winter	21	15	4	2
Melting season	22	16	4	2
Spring overturn	14	8	4	2

Table 3: Per-ANOVA table of results for total zooplankton species richness per sampling date for 2020-2021 winter campaign in Lake Simoncouche.

Source	df	SS	MS	Pseudo-F	P(MC)
Date	5	48.709	9.7418	2.1623	0.0755
Residuals	38	171.2	4.5053		
Total	43	219.91			

Zooplankton biomass in the integrated water column changed modestly between the sampling dates and remained stable until close to the ice-melt open-water transition, where the abundances of rotifers, copepods and cladocerans statistically increased (Figure 7).

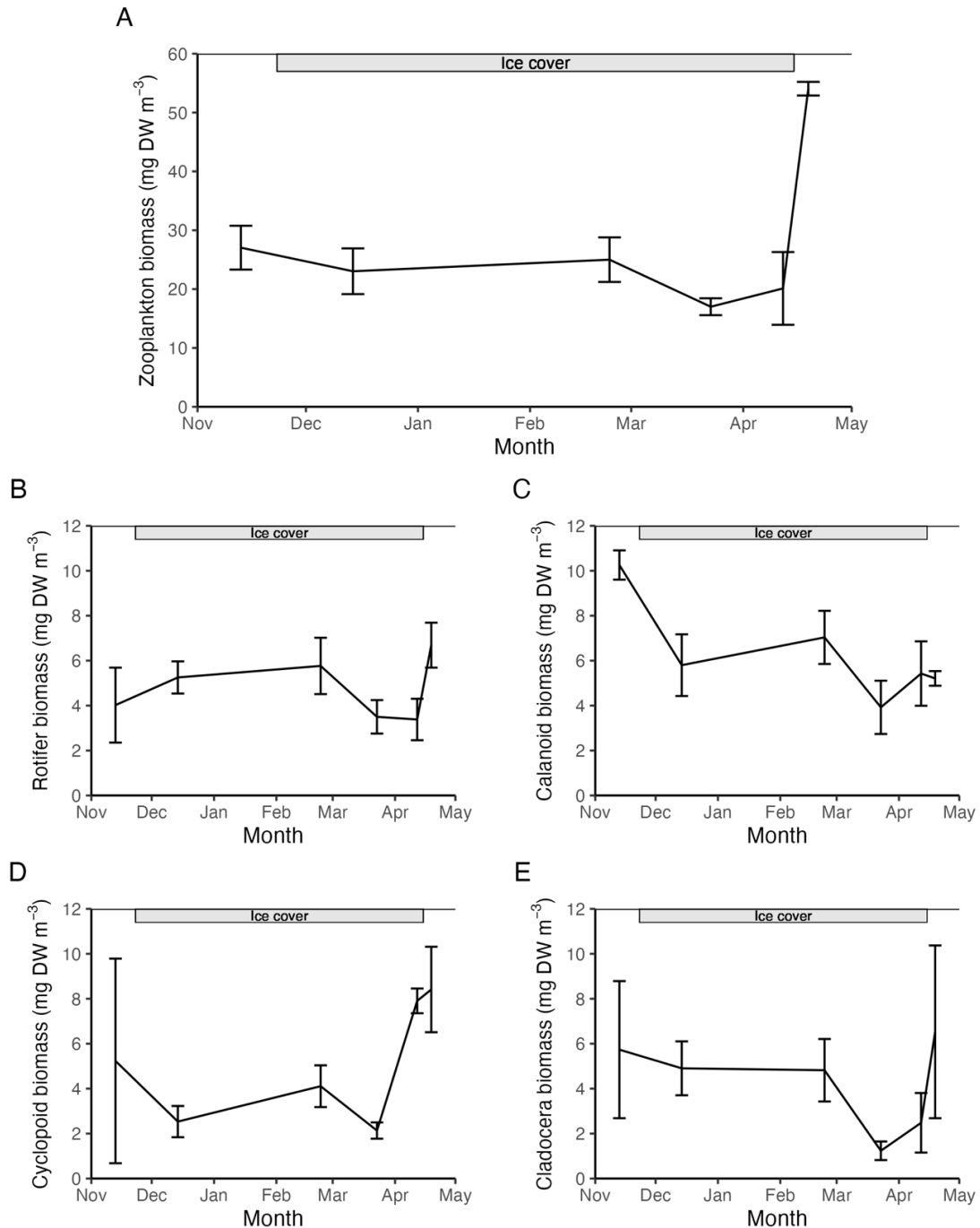


Figure 7: Average water column biomass (mg DW m⁻³ ± SE) of all zooplankton groups (A), rotifers (B), calanoid copepods (C), cyclopoid copepods (D) and cladocerans (E) during winter 2020-2021 in Lake Simoncouche.

The differences in zooplankton biomass and abundance between depths were substantial (Figure 8, Figure 9, Table 4, Table S1, Table S2). The vertical distribution of zooplankton followed a pattern that was characterized with the lowest biomasses in the surface and highest in the bottom layers of the lake (Figure 8). The biomass at 7 m was on average three times higher than at 0 m and 1.5 m and except for the early winter, the two surface layers had significantly lower biomass compared to the deepest stratum (PER-ANOVA all pairwise, $p < 0.001$) and characterised by low biomass. The biomasses in the middle depths 3 m and 4.5 m were for most of the sampling dates similar and higher than in the surface layers, but lower than 7 m values (Figure 8). Zooplankton abundance followed a similar overall vertical pattern as their biomass, with more individuals at greater depths and fewer in the top layer of the water column (Figure 9). Only in early winter the cladocerans were the most abundant at 0 m (Figure 9A). The differences among depths were least pronounced for calanoid copepods but they too avoided the 0 m at all times, except in late winter when the abundance at the surface was as high as at most other depths (Figure 9B). Cyclopoid copepods and rotifers were throughout the whole winter most abundant either at 4.5 m or at 7 m (Figure 9C-D).

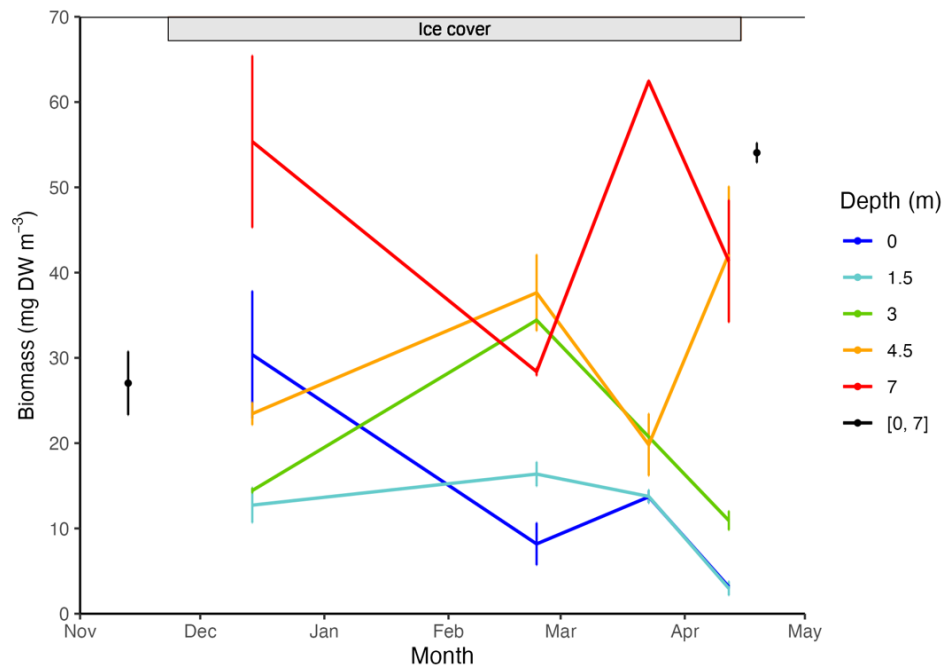


Figure 8: Seasonal variation in total zooplankton biomass ($\text{mg DW m}^{-3} \pm \text{SE}$) at five fixed depths under the ice in winter 2020-2021 in Lake Simoncouche. Biomass during the fall and spring overturn is shown as an average for the water column [0, 7] m. All taxa of cladocerans, copepods and rotifers are pooled together.

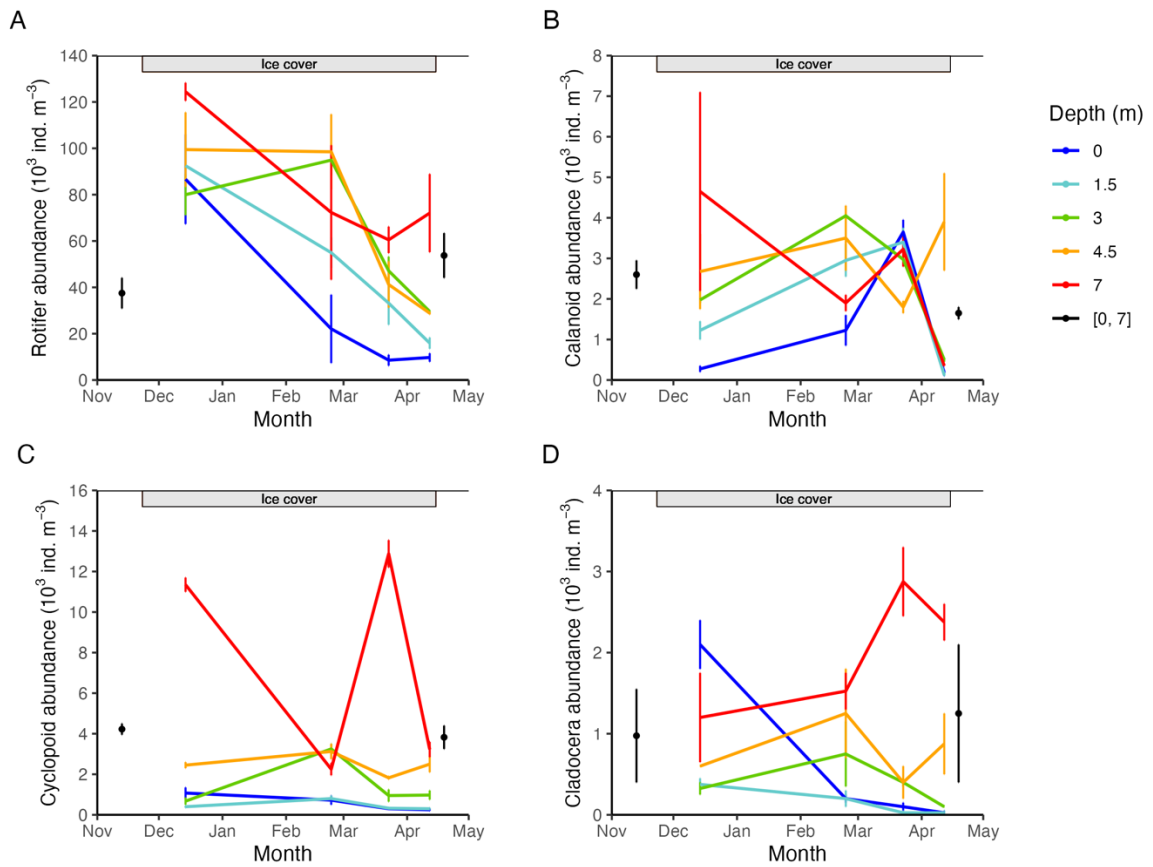


Figure 9: Seasonal and vertical abundance ($10^3 \text{ ind. m}^{-3} \pm \text{SE}$) variation of rotifers (A), calanoid copepods (B), cyclopoid copepods (C) and cladocerans (D) at five fixed depths under the ice 2020-2021 in Lake Simoncouche. Abundance during the fall and spring overturn is shown as an average for the water column [0, 7] m.

Table 4: Per-ANOVA table of results for total zooplankton biomass, cladocerans biomass, calanoid and cyclopoid copepod biomass, rotifer biomass, and HNF:PNF mass ratio.

Group	Source	df	SS	MS	Pseudo-F	perms	P(perm)
Pooled zooplankton biomass	Date	3	2.96*10 ⁸	9.88*10 ⁷	3.06	9949	0.0489
	Depth	4	6.74*10 ⁹	1.68*10 ⁹	52.25	9948	0.0001
	Date*Depth	12	3.47*10 ⁹	2.89*10 ⁸	8.95	9945	0.0001
	Residuals	20	6.45*10 ⁸	3.22*10 ⁷			
	Total	39	1.11*10 ¹⁰				
Rotifers	Date	3	11816.0	3938.5	14.98	9909	0.0001
	Depth	4	13666.0	3416.4	13.00	9912	0.0001
	Date*Depth	12	9741.1	811.8	3.09	9859	0.0001
	Residuals	20	5256.9	262.8			
	Total	39	40479.0				
Calanoids	Date	3	15977.0	5325.6	81.96	9951	0.0001
	Depth	4	6759.4	1689.9	26.01	9931	0.0001
	Date*Depth	12	10646.0	887.2	13.65	9903	0.0001
	Residuals	20	1299.5	65.0			
	Total	39	34682.0				
Cyclopoids	Date	3	3296.1	1098.7	5.32	9952	0.0004
	Depth	4	10303.0	2575.9	12.47	9944	0.0001
	Date*Depth	12	11880.0	990.0	4.79	9929	0.0001
	Residuals	20	4132.0	206.6			
	Total	39	29612.0				
Cladocerans	Date	3	5030.8	1676.9	6.87	9924	0.0002
	Depth	4	14187.0	3546.7	14.54	9933	0.0001
	Date*Depth	12	28378.0	2364.9	9.70	9924	0.0001
	Residuals	17	4146.7	243.9			
	Total	36	52153.0				
HNF:PNF	Date	5	25529.0	5105.9	41.46	9933	0.0001
	Residuals	60	7390.1	123.2			
	Total	65	32919.0				

The zooplankton community structure, including rotifer, copepod and cladoceran biomass, differed through time over the winter and with depth (PERMANOVA Pseudo-F_{12,20} = 4.5, p < 0.0001; Table 5, Figure 10, Table S3, Table S4). Changes in *Keratella cochlearis* and copepod nauplii biomass explained most of the differences in zooplankton community structure between early winter and the melting season (SIMPER Table S5). Of these taxa, *K. cochlearis* was most abundant in early winter, composing 20% of the total zooplankton biomass. The biomass of *K. cochlearis* population decreased with winter's progression and was about 9% of the total zooplankton biomass in the melting season. Copepod nauplii were nearly absent from the water column in early winter but became very abundant and contributed about 34% of the zooplankton community's biomass in the melting season. Similarly to seasonal differences, zooplankton community structure was different among depths; the community changed the most near the surface, while remaining more stable in the deeper strata (Figure 10B). The community at the two surface layers differed from the community in the bottom layer, except in mid-winter where zooplankton were similar at 0 m and 7 m but different between 1.5 m and 7 m (Table S3, pairwise). The middle layers (3 m and 4.5 m) shared similarity with both the surface and the bottom layers throughout winter. The zooplankton community during the fall and spring overturns was similar to the under-ice community at 7 m. The most important taxa separating the different sampling depths were *Ascomorpha* sp., *Cyclops scutifer*, and *K. longispina* (Table S5). *Ascomorpha* contributed to about 9% of the community's biomass at the surface (directly under ice), while *C. scutifer* and *K. longispina* were most abundant at the bottom of the water column, contributing about 15% and 9%, respectively, to the total biomass at 7 m (Table S5).

Table 5: Per-ANOVA table of results for zooplankton community in winter, including biomass from taxa of rotifers, copepods and cladocerans.

Source	df	SS	MS	Pseudo-F	Permutations	P(perm)
Date	3	10271	3423.6	18.203	9917	0.0001
Depth	4	12841	3210.3	17.069	9906	0.0001
Date*Depth	12	10163	846.91	4.5031	9856	0.0001
Residuals	20	3761.5	188.07			
Total	39	37036				

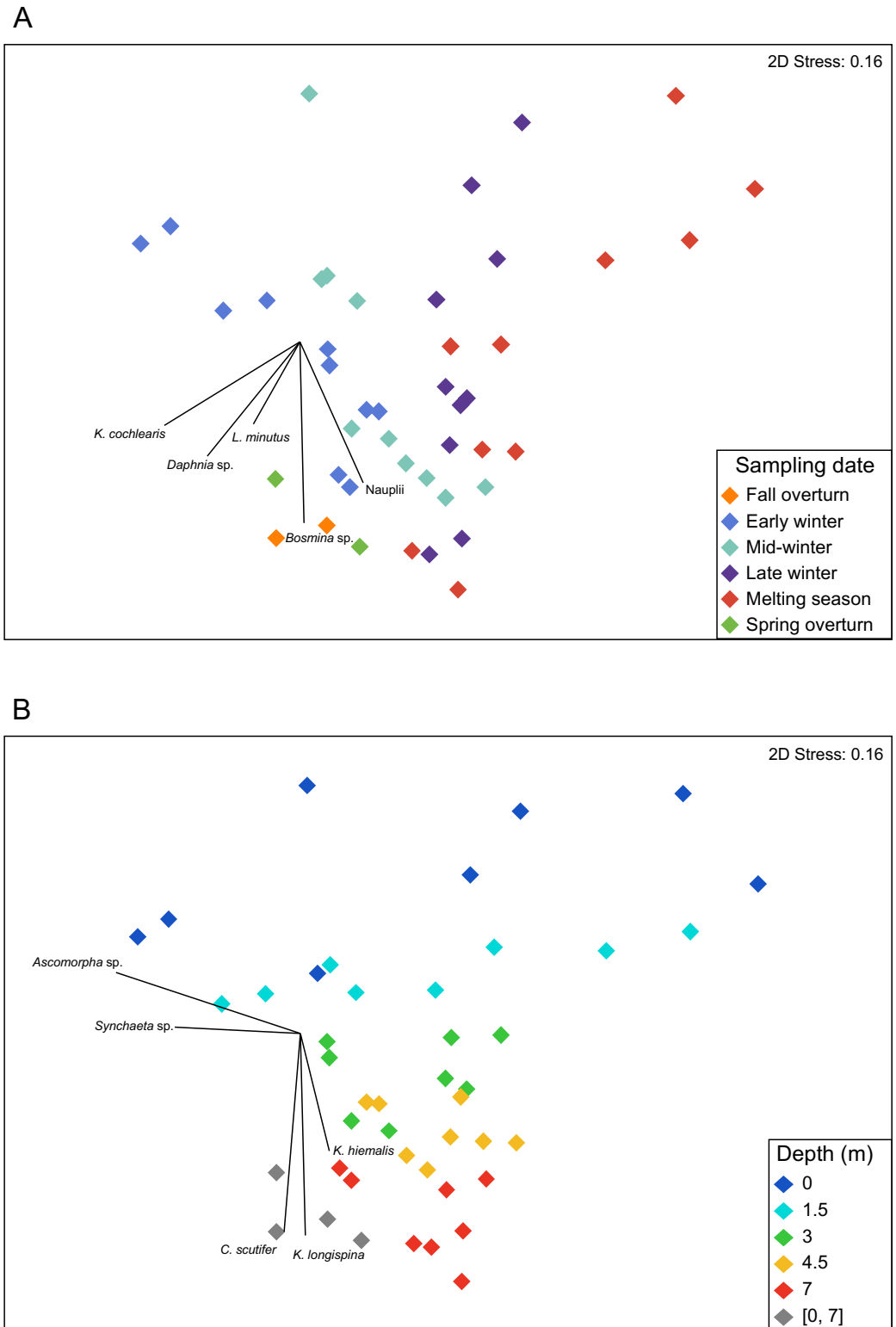


Figure 10: Non-metric multidimensional scaling (nMDS) of zooplankton (cladocerans, copepods and rotifers) community structure based on biomass and Bray-Curtis dissimilarity matrix, showing the differences among sampling dates (A) and depths (B). Shown vectors of taxa were selected based on their contribution as % of the difference between samples.

Zooplankton reproduction under the ice

Zooplankton reproduction occurred during winter 2020-2021, as rotifers, cladocerans and copepods produced eggs under the ice. For rotifers *Kellicottia* sp. and *Keratella* sp., the percentage of the population bearing an egg was high during the fall overturn then decreased until late winter, before increasing again during the melting season and the spring overturn, where it reached more than 20% of the total population of each *Kellicottia* and *Keratella* (Figure 11A). *L. minutus* showed signs of reproduction throughout winter, with between 1% and 6% of the population bearing eggs from the fall overturn to late winter. The proportion of reproducing individuals of *L. minutus* increased in the melting season and during the spring overturn, reaching 28% and 44%, respectively (Figure 11B). Copepod nauplii followed a similar pattern and were observed at low abundances until late winter, when the abundance increased more than threefold. Nauplii abundance was highest in the melting season and the larvae were mostly distributed in the lower strata of the lake (Figure 12). Cladocerans also showed signs of reproduction under ice, as the proportion of *Bosmina* sp. with eggs increased after ice-on in early winter. Egg production then decreased, and only 1.2% of individuals was egg-bearing in mid-winter. In late winter, *Bosmina* egg production increased to about 8% of the population (Figure 11C) and reached a maximum of 14% during the spring overturn. Juvenile *Bosmina* sp. were also observed in early and mid-winter but were absent from the water column thereafter. *Daphnia* sp. followed a similar pattern as *Bosmina* sp. and produced eggs under ice (Figure 11D). The proportion of *Daphnia* sp. with eggs decreased during early and mid-winter, completely stopped in late winter and increased again just before ice-off, to about 6% of the population. Egg production reached its peak during the spring overturn, with about 21% of the active *Daphnia* sp. population bearing eggs. The proportion of *Daphnia* sp. in the juvenile stages increased under the ice and was highest in late winter, then decreased in the melting season and during the spring overturn. Parthenogenic resting eggs were produced in the fall overturn and early winter, when 27% and 13%, respectively, of the *Daphnia* sp. population bore ephippia.

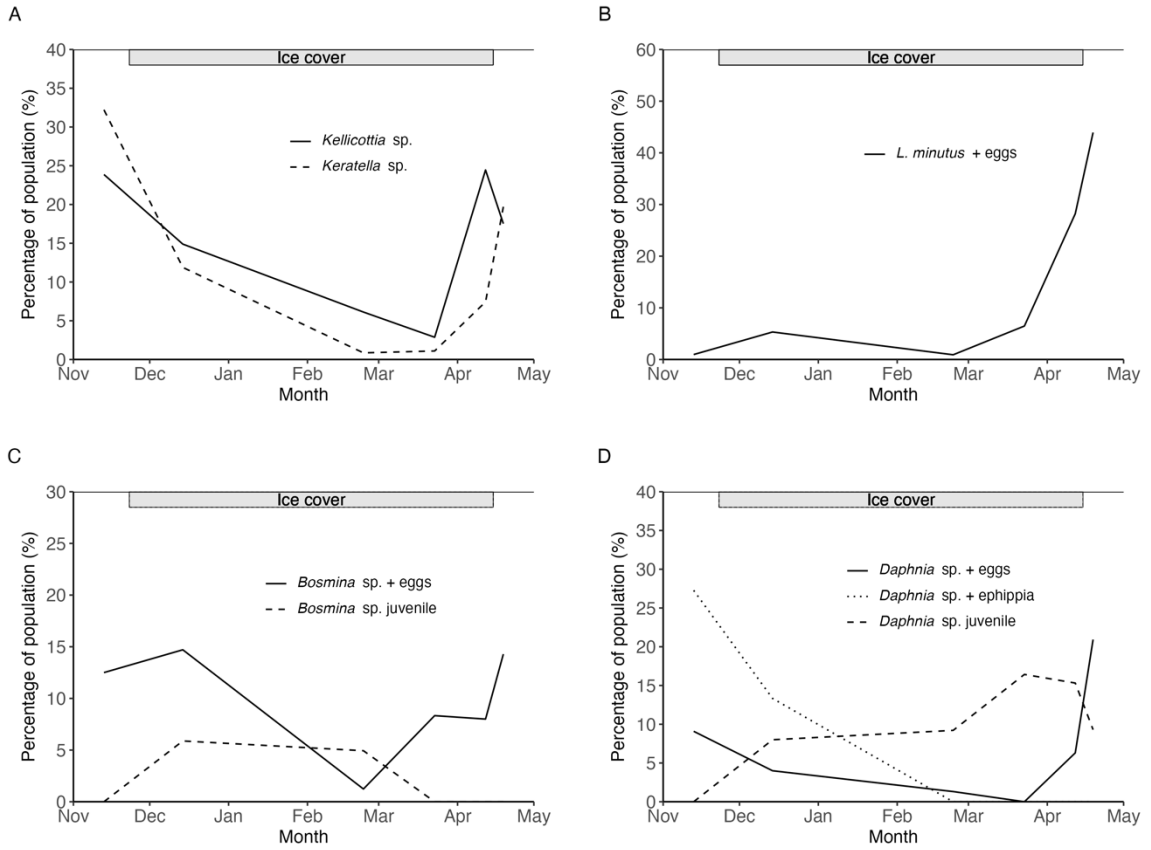


Figure 11: Zooplankton reproduction during winter, expressed as the percentage of rotifers (*Kellicottia* sp. and *Keratella* sp.) bearing eggs (A), percentage of *L. minutus* bearing eggs (B), percentage of *Bosmina* sp. bearing eggs or in the juvenile stage (C), percentage of *Daphnia* sp. bearing ephippia, eggs or in the juvenile stage (D).

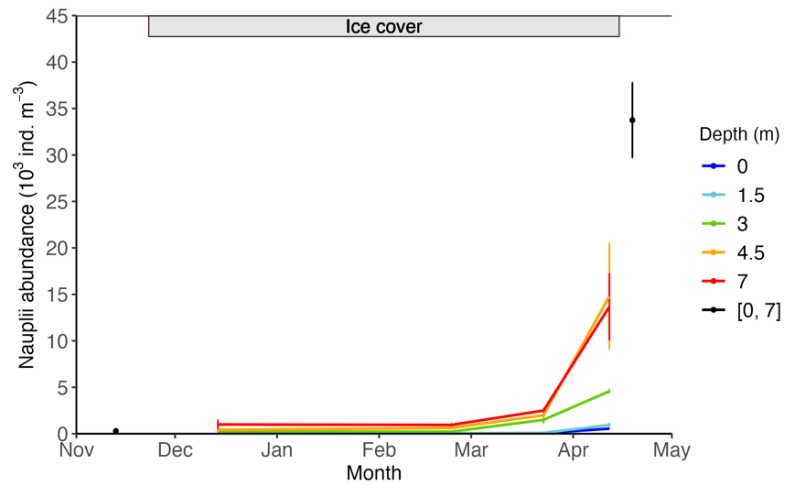


Figure 12: Copepod nauplii abundance ($10^3 \text{ ind. m}^{-3} \pm \text{SE}$) per depth during winter 2020-2021 in Lake Simoncouche.

Microbial biomass under the ice

Bacterioplankton biomass was highest in the fall overturn and was generally greater at deeper depths, except in mid-winter when more bacteria were counted near the surface (Table 6). The biomass of bacterioplankton decreased after the fall overturn and remained relatively low for the rest of the sampling period. A brief increase in biomass was observed during the melting season, after which it decreased again during the spring overturn to about 36% of the maximum biomass from the fall overturn (Table 6). Picoplankton biomass decreased more than twofold between the fall overturn and early winter, then reached a maximum during the melting season (Table 6). The spring overturn had the lowest picoplankton biomass, with about 35% of the maximum value. In early winter, cells were mostly distributed in the 1.5 m and 3 m strata of the water column. Their preferred depth then shifted downward for the rest of the sampling period, with a maximum at 4.5 m in mid-winter and at 7 m in late winter and during the melting season (Table 6). The biomass of HNF decreased between the fall overturn and early winter, before continually increasing for the rest of the sampling period, with a maximum biomass in the spring overturn, corresponding to an 835% increase between minimum and maximum values (Table 6). In early winter, heterotrophic nanoflagellate biomass was higher at 0 m (directly below ice), then they moved deeper from mid-winter, where the maximum biomass was at 1.5 m. During late winter and the melting season, the preferred depth of HNF was at 7 m (Table 6). PNF followed a different pattern, with the highest biomass occurring at the fall overturn and then decreasing until mid-winter, followed by a subsequent increase through the rest of the sampling dates (Table 6). PNF biomass increased almost six folds between the minimum in mid-winter and the maximum in spring overturn. The biomass of photo-autotrophic nanoflagellates was higher near the surface in early and mid-winter, then the highest values were at 7 m for late winter and the melting season (Table 6). Except for the fall overturn, HNF had a higher biomass than PNF throughout winter and the maximum value for HNF was almost six times higher than that of PNF (Table 6). The mass ratio of heterotrophic to photo-autotrophic nanoflagellates (HNF:PNF) increased during winter (Figure 13), as HNF became more abundant during the winter and spring overturn (Table 6). HNF:PNF mass ratio was low and significantly different between the fall overturn and early winter, and the remaining of the sampling period (Table 4).

Table 6: Seasonal and vertical variation of microbial biomass ($\mu\text{g C L}^{-1}$), including bacterioplankton, heterotrophic nanoflagellates (HNF), pigmented nanoflagellates (PNF) and autotrophic picoplankton during the key winter periods of Lake Simoncouche in 2020-2021. Values per depth are the mean of three replicates. The water column average is the mean (\pm SE) of all replicates for a given date.

Group	Depth (m)	Fall overturn	Early winter	Mid-winter	Late winter	Melting ice	Spring overturn
		Biomass ($\mu\text{g C L}^{-1}$)					
Bacteria	0	-	13.02	13.84	7.71	6.79	-
	1.5	-	12.54	10.19	7.34	8.61	-
	3	-	12.58	10.67	7.25	9.04	-
	4.5	-	13.66	12.12	7.34	11.62	-
	7	-	14.05	8.73	9.98	14.35	-
	Water column average \pm SE		26.58 \pm 1.67	13.17 \pm 0.26	11.11 \pm 0.68	7.92 \pm 0.37	10.08 \pm 0.86
HNF	0	-	43.88	39.68	44.17	71.33	-
	1.5	-	27.46	71.10	42.49	119.77	-
	3	-	23.19	44.84	36.83	130.70	-
	4.5	-	20.92	27.61	51.13	152.01	-
	7	-	18.32	31.97	126.85	233.36	-
	Water column average \pm SE		48.93 \pm 5.07	26.75 \pm 2.81	43.04 \pm 4.60	60.29 \pm 10.16	141.43 \pm 18.91
PNF	0	-	31.85	9.05	6.18	17.91	-
	1.5	-	22.75	9.59	5.64	16.23	-
	3	-	16.91	5.12	4.68	24.10	-
	4.5	-	16.36	3.06	6.95	44.39	-
	7	-	17.04	6.74	40.63	77.12	-
	Water column average \pm SE		77.10 \pm 6.36	20.98 \pm 1.98	6.71 \pm 0.93	12.82 \pm 3.82	35.95 \pm 8.42
Picoplankton	0	-	2.84	4.91	2.36	1.46	-
	1.5	-	5.25	3.04	4.20	3.87	-
	3	-	5.29	4.36	3.50	5.43	-
	4.5	-	3.59	10.53	4.77	9.59	-
	7	-	1.80	3.95	7.18	20.88	-
	Water column average \pm SE		6.99 \pm 0.55	3.91 \pm 0.40	5.24 \pm 0.67	3.99 \pm 0.37	7.25 \pm 1.41

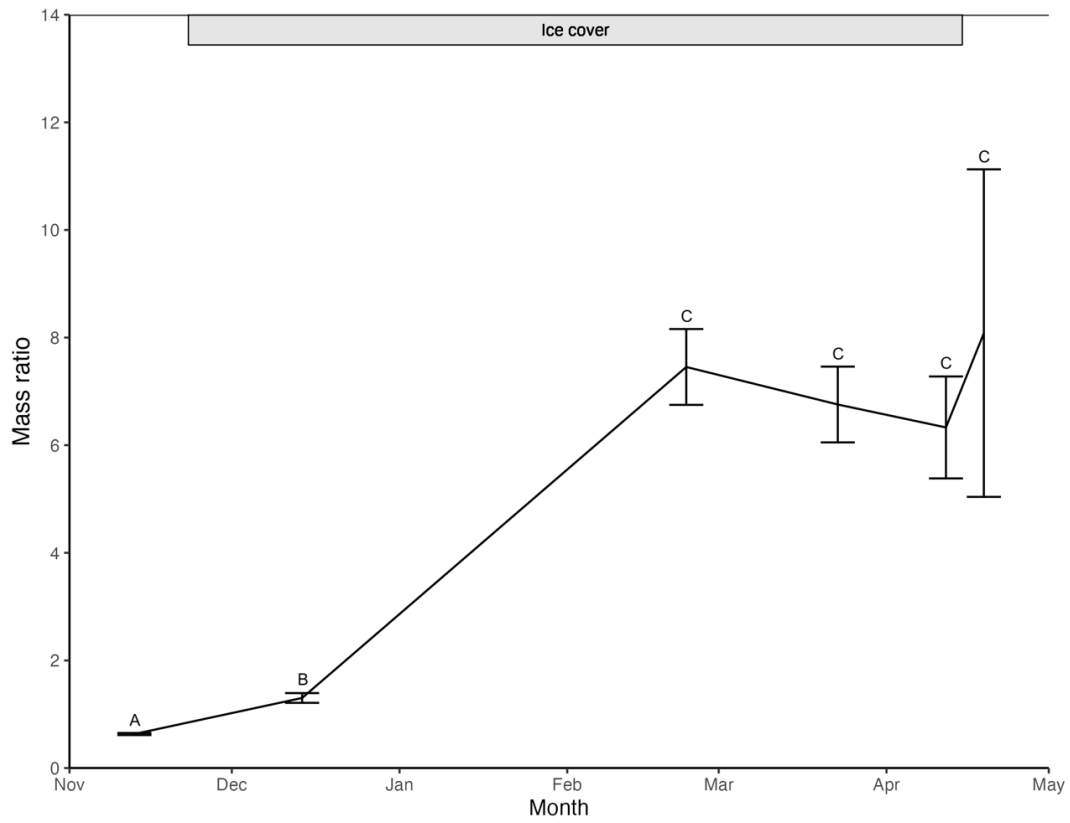


Figure 13: Mass ratio \pm SE of heterotrophic nanoflagellates (HNF) and pigmented nanoflagellates (PNF), as a proxy to study the energy transfers at the base of the food web during winter 2020-2021 in Lake Simoncouche.

Discussion

The results presented in this *memoire* show a change in the plankton community structure, following both the temporal winter seasonality from early winter to the melting season and the thermal stratification of the water column under the ice. Overall, the observed levels of zooplankton biomass and reproduction, as well as the microbial biomass indicate dynamic under-ice biological activity, arguably sustained by the decomposition and recycling of organic matter by the microbial loop in a rather dark under-ice environment, especially in mid-winter when the ice cover snowpack were thickest, and until the melting season, when light passes more easily through the thinning ice and can sustain primary production. The results of this project are in line with previous plankton studies from winter (Salonen *et al.* 2009; Hampton *et al.* 2017; Grosbois *et al.* 2020) that have shown active and abundant zooplankton communities thriving under the ice in boreal lakes. Very few studies have, however, studied the changes in vertical distribution of zooplankton under ice over a period of several months, and results from this project provide high-resolution, two-dimensional (time and depth) information on winter ecology of plankton in boreal lakes.

Zooplankton community under ice

All three zooplankton groups, including rotifers, copepods and cladocerans, were present under the ice of lake Simoncouche during winter 2020-2021. A total of 29 zooplankton taxa were identified during the sampling period, with an average of 17 in open water during the overturns, and 21 under the ice. Species richness was not significantly different between the shoulder seasons and the ice-covered period, as 14 to 22 taxa were identified at each of the sampling dates. Rotifers were largely dominant in abundance, but their biomass was in a range comparable with other groups, with values varying between 2 and 10 mg DW L⁻¹. This can be attributed to the relatively small body size of rotifers (50-200 µm length), in comparison to crustacean zooplankton (>200 µm length). Our results are in line with previous studies that have documented the presence of winter-active zooplankton in boreal lakes. Hampton *et al.* (2017) reported the activity of crustacean zooplankton under the ice, with an abundance of 25% of the open water average and with the most negatively affected group being the cladocerans, in a study of 101 temperate, boreal, and arctic lakes. In the present study, the

average under-ice pooled biomass of all zooplankton was about 60% of the open water average values during the shoulder seasons, with all taxonomic groups in the same range of proportions. The biomass of cladocerans was less affected by the ice cover than what is reported in literature and the under-ice biomass was about 62% of the ice-free average. However, the open water is only represented here by the fall and spring overturns, without data collected in the summer. In fact, previous studies from Simoncouche have reported other cladocerans such as *Holopedium gibberum*, *Leptodora kindtii* and different chydoridae during the warmest summer months (Grosbois *et al.* 2020), suggesting the open water diversity is higher than indicated by our overturn samples. Species richness during winter may then be different than summer, but similar to the overturns, suggesting that species that do not overwinter actively withdraw from the water column before the fall overturn. The high and stable cladoceran (*Bosmina* sp., *Daphnia* sp.) biomass throughout the winter suggests that species from this group may be well-adapted to winter conditions, allowing some to stay active under ice while others rest in the sediments (Mariash *et al.* 2016).

While the zooplankton species richness changed modestly during the winter, abundance and biomass decreased for all groups between the fall overturn and late winter. Minimal values of biomass were recorded in late winter, only few weeks before the ice-out. Environmental variables, such as temperature, oxygen saturation and chlorophyll-*a* concentration, denoted an overall decrease during the first months of winter, with values declining until mid-winter, suggesting less-favourable ecological conditions for zooplankton. Conditions then started to improve in late winter, when chlorophyll-*a* concentration increased in the water column as a result of increased light penetration through the ice. Additionally, a deep, lateral flow of oxygenated water was detected by the moored RBR sensors and the RBR profiles in late winter, which likely brought organisms and improved conditions at the bottom of the water column, where oxygen saturation increased to about 45%. Such patterns of under-ice currents have been reported previously (MacIntyre *et al.* 2018; Cortés and MacIntyre 2020), but their ecological consequences on plankton are not known (Jansen *et al.* 2021). There was a subsequent (non-significant) increase in the average water column zooplankton biomass during the melting season and through the spring overturn, as conditions in the water column became less harsh.

When vertical distribution in the water column was considered, the changes in zooplankton biomass patterns became clearer, with substantial differences between the depths. In general, the biomass of zooplankton (all taxa pooled) increased with depth and the top stratum immediately under the ice (0 m) was systematically avoided by all taxa, except for the rotifers in early winter. As winter progressed, the zooplankton community tended to shift deeper, where temperatures were higher. The seasonal variation of zooplankton biomass at the five sampling depths indicated clear preference for deeper, warmer waters, with a biomass at 7 m about twice of that at 0 m. Species preference for depth and zooplankton stratification in the water column during winter is not widely documented, but Perga *et al.* (2020) and Perga *et al.* (2021) have shown evidence of diel vertical migration. Factors such as water temperature, oxygen and light levels, and food availability are known to modulate zooplankton abundance in the water column (Huntley and Mai 1992; Brown *et al.* 2004; Hansen *et al.* 2014; Perga *et al.* 2021). The ability of zooplankton to move vertically in the water column and to select depths according to more favourable living conditions, could have led the community to deeper waters where temperatures were higher. Decreasing oxygen in the bottom of the water column until late winter represented a depth limitation to heterotrophic organisms and when the oxygen saturation was at its lowest at 7 m (2.5 %, 0.34 mg L⁻¹) in mid-winter, the zooplankton community was observed to avoid this depth and move upwards. An exception to this was the cladocerans, that seemed unbothered by the hypoxia and still had a higher abundance at the 7 m depth. Similar trends of high cladoceran abundance in hypoxic waters were reported earlier by Vanderploeg *et al.* (2009). The lateral flow of oxygenated water in late winter then caused O₂ saturation to increase at the 7m depth, where the zooplankton biomass became highest in the water column. The already greater temperature at 7 m, along with the newly brought dissolved oxygen from the current, may have caused the observed increase in zooplankton biomass, which was most pronounced for cyclopoids and cladocerans. The current likely transferred organic matter and nutrients from the littoral zone to the lower part of the pelagic water column, providing both heterotrophic organisms and primary producers with a fresh supply of nutritive inputs. Organisms may also have been carried by the current from the littoral zone to the 7 m sampling location. In general, the higher oxygen saturation in the cold upper part of the water column compared to the bottom likely favored cold-resistant taxa, while the

higher temperature at the bottom served as a refuge for thermo-sensitive species. The consistently higher biomass in deeper strata however suggests that organisms favor higher temperature over oxygen availability if oxygen saturation is sufficient for respiration. Some species could have also used the reduced light conditions at deeper depths as protection against visual predation during daytime (Pearre 2003). Crustacean zooplankton depth preference is known to follow the circadian rhythm of diel vertical migration under a frozen surface and is mostly linked with refuge seeking and visual predation avoidance (Pearre 2003; Perga *et al.* 2020). The irradiated under-ice epilimnion may have sustained low levels of photosynthesis, but despite the increased chlorophyll-*a* concentration in the upper water column and the reported increase in primary production during late winter and in the melting season (Kivilä *et al.* 2023), denoting a rise in photo-autotrophic bioactivity and thus food availability, no evidence of upward movement of the zooplankton community was observed. Instead, the organisms stayed in the lower water column where temperatures were still higher, underlining the importance of the thermal refuge for all taxa.

Rotifers were present at all depths and at all times during the ice-covered season and showed preference for deeper, warmer water (4.5 m and 7 m depths). Rotifers form a robust and diverse group of short living animals, that continuously feed on smaller organisms, such as nanoflagellates and picoplankton, and follow the availability of food resources (Bégin 2020). Rotifer biomass patterns were characterized by decreasing values throughout winter, with consistently higher biomass in deeper strata. Rotifers arguably used nanoflagellates as a food source and their biomass distribution roughly corresponds with that of the nanoflagellates, that declined in the upper water column but remained high and even increased near the bottom around late winter. The deep water was a thermal refuge and a zone of greater food abundance for rotifers. When the oxygen-rich current also brought food, lower strata were favourable for the species that actively feed. The combined abundance of rotifers had, however, a less drastic vertical stratification than other zooplankton taxa, and they used the water column at each sampling depths throughout winter. Although on a finer taxonomical scale, the rotifers had species-specific vertical distributions, with *Ascomorpha* sp. preferring the top strata, while others like *Polyarthra* sp. were mostly present in the mid water column. Some species of rotifers used specific parts of the water column at certain times while others were found at all depths, hinting

on their broad range of temperature tolerance and feeding strategies. *K. cochlearis* and *K. longispina* were the most abundant species of rotifers in most samples and were found at all depths, with a preference for deeper, warmer waters. Their generalist diet and their tolerance for low temperatures and low oxygen levels allowed these taxa to most efficiently use the water column during the ice-covered period. The vertical movement of rotifers in lakes over the course of the ice covered season is not broadly studied, but Perga *et al.* (2020) have shown that vertical movement can occur under ice in late winter.

The calanoid copepods, mostly composed of *Leptodiaptomus minutus*, were present at all depths with abundances declining until late winter. Previous studies have demonstrated the ability of *L. minutus* to stay active under ice by using lipid reserves accumulated during the fall (Grosbois *et al.* 2017; Schneider *et al.* 2017) and report that in winter *L. minutus* individuals were brightly coloured by carotenoids from algal derived diet, that were used as antioxidant to protect the fat reserves. Accumulated lipids allow overwintering and under-ice reproduction while reducing the reliance on active feeding. Despite no specific analysis on lipids in the context of the present study, the visible lipid bubbles and the bright orange colour of the individuals suggest that a similar mechanism was in action during this sampling campaign. The vertical preference of adult calanoid was not obvious from the results, supporting the notion of hiemal fasting, as their presence did not follow food availability. An exception to this pattern were the nauplii that were born in late winter and were most abundant in the melting season and spring overturn in deep water. The phenology of calanoid reproduction takes advantage of the under-ice spring bloom of nanoflagellates and picoplankton, which can be used by copepodites as an important food resource that allows for rapid growth and regeneration of the population (Vanderploeg *et al.* 1992). The nauplii displayed a clear preference for deep water and were most abundant at 7 m and 4.5 m. This corresponds with the vertical distribution of nanoflagellates and picoplankton biomass, that were highest at the 7 m depth during the melting season. An increase in the abundance of adult calanoid was observed during the melting season, likely caused by a lateral flow of water from the littoral zone carrying individuals.

The cyclopoid copepods, mostly represented by *Cyclops scutifer* and *Tropocyclops prasinus*, were also present in high abundance during the sampling period. Their seasonal variation was low,

but the preference for deeper water was significant. Cyclopoids were also coloured orange, similar to the calanoids, but their vertical distribution suggests a stronger importance of warmer temperature, with a preferred depth of 7 meters. The appearance of lipid bubbles, as observed with the microscope, suggests that a similar overwintering strategy was employed by both groups of copepods, wherein fat reserves reduced reliance on active feeding. Although *Cyclops* sp. under-ice feeding can target mixotrophic and benthic algae from the littoral zone, (Karlsson and Sävström 2009), lipidic reserves remain an important overwintering strategy for *Cyclops* (Perga *et al.* 2021). The preference of *C. scutifer* for deeper strata was likely caused by the higher water temperature and food availability.

Cladocerans were most abundant at the surface in early winter, after which the community shifted deeper in the water column. This taxonomic group is sensitive to low temperatures and can withstand low O₂ concentrations (Vanderploeg *et al.* 2009; Lampert *et al.* 2010). likely used deeper waters as a thermal shelter. Additionally, cladocerans and have been considered to need constant feeding and follow the availability of food resources, such as diatoms and other phytoplankton (Sommer *et al.* 1986), but studies have demonstrated the ability of species of *Daphnia* to survive the winter by using accumulated lipids (Slusarczyk 2009). Under the ice, diatom production is strongly limited and actively feeding *Daphnia* sp. might consume mixotrophic algae, which are high-quality food and an important alternative polyunsaturated fatty acids (PUFA) source (Vad *et al.* 2021). The higher temperature in lower strata of the lake, along with the high biomass of nanoflagellates and picoplankton in late winter, likely allowed part of the cladoceran population to overwinter by actively adapting to the changing conditions.

The community structure of zooplankton, including rotifers, copepods and cladocerans demonstrated a clear variation in species composition following the winter seasonality and depth in the water column. Shchapov and Ozersky (2023) report that zooplankton communities undergo significant changes between summer and winter, but depth-specific comparisons were not conducted. Our results show significant vertical heterogeneity in the community composition under the ice, underlining the relevance of depth as a factor modulating the community structure of zooplankton during winter. The community was most stable at 7 m and experienced the most changes at 0 m and 1.5 m. For all depths, early winter community was statistically different than that of the

melting season. The vertical heterogeneity was also significant, with the community at the surface being statistically different than at the bottom. The two seasonal overturns were similar in terms of species composition and were more similar to the communities at 7 m. The changing conditions in the water column did favour different species at different times and at different depths during the ice-covered period. The decreasing chlorophyll-*a* concentration in winter could indicate a reduced availability of food resources for grazers, such as cladocerans and certain rotifers. Their constant need for feeding would then make this group more sensitive to seasonality, and their sensitivity to low temperatures could result in a deeper vertical position of the population. The SIMPER analysis identified important species defining the community according to seasonality and depth. Taxa displaying the highest dissimilarity between early winter and the melting season were *K. cochlearis*, *Ascomorpha* sp., and nauplii sp.. *K. cochlearis* is a rotifer species that was found at all sampling dates and at all depths, but their abundance declined sharply after the onset of the ice cover. The genus *Ascomorpha* was abundant in early winter near the surface, but almost disappeared from the water column during winter. As for the nauplii, their abundance increased steeply after late winter. Differences between the surface and bottom samples were mainly attributed to the higher abundance of all taxa of zooplankton, particularly that of *C. scutifer* and *K. longispina* at the 7 m depth and were explaining the most dissimilarity.

Zooplankton reproduction under ice

Species of zooplankton from all three taxonomic groups reproduced under the ice, as observed by the presence of females bearing eggs and individuals in the juvenile stages. Similar patterns have been observed previously in boreal and sub-arctic lakes (Rautio *et al.* 2000; Schneider *et al.* 2017). The study presented in this *memoire* reports notable trends in reproductive behavior among rotifers belonging to the *Keratella* and *Kellicottia* genera. The production of eggs by these rotifers exhibited a discernible pattern beneath the ice, characterized by a gradual decrease over time followed by an upswing during the melting season, wherein nearly a quarter of the *Kellicottia* population was in an egg-bearing state. At the same time, near the open-water transition, food availability for zooplankton increased and a generalized spike of bioactivity happened in the water column. This underscores the dynamic nature of reproductive strategies in response to environmental cues such as periods of high

food availability, with the melting season potentially serving as a key trigger for heightened reproductive efforts among certain rotifer populations. Cladocerans exhibited a distinct temporal pattern in their reproductive strategies during winter. Notably, during early winter, a significant proportion of the cladoceran populations produced eggs and ephippia. There was a subsequent decline in egg production, reaching a minimum in late winter. Reproductive activity increased again immediately prior to the open water transition, when populations of *Daphnia* sp. and *Bosmina* sp. produced eggs in high proportion. The production of resting eggs is well-documented in the literature and is proposed as a mechanism triggered by low temperatures or reduced light conditions (Larsson and Wathne 2006). This reproductive strategy allows for the survival and subsequent emergence of a new generation of cladocerans when ice melts, coinciding with the spring bloom of phytoplankton. The cladocerans, however, did not disappear from the water column during winter and there was no significant effect of the seasonality on their integrated water column abundance, although the population shifted to deeper depths during the winter months. This behavioral shift is consistent with findings from Mariash *et al.* (2016), providing evidence that some cladocerans actively overwinter, challenging the conventional understanding that these organisms mostly withdraw from the water column during the winter season, after ensuring successful future cohort by the production of parthenogenic resting eggs. Juvenile *Daphnia* sp. exhibited an increasing abundance beneath the ice, followed by a subsequent reduction around the period of ice-off. The observed pattern suggests a potential delay in the growth and maturation of *Daphnia* sp. during the harsh winter period. This phenomenon may be indicative of a life history strategy wherein cladocerans allocate resources towards survival and maintenance rather than rapid growth during adverse environmental conditions. Considering copepods, previous studies in Lake Simoncouche have demonstrated that *L. minutus* produces two cohorts per year, one in early spring before ice-off and a second, much smaller one, in August (Schneider *et al.* 2017). Our results are in support of existing literature and suggest reproductive activity during the ice-covered period. The proportion of females with egg sacks was low during the first sampling dates but increased drastically in the melting season, with about 30% of the population being egg-bearing females. The phenology of *L. minutus* reproduction coincides with the under-ice algal spring bloom, where an upsurge in photosynthesis from increased light penetration

and nutrient circulation causes an overall increase in bioactivity in the water column, resulting in high food availability (Salmi and Salonen 2016; Kivilä *et al.* 2023). Larval stages of *L. minutus* became highly abundant around that time, as nauplii have access to resources for growth and development. For nauplii to be ready to feed before the spring overturn, adults must start producing egg sacks under the ice. Our results show a steep increase in nauplii abundance in the melting season and during the spring overturn, following calanoid egg production. The total abundance of *L. minutus* in various development stages (excluding nauplii) remained however mostly unchanged throughout the sampling period.

Microbial ecology under the ice

The microbial loop was a key constituent of the winter-active food web, as heterotrophy became the main energy pathway. The classical summer food web, fueled by sunlight and through primary producers, was hindered by the presence of ice and snow, leading to a decrease in chlorophyll-a concentration in the water column. This is in accordance with Kivilä *et al.* (2023), wherein light conditions were deemed insufficient to sustain photosynthesis ($< 10 \mu\text{mol m}^{-2} \text{s}^{-1}$) until late winter in lake Simoncouche. Despite a significant decrease in the abundance of primary producers, the under-ice food web remained active. This was likely enabled by the recycling of organic matter and dissolved carbon compounds by heterotrophic organisms, as suggested by the high biomass of heterotrophic nanoflagellates and bacteria. These observations underscore the seasonal dynamics of energy flow within the aquatic ecosystem during winter, emphasizing the role of the microbial loop in sustaining biological processes when photo-autotrophic energy pathways can no longer be sustained.

Bacterioplankton likely played an important role in fueling the under-ice food web, owing to its ability to decompose and recycle both terrestrial and available autochthonous organic matter such as excretions and remains of deceased organisms (Bertilsson *et al.* 2013; Bižić-Ionescu *et al.* 2014). Bacteria served as a vital food source for larger organisms such as heterotrophic and mixotrophic nanoflagellates, which were in turn consumed by organisms of to higher trophic levels (Bertilsson *et al.* 2013). The seasonal and vertical biomass patterns of bacterioplankton displayed minimal variation, with their presence documented near the surface in cold water, as well as at deeper depths where

temperature was warmer. Although the bacterial biomass presented in this *memoire* remained relatively stable during winter, Kivilä *et al.* (2023) report a succession in the bacterioplankton community during winter 2020-2021 in Lake Simoncouche. In early winter, autochthonous carbon was still present in the water column, and it was preferentially consumed by bacteria. In mid-winter, carbon from terrestrial sources was used, as autochthonous input was reduced. A fresh input of both terrestrial and autochthonous carbon occurred in the melting season, as material was brought to the lake from the catchment and as biological activity increased in the lake. The succession in bacterioplankton community followed the availability of the carbon components and the adaptation of different groups to efficiently use certain carbon fractions (Kivilä *et al.* 2023). Moreover, despite the relatively stable biomass, bacterial production has been shown to display considerable variations and to escalate before ice-off in Lake Simoncouche (Kivilä *et al.* 2023). This suggests that a top-down control on bacterioplankton occurs shortly before spring, as general bioactivity was rising in the water column. In other words, the stable bacterioplankton biomass during winter and between depths reported in the present study, along with the increased bacterial production near the end of winter as reported by Kivilä *et al.* (2023), suggest that bacterioplankton was effectively used as a food resource by larger heterotrophs. In fact, consumers of bacteria including mixotrophic nanoflagellates and rotifers, saw an increase in their biomass near the open-water transition, arguably partly fueled by the high bacteria production.

Heterotrophic nanoflagellates (HNF) are single celled organisms that do not produce photosynthetic pigments, but instead feed on bacteria. They are present in all algal groups and are defined by their size and energy uptake mode instead of taxonomy. Some mixotrophic nanoflagellates can change their metabolism under low light conditions and stop pigment production and photosynthesis, switching to a heterotrophic metabolism and a bacterial diet (Laybourn-Parry *et al.* 1995; Bertilsson *et al.* 2013). In this study, there was a steep increase of HNF in deep waters before ice-off, representing a vernal bloom of nanoflagellates following the fresh input of terrestrial materials from the catchment and the littoral (Kivilä *et al.* 2023), which consequently provided a high quality food resource for zooplankton. The by-products from their metabolic activity coupled with the release of senescent cells due to their short lives, represented a considerable input of autochthonous organic

matter that could be decomposed and recycled by bacterioplankton, further fueling the food web. Top-down control from zooplankton may have limited the extent of the nanoflagellate spring bloom, as the abundance of all plankton groups, including NF consumers, also increased before ice-off.

Pigmented nanoflagellates (PNF) and picoplankton, being unicellular autotrophic organisms capable of photosynthesis, served as proxies for photo-autotrophy in the water column. Their biomass steeply decreased after the onset of ice and exhibited a surface-dominant distribution until mid-winter, followed by a shift towards deeper, warmer waters with elevated nutrient and dissolved carbon concentrations. Under-ice spring blooms were observed for both PNF and picoplankton from the melting season to the spring overturn, with a vertical preference for deeper waters. The magnitude of the biomass increase during the spring bloom was less pronounced for picoplankton and PNF than for HNF but was still significant, suggesting that despite the increasing light supporting autotrophic production, the heterotrophic pathway was still more energetically efficient. This is consistent with previous studies of subarctic, Finnish lake Saanajärvi, where HNF were more abundant than photo-autotrophs in the under-ice epilimnion, just before ice-off (Rautio *et al.* 2011). Kivilä *et al.* (2023) report that primary production occurs in late-winter and in the melting season in Lake Simoncouche, with maximum values at 1.5 m depth. The chl-*a* concentrations followed this pattern in the context of project, with rising concentrations in the melting season at 1.5 m. The biomass of PNF and picoplankton was high in late winter and in the melting season, supporting the seasonal patterns reported in literature. The vertical distribution of these autotrophic organisms, however, did not totally coincide with findings from Kivilä *et al.* (2023) or with the measured chl-*a*, as the PNF biomass was significantly higher at 7 m and 4.5 m, at time when primary production and chl-*a* concentrations were low at these depths. A reason for the PNF biomass not to match with the chl-*a* distribution is their small overall biomass in relation to larger algal taxa. It is possible that large-size phototrophic organisms such as ciliates, diatoms and other algae dominated the phytoplankton and partially explained the chl-*a* distribution, as larger body-sized phytoplankton would have been filtered out during the sample preparation for nanoflagellates, which involved passing the lake water through a 50 µm sieve. Further, we suspect the high PNF abundance at the deeper depths was not dictated by the light intensity at the sampling site but that these autotrophic cells were carried by a laterally

flowing bottom current that we detected from the oxygen profiles and moored sensors. The affinity to 7m depth was then arguably a result of the lateral current, which brought PNF to the deepest point of the lake, at the sampling site. The origin of the deep water PNF in fact would have been the littoral where the nanoflagellates had been exposed to light and had built their autotrophic pigments. In the deeper, darker environment, the pigments may not have been efficiently used for photosynthesis until vertical mixing of the water column during the spring overturn or when enough light could reach the deeper strata. Alternatively, another explanation for the mismatch between the vertical distributions of the PNF and chl-*a* could be that the PNF in deep strata may have produced other pigments, such as carotenoids, which would not have been detected by our analyses.

The ratio of heterotrophic to pigmented nanoflagellates (HNF:PNF) increased with the progression of winter and with depth. This is mostly explained by the biomass of HNF increasing significantly near the end of the sampling period and by PNF biomass decreasing sharply in the dark and cold, ice-covered water column. Nutrient concentration in the water column (TN, TP), as well as dissolved organic carbon origin (autochthonous or allochthonous) may influence the metabolism of mixotrophic nanoflagellates. Generally, higher nutrient concentrations favor photo-autotrophic nanoflagellates when the light conditions are sufficient, and this can coincide with increased concentrations of autochthonous DOC. Conversely, heterotrophic nanoflagellates can digest both autochthonous and allochthonous DOC (Carney *et al.* 2016). According to Kivilä *et al.* (2023), TN and TP concentrations were higher in the bottom strata of the water column throughout winter, with the exception of early winter where maximum TN value was recorded at 0 m. The higher concentrations of TN near the surface in early winter coincides with the vertical distribution of PNF at this sampling date, when the population was mostly distributed in the upper strata. The subsequent vertical downward shift of PNF also corresponds with nutrient stratification. Autochthonous carbon concentration was also higher in early winter, then decreased throughout the ice-covered period (Kivilä *et al.* 2023). The reported analyses of dissolved organic carbon (DOC) composition and proportions from Kivilä *et al.* (2023) revealed that indicators of allochthonous carbon were consistently more present in deep water, whereas autochthonous carbon was proportionally more abundant in the upper water column. These findings are partly in line with the biomass patterns of nanoflagellates

reported here. HNF vertical distribution also partly matches with the allochthonous carbon data from Kivilä *et al.* (2023), with higher biomass at 4.5 m and 7 m depth after late winter. However, the present results indicate that the thermal refuge and nutrient availability were important factors determining the vertical distribution of the nanoflagellate population, as nanoflagellates did not entirely follow the DOC allochthony-autochthony proportions per depth. In fact, higher biomass of PNF was recorded near the bottom of the water column in late winter and in the melting season, contrasting with reported autochthonous carbon vertical patterns and chl-*a* concentrations from Kivilä *et al.* (2023). As suggested above, it is probable that other larger algae were present in the upper strata of the lake in the melting season, explaining the measured chlorophyll-*a* values and the distribution of autochthonous DOC. The increasing HNF:PNF mass ratio throughout winter indicates a shift in lake metabolism, steering from photo-autotrophy to heterotrophy, and underlines hiemal adaptations to energy flows of the lake's food web.

Conclusion

This *memoire* set out to study the dynamics of the zooplankton communities and the microbial loop in ice-covered boreal Lake Simoncouche during winter, focusing on three key objectives. First, we analyzed the temporal and vertical structure of winter-active zooplankton communities. Our results confirmed significant temporal and vertical heterogeneity, consistent with our hypotheses. Although there was an overall decline in the biomass of all three zooplankton groups, rotifers, copepods, and cladocerans were present in samples corresponding to all sampling dates throughout winter 2020-2021. Notably, while the cladoceran biomass decreased under the ice, the cladoceran population remained present in the water column and used deeper strata as a thermal refuge, supporting the concept of active overwintering for some species of *Daphnia* sp. and *Bosmina* sp.. Species of zooplankton that had been previously identified in Lake Simoncouche during summer were absent in winter, hinting at the challenging life conditions in the ice-covered water column. The cold temperatures, low luminosity, low oxygen levels and low food availability that prevail during boreal winters are harsh conditions for heterotrophic metabolism, and adaptations like lipid reserves, alternative diets or vertical migrations allowed more than 20 taxa of zooplankton to stay active for months under the ice while others sank in the sediments. These highly adapted winter-active species play an important ecological role and contribute to sustaining the winter food web of boreal lakes. Our objective regarding the reproductive patterns of the winter-active zooplankton species was built around the understanding that the life cycle of *L. minutus* is in tune with the phenology of the ice cover, in such a way that a new cohort develops under the ice before the algal spring bloom. Consistent with the hypothesis, copepod nauplii abundance increased towards the end of winter, indicating the under-ice reproduction of *L. minutus*. Furthermore, we expected that some species of rotifers would not only survive the winter but would rather thrive and reproduce. The production of eggs during the ice-covered season was reported here for four species, including two of the most abundant species of rotifers in Lake Simoncouche. Lastly, we examined energy transfer at the base of the food web using pigmented and heterotrophic nanoflagellates. As expected, we observed a decrease in photo-autotrophy with progressing winter and with depth, aligning with the hypothesis. Moreover, the increasing heterotrophy/autotrophy ratio throughout winter peaked before ice melt,

indicating heightened bioactivity within the water column, with heterotrophy as a preferred energy pathway. These three objectives provided a comprehensive framework to assess the community dynamics of plankton in ice-covered Lake Simoncouche, addressing the urgent need for baseline data in plankton research. In fact, the vast majority of all lakes on earth are affected to some degree by ice-cover formation during winter, and of all the 1.4 million lakes (>10 ha) on the planet, around 62% are in Canada. The duration of the ice-cover period is decreasing due to the warming effects of climate change, and the ecological outcomes of rapid perturbations in the seasonality and phenology of lake ice are still not fully understood. This research lays the groundwork for future studies that will address the long-term implications of climate change on ice-covered aquatic ecosystems.

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Supplementary material

Table S1: Significant differences ($p < 0.05$) for total zooplankton biomass between depths for each date, obtained from post-hoc pairwise tests. Groups with shared letters were not significantly different. This table relates to figure 8.

Depth (m)	Early winter	Mid-winter	Late winter	Melting season
0	AB	A	A	A
1.5	A	A	A	A
3	A	B	B	B
4.5	B	BC	AB	B
7	AB	C	C	B

Table S2: Significant differences ($p < 0.05$) for total zooplankton biomass between dates for each depth, obtained from post-hoc pairwise tests. Groups with shared letters were not significantly different. This table relates to figure 8.

Depth (m)	Early winter	Mid-winter	Late winter	Melting season
0	AB	AB	A	B
1.5	A	A	A	B
3	A	B	C	A
4.5	A	A	A	A
7	AB	A	B	AB

Table S3: Significant differences ($p < 0.05$) for zooplankton community between depths for each date, obtained from post-hoc pairwise tests. Groups with shared letters were not significantly different. This table relates to figure 10.

Depth (m)	Early winter	Mid-winter	Late winter	Melting season
0	A	AB	A	AB
1.5	B	A	A	A
3	BC	AB	A	B
4.5	CD	B	AB	C
7	D	B	B	C

Table S4: Significant differences ($p < 0.05$) for zooplankton community between dates for each depth, obtained from post-hoc pairwise tests. Groups with shared letters were not significantly different. This table relates to figure 10.

Depth (m)	Early winter	Mid-winter	Late winter	Melting season
0	A	AB	B	B
1.5	A	AB	B	C
3	A	B	BC	C
4.5	A	A	AB	B
7	A	B	B	B

Table S5. Summary of SIMPER (percentage of similarity) for zooplankton community structure between early winter (EW) and the melting season (MS). Table shows species that cumulatively contribute up to 70% to the dissimilarity between the dates. Av. abund.: average abundance; Av. diss.: average dissimilarity; Diss/SD: dissimilarity divided by standard deviation; Contrib.%: percentage of contribution; Cum.%: cumulated percentage of contribution.

Species	Av. abund. EW	Av. abund. MS	Av. diss	Diss/SD	Contrib. (%)	Cum. (%)
<i>Keratella cochlearis</i>	3.54	1.24	6.02	1.78	12.40	12.40
Nauplii spp.	0.44	2.25	4.17	1.82	8.59	21.00
<i>Ascomorpha</i> sp.	2.14	0.64	4.07	1.17	8.38	29.38
<i>Polyarthra</i> sp.	1.83	0.59	3.62	1.32	7.46	36.83
<i>Leptodiatomus minutus</i>	1.96	1.11	3.11	1.54	6.40	43.23
<i>Daphnia</i> sp.	1.57	1.03	3.06	1.40	6.31	49.54
<i>Synchaeta</i> sp.	1.75	0.67	3.04	1.26	6.27	55.81
<i>Cyclops scutifer</i>	2.26	1.70	3.01	1.18	6.20	62.01
<i>Kellicottia longispina</i>	1.73	1.71	2.71	1.33	5.58	67.59
<i>Tropocyclops prasinus</i>	1.67	1.56	2.52	1.56	5.19	72.78

Table S6. Summary of SIMPER (percentage of similarity) for zooplankton community structure between 1.5 m and 7 m. Table shows species that cumulatively contribute up to 70% to the dissimilarity between the dates. Av. abund.: average abundance; Av. diss.: average dissimilarity; Diss/SD: dissimilarity divided by standard deviation; Contrib.%: percentage of contribution; Cum.%: cumulated percentage of contribution.

Species	Av. abund. 1.5 m	Av. abund. 7 m	Av. diss	Diss/SD	Contrib. (%)	Cum. (%)
<i>Cyclops scutifer</i>	1.10	3.91	6.18	4.09	13.15	13.15
<i>Bosmina</i> sp.	0.07	2.06	4.37	2.48	9.29	22.44
<i>Daphnia</i> sp.	0.58	2.21	4.05	1.54	8.61	31.05
Nauplii spp.	0.37	1.89	3.43	2.24	7.29	38.35
<i>Kellicottia longispina</i>	1.24	2.61	3.13	1.72	6.65	45.00
<i>Kellicottia bostoniensis</i>	0.00	1.39	2.95	1.54	6.28	51.29
<i>Tropocyclops prasinus</i>	0.84	1.89	2.73	1.75	5.80	57.09
<i>Ascomorpha</i> sp.	1.46	0.61	2.69	2.38	5.71	62.80
<i>Keratella hiemalis</i>	1.95	3.13	2.65	1.72	5.63	68.43
<i>Filinia terminalis</i>	0.11	1.14	2.41	1.33	5.12	73.55