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The hypopagea: an unexplored habitat for winter-active insects

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ABSTRACT

Upon learning of a report of a giant water bug (*Lethocerus americanus* (Leidy, 1847)) swimming to a hole drilled in an ice-covered pond, we investigated the extent to which insects remain active in ice covered ponds in northern Nova Scotia, Canada. Insects were caught primarily with a novel *ice hole trap*, but also by dip netting in the holes and examining the wash around the hole soon after the drilling. Using a submerged camera further confirmed some insects were active and motile. Over 1,300 specimens (seven orders, 17 families, at least 45 species) were captured over six winters, from 2012 to 2017. These data suggest that the *hypopagea* (the freshwater environment below ice) is an unexplored habitat full of active insects.

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Water insects; ice hole trap; pond; Nova Scotia; Canada

Introduction

The widely held assumption that aquatic insects are inactive in ponds during the winter in northern climates appears logical, given the quenching effect of low temperatures on poikilothermic metabolism (Sinclair 2015). Exposure to sub-zero temperatures in air or in water is normally fatal to adult insects unless they possess adaptations to withstand freezing (Danks 1978). However, most research on insect survival in winter concerns terrestrial species; we know far less about cold adaptations in aquatic insects (Danks 2008). Aquatic insects generally appear to have limited capacity to withstand sub-zero temperatures by super-cooling (Moore and Lee 1991; Oswald, Miller, and Irons 1991) and non-dipteran larvae or adults frozen into ice show poor survival (Irons, Miller, and Oswood 1993; Mihalicz 2015).

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Avoiding ice by moving to deeper or moving water is the most common survival mechanism of aquatic insects (Olsson 1983; Irons et al. 1993; Lencioni 2004).

Even among insects that survive winter in water, near-zero temperatures, such as may occur in unfrozen streams or below winter ice, would be expected to severely suppress metabolic rates in adults and larval stages. Moreover, the combination of a potential reduced food supply and diversion of energy to resisting cold may cause a serious energy drain (Duffy and Liston 1985) and reduce survival over winter. Therefore, even those insects that do not pass the winter as eggs are generally assumed to enter some kind of dormancy or quiescence throughout the winter (e.g., Patenaude, Smith, and Fahrig 2015). The assumption that aquatic insects are dormant in winter is supported by the parallel assumption that shallow ponds freeze to the bottom, leaving no liquid water as a habitat. This assumption may be correct for shallow ponds, especially in alpine and Arctic climates (e.g., Daborn and Clifford 1974; Danks 2008; Srayko, Mihalicz, Jardine, Phillips, and Chivers 2023). However, in all but the most severe climates, ponds of significant depth (>50 cm) retain liquid water throughout the winter because surface ice and snow insulate the water beneath from heat loss to the atmosphere (Ashton 1989). Flowing water resists freezing, and open water leads may be found in shallow streams at temperatures as low as −30 °C (B. Taylor, personal observation). Oswood, Miller, and Irons (1991) reported that small streams in interior Alaska remain flowing and have large portions of unfrozen streambed in midwinter. In streams and rivers, nymphs and larvae of many species may be found in open leads and below ice, growing slowly throughout the winter (Bengtsson 1981; Danks 2008).

Ponds that do not freeze to the bottom maintain cold water under the ice layer. Although some mention has been made regarding the activity of adult insects in ice-covered lentic systems (Hussey 1921; Hungerford 1948; Griffith 1945; Sailer 1952), no systematic survey of this habitat has ever been made. For example, Hungerford (1948) reported that some Corixidae (Hemiptera) remain active under the ice, but that and similar reports were not sourced or quantified. Holmen (1987) and Larson, Alarie, and Roughley (2000) reported observations of Dytiscidae (Coleoptera) overwintering under the ice of frozen ponds but provide no details of the species involved or the extent of winter activity. Evans (2014) noted briefly that some species within the Dytiscidae may remain active under winter ice, again without elaboration. There have been a few reports of mayfly nymphs (Ephemeroptera) under ice in frozen ponds (Brittain and Nagell 1981; Oswood et al. 1991), but none of the immatures from other insect orders. Otherwise, the assumption that insects remain dormant in winter, perhaps augmented by the logistic difficulties of sampling frozen ponds, has prevented systematic investigations of the possibility that some pond insects may remain active under winter ice.

Spurred by a local citizen's report of seeing a giant water bug swimming in a drilled hole in a frozen pond, we began a study of winter-active aquatic insects in frozen ponds of northern Nova Scotia, Canada. We refer to this neglected environment under winter ice as the *hypopagea* (from Greek *hypo* – under, and *pagea* – icy). Our primary objective was to document the diversity of insects active under ice in ponds of varying character. Because of the novel environment being sampled, and the lack of established procedures for sampling under winter ice, developing and

refining collection methods became part of this study. This paper provides the first rigorous work on hypopagic communities in frozen ponds.

Material and methods

Study sites

Our 14 study ponds lie in or near Antigonish County, on the northern mainland of Nova Scotia, on the Atlantic coast of Canada (Figure 1). The rural landscape of the region comprises gently rolling hills dissected by shallow, wide river valleys. The underlying geology is complex but consists largely of unsorted glacial till over

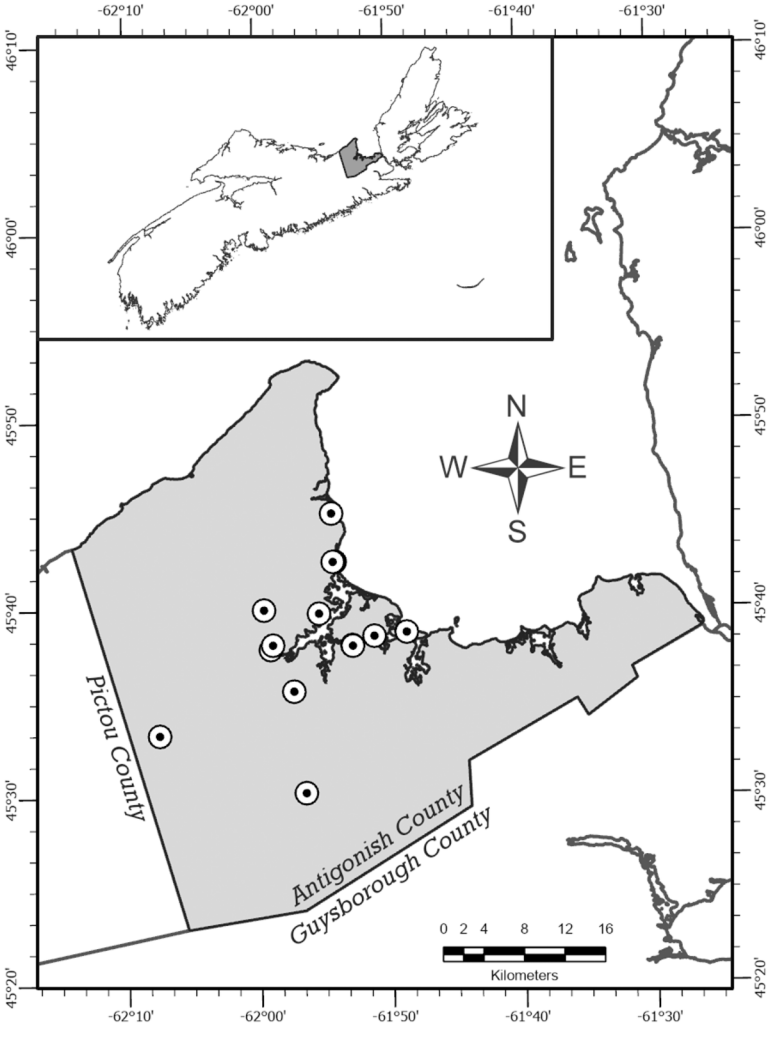

Figure 1. Locations of the ponds in this study. Only 12 marks are shown since in two cases, two ponds were very close together; each pair of closely neighbouring ponds is encompassed by one mark.

Table 1. Surface area, pH, and conductivity of the 14 ice covered ponds sampled in northern Nova Scotia, Canada in 2014, 2015, and 2017 (see Figure 1 for pond locations).

Pond Name	Surface Area (m^2)	pH (units)	Conductivity (µS/cm)	Year Sampled		
				2014	2015	2017
Frasers Mills	1426	5.5	52	X	X	X
Keppoch	235	5.6	37	X	X	X
Jewkes	454	6.3	125		X	X
Upper MacLean	238	5.5	78	X	X	X
Lanark	247	5.0	106			X
Silo	255	7.3	94		X	X
Saint Theresa's	664				X	X
Beech Hill	35,517	5.3	51		X	
Cloverville	919	4.7	35		X	X
Cribbons Beach Rd.	300	5.2	209		X	
Pomquet Beach Park	~10,000	5.9	289		X	
Bancroft	1000	*6.6*	*185*		X	
Sam Hills	902				X	
Lower MacLean	125	6.4	92			X

Conductivity and pH values represent average values calculated from one to three measurements taken in 2015 or 2017. Values *in italics* were measured in March 2020.

granitic bedrock (David and Browne 1996). Land use is primarily short-rotation Acadian forest intermittent with pasture fields, clear-cuts and country residences.

Nova Scotia has a modified continental climate, with long, snowy winters and short, cool summers; the annual mean temperature is 5.6 °C for 1916–1990, and −6.7 °C in January (Environment Canada 2002). Annual precipitation is about 1300 mm (David and Browne 1996). Autumn is delayed and extended by the maritime influence. Ice begins to form on local ponds in mid-December; in most years continuous ice cover extends from January to mid-April when daily mean temperatures rise above zero. High precipitation combined with impeded drainage through silty soils has led to the formation of numerous lakes, wetlands, and ponds throughout the region.

Insects were collected from a variety of natural, artificial, and modified ponds (Figure 1, Table 1). Most ponds range in size from 0.01 to 1.2 ha though the Beech Hill pond was 13 ha. Similarly, most ponds were known to be fishless, the exceptions being St. Theresa's Pond, which contained goldfish (*Carassius auratus* (L., 1758)) and the Beech Hill pond, which supported a variety of native fish. Most ponds were vegetated in the shallows by submerged grass, ericaceous shrubs and semi-aquatic, herbaceous plants, especially cattails. The site at Pomquet Beach Provincial Park is a slack, narrow, seasonally water-filled depression between ancient sand dunes.

Field methods

Sample periods

Both quantitative and qualitative sampling methods were employed. Systematic sampling was undertaken in the winters of 2014, 2015 and 2017, after preliminary sampling in 2012 and 2013 suggested that insects were abundant and active beneath the ice. Lanark pond was examined again in 2018. Sampling began in January of

each year when the ice was thick enough for safe access; sampling ended in late March or early April.

Boring the holes, habitat measurements

Either a 20 cm power auger (Rapala Enduro 51CC), a 15 cm manual auger, or (in the first three years) an axe was used to make one or more holes through the ice in each pond. Most insects were captured in traps placed in the boreholes, as described next, or observed with underwater cameras. Insects were also caught opportunistically at the boreholes in dip nets or retrieved from wash produced by drilling.

Ice thickness was measured at three evenly spaced points around each hole in 2015. The depth of the water from its surface to the substratum was also measured in the centre of each hole using a metre stick or a sounding line. Water temperature was measured (in 2015) in the ice hole with an alcohol thermometer. Dissolved oxygen concentration, conductivity and pH were measured in most ponds in 2015 and 2017 with calibrated electronic meters; because electronic instruments do not work reliably at sub-zero air temperatures, these measurements were made on 1-L water samples warmed in the laboratory to ~10 °C.

Trap design

Initial surveys, until mid-February 2014, used conventional minnow traps (Gee traps) for quantitative sampling of insects such as the larger predaceous diving beetles of the genus *Dytiscus* Linnaeus, 1758 (Coleoptera: Dytiscidae). At each site, after shovelling away surface snow, four holes were bored through the ice. Holes were generally bored close to shore, near vegetation, when possible, because that is where aquatic insects have been reported to spend most of their time (Merritt, Cummins, and Berg 2008). Holes were spaced at least 5 m apart. If the depth of water below the ice was <10 cm, a new hole was bored farther from shore, and the unused hole was filled with snow.

Ice fragments produced by the ice-borer were first cleared from the opened holes using an ice-fishing scoop. Insects in the water, visible on the ice, or among ice fragments (disturbed by boring) were collected, counted, and transported to the laboratory in cold water. Other invertebrates (e.g., gastropods, crustaceans) were transferred to 70% ethanol for storage and later identification.

Minnow traps (Promar; mesh size 7 × 11 mm) were lowered through the ice hole and positioned horizontally just under the surface of the ice. Traps were tethered to prevent sinking or were buoyed with an internal float. Traps were recovered 24 h later. Most traps were baited with chemical light sticks (OmniGlow, Brownsville, Texas, USA), usually red, because these had been found to be attractive to aquatic beetles (Lauff, MacDonnell, and Taylor 2018). However, the light sticks did not work reliably in ice-cold water and did not appreciably increase trapping success compared with unbaited traps.

Experience with the minnow traps suggested that capture success was not diminished if traps were inserted vertically into the ice hole without suspending them horizontally below the ice. An *ice hole trap* was developed and tested based on this observation. Half of a conventional minnow trap was lowered into the hole such

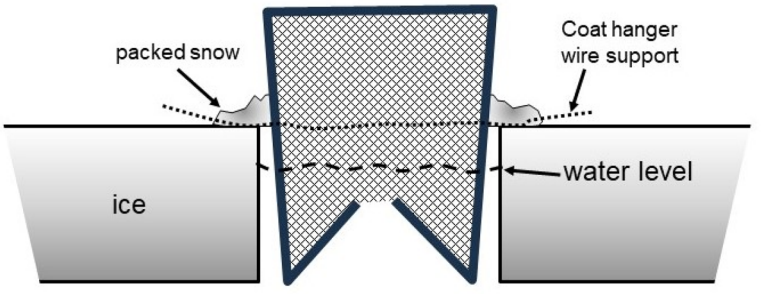

Figure 2. The ice hole trap. One half of a typical minnow trap is inserted into a hole bored in the ice and stabilised with snow. Sinking is prevented by a coat hanger wire fed through the trap, resting on the ice surface.

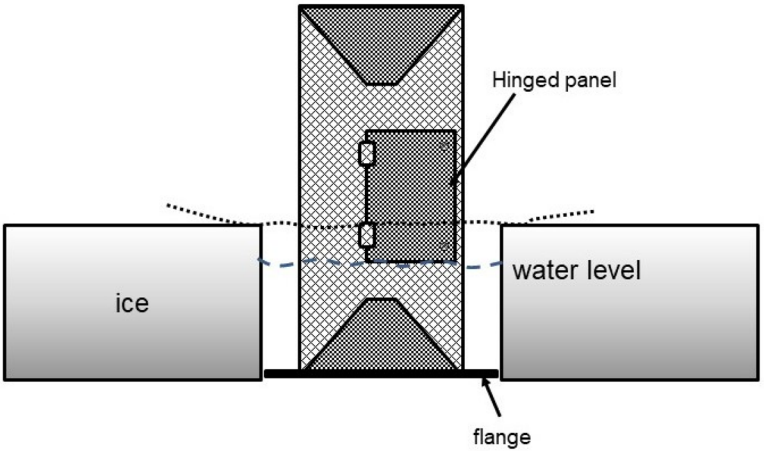

Figure 3. The modified ice hole trap. In addition to finer mesh to trap smaller insects, a flange was added to help direct swimming insects into the funnel and away from the space between the trap and the wall of the ice hole.

that the entrance cone was perpendicular to the ice surface and the cone's internal opening was approximately 5 cm under the water surface (Figure 2). A stiff wire (often a straightened coat hanger), protruding through, and extending onto the ice beyond the trap, prevented sinking. Ideally, the narrow rim of the trap (i.e., the end deeper in the water) was situated flush with the underside of the ice; this was not always possible, however, if the ice was too thin or too thick. These ice hole traps were used during February to April 2014 and January 2015.

The ice hole trap successfully captured large aquatic insects such as *Dytiscus* spp. and *Lethocerus americanus* but smaller insects were not retained. Hence, a finer mesh version of the ice hole trap (Figure 3) was designed to capture a wider diversity and size range of organisms. These traps consisted of a 2 mm mesh tube (41 cm long, 18 cm diameter) with funnel-shaped openings at each end, similar to a conventional minnow trap. The funnels were 10 cm deep with a 2.5-cm entrance hole at the apex. A hinged panel covered an access port of 24 × 16 cm along one side. A

1-cm plastic flange mounted at one end better filled the 20-cm ice auger holes and prevent insects swimming in between the side of the trap and the wall of the hole. Since the traps were left in place for 24 h, they inevitably froze into the sides of the bored hole. An axe was used to free the traps; removing the traps without damage was unexpectedly daunting, repairs to the traps were common. Sampling proceeded more or less weekly, from early January through to early April. Most ponds were sampled once per year, but a few were visited twice or more, especially the easily accessible pond in Frasers Mills.

Fine mesh traps were placed in the ice hole vertically with the submerged end intruding into the water below the ice surface, in the same manner as for the coarse-mesh ice hole traps. The end of the trap with the plastic flange was placed down. When possible, the traps did not protrude below the bottom surface of the ice, so insects on or near the undersurface of the ice could enter them more readily. Snow and ice were then packed into the holes to stabilize the traps. After 24 h, the traps were chopped out of the ice with an axe and transported to the laboratory in plastic totes. Once in the laboratory, the ice was allowed to thaw, after which the insects were removed. Adults were euthanized with ethyl acetate and pinned; immatures were euthanized and preserved in 70% ethanol.

Taxonomy

Beetle nomenclature is based on the classification in Oygur and Wolfe (1991) (Gyrinidae: *Gyrinus* Geoffroy, 1762), Vondel (2005) and Ashbee, Marshall, and Alarie (2017) (Haliplidae), Gustafson and Miller (2015) (Gyrinidae: *Dineutus* MacLeay, 1825), and Larson et al. (2000) and Nilsson and Hájek (2023) (Dytiscidae). Hungerford (1934) (Notonectidae) and Hungerford (1948) (Corixidae) were used to identify Hemiptera. Species identification of other insect orders (e.g., Ephemeroptera and Trichoptera) was based on Gordon (1933), Peckarsky, Fraissinet, Penton, and Conklin (1990), Wiggins (2000) and Merritt et al. (2008).

Many insect larvae cannot be identified below the genus by conventional methods. The species identity of the abundant and widespread caddisfly *Platycentropus* Ulmer, 1905 was determined by sequencing a fragment of cytochrome *c* oxidase subunit I (COI) from larvae which had been preserved for up to six months in 70% ethanol. Amplified DNA fragments were directly sequenced by The Centre for Applied Genomics at The Hospital for Sick Children, Toronto, Canada. Four sequences were used to query the BOLD CO1 Animal Identification database (http://www.boldsystems.org/index.php/IDS_OpenIdEngine). All queries returned *Platycentropus radiatus* (Say, 1824) with a probability of 99%. Therefore, we tentatively identify our caddisfly larvae as *P. radiatus*. Attempts to identify other larval taxa were frustrated by amplification inhibitors.

Results

Physical characteristics

Typical of surface waters of northern mainland Nova Scotia, water in the study ponds was very soft (mean conductivity 81.5 ± 50.5 µS/cm, $n = 15$) and circumneutral

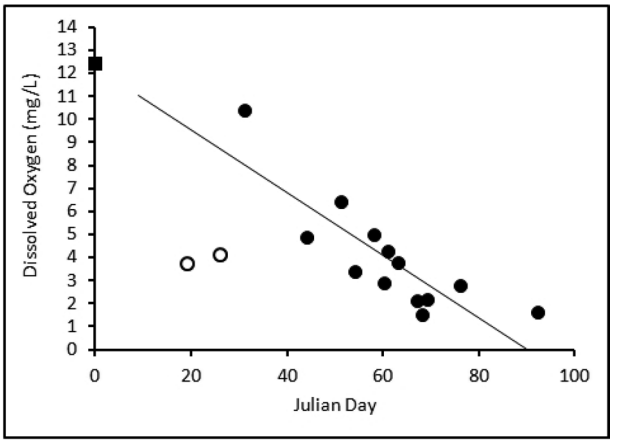

Figure 4. Decline in dissolved oxygen concentration under winter ice in Nova Scotia ponds, winters of 2015 and 2017. Regression line is based on 13 observations (filled circles) but excludes two ponds (open circles) with low oxygen concentrations in January. Oxygen concentration in Frasers Mills pond on 31 December 2019 (filled square) closely matches prediction from the regression line.

to mildly acidic (mean pH 5.7 ± 0.8) (Table 1). A pH above 7 was observed only twice. Mean oxygen tension was 3.9 ± 2.2 mg/L in 2015 and 3.2 ± 0.9 mg/L in 2017. Excepting two ponds which already suffered low oxygen tensions in January (2017), a decline in dissolved oxygen tensions with time under the ice was detectable across all ponds in 2015 and 2017 combined (R^2 = 0.70, p = 0.0003, n = 13). A water sample from the Frasers Mills pond on 31 December 2019 held 12.4 mg/L of dissolved oxygen, very near the 12.2 mg/L predicted by the regression (Figure 4). We did not observe anoxia in any pond. The regression model predicted anoxia on 31 March, near the end of the ice-cover season in our region.

Mean ice thickness increased throughout the winter of 2015, from <10 cm (the minimum for safe access) in December to 45 cm in March. Ice cover was well established by early January of 2017 (23–25 cm) and increased only modestly, to ~35 cm, by mid-March. Nevertheless, abundant free water persisted below the ice in all our ponds in all years. Combining data from 2015 and 2017, the average increase in ice thickness across all ponds over the winter (Figure 5a) can be approximated by a logarithmic regression model (R^2 = 0.72, p < 0.0001, n = 16). The trend is discernable despite that it is based on multiple ponds over two winters. Ice thickness on various ponds measured in three other winters all lie close to the regression line or an extrapolation of it (Figure 5a), suggesting that the general trend of increasing ice thickness is repeated each winter.

Ice accumulation depends on the loss of heat from the liquid water to the air and should be increasingly inhibit as the ice layer grows thicker (Ashton 1989). Consistent with this expectation, the rate of ice accretion in our ponds declined exponentially throughout the winter (Figure 5b). Maximum ice thickness probably does not exceed 45 cm, because mean daily air temperatures above zero usually arrive in early April (Environment Canada records from Tracadie, the nearest

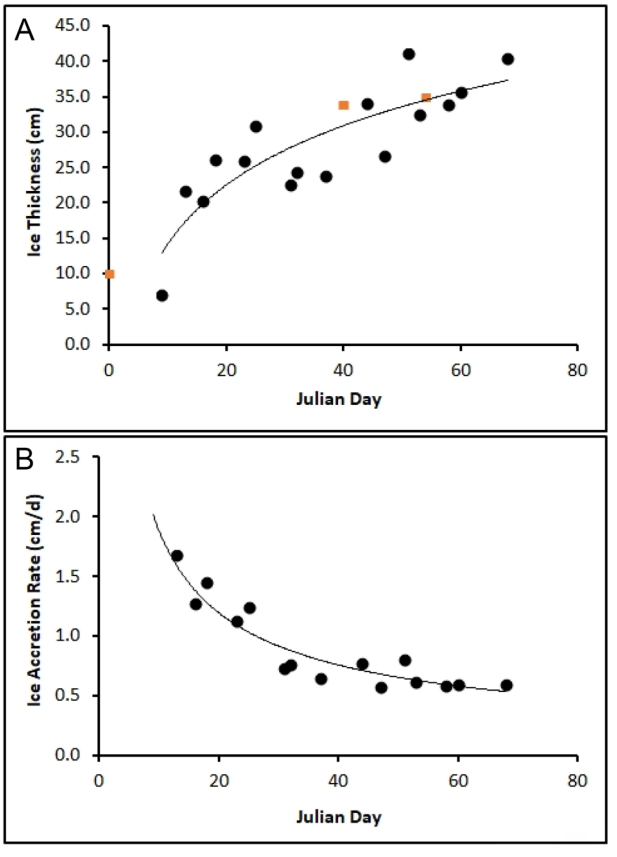

Figure 5. Winter ice accretion in Nova Scotia ponds: (A) Increase in ice thickness after 1 January. Regression is based on data from 2015 and 2017 (dark circles). Data from Frasers Mills pond in 2013, 2014 and 2019 (filled squares) suggest similar ice thickness. (B) Exponentially declining rate of ice accretion with time after 1 January, combining data from 2015 and 2017.

station). Hence, any pond deeper than ~ 50 cm would maintain free water below the ice. Neither diversity nor abundance of captured insects correlated significantly with pond depth, ice thickness, or depth of the free water layer.

Water temperature 5 cm below the surface was always 0 °C. There was no evidence of super-cooling or sediment warming in our ponds.

Insect sampling

A total of 1331 insects was caught over the duration of this study (Table 2). Of these, 1207 insects were caught in the new ice hole trap, the remainder were netted in the hole or retrieved from the wash. The greatest richness was found among beetles (24 species, 267 specimens; all adults except for one larva of *Ilybius* Erichson, 1832) followed by Hemiptera (ten species, 414 specimens). Five other orders (Diptera, Ephemeroptera, Odonata, Megaloptera and Trichoptera) contributed 650 specimens among at least nine species.

Table 2. Insect species recorded in 14 ice covered ponds in northern Nova Scotia, Canada in 2014, 2015 and 2017.

	Family	Species	Number of collected specimens
Coleoptera	Dytiscidae	*Acilius semisulcatus* Aubé, 1838	23
		Agabus anthracinus Mannerheim, 1852	3
		Colymbetes paykulli Erichson, 1837	2
		Colymbetes sculptilis Harris, 1829	4
		Coptotomus longulus LeConte, 1852	2
		Dytiscus fasciventris Say, 1824	43
		Dytiscus harrisii Kirby, 1837	4
		Dytiscus verticalis Say, 1823	37
		Graphoderus liberus (Say, 1825)	4
		Ilybius sp.	1
		Laccophilus maculosus Say, 1823	1
		Nebrioporus rotundatus (LeConte, 1863)	1
		Neoporus carolinus (Fall, 1917)	1
		Rhantus binotatus (Harris, 1828)	2
	Gyrinidae	*Dineutus assimilis* (Kirby, 1837)	56
		Dineutus nigrior Roberts, 1885	51
		Gyrinus affinis Aubé, 1838	4
		Gyrinus opacus C.R. Sahlberg, 1819	1
		Gyrinus pectoralis LeConte, 1868	4
		Gyrinus sayi Aubé, 1838	2
		Gyrinus sp.	1
	Haliplidae	*Haliplus connexus* Matheson, 1912	1
		Peltodytes edentulus (LeConte, 1863)	1
		Peltodytes litoralis Matheson, 1912	1
	Hydrophilidae	*Tropisternus mixtus* (LeConte, 1855)	17
Hemiptera	Corixidae	*Hesperocorixa atopodonta* (Hungerford, 1927)	13
		Hesperocorixa interrupta (Say, 1825)	49
		Hesperocorixa lobata (Hungerford, 1925)	47
		Hesperocorixa sp.	1
		Sigara compressoidea (Hungerford, 1926)	1
		Sigara penniensis Hungerford, 1928	6
	Notonectidae	*Notonecta insulata* Kirby, 1837	163
		Notonecta irrorata Uhler, 1879	3
		Notonecta undulata Say, 1832	97
	Nepidae	*Ranatra fusca* Palisot de Beauvois, 1820	30
	Belostomatidae	*Lethocerus americanus* (Leidy, 1947)	4
Ephemeroptera	Baetidae	*Callibaetis* spp.	95
	Leptophlebidae	*Leptophlebia* sp.	12
Diptera	Chaoboridae	*Chaoborus* spp.	15
	Chironomidae		1
Odonata	Coenagrionidae	*Nehalennia* spp.	3
	Lestidae	*Archilestes* sp.	1
		Lestes spp.	2
Megaloptera	Corydalidae	*Chauliodes rastricornis* Rambur, 1842.	3
		Chauliodes pectinicornis (Linnaeus, 1763)	2
Trichoptera	Limnephilidae	*Platycentropus radiatus* (Say, 1824)	447
		Platycentropus spp.	69
		Grand Total:	1331

The number and diversity of insects collected varied widely among ponds. A few ponds, notably Keppoch, Jewkes, and Frasers Mills, produced an assortment of that were abundant. Other ponds yielded only one or two species (Table 2).

Early trapping with the large-mesh minnow traps confirmed that dytiscid beetles (Coleoptera: Dytiscidae), particularly the large predaceous diving beetle *Dytiscus fasciventris* Say, 1824 were active beneath the ice in mid-winter. For example, seventeen specimens of *D. fasciventris* were caught in the Keppoch pond over five days in March 2014. The Frasers Mills and Upper MacLean ponds also yielded numerous

D. fasciventris along with occasional *D. harrisii* Kirby, 1837 and one *Graphoderus liberus* (Say, 1825). The gyrinid beetle *Dineutus assimilis* (Kirby, 1837) was captured in those same three ponds.

Discussion

A broad diversity of adult and immature insects was collected from the ice-covered ponds we sampled, although this was not expected. Essentially every major insect taxon that would be expected in ponds of this region in summer was also present, and active, in winter. This list includes beetles, bugs, mayflies, caddisflies, hellgrammites, and phantom midges; cladocerans were also collected. Insects must have been sufficiently active to find and enter the traps. Dytiscid and gyrinid beetles, in particular, appeared to swim about with the same vigour and speed as during the ice-free seasons. Mayflies were commonly observed swimming vigorously toward newly opened ice holes. Therefore, it was concluded that the insects observed were active in winter, and not merely dormant or quiescent forms which would be taken only by more active sampling.

Overwintering in most of the species caught here has not been studied, and previous reports of hypopagic activity have been scattered and anecdotal. Hussey (1921) reported that *Cymatia americana* Hussey, 1920 (Hemiptera, Corixidae) overwinters in groups of 10–50 individuals within cavities in the ice, but that other aquatic hemipterans hibernated deep within the mud at the bottom of ponds. In subsequent field visits, he reported catching *C. americana* under ice, along with several species of the corixid genus *Arctocorisa* Wallengren, 1894. Hungerford (1948) observed that Corixids of various species could be taken in midwinter swimming beneath the ice. Hilsenhoff (2017) summarises (without citations) the overwintering of *Dytiscus* as being in deeper aquatic habitats, though no notes on activity are given. However, more than 60% of his *Dytiscus* captures were in the spring with only partial ice cover present. Most other studies of overwintering in aquatic insects concern their capacity to survive freezing in ice or sediments (e.g., Mihalicz 2015), rather than their ability to remain active in cold water.

One major impediment to studying pond life in winter is the logistical difficulties involved in doing so. Previous experience (Lauff et al. 2018) suggested that modified minnow traps would successfully capture active adult insects from under ice. Because the adults do not fly during winter, our ice-hole trap was effective at capturing and retaining them; the insects could not escape by flying out of the traps.

Moreover, the mesh size on a standard minnow trap is too coarse to trap all but the largest insects. The fine-mesh trap we developed overcomes this limitation. Both traps (standard and fine-mesh) suffered strong refreezing to the surrounding ice as well as ice formation within the trap itself. They were difficult to remove and could be emptied only after thawing in the laboratory for several hours.

The hypopagea

The environment we are calling the hypopagea is defined by cold, darkness and isolation from the atmosphere. The ice layer approached 40 cm by late winter in our ponds, and given the non-linear pattern of ice accretion, would rarely reach

45 cm. Therefore, free water persisted even in the comparatively shallow ponds that were sampled.

Low oxygen tension in the hypopagea poses an additional challenge to organisms living there. Isolation from the atmosphere, coupled with decreased aquatic plant photosynthesis and continued oxygen demand for organic matter decomposition, would explain the low and declining oxygen tensions we observed. Winter anoxia is reportedly widespread in ponds in cold temperate regions (Daborn and Clifford 1974; Nagell and Brittain 1977; Lencioni 2004). While low temperatures reduce metabolic oxygen demands, non-dormant invertebrates would still require an oxygen supply to sustain activity. Air-breathing insects (i.e., any adult), moreover, and especially the very active Dytiscidae, would be denied access to their usual oxygen supply by ice cover; the water level in every drilled pond was always immediately under the ice, i.e., there was no air space between the water and the undersurface of the ice. Air bubbles trapped beneath the ice provide only a temporary oxygen supply because the partial pressure of oxygen in these air pockets would be in equilibrium with the water below them; by late winter, sub-ice air pockets would be a weak oxygen source. Tolerance of low oxygen tensions in frozen ponds has been reported for *Leptophlebia vespertina* (Linnaeus, 1758) (Brittain and Nagell 1981) and some species of chironomids (Danks 2008), but not for many of the adult insects we collected. Exploring the adaptations that allow so many insects to persist and remain active under these conditions would be a profitable avenue for future research.

Evidence that dissolved oxygen is potentially limiting in the hypopagea is suggested by the abrupt appearance of insects as holes are opened. Some of these animals were no doubt disturbed when the ice was cut, but we have observed beetles and mayflies (especially), actively swimming upward toward the surface in the ice hole. Nagell and Brittain (1977) reported finding a layer of slightly oxygenated water (0.8–1.7 mg/L) 5–10 cm below the ice in otherwise anoxic ponds in Norway, arising from intermittent meltwater seeping through minute cracks in the ice. Mayflies that are negatively phototactic in ice-free seasons may become positively phototactic in cold, hypoxic water (Brittain and Nagell 1981; Ward 1992) presumably to take advantage of the oxygenated sub-ice layer. Groundwater seepage may also relieve hypoxia near the pond edge. This distribution is consistent with the large number of mayflies observed on the lower ice surface, especially close to shore, in our ponds. Brittain and Nagell (1981) also reported that mayflies in these conditions were strongly attracted to air bubbles. It would be worth exploring whether oxygenation would prove an effective bait for insect traps beneath the ice. The success of the ice-hole trap may in part depend upon insects displaying a positive phototaxis, which would lead them to the influx of oxygen.

Aquatic insects are distributed very unevenly within and between ponds. There is clear evidence that the abundant caddisfly *Platycentropus radiatus* and to a lesser extent the two mayfly genera observed are strongly associated with aquatic vegetation, in particular old cattail stems.

It is tempting to suppose that the larvae were feeding on biofilm or algae on the ice (Frenette, Thibeault, Lapierre, and Hamilton 2008), but it is unlikely that sufficient light penetrated the snow-covered ice to support algal growth. Furthermore, if caddisflies were feeding on biofilm, larvae would be expected to be abundant

throughout the pond, rather than only among cattails. It is more likely, therefore, that caddisflies and other benthic organisms use the under-ice surface as a substratum or an oxygen refuge rather than a food source.

We found no correspondence between the distribution of insects among our ponds and any of the simple environmental parameters we measured. Water chemistry did not differ greatly among ponds, and all but two were fishless. We could not measure aquatic vegetation cover or composition, which may be important factors. Ponds connected to a drainage system may be more readily colonized, and suffer less hypoxia, but Frasers Mills pond, among others, which supported the greatest diversity of species and orders, has no surface inflow.

In addition to the numerous adult and larval insects collected, we also encountered high densities of planktonic *Chaoborus* Lichtenstein, 1800 in two ponds, especially Frasers Mills; these were not routinely caught presumably because they could fit through the mesh of the traps. *Chaoborus* is a generalist predator which feeds on a variety of cladocerans, copepods, rotifers, and large protists (Fedorenko 1975; Hare and Carter 1987). In the apparent absence of crustacean plankton in the oft-sampled Frasers Mills pond, the food source sustaining this dense population is an open question.

An unidentified cladoceran (possibly of the genus *Diaphanosoma* Fischer, 1850) was encountered in Lanark Pond. The cladoceran is of interest because populations of crustacean zooplankton are generally very low in winter (Agbeti and Smol 1995; Kalinowska and Grabowska 2016) and were not observed in any other pond.

Conclusions

This study showed the diversity of hypopagic insect activity is far greater than previously understood. This survey is just the underside of the iceberg in terms of comprehending this newly discovered subset of the freshwater ecosystem. More research is required to better understand the mechanism of attraction, the physiological coping mechanisms to ice cover, and the true environmental factors that influence hypopagic invertebrate diversity.

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Authors' contributions

RFL came up with the study, contributed to the design and critically revised the manuscript. He contributed to the field work and identified some Coleoptera and Hemiptera. He gave final approval for publication. BRT contributed to the design, came up with the *ice hole trap*,

and critically revised the manuscript. He contributed to the field work and identified the nymphs. YA and SC were the taxonomists who identified the vast majority of adult beetles and true bugs, respectively. LD, MD, and GM were the undergraduate students who did most of the field work, wrote the first draughts and reviewed the manuscript. All authors agree to be accountable for all aspects of the work.

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References

Agbeti, M.D., and Smol, J.R. (1995), 'Winter Limnology: A Comparison of Physical, Chemical and Biological Characteristics in Two Temperate Lakes During Ice Cover', *Hydrobiologia*, 304, 221–234.

Ashbee, H.V., Marshall, S.A., and Alarie, Y. (2017), 'Haliplidae of Eastern Canada', *Canadian Journal of Arthropod Identification*, 32, 80.

Ashton, G.D. (1989), 'Thin Ice Growth', *Water Resources Research*, 25, 564–566.

Bengtsson, B.E. (1981), 'The Growth of Some Ephemeropteran Nymphs During Winter In A North Swedish River', *Aquatic Insects*, 3, 199–208.

Brittain, J. E., and Nagell, B. (1981), 'Overwintering at Low Oxygen Concentrations In The Mayfly *Leptophlebia Vespertina*', *Oikos*, 36, 45–50.

Daborn, G.R., and Clifford, H.F. (1974), 'Physical and Chemical Features of an Aestival Pond in Western Canada', *Hydrobiologia*, 44, 43–59.

Danks, H.V. (1978), 'Modes of Seasonal Adaptations in the Insects: I. Winter Survival', *The Canadian Entomologist*, 110, 1167–1205.

Danks, H.V. (2008), 'Aquatic Insect Adaptations to Winter Cold and Ice', in Aquatic Insects: Challenges to Populations, pp. 1–19.

David, D.S., and Browne, S. (eds.). (1996), *Natural History of Nova Scotia.: Vol. 2. Theme Regions*, Halifax: Nimbus Publishing and Nova Scotia Museum. https://ojs.library.dal.ca/NSM/article/view/3775/3458.

Duffy, W.G., and Liston, C.R. (1985), 'Survival Following Exposure to Subzero Temperatures and Respiration in Cold Acclimatized Larvae of *Enallagma Boreale* (Odonata: Zygoptera)', *Freshwater Invertebrate Biology*, 4, 1–7.

Evans, A.V. (2014), *Beetles of Eastern North America*. Princeton: Princeton University Press.

Fedorenko, A.Y. (1975), 'Instar and Species-Specific Diets in Two Species of *Chaoborus*', *Limnology and Oceanography*, 20, 238–249.

Frenette, J.J., Thibeault, P., Lapierre, J.F., and Hamilton, P.B. (2008), 'Presence of Algae in Freshwater Ice Cover of Fluvial Lac Saint Pierre (St. Lawrence River, Canada)', *Journal of Phycology*, 44, 284–291.

Gordon, E.L. (1933), 'Notes on the Ephemerid Genus *Leptophlebia*', *Bulletin of the Brooklyn Entomological Society*, 8, 116–134.

Griffith, M.E. (1945), 'The Environment, Life History and Structure of the Water Boatman, *Ramphocorixa Acuminata* (Uhler) (Hemiptera, Corixidae)', *University of Kansas Science Bulletin*, 30, 241–365.

Gustafson, G.T., and Miller, K.B. (2015), 'The New World Whirligig Beetles of the Genus *Dineutus* Macleay, 1825 (Coleoptera, Gyrinidae, Gyrininae, Dineutini)', *ZooKeys*, 476, 1–135.

Hare, L., and Carter, J.C.H. (1987), 'Zooplankton Populations and the Diets of Three Chaoborus Species (Diptera, Chaoboridae) in a Tropical Lake', *Freshwater Biology*, 17, 275–290.
Hilsenhoff, W.L. (2017), 'Dytiscidae and Noteridae of Wisconsin (Coleoptera). II. Distribution, Habitat, Life Cycle, and Identification of Species of Dytiscinae', *The Great Lakes Entomologist*, 26, 35–53.
Holmen, M. (1987), *The aquatic Adephaga (Coleoptera) of Fennoscandia and Denmark*, New York, NY: Brill.
Hungerford, H.B. (1934), *The Genus* Notonecta *of the World* (Vol. 21), University of Kansas Science Bulletin, Lawrence, Kansas pp. 5–195.
Hungerford, H.B. (1948), *The Corixidae of the Western Hemisphere (Hemiptera)*, University of Kansas Science Bulletin, Lawrence, Kansas pp. 1–827.
Hussey, R. (1920), 'An American Species of Cymatia (Corixidae, Hemiptera)', *Bulletin of the Brooklyn Entomological Society*, 15, 80–83.
Hussey, R. (1921), 'Ecological Notes on *Cymatia Americana*', *Bulletin of the Brooklyn Entomological Society*, 16, 131–136.
Irons, J.G I., Miller, L.K., and Oswood, M.K. (1993), 'Ecological Adaptations of Aquatic Macroinvertebrates to Overwintering in Interior Alaska (U.S.A.) Subarctic Streams', *Canadian Journal of Zoology*, 71, 98–108.
Kalinowska, K., and Grabowska, M. (2016), 'Autotrophic and Heterotrophic Plankton under Ice in a Eutrophic Temperate Lake', *Hydrobiologia*, 777, 111–118.
Larson, D.J., Alarie, Y., and Roughley, R.E. (2000), *Predaceous diving beetles (Coleoptera: Dytiscidae) of the Nearctic Region, with emphasis on the fauna of Canada and Alaska*, Ottawa: National Research Council Research Press.
Lauff, R.F., MacDonnell, M., and Taylor, B.R. (2018), 'Chemical Light Sticks as Bait to Trap Predaceous Aquatic Insects: Effect of Light Colour', *Proceedings of the Nova Scotian Institute of Science*, 49, 235–252.
Lencioni, V. (2004), 'Survival Strategies of Freshwater Insects in Cold Environments', *Journal of Limnology*, 63, 45–55.
Merritt, R.W., Cummins, K.W., and Berg, M.B. (2008), *An Introduction to the Aquatic Insects of North America*, Dubuque, IO: Kendall/Hunt Publishing.
Mihalicz, J.E. (2015), 'An Observation of the Overwintering Aquatic Insects in a Prairie Pond in Saskatchewan, Canada', *University of Saskatchewan Undergraduate Research Journal*, 1, 99–106.
Moore, M.V., and Lee, R. E. (1991), 'Surviving the Big Chill: Overwintering Strategies of Aquatic and Terrestrial Insects', *American Entomologist*, 37, 111–118.
Nagell, B., and Brittain, J.E. (1977), 'Winter Anoxia – A General Feature of Ponds in Cold Temperate Regions', *Internationale Revue Der Gesamten Hydrobiologie*, 62, 821–824.
Nilsson, A.N., and Hájek, J. (2023), *A world catalogue of the family Dytiscidae, or the diving beetles (Coleoptera, Adephaga)*, Version 1.I.2023. Distributed as a PDF file via Internet. Available at http://www.waterbeetles.eu (accessed May 3, 2023).
Olsson, T.I. (1983), 'Seasonal Variation in the Lateral Distribution of Mayfly Nymphs in a Boreal River', *Ecography*, 6, 333–339.
Oswood, M.W., Miller, L.K., and Irons, J.G. (1991), 'Overwintering of freshwater benthic macroinvertebrates', in *Insects at Low Temperature*, eds. R.E. Lee Jr., and D.L. Denlinger, New York: Chapman and Hall.
Oygur, S., and Wolfe, G.W. (1991), 'Classification, Distribution, and Phylogeny of North American (North of Mexico) Species of *Gyrinus* Müller Coleoptera: Gyrinidae', *Bulletin of the American Museum of Natural History*, 207, 1–97.
Patenaude, T., Smith, A.C., and Fahrig, L. (2015), 'Disentangling the Effects of Wetland Cover and Urban Development on Quality of Remaining Wetlands', *Urban Ecosystems*, 18, 663–684.
Peckarsky, B.L., Fraissinet, P.R., Penton, M.A., and Conklin, D.J. (1990), *Freshwater Macroinvertebrates of Northeastern North America*, Ithaca, NY: Comstock Publishing Associates.

Sailer, R.I. (1952), 'Circumpolar Distribution of Water Boatmen (Hemiptera: Corixidae)', *The Canadian Entomologist*, 84, 280–280.
Sinclair, B.J. (2015), 'Linking Energetics and Overwintering in Temperate Insects', *Journal of Thermal Biology*, 54, 5–11.
Srayko, S.H., Mihalicz, J.E., Jardine, T.D., Phillips, I.D., and Chivers, D.P. (2023), 'Overwintering Capacity of Water Boatmen (Hemiptera: Corixidae) and Other Invertebrates Encased in the Ice of Shallow Prairie Wetlands', *Canadian Journal of Zoology*, 101, 434–447.
Vondel, B.J V. (2005), 'Haliplidae', in *World Catalogue of Insects*. eds. A.N. Nilsson, Stenstrup, Denmark: Apollo Books Aps.
Wallengren, H.D. (1894), 'Revision af Släktet *Corisa* Latr', *Beträffande Dess Skandinaviska Arter.', Entomologisk Tidskrift*, 15, 129–164.
Ward, J.V. (1992), *'Aquatic insect ecology. 1. Ecology and habitat'*, John Wiley and Sons, Inc. New York.
Wiggins, G.B. (2000), *Larvae of the North American Caddisfly Genera (Trichoptera)* (2nd ed.), Toronto: University of Toronto Press.