

Rapid Communication**First records of non-indigenous cyclopoid copepod *Thermocyclops crassus* (Fischer, 1853) in Eastern Canada**Linda M. Campbell¹, Kaylee MacLeod^{1,2}, Vincent Fugère³, Gilbert Cabana³, Lynne Witty⁴ and Olivier Morissette⁵¹Department of Environmental Science, Saint Mary's University, Halifax, Nova Scotia, Canada²Bluenose Coastal Action Foundation, Mahone Bay, Nova Scotia, Canada³Université du Québec à Trois-Rivières, Trois-Rivières, Québec, Canada⁴Identazoop, Capreol, Ontario, Canada⁵Département des sciences fondamentales, Université du Québec à Chicoutimi, Québec, CanadaCorresponding author: Olivier Morissette (omorisse@uqac.ca)

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Received: 2 May 2023**Accepted:** 4 September 2023**Published:** 29 November 2023**Handling editor:** Tatenda Dalu**Thematic editor:** Amy Fowler**Copyright:** © Campbell et al.This is an open access article distributed under terms of the Creative Commons Attribution License ([Attribution 4.0 International - CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)).**OPEN ACCESS****Abstract**

We report the first records for the Eurasian zooplankton species *Thermocyclops crassus* (Fischer, 1853) (Copepoda, Cyclopoida) from two freshwater sites in eastern Canada collected between 2020 and 2022. *Thermocyclops crassus* were found at high densities (between 8×10^3 and 2×10^5 adults/m³) in Lake St. Pierre on the St. Lawrence River, Québec, but at very low densities (between 0.3 and 0.7 adults/m³) in Wentzell's Lake on the LaHave River system, Nova Scotia. We discuss possible introduction pathways for both sites as well as the need for enhanced biosurveillance and increased public education in eastern Canada.

Key words: zooplankton, St. Lawrence River, Québec, LaHave River, Nova Scotia**Introduction**

The Eurasian *Thermocyclops crassus* (Fischer, 1853) (Copepoda, Cyclopoida) is a free-living, small (0.6–0.9-mm long) euryhaline cyclopoid copepod indigenous to warm brackish and freshwater ecosystems throughout Asia and parts of Europe (Maier 1989; Kobari and Ban 1998; Xiaoming 1999; Dahms et al. 2010; Chaicharoen et al. 2011; Bledzki and Rybak 2016; Verbitsky et al. 2018). The species is a thermophilic copepod with a preference for eutrophic habitats and thrives both in limnetic and pelagic zones of lakes, rivers and marshes, and densely vegetated habitats (Duchovnay et al. 1992).

Thermocyclops crassus has been reported as a non-indigenous species in Costa Rica in 1983 (Collado et al. 1984), northern Mexico in 1998 (Gutiérrez-Aguirre and Suárez-Morales 2000), eastern Ukraine in 2012 (Anufrieva and Shadrin 2016), and two lakes in eastern North America – Lake Champlain in 1991 (Duchovnay et al. 1992) and Lake Erie in 2014 (Connolly et al. 2017). Reports of *T. crassus* in North America remain scarce and likely underestimated, which could be linked to the few surveillance programs for zooplankton and the challenge to differentiate *T. crassus* from similar species congeners, such as the native species *Mesocyclops edax*. Here, we report

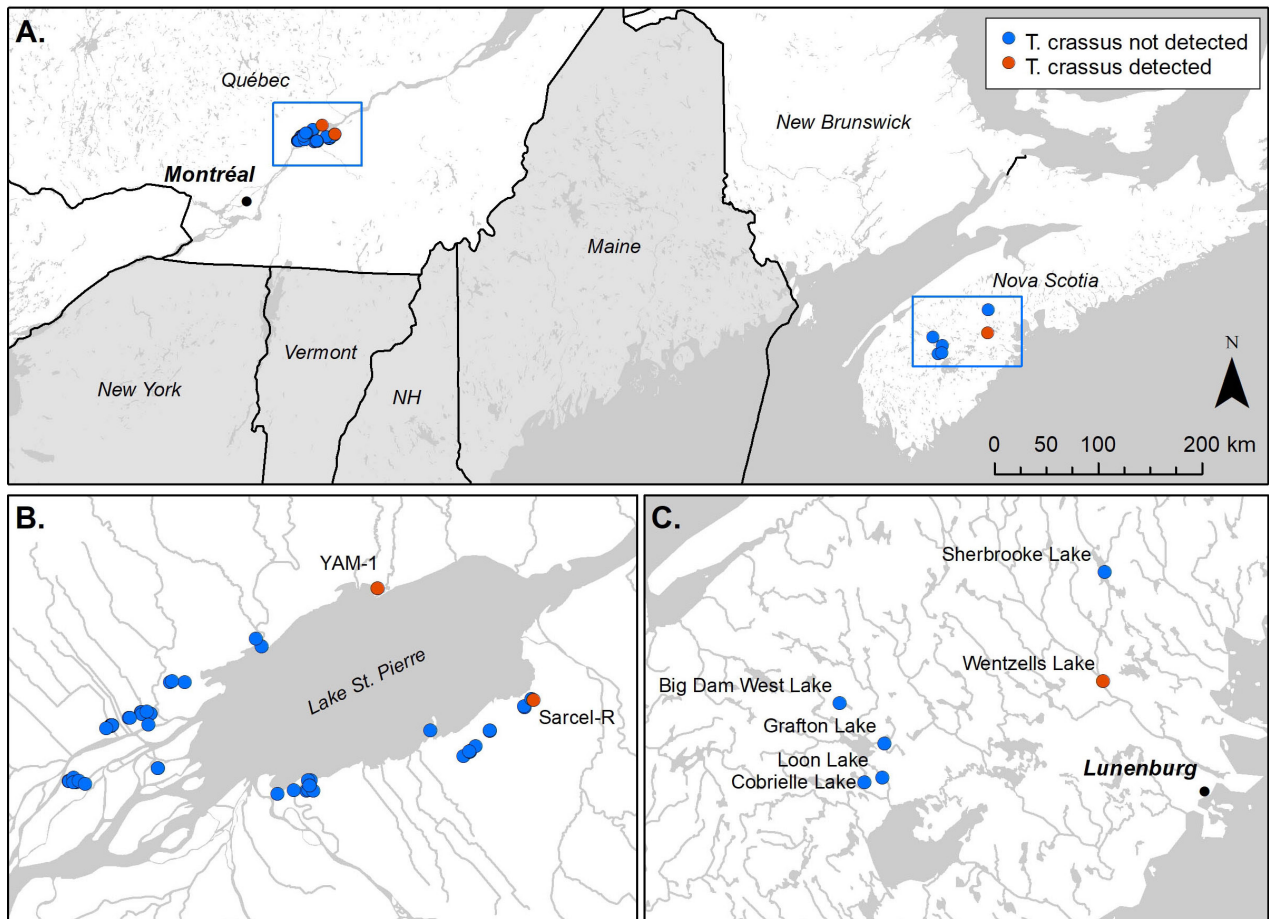


Figure 1. Location surveyed where *Thermocyclops crassus* were detected (red dots, GPS coordinates in Table 1) and not detected (blue dots) in eastern Canada (A.), with closer view on Lake St. Pierre (B.) and southeastern Nova Scotia (C.).

two new observations of *T. crassus*, which are now the two easternmost records of this species in North America. We also discuss the species' invasion history, its preferred habitats, and the probable introduction and secondary invasion pathways in Canada and elsewhere, and we comment on the gaps in aquatic invasive species surveillance for zooplankton and other plankton-size taxa in eastern Canada.

Materials and methods

Thermocyclops crassus was detected during two different projects led by our team of authors. In Québec, sampling was undertaken as part of a zooplankton survey in the Lake St. Pierre floodplain, the largest widening of the St. Lawrence River, roughly 800 km upstream of the St. Lawrence Gulf (Figure 1). Lake St. Pierre contains shallow and turbid freshwater non-tidal habitats and is a RAMSAR wetland of international importance (Ramsar Convention Secretariat 2023). The survey was part of a 3-year project examining zooplankton abundance and diversity during the low-water period (in 2020) and prolonged, significant flooding throughout the whole system (in 2022). In 2020, with only a short flooding period, two sites were sampled in the floodplain, where the water was held behind a levee; all other sites ($n = 6$) were situated in the littoral zone of the fluvial lake.

Table 1. Longitude and latitude coordinates and physiochemical parameters for sites where *Thermocyclops crassus* was collected in Lake St. Pierre (YAM1, Sarcel-1), Québec and in Wentzell’s Lake, Nova Scotia. Date of sampling, GPS coordinates (degree decimal, NAD83), *T. crassus* densities, air and surface-water temperature, dissolved oxygen (DO) and pH are shown. NA indicates no measurements collected.

Date	Location	GPS coordinates	<i>T. crassus</i> density (ind/m ³)	Air °C	Water °C	DO (% saturation)	DO (mg/L)	pH
25 May 2020	YAM1	46.26382, -72.81512	2600	26.2	NA	NA	NA	NA
11 June 2020	Sarcel-R	46.19252, -72.65260	2.20×10^5	20	17.98	20.9	1.88	7.27
14 July 2021	Wentzells Lake	44.47508, -64.62480	0.55	20	22	70.6	6.19	5.78
25 May 2022	YAM1	46.26382, -72.81512	500	NA	NA	NA	NA	NA
11 June 2022	Sarcel-R	46.19252, -72.65260	8.2×10^3	NA	NA	NA	NA	NA

In 2022, 31 floodplain sites and 6 main river sites were sampled in spring flood season. Zooplankton was collected by immersing carboys at depth of 60–100 cm, filtering 20 L of water through 65 µm mesh filters, and preserving sampled organisms in 10% buffered formalin. Site physiochemical parameters were measured using a YSI multiparameter probe (Table 1).

In Nova Scotia, mixed-species vertical zooplankton hauls were collected from six lakes across the province in July 2021 as part of a multi-lake food-web research project. Only one lake (Wentzell’s Lake; Figure 1) had *T. crassus* (MacLeod 2022), collected on midday of July 14, 2021. Wentzell’s Lake is a 4.5-ha freshwater lake in the LaHave River watershed in southeastern Nova Scotia (Coastal Action 2022). After measuring water quality parameters, a 30-cm diameter tow net with 243-µm mesh was deployed for 14 vertical hauls from a depth of 8 m (Table 1, Figure 1), and the pooled 14-haul sample was divided in 3 subsamples for stable isotope analyses, mercury analyses and zooplankton identification (10% buffered formalin).

Identification of zooplankton samples to species were performed by Lynne Witty (Figure 2). Specimens identified as *T. crassus* were differentiated from a similar native zooplankton species, *M. edax*, by the general smaller size of female *T. crassus* compared to *M. edax* (Reid and Williamson 2010), the lack of hairs on the inner margin of the caudal rami, the insertion of spines on the fifth legs and the presence of curled setae on the caudal rami (Reid 1989; Duchovnay et al. 1992).

Results and discussion

Between 2020 and 2022, *T. crassus* was detected in two distant locations (i.e., Québec and Nova Scotia, ~ 600 kms direct distance from each other), showing contrasting density, which could provide insight on the species’ invasion history, preferred habitats, and potential pathways of introduction. First, in Lake St. Pierre, *T. crassus* were found at high densities but at only two sites both in 2020 (low water year) and 2022 (flooding year) – in the Sarcel floodplain (Sarcel-R) and in the downstream River, Yamachiche (YAM-1). In Sarcel-R, 23 zooplankton taxa were recorded with *T. crassus* being the dominant species (61–63% of all adult copepods), with 2.20×10^5 adults/m³ in 2020 and 8.2×10^3 adults/m³ in 2022. In YAM-1, where 11 zooplankton taxa were found, cyclopoid copepods were the dominant taxon

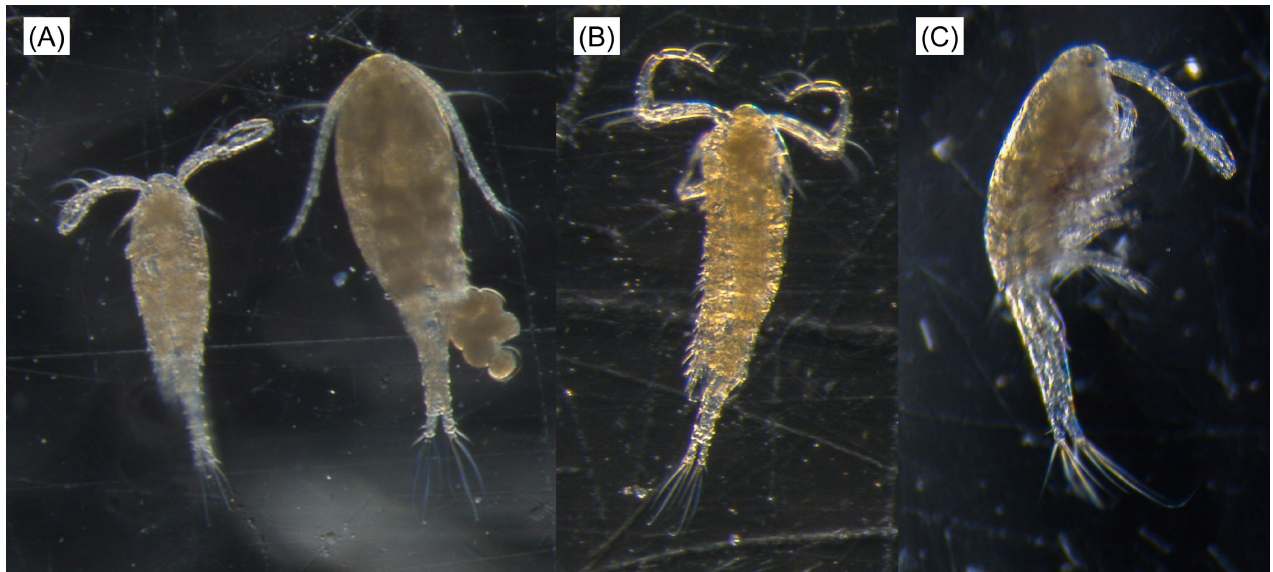


Figure 2. *Thermocyclops crassus* microphotographs. Lake St. Pierre, St. Lawrence River (2020), Québec specimens (A) male (left) and female (right) habitus in ventral view (B) Male habitus in ventral view (C) Wentzell's Lake, LaHave River, Nova Scotia specimen (2021) male in lateral view (Photos provided by Lynne Witty). All show males, apart from the large female with eggs on the right in Fig (A). Females generally are 0.8 to 1.0 mm while males are usually 0.6 to 0.7 mm.

with *T. crassus* found at densities of 2,600 adults/m³ in 2020 and 500 adults/m³ in 2022. Conversely, very low densities of *T. crassus* were found in the water column of Wentzell's Lake, Nova Scotia (Figure 2), the only Nova Scotian lake where the species was found (out of six sampled lakes). The Wentzell's Lake 14-haul set included 6 major zooplankton taxa, dominated by *Bosmina longirostris*. Only three *T. crassus* individuals were found, with resulting density of 0.367 and 0,734 individuals/m³. Those are among the lowest density reported for this species in North America to date.

In the early stages of the invasion process, abundance is generally correlated with time elapsed since invasion. Hence, the contrasting abundance of the species between the two regions may be indicative of invasion history and time since introduction. In other invaded regions, *T. crassus* density seems to increase with time. In Lake Erie, density changed from 1.3 individuals/m³ in 2014 to 35.5 individuals/m³ in 2016 (Connolly et al. 2017). Additionally, reports from introduced populations in China, Cambodia and Guinea indicate that *T. crassus* are consistently abundant and among the dominant zooplankton (~ 53 individuals/m³, 77% of captured copepods) taxon groups (Guiguemde et al. 1987; Xiaoming 1999; Chaicharoen et al. 2011). Therefore, *T. crassus* has the potential to become one of the dominant copepod species when established in new systems. The low abundance in Wentzell's Lake may suggest the invasion is very recent in this region given the lack of presence in samples from other Nova Scotia lakes. Interestingly, *T. crassus* density in Lake Champlain, Vermont, was estimated at 630 individuals/m³ at the time of the first detection in August 1991 (Duchovnay et al. 1992). The high abundance observed in Lake Champlain may be linked to late detection,

which is common for zooplankton species (Walsh et al. 2018), or possibly an especially productive habitat for *T. crassus* in Lake Champlain.

Environmental conditions may also be a predictor of *T. crassus* invasion success. Being a thermophilic species, *T. crassus* has a thermal optimum between 25 and 30 °C, with slow or no development under 20 °C, and does not produce eggs below 10 °C (Verbitsky et al. 2018). Water temperature for all sites with and without detected presences in the region are well within these limits, therefore temperature is likely not a variable to be considered here. On the other hand, the high density observed at the shallow, eutrophic Sarcel-R floodplain site is similar to densities reported in warm and shallow eutrophic lakes in France and Germany (Lacroix and Lescher-Moutoué 1984; Maier 1990). In contrast, we found low abundance in the mesotrophic (and deeper) Wentzell's Lake, which could be interpreted as *T. crassus* preferring shallow eutrophic waters. Interestingly, our results show that this species can be found in riverine and limnetic zones in substantial numbers, which is concordant with the known biology of the species (Duchovnay et al. 1992). Based on this observation, we suggest that floodplains could be sites of important production of *T. crassus*, which could then lead to a large export of adults, copepodites, and nauplii for the successful establishment of the species downstream, in the main river channel, at the end of the flooding period (Simberloff 2009). As no systematic sampling was performed for *T. crassus*, we cannot confirm if downstream unaided dispersal could indeed have influenced longitudinal variation in abundance and occurrence. Hence, *T. crassus* preference for warm and eutrophic systems call for higher vigilance (and surveillance program) in southern Canada, especially in anthropized habitats that are likely introduction pathways via small crafts and international shipping.

As our two study regions are very different habitats, the presence of *T. crassus* may also illustrate two different introduction or dispersal mechanisms at play (in addition to the abiotic and biotic differences in depth and productivity). Lake St. Pierre is part of the St. Lawrence River, a major shipping route between the Atlantic Ocean and the Great Lakes. As a result, natural passive dispersal or ship-mediated transport from near invaded lakes sharing hydraulic connections (e.g., Erie and Champlain) or international ballast water exchange sites are very likely (Duggan et al. 2005). Ships frequently use fresh or brackish water as ballast from their originating port for transoceanic voyages. The incoming transoceanic ships are required to exchange their ballast water for oceanic water to remove as many non-indigenous species as possible before entering the St. Lawrence Gulf. Currently, ballast water exchanges (BWE) of large ships can only be done in designated offshore zones, with replacement of water to a salinity of 30 parts per thousand (Department of Fisheries and Oceans 2013). Studies have shown that despite offshore BWE regulations, many large ships remain potential vectors of small organisms

such as dinoflagellates, zooplankton and phytoplankton (Roy et al. 2012; Department of Fisheries and Oceans 2013), which may present a constant risk for marine and freshwater habitats along the St. Lawrence River.

In contrast, Wentzell's Lake is a part of the LaHave River watershed which flows to the Atlantic Ocean, near Lunenburg, on the east coast of Nova Scotia. This area, unlike Lake St. Pierre, is not directly connected to transoceanic ships exchanging ballast water in the designated BWE Areas (Figure 2) in Atlantic Canada (Transport Canada 2019). This points to another potential introduction pathway for Nova Scotia: transport by small crafts and recreational boating activity. Overland transport of boats between freshwater ecosystems is a well-known vector of planktonic species introduction, e.g. for *Dreissena* mussel larvae (Johnson and Padilla 1996; Johnson et al. 2001; Kelly et al. 2013). Wentzell's Lake is a very popular sport fishing site frequented by recreational fishers (and their small crafts) from across northeast North America. Professional and amateur sports fishing tournaments with multiple stops on a scheduled circuit, especially those involving boats with fish wells, have been associated with the distribution of non-indigenous species (Kerr et al. 2005). Additionally, there are many locally organized sport fishing competitions in and around the LaHave watershed region which have become "destination events" for anglers and visitors outside of the region (Schramm Jr. et al. 1991; McNeill 1995; Kerr and Kamke 2003).

Once established, *T. crassus* may expand its distribution in both regions either by unaided dispersal or other introduction pathways, but another pathway may need consideration for future management. As *T. crassus* is an euryhaline species, small coastal and marine crafts entering and re-entering multiple coastal freshwater sites could also be vectors for aquatic species with similarly broad salinity tolerance ranges. Lunenburg, on the South Shore of Nova Scotia, Canada, is a significant UNESCO-recognized active marine port with long history of sailing and coastal fishery industries, and as such is a popular marine tourist destination while supporting coastal traffic (UNESCO World Heritage Centre 2022). Hence, small crafts from larger oceanic yachts, marine ships, and from other coastal locations entering freshwater sites are a common occurrence and may represent a potential pathway for further introduction of *T. crassus* in other coastal freshwater sites outside of Lunenburg. For instance, Lunenburg is only 37 kms from Wentzell's Lake. It is notable that small marine craft have already been identified as a significant transport mechanism for a number of coastal marine species, such as the clubbed tunicate and green crab, between sites along most of the eastern Canada's coastline (Darbyson et al. 2009).

Bird-mediated and wind-mediated transport of planktonic species (Havel and Shurin 2004; Green and Figuerola 2005; Pinceel et al. 2016) is another pathway which merits consideration. However, those mechanisms are likely insignificant (Jenkins and Underwood 1998; Reynolds et al. 2015) compared to small-craft and BWE-mediated transportation. Those are likely a tertiary

mode of dispersal after a planktonic species is already well established throughout the region (Havel and Shurin 2004). The lack of dedicated surveillance programs for zooplanktonic invasive species could exacerbate their dispersal, as unknown presence hinders the implementation of prevention measures. The emergence of participative science initiatives (Marchessaux et al. 2023), the AI-driven automated identification of zooplankton (Sosa-Trejo et al. 2023) and the lowering cost of metabarcoding approaches (Mychek-Londer et al. 2019) are all opportunities to implement better surveillance on exotic and invasive planktonic species.

Conclusion

In conclusion, we herein report the first documented cases of *T. crassus* in eastern Canada and suggest that there is a very real possibility of multiple undetected presence of *T. crassus* (and other non-indigenous zooplankton species) throughout North America. Large shipping transport routes through BWE sites, overland transport of small crafts between leisure and fishing locations and coastal small-craft traffic are all considered potential pathways for this non-indigenous zooplankton species to spread across eastern North America. The high density in Lake St. Pierre and the presence of the species in two sites over 1,000 km apart within a single year indicates a strong need to extend surveillance programs to assess the possible presence of *T. crassus*, as well as other non-indigenous planktonic species (such as *Dreissena* spp., including quagga and zebra mussel larvae), in the Great Lakes Basin and throughout eastern regions of Canada. Actions to further prevent the dispersal of existing non-indigenous species as well as minimize the introductions of new species are also urgently needed. The establishment of public outreach programs (e.g., Canadian Council on Invasive Species 2020) throughout eastern Canada, plus investment in regional resources (e.g. Ministère des Forêts, de la Faune et des Parcs 2022; NSISC 2022) for monitoring of planktonic and invertebrate species across eastern Canada are necessary.

Author's contribution

All authors participated to the writing of the initial manuscript. KM sampled and processed zooplankton from Wentzell's Lake, with significant field logistical support from Bluenose Coastal Action Foundation. LMC conceptualized and coordinated the multi-lake project. The Lake St. Pierre study was coordinated by GC, who also did field work and zooplankton processing. All zooplankton samples were identified by LW, who also connected all co-authors for this manuscript and verified zooplankton density values reported here. LMC prepared and edited this article, KM prepared the figures and tables, GC prepared the Lake St. Pierre paragraphs, and OM and VF edited the manuscript. OM conducted the revision and publication process.

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Statement of conflict of interest

There are no conflicts of interest identified with funders or the study.

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