

Research Article

The first documented occurrence and life history characteristics of the Chinese mystery snail, *Cipangopaludina chinensis* (Gray, 1834) (Mollusca: Viviparidae), in Alberta, Canada

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Abstract

The Chinese mystery snail *Cipangopaludina chinensis* (Gray, 1834), a species native to Asia, is documented for the first time in Alberta, Canada, in McGregor Lake Reservoir in 2019. Here, we describe the initial finding of *C. chinensis* in Alberta, Canada, and biological information that may aid management efforts. Collected specimens were confirmed as *C. chinensis* through DNA barcoding. Analysis of growth rate, fecundity, and infection by digenean trematodes was assessed. It is unknown how *C. chinensis* arrived in Alberta. However, this species' ability to withstand environmental stressors, such as desiccation, facilitates overland and long-distance transport via recreationists or deliberate release of *C. chinensis* into waterbodies. Snails collected from McGregor Lake Reservoir matched with GenBank results for *C. chinensis* from Korea. Analysis of digenean trematodes revealed that the population in McGregor Lake are not infected, as there were no cercariae present after 24 hours. Growth assessment over a period of 60 weeks revealed that shell length growth quickly outpaces growth in shell width. Upon emergence, *C. chinensis* are larger than many native snail species. The expansion of *C. chinensis* into Alberta poses potential negative consequences, such as decreased native snail biomass, increased nitrogen to phosphorus ratios, and additive impacts when paired with other invasive species.

Key words: aquatic invasive species, introduced species, non-native species, *Bellamya chinensis*, ecological integrity, eDNA, digenean trematodes

Introduction

The Chinese mystery snail, *Cipangopaludina chinensis* is a viviparid mollusc native to Asia (Jokinen 1982; Solomon et al. 2010). *Cipangopaludina chinensis* has two subspecies, *C. c. chinensis* (Gray, 1834) and *C. c. laeta* (Martens, 1860). Here, we focus on *C. chinensis* clade introduced into North America through San Francisco, California, in the 1890s (Kingsbury et al. 2021). *C. chinensis* are well established in the United States, with the largest populations in the upper midwest and northeastern states (McAlpine et al. 2016; Kipp et al. 2014). In Canada, *C. chinensis* are established in

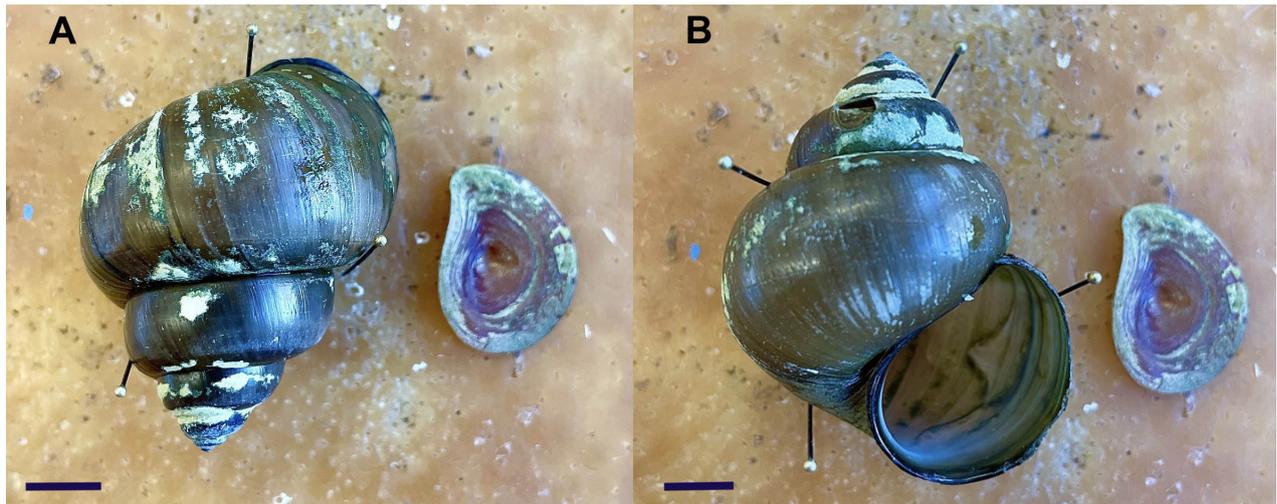


Figure 1. Shell of an individual *C. chinensis* from McGregor Lake Reservoir, Alberta, Canada. A. Abapertural view. B. Apertural view. Scale bar = 1 cm. Photo by Megan R. Edgar.

southern Ontario, including Lake Erie, western Lake Ontario, the Kawartha Lakes, and Crowe, Moira and Trent River drainages (Therriault and Kott 2002), Québec (Clarke 1981; Tornimbeni et al. 2014), British Columbia (Clarke 1981), and Atlantic Canada (Nova Scotia, New Brunswick Prince Edward Island, Newfoundland (McAlpine et al. 2016; Kingsbury et al. 2021)).

Cipangopaludina chinensis were first reported in 2019 in Alberta, Canada, from McGregor Lake Reservoir, near the Village of Milo. Date of introduction, population history, and extent of dispersal from the first introduction at the site are unknown. Before discovery, *C. chinensis* were listed under the *Fisheries Act of Alberta* as a prohibited species, making it illegal to possess, release, sell or transport the species within the province (Alberta Environment and Parks 2019). Further, federal legislation under *The Fisheries Act, Aquatic Invasive Species Regulations* (Government of Canada 2015) prohibits the possession and sale of *C. chinensis* in Alberta. Despite these prohibitions, *C. chinensis* are sold as a food item in Asian food markets and in the aquarium and pet trade across North America (Wyman-Grothem et al. 2018). Human dispersal of *C. chinensis* and seeding of water bodies are likely important in spreading this species in North America.

Research suggests that *C. chinensis* are challenging to eradicate once established. Difficulty in eradication is a direct consequence of the operculum, which protects the species internal viscera from desiccation (Figure 1). *Cipangopaludina chinensis* may survive out of water for up to nine weeks, and chemical means of population control, including traditional molluscicides such as rotenone and copper sulphate, are apparently ineffective (Haak et al. 2014; Unstad et al. 2013). According to Unstad et al. (2013), *C. chinensis* cannot be managed via culling methods or drawdowns. Burnett et al. (2018) found *C. chinensis* exhibits an upper thermal tolerance of 45 °C but they did not determine a lower lethal temperature limit, thus

further supporting its probable overwintering ability in regions with colder winter temperatures like Alberta. Within their indigenous range *C. chinensis* serves as a host for several helminth parasites that affect humans, including human intestinal fluke (Chung and Jung 1999). Conceivably, parasites that may negatively impact non-native ecosystems can be introduced from *C. chinensis* (Harried et al. 2015).

This paper reports our initial research and monitoring efforts on the occurrence and implications of *C. chinensis* in Alberta. Monitoring and tracking the occurrence and spread of non-native *C. chinensis* in Canada is key to mitigating potential ecological and economic harm (Lodge and Shrader-Frechette 2003). Research and monitoring often focus on charismatic and game species (Thomsen et al. 2014; Tensen 2018) such as sportfish or the organisms that impact them (i.e. whirling disease; Mayhood 2000; Bartholomew et al. 2005). This may allow less conspicuous organisms, such as benthic invertebrates, to spread unnoticed until they become problematic in an ecosystem.

Materials and methods

Cipangopaludina chinensis were first observed by one of the authors (RL) in McGregor Lake Reservoir, Alberta, in 2019. McGregor Lake Reservoir (50.5696°N; 112.8828°W; WGS84) is an off-stream storage reservoir built in 1920 next to the Village of Milo and situated approximately 30 kilometers east of the town of Vulcan in southern Alberta, Canada (Mitchell et al. 1990) (Figure 2). McGregor Lake Reservoir is part of the Oldman River drainage basin, although most water input is via diversion from the Bow River (Mitchell et al. 1990). Immediately downstream to the south and then south-east of McGregor Lake Reservoir are Travers and Little Bow Lake Reservoirs, respectively (Figure 2). The three reservoirs are part of the Carseland-Bow River Headworks system. Travers and Little Bow Reservoirs do not currently have any reports of *C. chinensis*.

In October 2019 and August 2020, visual shoreline surveys were conducted. *C. chinensis* were surveyed for at nine different locations around McGregor Lake Reservoir in 2019, resulting in the collection of twenty-six live snails (Supplementary material Table S1; Figure 1). In 2020, searches at McGregor Lake, Travers Lake, and Little Bow Reservoir were surveyed more intensively to determine the extent of *C. chinensis* occurrence, utilizing an Eckman dredge and kick samples to collect benthic samples. An additional 58 live snails were collected. Species was first identified morphologically following a key created by Lu et al. (2014). Broad similarities to other viviparid species, notably the Japanese mystery snail, *C. japonica* (von Martens, 1861), prompted molecular investigation, the preferred method for distinguishing the species (Kingsbury et al. 2021). The collected snails were further used to assess growth rate, fecundity, and digenean trematode infection.

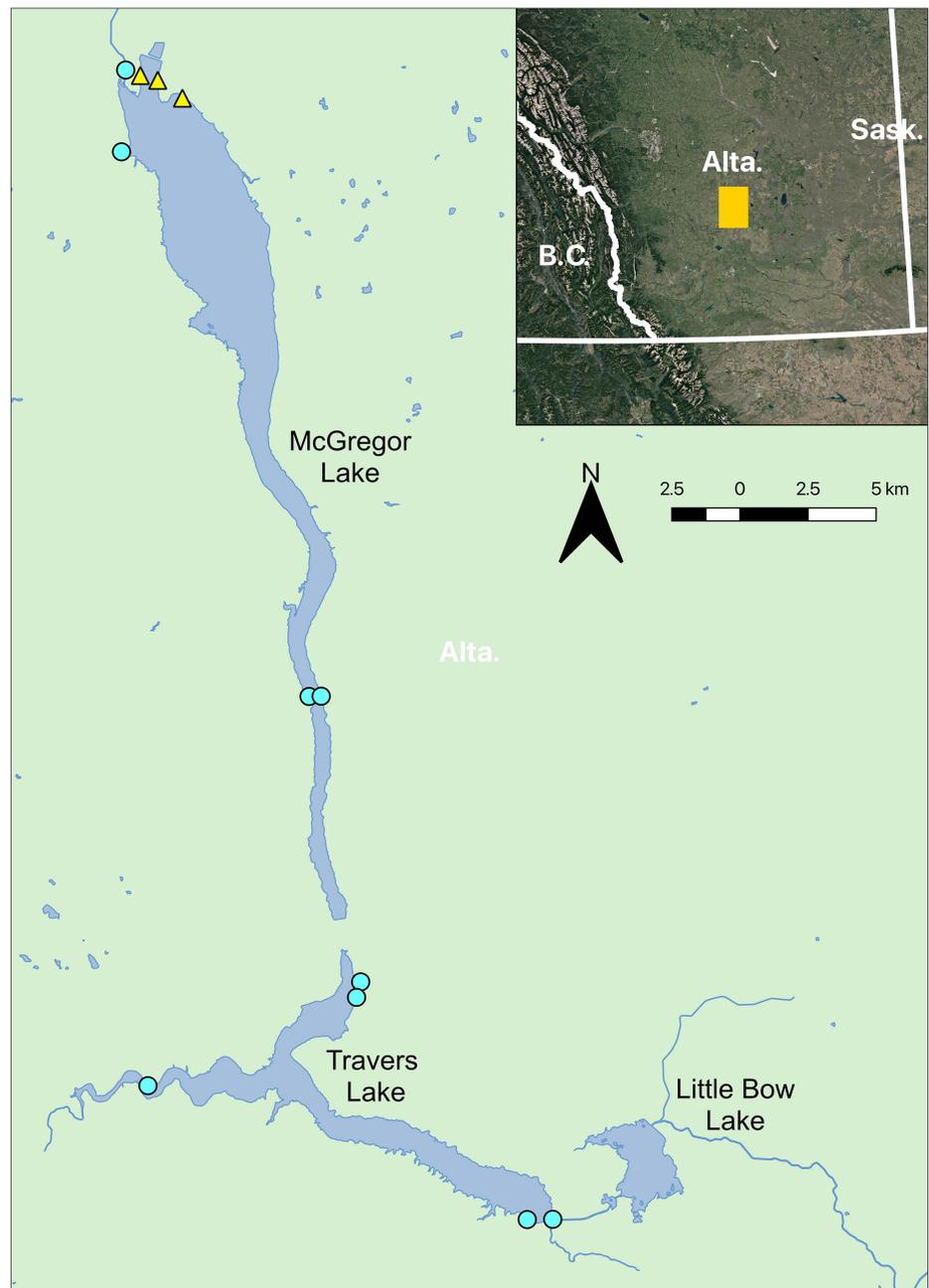


Figure 2. Location of sites surveyed for Chinese mystery snail in McGregor Lake and Travers Lake, Alberta, Canada. The inset map shows the survey area relative to British Columbia (B.C.), Saskatchewan (Sask.) and the United States of America boundary. Yellow triangles = *C. chinensis* detected; blue circles = *C. chinensis* not detected..

DNA barcoding

Genomic DNA from tentacle clips taken from three individual snails was extracted using the DNeasy Blood and Tissue kit (Qiagen, USA). A ~ 710 fragment of cytochrome *c* oxidase 1 (CO1) was amplified using the forward and reverse primer pairs published by Folmer et al. (1994): (HCO2198 and LCO 1490). Endpoint Polymerase Chain Reaction (PCR) was carried out in a 50 µl reaction mixture using the following cycling parameters: 92 °C for 2 min, 92 °C for 40 s, 51 °C for 1 min, 68 °C for 1 min and 68 °C for 7 min. Amplicons were visualized on 1% agarose gels, and bands at the expected

size were extracted and purified using the QIAquick Gel Extraction Kit (Qiagen, USA). Sanger sequencing using the HCO2198 and LCO1490 primers was carried out on the purified amplicons (Macrogen Inc, South Korea). Sequences were input into Geneious version 11.0.6 (Kearse et al. 2012; <http://www.geneious.com>), trimmed, aligned, and the resulting consensus sequences were queried against the BLASTn database (Zhang et al. 2000).

Phylogenetic analysis

All CO1 sequences were checked for quality by viewing chromatograms and quality scores in 4peaks (Nucleobytes) software. Primer regions were trimmed and transferred to Geneious Prime 2019 (<https://www.geneious.com>) to align the forward and reverse sequences. The three 659 base pair consensus sequences were identical to each other. The representative sequence (GenBank # OK147091) was then compared using BLASTn against the NCBI GenBank database. GenBank submissions representing *C. chinensis*, or closely related species were selected for subsequent phylogenetic analysis. When possible, submissions for which voucher specimens had been submitted were used. Geneious Prime was used to create nucleotide alignments for the selected sequences. Alignments were trimmed to the shortest sequence length (which was the 530 nucleotide outgroup CO1 sequence) prior to analysis. Bayesian inference (BI) reconstructions were generated using the Mr. Bayes plug-in (Ronquist and Huelsenbeck 2003) in Geneious Prime with a burn-in of 100,000, a chain length of 1,000,000, and sub-sampling frequency of 200. Maximum-likelihood (ML) analyses were run in the PhyML plug-in (Guindon et al. 2010) for separate genus-level analysis. The settings used were: 200 bootstraps, proportion of invariable sites was fixed at 0, the number of substitution rate categories was 4, the gamma distribution parameter was set to estimated and “topology/length/rate” was selected to be optimized. GTR + invgamma was the best-supported nucleotide substitution model available in the MrBayes plug-in in Geneious for BI analyses. The CO1 sequence for *Viviparus contectus* isolate 1Z33 (MK517422.1) was used as an outgroup for the phylogenetic tree.

Assessment of fecundity and growth rate

Snails collected in 2019 were assessed for the presence of offspring. Those *C. chinensis* with offspring (n = 6) were separated into individual aquaria in the lab and furnished with 10 cm of autoclaved substrate collected from Lake McGregor. Snails without offspring were dissected and sexed based on structures describe by Simone (2011) (16 males; 4 females). After offspring emerged, the adult female snails were euthanized and dissected to confirm that all offspring had emerged. Juvenile snails were kept in the same aquaria and individually marked using coloured enamel paint before

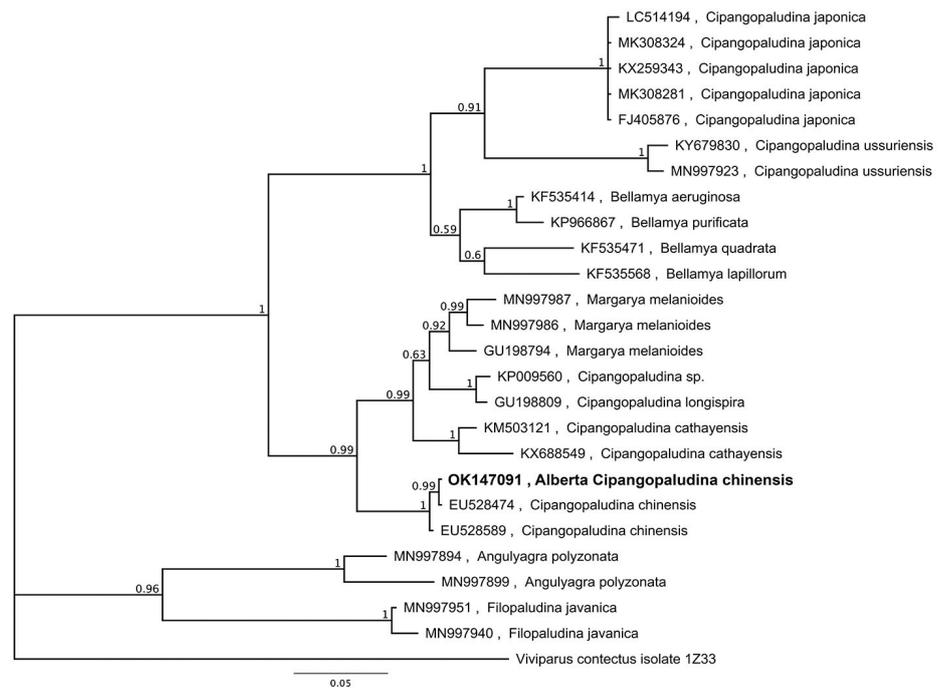


Figure 3. Maximum Likelihood (ML) tree of COI barcodes showing phylogenetic position of *Cipangopaludina chinensis* collected from McGregor Lake Reservoir in southern Alberta, Canada.

measuring shell width and length. Juvenile snails were fed algae pellets (Hikari, USA) weekly and kept at 17 °C in a room with a 12-hour day/night cycle. Shell morphometrics were collected weekly until all snails had died (60 weeks).

Assessment for infection by digenean trematodes

The 84 snails from 2019 and 2020 were isolated individually in containers with artificial spring water at a depth of ~ 3 cm for 24 hours in a 12 hour day-night cycle (Gordy and Hanington 2019), the water was then assessed to see if any trematode cercariae had emerged from the snails, using a Zeiss V16 stereomicroscope.

Results

Visual shoreline surveys in October 2019 confirmed the presence of *C. chinensis* at McGregor Lake at the north end of the Reservoir (Table S1). *Cipangopaludina chinensis* shells found on beaches south of the boat launch ranged from 5 to 50 mm in length. No *C. chinensis* were found in Travers Lake Reservoir (Figure 1; Table S2).

All three snails for which CO1 sequences were obtained returned a 100% nucleotide identity to GenBank submission # MN997925.1, identified as a *Cipangopaludina chinensis* specimen (voucher ZMB 192694) from Korea. The Alberta *C. chinensis* (OK147091) formed a monophyletic group with other *C. chinensis* (Figure 3), confirming the results of the BLASTn analysis.

Six female *C. chinensis* yielded offspring that were assessed for growth over 60 weeks. A total of 116 juvenile snails emerged from the females. The

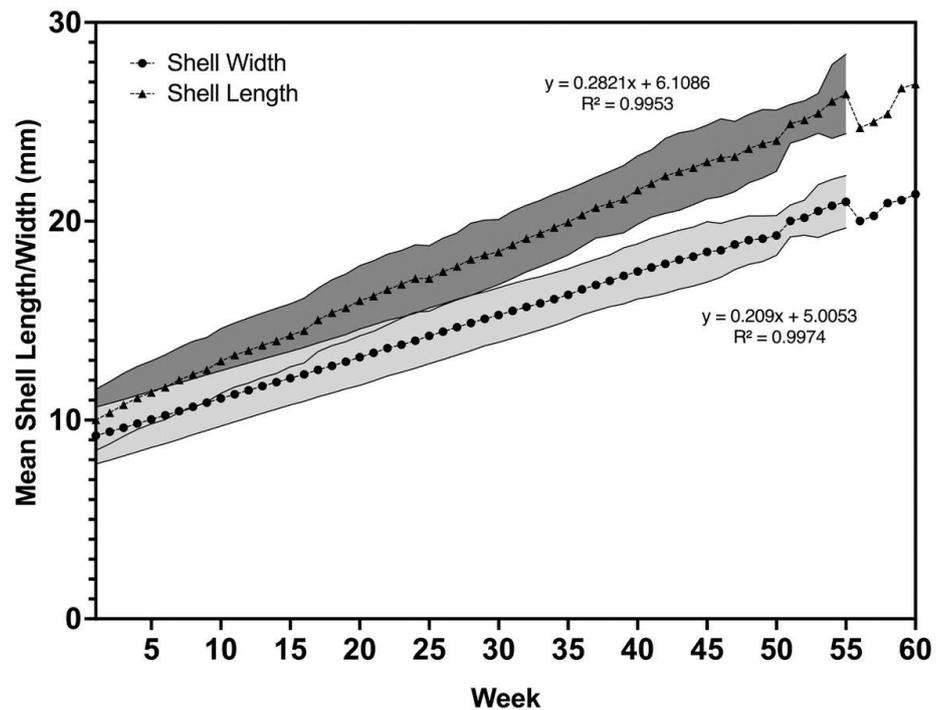


Figure 4. The growth of juvenile *Cipangopaludina chinensis* from McGregor Lake Reservoir, Alberta, Canada. Shell length (dark grey) rapidly outpaces an increase in shell width (light grey) over the first 60 weeks of life. Starting population size of this study was 116 juvenile snails originating from six females. No snails survived to week 60. The shaded areas represent the standard deviation.

mean number of juveniles was 19.3, with 25, 23, 23, 19, 13 and 13 individual juveniles emerging from each female (Table S2). Upon emergence, mean shell width was 5.13 ± 1.45 mm and mean shell length was 5.9 ± 1.6 mm. The width:length ratio on the day of emergence was 0.87 ± 0.02 ($n = 116$). Weekly measurement of shell width and length over a 60-week period indicates that shell length growth (slope of 0.2821) outpaces growth in shell width (slope of 0.209) with a width:length ratio of 0.76 ($n = 1$) measured on week 60 (Figure 4).

None of the 84 snails assessed for digenean trematode infection produced any cercariae after 24 hours.

Discussion

We confirm the presence of non-native *C. chinensis* in Alberta, Canada, notably at the north end of McGregor Lake Reservoir, where the boat launch recreation areas are located (Figure 2). We suggest that at McGregor Lake Reservoir *C. chinensis* has met criteria for four of the five stages of species invasion: arrival, establishment, growth, and reproduction (Lockwood et al. 2007). As of yet there is no evidence to confirm the fifth stage, dispersal, of *C. chinensis* from McGregor Lake Reservoir to other waterbodies in Alberta. The two closest waterbodies, Little Bow and Travers Lake Reservoirs, have been surveyed but to date found to be negative for *C. chinensis*. Should *C. chinensis* disperse to these adjacent water bodies, continuous monitoring at these sites will be required for early detection.

DNA barcoding of CO1 suggests that the snails invading McGregor Lake Reservoir are *C. chinensis*. This conclusion is supported by phylogenetic analysis that groups the Alberta CO1 sequences, which are identical to each other, in a monophyletic group with other *C. chinensis*. Our phylogenetic analysis closely resembles recently constructed phylogenies for *C. chinensis* (David and Cote 2019).

Our study determines that populations of *C. chinensis* in Alberta grow allometrically; shell width to shell height ratio decreases as the shell increases in size, from juvenile to adult life stages, as also reported from studies by Jokinen (1982). Growth assessment of *C. chinensis* affirms that juvenile *C. chinensis* emerge larger than some adult native species in Alberta (Clifford 1991), and that adult *C. chinensis* are larger than any native freshwater gastropod species (Liu et al. 1995; Kingsbury 2021). The large size of *C. chinensis*, when compared to native snails, may facilitate their avoidance of predators in Alberta waterbodies. A study by Johnson et al. (2009) saw that when non-native *C. chinensis* co-occurs with native snails, crayfish attack the native snails at a higher rate than *C. chinensis*, as a result of their larger size and thicker shell, exacerbating predation on native gastropods. Already, *C. chinensis* is documented to have facilitative interactions with invasive species in their introduced regions (Haak 2015). This suggests that *C. chinensis* may interact with Northern crayfish (*Faxonius virilis*), a crayfish that has recently expanded its native range from the Beaver River drainage of Alberta (Williams et al. 2011). Further, a behavioural study by Sura and Mahon (2011) determined that the presence of crayfish, as detected by chemical cues, causes native snails to decrease their feeding rates relative to the absence of crayfish; a costly behavioural change, as this affects metabolism, growth, and reproduction. A report from 2013 determined that 74% of freshwater gastropods are presently suffering declines (Johnson et al. 2013). Where *C. chinensis* is introduced, there is a potential that native snails will have much lower feeding rates, especially where crayfish are present (Sura and Mahon 2011), presenting a facilitative interaction between two non-natives (Ricciardi 2001).

Annual fecundity on average is between 25 and 30 juveniles per female (Stephen et al. 2013), with specimens from Alberta producing offspring numbers somewhat below this at 19.3 juveniles per female. However, previous research has shown that even one gravid female is sufficient to found a population, with *C. chinensis* producing young every year until death (Stephen et al. 2013). Female *C. chinensis* also grow larger than male conspecifics (Jokinen 1982), and because of predator aversion due to their larger size, and these characteristics could facilitate reproduction and survival in Alberta, and potential spread.

We did not detect any *C. chinensis* from McGregor Lake infected with larval digenean trematodes. Parasites detected in *C. chinensis* in North America include *Cyathocotyle bushiensis*, a parasite known to cause mortality

in dabbling ducks (Hoeve and Scott 1988), and *Aspidogaster conchicola*, a parasite known to infect fish, freshwater bivalves and turtles (Harried et al. 2015; Kingsbury 2021). According to Karatayez et al. (2012), parasite prevalence in *C. chinensis* is much lower than in native snail species. However, continual monitoring for parasites on *C. chinensis* is needed as warmer water temperature could result in an increase in the prevalence of parasites. Previous research has shown that metacercarial infections were found in snails in Lake Wabuman, Alberta, where water was kept warm year-round due to thermal effluents, at temperatures between 5 and 24 °C (Sankurathri and Holmes 1976).

Based on our findings, survey efforts focussed on water depths of 1–4 meters may be the most effective for detecting snail presence (Table S1). Extra consideration should be focussed on areas with artificial substrates, riprap, submerged vegetation (Chaine 2012), and where there are stagnant waters (Perron and Probert 1973). Vigilance should be taken near the northern end of McGregor Lake Reservoir, where the boat launch and recreation area are located, as those location types are speculated to be critical factors for habitat suitability (Solomon et al. 2010). In addition to potential impacts on native snails species, other documented and potential impacts of introduced *C. chinensis* in invaded water bodies include: increasing nitrogen to phosphorus ratios of surface water (Johnson et al. 2009); additive impacts to ecosystems when paired with other invasive species (Johnson et al. 2009; Olden et al. 2009); and potential food web effects (Sura and Mahon 2011; Kingsbury et al. 2020). Without management efforts that involve enforcing legislation now in place, public education, and continued monitoring, the range of the *C. chinensis* in Canada is only likely to expand.

Conclusions

Continued monitoring to track dispersal of *C. chinensis* in Alberta, Canada, is recommended. Further studies to document *C. chinensis* impact on aquatic systems in Canada and facilitate invasive species management are required. In Alberta, watershed managers should monitor Travers and Little Bow Lake Reservoirs adjacent to the McGregor Lake Reservoir, where *C. chinensis* is now established.

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Authors' contribution

MRE worked on investigation and data collection, result interpretation, writing, reviewing and editing. PCH worked on investigation and data collection, data analysis and interpretation, writing, reviewing and editing. RL was part of sample collection and initial identification of species. HP and MSP worked on reviewing and editing the manuscript. RZ worked on field survey, sample collection, and initial review and writing of the manuscript. NK helped with funding provisions, field survey, and sample collection.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Visual shoreline surveys for *C. chinensis* at seven locations on McGregor Lake and two at Travers Reservoir, conducted by AEP and ASERT in October 2019.

Table S2. Measurement data collected during dissections of *C. chinensis*, collected in 2020.

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