Predicting the distribution of the Chinese mystery snail, *Cipangopaludina chinensis*, a potentially invasive, non-indigenous species, in Atlantic Canada

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The Chinese Mystery Snail Project: predicting potential distribution of the Chinese mystery snail, *Cipangopaludina chinensis*, a potentially invasive, non-indigenous species, in Atlantic Canada

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ABSTRACT

The Chinese mystery snail, *Cipangopaludina chinensis*, a freshwater mollusc indigenous to Eastern Asia, introduced to North America and Europe. Currently, little is known about *C. chinensis* in North America. My thesis objectives were to: (1) synthesize relevant literature and confirm whether *C. chinensis* should be considered invasive in North America, (2) determine the known species occurrence in continental North America highlighting reporting gaps, and (3) create a species distribution model for *C. chinensis* in the Maritimes. The literature review indicated that *C. chinensis* should be considered invasive in North America. The largest number of reported occurrences were in southern Ontario and northern US along the Great Lakes with the lowest in the Maritimes, the Prairies, Quebec, and near Lake Superior. Finally, a random forest model was developed to predict *C. chinensis* distribution in Nova Scotia, with highest probable occurrence in the Halifax-area, the New Brunswick-Nova Scotia border, and Cape Breton.

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Special Note: This thesis is manuscript-based. Chapter 1 was submitted to *Environmental Reviews*. Chapter 2 has already been submitted for publication in *Aquatic Invasions.* Appendix B will be submitted for publication as part of Fraser et al. (in-prep). These manuscripts have yet to be accepted. Therefore, this thesis is still considered original work and does not require additional permissions to replicate. Also, note that the introduction for this thesis may be shorter than expected as Chapter 1 is essentially a detailed introduction to this species within North America.

To Alexandre, Amelia, and Benjamin Kingsbury-

Thank-you for always supporting curiosity.

TABLE OF CONTENT

Introduction:

The Chinese mystery snail, *Cipangopaludina chinensis*, is indigenous to eastern Asia and first appeared in North America in the 1890s in San Francisco, sold as an edible mollusc (McAlpine et al. 2016). Since its arrival, *C. chinensis* has spread across the Unite States of America (US), Canada, and now is being reported in Belgium and the Netherlands (Collas et al. 2017; Kipp et al. 2020; McAlpine et al. 2016). This species of mollusc is a freshwater, deciduous snail (Jokinen 1982). *C. chinensis* is very resilient, likely due to biological plasticity and the presence of an operculum which can seal the snail within its shell until adverse environment conditions have passed. Traditional invasive species management techniques such as chemical treatments and desiccation do not effectively manage this species (Burnett et al. 2018; Haak et al. 2014; Havel 2011; Unstad et al. 2013). One gravid female *C. chinensis* is able to establish a new population once introduced to an uninvaded waterbody. Female *C. chinensis* reproduce frequently, fecundity estimates range from approximately 27 offspring/female/year to 100 offspring/female/year (Jokinen 1982; Haak 2015; Stephen et al. 2013). As part of Vivipardae, *C. chinensis* gives birth to full-formed, live offspring (i.e. juvenile *C. chinensis* are born live with fully formed shells, including an operculum). Previous studies suggest that *C. chinensis* presence impacts chlorophyll-α concentrations, nitrogen and phosphorus speciation and concentrations, and downstream eutrophication (Bobeldyk 2009; Chen et al. 2012; Olden et al. 2013). Additionally, *C. chinensis* may shift food webs by altering the bacterial community composition (Olden et al. 2013), increasing inter-mollusc competition (due to multiple feeding techniques *C. chinensis* is in direct competition for food with indigenous snails and mussels), and offering indigenous predators with a novel prey source (Twardochleb & Olden 2016). In totality, *C. chinensis* has multiple negative environmental impacts on invaded ecosystems and socioeconomic consequences as this species fouls beaches, clogs water pipes, and blocks irrigation water intake screens. Therefore, understanding where *C. chinensis* is distributed in the Maritimes (New Brunswick, Nova Scotia, and Prince Edward Island) is essential for determining the management strategies needed to control the population, identifying area of greatness concern for future/continued spreading, and predicting habitat overlap with vulnerable species such as Species at Risk (SAR).

For this thesis, I have used random forest modeling to predict the distribution of *C. chinensis* in the Maritimes. Random forest models (RFM) are an assemblage of decision trees which randomly sub-sample training data, and randomly sub-sample and arrange model parameters to build many classification trees (Breiman 2001; Danisko & Hoffman 2018). Each tree "votes" on whether a background data point belongs to a category (in our case 0 for no *Cipangopaludina chinensis* presence or 1 for presence). The votes are counted to determine if the forest predicts *C. chinensis* presence or no-presence. The resulting probabilities are the percentage of trees that voted for presence, with higher probability indicating higher likelihood of *C. chinensis* presence. In this study, *C. chinensis* presence means likelihood of real *C. chinensis* presence or high probability of suitable habitat that could lead to successful *C. chinensis* establishment if the species were introduced.

The RFM variable importance plots, and specifically the decreased gini impurity plots, indicate the relative importance of each parameter used in the model creation. Decreased gini impurity is the ranking of parameters where parameters listed with larger mean decreased gini are more important for inclusion in the model formula. If a parameter with a high mean decreased gini were to be removed from the model formula then the probability of the decision trees in the forest to incorrectly classify a data point would increase (Koehrsen 2018). However, the mean decreased gini only ranks the parameters that were included in the model. An assumption is made that all model parameters represent diverse background data and/or are ecologically relevant data for the species in question. Researchers wishing to create RFM must have a thorough understanding of the datasets they intend to use and the biology of the species under study. For *C. chinensis* it is difficult to have a thorough understanding of the species because the information currently available is spread out over multiple forums (i.e. internet sites, peer-reviewed articles, grey literature which includes theses and government reports), multiple different languages, and multiple different taxonomic names. Therefore, the first step in the modeling process is to gather, analysis, and summarise all the relevant information on *C. chinensis* in a literature review. Then models can be constructed using predictive parameters that have been identified as important for this species. Also, a synthesis of available information on *C. chinensis* will provide a better understanding of what to expect once *C. chinensis* becomes established in a new water body and which indigenous species will be most impacted by *C. chinensis* presence.

Due to uncertainty the proper taxonomic naming of *C. chinensis*, the multiple different languages that literature is published in, and the multiple forms of information available (i.e. peer-reviewed journal articles, grey literature, reputable internet sources) it is challenging to fully understand the impacts that *C. chinensis* has on invaded ecosystems. Therefore, a summary of all these different sources was needed to understand if *C. chinensis* is invasive in North America. Likewise, the extent of the distribution of *C. chinensis* is currently unknown, hence the creation of a Canadian occurrences database is needed to identify areas with high *C. chinensis* establishment. The information gathered through reviewing literature on *C. chinensis* and collecting occurrence data was used to generate a species distribution model of *C. chinensis* potential distribution in the Maritimes, which was used to identify potential habitat overlap with SAR. More details about background information of *C. chinensis* can be found in Chapter 1 of this thesis and the predicted species distribution model is contained in Chapter 2.

The result objectives for this thesis were to (1) synthesis the relevant literature of *C. chinensis* and species occurrence reports of *C. chinensis* occurrences in continental North America to determine whether this species should be considered invasive and (2) to develop a species distribution model to identify areas in the Maritimes that are risk of *C. chinensis* invasion and to indicate possible habitat overlap between predicted *C. chinensis* distribution and SAR.

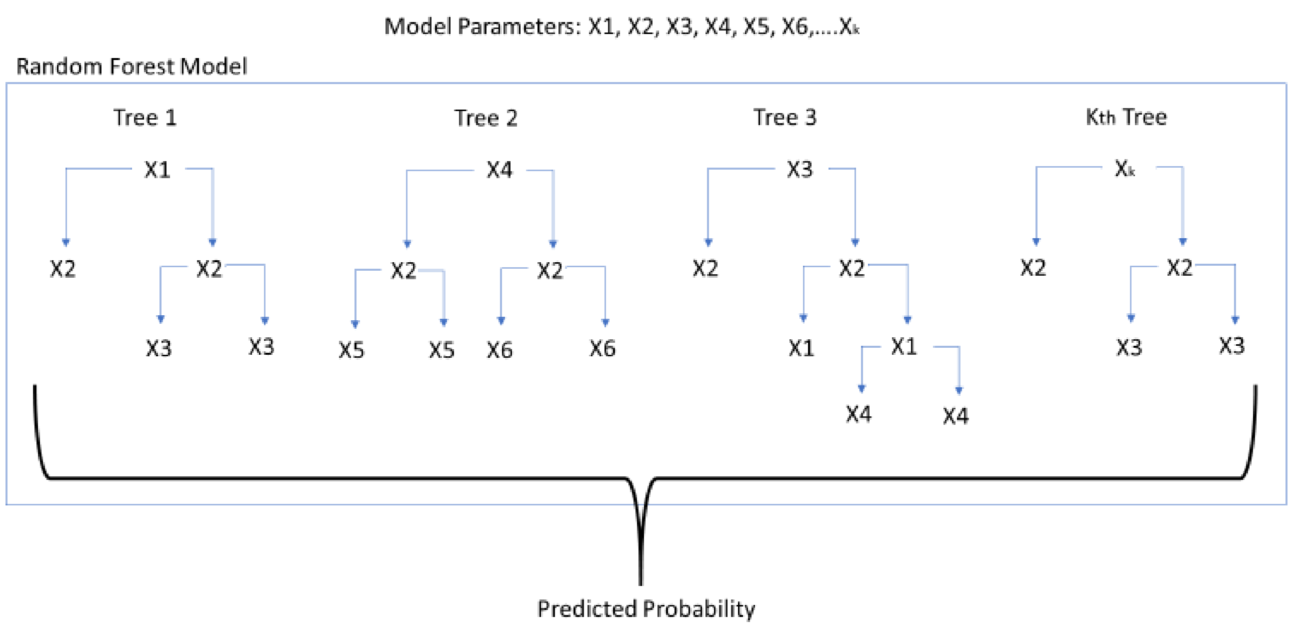

Figure 1: In random forest models (RFM), the available model parameters (K) are sub-sampled to create each tree (Kth) splits. Additionally, the dataset is sub-sampled and run through each tree. Each tree has a vote (0=no *Cipangopaludina chinensis* presence, 1=*C. chinensis* presence). The RFM tallies the votes to determine the percentage of trees that voted “yes” (i.e. 1). The error rates of each tree are compared to determine which trees were more correct with their classification of the training data. The trees with more accurate predictions are weighted with high relevance and the parameter order is taken into consideration. The decrease gini impurity is

created from this comparison of tree correctness. Trees that performed better have a better organization of parameters. Therefore, parameters placed most often at the top of these trees are more highly ranked for decrease gini impurity.

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CHAPTER 1: A review of the non-indigenous Chinese Mystery Snail, *Cipangopaludina chinensis* (Viviparidae), in North America, with emphasis on occurrence in Canada and the potential impact on indigenous aquatic species

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Abstract

Evidence suggests that the Chinese mystery snail, *Cipangopaludina chinensis*, a freshwater, dioecious, snail of Asian origin has become invasive in North America, Belgium, and the Netherlands. Invasive species threaten indigenous biodiversity and have socioeconomic consequences where invasive. The aim of this review is to synthesize the relevant literature pertaining to *C. chinensis* in Canada. In doing so, we (1) describe *C. chinensis* ecosystem interactions in both indigenous and non-indigenous habitats, (2) identify gaps in the literature, and (3) determine where the species potential distribution in North America requires further exploration. We also briefly discuss potential management strategies where this species may be identified as an aquatic invasive species (AIS) in Canada. Due to the much larger relative size of adult *C. chinensis*, multiple feeding mechanisms, and resistance to predation, *C. chinensis* can out-compete and displace indigenous freshwater gastropods and other molluscs. Furthermore, *C. chinensis* can affect food webs through bottom-up interactions with the bacterial and zooplankton communities by changing nitrogen and phosphorous concentrations. Also, the Chinese literature indicates the potential for *C. chinensis* to biotransfer contaminants between polluted ecosystems and consumers. In its indigenous range, *C. chinensis* has been identified as a host for numerous parasites harmful to human and animal consumers alike. A comparison of the Canadian geographical distribution of reported occurrences with that for the United States indicates several potential gaps in Canadian reporting which merits further investigation and consideration, especially in regard to federal and provincial non-indigenous monitoring and regulations. Southern Ontario had the highest number of reports which were mostly from web-based photo-supported sources. This suggests that interactive citizen science through popular apps backed by well-supported educational campaigns may be a highly effective means of tracking *C. chinensis* spread, which can be complementary to traditional methods using specimen-vouchered taxonomically-verified natural-history collections overseen by professional curators.

Key Words: *Bellamya chinensis,* Vivipardae, non-native/non-indigenous species, ecosystem impacts

Introduction

The Chinese Mystery Snail (CMS), *Cipangopaludina chinensis*, is a dioecious, freshwater, potentially invasive, viviparous mollusc introduced to North America around 1890 for consumption in the Asian food market (Stephen et al. 2013). The species' indigenous range is poorly defined but is believed to include Burma (Myanmar), Thailand, South Vietnam, China, Korea, Japan, the Philippines, and the Island of Java (Kohler & Jinghua 2001; Figure 1). Fecundity studies indicate *C. chinensis* produces shelled, fully-formed young at an estimated rate of 27-33 young per female per year (Stephen et al. 2013), although estimates of up to 100 young per female per year have been suggested (Haak 2015). The species feeds on zooplankton and phytoplankton (Indiana Department of Natural Resources 2005), either by grazing using a radula, or by filtering water (Olden et al. 2013). *Cipangopaludina chinensis* excrete large amounts of fecal matter which can lead to potential re-engineering of nitrogen and phosphorous cycling within aquatic ecosystems (Olden et al. 2013). The ability of *C. chinensis* to resist predation, out-compete indigenous mollusc species for resources, reproduce rapidly, and shift food-webs, suggests that *C. chinensis* may be an aquatic invasive species.

In Canada, the Department of Fisheries and Oceans (DFO), Canadian Food Inspection Agency, Canada Border Services Agency, Environment and Climate Change Canada, Transport Canada, and the Department of National Defense, in collaboration with every province and territory, haves a mandate to monitor and regulate invasive species in fresh and marine waters (Fisheries and Oceans Canada 2018). DFO defines aquatic invasive species (AIS) as: "*Fish, animal, and plant species that have been introduced into a new aquatic ecosystem and are having harmful consequences for the natural resources in the native aquatic ecosystem and/or the human use of the resource*" (Fisheries and Oceans Canada 2018). AIS threaten indigenous biodiversity and natural ecosystems, have significant financial consequences, and can become difficult to manage once established. Globally, invasive species are second only to habitat destruction as a cause of species endangerment and extinction (Pejchar and Mooney 2009). Many AIS threaten natural biodiversity through predation, parasitism, competition, and degradation or destruction of invaded ecosystems (Fisheries and Oceans Canada 2019). AIS may carry viruses, bacteria, and parasites and typically entail significant economic costs due to disruption of ecosystem services, impacts on aquaculture and fisheries, and damage to infrastructure (Fisheries and Oceans Canada 2019; Pejchar & Mooney 2009). In both Canada and United States, *C. chinensis* is not yet federally prioritized as a species needing close management and is labelled in both countries as “non-indigenous” versus “invasive” which may lead to further confusion on the species’ status (Fisheries and Oceans Canada 2019; Kipp et al. 2020). Many US states recognize the species as invasive and have taken steps to prevent sale and distribution of *C. chinensis.* In the USA, *C. chinensis* is federally categorized as a non-indigenous aquatic species, although the United States Geological Survey (USGS) currently offers a disclaimer that the listing information for the species is preliminary (Kipp et al. 2020). In Canada, under federal Aquatic Invasive Species Regulations, only Manitoba (where to date *C. chinensis* has not been recorded) seeks to prohibit the possession, transportation, and release of *C. chinensis*, and no provincial jurisdiction currently prohibits the importation of *C. chinensis* (see Aquatic Invasive Species Regulations, Part 2, available at http://laws-lois.justice.gc.ca/eng/regulations/SOR-2015-121/Fulltext.html; accessed 5 June 2020)

Cipangopaludina chinensis is reported to be widespread in freshwater systems across the USA, Canada, and Europe (Clarke 1981; Collas et al. 2017; McAlpine et al. 2016; Jokinen 1982; Kipp et al. 2020; Stephen 2017; Van den Neucker et al. 2017). McAlpine et al. (2016) reported established populations of *C. chinensis* in Canada in southern Ontario, British Columbia, Quebec, New Brunswick, Newfoundland, and Nova Scotia. In the USA, *C. chinensis* has been reported from 32 states (Jokinen 1982; Kipp et al. 2020). The main vectors for the spread of *C. chinensis* in North America are human activities including boating, aquarium releases, and the addition of *C. chinensis* to garden ponds (Haak 2015; Havel 2011; Indiana Department of Natural Resources 2005; Harried et al. 2015; Solomon et al. 2010). Unlike the USA, Canada has not created a national database for *C. chinensis*, and there is no risk assessment or predictive species distribution model for *C. chinensis* in Canada. Therefore, the true extent of this species distribution in Canada is unknown. Furthermore, the current literature dealing with the impact of *C. chinensis* on indigenous freshwater species, habitat preferences, and basic biological information, is sometimes contradictory (Haak 2015; Olden et al. 2013; Solomon et al. 2010; Stephen et al. 2013; Sura & Mahon 2011).

Policy makers need to consider AIS effects on each invaded ecosystem in totality when developing management strategies. However, this is difficult to accomplish when information on individual AIS is spread across multiple forums (i.e. peer-reviewed publications, grey literature, on-line and off-line databases, expert knowledge), and political jurisdictions. Literature reviews are therefore increasingly necessary to condense available knowledge, create conceptual frameworks of species impacts on invaded ecosystems, identify potential risk factors that could be important for future species risk assessments, and identify gaps in knowledge (Matthews et al. 2017).

The aim of this review is to synthesize relevant literature dealing with the Chinese mystery snail so as to (1) describe *C. chinensis* ecosystem interactions in both indigenous and non-indigenous habitats, (2) identify gaps in the literature, and (3) determine where the species potential distribution in North America requires further exploration. We also briefly discuss potential management strategies for this species, as an aquatic invasive species (AIS), in Canada.

Methods

Literature Review

Internet searches for published papers written in English, French, or translated to English (often from Chinese) and in Chinese, were conducted using Web of Science, Science Direct, PubMed, Google, Google Scholar, and China National Knowledge Infrastructure (CNKI, 中国知网). Reputable internet sources were also examined to determine the extent of information directed at efforts to enlist the public in limiting the species introduction to North American water bodies. References cited within all literature were reviewed, additional papers of interest were noted, and if relevant, included. Additionally, any works which cited these papers were reviewed for relevance.

The key words used for the searches were "Chinese mystery snail," "*Cipangopaludina chinensis*," "*Bellamya chinensis*," "*Cipangopaludina leucostoma," "Cipangopaludina diminuta," "Cipangopaluina wingatei," "Paludina malleata," "Paludina chinensis," "Vivipare chiniose," "Cipangopaludina chinensis malleata," "Cipangopaludina malleata," "Viviparus chinensis," "Viviparus malleata,"* and "mystery snail." The literature searches in Chinese were conducted using those commonly used terms for *Cipangopaludina chinensis*: "田螺科", "中华圆田螺", "中华圆田螺+天敌", "中华圆田螺+饲料" and "中华圆田螺".

For *C. chinensis,* condensing knowledge from older literature can be problematic because the taxonomy of the Chinese mystery snail has been contentious, with various scientific names applied to the species over the past few decades. Regardless of the name used in each reference, we follow ITIS conventions here and consistently use “Chinese mystery snail” and “*Cipangopaludina chinensis*” (ITIS Taxonomic Serial No. 70329) in this review. It is noted that *C. chinensis* and *Bellamya chinensis* are the more commonly used terms in modern English-language literature (Haak 2015; Haak et al. 2017; Johnson et al. 2009; Olden et al. 2013; Prezant et al. 2006; Smith 2000; Solomon et al. 2010; Stephen et al. 2013; Twardochleb & Olden 2016; Waltz 2008), while *Viviparis chinensis*, *Paludina chinensis*, and *Paludina malleata* reflect an older taxonomy before *C. chinensis* and *Cipangopaludina japonica* (see below) were considered separate species (Jokinens, 1982). *Vivpare chiniose* and *V. malleata* were used in French-language literature for *C. chinensis* (Forets, Faune et Parcs Québec 2018, Stańczykowska et al. 1971). The names *C. wingatei*, *C. leucostoma*, and *C. diminuta* are synonyms in Asian literature for *C. chinensis* (Lu *et al.* 2014). Other synonyms used for C. *chinensis* also have included *Cipangopaludina chinensis malleata* (a sub-species), *Cipangopaludina malleata*, and *Viviparus chinensis* (Kipp et al. 2020, Lu et al. 2014).

Taxonomic confusion between *C. japonica* (ITIS Taxonomic Serial No. 70332) and *C. chinesis* has also led to uncertainty in the North American literature with respect to both species. *Cipangopaludina japonica,* Japanese mystery snail, is another large, non-indigenous, viviparid (also established in North America and potentially invasive; citation needed). Older literature has suggested this species is conspecific with *C. chinensis*, each representing different ecophenotypes of the same species (Jokinen 1982; Kipp et al. 2020). Adding to the confusion, *C. japonica* also has an array of various taxonomic names (e.g., Kipp et al. 2020; = *Viviparus japonicus*). However, morphologic and genetic differences between the two species have been reported, and here we follow current consensus as per the Integrated Taxonomic Information System (ITIS, http://www.itis.gov/) and treat both species as distinct (Chiu et al. 2002; Clench &

Fuller 1965; David & Cote 2019; Hirano et al. 2015; Lu et al. 2014; Smith 2000; Wang et al. 2017).

While much of the older literature (pre-1990s) may contain information relating to one or both species (Jokinen 1982; Smith 2000), we have avoided literature that deals explicitly with *C. japonica*, and included literature pertaining to *C. japonica* only where genetic or morphologic distinction between the two is discussed (Burks et al. 2016; David & Cote 2019; Fox 2007; Hirano et al. 2015). Conversely, across eastern Asia, there are 16 species of Viviparidae, all relatively large. Hirano et al. (2015) identified 3 species of viviparids in Japan, Chiu et al. (2002) identified 2 in Taiwan, and Lu et al. (2014) identified 11 species and 2 sub-species within the genus *Cipangopaludina* in China. We also have avoided inclusion of those indigenous viviparids in our review, keeping the focus on *C. chinensis* in China where it is highly abundant, with sufficient scientific literature on its indigenous habitats and ecological interactions.

Our list of references includes peer-reviewed literature, grey literature (e.g. reports, theses), and reputable internet sources. For this review, the term "publication" includes both peer-reviewed and grey literature sources. Together, these publications comprise the literature assessing *C. chinensis* effects on indigenous species in North America, the species establishment and spread within North America, and the history and biology of the species in both indigenous and non-indigenous habitats. In regard to terminology, we use "non-indigenous" and "indigenous" as recommended by Colautti and MacIsaac (2004). Publications dealing with *C. chinensis* as non-indigenous species in Europe and as indigenous species in Asia were used to supplement the limited knowledge currently available for North America. Publications were identified as belonging to one of eight categories (Figure 2) including (category names noted in *italics* and are listed in Supplementary Table 1): *Ecology* (ecological studies)*; Reviews* (literature reviews)*; Management* (species management)*; Ecosystem Impacts* (disruption of systems by non-indigenous CMS); *Distribution* (both modelled output and absence/presence studies); *Commercial Use* (use of CMS for industrial use and for profit)*; Medical Use* (use of CMS in medical studies and applications); and *Biology* (anatomy, taxonomy, life cycle, morphology, and genetic studies). Reputable internet sources were included in this review to determine the extent and types of information readily available to the public, but these sources were separated from publications because no formal research methodologies were followed or reported. Internet sources were considered "reputable" if they were managed by a group of researchers studying aquatic invasive species, individual researchers, universities, or government organizations.

Conceptual diagrams

To predict and compare *C. chinensis* ecosystem interactions, conceptual diagrams presented here draw on literature dealing with *C. chinensis* across both its non-indigenous (Figure 3A) and indigenous (Figure 3B) ecosystems using literature from the *Ecosystem Impacts*, *Ecology*, and *Biology* categories, as well as suspected vectors of transport as a non-indigenous species in North America. The term "conceptual diagram" was selected because these diagrams provide a visual summary of the main conclusions derived from literature pertaining to *C. chinensis* biological needs, ecosystem impacts, and human interactions. Those were created with two goals; (1) to determine if *C. chinensis* meets Canadian federal government criteria as a non-indigenous "invasive" species, and (2) to identify ecosystem interactions (either positive or negative) that may occur in North America should *C. chinensis* populations become as widely distributed or as abundant as the species is across its indigenous range.

Geographical distribution of C. chinensis in Canada

We contacted multiple Canadian sources to gain access to and build a database of *C. chinensis* reported occurrences in Canada. An effort was made to include all Canadian reports of

C. chinensis occurrence, regardless of year reported (i.e. both historical and more recent reports). Natural history collections databases from across Canada were accessed for records of *C. chinensis*. The following are the natural history museums we contacted (and whether they had CMS reports): Royal British Columbia Museum (yes), Royal Alberta Museum (no), University of Saskatchewan Museum of Natural Sciences (no), Manitoba Museum (no), the Canadian Nature Museum (yes), the Royal Ontario Museum (yes), Ontario Natural Heritage Information Centre (yes), Quebec Biodome (no), New Brunswick Museum (yes), Nova Scotia Museum of Natural History (yes), and the Provincial Museum of Newfoundland and Labrador (yes). Additional reports were shared with us from the British Columbia Conservation Data Centre, Alberta Department of Environment and Parks, Environment and Climate Change Canada; Wildlife Systems Research, Fragile Heritage (Ontario), the Maritime Aboriginal People's Council (Nova Scotia, New Brunswick & Prince Edward Island), and our own unpublished records (Kingsbury et al. in-review). The popular online citizen-science natural-history social network, iNaturalist and Early Detection & Distribution Mapping System (EDDMapS) Ontario, which also includes an on-line app for smartphones, was also searched for confirmed observations of *C. chinensis*. All reports in our geographical distribution maps were verified from specimen vouchers, photographs, or from sources regarded as reliable, and geo-tags (latitude and longitude) confirmed. The geo-tagged report occurrences in Canada were compiled as a data layer in ArcGIS and compared with the United States *C. chinensis* data layer maintained by the USGS (https://nas.er.usgs.gov/viewer/omap.aspx?SpeciesID=1044). In keeping with our focus on Canada and continental North America, we also checked Mexican reports for *C. chinensis*. We did not find any *verified* species reports or reported presences (to date) in Mexican waterbodies. *C. chinensis* is not on the United Mexican States invasive species list or the Mexico Invasive Alien Species Action Plan (Mexico Secretariat of the Convention of Biological Diversity, n.d.).

Results & Discussion

Literature Review

This review includes 123 records across in 93 publications (Figure 2) which did include English-, French- and Chinese-language sources and thirty reputable internet sources (Supplementary Materials: Table1). Literature reporting *C. chinensis* occurrences for Canada was all published prior to 2001-2000s (Clarke 1981; Clench & Fuller 1965; Jokinen 1982; Smith 2000; Stańczykowska et al. 1971), except an article reporting the species for Atlantic Canada (McAlpine et al. 2016). The categories *Ecology*, *Biology*, *Medical Use*, and *Distribution* included two thirds of the publications available with 26, 18, 13, and 13 papers, respectively (Figure 2). Publications reporting the *Ecosystem Impacts* of *C. chinensis* on indigenous species (8), species management-*Management* (6), and *Commercial Use* (7) were relatively few.

The least common publication category was *Reviews* (2 publications), which includes a grey-literature review (Waltz 2008), and the second, while peer-reviewed, was written prior to the publication of the majority of the North American literature on *C. chinensis* (Jokinen 1982). Both reviews have been widely cited in a number of grey-literature documents, including federal government documents and theses (Bobeldyk 2009; Haak 2015; Rivera & Peters 2008), government and expert-run websites (Kipp et al. 2020), and peer-reviewed journal articles (Collas et al. 2017; Collas et al. 2017; Matthews et al. 2017; Rothlisberger et al. 2010).

The 30 reputable internet sources found were from the USA (17), Canada (10), Australia (1), and international organizations such as the International Union for Conservation of Nature and the Global Invasive Species Database (2) (see Supplementary Materials, Table 2 for a full list of internet links). Much of the on-line information available to the public appears to be reputable (i.e. written by a scientific specialist) and is presented on government supported websites. These websites offer concise biological information, scientific and common names, descriptions of indigenous and non-indigenous range, and likely vectors of dispersal for *C. chinensis* in North America. Generally, internet sources tended to focus on the biology of the snail, often including notes on its indigenous range, and typically targeted recreational boaters in an effort to enlist the public in limiting the species introduction to water bodies where *C. chinensis* is non-indigenous and potentially invasive.

Literature from both indigenous and non-indigenous ecosystems indicate similar biology and ecological characteristics in all systems. A single *C. chinensis* female carrying embryos may rapidly establish a population in freshwater ecosystems. Female *C. chinensis* give birth to live, shelled, young that are between 3-5 mm in shell diameter. Mature females can produce numerous offspring per brood (Fox 2007). Females reach sexual maturity at 6-12 months (Bobeldyk 2009; Zhang et al. 2017) and studies in North America estimate fecundity to be 27.2-33.3 young/female/year (Stephen et al. 2013). However, snail uteri have been documented holding as many as 102 embryos (Jokinen 1982; Waltz 2008). Chinese studies indicate that *C. chinensis* may reproduce 2-7 times/yearly (April-October) and brood 20 - 50 fertilized eggs at a time (Zhang et al. 2017). In North America, females are believed to release juvenile snails from June to late October (Jokinen 1982). Haak (2015) found that adult *C. chinensis* exposed to colder temperatures (≤12℃) ceased reproduction, while those held at warmer temperatures (27℃) increased reproduction when compared to those at ambient temperatures (20℃). The number of brooded embryos appears to be directly correlated to female shell size in *C. chinensis* (Stephen et al. 2013). *Cipangopaludina chinensis* is believed to be iteroparous, with females expected to produce multiple broods over a lifetime (Fox 2007; Jokinen 1982), estimated at 5 years (Jokinen 1982; Stephen et al. 2013). Thus, once introduced to a site, *C. chinensis* may undergo exponential population growth, especially in the absence of density-dependent factors that might limit the population (Stephen et al. 2013).

Although habitat can influence the size and morphology of *C. chinensis*, this species is one of the largest freshwater snails among the Vivipardae (Liu et al. 1995). Growth of *C. chinensis* is indeterminate, with both sexes of the species reaching much larger maxima (40-60mm) than any indigenous North America freshwater gastropod (Liu et al. 1995). Females appear to live longer than males (5 years versus 3-4 years) and therefore grow to larger size (Jokinen 1982). The larger shell size likely enables non-indigenous *C. chinensis* to avoid some predators that otherwise feed on aquatic North American gastropods (e.g. Yellow Perch, *Perca flavescens*) (Twardochleb & Olden 2016).

Both morphology and genetics suggest that *C. chinensis* is a species distinct from *C. japonica* (Burks et al. 2016; Chiu et al. 2002; Clarke 1981; Clench & Fuller 1965; David & Cote 2019; Hirano et al. 2015; Lu et al. 2014; Smith 2000; Wang et al. 2017). Nonetheless, the nomenclatural status of snails currently placed within the genus *Cipangopaludina* remains contentious (Clench & Fuller 1965; Smith 2000). This is especially the case across eastern Asia, where multiple subspecies of *C. chinensis* have been recognized and other species of *Cipangopaludina* co-occur (Lu et al. 2014). Lu et al. (2014) has develop an identification key for the 18 species of *Cipangopaludina* spp. recognized in China, which may be useful for tracking potentially invasive *Cipangopaludina* spp. in North America. Morphologically, two shell phenologies have been identified in *C. chinensis*; tall-spire and short-spire (Chiu et al. 2002). Short-spired shells are found in waterbodies with low pH and low calcium concentrations. Tallspired shells are found in higher pH, higher calcium concentration lakes (Chiu et al. 2002). The differences in *C. chinensis* shell morphology further confounds identification issues between *C. chinensis* and *C. japonicus* in North America suggesting that either species may be more prevalent than previously believed.

In Canada, public education about invasive and potentially invasive species, including *C. chinensis*, is limited (Matthews et al. 2017; Office of the Auditor General of Canada 2019). This has produced a lack of public engagement in terms of monitoring invasive species across much of Canada, with the possible exception of Ontario. This may explain some of the gaps in distribution currently present in Canada. A more complete understanding of *C. chinensis* impacts on freshwater ecosystems in North America is required, especially in terms of determining where *C. chinensis* is present, and which ecosystems are likely to be most affected. Also, species managers should consider expanding the terminology used to describe *C. chinensis* from merely "non-indigenous" or "non-native" to incorporate "invasive". This should encourage greater public support for, and assistance with, species containment and geographical analyses.

Conceptual Diagrams

The conceptual diagrams (Figure 3) indicate that *C. chinensis*' role in non-indigenous (Figure 3A) habitats versus its role in indigenous habitats (Figure 3B) are similar but the interactions between *C. chinensis* and its ecosystem are more proportional in the indigenous range than in non-indigenous habitats. The arrows in the diagram pointing towards *C. chinensis* (in the centre of each diagram) refer to ecosystem interactions on *C. chinensis*, arrows pointing away from *C. chinensis* indicate the snail's impact on the ecosystem. The arrow width indicates greater (thicker), lesser (thin), or equal (equal thickness) ecosystem interactions. The indigenous habitat conceptual diagram has nearly equal number of arrows pointing towards and away from

C. chinensis, this does not suggest that the expected ecosystem interactions are different between indigenous and non-indigenous habitats. Rather, the type of research being conducted in each geographic region is different (remembering that these conceptual diagrams are visual summaries of the literature). In North America and Europe, research is typically focused on determining *C. chinensis*' ability to spread, establish, and exhibit invasive behaviours.

Due to its ecological plasticity, *C. chinensis* has proven to be extremely adaptable to a variety of environments. Burnett et al. (2018) tested thermal tolerance in *C. chinensis*, establishing temperature tolerances ranging from 0°C to 45°C, with 0°C the lowest temperature tested (and not necessarily the lower thermal limit), these experiments assessed critical maximum temperature and incipient lower lethal temperature over acute exposures (i.e. relatively short-term exposure). *Cipangopaludina chinensis* survival decreases where oxygen concentration is below 15 mg/L, and 4-day survival rate drops to 38.3% at pH 5.5, suggesting the species is sensitive to oxygen and pH levels (Zhang et al. 2017). Molluscicides, such as copper sulphate and rotenone, are not effective at controlling this species (Haak et al. 2014). Although an aquatic mollusc, initial air exposure experiments found that adult snails can survive at least four weeks of air exposure and juvenile snails survive 3 - 14 days (Havel 2011). Other desiccation experiments suggest that adult *C. chinensis* can survive exposure for longer than nine weeks (Unstad et al. 2013). Finally, the concentration of calcium required by *C. chinensis*, at < 2 ppm (see Supplementary Material Table 3 for a complete list of ecological thresholds), is very low compared to other molluscs, such as the zebra mussel, quagga mussel, and other gastropods (Chiu et al. 2002; Latzka et al. 2015). Our conceptual diagrams therefore represent a conservative estimate of *C. chinensis* ecosystem impacts in North America.

Where *C. chinensis* is present in North America, especially where other freshwater invasive calcified species are established (i.e. snails, molluscs and crayfish), there is evidence of impacts on indigenous species. Studies by Sura and Mahon (2011) found that the presence of *C. chinensis* caused snails (*Helisoma trivolvis*) to increase feeding rates and suggested cascading changes to community structures due to higher rates of algal consumption by *C. chinensis* (Sura & Mahon 2011). Johnson et al. (2009) found that indigenous snails (*H. trivolvis, Lymnaea stagnalis,* and *Physa gyrina*) decreased in mass during in-lab exposure to *C. chinensis,* and in the presence of a second invasive species, the rusty crayfish (*Orconectes rusticus*), one indigenous mollusc species became extinct (Johnson et al. 2009). The thick shell and larger size of *C. chinensis* relative to indigenous gastropods leaves *C. chinensis* more resistant to predation by *O. rusticus* (Johnson et al. 2009). However, *O. rusticus* and other invasive crayfish species, such as red swamp crayfish (*Procambarus clarkii*) and northern crayfish (*Orconectes virilis*), will consume *C. chinensis,* although apparently smaller, juvenile snails. Indigenous signal crayfish (*Pacifastacus leniusculus)* seem to be particularly prone to prey on *C. chinensis* (Olden et al. 2009). Predation experienced by *C. chinensis* in mesocosm experiments with *P. clarkia*, *O. virilis*, and *P. leniusculus* decreased with increased *C. chinensis* shell size (Olden et al. 2009). As a result, *C. chinensis* has been documented to have impact on inter-species dynamics in regard to competition, stressors, and as a potential prey source for a limited number of indigenous predator species.

There is evidence that the diet, feeding mechanisms, and excretions of *C. chinensis* may alter freshwater bacterial and algal communities leading to changes in water chemistry and food webs. Previous studies have shown that *C. chinensis* ingest benthic organic matter, inorganic matter, and algae, primarily using the radula. Stomach contents of *C. chinensis* show that this species favours diatoms, but other studies indicate that *C. chinensis* does not feed selectively (Jokinen 1982; Olden et al. 2013; Plinski et al. 1978; Stańczykowska et al. 1971). Additionally, larger adult *C. chinensis* (≥44mm) can filter feed and will do so selectively when inter-snail competition is high (Olden et al. 2013). Due to the significant amounts of nitrogen and phosphorous this large gastropod excretes via fecal matter; it is possible for *C. chinensis* to alter the algal community in habitats occupied (Olden et al. 2013). Mesocosm experiments with high *C. chinensis* densities showed significant changes in bacterial community composition (Olden et al. 2013). Changes in bacterial community composition led to a decrease in bacterial community variability but did not decrease bacterial abundance (Olden et al. 2013).

Filtration rates of large *C. chinensis* (maximum 471 mL/ snail/ hr) are comparable to high-profile invasive freshwater and marine bivalves, including zebra mussel (*Dreissena polymorpha)*, quagga mussel (*Dreissena bugensis*), Asian clam (*Corbicula fluminea*), golden mussel (*Limnoperna fortune*), and blue mussel (*Mytilus edulis*) (Olden et al. 2013). Additionally, mesocosm studies with *C. chinensis* present at low and high densities showed increases of 54% in the amount of suspended chlorophyll-*α* concentrations at "low" snail density and a 115% increase at "high" snail density (Olden et al. 2013). Water column chlorophyll-α increases observed were likely due to elevated nitrogen and phosphorus concentrations produced by *C. chinensis* excreta that promote periphyton production (Olden et al. 2013). Future research should further investigate the food web implications of bacterial community shifts, the type of algae produced (whether *C. chinensis* presence may lead to higher probabilities of toxic algal blooms), and determine if *C. chinensis* requires particular algal densities before snail populations become established. The latter may impact the potential for *C. chinensis* spread. Also, previous studies link *C. chinensis* nutrient cycling, particularly nitrogen (N) and phosphorus (P) compounds to downstream eutrophication, with higher temperatures producing greater release rates of total-N, dissolved-N, total-P, dissolved-P, ammonia, and phosphate (Bobeldyk 2009; Chen et al. 2012). These studies suggest that *C. chinensis* may play an important role in eutrophication which warrants further research to determine *C. chinensis* population densities needed to cause eutrophication. More importantly, aquatic systems already under pressure of elevated nutrient levels such as from farmland run-off, should be protected against *C. chinensis* introduction to ensure that this species does not become the tipping point that leads to eutrophication of these vulnerable ecosystems.

It appears that in North America, parasite prevalence in *C. chinensis* is much lower than among indigenous gastropod species (Karatayev et al. 2012). Of 147 necropsied *C. chinensis* from lakes in Wisconsin, USA, only two contained trematode parasites (Harried et al. 2015). One snail contained *Cyathocotyle bushiensis*, a parasite of waterfowl known to cause mortality in dabbling ducks (Hoeve and Scott 1988), and the other contained nine aspidogastrean flatworms, *Aspindogaster conchicola* (Harried et al. 2015), a widespread parasite (North American, Europe, Africa) of freshwater bivalves, fish, and turtles. Karatayev et al. (2012) found that of 30 *C. chinensis* collected from the Great Lakes area, Canada, none were infected. In laboratory experiments where *C. chinensis* and two indigenous snail species, *Bithynia tentaculata* and *Physa gyrina*, were exposed to *Sphaeridiotrema pseudoglobulus*, an important waterfowl parasite, *C. chinensis* contained significantly fewer metacercariae than either of the two indigenous snail species (Harried et al. 2015). Moreover, those metacercarial cysts found in *C. chinensis* were typically encased within the mollusc’s shell and likely no longer viable (Harried et al. 2015). This limited information suggests that *C. chinensis* may be less vulnerable to infection by endoparasites commonly affecting indigenous gastropod species in North America.

However, it is concerning that *C. chinensis* has been found hosting *A. conchicola* as this parasite has been linked to decreased reproduction in North American freshwater mussels (Gangloff et al. 2008), a diverse group of organisms for which there is considerable conservation concern (Williams et al. 1993).

In North America, there have yet to be studies published on the potential for *C. chinensis* to transfer contaminants from environments with elevated contaminant concentrations to human consumers, but a few North American studies have shown that this species bioaccumulates contaminants, specifically mercury, arsenic, iron, manganese, zinc, copper, nickel, lead, cadmium, and chromium found in polluted sediments (Chapman et al. in prep; Tornimbeni et al. 2013). The study of *C. chinensis* acting as a biotransmitter of contaminants between contaminated sediments and consumers is a key focus of multiple Asian-origin literature because *C. chinensis* has acted as a biotransfer of contaminants there. Therefore, further North American research is needed on this subject because, even though it is less popular for humans to consume *C. chinensis* in North America than in Asian countries, North American wildlife still consume *C. chinensis* and may bioaccumulate harmful contaminants from this snail.

Cipangopaludina chinensis (Chinese taxonomic name: 中国圆田螺) within its indigenous range is wide-spread and is an important prey option to indigenous predators and a staple-food for people's diets in many regions of China and eastern Asia. The indigenous range of C. *chinensis*, commonly referred as 'mud snails' in China (Chinese common name: 田螺) extends across eastern Asia (Figure 1). *C. chinensis* occurs naturally in rice paddies, rivers, and lakes, where it is common and where positive ecosystem interactions have been documented (Dewi et al. 2017; Kurniawan et al. 2018; Liu et al. 1995) (Nakanishi et al. 2014). As a result, this species is an important component of managed rice paddy ecosystems where the presence of mud snails is correlated with a greater abundance and diversity of terrestrial arthropods, which leads to greater rice yields (Dewi et al. 2017). Also, within its indigenous range, *C. chinensis* is prey for multiple species (See Supplementary Materials Table 5), including waterfowl such as Muscovy duck (*Cairina moschata*, 番鸭), domestic duck (*Anas platyrhynchos domesticus*, 北京鸭), and Mallard duck (*Anas platyrhynchos*, 绿头鸭), and Asian carp such as black crap (*Mylopharyngodon piceus*, 青鱼) and Amur carp (*Cyprinus rubrofuscus*, 鲤鱼), Chinese softshell turtle (*Pelodiscus sinensis*, 中华鳖), and is sold in local wet markets for human consumption (Luo et al. 2012; Tian et al. 2012; Yan 2002; Zhang et al. 2017). *C. chinensis* also competes with golden apple snail (*Pomacea canaliculate*, 福寿螺) which is a non-indigenous invasive species in China (Luo et al. 2012; Zhang et al. 2017). In North America, comparative species reported preying on *C. chinensis* include largemouth bass (*Micropterus salmoides*), pumpkinseed sunfish (*Lepomis gibbosus*), signal crayfish (Olden et al. 2009; Twardochleb & Olden 2016), ringed crayfish (*Orconectes neglectus*), and as such, those aquatic species further exploration as potential biological controls of non-indigenous *C. chinensis*.

An array of human uses for *C. chinensis* have been documented in East Asia, although in North America, *C. chinensis* has not yet been exploited commercially. The species has been used as feed for larval fireflies (*Luciola ficta*) (Ho et al. 2010), and hatchery-cultured mud crab (*Scylla paramamosain*) (Gong et al. 2017). Fish farms in China use viviparid snails as commercial feed for Chinese carp (*Mylopharyngodon piceus* and *Cyprinus rubrofuscus)* (Yan 2002), Chinese softshell turtles (*Pelodiscus sinensis*) (Tian et al. 2012), and Chinese golden-coin turtles (*Cuora trifasciata)* (Luo et al. 2012). *Cipangopaludina chinensis* has been used to remediate rice paddy fields contaminated with sewage with elevated metals (Kurihara & Suzuki 1987; Kurihara et al. 1987; Xing et al. 2016).

In Asia, there have been recent efforts to develop pharmaceutical uses for *C. chinensis*. *Cipangopaludina chinensis* has been long used in traditional Korean medicine and is a component of traditional Korean medical knowledge used to treat indigestion (Kim et al. 2018). More recent medical studies have explored the efficacy of compounds derived from various *C. chinensis* tissues for protection against liver damage (Wang et al. 2015; Xiong et al. 2019), as an anti-inflammatory for joint pain caused immune responses (Lee et al. 1998; Shi et al. 2016), and as a supplement that protects against plaque build-up in arteries (Xiong et al. 2013, 2017, 2019). Additionally, *C. chinensis* has been used as a model organism in neurophysiological, hepatological, and reproductive studies testing pharmaceutical products and supplements (Swart et al. 2017; Wang et al. 2014; Wang et al. 2015). Finally, waste shells have been recycled to extract calcium which can be used for promoting bone growth (Zhou et al. 2016). Those methodologies have the potential for transfer and development in North America as a means of reducing non-indigenous *C. chinensis* populations.

East Asian research demonstrates that *C. chinensis* may act as an intermediate host for a number of human endoparasites of medical importance, including *Echinostoma cinetorchis* Ando and Ozaki(an Asian intestinal fluke), and *Angiostrongylus cantonensis* (a nervous system nematode) (Chao et al. 1993; Chung & Jung 1999; Jokinen 1982; Lü et al 2006; Sohn & Na 2017). Although of Asian origin, *A. cantonensis* has been identified as an emerging zoonotic pathogen in North America (York et al. 2015).

Much of the Asian ecological literature for *C. chinensis* is focused on contaminant bioaccumulation (Cui et al. 2012; Fang et al. 2001; Kurihara et al. 1987; Luo et al. 2016; Wu et al. 2001), because this is of particular human health concern where *C. chinensis* is widely eaten. For example, of 14 edible mollusc species purchased at food markets originating in the Pearl River Delta, China, *C. chinensis* was among those that had elevated concentrations of cadmium, copper, zinc, lead, nickel, chromium, antimony, and tin that exceeded the regulatory human consumption limits, especially chromium which exceeded the Chinese daily consumption limits of 1µg/g (Fang et al. 2001). Snails from the highly polluted Zhalong Wetland, China, had elevated lead (average concentration= 45.32 µg kg^{-1}), cadmium (average concentration= 2.45 µg kg^{-1}), and arsenic (average concentration= 11.48 µg kg^{-1}), due to grazing over highly contaminated sediments. (Luo et al. 2016). Additionally, *C. chinensis* has been documented to bioaccumulate zinc and copper from sewage sludge used to fertilize rice paddy fields (Kurihara et al. 1987). This ability to bioaccumulate contaminants may prove useful in bioremediation, or allow *C. chinensis* to serve as a bioindicator, there is also concern that *C. chinensis* may transfer contaminants to consumers where the species is widely eaten.

In summary, the introduction of a non-indigenous species into an ecosystem can have unpredictable outcomes. Our “non-indigenous” and “indigenous” conceptual diagrams are useful for assessing potential scenarios by summarizing previously published information on *C. chinensis* from both indigenous and non-indigenous habitats. Based on the conceptual diagram for “non-indigenous ranges” and applying the DFO definition for an AIS (Fisheries and Oceans Canada 2018), *C. chinensis* in North America should be regarded as an invasive snail.

Our conceptual diagram of *C. chinensis* ecosystem interactions in North America further support the argument that *C. chinensis* is an aquatic invasive species on the continent. *Cipangopaludina chinensis* negatively affects indigenous aquatic species by increasing environmental pressures on freshwater molluscs leading to decreased biomass and expiration of indigenous molluscs (Johnson et al. 2009; Karatayev et al. 2009; Sura & Mahon 2011). Indirect, negative, impacts on indigenous aquatic species may be caused by *C. chinensis* through shifts in food web structure (Olden et al. 2013), changes in water chemistry (Olden et al. 2013), and eutrophication (Bobeldyk, 2009). Also, *C. chinensis* has become a vector for indigenous *A. conchicola* which parasitize North American unionid bivalves of conservation concern (Harried et al. 2015).

The indigenous habitat conceptual diagram identifies areas where North American literature is incomplete. The indigenous range conceptual diagram flags some future concerns for growing *C. chinensis* populations and further distribution in North America. Particularly, consumption of *C. chinensis* by naïve indigenous species and human populations is troubling because there is numerous evidence of *C. chinensis* transferring parasites and contaminants to both human and wildlife consumers and co-existing mollusc species (Chao et al. 1993; Chung & Jung 1999; Cui et al. 2012; Fang et al. 2001; Kurihara et al. 1987; Luo et al. 2016; Sohn & Na 2017; Tornimbeni et al. 2013; Wu et al. 2001). We have yet to find studies, in North America or Asia, examining the possibility of *C. chinensis* to host fish and mussel parasites which poses additional uncertainty in terms of assessing potential risks to North American habitats, especially since previous studies have shown that North America fish do consume *C. chinensis* (Twardochleb & Olden 2016). Therefore, further research is needed to assess how or if *C. chinensis* can host and/or transfer parasites and contaminants into different consumers in freshwater food webs.

Additionally, our literature review and conceptual diagrams uncovered gaps in current knowledge for *C. chinensis* distribution and management across Canada. There are indications that *C. chinensis* is an invasive species with the potential to disrupt North American ecosystems and food webs and to reduce the economic benefits of freshwater habitats (Figure 5). Unfortunately, available data is based on a limited number of laboratory and field experiments and surveys (Bury et al. 2007; Chaine et al. 2012; Clarke 1981; Haak et al. 2017; Karatayev et al. 2009; Kipp et al. 2020; Latzka et al. 2015; McAlpine et al. 2016; Rothlisberger et al. 2010; Solomon et al. 2010; B. Stephen 2017). Also, there is limited literature regarding *C. chinensis* in its indigenous ranges used to supplement English-language literature in management decisions. While formal risk assessments may allow one to accurately assess the risks associated with C. *chinensis* presence in North America, data and studies are needed to support such a risk assessment. A systematic, continental-scale (North America) record-keeping approach to *C. chinensis* presence will enable predictions of habitat overlap with indigenous species of conservation concern (e.g. species at risk).

Geographical distribution of reported C. chinensis occurrences in Canada

We located 278 reports documenting *C. chinensis* in Canada (Figure 5), with the greatest number of reports from Ontario (77.7%). Canadian reports of *C. chinensis* occurrence by province included: British Columbia (12); Alberta (1); Ontario (216); Quebec (10); New Brunswick (7); Nova Scotia (30); Prince Edward Island (1); and the island of Newfoundland (1).We located no reports of *C. chinensis* from Saskatchewan, Manitoba, Yukon, Northwest Territories, Nunavut or Labrador (Table 1). The first reported occurrence in Canada was at St. John's, Newfoundland, in 1905, the second from Kawkawa Lake, British Columbia (1953). Since then there has been only sporadic reporting in Canada (Clarke 1981; Clench & Fuller 1965; Kalas et al. 1980; McAlpine et al. 2016; Plinski et al. 1978; Stańczykowska et al. 1971). The greatest number of Canadian *C. chinensis* occurrence reports were made between 2010-2019 (215 reports), 1990-1999 (33), followed by 2000-2009 (26), and very few reports pre-date

1990 (9). For example, *C. chinensis* was first noted in Quebec in 1971 (Jokinen 1982) but there has been very few subsequent reports for the province.

The source of *C. chinensis* reports varied and is associated with the level of public awareness in each province and accessible means of reporting. In British Columbia, *C. chinensis* reports were derived entirely from museum records. The Prairie provinces (Alberta, Saskatchewan, Manitoba) had no records or reports at the start of this project. But recently, Alberta Environment has confirmed a *C. chinensis* populations in a southern Alberta lake, and have now started an awareness campaign (Alberta Invasive Species Council 2018), suggesting all Prairie provinces may need to add this species to their non-indigenous monitoring programs. Ontario reporting methods include a preponderance of internet and phone app reports, likely the result of a high level of public awareness of invasive species through educational campaigns. Publicly accessible phone apps and internet databases, such as the Ontario Department of Natural Resources online database (EDDMaps Ontario; 33%) and iNaturalist (21%), were the most frequently used tools for reporting public sightings of *C. chinensis* in Ontario. For Quebec, the majority of *C. chinensis* reports were derived from previously published literature (71.4%) with a limited number of reports available at iNaturalist (28.6%). In Atlantic Canada (New Brunswick, Nova Scotia, Prince Edward Island, Newfoundland, and Labrador) scientists researching freshwater lakes (50%) were the most common source of *C. chinensis* records, followed by museum records (25%), and iNaturalist (11%).

Across Canada, established populations of C. *chinensis* have been reported from 146 locations: lakes (64 lakes), rivers (29). ponds (25), creeks (9), harbours (1), bays (5), streams (5), marshes (1), reservoirs (3), mills (1), beaches (1), canals (1), or basins (1). The number of freshwater bodies with reported *C. chinensis* occurrences by province include: British Columbia

(11), Alberta (1), Ontario (105), Quebec (9), New Brunswick (6), Nova Scotia (16), Prince Edward Island (1), the island of Newfoundland (1), (Table 1). The number of invaded lakes per provinces followed the same trend as the number of reported occurrences of *C. chinensis* with the highest number of invaded water bodies in ON (Table 1). Previous studies have suggested that the habitat preference of *C. chinensis* as slow or stagnant freshwater (Matthews et al. 2017; McAlpine et al. 2016; Rothlisberger et al. 2010) . Our review of Canadian *C. chinensis* reports indicate that *C. chinensis* also inhabit rivers, harbours, and bays, which is confirmed by the Asian literature as indigenous habitats. The assumptions about *C. chinensis* habitats being confined to slow-moving freshwater ecosystems only can be risky – for example, in 2019, *C. chinensis* was confirmed in a brackish tidal stream (conductivity=349.8 µS/cm) which is directly connected with Cow Bay (conductivity=36,700 µS/cm) in Halifax Regional Municipality (M. Fraser personal observation 2019), which leads to questions about salinity tolerance and thresholds for this species in such locations where seasonal salt and freshwater mixing may occur.

This is the first country-wide summary to date for *C. chinensis* in Canada. A federally-maintained Canadian database would allow researchers and managers to identify what will likely be an expanding distribution, gaps in distributional survey effort, and most importantly, will contribute to predictive distribution models (Haak et al. 2017; Papes et al. 2016) that may highlight areas of concern (e.g. species and habitats at risk, areas of ecotourism, recreational fishing spots). Species distribution modelling for *C. chinensis* in Atlantic Canada and other parts of North America suggest that water chemistry, ecosystem composition, and human-mediated transfers between waterbodies are significantly correlated with *C. chinensis* spread (Haak 2015; Haak et al. 2017; Kingsbury et al, in review; Latzka et al. 2015; Papes et al. 2016). However, these studies are limited, in terms of geographic area and in modeling success, due to the lack of knowledge of ecological thresholds ecosystem interactions between *C. chinensis* and other indigenous or invasive species, inconsistency in water chemistry databases and inconsistent coverage of water chemistry databases over entire political jurisdictions (e.g. a whole province or state). Research and improved monitoring efforts of both species occurrence and habitat characteristics are clearly needed to expand our current understanding of how ecological thresholds influence habitat selection in *C. chinensis* as it will influence presence/absence surveys and predictive distributional modeling for the species.

Not surprisingly, current Canadian *C. chinensis* distribution is concentrated in areas where human population is most dense. This may reflect actual distribution but may also be reflective of reporting opportunity. The distribution of *C. chinensis* in Canada is essentially a northward extension of the species range in the USA, so the US spatial trends for this species merits an examination when examining Canadian trends. The geographical distribution of North American *C. chinensis* reports in both Canada and USA identified a few likely reporting gaps in western Canada, around Lake Superior in Ontario, southern Quebec, and eastern Canada (Figure 5). The prevalence of pleasure craft, developments that redirect and change water flow, and the significant likelihood for aquarium releases, suggest a wider establishment for *C. chinensis* in southern Quebec since 1971, the year of first report (Matthews et al. 2017). In Alberta, there were no reports at the start of this review project, but subsequently, a discovery of a previously undocumented, but well-established, population of *C. chinensis* was confirmed in Lake McGregor, an important reservoir connected to two main rivers in southern Alberta - the first occurrence for the province has led to a province-wide public education campaign. Public education of *C. chinensis* as a potential problem for indigenous ecosystems, in parallel with an accessible reporting system (e.g. smartphone apps, email, mobile texting and phone contacts), should lead to increased reports of occurrence. We recommend that a Canada-wide database of *C. chinensis* occurrences, using the one we have started here as a foundation, be established, and perhaps maintained by DFO. These occurrence reports can then be included with other geo-tagged data layers for non-indigenous species, vulnerable species and species-at-risk and vulnerable habitats to determine areas of conservation management priority.

Management

Commonly employed molluscicides, including rotenone and copper sulphate, are not effective in controlling or eliminating *C. chinensis* (Haak et al. 2014) and can be toxic to other invertebrates. Drawdowns, in which the water is removed from a specific water body, will likely not eradicate *C. chinensis* because the species can survive periods of desiccation exceeding nine weeks (Unstad et al. 2013). Furthermore, long periods of air exposure, or increased predator pressure, has only been found to elevate *C. chinensis* reproductive rates (Prezant et al. 2006; Unstad et al. 2013; and personal observation of laboratory cultures). Juvenile *C. chinensis* typically hide under rocks and burrow into sediment, so it is impractical to manually cull populations of *C. chinensis* (Jokinen 1982).

Nonetheless, approaches to management have not been thoroughly explored, with only five publications available (Collas et al. 2017; Haak et al. 2014; Matthews et al. 2017; Rothlisberger et al. 2010; Unstad et al. 2013). Only two of these publications conducted experiments to examine *C. chinensis* response to commonly used AIS management approaches (Haak et al. 2014; Unstad et al. 2013). Two publications focused on public education, placing controls on species movements, and early species detection (Matthews et al. 2017; Rothlisberger et al. 2010). Matthews et al. (2017) noted that there are no ongoing case studies for eradication of *C.*

chinensis, perhaps due to the perception that *C. chinensis* in North America is always present at relatively low densities and is non-problematic. Eradication of AIS (by chemical, manual, biological, or mechanical means) is generally only effective when a species is recently introduced to an aquatic system (Government of Canada 2019a), supporting the need for an effective monitoring and modelling program across Canada. There is one study of *C. chinensis* ecological preference for slow moving water which noted that perhaps increasing water flow via installation of a culvert between connected water bodies may limit the upstream spread of *C. chinensis*, however these results were only observational and require further investigation (Rivera & Peters 2008) – especially as our review indicate that *C. chinensis* can establish in a variety of freshwater and brackish habitats.

Some governments have already taken steps to limit the spread of *C. chinensis* by preventing new introductions. In Canada, Manitoba and Alberta both have regulations controlling the sale, distribution, and reporting of *C. chinensis* and other non-indigenous viviparid snail species. These Canadian provinces have also implemented required boat cleaning and AIS reporting programs to ensure AIS is not accidently transferred between waterbodies and to improve early AIS detection (Government of Alberta 2015; Government of Manitoba 2015). The Department of Fisheries and Oceans (DFO), having federal oversight of AIS in Canada, operates under the umbrella of the *Fisheries Act* which enforce AIS management through the *Aquatic Invasive Species Regulations*. Section 10 of the *Aquatic Invasive Species Regulations* prohibits any person from introducing a species that is non-indigenous to any region or water body frequented by fish unless authorized under provincial or federal law (Canada Legislative Services Branch 2019). *Cipangopaludina chinensis* is listed under the *Aquatic Invasive Species Regulations*, and is therefore, subject to management under these regulations. However, previous risk assessments by DFO have assessed *C. chinensis* impacts to be negligible, which has lowered the priority for management of *C. chinensis* compared to other AIS in Canada (Schroeder et al. 2013). Based on information reviewed here, we believe that jurisdictions may be underestimating impacts. In the US, examples of jurisdictions that do seek control *C. chinensis* include Missouri and Minnesota. *Cipangopaludina chinensis* is listed as a "regulated invasive species" in Minnesota, which allows for possession, sale, purchase, and transportation of this species, but prohibits the introduction of live *C. chinensis* into new ecosystems such as through aquarium release or garden ponds (Minnesota Department of Natural Resources 2020). In Missouri, *C. chinensis* is labelled as a "prohibited species," meaning that no person may possess, purchase, sell, transport, import, or export this species (Missouri Department of Conservation 2020). Within the European Union, a risk assessment was completed which recommended that targeting the pet and aquarium trade and increasing public education/engagement will assist in controlling the further spread of *C. chinensis* (Matthews et al. 2017). To conclude, regulatory action on preventing the sale and transport of viviparid snail species in the pet and aquarium trade, and public education and engagement, are essential for early detection and slowing the spread of *C. chinensis* in North America.

Conclusion

We have reviewed 123 literature and internet sources (86 peer-reviewed journal articles, 7 grey literature, and 30 internet sources) for both indigenous and non-indigenous *C. chinensis* habitats, compiled North American reported occurrences and created two conceptual diagrams for both indigenous and non-indigenous habitats. Evidence suggests that *Cipangopaludina chinensis* meets the current federal Canadian definition of an aquatic invasive species.

While we believe the map showing the geographical distribution of reported occurrences (Figure 5) underrepresents actual *C. chinensis* distribution in Canada, it does indicate that *C. chinensis* is already widespread in Canada. Our conceptual diagrams (Figure 3) suggest freshwater ecosystems in North America may be vulnerable to negative impacts from *C. chinensis*. The conceptual diagram of *C. chinensis* in its indigenous habitat raises concerns of hosting parasites and transferring contaminants from polluted environments to naïve consumers (fish and humans included). With global temperatures predicted to rise, and given the species adaptability and fecundity, *C. chinensis* can be expected to expand its range northward in North America. Rising temperatures will also further elevated fecundity, enhancing dispersal.

Due to the difficulty of eradicating *C. chinensis* once established, preventing introduction of the species is the most cost-effective management strategy. This will require greater public education and stricter regulations, including stricter enforcement on existing AIS regulations, on the sale and release of *C. chinensis* in the aquarium and water garden trade. Canada lags in terms of research on this species, with most of the northern temperate literature based on studies from the USA and Europe. Further research is needed in North America to better document *C. chinensis* presence and to establish predicted impacts. Impacts may vary geographically, and those in Canada may depart from impacts documented to date for the southern USA. In summary, we recommend that *C. chinensis* be recognized as an aquatic invasive species in Canada and across the USA with stricter management of the species. This should assist in expanding funding opportunities for research, public education, and AIS species management.

CHAPTER 1: Figures

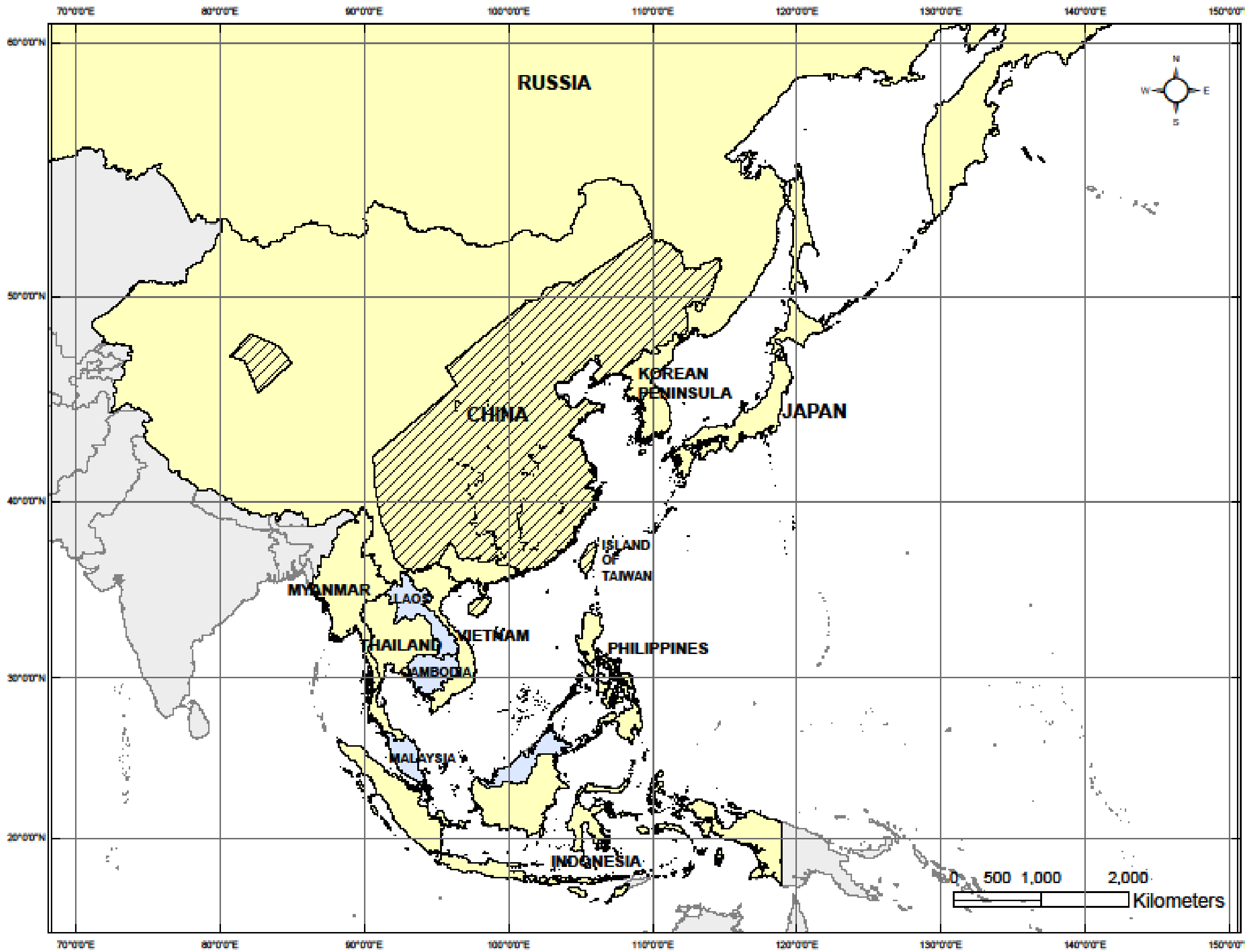

Figure 1: *Cipangopaludina chinensis* is reportedly indigenous to China (Dewi et al. 2017; Liu et al. 1995) with specific bio-geographic represented by the hatched lines (Liu et al. 1995), Japan (Dewi et al. 2017; Hirano et al. 2015; Kurihara et al. 1987), the Korean Peninsula (Chung & Jung 1999; Dewi et al. 2017; Hirano et al. 2015), the Island of Taiwan (Chiu et al. 2002; Dewi et al. 2017; Hirano et al. 2015), eastern Russia (Kipp et al. 2020), Indonesia, Thailand, Myanmar (Burma), Vietnam, and general south-eastern Asia (Global Invasive Species Database 2020; Kohler & Jinghua 2012). Regions highlighted in yellow are confirmed by the previously listed sources; and the areas in light blue we suspect are “highly-probable” countries of *C. chinensis* origins. The species’ indigenous range is poorly defined, perhaps due to unresolved taxonomic issues with other closely related viviparids. The 2009 IUCN Red List reports that the assessment of *C. chinensis* is in need of updating (Köhler, & Jinghua 2012).

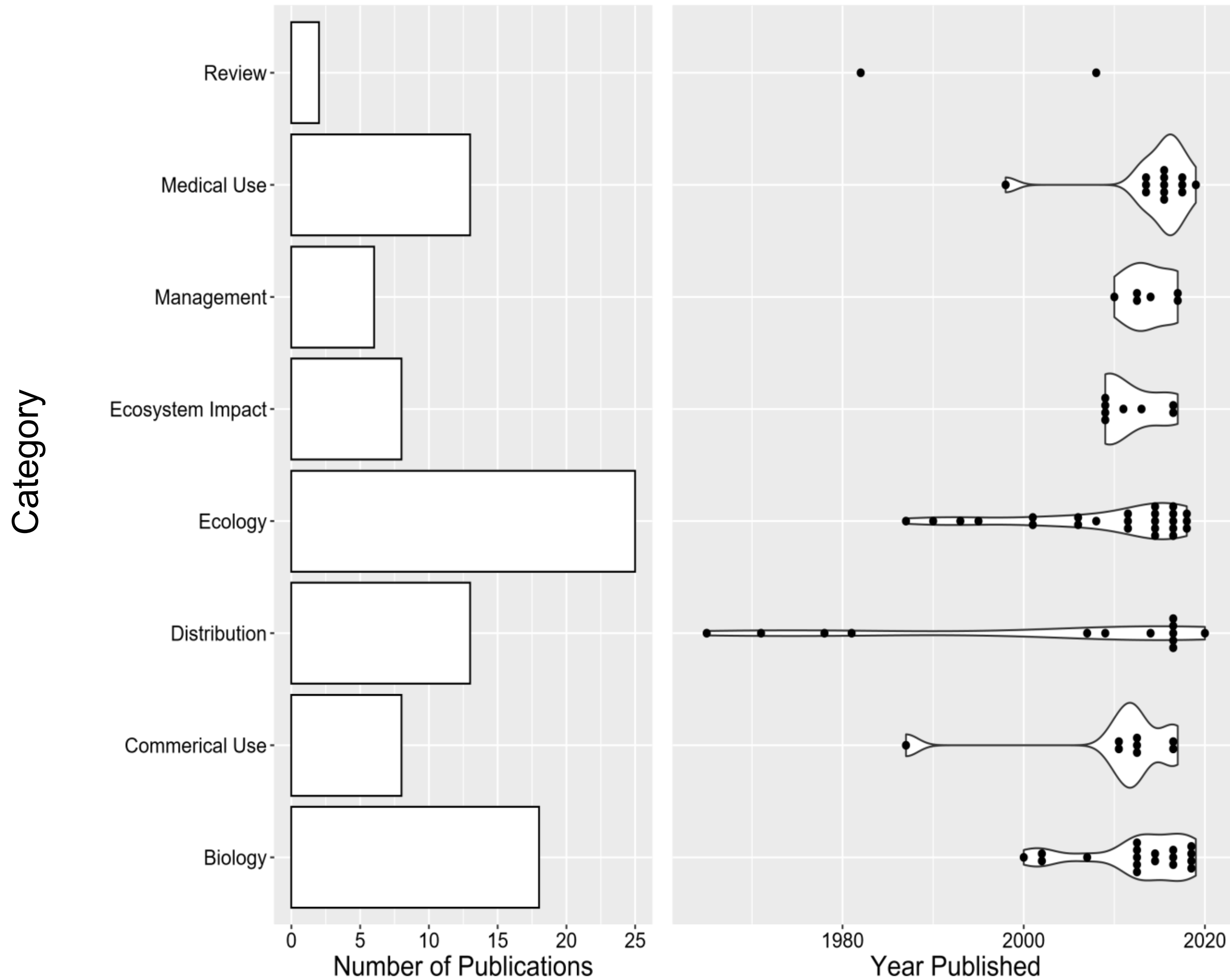

Figure 2: Literature located for this review (n=93) dealing with *Cipangopaludina chinensis* and organized by nature of content. Publication sources were peer-reviewed journal articles (n=86) and grey literature (n=7). Additionally, source year of publication is plotted using a violin plot to visualize the relevance of the data. Most literature were published 2010-2020, suggesting an increased interest in *C.* chinensis. All literature are listed in Supplementary Information Table 1.

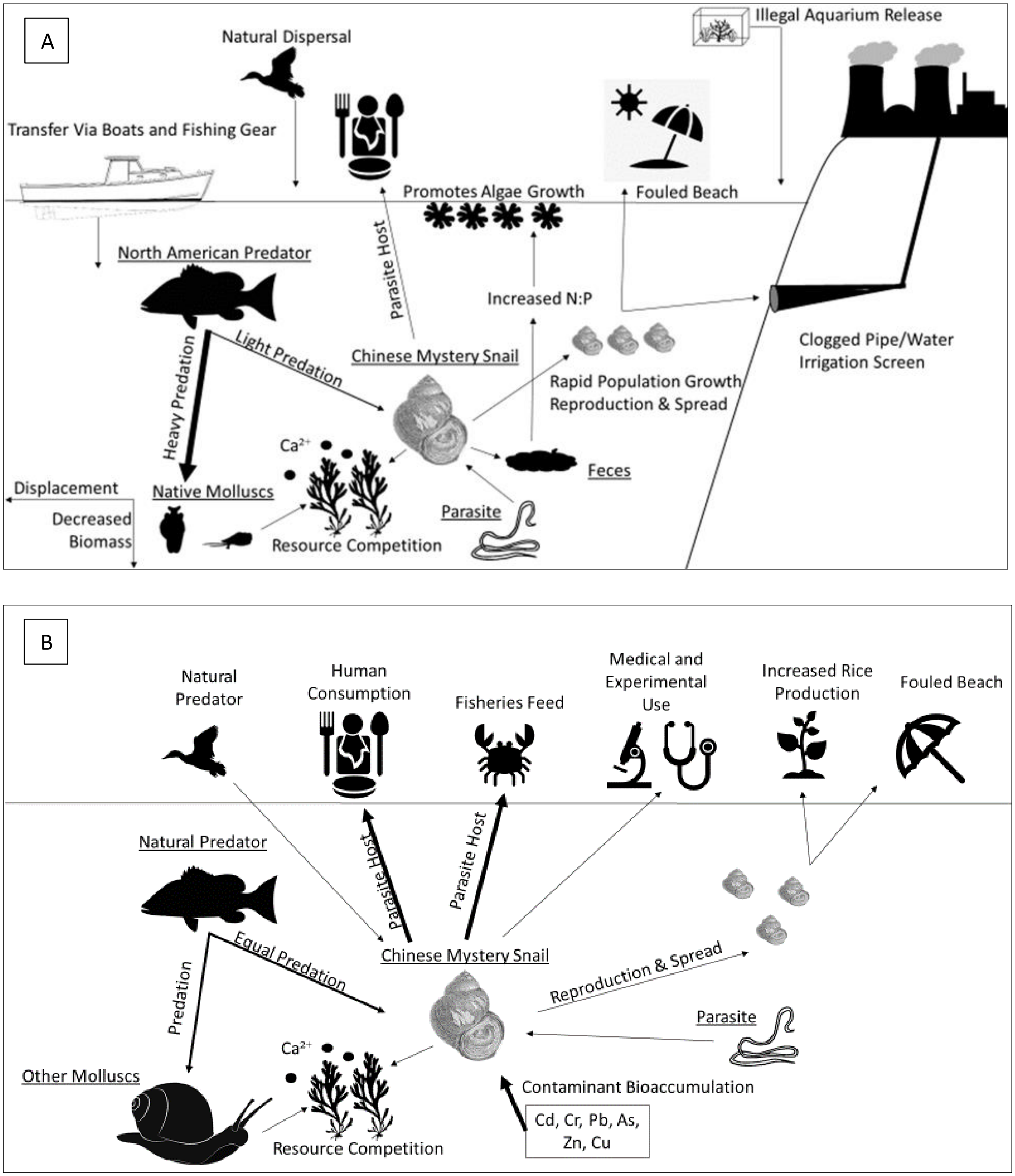

Figure 3: Figure 3A. Conceptual diagram summarizing *Cipangopaludina chinensis* ecosystem impacts within the non-indigenous range in North America and Europe, based on a review of current North American literature. Figure 3B. Conceptual diagram summarizing ecosystem interactions for *C. chinensis* across indigenous range based on current Asian literature. Arrows represent *C. chinensis* impacted by the ecosystem (towards) or impacting the ecosystem (away), thickness indicated the severity of the impacts (thicker arrow=increased effect).

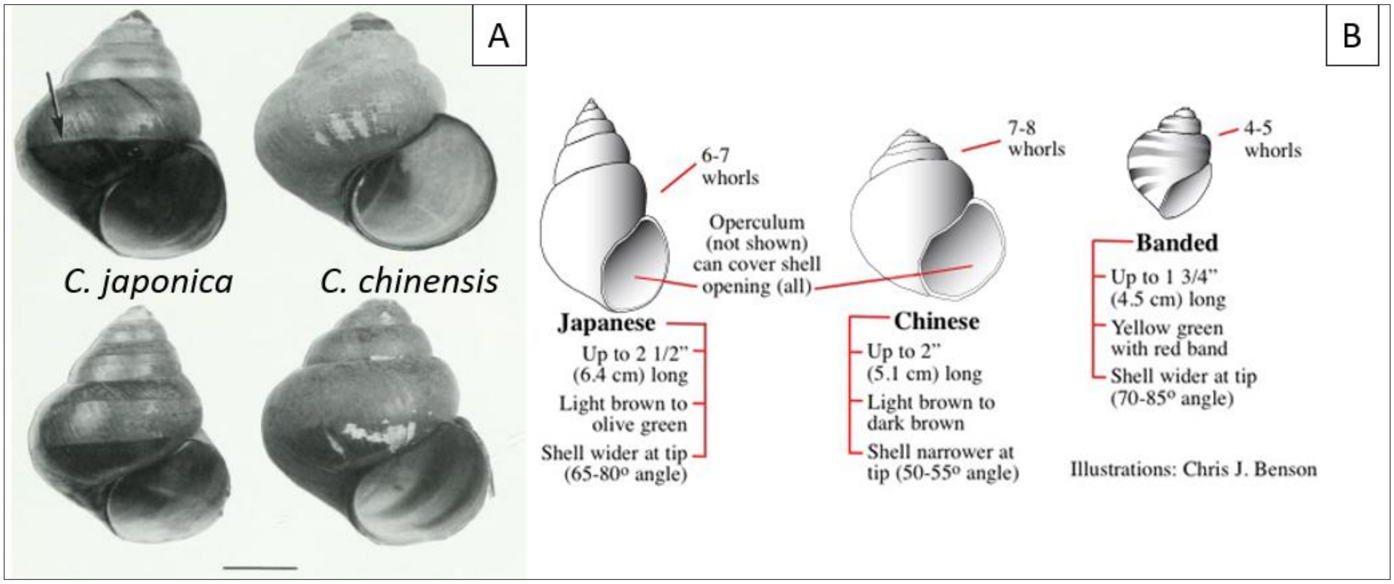

Figure 4: (A) Modified from Smith (2000) showing *C. japonica* and *C. chinensis*. Arrow marks carina, which is believed to distinguish *C. japonica.* This species also has a taller spire, a more acute angle and finer carina on the shell's surface, compared to *C. chinensis*. *C. chinensis* is broader than *C. japonica*. However, these distinguishing features may be more plastic than previously realised and shell morphology may vary considerably. (B) Morphological features distinguishing the three types of non-indigenous mystery snails commonly found in North America (Minnesota Sea Grant 2016). Banded mystery snails, *Viviparus georgianus*, is also found throughout North America (especially in the Great Lakes region).

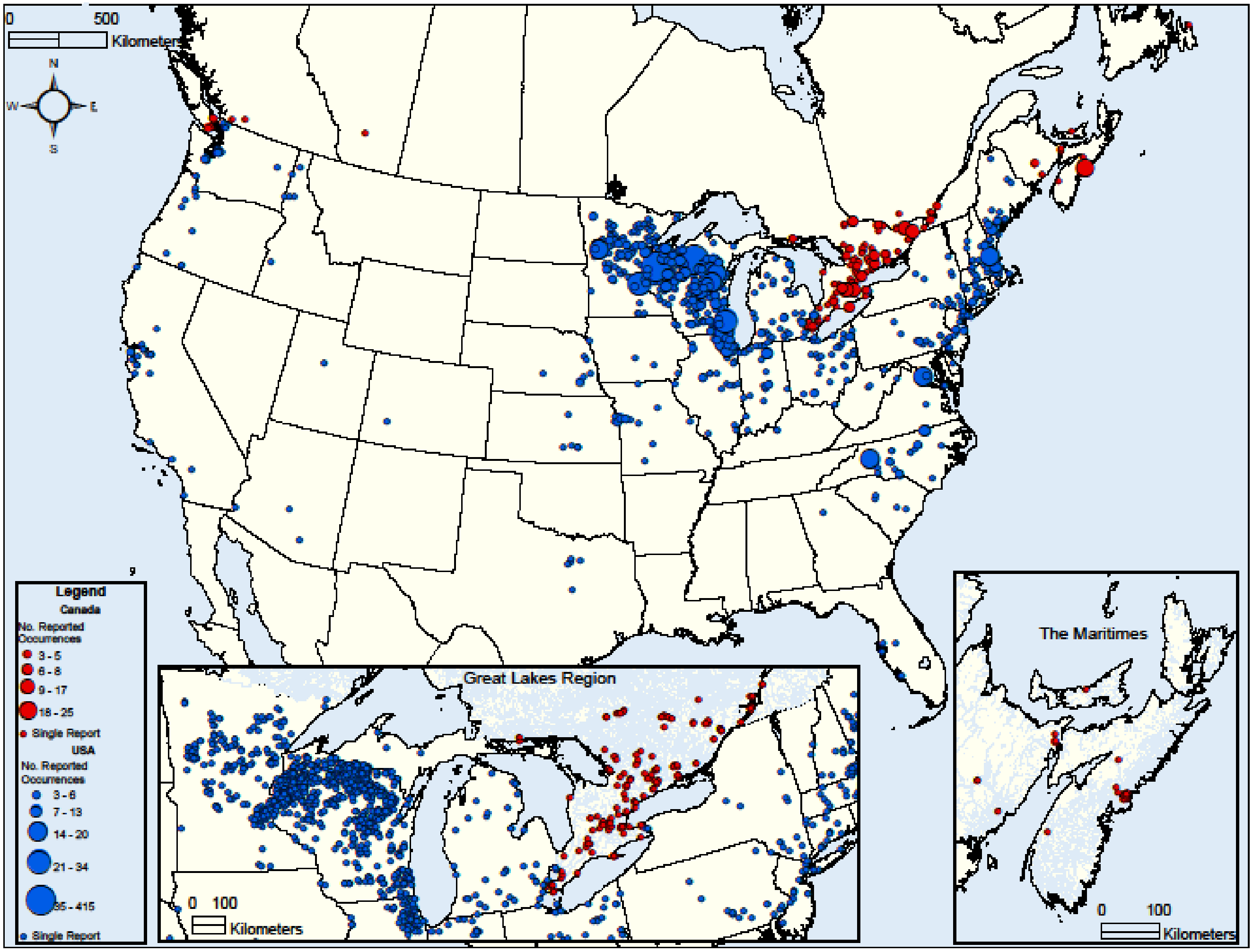

Figure 5: *Cipangopaludina chinensis* occurrences in Canada and the continental United States of America (USA). USA records (blue) are taken from Kipp et al. (2020) except those records in Hawaii which does have reported occurrences of *C. chinensis*. Hawaii is the first ever reported case of *C. chinensis* in North America (year 1800), has 4 reports total (Hawaii, Kauai, Molokai, and Oahu), but has no further reports since 1997 (Kipp et al. 2020). Canadian records (red) were assembled for this study (see text for sources). Species occurrence points are aggregated to within 10 km.

CHAPTER 1: Tables

Table 1: Canadian *Cipangopaludina chinensis* reports by province, and the total number of water bodies with documented *C. chinensis* occurrence by province.

Provincial Data		
Province	**Number of Reports**	**No. Invaded Waterbodies**
British Columbia	12	11
Alberta	1	1
Saskatchewan	0	0
Manitoba	0	0
Ontario	216	105
Quebec	10	9
New Brunswick	7	6
Nova Scotia	30	16
Prince Edward Island	1	1
Island of Newfoundland	1	1
Labrador	0	0
Yukon	0	0
Northwest Territories	0	0
Nunavut	0	0
total:	**231**	**150**

CHAPTER 1: Reference List

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CHAPTER 1: Supplementary Materials

Table 1: All sources (peer-review publications, grey literature, and internet) consulted for the literature review reported here. Sources are organized by categories used in this review. Note that sub-categories further specify source content. Categories could be broken down into sub-categories, for example "biology" included studies on morphology (Chiu et al. 2002; Lu et al. 2014; Smith 2000), genetics (Burks et al. 2016; David & Cote 2019; Hirano et al. 2019; Ueda et al. 2018; Wang et al. 2017; Yang et al. 2012), evolutionary history (Hirano et al. 2015; Li et al. 2018), and taxonomy (Fox 2007; Smith 2000).

Paper Title	**Author(s)**	**Year Published**	**Sub-Category**	**Category**	**Country of Origin**	**Species Names Used for the Chinese mystery snail**
First record of Japanese Mystery Snail *Cipangopaludina japonica* (von Martens, 1861) in Texas	Burks et al.	2016	Genetic	Biology	USA	Non-native species Invasive species *Cipangopaludina chinensis*
Morphometric analysis of shell and operculum variations in the viviparid snail, *Cipangopaludina chinensis* (Mollusca: Gastropoda), in Taiwan	Chiu et al.	2002	Morphology	Biology	Taiwan	*Cipangopaludina chinensis* *C. c. fluminalis* *C. c. aubryana* *C. c. longispira* *C. c. hanianensis*
Genetic evidence confirms the presence of the Japanese mystery snail, *Cipangopaludina japonica* (von Martens, 1861) (Caenogastropoda:	David and Cote	2019	Genetic	Biology	USA	Asian mystery snail Invasive species *Cipangopaludina chinensis*

Viviparidae) in northern New York						
Bellamya japonica: Japanese mystery snail	Fox	2007	Grey Literature	Biology	USA	No longer accessible
Enigmatic incongruence between mtDNA and nDNA revealed by multi-locus phylogenomic analyses in freshwater snails	Hirano et al.	2019	Genetic	Biology		*Cipangopaludina chinensis* *C. c. chinensis* *C. c. laeta*
Phylogeny of freshwater viviparid snails in Japan	Hirano et al.	2015	Evolution	Biology	Japan	*Cipangopaludina chinensis* *C. c. chinensis* *C. c. laeta*
Molecular analysis of intestinal bacterial communities in *Cipangopaludina chinensis* used in aquatic ecology restorations	Li	2012	Biology	Biology	China	Mud-snail *Cipangopaludina chinensis*
Mid-Neolithic Exploitation of Mollusks in the Guanzhong Basin of Northwestern China: Preliminary Results	Li et al.	2013	Evolution	Biology	China	*Cipangopaludina chinensis*
Radiocarbon dating of aquatic gastropod shells and its significance in reconstructing Quaternary environmental changes in the Alashan Plateau of northwestern China	Li et al.	2018	Evolution	Biology	China	*Cipangopaludina chinensis*
Morphological analysis of the *Chinese Cipangopaludina* species (Gastropoda; Caenogastropoda: Viviparidae)	Lu et al.	2014	Morphology	Biology	China	*Cipangopaludina chinensis* *C. wingatei* *C. fluminalis*

Identification of Carotenoids in the Freshwater Shellfish *Unio douglasiae nipponensis*, *Anodonta lauta*, *Cipangopaludina chinensis laeta*, and *Semisulcospira libertina*	Maoka et al.	2012	Biology	Biology	Japan	*Cipangopaludina chinensis laeta*
Notes on the taxonomy of introduced Bellamya (Gastropoda: Viviparidae) species in northeastern North America	Smith	2000	Morphology	Biology	USA	Introduced species *Bellamya chinensis* *Cipangopaludina chinensis*
Fecundity of the Chinese mystery snail in a Nebraska reservoir	Stephen et al.	2013	Biology	Biology	USA	Mystery snail Chinese mystery snail Non-indigenous species Invasive species *Bellamya chinensis* *Viviparus malleatus* *Cipangopaludina malleatus* *Cipangopaludina chinensis*
Heterologous expression and characterization of a cold-adapted endo-1,4−β−glucanase gene from *Bellamya chinensis laeta*	Ueda et al.	2018	Genetic	Biology	Japan	*Bellamya chinensis laeta*

Sequencing of the complete mitochondrial genomes of eight freshwater snail species exposes pervasive paraphyly within the Viviparidae family (Caenogastropoda).	Wang et al.	2017	Genetic	Biology	China	*Cipangopaludina chinensis*
A study on the sub-microscopic structure of mature sperm and spermatogenesis in *Cipangopaludina chinensis* （中国圆田螺成熟精子和精子发生的超微结构研究）	Yan	2002	Biology	Biology	China	*Cipangopaludina chinensis*
LPS-induced TNF alpha factor (LITAF) in the snail *Cipangopaludina chinensis*: Gene cloning and its apoptotic effect on NCI-H446 cells	Yang et al.	2012	Genetic	Biology	China	*Cipangopaludina chinensis*
Research progress on the interaction between alien invasive snail *Pomacea canaliculata* and native species （外来入侵生物福寿螺与本地生物的互作影响研究进展）	Zhang et al.	2017	Biology	Biology	China	*Cipangopaludina chinensis*
Remediation of Eutrophic Water Body by Zoobenthos	Chen et al.	2011	Bioremediation	Commercial Use	China	*Cipangopaludina chinensis*
Reponses of Hatchery-Cultured Mud Crab (*Scylla paramamosain*) Instar	Gong et al.	2017	Commercial USe	Commercial Use	Israel	Chinese mystery snail

Fed Natural Prey and an Artificial Feed						*Cipangopaludina chinensis*
Life cycle of the aquatic firefly *Luciola ficta* (Coleoptera: Lampyridae)	Ho et al.	2010	Commercial USe	Commercial Use	China	Water-snail *Cipangopaludina chinensis*
Removal of heavy-metals and sewage-sludge using the mud snail, *Cipangopaludina chinensis malleata* reeve, in paddy fields as artificial wetlands	Kurihara and Suzuki	1987	Bioremediation	Commercial Use	Japan	Mud-snail *Cipangopaludina chinensis malleata*
Nutritional components and utilization values of golden apple snails (*Pomacea canaliculata*) in different habitats（不同生境福寿螺的营养成分及其利用价值）	Luo et al.	2012	Commercial Use	Commercial Use	China	*Cipangopaludina chinensis*
Effects of Different Culturing Modes on Pond Environment and Output of *Trionyx sinensis*（中华鳖不同生态养殖模式对池塘水环境及养殖效果的影响）	Tian et al.	2012	Commercial Use	Commercial Use	China	*Cipangopaludina chinensis*
Heavy metal concentrations in *Cipangopaludina chinensis* (Reeve, 1863) and relationships with sediments in Saint-Augustin Lake, Quebec City (Qc, Canada).	Tornimbeni et al.	2013	Bioremediation	Commercial Use	Canada	*Cipangopaludina chinensis*

Biosorption of Pb(II) by the shell of viviparid snail: Implications for heavy metal bioremediation	Xing et al.	2016	Bioremediation	Commercial Use	China	*Cipangopaludina chinensis*
Distribution of the on-native viviparid snails, *Bellamya chinensis* and *Viviparus georgianus*, in Minnesota and the first record of *Bellamya japonica* from Wisconsin	Bury et al.	2007	Distribution	Distribution	USA	Asian freshwater snail Non-native species *Bellamya chinensis* *Cipangopaludina chinensis*
Asian Apple Snail, *Cipangopaludina chinensis* (Viviparidae) in Oneida Lake, New-York	Clarke	1978	Distribution	Distribution	USA	Asian Apple Snail *Cipangopaludina chinensis*
The freshwater molluscs of Canada	Clarke	1981	Distribution	Distribution	Canada	Oriental mystery snail *Cipangopaludina chinensis* *Viviparus japonicus* *V. malleatus*
The Genus Viviparus (Viviparidae) in North America	Clench and Fuller	1965	Distribution	Distribution	USA	Oriental snail *Cipangopaludina chinensis* *Paludina malleata* *Viviparus chinensis malleata* *Viviparus malleatus*

Coupling ecological and social network models to assess "transmission" and "contagion" of an aquatic invasive species	Haak et al.	2017	Distribution Modeling	Distribution	USA	Chinese mystery snail Invasive species *Bellamya chinensis*
Introduction, distribution, spread, and impacts of exotic freshwater gastropods in Texas	Karatayev et al.	2009	Distribution	Distribution	USA	Chinese mysterysnail Exotic species *Cipangopaludina chinensis*
Cipangopaludina chinensis (Gray, 1834): U.S. Geological Survey, Nonindigenous Aquatic Species Database	Kipp et al.	2020	Distribution	Distribution	USA	Chinese mysterysnail Oriental mysterysnail Asian applesnail Chinese applesnail Non-indigenous species *Viviparus malleatus* *V. chinensis malleatus* *Bellamya chinensis* *B. chinensis malleatus* *Cipangopaludina chinensis* *C. chinensis malleatus*
Occurrence of the Chinese mystery snail,	McAlpine et al.	2016	Distribution	Distribution	Canada	Chinese mystery snail

Cipangopaludina chinensis (Gray, 1834) (Mollusca: Viviparidae) in the Saint John River system, New Brunswick, with review of status in Atlantic Canada						Non-native mollusc *Cipangopaludina chinensis* *Bellamya chinensis*
Population dynamics of the non-native freshwater gastropod, *Cipangopaludina chinensis* (Viviparidae): a capture-mark-recapture study	McCann	2014	Distribution	Distribution	USA	Chinese mystery snail Non-native species *Cipangopaludina chinensis* *Bellamya chinensis*
Using Maximum entropy to predict the potential distribution of an invasive freshwater snail	Papes et al.	2016	Distribution Modeling	Distribution	USA	Chinese mystery snail Invasive freshwater snail *Cipangopaludina chinensis* *C. c. malleata* *Bellamya chinensis*
Etude de trois populations de *Viviparus malleatus* (Reeve) (Gastropoda, Prosobranchia) de la région de Montréal. I. Croissance, fécondité, biomasse et production annuelle	Stańczykowska et al.	1971	Distribution	Distribution	Canada	*Viviparus malleatus*
Distribution and Conservation Status of the freshwater	Stephen	2017	Distribution	Distribution	USA	Chinese mystery snail

gastropods of Nebraska						Non-indigenous species *Bellamya chinensis*
The invasive Chinese mystery snail *Bellamya chinensis* (Gastropoda: Viviparidae) expands its European range to Belgium.	Neucker et al.	2017	Distribution	Distribution	Belgium	Chinese mystery snail Freshwater snail *Bellamya chinensis* *Cipangopaludina chinensis*
Thermal tolerance limits of the Chinese mystery snail (*Bellamya chinensis*): Implications for management	Burnett et al.	2018	Ecological threshold	Ecology	USA	Chinese mystery snail Invasive species *Bellamya chinensis*
Prevalence of larval Helminths in fresh-water snails of the Kinmen Islands.	Chao et al.	1993	Parasitology	Ecology	Taiwan	*Cipangopaludina chinensis*
Cipangopaludina chinensis malleata (Gastropoda: Viviparidae): A new second molluscan intermediate host of a human intestinal fluke *Echinostoma cinetorchis* (Trematoda: Echinostomatidae) in Korea	Chung and Jung	1990	Parasitology	Ecology	South Korea	Rice field snail Viviparid snail *Cipangopaludina chinensis malleata*
Effect of periphyton community structure on heavy metal accumulation in mystery snail (*Cipangopaludina chinensis*): A case	Cui et al.	2012	Chinese Literature	Ecology	China	Mystery snail Chinese mystery snail *Cipangopaludina chinensis*

study of the bai River, China						
Heavy metal concentrations in edible bivalves and gastropods available in major markets of the Pearl River Delta	Fang et al.	2001	Chinese Literature	Ecology	China	*Cipangopaludina chinensis*
Bioenergetics and habitat suitability models for the Chinese mystery snail (*Bellamya chinensis*).	Haak	2015	Grey Literature	Ecology	USA	Chinese mystery snail Aquatic invasive species *Bellamya chinensis*
Assessing infection patterns in Chinese mystery snails from Wisconsin, USA using field and laboratory approaches	Harried et al.	2015	Parasitolo gy	Ecology	USA	Invasive snail Exotic snail *Bellamya chinensis* *Cipangopaludina chinensis*
Survival of the exotic Chinese mystery snail (*Cipangopaludina chinensis malleata*) during air exposure and implications for overland dispersal by boats	Havel	2011	Ecologica l threshold	Ecology	USA	Chinese mystery snail Exotic snail *Bellamya chinensis* *Cipangopaludina chinensis malleata*
Resistance to desiccation in aquatic invasive snails and implications for their overland dispersal	Havel et al.	2014	Ecologica l threshold	Ecology	USA	Invasive aquatic snail Chinese mystery snail *Bellamya chinensis* *Cipangopaludina chinensis*

EXOTIC MOLLUSCS IN THE GREAT LAKES HOST EPIZOOTICALLY IMPORTANT TREMATODES	Karatayev et al.	2012	Parasitology	Ecology	Canada/ USA	Exotic mollusc Chinese mystery snail *Cipangopaludina chinensis*
Size-Selective Predation By Ringed Crayfish (*Orconectes Neglectus*) On Native And Invasive Snails	Kelley	2016	Grey Literature	Ecology	USA	Invasive snail Chinese mystery snail *Cipangopaludina chinensis*
Incorporation of heavy-metals by the mud snail, *Cipangopaludina chinensis malleata* reeve, in submerged paddy soil treated with composted sewage-sludge	Kurihara et al.	1987	Chinese Literature	Ecology	Japan	Mud snail *Cipangopaludina chinensis malleata*
Effects of ambient temperature and the mud snail *Cipangopaludina chinensis laeta* (Architaenioglossa: Viviparidae) on performance of rice plants	Kurniawan et al.	2018	Chinese Literature	Ecology	Japan	Mud snail *Cipangopaludina chinensis laeta*
Representing calcification in distribution models for aquatic invasive species: surrogates perform as well as CaCO3 saturation state	Latzka et al.	2015	Ecological threshold	Ecology	USA	Aquatic invasive species Chinese mystery snail *Bellamya chinensis* *Cipangopaludina chinensis*
Distribution of the family Viviparidae	Liu et al.	1995	Chinese Literature	Ecology	China	*Cipangopaludina chinensis*

from China (Mollusca: Gastropoda) (中国田螺科的地理分布)						
Experimental study on compatibility of three species of freshwater snails with *Angiostrongylus cantonensis* （三种淡水螺与广州管圆线虫相容性的实验研究）	Lü et al.	2006	Chinese Literature	Ecology	China?	*Cipangopaludina chinensis*
Investigation on the animal food contaminated with parasites in markets in Huangpu District of Shanghai from 2015 to 2017 （2015-2017年上海市黄浦区市售动物食品寄生虫感染情况调查）	Lu et al.	2018	Chinese Literature - Parasitology	Ecology	China	*Cipangopaludina chinensis*
Trace Element (Pb, Cd, and As) Contamination in the Sediments and Organisms in Zhalong Wetland, Northeastern China	Luo et al.	2016	Chinese Literature	Ecology	China	*Cipangopaludina chinensis*
Impacts of environmental factors in rice paddy fields on abundance of the mud snail (*Cipangopaludina chinensis laeta*).	Nakanishi et al.	2014	Chinese Literature	Ecology	Japan	Mud snail *Cipangopaludina chinensis laeta*
Land Use Effects on Benthic Macroinvertebrate Communities in Conesus, Hemlock,	Owens	2017	Grey Literature	Ecology	USA	Potentially invasive species Invasive snail

Canadice, and Honeoye Lakes						*Cipangopaludina chinensis malleata*
In utero predator-induced response in the viviparid snail *Bellamya chinensis*	Prezant et al.	2006	Ecology	Ecology	USA	Viviparid snail *Bellamya chinensis* *Cipangopaludina chinensis*
Obstruction of the upstream migration of the invasive snail *Cipangopaludina chinensis* by high water currents	Rivera and Peters	2008	Grey Literature	Ecology	USA	Viviparid snail Asian invasive species *Cipangopaludina chinensis*
Echinostoma macrorchis (Digenea: Echinostomatidae): Metacercariae in *Cipangopaludina chinensis malleata* Snails and Adults from Experimental Rats in Korea	Sohn and Na	2017	Parasitology	Ecology	South Korea	*Cipangopaludina chinensis malleata*
Bioaccumulation of polychlorinated dibenzo-p-dioxins and dibenzofurans in the foodweb of Ya-Er Lake Area, China	Wu et al.	2001	Chinese Literature	Ecology	China	*note this study focused on a very similar species to *C. chinensis* which is *Bellamya aeruginosa*
Tolerance values of macroinvertebrate taxa in Liao River basin （辽河流域大型底栖动物耐污值）	Zhao et al.	2016	Chinese Literature	Ecology	China	*Cipangopalduina chinensis*
Spread and impact of freshwater invasive invertebrates in North America	Bobeldyk	2009	Grey Literature	Ecosystem Impacts	USA	Chinese mystery snail *Bellamya chinensis* *Cipangopaludina chinensis*

Effects of a mud snail *Cipangopaludina chinensis laeta* (Architaenioglossa: Viviparidae) on the abundance of terrestrial arthropods through rice plant development in a paddy field	Dewi et al.	2017	Ecosystem Impacts	Ecosystem Impact	Japan	Mud snail *Cipangopaludina chinensis laeta*
Interactions among invaders: community and ecosystem effects of multiple invasive species in an experimental aquatic system	Johnson et al.	2009	Ecosystem Impacts	Ecosystem Impact	USA	Chinese mystery snail Invasive species *Bellamya chinensis*
Home-field advantage: native signal crayfish (*Pacifastacus leniusculus*) out consume newly introduced crayfishes for invasive Chinese mystery snail (*Bellamya chinensis*)	Olden et al.	2009	Ecosystem Impacts	Ecosystem Impact	USA	*Bellamya chinensis*
Filtration rates of the non native Chinese mystery snail (*Bellamya chinensis*) and potential impacts on microbial communities	Olden et al.	2013	Ecosystem Impacts	Ecosystem Impact	USA	Chinese mystery snail Non-native species *Bellamya chinensis*
Distribution and community-level effects of the Chinese mystery snail (*Bellamya chinensis*) in northern Wisconsin lakes	Solomon et al.	2009	Ecosystem Impacts	Ecosystem Impact	USA	Chinese mystery snail Invasive gastropod *Bellamya chinensis* *Cipangopaludina chinensis*

Effects of Competition and Predation on the Feeding Rate of the Freshwater Snail, *Helisoma trivolvis*	Sura and Mahon	2011	Ecosystem Impacts	Ecosystem Impact	USA	Chinese mystery snail Invasive species *Cipangopaludina chinensis*
Non-native Chinese mystery snail (*Bellamya chinensis*) supports consumers in urban lake food webs	Twardochleb and Olden	2016	Ecosystem Impacts	Ecosystem Impact	USA	Chinese mystery snail Non-native species *Bellamya chinensis*
Population estimate of Chinese mystery snail (*Bellamya chinensis*) in a Nebraska reservoir	Chaine et al.	2012	Management	Management	USA	Chinese mystery snail Aquatic invasive species *Bellamya chinensis*
Invasion biology and risk assessment of the recently introduced Chinese mystery snail, *Bellamya* (*Cipangopaludina*) *chinensis* (Gray, 1834), in the Rhine and Meuse River basins in Western Europe	Collas et al.	2017	Management	Management	The Netherlands	Chinese mystery snail Introduced species *Bellamya chinensis* *Cipangopaludina chinensis*
Toxicity of copper sulfate and rotenone to Chinese mystery snail (*Bellamya chinensis*)	Haak et al.	2014	Management	Management	USA	Chinese mystery snail *Bellamya chinensis* *Cipangopaludina chinensis*
Management approaches for the alien Chinese mystery	Matthews et al.	2017	Management	Management	The Netherlands	Chinese mystery snail Alien species

snail (*Bellamya chinensis*)						*Bellamya chinensis*
INTRODUCED SPECIES Aquatic Invasive Species Transport via Trailered Boats: What Is Being Moved, Who Is Moving It, and What Can Be Done	Rothlisberger et al.	2010	Management	Management	USA	Nonindigenous species *Cipangopaludina chinensis malleata*
Survival and behavior of Chinese mystery snails (*Bellamya chinensis*) in response to simulated water body drawdowns and extended air exposure	Unstad et al.	2013	Management	Management	USA	Chinese mystery snail Invasive aquatic snail *Bellamya chinensis* *Cipangopaludina chinensis malleata*
Food utilization of shell-attached algae contributes to the growth of host mud snail, *Bellamya chinensis*: Evidence from fatty acid biomarkers and carbon stable isotope analysis	Fujibayashi et al.	2016	Medical Study	Medical Use	Japan	Freshwater mud snail *Bellamya chinensis* *Cipangopaludina chinensis*
Preliminary characterization and potential hepatoprotective effect of polysaccharides from *Cipangopaludina chinensis*	Jiang et al.	2013	Medical Study	Medical Use	China	Viviparid gastropod *Cipangopaludina chinensis*
Ethnopharmacological implications of quantitative and network analysis for traditional knowledge regarding the medicinal use of animals by indigenous	Kim et al.	2018	Medical Study	Medical Use	South Korea	*Cipangopaludina chinensis malleata* Nonureongi

people in Wolchulsan National Park, Korea						
Isolation and identification of chondroitin sulfates from the mud snail	Lee et al.	1998	Medical Study	Medical Use	South Korea	*Cipangopaludina chinensis* Mud snail
Characterization of a novel purified polysaccharide from the flesh of *Cipangopaludina chinensis*	Shi et al.	2016	Medical Study	Medical Use	China	*Cipangopaludina chinensis*
Lifelong neurogenesis in the cerebral ganglion of the Chinese mud snail, *Cipangopaludina chinensis*	Swart et al.	2017	Medical Study	Medical Use	USA	Chinese Mud snail Viviparid snail *Bellamya chinensis* *Cipangopaludina chinensis*
The effects of Roxithromycin on the Activity of APND and GST in *Cipangopaludina chinensis*	Wang et al.	2015	Medical Study	Medical Use	China	*Cipangopaludina chinensis*
Effects of nonylphenol on expression levels of GnEH-like polypeptide in *Cipangopaludina chinensis*	Wang et al.	2016	Medical Study	Medical Use	China	*Cipangopaludina chinensis*
Effect of Roxithromycin on the morphology of tissues in the liver of *Cipangopaludina chinensis*	Wang et al.	2014	Medical Study	Medical Use	China	*Cipangopaludina chinensis*
Anti-inflammatory and anti-angiogenic activities of a purified polysaccharide from flesh of	Xiong et al.	2017	Medical Study	Medical Use	China	Edible mollusc *Cipangopaludina chinensis*

Cipangopaludina chinensis						
Purification, characterization and immunostimulatory activity of polysaccharide from *Cipangopaludina chinensis*	Xiong et al.	2013	Medical Study	Medical Use	China	Viviparid gastropod *Cipangopaludina chinensis*
Protective activities of polysaccharides from *Cipangopaludina chinensis* against high-fat-diet-induced atherosclerosis via regulating gut microbiota in ApoE-deficient mice	Xiong et al.	2019	Medical Study	Medical Use	China	*Cipangopaludina chinensis*
Preparation of Chinese mystery snail shells derived hydroxyapatite with different morphology using condensed phosphate sources	Zhou et al.	2016	Medical Study	Medical Use	China	Chinese mystery snail Popular aquatic food *Cipangopaludina chinensis*
Cipangopaludina chinensis (Gastropoda: Viviparidae) in North America, Review and Update	Jokinen	1982	Review	Review	USA	*Bellamya chinensis* *Cipangopaludina chinensis* Chinese mystery snail
Chinese Mystery Snail (*Bellamya chinensis*) Review	Waltz	2008	Grey Literature	Reviews		Chinese mystery snail Chinese vivipara Tanisha Rice snail Chinese apple snail

						Asian apple snail *Bellamya chinensis* *B. c. malleata* *Cipangopaludina chinensis* *C. c. malleata* *Viviparus malleatus*
Chinese Mystery Snail Alert	BC Government	2019	Canada Internet Source	Internet Source	Canada	Chinese mystery snail Invasive species *Cipangopaludina chinensis*
Vivipare chinoise (*Cipangopaludina/Bellamya chinensis*).	Forets, Faune et Parcs Québec	2018	Canada Internet Source	Internet Source	Canada	Espèce exotique (Exotic Species) La vivipare chinoise (Chinese viviparid) *Bellamya chinensis* *Cipangopaludina chinensis* *Vivipare chinoise*
Aquatic Invasive Species Regulations	Government of Canada	2015	Canada Internet Source	Internet Source	Canada	Chinese mystery snail *Cipangopaludina chinensis*
Invasive Species	Severn Sound Environmental Association	NA	Canada Internet Source	Internet Source	Canada	Alien species Non-native species Invasive species

						Chinese mystery snail Invasive snail *Bellamya chinensis* *Cipangopaludina chinensis*
Invasive Snails	Ontario's Invasive Species Awareness Program	2012	Canada Internet Source	Internet Source	Canada	Chinese Mysterysnail Invasive snail *Bellamya chinensis*
Watershed Invasive Species Watch-The Chinese Mystery Snail	Bonnechere River Watershed Project	2011	Canada Internet Source	Internet Source	Canada	Chinese mystery snail Chinese vivipara Tanisha Rice snail Chinese apple snail Asian apple snail *Cipangopaludina chinensis*
Invasive Species Control	South Nation Conservation	2018	Canada Internet Source	Internet Source	Canada	Chinese mystery snail Invasive species
Chinese Mystery Snail	Alberta Invasive Species Council	2018	Canada Internet Source	Internet Source	Canada	Chinese mystery snail *Cipangopaludina chinensis* *C. malleata*

						C. c. malleata *Viviparus malleata* *V. japonicus* *Paludina malleata* *Bellamya chinensis*
Chinese mystery snails in Atlantic Canada	Dynamic Environment & Ecosystem Health Research Group	2019	Canada Internet Source	Internet Source	Canada	Chinese mystery snail Aquatic invasive invertebrate *Bellamya chinensis* *Cipangopaludina chinensis* *Paludina chinensis*
Cipangopaludina chinensis	Köhler et al.	2012	Internet Source-International	Internet Source	Based in USA	Chinese mystery snail *Bellamya chinensis* *Cipangopaludina chinensis* *Paludina chinensis*
Australian Freshwater Molluscs	Ponder et al.	2016	Internet Source-Australia	Internet Source	Australia	Chinese mystery snail Chinese apple snail *Cipangopaludina chinensis*

Chinese Mystery Snail	Indiana Government	1993	US Internet Source	Internet Source	USA	Chinese mystery snail Japanese mystery snail Japanese black snail Japanese trapdoor snail *Cipangopaludina chinensis* *C. malleata* *C. c. chinensis* *C. c. malleata* *Viviparus chinensis malleatus* *V. japonicus* *V. malleatus* *V. stelmaphora* *Paludina malleata* *P. japonicus*
Chinese Mystery Snail	Cornell Cooperative Extension	NA	US Internet Source	Internet Source	USA	Chinese mystery snail Aquatic invasive species Nonindigenous species *Cipangopaludina chinensis*

How to Prevent the Spread of Chinese Mystery Snails | Lake George Invasive Species	Lake George Association	NA	US Internet Source	Internet Source	USA	Chinese mystery snail Japanese mystery snail Oriental mystery snail Invasive species *Cipangopaludina chinensis malleata*
Mystery Snails (Chinese, Japanese and Banded).	Minnesota Sea Grant	2016	US Internet Source	Internet Source	USA	Chinese mystery snail *Cipangopaludina chinensis*
Chinese Mystery Snail: Texas Invasive Species Institute	Texas Government	NA	US Internet Source	Internet Source	USA	Chinese mystery snail *Cipangopaludina chinensis malleata*
Chinese Mystery Snail, *Cipangopaludina chinensis malleatus* - invasive	The Maine Invasion	NA	US Internet Source	Internet Source	USA	*Cipangopaludina chinensis malleatus* Chinese mystery snail
Chinese Mystery Snail	University of Wisconsin Sea Grant	NA	US Internet Source	Internet Source	USA	Chinese mystery snail *Cipangopaludina chinensis*
Chinese mystery snail	Vander Zanden Lab Center for Limnology	NA	US Internet Source	Internet Source	USA	Chinese mystery snail *Cipangopaludina chinensis*
Chinese mystery snail (*Cipangopaludina chinensis*) Ecological Risk Screening Summary	U.S. Fish & Wildlife Services	2011	US Internet Source	Internet Source	USA	Chinese mystery snail Mystery snail

						Oriental mystery snail *Cipangopaludina chinensis* *C. c. malleata* *C. c. malletus* *Bellamya chinensis* *B. c. malleatus* *Viviparus malleatus* *V. chinensis malleatus*
Aquatic Invasive Species Quick Guide Chinese mystery snail (*Cipangopaludina chinensis* Reeve)	Golden Sands Resource Conservation & Development (RC&D) Council, Inc.	NA	US Internet Source	Internet Source	USA	Chinese mystery snail Aquatic invasive species *Cipangopaludina chinensis*
SESWIC	Southeastern Wisconsin Invasive Species Consortium, Inc.	NA	US Internet Source	Internet Source	USA	Chinese mystery snail Large invasive snail *Bellamya chinensis*
Chinese Mystery Snail	Sea Grant University of Wisconsin	2018	US Internet Source	Internet Source	USA	Chinese mystery snail
CHINESE MYSTERY SNAIL *Cipangopaludina chinensis malleata* (syn. *Bellamya chinensis*)	Missouri Department of Conversation	NA	US Internet Source	Internet Source	USA	Chinese mystery snail Asian apple snail Oriental mystery snail

						Invasive species *Bellamya chinensis* *Cipangopaludina chinensis malleata* Chinese mystery snail
Chinese Mystery Snail (*Cipangopaludina chinensis*)	Minnesota Department of Natural Resources	NA	US Internet Source	Internet Source	USA	Chinese mystery snail Invasive species *Cipangopaludina chinensis*
Cary Institute of Ecosystem Studies-Invasive Species	Dr. David L. Strayer	2013	US Internet Source	Internet Source	USA	Chinese mystery snail Invasive species
CHINESE MYSTERY SNAIL *Cipangopaludina chinensis malleata*	Texas Invasive Species Institute	2014	US Internet Source	Internet Source	USA	Chinese mystery snail *Cipangopaludina chinensis malleata*
Pennsylvania's Field Guide to Aquatic Invasive Species	Sea Grant Pennsylvania	2013	US Internet Source	Internet Source	USA	Chinese mystery snail Aquatic invasive species *Cipangopaludina chinensis*
Chinese Mystery Snails	Lake Stewards of Maine	NA	US Internet Source	Internet Source	USA	Chinese mystery snail *Cipangopaludina chinensis malleatus*
Species Profile: *Cipangopaludina chinensis*	Global Invasive Species Database	2011	Internet Source-International	Internet Source	Based in USA	Chinese mystery snail Trapdoor snail

						Chinese mysterysnail Mystery snail Oriental mystery snail Asian freshwater snail Asian apple snail *Viviparus malleatus* *V. japonicus* *V. stelmaphora* *V. chinensis malleatus* *Paludina malleata* *Paludina japonicus* *Bellamya chinensis* *Cipangopaludina chinensis* *C. c. malleata* *C. malleata*

Table 2: List of the internet sources referred to in SS Table 1 with corresponding website links (active as of February 2020). Those sites are also archived on the Internet Archive "Wayback Machine" (https://archive.org/web/web.php).

Website Title	**Author**	**Website Link**

Chinese Mystery Snail Alert	BC Government	https://www2.gov.bc.ca/assets/gov/environment/plants-animals-and-ecosystems/invasive-species/alerts/chinese_mystery_snail_alert.pdf
Vivipare chinoise (Cipangopaludina/Bellamya chinensis).	Forets, Faune et Parcs Québec	https://mffp.gouv.qc.ca/la-faune/especes/envahissantes/vivipare-chinoise/
Aquatic Invasive Species Regulations	Government of Canada	https://laws-lois.justice.gc.ca/eng/regulations/SOR-2015-121/page-4.html
Invasive Species	Severn Sound Environmental Association	https://www.severnsound.ca/programs-projects/wildlife-habitat/invasive_species
Invasive Snails	Ontario's Invasive Species Awareness Program	http://www.invadingspecies.com/invasive-snails/
Watershed Invasive Species Watch-The Chinese Mystery Snail	Bonnechere River Watershed Project	http://www.bonnechereriver.ca/brwp-news/watershedinvasivewatch-thechinesemysterysnail
Invasive Species Control	South Nation Conservation	https://www.nation.on.ca/water/projects/invasive-species-control
Chinese Mystery Snail	Alberta Invasive Species Council	https://abinvasives.ca/wp-content/uploads/2018/05/FS-Chinese-Mystery-Snail.pdf
Chinese mystery snails in Atlantic Canada	Dynamic Environment & Ecosystem Health Research Group	http://www.ap.smu.ca/~lcampbel/CMS.html
Cipangopaludina chinensis	Köhler et al. (IUCN, International Union for Conservation of Nature)	https://www.iucnredlist.org/species/166265/1124988
Australian Freshwater Molluscs	Ponder et al.	https://keys.lucidcentral.org/keys/v3/freshwater_molluscs/Freshwater_Oct18/Media/Html/cipangopaludina_chinensis.htm
Chinese Mystery Snail	Indiana Government	https://www.in.gov/dnr/files/CHINESE_MYSTERY_SNAIL.pdf

Chinese Mystery Snail	Cornell Cooperative Extension	http://monroe.cce.cornell.edu/environment/invasive-nuisance-species/aquatic-invasives/chinese-mystery-snail
How to Prevent the Spread of Chinese Mystery Snails | Lake George Invasive Species	Lake George Association	https://www.lakegeorgeassociation.org/educate/science/lake-george-invasive-species/chinese-mystery-snail/
Mystery Snails (Chinese, Japanese and Banded).	Minnesota Sea Grant	http://www.seagrant.umn.edu/ais/mysterysnail
Chinese Mystery Snail: Texas Invasive Species Institute	Texas Government	http://www.tsusinvasives.org/home/database/cipangopaludina-chinensis-malleata
Chinese Mystery Snail, Cipangopaludina chinensis malleatus - invasive	The Maine Invasion	https://sites.google.com/a/rsu5.org/invasive/maine-invasive-species/chinese-mystery-snail-cipangopaludina-chinensis-malleatus
Chinese Mystery Snail	University of Wisconsin Sea Grant	https://www.seagrant.wisc.edu/our-work/focus-areas/ais/invasive-species/invasive-species-fact-sheets/mollusks/chinese-mystery-snail/
Chinese mystery snail	Vander Zanden Lab Center for Limnology	https://www.jakevzlab.net/chinese-mystery-snail.html
Chinese mystery snail (Cipangopaludina chinensis) Ecological Risk Screening Summary	U.S. Fish & Wildlife Services	https://www.fws.gov/fisheries/ans/erss/highrisk/ERSS-Cipangopaludina-chinensis-FINAL-March2018.pdf
Aquatic Invasive Species Quick Guide Chinese mystery snail (Cipangopaludina chinensis Reeve)	Golden Sands Resource Conservation & Development (RC&D) Council, Inc.	https://www.uwsp.edu/cnr-ap/UWEXLakes/Documents/programs/CLMN/AISfactsheets/05ChineseMysterySnail.pdf

SESWIC	Southeastern Wisconsin Invasive Species Consortium, Inc.	https://sewisc.org/invasives/invasive-animals/220-chinese-mystery-snail
Chinese Mystery Snail	Sea Grant University of Wisconsin	https://www.seagrant.wisc.edu/our-work/focus-areas/ais/invasive-species/invasive-species-fact-sheets/mollusks/chinese-mystery-snail/
CHINESE MYSTERY SNAIL Cipangopaludina chinensis malleata (syn. Bellamya chinensis)	Missouri Department of Conversation	https://nature.mdc.mo.gov/discover-nature/field-guide/chinese-mystery-snail
Chinese Mystery Snail (Cipangopaludina chinensis)	Minnesota Department of Natural Resources	https://www.dnr.state.mn.us/invasives/aquaticanimals/chinese-mystery-snail/index.html
Cary Institute of Ecosystem Studies-Invasive Species	Dr. David L. Strayer	https://www.caryinstitute.org/news-insights/feature/mysterious-mollusks-multiplying-valley
CHINESE MYSTERY SNAIL Cipangopaludina chinensis malleata	Texas Invasive Species Institute	http://www.tsusinvasives.org/home/database/cipangopaludina-chinensis-malleata
Pennsylvania's Field Guide to Aquatic Invasive Species	Sea Grant Pennsylvania	https://seagrant.psu.edu/sites/default/files/AIS%20Field%20Guide_Finalweb_0.pdf
Chinese Mystery Snails	Lake Stewards of Maine	https://www.lakestewardsofmaine.org/programs/other-programs/chinese-mystery-snails/
Species Profile: *Cipangopaludina chinensis*	Global Invasive Species Database	http://www.iucngisd.org/gisd/species.php?sc=1812

Table 3: Biological and ecological information for *C. chinensis* derived from English, French, and Chinese language literature

Parameter	Range	Literature Reference	Additional Notes
Thermal tolerance	Upper limit: between 40-45℃ Lower limit: <0℃	Burnett et al., 2018	Researchers were not able to find the lower limit because the experiment went only to 0℃. Exposure time varied because treatments were either heated or cooled (1℃/hr) until water temperature reach the testing temperature then aquaria were removed from testing chamber and allowed to recover to room temperature for 48 hr.
Water Temperature	0-30℃	Karatayev et al., 2009	Not actually testing thermal tolerance, simply reporting on water body temperatures where the species was found.
	19-21.4℃	Collas et al., 2017	Not actually testing thermal tolerance, simply reporting on water body temperatures where the species was found.
Air Exposure	Adult + mid-large size juveniles: +4 weeks Small juveniles: < 2 weeks	Havel, 2011	Havel's initial work found that *C. chinensis* could survive impress amounts of air exposure. These discoveries were later updated by Havel's student, Unstad.
	>9 weeks	Unstad et al., 2013	

$CaCO_3$ Saturation	0.00015	Latzka et al., 2015	These thresholds were determined using a study set of lakes in the North Highland District of Wisconsin. Therefore, these results were not true ecological threshold tests and only represent ecological ranges where this species was found.
Max depth	<15 m		
Conductivity	≥67 µS/cm		
Distance to HWY	<28 km		
Total P	No significant difference between lakes with *C. chinensis* and lakes without	Twardochleb & Olden, 2016	
Water Clarity			
Predators	Largemouth Bass and Pumpkinseed sunfish are able to eat *C. chinensis*, but yellow perch may not.		
pH	6.5-8.4	Jokinen, 1982	Reporting pH values where the species was found, no laboratory analysis was conducted to determine these ranges.
	4-10	Haak, 2015	Part of a larger PhD dissertation. Exposure duration: 4 weeks
	~4.2-8	Fraser et al. unpublished	Exposure duration: 14 days
Salinity	0.03-0.08ppt	Collas et al., 2017	Reporting salinity values where the species was found, no laboratory experiments were

			conducted to establish these ranges.
	0-10 ppt	Fraser et al. unpublished	Exposure duration: 14 days
Calcium Concentration	5-97 ppm	Jokinen, 1982	Noting calcium concentration range where the species occurs. Therefore, not a true ecological threshold test.
	<2- >20 ppm	Chiu et al., 2002	Noting calcium concentration range where the species occurs. Therefore, not a true ecological threshold test.
	2-120 ppm	Haak, 2015	Part of a larger PhD dissertation. Exposure duration: 4 weeks
Conductivity	63-400 us/cm	Jokinen, 1982	Noting conductivity range where the species occurs. Therefore, not a true ecological threshold test.
	4-9 us/cm	Chiu et al., 2002	Noting conductivity range where the species occurs. Therefore, not a true ecological threshold test.
	140-437 us/cm	Collas et al., 2017	Noting conductivity range where the species occurs. Therefore, not a true ecological threshold test.
Magnesium concentration	13-31 ppm	Jokinen, 1982	Noting magnesium range where the species occurs. Therefore, not a true ecological threshold test.
Sodium concentration	2-49 ppm	Jokinen, 1982	Noting salinity range where the species occurs. Therefore, not a true ecological threshold test.

Oxygen concentration	7-11 ppm	Jokinen, 1982	Noting dissolved oxygen concentration range where the species occurs. Therefore, not a true ecological threshold test.
Flow Rate	0.03-0.08 m/s	Collas et al., 2017	This is the suspected tolerance of water flow rate for habitats inhabited by *C. chinensis*.
Snail Density (individuals/m^2)	2.62-3.92	Stephen et al., 2013	
	<1-40	McCann, 2014	
	100	Karatayev et al., 2009	
	38	Solomon et al., 2010)	
	<0.5	Soes et al., 2011	This reference was from Collas et al. (2017).
	0.33	Collas et al., 2017	
	0.25-30	Nakanishi et al., 2014	

Filtration Rate	106-113 mL/Snail x h (max of 471 mL/ snail x h)	Olden et al., 2013 *This study also suggests that *C. chinensis* is related to Chlorophyll-a concentrations.	Similar to zebra mussel (22-375), quagga mussel (40-310), Asian clam (347-567), golden mussel (19-350), and blue mussel (17-2767). However, the study points out that it is difficult to compare filtration rates between different experiments that the setups will vary.
Reproduction rate based on fecundity	27.2-33.3 young per year per female	Stephen et al., 2013	This value varies greatly and has been estimated to be as high as 100 young per year per female.
Feeding Mechanism	Grazer or filter feeder	Olden et al., 2013	
Taxa Tolerance Values for Viviparidae	Liao River, Northeast China: 8.4 East China: 5 Idaho, USA: 6	Zhao et al., 2015	This study was a comparison of the abundance of various Viviparidae snails commonly found in China and the US in different habitat quality. Tolerance values are used for bioassessments where different species' assemblage can be indicative of the habitat health (e.g. areas of high contamination may have low biodiversity and are only inhabited by more tolerant species). The TV for *C. chinensis* indicates that in the Northeast Liao River of China, it has intermediate tolerance to poor water quality.
Taxa Tolerance Values for *Cipangopaludina chinensis*	Liao River, Northeast China: 4.6		
Land-use	No correlation found between different land-use (state protected forest vs	Owens 2017	

	developed housing) and *C. chinensis* presence		

Table 5: Predators of, and species competing with, *C. chinensis* as reported in the literature

Common Name in English	**Common Name in Chinese**	**Scientific name**	**Reference**	**Note**
Large-mouth Bass	大口黑鲈	*Micropterus salmoides*	Twardochleb & Olden, 2016	Original literature is in English
Pumpkinseed Sunfish	驼背太阳鱼	*Lepomis gibbosus*	Twardochleb & Olden, 2016	Original literature is in English
Ringed Crayfish		*Orconectes neglectus*	Kelley, 2016	Original literature is in English
Signal Crayfish	信号小龙虾	*Pacifastacus leniusculus*	Olden et al, 2009	Original literature is in English
Black Carp Fish	青鱼	*Mylopharyngodon piceus*	Yan, 2002	Original literature is in Chinese
Amur Carp Fish	鲤鱼	*Cyprinus rubrofuscus*	Yan, 2002	Original literature is in Chinese
Chinese Softshell Turtle	中华鳖	*Pelodiscus sinensis*	Tian et al, 2012	Original literature is in Chinese

<table>
<tr><td>Golden Apple Snails</td><td>福寿螺</td><td>Pomacea canaliculata</td><td>Luo et al, 2012;

Zhang et al, 2017</td><td>Original literature is in Chinese Pomacea canaliculata is a competing species to C. chinensis.</td></tr>
<tr><td>Muscovy Duck</td><td>番鸭</td><td>Cairina moschata</td><td>Luo et al, 2012;

Zhang et al, 2017</td><td rowspan="3">Original literature is in Chinese. Cairina moschata, Anas platyrhynchos domesticus, and Anas platyrhynchos are all referred as "ducks" (鸭) in Chinese literature.</td></tr>
<tr><td>Domestic Duck</td><td>北京鸭</td><td>Anas platyrhynchos domesticus</td><td>Luo et al, 2012;

Zhang et al, 2017</td></tr>
<tr><td>Mallard Duck</td><td>绿头鸭</td><td>Anas platyrhynchos</td><td>Luo et al, 2012;

Zhang et al, 2017</td></tr>
</table>

CHAPTER 2: Assessing the probable distribution of the potentially invasive Chinese Mystery Snail, *Cipangopaludina chinensis*, in Nova Scotia using a random forest model approach

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Abstract

Non-native species that become invasive threaten natural biodiversity and can lead to socioeconomic impacts. Prediction of invasive species distributions is important to prevent further spread and protect vulnerable habitats and species at risk (SAR) from future invasions. The Chinese mystery snail, *Cipangopaludina chinensis*, native to Eastern Asia, is a non-native, potentially invasive, freshwater snail now widely established across North America, Belgium, and the Netherlands. This species was first reported in Nova Scotia, eastern Canada in 1955, but was not found to be established until the 1990's and now exists at high densities in several urban lakes. Nonetheless, the presence and potential distribution of this species in Nova Scotia remains unknown. Limited resources make it difficult to do a broad survey of freshwater lakes in Nova Scotia, however a species distribution probability model has the potential to direct focus to priority areas. We apply a random forest model in tandem with a combination of water quality, fish community, anthropogenic water use, and geomorphological data to predict *C. chinensis* habitat in Nova Scotia (NS), Canada. All predicted probabilities of suitable *C. chinensisi* habitats in Nova Scotia were >50% and include Cape Breton Island, the Nova Scotia-New Brunswick border, and the Halifax Regional Municipality. Suitable habitats predicted for *C. chinensis* overlap with many SAR habitats, most notably brook floater mussel, *Alasmidonta varicosa*, and yellow lampmussel, *Lampsila cariosa*. Our results indicate that *C. chinensis* could become widespread throughout NS, appearing first in the aforementioned areas of highest probability. Further research is required to test *C. chinensis* ecological thresholds in order to improve the accuracy of future species distribution and habitat models, and to determine *C. chinensis* impacts on native freshwater mussel populations of conservation concern.

Introductions

The Chinese mystery snail, *Cipangopaludina* (= *Bellamya*) *chinensis* (Gray 1834), is a non-native, freshwater, gastropod native to Eastern Asia. The species was introduced to North America in the 1890s via the Asian food market and has since become widespread in natural environments in Canada, the United States of America (USA), Belgium, and the Netherlands (Benson 2011; Collas et al. 2017; Jokinen 1892; Matthews et al. 2017; McAlpine et al. 2016; Kingsbury, personal observation). It is suspected that *C. chinensis* is spreading through illegal aquarium releases and accidental boat transfers between infected and uninfected water bodies (Collas et al. 2017; Matthews et al. 2017; Rothlisberger et al. 2010). *C. chinensis* adults can survive more than nine weeks of air exposure, making long range, over-land dispersal possible (Unstad et al. 2013). *C. chinensis* reported occurrences in continental North America suggest that this species is far more widespread than currently documented.

Once introduced, *C. chinensis* presence may lead to blocked water pipes, fouled beaches, altered nitrogen: phosphorus ratios which can elevate eutrophication, and altered food webs through changes in bacterial community composition (Chao et al. 1993; Collas et al. 2017; Cui et al. 2012; Fang et al. 2001; Hanstein 2012; Harried et al. 2015; Xing et al. 2016). Pressure on native mollusc species may also occur through competition for resources (e.g. food, space, dissolved calcium). Female *C. chinensis* produce large broods of live young. Estimates of >100 offspring / female/ year have been suggested (Haak 2015; Havel 2011; Stephen et al. 2013; Unstad et al. 2013). Being more resistant to predation than native molluscs due to large size and the presence of a protective operculum “trapdoor” (Haak 2015; Johnson et al. 2009; Karatayev et al. 2009; Olden et al. 2013; Plinski et al. 1978; Sura & Mahon 2011), *C. chinensis* can become established rapidly within new water bodies. Although there is recognition in some provinces that *C. chinensis* introduction to Canada should be of concern, the species has not been identified by federal agencies as potentially invasive across the country, due to lack of published data for Canada (McAlpine et al. 2016; Schroeder et al. 2013).

It is difficult to manage *C. chinensis* once a population is established because the species is resistant to typical species management techniques such as heat (Burnett et al. 2018), desiccation (Unstad et al. 2013), and chemical treatments (Haak et al. 2014). Therefore, it is important to predict future *C. chinensis* distribution trends so as to proactively prevent introductions, especially to vulnerable habitats where species at risk (SAR) are present. Species distribution models (SDMs) are based on simulations that predict a species future distribution using habitat variables from areas of known species presences and real- or pseudo-absences (Hijmans & Elith 2016; Jackson et al. 2000; Law & Kelton 1991). SDMs have become important for freshwater ecosystem management as a means to predict aquatic invasive species (AIS) introductions and spread (Rodríguez-Rey et al. 2019; Drake & Bossenbroek 2009; Love et al. 2015; Phillips et al. 2004; Robbins 2004; Václavík & Meentemeyer 2009). SDMs have been used to predict the distributions of native species, rare species, marine ecosystem assemblages, and to forecast plant dispersal (Baldwin 2009; Best et al. 2007; DeAngelis & Grimm 2014; Evans et al. 2011; Joy & Death 2004; Merow et al. 2013; Mi et al. 2017; Phillips et al. 2004; Roberts et al. 2010). However, modeling AIS distributions can be challenging because AIS monitoring often excludes absence reports (Václavík & Meentemeyer 2009, 2012). Other AIS modeling challenges include selecting the right model for the species (Mi et al. 2017), choosing appropriate absence data (whether choosing to use true-absences or pseudo-absence or a combination of the two) (Václavík & Meentemeyer 2012), and balancing the assumptions made to fit a model to the current known species distribution without overfitting the model so that it prevents accurate prediction (DeAngelis & Grimm 2014; Hijmans & Elith 2016; Jackson et al. 2000; Merow et al. 2013; Rodríguez-Rey et al. 2019). *C. chinensis* is particularly difficult to accurately model because little is known about the species ecological thresholds or biological needs (Kingsbury et al. in prep).

Previous studies have used either generalized linear models (glm), Ecopath, or MaxEnt to predict *C. chinensis* distribution or environmental parameters important for its invasion success. However, all the previous modeling attempts for *C. chinensis* have been restricted to a single well-studied area of Wisconsin, USA. (Haak 2015; Haak et al. 2017; Latzka et al. 2015; Papes et al. 2016) and may not translate to other geographic regions that are less well monitored. Moreover, model type selection such as MaxEnt and glm, are problematic when applied specifically to AIS because MaxEnt has been misapplied (i.e. used to predict species distribution when MaxEnt is a tool for assessing habitat suitability) and glms assume linear relationships in data (Phillips et al. 2004; Richmond 2019). Finally, studies have yet to combine water chemistry, physical environmental parameters, ecosystem composition, and anthropogenic aquatic ecosystem interactions into one statistically robust species distribution model. Perhaps, due to gaps in freshwater monitoring data, or the limitations of models that tend to overfit when extreme numbers of parameters are included (e.g. glm), previous modeling attempts have been unable to incorporate multiple parameter types. As demonstrated previously in conservation science (Balden et al. 2020; Evans et al. 2011; Houngnandan et al. 2020; Maguire & Mundle 2020; Mi et al. 2017; Pearman et al. 2020), random forest models (RFMs) have the potential to be more accurate than other model types currently used (e.g. TreeNet, glm, MaxEnt). Nonetheless, RFMs have yet to become widely used for freshwater species, including invasive forms. A new statistically robust predictive modeling approach using species specific data is needed for more accurate predictions of *C. chinensis* distributions.

Random forest models (RFMs) build hundreds or thousands of decision trees by random selection of variables from different database subsets in various orders. Each tree is incorporated to build a 'forest' that is resilient to background noise, handles large datasets well, and allows for parameter-type (e.g. water chemistry, ecosystem composition, etc.) variation (Breiman 2001; Koehrsen 2018). RFMs average predictions made by each tree in the forest to give one prediction encompassing as many parameter interactions as possible within that forest size (Breiman 2001; Koehrsen 2018). RFMs are a collection of many individual regression trees, they do not assume linearity and, therefore, can identify important variables for accurate predictions (Breiman 2001). This model may be suitable for *C. chinensis* SDM as it can incorporate a number of variables, leading to predictions that are more robust, reliable, and flexible than other models..

Finally, RFM will indicate which model variables are highly correlated with *C. chinensis* presence, suggesting the most important factors for *C. chinensis* spread. This study aims to (1) predict suitable *C. chinensis* habitats in Atlantic Canada using a random forest model approach to indicate future distribution, (2) determine potential overlap between areas of high-probability for *C. chinensis* invasion and SAR habitat, and more generally, (3) determine the feasibility of using RFM for freshwater AIS distribution modeling.

Methods

Data Collection:

Habitat parameter datasets were obtained from various sources (e.g. Atlantic Data Stream, provincial government websites/databases, Boat Launches Canada, Google Maps) for 343 freshwater bodies (i.e. lakes, rivers, ponds, harbours, and bays) from six Canadian provinces: Nova Scotia (NS) (n=250), New Brunswick (NB) (n=44), Prince Edward Island (PEI) (n=3), Ontario (ON) (n=40), Alberta (AB) (n=1), and British Columbia (BC) (n=5). We logged 36 parameters, including 3 that identified sites (water body name, county name/station number, and data source), 2 that recorded spatial position (latitude and longitude), 17 water quality parameters (e.g. salinity, alkalinity), 6 geomorphic features (surface area, depths, shoreline development, and water body connectedness), 3 that were reflective of human perturbation (stocking frequency indicated greater recreational fishing pressures, number of public boat launches indicate recreational use, and distance to a highway from a boat launch indicate accessibility), and 2 that were indicative of current ecosystem make-up (number of fish species recorded and number of invasive species). See Supplementary Material Table 1 for a list of data sources and Figure 1 for a brief overview of the database. Surface area refers to the water body surface area (km^2). Depths reported both maximum depth (m) and mean depth (m). Water body connectedness was ascertained by counting the number of freshwater bodies directly connected to the water body in question (e.g. Lake Banook in Dartmouth, NS has two water bodies directly connected to it, Sullivan's Pond and Lake Micmac, therefore, the number of connected water bodies is 2).

As a means of quality assurance/quality control, each water body was searched using Google Maps to ensure that the name and location data were identical to the original dataset. Water bodies were excluded from the database if the water body name and latitude/longitude data did not match, if the given latitude/longitude did not align with any water body, or if it was not possible to confirm the location and water body name. See Supplementary Material Table 2 for a complete list of parameters recorded. To gather the data on each parameter, multiple data sources were used and combined from the year 2000 forward. If multiple sources of information were available for a single water body, the most recent data were used.

CMS = probability of *C. chinensis* presence, Lat = latitude, Lon = Longitude, Ca = calcium concentration in mg/L, Cond= conductivity, Na = sodium, No. Stocking = number of annual fish stocking events, no fish species = number of fish species recorded, no boat launches = number of public boat launches, Connected lakes = number of water bodies connected to a specific water body, Invasives = number of invasive species recorded. Note that species labelled as “invasive” are those identified as such by each respective province. Also, the number of recorded fish species varies depending on the individual provincal desire to monitor, stock, or record the number of native fish species in freshwater bodies.

Training the Model

RFMs were created to analyse water bodies with known *C. chinensis* presence/absence (or pseudo-absences) and predict *C. chinensis* probability of occurrence in water bodies where potential presence/absences were unknown (Table 1). Of the 343 water bodies, 46 had *C. chinensis* presence/absence data or pseudo-absence data which were used to train the RFMs. Based on preliminary work, we found data on the model parameters for 40 water bodies with known *C. chinensis* occurrences from ON (n=21), BC (n=5), and AB (n=1). Generated models were coded as RFM#, the number denoting the order in which each model was generated (RFM1, RFM2,....RFM12).The NS *C. chinensis* presence data (n=13) and true absence data (n=5) was kept separate for model validation (RFM 9-12) or was mixed with the ON data which were randomly subdivided into training and validation sets (RFM 2-8). We followed the MaxEnt method of selecting pseudo-absences, making our selection from a geographical area that is limited to the region with the greatest number of known species occurrences (in this case, ON). This allowed us to select water bodies with estimated *C. chinensis* absences that could then be used to train our models (Baldwin 2009; Phillips et al. 2004; Richmond 2019).

We semi-randomly selected 19 pseudo-absences from ON (in the Kingston-Toronto area). The known presence points from Kingsbury et al. (in prep), were mapped using ESRIs ArcGIS (ArcMap 10.7). The species presence points were aggregated (within a 35 km radius) and spatially joined to the aggregate polygon (hereafter called the TK polygon) in order to determine the area with the most reported *C. chinensis* presences. The background data points from our water body database were loaded as a layer (water bodies with unknown *C. chinensis* presence/absence). The background points that fell within the TK polygon were selected and exported into an Excel spreadsheet and randomly ranked. Points 1 through 30 were selected as potential pseudo-absence points. Points were excluded if, when cross-checked with known *C. chinensis* presence, they were located in water bodies with known populations. Hence, semirandomness of selected pseudo-absences was used to train our model. The final training dataset, containing 27 presence points and 19 absence points (total n=46), was built from as many water bodies as possible.

Each model used different combinations of formulae (Table 1) and datasets (i.e. different combinations of normalized or non-normalized data, Atlantic Canada + ON data or Atlantic Canada + ON + AB + BC, and with/without pseudo-absences). The error rates denoted in Table 2 are the out-of-bag errors, which represent a percentage of trees within the forest that incorrectly classified water bodies from the training or validation datasets. The number of predictions made by each model (Table 2) vary depending on the ability of that model to make predictions. Some models made fewer predictions than others due to data not being normalized on a scale of 0 to 1 (i.e. models that were confused by variations in parameter magnitude), model formulae including a significant number of parameters missing large amounts of data, or the training dataset being relatively smaller than that which was used to train models that produced a greater number of predictions.

Model Validation

A variety of validation methods were used. RFM1 was not validated as all of the *C. chinensis* presence/absence data were used to train the model. Although this provided an understanding of relative parameter importance for *C. chinensis* establishment success in NS, it was not statistically reliable. For this reason, we have chosen not to use RFM1 to represent predicted probabilities in NS. RFM2-8 used the k-fold cross validation technique (Al-Mukhtar 2019; Guillaumot et al. 2019). RFM9-12 were validated on *C. chinensis* presence/absence data including BC, AB, ON, and NS data. Validation error and the number of predictions each model was able to make were considered when selecting the final model (RFM9). Note that the model validations conducted are internal model validations (i.e. the model used data from our dataset). External model validation is recommended for future studies. Future occurrence reports of *C. chinensis* in Nova Scotia could be used as an external model validation.

Mapping Predicted C. chinensis Habitat Suitability, Species at Risk (SAR), and Significant Habitat

Model prediction probabilities were mapped using ArcGIS (Figure 2). The predicted probabilities and their associated latitude/longitude were saved as a csv file, imported into ArcMap, and categorized based on four percentile groupings starting at the lowest predicted probability of 0.5 (50%). The predicted *C. chinensis* distribution was mapped separately from the SAR and Significant Habitat maps (Figure 3) to maximize clarity when comparing the geographic locations of each and to delineate the actual distribution of SAR (current context) versus the predictions (future context).

SAR and significant habitat were compared to establish any areas within regions of general overlap with predicted *C. chinensis* distribution. Federally-recognized SAR in

NS include *Salmo salar* (Atlantic salmon), *Thamnophis sauritus* (eastern ribbon snake), *Osmerus mordax* (rainbow smelt), *Hydrocotyle umbellata* (water pennywort), *Coregonus huntsmanii* (Atlantic whitefish), *Alasmidonta varicosa* (brook floater mussel), *Emydoidea blandingii* (Blandings turtle) ,and *Lampsilis cariosa* (yellow lampmussel). SAR and significant habitat polygons were created based on species recovery plans, point distribution, and geospatial files accessed through multiple government organizations (see Supplementary Material Table 3 for details). Note that our SAR and significant habitat map only included freshwater aquatic habitats. Our study focused only on aquatic species or semi-aquatic species (e.g. *T. sauritus*) as these are the species predicted to be most impacted by *C. chinensis* presence.

Results

The final model (RFM9) predicted 169 of 279 NS water bodies to have a >50% likelihood of suitability for *C. chinensis* distribution (Figure 4). Among the models generated, none were able to provide predictions for water bodies in NB or PEI (NB predictions: 0/44, PEI: 0 /6). The model results listed in Table 2 therefore include only predictions among the 250 NS water bodies examined. Lack of model ability to make predictions for NB or PEI, may be because parameters measured in these provinces did not adequately match parameters used to train the model in terms of data availability, or perhaps due to small sample size. This would appear to be the result of a lack of standardization in freshwater parameters reported across jurisdictions.

Three regions with the highest probability of *C. chinensis* distribution include (a) Cape Breton, NS (the north-eastern Nova Scotian peninsula), (b) along the NS -NB border, (c) and the Halifax Regional Municipality (HRM) (Figure 2). The lowest predicted probability, at 51.6%, is located at the tip of Digby, NS (the south-western edge of NS) on the Bay of Fundy, where high salinity is expected. The highest predicted probability of *C. chinensis* invasion was 85.2% at Lake Ainslie, NS in Cape Breton. Water bodies in northern NS typically have a higher pH and alkalinity than elsewhere in NS and have a higher probability of *C. chinensis* presence, whereas water bodies in southern NS tend to be acidic and dystrophic and not support calcium-bearing invertebrates. It is important to note that all models (RFM1-12) followed similar trends (Figure 5).

The most important predictive parameters for *C. chinensis* distribution in NS are the number of fish species present, alkalinity, presence of other invasive species, number of publicly accessible boat launches, and number of connected freshwater bodies (Figure 6). Longitude and latitude are also considered relatively important parameters for the model, but we found national datasets were biased with high proportion of ON reports. Sodium concentration, dissolved calcium concentration, conductivity, pH, and stocking frequency were found to be relatively less important for predicting *C. chinensis* distribution. Multiple areas with high probability of *C. chinensis* distribution were identified in regions supporting habitat for SAR (Figure 3), especially the two freshwater mussel species (brook floater and yellow lampmussel) and Atlantic salmon. Habitats for other species (pennywort, Blanding's turtle, eastern ribbon snake, and the Petite Riviere Watershed which is inhabited by Atlantic whitefish) had low-predicted probability of suitable habitat for *C. chinensis*.

Discussion

The predicted presence of *C. chinensis* in NS was highest in areas with high alkalinity, neutral pH (7-8), high recreational fishing and boating, other invasive species already present in the watershed, and lower fish diversity. This is in line with the results from social network models of *C. chinensis* in Nebraska lakes (Haak 2015; Haak et al. 2017) and classification tree and maximum entropy models of *C. chinensis* in Wisconsin, USA (Latzka et al. 2015; Papes et al. 2016). Predicted *C. chinensis* distribution for NS was high (>50%) throughout the entire province. Previous research has indicated that *C. chinensis* can tolerate a range of pH values (Haak 2015), salinity concentrations (personal observation), dissolved calcium levels (Haak 2015; Latzka et al. 2015), water temperatures (Burnett et al. 2018), and nutrient concentrations (personal observation). *C. chinensis* is a habitat generalist and an opportunistic feeder, and is therefore adaptable to a variety of environments (Ricciardi 2013). In Atlantic Canada, most freshwater bodies are at risk of *C. chinensis* establishment, especially given ongoing anthropogenic disturbance to wetland habitat and climate change leading to range expansion in potential AIS (Bellard et al. 2013; Lassuy & Lewis 2013; Lozon & MacIsaac 1997; Pyšek et al. 2010; Ricciardi 2007; Spear et al. 2013). Many studies have predicted that NS may become a "hot-spot" for invasive species in the future, highlighting the importance of developing effective SDMs for AIS (Barbosa et al. 2013; Bellard et al. 2013; Gallardo et al. 2015; González-Moreno et al. 2015; Lowry et al. 2013; Pyšek et al. 2010; Robbins 2004; Spear et al. 2013).

The principal means of *C. chinensis* introductions to Atlantic Canada is thought to be chiefly boater transfers and aquarium releases (McAlpine et al. 2016). The distribution of *C. chinensis* across multiple locations within the Shubenacadie watershed suggests that this species may also be spreading naturally. Our own observations of juvenile *C. chinensis* in laboratory cultures demonstrate that juveniles may be carried and spread via water currents. Even though it is likely that many of the initial *C. chinensis* introductions are the result of aquarium releases, it is reasonable to conclude that natural dispersal is now a pathway for the continued spread of *C. chinensis* within NS watersheds. Unfortunately, there is a large temporal-gap (~1955-1990) in *C. chinensis* occurrence data for NS, so it has been impossible to track distributional change within the province, except to some degree over the past two decades. And even then, data is patchy. *C. chinensis* continues to be sold in Canada in the water garden and aquarium trade. For regions where models predict high probability for future distribution, strong provincial and federal regulations need to be enacted for boater cleaning (e.g. clean-drain-dry programs). Legally preventing the sale or release of *C. chinensis* is of utmost importance. For regions with established *C. chinensis* populations, including the NS-NB border and

HRM, and where our model has predicted especially high probability of continued *C. chinensis* spread, containment of existing populations must be ensured. Active programs of public education and boater awareness may help in this regard. Furthermore, additional modeling approaches may improve predictability by including distance to urban areas, where *C. chinensis* is likely to be sold, and proximity to established *C. chinensis* populations. Such data may help reveal further information about the pathways by which *C. chinensis* has spread.

C. chinensis cannot be effectively managed through culling, drawdowns (Unstad et al. 2013), or chemical treatments (Haak et al. 2014). Our best model indicates that ecosystem composition (defined by number of fish species and number of invasive species known to inhabit a water body), alkalinity, and presence of publicly accessible boat launches were relatively important in predicting *C. chinensis* presence. Hence, as already mentioned, the importance of public education and boater clean-drain-dry programs (Matthews et al. 2017; Rothlisberger et al. 2010). Increasing public involvement through citizen science programs can assist in species monitoring and can increase awareness of the threats that non-native species present to water bodies. Such approaches have been identified as essential elements in *C. chinensis* monitoring and control (citation?). Previous public involvement in AIS management and prevention has slowed the spread of zebra mussels in North America, and helped prevent Asian carp (*Cyprinus carpio*), invasion of the Great Lakes (Government of Canada 2019; Meyers 2016). A better understanding of how humans impact and enable *C. chinensis* establishment in North America is required so that critical pathways of introduction can be targeted (Barbosa et al. 2013; Gallardo et al. 2015; González-Moreno et al. 2014; Lassuy & Lewis 2013; Lozon & MacIsaac 1997; Pyšek et al. 2010; Robbins 2004; Spear et al. 2013).

The predicted *C. chinensis* distribution in NS is concerning because it frequently overlaps with SAR occurrence and significant habitat for these species. In particular, the predicted habitat overlap between *C. chinensis* and yellow lampmussel, is of special concern. Nova Scotia supports only three populations of yellow lampmussel, the species distribution in Canada is restricted to NS and NB, and the species is believed to be in decline (Government of Canada 2015). Although the protective measures in place at some yellow lampmussel habitats in NS appear to be robust (e.g. Pottle Lake is a municipal water supply and has many restriction on use), and may decrease the likelihood of *C. chinensis* introduction, other sites are less well protected (e.g. Blacketts Lake). The yellow lampmussel is sensitive to changes in water quality (Government of Canada 2015) and sedimentation and eutrophication (Sabine et al. 2004). In the Saint John River system, NB, *C. chinensis* and yellow lampmussel have already been shown to overlap in distribution (Sabine et al. 2004, McAlpine et al. 2016), although any impacts on the latter are unknown. From our model, the most notable areas of concern are yellow lampmussel habitat in Cape Breton, brook floater habitats in northern and northwestern NS, the Shubenacadie Watershed connecting HRM and the Bay of Fundy, and the St. Mary's

River in Guysborough Co, where predicted *C. chinensis* distribution is high (>70%). The SAR mussel populations are of concern because, should *C. chinensis*, densities become sufficiently high, this may directly impact mussel populations through competition for food and calcium necessary for shell development. As an example, the Shubenacadie Watershed, parts of which are already occupied by *C. chinensis*, has great historical and economic importance for NS, and is at high risk of expanded *C. chinensis* occupation. Within this watershed, Shubenacadie Grand Lake is predicted to have an 84.2% likelihood of future *C. chinensis* presence.

At high densities, *C. chinensis* can be considered an ecosystem engineer as it alters habitat to *C. chinensis* benefit. *C. chinensis* prefers diatoms as food and *C. chinensis* presence encourages the growth of diatoms over other algal forms (Gonzalez et al. 2008; Olden et al. 2013). *C. chinensis* alters nitrogen:phosphorous ratios leading to higher concentrations of chlorophyll-a, which can lead to eutrophication (Bobeldyk 2009). Furthermore, filter feeding *C. chinensis* are likely to be in direct competition with mussel species for food resources (Olden et al. 2013). Other SAR are also considered to be sensitive to water quality changes, including Atlantic salmon, eastern ribbon snake, rainbow smelt, and water pennywort (Government of Canada 2016, 2019; Government of Canada 2011, 2014; Parks Canada 2012). Alterations in water chemistry or food web structure may negatively impact other species that rely on aquatic ecosystems, including Atlantic whitefish and brook floater mussel (Bredin et al. 2009; Government of Canada 2018). Of the eight SAR listed and mapped in our study, only one, Blandings turtle, is at low risk of being affected by *C. chinensis* presence in NS. The Blandings turtle is apparently less sensitive to changes in water quality or impacted by habitat perturbation than the other species we considered in our analysis. Conversely, Kejimkujik National Park and National Historic Site, where the NS population of Blandings turtles is concentrated, showed relatively low probabilities for future *C. chinensis* occurrence (generally < 70%) (Government of Canada 2011b), due to the acidic water chemistry (pH 4 – 5) of many of those dystrophic lakes. Further research is required to determine the probable consequences of *C. chinensis* presence for each SAR in NS, although we suggest that the best strategy is to prevent *C. chinensis* introductions and to protect the critical habitats of SAR.

Conclusion

The non-native and potentially invasive freshwater mollusc, *C. chinensis*, currently has a limited, although widespread, distribution throughout Atlantic Canada. Our RFM predicted, with >50% probability, *C. chinensis* establishment throughout NS, with the highest predicted probability in northern-NS, the Halifax Regional Municipality (HRM), and near the NS -NB border. When SAR habitat is compared with predicted *C. chinensis* distribution, overlaps with SAR habitat were identified. Most concerning is potential overlap that *C. chinensis* may have with the yellow lampmussel, a mussel species of conservation concern in Canada. Further research is needed to define the ecological thresholds of *C. chinensis* and to identify the manner in which human factors influence the distribution of this species across its non-native range. Although our model was successfully applied to NS, we were not able to make any predictions for NB or PEI. This was likely due to a lack of standardization in freshwater parameters reported across jurisdictions. Future modelling exercises for AIS in Canada would benefit from greater conformity across provinces in the collection and reporting of water quality and other parameters important to SDMs. Due to the high probability of expanding C. *chinensis* range in Atlantic Canada, and the potential negative impacts this species may have on SAR, it is important that *C. chinensis* be recognized as potentially invasive in Canada and managed as such.

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CHAPTER 2 Figures:

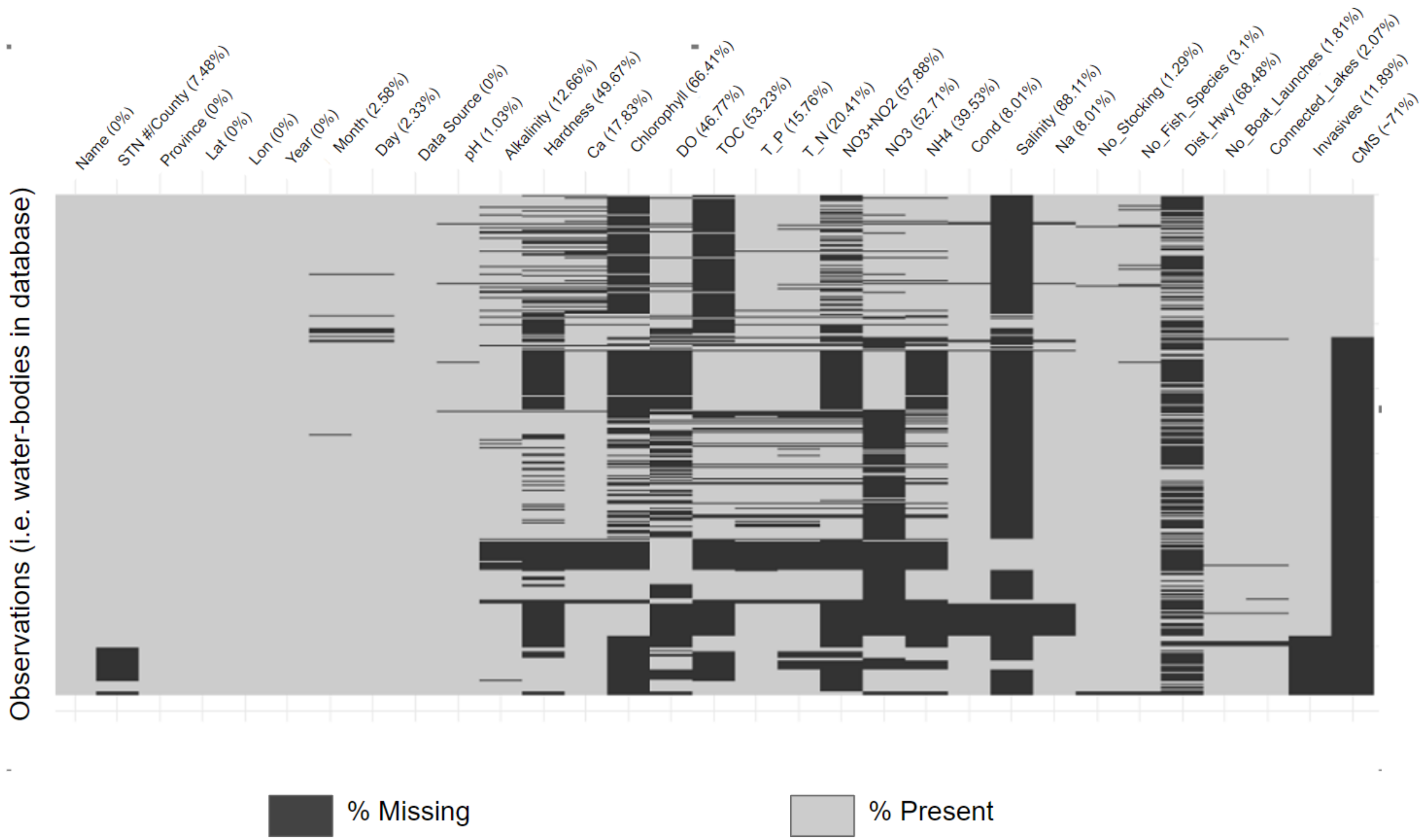

Figure 1. Data categories used to develop Random Forest Models to predict *Cipangopaludina chinensis* distribution in Maritime Canada. Data absences are the result of a lack of standardization in freshwater parameters collected across Canadian jurisdictions. Note, the geomorphologic data is not included in this figure because the relatively large amounts of missing data lead to the exclusion of these parameters from all models. The parameters along the y-axis are: name (name of water body), STN #/County (the station number or county used to identify the water body), province, Lat (latitude in decimal degrees), Lon (longitude in decimal degrees), Year (year of water sampling), Month (month of sampling), Day (day of sampling), Data Source (the source of data, see Supplementary Materials Table 1), pH, Alkalinity, Hardness (water hardness), Ca (calcium concentration), Chlorophyll (chlorophyll-a concentration), DO (dissolved oxygen concentration), TOC (total organic carbon concentration), T_P (total phosphorous concentration), T_N (total nitrogen concentration), NO3+NO2 (nitrate plus nitrite concentration), NO3 (nitrate concentration), NH4 (ammonia concentration), Cond (conductivity), salinity (salinity concentration), Na (sodium concentration), No stocking (number of annual fish stocking events), No Fish_Species (number of fish species recorded), Dist Hwy (distance to nearest highway), No Boat Launches (number of public boat launches), Invasives (number of known invasive species reported), CMS (known *C. chinensis* presence/absence).

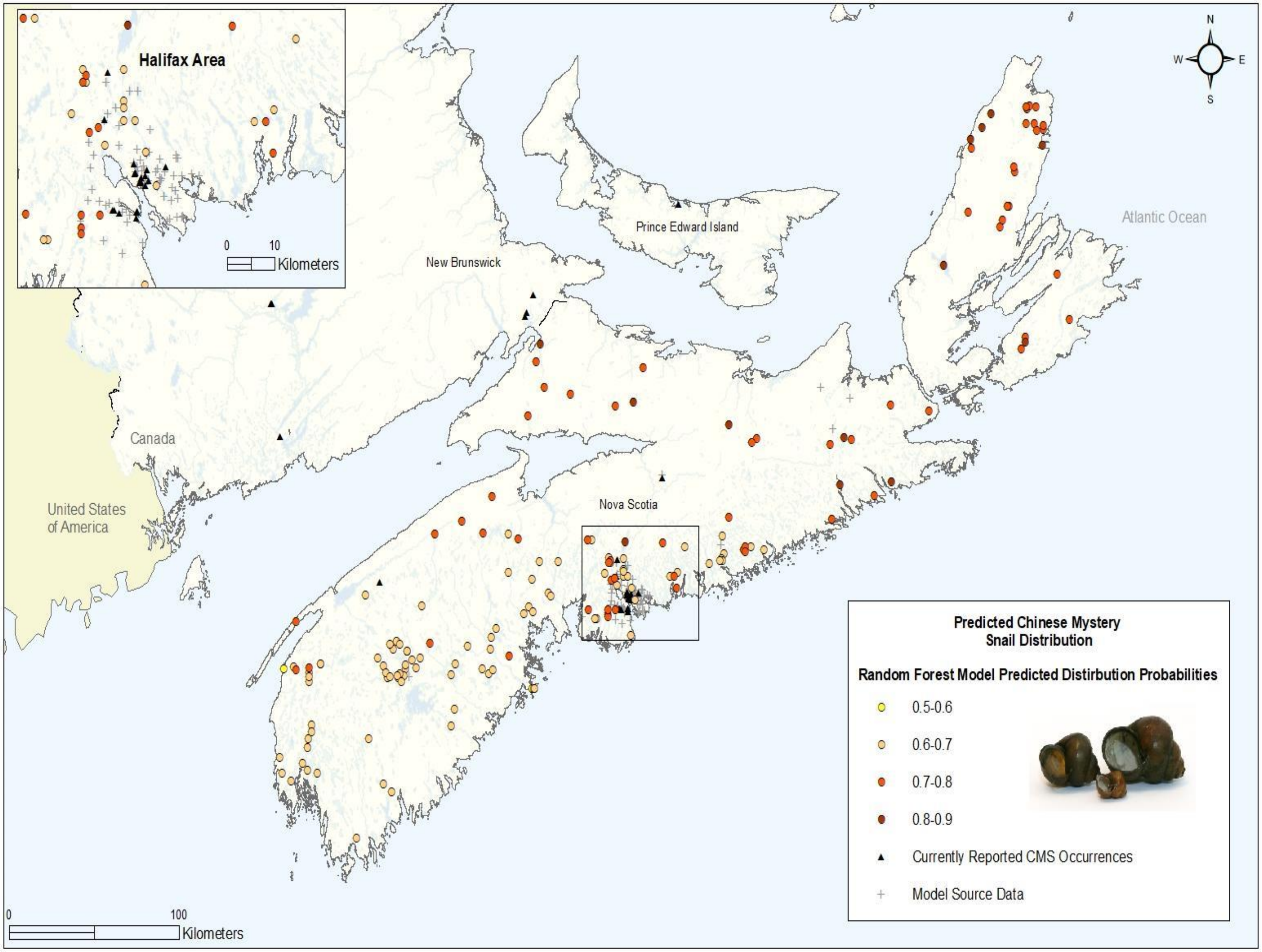

Figure 2: Known and predicted distribution of *Cipangopaludina chinensis* in the Maritimes. Currently confirmed reports for *C. chinensis* occurrences in the Maritimes are shown as black circles. Predicted *C. chinensis* distribution with probabilities are shown as red circles. Crosses mark source data for development of random forest models.

. Cape Breton, the northeaster peninsula in Nova Scotia, had the high predicted probabilities and Digby, the southern tip, had the lowest.

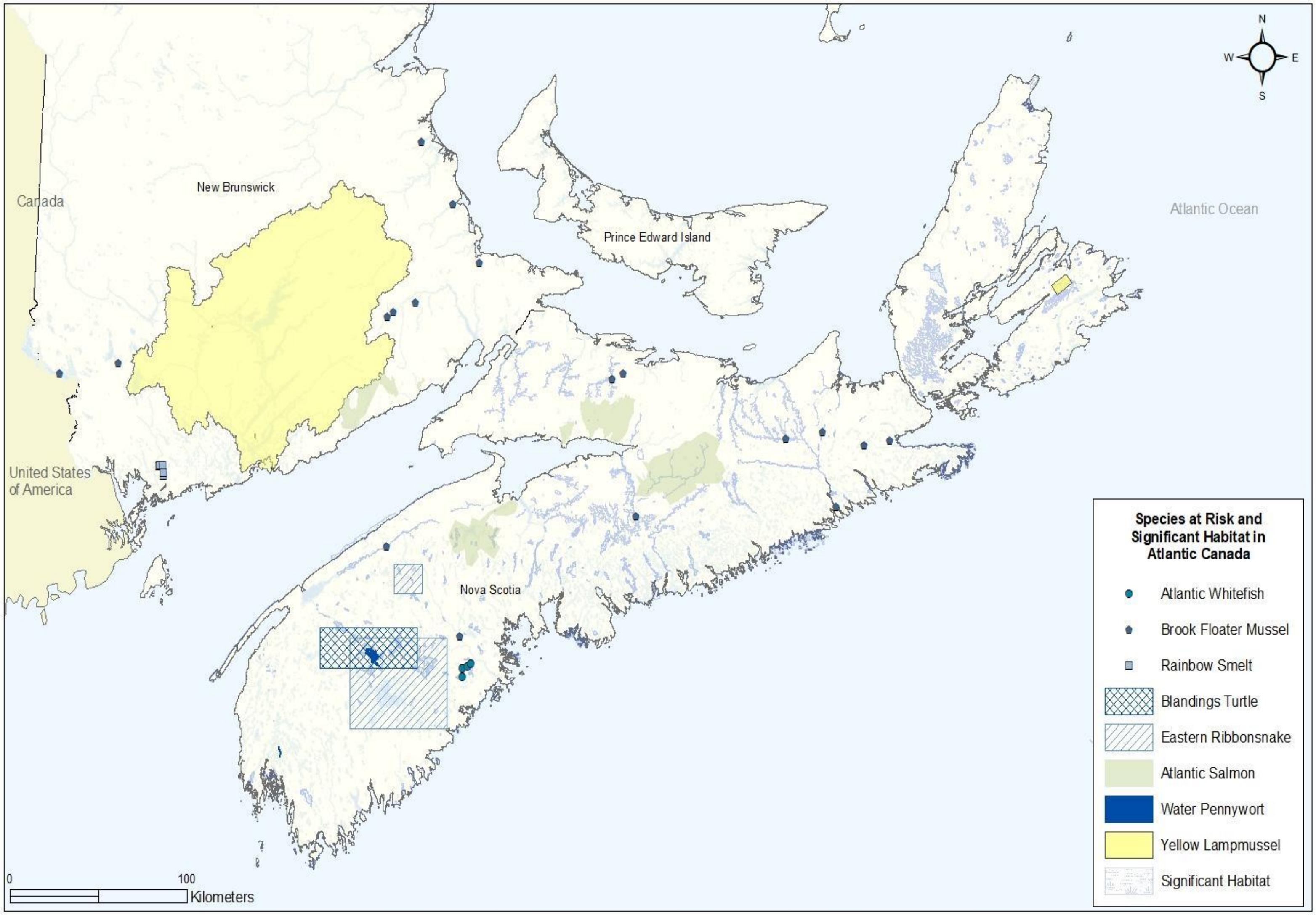

Figure 3: Distribution of selected species at risk (SAR) habitat and significant habitat in Nova Scotia and New Brunswick. See Supplementary Materials, Table 3 for a list of various Government of Canada SAR conservation websites where the data for this map was obtained.

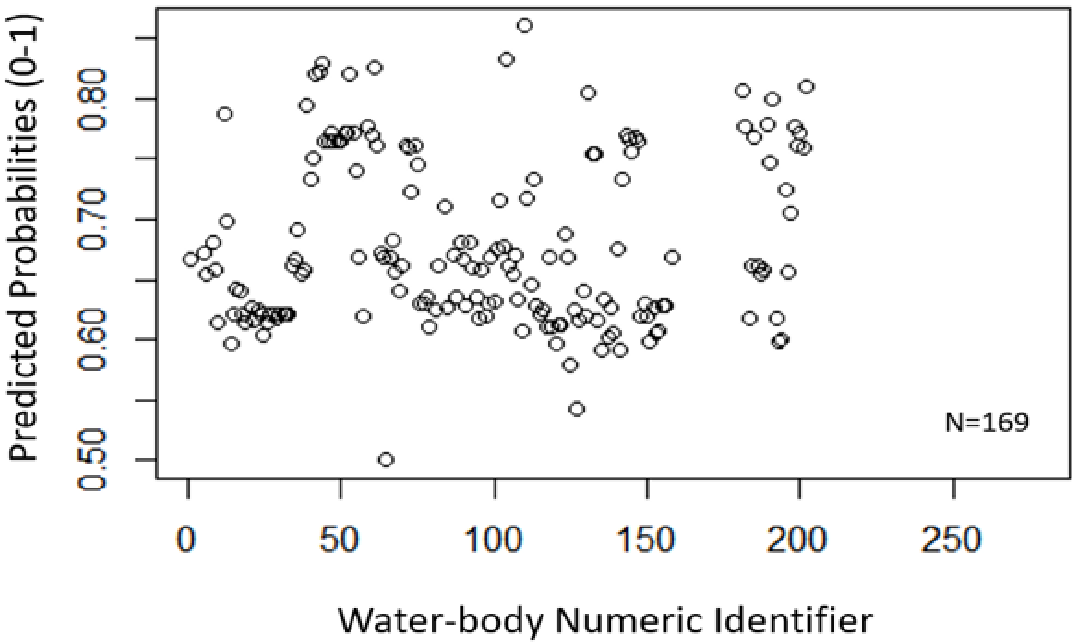

Figure 4: Predicted probability of *Cipangopaludina chinensis* in 169 Nova Scotia (NS) water bodies based on a random forest model. Each water body was given its own numeric identifier (see Supplementary Materials for list of all lakes with its numeric identifier). All predicted probabilities for NS suggest presence of *C. chinensis* with >50% probability.

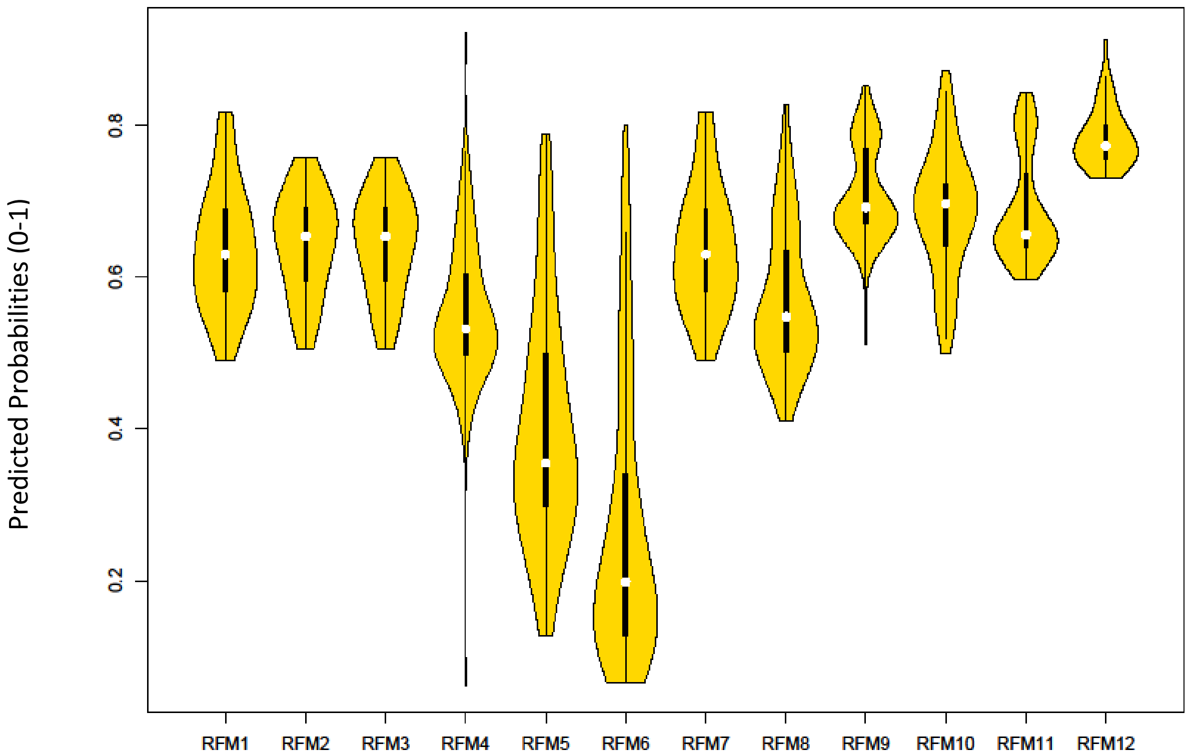

Figure 5: Violin plots comparing each of twelve random forest models (RFM) developed (RFM1-12) to predict *Cipangopaludina chinensis* distribution in Maritime Canada. The x-axis lists RFM developed along with sequential numbering (e.g. RFM1, RFM2). The y-axis shows the predicted probability (0-1) of water bodies in the dataset having suitable habitat for *C. chinensis*. See Table 1 for the list of the RFM equations and parameters. Note that RFM5 and RFM6 included pseudo-absence data and data were not normalized in these models. We selected RFM9 to represent the model predictions seen in RFM1-12 because RFM9 contained data from a national context, data were normalized, the training error and validation error were relatively low and similar, and RFM9 was able to make the largest number of predictions (n=169) of all models developed.

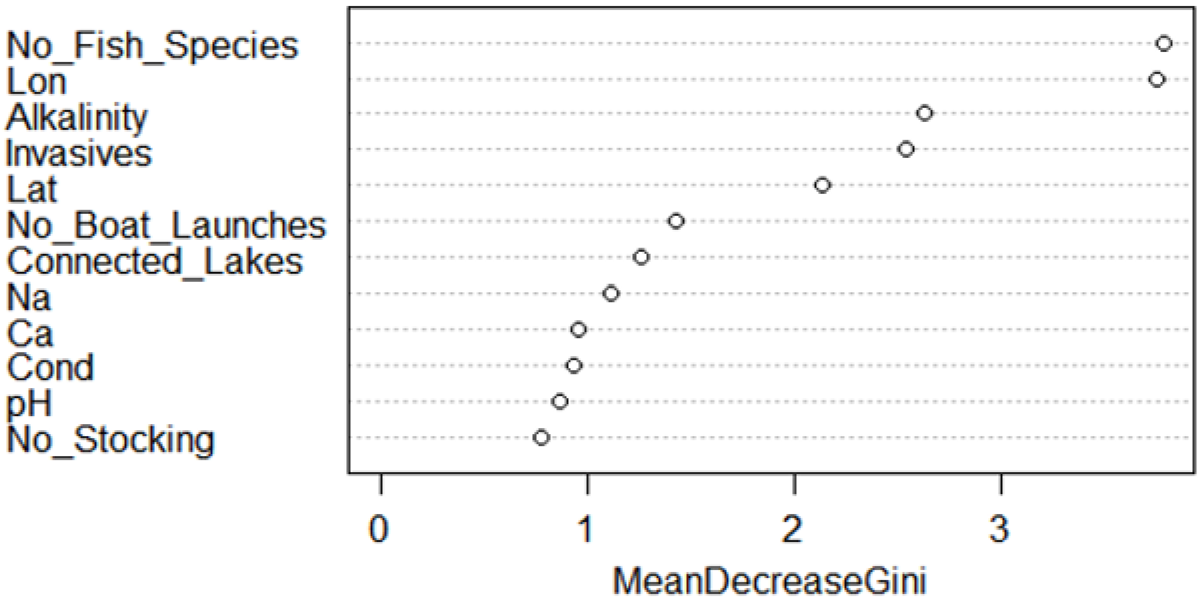

Figure 6: The importance of selected parameters to the preferred Random Forest Model used to predict the distribution of *Cipangopaludina chinensis* in Nova Scotia. The higher mean decrease gini, the more important the variable in predicting *C. chinensis* presence in Nova Scotia. The parameters along the y-axis are: No Fish_Species = number of fish species recorded; Lon = longitude; Invasives = number of known invasive species reported; Lat = latitude; No Boat Launches = number of public boat launches; Na =sodium; Ca= calcium concentration; Cond = conductivity; No stocking = number of annual fish stocking events. Mean decrease gini stems from the gini impurity which is used in decisions trees to determine the importance of random selected variables to correctly split/subset the data. Because RFMs are multiple decision trees which makes a forest, the mean decrease gini is the average of each tree's gini impurity for each parameter. Gini impurity is the measure of probability of misclassifying *C. chinensis* absence if each variable were introduced to the prediction model formula.

Chapter 2: Tables

Table 1: Twelve (numbered 1 through 12) random forest model (RFM) itinerations were generated using different combinations of model formula (see footnotes) and datasets used to train and validate the models. Only RFM1 was unvalidated, all other models were validated using subsets of the training datasets. Additionally, the RFM1-8 models were trained on unnormalized data ((all units retained). The models RFM9-12 were normalized, with all units converted to a scale of 0 to 1. For most models, all waterbodies that had data were included, regardless the amount of data, while for RFM7, only waterbodies that had more than 50% of the characteristics in the dataset were included. In regards to the Formulas 1 – 4 below, 'CMS' is the verified presence /absence or pseudo-absence of *Cipangopaludina chinensis*; 'Lat' is latitude; 'Lon' is longitude; 'Ca' is dissolved water calcium concentration; 'T_P' is total phosphorous concentration; 'T_N' is total nitrogen concentration; 'Cond' is conductivity; 'Na' is water sodium concentration; 'No_Stocking' is number of times per year a water body is stocked with fish; 'No_Fish_Species' is the number of reported fish species per water body; 'Dist_Hwy' is the distance to the nearest highway from a publicly accessible boat launch; 'No_Boat_Launches' is the number of publicly accessible boat launches per water body; 'Connected_Lakes' is the number of freshwater bodies connected to the water body in the dataset; and 'Invasives' is the number of invasive species reported in each water body.

Model phase	Normalized ?	Datasets	Waterbodies	RFM Formula No.	RFM Itineration
Unvalidated	No	Verified Presence/Absence data from ON & NS	All waterbodies having data	1	RFM1
Validated	No	Verified Presence/Absence data from ON & NS	All waterbodies having data	1	RFM2
Validated	No	Verified Presence/Absence data from ON & NS	All waterbodies having data	2	RFM3
Validated	No	Verified Presence/Absence data from ON & NS	All waterbodies having data	3	RFM4
Validated	No	Verified Presence/Absence data from ON & NS Pseudo-absence data from ON	All waterbodies having data	1	RFM5

Validated	No	Verified Presence/Absence data from ON & NS Pseudo-absence data from ON	All waterbodies having data	2	RFM6
Validated	No	Verified Presence/Absence data from ON & NS Pseudo-absence data from ON	Waterbodies with large amounts (>50%) of missing data removed from dataset	1	RFM7
Validated	No	Verified Presence/Absence data from ON & NS Pseudo-absence data from ON	Waterbodies with large amounts (>50%) of missing data removed from dataset	2	RFM8
Validated	Yes	Verified Presence/Absence data from BC, AB, ON & NS Pseudo-absence data from ON	All waterbodies having data	2	RFM9
Validated	Yes	Verified Presence/Absence data from BC, AB, ON & NS Pseudo-absence data from ON	All waterbodies having data	1	RFM10
Validated	Yes	Verified Presence/Absence data from BC, AB, ON & NS Pseudo-absence data from ON	All waterbodies having data.	3	RFM11

Validated	Yes	Verified Presence/Absence data from BC, AB, ON & NS Pseudo-absence data from ON	All waterbodies having data.	4	RFM12

1. CMS ~ Lat + Lon + pH + Alkalinity + Ca + T_P + T_N + Cond + Na + No_Stocking + No_Fish_Species + Dist_Hwy + No_Boat_Launches + Connected_Lakes + Invasives
2. CMS ~ Lat + Lon + pH + Alkalinity + Ca + Cond + Na + No_Stocking + No_Fish_Species + No_Boat_Launches + Connected_Lakes + Invasives
3. CMS ~ Lat + Lon + pH + Alkalinity + Ca + T_P + Cond + Na + No_Stocking + No_Fish_Species + No_Boat_Launches + Connected_Lakes + Invasives
4. CMS ~ pH + Alkalinity + Ca + T_P + T_N + Cond + Na + No_Stocking + No_Fish_Species + Dist_Hwy + No_Boat_Launches + Connected_Lakes + Invasives

Table 2: Error rates, number of predictions and formula used to develop Random forest models used to predict *Cipangopaludina chinensis* distribution in Maritime Canada. Model 9 (RFM9) was selected for use as it was able to predict presence/absence at the greatest number of water bodies while maintaining relatively low training and validation error rates.

Model	Error Rate (Training)	Error Rate (Validation)	Number of Predictions	Formula Used
RFM1	18.92	NA	43	1
RFM2	24	25	44	1
RFM3	24	60	168	2
RFM4	24	40	142	3
RFM5	15.62	33	44	1
RFM6	21.88	22.22	162	2
RFM7	27.78	100	43	1

RFM8	27.78	42.86	141	2
RFM9	10.87	13.16	169	2
RFM10	8.7	7.69	46	1
RFM11	10.87	13.16	142	3
RFM12	28.26	7.69	46	4

Table 3: List of acronyms used in the manuscript text.

Acronym	Long-version
AB	Alberta
AIS	Aquatic Invasive Species
BC	British Columbia
NB	New Brunswick
NS	Nova Scotia
ON	Ontario
PEI	Prince Edward Island
RFM	Random Forest Model
SAR	Species at Risk
SDM	Species Distribution Model

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CHAPTER 2: Supplementary Material

Table 1: Data Sources by Province

Province	Data Source
Alberta (AB)	AB Environment and Parks data for McGregor Lake
	Boat Launches Canada
	Google Maps
British Columbia (BC)	BC Invasive Alien Plant Program (IAPP) data
	BC Ministry of Environment and Climate Change Strategy Surface Water Monitoring data
	Freshwater Fisheries Society of BC Stocking Reports
	Boat Launches Canada
	Google Maps
New Brunswick (NB)	New Brunswick Department of Environment and Local Government
	Nashwaak Watershed Water Quality Data
	Long Term Water Quality Monitoring Program- Tantramar River Watershed
	Kennebecasis Watershed Restoration Data
	Belleisle Water Coalition Data
	Maritime Coastal Basin Long-Term Water Quality Monitoring Data

	McAlpine et al. (2016)
	Boat Launches Canada
	Google Maps
Nova Scotia (NS)	Nova Scotia lake chemistry data from the NS Department of the Environment
	NS Government Lake Survey Data
	NS Fisheries and Aquaculture Fish Species Distribution List
	Nova Scotia Fisheries and Aquaculture Spring and Fall Recreational Fishing Trout Stocking List
	Fisheries and Oceans Canada 2011 HRM Lake Data
	Maritime Coastal Basin Long-Term Water Quality Monitoring Data
	Clean Foundation Watershed Restoration Monitoring Data
	BARA-Dartmouth, NS-Sawmill River Watershed-Baseline YSI Study Data
	McAlpine et al. (2016)
	Kingsbury et al. (unpublished)
	Boat Launches Canada
	Google Maps
Ontario (ON)	ON Provincial (Streams) Water Quality Monitoring Network Data
	Government of ON- Ontario’s fishing stocking program data
	EDD MapS Ontario
	Boat Launches Canada
	Google Maps
Prince Edward Island	Maritime Coastal Basin Long-Term Water Quality Monitoring Data

	South Shore Watershed Group Data
	PEI Government
	Boat Launches Canada
	Google Maps

Table 2: Random Forest Model Parameters

Parameter	**Unit of Measurement**
water body name	--
county name and/or sampling station reference number	--
data sampled	year, month, and day
location (latitude and longitude)	Decimal degrees
data source	Organization and/or dataset name
pH	0-14
alkalinity	mg/L
hardness	mg/L
dissolved calcium	mg/L
dissolved oxygen	mg/L
chlorophyll-a concentration	ug/L
total organic carbon	mg/L
total phosphorus concentration	mg/L
total nitrogen concentration	mg/L
nitrate concentration	mg/L
nitrite concentration	mg/L
nitrate + nitrite concentration	mg/L
ammonia concentration	mg/L
specific conductivity	us/cm
sodium concentration	mg/L

salinity	Ppt
total dissolved solids	mg/L
surface area	km^2
maximum depth	m
mean depth	m
secchi depth	m
shoreline development score	no units provided in dataset
stocking frequency	number of times per year
number of fish species	number of fish species recorded in the water body (e.g. Ontario) or number of fish that were recorded as added through stocking programs (e.g. Nova Scotia)
number of public boat launches	number of boat launches that were accessible by the public (i.e. not privately owned) which included marinas
distance to the nearest highway from a public boat launch	km
number of invasive species	the number of invasive species recorded to inhabit a certain water body
connected water bodies	the number of water bodies connected to a specific water body

Table 3: Species at risk (SAR) and Significant Habitat Data Sources

Species	Data Source	Shapefile Type
Atlantic salmon (inner Bay of Fundy population)	(Government of Canada, 2011a)	Watershed polygon
Atlantic whitefish	(Government of Canada, 2018)	Central point of lakes

Blanding's turtle (Nova Scotia population)	(Government of Canada, 2011b)	Generalized critical habitat polygon
Brook floater	(Bredin et al., 2009)	Known subpopulation points
Eastern ribbonsnake (Atlantic population)	(Parks Canada, 2012)	Generalized critical habitat polygon
Lake Utopia rainbow smelt (Large- and small-bodied Population)	(Government of Canada, 2016)	Central point of Lake Utopia critical habitat
Yellow lampmussel	(Government of Canada, 2015)	Overview polygon of watershed with known populations
Water pennywort	(Government of Canada, 2014)	Critical habitat polygon
Significant Habitat	(Government of Nova Scotia, 2013)	Significant habitat polygon

Notes on Model Assumptions Made:

One assumption of RFM, is that the dataset used to train and validate the model is complete (i.e. the dataset does not contain missing data). The dataset of Canadian freshwater-bodies used to train and validate the RFMs in this study did contain missing data. To deal with the problem of missing data, the code na.action=na.roughfix was included (see Appendix D for the full code used). The na.roughfix code estimates the missing data by continuously comparing the missing data cell with data of other waterbodies that have complete data. The code will continue to do these comparisons until it reaches a converging value which is then assigned to the missing data cell. This is another reason why mean decrease gini should be interpreted with caution because parameters with more data present are more likely to be ranked with a higher mean decrease gini. Also, the RFM cannot make predictions on waterbodies that have large amounts of missing data, especially if the missing data involves parameters that were ranked high for mean decrease gini (because those are the most important parameters for model training). This may lead to low predictive power (number of possible predictions made by the model) in areas that are "data poor." By standardizing water sampling regimes, it is possible to make larger databases that include multiple geographic regions that have complete data.

Another difficulty with invasive species modeling is the lack of true absence reports. This forces ecologists to use pseudo-absences for models. Pseudo-absences are points randomly selected from the background data points (i.e. the model testing dataset) and are assumed to have no species presence. When only presence data is available for modeling plus the additional background points where species presence/absence is unknown, species absences must be estimated (Jackson et al. 2000; Rodríguez-Rey et al. 2019; Václavík & Meentemeyer 2009, 2012). Randomly selecting pseudo-absences from background points assumes that the species is in equilibrium with its environment meaning that the species occupies all possible habitat that it can. For invasive or non-indigenous species this assumption might not be true, it may be that the non-indigenous species has not been introduced into all available/suitable habitats yet (Václavík & Meentemeyer 2012). The issues with pseudo-absences have been noted in the literature. Some modeling approaches, such as MaxEnt, have tried different ways of selecting pseudo-absences in an effort to minimize assumptions. MaxEnt geographically confines pseudo-absence selection to areas with the highest reported species presence because it is more likely to be at or close to equilibrium in these areas (Merow et al. 2013; Phillips et al. 2004; Richmond 2019). This study used the MaxEnt approach to select pseudo-absences. Also, attempts were made to collect "true" absence points in Nova Scotia (NS) through field surveys and public inquiries. Unfortunately, there was low public participation in the study and the field surveys represent a small transect of available NS habitat, which closely resembled neighbouring habitats where *C. chinensis* was present. Hence, it seems that our “true” absence data collected from the Halifax Regional Municipality are ideal habitats for future *C. chinensis* establishment, awaiting only the species’ introduction. The collection of species absence data is equally as important for modeling as is presence data, therefore, public engagement programs need to include the desire to receive suspected absence reports too.

Another assumption made by RFM is that the data used to train and validate the model contains sufficient variety (the greater the variety the stronger the model). However, this study used only Canadian data, although attempts were made to secure datasets from the United States of America, those enquires were not successful. The majority of data used to train models in this study were from Ontario. There may be inter-provincial inconsistencies between freshwater parameters monitored (each province has its own freshwater monitoring goals and priorities) and between the ranges for water chemistry parameters (i.e. Ontario habitats differ from Atlantic Canada habitats). More data is needed from Atlantic Canadian for freshwater ecosystems so models may be based on data that is clearly consistent with Maritime ecosystems. Additionally, data from across the globe, including from the species’ indigenous range, is required to ensure greater data variability.

Finally, the gini impurity index of model parameters not only assumes that all model parameters are ecologically or biologically important for the species’ establishment, but also that each parameter is not missing large amounts of data. Therefore, generated RFMs may bias towards parameters which have “complete” data (i.e. no missing data). In

To further explain how gini impurity is impacted by missing data:

$$G = \sum [pk\ (1 - pk)]$$

Where G is the gini index over all classes and pk is the portion of training data in class k.

For this study, we have two classes in the training dataset, “YES” indicating *C. chinensis* presence and “NO” indicating *C. chinensis* absence. Let p_1=class 1 (i.e. the “YES” values) and p_0=class 0 (i.e. “NO” values).

$$G = 1 - (p_1^2 + p_0^2)$$

The gini index (G) calculated for each node is weighted by the total number of instances in the parent node. The gine score for a chosen split for binary classificants (e.g. 0 and 1) is:

$$G = [\left(1 - \left(g_{1\_1}^2 + g_{1\_0}^2\right)\right)\left(\frac{ng_1}{n}\right)] + [\left(1 - \left(g_{0\_0}^2 + g_{0\_1}^2\right)\right)\left(\frac{ng_0}{n}\right)]$$

Where g_{1_1} is the instances of class 1 in group 1 (i.e. the true positives), g_{1_0} is the instances of class 0 in group 1 (i.e. the false positives), g_{0_0} is the instances of class 0 in group 0 (i.e. the true negatives), and g_{0_1} is the instances of class 1 in group 0 (i.e. the false negatives); n is the total number of instances in the training data, ng_1 is the portion of training date in class 1 and ng_0 is the portion of training data in class 0.

Considering our dataset which as a series of data in either class 0 (no *C. chinensis* presence) and class 1 (*C. chinensis* presence), we have a series of zeros and ones. At each split in classification and regression trees (CART), the model asks a question: *which parameter at what value will effectively separate all of the data of class 0 into one group and all the data of class 1 into a* *different group?* When it is not possible to separate the training data into these two distinct groups, the model continues to build the tree by adding daughter nodes and reassesses this essential question. A RFM buildings hundreds or thousands of these trees which enables the forest to be more robust and less likely to overfit to training data than a single tree. However, it is important to consider that each tree is built in this fashion and the trees will opt to use parameters with more complete data (i.e. less missing data) because the resulting node will be more effective at separating data into their respective groups.

Random forest model sensitive testing and trend analysis

Summary**:**

Random forest models (RFMs) can be applied to ecological predictive models by combining a variety of parameter types (e.g. climatic, water quality, etc.) to give robust predictions. Twelve RFM were developed to predict Chinese mystery snail, *Cipangopaludina chinensis*, suitable habitat in Nova Scotia. The nineth model, RFM9, was chosen as the best model to represent the probability of Nova Scotia habitats to be suitable for successful *C. chinensis* establishment. The parameters used to create RFM9 were ranked in relative importance. In order to test the model's sensitivity, parameters of lowest importance were sequentially excluded from the model (RFM13-RFM15) until the error rate was larger than that of the initial model (RFM9). RFM13 had a better error rate than RFM9 and was able to predict on more waterbodies (i.e. greater predictive power) in the testing dataset. However, RFM13 was not chosen to represent the predicted suitable habitats because it is likely that the improved capabilities of RFM13 over RFM9 is due to the parameters used having more data presence than those included in RFM9 (data presence referring to Figure 1, Chapter 2). Additionally, if RFM13 was chosen as the "best" model then some ecological important parameters would be excluded (e.g. pH, calcium concentration, conductivity, and sodium concentration). Previous studies

testing *C. chinensis* ecological thresholds for pH, salinity, and calcium concentration, have already noted that, although *C. chinensis* may have a large tolerance range for each of these parameters, they are all still important considerations for suitable habitat. Therefore, further research is needed to determine how each of the parameters used in RFM9 interact and impact or are impacted by *C. chinensis* presence.

Methods:

The same dataset used to generate RFM9 (the same training and validation data) was used (refer to Chapter 2 for more details about the data) for the model sensitivity test models (RFM13-15). The relative importance plot for RFM9 (see Chapter 2, Figure 5) ranked parameters from formula 2, which was used for RFM9, was used to determine which parameters were sequentially excluded to create three additional models (RFM13-15) using fewer and fewer parameters each time (formula 5-7). The models generated to test RFM9 sensitivity (RFM13-15) predicted on the same background dataset as RFM9. The error rates and number of predictions made of RFM1-15 were compared.

Additionally, a Mann Kendall test was used to compare models to determine if there were any statistical differences. A MANOVA was performed to identify which models were statistically different. Also, a Shapiro-Wilk normality test was carried out to ensure that the predicted probabilities were normally distributed.

Results:

Of the RFM generated to test RFM9 sensitivity, RFM13 yielded a higher predictive power (i.e. was able to make more predictions than RFM9) and a better error rate. However, RFM13 excluded important ecological parameters that have previously been determine as potentially impacting *C. chinensis* and other mollusc species. Therefore, RFM9 was still a better choice than RFM13 because it includes ecologically relevant parameters within the model formula.

Predicted probabilities were normally distributed (Shapiro-Wilk normality test p value=0.00032). All models (RFM1-15) followed the same prediction trends except RFM9 (p=0.0497) and RFM11 (p=0.007819). Notably, RFM4 was semi-significantly different from other models (p=0.05751).

Shapiro-Wilk Normailty Test Results:

```
data:  Z
W = 0.71018, p-value = 0.00032
```

Mann Kendall Results:

```
Score =  385772 , Var(Score) = 532006688
denominator =  1414825
tau = 0.273, 2-sided pvalue =< 2.22e-16
```

MANOVA Results:

```
Response 1 :
            Df    Sum Sq   Mean Sq F value Pr(>F)
number       1 0.0059281 0.0059281  1.5318 0.3414
Residuals    2 0.0077399 0.0038700

 Response 2 :
            Df    Sum Sq   Mean Sq F value Pr(>F)
number       1 0.0027343 0.0027343  0.3945 0.5941
Residuals    2 0.0138607 0.0069303

 Response 3 :
            Df   Sum Sq  Mean Sq F value Pr(>F)
number       1 0.007304 0.007304  2.3158 0.2675
Residuals    2 0.006308 0.003154

 Response 4 :
            Df    Sum Sq   Mean Sq F value  Pr(>F)
number       1 0.0108824 0.0108824  15.903 0.05751 .
Residuals    2 0.0013686 0.0006843
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

 Response 5 :
            Df   Sum Sq  Mean Sq F value Pr(>F)
number       1 0.129443 0.129443  7.0999 0.1167
Residuals    2 0.036464 0.018232
```

```
 Response 6 :
            Df   Sum Sq  Mean Sq F value Pr(>F)
number       1 0.022577 0.022577  0.3531 0.6126
Residuals    2 0.127890 0.063945

 Response 7 :
            Df    Sum Sq   Mean Sq F value Pr(>F)
number       1 0.0212578 0.0212578  6.2283   0.13
Residuals    2 0.0068262 0.0034131

 Response 8 :
            Df    Sum Sq   Mean Sq F value Pr(>F)
number       1 0.0117971 0.0117971  7.8466 0.1073
Residuals    2 0.0030069 0.0015035

 Response 9 :
            Df   Sum Sq   Mean Sq F value Pr(>F)
number       1 0.004891 0.0048910  18.633 0.0497 *
Residuals    2 0.000525 0.0002625
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

 Response 10 :
            Df   Sum Sq  Mean Sq F value Pr(>F)
number       1 0.002035 0.002035  0.2131 0.6897
Residuals    2 0.019096 0.009548

 Response 11 :
            Df    Sum Sq   Mean Sq F value   Pr(>F)
number       1 0.0034366 0.0034366  126.39 0.007819 **
Residuals    2 0.0000544 0.0000272
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

 Response 12 :
            Df    Sum Sq   Mean Sq F value Pr(>F)
number       1 0.0010752 0.0010752  0.6962 0.4919
Residuals    2 0.0030888 0.0015444

 Response 13 :
            Df    Sum Sq   Mean Sq F value Pr(>F)
number       1 0.0065197 0.0065197  0.5865 0.5238
Residuals    2 0.0222323 0.0111161

 Response 14 :
            Df    Sum Sq   Mean Sq F value Pr(>F)
number       1 0.0035944 0.0035944   0.282 0.6485
Residuals    2 0.0254966 0.0127483

 Response 15 :
            Df   Sum Sq   Mean Sq F value Pr(>F)
number       1 0.009993 0.0099926   0.604 0.5184
Residuals    2 0.033090 0.0165452
```

Table 1: Error rates, number of predictions and formula used to develop random forest model 9 and the models used to test the model’s sensitivity used to predict *Cipangopaludina chinensis* distribution in Maritime Canada. Model 9 (RFM9) was selected for use as it was able to predict presence/absence at the greatest number of water bodies while maintaining relatively low training and validation error

rates; and maintaining parameters of ecological importance previously identified in the literature (Fraser et al. in-prep; Haak et al. 2017; Latzke et al. 2015; Papes et al. 2016).

Model	Error Rate (Training)	Error Rate (Validation)	Number of Predictions	Formula Used
RFM9	8.7	13.16	169	2
RFM13	6.52	10	196	5
RFM14	15.22	13.73	200	6
RFM15	10.87	16	198	7

List 1: Formulas used to create RFM9, RFM13, RFM14, and RFM15

2. CMS ~ Lat + Lon + pH + Alkalinity + Ca + Cond + Na + No_Stocking + No_Fish_Species + No_Boat_Launches + Connected_Lakes + Invasives
5. CMS ~ Lat + Lon + Alkalinity + No_Fish_Species + No_Boat_Launches + Connected_Lakes + Invasives
6. CMS ~ Lat + Lon + Alkalinity + No_Fish_Species + Invasives
7. CMS ~ Lat + Lon + Alkalinity + No_Fish_Species + No_Boat_Launches + Invasives

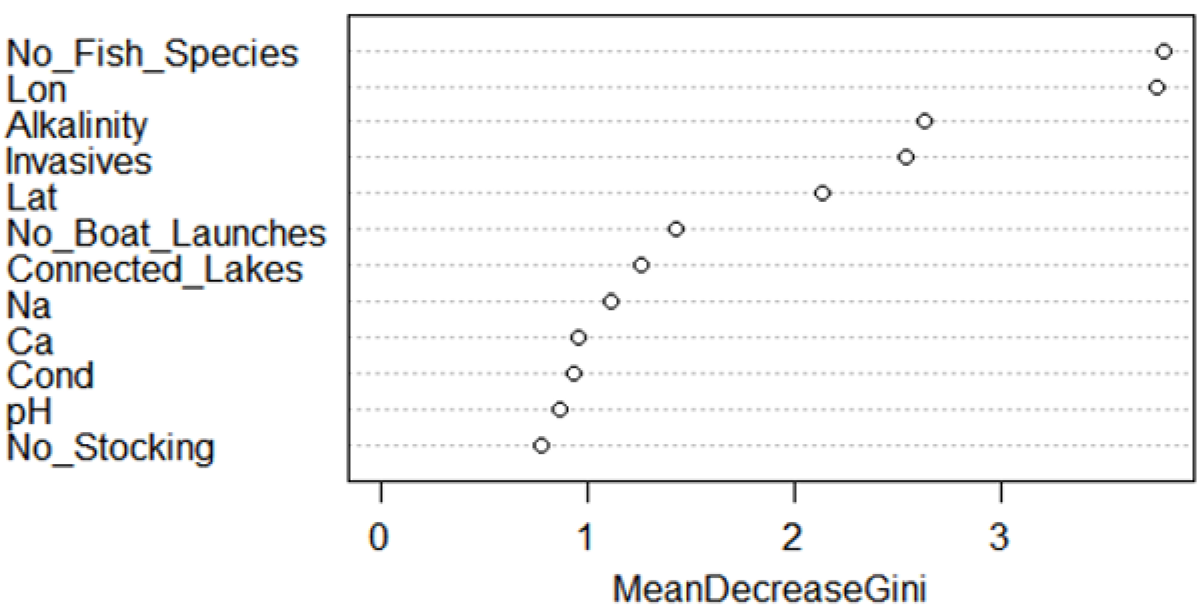

Figure 1: The importance of selected parameters to the preferred Random Forest Model used to predict the distribution of *Cipangopaludina chinensis* in Nova Scotia. The higher the Mean

Decrease Gini, the more important the variable in predicting *C. chinensis* presence in Nova scotia; No Fish_Species = number of fish species recorded, Lon = longitude, Invasives = number of known invasive species reported, Lat = latitude, No Boat Launches = number of public boat launches; Na =sodium, Ca= calcium concentration, Cond = conductivity, No stocking = number of annual fish stocking events.

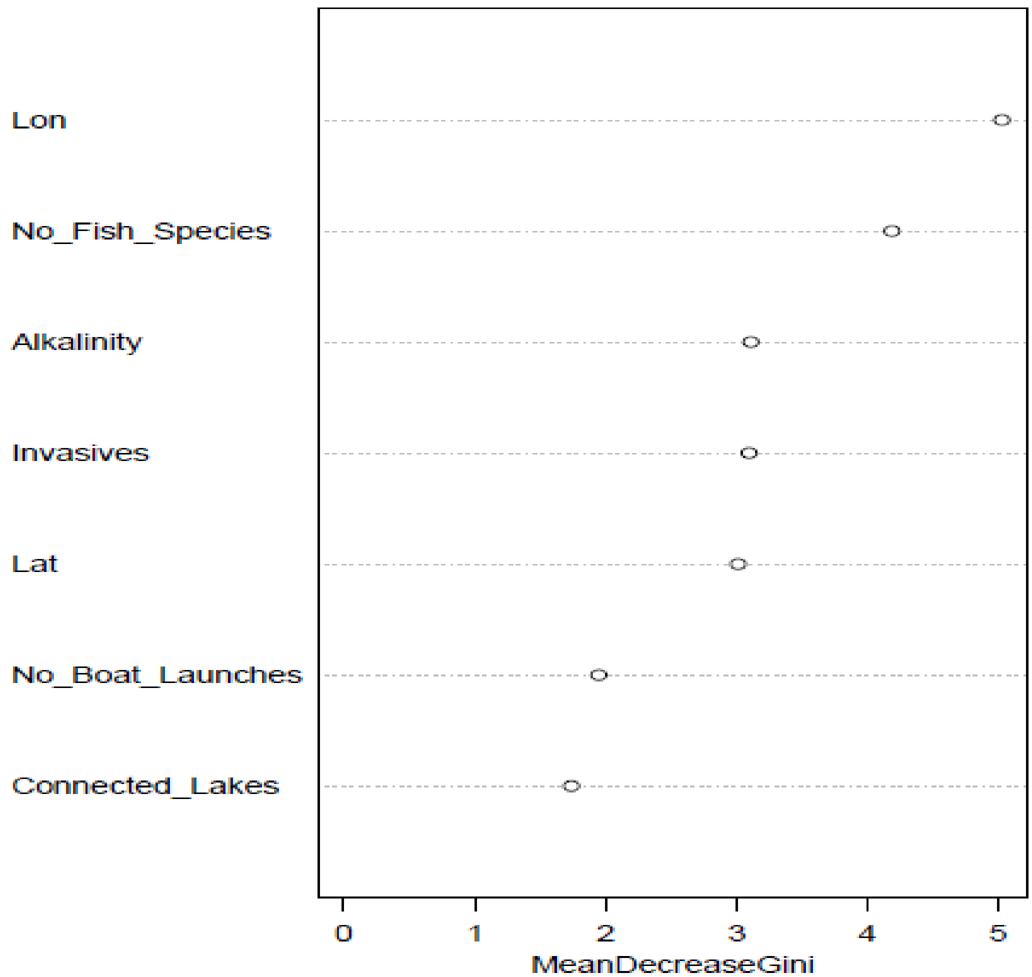

Figure 1: The mean decrease gini plot for RFM13 seems very similar to that of RFM9 except for No_Fish_Species (number of recorded fish species) and Lon (longitude) which switched positions. This may indicate that either No_Stcoking (number of time per year a waterbody is stocked with fish), pH, Cond (conductivity), Ca (calcium concentrations), and Na (sodium concentrations) are not ecologically important for determining *Cipangopaludina chinensis* successful establishment or may indicate that the created random forest model's (RFM) error rates and predictive power (i.e. the ability to make predictions) is dependent on data presence. Additionally, the lack of need for ecological parameters (i.e. pH, Cond, Ca, and Na) may be the result of not having a wide variety of measurements for each ecological parameter in the training data. Previous studies have found that there is some dependence on pH, salinity (indicative of Na

and Cond), and Ca for *C. chinensis* habitat suitability which means that either the model is highly dependent on data presence or the band-width for parameter measurements was fairly small.

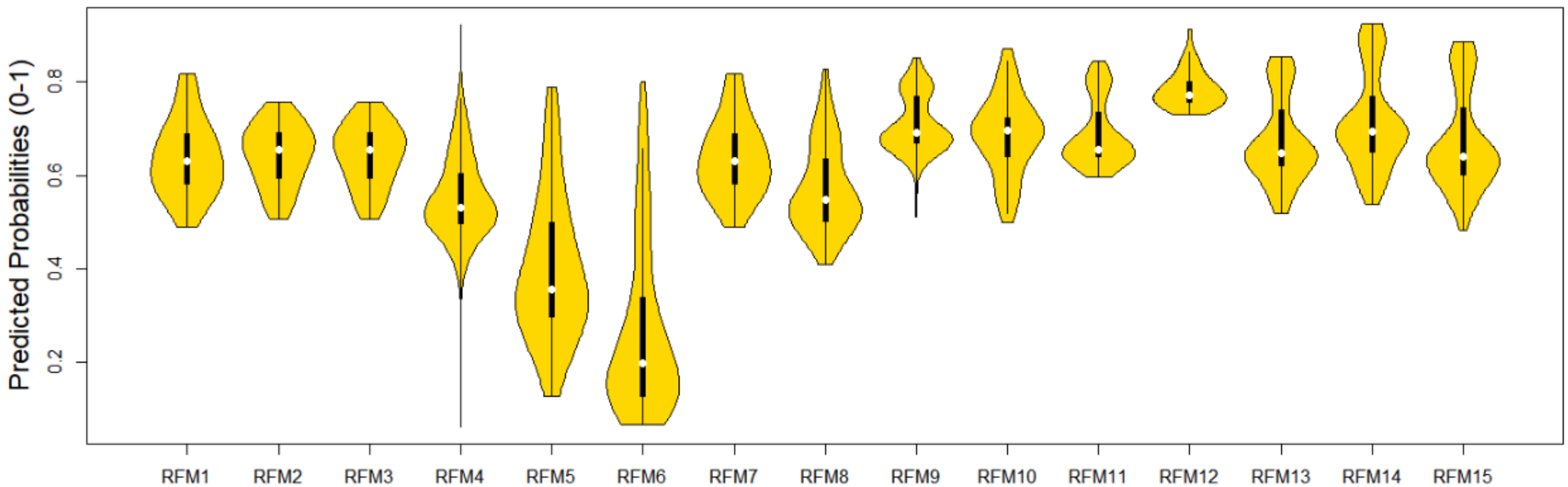

Figure 2: The predicted probabilities of the random forest models (RFM) created to test the sensitivity of RFM9, which was the model selected to best represent the predicted probabilities of suitable habitat for *Cipangopaludina chinensis* establishment in Nova Scotia, are noted as RFM13, RFM14, and RFM15. Out of all the models plotted here, only RFM13 seemed to perform better than RFM9 in terms of error rate and predictive power, but RFM13 was not selected as the "best" model because it excluded important ecological parameters in the model formula that were previously discovered by other studies to be important indicators of *C. chinensis*' invasion success.

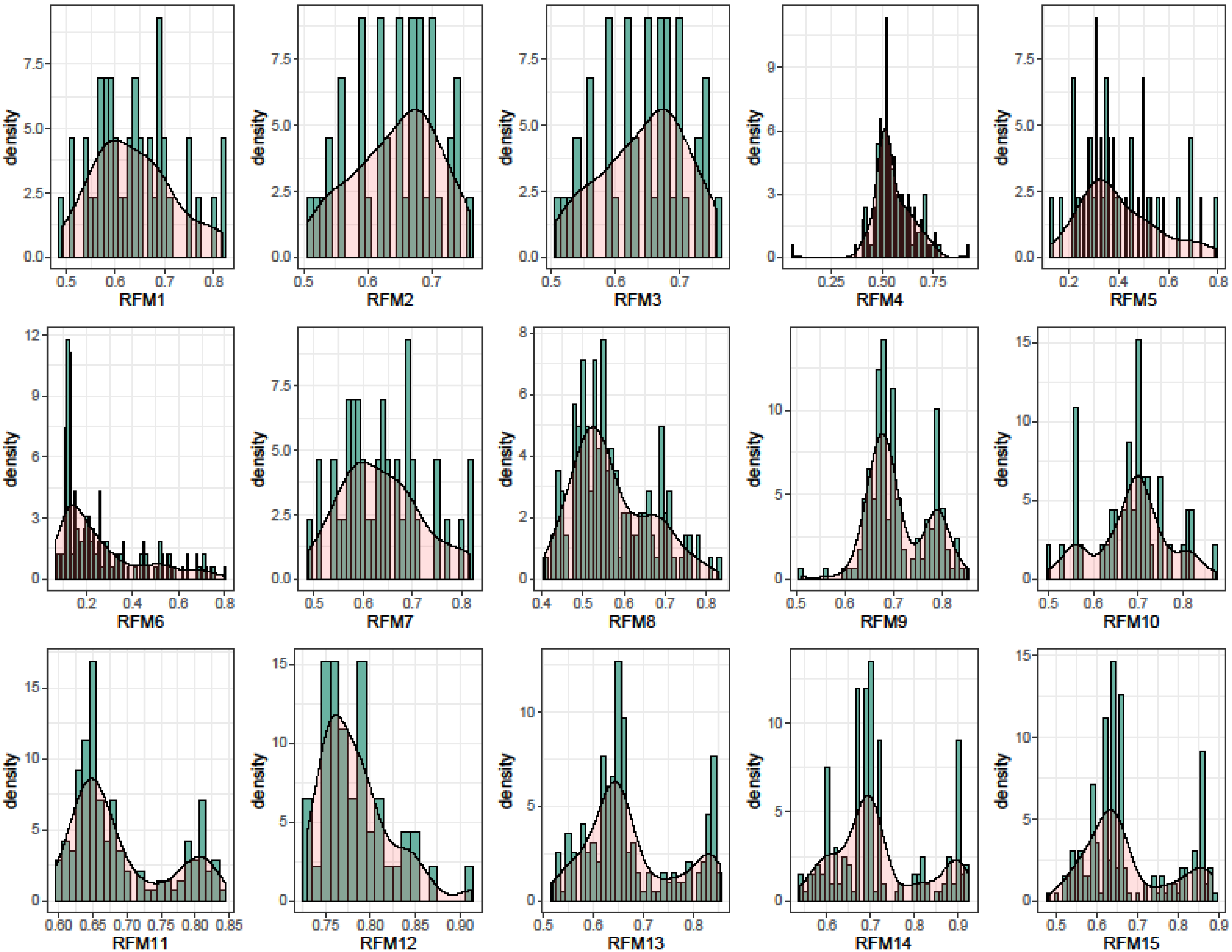
density
RFM1
RFM2
RFM3
RFM4
RFM5
RFM6
RFM7
RFM8
RFM9
RFM10
RFM11
RFM12
RFM13
RFM14
RFM15

Figure 3: Of all the models generated, RFM9 ($p=0.0497$) and RFM11 ($p=0.0078$) were significantly different from other models, RFM4 ($p=0.05751$) was semi-significantly different. Models were compared using a MANOVA and normality was confirmed via Shapiro-Wilk normality test. The statistical differences between RFM9/RFM11 and the other models may be caused by the selection and subsequent ranking of model parameters which causes the differences in model prediction densities. Both RFM9 and RFM11 prioritized location data (latitude and longitude), ecosystem composition parameters (number of recorded fish species, number of known invasive species), and waterbody accessibility (number of public boat launches, number of connected waterbodies) higher than water chemistry parameters in the decreased gini impurity plots. These results reinforce the need for further research into how *Cipangopaludina chinensis* presence impacts ecosystem composition as fish populations may be influenced by *C. chinensis* presence. The last three models (RFM13, RFM14, and RFM15) have similar density plots as RFM9 and RFM11 which was expected because the model formulas for RFM13-15 were developed from RFM9.

Conclusion:

In conclusion, the literature review (Chapter 1) found that *C. chinensis* does fit the federal definition for invasive species used in Canada and this species should be prioritized for future species management. The establishment of *C. chinensis* will negatively impact other indigenous mollusc species as *C. chinensis* presence increases inter-mollusc competition, shifts food webs by changing the bacterial community structure, and degrades water quality which promotes algal growth and eutrophication. Furthermore, *C. chinensis* may impact predators that naively consume this species as *C. chinensis* can host harmful parasites and act as a biotransfer for contaminants. Additionally, *C. chinensis* has been shown to host a parasite linked to decreased freshwater mussel reproduction in North America. The random forest modeling (Chapter 2) indicated that Halifax Regional Municipality, Cape Breton, and near the Nova Scotia-New Brunswick border where areas at high risk of future *C. chinensis* establishment. The areas identified by our models as at high-risk of *C. chinensis* invasion are also common habitats for endangered freshwater mussels. This is particularly concerning because our literature review's conceptual diagrams indicated that freshwater molluscs would be negatively impacted by *C. chinensis* presence. It is recommended that aquatic invasive species managers engage the public in collecting species occurrence data. The map of *C. chinensis* distribution in North America indicated that more reports of *C. chinensis* were collected from provinces with invasive species education and smart phone reporting apps, perhaps these techniques could be implemented elsewhere in Canada to augment ongoing efforts. Also, further research on *C. chinensis* ecological thresholds is needed to help interpret model predictions. Lastly, future studies should examine the ways in which humans facilitate species introductions and establishments as this may increase the probability of successful *C. chinensis* introductions and establishments, which will play a role in introduction of *C. chinensis* to habitats with endangered freshwater mussels.

Appendix A: Nutrient cycling experiment and tolerance of *Cipangopaludina chinensis* to various environments

Abstract:

This experiment was completed in support of Chapter 2 to determine the ecological requirements of the Chinese mystery snail, *Cipangopaludina chinensis*, for nutrient concentrations. Initial random forest models created for *C. chinensis*, which were trained only on true presence and field-confirmed and assumed absence data from Nova Scotia, identified that chlorophyll-a concentrations were highly correlated with *C. chinensis* presence. Therefore, this experiment was done to determine if *C. chinensis* needed a specific chlorophyll-a concentration (i.e. perhaps *C. chinensis* can not survive in nutrient poor water), or if *C. chinensis* presence had increased chlorophyll-a concentrations. The latter would indicate *C. chinensis* presence if chlorophyll-a concentrations were higher than usual. Adult and juvenile *C. chinensis* were exposed for 14 days to a variety of environments: high nutrient concentration, natural concentration, and low concentration. Additionally, a parallel experiment exposing adult *C. chinensis* to different snail population densities (0,1, 2, and 4 snails per jar) to determine if the strong correlation seen in earlier models between increased chlorophyll-a concentration and *C. chinensis* presence was indicative of either *C. chinensis* presence (i.e. 1 snail per jar would be statistical different than 0 snails per jar) or population size (i.e. only larger populations cause increased chlorophyll-a concentrations). There was no significant difference observed for changes in *C. chinensis* wet weights between the natural- and low-nutrient environment treatments indicating that *C. chinensis* did not need a specific concentration of chlorophyll-a to survive. However, all adult *C. chinensis* and some juveniles perished in the enriched treatments, which had relatively low dissolved oxygen percentage. This indicates that dissolved oxygen concentrations may be a limiting factor for *C. chinensis* establishment. Additionally, in the snail

density experiment, treatments with higher snail density had higher chlorophyll-a concentrations, particularly 2 snails per jar which was significantly higher than controls ($p=0.0141$). Our results suggest that the correlation between chlorophyll-a concentrations and *C. chinensis* presence in Nova Scotian waterbodies was not indicative of *C. chinensis* establishment but may be caused, in part, by *C. chinensis* presence.

Methods:

Chinese mystery snails ($n=40$) were caught from Loon Lake, Dartmouth, Nova Scotia, Canada during the second week of October and transported in a cooler back to the Dynamic Environments & Ecosystem Health Research Group Laboratory at Saint Mary's University in Halifax, Nova Scotia (about a 30 min drive). The snails were left in the cooler overnight to adjust to lab settings. The following day, all snails were removed from the cooler, lightly scrubbed clean with a toothbrush and placed in a pre-prepared 30-gallon aquarium. The aquarium was prepared three days earlier with sieved (with a 2mm stainless steel sieve) Loon Lake sediment and dechlorinated water. The aquarium was fitted with an aquarium filter, thermometer, and hood light (100-1000 lux) and had a light cycle of 16h light: 8h dark. Liquid calcium was added to the aquarium (3mL added on day 1 of setup). *C. chinensis* once added to the aquarium, were given 3 discs of algae wafers. Water changes were conducted three times a week; and food was administered and any juveniles produced by the adult *C. chinensis* population were removed and placed into their own, small aquarium with the same conditions as the adult aquarium (i.e. with the sieved Loon Lake sediment, dechlorinated water, same light cycle and intensity, same food was given, and same schedule for water change). Adult *C. chinensis* were given three weeks in the lab aquarium to adjust to the lab setting (i.e. both the experiment and the culture aquarium were housed in the same lab).

Before adding *C. chinensis* (both juvenile and adult snails) to experiments, each snail was painted with nail polish in order to facilitate individual identification. Each snail shell was measured, height was measured from the tip of the apex to the opening of the operculum and width was measured across the first whorl, using a digital calliper. Also, snails were removed from aquariums, left out of water for 2h, dried with a Kim wipe, and weighed using a microbalance.

Snail Density Experiment

Four snail density treatments were tested, zero snails in 2L, 1 snail in 2L, 2 snails in 2L, and 4 snails in 2L. Each treatment had three replicates carried out in 2L glass jars filled with dechlorinated water (i.e. the same type of water that the snails were adapted to from the lab culture aquariums) with approximately 2 cm (~500 g ww) of sieved (with 2 mm sieve) Loon Lake sediment. The appropriate number of adult *C. chinensis* (i.e. 0, 1, 2, or 4) were added to each jar. The jars were covered with parafilm (to mitigate water evaporation), an air bubbler (to ensure consistent air circulation) and placed within a water bath (to maintain a consistent temperature 18-22°C). The baths with jars were placed under a light with a light intensity of 400-600 lux and a cycle of 16h light: 8h dark. There was a total of 12 jars with various snail densities (0-4 snails) for a total of 21 adult *C. chinensis*. The experiment's duration was 14 days.

Once a week (day 0, 7, and 14) 250 mL water was exacted from each jar, below the water's surface, using a 60 mL plastic syringe. The specific conductivity (µs/cm), total dissolved solids (mg/L), dissolved oxygen (%), temperature (°C), salinity (ppt), and pH were measured with a WetPro multiprobe. Water samples were filtered through a glass fiber filter following the EPA Method 445.0 *In Vitro* Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorometer Protocol (https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NERL&dirEntryId=309417) for Chlorophyll-a analysis. The filters were frozen at -20℃ and analysed within one week of filter extraction.

At the end of the experiment (day 14), all *C. chinensis* were removed from their jars and re-weighed and re-measured (following the same methodology previously described). The initial and final weights of each *C. chinensis* were compared to indicate whether the specimen had decreased health (i.e. *C. chinensis* experienced significant weight loss).

<u>Environment Suitability and Thresholds:</u>

Adult *C. chinensis* and juvenile *C. chinensis* were added to 2L jars (either 1 adult snail per jar or 4 juvenile snails per jar) that either mimicked an enriched environment, a natural environment, or a sterile/very low nutrient environment with 3 replicates each (9 jars with adult *C. chinensis* and 9 jars with juvenile *C. chinensis*). The experiment lasted for 14 days with water quality, specific conductivity, dissolved oxygen, pH, total dissolved solids, salinity, water temperature, measured on days 0, 7, and 14. The exacted water was replaced with fresh dechlorinated water (or in the case of the low-nutrient treatment, replaced with autoclaved dechlorinated water). The extracted water samples, once tested for water quality, was filtered through a glass fiber filter (0.7 µm pore size) and filters were analysed for chlorophyll-a concentration for day 0 (all jars) and day 14 (for low-nutrient environment jars only). The jars were placed in a water bath to maintain an appropriate temperature (19-23 ℃) and water baths were placed under a fluorescent light (400-600 lux) with a 16h light: 8h dark cycle. Jars were fitted with an air bubbler and covered with parafilm to decrease evaporation.

Each day jars were checked for mortality and/or birth(s). Nutrient-rich jars had poor water clarity such that if snails were dead within the jar, they were not detected as dead until a water change. Once removed from the jars, each snail was re-weighed for comparison with initial weight. Weights reported here are wet weight.

Statistical Analysis

One-way ANOVA was used to assess the statistical relationships between:

(1) the change in weight of adult or juvenile *C. chinensis* and treatment environment (either enriched, natural, or sterilized).; and
(2) the changes in chlorophyll-a concentration and the density of snails (i.e. number of snail per jar).

Hypotheses:

(1) *C. chinensis* exposed to the sterile treatments will loss significantly more weight than those in the natural treatments and *C. chinensis* in the enriched treatments will loss less weight than those in the natural treatments.

(2) The chlorophyll-a concentrations will increase with the increase in snail density. Therefore, treatments with greater snail densities (i.e. 2 and 4 *C. chinensis*/jar) will be significantly greater than the control (i.e. 0 *C. chinensis*/jar).

(3) *C. chinensis* in the different snail density treatments will change their weight proportional to the number of snails per jar.

Results:

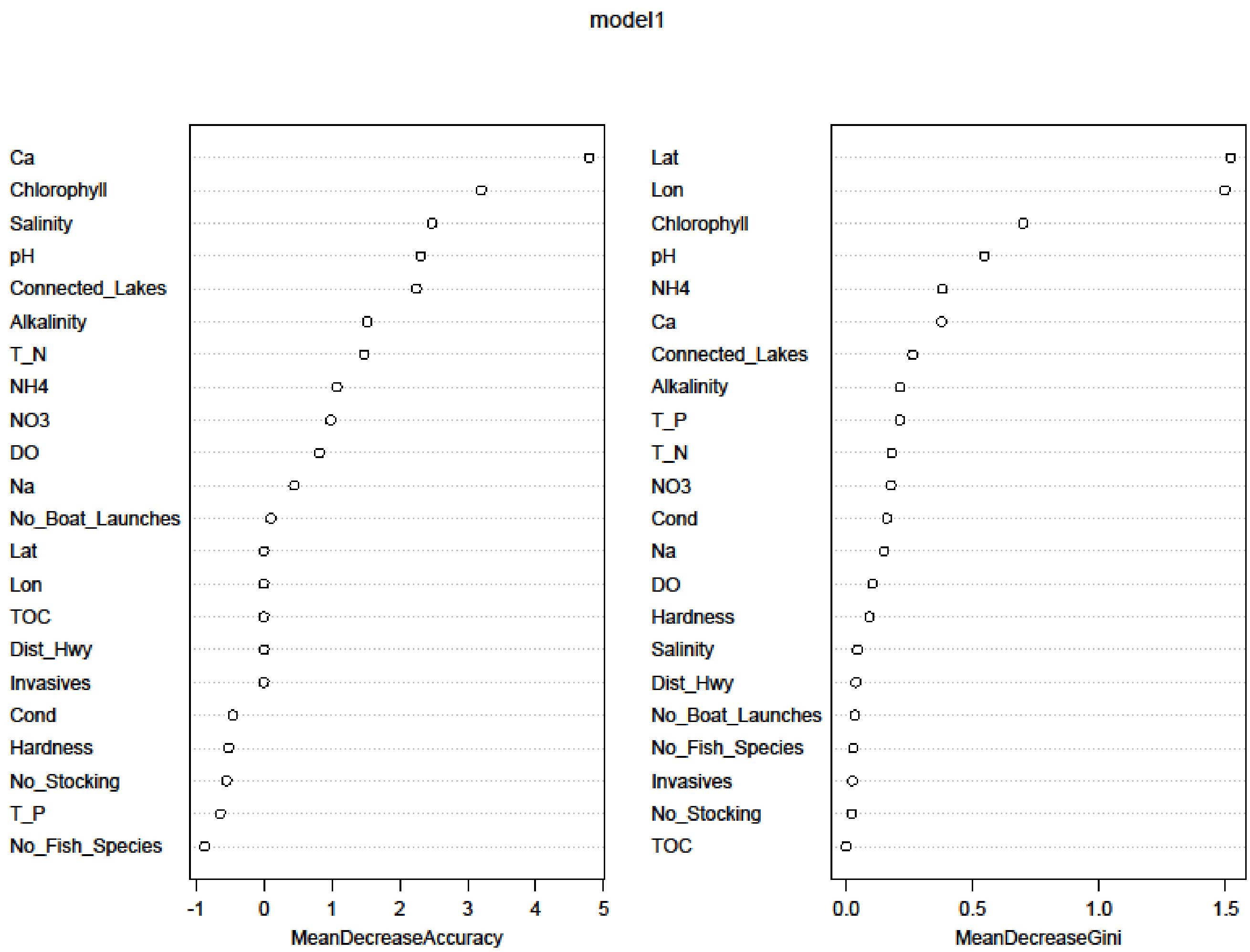

Figure 1: The mean decrease in accuracy indicative of the relative importance of each parameter in completing accurate predictions and mean decrease gini is the score of relative importance of each parameter used to create the model. This model (i.e. "model 1") was trained using data collected from the Department of Fisheries and Oceans Canada 2011 Halifax Regional Municipality (HRM) Synoptic data and only Nova Scotia true presence and absence data for *Cipangopaludina chinensis*. Notably, chlorophyll-a concentration ("Chlorophyll") was ranked near the top of the mean decrease gini plot meaning that this variable was strongly correlated with *C. chinensis* presence in HRM waterbodies. However, it is not clear from this plot whether the correlation is because *C. chinensis* requires certain concentrations for successful establishment, or if *C. chinensis* presence causes increased chlorophyll-a concentration (i.e. chlorophyll-a concentration may be influenced by *C. chinensis* presence). One-way ANOVA Results for Chlorophyll-a concentration for treatment of various snail densities.Residual standard error: 3.136 on 32 degrees of freedom, Multiple R-squared: 0.2259, Adjusted R-squared: 0.1533, F-statistic: 3.112 on 3 and 32 DF, p-value: 0.03991

* indicates significant difference ($p<0.05$):

Source	Estimate Std.	Error	T-value	p-value
Control	0.0581	1.0453	0.056	0.9560
Control: 1 snail per jar	0.2689	1.4783	0.182	0.8568
Control: 2 snails per jar	3.8374	1.4783	2.596	0.0141*
Control: 4 snails per jar	2.5377	1.4783	1.717	0.0957

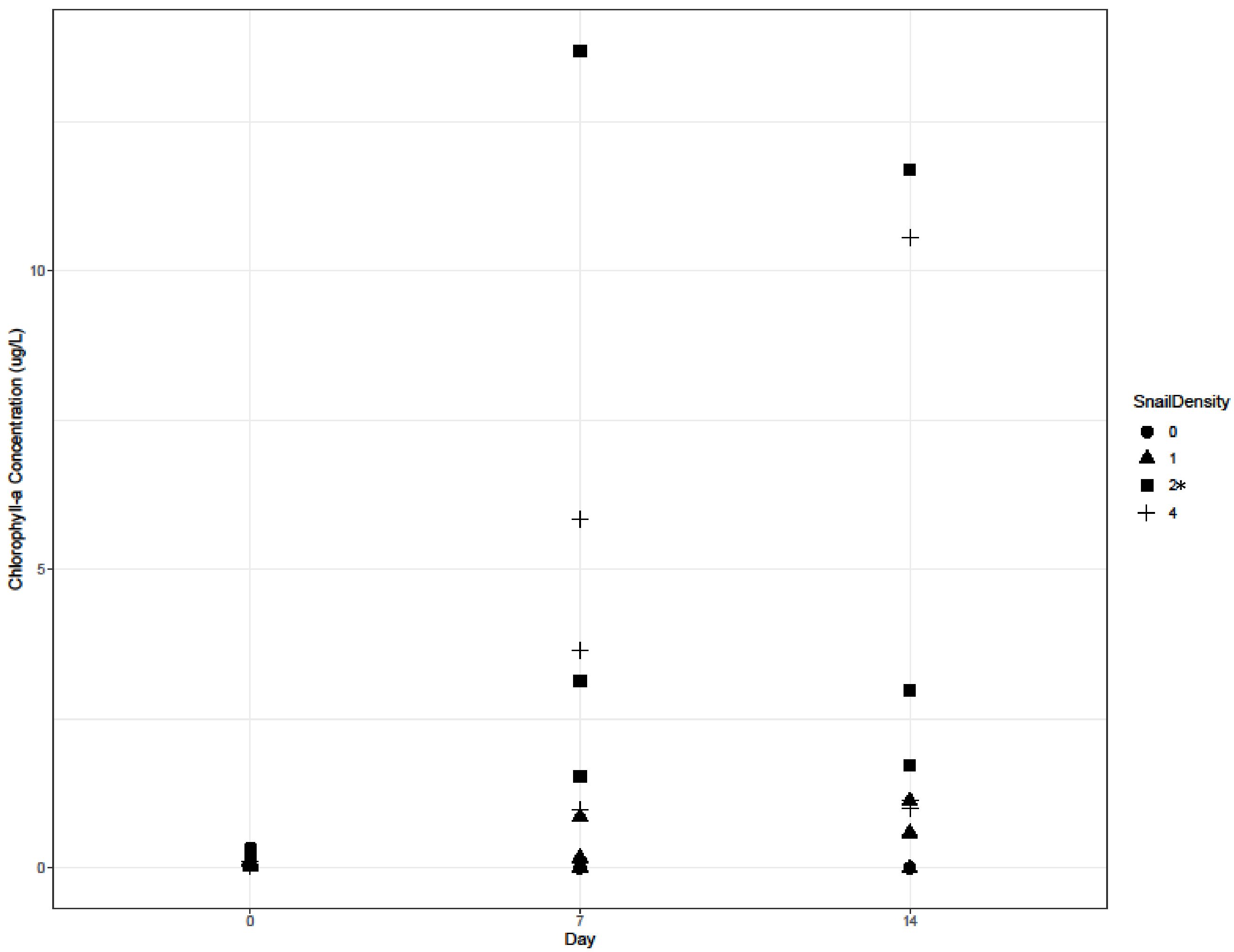

Figure 2: The density of snails per jar did have an effect on the chlorophyll-a concentrations. However, only 2 snails per jar had chlorophyll-a concentrations that were significantly (p=0.0141) higher than the control group (i.e. 0 snails per jar). All other treatments were not significantly different from the control. Notably, 4 snails per jar did have increased chlorophyll-a concentrations for some replicates, but these high concentrations were not seen throughout all

jars and were not sustained (i.e. decreased seen in chlorophyll-a concentration between days 7 and 14). Possibly, 4 snails per jar both produced and consumed large amounts of chlorophyll-a. Further studies are needed to determine at what snail density chlorophyll-a production and consumption are equivalent (if possible), and what the typical snail density in a given waterbody is (presumably this will vary depending on population size and waterbody size).

One-way ANOVA Results for weight change of juvenile *C. chinensis* in various treatment environments (Intercept=NE= Natural Environment, Order 1=SE= Sterilized Environment, Order 2=EE= Enriched Environment).

Juvenile snails: Residual standard error: 0.05435 on 33 degrees of freedom, Multiple R-squared: 0.004542, Adjusted R-squared: -0.05579, F-statistic: 0.07529 on 2 and 33 DF, p-value: 0.9276

Adult snails: Residual standard error: 3.797 on 6 degrees of freedom, Multiple R-squared: 0.5666, Adjusted R-squared: 0.4222, F-statistic: 3.923 on 2 and 6 DF, p-value: 0.08139

* indicates significant difference (p<0.05):

Source	Estimate Std.	Error	T-value	p-value
Juvenile snails				
NE	0.029833	0.015689	1.902	0.066
NE:SE	-0.005917	0.022187	-0.267	0.791
NE:EE	0.002458	0.022187	0.111	0.912
Adult snails				
NE	2.711	2.192	1.237	0.2623
NE:SE	-2.889	3.100	-0.932	0.3874
NE:EE	-.8536	3.100	-2.753	0.0331*

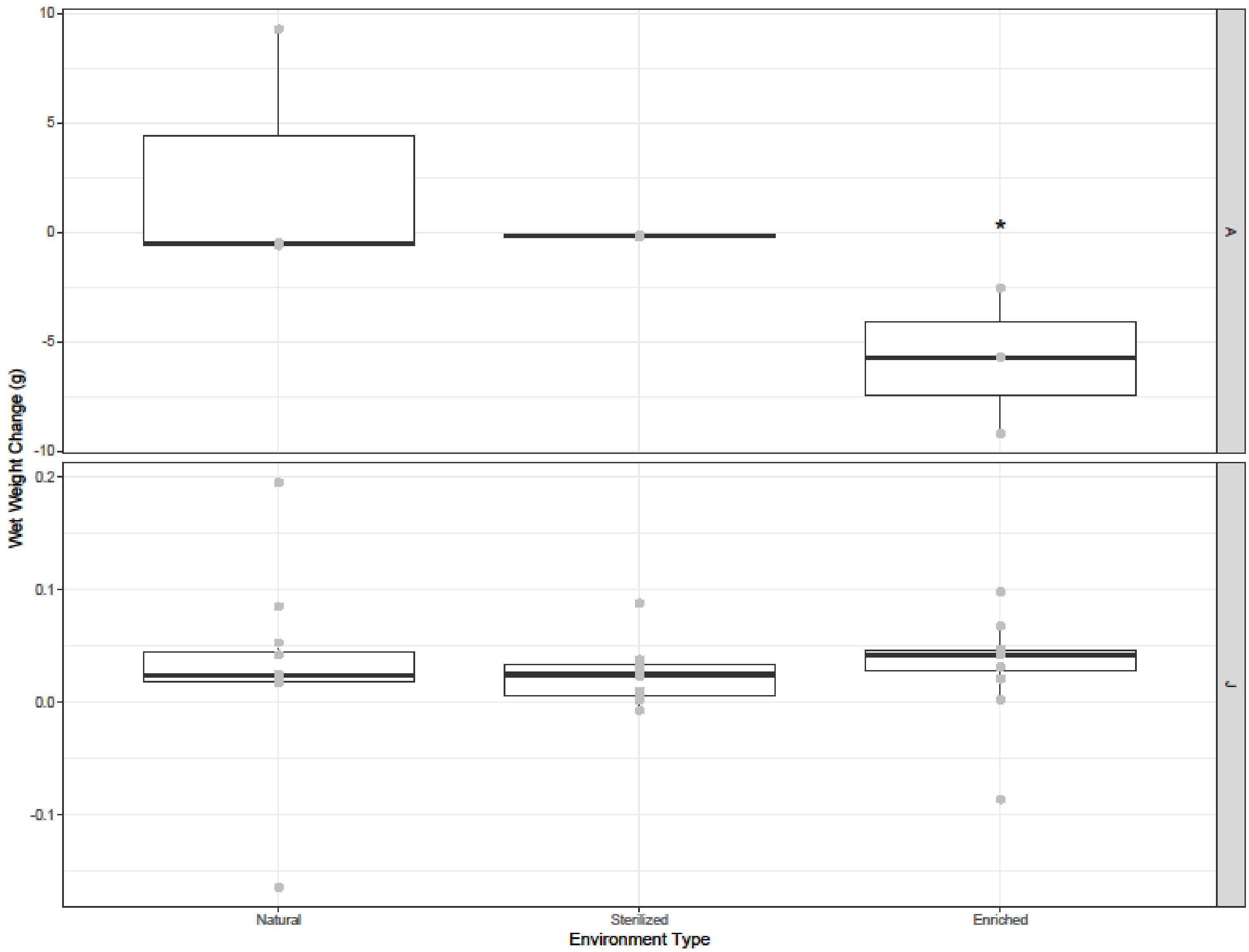

Figure 3: *Cipangopaludina chinensis* did not suffer significant weight loss or death in the sterile environment treatments compared to the natural environment treatments which indicated that *C. chinensis* did not require a specific concentration of chlorophyll-a for survival or establishment. Yet, all adult and some juvenile snails perished in the enriched treatments and average adult *C. chinensis* weight loss was significantly greater (p=0.0331) than snails in the natural treatment. This result may represent a possible ecological threshold in terms of dissolved oxygen concentration which could be a limiting factor for future *C. chinensis* population success.

One-way ANOVA Results for weight change of adult *C. chinensis* in various snail density treatments (Intercept= 1 snail per jar= SD1, Order 2= 2 snails per jar= SD2, Order 4= 4 snails per jar= SD4). Residual standard error: 0.4037 on 18 degrees of freedom, Multiple R-squared: 0.08391, Adjusted R-squared: -0.01788, F-statistic: 0.8243 on 2 and 18 DF, p-value: 0.4544

Source	Estimate Std.	Error	T-value	p-value
SD1	-0.43033	0.23307	-1.846	0.0813
SD1:SD2	0.07817	0.28545	0.274	0.7873
SD2:SD4	0.27542	0.26058	1.057	0.3045

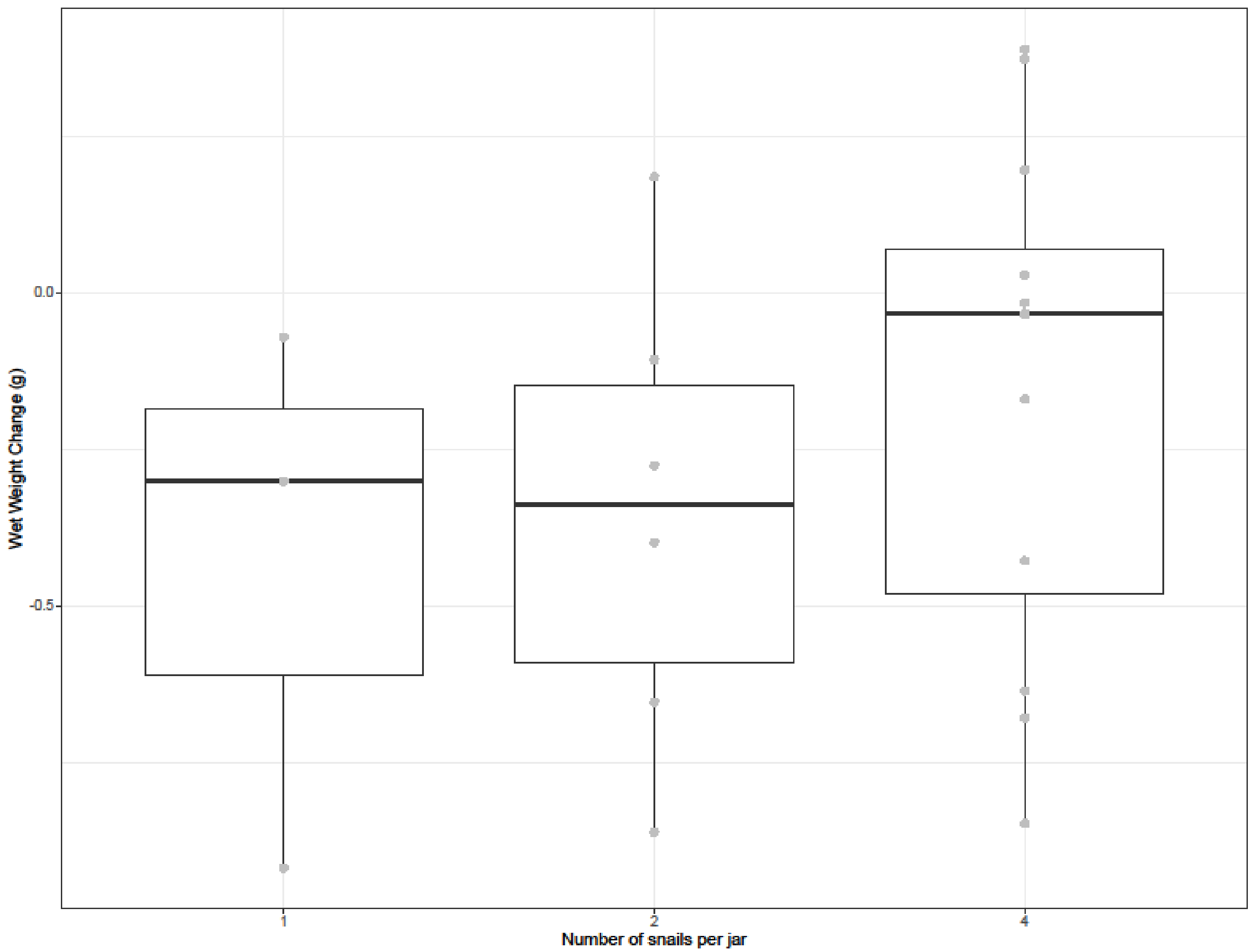

Figure 4: There was no observed significant difference in weight loss due to snail density. The snails in the density experiment were not fed for the experiment duration, therefore, some weight loss was expected. *Cipangopaludina chinensis* in higher snail densities proportionally loss less weight (i.e. snails in the 2 snail per jar treatments loss less weight than 1 snail per jar treatments but more than 4 snail per jar treatment). This further supports the theory that snails in the 4 snails per jar treatments were both producing and consuming relatively large amounts of chlorophyll-a.

Appendix B: Links to Database, R Code, and Additional Model Analyses

Databases and R Code: http://doi.org/10.6084/m9.figshare.12294743

Master database: http://doi.org/10.6084/m9.figshare.12295463

Raw model outputs: http://doi.org/10.6084/m9.figshare.12295925

Note: all these items listed above can be found on FigShare under "The Chinese Mystery Snail Project" at: https://figshare.com/account/home#/projects/80522.