COSEWIC
Assessment and Status Report

on the

Rainbow
Villosa iris

in Canada

SPECIAL CONCERN
2015

COSEWIC
Committee on the Status
of Endangered Wildlife
in Canada

COSEPAC
Comité sur la situation
des espèces en péril
au Canada

COSEWIC status reports are working documents used in assigning the status of wildlife species suspected of being at risk. This report may be cited as follows:

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Previous report(s):

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COSEWIC
Assessment Summary

Assessment Summary – November 2015

Common name
Rainbow

Scientific name
Villosa iris

Status
Special Concern

Reason for designation
This small mussel is widely distributed in southern Ontario. Surveys since the previous assessment in 2006 have found large numbers of individuals in previously unknown localities, especially at headwaters of larger rivers. There is strong evidence of recent recruitment in six of the seven subpopulations examined. Although it has been lost from Lake Erie and the Detroit and Niagara Rivers, it was apparently never common in these waters. Two subpopulations (Ausable River and Lake St. Clair) have low abundance and are showing signs of continued decline. Ongoing threats to some subpopulations include invasive species (dreissenid mussels and Round Goby) and pollution (household sewage and urban wastewater as well as agricultural effluents). The species may become Threatened if threats are not effectively managed or mitigated.

Occurrence
Ontario

Status history
Designated Endangered in April 2006. Status re-examined and designated Special Concern in November 2015.

COSEWIC
Executive Summary

Rainbow
Villosa iris

Wildlife Species Description and Significance

Rainbow is a small freshwater mussel (average length in Canada about 55 mm) with a compressed, elongate-elliptical shape. The shell is yellowish, yellowish-green, or brown (in old specimens) with numerous narrow and/or wide broken dark green rays that cover the whole surface of the shell. Rays may be absent from the anterior portion of the shell. The nacre (inside of the shell) is silvery white and iridescent, which is the origin of the species' common name. There are 18 species in the genus *Villosa* in North America, but only *Villosa iris* (Rainbow) and *Villosa fabalis* have ranges that extend into Canada. Only two species in the genus are listed as secure (G5) in North America, one of which is *V. iris*. Freshwater mussels are sensitive indicators of ecosystem health, including water and habitat quality and the fish community on which they depend. Rainbow may be a particularly good indicator because of its sensitivity to toxic chemicals.

Distribution

Rainbow was once widely distributed in North America from New York and Ontario west to Wisconsin and south to Oklahoma, Arkansas and Alabama. In Canada, there are records from the Ausable, Bayfield, Detroit, Grand, Maitland, Moira, Niagara, Salmon, Saugeen, Sydenham, Thames and Trent river watersheds, as well as Lakes Ontario, Erie and St. Clair. The species appears to have been lost from the lower Great Lakes and connecting channels, except for the delta area of Lake St. Clair, and is still extant in all rivers, with the exception of the Detroit and Niagara rivers.

Habitat

Rainbow is most abundant in small to medium-sized rivers, but can also be found in inland lakes. It once occurred in small numbers throughout the shallow nearshore areas of the lower Great Lakes and connecting channels in firm sand or gravel substrates. In rivers, Rainbow is usually found in or near riffles and along the edges of emergent vegetation in moderate to strong current. It occupies substrate mixtures of cobble, gravel, sand and occasionally mud or boulder. Rainbow is most numerous in clean, well-oxygenated reaches at depths of less than one metre.

Biology

Rainbow has separate sexes, but males and females differ only slightly in shell shape and are difficult to tell apart. Rainbow glochidia (larvae), like those of most other freshwater mussels, are parasitic on fish. Rainbow is a long-term brooder that spawns in the late summer, broods its glochidia over the winter and releases them in the early spring. Sexual maturity occurs between 5 – 9 years of age and individuals can live up to 43 years. Generation time is estimated to be 15 years. Hosts for Rainbow in Canada include Striped Shiner, Smallmouth Bass, Largemouth Bass, Green Sunfish, Greenside Darter, Rainbow Darter, Yellow Perch, Mottled Sculpin and Rock Bass. Adult Rainbow feed on bacteria, algae and other organic particles that they filter from the water column. Juvenile Rainbow live completely burrowed in the substrate, where they feed on similar food items obtained directly from the substrate or interstitial water.

Population Sizes and Trends

Rainbow has likely been extirpated from the Niagara and Detroit rivers and most previously inhabited areas of Lake Erie and Lake St. Clair. A small population estimated at 1,500 individuals occupies the Canadian waters of the St. Clair River delta, but it is declining at an estimated rate of 7% per year based on data collected from 9 sites in 2001 and 2003 and has been eliminated from the Pocket Bay area as of 2011. Populations in the Ausable, Bayfield, Grand, Sydenham and Thames rivers are small, with population estimates of 12,000, 74,000, 25,000, 17,000 and 71,000 individuals, respectively. The population in the Ausable River appears to be declining. The Maitland, Saugeen and Trent river watersheds have the largest Rainbow populations estimated to be 4,200,000, 700,000 and 330,000 individuals. The Maitland River supports the largest and healthiest population in Canada.

Threats and Limiting Factors

The two greatest threats to Rainbow are invasive species (mainly the eastern locations and Lake. St. Clair) and pollution (mainly the southwestern locations). Dreissenids (*Dreissena polymorpha* and *D. rostiformis*) have established in waterways where Rainbow reside, including the Thames, Trent and Moira rivers and Lake St. Clair. This is most notable in the Trent River watershed where dreissenids can form blankets of shells effectively smothering anything below. The invasive Round Goby (*Neogobius melanostomus*) can also negatively impact Rainbow populations by disrupting the host fish relationship, in effect acting as a sink for glochidia and competing with hosts. Southwestern populations of Rainbow are surrounded primarily by agricultural land and urban centres fitted with wastewater treatment facilities. As such, their watersheds are prone to run-off of known toxins to Rainbow (road salt, endocrine disruptors, ammonia, mercury and copper), phosphorus/nitrogen and increased erosion. In addition, the Moira River has had chronic loading of arsenic and copper from mining activities upstream of where Rainbow reside. Other threats include damming and other system modifications, the severity of which are unknown. Host fishes and anthropogenic-induced changes in predation are considered minor limiting factors for Rainbow populations.

Protection, Status and Ranks

Rainbow was assessed as Endangered in 2006 by COSEWIC and listed as Endangered under the federal *Species at Risk Act* (*SARA*) in 2013. It was listed as Threatened under Ontario's *Endangered Species Act* (*ESA*) in 2007. Rainbow has a designated national ranking of N2N3 (nationally vulnerable – imperilled) in Canada and S2S3 in Ontario, the only province where it occurs. Globally, the species is listed as G5, defined as demonstrably widespread, abundant, and secure. Generally the species is considered secure in the U.S. (N5), but there are several states where Rainbow is legally considered Endangered (Alabama (S3), Arkansas (S2S3), Illinois (S1), Indiana (S3), Michigan (S2S3), New York (S2S3), North Carolina (S1), Oklahoma (S1), Pennsylvania (S1), West Virginia (S2), Wisconsin (S1)).

TECHNICAL SUMMARY

Villosa iris

Rainbow *Villeuse irisée*

Range of occurrence in Canada (province/territory/ocean): ON

Demographic Information

Generation time (average age of parents in the population)	15 yrs
Is there an [observed, inferred, or projected] continuing decline in number of mature individuals?	unknown
Estimated percent of continuing decline in total number of mature individuals within [5 years or 2 generations]	unknown
[Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over the last [10 years, or 3 generations].	unknown
[Projected or suspected] percent [reduction or increase] in total number of mature individuals over the next [10 years, or 3 generations].	unknown
[Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over any [10 years, or 3 generations] period, over a time period including both the past and the future.	unknown
Are the causes of the decline a. clearly reversible and b. understood and c. ceased?	a. No b. Yes c. No
Are there extreme fluctuations in number of mature individuals?	No

Extent and Occupancy Information

Estimated extent of occurrence	48,051 km²
Index of area of occupancy (IAO) (Always report 2x2 grid value).	2,532 km²
Is the population "severely fragmented" ie. is >50% of its total area of occupancy in habitat patches that are (a) smaller than would be required to support a viable population, and (b) separated from other habitat patches by a distance larger than the species can be expected to disperse?	a. No b. Yes
Number of "locations"* (use plausible range to reflect uncertainty if appropriate)	14 (range 11 – 17). Based on High impact threat of Invasive non-native/alien species (8.1) (in particular dreissenids and Round Goby) and the Medium-High impact threats of Household sewage and urban wastewater (9.1) and Agricultural and forestry effluents (9.3).

* See Definitions and Abbreviations on COSEWIC website and IUCN (Feb 2014) for more information on this term

Is there an [observed, inferred, or projected] decline in extent of occurrence?	Observed past decline of 38%.Inferred continuing decline based on continuing threats (pollution and invasive species). St. Clair delta location is very small and apparently still declining. If lost this will result in a reduction in EOO
Is there an [observed, inferred, or projected] decline in index of area of occupancy?	Observed past decline of 30%. Inferred continuing decline based on continuing threats (pollution and invasive species). St. Clair delta location is very small and apparently still declining. If lost this will result in a reduction in IAO
Is there an [observed, inferred, or projected] decline in number of subpopulations?	Inferred continuing decline based on continuing threats (pollution and invasive species). St. Clair delta location is very small and apparently still declining. If lost this will result in a reduction of number of subpopulations.
Is there an [observed, inferred, or projected] decline in number of "locations"*?	Inferred continuing decline based on continuing threats (pollution and invasive species). St. Clair delta location is very small and apparently still declining. If lost this will result in a reduction of locations.
Is there an [observed, inferred, or projected] decline in [area, extent and/or quality] of habitat?	Observed decline in quality of habitat.
Are there extreme fluctuations in number of subpopulations?	No
Are there extreme fluctuations in number of "locations"∗?	No
Are there extreme fluctuations in extent of occurrence?	No
Are there extreme fluctuations in index of area of occupancy?	No

Number of Mature Individuals (in each subpopulation)

Subpopulations (give plausible ranges)	**N Mature Individuals**
Ausable River	5,900-18,000
Bayfield River	74,000
Grand River	4,700-45,000
Lake St Clair	1,500
Maitland River	2,000,000 - 6,500,000
Moira River	Unknown
Salmon River	Unknown
Saugeen River	520,000 – 880,000
Sydenham River	17,000 – 18,000
Thames River	48,000 – 94,000
Trent River	Unknown

[*] See Definitions and Abbreviations on COSEWIC website and IUCN (Feb 2014) for more information on this term

Quantitative Analysis

Probability of extinction in the wild is at least [20% within 20 years or 5 generations, or 10% within 100 years].	N/A

Threats (actual or imminent, to populations or habitats, from highest impact to least)

i. Invasive Species (dreissenids, Round Goby)
ii. Pollution (ammonia, chloride, heavy metals, pharmaceuticals, wastewater effluent, nutrient and sediment loading)

Rescue Effect (immigration from outside Canada)

Status of outside population(s) most likely to provide immigrants to Canada.	Michigan S2S3 Pennsylvania S1 Ohio SNR New York S2S3
Is immigration known or possible?	Possible for St. Clair delta location, unlikely for all others
Would immigrants be adapted to survive in Canada?	Likely
Is there sufficient habitat for immigrants in Canada?	Likely
Are conditions deteriorating in Canada?[+]	Yes
Are conditions for the source population deteriorating?[+]	Unknown
Is the Canadian population considered to be a sink?[+]	No
Is rescue from outside populations likely?	No

Data Sensitive Species

Is this a data sensitive species? No

Status History

COSEWIC Designated Endangered in April 2006. Status re-examined and designated Special Concern in November 2015.

[+] See Table 3 (Guidelines for modifying status assessment based on rescue effect)

Status and Reasons for Designation:

Status: Special Concern	**Alpha-numeric codes:** Not applicable
Reasons for designation: This small mussel is widely distributed in southern Ontario. Surveys since the previous assessment in 2006 have found large numbers of individuals in previously unknown localities, especially at headwaters of larger rivers. There is strong evidence of recent recruitment in six of the seven subpopulations examined. Although it has been lost from Lake Erie and the Detroit and Niagara Rivers, it was apparently never common in these waters. Two subpopulations (Ausable River and Lake St. Clair) have low abundance and are showing signs of continued decline. Ongoing threats to some subpopulations include invasive species (dreissenid mussels and Round Goby) and pollution (household sewage and urban wastewater as well as agricultural effluents). The species may become Threatened if threats are not effectively managed or mitigated.	

Applicability of Criteria

Criterion A (Decline in Total Number of Mature Individuals): Does not meet criteria. The number of mature individuals is unknown but estimated to be over 7 million. Although there is an observed decline in EOO and IAO, inferred declines based on these changes in number of individuals are below threshold values.
Criterion B (Small Distribution Range and Decline or Fluctuation): Does not meet criteria. Both the EOO (48,051 km²) and IAO (2,532 km² continuous within water bodies) are above the thresholds for Threatened (<20,000 km² and < 2,000 km², respectively), the population is not severely fragmented (although subpopulations are separated from one another that makes dispersal unlikely), and there are greater than 10 locations. However, an inferred continuing decline in EOO, IAO, area, extent and quality of habitat, and number of locations or subpopulations is inferred due to continuing high impact threats (pollution and invasive species).
Criterion C (Small and Declining Number of Mature Individuals): Does not meet criteria. The number of mature individuals is unknown but estimated to be over 7 million.
Criterion D (Very Small or Restricted Population): D1 is not applicable as the population is not very small or restricted. Does not meet criteria for Threatened D2 with both the IAO and number of locations being well above the typical thresholds. Although the species is subject to threats caused by human activities such as invasive species and pollution, the effects of these threats will not lead to critical endangerment or extinction within 1 or 2 generations after the threats occur.
Criterion E (Quantitative Analysis): Not applicable. Analyses have not been done.

PREFACE

Since Rainbow was assessed as Endangered by COSEWIC in 2006, much new information has been gathered concerning the species' distribution. These new data significantly influence estimates of extent of occurrence, area of occupancy and number of locations. In particular, the species was found to inhabit the headwaters of many rivers including the Bayfield, Grand, Maitland, Saugeen, and Thames rivers as well as tributaries of the Trent River and main body of the Moira and Salmon rivers. Declines in abundance have been noted for two locations, namely the St. Clair delta population and the Ausable River population, with the St. Clair delta decline being more extreme (declines in abundance and distribution). Population size estimates, based on quantitative sampling, are now available for most rivers.

COSEWIC HISTORY

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) was created in 1977 as a result of a recommendation at the Federal-Provincial Wildlife Conference held in 1976. It arose from the need for a single, official, scientifically sound, national listing of wildlife species at risk. In 1978, COSEWIC designated its first species and produced its first list of Canadian species at risk. Species designated at meetings of the full committee are added to the list. On June 5, 2003, the *Species at Risk Act* (SARA) was proclaimed. SARA establishes COSEWIC as an advisory body ensuring that species will continue to be assessed under a rigorous and independent scientific process.

COSEWIC MANDATE

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) assesses the national status of wild species, subspecies, varieties, or other designatable units that are considered to be at risk in Canada. Designations are made on native species for the following taxonomic groups: mammals, birds, reptiles, amphibians, fishes, arthropods, molluscs, vascular plants, mosses, and lichens.

COSEWIC MEMBERSHIP

COSEWIC comprises members from each provincial and territorial government wildlife agency, four federal entities (Canadian Wildlife Service, Parks Canada Agency, Department of Fisheries and Oceans, and the Federal Biodiversity Information Partnership, chaired by the Canadian Museum of Nature), three non-government science members and the co-chairs of the species specialist subcommittees and the Aboriginal Traditional Knowledge subcommittee. The Committee meets to consider status reports on candidate species.

DEFINITIONS
(2015)

Wildlife Species	A species, subspecies, variety, or geographically or genetically distinct population of animal, plant or other organism, other than a bacterium or virus, that is wild by nature and is either native to Canada or has extended its range into Canada without human intervention and has been present in Canada for at least 50 years.
Extinct (X)	A wildlife species that no longer exists.
Extirpated (XT)	A wildlife species no longer existing in the wild in Canada, but occurring elsewhere.
Endangered (E)	A wildlife species facing imminent extirpation or extinction.
Threatened (T)	A wildlife species likely to become endangered if limiting factors are not reversed.
Special Concern (SC)*	A wildlife species that may become a threatened or an endangered species because of a combination of biological characteristics and identified threats.
Not at Risk (NAR)**	A wildlife species that has been evaluated and found to be not at risk of extinction given the current circumstances.
Data Deficient (DD)***	A category that applies when the available information is insufficient (a) to resolve a species' eligibility for assessment or (b) to permit an assessment of the species' risk of extinction.

* Formerly described as "Vulnerable" from 1990 to 1999, or "Rare" prior to 1990.

** Formerly described as "Not In Any Category", or "No Designation Required."

*** Formerly described as "Indeterminate" from 1994 to 1999 or "ISIBD" (insufficient scientific information on which to base a designation) prior to 1994. Definition of the (DD) category revised in 2006.

Environment Canada / Environnement Canada
Canadian Wildlife Service / Service canadien de la faune

Canada

The Canadian Wildlife Service, Environment Canada, provides full administrative and financial support to the COSEWIC Secretariat.

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WILDLIFE SPECIES DESCRIPTION AND SIGNIFICANCE

Name and Classification

Scientific name: *Villosa iris* (Lea, 1829)

English common name: Rainbow

French common name: Villeuse irisée

The recognized authority for the classification of aquatic molluscs in the United States and Canada is Turgeon *et al*. (1998) and the Integrated Taxonomic Information System (2015). The current accepted classification of this species is as follows:

Kingdom: Animalia
Phylum: Mollusca
Class: Bivalvia
Subclass: Paleoheterodonta
Order: Unionoida
Superfamily: Unionoidea
Family: Unionidae
Subfamily: Lampsilinae
Genus: *Villosa*
Species: *Villosa iris*

Parmalee and Bogan (1998) provide a complete list of synonyms for this species. They note that *Villosa iris* is a species complex likely composed of several valid species that cannot be resolved by morphological characteristics alone (Watters *et al.* 2009). Dr. G. Thomas Watters has used shell characteristics to separate *Villosa iris* into three subspecies across its range, namely, *V. iris iris*, *V. iris novieboraci* and *V. iris* "Missouri" (Ohio State University 2004). The *V. iris novieboraci* (Lea, 1838) form occurs in the Laurentian system as well as the Wabash and upper Mississippi river systems and is therefore the only form found in Canada.

Morphological Description

Rainbow is a small freshwater mussel that was first described by I. Lea in 1829 (Figure 1). The type locality is an unidentified waterbody in Ohio. The following description of the species was adapted from Clarke (1981), Strayer and Jirka (1997) and Parmalee and Bogan (1998). The shell is elongate-elliptical in shape, laterally compressed, and moderately thick anteriorly but becoming quite thin posteriorly. The posterior ridge is low and rounded. Male shells are bluntly pointed posteriorly whereas female shells are expanded and more broadly rounded, although the differences are subtle and visual separation of the sexes is difficult. The beaks are low and compressed; beak sculpture consists of four to six distinct bars – the first concentric and the rest becoming double-looped or irregular and nodulous. The hinge teeth are medium-sized, well developed and complete. Pseudocardinal teeth are elevated, a little compressed, conical and serrated. Lateral teeth are long, straight and thin. The surface of the shell is smooth with well-marked growth rests. The periostracum is yellowish, yellowish-green or brown (in old specimens) with numerous wide, or both narrow and wide, broken dark green rays that cover the whole surface of the shell or are absent anteriorly. Rays may become obscured in older specimens. The nacre is silvery white and iridescent, which is the origin of the common name.

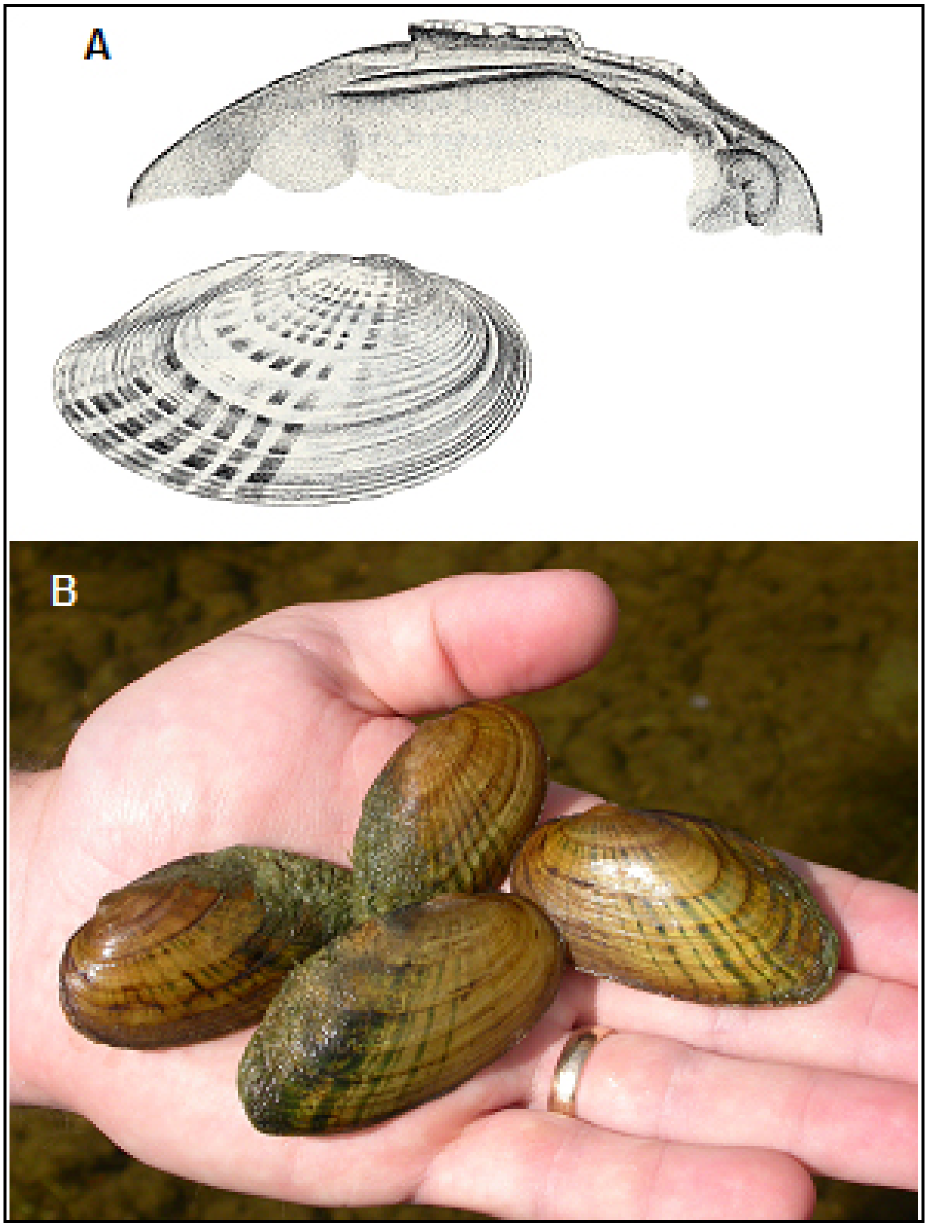

Figure 1. (A) Line drawing of the external features of the shell and internal structure of the left valve of Rainbow. Reproduced with permission from Burch (1975). (B) Photograph of live specimens collected from the Maitland River near Wingham, Ontario, in 2003. (Photo credit: D. McGoldrick, NWRI.)

Rainbow reaches a maximum length of about 90 mm in Canada. The average length of an adult shell is approximately 55 mm based on over 300 live specimens measured by the report writers and their associates between 1997 and 2004. Rainbow can be distinguished from all other species of freshwater mussel in Canada by its small size, narrow elliptical shape and interrupted green rays.

Population Spatial Structure and Variability

There is no information pertaining to the population structure of Rainbow. The geographical, ecological and behavioural barriers to movement that would create demographic isolation remain unknown.

Designatable Units

All Rainbow within Canada are found in the Great Lakes-Upper St. Lawrence National Freshwater Biogeographic Zone. There are no known differences among populations that would justify designation other than *V. iris novieboraci* (**see Name and Classification**); therefore, there is a single DU in Canada.

Special Significance

There are 18 species in the genus *Villosa* (recognized by Turgeon *et al*. 1998), but only *Villosa iris* and *V. fabalis* (Rayed Bean) have ranges that extend into Canada. Rainbow may be a particularly good indicator of ecosystem health because it is more sensitive to environmental contaminants than most other mussel species tested to date (Mummert *et al*. 2003) (see **LIMITING FACTORS**).

DISTRIBUTION

Global Range

Rainbow was once widely distributed in eastern North America from New York and Ontario west to Wisconsin and south to Oklahoma, Arkansas and Alabama. In the United States it has been recorded from Alabama, Arkansas, Illinois, Indiana, Kentucky, Michigan, Missouri, New York, North Carolina, North Dakota, Ohio, Oklahoma, Pennsylvania, Tennessee, Virginia, West Virginia and Wisconsin (Figure 2). The current distribution of Rainbow is similar to its historical distribution, but the species has been declining across the western part of its range in the United States (Cummings and Mayer 1992; Haag 2012).

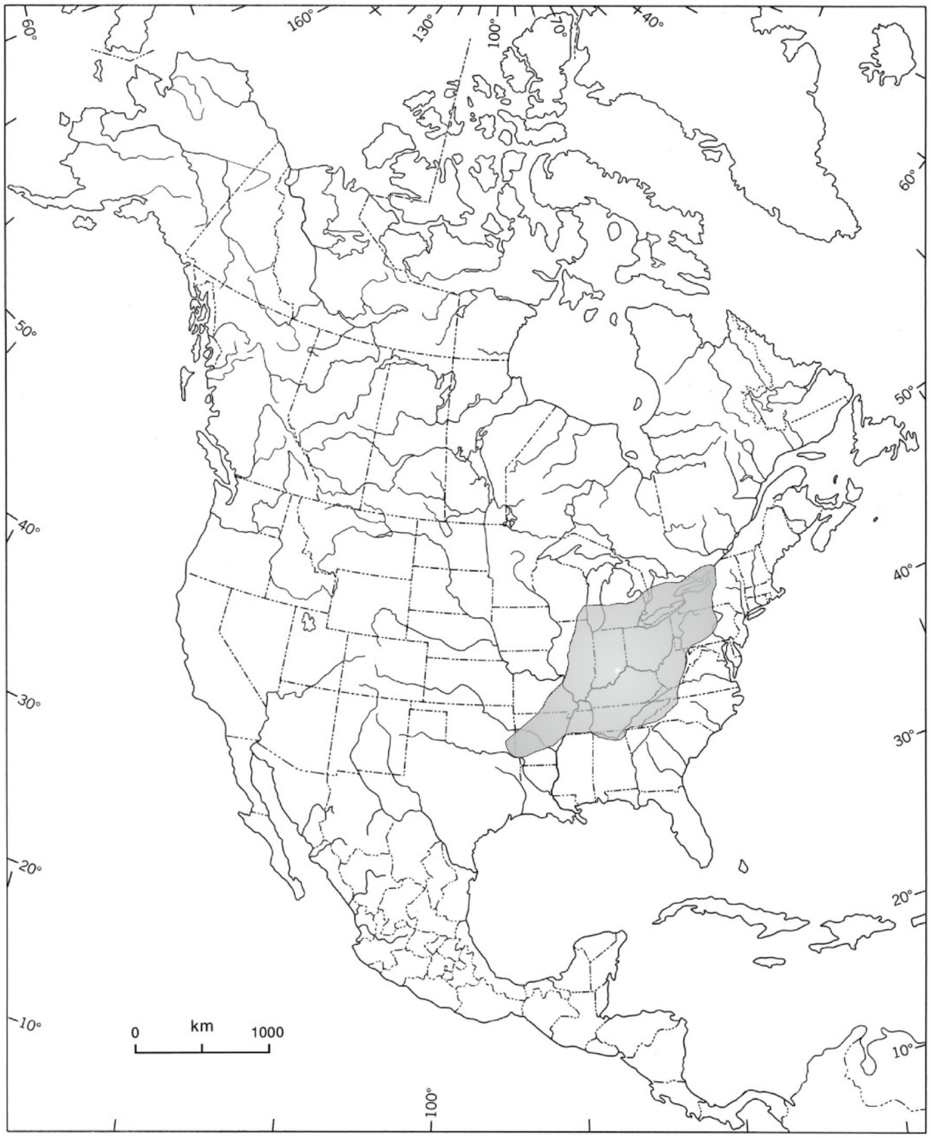

Figure 2. North American distribution of Rainbow (based on information provided by jurisdictions).

Canadian Range

In Canada, Rainbow is known only from southern Ontario. The earliest records of this species were reported in the 1890s by J. Macoun, who found specimens in the Detroit River near Windsor, the Grand River near Cayuga, and the Thames River near Chatham (Lower Great Lakes Unionid Database 2014; see **COLLECTIONS EXAMINED**). Since then, 425 records of just over 3000 live Rainbow have been reported.

Prior to 1997, Rainbow was collected from the Ausable, Detroit, Grand, Maitland, Moira, Niagara, Salmon, Saugeen, Sydenham, Thames and Trent rivers, as well as lakes Ontario, Erie and St. Clair. However, most of this sampling was opportunistic or semi-quantitative and in most cases, focused on lower river sites or lake shorelines. More recent survey efforts have begun to examine a wider range of habitat types, particularly headwater and tributary sites. Based on this new information, the known range of occurrence for Rainbow has expanded to include many tributaries of the Ausable, Bayfield, Grand, Saugeen and Thames rivers as well as areas in eastern Ontario including the Trent and Salmon rivers (Figure 3). The historical distribution of Rainbow in Ontario is based on 85 records (mostly from qualitative surveys) collected between 1890 and 1996 (Figure 4). The current distribution of the species is based on 340 records (live animals and shells) collected between 1997 and 2014 (Figure 4). Nine historical and one current record lack coordinates and are not included in the maps.

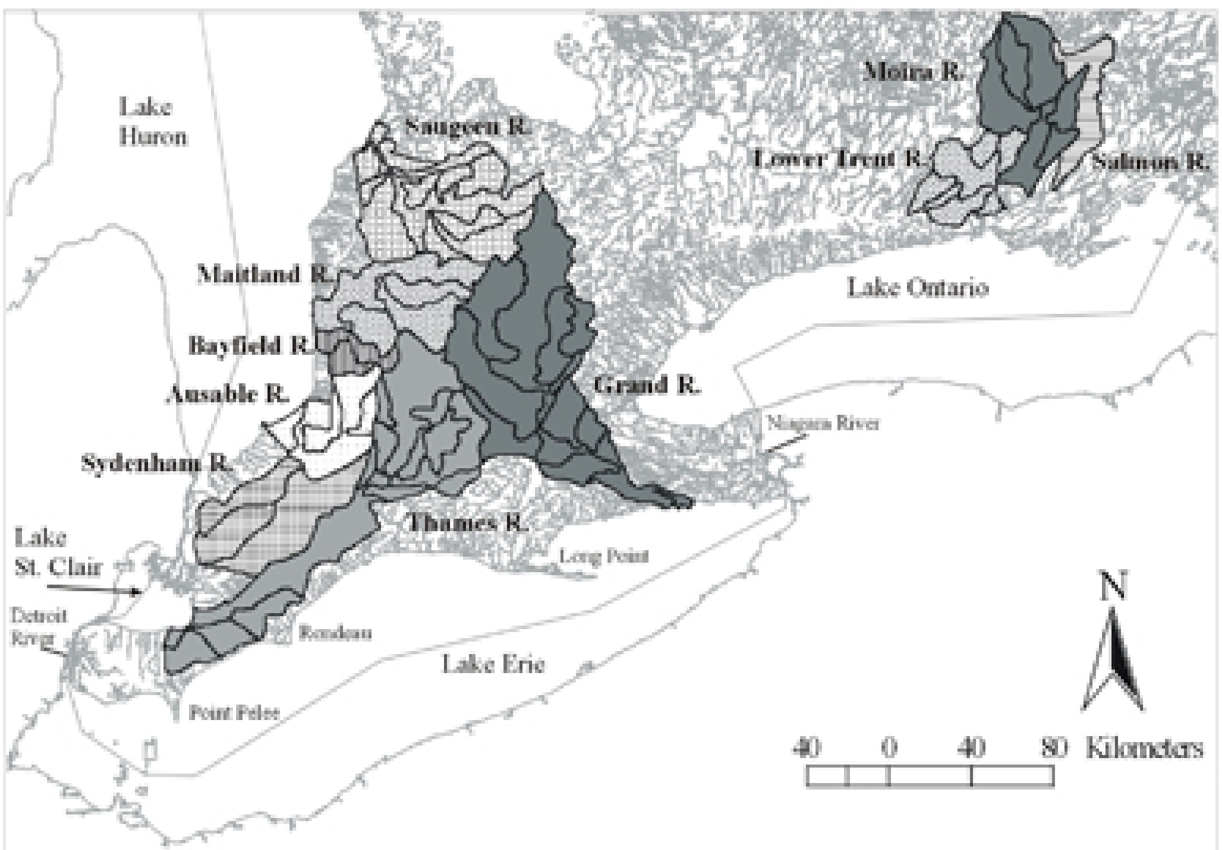

Figure 3. Watersheds where Rainbow occurs or occurred historically in Ontario.

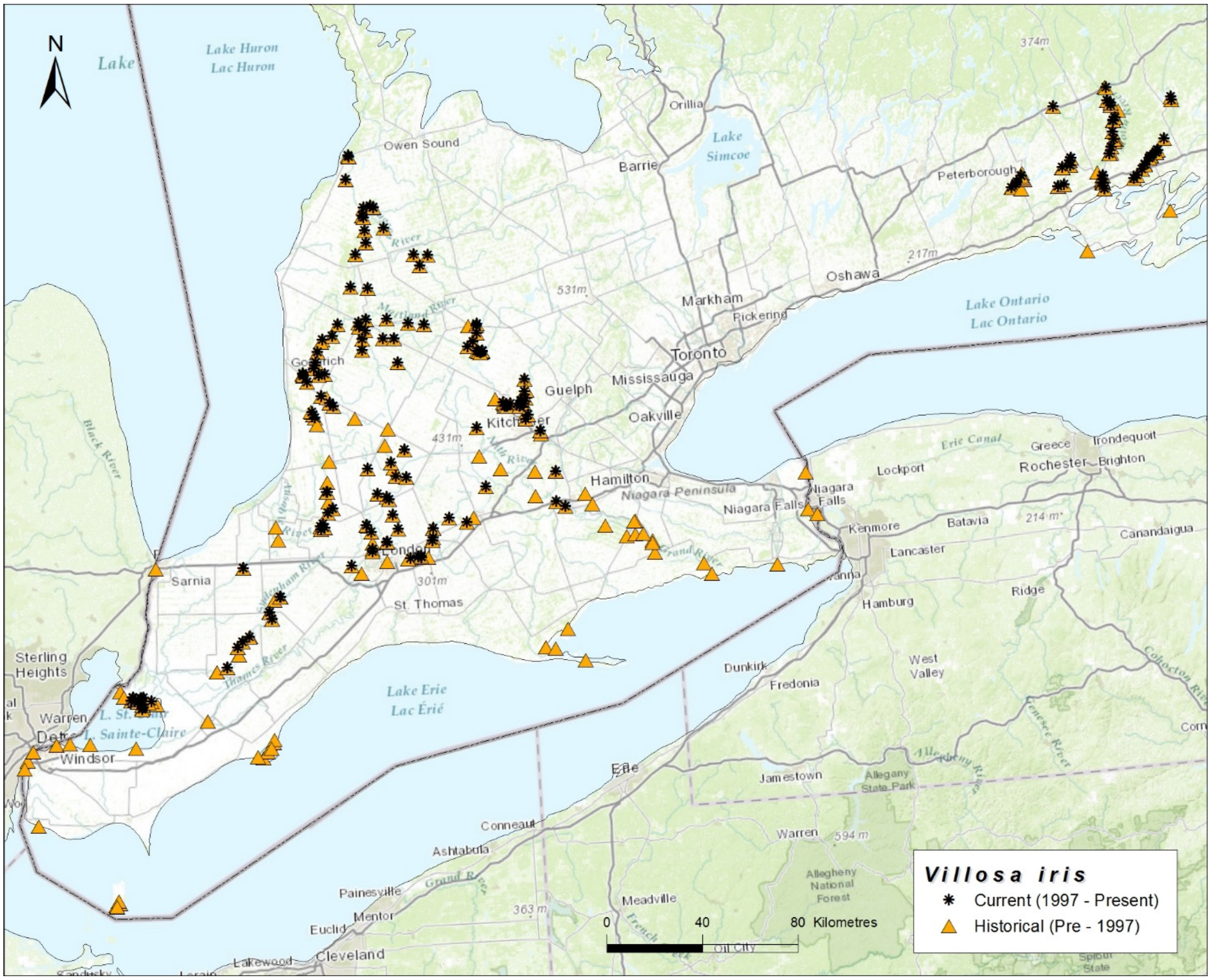

Figure 4. Historical (1890-1996) and current distribution (1997-2014) of Rainbow in Ontario (based on records from the Lower Great Lakes Unionid Database). Current records include live or fresh shells collected from 1997 up until the present whereas historical records include all shells (live, fresh dead, weathered) collected pre-1997 as well as any weathered shells collected post-1997.

Ausable River

The first record was made in 1950 by I.G. Reiman on the main branch near Springbank (Lower Great Lakes Unionid Database 2014). Only 39 live Rainbow have been discovered since then, all within the upper reaches spanning Brinsley to Nairn, as well as two tributaries: Narin Creek and the Little Ausable River (Lower Great Lakes Unionid Database 2014). In total there are five sites where Rainbow currently reside and eight additional sites with records of weathered and fresh shells.

Bayfield River

No historical information pertaining to Rainbow in the Bayfield River exists, the earliest collection record being in 2005 when a single fresh shell was discovered incidentally by the Ausable Bayfield Conservation Authority (ABCA; Lower Great Lakes Unionid Database 2014). Exploratory surveys completed in 2007 and 2011 by Fisheries and Oceans Canada detected Rainbow at nine sites (of 18 total): two on the Bannockburn River, three on the upstream reaches of main channel (spanning Clinton to Vanastra), as well as four headwater sites with shells only (33 live individuals; Morris *et al.* 2012). No individuals or shells were found in the lower reaches of the river. These surveys represent the first formal examination of unionids in the Bayfield River watershed and confirm an extant population of Rainbow.

Detroit River

Surveys in 1992 confirm that Rainbow resided in the Canadian waters of the Detroit River when three live individuals were found at one of the four sites surveyed (Schloesser *et al.* 1998). The only other Canadian record identified 34 fresh Rainbow shells in 1890 (Lower Great Lakes Unionid Database 2014). Detailed and long-term surveys have shown that all unionids are extirpated from the main branch (Schloesser *et al.* 2006; Zanatta *et al.* 2015) on the American side, with no Rainbow collected during the last surveys in 1998 or 2015. Since the American sites are in close proximity to the Canadian sites, it is reasonable to assume that this extirpation has also occurred in the Canadian waters.

Grand River

Although Kidd (1973) collected Rainbow shells from many sites in the Lower Grand River there have only ever been five records of live Rainbow from this area despite survey efforts at over 100 sites (Lower Great Lakes Unionid Database 2014). Four of these collections come from the 1970s: one site on each of Boston and Fairchild creeks with 10 and one individual, respectively, and one individual recorded from each of two sites on MacKenzie Creek (Kidd 1973). The fifth record is of one live specimen found near Brantford in 2012 (Lower Great Lakes Unionid Database 2014).

More recent and wide-spanning surveys have been conducted in the Grand River headwaters and tributaries. Since the 1970s, 40 live Rainbow have been found in these regions, 34 of which were discovered in the last decade. Three of these specimens are located in the Mallet River, 14 in the Conestogo River and 17 in the upper portions of the main branch. The Mallet, Conestogo and Nith rivers have numerous records of fresh and weathered shells (mostly from shoreline searches) further suggesting that this area sustains widespread Rainbow populations. Rainbow distributions likely extend further upstream in the watershed to areas that have gone unsearched. Previously, headwater systems were not targeted for Rainbow survey efforts. It is possible that the abundance of shells detected in the lower river during the historical period represent washout of shells from these upstream but unsampled (at the time) areas.

Lake Erie

Rainbow shells were collected from Long Point Bay, Rondeau Bay and Pelee Island in Lake Erie in the 1930s, 1960s and 1970s. D. Zanatta and D. Woolnough surveyed six sites (totalling 12 person-hours searched or PH) in Rondeau Bay in 2001. They found one live mussel (*Amblema plicata*) and weathered shells of 15 other species, but none were Rainbow. In 2014, the report writers in combination with the Ontario Ministry of Natural Resources and Forestry (OMNRF) instigated a more thorough resurvey of Rondeau Bay, expending 54 PH in visual-tactile searches across 12 sites including historical sites. One weathered Rainbow valve was detected at a site located between the 1934 and 1961 historical sites. In total, 2227 shells or shell fragments encompassing 24 species were collected with a substantial number representing weathered valves, indicating a high abundance of historical mussels but very few in the present day. Only three live animals were found: one Round Pigtoe (*Pleurobema sintoxia*), one Threeridge (*Amblema plicata*), and one Giant Floater (*Pyganodon grandis*). Long Point has received limited recent mussel survey effort with no Rainbow detected while Pelee Island has not been surveyed recently. American waters of the western basin and around the Bass Islands were surveyed in 1998 (33 sites) and again from 2004 - 2009 (17 sites), and no live Rainbow were observed (Ecological Specialists 1999; Crail *et al.* 2011). In fact, no live Rainbow has ever been documented from the Canadian waters of Lake Erie; only shells, the last known fresh shell being in 1966.

Lake Ontario

Rainbow has been collected twice in Lake Ontario: the first in the Bay of Quinte in 1932 and the second at a beach south of Hillier in 1996 (Lower Great Lakes Unionid Database 2014). Neither record has any further information regarding numbers collected or search effort/method. An extensive amount of effort encompassing 51 sites has been expended in the coastal wetlands of Lake Ontario in the past 10 years with a variety of techniques (snorkelling, visual search and half-hectare methods using visual-tactile and scoops) and have yielded no Rainbow specimens or shells (Lower Great Lakes Unionid Database 2014). The Bay of Quinte and Hillier areas were included in these searches. Although fairly extensive surveys have been completed in the coastal wetlands of Lake Ontario, the majority of this work has targeted Eastern Pondmussel (*Ligumia nasuta*). These surveys have focused on areas with soft, silty substrate and generally do not represent the preferred habitat of Rainbow.

Lake St. Clair

Zanatta *et al*. (2002) surveyed 95 sites in nearshore areas around Lake St. Clair between 1999 and 2001 and found live mussels at 33 sites. Rainbow was found at 13 of these, all within Canadian waters, namely Mitchell's Bay and Johnston Bay in the Walpole Island area. Two years later, Metcalfe-Smith *et al*. (2004) surveyed 28 sites (stake and rope and timed search methods) in the delta and found the species to be much more common in U.S. than Canadian waters (70% vs. 30% of sites sampled, respectively). Resurveys in 2004 – 2006 and 2011 revealed that live Rainbow only occupied three of 17 sites in

Canadian waters, with the most recent stake and rope searches in 2006 and 2011 having the least, at 0% (0 of 2 sites) and 11% (1 of 9 sites) site occupancy. In the 2011 surveys, Rainbow were only located in the Squirrel Island/Bass Bay area, and none were found in Pocket Bay where they had previously been found in low numbers. Rainbow was not recorded from the offshore waters of Lake St. Clair either before or after the dreissenid invasion (Nalepa *et al*. 1996), although one historical record exists for Rainbow in the St. Clair River (Lower Great Lakes Unionid Database 2014).

Maitland River

The first observations of Rainbow in the Maitland River occurred near Wingham, Auburn (two sites) and Seaforth in the 1930s (Lower Great Lakes Unionid Database 2014). However, the first known live specimen was collected in 1998 by S. Staton in a 1.5 PH timed search. In total there have been 33 sites surveyed throughout the Maitland watershed between 1998 and 2012, over half of which sustain Rainbow populations at high densities. Ten of the sites with live Rainbow are on tributaries representing all branches of the river (Middle Maitland, South Maitland and Little Maitland rivers). Overall, the Maitland River still appears to support the largest remaining population of Rainbow in Canada.

Moira River

Between 1938 and 1964 six historical sites were reported for Rainbow on the Moira and Skootamatta rivers. Surveys carried out by Environment Canada in 1996 revealed 32 live Rainbow at three of six sites in the Moira River. In 2014, DFO and the OMNRF jointly revisited both rivers with the purpose of expanding surveys into unsearched areas. Rainbow is highly widespread in the Moira River and is distributed throughout the stretch from Tweed to just upstream of the mouth of the river in the most developed areas in Belleville. However, it would be worth noting that there are large stretches of habitat that are likely unsuitable to Rainbow (deep and highly vegetated) including lakes. The one site surveyed on the Skootamatta River also contained Rainbow, but in lower numbers.

Niagara River

Three historical records exist for Rainbow within Canadian waters of the Niagara River. One for Niagara on the Lake in 1936, one just upstream of Niagara Falls in 1934 and one on the west side of Navy Island in 1983, which is the only record confirming a live specimen (Lower Great Lakes Unionid Database 2014). Ten years later, surveys for the New York Power Authority confirmed that no live Rainbow or shells were found in proximity to this area (Riveredge Associates 2005). Zanatta *et al.* (2015) also found no evidence of Rainbow in their recent surveys of the Niagara River.

Salmon River

Throughout 1998 – 2008 Fred Schueler reported sightings of Rainbow shells in the Lonsdale, Kingsford and Milltown areas of the Salmon River. In 2010 - 2011 the first live Rainbow was found incidentally by OMNRF staff while conducting unrelated work. They conducted timed search surveys in 2014 with DFO and discovered live Rainbow at five of seven sites (459 live in 22.5 PH, although this total may be an overestimate due to repeated sampling throughout the field season). It was not found at the two most upstream sites sampled (just south of Tamworth). Kingsford is farthest upstream that Rainbow were detected and their distribution continues downstream towards the most southern site searched between Lonsdale and Milltown.

Saugeen River

There are 18 sites where live Rainbow or shells have been reported in this watershed. This includes one historical record of a whole shell found within a pond in Southampton as well as one and five individuals at two sites along the main branch of the river in 1993 and 1998, respectively (Lower Great Lakes Unionid Database 2014). Since then, more widespread and thorough sampling of the river and tributaries has been undertaken across 15 new sites by DFO. An impressive 68% of Rainbow individuals found in the watershed reside in the Teeswater River, even though it possesses only one-third of the sites searched. Other tributaries with Rainbow include the North Saugeen, South Saugeen and Beatty Saugeen rivers. Morris *et al.* (2007) found that Rainbow is widely distributed, but the species is numerically rare (accounting for less than 5% of all mussels). Interestingly, at one of the sites it was the only species found.

Sydenham River

The Sydenham River is one of the most well studied systems in Canada in terms of unionid richness and abundance. This system has several historical records for Rainbow, although the species appears to be in low abundance with only one to two individuals at each site (10 live Rainbow across 12 sites; Lower Great Lakes Unionid Database 2014). Only a few specimens were found alive at two of twelve sites sampled quantitatively in 1999-2003 (Metcalfe-Smith *et al*. 2003) and four of 10 sites resampled in 2012-2013. Very few records (historical or current) account for more than one individual, the bulk of which occur in the east Sydenham River and in lower numbers, Bear Creek (north branch). It appears that Rainbow has never occurred in significant numbers within this river.

Thames River

Rainbow is currently restricted to the North Thames River and several of its tributaries, a small reach of the Middle Thames River and two sites on the South Thames River near Innerkip and Dorchester (Mackie 2011b; Lower Great Lakes Unionid Database 2014). Most historical records are from the Middle Thames River including the first live collection, when 108 individuals were found near Thamesford in 1977 (Salmon and Green 1983). Morris and Edwards (2007) report that the lower reaches of the Thames River support the most diverse and abundant mussel communities; however, the upper river and its tributaries (above London) are where Rainbow populations can be found. The headwaters have some of the highest Rainbow abundances of the watershed, most notably Fish and Otter creeks, followed by Medway and Stoney creeks, and to a lesser degree, Black, Dingman and Oxbow creeks where the species represents 0.6-25% of the mussel community (Morris and Edwards 2007). It appears that Rainbow occurs infrequently in the Thames River, but is occasionally abundant in the most upstream reaches.

Trent River

Recent and wide-spanning surveys (semi-quantitative and quantitative) have been executed throughout eastern Ontario, most notably in the Trent River watershed (53 sites and 109 live Rainbow, with repeated sampling) within the past year (2013). This was instigated by the discovery of live and weathered shells of Rainbow in Mill Creek by F. Schueler in 1996 and several other Species at Risk in the vicinity. Live Rainbow are primarily found in the tributaries of the Trent River watershed; the most productive water bodies being Rawdon Creek, with findings also occurring in Burnley, Cold and Percy creeks. Although no live Rainbow were found in Salt Creek, this tributary evidently had previous populations as indicated by three weathered shells. In general, very few unionids were found along the river proper, with only four live Rainbow in total across nine sites. However, many Rainbow shells were found, with up to 40 weathered shells per site.

Extent of Occurrence and Area of Occupancy

Extent of occurrence (EOO) was calculated using the minimum convex polygon technique. The historical or maximal extent (1890 – 2014) of Rainbow's distribution was estimated as 77,182 km^2 (compared to the current (1997-2014) EOO of 48,051 km^2, enumerating to a 38% reduction. The index of area of occupancy (IAO) was calculated using a continuous 2 km x 2 km grid overlay technique, in keeping with the critical habitat maps in the Rainbow recovery strategy (DFO 2014). Maximal IAO was estimated as 3,604 km^2 compared to the current IAO of 2,532 km^2, representing a loss of 30% since 1997. It should be noted that the bulk of the decline in both EOO and IAO can be attributed to retractions from areas (Detroit River, Lake Erie, Lower Grand River, and Niagara River) where there is little evidence that Rainbow was ever widespread or abundant. When compared to the 2006 status report, it would appear that the IAO has increased substantially from 10.87 km^2 to 2,532 km^2. This is due to surveys in new areas since 2006 and a change in calculation methods (a 2 x 2 km continuous grid compared to a biological AO in 2006). The current EOO (48,051 km^2 versus 53,700 km^2 in 2006) and maximal EOO estimates (77,182 km^2 versus 76,500 km^2 in 2006) have changed very little and therefore the increase in IAO is not representative of a range expansion but of new detection.

Search Effort

There are 94 historical records (1890-1996) of Rainbow in the Lower Great Lakes Unionid Database showing distributions in the Ausable, Bayfield, Detroit, Grand, Maitland, Moira, Niagara, Salmon, Saugeen, Sydenham, Thames and Trent rivers, as well as lakes Ontario, Erie and St. Clair. The majority of collections are assumed to be made by shoreline searches and the accompanying documentation is often composed of very little information. Only 7% of records are complete, while most are missing vital data including specimen condition (dead or live), site coordinates, search effort or methods. A mere 27% percent of historical Rainbow records are based on known live animals. Records based only on shell collections should be interpreted with caution particularly where known populations exist upstream.

Three hundred and forty-one current records (1997-2014) indicate that Rainbow still resides throughout the majority of the 14 historical waterbodies as well as the Bayfield River. The year 1997 marks the beginning of the current timespan as this is when systematic, targeted and (semi)quantitative sampling of watersheds began for mussel fauna. Table 1 provides a summary of the sampling methods/effort and current distribution of Rainbow.

Table 1. Summary of current (1997-2014) mussel sampling effort within the range of Rainbow. PH refers to the number of person-hours searched and numbers in superscript signify the additional sites where only shells were found.

Waterbody	# of sites where live individuals occurred/Total # of sites surveyed	Year	Effort (per site)	Notes	Source
Ausable River	1/4	1998	2.5 – 4.5 PH timed search		Fisheries and Oceans Canada
	2/5	2002	4.5 PH timed search		Fisheries and Oceans Canada
	0/1	2004	4.5 PH timed search		Fisheries and Oceans Canada
	3/7	2006	69 - 75 x 1 m^2 quadrats		Upsdell *et al.* 2012
	0/1	2009	75 x 1 m^2 quadrats		Ausable Bayfield Conservation Authority unpubl. data
	1/1	2010	15 PH timed search and excavation	Found while searching for other species. Resurvey of 2002 site.	J. Vanden Byllaardt unpubl. data
	2/7	2011	74 – 80 x 1 m^2 quadrats	Sites previously surveyed in 2006	Upsdell *et al.* 2012

Waterbody	# of sites where live individuals occurred/Total # of sites surveyed	Year	Effort (per site)	Notes	Source
	1/1	2012	1 x 300 m^2 visual search	Found in plot during behavioural study, Resurvey of 2006 site.	T.J. Morris unpubl. data
Bayfield River	0/1	2005	Unknown	Found shell incidentally	ABCA unpubl. data
	5/18	2007	4.5 PH timed search	Targeted surveys for SAR	Morris *et al.* 2012
	1/1	2011	75 x 1 m^2 quadrats	Targeted survey for Rainbow	T.J. Morris unpubl. data
Detroit River	0/1	1997	4 x 120 m^2 line transects	Sites where live unionids were observed in 1990	Schloesser *et al.* 2006 and unpubl. data
	0/4	1998	Two 500 m^2 plots searched for 25 and 60 minutes using SCUBA	Sites where live unionids were observed in 1992 and 1994	Schloesser *et al.* 2006
	0/1	1998	10 random quadrats within a 10 m^2 grid excavated to 30 cm depth	Sites where live unionids were observed in 1987	Schloesser *et al.* 2006 and unpubl. data
Grand River	0/15[3]	1997	Visual and shoreline searches	Targeted surveys for Rainbow	A. Timmerman unpubl. data
	0/7	1997	Unknown		M.J. Oldham unpubl. data
	0/17[4]	1997	4.5 PH (wading)	Upper and Lower Grand	Metcalfe-Smith *et al*. 1998
	0/1	1998	Visual and shoreline searches	Targeted surveys for Rainbow	A. Timmerman unpubl. data
	1/7[2]	1998	4.5 PH (wading)	Upper and Lower Grand	Metcalfe-Smith *et al*. 1999
	0/2	2001	Unknown		J.L. Metcalfe-Smith unpubl. data
	0/4	2004	Visual and shoreline searches	Targeted surveys for Rainbow	A. Timmerman unpubl. data
	2/6[1]	2004	4.5 PH timed search		J.L. Metcalfe-Smith unpubl. data
	0/1	2005	2.5-3 PH timed search		J.L. Metcalfe-Smith unpubl. data

Waterbody	# of sites where live individuals occurred/Total # of sites surveyed	Year	Effort (per site)	Notes	Source
	0/2	2005	Visual search (viewing boxes)	Searching for gravid SAR females. Resurveyed in 2007, 2008, 2009, 2010, 2011	K.A. McNichols-O'Rourke pers. comm. 2014
	1/7[4]	2006	Visual and shoreline searches	Targeted surveys for Rainbow	A. Timmerman unpubl. data
	0/2	2006	330 x 1 m^2 quadrats	Grand River relocation project (Thurber Engineering)	Mackie 2006
	2/11[4]	2007	Visual and shoreline searches	Targeted surveys for Rainbow	A. Timmerman unpubl. data
	0/2	2007	Hand searching and excavation		G.L. Mackie unpubl. data
	0/4	2007	48-65 x 1 m^2 quadrats	All sites included in Metcalfe-Smith *et al.* 2000	T.J. Morris unpubl. data
	3/11[5]	2008	Visual and shoreline searches	Targeted surveys for Rainbow	A. Timmerman unpubl. data
	0/7	2008	Unknown		D. Zanatta unpubl. data
	1/1	2008	825 x 1 m^2 quadrats	Grand River relocation project (Region of Waterloo and Stantec)	Mackie 2008a
	1/5[4]	2009	Visual and shoreline searches	Targeted surveys for Rainbow	A. Timmerman unpubl. data
	0/1	2009	1525 x 1 m^2 quadrats	Grand River relocation project (Bot Construction)	Mackie 2009
	1/4[3]	2010	Visual and shoreline searches	Targeted surveys for Rainbow	A. Timmerman unpubl. data
	0/1	2010	3.8 PH timed search		T.J. Morris unpubl. data
	0/2	2010	78 and 93 x 1 m^2 quadrats	Grand River relocation project (Region of Waterloo and Ecoplans)	Mackie 2010a
	0/4	2011	6 PH timed search		T.J. Morris unpubl. data
	0/6	2011	4.5-6 PH timed search	Resurveys	T.J. Morris unpubl. data

Waterbody	# of sites where live individuals occurred/Total # of sites surveyed	Year	Effort (per site)	Notes	Source
	0/1	2011	10 PH timed search		P.L. Gillis unpubl. data
	1/1	2011	435 x 1 m^2 quadrats	Grand River relocation project (Natural Resource Solutions)	Mackie 2011a
	8/15[7]	2012	Visual and shoreline searches	Targeted surveys for Rainbow	A. Timmerman unpubl. data
	0/1	2012	Timed search 3 PH	Resurvey	T.J. Morris unpubl. data
	2/2	2012	8 PH timed search		P.L. Gillis unpubl. data
	0/1	2012	3640 x 1 m^2 quadrats	Grand River relocation project (Natural Resource Solutions)	Mackie 2012a
	0/1	2012	289 x 1 m^2 quadrats	Grand River relocation project (Ontario Ministry of Transportation and Dufferin Construction)	Mackie *et al.* 2012
	0/1[1]	2013	Visual search	Informal survey before potential relocation project	G.L. Mackie pers. comm. 2014
	0/2	2013	2.25 and 8 PH timed search	Resurvey	J.D. Ackerman unpubl. data
	0/1	2013	5 PH timed search	Resurvey	T.J. Morris unpubl. data
	0/1	2014	30 PH timed search	Resurvey	T.J. Morris unpubl. data
Lake Erie	0/33[1]	1998	0.1 – 1.18 PH SCUBA	U.S. side	Ecological Specialists 1999
	0/6	2001	2 PH snorkelling	Rondeau Bay	D. Zanatta and D. Woolnough unpubl. data
	0/17	2005	1.5 PH snorkelling and beach search	Pelee Island, Point Pelee, Sunset and Lakewood beaches, Holiday Beach Conservation area and the Meadows	D. McGoldrick unpubl. data
	0/3	2007	Unknown	Longpoint	J. Gilbert unpubl. data
	0/1	2009	4.5 PH timed search	Targeted survey for Eastern Pondmussel	K.A. McNichols-O'Rourke pers. comm. 2014

Waterbody	# of sites where live individuals occurred/Total # of sites surveyed	Year	Effort (per site)	Notes	Source
	0/17	2007-2009	20-60 min timed search and visual search of four random 100 m^2 quadrats	U.S. side	Crail *et al.* 2011
	0/12[1]	2014	4.5 PH visual-tactile search with viewers	Includes historical sites of Rondeau Bay	S. Reid and T.J. Morris unpubl. data
Lake Ontario	0/1	1998	Unknown	Cootes Paradise	S. Staton unpubl. data
	0/1	2000	Visual search along shoreline	Sunfish Pond	P. Smith unpubl. data
	0/15	2005	0.5 - 2 hrs snorkelling, visual search or beach surveying	Bay of Quinte, Consecon, East and West lakes	McGoldrick 2005
	0/9	2011	10 PH visual tactile and 10 PH scoops	Wetlands/marshes	S. Reid and T.J. Morris unpubl. data
	0/3	2011	3.5 PH scoops	Sunfish Pond, Grindstone Creek, Carrolls Bay	T.J. Morris unpubl. data
	0/18	2012	10 PH visual tactile and 10 PH scoops	Wetlands/marshes	S. Reid and T.J. Morris unpubl. data
	0/13	2013	10 PH visual tactile and 10 PH scoops	Wetlands/marshes	S. Reid and T.J. Morris unpubl. data
	0/2	2014	1 and 10 PH timed search with scoops	Jordan Harbour	T.J. Morris unpubl. data
Lake St. Clair	0/30	1998	10 transects at 1, 2.5, and 4 m depths with 5 x 1 m^2 quadrats and 20 Ekman grabs in each transect		Zanatta *et al.* 2002
	11/77	1999	Sites < 2 m deep = 0.75 PH snorkelling and if mussels present an additional 0.75 PH; sites > 2 m deep = 0.5 PH SCUBA	Includes 10 sites surveyed in 1998	Zanatta *et al.* 2002
	2/10	2000	1.5 PH snorkelling, 10 x 1 m^2 quadrats	Includes 10 most abundant sites from 1999	Zanatta *et al.* 2002

Waterbody	# of sites where live individuals occurred/Total # of sites surveyed	Year	Effort (per site)	Notes	Source
	5/9	2001	5-21 x 65 m^2 circular plots snorkelling	Includes 4 previously sampled sites	Zanatta *et al.* 2002
	12/18[2]	2003	16 - 30 x 65 m^2 circular plots snorkelling	9 sites in Canadian waters of delta, 9 sites in U.S. waters, includes 9 previously sampled sites from 2001	Metcalfe-Smith *et al.* 2004
	3/10[3]	2003	0.5 - 3 PH snorkelling	2 sites in Canadian waters of delta, 8 sites in U.S. waters	Metcalfe-Smith *et al.* 2004
	1/2	2004	29 and 138 x 65 m^2 circular plots snorkelling	Resurveys of 2003 sites	Metcalfe-Smith *et al*. 2005a
	1/4[1]	2005	3-4 PH snorkelling	Chematogan, Horeshoe and St. Anne's bays and Johnson Channel.	Metcalfe-Smith *et al.* 2005b
	0/2	2006	~ 9 PH snorkelling	Targeted search for SAR, both previously searched (Metcalfe-Smith *et al.* 2005b)	K.A. McNichols-O'Rourke pers. comm. 2014
	1/9[1]	2011	5 – 101 x 65 m^2 circular plots snorkelling, or 2 - 3 PH timed search	Resurveys of Metcalfe-Smith *et al.* 2004 sites	T.J. Morris unpubl. data
Maitland River	1/1	1998	1.5 PH timed search		McGoldrick and Metcalfe-Smith 2004
	8/11[2]	2003	4.5 PH timed search	One was a resurvey of the 1998 site	McGoldrick and Metcalfe-Smith 2004
	9/10	2004	4.5 PH timed search		J.L. Metcalfe-Smith unpubl. data
	1/1	2008	Timed search		J.D. Ackerman unpubl. data
	6/6	2008	60 – 63 x 1 m^2 quadrats	Resurvey of 1998, 2003 and 2004 sites	T.J. Morris unpubl. data
	0/1	2009	6 - 8 PH timed search	Vertical migration study; resurvey of 2003 site	T.J. Morris unpubl. data
	1/1	2010	1.5 PH timed search	Resurvey of Ackerman 2009 site	J.D. Ackerman unpubl. data
	1/1	2012	17 PH timed search	Survey with Wingham Highschool; resurvey of 2003 site	T.J. Morris unpubl. data

Waterbody	# of sites where live individuals occurred/Total # of sites surveyed	Year	Effort (per site)	Notes	Source
	8/11	2012	6 – 8 PH timed search	One was a resurvey of a 2003 site	Epp *et al.* 2013
	0/1[1]	2012	Shoreline search		A. Timmerman unpubl. data
Moira River	2/2	2011	Incidental	Found incidentally during eel surveys	MNR unpubl. data
	15/18	2014	4.5 PH timed search, sites resurveyed twice during the season	One site on the Skootamatta River	S. Reid and T.J. Morris unpubl. data
Niagara River	0/22[3]	2001-2002	4.5 PH timed search	U.S. Waters	Riveredge Associates 2005
Salmon River	0/1[1]	1998	Shoreline search		F. and J. Schueler unpubl. data
	0/5[5]	2005-2007	1.75 PH shoreline search	Up to 116 Rainbow shells were found.	F. Schueler unpubl. data
	2/6[4]	2010-2011	Incidental	Found during eel sampling	S. Reid and T.J. Morris unpubl. data
	5/7	2014	4.5 PH timed search, sites resurveyed twice during the season		S. Reid and T.J. Morris unpubl. data
Saugeen River	1/1	1998	Shoreline search		F. Scheuler unpubl. data
	0/1	2005	Incidental	Conducting other studies	D. Halliwell, B. Upsdell, M. Benner and D. McGoldrick unpubl. data
	1/4[3]	2006-2007	Shoreline search	Includes South Saugeen River	A. Timmerman unpubl. data
	5/8	2006	4.5 PH timed search	Includes Beatty Saugeen River and North Saugeen River.	Morris *et al*. 2007
	0/1[1]	2010	Informal survey		E. Vokey unpubl. data
	6/11[2]	2011	0.5 - 4.5 PH timed search	Mostly Teeswater, North Saugeen and Beatty Saugeen rivers. Four sites previously searched in 1993-1994 and in 2006	T.J. Morris unpubl. data

Waterbody	# of sites where live individuals occurred/Total # of sites surveyed	Year	Effort (per site)	Notes	Source
	2/4	2011	75 – 78 x 1 m^2 quadrats	Includes Teeswater and Beatty Saugeen rivers. Resurveys of 1993-1994, 2006 and 2011 sites.	T.J. Morris unpubl. data
Sydenham River	3/17[8]	1997-1998	4.5 PH timed search		Metcalfe-Smith *et al.* 2003
	2/15	1999-2003	60 - 80 x 1 m^2 quadrats	Includes 12 sites surveyed in 1997 - 1998	Metcalfe-Smith *et al.* 2007
	0/15	2001	4.5 PH timed-search	Nine sites previously surveyed in 1997-1998	D. Woolnough 2002
	2/11	2002	> 110 PH timed search with excavation	Targeted surveys for SAR females, 10 sites previously surveyed in 1999 - 2003	K.A. McNichols-O'Rourke pers. comm. 2014
	0/7	2003	~ 212 PH timed search with excavation	Targeted surveys for SAR females, all sites previously surveyed in 1999 - 2003	K.A. McNichols-O'Rourke pers. comm. 2014
	0/7	2004	~ 176 PH timed search with excavation	Targeted surveys for SAR females, all sites previously surveyed in 1999 - 2003	K.A. McNichols-O'Rourke pers. comm. 2014
	0/6	2005	~ 120.5 PH timed search with excavation	Targeted surveys for SAR females, all sites previously surveyed in 1999 - 2003	K.A. McNichols-O'Rourke pers. comm. 2014
	0/2	2005	Excavation	Mussel identification course; all sites previously surveyed in 1999-2003	T.J. Morris unpubl. data
	0/4	2006	~ 47.5 PH timed search with excavation	Targeted surveys for SAR females, all sites previously surveyed in 1999 - 2003	K.A. McNichols-O'Rourke pers. comm. 2014
	0/2	2006	Excavation	Mussel identification course; all sites previously surveyed in 1999-2003	T.J. Morris unpubl. data

Waterbody	# of sites where live individuals occurred/Total # of sites surveyed	Year	Effort (per site)	Notes	Source
	0/4	2007	~ 20 PH timed search with excavation	Targeted surveys for SAR females, all sites previously surveyed in 1999 - 2003	K.A. McNichols-O'Rourke pers. comm. 2014
	0/2	2007	Excavation	Mussel identification course; all sites previously surveyed in 1999-2003	T.J. Morris unpubl. data
	0/4	2008	~ 41 PH timed search with excavation	Targeted surveys for SAR females, all sites previously surveyed in 1999 - 2003	K.A. McNichols-O'Rourke pers. comm. 2014
	0/2	2008	Excavation	Mussel identification course; all sites previously surveyed in 1999-2003	T.J. Morris unpubl. data
	0/1	2008	167 x 1 m^2 quadrats	Sydenham River relocation project (Wallaceburg Community Task Force and Chatham-Kent Economic Development Services)	Mackie 2008b
	0/at least 11	2008-2009	unknown		D. Zanatta unpubl. data
	0/3	2009	~ 35 PH timed search with excavation	Targeted surveys for SAR females, all sites previously surveyed in 1999 - 2003	K.A. McNichols-O'Rourke pers. comm. 2014
	0/2	2009	Excavation	Mussel identification course; all sites previously surveyed in 1999-2003	T.J. Morris unpubl. data
	0/3	2010	~ 39 PH timed search with excavation	Targeted surveys for SAR females, all sites previously surveyed in 1999 - 2003	McNichols-O'Rourke pers. comm. 2014
	0/2	2010	Excavation	Mussel identification course; all sites previously surveyed in 1999-2003	T.J. Morris unpubl. data

Waterbody	# of sites where live individuals occurred/Total # of sites surveyed	Year	Effort (per site)	Notes	Source
	0/2	2011	~ 61 PH timed search with excavation	Targeted surveys for SAR females, all sites previously surveyed in 1999 - 2003	K.A. McNichols-O'Rourke pers. comm. 2014
	0/2	2011	Excavation	Mussel identification course; all sites previously surveyed in 1999-2003	T.J. Morris unpubl. data
	0/1	2011	4.5 PH timed search		T.J. Morris unpubl. data
	4/10	2012 - 2013	23 – 25 x 1 m^2 quadrats	All sites previously surveyed in 1999 - 2003	T.J. Morris unpubl. data
	0/2	2013	60 and 60.5 PH timed search	Targeted surveys for SAR, all sites previously surveyed in 1999 - 2003	J.D. Ackerman unpubl. data
Thames River	2/16[6]	1997-1998	4.5 PH timed search		J.L. Metcalfe-Smith unpubl. data
	0/1	1998	Shoreline search		A. Timmerman unpubl. data
	3/at least 3	2003-2004	Timed search		Upper Thames River Conservation Authority unpubl. data
	7/37[3]	2004-2005	4.5 PH timed search	27 sites on upper Thames River, 10 sites on lower Thames River	Morris and Edwards 2007
	1/12	2004-2005	60 - 80 x 1 m^2 quadrats	Sites included in Morris and Edwards 2007	T.J. Morris unpubl. data
	0/2	2006	720 x 1 m^2 quadrats (360 at each site)	Medway Creek relocation project (Stantec)	G.L. Mackie pers. comm. 2013
	0/1	2006	~ 3 PH timed search	Targeted surveys for SAR females	K.A. McNichols-O'Rourke pers. comm. 2014
	1/2	2007	729 x 1 m^2 quadrats (561 quadrats at one site and 168 quadrats at the other site)	Medway Creek relocation project (Stantec)	Mackie 2007
	0/1	2008	1 x 444 m^2 quadrats	Plot sampled 14 times between May and October	T.J. Morris unpubl. data TM-10 of Morris and Edwards (2007)

Waterbody	# of sites where live individuals occurred/Total # of sites surveyed	Year	Effort (per site)	Notes	Source
	0/17[1]	2008	4 – 4.5 PH timed search	Targeted searches for Rayed Bean, one site previously searched in 1997-1998	D. Zanatta, D. Woolnough and T.J. Morris unpubl. data
	0/1[1]	2008	4.5 PH timed search (viewing boxes or raccooning)	Targeted surveys for SAR females, previously searched in Morris and Edwards 2007	K.A. McNichols-O'Rourke pers. comm. 2014
	0/1	2009	Visual search	Targeted surveys for SAR females, previously searched in Morris and Edwards 2007	K.A. McNichols-O'Rourke pers. comm. 2014
	1/6	2010	45 - 78 x 1 m^2 quadrats	Sites previously surveyed in Morris and Edwards 2007	T.J. Morris unpubl. data
	0/3	2010	1830 x 1 m^2 quadrats (630, 750 and 450 at each site)	Medway Creek relocation project (Stantec and City of London)	Mackie 2010b
	0/1	2010	1 PH timed search	Targeted surveys for SAR females	K.A. McNichols-O'Rourke pers. comm. 2014
	0/2	2010	4.5 PH timed search		T.J. Morris unpubl. data
	2/4	2011	32 PH timed search (viewing boxes or raccooning)	Targeted surveys for SAR females	K.A. McNichols-O'Rourke pers. comm. 2014
	1/1	2011	435 x 1 m^2 quadrats	Thames River relocation project (County of Middlesex)	Mackie 2011b
	0/5	2011	3 – 13 PH timed search		J.D. Ackerman unpubl. data
	0/1	2011	Timed search		Trout Unlimited unpubl. data
	1/1	2012	~ 300 m^2 (20 m long x 15 m wide) SCUBA	Stoney Creek emergency relocation (Delcan)	Mackie and Beneteau 2012
	1/1	2012	318 x 1 m^2 quadrats	Thames River relocation project (Ministry of Transportation Ontario and Delcan)	Mackie 2012b
	1/3	2013	1 - 33 PH timed search		J.D. Ackerman unpubl. data

Waterbody	# of sites where live individuals occurred/Total # of sites surveyed	Year	Effort (per site)	Notes	Source
Trent River	9/51[8]	2013	4.5 PH timed search, sites resurveyed twice during the season	41 sites on tributaries: Burnley Hoards, Cold, Percy, Rawdon, Salt and Trout creeks	S. Reid and T.J. Morris unpubl. data
	1/1	2013	75 x 1 m^2 quadrats	Rawdon Creek	S. Reid and T.J. Morris unpubl. data

HABITAT

Habitat Requirements

Rainbow is most abundant in headwater systems, including small to medium-sized rivers and creeks (van der Schalie 1938; Strayer 1983; Parmalee and Bogan 1998; Watters *et al*. 2009). It can also be found in inland lakes and once occurred throughout the shallow nearshore areas of the lower Great Lakes and connecting channels in firm sand or gravel substrates (Clarke 1981; Strayer and Jirka 1997; Zanatta *et al*. 2002). In rivers, Rainbow is usually found in or near riffles and along the edges of emergent vegetation in moderate to strong current. The species occupies substrate mixtures of cobble, gravel, sand and occasionally mud or boulder. Rainbow is most numerous in clean, well-oxygenated reaches at depths of less than 1 m (van der Shalie 1938; Gordon and Layzer 1989; Parmalee and Bogan 1998).

Habitat Trends

The invasion of the Great Lakes by dressenid mussels began in 1986 (Hebert *et al*. 1989) and resulted in the near extirpation of native mussels from lakes Erie, St. Clair and Ontario as well as the Detroit and Niagara rivers by the mid-1990s (Schloesser *et al*. 1998, 2006; Riveredge Associates 2005). Much of the historical habitat in the Great Lakes has been rendered uninhabitable as a result of this invasion. Only isolated communities with reduced species richness and low abundance still survive in several bays and marshes along the shore of Lake Erie and in the delta area of Lake St. Clair where dreissenid densities are low. This loss of nearshore lake habitat has a substantial effect on EOO and IAO calculations; however, it appears that Rainbow was never abundant in these habitats and the overall impact on the species' status is likely less than that observed for other species (e.g., Eastern Pondmussel, *Ligumia nasuta*), which prefer this type of habitat.

Mussel communities in the Grand River declined dramatically from a historical total of 32 species to only 17 by the early 1970s. Kidd (1973) attributed this decline to pollution, siltation and the presence of dams. He found few mussels living below dams or in reservoirs and noted that none of the dams had fishways. He also found that dissolved oxygen concentrations were low and turbidity was high in the lower reaches of the river, most likely due to agricultural runoff. Sewage pollution was probably the major cause of the decline of mussels in this river (Metcalfe-Smith *et al*. 2000). At the time of Kidd's surveys, only seven of the river's 22 sewage treatment plants (STPs) had had secondary treatment in place for the previous 10 years, seven others had upgraded from no treatment to secondary treatment during that time and eight were in the process of installing treatment facilities for the first time. Twenty-five years later, Metcalfe-Smith *et al*. (2000) found that the mussel communities of the river had rebounded – most likely in response to significant improvements in water quality and a corresponding increase in the number of warmwater fish species (including hosts Rock Bass (*Ambloplites rupestris*) and Yellow Perch (*Perca flavescens*)) from 16 to 26 species (Coleman 1991). McNichols *et al.* (2012) confirm that 26 mussel species can be found alive in the Grand River as of 2011, including four federally Endangered species. Five of the six species which appear to have been lost from the Grand River are also assessed as at-risk. The human population of the watershed almost tripled from 375,000 to 985,000 between 1971 and 2014 and is expected to grow to 1.53 million by 2051 (GRCA 1997, 2014). The percentage of the minimum daily flow consisting of treated effluent from STPs ranged from 1% to 22% in 1993 and the capacity of the river to receive additional wastewater at reasonable cost is in question. The proportion of the Grand River basin in agricultural use increased from 68% in 1976 to 75% by 2014 (GRCA 2014). Row crop farming has increased, and along with it the potential for greater soil erosion and runoff of pesticides and fertilizers. Livestock production has changed, becoming more concentrated and specialized, and focusing on pigs and sheep rather than cattle, which may result in less trampling. There has also been a change in manure handling from solid to liquid, and inadequate management of these liquid wastes has become a problem (e.g., runoff) in some areas (GRCA 1998).

Habitat trends for the Sydenham River watershed are summarized from Staton *et al*. (2003) and SCRCA (2013a,b, c). Prior to European settlement, the Sydenham River watershed was 70% forest and 30% swamp. By 1983, 81% of the land area was being used for intensive agricultural practices (mainly corn and soybean crops), with only 12% forest and <1% swamp remaining. Sixty percent of the watershed is tile drained. Total phosphorus (TP) levels have consistently exceeded the provincial water quality objective (PWQO) over the past 30 years, but remain steady or slightly improved in the lower and middle East branch. Most of the phosphorus is associated with particulate material that probably originates from agricultural runoff. Chloride levels have been relatively low but are slowly increasing – a widespread pattern that has been attributed to the increased use of road salt. Sediment loadings from overland runoff and tile drains are high, but the surface water quality of the north branch has been steady. Wooded riparian zones, which are important for bank stabilization and interception of nutrients and sediments from overland runoff, are very limited. The human population of the Sydenham River watershed is small (74,000), with 50% rural and 50% living in towns and villages. Despite a modest rate of population growth, all municipalities have upgraded their sewage treatment facilities over the past 30 years. However, leakage of nutrients and contaminants from rural septic systems is a significant and ongoing problem, especially in the north branch.

Habitat trends for the Thames River watershed are summarized from Taylor *et al*. (2004), UTRCA (2012) and LTVCA (2012). Agriculture is the dominant form of land use in the Thames River watershed, with 75% of the land area in the upper Thames and 88% in the lower Thames in agricultural use. Forested areas have been reduced to 11.3% of the land area in the upper Thames and 10% in the lower Thames. Ten percent of the watershed is classified as urban, with an overall population of 515,640 concentrated in the cities of London, Stratford and Woodstock in the upper watershed and Chatham in the lower watershed. As the land was cleared, flooding became a serious problem. Three large dams and reservoirs were constructed in the upper watershed between 1952 and 1965. Numerous private dams and weirs have been installed since the 1980s and there are now 177 structures in the upper watershed and 65 in the lower watershed. Dreissenids were discovered in Fanshawe and Springbank reservoirs in 2003 and have since spread downstream where they were found attached to native mussels in 2004 (Morris unpubl. data). Fortunately, these two reservoirs are located downstream of the existing populations of Rainbow. Tile drainage dominates 56% of the land in the watershed. Water quality data collected since the 1960s show that concentrations of phosphorus and heavy metals are declining while nitrate and chloride levels are on the rise. The number of pollution spills has risen to 670 reported incidents in 2006 - 2010 from 380 incidents in a timespan five years earlier. The upper Thames River hosts 22 wastewater treatment facilities. The upper Thames River where Rainbow mainly occurs is moderately turbid, while the lower Thames is highly turbid. Soil conservation remains a serious issue in the watershed.

Habitat trends for the Ausable River watershed are summarized from Nelson *et al*. 2003 and ABCA (2013a,b). Mussel habitat in the Ausable River has been dramatically altered over time. Prior to European settlement, 80% of the basin was covered in forest, 19% was in lowland vegetation and 1% was marsh. By 1983, 85% of the land area was in agriculture (70% in row crops), and only 13% remained in small unconnected woodlots, similar to the current amount (14%). Over 70% of the basin is now in tile drainage and TP concentrations have increased in at least the upper Ausable River. The natural course of the lower portion of the river was destroyed in the late 1800s, when it was diverted in two places to alleviate flooding. The Ausable River has been described as “event responsive”, which means that there are large increases in flow during runoff events following storms. The nearby Sydenham, Thames and Maitland rivers are more stable in this regard (Richards 1990). There are 21 dams in the watershed that cause sediment retention upstream and scouring downstream. Nitrate levels currently exceed federal guidelines for the prevention of eutrophication and the protection of aquatic life and are slowly rising, similar to chloride levels (from road salt), which are approaching 250 mg/L (ABCA 2013b). Mean total suspended solid concentrations in the lower Ausable River exceed levels required for good fisheries. The Bayfield River falls into the care of the Ausable Bayfield Conservation Authority and fares worse than the Ausable River in some aspects of habitat trends. For example, the amount of forest cover is less, at 7.2% in 2012. In terms of pollution, total phosphorus levels are lower in the Bayfield River headwaters than the watershed average, similar to the amount of area occupied by agriculture (83%; ABCA 2013b).

There have been significant land use changes in the Maitland River watershed over the past 30 years (Malhiot pers. comm. 2004). Although there have been some minor impacts from urban and industrial expansion, these are greatly overshadowed by technological changes in the agricultural industry. Typical farming in the 1960s and 1970s focused on pasture and hay crops. Small grains were rotated through the grass fields and corn was cropped on the better lands. An extensive tile drainage system was installed during the 1970s. Better outlets were required to accommodate the improved drainage, which necessitated the installation or improvement of open drains, especially in wetlands. There was also a move towards larger farm implements in the 1970s and this required the expansion of field size through the clearing of fencelines/hedgerows and the straightening of field edges. It is now possible to grow corn and beans on lands that had only been suitable for grazing and hay in the past. The amount of row cropping greatly expanded through the 1980s as improved seed varieties were developed. The overall impact of these technological changes would have resulted in more nutrients, pesticides and sediment entering watercourses. As land prices increased due to improved crop values, there was also a move towards cattle feedlots. Factory farming for hogs expanded significantly in the 1990s. These two changes resulted in fewer livestock having access to watercourses (with presumably less trampling in the watercourse), but there were now new impacts in the form of liquid manure applications on tiled crop lands. Environmental programs introduced to keep pace with these changes have had some success through efforts in conservation tillage, watercourse rehabilitation (fencing livestock and reforestation) and most recently with nutrient management. A recent watershed report card indicates that the level of forest cover is greater than most other watersheds (up to 26.95%) within Rainbow distribution and the Lower Maitland River sub-basin received the best ratings for current phosphorus levels (MVCA 2013).

Land use changes have also occurred in the Saugeen River basin (Nichol pers. comm. 2005). Parts of the watershed (Huron-Kinloss, Kincardine) are undergoing a change to more intensive agricultural operations. More systematic tile drainage is being installed in the western portions of the basin and there is continuing development around existing urban areas and along the Lake Huron shoreline. Many landowners are implementing Best Management Practices that would improve water quality, but only when incentives are available. Nitrate/nitrite and TP levels have dropped on average in the past 10 years and are all below their safe water quality objective in the Lower Main Saugeen River watershed. In 2013, 19.5% was forested, which is a slight increase from 2006. There are currently 21 dams in the watershed, four sewage treatment facilities, and 76% of the land is used for agricultural purposes (SVCA 2013a). The Teeswater River watershed fares well, with only 66% of land dedicated to agriculture, 29% to forest and 0.6% to urban development (SVCA 2013b). It has no sewage treatment facilities and 14 dams. Total phosphorus has decreased in the past 10 years. Nitrite and nitrate levels have increased four times over a 10 year timespan, but still remain well below the drinking water standards. Chloride levels from input of road salt range from 1.0 -14 mg/L (SVCA 2013a,b).

Although two-thirds of the Moira River watershed (upper portion) is on the Canadian Shield where agricultural activity is limited (Sprague pers. comm. 1997), Rainbow is also found in the lower part of the watershed, which is subject to agricultural activity. Dreissenids are known to occur in Moira and Stoco lakes, just upstream of Rainbow populations in the Moira River. Contamination of the river with metals and arsenic due to a long history of mining and smelting activities is likely the main source of stress to the aquatic community (QC 2008). The Moira and Salmon rivers are situated in the Quinte Conservation jurisdiction, which has 59% forest coverage (QC 2013). Recent studies have revealed watershed trends of earlier wintertime snowmelt runoff events, greater flows in winter months and lower than average flows in the summer (QC 2013). These changes are congruent with the high water levels and flooding events of the Moira, Salmon and Trent rivers in spring 2014, a result of the large amount of snow received in the watershed. The Moira and Salmon rivers generally have low levels of road salt input, with chloride levels averaging 7 and 9 mg/L, respectively, although concentrations can reach as high as 138 mg/L locally (QC 2008).

The Trent River watershed covers 2,121 km^2 and encompasses 12 subwatersheds and eight main tributaries (LTCA 2013). Overall, 35% of the watershed is forested. The best conditions are in Rawdon Creek with approximately 50% of forest cover, in contrast to Trout Creek which has the least amount of forest cover, and is primarily agricultural. The watershed contains a portion of the Oak Ridges Moraine, a glacial landform consisting of sand and gravel layers, which retains water and feeds 65 rivers and streams. There is a lack of long-term, consistent data to evaluate the total phosphorus, nitrate and nitrate trends; however, two monitoring wells indicate elevated levels of chloride, nitrates and nitrites, exceeding the drinking water standards. The Trent River is occasionally subject to flooding and high water levels, particularly in spring 2014. The Trent-Severn waterway contains a series of 45 locks, several of which are in the vicinity of where Rainbow reside, including Percy Reach, Glen Ross and Frankford. Not surprisingly, dreissenids can be found throughout the system, including at Rainbow occupied sites, due to the large amount of boat traffic in this area.

BIOLOGY

Rainbow, like all freshwater mussels, is a sedentary animal that buries itself partially or completely in the substrates of rivers, streams or lakes. Adult freshwater mussels are filter-feeders that obtain nourishment by siphoning particles of organic detritus, algae (of select sizes, see Beck and Neves 2003) and bacteria from the water column and, as recently shown, from the sediment (Nichols *et al*. 2005). Juvenile Rainbow live completely buried in the substrate, where they feed on similar food items obtained directly from the substrate or from interstitial water (Yeager *et al*. 1994; Gatenby *et al*. 1997). Aspects of the life history of the species, summarized in the following sections, were derived from a review of the available literature as well as the report writers' knowledge of the species.

Life Cycle and Reproduction

The life cycle of Rainbow is similar to that of all freshwater mussels and is described as follows (adapted from Strayer 2008; Watters *et al.* 2009; Haag 2012): during spawning, males release sperm into the water and females living downstream filter the sperm out of the water with their gills. Ova are fertilized in specialized regions of the female gills, called marsupia, where they are held until they reach a larval stage called the glochidium (plural = glochidia). Recent evidence suggests that the female broods can be fertilized by numerous males (Christian *et al.* 2007). The female mussel then releases the glochidia, which must attach to an appropriate host – usually a fish. The glochidia become encysted on the host and are nourished by its body fluids until they metamorphose into juveniles. The juveniles then release themselves from the host and fall to the substrate to begin life as free-living mussels. The proportion of freshwater mussel glochidia that survive to the juvenile stage is estimated to be as low as 0.000001% (Bauer and Wächtler 2001). Mussels overcome the extremely high mortality associated with this life cycle by producing large numbers of glochidia – often more than a million. Juvenile mussels are difficult to find because of their small size and because they quickly burrow into the sediment upon release. They remain buried until they are nearly sexually mature, at which time they move to the surface for the dispersal/intake of gametes (Watters *et al*. 2001).

Rainbow is believed to be dioecious, but is occasionally reported as being hermaphroditic. There are subtle differences in the external shell features of males and females (see **Morphological description**). The species is bradytictic (long-term brooders); that is, they spawn in late summer, brood their glochidia over the winter, and release them in the early spring (Watters *et al.* 2009). Glochidia are semi-elliptical, large, with a short hinge line and measure approximately 230 µm in length and 290 µm in height (Clarke 1981; Watters *et al.* 2009). Based on studies conducted in the United States, Rainbow parasitizes an array of widely distributed fishes including the Striped Shiner (*Luxilus chrysocephalus*), Streamline Chub (*Erimystax dissimilis*), Smallmouth Bass (*Micropterus dolomieu*), Largemouth Bass (*Micropterus salmoides*), Green Sunfish (*Lepomis cyanellus*), Bluebreast Darter (*Etheostoma camurum*), Greenside Darter (*Etheostoma blennioides*), Rainbow Darter (*Etheostoma caeruleum*) and Yellow Perch (*Perca flavescens*) (Watters and O'Dee 1997; O'Connell and Neves 1999). All species except for the Streamline Chub and Bluebreast Darter occur in Ontario throughout the range of Rainbow and therefore have the potential to serve as glochidial hosts in Canadian waters. Specific hosts for Canadian populations have been verified as Mottled Sculpin (*Cottus bairdii*), Largemouth Bass, Yellow Perch and Rock Bass (*Ambloplites rupestris*; Woolnough *et al.* 2007; McNichols *et al*. 2008). Female Rainbow have modified mantle flaps that mimic a crawling crayfish in both shape and movement (Figure 5). When the glochidia are ready for release, the female mussel displays a crayfish-like "lure" in order to attract the host fish. This display is accompanied by a rocking behaviour of the mussel and fluttering of long tentacle-like extensions (Haag 2012). The glochidia are discharged when the fish approaches close enough to touch the lure.

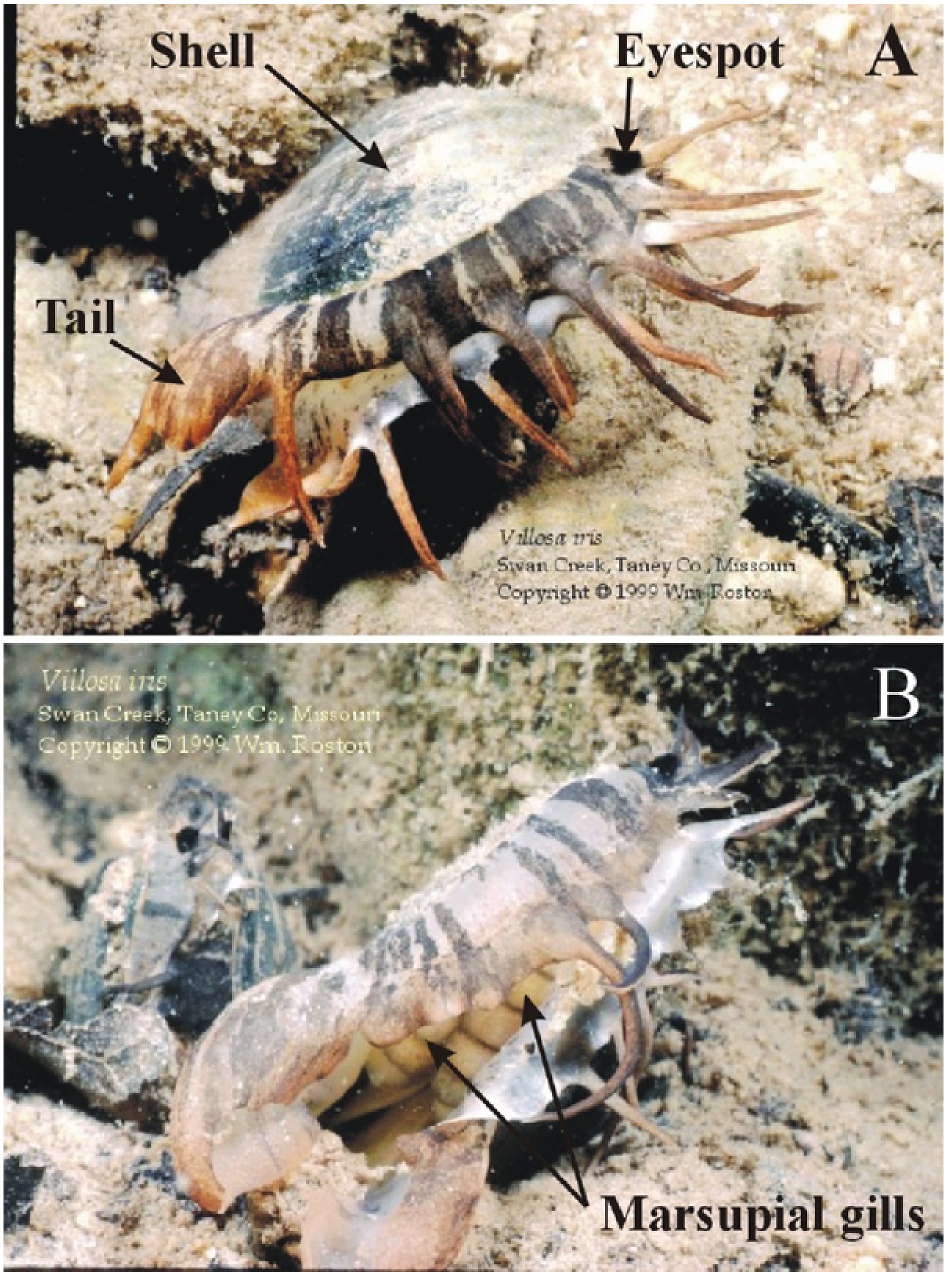

Figure 5. Photographs of a gravid female Rainbow: (A) displaying a crayfish-like lure to attract its host and (B) showing the marsupial gills filled with glochidia. Reproduced with permission from M.C. Barnhart, Southwest Missouri State University, Springfield, MO.

Using the average age of adult Rainbow from examination of internal growth rings, Vanden Byllaardt *et al.* (2013) estimate a generation time of 15 years. Their data indicate that sexual maturity can occur as early as five to nine years of age, based on the measured age at which females begin to differentiate morphologically from males, and reveal life spans of up to 43 years for this species. This is in contrast to data collected from counterparts in Ohio, where specimens over 15 years are reportedly rare and sexual maturity appears to occur as early as three years of age (Watters *et al.* 2009).

Physiology and Adaptability

Freshwater mussels are sensitive indicators of environmental conditions in rivers and lakes because many species require optimal water and habitat quality for survival (see **THREATS AND LIMITING FACTORS**). Generally, Rainbow is most commonly found in small creeks with sandy cobble and fast flowing water (Watters *et al.* 2009). The species is particularly sensitive to low oxygen conditions, especially at high temperatures (24.5º C; Chen *et al.* 2001). At 16.5º C individuals can respire normally and regulate their oxygen consumption better than at 24.5º C. (Chen *et al*. 2001). Other specific environmental requirements (e.g., water velocity, pH, etc.) of Rainbow are unknown.

Freshwater mussels are particularly sensitive to environmental perturbations because of their complicated life cycle. They are threatened not only by disturbances that impact them directly, but also by those that affect their host fish populations. Recent successes in the captive-rearing of several species of freshwater mussels have been reported (e.g., Hanlon and Neves 2000). The release of artificially reared juvenile Rainbow, and several other species, has taken place on a trial basis in the United States with some indications of success (Barnhart 2004; Fraley 2014), but the long-term outcome of such releases is still being evaluated.

Dispersal and Migration

Freshwater mussels are basically sedentary as adults, with movement limited to a few metres of the lake or river bottom (Strayer 2008). If local habitat becomes unsuitable (e.g., a drop in water level), some species are capable of moving up to several metres a day (Strayer 2008). The only time that significant dispersal can take place is during the parasitic phase. Infected host fishes can transport larval unionids hundreds of metres or kilometres into new habitats and replenish depleted populations with new individuals. Although the absolute distance travelled by known Rainbow host fishes is small (67 m on average; Schwalb *et al.* 2011), the widespread distribution of Rainbow and prevalence of its hosts indicate that Rainbow is not likely experiencing dispersal limitations. Dispersal is particularly important for genetic exchange between populations (Nedeau *et al*. 2000).

Interspecific Interactions

The larvae of Rainbow are obligate parasites on fish. Specific host fishes for Canadian populations of this species have been identified as Mottled Sculpin, Largemouth Bass, Yellow Perch and Rock Bass, as well as Striped Shiner, Smallmouth Bass, Green Sunfish, Greenside Darter, Rainbow Darter, as confirmed through American studies (**see Lifecycle and Reproduction**).

Predation can have significant effects on freshwater mussels. Important predators include Muskrat (*Ondatra zibethicus*) and Raccoon (*Procyon lotor*) (Fuller 1974; Tyrrell and Hornbach 1998). Invasive Round Goby (*Neogobius melanostomus*) may also prey upon juvenile mussels as well as cause disruption to the fish host populations.

POPULATION SIZES AND TRENDS

Sampling Effort and Methods

Historical surveys

Approximately 70% of the historical records for Rainbow in Canada are based on either museum specimens or occurrence data. For most of these records, there is little if any information on sampling method, search effort, numbers of sites visited where the species did *not* occur, or even whether the animals were dead or alive when collected. Abundance data from this period are extremely limited. Estimates of relative abundance (Catch-Per-Unit-Effort or CPUE) are available from timed-search surveys of several sites on the Sydenham River in the 1960s (Stein pers. comm. 1996) and 1991 (Clarke 1992). These sites were revisited in 1997-98 (Metcalfe-Smith *et al.* 1998, 1999) and the results can be compared. Kidd (1973) surveyed 68 sites on the Grand River in 1970-72 and 14 of these sites were re-surveyed 25 years later using a similar sampling protocol (Metcalfe-Smith *et al.* 2000). One site on the Thames River was sampled quantitatively in 1977 and a nearby site was sampled using the same method in 2004. In the past three years, focus has been given to resampling historical sites (1960s) in lakes Ontario (Hillier and Bay of Quinte) and Erie (Rondeau Bay), in addition to new neighbouring sites using timed search methods. Few eastern Ontario historical sites exist, with the exception of the Moira River (sites founded in 1938-1964). Although quantitative comparisons cannot be made, resampling efforts of this watershed demonstrate that Rainbow subpopulations are persisting.

Recent surveys

Surveys conducted between 1997 and 2014 within the range of Rainbow in Ontario have been either semi-quantitative (timed-searches, stake and rope and visual-tactile) or quantitative (quadrat surveys). The same sampling methods were used throughout and are described below.

Semi-quantitative surveys

Timed search:

In rivers, surveys were conducted using an intensive timed-search technique for detecting rare species of mussels. The technique is described in detail in Metcalfe-Smith *et al.* (2000). Briefly, the riverbed is visually searched by a team of three or more persons using waders, polarized sunglasses, and underwater viewers for a total of 4.5 person-hours (PH) of sampling effort. Where visibility is poor, searching is done by feel. The length of reach searched varies depending on river width, but is generally 100 to 300 m. Live mussels are held in the water in mesh diver's bags until the end of the search period when they are identified to species, counted, measured (shell length), sexed (if sexually dimorphic) and returned to the riverbed. Over the past 10 years, such surveys have been conducted in the Ausable, Bayfield, Grand, Maitland, Moira, Salmon, Saugeen, Sydenham, Thames and Trent rivers as well as Lake Erie by several different researchers. Sampling efforts of other researchers vary between 1.0 and 15 PH per site.

In Lake St. Clair, timed searches at water depths greater than 2 m were conducted by two SCUBA divers for a total effort of 0.5 PH whereas searches at depths less than 2 m were conducted by three people using mask and snorkel for a total of 0.75 PH (Zanatta *et al.* 2002). At sites where live mussels were found (all were shallow), snorkel searches were extended to a total of 1.5 PH.

Stake and Rope:

Stake and rope surveys were also conducted in the delta area of Lake St. Clair. At each site, sampling was performed by several (usually three) 2-person teams, with each team consisting of a snorkeler and a helper to carry the gear and mussels. Each snorkeler swam until a mussel was seen, then placed a stake in the water and surveyed a 65-m^2 circular area around the mussel and collected any other live mussels found. Each team surveyed 10 such circle plots. All live mussels were identified, counted, measured, sexed and returned to the lake bottom. Methods are described in detail in Metcalfe-Smith *et al.* (2004). Such surveys were conducted in 2001, 2003, 2004 and 2011.

Visual-Tactile Searches:

Areas with particularly soft substrate and poor visibility, like the Lake Ontario coastal wetlands, were sampled via visual-tactile techniques. This involved a team of two to three people equipped with wetsuits and floatation devices. Prior to sampling, a grid would be placed on a site map and 10 random points would be chosen for sampling. At each point, a team member would position themselves on a floatation device and gently disturb the sediment with their feet and any mussels found would be brought to the surface to be identified, measured and sexed, if possible. At the same sampling points, mussels were collected by scooping sediment into a clam rake for one hour. This type of surveying has been conducted across Lake Ontario wetlands from 2011-2013 (Reid *et al.* 2014).

Quantitative surveys

Surveys in rivers employed a time- and labour-intensive quantitative sampling technique that would allow the generation of precise estimates of demographic variables such as density, size class frequencies and recruitment levels. The monitoring protocol was developed in consultation with Dr. David R. Smith, a biostatistician with the U.S. Geological Survey for assessing the impacts of development projects on federally endangered mussels in the United States. Dr. Smith and Dr. David L. Strayer, another American mussel expert, were commissioned by the Guidelines and Techniques Committee of the Freshwater Mollusk Conservation Society to prepare a guide to sampling freshwater mussel populations. This guide (Strayer and Smith 2003) includes a description of the protocol, which is summarized below.

Sampling employed a two- to six-person search team including a data recorder and required approximately 2-3 days of work per site. At each site, roughly 400 m^2 of the most productive portion of the reach (usually a riffle) was selected for sampling. Quantitative sampling was conducted using 1-m^2 quadrats and a systematic sampling design with three random starts. The area to be sampled was divided into blocks of equal size (5 m long × 3 m wide) and each block was further divided into 15 - 1-m^2 quadrats. The same three randomly chosen quadrats were sampled in each block; thus, 20% (75 m^2) of the 400 m^2 area was sampled at each site. Each quadrat was searched by two people until all live mussels had been recovered (~ 8 minutes). All embedded stones (except large boulders) were removed and the substrate was excavated to a depth of 10-15 cm in order to obtain juveniles. Young mussels are known to burrow deeply in the substrate for the first three years of life. All live mussels found in each quadrat were identified, counted, measured, sexed where possible and returned to the riverbed. Several habitat variables (e.g., depth, current velocity, substrate composition) were also measured. To date, quantitative surveys have been conducted at eight rivers: the Ausable, Bayfield, Grand, Maitland, Saugeen, Sydenham, Thames and Trent rivers. Due to the large amount of effort required to conduct quadrat surveys, typically only a few sites per river are surveyed with this technique. Some of the Grand River surveys were part of mussel relocations and were even more intensive (up to 3640 1-m^2 quadrats searched and teams of up to 15 people across a two week span).

Abundance

Quantitative estimates of abundance can be made for the Ausable, Bayfield, Grand, Maitland, Saugeen, Sydenham and Thames rivers, as well as Lake St. Clair. The Salmon and Moira rivers are believed to contain large amounts of Rainbow, but these systems have yet to be surveyed quantitatively (Rainbow comprises 11% and 12% of the mussel community, respectively) (Lower Great Lakes Unionid Database, 2015). Only one site on the Trent River has been examined quantitatively (Rawdon Creek) in which Rainbow are thought to be in high abundance. To extrapolate this density over the river length would not produce an accurate abundance estimate and therefore this river was not included (S. Reid pers. comm. 2015). Abundance was enumerated by measuring the distance between the most upstream and downstream sites and multiplying this by the average stream width and density of Rainbow. Connectivity of sites and tributaries was based on critical habitat, which has been recently delineated for the species (DFO 2014). Population estimates should only be used in a relative sense, as they are often based on limited quantitative sampling.

Extirpated Populations

Based on current information (Lower Great Lakes Unionid Database, 2015), Rainbow is no longer present in the Detroit River, Lake Erie, Niagara River or Lake Ontario.

High Abundance Populations (Maitland and Saugeen rivers)

The Maitland River watershed sustains the largest population and accounts for 78% of Rainbow in Canada, with abundance estimates totalling 4.2 million individuals (Table 2). This system has the highest known densities (2.4 individuals/m^2) throughout the second largest area of occupancy (5.69 km^2), with the most impressive catch being 115 individuals found in a 4.5 PH timed search (Lower Great Lakes Unionid Database 2014). In total, 898 live Rainbow have been discovered in this system, of which 440 have been found in the past 10 years, spanning all but the lowest reaches. They can be found throughout all branches of the river (Middle Maitland, South Maitland and Little Maitland rivers), where they also appear to be quite productive (density of up to 1.4 individuals/m^2 and catch per unit effort of 26 individuals/PH). The Saugeen and Trent river watersheds contain the second and third largest subpopulations with 700,000 and 330,000 estimated Rainbow, respectively. The species is found throughout the main channel, North and South Saugeen as well as the Teeswater River, occupying 2.2 km^2 of river reach in the watershed.

Table 2. Comparisons of recent population strength for Rainbow based on quantitative (quadrat) surveys.

Waterbody	Average Density (individuals/m^2 ± SE)	Area of Occupancy (km^2)	Estimated population size	Year(s) of surveys
Ausable River	0.07 ± 0.03	0.18	5,900-18,000	1998-2012
Bayfield River	0.28 ± na	0.26	74,000	2005-2011
Grand River	0.01 ± 0.01	3.89	4,700-45,000	1997-2013
Lake St. Clair	0.0002 ± na*	6.82	1,500	1999-2011
Maitland River	0.74 ± 0.40	5.69	2,000,000 - 6,500,000	2003-2012
Saugeen River	0.31 ± 0.08	2.28	520,000 – 880,000	1997-2011
Sydenham River	0.01 ± 0.00	1.24	17,000 – 18,000	1997-2013
Thames River	0.06 ± 0.02	1.27	48,000 – 94,000	1997-2013

na = not applicable
*Density estimate from stake and rope surveys

Moderate Abundance Populations (Bayfield and Thames rivers)

Moderate-sized populations can be found in the Bayfield and Thames rivers (Table 2). A small amount of area (0.26 km^2) is inhabited by Rainbow in the Bayfield and Bannockburn rivers, but at a high density (0.28 individuals/m^2), amounting to 74,000 estimated individuals and 2% of all mussels found. The vast majority of Rainbow in the Thames River watershed resides in the North Thames and Middle Thames rivers, as well as Fish, Otter and Medway creeks and overall totals 71,000 individuals. Several tributaries including Stoney, Oxbow, Black and Dingman creeks have not been assessed quantitatively and/or only have one site and are excluded. The South Thames River has sparse records of single live individuals intermingled with records of weathered shells.

Low Abundance Populations (Grand, Ausable and Sydenham rivers)

The smallest subpopulations belong to the Grand, Ausable and Sydenham rivers (Table 2). All Rainbow in the Grand River are contained within the upper Grand and its tributaries such as the Mallet and Conestogo rivers. Although the watershed has one of the largest areas of occupancy (3.89 km^2), the abundance enumerates to 25,000 individuals, due to the low density of the species (0.01 individuals/m^2). A short 0.18 km^2 stretch of the Ausable River contains Rainbow at a low density (0.07 individuals/m^2), resulting in the abundance estimate of 12,000 individuals (1% of all mussels found; Upsdell *et al.* 2012). A comparatively larger 1.24 km^2 continuous stretch of the East Sydenham River at a density of 0.01 individuals/m^2 calculates to an estimated population size of 17,000 individuals. The species is also present in Bear Creek, but is restricted to one site and is not included in the estimate.

Very Low Abundance Population (St. Clair delta)

The St. Clair delta contains the smallest remaining extant Rainbow subpopulation in Canada with an estimated size of only 1,500 individuals. Although this system has the largest Rainbow area of occupancy (6.82 km^2), it also has the lowest densities throughout Canada at 0.0002 individuals/m^2 (Table 2). Resurveys in 2011 confirmed that Rainbow still occupies the Squirrel Island area of the delta, but no longer inhabits Pocket and Horseshoe bay areas. Mitchell and Johnston bays contained Rainbow in 2001-2005 surveys, and it is believed that Rainbow still persist in these areas although formal surveys have not been undertaken since.

Fluctuations and Trends

Ausable River

Upsdell *et al*. (2012) implemented a five-year study examining the change in the Ausable River mussel community composition, beginning in 2006. They found that 71% of their monitoring stations (five of seven) had significant density declines for all mussel species present and 43% (3 of 7) had declines in species at risk (SAR). The stations were widespread and located throughout the upper, middle and lower Ausable River subcatchments. Rainbow was found at three of seven sites in 2006 and only two in 2011. Rainbow density declined at both sites, from 0.21 to 0.01 individuals/m^2 (Little Ausable River) and 0.12 to 0.03 individuals/m^2 (main channel), representing an 85% loss on average. They also report that while Rainbow juveniles (< 30 mm in length) were found in 2006, none were found in the 2011 surveys indicating recruitment may be declining (Figure 6). Likewise, one site on Nairn Creek in 2010 was resurveyed using a timed search technique and shows a decrease in catch per unit effort (CPUE) of 0.22 to 0.13 individuals/PH over the span of eight years (Vanden Byllaardt unpubl. data). It appears that Rainbow is not a large component of the Ausable River mussel community and may be declining throughout.

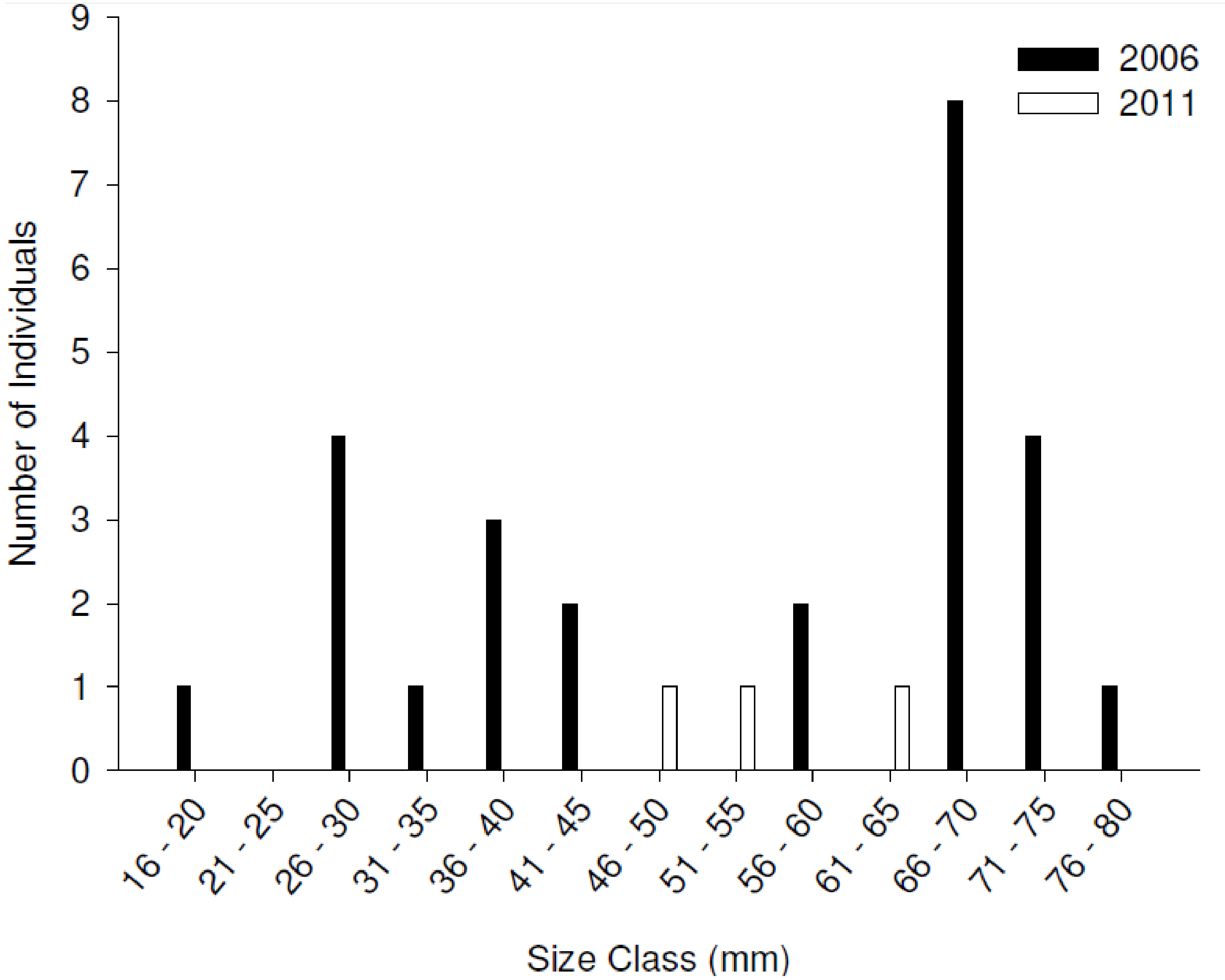

Figure 6. Size distribution of Rainbow across seven sites in the Ausable River in 2006 (*n* = 26) and 2011 (*n* = 3). Reproduced from Upsdell *et al*. (2012).

Bayfield River

All data concerning Rainbow in the Bayfield River has been obtained within the past seven years. There is no temporal information to analyze trends through time. One resampling event has occurred in 2011, but no direct comparison can be made because of a change in survey techniques. Morris *et al.* (2012) confirmed the presence of a Rainbow population undergoing active reproduction and recruitment as demonstrated by representation in a wide range of size classes (Figure 7).

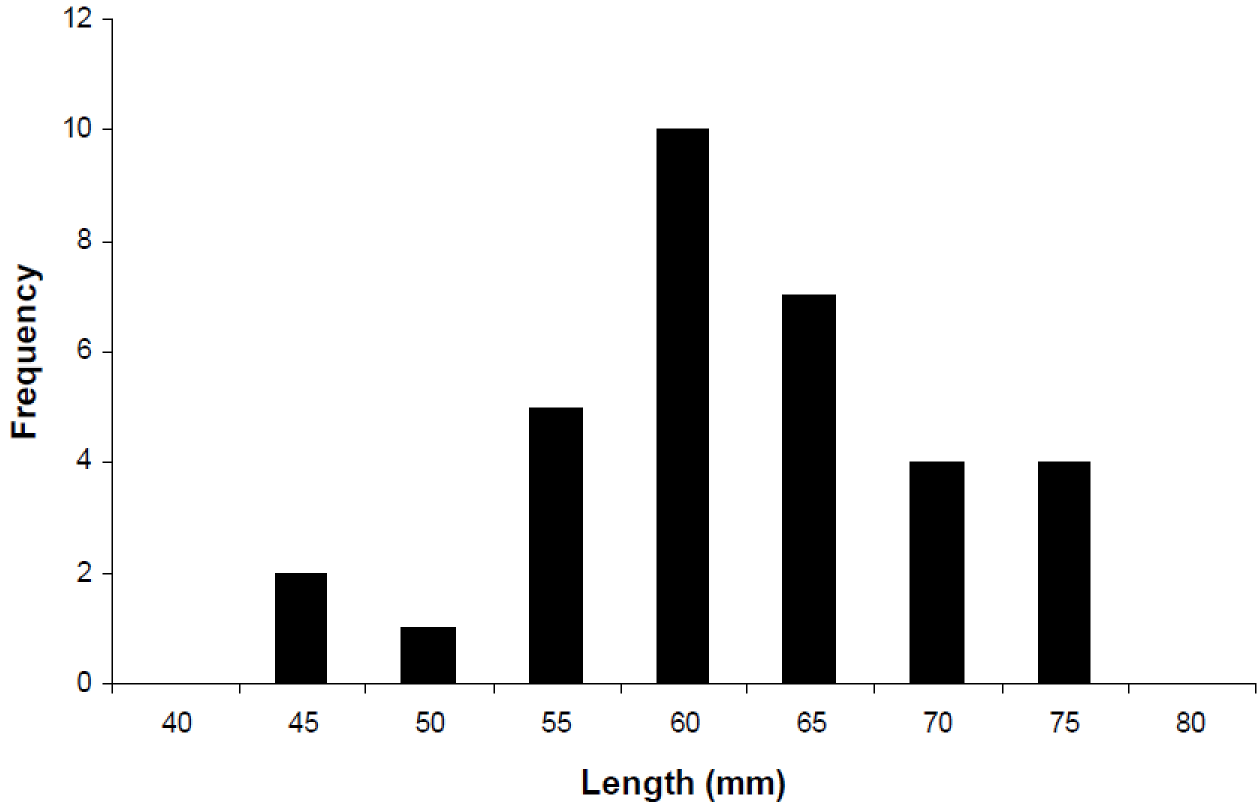

Figure 7. Size distribution of Rainbow in the Bayfield River in 2007 (n = 33). Reproduced from Morris *et al.* (2012).

Detroit River

The Detroit River unionid community has undergone drastic declines beginning in the 1990s and leading up to their current extirpation. Long-term studies by Schloesser *et al.* (2006) have documented this decline. In their earliest surveys (1987 and 1992), they found over 1000 live unionids across six sites, which reduced to five live individuals in 1997-1998 resurveys, even though they searched twice the amount of area. Although Rainbow was never a large component of the mussel community (2% in 1992), their numbers continually dropped from 14 live individuals in 1992 to two in 1994 and zero in 1998. The study concludes that total unionid densities are too low (<0.01 individuals/m^2) to support viable populations in the Detroit River. Zanatta *et al.* (2015) recently reconfirmed this as well.

Grand River

It was previously believed that Rainbow only occupied a fraction of its historical range in the Grand River system. This assertion was largely based on Kidd's (1973) survey of the Grand River in 1970-72 and the re-survey efforts of Metcalfe-Smith *et al.* (2000) 25 years later. During these surveys only four live Rainbow were detected across 94 sites (0.02 individuals/PH) between 1995 and 1997-98 versus 15 across 68 sites (0.05 individuals/PH) in Kidd's (1973) surveys, although the majority of Kidd's (1973) Rainbow were contained at one site. These sampling events gave much focus to the lower and mid-river regions along with their tributaries, but neglected to effectively survey the upper river and tributaries to their full extent. The lower and mid-portions of the river appear to have never supported large numbers of Rainbow, even historically. This is consistent with what is seen in other nearby and large river systems such as the Thames River.

In the last ten years, more focus has been given to exploring the unionid communities in the tributaries and upper portion of the Grand River with opportunistic sampling in the Conestogo and Mallet rivers, and their confluences with the upper Grand River. Since 2004, 35 live Rainbow have been recorded in these regions. Two recent timed searches in the upper Grand River in 2012 revealed an average CPUE of 0.31 individuals/PH, which is much higher than that of previous studies and other sites in this river (Lower Great Lakes Unionid Database 2014). In addition, seven mussel relocations have been deployed since 2006, two of which have detected Rainbow with an average density of 0.01 individuals/m^2 in the upper regions (Mackie 2008a, 2011a). The upper Grand River and tributaries appear to house the majority of Rainbow within this system and continued monitoring will be needed to accurately detect fluctuations in numbers.

Lakes Erie and Ontario

Rainbow has been essentially eliminated from Lake Erie and Lake Ontario, although these systems likely never supported large populations. Areas with historical records of Rainbow have been recently (2011-2014) and heavily resurveyed in both lakes, and have yielded no evidence of this species beyond a weathered valve in Rondeau Bay in 2014 (Ecological specialists 1999; McGoldrick 2005; Crail *et al.* 2011; Fisheries and Oceans Canada and Ontario Ministry of Natural Resources and Forestry unpubl. data).

Lake St. Clair

Eighteen sites were surveyed (stake and rope method) in the delta area of Lake St. Clair in 2003: nine sites in Canadian waters and nine in U.S. waters (Metcalfe-Smith *et al*. 2004). Rainbow was found at three of the nine sites in Canadian waters at an average density of 0.002 individuals/m^2. Since then, more search effort has been required to obtain specimens as densities appear to be declining, as evidenced in the Squirrel Island sites. Densities decreased by more than an order of magnitude over an eight year period and ranged from 0.003 individuals/m^2 in 2003 (five individuals in 1950 m^2) to 0.001 individuals/m^2 in 2004 (ten individuals in 8970 m^2) and 0.0002 individuals/m^2 in 2011 (one individual in 6565 m^2), representing a 12% loss per year. Rainbow never comprised a large component of the mussel community on the Canadian side (1%) and their numbers seem to be declining in number in addition to contracting to areas like Squirrel Island. Surveys in 2011 revealed that Rainbow are no longer found in the Pocket Bay area of the delta. Throughout all the surveys, it was noted that dreissenids continue to use unionids as substratum in these areas.

Maitland River

Since survey efforts in the Maitland River are relatively recent (significant effort beginning in 2003), not much can be said regarding trends and fluctuations of its Rainbow population. Four major sampling events have occurred on the Maitland: three using timed searches (2003, 2004, 2012) encompassing new sites and one resurveying event (2008) using quantitative methods (quadrats). Rainbow can be found consistently at 70% or more of sites for each sampling event and is the most abundant species present in this system (61% relative abundance), with densities ranging from 0.2 – 2.4 individuals/m^2. These densities are up to three orders of magnitude greater than that of any other waterbody, which makes the Maitland River home to the largest and most productive Rainbow subpopulation.

Moira River

Although records of Rainbow date back to 1938 in the Moira River, no information regarding effort was recorded making it difficult to make any comparison to current records. However, some of the sites resurveyed in 1996 and 2014 were at or in proximity of the earliest records (near to Stoco, Latta and Chisholm Mills) and demonstrate that the species is persisting well. In 1996, CPUE in Stoco and Chisholm Mills was 5.33 and 0.88 individuals/PH which increased to 7.70 and 1.78 individuals/PH in 2014 (averaged across three surveys/field season), respectively. Unlike the two former sites, Latta exhibited a decrease from 29.33 to 4.96 individuals/PH in the near 20 year timespan. The 8-11% increase in abundance per year at some sites and 5% decrease at others may reflect the slow migration of mussel beds rather than true losses and gains. The size distributions of males and females across the watershed demonstrate a healthy and productive Rainbow population exhibiting recruitment (Figure 8).

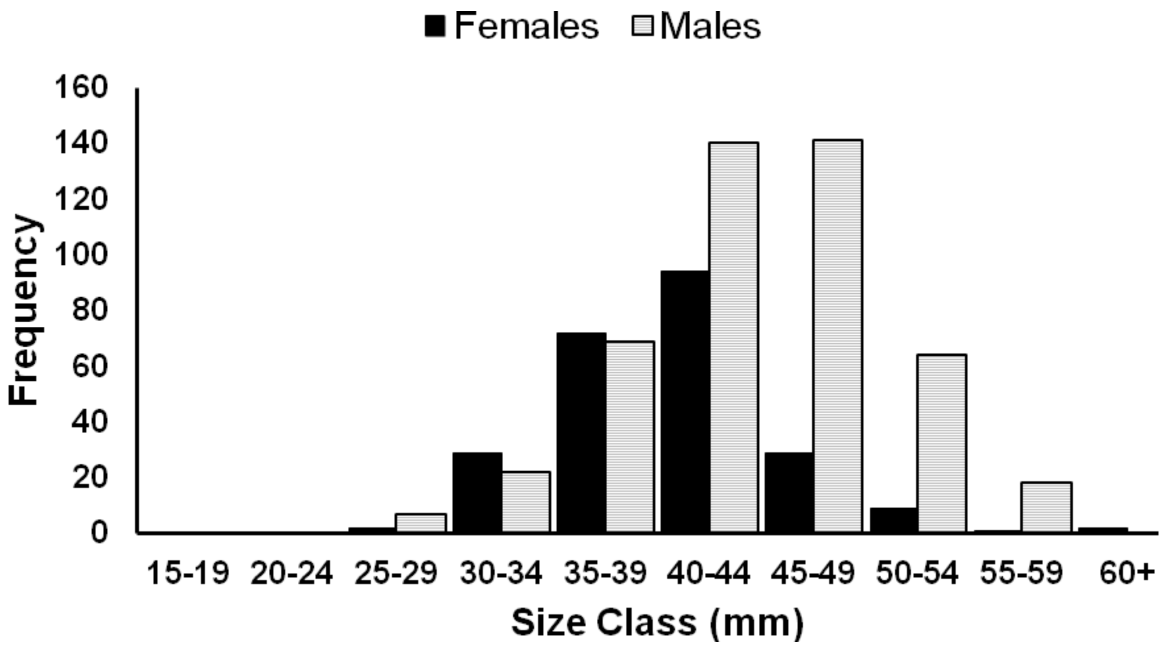

Figure 8. Size distribution of Rainbow collected from the Moira River watershed in 2014 (female *n* = 238, male *n* = 461). Fisheries and Oceans Canada and the Ministry of Natural Resources unpubl. data.

Niagara River

The only evidence of live Rainbow in the Canadian waters of the Niagara River comes from surveys undertaken in 1983. Ten years after, surveys for the New York Power Authority demonstrated that Rainbow and other unionids no longer exist in these areas (Riveredge Associates 2005). This elimination of native mussels seems to be the result of colonization by invasive dreissenids that are known to be situated within a kilometre of the former Canadian sites.

Salmon River

No quantitative or semi-quantitative data exist for the Salmon River prior to 2014. Length-frequency plots of males and females show a healthy size distribution and that the population is undergoing active recruitment (Figure 9).

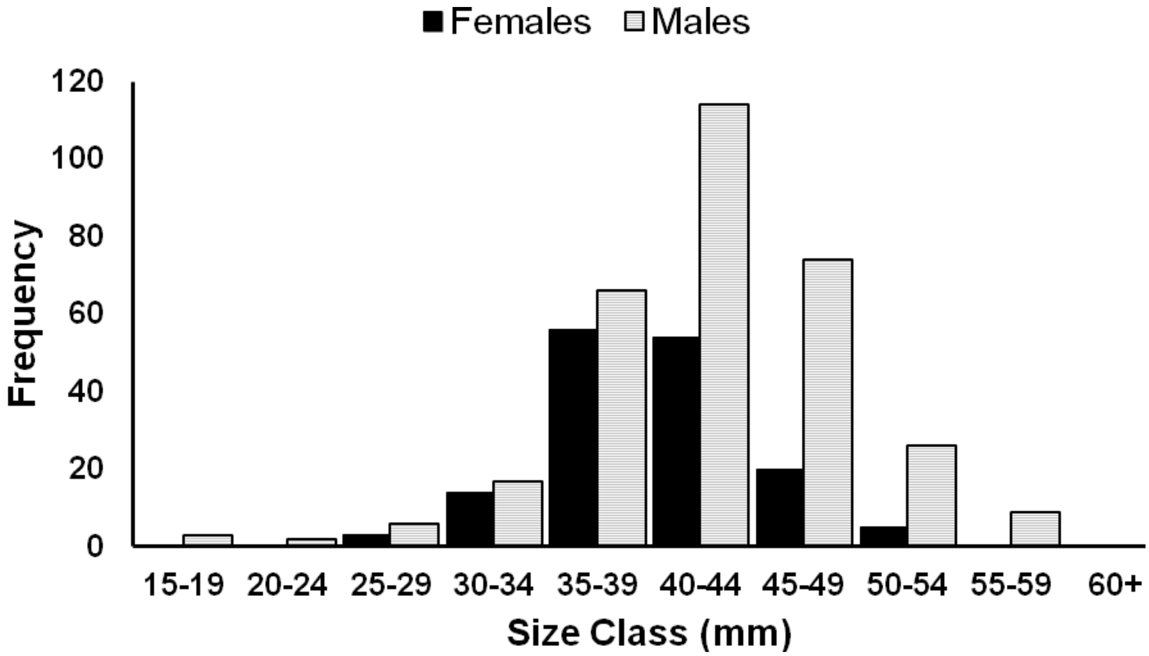

Figure 9. Size distribution of Rainbow collected from the Salmon River in 2014 (female *n* = 152, male *n* = 317). Fisheries and Oceans Canada and the Ministry of Natural Resources unpubl. data.

<u>Saugeen River</u>

Sampling efforts have been relatively recent in this watershed. Formal surveys undertaken by Morris *et al.* (2007) in 2006 revealed that 63% (five of eight) of sites searched contained live Rainbow, and this statistic has remained relatively constant during follow-up surveys in 2011 (present at six of 11 sites). Of the four sites that were resampled in 2011, one had a decrease in CPUE from 7.3 to 0.0 individuals/PH over a five year span (Teeswater River) and one increased from 1 to 6.6 individuals/PH over a twenty-one year span (North Saugeen River). Lack of information limits what more can be said about fluctuations, but it appears that Rainbow is widespread and recruiting in this watershed (Figure 10) and more abundant than in most other systems (densities up to 0.39 individuals/m^2).

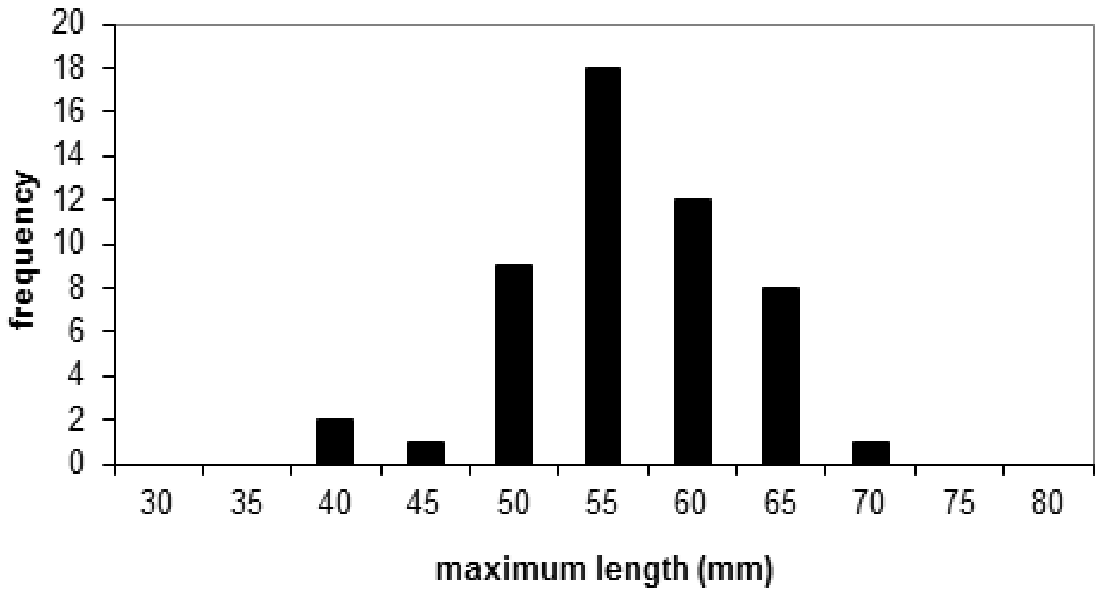

Figure 10. Size distribution of Rainbow collected in 2006 from the Saugeen River watershed. Reproduced from Morris *et al*. (2007).

Sydenham River

Previously, it was discovered that Rainbow has declined in the Sydenham River, especially the east branch where it was found at 33% of 15 sites surveyed between 1929 and 1991 vs. 17% of 12 sites surveyed in the same reaches in 1997-1999 (Metcalfe-Smith *et al*. 2003). Fifteen years later, resurveys revealed a 40% site occupancy (4 of 10 sites). This expansion was produced by the discovery of live Rainbow at two sites that were previously known to have fresh shells. The apparent range contraction from five historical sites to two sites and then expansion to four sites is likely an artifact of the ability to detect rare species in a mussel bed. Rainbow is currently supported at three sites in the northern reaches of East Sydenham River and at one site on Bear Creek. Densities seem to be declining based on two sites that were quantitatively resampled, from 0.03 individuals/m^2 on average in 1997-1999 to 0.01 individuals/m^2 in 2012-2013.

Thames River

Rainbow is a small component of the Thames River mussel community (1.5% of all species present) and is occasionally abundant, particularly in tributaries. Salmon and Green (1983) sampled a site on the Middle Thames River above Thamesford in 1977 and T.J. Morris (unpubl. data 2004) sampled a nearby site in 2004. Quadrat sampling was used for both surveys. The density of Rainbow was an order of magnitude lower in 2004 than in 1977 (0.09 vs. 0.9 individuals/m^2, respectively), suggesting that the species may be declining in this region. However, the frequency of occurrence across sites has slightly increased since the 1998 surveys (12.5% versus 18.9%) indicating a range expansion in the upper Thames and tributaries. This could be interpreted as evidence of a small but stable population with evidence of recruitment (Figure 11).

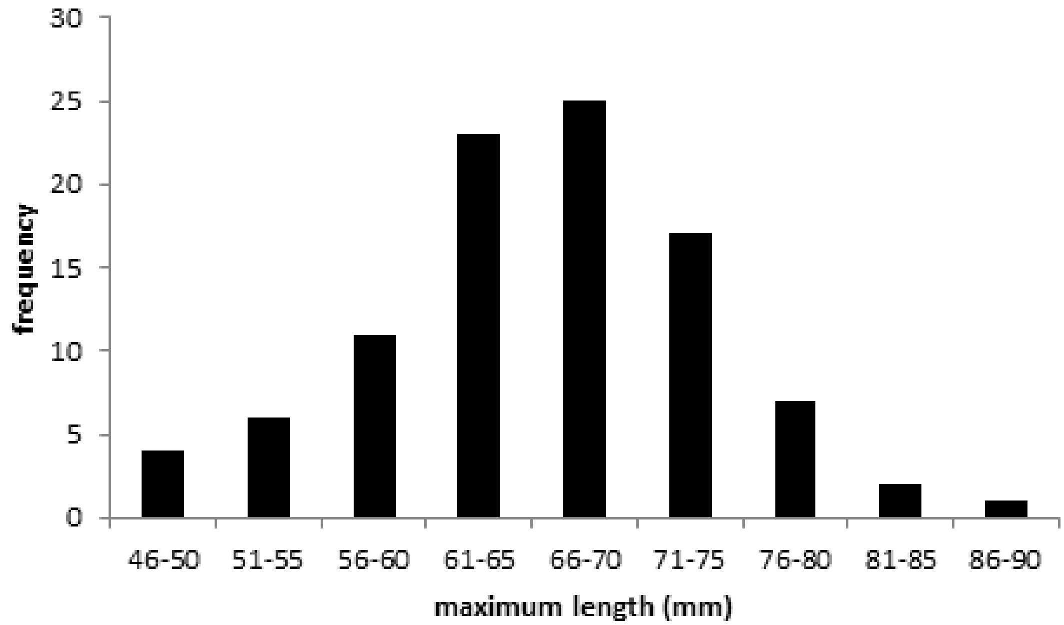

Figure 11. Size distribution of Rainbow collected from the Thames River watershed during the 2004-2005 surveys. Reproduced from Morris and Edwards (2007).

Trent River

A paucity of data prevents the discussion of Rainbow population fluctuations and trends in the Trent River watershed. The first formal surveys were completed in 2013 and demonstrated that Rainbow populations persist in the creeks and tributaries and a portion of the main branch, despite the establishment of dreissenids in these areas. Density at a single Rawdon Creek site is estimated as 0.24 individuals/m^2 and size distributions also confirm that Rainbow are actively reproducing in this watershed (Figure 12). Along the Trent River, thick mats of dreissenid shells (covering approximately 70% of the substrate) made it difficult to detect unionids in some areas (J. Epp pers. comm. 2014).

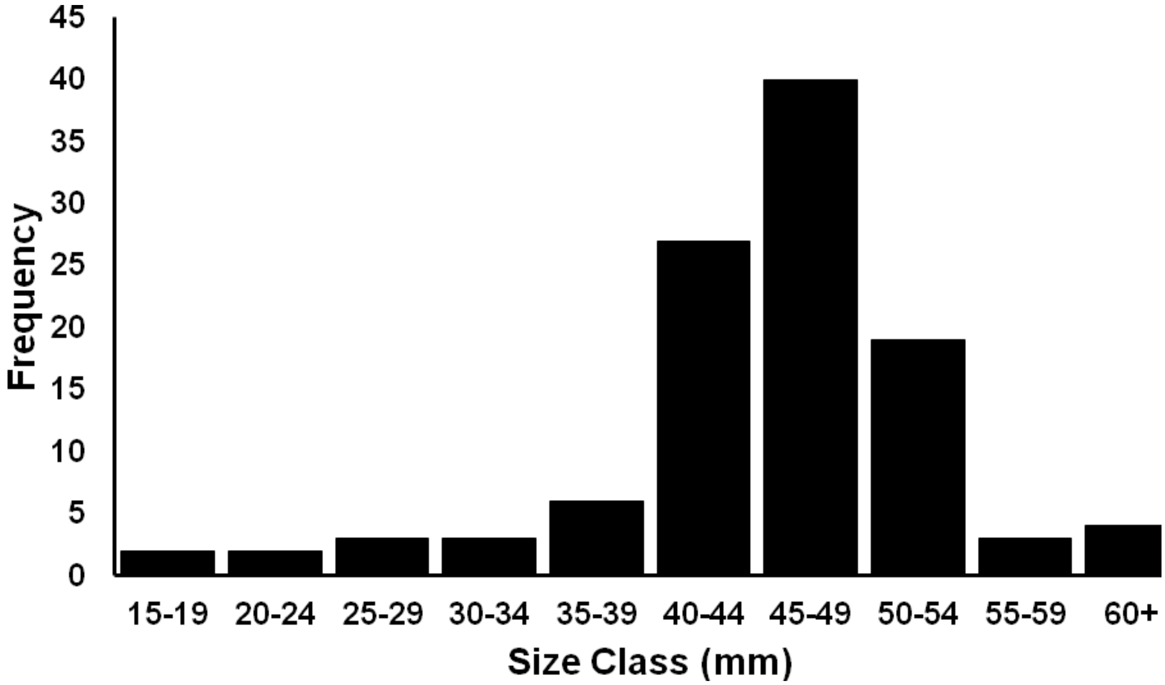

Figure 12. Size distribution of Rainbow collected from the Trent River watershed in 2013 (*n* = 109). Fisheries and Oceans Canada and Ministry of Natural Resources unpubl. data.

Overall, Rainbow seems to be persisting in the Maitland, Moira, Saugeen, Thames, Trent, Ausable and Sydenham river watersheds as well as Lake St. Clair, although they may be undergoing declines in the latter three. New information has been gained for the Grand River, demonstrating extant populations in the headwaters. Rainbow no longer occur in the Detroit and Niagara rivers, and lakes Ontario and Erie, despite the fact that they were never a large component of the historical mussel communities. Due to lack of quantitative data, the Bayfield and Salmon rivers could not be assessed, but widespread occurrences of Rainbow have been documented for these systems.

Rescue Effect

Rainbow occurs in four Great Lakes states (Michigan, New York, Ohio and Pennsylvania) that are connected to Ontario waterways via Lakes Ontario, Erie, St. Clair and Huron. Rescue of riverine populations by natural immigration of animals from the United States would be highly unlikely. Movement of infected host fishes over the large distances required to traverse the open water Great Lakes habitats and then to proceed up river to areas of suitable Rainbow habitat is unlikely. There is a large population of Rainbow in the U.S. waters of the St. Clair delta which is in close proximity to a smaller population in Canadian waters. Rescue of Rainbow population in the Canadian waters of the St. Clair delta is possible given this close proximity although these populations do not appear to be currently interacting.

THREATS AND LIMITING FACTORS

Threats

The two greatest threats to Rainbow are invasive species (eastern subpopulations) and pollution (southwestern subpopulations). Additional threats include natural system modifications (including damming, construction, changes to physical habitat from invasive species), but the severity of these activities remain unknown. Threats identified here follow those discussed in a peer-reviewed Recovery Potential Assessment for Rainbow in 2011 (DFO 2011) and a Threats Calculator call on 19 March 2015 (Appendix 1). Taking an ecosystem approach, these threats may interact to directly or indirectly influence one another. However, at this point in time it is difficult to define the overlap between them and therefore each one is discussed in singularity.

High Impact Threats

Invasive and other problematic species and genes (8.1 Invasive non-native/alien species)

The threat of dreissenid mussels (Zebra and Quagga mussels) to native unionids is substantial. These invasive species compete with native mussels for physical habitat and food, and can also attach to unionids as their substratum, thereby restricting unionid movement, burrowing, feeding, respiration, and reproduction. In heavily infested areas, dreissenids form blankets of live and spent shells over river and lake bottom, effectively smothering any animals below. The fouling effects, food limitation and virtual elimination of native mussels following the invasion of dreissenid mussels is well documented in southern Ontario and across North America (Strayer 2008).

Dreissenids have established in the watercourses where Rainbow reside, especially in eastern Ontario and the Great Lakes. The lower Trent River is subject to a constant infusion of veligers (dreissenid free-living larval stage) from Percy's Reach, Seymour Lake and Rice Lake upstream. During the 2013 sampling of the watershed, live dreissenids were found at all the main channel sites as well as one live individual at Rawdon Creek, and spent shells at Cold Creek (J. Epp pers. comm. 2014). The mats of dreissenid shells were so dense at some main channel sites that the river bottom could not be seen and a Rainbow was discovered lying on top of a mound of their shells (J. Epp pers. comm. 2014). Based on the CPUE, Rawdon Creek has the most Rainbow in this system, and the persistence of dreissenids in this tributary would severely threaten the Trent River subpopulation. The Moira River also contains dreissenid subpopulations, likely sourced from Moira and Stoco lakes since 2006 (QC 2008). All but two sites sampled in the Moira River during 2014 had infestations, the heaviest being in Stoco and Rapids Road sites. These two sites are downstream of, and in very close proximity to, Stoco and Moira lakes, which likely explains the high dreissenid density at these localities. Interestingly, Stoco has one of the highest Rainbow CPUE of the river, but Rapids Road is one of three sites (18 total) without Rainbow (shells or live). The Skootamatta River has no evidence of dreissenid colonization. The Salmon River hosts no known dreissenid subpopulations in the main channel, but they have been confirmed in the watershed at Beaver Lake (F. Scheuler unpubl. data) since 2006 (QC 2008).

The introduction and spread of the non-native dreissenid mussels throughout the Great Lakes has led to dramatic declines of native freshwater mussels in colonized areas (Schloesser *et al*. 1998, 2006; Riveredge Associates 2005). Nearly 50% of sites where Rainbow was known to occur historically are now infested with dreissenids. These biofouling organisms continue to threaten the population in the delta area of Lake St. Clair which acts as a refuge for many unionid species. Metcalfe-Smith *et al*. (2004) reported that densities of Rainbow declined between 2001 and 2003 and this trend has continued through to 2011 (Fisheries and Oceans unpubl. data). Rainbow was the most heavily infested of 10 species of unionids collected from the Squirrel Island/Bass Bay in the Canadian waters of the delta in 2004 (Metcalfe-Smith *et al*. unpubl. data). Resurveys of the delta in 2011 indicate that Rainbow no longer inhabits the Pocket Bay areas, and only one site still supports the species in the Squirrel Island/Bass Bay area, albeit not in high abundance. Live dreissenids were reported for all sites sampled; however, Johnston and Mitchell's bays were not recently resurveyed. In contrast, unionid subpopulations on the American side of the delta seem to be stable, despite the infestation of dreissenids (Zanatta *et al.* 2015); the reasons for this remain unknown.

Dreissenid mussels are unlikely to endanger the largest subpopulation of Rainbow in Ontario, i.e., the subpopulation in the Maitland River, because the watercourse is not navigable by boats and has few impoundments that could support a permanent colony. Likewise, there are no known records of this species in the Ausable, Bayfield, Saugeen or Sydenham rivers. Reservoirs with retention times greater than 20-30 days allow dreissenid veligers to develop and settle, after which the impounded populations will seed downstream reaches on an annual basis (Metcalfe-Smith *et al*. 2000). For example, dreissenids have established in the Fanshawe and Springbank reservoirs in the upper reaches of the Thames River system, and can be found within the river at most sites downstream of these reservoirs (Morris and Edwards 2007). Although highly abundant in the reservoirs, dreissenids have not reached high densities in the river below and have co-existed with Rainbow for over 15 years. The Grand River is another highly impounded watercourse, with 34 dams/weirs located throughout (Metcalfe-Smith *et al.* 2000). Dreissenid establishment in the Luther, Belwood, Guelph or Conestogo reservoirs could impact the population in the headwaters (Metcalfe-Smith *et al.* 2000).

There is also the potential for invasive fishes to negatively impact Rainbow subpopulations by disrupting the host fish relationship. Recent evidence has confirmed the potential of the Round Goby (*Neogobius melanostomus*) to be infested with Rainbow glochidia and potentially act as a reproductive sink (Tremblay 2012). Rainbow glochidia are able attach to wild and laboratory-kept Round Gobies, but generally do not transform (Tremblay 2012). In other studies native fishes were found to decline in abundance and lack recruitment in the presence of the Round Goby in the Great Lakes basin (Dubs and Corkum 1996; Janssen and Jude 2001). It is unknown whether this invasive species competes with the host fishes of Rainbow, but it has been documented in areas where Rainbow subpopulations reside including the Grand River (Poos *et al.* 2010) and the lower Sydenham, Ausable, Thames and Trent rivers. In addition, there is the potential for Round Gobies to ingest juvenile unionids although there has been no documented evidence of targeted Rainbow predation.

Medium-High Impact Threats

<u>Pollution (9.1 Household sewage and urban waste water,9.2 Industrial and military effluents, 9.3 Agricultural and forestry effluents)</u>

Aquatic pollution likely affects southwestern Rainbow subpopulations more so than the eastern subpopulations due to the larger agricultural presence and urban centres in these watersheds. Household sewage and urban pollution often includes contaminants that Rainbow are particularly sensitive to including ammonia, mercury, chlorine from road salt and pharmaceuticals (Gagné *et al*. 2012, Gillis 2012**)**. Industrial sources can additionally include toxins such as arsenic and copper (Jorge *et al.* 2013). Excess sediment, nitrogen and phosphorus loading can be a direct threat to Rainbow and are largely caused by agricultural activities (Mummert *et al.* 2003). Although a variety of substances have been deemed toxic to Rainbow, the species could also be sensitive to other substances that have not yet been tested.

9.1 Household sewage and urban waste water

Ammonia and mercury:

Freshwater mussels are among the most sensitive aquatic organisms to environmental contaminants and there is growing evidence that Rainbow may be particularly sensitive to ammonia. For example, Goudreau *et al*. (1993) reported that the glochidia of Rainbow were more sensitive to ammonia (24-hour LC50 = 0.284 mg/L) and monochloramine (24-hour LC50 = 0.084 mg/L) than many other species of invertebrates, including other molluscs. Similarly, Mummert *et al*. (2003) found that juvenile Rainbow and Wavyrayed Lampmussels (*Lampsilis fasciola*) were among the most sensitive aquatic organisms to unionized ammonia, with Rainbow being more sensitive than the Wavyrayed Lampmussel (96-hour LC50s = 0.11 and 0.26 mg/L NH_3-N, respectively). Based on reported levels of unionized ammonia in the aquatic environment, this contaminant may limit the distribution of Rainbow and other freshwater mussels in some systems (Mummert *et al*. 2003). Juvenile freshwater mussels remain buried in the sediment for the first few years of life, where they feed exclusively on particles in the interstitial water. Such behaviour may increase their exposure to ammonia as the contaminant is produced from decomposition of organic matter in sediments in the absence of dissolved oxygen. This would be further exacerbated with rising water temperature and pH, conditions that naturally lead to increased amounts of unionized ammonia. Ammonia production is also enhanced under conditions of increased silt and clay, the compactness of which helps reduce interstitial oxygen. Therefore, anthropogenic inputs of organic material, organic pollution and nutrients could influence ammonia concentrations (see Strayer 2008).

One study has found mercury to be both acutely and chronically toxic to Rainbow in the juvenile and glochidial life stages. Valenti *et al.* 2005 revealed through lab experimentation that glochidia are more responsive to acute exposure to mercury than juveniles, with 72-hour LC50s of 14 µg/L and 114 µg Hg/L, respectively. Chronic exposure led to sublethal effects including stunted growth for juveniles exposed to levels as low as 8 µg/L over 21 days. Very little is known of the mercury levels in watersheds where Rainbow resides. Presumably releases of mercury would be related to leaching from landfills (improperly disposed of batteries and fluorescent lamps, for example) or, previously, from old but currently banned pesticides.

Road salt:

Acute sensitivity to sodium chloride levels is most notable for the early life stages of unionids. Gillis (2011) has pioneered this body of research and has shown that chloride from road salt is an increasing threat for the glochidia of the Wavyrayed Lampmussel. She reports surface water levels greater than 1300 mg/L within the species' distribution, levels which would be toxic to the young. Rainbow distribution overlaps with that of the Wavyrayed Lampmussel and is therefore also subject to excessive and chronic chloride inputs. Rainbow glochidia and juveniles have 24-96 hr LC50s ranging between 1660-2300 mg NaCl/L and no observed effect concentrations (NOECs) ranging between 500-2000 mg NaCl/L (Pandolfo *et al.* 2012). National water quality guidelines for the protection of aquatic life have been set at 120 mg/L of chloride (CCME 2011); however, in some cases, levels are far exceeding this standard and protection for species at risk is limited. Recently, the ABCA (Ausable Bayfield Conservation Authority) reports chloride levels approaching 250 mg/L (ABCA 2013b). Data are also available for the Saugeen River watershed (including the Teeswater River) with chloride levels ranging from 1.0 -14 mg/L (SVCA 2013a,b). Todd and Kaltenecker (2012) further demonstrate that baseline chloride levels are increasing in mussel habitats across southern Ontario (96% of 24 monitoring stations) and suggest that this will affect recruitment of mussel species at risk in the future. This is in contrast to the eastern part of the province where chloride levels are steadying (QC 2008). The Moira and Salmon river watersheds average 7 and 9 mg/L respectively, but levels can reach as high as 138 mg/L locally (QC 2008). Hard water (typical of southern Ontario) acts as a natural buffer to chloride retention, but only to a limited degree (Gillis 2011).

Wastewater discharge:

Wastewater discharge is inherently implicated in ammonia, heavy metal, nitrogen and phosphorus loading, but recent evidence shows sewage pollution also releases endocrine disruptors including human pharmaceuticals, organochlorine pesticides and paint by-products, which may have effects in small quantities including the feminization of aquatic organisms and disruption of gonad physiology (Strayer 2008). Gagné *et al.* (2011) have ascertained that mussels downstream of a municipal effluent outlet had two and a half times more females than males, in addition to males displaying female-specific proteins related to egg production. However, the effects are not limited to mussels; host fishes could also be impacted. Experimental addition of endocrine disruptors at environmentally detectable levels influenced Fathead Minnow (*Pimephales promelas*) reproduction so harshly that the entire population disappeared from a lake (Kidd *et al.* 2007), whereas Tetreault *et al.* (2011) and Tanna (2012) observed feminization and intersex qualities (lowered testosterone and female oocytes in male testicular tissue) of Grand River fishes (including Rainbow hosts Greenside Darter and Rainbow Darter). Other contaminants include chemicals (Perfluoroalkyl acids) that are added to household items and fabric to provide water repellency. These are detectable (at the ng/L to 2,200 µg/L range) in southern Ontario waterways (Moody *et al.* 2001; Oakes *et al.* 2010) and have demonstrated effects on the duration of glochidia viability and probability of metamorphosis (Hazelton *et al.* 2012). Although it is known that previous aquatic pollution has had generally detrimental effects on unionid reproduction and distribution, this body of information is still incomplete and more data are needed on the precise impacts for unionid distribution and abundance.

Mackie (1996) suggested that most of the harm inflicted upon freshwater mussel communities was instigated by poor wastewater management, particularly in the Grand River. Studies by Gillis (2012) and Gillis *et al.* (2014) confirmed that the Grand River subpopulation is chronically exposed to an array of contaminants including ammonia, chloride and metals such as copper, lead and zinc. Furthermore they discovered a dead zone immediately downstream of a wastewater facility effluent outlet where no mussels were detected for several kilometres (P. Gillis unpubl. data). Rivers in southern Ontario usually have many sewage treatment facilities along their course; for example, the Grand River has 29 and Thames River has 22. Eastern Ontario has fewer municipal treatment facilities, for instance the Moira River has three located in Belleville, Tweed and Madoc, as half of the population in the watershed relies on private or communal septic systems, especially in rural areas (QC 2008). Rainbow in the Trent River are subject to effluent from upwards of 50+ wastewater treatment plants; however only nine are in the vicinity of current populations.

9.2 Industrial and military effluents

Arsenic and Copper:

Mining and aggregate extraction have been the focus of development activities in eastern Ontario where mineral formations are bountiful. These activities have particularly affected the Moira River, where the Deloro Gold Mine has left a 150 year legacy of aquatic pollution (see QC 2008). The two largest concerns regarding Rainbow are the leaching of arsenic (a by-product of smelting copper, gold and silver ore) and refined copper from the site, although radioactive waste (uranium used in extraction process) and cobalt are also seeping from contaminated soils (QC 2008). Specific information regarding the toxicity of arsenic to Rainbow is not available; however, a study soon after the abandonment of the site in 1961 showed unprecedented declines in mussels and aquatic life in general several kilometres downstream of the mine (Owen and Galloway 1969). Currently Rainbow resides 20-30 km downstream of Deloro, but whether this is a result of the contamination or a natural occurrence is unknown. In 1979 the Ontario Ministry of the Environment began a cleanup of the site which currently continues. Despite an 80% reduction in contamination following these efforts, elevated arsenic levels are still an ongoing issue for the river as the element tends to bind in the sediment, releasing upon disturbance, and continues to leach. Concentrations between 0.1 and 8.2 mg/L still occur downstream and there is a regulated ban on dredging the Moira River anywhere downstream of the town of Deloro (QC 2008). In comparison, neighbouring watersheds have naturally occurring arsenic concentrations around 0.001 mg/L on average.

Unionids are also particularly sensitive to copper levels, because as with arsenic, the unionized forms tend to accumulate in the interstitial water of river bottom habitat (Strayer 2008). While dissolved organic carbon (DOC) can act as a natural buffer to copper bioavailability, systems without inherently high DOC inputs or hardwater would offer little protection to freshwater mussels (Gillis *et al.* 2008, 2010). Rainbow is more sensitive to copper than other aquatic invertebrates (in terms of growth and reproduction) and has a 4-day LC50 of 15 µg/L under conditions of low DOC (0.5 mg DOC/L; Wang *et al.* 2011). While the concentration of bioavailable copper in southern Ontario rivers is generally low, given the protection of high DOC, copper can also be released in spikes from wastewater and mining effluents. The Moira and Salmon rivers average 0.002 mg/L (QC 2008) and the Thames River exceeds the federal guidelines (0.005 mg/L) in all sub-basins (Metcalfe-Smith *et al.* 2000; Morris *et al.* 2008).

Pipelines and Oil Spills:

There is growing concern that oil leaks from pipelines in southern Ontario would present a particular risk to species at risk in the region. The recent approval of the Line 9 reversal has especially fostered this. Line 9 is a pipeline established in the 1960s that extends over 700 km from Sarnia to Montréal in some of the most populated areas of southern Ontario. It has a capacity of 240,000 barrels per day, providing Quebec and Ontario refineries with predominately light crude oil, but is proposed to also carry heavy crudes including diluted bitumen. Although currently flowing westward, the reversal would bring the flow back to its original eastward direction, as it was prior to 1998. There is concern that with aging infrastructure, there is the potential for leaks; however, there has been no known occurrence of leaks from this pipeline to date (see Enbridge 2015). Nonetheless, if a spill were to occur, depending on the location, it could have negative impacts on the Ausable, Thames or Grand rivers in the southwest and the lower reaches of the Trent, Moira or Salmon rivers in the east, within the vicinity and upstream of Rainbow subpopulations. Pipeline ruptures carrying diluted bitumen in the United States have had devastating and costly consequences (e.g., the Kalamazoo River in Michigan) including dredging and sediment removal in the areas where Rainbow have been found (Sherman Mulcrone and Mehne 2001; EPA 2015).

A second potential spill route would be from railcars carrying oil and other potentially hazardous substances. Railroad crossings intersecting rivers are common in southern Ontario although those used primarily for oil transportation are not commonly known.

9.3 Agricultural and forestry effluents

Erosion:

Anthropogenic stressors such as high turbidity and sediment loading originating from urban and agricultural sources are potential problems in southern Ontario where Rainbow occurs. High levels of entrained silt and sand particles are problematic in that they clog the siphons, gills, and digestive tracts of mussels when they are gaping. Although mussels have an innate sorting system whereby organs (labial palps) remove inorganic particles before they enter the digestive tract (lodging them out the inhalant siphon as pseudofaeces), the animal's feeding structures can saturate whereby the animal will close and postpone filtration until conditions improve (Wildish and Kristmanson 1997; Cummings and Graf 2010). Long duration of valve closure could potentially cease feeding, movement, and reproductive behaviours such as lure display and release of glochidia. Large pebbles can also be problematic when they lodge between the valves in such a way that it prevents closing (J. Vanden Byllaardt pers. obs.).

Erosion and siltation resulting from intensive agriculture has fouled many of the sand and gravel riffles in rivers inhabited by this species. Tile drains, livestock access to streams, and the reduction or elimination of riparian buffer strips have all contributed to this problem. The watershed with the highest percentage of agricultural land use within range of Rainbow is the Ausable (85%), followed by the Sydenham (81%), Saugeen (76%), Grand (75%) and Thames (75% for Upper) river watersheds (**see Habitat Trends**). Data were not available for the Maitland, Moira and Trent rivers, but, in contrast, these have notably higher than average forest cover. Rainbow abundance seems to coincide with high forest cover in many systems (**see Habitat Trends**). Particularly, the Maitland and Trent river watersheds have the largest and third largest subpopulations in combination with high forest cover (35% and 26.95%, respectively). Likewise, the Moira and Salmon rivers seem to have high numbers of Rainbow based on CPUE and are also heavily forested (59%). Several other areas also fit this trend, including Rawdon Creek and the Teeswater River (50% and 29% forested, respectively) and are the most heavily populated areas within their watersheds.

Common to all the watersheds, large installations of tile drainage, application of liquid manure and increases in row crops have led to noticeable increases in turbidity. This is particularly true of the north branch of the Sydenham River and lower Thames River. Suspended solids have been discovered in concentrations as high as 900 mg/mL, a level which would be potentially harmful to mussel communities if it were sustained through time (Bouvier and Morris 2011). The rivers most protected from agricultural influence seem to be in the eastern Ontario region, where the Canadian Shield makes it difficult to farm and the rivers have a higher percentage of forested riparian zones (up to 62%; QC 2013). The Lake St. Clair Rainbow subpopulation is afforded protection from degradation of terrestrial surroundings by the Walpole Island First Nation Territory.

Phosphorus and Nitrogen:

Total phosphorus (TP) and nitrates/nitrites are not directly toxic to mussels but it is well known that excessive nutrient loading leads to eutrophication of water bodies, which results in anoxic conditions and potential release of algal toxins leading to mussel die-offs (Augsberger *et al.* 2003). Phosphorus is often the limiting nutrient. The Ontario provincial government considers streams with TP greater than 0.03 mg/L eutrophic (lakes > 0.02 mg/L), as well as waterbodies exceeding 0.10 mg/L nitrate (PWQO 2015). Farming practices have the duality of increasing turbidity as well as increasing nutrient loads. In Ontario, this is primarily due to tile drainage and application of fertilizers and liquid manure. Three watersheds have available data on percentage of tile drainage, the highest being the Ausable River, followed by the Sydenham and Thames rivers (at 70%, 60% and 56%, respectively; **see Habitat Trends**).

The watersheds have varying amounts of information available about TP and nitrate/nitrites, but the Ausable, Thames and Sydenham rivers, in addition to the St. Clair delta, seem to fare the worst. The Ausable and Sydenham river watersheds have up to seven times the recommended concentrations of phosphorus (up to 0.22 mg/L, ABCA 2013b; SCRCA 2013). The Upper Thames has poor water quality, with the phosphorus and nitrogen loadings being some of the highest in the Great Lakes Basin (Bouvier and Morris 2011), although areas inhabited by the Rainbow fare somewhat better (i.e., Middle Thames, Fish, Black and Otter creeks; UTRCA 2012). The phosphorus levels in the St. Clair watershed average 0.13 mg/L, classifying it as eutrophic. In comparison, the Maitland and Saugeen river watersheds seem to have less phosphorus input (0.0163 - 0.03 mg/L) and nitrite/nitrate levels remain well below the standard of 0.10 mg/L (MVCA 2013; SVCA 2013a,b), which implies that the threat of nutrient loading is not as high for the largest Rainbow subpopulation in Canada. Of the eastern Ontario subpopulations, information is vague, but reports indicate that the Trent River watershed surface water quality is good, with the highest ratings for Percy and Burnley creeks (LTRCA 2013). Surface water quality is excellent in the northern third of the Quinte watershed, but is only in fair condition in the lower portion, especially in proximity to urbanized areas (QC 2013). The Salmon River has estimated phosphorus concentrations ranging from 0.01-0.03 mg/L and the Moira River has 0.06 mg/L (QC 2008).

Threats with Unknown Severity

Natural System Modifications (7.2 Dams and water management/use, 7.3 Other ecosystem modifications)

7.2 Dams and water management/use

Damming can result in altered flow, scouring of the downstream riverbed, increased turbidity, temperature and the physical separation of mussel and host. The severity of the effects is unknown because not much is understood about how unionids respond to these activities and what the actual extent of the impacts is. The spatial footprint of the dam's effects can be large but, again, this is not well-known. Unionids often have species-specific requirements for flow, which is primarily determined by their native habitat (Vanden Byllaardt and Ackerman 2014). Disturbances to the native flow regimes could negatively influence populations especially at the extremes. Unnaturally low flow conditions prevent the replenishment of food sources and promote low oxygen levels, increased temperatures, and desiccation, whereas high flow rates could dislodge mussels, prevent larvae settlement, increase turbidity and cause the mussels to expend more energy by burrowing. Freshwater mussels have shown sensitivity to altered flow regimes, evidenced by entire populations going extinct after the construction of dams (Theler 1987; Layzer *et al.* 1993). The particular flow requirements or turbidity tolerance of Rainbow are not known, beyond the fact that they are often found in fast-flowing riffle habitat. Mussels generally have preference for shallow water (10 – 20 cm; Metcalfe-Smith *et al*. 2007) and their growth has been linked to mean annual stream flow (Rypel *et al*. 2008). Both reservoirs and impoundments also alter downstream flow and temperature patterns, and promote the establishment of dreissenid mussel larvae (see Invasive and other problematic species and genes), but impoundments further act as a physical barrier to host fishes. Moreover, the disturbance of river bottom by dredging and construction activities would lead to direct loss of physical habitat and cause siltation and greater turbidity of ambient and downstream waters.

While there are not many dams on the Maitland and Sydenham rivers, there are many on the Trent, Moira, Grand and Thames rivers. For example, the upper Thames River has a multitude of water control structures, totalling upwards of 170 dams and weirs. There are 21 dams throughout the Ausable River, two of which are large enough to create small reservoirs of the Rainbow subpopulation, near Exeter. The Saugeen River hosts 21 dams and Teeswater River has 14. The Trent River likely has the highest level of flow alterations with large-scale navigational locks throughout. The Bayfield, Ausable, Salmon, Moira and Thames rivers are considerably more 'flashy' and often experience flooding, the impact of which is unknown.

7.3 Other ecosystem modifications

Invasive species not only compete with native mussels for resources, they also tend to modify their habitat in suboptimal ways. For example, dreissenid mussels smother other organisms by forming blankets over lake and river bottoms. This was especially noticeable in the Trent River, where in some cases, Rainbow sites were completely covered in shells and the river bottom could not be seen (J. Epp. pers. comm. 2014). Dreissenids are also present in the Thames and Moira rivers and Lake St. Clair, but not as pervasively. Lastly, clearing of riparian vegetation may increase water temperature by reducing shade, and removing snags in navigable sections of rivers, also are concerns for Rainbow habitat.

Limiting Factors

Host Fishes

The most significant natural control on the size and distribution of mussel populations are the distribution and abundance of their host fishes. Unionids cannot complete their life cycle without access to the appropriate glochidial host. If host fishes' populations disappear or decline in abundance to levels below that which can sustain a mussel population (e.g., due to anthropogenic pollution, see **Habitat Trends**), recruitment will no longer occur and the mussel species may become functionally extinct (Bogan 1993). As noted earlier (**Life cycle and Reproduction**), several fishes known to be glochidial hosts for the Rainbow are common throughout the species' range in Canada; therefore, access to hosts is not currently a major limiting factor for this species in Ontario.

Predation

Freshwater mussels are known to be food sources for a variety of mammals and fishes (Fuller 1974). Predation by Muskrat, in particular, may be a limiting factor for some mussel species, especially in urbanized areas. Hanson *et al*. (1989) and Tyrrell and Hornbach (1998) have shown that Muskrats are both size- and species-selective in their foraging, and can therefore significantly affect both the size structure and species composition of mussel communities. There have been several studies of Muskrat predation on freshwater mussels (Neves and Odum 1989; Watters 1993-1994; Tyrrell and Hornbach 1998). None of these studies reported the presence of Rainbow shells in Muskrat middens, suggesting that this mussel is not a preferred prey species. Raccoon is another potential predator. Although the report writers are not aware of any studies on raccoon predation, they have observed Raccoons feeding on mussels in the field and there is a need to study the impacts of Raccoon predation on freshwater mussels in Ontario, especially in increasingly urbanized areas where they are becoming more populous.

Number of Locations

The number of locations was determined by selecting the most serious and plausible threat that affect the species' distribution. In cases where there were more than two main threats, the number of locations was based on the threat that results in the lowest number of locations. When considering just invasive species, dreissenids are the major threat for the eastern populations (Moira, Salmon and Trent rivers) and the St. Clair River Delta, whereas Round Goby is the major invasive threat collectively for the subpopulations in the southwestern watersheds (Ausable Bayfield, Grand, Maitland and South Maitland, Teeswater, North Thames, South and Middle Thames, Saugeen, and Sydenham rivers) but not the eastern watersheds (Moira, Salmon and Trent rivers). By contrast agriculture and urban threats are most important for the southwestern watersheds and less so for those in the east. Considering the high impact of invasive species (dreissenids and Round Goby) and aquatic pollution (from the combination of sediment and nutrient loading and contaminants from urban development) 14 locations resulted: (1) Salmon River, (2) Moira River, (3) Trent River, (4) Grand River, (5) North Thames River (including Fish and Medway creeks), (6) South Thames and Middle Thames rivers, (7) Ausable River, (8) Sydenham River, (9) Bayfield River, (10) Teeswater River, (11) Saugeen River, (12) South Maitland River, (13) Maitland River and (14) the St. Clair River Delta.

PROTECTION, STATUS AND RANKS

Legal Protection and Status

Rainbow was assessed as Endangered in 2006 by COSEWIC and listed as Endangered under the federal *Species at Risk Act* (*SARA*) in 2013 and Threatened under Ontario's *Endangered Species Act* (*ESA*) in 2007. The two acts afford Rainbow legal protection through prohibitions to direct harm of the animals or significant disturbance to their habitat. Changes to the *Fisheries Act* in 2012 eliminated protection for freshwater mussels. However, Rainbow host fishes are allotted protection as several species contribute to recreational fisheries (Largemouth Bass, Yellow Perch, Rock Bass, Green Sunfish and Smallmouth Bass) or support such a fishery (Mottled Sculpin, Greenside and Rainbow Darters). All of these hosts are protected from serious harm, defined as death or permanent alteration/destruction of habitat as stated in subsection 35(1) (D. Ming pers. comm. 2015). Furthermore, subsection 36(3) of the Fisheries *Act* prohibits the deposition of harmful substances (including sediment) in any water frequented by these host fishes. A recovery strategy is being developed for Rainbow, which identifies critical habitat and key actions that will be implemented. It is not currently listed or proposed for listing under the U.S. *Endangered Species Act.* Rainbow is listed as endangered in Illinois and Wisconsin (Cummings and Mayer 1992), special concern in Michigan (Badra and Goforth 2003) and North Carolina (Bogan 2002) and proposed for endangered status in Pennsylvania (Crabtree pers. comm. 2004) and is therefore afforded some protection in these states.

Non-Legal Status and Ranks

Rainbow has a designated national ranking of N2N3 (nationally vulnerable – imperilled) in Canada and S2S3 in Ontario, the only province where it occurs (NatureServe 2014). The national general status of freshwater mussels in Canada was updated in 2010 (CESCC 2011) and Rainbow was ranked as 1 (At Risk) nationally and in Ontario. Globally, the species is listed as G5, defined as demonstrably widespread, abundant, and secure. Generally the species is also considered secure in the U.S. (N5). According to NatureServe (2014), current state ranks for the Rainbow are: Alabama (S3), Arkansas (S2S3), Illinois (S1), Indiana (S3), Kentucky (S4S5), Michigan (S2S3), Missouri (S4), New York (S2S3), North Carolina (S1), North Dakota (SNR), Ohio (S5), Oklahoma (S1), Pennsylvania (S1), Tennessee (S5), Virginia (S4), West Virginia (S2) and Wisconsin (S1). It does not appear on the IUCN Red List of Threatened Species.

A portion of Rainbow population in Lake St. Clair occurs within the territory of the Walpole Island First Nation (WIFN). Special user permits are required to access First Nation territory and waters, thus limiting human disturbance in the area. The WIFN drafted the Walpole Island Recovery Strategy which has the following goal: “To conserve and recover the ecosystems of the Walpole Island Territory in a way that is compliant with the Walpole Island First Nation Environmental Policy Statement and provides opportunities for cultural and economic development and protection for Species at Risk” (Bowles 2004).

Habitat Protection and Ownership

Land ownership along the reaches of the Sydenham, Thames, Ausable, Maitland, Grand and Saugeen rivers currently occupied by Rainbow is mainly private and in agricultural use. Only two small properties in the Sydenham River watershed, the 7 ha Shetland Conservation Area and the 20 ha Mosa Township forest, are publicly owned and somewhat protected (Andreae pers. comm. 1998). There are 21 natural areas totalling 6,200 ha in the Thames River watershed and most of these are in the upper reaches where Rainbow occurs (Thames River Background Study Research Team 1998). Four First Nations Reserves occupy over 6,700 ha of land along ~ 45 km of the Thames River downstream of the City of London, but Rainbow has never been found in this area. The Ausable Bayfield Conservation Authority owns a number of properties totalling 1,830 ha throughout the basin (Snell and Cecile Environmental Research 1995). The Maitland Valley Conservation Authority owns 28 conservation areas covering 1,750 ha, but these areas represent only about 0.5% of the land in the Maitland River watershed (Kenny pers. comm. 2005). Less than 3% of the land in the Grand River watershed is publicly owned (GRCA 1998). There are 11 conservation areas, one of which (Elora Gorge) is about 10 km upstream of the reach occupied by Rainbow. Saugeen Conservation owns over 8,498 ha of Conservation Areas and Lands composed of wetland complexes, managed forests and recreation parks (Nicol pers. comm. 2005). It should be noted that recovery strategies and action plans have been developed and implemented for the Sydenham, Thames and Ausable river aquatic ecosystems to protect and recover aquatic and semi-aquatic species at risk including fishes, mussels, turtles and snakes (Dextrase *et al.* 2003; ARRT 2005; TRRT 2005; DFO 2012, 2015). Many landowners are participating in riparian rehabilitation projects and improved land use practices that will ultimately benefit all aquatic species.

The Trent River is part of the Trent-Severn Waterway, one of seven national historical canals that are managed and protected by Parks Canada. Parks Canada is developing policies for in-water and shoreline works and related activities that will "ensure that the (natural and cultural) heritage and recreational values of the waterways will continue to be sustained…" (Parks Canada 2014). Information was not available for the Moira and Salmon rivers, although it is known that conservation authorities manage some of the land adjacent to the rivers.

Occupied habitats in Canadian waters of the Lake St. Clair delta fall within the territory of the Walpole Island First Nation. These areas are primarily used for hunting and fishing and are protected from urban development as well as certain recreational uses (e.g., jet skis are prohibited). Walpole Island contains over 12,000 ha of World Class Wetlands – one of the largest wetland complexes in the Great Lakes Basin (Bowles 2004) – and freshwater mussels occupy the transition zone between these wetlands and the open waters of Lake St. Clair. Rainbow is currently more abundant in U.S. waters (see Zanatta *et al.* 2015) where habitat protection is minimal because the shoreline is almost completely urbanized and the waters are heavily utilized for recreational purposes. It is not known why Rainbow is more abundant in U.S. waters than in Canadian waters, as substrate type and dreissenid infestation rates are similar in both areas of the delta.

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BIOGRAPHICAL SUMMARY OF REPORT WRITER(S)

Julie Vanden Byllaardt is an Aquatic Science Technician responsible for leading studies on aquatic invasive species via ballast water of ships. Previously, she was employed by Fisheries and Oceans Canada to conduct field collections, analyze data and write first-author publications regarding the life history and reproduction of mussel species at risk (including Rainbow). She completed a Master's thesis (University of Guelph, Integrative Biology) on the feeding ecology of Ontario unionids and has been working with freshwater mussels since 2008. She has collaborated with Dr. Gerry Mackie (Water Systems Analysts) to conduct mussel relocations and has experience teaching mussel identification at the DFO mussel ID course, to consulting companies and a University of Guelph biology course. She also has a BSc (Hons) in Botany (University of Toronto) and is a member of the Ontario Freshwater Mussel Recovery Team.

Dr. Todd Morris is a Benthic Research Scientist with Fisheries and Oceans Canada responsible for leading a scientific research program focusing exclusively on molluscs with a major emphasis on unionid species at risk. He has a BSc (Hons) in Zoology (1993) from the University of Western Ontario, an MSc in Aquatic Ecology from the University of Windsor (1995) and a PhD in Zoology from the University of Toronto (2002). His research interests centre on the biotic and abiotic factors structuring the distributional patterns of freshwater invertebrates of the Great Lakes basin. He began working on the unionid fauna of Ontario in 1993 and is a member of the COSEWIC Mollusc SSC, the American Fisheries Society Endangered Mussel Subcommittee and a founding member and co-Chair of the Ontario Freshwater Mussel Recovery Team.

COLLECTIONS EXAMINED

In 1996, all available historical and recent data on the occurrences of freshwater mussel species throughout the Lower Great Lakes drainage basin were compiled into a computerized, GIS-linked database referred to as the Lower Great Lakes Unionid Database. The database is housed at the Fisheries and Oceans Canada’s Great Lakes Laboratory for Fisheries and Aquatic Sciences in Burlington, Ontario. This database now contains over 160,000 unique records collected from nearly 1300 sites in southern Ontario. Data sources included the primary literature, natural history museums, federal, provincial, and municipal government agencies (and some American agencies), conservation authorities, Remedial Action Plans for the Great Lakes Areas of Concern, university theses and environmental consulting firms. Mussel collections held by six natural history museums in the Great Lakes region (Canadian Museum of Nature, Ohio State University Museum of Zoology, Royal Ontario Museum, University of Michigan Museum of Zoology, Rochester Museum and Science Center, and Buffalo Museum of Science) were the primary sources of information, accounting for over two-thirds of the data acquired. Previous report writers (J.L. Metcalfe-Smith) personally examined the collections held by the Royal Ontario Museum, University of Michigan Museum of Zoology and Buffalo Museum of Science, as well as smaller collections held by the Ontario Ministry of Natural Resources. The current status report writers have personally verified live specimens from all subpopulations presented in this report.

Appendix 1. Threats Assessment Worksheet for Rainbow (*Villosa iris*)

THREATS ASSESSMENT WORKSHEET

Species or Ecosystem Scientific Name	*Villosa iris* (Rainbow)			
Element ID			**Elcode**	
Date (Ctrl + ";" for today's date):	19/03/2015			
Assessor(s):	Molluscs SSC members: Joe Carney (co-chair), Suzanne Dufour; Daelyn Woolnough, Dave Zanatta. Status report writers: Todd Morris, Julie vanden Bylaardt. Jurisdictional reps (ON): Sarah Hogg, Scott Reid. Facilitator: Dwayne Leptizki. COSEWIC secretariat: Bev McBride			
References:	Draft COSEWIC status report on the Rainbow, *Villosa iris*; Teleconference held March 19, 2015, duration 2:20			

Overall Threat Impact Calculation Help:

Threat Impact		Level 1 Threat Impact Counts: high range	Level 1 Threat Impact Counts: low range
A	Very High	0	0
B	High	2	1
C	Medium	0	1
D	Low	0	0
Calculated Overall Threat Impact:		Very High	High

Assigned Overall Threat Impact:	**High**
Impact Adjustment Reasons:	
Overall Threat Comments	Based on experience with other species, the Rainbow would fall closer to the high than the very high overall threat impact. Based on where it is found in the rivers (headwater) it is further removed from the main threats (it is above the urban and agricultural impacts and invasives). As well, the eastern Ontario populations face different threats which are not as severe as those in other parts of the range.

Threat		**Impact (calculated)**		**Scope (next 10 Yrs)**	**Severity (10 Yrs or 3 Gen.)**	**Timing**	**Comments**
1	Residential & commercial development						
1.1	Housing & urban areas						
1.2	Commercial & industrial areas						
1.3	Tourism & recreation areas						
2	Agriculture & aquaculture		Negligible	Negligible (<1%)	Extreme (71-100%)	High (Continuing)	
2.1	Annual & perennial non-timber crops		Negligible	Negligible (<1%)	Extreme (71-100%)	Unknown	Tile drainage in headwater areas is a specific concern, but we could not predict whether there would be new instances of this or other new or changed farm practices (e.g. hog barns). The authors will investigate.

Threat		Impact (calculated)		Scope (next 10 Yrs)	Severity (10 Yrs or 3 Gen.)	Timing	Comments
2.2	Wood & pulp plantations						
2.3	Livestock farming & ranching		Negligible	Negligible (<1%)	Extreme (71-100%)	High (Continuing)	Trampling in streams is a concern in watersheds where livestock still have access to streams (such as Thames, Sydenham, Salmon, Moira and Trent systems).
2.4	Marine & freshwater aquaculture						
3	Energy production & mining						
3.1	Oil & gas drilling						
3.2	Mining & quarrying						Gravel extraction takes place near the Maitland River but not directly in the stream. Also, see pollution section.
3.3	Renewable energy						
4	Transportation & service corridors		Negligible	Negligible (<1%)	Moderate (11-30%)	High (Continuing)	
4.1	Roads & railroads		Negligible	Negligible (<1%)	Moderate (11-30%)	High (Continuing)	Ongoing road, bridge and culvert maintenance or installation throughout range. Some mitigation measures are in place, such as translocation.
4.2	Utility & service lines						Daelyn W. and Todd M. will investigate cases where pipelines might be crossing water courses. This section may be updated following more information-gathering.
4.3	Shipping lanes						
4.4	Flight paths						
5	Biological resource use		Negligible	Negligible (<1%)	Unknown	High (Continuing)	
5.1	Hunting & collecting terrestrial animals						
5.2	Gathering terrestrial plants						
5.3	Logging & wood harvesting						Pollution from these activities would be covered in the pollution section.
5.4	Fishing & harvesting aquatic resources		Negligible	Negligible (<1%)	Unknown	High (Continuing)	Threat is to potential fish hosts. Possible host species are subject to recreational fishery (smallmouth and largemouth bass on the Grand R.), maybe also as bait species, not really enough known about what species serve as hosts.
6	Human intrusions & disturbance		Negligible	Large (31-70%)	Negligible (<1%)	High (Continuing)	
6.1	Recreational activities		Negligible	Large (31-70%)	Negligible (<1%)	High (Continuing)	Many populations are subject to recreational waterway users in ATVs, canoes, jet skis and other boats.

Threat		Impact (calculated)		Scope (next 10 Yrs)	Severity (10 Yrs or 3 Gen.)	Timing	Comments
6.2	War, civil unrest & military exercises						
6.3	Work & other activities		Negligible	Small (1-10%)	Negligible (<1%)	High (Continuing)	Includes research activities (e.g. sampling) which will occur in most to all areas. Scope is small and severity negligible because relatively very few individuals and only a small fraction of area will be affected.
7	Natural system modifications		Unknown	Large (31-70%)	Unknown	High (Continuing)	
7.1	Fire & fire suppression						
7.2	Dams & water management/use		Unknown	Large - Restricted (11-70%)	Unknown	High (Continuing)	Severity is unknown because little is known about how unionids respond to these activities and what the actual extent of the impacts is. The spatial footprint of the dam's effects can be large but, again, this is not well-known. While there are not many dams on the Maitland and Sydenham rivers, there are many on the Trent, Moira, Grand and Thames.
7.3	Other ecosystem modifications		Unknown	Large (31-70%)	Unknown	High (Continuing)	Dreissenid mussels (Zebra, Quagga) modify habitat by forming a blanket over lake and river bottoms. In addition to smothering Rainbow, this may change the spawning habitat of the host fish species and may also smother other organisms. The Round Goby's negative effects on host fish species constitute an impact to habitat for glochidia. Removal of riparian vegetation (snag removal) is also a concern. Dreissenids are present in the Trent and Moira systems (although not pervasively). Spread of Round Goby is expected in the Sydenham, Thames and Grand rivers and they are in the Bay of Quinte. The impact of Dreissinids is better known than that of Round Goby. Severity is unknown because the specific impacts are not well known.
8	Invasive & other problematic species & genes	B	High	Large (31-70%)	Extreme - Serious (31-100%)	High - Moderate	
8.1	Invasive non-native/alien species	B	High	Large (31-70%)	Extreme - Serious (31-100%)	High - Moderate	Dreissenid mussels (Zebra, Quagga) may affect Rainbow by attaching directly to them. This can smother, compromise feeding and burrowing, and ultimately kill the Rainbow. Round Goby may eat young Rainbow and may also be a "sink" as young Rainbow can attach to the fish (shown in labs) but do not transform into adults. Timing is variable depending on location hence the range of High - Moderate.

Threat		Impact (calculated)		Scope (next 10 Yrs)	Severity (10 Yrs or 3 Gen.)	Timing	Comments
8.2	Problematic native species		Negligible	Negligible (<1%)	Extreme (71-100%)	High (Continuing)	Raccoons and muskrat numbers increase due to human activity. Severity is extreme because individual Rainbow are killed for food.
8.3	Introduced genetic material						
9	Pollution	BC	High - Medium	Pervasive (71-100%)	Serious - Moderate (11-70%)	High (Continuing)	
9.1	Household sewage & urban waste water	BC	High - Medium	Large (31-70%)	Serious - Moderate (11-70%)	High (Continuing)	Includes or may include road salt, discharge from wastewater plants, chemicals, pharmaceuticals, oil from roads, industrial plants. Upper reaches and areas closer to urban areas and highways are especially affected by road runoff. Eastern Ontario streams are not as badly affected as those in southwestern Ontario.
9.2	Industrial & military effluents	D	Low	Small (1-10%)	Serious (31-70%)	Moderate (Possibly in the short term, < 10 yrs)	Spills from pipelines, rail accidents etc. -- we will know more once we look into crossing and other locations. Possibility of contamination (arsenic, copper) from the DeLoro mine clean up on the Moira River upstream in the Bay of Quinte basin. Uncertainty as to whether these impacts are where the Rainbow are.
9.3	Agricultural & forestry effluents	BC	High - Medium	Pervasive (71-100%)	Serious - Moderate (11-70%)	High (Continuing)	Sedimentation from soil erosion; contamination from fertilizer runoff. Agricultural activities present in all the watersheds. Less severe in Bay of Quinte drainage where some land is no longer in agriculture.
9.4	Garbage & solid waste						
9.5	Air-borne pollutants						
9.6	Excess energy						
10	Geological events						
10.1	Volcanoes						
10.2	Earthquakes/tsunamis						
10.3	Avalanches/landslides						
11	Climate change & severe weather		Not Calculated (outside assessment timeframe)	Large - Restricted (11-70%)	Serious (31-70%)	Low (Possibly in the long term, >10 yrs)	
11.1	Habitat shifting & alteration						See Droughts and Storms sections.

Threat		Impact (calculated)		Scope (next 10 Yrs)	Severity (10 Yrs or 3 Gen.)	Timing	Comments
11.2	Droughts		Not Calculated (outside assessment timeframe)	Large - Restricted (11-70%)	Serious (31-70%)	Low (Possibly in the long term, >10 yrs)	Water levels in Great Lakes have gone down (to be confirmed). Occurs in shallow headwater areas. May be affected but unknown for now. Authors will address.
11.3	Temperature extremes						Species is found in very warm water in some parts of range.
11.4	Storms & flooding		Not Calculated (outside assessment timeframe)	Large - Restricted (11-70%)	Slight (1-10%)	Low (Possibly in the long term, >10 yrs)	Maitland River is prone to flash flooding. See Spooner *et al*. (2008). Increased storms may be likely but there is lots of uncertainty on this. Can get washed down to another location, but mussels, generally speaking, seem to survive flood events.

Classification of Threats adopted from IUCN-CMP, Salafsky *et al*. (2008).