Fisheries and Oceans Canada — Pêches et Océans Canada
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Research Document 2008/083 — **Document de recherche 2008/083**

Pre-COSEWIC assessment of the Wavyrayed Lampmussel (*Lampsilis fasciola*)

Évaluation pré-COSEPAC de la lampsile fasciolée (*Lampsilis fasciola*)

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ISSN 1499-3848 (Printed / Imprimé)

Canada

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ABSTRACT

A regional science peer review meeting was held on November 14, 2008 in Burlington Ontario at the Canada Centre for Inland waters. The purpose of the meeting was to peer-review information relevant to the 10-year update for the original COSEWIC status assessment for the Wavyrayed Lampmussel (*Lampsilis fasciola*) in Canadian waters. This freshwater mussel was originally assessed as Endangered by COSEWIC in 1999 and subsequently added to Schedule 1 under the *Species at Risk Act* in 2003. A draft of this Research Document was presented as a working paper at the workshop and was intended to provide a summary of all available new data related to the status and trends of the species and its habitat as well as threats to this species inside and outside of Canadian waters. A large amount of new data has been collected since the initial COSEWIC assessment in 1999 and considerably more is known about the biology of all life stages and of the threats to the species. Limited trend data suggest that the species is likely expanding its range in the Grand and Thames rivers where it has become a significant component of the mussel community (20 – 50% numerically). A new large population has been confirmed in the Maitland River and a small population still persists in the Ausable River. Intensive efforts in the Sydenham River have failed to detect any live individuals. Reproduction and a range of size/age classes have been confirmed for all extant populations.

RÉSUMÉ

Le 14 novembre 2008, une réunion régionale d'examen scientifique par des pairs a eu lieu à Burlington, en Ontario, au Centre canadien des eaux intérieures. Le but de la réunion était d'effectuer un examen par des pairs de l'information pouvant servir à la mise à jour décennale de la première évaluation, par le COSEPAC, de la situation de la lampsile fasciolée (*Lampsilis fasciola*) dans les eaux canadiennes. Cet anodonte, qui a été considéré comme étant « menacé » dans la première évaluation du COSEPAC, en 1999, a par la suite été inscrit à l'annexe 1 de la *Loi sur les espèces en péril*. Une ébauche du présent document de recherche a été utilisée comme document de travail à l'atelier afin de résumer les nouvelles données disponibles sur la situation de l'espèce et de son habitat et les tendances s'y rapportant ainsi que les menaces pesant sur l'espèce à l'intérieur et à l'extérieur des eaux canadiennes. Une grande quantité de données nouvelles ont été recueillies depuis la première évaluation du COSEPAC, en 1999, et les connaissances ont augmenté considérablement à propos de la biologie et des stades de développement de l'espèce et des menaces pesant sur celle-ci. Selon les données limitées sur les tendances dont on dispose, l'aire de répartition de l'espèce s'agrandirait dans les rivières Grand et Thames, où la lampsile constitue un composant important de la communauté de mollusques (20 à 50 % en nombre). On a observé une importante population de lampsiles dans la rivière Maitland, et une petite population persiste dans la rivière Ausable. Les efforts intensifs entrepris dans la rivière Sydenham afin de trouver des individus vivants n'ont donné aucun résultat. Les populations connues se reproduisent, et on a observé un éventail de classes d'âges et de tailles au sein de celles-ci.

INTRODUCTION

In North America, Wavyrayed Lampmussel (*Lampsilis fasciola*) was found throughout much of the Ohio and Mississippi River systems, the upper Allegheny River, and Lake Ontario, Lake Erie Lake St. Clair and lower Lake Huron as well as their tributaries and connecting channels. There have been substantial declines in the populations of *L. fasciola* in the midwestern United States due in large part to poor water quality and habitat losses. In the lower Great Lakes and their connecting channels, populations of unionids have suffered significant declines due to the impacts of invasive dreissenid mussels (Gillis and Mackie 1994, Schloesser and Nalepa 1994, Nalepa *et al*. 1996, Schloesser *et al*. 2006). In Canada, *L. fasciola* is still extant in the Ausable, Grand, Maitland and Thames rivers and a small remnant of the Great Lakes population survives in the delta area of Lake St. Clair, mainly within the territory of the Walpole Island First Nation (Metcalfe-Smith and McGoldrick 2003) (**Figure 1**).

The Wavyrayed Lampmussel was assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 1999 and assigned a status of Endangered. Subsequent listing to Schedule 1 of Canada's *Species at Risk Act* (SARA) in 2003 instigated the development of a national recovery strategy for this species which was completed in 2006 (Morris 2006). The recovery strategy identified many high priority research activities that were determined to be necessary to fill identified knowledge gaps and to provide a sound scientific basis for managing the future recovery of the species.

Today, many activities identified in the recovery strategy for the Wavyrayed Lampmussel have been acted upon and a plethora of new information is available as we approach the scheduled COSEWIC reassessment of the species in 2010. This document collates all new and relevant information generated since the last assessment; provides a critical scientific review of its quality, and will ensure that the best available information is available to inform the reassessment of *L. fasciola* by COSEWIC.

LIFE HISTORY

Lampsilis fasciola, like all freshwater mussels, is a sedentary animal that buries itself partially or completely in the substrates of lakes, rivers or streams. Adult freshwater mussels are filter-feeders that obtain nourishment by siphoning particles of organic detritus, algae and bacteria from the water column and sediments (Nichols *et al*. 2005). The juveniles of most freshwater mussels live completely buried in the substrate where they feed on similar foods obtained directly from the substrate or from interstitial water (Yeager *et al*. 1994; Gatenby *et al*. 1997).

Life cycle and reproduction

The life cycle of the Wavyrayed Lampmussel is similar to that of all freshwater mussels and is described as follows (adapted from Kat 1984, Watters 1999, and Nedeau *et al*. 2000): 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 a specialized region of the female gills, called marsupia, where they are held until they reach a larval stage called the glochidium. 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 - a process that can last from as little as a few weeks to several months. The juveniles then release themselves from the host and fall to the substrate to begin life as free-living mussels. The proportion of glochidia that survive to the juvenile stage is estimated to be as low as 0.000001%. Mussels overcome the extremely high mortality associated with this life cycle by producing large numbers of glochidia – often more than a million per female. Juvenile mussels are difficult to find because of their small size and because they quickly burrow into the sediment upon release. Juvenile mussels remain buried until they are sexually mature, at which point they move to the surface for the dispersal/intake of gametes (Watters *et al*. 2001).

Wavyrayed Lampmussels are reportedly bradytictic (long-term brooders); that is, they spawn in mid to late summer, brood their glochidia over the winter, and release them in the following summer (Clarke 1981). In a study of the of the Thames and Grand River Wavyrayed Lampmussel populations in 2008, one of the authors (TJM unpubished data) found gravid, displaying females at the substrate surface continually from May 15th through September 24th although abundance showed two distinct peaks (**Figure 2**). These peaks occurred in late May to early June (peak one) and early to late July (peak two) although both peaks occurred earlier in the Grand River population. Females typically appeared in one peak or the other but not both – only 18% of Grand River females and 27% of Thames River females were common to both peaks. In the same study it was found that male abundance at the surface peaked during late June just prior to the second female peak. At this time male abundance was approximately 3 – 4 times higher than at other points during the sampling period. It is likely that males are coming to the surface to release sperm during this time period.

The glochidia are small (mean height = 302 µm, mean length = 246 µm; mean hinge length = 112 µm) and lack hooks indicating they are likely acting as gill parasites (**Figure 3**). McNichols (2007) reported a mean number of glochidia per female of 34,192 with a range of 6075 – 76667. Glochidial hosts for the Wavyrayed Lampmussel in Canada, as identified through laboratory infestation experiments, include: smallmouth bass (*Micropterus dolomieu*), largemouth bass (*M. salmoides*), mottled sculpin (*Cottus bairdi*) and brook stickleback (*Culaea inconstans*) (McNichols *et al*. 2004).

The functional host(s) of *Lampsilis fasciola* in natural settings are most likely visual predators (i.e. bass), as female *L. fasciola* have developed a specialized “lure” which they use to attract suitable hosts and facilitate the infestation of the fish with their glochidia. The authors have observed at least four distinct lure morphs in female *L. fasciola* in Ontario (**Figure 4**). One lure morph is completely black; one is completely red; another effectively mimics a small fish – complete with eyespots, lateral line and tail; and the fourth, termed a flamboyant attractor, is similar to the fish lure but lacks much of the complex pigmentation. All four lure morphs have been observed occurring within single beds in the Grand and Thames rivers (TJM, unpublished data). When a fish strikes the lure the female releases a pulse of glochidia which attach themselves and encyst within the gill tissues of the fish.

Dispersal and migration

Freshwater mussels are basically sedentary as adults, with movement limited to a few metres of the lake or river bottom. The only time that significant dispersal can take place is during the parasitic phase. Infected host fishes can transport larval unionids into new habitats and replenish depleted populations with new individuals. Dispersal is particularly important for genetic exchange between populations (Nedeau *et al*. 2000).

In a study of the relationship between the Wavyrayed Lampmussel and its glochidial host in the upper Grand River, Morris and Granados (2007) determined that *L. fasciola* glochidia occurred at relatively low densities in the drift (mean of < 0.08 animals per m^3) but that their abundance did show a seasonal trend peaking at all three stations between mid July and late August. This pattern of low glochidial abundance in the drift is consistent with the expectations from a species which uses a luring technique. Glochidia of luring species are generally released in close proximity to the host and would not require an extended period of buoyancy in the water column to permit host attachment.

Morris and Granados (2007) also examined glochidial infestation rates on smallmouth bass captured over beds containing Wavyrayed Lampmussels and reported relatively high infestation rates. Thirty-four percent of all smallmouth bass captured during the study showed signs of glochidial infestation with infestation rates ranging from 1 to 196 glochidia per fish. These authors also sampled larger smallmouth targeted by anglers during the Optimist Club Bass Derby and showed an infestation rate of 47% (Morris and Granados 2007).

HABITAT

Biological

The parasitic life stage of *Lampsilis fasciola* is an obligate step in the life cycle of the species, and successful reproduction will not occur in the absence of suitable host fishes. Recent work has shown that largemouth bass can acquire immunity to the glochidia of a closely related species, *Lampsilis siliquoidea*, after repetitive infestations (O'Dee and Watters 2005). Furthermore, this host-acquired resistance to glochidial infestation can extend across mussel genera (Dodd *et al*. 2005). Implications of these studies on the successful reproduction of *Lampsilis fasciola* are profound. Individual largemouth or smallmouth bass may become less suitable as hosts with each repeated infestation, regardless of which species of mussel is first to infest them. In river reaches with depauperate populations of largemouth and/or smallmouth bass, the competition for naive hosts may be a significant factor limiting the reproduction of *L. fasciola*. As such, healthy and recruiting populations of largemouth and/or smallmouth bass are crucial "habitat" for the larval stage of *Lampsilis fasciola*.

Physiochemical

<u>Physical Habitat</u>

Adult *Lampsilis fasciola* inhabit clear, hydrologically stable streams and rivers. They are typically found in and around riffle/run areas in sand/gravel substrates that are stabilized by larger material (rubble or boulder) which represent an average of approximately 40% of the substrate (Metcalfe-Smith *et al*. unpublished data). In the U.S., Wavyrayed Lampmussels are most abundant in small (2^{nd} to 4^{th} order) to medium-sized (5^{th} to 7^{th} order) streams (Dennis 1984). The last known population remaining in the Canadian waters of the Laurentian Great Lakes inhabits the shallow sand flats of the Lake St. Clair delta (Metcalfe-Smith *et al*. 2004).

The physical habitat preferences of juvenile mussels are poorly understood, due to their habit of remaining completely buried in the substrate until they reach several years of age. It is not conclusively known if adult and juvenile *Lampsilis fasciola* share the same physical habitats however it is clear that sampling methods employed to detect adults do periodically detect juveniles in the same habitats. *see **Population size and structure**).

Water Quality

Certain life-history characteristics of freshwater mussels make them particularly sensitive to water and sediment pollution in rivers: they live in close association with sediments and they obtain food principally by filter-feeding. Juvenile mussels spend their early years completely buried in the substrate where they feed on particles associated with sediments and pore water (Yeager *et al*. 1994; Gatenby *et al*. 1997). Consequently, all life stages of *Lampsilis fasciola* are exposed to contaminants that are dissolved in the water, associated with suspended particles and deposited in sediments.

Chemical Contaminants

The majority of the data described below are derived from controlled laboratory studies. These studies typically focus on the sensitivity of an organism to one contaminant at a time and although these studies are critical to the derivation of water quality criteria for an individual chemicals or stressor, they do not necessarily indicate how an organism would respond to the simultaneous exposure of multiple stressors as is often the situation in the natural environment. In order to fully understand the threat that complex mixtures of environmental contaminants pose to *L. fasciola* further field-based studies are required.

i) Ammonia and Copper

While freshwater mussels, as a group, appear to be sensitive to poor water quality, two contaminants, ammonia and copper stand out as being particularly concerning for the sensitive early life stages (glochidia and juveniles). A number of studies have shown that *L. fasciola,* and most freshwater mussels, are very sensitive to ammonia and copper (Jacobsen *et al*. 1997; Mummert *et al*. 2003; Wang *et al*. 2007; Gillis *et al*. 2008). In fact, Gillis *et al.* (2008) reported that glochidia from the six endangered species tested (including *L. fasciola*) were significantly more sensitive to copper than the three common species tested. Furthermore, Augspurger *et al.* (2007) suggested that ammonia should be considered among the factors that may be limiting the survival and recovery of freshwater mussels. Glochidia and newly transformed juveniles (including *L. fasciola*) have been shown to be more sensitive to copper and ammonia than routinely tested aquatic organisms such as *Daphnia magna* (cladoceran) and *Pimephales promelas* (fathead minnow) (Wang *et al.* 2007). This is important because toxicity data from the routinely tested organisms were used to derive water quality regulations before the majority of the early life stage data were available. A number of studies have questioned whether current North American water quality guidelines for copper (Wang *et al*. 2007; March *et al.* 2007; Gillis *et al.* 2008) and ammonia (Augspurger *et al.* 2007) will adequately protect freshwater mussels. Augspurger *et al.* (2007) and March *et al*. (2007) both concluded that the current U.S. Environmental Protection Agency (USEPA) criteria for these contaminants would be lower if the recently published data had been included in their derivation. Now that standardized test methods (ASTM 2006) are available to assess sensitivity of the early life stages of freshwater mussels to waterborne contaminants, any future revisions of the water quality guidelines for copper and ammonia would include these data and thus should result in guidelines and criteria which will better protect freshwater mussels. A summary of the published copper and ammonia toxicity data for *L. fasciola* along with the current water quality guidelines for the protection of aquatic life are presented in **Table 1**.

It is important to note that water chemistry has a significant effect on metal sensitivity of aquatic organisms. Gillis *et al*. (2008) found that *L. fasciola* glochidia were significantly more sensitive to copper (suffered mortality at a lower concentration) when they were exposed to copper in soft water compared to hard water. Also, *L. fasciola* glochidia survived higher concentrations of copper in water with elevated levels of dissolved organic carbon. These results indicate that the risk of toxic copper exposure will vary significantly with the water composition of a mussel's habitat. For instance, *L. fasciola* which inhabit soft water with low dissolved organic carbon would be the most vulnerable to acute copper toxicity.

ii) Pesticides

A number of studies have investigated the sensitivity of freshwater mussels to pesticides. Bringhoff *et al*. (2007) examined the toxicity of technical grade atrazine, pendimethalin, fipronil, and permethrin to five species of glochidia, including *L. fasciola*. Although they found that the relative risk associated with acute exposure of early life stages to these pesticides is likely low, the decreased growth and survival observed during chronic (21 days) exposures with juvenile *L. siliquoidea* indicate that long term exposure to high concentrations (3.8 mg/L) of atrazine may have the potential to impact mussel populations. Milam *et al*. (2005) also investigated the toxicity of pesticides to six species of glochidia and although they did not specifically test *L. fasciola*, they concluded that the risk of acutely toxic exposures of carbaryl, 4-nonylphenol, permethrin, 2,4-D, and pentachlorophenol to freshwater mussels in the natural environment is relatively low. Bringhoff *et al*. (2007) also examined the toxicity of fungicides (chlorothalonil, propiconazole, and pyraclostrobin) to *L. siliquoidea* glochidia and found that while glochidia were extremely sensitive to these chemicals, they experienced acute toxicity at concentrations similar to other commonly tested aquatic organisms. Because the early life stages of unionids are not uniform in their sensitivity to pesticides and sensitivity is species- and chemical- (or possibly chemical class) specific (Bringhoff *et al*. 2007), caution should be used when extrapolating the sensitivity of one species of mussel or chemical to another.

iii) Fluoride

Keller and Augspuger (2005) tested the sensitivity of glochidia and juvenile mussels to fluoride. They found *L. fasciola* juveniles had an LC50 (172 mg/L) which was comparable to the other two species tested, but based on measured fluoride concentrations in a fluoride impacted stream (1.5-8 mg/L), they concluded that acute fluoride toxicity in the natural environment was unlikely.

iv) Emerging Contaminants (Nanoparticles, Municipal Effluents, Road Salt)

Gangé *et al*. (2007) reported that cadmium nanoparticles (cadmium–telluride) were immunotoxic to adult *Elliptio complanata* and led to oxidative stress in gills and DNA damage. The effect of nanoparticles on other life stages or species of mussels is unknown.

Exposure to municipal effluents has also been shown to negatively affect the health of freshwater mussels. Gangé *et al*. (2004) demonstrated that *Elliptio complanata* caged downstream of a municipal outflow for one year displayed a complex but characteristic pattern of responses that could lead to harmful health effects including neuroendocrine disruption of reproduction. They suggested that mussels were likely exposed to estrogenic chemicals in the effluent plume. Similarly, Gangon *et al*. (2006) reported that *Elliptio complanata* caged downstream of a municipal effluent for 90 days accumulated metals from both waterborne and dietary sources. Both studies found that the exposed mussels exhibited numerous biomarkers of toxic stress (Gangé *et al*. 2004; Gangon *et al*. 2006). Since many of the Canadian endangered freshwater mussels inhabit urbanized rivers which receive input from municipal treatment plants, further investigation is required to fully understand the effect of this exposure. Of particular concern for *L. fasciola* is the Grand River, a river which receives effluent from more than twenty municipal treatment plants.

Gillis (unpublished data) found that glochidia, including those of *L. fasciola* are very sensitive to chloride (Cl) salts (*L. fasciola* LC50, 100 mg Cl/L). Although Canada does not currently have a water quality guideline for chloride, neither the USEPA criteria (230 mg/L) nor the British Columbia Environment guideline (600 mg/L) would protect *L. fasciola* glochidia from acute chloride toxicity. The concentration of chloride in North American rivers has been shown to be correlated with the percentage of impermeable surfaces in the watershed (Kaushal *et al.* 2005). Therefore the increased salinization of freshwater due to application of road salt may be of particular concern for Canada's endangered freshwater mussels whose ranges are limited to streams and rivers in road dense Southern Ontario.

Water Quality and Composition

i) Dissolved Oxygen

Low dissolve oxygen (DO) events usually result from spills of organic material (e.g., agricultural wastes and untreated sewage) and can kill fish and mussels for several kilometers downstream. A spill of agricultural waste into Big Darby Creek, Ohio in 2000 resulted in an event where levels of DO approached zero and remained low for a period of 1 week (Tetzloff 2001). Thousands of fish and mussels were killed as a result of this spill. Most species of mussels showed some response to the event, but the rates of survival among species varied considerably. Almost all individuals of some species, such as *Amblema plicata* and *Fusconaia flava*, survived the event but *Lampsilis fasciola* was amongst the most sensitive of the 18 species present, only 5% of the *Lampsilis fasciola* survived the event.

ii) Phosphorus, Nitrates/Nitrites, Turbidity

Most contaminants do not have published toxicological information available for freshwater mussels, yet many of these contaminants may have deleterious effects on *Lampsilis fasciola*. We conducted water quality sampling at 66 sites in the Ausable, Grand, Maitland, Sydenham and Thames rivers over a two week period in mid-September, 1998. Each of these sites had been surveyed for mussels in 1997 or 1998. In 2004, we repeated the water quality sampling at 16 of these sites and sampled an additional 20 sites in the same rivers that were found to support live *L. fasciola* during mussel surveys conducted between 1998 and 2004. A principal component analysis using several water quality parameters along with Wavyrayed Lampmussel abundance as variables was conducted for both sampling events (**Figures 5 and 6**). The results for each analysis were similar and showed that the abundance of *L. fasciola* at a site was negatively correlated with the concentration of total phosphorus (TP), nitrates/nitrites, total Kjeldahl nitrogen (TKN), and turbidity. *Lampsilis fasciola* were not found alive at sites with TP concentrations greater than 0.10 mg/L and were most abundant at sites with concentrations less than 0.05 mg/L (**Figure 7**). Wavyrayed Lampmussels were also more abundant at sites with nitrate/nitrite concentrations of less than 3 mg/L (**Figure 8**), and live animals were not found at sites with turbidity levels greater than 8 JTU (**Figure 9**). Increased concentrations of nutrients in the form of phosphorus and nitrogen compounds contribute to higher levels of turbidity in aquatic systems and it is likely that the impacts of these contaminants on *L. fasciola* are linked. The water quality guideline to prevent excessive algal growth in flowing waters is

0.03 mg/L TP (PWQO 2005), which should be sufficient to protect *L. fasciola*. There are currently no guidelines for nitrate/nitrite or turbidity in Ontario waters.

iv) Potassium

Another naturally occurring but potentially toxic metal in Ontario rivers that receives little attention (in terms of the protection of aquatic life) is potassium (K). Imlay (1973) observed that only 2 of 10 rivers in the United States with baseline K concentrations of greater than 4 mg/L supported freshwater mussels whereas 28 of 39 rivers with levels less than 4 mg/L were found to support mussels. He confirmed this relationship through laboratory exposures and found that concentrations of 11 mg/L were lethal to 90% of the adult mussels after exposures of 36-52 days. Preliminary data (Gillis *et al.* unpublished) indicates that the early life stages of freshwater mussels are also very sensitivity to K. The 24 h LC50 for *L. fasciola* glochidia was 10 mg K/L (Gillis *et al.* unpublished). Our observation that *L. fasciola* are not abundant at sites with K concentrations greater than 6 mg/L (**Figure 10**) is consistent with these findings. These laboratory studies and field observations suggest that levels of K in Ontario waters (1-55 mg/L, Ontario Ministry of the Environment, 2008) may indeed be a threat to the recovery of freshwater mussels including *L. fasciola*. There are currently no water quality guidelines for potassium concentrations in rivers or lakes in Ontario.

Habitat Trends in Ontario

Host population dynamics may have contributed to the observed changes in Wavyrayed Lampmussel populations, particularly in the Thames and Sydenham rivers. In all watersheds where the Wavyrayed Lampmussel is found smallmouth bass are numerically dominant to largemouth and likely function as the primary functional host species. Smallmouth populations in the Maitland River are believed to be healthy and stable (pers. comm. D. Kenny, Maitland Valley Conservation Authority); populations in the Ausable River are also healthy and at their highest abundance in the stretch of river currently occupied by the Wavyrayed Lampmussel (pers. comm. K. Killins, Ausable Bayfield Conservation Authority). In the Sydenham River, smallmouth populations are only considered to be fair but have remained relatively stable over the last 20 years (pers. comm. P. Hunter, Ontario Ministry of Natural Resources (OMNR)). Poos (2004) reported very low capture rates for smallmouth bass from the Sydenham River. In the upper Thames River, where Wavyrayed Lampmussels are thriving, smallmouth bass populations have improved from good to robust over the last 20 years, particularly in the North Thames River upstream of Fanshawe Reservoir (pers. comm. P. Hunter, OMNR). The upper Grand River supports a healthy smallmouth bass population (pers. comm.. W. Yerex, Grand River Conservation Authority).

Intense agricultural activity in the Ausable, Grand, Thames and Maitland Rivers have increased sediment and nutrient loading, and the practice of systematically installing drainage tile in crop fields has altered the hydrological regime in these watersheds. The increase in turbidity and decrease in stable silt-free riffle/run habitats associated with these impacts have reduced the quantity and quality of physical habitat available to *Lampsilis fasciola* across its range in Ontario. The habitat quality in the Lake St. Clair delta in areas still occupied by *L. fasciola* is poor due to the impacts of dreissenid mussels.

Water quality data collected since 1965 in the Ausable River watershed show that TP levels are consistently above the water quality guidelines and have decreased only marginally over the past 35 years. Nitrate levels currently exceed federal guidelines for the prevention of eutrophication and the protection of aquatic life and are slowly rising (Nelson *et al*. 2003). This trend of decreasing concentrations of TP and increasing concentrations of nitrates/nitrites is also evident in long term monitoring data from the Maitland River (Malhiot pers. comm. 2004), the Grand River (GRCA 1998) and the Thames River (Taylor *et al*. 2004).

In the Grand and Thames Rivers, concentrations of TP, nitrates/nitrites and turbidity exceed the tolerance thresholds for *L. fasciola* (see previous section) more often in areas that were historically occupied by the species than in areas that currently support viable populations (**Table 2**). The general observance of elevated nitrate/nitrite concentrations in southwestern Ontario surface waters may be negatively impacting the suitability of habitat currently occupied by *L. fasciola*. However, an analysis of trends in concentrations of several key components (chloride, copper, potassium, nitrates and phosphorus) indicate that recent conditions may be stabilizing. Tables 3-7 provide watershed based comparisons for these components over two time periods corresponding to the 10 year period (approximately 1 generation) prior to the initial COSEWIC assessment (1988-1998) and the 10 year period between the initial COSEWIC assessment and the present time (1999-2008). Phosphorus and copper levels are stable to declining in all watersheds while nitrates are stable in the Maitland, Ausable and Sydenham rivers but increasing slightly in the Grand and Thames rivers. Chloride values appear to be increasing in all watersheds except the Thames.

CURRENT DISTRIBUTION AND ABUNDANCES IN ONTARIO

Survey methods

Surveys conducted between 1993 and 2008 within the range of *Lampsilis fasciola* in Ontario have been either semi-quantitative (timed-searches) or quantitative (quadrat surveys). The same sampling methods were used throughout and are described below.

Timed-searches surveys:

Since 1997, surveys in rivers were conducted using an intensive timed-search technique developed by Janice Metcalfe-Smith and her team for detecting rare species of mussels. The technique is described in its entirety in Metcalfe-Smith *et al*. (2000b). Briefly, the riverbed is visually searched by a team of 3 or more persons using waders, polarized sunglasses, and underwater viewers for a total of 4.5 person-hours (p-h) 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 100-300 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.

Surveys conducted prior to 1997 followed a protocol similar to the one described in the previous paragraph, but the amount of search effort (i.e. the time spent searching for mussels) varied considerably among surveyors.

Quantitative surveys:

Surveys in rivers employed an intensive quantitative sampling technique that would allow the generation of precise estimates of demographic variables such as density, size class frequencies and recruitment levels. Strayer and Smith (2003) describe this technique, which is summarized below:

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% of the 400 m^2 area was sampled at each site. Each quadrat was searched until all live mussels had been recovered. 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 and recorded. Quantitative surveys have now been conducted on all riverine populations.

Quantitative surveys were also conducted in the delta area of Lake St. Clair using a different protocol. 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 they encountered a live mussel. A stake was planted at this point and a circular area of 65 m^2 surrounding the point was searched. All live mussels within the area were collected. 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).

An additional source of quantitative data results from recent relocation efforts related to development projects within the range of the Wavyrayed Lampmussel. Since Canada's *Species at Risk Act* came into force in 2003 it has been illegal to kill or harm this species. As a result of these prohibitions, activities (bridge crossings, pipeline installations, dredging operations) proposed within the known range of the species must ensure that Wavyrayed Lampmussels are not negatively impacted. To achieve this end, Fisheries and Oceans Canada has developed a protocol (Mackie *et al*. 2008) for undertaking relocations of listed mussel species when deemed appropriate. When followed, this protocol produces data equivalent to that resulting from the quantitative surveys outlined above. To date there have been six relocations undertaken within the range of the Wavyrayed Lampmussel (citations). Of these six, two have resulted in relocations of significant numbers (13-295) of Wavyrayed Lampmussels, both in the Grand River.

Distribution and area of occupancy in Ontario

The following descriptions of the distribution of *Lampsilis fasciola* for each waterbody are based on the occurrence of live animals in surveys conducted by the authors and their colleagues since 1993. The upper and lower bounds of the area of occupancy described for each waterbody were determined to be sites where *L. fasciola* was not found alive immediately upstream or downstream of sites where it was found alive. The lengths of the occupied area for each watershed were determined using ArcView GIS v.3.3.

<u>Ausable River</u>

The current distribution of *Lampsilis fasciola* in the Ausable River is limited to the bottom 3 km of the Little Ausable River and 84 km of the main stem from Brinsley to Nairn. The average width of the river along both reaches is 7.5 m; thus, the area of occupancy (AO) for *L. fasciola* in the Ausable River watershed is ~ 0.7 km^2 (**Table 3**).

Grand River

In the Grand River watershed, *Lampsilis fasciola* occurs along 77 km of the main stem from Inverhaugh (north of Waterloo) downstream to Glen Morris (south of Cambridge). Live animals were found at every site surveyed within this section of the river. *Lampsilis fasciola* is also found in three tributaries of the Grand River; i.e., in the lower 11 km of the Conestogo River from St. Jacobs to the confluence with the Grand River; in a 30 km stretch of the Nith River between Drumbo and the confluence with the Grand and in the lower portion of the Speed River below Highway 8. The average river width at sites occupied by *L. fasciola* is 63 m; therefore, the AO for *L. fasciola* in the Grand River watershed is ~ 7.5 km^2 (**Table 3**).

Maitland River

Lampsilis fasciola occurs in all 4 branches of the Maitland River watershed. In the Middle Maitland River it is found alive along 23 km of the river from the junction of Morris Rd. (County Rd. 16) and Clyde Line to the confluence with the main stem in Wingham. It also occurs in 15 km of the Little Maitland River from Jamestown to the confluence with the Middle Maitland River just south of Wingham. In the main stem, *L. fasciola* occurs from Wingham downstream to Benmiller (54 km) and in the South Maitland River the species occurs from Londesborough to the confluence with the main stem (10 km). The average river width at sites supporting live *L. fasciola* is 31 m, so the AO is ~ 3.2 km^2 (**Table 3**).

Thames River

Lampsilis fasciola occurs in the North, South and Middle Thames Rivers upstream of the City of London. In the North Thames River *L. fasciola* is found along 34 km of river starting near Motherwell extending downstream to the Fanshawe Lake reservoir. The species is also found in two tributaries (Fish and Medway creeks) of the North Thames River. In Fish Creek, it is found from RR #151 to the confluence with the North Thames River and in Medway Creek from Fanshawe Park Rd downstream to the confluence with the North Thames. In the Middle Thames River *L. fasciola* can be found from just upstream of Thamesford through to the confluence then along the South Thames River to Airport Rd. in the City of London, a reach of river spanning 44 km. The average river width at sites with live *L. fasciola* is 32 m in the Thames River watershed; thus, the AO for *L. fasciola* is ~ 2.5 km^2 (**Table 3**).

Sydenham River

Lampsilis fasciola has not been found alive in the Sydenham River since 1971, despite more than 600 person-hours of search effort conducted by the authors and their associates from 1997 to 2004. *Lampsilis fasciola* historically occurred in 42 km of the middle reach of the East Sydenham River from Rokeby downstream to Florence.

Lake St. Clair

The delta area of Lake St. Clair may support the most intact freshwater mussel community remaining in the lower Great Lakes and their connecting channels (Zanatta *et al*. 2002; McGoldrick *et al*. in press). *Lampsilis fasciola* continues to persist in 12 km^2 of the shallow nearshore areas of the delta within the territory of the Walpole Island First Nation; however, 12 km^2 is not an appropriate AO for *L. fasciola* because it was only found at a few sites. The AO was therefore calculated as follows using data from Metcalfe-Smith *et al*. (2004): the total area of lake bottom searched at the 9 sites surveyed was 14,560 m^2. *Lampsilis fasciola* was found at 4 sites where the total area searched was 6,760 m^2, or about 46% of the total area searched. Assuming that these sampling sites are representative of the entire area of habitat, *L. fasciola* occupies 46% of the area or 5.5 km^2 (**Table 4**).

Population size and structure

Abundance

Quantitative surveys for freshwater mussels in Ontario have now been conducted in all localities where *Lampsilis fasciola* currently occurs or was historically known. These quantitative surveys include targeted efforts designed to monitor recovery (Sydenham River (2001-2004) (Metcalfe-Smith *et al*. 2007); Thames River (2004) (TJM, unpublished data); Ausable River (2006-2008) (S. Staton, Fisheries and Oceans Canada, unpublished data); Grand River 2007; Maitland River 2008 (TJM, unpublished data)) and one-off events associated with mussel relocations arising from development activities (e.g., Grand River (Mackie 2008)). All quantitative efforts involved complete census of the mussel community and population estimates derived from these efforts should be considered free of the size and sex bias commonly associated with timed-searches. Population estimates derived from these quantitative sampling efforts indicate that the Grand River supports the largest remaining population in Canada while the Thames and Maitland river populations are similar to one another but an order of magnitude smaller than the Grand River. The Ausable River and St. Clair delta still support remnant populations two to three orders of magnitude smaller than the Grand River. (**Table 4**).

Since 1997, semi-quantitative surveys have been conducted in the Ausable, Grand, Maitland and Thames Rivers using the same timed-search method described previously. As method and effort were consistent in these surveys we can compare the relative abundance based on the Catch per Unit Effort (CPUE) of *L. fasciola* in these 4 watersheds (**Table 3**). The largest population of Wavyrayed Lampmussels occurs in the Grand River (CPUE = 0.37) followed by the Thames (CPUE = 0.30) and Maitland Rivers (CPUE = 0.22). Only 2 live animals were found in these surveys of the Ausable River and the Lake St. Clair delta supports a small and sparse population (density = 0.0006 animals/m^2). It is not clear whether the populations in the Ausable River and Lake St. Clair are viable in their present condition.

Abundance Trends

There has been limited repeated sampling at sites within the range of the Wavyrayed Lampmussel using methods that make abundance comparisons possible. However there are two sites along the North Thames River for which comparisons can be readily made (**Table 5**). Morris (1996) sampled a site where Elginfield Road crosses the North Thames River in 1995 employing an effort of 1 p-h and again in 2004 (Morris and Edwards 2007) with an effort of 4.5 p-h. The 1995 sampling event produced no evidence (live animals or shells) of *L. fasciola* whereas the 2004 sampling detected 15 individuals (CPUE = 3.33). Morris (unpublished data) also conducted quantitative sampling at the site in 2004 producing a density estimate of 0.12 animals/m^2 (includes burrowed and unburrowed animals). In 2008, Morris again visited this site, to undertake a study of gravid female Wavyrayed Lampmussels (data contained in Figure 1), and examined an area of 444 m^2 adjacent to the location sampled in 2004 detecting 136 animals for a density of 0.31 animals/m^2 (does not include burrowed animals).

Multiple years of data also exist for a site on the North Thames at Plover Mills. Metcalfe-Smith (Environment Canada, unpublished data contained in the Lower Great Lakes Unionid Database) surveyed the site in 1998 using an effort of 4.5 p-h and detected a single animal (CPUE = 0.22). When the site was revisited in 2008 (T. Morris and D. Woolnough, Fisheries and Oceans Canada/Central Michigan University, unpublished data) 14 live Wavyrayed Lampmussels were detected (CPUE = 2.8) (**Table 5**).

Although no directly comparable surveys have been conducted in other watersheds it is possible to make some assessment of trends within the Grand River where much additional work has been conducted in recent years. Metcalfe-Smith *et al*. (2000) surveyed a site on the Grand River in Kitchener near Doon in 1998 and found 8 live Wavyrayed Lampmussels (CPUE = 1.77). Morris (unpublished data) undertook quantitative sampling at this same site in 2007 and found 46 animals in an area of 63 m^2 (density = 0.73/m^2) making it the most abundant species at the site. In 2008 Morris sampled an adjacent patch at this site as part of his gravid study and found 87 animals in an area of 450 m^2 (density = 0.19/m^2). Given that this 2008 estimate represents only animals that were detected at the surface it is likely comparable to the overall estimate reported for 2007. The locations of both the 2007 and 2008 work were contained within the survey area of Metcalfe-Smith (2000).

Size and Age Class Distribution

Length frequency distributions, based on maximum shell length, are provided by sex for *Lampsilis fasciola* from the Grand River (**Figure 11**) and from the Thames River (**Figure 12**). For each of the populations, animals from a wide range of size classes are represented and the shell lengths appear to be approximately normally distributed. Length frequency distributions have not been presented for the remaining populations as the small sample sizes make interpretation difficult. However, even for these small populations, samples have produced individuals of multiple size classes indicating recent reproduction (Table 6). Even though the St. Clair samples do not include excavation which would typically produce representatives of the smaller size classes the length frequency distribution for *L. fasciola* from Lake St. Clair is shifted to the left in comparison to the other riverine populations (**Table 6**). This is likely because freshwater mussels tend to grow rounder and shorter in sheltered areas such as lakes and reservoirs than in moving water (Green 1972; Bailey & Green 1988).

One of the authors (TJM, unpublished data) has developed length-at-age curves for *L. fasciola* males and females from the Grand and Thames rivers following the methods of Neves and Moyer (1988). We present here age distributions derived from these curves for both populations (**Figures 13 & 14**). It can be seen from these figures that the age structure differs between the two populations. Although both populations follow a skewed normal distribution the median age of Thames River animals is approximately 4 years older than the Grand River population. In general there is a wider age distribution within the Thames River animals however the maximum ages are similar in both watersheds. In both rivers it appears that females can be readily discerned from males by about 3-4 years of age. Since the shell morphology which allows this distinction of the sexes is a direct result of the brooding behaviour of the female this suggests that females are reproductively active by this time.

COSEWIC (2006) defines the generation time of a population as the average age of parents within the population. Applying this definition to the Thames and Grand river populations yields two distinct generation times. The Grand River population can be described by a generation time of 6.3 years whereas the Thames population has a generation time of 10.4 years.

Sex Ratios

Sex ratios (male:female) are presented for data collected during timed-searches (**Table 7**) and for collections made during quantitative quadrat excavation studies (**Table 8**). Ratios derived from timed-search data should be interpreted with caution. These collections are known to be biased by animal size (juveniles are rarely detected), vertical position in the substrate (burrowed vs. unburrowed) and other features which might make one sex more obvious to the observer (i.e. the lure attractants of female Wavyrayed Lampmussels). During quantitative surveys of the Grand and Maitland rivers in 2007-2008 one of the authors (TJM, unpublished data) recorded the vertical position of each Wavyrayed Lampmussel (surface vs. burrowed) and determined that 18% of animals in the Grand River and 17% of animals in the Maitland River were at the surface during the sampling period (August). These data indicate that timed-searches are likely missing 4-5 times as many animals as they are detecting and, given the differences in vertical distributions of the sexes (Figure 1), likely missing more males than females. We present them here only because we have much more data for timed-searches than quadrat excavations and because we do not have true excavation data for the St. Clair delta population. In an attempt to minimize the potential biases of timed searches we have included the sex ratio of shells of *L. fasciola* that were collected while conducting the surveys since there would not be a detection bias based on behavioral differences between males and females.

In contrast, sex ratios derived from quantitative quadrat excavation data are believed to be highly representative of the true ratio at the site as capture rates are unbiased. For all populations where quantitative data are available the sex ratios appear nearly balanced indicating a healthy population. This is true even though the total sample sizes are relatively small (e.g., n = 24 in the Maitland River). For the St. Clair delta population where excavation data is lacking the sex ratio still remains relatively close to balanced (0.85).

Phylogenetic systematics and genetic population structure

Lampsilis faciola belongs to the diverse clade of North American unionoids called the Lampilini. In molecular phylogenetics have shown that *L. fasciola* is most closely related to “true” *Lampsilis* (including *L. cardium*, *L. ovata*, and *L. ornata*), forming a well resolved and supported clade (Zanatta and Murphy 2006).

In a population-level molecular analysis, the polymorphic mantle displays in *L. fasciola* were found to be genetically indistinguishable using a suite of microsatellite loci, their diversity was correlated with genetic diversity (Zanatta *et al.* 2007). This absence of some display morphologies in Ontario could be a reflection of the low number of specimens collected from some localities. Further molecular analysis may be required to determine how these polymorphisms occur. In managing populations of *L. fasciola* for propagation, augmentation, and translocation, Zanatta *et al.* (2007) recommended that polymorphic lures be represented in approximate proportions to what is observed in wild populations.

Moderate to high gene flow appeared to have been recently occurring between all of the sampling localities (**Figure 15**; Zanatta *et al.,* 2007). Within drainage gene flow was highest and sampling localities within the Ontario drainages displayed panmixia. The relatively recent construction of impoundments on the Grand and Thames rivers and the introduction of dreissenid mussels have further isolated the remaining populations in Canada. As such, many of the intervening riverine and lacustrine habitats are now inhospitable to *L. fasciola*. Although not detectable today, this will ultimately lead to ever-increasing genetic divergence and isolation due to drift (Zanatta *et al.* 2007).

Populations of *L. fasciola* in the Thames River (North Thames and Middle Thames) showed limited evidence (significance under two of four models) of a recent genetic bottleneck (Zanatta *et al.* 2007). This could be evidence of a rapid decline of *L. faciola* in the Thames River drainage to very small numbers, followed by a recovery in the number of individuals. Although historical data are not available in the Thames River, the mussel populations in the Grand River have shown evidence of recovery in recent decades (Metcalfe-Smith *et al.* 2000). Like recovery of unionids in the Grand River, a possible recovery of *L. fasciola* after a genetic bottleneck in the Thames River could be attributed to improvements in water quality in recent decades.

The importance of maintaining genetic diversity is well recognized. Because Canadian populations of *L. fasciola* are in the same geographic area (lower Great Lakes drainage) facing similar threats to their status, they likely do not merit listing by COSEWIC as separate designatable units (COSEWIC 2005; Green 2005). However, based on moderate F_{ST} values, moderately high genetic distances (**Figure 16**), and nearly no misclassification between drainages in the assignment test, Zanatta *et al.* (2007) recommended that populations in each of the drainages sampled in Ontario be treated as separate management units (MU; *sensu* Moritz 1994) by managers responsible for their conservation.

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Table 1. Toxicity of ammonia and copper to the glochidia and juveniles of *Lampsilis fasciola*.

Contaminant	Life stage tested	Result	Source	Water Quality Guidelines
Total Ammonia (as mg/L NH_3)	Glochidia	24h EC50: 4-6 mg/L[1]	[1]Wang *et al*. (2007) (20°C at pH 8)	CCME[a]: 1 mg/L (10°C at pH 8) 0.5 mg/L (20°C at pH 8)
	Juvenile	48h EC50: 6-9 mg/L[1]	[2]Mummert *et al*. (2003)[b]	10 mg/L (10°C at pH 7) 5 mg/L (20°C at pH 7)
		96h LC50: 7.2 mg/L[2] (12°C,pH 7.8) 10.9 mg/L[2] (21°C, pH 8)		
		4d EC50: 7.4 mg/L[1]		PWQO: N/A
		10d EC50: 1.7 mg/L[1]		
Copper	Glochidia	24h LC50: 26-48 µg/L[1]	[1]Jacobsen *et al*. (1997)	CCME: 2-4 µg/L
		24h EC50: 16-18 µg/L[2] 18 µg/L[3]	[2]Wang *et al*. (2007)	PWQO[c]: 5 µg/L
		48h EC50: 7 µg/L[2] 12 µg/L[3]	[3]Gillis *et al*. (2008)	
	Juvenile	10d EC50: 5-7 µg/L[2]		

[a] Canadian Council of Ministers of the Environment Guideline (CCME) for total ammonia is temperature and pH dependant. For full listing of the ammonia guidelines see CCME (2000).
[b] The Mummert *et al*. (2003) data was normalized to pH 8 and expressed as total ammonia (by Augspurger *et al.* 2007).
[c] Ontario Provincial Water Quality Objectives (PWQO 2005)

Table 2. Trends in several water quality parameters in the Thames and Grand Rivers

		% exceedence of threshold since 1990[1]				
		Cu (4.7 µg/L)[2]	TP (0.03 mg/L)[3]	NO_3/NO_2 (3 mg/L)[4]	Turbidity (8 JTU)[4]	K (6 mg/L)[4]
Thames River	IN[a]	3%	85%	78%	31%	15%
	OUT[b]	28%	99.5%	85%	52%	8%
Grand River	IN	0.9%	71%	31%	28%	0%
	OUT	5%	96%	49%	54%	0%

[1] Percentage of samples analysed since 1990 that were greater than the identified concentration for each parameter. Data from the Ontario Provincial Water Quality Monitoring Network

[2] EC50 value obtained from C. Ingersoll (unpublished data)

[3] Ontario Provincial Water Quality Objective

[4] Threshold concentration identified in this report

[a] Samples from sites in the current occupied range of *Lampsilis fasciola* in each watershed

[b] Samples from sites outside the current occupied range of *Lampsilis fasciola* in each watershed

Table 3. Comparison of selected water quality parameters within the reaches occupied by *Lampsilis fasciola* in the Thames River watershed. Results of ANOVA comparing the two time periods (1988-1998 & 1999-2008) are included.

	1988-1998			1999-2008			ANOVA result
Parameter	# Stations	# Samples	Median (quartile range)	# Stations	# Samples	Median (quartile range)	F-ratio (p-value)
Chloride (mg/L)	6	531	42.6 (33.3 - 60.1)	5	168	42.2 (34.0 - 58.7)	0.02 (0.901)
Copper (ug/L)	5	454	2.0 (1.2 - 2.9)	5	147	1.3 (0.97 – 1.7)	45.3 (<0.001)
Potassium (mg/L)	2	19	3.9 (3.0 – 5.1)	5	131	3.7 (3.1 – 4.7)	2.3 (0.132)
Nitrates (mg/L)	6	564	5.4 (3.1 – 7.4)	5	167	6.2 (4.1 – 8.5)	6.1 (0.014)
Total Phosphorus (mg/L)	6	531	0.065 (0.043 – 0.106)	5	168	0.045 (0.029 – 0.084)	12.4 (<0.001)

Table 4. Comparison of selected water quality parameters within the reaches occupied by *Lampsilis fasciola* in the Grand River watershed. Results of ANOVA comparing the two time periods (1988-1998 & 1999-2008) are included.

	1988-1998			1999-2008			ANOVA result
Parameter	# Stations	# Samples	Median (quartile range)	# Stations	# Samples	Median (quartile range)	F-ratio (p-value)
Chloride (mg/L)	4	568	34.3 (21.2 – 65.3)	4	308	38.2 (26.6 – 87.4	10.7 (<0.001)
Copper (ug/L)	4	466	1.8 (1.0 – 2.7)	4	282	1.8 (1.1 – 2.5)	0.06 (0.801)
Potassium (mg/L)	4	232	2.8 (2.3 – 3.3)	4	240	3.2 (2.3 – 3.9)	9.5 (0.002)
Nitrates (mg/L)	4	468	2.4 (1.6 – 3.4)	4	304	2.9 (1.8 – 4.1)	12.6 (<0.001)
Total Phosphorus (mg/L)	4	467	0.051 (0.029 – 0.079)	4	308	0.051 (0.025 – 0.084)	0.21 (0.647)

Table 5. Comparison of selected water quality parameters within the reaches occupied by *Lampsilis fasciola* in the Maitland River watershed. Results of ANOVA comparing the two time periods (1988-1998 & 1999-2008) are included.

	1988-1998			1999-2008			ANOVA result
Parameter	# Stations	# Samples	Median (quartile range)	# Stations	# Samples	Median (quartile range)	F-ratio (p-value)
Chloride (mg/L)	4	351	18.2 (15.5 – 21.8)	3	170	23.2 (20.4 – 27.1)	68.7 (<0.001)
Copper (ug/L)	4	264	1.5 (1.0 – 2.1)	3	124	1.2 (0.89 – 1.6)	11.5 (<0.001)
Potassium (mg/L)	2	10	3.0 (2.3 – 4.3)	3	132	2.6 (2.1 – 3.2)	2.4 (0.118)
Nitrates (mg/L)	4	342	4.6 (2.3 – 6.2)	3	170	4.1 (1.6 – 6.2)	1.5 (0.222)
Total Phosphorus (mg/L)	4	351	0.029 (0.020 – 0.044)	3	169	0.024 (0.016 – 0.036)	9.4 (0.002)

Table 6. Comparison of selected water quality parameters within the reaches occupied by *Lampsilis fasciola* in the Ausable River watershed. Results of ANOVA comparing the two time periods (1988-1998 & 1999-2008) are included.

	1988-1998			1999-2008			ANOVA result
Parameter	# Stations	# Samples	Median (quartile range)	# Stations	# Samples	Median (quartile range)	F-ratio (p-value)
Chloride (mg/L)	2	156	21.9 (19.1 – 27.4)	2	118	27.4 (24.7 – 31.8)	29.3 (<0.001)
Copper (ug/L)	2	156	2.0 (1.1 – 3.0)	2	79	1.7 (1.2 – 2.4)	3.4 (0.068)
Potassium (mg/L)	-	-	-	2	80	2.5 (1.9 – 3.3)	-
Nitrates (mg/L)	2	156	5.1 (1.3 – 7.3)	2	117	5.0 (1.5 – 8.6)	1.3 (0.263)
Total Phosphorus (mg/L)	2	156	0.065 (0.041 – 0.110)	2	118	0.047 (0.027 – 0.079)	16.7 (<0.001)

Table 7. Comparison of selected water quality parameters within the reaches formerly occupied by *Lampsilis fasciola* in the Sydenham River watershed. Results of ANOVA comparing the two time periods (1988-1998 & 1999-2008) are included.

	1988-1998			1999-2008			ANOVA result
Parameter	# Stations	# Samples	Median (quartile range)	# Stations	# Samples	Median (quartile range)	F-ratio (p-value)
Chloride (mg/L)	2	178	30.2 (26.7 – 34.9)	2	105	43.1 (35.9 – 49.1)	104 (<0.001)
Copper (ug/L)	2	169	2.6 (1.7 – 4.2)	2	77	1.5 (1.1 – 2.0)	34.2 (<0.001)
Potassium (mg/L)	-	-	-	2	77	4.6 (3.2 – 5.8)	-
Nitrates (mg/L)	2	175	4.1 (2.7 – 6.1)	2	105	4.3 (2.7 – 5.4)	0.1 (0.789)
Total Phosphorus (mg/L)	2	181	0.099 (0.067 – 0.140)	2	105	0.068 (0.045 – 0.090)	29.1 (<0.001)

Table 8. Population strengths of *Lampsilis fasciola* determined from semi-quantitative surveys of four southern Ontario watersheds.

Watershed	# of sites surveyed	Effort (person-hours)	# of extant sites	Catch per Unit Effort (animals/person-hour)	Area of Occupancy (km^2)
Ausable River	25	112.5	2	0.017	0.7
Grand River	33	143	12	0.37	7.5
Maitland River	21	94.5	9	0.22	3.2
Thames River	40	180	13	0.30	2.5

Table 9. Estimated population sizes for *Lampsilis fasciola* based on quantitative surveys within the area of occupancy.

Waterbody	# of sites surveyed	Density ($\#/m^2$)	Area of Occupancy (km^2)	Estimated population size
Ausable River	4	0.048	0.7	33,600
Grand River	4	0.28	7.5	2,100,000
Thames River	5	0.13	2.5	325,000
Maitland	4	0.096	3.2	310,000
Lake St. Clair delta	18	0.0006	5.5	3,575

Table 10. Trends in *Lampsilis fasciola* abundance in the Thames River.

Site	Year surveyed	Abundance (#)	Effort (hours)	CPUE
Elginfield Road[1]	1995	0	1	0
Elginfield Road[2]	2004	15	4.5	3.33
Plover Mills[3]	1998	1	4.5	0.22
Plover Mills[4]	2008	14	5	2.8

[1] Morris 1996.
[2] Morris and Edwards 2007.
[3] J. L. Metcalfe-Smith, Environment Canada, unpublished data.
[4] T. Morris and D. Woolnough, Fisheries and Oceans Canada/Central Michigan University, unpublished data.

Table 11. Shell length for *Lampsilis fasciola* populations from the Ausable and Maitland rivers and St. Clair delta. Ausable and Maitland river samples include excavation studies while St. Clair samples do not.

Population	sample size	mean (SE)	minimum	maximum
Ausable (male/juvenile)	13	53.7 (4.89)	22	80
Ausable (female)	5	59.4 (7.35)	45	83
Maitland (male/juvenile)	11	52.3 (5.34)	29	79
Maitland (female)	13	57.8 (3.19)	39	77
St. Clair (male/juvenile)	5	51.0 (2.66)	46	61
St. Clair (female)	10	48.0 (2.64)	35	59

Table 12. Sex ratios for *Lampsilis fasciola* found in semi-quantitative surveys of Ontario waters 1997-2008.

	Live animals			Shells + Live animals		
Population	male	female	ratio	male	female	ratio
Ausable River	0	2	0.00	12	5	2.40
Maitland River	13	11	1.18	46	32	1.44
Thames River	70	155	0.45	79	168	0.47
Grand River	42	94	0.45	108	147	0.73
Lake St. Clair	13	17	0.76	17	20	0.85
Sydenham River	0	0	-	3	9	0.33

Table 13. Sex ratios for *Lampsilis fasciola* found during quantitative sampling of watersheds where the species occurs between 2004 and 2008.

Population	male	female	ratio
Ausable River	7	5	1.40
Maitland River	11	13	0.85
Thames River	17	16	1.06
Grand River	128	93	1.28

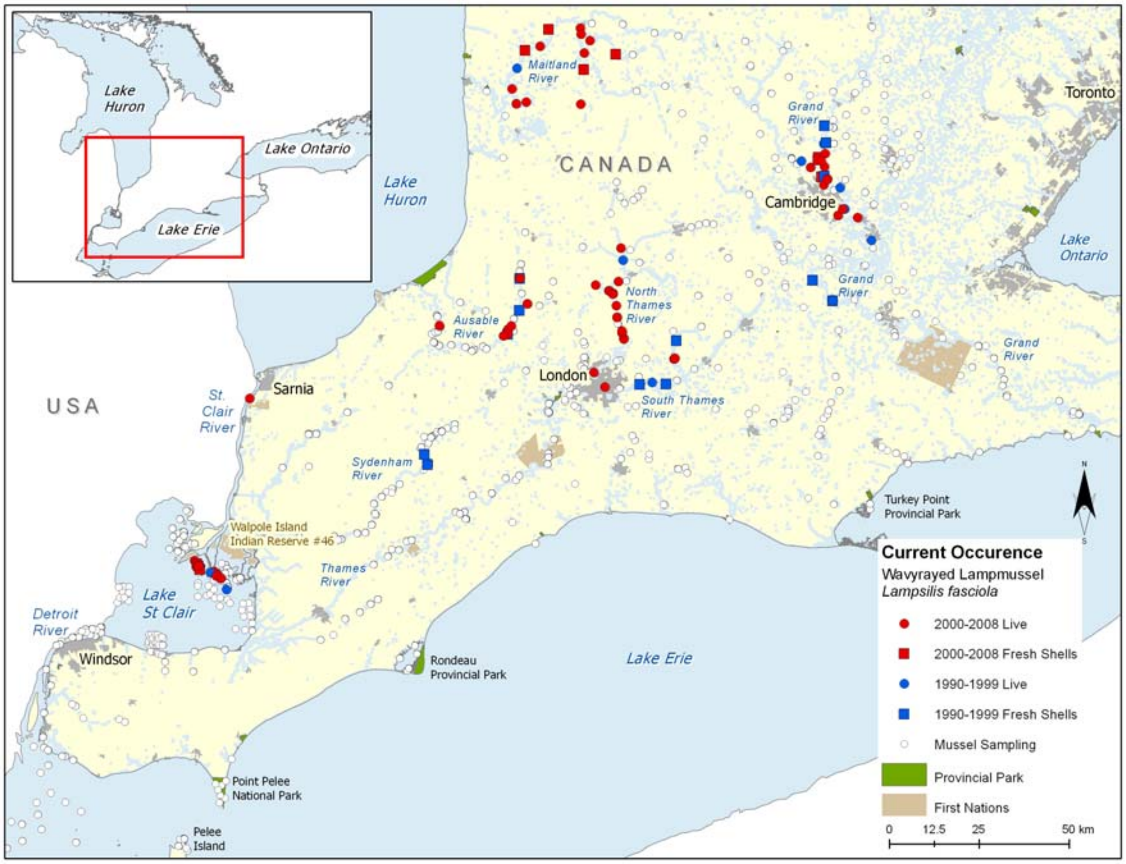


Figure 1. Distribution of the Wavyrayed Lampmussel in Canadian waters.

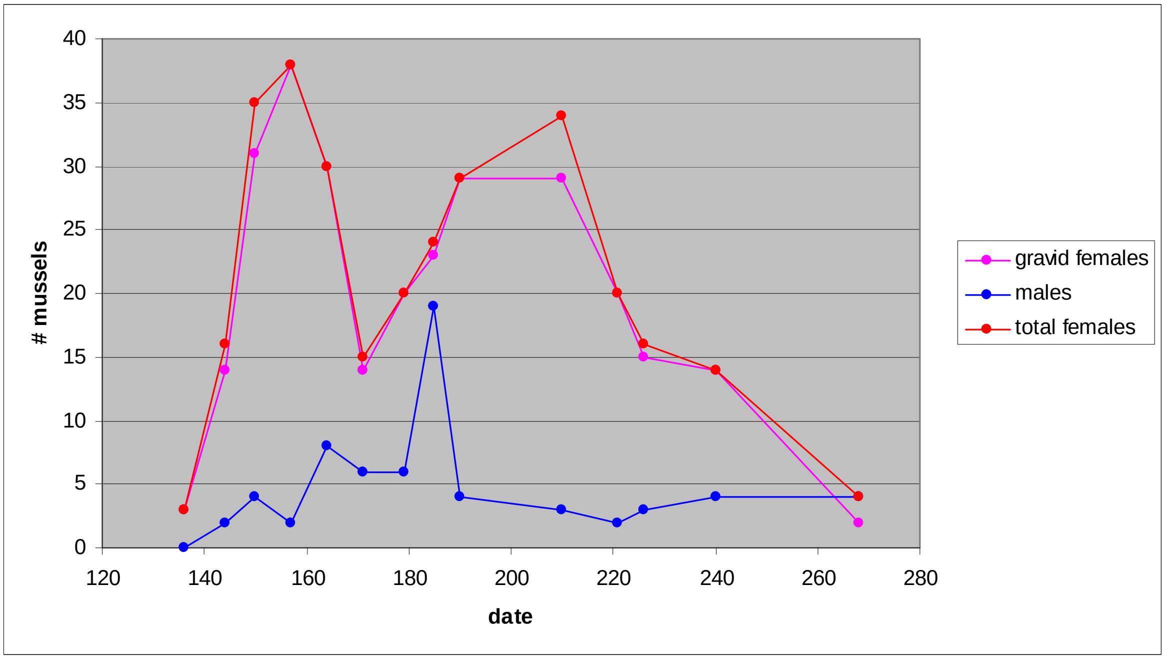
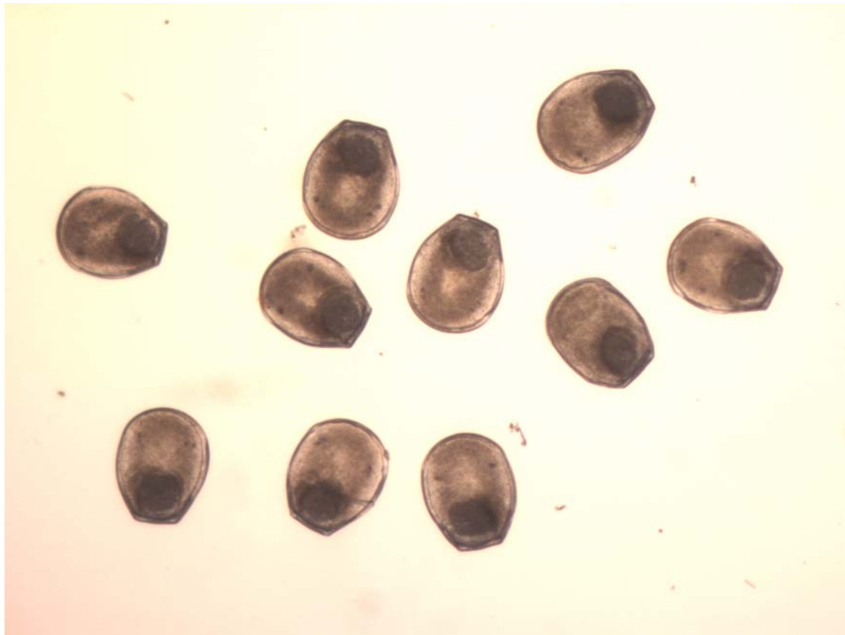


Figure 2. Abundance of Wavyrayed Lampmussels at the substrate surface for the Thames River population during the open water season of 2008.



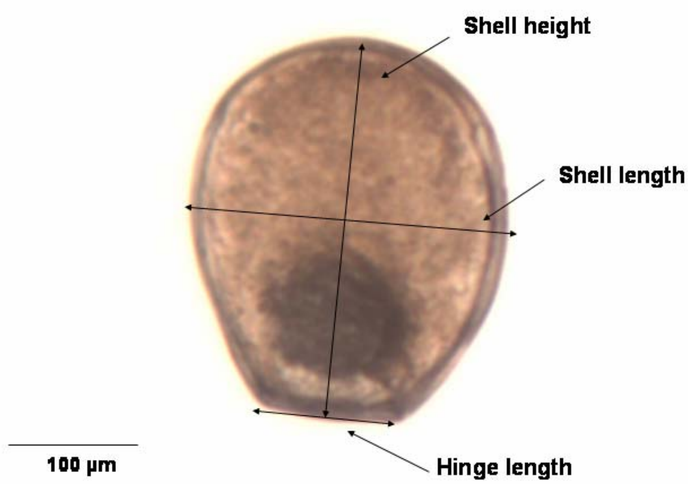


Figure 3. Glochidia of *Lampsilis fasciola*.

Figure 4. Lure morphs observed in the Thames (A, B, C) and Grand (D) rivers of southwestern Ontario. A. Red morph. B. Black morph. C. Fish morph. D. Flamboyant attractor.

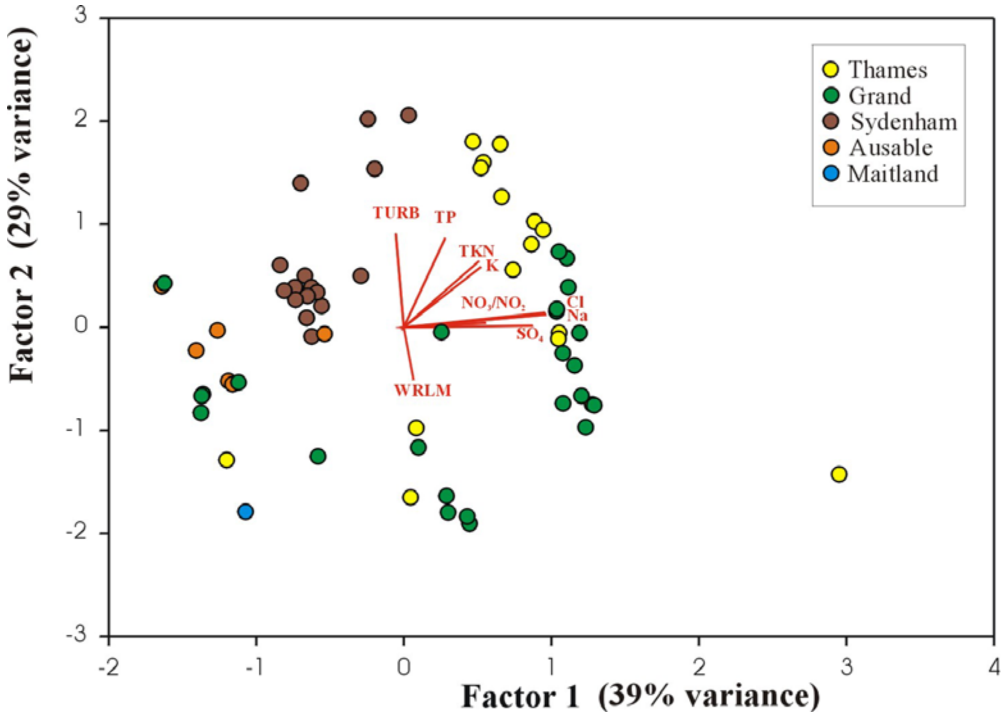


Figure 5. Principal component analysis of several water quality parameters and the abundance of the Wavyrayed Lampmussel (WRLM) in southern Ontario rivers in 1998.

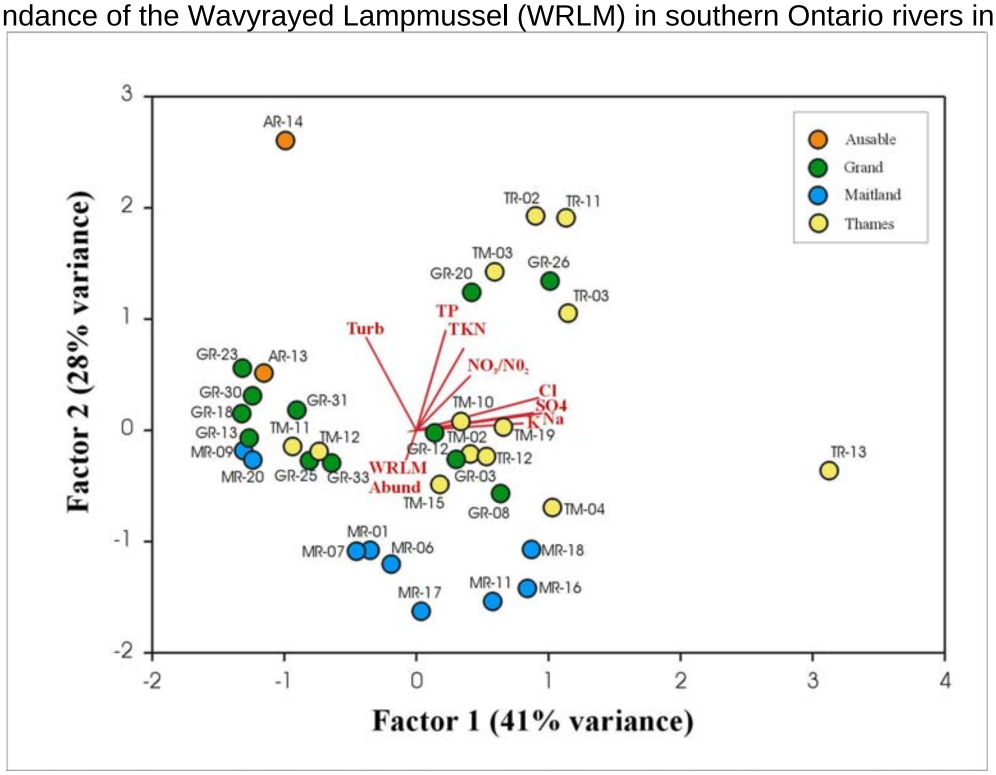


Figure 6. Principal component analysis of several water quality parameters and the abundance of the Wavyrayed Lampmussel (WRLM) in southern Ontario rivers in 2004.

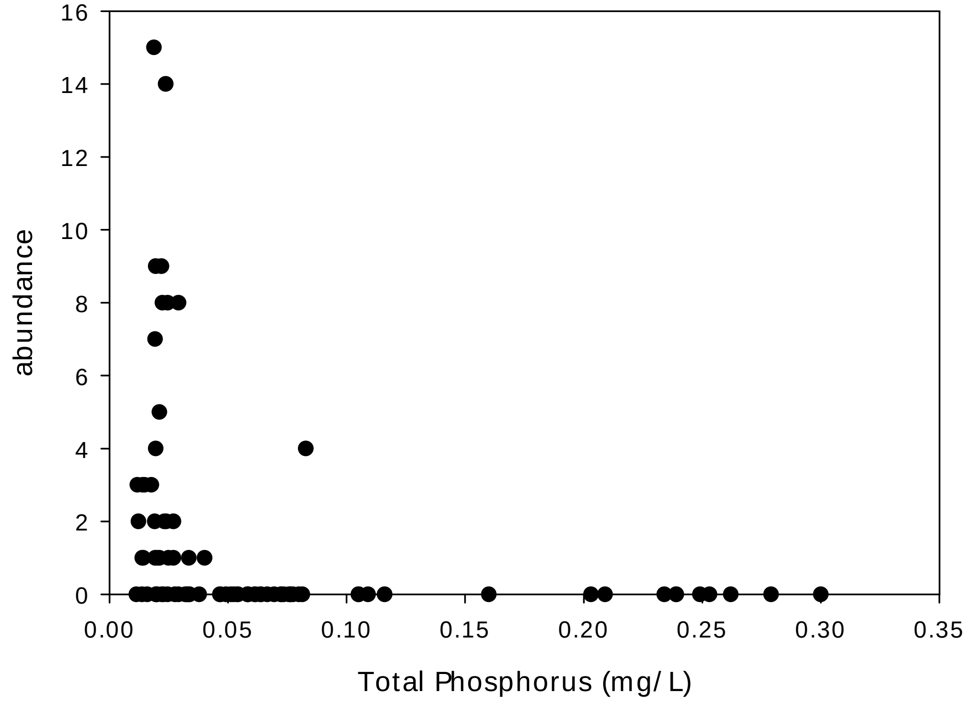


Figure 7. Abundance of *Lampsilis fasciola* versus the concentration of total phosphorus at sites in southern Ontario rivers.

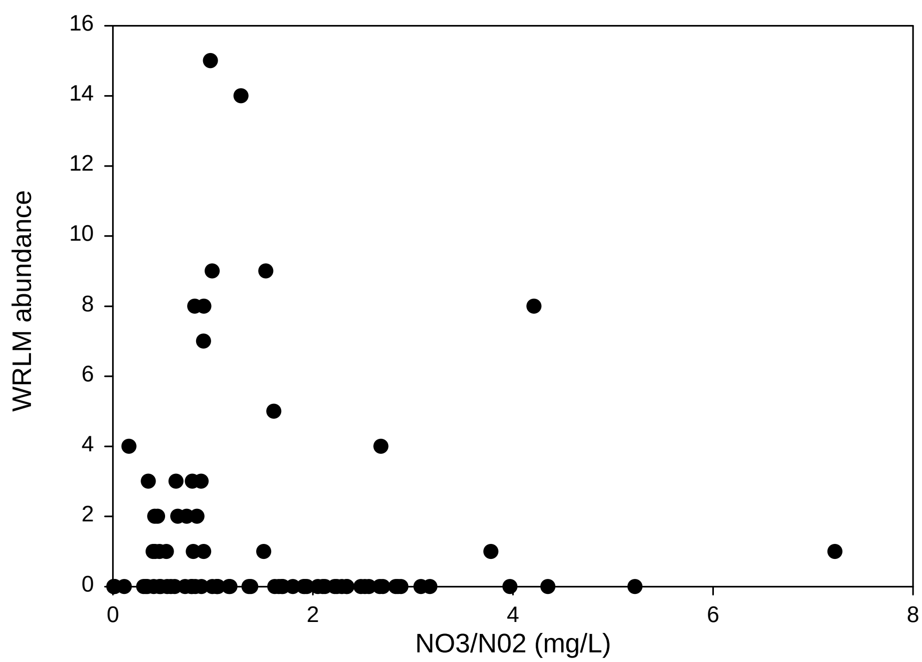


Figure 8. Abundance of *Lampsilis fasciola* versus the concentration of nitrate and nitrite at sites in southern Ontario rivers.

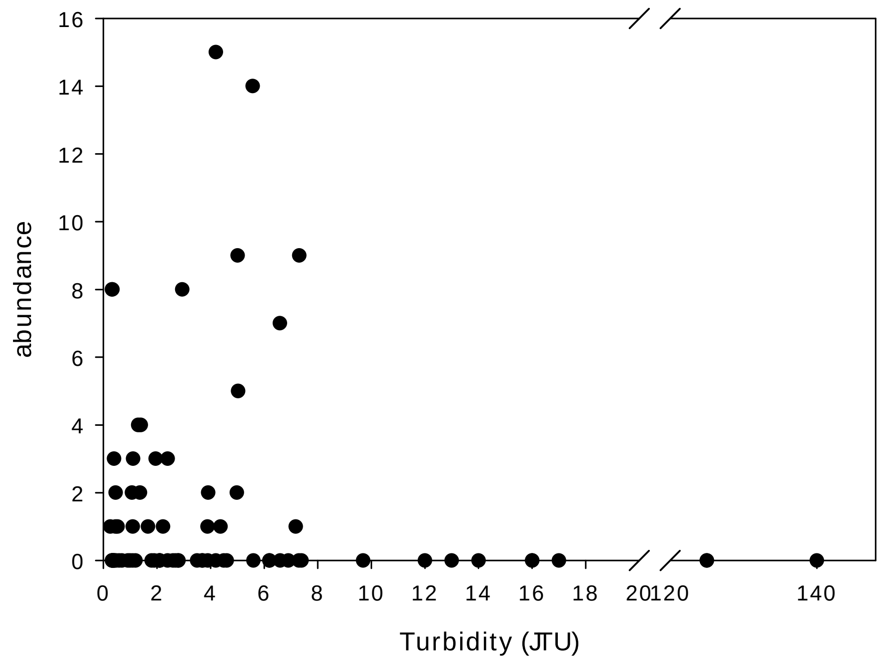


Figure 9. Abundance of *Lampsilis fasciola* versus turbidity at sites in southern Ontario rivers.

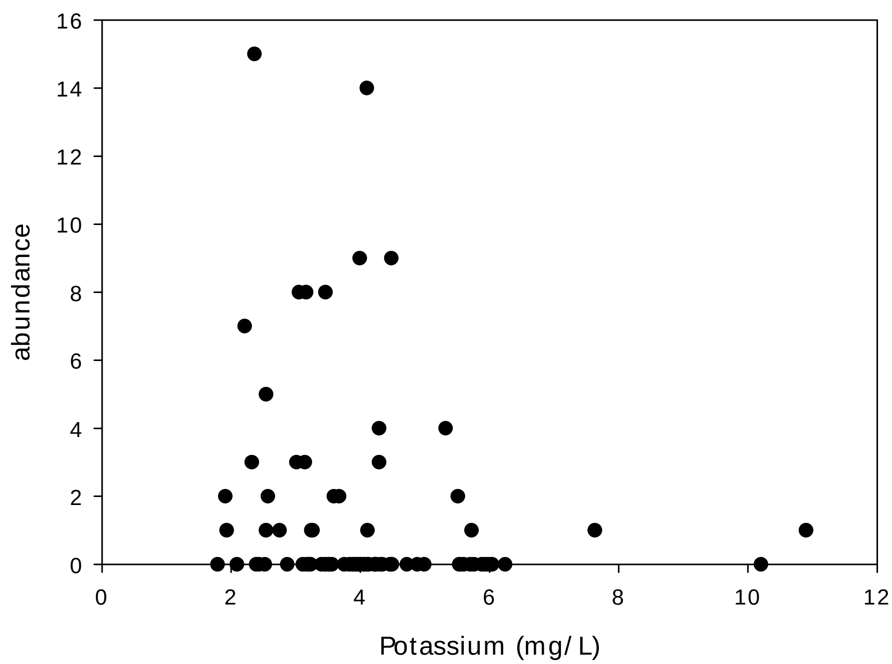


Figure 10. Abundance of *Lampsilis fasciola* versus the concentration of potassium at sites in southern Ontario rivers.

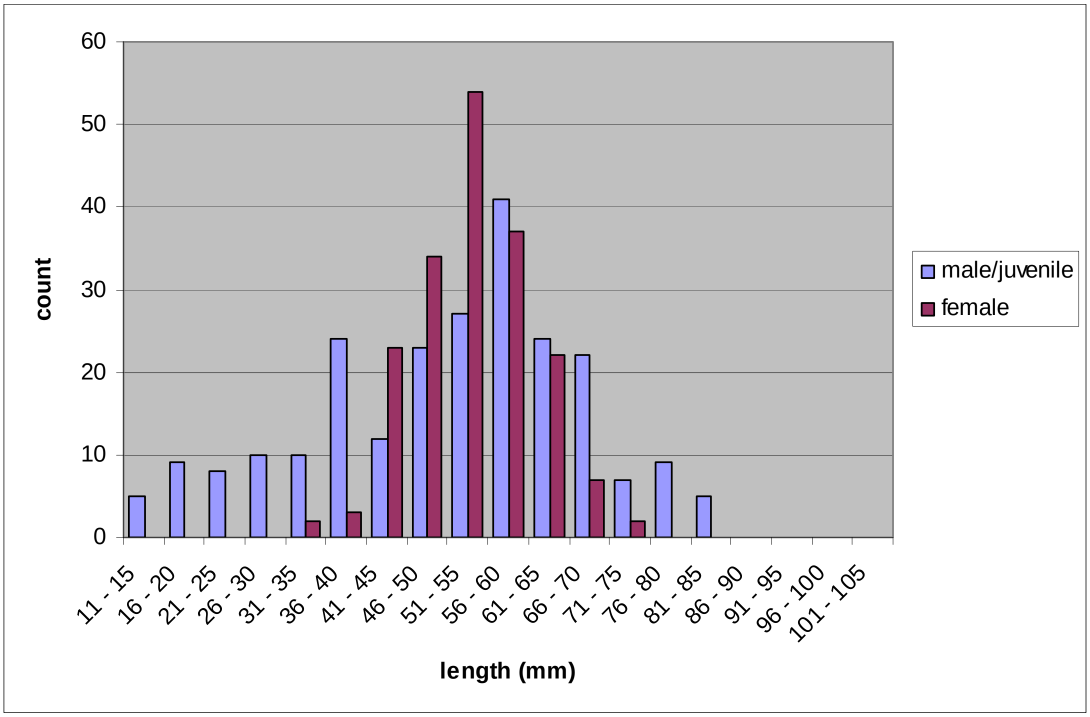


Figure 11. Size class distribution of *Lampsilis fasciola* collected in the Grand River between 1997 and 2008. Male category includes juveniles.

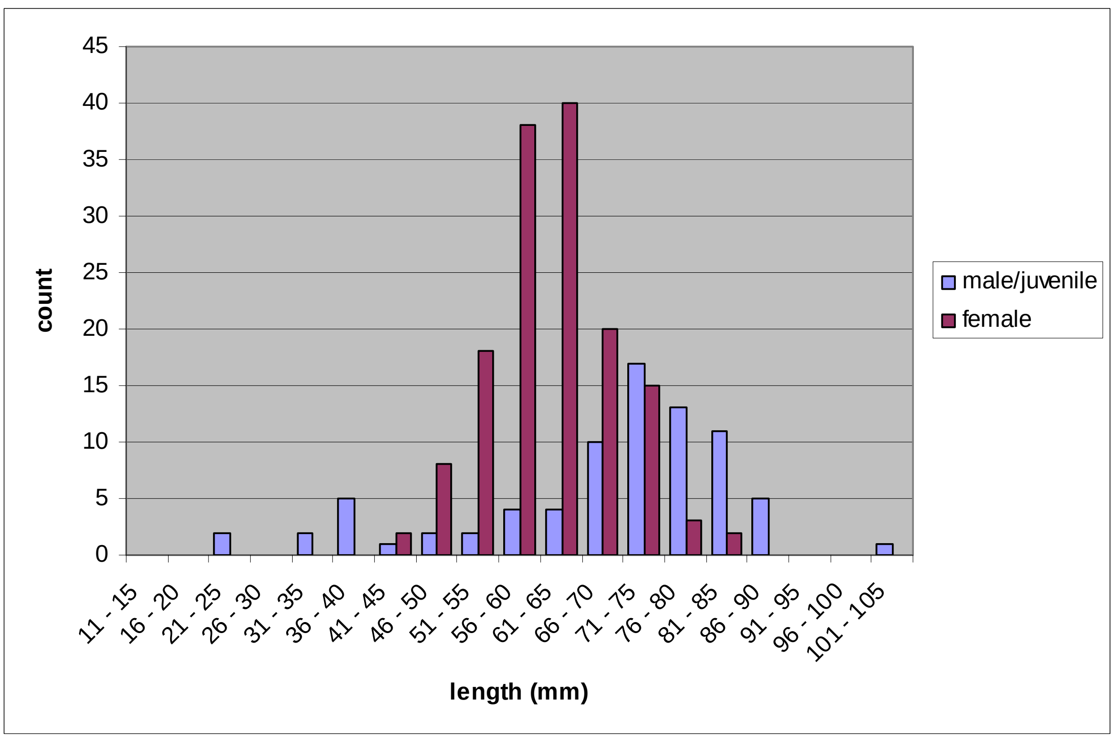


Figure 12. Size class distribution of *Lampsilis fasciola* collected from the Thames River between 1997 and 2008.

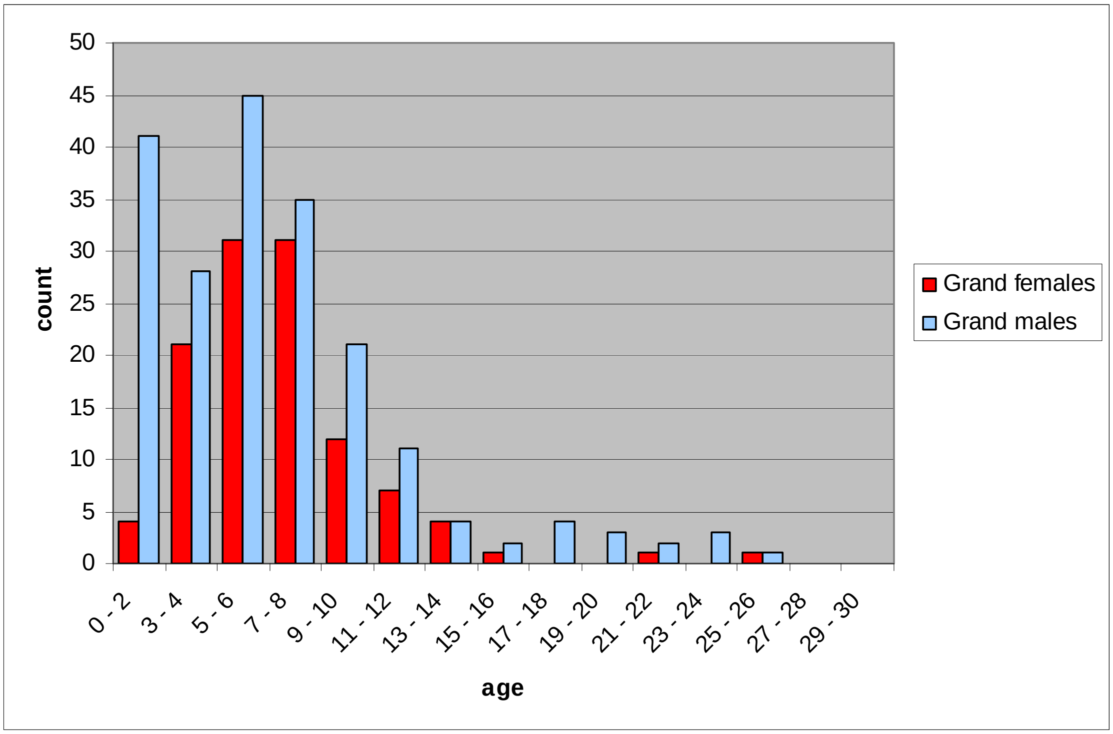


Figure 13. Age distribution of *Lampsilis fasciola* collected from the Grand River between 1997 and 2008. Male category includes juvenile animals.

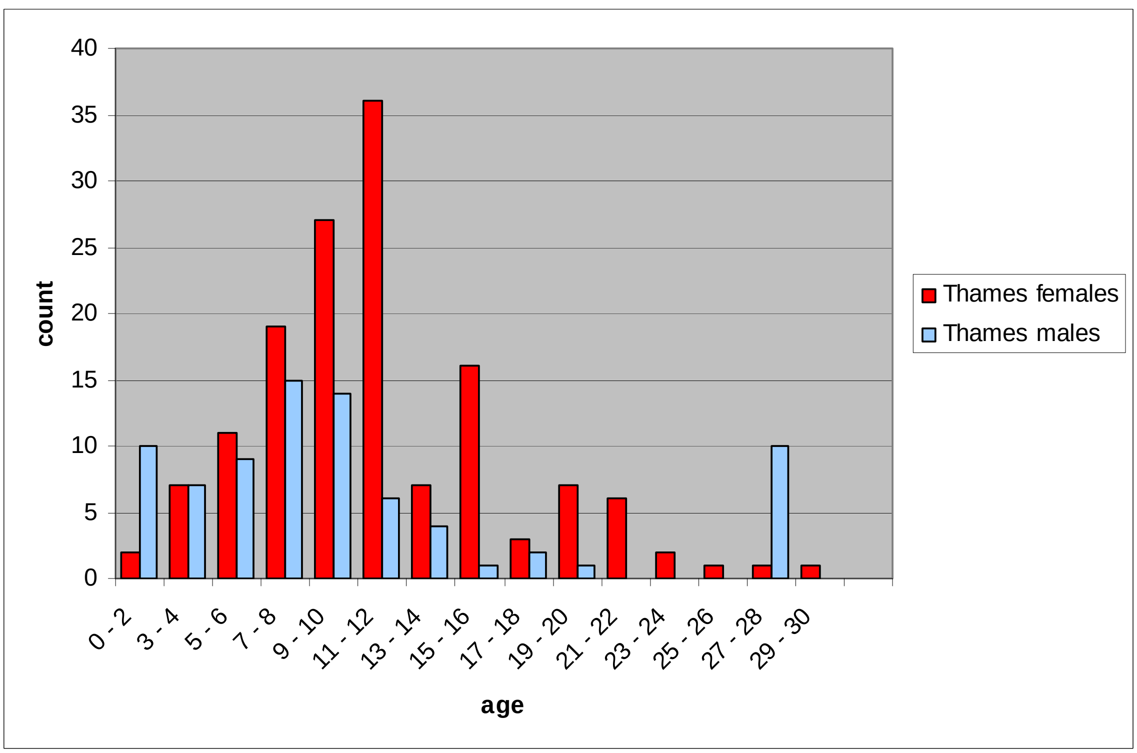


Figure 14. Age distribution of *Lampsilis fasciola* collected from the Thames River between 1997 and 2008. Male category includes juvenile animals.

Figure 15. The distribution of populations where tissue collections were made for *Lampsilis fasciola* (Zanatta *et al.*, 2007). Sample site localities: Grand River (UG) near Waterloo, ON (43°29’39.61”N, 80°28’15.17”W); Grand River (LG) near Kitchener, ON (43°24’13.58”N, 80°25’58.17”W); North Thames River (NT) near St. Mary’s, ON (43°12’31.04”N, 81°12’26.10”W); Middle Thames River (MT) near Thamesford, ON (43°2’49.56”N, 80°59’37.41”W); South Maitland River (SM) near Summerhill, ON (43°41’4.56”N, 81°32’27.64”W); Middle Maitland River (MM) near Wingham, ON (43°51’35.92”N, 81°19’9.87”W). From Zanatta *et al.* (2007).

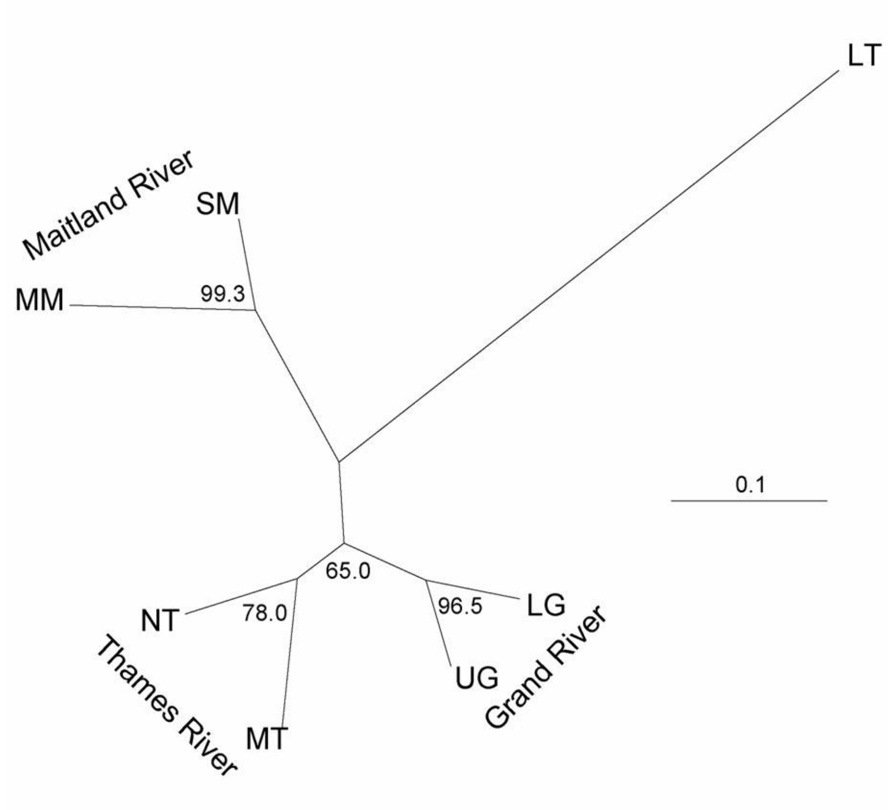


Figure 16. An unrooted neighbour-joining network based on *Nei* D_A (Nei *et al.*, 1983) genetic distance for 7 populations of *Lampsilis fasciola* (LT are samples from the Little Tennessee River in North Carolina, USA). Numbers indicate nodes with bootstrap support of more than 50% for 1000 replications. From Zanatta *et al.* (2007).