Rocky Mountain ridged mussel (*Gonidea angulata*) in the Okanagan Valley, B.C.:

Final report on host fish field sampling, mussel surveys, genetic analyses, and maximum age determination.

April 29th, 2017

Photo: Roxanne Snook

On behalf of the University of British Columbia – Okanagan Campus,

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Summary

The Rocky Mountain ridged mussel (RMRM; *Gonidea angulata*, Lea 1839) is COSEWIC assessed as "Endangered" and listed as a "Species of Special Concern", under schedule one of the Species at Risk Act (SARA), in Canada, due to its limited distribution and relatively small population size, past and ongoing habitat impacts, and effects of introduced species. It is only found within the Okanagan Valley of British Columbia (B.C.) in Canada, and the province has listed it as "Imperiled". However, very little is known about the biology of this mussel in general. From a conservation perspective, priority has been placed on improving knowledge of the mussel's biology, identifying threats to the species, and developing appropriate mitigation to reduce those threats. In this project, we investigated four aspects of RMRM biology: 1. Host fish suitability. 2. RMRM distribution. 3. RMRM genetics. 4. RMRM maximum age.

Determining the host fish of RMRM is essential to evaluate the threats against this species. One has to know the identity of the mussel's host fish to determine if the lack of host fish availability is a threat to the mussel. A reduction in host fish availability could stem from hydromorphological changes, altering habitat availability to the fish, and/or from the introduction of new fish species, outcompeting and/or predating on native host fish. Experimental studies in the United States of America (U.S.) have confirmed that sculpin (*Cottus* spp.) serve as the primary host for RMRM. Field studies from two sites in Okanagan Lake support the conclusion that prickly sculpin (*Cottus asper,* Richardson 1836) is the primary host in the valley. In addition, these studies suggest that longnose dace (*Rhinichthys cataractae*, Valenciennes 1842), leopard dace (*Rhinichthys falcatus*, Eigenmann and Eigenmann 1893), and, potentially, northern pikeminnow (*Ptychocheilus oregonensis*, Richardson 1836) function as secondary hosts. In this project, we sampled for host fish at four new sites within the Okanagan Valley. Our findings support prickly sculpin being the primary host for RMRM in this system and suggest that northern pikeminnow functions as a secondary host. In addition, our findings excluded several introduced fish species as potential hosts for RMRM. These findings show that introduced fish species may pose a threat to RMRM, as they may outcompete and/or predate on native host fish in some parts of the Okanagan. However, unlike other native hosts for RMRM, the northern pikeminnow is common in the parts of the Okanagan that contain substantial numbers of introduced fish. Therefore, the identification of this species as a likely host may negate this threat to some extent.

Knowing the location of RMRM and its habitat is essential in protecting the species. The lakes, rivers, and streams of the Okanagan Valley have been extensively surveyed, resulting in the mussel being found intermittently from the northern end of Osoyoos Lake, in the south, to the northern end of Okanagan Lake, in the north. In this project, we surveyed 32 new sites for the mussel. We found RMRM at five of those sites. In total, we found 155 live mussels. Identifying these sites will help protect RMRM and its habitat. However, there are still sites with potential mussel habitat that have not been surveyed.

Analyzing the genetic makeup of RMRM within the Okanagan Valley has several potential benefits. It can determine if mussels from the Okanagan differ genetically from U.S. populations, and if there are different populations of the mussel within the valley. If the mussels within the system are genetically unique, this will increase their conservation value. In this project, we undertook several forms of genetic analyses. Mitochondrial sequencing did not show great differences in genetic makeup between the Okanagan and U.S. populations, although it did reveal one haplotype that is unique to the Canadian population. The mitochondrial haplotypes also indicate that there is a gradient in genetic diversity between sites within the Okanagan. Microsatellites have been developed to further investigate this aspect of the genetic makeup of RMRM in the system. However, further analysis and testing is necessary before any conclusions can be reached on this topic. This work will be completed in the 2017-2018 fiscal year. During the coming year eDNA markers will also be developed. These markers can be used to identify RMRM within other watersheds in B.C., identify life history events such a sperm and glochidial releases, and any major die-offs among the mussels.

Determining the maximum age of RMRM within the Okanagan Valley is important in evaluating whether the level of juvenile recruitment is sufficient to maintain mussel numbers. Juvenile recruitment has previously been evaluated in the Okanagan. However, not knowing the maximum age of the mussel in the system creates some uncertainty with respect to this evaluation. Therefore, determining the maximum age of the mussel will contribute to a better understanding of recruitment levels in the Okanagan. In this project, our aim was to determine the maximum age of RMRM in the Okanagan, with certainty, to better evaluate recruitment levels. We collected shells from the system. The age of the shells were evaluated through counting of external growth rings and internal growth rings through thin sectioning. Counting of external growth rings was shown to be unsuitable for determining the age of RMRM. Thin sectioning is being explored as a more reliable source for determining the maximum age of the mussel. Completion of the maximum age determination will allow us to better evaluate whether the level of juvenile recruitment is sufficient to maintain RMRM within the Okanagan.

Based on the findings from this project, it is recommended that: 1. Host fish use and the impact of introduced fish species should be further investigated. The host fish use should be confirmed via completion of a host infection experiment. In addition, the host fish use in the Okanagan River should be further investigated. An invasive fish species assessment, with a special emphasis on smallmouth bass, should also be conducted to determine the presence and impact of invasive fish species in the Okanagan Valley. This would increase certainty regarding the threats from lack of host fish and introduced fish species. 2. Further mussel surveys should be undertaken in the Okanagan Valley, as not all potential RMRM habitat has been surveyed. This especially applies to Okanagan Lake. 3. The genetics work should be completed. Determining if there is more than one population of RMRM in the Okanagan will provide information on the conservation value of the individual mussel beds within the valley. Developing eDNA for RMRM will allow for easier detection of the mussel within other watersheds in Canada and the U.S. In addition, it can be used to detect life history events and major die-offs. 4. The determination of the maximum age of RMRM in the Okanagan Valley should be completed, which will allow us to better evaluate whether juvenile recruitment is sufficient to maintain mussel numbers.

Introduction

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The Rocky Mountain ridged mussel (RMRM; *Gonidea angulata*) is found west of the Rocky Mountains in the United States of America (U.S.) and Canada, from California in the south to British Columbia (B.C.) in the north. Unfortunately, the mussel is known to be in decline throughout most of its range (Jepsen *et al.* 2010a). In Canada the distribution is limited to only the Okanagan Valley, B.C. (Stanton *et al.* 2012). In the valley, the mussel has been declining in numbers and distribution (Stanton *et al.* 2012), which has led to it being listed as "Imperiled" by the province (B.C. Conservation Data Centre 2015a, b). On a national level, it has recently been reassessed as "Endangered" by COSEWIC (COSEWIC 2010) and, following an earlier assessment (COSEWIC 2003), it was listed as a "Species of Special Concern" under Schedule 1 of Species at Risk Act (SARA) (Fisheries and Oceans Canada 2010).

Despite the decline of the Rocky Mountain ridged mussel, very little is known about its biology and the threats to it (COSEWIC 2003, 2010, Fisheries and Oceans Canada 2010, 2011a, Jepsen *et al.* 2010a, B.C. Conservation Data Centre 2015b). In fact, the management plan for the species in Canada points out that this lack of knowledge is one of the main threats to this species. It emphasizes the importance of doing research on the mussel to protect and implement its recovery in Canada. More specifically, the plan states that "[p]riority research [on RMRM] will focus on life history and host fish(s), habitat mapping, clarification of threats to both the species and the host fish(s), and inventory throughout the species range in Canada" (Fisheries and Oceans Canada 2010).

In this project, we investigated four aspects of RMRM biology: 1. Host fish suitability. 2. RMRM distribution. 3. RMRM genetics. 4. RMRM maximum age. For further details, see the subsequent sections.

Host Fish Field Sampling

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Introduction

One of the areas of Rocky Mountain ridged mussel (RMRM; *Gonidea angulata*) biology that still contains knowledge gaps is the host fish use by the mussel in the Okanagan Valley, British Columbia (B.C.) (COSEWIC 2003, 2010, Fisheries and Oceans Canada 2010, 2011a). Only laboratory studies can confirm the host use of RMRM, but field data can suggest the likely host species (O'Brien *et al.* 2013). Laboratory studies from the United States of America (U.S.) have confirmed sculpin (*Cottus* spp.) as the primary host for the mussel in these systems (Spring Rivers 2007, O'Brien *et al.* 2013). Field data from two sites in Okanagan Lake point in the same direction, strongly suggesting that Prickly sculpin (*Cottus asper,* Richardson 1836) is the primary host for RMRM in this lake (Stanton *et al.* 2012, Mageroy 2015). In addition, field data also point to Longnose dace (*Rhinichthys cataractae*, Valenciennes 1842) and Leopard dace (*Rhinichthys falcatus*, Eigenmann and Eigenmann 1893) as likely secondary hosts (Mageroy 2015), and Northern pikeminnow (*Ptychocheilus oregonensis*, Richardson 1836) as a possible secondary host (Stanton *et al.* 2012) for the mussel. However, there are no data on host use for RMRM in other parts of the Okanagan. To evaluate whether the lack of host fish is a threat to the mussel it is important to identify other likely host fish. It is especially important to gather information about host use in the southern Okanagan, since data indicate that introduced fish species have displaced the known native host fish at the mussel beds in this part of the system (Mageroy 2016). Therefore, it is important to evaluate whether introduced fish or the native fish that are still present at the mussel beds can serve as

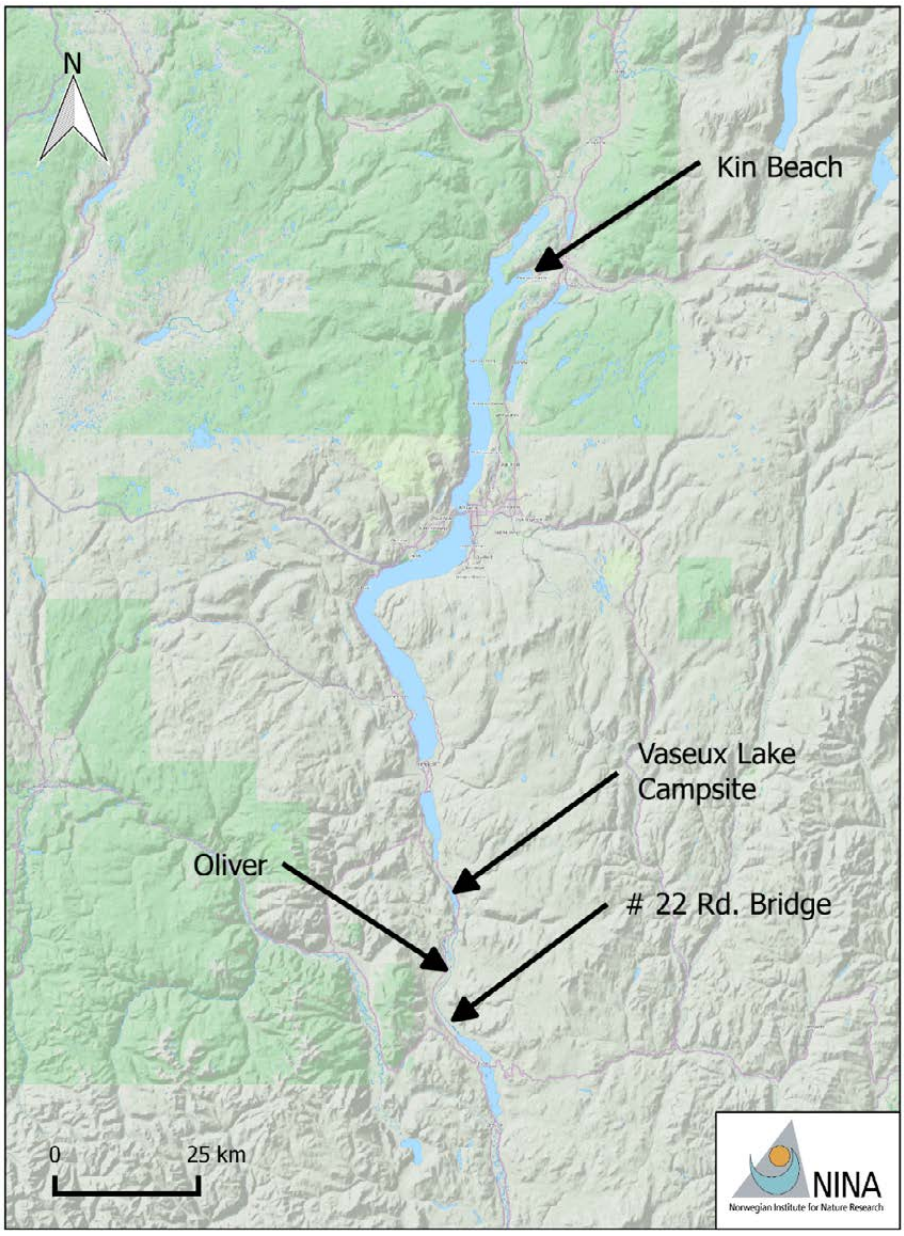

Figure 1. Host fish sampling sites. The map was generated in QGIS 2.16.1 (QGIS Developmental Team 2016) and the basemap is from OpenStreetMap (2017).

host fish for RMRM. In this project, our aim was to identify additional host fish for the mussel and determine if introduced fish species may function as hosts.

Methods

For the field sampling of potential host fish, we chose four sites that have relatively high densities of RMRM (Mageroy 2015, 2016) and were geographically distant from the previously sampled

sites in Okanagan Lake (Stanton *et al.* 2012, Mageroy 2015) (see Figure 1, for further details see Appendix 1 Table 1). Since gaining information about host fish use is especially important in the southern Okanagan Valley, three of the four sites were located in this part of the system. We, generally, followed the methodology described by Mageroy (2015, 2016). The timing of fish collection was determined by snorkeling the Kin Beach and Vaseux Lake Campsite sites, and surveying for the release of RMRM conglutinates (packages of glochidia (mussel larvae)). These surveys were started at the beginning of May, as the first conglutinates are typically released during this month in the Okanagan Valley (Mageroy 2015, 2016). Fish sampling was undertaken during the entire period of conglutinate release. All fish sampling was completed between May 9th and June 9th. Sites were fished between five and seven times during this period.

The fish collection methods were adapted to the site in question. At the lake sites (Kin Beach and the Vaseux Lake Campsite) beach seines and minnow traps were used, while at the river sites (Oliver and # 22 Rd. Bridge) minnow traps were used. Electrofishing was also used, by Greg Wilson with the B.C. Ministry of Environment, at the Vaseux Lake Campsite and the two river sites. Unfortunately, at the two river sites water flow and depth was too great to successfully collect fish using this method. All methods follow or are modified from the recommendations made by the British Columbia Resources Information Standards Committee (RISC) (B.C. Ministry of Environment, Lands, and Parks 1997). Minnow traps were baited with sardines and left overnight (ca. 16 hrs.). We set three traps at each site. Beach seines were set and pulled during the morning hours. We completed 3 to 15 seine pulls at each site, depending on the number of fish caught during each pull. Electrofishing was completed using a portable (backpack) unit and fish were collected using dip nets. The Vaseux Lake Campsite was electrofished for ca. one hour. Any fish that were caught, using the various collection methods, were euthanized immediately. The fish were euthanized using buffered MS-222 and preserved in 70 % ethanol. For further details on the fish collection methods, see Mageroy (2015, 2016) and the RISC guidelines (B.C. Ministry of Environment, Lands, and Parks 1997).

Subsequently, the fish gills were examined to determine prevalence, intensity, and whether the glochidia were encysted or not, for each fish species. The latter was done because previous studies have shown that glochidial encapsulation, on the gills of host fish, is necessary for successful metamorphosis to juvenile mussels (O'Brien *et al.* 2013). Therefore, only fish species with encapsulated glochidia are potential host fish. The gill filaments were excised individually, using a scalpel and forceps. They were placed on a microscope slide, and each side of the gill filament was inspected under the microscope. Any

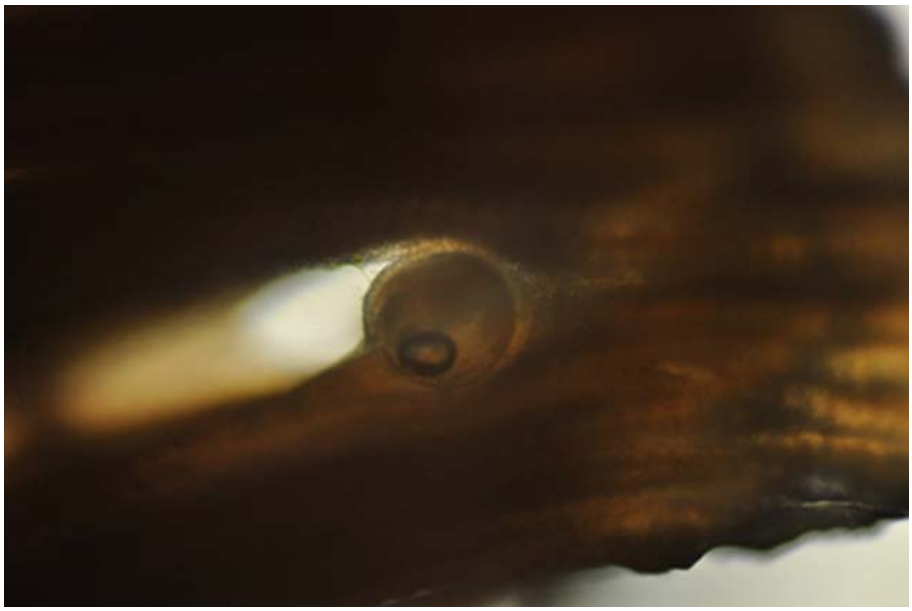

Figure 2. RMRM glochidium. The photo shows the glochidium attached to a gill filament. (Photo: Madalyn Laslett.)

glochidia present (Figure 2) were counted. Glochidia on both gills were quantified during this process. Encysted and non-encysted glochidia were quantified separately. Based on these analyses, the prevalence and intensity of glochidia were calculated.

Results

Unfortunately, no fish were caught at the two river sites. In total, 86 fish were caught at the Kin Beach and the Vaseux Lake Campsite. At Kin Beach, 28 northern pikeminnow and 4 prickly sculpin were caught. At the Vaseux Lake Campsite, 2 brown bullhead (*Ameiurus nebulosus*, Lesueur 1819), 2 largemouth bass (*Micropterus salmoides*, Lacepede 1802), 14 prickly sculpin, 11 pumpkinseed sunfish (*Lepomis gibbosus* L. 1758), 16 smallmouth bass (*Micropterus dolomieu*, Lacepede 1802), and 9 yellow perch (*Perca flavescens*, Mitchill 1814) were caught. Note that the yellow perch were found dead at the site, but 6 were in sufficient condition to have their gills examined. Most of these fish species are present in the Okanagan River, and Osoyoos, Vaseux, Skaha, and Okanagan Lakes (McPhail 2007, Mageroy 2016, iMapBC 2017, Jerry Mitchell *Pers. com.*). Exceptions are smallmouth bass, which only recently has been found in Okanagan Lake (iMapBC 2017, Jerry Mitchell *Pers. com.*), and brown bullhead and largemouth bass, which have not been found in Okanagan Lake (iMapBC 2017, Jerry Mitchell *Pers. com.*). Among these species, only the sculpin and pikeminnow are native to the Okanagan Valley (McPhail 2007) and

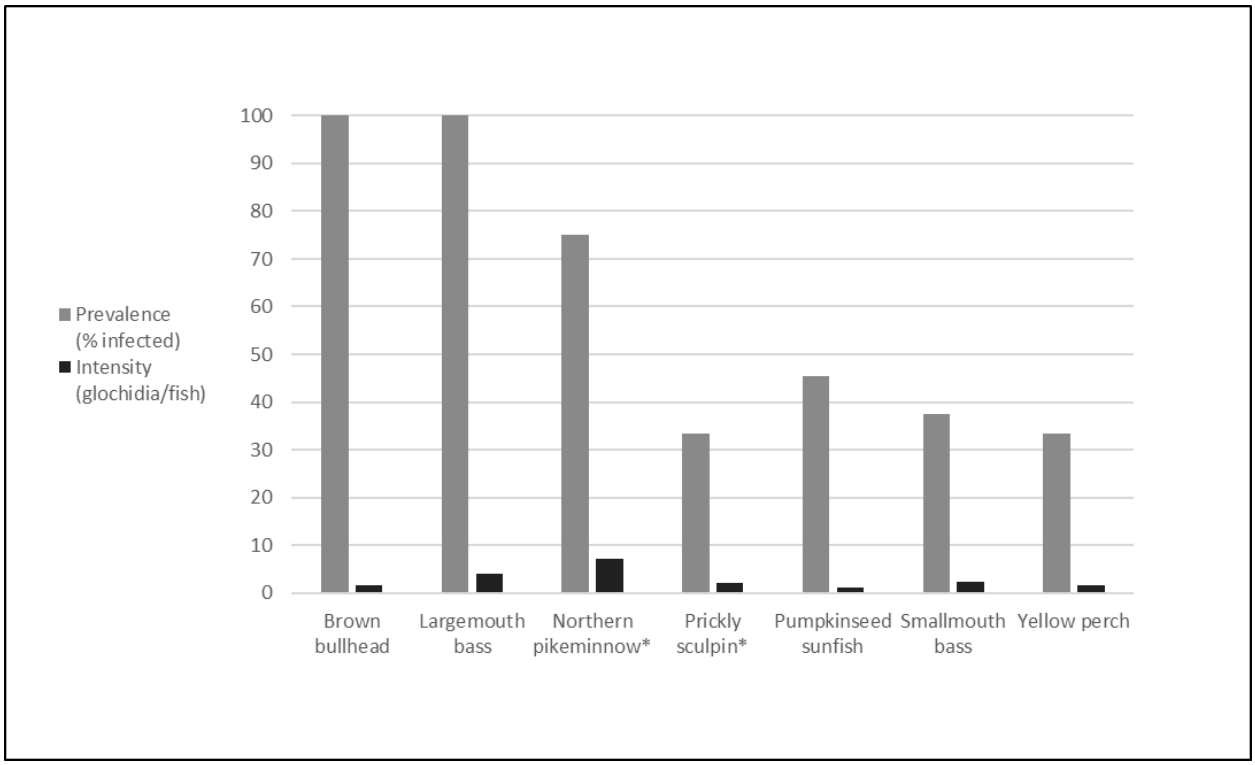

Figure 3. Host fish prevalence and intensity. The asterisk indicates that only northern pikeminnow and prickly sculpin had encapsulated RMRM glochidia on their gills. All other species of fish only had non-encapsulated glochidia. Note that prickly sculpin was the only fish species caught at both Kin Beach and the Vaseux Lake Campsite, northern pikeminnow was only caught at Kin Beach, and the other species were only caught at the Vaseux Lake Campsite. The figure was created using Microsoft Excel 2016.

only these two species had RMRM glochidia encapsulated on their gills. Non-encapsulated glochidia were found on all other species of fish. This shows that all the fish species, present at the mussel beds, are exposed to the glochidia, but the glochidia are only able to successfully encapsulate on some species. See Figure 3, for an overview of prevalence and intensity of the glochidia. For separate data for Kin Beach and the Vaseux Lake Campsite, see Appendix 1 Table 2.

Discussion

Our data strongly suggest that introduced fish species cannot function as hosts for RMRM in the Okanagan Valley, as we did not find encapsulated glochidia on the gills of any of the introduced fish

species (Figure 3). This supports previous findings from the Okanagan (Mageroy 2015) and the U.S. (Spring Rivers 2007, O'Brien *et al.* 2013), which also found no evidence for this mussel species being able to use introduced fish species as hosts. Mageroy (2016) showed that the previously identified likely host species, prickly sculpin and two species of dace (Stanton *et al.* 2012, Mageroy 2015), were quite common in Okanagan Lake, but absent or almost absent from the southern Okanagan. Therefore, our findings support prior concerns that introduced host fish are a threat against RMRM in parts of the system. Mageroy (2016) also suggested that the lack of native host fish, in the southern Okanagan Valley, is the result of competition and/or predation from introduced fish species, especially the extremely common smallmouth bass. Smallmouth bass has recently been found in Okanagan Lake (iMapBC 2017, Jerry Mitchell *Pers. com.*), and it is likely to establish in the bays of larger lakes it invades, according to the invasive species risk assessment for this species in British Columbia (Fisheries and Oceans Canada 2011b). Several of the RMRM beds are found in the bays of Okanagan Lake (EcoCat 2015, Snook 2015, Mageroy 2016). Therefore, a lack of host fish could also become a threat to RMRM in the northern part of the system.

However, we also found that northern pikeminnow likely is an important host to RMRM in the Okanagan Valley. The prevalence and intensity of encapsulated glochidia were relatively high on this species (Figure 3), and encapsulation is necessary for a fish to serve as a host (O'Brien *et al.* 2013). Although data from Stanton *et al.* (2012) suggested the possibility that this species might be a host for RMRM, our data provide evidence that it is a likely host for the mussel. No other studies have shown that pikeminnow species may serve as hosts for this species (Spring Rivers 2007, O'Brien *et al.* 2013). Therefore, this finding needs confirmation through laboratory studies (O'Brien *et al.* 2013). This finding is important, given the availability of host fish in the southern Okanagan. Mageroy (2016) showed that this species, unlike prickly sculpin and dace, was quite common in this part of the system, at least in the Okanagan River. Therefore, the northern pikeminnow is clearly capable of coexisting with the introduced fish in the system and may serve as the main host for RMRM when the other host species are absent or almost absent. However, we do not know if this applies to all size classes of pikeminnow, and it is likely that juveniles would be more important host fish, as they are more likely to forage near the shore (Scott & Crossman 1973, Coker *et al.* 2001) where the mussel beds are (Stanton *et al.* 2012). If smallmouth bass establish in Okanagan Lake and reduce the numbers of the other native host fish, the pikeminnow may become an even more important host in this part of the system.

Our data also lend further evidence towards prickly sculpin being the primary host in the Okanagan Valley. We found that prickly sculpin were infected with encapsulated glochidia, although the prevalence and intensity we found on the sculpin were relatively low (Figure 3). The latter can be explained by the fact that we only caught four prickly sculpin at Kin Beach (see Appendix 1 Table 2), which has relatively high densities of RMRM (Mageroy 2015), and that the densities of the mussel are quite low at the Vaseux Lake Campsite (Mageroy 2015). These findings contribute to the combined evidence, from the Okanagan (Stanton *et al.* 2012, Mageroy 2015) and from the U.S. (Spring Rivers 2007, O'Brien *et al.* 2013), for sculpin species being the primary hosts for RMRM. Prickly sculpin is clearly the most common host species at mussel beds in Okanagan Lake (Mageroy 2016). Although it is almost absent from mussel beds in the southern Okanagan, our data from the Vaseux Lake Campsite show that it does have some functional value as a host, despite the presence of smallmouth bass and other introduced species at this site (Mageroy 2016, our data).

Overall, our findings show that introduced fish species are a threat to RMRM in the Okanagan Valley. However, the identification of northern pikeminnow as a likely host fish may alleviate this problem somewhat, as it seems to be able to coexist with the introduced fish. As mentioned, it needs to be confirmed that the pikeminnow can serve as a host, through laboratory studies (O'Brien *et al.* 2013). In addition, more information on host fish use in the Okanagan River need to be collected, as our attempts failed. Finally, we recommend that an introduced fish species assessment be completed. Such an assessment should determine the presence of introduced fish at mussel beds throughout the Okanagan Valley and determine to what extent smallmouth bass is establishing in RMRM habitats in Okanagan Lake. These studies will shed light on how great of a threat introduced species are to RMRM in the Okanagan Valley, and whether the northern pikeminnow alleviates some of the host fish issues in the system.

Mussel Surveys

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Introduction

Another aspect of Rocky Mountain ridged mussel (RMRM; *Gonidea angulata*) biology that still requires further study is its distribution within the Okanagan Valley, British Columbia (B.C.) (COSEWIC 2003, 2010, Fisheries and Oceans Canada 2010, 2011a). Over recent years, the survey effort has increased in the valley (Stanton et al. 2012, Mageroy 2015, 2016, Snook 2015). It is now known that the mussel is found intermittently from the northern end of Osoyoos Lake to the northern end of Okanagan Lake (Figure 4) (EcoCat 2015, Snook 2015, Mageroy 2016). However, there are still many areas with potential RMRM habitat that have not been surveyed. Identifying the sites with the mussel is essential to protect it, to aid its recovery (COSEWIC 2003, 2010, Fisheries and Oceans Canada 2010, 2011a), and to identify its critical habitat (Snook *In prep.* a). In this project, our aim was to identify additional sites with RMRM.

Methods

For the mussel surveys, we chose sites that were deemed to be likely RMRM habitat, based on our previous survey experience (Mageroy 2015, 2016, Snook 2015), the habitat variables identified through modelling of the mussel's habitat in Okanagan Lake, and by limited modelling results applied to

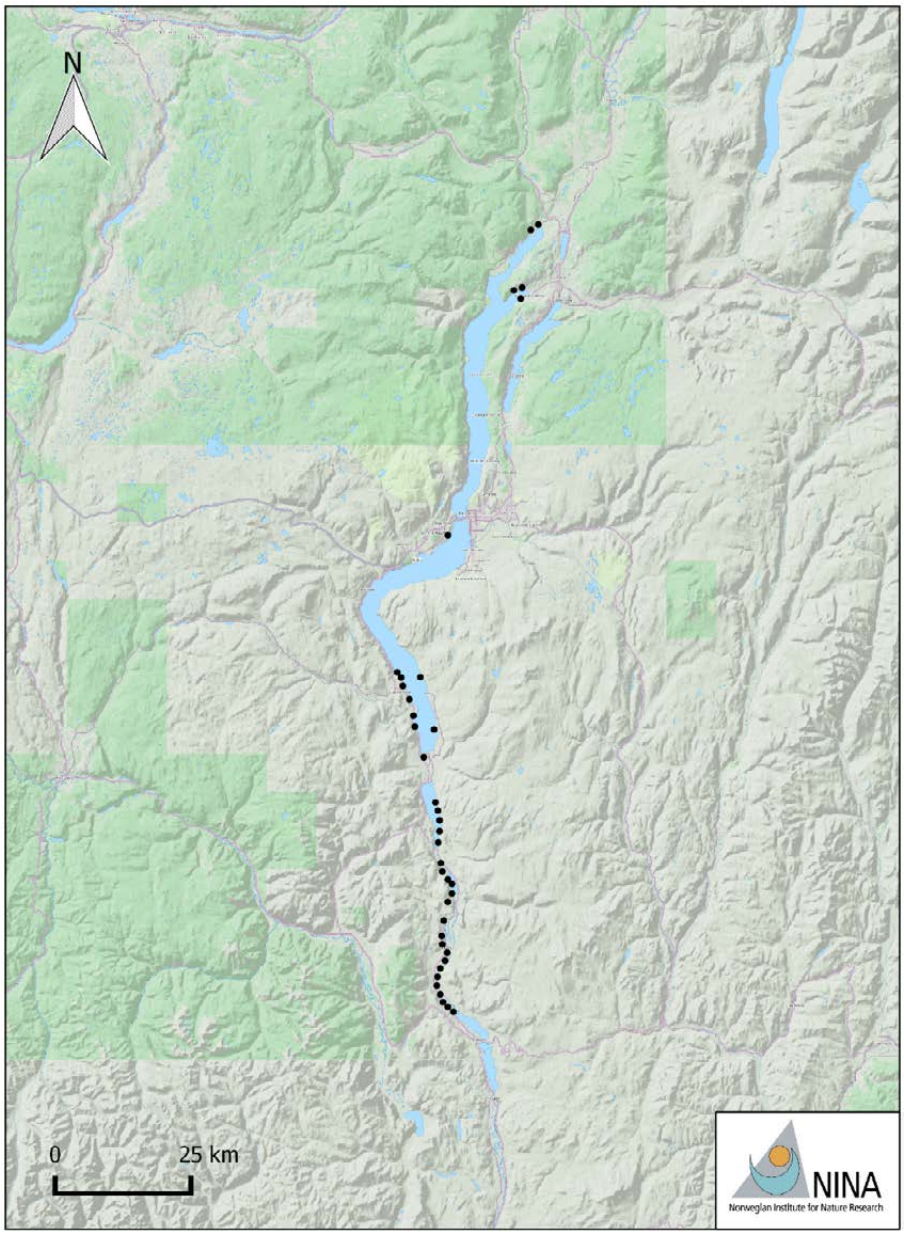

Figure 4. RMRM distribution in the Okanagan Valley, B.C. The distribution is based on data maintained by the B.C. Ministry of Environment (EcoCat 2015) and data from recent survey efforts (Snook 2015, Mageroy 2016). The map was generated in QGIS 2.16.1 (QGIS Developmental Team 2016) and the basemap is from OpenStreetMap (2017).

the lake (Snook 2015). We surveyed 32 sites (Figure 5, see Appendix 2 Table 1a-g for further details). The methodology generally followed the methodologies described by Mageroy (2015, 2016). All sites were surveyed by two surveyors. For lake sites, the surveyors swam in a grid pattern to cover the sites as thoroughly as possible, from the shoreline until the depth was too great to see the bottom. For tributary sites, the surveyors walked or crawled up the tributary, immersed themselves when possible and looked for mussels. Surveying efforts continued upstream until either the habitat became inappropriate for mussels or access was no longer possible. For each survey, the numbers of live RMRM and empty shells were recorded. The surveys were completed between July 8th and August 26th, 2016.

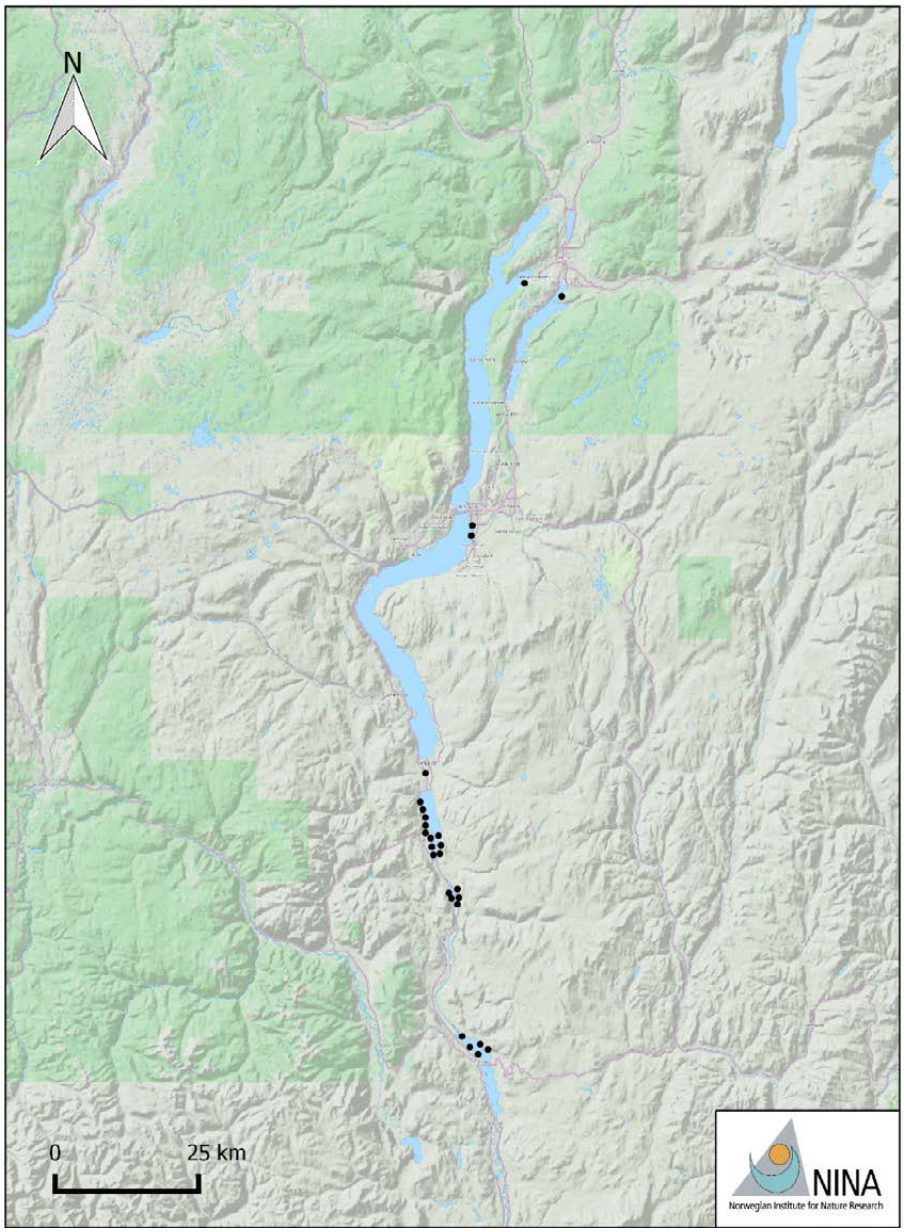

Figure 5. Mussel survey sites. Circles indicate survey locations. Note that each location typically represents more than one survey site. The map was generated in QGIS 2.16.1 (QGIS Developmental Team 2016) and the basemap is from OpenStreetMap (2017).

Results

We found 155 live RMRM and 99 RMRM shells. The live mussels were distributed across only five sites and the vast majority (136) were found at Vaseux Island in Vaseux Lake. The shells were distributed across six sites and the vast majority (82) were found at Vaseux Island. One of the sites with live mussels did not contain shells, and two of the sites with shells did not contain live mussels. See Table 1 and

Appendix 2 Figures 1a-c, for an overview of the surveys that resulted in the detection of live RMRM and RMRM shells. See Appendix 2 Table 1a-g for details on all survey results.

Discussion

Our mussel surveys contributed to increased knowledge concerning the distribution of RMRM in the Okanagan Valley. In Vaseux Lake, the surveys revealed that the mussel was much more widely

Table 1. Mussel survey results. The table only shows the results from sites where live RMRM and RMRM shells were found. Note that the shells at Vaseux Island were not found in the survey area, but when snorkeling back from the island to the mainland.

Site	Description	Water body	Start location (UTM)	End location (UTM)	Live RMRM #	RMRM shell #
Okanagan Landing	Vernon Yacht Club to O'Keefe's Landing	Okanagan Lake	11U 331360 5567278	11U 330985 5567008	7	0
Western Skaha 4	Banbury Green Point to shoreline parallel to Hemlock Rd.	Skaha Lake	11U 311574 5476548	11U 311547 5475620	0	6
Western Skaha 5	Shoreline parallel to Hemlock Rd. to Kaleden Hotel Regional Park	Skaha Lake	11U 311547 5475620	11U 312021 5474848	0	2
Eastern Skaha 1	Parsons Rd. to Devon Dr.	Skaha Lake	11U 314072 5473393	11U 313761 5472822	1	5
Western Vaseux	Southern 80 % of the western shoreline	Vaseux Lake	11U 315266 5463634	11U 316026 5460852	7	3
Eastern Vaseux	Vaseux Lake Campsite to southern point	Vaseux Lake	11U 316101 5463496	11U 316510 5462310	4	1
Vaseux Island	Circumference of Vaseux Island	Vaseux Lake	11U 316176 5461554	11U 316176 5461554	136	82

distributed and that the numbers of mussels were higher than previously known. The two new sites in Okanagan Lake and Skaha Lake added to the understanding of the local distribution in areas that were already known to contain the mussel (EcoCat 2015). The finding of two sites with RMRM shells along the western shore of Skaha Lake shows that the species has been present in this area, which corroborates previous findings (EcoCat 2015). However, the fact that we found no live RMRM in this area suggests that the species has been extirpated from the area. This seems to be a general trend in Skaha as the mussel also has disappeared or declined in numbers along the eastern shore (EcoCat 2015).

Knowing the distribution of RMRM within the Okanagan Valley is essential for protecting the species. For example, recent survey efforts (Mageroy 2015, 2016, Snook 2015), including the Vaseux Lake surveys in this study, have already facilitated a ban on rototilling to control Eurasian watermilfoil in areas where RMRM is present (Lora Nield *Pers. com.*). Rototilling is known to harm the mussel (Mageroy 2015). Survey efforts have also lead to changes in other in-stream activities, such as dredging of harbours and building of docks (Lora Nield *Pers. com.*). Knowledge of the RMRM distribution is also being used to determine the critical habitat of the species in the Okanagan Valley (Snook *In prep.* a). In addition, re-surveying may reveal changes in distribution patterns, reflecting increases and declines in the RMRM population, as shown for Skaha Lake.

Although there has been an extensive survey effort for RMRM over the last few years and we surveyed an additional 32 sites, there are still sites with potential mussel habitat in the Okanagan Valley that have not been surveyed. This especially applies to Okanagan Lake. For this lake, Roxanne Snook is applying a habitat model (Snook 2015) to identify sites with suitable habitat for RMRM (Snook *In prep.* b). We recommend that further surveys be undertaken in Okanagan Lake, once the application of the model has been completed.

Genetic Analyses

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Introduction

Nothing is known about the genetics of the Rocky Mountain ridged mussel (RMRM; *Gonidea angulata*) in the Okanagan Valley, British Columbia (B.C.). Analyzing the genetic makeup of RMRM within the valley has several potential benefits. It can determine if mussels from the Okanagan differ genetically from populations in the United States of America (U.S.). The RMRM in the Okanagan make up the northernmost extent of the species' distribution (COSEWIC 2010, Fisheries and Oceans Canada 2010, Jepsen *et al.* 2010a, B.C. Conservation Data Centre 2015a, Stanton et al. 2012), raising the possibility that these populations may be genetically isolated and locally adapted. Genetic analyses can be used to detect population differentiation at the region and population levels in the Okanagan. The southernmost and northernmost mussel beds within the Canadian part of the Okanagan Valley are more than 150 river km apart. Even within this area, some of the RMRM beds are more than 30 river km apart (Calculated using Google Earth, based on the known RMRM distribution within the Okanagan (EcoCat 2015).). Depending on the migration potential of host fish, these distances could lead to genetic differentiation within the system. In addition, RMRM use both lakes and the Okanagan River as habitat (Mageroy 2015,

2016). This differentiation in habitat use may also have created genetic differentiation. If the mussels within the Okanagan Valley are genetically unique, compared to each other or to mussels in the U.S., it increases their conservation value.

In this project, we undertook mitochondrial DNA analysis to investigate the genetic relationship between RMRM in the Okanagan and the U.S., and initiated the development of nuclear microsatellite markers to investigate the genetic relationship within the system. Mitochondrial sequence data can be used to detect deep subdivisions within species or genera (e.g., Chong *et al.* 2008), but hypervariable nuclear microsatellite loci are the tools of choice for detection of population structuring, population diversity metrics, and inbreeding metrics (Mock *et al.* 2010, 2013).

Methods

Forty RMRM were collected from five different sites within the Okanagan Valley (Figure 6), initially frozen, and later preserved in 95 % ethanol. All mussels were collected between May 21st and September 12th, 2016. Foot tissue samples were collected from each mussel and re-preserved in 95 % ethanol. These samples were shipped to the Molecular Ecology Laboratory at the Quinney College of Natural Resources, Utah State University, which performed all genetic analyses. Due to shipping regulations, samples were mailed with 95 % ethanol removed. Upon arrival, 5 mL of 95 % ethanol was added to each sample. Tissue subsets were dissected from all samples, placed in 1.7 mL microfuge tubes, and dried at 56 °C for 10 minutes to remove ethanol. The remainder of the tissue samples remained in 95 % ethanol and were archived in the lab. Genomic DNA was extracted from each of the subsampled tissues using a Qiagen DNeasy Blood and Tissue kit following the manufacturer protocol, with negative controls.

Mitochondrial Sequencing

We included five RMRM samples from each site in the mitochondrial analysis. This number of samples per population is generally considered adequate for detection of pronounced subdivision, since population-level mitochondrial diversity is not expected to be high enough to warrant large sample numbers. For each sample, we amplified an approximately 650 base pair (bp) region of the mitochondrial

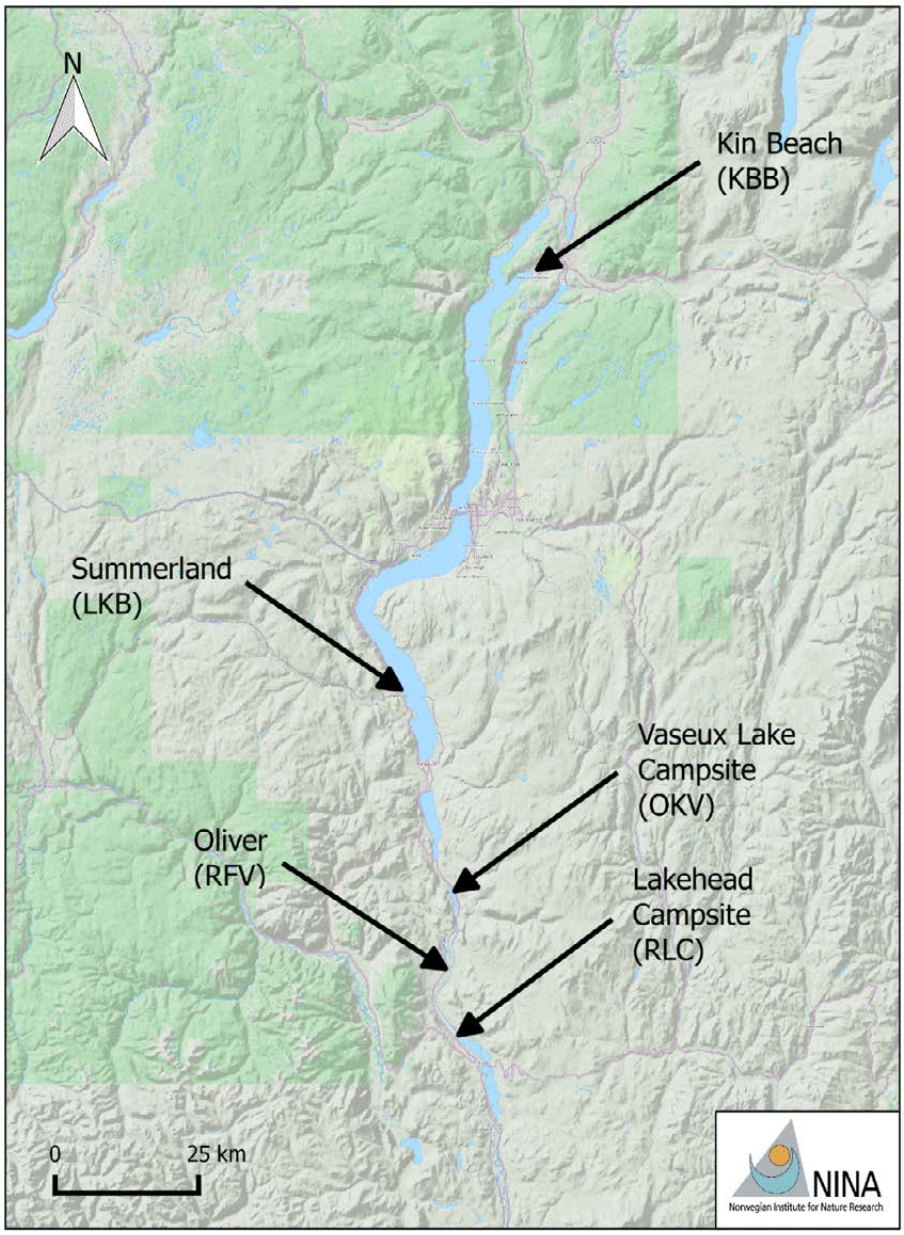

Figure 6. Genetic sampling sites. The short forms, in parentheses, are the notations used to identify the site the samples were collected from (See Figure 7, Table 2 and Appendix 3 Table 1a-e.). The map was generated in QGIS 2.16.1 (QGIS Developmental Team 2016) and the basemap is from OpenStreetMap (2017).

F-lineage cytochrome c oxidase I subunit (COI). Amplification was performed using the HCO700dy (Hoeh *et al.* 2002; 5' TCAGGGTGACCAAAAAATCA, HCO2198 first 5' end 6 bases removed) and LCO1490 (Folmer *et al.* 1994; 5' GGTCAACAAATCATAAAGATATTGG) primers. Amplification reactions contained 1x MyTaq HS Master Mix (Bioline), 0.1 mg/mL BSA, 0.5 µM of each forward and reverse primer, and approximately 15 ng genomic DNA. The reactions were denatured at 95 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 50 °C for 60 s, 72 °C for 90 s, with a final extension step of 72 °C for 5 minutes. Reactions were then analyzed for quality assurance via 1.4 % agarose gel prior to sequencing, which was performed by the

Center for Integrated Biosystems, Utah State University. Bidirectional sequences were obtained using primer pairs HCO700dy and internal primer LCO1550 (Chong *et al.* 2008).

Contiguous COI sequences, using forward and reverse sequencing primers, were constructed using Geneious software for alignment, editing, and quality assessment (Kearse *et al.* 2012). Contiguous sequences for each individual were aligned using MEGA software (Tamura *et al.* 2007) and trimmed to be comparable to pre-existing sequence data (a compilation of unique haplotypes compiled from 98 reference sequences from the Columbia, Chehalis, Klamath, and Pit River basins, representing 8 unique haplotypes (Karen Mock *Unpubl. data*)). This was done to determine whether the sequences represented divergent or distinct haplotypes, and whether the observed haplotypes were unique to the sampling sites. The alignment was trimmed to 537 bp and included the 8 previously identified haplotypes described above.

Microsatellite Development

Microsatellites, or simple sequence repeats, are regions of the nuclear genome that are hypervariable and biparentally inherited, so they are excellent genetic loci for assessing population divergence, gene flow, and inbreeding. These loci require initial development from 'shotgun' genomic sequences, followed by refinement, primer design, and population-level assessment. We performed the library preparation and initial 'shotgun' sequencing of one RMRM from each of the five sites (pooled) using an Illumina MiSeq® platform, following the manufacturer's instructions. We used a 600-cycle v3 kit with a 2X300 paired-end configuration with a single run.

Results

Mitochondrial Sequencing

The alignment contained eight variable locations: Seven were synonymous and in third codon positions, and one was in a first codon position and resulted in an amino acid replacement. The alignment revealed a total of 4 haplotypes (GonA, GonB, GonD and Unique) represented among the 25 mitochondrial COI sequences. The most common haplotype, GonD, was present at all five sample sites

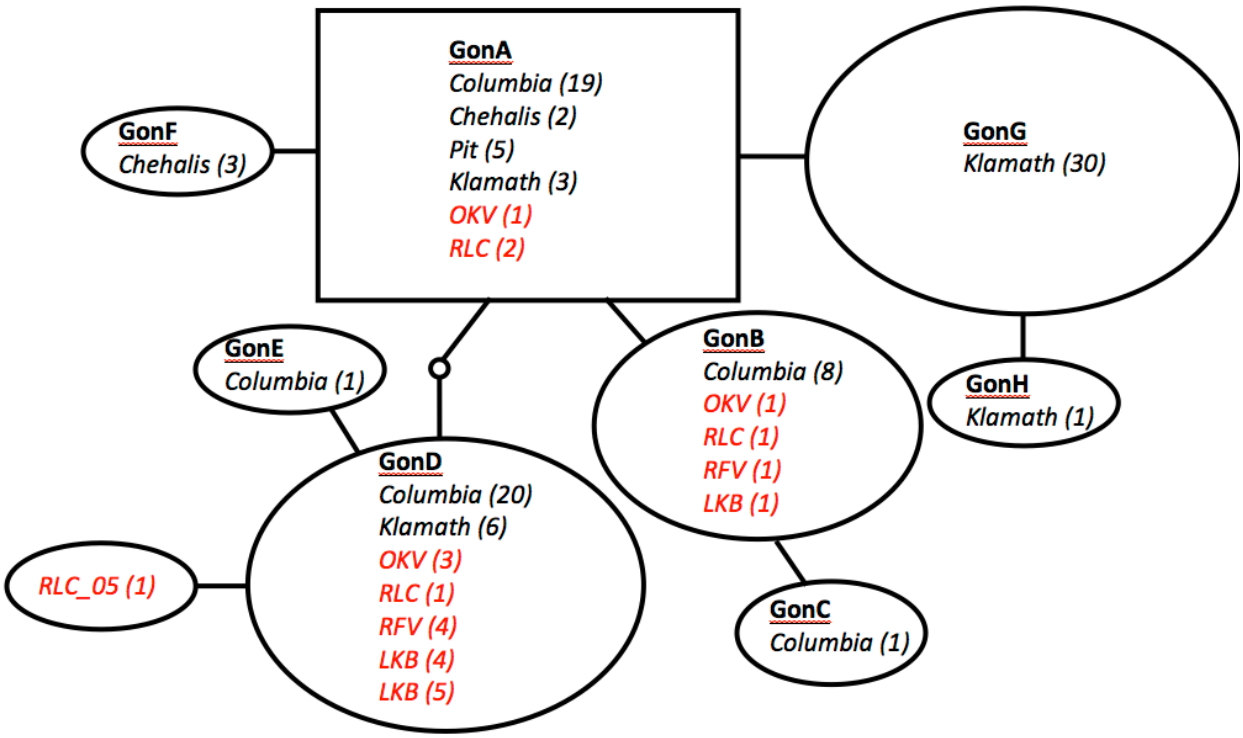

Figure 7. Network representation of RMRM haplotype data. Populations in red font represent sites sampled in the Okanagan Valley for this project. Kin Beach (KBB) = RLC_05 (1) in this diagram, Summerland = LKB, Vaseux Lake Campsite = OKV, Oliver = RFV, and Lakehead Campsite = RLC. The remaining are known haplotypes from western U.S. populations (Karen Mock *Unpubl. data*). Lines represent single base pair differences. Circles and squares represent individual observed haplotypes. The square symbol represents the presumed ancestral haplotype. For further details on the results, see Appendix 3 Table 1a-e. The figure was created using TCS software (Clement *et al.* 2000).

and was the only haplotype represented at Kin Beach (KBB). A haplotype unique to the Okanagan Valley was found at the Lakehead Campsite (RLC). See Figure 7 and Table 2. See Appendix 3 Table 1a-e for further details. Note that one RMRM sequence (RVF_08, see Appendix 3 Table 1d) had a reverse read of poor quality and had only 464 bp included in the alignment.

Microsatellite Development

The library preparation and initial 'shotgun' sequencing have been performed. This sequencing yielded over 20 million sequences of varying length. These sequences were filtered using SSR_Pipeline

(Miller *et al.* 2013) and custom bash scripts to yield a subset of 64,942 sequences, containing between 10 and 89 repeats of 4-bp motifs (e.g., ATGT), and 47,480 sequences, containing between 10 and 78 repeats of 3-bp motifs (e.g., TTC). Sequences were further filtered and motifs with ≥16 repeats, contained ≥1 'G' or 'C', and 4-mer compound motifs were removed to yield a subset of 19,446 sequences (1,602 3-mer, and 17,844 4-mer motifs). Sequences were then analyzed via BatchPrimer3 (http://primer3.sourceforge.net/) to identify suitable primer pairs in flanking microsatellite sequences. Primers selected by BatchPrimer3 were selected with a primer Tm of 57-63 °C, 2 °C difference between primers, 18-23 bp primer length, contained 45-55 % GC content, and a product size of 100-500 bp. All other parameters were set to default by BatchPrimer3. 13,420 sequences met these criteria and contained primer pairs. Both raw data and sequences are available upon request from the Molecular Ecology Laboratory at the Quinney College of Natural Resources, Utah State University. The sequences will be the focus of the next stage in microsatellite development, which will include testing for high quality single-locus amplification and population-level polymorphism.

Discussion

Mitochondrial sequencing did not show great differences in genetic makeup between the Okanagan and U.S. RMRM populations, as three of the four haplotypes also have been found in the U.S. However, one of the haplotypes was unique to the Okanagan Valley, although it only showed a one bp difference from the previously known GonD haplotype (Figure 7). This genetic uniqueness, although slight, increases the conservation value of the RMRM in the Okanagan. It may also signify genetic divergence, which will be better detected using microsatellite data. In addition, the mitochondrial haplotypes suggest that there is a genetic gradient in the system (Table 2), increasing from the northern

Table 2. Distribution of mitochondrial haplotypes across sites.

Site	Mitochondrial haplotypes
Kin Beach (KBB)	GonD
Summerland (LKB)	GonB, GonD
Vaseux Lake Campsite (OKV)	GonA, GonB, GonD
Oliver (RFV)	GonB, GonD
Lakehead Campsite (RLC)	GonA, GonB, GonD, Unique

to the southern sites. This corresponds to the genetic diversity increasing downriver, as has been shown for other freshwater mussels in the U.S. Columbia River basin (Mock *et al.* 2013). Microsatellite analysis, along with larger sample numbers, will provide a more thorough description of this pattern. To further evaluate the genetic differences within the Okanagan Valley, and between the RMRM in the Okanagan and in the U.S., additional mitochondrial DNA and nuclear microsatellite analyses are planned for the 2017-2018 fiscal year. The intent is to collect mussels from one additional site in Okanagan Lake. In addition, the intent is to increase the numbers of samples analyzed from each site to 20 mussels. These analyses will potentially identify further unique mitochondrial haplotypes and will improve the understanding of the conservation value of RMRM in the Okanagan Valley.

It is also possible to develop eDNA markers for freshwater mussels (e.g., Deiner & Altermatt 2014, Stoeckle *et al.* 2015, Cho *et al.* 2016). These markers can be a useful tool for research on RMRM, because they allow for efficient detection of these mussels without the need to sample, or even visualize them (which generally requires snorkeling and an experienced technician). eDNA markers have already be used to detect other freshwater mussel species in the field (Deiner & Altermatt 2014, Stoeckle *et al.* 2015). The mussel is currently only known from the Okanagan Valley in B.C., but there are historical records that indicate that it may have been found in other watersheds within the province (COSEWIC 2010, Fisheries and Oceans Canada 2010, Jepsen *et al.* 2010a, B.C. Conservation Data Centre 2015a, Stanton et al. 2012). The markers could be used to identify other watersheds, within B.C. and the U.S. that contain RMRM. eDNA markers may also be useful in quantifying the functional biomass of organisms (e.g., Pilliod *et al.* 2013, Baldigo *et al.* 2017). Therefore, the markers could also be used to suggest the quantity of RMRM in areas without survey data.

It has also been proposed that eDNA can be used to detect a variety of life history events (e.g., Barnes & Turner 2016, de Souza *et al.* 2016, Erickson *et al.* 2016). For example, it has been shown that quantities of eDNA increase during reproductive events (Spear *et al.* 2015). Therefore, RMRM sperm and glochidial release are likely to be associated with increased detectability of eDNA in the water. Sperm release is thought to occur during early spring in the U.S. (Spring Rivers 2007, O'Brien *et al.* 2013), and glochidial release is known to occur during late spring/early summer in the Okanagan (Mageroy 2015, 2016, our data). Sampling for eDNA during these time periods would allow for easy determination of when these life history events actually occur. Better knowledge of such reproductive events would allow us to determine periods when the mussel is especially sensitive and when it is necessary that host fish are present at the mussel beds. It has been suggested that die-offs also will result in increased eDNA

detectability (Barnes & Tuner 2016). Therefore, regular sampling could detect RMRM die-offs and be used to further investigate the cause of the die-offs. eDNA markers for RMRM will be developed in the 2017-2018 fiscal year, based on the mitochondrial sequences detected.

Overall, we recommend completing the genetic analyses outlined in the previous paragraphs, as it will contribute to a better understanding of the conservation value of RMRM in the Okanagan Valley, the detectability of the mussel outside of the system, and the detectability of life history events and die-offs.

Maximum Age Determination

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Introduction

One of the most important facts to assess when evaluating a freshwater mussel population is whether juvenile mussels are being recruited into the population (e.g., Larsen 1997, Stanton *et al.* 2012). The reason for this is that adults are known to survive even if they cannot reproduce and/or juveniles cannot survive (e.g., Larsen 1997, Jepsen 2010b, Stanton *et al.* 2012). Since freshwater mussels, including Rocky Mountain ridged mussels, are relatively long lived (e.g., Larsen 1997, Jepsen 2010a, b) populations can persist for long periods of time without reproduction and/or juvenile recruitment (Larsen 1997, Jepsen 2010a). Therefore, if only investigating adult mussels one might conclude that the mussel population is healthy, despite environmental factors having eliminated reproduction and/or recruitment.

Mageroy (2015) evaluated the juvenile recruitment among Rocky Mountain ridged mussel (RMRM; *Gonidea angulata*) in the Okanagan Valley, British Columbia (B.C.). The author concluded that recruitment was sufficient to maintain mussel numbers at some, but not all sites. However, there were some uncertainties associated with the conclusions. The evaluation was based on findings by Young *et al.* (2001). Their methodology was based on knowing the maximum age of the mussel populations. However, the maximum age of RMRM in the Okanagan is not known with certainty. Mageroy (2015) did estimate the maximum age, both based on shells collected in the system and based on a literature review. However, the method used is not very reliable for determining the age of older mussels (Neves

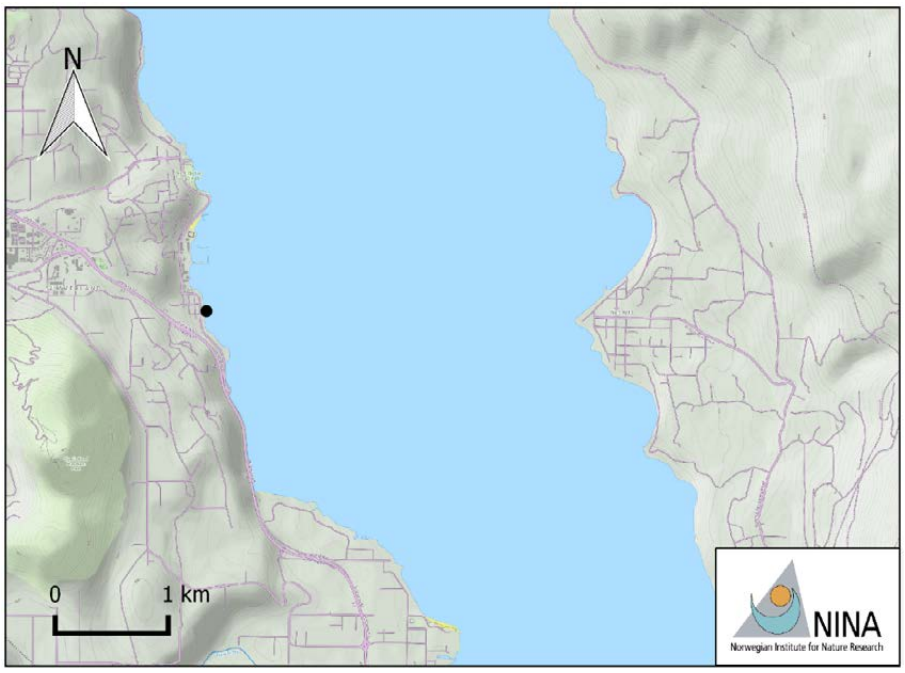

Figure 8. Shell sampling site in Summerland. The circle indicates the shell sampling site. The map was generated in QGIS 2.16.1 (QGIS Developmental Team 2016) and the basemap is from OpenStreetMap (2017).

and Moyer 1988, Downing *et al.* 1992), and the longevity of mussels varies among locations (e.g., Larsen 1997). Determining the maximum age of the mussel in the Okanagan would therefore provide greater certainty in evaluating whether juvenile recruitment in the system is sufficient to maintain mussel numbers, by reanalyzing the data from Mageroy (2015). In this project, our aim was to determine the maximum age of RMRM in the Okanagan with certainty and reanalyze the Mageroy (2015) data.

Methods

Approximately 20 empty shells of adult RMRM were collected in Okanagan Lake, by Summerland (Figure 8). The shells were collected at random. Two methods were used to evaluate the age of the shells: Counting of external growth rings and counting of internal growth rings through thin sectioning.

Counting of External Growth Rings

Even though counting external growth rings is known to be unsuitable for determining the age of older freshwater mussels (Neves & Moyer 1988, Downing *et al.* 1992), we evaluated whether this

Figure 9. Thin sectioning axes. The photo shows the thin sectioning of a RMRM valve along two axes. (Photo: Barbara Campbell

method is suitable for ageing older RMRM. We attempted to age ten valves (shells) via this method. For each valve, the growth rings were counted and the width of the rings were measured. Attempts were made to remove the periostracum from a few valves to improve the accuracy of surface aging. In addition, some valves were soaked in water/soap or water/bleach solution for 24 hrs. and scrubbed to improve the accuracy of surface aging.

Counting of Internal Growth Rings through Thin Sectioning

We attempted to age five valves via thin sectioning. Thin sections (~1mm) were removed from epoxied valves of various lengths, following the axis of maximum growth from the umbo to shell margin, and mounted on glass slides with thermo-plastic adhesive (Figure 9). On one of the five valves, another thin section was removed at a 90˚ angle to the first cut (Figure 9) to compare the growth pattern along the 2 axes. Sections were then ground and polished on both sides, until the growth pattern was clearly visible using a dissecting microscope with transmitted light. The internal growth rings were counted both in the chondrophore and mid-section areas (Figure 10).

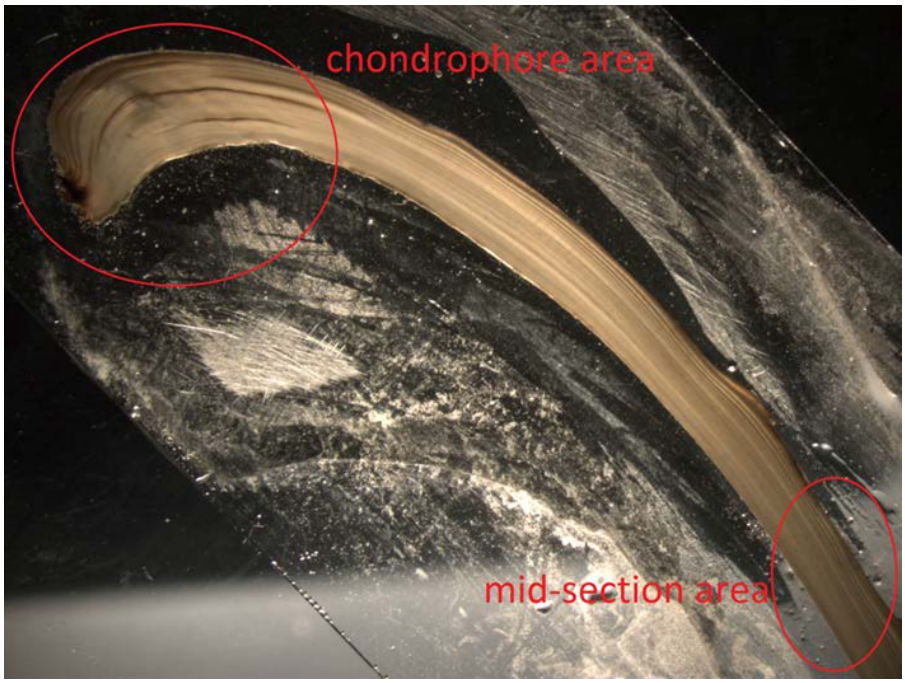

Figure 10. RMRM growth rings in the chondrophore and mid-section area. The photo shows both areas within a thin section mounted on a glass slide. (Photo: Barbara Campbell.)

Results

Counting of External Growth Rings

The counting of external growth rings was shown to be unsuitable for determining the maximum age of RMRM, as the outer growth rings became indistinguishable from each other. This was the case independent of preparation method.

Counting of Internal Growth Rings through Thin Sectioning

The counting of internal growth rings, through thin sectioning, is being explored for determining the depositional periodicity of growth zones for RMRM. Thin sections exhibited growth patterns that were reasonably clear and encouraging for age estimation of mussels (Figures 11 and 12). The thin section following the axis of maximum growth, from the umbo to shell margin, provided clearer patterns than the thin section removed at a 90˚ angle to the first cut. However, the methods need fine tuning to

Figure 11. RMRM growth rings in the chondrophore. The photo shows a back lit thin section of the area. (Photo: Barbara Campbell.)

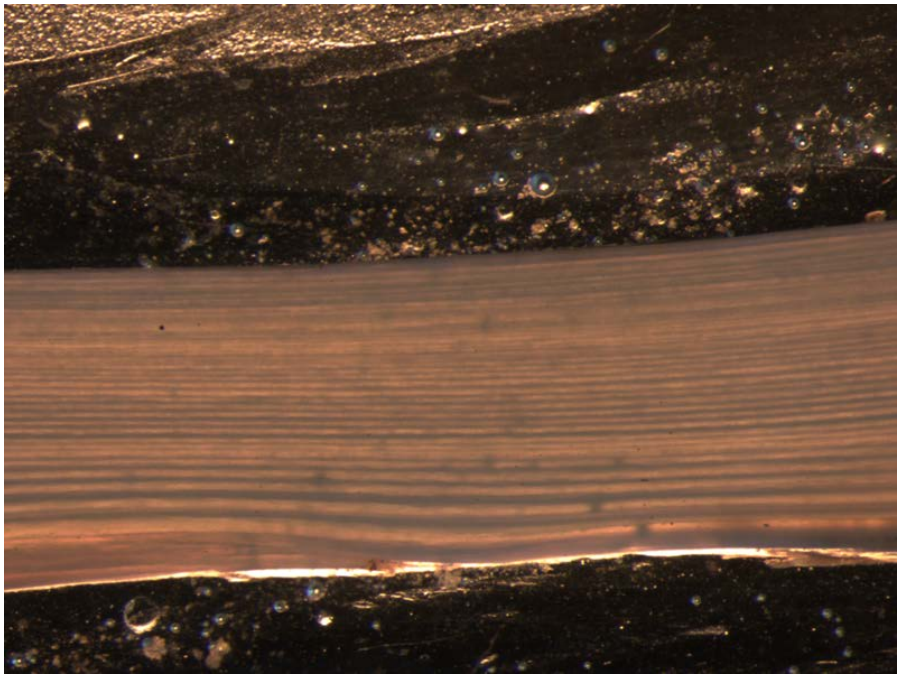

Figure 12. RMRM growth rings in the mid-section area. The photo shows a back lit thin section of the area. (Photo: Barbara Campbell.)

determine the exact axis to use for thin sectioning and whether to use the chondrophore (Figure 11) or mid-section area (Figure 12) for age estimation. In addition, validation studies need to be employed to confirm the annual periodicity of deposited microstructural growth. Studies such as bomb carbon, lead-radium, and oxygen isotopes (to name a few) produce a chemical age. This age, when compared to the microstructural age, can validate both the structure and technique employed, if similar age estimates are

produced. Based on recommendations by Stephen Wischniowski (*Pers. com.*), we propose an oxygen isotopic study by way of Ion Microprobe/Secondary Ion Mass Spectrometry (SIMS) technology to validate age estimates of RMRM.

Discussion

Age determination of RMRM within the Okanagan Valley will continue to be explored during 2017-2018 fiscal year. The completion of this analysis will allow us to better evaluate the juvenile recruitment within the system. Knowing the maximum age with certainty will allow us to apply the criteria developed by Young et al. (2001) more accurately to the data collected by Mageroy (2015). Reanalyzing the data, with the new maximum age in mind, will allow us to determine if RMRM juvenile recruitment is sufficient to maintain the mussel in the Okanagan, as a whole, with more certainty. It will also allow us to determine if juvenile recruitment is sufficient to maintain mussel numbers at the individual mussel beds, sampled by Mageroy (2015).

We recommend that the maximum age determination is completed and that the juvenile recruitment among RMRM in the Okanagan Valley is re-evaluated, as planned.

Conclusions and Recommendations

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Overall, this project has enhanced our understanding of Rocky Mountain ridged mussel (RMRM; *Gonidea angulata*) biology in the Okanagan Valley, British Columbia (B.C.). Our findings have contributed to an increased understanding of host fish use by the mussel, the distribution of the mussel within the Okanagan, the genetic characteristics of the population, and the maximum age of the population. Some of these investigations are complete and some are not, at this time. When all the investigations have been completed, our findings will allow us to better understand if host fish availability is a threat to RMRM, protect sites with the mussel and its habitat, evaluate the conservation value of the mussel beds, and evaluate whether juvenile recruitment is sufficient to maintain mussel numbers in the Okanagan Valley.

The host fish field sampling revealed that introduced fish do not function as hosts for RMRM in the Okanagan Valley. Therefore, our data support previous findings that suggest that introduced fish pose a threat to the mussel, by displacing native host fish from the mussel beds. This especially applies to sites in the southern Okanagan (Mageroy 2016). However, our data show that the northern pikeminnow is a likely secondary host for the mussel. This species is quite common in the southern Okanagan (Mageroy 2016) and clearly is able to coexist with these introduced species. Therefore, it is likely to be a very important host in this part of the system and may contribute to threat reduction from introduced fish. As introduced fish establish in the northern Okanagan, it may also become an even more important host species in this part of the system.

To gain a better understanding of how great a threat to RMRM introduced fish are and whether the northern pikeminnow contributes to alleviating this threat, further studies are needed. Our field data can only strongly suggest that northern pikeminnow is a host for the mussel. This suggestion needs to be confirmed through laboratory studies (O'Brien *et al.* 2013). In addition, more information on host fish use in the Okanagan River needs to be collected, as our attempts failed. Finally, we also recommend that

an introduced fish species assessment be completed. Such an assessment should determine the presence of introduced fish at mussel beds throughout the Okanagan Valley and to what extent smallmouth bass is establishing in RMRM habitats in Okanagan Lake.

The mussel surveys resulted in detecting 155 RMRM at 5 sites that had previously not been surveyed. These sites were in areas known to contain the mussel. However, the Vaseux Lake surveys revealed that the distribution and numbers within this lake were much greater than previously known. Findings of shells, but no live RMRM, along the western shore of Skaha Lake supports previous findings that show that the mussel is in decline in this lake. Knowing the distribution of RMRM within the Okanagan Valley is essential in protecting the species, from a variety of human activities (COSEWIC 2003, 2010, Fisheries and Oceans Canada 2010, 2011a). Knowledge of the RMRM distribution is also being used to determine the critical habitat of the species within the Okanagan (Snook *In prep.* a). In addition, re-surveying may reveal changes in distribution patterns, reflecting increases and declines in the RMRM population, as shown for Skaha Lake.

Although our findings contribute to the extensive survey efforts for RMRM over the last few years, there are still sites with potential mussel habitat that have not been surveyed. This especially applies to Okanagan Lake. For this lake, we suggest that further studies are undertaken based on the application of a habitat model (Snook 2015) that will identify sites with habitat suitable for RMRM (Snook *In prep.* b).

The mitochondrial DNA analysis revealed that there are genetic differences between the mussels in the Okanagan Valley and mussels in the United States of America (U.S.), although these differences are only slight. In addition, the analysis suggests that there is a genetic gradient within the Okanagan. The findings suggest that the genetic diversity increases downriver. However, microsatellite analyses are better suited for studying such a gradient. Such analyses are under way, but no conclusions can be drawn at this point in time. Even so, the genetic analyses indicate that there are genetic differences between RMRM beds within the Okanagan Valley, and between the mussels in the Okanagan and the U.S.

We recommend that the genetic analyses described above be completed. An additional site and additional samples are planned to be included in both the mitochondrial DNA and microsatellite analyses, and the latter needs further development before it can answer questions about the genetic relatedness among mussels within the Okanagan Valley. In addition, we recommend developing eDNA markers for RMRM. These markers can be used to detect the mussel outside of the Okanagan, and to

detect life history events and mussel die-offs. It is the intent to complete all of these genetic analyses during the 2017-2018 fiscal year.

Our project suggests that counting internal growth rings through thin sectioning is suitable, while counting external growth rings is unsuitable, for estimating the maximum age of RMRM. However, the methodology is still being developed and needs independent validation, via other techniques such as isotopic oxygen analysis of growth patterns. The age determination research will continue during the 2017-2018 fiscal year.

We recommend that the maximum age determination be completed. This will allow us to reanalyze the data on juvenile recruitment from Mageroy (2015) with greater certainty. The reanalysis will allow us to determine if juvenile recruitment is sufficient to maintain RMRM numbers within the individual mussel beds in the Okanagan Valley.

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Second, we would like to thank all of those who contributed to the project through practical help, advice, feedback on reporting, etc.: Sean MacConnachie provided feedback on the host fish and mussel methodology, and on all chapters of the finalized report. Lora Nield (British Columbia Ministry of Forests, Lands, and Natural Resource Operations) contributed to developing the host fish and mussel survey methodology, and provided some of the equipment necessary for the project. Greg Wilson provided feedback on the host fish and mussel methodology, and on all the chapters of the finalized report. In addition, he contributed to the host fish sampling through electrofishing. Stephen Wischniowski (DFO) provided scientific advice on the ageing of the mussels and gave feedback on the maximum age determination chapter.

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Appendices

Appendix 1: Host Fish Field Sampling

Methods

For specific location details, including UTMs, and description of the host fish sampling sites, see Appendix 1 Table 1.

Results

For detailed results on fish collection, prevalence, and intensity, see Appendix 1 Table 2. Note that fish only were caught at Kin Beach and the Vaseux Lake Campsite.

Appendix 1 Table 1. Fish collection sites.

Site	**Type**	**Location (UTM)**	**Description**
Kin Beach	Lake	11U 332259 5568979	Northeastern corner of beach
Vaseux Lake Campsite	Lake	11U 316049 5463620	Southern end of the campsite
Oliver	River	11U 314236 5451625 to 11U 314407 5450979	Between pedestrian bridge and Fairview Rd. Bridge
# 22 Rd. Bridge	River	11U 314907 5440470 to 11U 315238 5440062	Between # 22 Rd. Bridge and Weir # 1

Appendix 1 Table 2. Fish collection data. The asterisk indicates that only northern pikeminnow and prickly sculpin had encapsulated RMRM glochidia on their gills. All other species of fish only had non-encapsulated glochidia. Note that the yellow perch were found dead at the site, but six were in sufficient condition to have their gills examined.

Fish species	Kin Beach			Vaseux Lake Campsite		
	Total caught	Prevalence (% infected)	Intensity (glochidia/fish)	Total caught	Prevalence (% infected)	Intensity (glochidia/fish)
Brown bullhead	0	NA	NA	2	100	1.5
Largemouth bass	0	NA	NA	2	100	4
Northern pikeminnow*	28	75.0	7.1	0	NA	NA
Prickly sculpin*	4	0	0	14	42.9	2.2
Pumpkinseed sunfish	0	NA	NA	11	45.5	1.2
Smallmouth bass	0	NA	NA	16	37.5	2.3
Yellow perch	0	NA	NA	9	33.3	1.5

Appendix 2: Mussel Surveys

Methods

For specific location details, including UTMs, for the mussel survey sites, see Appendix 2 Table 1a-g.

Results

For detailed results for each survey site, see Appendix 2 Table 1a-g. For an overview of the surveys that resulted in the detection of RMRM, see Appendix 2 Figures 1a-c.

Appendix 2 Table 1a. Okanagan Lake mussel survey sites with results.

Location	Description	Start location (UTM)	End location (UTM)	Live RMRM #	RMRM shell #
Okanagan Landing	Vernon Yacht Club to O'Keefe's Landing	11U 331360 5567278	11U 330985 5567008	7	0
Kelowna 1	Maude Roxby to Watt Park	11U 320407 5527269	11U 320497 5526077	0	0
Kelowna 2	Watt Park to Mission Creek	11U 320497 5526077	11U 320665 5524206	0	0

Appendix 2 Table 1b. Kalamalka Lake mussel survey sites with results.

Location	Description	Start location (UTM)	End location (UTM)	Live RMRM #	RMRM shell #
Kalamalka Lake 1	Western to eastern end of Kaloya Regional Park	11U 330547 5554205	11U 330696 5554200	0	0
Kalamalka Lake 2	Ponderosa Way Point to Juniper Bay Point	11U 337042 5564087	11U 336833 5563725	0	0

Appendix 2 Table 1c. Penticton Channel tributaries mussel survey sites with results.

Location	Description	Start location (UTM)	End location (UTM)	Live RMRM #	RMRM shell #
Penticton Tributary 1	Shingle Creek mouth	11U 311779 5484001	NA	0	0
Penticton Tributary 2	Ellis Creek mouth	11U 311872 5483723	NA	0	0

Appendix 2 Table 1d. Western Skaha Lake mussel survey sites with results.

Location	Description	Start location (UTM)	End location (UTM)	Live RMRM #	RMRM shell #
Western Skaha 1	Shoreline parallel to the southern part of the Penticton Oliver Hwy.	11U 310764 5478761	11U 310926 5478372	0	0
Western Skaha 2	Shoreline from the Penticton Oliver Hwy. to N. Pineview Dr.	11U 310926 5478372	11U 311315 5477082	0	0
Western Skaha 3	Shoreline from N. Pineview Dr. to Banbury Green Point	11U 311315 5477082	11U 311574 5476548	0	0
Western Skaha 4	Banbury Green Point to shoreline parallel to Hemlock Rd.	11U 311574 5476548	11U 311547 5475620	0	6
Western Skaha 5	Shoreline parallel to Hemlock Rd. to Kaleden Hotel Regional Park	11U 311547 5475620	11U 312021 5474848	0	2
Western Skaha 6	Kaleden Hotel Regional Park to N. Alder Ave.	11U 312021 5474848	11U 312394 5474136	0	0
Western Skaha 7	N. Alder Ave to 6th St. Boat Launch	11U 312394 5474136	11U 312731 5473420	0	0
Western Skaha 8	6th St. Boat Launch to Old Kaleden Rd.	11U 312731 5473420	11U 312759 5470497	0	0
Western Skaha 9	Old Kaleden Rd. to Kettle Valley Trail Bridge	11U 312759 5470497	11U 312562 5469490	0	0

Appendix 2 Table 1e. Eastern Skaha Lake mussel survey sites with results.

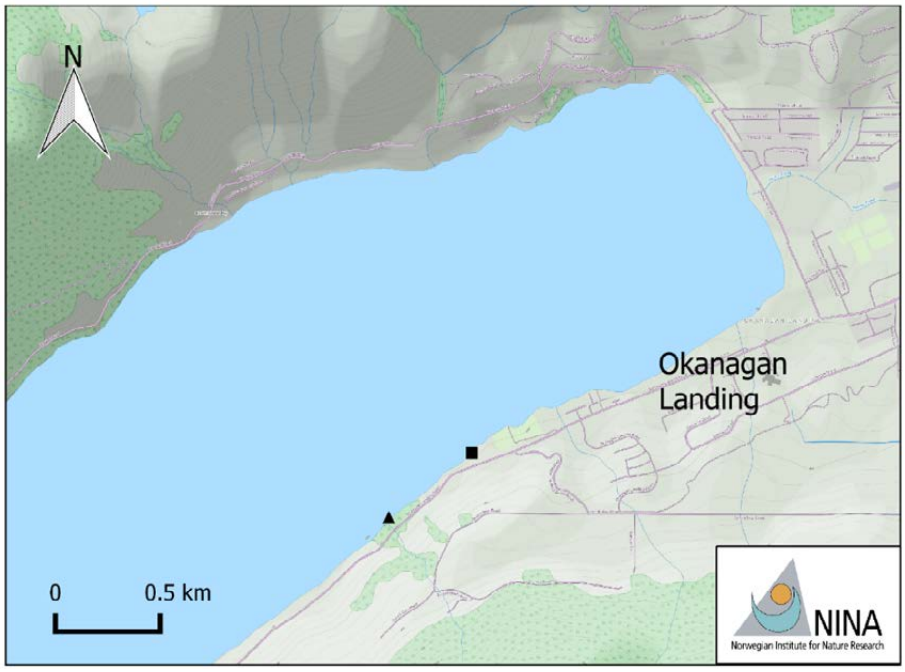
Location	Description	Start location (UTM)	End location (UTM)	Live RMRM #	RMRM shell #
Eastern Skaha 1	Parsons Rd. to Devon Dr.	11U 314072 5473393	11U 313761 5472822	1	5
Eastern Skaha 2	Devon Dr. to Camberly Cove	11U 313761 5472822	11U 313507 5472208	0	0
Eastern Skaha 3	Camberly Cove to Echo Bay	11U 313507 5472208	11U 313214 5471505	0	0
Eastern Skaha 4	Echo Bay	11U 313214 5471505	11U 313333 5471402	0	0
Eastern Skaha 5	Echo Bay to Kipper Cove	11U 313333 5471402	11U 313362 5471317	0	0
Eastern Skaha 6	Kipper Cove	11U 313362 5471317	11U 313390 5471084	0	0
Eastern Skaha 7	Kipper Cove to Skaha Water Gardens	11U 313390 5471084	11U 313550 5470281	0	0
Eastern Skaha 8	Skaha Water Gardens to Holy Rd. Point	11U 313550 5470281	11U 313393 5469715	0	0

Appendix 2 Table 1f. Vaseux Lake mussel survey sites with results.

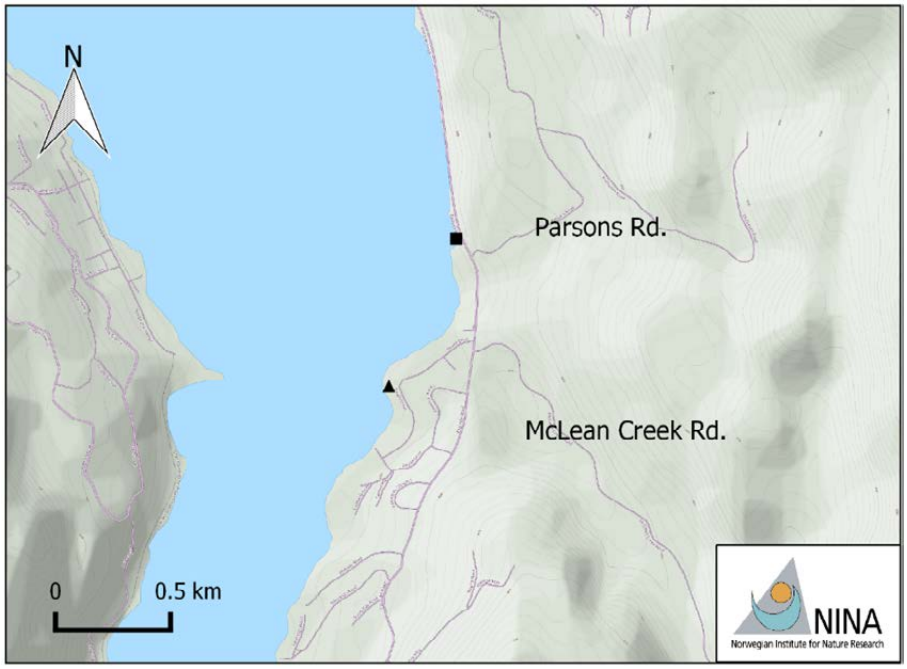
Location	Description	Start location (UTM)	End location (UTM)	Live RMRM #	RMRM shell #
Western Vaseux	Southern 80 % of the western shoreline	11U 315266 5463634	11U 316026 5460852	7	3
Eastern Vaseux	Vaseux Lake Campsite to southern point	11U 316101 5463496	11U 316510 5462310	4	1
Vaseux Island	Circumference of Vaseux Island	11U 316176 5461554	11U 316176 5461554	136	82

Appendix 2 Table 1g. Osoyoos Lake mussel survey sites with results.

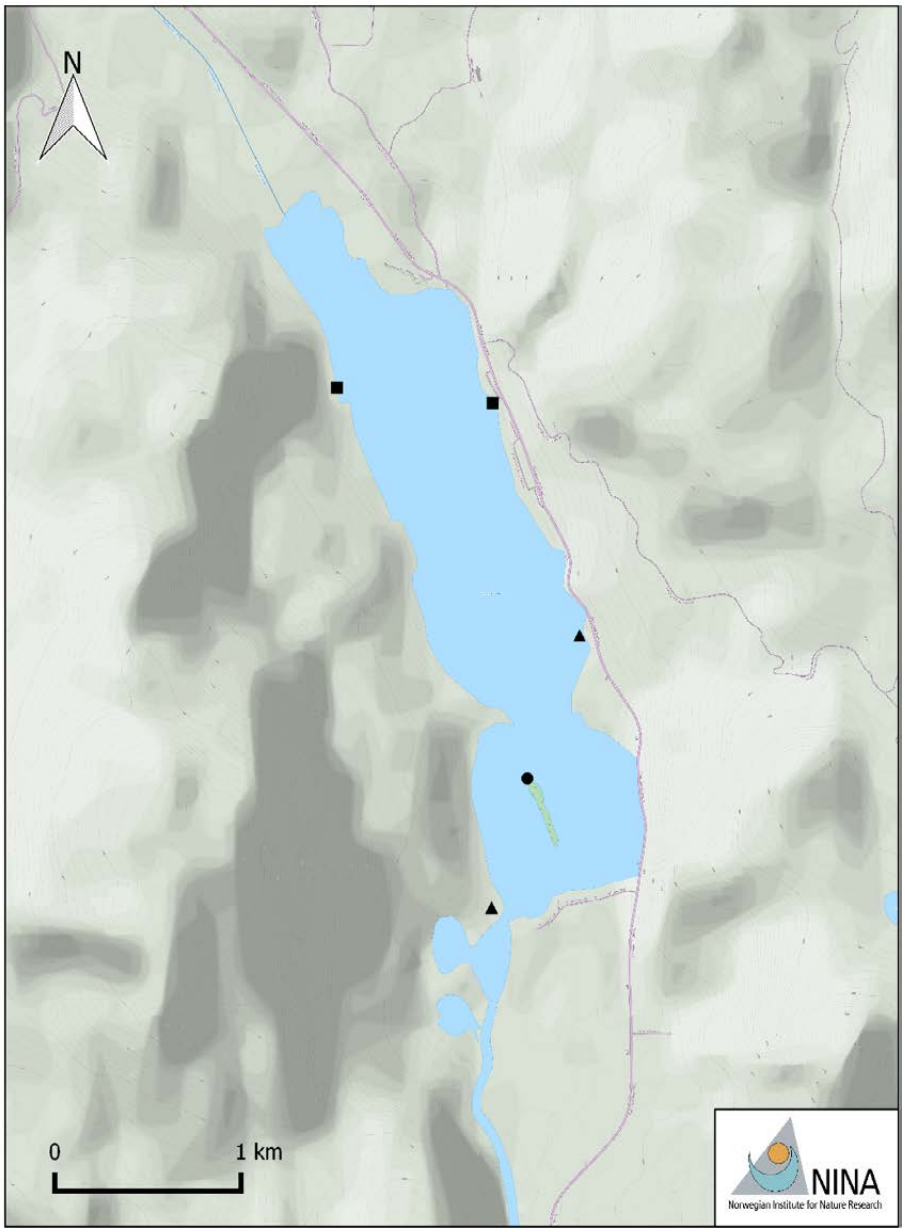
Location	Description	Start location (UTM)	End location (UTM)	Live RMRM #	RMRM shell #
Eastern Osoyoos 1	Unnamed Rd. Point to northern White Sands Point	11U 319176 5437250	11U 319729 5436214	0	0
Eastern Osoyoos 2	Northern White Sands Point to southern White Sands Point	11U 319729 5436214	11U 320143 5436024	0	0
Western Osoyoos 1	148th Ave. to Northern Spartan Dr.	11U 317738 5436774	11U 319879 5435065	0	0
Western Osoyoos 2	Northern Spartan Dr. to Park Pl.	11U 319879 5435065	11U 320141 5434116	0	0
Osoyoos Tributary	Nk'Mip Creek mouth	11U 317028 5438300	NA	0	0



Appendix 2 Figure 1a. Okanagan Landing mussel survey. The rectangle and the triangle represent the start and end of the survey, respectively. Seven live RMRM were found during this survey. The map was generated in QGIS 2.16.1 (QGIS Developmental Team 2016) and the basemap is from OpenStreetMap (2017).



Appendix 2 Figure 1b. Parsons Rd. mussel survey. The rectangle and the triangle represent the start and end of the survey, respectively. One live RMRM was found during this survey. The map was generated in QGIS 2.16.1 (QGIS Developmental Team 2016) and the basemap is from OpenStreetMap (2017).



Appendix 2 Figure 1c. Vaseux Lake mussel surveys. The rectangles and the triangles represent the start and end of the western and eastern shore surveys, respectively. The circle indicates both the start and the end of the island survey, as the entire shoreline was searched. 7, 4, and 136 live RMRM were found during the western, eastern, and island surveys, respectively. The map was generated in QGIS 2.16.1 (QGIS Developmental Team 2016) and the basemap is from OpenStreetMap (2017).

Appendix 3: Genetic Analyses

Results

For the results, with respect to the mitochondrial haplotype for each RMRM sampled, see Appendix 3 Table 1a-e.

Appendix 3 Table 1a. Mitochondrial haplotype data from Kin Beach. KBB is the notation used to identify that the samples were collected from this site.

Sample ID	Haplotype	# of bp from GonD
KBB_01	GonD	0
KBB_04	GonD	0
KBB_05	GonD	0
KBB_08	GonD	0
KBB_10	GonD	0

Appendix 3 Table 1b. Mitochondrial haplotype data from Summerland. LKB is the notation used to identify that the samples were collected from this site.

Sample ID	Haplotype	# of bp from GonD
LKB_01	GonD	0
LKB_05	GonD	0
LKB_06	GonD	0
LKB_07	GonB	3
LKB_08	GonD	0

Appendix 3 Table 1c. Mitochondrial haplotype data from the Vaseux Lake Campsite. OKV is the notation used to identify that the samples were collected from this site.

Sample ID	Haplotype	# of bp from GonD
OKV_01	GonA	2
OKV_02	GonD	0
OKV_03	GonD	0
OKV_04	GonD	0
OKV_06	GonB	1

Appendix 3 Table 1d. Mitochondrial haplotype data from Oliver. RFV is the notation used to identify that the samples were collected from this site.

Sample ID	Haplotype	# of bp from GonD
RFV_01	GonD	0
RFV_03	GonD	0
RFV_06	GonD	0
RFV_07	GonD	0
RFV_08	GonB	3

Appendix 3 Table 1e. Mitochondrial haplotype data from the Lakehead Campsite. RLC is the notation used to identify that the samples were collected from this site.

Sample ID	Haplotype	# of bp from GonD
RLC_01	GonD	0
RLC_03	GonA	2
RLC_04	GonA	2
RLC_05	Unique	1
RLC_08	GonB	3