Rocky Mountain ridged mussel (*Gonidea angulata*) in the Okanagan Valley, BC:

Final report on juvenile recruitment, host fish field sampling, and the impact of rototilling against Eurasian watermilfoil (*Myriophyllum spicatum*).

March 15, 2014

Photo: Roxanne Snook

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On behalf of the University of British Columbia – Okanagan,

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Introduction

The Rocky Mountain ridged mussel (*Gonidea angulata*) is COSEWIC listed as endangered (COSEWIC 2011) and SARA listed as a Species of Concern (Fisheries and Oceans Canada, 2010) in Canada. It is only found within the Okanagan Valley in British Columbia (Stanton *et al.* 2012), and the province has red-listed it with a S1 status (BC Conservation Data Centre 2012a). However, very little is known about the biology of this mussel in general (reviewed in Jepsen *et al.* 2010b, COSEWIC 2011, Fisheries and Oceans Canada 2010, BC Conservation Data Centre 2012b). Even less is known about its current status and the threats to its survival in BC (see discussions in COSEWIC 2011, Fisheries and Ocean Canada 2010, BC Conservation Data Centre 2012a,b, Stanton *et al.* 2012).

Due to the lack of knowledge about Rocky Mountain ridged mussel, the *Species at Risk* Management Plan for the mussel (Fisheries and Oceans Canada 2010) emphasizes the importance of doing research to provide the necessary information to help protect the mussel and contribute to its recovery in Canada. Specifically, it states that "[p]riority research [on the mussel] 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" (p. 33 in Fisheries and Oceans Canada 2010). Among the potential threats against this mussel in the Okanagan Valley are in-stream development, historic channelization of the Okanagan River, water flow alterations, introduced species, host species availability, land-use pollution, activities with direct harmful impact (e.g. mussel collection), and climate change (see COSEWIC 2011, Fisheries and Oceans Canada 2010). Thus, the research efforts on the mussel should focus on understanding its basic biology and these threats.

One of the most important facts to determine when evaluating a freshwater mussel population is whether juvenile mussels are being recruited into the population (see e.g. review in Larsen 1997, discussion in 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 (reviewed in e.g. Larsen 1997, Jepsen 2010a, Stanton *et al.* 2012). Since freshwater mussels, including Rocky Mountain ridged mussel, are relatively long lived (reviewed in e.g. Larsen 1997, Jepsen 2010a,b) populations can persist for a long period of time without reproduction and/or juvenile recruitment (reviewed in e.g. Larsen 1997, Jepsen 2010a). Thus, if only investigating adult mussels one might come to the conclusion that the mussel population is healthy, despite environmental factors having eliminated reproduction and/or recruitment. Although it is known that Rocky Mountain ridged mussels reproduce within the Okanagan Valley (Fisheries and Oceans Canada 2010, Stanton *et al.* 2012), very little is known about the recruitment of juveniles into the population (Lora Nield *Pers. com.*). The few surveys that have been undertaken have only revealed larger mussels (Lora Nield *Pers. com.*, Stanton *et al.* 2012). Thus, it is essential to investigate whether recruitment is occurring in the Okanagan Valley, where it is occurring, and whether the recruitment is occurring at a level sufficient to sustain the population. If this is not the case, a juvenile stocking program may be necessary (for examples, see reviews in Neves 2004, Thomas *et al.* 2010).

Among the potential threats to Rocky Mountain ridged mussel in the Okanagan Valley is the lack of fish host availability (COSEWIC 2011, Fisheries and Oceans Canada 2010). A lack of fish hosts may, among other things, be the result of invasive fish species displacing suitable native host fish from the mussel beds (COSEWIC 2011, Fisheries and Oceans Canada 2010). From the US it is known that the mussel's glochidia only can metamorphose into juvenile mussels on a few host species (Spring Rivers 2007, O'Brien *et al.* 2013). However, the host is unknown in Canada, although limited field sampling suggests that both northern pikeminnow (*Ptychochelius oregonensis*) and prickly sculpin (*Cottus asper*) may be host fish (Stanton *et al.* 2012). Without knowing the host for the mussel, it is impossible to determine whether the lack of fish host availability is a threat to the mussel in the Okanagan Valley. Similarly, it is impossible to determine if invasive fish are a threat to the mussel by displacing suitable host fish from the mussel beds.

Another potential threat to Rocky Mountain ridged mussel in the Okanagan Valley is associated with the invasive plant Eurasian watermilfoil (*Myriophyllum spicatum*). The species may potentially be a threat, in itself, as it alters the littoral habitat (COSEWIC 2011, Fisheries and Canada 2010). Thus, treating against this plant may be beneficial to the mussel. However, one of the treatment methods in use encompasses rototilling the substrate (for details, see Dunbar 2009). This method may be a threat to the mussel (COSEWIC 2011, Fisheries and Oceans Canada 2010), as it may directly crush the mussel or bury it, which is known to negatively impact the mussel (Krueger *et al.* 2007). Further, it may also alter the substrate (Dunbar 2009), which can affect the mussel. Thus, treating against watermilfoil may be positive or negative for the mussel, depending on the methods used.

The BC Ministry of Forests, Lands, and Natural Resource Operations, the BC Ministry of Environment, and the University of British Columbia - Okanagan have launched a research project on Rocky Mountain ridged mussel. The overall goal of the project is to improve the management of this endangered species. This project includes research on, among other things, these three areas related to the conservation of the mussel in the Okanagan Valley: 1) Determining whether Rocky Mountain ridged mussel recruitment is occurring, where it is occurring, and if it is sufficient to maintain the population. 2) Investigating the host fish use of Rocky Mountain ridged mussel based on field sampling. 3) Evaluating the impact of rototilling against Eurasian watermilfoil on Rocky Mountain ridged mussel. To achieve these goals a variety of methods, including surveys and fish sampling, will be used.

Methods

Recruitment of juvenile Rocky Mountain ridged mussels

The goal of the surveys on juvenile recruitment is to determine whether juvenile Rocky Mountain ridged mussels are being recruited in the Okanagan Valley, where this recruitment is occurring, and if it is sufficient to maintain the mussel population. To achieve the two former goals it is sufficient to detect juveniles. However, to determine whether recruitment is occurring at a sufficient rate is more complicated. For the eastern pearlshell (*Margaritifera margaritifera*), it has been suggested that a healthily recruiting population contains 20 % mussels 20 years old or younger, and some mussels 10 years old or younger (Young *et al.* 2001). However, since Rocky Mountain ridged mussels live shorter than pearlshells (see reviews in e.g. Larsen 1997, COSEWIC 2011, Fisheries and Oceans Canada 2010, Jepsen *et al.* 2010a,b) these ages will have to be adjusted. To determine the approximate age of Rocky Mountain ridged mussels in the Okanagan Valley, the number of growth rings in the mussels shells will be counted (see e.g. review in Larsen 1997, Ruppert *et al.* 2004). Based on the maximum age of the mussel, the percentage of mussels that need to be under a certain age to maintain a healthy population will be established. These methods are adapted from Larsen and Hartvigsen (1999).

To maximize the likelihood of finding juveniles and due to the importance of high density locations to overall population numbers, surveys were limited to locations with a substantial

Table 1 Overview of locations for Rocky Mountain ridged mussel juvenile recruitment surveys. See Appendix C for more detailed overview of each location.

Location	Town	Waterbody	UTM
Kin Beach, Vernon Arm	Vernon	Okanagan Lake	11U 332244 5568992
Peach Orchard Beach	Summerland	Okanagan Lake	11U 308430 5498588
Dog Beach	Summerland	Okanagan Lake	11U 308563 5498378
Kinsman Park	Summerland	Okanagan Lake	11U 308458 5497453
Pumphouse	Summerland	Okanagan Lake	11U 308469 5497360
South Okanagan Sailing Association	Summerland	Okanagan Lake	11U 308569 5497093
Three Mile Beach, Naramata Benchlands	Penticton	Okanagan Lake	11U 313605 5490390
Vaseux Campsite	Oliver	Vaseux Lake	11U 316027 5463628
Downtown Oliver	Oliver	Okanagan River	11U 314292 5451415

number of adult Rocky Mountain ridged mussels. Based on these selection criteria nine locations were selected and surveyed (see Table 1 and Figure 1).

Detecting juvenile freshwater mussels is difficult due to their small size (reviewed in e.g. Larsen and Hartvigsen 1997, Stanton *et al.* 2012) and the fact that they are typically buried in the substrate (reviewed in e.g. Larsen 1997, Strayer *et al.* 2004, Jepsen 2010a). To maximize the chance of finding a representative age distribution of Rocky Mountain ridged mussels, transect surveys were undertaken at each location. The transects were placed at regular intervals throughout the mussels beds and ran from the shoreline to the end of the mussel beds. Both visible and buried mussels were measured as a proxy for age. The buried mussels were detected by removing rocks from within the transects and carefully fanning away the rest of the substrate. The substrate was removed as far down as possible, which was typically down to a layer of clay

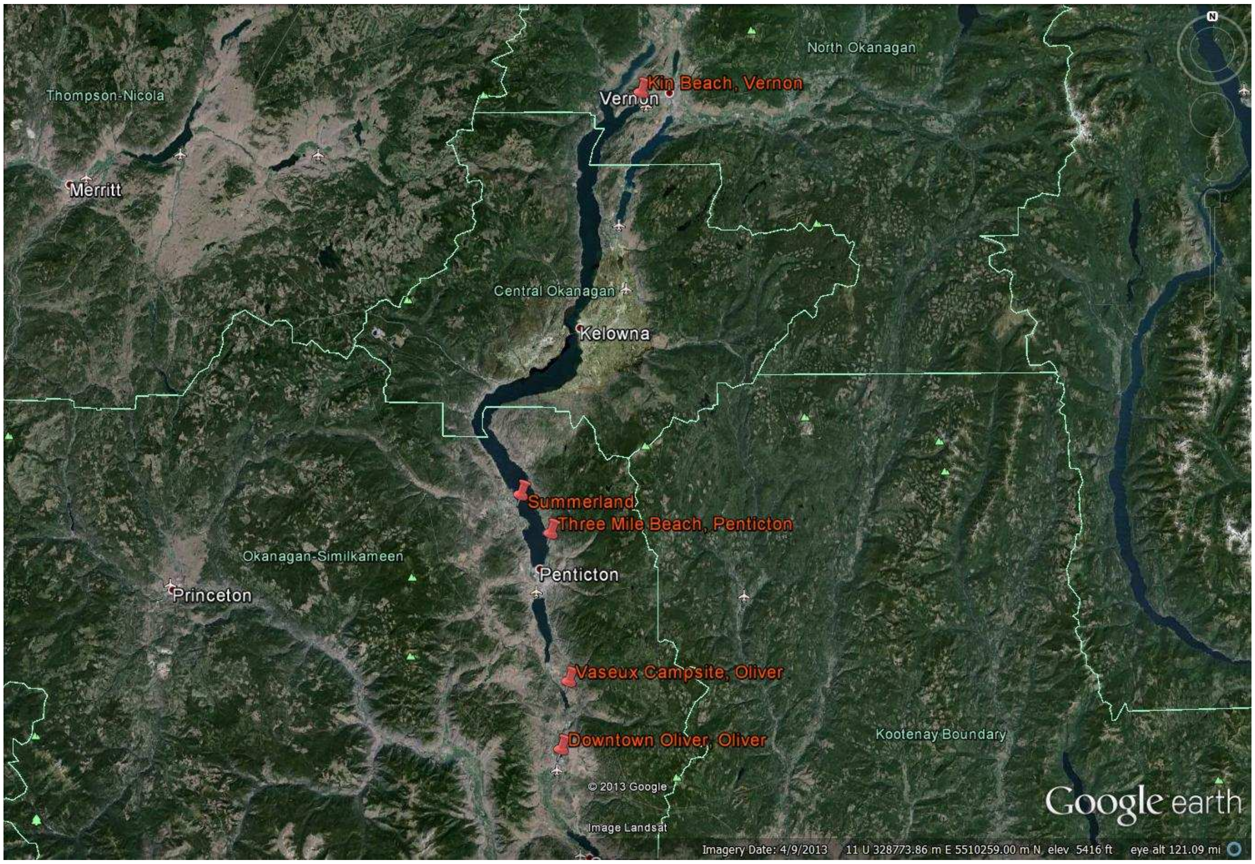

Figure 1 Overview of locations for Rocky Mountain ridged mussel juvenile recruitment surveys. Note that there are several different survey locations within Summerland. See Appendix C for more detailed overview of each location.

between 20 and 40 cm below the lake bottom. Any younger mussels found were aged by counting growth rings (see review of methods in e.g. Larsen 1997, Ruppert *et al.* 2004). Over 100 mussels were measured at each location, with the exception of a few low density locations. In addition, the approximate maximum age of the mussel in the Okanagan was established by estimating the age of at least 25 older mussels at each of two high density locations (Dog Beach and Kinsmen Park, Summerland). These methods are adapted from Larsen and Hartvigsen (1997), and Mageroy (2005). All surveys were completed by snorkellers between August 12th and September 10th, 2013.

For methodology and results with respect to statistical comparison between locations when it comes to frequency of juvenile mussels, mussel growth, and mussel length, see Appendix A.

Although not technically a part of the project, the juvenile recruitment surveys can also be used to estimate density and mussel numbers at the selected locations. Methodology, results and discussion of such use of the data has been included in Appendix B.

Field data on Rocky Mountain ridged mussel fish host use

The goal of collecting field data on Rocky Mountain ridged mussel fish host use is twofold:

1) To suggest which fish hosts Rocky Mountain ridged mussels use. This will be achieved by collecting fish during the period that the mussel releases conglutinates with glochidia. The fish gills will be investigated with respect to prevalence and intensity, which will show which fish are exposed to the mussel's glochidia the most. Determining if the glochidia get encysted on the fish gills will also provide another clue to the host fish, since encystment has been shown to be necessary for glochidia metamorphosing to juvenile mussels (see discussion in O'Brien *et al.* 2013). However, as mussel glochidia can attach to and encyst on unsuitable host species (reviewed in e.g. Larsen 1997, discussed in e.g. O'Brien *et al.* 2013), such findings can only suggest potential host species. Glochidial growth will only be expected to occur on suitable host fish and will provide a stronger suggestion with respect to the mussel's host use. This growth will be determined by comparing the size of encysted glochidia on the fish gills to the size of glochidia in the conglutinates released by the mussel.

2) To provide information necessary to complete an experiment infecting fish with Rocky Mountain ridged mussel glochidia. Although glochidial growth would strongly suggest which fish species serve as hosts for the mussel, only the observation of glochidia metamorphosing into juvenile mussels can confirm such field findings (see Spring Rivers 2007, O'Brien *et al.* 2013). Thus, it will be necessary to complete an experiment for such a confirmation. In designing such an experiment, the field data on prevalence, intensity, encystment, and glochidial growth is important in determining which fish species to include in the experiment. In addition, it is important to gain some understanding of the length of the infection period. This will be achieved by comparing the observation of conglutinates to the observation of glochidia infecting fish, as conglutinate rates are known to occur in peaks in the Okanagan Valley (Stanton *et al.* 2012). Timing, maximal prevalence, and maximal intensity is expected to lag behind the timing and

maximal conglutinate release, and the lag should suggest the approximate length of the infection period.

All data on Rocky Mountain ridged mussel fish host use were collected from Kinsmen Park and Dog Beach in Summerland, Okanagan Lake (see Figure 1 for location). These locations were selected to maximize prevalence and intensity, as they have two of the largest populations of the mussel in the Okanagan Valley (Lora Nield *Pers. com.)*.

The BC Ministry of Forests, Lands, and Natural Resource Operations had a contractor survey these locations for the release of Rocky Mountain ridged mussel conglutinates from the middle of May until conglutinate release approached minimal levels. University of British Columbia - Okanagan personnel also completed supplementary surveying of conglutinate releases at the same locations during the same time period. The results of these surveys were used to determine the rates of conglutinate release and when fish should be collected from the lake.

BC Ministry of Forests, Lands, and Natural Resource Operations, BC Ministry of Environment, and University of British Columbia – Okanagan personnel took part in the fish collection. The collection took place approximately once a week between June 17th and July 12th, 2013. In addition, the BC ministries provided fish sampled in their collection program between May 31st and July 3rd, 2013. The fish were collected using minnow traps, seines, and gillnets. Traps and gillnets were set over night. The fish were euthanized using buffered MS-222 and preserved in 70 % ethanol. In addition, conglutinates were sampled from 5 mussels each at Dog Beach and Kinsmen Park. The conglutinates were also preserved in 70 % ethanol.

Subsequently, the fish gills were examined to determine prevalence, intensity and whether the glochidia were encysted or not, for each fish species. Prevalence was exclusively determined based on the analysis of the left gills of fish. For fish with larger sample sizes (sculpin and lake whitefish), intensity was also based exclusively on the analysis of the left gills of fish. However, for fish with lower sample sizes (lake chub, redside shiner, northern pikeminnow, leopard dace, and longnose dace), the intensity was based on an average of glochidial numbers on both the right and left gills. Therefore, all intensities reported represents the intensity for one gill. Overall, intensity should be assumed to be twice as high, on average. For statistical methodology, results, and detailed discussion with respect to glochidial prevalence and intensity, see Appendix D.

Further, glochidia from conglutinates and glochidia encysted on fish gills were measured to determine if the glochidia had grown on the fish. Three measurements of glochidial size were taken: Width was measured at the widest point, from the posterior to anterior end. Hingelength was measured from one to the other end of the hinge. Length was measured from the hinge to the ventral point of the shell. These measurements were based on photographs. The photographs were taken at either 100 X or 200 X magnification. The measurements were taken using ImageJ (Rasband 2013), and the correct conversions were made from either magnification. For statistical methodology, results, and detailed discussion with respect to glochidial growth, see Appendix D.

Effects of Eurasian watermilfoil rototilling on Rocky Mountain ridged mussel

The overall goal of the investigation into treatment for Eurasian watermilfoil is to determine whether rototilling has a negative impact on Rocky Mountain ridged mussel. This impact on the mussel will be evaluated using two methods: 1) Locations that are being or have been rototilled against Eurasian watermilfoil, and are potential Rocky Mountain ridged mussel locations, will be surveyed to evaluate the extent of the potential current conflict between conservation of the mussel and rototilling against the plant. 2) Evaluation of the direct effect of rototilling on Rocky Mountain ridged mussel by exposing mussels to rototilling.

The attempt at using artificial mussels instead of live Rocky Mountain ridged mussels for evaluating the direct effects of rototilling on the mussel (see Mageroy 2013a,b) had to be abandoned, due to difficulties in mimicking the qualities of live mussels (e.g. strength, a foot, etc.). See Appendix D for results of the strength tests on live mussels associated with the attempted development of artificial mussels. The comparison studies (see Mageroy 2013b) also had to be abandoned, due to the low number of rototilling locations with mussels found during surveying (see results).

Surveys for Rocky Mountain ridged mussel in Eurasian watermilfoil rototilling polygons

The overall goal of these surveys was to determine the current geographic scope of the potential conflict between Rocky Mountain ridged mussel conservation and rototilling against Eurasian watermilfoil. The surveys were used to determine the presence or absence of both live mussels and empty shells. They were only performed in watermilfoil polygons that are or have

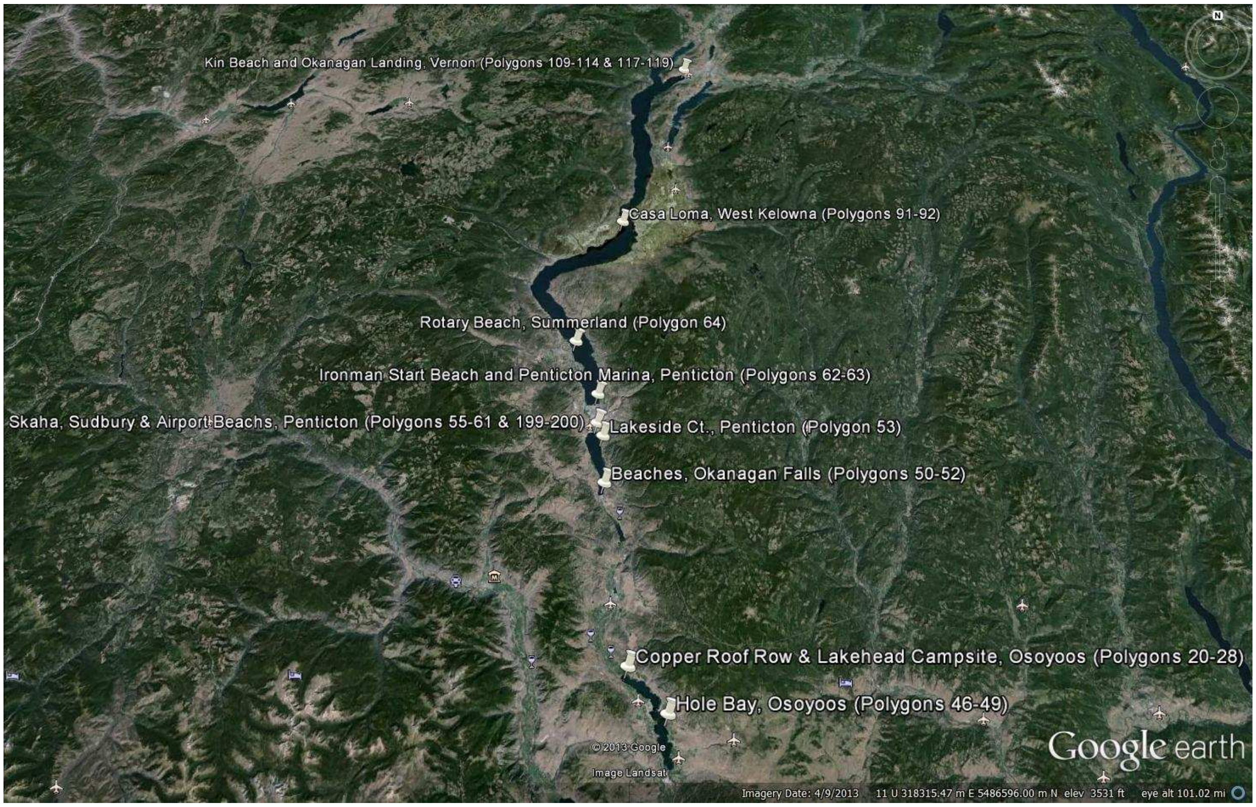

Figure 2 Overview of locations for Eurasian watermilfoil rototilling surveys. See Appendix G for a more detailed overview of each location.

been rototilled, since harvesting (for details, see Dunbar 2009) is likely to have a positive impact on the mussel (see introduction).

Survey locations were selected in consultation with Lora Nield, BC Ministry of Forests, Lands, and Natural Resource Operations, and James Littley, Ian Horner, Dave Caswell, and Pat Field, Okanagan Basin Water Board. The locations were selected based on the proximity of Eurasian watermilfoil rototilling polygons to Rocky Mountain ridged mussel find sites (both live mussels and empty shells), perceived habitat suitability for the mussel, previous survey efforts, ease of access, and current and past watermilfoil treatment practices. Based on these selection criteria, 40 Eurasian watermilfoil rototilling polygons were selected (see Table 2 in Results and Figure 2).

The surveys were completed by snorkellers. They were conducted using a grid pattern to cover the Eurasian watermilfoil rototilling polygons as thoroughly as possible, from the shoreline until the depth was too great to see the bottom. For each survey, the numbers of live Rocky

Mountain ridged mussels and empty shells were recorded. The surveys were completed between June 28th and September 16th, 2013.

Direct effects of Eurasian watermilfoil rototilling on Rocky Mountain ridged mussel

The goal of this experiment was to directly evaluate the impact of Eurasian watermilfoil rototilling on Rocky Mountain ridged mussel. To achieve this, live mussels were exposed to rototilling to determine whether this treatment crushes and/or buries the mussels.

Possible locations for the experiment were evaluated based on the presence of Rocky Mountain ridged mussel in the area and the possibility of placing the mussels at two adjacent sites, one inside and the other outside of a rototilling polygon. Based on these criteria, we selected Casa Loma, West Kelowna (polygon 91) (see Figure 2 for location). This location is a rototilling location and is also known RMRM habitat, but the density is sow low that it was easy to find sites without mussels to relocate mussels to for the experiment.

At Casa Loma, two sites were chosen for the experimental and control treatments. The two sites were chosen based on having substrate suitable for Rocky Mountain ridged mussel, the ability of the rototiller to rototill the sites (depth and distance from obstacles), and their location inside or outside the rototilling polygon. Based on these criteria, we chose an experimental site in front of Casa Loma Resort, 10 m north of the northern Casa Loma Resort dock between stalls 15 and 17 (UTM: 11U 0317695 5529809, see Figure 3), and a control site in front of the public access point north of Casa Loma Resort, 5 m north and 20 m east of the dock south of the public access point (UTM: 11U 0317716 5525859, see Figure 3).

Kinsmen Park, Summerland (see Table 1 and Figure 1 for location), was chosen as a donor location for the mussels to be used in the experiment, due to the high Rocky Mountain ridged mussel numbers at this location.

The timing of the experiment, as described below, was chosen based generally on when rototilling takes place in the Okanagan Valley and, more specifically, on when the rototiller was rototilling in the area close to Casa Loma.

On February 25th, 100 Rocky Mountain ridged mussels were collected from Kinsmen Park, Summerland. The mussels were transported in tanks to Casa Loma. There, 50 mussels were randomly assigned to the experimental site, and the other 50 mussels were assigned to the control site. The mussels were placed in a 5 x 5 m area at the chosen site (see Figure 3). Each mussel

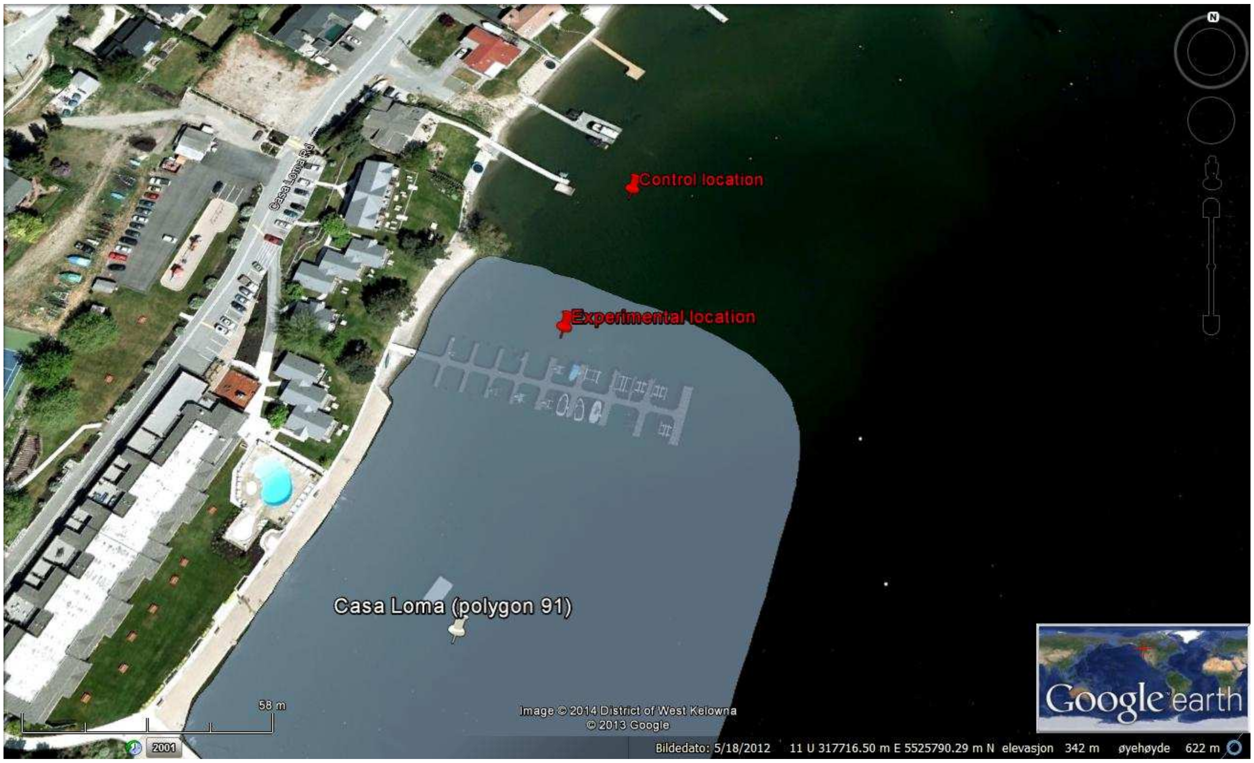

Figure 3 Rototilling experimental location, Casa Loma, West Kelowna. The red pins indicate the experimental and control sites. The white pin and grey polygon indicate the Eurasian watermilfoil treatment polygon number and area.

was placed individually and inserted into the substrate in a natural position (anterior end in the substrate, tilted approximately 45° towards the ventral size) to minimize the stress to the mussels. Also to minimize stress to the mussels, they were maintained in water and never exposed to air during the relocation process.

On February 26th, the Rocky Mountain ridged mussels were surveyed by snorkellers to evaluate the mussels for stress, based on their positioning and siphoning activity.

On February 27th, the experimental site was rototilled.

On February 28th, the Rocky Mountain ridged mussels were surveyed by snorkellers. The surveys included the 5 x 5 m site which the mussels were originally relocated to and the surrounding area, 10 meters to either side of the original relocation site. The number of recovered mussels was recorded, and the mussels were inspected for cracks and breakage. Both the experimental and control sites were re-surveyed three times to maximize the chance of recovering mussels.

For statistical methodology and results with respect to the rototilling experiment, see Appendix E.

Results

Recruitment of juvenile Rocky Mountain ridged mussels

Overall, we measured a total of 1049 Rocky Mountain ridged mussels as a part of our juvenile recruitment surveys and 53 mussels when trying to establish maximum age among the mussels. The youngest mussels found were two years old, while the oldest mussel was estimated to be 30 years old. The shortest mussel found was 16 mm, while the longest was 120 mm. 1.3 %, 5.3 %, and 23.0 % of the mussels were 3, 6 or 10 years and younger, respectively (see Figure 4a). The reduction in growth among seven year old mussels (see Figure 5a) suggests that these mussels mature when they are approximately at this age (see discussion of the relationship between growth and sexual maturation in Larsen 1997). If we make this assumption, the mussels 6 years old and younger make up the juvenile percentage of the population. Overall, 23.7 % of the mussels were found buried in the substrate, while 77.6 % of juveniles were buried. See Figure 6a for the overall length distribution.

Kin Beach, Vernon, Vernon Arm, Okanagan Lake: A total of 106 Rocky Mountain ridged mussels were measured. The youngest mussel was two years old, and the mussels ranged in size from 21 to 112 mm in length. 0.9 %, 1.9 %, and 2.8 % of the mussels were 3, 6, or 10 years and younger, respectively (see Figure 4b). 24.4 % of the mussels were buried. See Figure 5b for growth curve and Figure 6b for length distribution. Note that for 27% of the quadrants, the buried mussels could not be investigated due to silt from digging resulting in too low a visibility. If assuming that there were as many juveniles mussels in these quadrants as in the quadrants that could be investigated for buried mussels, the corrected percentage of mussels 3, 6, and 10 year old or younger would be 1.2 %, 2.5 %, and 3.4 %, respectively. Note that mussels 7 years old and older are considered adults, and assumed to not be over-represented among buried mussels. Therefore, the increase in mussels 10 years old or younger is assumed to equal the increase in mussels 6 years old or younger.

Peach Orchard Beach, Summerland, Okanagan Lake: A total of 22 Rocky Mountain ridged mussels were measured. The youngest mussel was five years old, and the mussels ranged in size from 45 to 93 mm in length. 0.0 %, 9.1 %, and 27.3 % of the mussels were 3, 6 or 10 years and younger, respectively (see Figure 4c). 22.7 % of the mussels were buried. See Figure

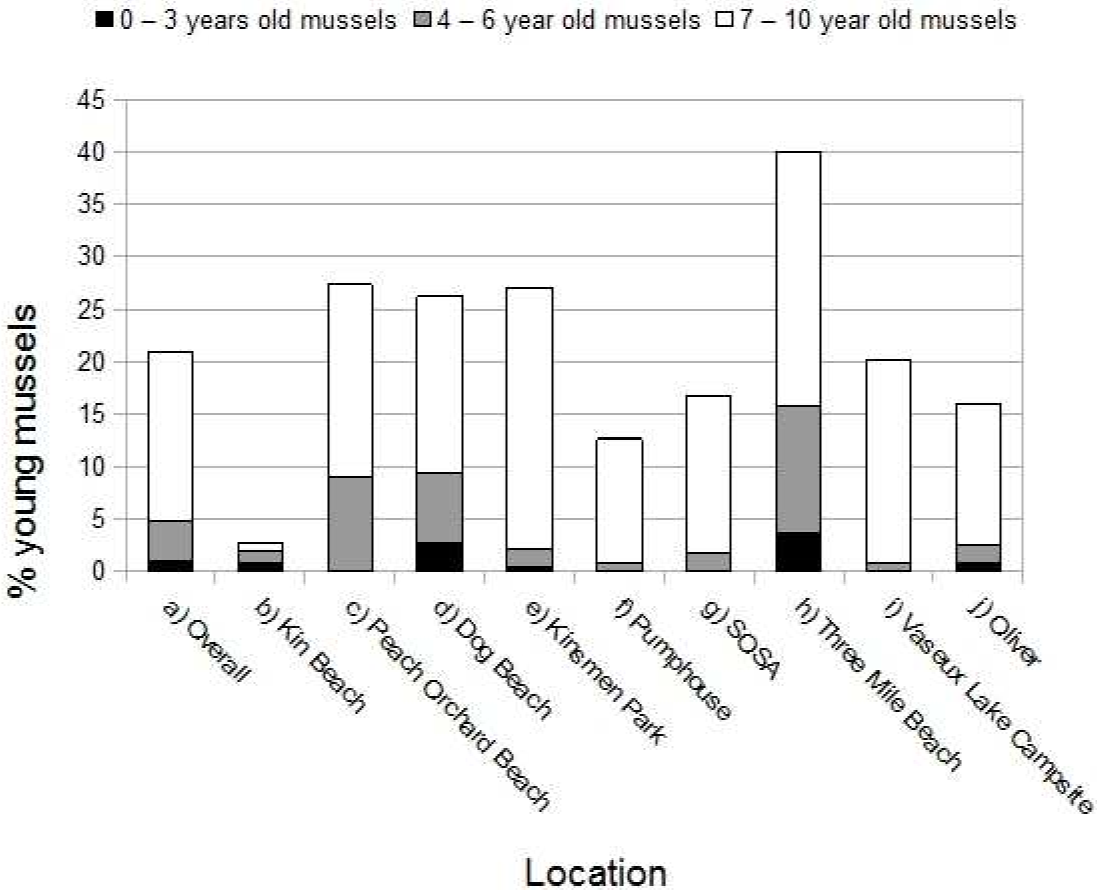

Figure 4 Percentage young Rocky Mountain ridged mussels. Each bar represents cumulative percentages of juvenile mussels at juvenile recruitment locations. Each section of each bar represents mussels 0-3 (black), 4-6 (grey), and 7-10 (white) years old, respectively.

5c for growth curve and Figure 6c for length distribution.

Dog Beach, Summerland, Okanagan Lake: A total of 111 Rocky Mountain ridged mussels were measured. The youngest mussel was two years old, and the mussels ranged in size from 19 to 96 mm in length. 2.8 %, 9.4 %, and 26.2 % of the mussels were 3, 6 or 10 years and younger, respectively (see Figure 4d). 23.4 % of the mussels were buried. See Figure 5d for growth curve and Figure 6d for length distribution.

Kinsmen Park, Summerland, Okanagan Lake: A total of 194 Rocky Mountain ridged mussels were measured. The youngest mussel was three years old, and the mussels ranged in size

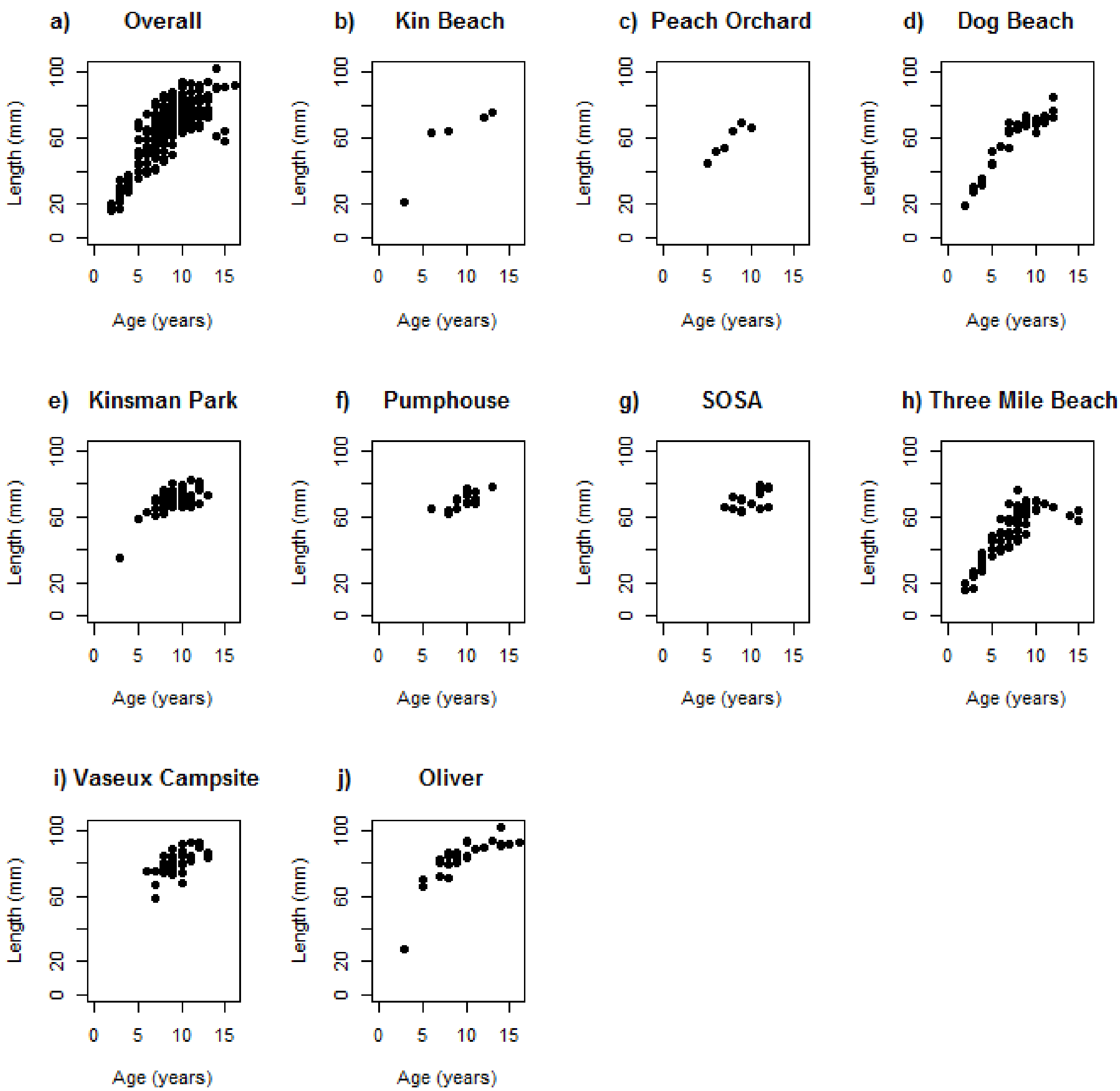

Figure 5 Growth of young Rocky Mountain ridged mussels. Growth of the mussels overall and at individual locations. The length of different age classes is considered a proxy for mussel growth.

from 35 to 100 mm in length. 0.5 %, 2.1 %, and 27.3 % of the mussels were 3, 6 or 10 years and younger, respectively (see Figure 4e). 22.3 % of the mussels were buried. See Figure 5e for growth curve and Figure 6e for length distribution. Note that for 58% of the quadrants, the buried mussels could not be investigated due to silt from digging resulting in too low a visibility. If assuming that there were as many juveniles mussels in these quadrants as in the quadrants that could be investigated for buried mussels, the corrected percentage of mussels 3, 6, and 10 year old or younger would be 1.2 %, 5.0 %, and 30.2 %, respectively. Note that mussels 7 years old and older are considered adults, and assumed to not be over-represented among buried mussels. Therefore, the increase in mussels 10 years old or younger is assumed to equal the increase in mussels 6 years old or younger.

Pumphouse, Summerland, Okanagan Lake: A total of 111 Rocky Mountain ridged mussels were measured. The youngest mussel was six years old, and the mussels ranged in size from 61 to 104 mm in length. 0.0 %, 0.9 %, and 12.6 % of the mussels were 3, 6 or 10 years and younger, respectively (see Figure 4f). 19.8 % of the mussels were buried. See Figure 5f for growth curve and Figure 6f for length distribution. Note that for 39% of the quadrants, the buried mussels could not be investigated due to silt from digging resulting in too low a visibility. If assuming that there were as many juveniles mussels in these quadrants as in the quadrants that could be investigated for buried mussels, the corrected percentage of mussels 3, 6, and 10 year old or younger would be 0.0 %, 1.5 %, and 13.2 %, respectively. Note that mussels 7 years old and older are considered adults, and assumed to not be over-represented among buried mussels. Therefore, the increase in mussels 10 years old or younger is assumed to equal the increase in mussels 6 years old or younger.

South Okanagan Sailing Association, Summerland, Okanagan Lake: A total of 64 Rocky Mountain ridged mussels were measured. The youngest mussel was four years old, and the mussels ranged in size from 34 to 96 mm in length. 0.0 %, 1.7 %, and 16.7 % of the mussels were 3, 6 or 10 years and younger, respectively (see Figure 4g). 2.2 % of the mussels were buried. See Figure 5g for growth curve and Figure 6g for length distribution. Note that for 35% of the quadrants, the buried mussels could not be investigated due to silt from digging resulting in too low a visibility. If assuming that there were as many juveniles mussels in these quadrants as in the quadrants that could be investigated for buried mussels, the corrected percentage of mussels 3, 6, and 10 year old or younger would be 0.0 %, 2.6 %, and 17.6 %, respectively. Note that mussels 7 years old and older are considered adults, and assumed to not be over-represented among buried mussels. Therefore, the increase in mussels 10 years old or younger is assumed to equal the increase in mussels 6 years old or younger.

Three Mile Beach, Naramata Benchlands, Penticton, Okanagan Lake: A total of 198 Rocky Mountain ridged mussels were measured. The youngest mussel was two years old, and the mussels ranged in size from 16 to 95 mm in length. 3.6 %, 15.7 %, and 40.0 % of the mussels were 3, 6 or 10 years and younger, respectively (see Figure 4h). 31.8 % of the mussels were

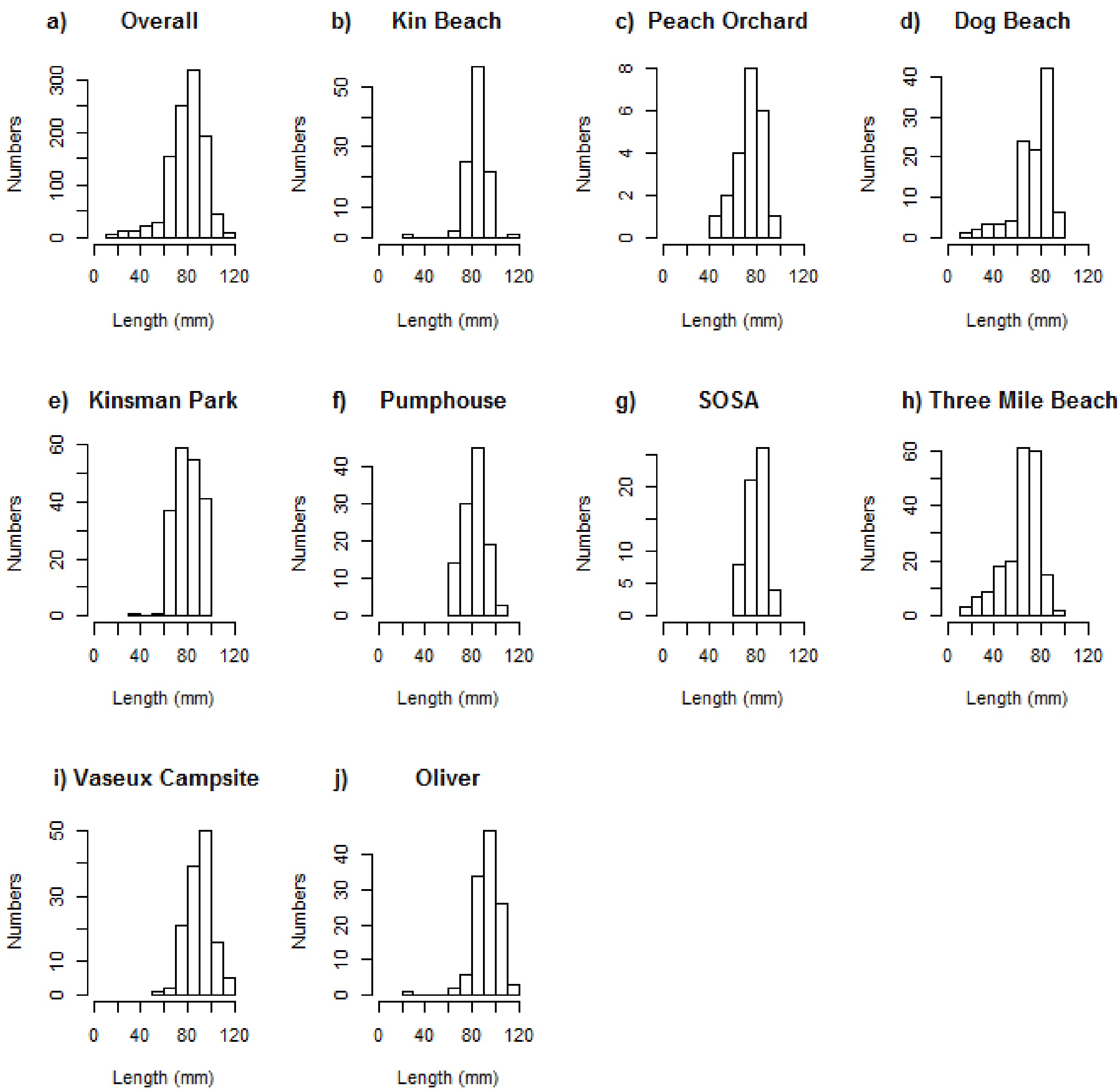

Figure 6 Length distribution of Rocky Mountain ridged mussels. Length distribution overall and at individual locations. Note that at many of the locations the buried mussels could only be partially surveyed (b, e, f, and g) or not surveyed at all (i and j).

buried. See Figure 5h for growth curve and Figure 6h for length distribution.

Vaseux Lake Campsite, Vaseux Lake: A total of 138 Rocky Mountain ridged mussels were measured. The youngest mussel was six years old, and the mussels ranged in size from 59 to 120 mm in length. 0.0 %, 0.8 %, and 20.2 % of the mussels were 3, 6 or 10 years and younger, respectively (see Figure 4i). See Figure 5i for growth curve and Figure 6i for length distribution. Note that for this location, the buried mussels could not be investigated due to silt from digging resulting in too low a visibility.

Pedestrian bridge to Fairview Rd., Oliver, Okanagan River: A total of 116 Rocky Mountain ridged mussels were measured. The youngest mussel was three years old, and the mussels ranged in size from 28 to 116 mm in length. 0.8 %, 2.5 %, and 16.0 % of the mussels were 3, 6 or 10 years and younger, respectively (see Figure 4j). See Figure 5j for growth curve and Figure 6j for length distribution. Note that for this location, with the exception of a few quadrants, the buried mussels could not be investigated due to the high current.

Overall, there were great differences in youngest Rocky Mountain ridged mussel found, percentage buried, frequency of juveniles, growth, and length distributions among the different survey locations.

With respect to the frequency of juveniles Rocky Mountain ridged mussels (see Figure 4), the statistical analysis showed that Three Mile Beach had a significantly higher frequency than at Kin Beach, Kinsmen Park, Vaseux Lake Campsite, and Oliver ($p \leq 0.005$). In addition, it was borderline significantly higher than at Pumphouse ($p=0.07$). However, the frequency did not differ significantly from Peach Orchard Beach, Dog Beach, and South Okanagan Sailing Association ($p \geq 0.88$). The p-values suggest that the locations should be ordered as follows when it comes to juvenile frequencies: Three Mile Beach > Peach Orchard Beach = Dog Beach > South Okanagan Sailing Association > Pumphouse > Kin Beach = Kinsmen Park = Vaseux Lake Campsite = Oliver. For further details, see Appendix A.

When considering Rocky Mountain ridged mussel juvenile growth (see Figure 5) it is not so easy to rank locations. The mussels grow significantly faster at Vaseux Lake Campsite and Oliver than the other locations ($p<0.001$). If excluding Kin Beach and Peach Orchard Beach due to their low sample sizes, the p-values suggest that the remaining locations should be ordered as follows: Three Mile Beach < Dog Beach < Pumphouse = South Okanagan Sailing Association < Kinsmen Park. Note that there is not a significant difference between subsequent location in this order (e.g. mussels at Dog Beach do not grow significantly slower than mussels at Pumphouse and South Okanagan Sailing Association ($p \geq 0.94$), but grow significantly slower than at Kinsmen Park ($p<0.004$)). For further details, see Appendix A.

With respect to the length (see Figure 6), the Rocky Mountain ridged mussels at Three Mile Beach were significantly shorter than at any other location ($p \leq 0.003$), and the mussels at

Vaseux Lake Campsite and Oliver were significantly longer than at any other location ($p \leq 0.002$). When comparing the remaining locations, the p-values suggest that the locations should be ordered as follows: Dog Beach < Peach Orchard Beach < Kinsmen Park = South Okanagan Sailing Association < Pumphouse = Kin Beach. Note that there is not a significant difference between subsequent locations in this order (e.g. mussels at Dog Beach are not significantly shorter than at Peach Orchard Beach, but are significantly shorter than at all other locations). For further details, see Appendix A.

Although not technically a part of the project, the juvenile recruitment surveys can also be used to estimate Rocky Mountain ridged mussel density and numbers at the selected locations. Methodology for, results from, and discussion of such use of the data has been included in Appendix B.

Field data on Rocky Mountain ridged mussel fish host use

A total of 395 fish were caught during the fish sampling. Of these fish, 259 were caught by UBCO, BC Ministry of Forests, Lands, and Natural Resource Operations, and BC Ministry of Environment personnel between the June 14th and July 12th. The remainder (136 fish) were provided by the BC Ministry of Forests, Lands, and Natural Resource Operations, and the BC Ministry of Environment. These fish were caught as a part of their sampling program, between the 30th of May and the 3rd of July. The fish caught, included 167 sculpin (*Cottus asper* or *C. cognatus*), 100 lake whitefish (*Coregonus clupeaformis*), 33 redside shiners (*Richardsonius balteatus*), 29 longnose dace (*Rhinichthys cataractae*), 26 lake chub (*Couesius plumbeus*), 14 northern pikeminnow (*Ptychocheilus oregonensis*), 12 suckers (*Catostomus catostomus* or *C. macrocheilus*), 6 leopard dace (*Rhinichthys falcatus*), 6 yellow perch (*Perca flavescens*), and 2 common carp (*Cyprinus carpio*).

Of the fish caught, 164 sculpin, 86 whitefish, 30 shiners, 28 longnose dace, 13 chub, 3 pikeminnow, 0 suckers, 6 leopard dace, 0 perch and 0 carp were in a condition that allowed them to be screened for Rocky Mountain ridged mussel glochidial infection. The remainder had deteriorated, and unfortunately this deterioration was more common among our larger fish, which typically had a lower sample size.

Rocky Mountain ridged mussel glochidia were found on sculpin, longnose dace, leopard dace, and whitefish. The glochidia were encysted on the three former species, but not on the

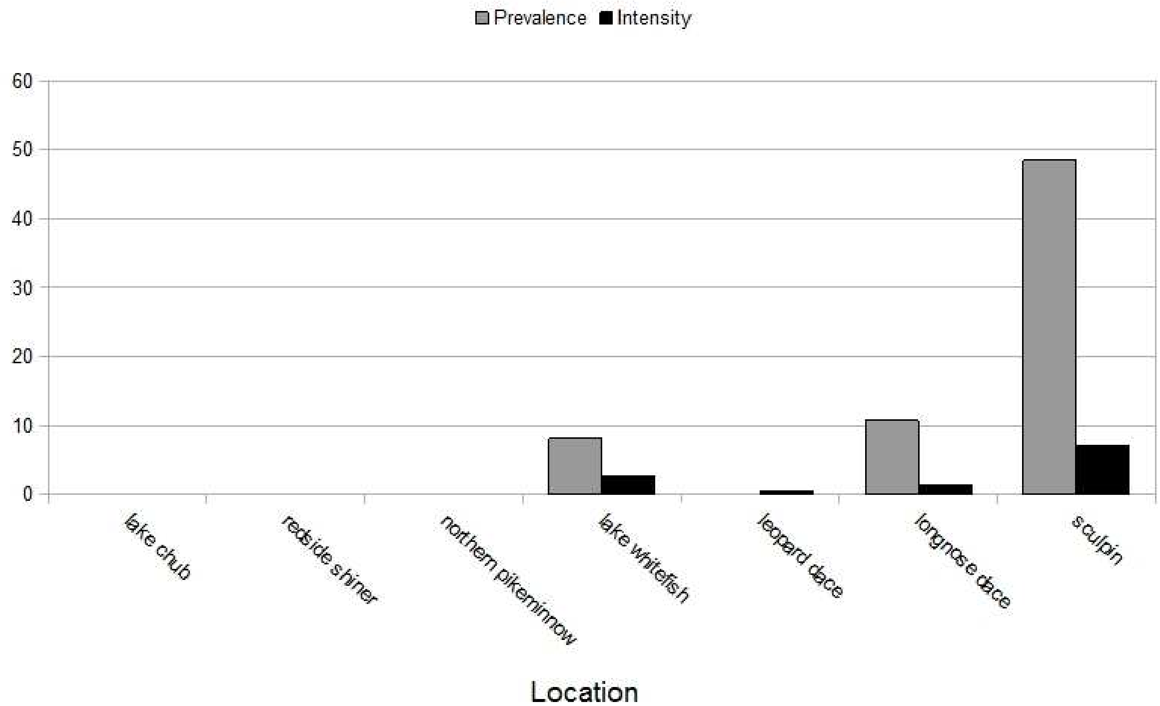

Figure 7 Rocky Mountain ridged mussel prevalence and intensity on wild caught fish in Summerland, Okanagan Lake. Note that the glochidia were not encysted on lake whitefish. In addition, note that the prevalence was exclusively based on glochidial numbers on the left gills of the fish, while intensity was based on an average of glochidial numbers on both the right and left gills of fish with lower sample size (chub, shiner, pikeminnow, leopard dace, and longnose dace). Therefore, all intensities reported represents the intensity for one gill. Overall intensity should be assumed to be twice as high, on average. In addition, this explains the apparent discrepancy between prevalence and intensity in leopard dace. Also note that the sample sizes were very low for leopard dace and pikeminnow (6 and 3, respectively).

latter. For prevalence and intensity, see Figure 7. Note that the sample sizes for leopard dace and pikeminnow were very low (6 and 3, respectively). The statistical analyses of prevalence and intensity were not very helpful in providing insight into differences between different fish species. For methodology for, results of, and discussion of these analyses, see Appendix D.

The size (width, hingelength, and length) of Rocky Mountain glochidia were only measured on sculpin (43 infected fish with measurable encysted glochidia), due to the low number of infected fish among other species. The size of the encysted glochidia were not consistently greater than for the free glochidia (see Figure 8). For further details on statistical methodology, results, and discussion, see Appendix D.

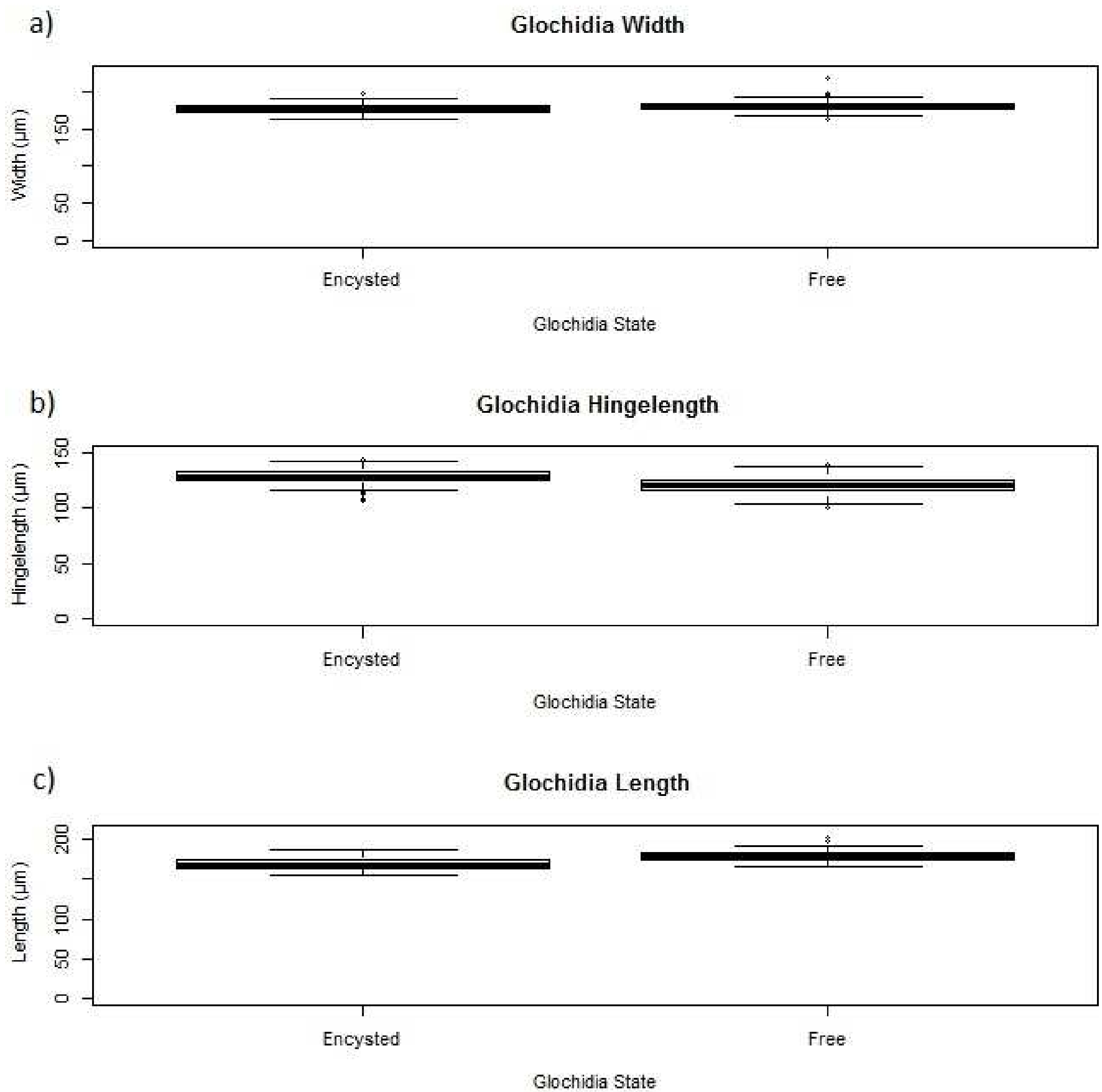

Figure 8 Rocky Mountain ridged mussel glochidial size dependent on its 'state'. A) Width was measured at the widest point, from the posterior to anterior end. B) Hingelength was measured from one to the other end of the hinge. C) Length was measured from the hinge to the ventral point of the shell. For A), B), and C): 'Encysted' indicates that the glochidia were found encysted on sculpin gills. 'Free' indicates that the glochidia were collected directly from conglutinates released by the mussels. The box plot portrays the median, and first and third quartile values. The whiskers represent min. and max. values, with the exception of outliers. Open circles indicate outliers, which are more than 1.5 times the length of the box away from the box.

Rocky Mountain ridged mussel conglutinates were found between May 23rd and June 28th (end of the survey period). At Kinsmen Park, Summerland, the maximal numbers of conglutinates were observed between May 23rd and June 3rd, with numbers tapering off after the latter date. At Dog Beach, Summerland, the maximal numbers of conglutinates were observed between May 24th and June 7th, with lower numbers before and after these dates, respectively. Attached glochidia were found during the entire sampling period (May 31st to July 12th). At both Kinsmen Park and Dog Beach the maximal prevalence and intensities were observed between June 13th and July 3rd, with lower numbers before and after these dates, respectively. The timing of conglutinate observations and glochidial attachment suggest that the infection period lasts at least 13 days. However, the time lags between the maximal observations suggest that the glochidia may be attached approximately 24 days.

Effects of Eurasian watermilfoil rototilling on Rocky Mountain ridged mussel

Survey results for Rocky Mountain ridged mussel in Eurasian watermilfoil rototilling polygons

Overall, live Rocky Mountain ridged mussels were found in or associated with 10 out of 40 Eurasian watermilfoil rototilling polygons that were surveyed (see Table 2 and Appendix G Figures 1-8). Out of these polygons, four are still being rototilled. In addition, we found shells of the mussel in another two polygons. These two polygons are not currently being rototilled.

Direct effects of Eurasian watermilfoil rototilling on Rocky Mountain ridged mussel

Surveying of the relocated Rocky Mountain ridged mussels, before rototilling, showed no apparent signs of stress among the mussels. They were all located in the position they were placed in the substrate during relocation, although there were signs of some mussels burying deeper. No mussels were found on their side. The mussels were either closed up or sitting with their siphons open, similarly to what we observed when collecting the mussels for relocation.

See Table 3 for results. Note that the number of recovered Rocky Mountain ridged mussels was significantly higher at the control than the experimental site ($p<0.001$), but there was no significant difference in crushed mussels ($p=0.18$). All mussels were recovered at or close to the site they were originally relocated to. No mussels were recovered far from the relocation site. See Appendix E for details on the statistical analyses.

Table 2 Overview of Rocky Mountain ridged mussel surveys in Eurasian watermilfoil rototilling polygons.

Polygon #	Lake	Location	Start UTM	Stop UTM	Live RMRM	Shells of RMRM	Last Rototilled	Current Watermilfoil Treatment
20	Osoyoos	Copper Roof Row, Osoyoos	11U 316105 5437807	11U 316066 5437919	0	20	2009	Harvesting
21	Osoyoos	Copper Roof Row, Osoyoos	11U 316066 5437919	11U 316044 5437947	0	4	2009	Harvesting
22	Osoyoos	Lakehead Campsite, Osoyoos	11U 315875 5438038	11U 315582 5438406	1	0	2012	Harvesting and Rototilling
23	Osoyoos	Lakehead Campsite, Osoyoos	11U 315582 5438406	11U 315484 5438723	0	0	2012	Harvesting and Rototilling
24	Osoyoos	Lakehead Campsite, Osoyoos	11U 315484 5438723	11U 315502 5438749	0	0	2012	Harvesting and Rototilling
25	Osoyoos	Lakehead Campsite, Osoyoos	11U 315502 5438749	11U 315629 5438872	0	0	2012	Harvesting and Rototilling
26	Osoyoos	Lakehead Campsite, Osoyoos	11U 315629 5438872	11U 315732 5438974	0	0	2010	Harvesting
27	Osoyoos	Lakehead Campsite, Osoyoos	11U 315732 5438974	11U 315925 5438942	1	0	2010	Harvesting
28	Osoyoos	Lakehead Campsite, Osoyoos	11U 315925 5438942	11U 316102 5438952	8	1	2010	Harvesting
46	Osoyoos	Hole Bay, Osoyoos	11U 321641 5431187	11U 321562 5431245	0	0	2011	Harvesting and Rototilling
47	Osoyoos	Hole Bay, Osoyoos	11U 321656 5431137	11U 321641 5431187	0	0	2011	Harvesting and Rototilling
48	Osoyoos	Hole Bay, Osoyoos	11U 321718 5430922	11U 321634 5431018	0	0	2012	Harvesting and Rototilling
49	Osoyoos	Hole Bay, Osoyoos	11U 321919 5430754	11U 321714 5430860	0	0	2012	Harvesting and Rototilling
50	Skaha	Beaches, Okanagan Falls	11U 312787 5469243	11U 312922 5469283	13	1	2003	Harvesting
51	Skaha	Beaches, Okanagan Falls	11U 312951 5469344	11U 313088 5469297	0	0	2003	Harvesting
52	Skaha	Beaches, Okanagan Falls	11U 313163 5469332	11U 313373 5469490	3	1	2003	Harvesting
53	Skaha	Lakeside Ct., Penticton	11U 313192 5478150	11U 313104 5478692	2	28	2003	Harvesting
55	Skaha	Skaha Beach, Penticton	11U 312922 5480918	11U 312919 5480780	0	0	2012	Rototilling
56	Skaha	Skaha Beach, Penticton	11U 312413 5481036	11U 312922 5480918	0	0	2012	Rototilling
57	Skaha	Skaha Beach, Penticton	11U 312253 5481022	11U 312413 5481036	0	0	2012	Rototilling

Table 2 Continued Overview of Rocky Mountain ridged mussel surveys in Eurasian watermilfoil rototilling polygons.

Polygon #	Lake	Location	Start UTM	Stop UTM	Live RMRM	Shells of RMRM	Last Rototilled	Current Watermilfoil Treatment
58	Skaha	Sudbury Beach, Penticton	11U 311819 5480974	11U 312056 5480999	0	0	2012	Rototilling
59	Skaha	Airport Beach, Penticton	11U 311588 5480986	11U 311819 5480974	0	0	2012	Rototilling
60	Skaha	Airport Beach, Penticton	11U 311223 5480922	11U 311256 5480930	0	0	2012	Rototilling
61	Skaha	Airport Beach, Penticton	11U 310997 5480836	11U 311223 5480922	0	0	2012	Rototilling
62	Okanagan	Ironman Start Beach, Penticton	11U 311845 5486542	11U 311890 5486654	0	0	Unknown	Rototilling
63	Okanagan	Penticton Marina, Penticton	11U 312661 5486868	11U 312955 5486941	0	0	2012	Rototilling
64	Okanagan	Rotary Beach, Summerland	11U 308396 5497948	11U 308571 5498316	656	531	1993	None
91	Okanagan	Casa Loma, West Kelowna	11U 317592 5525622	11U 317668 5525831	1	0	2008	Rototilling
92	Okanagan	Casa Loma, West Kelowna	11U 317957 5526175	11U 317940 5526591	0	0	2012	Rototilling
109	Okanagan	Kin Beach, Vernon	11U 332558 5568891	11U 332508 5568953	1	1	2012	Harvesting and Rototilling
110	Okanagan	Kin Beach, Vernon	11U 332750 5568568	11U 332558 5568891	0	0	2012	Harvesting and Rototilling
111	Okanagan	Kin Beach, Vernon	11U 332820 5568385	11U 332750 5568568	0	0	2012	Harvesting and Rototilling
112	Okanagan	Kin Beach, Vernon	11U 332837 5568349	11U 332820 5568385	0	0	2012	Harvesting and Rototilling
113	Okanagan	Kin Beach, Vernon	11U 332920 5568045	11U 332837 5568349	0	0	2012	Harvesting and Rototilling
114	Okanagan	Kin Beach, Vernon	11U 332780 5567909	11U 332920 5568045	0	0	2012	Harvesting and Rototilling
117	Okanagan	Okanagan Landing, Vernon	11U 331763 5567484	11U 332057 5567563	1	0	2012	Harvesting and Rototilling
118	Okanagan	Okanagan Landing, Vernon	11U 331576 5567402	11U 331763 5567484	0	0	2012	Harvesting and Rototilling
119	Okanagan	Okanagan Landing, Vernon	11U 331521 5567380	11U 331576 5567402	0	0	2012	Harvesting and Rototilling
199	Skaha	Sudbury Beach, Penticton	11U 312056 5480999	11U 312253 5481022	0	0	2012	Rototilling
200	Skaha	Airport Beach, Penticton	11U 311256 5480930	11U 311588 5480986	0	0	2012	Rototilling

Table 3 Rototilling experiment.

Site	Relocated mussel #	Recovered mussel #	Crushed mussel #
Experimental	50	19	2
Control	50	41	0

See Appendix F for results of the strength tests on live Rocky Mountain ridged mussels associated with the attempted development of artificial mussels.

Discussion

Recruitment of juvenile Rocky Mountain ridged mussels

Description and comparison of Rocky Mountain ridged mussel juvenile recruitment locations

It is good news that juvenile Rocky Mountain ridged mussels as young as two or three years old were found at a majority of the locations surveyed, and mussels seven years or younger were found at all locations (see Figure 5). These findings show that juveniles have been recruited into all of these populations fairly recently. It is also important to consider that juvenile mussels are very difficult to find due to their small size (reviewed in e.g. Larsen and Hartvigsen 1997, Stanton *et al.* 2012) and the fact that they are typically buried in the substrate (reviewed in e.g. Larsen 1997, Strayer *et al.* 2004, Jepsen 2010a). Thus, the youngest ages are likely to be overestimates. Similarly, the percentages of young mussels are likely to be underestimates.

The youngest Rocky Mountain ridged mussel found, percentages of young mussels (Figure 4), young mussel growth (Figure 5), and length distributions (see Figure 6) vary greatly between locations. It is important to note that there were significant differences in growth between the locations, which may be explained by higher temperatures and increased food availability favouring increased growth in mussels (see e.g. Larsen 1997). This fact illustrates how length distributions can provide a false impression of the status of juvenile recruitment when comparing locations. Therefore, it is important to age mussels when considering the health of mussels populations. Ageing mussels allows one to consider factors such as youngest mussel found and, more importantly, frequency of juveniles when considering the status of juvenile recruitment at the different locations in the Okanagan Valley.

Investigating Rocky Mountain ridged mussel age showed that Peach Orchard Beach, Dog Beach and Three Mile Beach house the youngest mussel populations among the locations we surveyed. This fact, in combination with the high numbers of mussels at the latter two locations (see Appendix B Table 1), suggest that these two locations should be considered of special importance to the conservation of the mussel in the Okanagan Valley. For the other locations, there was no significant difference in the frequencies of juvenile mussels, but the locations differed (although not statistically tested) in mussels younger than 3 and 10 years old. Taking these percentages into consideration, it seems that Kin Beach houses the oldest population, which suggests that it may be the most threatened population among the ones we surveyed. The other locations seem to house populations that are between these two extremes in their age distributions.

However, certain considerations have to be taken when discussing these conclusions. Many of the locations with higher youngest ages and lower percentages of young Rocky Mountain ridged mussels could not be surveyed or only partially surveyed for buried mussels, due to poor visibility when digging or high currents. Overall, we found that 23.7 % of the mussels were buried in the substrate, while 77.6 % of juveniles were buried. This suggests that our inability to complete surveys for buried mussels may explain why we didn't find more young mussels at these locations. It is possible to correct for the quadrants that could not be investigated with respect to buried mussels, by using the percentages of 3 and 6 year old mussels in the quadrants that could be investigated for buried mussels. Such corrections increase the percentages of 3, 6, and 10 year old mussels at Kin Beach, Kinsmen Park, Pumphouse, and South Okanagan Sailing Association, but do not change our overall understanding of the status of these locations. Note that no such corrections could be made for Vaseux Lake Campsite and Oliver, due to the extremely low number of quadrants that could be investigated for buried mussels.

Alternatively, the silt may not only have prevented us from finding young Rocky Mountain ridged mussels, but it may also have prevented young mussels from being recruited into the population at these sites. This may be the case since juveniles of freshwater mussels have been found to be more sensitive to siltation than adults (e.g. reviewed in Larsen 1997) and since Rocky Mountain ridged mussels typically do not favour too high a level of siltation (see reviews in COSEWIC 2011, Fisheries and Canada 2010, Jepsen 2010a). With this in mind, it is interesting to note that Dog Beach and Three Mile Beach are the two location most exposed to wave action of any of the locations we surveyed (*Pers. obs.*).

Evaluation of whether juvenile recruitment is sufficient to maintain Rocky Mountain ridged mussel numbers

Young *et al.* (2001) proposes that the ideal eastern pearlshell *(Margaritifera margaritifera*) population contains 20 % of mussels 20 years old or younger and some mussels 10 years old or younger. However, their calculations were based on populations containing mussels up to approximately 120 years old. The oldest Rocky Mountain ridged mussel we found was 30 years old. However, it has been shown that the methods we used for determining the age of the mussels underestimates the maximum age by approximately 100 % (Neves and Moyer 1988, Downing *et al.* 1992). Assuming a maximum age of 60 years for Rocky Mountain ridged mussel in the Okanagan Valley, an ideal population should contain 20 % of mussels under 10 years and some mussels under 5 years old (in this case I will use mussels 6 years old, i.e. juveniles).

Assuming a maximum age of 60 years old for Rocky Mountain ridged mussels in the Okanagan Valley, the juvenile recruitment surveys show that Peach Orchard Beach, Dog Beach, Kinsmen Park, and Three Mile Beach clearly fulfil the criteria for an ideal mussel population (see Figure 4 for overview of percentages). Vaseux Lake Campsite fulfils the criterion of 20 % of the mussels being 10 years old or younger, but only 0.8 % of the mussels were 6 years old or younger. Note that this is one of the locations were we could not investigate buried mussels. Pumphouse (12.6 %), South Okanagan Sailing Association (16.7 %), and Oliver (16.0%) all have percentages of mussels 10 years old or younger below the criterion. Note that this is the case for Pumphouse and South Okanagan Sailing Association when correcting for the inability to investigate some transects for buried mussels, while Oliver could not be investigated for buried mussels with the exception of a few quadrants. Kin Beach had a percentage of mussels 10 years old or younger (2.8 % or 3.4 % if correcting for the inability to investigate buried mussels in some quadrants) far below the criterion.

Overall, this evaluation shows that approximately half the locations have a high enough juvenile recruitment to maintain Rocky Mountain ridged mussel numbers. However, some locations show recruitment somewhat below the necessary threshold to maintain the numbers. Further, the recruitment at Kin Beach is substantially bellow the necessary threshold, and the number of mussels at this location seems likely to be reduced dramatically when the older mussels eventually die off. Note that due to the uncertainty with respect to the maximum age of Rocky Mountain ridged mussels in the Okanagan Valley, these conclusions have to be considered with this uncertainty in mind.

Field data on Rocky Mountain ridged mussel fish host use

Conclusions about Rocky Mountain ridged mussel fish host use

The field data on Rocky Mountain ridged mussel fish host use strongly suggest that sculpin (*Cottus asper* and/or *C. cognatus*) serve as the main hosts for the mussel, and that longnose dace (*Rhinichthys cataractae*) and leopard dace (*Rhinichthys falcatus*) are potential additional hosts. We found encysted glochidia on these species, which shows that these fish may serve as hosts (see discussion in O'Brien *et al.* 2013). In addition, the glochidial prevalence and intensity (Figure 7) was substantially greater on sculpin than on the other species, suggesting that sculpin are the main hosts for the mussel in the Okanagan Valley. Unfortunately, we gained no further support for these findings through our analysis of glochidial growth (see Figure 8 and Appendix D). Unlike during previous sampling (Stanton *et al.* 2012), we did not find any glochidia on northern pikeminnow. However, we could only screen three specimens. Therefore, this species should still be considered a potential host fish for the mussel in the Okanagan Valley. Overall, sculpin should be considered the likely major hosts for the mussel in the Okanagan Valley, while longnose dace, leopard dace, and northern pikeminnow should be considered potential hosts.

Our findings also eliminate some species as potentially hosts for Rocky Mountain ridged mussel. We found non-encysted glochidia on lake whitefish (*Coregonus clupeaformis*). However, encystment is necessary for a fish to serve as host (see discussion in O'Brien *et al.* 2013). In combination with the large number of fish screened, this finding suggests that we can eliminate this species as a potential host. In addition, we can can eliminate redside shiner (*Richardsonius* *balteatus*) and lake chub (*Couesius plumbeus*), due to not finding glochidia on any of the large number of fish screened. However, we cannot eliminate suckers (*Catostomus catostomus* or *C. macrocheilus*), yellow perch (*Perca flavescens*), or common carp (*Cyprinus carpio*), as we were not able to screen any fish belonging to these species.

When discussing these findings, one should consider the fact that these data were all collected from two locations in Summerland, Okanagan Lake. The other mussel locations in Okanagan Lake and other parts of the Okanagan Valley could potentially represent different populations of Rocky Mountain ridged mussels, due to great geographic distances, isolation of host fish populations through the building of dams, and differences in habitat use. If these locations do represent different populations, there may be differences in host use between them as Rocky Mountain ridged mussel populations are know to differ in their host use (compare this study, Spring Rivers 2007, O'Brien *et al.* 2013). Therefore, further investigation into the host use of the mussel at other locations is necessary. This is especially the case for mussel locations in the South Okanagan where numerous introduced fish species (*Pers. obs.*) present the mussel with a much wider array of potential hosts.

Implications for Rocky Mountain ridged mussel infection experiment

Although our findings strongly suggest that sculpin are the main hosts of Rocky Mountain ridged mussel in the Okanagan Valley, they do not confirm this. In addition, our findings also add longnose and leopard dace to northern pikeminnow (Stanton *et al.* 2012) on the list of potential additional hosts. To confirm or eliminate these species as hosts, an infection experiment is needed (see methodology in this study and introduction, methodology, and discussion in O'Brien *et al.* 2013). Further, the South Okanagan is dominated by invasive fish species such as smallmouth bass, yellow perch, pumpkinseed, common carp, etc. (*Pers. obs.*), and there is no information on their suitability as hosts for the mussel.

Based on the information above, any infection experiment with Rocky Mountain ridged mussel glochidia should include all the fish species mentioned in the previous paragraph. In addition, suckers should be included since we were not able to eliminate them as potential hosts. Further, the glochidia should be collected from mussels at several locations in the Okanagan Valley. This would allow one to determine whether there are differences in fish host use between locations. It would also suggest whether there are more than one population of the mussel in the system. Such an experiment would likely have to last for up to a month, as our data on the timing and maxima of conglutinate release and fish infection suggest that the glochidia stay on the fish for up to approximately 24 days.

Effects of Eurasian watermilfoil rototilling on Rocky Mountain ridged mussel

Survey results for Rocky Mountain ridged mussel in Eurasian watermilfoil rototilling polygons

Our surveys show that rototilling against Eurasian watermilfoil is only occurring in four polygons that contain or are associated with live Rocky Mountain ridged mussels (see Table 2). Therefore, the current level of conflict between rototilling and the mussel seems rather limited. However, it is almost impossible to determine whether the absence of the mussel in a rototilling polygon is due to harmful effects of rototilling or simply that these locations are not suitable habitat for the mussel. With this in mind, it is interesting to note that we found live mussels in or associated with six of the polygons that are no longer being rototilled. In addition, we found empty shells in another two polygons that are no longer being rototilled. It is also interesting to note that mussels were found in very low numbers in these polygons, with the exception of the polygon which has been not been rototilled since approximately 1993 (Polygon 64, Rotary Beach, Summerland). Further, it is interesting to note that in the other polygons, the mussels were often found right along the edges (see Appendix G Figures 2, 3, and 6) of the rototilling area, under docks (see Appendix G Figures 3 and 6) or close to shore (see Appendix G Figures 1, 2, 3, 7, and 8). These are all sites that could not have been or are unlikely to have been rototilled. Such findings suggest that rototilling may have eliminated the mussel from some of its habitat. Whether this could have happened due to direct harm to the mussels or alterations of the substrate (see discussion of the impact of rototilling in Dunbar 2009) is impossible to know.

Direct effects of Eurasian watermilfoil rototilling on Rocky Mountain ridged mussel

The experimental rototilling of Rocky Mountain ridged mussels shows clearly that rototilling is harmful to the mussel (Table 3). Two of the recovered mussels at the experimental site were crushed. Although this was not significantly different from the number of crushed mussels at the control site (0), it is safe to conclude that rototilling crushes some of the mussels.

Therefore, we can conclude that rototilling causes direct harm to the mussels. In addition, we recovered a significantly greater percentage of mussels at the control than the experimental site (Note that the recovery of less than 100 % of the mussels at the control site indicates the limitations of snorkel surveys, as discussed in Appendix B.). Since we did not recover any mussels in the area outside of the original relocation site, it is safe to assume that the majority of the remaining mussels that we could not recover had been buried by the rototilling. This strongly suggests that rototilling also indirectly causes harm to the mussels, as it has been shown that burial of Rocky Mountain ridged mussels causes mortality among the mussels (Krueger *et al.* 2007).

Implications for Rocky Mountain ridged mussel conservation and management

Overall, our findings have several implications for the conservation and management of Rocky Mountain ridged mussel in the Okanagan Valley:

1) The fact that recruitment is occurring at a sufficient level to maintain Rocky Mountain ridged mussel numbers at half of the locations and at some level at the other locations, shows that there is no immediate need to start a mussel propagation program (see Neves 2004, Thomas *et al.* 2010) in the Okanagan Valley. However, the fact that recruitment is too low to maintain mussel numbers at some locations and nearly non-existent at Kin Beach highlights the negative trend for the mussel in the system. Therefore, it is important to monitor recruitment of juveniles mussels on a regular basis (i.e. every 10 years).

2) Our findings suggest that sculpin function as the most important hosts for Rocky Mountain ridged mussel in the Okanagan Valley. Due to the high numbers of sculpin at the mussel locations in Okanagan Lake (*Pers. obs.*), there is no reason to assume that the mussel is host limited in the lake. However, at the mussel locations in the South Okanagan we hardly observed any sculpin (*Pers. obs.*). Therefore, the mussel could potentially be host limited at these locations. To determine if this is the case, it is important to investigate whether the invasive fish species that dominate these locations (such as smallmouth bass, yellow perch, pumpkinseed sunfish, and common carp) (*Pers. obs.*) or some of the native fish that are present (such as northern pikeminnow and suckers) (*Pers. obs.*) can function as hosts for the mussel.

3) The rototilling experiment clearly showed that rototilling is harmful to Rocky Mountain ridged mussel. Therefore, it is important to consider the conservation needs of the mussel when determining if rototilling should be allowed in an area with mussels.

Acknowledgements

First of all, I would like to thank Stephen Brownlee and Roxanne Snook for all their assistance in the field, and Ian Walker for facilitating both field and laboratory work (all at the University of British Columbia – Okanagan). I would also like to thank John Richardson (UBC - Vancouver) for providing office and lab space in Vancouver. Further, I would like to thank Ryan Mandau and Russell LaMountain (both at UBCO) with help in strength testing live and artificial Rocky Mountain ridged mussels.

Among contributors outside of UBC, I would like to thank Lora Nield (BC Ministry of Forests, Lands and Natural Resource Operations) for all her help in planning the project, for familiarizing me with Rocky Mountain ridged mussels, for her help with fish collection, and providing additional fish. Similarly, I would like to thank Jerry Mitchell (BC Ministry of Environment) for his help with fish collection, providing additional fish, and help in identifying the fish. Further, I would like to thank James Littley, Dave Caswell, Pat Field, and Ian Horner (all with the Okanagan Basin Water Board) for providing information with respect to the historical and current practices of the Eurasian watermilfoil management program. Ian Horner I would also like to thank for performing the rototilling during the rototilling experiment.

Finally, I would like to thank the BC Ministry of Forests, Lands and Natural Resource Operations, the BC Ministry of Environment, and Joerg Bohlmann (UBC – Vancouver) for providing funding for this project.

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Appendices

Appendix A:
Statistical comparison between Rocky Mountain ridged mussel juvenile recruitments locations when it comes to frequency of juveniles, mussel growth, and mussel length.

Methods

For details on the collection of the data, see the juvenile recruitment methodology section. All statistical analyses were completed using R version 3.02. (R Development Core Team, 2013).

To compare frequencies of juvenile Rocky Mountain ridged mussels between the locations, we used a Generalized Linear Model (GLM). The full R syntax for the model was: *glm(Juvenile~Location, binomial)*. In this model, *Juvenile* is a binomial response variable that indicates whether the mussels were juveniles or adults. *Location* is a categorical predictor variable that represents the location the mussels were collected from. A *binomial* distribution was used due to the binomial nature of the data.

For comparing Rocky Mountain ridged mussel growth between the locations, the length of young mussels was compared while correcting for mussel age. This was done using a two-way linear ANOVA (LM) with this full R syntax: *lm(Length~Location+Age)*. In this model, *Length* is a continuous response variable that represents the length of the mussels. *Location* is the same predictor variable as described above. *Age* is a continuous predictor variable that represents the age of the mussels.

To compare overall Rocky Mountain ridged mussel length between the locations, we used a two-way LM. The full R syntax for this model is: *lm(Length~Location)*. In this model, *Length and Location* are the same variables as described above.

Results

Frequencies of juvenile Rocky Mountain ridged mussels

Overall, the analysis of frequencies of juvenile Rocky Mountain ridged mussels showed that *Location* did have a significant impact on the frequencies of juvenile mussels ($p<0.001$). A multiple comparisons of means using Tukey contrasts gave the results below:

Kin Beach, Vernon, Vernon Arm, Okanagan Lake: A total of 106 Rocky Mountain ridged mussels were measured, including 2 juveniles and 104 adults. The frequency of juvenile mussels at this location was significantly lower than at Three Mile Beach ($p=0.05$). It did not differ significantly from any other location ($p\geq0.36$).

Peach Orchard Beach, Summerland, Okanagan Lake: A total of 22 Rocky Mountain ridged mussels were measured, including 3 juveniles and 19 adults. The frequency of juvenile mussels at this location did not differ significantly from any other location ($p\geq0.42$).

Dog Beach, Summerland, Okanagan Lake: A total of 111 Rocky Mountain ridged mussels were measured, including 10 juveniles and 101 adults. The frequency of juvenile mussels at this location did not differ significantly from any other location ($p\geq0.13$).

Kinsmen Park, Summerland, Okanagan Lake: A total of 194 Rocky Mountain ridged mussels were measured, including 4 juveniles and 190 adults. The frequency of juvenile mussels at this location was significantly lower than at Three Mile Beach ($p=0.02$). It did not differ significantly from any other location ($p\geq0.13$).

Pumphouse, Summerland, Okanagan Lake: A total of 111 Rocky Mountain ridged mussels were measured, including 1 juvenile and 110 adults. The frequency of juvenile mussels at this location was borderline significantly lower than at Three Mile Beach ($p=0.07$). It did not differ significantly from any other location ($p\geq0.29$).

South Okanagan Sailing Association, Summerland, Okanagan Lake: A total of 64 Rocky Mountain ridged mussels were measured, including 1 juvenile and 63 adults. The frequency of juvenile mussels at this location did not differ significantly from any other location ($p=1.00$).

Three Mile Beach, Naramata Benchlands, Penticton, Okanagan Lake: A total of 198 Rocky Mountain ridged mussels were measured, including 30 juveniles and 168 adults. The frequency of juvenile mussels at this location was significantly higher than at Kin Beach, Kinsmen Park, Vaseux Lake Campsite, and Oliver ($p \leq 0.005$). It was borderline significantly higher than at Pumphouse ($p=0.07$). The frequency did not differ significantly from any other location ($p \geq 0.88$).

Vaseux Lake Campsite, Vaseux Lake: A total of 138 Rocky Mountain ridged mussels were measured, including 1 juvenile and 137 adults. The frequency of juvenile mussels at this location was significantly lower than at Three Mile Beach ($p=0.04$). It did not differ significantly from any other location ($p \geq 0.29$).

Pedestrian bridge to Fairview Rd., Oliver, Okanagan River: A total of 116 Rocky Mountain ridged mussels were measured, including 3 juveniles and 116 adults. The frequency of juvenile mussels at this location was significantly lower than at Three Mile Beach ($p=0.03$). It did not differ significantly from any other location ($p \geq 0.29$).

Growth of young mussels

Overall, the ANOVA output showed that there were significant differences in Rocky Mountain ridged mussel *Length* between the locations ($p<0.001$) and that length was significantly influenced by the *Age* of the mussels ($p<0.001$). When correcting for the age of the mussels, a multiple comparisons of means using Tukey contrasts gave the results below:

Kin Beach, Vernon, Vernon Arm, Okanagan Lake: A total of 5 young mussels were measured and could be aged. The length of the mussels (a proxy for growth) was significantly lower than at Vaseux Lake Campsite and Oliver ($p<0.001$). It was not significantly different compared to any of the other locations ($p \geq 0.28$).

Peach Orchard Beach, Summerland, Okanagan Lake: A total of 7 young mussels were measured and could be aged. The length of the mussels (a proxy for growth) was significantly lower than at Vaseux Lake Campsite and Oliver ($p<0.001$). It was not significantly different compared to any of the other locations ($p \geq 0.22$).

Dog Beach, Summerland, Okanagan Lake: A total of 36 young mussels were measured and could be aged. The length of the mussels (a proxy for growth) was significantly lower than at Kinsmen Park, Vaseux Lake Campsite, and Oliver ($p \leq 0.004$). It was significantly higher than at Three Mile Beach ($p=0.001$). The length of the mussels was not significantly different compared to any of the other locations ($p \geq 0.93$).

Kinsmen Park, Summerland, Okanagan Lake: A total of 64 young mussels were measured and could be aged. The length of the mussels (a proxy for growth) was significantly lower than at Vaseux Lake Campsite and Oliver ($p<0.001$). It was significantly higher than at Dog Beach and Three Mile Beach ($p \leq 0.004$). The length of the mussels was not significantly different compared to any of the other locations ($p \geq 0.22$).

Pumphouse, Summerland, Okanagan Lake: A total of 18 young mussels were measured and could be aged. The length of the mussels (a proxy for growth) was significantly lower than at Vaseux Lake Campsite and Oliver ($p<0.001$). It was significantly higher than at Three Mile Beach ($p<0.001$). The length of the mussels was not significantly different compared to any of the other locations ($p \geq 0.76$).

South Okanagan Sailing Association, Summerland, Okanagan Lake: A total of 19 young mussels were measured and could be aged. The length of the mussels (a proxy for growth) was significantly lower than at Vaseux Lake Campsite and Oliver ($p<0.001$). It was significantly higher than at Three Mile Beach ($p=0.001$). The length of the mussels was not significantly different compared to any of the other locations ($p \geq 0.40$).

Three Mile Beach, Naramata Benchlands, Penticton, Okanagan Lake: A total of 85 young mussels were measured and could be aged. The length of the mussels (a proxy for growth) was significantly lower than at Dog Beach, Kinsmen Park, Pumphouse, South Okanagan Sailing Association, Vaseux Lake Campsite, and Oliver ($p \leq 0.001$). It was not significantly different compared to any of the other locations ($p \geq 0.76$).

Vaseux Campsite, Vaseux Lake: A total of 37 young mussels were measured and could be aged. The length of the mussels (a proxy for growth) was significantly higher than at all other locations ($p<0.001$), with the exception of Oliver ($p=0.93$).

Pedestrian bridge to Fairview Rd., Oliver, Okanagan River: A total of 26 young mussels were measured and could be aged. The length of the mussels (a proxy for growth) was significantly higher than at all other locations ($p<0.001$), with the exception of Vaseux Lake Campsite ($p=0.93$).

Length distribution of mussels

Overall, the ANOVA output showed that there were significant differences in Rocky Mountain ridged mussel *Length* between the locations ($p<0.001$). A multiple comparisons of means using Tukey contrasts gave the results below:

Kin Beach, Vernon, Vernon Arm, Okanagan Lake: A total of 106 Rocky Mountain ridged mussels were measured. The mean length of the mussels (84.6 mm) was significantly shorter than at Vaseux Lake Campsite and Oliver ($p\leq0.002$). It was significantly longer than at Peach Orchard Beach, Dog Beach, and Three Mile Beach ($p\leq0.002$). There was no significant difference in length compared to any other location ($p\geq0.07$).

Peach Orchard Beach, Summerland, Okanagan Lake: A total of 22 Rocky Mountain ridged mussels were measured. The mean length of the mussels (73.1 mm) was significantly shorter than at Kin Beach, Pumphouse, Vaseux Lake Campsite, and Oliver ($p\leq0.004$). It was significantly longer than at Three Mile Beach ($p=0.03$). There was no significant difference in length compared to any other location ($p\geq0.17$).

Dog Beach, Summerland, Okanagan Lake: A total of 111 Rocky Mountain ridged mussels were measured. The mean length of the mussels (74.3 mm) was significantly shorter than at Kin Beach, Kinsmen Park, Pumphouse, South Okanagan Sailing Association, Vaseux Lake Campsite, and Oliver. It was significantly longer than at Three Mile Beach ($p=0.001$). There was no significant difference in length compared to Peach Orchard Beach ($p=1.00$).

Kinsmen Park, Summerland, Okanagan Lake: A total of 194 Rocky Mountain ridged mussels were measured. The mean length of the mussels (80.3 mm) was significantly shorter than at Vaseux Lake Campsite and Oliver ($p<0.001$). It was significantly longer than at Dog Beach and Three Mile Beach ($p\leq0.001$). There was no significant difference in length compared to any other location ($p\geq0.07$).

Pumphouse, Summerland, Okanagan Lake: A total of 111 Rocky Mountain ridged mussels were measured. The mean length of the mussels (82.1 mm) was significantly shorter than at Vaseux Lake Campsite and Oliver ($p<0.001$). It was significantly longer than at Peach Orchard Beach, Dog Beach, and Three Mile Beach ($p\leq0.004$). There was no significant difference in length compared to any other location ($p\geq0.84$).

South Okanagan Sailing Association, Summerland, Okanagan Lake: A total of 64 Rocky Mountain ridged mussels were measured. The mean length of the mussels (80.4 mm) was significantly shorter than at Vaseux Lake Campsite and Oliver ($p<0.001$). It was significantly longer than at Dog Beach and Three Mile Beach ($p \leq 0.004$). There was no significant difference in length compared to any other location ($p \geq 0.27$).

Three Mile Beach, Naramata Benchlands, Penticton, Okanagan Lake: A total of 198 Rocky Mountain ridged mussels were measured. The mean length of the mussels (64.2 mm) was significantly shorter than at all other locations ($p \leq 0.003$).

Vaseux Campsite, Vaseux Lake: A total of 138 Rocky Mountain ridged mussels were measured. The mean length of the mussels (90.8 mm) was significantly longer than at all other locations ($p \leq 0.002$), with the exception of Oliver ($p=0.64$).

Pedestrian bridge to Fairview Rd., Oliver, Okanagan River: A total of 116 Rocky Mountain ridged mussels were measured. The mean length of the mussels (93.5 mm) was significantly longer than at all other locations ($p \leq 0.001$), with the exception of Vaseux Lake Campsite ($p=0.64$).

Appendix B:
Use of juvenile recruitment data for estimating Rocky Mountain ridged mussel density and numbers.

Methods

For data collection methods, see description of juvenile recruitment methods. Density for each location was determined by dividing the total number of Rocky Mountain ridged mussels found by the total area covered by the transects. The total number of mussels for the location was estimated by multiplying the density by the length of shoreline between the two outer transects and by the mean length of the transects.

Results

See Table 1.

Discussion

Overall, the Rocky Mountain ridged mussel number estimates from the juvenile recruitment surveys are substantially higher than those recorded from snorkel surveys from the same locations. This is the case even though the juvenile recruitment surveys typically cover a smaller area than the snorkel surveys. The discrepancy in mussel numbers may partially be explained by the fact that the mussel number estimates also include buried mussels, which encompass approximately a quarter of the mussels, but differs between locations (see juvenile recruitment results). However, this cannot explain the magnitude of the difference between the estimated and the surveyed mussel numbers. Therefore, the mussel number estimates highlight that snorkel surveys are likely to substantially underestimate the numbers of mussels present at a location. It also suggests that the numbers of Rocky Mountain ridged mussels are substantially higher in the Okanagan Valley than suggested by snorkel surveys.

Table 1 Appendix B Overview of Rocky Mountain ridged mussel number estimates for juvenile recruitment locations. The densities and mussel number estimates are from juvenile recruitment surveys. The mussel survey numbers are compiled from survey data provided by Lora Nield (*Pers. com.*), by Roxanne Snook (*Pers. com.*), and from Stanton *et al.* (2012). Note that the estimate for the Okanagan River in downtown Oliver is only for one river bank.

Location	Density (mussels/m^2)	Shoreline Covered (m)	Mussel# Estimate	Mussel Survey #
Kin Beach, Vernon Arm	1.23	38	1340	324
Peach Orchard Beach, Summerland	0.28	31	110	181
Dog Beach, Summerland	0.99	34	940	1146
KinsmenPark, Summerland	3.46	43	2780	123
Pumphouse, Summerland	1.34	106	2350	150+
South Okanagan Sailing Association, Summerland	0.23	198	550	150+
Three Mile Beach, NaramataBenchlands	2.64	43	2840	200+
VaseuxCampiste, VaseuxLake	1.05	16	140	69
Downtown Oliver, Oliver	1.35	358	2310	369

Appendix C:

Sketches of survey transects at

Rocky Mountain ridged mussel juvenile recruitment locations.

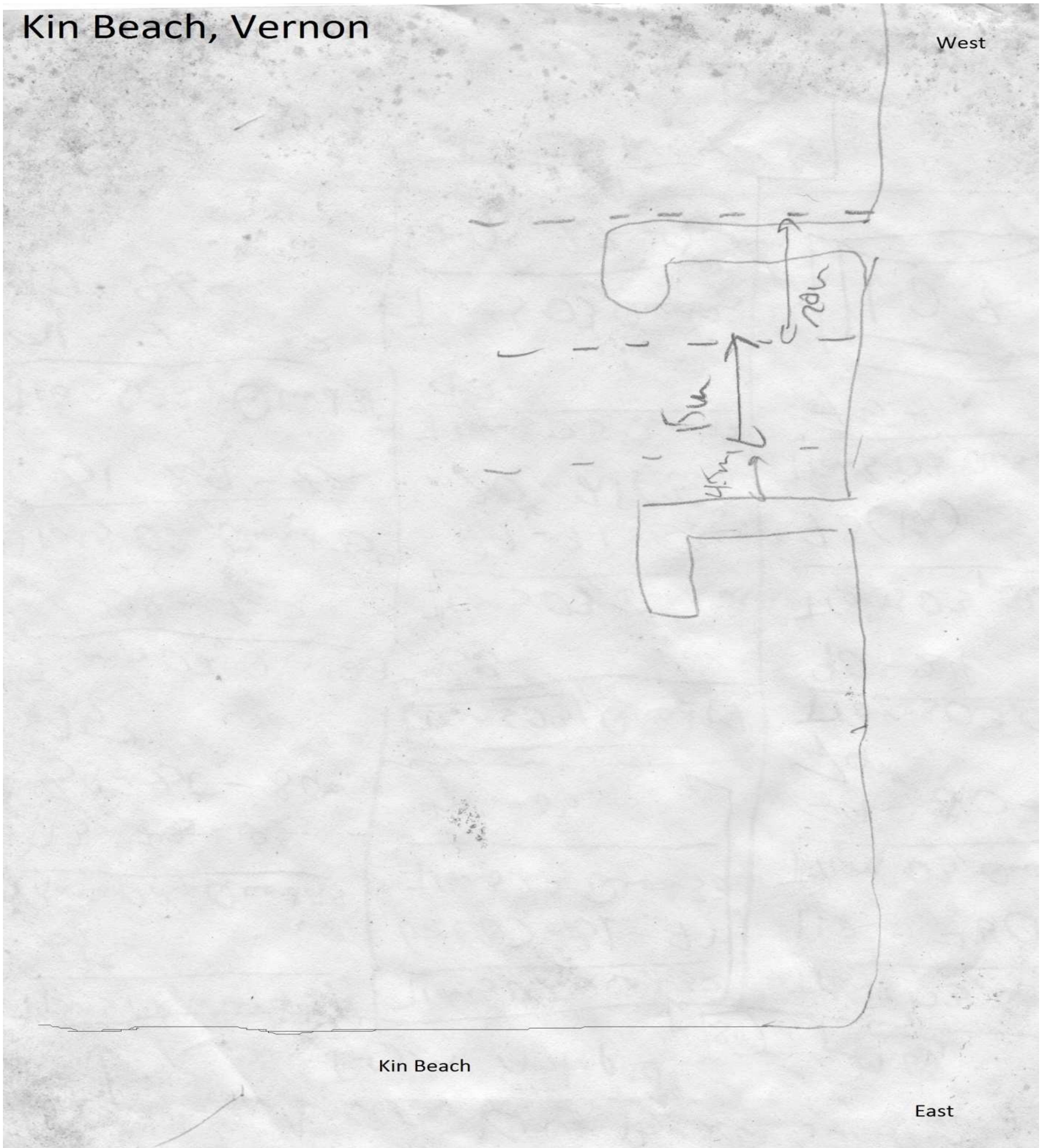

Figure 1 Appendix C Kin Beach, Vernon Arm, Okanagan Lake. Dashed lines indicate transects. Distances between transects are indicated on the sketch.

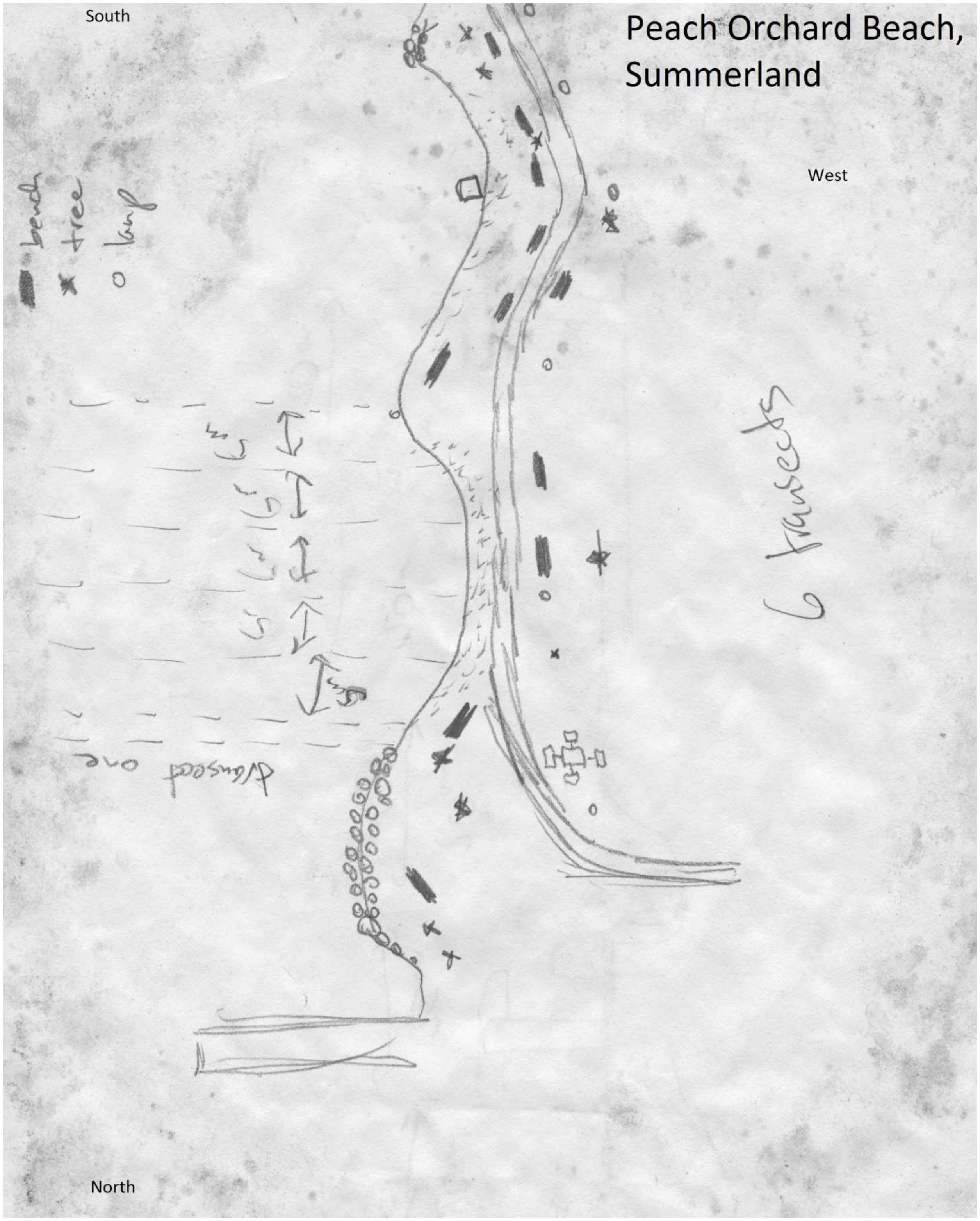

Figure 2 Appendix C Peach Orchard Beach, Summerland, Okanagan Lake. Dashed lines indicate transects. Distances between transects are indicated on the sketch.

Figure 3 Appendix C Dog Beach, Summerland, Okanagan Lake. Dashed lines indicate transects. Distances between transects are indicated on the sketch.

Figure 4 Appendix C Kinsmen Park, Summerland, Okanagan Lake. Dashed lines indicate transects. Distances between transects are indicated on the sketch.

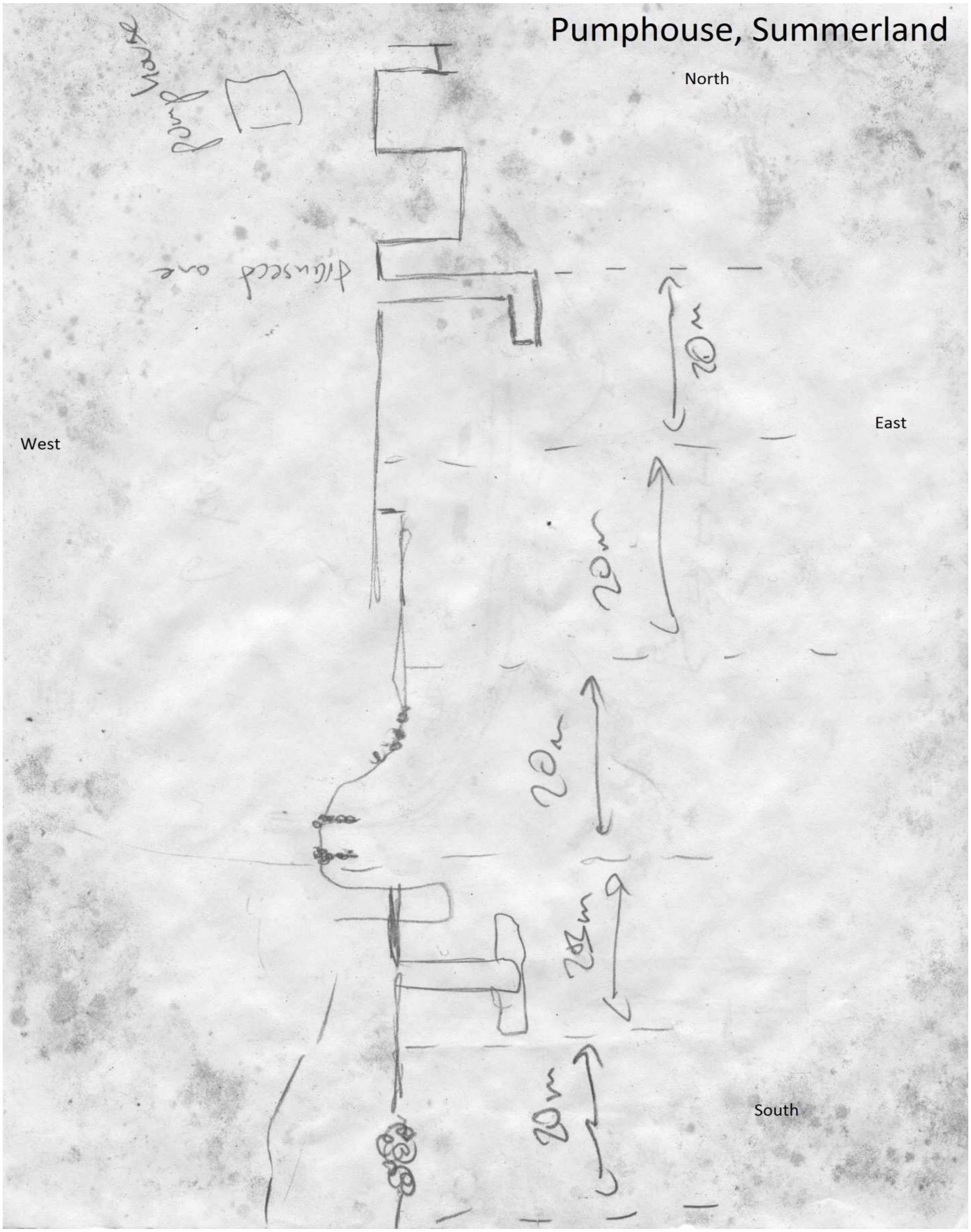

Figure 5 Appendix C Pumphouse, Summerland, Okanagan Lake. Dashed lines indicate transects. Distances between transects are indicated on the sketch.

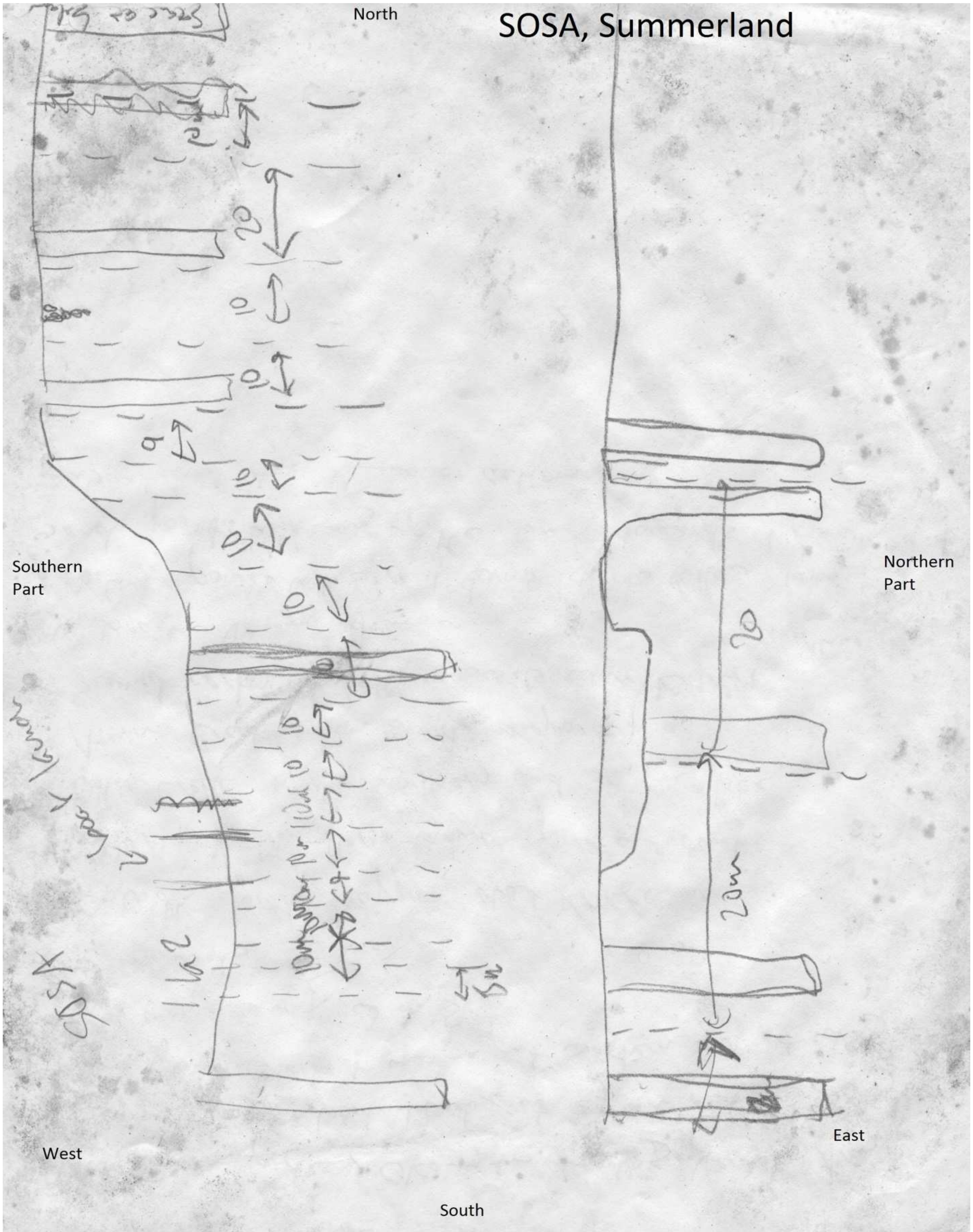

Figure 6 Appendix C South Okanagan Sailing Association, Summerland, Okanagan Lake. Dashed lines indicate transects. Distances between transects are indicated on the sketch.

Figure 7 Appendix C Three Mile Beach, Naramata Benchlands, Okanagan Lake. Dashed lines indicate transects. Distances between transects are indicated on the sketch.

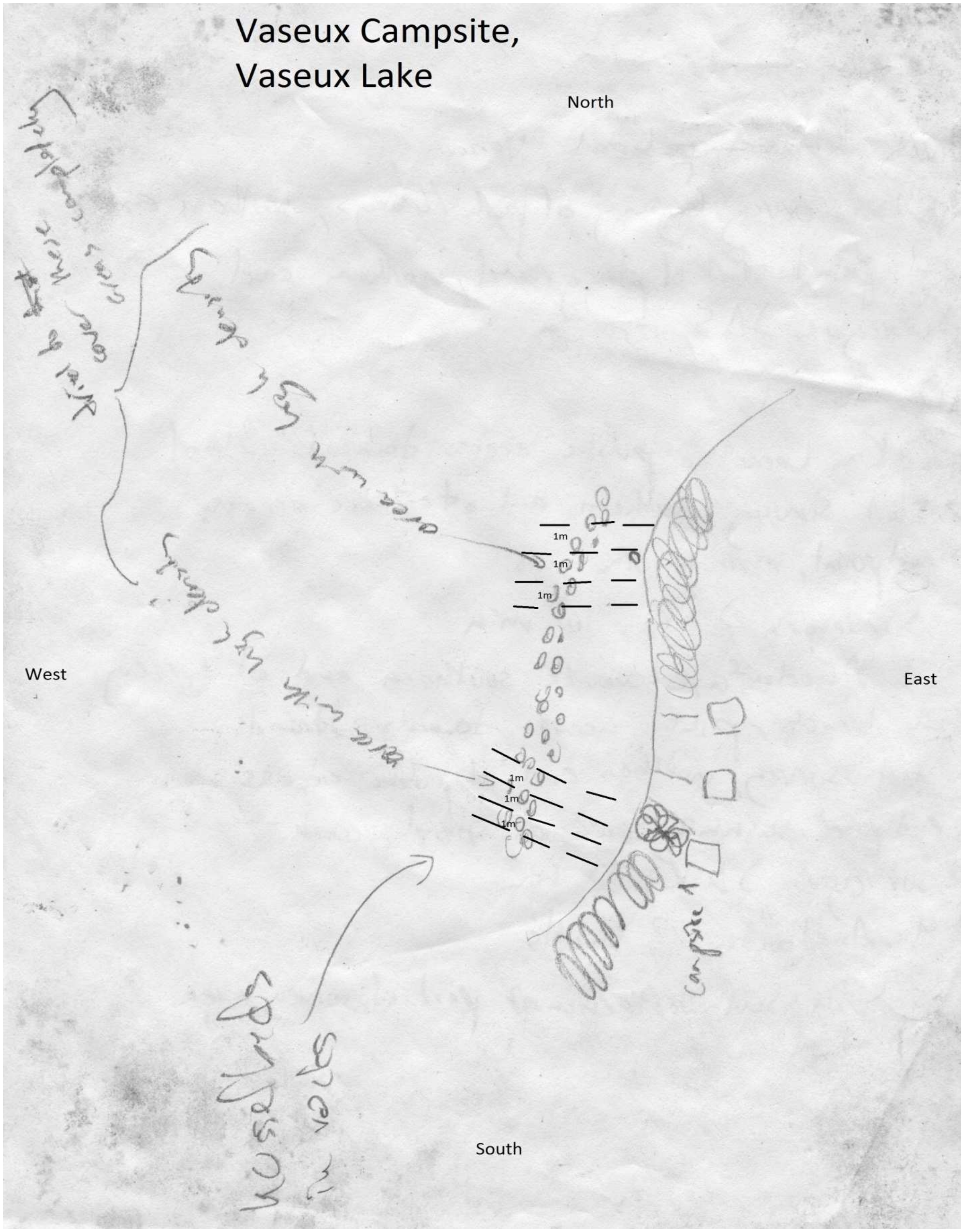

Figure 8 Appendix C Vaseux Lake Campsite, Vaseux Lake. Dashed lines indicate transects. Distances between transects are indicated on the sketch.

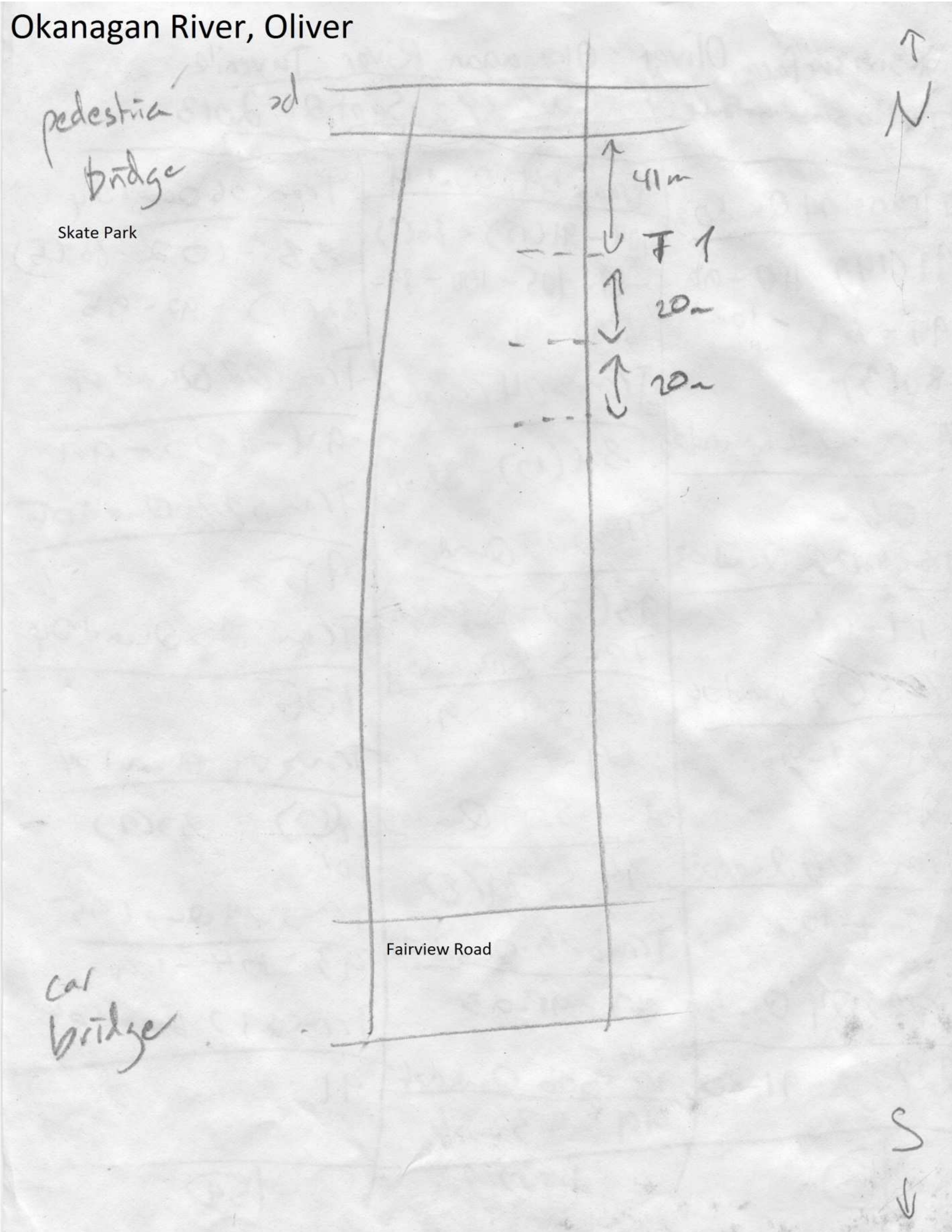

Figure 9 Appendix C Downtown Oliver, Oliver, Okanagan River. Dashed lines indicate transects. Distances between transects are indicated on the sketch.

Appendix D:
Statistical analyses of Rocky Mountain ridged mussel host fish use field data

Methods

For details on the collection of the data, see the fish host field data methodology section. All statistical analyses were completed using R version 3.02. (R Development Core Team, 2013).

To compare the Rocky Mountain ridged mussel glochidial prevalence between fish species a Generalized Linear Model (GLM) was used. The full R syntax for this model was: *glm(Infected~Species, binomial)*. In this model *Infected* is a binomial response variable that indicates whether the fish was infected with glochidia or not. *Species* is a categorical predictor variable that represents the different fish species. A *binomial* distribution was used due to the binomial nature of the data.

When analysing for differences in Rocky Mountain ridged mussel glochidial intensity, we only included fish that were infected (n=92). To complete this analysis, a GLM with this full R syntax was used: *glm(Intensity~Species, quasipoisson)*. In this model *Intensity* is a categorical response variable that represents the glochidial intensity on the fish. *Species* is the same predictor variable as above. A *quasipoisson* distribution was used due to the data being over-dispersed count data.

To determine whether Rocky Mountain ridged mussel glochidia grew on fish, we compared the size of glochidia found in conglutinates and glochidia encysted on fish. Non-encysted glochidia were excluded from the analysis since they are not expected to show any growth on fish (see discussion of encystment of glochidia in O'Brien *et al.* 2013). Only sculpin where included in this analysis due to the low number of infected fish among the other fish species. The comparison was made using two-way linear ANOVAs (LMs). The models were identical whether investigating the width, hingelength or length of the glochidia. For these models, the full R syntax was: *lme(Size~State, random=~+1|Source)*. In this model, *Size* is a continuous response variable that represents either the width, hingelength, or the length of the glochidia. *State* is a categorical predictor variable that represents whether the glochidia were encysted on fish gills or freely present in conglutinates. *Source* is a categorical predictor variable that represents the source of the glochidia. For encysted glochidia, this source is the fish the glochidia were attached to. For free glochidia, this is the mussel the conglutinates came from.

Results

When analysing the data with respect to Rocky Mountain ridged mussel glochidial prevalence on the different fish species, the overall analysis showed that *Species* had a significant impact on prevalence ($p<0.001$). A multiple comparisons of means using Tukey contrasts gave these results: Sculpin had significantly higher prevalence than whitefish ($p<0.001$) and longnose dace ($p=0.05$). There was no significant difference in prevalence for any other combination of fish species ($p\geq0.77$).

The overall analysis of Rocky Mountain ridged mussel glochidial intensity on the different fish species showed no significant differences in intensity between the fish species ($p=0.26$).

When analysing the data with respect to Rocky Mountain ridged mussel glochidial size, the ANOVA output for width showed a significant effect of *State* ($p<0.001$), i.e. that the width was significantly shorter for encysted than free glochidia. In addition, the analysis showed that there was a significant effect of *Source*. ($p<0.001$). With respect to Rocky Mountain ridged mussel glochidial hingelength, the ANOVA output showed a significant effect of *State* ($p<0.001$), i.e. that the hingelength was significantly longer for encysted than free glochidia. In addition, the analysis showed that there was a significant effect of *Source*. ($p<0.001$). The ANOVA output for Rocky Mountain ridged mussel glochidial length showed a significant effect of *State* ($p<0.001$), i.e. that the length was significantly shorter for encysted than free glochidia. In addition, the analysis showed that there was a significant effect of *Source*. ($p<0.001$).

Discussion

The analysis of glochidial prevalence was not very helpful. Almost all of the fish species were found to not differ in prevalence, which is likely explained by the low sample size for many of the species. In fact, the only significant differences were found between sculpin and whitefish, and sculpin and longnose dace, which were among the species with the highest sample sizes.

Similarly, the analysis of glochidial intensity was not very helpful. It showed not significant effect of *Species* on intensity, which is likely explained by the low number of infected fish among all the species, except for sculpin.

The only measurement that showed the expected results that encysted glochidia were significantly larger than free glochidia was hingelength. For the other two measurements, the encysted glochidia were significantly smaller than the free glochidia. The discrepancy in the findings suggest that the significant differences are likely the result of errors in the measurement of the glochidia (they were measured at different times, using different microscopes and different cameras). However, such errors should have been overshadowed by growth on the glochidia on the sculpin, if such a growth occurred. Therefore, these results suggest that there was no substantial growth of the encysted glochidia on the fish. Given these findings, one might question whether Rocky Mountain ridged mussel truly is parasitic on fish, as one would expect to see glochidial growth on the gills if this is the case. However, further investigations are needed to answer this question.

Appendix E:
Statistical analyses of rototilling experiment.

Methods

For details on the collection of the data, see the rototilling experiment methodology section. All statistical analyses were completed using R version 3.02. (R Development Core Team, 2013). The comparison of frequencies of recovered vs. relocated mussels between the experimental and control sites was completed using a Fisher's Exact Test. An identical analysis was performed for comparing the frequencies of crushed to recovered mussels between the two sites.

Results

The frequency of recovered mussels was significantly higher at the control site ($p<0.001$). There was no significant difference in the frequency of crushed mussels between the two sites ($p=0.18$).

Appendix F:
Strength testing of live Rocky Mountain ridged mussels.

Methods

20 live Rocky Mountain ridged mussels were collected from Kinsmen Park, Summerland. The mussels were transported in water to the University of British Columbia – Okanagan. At the University the mussels were weighed (wet) and measured (from anterior to posterior end). Subsequently, the strength of the mussels were tested using an Instron 3385H tension and compression tester. The mussels were exposed to a compression of 25 mm/min and the point at which the shells cracked (a marked decrease in force measured) were recorded (in Newtons). 10 mussels were mounted in a foam mount to allow for lateral compression. The 10 other mussels were mounted in a foam mount to allow for ventrodorsal compression.

Results

See Table 1.

Table 1 Appendix F Strength testing of Rocky Mountain ridged mussels. Note that weights are wet weights.

Mussel #	Mussel Length (mm)	Mussel Weight (g)	Compression Type	Mussel Strength (N)
1	93	72.5	Lateral	1072
2	76	42.3	Lateral	763
3	75	34.3	Lateral	547
4	89	73.5	Lateral	1595
5	86	64.9	Lateral	1136
6	90	72.8	Lateral	1349
7	84	72.2	Lateral	1528
8	84	57.4	Lateral	831
9	76	44.5	Lateral	979
10	80	59.5	Lateral	1222
Mean	*83*	*59.4*	*Lateral*	*1102*
11	77	41.6	Ventrodorsal	583
12	83	59.3	Ventrodorsal	849
13	96	108.5	Ventrodorsal	1165
14	97	110.0	Ventrodorsal	859
15	90	69.2	Ventrodorsal	721
16	85	58.4	Ventrodorsal	687
17	90	72.9	Ventrodorsal	1046
18	84	58.7	Ventrodorsal	568
19	73	35.3	Ventrodorsal	557
20	68	29.9	Ventrodorsal	275
Mean	*84*	*64.4*	*Ventrodorsal*	*731*

Appendix G:

Figures showing Rocky Mountain ridged mussel find sites in and associated with Eurasian watermilfoil rototilling polygons.

Figure 1 Appendix G Polygon 22 Rocky Mountain ridged mussel find site. The find site is in the northwestern corner of Osoyoos Lake (Lakehead Campsite, Osoyoos). The white pins and grey polygons indicate the Eurasian watermilfoil treatment polygon numbers and areas. Within the polygons, the yellow area indicates the area surveyed for the mussel. The red polygon and pin indicates the area in which mussels were found and the number of mussels found.

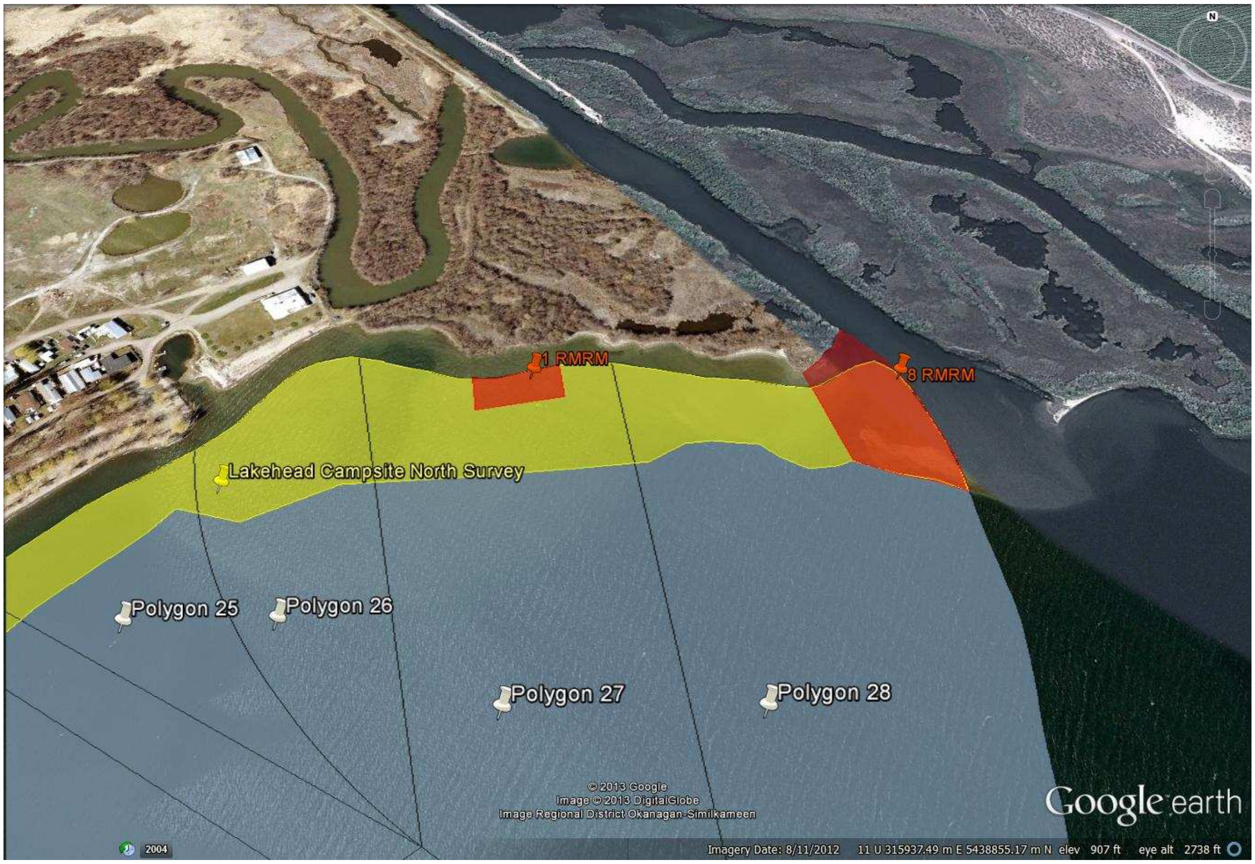

Figure 2 Appendix G Polygons 27 and 28 Rocky Mountain ridged mussel find sites. The find sites are at the northern end of Osoyoos Lake (Lakehead Campsite, Osoyoos). The white pins and grey polygons indicate the Eurasian watermilfoil treatment polygon numbers and areas. Within the polygons, the yellow area indicates the area surveyed for the mussel. The red polygons and pins indicate the areas in which mussels were found and the number of mussels found.

Figure 3 Appendix G Polygons 50 and 52 Rocky Mountain ridged mussel find sites. The find sites are at the southern end of Skaha Lake (Beaches, Okanagan Falls). The white pins and grey polygons indicate the Eurasian watermilfoil treatment polygon numbers and areas. Within the polygons, the yellow areas indicate the areas surveyed for the mussel. The red polygons and pins indicate the areas in which mussels were found and the number of mussels found.

Figure 4 Appendix G Polygon 53 Rocky Mountain ridged mussel find site. The find site is towards the northern end of the eastern shore of Skaha Lake (Lakeside Ct., Penticton). The white pin and grey polygon indicates the Eurasian watermilfoil treatment polygon number and area. Within the polygon, the yellow area indicates the area surveyed for the mussel. The red polygon and pin indicate the area in which mussels were found and the number of mussels found.

Figure 5 Appendix G Polygon 64 Rocky Mountain ridged mussel find sites. The find sites are towards the southern end of the western shore of Okanagan Lake (Rotary Beach, Summerland). The white pin and grey polygon indicates the Eurasian watermilfoil treatment polygon number and area. Within the polygon, the yellow area indicates the area surveyed for the mussel. The red polygons and pins indicate the areas in which mussels were found and the number of mussels found.

Figure 6 Appendix G Polygon 91 Rocky Mountain ridged mussel find sites. The find sites are along the central part of the western shore of Okanagan Lake (Casa Loma, West Kelowna). The white pins and grey polygons indicate the Eurasian watermilfoil treatment polygon number and area. Within the polygon, the yellow area indicates the area surveyed for the mussel. The red polygons and pins indicate the areas in which mussels were found and the number of mussels found.

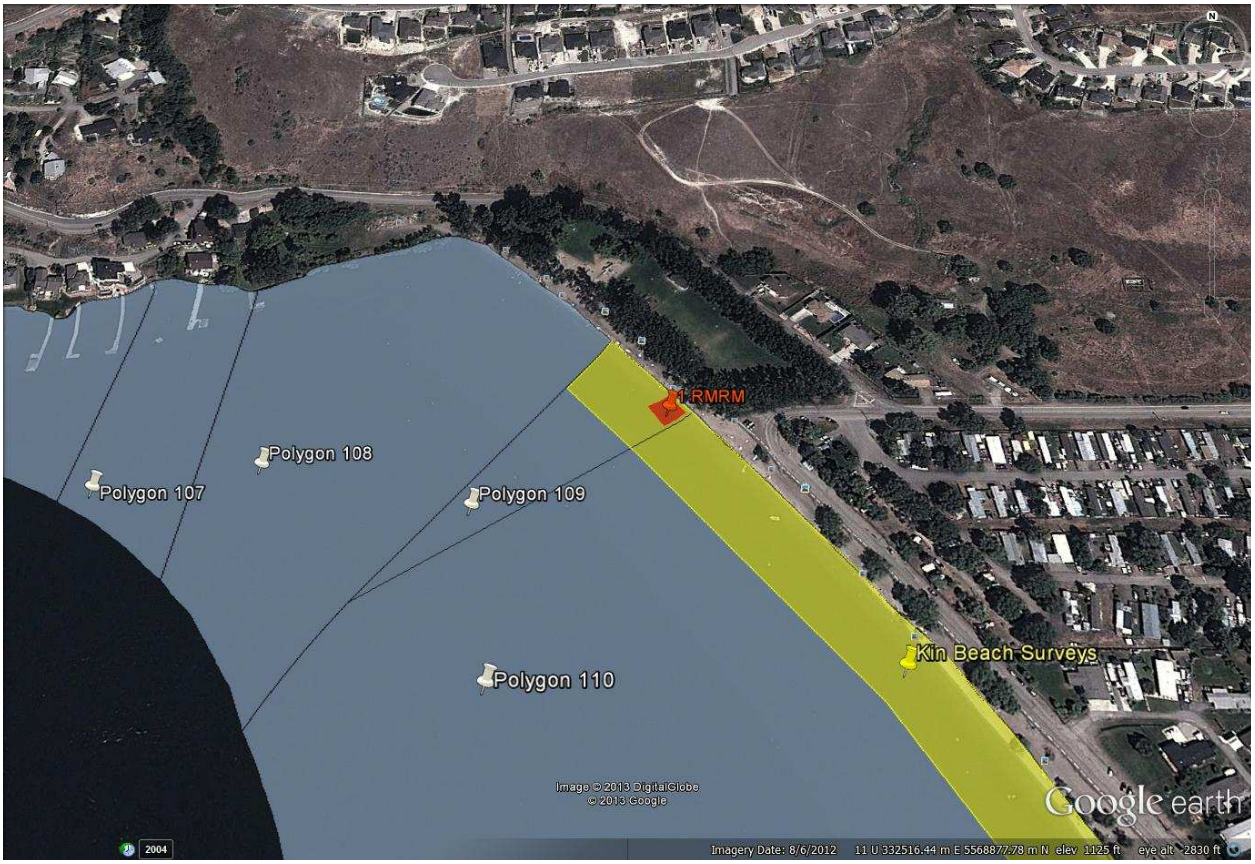

Figure 7 Appendix G Polygon 109 Rocky Mountain ridged mussel find site. The find site is at the eastern end of the Vernon Arm of Okanagan Lake (Kin Beach, Vernon). The white pins and grey polygons indicate the Eurasian watermilfoil treatment polygon numbers and areas. Within the polygons, the yellow area indicates the area surveyed for the mussel. The red polygon and pin indicates the area in which mussels were found and the number of mussels found.

Figure 8 Appendix G Polygon 117 Rocky Mountain ridged mussel find site. The find site is along the southern shore of the Vernon Arm of Okanagan Lake (Okanagan Landing, Vernon). The white pins and grey polygons indicate the Eurasian watermilfoil treatment polygon numbers and areas. Within the polygons, the yellow area indicates the area surveyed for the mussel. The red polygon and pin indicates the area in which mussels were found and the number of mussels found.