

**2011 St. Croix National Scenic Riverway
Zebra Mussel Monitoring and
Support of Federally Listed
Endangered Mussel Species**



Report Prepared by:

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March, 2012

Report Prepare for:

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Introduction:

The St. Croix National Scenic Riverway was the first unit of the National Park System included in the Wild and Scenic Rivers Act of 1968. The Riverway is considered a nationally significant resource for its richness and abundance of freshwater mussels (~40 species, the greatest in the Upper Mississippi watershed) and is recognized for its outstanding recreational and biological assets. The diversity of unionids within the Riverway is well documented and many threats to that diversity have been identified. This faunal group could be severely impacted by a zebra mussel (*Dreissena polymorpha*) infestation and from other invasive species. Freshwater mollusks are a keystone faunal group of freshwater systems and their potential loss is unacceptable.

In order to understand the invasion of zebra mussels into the St. Croix, measurements of density have been taken since 2004 within the known infestation zone (the last 21 miles of river). Anecdotal evidence from the Upper Mississippi River suggests zebra mussel colonization predominates on native mussel beds, especially when substrates are less favorable for recruitment (e.g., sand, silt, etc.). Therefore, sample locations were chosen based on native mussel bed survey work previously conducted by the Minnesota Department of Natural Resources (MN DNR). Six locations were identified from Stillwater, MN, to Prescott, WI, reflecting the range of habitats and hydrology found in the infestation zone. In addition, the endangered Higgin's eye pearly mussel Essential Habitat Areas of Hudson and Prescott were included. Thirty 1/8-meter quadrat samples were collected by divers at each of the locations. These samples were processed on site, frozen and examined under magnification. Data collected continues to aid managers who are creating policy based on the spread and intensity of the invasion.

In addition to understanding relative abundance of zebra mussels in Lake St. Croix, other St. Croix Basin activities for 2011 related to zebra mussels and work with endangered native mussels follows.

Qualitative zebra mussel sampling was performed in 2011 using two methods. First, throughout the watershed, including 16 locations on the main stem of the St. Croix and Namekagon and several area lakes, plate samplers with glass slides were placed in the water to encourage initial settlement of any floating veligers in the system. A second, more active sampling method involves shoreline searches in areas of the river/basin lakes (15) with higher probability of zebra mussel colonization (boat landings), but where none, to date, have been found. During 2011, these activities were supported by the U.S. Fish and Wildlife Service (Fisheries Resources Office, Onalaska, WI).

Associated quantitative and qualitative veliger collection continued this year as part of a broader determination of reproduction of zebra mussels throughout the Upper Mississippi River system. This monitoring is critical as a complement to other projects in the basin in determining effects of the infestation. For the second consecutive year, veliger samples were collected at 15 lakes within the St. Croix River watershed in Minnesota (6) and Wisconsin (9) identified as high risk for zebra mussel infestation. This includes Bass Lake, Polk Co. Wisconsin, added to the list of lakes sampled for veligers/adults in 2011.

Finally, the St. Croix Riverway Scuba Dive Team participated in several activities related to the propagation and recovery of the winged mapleleaf and Higgin's eye pearly mussel (both federally listed as endangered). These activities included propagation (fish cages and lab infestations), developing mussel caches and gravid female recovery, placing and checking juvenile rearing cages, and relocation habitat assessments.

Lake St. Croix Zebra Mussel Densities (*USACOE SOW Task 1*)

Quantitative samples were collected at eight locations (established native mussel beds) to determine zebra mussel densities at various places within the last 21 miles of the river (Fig. 1). The locations included the Essential Habitat Areas designated for the Higgins eye mussel and representative of each of the pools and narrows of Lake St. Croix. From the confluence with Mississippi River, upstream:

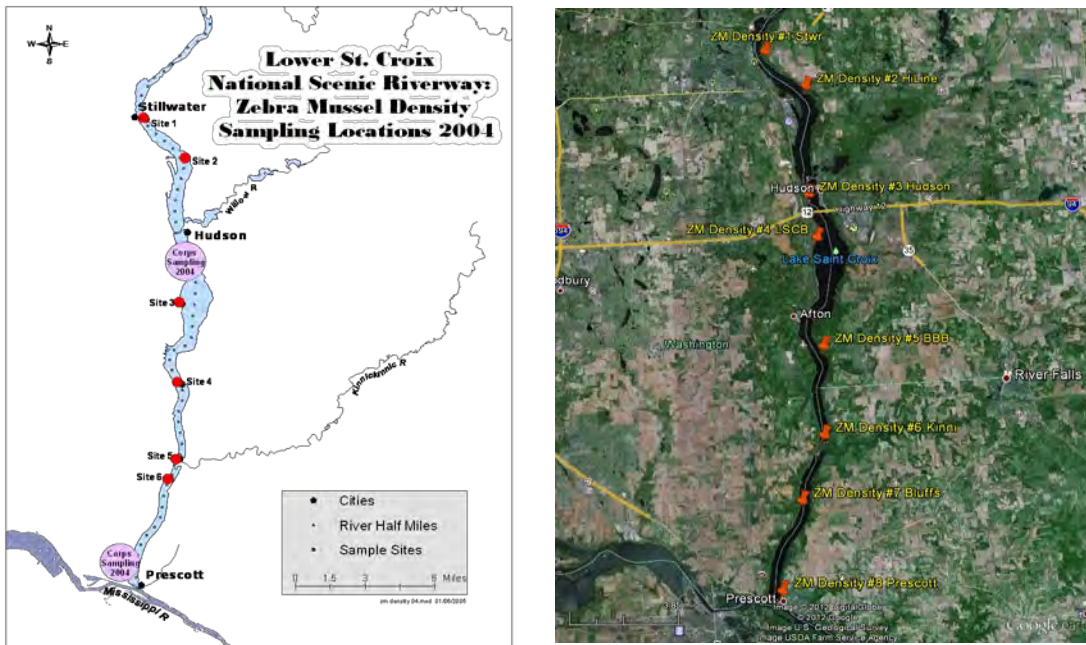
- 1) Prescott Higgins eye Essential Habitat Area (EHA) (River Mile Right 0.2),
- 2) St. Croix Bluffs (RM R5)
- 3) Kinnickinnic Narrows (RM L6)
- 4) Black Bass Bar (RM L10)
- 5) Lake St. Croix Beach (RM R14)
- 6) Hudson Higgins' eye Essential Habitat Area (RM R17)
- 7) South Highline Beach (RM L21)
- 8) Stillwater Mussel Relocation Site (RM L23).

These same eight sites were sampled in 2005-10 by NPS/FWS. In 2004, the EHAs were sampled by the USACE and WI DNR.

At each site, 30 1/8th meter-squared quadrates were sampled by haphazardly tossing the metal squares from the boat. A test dive was performed to mark suitable habitat and native mussel densities. Within the location perimeter, the boat was moved to ensure full coverage of the native mussel bed. The dive boat was positioned in as close proximity as possible to previously sampled sub-sites locations via GPS. After all samples from the location were collected, an appropriate shore site was determined and a crew of 4-6 staff and volunteers processed each sample through 3mm screen mesh using a low pressure pump of river water. This technique (though potentially labor intensive) allowed for completion of time consuming flushing of sand, mud and small organic matter on site, without tedious transport. On site processing reduces off-site storage and contamination, aids in sample preservation, and improves efficiency.

In the laboratory, sample material was thawed and removed from the one gallon zip-locked baggies and spread onto a sorting tray. Larger debris was checked under handheld magnification and zebra mussels (if any) removed by hand or forceps. Next, the remaining material was rewashed in a 250 μ m sieve then spread evenly throughout a dissecting scope and analyzed. If as in previous years abundance is high, sub-samples are used to obtain density estimates. This year's material used for density estimates was fully counted. Zebra mussels were not measured for length in the 2011. In 2010 and 2011, a USGS predator/competition study began to assess growth patterns in the Lower St. Croix River from Stillwater to Prescott. Preliminary results from this study should be available later this year.

Figure 1. Maps of eight long term zebra mussel monitoring sites established in 2004.



Only zebra mussels that were alive or considered living during collection were counted. All zebra mussels were placed into 4 groups: zebra mussels found attached to living native mussels were categorized for infestation rates (Fig. 2) and included for density, live zebra mussels found elsewhere in the sample quadrat were added for total density. Live native mussels (identified to species) were used to calculate bed densities (Fig. 3). The total number of live mussels collected and shells from recently dead animals included in Figure 2 of Appendix A. While the overall density trend in Lake St. Croix is similar whether or not dead shells are included, it does highlight slight location reductions at St. Croix Bluffs and the Kinni Narrows.

Figure 2. Density (m^2) of zebra mussel attached to native mussels, by location.

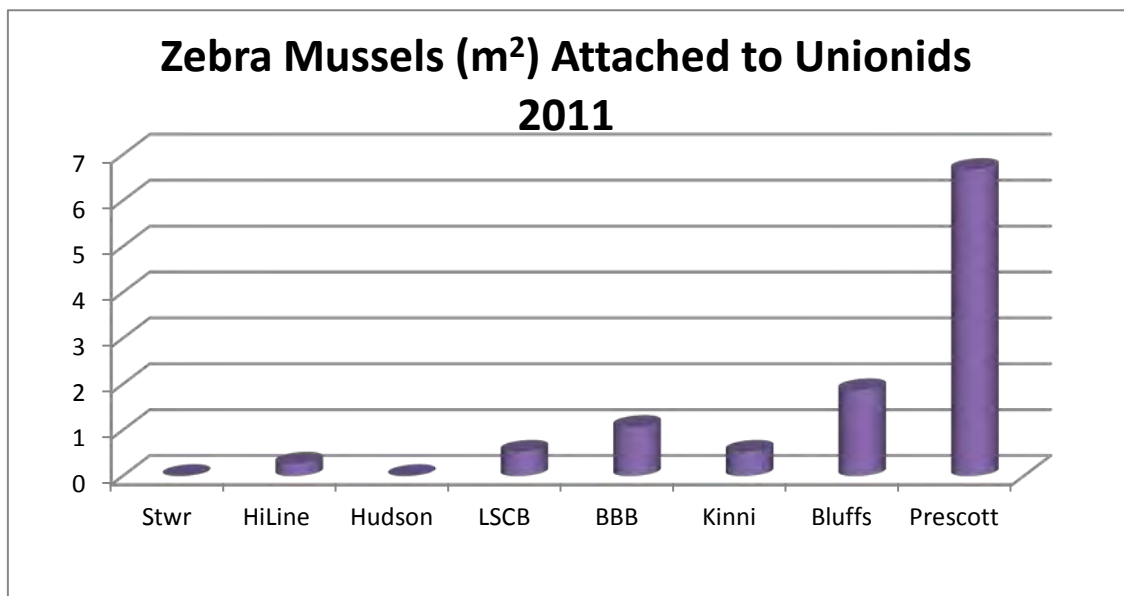
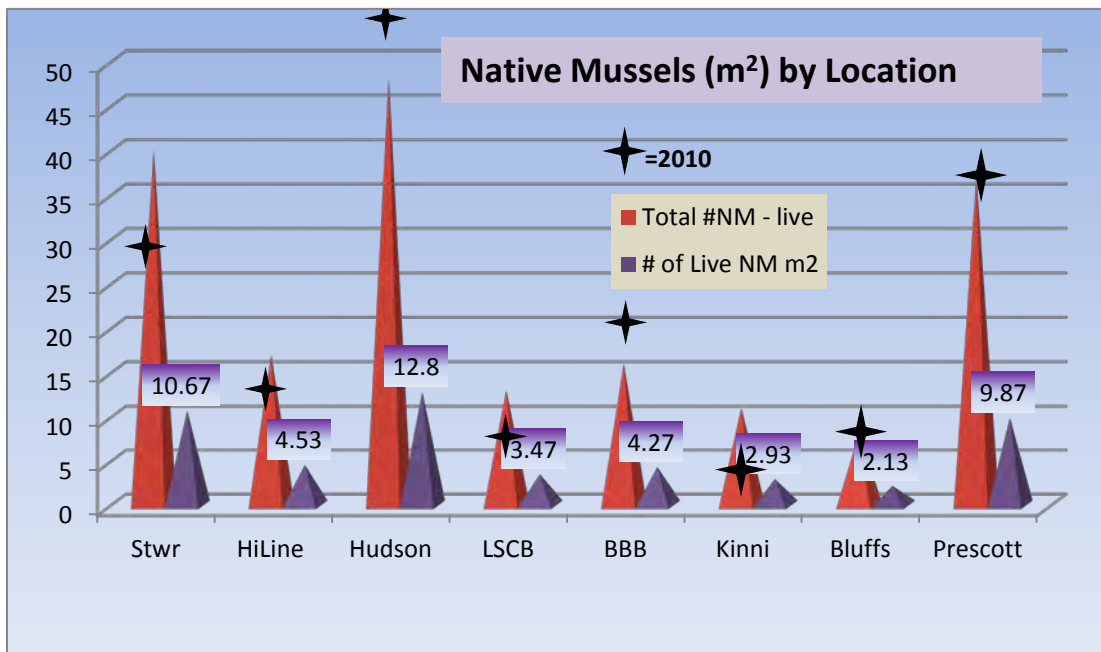


Figure 3. Live native mussels (m^2) collected at eight long term monitoring sites.

At all of the eight locations sampled this year, infestation rates by zebra mussels onto native mussels were insignificant. At the location with the highest number of attached invasive mussels (Prescott= $6.67m^2$), the native mussel bed density was great enough that the average less than one zebra mussel per native is not considered to be significant.

The overall density results for 2011 reflect a dramatic decline in live zebra mussels of any size (Fig. 4). Results of zebra mussel densities since 2004 had shown a steady increase, with a dramatic spike in 2007 and 2008 (Fig. 6). However, during these field seasons, addition data collected from three sites within the Prescott Pool, suggested heavy predation on adult zebra mussels at mid-summer (see the 2010 report). Reproduction within the river was apparently sufficient to maintain a robust population, but suddenly the population structure was restricted to the previous year's class—which disappeared mid-summer at an average size of 2cm—and the newly settled cohort of <1mm animals. As Figure 6 shows, densities at some upstream locations actually increased in 2009 and 10, but possible ecological factors, such as predation, may have accounted for the recent lower densities at the downstream most locations in the river. Hydrological conditions probably drives settlement opportunity within the various river pools.

The rank of each of the eight locations by zebra mussel density did not change significantly in 2011 (Fig. 5), but the overall reduction may be explained by two factors. As has been shown over the last several years, adult zebra mussels age 1 year (15mm) die-off, crushed shells in the substrate the only remains. This appears to be the result of fish predation – most of the live native mussels in the pool are still captured with multiple byssus affixed – and this phenomena is currently being explored in a related USGS Study. Second, 2011 was record setting in the depth and length of time high water remained in the St. Croix and much of the Upper Mississippi Basin. The three year hydrographic data presented in Appendix A reflects the unusual events. It

may be possible that the typical mid-summer predation concurrent with a reproductive pulse that has typically be the pattern was disrupted by the high water and settlement within the St. Croix could not occur. While size distribution is not part of this study, we have typically counted hundreds of less than 1mm animals absent in these 2011 samples. Data collected and currently being analyzed by the USGS, may provide additional insight.

Figure 4. Total number of live zebra mussels collected in substrate samples 2011. (Includes animals pulled from native mussels and other hard substrate).

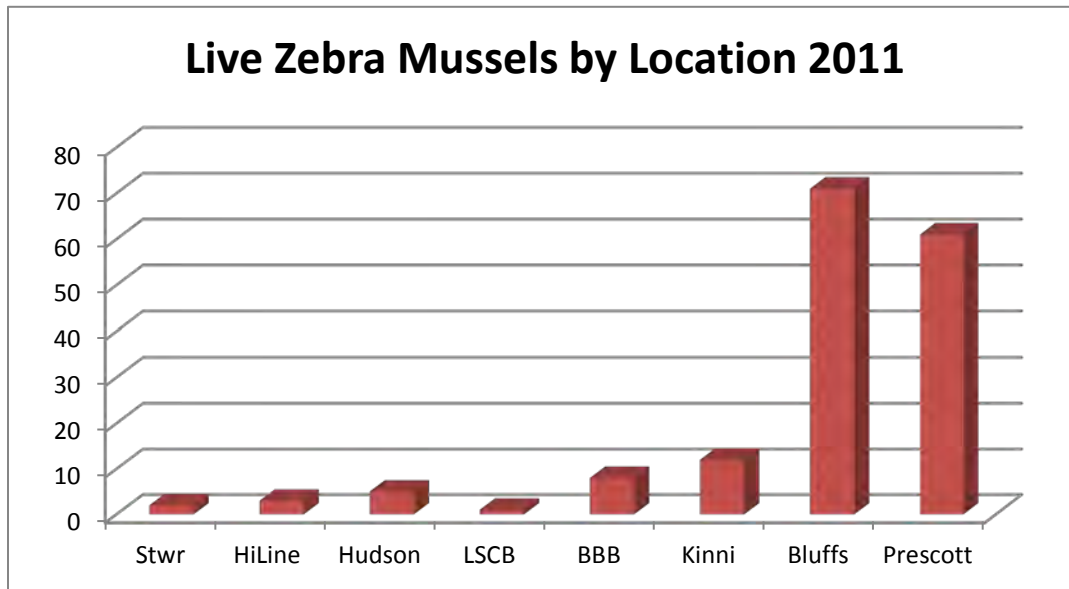
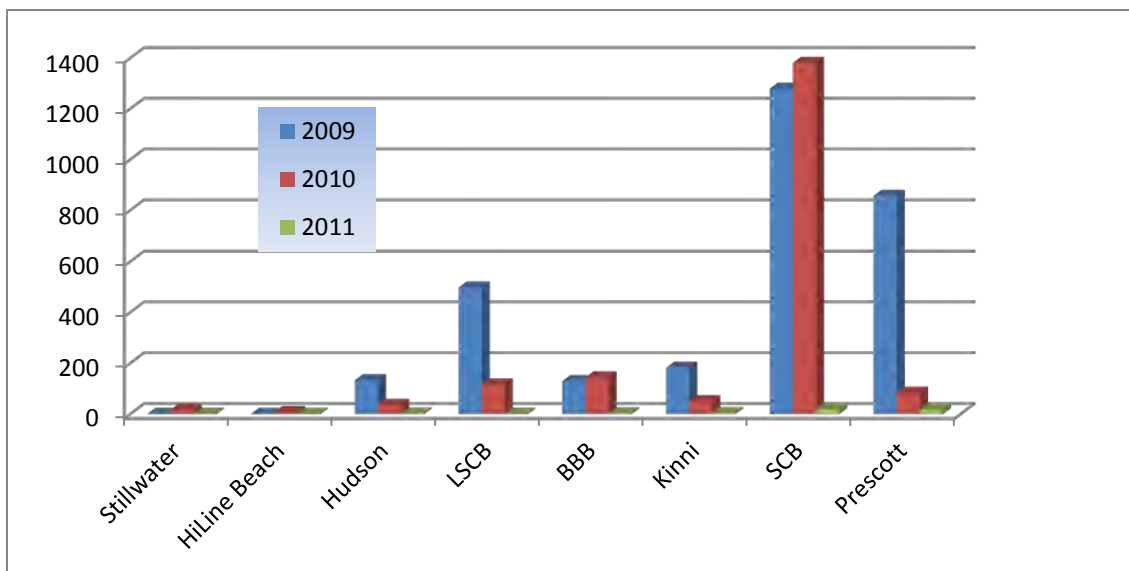


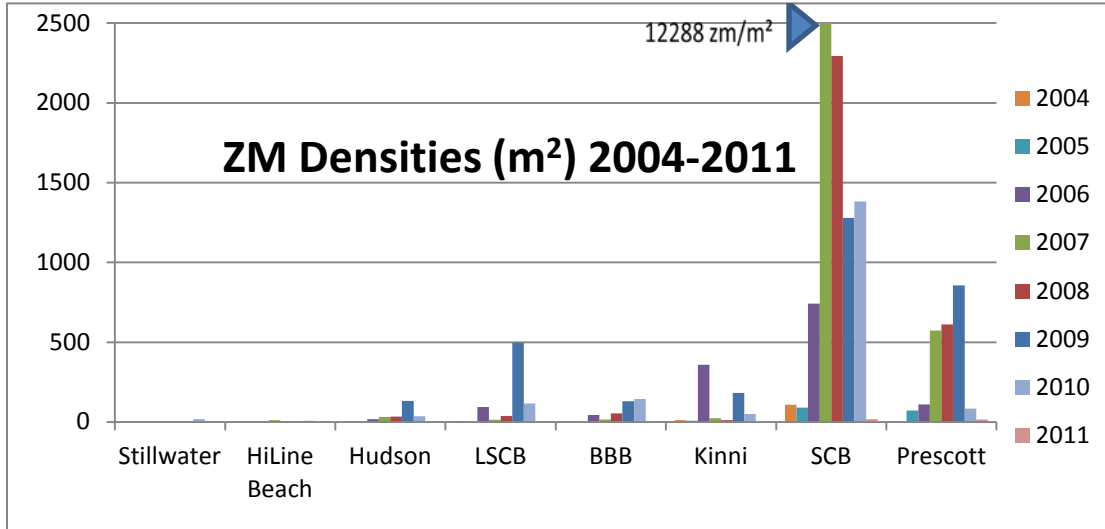
Figure 5. Zebra Mussel Densities by Location, 2009 to 2011



Though zebra mussel densities have dramatically declined since their 2007 peak in the Prescott Pool, the amount of shells-as-substrate remains stable, Appendix A. In the middle of this pool, at

St. Croix Bluffs, shell densities, remain high. The Prescott sampling site saw a dramatic decrease in live zebra mussels, however empty shells continue to be plentiful at both location.

Figure 6. Densities (m²) of zebra mussel populations at 8 long term sites, 2004-2010.



As in the past additional data was collected as zebra mussel densities were determined. These included Asian clams (*Corbicula fluminea*), any species of snails and dead mussels or their shells. The results of the clam and snail enumeration are pending in the Appendix D. The Asian clam data showed an unusual spike in the number of shells collected at the St. Croix Bluffs location in 2009 and 10, but may reflect the Prescott pool zebra mussel die off in 2011. The vast majority of the snails collected were of the Family Pleuroceridae.

Zebra Mussel Veliger Qualitative Monitoring (USACOE SOW Task 2)

The FWS continues to add to the efforts of the NPS by providing PVC samplers at locations on the river and at Wisconsin lakes at high risk to the river (information pending). These samplers are in addition to the sixteen PVC samplers with glass slides deployed by the NPS during the summer season. None of the NPS samplers (located from Stillwater to the headwaters and includes the Namekagon) had a positive detection in 2011. The Fish and Wildlife efforts include several samplers at marina on the lower St. Croix. No zebra mussels have been found by passive sampler or active scuba north of the lift bridge.

FWS staff also deployed PVC samplers at eleven Wisconsin lakes within the St. Croix watershed and draining directly into the river. These samplers were checked 15 times from April through October (Table1). No zebra mussel juveniles were detected. Also during site visits, staff engaged in outreach related to aquatic invasive species and ways to reduce their spread. As in the past, no zebra mussels were found on samplers upstream of the Stillwater Lift Bridge. Also of note, zebra mussels died-off in considerable numbers during the summer. The cause is yet to be determined.

Table 1. USFWS qualitative zebra mussel veliger monitoring

2011 Zebra Mussel Plate Sampler Monitoring/ANS Outreach-LaCrosse FWCO USFWS									
Location	# Samplers		ZM's Found-Comments	#Public Contacts					
Lower St Croix River:									
Afton Marina-Washington-2			ZM's Found-considerable summer die-off noted.						2
Hudson Marina-St Croix---3			ZM's Found-considerable summer die-off noted.						8
Bayport Marina-Wash.-----3			ZM's Found-Considerable summer die-off noted.						12
Sunnyside Marina-Wash.---2			Few Found-Summer die-off noted						9
Stillwater Marina-----3			None						6
Wolf Marina-----3			None						8
Boomsite Landing-----1			None						22
Total Sites=7		17	ZM's found at 4 Sites						67
WI Lakes:									
Mallalieu-St Croix		2	None						13
Bass-St Croix		2		1	Adult	Notified NPS/Karns			9
Cedar-St Croix/Polk		2	None						11
Big Lake-	Polk	2	None						8
Wapagassett-Polk		2	None						21
Deer-Polk		2	None						17
Long-Polk		3	None						4
Balsam-Polk		6	None						38
Half Moon-Polk		3	None						9
Bone-Polk		3	None						13
Big Round-Polk		2	None						11
Big Butternut-Polk		3	None						12
Clam Lake-Burnett		2	None						25
Total Lakes=13		34				1 ZM=Bass Lake-St Croix County			191

Dates Sampled:	April 27/28, May 13/14, 24/25, June 7/8, 21/22, 30.
15 2-day trips.	July 1, 14/15, 28/29, Aug 8/9, 17/18, 29/30
	Sept 8/9, 14/15, Oct 12/13, 27/28.

Table 2. Qualitative veliger sampling using plankton tows at fifteen lakes within the St. Croix Basin, Summer 2011.

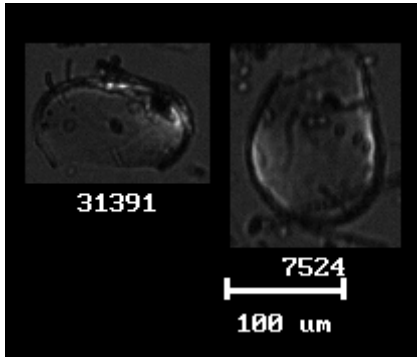
Qualitative Veliger Sampling, St. Croix Basin 2011				
State and County	Water Body	Date Collected	Results	Notes
MN				
Chisago County	St. Croix Flowage	8/10/2011	ND	
Chisago County	Impoundment-Kost Dam (Sunrise R.)	8/10/2011	ND	
Chisago County	North Center Lake (Sunrise R.)	8/25/2011	ND	
Chisago County	Rush River	8/25/2011	ND	
Pine County	Pokegama Lake (Snake R.)	7/13/2011	ND	
Pine County	Cross Lake Snake R.)	7/13/2011	ND	
WI				
Burnett County	Minerva Lake	7/19/2011	ND	
Burnett County	Yellow Lake (Yellow R.)	7/19/2011	ND	
Burnett County	Clam River Flowage	7/26/2011	ND	
Burnett County	Memory Lake (Wood Creek)	7/26/2011	ND	
Polk County	Big Trade Lake	7/26/2011	ND	
Polk County	Bone	8/23/2011	ND	
Polk County	Balsam	8/23/2011	ND	
Polk County	Deer	8/23/2011	ND	
Polk County	Bass Lake	6/17/2011	D: Low Concentrations	WI DNR Confirmed

Again in 2011, various lakes within the watershed and on both sides of the river were tested for veliger presence using new technology associated with the FlowCAM (Fig. 8). This microscope speeds the processing of sampling remarkably and provides surprisingly fast results. NPS staff collected samples in July, using protocols developed by Clean Lakes, Inc. These samples were shipped overnight to company facilities and a report was generated with a couple of weeks. An example is provided below (Fig. 7). Samples were collected using a 20cm Wisconsin tow net, a 1 to 3 meter vertical pull at three locations per lake (mid and 2 littoral), integrated and preserved in ethanol. During these events, shoreline searches were conducted to detect for the presence of adult zebra mussels. No adults or shells were found along the shore. No veligers were detected by Clean Lakes from the 15 samples provided (Table 2). This strategy will be expanded during the 2011 field season. The cover page of the Clean Lakes report is provided in Appendix B. The full report is available as a pdf upon request.

Figure 7. Descriptive analysis of veliger samples collected in July 2010 by NPS and processed by Clean Waters, Inc.

SC 10001 – Yellow Lake

This sample has been labeled as a No Detect result w/ Inconclusive Images. A positive identification picture from a Lake Mead Sample is shown above in Figure 2. The images below are from Yellow Lake and an explanation as to why we believe each image is or is not a Dressinid veliger. Some of these images are meant to show the difficulties with providing positive identification as well as some pictures commonly found in the samples.



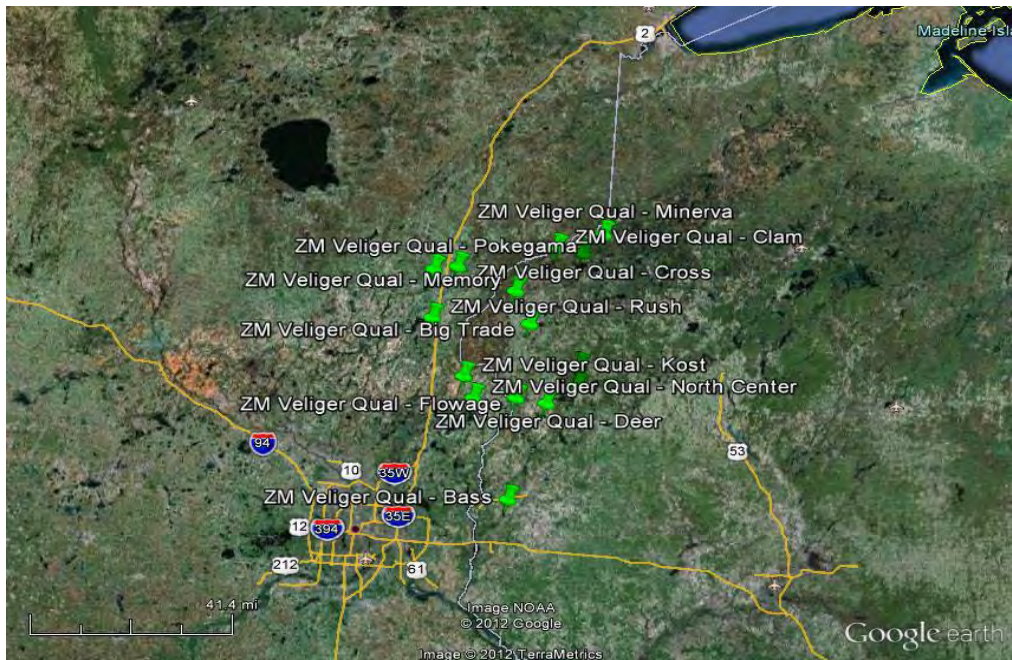
Sample Information:

Client Sample ID, Date Sample Collected, Date Sample Received, Detail Sample Location, Clean Lakes Sample Number, Date Sample Analyzed, Amount Tested (mL)

Image 31391 is a similar shape to a bivalve, but is exhibiting weak birefringence lacking the Maltese Cross pattern, is translucent, and is not quite the characteristic “D” shape of a Dressinid veliger. This is not a Dressinid veliger.

Image 7524 is a similar shape to a bivalve, but is exhibiting weak birefringence lacking the Maltese Cross pattern, is translucent, and is not quite the characteristic “D” shape of a Dressinid veliger. This is not a Dressinid veliger.

Figure 8. Locations of 15 Qualitative Veliger Samples.

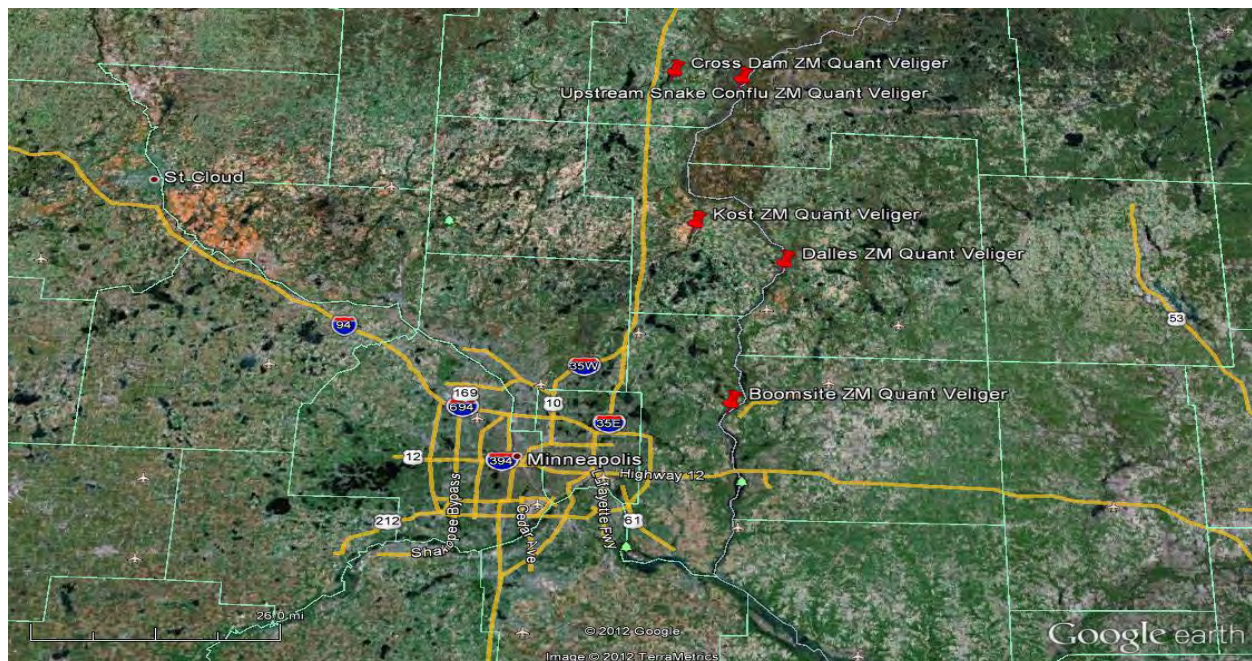


Zebra Mussel Veliger Quantitative Monitoring (*USACOE SOW Task 3*)

A multi-agency effort to collect water samples to quantify zebra mussel is conducted in July and August each summer. Again this summer, staff from NPS visited the long term monitoring locations affect the St. Croix River (Fig. 9). Results are pending and will be contained in a separate report provided by the ACOE, ERDC office.

Figure 9. Locations of CORPS Veliger Sites in the St. Croix Basin of NPS Responsibility

Quantitative Veliger Sampling - St. Croix Basin (Miss Tributary) 2011			
Dates	Tributaries		Notes
July 13, Aug 10	St. Croix R. - Prescott	St. Croix R. Mouth at Prescott	CORPS
July 13, Aug 10	St. Croix R. - Hudson	St. Croix R. at Hudson	CORPS
July 13, Aug 10	St. Croix River - Stillwater	Mile Long Island - Boomsite	aka Boomsite
July 13, Aug 10	St. Croix River -St. Croix Falls	Below Falls - Interstate SP	aka Dalles
July 13, Aug 10	St. Croix R. North of Snake R.	Snake River Landing	High Water, No data
July 13, Aug 10	Snake River	Above Confluence with St. Croix	Cross Dam
July 13, Aug 10	Sunrise River	Above Confluence with St. Croix	Kost Dam



Native Mussel Propagation and Management

Appendix C has the final report of activities associated with endangered mussel management and protection on the St. Croix River in 2011.

APPENDIX A

Figure 1. Hydrograph Downstream End of Lake St. Croix 2009-11.

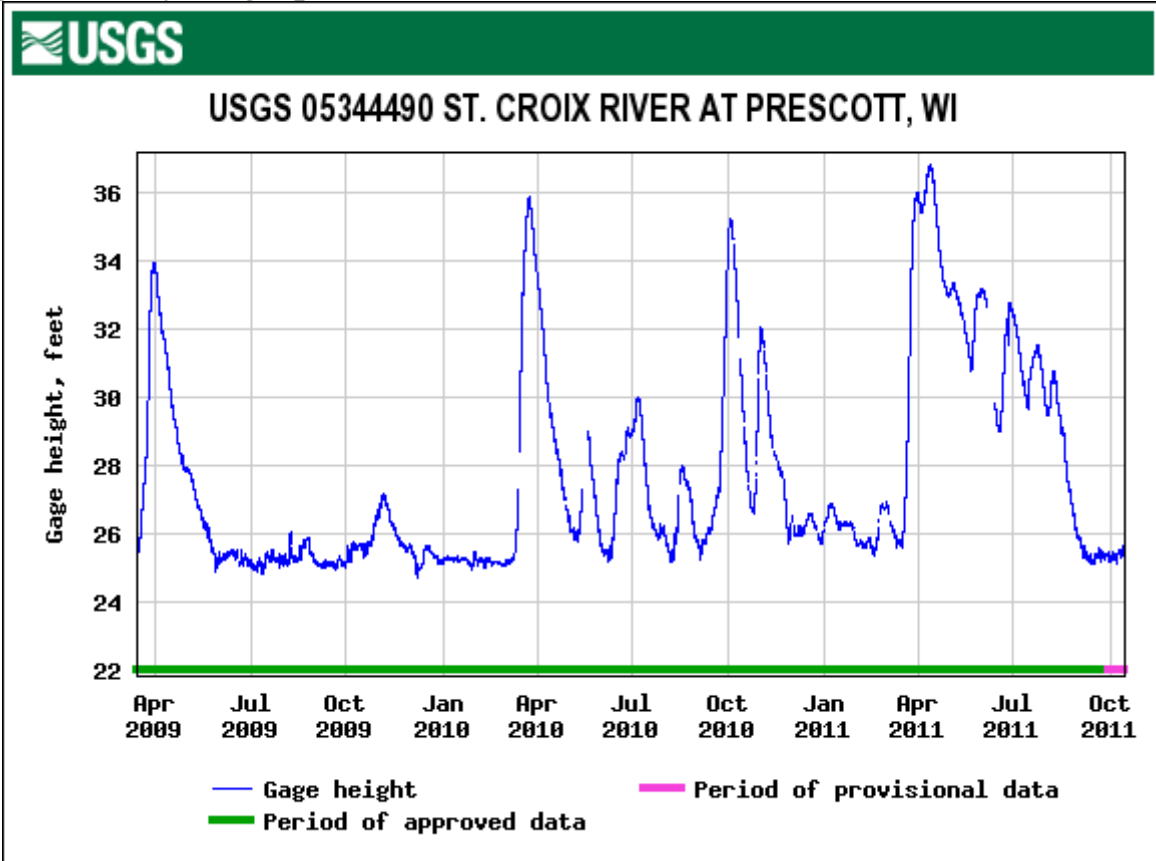


Figure 2. Total Live/Dead Animals Found, 2011.

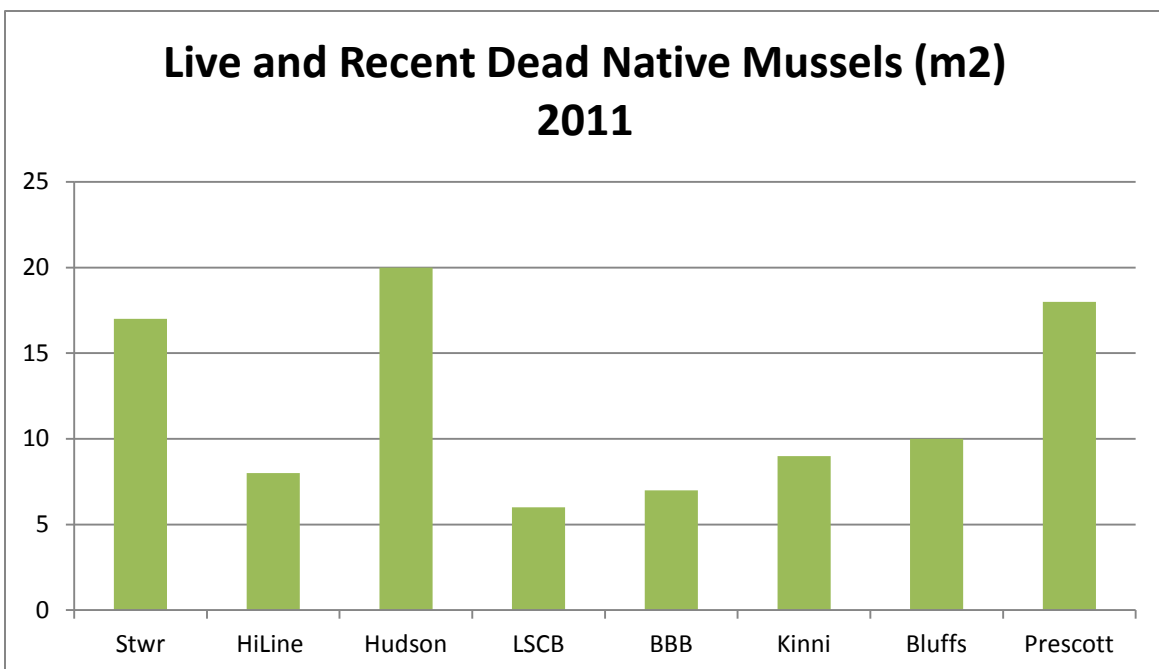


Figure 3. Total Zebra Mussels found in 2011, by Location.

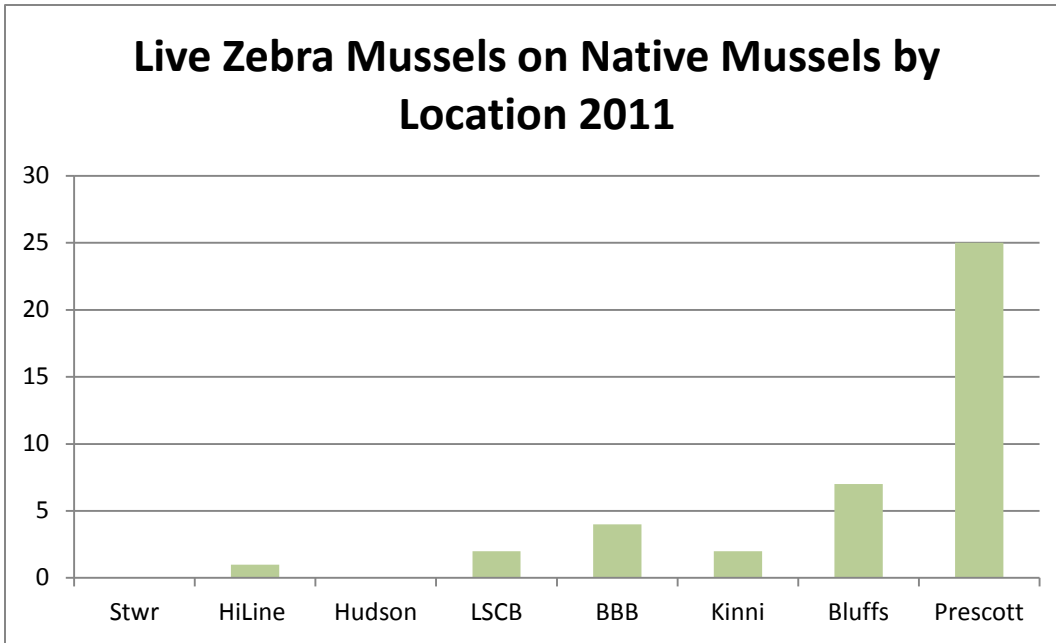
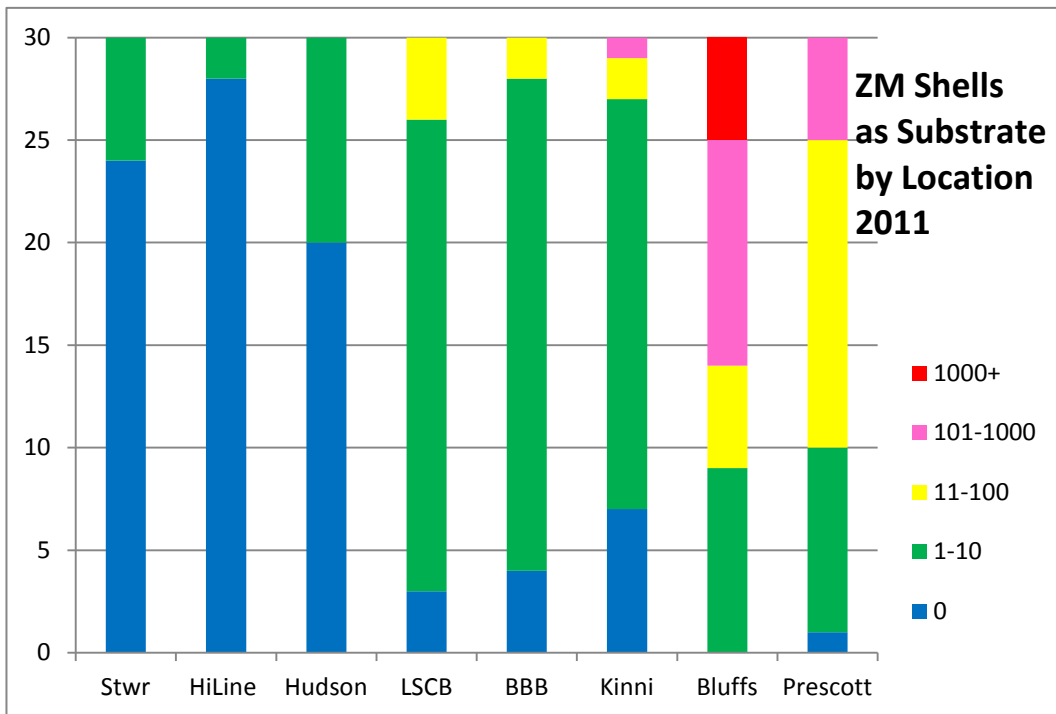


Figure 4. Amount of Zebra Mussel Shells within Each Sample, by Location.



APPENDIX B

**Dressinid Veliger Monitoring Report
For
St. Croix National Scenic Riverway
Submitted to
St. Croix National Scenic Riverway
c/o Byron Karns
401 Hamilton Way
St Croix Falls, WI 54024
Submitted by
1770 West State Street #125
Boise, ID 83702**

INTRODUCTION: Clean Lakes, Inc. recently completed analysis of fourteen (14) water samples for the St. Croix National Scenic Riverway to determine the presence or absence of Dressinid veligers. Clean Lakes, Inc. utilized FlowCAM® technology with a 300um x Field of Vision flow cell, 4 x objective, and cross polarized light source optimized for maximum veliger birefringence. During visual examination of the sample picture files, **we did detect birefringent organisms, though no positive Dressinid veliger identification was possible.** We are including several images that are representative of organisms with similar features to Dressinid veligers with an explanation of features that disqualify each from positive identification.

BACKGROUND INFORMATION: Positive identification of Dressinid veligers (Zebra and Quagga mussels) using standard or FlowCAM® microscopy has certain limitations which require further testing using PCR technology for final confirmation of species. The main limiting factor for positive identification using microscopic based technologies is the wide variety of common bivalves that may exist in a waterbody that can have similar characteristics to the Dressinid family of bivalves. Of particular concern are Ostracods and Corbicula bivalves, both of which share similar traits with zebra and quagga mussels, including birefringence. However, there are physical characteristics that can be used to differentiate between the species, with shell shape being the primary characteristic. A good graphic showing the difference between Dressinid mussel veligers and Ostracods is shown in Figure 1 with descriptions of differentiating characteristics. Figure 1 also depicts other challenges to providing 100% positive identification, including mussels on edge and other particles such as sand that can produce birefringence.

Note: Results table included in body of the report

2011 Field Season

APPENDIX C

Project – 2011 Final Report

Submitted to:

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Dan Kelner

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January 2012

1.0 Executive Summary

We completed two objectives to improve winged mapleleaf conservation efforts in the St. Croix River during 2011. Over the past century the winged mapleleaf has experienced reductions in range and abundance and the federal government responded by listing it as endangered in 1991. The Winged Mapleleaf Mussel Recovery Plan lists, among other activities, re-establishing winged mapleleaf populations. We undertook the following projects to assist with conservation efforts: (1) aggregate winged mapleleaf in the St. Croix River at Interstate State Park for use in propagating juveniles, and (2) collect brooding winged mapleleaf for use in propagating juveniles at Genoa National Fish Hatchery. We followed standard methods to conduct these projects. SCUBA was used to aggregate and collect winged mapleleaf at Interstate State Park that were used in the 2011 juvenile winged mapleleaf propagation program at Genoa National Fish Hatchery.

2.0 Introduction

Winged mapleleaf (*Quadrula fragosa*), a native mussel once found throughout much of the upper Mississippi River, has become extirpated throughout most of its historic range and is in need of conservation efforts. Listed as federally endangered in 1991 (USFWS 1991) the Winged Mapleleaf Recovery Plan lists several activities needed to delist the species including re-establishing and strengthening winged mapleleaf populations (e.g., using propagated juveniles). This project addressed these conservation needs through the following objectives: (1) aggregate winged mapleleaf in the St. Croix River at Interstate State Park for use in juvenile propagation efforts, and (2) collect brooding winged mapleleaf from the St. Croix River for the production of juvenile winged mapleleaf at Genoa National Fish Hatchery.

3.0 Methods

3.1 Winged mapleleaf aggregation

We worked with employees of the US Fish and Wildlife Service (USFWS), National Park Service (NPS), and Minnesota Department of Natural Resources (MN DNR) to aggregate winged mapleleaf in the St. Croix River at Interstate State Park for use in juvenile mussel propagation at Genoa National Fish Hatchery, Genoa, WI. We used SCUBA and snorkeling equipment in July 2011 to collect winged mapleleaf and relocate them to pre-established USFWS aggregations located just downstream of the Folsom Island wing dam, Interstate State Park.

3.2 Winged mapleleaf retrieval

Working with USFWS, NPS, and MN DNR employees we retrieved winged mapleleaf from aggregation sites at Interstate State Park, as well as finding new winged mapleleaf not in the aggregations, for use in juvenile mussel propagation. Mark Hove assisted with winged mapleleaf collection dives weekly during September 2011 in the St. Croix River.

4.0 Results

4.1 Winged mapleleaf aggregation

Working with the USFWS and NPS we collected and relocated 108 winged mapleleaf to aggregations downstream of the wing dam at Folsom Island, Interstate State Park, St. Croix River. We spent three days assisting with this effort. The USFWS, Twin Cities Field Office kept a tally of winged mapleleaf that were collected and relocated.

4.2 Winged mapleleaf retrieval

Mark Hove worked with USFWS and NPS employees to check winged mapleleaf aggregations regularly during the fall of 2011. Seven displaying winged mapleleaf were collected and brought to Genoa National Fish Hatchery for use in propagating juveniles.

5.0 Discussion

Efforts to aggregate winged mapleleaf during summer 2011 were successful as was the work to bring gravid winged mapleleaf to Genoa National Fish Hatchery. This year we visited winged mapleleaf aggregation areas more frequently than in the past in order to increase the likelihood of collecting more brooding animals. The team searching for brooding winged mapleleaf this year found seven brooding individuals, six of which released glochidia, and glochidia were placed on 286 channel catfish at Genoa National Fish Hatchery in hopes of producing juveniles for future restocking efforts.

6.0 Acknowledgments

We thank the St. Paul District Army Corps of Engineers for project funding and the National Park Service for administering the funds.

7.0 Literature Cited

U.S. Fish and Wildlife Service. 1991. Determination of endangered status for the winged mapleleaf freshwater mussel. *Federal Register*, **56**: 28345-28349.

APPENDIX D

Figure 1. Asian Clams collected 2009-2011, by Location

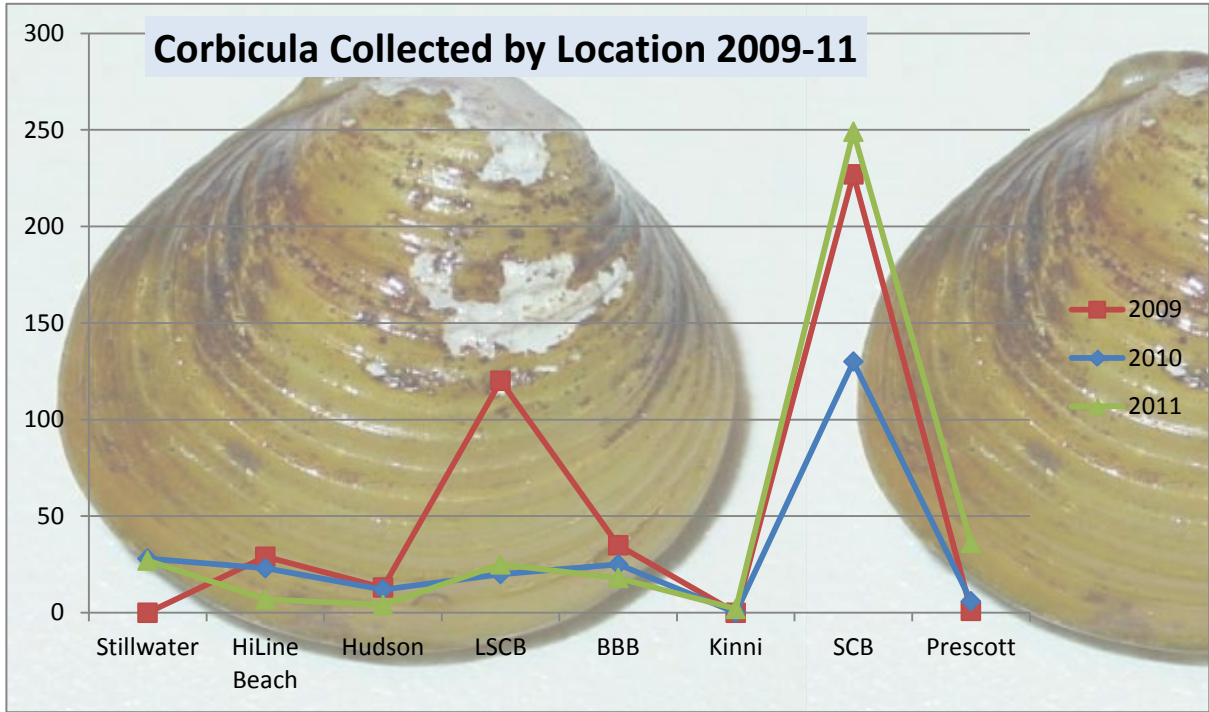


Figure 2. Snails collected in 2010 and 2011.

