

An account of activities associated with efforts to propagate and repatriate *Lampsilis higginsii* in the Mississippi River, Minnesota Minnesota Department of Natural Resources (Mike Davis - Biologist)

2005 FIELD SEASON



Hudson, Wisconsin 4/11/05. *Lampsilis higginsii* female collection effort – gravid *Lampsilis cardium* shown with scarred mantle flaps indicating previous fish host inflicted injury.

<u>April 11, 2005</u>: Nine gravid female *Lampsilis higginsii* were collected from the St. Croix River mussel bed near Hudson, Wisconsin and transported to Genoa National Fish Hatchery. All of these females were observed to be displaying their lures.

<u>April 12, 2005</u>: Sixteen female *Lampsilis higginsii* were collected from the aggregation site near Cordova, Illinois. High water and swift current made collecting the target number of xx impossible on this date. The aggregation site locating line was not found, probably ripped loose by a large submerged tree that was located downstream a few meters.

<u>April 13, 2005</u>: Six female *Lampsilis higginsii* were collected from the aggregation area at Cassville, Wisconsin. High water and swift current made collecting all of the needed females impossible on this date.

April 18-19, 2005: Largemouth bass were inoculated with *L. higginsii* glochidia at the Genoa National Fish Hatchery.

<u>April 28, 2005</u>: 13 *Lampsilis higginsii* females were collected from the aggregation site at Cordova, IL and taken to the Genoa National Fish Hatchery.

<u>April 29, 2005</u>: All remaining female *Lampsilis higginsii* were used to inoculate largemouth bass with glochidia at the Genoa National Fish Hatchery.

<u>May 9, 2005</u>: Fifteen closed bottom cages containing a total of 450 largemouth bass inoculated with *L. higginsii* larvae obtained from St, Croix River at Hudson, WI were placed in Lake Pepin at the Frontenac, MN site. Fifty closed bottom cages containing 1,320 largemouth bass inoculated with *Lampsilis higginsii* larvae obtained from the Cordova, IL aggregation site were placed at this site also. The site used last year at the Lake City DNR office was abandoned due to excessive zebra mussel colonization of the propagation cages and low numbers of juveniles produced.



Cages are readied for placement at the Frontenac propagation site

<u>May 26, 2005</u>: Thirty six closed bottom cages containing a total of 689 largemouth bass inoculated with *Lampsilis higginsii* collcted from the Cassville, WI aggregation site were placed at the Frontenac, MN site.

June 23, 2005: Juvenile relocation grids at the Lower Pool 2 reintroduction site were checked. Three sub sites were established here in September 2004. At site 1 a 1x4 meter grid was placed and covered with a plastic blanket in 2004. We removed the blanket and collected all mussels from the four grids. Grid one was in the shallowest water, grid 4 had 4-5 cm silt deposit in it.

Grid	Median	Mean	Length	#	#dead	#placed in	Mean
#	Length mm	Length	Range mm	live		2004	length in
		mm					2004
1	43	44.7	34-58	23	1	30	?
2	43	43.4	34-58	21	0	30	?
3	51	50.2	40-60	20	0	30	53
4	44.5	42	33-60	31	3	30	?

Grid#	Mean	Growth	Comments
	Growth	Range	
	9/2004-		
	6/2005		
3 - 20	- 2.8	+9 to	Negative growth is likely due to inaccurate
individuals	mm	-19mm	measuring or tag # recording prior to placement in
			2004. The mean length of the tagged animals is
			comparable to the non-tagged individuals in the other
			grids.



Possible error in length or tag # recording in 2004.



Live L. higginsii from the sub site 1 blanket grid on 6/23/2005

At sub site 2 another 1X4 meter grid was placed and covered with a plastic blanket. This grid had been buried in 5-10 cm of sand that apparently drifted in from the adjacent shallow sand bar, probably due to wave action. After locating the buried grid, the blanket was removed and the grid thoroughly searched and all L. higginsii were removed. Although only 10 live were found in the buried grid only 8 empty shells were found, most of the 120 animals placed in 2004 were simply missing, perhaps indicating that they had moved away from the grid as the sand filled it. Also at site 2 an open grid with 2002 cohort *L. higginsii* was checked and the presence of live animals verified.

At sub site 3 a simple check to verify that the two blanket grids and one open grid were in place and contained live animals was done.

June 24, 2005: All *L. higginsii* propagation cages placed at Frontenac in May were opened and the fish released.

<u>July 5-6, 2005</u>: Two mussel beds in Lake Pepin upstream of the Frontenac cage site were evaluated for their potential as alternate cage propagation sites.



Fredericks Point and Methodist Camp sites in relation to the existing Frontenac cage site. Fredericks Point and Methodist Camp sites were evaluated for zebra mussel presence, native mussel density, native mussel species abundance and evidence of ongoing native mussel recruitment.

Fronteac cage site: 15 quadrats collected in the area of past cage placement. No live or dead *Lampsilis higginsii* were collected. Apparently few if any of the propagated *L. higginsii* have escaped to become established at this site.

Mussel Species	Mean #/M ²
Amblema plicata	3.467
Obliquaria reflexa	1.067
Fusconaia flava	0.533
Total Density	5.067
Zebra mussels	9.3

Zebra mussel density at the Frontenac cage site in 2005 was $9.3/M^2$. Last year a timed search at this site resulted in eight live species collected amongst a total of 335 live mussels for a CPUE of 310 mussels/minute.



Random placement of ¹/₄ Meter square quadrat sampler.

Fredrich's Point. This site is near shore on the downstream side of the point. Substrate ranged from sand and cobble in 2-4 foot depth to silt at greater depth. Mussel density was higher in the silty substrate found in deeper water (5-8 feet). Nine species of native mussels were collected. Mean zebra mussel density at Fredrich's Point was $3.5 / M^2$.

The Tables below summarize the mussel fauna and zebra mussel abundance at this site.

Fredrich's Point Site 7/06/05 – Jata from 35 ¼ Meter square quadrats							
Mussel Species	# individuals	Mean density/M ²	Standard Deviation	Zebra mussel/M ²			
Amblema plicata	296	8.46	6.56				
Obliquaria reflexa	148	4.23	5.65				
Fusconaia flava	52	1.49	2.58				
Utterbackia imbecillis	12	0.34	1.14				
Quadrula pustulosa	8	0.23	0.94				
Pyganodon grandis	8	0.23	1.35				
Truncilla truncata	4	0.11	0.68				
Lampsilis cardium	4	0.11	0.68				
Total Natives	532	15.20	11.31	3.5			

Frederich's Point Timed Search Results 7/06/05	CPUE	(#/Hr)
30 minute search		
2 divers		
Mussel Species		238
Amblema plicata	158	
Obliquaria reflexa	34	
Fusconaia flava	30	
Quadrula pustulosa	9	
Quadrula quadrula	5	
Truncilla truncata	1	
Lampsilis cardium	1	
TOTAL MUSSELS	238	

Methodist Camp site. Depth here ranged from 3-6 feet and substrate was silt or silty sand. A timed search was not done at this site. At this site 127 mussels were collected and 71 zebra mussels. Mean zebra mussel density was $8.1/M^2$, very similar to the 9.3 zebra mussels/M² recorded at the Frontenac cage site.

QUADRAT DATA	
Methodist Camp	Density
Mussel Species	Mean
Amblema plicata	7.771
Fusconaia flava	3.429
Obliquaria reflexa	2.743
Quadrula pustulosa	0.114
Truncilla truncata	0.114
Total Density	14.171
Zebra mussels	8.1

Of these two sites, Fredrich's Point seemed to be the best mussel habitat and supported fewer zebra mussels. However the shoreline here is privately owned and access for cage propagation would need to be arranged. Cage placement could interfere with recreation by local residents. Methodist Camp site offers adequate mussel habitat with low zebra mussel density. The shoreline is private although not developed and there was no evidence that cage placement would be likely to interfere with recreation by local residents. Access would need to be arranged with the land owner(s).

Our existing Frontenac cage site, while apparently supporting fewer native mussels, is not greatly different from the other sites in terms of zebra mussel risk. Its advantages include ease of access and a publicly owned shoreline that offers little conflict with recreational use, probability of less fine particle deposition due its location farther downstream in Lake Pepin, and its location in relation to wind driven waves. The points protruding into Lake Pepin upstream and downstream of this site protect it from NW and SE winds, the two most prevalent wind directions. Methodist camp is better protected still, and Frederich's Point, while protected from SE winds may be more vulnerable to N and NW

winds. Frederich's point is also nearest the point where the river flows into the lake and thus has the highest exposure to silt laden water.



August 22-24: Lake Pepin zebra mussel monitoring

DENSITY OF ZEBRA MUSSELS AT LAKE PEPIN SITES 8/23/2005

Depth contour					
Site	0-1meter	1-2meter 2	2-3meter 3-	4meter	
WI 1-8	0	128	10.6		
MN 1-9	present	0	0		
Fredrichs Pt	0	3.5			
WI 2-11	present	829.3	10.6		
Methodist Camp	0	8.5			
Frontenac Cage Site	present	42.6	110.6		
Sandpoint	0	41.3	6.6		
MN 3-3	abundant	336	248	0	
WI 3-12	abundant	85.3	76pr	esent	
Lake City Office	present	33.3	337.3pr	esent	
Erickson Pt	abundant	104	7.5		
MN 4-7	abundant	2672	1486sh	nells only	
Lacupolis			3584		

Zebra mussel density in Lake Pepin has been highest in the lower portion of the lake and generally along the Minnesota shore since survey work began in 1996. For convenient reference, we divided the lake into 4 sampling quadrants, numbers 1-4, with number 1 being at the upstream end of the lake. The pattern of zebra mussel distribution has remained similar over the years. Highest density is found in quadrant 4 or the outlet (LaCupolis) and low density or zebra mussel absence has been found at sites in quadrant 1. Given the predominantly southeastern winds during the summer reproduction months, it is likely that the lower lake quadrants have been a source of larvae for zebra mussel colonization of the upper lake. No reproducing population of zebra mussels existed upstream of Lake Pepin prior to the colonization of Lake St. Croix around 2000-2001.





Mean density of zebra mussels by quadrant in Lake Pepin

Year	MN-1	WI-	1 MN-2	WI-2	MN	1-3 WI-3	MN-4	WI	-4
	1996	0	0	0	181	142	97	11106	176
	1997	81	4	27	356	1800	10	22595	2242
	2003No data		352No data		924	1814	48	9524	91
	2004	4	153	64	405	8497	82	15430	2793
	2005	1.7	69	42	420	238	68	2080	3584

Highest zebra mussel density over most sampling years was observed at site MN 4-7. This site had been sampled four times prior to 2005 allowing for a good comparison of zebra mussel density and population demography over time. The table below contains information on density at this site.

Lake Pepin – Site MN 4-7 Depth zone	1996 zebra mussel average density (#/M ²)	1997 zebra mussel average density (#/M ²)	2003 zebra mussel average density (#/M ²)	2004 zebra mussel average density (#/M ²)	2005 zebra mussel average density (#/M ²)
0-1 meters	2,079	2,963	present	present	Present/abundant/small
1-2 meters	11,106	22,595	9,524	15,430	2,672
2-3 meters	6,915	13,120	present	1,914	1,486
3-4 meters	697	10,803	present	A few small zebes present, not on old zebe shells, just on unionid shells, wood or rock	none
4-5 meters	188	3,424	present	Not checked	Not checked
5-6 meters	0	3,962	present	Not checked	Not checked
6-7 meters	0	1,048	present	Not checked	Not checked
7-8 meters	0	684	present	Not checked	Not checked



While the density of zebra mussels has remained higher in the lower part of Lake Pepin over the years, size distributions at sites have changed.

The length frequency of zebra mussels present live at Site MN 4-7 has changed over time. The series of charts below illustrates the size demography of the zebra mussel population at this site for each of the years measured. Note that sampling times occur during August in 2003, 2004 and 2005 and in June in 1996, young of the year zebra mussels are unlikely to be detected in June.



2005 cohort zebra mussels at site MN 4-7.



In 2005 nearly all the live zebra mussels at site MN 4-7 were of a size consistent with their recruitment during 2005 as were zebra mussels in the upper two quadrants. In the third quadrant of the lake the size distribution includes adult size individuals that are likely 2004 recruits. It may be possible that these adults were the source of larvae in the lake in 2005.







Zebra mussel density in 2005 was highest at the outlet of the Lake (LaCupolis site). This is also the site of a high density and species rich native mussel bed that has been monitored periodically since 1991. The LaCupolis bed is unique in its abundance of *Elliptio dilatata* and the presence of 29 species of native mussels. However zebra mussel infestations in the late 1990s and in 2000 reduced the abundance of native mussels significantly. It appears that this mussel bed is once again suffering from very high zebra mussel density that threatens to suffocate the remaining native mussels (see photos below).





September 7, 2005: Sub-Adult monitoring

Monitoring of L. higginsii placed at the Sturgeon Lake reintroduction site was completed on 9/7/06. This site was established in 2003 using 2001 cohort individuals that had been raised in Lake St. Croix. A single propagation cage containing 16 sub adults was also placed here at that time; this cage was removed in 2005 due to deterioration. All 16 mussels were recovered live.

Many of the females were gravid indicating that reproduction among the reintroduced population was occurring. Presumably then, host fish present in this area could become infected with Lampsilis higginsii larvae, carry them on their gills to excystment, and start new populations throughout the area. Evidence for this occurring has not yet been observed.

The table below compares growth rates and gravidity rates between the sub adults placed in open grids to those in held in the protective cage.

Sturgeon Lake 2005 - growth of 2001 cohort tagged mussels placed in 2003

	Inside cage	In open grid
Average increase (mm)	27.3	22.9
Range of increased length	20-40	15-31
average length 05	67.8	66.3
average length 03	40.5	30.3
range of lengths 05	56-84	50-81
range of lengths 03	32-47	35-57
percent female	68.8	43.8
Percent of females gravid	18.2	71.4
percent females not gravid	18.2	?

Individuals that remained in the cage since 2003 increased in length at a faster rate than those in the open grids. More females were found among the caged individuals but fewer of them were gravid than in grids in 2005. Although the cause for these differences remains undetermined there are several potential explanations.

Growth: 1) caged animals are protected and therefore suffer fewer disturbances per year resulting in more time feeding and greater growth rate. 2) water quality along the river bottom is poorer than in the cage that is situated several cm off the bottom creating stress effects that reduce growth rate. 3) the higher percent gravidity of females in the grids resulted in slower growth.

Gravidity: 1) the greater ratio of females to males in the cage led to lower fertilization rates. 2) the cage interfered with sperm finding its way to females inside.



2001 cohort mussels at the Sturgeon Lake reintroduction site in 2005.



Sturgeon Lake reintroduction site, 2005





September 8: Wisconsin Channel, Pool 4 reintroduction site monitoring

At the 4th Cut site all cells in the open grid containing 2002 cohort propagules were searched and emptied of *L. higginsii* mussels or empty shells. Results appear in the table below. Predation in the grid was very high, only 5 live *L. higginsii* were recovered of the 262 placed in there in 2004. Many crushed shell fragments were found, some still bearing tags. The grid was removed and this site abandoned as a place for unprotected release of propagated mussels.

An aluminum framed, wire mesh protective blanket was also installed at this site in 2004. Beneath it 300 2003-cohort L. higginsii were placed in an effort to protect them from predation. Predators seeking the mussels had excavated bottom soil all around the perimeter of this blanket. By reaching beyond this excavated area into the middle of the blanket, the diver was able to feel many live L. higginsii. Predators no doubt consumed an as yet unknown number of mussels from around the outside edge of the blanket.



Shell fragments from grid, probably crushed by fish's pharyngeal teeth.

At the Goose Lake outlet site the open grid containing 2002 cohort *L. higginsii* placed in 2004 was searched and all mussels were removed from six of the cells for enumeration and measuring. Although just 27 of the 60 mussels placed in these cells were recovered, no crushed or empty shells were found. Perhaps the 33 missing individuals simply moved out of the grid cells. Three live and two dead *L. higginsii* from the 2003 placement of 2001 cohort mussels were found in that grid. A cage placed at this site in 2003 was retrieved and 43 live L. higginsii were removed and lengths recorded. Survival of caged mussels was 100%. The wire mesh blanket placed at this site in 2004 was similarly excavated around the edges, live mussels could be felt inside this area where they were beyond the reach of predators. Wire mesh blankets at both sites appeared to be stable with little sediment or debris accumulations.

Length of mussels from Goose Lake grid vs. those kept in the cage is shown in the table below.

Source of shells (2002 cohort)	Mean length	Max length	Min length
Open grid	60.8	85	51
Cage	59.9	78	45

Growth	of L	. higginsi	<i>i</i> from	placen	nent in 200	04 throu	1gh 9/8/2005 –	Goose Lake outlet.

Source of shells	Mean length 2004	Mean length 2005	Growth (mm)
2002 cohort	54.21	60.86	6.51
2003 cohort	45.02	Not assessed in 05	

September 9: Lower Pool 2 monitoring

Two sub sites at the Lower Pool 2 relocation area were examined. Sub site one was checked in June. At sub site 2 one grid with a plastic blanket was also checked in June and removed. The open grid at sub site two was examined. This grid contained 100 2002 cohort *L. higginsii* placed in 10 cells in September 2004. We collected 61 live *L. higginsii* and one empty shell from 8 cells. Lengths were recorded for all mussels. Eleven individuals appeared to be females and of those 9 were found to be gravid.

At sub site 3 two blanket grids and one open grid were established in September 2004. Blanket covered grids contained 2003 cohort L. higginsii. These blankets had been excavated around the perimeter by predators much like those at the Wisconsin Channel sites. These blanket grids are much farther from shore than at the other sites and it is likely that fish are the predator involved. Because the excavations are characterized by deep pockets one after another it suggests the feeding behavior of common carp. Thirty one live L. higginsii were collected from beneath the blankets and 4 empty shells were retrieved. Like at the Wisconsin Channel sites, many live mussels could be felt in the middle of the blanket grids where they were out of reach of predators. Seven individuals were identified as female based on shell morphology, of these 2 were found to be gravid. No zebra mussels were found.

Four of the ten cells in the open grid containing 100 2002 cohort L. higginsii were searched and all mussels and shells were enumerated and lengths recorded. Thirty five of the forty live L. higginsii placed in the cells were retrieved live, no empty shells were found. Of 13 positively identified females, 8 were gravid. No zebra mussels were found.

Growth of L. higghish placed in 2004 through 9/9/2005 – Lower 1 001 2 sub site 5					
Source of shells	Mean length 2004	Mean length 2005	Growth (mm)		
2002 cohort	52.1	63.5	11.4		
2003 cohort	42.6	55.0	13.6		

Growth of L. higginsii placed in 2004 through 9/9/2005 - Lower Pool 2 sub site 3

September 8-9: Hidden Falls adult reintroduction site quadrat sampling.



Forty ¹/₄ square meter quadrat samples were collected from the mussel bed in the area of L. higginsii adult reintroduction along the right descending bank of the river at RMxxx. In total, 206 live mussels of 13 species were collected for an average density at this site of 21 mussels/meter square. The 40 samples averaged 5.15 mussels in each, the standard

deviation in this group of samples is 4.30 giving 95% confidence that the mean is 5.15 animals +/-1.59.

No recently recruited L. higginsii were collected in the samples, however two of the adults placed here in 2001 were. Four adult *Ellipsaria lineolata* and one *Arcidens confragosus* that were placed in this area in 2000 and 2001 were collected during sampling.

Species	Number Collected	% of population	Rank
Obliquaria reflexa	94	45.63	1
Amblema plicata	32	2 15.53	2
Truncilla truncata	21	10.19	3
Quadrula quadrula	15	5 7.28	4
Quadrula pustulosa	13	6.31	5
Fusconaia flava	12	2 5.83	6
Lampsilis cardium	6	6 2.91	7
Ellipsaria lineolata	2	1.94	8
Quadrula nodulata	3	3 1.46	9
Lampsilis higginsi		2 0.97	10
Leptodea fragilis		2 0.97	11
Arcidens confragosus	Î	0.49	12
Truncilla donaciformis	Î	0.49	13
Total	206	6 100.00	l

Length distribution of individuals collected was plotted for the three most abundant species. Some ongoing recruitment is indicated but most of the population of each species falls into the adult range of lengths.





September 20: Lake Pepin Frontenac cage checks

Several 2003 and 2004 propagation cages were retrieved from the lake. It was noted that 2003 cages that were not opened in 2004 had many more zebra mussels in them. Five St. Croix stock 2004 cages were opened and the mussels within counted and placed back into cages for growing until fall of 2006. Survival in these cages was near 100% with 4 empty shells out of 1,349 live mussels. One Cassville stock 2004 cage was retrieved, inside were 252 live *L. higginsii* and 15 empty shells of the same species. Growth of the two stock sources are compared in the table below. Also compared in the table below is the difference in length of animals that were checked and cleaned in 2004 and those in cages that were left undisturbed.

Source			Checked in 04	Not checked	Checked in 04	Not checked
	# Cages checked	Total live	Avg/size(mm)	Avg size(mm)	Range(mm)	Range(mm)
St Croix	5	1,349	37.2	34.2	25-50	24-49
Cassville	1	252	34.4		28-41	
Cordova	None					

September 26: Lake Pepin cage harvesting.

Cages placed in 2005 at the Frontenac propagation site in Lake Pepin were checked for production during this week. One of the cages containing Cordova derived juveniles appeared to consist of about 40% *Lampsilis cardium*, based on beak sculpture characteristics. This has been verified by DNA analysis by Dr. Bonnie Bowen and means that the actual production of *L. higginsii* for the Cordova propagules is unknown at this time. All juveniles from retrieved cages were placed back into cages at the Frontenac site.

2005 pro				
Source	# Cages checked	Total live	Avg/cage	Range
St Croix	1 of 15	1,201	1201	1201
Cassville	3 of 36	208	69	28-112
Cordova	3 of 50	457	152	103-194

September 27-29:

Juveniles produced in 2003 were collected from cages and either given a unique numbered tag and measured for total length or had a black dot placed on their shell to distinguish them as propagated individuals.





2003 cohorts in cages that were not cleaned in fall 2004 had high numbers of zebra mussels inside.

These sub adults were then taken to the previously established reintroduction sites in Pools 2, 3 and 4 to augment the individuals placed in previous years. Their distribution among those sites is shown in the table below. 6,800 of the 7,400+2003 cohort sub adults have now been placed at reintroduction sites around the Midwest, the exception being those that died in the propagation cages between 2003 and 2005 (283 found dead in cages this year). All of the 600 remaining 2003 sub adults are in a rearing sled at the Frontenac cage site.

Sub Adult Site	# Blanket	# Open grid	Random and/or along	Total
			transect	
Sturgeon Lake		200	1200	1400
WI Channel – 4 th	500			500
WI Channel –	500			500
Goose				
Lower Pool 2 - 1				0
Lower Pool 2 - 2		100	300	400
Lower Pool 2 - 3		100	300	400
Grand totals	1000	400	1800	3200

2005 Placement of 2003 cohort L. higginsii.



Pool 3 reintroduction site in Sturgeon Lake



Upper Pool $4 - 4^{th}$ cut reintroduction site





Lower Pool 2 reintroduction site for propagated juvenile *L. higginsii*. Black bars indicate approximate positions of grids.