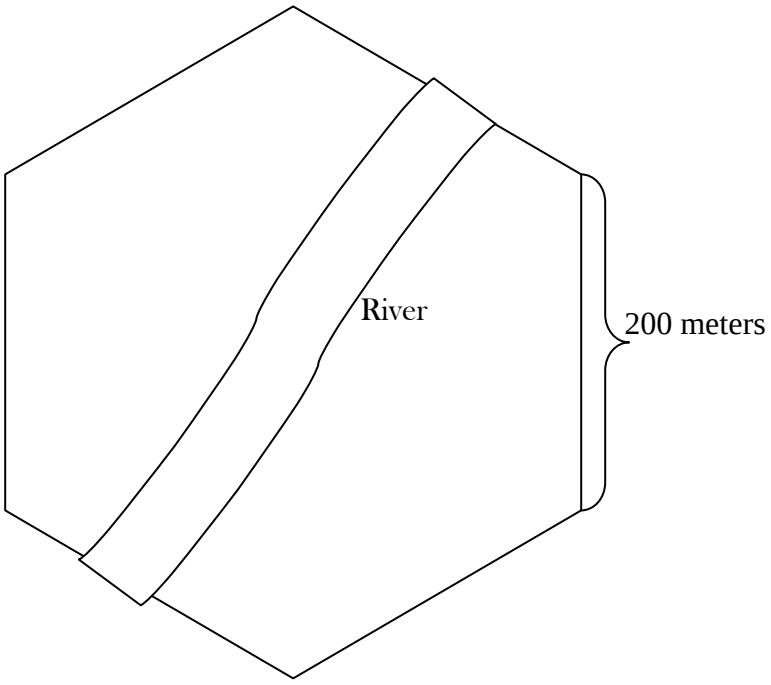
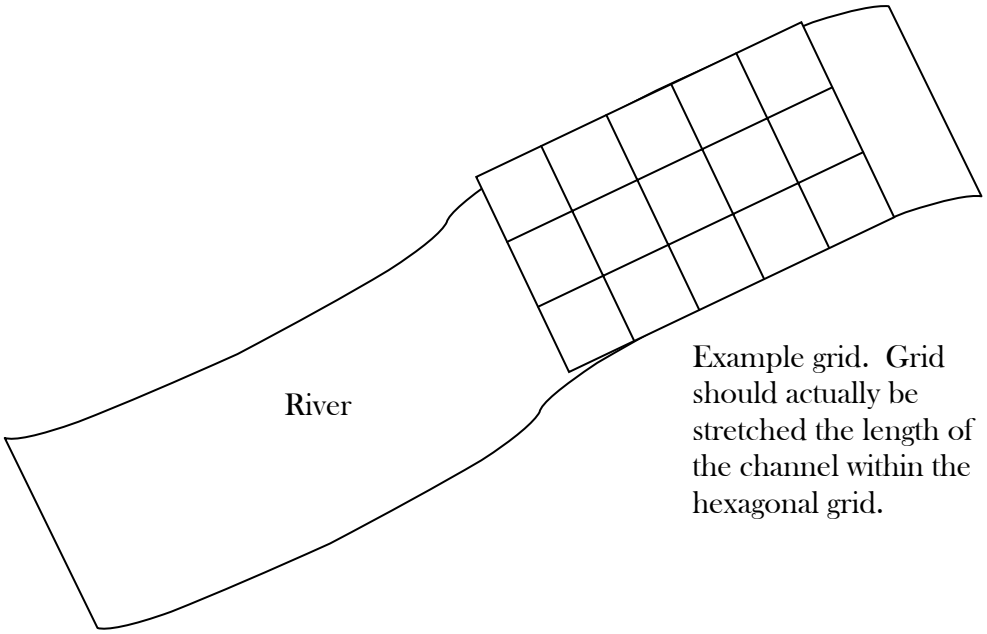
Chapter Eighteen
Mussel Monitoring Protocol

IOWA MUSSEL MONITORING:
Mussels are dependent upon host fish for dispersal and therefore areas to be searched for mussels will be restricted to those to which fish have access, as documented by the appropriate fish survey which will be conducted on each permanent sampling plot at a time earlier in the year to the timeframe recommended for mussel surveys. However, as it is possible that fish may pass through some wetlands without inhabiting them, fish presence is not a requirement for mussel surveys.



SURVEY METHODS:
Surveys are to be conducted between the beginning of August and the end of September (8 weeks and 5 days). This time frame will allowed high flow and cold temperature waters to be avoided.

A sampling grid should be established using the side of the channel as one direction. The other direction should be perpendicular to the stream bank such that it crosses the channel. It may be best to establish this grid by fully extending a surveyors tape along the stream side as a reference guide. GPS the location of the starting end of the grid, along with the starting points of the longitudinal transects. This grid should be established in both deep waters requiring scuba diving (>3 feet deep) and wade able waters < 3 feet deep. The only difference will be that it will be more difficult to establish the grid spacing in a major river (like the Mississippi or Missouri). The grid may have to be placed based on GPS in these systems. Established quadrats will be 1 m^2.



Example grid. Grid should actually be stretched the length of the channel within the hexagonal grid.

A total of 100 meters of channel length should be searched. This 100 m can be divided into two sections of 50 m each. The location of these 2 sections of the same water body within (or nearby) the permanent hexagonal sampling plot should be placed such that as diverse of habitats as possible are surveyed. Once the starting points have been established, each technician should spend 15-20 minutes randomly searching the entire area for as many species as possible. If there are fewer than 3 technicians in the water, then increase the amount of time each spends randomly searching so that at least 1 hour of total time is spent in the random search. One person should remain on shore to record the species as they are called out.

Once the random timed search has been completed, begin at the furthest downstream location for the quadrat sampling. Depending on stream width, 50 to 80 quadrats should be sampled at each location following a systematic design. The following formula is adapted from Strayer and Smith (2003) and can be used to determine the number of quadrats that should be 'skipped' between those that are searched:

$$q = (L * W) \,/\, (n/k)$$

Where q is the number between quadrats, L is the length of the area to be searched, W is the width of the area to be searched, n is the number of quadrats to be searched, and k is the number of starts (e.g. the number of technicians searching). So, for example, if a river is 10 m wide, 50 m in length, and 25 (50 quadrats divided by 2 stream sections) quadrats should be searched by 3 technicians, then the spacing between quadrats should be equal to 60 quadrats. So, if 3 technicians (***X***, **Y**, and Z) randomly choose 4, 12, and 25 as starting locations, then the following table would illustrate the quadrats each would search given the above length and width measurements.

↓ *Riverwidth* → *Riverlength*

1	11	21	31	41	51	61	71	81	91	101	111	121	131	141	151
2	**Y**	22	32	42	52	62	**Y**	82	92	102	112	122	**Y**	142	152
3	13	23	33	43	53	63	73	83	93	103	113	123	133	143	153
X	14	24	34	44	54	***X***	74	84	94	104	114	***X***	134	144	154
5	15	25	35	45	55	65	75	Z	95	105	115	125	135	Z	155
6	16	26	36	46	56	66	76	86	96	106	116	126	136	146	156
7	17	27	37	47	57	67	77	86	97	107	117	127	137	147	157
8	18	28	38	48	58	68	78	88	98	108	118	128	138	148	158
9	19	29	39	49	59	69	79	89	99	109	119	129	139	149	159
10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160

↓ *Riverwidth* → *Riverlength*

161	171	181	191	201	211	221	231	241	251	261	271	281	291	301
162	172	182	192	202	212	222	232	242	252	262	272	282	292	302
163	173	183	**Y**	203	213	223	233	243	**Y**	263	273	283	293	303
164	174	***X***	194	204	214	224	234	***X***	254	264	274	284	294	***X***
165	175	185	195	Z	215	225	235	245	255	Z	275	285	295	305
166	176	186	196	206	216	226	236	246	256	266	276	286	296	306
167	177	187	197	207	217	227	237	247	257	267	277	287	297	307
168	178	188	198	208	218	228	238	248	258	268	278	288	298	308
169	179	189	199	209	219	229	239	249	259	269	279	289	299	309
170	180	190	200	210	220	230	240	250	260	270	280	290	300	310

↓ *Riverwidth* → *Riverlength*

311	321	331	341	351	361	371	381	391	401	411	421	431	441	451
312	322	332	342	352	362	372	382	392	402	412	422	432	442	452
Y	323	333	343	353	363	**Y**	383	393	403	413	423	**Y**	443	453
314	324	334	344	354	***X***	374	384	394	404	414	***X***	434	444	454
315	Z	335	345	355	365	375	Z	395	405	415	425	435	Z	455
316	326	336	346	356	366	376	386	396	406	416	426	436	446	456
317	327	337	347	357	367	377	387	397	407	417	427	437	447	457
318	328	338	348	358	368	378	388	398	408	418	428	438	448	458
319	329	339	349	359	369	379	389	399	409	419	429	439	449	459
320	330	340	350	360	370	380	390	400	410	420	430	440	450	460

↓ *Riverwidth* → *Riverlength*

461	471	481	491
462	472	482	492
463	473	483	**Y**
464	474	***X***	494
465	475	485	495
466	476	486	496
467	477	487	497
468	478	488	498
469	479	489	499
470	480	490	500

Each technician should randomly choose a starting quadrat within the first 5 m from the starting downstream location. The technician may not always move in a straight line, depending upon how straight the channel bed is. As another example, a stretch that is *an average* of 4 m wide and 50 m in length, for 25 quadrats and 3 technicians, (and assuming the 3 technicians (***X***, **Y**, and Z) again randomly choose 2, 12, and 15 as starting locations,would result in every 24th quadrat being searched as depicted below:

↓ *Riverwidth* → *Riverlength*

1	5	9	13	17	21	25	29	33	37	41	45	49	53	57
X	6	10	14	18	22	***X***	30	34	38	42	46	***X***	54	58
3	7	11	**Y**	19	23	27	31	35	**Y**	43	47	51	55	59
4	8	Z	16	20	24	28	32	Z	40	44	48	52	56	Z

↓ *Riverwidth* → *Riverlength*

61	65	69	73	77	81	85	89	93	97	101	105	109	113	117
62	66	70	***X***	78	82	86	90	94	***X***	102	106	110	114	118
Y	67	71	75	79	83	**Y**	91	95	99	103	107	**Y**	115	119
64	68	72	76	80	Z	88	92	96	100	104	Z	112	116	120

↓ *Riverwidth* → *Riverlength*

										161	166	171		
121	125	129	133	137	141	145	149	153	157	162	167	172	176	Z
X	126	130	134	138	142	***X***	150	154	158	163	168	173	177	181
123	127	131	**Y**	139	143	147	151	155	**Y**	164	169	174	178	182
124	128	Z	136	140	144	148	152	Z	160	165	***X***	175	179	**Y**

↓ *Riverwidth* → *Riverlength*

	188	193	198	Z
184	189	***X***	200	205
185	190	195	201	206
186	191	196	202	**Y**
187	192	197	203	208

If the above example were a straight channel, then only 200 quadrats would be available for sampling. With the extra width in some sections, the number of quadrats increases to 208, with a resulting 2 extra quadats (27 as opposed to 25) being available for the survey. If time permits, these quadrats should be searched in addition to the first 25.

It may be easiest to use yellow ropes, delineated in 1 m increments, stretched across the river. These ropes should be held in place with rebar and spaced at 1 m increments as well. The technician on shore (along with one of the technicians in the water) can move the ropes to keep ahead of the quadrat searchers. Eight to 10 ropes may be needed depending on the width of the stream. Do not leave ropes unattended. Alternatively, it may be necessary to use meter tapes instead of ropes, especially in rivers with excessive meandering.

To search each chosen 1 m^2 quadrat, the technicians use their hands to feel for mussels along the surface of the channel bed. The quadrat should also be excavated to a depth of 10 cm (or deeper if mussels are found that deep) using a small hand trowel or possibly a shovel in some situations. This step is important to remove larger cobble that may impede the search. If necessary, use a sieve to sort through the substrate to search for mussels.

In water over 3 feet deep, it will be necessary to SCUBA dive to collect the mussels. The same transect-grid should be established along with the same methods of selecting the 1 m^2 quadrats to be searched and excavated. The only difference being that dive equipment is needed to collect the mussels. Weighted lead lines or a person guiding from the water surface will be needed in order to maintain proper spacing between quadrats. In Iowa, water in river channels moves very fast. It is critical that only fast river qualified/certified divers be used in these situations. It is probably that GPS will be needed to find the location for the quadrats in this situation.

Iowa has been working on a mussel re-introduction program. Information on this program should be read by the technicians and notes should be made on their data sheets as to the possibility of re-introductions in the area to be examined (USFWS 2004, MCT 2003).

Mussel Handling

All mussels should be kept wet in a dive bag until measurements have been completed and the mussels can be replaced at the site from which they were discovered. Mussels should be removed from the water for the shortest amount of time possible to minimize disturbance and mortalities.

In addition to species identification, the length of each mussel should be recorded. This length is measured from the posterior to the anterior margin of the shell (the longest length of the shell). Also, in some species, it should be possible to tell the sex of the mussel by examining the shell. Mussel shells are differently shaped between the sexes. The sex of each individual should also be recorded, if possible.

If possible, mark each mussel with a bee tag and dental adhesive. It is not necessary to mark the mussels, as the quadrats are excavated and the site is visited only once per year. However, marking is not time consuming, or particularly expensive. Mussels are capable of moving 20 meters upstream or >40 meters downstream, so estimating survival between years using mark-recapture and searching the same quadrats may be difficult, but not impossible.

The coordinates of the ends of the overall grid should be recorded using a GPS unit so that the locations can be found for later surveys. In addition, record the location of each excavated quadrat.

HABITAT AND PLANT SPECIES COMPOSITION DATA COLLECTION:

It is expected that the Aquatic Habitat Monitoring Protocol (Chapter 20) will acquire all necessary data in the area searched for mussels. Any additional water body (i.e. creeks, streams, ponds, etc) in the sampling plot which was surveyed for mussels would also need to

have aquatic habitat characteristics measured. These measurements should be collected as outlined in Chapter 20.

Included in these measurements are data that can be determined from GIS coverages in the lab prior to field work (Chapter 3, Landscape Characteristics). Measurements include amount of roads and other impacted soils adjacent to the water body, locations of, and numbers of water bodies. These parameters will still need to be ground-truthed in the field.

EQUIPMENT LIST:
Plastic calipers
Chest waders
Inflatable life jackets
Knee pads
Small spade for excavation
Mesh dive bags
Buckets
Gloves (E.g. dish washing gloves)
Delineated ropes
Rebar
Flagging tape
Sieve
SCUBA equipment for water > 3 feet deep
Standard field kit: Clip board, pencils, ruler, small scissors, Sharpie markers, hand sanitizer, & rite-in-rain data sheets.

STAFF & TRAINING:
A crew of 4 people will allow one person to stay on land as the data recorder as the other technicians call out the information from the water.

Mussel identification must be learned with hands on experience. The best starting place will be a museum collection or a short course with a malecologist. Two weeks of training (beginning in mid-July) is recommended and should include 1) field guide use and id, 2) trips to University museums to discuss defining species characteristics, 3) field practice with an experienced observer, 4) proficiency testing, and 5) habitat data collection.

SCUBA divers will be needed to conduct surveys in water > 3 feet deep. In fast flowing water (most of the rivers of Iowa) these certified SCUBA divers should be qualified to handle conditions in fast flowing water. It may be that the IDNR will need to contract these surveys to the USGS or a consulting firm (e.g. Ecological Specialist Consultants from Missouri).

DATA QUALITY & MANAGEMENT:

Voucher Specimens

Shells of mussels may be collected and catalogued at a willing museum. No live mussels should be collected without written permission from the Iowa DNR endangered species coordinator. For individuals difficult to identify in the field (and also *in lieu* of collecting living organisms), digital photo vouchers should be made. To photograph a mussel for use as a voucher, take pictures of both sides of the mussel after it has been cleaned as much as possible

(i.e. wipe off mud and algae). Also take a photograph of the beak - the raised part of the dorsal margin of the shell. This structure is also called the umbo. This photograph should be taken looking straight onto the beak, so, for example, hold the mussel so that each side is touching one of your knees.

Once the first year of data collection is finished, the 1 m^2 size of the quadrat should be reconsidered. It may be that this size should be increased or decreased depending upon the density of mussels found in Iowa waters.

DATA ANALYSIS:
By using the quadrat design, density of mussel species will be able to be computed. Since the sex of each individual will be recorded, inferences as to sex ratios can be made as well. The basic information should allow the creation of a species list for each site, and data should at least be used to estimate the proportion of sites occupied using program PRESENCE or program MARK. For additional information on the PAO techniques, see Chapter 5 (Data Analysis).

Data collected under this protocol could also be used to examine recruitment, size class distribution, and habitat preferences, depending on the number of mussels found.

SAFETY CONSIDERATIONS:
As with all other protocols, basic hygiene, including washing hands prior to eating or face touching should be followed by all personnel. In searching through sediments by hand, technicians are at risk for injury due to broken glass and sharp rocks scattered along the channel bed. Iowa water is often murky with low visibility. Therefore it is advised that technicians wear gloves, for example the yellow dishwashing gloves available at grocery stores, to protect their hands. All technicians should have current tetanus shots before beginning work.

Working in aquatic situations can be dangerous. Technicians should be cautious of slippery substrates and be aware of the speed of the river flow. Sampling should be suspended during inclement weather, including heavy rain or lightning storms. If a person is swept off their feet when wearing chest waders, it is possible that the air trapped in the bottom of the waders will force the person to travel down the channel upside down with their head below water. Therefore, it is recommended that chest waders have release snaps in the front of the bib to allow the technician to escape in that situation. It would also be advisable to wear an inflatable life jacket underneath the bib of the chest waders.

Care should be taken to decrease the probability of spreading an infectious agent, such as a fungus or virus, between wetlands. An additional concern is the potential spread of zebra mussels, an exotic species. One way to reduce the chance of spreading an infectious agent between wetlands is to allow the equipment to dry for 3-4 days between sites. This may be impractical given the short time frame available for mussel surveying in Iowa. As an alternative, it may be best to rinse the waders and all equipment with a solution of hot water and bleach (Miller and Payne 1998).

TARGET SPECIES:
The following list of target species represents the species of greatest conservation need as chosen by the Steering committee for the Iowa Wildlife Action Plan (Zohrer et al. 2005). Limited distribution maps for these species can be found in Cummings and Mayer (1992) with additional information provided in Arbuckle (2000). Appendix 1 contains a list of additional, more common, mussel species which may be encountered during the monitoring efforts.

Target mussel species:

Common Name	Scientific Name	Habitat
Elktoe	*Alasmidonta marginata*	NE ¾ Iowa
Slippershell	*Alasmidonta viridis*	East Iowa
Flat floater	*Anodonta suborbiculata*	Mississippi River
Cylinder	*Anodontoides ferussacianus*	North central Iowa
Rock poctetbook	*Arcidens confragosus*	Mississippi River
Spectacle case	*Cumberlandia monodonta*	Mississippi River
Purple pimpleback	*Cyclonaias tuberculata*	SE Iowa
Butterfly	*Ellipsaria lineolata*	Mississippi & Cedar Rivers
Spike	*Elliptio dilatata*	NE ¾ Iowa
Ebony shell	*Fusconaia ebena*	Mississippi River
Higgin's eye pearlymussel	*Lampsilis higginsi*	Mississippi River & tributaries
Yellow sandshell	*Lampsilis teres anodontoides*	NE 2/3 Iowa
Slough sandshell	*Lampsilis teres teres*	NE 2/3 Iowa
Creek heelsplitter	*Lasmigona compressa*	NE 2/3 Iowa
Fluted shell	*Lasmigona costata*	NE ¾ Iowa
Pondmussel	*Ligumia subrostrata*	Des Moines & Iowa Rivers
Hickorynut	*Obovaria olivaria*	Mississippi River
Bullhead (Sheepnose)	*Plethobasus cyphus*	Mississippi & Des Moines Rivers
Round pigtoe	*Pleurobema sintoxia*	NE ¾ Iowa
Monkeyface	*Quadrula metanerva*	NE 2/3 Iowa
Wartyback	*Quadrula nodulata*	Mississippi River
Strange floater (Squawfoot)	*Strophitus undulates*	NE ¾ Iowa
Lilliput	*Toxoplasma parvus*	NE 2/3 Iowa
Pistolgrip	*Tritogonia verrucosa*	Mississippi, Iowa, & Des Moines Rivers
Fawnsfoot	*Truncilla donaciformis*	East Iowa
Pondhorn	*Uniomerus tetralasmus*	South central Iowa
Paper pondshell	*Utterbackia imbecillis*	NE ¾ Iowa
Ellipse	*Venustaconcha ellipsiformis*	East 2/3 Iowa
Fingernail clams	*Musculium sp.* *Pisidium sp.* *Sphaerium sp.*	

ADDITIONAL METHODS FOR SPECIAL LOCATIONS:

The USGS is currently monitoring Mississippi River Pools 8 & 13. This survey covers sections of meandering, channelized, and pool classes within each Pool as part of a Long Term Resource Monitoring project.

LITERATURE CITED:

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Miller, AC and BS Payne. 1998. *Effects of Disturbance on Large-River Messel Assemblages*. Regulated Rivers Research and Management. 14: 179-190.

Strayer, DL, and DR Smith. 2003. A Guide to Sampling Freshwater Mussel Populations. American Fisheries Society, Monograph 8. Bethesda, MD.

USFWS. 2004. Higgins' Eye Pearly Mussel (Lampsilis higginsii) Recovery Plan: First Revision. 126 Pp.

Zohrer et al. 2005. The Iowa Wildlife Action Plan. http://www.iowadnr.com/wildlife/files/IAcomprehensive_plan.html

Mussel survey data sheet. DATE:_______ OBS:_____________ LOCATION:________________ WATER BODY NAME:_________
GPS Coordinates of downstream starting point:____ START TEMP:______END TEMP:______
Rain:______ % CLOUDS:______ STREAM SECTION (1 or 2):_________________ Always have at least 2 data sheets (1/stream section)
Quad #__:Start Time:____End time:____ Q#__:ST:____ET:____ Q#__:ST:____ET:____ Q#__:ST:____ET:____ Q#__: ST:____ ET:____
Q#__:ST:____ET:____ Q#__:ST:____ET:____ Q#__:ST:____ET:____ Q#__:ST:____ET:____ Q#__:ST:____ET:____
Quad=quadrat #; length in mm; S=Sex

Quad	Species	Length	S	Quad	Species	Length	S	Quad	Species	Length	S