

Welke
TM 995
VA
Cooks
unsub,
J. Welke

March 21, 1995 (first day of spring !)

TO; Ad Hoc Members

From: Kurt Welke

RE: Genoa protocol

USFWS	
ASHEVILLE, NC	
BPC	NAM
4/11	JAR
RGB	NC
RRC	LR
JAF	SE
JLN	
VGH	

Folks - here 'tis...the operating plan and schedule for Genoa.

Please review, commit to memory, and mark calenders. The collection is slated for 5/1 and we will need all available hands for diving (Glenn, Lissie, Mike, etc, along with 2 LaCrosse divers and perhaps the contract diver) scrubbing, measuring and the whole 9 yards. Everyone has a role and all takers are welcomed. If you can swing out of state travel, I am sure that we can do a group bunk-a-thon at my house (I have beaucoup room) and keep expenses low by having a grill and informal grocery arrangements.

RSVP with your eye or nay, and how much time you can commit - I expect that if we get going by noon on Monday, that y'all will be driving home by noon on Wednesday (with luck).

This is our chance to shine - lets rock !

Regards

Kurt

PS - Someone bring a Boom Box !

For those wanting a "natural" experience, bring sleeping bags & tents - I have 20 acres on my palacial estate !

An Evaluation of Relocation and Holding of Unionid Mussels in an Artificial Pond

- Purpose: To obtain quantitative data on the growth and survival of freshwater mussels following removal from the UMR and subsequent relocation into an artificial pond.
- Methods:
- . We will collect 768 mussels, representing 4 species, from Pool 9, UMR, in May 1995.
 - . 368 mussels will be placed into 1 of 4 treatment options and placed into the artificial pond at Genoa National Fish Hatchery.
 - . 368 mussels will be placed into 1 of 4 treatment options and returned to Pool 9, UMR.
 - . All mussels will be individually marked and measured for shell length and height.
 - . All mussels will be randomly assigned to each treatment.
- Precautions:
- . Each mussel will be scrubbed by two people, independently, to remove zebra mussels.
 - . The scrub brush will be washed in a dilute chlorine solution between each mussel.
 - . A subsample of mussels from Pool 9 will be analyzed for the viruses VHSV, IHNV, and IPNV, and for the bacteria *Aeromonas salmonicida* and *Yersinia ruckeri*.
 - . Mussels to be relocated to Genoa NFH and Pool 9 will be held in an artificial pond at the UMSC for 30 days and then re-inspected for zebra mussels prior to relocation.

- Treatments:
- . Artificial Pond and Upper Mississippi River. At each site we will have 4 placement options:
 1. Suspended substrate-filled tubs
 2. Buried substrate-filled tubs
 3. Buried hardware cloth cages
 4. Vertically-suspended nylon mesh pockets
 - . Each placement option is replicated four times.

- Measures:
- . **Water quality** done biweekly during the ice-free season.
 - . temperature
 - . dissolved oxygen
 - . pH
 - . conductivity
 - . total alkalinity
 - . total hardness
 - . total ammonia nitrogen
 - . **Sediment characterization** done annually in May.
 - . dry weight:wet weight ratio
 - . organic carbon content
 - . **Mussel biometrics** done annually in May and Sept.
 - . number alive and number dead
 - . shell length
 - . shell height
 - . shell condition (observational)
 - . glycogen (sacrifice one individual of one species from each of the 32 treatment combinations in May 95, June 95, Sept 95, May 96, and Sept 96)

- Study
Duration:
- . Minimum of two years

Statistical
Analyses:

- . Raw data includes: mean shell length, shell height, and the number of alive and dead individuals of each species from each treatment combination.
- . Variation in growth and survival of each species within a treatment will be compared by one-way ANOVA.
- . H_0 : Growth and survival of mussels do not vary among treatments or species.

Funding:

- . \$20,000 MICRA
 - . 5,000 Mussel Mitigation Trust
 - . 2,500 Shell Exporters Association
- \$27,500

Experimental Design

Treatment	Genoa Hatchery Pond				Pool 9, UMR			
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 1	Rep 2	Rep 3	Rep 4
Suspended substrate-filled tub	n = 23 ^a	n = 23	n = 23	n = 23	n = 23	n = 23	n = 23	n = 23
Buried substrate-filled tub	n = 23	n = 23	n = 23	n = 23	n = 23	n = 23	n = 23	n = 23
Buried hardware cloth cage	n = 23	n = 23	n = 23	n = 23	n = 23	n = 23	n = 23	n = 23
Vertically-suspended nylon mesh pocket	n = 23	n = 23	n = 23	n = 23	n = 23	n = 23	n = 23	n = 23

^aOf the 23 mussels in each treatment combination:

20 mussels (5 individuals from each of 4 species) will be used to measure survival, length, and height, and then replaced into their respective treatment option (640 mussels total).

3 mussels (one species only) will be used for tissue glycogen levels. One mussel from each treatment combination will be sacrificed in Sept 95, May 96, and Sept 96 (128 mussels total).

Procedures for Relocation of Unionid Mussels

Preparation

1. Fill a 0.1 acre concrete pond at the UMSC with well water in April 1995. Fertilize weekly to get food resources??.
2. Devise a holding scheme that will suspend 960 mussels (need 768) above the pond bottom. Ensure that mussels to be relocated to GNFH are held separate from mussels to be relocated to Pool 9 (i.e., different holding racks). This will facilitate the actual movement of mussels to the treatment location.
3. Ensure that all 4 treatment options are in place in Pool 9 and at GNFH prior to relocating mussels. I suggest we do this one day prior to actually placing the mussels in Pool 9 and GNFH, i.e. on day 29 (about June 5, 1995).
- * 4. Set up pond or well water holding tanks in the boat(s) so that once the mussels are scrubbed free of zebra mussels, they are not exposed to river water again. We will need 4 tanks, each capable of holding between 200 and 360 mussels; one for each of the four species.

Procedure

1. Motor to preselected mussel bed in Pool 9. Need a minimum of 2 boats.
2. Number of mussels needed:
 - a. NOTE: We need to get extra mussels over what we need to account for mortality. I suggest that we get 25% over what we need (Based on Dick Neves 1-yr overall survival rate of 72%).
 - b. For species A, B, and C (not used in glycogen analyses), we will need $5 \times 32 = 160$ individuals of each species; we will want to get 200 individuals of each species.
 - c. For species D (used in glycogen analysis) we will need $9 \times 32 = 288$ individuals of this species; we will want to get 360 individuals of this species. If we cannot get this number of individuals from this bed, we will simply drop the glycogen portion and get the amounts listed in step 2.b.
 - d. Thus, we need 768 mussels for the relocation effort, with the extra 25% that's a total of 960 mussels.
3. Set up 12 mesh bags and suspend over the side of the boat--three bags for each of the four mussel species.
 - a. Six of the eight bags (Species A, B, and C) will contain 100 mussels each (need 80).
 - b. The seventh and eighth bags (Species D) will contain 180 mussels each (need 144).

- c. Bags nine, ten, eleven, and twelve will be extra bags (one for each species) for mussels that are brought to the surface but are just outside of the specified size range. These mussels will be used only if the correct number of mussels in the specified size range cannot be obtained.
4. Send divers down to obtain mussels. We will have pre-selected a tight, but realistic, size range (mm) for each species by this time. How do we do this?.
5. As mussels come to the surface, place them in the appropriate mesh bag, making sure to keep a count of the number of mussels placed into each bag.
6. As the mussels accumulate, designate two individuals as scrubbers. Remove one mussel from a mesh bag, have one person scrub the valves thoroughly. Rinse the scrub brush in a dilute solution (10%??) of bleach and then into water (anything but river water). Have the second person scrub the valves again.
7. Place this newly-scrubbed mussel into the appropriate container of pond or well water for holding until transfer to UMSC (see preparation step 4). These containers need to be well aerated.
8. Transfer mussels from pond or well water tank into the appropriate holding device in the concrete pond at UMSC (see preparation step 2). Again, remember to keep the "GNFH-relocated mussels" separate from the "Pool 9-relocated mussels".
9. Mussels will be held, undisturbed, in this pond at the UMSC for 30 days.
10. On day 28 or 29 (about June 5, 1995) we will need to set up the structures that will house our racks and tubs in the river and at GNFH (i.e pound in stakes). Also, we need to obtain riverine sediment to fill the substrate-filled tubs. Transport the tubs filled with sediment to the GNFH and place in the pond for "storage" until we need them--in 1 to 3 days. Lets get a few extra tubs containing sediment in case we spill or otherwise mess up.
11. On days 30 and 31, begin to process the mussels in the UMSC pond. I think it is unrealistic to expect that we can process and relocate all 768 mussels in one day. Thus, I propose that we separate the processing and relocation effort into two consecutive days--one for mussels going to Pool-9 and one for mussels going to GNFH. Furthermore, lets start with GNFH--I think it will be the easier of the two and give us time to work out the bugs. Either way, the mussels will be worked up as follows:
 - a. On day 30, transport the 460 (need 368) mussels to be relocated to GNFH from UMSC to GNFH. Work up the mussels at GNFH.
 1. Before these mussels are removed from the UMSC, I will sacrifice 32 mussels of species D for glycogen analysis.

2. We need to set up four "tubs" of mussels; one for each species. Tubs containing species A, B, and C will contain 100 (need 80) mussels each. The one tub containing species D will contain 160 (need 128) mussels.
3. I will generate four random number tables, one for each species. Tables for species A, B, and C will contain 80 random numbers. The table for species D will contain 128 random numbers. These tables will be used to decide which position (in which treatment option and which replicate) each mussel will be placed (Figure 1).
4. Start with species A. Randomly, remove one mussel and visually inspect and scrub the mussel. Record the number of zebra mussels, if any. Mark this mussel with a dremel tool so that each individual mussel can be identified, such as A1. (NOTE: Might mark A1-A200 for species A, B1-B200 for species B, C1-C200 for species C, D1-D360 for species D. These numbers include all mussels going to GNFH and to Pool 9).

Measure shell height and shell length to the nearest 0.1 mm with a digimatic caliper. Record this data on a data sheet. Record a visual description of shell condition (i.e. beaks eroded?, any dents in the shell?).

After an individual mussel is scrubbed, marked, and measured, look at the random number table for that species to identify into which position it will be placed (Figure 1). Physically place the mussel into the correct position in one of the 16 treatment options.

5. Repeat step 4 with one mussel from Species B.
 6. Repeat step 4 with one mussel from Species C.
 7. Repeat step 4 with one mussel from Species D.
 8. Repeat step 4 again with one mussel from species A. So the order is mussel 1 from species A, mussel 1 from species B, mussel 1 from species C, mussel 1 from Species D, mussel 2 from species A, mussel 2 from species B, mussel 2 from species C, mussel 2 from species D, mussel 3 from species A, etc.....
 9. Continue until all 368 mussels have been placed into one of the 368 positions in the 16 treatment options. Place all treatment options into the pond at GNFH.
- b. On day 31, transport the 460 (need 368) mussels to be relocated to Pool 9 from UMSC to Pool 9. Work up the mussels on a boat in Pool 9, repeating steps 11.a.2 to 11.a.9.
 - c. Return extra mussels to the river.
12. Breathe. Relax until September 1995.

13. In Sept 1995, May 1996, and Sept 1996, randomly retrieve one of the 16 treatment options from GNFH. Remove all 23 mussels and measure shell height, shell length, shell condition, and number of dead unionids. Sacrifice one mussel of Species D from each treatment option for glycogen analysis. Replace treatment option. Continue in this manner until all 16 treatment options have been inspected. Repeat this process at Pool 9.

	Rep 1				Rep 2				Rep 3				Rep 4			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Trt 1	1	1	1	1	6	6	6	9	11	11	11	17	16	16	16	25
	2	2	2	2	7	7	7	10	12	12	12	18	17	17	17	26
	3	3	3	3	8	8	8	11	13	13	13	19	18	18	18	27
	4	4	4	4	9	9	9	12	14	14	14	20	19	19	19	28
	5	5	5	5	10	10	10	13	15	15	15	21	20	20	20	29
								14				22				30
								15				23				31
								16				24				32
Trt 2	21	21	21	33	26	26	26	41	31	31	31	49	36	36	36	57
	22	22	22	34	27	27	27	42	32	34	32	50	37	37	37	58
	23	23	23	35	28	28	28	43	33	33	33	51	38	38	38	59
	24	24	24	36	29	29	29	44	34	34	34	52	39	39	39	60
	25	25	25	37	30	30	30	45	35	35	35	53	40	40	40	61
			38				46				54					62
			39				47				55					63
			40				48				56					64
Trt 3	41	41	41	65	46	46	46	73	51	51	51	81	56	56	56	89
	42	42	42	66	47	47	47	74	52	52	52	82	57	57	57	90
	43	43	43	67	48	48	48	75	53	53	53	83	58	58	58	91
	44	44	44	68	49	49	49	76	54	54	54	84	59	59	59	92
	45	45	45	69	50	50	50	77	55	55	55	85	60	60	60	93
			70				78				86					94
			71				79				87					95
			72				80				88					96
Trt 4	61	61	61	97	66	66	66	105	71	71	71	113	76	76	76	121
	62	62	62	98	67	67	67	106	72	72	72	114	77	77	77	122
	63	63	63	99	68	68	68	107	73	73	73	115	78	78	78	123
	64	64	64	100	69	69	69	108	74	74	74	116	79	79	79	124
	65	65	65	101	70	70	70	109	75	75	75	117	80	80	80	125
			102				110				118					126
			103				111				119					127
			104				112				120					128

Figure 1. Randomization of four species (A, B, C, and D) of unionid mussels into specific position (identified with numerals) within each treatment and replicate.

Sampling Event and Time

Number and Fate of Mussels

Mussel Collection, Pool 9
May 1-5, 1995

Mussel Storage at UMSC Pond
May 5-June 5, 1995

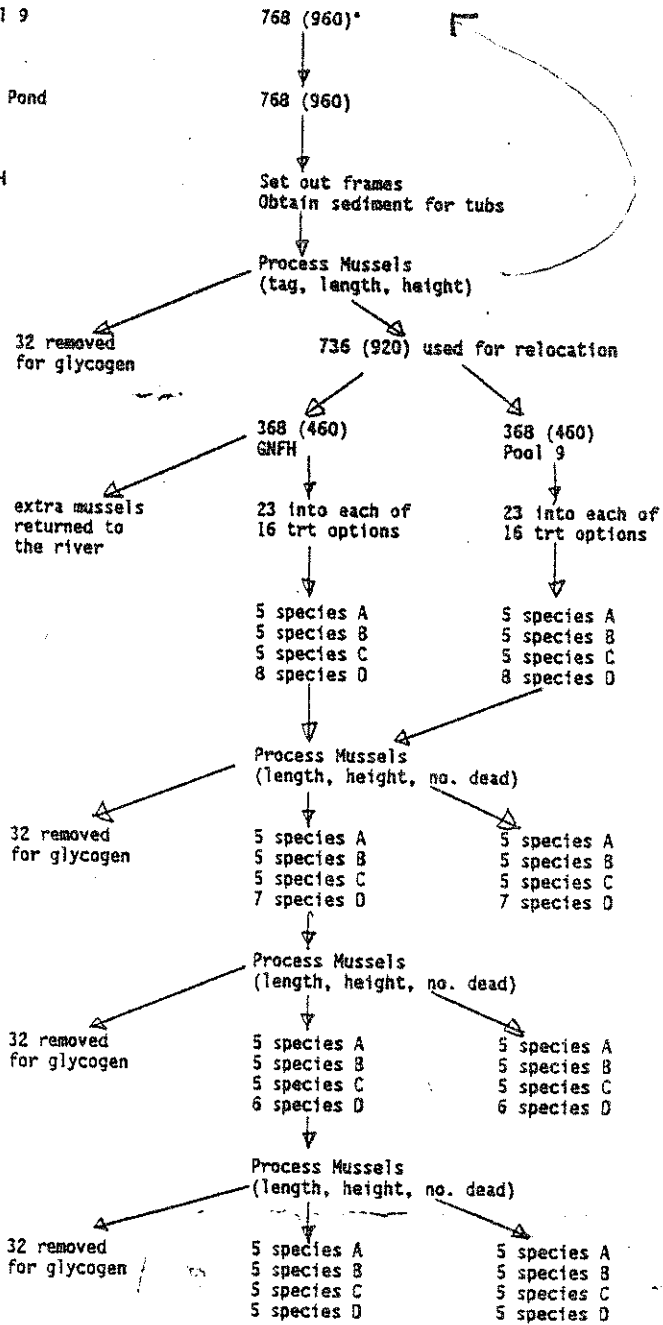
Day 29, Pool 9 and GNFH

Days 30 and 31,
GNFH, and Pool 9

September 1995

May 1996

September 1996



* Number in parentheses is 25% over what we need.
Will also collect about 10 specimens of species D for initial tissue glycogen levels.

Qualitative Mussel Sampling in Pool 9
 Victory, Wisconsin; February 22, 1995

Species	10 mm Shell Length Increment																										Total
	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260			
A. plicata	1	2		1	7	10	6	1	1						1			4	1	2						40	
A. complanata																										2	
L. cardium								1																		2	
H. nervosa							1								1	4										8	
A. grandis															1							1				2	
P. alatus											1	1						1								3	
Q. quadrula	1				2	1	1																			5	
L. fragilis					1	2	2	1	1	1																7	
E. dilatata										1	1															2	
T. truncata	3	8	2																							13	
L. recta															2											2	
O. olivaria							1																			2	
F. flava					2	3																				5	
O. reflexa		2			2																					4	
E. lineolata		1			1																					2	
A. confragosus			1																							2	
Total (16 sp.)																										101	

A. plicata = 39.6% (best size group 70 to 100 mm)
 T. truncata = 12.9% (best size group 30 to 60 mm)
 M. nervosa = 7.9% (best size group 110 to 140 mm)
 L. fragilis = 6.9% (best size group 70 to 100 mm)