SEMI-ANNUAL PROGRESS REPORT

NATIONAL POLLUTION DISCHARGE ELIMINATION SYSTEM

TOXICITY OF THE MAJOR SALT (SODIUM BICARBONATE) FROM COALBED METHANE PRODUCTION TO FISH IN THE TONGUE AND POWDER RIVER DRAINAGES IN MONTANA

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SUMMARY

This progress report describes the activities of MFWP and USGS during the period of September 2004-October 2005. All data included in this report is considered provisional, pending completion of the quality assurance and technical review process of the USGS, Columbia Environmental Research Center. The work plans for both the MFWP and the USGS stated that chronic and acute toxicity experiments would be conducted on pallid sturgeon. These efforts, conducted in the summer and fall 2003, were largely unsuccessful and the details of those efforts were provided in the March 2004 progress report. Additional efforts to successfully maintain shovelnose sturgeon fry at the USGS Jackson Field Research Station were conducted in July 2004 and survival of the sturgeon during these attempts was not adequate to perform a chronic experiment.

Subsequent to the unsuccessful efforts to conduct chronic toxicity experiments with pallid sturgeon, fathead minnows were chosen as an alternative species, and successful acute and chronic experiments were conducted in the fall of 2003. The initial results of the experiments were provided in a March 2004 Progress Report to the EPA. Additional results of the chronic experiment with fathead minnows are provided in this report, including histological observations, Na/K ATPase, and whole body ion measurements. Also, this report provides the results of follow-up acute and chronic toxicity experiments that were performed with white sucker in the spring and summer of 2005.

Finnally, the results of field sampling conducted in July 2004 at 15 sites in the Tongue and Powder River drainages are discussed in this report. Initially, the objective for field sampling site selection (as described in the work plans) was to locate sites at regular intervals on the mainstem Tongue and Powder rivers and in the major tributaries of both drainages. This objective was modified so that sites were co-located with USGS long-term water quality sampling stations. The work plans also stated that habitat measurements would be collected at each site using EPA Rapid Bioassessment protocols. These measurements were originally intended to be used to generate IBI values for each site (per models developed by Bramblett et al 2003). Subsequent to this, we also have decided to use the Wyoming Warmwater Fisheries Assessment (Quist et al. 2004), which is a tool for predicting fish species occurrence at a site based on habitat conditions.

The Results section of this report is divided into subsections relating to the Tasks identified in our contracts: Task 1 provides a summary of available data from both the original and follow-up 60-day chronic toxicity experiments with fathead minnows conducted by the USGS. Task 2 provides a summary of the fathead minnow acute toxicity experiments conducted by MFWP. Task 3 provides a summary of fish and habitat surveys conducted in the Tongue and Powder River drainages.

RESULTS

Task 1. Investigate the chronic toxicity of sodium bicarbonate for the egg/larvae life stages of fish.

Task 1a. Fathead Minnow chronic experiments.

Hatch, Survival and Growth: We conducted two flow-through chronic toxicity experiments beginning with freshly fertilized FHM eggs. The FHM were exposed to concentrations ranging from 500 to 1400 mg $NaHCO_3$/L in experiment 1 and from 300 to 625 mg $NaHCO_3$/L in experiment 2 Both experiments were terminated at 60 d post hatch (a 65-d total exposure). Percentage hatch of the eggs were significantly smaller compared to the controls at 1400 and 625 mg $NaHCO_3$/L during experiments 1 and 2, respectively. We also observed a 15% decrease in survival during the swim-up stage (~5-8 d post hatch) during both experiments (data provided in 2004 progress report). For Experiment 1, survival decreased significantly at all concentrations $\geq$800 mg $NaHCO_3$/L. Survival did not decrease significantly in Experiment 2 because it was conducted at lesser $NaHCO_3$ concentrations to better determine sublethal effects. Lengths and weights recorded for individual fish did not decrease significantly in either experiment (data provided in 2004 progress report).

Histology: Rodlet cells were found closely associated with cranial meninges in all groups of fish and appeared to increase with concentrations or the number of days of exposure. Similarily, focal degeneration in ovarian tissue appeared to be more common in fish from the larger $NaHCO_3$ exposure concentrations, but no degenerative changes of this type were observed in fish from the control and 500 mg $NaHCO_3$/L treatments.

Gill lesions (epithelial hypertrophy, edema, mucus and necrosis) increased in severity with $NaHCO_3$ concentration or the number of days of exposure. For example, at 30 d, fish from the control had no necrosis in the gills, while fish from the 1100 mg $NaHCO_3$ /L exposure experienced moderate changes and contained focal areas of necrosis in the gills. At 60 d, large necrotic lesions were noted in both gills of fish from the control and 500 mg $NaHCO_3$/L treatment, but were more numerous in the 500 mg/L NaHCO3 treatment. In the kidney, changes were not observed at Day 30, but at Day 60 the numbers of regenerating tubules were slightly greater in fish from the 500 mg $NaHCO_3$/L compared to the controls.

Na/K Atpase and Whole Body Ions: The methods used to measure Na/K Atpase was adapted from McCormick (1993). Na/K ATPase activity decreased as exposure concentration increased (Figure 1). Whole body Ca, Na, K, and Mg were measured with flame atomic absorption spectrphotometry (Figure 2). Statistical evaluations of these data have not yet been performed.

Summary: For fathead minnows, mortality was significant in all concentrations above 400 mg/L NaHCO3; however, in the treatments without significant mortality, growth was not affected. As concentration and exposure time increased, so did the occurrence and severity of the microscopic lesions. After 60 d, fish from the 500 mg $NaHCO_3$/L treatment had slightly increased incidence of kidney damage compared to the controls as defined by the greater number of regenerating tubules and focal degeneration that were observed in the kidneys of fish exposed to 500 mg $NaHCO_3$/L.

Task 1b. White Sucker chronic experiment.

White suckers (*Catostomus commersoni*) were exposed to $NaHCO_3$ in a flow through diluter system. The diluter system contained 12 16L glass aquaria that were supplied with reconstituted water representative of the Tongue River. The study was started on May 20, 2005 and continued 63-days (51 days post-hatch). Each 16L aquarium was divided into 4 4L chambers. The concentrations included a control, 450, 800, and 1400 mg $NaHCO_3$/L. The concentrations were replicated three times and randomly assigned across the 12 glass aquaria. The exposure concentrations were obtained by adding appropriate volumes of a 100g $NaHCO_3$/L solution dissolved in reverse osmosis water to the reconstituted Tongue River water. Then appropriate volumes of the reconstituted Tongue River water that contained $NaHCO_3$ was added to each replicate exposure chamber about every 15 min. Complete water replacement in each tank occurred approximately every 4 hours.

The white suckers for the experiment were obtained from a private pond near Dillon, Montana. The artificial pond receives water from the Beaverhead River via an irrigation ditch. On May 20, 2005, the pond water contained a pH of 8.69, a total alkalinity of 136 mg/L as CaCO3 and a specific conductance of 416 µS/cm. Sexually mature suckers were captured in trap nets that were set overnight on multiple occasions. On May 20, 2005, eggs from three females were fertilized with sperm from seven males in reconstituted Tongue River water, and then soaked in a solution of 4.75 ml iodine dissolved in 2 L reconstituted Tongue river water for 30 minutes. The eggs were then rinsed and transported to the USGS – CERC - Jackson Field Research Station in a plastic cooler filled with reconstituted Tongue River water. Upon arrival at the Field Station the eggs were tempered from 16 ^{0}C to 18 ^{0}C and approximately 50-60 eggs were placed in each 4L chamber containing the exposure concentrations of $NaHCO_3$ and in each replicate of the control. Conductivity, dissolved oxygen, and temperature were monitored at least once daily. Alkalinity, hardness, and pH were monitored multiple times throughout the experiment. Water samples were collected from each concentration on 5/23, 6/9, 6/17, 6/29, 7/15 and 7/22, 2005 for analyses of total $NaHCO_3$.

After 30 days, surviving fry were culled to $\geq$ 15 fish per 4L replicated chamber. The culled fish were anesthetized with ice, measured for length and weight, and samples were collected for histological evaluations and whole body ion measurements. Percent hatch was 23% in the control, 21% in the 450, 29% in the 800, and 30% in the 1400 mg/L $NaHCO_3$ treatments. After 30 days, the survival of white sucker fry that hatched was 59% in the control, 75% in the 450, 86% in 800, and 87% in the 1400 mg/L $NaHCO_3$ exposures. Overall survival of eggs to 60-day old fry (including mortalities to hatch) was 13% for the control, 19% for the 450, 24% for the 800 and 27% for the 1400 mg/L $NaHCO_3$ exposures.

A second experiment was performed to validate the repeatability of the hatching success of white suckers. On June 11, 2005, 10 female and 14 male white suckers from the Beaverhead River pond were spawned and the eggs transported to the Jackson Field Research Station. At least 250 eggs were placed in a vacant 4L glass aquaria and exposed to $NaHCO_3$ using the methods described above. Hatching success and survival of the second group of eggs spawned on 6/11/2005 was similar to the first group and these data will be further analyzed and reported later.

Task 2. Determine the acute toxicity of sodium bicarbonate to white suckers.

Task 2a. Newly hatched fry. White sucker fry were obtained from the USGS CERC Jackson Field Research Station, Jackson, Wyoming, where the eggs had been incubated and hatched and the fry were being kept in water reconstituted to simulate Tongue River water. The first hatchlings were observed on June 11, and exogeneous feeding was first observed on June 20. The fish were transferred from Jackson, WY to Helena, MT on June 30, 2005 where they were held in water of a similar quality (the constituents of this water were the same as described for the pallid sturgeon in the March 2004 Progress report). Beginning on July 2, 2005, the fry (22-days old at the beginning of the experiment) were exposed to nominal sodium bicarbonate concentrations of 864, 1440, 2400, 4000 and 6666 mg/L for 96 hours. The control chambers contained a nominal sodium bicarbonate level of 267 mg/L (to simulate Tongue River conditions) or 323 mg/L (to simulate Powder River conditions). Exposure chambers were glass mason jars containing 200 mL of reconstituted water. For the Tongue River experiment, ten fish were placed into each of three replicate jars for each exposure concentration. For the Powder River experiment, eight fish were placed in each of two replicate jars. Water temperature was maintained at $20 \pm 2^{o}C$, and lighting was natural supplemented with fluorescent lights held on a light:dark cycle of 16:8 hours. The fish were exposed under static renewal conditions for 96 hours, with the fish being transferred to fresh solution after 24, 48 and 72 hours. Fish were fed commercial Rangen Trout Starter fish feed on the two days prior to the experiment, and for one hour prior to transfer during the experiment.

The mean length and weight of the white sucker fry during these experiments was 13.6 mm and 0.014 g, respectively. There was greater mortality among fish exposed in the Tongue River water than those exposed in the Powder River water. The EPA Probit Analysis Program was used to calculate a 96-h LC50 for the Tongue River water of 5,121 mg $NaHCO_3$/L with upper and lower 95% confidence intervals of 6,678 and 4,049 mg $NaHCO_3$/L. The Trimmed Spearman-Karber Program was used to calculate a 96-h LC50 for the Powder River water of 5,421 mg $NaHCO_3$/L without reliable confidence intervals.

Task 2b. Older fry. Additional fish were transported from Jackson, WY to Helena, MT on August 16 to evaluate the toxicity of sodium bicarbonate to older fry. Testing began on August 18 when the fish were 69 days old, and had a mean length and weight of 19.3 mm and 0.038 g, respectively. Fish were exposed to reconstituted Tongue River water (as described above). The fish were exposed in a 96 hour static renewal experiment with the fish being transferred to fresh solution after 24, 48 and 72 hours. Fish were fed commercial Rangen Trout Starter fish feed on the two days prior to the experiment, and for one hour prior to transfer to fresh solutions during the experiment. Due to the limited supply of fish, only three experimental concentrations were used (control, 4000, and 6666 mg/L NaHCO3) with 20 fish being exposed at each concentration (in 2 replicates of 10 fish). This limited number of exposure levels did not allow for the calculation of an LC50, but mortality rates were 9.5%, 13.6% and 50% for the control, 4000 mg/L and

6666 mg/L, respectively. This result suggests that the older fry were somewhat more tolerant of the sodium bicarbonate than were the younger fish.

Task 3. Define presence/absence and relative abundance of fathead minnows and other resident species in the Powder and Tongue drainages relative to the results of chronic toxicity experiments.

2004 Sampling Effort. Fish and habitat surveys were conducted at fifteen sites in the Tongue and Powder River basins between July 19 and 25, 2004. Fish and habitat measurements were taken in general conformity to EPA Rapid Bioassessment protocols. The method of fish capture was typically seining, with electrofishing used at a limited number of sites. Habitat measurements were modified somewhat so data could be used in both the Wyoming Warmwater Fish Assessment (Quist et al. 2004) and Montana Prairie Stream IBI (Bramblett et al. 2003).

Six sites were sampled in the Powder River drainage, three were on the mainstem, and three in major tributaries (Table 3). The number of fish species was highest in Clear Creek near Arvada, WY (13 species) and lowest in the Powder River at Locate, MT, Moorhead, MT and Sussex, WY (5 species). Total number of individuals was highest at Crazy Woman Creek, WY (1216) and lowest at Clear Creek, WY (221). In the Tongue River drainage, six sites were on the mainstem and three were on major tributaries (Table 4). Number of species ranged from a low of four at Prairie Dog Creek, WY to a high of 12 on the Tongue River at Miles City MT, Brandenburg Bridge MT, and Stateline. Number of individuals ranged from a low of 22 on the Tongue River below the reservoir (due to limited sampling) to a high of 256 on Hanging Woman Creek. More detail is provided for this sampling effort in the previous progress report (November 2004).

In our final report, we will determine the degree correlation between the presence of fish species and conductivity at each of these 15 sites. This will provide a visual representation of the tolerance of each species to different levels of conductivity. Also, the plot will show the conductivity at sites where native species were not present but would be expected on the basis of physical habitat. This type of analysis will help define the upper and lower thresholds of conductivity for the different species. The Wyoming Warmwater Stream Assessment (WSA) protocol is being used to predict the presence or absence of a species at a site on the basis of historic distribution and physical habitat. The absence of a species at a site may be related to poor physical habitat rather than high salinity. The WSA approach allows us to qualify the "absence" side of the equation. The framework for the WSA assessment is described by Quist et al. (2005), and Quist et al. (2004) is used as an example. These methods provide information on the probable historic native fish distribution in a stream reach, which can be compared to the current (or sampled) native fish assemblage. An understanding of the expected fish assemblage in a stream reach can be used to evaluate factors that may be acting on the current fish assemblage. The comparison of predicted to observed fish assemblages can indicate changes in community structure due to natural or anthropogenic influences (Lipsey 2001). For example, if a fish species is predicted to occur in a stream reach based on its historic distribution and its elevation, stream size and habitat requirements, but is not represented in a sample of the fish community, then other factors may be affecting the occurrence of that species (e.g. water quality, predators, land management).

Information on the current and historic distribution of native fishes in the Powder and Tongue River drainages was taken from Elser et al. (1980), Holton (1990), Baxter and Stone (1995), and Patton (1997). This provides the first filter in predicting the occurrence of fish species (Tables 3 and 4).

Because fish often have specific habitat requirements, their occurrence can be predicted based on the presence of habitat characteristics in a stream reach. General habitat characteristics required and preferred by native fishes in the Powder and Tongue rivers were taken from Lee et al. (1980), Holton (1990), and Baxter and Stone (1995).

Stream reaches were evaluated based on conformity between the habitat requirements of native fishes (Tables 3 and 4) and the habitat characteristics present in the stream reach (Table 5). This information can be used to predict what fishes one may expect to encounter in a stream reach.

Additionally, the Index of Biotic Integrity (IBI) developed for Montana prairie streams by Bramblett et al (2003) will be used to aid in the interpretation of the presence or absence analyses. The IBI scores have been calculated for the 15 sites we sampled in 2004 (Table 6). Sites with relatively low IBI scores are typically interpreted to have native fish assemblages that are impacted by anthropogenic influences such as habitat, water quality degradation and the presence of non-native fish species. As appropriate, the IBI scores will be used to help interpret the presence/absence analyses.

2005 Sampling Effort. In order to build the database of sites used for the fish species presence/absence graphic analyses (or plots), FWP funded two efforts. The first was work done by the MFWP Prairie Fish Crew. Fish were sampled from nine locations in the Powder/Tongue/Rosebud Creek drainages in late April and again in mid-July. Water quality measurements were taken at the same time. Locations of the sites and field measurements are shown in Table 7. Laboratory measurements of major ions are shown in Table 8. When the Prairie Fish Crew finishes their report for 2005, the fish occurrence data for these sites will be combined with the conductivity data and incorporated into the presence/absence plots.

The second effort that was funded was sampling work done by the USGS in Cheyenne, WY. We funded the collection of major ions in the water at 15 fish sampling sites in the Powder and Tongue River drainages in Wyoming in the summer of 2005. Once their reports are complete, the fish presence data will be combined with the conductivity data and incorporated into our presence/absence plots. Their sampling methods, as well as the methods of the MFWP Prairie Fish Crew, are similar to those we employed in 2004, which will make all the data described above comparable in terms of effort used at a given site to determine occurrence.

Table 1. Native fish species expected to occur and present or absent, and non-native fish present at sites in the Tongue River drainage.

Site	Present	Expected/Absent	Present/Non-native
Tongue River at Monarch (TRN)	Shorthead redhorse Longnose sucker Longnose dace Stonecat Black bullhead	Mounain sucker Fathead minnow White sucker	Green sunfish Rock bass Common carp
Tongue River at MT/WY stateline (TRSL)	Black bullhead White sucker Shorthead redhorse Stonecat Yellow bullhead	Lake chub Longnose dace Fathead minnow Channel catfish Goldeye River carpsucker Sauger Emerald shiner	Rock bass Spottail shiner Smallmouth bass Chite crappie Common Carp
Tongue River below reservoir (TRBD)	Black bullhead Longnose sucker Yellow bullhead White sucker River carpsucker	Lake chub Mountain sucker Longnose dace Stonecat Shorthead redhorse Emerald shiner	Green sunfish Pumpkinseed Common carp Smallmouth bass
Tongue River at Birney Bridge (TRBB)	White sucker Shorthead redhorse Yellow bullhead Stonecat Longnose dace	Lake chub Mountain sucker Longnose dace Fathead minnow River carpsucker Sauger Brassy minnow	Smallmouth bass Rockbass Common carp
Tongue River at Barndenburg Bridge (TRBR)	Channel catfish White sucker Flathead chub Longnose dace Sand shiner Shorthead redhorse River carpsucker Yellow bullhead Fathead minnow Stonecat	Lake chub Goldeye Sauger Shovelnose sturgeon	Green sunfish Common carp
Tongue River at Miles City (TRMC)	Western silvery minnow Stone cat Flathead chub White sucker Longnose dace Shorthead redhorse Sand shiner Emerald shiner	Fathead minnow Black bullhead Plains minnow Sauger Yellow bullhead	Common carp

	River carpsucker Channel catfish Goldeye		
Goose Creek (GCR)	Shorthead redhorse White sucker Stonecat Black bullhead Fathead minnow	Lake chub Mountain sucker Longnose dace Longnose sucker	Smallmouth bass Common carp Rockbass
Prairie Dog Creek (PDCR)	White sucker Creek chub Shorthead redhorse	Lake chub Stonecat Goldeye	White crappie
Hanging Woman Creek (HWCR)	Fathead minnow Black bullhead White sucker	Lake chub Yellow bullhead Brassy minnow	Green sunfish Common carp

Table 2. Native fish species expected to occur and present or absent, and non-native fish present at sites in the Powder River drainage.

Site	Present	Expected/Absent	Present/Non-native
Powder River at Sussex (PRSX)	Plains minnow Sand shiner Flathead chub White sucker	Lake chub Fathead minnow Burbot Black bullhead Channel catfish Goldeye River carpsucker Sauger Sturgeon chub Western silvery minnow	Plains killifish
Powder River at Moorehead (PRMD)	Sand shiner Flathead chub Longnose dace Stonecat Channel catfish	Goldeye	
Powder River at Locate (PRLM)	White sucker Sand shiner Flathead chub	Goldeye Plainsminnow	Plains killifish
Clear Creek (CLCR)	Sand shiner Goldeye White sucker Stonecat Shorthead redhorse Creek chub Flathead chub River carpsucker	Lake chub Longnose dace Burbot	Rock bass Common carp Green sunfish Smallmouth bass
Crazy Woman Creek (CWCR)	Sand shiner Fathead minnow Black bullhead White sucker Longnose dace Flathead chub Plains minnow	Channel catfish goldeye	
Little Powder River (LPR)	River carpsucker Channel catfish Shorthead redhorse White sucker Flathead chub Longnose dace Sand shiner Fathead minnow	Lake chub Black bullhead Goldeye Yellow bullhead	Common carp Green sunfish

Table 3. Habitat characteristics associated with native fish distributions in the Tongue River watershed, Montana/Wyoming

		Stream Characteristics																				
		Intermittent		Turbidity		Pools (> 0.5 m)					Pools (> 0.5)			Riffles			Runs			Backwater/ Sidechannel		Predators absent
Species	Width*	Y	N	H	L	SA	GR	CB	BL	cover	SA+ Cover	GR+ cover	CB+ cover	GR	CB	BL	SA	GR	CB	present	w/AV	
CKC	S-L				x							x	x									x
LKC	S-XL					x	x															
MTS	S-L				x		x	x	x					x	x	x		x	x			
LND	S-L													x	x	x		x	x			
LNS	S-L				x		x	x	x					x	x	x		x	x			
STC	S-L														x	x						
FHM	S-L									x											x	
SDS	S-L					x											x	x		x		x
WHS	S-L						x	x	x					x	x	x		x	x			
BBH	S-L	x		x						x												x
CCF	M-XL			x						x												
NRH	M-XL						x	x	x					x	x	x		x	x			
GDE	M-XL			x		x											x					
FHC	S-L	x		x		x											x					x
PMN	M-XL	x		x		x											x					x
RCS	L-XL					x	x										x	x				
SAR	L-XL			x						x												
SGC	L-XL	x		x										x			x	x				x
SMN	L-XL	x		x		x											x			x		x
SNS	XL			x		x	x										x	x				
YBH**	S-L									x												
BMN**	S-XL				x	x	x			x	X	x										
EMS**	XL					x	x										x	x				

*Stream widths: small 1-5 m (S), medium 5-10 m (M), large 10-20 m (L), extra-large > 20 m (XL)
**Indicates fish found only in Montana

Table 4. Habitat characteristics associated with fish distributions in the Powder/Little Powder watersheds, Montana/Wyoming

Stream Characteristics																						
		Intermittent		Turbidity		Pools (> 0.5 m)					Pools (> 0.5)			Riffles			Runs			Backwater/ Sidechannel		Predators absent
Species	Width*	Y	N	H	L	SA	GR	CB	BL	cover	SA+ Cover	GR+ cover	CB+ cover	GR	CB	BL	SA	GR	CB	present	w/AV	
BMN**	S-XL				x	x	x			x	X	x										
CKC	S-L				x							x	x									x
LKC	S-XL					x	x															
MTS	S-L				x		x	x	x					x	x	x		x	x			
LND	S-L													x	x	x		x	x			
LNS	S-L				x		x	x	x					x	x	x		x	x			
STC	S-L														x	x						
FHM	S-L									x											x	
SDS	S-L					x											x	x		x		x
WHS	S-L						x	x	x					x	x	x		x	x			
BBT	L-XL					x	x															
BBH	S-L	x		x						x												x
CCF	M-XL			x						x												
NRH	M-XL						x	x	x					x	x	x		x	x			
GDE	M-XL			x		x											x					
FHC	S-L	x		x		x											x					x
PMN	M-XL	x		x		x											x					x
RCS	L-XL					x	x										x	x				
SAR	L-XL			x						x												
SGC	L-XL	x		x										x			x	x				x
SMN	L-XL	x		x		x											x			x		x
SNS	XL			x		x	x										x	x				
YBH**	S-L									x												

*Stream widths: small 1-5 m (S), medium 5-10 m (M), large 10-20 m (L), extra-large > 20 m (XL)
**Indicates fish found only in Montana

Table 5. Habitat characteristics by stream reach.

Stream Characteristics																						
		Intermittent		Turbidity		Pools (> 0.5 m)					Pools (> 0.5)			Riffles			Runs			Backwater/ Sidechannel		Predators absent
Reach	Width (m)	Y	N	H	L	SA	GR	CB	BL	cover	SA+ Cover	GR+ cover	CB+ cover	GR	CB	BL	SA	GR	CB	present	w/AV	
TRM	21		x		x			x	x					x	x			x	x	x	x	x
TRSL	35		x	x		x	x			x	X			x	x		x	x		x	x	
TRBD	50		x		x			x	x						x	x			x	x	x	
TRBB	50		x		x		x			x		x		x	x			x				
TRBR	26			x		x	x	x		x	X			x	x		x	x		x		
TRMC	19	x		x			x		x	x		x			x		x	x	x	x		
GCR	15		x		x	x	x	x	x		X				x	x			x			
PDCR	5.1		x	x		x	x	x						x	x			x	x			
HWCR	2.9	x			x	x				x	X			x			x					
PRSX	13	x		x		x	x			x	X			x			x			x		x
PRMD	32		x	x										x	x		x	x	x	x		
PRLM	8.3	x		x										x			x	x		x		x
CLCR	14	x		x		x	x	x						x	x		x	x		x		
CWCR	4.8	x		x		x				x	X			x	x		x					x
LPR	7.9			x		x				x	X			x			x	x		x	x	

Table 6. Index of Biotic Integrity (Bramblett et al 2003) scores for fish assemblages on Powder and Tongue River sections.

River/Section	Drainage area (km2)	IBI score
Tongue River at Miles City	13,998	63*
Tongue River at Brandenburg Bridge	10,041	52*
Tongue River near Birney	6,824	53**
Hanging Woman Creek	1,210	35
Tongue River at Stateline	3,753	34
Prairie Dog Creek	915	56
Goose Creek	1,180	52
Tongue River near Monarch	1,134	49
Powder River near Locate	33,849	38*
Little Powder River at Broadus	5,076	56
Powder River at Moorhead	20,832	43*
Powder River at Sussex	5,470	55
Clear Creek near Arvada	2,991	65
Crazy Woman Creek near Arvada	2,477	66

*Scores considered to be unreliable because the drainage size was much larger than that used by Bramblett et al to develop their model.

**Score considered unreliable because only a side channel was sampled.

Table 7. Field measurements of physical and chemical attributes of sites sampled for fish by MFWP Region 7 Prairie Fish Survey crew in 2005.

Site Name	Date sampled	Time	Latitude	Longitude	Water Temperature (°C)	pH (s.u.)	Dissolved oxygen (mg/L)	Specific Conductivity (uS/cm)
Deer Creek	4-27-05	1045	45.05529	106.70295	7.4	8.48	6.3	3,795
Deer Creek	7-19-05	1500	45.05529	106.70295	34.7	9.28	0.11	12,110
Hanging Woman Creek	4-26-05	1430	45.22817	106.49747	13.3	9.03	4.14	2,744
Hanging Woman Creek	7-20-05	1000	45.22764	106.49904	20.2	8.5	3.60	3,145
Little Powder River	4-28-05	1100	45.10371	105.33128	4.8	8.75	9.59	1,728
Pumpkin Creek	4-28-05	0945	46.07593	105.55361	10.4	8.70	3.71	774
Pumpkin Creek	7-20-05	1600	46.075	105.5637	20.9	8.85	2.23	2,101
Rosebud Creek	7-18-05	1530	45.21363	107.00193	16.6	7.76	1.78	1,228
Rosebud Creek	7-18-05	1300	45.22036	106.95095	15.4	6.39	1.60	16,130
Rosebud Creek (park)	7-19-05	1130	45.21648	106.99321	18.8	7.72	0.10	1,654
Spring Creek	5-02-05	1345	45.82289	104.85794	9.9	8.99	9.52	1,224
Spring Creek	7-21-05	1330	45.823602	104.86151	31.8	9.03	0.21	2,906
Waddle Creek	4-26-05	1700	45.04868	106.44972	12.6	9.5	7.88	1,613
Waddle Creek	7-19-05	N/a	45.04982	106.45166	32.1	9.82	3.27	45,310
Youngs Creek	4-27-05	0845	45.01468	106.97492	3.6	9.28	9.56	813
Youngs Creek	7-19-05	N/a	45.01468	106.97492	14.5	6.90	7.05	843

Table 8. Laboratory measurements of major ion composition (mg/L) of surface waters sampled by MFWP Region 7 Prairie Fish Survey crew, 2005.

Site Name	Date sampled	pH (s.u.)	Ca	Cl	K	Mg	Na	SO4	HCO3	CO3
Deer Creek	4-27-05	8.03	356	27.0	24.4	436	1,030	4,540	538	<1.00
Deer Creek	7-19-05	8.89	391	62.1	69.0	1,140	2,940	11,200	168	56.0
Hanging Woman Creek	April??	8.16	126	17.5	19.7	174	507	1,620	488	<1.00
Hanging Woman Creek	7-20-05	8.92	40.0	15.0	22.2	166	493	1,390	272	56.0
Little Powder River	4-28-05	8.22	144	50.8	19.2	86.3	436	1,210	386	<1.00
Pumpkin Creek	4-28-05	7.79	41.7	1.53	7.90	14.4	137	127	230	<1.00
Pumpkin Creek	7-20-05	8.83	48.2	6.53	15.1	63.6	409	807	332	48.0
Rosebud Creek	7-18-05	8.26	77.1	1.18	6.20	104	33.4	204	450	<1.00
Rosebud Creek	7-18-05	7.77	122	2.29	7.70	155	52.8	469	526	<1.00
Rosebud Creek (park)	7-19-05	7.90	109	2.85	9.20	137	41.8	342	528	<1.00
Spring Creek	5-02-05	8.51	47.2	4.33	7.10	32.3	304	439	460	32.0
Spring Creek	7-21-05	8.92	20.7	3.74	10.1	21.8	10.1	602	500	64.0
Waddle Creek	4-26-05	8.37	359	66.9	42.0	987	3,220	11,100	700	24.0
Waddle Creek	7-19-05	9.16	545	193	138	3640	11,000	40,800	280	304
Youngs Creek	4-27-05	8.34	115	4.51	12.3	129	84.1	516	458	8.00
Youngs Creek	7-19-05	8.36	76.1	1.54	8.10	61.6	25.1	91.1	384	8.00

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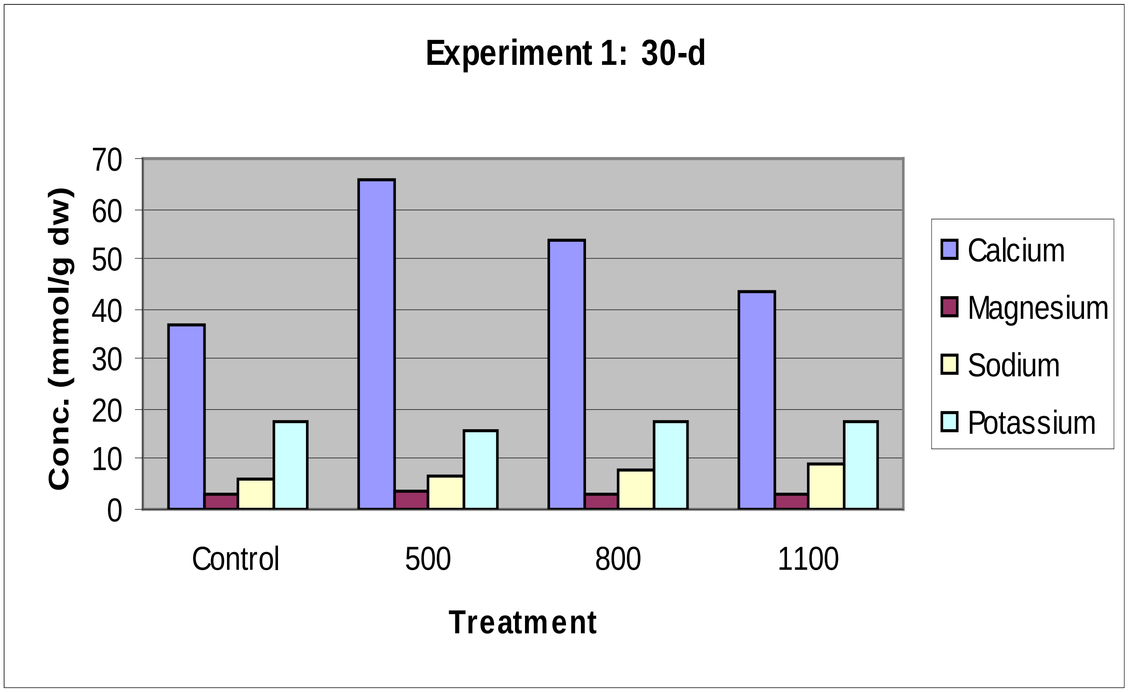
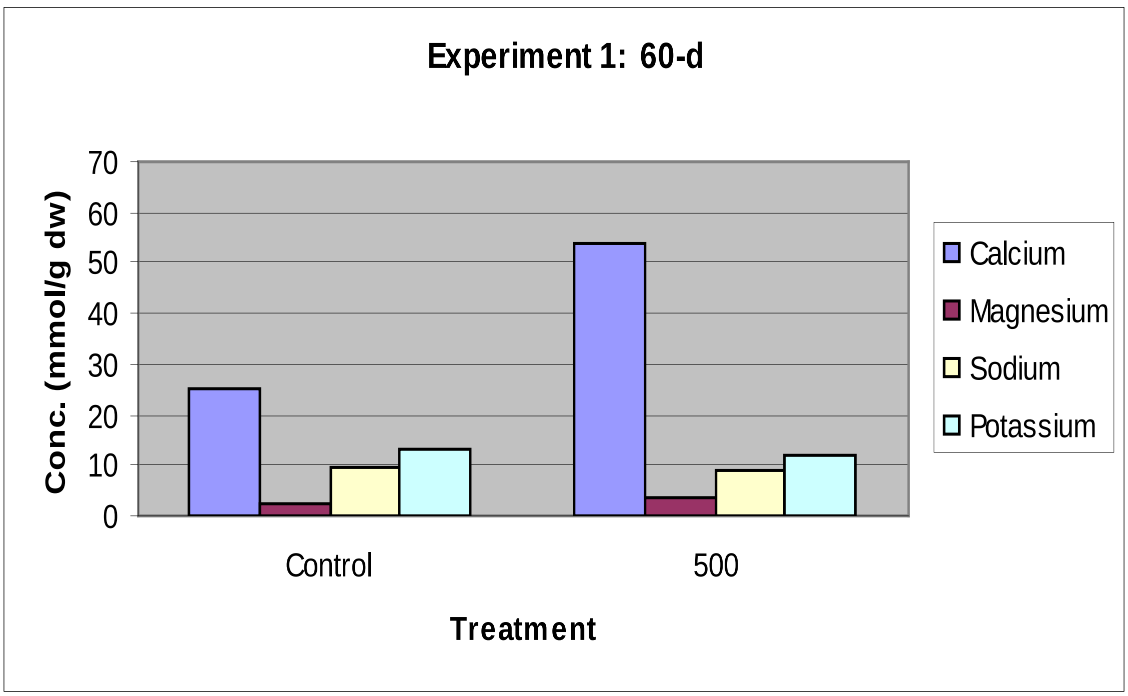
Figure 1. Na/K ATPase activity measured in fathead minnows exposed to $NaHCO_3$ for 30 days.

Figure 2: Whole body ion concentrations

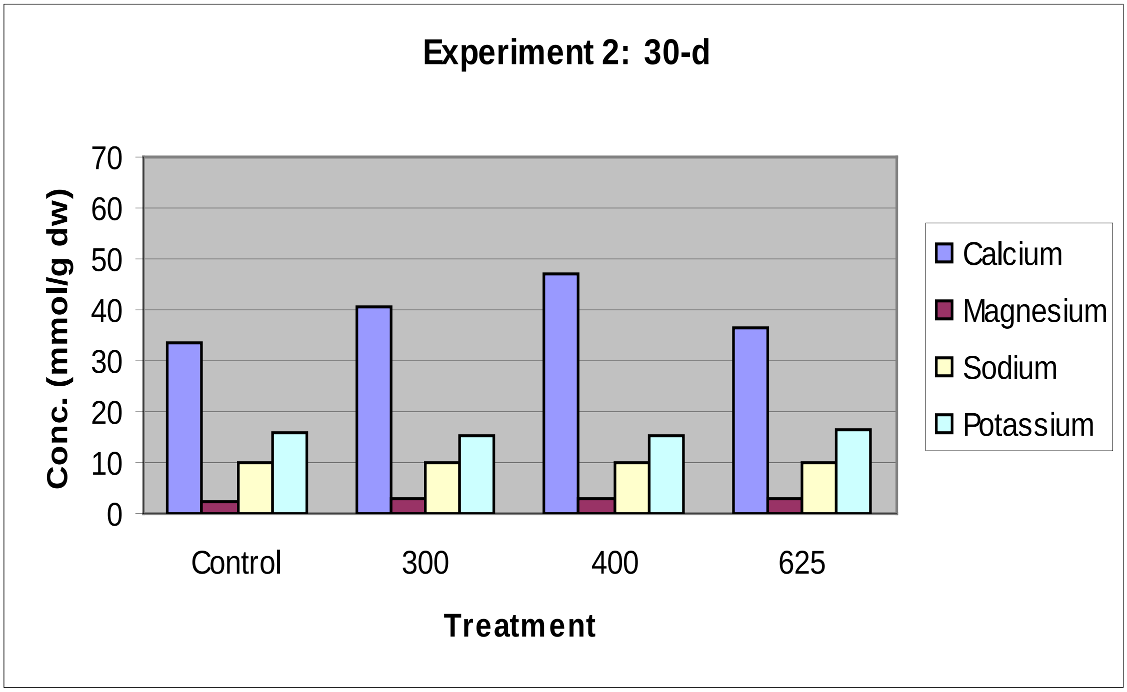
A.



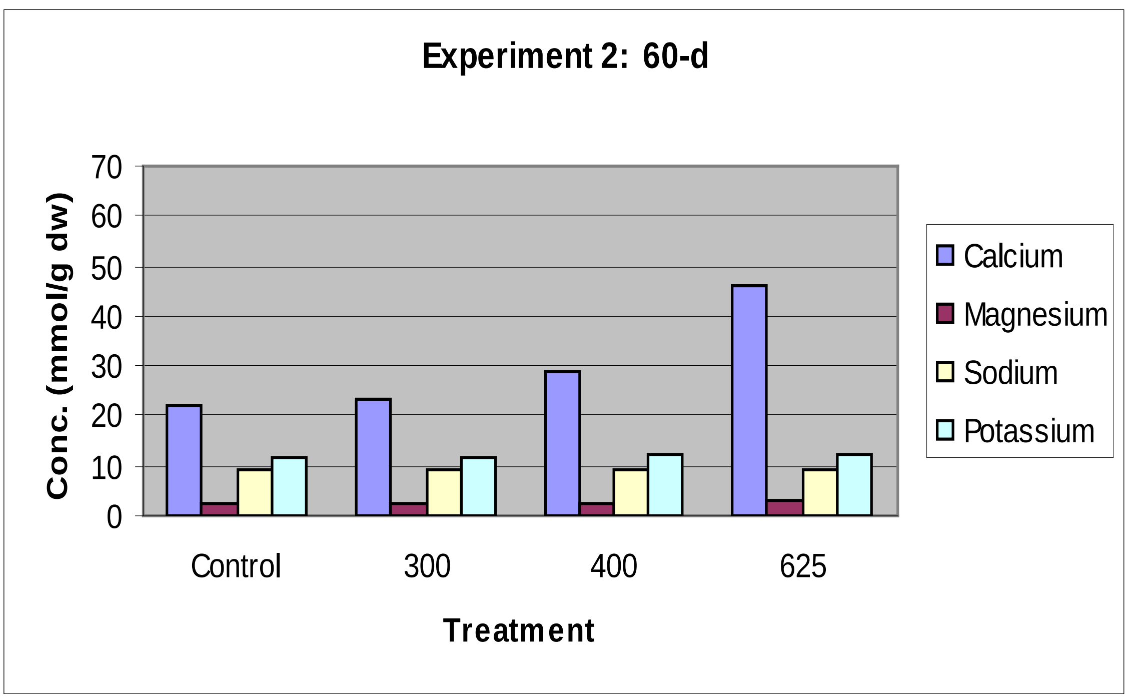
B.



C.


Experiment 2: 30-d
Conc. (mmol/g dw)
70
60
50
40
30
20
10
0
Control
300
400
625
Treatment
Calcium
Magnesium
Sodium
Potassium

D.


Experiment 2: 60-d
Conc. (mmol/g dw)
70
60
50
40
30
20
10
0
Control
300
400
625
Treatment
Calcium
Magnesium
Sodium
Potassium