# LONGITUDINAL CHANGES IN POTENTIAL TOXICITY OF COALBED NATURAL GAS PRODUCED WATER ALONG BEAVER CREEK IN THE POWDER RIVER BASIN, WYOMING

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In the Powder River Basin (PRB) in northeast Wyoming, coalbed natural gas (CBNG) is extracted from coal seams at depths of up to 500 m. Some concurrently produced waters are discharged into ephemeral drainages like Beaver Creek, a tributary to the Powder River. Total ammonia, $CO_2$, $Na^+$ and $HCO_3^-$ concentrations are high in waters from some of the deeper coal seams. I evaluated the fate and effects of ammonia in CBNG produced water discharged into Beaver Creek from August 2006 through March 2007. The study included (1) observation of fish and amphibians, (2) in-stream toxicity tests at 6 sites using caged fathead minnow larvae (FHM; *Pimephales promelas*), (3) concurrent ambient-pH and $CO_2$ pH-controlled laboratory toxicity tests using the same site waters, (4) analyses of water quality parameters in the effluent and the receiving water, and (5) evaluation of ammonification processes occurring during transport from the stream to the laboratory. Although 96-h survival of FHM in-stream and in the laboratory was comparable to controls, 144-h survival was lower in some ambient-pH exposures in the laboratory as pH increased due to $CO_2$ degassing. In Beaver Creek, pH gradually increased along a downstream gradient from initially circumneutral values, primarily due to $CaCO_3$ buffering. Because ammonia decreased along the same gradient, presumably assimilated by plants and microbes, the potential toxicity also decreased. I conclude that $CO_2$ pH-controlled toxicity test methods are preferred laboratory toxicity tests because this method prevents the degassing and artifactual increase in pH. Yet based on the evaluation of ammonia changes in unpreserved CBNG effluent transported from the discharge point to the laboratory, I also conclude laboratory toxicity tests may be biased.

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# CHAPTER 1

## Introduction

## COALBED NATURAL GAS AND PRODUCED WATERS

In the late 1990s, coalbed natural gas (CBNG) production increased dramatically in Wyoming, particularly in the Powder River Basin (PRB) in northeast Wyoming. Coalbed natural gas, predominantly methane ($CH_4$), is a by-product of reduction-oxidation processes that occur during coalification. Coalification is the conversion of plants to coal, which began in the PRB during the late Paleocene (Nichols 1999) when ancient bogs and marshes were repeatedly buried. Through time and as temperature of the buried strata increased, different ranks of coal formed, starting with lignite, and followed by sub-bituminous and bituminous coal (De Bruin et al. 2004). During these different stages of coalification, various by-products including $CH_4$, carbon dioxide ($CO_2$), nitrogen-containing compounds (e.g., ammonia - $NH_3$) and water are released (De Bruin et al. 2004).

The composition of the by-product mixture varies, depending on the coalification processes (biogenic or thermogenic) and the water-coal interactions (Rightmire 1984, Rice et al. 2000). Biogenic methane is a by-product of bacteria reducing $CO_2$ to $CH_4$ during anaerobic respiration (Rice and Claypool 1981). Typically, biogenic $CH_4$ production begins in shallow coal beds with lignite and sub-bituminous coal ranks at 20 to 50 °C. Primarily $CO_2$ and $H_2O$ are produced along with the biogenic methane from these shallow coal ranks (Rightmire 1984). When the temperature exceeds that in which most bacteria can live (~50 °C), thermogenic processes begin (Rightmire 1984, De Bruin et al. 2004). Thus, deeper coals with higher rank and increased temperatures could conceivably produce more $CH_4$, $CO_2$, nitrogen-containing compounds, and $H_2O$. The predominant coal rank in the PRB is sub-bituminous, with primarily biogenic processes

occurring; however, thermogenic processes could also occur in the deeper coal seams (personal communication, Nicholas Jones, Wyoming Geological Survey, Laramie, Wyoming, USA; May 2, 2007 – telephone interview). I propose that the organic nitrogen by-products of either coalification process could be a possible source of the ammonia that is present in some CBNG produced waters.

In the PRB, $CH_4$ gas and produced water are pumped out of the coal bed via separate collection systems. The produced water must be removed from the coal bed to release the gas, which is piped to a compressor unit and then shipped via pipeline to various end-users (power generation, home heating, etc.). The produced water is discharged into an impoundment or directly into an ephemeral or perennial drainage. Typically, produced water from multiple wells is piped into an ephemeral drainage via a central discharge point or outfall, resulting in year-round effluent-dominated flow. I suggest that organic matter in these collection systems could decompose to form ammonia via ammonification, although this has not been quantitated. Therefore, ammonia concentrations in produced water could conceivably increase from the decomposition of organic matter in the coal bed, ammonification occurring in the collection systems, or a combination of both processes.

Along with the measurable concentrations of $CO_2$ and total ammonia, concentrations of total dissolved solids (TDS) are higher in CBNG produced waters in the PRB than in many surface waters (Meyer et al. 2007: pp. 10-16). Rice et al. (2000) reported that TDS concentrations at CBNG wellheads ranged from 370 to 1940 mg/L, generally a result of increased sodium ($Na^+$) and bicarbonate ($HCO_3^-$). For comparison, the national drinking water standards recommendation for potable water is 500 mg/L and

seawater is 30,000 mg/L (Rice et al. 2000). Water-mineral interactions or changes in the ash content of the coal could be two reasons for the higher TDS concentrations, which increase from south to north and from east to west in the PRB coal seams (Rice et al. 2000).

Therefore, CBNG produced waters from deeper coal seams in the PRB have measurable concentrations of $CO_2$, $Na^+$, $HCO_3^-$, and total ammonia nitrogen (TA-N). Reported ranges of these constituents across the entire PRB are: $Na^+$ = 110 to 994 mg/L, and $HCO_3^-$ = 290 to 2,440 mg/L as $CaCO_3$ (Clearwater et al. 2002); TA-N = 0.9 to 4.4 mg N/L at wellhead (Rice et al. 2000); TA-N = 0.09 - 6.60 mg N/L at outfalls (unpublished data made available by permission of Anadarko Petroleum Corporation, Lance Oil and Gas, and Williams Production RMT Company; Appendix A). Aquatic ecosystems with elevated concentrations of TA-N, $Na^+$ and $HCO_3^-$ are potentially toxic to fish and other aquatic life. However, fish have been observed swimming in effluent-dominated tributaries of the Powder River, such as Beaver Creek, many km upstream from their confluences with the Powder River and immediately downstream of the effluent discharges (personal communication, Todd Adams, CBM Associates, Inc., Laramie, Wyoming, USA; February 2006).

## AMMONIA

Nitrogen (N) is an important element that is biologically incorporated into various N-containing compounds. Ammonification is the formation of ammonium ($NH_4^+$) during decomposition of N-containing organic compounds, nitrification is the conversion of $NH_4^+$ to nitrite ($NO_2^-$) and nitrate ($NO_3^-$), and denitrification is the respiratory reduction

of $NO_3^-$ to $N_2$ or $N_2O$ gas (Meyer and Gächter 2005). Although $NO_2^-$ is a relatively toxic species (Russo 1985), it can be converted to $NO_3^-$ under normal aerobic conditions by ubiquitous *Nitrobacter* bacteria (Meyer and Gächter 2005). Bacteria and algae assimilate $NH_4^+$ and $NO_3^-$ as nitrogen sources, therefore increasing primary production in an ecosystem.

Although nitrogen is essential to primary production of ecosystems, ammonia is one of the most important pollutants in the aquatic environment because it is widespread and relatively toxic to aquatic organisms (Russo 1985). Acute exposures of fish to high concentrations of ammonia damage the central nervous system, indicated by an increase in gill ventilation, hyperexcitability, convulsions, and then death; whereas chronic exposures to lower ammonia concentrations decrease reproduction, decrease growth and morphological development, and increase disease susceptibility (Russo 1985).

In aqueous solutions, ammonia assumes two chemical forms, the positively charged (ionized) ammonium ($NH_4^+$) and the neutral un-ionized ammonia ($NH_3$ or UIA). These two forms of ammonia interconvert through the processes of deprotonation (left to right in Eqn 1) and protonation (right to left in Eqn. 1).

$$NH_4^+ \leftrightarrow NH_3 + H^+ \qquad \text{(Eqn. 1)}$$

Total ammonia nitrogen is the summation of the two forms (TA-N = $NH_3 + NH_4^+$). Un-ionized ammonia is more toxic than $NH_4^+$ because $NH_3$ more readily diffuses across epithelial membranes and into aquatic organisms than does $NH_4^+$ (Russo 1985).

The percentages of $NH_4^+$ and $NH_3$ in a freshwater system are a function of pH, temperature, and ionic strength (USEPA 1999). As pH increases (i.e., as $H^+$ concentration decreases), the equilibrium is shifted toward $NH_3$, increasing toxicity (Eqn. 1). The $pK_a$ of the reaction in Equation 1 (i.e., the pH at which 50% of the TA-N is $NH_3$ and 50% is $NH_4^+$) is ~9.3 at 25 °C (Emerson et al. 1975). Additionally, because the $pK_a$ of the reaction decreases as temperature increases, the percentage of $NH_3$ increases as temperature increases. However, because increasing the ionic strength of water shifts the equilibrium towards the ammonium species ($NH_4^+$), ammonia toxicity decreases as ionic strength of fresh water increases (Russo 1985). Although $NH_4^+$ is not as toxic as $NH_3$, it can still contribute to toxicity if present in high concentrations (USEPA 1999).

The pH of a CBNG effluent plays a vital role in potential ammonia toxicity. Rice et al. (2000) reported the pH of CBNG produced water analyzed from wellheads in the PRB ranged from 6.8 to 7.7. The pH range of the CBNG effluent directly discharged into my study reach (Site 1) after traveling through a collection system was 7.3 to 8.2 (Chapter 2). Although these CBNG produced waters contain high concentrations of $HCO_3^-$ and thus might be expected to have higher pH, the $CO_2$-super-saturated water maintains near circumneutral pH. Figure 1-1 shows significant geochemical equilibria that should be occurring in CBNG produced waters. Primarily, as $CO_2$ degasses from the effluent, the $H^+$ concentration decreases; thus, the pH of the water increases.

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$$

$$HCO_3^- \rightarrow CO_3^{2-} + 2H^+$$

$$CaSO_4 \leftrightarrow Ca^{2+} + SO_4^-$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3$$

Figure 1-1. Dominant carbonate-related equilibria that should occur in CBNG effluents.

In the central to northwestern portion of the PRB, produced waters tend be dominated by $Na^+$ and $HCO_3^-$. If these produced waters contact soils containing gypsum ($CaSO_4$) in stream beds, calcium ($Ca^{2+}$) and sulfate ($SO_4^{2-}$) should be mobilized. In turn, in addition to $Ca^{2+}$ already in the produced water, the increased $Ca^{2+}$ concentrations should cause calcium carbonate ($CaCO_3$) to precipitate (evidenced by a thin white coating on the stream beds), with a concomitant buffering of the pH at ~8.0 to ~8.5. Therefore, the pH of the produced water should increase as it flows downstream, but at a slower rate than if the produced water did not dissolve gypsum -- because of the $CaCO_3$ buffering. If the pH remains circumneutral, the total ammonia nitrogen predictably would be dominated by the less toxic form ($NH_4^+$) at or near the outfall.

The temperature of CBNG produced water discharged from wellheads averages ~20 °C (Rice et al. 2000), and the average temperature of the effluent discharged into Beaver Creek during this study at Site 1 was 18 °C. The average dissolved oxygen measured at the same location was 5.6 mg/L. Because (1) the chronic toxic effects of ammonia increase as temperature increases (USEPA 1999) and (2) dissolved oxygen concentration decreases as water temperature increases, the combination of warmer water temperatures and lowered dissolved oxygen concentrations of the effluent as it enters the creek might increase stress to aquatic organisms.

The effects of ionic composition on ammonia toxicity are poorly understood (USEPA 1999). Some studies indicate that $Ca^{+}$ might decrease gill permeability by binding to gill sites and therefore decrease the diffusion of $NH_3$, decreasing toxicity (Tomasso et al. 1980). Soderberg (1991) suggested that increased salinity might have mitigating effects in waters by increasing ionization of ammonia. In contrast, high concentrations of major ions ($Ca^{2+}$, $Mg^{2+}$, $Na^{+}$, $K^{+}$, $HCO_3^{-}$, $Cl^{-}$, and $SO_4^{-}$) in freshwater systems can adversely impact aquatic life (Mount et al. 1997). For example, the 96-h LC50s (median lethal concentrations) for fathead minnows (FHM; *Pimephales promelas*) exposed to $NaHCO_3$ were < 310 to 1220 mg/L (i.e., < 85 to 334 mg $Na^{+}$/L and < 225 to 886 mg $HCO_3^{-}$ /L; Mount et al. 1997).

The U.S. Environmental Protection Agency (USEPA) water quality criteria document for ammonia recognizes a joint toxicity of both $NH_4^{+}$ and $NH_3$; therefore, the criteria are based on TA-N concentrations rather than UIA concentrations (USEPA 1999). The acute criterion (Criterion Maximum Concentration - CMC) is the highest average TA-N concentration to which at least 95% of aquatic species can be exposed for 1 h and survive. The chronic criterion (Criterion Continuous Concentration – CCC) is the highest average concentration to which at least 95% of aquatic species can be exposed for 4 d with no adverse effects to growth, reproduction and survival. The CMC (expressed in terms of TA-N concentration) decreases as pH increases; and the CCC (expressed in terms of TA-N concentration) decreases as temperature and pH increase. For example, at conditions that are relevant in a PRB ephemeral drainage, the USEPA CMC is 8.40 mg TA-N/L at pH 8.0 for non-salmonid freshwater species and the CCC is 1.71 mg TA-N/L at pH 8.0 and 20 °C (USEPA 1999).

Because the ammonia criteria are a function of pH and temperature, calculation of a weighted average can be complicated (USEPA 1999). For some purposes, the calculation of an average pH and temperature can be avoided. For example, if samples are obtained from a receiving water over a period of time during which pH and/or temperature is not constant, the pH, temperature, and the concentration of total ammonia in each sample should be determined. For each sample, the criterion should be determined at the pH and temperature of the sample, and then the concentration of total ammonia nitrogen in the sample should be divided by the criterion to determine a quotient. The criterion is attained if the mean of the quotients is less than 1 over the duration of the averaging period (USEPA 1999).

## TOXICITY OF CBNG PRODUCED WATERS AND MAJOR CONSTITUENTS

Before this study, limited data were available about the toxicity of CBNG produced waters to aquatic organisms. Three other studies within the past few years have evaluated the toxicity of CBNG-related waters collected from streams in the PRB. In 2001, Forbes (2003) tested several CBNG well-head produced waters and nearby stream waters from the PRB and the Tongue River Basin of Wyoming to determine if they were acutely or chronically toxic to two aquatic invertebrates (*Ceriodaphnia dubia* and *Daphnia magna*) and to a warm water fish (the fathead minnow, *Pimephales promelas*). She also compared the observed toxicity to the toxicity predicted by a regression model of major inorganic ions (Mount et al. 1997) to determine if the model would be a useful regulatory screening tool. Forbes (2003) concluded that 2 out of 30 waters were acutely toxic to FHM, of which both waters were collected from streams that had unknown

CBNG effluent contribution. She also concluded the model over-predicted the toxicity of the various waters in most cases.

In 2003, the Wyoming Department of Environmental Quality/Water Quality Division (WDEQ/WQD) implemented a study to examine the potential toxicity of CBNG produced water, particularly in light of the multiple discharges to single drainages or tributaries that could result in cumulative impacts (WDEQ 2003). WDEQ collected water samples for acute and chronic whole effluent toxicity (WET) tests from 10 sites in the PRB representing various coal seams and drainages. Based on this study, WDEQ concluded that any toxicity issues related to CBNG produced water are limited to areas of the Big George coal seam (WDEQ 2004). Acute and chronic WET tests were incorporated into WYPDES permits that discharge from the Big George coal seam beginning in 2004, using *Daphnia magna* and FHM as the appropriate species for CBNG produced waters.

Since 2003, Dr. Aïda Farag with United States Geological Survey (USGS) has been evaluating the potential toxicity of $NaHCO_3$ in CBNG effluents by conducting laboratory toxicity tests on simulated CBNG water from the Tongue River and the Powder River watersheds (USGS 2006). Farag et al. (2007) also conducted a field study using in-situ caged fish similar to the approach used in this study. Although the research is on-going, she concluded that 96-h LC50s (median lethal concentrations) for early life stage fish exposed to $NaHCO_3$ in simulated Tongue River and Powder River waters were 1100 to 1600 mg $NaHCO_3$/L (USGS 2006). Farag et al. (2007) reported significant mortality of some in-situ caged FHM in the summer of 2006, particularly with FHM larvae <24 h old.

## WYOMING REQUIREMENTS

Along with the USEPA, the Wyoming Department of Environmental Quality/Water Quality Division (WDEQ/WQD) establishes ambient water quality criteria to protect aquatic organisms and human health (WDEQ 2001). Wyoming has developed specific water quality standards related to CBNG produced water discharges under the Wyoming Pollution Discharge Elimination System (WYPDES) program. Discharge limitations vary, depending on whether the water is discharged to an impoundment (reservoir) or directly into an ephemeral or perennial drainage. Since the initiation of CBNG developments in the PRB, an increasing number of ephemeral drainages are becoming effluent-dominated. In general, effluent-dominated streams have unique water quality characteristics that, in most cases, differ from normal freshwater stream conditions at regional reference sites (Brooks et al. 2006). These varying water quality characteristics paired with the natural geology of the drainage can create adverse or mitigating effects to the resident aquatic life. Also, continuous flow augmentation of intermittent streams by effluent discharges can modify available habitat, temperature, dissolved oxygen concentrations, nutrient and chemical-constituent loading, and in-stream toxicity (Brooks et al. 2006).

Since 2004, the WYPDES program monitors toxicants of concern, like ammonia and bicarbonate, in CBNG effluents with acute and chronic static-renewal whole effluent toxicity (WET) tests (USEPA 2002a, 2002b). These tests evaluate the total toxic effect of an effluent using aquatic organisms in a laboratory setting. The endpoint for an acute WET test is survival, whereas a chronic WET test evaluates the aquatic organism's

survival, growth and reproduction. As of 2007, WDEQ/WQD has established ammonia limits in effluents that are discharged within 1.6 km of the Powder River and that are permitted within a watershed general permit. The ammonia limits are watershed specific and are based on numeric chronic criteria for ammonia (CCC) for fish with early life stages present from data found in Chapter 1, Appendix C of the Wyoming Water Quality Rules and Regulations (WDEQ 2001). The ammonia criteria in Chapter 1 are the same as the USEPA (1999) criteria. The WDEQ/WQD uses average pH and temperature obtained from the nearest Powder River USGS gaging station to calculate the CCC. This CCC is used in a waste load allocation calculation to derive the general permit's seasonal ammonia limit. For example, the Willow Creek watershed's TA-N limits for July, August and September are 3.14, 2.20, and 3.49 mg N/L, respectively (personal communication, Jason Thomas, CBNG permit writer, WDEQ/WQD, Cheyenne, Wyoming, USA; July 10, 2007 – email correspondence).

## WHOLE EFFLUENT TOXICITY TESTS

Standard, static-renewal WET tests expose a specified number of test organisms in varying concentrations of effluent for a specified time. These organisms are placed in beakers of effluent that are then placed in a 20 or 25 °C water bath and are exposed to the ambient atmosphere. In effluents, as in pure water, the concentration of $H^+$ (and therefore pH) is partly controlled at equilibrium by the partial pressure of $CO_2$ ($pCO_2$) in the atmosphere above the solution. The higher the $pCO_2$, the more protons are produced and the lower the pH (Mount and Mount 1992). In this study, tests in which the beakers

were open to ambient atmosphere in the laboratory were termed ambient-pH WET tests because the pH depended in part on the atmospheric or ambient conditions in the room.

If $CO_2$ degasses from open containers, the pH drifts upward (Fig. 1-1). Although the average pH of a variety of CBNG effluents in the PRB at the point of discharge (top-of-pipe) was ~7.8 (CBMA 2007), the pH can drift upward to ~8.5 and higher in ambient-pH laboratory tests if $CO_2$ is allowed to degas (CBMA 2007). Drift in pH can be very important. For example, increasing the pH from 7.8 to 9.0 increases the percentage of un-ionized ammonia by approximately an order of magnitude. Therefore, the use of ambient-pH toxicity test methods could cause larger increases of pH values in the beaker than would occur in-stream; consequently, ammonia toxicity of CBNG effluent in a laboratory might not be completely representative of in-stream conditions.

The USEPA chronic WET guidelines allow the regulatory authority to approve $CO_2$ pH-controlled toxicity methods to maintain the pH of WET test waters to more closely approximate in-stream exposure conditions (USEPA 2002a). A $CO_2$ pH-controlled WET test is similar to the ambient-pH method but is conducted within a sealed glass chamber blanketed with an air mixture containing at least 2 to 3% $CO_2$, added as needed to maintain pH. Beginning in 2006, WDEQ/WQD allowed a $CO_2$ pH-controlled method for WYPDES required WET tests conducted on CBNG effluents collected in the PRB (personal communication, Jason Thomas, CBNG permit writer, WDEQ/WQD, Cheyenne, Wyoming, USA; August 30, 2007 – telephone communication).

Therefore in this study, I compared results of ambient-pH and $CO_2$ pH-controlled WET tests with CBNG effluent and stream waters, to determine if toxicity was biased upward in the ambient-pH tests

## AMMONIFICATION

Assuming the in-stream pH can be maintained in the laboratory using a $CO_2$ pH-controlled method, laboratory ammonia and in-stream ammonia concentrations might still differ due to ammonification that might occur in the effluent during transport and storage. The in-transit transformation could easily occur because, unlike water samples for ammonia analyses that should be acidified and stored on ice to prevent ammonification (ELI 2001), water samples that are to be used for WET tests should not be preserved by addition of acid or other chemicals.

The possible ammonification can be evaluated by calculating a nitrogen budget and determining how much organic nitrogen might be in a water sample. A nitrogen budget represents the nitrogen species that constitute the total nitrogen (TN) concentration, which equals the sum of $NH_4^+$, $NH_3$, $NO_3^-$, $NO_2^-$, and all other inorganic and organic nitrogen species. In this study, the TN concentrations are not completely accounted for by summation of TA-N, $NO_3^-$, and $NO_2^-$ concentrations, indicating one or more organic nitrogen species might be in the effluent (Chapter 3). The organic nitrogen could conceivably transform to ammonia via ammonification during transit from the field to the laboratory, thus increasing the ammonia concentration in the effluent used in laboratory WET tests above the in-stream ammonia concentration. This could create another bias, similar to the pH-drift scenario, inducing artifactual ammonia toxicity in laboratory WET tests.

Therefore, in this study I examined potential ammonification during transit of stream waters from the field to various analytical laboratories with various preservation treatments.

## IN-STREAM EXPOSURE ASSESSMENTS

The objective of laboratory toxicity tests is to determine whether chemicals are harmful to organisms. However, dynamic physical, chemical and biological processes that are interrelated in the aquatic environment are difficult, if not impossible, to replicate in the laboratory (Chappie and Burton 2000). Therefore, in-situ or in-stream assessments provide additional lines of evidence to traditional laboratory toxicity tests and chemical analyses. In-situ exposures provide unique information that is complementary to traditional lab-based toxicity results and are also more realistic than laboratory testing, thus decreasing the gap in laboratory-to-field extrapolations. Decreasing that gap should decrease uncertainties and allow more direct interpretations of toxicity tests (Burton 2005).

Therefore, in this study I conducted in-stream exposures of caged FHM, to help interpret potential toxicity of the CBNG effluent and to test for biases in the results of the laboratory toxicity tests.

## HYPOTHESES

Toxicity studies combining field observations and in-situ studies, lab toxicity tests and chemical analyses provide more comprehensive answers to questions of potential toxicity in aquatic systems. To evaluate fully the potential acute toxicity of CBNG effluent, I combined all four lines of evidence in this thesis. I hypothesized that in-stream biogeochemical changes in CBNG produced water alter its toxicity and thus allow aquatic organisms to inhabit ephemeral drainages at predictable distances

downstream from discharge points for CBNG produced water. Second, I hypothesized that ambient-pH laboratory toxicity test methods artifactually increase ammonia toxicity; thus, the use of a $CO_2$ pH-controlled method will more closely approximate results that could be obtained in the toxicity studies conducted in the field. Finally, I hypothesized that ammonification occurs during transport and storage of unpreserved effluent samples before laboratory toxicity tests are begun.

## SCOPE OF THE STUDY

I tested these hypotheses by conducting a four-tiered study in Beaver Creek. I conducted seasonal studies from July 30 to August 3, 2006; October 15 to 19, 2006; and from January 23 to 27, 2007. In Chapter 2, which is prepared as a manuscript to be submitted to *Environmental Toxicology and Chemistry*, I present results of (1) in-stream toxicity tests using caged FHM placed along a longitudinal gradient downstream from a CBNG discharge point, (2) concurrent laboratory toxicity tests in ambient-pH and $CO_2$ pH-controlled conditions with the same batch of FHM exposed to water collected at the same in-stream locations as the caged tests, (3) geochemical analyses of the effluent-dominated water, and (4) complementary studies that sampled fish, amphibian, and reptile populations along Beaver Creek during summer 2006. In this thesis study, I only evaluated acute toxicity effects (survival) in the CBNG effluent-dominated stream, Beaver Creek. Additionally, although the potential toxicant emphasized in this study is ammonia, I will also compare my data to published toxicity effects of $Na^+$ and $HCO_3^-$.

In Chapter 3, which is prepared as a manuscript to be submitted to *Environmental Toxicology and Chemistry*, I present results of two studies that examine if

ammonification is occurring while water samples are transported from the in-stream environment to a laboratory setting. Understanding these processes is valuable in the interpretation of potential ammonia toxicity and consequent regulation of discharges of CBNG produced water. Because both Chapter 2 and 3 are written to be individually submitted for publication, some of the information is repetitive.

In Chapter 4, I integrate the in-stream toxicity studies, laboratory toxicity studies, biogeochemical data, aquatic life observations, and ammonification studies to draw conclusions about the potential for ammonia toxicity and about current regulatory processes in the Beaver Creek. Oil and gas industries, environmental groups, and state agencies could use these results and conclusions to more accurately assess the risks of CBNG produced waters, the fate and effects of effluents in ephemeral drainages, and the appropriateness of current WET procedures.

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# CHAPTER 2

## Is Coalbed Natural Gas Produced Water Toxic to Fathead Minnows?

**ABSTRACT**

Coalbed natural gas produced waters extracted from coal seams in the Powder River Basin of Wyoming contain measurable concentrations of potential toxicants like ammonia, $CO_2$, $Na^+$, and $HCO_3^-$. I evaluated the fate and effects of ammonia discharged into ephemeral Beaver Creek, a Powder River tributary, from August 2006 through January 2007. The study included (1) in-stream monitoring of fish and amphibians, (2) in-stream toxicity tests at 6 sites using caged fathead minnow (FHM; *Pimephales promelas*) larvae, (3) concurrent ambient-pH and $CO_2$ pH-controlled laboratory toxicity tests using the same site waters, and (4) analyses of water quality parameters in the effluent and the receiving water. Although 96-h survival in-stream and in the laboratory did not decrease significantly compared to controls, 144-h survival decreased in some ambient-pH exposures in the laboratory as pH increased due to $CO_2$ degassing. In Beaver Creek, pH increased longitudinally downstream while ammonia concentrations decreased. Geochemical processes occurring between the effluent and the stream bed (i.e., dissolution of $CaSO_4$ and precipitation of $CaCO_3$) mitigated potential ammonia toxicity. I conclude that toxicity testing requirements should be evaluated considering the pH drift in laboratory toxicity tests and that in-stream conditions should be considered when applying regulatory criteria.

Key words: CBNG, ammonia, $CO_2$, fathead minnow, toxicity.

# INTRODUCTION

In the late 1990s, coalbed natural gas (CBNG) production increased dramatically in Wyoming, particularly in the Powder River Basin (PRB) in northeast Wyoming. Thermogenic and biogenic coalification processes generate by-products including methane ($CH_4$), carbon dioxide ($CO_2$), nitrogen-containing compounds (e.g., ammonia- - $NH_3$), and water (De Bruin et al. 2004). Along with the relatively high concentrations of $CO_2$ are elevated concentrations of total dissolved solids (TDS), dominated by sodium ($Na^+$) and bicarbonate ($HCO_3^-$) ions, compared to many surface waters (Meyer et al. 2007: pp. 10-16). For example, total dissolved solids collected from wellheads in the PRB range from 370 to 1940 mg/L; in comparison to the national drinking water standards recommendation for potable water being 500 mg/L (Rice et al. 2000). Additionally, total ammonia is measurable and could contribute to toxicity to aquatic organisms, depending on the pH and temperature of the receiving water. Reported ranges of concentrations of $Na^+$, $HCO_3^-$, and total ammonia nitrogen (TA-N) in the PRB produced waters are: $Na^+$ = 110 to 994 mg/L and $HCO_3^-$ = 290 to 2440 mg/L as $CaCO_3$ (Clearwater et al. 2002); TA-N = 0.9 to 4.4 mg N/L at the wellhead (Rice et al. 2000); TA-N = 0.09 to 6.60 mg N/L at the discharge outfalls (unpublished data made available by permission of Anadarko Petroleum Corporation, Lance Oil and Gas, and Williams Production RMT Company; Appendix A). Because groundwaters in deeper coal seams (e.g., the Big George coal seam) contain the higher concentrations of these by-products and because elevated concentrations of $Na^+$, $HCO_3^-$, and TA-N can be toxic to fish and other aquatic life, disposal of CBNG produced water is a concern (Clearwater et al. 2002).

In the PRB, $CH_4$ gas and produced water are pumped out of the coal bed via separate collection systems. The produced water must be removed from the coal bed to release the gas, which is piped to a compressor unit and then shipped via pipeline to various end-users (power generation, home heating, etc). The produced water is discharged either into an impoundment or directly into an ephemeral or perennial drainage. Often, produced water from multiple wells is piped into an ephemeral drainage via a central discharge point or outfall, resulting in year-round, effluent-dominated flow. Although water quality of some CBNG effluents might be predicted to adversely affect aquatic life in these drainages (Clearwater et al. 2002), fish have been observed swimming in CBNG effluent-dominated streams, such as Beaver Creek, many km upstream from their confluences with the Powder River (personal communication, Todd Adams, CBM Associates, Inc., Laramie, Wyoming, USA; February 2006).

The potential toxicant emphasized in this study is total ammonia, although the potential toxic effects of $Na^+$ and $HCO_3^-$ will also be discussed. Ammonia is one of the most important pollutants in the aquatic environment because it is widely distributed and relatively toxic to aquatic organisms (Russo 1985). In aqueous solutions, ammonia assumes two chemical forms, the positively charged (ionized) ammonium ($NH_4^+$) ion and the neutral (un-ionized) ammonia molecule ($NH_3$, also referred to herein as UIA). Total ammonia nitrogen is the summation of these two forms. The percentages of $NH_4^+$ and $NH_3$ in a freshwater system are a function of pH, temperature, and ionic strength (Russo 1985).

The pH of the CBNG produced water plays an important role in the potential ammonia toxicity. Rice et al. (2000) reported the pH of product water analyzed from

wellheads in the PRB ranged from 6.8 to 7.7. The pH range of the CBNG effluent measured at the flume (Site 1) that directly discharged into my study reach after traveling through two retention ponds was 7.3 to 8.2 (see below). Although these CBNG produced waters contain high concentrations of $HCO_3^-$ and thus usually would have higher pH, the $CO_2$-super-saturated water from deep wells maintains near circumneutral pH. As $CO_2$ degasses from the super-saturated waters when they contact the atmosphere, their $H^+$ concentration decreases; thus, the pH of CBNG effluents in general increases as the effluents flow downstream and $CO_2$ degasses.

Produced waters tend be dominated by various ions like $Na^+$, $Ca^{2+}$, $Mg^{2+}$ and $HCO_3^-$. When those waters contact stream beds containing gypsum soils ($CaSO_4$), $Ca^{2+}$ and $SO_4^-$ can be mobilized. In turn, in addition to $Ca^{2+}$ already in the produced water, the increased $Ca^{2+}$ concentrations should cause calcium carbonate ($CaCO_3$) to precipitate (e.g., evidenced by a thin white coating on the stream beds in Beaver Creek), with a concomitant buffering of the pH at ~8.0 to ~8.5. Therefore, the pH of the produced water should increase as it flows downstream, but at a slower rate than if the produced water did not dissolve gypsum -- because of the $CaCO_3$ buffering. If the pH remains circumneutral at or near the outfall, the TA-N predictably would be dominated by the less toxic form ($NH_4^+$).

The U.S. Environmental Protection Agency (USEPA) water quality criteria document for ammonia recognizes a joint toxicity of both $NH_4^+$ and $NH_3$ (USEPA 1999). For example, at conditions that are relevant in a PRB ephemeral drainage, the USEPA Criterion Maximum Concentration (CMC) is 8.40 mg TA-N/L at pH 8.0 for non-

salmonid freshwater species and the Criterion Continuous Concentration (CCC) is 1.71 mg TA-N/L at pH 8.0 and 20 °C (USEPA 1999).

In 2007, the Wyoming Department of Environmental Quality/Water Quality Division (WDEQ/WQD) established seasonal ammonia limits in effluents that are discharged within 1.6 km of the Powder River and are permitted within a watershed general permit. The limits vary depending on the pH, temperature and flow from the nearest Powder River USGS gaging station. For example, the TA-N limits for produced water discharging within 1.6 km of the Powder River and within the Willow Creek watershed are 3.14, 2.20, and 3.49 mg TA-N/L for July, August and September, respectively. The ammonia limits are based on numeric chronic criteria for ammonia (criteria continuous concentrations -- CCCs) for fish with early life stages present [from data found in Chapter 1, Appendix C of the Wyoming Water Quality Rules and Regulations (WDEQ 2001, USEPA 1999)], using average pH and temperature obtained from the gaging station. This CCC, along with an average monthly flow from the gaging station, is used in a waste load allocation calculation to derive the watershed's seasonal ammonia limits (personal communication, Jason Thomas, CBNG permit writer, WDEQ/WQD, Cheyenne, Wyoming, USA, July 10, 2007 – email correspondence). As of Fall 2007, ammonia limits only apply to discharges that are permitted under a general watershed discharge permit, not individual discharge permits.

As of 2004, Wyoming Pollution Discharge Elimination System (WYPDES) permits monitor toxicants of concern like ammonia and bicarbonate in some CBNG effluents with acute and chronic static-renewal whole effluent toxicity (WET) tests (USEPA 2002a, 2002b). Standard static-renewal WET tests expose pre-determined

organisms in beakers of effluent, which are placed in a 20 or 25 °C water bath and are exposed to the ambient atmosphere. The pH of the effluent exposed to the ambient atmosphere is partly controlled at equilibrium by the partial pressure of $CO_2$ ($pCO_2$) in the atmosphere over the solution. The higher the $pCO_2$, the more protons are produced and the lower the pH (Mount and Mount 1992). For my study, the beakers of fish exposed to ambient atmosphere in the laboratory are termed ambient-pH because the pH depended in part on the ambient room-atmosphere conditions.

If $CO_2$ degasses from the open containers (ambient-pH beakers), the pH drifts upward. Drift in pH can be very important in relation to ammonia toxicity. For example, increasing the pH from 7.8 to 9.0 increases the percentage of unionized ammonia by approximately an order of magnitude. Therefore, use of ambient-pH toxicity test methods could artifactually increase the pH and the ammonia toxicity of CBNG effluent. The use of USEPA approved $CO_2$ pH-controlled WET test methods to maintain the pH of the effluent at stream conditions would more closely approximate in-stream exposure conditions (USEPA 2002a). $CO_2$ pH-controlled methods are similar to the ambient-pH methods but are conducted within sealed glass chambers blanketed with an air mixture containing at least 2 to 3% $CO_2$. In this study, I compared the ambient-pH and $CO_2$ pH-controlled toxicity methods.

Before this study, limited data were available about the toxicity of CBNG produced waters to aquatic organisms. Three other studies within the past few years evaluated streams influenced by CBNG product water in the Tongue and Powder River watersheds of Wyoming. In 2001, Forbes (2003) tested several CBNG wellhead produced waters and nearby stream waters to determine if they were acutely or

chronically toxic to two aquatic invertebrates (Ceriodaphnia dubia and Daphnia magna) and to FHM (Forbes 2003). Forbes (2003) concluded that 2 out of 30 waters were acutely toxic to FHM, of which both waters were collected from streams that had unknown CBNG effluent contribution. She also concluded the model over-predicted the toxicity of the various waters in most cases.

In 2003, WDEQ/WQD implemented a study to examine the potential toxicity of the produced water by collecting water samples for acute and chronic WET tests from 10 sites in the PRB representing various coal seams and drainages (WDEQ 2003). Based on this study, WDEQ concluded that any toxicity issues related to CBNG produced water are limited to areas of the Big George coal seam (WDEQ 2004). Acute and chronic WET tests were incorporated into WYPDES permits that discharge from the Big George coal seam beginning in 2004, using *Daphnia magna* and FHM as the appropriate species for CBNG produced waters.

Finally since 2003, the United States Geological Survey (USGS) has been evaluating the potential toxicity of $NaHCO_3$ in streams influenced by CBNG produced water. Those researchers have conducted laboratory toxicity tests with $NaHCO_3$ added to simulated Tongue River and Powder River waters and have conducted in-situ seasonal caged studies similar to the approach used in this study (USGS 2006, Farag et. al. 2007). Although the research is on-going, they concluded that 96-h LC50s (median lethal concentrations) for early life stage fish exposed to $NaHCO_3$ in simulated Tongue River and Powder River waters were 1100 to 1600 mg $NaHCO_3$/L (USGS 2006). Farag et al. (2007) reported significant mortality of some in-situ caged FHM in the summer of 2006, particularly with FHM larvae <24 h old.

The objective of laboratory toxicity tests is to determine whether environmental chemicals are harmful to organisms. Often it is difficult to replicate in a laboratory setting the dynamic physical, chemical and biological processes that are interrelated in the aquatic environment (Chappie and Burton 2000). In-situ or in-stream assessments provide additional lines of evidence to traditional laboratory toxicity tests and chemical analyses. Toxicity studies combining field observations and in-situ studies, lab toxicity tests and chemical analyses provide more comprehensive answers to questions of potential toxicity in aquatic systems. In this chapter, I combined all four lines of evidence to evaluate the potential acute toxicity of CBNG effluent. I also suggest additional research using both acute and chronic toxicity tests to more fully evaluate long-term survival, reproduction and growth effects in the CBNG effluent-dominated streams.

I hypothesized that in-stream biogeochemical changes in the CBNG produced water alter its toxicity and thus allow aquatic organisms to inhabit ephemeral drainages at predictable distances downstream from CBNG produced water discharge points. Second, I hypothesized that ambient-pH laboratory toxicity test methods artifactually increase ammonia toxicity.

I tested these hypotheses by conducting a four-tiered study in Beaver Creek, tributary to the Powder River, Wyoming. I conducted seasonal studies from July 30 to August 3, 2006; from October 15 to 19, 2006; and from January 23 to 27, 2007. The studies included (1) in-stream toxicity tests using caged FHM placed along a longitudinal gradient downstream from a CBNG discharge point, (2) concurrent laboratory toxicity tests in ambient-pH and $CO_2$ pH-controlled conditions with the same batch of FHM

exposed to water collected at the same in-stream locations as the caged tests, (3) geochemical analyses of the effluent-dominated water, and (4) complementary studies that sampled fish, amphibian, and reptile populations along Beaver Creek during summer 2006.

## MATERIALS AND METHODS

### Study Site

The study area was along Beaver Creek in Campbell County in northeast Wyoming (T48N R76W). Semi-arid northeast Wyoming has an average of 40 cm precipitation (http://www.wrcc ), with warm summers (average 29 °C) and cold winters (average 12 °C) (http://www.weather.com). The vegetation along Beaver Creek includes small shrubs, sage brush, and short prairie grasses. Land uses include livestock grazing, wildlife habitat, oil and gas development, and recreation. Historically, Beaver Creek was an ephemeral drainage, with seasonal intermittent flow before CBNG development (personal communication, John Iberlin (landowner) Iberlin Land and Livestock, Gillette, Wyoming; February 18, 2007). It flows ~65 km from the headwaters to the Powder River (Fig. 2-1). The CBNG effluent I studied discharged into Beaver Creek ~25 km upstream from the Powder River. The discharge point initiating the flow is operated by Williams Production RMT Company (WYPDES WY0046922-001). The study reach was ~14 stream km long, extending from S4 T47N R76W to S25 T48N R77W.

Approximately 150 to 180 CBNG wells piped produced water to the original discharge point that was 300 m from Beaver Creek. At this point (top-of-pipe), the water surfaced and flowed into two consecutive retention ponds (Fig. 2-2). The retention ponds

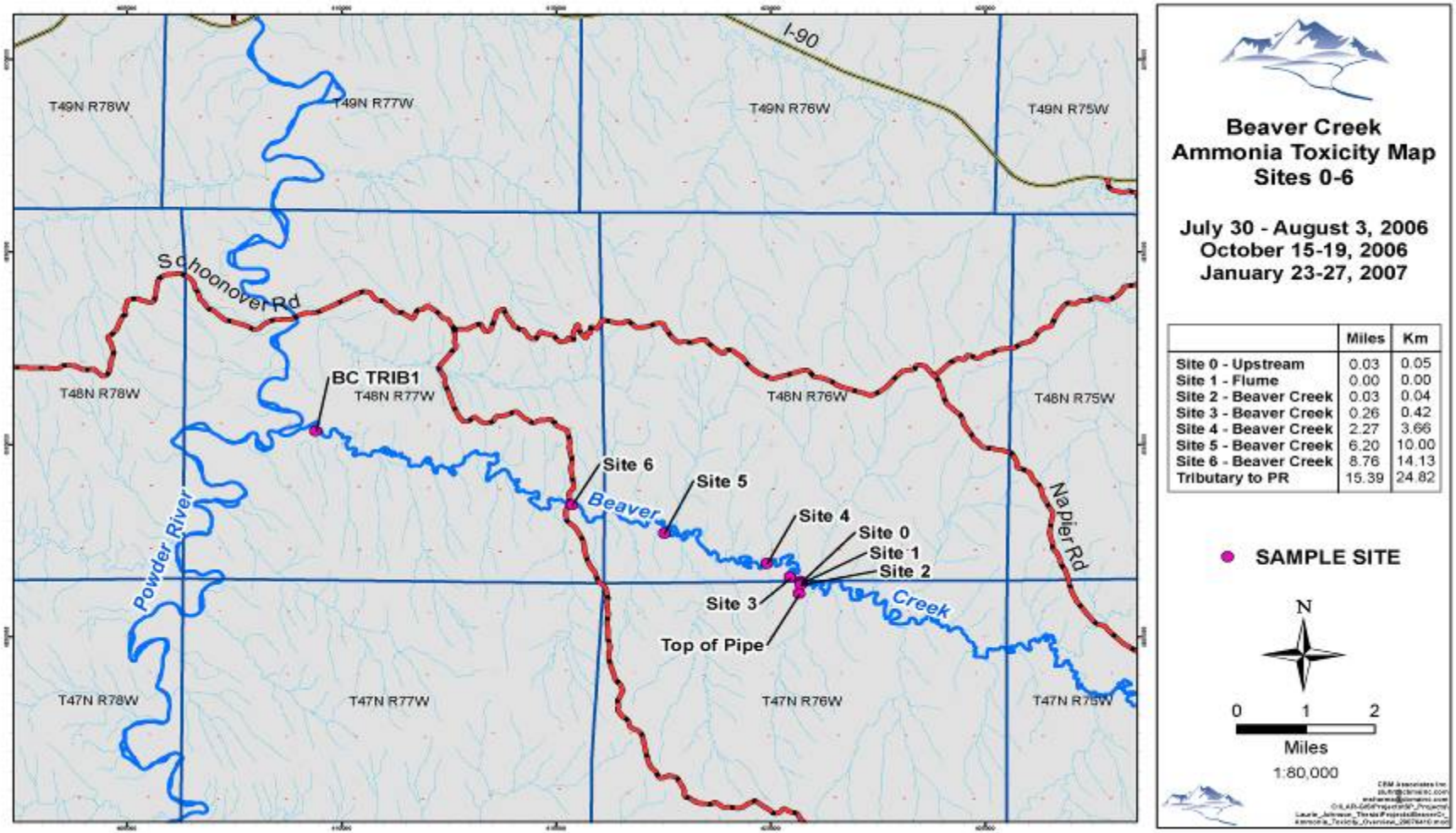



Figure 2-1. Beaver Creek is a tributary to the Powder River in northeast Wyoming. In-stream toxicity tests were conducted at Sites 1 to 6, which were 24.8, 24.8, 24.4, 21.1, 14.8, and 10.7 km, respectively, from the confluence with the Powder River.

Figure 2-2. CBNG produced water from multiple wells operated by Williams Production RMT Company discharges from “top-of-pipe” prior to flowing into two retention ponds and subsequently is piped underground ~300 m into Beaver Creek (photo taken May 2006).

Figure 2-3. CBNG produced water that traveled through an underground collection system from the retention ponds was discharged down the concrete flume into Beaver Creek (Site 1). The above CBM facility, WY0046922-001, is operated by Williams Production RMT Company (photo taken May 2006).

were ~15 x ~15 m wide and ~2.5 to ~3.0 m deep. The effluent entered the first pond and then flowed into the second pond before entering an underground collection system, from which the water was piped to the top of a concrete flume that discharged into Beaver Creek (Fig. 2-3).

In this study, the top of the concrete flume was Site 1, which was 24.8 km from the confluence with the Powder River. Sites 2 to 6 were 0.04, 0.4, 3.7, 10.0, and 14.1 km, respectively, downstream from the end of the concrete flume and 24.8, 24.8, 24.4, 21.1, 14.8, and 10.7 km, respectively, upstream from the confluence with the Powder River (Fig. 2-4). I sampled Site 0 directly upstream from the flume only during the July/August 2006 field bout, but no in-stream toxicity tests were conducted at this site. In-stream toxicity tests using caged FHM were conducted at Sites 1 to 6; water was collected for laboratory toxicity tests and chemical analyses at the same sites.

Flow into Beaver Creek was measured using a 9”- Montana flume manufactured by Tracom. Flow depth was measured and recorded using a pressure transducer linked to Williams Production RMT Company through its telemetry system. The stream dimensions (~30 cm deep x ~4 m wide) varied with the flow and seasonal vegetation (Davis 2007). The stream bed consisted of fine sediment at Site 1 near the outfall to fine gravel near Site 6. In July/August 2006, the vegetation changed from algae near the outfall to short wetland grasses near Site 3 to taller wetland grasses at Site 5. Beaver Creek opened into a meadow where cows grazed at Site 6 (Fig. 2-5).

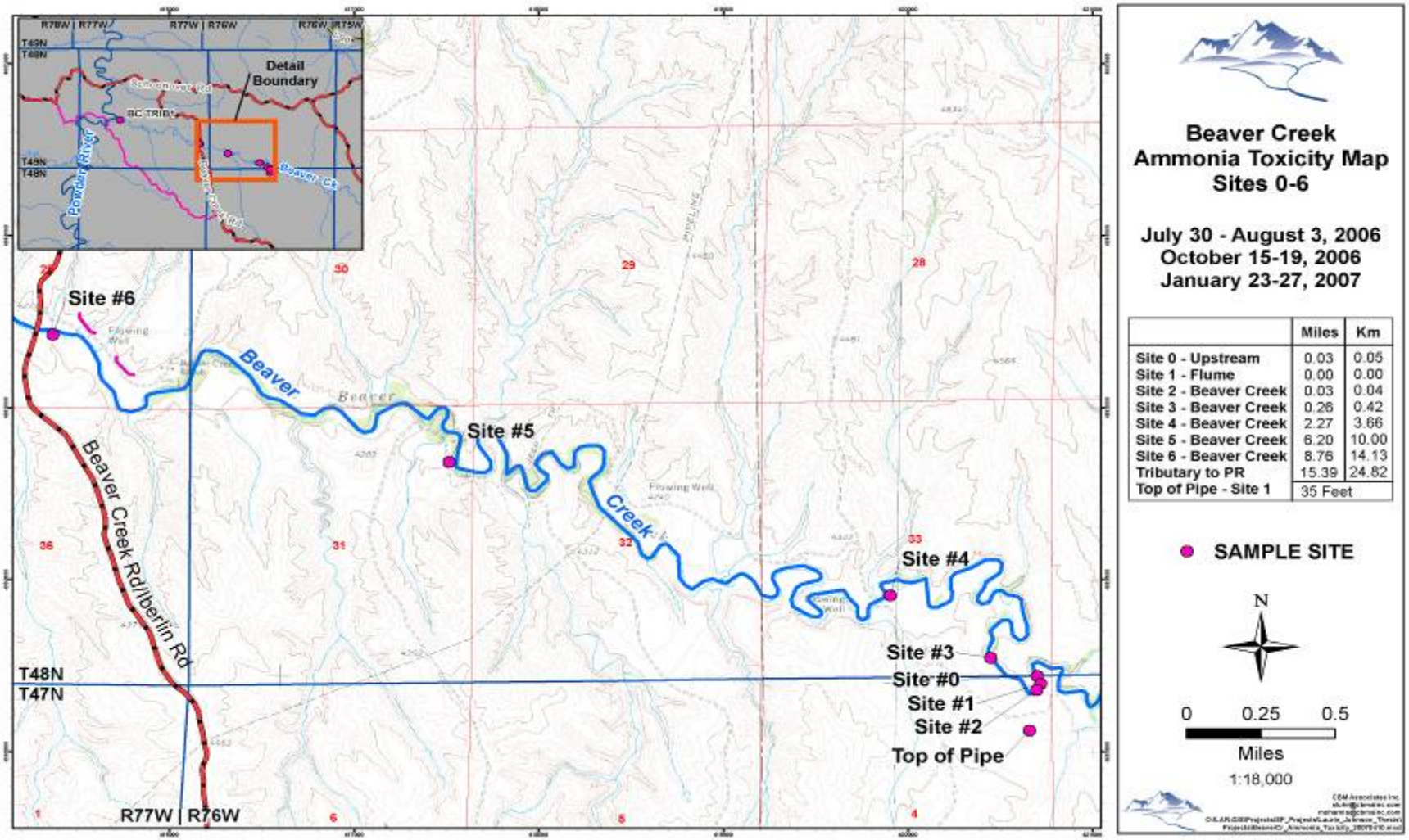
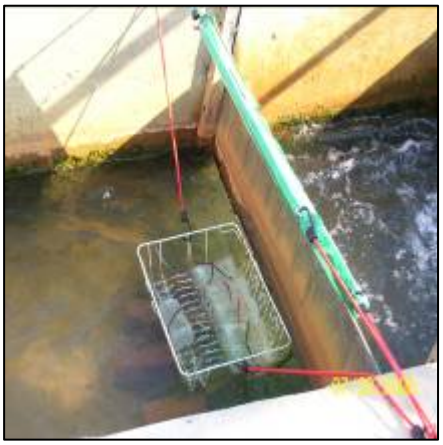



Figure 2-4. Six study sites used in seasonal studies located longitudinally downstream from Williams Production RMT Company outfall WY0046922-001 that discharges into Beaver Creek, Wyoming.

Figure 2-5. Photographs of sites 1 to 6 located longitudinally downstream from Williams Production RMT Company facility, WY0046922-001, along Beaver Creek, Wyoming (photos taken in July/August 2006 for Sites 1, 3 to 6 and in January 2007 for Site 2). During each field week at each accessible site, caged fathead minnow (*Pimephales promelas*) larvae were used to conduct in-stream toxicity tests. Also, water was collected for laboratory toxicity tests and selected chemical analyses. (a.) Site 1, in the concrete flume; (b.) Site 2, in Beaver Creek 40 m downstream from the end of the concrete flume; (c.) Site 3, in Beaver Creek 420 m downstream from Site 1; (d.) Site 4, in Beaver Creek 3.7 km downstream from Site 1; (e.) Site 5, in Beaver Creek 10.0 km downstream from Site 1; and (f.) Site 6, in Beaver Creek 14.1 km downstream from Site 1.

**a.**



**b.**

**c.**

**d.**

**e.**

**f.**

**Toxicity Tests**

In-stream toxicity tests were conducted according to Burton (2005b). Concurrent toxicity tests were conducted at the University of Wyoming's Red Buttes Environmental Biology Laboratory (RB) following standard 96-h static-renewal toxicity test methods (USEPA 2002b). In Table 2-1, the test conditions of the in-stream and laboratory toxicity tests are compared to the test conditions listed in the USEPA (2002b) 96-h WET test manual. Both the in-stream and the laboratory toxicity tests used the same batch of taxonomically verified FHM (batch #042105; personal communication Anita Rehner, ENSR Consulting Inc., Fort Collins, CO; June 6, 2006) hatched at ENSR Analytical Laboratory, Fort Collins, Colorado. The hatch dates were July 18, 2006, October 2, 2006, and a combination of January 9 and 12, 2007. The ages of the fish at initiation of the in-stream tests were 12 d, 13 d, and combination 11 and 14 d during the July/August, October, and January field bouts, respectively. The ages of the fish at laboratory test initiation were 14 d, 15 d, and a combination of 12 and 15 d during July/August, October, and January field bouts, respectively. For in-stream tests, Burton (2005b) recommended using 7- to 14-d-old FHM larvae because 1- to 7-d-old larvae had decreased survival using this in-situ test design. Because younger larvae tend to be more sensitive to toxins, the desired age of fish would have been closer to 7 d, but difficulties coordinating the hatch dates and the field weeks precluded using the younger fish. I selected FHM as the test organism because they have a sufficient toxicity database (Burton 2005b) and are found in ephemeral drainages of the PRB (Davis 2007).

Table 2-1. Comparison of 96-h whole effluent toxicity (WET) test conditions for fathead minnows (*Pimephales promelas)* recommended by USEPA (2002b) to test conditions used in laboratory and in-stream toxicity studies performed with CBNG produced water or water collected from Beaver Creek (a tributary to the Powder River, Wyoming) during the July/August 2006, October 2006 and January 2007 field studies.

| Test conditions | EPA (2002) WET test | Red Buttes WET tests | Beaver Creek in-stream tests |
|---|---|---|---|
| Test type | Static-renewal | Static-renewal | Flow-through |
| Test duration | 96 h | 96 h | 96 h |
| Temperature | 20 or 25 °C | 20 °C - July/August and October;<br>25 °C - January | Variable |
| Light quality | Ambient lab illumination | Ambient lab illumination | Seasonal field conditions |
| Photoperiod | 16 h daylight, 8 h dark | 16 h daylight, 8 h dark | Seasonal field conditions |
| Test chamber size | 250-ml beakers, minimum | 250-ml beakers | 12.7-cm x 6.7-cm tube |
| Test solution volume | 200 ml, minimum | 200 ml | 448 ml |
| Renewal of test solutions | After 48 h | After 48 h | Flow-through |
| Age of organism | 1- to14-d-old | July/August – 14-d-old<br>October – 15-d-old<br>January – 12- & 15-d-old | July/August – 12-d-old<br>October – 13-d-old<br>January – 11- & 14-d-old |
| No. organisms per test chamber | 10 | July/August - 10<br>October - 8<br>January - 10 | All weeks - 10 |
| No. of replicates | 4 minimum | 5 | 5 |
| No. organisms per concentration | 20 minimum | 40-50 | 50 |
| Feeding regime | *Artemia* nauplii during holding | *Artemia* nauplii during holding | None |

The larvae were transported from ENSR to RB in culture water. At RB, the fish were acclimated to the hardness of RB well water prior to field deployment by mixing 1 part ENSR culture water (hardness of 95 mg/L as $CaCO_3$) and 1 part RB well water (hardness of 215 mg/L as $CaCO_3$). The hardness of this 1:1 mixture was not analyzed but the calculated average is 155 mg/L as $CaCO_3$. Because the average of water collected from Site 1 during a pilot study was ~160 mg/L as $CaCO_3$, the acclimation for hardness was appropriate. Each batch of fish was split at RB, with one-third transported to the PRB in oxygenated, temperature-monitored coolers. The remaining two-thirds were held at RB to be used in the ambient-pH and $CO_2$ pH-controlled WET tests.

Temperature was measured at the outfall approximately one week prior to each deployment. During transport from Red Buttes to the creek, the fish were slowly acclimated to within 5 °C of the determined field temperature. At each site during each field bout, I acclimated the fish to the temperature of the site water by placing the bag of fish that were in the culture water into a tub of site water until the water temperature of the culture water was within 2 °C of the site water. After temperature acclimation, I pipetted 10 FHM into an in-situ cage that was set on end in a tub of site water. The in-situ cages were constructed of cellulose acetate/butyrate transparent core tubing (12.7 cm long, 6.7 cm diameter, 448 ml total volume; Fig. 2-6). Removable polyethylene closures capped each end; two rectangular windows (4 cm x 8 cm) cut on opposite sides were covered with 250-µm nylon mesh, allowing water to flow through. The labeled cages were submerged below the water surface with the mesh windows perpendicular to the stream current and emptied of air by gently tapping the sides. Six replicate cages secured in plastic-covered wire baskets (dish drainers) were positioned according to a

randomization grid (Table 2-2, Fig. 2-7). The end of each basket was weighted with bricks, secured by plastic zip ties. A tarp-covered diversion was placed directly upstream from the basket to control flow and prevent debris from clogging the cages.

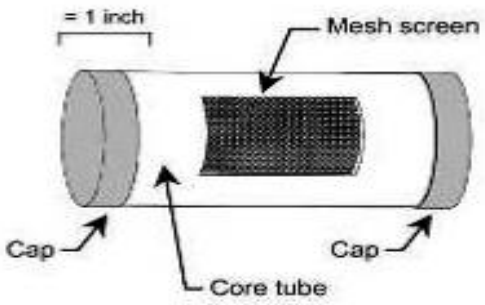



Figure 2-6. Diagram of the cages (12.7 cm long x 6.7 cm diameter) used in in-stream toxicity tests. Removable caps on both ends and rectangular mesh side windows allowed easy access and flow through. Six cages were tied into a basket at each site during each field bout (Burton 2005a).

Table 2-2. Randomization of replicates at each field site determined by numbers drawn from a hat, placement left to right. FC = field control, HC = handling control, and LC = lab control.

| Test method | Site | Position 1 | Position 2 | Position 3 | Position 4 | Position 5 | Position 6 | Position 7 | Position 8 |
|---|---|---|---|---|---|---|---|---|---|
| In-stream | 1 | 5 | 2 | 3 | 6 | 1 | 4 | | |
| | 2 | 3 | 4 | 5 | 1 | 2 | 6 | | |
| | 3 | 5 | 6 | 2 | 1 | 3 | 4 | | |
| | 4 | 2 | 1 | 4 | 6 | 3 | 5 | | |
| | 5 | 1 | 6 | 3 | 4 | 5 | 2 | | |
| | 6 | 1 | 2 | 6 | 4 | 3 | 5 | | |
| | FC | 2 | 3 | 6 | 1 | 5 | 4 | | |
| | HC | 1 | 3 | 2 | | | | | |
| Ambient-pH | 1 | 1 | 3 | 5 | 2 | 4 | | | |
| | 2 | 5 | 4 | 2 | 1 | 3 | | | |
| | 3 | 1 | 5 | 2 | 4 | 3 | | | |
| | 4 | 1 | 3 | 4 | 5 | 2 | | | |
| | 5 | 3 | 5 | 4 | 1 | 2 | | | |
| | 6 | 2 | 1 | 5 | 3 | 4 | | | |
| | LC | Randomized at discretion of laboratory technician | | | | | | | |
| $CO_2$ pH-controlled | 1 | 4 | 3 | LC1 | LC2 | 5 | 1 | Empty | 2 |
| | 2 | LC2 | Empty | 2 | 1 | LC1 | 3 | 5 | 4 |
| | 3 | LC1 | LC1 | Empty | 3 | 5 | 2 | 4 | 1 |
| | 4 | 1 | LC2 | 5 | LC1 | 3 | Empty | 2 | 4 |
| | 5 | 3 | 1 | LC1 | 4 | 5 | LC2 | 2 | Empty |
| | 6 | 2 | 3 | LC1 | 1 | 5 | Empty | 4 | LC2 |

Figure 2-7. Six cages of FHM larvae were secured into wire baskets and placed at specified sites longitudinally downstream from the Williams Production RMT Company outfall WY0046922-001on Beaver Creek, in the Powder River Basin, Wyoming. The above photo was taken at Site 2 during the July/August 2006 field bout.

Before each deployment, I measured pH (Oakton 10 Series meter), temperature (Oakton 10 Series meter), and dissolved oxygen concentration (YSI model 95 meter). Cages were deployed at Sites 1 to 6 for the July/August and October 2006 field bouts. In January 2007, cages of FHM were deployed only at Sites 1 to 4, because Sites 5 and 6 were frozen. Cage 3 was not deployed at Site 3 during January because I did not have enough acclimated fish. Because temperature fluctuations seemed to adversely affect the in-stream tests during the October bout, I attached temperature probes (Madge Tech Temp 1000) to the baskets located at Sites 2, 3 and 4 during the January bout.

Cages 1 to 5 were not opened and were left primarily undisturbed for 96 h as suggested in the Burton (2005b) protocol. The only exception was the mesh was cleaned off daily to ensure adequate water flow through the cages. Cage 6 was opened at 48 h and survival was recorded, but this record was not used in 96 h survival statistics. At 96 h, I recorded final pH, temperature, and dissolved oxygen concentration before counting the number of fish alive and dead in every cage. The final count at each site was verified by at least two people.

I incorporated a field and handling control into each seasonal study. The field control, a plastic tub filled with RB well water, had 4 to 5 replicate cages of FHM. The tub of well water was placed in the creek between Sites 1 and 2 to maintain in-stream temperature and lighting. Water chemistry was measured at least daily in the field control, and the water was renewed at 48 h with RB well water adjusted to the current in-stream temperature. The handling control determined if the transport and handling of the fish adversely impacted survival. After the last site deployment, I pipetted 10 FHM into at least three cages and had them transported to RB in a cooler of aerated well water, within 24 h of deployment. At RB, these handling-control cages of fish were acclimated in ~3 L of well water before being placed into the same water bath containing the laboratory toxicity test beakers. These cages were monitored for pH, temperature and dissolved oxygen along with the other WET test beakers. Survival was recorded at time of receipt and at the end of 96 h.

On either the day of deployment in the January field bout or the next day in the July/August and October field bouts, two 4-L cubitainers of unfiltered, unpreserved water was collected with no air space from each test site and transported on ice the same day to

Red Buttes Laboratory. After they arrived at the laboratory, the cubitainers were refrigerated overnight; and static-renewal 96-h WET tests (USEPA 2002b) were initiated with one cubitainer from each site within 36 h of collection. The second cubitainer of site water was used to renew the exposure solutions in the WET test at 48 h.

The remaining batch of FHM delivered from ENSR to RB were held in well water in flow-through plastic tubs and fed twice daily with 24-h old brine shrimp (*Artemia* sp., San Francisco strain, Brine Shrimp Direct, Ogden, Utah, USA) until test initiation. During the July/August and January field bouts, 10 fish were added to each beaker in the ambient-pH test and 10 fish were added to each beaker in the $CO_2$ pH-controlled test, until all the beakers had fish. During the October field bout, only 8 fish were used in the laboratory WET-test beakers because too few fish were hatched by ENSR. Although the static-renewal guidelines (USEPA 2002b) require that fish be randomly placed into all the beakers, the larvae were not randomized order to assure the test would start within the required time period (36 h post collection; USEPA 2002b). In July/August and October 2006 the WET tests were conducted at 20 °C; in January 2007, the tests were inadvertently conducted at 25 °C (Table 2-1).

To prepare the ambient-pH WET tests, 5 randomly placed replicate beakers of each undiluted site water and 5 replicate beakers of well water (lab control), all with fish added, were incubated in 20 or 25 °C water baths open to room air (Fig. 2-8, Table 2-2). For the $CO_2$ pH-controlled tests, each site water had 5 randomly placed replicate beakers of undiluted water and 2 replicates of well water (lab control), all with fish, placed in a $CO_2$ chamber. During the October and January studies, 1 beaker of each water (well and site) in each treatment (ambient-pH and $CO_2$ pH-controlled) was incubated without fish

to determine the effect the fish had on the ammonia concentrations in the exposure waters.

All beakers used in the $CO_2$ pH-controlled studies were placed into sealed glass chambers (41 cm long x 20 cm wide x 14 cm deep). A separate glass chamber was used for each site (Fig. 2-8). Eight 2-cm-diameter holes were drilled into each removable cover to allow access to the beakers inside the chambers without breaking the silicone seal. An air mixture containing 2 to 3% $CO_2$ was injected through one hole at ~0.3 psi for about 5 seconds, and then the hole was immediately sealed. Daily, pH of the chamber was recorded; and additional $CO_2$ gas was added if the pH drifted higher than 0.3 units from the initial pH. Additional $CO_2$ gas was not added after initial 96 h.

In the laboratory, temperature, dissolved oxygen, and pH were monitored daily from one replicate of each treatment. Additionally, fish survival was recorded daily for at least 96 h; and survival was recorded daily for 144 h during the October and January bouts. To monitor pH drifts, the remaining beakers in both treatments were incubated an additional 48 h after the end of each 96 h test. Because the second 4-L cubitainer was used to renew all beakers at the original 48 h, this additional time resulted in a 96 h un-renewed extended exposure time.

Figure 2-8. Laboratory whole effluent toxicity test chambers incubated in 20 or 25 °C water baths. Ambient-pH beakers were open to the atmosphere while $CO_2$ pH-controlled beakers were contained in sealed glass chambers blanketed with air containing 2 to 3% $CO_2$.

**Chemical Analyses**

In the field, temperature, pH, conductivity, turbidity and dissolved oxygen were recorded daily or twice daily at all sampling locations (Oakton 10 Series - Combination Temperature/pH/Conductivity Meter; Hach 2100 – turbidity; and YSI 95 - DO meter). Along with the toxicity test water, water samples were collected and transported to RB for analysis. Water samples for anions, cations, and dissolved organic carbon were filtered on-site using a 0.45 μm filter. At RB analyses of major inorganic anions ($Cl^-$, $NO_2^-$, $NO_3^-$, $SO_4^{2-}$; by ion chromatography), major inorganic cations ($Ca^{2+}$, $K^+$, $Mg^{2+}$, $Na^+$; by flame atomic absorption spectrophotometry), alkalinity (by $H_2SO_4$ titration), TA-N (by colorimetry), TN (digestion followed by colorimetry), dissolved organic carbon (by combustion), and total barium (by inductively coupled plasma -mass spectrometry) were conducted according to standard methods (APHA et al. 1995; Table 2-3). Bicarbonate was computed using a geochemistry speciation program (AquaChem Version 3.7, Scientific Software Group, Sandy, Utah, USA). Since sodium adsorption ratio (SAR) is a common parameter used to monitor water quality in CBNG produced waters, the SAR was calculated (Eqn. 2) using the $Na^+$, $Ca^{2+}$, and $Mg^{2+}$ values.

Eqn. 2:

$$SAR = \frac{Na^+}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}}$$

Concentrations of UIA were calculated from temperature, pH, and TA-N concentrations, according to USEPA (1999). Duplicate preserved ($H_2SO_4$ to pH < 2.0) samples were collected from the same location in the stream and analyzed at Energy Labs

Table 2-3. Collection and preservation requirements, along with the analytical method for selected chemical analyses performed at University of Wyoming's Red Buttes Laboratory (RB) and at Energy Labs Inc.(ELI) (APHA et al. 1995, ELI 2001).

| Lab | Analyte | Holding time | Filtration Y/N | Preservative | Analytical method |
|---|---|---|---|---|---|
| RB | Alkalinity | 24 h | N | Refrigerate | $H_2SO_4$ titration |
| RB | Hardness | 7 d | N | Refrigerate | EDTA titration |
| RB and ELI | Chloride | 28 d | Y | Refrigerate or freeze | Ion chromatography |
| RB and ELI | Nitrite | 48 h | Y | Refrigerate or freeze | Ion chromatography |
| RB and ELI | Nitrate | 48 h | Y | Refrigerate or freeze | Ion chromatography |
| RB and ELI | Sulfate | 28 d | Y | Refrigerate or freeze | Ion chromatography |
| RB | Calcium | 6 mo | Y | $HNO_3$ to pH <2 | Flame atomic absorption spectrophotometry |
| RB | Magnesium | 6 mo | Y | $HNO_3$ to pH <2 | Flame atomic absorption spectrophotometry |
| RB | Potassium | 6 mo | Y | $HNO_3$ to pH <2 | Flame atomic absorption spectrophotometry |
| RB | Sodium | 6 mo | Y | $HNO_3$ to pH <2 | Flame atomic absorption spectrophotometry |
| RB | Total ammonia nitrogen | 28 d | N | Refrigerate or freeze | Colorimetric |
| ELI | Total ammonia nitrogen | 28 d | N | Refrigerate, $H_2SO_4$ to pH < 2.0 | Colorimetric |
| RB | Total nitrogen | 28 d | N | None | Digestion by $K_2S_20_8$, colorimetry |
| ELI | Total nitrogen | 28 d | N | Refrigerate, $H_2SO_4$ to pH < 2.0 | Kjeldahl |
| RB | Dissolved organic carbon | 28 d | Y | HCl to pH < 3.0 | Combustion |
| RB | Total barium | 6 mo | N | $HNO_3$ to pH <2 | ICP-mass spectrometry |

Incorporated (ELI) in Gillette, Wyoming and in Billings, Montana for TA-N (by colorimetry), $NO_3^-$ (by ion chromatography), $NO_2^-$ (by ion chromatography), and TN (by Kjeldahl digestion). Blind samples of known standards of TA-N, $NO_3^-$ and $NO_2^-$ were analyzed at both RB and ELI to test inter-laboratory precision. In both WET test environments, one beaker daily from each site was monitored for pH (Orion Model 210), temperature (Fisher 76 immersion thermometer) and dissolved oxygen (YSI model 50B meter).

**Field Observations of Biota**

I recorded observations of fish, frogs and snakes during each study week. The Wyoming Department of Game and Fish (WDGF) conducted an amphibian-and-reptile survey on June 28, 2006 (Turner 2007). Windy Davis, a graduate student at Montana State University, seined (heavy delta ¼ inch mesh) four 300 m reaches along Beaver Creek on July 26 and 27, 2006 for her thesis research (Davis 2007). Davis's Site A was downstream near the mouth of the Powder River, Site B was Site 6 in my study, Site C was Site 5 in my study, and Site D was between my Sites 2 and 3 in my study. She calculated richness and diversity of the fish assemblages along the drainage.

**Data Analyses**

To evaluate in-stream toxicity, the survival at each site was compared to the survival of the field control for each season. Similarly, the survival in each beaker of both laboratory methods (ambient-pH WET tests and $CO_2$ pH-controlled WET tests) was compared to the survival of each method's well water control. Following the USEPA flowchart (USEPA 2002a) for analysis of multi-effluent-concentration test data, the percentage survival data were transformed using an angular transformation (i.e., arc sine

square root). If the number of replicates were unequal, a Wilcoxon Rank Sum test with Bonferroni adjustment was used to determine significance using Toxcalc 5.0.23F (Tidepool Scientific Software, McKinleyville, California, USA). If the toxicity data had equal replicates, a Steel's Many-One non-parametric rank test in Toxcalc 5.0.23F was used to determine significance. A site was considered non-toxic if survival did not differ significantly from the control ($P > 0.05$). In the field during the July/August 2006 field bout, not all the fish were accounted for at 96 h because muskrats tore into cages at Sites 2 and 3, presumably allowing the fish to escape. Also, since some of the fish were smaller they could not be found at some sites when the cages were pulled from the water, likely caught in the mud on the side of the cages. Therefore, statistics for these replicates were determined based on the number of fish that were confirmed alive or dead at 96 h. For example, during the July/August field bout at Site 3, cage 2 had 6 FHM alive at 96 h and four missing, with no carcasses found; therefore I computed this as 6/6 (or 100%) survival.

The CMC and CCC for ammonia were calculated using Equations 3 to 5 from USEPA 1999: page 83). The CMC equation for salmonid cold-water species absent is listed below in Eqn. 3 because salmonid species are not normally found in the ephemeral Beaver Creek drainage. I calculated the ratio of TA-N (analyzed at ELI) divided by the CMC or the CCC. During July/August 2006, I calculated the 4 d mean of the TA-N/CCC quotient at Sites 3 and 4 to determine if the mean of the quotient was <1.

CMC for salmonids absent (Eqn. 3):

$$CMC = \frac{0.411}{1+10^{7.204-pH}} + \frac{58.4}{1+10^{pH-7.204}}$$

CCC when fish early life stages are present (Eqn. 4):

$$CCC = (\frac{0.0577}{1+10^{7.688-pH}} + \frac{2.487}{1+10^{pH-7.688}}) * MIN(2.85,\ 1.45*10^{0.028*(25-T)})$$

CCC when fish early life stages are absent (Eqn. 5):

$$CCC = (\frac{0.0577}{1+10^{7.688-pH}} + \frac{2.487}{1+10^{pH-7.688}}) * 1.45*10^{0.028*(25-MAX(T,7))}$$

Using analysis of variance (ANOVA) followed by Tukey HSD pairwise comparisons (Statistical Analysis System (SAS) PROC GLM), I determined the significance of differences among the sites for each field bout for TN, TA-N, and UIA using only the ELI results. I also determined the significance of differences between both ELI and RB laboratories for these parameters. For each field bout, I regressed the natural logarithm ammonia concentrations on pH, temperature, and distance and performed a multiple regression of ammonia concentrations on temperature and distance combined. For the ANOVA's, the null hypothesis was that the specified dependent variable did not change among sites ($\alpha = 0.05$); for the regressions, the null hypothesis was that the specified parameter did not change as distance downstream from the CBNG outfall increased ($\alpha = 0.05$).

Finally, I determined if the TA-N values measured along Beaver Creek would comply with the ammonia limits established for the Willow Creek watershed general

permit using the waste load allocation (WLA) equation WDEQ/WQD provided (personal communication, Jason Thomas, CBNG permit writer, WDEQ/WQD, Cheyenne, Wyoming, USA; August 30, 2007 -- email correspondence; Eqn. 6). The average pH, temperature and flow designated by WDEQ/WQD for the watershed of which Beaver Creek is a part of were not available at the time of thesis completion. However, Jason Thomas of WDEQ/WQD stated the data at the Sussex USGS gaging station used for the Willow Creek watershed were similar and could be used for the purpose of comparison.

$$\text{WLA} = \frac{(\text{Combined flow} * \text{Allowable standard}) - (\text{Low flow (7Q10)} * \text{Background})}{\text{CBNG discharge rate}}$$

(Eqn. 6)

where:
Combined flow = Low flow (7Q10) in receiving stream + CBNG discharge rate
Low flow (7Q10) = 10-year low-flow average
Allowable standard = 20% of assimilative capacity for ammonia + background ammonia
Assimilative capacity = CCC – background ammonia concentration
Background = background ammonia concentration

## RESULTS

The maximum air temperatures were 38, 22 and 11 °C and the minimum air temperatures were 20, 3, and 0° C, respectively during July/August, October and January (http://www.wunderground.com). No significant rain or snow events occurred during the three field bouts. The flows measured at the flume (Site 1) were 0.045, 0.024, and 0.061 $m^3/s$ during July/August, October and January, respectively (personal communication, Eric Sandberg, Williams Production RMT Company, Gillette, Wyoming; February 22, 2007-- email correspondence).

The closest CBNG discharges entering Beaver Creek were 30 km upstream and 14 km downstream. During the study period, Beaver Creek did not flow immediately upstream of the discharge point; instead, in July/August only, stagnant water backed up ~45 m above the discharge point (Site 0). During October and January, no water was in the stream bed upstream from the discharge point. Therefore, the reach along Beaver Creek was only influenced by the discharge at Site 1. Downstream of my study site, Beaver Creek was effluent-dominated and hydraulically connected to the Powder River during the entire study period.

The effluent-dominated water did not appear to cause acute toxicity within the 14-km reach of Beaver Creek (Table 2-4 and Appendix Tables B-1 to B-5). Significant losses of fishes occurred only at Sites 2 and 3 during the July/August field bout, at Sites 5 and 6 during the October field bout, and at Site 4 during the January field bout; and all those losses appeared to be caused by factors not related to water chemistry (see following paragraphs). The field controls and the handling controls had >80% survival for all field bouts, indicating the fish in the in-stream tests were viable and were not adversely affected by handling and transport.

At Sites 2 and 3 during the July/August study, muskrats scratched holes in the cages, allowing fish to escape while all the remaining fish I could find were alive and active. At Sites 5 and 6 during October, the water temperature was <0.7 °C one morning when temperature was measured before noon (between 7:00 and 8:00 AM on 18 October; Appendix Table C-1); and at Site 4 during January, the water temperature was ≤2 °C on the last morning of the 96-h exposure period (on 26 and 27 January; Appendix Table C-1 and Appendix E).

Hart (1947) reported the lower incipient lethal temperature (LILT; i.e., the minimum temperature at which 50% of the fish died) for FHM acclimated to 20 °C water was 1.5 °C, and the LILT for FHM acclimated to 30 °C was 10.5 °C. The FHM in that study were 1- to 2-year-old fish collected from the Don River in Thornhill, Ontario and then acclimated to 10, 20 or 30 °C water for 24 h. The FHM acclimated to 10 °C survived all low temperatures tested (i.e., the LILT was <0 °C). I am unaware of other LILTs studies for FHM.

For several reasons, it is difficult to definitively conclude from Hart's (1947) study whether the low temperatures in Beaver Creek on some mornings killed my fish. For example, Hart's fish were much older than the FHM larvae in my study and thus might have been less sensitive to low temperatures; therefore, the LILT for my FHM larvae might have been >1.5 °C. Additionally, Hart's fish were acclimated to a single temperature for 24 h before being exposed to low temperatures, whereas my caged larvae in Beaver Creek were exposed to highly variable temperatures during the 96-h exposures (<0.7-11.9 °C at Site 5 in October, <0.7-12.4 °C at Site 6 in October, and 1.0-8.5 °C at Site 4 in January; Appendix Table C-1). In October, I do not know how long my FHM larvae were exposed to the very low temperatures (e.g., ≤2.0 °C) at Sites 5 and 6 in Beaver Creek; but in January, the FHM larvae were exposed to temperatures ≤2.0 ° for ~2 h on day 4 (Appendix E). These uncertainties make the field data alone difficult to interpret. However, because (1) I used a presumably more sensitive life stage in my study than was used in Hart (1947) and (2) FHM larvae exposed to water collected from the same sites at the beginning of each field bout and maintained at 20 or 25 °C in the laboratory had ≥90% survival (Appendix Tables B-2 and B-4), I conclude based on a

weight of evidence that cold water temperatures (not the water quality) in Beaver Creek were the most likely cause of the deaths of my caged FHM larvae at Sites 5 and 6 in October and at Site 4 in January.

Similarly, all lab controls had >90% survival, indicating acceptable exposure conditions in the laboratory studies. The pH of the water in the $CO_2$ pH-controlled test chambers did not increase more than 0.3 units from pH at the beginning of the test to the end of the 96 h period indicating successful pH control. No acute toxicity occurred in the ambient-pH and $CO_2$ pH-controlled toxicity tests at 96 h (Table 2-4 and Appendix Tables B-2 and B-4). However, mortality in the ambient-pH beakers maintained in the water bath for an additional 48 h (i.e., for 96 h un-renewed) was greater than in the $CO_2$ pH-controlled beakers held an additional 48 h (Table 2-4 and Appendix Tables B-3 and B-5). The pH spikes and increased UIA in the ambient-pH beakers at 144 h (96 h since last renewal; Tables 2-5 to 2-8) suggest the increased mortality probably was caused by ammonia (Tables 2-4). Un-ionized ammonia nitrogen concentrations could not be calculated for some waters because either pH, temperature or TA-N was not measured. Although 144 h exceeds the USEPA (2002a) recommendations for a 96-h static-renewal test, survival in the beakers of well water (control) and the $CO_2$ pH-controlled test beakers was similar at 96 and 144 h.

Table 2-4. Average percent survival of fathead minnow (*Pimephales promelas*) larvae among all replicates in the in-stream, ambient-pH, and $CO_2$ pH-controlled toxicity tests with water from Beaver Creek in the Powder River Basin, Wyoming. FC = field control, LC# = lab control, NA = not applicable. *Statistically different from control (P>0.05).

| Month | Site | Survival (%) | | | | |
|---|---|---|---|---|---|---|
| | | In-stream test 96 h | Ambient-pH 96 h | Ambient-pH 144 h | pH-controlled 96 h | pH-controlled 144 h |
| July/August | FC | 96 | NA | NA | NA | NA |
| | LC1 | NA | 100 | 100 | 100 | 100 |
| | LC2 | NA | NA | NA | 95 | 95 |
| | 1 | 75 | 100 | 34* | 100 | 100 |
| | 2 | 78 | 94 | 28* | 100 | 100 |
| | 3 | 98 | 100 | 44* | 100 | 100 |
| | 4 | 100 | 94 | 88* | 98 | 98 |
| | 5 | 98 | 94 | 92 | 96 | 96 |
| | 6 | 100 | 94 | 94 | 90 | 90 |
| October | FC | 100 | NA | NA | NA | NA |
| | LC1 | NA | 100 | 100 | 100 | 100 |
| | LC2 | NA | NA | NA | 100 | 100 |
| | 1 | 100 | 100 | 85 | 100 | 100 |
| | 2 | 100 | 100 | 83 | 100 | 100 |
| | 3 | 100 | 100 | 80 | 100 | 100 |
| | 4 | 88 | 100 | 100 | 100 | 100 |
| | 5 | 12*[a] | 100 | 100 | 100 | 100 |
| | 6 | 15*[a] | 100 | 100 | 100 | 100 |
| January | FC | 90 | NA | NA | NA | NA |
| | LC1 | NA | 100 | 100 | 100 | 90 |
| | LC2 | NA | NA | NA | 100 | 100 |
| | 1 | 76 | 98 | 44* | 100 | 100 |
| | 2 | 92 | 96 | 16* | 100 | 94 |
| | 3 | 95 | 98 | 36* | 100 | 98 |
| | 4 | 0*[a] | 90 | 88 | 98 | 96 |

[a] Water temperature decreased below 2 °C; however, no significant mortality occurred in laboratory toxicity WET tests with water collected from these sites.

Table 2-5. pH measured in Beaver Creek in the Powder River Basin, Wyoming; in stream water, on arrival at Red Buttes Laboratory in Laramie, Wyoming, and in one beaker of each method at the time of WET test initiation, at 96 h, and at 144 h. NA = measurements not available; Y = yes; N = no.

| Month | Site | Fish in beaker | pH | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Field | Arrival at Red Buttes | Initial 0 h | Ambient-pH 96 h | Ambient-pH 144 h | pH-controlled 96 h | pH-controlled 144 h |
| July/August | 1 | Y | 7.83 | 7.79 | 7.99 | 8.78 | 8.93 | 8.09 | 8.18 |
| | 2 | Y | 8.00 | 7.95 | 8.06 | 8.74 | 8.97 | 8.12 | 8.18 |
| | 3 | Y | 8.24 | 8.25 | 8.37 | 8.83 | 9.03 | 8.22 | 8.26 |
| | 4 | Y | 8.74 | 8.58 | 8.64 | 9.00 | 9.08 | 8.32 | 8.26 |
| | 5 | Y | 8.95 | 8.76 | 8.76 | 9.08 | 9.13 | 8.56 | 8.44 |
| | 6 | Y | 9.09 | 8.88 | 8.90 | 9.06 | 9.18 | 8.48 | 8.48 |
| October | 1 | Y | 7.39 | 7.71 | 8.00 | 8.82 | 9.05 | 8.06 | 8.10 |
| | 1 | N | 7.39 | 7.71 | 8.00 | 9.11 | NA | 8.24 | NA |
| | 2 | Y | 7.67 | 7.85 | 8.02 | 8.75 | 9.09 | 8.10 | 8.06 |
| | 2 | N | 7.67 | 7.85 | 8.02 | 9.16 | NA | 8.20 | NA |
| | 3 | Y | 7.91 | 8.10 | 8.27 | 8.90 | 9.07 | 8.27 | 8.30 |
| | 3 | N | 7.91 | 8.10 | 8.27 | 9.19 | NA | 8.32 | NA |
| | 4 | Y | 8.24 | 8.47 | 8.44 | 8.96 | 9.14 | 8.39 | 8.38 |
| | 4 | N | 8.24 | 8.47 | 8.44 | 9.18 | NA | 8.47 | NA |
| | 5 | Y | 8.45 | 8.68 | 8.66 | 9.05 | 9.10 | 8.58 | 8.35 |
| | 5 | N | 8.45 | 8.68 | 8.66 | 9.23 | NA | 8.55 | NA |
| | 6 | Y | 8.56 | 8.80 | 8.85 | 9.07 | 9.17 | 8.70 | 8.63 |
| | 6 | N | 8.56 | 8.80 | 8.85 | 9.25 | NA | 8.71 | NA |
| January | 1 | Y | 7.33 | NA | 8.12 | 9.08 | 9.23 | 8.13 | 8.17 |
| | 1 | N | 7.33 | NA | 8.12 | 9.38 | 9.45 | NA | NA |
| | 2 | Y | 7.91 | NA | 7.93 | 9.03 | 9.22 | 8.20 | 8.09 |
| | 2 | N | 7.91 | NA | 7.93 | 9.35 | 9.43 | NA | NA |
| | 3 | Y | 7.96 | NA | 8.24 | 9.06 | 9.28 | 8.21 | 8.25 |
| | 3 | N | 7.96 | NA | 8.24 | 9.38 | 9.44 | NA | NA |
| | 4 | Y | 8.32 | NA | 8.41 | 9.12 | 9.29 | 8.32 | 8.22 |
| | 4 | N | 8.32 | NA | 8.41 | 9.37 | 9.45 | NA | NA |

Table 2-6. Temperature measured in Beaver Creek in the Powder River Basin, Wyoming (range measured over 96 h in stream); on arrival at Red Buttes Laboratory in Laramie, Wyoming; and in one beaker of each test method at the time of WET test initiation, at 96 h, and at 144 h. NA = measurements not available; Y = yes; N = no.

| Month | Site | Fish in beaker? | Temperature (°C) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Field | Arrival at Red Buttes | Initial 0 h | Ambient-pH 96 h | Ambient-pH 144 h | pH-controlled 96 h | pH-controlled 144 h |
| July/August | 1 | Y | 20.2 – 23.4 | 12.5 | 21.5 | 20.5 | 21.0 | 20.5 | 21.0 |
| | 2 | Y | 19.9 – 23.9 | 17.0 | 20.0 | 20.3 | 21.0 | 20.8 | 21.0 |
| | 3 | Y | 18.2 – 26.4 | 16.2 | 20.0 | 20.3 | 21.0 | 20.5 | 21.0 |
| | 4 | Y | 16.0 – 26.7 | 16.2 | 20.5 | 20.3 | 21.0 | 20.8 | 21.0 |
| | 5 | Y | 14.4 – 29.1 | 19.5 | 21.0 | 20.0 | 21.0 | 20.8 | 21.0 |
| | 6 | Y | 14.2 – 31.4 | 12.5 | 21.0 | 20.0 | 21.0 | 20.8 | 21.0 |
| October | 1 | Y | 13.9 – 19.2 | 5.3 | 20.0 | 20.3 | 20.3 | 20.5 | 20.5 |
| | 1 | N | 13.9 – 19.2 | 5.3 | 20.0 | 20.0 | 20.3 | 20.5 | NA |
| | 2 | Y | 13.5 – 19.0 | 5.6 | 19.0 | 20.0 | 20.3 | 20.5 | 20.5 |
| | 2 | N | 13.5 – 19.0 | 5.6 | 19.0 | 20.0 | 20.3 | 20.5 | NA |
| | 3 | Y | 10.5 – 18.9 | 4.0 | 19.5 | 20.3 | 20.3 | 20.5 | 20.8 |
| | 3 | N | 10.5 – 18.9 | 4.0 | 19.5 | 20.0 | 20.3 | 20.5 | NA |
| | 4 | Y | 2.2 – 12.4 | 4.0 | 19.8 | 20.2 | 20.3 | 20.5 | 20.8 |
| | 4 | N | 2.2 – 12.4 | 4.0 | 19.8 | 20.0 | 20.3 | 20.5 | NA |
| | 5 | Y | < 0.0[a] – 11.9 | 3.8 | 20.2 | 20.3 | 20.3 | 20.2 | 20.8 |
| | 5 | N | < 0.0[a] – 11.9 | 3.8 | 20.2 | 20.0 | 20.3 | 20.2 | NA |
| | 6 | Y | < 0.0[a] – 12.4 | 3.5 | 20.2 | 20.5 | 20.6 | 20.2 | 20.8 |
| | 6 | N | < 0.0[a] – 12.4 | 3.5 | 20.2 | 20.0 | 20.3 | 20.2 | NA |
| January | 1 | Y | 14.0 – 15.9 | 0.8 | 22.0 | 25.0 | 25.0 | 26.0 | 25.0 |
| | 1 | N | 14.0 – 15.9 | 0.8 | 22.0 | 25.0 | 25.0 | NA | NA |
| | 2 | Y | 13.9 – 15.7 | 1.2 | 22.0 | 25.0 | 25.0 | 26.0 | 25.8 |
| | 2 | N | 13.9 – 15.7 | 1.2 | 22.0 | 25.0 | 25.0 | NA | NA |
| | 3 | Y | 12.3 – 14.7 | 3.2 | 22.0 | 25.0 | 25.0 | 26.0 | 26.0 |
| | 3 | N | 12.3 – 14.7 | 3.2 | 22.0 | 25.0 | 25.0 | NA | NA |
| | 4 | Y | 1.0 – 8.5 | 4.0 | 22.0 | 25.3 | 25.3 | 26.0 | 26.0 |
| | 4 | N | 1.0 – 8.5 | 4.0 | 22.0 | 25.3 | 25.3 | NA | NA |

[a] Below range detectable on combination temperature/pH/conductivity meter.

Table 2-7. Total ammonia nitrogen (TA-N) concentrations collected in Beaver Creek in the Powder River Basin, Wyoming and analyzed on arrival to Red Buttes Laboratory in Laramie, Wyoming, at 96 h, and at 144 h. NA = measurements not available; Y = yes; N = no.

| Month | Site | Fish in beaker? | TA-N (mg N/L) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Arrival at Red Buttes | Ambient-pH | | $CO_2$ pH-controlled | |
| | | | | 96 h | 144 h | 96 h | 144 h |
| July/August[a] | 1 | Y | 2.41 | NA | 2.22 | NA | 2.86 |
| | 2 | Y | 2.38 | NA | 2.20 | NA | 2.86 |
| | 3 | Y | 2.35 | NA | 2.07 | NA | 2.44 |
| | 4 | Y | 0.56 | NA | 0.79 | NA | 1.09 |
| | 5 | Y | 0.00 | NA | 0.50 | NA | 0.54 |
| | 6 | Y | 0.00 | NA | 0.54 | NA | 0.43 |
| October[a] | 1 | Y | 2.70 | 2.32 | 3.13 | 2.78 | 3.07 |
| | 1 | N | 2.70 | 2.07 | 0.84 | 2.46 | 2.32 |
| | 2 | Y | 2.71 | 2.63 | 2.57 | 2.81 | 3.05 |
| | 2 | N | 2.71 | 1.37 | 0.53 | 2.34 | 2.31 |
| | 3 | Y | 2.65 | 2.51 | 2.60 | 2.80 | 2.95 |
| | 3 | N | 2.65 | 1.58 | 0.91 | 2.38 | 2.02 |
| | 4 | Y | 1.50 | 1.30 | 1.01 | 1.42 | 1.02 |
| | 4 | N | 1.50 | 0.94 | 0.44 | 0.95 | 0.32 |
| | 5 | Y | 0.73 | 0.91 | 0.85 | 0.45 | 0.72 |
| | 5 | N | 0.73 | 0.46 | 0.29 | 0.59 | 0.52 |
| | 6 | Y | 0.32 | 0.38 | 0.81 | 0.45 | 0.72 |
| | 6 | N | 0.32 | 0.25 | 0.15 | 0.25 | 0.22 |
| January[b] | 1 | Y | 2.58 | 2.66 | 2.63 | 3.56 | 3.78 |
| | 1 | N | 2.58 | 0.67 | 0.17 | 2.11 | 1.93 |
| | 2 | Y | 2.55 | 2.62 | 2.80 | 3.37 | 3.90 |
| | 2 | N | 2.55 | 0.74 | 0.31 | 2.17 | 2.00 |
| | 3 | Y | 2.53 | 3.26 | 2.92 | 2.94 | 3.47 |
| | 3 | N | 2.53 | 1.12 | 0.33 | 2.07 | 1.16 |
| | 4 | Y | 2.16 | 2.06 | 0.19 | 2.53 | 1.29 |
| | 4 | N | 2.16 | 0.06 | 0.03 | 0.08 | 0.01 |

[a]Toxicity tests conducted at 20 °C.
[b]Toxicity tests conducted at 25 °C.

Table 2-8. Un-ionized ammonia nitrogen (UIA) concentrations collected in Beaver Creek in the Powder River Basin, Wyoming and analyzed on arrival to Red Buttes Laboratory in Laramie, Wyoming, at 96 h, and at 144 h. NA = measurements not available; Y = yes; N = no.

| Month | Site | Fish in beaker? | Arrival at Red Buttes | Ambient-pH | | $CO_2$ pH-controlled | |
|---|---|---|---|---|---|---|---|
| | | | | 96-h | 144-h | 96-h | 144-h |
| July/August[a] | 1 | Y | 0.033 | NA | 0.592 | NA | 0.174 |
| | 2 | Y | 0.066 | NA | 0.627 | NA | 0.174 |
| | 3 | Y | 0.119 | NA | 0.650 | NA | 0.176 |
| | 4 | Y | 0.057 | NA | 0.268 | NA | 0.079 |
| | 5 | Y | 0.000 | NA | 0.183 | NA | 0.057 |
| | 6 | Y | 0.000 | NA | 0.212 | NA | 0.049 |
| October[a] | 1 | Y | 0.018 | 0.491 | 0.980 | 0.126 | 0.151 |
| | 1 | N | 0.018 | 0.701 | NA | 0.164 | NA |
| | 2 | Y | 0.025 | 0.480 | 0.856 | 0.139 | 0.138 |
| | 2 | N | 0.025 | 0.500 | NA | 0.143 | NA |
| | 3 | Y | 0.038 | 0.612 | 0.840 | 0.200 | 0.229 |
| | 3 | N | 0.038 | 0.602 | NA | 0.189 | NA |
| | 4 | Y | 0.049 | 0.349 | 0.363 | 0.130 | 0.094 |
| | 4 | N | 0.049 | 0.352 | NA | 0.103 | NA |
| | 5 | Y | 0.037 | 0.284 | 0.288 | 0.060 | 0.062 |
| | 5 | N | 0.037 | 0.185 | NA | 0.074 | NA |
| | 6 | Y | 0.016 | 0.124 | 0.308 | 0.076 | 0.110 |
| | 6 | N | 0.016 | 0.103 | NA | 0.043 | NA |
| January[b] | 1 | Y | NA | 1.081 | 1.293 | 0.271 | 0.293 |
| | 1 | N | NA | 0.387 | 0.105 | NA | NA |
| | 2 | Y | NA | 0.992 | 1.360 | 0.297 | 0.269 |
| | 2 | N | NA | 0.415 | 0.188 | NA | NA |
| | 3 | Y | NA | 1.288 | 1.519 | 0.265 | 0.340 |
| | 3 | N | NA | 0.646 | 0.201 | NA | NA |
| | 4 | Y | NA | 0.894 | 0.101 | 0.286 | 0.119 |
| | 4 | N | NA | 0.035 | 0.019 | NA | NA |

[a]Toxicity tests conducted at 20 °C.
[b]Toxicity tests conducted at 25 °C.

In the field, pH, temperature, conductivity, dissolved oxygen and turbidity varied between sites and from month to month (Table 2-9, Appendix C). Differences among sites for a given parameter could have been artifacts of varying sampling times and field temperatures, not necessarily due to the water quality of the certain site.

In all three field bouts, $Ba^{2+}$ decreased downstream from the outfall as $SO_4^{2-}$ increased. Calcium also decreased downstream in July/August and October; however, a similar decrease was not evident in January because Sites 5 and 6 (where the decreases were most pronounced in previous months) were frozen. Sodium and $Mg^{2+}$ increased downstream from Sites 1 to 6 during all three field bouts. Alkalinity, $HCO_3^-$, SAR, $F^-$, $Cl^-$, $K^+$, and DOC varied little from Site 1 to Site 6. Water quality at Site 0, with stagnant water upstream from the discharge point and only sampled in July/August, differed considerably from the other sites (Table 2-10).

Total nitrogen concentrations analyzed at RB and ELI were similar in January but different in October (Table 2-11). Total ammonia nitrogen concentrations differed significantly between the two laboratories in all three months (Tables 2-12). However, blind analyses of known TA-N standards differed by only 1.5% (RB = 4.91 mg N/L; ELI = 4.98 mg N/L). Nitrite (all values <0.30 mg N/L) and nitrate (all values <0.58 mg N/L) were not statistically compared but are reported in Appendix D. Comparing only ELI values, TN differed significantly among sites in October but not in January (Table 2-11). Total ammonia nitrogen differed significantly among sites in July/August and October but not in January (Table 2-12). Un-ionized ammonia differed significantly among sites in July/August and January but not in October (Table 2-13).

Table 2-9. Average daily field measurements ± standard deviation of pH, temperature, electrical conductivity, dissolved oxygen and turbidity in Beaver Creek in the Powder River Basin, Wyoming, at Sites 0 to 6 during July/August 2006, Sites 1 to 6 during October 2006, and Sites 1 to 4 during January 2007. All field data are compiled in Appendix C[a].

| Month | Site | pH[a] | Temperature (°C) | Conductivity (μS/cm) | Dissolved oxygen (mg/L) | Turbidity (ntu) |
|---|---|---|---|---|---|---|
| July/August | 0 | 9.04 (± 0.33, n = 8)$^{A}$ | 19.30 (± 2.42, n = 8)$^{A}$ | 2915 (± 15.71, n = 8)$^{A}$ | 8.49 (± 2.21, n = 6)$^{A}$ | 252 (± 13.09, n = 6)$^{A}$ |
| | 1 | 7.63 (± 0.37, n = 9)$^{D}$ | 21.31 (± 1.00, n = 9)$^{A}$ | 2098 (± 3.92, n = 9)$^{D}$ | 4.79 (± 0.75, n = 8)$^{B}$ | 9 (± 0.99, n = 7)$^{C}$ |
| | 2 | 7.84 (± 0.38 , n = 9)$^{D}$ | 21.34 (± 1.15, n = 9)$^{A}$ | 2098 (± 3.92, n = 9)$^{D}$ | 5.13 (± 0.81, n = 8)$^{B}$ | 9 (± 1.09, n = 8)$^{C}$ |
| | 3 | 8.20 (± 0.42, n = 9)$^{C}$ | 21.80 (± 1.56, n = 9)$^{A}$ | 2094 (± 4.17, n = 9)$^{D}$ | 7.48 (± 1.44, n = 8)$^{A}$ | 14 (± 2.57, n = 6)$^{BC}$ |
| | 4 | 8.59 (± 0.38, n = 9)$^{B}$ | 20.77 (± 1.79, n = 9)$^{A}$ | 2136 (± 4.07, n = 9)$^{C}$ | 8.19 (± 1.45, n = 8)$^{A}$ | 37 (± 4.09, n = 8)$^{B}$ |
| | 5 | 8.90 (± 0.40, n = 9)$^{A}$ | 21.32 (± 2.08, n = 9)$^{A}$ | 2203 (± 4.79, n = 9)$^{B}$ | 9.86 (± 1.28, n = 8)$^{A}$ | 4 (± 0.89, n = 7)$^{C}$ |
| | 6 | 9.01 (± 0.45, n = 9)$^{A}$ | 22.96 (± 2.31, n = 9)$^{A}$ | 2246 (± 5.92, n = 9)$^{B}$ | 9.10 (± 1.23, n = 8)$^{A}$ | 38 (± 4.09, n = 6)$^{B}$ |
| October | 1 | 7.78 (± 0.51, n = 9)$^{D}$ | 16.39 (± 1.41, n = 9)$^{A}$ | 2096 (± 7.57, n = 8)$^{B}$ | 6.08 (± 0.99, n = 9)$^{B}$ | 11 (± 2.09, n = 7)$^{B}$ |
| | 2 | 7.95 (± 0.51, n = 9)$^{CD}$ | 16.02 (± 1.45, n = 9)$^{A}$ | 2097 (± 6.73, n = 8)$^{B}$ | 6.87 (± 0.97, n = 7)$^{B}$ | 10 (± 1.18, n = 7)$^{B}$ |
| | 3 | 8.21 (± 0.48, n=10)$^{BC}$ | 14.56 (± 1.80, n= 10)$^{A}$ | 2113 (± 8.20, n = 9)$^{B}$ | 8.08 (± 0.98, n = 9)$^{AB}$ | 30 (± 3.31, n = 4)$^{B}$ |
| | 4 | 8.48 (± 0.45, n = 9)$^{AB}$ | 7.79 (± 1.84, n = 9)$^{B}$ | 2317 (± 10.8, n = 8)$^{A}$ | 9.42 (± 1.26, n = 8)$^{A}$ | 88 (± 5.18, n = 6)$^{A}$ |
| | 5 | 8.67 (± 0.47, n = 9)$^{A}$ | 6.43 (± 1.94, n = 9)$^{B}$ | 2376 (± 11.3, n = 8)$^{A}$ | 10.48 (± 1.07, n = 8)$^{A}$ | 69 (± 6.16, n = 7)$^{A}$ |
| | 6 | 8.54 (± 0.78, n = 8)$^{AB}$ | 6.96 (± 2.04, n = 8)$^{B}$ | 2328 (± 9.3, n = 7)$^{A}$ | 10.82 (± 0.93, n = 7)$^{A}$ | 84 (± 4.67, n = 6)$^{A}$ |
| January | 1 | 7.58 (± 0.34, n = 6)$^{D}$ | 14.88 (± 0.83, n = 6)$^{A}$ | 2501 (± 4.51, n = 6)$^{B}$ | 4.87 (± 0.88, n = 6)$^{C}$ | 10 (± 2.05, n = 6)$^{B}$ |
| | 2 | 7.83 (± 0.23, n = 6)$^{C}$ | 14.66 (± 0.81, n = 6)$^{A}$ | 2515 (± 5.52, n = 6)$^{B}$ | 6.36 (± 0.87, n = 6)$^{B}$ | 9 (± 1.65, n = 6)$^{B}$ |
| | 3 | 8.12 (± 0.32, n = 6)$^{B}$ | 13.47 (± 0.95, n = 6)$^{A}$ | 2541 (± 4.90, n = 6)$^{B}$ | 6.10 (± 0.69, n = 6)$^{B}$ | 22 (± 3.58, n = 6)$^{B}$ |
| | 4 | 8.59 (± 0.40, n = 6)$^{A}$ | 4.33 (± 1.65, n = 6)$^{B}$ | 2760 (± 8.12, n = 6)$^{A}$ | 9.48 (± 1.08, n = 6)$^{A}$ | 96 (± 4.45, n = 6)$^{A}$ |

[a] Within the same column and same month, the same letter indicates that the means of the sites did not differ significantly (P>0.05).

Table 2-10. Water quality in Beaver Creek in the Powder River Basin, Wyoming during July/August and October 2006, and January 2007 and analyzed at the University of Wyoming's Red Buttes Laboratory in Laramie, Wyoming. Site 0 was only sampled in July/August 2006 because no upstream water was present during October 2006 and January 2007 field bouts. Sites 2 to 6 were 0.04, 0.4, 3.7, 10.0, 14.1 km, respectively, downstream from Site 1, where CBNG effluent discharged into Beaver Creek. SAR = sodium adsorption ratio (calculated), DOC = dissolved organic carbon (mg/L). $HCO_3^-$ was computed in a geochemical speciation program (Aquachem version 3.7, Scientific Software Group, Sandy, Utah, USA).

| Month | Site | Date | Time | Field pH | Concentration (mg/L) | | | | | | | | | | | SAR[a] | DOC (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hardness mg/L as $CaCO_3$ | Alkalinity mg/L as $CaCO_3$ | $HCO_3^-$ | $F^-$ | $Cl^-$ | $SO_4^-$ | $Ba^{2+}$ | $Ca^{2+}$ | $K^+$ | $Mg^2$ | $Na^+$ | | |
| July/August | 0 | 7/31/06 | 0555 | 8.90 | NA | NA | 1785 | 2.6 | 19.6 | 137 | 0.12 | 12 | 13 | 25 | 690 | 26 | 11.9 |
| | 1 | 7/31/06 | 0639 | 7.83 | 160 | 1414 | 1672 | 2.3 | 15.5 | 2 | 0.84 | 27 | 14 | 18 | 576 | 21 | 4.0 |
| | 2 | 7/31/06 | 0655 | 8.00 | 163 | 1409 | 1667 | 2.2 | 16.4 | <1 | 0.86 | 26 | 14 | 18 | 575 | 21 | 4.3 |
| | 3 | 7/31/06 | 0730 | 8.24 | 164 | 1435 | 1651 | 2.0 | 13.5 | <1 | 0.85 | 26 | 13 | 18 | 567 | 21 | 4.8 |
| | 4 | 7/31/06 | 0755 | 8.74 | 135 | 1406 | 1658 | 2.3 | 15.6 | 33 | 0.46 | 14 | 14 | 19 | 599 | 25 | 3.9 |
| | 5 | 7/31/06 | 0835 | 8.95 | 130 | 1454 | 1669 | 2.3 | 16.6 | 51 | 0.31 | 12 | 14 | 21 | 611 | 25 | 6.1 |
| | 6 | 7/31/06 | 0905 | 9.09 | 126 | 1472 | 1759 | 2.3 | 17.1 | 52 | 0.26 | 10 | 15 | 22 | 646 | 26 | 4.3 |
| October | 1 | 10/16/06 | 0730 | 7.39 | 159 | 1447 | 1765 | 2.1 | 15.4 | <1 | 0.85 | 30 | 16 | 20 | 603 | 21 | 3.4 |
| | 2 | 10/16/06 | 0805 | 7.67 | 159 | 1479 | 1752 | 2.0 | 15.3 | <1 | 0.84 | 30 | 16 | 20 | 598 | 21 | 4.1 |
| | 3 | 10/16/06 | 0845 | 7.91 | 167 | 1452 | 1746 | 2.1 | 14.8 | 8 | 0.82 | 31 | 16 | 20 | 598 | 21 | 4.7 |
| | 4 | 10/16/06 | 0930 | 8.24 | 177 | 1436 | 1714 | 2.0 | 15.9 | 74 | 0.56 | 29 | 16 | 24 | 613 | 20 | 4.3 |
| | 5 | 10/16/06 | 1020 | 8.45 | 183 | 1383 | 1660 | 2.1 | 14.4 | 136 | 0.35 | 22 | 16 | 29 | 620 | 20 | 4.9 |
| | 6 | 10/16/06 | 1100 | 8.56 | 168 | 1367 | 1637 | 2.1 | 16.5 | 144 | 0.28 | 18 | 15 | 28 | 623 | 22 | 5.2 |
| January | 1 | 1/23/07 | 0725 | 7.33 | 171 | 1494 | 1768 | 1.9 | 12.2 | <1 | 0.85 | 31 | 16 | 20 | 601 | 21 | 4.0 |
| | 2 | 1/23/07 | 0900 | 7.91 | 173 | 1483 | 1784 | 2.1 | 12.3 | <1 | 0.85 | 30 | 16 | 20 | 608 | 21 | 4.3 |
| | 3 | 1/23/07 | 1015 | 7.96 | 181 | 1494 | 1818 | 1.8 | 12.8 | 17 | 0.84 | 32 | 16 | 21 | 617 | 21 | 4.1 |
| | 4 | 1/23/07 | 1215 | 8.32 | 200 | 1457 | 1752 | 1.9 | 14.8 | 76 | 0.68 | 34 | 16 | 26 | 618 | 19 | 5.1 |

[a] SAR calculated with $Na^+$, $Ca^{2+,}$ and $Mg^{2+}$ (meq/L)

Table 2-11. Total nitrogen (TN) in Beaver Creek in the Powder River Basin, Wyoming analyzed at the University of Wyoming's Red Buttes Laboratory in Laramie, Wyoming (RB) and at Energy Labs Inc. in Billings, Montana (ELI). Total nitrogen was not analyzed at either lab during July/August 2006. Duplicate samples were collected into two different bottles, one preserved (ELI) and one un-preserved (RB) from the same location in the creek. Additional preserved samples collected throughout the study week were analyzed at ELI. The complete dataset is compiled in Appendix D.

| | | Red Buttes Lab[a] | | Energy Labs Inc.[a] | | | |
|---|---|---|---|---|---|---|---|
| Month | Site | n | TN (mg N/L) | n | Average TN (mg N/L)[b] | S.D. | Between Lab Comparison |
| October | 1 | 1 | 3.86 | 2 | 3.60[A] | 0.14 | $F_{1,11} = 6.61$, $P = 0.03$ |
| | 2 | 1 | 3.49 | 2 | 3.50[A] | 0.00 | |
| | 3 | 1 | 3.90 | 2 | 3.55[A] | 0.07 | |
| | 4 | 1 | 2.53 | 2 | 2.70[B] | 0.14 | |
| | 5 | 1 | 2.00 | 2 | 1.70[C] | 0.00 | |
| | 6 | 1 | 1.56 | 2 | 1.25[C] | 0.07 | |
| January | 1 | 1 | 3.34 | 3 | 3.23[A] | 0.15 | $F_{1,11} = 3.03$, $P = 0.11$ |
| | 2 | 1 | 3.52 | 3 | 3.13[A] | 0.25 | |
| | 3 | 1 | 3.62 | 3 | 3.17[A] | 0.38 | |
| | 4 | 1 | 3.06 | 3 | 3.03[A] | 0.21 | |

[a] RB analyzed TN using digestion by $K_2S_20_8$, followed by colorimetry; whereas ELI analyzed TN using a Kjeldahl method.
[b] The same letter following the value indicates that the means of the ELI analyses did not differ significantly (P>0.05) among sites within the month.

Table 2-12. Total ammonia nitrogen (TA-N) analyzed at Red Buttes Laboratory - Laramie, Wyoming (RB) and Energy Labs Inc. - Billings, Montana (ELI). Duplicate samples were collected into two different bottles, one preserved (ELI) and one un-preserved (RB) from the same location in the creek. Additional preserved samples collected throughout the study week were analyzed at ELI. ANOVA analysis with a Tukey's mean separation test was used to determine differences between sites using only ELI data. Refer to Appendix D for a complete dataset.

| Month | Site | Red Buttes Lab[a] n | Red Buttes Lab[a] TA-N (mg N/L) | Energy Labs Inc.[a] n | Energy Labs Inc.[a] Ave. TA-N (mg N/L)[b] | Energy Labs Inc.[a] S.D. | Between Labs Comparison |
|---|---|---|---|---|---|---|---|
| July/August | 0 | 1 | 0.11 | 2 | <0.1[C] | NA | $F_{1,11} = 10.35$, $P = 0.01$ |
| | 1 | 1 | 2.40 | 2 | 1.45[A] | 0.21 | |
| | 2 | 1 | 2.40 | 2 | 1.35[A] | 0.21 | |
| | 3 | 1 | 2.33 | 2 | 1.20[A] | 0.00 | |
| | 4 | 1 | 0.58 | 2 | 0.50[B] | 0.28 | |
| | 5 | 1 | 0.00 | 2 | <0.1[C] | NA | |
| | 6 | 1 | 0.00 | 2 | <0.1[C] | NA | |
| October | 1 | 1 | 2.68 | 3 | 1.47[A] | 0.12 | $F_{1,17} = 42.30$, $P = <0.001$ |
| | 2 | 1 | 2.68 | 3 | 1.50[A] | 0.00 | |
| | 3 | 1 | 2.66 | 3 | 1.37[A] | 0.06 | |
| | 4 | 1 | 1.49 | 3 | 0.97[B] | 0.15 | |
| | 5 | 1 | 0.75 | 3 | 0.47[C] | 0.06 | |
| | 6 | 1 | 0.36 | 3 | 0.20[C] | 0.10 | |
| January | 1 | 1 | 2.60 | 3 | 1.40[A] | 0.20 | $F_{1,11} = 190.93$, $P = <0.001$ |
| | 2 | 1 | 2.62 | 3 | 1.30[A] | 0.20 | |
| | 3 | 1 | 2.63 | 3 | 1.27[A] | 0.21 | |
| | 4 | 1 | 2.32 | 3 | 1.07[A] | 0.12 | |

[a] Red Buttes Laboratory and ELI measured TA-N using colorimetry.
[b] The same letter following the value indicates that the means of the ELI analyses did not differ significantly (P>0.05) among sites within the month.

Table 2-13. Un-ionized ammonia nitrogen (UIA) analyzed at Red Buttes Laboratory - Laramie, Wyoming (RB) and Energy Labs Inc. - Billings, Montana (ELI). Duplicate samples were collected into two different bottles, one preserved (ELI) and one un-preserved (RB) from the same location in the creek. Additional preserved samples collected throughout the study week were analyzed at ELI. ANOVA analysis with a Tukey's mean separation test was used to determine differences between sites using only ELI data. Refer to Appendix D for a complete dataset.

| Month | Site | Red Buttes Lab[a] | | Energy Labs Inc.[a] | | | Between Labs Comparison |
|---|---|---|---|---|---|---|---|
| | | n | UIA (mg N/L) | n | Ave. UIA (mg N/L)[b] | S.D. | |
| July/August | 0 | 1 | 0.022 | 2 | <0.001[B] | NA | $F_{1,11} = 1.17$, $P = 0.30$ |
| | 1 | 1 | 0.063 | 2 | 0.036[A] | 0.008 | |
| | 2 | 1 | 0.091 | 2 | 0.051[A] | 0.009 | |
| | 3 | 1 | 0.132 | 2 | 0.083[A] | 0.020 | |
| | 4 | 1 | 0.105 | 2 | 0.111[A] | 0.080 | |
| | 5 | 1 | 0.000 | 2 | <0.001[B] | NA | |
| | 6 | 1 | 0.000 | 2 | <0.001[B] | NA | |
| October | 1 | 1 | 0.024 | 3 | 0.027[A] | 0.013 | $F_{1,17} = 0.02$, $P = 0.88$ |
| | 2 | 1 | 0.044 | 3 | 0.034[A] | 0.011 | |
| | 3 | 1 | 0.070 | 3 | 0.049[A] | 0.014 | |
| | 4 | 1 | 0.044 | 3 | 0.047[A] | 0.026 | |
| | 5 | 1 | 0.033 | 3 | 0.033[A] | 0.018 | |
| | 6 | 1 | 0.020 | 3 | 0.021[A] | 0.020 | |
| January | 1 | 1 | 0.015 | 3 | 0.013[B] | 0.005 | $F_{1,11} = 7.19$, $P = 0.02$ |
| | 2 | 1 | 0.056 | 3 | 0.026[AB] | 0.003 | |
| | 3 | 1 | 0.060 | 3 | 0.041[A] | 0.012 | |
| | 4 | 1 | 0.063 | 3 | 0.048[A] | 0.022 | |

[a] Un-ionized ammonia was calculated using field pH and temperature measured at collection.
[b] The same letter following the value indicates that the means of the ELI analyses did not differ significantly (P>0.05) among sites within the month.

Natural logarithm of total ammonia nitrogen was significantly negatively related to the distance downstream from the discharge point in all three months (Table 2-14). Natural logarithm total ammonia nitrogen was significantly positively related to water temperature in October and January but not in July/August. A consistent negative relationship existed between ln(TA-N) and pH in all three months, although it was only significant in July/August and October. When all three seasons were combined ln(TA-N) was significantly related to a combination of distance and temperature (Table 2-14). If the regression coefficients from the multiple linear regression are applied to the drainage, on average ammonia concentrations decrease by ~18%/km (i.e., $e^{-0.201} = 0.818$), and by approximately 6%/° C (i.e., $e^{-0.058} = 0.944$). This regression coefficient for distance indicates that 90% of the original TA-N in the effluent would be removed after the effluent flowed 11.5 km downstream.

The means of the ratio of TA-N in Beaver Creek to the CMC or CCC less than one would indicate compliance with USEPA (1999) ammonia criteria. The acute criterion ratio (TA-N/CMC) was less than one at all sites during all three field weeks (Table 2-15, Fig. 2-9). The chronic criterion ratio (TA-N/CCC) with early life stage (ELS) present or absent, was greater than one at Site 3 and 4 during July/August but only the TA-N/CCC with ELS present ratio was greater than one at Site 4 during October and January. Both TA-N/CCC with ELS present and absent exceeded the chronic criteria in July/August at Sites 3 and 4 in the afternoon, but not in the morning at the same location. The mean of the ratio of the morning and afternoon single grab samples for TA-N/CCC at Sites 3 and 4 over the 4 d period was >1.0 (1.1 and 1.2, respectively; n = 2).

Table 2-14. Natural logarithm of total ammonia nitrogen (TA-N) concentration in Beaver Creek in the Powder River Basin, Wyoming, was negatively related to the pH and distance downstream from the discharge point in all three field weeks, (i.e., ammonia decreased as pH and distance from the outfall increased). Natural logarithm of total ammonia nitrogen decreased as the temperature decreased in October 2006 (O) and January 2007 (J) but not in July/August 2006 (JA). When all three seasons were combined, ln(TA-N) decreased as the combination of distance from the outfall and water temperature increased. Standard error listed parenthetically.

| Regression | Month | Intercept | Slope | $t_{slope}$ | $r^2$ | P |
|---|---|---|---|---|---|---|
| ln(TA-N) on distance | JA | 0.243 (0.347) | -0.261 (0.048) | -12.22 | 0.94 | <0.0001 |
| | O | 0.415 (0.081) | -0.136 (0.011) | -12.12 | 0.90 | <0.0001 |
| | J | 0.281 (0.046) | -0.061 (0.025) | -2.45 | 0.38 | 0.034 |
| ln(TA-N) on temperature | JA | -1.898 (3.493) | 0.042 (0.159) | 0.26 | 0.00 | 0.798 |
| | O | -1.809 (0.448) | 0.118 (0.032) | 3.72 | 0.46 | 0.002 |
| | J | -0.088 (0.095) | 0.025 (0.007) | 3.43 | 0.54 | 0.006 |
| ln(TA-N) on pH | JA | 21.382 (3.846) | -2.655 (0.456) | -5.83 | 0.77 | <0.0001 |
| | O | 10.163 (2.578) | -1.262 (0.313) | -4.04 | 0.50 | 0.001 |
| | J | 2.133 (0.756) | -0.239 (0.094) | -2.54 | 0.39 | 0.26 |
| ln(TA-N) on distance and temperature during all months | __ | 1.316 (0.203) | | | 0.85 | <0.0001 |
| --distance (as km) | | | -0.201 (0.013) | -15.36 | | |
| --temperature (as °C) | | | -0.058 (0.011) | -5.11 | | |

Table 2-15. Criterion maximum concentration (CMC), criterion continuous concentration (CCC), the ratio of TA-N/CMC and the ratio of TA-N/CCC are listed below. All TA-N analyses were performed at Energy Labs – Billings, Montana. Ratios >1 would indicate the criteria were exceeded (EPA 1999). TA-N = total ammonia nitrogen, Salm = salmonid fish, ELS = Early life stage.

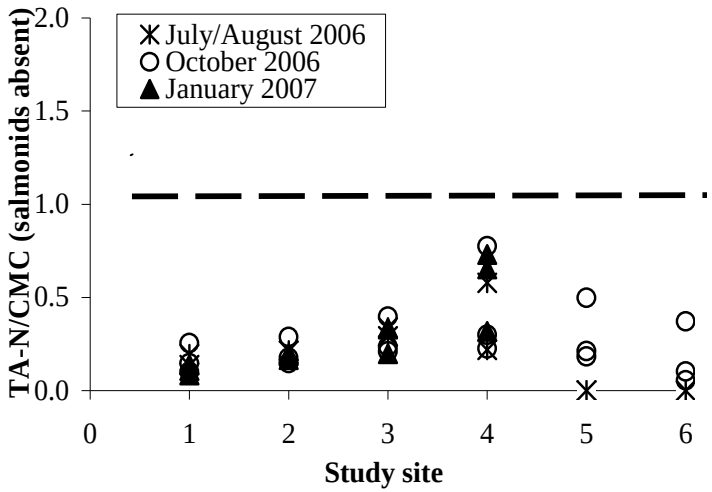
| | | | | | | | | CMC (mg N/L) | | CCC (mg N/L) | | TA-N/CMC | | TA-N/CCC | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Month | Site | Date | Time | Field pH | Field temp (˚C) | $pK_a$ | TA-N | Salm. absent | Salm. present | ELS absent | ELS present | Salm. absent | Salm. present | ELS absent | ELS present |
| July/August | 1 | 7/31/06 | 0623 | 7.91 | 20.2 | 9.40 | 1.30 | 9.95 | 6.64 | 1.91 | 1.91 | 0.13 | 0.20 | 0.68 | 0.68 |
| | 1 | 8/3/06 | 1500 | 7.58 | 23.4 | 9.30 | 1.60 | 17.58 | 11.74 | 2.29 | 2.29 | 0.09 | 0.14 | 0.70 | 0.70 |
| | 2 | 7/31/06 | 0655 | 8.00 | 19.9 | 9.41 | 1.20 | 8.41 | 5.62 | 1.72 | 1.72 | 0.14 | 0.21 | 0.70 | 0.70 |
| | 2 | 8/3/06 | 1510 | 7.88 | 23.9 | 9.28 | 1.50 | 10.51 | 7.02 | 1.57 | 1.57 | 0.14 | 0.21 | 0.96 | 0.96 |
| | 3 | 7/31/06 | 0728 | 8.24 | 18.2 | 9.46 | 1.20 | 5.30 | 3.54 | 1.33 | 1.33 | 0.23 | 0.34 | 0.90 | 0.90 |
| | 3 | 8/3/06 | 1445 | 8.16 | 26.0 | 9.22 | 1.20 | 6.19 | 4.13 | 0.91 | 0.91 | 0.19 | 0.29 | 1.32 | 1.32 |
| | 4 | 7/31/06 | 0755 | 8.74 | 20.2 | 9.40 | 0.30 | 2.05 | 1.37 | 0.51 | 0.51 | 0.15 | 0.22 | 0.59 | 0.59 |
| | 4 | 8/3/06 | 1425 | 8.81 | 22.8 | 9.31 | 0.70 | 1.81 | 1.21 | 0.38 | 0.38 | 0.39 | 0.58 | 1.84 | 1.84 |
| | 5 | 7/31/06 | 0835 | 8.95 | 17.9 | 9.47 | 0.00 | 1.43 | 0.96 | 0.42 | 0.42 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 5 | 8/3/06 | 1410 | 8.83 | 24.6 | 9.26 | 0.00 | 1.75 | 1.17 | 0.33 | 0.33 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 6 | 7/31/06 | 0905 | 9.09 | 17.9 | 9.47 | 0.00 | 1.16 | 0.77 | 0.34 | 0.34 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 6 | 8/3/06 | 1345 | 8.93 | 26.0 | 9.22 | 0.00 | 1.48 | 0.99 | 0.26 | 0.26 | 0.00 | 0.00 | 0.00 | 0.00 |

Table 2-15 (cont.)

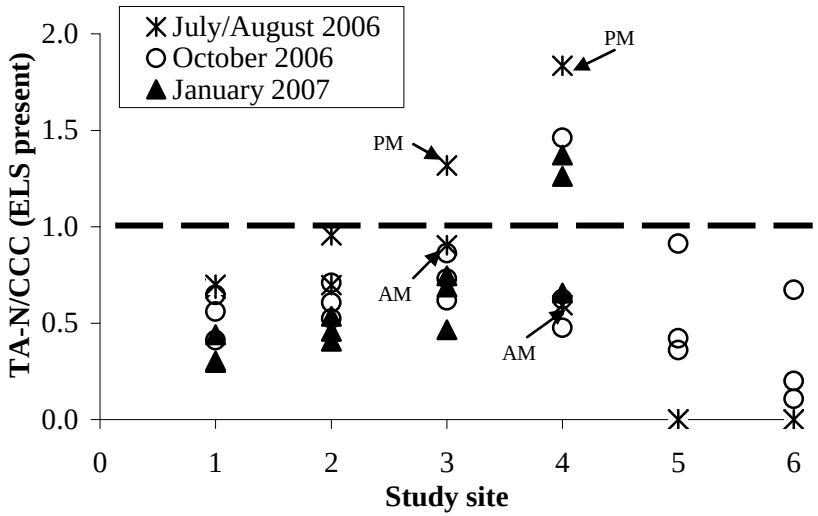
| Month | Site | Date | Time | Field pH | Field temp (˚C) | $pK_a$ | TA-N | CMC (mg N/L) | | CCC (mg N/L) | | TA-N/CMC | | TA-N/CCC | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Salm. absent | Salm. present | ELS absent | ELS present | Salm. absent | Salm. present | ELS absent | ELS present |
| October | 1 | 10/16/06 | 0730 | 7.47 | 18.8 | 9.44 | 1.40 | 20.79 | 13.89 | 3.40 | 3.40 | 0.07 | 0.10 | 0.41 | 0.41 |
| | 1 | 10/16/06 | 1300 | 7.63 | 19.2 | 9.43 | 1.60 | 16.23 | 10.84 | 2.85 | 2.85 | 0.10 | 0.15 | 0.56 | 0.56 |
| | 1 | 10/19/06 | 1020 | 8.01 | 16.1 | 9.53 | 1.40 | 8.25 | 5.51 | 2.17 | 2.17 | 0.17 | 0.25 | 0.65 | 0.65 |
| | 2 | 10/16/06 | 0800 | 7.67 | 18.5 | 9.45 | 1.50 | 15.19 | 10.14 | 2.86 | 2.86 | 0.10 | 0.15 | 0.52 | 0.52 |
| | 2 | 10/16/06 | 1330 | 7.77 | 19.0 | 9.43 | 1.50 | 12.80 | 8.55 | 2.47 | 2.47 | 0.12 | 0.18 | 0.61 | 0.61 |
| | 2 | 10/19/06 | 1040 | 8.04 | 15.8 | 9.54 | 1.50 | 7.79 | 5.21 | 2.11 | 2.11 | 0.19 | 0.29 | 0.71 | 0.71 |
| | 3 | 10/16/06 | 0845 | 7.91 | 17.6 | 9.48 | 1.40 | 9.95 | 6.64 | 2.26 | 2.26 | 0.14 | 0.21 | 0.62 | 0.62 |
| | 3 | 10/16/06 | 1400 | 7.97 | 18.9 | 9.44 | 1.40 | 8.90 | 5.94 | 1.92 | 1.92 | 0.16 | 0.24 | 0.73 | 0.73 |
| | 3 | 10/19/06 | 1130 | 8.28 | 15.2 | 9.56 | 1.30 | 4.90 | 3.27 | 1.51 | 1.51 | 0.27 | 0.40 | 0.86 | 0.86 |
| | 4 | 10/16/06 | 0930 | 8.24 | 11.1 | 9.69 | 0.80 | 5.30 | 3.54 | 2.10 | 1.68 | 0.15 | 0.23 | 0.38 | 0.48 |
| | 4 | 10/16/06 | 1430 | 8.27 | 12.0 | 9.66 | 1.00 | 5.00 | 3.34 | 1.88 | 1.60 | 0.20 | 0.30 | 0.53 | 0.62 |
| | 4 | 10/19/06 | 1230 | 8.72 | 6.6 | 9.85 | 1.10 | 2.13 | 1.42 | 1.22 | 0.75 | 0.52 | 0.77 | 0.90 | 1.46 |
| | 5 | 10/16/06 | 1010 | 8.45 | 8.5 | 9.78 | 0.50 | 3.53 | 2.35 | 1.75 | 1.19 | 0.14 | 0.21 | 0.29 | 0.42 |
| | 5 | 10/16/06 | 1500 | 8.49 | 11.1 | 9.69 | 0.40 | 3.26 | 2.18 | 1.38 | 1.11 | 0.12 | 0.18 | 0.29 | 0.36 |
| | 5 | 10/19/06 | 1330 | 8.92 | 7.0 | 9.83 | 0.50 | 1.51 | 1.01 | 0.89 | 0.55 | 0.33 | 0.50 | 0.56 | 0.91 |
| | 6 | 10/16/06 | 1100 | 8.55 | 8.5 | 9.78 | 0.20 | 2.91 | 1.95 | 1.48 | 1.00 | 0.07 | 0.10 | 0.14 | 0.20 |
| | 6 | 10/16/06 | 1515 | 8.59 | 11.1 | 9.69 | 0.10 | 2.70 | 1.80 | 1.17 | 0.94 | 0.04 | 0.06 | 0.09 | 0.11 |
| | 6 | 10/19/06 | 1435 | 9.06 | 7.4 | 9.82 | 0.30 | 1.21 | 0.81 | 0.71 | 0.45 | 0.25 | 0.37 | 0.42 | 0.67 |

| | | | | | | | | CMC (mg N/L) | | CCC (mg N/L) | | TA-N/CMC | | TA-N/CCC | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Month | Site | Date | Time | Field pH | Field temp (˚C) | $pK_a$ | TA-N | Salm. absent | Salm. present | ELS absent | ELS present | Salm. absent | Salm. present | ELS absent | ELS present |
| January | 1 | 1/23/07 | 0755 | 7.33 | 15.3 | 9.55 | 1.40 | 25.23 | 16.85 | 4.73 | 4.73 | 0.06 | 0.08 | 0.30 | 0.30 |
| | 1 | 1/24/07 | 1650 | 7.60 | 15.9 | 9.53 | 1.60 | 17.03 | 11.37 | 3.64 | 3.64 | 0.09 | 0.14 | 0.44 | 0.44 |
| | 1 | 1/27/07 | 0920 | 7.61 | 14.0 | 9.60 | 1.20 | 16.76 | 11.19 | 4.07 | 3.94 | 0.07 | 0.11 | 0.29 | 0.30 |
| | 2 | 1/23/07 | 0850 | 7.91 | 14.8 | 9.57 | 1.10 | 9.95 | 6.64 | 2.71 | 2.71 | 0.11 | 0.17 | 0.41 | 0.41 |
| | 2 | 1/24/07 | 1630 | 7.84 | 15.7 | 9.54 | 1.50 | 11.30 | 7.55 | 2.80 | 2.80 | 0.13 | 0.20 | 0.53 | 0.53 |
| | 2 | 1/27/07 | 0910 | 7.89 | 13.9 | 9.60 | 1.30 | 10.32 | 6.89 | 2.95 | 2.84 | 0.13 | 0.19 | 0.44 | 0.46 |
| | 3 | 1/23/07 | 1015 | 7.96 | 14.2 | 9.59 | 1.20 | 9.06 | 6.05 | 2.63 | 2.58 | 0.13 | 0.20 | 0.46 | 0.47 |
| | 3 | 1/24/07 | 1540 | 8.12 | 14.7 | 9.57 | 1.50 | 6.69 | 4.47 | 2.01 | 2.01 | 0.22 | 0.34 | 0.75 | 0.75 |
| | 3 | 1/27/07 | 0940 | 8.27 | 12.3 | 9.65 | 1.10 | 5.00 | 3.34 | 1.85 | 1.60 | 0.22 | 0.33 | 0.60 | 0.69 |
| | 4 | 1/23/07 | 1115 | 8.30 | 5.8 | 9.88 | 1.00 | 4.71 | 3.15 | 2.47 | 1.52 | 0.21 | 0.32 | 0.40 | 0.66 |
| | 4 | 1/24/07 | 1530 | 8.58 | 8.5 | 9.78 | 1.20 | 2.75 | 1.84 | 1.40 | 0.95 | 0.44 | 0.65 | 0.86 | 1.26 |
| | 4 | 1/27/07 | 1000 | 8.74 | 1.0 | 10.05 | 1.00 | 2.05 | 1.37 | 1.18 | 0.73 | 0.49 | 0.73 | 0.85 | 1.37 |

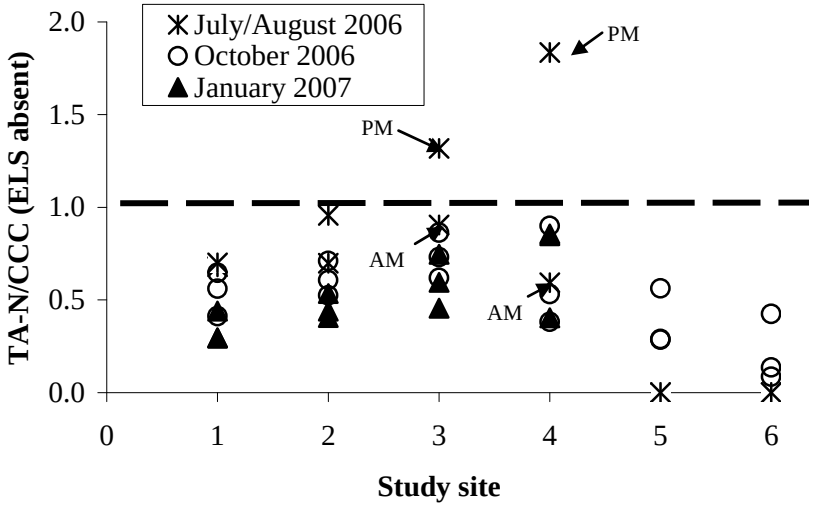
Figure 2-9. Graphical representations of water collected from Beaver Creek in the Powder River Basin, Wyoming during July/August 2006, October 2006 and January 2007 field bouts. (a) Ratio of total ammonia nitrogen/criterion maximum concentration (TA-N/CMC) with salmonids absent indicating no exceedances of the acute criteria during all three field bouts (b) ratio of total ammonia nitrogen/criterion continuous concentration (TA-N/CCC) with early life stage (ELS) present, indicating exceedances of the chronic criteria at Site 4 for all three field bouts (c) TA-N/CCC with ELS absent, indicating exceedances of the chronic criteria in July at Sites 3 and 4 only in the afternoon when pH increased due to photosynthesis. In July/August 2006 field bout, the TA-N/CCC with ELS absent or present from the same site sampled in the morning did not exceed the criteria.


July/August 2006
October 2006
January 2007
TA-N/CMC (salmonids absent)
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Study site

a.)


July/August 2006
October 2006
January 2007
TA-N/CCC (ELS present)
PM
PM
AM
AM
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Study site

b.)


July/August 2006
October 2006
January 2007
TA-N/CCC (ELS absent)
PM
PM
AM
AM
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Study site

c.)

I compared the FHM UIA LC50s in Appendix 4 of USEPA (1999) to the UIA values in Beaver Creek during this study. The UIA concentrations along Beaver Creek were well below the FHM LC50s (Figure 2-10). Also, I compared preserved TA-N analyses (ELI) collected in July/August 2006 (0.0 - 1.6 mg N/L) to the seasonal ammonia limits in the Willow Creek watershed general permit (3.1 mg N/L in July and 2.2 mg N/L in August). Although, the Beaver Creek discharge used in my study is greater than 1.6 km from the Powder River and therefore watershed general permit limits do not apply, the TA-N values were in compliance (Table 2-16).

Fish were seen at all sites in July/August and at Sites 2 and 3 during October and January (Appendix C). Toad metamorphs, toads, bull snakes, intermountain wandering garter snakes, and muskrats were also observed along the drainage during the study periods. Turner (2007) observed early life stage boreal chorus frogs (*Pseudacris maculate*) and Woodhouse toads (*Bufo woodhousii*) near the outfall (Site 2). He concluded the amphibians were using portions of the river as breeding sites, where habitat was suitable, with special notation of later stage metamorphosis in the boreal chorus frogs (Gosner stage 41). He also stated the water in the arid landscape was a possible attractant and resource to the aquatic community (personal communication, Bill Turner, Wyoming Game and Fish, Herpetological Coordinator, Laramie, Wyoming; February 1, 2007 – email correspondence). Davis (2007) found seven fish species along Beaver Creek (Table 2-17). Fathead minnows were found in various sizes at every site, suggesting growth and reproduction (Fig. 2-11).

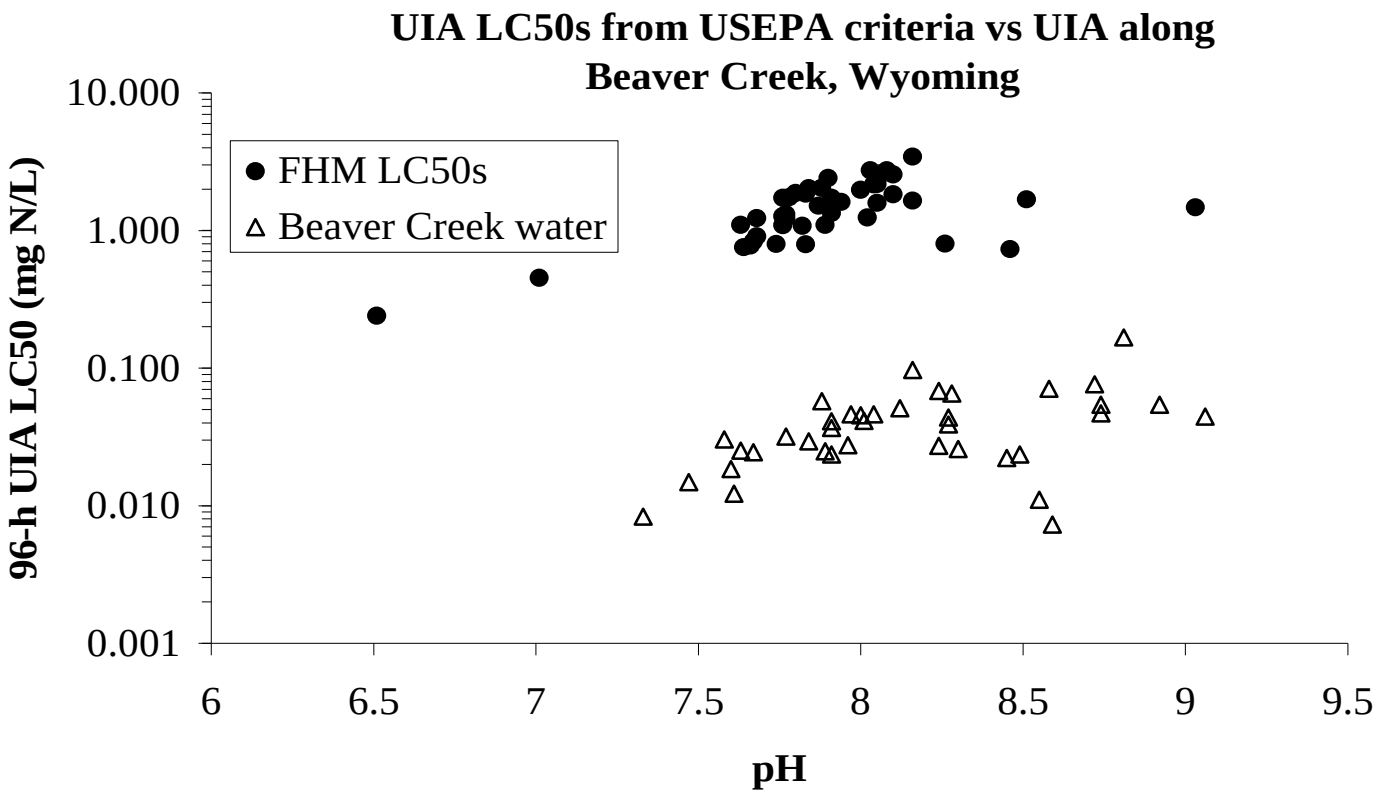



Figure 2-10. Un-ionized ammonia (UIA) LC50s for fathead minnow (*Pimephelas promelas*) tabulated in USEPA (1999) compared to UIA and pH along Beaver Creek. UIA calculated from Energy Labs Inc. data. The UIA concentrations along Beaver Creek are ≥10X below the fathead minnow LC50s used to develop freshwater criteria for ammonia.

Table 2-16. Wyoming Department of Environmental Quality/Water Quality Division (WDEQ/WQD) derived the below total ammonia nitrogen (TA-N) limits for the Willow Creek watershed based general permit using pH, temperature and flow averages of USGS gaging station at Sussex along the Powder River in a waste load allocation calculation (WLA). These calculations are similar to values that will be used in the WLA for Beaver Creek (personal communication, Jason Thomas, WDEQ/WQD, August 30, 2007). All TA-N collected 7/31/06 and 8/3/06 at Sites 1 to 6 were below the corresponding July and August limits (0.0 - 1.6 mg N/L).

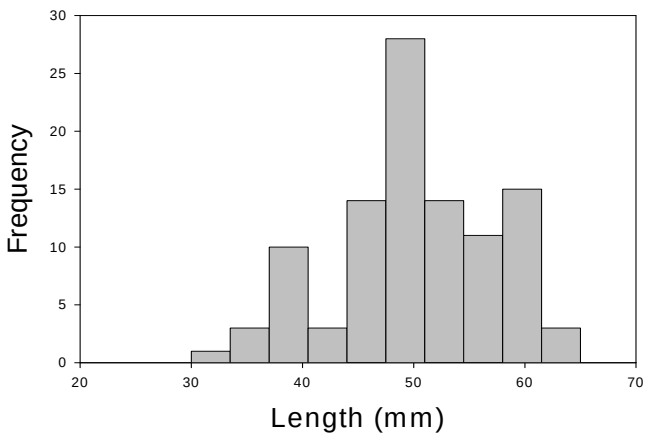
| Month | Powder River (7Q10) low flow (MGD) | CBNG discharge rate (MGD) | Combined flow (MGD) | Chapter1 water quality standard, chronic (CCC, mg TA-N/L) | Powder River background concentration (mg TA-N/L) | Assimilative capacity of stream (mg TA-N/L) | TA-N limit at a CBNG discharge (mg TA-N/L) |
|---|---|---|---|---|---|---|---|
| July | 5.4 | 0.36 | 5.71 | 1.06 | 0.11 | 0.95 | **3.1** |
| August | 3.1 | 0.36 | 3.46 | 1.20 | 0.11 | 1.09 | **2.2** |

Table 2-17. Numbers of fish seined in Beaver Creek in the Powder River Basin, Wyoming on July 26 and 27, 2006 (Davis 2007). Percentage of total fish per site is in parentheses.

| | | Site | | | |
|---|---|---|---|---|---|
| | Davis (2007) [a] | D | C | B | A |
| Species | This Study [b] | 2 | 5 | 6 | ---- |
| Fathead minnow (*Pimephales promelas*) | | 370 (74.9%) | 148 (39.3%) | 391 (59.2%) | 582 (72.8%) |
| Sand shiner (*Notropis stramineus)* | | 70 (14.2%) | 120 (31.8%) | 35 (5.3%) | 207 (25.9%) |
| Plains killifish (*Fundulus zebrinus*) | | 51 (10.3%) | 108 (28.6%) | 227 (34.3%) | 4 (0.5%) |
| White sucker (*Catostomus commersoni)* | | | 1 (0.3%) | | 4 (0.5%) |
| Black bullhead (*Ameiures melas*) | | 1 (0.2%) | | 7 (1.1%) | 3 (0.3%) |
| Green sunfish (*Lepomis cyanellus*) | | | | 1 (0.1%) | |
| Creek chub (*Semotilus atromaculatus*) | | 2 (0.4%) | | | |
| | Total | 494 | 377 | 661 | 800 |

[a] Site A, B, C, and D were 1.6, 10, 15, and 24 km upstream from Powder River, respectively.
[b] Site 2, 5, and 6 were 0.04, 10, and 14 km downstream from Site 1 in this study.

Figure 2-11. Fathead minnow (*Pimephales promelas*) length-frequency histograms seined (1/4“mesh) July 26-27, 2006 at four sites along Beaver Creek in the Powder River Basin, Wyoming, from Williams Production RMT Company discharge point WY0046922-001 to the confluence with the Powder River (PR) (Davis 2007). a) Davis Site A, no correlating Site (~1.6-km from PR) b) Davis Site B, my Site 6 (~10-km from PR), c) Davis Site C, my Site 5 (~15-km from PR, and d) Davis Site D, my Site 2 (~24- km from PR).


Frequency
Length (mm)
0
5
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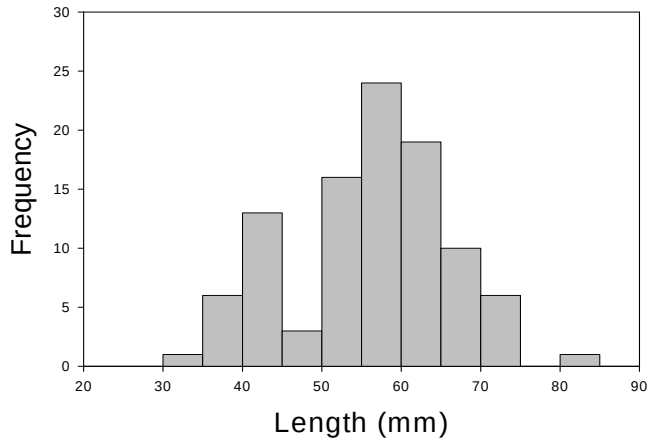
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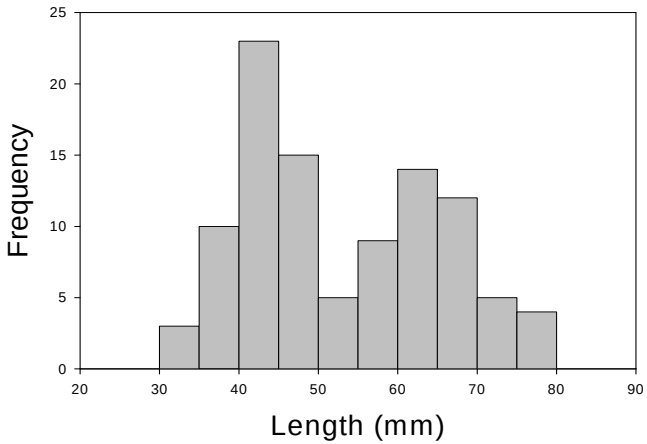
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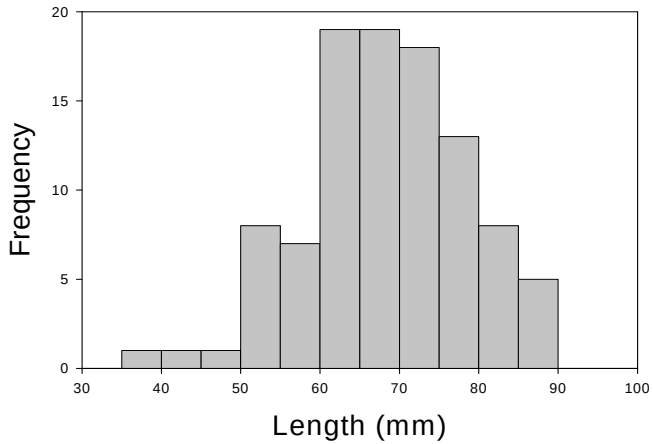
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## DISCUSSION

Comparison of Field to Laboratory Environments

When losses of fish due to damage of cages by muskrats in July/August and due to low water temperatures in October 2006 and January 2007 are factored out, survival of FHM in Beaver Creek and in both types of laboratory toxicity tests indicate no apparent acute toxicity within the 14 km study reach. However, some fish died as pH and, thus, un-ionized ammonia concentrations increased when exposure water in the ambient-pH tests was not renewed for 96 h. This supports my hypothesis that pH drift can artifactually increase the toxicity of ammonia in CBNG produced waters tested in the laboratory. Because pH did not drift as much in the $CO_2$ pH-controlled tests, fish did not die when the exposure waters in those tests were not renewed for 96 h. Therefore, the $CO_2$ pH-controlled method appears to be the most acceptable method for testing CBNG produced water in the laboratory.

As expected, pH increased as the $CO_2$-saturated produced water flowed downstream in Beaver Creek, but at a much slower rate than would occur if the same water had degassed in a glass beaker in the laboratory. The pH increased gradually as the TA-N decreased, most likely due to $CaCO_3$ formation and consequent pH buffering while the ammonia probably was assimilated by plants and microbes in the stream. The TA-N would be 90% assimilated after 11.5 km per the multiple linear regression equation which would decrease the toxicity of the water and increase primary production in the aquatic ecosystem. Calcium and $Ba^{2+}$ decreased longitudinally from the outfall as $SO_4^{2-}$ increased, probably due to dissolution of gypsum ($CaSO_4$) followed by precipitation of $CaCO_3$ and barite ($BaSO_4$). Alkalinity, $HCO_3^-$, SAR, $F^-$, $Cl^-$, $K^+$, and DOC varied little

from Site 1 to Site 6, indicating little groundwater or precipitation influence during the study period. Alkalinity was primarily in the form of $HCO_3^-$ at pH 7.3 to 8.2. Overall, interactions with the atmosphere and stream substrate not replicated in a laboratory environment provide mitigating factors that help decrease toxicity of the CBNG produced water to aquatic organisms.

Field Observations

Observations of fish of varying species and lengths and of other aquatic life apparently breeding near this CBNG outfall further indicate CBNG water in this arid landscape is a possible attractant and wildlife resource. The conclusions of the Wyoming Department of Game and Fish (Turner 2007), the richness and diversity determined by Davis (2007) and my field observations of aquatic plants and animals suggest no overt adverse effects from CBNG effluent discharged to Beaver Creek during the 2006 to 2007 study periods. However, the acute toxicity at Beaver Creek water to 2-d-old FHM larvae reported by Farag et al. (2007) indicates at least part of the aquatic community might be adversely affected by constituent(s) in the water. Inclusive eco-assessments would be required to determine community level effects of CBNG effluent.

Comparison of Ammonia from Stream to Laboratory

Total ammonia nitrogen concentrations analyzed at RB on unpreserved samples were generally higher than paired analyses of acid-preserved samples performed at ELI (Table 2-11), possibly due to ammonification during transport and storage of the unpreserved samples. This would imply that analyses of acid-preserved ($H_2SO_4$ to pH < 2.0) samples probably more accurately represent in-stream ammonia concentrations. Unfortunately, WET tests cannot be conducted using acid-preserved

waters because the acid will be toxic to the aquatic organisms. Therefore, based on these results and those presented in Chapter 3, I conclude that static-renewal WET tests using unpreserved CBNG produced water might be biased by artifactually elevated TA-N concentrations.

Comparison to Current Regulatory Standards

Because the UIA concentrations along Beaver Creek were ≥10× below the UIA LC50s for FHM that are tabulated in the USEPA (1999) ammonia criteria document, un-ionized ammonia should not have been a major contributor to acute toxicity of the CBNG effluent-dominated water in Beaver Creek to FHM in this study. Also, because the TA-N/CMC ratio at all sites for all study periods was less than 1.0, >95% of the aquatic community is nominally being protected from acute effects in Beaver Creek. The TA-N/CCC with early life stages present ratio was greater than 1.0 at Site 3 during July/August and at Site 4 during all field bouts, indicating possible chronic toxic effects to >5% of the aquatic community. Additionally, the TA-N/CCC ratio was greater than 1.0 in the afternoon at Sites 3 and 4 in July/August, less than 1.0 at the same site in the morning, and yet the mean of the quotients over the 4 d averaging period was slightly greater than 1.0, indicating non-compliance with the chronic criteria.

According to current WDEQ/WQD policy, the CCC for ammonia that is used in a WLA calculation of CBNG effluent flowing into a tributary of the Powder River is based on the average pH, temperature, and flow measured at the closest USGS gaging station in the Powder River (i.e. measurements at USGS gaging stations on the Powder River). Because current regulatory limits for ammonia apply only to discharges <1.6 km from the main stem of the Powder River and the pH and temperature for the calculation are from

the Powder River, it would seem the WDEQ/WQD is only protecting the aquatic community in the Powder River and not in the ephemeral drainages. If the aquatic community of the ephemeral drainage is to be protected, the temperature and pH of the CBNG discharge should be used to calculate a waste load allocation. However, because the flow of the ephemeral drainage is intermittent by definition, the protection of aquatic life may not be necessary at distances >1.6 km from the Powder River.

Comparison to Other Toxicity Studies

Three previous studies have been conducted on the aquatic toxicity of CBNG produced water. Forbes (2003) concluded that only 2 out of 30 CBNG-related water samples collected from outfalls and PRB streams were acutely toxic to FHM. Because the two CBNG-related waters that caused significant acute toxicity in the laboratory were stream samples that had unknown CBNG effluent contribution, it cannot be concluded that a CBNG effluent contributed to their toxicity (Table 2-18). Also, samples collected from the same locations 21 d after the original samples were not acutely toxic to FHM. Forbes (2003) indicated it was unlikely the differences in toxicity were biologically significant but rather were statistical artifacts based on differences in control mortality. She also noted the un-ionized ammonia concentrations for the two stream samples (both 0.01 mg N/L) were well below published acute LC50s at the sample pH (0.73-3.44 mg/L at pH 8.0-8.5 and 1.68 mg N/L at pH 8.5-9.0; USEPA 1999), also implying ammonia did not contribute to the observed toxicity. Therefore, if the small differences in mortality were not biologically significant, my findings would correlate with Forbes (2003).

Table 2-18. Comparison of acute toxicity of CBNG-related waters to fathead minnows (*Pimephelas promelas*). Forbes (2003) had significant mortality in 96-h laboratory WET tests in two of 30 CBNG-related samples from the Powder River Basin (PRB), Wyoming, and Farag et al. (2007) had significant mortality using in-situ cages placed in Beaver Creek in the PRB. * = statistically different from control.

| Study | Sample location | Date | pH | TA-N (mg N/L) | $Na^+$ (mg/L) | $HCO_3^-$ (mg/L) | WET test | Age of FHM (d) | 96-h survival |
|---|---|---|---|---|---|---|---|---|---|
| Forbes (2003) | Spotted Horse Creek - N | 6/4/2001 | 8.87 | 0.10 | 604.7 | 1077 | Lab | < 1 | 92* |
| Forbes (2003) | Powder River below Spotted Horse Creek - M | 6/4/2001 | 8.45 | 0.07 | 513.3 | 251 | Lab | < 1 | 88* |
| Farag (2007) | Upper Beaver Creek (~40 m downstream from Site 6 in this study) | July/August 2006 | 9.0 (n = 4) | 0.5 | 664 | 1646 | Stream | 2 | 37* |
| Farag (2007) | Upper Beaver Creek (~40 m downstream from Site 6 in this study) | July/August 2006 | 9.0 (n = 4) | 0.5 | 664 | 1646 | Stream | 6 | 75 |
| This study | Site 6 (14-km from CBNG outfall) | July/August 2006 | 8.88 (on arrival) | < 0.10 | 646 | 1759 | Lab | 13 | 94 |
| This study | Site 6 (14-km from CBNG outfall) | July/August 2006 | 9.0 (n = 11) | < 0.10 | 646 | 1759 | Stream | 11 | 100 |

Farag et al. (2007) conducted in-stream toxicity tests in Beaver Creek during the same week as my July/August field bout. They also placed in-situ test cages in perennial Clear Creek and in the Powder River near Morehead, Montana as reference control sites. The Farag et al. (2007) location labeled "Upper Beaver" was ~ 40 m downstream from my Site 6, but another CBNG outfall discharged into Beaver Creek between the two sites. Bicarbonate and TA-N concentrations were 1646 mg/L and 0.5 mg N/L, respectively, at their site compared to $HCO_3^-$ and TA-N concentrations at Site 6 during my July/August field bout of 1759 mg/L and <0.10 mg N/L, respectively. The increase in ammonia at the Farag et al. site was most likely due to the influx of new CBNG water between Site 6 and their site. Because $HCO_3^-$ at Site 6 was greater than the Farag et al.'s at "Upper Beaver" site, the difference in $HCO_3^-$ concentration between the two sites could not be the cause of the higher toxicity reported by Farag et al.

Farag et al. (2007) reported 96-h toxicity (only 37% survival) to 2-d-old FHM larvae in in-situ cages in Beaver Creek but no significant toxicity (75% survival) to 6-d-old FHM larvae at the same site, both studies were compared to pooled reference fish in in-situ cages placed in Clear Creek and the Powder River (Table 2-18). In contrast, my in-stream toxicity test at Site 6 during the July/August 2006 bout had 100% survival (Table 2-18). The in-stream cages and protocol (Burton 2005a) that Farag et al. (2007) followed were the same as I used with the exceptions that Farag (2007) had smaller size mesh on the cages, the cages were kept in a covered container (no light) and they were opened daily to document survival. Additionally, my FHM larvae were older than the larvae used by Farag et al. (2007).

Generally, younger fish are more sensitive to toxins than are older fish (USEPA 2002b). USEPA acute criteria guidelines recommend 1- to14-d-old larvae for laboratory acute toxicity tests (USEPA 2002b), and Burton (2005a) recommended FHM larvae for in-situ caged tests be greater than 7-d-old. The larvae in my July/August field bout were 11-d-old at test initiation compared to 2- and 6-d-old larvae in Farag et al. (2007). Therefore, significant differences in the mortality of the 2-d-old larvae compared to the 6-d-old larvae at the Farag et al. (2007) site on Beaver Creek probably was caused by an age-specific difference in sensitivity, but the difference in mortality between the 2-d-old larvae at Farag et al.'s (2007) and my 11-d-old larvae at Site 6 might have been caused by a combination of age-specific differences in sensitivity, site-specific differences in ammonia concentration, or differences in the study design. Farag and co-workers plan to conduct additional field studies to determine if younger larvae in a natural environment are more susceptible than older larvae to possible toxicants in the CBNG produced water.

In laboratory waters that were prepared to mimic water quality in the Tongue River and the Powder River, 96-h LC50s for early life stage FHM exposed to $NaHCO_3$ were 1100 to 1600 mg $NaHCO_3$ /L (USGS 2006). A major-ion toxicity model (Mount et al. 1997), predicted acute toxicity of $NaHCO_3$ in simulated salt water as low as 310 mg/l using 1 to 7-d-old fish (LC50 < 310 – 1220 mg/L, Mount et al. 1997). In contrast, I measured $Na^+$ concentrations > 600 mg/L and $HCO_3^-$ concentrations > 1800 mg/L in Beaver Creek with no observed acute toxicity. This would seem to indicate that either (1) other components in Beaver Creek water or sediment mitigate toxic effects of $NaHCO_3$ to FHM or (2) the younger FHM larvae used by USGS (2006) and Mount et al. (1997) are more sensitive to $NaHCO_3$. The varying FHM life stages seined along a 24-km reach of

Beaver Creek (Davis 2007, Fig. 2-11) demonstrate that FHM grow and probably reproduce in the CBNG product-water-dominated stream, yet negative effects cannot be excluded. Additional field studies of the chronic effects (growth and reproduction) of CBNG product water using younger FHM larvae are needed to investigate that possibility.

In summary, although TA-N, $Na^+$ and $HCO_3^-$ are present at measurable concentrations in this CBNG effluent, the potential toxic effects of TA-N (and possibly $Na^+$ and $HCO_3^-$ ) are mitigated by biogeochemical interactions between the effluent and the stream ecosystem. However, because potential ammonification during transport of stream or outfall water to a laboratory and potential pH drift due to $CO_2$ degassing in the laboratory can bias the results of WET tests conducted in the laboratory, results of any WET test conducted in the laboratory (or even any static-renewal test conducted in the field) with CBNG-related water should be interpreted cautiously. The only reliable WET test would be an on-site, flow-through toxicity test using water pumped directly into test chambers in a bioassay trailer or other type of toxicity-testing environment.

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# CHAPTER 3

## Does Ammonification Occur during Transport of Water from Stream to Lab?

## ABSTRACT

Coalbed natural gas (CBNG) produced water extracted from deep coal seams in the Powder River Basin (PRB) of Wyoming contains measurable concentrations of ammonia. In some whole effluent toxicity (WET) tests required for CBNG effluent discharge permits, ammonia concentrations in unpreserved effluent have been higher than ammonia concentrations analyzed in acid-preserved samples of the same water; however, WET tests should not be conducted in such acidic conditions. Ammonia concentration in an effluent might increase during transport or storage of unpreserved samples due to transformation of organic nitrogen to ammonia via ammonification, thus increasing the ammonia concentration higher than in-stream concentrations and potentially biasing WET tests. To investigate this possibility, I compared collection and storage procedures, ammonia analytical methods, and nitrogen budgets in CBNG produced water discharging into Beaver Creek, Wyoming. In October 2006, few significant differences occurred among a variety of collection, storage, and analytical methods. However, in March 2007, ammonia concentrations differed considerably between preserved and unpreserved samples, implying ammonification during transport. This increase in ammonia in transported samples and/or in static-exposure testing chambers will substantially bias laboratory toxicity tests. Understanding these processes is valuable in interpretation and regulation of the potential toxicity of ammonia in CBNG produced waters.

# INTRODUCTION

Coalbed natural gas (CBNG) produced waters extracted from deep coal seams in the Powder River Basin of Wyoming contain measurable concentrations of ammonia (Chapter 2). Nitrogenous by-products of organic matter decomposition are probably the main source of ammonia in CBNG produced water. Because ammonia is a major toxicant of concern to aquatic organisms (Russo 1985), it is a toxicant of concern in CBNG effluents. Whole effluent toxicity (WET) tests, mandated by the Wyoming Department of Environmental Quality/Water Quality Division (WDEQ/WQD) on some effluents extracted from deeper coal seams in the PRB (WDEQ 2004), evaluate the total toxic effect of an effluent using aquatic organisms in a laboratory setting. The endpoint for acute WET tests is survival (USEPA 2002a), whereas chronic WET tests evaluate an aquatic organism's survival, growth and reproduction (USEPA 2002b).

Some CBNG produced waters from the PRB have failed laboratory WET tests (WDEQ 2003), and ammonia has been identified as one possible toxicant of concern in laboratory toxicity tests (CBMA 2007). Analysis of ammonia concentrations in CBNG product waters produced varying results when waters preserved with acid were compared to unpreserved waters. On some concurrent analyses of the same source water (one sample preserved and the other unpreserved), ammonia was observed to be higher after transport to the WET laboratory, indicating a possible transformation of nitrogen in the effluent during transport from the stream to the laboratory (personal communication , Dr. Frank Sanders, CBM Associates, Inc., Laramie, Wyoming, USA; April 2007). For example, unpreserved water for a WET test was collected at WY0051594 (Williams Production RMT Company) on January 3, 2005 and shipped to the toxicity testing

laboratory at Parametrix Environmental in Albany, Oregon. On receipt of the water, Parametrix measured a total ammonia nitrogen concentration of 8.5 mg N/L using an ion-selective electrode. In a duplicate sample of the same site water collected at the same time but acid-preserved, Energy Labs Inc. in Billings, Montana measured 2.0 mg TA-N/L using a colorimetric method (CBMA 2007, Appendix A). This increase during shipment of the unpreserved water could be explained if dissolved or particulate nitrogen-containing organic matter had been transformed to ammonia via ammonification. If ammonia concentrations at a laboratory are elevated above the in-stream concentrations, WET tests conducted in the laboratory could be biased. Although, this does not obviate the potential need to mitigate high concentrations of ammonia in CBNG effluents, knowledge about ammonification occurring during transport and storage of CBNG waters might be helpful in evaluating the extent of mitigation required to meet the regulatory requirements.

Various questions arise concerning ammonification in CBNG effluent. Does filtration, preservation, transport, or storage change the ammonia concentration from the stream to the lab? Is one TA-N analytical method more reliable with $CO_2$-saturated CBNG product water than another? Can reliable WET tests be conducted if ammonification does occur?

Approved TA-N analyses are performed using colorimetric (CM) or ion-selective-electrode (ISE) methods on unfiltered, acid-preserved ($H_2SO_4$ to pH < 2.0) samples (APHA et al. 1995), whereas WET tests are performed on unfiltered, unpreserved samples transported in a sealed container at 4 °C (USEPA 2002). The primary objective of this study is to determine if collection methods (filtration, preservation, transport and

storage) or analytical methods (CM and ISE) alter concentrations of TA-N. My hypothesis is that TA-N concentrations can change in produced water transported from the stream to the testing laboratory, particularly in unpreserved samples.

To test this, I conducted factorial studies analyzing nitrogen species in October 2006 and March 2007. In October, the water samples were filtered or unfiltered, acidified or non-acidified, stored in darkness or in light, and analyzed using the colorimetric or ISE method; in March the water samples were filtered or unfiltered, acidified or non-acidified, transported by ground or air, and analyzed using the colorimetric or ISE method. I used analytical data from both studies to calculate nitrogen budgets, which represented the nitrogen species that constituted the total nitrogen (TN) concentration. The TN concentration equals the sum of ionized ammonia nitrogen ($NH_4^+$-N), un-ionized ammonia nitrogen ($NH_3$-N, or UIA), nitrate ($NO_3^-$), nitrite ($NO_2^-$) and all other inorganic and organic nitrogen species. If inorganic and organic nitrogen species present in the CBNG effluent transform to ammonia during transport and storage, then laboratory TA-N concentrations would be higher than in-stream TA-N concentrations.

## STUDY AREA

The study area was along Beaver Creek, tributary to the Powder River, in Campbell County in northeast Wyoming (T48N R76W). Historically, Beaver Creek was an ephemeral drainage, with seasonal intermittent flow before CBNG development (personal communication, John Iberlin (landowner), Iberlin Land and Livestock, Gillette, Wyoming; February 18, 2007). It flows ~65 km from the headwaters to the Powder

River.

Approximately 150 to 180 CBNG wells are piped to a discharge point located 300 m from Beaver Creek (WY0046922, operated by Williams Production RMT Company). At this point (top-of-pipe), the water surfaced and flowed into two consecutive retention ponds. The effluent traveled through both ponds prior to entering an underground collection system. The water was piped to the top of a concrete flume that discharged into Beaver Creek. In the October 2006 study, I sampled water from the top-of-pipe; during March 2007, I sampled water from the top-of-pipe and in Beaver Creek, ~40 m downstream from the discharge of the CBNG water into Beaver Creek. During the two study periods, no water flowed in Beaver Creek upstream of the CBNG discharge point.

## WATER SAMPLING AND PROCESSING

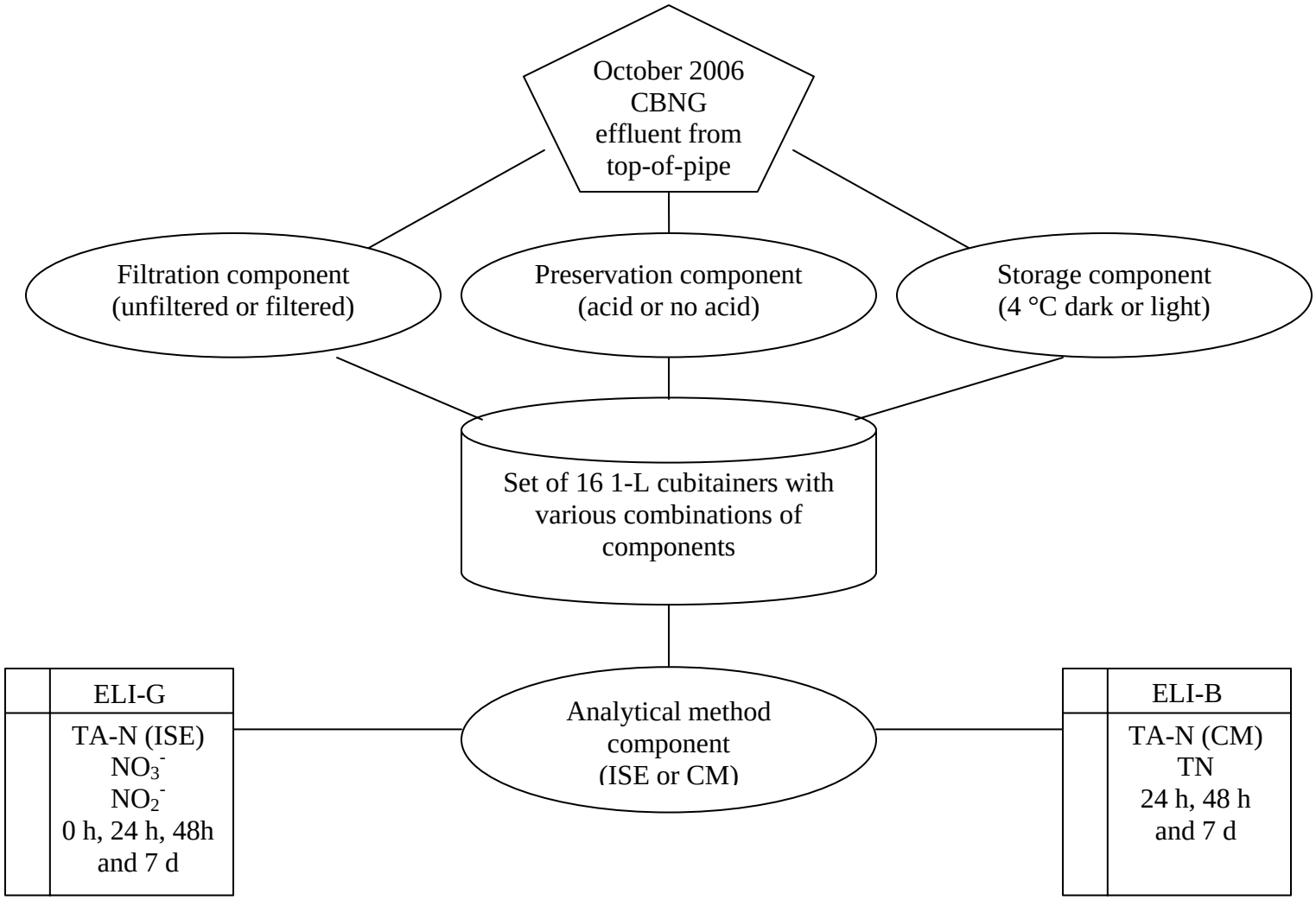
### October 2006 Study

The October study design incorporated filtration, preservation and storage components from one discharge point (top-of-pipe). Samples were unfiltered or filtered (0.45 μm Geotech Dispos-a-filter) on-site to determine if particulate versus dissolved organic nitrogen significantly increased the TA-N concentrations. Also on-site, one set of samples were acidified ($H_2SO_4$ to pH < 2.0) to determine if preserved versus unpreserved samples produced significant differences in TA-N concentrations. Finally, after the samples were received at the testing laboratories, sets were stored at 4 °C in either dark or light conditions to test the influence of storage condition. Colorimetric and

ISE analytical methods were conducted on designated sub-samples to determine if one method was preferred for use with CBNG produced water.

At top-of-pipe, water was collected into sixteen 1-L cubitainers using the preservation and filtration scheme diagram in Figure 3-1. In order to ensure pH < 2.0, $H_2SO_4$ was added to the designated cubitainers and the pH measured using a Model IQ125 Professional pH meter. The cubitainers were transported on ice as soon as possible to Energy Labs Inc. in Gillette, Wyoming (ELI-G) and Billings, Montana (ELI-B). After receipt at ELI-G, a sample from each bottle was analyzed for TA-N using the ISE (Orion 95-12 electrode/EPA A4500D) method at 0 h (time of receipt), 24 h, 48 h and 7 d after arrival at the laboratory. Also at ELI-G, $NO_3^-$ and $NO_2^-$ (ion chromatography) were analyzed on all samples. An identical set of sub-samples was shipped overnight at 4 °C to ELI-B, where TA-N was analyzed at 24 h, 48 h and 7 d by colorimetry (EPA method 350.1). Also, at ELI-B, TN (by Kjeldahl method) was measured to help calculate a nitrogen budget. At each lab, one set of cubitainers was stored at 4 °C in the dark and opened in such a way as to minimize air space that could alter the pH. The second set was stored under fluorescent lighting at 4 °C in an identical air-tight system.

Figure 3-1. Flow chart of the ammonification study conducted in October 2006 with effluent from a collection system for coalbed natural gas (CBNG) produced water adjacent to Beaver Creek in the Powder River Basin, Wyoming. Sub-samples were collected combining various collection methods (acid-preservation and filtration), storage conditions (light and dark) and analytical methods (ion selective electrode (ISE) and colorimetry (CM)). Total ammonia nitrogen (TA-N) by ISE along with nitrate ($NO_3^-$) and nitrite ($NO_2^-$) by ion chromatography were analyzed at Energy Labs Inc. in Gillette, Wyoming (ELI-G). Total ammonia nitrogen by CM and total nitrogen (TN) using a Kjeldahl method were analyzed at Energy Labs Inc. in Billings, Montana (ELI-B). Samples were collected at top-of-pipe and analyzed at 0 h (time of receipt, ELI-G only), 24 h, 48 h, and 7 d.

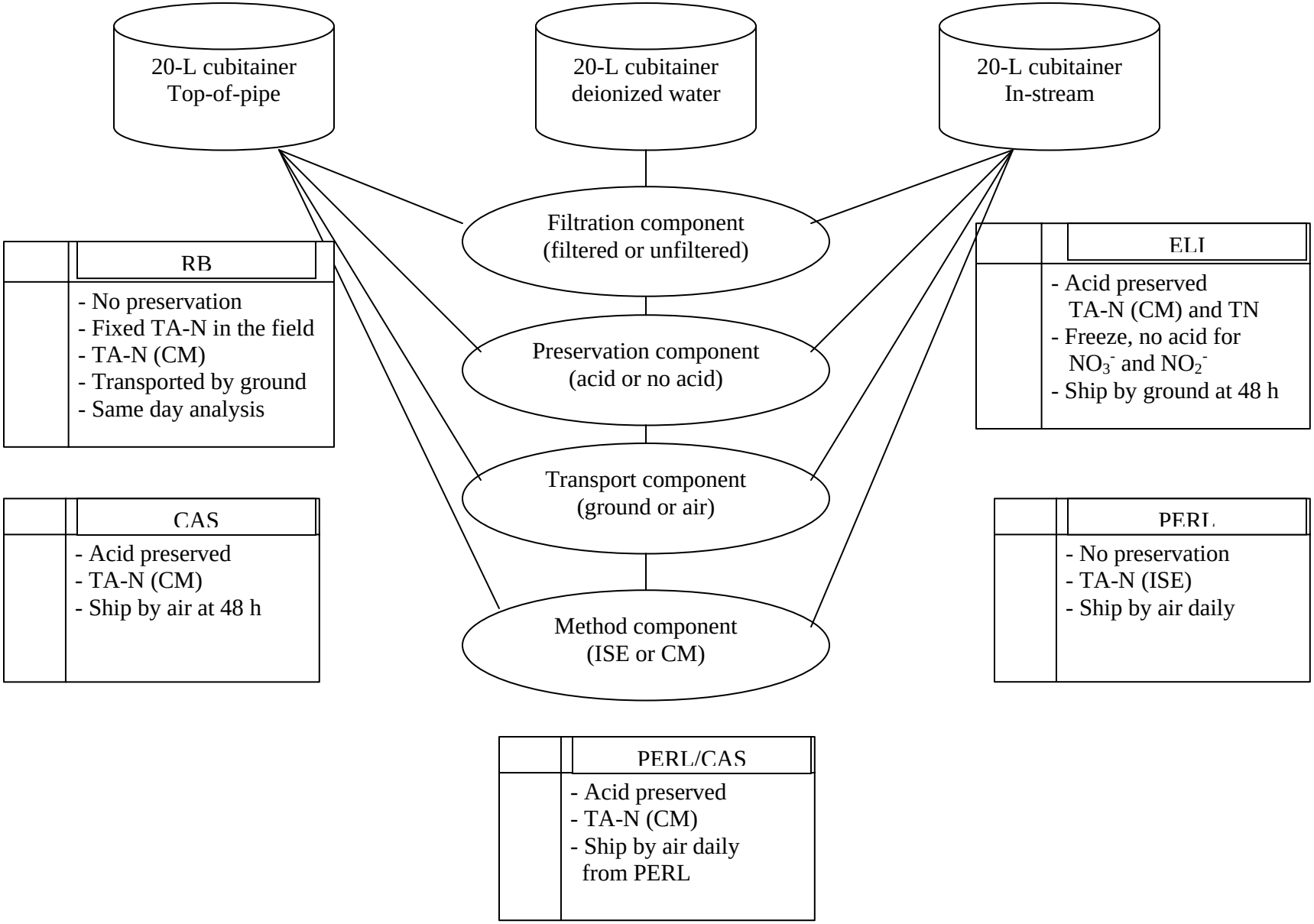

October 2006
CBNG
effluent from
top-of-pipe
Filtration component
(unfiltered or filtered)
Preservation component
(acid or no acid)
Storage component
(4 °C dark or light)
Set of 16 1-L cubitainers with
various combinations of
components
Analytical method
component
(ISE or CM)
ELI-G
TA-N (ISE)
$NO_3^-$
$NO_2^-$
0 h, 24 h, 48h
and 7 d
ELI-B
TA-N (CM)
TN
24 h, 48 h
and 7 d

**March 2007 Study**

After the October study, I conducted another study in March 2007 to compare results among more laboratories, to compare other shipment and handling methods, and to determine if ammonification occurs between time of sampling at the stream and receipt of samples at a lab a few hours to days later. In the March study I intended to determine real-time ammonia concentrations by fixing the sample (and presumably the ammonia concentration) with a colorimetric reagent at the time of collection or sub-sampling, using smaller time increments between sub-sampling than were used in the October study (Fig. 3-2). Also different from the October study, water was collected into one 20-L cubitainer that then was sub-sampled over time into various lab-specific sample bottles with various preservation and filtration components. Total ammonia nitrogen concentrations at various sub-sampling times were evaluated among samples sent to Energy Labs Inc. in Gillette, Wyoming and Billings, Montana (ELI) , Parametrix Environmental Research Laboratory in Albany, Oregon (PERL), Columbia Analytical in Kelso, Washington (CAS) and University of Wyoming Red Buttes Environmental Biology Lab in Laramie, Wyoming (RB). Total nitrogen, $NO_3^-$ and $NO_2^-$ were performed on samples sent to ELI.

In the laboratory, I filled a 20-L cubitainer with deionized water (Hydropure system) to monitor contamination during collection and transport. In the field, I also filled 20-L cubitainers with water collected from the top-of-pipe and from ~ 40 m downstream in Beaver Creek (in-stream). These original cubitainers were sub-sampled at designated time intervals (0, 1, 2, 8, 24, and 48 h) in such a way as to minimize air input and to maintain their original pH. Samples designated Time 0 h and 1 h were sub-

Figure 3-2. Flow chart of the ammonification study conducted in March 2007 with effluent from a collection system for coalbed natural gas (CBNG) produced water adjacent and within Beaver Creek in the Powder River Basin, Wyoming. This study compared various collection (preservation and filtration), shipment (air and ground), and analytical methods (ion selective and colorimetric). Nitrogen species were analyzed at the University of Wyoming's Red Buttes Laboratory in Laramie, WY (RB); Energy Laboratories, Inc. in Gillette, WY and Billing, MT (ELI); Parametrix Environmental Research Laboratory in Albany, OR (PERL); and Columbia Analytical Services - Kelso, WA (CAS). The original water samples were collected in 20-L cubitainers that subsequently were sub-sampled at 0, 1, 2, 8, 24, and 48 h. ISE = ion-selective electrode analytical method, CM = colorimetric analytical method.


20-L cubitainer
Top-of-pipe
20-L cubitainer
deionized water
20-L cubitainer
In-stream
Filtration component
(filtered or unfiltered)
Preservation component
(acid or no acid)
Transport component
(ground or air)
Method component
(ISE or CM)
RB
- No preservation
- Fixed TA-N in the field
- TA-N (CM)
- Transported by ground
- Same day analysis
ELI
- Acid preserved
TA-N (CM) and TN
- Freeze, no acid for
$NO_3^-$ and $NO_2^-$
- Ship by ground at 48 h
CAS
- Acid preserved
- TA-N (CM)
- Ship by air at 48 h
PERL
- No preservation
- TA-N (ISE)
- Ship by air daily
PERL/CAS
- Acid preserved
- TA-N (CM)
- Ship by air daily
from PERL

sampled in the field, Time 2 was sub-sampled in Gillette (enroute to Laramie), and Time 8 h, 24 h and 48 h were sub-sampled at RB. The cubitainer and all sub-samples were stored in an ice-filled cooler during transport to RB, where they then were stored in a refrigerator (4-6 °C).

The following procedure was followed at each time interval to sub-sample the cubitainers of the top-of-pipe effluent and the in-stream water into labeled sample bottles. A separate set of tygon tubing (one for filtered and one for unfiltered collections) were used for sub-sampling each cubitainer. First, for the unfiltered samples, water from the cubitainer was flushed through the tygon tubing into a small flask and this was used to rinse the sample bottles. Next, filtered and unfiltered sub-samples were withdrawn from the cubitainer into bottles that either received no acid or $H_2SO_4$. To collect the filtered samples, a piece of tubing was connected from the cubitainer to a new filter (0.45 μm Pall AquaPrep 600) that was inserted into a 1-L plastic flask (Fig. 3-3) and ~300 ml of the sample was hand pumped through the filter to use as a rinse for the 'filtered' bottles. Finally, ~650 ml sample was collected through the filtration system and divided among the bottles labeled 'filtered', and acid ($H_2SO_4$ to pH < 2.0) was added to the bottles labeled 'filtered, acid'. Unfiltered water was similarly acidified or not acidified in appropriately labeled bottles. After the addition of acid to the appropriate samples, the pH < 2.0 was confirmed with a Model IQ125 Professional pH meter. The pH, temperature (Fisher 76 immersion thermometer), and time of sub-sampling were documented at each sample interval. In addition to sub-sampling for TA-N analyses, we froze unfiltered, unpreserved samples to be analyzed for $NO_3^-$ and $NO_2^-$ at ELI.

At each time interval, samples from the cubitainer of hydropure water (that was handled in the same way as the cubitainers of effluent and stream water) were collected using the filtration and preservation procedure above and were labeled as 'lab blanks'. Total ammonia nitrogen was analyzed in the lab blanks at designated labs for artifactual elevation of TA-N concentration due to contamination.

Figure 3-3. Apparatus used in the March 2007 study to filter water collected from top-of-pipe (Williams Production RMT Company permit WY0046922) and 40 m downstream in effluent-dominated Beaver Creek in the Powder River Basin, Wyoming.

The role a given laboratory plays in actual CBNG water chemistry analyses and toxicity testing determined what collection and shipping methods were to be used for that laboratory. For example, PERL is a toxicity lab that routinely conducts WET tests on effluent discharged from CBNG outfalls in the PRB, using unfiltered, unpreserved samples that are shipped to it via air. They analyze TA-N with the ISE method and have previously sent acid-preserved sub-samples to CAS for TA-N analysis by colorimetry.

Therefore I daily shipped by air to PERL, two refrigerated bottle sets (one bottle in each set was unfiltered and unpreserved, and the other bottle was filtered and unpreserved) to be analyzed at PERL using the ISE method on one set and the other set was to be acid-preserved by PERL and then shipped overnight to CAS for TA-N analysis by colorimetry. These sub-samples were designated as PERL/CAS. Similarly, acid-preserved samples are sometimes collected at the same time as water for a WET test and shipped by ground to ELI for TA-N analysis by colorimetry. In this study, I shipped refrigerated acid-preserved filtered and acid-preserved unfiltered samples for TA-N and TN analyses along with frozen unpreserved, unfiltered samples for $NO_3^-$ and $NO_2^-$ to ELI via ground transportation. Also, with the original intent to check if transportation choice (ground versus air) had an effect on TA-N concentration, I packaged 48-h refrigerated acid-preserved filtered and acid-preserved unfiltered samples to be shipped overnight by air to CAS; however, due to a shipping error by the University of Wyoming, those samples were sent via ground transportation. Finally, sub-samples taken directly from the unpreserved ELI bottle at each collection time were fixed with colorimetric reagent and stored on ice in a dark cooler, for same-day TA-N analysis at RB. Deionized water containing known standard concentrations of TN, TA-N, nitrate and nitrite were sent as blind samples to ELI, and blind samples of TA-N were sent to CAS and PERL to check inter-laboratory accuracy.

# RESULTS

## October 2006

In October, there were few differences in ammonia concentrations due to filtration and few differences among storage methods. Ammonia concentrations differed among the analytical methods, and the differences between preserved and unpreserved samples were variable (Table 3-1). Because there was only one analysis per observation time, I did not statistically compare the difference of TN and TA-N among the various collection, storage and analytical methods (Table 3-2, Fig. 3-4 to 3-5). The difference between filtered and unfiltered samples between samples stored in the dark and those stored under fluorescent lighting was <10 and 12%, respectively. The difference between acid–preserved and unpreserved samples ranged from 0% at 24 h to 17% at 7 d.

The ISE method for TA-N was more variable than the colorimetric method possibly due to the high ionic concentration of the CBNG effluent (personal communication, Eric Van Genderen, Parametrix Environmental Research Laboratory, Albany, Oregon, USA; February 2007). The 0 h dark treatment, unpreserved ISE analyses are not reliable because the TA-N concentrations were greater than the corresponding TN concentrations. The TN concentration from top-of-pipe in October was 3.4 to 4.1 mg/L, and TA-N concentration (colorimetric) was 2.4 to 3.0 mg N/L. Nitrites and nitrates were not detected. Based on these calculations, ~30% (1.0 to 1.1 mg N/L) of the TN could be organic nitrogen and available for ammonification (Table 3-1, Figs. 3-4 and 3-5).

Table 3-1. Concentrations of N-containing species collected from top-of-pipe during the October 2006 ammonification study comparing collection, preservation, and storage methods. F = filtered, UF = unfiltered, D = stored in the dark, L = stored under room lights P = acid-preserved ($H_2SO_4$ to pH < 2.0), CM = colorimetric analytical method, ISE = ion-selective electrode analytical method, TN = total nitrogen, TA-N = total ammonia nitrogen, $NO_3^-$ = nitrate, $NO_2^-$ = nitrite, and NA = not available. *Questionable data because TA-N greater than TN.

| | | | 0 h | | 24 h | | 48 h | | 7 d | |
|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Method | Treatment | F | UF | F | UF | F | UF | F | UF |
| TN | Kjeldahl | D-P | NA | NA | 4.1 | 3.7 | 3.9 | 3.8 | 3.7 | 3.6 |
| | | D | NA | NA | 3.7 | 3.7 | 3.7 | 3.9 | 3.6 | 3.4 |
| | | L-P | NA | NA | 3.8 | 3.8 | 4.1 | 3.9 | 3.7 | 3.8 |
| | | L | NA | NA | 3.8 | 3.6 | 4.0 | 3.6 | 3.6 | 3.4 |
| TA-N | ISE | D-P | 2.9 | 2.6 | 2.9 | 3.3 | 2.9 | 2.9 | 3.4 | 3.2 |
| | | D | 4.5* | 4.1* | 2.6 | 3.0 | 2.4 | 2.4 | 2.8 | 2.9 |
| | | L-P | 2.7 | 3.2 | 2.7 | 3.0 | 3.0 | 3.1 | 3.3 | 3.8 |
| | | L | 3.3 | 3.7 | 2.5 | 2.6 | 2.5 | 2.7 | 2.9 | 2.9 |
| TA-N | CM | D-P | NA | NA | 2.5 | 2.6 | 2.6 | 2.7 | 2.8 | 3.0 |
| | | D | NA | NA | 2.5 | 2.6 | 2.4 | 2.5 | 2.4 | 2.5 |
| | | L-P | NA | NA | 2.7 | 2.8 | 2.7 | 2.6 | 2.7 | 2.8 |
| | | L | NA | NA | 2.5 | 2.6 | 2.4 | 2.4 | 2.6 | 2.8 |
| $NO_3^-$ | IC | D | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 |
| | | L | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 |
| $NO_2^-$ (mg N/L) | IC | D | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 |
| | | L | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 |

Table 3-2. Concentrations of N-containing species analyzed in March 2007 ammonification study. Analytical tests were conducted with various collection methods at the University of Wyoming's Red Buttes Lab in Laramie, Wyoming (RB); Energy Laboratories, Inc. in Gillette, Wyoming and Billings, Montana (ELI); Parametrix Environmental Research Laboratory in Albany, Oregon (PERL); and Columbia Analytical Services in Kelso, Washington (CAS). Samples were shipped from PERL to CAS (PC). Collection points were top-of-pipe and 40 m downstream from CBNG effluent entrance into Beaver Creek (in-stream) in the Powder River Basin, Wyoming, during March 2007. Sub-samples were withdrawn from original cubitainer at 0, 1, 2, 8, 24, and 48 h (RB and ELI) and at 0, 24, and 48 h (CAS, PERL, PC). TP = Top-of-pipe, IS = In stream, TA-N = total ammonia nitrogen, TN = total nitrogen, $NO_3^-$ = nitrate, $NO_2^-$ = nitrite, UIA = unionized ammonia, CM = colorimetric, ISE = ion-selective electrode, K = Kjeldahl, Calculated = calculated from TA-N, pH and temperature, Y = acidified with $H_2SO_4$ to < pH 2.0, N = not acidified, F = filtered, UF = unfiltered, NA = not available.

| | | | | | 0 h[a] | | | 1 h[b] | | | 2 h[c] | | | 8 h[d] | | | 24 h[e] | | | 48 h[f] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | mg N/L | | | mg N/L | | | mg N/L | | | mg N/L | | | mg N/L | | | mg N/L | |
| Site | Parameter | Method | Lab | Acid | pH | F | UF | pH | F | UF | pH | F | UF | pH | F | UF | pH | F | UF | pH | F | UF |
| TP | TA-N | CM | ELI | Y | <2 | 2.10 | 2.00 | <2 | 1.10 | 1.40 | <2 | 2.10 | 2.40 | <2 | 1.90 | 1.80 | <2 | 2.10 | 1.80 | <2 | 1.70 | 1.70 |
| | TA-N | CM | RB | N | NA | 3.19 | 3.25 | NA | 3.28 | 3.29 | NA | 3.12 | 3.11 | NA | 3.26 | 3.30 | NA | 3.28 | 3.31 | NA | 3.21 | 3.16 |
| | TA-N | CM | CAS | Y | <2 | 2.98 | 2.97 | | | | | | | | | | <2 | 3.04 | 2.94 | <2 | 2.88 | 2.89 |
| | TA-N | CM | PC | Y | <2 | 2.97 | 2.97 | | | | | | | | | | <2 | 2.92 | 2.92 | <2 | 2.94 | 2.88 |
| | TA-N | ISE | PERL | N | 7.60 | 2.06 | 2.39 | | | | | | | | | | 7.60 | 2.06 | 2.14 | 7.50 | 1.48 | 1.40 |
| | TN | K | ELI | Y | <2 | 3.80 | 3.90 | <2 | 3.90 | 3.90 | <2 | 3.70 | 3.70 | <2 | 3.90 | 3.70 | <2 | 3.90 | 3.70 | <2 | 3.80 | 3.80 |
| | $NO_3^-$ | IC | ELI | N | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 |
| | $NO_2^-$ | IC | ELI | N | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 |
| | UIA | Calculated | ELI | Y | <2 | 0.01 | 0.01 | <2 | 0.01 | 0.01 | <2 | 0.01 | 0.01 | <2 | 0.00 | 0.00 | <2 | 0.01 | 0.01 | <2 | 0.00 | 0.00 |
| IS | TA-N | CM | ELI | Y | <2 | 1.00 | 0.50 | <2 | 1.30 | 1.20 | <2 | 1.50 | 1.30 | <2 | 1.20 | 1.10 | <2 | 0.90 | 1.10 | <2 | 1.10 | 1.10 |
| | TA-N | CM | RB | N | NA | 1.93 | 1.93 | NA | 1.94 | 1.97 | NA | 1.89 | 1.87 | NA | 1.92 | 1.93 | NA | 1.90 | 1.86 | NA | 1.85 | 1.90 |
| | TA-N | CM | CAS | Y | <2 | 1.79 | 1.77 | | | | | | | | | | <2 | 1.76 | 1.73 | <2 | 1.72 | 1.68 |
| | TA-N | CM | PC | Y | <2 | 1.71 | 1.70 | | | | | | | | | | <2 | 1.72 | 1.71 | <2 | 1.71 | 1.70 |
| | TA-N | ISE | PERL | N | 8.10 | 1.32 | 1.15 | | | | | | | | | | 8.10 | 1.32 | 1.24 | 8.10 | 0.82 | 0.82 |
| | TN | K | ELI | Y | <2 | 2.50 | 2.80 | <2 | 2.60 | 2.70 | <2 | 2.30 | 2.70 | <2 | 2.50 | 2.50 | <2 | 2.50 | 2.30 | <2 | 2.40 | 2.40 |
| | $NO_3^-$ | IC | ELI | N | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 |
| | $NO_2^-$ | IC | ELI | N | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 | NA | <0.1 | <0.1 |
| | UIA | Calculated | ELI | Y | <2 | 0.03 | 0.01 | <2 | 0.03 | 0.03 | <2 | 0.02 | 0.02 | <2 | 0.01 | 0.01 | <2 | 0.01 | 0.02 | <2 | 0.01 | 0.01 |

[a] Field measurements for the top-of-pipe 0 h -- pH = 7.30, temperature = 14.9 °C; in-stream 0 h -- pH = 8.10, temperature = 11.2 °C.
[b] Field measurements for the top-of-pipe 1 h -- pH = 7.50, temperature = 13.7 °C; in-stream 1 h -- pH = 8.00, temperature = 12.8 °C.
[c] Field measurements for the top-of-pipe 2 h -- pH = 7.20, temperature = 9.1 °C; in-stream 2 h -- pH = 7.90, temperature = 11.4 °C.
[d] Field measurements for the top-of-pipe 8 h -- pH = 7.20, temperature = 7.8 °C; in-stream 8 h -- pH = 8.00, temperature = 7.8 °C.
[e] Field measurements for the top-of-pipe 24 h -- pH = 7.40, temperature = 7.9 °C; in-stream 24 h -- pH = 8.10, temperature = 11.2 °C.
[f] Field measurements for the top-of-pipe 48 h -- pH = 7.20, temperature = 8.1 °C; in-stream 48 h -- pH = 7.90, temperature = 9.0 °C.

Table 3-3. Blind samples of known concentration standards prepared at RB were sent to the laboratory at 24 h (PERL) and 48 h (ELI and CAS).

| | | | | | 24 h | | | 48 h | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | mg N/L | | | mg N/L | |
| Standard | Parameter | Method | Lab | Acid | pH | F | UF | pH | F | UF |
| 0.00 mg N/L Std. | TA-N | CM | ELI | Y | | | | <2.00 | | <0.10 |
| | TA-N | CM | RB | N | | | <0.10 | | | <0.10 |
| | TA-N | CM | CAS | Y | | | | <2.00 | | <0.10 |
| | TA-N | ISE | PERL | N | 5.40 | | <0.50 | | | |
| | TN | Kjeldahl | ELI | Y | | | | <2.00 | | <0.50 |
| | TN | Digestion, colorimetry | RB | Y | | | | <2.00 | | <0.10 |
| | $NO_3^-$ | IC | ELI | N | | | | | | <0.10 |
| | $NO_3^-$ | IC | RB | N | | | | | | <0.10 |
| | $NO_2^-$ | IC | ELI | N | | | | | | <0.10 |
| | $NO_2^-$ | IC | RB | N | | | | | | <0.10 |
| 2.00 mg N/L Std. | TA-N | CM | RB | N | | | 1.61 | | | |
| | TA-N | ISE | PERL | N | 5.50 | | 1.15 | | | |
| | TA-N | CM | RB | N | | | | | | 1.92 |
| | TA-N | CM | ELI | Y | | | | <2.00 | | 1.80 |
| | TA-N | CM | CAS | Y | | | | <2.00 | | 1.91 |
| 5.00 mg/L Std. | TN | Kjeldahl | ELI | Y | | | | <2.00 | | 5.50 |
| | TN | Digestion, colorimetry | RB | Y | | | | <2.00 | | 1.10 |
| 1.00 mg N/L Std. | $NO_3^-$ | IC | ELI | N | | | | | | 0.90 |
| | $NO_3^-$ | IC | RB | N | | | | | | 0.94 |
| | $NO_2^-$ | IC | ELI | N | | | | | | 1.00 |
| | $NO_2^-$ | IC | RB | N | | | | | | 1.36 |

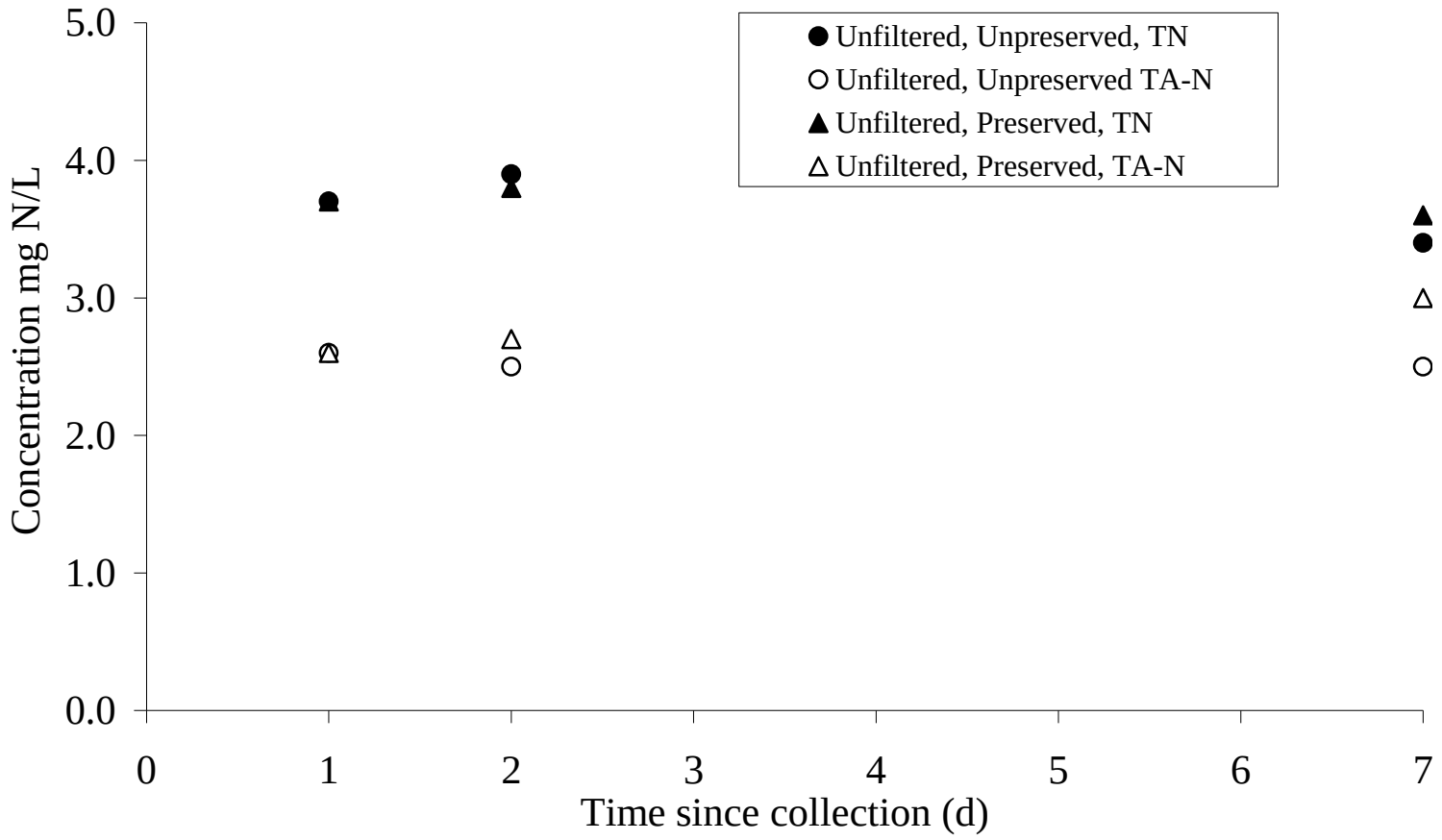



Figure 3-4. Concentrations of total nitrogen (TN) and total ammonia nitrogen (TA-N) collected from top-of-pipe during the October 2006 ammonification study.

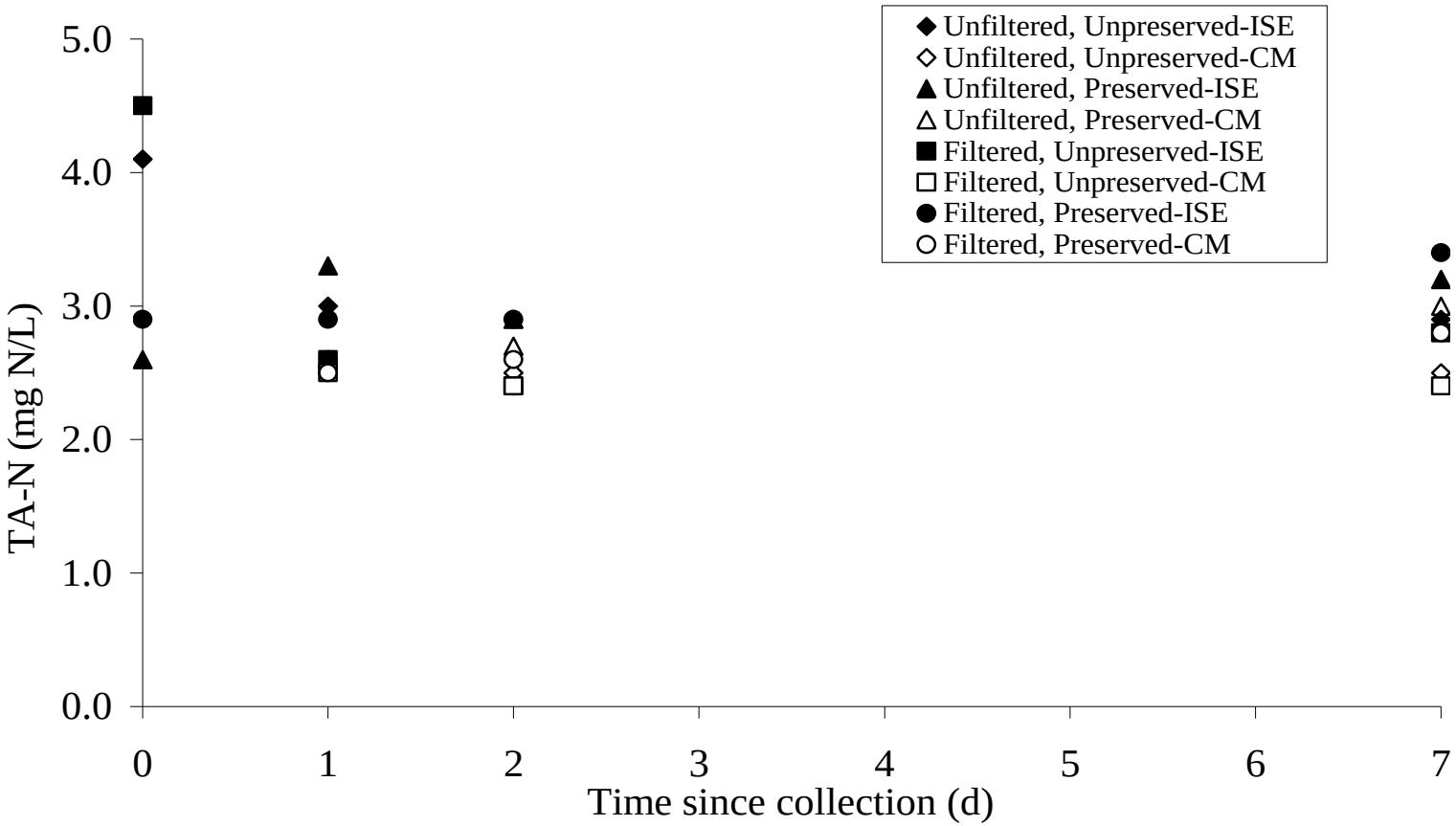



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Figure 3-5. Concentrations total ammonia nitrogen (TA-N) in October 2006 ammonification study. Analyses from samples stored at 4 °C in the dark comparing filtration, preservation, and analytical methods. ISE = ion selective electrode method, CM = colorimetric method

**March 2007**

In March 2007, TA-N concentrations analyzed at ELI on preserved samples were consistently lower than analyses conducted at PERL, PERL/CAS, and RB on unpreserved samples (Table 3-4, Figs. 3-6 to 3-9). Total ammonia nitrogen analyses on preserved samples at ELI were lower than preserved samples sent to CAS. ELI had 30 to 286% less TA-N than comparative samples at the other labs. The PERL ion-selective electrode TA-N analyses generally were higher than ELI but lower than RB, PERL/CAS and CAS (~ 30% lower). The filtration effect on average was <5%, correlating with the October study.

Similar to the October study, the nitrite and nitrate were below detection limits in ELI analyses. Un-ionized ammonia concentrations at top-of-pipe ranged from 0.004 to 0.011 mg N/L and at Site 2 from 0.012 to 0.029 mg N/L (Appendix D). The ELI TA-N (colorimetric) over the 48 h period ranged from 1.7 to 2.1 mg N/L. The TN concentration from top-of-pipe in March ranged from 3.7 to 3.9 mg/L over the 48 h period (Table 3-2, Fig. 3-6 to 3-7). Therefore, ~50% of the TN (1.6 to 2.2 mg N/L) might have been organic nitrogen and available for ammonification. Results were similar with the in-stream sample, for which TA-N ELI (colorimetric) over the 48-h period ranged from 0.5 to 1.5 mg N/L and the TN concentration ranged from 2.3 to 2.7 mg/L. Therefore, on average ~60% of the TN from the in-stream sample might have been organic nitrogen and available for ammonification (Tables 3-2, Figs. 3-8 to 3-9).

Table 3-4. Total ammonia nitrogen (TA-N) collected during March 2007 from the top-of-pipe and 40 m downstream from CBNG effluent entrance into Beaver Creek (in-stream) in the Powder River, Wyoming. Comparison of filtration (0.45 mm), preservation ($H_2SO_4$ to pH < 2.0), and analytical methods (ion-selective electrode and colorimetric) between the University of Wyoming's Red Buttes Lab in Laramie, Wyoming (RB); Energy Labs, Inc. in Billings, Montana (ELI); Parametrix Environmental Research Laboratory in Albany, Oregon (PERL); and Columbia Analytical Services in Kelso, Washington (CAS). One set of unpreserved sub-samples sent to PERL were acidified and shipped to CAS (PERL/CAS). % A = % difference between filtered and unfiltered analyses at same lab, % B = % difference between unfiltered analysis from specific lab compared to the unfiltered analysis from ELI, % C = % difference between unfiltered samples analyzed at ELI, RB, CAS and PERL compared to expected standard concentration (i.e. 0.00 and 2.00 mg /L).TP = Top-of-pipe, IS = In stream, TA-N = total ammonia nitrogen, CM = colorimetric, ISE = ion-selective electrode, K = Kjeldahl, Calculated = calculated from TA-N, pH and temperature, Y = acidified with $H_2SO_4$ to < pH 2.0, N = not acidified, F = filtered, UF = unfiltered.

| | | ELI - CM, Y | | | | RB - CM, N | | | | | CAS - CM, Y | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg TA-N/L | | | | mg TA-N/L | | | | | mg TA-N/L | | | | |
| Site | Time | F | UF | %A | %C | F | UF | %A | %B | %C | F | UF | %A | %B | %C |
| Top-of-pipe | 0 h | 2.10 | 2.00 | 5% | | 3.19 | 3.25 | 2% | 63% | | 2.98 | 2.97 | 0% | 49% | |
| | 1 h | 1.10 | 1.40 | 27% | | 3.28 | 3.29 | 0% | 135% | | NA | NA | | | |
| | 2 h | 2.10 | 2.40 | 14% | | 3.12 | 3.11 | 0% | 30% | | NA | NA | | | |
| | 8 h | 1.90 | 1.80 | 5% | | 3.26 | 3.30 | 1% | 83% | | NA | NA | | | |
| | 24 h | 2.10 | 1.80 | 14% | | 3.28 | 3.31 | 1% | 84% | | 3.04 | 2.94 | 3% | 63% | |
| | 48 h | 1.70 | 1.70 | 0% | | 3.21 | 3.16 | 2% | 86% | | 2.88 | 2.89 | 0% | 70% | |
| In-stream | 0 h | 1.00 | 0.50 | 50% | | 1.93 | 1.93 | 0% | 286% | | 1.79 | 1.77 | 1% | 254% | |
| | 1 h | 1.30 | 1.20 | 8% | | 1.94 | 1.97 | 2% | 64% | | | | | | |
| | 2 h | 1.50 | 1.30 | 13% | | 1.89 | 1.87 | 1% | 44% | | | | | | |
| | 8 h | 1.20 | 1.10 | 8% | | 1.92 | 1.93 | 1% | 75% | | | | | | |
| | 24 h | 0.90 | 1.10 | 22% | | 1.90 | 1.86 | 2% | 69% | | 1.76 | 1.73 | 2% | 57% | |
| | 48 h | 1.10 | 1.10 | 0% | | 1.85 | 1.90 | 3% | 73% | | 1.72 | 1.68 | 2% | 53% | |
| 0.00 Std | 24 h | | | | | | <0.10 | | | 0% | | | | | |
| 0.00 Std | 48 h | | <0.10 | | 0% | | <0.10 | | | 0% | | <0.10 | | | 0% |
| 2.00 Std | 24 h | | | | | | 1.61 | | | 20% | | | | | |
| 2.00 Std | 48 h | | 1.80 | | 10% | | 1.61 | | | 20% | | 1.91 | | | 5% |

Table 3-4 (cont.)

| | | PERL - ISE, UP | | | | | PERL/CAS - CM, P | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | mg TA-N/L | | | | | mg TA-N/L | | | |
| Site | Time | FY | FN | %A | %B | %C | FY | FN | %A | %B |
| Top-of-pipe | 0 h | 2.06 | 2.39 | 16% | 20% | | 2.97 | 2.97 | 0% | 49% |
| | 1 h | NA | NA | | | | NA | NA | | |
| | 2 h | NA | NA | | | | NA | NA | | |
| | 8 h | NA | NA | | | | NA | NA | | |
| | 24 h | 2.06 | 2.14 | 4% | 19% | | 2.92 | 2.92 | 0% | 62% |
| | 48 h | 1.48 | 1.40 | 5% | 18% | | 2.94 | 2.88 | 2% | 69% |
| In-stream | 0 h | 1.32 | 1.15 | 13% | 130% | | 1.71 | 1.70 | 1% | 240% |
| | 1 h | | | | | | | | | |
| | 2 h | | | | | | | | | |
| | 8 h | | | | | | | | | |
| | 24 h | 1.32 | 1.24 | 6% | 13% | | 1.72 | 1.71 | 1% | 55% |
| | 48 h | 0.82 | 0.82 | 0% | 25% | | 1.71 | 1.70 | 1% | 55% |
| 0.00 Std | 24 h | | <0.5 | | | 0% | | | | |
| 0.00 Std | 48 h | | | | | | | | | |
| 2.00 Std | 24 h | | 1.15 | | | 43% | | | | |
| 2.00 Std | 48 h | | | | | | | | | |

Figure 3-6. Total ammonia nitrogen (TA-N) in coalbed natural gas water collected unfiltered from **top-of-pipe** (Williams Production RMT Company., WY0046922 on Beaver Creek in the Powder River Basin, Wyoming) analyzed at various laboratories during the March 2007 ammonification study. RB = University of Wyoming's Red Buttes Laboratory in Laramie, Wyoming; ELI = Energy Labs, Inc. in Billings, Montana; PERL = Parametrix Environmental Research Laboratory in Albany, Oregon; CAS = Columbia Analytical Services in Kelso, Washington; and PERL-CAS = Parametrix Environmental Research Laboratory shipped to Columbia Analytical Services.

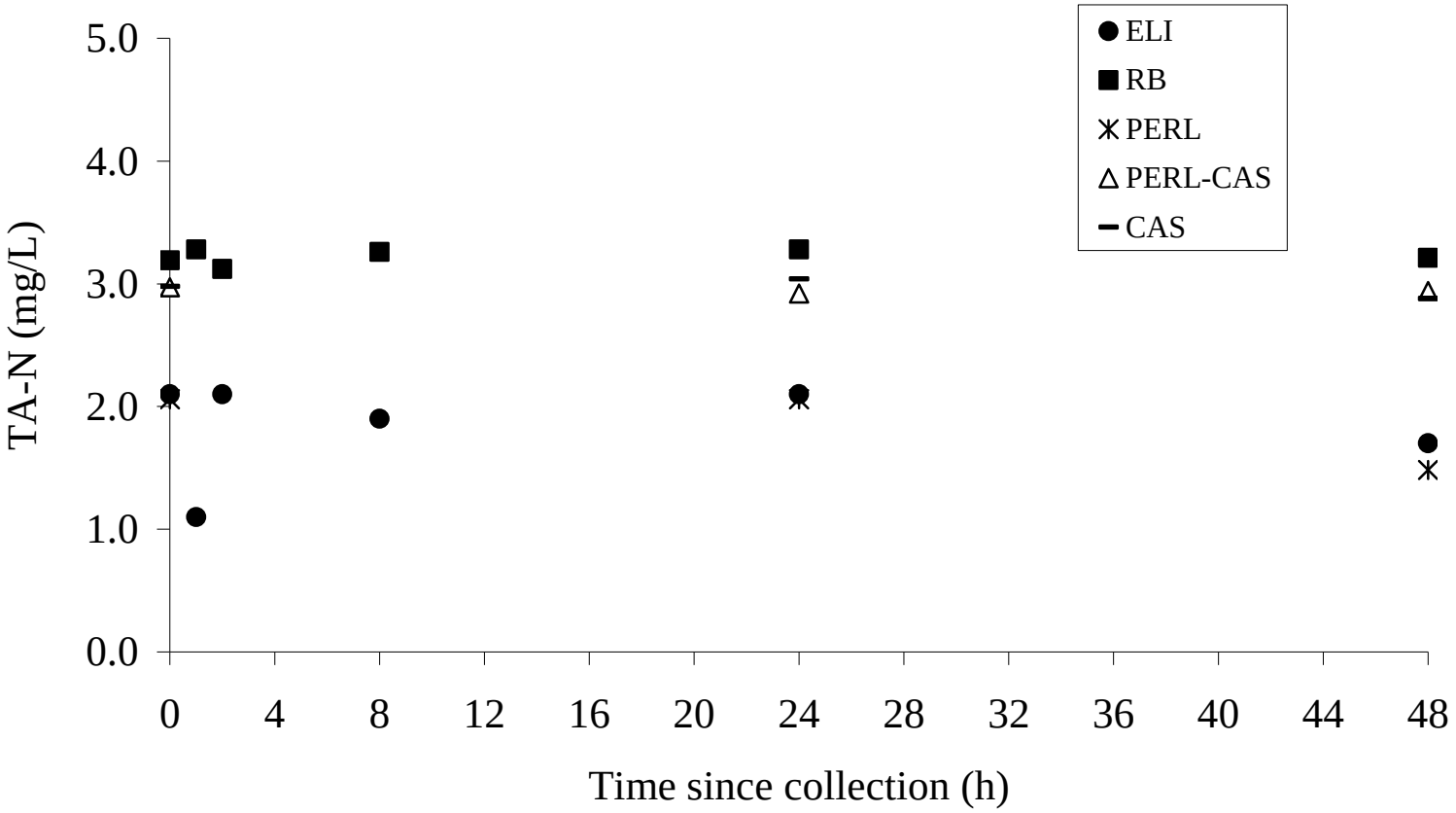

5.0
4.0
3.0
2.0
1.0
0.0
TA-N (mg/L)
0
4
8
12
16
20
24
28
32
36
40
44
48
Time since collection (h)
ELI
RB
PERL
PERL-CAS
CAS

Figure 3-7. Concentrations of N-containing species in coalbed natural gas water collected unfiltered from **top-of-pipe** (Williams Production RMT Company., WY0046922 on Beaver Creek in the Powder River Basin, Wyoming) analyzed at various laboratories during the March 2007 ammonification study. RB = University of Wyoming's Red Buttes Laboratory in Laramie, Wyoming; ELI = Energy Labs, Inc. in Billings, Montana; PERL = Parametrix Environmental Research Laboratory in Albany, Oregon; CAS = Columbia Analytical Services in Kelso, Washington; and PERL-CAS = Parametrix Environmental Research Laboratory shipped to Columbia Analytical Services. Analyses conducted at Energy Labs Inc., Gillette-Wyoming and Billings, Montana. TN = total nitrogen, TA-N = total ammonia nitrogen, UIA = un-ionized ammonia, $NO_3$.

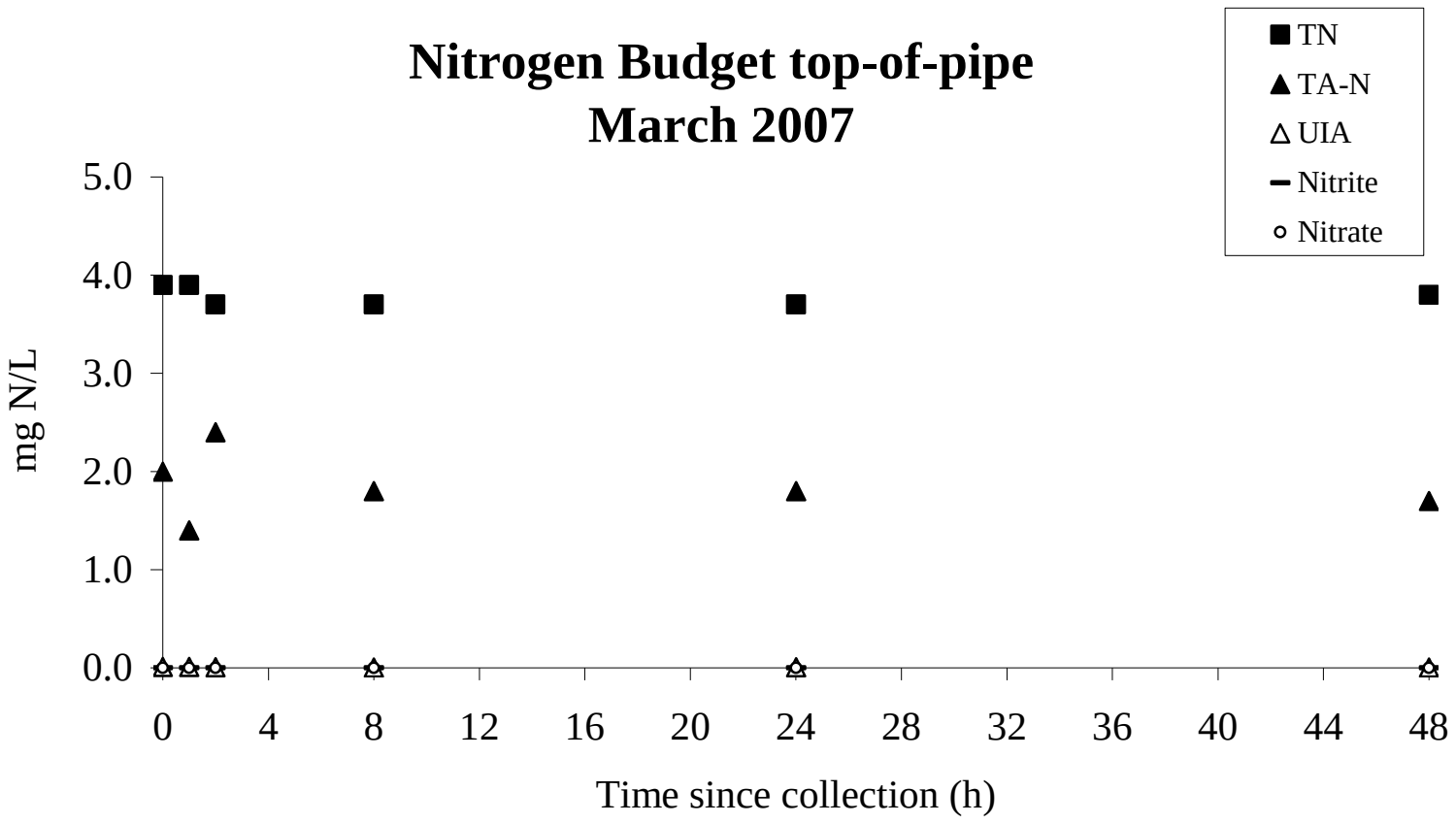

Nitrogen Budget top-of-pipe
March 2007
TN
TA-N
UIA
Nitrite
Nitrate
mg N/L
5.0
4.0
3.0
2.0
1.0
0.0
0
4
8
12
16
20
24
28
32
36
40
44
48
Time since collection (h)

Figure 3-8. Total ammonia nitrogen (TA-N) in coalbed natural gas water collected unfiltered from **in-stream** (Williams Production RMT Company., WY0046922 on Beaver Creek in the Powder River Basin, Wyoming) analyzed at various laboratories during the March 2007 ammonification study. RB = University of Wyoming's Red Buttes Laboratory in Laramie, Wyoming; ELI = Energy Labs, Inc. in Billings, Montana; PERL = Parametrix Environmental Research Laboratory in Albany, Oregon; CAS = Columbia Analytical Services in Kelso, Washington; and PERL-CAS = Parametrix Environmental Research Laboratory shipped to Columbia Analytical Services.

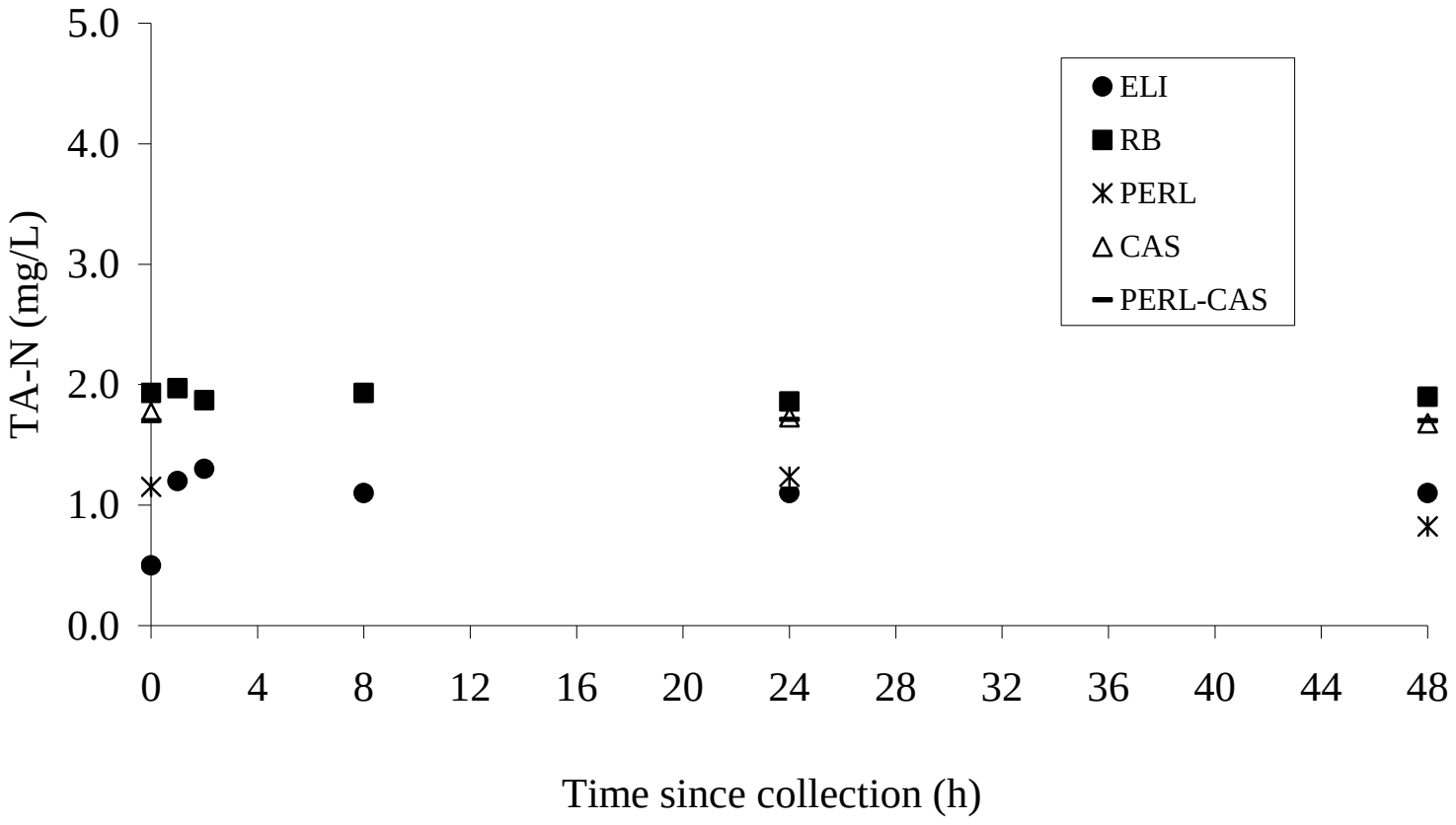
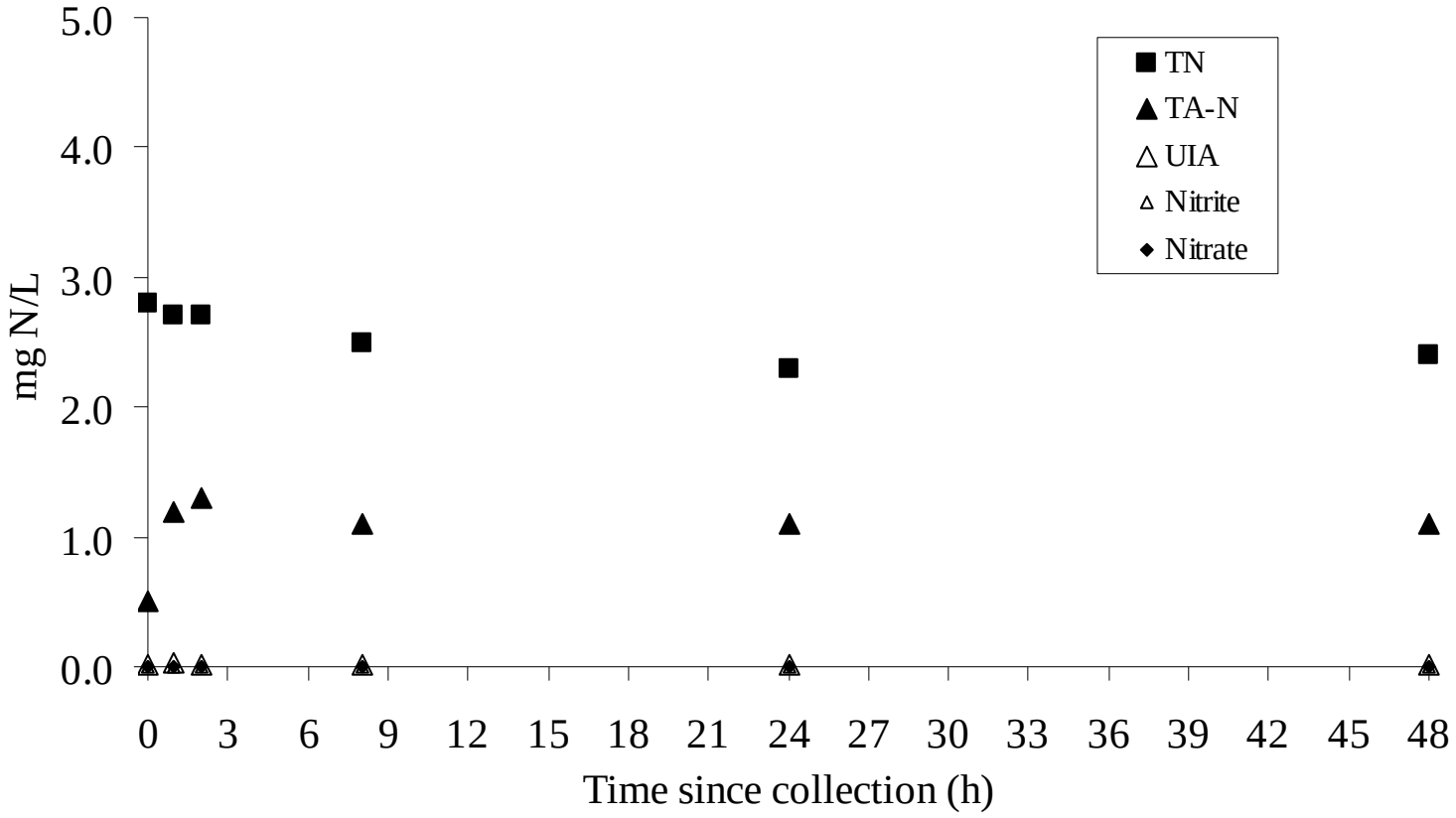

5.0
4.0
3.0
2.0
1.0
0.0
TA-N (mg/L)
0
4
8
12
16
20
24
28
32
36
40
44
48
Time since collection (h)
ELI
RB
PERL
CAS
PERL-CAS

Figure 3-9. Concentrations of N-containing species in coalbed natural gas water collected unfiltered from **in-stream** (Williams Production RMT Company., WY0046922 on Beaver Creek in the Powder River Basin, Wyoming) analyzed at various laboratories during the March 2007 ammonification study. RB = University of Wyoming's Red Buttes Laboratory in Laramie, Wyoming; ELI = Energy Labs, Inc. in Billings, Montana; PERL = Parametrix Environmental Research Laboratory in Albany, Oregon; CAS = Columbia Analytical Services in Kelso, Washington; and PERL-CAS = Parametrix Environmental Research Laboratory shipped to Columbia Analytical Services. Analyses conducted at Energy Labs Inc., Gillette-Wyoming and Billings, Montana. TN = total nitrogen, TA-N = total ammonia nitrogen, UIA = un-ionized ammonia, $NO_3$.


5.0
4.0
3.0
2.0
1.0
0.0
mg N/L
0
3
6
9
12
15
18
21
24
27
30
33
36
39
42
45
48
Time since collection (h)
TN
TA-N
UIA
Nitrite
Nitrate

**Quality Control and Blind Analysis of Known-concentration Standards**

The concentrations of the 5.0 mg/L TN standard reported by RB was 1.0 mg/L (i.e., 80% low) but the concentration reported by ELI was 5.5 mg/L (only 10% higher), only the ELI TN concentrations were used in nitrogen budget calculations. The percentage differences from the 2.0 mg N/L standards for TA-N analyses submitted at 48-h to ELI, RB, and CAS were 10, 20, and 5%, respectively; however, the blind TA-N sample submitted to PERL (ISE) at 24 h was 43% lower than the 2.0 mg/L TA-N standard. On average, the PERL TA-N results using the ISE method were ~40% lower than similarly collected and transported PERL/CAS samples that were acidified at PERL, shipped to CAS, and then analyzed using colorimetry. Assuming the 40% underestimate of TA-N by PERL was due to the method differences, the PERL TA-N analyses would trend along with the CAS concentrations after adjustment for the PERL bias (Figs. 3-6, 3-8). The known-concentration standards blindly submitted to ELI, PERL, and CAS generally indicated acceptable quality control.

Spike recovery data provided by CAS, ELI and RB was acceptable, and no contamination was measured in lab blank samples submitted to RB and ELI. PERL did not perform spike recovery data because it is not required for a toxicity lab, but did have acceptable quality control analyzing known calibration standards and controls for the ISE method.

The cooler containing acid-preserved samples for CAS packed at <4 °C was supposed to arrive overnight by air to CAS; however, due to the shipping error, it arrived 5 d later on a truck with a temperature in the cooler of ~9 °C. The TA-N concentrations reported by CAS were 0.9 to 1.3 mg N/L higher than those reported by ELI (Table 3-3).

Therefore although the sample was preserved, the increased temperature and delay of transport appear to have contributed to an increase in TA-N most likely from ammonification.

## DISCUSSION

Based on results from the October 2006 study, I could not conclude that ammonification occurred in unpreserved samples; however, results from the March 2007 study clearly demonstrate that ammonification can occur in unpreserved or improperly transported samples. The significant difference between the two studies was the timing of the analyses, with results of the March study suggesting ammonification occurs within 24 h. In October 2006, preserved and unpreserved samples had similar TA-N concentrations after 24 h, and the preserved samples had increased TA-N concentrations after 7 d. In contrast in March 2007, the TA-N concentrations in unpreserved samples were consistently higher than in well-preserved samples. The nitrogen budgets from both studies indicated that one or more organic nitrogen species might be a significant component of the TN because the summations of TA-N, UIA, $NO_3^-$ and $NO_2^-$ concentrations were less than TN. Therefore, ammonification in unpreserved CBNG effluent probably increased the TA-N concentrations during transport and/or storage.

Variation in dark/light storage and filtered/unfiltered collection did not significantly alter the TA-N concentration. Although the transport component of the March 2007 study cannot be completely evaluated because the CAS sample designed to be shipped by air was mistakenly shipped by ground, it appears that delayed transport at a temperature >4 °C can warm the sample and promote ammonification, which increases the TA-N concentration.

The original intent of the March 2007 study was to determine real-time TA-N concentrations using the RB samples, with the expectation that the TA-N concentration would be 'fixed' in the field by the addition of the colorimetric reagent (i.e., maintained until analyzed at RB). I thought the 'fixed' RB TA-N analyses would match the acid-preserved ELI TA-N analyses, but the RB samples not acidified had higher TA-N concentrations indicating ammonification probably occurred even after addition of the colorimetric reagents to the water.

As expected, refrigerated unpreserved samples shipped overnight to PERL had higher TA-N concentrations than refrigerated acid-preserved samples sent to ELI at 48 h. Although the PERL/CAS samples were acid-preserved after receipt at PERL but before subsequent shipment by PERL to CAS, the TA-N concentration was higher in the acid-preserved PERL/CAS sample than in the acid-preserved ELI sample. Presumably, the initial 24 h transport of the unpreserved sample from RB to PERL allowed enough time for ammonification in the PERL samples and, thus, in the PERL/CAS samples. Total ammonia nitrogen concentrations analyzed at PERL/CAS and RB, both using colorimetry and both transported unpreserved before analysis or acid preservation, were similar.

The PERL ISE TA-N analyses were higher than ELI but lower than RB, PERL/CAS and CAS, possibly due to the variability (< 30%) of the ISE method with the high ionic CBNG effluent. Because ELI, RB and CAS had excellent spike recoveries and the colorimetric method does not appear to be affected by the ionic strength of the effluent, their data appear to be comparable. Therefore, based on these studies, refrigerated unfiltered, acid-preserved samples transported within 24 h of collection and analyzed using colorimetry appear to have TA-N concentrations closest to those in the

in-stream environment.

Although the actual time required to induce ammonification from an in-stream environment to a laboratory setting was not determined as I expected, because the samples fixed in the field for colorimetry were unpreserved, this study strongly suggests ammonification occurs. In the analyses conducted in March, it appears that the transformation was complete within 24 h under these sampling conditions. Although, my results do not allow me to evaluate whether ammonification also occurred in the acid-preserved samples analyzed at ELI, I conclude that acid preservation at least minimized the amount of ammonification.

In summary, ammonification of what appears to be organic nitrogen in CBNG effluents can occur during transport and storage of unpreserved samples, thus potentially biasing WET tests by increasing ammonia concentrations in the water by the time they arrive at the laboratory. The apparent ammonification that occurred in ≤ 24 h during my March 2007 study indicates that even static-renewal WET tests started immediately after an effluent or stream water is collected will have elevated TA-N concentration even before the end of the first 24 h of exposure, much less before the end of the customary 48 h exposure-water renewal period. Because WET tests should not be conducted using acid-preserved effluents, regulatory and management agencies should consider possible ammonification from the stream to the laboratory when selecting appropriate tests to measure toxicity in CBNG produced waters.

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# CHAPTER 4

# Conclusions

This study provided a multi-faceted evaluation of potential acute toxicity in coalbed natural gas (CBNG) effluent-dominated Beaver Creek in the Powder River Basin, Wyoming. I compared the acute toxic effects of an effluent containing measurable concentrations of ammonia and sodium bicarbonate ($NaHCO_3$) discharged from a CBNG outfall, using in-stream, ambient-pH, and $CO_2$ pH-controlled toxicity tests methods. Additionally, I complemented the toxicity studies with field observations and water chemistry analyses.

The results of the in-stream and laboratory toxicity tests suggest no apparent acute toxicity at 96 h. Although another in-situ study conducted within the same drainage suggests possible $NaHCO_3$ toxicity to younger fathead minnow (*Pimephales promelas*), no toxicity occurred in my study with slightly higher $HCO_3^-$ concentrations. However, FHM are not among the most sensitive species to ammonia and $NaHCO_3$, and the ages of the larvae I used might not have been as sensitive to ammonia as younger larvae. Field observations of multiple fish species including FHM, a range of body lengths of individual species, amphibian reproduction, and observation of aquatic vegetation at or near the outfall also indicates no overt toxicity by the CBNG effluent.

My results support my hypothesis that biogeochemical interactions mitigate ammonia toxicity in CBNG effluent-dominated drainages. The super-saturated-$CO_2$ effluent maintained a lower pH at the outfall, where total ammonia was elevated. As the water flowed downstream, the $CO_2$ degassed and the pH began to increase, but gradually due to precipitation of $CaCO_3$ and subsequent buffering. Dissolution of gypsum ($CaSO_4$) resupplied the $Ca^{2+}$, allowing more $CaCO_3$ precipitation and formation of barite ($BaSO_4$), thus decreasing $Ba^+$ as the effluent flowed toward the Powder River. The $CaCO_3$

precipitation buffered the effluent-dominated stream to pH 8.0 – 8.5. This compares to pH 9.0 – 9.5 in the laboratory ambient-pH beakers, in which the pH continued to increase as the $CO_2$ degassed -- because not much $CaCO_3$ could precipitate and $Ca^{2+}$ was not resupplied in the beaker by dissolution of $CaSO_4$.

The ambient-pH and $CO_2$ pH-controlled whole effluent toxicity (WET) tests had similar acceptable survival at 96 h, but at 144 h (i.e., 96 h after renewal of the exposure water), the fish in the ambient-pH treatments had increased mortality probably due to pH drift and increased un-ionized ammonia concentrations. Based on these results, I recommend that the $CO_2$ pH-controlled test method is more appropriate for this type of CBNG produced water because it maintains pH closer to in-stream conditions than the ambient-pH test method.

Finally, ammonia concentrations increased in unpreserved effluent and stream samples, indicating organic matter are ammonified during transport from the field to the laboratory. Assuming ammonification occurs during transport and storage of CBNG produced waters, standard WET tests could be biased if the toxicant of concern is ammonia. Additional studies may be needed to 1) determine the conditions leading to ammonification and 2) determine the rates at which the ammonification occurs. However, results of WET tests conducted with any CBNG-related effluent or stream water should be interpreted with caution, unless the tests are conducted in flow-through chambers with the exposure waters pumped directly from the effluent discharge point or stream into the exposure chambers.

**Summary of Conclusions**

1. CBNG effluent and Beaver Creek water did not cause acute toxicity to 11- to 15-d-old FHM larvae after 96 h, in in-stream and in laboratory toxicity tests.
2. Field observations of aquatic plants and animals suggest no overt adverse effects from CBNG effluent discharged to Beaver Creek during the 2006 - 2007 study periods. Because Beaver Creek was historically ephemeral and did not support aquatic life prior to CBNG production, the year-round discharge provides a resource to support aquatic life.
3. $CO_2$ pH-controlled WET tests that maintain the pH of the original sample are more appropriate than ambient-pH WET tests for CBNG produced waters in the Powder River Basin of Wyoming.
4. The biogeochemistry of the interactions between the CBNG produced water and the Beaver Creek ecosystem helped mitigate toxic effects to aquatic organisms by buffering the pH gradually downstream from the outfall. Because increased pH (especially > 9.0) increases the percentage of un-ionized ammonia (the toxic form of ammonia in water), the $CO_2$-saturated effluent and the precipitation of $CaCO_3$ help maintain a lower pH than expected in the bicarbonate-rich CBNG-related waters.
5. In this study, total ammonia nitrogen concentrations decreased at a rate of 18% per km along a downstream gradient in Beaver Creek, probably due to assimilation by bacteria and algae -- thus increasing primary production in the drainage. Because ammonia is toxic at higher pH and the pH along Beaver Creek did not increase significantly before the ammonia decreased

considerably, the ammonia might be interpreted from one perspective as a benefit to the ecosystem. However, because the potentially most toxic combinations of pH and ammonia occurred at intermediate distances downstream from the discharge point in my study reach, the USEPA (1999) chronic criterion for ammonia was sometimes exceeded.

6. Current WDEQ/WQD waste load allocation calculations using flow, pH and temperature of the nearest USGS station to establish ammonia limits for CBNG discharges <1.6 km from the Powder River indicate the WDEQ/WQD's primary concern is the protection of the Powder River's aquatic community, not the aquatic community of the ephemeral drainages. However, based on my results for Beaver Creek, only ~28% of the ammonia would be lost in 1.6 stream km -- indicating that CBNG discharges further than 1.6 km from the Powder River might contribute considerably to the ammonia loading if the in-stream processes are similar to those along my study reach.
7. If the aquatic communities of ephemeral drainages are to be protected, the temperature and pH of the ephemeral streams should be considered in waste load allocation calculations. However, because the flow of ephemeral streams is intermittent by definition in the absence of the CBNG discharges, protection of aquatic life might not be necessary at distances >1.6 km from the Powder River in ephemeral drainages.
8. Based on the March 2007 study, ammonification can occur during transport and storage of unpreserved CBNG produced water and thus can bias laboratory toxicity tests, apparently even if the water is acidified but not maintained to

≤4 °C.

9. In this study, I only considered acute toxicity. Chronic studies will be needed to examine the effects of CBNG-related waters on reproduction and growth of aquatic organisms.

# Appendix A

## Supplemental Total Ammonia Nitrogen Collected from CBNG Outfalls in the Powder River Basin, Wyoming

Table A-1. Total ammonia nitrogen (TA-N) concentrations analyzed in coalbed natural gas produced-waters for informational purposes only, because these Wyoming Pollution Discharge Elimination System (WYPDES) permits do not require ammonia to be monitored and therefore have no associated regulatory enforcement. These data are made available for use by permission of Anadarko Petroleum Corporation (APC) and Lance Oil and Gas (LOG), 1099 18th Street Suite 1200, Denver, Colorado, USA, and Williams Production RMT Company (WPC), 300 North Works Avenue, Gillette, Wyoming, USA.

| Operator | Permit number | Outfall | Sample date | TA-N (mg N/L) |
|---|---|---|---|---|
| APC | WY0042013 | 006 | 07/24/06 | 1.80 |
| APC | WY0042013 | 006 | 10/23/06 | 2.60 |
| APC | WY0042013 | 006 | 12/13/06 | 2.70 |
| APC | WY0042013 | 008 | 07/24/06 | 2.60 |
| APC | WY0042013 | 008 | 09/18/06 | 4.70 |
| APC | WY0042013 | 008 | 12/11/06 | 2.40 |
| APC | WY0042013 | 009 | 07/24/06 | 2.70 |
| APC | WY0042013 | 009 | 10/23/06 | 3.20 |
| APC | WY0042013 | 009 | 12/14/06 | 3.10 |
| APC | WY0042013 | 017 | 07/24/06 | 1.50 |
| APC | WY0042013 | 031 | 07/24/06 | 3.50 |
| APC | WY0042013 | 031 | 09/20/06 | 3.50 |
| APC | WY0042013 | 034 | 08/07/06 | 2.80 |
| APC | WY0042013 | 034 | 09/18/06 | 5.30 |
| APC | WY0042030 | 010 | 07/24/06 | 1.90 |
| LOG | WY0047317 | 001 | 10/18/04 | <0.1 |
| LOG | WY0047317 | 001 | 11/18/05 | 1.10 |
| LOG | WY0048381 | 009 | 10/25/04 | <0.1 |
| LOG | WY0048381 | 010 | 02/13/06 | 1.60 |
| LOG | WY0048381 | 012 | 01/03/05 | <0.1 |

Table A-1 (cont.)

| Operator | Permit number | Outfall | Sample date | TA-N (mg N/L) |
|---|---|---|---|---|
| LOG | WY0051276 | 001 | 03/25/05 | <0.1 |
| LOG | WY0051276 | 005 | 12/12/06 | 2.10 |
| LOG | WY0052833 | 012 | 04/24/06 | 4.60 |
| WPC | WY0046922 | 001 | 04/06/05 | 1.00 |
| WPC | WY0048259 | 001 | 09/25/06 | 0.40 |
| WPC | WY0048534 | 002 | 09/26/06 | 0.09 |
| WPC | WY0048569 | 002 | 09/06/06 | 0.30 |
| WPC | WY0048577 | 004 | 09/11/06 | 0.40 |
| WPC | WY0051284 | 001 | 02/27/06 | 3.80 |
| WPC | WY0051284 | 004 | 03/19/07 | 6.60 |
| WPC | WY0051357 | 001 | 10/05/04 | 1.60 |
| WPC | WY0051357 | 001 | 04/19/05 | 2.10 |
| WPC | WY0051357 | 001 | 04/26/05 | 2.80 |
| WPC | WY0051357 | 001 | 07/10/06 | 4.20 |
| WPC | WY0051594 | 001 | 01/03/05 | 2.00 |
| WPC | WY0051594 | 001 | 02/15/05 | 2.20 |
| WPC | WY0051594 | 001 | 12/12/05 | 4.90 |
| WPC | WY0051594 | 001 | 06/19/06 | 3.40 |
| WPC | WY0051594 | 001 | 08/14/06 | 4.40 |
| WPC | WY0051713 | 001 | 03/14/05 | 1.80 |
| WPC | WY0051713 | 001 | 12/12/05 | 3.40 |
| WPC | WY0051713 | 001 | 06/19/06 | 2.70 |
| WPC | WY0051713 | 001 | 08/14/06 | 4.40 |
| WPC | WY0053171 | 005 | 09/19/05 | 2.30 |
| WPC | WY0053783 | 004 | 10/30/06 | 3.60 |
| WPC | WY0053783 | 004 | 12/04/06 | 2.00 |
| WPC | WY0053783 | 004 | 03/19/07 | 3.70 |
| WPC | WY0053783 | 010 | 06/05/06 | 2.60 |
| WPC | WY0054593 | 015 | 01/22/07 | 1.40 |

# Appendix B

## Raw Data of Percent Survival for In-Stream, Ambient-pH and $CO_2$ pH-Controlled Toxicity Tests

Table B-1. Survival of fathead minnows (*Pimephales promelas*) larvae in replicate exposure cages during **96-h in-stream** toxicity tests in Beaver Creek in the Powder River Basin, Wyoming during the July/August 2006, October 2006 and January 2007 field bouts. FC = field control, CV = coefficient of variation, N/A = not available.

| | | Survival % | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Cage | | | | | | |
| Month | Site | 1 | 2 | 3 | 4 | 5 | Average | CV |
| July/August 2006 | FC | 100 | 100 | 100 | 100 | 80 | 96 | 10.1 |
| | 1 | 0 | 100 | 100 | 100 | 78 | 76 | 48.6 |
| | 2 | 89 | 67 | 44 | 88 | 100 | 78 | 23.7 |
| | 3 | 90 | 100 | 100 | - - -[a] | 100 | 98 | 4.7 |
| | 4 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 5 | 100 | 100 | 90 | 100 | 100 | 98 | 5.2 |
| | 6 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| October 2006 | FC | 100 | 100 | 100 | 100 | N/A | 100 | 0.0 |
| | 1 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 2 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 3 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 4 | 100 | 70 | 90 | 80 | 100 | 88 | 15.1 |
| | 5 | 11 | 10 | 0 | 11 | 30 | 12[b] | 43.2 |
| | 6 | 0 | 10 | 20 | 33 | 10 | 15[b] | 45.7 |
| January 2007 | FC | 90 | 80 | 100 | 90 | N/A | 90 | 9.9 |
| | 1 | 0 | 80 | 100 | 100 | 100 | 76 | 49.3 |
| | 2 | 90 | 100 | 100 | 70 | 100 | 92 | 14.2 |
| | 3 | 80 | 100 | N/A | 100 | 100 | 95 | 11.4 |
| | 4 | 0 | 0 | 0 | 0 | 0 | 0[b] | 7.6 |

[*] Because 10 out of 10 fish were missing from the muskrat-torn cage 3 at Site 3, this replicate was removed from the survival analysis.
[a] Water temperature dropped below 2 °C which is the lower lethal temperature for fathead minnows (Hart 1947).

Table B-2. Survival of fathead minnows (*Pimephales promelas*) larvae in replicate exposure cages during **96-h ambient-pH** toxicity tests conducted at the University of Wyoming's Red Buttes Laboratory in Laramie, Wyoming with water from Beaver Creek in the Powder River Basin, Wyoming during the July/August 2006, October 2006 and January 2007 field bouts. LC = well-water lab control.

| | | Survival % | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Beaker | | | | | | |
| Month | Site | 1 | 2 | 3 | 4 | 5 | Average | CV |
| July/August 2006 | LC | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 1 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 2 | 90 | 90 | 90 | 100 | 100 | 94 | 10.96 |
| | 3 | 100 | 100 | 100 | 100 | 100 | 100 | 0.00 |
| | 4 | 100 | 90 | 100 | 90 | 90 | 94 | 11.0 |
| | 5 | 90 | 100 | 80 | 100 | 100 | 94 | 14.0 |
| | 6 | 90 | 80 | 100 | 100 | 100 | 94 | 14.0 |
| October 2006 | LC | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 1 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 2 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 3 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 4 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 5 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 6 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| January 2007 | LC | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 1 | 100 | 90 | 100 | 100 | 100 | 98 | 8.3 |
| | 2 | 100 | 90 | 100 | 90 | 100 | 96 | 10.5 |
| | 3 | 100 | 100 | 100 | 90 | 100 | 98 | 8.3 |
| | 4 | 100 | 80 | 100 | 100 | 70 | 90 | 19.6 |

Table B-3. Survival of fathead minnows (*Pimephales promelas*) larvae in replicate exposure cages during **144-h ambient-pH** toxicity tests conducted at the University of Wyoming's Red Buttes Laboratory in Laramie, Wyoming with water from Beaver Creek in the Powder River Basin, Wyoming during the July/August 2006, October 2006 and January 2007 field bouts. LC = well-water lab control.

| | | Survival % | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Beaker | | | | | | |
| Month | Site | 1 | 2 | 3 | 4 | 5 | Average | CV |
| July/August 2006 | LC | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 1 | 20 | 40 | 40 | 50 | 20 | 34 | 23.6 |
| | 2 | 30 | 30 | 30 | 10 | 40 | 28 | 24.6 |
| | 3 | 30 | 60 | 50 | 50 | 30 | 44 | 19.0 |
| | 4 | 90 | 90 | 90 | 80 | 90 | 88 | 5.1 |
| | 5 | 90 | 100 | 80 | 100 | 90 | 92 | 13.9 |
| | 6 | 90 | 80 | 100 | 100 | 100 | 94 | 14.0 |
| October 2006 | LC | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 1 | 100 | 100 | 63 | 100 | 63 | 85 | 0.0 |
| | 2 | 75 | 75 | 75 | 100 | 88 | 83 | 17.5 |
| | 3 | 75 | 75 | 100 | 88 | 63 | 80 | 20.4 |
| | 4 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 5 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 6 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| January 2007 | LC | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 1 | 0 | 70 | 80 | 0 | 70 | 44 | 84.5 |
| | 2 | 0 | 80 | 0 | 0 | 0 | 16 | 180.8 |
| | 3 | 60 | 10 | 0 | 10 | 100 | 36 | 94.9 |
| | 4 | 100 | 80 | 100 | 100 | 60 | 88 | 22.7 |

Table B-4. Survival of fathead minnows (*Pimephales promelas*) larvae in replicate exposure cages during **96-h $CO_2$ pH-controlled** toxicity tests conducted at the University of Wyoming's Red Buttes Laboratory in Laramie, Wyoming with water from Beaver Creek in the Powder River Basin, Wyoming during the July/August 2006, October 2006 and January 2007 field bouts. LC = well-water lab control. Two lab controls were maintained in each site-specific $CO_2$ chamber.

| | | Survival % | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Beaker | | | | | | | | |
| Month | Site | 1 | 2 | 3 | 4 | 5 | LC1 | LC2 | Average[a] | CV[a] |
| July/August 2006 | 1 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 2 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 4 | 100 | 100 | 100 | 100 | 90 | 90 | 100 | 98 | 8.3 |
| | 5 | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 96 | 12.9 |
| | 6 | 90 | 80 | 90 | 100 | 90 | 100 | 90 | 90 | 11.8 |
| October 2006 | 1 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 2 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 4 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 5 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 6 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| January 2007 | 1 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 2 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 4 | 100 | 90 | 100 | 100 | 100 | 100 | 100 | 98 | 8.3 |

[a] Average and CV calculating using the five beakers of site water only, not controls.

Table B-5. Survival of fathead minnows (*Pimephales promelas*) larvae in replicate exposure cages during **144-h $CO_2$ pH-controlled** toxicity tests conducted at the University of Wyoming's Red Buttes Laboratory in Laramie, Wyoming with water from Beaver Creek in the Powder River Basin, Wyoming during the July/August 2006, October 2006 and January 2007 field bouts. LC = well-water lab control. Two lab controls were maintained in each site-specific $CO_2$ chamber.

| | | Survival % | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Beaker | | | | | | | | |
| Month | Site | 1 | 2 | 3 | 4 | 5 | LC1 | LC2 | Average[a] | CV[a] |
| July/August 2006 | 1 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 2 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 4 | 100 | 100 | 100 | 100 | 90 | 90 | 100 | 98 | 8.3 |
| | 5 | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 96 | 12.9 |
| | 6 | 90 | 80 | 90 | 100 | 90 | 100 | 90 | 90 | 11.8 |
| October 2006 | 1 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 2 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 4 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 5 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| | 6 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.0 |
| January 2007 | 1 | 100 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 0.0 |
| | 2 | 100 | 100 | 100 | 90 | 80 | 100 | 100 | 94 | 14.0 |
| | 3 | 100 | 90 | 100 | 100 | 100 | 100 | 100 | 98 | 8.3 |
| | 4 | 100 | 90 | 100 | 90 | 90 | 100 | 100 | 96 | 10.5 |

[a] Average and CV calculating using the five beakers of site water only, not controls.

# Appendix C

## Field Sheets with Raw Data of Field Parameters and Comments

Table C-1. Daily field measurements of pH, temperature, electrical conductivity, dissolved oxygen, and turbidity in Beaver Creek in the Powder River Basin, Wyoming, and comments recorded during July/August 2006, October 2006, and January 2007 field bouts. FC = field control, $T_0$ = initiation time of in-stream toxicity test.

| Month | Site | Sample date | Time | pH | Temp. (˚C) | Conductivity (µS/cm) | Dissolved oxygen (mg/L) | Turbidity (ntu) | Comments |
|---|---|---|---|---|---|---|---|---|---|
| July-August | Site 0 | 7/30/2006 | 1342 | 9.19 | 29.3 | 2760 | 14.22 | 261 | Temperature 38+ °C on 7/30/06 |
| July-August | Site 0 | 7/31/2006 | 0555 | 8.90 | 16.9 | 2600 | | | Outside temp 21-29 °C on 7/31/06 |
| July-August | Site 0 | 7/31/2006 | 1315 | 8.99 | 21.1 | 2650 | | | |
| July-August | Site 0 | 8/1/2006 | 0845 | 8.94 | 15.4 | 3210 | 5.85 | 158 | |
| July-August | Site 0 | 8/1/2006 | 1318 | 9.14 | 18.4 | 3140 | 11.90 | 147 | |
| July-August | Site 0 | 8/2/2006 | 0840 | 8.97 | 14.3 | 3060 | 3.84 | 183 | Recalibrated DO and it was same low DO |
| July-August | Site 0 | 8/2/2006 | 1722 | 9.01 | 26.2 | 2760 | 12.31 | 592 | |
| July-August | Site 0 | 8/3/2006 | 0720 | 9.14 | 12.8 | 3140 | 2.82 | 169 | |
| July-August | Site 1 | 7/30/2006 | 0705 | 7.59 | 21.3 | 2100 | 3.95 | 9 | Temp at time of fish deployment=23.1 °C. $T_0$=0742 |
| July-August | Site 1 | 7/30/2006 | 1320 | 7.75 | 23.1 | 2100 | 5.82 | 9 | |
| July-August | Site 1 | 7/31/2006 | 0629 | 7.83 | 20.2 | 2120 | 4.68 | | |
| July-August | Site 1 | 7/31/2006 | 1320 | 7.85 | 21.4 | 2110 | | | |
| July-August | Site 1 | 8/1/2006 | 0905 | 7.64 | 20.4 | 2080 | 4.93 | 8 | |
| July-August | Site 1 | 8/1/2006 | 1310 | 7.64 | 21.0 | 2070 | 4.85 | 11 | |
| | | | | | | | | | |

| Table C-1 (cont.) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Month | Site | Sample date | Time | pH | Temp. (˚C) | Conductivity (µS/cm) | Dissolved oxygen (mg/L) | Turbidity (ntu) | Comments |
| July-August | Site 1 | 8/2/2006 | 0830 | 7.42 | 20.9 | 2100 | 4.38 | 9 | All cages had vacuum and did not drain easily when lifted basket out of water. Took individual cages and gently lifted out till drained well and then thumped side of cage to remove air bubbles per ASTM protocol |
| July-August | Site 1 | 8/2/2006 | 1710 | 7.42 | 22.8 | 2110 | 5.22 | 10 | |
| July-August | Site 1 | 8/3/2006 | 0730 | 7.54 | 20.7 | 2100 | 4.50 | 10 | |
| July-August | Site 2 | 7/30/2006 | 0854 | 7.92 | 21.6 | 2100 | 4.97 | 9 | Temp fish=23.1 $T_0$=0900 Stepped out from Site 2 to flume entrance = 31 m |
| July-August | Site 2 | 7/30/2006 | 1331 | 8.01 | 23.7 | 2110 | 6.42 | 8 | When flushed, sediment inside cages |
| July-August | Site 2 | 7/31/2006 | 0655 | 8.00 | 19.9 | 2120 | | | |
| July-August | Site 2 | 7/31/2006 | 1340 | 7.94 | 21.5 | 2100 | 5.60 | 7 | Two DO tubes broken off by muskrats |
| July-August | Site 2 | 8/1/2006 | 0935 | 7.72 | 20.2 | 2080 | 4.91 | 9 | |
| July-August | Site 2 | 8/1/2006 | 1305 | 7.90 | 20.8 | 2070 | 4.64 | 8 | |
| July-August | Site 2 | 8/2/2006 | 0820 | 7.63 | 20.7 | 2100 | 4.43 | 11 | |
| July-August | Site 2 | 8/2/2006 | 1655 | 7.70 | 23.2 | 2110 | 5.47 | 9 | |
| July-August | Site 2 | 8/3/2006 | 0750 | 7.72 | 20.5 | 2100 | 4.57 | 8 | |
| July-August | Site 3 | 7/30/2006 | 0929 | 8.27 | 22.5 | 2090 | 6.69 | 26 | Possibly stirred up since put in cage upstream Fish temp=22.7 $T_o$=9.46 |
| July-August | Site 3 | 7/30/2006 | 1405 | 8.50 | 26.4 | 2110 | 10.97 | 9 | |
| July-August | Site 3 | 7/31/2006 | 0728 | 8.24 | 18.2 | 2120 | | | |
| July-August | Site 3 | 7/31/2006 | 1404 | 8.31 | 22.8 | 2100 | 9.37 | 10 | |
| July-August | Site 3 | 8/1/2006 | 1030 | 8.09 | 19.4 | 2070 | 5.66 | | |

| Table C-1 (cont.) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Month | Site | Sample date | Time | pH | Temp. (˚C) | Conductivity (µS/cm) | Dissolved oxygen (mg/L) | Turbidity (ntu) | Comments |
| July-August | Site 3 | 8/1/2006 | 1405 | 8.35 | 21.2 | 2070 | 7.58 | 15 | Monitored downstream to upstream but same turbidity |
| July-August | Site 3 | 8/2/2006 | 0800 | 7.99 | 19.5 | 2090 | 4.82 | 17 | Forgot to do turbidity |
| July-August | Site 3 | 8/2/2006 | 1640 | 8.01 | 23.4 | 2110 | 6.16 | NA | |
| July-August | Site 3 | 8/3/2006 | 1030 | 8.01 | 22.8 | 2090 | 8.61 | 9 | |
| July-August | Site 4 | 7/30/2006 | 1030 | 8.61 | 23.6 | 2140 | 7.46 | 36 | Fish temp=25.4 $T_o$=1047 |
| July-August | Site 4 | 7/30/2006 | 1340 | 8.81 | 26.7 | 2130 | 11.70 | 19 | |
| July-August | Site 4 | 7/31/2006 | 0755 | 8.74 | 20.2 | 2140 | | | Collected downstream to upstream |
| July-August | Site 4 | 7/31/2006 | 1425 | 8.67 | 21.6 | 2160 | 9.94 | 27 | Turbidity may be higher since just sampled upstream |
| July-August | Site 4 | 8/1/2006 | 1100 | 8.50 | 18.1 | 2140 | 6.26 | 57 | |
| July-August | Site 4 | 8/1/2006 | 1423 | 8.58 | 18.5 | 2160 | 7.51 | 34 | |
| July-August | Site 4 | 8/2/2006 | 0737 | 8.48 | 16.0 | 2110 | 5.15 | 59 | |
| July-August | Site 4 | 8/2/2006 | 1613 | 8.54 | 22.4 | 2120 | 9.34 | 26 | |
| July-August | Site 4 | 8/3/2006 | 1145 | 8.38 | 19.8 | 2130 | 8.13 | 40 | |
| July-August | Site 5 | 7/30/2006 | 1121 | 8.89 | 24.0 | 2210 | 12.65 | 4 | Fish temp=26.2 $T_o$=1145 |
| July-August | Site 5 | 7/30/2006 | 1420 | 9.19 | 29.1 | 2210 | 10.95 | NA | afternoon observation so increased pH due to photosynthesis |
| July-August | Site 5 | 7/31/2006 | 0835 | 8.95 | 17.9 | 2230 | | | |
| July-August | Site 5 | 7/31/2006 | 1442 | 9.06 | 22.7 | 2200 | 10.69 | 4 | |
| July-August | Site 5 | 8/1/2006 | 1125 | 8.80 | 18.1 | 2210 | 9.10 | 4 | |
| July-August | Site 5 | 8/1/2006 | 1442 | 8.95 | 20.0 | 2210 | 9.46 | 4 | |
| July-August | Site 5 | 8/2/2006 | 0720 | 8.60 | 14.4 | 2220 | 7.05 | 6 | |
| July-August | Site 5 | 8/2/2006 | 1558 | 8.88 | 24.1 | 2190 | 9.25 | 4 | |
| July-August | Site 5 | 8/3/2006 | 1240 | 8.77 | 21.6 | 2150 | 9.73 | 5 | |

Table C-1 (cont.)

| Month | Site | Sample date | Time | pH | Temp. (˚C) | Conductivity (µS/cm) | Dissolved oxygen (mg/L) | Turbidity (ntu) | Comments |
|---|---|---|---|---|---|---|---|---|---|
| July-August | Site 6 | 7/31/2006 | 0905 | 9.09 | 17.9 | 2280 | | | Fish temp=27.2 $T_o$=1230 |
| July-August | Site 6 | 7/31/2006 | 1510 | 9.16 | 25.1 | 2250 | 9.89 | NA | afternoon observation so increased pH due to photosynthesis |
| July-August | Site 6 | 8/1/2006 | 1205 | 8.95 | 19.0 | 2240 | 8.24 | | |
| July-August | Site 6 | 8/1/2006 | 1500 | 9.03 | 21.2 | 2230 | 8.23 | 64 | |
| July-August | Site 6 | 8/2/2006 | 0700 | 8.62 | 14.2 | 2300 | 6.73 | 34 | No cows present, unusual for week |
| July-August | Site 6 | 8/2/2006 | 1540 | 8.86 | 25.0 | 2220 | 8.78 | 37 | Fish temp=23.1 $T_o$=0821 |
| July-August | Site 6 | 8/3/2006 | 1315 | 8.89 | 25.4 | 2180 | 8.80 | 14 | |
| July-August | FC | 7/30/2006 | 0819 | 8.02 | 23.0 | 432 | 6.89 | | Sampled soil and algae |
| July-August | FC | 7/30/2006 | 1328 | 8.24 | 23.4 | 445 | 7.78 | 6 | Placed RB water carboys in BC to equilibrate; Carboy1 temp=20.8 at 1003; Carboy#2=27° left in creek till 1240 |
| July-August | FC | 7/31/2006 | 1320 | 8.47 | 19.2 | 476 | 6.22 | | |
| July-August | FC | 8/1/2006 | 1003 | 8.06 | 17.2 | 517 | 5.32 | | |
| July-August | FC | 8/1/2006 | 1248 | 8.50 | 20.9 | 425 | 6.24 | 10 | |
| July-August | FC | 8/2/2006 | 0824 | 8.15 | 17.1 | 462 | 6.46 | 2 | |
| July-August | FC | 8/2/2006 | 1700 | 8.04 | 21.7 | 464 | 5.98 | 2 | |
| July-August | FC | 8/3/2006 | 0740 | 8.05 | 17.6 | 473 | 6.21 | 2 | |
| October | Site 1 | 10/15/2006 | 0920 | 7.44 | 18.6 | 2080 | 5.58 | 9 | $T_0$=1020, No acclimation since fish temp=18 Per pumper (Jeremy), Tuesday 10/17/06 at ~5 am WPC shut down water to tie in gas lines |

| Table C-1 (cont.) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Month | Site | Sample date | Time | pH | Temp. (˚C) | Conductivity (µS/cm) | Dissolved oxygen (mg/L) | Turbidity (ntu) | Comments |
| October | Site 1 | 10/16/2006 | 0730 | 7.39 | 18.8 | 2080 | 5.12 | 9 | |
| October | Site 1 | 10/16/2006 | 1300 | 7.63 | 19.2 | 2080 | 4.98 | 9 | Cages almost completely out of water, removed lower drainer |
| October | Site 1 | 10/17/2006 | 0815 | 7.60 | 15.6 | 2190 | 6.47 | 8 | |
| October | Site 1 | 10/17/2006 | 1015 | 7.92 | 15.3 | 2180 | 6.29 | 8 | |
| October | Site 1 | 10/17/2006 | 1505 | 8.16 | 14.2 | | 8.28 | | |
| October | Site 1 | 10/18/2006 | 0950 | 7.92 | 13.9 | 2070 | 5.91 | | |
| October | Site 1 | 10/18/2006 | 1410 | 8.01 | 16.1 | 2030 | 6.36 | 17 | |
| October | Site 1 | 10/19/2006 | 0950 | 7.94 | 15.8 | 2060 | 5.76 | 18 | |
| October | Site 2 | 10/15/2006 | 0950 | 7.60 | 18.2 | 2080 | 5.30 | 9 | No acclimation required. Fish temp within 2 degrees of creek. Small pools of fish that appeared to be FHM between site 2 and outfall. $T_0$=1100 |
| October | Site 2 | 10/16/2006 | 0805 | 7.67 | 18.5 | 2080 | 6.41 | 9 | Pools of fish sited near SCC |
| October | Site 2 | 10/16/2006 | 1330 | 7.77 | 19.0 | 2080 | 6.44 | 12 | |
| October | Site 2 | 10/17/2006 | 0830 | 7.92 | 14.9 | 2170 | 7.72 | 8 | |
| October | Site 2 | 10/17/2006 | 1035 | 8.13 | 14.5 | 2170 | 8.11 | 9 | |
| October | Site 2 | 10/17/2006 | 1455 | 8.33 | 13.6 | | | | |
| October | Site 2 | 10/18/2006 | 0845 | 8.02 | 13.5 | 2070 | 6.89 | 9 | |
| October | Site 2 | 10/18/2006 | 1430 | 8.10 | 16.2 | 2070 | | | |
| October | Site 2 | 10/19/2006 | 1040 | 8.04 | 15.8 | 2060 | 7.20 | 11 | |
| October | Site 3 | 10/15/2006 | 1135 | 7.89 | 18.6 | 2030 | 7.66 | 35 | Fish seen, $T_0$=1210<br>No acclimation required |
| October | Site 3 | 10/16/2006 | 0845 | 7.91 | 17.6 | 2100 | 6.80 | 30 | DO meter replaced 10/16/06 |
| October | Site 3 | 10/16/2006 | 1400 | 7.97 | 18.9 | 2090 | 7.04 | 14 | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |

| Table C-1 (cont.) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Month | Site | Sample date | Time | pH | Temp. (˚C) | Conductivity (µS/cm) | Dissolved oxygen (mg/L) | Turbidity (ntu) | |
| October | Site 3 | 10/17/2006 | 0950 | 8.27 | 12.0 | 2220 | 8.23 | | Only pH and EC due to cold weather coming |
| October | Site 3 | 10/17/2006 | 1115 | 8.48 | 11.5 | 2230 | 8.80 | 39 | |
| October | Site 3 | 10/17/2006 | 1435 | 8.53 | 10.8 | | | | |
| October | Site 3 | 10/18/2006 | 0830 | 8.30 | 10.5 | 2110 | 7.75 | | Fish sited |
| October | Site 3 | 10/18/2006 | 1150 | 8.20 | 14.4 | 2090 | 9.92 | | |
| October | Site 3 | 10/18/2006 | 1500 | 8.31 | 16.1 | 2080 | 8.63 | | |
| October | Site 3 | 10/19/2006 | 1130 | 8.28 | 15.2 | 2070 | 7.93 | | |
| October | Site 4 | 10/15/2006 | 1240 | 8.13 | 12.4 | 2170 | 8.54 | 60 | Acclimated fish to 15 degrees by placing bag in tub of creek water for about 20 minutes. Fish temp=15 prior to deployment $T_0$=1300 |
| October | Site 4 | 10/16/2006 | 0930 | 8.24 | 11.1 | 2200 | 6.91 | 103 | |
| October | Site 4 | 10/16/2006 | 1430 | 8.27 | 12.0 | 2200 | 8.58 | 67 | |
| October | Site 4 | 10/17/2006 | 1200 | 8.60 | 6.7 | 2280 | 9.77 | | Outside temp=31° F per Lesley truck and windy |
| October | Site 4 | 10/17/2006 | 1425 | 8.70 | 6.2 | | | | |
| October | Site 4 | 10/18/2006 | 0810 | 8.55 | 2.2 | 2440 | 9.07 | 88 | DO temp =3.1 |
| October | Site 4 | 10/18/2006 | 1211 | 8.51 | 5.6 | 2420 | 11.60 | | |
| October | Site 4 | 10/18/2006 | 1510 | 8.59 | 7.3 | 2410 | 11.58 | 77 | |
| October | Site 4 | 10/19/2006 | 1230 | 8.72 | 6.6 | 2420 | 9.34 | 133 | |
| October | Site 5 | 10/15/2006 | 1330 | 8.35 | 11.9 | 2230 | 8.16 | 45 | $T_0$=1345; fish sited and frog sited; lots of leaves and grasses Acclimated fish to 14 degrees by placing bag in tub of creek water for about 20 minutes. Fish temp=14° prior to deployment |
| October | Site 5 | 10/16/2006 | 1020 | 8.45 | 8.5 | 2270 | 10.14 | 49 | WET collected at 1010 |

| Table C-1 (cont.) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Month | Site | Sample date | Time | pH | Temp. (˚C) | Conductivity (µS/cm) | Dissolved oxygen (mg/L) | Turbidity (ntu) | Comments |
| October | Site 5 | 10/16/2006 | 1500 | 8.49 | 11.1 | 2240 | 9.84 | 43 | |
| October | Site 5 | 10/17/2006 | 1300 | 8.81 | 4.7 | 2340 | 11.18 | 91 | Cages were under water |
| October | Site 5 | 10/17/2006 | 1410 | 8.87 | 4.6 | | | | DO meter reads 0.7 degrees; cages half out of water so took off bottom basket and resubmerged |
| October | Site 5 | 10/18/2006 | 0750 | 8.64 | Under range | 2550 | 10.52 | 147 | Under range = below detectable range on the temperature meter |
| October | Site 5 | 10/18/2006 | 1230 | 8.69 | 3.3 | 2400 | 11.97 | | |
| October | Site 5 | 10/18/2006 | 1530 | 8.79 | 6.8 | 2440 | 10.83 | 52 | |
| October | Site 5 | 10/19/2006 | 1330 | 8.92 | 7.0 | 2540 | 11.22 | 61 | |
| October | Site 6 | 10/15/2006 | 1430 | 8.44 | 12.4 | 2220 | 9.38 | 67 | $T_0$=1500<br>same deployment at Site 5; fish temp=15.2 at time of deployment |
| October | Site 6 | 10/16/2006 | 1100 | 8.56 | 8.5 | 2270 | 10.72 | 65 | WET collected at 1100 |
| October | Site 6 | 10/16/2006 | 1515 | 8.59 | 11.1 | 2250 | 10.64 | 64 | |
| October | Site 6 | 10/17/2006 | 1345 | 8.96 | 3.6 | 2340 | 10.75 | | |
| October | Site 6 | 10/17/2006 | 1355 | 8.96 | 3.8 | | | | Outside temp 33 ° F |
| October | Site 6 | 10/18/2006 | 0720 | 8.70 | Under range | 2420 | 10.67 | 103 | Under range = below detectable range on the temperature meter, DO meter reads 0.7 °C |
| October | Site 6 | 10/18/2006 | 1550 | 7.10 | 8.9 | 2350 | 12.24 | 93 | |
| October | Site 6 | 10/19/2006 | 1435 | 9.06 | 7.4 | 2450 | 11.32 | 114 | Cages half out so took bottom basket off |
| October | FC | 10/15/2006 | 1610 | 7.64 | 18.0 | 419 | | 1 | |
| October | FC | 10/16/2006 | 0750 | 7.71 | 16.3 | 439 | 7.13 | | |
| October | FC | 10/16/2006 | 1320 | 7.89 | 16.4 | 449 | 6.68 | 2 | Renewal water equilibrated in creek |
| | | | | | | | | | |

| Table C-1 (cont.) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Month | Site | Sample date | Time | pH | Temp. (˚C) | Conductivity (µS/cm) | Dissolved oxygen (mg/L) | Turbidity (ntu) | Comments |
| October | FC | 10/17/2006 | 1500 | 8.37 | 7.8 | 477 | 8.28 | | |
| October | FC | 10/17/2006 | 1515 | 8.07 | 11.0 | 423 | 7.37 | 5 | |
| October | FC | 10/18/2006 | 0955 | 8.12 | 8.1 | 445 | 8.46 | | |
| October | FC | 10/18/2006 | 1435 | 8.11 | 13.5 | 438 | 8.31 | | |
| October | FC | 10/19/2006 | 1530 | 8.68 | 15.5 | 453 | 6.91 | 5 | |
| January | Site 1 | 1/23/2007 | 0725 | 7.33 | 15.3 | 2510 | 4.01 | 6 | Picked up fish 1/23 from ENSR, took to RB to acclimate to hardness. Transported in 50/50 |
| January | Site 1 | 1/24/2007 | 0810 | 7.70 | 15.1 | 2470 | 5.00 | 10 | |
| January | Site 1 | 1/24/2007 | 1650 | 7.60 | 15.9 | 2490 | 4.70 | 18 | |
| January | Site 1 | 1/25/2007 | 0820 | 7.67 | 14.6 | 2510 | 5.37 | 11 | |
| January | Site 1 | 1/26/2007 | 0950 | 7.56 | 14.4 | 2530 | 4.12 | 8 | |
| January | Site 1 | 1/27/2007 | 0850 | 7.61 | 14.0 | 2500 | 6.04 | 8 | |
| January | Site 2 | 1/23/2007 | 0900 | 7.91 | 14.8 | 2510 | 5.86 | 7 | |
| January | Site 2 | 1/24/2007 | 0845 | 7.79 | 14.8 | 2500 | 6.19 | 9 | |
| January | Site 2 | 1/24/2007 | 1630 | 7.84 | 15.7 | 2490 | 5.61 | 7 | Fish sited in stream |
| January | Site 2 | 1/25/2007 | 0730 | 7.74 | 14.3 | 2490 | 6.96 | 11 | |
| January | Site 2 | 1/26/2007 | 0900 | 7.81 | 14.1 | 2570 | 5.96 | 13 | |
| January | Site 2 | 1/27/2007 | 0910 | 7.89 | 13.9 | 2530 | 7.60 | 6 | |
| January | Site 3 | 1/23/2007 | 1015 | 7.96 | 14.2 | 2550 | 5.60 | 11 | |
| January | Site 3 | 1/24/2007 | 0915 | 8.04 | 13.7 | 2530 | 6.09 | 23 | Fish sited in stream |
| January | Site 3 | 1/24/2007 | 1545 | 8.12 | 14.7 | 2530 | 5.49 | 9 | |
| January | Site 3 | 1/25/2007 | 0830 | 8.18 | 12.9 | 2550 | 6.25 | 19 | |
| January | Site 3 | 1/26/2007 | 0845 | 8.13 | 13.0 | 2580 | 6.57 | 44 | |
| January | Site 3 | 1/27/2007 | 0940 | 8.27 | 12.3 | 2510 | 6.60 | 28 | |
| January | Site 4 | 1/23/2007 | 1215 | 8.32 | 5.8 | 2700 | 8.38 | 112 | Acclimated bag of fish to 6.9 and stream was 5.5. Confident adequate acclimation occurred |

| Table C-1 (cont.) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Month | Site | Sample date | Time | pH | Temp. (˚C) | Conductivity (µS/cm) | Dissolved oxygen (mg/L) | Turbidity (ntu) | Comments |
| January | Site 4 | 1/24/2007 | 0930 | 8.51 | 5.2 | 2770 | 8.27 | NA | |
| January | Site 4 | 1/24/2007 | 1530 | 8.58 | 8.5 | 2670 | 9.40 | 77 | |
| January | Site 4 | 1/25/2007 | 0900 | 8.60 | 3.5 | 2770 | 9.12 | 79 | |
| January | Site 4 | 1/26/2007 | 0830 | 8.77 | 2.0 | 2850 | 10.50 | 90 | |
| January | Site 4 | 1/27/2007 | 1000 | 8.74 | 1.0 | 2800 | 11.20 | 121 | |
| January | FC | 1/24/2007 | 0830 | 8.12 | 13.1 | 431 | 7.55 | | |
| January | FC | 1/25/2007 | 0800 | 8.25 | 12.8 | 441 | 7.06 | | |
| January | FC | 1/26/2007 | 0915 | 8.08 | 12.6 | 488 | 5.63 | | |
| January | FC | 1/26/2007 | 0930 | 8.11 | 10.1 | 478 | | | |

# Appendix D

## Raw Data of Nitrogen Containing

## Species Analyzed at Energy Labs Inc.

Table D-1. Nitrogen-containing species in water from Beaver Creek in the Powder River Basin, Wyoming analyzed at Energy Labs Inc. in Gillette, Wyoming and Billings, Montana. Samples were collected and preserved ($H_2SO_4$ to pH < 2.0) twice during the July/August 2006 field bout and three times during the October 2006 and January 2007 field bouts. TN = total nitrogen, TA-N = total ammonia nitrogen, UIA = unionized ammonia nitrogen, NA = not available.

| | | TN (mg N/L) | | | TA-N (mg N/L) | | | UIA (mg N/L) | | | $NO_3^-$ (mg N/L) | | | $NO_2^-$ (mg N/L) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Month | Site | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| July/August 2006 | 0 | NA | NA | | <0.10 | <0.10 | | 0.000 | 0.000 | | <0.10 | <0.10 | | <0.10 | <0.10 | |
| | 1 | NA | NA | | 1.30 | 1.60 | | 0.041 | 0.030 | | <0.10 | <0.10 | | <0.10 | <0.10 | |
| | 2 | NA | NA | | 1.20 | 1.50 | | 0.045 | 0.057 | | <0.10 | <0.10 | | 0.10 | <0.10 | |
| | 3 | NA | NA | | 1.20 | 1.20 | | 0.068 | 0.097 | | <0.10 | <0.10 | | <0.10 | <0.10 | |
| | 4 | NA | NA | | 0.30 | 0.70 | | 0.054 | 0.167 | | 0.10 | 0.20 | | 0.30 | 0.20 | |
| | 5 | NA | NA | | <0.10 | <0.10 | | 0.000 | 0.000 | | <0.10 | 0.40 | | <0.10 | <0.10 | |
| | 6 | NA | NA | | <0.10 | <0.10 | | 0.000 | 0.000 | | <0.10 | <0.10 | | <0.10 | <0.10 | |
| October 2006 | 1 | 3.70 | 3.50 | | 1.40 | 1.60 | 1.40 | 0.015 | 0.025 | 0.041 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 |
| | 2 | 3.50 | 3.50 | | 1.50 | 1.50 | 1.50 | 0.024 | 0.032 | 0.046 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 |
| | 3 | 3.50 | 3.60 | | 1.40 | 1.40 | 1.30 | 0.037 | 0.046 | 0.065 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 |
| | 4 | 2.60 | 2.80 | | 0.80 | 1.00 | 1.10 | 0.027 | 0.039 | 0.076 | 0.20 | 0.20 | 0.30 | 0.20 | 0.10 | 0.10 |
| | 5 | 1.70 | 1.70 | | 0.50 | 0.40 | 0.50 | 0.022 | 0.024 | 0.054 | 0.50 | 0.50 | 0.50 | 0.10 | 0.20 | <0.10 |
| | 6 | 1.20 | 1.30 | | 0.20 | 0.10 | 0.30 | 0.011 | 0.007 | 0.044 | 0.50 | 0.40 | 0.40 | <0.10 | <0.10 | <0.10 |
| January 2007 | 1 | 3.20 | 3.10 | 3.40 | 1.40 | 1.60 | 1.20 | 0.008 | 0.018 | 0.012 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 |
| | 2 | 3.10 | 2.90 | 3.40 | 1.10 | 1.50 | 1.30 | 0.024 | 0.029 | 0.025 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 |
| | 3 | 3.00 | 2.90 | 3.60 | 1.20 | 1.50 | 1.10 | 0.027 | 0.051 | 0.044 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 |
| | 4 | 3.10 | 2.80 | 3.20 | 1.00 | 1.20 | 1.00 | 0.026 | 0.071 | 0.047 | 0.20 | 0.30 | 0.10 | <0.10 | <0.10 | <0.10 |

# Appendix E

## Madge Tech Data Logger Graph of Temperature

## during January Study
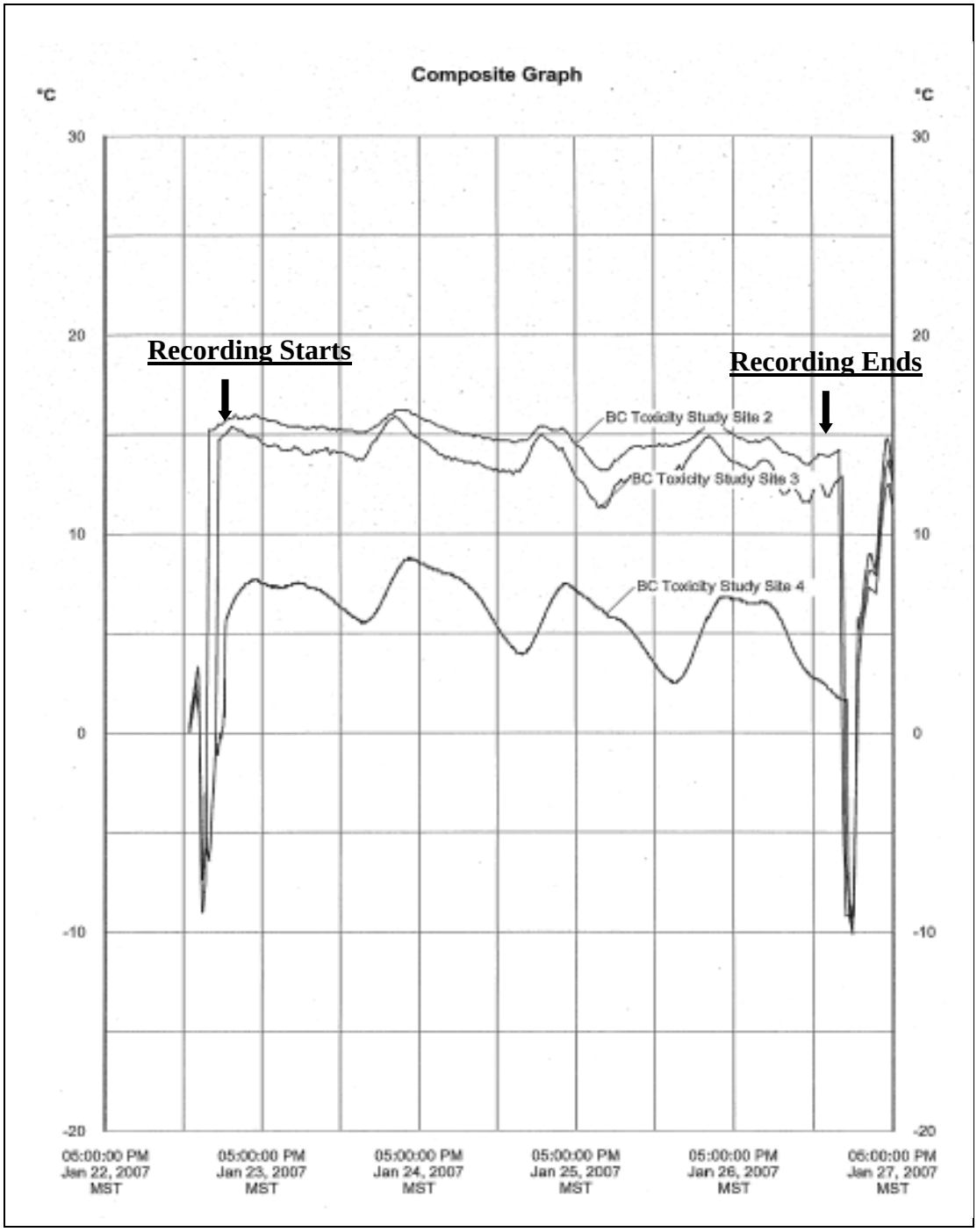
Figure E-1. The plotted temperatures below were from the continuous temperature recorder (Madge Tech Temp 1000) at Sites 1, 2, 3 and 4 in January 2007. The temperatures at Site 4 were 0.5 °C higher than those recorded daily in the field using the Oakton 10 Series hand-held meter.