SWMA

# United States Department of the Interior

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In Reply Refer To:
FWS/Region 8/ORS

4 JAN 1990

Memorandum

To: Regional Director, Region 1

From: Regional Director, Region 8

Subject: Progress Report - Stillwater National Wildlife Refuge Drainwater Studies

Attached is the research progress report "Investigations on the Combined Toxicity of Trace Elements and Salinity to Aquatic Organisms at Stillwater National Wildlife Refuge". This progress report describes research conducted by the National Fisheries Contaminants Research Center which was funded by Region 1 (account #10120-1902-A3) in FY 1989. A final report will be submitted in early 1990 after trace element analysis and final interpretation of the data are completed.

/s/ John D. Buffington

Attachment

cc: Richard Stroud, Region 1
Region 8 reading file
ARD - A reading file
Bob Hallock, Region 1
DRD chron

FWS:R8:ORS:CJohnson:12/29/89:WPORS2:CJ-PRSTILLWATER

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# INVESTIGATIONS ON THE COMBINED TOXICITY OF TRACE ELEMENTS AND SALINITY TO AQUATIC ORGANISMS AT STILLWATER NATIONAL WILDLIFE REFUGE

A Research Progress Report

Submitted To:

Regional Director
Region 1
U.S. Fish and Wildlife Service
Portland, OR

Submitted By:

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December 11, 1989

## EXECUTIVE SUMMARY

Agricultural drain water poses a threat to fish and wildlife resources at several sites administered by the U.S. Department of Interior (DOI) including Stillwater National Wildlife Refuge (SNWR). The SNWR is located in western Nevada, Churchill County, outside of the city of Fallon and receives agricultural drain waters from the Truckee-Carson Irrigation District. Often the drain water has elevated salinity and concentrations of trace elements including arsenic, boron, copper, lithium, molybdenum, and strontium. Recognizing the need to address the environmental hazard of elevated salinities and trace elements Region 1, U.S. Fish and Wildlife Service appropriated funds to the National Fisheries Contaminant Research Center (NFCRC) to conduct research on the toxicity of trace elements and salinities found at SNWR.

During FY88, effluent toxicity studies conducted with fish and aquatic invertebrates showed that many of the drain waters were toxic (Ingersoll, et. al.; 1988, Finger et. al., 1989). Studies conducted by NFCRC during FY89 addressed questions which arose from these studies. Studies were conducted with both fresh and salt water organisms. The use of both freshwater and saltwater organisms allowed for the separation of toxic effects due to salinity from the toxic effects of trace elements. Toxicological data generated includes: (1) the importance of ion ratios in addition to trace elements on the toxicity of drain

waters to aquatic organisms; (2) the increased toxicity of mixtures of individual trace elements; and (3) the high toxicity of groundwater that enters into SNWR surface waters. The following information is preliminary as trace element analyses are in progress. Results from the FY89 studies will be helpful in managing limited freshwater resources at SNWR.

## BACKGROUND

Contaminants in agricultural irrigation drain water pose a threat to fish and wildlife resources at several sites administered by the U.S. Department of the Interior (DOI). Recently, a DOI Task Group on Irrigation Water Quality was given the charge to evaluate existing and potential drain water related contaminant problems on DOI lands throughout the western United States. The results of initial field investigations indicate a need to further assess biological impacts at four locations: Stillwater in Nevada, Ouray in Utah, Salton Sea in California, and Kendrick in Wyoming (Hoffman et al., 1988).

Stillwater National Wildlife Refuge (SNWR) is part of the Stillwater Wildlife Management Area (SWMA) located in Churchill County, in the lower Carson River Basin, about 50 miles east of Reno, NV. The SWMA was established in 1948 by a cooperative agreement with the U.S. Fish and Wildlife Service (Service), the Nevada Fish and Game Commission, and the Truckee-Carson Irrigation District. Historically, the arid region of northwestern Nevada contained about 171,000 acres of freshwater wetland. However, since 1905, the quality and quantity of water reaching the wetlands has declined primarily due to extensive irrigation (Hoffman et al., 1988).

The Service is responsible for the physical management of SWMA. SNWR receives agricultural drain water which has elevated salinity and trace element concentrations including arsenic, boron, chromium, lithium, mercury, molybdenum, strontium, vanadium, and zinc (Hoffman, et al., 1988). As the overall flow to the Refuge decreases the relative volume of potentially contaminated agricultural drain water increases. Water entering SNWR is terminal and is eventually lost to evapotranspiration which leaves most of the dissolved solids load in the basin. The wetland management strategy for SNWR has been to minimize the impact of these salts through the use of sequentially connected ponds. The first pond, which receives the freshest water, experiences some elevation of dissolved solids due to evapotranspiration and potential input of dissolved solids from subsurface water. Water from the first pond is discharged into a second pond where dissolved solids are further concentrated. A third pond in turn receives highly concentrated dissolved solids and eventually becomes unsuitable for wildlife management. This tertiary pond is maintained only if water is available. When water is available the management strategy is to discharge the water into Carson Sink or add fresh water when desirable aquatic macrophytes can no longer tolerate the high dissolved solid concentrations. More predictive monitoring tools are needed to assess water quality at SNWR.

Fish and invertebrate toxicity tests are sensitive indicators of either elevated trace elements or salinity below the water quality conditions that affect aquatic macrophytes (Ingersoll et al., 1988; Finger et al., 1989). Both fresh and salt water organisms can be used to assess the toxicity of drain waters. The use of both fresh and salt water organisms allows for the separation of salinity effects from the toxic effects of trace elements associated with irrigation drain waters.

Previous studies conducted by the National Fisheries Contaminant Research Center (NFCRC) assessed the whole effluent toxicity of agricultural irrigation drain water entering SNWR (Ingersoll et al., 1988; Finger et al., 1989). Many of the drain waters tested were toxic to both fresh and salt water organisms. While the drain water effluent samples contained elevated concentrations of several inorganic contaminants, none of these elements were present at levels expected to be acutely lethal based on reported literature values for single-compound toxicity tests. The complicating factor of trace element-salinity interactions may result in literature values that are not sufficiently protective. In addition, the toxic mechanism occurring within complex chemical mixtures cannot always be identified on the basis of single-compound toxicity values. The objective of NFCRC Research Plans I and II was to address salinity and trace element interactions of drain waters.

In addition to elevated concentrations of trace elements in the surface waters of SNWR, subsurface waters also contain high levels of dissolved salts. As a consequence of irrigating this desert land, the ground water in the Fallon agricultural area has risen about 60 feet in the past century (Hoffman et al., 1988). The water table rises and falls in response to irrigation and during times of low flows groundwater seepage into the drains may be significant. The objective of NFCRC Research Plan III was to provide an estimate of the relative importance of groundwater seepage to the overall toxicity of surface water at SNWR. Results from each Research Plan will help establish needed water quality data on which to base improved management and protection of fish and wildlife resources at SNWR. The following information is preliminary as trace element analyses are in progress.

RESEARCH PLAN I. DETERMINATION OF THE INFLUENCE OF SALINITY ON THE TOXICITY OF MULTIPLE TRACE ELEMENT CONTAMINANTS IDENTIFIED AT SNWR.

## INTRODUCTION

Water at Stillwater National Wildlife Refuge (SNWR) is generally discharged from tertiary ponds when aquatic macrophytes and vegetation can no longer tolerate high concentrations of dissolved solids. More predictive monitoring tools are needed to better assess water quality. Fish and invertebrate toxicity tests can be sensitive indicators of either elevated trace elements or salinity well below the water quality conditions that might affect aquatic macrophytes (Ingersoll, et. al., 1988).

During FY88 four drain waters (T.J. Drain, Hunter Drain, Pintail Bay, and Lead Lake) were collected refuge personnel and tested at the National Fisheries Contaminant Research Center (NFCRC). Only one site, Pintail Bay, was acutely toxic to both freshwater and saltwater acclimated or cultured organisms. These results suggested that the toxicity of Pintail Bay effluent was not solely the result of elevated salinity alone (Ingersoll, et. al., 1988). Additional tests conducted in FY88 revealed that the major ion concentrations (Ca, Mg, K, Na, $CO_3$, $SO_4$, Cl) present in the Pintail Bay water alone were toxic and a trace element mixture added to this toxicity. Results from these studies suggested that more data were needed on toxicological interactions of multiple trace element contaminants and ionic composition of the water.

The objective of this Research Plan was to investigate the toxicity of multiple trace element contaminants as influenced by salinity and water hardness. These investigations provided information on the toxic interactions of multiple trace elements with salinity and hardness to provide a management tool which could be used to monitor water quality at SNWR. The following describes studies using four reconstituted waters with striped bass Morone saxatilis, the amphipod Hyalella azteca, and the cladoceran Daphnia magna to address the toxic interactions among multiple trace elements, salinity, and hardness.

## MATERIALS AND METHODS

Acute toxicity tests were conducted which generally followed EPA (EPA/660/3-75/009) and ASTM (ASTM, 1988) testing procedures. Four different test waters (low, medium, and high hardness; and Instant Ocean) with similar salinities (22 g/L) were tested with and without a mixture of 6 trace elements (As, B, Cu, Li, Mo, and Sr) identified from studies conducted during FY88 (Ingersoll et. al., 1988). Table 1 and 2 describe the salts used in mixing the test waters. Each test water was diluted in a 70% series with reconstituted water which simulated Lahonton Reservoir water (Table 1); a source of fresh water at SNWR. All studies were conducted at 20°C and organisms were not fed during the tests. Biological observations were made every 24 h for the duration of each test.

## Water Chemistry

Chemical analyses were conducted on 100% test waters with and without trace elements. Samples for inductively coupled argon plasma emission spectrophotometry (ICAPES) analysis were filtered using a Millipore 0.4 $\mu m$ polycarbonate filter, acidified with $HNO_3$ to pH < 2, and stored at $4^oC$. In addition, water samples were analyzed for conductivity, salinity, pH, total water hardness (as $CaCO_3$), alkalinity (as $CaCO_3$), and chloride. The pH, dissolved oxygen, conductivity, and salinity were measured on test water dilutions 100%, 49%, and 0%.

## Invertebrates

Daphnia magna, less than 24-h old, were obtained from NFCRC freshwater cultures (Ingersoll et. al., in-press). Exposures were conducted for 48 hours in 250-mL beakers containing 200 mL of test water. Photoperiod for the test was 16:8 light:darkness, with a light intensity of about 50 footcandles.

Hyalella azteca, less than 3 mm long, were obtained from NFCRC saltwater cultures (Ingersoll and Nelson, in-press). Individual Hyalella azteca were exposed for 72 hours in polycarbonate containers (about 30 mL) holding 20 mL of test water. There were 10 replicates per exposure concentration. Tests were conducted under ambient light conditions.

## Fish

Striped bass were obtained from the California Fish and Game, Central Valley Fish Hatchery. Ten striped bass weighing approximately 0.64 grams were exposed for 96 hours in 19.6-L glass test chambers containing 14 L of test water. The tests were conducted under ambient light conditions.

Statistical Analyses

An EC50 or a LC50 was calculated for each of these tests. Mortality was the biological endpoint observed for the tests conducted with striped bass (96-h LC50). The Daphnia magna test observations included lethality and immobility to determine the 48-h EC50. For the Hyalella azteca exposures lethality, immobility, and floating were the biological endpoints (72-h EC50). Hyalella azteca is an epibenthic invertebrate and should remain in the water column or near the bottom of the container when substrate is not available. For this reason, amphipods observed floating on the surface of the water were considered to be stressed and included in the EC50 calculation. Calculations were made using probit, Spearman-Karber, or moving average procedures. Where data were incomplete, (i.e., no partial mortality occurred) a range was provided (highest concentration with 0% mortality and lowest concentration with 100% mortality) which was a conservative estimate of the LC50 confidence interval.

## RESULTS

Water Chemistry

The water quality of the different test waters is shown in Table 3. In general, the low, medium, and high hardness waters had similar ion ratios; however, as hardness increased (increased calcium and magnesium concentrations), sodium concentrations decreased. We encountered difficulty reconstituting a water with a hardness as high as Instant Ocean. Three attempts to mix a hard water with a hardness equal to the Instant Ocean water were unsuccessful. A white precipitate was observed in the mixing tanks which we believe was $CaCO_3$ as shown by the decrease in measured alkalinity in the high hardness water. Instant Ocean, has a high hardness but low alkalinity (compared to our reconstituted high hardness water) which is typical of seawater. ICAPES analysis on the test waters are in progress.

Invertebrates

From the invertebrate tests we were able to identify EC50 ranges for all of the test waters.

Daphnia magna responded primarily to elevated salinity. In all 4 waters with and without trace metals, there was 100% response at salinities between 10 and 11.5 g/L (Table 4). In the 34% dilution water (salinity 7.5 g/L), 100% of the organisms were affected in the low and medium hardness waters, but very little effect was observed in the high hardness water. In the Instant Ocean 34% dilution water (salinity 8.0 g/L), we observed a large number of dead and immobile organisms. There was no consistent difference between test waters with and without the trace element addition.

The Hyalella azteca (Table 5) responded similarly to the Daphnia magna in the low, medium, and high hardness waters, with the majority of the organisms exhibiting a response in dilutions of 49% and above (salinity > 10.0 g/L). There was no consistent difference between the test waters with and without the trace element addition.

### Fish

Striped bass exhibited a more graded response to the test waters compared to the invertebrates (Table 6). In the 100% test water (average salinity 20 g/L), as the hardness increased the toxicity decreased. In addition, the low, medium, and high hardness waters were more toxic with addition of the trace element mixture than the test waters without the trace element mixture. The Instant Ocean test water was not toxic to the striped bass with or without addition of the trace elements.

## DISCUSSION

Ingersoll, et. al., 1988 reported atypical anion and cation ratios in water collected from Pintail Bay. Using these data, we reconstituted the Pintail Bay water (low hardness water), and this same water with an elevated hardness. We also utilized a commercially available saltwater, Instant Ocean, which has ion ratios typical of natural salt water. Most of the ion concentrations in our reconstituted waters were similar to the Instant Ocean water, with the exception of hardness (Ca and Mg) which was one of our experimental variables.

### Invertebrates

The response of Daphnia magna was related to the salinities of these different waters. Daphnia magna has a reported salinity tolerance of about 6.8 to 7.5 g/L (Meyer et. al., 1985). In all four reconstituted waters at 49% dilution, salinities were 10 g/L or higher. For the low, medium, and high hardness test waters at 34% dilution, salinity was 7.5 g/L which would be near the daphnids maximum salinity tolerance. The Instant Ocean water had a higher salinity (8 g/L at 34% dilution), and was above the salinity tolerance of Daphnia magna. At the 34% dilution, hardness appeared to moderate toxicity , with the high hardness water causing less toxicity.

Although H. azteca are found in brackish habitats up to 22.5 g/L salinity, research at NFCRC has indicated that reconstituted waters with salinities greater than 18 g/L may cause acute toxicity. Hyalella azteca exhibited a response to all waters at a 49% dilution (10 g/L salinity), but there was a slight decrease in toxicity at 34% dilution (7.5 - 8.0 g/L salinity) for the high hardness and Instant Ocean waters. Therefore, for Daphnia magna and Hyalella azteca it appears salinity is the most critical water quality parameter but, as salinity decreases hardness may also influence survival.

Fish

Striped bass inhabit freshwater, brackish, and marine environments. The reported acute salinity tolerance range of striped bass 30 days and older is 0 - 34 g/L (Lal et. al., 1977). Fish used in our tests were 30 days and older and should have been able to tolerate salinities of at least 34 g/L. Decreases in hardness increased the toxicity of the saline waters. With the addition of the trace element mixture (As, B, Cu, Li, Mo, and Sr), the Instant Ocean water induced no acute mortality, while the high, medium, and low hardness waters had increased mortalities. There was no survival of fish exposed to the mixture in the low, medium, and high hardness waters at a 70% dilution (14 g/L salinity). However, there was 100% survival by striped bass in 70% dilution Instant Ocean (15 g/L) water. Therefore, we conclude that the ion composition of the water can be stressful to aquatic organisms, and trace element contaminants would further add to the toxicity of the water.

RESEARCH PLAN II. DETERMINATION OF THE TOXICITY OF INDIVIDUAL TRACE ELEMENT CONTAMINANTS IN WATER QUALITY REPRESENTATIVE OF SNWR.

## INTRODUCTION

Several inorganic contaminants are elevated in drain water entering SNWR. However, the concentrations of these contaminants are often below reported acutely lethal concentrations for single-compound toxicity tests. In some cases the acutely lethal concentration of the inorganic contaminants is unknown. In addition, the toxicity of trace elements may be influenced by changes in salinity and major cation and anion concentrations. Studies were needed to provide more specific data on the contribution of individual toxic components to the overall toxicity of SNWR surface waters. The objective of this Research Plan was to investigate the acute toxicity of arsenic, lithium, molybdenum, and strontium in a saline water (22 g/L Instant Ocean) to striped bass.

## MATERIALS AND METHODS

Static acute toxicity tests (96-h LC50) were conducted which generally followed EPA (EPA 660/3-75/009) and ASTM (ASTM, 1988) procedures. Tests with arsenic (arsenic pentoxide), lithium (lithium chloride), molybdenum (sodium molybdenate), and strontium (strontium chloride) were conducted in 22 g/L salt water (Instant Ocean).

### Water Chemistry

Trace element residues were analyzed on the high, medium, low, and control test waters unless the LC50 was greater than 100 mg/L, in which case only the high concentration was analyzed. A 100 mL volume from each replicate exposure jars was pooled for analysis. Samples taken for ICAPES analysis were filtered using a Millipore 0.4 $\mu$m polycarbonate filter, acidified with $HNO_3$ to pH <2, and stored at 4$^{o}$C. In addition, water samples were taken for measurement of conductivity, salinity, total water hardness (as $CaCO_3$), alkalinity (as $CaCO_3$), and chloride. Dissolved oxygen and pH were measured on the high, medium, low, and control test waters.

Fish

Striped bass were obtained from Central Valley Fish Hatchery, CA, and Cedarville Hatchery, MD, and had an average weight of 1.83 g. Striped bass were acclimated over 48 hrs to the test water without trace element addition.

Toxicity tests were conducted in 19.6-L glass jars containing 15 L of test water. Five striped bass were stocked into each of two replicates per exposure. The tests were conducted at 20$^{o}$C with ambient light conditions. Biological observations were made every 24 hours and dead fish removed. Fish were not fed during conduct of the tests.

Statistical Analyses

In exposure studies where mortality occurred, a 96-h LC50 was calculated using probit, Spearman-Karber, or moving average procedures. Where data were incomplete (i.e. no partial mortality occurred), a range was provided (highest concentration with 0% mortality and lowest concentration with 100% mortality).

This provides a conservative estimate of the 96-h LC50 confidence interval. If less than 50% mortality occurred at 100 mg/L, then the LC50 is reported as >100 mg/L.

## RESULTS

The Instant Ocean test water had the following water quality characteristics: salinity 21 g/L; conductivity 36,000 umhos/cm; hardness 4560 mg/L as $CaCO_3$; alkalinity 59 mg/L as $CaCO_3$; and pH 8.04. This water quality was similar to Research Plan I tests conducted with Instant Ocean (Table 3). The 96-h LC50 for strontium, lithium, and molybdenum was greater than 100 mg/L (Table 7). The 96-h LC50 for arsenic was 10.3 mg/L. ICAPES analyses are in progress.

## DISCUSSION

The acute toxicity tests with As, Li, Mo, and Sr were conducted in Instant Ocean to minimize stress to the organisms by the ionic composition of the water. Li, Mo and Sr each exhibited low toxicity. The LC50 determined for As agrees well with LC50s reported by other investigators (Table 8). The LC50 calculated for As was approximately 10 times greater than the As concentration used in the trace element mixture studies in Research Plan I.

The reported acute toxicity (6-h) of boron (as boric acid) to fathead minnows (<u>Pimephales promelas</u>) was 19,000 to 19,500 mg/L in hard water (Le Clerc and Devlaminck, 1955; Le Clerc, 1960). Also, Hamilton and Buhl (1990) reported a 96-h LC50 for chinook salmon, <u>Oncorhynchus tschawytscha</u>, exposed in 1.2 g/L saline water at 600 (511 - 706) mg/L. While B concentrations in the mixture were high compared to the other trace elements, B individually appears to be relatively nontoxic.

Of the six trace elements tested, Cu was the most toxic. The reported 96-h LC50 for Cu to striped bass in 1 g/L saline water was 0.19 (0.13 - 0.27) mg/L (Palawski, et al., 1985). While Cu is more toxic, the concentration used in the trace element mixture is 4 times less than this reported concentration. Other reported static acute toxicity tests for fish are listed in Table 9. The LC50s reported from these studies are also greater than the Cu concentration used in the trace element mixture.

In tests conducted in Research Plan I, concentrations of trace elements were below acute toxicity concentrations determined for the individual components. However, the mixture was still toxic in all of the waters except the Instant Ocean water. These findings support the importance of determining the ionic composition of agricultural drainwaters as they may contribute to trace element toxicity. Further, from the results of the high hardness and Instant Ocean test waters, measurement of hardness alone may not be enough to identify a water as being stressful to aquatic life.

RESEARCH PLAN III. **Whole Effluent Toxicity of Subsurface Water entering SNWR to Aquatic Invertebrates and Fish.**

## INTRODUCTION

In addition to elevated concentrations of trace elements and salts in the surface waters of SNWR, subsurface water also contains high levels of inorganic contaminants (Hoffman et al., 1986). Water samples from subsurface flow collected immediately up-gradient of Stillwater Wildlife Management Area (SWMA) and Carson Lake contain elevated concentrations of total dissolved solids and several trace elements. To investigate the contribution of ground water contamination to SNWR surface waters, static acute toxicity tests were conducted with fresh and saltwater organisms and selected well water samples. Water was collected from six U.S. Geological Survey (USGS) monitoring sites which were selected according to their proximity to drains or ponds at SNWR.

## MATERIALS AND METHODS

Groundwater samples were collected in polyethylene carboys, placed in coolers with ice packs (4$^{o}$C), and shipped by air freight to the NFCRC. These waters were tested as described for effluent toxicity tests (EPA/600/4-85/013). Sites chosen were: Site 1: Lead Lake well #6; Site 2: Lead Lake well #4; Site 3: Stwr Div Canal well; Site 4: Hunter Road well; Site 5: Harmon Lake well; and Site 6: FWS Flowing well.

All carboys from a single sample location were mixed into a common container. Serial dilutions were made of each 100% site water with reconstituted Lahonton Reservoir water. The four dilutions tested were 100%, 50%, 25%, and 12.5%, and dilution water control. Two additional controls (negative controls not expected to elicit a response) included NFCRC well water (280 mg/L total water hardness as $CaCO_3$), and 10 g/L Instant Ocean$^R$ water (2000 mg/L total water hardness as $CaCO_3$). Reconstituted Pintail Bay water (Ingersoll, et. al., 1988) was tested as a positive control at 23 g/L salinity. See Table 1 for nominal ion concentrations for reconstituted Lahonton Reservoir and Pintail Bay waters.

Water Chemistry

Unfiltered and filtered water samples collected on-site and from laboratory containers were submitted for ICAPES analysis. Effluent was filtered using Millipore prefilters (type AP), followed by Millipore 0.4 $\mu$m polycarbonate filters. Water quality parameters of pH, total water hardness (as $CaCO_3$), alkalinity (as $CaCO_3$), salinity, conductivity, and chloride were measured. Also, conductivity, salinity, pH, and dissolved oxygen were measured on dilutions 100%, 25%, and controls. These tests were maintained at 20$^oC$ and biological observations were recorded every 24 hours and dead fish removed. Test animals were not fed during the test.

Invertebrates

Daphnia magna, less than 24 h old, were exposed for 48 h in

250-mL test chambers with 200 mL of test water. Ten D. magna, cultured at NFCRC (Ingersoll, et. al., 1990), were put into each chamber without replication. These tests were conducted with a photoperiod of 16:8 light:darkness at a light intensity of about 50 footcandles.

Fish

Two exposure tests with fish were conducted: fathead minnows cultured in NFCRC well water, and striped bass acclimated to 10 g/L salinity water (as Instant Ocean). Fathead minnows were approximately 48 h old and were obtained from NFCRC cultures. Striped bass weighed about 0.33 g, and were obtained from the Central Valley Fish Hatchery, California. Fathead minnow tests were conducted in 1-L beakers using 750 mL of test water, and striped bass tests were conducted in 19.6-L glass jars using 5 L of test water. Ten fish were placed in each exposure chamber for both fathead minnow and striped bass tests. Dissolved oxygen was measured daily in each exposure chamber and control exposures.

## RESULTS

Water Chemistry

Measured alkalinity (as $CaCO_3$), pH, hardness (as $CaCO_3$), conductivity, salinity, dissolved oxygen, and chloride for each exposure are listed in Table 10. Salinities of 100% Site 1, Site 2, and Site 4, were greater than 40 g/L. Temperature, salinities, and pH remained relatively constant in all studies. Results from ICAPES analyses are in progress.

Invertebrates

All water samples except for Site 3 were acutely toxic to *Daphnia magna* (Table 11). The 48 h LC50 was less than 12.5% for Site 1 and 4 with corresponding salinities of 10 g/L, and 8 g/L, respectively. The LC50s for sites 2 and 5 were between 12.5 and 25% with corresponding salinities (g/L) of 7 - 12 and 5 - 8 respectively. The LC50 for Site 6 was between 25 and 50% (salinity 7 - 12 g/L). Additionally, no animals survived in the 10 g/L Instant Ocean positive control.

Fish

Fathead minnow survival response was generally similar to *Daphnia magna*; however, in Site 5, *D. magna* were slightly more sensitive than fathead minnows. See Table 12 for 96-h LC50 calculations.

The response of striped bass to Site 2, 3, and 4 test waters was similar to both *D. magna* and fathead minnow. However, striped bass were less sensitive to the effluent collected from Site 1 (35 g/L salinity - 100% effluent), Site 5 (15 g/L - 100% effluent), and Site 6 (22 g/L - 100% effluent, see Table 13).

## DISCUSSION

Water collected from five of the six wells at SNWR exhibited some degree of toxicity. Only Site 3 was found not to be acutely toxic to the fresh and salt water organisms tested.

For the *D. magna* and the fathead minnows, the salinity at the lowest dilution (12.5%) of Site 1 water was above their reported salinity tolerance of 6 - 7 g/L (Meyer et. al. 1985);

further this site was lethal to striped bass at a 50% dilution (35 g/L). Although striped bass should tolerate salinities up to full strength seawater (34 g/L), higher salinities may be stressful. At the 50% dilution of Site 1, the calculated water hardness is about 4200 mg/L (as $CaCO_3$), while Instant Ocean water at a similar salinity is about 6200 mg/L. Based on results from Research Plan I, ion ratios and trace element concentrations could be contributing to this toxicity. Results from ICAPES analysis are in progress and will help to better distinguish between toxic effects of trace elements from toxic effects of atypical ion ratios.

Well water from Site 4 was more toxic than Site 1. The salinity at Site 4 at the 25% dilution was 15 g/L. Again, elevated salinity probably accounts for the mortality of the freshwater organisms, but water from this site is also acutely lethal to striped bass. Without ICAPES results, the cause of this response can not be evaluated. However, as in Site 1, the calculated water hardness is low (1960 mg/L as $CaCO_3$) compared to Instant Ocean (3290 mg/L as $CaCO_3$) at a similar salinity. This data also suggests ion stress may have occurred in the toxicity tests.

Site 5 water was less toxic than water from either Sites 1 or 4. The Site 5 100% dilution water had a salinity of 28 g/L, but was toxic to striped bass. As in Sites 1 and 4 the hardness was low (2140 mg/L as $CaCO_3$) compared to the hardness of Instant Ocean (5108 mg/L as $CaCO_3$) at a similar salinity. At 25%

dilution, the salinity was 8 g/L and this water was toxic to both D. magna and fathead minnows.

Water from Sites 2 and 6 was also toxic to striped bass at a salinity within the tolerance range of these animals. Sites 2 and 6 differ from Sites 1, 4, and 5 in that the water hardness was close to the expected hardness of similar salinity Instant Ocean water. For Site 2, Daphnia magna, fathead minnows, and striped bass responded in a similar manner suggesting a contaminant problem. Toxicity to freshwater organisms of the water from Site 6 is probably related to salinity, but 100% dilution water (salinity 22 g/L) was also toxic to striped bass. ICAPES data are needed in order to better determine the cause of the toxicity in these tests.

## CONCLUSIONS

Toxicity investigations tests conducted as part of Research Study Plans I, II, and III have identified several key factors influencing water quality at SNWR, including: (1) the importance of ionic composition of the water; (2) the increase in toxicity of mixtures compared to individual trace inorganic contaminants; and (3) the potential contribution of ground water to drain water toxicity during times of low flow.

Finger et. al. (1989) identified conductivity as a tool for monitoring the toxicity of drain water. They concluded that increases in salinity corresponded with elevated concentrations of As, B, Li, and Mo. Results from the present studies suggest that in addition to salinity, the ionic composition of the water must also be considered. Furthermore, our results suggest that it is difficult to predict the toxicity of inorganic mixtures based on the toxicity of individual compounds. The ionic composition in combination with the salinity of the water can be stressful to organisms and further influences the toxicity of inorganic mixtures. The management implications of these findings include: 1) a greater need for fresh water than previously believed since more dilution of the drain waters may be necessary; 2) toxicity assessments of fresh waters present or made available to SNWR should be conducted since individual trace element analysis will not provide information regarding the interactive effects of trace element mixtures and atypical ion ratios; and 3) the need to measure more than just the ionic strength (salinity/conductivity) of the waters at SNWR since other measures such as hardness may provide insight into the acceptability of a water for supporting aquatic life.

Additionally subsurface waters appear to be quite toxic, and may substantially contribute to the toxicity of the drain water when surface flows are low and groundwater seepage into the drains is high. It may be necessary to provide some continuous flows of fresh water through the drains in order to continually dilute the groundwater which seeps into the drains.

The toxicity tests conducted during FY88 and FY89 have been of relatively short duration (10 days or less), and have identified a number of important factors contributing to the acute lethality of SNWR water sources. To more thoroughly assess the potential suitability of drain water for supporting aquatic life the long term effects of low levels of salinity and trace elements in combination with atypical ion ratios should be investigated.

Table 1. Inorganic salts and concentrations used for reconstituting various test waters used in Research Plans I, II, and III.

| | |
|---|---|
| **High hardness water** | |
| $CaSO_4 \cdot 2H_2O$ | 116.4 mg/L |
| $MgSO_4 \cdot 7H_2O$ | 1854.8 mg/L |
| $NaHCO_3$ | 1474.5 mg/L |
| KCl | 476.3 mg/L |
| $Na_2SO_4$ | 2379.4 mg/L |
| $CaCL_2 . 2H_2O$ | 488.0 mg/L |
| $MgCl_2 . 6H_2O$ | 5968.0 mg/L |
| NaCl | 12485.0 mg/L |
| **Medium Hardness** | |
| $CaSO_4 \cdot 2H_2O$ | 116.4 mg/L |
| $MgSO_4 \cdot 7H_2O$ | 1854.8 mg/L |
| $NaHCO_3$ | 1474.5 mg/L |
| KCl | 476.3 mg/L |
| $Na_2SO_4$ | 2379.4 mg/L |
| $CaCl_2 \cdot 6H_2O$ | 120.7 mg/L |
| $MgCl_2 \cdot 6H_2O$ | 1872.2 mg/L |
| NaCl | 14868.2 mg/L |
| **Low hardness water*** | |
| $CaSO_4 \cdot 2H_2O$ | 116.4 mg/L |
| $MgSO_4 \cdot 6H_2O$ | 1854.8 mg/L |
| $NaHCO_3$ | 1474.5 mg/L |
| KCl | 476.3 mg/L |
| $Na_2SO_4$ | 2379.4 mg/L |
| NaCl | 15992.2 mg/L |
| **Instant Ocean salt** | 27000. mg/L |
| **Dilution water:** | **Lahonton Reservoir** |
| $NaHCO_3$ | 48.3 mg/L |
| KCl | 3.8 mg/L |
| $Na_2SO_4$ | 14.0 mg/L |
| NaCl | 23.6 mg/L |
| Well:DI | 1:2.94 |

* This reconstituted water simulates Pintail Bay water identified from studies conducted during FY88.

Table 2. Concentrations of trace elements used in trace element mixture 100% dilution.

| Element | Nominal Test Concentration (mg/L) | Chemical Form |
|---|---|---|
| As | 0.8 | $As_2O_5 \cdot 5H_2O$ |
| B | 36.1 | $H_3BO_3$ |
| Cu | 0.04 | $CuCl_2$ |
| Li | 0.93 | $LiCl$ |
| Mo | 0.3 | $Na_2MoO_4 . 2H_2O$ |
| Sr | 2.42 | $SrCl_2 \cdot 6H_2O$ |

Table 3. Water quality parameters of test waters and dilution water prior to starting toxicity tests to determine the toxicity of waters with different hardnesses with and without a trace element mixture addition.

| Test Water | | pH | Alkalinity (mg/L*) | Hardness (mg/L*) | Conductivity (µmhos/cm) | Salinity (g/L) | D.O. (mg/L) | Chloride (mg/L) |
|---|---|---|---|---|---|---|---|---|
| Low hardness | 34% | | | | 13,500 | 7.5 | 8.3 | |
| | 49% | 9.18 | | | 18,500 | 10.0 | 8.5 | |
| | 70% | | | | 25,000 | 14.0 | 8.0 | |
| | 100% | 9.14 | 850 | 840 | 32,100 | 19.5 | 8.1 | 109,800 |
| Medium hardness | 34% | | | | 13,700 | 7.5 | 8.3 | |
| | 49% | 9.12 | | | 18,500 | 10.0 | 8.1 | |
| | 70% | | | | 25,000 | 14.5 | 7.8 | |
| | 100% | 9.05 | 900 | 1768 | 33,500 | 19.5 | 8.0 | 110,700 |
| High Hardness | 34% | | | | 12,500 | 7.5 | 8.2 | |
| | 49% | 8.57 | | | 17,000 | 10.5 | 7.5 | |
| | 70% | | | | 23,500 | 14.5 | 7.6 | |
| | 100% | 8.43 | 540 | 3588 | 31,500 | 20.0 | 7.8 | 102,000 |
| Instant Ocean | 34% | | | | 14,500 | 8.0 | 8.1 | |
| | 49% | 8.16 | | | 19,500 | 11.0 | 7.8 | |
| | 70% | | | | 26,000 | 15.5 | 8.1 | |
| | 100% | 8.27 | 38 | 4220 | 34,000 | 21.0 | 8.2 | 139,800 |
| Dilution water: Lahonton Reservoir | | 8.42 | 110 | 76 | 350 | 0.0 | 7.2 | 29 |

* as $CaCO_3$

Table 4. Percent of *Daphnia magna* dead and immobile at 48 hours of exposure to three different hardness waters and Instant Ocean at a constant salinity with and without a trace element mixture addition.

| | Dilution | | | |
|---|---|---|---|---|
| | 34% | 49% | 70% | 100% |
| Without trace element addition | | | | |
| Low | 100 | 100 | 100 | 100 |
| Medium | 100 | 100 | 100 | 100 |
| High | 20 | 100 | 100 | 100 |
| Instant Ocean | 100 | 100 | 100 | 100 |
| With trace element addition | | | | |
| Low | 100 | 100 | 100 | 100 |
| Medium | 100 | 100 | 100 | 100 |
| High | 20 | 100 | 100 | 100 |
| Instant Ocean | 70 | 100 | 100 | 100 |

Dilution water control (average): 0

Table 5. Percent of *Hyalella azteca* dead, immobile, or floating at 72 hours of exposure to three different hardness waters and Instant Ocean at a constant salinity with and without a trace element mixture addition.

| | Dilution | | | |
|---|---|---|---|---|
| | 34% | 49% | 70% | 100% |
| Without trace element addition | | | | |
| Low | 100 | 100 | 100 | 100 |
| Medium | 80 | 100 | 100 | 100 |
| High | 50 | 80 | 100 | 100 |
| Instant Ocean | 20 | 80 | 30 | 100 |
| With trace element addition | | | | |
| Low | 40 | 90 | 100 | 100 |
| Medium | 70 | 100 | 100 | 100 |
| High | 30 | 80 | 80 | 100 |
| Instant Ocean | 10 | 50 | 50 | 100 |

Dilution water control (average): 21

Table 6. Percent mortality of striped bass at 96 hours of exposure to three different hardness waters and Instant Ocean at a constant salinity with and without a trace element mixture addition.

| | Dilution | | | |
|---|---|---|---|---|
| | 34% | 49% | 70% | 100% |
| Without trace element mixture | | | | |
| Low | 0 | 0 | 50 | 100 |
| Medium | 0 | 0 | 0 | 100 |
| High | 0 | 0 | 0 | 0 |
| Instant Ocean | 0 | 0 | 0 | 0 |
| With trace element mixture | | | | |
| Low | 0 | 70 | 100 | 100 |
| Medium | 0 | 40 | 100 | 100 |
| High | 0 | 0 | 100 | 100 |
| Instant Ocean | 0 | 0 | 0 | 0 |

Dilution water control (average): 6

Table 7. Acute toxicity of Arsenic, Lithium, Molybdenum, and Strontium to striped bass.

| Element | 96 h LC50 (mg/L) | Confidence Interval (95%) |
|---|---|---|
| As | 10.3 | 6.4 - 13.5 |
| Li | >100.0 | - |
| Mo | >100.0 | - |
| Sr | >100.0 | - |

Table 8. Static acute toxicity of arsenic to fish.

| Chemical Form | Species | 96-LC50 (mg/L) | Test Conditions | Notes | References |
|---|---|---|---|---|---|
| Pentoxide | Striped bass | 30.5 | Static hardness=285 mg/L* | 35-80 day old | Palawski et al., 1985 |
| Sodium arsenate | Rainbow trout | 108 | | 60 day old | Hale, 1977 |

* as $CaCO_3$

Table 9. Static acute toxicity of copper to various fish species.

| Chemical Form | Species | 96-h LC50 (mg/L) | Test Conditions | Notes | References |
|---|---|---|---|---|---|
| Sulfate | Striped bass | 0.10 | static hardness=40 mg/L* | 35-80 day old | Palawski et al., 1985 |
| | Striped bass | 0.27 | static hardness=285 mg/L* | 35-80 day old | Palawski et al., 1985 |
| | Striped bass | 0.19 | static, saltwater | 35-80 day old | Palawski et al., 1985 |
| | Rainbow trout | 0.102 | static | | Fogels and Sprague, 1977 |
| | Rainbow trout | 0.250-0.680 | static, well water | | Lett et al., 1976 |
| | Fathead minnow | 0.838 | static hardness=272 mg/L* | 1.2 g | Mayer and Ellersieck, 1986 |
| | Green sunfish | 3.40 | static hardness=272 mg/L* | 1.1 g | Mayer and Ellersieck, 1986 |
| | Bluegill | 7.34 | static hardness=272 mg/L* | 1.5 g | Mayer and Ellersieck, 1986 |

* as $CaCO_3$

Table 10. Water quality parameters of dilutions of the six wells collected from SNWR prior to start of the toxicity tests.

| Site/Control | | pH | Alkalinity (mg/L) | Hardness (mg/L) | Conductivity ($\mu$mhos/cm) | Salinity (g/L) | D.O. (mg/L) | Chloride (mg/L) |
|---|---|---|---|---|---|---|---|---|
| Site 1 | 12.5% | | | | 10,800 | 10 | 7.4 | |
| | 25% | 8.12 | | | 32,000 | 20 | 7.5 | |
| | 50% | | | | >50,000 | 35 | 7.7 | |
| | 100% | 7.87 | 334 | 8340 | >50,000 | >40 | 8.2 | 39,300 |
| Site 2 | 12.5% | | | | 11,000 | 7 | 7.3 | |
| | 25% | 8.35 | | | 20,000 | 12 | 7.5 | |
| | 50% | | | | 34,000 | 22 | 7.7 | |
| | 100% | 8.06 | 1004 | 4800 | 45,500 | >40 | 8.3 | 27,600 |
| Site 3 | 12.5% | | | | 1,800 | <1 | 7.3 | |
| | 25% | 8.47 | | | 2,200 | 1 | 7.5 | |
| | 50% | | | | 3,600 | 2 | 7.8 | |
| | 100% | 8.50 | 215 | 84 | 4,900 | 4 | 8.4 | 2,650 |
| Site 4 | 12.5% | | | | 13,500 | 8 | 8.4 | |
| | 25% | 8.22 | | | 24,500 | 15 | 8.4 | |
| | 50% | | | | 42,000 | 27 | 8.8 | |
| | 100% | 8.23 | 336 | 7560 | >50,000 | >40 | 10.0 | 33,500 |
| Site 5 | 12.5% | | | | 7,000 | 5 | 8.3 | |
| | 25% | 8.29 | | | 13,000 | 8 | 8.4 | |
| | 50% | | | | 22,500 | 15 | 8.8 | |
| | 100% | 8.28 | 452 | 2140 | 33,500 | 28 | 10.4 | 18,100 |
| Site 6 | 12.5% | | | | 6,000 | 4 | 8.2 | |
| | 25% | 8.54 | | | 11,000 | 7 | 8.4 | |
| | 50% | | | | 18,500 | 12 | 9.0 | |
| | 100% | 8.49 | 1012 | 5880 | 28,000 | 22 | 10.3 | 28,000 |
| Dilution water: | | 8.58 | 98 | 93 | 309 | 0 | 7.7 | 30 |

Table 11. Percent toxic response and LC50 estimates for *Daphnia magna* exposed to subsurface waters collected from SNWR.

| | Dilutions | | | | |
|---|---|---|---|---|---|
| | 12.5% | 25% | 50% | 100% | 48-hr LC50 |
| Site 1 | 100 | 100 | 100 | 100 | < 12.5 |
| Site 2 | 0 | 100 | 100 | 100 | 12.5 - 25 |
| Site 3 | 0 | 0 | 10 | 0 | > 100 |
| Site 4 | 60 | 100 | 100 | 100 | < 12.5 |
| Site 5 | 0 | 90 | 100 | 100 | 12.5 - 25 |
| Site 6 | 0 | 10 | 100 | 100 | 25 - 50 |

Dilution water control (average): 3

Table 12. Percent mortality and LC50 estimates for fathead minnows exposed to subsurface waters collected from SNWR.

**Fathead Minnows**

| | Dilution | | | | |
|---|---|---|---|---|---|
| | 12.5% | 25% | 50% | 100% | 96-hr LC50 |
| Site 1 | 100 | 100 | 100 | 100 | < 12.5 |
| Site 2 | 0 | 100 | 100 | 100 | 12.5 - 25 |
| Site 3 | 10 | 30 | 0 | 10 | > 100 |
| Site 4 | 10 | 100 | 100 | 100 | 12.5 - 25 |
| Site 5 | 0 | 50 | 100 | 100 | 25 - 50 |
| Site 6 | 0 | 0 | 100 | 100 | 25 - 50 |

Dilution water control (average): 13

Table 13. Percent mortality and LC50 estimates for striped bass exposed to subsurface waters collected from SNWR

| | Dilution | | | | |
|---|---|---|---|---|---|
| | 12.5% | 25% | 50% | 100% | 96-hr LC50 |
| Site 1 | 10 | 0 | 100 | 100 | < 50 |
| Site 2 | 0 | 100 | 100 | 100 | 12.5 - 25 |
| Site 3 | 10 | 0 | 10 | 50 | > 100 |
| Site 4 | 40 | 100 | 100 | 100 | < 50 |
| Site 5 | 0 | 10 | 0 | 100 | 50 - 100 |
| Site 6 | 10 | 0 | 10 | 100 | 50 - 100 |

Dilution water control (average): 8

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