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Scientific Investigations Report 2011–5061

U.S. Department of the Interior
U.S. Geological Survey

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Conversion Factors

SI to Inch/Pound

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	.03937	inch (in.)
micrometer (µm)	.00003937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	.6214	mile (mi)
	Volume	
milliliter (mL)	0.034	ounce, fluid (fl. oz
liter (L)	1.057	quart (qt)
liter (L)	.2642	gallon (gal)
	Flow rate	
centimeter per second (cm/s)	0.3937	inch per second (in/s)
centimeter per second (cm/s)	.0328	foot per second (ft/s)
cubic meter per second (m^3/s)	35.31	cubic foot per second (ft^3/s)
	Mass	
milligram (mg)	0.00003527	ounce, avoirdupois (oz)
micrograms (µg)	.00000003527	ounce, avoirdupois (oz)
gram (g)	.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound, avoirdupois (lb)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F=(1.8\times°C)+32$$

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25°C).

Turbidity is given in Nephelometric Turbidity Units (NTU).

pH is given in standard units (SU).

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter (µg/L).

Concentrations of chemical constituents in fish tissue are given either in milligrams per gram (mg/g) or micrograms per gram (µg/g).

Horizontal coordinate information is referenced to North American Datum of 1983 (NAD 83).

On-Site Evaluation of the Suitability of a Wetted Instream Habitat in the Middle Rio Grande, New Mexico, for the Rio Grande Silvery Minnow (*Hybognathus amarus*)

By Kevin J. Buhl

Abstract

Two in-situ exposure studies were conducted with the federally-listed endangered Rio Grande silvery minnow (*Hybognathus amarus*). One-year-old adults were exposed in cages deployed at three sites in the Middle Rio Grande, N. Mex., for 4 days to assess survival and for 26 days to evaluate survival, growth, overall health, and whole-body elemental composition. The test sites were located on the Pueblo of Isleta in the (1) main channel of the Middle Rio Grande, (2) 240-Wasteway irrigation return drain, and (3) wetted instream habitat created below the outfall of the 240-Wasteway irrigation return drain. During the cage exposures, temperature, dissolved oxygen, pH, conductivity, and turbidity were monitored continuously (15-minute intervals) and common constituents, nutrients, carbons, metals, and pesticides were measured at discrete intervals. In both studies, there were statistical differences in several water-quality parameters among sites; and except for turbidity, these differences were small and were not considered to be biologically significant. The cages used in the 4-day exposure study were ineffective at preventing access to the fish by predators, and survival was highly variable (20 percent to 90 percent) across sites. In the 26-day chronic exposure study, weight and condition factor of caged-exposed fish at all sites were significantly lower than those at test initiation. After 26 days of exposure, there were no significant differences in survival, total length, weight, or condition factor of fish across sites, but absolute weight loss and relative reduction in condition factor were significantly greater in fish at the wetted instream habitat site compared to those at the Middle Rio Grande site. There were no statistical differences in health assessment indices, mesenteric fat indices, or prevalence of abnormalities in cage-exposed fish among sites. Cage-exposed fish had higher health assessment indices and prevalence of fin anomalies and a lower mesenteric fat indices compared to pre-exposed fish. Prevalence of macrophage aggregates in the kidney, liver, and spleen of caged-exposed fish was similar across sites and also was similar to those in pre-exposed fish. Absolute and relative weight loss and relative reduced condition factors were inversely correlated with water depth in the cages, which were the lowest at the WIH site.

Introduction

Recent discussions in the Middle Rio Grande Endangered Species Act Collaborative Program subcommittees and working groups have identified the need to create refuge habitat for the Rio Grande silvery minnow in the Isleta and San Acacia Reaches of the Middle Rio Grande, N. Mex. These two reaches of the river are particularly susceptible to drying events during which segments of the river become completely dewatered resulting in fish kills (U.S. Fish and Wildlife Service, 2007, 2010). The construction of refugial habitats in these reaches would facilitate short-term survival of Rio Grande silvery minnows during channel drying events (Cowley, 2003). The Middle Rio Grande Endangered Species Act Collaborative Program (2005) has specifically identified the use of wooden debris anchored in the river channel as a technique for the creation of deeper pools with low velocity flow that can serve as over winter habitat for Rio Grande silvery minnows as well as refugia during periods of river intermittency. The structures will encourage fluvial processes such as the scouring of sand substrate to create deep pools with low velocity river flows that are noted to be favored by Rio Grande silvery minnows (U.S. Fish and Wildlife Service, 2007, 2010). The wetted instream habitats created by these structures will be located below the outfalls of irrigation drains that contribute flow to the river throughout the year. Cowley (2003) proposed the concept of developing naturalized refugial habitats for silvery minnows in irrigation ditches and drains and provided a conceptual model for their deployment in an irrigation conveyance. Cowly and others (2007) collected 122 Rio Grande silvery minnows from the conveyance-return canal and 4 specimens from the drain-return canal of the Peralta irrigation canal system when the river channel in the Isleta Reach was dry. Similarly, U.S. Fish and Wildlife Service (USFWS) personnel have collected or observed thousands of Rio Grande silvery minnows in the Peralta Drain (Mike Hatch, U.S. Fish and Wildlife Service, Albuquerque, N. Mex., personal commun.). These findings suggest that the drains and their outfalls into the Middle Rio Grande can function as refugial habitats for Rio Grande silvery minnows and other fishes during periods of river channel desiccation.

In-situ bioassays with caged fish have been used to assess the toxicity of ambient surface waters and bioaccumulation of selected contaminants from different media (Chappie and Burton, 2000; Burton and others, 2005). In-situ exposure studies integrate the dynamic physical and chemical factors at a site and provide a measure of the cumulative effects of these fluctuating field conditions on the organisms of interest. This approach differs from standardized laboratory toxicity testing where most of the nontreatment factors are controlled and from fish community evaluations, which typically do not provide information on the condition of test organisms before and after a defined exposure period (Chappie and Burton, 2000).

The objectives of this research were to:

1. Evaluate the suitability of a wetted instream habitat created in the river for Rio Grande silvery minnows as related to ambient water quality and quantity during the late irrigation season when river channel drying events occur.

2. Evaluate the use of cages as a tool for assessing habitat quality for Rio Grande silvery minnows.

Methods

Experimental Sites

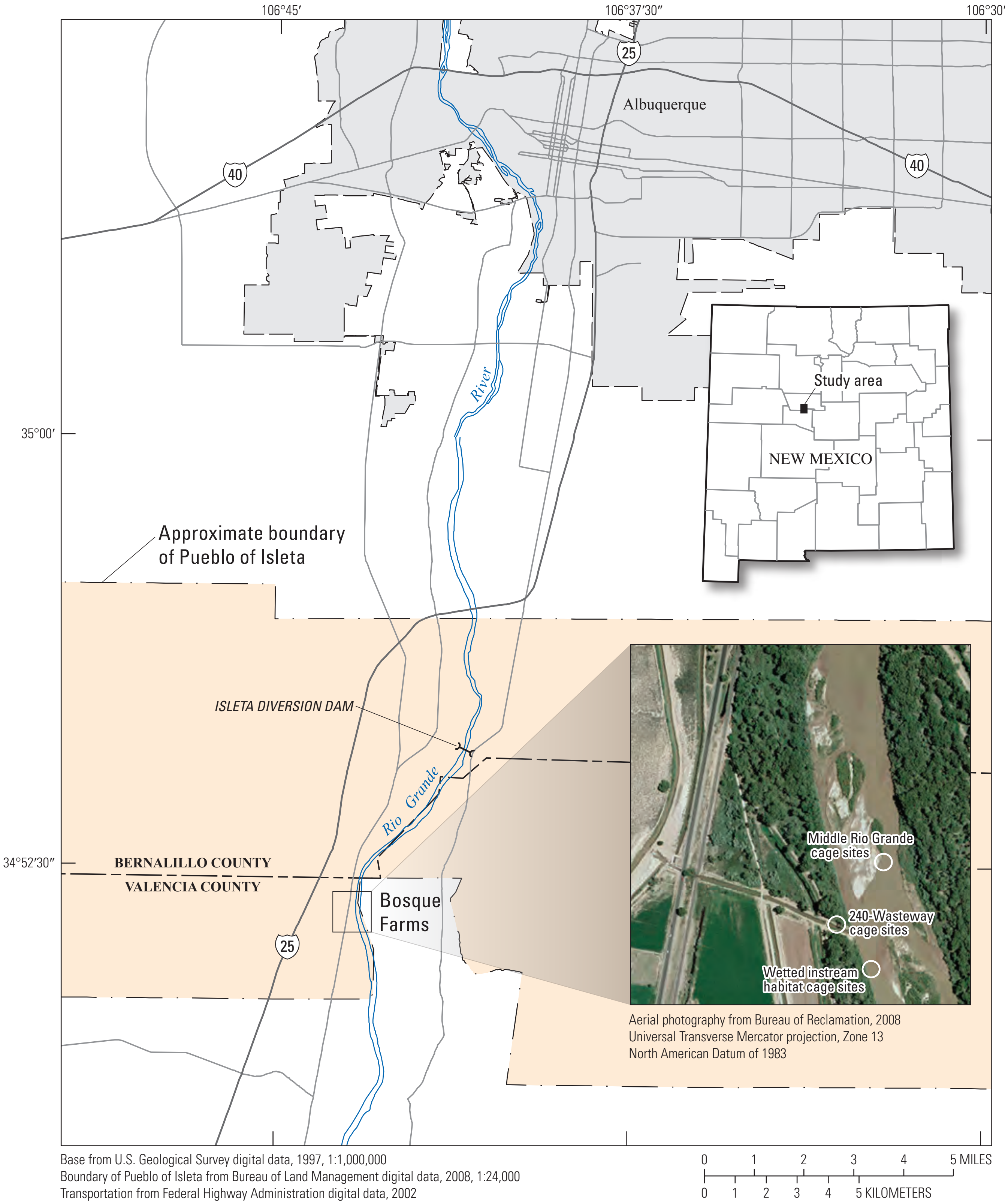
In both studies, three cages were deployed at each of three sites on the Pueblo of Isleta (fig. 1). Site locations were documented by a hand-held global positioning system (GPS) receiver (position accuracy plus or minus [±] 15 meters (m); Garmin GPS III Plus, Olathe, Kans.). The sites and locations were as follows: (1) Middle Rio Grande (MRG) site, along the west shore of the east channel about 170 m upstream from the 240-Wasteway outfall, Universal Transverse Mercator (UTM)-North American Datum of 1983 (NAD 83) coordinates, Easting 13S 0342753 Northing 3858711; (2) 240-Wasteway (240-WW) channel site, approximately 20 m upstream from the confluence with the Middle Rio Grande, UTM-NAD 83 coordinates, Easting 13S 0342665 Northing 3858580; and (3) furthest downstream portion of the wetted instream habitat (WIH) site in the west channel of the MRG about 61 m below the outfall of the 240-WW, UTM-NAD 83 coordinates, Easting 13S 0342771 Northing 3858492. The 240-WW conveys irrigation return flows back to the river and is regulated by a headgate with the flows adjusted to about 0.1 m^3/s during both studies (David Gensler, Middle Rio Grande Conservancy District, Albuquerque, N. Mex., personal commun.). The WIH site receives either full strength drain water from the 240-WW or a mixture of drain and river water when sufficient flows are present in the MRG.

The design of the WIH structures is given in Wesche (2006). Briefly, the WIH was created in 2007 by installing three large cottonwood (*Populus* sp.) snag structures, each consisting of a pair of cottonwood logs about 7–9 m long and 0.6–0.9 m in diameter with the rootwads attached. The tops of the logs were anchored in the riverbank and the rootwads extended about 6 m into the river channel at a slight angle facing upstream. The first pair of logs was placed about 5 m (at center of pair) below the outfall of the 240-WW, the second pair was placed about 27 m (from the middle of each pair) downstream from the first pair, and the third pair was placed about 29 m (from the middle of each pair) downstream from the second pair.

Fish

Rio Grande silvery minnows used in the cage exposures were 1-year-old adults obtained from the Rio Grande Silvery Minnow Rearing and Breeding Facility (lot #ABP07-017) at the city of Albuquerque Biological Park and Aquarium, Albuquerque, N. Mex. (hereinafter referred to as the BioPark). The fish originated from wild-caught eggs collected in 2006 in the MRG. The eggs were hatched at the BioPark and later transported to Dexter National Fish Hatchery and Technology Center (DNFH&TC), Dexter, N. Mex., where they were reared to adults in a pond. The fish were transported back to the BioPark on August 22, 2007, and stocked in an outdoor circular tank (Tank #22), 9.15 m in diameter and filled with about 60,000 liters (L) of water. The fish were under the care of BioPark personnel.

The test fish for the field exposures were collected from the outdoor circular tank and held in two large net cages with covers. The net cages were constructed of 0.32-centimeter (cm) delta mesh (Memphis Net and Twine, Memphis, Tenn.) and measured 2 m long × 1 m wide × 1 m high. The net cages were attached to frames constructed of 2.54-cm schedule 40 polyvinyl chloride (PVC) pipe and fittings and were set at a depth of 90–95 cm in the outdoor circular tank. The test fish were culled from a sample of about 1,000 fish that were collected by seining and distributed among five 189-L polyethylene holding tanks filled with about 150 L of water from the culture tank. Twenty fish were impartially collected from each holding tank, anesthetized with tricaine methanesulfonate (MS-222), measured for total length (TL), and then released back into the outdoor circular tank. The initial size range in TL of the test fish, determined from the TL-frequency distribution of the 100-fish sample, was 62 to 73 millimeters (mm). The remaining fish were measured for TL as described above and fish of the desired TL were placed in one of two holding cages (310 fish per cage). The other fish were released back into the outdoor circular tank. Fish used in the acute exposure study were held for 3 days, and fish used in the chronic test were held for 24 days in the net cages. The test fish were fed daily



Base from U.S. Geological Survey digital data, 1997, 1:1,000,000
Boundary of Pueblo of Isleta from Bureau of Land Management digital data, 2008, 1:24,000
Transportation from Federal Highway Administration digital data, 2002
Albers Equal-area Conic projection
Standard parallels 33°N and 36°N
Central meridian 106°W

Figure 1. Location of the three study sites in the Middle Rio Grande on the Pueblo of Isleta, New Mexico.

by BioPark personnel and mortality in both holding cages was <1 percent. Water quality in the holding cages was measured (described below) on the days when the test fish were collected for the field exposures and had the following characteristics (number of samples [n] = 2): hardness, 80–92 milligrams per liter (mg/L) as calcium carbonate ($CaCO_3$); alkalinity, 110 mg/L as $CaCO_3$; calcium, 26 mg/L; magnesium, 4–7 mg/L; pH, 8.66–8.90; dissolved oxygen, 7.7–10.0 mg/L; temperature, 18.8–20.8 degrees Celsius (ºC); conductivity, 427–438 microsiemens per cm (µS/cm) at 25ºC; and total ammonia, <0.1 mg/L as nitrogen (N).

Cage Design

Cages used in the acute exposures were obtained from the USFWS New Mexico Ecological Services Field Office (NMESFO), Albuquerque, N. Mex., and were constructed of 1.90-cm schedule 40 PVC pipe and fittings and 0.32-cm delta mesh netting sewn into a rectangular box with a cover sewn along one of the long sides. The PVC frame measured 60 cm long × 34 cm wide × 35 cm high and was placed inside the net box. The covers were secured to the cage using nylon cord and nylon cable ties. These covers were found to be ineffective in preventing access to the fish by predators (see below). Prior to use, the cages were washed with laboratory detergent, sequentially rinsed with tap water and deionized (DI) water, and air dried. The cages were deployed by fastening the top of the frame to two steel fence posts, placed at opposite ends, with nylon cable ties.

The cages used for the chronic exposures were 0.32-cm delta mesh net boxes (Memphis Net and Twine) placed over a PVC frame constructed of 2.54-cm schedule 40 PVC pipe and fittings. The outside dimensions of the PVC frame were 100 cm long × 50 cm wide × 56 cm high. No adhesives were used as the net box helped hold the frame together. To minimize the chances of fish getting impinged between the frame and netting, the netting was secured tightly to the frame with nylon cable ties. The net boxes had sleeves sewn along the outside of the vertical seams for use in deployment. The long sides of the cages were mounted on two 110-cm pieces of 2.54-cm schedule 40 pipe capped at both ends with nylon cable ties. These outside pipes provided additional support for the cages and served as handles for lifting the cages, especially when they became partly buried by sediment.

The covers consisted of a piece of 0.32-cm delta mesh netting that was sewn to the top of the net box along one of the long ends. Velcro® strips (5.1-cm wide) were fastened along the other three edges of the cover and along the outside of the corresponding sides of the net box. In addition, nylon cable ties were used to secure the covers at the corners. This design provided covers that were relatively easy to open and close and also were effective in preventing access to the fish by predators and the escape of fish when sporadic high flows submerged the cages. Prior to final assembly, the cages, covers, and PVC frames were washed with laboratory detergent, sequentially rinsed with tap water and DI water, and air dried.

For deployment, the cages were attached to two 91.4-cm steel fence posts placed at opposite corners of the cage. The posts were driven into the sediment to a depth where the top of the posts was about 2.54 cm above the top of the cage. A 53-cm long piece of 3.17-cm PVC pipe was inserted into two of the outside sleeves at opposite corners and secured to the netting with nylon cable ties. The cages were set by placing the pipes over the fence posts. The tops of the sleeves were secured to the fence posts with nylon cable ties. This arrangement allowed the cages to be lifted up for daily observations of the fish and reset with minimal effort by removing and reattaching the cable ties at the corners.

To provide structure inside the cage, a 61-cm long flattened tube (about 13 cm wide × 11 cm high) constructed of 0.64-cm mesh plastic netting was attached diagonally across the bottom of the cage with nylon cable ties. The tube was centered at the middle and set slightly diagonally to the long axis. A large plastic aquarium plant was attached at the top of the tube near the center to provide additional structure. Prior to use, the tubes and plants were cleaned by sequential washing with laboratory detergent and 20 percent hydrochloric acid and then soaked overnight in DI water.

In both studies, the cages were deployed on the day before the fish were stocked. The exposed parts of the cages were covered with camouflage burlap cloth to provide concealment and shade. The burlap covers were placed over cages and draped to the water surface and secured with twine. Prior to use, the burlap covers were washed with laboratory detergent, rinsed with tap water, soaked overnight in tap water, rinsed in DI water, and then air dried.

Acute Field Study

For the acute study, 10 fish were stocked in each cage (total of 90 fish) and exposed for four days (September 14–18, 2007) to assess survival. The fish were not fed for 48 hours prior to the test or during the study. To start the study, approximately 150 fish were collected from one of the holding cages and placed in a 189-L polyethylene holding tank filled with about 150 L of tank water. Ten fish were impartially stocked, one at a time, into each of 10 doubled plastic fish shipping bags filled with about 8 L of tank water and placed inside separate Styrofoam® insulated corrugated boxes. The inner bags were inflated with oxygen and both bags were sealed independently with rubber bands. The bags were packed in one of three large coolers and driven to the test location within 2 hours. The bags were randomly assigned to one of the nine cages or as the field control to assess effects of handling and transport. At the test sites, the bags were placed in the randomly assigned test cages to acclimate the fish to the ambient temperature. The bag containing the field control fish was carried to each site and kept in the cooler. After the temperatures equilibrated, about 8 L of site water was added to the bags to allow for partial acclimation to the ambient water quality. After 20 minutes, the fish were released into the cages to initiate the test. The field control fish were returned to the BioPark, and the bag was placed in one of the holding cages. On the following day, the field control fish were examined for mortality (0 percent) and abnormal behavior (none observed), and then released into the holding cage.

Survival was monitored daily; dead fish were placed in a plastic bag on ice, returned to the laboratory at the NMESFO, and preserved in 10 percent neutral buffered formalin (NBF). At the end of the exposure, all live fish were packed in fish shipping bags (as described above), returned to the laboratory, euthanized in MS-222, measured for TL (to the nearest 1 mm), weighed (to the nearest 0.001 gram [g]), and preserved in 10 percent NBF.

Chronic Field Study

Test Conditions

For the chronic study, 25 fish were stocked in each cage and exposed for 26 days (October 5–31, 2007) to assess survival, growth, overall condition, and accumulation of selected elements. The test fish were collected from one of the holding cages 2 days before test initiation as follows: Groups of about 50 fish were netted from the cage into a 189-L polyethylene holding tank filled with about 150 L of tank water. Five fish were impartially collected from the tank, anesthetized with MS-222, measured for TL, weighed, and then stocked into 1 of 10 randomly assigned holding tubs (1 tub for each cage plus a field control to assess effects of handling and transport). After all tubs were stocked with 5 fish, the process was repeated four times (25 fish/tub). The fish in each tub were netted into labeled doubled fish shipping bags filled with about 8 L of tank water and placed inside separate Styrofoam® insulated corrugated boxes. The inner bags were inflated with oxygen and both bags were sealed independently with rubber bands. The bags were then placed in one of the holding cages until transported to the test sites. On the following day, the water in the bags was renewed by replacing about one-half of the water with an equal volume of water from the tank. The bags were inflated with oxygen as described above and placed back in the holding cage. An additional 55 fish were euthanized with a lethal dose of MS-222, measured for TL, weighed, and processed as follows: 30 fish were subjected to a necropsy-based fish health assessment by DNFH&TC personnel, 5 fish were preserved in separate 30-mL vials filled with 10 percent NBF (after the abdominal cavity was slit) for histopathology; and 10 fish (two whole-body composites of 5 fish) were frozen for initial whole-body elemental composition.

To start the test, the doubled bags containing the test fish were packed in one of three large coolers and driven to the test location. The coolers were carried to the sites and the bags were placed in their randomly assigned test cages (one bag/cage). The bags were left unopened for at least 1 hour to acclimate the fish to site water temperatures. The bags were opened and the fish were held in a 1:2 mixture of site water to BioPark water (added about 4 L of site water to the bag) for at least 30 minutes to partially acclimate them to the site water quality. The fish were then released into the cages to initiate the test. The bag with the field-control fish was carried to each site but was left in the cooler. At the last site, this bag was transferred to a second cooler, returned to the BioPark, and placed in one of the holding cages. On the following day, the field-control fish were examined for mortality (0 percent) and abnormal behavior (none observed) and then released into the holding cage. The test fish were not fed on the day before collection or during the 2-day holding period.

Survival and overt abnormal behaviors were monitored daily. Dead fish were placed in plastic bags on ice, returned to the laboratory, measured for TL, weighed, and preserved in 10 percent NBF. On days 2 through 25 of exposure, the fish were fed the same formulated flake diet they received at the BioPark at 3 percent body weight (based on initial weights and adjusted for mortality) after all sampling was completed. The cages were checked daily for damage and sediment accumulation and were brushed as needed. Cages becoming partly buried were lifted out of the sediment and set on the substrate to maintain sufficient water volume.

At the end of the study, the cages were removed from the site water (one at a time) and placed into a portable shallow rectangular pool set up on shore and filled with site water. The pool was constructed of a large white polyethylene sheet placed inside a PVC frame (2.54-cm schedule 40 PVC pipe and fittings). The cage was inverted in the pool, and the fish were collected with a dip net and placed in labeled doubled fish shipping bags. The bags were inflated with oxygen and sealed as described above and transported to the laboratory in coolers. In the laboratory, fish from a given cage were euthanized with MS-222, measured for TL (to the nearest 1 mm), weighed (to the nearest 0.001 g), and then sampled as follows: five fish for a necropsy-based fish health assessment by DNFH&TC personnel (each fish was euthanized just prior to examination), three fish for histopathology, and five fish for whole-body elemental analyses. Any remaining fish were placed in plastic bags and frozen. Fish collected for histopathology were placed in separate 30-mL vials filled with 10 percent NBF after the abdominal cavity was cut open. The preserved fish were placed in fresh 10 percent NBF on the following day. Fish collected for whole-body elemental analysis were composited directly into Whirl-pak® bags and placed in the freezer.

Necropsy-Based Fish Health Assessments

A necropsy-based health assessment was performed on 30 fish randomly sampled from the lot used to stock the cages (pre-exposure group) and 5 live fish randomly sampled from each cage after 26 days of exposure. The results of the assessments were used to characterize and compare the health status of Rio Grande silvery minnows prior to and after in-situ exposures to the site waters. Examinations on both groups of fish were performed by the same experienced fish biologist from the DNFH&TC. The examinations involved systematic observations on a selected set of external and internal anatomical features for parasites, visible abnormalities, and overall condition using a standardized data form. The external features examined (and rated as) were the eyes (normal, exopthalmic, hemorrhagic, opaque, embolic, or missing); head and body surface (normal, tumors, lesions, or parasites); fins (normal, eroded [mild or severe], frayed, embolic, or hemorrhagic); gills (normal, frayed, clubbed, marginate, pale, or parasites); and opercles (normal, slight shortening, or severe shortening). The internal organs examined (and rated as) were the liver (normal, tan, general discoloration, focal discoloration, or nodular); spleen (normal, granular, nodular, or enlarged); and kidney (normal, swollen, mottled, granular, or utolithiasis). In addition, the gonads were examined to determine gender. Values were assigned to each of the seven variables according to the type and severity of the abnormality observed based on the modified protocol of Adams and others (1993) described in Blazer and others (2002). A health assessment index (HAI) was calculated for each fish by summing the numerical values for the features examined. A HAI was only computed for fish having observations on all seven features.

The quantity of mesenteric fat and presence and color of bile in each fish were categorized by index values described in Goede and Barton (1990). The extent of mesenteric fat coverage was ranked as 0 for no fat deposits, 1 for less than (<)50 percent coverage, 2 for 50 percent coverage, 3 for greater than (>)50 percent coverage, and 4 for complete coverage to derive a mesenteric fat index (MFI). Bile color-fullness indices (BCFI) for the gall bladder were derived from ratings of 0 for straw-yellow bile and bladder partly full or empty; 1 for yellow bile and bladder full, 2 for light- to grass-green bile and bladder full, and 3 for dark-green to blue-green bile and bladder full.

Histopathological Assessments

A total of 32 preserved fish (27 exposed and 5 pre-exposed) were submitted to a contract lab (Colorado Histo-Prep Inc, Fort Collins, Colo.) for histopathological evaluation. The tissues examined for pathology were the brain, gastrointestinal tract, gills, gonads, kidneys, liver, muscle, and spleen. The tissues were processed and embedded into paraffin blocks, microtomed into 5-micron sections, and placed on glass slides. Two sections from each paraffin block were taken at different depths and placed on the same slide. The slides were then stained with hematoxylin and eosin and submitted for histopathological evaluation. Tissues were evaluated by a Certified Fish Pathologist (American Fisheries Society) for overall health and condition. The observations on conditions and lesions were scored on a scale of 0 (none) to 6 (severe). All tissues were examined for the presence of parasites and bacterial infections.

Field Parameters and Water Sampling

Multiparameter water-quality sondes (YSI model 6600 V2 water-quality sondes, Yellow Springs Instruments, Yellow Springs, Ohio) were deployed at each site during both studies to obtain measurements of conductivity, dissolved oxygen (DO), pH, temperature, and turbidity at 15-minute intervals. The water-quality sondes (hereinafter referred to as sondes) were calibrated prior to deployment and post-calibrated after retrieval (to assess performance and accuracy) according to the manufacturer's user manual and software (Yellow Springs Instruments, 2006). The sondes were placed in a protective PVC housing and deployed at mid-depth just upstream from the cages. During the chronic study, a second sonde was deployed at the WIH site on day 3 of exposure because river water from the East channel had started flowing into the WIH site, and there was concern that the first sonde may become buried in sediment. This sonde was deployed between the second and third cage because there was not sufficient depth below the third cage. In both studies, there were problems with the sondes deployed at the WIH site. In the acute study, the sonde only collected data for the last 9 hours of the study. During the chronic study, the first deployed sonde stopped collecting data on day 11 of exposure.

In both tests, DO, pH, conductivity, and temperature were measured daily in the cages and near the sonde with portable meters (YSI model 58 dissolved oxygen meter; Orion model 250A pH meter with an Orion model 9107 pH electrode, and Orion model 115 conductivity meter with a model 11510 conductivity cell, Orion Research, Boston, Mass.) calibrated in the field. Measurements were taken at mid-depth near the sonde and in the center of the cages.

Water samples were collected at each site as subsurface grabs in dedicated (precleaned) polyethylene bottles for general water-quality analyses. The samples were placed in coolers for transport back to the laboratory. For the acute study, water samples were collected daily near the sonde. For the chronic study, water samples were collected at test initiation and every 2–3 days thereafter at the sondes or in one of the cages. Additional water samples were collected at the sondes at the beginning of both studies and every 5–7 days during the chronic study for analyses of dissolved and total organic carbon, biological and chemical oxygen demand, nutrients (total and orthophosphorus, nitrate, nitrite, and total nitrogen), and 34 elements (chronic test only). Another set of samples was collected at the beginning of both studies and at the end of the chronic study for analysis of 10 chlorinated herbicides and 18 organophosphorous pesticides. These samples (three per site) were collected directly into precleaned 1-L amber glass bottles provided by the contract laboratory.

After water-quality sampling was completed, depth and water flow were measured daily in the cages and near the sonde with a portable flowmeter (Marsh-McBirney model 2000 flowmeter, Frederick, Md.) and wading rod. Depth was measured near the front and back of each cage, and the flow was measured near the front of the cage at 0.6 of the depth (from surface) following the operations manual (Marsh-McBirney, 1990).

Water-Quality Analysis

For each sample collected, a set of subsamples was filtered through 0.45-µm versapor filters (Geotech dispos-a-filter™, Denver, Colo.). One subsample of filtered water was analyzed within 6 hours of collection for calcium, total alkalinity, and total hardness by standard titrimetric methods of American Public Health Association (1995); magnesium was calculated as the difference between total hardness and calcium. Quality-control measures involved analysis of duplicate samples and DI water spikes with each set of samples. Another subsample of filtered water was collected in a 125-mL polyethylene bottle and held under refrigeration until transported to the U.S Geological Survey (USGS) Yankton Field Research Station (FRS) for analysis of chloride and sulfate. For samples collected during the acute study and during the first 10 days of the chronic study, chloride was measured in triplicate with a Buchler model 4-2500 chloridometer (Buchler Instruments, 1978) and sulfate was measured in duplicate by the modified turbidimetric method in Hach (1997) with a Hach model DR/2000 spectrophotometer (Hach Company, Loveland, Colo.) using a Pour-Thru Cell Kit and Hach SulfaVer 4 reagent at the USGS Yankton FRS. For samples collected on days 12 to 25 of the chronic study, chlorides and sulfates were measured by U.S. Environmental Protection Agency (1983a) method 300.0A (ion chromatography) at the Water and Environmental Engineering Research Center (WEERC) at South Dakota State University, Brookings, S. Dak. The samples were analyzed in duplicate along with spiked reagent water. The samples were transported to the WEERC in a cooler with wet ice.

One unfiltered subsample (125–250 mL) of each site water was collected in a polyethylene bottle, acidified in 0.4 percent reagent grade sulfuric acid (pH <2) and held under refrigeration until transported to the USGS Yankton FRS for analysis of ammonia. Total ammonia as N was measured with an ion-specific electrode following the procedures for low concentration measurements of the electrode manufacturer (Orion Research, 1990) and standard methods (American Public Health Association, 1995). Quality-control measurements included analysis of duplicate and spiked samples. Un-ionized ammonia concentrations were calculated from the total ammonia concentrations using the ammonia equilibrium equations of Emerson and others (1975) and the pH and temperature measured at the site.

A second unfiltered subsample was collected in a 500- or 1,000-mL polyethylene bottle for measurement of turbidity, total suspended solids, volatile suspended solids, and fixed suspended solids. Turbidity was measured on 2–4 subsamples collected from the bottle. The remaining sample was held under refrigeration until transported to the USGS Yankton FRS. Turbidity was measured with a Hach model 2100P turbidimeter and cuvettes following the procedures of the manufacturer (Hach, 2004). Suspended solids (total, fixed, and volatile) concentrations were determined gravimetrically according to standard methods (American Public Health Association, 1995). One duplicate analysis of the suspended solids (total and fixed) was performed with each set of samples. Samples sent to the USGS Yankton FRS were packed with wet ice in a cooler.

Subsamples of water collected at the sondes were submitted to contract laboratories for more extensive chemical characterizations. Filtered samples (described above) for dissolved organic carbon (DOC) and unfiltered samples for total organic carbon (TOC), total inorganic carbon (TIC), total Kejldahl nitrogen (TKN), total phosphorus (TP), and chemical oxygen demand (COD) were collected in glass bottles containing a small aliquot of sulphuric acid provided by the contract laboratory. Unfiltered samples for anions and biological oxygen demand (BOD) were collected in polyethylene bottles. The samples were packed in a cooler with wet ice and shipped by overnight courier to Test America Laboratories (formerly Severn Trent Laboratories), Arvada, Colo. The samples for anions were not filtered prior to shipment because they were filtered at Test America before analyses. These parameters were measured according to U.S. Environmental Protection Agency (1983a) methods listed in appendix 1. Quality control measures included analyses of procedural blanks, duplicate samples, and duplicate laboratory and matrix spikes.

A second set of filtered samples was collected in 125-mL polyethylene (acid-cleaned) bottles for analysis of 34 elements. The samples were acidified with 1 percent ultrapure nitric acid and stored frozen until shipment to Trace Element Research Laboratory (TERL), Texas A&M University, College Station, Tex. A DI water field blank was also filtered and preserved as above. The water and whole-body fish samples (described above) were packed with dry ice in a cooler and shipped by overnight courier to TERL.

Elemental Analysis

Water and fish tissue (whole-body composites of three to five fish) samples submitted to TERL for analysis of 34 elements were prepared for instrumental analysis according to USEPA method 200.2 (Martin and Creed, 1992). Tissue samples were freeze dried prior to digestion, and the moisture content was determined gravimetrically. Elemental concentrations were measured by one of four instrumental methods. Concentrations of antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), boron (B), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), lithium (Li), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), silver (Ag), thallium (Tl), thorium (Th), tin (Sn), uranium (U), vanadium (V), and zinc (Zn) were determined by inductively coupled plasma-mass spectroscopy (ICP-MS) according to USEPA method 200.8 (Long and Martin, 1992). Concentrations of aluminum (Al), calcium (Ca), magnesium (Mg), iron

(Fe), phosphorus (P), potassium (K), silicone (Si), sodium (Na), strontium (Sr), and titanium (Ti) were measured by ICP-optical emissions spectroscopy (ICP-OES) following USEPA method 200.7 (Martin and others, 1992). Total mercury (Hg) in water was measured by cold vapor atomic absorption (CVAAS) based on U.S. Environmental Protection Agency (1983b) method 245.2 and in tissues by combustion trapping atomic absorption spectroscopy (C-T-AAS) based on U.S. Environmental Protection Agency (2007) method 7473.

The quality-control measures included the analysis of procedural blanks (to measure contamination during sample preparation and analysis and to determine detection limits), duplicate samples (to measure precision), fortified (spiked) samples (to measure matrix interferences), and laboratory standards and certified reference material (to measure accuracy). The reference materials analyzed were Standard Reference Material (SRM)1640-Trace Elements in Water and SRM 1641d-Mercury in Water obtained from the National Institute of Standards and Technology (Gaithersburg, Md.) and certified dogfish liver DOLT-4 (10 elements) and dogfish muscle DORM-2 (mercury only) obtained from the National Research Council Canada (Ottawa, Ontario, Canada).

Method detection limits (MDL) were determined individually for each analyte in each sample**.** Elements in water were reported as dissolved concentrations (mg/L or micrograms per liter [µg/L]), and elements in whole-body fish tissues were reported as dry-weight (dw) and wet-weight (ww) concentrations (milligrams per gram [mg/g] or micrograms per gram [µg/g]); the latter was calculated from the moisture content of the sample.

Semivolatile Organic Compounds

Samples destined for analysis of 10 chlorinated herbicides and 18 organophosphorous compounds were packed in wet ice and shipped to Test America Laboratories. Chlorinated herbicides were measured by gas chromatography/mass spectrometry (GC/MS) according to U.S. Environmental Protection Agency (1986) method 8151AB. Organophosphorous compounds were measured by GC/MS following U.S. Environmental Protection Agency (1986) method 3510 for sample preparation and method 8141A for instrumental analysis. Quality-control measures included method blanks, duplicate analysis of spiked laboratory control samples, and analysis of surrogate organic compounds.

Statistical Analysis

Data were analyzed using Statistical Analysis System (SAS) for Windows software, version 9.2 (SAS Institute, Cary, N.C.). Prior to final statistical analysis of the quantitative variables, the assumptions of normality and equal variance were formally tested by the Shapiro-Wilk and Levene's test, respectively. Data that did not meet these assumptions were log 10 transformed and retested. If the log-transformation did not satisfy the assumptions, the data were transformed to ranks and the appropriate statistical test was applied to the ranks (Conover and Iman, 1981). Statistical significance probability (p) was set at $p < 0.05$ for all tests.

In-situ water-quality data collected by the sondes and laboratory-derived water-quality data were compared among sites using two-factor analysis of variance (ANOVA) without replication, with site and time as main effects. Tukey's Honestly Significant Difference (HSD) test (for equal sample sizes) or Tukey-Kramer tests (for unequal sample sizes) were used for post hoc pairwise comparisons between sites (SAS, 1990). For constituents having censored values (values below the detection limit), a value of one-half the MDL was substituted for the censored value in the statistical analyses.

Physical habitat data collected daily in the cages were compared by repeated-measures ANOVA using the MIXED procedure in SAS, with time as the repeated factor and cage as the subject. A compound symmetric covariance structure was selected for all analyses based on Akaike's Information Criteria and Schwarz Bayesian Criterion (Littell and others, 1996). The least square means were compared using Tukey's adjustment.

Total length, body weight , condition factors, HAI, MFI, BFCI, and histological ratings for Rio Grande silvery minnows were compiled into means for each cage (experimental unit) prior to statistical analyses. Fulton-type condition factors were calculated for each fish by the formula of Anderson and Gutreuter (1983) as follows:

$$\text{Condition factor} = [\text{body weight (g)}/\text{total length (mm)}^3] \times 100{,}000$$

Changes in growth metrics were calculated from cage-average values. Cage-average absolute growth and relative growth for TL (mm) and weight (g) were calculated according to Ricker (1979) as follows:

$$\text{absolute growth-metric} = (\text{final mean metric} - \text{initial mean metric})$$

$$\text{relative growth-metric} = (\text{final mean metric} - \text{initial mean metric})/(\text{initial mean metric})$$

Cage-average survival, growth metrics, health indices, and whole-body concentrations of major, minor, and trace elements were subjected to one-way ANOVA using the general linear models procedure in SAS. When statistical significance was found, multiple comparisons between sites were performed by Tukey's HSD test. Percent survival data were arcsine square root transformed prior to analysis. Proportions of fish with a given anomaly or lesion were compared using contingency tables and applying Fisher's exact test when any expectation was <5. Other statistical methods used are described below where appropriate.

Results and Discussion

Quality Control

Multiprobe Sonde

Post sampling checks of the sondes after the acute study showed little drift in the probes; pH readings were within plus or minus [±]0.11 unit of the buffers, conductivity was within 0.6 percent and turbidity was within 1.4 percent of certified values, and optical DO was ±0.2 mg/L and ±1.3 percent saturation of the expected values. After the chronic study, post sampling readings showed little drift in pH (±0.23 unit), conductivity (±0.3 percent), and optical DO (±0.3 mg/L, ±4.0 percent saturation). The post sampling turbidity readings were about 12 to 25 percent higher than the low standard (100 nephelometric turbidity units [NTU]), but were only 1 to 4 percent lower than the high standard (1,000 NTU).

General Water Quality

The recoveries of calcium, measured as hardness (mg/L as $CaCO_3$) and calcium (mg/L), in spiked DI water were 100 percent for all analyses and those of alkalinity ranged from 98 to 102 percent. The relative percent difference (RPD) for duplicate samples ranged from 0 to 0.8 percent for alkalinity, 0 to 3.7 percent for calcium, and 0 to 1.4 percent for hardness. Because duplicate analyses were performed on each batch of samples, the average of the duplicate readings was reported for these samples. Recoveries of total ammonia as N in samples spiked prior to preservation ranged from 90.3 to 100.0 percent. The RPD for duplicate samples was not calculated because the total ammonia concentrations were below the method detection limit of 0.1 mg/L as N in all samples. For the solids, the RPD ranged from 0.7 to 4.2 percent for total suspended solids and 0.5 to 9.9 percent for fixed suspended solids. Duplicate analyses and reagent water spikes performed on samples for chloride and sulfate at WEERC were within the laboratory's control limits.

For water samples analyzed at Test America Laboratories, nitrate, total phosphorus, sulfate, TOC, and TIC were occasionally detected in the blanks, but the concentrations were always below the reporting limit (appendix 1). Method accuracy and precision were demonstrated for all analytes by the acceptable recoveries of reagent water spikes (81 to 112 percent) and acceptable RPD for reagent water spike duplicates (0 to 5.2 percent) and sample spike duplicates (<0.1 to 8.7 percent). Recoveries of matrix spikes performed on USGS Yankton FRS samples were within established control limits for 9 of 12 analytes. Recoveries of orthophosphate and sulfate were above the control limits (80 to 120 percent) in two sample runs and those of TIC were below the control limits (90 to 110 percent) in four sample runs. The high recoveries of orthophosphate were in samples where the orthophosphate concentrations were below the detection limit. The validity of one set of spike recoveries for sulfate (that exceeded the upper control limit) was questionable because of the low amount of analyte spiked relative to that in the original sample. The low matrix spike recoveries for TIC were based on estimated results, as the concentration of the spiked samples exceeded the calibration range. Because method accuracy and precision were verified by acceptable reagent water spikes and reagent water spike duplicates, the contract laboratory deemed that corrective action was unnecessary for these results.

Elemental Analyses

For the water samples analyzed for 34 elements by TERL, background concentrations in the procedural blanks were below the MDL (appendix 2), except for Ca (0.03 mg/L), K (0.01 mg/L), P (0.11 mg/L), Na (0.2 mg/L), and S (0.07 mg/L). The concentrations of these elements detected in the blanks were within a factor of two of their MDL and were considered to be within the acceptable control limits at TERL. Concentrations of 10 elements in duplicate samples were below the MDL and thus no measure of the method precision was calculated for these elements. The RPD measured for the remaining elements, except for Hg, in the duplicate samples averaged 2.4 percent with a range of 0 to 10.5 percent. The high RPD of 18.2 percent for Hg was because of the low concentrations (0.005 and 0.006 µg/L) in the duplicate samples, which were at or just above the MDL (0.005 µg/L). The percent recovery of 32 elements from spiked reagent water averaged 98 percent with a range of 83 to 115 percent. The percent recovery of the same 32 elements in a fortified water sample averaged 101 percent and ranged from 91 to 116 percent. The analyses of 27 elements in standard reference water were within 84 to 111 percent of certified values, which indicated that these elements were accurately measured in the water samples. Several elements (Ca, Mg, Hg, P, Na, Si, Zn) were detected in the field blank and, except for Ca and Si, the concentrations detected were less than or equal to (≤) 2 times the MDL. The concentrations of Ca (0.2 mg/L) and Si (0.06 mg/L) in the blank were <1 percent of those in the water samples and were not considered to have a significant effect on the results.

Dry weight MDL for all elements were determined for each tissue sample and thus varied because of differences in the mass of the sample analyzed (appendix 3). Concentrations of Al, Ca, Mn, K, Si, and Zn were detected in the reagent blank; however, the concentrations of Ca and Zn were 1.5 times higher than the MDL and concentrations of Al, K, Mn, and Si were 2.1 to 2.9 times higher than their MDL. These reagent blank concentrations were not considered to have a significant effect on the sample results because these concentrations were at least an order of magnitude lower than those measured in the samples. The RPD for the 26 elements that were detectable in the duplicate samples averaged 3.2 percent with a range of 0.2 to 13.1 percent. The recovery of 27 elements added to reagent water averaged 102 percent and ranged from 89 to 112 percent. Except for Ca and Sr, the recoveries of 26 elements from a fortified tissue sample averaged 100 percent with a range of 80 to 113 percent. The high spiked sample recoveries observed for Ca (341percent) and Sr (134 percent) were not considered to be valid by TERL because of the low concentration of the analyte spiked relative to that in the original sample. The analysis of 10 elements in standard reference tissue samples were within the range of certified values.

Semivolatile Compounds

None of the 28 target analytes (appendix 4) were detected in the reagent blanks and recoveries of laboratory control spikes and laboratory control spike duplicates were within the laboratory's control limits. The RPD (not given in appendix 4) for duplicate laboratory control spikes were within acceptable limits, except for 2,4-D in one sample run. However, the recoveries of both laboratory control spikes for 2,4-D were within acceptable limits. In one sample run, the recovery of the surrogate compound (triphenyl phosphate) was above the quality control limit, which indicated that the data may be biased high. However, because no detectable concentrations of organophosphorous pesticides were present in the samples, no corrective action was deemed necessary by the analytical laboratory.

Acute Field Study

In-Situ Monitoring

Initial ANOVA tests on water-quality parameters measured daily in the cages and at the sondes did not detect significant differences in temperature, DO, pH, or conductivity among the cages and sonde at a given site (appendix 5). These results indicate that the water-quality parameters measured at the sonde were representative of those in the cages at a given site.

Daily average, minimum, and maximum values for the in-situ water-quality parameters measured at 15-minute intervals by the sondes are given in appendix 6 and summarized in table 1. The sonde deployed at the WIH site malfunctioned after 9 hours and these data were not used in the analyses. Over the course of the study, daily average temperatures and DO concentrations were similar at the MRG and 240-WW sites, whereas, the mean daily average pH, conductivity, and turbidity were statistically higher at the MRG site than at the 240-WW site. The mean daily minimum and daily maximum values for each parameter differed statistically between sites, except for daily maximum values of DO as milligrams per liter and turbidity. The diel variations (daily ranges) in temperature, DO, and pH were significantly larger at the MRG site, whereas, the daily variations in turbidity were larger at the 240-WW site. Temperature was the only parameter where the effect of time was significant; the mean average and mean minimum temperatures decreased over time.

None of the temperatures (≤31.0ºC) exceeded the Pueblo of Isleta (2002) maximum temperature standard of 32.2 ºC for warmwater fishery use. All DO concentrations ≥6.1 mg/L were above the Pueblo of Isleta (2002) minimum DO standard of 5 mg/L and all pH readings (7.91–8.74) were within the Pueblo of Isleta standard pH range of 6.0–9.0 units.

The Pueblo of Isleta (2002) water-quality standard for turbidity is based on the background value and for background turbidities above 50 NTU, the standard is 10 percent higher than the background value. Using turbidities measured at the MRG site as the background values, there were no exceedences at the 240-WW site based on daily average turbidities or in daily grab samples (appendix 6; tables 1 and 2). Further analysis of the sonde data revealed that only 2 of 359 measurements at the 240-WW site exceeded the background turbidity standard. The exceedences of 116 compared to 75 NTU or 54.7 percent above MRG site turbidity and 105 compared to 81 NTU or 29.6 percent above MRG site turbidity occurred on the first and last day of the study, respectively.

General Water Quality and Physical Conditions

Most water-quality parameters differed statistically among sites (table 2). Hardness, alkalinity, calcium, and sulfate were significantly higher and chloride was significantly lower at the 240-WW and WIH sites than at the MRG site. There were no significant differences in the concentrations of the six chemical parameters measured daily between the 240-WW and WIH sites. Total suspended solids, volatile suspended solids, and turbidity at the WIH site and volatile suspended solids and turbidity at the MRG site were significantly higher than those at the 240-WW site. The volatile suspended solids, which provide a rough approximation of the amount of organic matter present in the solids, comprised only about 10 to 12 percent of the total suspended solids measured at all sites. Turbidity was significantly correlated with total suspended and fixed suspended solids (Spearman's rho, $r \leq 0.975$, $p \leq 0.005$) at the MRG and WIH sites but not at the 240-WW site.

Total ammonia concentrations in samples collected daily were all below the detection limit (table 2). Concentrations of the other constituents that were measured at test initiation were below or within a factor of two of their reporting limit (appendix 1).

The Pueblo of Isleta have established water-quality standards for chlorides and sulfates based on naturally occurring levels. Concentrations of these constituents should not exceed background levels by more than 33 percent. Using the concentrations measured at the MRG site as the background values, there were no exceedences for chlorides or sulfates.

Concentrations of fluoride (≤0.54 mg/L) and total inorganic N (≤1.00 mg N/L; calculated as the sum of total ammonia, nitrate, and nitrite) were below Pueblo of Isleta (2002)

Table 1. Mean and range of daily average, minimum, maximum, and range of in-situ parameters computed from readings taken at 15-minute intervals by sondes at two sites in the Middle Rio Grande, New Mexico, during acute cage exposures of Rio Grande silvery minnows, September 14–18, 2007.

[Sample size was 5. Means within a row sharing the same uppercase letter are not significantly (p <0.05) different. p, probability; <, less than; MRG, Middle Rio Grande; 240-WW, 240-Wasteway; °C, degrees Celsius; mg/L, milligrams per liter; %, percent; SU, standard units; µS/cm at 25°C, microsiemens per centimeter at 25 degrees Celsius; NTU, nephelometric turbidity unit]

Daily	Site (fig. 1)			
	MRG		240-WW	
	Mean	Range	Mean	Range
Temperature (ºC)				
Average	22.0[A]	17.9 – 25.0	21.5[A]	19.7 – 23.3
Minimum	17.9[B]	15.7 – 20.9	19.9[A]	18.6 – 21.6
Maximum	27.8[A]	23.4 – 31.0	23.4[B]	21.5 – 24.3
Range	9.9[A]	6.8 – 14.0	3.5[B]	2.6 – 4.8
Dissolved oxygen (mg/L)				
Average	7.06[A]	6.39 – 7.42	7.17[A]	7.10 – 7.26
Minimum	6.34[B]	6.08 – 6.70	6.94[A]	6.81 – 7.07
Maximum	8.12[A]	7.09 – 8.70	7.36[A]	7.28 – 7.43
Range	1.78[A]	1.01 – 2.33	.42[B]	0.36 – 0.51
Dissolved oxygen (% saturation)				
Average	80.9[A]	77.6 – 84.4	81.3[A]	78.8 – 85.2
Minimum	72.5[B]	72.2 – 73.0	78.0[A]	76.5 – 81.4
Maximum	100.9[A]	92.8 – 109.3	84.8[B]	80.4 – 87.6
Range	28.4[A]	20.6 – 37.0	6.8[B]	2.3 – 10.7
pH (SU)				
Average	8.47[A]	8.32 – 8.58	8.01[B]	7.97 – 8.05
Minimum	8.32[A]	8.28 – 8.44	7.93[B]	7.91 – 7.95
Maximum	8.64[A]	8.45 – 8.74	8.11[B]	8.03 – 8.16
Range	.32[A]	0.17 – 0.45	.18[B]	0.10 – 0.25
Conductivity (µS/cm at 25ºC)				
Average	500[A]	475 – 515	436[B]	424 – 451
Minimum	482[A]	466 – 507	424[B]	413 – 438
Maximum	515[A]	487 – 530	455[B]	430 – 483
Range	33[A]	20 – 46	31[A]	6 – 53
Turbidity[a] (NTU)				
Average	81[A]	77 – 85	55[B]	51 – 58
Minimum	71[A]	69 – 74	47[B]	43 – 52
Maximum	89[A]	83 – 97	86[A]	65 – 120
Range	18[B]	13 – 26	39[A]	22 – 70

[a]Turbidity values were rounded following U.S. Geological Survey guidelines (Wilde, variously dated).

Table 2. Water quality measured in samples collected at the sondes and physical characteristics measured in cages during acute cage exposures of Rio Grande silvery minnows in the Middle Rio Grande, New Mexico, September 14–18, 2007.

[Water-quality data are mean±1 standard deviation and range in parenthesis and physical characteristics are the least square mean±1 standard error and range in parenthesis. Means within a row sharing the same uppercase letter are not significantly (*p* <0.05) different. ±, plus or minus; *p*, probability; <, less than; MRG, Middle Rio Grande; 240-WW, 240-Wasteway; WIH, Wetted Instream Habitat; n, number of samples; mg/L, milligrams per liter; $CaCO_3$, calcium carbonate; NTU, nephelometric turbidity unit; N, nitrogen; P, phosphorus; cm, centimeter; cm/sec, centimeter per second]

Parameter (unit)	Site (fig. 1)		
	MRG	240-WW	WIH
Water quality measured daily (n = 5)			
Hardness (mg/L as $CaCO_3$)	$138^{B} \pm 1$ (136 – 139)	$156^{A} \pm 3$ (152 – 161)	$157^{A} \pm 5$ (150 – 164)
Alkalinity (mg/L as $CaCO_3$)	$130^{B} \pm 1$ (129 – 130)	$142^{A} \pm 3$ (137 – 146)	$143^{A} \pm 4$ (138 – 148)
Calcium (mg/L)	$45^{B} \pm 1$ (45 – 46)	$52^{A} \pm 2$ (50 – 54)	$52^{A} \pm 3$ (50 – 58)
Chloride (mg/L)	$24^{A} \pm 1$ (23 – 25)	$16^{B} \pm 1$ (15 – 17)	$16^{B} \pm 1$ (15 – 17)
Magnesium (mg/L)	$6^{A} \pm 0$ (6)	$7^{A} \pm 1$ (6 – 7)	$6^{A} \pm 1$ (5 – 7)
Sulfate (mg/L)	$60^{B} \pm 1$ (59 – 61)	$63^{A} \pm 2$ (60 – 66)	$65^{A} \pm 1$ (63 – 66)
Total suspended solids (mg/L)	$140^{AB} \pm 10$ (125 – 151)	$123^{B} \pm 9$ (110 – 133)	$170^{A} \pm 42$ (125 – 222)
Fixed suspended solids (mg/L)	$123^{A} \pm 10$ (108 – 134)	$110^{A} \pm 9$ (98 – 120)	$153^{A} \pm 40$ (111 – 202)
Volatile suspended solids (mg/L)	$16.6^{A} \pm 1.2$ (15.0 – 18.0)	$13.6^{B} \pm 1.0$ (12.4 – 14.7)	$17.2^{A} \pm 2.0$ (14.0 – 19.6)
Turbidity[a] (NTU)	$180^{A} \pm 19$ (150 – 200)	$120^{C} \pm 11$ (110 – 130)	$150^{B} \pm 25$ (120 – 190)
Ammonia, total (mg/L as N)	< 0.1	< 0.1	< 0.1
Water quality measured at test initiation (n = 1)			
Nitrate (mg/L as N)	0.93	0.41[b]	0.40[b]
Nitrite (mg/L as N)	.069[b]	<.049	<.049
Orthophosphate (mg/L as P)	1	<.19	<.19
Biochemical oxygen demand (mg/L)	< 0.3	< .3	2.1
Bromide (mg/L)	.12[b]	<.11	<.11
Fluoride (mg/L)	.54	.44[b]	.43[b]
Physical characteristics in cages (n = 6)			
Depth (cm)	$26^{A} \pm 1$ (23 – 27)	$25^{A} \pm 1$ (22 – 30)	$20^{B} \pm 1$ (16 – 24)
Flow (cm/sec)	$1.9^{B} \pm 0.4$ (1.2 – 2.1)	$4.1^{A} \pm 0.4$ (2.1 – 5.5)	$2.6^{AB} \pm 0.4$ (1.2 – 4.6)

[a]Turbidity values were rounded following U.S. Geological Survey guidelines (Wilde, variously dated).

[b]Result is below the reporting limit (nitrate and nitrite, 0.50 mg N/L; bromide, 0.20 mg/L; flouride, 0.50 mg/L).

water-quality standards of 4.0 and 10.0 mg/L, respectively, for waters designated for primary contact ceremonial use. There were no detectable concentrations of the 28 target pesticides at any of the sites (appendix 4).

Although an attempt was made to deploy the cages at locations with similar morphology and hydrology, results of repeated-measures ANOVA found that depth and flow differed among sites (table 2), but not over time, and the interaction of site and time was not significant. The lowest depths were observed in cages at the WIH site and the lowest flows were in cages at the MRG site.

Exposure Endpoints

The recovery of live fish in cages after 4 days of exposure at each site was highly variable within and among sites and ranged from 20 to 60 percent at the MRG site, 20 to 90 percent at the 240-WW site, 70 to 90 percent at the WIH site. In cages where survival was <80 percent, the number of dead fish found did not completely account for the number of expected mortalities (based on survival). Evidence existed of predation by other aquatic organisms (probably crayfish) as parts of fish were found and of escape because of holes found in some of the cages that were not present prior to deployment. These results led to the redesign of the cages used for the chronic exposures. There were no differences in TL or weight of the surviving fish (n=55) among sites; mean (±1 standard error [SE]) TL was 67.9±0.4 mm with a range of 63–74 mm and mean (±1 SE) weight was 2.477±0.047 g with a range of 1.871–3.437 g.

Chronic Field Study

In-Situ Monitoring

The data collected by the two sondes at the WIH site were averaged for statistical analyses. As was observed in the acute exposure study, there were no significant differences among the cages and the sonde for any of the water-quality parameters measured daily (appendix 7). These results indicate that the water-quality parameters measured at the sonde were representative of those in the cages.

Daily average, minimum, and maximum values for the in-situ water-quality parameters monitored at 15-minute intervals by the sondes are given in appendix 8 and summarized in table 3 and figure 2. Daily average DO concentrations, pH, and turbidities were statistically higher and temperatures were lower at the MRG site than at the other two sites, but the magnitudes of the differences were small. Mean conductivity was statistically higher at the WIH site compared to the other sites, but the magnitude of difference was small ≤8.3 percent. Average diel variation (range) in temperature during the exposure was about 2.5-times greater at the MRG site (10.0ºC) compared to that at the 240-WW (3.9ºC) and WIH (4.0ºC) sites. Turbidity was highly variable at each site; mean daily ranges (maximum-minimum) were greater than the mean daily average values. The highest turbidities were observed at the 240-WW site.

There was a significant effect of time in the analyses of daily average values for all parameters except conductivity (fig. 2). The Mann-Kendall test was applied to the sonde data to identify trends in water-quality parameters at each site using the computer program of Helsel and others (2006). Analysis of the daily average values revealed statistically significant trends of decreasing temperature (Mann-Kendall tau [τ] ≤-570, p <0.001) and turbidity (τ ≤-0.450, p ≤0.001) and increasing DO as milligrams per liter (τ ≥0.366, p ≤0.009) and pH (τ ≥0.376, p ≤0.006) at all sites during the 26-day chronic study. An increasing trend in conductivity was observed at the MRG (τ=0.362, p=0.009) and WIH (τ=0.345, p=0.012) sites and in DO as percent saturation (τ=0.570, p<0.001) at the MRG site. Daily average water temperatures generally tracked the daily mean air temperatures recorded in Albuquerque, N. Mex., during the period of study (fig. 2). Similar trends were observed for the daily minimum and maximum values at all sites; temperature (τ ≤-0.444, p ≤0.001) and turbidity (τ ≤-0.442, p ≤0.001) showed decreasing trends, and DO as milligrams per liter (τ ≥0.271, p ≤0.050) showed increasing trends over time. Significant increasing trends in daily minimum and maximum values were observed for pH at the 240-WW (τ=0.348, p=0.010) and WIH (τ=0.724, p <0.001) sites and for conductivity at the MRG (τ=0.348, P=0.012) and WIH (τ=0.336, p=0.015) sites.

There were no exceedences of Pueblo of Isleta (2002) water-quality standards protective of warmwater fishery use for high water temperature (≤25.5ºC compared to 32.2 ºC), low DO concentration (≥5.7 mg/L compared to 5.0 mg/L), or pH (7.84–8.78 compared to 6.0–9.0 units). Using turbidities measured at the MRG site as the background values, there were no exceedences (turbidity >10 percent of MRG value) at the 240-WW and WIH sites based on daily average turbidities (appendix 8). However, the daily maximum turbidities exceeded the background standard on 15 days at the 240-WW site and on 7 days at the WIH site during the chronic study. Also, the grab sample collected at the WIH site on day 1 of the chronic study exceeded the background turbidity standard (530 compared to 400 NTU, 32.5 percent above background).

Further examination of the turbidity data collected by the sonde at the 240-WW site revealed a total of 184 instantaneous exceedences (about 7.5 percent of all sampling points) that occurred on 20 days. On 13 days, the exceedences were brief, occurring at only 1 to 5 (1 to 5 percent occurrence rate) of the 96 daily sampling points and the exceedence durations were ≤0.50 hour. Four days had 9–12 exceedences (7 to12 percent occurrence rate) with continuous excursion durations <1.50 hours. One day had 22 exceedences with a maximum continuous duration of 4.50 hours. There was one episode where 81 exceedences occurred within a 23-hour period (92 sampling points) that extended over 2 consecutive calendar days (October 24–25, 2007), the longest continuous

Table 3. Mean and range of daily average, minimum, maximum, and range of in-situ parameters computed from readings taken at 15-minute intervals by sondes at three sites in the Middle Rio Grande, New Mexico, during chronic cage exposures of Rio Grande silvery minnows, October 5–31, 2007.

[Sample size was 27. Means within a row sharing the same uppercase letter are not significantly (p <0.05) different. p, probability; <, less than; MRG, Middle Rio Grande; 240-WW, 240-Wasteway; WIH, Wetted Instream Habitat; °C, degrees Celsius; mg/L, milligrams per liter; %, percent; SU, standard units; µS/cm at 25°C, microsiemens per centimeter at 25 degrees Celsius; NTU, nephelometric turbidity unit]

Daily	Site (fig. 1)					
	MRG		240-WW		WIH	
	Mean	Range	Mean	Range	Mean	Range
Temperature (ºC)						
Average	14.9[C]	10.3 – 21.5	15.3[A]	12.0 – 20.4	15.1[B]	11.8 – 19.9
Minimum	10.5[B]	4.9 – 18.2	13.5[A]	10.1 – 19.3	13.2[A]	10.0 – 19.1
Maximum	20.4[A]	12.3 – 25.5	17.4[B]	13.9 – 21.6	17.2[B]	13.5 – 22.9
Range	10.0[A]	3.0 – 12.2	3.9[B]	1.6 – 5.2	4.0[B]	1.9 – 7.0
Dissolved oxygen (mg/L)						
Average	8.21[A]	6.79 – 9.08	7.84[B]	7.05 – 8.46	7.85[B]	6.91 – 8.38
Minimum	7.39[A]	6.66 – 8.15	7.36[A]	6.66 – 7.95	7.27[A]	5.66 – 7.92
Maximum	9.20[A]	7.01 – 10.8	8.48[B]	7.34 – 9.25	8.46[B]	7.06 – 9.64
Range	1.81[A]	0.35 – 3.18	1.12[B]	0.52 – 1.53	1.19[B]	0.30 – 3.92
Dissolved oxygen (% saturation)						
Average	81.0[A]	76.5 – 86.9	78.2[B]	74.3 – 79.7	78.0[B]	73.3 – 81.2
Minimum	74.1[A]	71.5 – 76.3	73.0[B]	70.5 – 75.6	72.2[A B]	52.1 – 76.4
Maximum	95.9[A]	77.7 – 117.4	86.3[B]	78.3 – 91.0	85.6[B]	75.8 – 90.9
Range	21.8[A]	1.5 – 42.5	13.3[B]	5.2 – 18.6	13.4[B]	3.6 – 35.5
pH (SU)						
Average	8.41[A]	8.32 – 8.55	8.25[C]	8.14 – 8.31	8.28[B]	7.98 – 8.43
Minimum	8.34[A]	8.25 – 8.41	8.17[B]	8.02 – 8.23	8.18[B]	7.84 – 8.36
Maximum	8.49[A]	8.34 – 8.78	8.36[C]	8.19 – 8.45	8.38[B]	8.01 – 8.54
Range	.16[B]	0.03 – 0.41	.19[A]	0.11 – 0.25	.20[A]	0.10 – 0.40
Conductivity (µS/cm at 25ºC)						
Average	476[B]	397 – 533	470[C]	445 – 502	509[A]	461 – 553
Minimum	456[B]	384 – 513	459[B]	433 – 490	492[A]	410 – 534
Maximum	493[B]	405 – 550	482[C]	456 – 516	528[A]	467 – 565
Range	38[A]	15 – 111	23[B]	5 – 61	36[A]	9 – 133
Turbidity[a b] (NTU)						
Average	92[A]	42 – 250	68[B]	25 – 220	58[B]	27 – 130
Minimum	70[A]	29 – 210	38[B]	19 – 110	39[B]	3 – 91
Maximum	270[A B]	66 – 950	370[A]	36 – 1,150	230[B]	45 – 920
Range	200[A B]	31 – 910	330[A]	15 – 1,150	190[B]	14 – 890

[a]Turbidity values were rounded following U.S. Geological Survey guidelines (Wilde, variously dated).

[b]Turbidities greater than 1,000 NTU were above the highest calibration standard for the instrument and considered to be estimates.

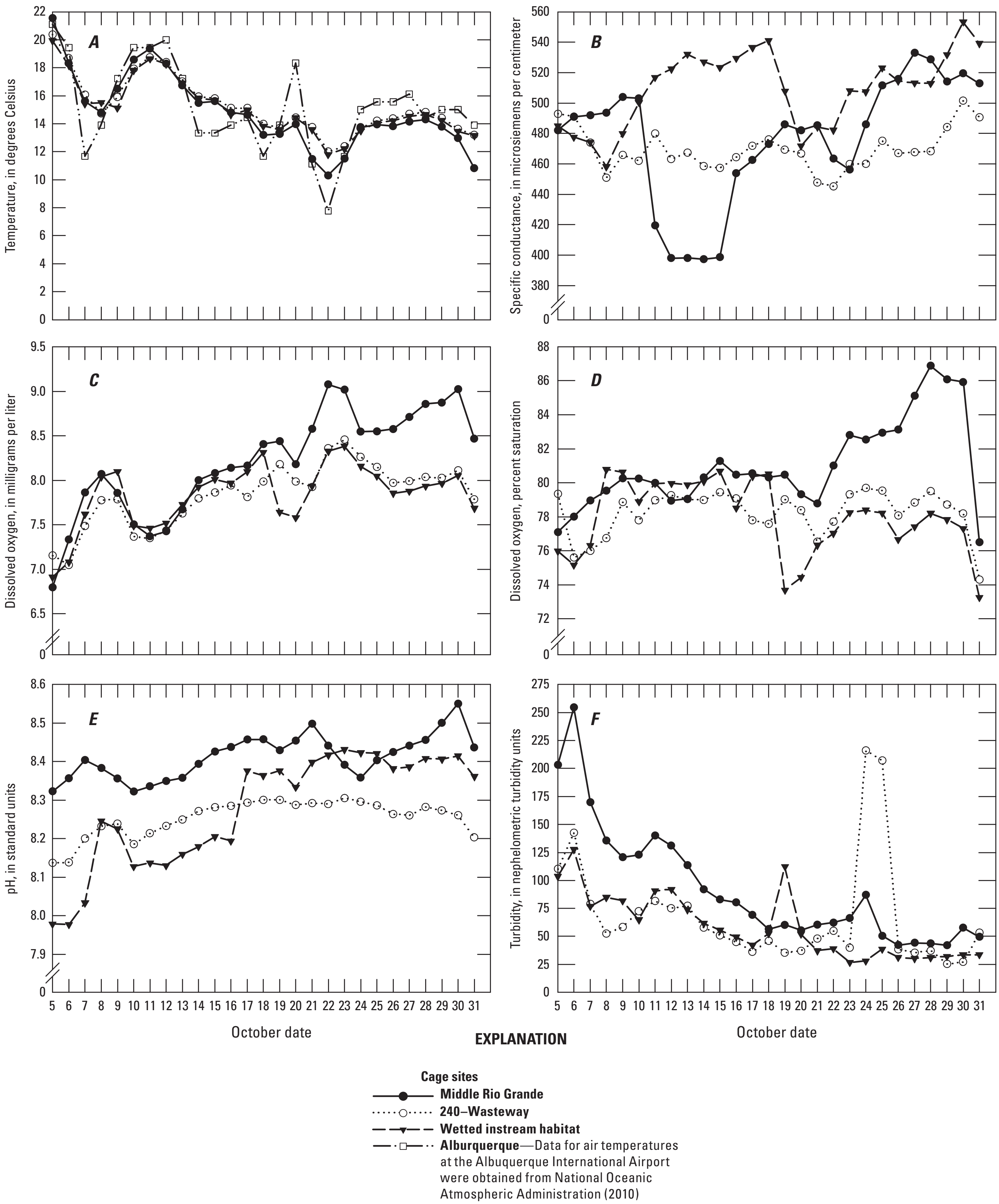


Figure 2. In-situ water quality monitored continuously during chronic cage exposures of Rio Grande silvery minnows at three sites in the Middle Rio Grande, New Mexico. The data are daily averages of *A*, Water temperature and air temperature at Albuquerque, N. Mex. as degrees Celsius; *B*, Specific conductance as microsiemens per centimeter; *C*, Dissolved oxygen as milligrams per liter; *D*, Dissolved oxygen as percent air saturation; *E*, pH as standard units; and *F*, Turbidity as nepholemetric turbidity units.

exceedence duration was 10.25 hours. At the WIH site, 128 exceedences (about 5.2 percent occurrence rate) of the background turbidity criteria were recorded on 19 days. On 16 days, the number of exceedences ranged from 1 to 7 and the excursion durations were ≤0.50 hour. During one 3-day period (October 18–20, 2007), there were 87 exceedences with continuous excursion durations of 1.00, 1.50, 3.50, 3.75, and 5.75 hours. The magnitudes of the exceedences at the 240-WW site were generally larger than those at the WIH site. The median (and interquartile range) of the exceedences were 240 percent (65.7 to 641 percent) above background (MRG site) at the 240-WW site compared to 68.7 percent (27.1 to 220 percent) above background at the WIH site. It is noteworthy that the turbidities measured at the MRG site characterized the exposure conditions for the test fish, but these values may not accurately reflect the turbidities in this reach of the MRG. The sampling occurred at one point along the shore in a low flow area compared to cross-sectional depth integrated sampling typically used in wadeable rivers (U.S. Geological Survey, 2006a). Consequently, the validity of using these site-specific turbidities as true background values is questionable.

General Water Quality and Physical Conditions

Six samples were analyzed for chlorides and sulfates at the USGS Yankton FRS and Test America Laboratories. Paired t-tests indicated no significant differences in concentrations measured between the laboratories. Consequently, the average value of the two analyses was reported for those samples. Fifteen samples were analyzed for Ca and Mg at the USGS Yankton FRS and TERL. The data were non-normal and were compared with the Wilcoxon Signed Rank test. The average difference in concentration of Ca and Mg between the two laboratories was significantly different from zero (p=0.021 for Ca, p <0.001 for Mg), and both values were reported.

Results for the major ions were evaluated by calculating anion-cation balances ([sum of cations – sum of anions] ÷ [sum of cations + sum of anions] × 100, where the ions are expressed as milliequivalents/L). Separate anion-cation balances were calculated using Ca and Mg values obtained at the USGS Yankton FRS and TERL. The anion-cation balance for the 15 samples in which sufficient water chemistry was conducted ranged from -0.63 to 2.19 percent using USGS Yankton FRS data and -0.05 to 3.71 percent using TERL data. These values were less than the attention value of ±4 percent used by the USGS National Water Quality Laboratory for flagging samples needing further quality control review (Blackburn, 1992).

General water-quality characteristics measured in samples collected in the cages and at the sondes are given in appendix 9 and summarized in table 4. Initial ANOVA testing of the water quality within a given site found no significant differences in any of the characteristics among the cages and sonde. These findings indicate that the water quality measured at the sonde was representative of that in the cages.

There were no statistical differences in any of the water-quality parameters measured at the 240-WW and the WIH sites. All of the paramenters measured at the MRG site were significantly different from those at the 240-WW and WIH sites, except for silicone (table 4). Hardness, alkalinity, the divalent ions (calcium, magnesium [measured at TERL], and sulfate), and sulfur were higher, and the monovalent ions (chloride, potassium, and sodium), suspended solids (total, fixed, and volatile), and turbidity were lower at the 240-WW and WIH sites than at the MRG site. For most parameters, differences in concentration between sites were <20 percent. Calcium was the most abundant cation followed by sodium; these elements comprised, on average, 53 percent and 31 percent of the total cations, respectively. Bicarbonate, calculated from the alkalinity, was the major anion followed by sulfate, which comprised, on average, 59 percent and 28 percent of the summed anions, respectively. No detectable total ammonia concentrations were observed during the study.

The composition of suspended sediments was similar at all sites (table 4); the average percentage of volatile and fixed solids ranged from 11.2 to 11.9 percent and 88.2 to 88.8 percent, respectively. As expected, turbidity was strongly correlated with total suspended solids at each site (Spearman's r ≥0.949, p <0.0001, n=12–23).

A significant effect of site, time, and site × time interaction was observed for depth and flow measured in the cages (table 4). The site × time interaction indicated that the differences in depth and flow among sites varied temporally. Water depth in the cages was the shallowest at the WIH site and deepest at the MRG site. The highest flows occurred at the 240-WW site and lowest flows occurred at the MRG site. The lower flows at the MRG site during the chronic study (mean, 0.6 centimeter per second [cm/sec]) compared to the acute study (mean, 1.9 cm/sec; table 2) may have resulted from a shift in the thalweg of the river channel. Daily average river flows measured at USGS gaging station 8331160 on the Rio Grande near Bosque Farms, N. Mex. (U.S. Geological Survey, 2009; about 1.5 kilometers [km] upstream from the site), during the chronic study (mean of daily averages, 1.20 cubic meters per second [m^3/s]; range, 0.76–1.56 m^3/s) were higher than those during the acute study (mean of daily averages, 0.67 m^3/s, range, 0.65–0.71 m^3/s).

To be in compliance with the Pueblo of Isleta (2002) water-quality standards, concentrations of chlorides and sulfates should not exceed background levels by more 33 percent. Using the concentrations measured at the MRG site as the background values, there were no exceedences of the criterion for chlorides, but one exceedence occurred for sulfates during the chronic study. The sulfate concentrations at the 240-WW and WIH sites on day 10 (70–71 mg/L) were 63 to 65 percent greater than that at the MRG site (43 mg/L). The high sulfate concentrations are not believed to be of significance when compared to proposed sulfate standard for the State of Iowa. The States of Illinois and Iowa are proposing the use of equation-derived sulfate standards based on hardness and chloride concentrations for the protection of aquatic life

Table 4. Water quality measured in samples collected in the cages and at the sondes and physical characteristics measured in cages during chronic cage exposures of Rio Grande silvery minnows in the Middle Rio Grande, New Mexico, October 5–31, 2007.

[Water quality data are mean±1 standard deviation and range in parenthesis and physical characteristics are the least square mean±1 standard error and range in parenthesis. Means within a row sharing the same uppercase letter are not significantly (*p* <0.05) different. ±, plus or minus; *p*, probability; <, less than; n, number of samples; MRG, Middle Rio Grande; 240-WW, 240-Wasteway; WIH, Wetted Instream Habitat; mg/L, milligrams per liter; $CaCO_3$, calcium carbonate; N, nitrogen; NTU, nephelometric turbidity unit; cm, centimeter; cm/sec, centimeter per second]

Characteristic (unit)	n	Site (fig. 1)		
		MRG	240-WW	WIH
Water quality in cages and at sondes				
Hardness (mg/L as $CaCO_3$)	12	$143^{B} \pm 5$ (138 – 156)	$168^{A} \pm 11$ (154 – 196)	$165^{A} \pm 14$ (140 – 192)
Alkalinity (mg/L as $CaCO_3$)	12	$131^{B} \pm 2$ (129 – 134)	$150^{A} \pm 9$ (140 – 174)	$148^{A} \pm 11$ (129 – 172)
Calcium, TERL[a] (mg/L)	5	$48.5^{B} \pm 1.0$ (47.6 – 50.2)	$58.2^{A} \pm 5.5$ (53.1 – 67.3)	$57.2^{A} \pm 6.5$ (48.5 – 66.8)
Calcium, YFRS[b] (mg/L)	12	$48^{B} \pm 1$ (46 – 49)	$57^{A} \pm 3$ (52 – 64)	$55^{A} \pm 5$ (47 – 64)
Chloride (mg/L)	12	$27^{A} \pm 1$ (24 – 29)	$18^{B} \pm 2$ (16 – 21)	$19^{B} \pm 3$ (16 – 26)
Magnesium, TERL[a] (mg/L)	5	$7.16^{B} \pm 0.28$ (6.89 – 7.62)	$8.18^{A} \pm 0.65$ (7.41 – 9.15)	$8.00^{A} \pm 0.75$ (6.92 – 9.00)
Magnesium, YFRS[b] (mg/L)	12	$6^{A} \pm 1$ (4 – 9)	$6^{A} \pm 1$ (4 – 9)	$6^{A} \pm 1$ (5 – 8)
Potassium (mg/L)	5	$6.47^{A} \pm 0.31$ (6.07 – 6.83)	$5.07^{B} \pm 0.25$ (4.63 – 5.26)	$5.32^{B} \pm 0.69$ (4.74 – 6.52)
Sodium (mg/L)	5	$39.1^{A} \pm 1.1$ (37.8 – 40.6)	$34.8^{B} \pm 3.1$ (31.9 – 39.9)	$35.9^{AB} \pm 3.1$ (32.6 – 39.6)
Silicone (mg/L)	5	$10.39^{A} \pm 0.32$ (9.96 – 10.80)	$10.18^{A} \pm 0.55$ (9.61 – 10.90)	$10.21^{A} \pm 0.42$ (9.63 – 10.70)
Sulfate (mg/L)	12	$58^{B} \pm 5$ (43 – 64)	$66^{A} \pm 8$ (44 – 79)	$68^{A} \pm 5$ (58 – 77)
Sulfur (mg/L)	5	$20.0^{B} \pm 0.5$ (19.5 – 20.6)	$23.2^{A} \pm 2.3$ (20.8 – 27.0)	$22.8^{A} \pm 2.4$ (20.0 – 26.5)
Ammonia, total (mg/L as N)	12	<0.1	<0.1	<0.1
Total suspended solids (mg/L)	12	$190^{A} \pm 107$ (59 – 442)	$96^{B} \pm 57$ (36 – 237)	$109^{B} \pm 83$ (38 – 340)
Fixed suspended solids (mg/L)	12	$168^{A} \pm 94$ (50 – 391)	$86^{B} \pm 52$ (31 – 216)	$97^{B} \pm 75$ (34 – 306)
Volatile suspended solids (mg/L)	12	$21.3^{A} \pm 12.5$ (7.0 – 50.6)	$10.6^{B} \pm 5.1$ (4.8 – 21.8)	$12.2^{B} \pm 8.2$ (4.0 – 34.0)
Turbidity[c] (NTU)	12	$250^{A} \pm 160$ (63 – 630)	$87^{B} \pm 66$ (30 – 270)	$120^{B} \pm 140$ (35 – 530)
Physical characteristics in cages				
Depth (cm)	81	$28^{A} \pm 0.3$ (22 – 33)	$25^{B} \pm 0.3$ (14 – 35)	$19^{C} \pm 0.3$ (8 – 30)
Flow (cm/sec)	81	$0.6^{C} \pm 0.2$ (<0.3 – 2.7)	$4.1^{A} \pm 0.2$ (1.2 – 11.3)	$2.8^{B} \pm 0.2$ (<0.3 – 8.2)

[a]Samples analyzed by Trace Element Research Lab, College Station, Texas.

[b]Samples analyzed by U.S. Geological Survey, Yankton Field Research Station, Yankton, South Dakota.

[c]Turbidity values were rounded following U.S. Geological Survey guidelines (Wilde, variously dated).

(Iowa Department of Natural Resources, 2009), because both constituents have been shown to ameliorate sulfate toxicity to aquatic invertebrates (Soucek and Kennedy, 2005). Using the appropriate equation given in Iowa Department of Natural Resources (2009) and the lowest hardness (138 mg/L as $CaCO_3$) and chloride concentration (25 mg/L) observed at the MRG site, the calculated protective criterion for sulfate is 1,300 mg/L, which is at least 16 times greater than the highest sulfate concentrations at the 240-WW (79 mg/L) and WIH (77 mg/L) sites.

Nutrients and Organic Matter

Only two samples had detectable concentrations of nitrite and these concentrations were below the reporting limit of 0.5 mg N/L (table 5). Concentrations of the different forms of N and TP were statistically higher at the MRG site than at other two sites. Nitrate was the only form of N detected in all samples and most of the detectable concentrations of nitrate, TKN, and orthophosphate at the 240-WW and WIH sites were below the reporting limit (0.5 mg/L). All total inorganic N (≤1.1 mg N/L; calculated as the sum of nitrate, nitrite, and total ammonia) concentrations were below the Pueblo of Isleta (2002) standard of 10.0 mg/L for primary contact ceremonial use.

The highest nitrite concentration of 0.34 mg N/L measured on day 1 of the study at the MRG site was at least 7 times greater than all of the other nitrite measurements (table 5), but this value was below the reporting limit. Currently there are no Pueblo of Isleta or Federal aquatic life water-quality standards or criteria, respectively, for nitrite, but all nitrite values measured in this study were below the State of Colorado (U.S. Environmental Protection Agency, 1988) standard of 0.50 mg/L as N for warmwater aquatic life use.

Most of the organic matter measured in the site waters was in the dissolved phase based on the high ratios of DOC to TOC that ranged from 0.65 to 1.18 and averaged 0.93 (n=15). All oxygen demand concentrations in the site waters were

Table 5. Concentrations of nutrients and carbons measured in water samples collected at the sondes during chronic cage exposures of Rio Grande silvery minnows in the Middle Rio Grande, New Mexico, October 5–31, 2007.

[Data are mean±1 standard deviation with range in parenthesis. Number of samples was 5. Means within a row sharing the same uppercase letter are not significantly (p <0.05) different. ±, plus or minus; p, probability; <, less than; MRG, Middle Rio Grande; 240-WW, 240-Wasteway; WIH, Wetted Instream Habitat; mg/L, milligrams per liter; N, nitrogen; P, phosphorus; nc, not calculated]

Constituent (unit)	Site (fig. 1)		
	MRG	240-WW	WIH
Nitrate (mg/L as N)	$0.95^{A} \pm 0.12$ (0.77 – 1.10)	$0.45^{B} \pm 0.04$ (0.39 – 0.49)	$0.55^{B} \pm 0.22$ (0.37 – 0.93)
Nitrite (mg/L as N)	(<0.049 – 0.340) [4][a]	<0.049 [5]	(<0.049 – 0.049) [4]
Total Kjeldahl nitrogen (mg/L)	$0.80^{A} \pm 0.13$ (0.64 – 0.98)	$0.25^{B} \pm 0.12$ (<0.25 – 0.39) [2]	nc (<0.25 – 0.80) [3]
Orthophosphate (mg/L as P)	$0.34^{A} \pm 0.15$ (<0.19 – 0.46) [1]	nc (<0.19 – 0.22) [4]	$0.18^{A} \pm 0.08$ (<0.19 – 0.25) [2]
Total phosphorus (mg/L as P)	$0.53^{A} \pm 0.16$ (0.34 – 0.70)	$0.29^{B} \pm 0.16$ (0.16 – 0.55)	$0.27^{B} \pm 0.15$ (0.15 – 0.50)
Dissolved organic carbon (mg/L)	$2.2^{A} \pm 0.3$ (2.0 – 2.6)	$2.0^{A} \pm 0.3$ (1.7 – 2.5)	$2.0^{A} \pm 0.3$ (1.8 – 2.6)
Total organic carbon (mg/L)	$2.4^{A} \pm 0.6$ (1.7 – 2.9)	$2.2^{A} \pm 0.5$ (1.7 – 2.7)	$2.3^{A} \pm 0.5$ (1.7 – 2.8)
Total inorganic carbon (mg/L)	$30^{B} \pm 1$ (28 – 31)	$35^{A} \pm 3$ (33 – 40)	$35^{A} \pm 3$[b] (32 – 39)
Biochemical oxygen demand (mg/L)	< 0.3 [5]	(< 0.3 – 1.7) [4]	< 0.3 [5]
Chemical oxygen demand (mg/L)	$7.3^{A} \pm 5.5$ (<4.1 – 14.0) [2]	nc (<4.1 – 18.0) [4]	$5.0^{A} \pm 3.3$ (<4.1 – 10.0) [2]

[a]Number of samples below the detection limit given in brackets.

[b]Number of samples was 4.

below the reporting limits (BOD, 2.0 mg/L; COD, 20 mg/L) and most were less than the laboratory detection limits. The detectable COD concentrations were about 2–7 times higher than the corresponding TOC concentration.

The BOD is used as a measure of organic pollution, and BOD values of 5 mg/L or lower have been reported for unpolluted natural waters (Missouri Department of Natural Resources, 2010). Using 5 mg/L as a criterion suggests that the site waters (BOD ≤1.7 mg/L) were not enriched with biodegradable organic matter.

Minor and Trace Elements and Minor Anions

Of the 27 target minor and trace elements, 21 were present at detectable concentrations and 15 of these were present at concentrations ≥2 times their MDL (table 6). Water at the MRG site contained significantly higher concentrations of Sb, As, B, Cd, Co, and V and lower concentrations of Li and U than waters at the 240-WW and WIH sites. No significant differences in trace element concentrations were observed between the 240-WW and WIH sites. Overlapping statistical similarities were observed for four elements (Cu, Mn, Sr, and Zn), and the mean concentrations of four other elements (Al, Ba, Mo, and Ni) were not significantly different across sites. Maximal differences in the concentration of a given element among sites were ≤2.2-fold.

Mercury, Pb, Se, Ag, and Fe were detected in ≤33 percent of the samples (table 6) and the detectable concentrations were ≤2 times the MDL (appendix 2). The data for Hg, Pb, Se, Ag, and Fe were not included in the statistical analysis because of the large number of censored values for these elements. Six elements (Be, Cr, Tl, Th, Sn, and Ti) were not detected in any of the samples.

Measured concentrations of the toxic metal and metalloid pollutants were all below the Pueblo of Isleta (2002) and State of New Mexico (New Mexico Environment Department, 2007) surface water-quality standards for protection of aquatic life (table 7). The aquatic life criterion concentrations of Cd, Cr, Cu, Pb, Ni, Ag, and Zn were adjusted to the lowest water hardness of 138 mg/L measured at the MRG site. Currently, there are no Pueblo of Isleta aquatic life criteria for Sb, Ba, Be, B, Co, Li, Mo, Tl, or V, but their criterion concentrations for agricultural water supply (livestock and irrigation) or primary contact ceremonial use are considerably higher than those measured at the cage sites. The water-quality criteria or guidelines adopted by British Columbia for total cobalt (30-d average, 4 µg/L; maximum, 110 µg/L) and total molybdenum (30-d average, 1, 000 µg/L; maximum, 2,000 µg/L) are higher than the concentrations measured at the study sites in this study.

The New Mexico Environment Department (New Mexico Environment Department, 2009) measured dissolved concentrations of 21 metals and metalloids in water collected during 8 sampling events in 2006–08 at several sites in the MRG. Two of the sites are relatively close to the cage sites; Rio Grande at the I-25 Bridge (approximately 9.3 km upstream from cage sites) and Rio Grande at Highway 6 at Los Lunas (approximately 4.5 km downstream from the cage sites). Of the 14 elements detected, the maximum concentrations of As, Ba, B, Mn, Mo, Ni, U, and V at both sites and Al and Zn at the Los Lunas site are within a factor of two of those measured at the cage sites. The New Mexico Environment Department (2009) obtained higher maximum concentrations of Cu (18-fold) at both sites; Al (10-fold), Cr (>19-fold), and Zn (6-fold) at the Interstate-25 site; and Co (8-fold) and Ag (81-fold) at the Los Lunas site compared to those observed at the cage sites. However, New Mexico Environment Department (2009) only detected Ag in one sample at 1.6 µg/L and Cr in two samples at 3.4 and 3.8 µg/L.

Samples from the 240-WW and WIH sites contained the same mean fluoride concentration, which was significantly lower than that at the MRG site (table 6). All fluoride concentrations measured at the cage sites were below the Pueblo of Isleta (2002) water quality criteria of 1,000 µg/L for irrigation and 2,000 µg/L for livestock watering usages. Two of five samples collected from the 240-WW and WIH sites contained detectable bromide concentrations, but these were below the laboratory's reporting limit of 0.20 mg/L.

Semivolatiles

Concentrations of all 18 organophosphorous pesticides (OP) and 10 chlorinated herbicides in water samples from the three sites were below detection (appendix 4). The MDL for all organophosphorous pesticides and 8 of 10 chlorinated herbicides ranged from 0.10 to 0.98 µg/L and averaged 0.39 µg/L. The highest MDL measured were for MCPA (64 µg/L) and MCPP (46 µg/L).

The New Mexico Environment Department (2009) monitored several of the same organophosphorous pesticides and chlorinated herbicides at 10 sites in the MRG, six upstream and four downstream of the cage sites. Of the seven to eight samples collected at each site in 2006–08 by the New Mexico Environment Department (2009), 2,4-D was detected in one sample from the Interstate-25 site (approximately 9.3 km upstream from the cages) at a concentration (0.297 µg/L) slightly above the sample detection limit (0.253 µg/L).

Water-Quality Synopsis

Overall, none of the water-quality parameters measured during this study, with the possible exception of turbidity, were at levels that individually would pose a hazard to the health and well being of Rio Grande silvery minnows. Based on the turbidities measured by the sondes during the chronic study (appendix 8, fig. 2) and strong correlation between suspended solids (SS) concentration and turbidity, Rio Grande silvery minnows were exposed periodically to high concentrations of SS that varied in magnitude and duration at the 240-WW and WIH sites. Applying regression models derived from turbidities and SS measured in the same grab samples, the highest

Table 6. Concentrations of minor and trace elements (micrograms per liter) and minor anions (milligrams per liter) in site waters collected at the sondes during chronic cage exposures of Rio Grande silvery minnows in the Middle Rio Grande, New Mexico, October 5–31, 2007.

[Data are mean±1 standard deviation and range in parenthesis. Number of samples was 5. Means within a row sharing the same uppercase letter are not significantly (p <0.05) different. Only ranges reported are for data sets with ≥60 percent of the values below the detection limit. Element concentrations without letters were not compared. ±, plus or minus; p, probability; <, less than; ≥, greater than or equal to; MRG, Middle Rio Grande; 240-WW, 240-Wasteway; WIH, Wetted Instream Habitat; µg/L, micrograms per liter; mg/L, milligrams per liter; nc, not calculated]

Element (symbol)	Site (fig. 1)		
	MRG	240-WW	WIH
	Minor and trace elements[a] (µg/L)		
Aluminum (Al)	$17^{A} \pm 5$ (13 – 24)	$12^{A} \pm 5$ (6 – 20)	$12^{A} \pm 4$ (7 – 19)
Antimony (Sb)	$0.19^{A} \pm 0.02$ (0.17 – 0.22)	$0.14^{B} \pm 0.02$ (0.12 – 0.16)	$0.15^{B} \pm 0.04$ (0.12 – 0.21)
Arsenic (As)	$4.5^{A} \pm 0.4$ (3.8 – 4.9)	$3.9^{B} \pm 0.3$ (3.6 – 4.3)	$3.8^{B} \pm 0.5$ (3.3 – 4.5)
Boron (B)	$103^{A} \pm 6$ (95 – 111)	$85^{B} \pm 9$ (78 – 100)	$85^{B} \pm 11$ (75 – 100)
Barium (Ba)	$78.2^{A} \pm 3.5$ (74.2 – 83.2)	$85.4^{A} \pm 5.7$ (80.1 – 95.2)	$84.2^{A} \pm 5.6$ (78.5 – 92.5)
Cadmium (Cd)	$0.03^{A} \pm 0.005$ (0.03 – 0.04)	$0.02^{B} \pm 0.004$ (<0.02 – 0.02) [1][b]	$0.02^{B} \pm 0.012$ (<0.02 – 0.04) [2]
Cobalt (Co)	$0.55^{A} \pm 0.17$ (0.43 – 0.85)	$0.33^{B} \pm 0.08$ (0.24 – 0.43)	$0.38^{B} \pm 0.13$ (0.28 – 0.54)
Copper (Cu)	$1.1^{A} \pm 0.17$ (0.9 – 1.3)	$1.0^{AB} \pm 0.09$ (0.9 – 1.1)	$0.9^{B} \pm 0.16$ (0.8 – 1.2)
Iron (Fe)	(<10 – 20) [4]	(<10 – 10) [4]	<10 [5]
Lithium (Li)	$35^{B} \pm 2$ (32 – 38)	$43^{A} \pm 5$ (40 – 51)	$40^{A} \pm 4$ (37 – 47)
Lead (Pb)	(<0.05 – 0.06) [3]	(<0.05 – 0.07) [4]	(<0.05 – 0.06) [4]
Manganese (Mn)	$9.76^{A} \pm 6.21$ (2.79 – 17.70)	$6.78^{AB} \pm 4.49$ (1.61 – 11.40)	$4.36^{B} \pm 1.83$ (2.31 – 6.70)
Molybdenum (Mo)	$5.7^{A} \pm 0.2$ (5.4 – 5.9)	$5.5^{A} \pm 0.3$ (5.2 – 6.0)	$5.6^{A} \pm 0.3$ (5.2 – 5.9)
Mercury (Hg)	(<0.005 – 0.006) [3]	(<0.005 – 0.009) [3]	(<0.005 – 0.008) [4]
Nickel (Ni)	$1.7^{A} \pm 0.1$ (1.6 – 1.8)	$1.6^{A} \pm 0.1$ (1.5 – 1.7)	$1.6^{A} \pm 0.2$ (1.4 – 1.9)
Selenium (Se)	(<0.5 – 0.6) [4]	<0.5 [5]	<0.5 [5]
Silver (Ag)	<0.01 [5]	(<0.01 – 0.02) [4]	<0.01 [5]
Strontium (Sr)	$372^{B} \pm 11$ (360 – 384)	$416^{A} \pm 32$ (389 – 466)	$411^{AB} \pm 26$ (385 – 455)
Uranium (U)	$2.06^{B} \pm 0.03$ (2.04 – 2.12)	$2.51^{A} \pm 0.29$ (2.21 – 2.98)	$2.42^{A} \pm 0.24$ (2.13 – 2.77)
Vanadium (V)	$5.0^{A} \pm 0.7$ (4.2 – 5.8)	$3.5^{B} \pm 0.5$ (3.1 – 4.1)	$3.9^{B} \pm 1.1$ (3.2 – 5.7)
Zinc (Zn)	$3.4^{A} \pm 0.3$ (3.1 – 3.8)	$1.5^{B} \pm 0.21$ (1.3 – 1.8)	$1.9^{AB} \pm 0.9$ (1.3 – 3.5)

Table 6. Concentrations of minor and trace elements (micrograms per liter) and minor anions (milligrams per liter) in site waters collected at the sondes during chronic cage exposures of Rio Grande silvery minnows in the Middle Rio Grande, New Mexxico, October 5–31, 2007.—Continued

[Data are mean±1 standard deviation and range in parenthesis. Number of samples was 5. Means within a row sharing the same uppercase letter are not significantly (*p* <0.05) different. Only ranges reported are for data sets with ≥60 percent of the values below the detection limit. Element concentrations without letters were not compared. ±, plus or minus; *p*, probability; <, less than; ≥, greater than or equal to; MRG, Middle Rio Grande; 240-WW, 240-Wasteway; WIH, Wetted Instream Habitat; µg/L, micrograms per liter; mg/L, milligrams per liter; nc, not calculated]

Element (symbol)	Site (fig. 1)		
	MRG	240-WW	WIH
Minor anions (mg/L)			
Bromide (Br)	0.16 ± 0.14 (<0.11 – 0.41) [1]	nc (<0.11 – 0.13) [3]	nc (<0.11 – 0.13) [3]
Fluoride (F)	$0.52^{A} \pm 0.13$ (0.43 – 0.75)	$0.43^{B} \pm 0.02$ (0.42 – 0.46)	$0.43^{B} \pm 0.04$ (0.38 – 0.47)

[a]All concentrations of beryllium (Be, <0.05 µg/L); chromium (Cr, <0.2 µg/L); thallium (Tl, <0.01 µg/L); thorium (Th, <0.05 µg/L); tin (Sn, <0.1 µg/L); and titanium (Ti, <5 µg/L) were below the method detection limit.

[b]Number of samples below the detection limit given in brackets.

predicted SS concentrations were 1,296 and 916 mg/L at the 240-WW and WIH sites, respectively. These SS concentrations are much lower than the acutely toxic concentrations of river sediment (96-h median lethal concentrations, 8,000–31,000 mg/L) reported for juvenile Pacific salmonids (*Oncorhynchus kisutch*, Servizi and Martens, 1991; *O. nerka*, Servizi and Martens, 1987; *O. tshawytscha*, Servizi and Gordon, 1990). Considering that Rio Grande silvery minnows have evolved in naturally turbid waters, there is little doubt that silvery minnows are more tolerant than salmonids to SS. However, it is not known what sublethal effects, if any, may occur in Rio Grande silvery minnows as a result of exposures to these increased SS concentrations. Horkel and Pearson (1976) observed increased, but not sustained, ventilation rates in green sunfish (*Lepomis cyanellus*) exposed to clay suspensions with turbidities of 800–1,800 and 1,500–2,500 Formazin Turbidity Units (nearly equivalent to NTU; U.S. Geological Survey, 2006b). The increased ventilation rate observed in the fish may be a means of compensating for reduced respiratory efficiency (Horkel and Pearson, 1976). Neumann and others (1982) also observed respiratory effects in fish exposed to sublethal concentrations of suspended sediments. In the study by Neumann and others (1982), oxygen consumption was reduced in striped bass (*Morone saxatilis*) during swimming trials in waters containing 790 mg/L of fuller's earth or 1,320 mg/L of natural sediment.

Increased ventilation rates in fish exposed to turbid water increases the amount of water and particles contacting the gills, which increases the chance of clogging and abrading the gills with particles that may damage gill tissue and result in increased susceptibility to disease or other stressors. Also, increased ventilation has a metabolic cost that may divert energy from other functions or may be compensated for by a reduction in activity (Horkel and Pearson, 1976; Neumann and others, 1982).

Exposure Endpoints

Survival And Growth

After 26 days of exposure, no significant differences in survival of caged fish were observed among the three sites (table 8). However, survival was highly variable within each site and one cage at the 240-WW and WIH sites had <50 percent survival after 26 days. No significant differences in TL, weight, or condition factor of fish were observed among the sites at the beginning or end of the chronic study. Comparisons of the growth metrics (based on cage averages) at the beginning and end of the study showed that body weight and condition factor of caged fish at all three sites were significantly reduced after 26 days of exposure. Absolute weight loss (expressed as the difference between initial and final weights) and relative weight loss (expressed as a percentage of initial weight) in fish were the highest at the WIH site (0.400 g, 15.6 percent), intermediate at the 240-WW site (0.343 g, 13.1 percent), and lowest at the MRG site (0.276 g, 11.0 percent). Differences in absolute weight loss were statistically significant (p=0.032), and those for relative weigh loss were close to being significant (p=0.053). The percent decrease in condition factors was statistically larger for fish exposed at the WIH than those at the MRG site.

Health Assessment

Of the 30 pre-exposed fish examined for overall health, 21 (70.0 percent) were females and 9 (30.0 percent) were males. The Wilcoxon rank-sum testing indicated that the HAI did not differ statistically (p=0.628) between sexes. A spleen was not found in one female and the HAI for this fish was not included in the analyses. The HAI averaged 22.4 and ranged

Table 7. Comparison of dissolved concentrations (micrograms per liter) of toxic metals/metalloids at cage sites in the Middle Rio Grande, New Mexico, with Pueblo of Isleta and State of New Mexico surface water-quality standards specific to designated uses.

[Pueblo of Isleta (2002). State of New Mexico (New Mexico Environment Department, 2007) standards for surface waters are given in brackets if different from those of the Pueblo of Isleta. Number of samples for this study was 15; ns, no standard for the designated use; <, less than; mg/L, milligrams per liter; $CaCO_3$, calcium carbonate]

Element	This study		Designated use				
			Aquatic life		Agriculture water supply		Primary contact/ ceremonial
	Geometric mean	Maximum	Chronic	Acute	Livestock	Irrigation	
Aluminum	13	24	87	750	5,000	5,000	ns
Arsenic	4.0	4.9	150	340	200	[100]	ns
Antimony	.16	.22	ns	ns	ns	ns	6
Barium	82.4	95.2	ns	ns	ns	ns	2,000
Beryllium	<.05	<.05	[5.3]	[130]	ns	ns	4
Boron	90	111	ns	ns	5,000	750	ns
Cadmium[a]	.02	.04	.32 [.31]	2.88 [2.75]	50	[10]	5
Chromium[a]	<.2	<.2	96	742	1,000	[100]	100
Cobalt	.40	.85	4[b]	110[b]	1,000	50	ns
Copper[a]	1.0	1.3	11.8	18.2	500	[200]	ns
Iron	<10	20	1,000	ns	ns	ns	ns
Lithium	39	51	ns	ns	ns	2,500	ns
Lead[a]	<.05	.07	3.57	91.54	[100]	[5,000]	ns
Mercury	<.005	.009	.012[c] [.77]	2.4[c] [1.4]	10[c]	ns	2[c]
Molybdenum	5.6	6.0	1,000[d]	2,000[d]	ns	10 -1,000	ns
Nickel[a]	1.6	1.9	68.3	615	ns	ns	ns
Selenium	<.5	.6	5[e]	20[e]	50[e] [50]	ns	50[e]
Silver[a]	<.01	.02	ns	6	ns	ns	ns
Thallium	<.05	<.05	ns	ns	ns	ns	2
Vanadium	4.0	5.8	ns	ns	100	100	ns
Zinc[a]	2.1	3.8	155	154	[25,000]	[2,000]	ns

[a]Aquatic life criterion concentration adjusted to a hardness of 138 mg/L as $CaCO_3$.

[b]British Columbia ambient water quality guideline for cobalt based on total concentration (Nagpal, 2004).

[c]Total concentration.

[d]British Columbia water quality criteria for molybdenum based on total concentration (British Columbia Ministry of Environment, 1986).

[e]Total recoverable concentration.

Table 8. Survival and growth metrics of Rio Grande silvery minnows exposed in cages for 26 days at three sites in the Middle Rio Grande, New Mexico.

[Data are the mean±1 standard error with range in parenthesis of cage-average values. Number of samples was 3. Means within a row sharing the same upper-case letter are not significantly (p <0.05) different. ±, plus or minus; p, probability; <, less than; MRG, Middle Rio Grande; 240-WW, 240-Wasteway; WIH, Wetted Instream Habitat; %, percent; mm, millimeters; g, grams]

Metric (unit)	Index	Site (fig. 1)		
		MRG	240-WW	WIH
Survival (%)	Final	$78.7^{A} \pm 7.3$ (44.0 – 96.0)	$76.0^{A} \pm 14.0$ (48.0 – 92.0)	$73.3^{A} \pm 3.5$ (68.0 – 80.0)
Total length (mm)	Initial	$67.4^{A} \pm 0.2$ (67.1 – 67.8)	$68.1^{A} \pm 0.2$ (67.7 – 68.3)	$67.8^{A} \pm 0.2$ (67.5 – 68.2)
	Final	$68.0^{A} \pm 0.3$ (67.4 – 68.4)	$68.4^{A} \pm 0.4$ (67.5 – 68.9)	$67.8^{A} \pm 0.4$ (67.1 – 68.4)
	Absolute[a]	$0.5^{A} \pm 0.2$ (0.3 – 1.0)	$0.3^{A} \pm 0.2$ (-0.2 – -0.6)	$0^{A} \pm 0.2$ (-0.4 – 0.2)
	Relative[b] (%)	$0.8^{A} \pm 0.3$ (0.4 – 1.5)	$0.6^{A} \pm 0.2$ (0.3 – 0.9)	$0.4^{A} \pm 0.1$ (0.3 – 0.6)
Weight (g)	Initial	$2.523^{A} \pm 0.016$ (2.492 – 2.543)	$2.630^{A} \pm 0.036$ (2.559 – 2.678)	$2.563^{A} \pm 0.048$ (2.474 – 2.638)
	Final	$2.247^{A} \pm 0.047$ (2.181 – 2.338)	$2.287^{A} \pm 0.057$ (2.174 – 2.346)	$2.163^{A} \pm 0.047$ (2.075 – 2.237)
	Absolute[a]	$-0.276^{A} \pm 0.036$ (-0.313 – -0.205)	$-0.343^{AB} \pm 0.022$ (-0.385 – -0.308)	$-0.400^{B} \pm 0.001$ (-0.401 – -0.399)
	Relative[b] (%)	$-11.0^{A} \pm 1.5$ (-12.5 – -8.1)	$-13.1^{A} \pm 1.0$ (-15.0 – -11.6)	$-15.6^{A} \pm 0.3$ (-16.1 – -15.2)
Condition factor[c]	Initial	$0.818^{A} \pm 0.005$ (0.808 – 0.823)	$0.828^{A} \pm 0.007$ (0.816 – 0.840)	$0.818^{A} \pm 0.009$ (0.801 – 0.831)
	Final	$0.711^{A} \pm 0.009$ (0.696 – 0.728)	$0.710^{A} \pm 0.007$ (0.698 – 0.722)	$0.690^{A} \pm 0.004$ (0.683 – 0.697)
	Relative[b] (%)	$-13.0^{A} \pm 0.7$ (-13.9 – -11.5)	$-14.2^{AB} \pm 0.1$ (-14.5 – -14.0)	$-15.6^{B} \pm 0.4$ (-16.1– -14.7)

[a]Absolute = final value-initial value.

[b]Relative = (final value-initial value)/initial value x 100.

[c]Fulton-type condition factor, weight (g)/total length^3 (mm) x 100,000.

from 0 to 70 (table 9). Of the 29 fish scored, only 1 (3.4 percent) fish was judged to be normal (HAI=0), 15 (51.7 percent) fish had one abnormality (HAI=10), 9 (31.0 percent) fish had two abnormalities (HAI=20–40), and 4 (13.8 percent) fish had three abnormalities (HAI=50–70). Shortening of the opercules was the most common abnormality and was observed in 27 of 30 fish (90.0 percent). The severity of shortening was rated as slight in 25 (92.6 percent) of the 27 affected fish, and the anomaly was predominately bilateral (88.9 percent). In the two fish where shortened opercula were rated as severe, this condition was unilateral and the other opercle was rated as slightly shortened. These two fish also received the highest HAI scores (70). No abnormal conditions were observed for eyes, head and body, gills (despite the predominance of shortened opercules), or spleen and only one fish had an abnormal liver.

The sex was identified in 40 of the 45 (88.9 percent) cage-exposed fish examined and in contrast to the pre-exposed fish, there were more males (25 fish, 62.5 percent) than females (15 fish, 37.5 percent). Preliminary statistical analyses indicated that HAI for these fish did not differ significantly between sexes (p=0.670) and the data were pooled for comparisons among sites. There were no significant differences (p=0.971) in cage-average HAI between sites, and the mean HAI at each site ranged from 46.7 to 49.3 (table 9). Of the 45 fish examined (15 fish/site), none were rated as normal (HAI=0) or as having only one abnormality, 17 (37.8 percent) fish had two abnormalities (HAI=20–40), 20 (44.4 percent) fish had three abnormalities (HAI=30 for 1 fish and HAI=50–70 for 19 fish), 7 (15.6 percent) fish had four abnormalities (HAI=80–100), and 1 (2.2 percent) fish had seven abnormalities and received a HAI of 150.

The prevalence of abnormalities (percentage of fish in which an abnormal condition was observed) in a given tissue or organ of cage-exposed fish did not differ significantly

Table 9. Health assessment condition of organs and tissues of Rio Grande silvery minnows exposed in cages for 26 days at three sites in the Middle Rio Grande, New Mexico, and pre-exposed fish.

[Indices are the mean±1 standard deviation (range in parenthesis) and prevalence of abnormality values are percentages with number affected in brackets. Data for cage-exposed fish were pooled for comparison with pre-exposed fish; index values were compared by Wilcoxon rank sum test and prevalence values by Fisher's exact test. ±, plus or minus; MRG, Middle Rio Grande; 240-WW, 240-Wasteway; WIH, Wetted Instream Habitat; n, number of samples; % percent; *p*, probability; <, less than]

Condition or feature measured	Site (fig. 1)				Pre-exposed fish (n=30)
	MRG (n=15)	240-WW (n=15)	WIH (n=15)	Pooled (n=45)	
Health assessment index	46.7 ± 22.3 (20 – 80)	47.3 ± 23.1 (20 – 100)	49.3 ± 36.3 (20 – 150)	47.8 ± 27.4 (20 – 150)	22.4[a] ± 19.0[b] (0 – 70)
Mesenteric fat index	1.3 ± 0.8 (0 – 3.0)	1.9 ± 1.4 (0 – 4.0)	1.0 ± 1.0 (0 – 3.0)	1.4 ± 1.2 (0 – 4.0)	2.8[a] ± 1.2 (0 – 4.0)
Bile color-fullness index[c]	0.5 ± 0.5 (0 – 1.0)	0.6 ± 0.7 (0 – 2.0)	0.6 ± 0.7 (0 – 2.0)	0.5 ± 0.6 (0 – 2.0)	0.8 ± 0.6 (0 – 2.0)
Prevalence of abnormality (%)					
Body surface (parasites)	26.7 [4]	33.3 [5]	40.0 [6]	33.3 [15]	0[a] [0]
Fins	93.3 [14]	86.7 [13]	100 [15]	93.3 [42]	36.7[a] [11]
Eyes	6.7 [1]	6.7 [1]	13.3 [2]	8.9 [4]	0 [0]
Opercles	100 [15]	86.7 [13]	100 [15]	95.6 [43]	90.0 [27]
Gills	0 [0]	0 [0]	13.3 [2]	4.4 [2]	0 [0]
Liver	6.7 [1]	20.0 [3]	20.0 [3]	15.6 [7]	3.3 [1]
Spleen	46.7 [7]	40.0 [6]	20.0 [3]	35.6 [16]	24.1 [7][b]
Kidney	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]

[a]Mean value for pre-exposed fish is significantly (p <0.05) different from the pooled mean value for cage-exposed fish.

[b]Number of samples was 29.

[c]Number of samples were 14 for MRG; 9 for 240-WW; 10 for WIH; 33 for pooled; and 27 for pre-exposed fish.

between sites (2 x 3 contingency tables using Fisher's exact test, $p \geq 0.318$), and these data were combined for comparison with those for the pre-exposed fish (table 9). The most common abnormalities observed were shortened opercula (95.6 percent of fish, all rated as slightly shortened) and fin anomalies (93.3 percent of fish) that were primarily mild erosion of the caudal fin and deformed rays on the pectoral and pelvic fins. Shortened opercula were primarily bilateral (83.7 percent of affected fish), and it is noteworthy that in all cases where this anomaly was unilateral, it was only observed on the right opercle. Despite the high incidence of shortened opercules, gill anomalies (pale color) were only observed in two fish. Parasites, mainly *Lernea* sp., were observed in 15 (33.3 percent) fish, and the numbers of infected fish were similar across sites; 4 fish at MRG, 5 fish at 240-WW, and 6 fish at WIH. Parasites were found in one to three fish from each cage, except for cage 1 at the 240-WW site where no parasites were observed. Among the three internal variables, anomalies in the spleen (mainly nodules) and liver (mainly pale in color) were observed in 16 (35.6 percent) and 7 (15.6 percent) fish, respectively, and no abnormal changes were observed in the kidneys.

Comparison of the mean HAI for exposed fish (47.8) to that of pre-exposed fish (22.4) indicated that health of the fish deteriorated during the 26-day in-situ exposures (table 9). The statistically higher HAI scores (p <0.0001) for exposed fish compared to those for pre-exposed fish were attributable primarily to higher proportions of fish with abnormal fins (93.3 percent compared to 36.7 percent) and to the presence of parasites (33.3 percent compared to 0 percent). Fin damage in cage-exposed fish was likely a caging artifact attributable to abrasion associated with physical contact with the interior surfaces of cages. Cage-exposed fish had a higher prevalence of abnormal livers (15.6 percent) and spleens (35.6 percent) compared to those in pre-exposed fish (3.3 percent and 24.1 percent, respectively), but the differences were not statistically significant.

Papoulias and others (2009) and Davis (2010) conducted a fish health assessment on wild-caught Rio Grande silvery minnows collected seasonally (July, October, January, and April) in 2006–07 and 2007–08 at six sites in the MRG using the same examination procedure and scoring system as in this study. The mean HAI scores of 53 for fish collected in 2006–07 and 65 for fish collected in 2007–08 reported by Papoulias and others (2009) and Davis (2010) are higher than the mean score of 48 for caged fish in this study. Papoulias and others (2009) and Davis (2010) also reported that the HAI for Rio Grande silvery minnows varied significantly between years, seasons, and location with the lowest HAI occurring for fish collected in October 2006 and for those collected at the two furthest upstream sites above the city of Albuquerque. At the two sites closest to the cage sites in this study, the estimated mean (across seasons) HAI scores, based on visual interpretation of the graphs in Davis (2010), are about 60 for Rio Grande silvery minnows collected at the Los Lunas site (about 4.6 km downstream from the cage sites) in both years and about 56 in year 1 and 75 in year 2 at the Los Padillas site (about 14.1 km upstream from the cage sites).

The high prevalence of shortened opercula observed in the fish (pre-exposed and cage-exposed) in this study (90 to 96 percent) was very similar to that observed in captive-reared Rio Grande silvery minnows at three propagation facilities. During general fish health inspections, shortened opercula were found in 98 percent of fish at DNFH&TC in March 2007, 88 percent of fish at New Mexico State University, A-Mountain Aquatic Research Facility, Las Cruces, N. Mex., in March 2007, and 98 percent of fish at the BioPark in March 2010 (Catherine Sykes, U.S. Fish and Wildlife Service, Dexter, N. Mex., written commun.). It is noteworthy that the same fish biologist performed all four fish health assessments mentioned above.

In contrast to the captive-reared Rio Grande silvery minnows, Papoulias and others (2009) and Davis (2010) observed considerable temporal variation in the prevalence of shortened opercules in wild-caught fish, ranging from 38 percent in fish collected in January 2007 to 98 percent in fish collected in January 2008. Also, the frequency of bilaterally shortened opercules varied temporally and averaged 83.2 percent in year 1 and only 40.9 percent in year 2 of their investigation.

Abnormalities of the opercular complex have been observed in other fish species in the wild and under culture (Koumoundouros and others, 1997; Beraldo and others, 2003). The exact significance and causes of opercular abnormalities in Rio Grande silvery minnows and in other fishes are not known. Possible causative factors, either individually or in combination, include vitamin C or other nutritional deficiencies, contaminant exposures, turbulence, and genetics (Papoulias and others, 2009; Davies, 2010). Studies on opercular deformities suggest these defects were induced during early developmental (embryonic or larval) stages (Koumoundouros and others, 1997) and the defect was not inherited (Tave and Handwerker, 1994; Handwerker and Tave, 1994; Beraldo and others, 2003).

The prevalence of the type of abnormalities observed in cage-exposed fish differed from those reported in wild-caught Rio Grande silvery minnows by Papoulias and others (2009) and Davis (2010). In comparison with wild-caught fish, cage-exposed fish had a lower prevalence of abnormal gills (4 percent compared to 35 to 50 percent), livers (16 percent compared to 45 to 59 percent), and kidneys (0 percent compared to 16 to 24 percent) and a higher prevalence of external anomalies, primarily the fins (96 percent [93 percent for fins] compared to 13 to 25 percent), and abnormal spleens (36 percent compared to 11 to 24 percent).

The extent of mesenteric fat deposits was evaluated on an index of 0 (no fat deposits) to 4 (abdominal organs completely covered). For the pre-exposed fish, the Wilcoxon rank-sum test provided moderate evidence that the fat index differed (p=0.0501) between sexes. Average fat indices were 2.5 for females and 3.4 for males, and the arithmetic average for both sexes was 2.8. The level of fat coverage was ≥ 50 percent in 88.9 percent of males and 47.6 percent of females. The lower fat content in females compared to males may be partly attributable to their post-spawning condition. Only one fish (female) received an index of 0 (no fat).

Preliminary statistical analysis indicated that MFI in cage-exposed fish did not differ between sexes (Wilcoxon test, p=0.369), and these data were combined for comparisons among sites (table 9). Differences in mean MFI across sites were close to being statistically significant (p=0.052); mean MFI were the highest in fish at the 240-WW site (1.9; cage averages, 1.8–2.0), intermediate in fish at the MRG site (1.3; cage-averages, 0.8–1.6), and lowest in fish at the WIH site (1.0; cage-averages, 1.0). No fat deposits were observed in 10 (22.2 percent) fish, 17 (37.8 percent) fish received a rating of 1 (<50 percent coverage), 10 fish (22.2 percent) received a rating of 2 (50 percent coverage), and 8 (17.8 percent) fish received a rating of 3 or 4 (>50 percent or completely covered). The MFI was significantly correlated (n=45) with weight (Spearman's r=0.340, p=0.022) and condition factor (Spearman's r=0.390, p=0.008). The pooled mean MFI of 1.4 for the three sites was one-half that for the pre-exposed fish.

A gall bladder was observed in 27 of the 30 pre-exposed fish and the BCFI averaged 0.8 with a range of 0 to 2.0 and did not differ between sexes (p=0.381) (table 9). A relatively high proportion (88.9 percent) of the fish had an index rating of 0 or 1. The color and fullness of the bile in the gall bladder is a good short-term indicator of feeding activity in fish (Love, 1980). Index ratings of 0 and 1 are indications that the fish has fed within the previous couple of days, and a rating of 2 indicates feeding within the previous week (Goede and Barton, 1990).

A gall bladder was found in 33 of 45 (73.3 percent) of the cage-exposed fish and the BCFI did not differ between sexes (p=0.252) nor among sites (p=0.989). The pooled arithmetic average BCFI for all cage-exposed fish of 0.5 (table 9) was similar to that for pre-exposed fish (0.8). Nearly all of the gall bladders (93.9 percent) observed received a rating of 0 or 1, which is indicative of recent feeding activity by these fish (Goede and Barton, 1990).

Histopathology

Tissues collected from the five pre-exposure fish were judged to be normal, but numerous macrophage aggregates (MA) were present in all spleen and kidney samples examined. Macrophage aggregates (also referred to as melanomacrophage centres) are discrete, encapsulated foci that are found near blood vessels and serve as repositories for end products of cell breakdown. In advanced boney fishes, MA are the most abundant in the spleen and kidney (haemopoitic organs) and also are found in the liver, gonads, thyroid, and thymus (Ferguson, 2006). The occurrence of MA may depend on the organ, age, nutritional state, and health of the fish (Haaparanta and others, 1996; Agius and Roberts, 2003). Fish that are chronically stressed, in poor health, or have nutritional deficiencies tend to have more and larger MA (Agius, 1980; Agius and Roberts, 1981, 2003; Ferguson, 2006). Wolke and others (1985) suggested using MA as a potential biomarker of chronic exposure to environmental pollution. Atretic ova were observed in ovaries of all three pre-exposed females examined. Mild gill lesions were observed in one female and an encysted parasite was seen in the lower jaw of another female.

The most common finding of the histopathological examination of tissue samples from the cage-exposed fish was the presence of MA. No gender differences were found for MA prevalence in the somatic tissues examined, and these data were pooled for additional analyses. Prevalence of MA in these tissues did not differ statistically among sites, and these data were combined for comparisons with those for the pre-exposed fish (table 10). A high prevalence of MA was observed in the ovaries (91.7 percent), spleen (91.3 percent), and kidney (74.1 percent) and a lower prevalence was found in the testes (20.0 percent) and liver (14.8 percent). Prevalence of MA in somatic tissues of cage-exposed fish was not statistically different from those of pre-exposed fish.

A qualitative assessment of the severity of MA infiltration (relative numbers and size) in the tissues examined was scored on a scale of 1 (minimal) to 6 (very severe). The MA scores in the kidney, spleen, and ovaries of cage-exposed fish did not differ statistically among sites and averaged (pooled data) 2.2, 3.2, and 3.1, respectively (table 10). In cage-exposed fish, the combined MA score for the kidney was lower, and those for the spleen and ovaries were similar to their respective MA scores in pre-exposed fish. The low prevalence of MA in the liver and testes precluded any statistical comparisons. The average MA score in ovaries (3.1) of cage-exposed fish was three times higher than that in testes (1.0). In general, the tissues in which MA were present did not show degenerative changes, and thus were not considered to be a significant finding.

Atretic ova were observed in females from all cages at each site. Mild gill lesions (hypertrophy) were seen in four fish exposed at the WIH site and one fish at the MRG site. Very few parasites (trichodina in gills of one fish at MRG and two fish at WIH) were observed in the tissues. Overall, most of the tissues were judged to be normal. It is of interest to note the low occurrence and severity of gill lesions in cage-exposed fish despite the high prevalence of shortened opercules (87 to 100 percent) and periodic exposures to high turbidities.

Whole-Body Elements

Of the 34 target elements measured in whole-body samples of cage-exposed fish (table 11), 4 (Sb, Be, Li, and Sn) were below the MDL in all samples, and 5 (B, Mo, Ag, Tl, and Th) were detected in only one or two samples. These elements were not compared statistically. Detectable concentrations of Cd and Cr were ≤2 times their MDL, and those of V were ≤3 times the MDL. Individual ANOVA tests found no significant

Table 10. Prevalence and severity of macrophage aggregates in tissues of Rio Grande silvery minnows exposed in cages for 26 days at three sites in the Middle Rio Grande, New Mexico, and pre-exposed fish.

[Prevalence values are percentages with number affected/number examined in brackets and scores are the mean with the range in parenthesis. Data for cage-exposed fish were pooled for comparison with pre-exposed fish; prevalence values were compared by Fisher's exact test and scores by Wilcoxon rank sum test. MA, macrophage aggregates; MRG, Middle Rio Grande; 240-WW, 240-Wasteway; WIH, Wetted Instream Habitat; ns, not scored; *p*, probability; <, less than]

Tissue	MA	Site (fig. 1)				Pre-exposed fish
		MRG	240-WW	WIH	Pooled	
Kidney	Prevalence	88.9 [8/9]	44.4 [4/9]	88.9 [8/9]	74.1 [20/27]	100 [5/5]
	Score	1.9 (1–3)	2.2 (1–4)	2.4 (1–4)	2.2 (1–4)	3.0[a] (2–4)
Liver	Prevalence	22.2 [2/9]	22.2 [2/9]	0 [0/9]	14.8 [4/27]	0 [0/5]
	Score	1.0 (1–1)	2.0 (2–2)	ns	1.5 (1–2)	ns
Spleen	Prevalence	100 [8/8]	77.8 [7/9]	100 [6/6]	91.3 [21/23]	100 [5/5]
	Score	2.8 (1–4)	3.3 (2–4)	3.7 (3–5)	3.2 (1–5)	3.2 (1–6)
Ovary	Prevalence	100 [3/3]	66.7 [2/3]	100 [6/6]	91.7 [11/12]	100 [3/3]
	Score	2.7 (2–4)	3.5 (3–4)	3.2 (1–4)	3.1 (1–4)	2.0 (2–2)
Testes	Prevalence	33.3 [2/6]	16.7 [1/6]	0 [0/3]	20.0 [3/15]	0 [0/2]
	Score	1.0 (1–1)	1.0 (1–1)	ns	1.0 (1–1)	ns

[a]Mean value for pre-exposed fish was significantly (p <0.05) different from the pooled mean value for cage-exposed fish.

Table 11. Whole-body concentrations of elements in Rio Grande silvery minnows exposed in cages for 26 days at three sites in the Middle Rio Grande, New Mexico, and pre-exposed fish.

[Data for cage-exposed fish are the mean±1 standard deviation with the range in parenthesis of 3 samples and data for pre-exposed fish are the mean and range in parenthesis of 2 composite samples. There were no significant differences in the mean concentrations of a given element in cage-exposed fish among sites. ±, plus or minus; MRG, Middle Rio Grande; 240-WW, 240-Wasteway; WIH, Wetted Instream Habitat; mg/g, milligram per gram; wt, weight; µg/g, microgram per gram; <, less than; %, percent]

Element (Symbol, unit)	Unit basis	Site (fig. 1)			Pre-exposed fish
		MRG	240-WW	WIH	
		Major			
Calcium (Ca, mg/g)	Dry wt	41.1 ± 17.6 (20.8 – 51.3)	51.5 ± 3.4 (49.0 – 55.3)	52.2 ± 2.3 (50.1 – 54.7)	47.2 (37.6 – 56.8)
	Wet wt	10.9 ± 4.2 (6.0 – 13.5)	13.5 ± 0.5 (13.0 – 14.1)	14.0 ± 1.2 (12.8 – 15.2)	13.8 (8.1 – 19.6)
Magnesium (Mg, mg/g)	Dry wt	1.10 ± 0.47 (0.55 – 1.38)	1.35 ± 0.07 (1.29 – 1.42)	1.36 ± 0.08 (1.30 – 1.45)	1.22 (1.03 – 1.40)
	Wet wt	0.29 ± 0.11 (0.16 – 0.36)	0.35 ± 0.01 (0.34 – 0.37)	0.36 ± 0.01 (0.35 – 0.37)	0.35 (0.22 – 0.48)
Potassium (K, mg/g)	Dry wt	7.94 ± 3.61 (3.77 – 10.10)	10.02 ± 0.75 (9.15 – 10.50)	9.99 ± 0.61 (9.59 – 10.70)	9.00 (7.99 – 10.00)
	Wet wt	2.10 ± 0.88 (1.09 – 2.62)	2.62 ± 0.08 (2.54 – 2.70)	2.68 ± 0.06 (2.62 – 2.73)	2.58 (1.72 – 3.45)
Phosphorus (P, mg/g)	Dry wt	19.7 ± 8.1 (10.4 – 24.6)	24.2 ± 1.3 (23.1 – 25.7)	24.5 ± 0.9 (23.9 – 25.5)	22.4 (18.7 – 26.0)
	Wet wt	5.2 ± 1.9 (3.0 – 6.4)	6.4 ± 0.2 (6.2 – 6.6)	6.6 ± 0.5 (6.2 – 7.1)	6.5 (4.0 – 9.0)
Sodium (Na, mg/g)	Dry wt	2.97 ± 1.41 (1.37 – 4.04)	3.54 ± 0.34 (3.22 – 3.89)	3.50 ± 0.09 (3.42 – 3.60)	3.72 (2.71 – 4.72)
	Wet wt	0.79 ± 0.35 (0.40 – 1.06)	0.93 ± 0.02 (0.91 – 0.94)	0.94 ± 0.03 (0.92 – 0.97)	1.11 (0.58 – 1.63)
Sulfur (S, mg/g)	Dry wt	5.00 ± 2.27 (2.38 – 6.40)	5.99 ± 0.49 (5.43 – 6.31)	5.93 ± 0.34 (5.69 – 6.32)	5.50 (4.93 – 6.06)
	Wet wt	1.32 ± 0.55 (0.69 – 1.64)	1.57 ± 0.05 (1.53 – 1.62)	1.59 ± 0.03 (1.55 – 1.61)	1.58 (1.06 – 2.09)
		Minor and trace			
Aluminum (Al, µg/g)	Dry wt	56.1 ± 27.0 (37.6 – 87.1)	89.6 ± 55.7 (32.6 – 144.0)	114.0 ± 96.0 (18.0 – 210.0)	4.52 (3.14 – 5.89)
	Wet wt	15.1 ± 7.0 (9.7 – 22.9)	24.0 ± 16.5 (8.5 – 41.3)	29.9 ± 24.3 (5.0 – 53.6)	1.35 (0.68 – 2.03)
Antimony (Sb, µg/g)	Dry wt	<0.046 [3][a]	<0.047 [3]	<0.047 [3]	<0.046 [2]
	Wet wt	<0.0130 [3]	<0.0125 [3]	<0.0131 [3]	<0.0156 [2]
Arsenic (As, µg/g)	Dry wt	2.17 ± 0.67 (1.40 – 2.62)	2.13 ± 0.29 (1.81 – 2.39)	2.53 ± 0.63 (1.83 – 3.04)	1.68 (1.65 – 1.71)
	Wet wt	0.58 ± 0.15 (0.40 – 0.69)	0.56 ± 0.08 (0.47 – 0.63)	0.68 ± 0.16 (0.51 – 0.83)	0.47 (0.35 – 0.59)
Boron (B, µg/g)	Dry wt	<0.93 [3]	(<0.87 – 1.23) [2]	(<0.92 – 0.97) [2]	(<0.92 – 1.17) [1]
	Wet wt	<0.26 [3]	(<0.25 – 0.30) [2]	(<0.25 – 0.27) [2]	(<0.20 – 0.40) [1]
Barium (Ba, µg/g)	Dry wt	8.66 ± 3.61 (4.49 – 10.80)	10.18 ± 1.47 (8.94 – 11.80)	10.99 ± 1.42 (9.57 – 12.40)	5.17 (4.96 – 5.38)

Table 11. Whole-body concentrations of elements in Rio Grande silvery minnows exposed in cages for 26 days at three sites in the Middle Rio Grande, New Mexico, and pre-exposed fish.—Continued

[Data for cage-exposed fish are the mean±1 standard deviation with the range in parenthesis of 3 samples and data for pre-exposed fish are the mean and range in parenthesis of 2 composite samples. There were no significant differences in the mean concentrations of a given element in cage-exposed fish among sites. ±, plus or minus; MRG, Middle Rio Grande; 240-WW, 240-Wasteway; WIH, Wetted Instream Habitat; mg/g, milligram per gram; wt, weight; µg/g, microgram per gram; <, less than; %, percent]

Element (Symbol, unit)	Site (fig. 1)				Pre-exposed fish
	Unit basis	MRG	240-WW	WIH	
		Minor and trace—Continued			
Barium (Ba, µg/g)	Wet wt	2.30 ± 0.87 (1.30 – 2.84)	2.66 ± 0.29 (2.32 – 2.86)	2.94 ± 0.26 (2.66 – 3.16)	1.46 (1.07 – 1.86)
Beryllium (Be, µg/g)	Dry wt	<0.046 [3]	<0.047 [3]	<0.047 [3]	<0.046 [2]
	Wet wt	<0.013 [3]	<0.013 [3]	<0.013 [3]	<0.016 [2]
Cadmium (Cd, µg/g)	Dry wt	0.021 ± 0.010 (<0.018 – 0.028) [1]	0.023 ± 0.005 (0.020 – 0.028)	0.024 ± 0.003 (0.021 – 0.026)	0.022 (0.020 – 0.025)
	Wet wt	0.006 ± 0.003 (<0.005 – 0.007) [1]	0.006 ± 0.001 (0.006 – 0.007)	0.007 ± 0.001 (0.006 – 0.007)	0.006 (0.004 – 0.009)
Chromium (Cr, µg/g)	Dry wt	0.164 ± 0.065 (<0.180 – 0.206) [1]	0.181 ± 0.088 (<0.174 – 0.262) [1]	0.222 ± 0.130 (<0.188 – 0.353) [1]	0.388 (<0.184 – 0.683) [1]
	Wet wt	0.044 ± 0.015 (<0.052 – 0.053) [1]	0.048 ± 0.026 (<0.045 – 0.075) [1]	0.059 ± 0.032 (<0.052 – 0.090) [1]	0.128 (<0.040 – 0.236) [1]
Cobalt (Co, µg/g)	Dry wt	0.154 ± 0.063 (0.081 – 0.195)	0.183 ± 0.028 (0.153 – 0.208)	0.194 ± 0.037 (0.156 – 0.230)	0.131 (0.110 – 0.152)
	Wet wt	0.041 ± 0.015 (0.024 – 0.051)	0.048 ± 0.007 (0.040 – 0.054)	0.052 ± 0.008 (0.043 – 0.059)	0.038 (0.024 – 0.052)
Copper (Cu, µg/g)	Dry wt	2.43 ± 1.14 (1.12 – 3.12)	2.72 ± 0.23 (2.51 – 2.97)	2.56 ± 0.27 (2.26 – 2.78)	2.42 (2.35 – 2.50)
	Wet wt	0.64 ± 0.28 (0.32 – 0.80)	0.71 ± 0.01 (0.69 – 0.72)	0.69 ± 0.05 (0.63 – 0.72)	0.68 (0.51 – 0.86)
Iron (Fe, µg/g)	Dry wt	95.2 ± 36.6 (59.9 – 133.0)	144.5 ± 44.4 (96.4 – 184.0)	171.0 ± 95.8 (80.0 – 271.0)	65.8 (57.9 – 73.6)
	Wet wt	25.4 ± 8.9 (17.3 – 35.0)	38.3 ± 13.9 (25.1 – 52.8)	45.2 ± 23.4 (22.2 – 69.1)	18.9 (12.4 – 25.4)
Lithium (Li, µg/g)	Dry wt	<0.929 [3]	<0.946 [3]	<0.942 [3]	<0.920 [2]
	Wet wt	<0.260 [3]	<0.251 [3]	<0.262 [3]	<0.311 [2]
Lead (Pb, µg/g)	Dry wt	0.183 ± 0.073 (0.100 – 0.236)	0.240 ± 0.065 (0.165 – 0.285)	0.264 ± 0.077 (0.189 – 0.343)	0.134 (0.123 – 0.145)
	Wet wt	0.048 ± 0.017 (0.029 – 0.062)	0.063 ± 0.018 (0.043 – 0.077)	0.070 ± 0.017 (0.053 – 0.087)	0.038 (0.026 – 0.050)
Manganese (Mn, µg/g)	Dry wt	9.75 ± 3.48 (5.74 – 12.00)	23.53 ± 12.20 (9.80 – 33.10)	25.53 ± 14.16 (12.20 – 40.40)	7.48 (7.08 – 7.88)
	Wet wt	2.59 ± 0.81 (1.66 – 3.08)	6.25 ± 3.50 (2.55 – 9.50)	6.75 ± 3.46 (3.39 – 10.30)	2.12 (1.52 – 2.72)

Table 11. Whole-body concentrations of elements in Rio Grande silvery minnows exposed in cages for 26 days at three sites in the Middle Rio Grande, New Mexico, and pre-exposed fish.—Continued

[Data for cage-exposed fish are the mean±1 standard deviation with the range in parenthesis of 3 samples and data for pre-exposed fish are the mean and range in parenthesis of 2 composite samples. There were no significant differences in the mean concentrations of a given element in cage-exposed fish among sites. ±, plus or minus; MRG, Middle Rio Grande; 240-WW, 240-Wasteway; WIH, Wetted Instream Habitat; mg/g, milligram per gram; wt, weight; µg/g, microgram per gram; <, less than; %, percent]

Element (Symbol, unit)	Site (fig. 1)				Pre-exposed fish
	Unit basis	MRG	240-WW	WIH	
Minor and trace—Continued					
Molybdenum (Mo, µg/g)	Dry wt	<0.093 [3]	<0.095 [3]	<0.094 [3]	(<0.092 – 0.100) [1]
	Wet wt	<0.026 [3]	<0.025 [3]	<0.026 [3]	(<0.020 – 0.034) [1]
Mercury (Hg, µg/g)	Dry wt	0.027 ± 0.004 (0.022 – 0.029)	0.030 ± 0.006 (0.024 – 0.036)	0.031 ± 0.006 (0.027 – 0.038)	0.029 (0.021 – 0.037)
	Wet wt	0.007 ± 0.001 (0.006 – 0.008)	0.008 ± 0.001 (0.007 – 0.009)	0.008 ± 0.001 (0.007 – 0.010)	0.009 (0.004 – 0.013)
Nickel (Ni, µg/g)	Dry wt	1.00 ± 0.42 (0.53 – 1.32)	1.17 ± 0.11 (1.07 – 1.29)	1.28 ± 0.06 (1.23 – 1.34)	1.32 (0.78 – 1.85)
	Wet wt	0.27 ± 0.10 (0.15 – 0.34)	0.31 ± 0.03 (0.28 – 0.33)	0.34 ± 0.02 (0.32 – 0.37)	0.40 (0.17 – 0.64)
Selenium (Se, µg/g)	Dry wt	3.05 ± 1.02 (1.94 – 3.95)	3.93 ± 0.34 (3.61 – 4.29)	3.79 ± 0.17 (3.61 – 3.95)	3.30 (3.16 – 3.44)
	Wet wt	0.81 ± 0.24 (0.56 – 1.04)	1.03 ± 0.09 (0.94 – 1.12)	1.02 ± 0.04 (0.99 – 1.06)	0.93 (0.68 – 1.19)
Silicone (Si, µg/g)	Dry wt	161 ± 85 (105 – 259)	282 ± 183 (97 – 462)	353 ± 297 (51 – 644)	36.1 (31.1 – 41.1)
	Wet wt	43 ± 22 (27 – 68)	76 ± 54 (25 – 133)	93 ± 75 (14 – 164)	10.4 (6.7 – 14.2)
Silver (Ag, µg/g)	Dry wt	(<0.009 – 0.010) [2]	<0.009 [3]	<0.009 [3]	<0.009 [2]
	Wet wt	<0.0025 – 0.0025 [2]	<0.0025 [3]	<0.0026 [3]	<0.0031 [2]
Strontium (Sr, µg/g)	Dry wt	250 ± 107 (126 – 316)	307 ± 25 (288 – 335)	313 ± 15 (302 – 330)	281 (232 – 330)
	Wet wt	66 ± 26 (36 – 81)	80 ± 3 (77 – 83)	84 ± 7 (77 – 92)	82 (50 – 114)
Thallium (Tl, µg/g)	Dry wt	(<0.009 – 0.012) [2]	<0.009 [3]	<0.009 [3]	<0.009 [2]
	Wet wt	(<0.003 – 0.003) [2]	<0.003 [3]	<0.003 [3]	<0.003 [2]
Thorium (Th, µg/g)	Dry wt	<0.046 [3]	(<0.047 – 0.050) [2]	(<0.047 – 0.076) [2]	<0.046 [2]
	Wet wt	<0.013 [3]	(<0.011 – 0.014) [2]	(<0.013 – 0.019) [2]	<0.016 [2]
Tin (Sn, µg/g)	Dry wt	<0.093 [3]	<0.095 [3]	<0.094 [3]	<0.092 [2]
	Wet wt	<0.026 [3]	<0.025 [3]	<0.026 [3]	<0.031 [2]
Titanium (Ti, µg/g)	Dry wt	12.62 ± 5.23 (6.76 – 16.80)	16.17 ± 3.81 (12.80 – 20.30)	15.37 ± 3.10 (12.00 – 18.10)	14.20 (10.90 – 17.50)

Table 11. Whole-body concentrations of elements in Rio Grande silvery minnows exposed in cages for 26 days at three sites in the Middle Rio Grande, New Mexico, and pre-exposed fish.—Continued

[Data for cage-exposed fish are the mean±1 standard deviation with the range in parenthesis of 3 samples and data for pre-exposed fish are the mean and range in parenthesis of 2 composite samples. There were no significant differences in the mean concentrations of a given element in cage-exposed fish among sites. ±, plus or minus; MRG, Middle Rio Grande; 240-WW, 240-Wasteway; WIH, Wetted Instream Habitat; mg/g, milligram per gram; wt, weight; µg/g, microgram per gram; <, less than; %, percent]

Element (Symbol, unit)	Unit basis	Site (fig. 1) MRG	240-WW	WIH	Pre-exposed fish
		Minor and trace—Continued			
Titanium (Ti, µg/g)	Wet wt	3.35 ± 1.26 (1.95 – 4.42)	4.31 ± 1.39 (3.10 – 5.83)	4.11 ± 0.68 (3.34 – 4.62)	3.76 (3.76 – 3.76)
Uranium (U, µg/g)	Dry wt	0.225 ± 0.101 (0.110 – 0.301)	0.299 ± 0.067 (0.259 – 0.376)	0.299 ± 0.034 (0.261 – 0.324)	0.260 (0.197 – 0.324)
	Wet wt	0.060 ± 0.025 (0.032 – 0.079)	0.078 ± 0.012 (0.068 – 0.091)	0.081 ± 0.012 (0.067 – 0.088)	0.077 (0.042 – 0.112)
Vanadium (V, µg/g)	Dry wt	0.696 ± 0.409 (<0.450 – 0.955) [1]	1.096 ± 0.146 (0.927 – 1.190)	1.217 ± 0.251 (1.020 – 1.500)	0.802 (0.685 – 0.918)
	Wet wt	0.183 ± 0.103 (<0.130 – 0.251) [1]	0.288 ± 0.047 (0.241 – 0.336)	0.325 ± 0.051 (0.284 – 0.382)	0.232 (0.147 – 0.317)
Zinc (Zn, µg/g)	Dry wt	187 ± 76 (99 – 238)	238 ± 21 (214 – 251)	236 ± 10 (225 – 243)	206 (191 – 220)
	Wet wt	50 ± 18 (29 – 61)	62 ± 3 (60 – 65)	63 ± 2 (62 – 66)	58 (41 – 76)
Moisture content (%)		73.0 ± 1.7 (71.1 – 74.3)	73.7 ± 2.3 (71.3 – 75.8)	73.1 ± 1.2 (72.2 – 74.5)	72.0 (65.5 – 78.5)

[a]Number of samples below the method detection limit given in brackets.

differences in whole-body concentrations of any of the elements in caged-exposed fish among the three test sites. For most elements, the lowest whole-body concentrations were observed in fish exposed at the MRG site, and differences in concentrations between sites were the smallest for fish exposed at the 240-WW and WIH sites.

Whole-body element concentrations in fish sampled at the BioPark before test initiation are given for comparison (table 11), but were not included in the statistical analyses because only one sample consisting of two composite samples from the same group of fish was analyzed. Whole-body concentrations of the six major elements (Ca, Mg, K, P, Na, and S; operationally defined as those present at concentrations at or above 1 mg/g dry weight) in cage-exposed fish were similar to those of pre-exposed fish.

Mean whole-body concentrations of Al and Si in cage-exposed fish were substantially higher (4.5- to 25-fold) than those in pre-exposed fish (table 11). Mean whole-body concentrations of the other minor and trace elements in cage-exposed fish were within a factor of two of those in pre-exposed fish, except for Fe and Mn. The mean whole-body concentrations of Fe in fish at the WIH site and Mn in fish at the 240-WW and WIH sites were within a factor of three of those in pre-exposed fish. The similarities in whole-body residues between cage-exposed and pre-exposed fish indicates that little bioaccumulation of these elements occurred during the exposures.

Differences in whole-body residues of Al and Si between cage-exposed and pre-exposed fish, and also among cage-exposed fish within and between sites may be partly attributable to the amount of sediment ingested or adsorbed to the mucus. Rio Grande silvery minnow adults are believed to be benthic foragers consuming diatoms and other organic materials on the river substrate and ingest sediment incidentally to taking organic matter (Shirey, 2004; Cowley and others, 2006). The fish were not voided (of sediment) nor were the gut contents removed before sampling and analysis and these elements naturally occur at high concentrations in bottom sediments. Bottom sediments were not analyzed in this study, but the New Mexico Environment Department (2009) reported wet weight concentrations of 1,000–13,800 µg Al/g and 520–1,670 µg Si/g in bed sediments collected in 2006–08 at two sites in the MRG; one about 9 km upstream and the other about 4.5 km downstream from the cage sites. These sediment concentrations are substantially greater than the whole-body concentrations measured in cage-exposed fish reported in this study. Among the 34 elements measured in cage-exposed fish, whole-body concentrations of Al and Si had the largest coefficients of variation (48 to 84 percent) at each site. Brumbaugh and Kane (1985) reported that whole-body Al concentrations in smallmouth bass (*Micropterus dolomieui*) were higher and more variable in fish analyzed with the gastrointestinal tract intact compared to those with the gut contents removed, presumably because of sediment in the gut.

The elements that were present at concentrations ≥3 times their MDL in cage-exposed fish were significantly intercorrelated with at least three other elements, except for Zn and Se (appendix 10). Zinc was correlated with only K, and Se was correlated with only Na and K. The strongest element:element correlations were observed for Al:Si (Spearman's r=1.000), followed by Ba:Co (r=0.979), Ca:U (r=0.975), Ca:Sr (r=0.958), Co:Pb (r=0.946), and P:Sr (r=0.945).

Mean and maximum whole-body concentrations of the toxic trace metals or metalloids measured in caged-exposed Rio Grande silvery minnows were compared to no observed effect concentrations and toxic threshold concentrations observed in waterborne and dietary exposure studies with fish (table 12). The majority of the tissue residue/effects data were obtained from the extensive compilation of Jarvinen and Ankley (1999) and references cited therein. Whole-body concentrations of As, Sb, Cd, Cr, Cu, Pb, Hg, Ni, Ag, and V in Rio Grande silvery minnows were lower than their respective no effect and toxic threshold concentrations in other fishes.

One sample of Rio Grande silvery minnows from the 240-WW site contained a whole-body Se concentration of 4.29 µg/g dw that exceeded the proposed whole-body toxicity threshold of 4 µg/g dw advocated by Lemly (1996) and Hamilton (2002, 2003). A less conservative whole-body toxic threshold of 9 µg/g dw for warmwater fish has been proposed by DeForest and others (1999). The U.S. Environmental Protection Agency (2004) has recommended an intermediate fish whole-body Se criterion of 7.91 µg/g dw with a caveat that if whole-body fish tissues exceed 5.85 µg/g dw during summer or fall, fish tissues should be monitored in the winter to determine whether the tissue concentration exceeds 7.91 µg/g dw. Average whole-body Se concentrations in cage-exposed Rio Grande silvery minnows at each site (3.05–3.93 µg/g dw) were below the lowest proposed threshold concentration (4 µg/g dw). All whole-body Se residues in Rio Grande silvery minnows were lower than those linked with reduced growth (5.4–7.0 µg/g dw) in fathead minnows (*Pimephales promelas*) exposed to foodborne Se (Ogle and Knight, 1989). A large proportion of the Se body burden in cage-exposed Rio Grande silvery minnows was likely acquired prior to the exposure, as the average whole-body Se concentration in pre-exposed fish reared at DNFH&TC and the BioPark was 3.30 ug/g dw.

Mean whole-body concentrations of Al and Zn in cage-exposed Rio Grande silvery minnows were higher than those reported to be associated with reduced survival or growth in fish (table 12). As discussed above, the Al content in cage-exposed Rio Grande silvery minnows may consist of Al associated with ingested sediment in the gut and Al incorporated into the tissues. The low adverse effect tissue concentration for Al of 20 µg/g ww was obtained for Atlantic salmon (*Salmo salar*) alevins exposed to Al at a pH of 4.8 to 5.0 (Peterson and others, 1989). Considering that Al is a gill toxicant and accumulation in and damage to the gill is greater in acidic water than in neutral or basic waters (Gensemer and Playle, 1999), the toxicity whole-body residue relation for Al under acidic conditions may not be representative of that under basic conditions in the MRG.

Cleveland and others (1986) exposed 37-day old brook trout (*Salvelinus fontinalis*) to 300 µg/L Al at a neutral pH (7.2) and observed that after 15 days of exposure the fish had accumulated a whole-body residue of 56 µg/g ww with no reduction in survival or growth; but after 30 days, growth was reduced and their whole-body residue had decreased to 33 µg/g ww. In the same study (Cleveland and others, 1986), brook trout exposed to the same Al concentration at a pH of 5.5 showed reduced survival and growth after 15 and 30 days of exposure with corresponding whole body residues of 52 and 112 µg/g ww, respectively. The mean whole-body Al concentrations in cage-exposed Rio Grande silvery minnows (15–30 µg/g ww) in this study are lower than those associated with toxic effects in brook trout (33–112 µg/g ww; Cleveland and others, 1986). The results of Cleveland and others (1986) coupled with the possibility that a portion of the Al concentrations measured in whole-body Rio Grande silvery minnows may have come from ingested or sorbed sediment provide evidence that the whole-body Al concentrations in caged-exposed fish were not elevated to concentrations of concern. Moreover, the whole-body Al residues in cage-exposed fish in this study are similar to or lower than those in wild-caugh fishes collected from the MRG (discussed below).

Average whole-body Zn residues in cage-exposed and pre-exposed Rio Grande silvery minnows (50-63 µg/g ww) are about 1.2 to 1.6 times higher than that (40 µg/g ww) associated with reduced growth in flagfish (*Jordanella florida;* table 12). In the study with Zn and flagfish, Spehar (1976) tested larvae that were initially exposed to Zn as embryos along with larvae naïve to Zn exposure and observed that reduced growth and survival occurred at lower waterborne Zn concentrations in naïve larvae compared to previously exposed larvae, even though both groups had accumulated similar whole-body Zn residues. These results indicate that preexposures to Zn can affect the toxicity of a given whole-body Zn residue. Farmer and others (1979) conducted an 80-day waterborne exposure test with Atlantic salmon juveniles that were fed different rations; neither survival nor growth of the Zn-exposed fish were affected, and the corresponding whole-body Zn residues calculated from their Zn accumulation equation were 29–31 µg/g ww in the controls and 57-60 µg/g ww in the high Zn exposure treatment. Mount and others (1994) exposed rainbow trout (*O. mykiss)* fry to Zn-contaminated diets (*Artemia* sp.) for 60 days and found no adverse effects on survival or growth at whole-body Zn residues ranging from 163 to 303 µg/g dw, which are similar to or higher than those in caged-exposed Rio Grande silvery minnows (range of means,

Table 12. Comparison of whole-body metal/metalloid concentrations (micrograms per gram) in cage-exposed Rio Grande silvery minnows with those associated with no effects and adverse effects observed in laboratory studies with fish.

[wt, weight; ATS, Atlantic salmon (*Salmo salar*); BKT, brook trout (*Salvelinus fontinalis*); RBT, rainbow trout (*Oncorhynchus mykiss*); Carp (*Cyprinus carpio*); FHM, fathead minnow (*Pimephales promelas*); FCS, fall run chinook salmon (*Oncorhynchus tshawytscha*); Flagfish (*Jordanella floridae*); nr, not reported]

Element	Unit basis	This study		Residue linked with			Endpoint	Species	Reference[a]
		Mean	Maximum	Controls	No effect	Adverse effect			
Aluminum	Wet wt	23	54	nr	1	20	Survival	ATS	1
				4.4	12	33	Growth (pH, 7.2)	BKT	2
Arsenic	Wet wt	.61	.83	nr	1	3	Survival, growth	RBT	1
Antimony	Wet wt	<.013	<.013	nr	5	9	Survival	RBT	1
Cadmium	Wet wt	.006	.007	nr	.06	.12	Growth	ATS	1
				nr	.54	.96	Growth	RBT	1
	Dry wt	.023	.028	0.11	.65	1.26	Growth	BKT	3
Chromium	Wet wt	.05	.09	.287[b]	.583[b]	nr	Survival	RBT	1, 4
Copper	Wet wt	.68	.8	nr	7.4	11.1	Survival	Carp	1
	Dry wt	2.57	3.12	15.3[b]	17.0[b]	nr	Survival, growth, reproduction	BKT	5
Lead	Dry wt	.229	.343	0.06–0.36	12.7–25.3	20.1–43.8	Growth	BKT	6
Mercury	Wet wt	.008	.01	.12	.8	1.31	Growth	FHM	7
				.32	nr	1.36	Reproduction	FHM	7
	Dry wt	.029	.038	.48	nr	3.4	Reproduction	FHM	8
Nickel	Wet wt	.31	.37	.46[b]	.82[b]	nr	Survival	RBT	1, 4
Selenium	Dry wt	3.59	4.29	.8	2.6–2.7	4.0–5.4	Survival, growth	FCS	9
				2.7	5	5.4	Growth	FHM	10
Silver	Dry wt	<.009	.01	0.28–1.07	1.46–2.82	3.63	Growth	FHM	11
Vanadium	Dry wt	1	1.5	.6	2.85	11.1	Growth, reproduction	Flagfish	12
Zinc	Wet wt	58.4	65.5	nr	34	40	Growth	Flagfish	1
				29–31	57–60	nr	Survival, growth	ATS	13
	Dry wt	220	251	93–111	303–392	nr	Survival, growth	RBT	14

[a]1–Jarvinen and Ankley, 1999; 2–Cleveland and others, 1986; 3–Benoit and others, 1976; 4–Calamari and others, 1982; 5–McKim and Benoit,1974; 6–Holcombe and others, 1976; 7–Snarski and Olson, 1982; 8–Hammerschmidt and others, 2002; 9–Hamilton and others, 1990; 10–Ogle and Knight, 1989; 11–Naddy and others, 2007; 12–Holdway and others, 1983; 13–Farmer and others, 1979; 14–Mount and others, 1994.

[b]Measured in muscle.

187–238 µg/g dw). Based on the findings of Farmer and others (1979) and Mount and others (1994) and similarities between whole body Zn residues in cage-exposed and pre-exposed Rio Grande silvery minnows (0.9- to 1.2-fold difference), the Zn residues in caged-exposed Rio Grande silvery minnows were probably not elevated to concentrations of concern.

Because of the site-specific nature of the present study, comparisons of elemental tissue concentrations between cage-exposed and wild-caught Rio Grande silvery minnows and other cyprinids were limited to sites that were relatively close to the cage sites. As part of a larger investigation to assess the health of Rio Grande silvery minnows throughout their critical habitat, Lusk (2007) and Lusk and others (2010) collected silvery minnows at six sites in the MRG over a 2-year period (2006–08) and had the carcasses (whole body minus the gonads and some body fluid) of necropsied fish analyzed for a suite of inorganic and organic constituents (Trace Element Research Laboratory, 2010). Two of the sites were in close proximity to the cage sites in the present study. Based on the GPS coordinates measured during fish collection (Trace Element Research Laboratory 2010), the Los Padillas site was approximately 14 km upstream and the Los Lunas site was approximately 4.6 km downstream from the cage sites. All 19 elements measured in Rio Grande silvery minnows collected at these two sites by Trace Element Research Laboratory (2010) were measured in cage-exposed silvery minnows in this study and 14 of the 19 elements were present at detectable concentrations in most or all of the samples. The mean and range of Al, As, Ba, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sr, and Zn concentrations in whole-body samples of caged-exposed Rio Grande silvery minnows are similar to those in whole-carcass samples of wild-caught conspecifics. Differences in mean values for these 12 elements were generally <2.5-fold, and the ranges overlapped. Wild-caught silvery minnows contained higher concentrations of Ba (4–15 fold) and Hg (2–13 fold) than cage-exposed fish. Concentrations of B, Be, and Mo were below the detection limits in 78 to 100 percent of samples analyzed in this study and by Trace Element Research Laboratory (2010). Cadmium was detected in only one sample of wild-caught fish (0.037 µg/g dw) and that concentration is similar to the maximum concentration in cage-exposed fish (0.028 µg/g dw). Chromium was detected in 5 of 12 wild-caught fish, and the detectable concentrations (0.513–2.58 µg/g dw) are 1.5 to 7.3 times higher than the maximum concentration in cage-exposed fish (0.353 µg/g dw).

The average moisture content in the pre-exposed (72.0 percent) and cage-exposed (73.3 percent) Rio Grande silvery minnows in this study are very similar to those in wild-caught conspecifics collected at six sampling sites (grand mean, 72.6 percent; range of means, 71.6 to 73.7 percent) in the MRG (Trace Element Research Laboratory, 2010). Based on the results of this study and that of Trace Element Research Laboratory (2010), a conversion factor based on 73 percent moisture is appropriate for converting between whole-body dry weight concentrations and wet weight concentrations in adult Rio Grande silvery minnows when the moisture content is not given.

Abeyta and Lusk (2004) measured whole-body concentrations of 30 elements in small bodied cyprinids collected at 11 sites within the main stem of the Rio Grande and at 3 sites in adjacent outfalls in 2002–03. The most comparable data of Abeyta and Lusk (2004) to this study are from the two main stem water-quality monitoring sites that bracketed the location of the cage sites; the Rio Grande at Isleta below the Railroad Bridge site was approximately 9.5 km upstream and Rio Grande at Los Lunas site was approximately 4.5 km downstream from the cages. The location of the Los Lunas site is close to the sampling site of Lusk and others (2010) described above.

All 30 elements measured in feral cyprinids by Abeyta and Lusk (2004) were measured in Rio Grande silvery minnows in this study. The whole-body concentrations were compared on a wet weight basis because the moisture content was not reported for samples where the concentrations were given on a wet weight basis, but moisture content was given for samples reported on a dry weight basis. For 16 of 21 elements that were detected in the majority of samples in both studies (Ca, K, Mg, Na, Al, As, Cd, Cu, Fe, Pb, Mn, Ni, Se, Ti, U, and Zn), the range of whole-body concentrations in cage-exposed Rio Grande silvery minnows overlapped those found in fathead minnows and red shiners (*Cyprinella lutrensis*) by Abeyta and Lusk (2004). Whole-body concentrations of four elements (Ba, Cr, Co, and Hg) were lower, and one element (Sr) was higher in cage-exposed silvery minnows compared to those in fathead minnows and red shiners (Abeyta and Lusk 2004). Differences in mean whole-body concentrations for these 21 elements between the two studies were within a factor of four, except for Al and Cr where the mean whole-body concentrations were about eight times higher in the wild-caught cyprinids compared to cage-exposed Rio Grande silvery minnows.

The detectable concentrations of V in whole-body samples of fathead minnows at the Los Lunas site (0.40 µg/g ww) and in red shiners at the Isleta site (0.58 and 1.1 µg/g ww) reported by Abeyta and Lusk (2004) are within a factor of three of the maximum concentration measured in cage-exposed Rio Grande silvery minnows (0.38 µg/g ww) in this study. Abeyta and Lusk (2004) detected Ag, Sn, and Tl in whole-body samples of wild-caught cyprinids, but all detectable concentrations were below the reporting limit. In this study, Ag and Tl were only detected in one sample and Sn was not detected in any whole-body samples of cage-exposed Rio Grande silvery minnow.

Exposure Synopsis

There are artifacts associated with caging fish at a site, which include reduced food availability, stresses associated with acclimation and transport, variable flows, and mechanical damage (Chappie and Burton, 2000). Caged exposures of adult Rio Grande silvery minnows at all three test sites resulted in a loss of weight, reduced condition factor, lower MFI, and a higher incidence of fin lesions and parasites compared to pre-exposed fish. Some weight loss and reduced MFI were expected as a caging artifact. The fish were transferred from a large circular culture tank maintained under conditions that maximize survival, growth, and health to a confined space in ambient site waters with variable temperatures and flows, reduced food availability, and natural parasites. Although the ration provided to the fish was comparable to that fed at the BioPark, it was fed at one time (compared to being split over two to three feedings per day); and some of the food may have been flushed out of the cages by the current.

Observations on the gall bladder (BCFI of 0-1 in most fish) indicated that the caged-exposed fish were feeding, but the type and amount of food consumed was not determined. In addition, the observation that the prevalence and severity of MA in the kidney and spleen of cage-exposed fish was similar to or less than those observed in pre-exposed fish suggests that the cage-exposed fish were not being starved relative to the pre-exposed fish. Fish under starvation tend to have more and larger MA (Ferguson, 2006). Agius and Roberts (1981) reported that rainbow trout fry starved for 3 weeks had a higher incidence of MA in the spleen and kidney compared to the fed controls.

The decline in condition factor accompanied the loss in weight because it was computed from length-weight equations and there was little or no change in TL of the fish after the exposures. The lower MFI in cage-exposed fish compared to pre-exposed fish provided clinical evidence of depleted fat reserves in cage-exposed fish.

The increased occurrence of fin anomalies, primarily as deformed rays of the pectoral and pelvic fins, in cage-exposed fish (compared to pre-exposed fish) was likely a caging artifact. Some of the damage may have occurred when the cages were lifted up (daily) to observe the fish; lifting the cages caused the fish to rapidly dart along the bottom from side to side. Some damage also may have occurred when the fish were collected at the end of the exposure.

Routine monitoring of DO, pH, conductivity, and ammonia indicated that the loading densities of Rio Grande silvery minnows did not adversely impact water quality in the cages. Initial and final loading densities of Rio Grande silvery minnows in the cages ranged from 0.39 to 0.78 g/L and 0.19 to 1.08 g/L, respectively, and were below the maximum recommended instantaneous rate of 5 g/L for flow-through toxicity tests. Water flow through the cages far exceeded the recommended rate of 1 L per 0.5 g of fish per 24-hour period (American Society for Testing and Materials, 1996).

The results of the chronic study provided some evidence that the exposure conditions at the WIH site may have had a greater adverse effect on caged Rio Grande silvery minnows than those at the other sites. Absolute weight loss and the magnitude of the decline in condition factor of fish exposed at the WIH site were statistically greater than for those at the MRG site and numerically greater than those at the 240-WW site. Although not statistically different, fish exposed at the WIH site also had the highest relative weight loss and prevalence of external abnormalities and the lowest weights, condition factors, and MFI compared to fish at the other two sites.

The causative factors for the greater weight loss and reduced condition factor of fish at the WIH site relative to those exposed at the other two sites are not known. The physical features of the sites may have had an influence on the fish. Weight loss (absolute and relative) and relative reduced condition factor of fish were inversely correlated with depth (Spearman's $r=^{-}0.824$ to $^{-}924$, $p=0.0064$ to 0.0004, n=9). Water depth was significantly lower in cages at the WIH site compared to the other sites, and the shallow depth may have placed an additional stress on the fish.

Summary and Conclusions

The results of chronic study indicated that the conditions at the WIH site during low flow periods in October were the least favorable to the health and condition of Rio Grande silvery minnows compared to the other sites. Fish exposed for 26 days at the WIH site exhibited highest weight loss and prevalence of external abnormalities and the lowest condition factors and MFI compared to fish at the other two sites. Water quality at the WIH site was similar to that at the 240-WW site and probably was not a contributing factor to the observed effects. The WIH site had the shallowest depths and the confinement in shallow waters may have imposed an additional stress on the fish that further impaired their health compared to the other sites. In addition, the shallow waters at the WIH site provided less cover (for free-ranging fish) from herons (*Ardea* spp.) and other predators compared to the 240-WW site. If deeper pools were created below the woody debris at the WIH site during high rivers flows, they were subsequently filled with sediment in September and October. Additional studies are needed to determine if free-ranging silvery minnows utilize the WIH and exhibit any preferences for WIH or 240-WW during river drying events.

The use of cages provided a means of continuously exposing Rio Grande silvery minnows to the ambient physical and chemical conditions at the sites, which could not be replicated in laboratory studies. However, there were drawbacks from the caging artifacts of weight loss and fin damage in cage-exposed fish. The size and shape of the cages used in this study were based on physical features of the test sites (WIH and 240-WW), which were relatively narrow and shallow. One approach that may have reduced or alleviated weight loss would have been to condition the cages to the site by deploying them for a longer period of time prior to stocking the fish, which may have allowed for the colonization of naturally occurring food organisms (aufwuch) in and on the cages. Also, the use of larger cages with more surface area (dependent on the site) may be advantageous.

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Appendixes 1–10

The Excel spreadsheet *Appendixes1–10.xlsx* contains the appendixes 1 through 10 listed below.

Appendix 1. Analytical methods and quality-control measures for general chemical analysis of water samples performed by Test America Laboratories, Arvada, Colorado.

Appendix 2. Analytical methods and quality-control measures for water samples analyzed for major, minor, and trace elements by Trace Element Research Laboratory, College Station,Texas.

Appendix 3. Quality-control measures for tissue samples analyzed for major, minor, and trace elements by Trace Element Research Laboratory, College Station, Texas.

Appendix 4. Organophosphorous and chlorinated herbicides measured in the water samples from three study sites in the Middle Rio Grande, New Mexico, during cage exposures of Rio Grande silvery minnows.

Appendix 5. In-situ field parameters measured daily in the cages and at the sondes during acute cage exposures of Rio Grande silvery minnows at three sites in the Middle Rio Grande, New Mexico, September 14–18, 2007.

Appendix 6. Daily average, minimum, and maximum of in-situ parameters measured at 15-minute intervals by sondes at two sites in the Middle Rio Grande, New Mexico, during acute cage exposures of Rio Grande silvery minnows, September 14–18, 2007.

Appendix 7. In-situ field parameters measured daily in the cages and at the sondes during chronic cages exposures of Rio Grande silvery minnows at three sites in Middle Rio Grande, New Mexico, October 5–31, 2007.

Appendix 8. Daily average, minimum, and maximum of in-situ parameters measured at 15-minute intervals by sondes at three sites in the Middle Rio Grande, New Mexico, during chronic cage exposures of Rio Grande silvery minnows, October 5–31, 2007.

Appendix 9. Water-quality characteristics measured in samples collected in the cages and at the sondes during chronic cage exposures of Rio Grande silvery minnows at three sites in the Middle Rio Grande, New Mexico, October 5–31, 2007.

Appendix 10. Correlation matrix (Spearman's rho values) of whole-body element concentrations in Rio Grande silvery minnows exposed for 26 days in cages at three sites in the Middle Rio Grande, New Mexico.

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