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Environmental contaminants and biomarker responses in fish from the Rio Grande and its U.S. tributaries: Spatial and temporal trends

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Abstract

We collected, examined, and analyzed 368 fish of seven species from 10 sites on rivers of the Rio Grande Basin (RGB) during late 1997 and early 1998 to document temporal and geographic trends in the concentrations of accumulative contaminants and to assess contaminant effects on the fish. Sites were located on the mainstem of the Rio Grande and on the Arroyo Colorado and Pecos River in Texas (TX), New Mexico (NM), and Colorado. Common carp (*Cyprinus carpio*) and largemouth bass (*Micropterus salmoides*) were the targeted species. Fish were examined in the field for internal and external visible gross lesions, selected organs were weighed to compute ponderal and organosomatic indices, and samples of tissues and fluids were obtained and preserved for analysis of fish health and reproductive biomarkers. Whole fish from each station were composited by species and gender and analyzed for organochlorine chemical residues and elemental contaminants using instrumental methods, and for 2,3,7,8-tetrachloro dibenzo-*p*-dioxin-like activity (TCDD-EQ) using the H4IIE rat hepatoma cell bioassay. Overall, fish from lower RGB stations contained greater concentrations of organochlorine pesticide residues and appeared to be less healthy than those from sites in the central and upper parts of the basin, as indicated by a general gradient of residue concentrations and biomarker responses. A minimal number of altered biomarkers and few or no elevated contaminant concentrations were noted in fish from the upper RGB. The exception was elevated concentrations [up to 0.46 μg/g wet-weight (ww)] of total mercury (Hg) in predatory species from the Rio Grande at Elephant Butte Reservoir, NM, a condition documented in previous studies. Arsenic (As) and selenium (Se) concentrations were greatest in fish from sites in the central RGB; Se concentrations in fish from the Pecos River at Red Bluff Lake, TX and from the Rio Grande at Langtry, TX and Amistad International Reservoir, TX exceeded published fish and wildlife toxicity thresholds. In the lower RGB, residues of

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p,p′-DDT metabolites (≤1.69 μg/g ww), chlordane-related compounds (≤0.21 μg/g ww), dieldrin (≤0.0.05 μg/g ww), and toxaphene (≤2.4 μg/g ww) were detected in fish from most sites; maximum concentrations were in channel catfish (*Ictalurus punctatus*) from the Arroyo Colorado at Harlingen, TX. Concentrations of one or more residues exceeded toxicity thresholds for fish and wildlife in fish from this site and from the Rio Grande at Mission, TX and Brownsville, TX; however, concentrations were lower than those reported by previous studies. In addition, the proportional concentrations of *p,p′*-DDT at all sites were low, indicating weathered DDT rather than the influx of new material. Concentrations of total PCBs (<0.05 μg/g ww) and TCDD-EQ (≤6 pg/g ww) were comparatively low in all samples. Hepatic ethoxyresorufin *O*-deethylase (EROD) activity in some fish was elevated relative to reference rates at most sites, but was generally lower than previously reported activity in fish from heavily contaminated locations. The comparatively low PCB and TCDD-EQ concentrations together with elevated EROD activity may reflect exposure to polycyclic aromatic hydrocarbons. Reproductive biomarkers were consistent with chronic contaminant exposure at lower RGB sites; comparatively large percentages of intersex male largemouth bass, relatively low gonadosomatic indices, and elevated plasma vitellogenin concentrations in male fish were noted at three of the four stations. Large percentages of atretic eggs were also observed in the ovaries of female common carp from the Rio Grande at Brownsville, TX. Although many of the conditions noted may have other causes in addition to contaminant exposure, the biomarker results for the lower RGB sites are consistent with subtle responses of fish to contaminants, an interpretation supported by the chemical data of this and other investigations.

Keywords: Arsenic; Selenium; Mercury; Pesticides; Organochlorine chemicals; Ethoxyresorufin *O*-deethylase (EROD) activity; Health assessment index, (HAI); Biomarkers; Ovotestis; Vitellogenin

1. Introduction

The Rio Grande is the second longest U.S. river; it is approximately 3059 km long and drains an area of some 924,300 km^2 (Texas Natural Resources Conservation Commission (TNRCC), 1997; Fig. 1). The Rio Grande also represents the international boundary between the United States and Mexico from El Paso, Texas (TX) to the Gulf of Mexico, a distance of about 2053 km (Fig. 1). About 69% (231,317 km^2) of the Rio Grande Basin (RGB) lies within the United States, with the remainder in Mexico. Although much of the RGB is desert, the area supports a rapidly expanding human population conservatively estimated at 10 million in 1992 (TNRCC, 1997) as well as a unique river-dependent biota. The Rio Grande and its tributaries therefore represent a vital source of water to both the human population and the ecological resources of the region. Heavy demands are placed on the Rio Grande and its tributaries for irrigation and for the water supply and waste disposal needs of population centers in the United States and Mexico. Water quality is affected by natural, agricultural, industrial, and urban erosional processes, which contribute to high sediment loads, and dams and diversions have dramatically altered flow regimes (e.g., Ong et al., 1991; TNRCC, 1994a, 1997; Davis et al., 1995; Levings et al., 1998). Chemically intensive irrigated agriculture is practiced in much of the RGB, parts of which are also highly mineralized and underlain by petroleum-rich geologic formations. Consequently, pesticides, oil, and potentially toxic trace elements such as arsenic (As), selenium (Se), and heavy metals are available for mobilization and transport. The rates of the processes controlling the release and distribution of these constituents have been profoundly altered by human activities such as irrigation, mining, oil and gas extraction, and complex systems of dams and diversions, which have profoundly affected the flux of water and sediments and their associated contaminants. Elevated concentrations of metals, metalloids, and organochlorine pesticides have been documented in sediments and river-dependent organisms throughout the RGB (Gamble et al., 1988; Ong et al., 1991; U.S. Environmental Protection Agency (USEPA), 1992; TNRCC, 1994a,b, 1997; Davis et al., 1995; Carter and Anderholm, 1997; Mora, 1997; Mora et al., 1997; Van Metre et al., 1997; Mora and Wainwright, 1998; Levings et al., 1998; Schmitt et al., 1999b; Moring, 1999).

We sampled the largest U.S. rivers in the RGB during late 1997 and early 1998 as part of the U.S. Geological Survey (USGS) Biomonitoring of Environmental Status and Trends program, which monitors

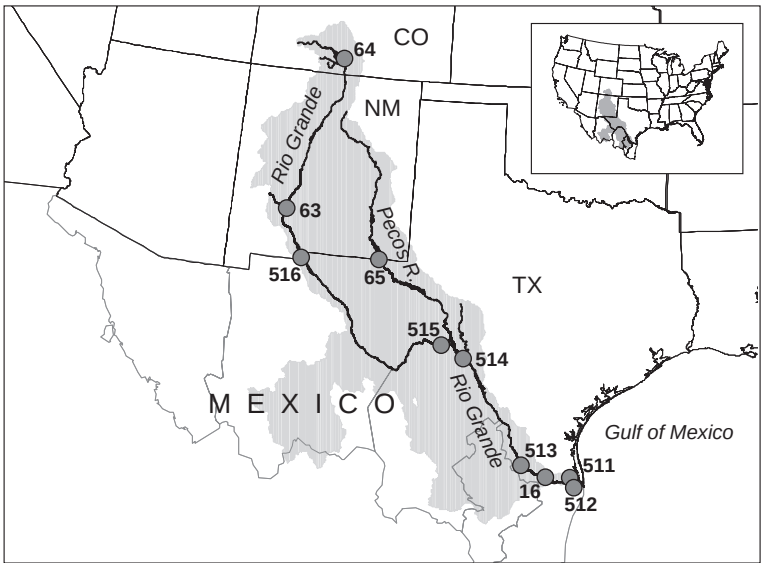

Fig. 1. Map of the Rio Grande basin illustrating waterways and impoundments, state and international boundaries, and locations sampled.

and evaluates environmental contaminants and their effects on fish throughout the United States (Schmitt, 2002a). Our primary objective was to document and assess spatial and temporal trends in the concentrations of environmental contaminants and their effects in RGB fish. Secondary objectives were to compare results from the RGB to other major U.S. rivers, and to further define benchmarks for the quantification of long-term trends and interpretation of biomarker results. In this paper we summarize the most pertinent findings of the RGB study, which are reported in greater detail by Schmitt et al. (2004). All raw data from this and related investigations are available at <http://www.cerc.usgs.gov/data/data.htm>.

2. Materials and methods

The chemical and biological methods used to assess exposure of fish to contaminants and the effects of exposure were selected to span multiple levels of biological organization. The methods included exposure indicators [concentrations of elemental contaminants, organochlorine chemical residues, and 2,3,7,8-tetrachloro dibenzo-*p*-dioxin equivalents (TCDD-EQ); and hepatic ethoxyresorufin *O*-deethylase (EROD) activity], fish health indicators [ponderal and somatic indices, external lesions, health assessment index (HAI), and general histopathology], and reproductive biomarkers [gonadosomatic index (GSI), gonadal histopathology, and plasma vitellogenin (vtg) concentrations]. Detailed descriptions of all procedures and quality assurance (QA) results are presented elsewhere (Schmitt et al., 1999a; Schmitt and Dethloff, 2000; Schmitt, 2002a,b, 2004).

2.1. Sampling and field procedures

Fish were collected at 10 sites located on the mainstem of the Rio Grande in Colorado (CO), New Mexico (NM), and Texas ($n=8$); the Arroyo Colorado, a distributary of the Rio Grande, in South Texas ($n=1$); and the Pecos River, the largest U.S. tributary of the Rio Grande, near the Texas–New Mexico boundary ($n=1$; Fig. 1; Table 1). To ensure spatial and temporal continuity with historical data and facilitate trend analysis, four sites were National Contaminant Biomonitoring Program (NCBP) stations where contaminants in fish had been monitored historically (Schmitt et al., 1999b). Most fish were collected between late September and early December 1997, but Station 65 was not sampled until January 1998 (Table 1). Sampling was completed during one visit spanning 2–3 d at most sites, but two visits were

Table 1
Location and fish collection dates of sampling stations in the Rio Grande (RG) basin (listed upstream to downstream)

Sub-basin and river	Station number	Location	Collection date(s)	Latitude, longitude
Upper RGB				
RG	64[a]	Alamosa, CO	09/23/97–10/21/97	37°25′06.42″ N, 105°46′48.48″ W
RG	63[a]	Elephant Butte Reservoir, NM	10/22/97–10/24/97	33°12′48.55″ N, 107°13′27.26″ W
RG	516	El Paso, TX	10/30/97–12/03/97	31°47′55.00″ N, 106°32′25.08″ W
Middle RGB/Pecos				
Pecos	65[a]	Red Bluff Lake, TX	01/21/98–01/22/98	32°00′00.00″ N, 103°58′56.28″ W
RG	515	Foster Ranch, Langtry, TX	11/06/97	29°46′40.91″ N, 101°45′13.22″ W
RG	514	Below Amistad Reservoir, TX	11/04/97–11/05/97	29°26′49.06″ N, 101°03′22.44″ W
Lower RGB				
RG	513	Below Falcon Dam, TX	11/18/97–11/19/97	20°08′06.66″ N, 99°08′06.42″ W
RG	16[a]	Mission, TX	12/02/97–12/03/97	26°09′28.74″ N, 98°20′02.82″ W
Arroyo Colorado	511	Harlingen, TX	09/30/97–10/02/97	26°11′44.28″ N, 97°36′20.52″ W
RG	512	Brownsville, TX	10/28/97–11/25/97	25°52′12.96″ N, 97°27′06.30″ W

[a] National Contaminant Biomonitoring Program (NCBP) site (Schmitt et al., 1999b).

required to complete the collections at Stations 64, 512, and 516 (Table 1).

Common carp (*Cyprinus carpio*, henceforth carp) and largemouth bass (*Micropterus salmoides*) were the preferred species, with a collection target of 10 (each) males and females of each taxon (total of 40 fish) per site. Adult fish of a size representative of those believed to be present at the sites based on extant information were sought, and extremely large or small fish were avoided.

Fish were collected by electrofishing, with the following exceptions: smallmouth bass (*Micropterus dolomieui*) were netted at Station 63, as were all fish from Stations 511 and 515 except blue catfish (*Ictalurus furcatus*), which were captured by hand. Fish were held in aerated live wells and transported to shore for processing, usually within a few hours of collection. Some fish were held alive overnight in aerated tanks containing ambient water or in situ in net pens following night collections. Fish processing began with the collection of a blood sample from the posterior caudal artery and vein with a heparinized needle and syringe. The blood sample was chilled on [wet] ice, and the fish was weighed, measured, and subdued with a blow to the head. Observations of external features were recorded, and grossly visible anomalies were removed by dissection and preserved in 10% neutral buffered formalin (NBF) for histopathological analysis. The abdominal cavity of the fish was dissected open and the liver (in species other than carp), spleen, and gonads were removed and weighed. The liver, gall bladder, posterior and anterior kidneys, gonads, mesenteric fat, and spleen were examined, and the gender of the fish was determined by gonadal observation. Pieces of liver were collected and immediately flash-frozen in a dry ice–ethanol slush for EROD analysis. Samples of gonad (posterior tips), kidney, spleen, and additional pieces of liver were collected and preserved in 10% NBF for histopathological examination, which included gender confirmation (gonad) and macrophage aggregate quantification (spleen). Scales (spines from ictalurids) were collected for age determination. All remaining tissues (those not frozen or fixed) were wrapped in aluminum foil, chilled, and later frozen for analysis of organochlorine chemical residues, elemental contaminants, and TCDD-EQ, the latter using the H4IIE bioassay (Tillitt et al., 1991). Between specimens all contact instruments and work surfaces were thoroughly cleaned to prevent cross-contamination. Following fish processing, blood samples were centrifuged and the plasma was aspirated and frozen in dry ice for vtg analysis. Cryogenically frozen liver and plasma samples were shipped frozen (in nitrogen dry shippers) and stored at −80 °C. Fish carcasses were shipped frozen in dry ice to the analytical laboratory and stored at −20 °C until prepared for analysis.

2.2. Laboratory analyses

Composite carcass samples were prepared for chemical and H4IIE analyses by band-sawing and grinding with a commercial meat grinder. The ground fish comprising each sample was subsampled and re-frozen (−20 °C). One subsample (100 g) was freeze-dried; following determination of moisture content (as moisture loss during lyophilization), a portion was acid-digested and analyzed for 19 elemental contaminants by atomic absorption spectroscopy and inductively coupled plasma emission spectroscopy. Quality assurance measures for the elemental analyses included the analysis of reagent blanks, duplicate samples, certified reference materials, and fortified samples. Dry-weight (dw) limits-of-detection (LODs) were determined individually for each element in each sample; for the elements reported here the nominal LODs (all dw) were 0.1 μg/g for cadmium (Cd) and total mercury (Hg), 0.2 μg/g for lead (Pb), 0.5 μg/g for As and Se, and 1.5 μg/g for zinc (Zn). For statistical analysis and reporting, all elemental concentrations and LODs were converted to wet-weight (ww) values using the moisture content of each sample. A second subsample (10 g) was solvent-extracted and analyzed gravimetrically for lipid content and by gas chromatography with electron capture detection for 21 organochlorine pesticide residues and total polychlorinated biphenyls (PCBs). Quality assurance measures for the organochlorine analyses included the analysis of duplicate and fortified samples and the confirmation of residue identities in selected samples by gas chromatography–mass spectrometry. Recovery efficiency ranged from 60.2% for hexachlorobenzene (HCB) to 94.4% for mirex, but was 85–92% for most analytes. Nominal LODs were 0.01 μg/g wet-weight (ww) for individual compounds and 0.05 μg/g for toxaphene and total PCBs. Residue concentrations were not adjusted for recovery efficiency. A third subsample (10 g) was solvent-extracted and subjected to reactive cleanup for use in the H4IIE bioassay (Tillitt et al., 1991) as modified for 96-well microtiter plates (Tysklind et al., 1994). Concentrations of TCDD-EQ (pg/g ww) were determined by slope ratio assay (Finney, 1980) as described by Ankley et al. (1991). Quality assurance measures for the H4IIE bioassay included the analysis of duplicate samples and reference materials. Limits-of-quantitation (LOQs) and LODs for TCDD-EQ were computed separately for each group of samples; LODs were 0–1 pg/g and all LOQs were 1 pg/g.

Hepatic EROD activity was determined in 96-well microtiter plates using kinetic assays performed on triplicate 5-μL portions of microsomal fractions prepared daily from each fish liver sample (Whyte et al., 2000). Protein content was determined using the fluorescamine protein assay (Lorenzen and Kennedy, 1993) in the same 96-well plate as the EROD analyses and used to normalize EROD activity in each well. Activity was reported as the mean of the triplicate determinations. LODs were 0–0.15 pmol/min/mg; LOQs were 0–0.35 pmol/min/mg. In addition to the triplicate determinations, QA measures for the EROD assay included the analysis of reference materials and duplicate samples.

Fixed tissues (liver, kidney, spleen, gill, gonad, and grossly visible lesions) were prepared for histopathological analysis as described by Blazer et al. (2002). Paraffin-embedded tissue sections (6-μm) mounted on glass slides were stained with hematoxylin and eosin (H and E) for microscopic examination. Macrophage aggregates (MA) and MA pigments in spleen sections were stained using Perl's method (Luna, 1992). All MA measurements were made with a computer-based image analysis system, and included the number of aggregates in 2 mm^2 of tissue (MA-#) and the mean area occupied by those aggregates (MA-A). The percentage of tissue occupied by MA (MA-%) was computed from these measurements. Transverse ovary sections were assigned to developmental stages 0–5 based on the predominant size and appearance of oocytes (Rodriguez et al., 1995; Nagahama, 1983; Treasurer and Holliday, 1981; McDonald et al., 2000; Blazer, 2002), and oocyte atresia was quantified. Transverse testes sections were similarly classified into developmental stages 0–4 (Nagahama, 1983; Blazer, 2002). Gonadal tissue was also examined microscopically for any abnormalities such as intersex, parasites, neoplasia, and pigmented cell accumulations (i.e., ceroid/lipofuscin deposits). Pigment deposits were categorized as absent, present, or abundant. Male fish were identified as intersex when individual or small foci of undeveloped oocytes were observed within testicular tissue (i.e., when an ovotestis condition was detected).

Concentrations of vtg in largemouth and smallmouth bass (henceforth bass) and carp plasma were determined by ELISA (Denslow et al., 1999). The LOD was 0.002 mg/mL for bass and 0.005 mg/mL for carp. All assays were performed in triplicate and reported as the mean of the three measurements; the coefficient of variation was <10% for all samples analyzed. Inter-assay variability was <10% as determined by routinely analyzing controls on several plates.

2.3. Data set composition and statistical analyses

A total of 368 fish representing eight species were collected and examined. Carp ($n=207$) and bass ($n=75$) together represented 77% of the total. Carp were obtained at all 10 stations and bass at five; the other species [blue catfish, channel catfish (*Ictalurus punctatus*), striped bass (*Morone saxatilis*), white bass (*M. chrysops*), and northern pike (*Esox lucius*)] were obtained at only one or two stations. Only three smallmouth bass were collected—two females at Station 63 and one male at Station 514. All other bass were largemouth. Of the species other than carp and bass, only channel catfish ($n=42$) accounted for more than 10% of the total. Composite samples ($n=47$) from 10 stations were analyzed for organochlorine chemical residues, elemental contaminants, and TCDD-EQ. Of these, 22 samples (47%) representing all ten stations were carp and 12 samples (25.5%) from five stations were bass. The remaining 13 samples (26%) comprised channel catfish ($n=5$, two stations), northern pike ($n=4$, one station), striped bass ($n=1$, one station), blue catfish ($n=1$, one station), and white bass ($n=2$, one station).

The occurrence of gross external pathological disorders was determined by assigning numerical values to field observations. Lesions were rated as present (1) or absent (0). For consistency with previous studies (e.g. Fournie et al., 1996, 2001; Blazer et al., 2002), only the following observations were included: grossly visible disorders of the eye (exopthalmia, hemorrhage, opacity, emboli, missing), opercles (shortening, deformities, parasites), body surface (ulcers, parasites, and raised or discolored areas), fins (hemorrhage, fraying, eroded), and skeleton (curvature). A necropsy-based HAI score was also calculated for each fish by assigning numerical values to gross internal and external lesions (Adams et al., 1993; Blazer et al., 2002), then summing the values for all organs observed. An HAI score was computed for a fish only if observations were present for all components.

Body and organ weights were used to compute condition factor (CF) and organosomatic indices (Dethloff and Schmitt, 2000; Blazer et al., 2002) according to the following formulae: CF = body weight/length^3; hepatosomatic index (HSI) = liver weight/(total body weight − gonad weight) × 100; splenosomatic index (SSI) = spleen weight/(total body weight − gonad weight) × 100; GSI = gonad weight/total body weight × 100. The weight of the gonads was subtracted from the body weight in the computation of HSI and SSI to minimize the effect of the reproductive cycle on these indices (Dethloff and Schmitt, 2000).

Some fish were grouped at the genus level for statistical analysis. Most biomarker results were analyzed using analysis-of-variance (ANOVA) and analysis-of-covariance (ANCOVA) to test for differences among sites and to examine for effects due to age, gender, and gonadal stage. Least-squares means, which are adjusted for all effects in the model, were tested. Transformations were applied to approximate the normality and homogeneity-of-variance required for the application of these parametric statistical methods. Contaminant concentrations (including TCDD-EQ) in composite samples and EROD activities and vtg concentrations in individual fish (females only for vtg) were $\log_{10}$-transformed; and HAI scores were rank-transformed. The length, weight, and age data were not transformed. External lesion frequencies were not analyzed statistically but were accounted for in the HAI scores. All computations and statistical analyses were performed with Version 8 of the Statistical Analysis System (SAS Institute, 1999).

Fish from which only regenerated scales were collected (22 carp, one largemouth bass) were excluded from all analyses that included age as a factor. Fish for which the field gender identification could not be verified histologically (including four fish of the targeted species) were also excluded from analyses that included gender as a factor. Biomarker data for carp and bass were summarized and are presented in more detail than other species.

All results for analytes in composite samples were converted to, reported as, and analyzed statistically as ww concentrations. A value of one-half the LOD was substituted for censored values in all statistical analyses and graphs. Concentrations of many analytes in composite samples were <LOD, which limited the extent and rigor of the statistical analyses that could be performed. Geographic differences in concentrations of *p,p′*-DDE, As, Hg, Se, and Zn were examined statistically using ANOVA, as were temporal differences by combining the results of our study with 1970–1986 NCBP data from Stations 16, 63, 64, and 65 (Schmitt et al., 1999b). Because concentrations of total Hg in predatory fish increase with size, age, or both (see for example Lange et al., 1993; Wiener et al., 2002), temporal and geographic differences in log-transformed length-adjusted (HgL) and weight-adjusted (HgW) concentrations computed as described by Brumbaugh et al. (2001) were also tested by ANOVA. A nominal α-level of 0.05 was used in all statistical tests unless otherwise indicated. Details of the statistical procedures are given by Schmitt et al. (2004).

3. Results

3.1. Lipid and moisture content (data not shown)

Most composite samples were 1–5% lipid, but lipid content varied among sites and species. Carp samples generally contained 2–7% lipid, but those from Stations 513 and 514 were 8.6–10.6%. Lipid in channel catfish from Station 516 was 2.2–4.5%, but at Station 511 it was 9.1–13.6%. The blue catfish sample from Station 515 was also 10% lipid. Conversely, lipid in striped bass from Station 63 was only 0.2%. Most samples contained 70–78% water, but moisture content ranged from 65.3% in a carp sample from Station 514 to 79.8% in the striped bass from Station 63. Except for the striped bass, lipid and moisture values were typical for these taxa.

3.2. Exposure indicators

3.2.1. Elemental contaminants

Arsenic concentrations were >LOD (~0.05 μg/g ww) in 38 samples (81%) representing all ten stations. The greatest concentrations (0.30–0.55 μg/g) were in carp and white bass from Stations 65 and 514, in the middle RGB; and in bass from Station 512, in the lower RGB (Fig. 2; Table 2). Elsewhere, concentrations in carp, bass channel catfish, and white bass were lower and generally similar (0.13–0.15 μg/g), but were all <LOD in northern pike from Station 64 (Fig. 2). Differences among stations were statistically significant in carp and bass, but not in blue or channel catfish (henceforth catfish) or in striped or white bass (*Morone* sp.; Table 2). Concentrations of As in 1997 differed significantly from historical levels in at least one taxon at Stations 63, 64, and 65, but there was only one obvious temporal trend—declining concentrations in *Morone* sp. at Station 65 (Table 3).

Selenium was detected in all samples at concentrations of 0.17–1.87 μg/g, the latter in white bass from Station 65 (Fig. 2). Concentrations ≥0.5 μg/g were present in at least one sample from all stations, and all samples from Station 514 and two from each of Stations 515 and 65 contained >1.0 μg/g (Fig. 2). Differences among or between stations were statistically significant in all taxa except catfish (Table 2), but the only statistically significant temporal difference was a downward trend in *Morone* sp. at Station 65 (Table 3).

Total Hg concentrations were >LOD (~0.02 μg/g ww) in 45 of 47 samples (96%) representing all 10 stations, and were generally greater in piscivores than in benthivores (Fig. 2). Greatest concentrations (0.25 μg/g) were in largemouth bass from Stations 63 and 514 and striped bass from Station 63; those in smallmouth bass and carp from Station 63 were lower (<0.20 μg/g; Fig. 2). Differences among stations were statistically significant only in *Morone* sp. and carp, however (Table 2). Total Hg and HgL concentrations in *Morone* sp. were significantly greater at Station 63 than at Station 65, but HgW differences were only significant in carp (Fig. 2; Table 2). Temporal Hg differences were significant in some taxa, and these differences were consistent for total Hg, HgL, and HgW (Table 3). Overall, Hg concentrations in bass at Station 63, *Morone* sp. at Stations 63 and 65, and carp at Station 64 differed significantly among years, but there were no clearly evident temporal trends at any of these sites (Table 3).

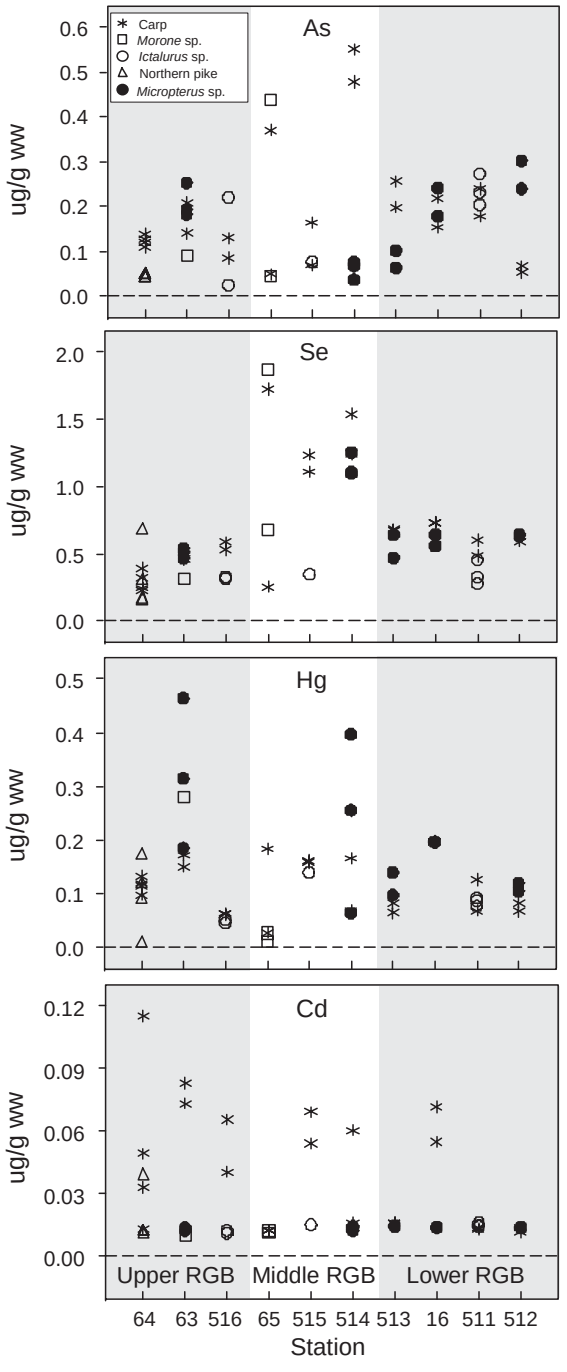

Fig. 2. Concentrations of arsenic (As), selenium (Se), and mercury (Hg) in composite samples of whole fish, by sub-basin, station, and taxon. (Note: censored values are plotted as 50% of LOD.) *Micropterus* sp., largemouth or smallmouth bass; *Morone* sp., white or striped bass; *Ictalurus* sp., blue or channel catfish.

Lead concentrations were >LOD (~0.03 μg/g ww) in 29 of 47 samples (62%) representing eight stations; of the 29, 16 (55%) were carp. Measured Pb concentrations ranged from 0.04 to 0.83 μg/g, the latter in female bass from Station 16 (data not shown). In addition to the bass from Station 16, concentrations were generally greatest in carp from Stations 511, 512, and 513 and channel catfish from Station 511. Mean concentrations were lowest at Stations 63, 65 and 515 (Table 2), and most 1997 concentrations were lower than in the past (Table 3). The exception was Station 16, where the 1997 concentrations in bass were greater than in all previous collections (Table 3). Lead was not analyzed statistically because of the many censored values.

Cadmium concentrations were uniformly low; they exceed the LOD (~0.02 μg/g ww) in only 13 of 47 samples (28%) and were also not analyzed statistically because of the many censored values. Of the 13 measured concentrations, 12 were in carp from six stations (Fig. 2). The maximum concentration was 0.12 μg/g, in a carp from Station 64 (Fig. 2). Mean concentrations in carp were ≥0.05 μg/g only at Stations 16, 63, 515, and 516 (Table 2). Concentrations in all other taxa were lower. Temporal changes were not evident in any taxa (Table 3).

Zinc was detected in all samples and was generally greater in carp and northern pike than in other taxa (data not shown). Zinc concentrations ranged from 11.1 to 83.6 μg/g, the latter in carp from Station 511. Differences among stations were statistically significant only in carp; concentrations were significantly greater at Stations 16, 63, 511, and 515 than elsewhere (Table 2). The only significant temporal change was in white bass at Station 65, where concentrations were about two-fold greater in 1997 than in all previous collections (Table 3).

3.2.2. Organochlorine pesticides

Residues of comparatively few compounds [dichlorodiphenyltrichloroethane (DDT) and its metabolites, chlordane components, dieldrin, and toxaphene)] were detected in fish. These were found primarily at lower RGB sites (Fig. 3).

Concentrations of *p,p′*-DDT, the active ingredient in commercial DDT, were <LOD (0.01 μg/g) in all 1997 samples (data not shown). In contrast, residues of *p,p′*-DDE, the most persistent metabolite of *p,p′*-

Table 2
Least-squares geometric mean concentrations[a] (all wet-weight) of *p,p′*-DDE (DDE), total mercury (Hg), length-adjusted total Hg (HgL), weight-adjusted total Hg (HgW), arsenic (As), cadmium (Cd)[b], lead (Pb)[b], zinc (Zn), and selenium (Se) in composite samples of whole fish

Taxon, station (*n*)	DDE (μg/g)	Hg (μg/g)	HgL (μg/g/m)	HgW (μg/g/kg)	As (μg/g)	Cd (μg/g)	Pb (μg/g)	Zn (μg/g)	Se (μg/g)
Carp (22)									
16 (2)	0.39a	0.20a	0.34a	0.07ab	0.18abc	0.06	0.09	67.7a	0.73abc
63 (2)	0.02b	0.16a	0.34a	0.13a	0.17abc	0.08	0.07	69.1a	0.47ad
64 (4)	0.05c	0.12a	0.24a	0.07ab	0.12bc	0.04	0.10	62.6a	0.29d
65 (2)	0.02b	0.07a	0.21a	0.13a	0.14abc	0.01	0.04	32.3b	0.66abc
511 (2)	0.52a	0.09a	0.19a	0.06abc	0.21abc	0.01	0.24	67.0a	0.54ac
512 (2)	0.07c	0.08a	0.14a	0.04abc	0.06b	0.01	0.15	61.5ab	0.61ab
513 (2)	0.21ad	0.07a	0.11a	0.02c	0.23ac	0.02	0.15	46.8ab	0.68abc
514 (2)	0.09d	0.11a	0.17a	0.03bc	0.51a	0.03	0.10	48.8ab	1.38b
515 (2)	0.15d	0.16a	0.25a	0.04abc	0.11bc	0.06	0.04	69.7a	1.17bc
516 (2)	0.29ad	0.06a	0.11a	0.03bc	0.11bc	0.05	0.11	56.9ab	0.56ac
Ictalurus sp. (6)									
511 (3)	1.43a	0.09a	0.22a	0.14a	0.24a	0.02	0.18	13.8a	0.35a
515 (1)	0.10b	0.14a	0.37a	0.16a	0.08a	0.02	0.05	16.6a	0.35a
516 (2)	0.11b	0.05a	0.12a	0.08a	0.07a	0.01	0.04	21.3a	0.32a
Micropterus sp. (12)									
16 (2)	0.38a	0.20a	0.52a	0.24a	0.21ab	0.01	0.47	13.2a	0.60ab
63 (3)	<0.01b	0.30a	0.79a	0.37a	0.21ab	0.01	0.04	15.3a	0.51a
512 (2)	0.09cd	0.11a	0.38a	0.25a	0.27b	0.01	0.04	15.8a	0.64ab
513 (2)	0.15d	0.12a	0.31a	0.13a	0.08ab	0.01	0.08	13.8a	0.55ab
514 (3)	0.05c	0.19a	0.62a	0.32a	0.06a	0.01	0.03	12.7a	1.15c
Morone sp. (3)									
63 (1)	<0.01a	0.28a	0.39a	0.12a	0.09a	0.01	0.03	19.2a	0.32a
65 (2)	0.03b	0.02b	0.07b	0.10a	0.14a	0.01	0.04	31.5a	1.13b
Northern pike (4)									
64 (4)	0.06	0.07	0.14	0.06	0.05	0.02	0.06	41.7	0.29
ANOVA									
F	41.67**	5.44**	5.97**	7.10**	4.21**	ND[b]	5.79	ND[b]	7.03**
df	67,45	61,42	61,42	61,42	54,40	ND[b]	54,40	ND[b]	48,23

Within each taxon-station group, means followed by the same letter are not significantly different ($P<0.01$, Fisher's protected LSD). Also shown are ANOVA *F*-values, degrees-of-freedom (*df*), and statistical significance (**$P\leq0.01$).

[a] Censored values were represented by 50% of the LOD in all computations.

[b] Cd and Pb not tested statistically due to large numbers of censored (i.e.,<LOD) values.

DDT, were widespread; they were detected (>0.01 μg/g) in 43 of the 47 samples analyzed (91%) and in at least one sample from all stations (Fig. 3). The greatest DDT concentrations (primarily as *p,p′*-DDE) were from Stations 16 and 511, in the lower RGB (Fig. 3, Table 2). Individual samples containing comparatively high concentrations of *p,p′*-DDE (≥0.5 μg/g) included carp from Station 16 (0.50 μg/g) and carp and channel catfish from Station 511 (0.67–1.60 μg/g; Fig. 2). Concentrations of *p,p′*-DDE differed significantly among stations in all taxa and were greatest in fish from lower RGB sites (Fig. 3, Table 2). The exception was Station 512, where concentrations were comparatively low. In addition to *p,p′*-DDE, residues of *p,p′*-DDD (TDE) were detected (>0.01 μg/g) in 23 of 47 samples representing six stations, but concentrations were uniformly low (≤0.084 μg/g; data not shown). Traces of *o,p′*-DDT (0.03–0.06 μg/g) and *o,p′*-DDD (TDE; 0.02–0.03 μg/g) were present exclu-

Table 3
Least-squares geometric mean concentrations[a] (all wet-weight) of *p,p′*-DDE, total mercury (Hg), length-adjusted total Hg (HgL), weight-adjusted total Hg (HgW), arsenic (As), cadmium (Cd)[b], lead (Pb)[b], zinc (Zn), and selenium (Se) in fish collected from 1970 to 1997 at National Contaminant Biomonitoring Program (NCBP) stations (Schmitt et al., 1999b) in the Rio Grande basin

Station, location, taxon	Year	DDE (μg/g)	Hg (μg/g)	HgL (μg/g/m)	HgW (μg/g/kg)	As (μg/g)	Cd (μg/g)	Pb (μg/g)	Zn (μg/g)	Se (μg/g)
Station 16, Rio Grande at Mission, TX										
Carp	1981	0.44	0.05*	0.11	0.04	0.15	0.03	0.10	41.0	0.40
	1997	0.39	0.20	0.34	0.07	0.18	0.06	0.09	67.7	0.73
Micropterus sp.	1980	2.57**	0.24	0.64	0.29	0.06	0.01	0.10	12.7	0.57
	1984	2.09**	0.18	0.50	0.26	0.11	0.01	0.07	13.2	0.44
	1986	2.73**	0.26	0.57	0.17	0.17	<0.01	0.12	11.6	0.49
	1997	0.38	0.20	0.52	0.24	0.21	0.01	0.47	13.2	0.60
Station 63, Rio Grande at Elephant Butte Reservoir, NM										
Carp	1972	0.11**	0.16	0.51	0.35	0.13	0.03	0.17	ND	0.64*
	1973	0.11**	0.11	0.30	0.20	0.05	0.03	0.05	ND	0.23
	1974	0.09**	ND	ND	ND	ND	ND	ND	ND	ND
	1978	0.07**	0.26	0.67	0.38*	0.04*	0.13	0.28	84.7	0.50
	1980	0.04*	0.16	0.39	0.20	0.12	0.08	0.30	68.9	0.41
	1984	0.01	0.11	0.24	0.06	0.08	0.07	0.25	51.8	0.29
	1986	0.01	0.13	0.31	0.13	0.14	0.04	0.18	52.2	0.34
	1997	0.02	0.16	0.34	0.13	0.17	0.08	0.07	69.1	0.47
Micropterus sp.	1970	0.23**	0.52	1.10	0.25	ND	ND	ND	ND	ND
	1971	0.15**	0.60	1.41	0.48	0.13	0.03	0.05	ND	ND
	1972	0.09**	0.28	0.73	0.31	0.27	0.03	0.05	ND	0.66
	1973	0.05**	0.05**	0.16**	0.08**	0.17	0.03	0.05	ND	0.20*
	1978	0.10**	0.65	1.69	0.57	0.16	0.01	0.18	16.3	0.56
	1980	0.03**	0.17	0.50	0.29	0.27	0.01	0.10	12.9	0.41
	1984	0.01	0.12	0.45	0.44	0.27	<0.01	0.01	13.8	0.36
	1997	<0.01	0.30	0.79	0.37	0.21	0.01	0.04	15.3	0.51
Morone sp.	1971	0.17**	0.63	1.55	0.69**	0.18	0.03	0.05	ND	ND
	1997	<0.01	0.28	0.39	0.12	0.09	0.01	0.03	ND	ND
Station 64, Rio Grande at Alamosa, NM										
Carp	1970	0.23**	0.03*	0.06*	0.02*	ND	ND	ND	ND	ND
	1971	0.11**	0.08	0.30	0.34**	0.04*	0.03	0.19	ND	ND
	1972	0.24**	0.05	0.13	0.05	0.08	0.03	4.65	ND	0.38
	1997	0.05	0.12	0.24	0.07	0.12	0.04	0.10	ND	0.29
Station 65, Pecos River at Red Bluff Lake, TX										
Carp	1986	0.08**	0.04	0.13	0.09	0.01**	0.01	0.28	52.9	0.63
	1997	0.02	0.07	0.21	0.13	0.14	0.01	0.04	32.3	0.66
Morone sp.	1974	0.47**	ND	ND	ND	ND	ND	ND	ND	ND
	1978	0.17**	0.12**	0.33*	0.15	0.66*	0.01	0.10	20.1	3.05*
	1980	0.14**	0.06	0.14	0.05	0.60*	0.01	0.10	11.2**	1.96
	1984	0.08*	0.06	0.22	0.22	0.24	<0.01	0.06	14.2*	1.50
	1986	0.16**	0.14**	0.31*	0.09	0.32	<0.01	0.14	13.0*	1.00
	1997	0.03	0.02	0.07	0.10	0.14	0.01	0.04	31.5	1.13
ANOVA-*F*	–	41.67**	5.44**	5.97**	7.10**	4.21**	ND[b]	ND[b]	13.45**	7.03**
df	–	67,45	61,42	61,42	61,42	54,40	ND[b]	ND[b]	37, 28	48, 23

Within each group of station-taxon means, values followed by asterisks (*) differ significantly (*$0.01 < P \leq 0.05$;**$P \leq 0.01$, Fisher's protected LSD) from 1997 means. Also shown are ANOVA *F*-values, degrees-of-freedom (*df*), and significance levels. ND, no data/not measured.

[a] Censored values (i.e.,<LOD) were represented by 50% of the LOD in all computations.

[b] Cd and Pb not tested statistically due to large numbers of censored values.

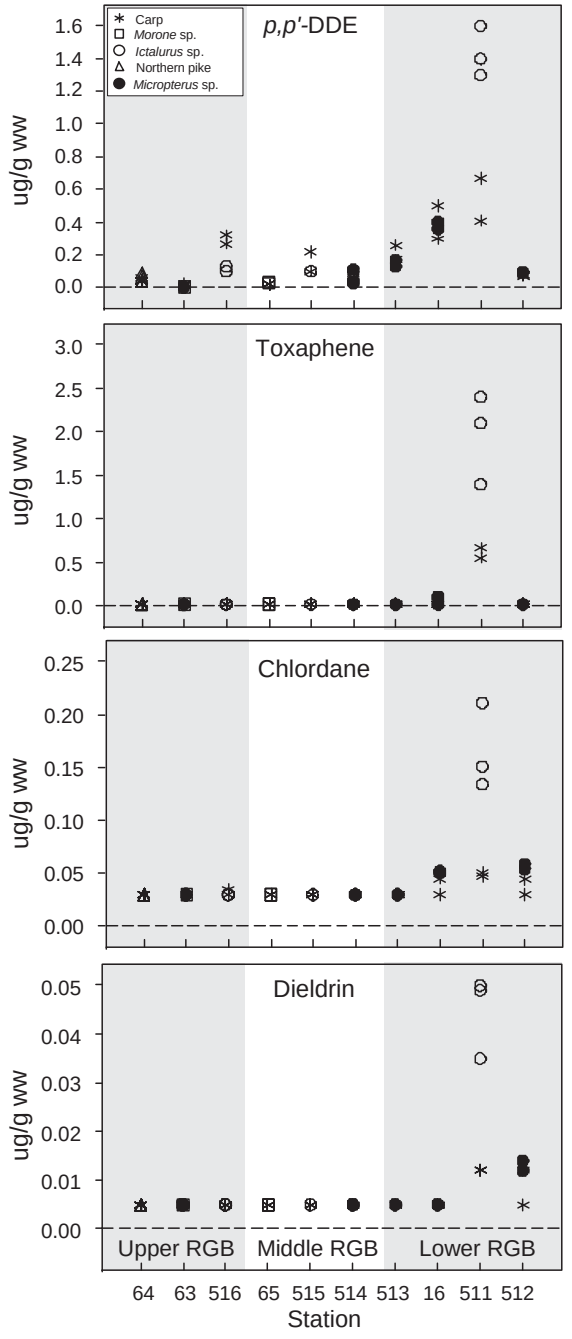

Fig. 3. Concentrations of *p,p*-DDE, total chlordanes (sum of *cis*-and *trans*-chlordanes and nonachlors, oxychlordane, and heptachlor epoxide), dieldrin, and toxaphene in composite samples of whole fish, by sub-basin, station, and taxon. (Note: censored values are plotted as 50% of LOD.) *Micropterus* sp., largemouth or smallmouth bass; *Morone* sp., white or striped bass; *Ictalurus* sp., blue or channel catfish.

sively in the three channel catfish samples from Station 511, but *o,p′*-DDE residues were not detected (<0.01 μg/g) in any samples (data not shown). Concentrations of *p,p′*-DDE declined significantly in at least one taxon at all four NCBP stations sampled in 1997 (Table 3).

Six chlordane-related compounds (*cis*- and *trans*-chlordanes and nonachlors; heptachlor epoxide; and oxychlordane) were measured. Only *cis*- and *trans*-chlordanes and nonachlors were detected and only in samples from Stations 16, 511, 512, and 516 (Fig. 3). Concentrations of oxychlordane and heptachlor epoxide were universally <LOD (0.01 μg/g). Chlordane concentrations (sum of all components) were greatest in channel catfish from Station 511 (Fig. 3). *Trans*-nonachlor was the most frequently detected chlordane constituent; it was detected (0.036–0.073 μg/g) in 12 samples (26%) representing four stations (16, 511, 512, and 516). It was also the most abundant component except at Station 511, where *cis*-chlordane was most abundant and was detected (0.017–0.073 μg/g) in both channel catfish and carp. Traces (<0.02 μg/g) of *cis*-chlordane were also detected in bass from Station 512 and carp from Station 16. *Trans*-chlordane (0.015–0.022 μg/g) and *cis*-nonachlor (0.019–0.033 μg/g) were detected only in channel catfish from Station 511.

Dieldrin was detected (>0.01 μg/g) in only 17% of the samples representing two sites (Fig. 3). Concentrations were greatest (0.035–0.05 μg/g) in channel catfish from Station 511. Both carp samples from Station 511 and three of the four samples from Station 512 (carp and largemouth bass) also contained traces of dieldrin (<0.015 μg/g).

Toxaphene was detected (>0.05 μg/g) in only six samples representing two lower RGB stations (16 and 511; Fig. 3). Concentrations at Station 16 were low (<0.1 μg/g) whereas those in fish from Station 511 were 0.56–2.40 μg/g, with those in channel catfish greatest.

3.2.3. Total PCBs and TCDD-EQ (data not shown)

Total PCB concentrations were <LOD (0.05 μg/g) in all 1997 samples. The H4IIE bioassay also detected relatively low dioxin-like activity; TCDD-EQ was ≤6 pg/g in all samples, and was ≥4 pg/g in only three samples (one from Station 511, two from Station 512).

3.2.4. Hepatic ethoxyresorufin O-deethylase (EROD) activity

In carp, statistically significant ANOVA models containing the factors station, gender, gonadal stage, and their interactions explained 54–56% of the total variation in EROD activity ($F_{28,\ 163}=7.53$, $P<0.01$). Differences among stations were highly significant ($F_{9,\ 172}=19.62$, $P<0.01$) as were those between genders ($F_{1,\ 172}=13.24$, $P<0.01$); activity was greater in males than in females. Station–gender interaction was not significant ($F_{9,\ 172}=1.00$, $P>0.05$), indicating that most among-station differences were similar in both genders; EROD activity was elevated at Stations 511 and 512 (means=8.70–32.6 pmol/min/mg) and lowest at Station 64 (0.25–0.34 pmol/min/mg) in both genders (Table 4). However, EROD activity was also elevated relative to most sites in male carp from Station 516 (mean=9.24 pmol/min/mg), but not in female carp (Table 4). Slightly elevated EROD rates (>4 pmol/min/mg) characterized at least one carp from all sites. In addition, one carp from Station 65 and most from Stations 511 and 512 were >10 pmol/min/mg.

In bass, ANOVA models containing the factors station, gender, gonadal stage, and their interactions were also statistically significant ($F_{15,\ 56}=4.91$, $P<0.01$) and explained 57% of the total variation in EROD activity. Of 30 male bass analyzed, only two had EROD rates <20.0 pmol/min/mg and most females were >16.0 pmol/min/mg (Table 4). Activity in bass was generally greatest at Stations 63 and 512 in both males and females; however, bass were not obtained at Stations 511 and 516, where elevated EROD rates were detected in carp, and only female bass were obtained at Station 63. All values in bass from Stations 63 and 512 were >25.0 pmol/min/mg (Table 4). In largemouth bass, differences among stations were highly significant ($F_{4,\ 63}=8.90$, $P<0.01$) as were those between genders ($F_{1,\ 63}=4.85$, $P<0.05$). As was true for carp, activity was significantly greater in males than in females and interactions between station and gender were not significant ($F_{4,\ 63}=0.96$, $P>0.05$), indicating that the geographic differences were consistent between genders. Activity was significantly lower in largemouth bass of both genders from Station 514 than from all other sites and was greatest at Station 512 (Table 4).

In addition to carp and bass, EROD activity was measured in 47 fish representing five other species (channel catfish, blue catfish, northern pike, white bass, and striped bass) from five of the 10 stations sampled (Table 4). In channel catfish, EROD rates differed significantly among stations ($F_{1,\ 37}=9.46$, $P<0.01$) but not genders ($F_{2,\ 37}=0.26$, $P>0.05$). The interaction of station and gender was also not significant ($F_{2,\ 37}=2.33$, $P>0.05$). Activity was several-fold greater in channel catfish from Station 511 than in either channel catfish from Station 516 or blue catfish from Station 515, and the channel catfish differences were consistent for males, females, and juveniles (Table 4).

3.3. Fish health indicators

3.3.1. External lesions and health assessment index (HAI)

Of the 368 fish examined, 28% had some type of external lesion, most of which were identified as eroded, frayed, or hemorrhagic fins. Lesion frequencies (all species combined) ranged from 2% at Station 65 to 75% at Station 515 (data not shown). Of the 207 carp examined, 29% had external lesions. Percentages for carp were lowest at Stations 16, 65, and 512 and highest at Stations 516 and 515. No carp from Station 65 had external lesions where occurrence was 90% at Station 515, which was 3–9 times greater than most other stations. Of the 75 bass examined, 27% had external lesions. Percentages for bass were lowest at Stations 16 (1%) and 513 (14%) but were ≥50% at Stations 512 and 514.

Most (90%) of the HAI scores for carp were between 20 and 90, and all station means except for Station 65 were between 30 and 70; Station 65 scored lower than most (Fig. 4). Station means were >60 only at Stations 511 and 515, and except for Station 515, 80–100% of the carp from each station scored <100 (Fig. 4). For bass, most HAI scores (90%) were between 10 and 110, but only the Station 512 mean was <50; means for Stations 514 and 63 were >60, and no individual bass from these sites scored <40. The greatest means for carp and bass also did not occur at the same stations, but bass were not collected at Stations 511 or 515, where scores in carp were generally greatest. Statistically significant ANOVA models that included the factors station, gender, stage, and their interactions accounted for 28% of the total HAI variation (rank-transformed) in carp and 41% in

Table 4
Geometric means[a] and ranges of hepatic ethoxyresorufin *O*-deethylase (EROD) activity (all in pmol/min/mg protein), by species, station, and gender

Species and station	Female			Male			Juvenile[b]		
	n	Range	Mean	*n*	Range	Mean	*n*	Range	Mean
Carp[c]									
16	10	0.76–3.35	1.31a	10	0.72–4.47	2.89b	0	–	–
63	8	0.18–4.95	1.81a	9	0.18–27.2	4.76c	0	–	–
64	19	0.07–2.72	0.25b	14	0.08–7.84	0.34a	1	–	0.07
65	10	0.03–55.5	1.90a	10	0.03–7.38	1.54b	0	–	–
511	13	0.06–161	16.8c	7	20.1–54.2	32.6e	0	–	–
512	6	3.19–15.1	9.44c	8	2.0–18.8	8.70df	0	–	–
513	11	0.49–7.98	1.80a	9	2.58–6.88	3.97bc	0	–	–
514	10	0.12–3.42	1.42a	10	0.06–8.74	3.46bc	0	–	–
515	6	0.02–2.98	0.86a	4	0.57–4.50	2.13bcd	0	–	–
516	10	0.81–2.83	1.49a	8	2.26–28.0	9.24ef	0	–	–
Largemouth bass[d]									
16	11	9.09–58.5	28.6ab	10	33.5–132	54.8a	0	–	–
63	4	38.5–81.0	57.7c	0	–	–	2	62.9–130	90.3
512	7	28.1–107	67.4c	8	46.2–107	75.9a	1	–	111.2
513	13	15.5–96.6	37.2b	9	19.7–83.5	50.6a	0	–	–
514	5	5.00–44.3	21.3a	2	13.0–22.3	17.0b	0	–	–
Smallmouth bass									
63	2	79.6–147	108	0	–	–	0	–	–
514	0	–	–	1	–	52.6	0	–	–
Striped bass									
63	1	–	<0.1	0	–	–	0	–	–
White bass									
65	18	2.50–22.4	9.52	3	2.77–8.36	4.34	0	–	–
Blue catfish									
515	3	7.19-17.2	10.3	0	–	–	3	9.75–12.2	11.0
Channel catfish[e]									
511	8	17.9–71.7	40.6a	3	12.8–27.9	20.3a	9	18.9–111	38.9a
516	11	6.12–23.1	12.2b	2	15.8–19.5	17.5a	9	9.3–25.4	14.1a
Northern pike									
64	6	0.14–1.77	0.52	10	0.18–2.30	0.69	0	–	–

Within each species–gender group for common carp, largemouth bass, and channel catfish, means followed by the same letter are not significantly different ($P>0.05$, Fisher's protected LSD).

[a] Censored values (i.e.,<LOD) were represented by 50% of the limit-of-quantitation in the computation of geometric means.

[b] May include fish of undetermined gender from which no gonad sample was obtained.

[c] ANOVA $F_{(19,\ 172)}=10.68$, $P<0.01$, $R^2=0.54$.

[d] ANOVA $F_{(8,\ 63)}=5.58$, $P<0.01$, $R^2=0.42$.

[e] ANOVA $F_{(5,\ 32)}=9.46$, $P<0.01$, $R^2=0.60$.

bass. Differences among gonadal stages were statistically significant in both taxa, as was the interaction of station, stage, and gender in bass. However, after accounting for all other effects, differences among stations were not significant in carp and only approached significance in bass ($F_{4,\ 56}=2.01$, $P=0.11$). Differences between genders were not significant in either carp or bass.

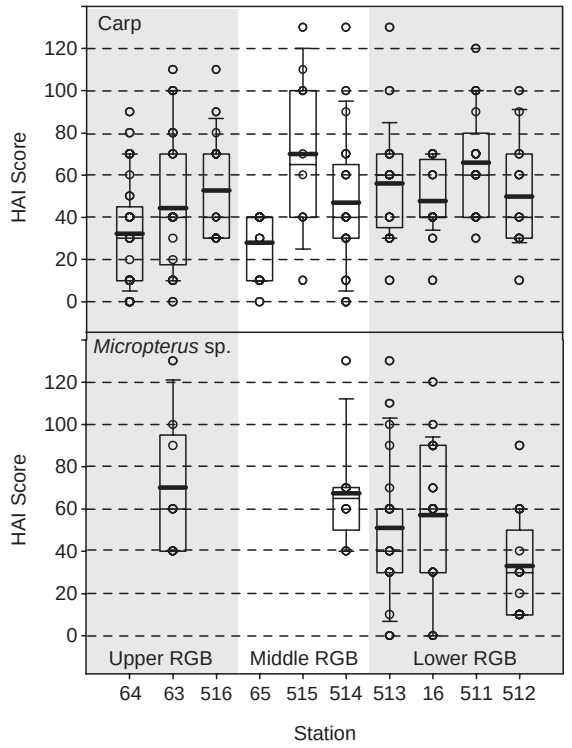

Fig. 4. Health assessment index (HAI) scores of carp and bass (*Micropterus* sp.), by sub-basin and station. Shown for each group are points representing individual fish and the mean (heavy horizontal line), median (light horizontal line), interquartile range (box), and the 10th and 90th percentiles (whiskers). Stations are ordered from upstream to downstream and are grouped by sub-basin.

Internal and external lesions contributing to elevated HAI ratings in carp and bass were examined histopathologically to determine their cause. Overall, most microscopic lesions were diagnosed as inflammatory and were usually associated with parasites. In bass, abnormal ratings at Station 63 were primarily due to frayed or marginate gills and abnormal livers; the latter were all associated with helminth parasites. Most of the abnormal ratings for Station 16 bass resulted from internal nodules, focal discolorations, or spots on the liver, kidney, and spleen, which were also determined to be parasite-induced. Many Station 513 bass kidneys were rated in the field as urolithic; however, histologically no calcifications were noted and these lesions were also determined to be parasite-induced. In carp, high HAI scores at Station 515 were primarily due to external lesions; the only abnormal internal ratings were for tan livers, which were also noted at Stations 511, 513, and 516. Two Station 515 carp had unusual inflammatory reactions in the epidermis or hypodermis associated with refractile, crystalline material (Fig. 5A, B). In addition, two carp from each of Stations 516 and 514 had papillomas of unknown etiology on the body surface or oral cavity (Fig. 5C). Histologically, the tan livers were found to contain hepatocytes with increased vacuolization, and ceroid/lipofuscin deposits were often contained within or replacing hepatocytes. Ceroid-containing cells were also present within the blood vessel walls in the livers of many carp from these stations (Fig. 5D). Four of 14 carp from Station 512 had abnormal kidneys rated as granular. In these four fish (and in one not identified as granular in the field), and in contrast to the bass described previously, histopathological analysis revealed urolithiasis or nephrocalcinosis (Fig. 5E).

Among the changes detected during histopathological examination was an apparent proliferation of thyroid follicles in the posterior kidney of carp from some sites (Fig. 5F); these were not evident during gross examination. Carp kidneys from Stations 63, 64 and 516 contained few thyroid follicles and those that were present tended to be small whereas greater numbers and larger size characterized the follicles of carp from stations further downstream, and the kidneys of one carp from Station 514 contained a large area of abnormal-appearing thyroid follicles. Further analyses are being conducted to determine thyroid hormone status and to provide additional quantitative and qualitative information on the kidney thyroid follicles.

3.3.2. Condition factor and organosomatic indices (data not shown)

Condition factor differences in carp were not statistically significant; an ANOVA model that included the factors station, gender, gonadal stage, and their interactions accounted for only 9% of the total variation ($F_{28,\ 176}=0.67$, $P>0.05$). Station means ranged from 1.2 at Stations 63 and 512 to 1.6 at Stations 513 and 514, and 90% of the individual values were between 1.1 and 1.8. In contrast to carp, ANOVA was highly significant ($F_{15,\ 56}=12.94$, $P<0.01$) and explained 78% of CF variation in bass; among-station differences were also significant ($F_{4,\ 56}=3.78$, $P<0.01$). Station means ranged from 1.4 at Stations

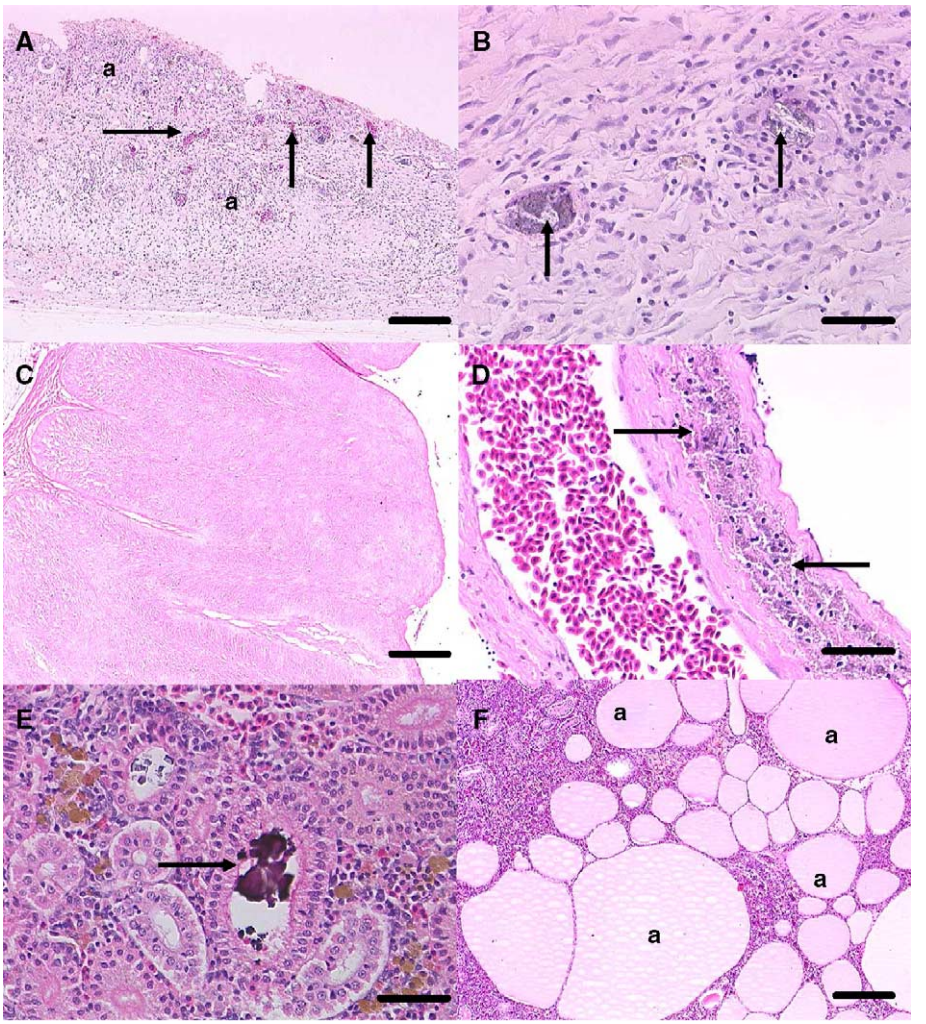

Fig. 5. A. Histologic appearance of external gross skin lesions observed in carp from station 515 in the Rio Grande basin. Inflammatory responses included chronic inflammation (a) and congestion (thin arrows) throughout a thickened epidermis and dermis. Scale bar=200 μm. B. Higher magnification of a portion of (A) illustrating the refractile, crystalline material that appears to elicit this inflammatory response (arrows). Scale bar=50 μm. C. Papilloma in the skin of carp from Station 514. Scale bar=200 μm. D. Pigmented cell accumulations within the hepatic blood vessel walls (arrows) typical of carp from Stations 515, 511, 513 and 516. Scale bar=50 μm. E. Nephrocalcinosis or mineral deposition within tubules (arrow) was observed at Station 512. Scale bar=50 μm. F. Posterior kidney tissue of carp from station 513 illustrating accumulations of enlarged thyroid follicles (a). Scale bar=200 μm. H and E stain.

16 and 63 to 1.8 at Station 514, and 90% of the bass examined had CF values of 1.2–2.0.

Hepatosomatic index differences (bass only) were not statistically significant; an ANOVA model that included the factors station and gender and their interactions explained only 12% of the total variation ($F_{7,\ 57}=1.10$, $P>0.05$). Station means for HSI ranged from 0.9% at Station 16 to 1.1% at Station 514. Most individual values were 0.6–1.5%, and only one fish from each of Stations 513 and 514 were >1.5%.

Splenosomatic index in carp differed significantly among stations ($F_{9,\ 183}=6.26$, $P<0.01$) and between genders ($F_{1,\ 183}=11.81$, $P<0.01$); ANOVA explained 31% of the total variation. In male carp, SSI station means ranged from 0.15% (Station 515) to 0.44% (Station 516). The lowest individual values (0.01%)

occurred at Stations 512 and 515; all others were ≥0.08% whereas individual male carp with SSI values >0.5% were captured at Stations 64, 514, and 516. In female carp, SSI station means ranged from 0.13% at Station 512 to 0.28% at Station 514, and 90% of the individual values were between 0.08% and 0.32%. Very low individual values (0.01%) also characterized one female carp from each of Stations 512 and 515 whereas the maximum value (0.80%) was represented by a fish from Station 64. In contrast to carp, SSI differences in bass only approached statistical significance ($F_{8,\ 63}=1.92$, $P=0.07$); ANOVA explained only 19% of the total variation. Values ranged from 0.01% in several fish from Stations 16, 512, and 513 to 0.8% in a largemouth bass from Station 16, but SSI varied greatly within stations. Station means ranged from 0.07% at Station 512 to 0.22% at Station 514.

3.3.3. Macrophage aggregates

All three MA parameters differed significantly ($P<0.05$) among stations in carp, but only MA-# and MA-% differences were significant in bass (Table 5). In addition, age significantly ($P<0.05$) affected MA-A and MA-% in both taxa (Table 5). With the genders combined, a 1-y increase in age was associated with a multiplicative change of 1.17 (16%) in median MA-A in bass and 1.45 (44%) in carp. For MA-%, a 1-y increase in age was associated with a multiplicative change of 1.25 (24%) in bass and 1.23 (23%) in carp. Overall, age-adjusted MA parameters in carp were universally smallest at

Table 5
Age-adjusted station means for splenic macrophage aggregate (MA) parameters in carp and bass (*Micropterus* spp.; none collected at Stations 64, 65, 511, 515, or 516)

Sub-basin and station	Carp			Bass		
	MA-# (no./mm^2)	MA-A (μm^2)	MA-% (%)	MA-# (no./mm^2)	MA-A (μm^2)	MA-% (%)
Upper Rio Grande						
64	9.15 cd	3431 bc	2.99 c	–	–	–
63	8.97 bcd	4063 bcd	3.22 c	5.37 b	3364 a	1.66 abc
516	6.54 bc	3989 bc	2.46 bc	–	–	–
Middle Rio Grande/Pecos						
65	10.09	1911 ab	1.73 bc	–	–	–
515	12.29 cde	3945 cd	4.73 cd	–	–	–
514	3.46 a	1430 a	0.30 a	4.31 b	2477 a	0.89 bc
Lower Rio Grande						
513	2.95 a	1484 a	0.30 a	4.91 b	2984 a	1.25 bc
16	8.35 bcd	1756 ab	1.11 bc	7.70 a	3685 a	2.67 a
511	7.82 bcd	3258 bc	1.79 bc	–	–	–
512	14.51 e	6393 d	8.49 d	4.97 b	3146 a	1.44 bc
ANCOVA						
Model	9.95**[a]	9.18**[a]	14.50**[a]	5.73**[b]	4.03**[b]	5.23**[b]
Station	10.38**[c]	7.70**[c]	13.19**[c]	5.65**[d]	1.10ns[d]	3.16*[d]
Age	2.18ns[e]	15.41**[e]	18.01**[e]	1.68ns[f]	9.99**[f]	6.64*[f]

Shown are arithmetic mean MA density (MA-#) and geometric mean MA area (MA-A) and percent tissue occupied (MA-%) adjusted to the basin-wide mean age for each species (3.2 y for carp, 1.8 y for bass) using analysis-of-covariance (ANCOVA). Also shown are ANCOVA *F*-values and degrees-of-freedom (*df*) for the analyses (**$P\leq0.01$; *$0.01<P\leq0.05$; ns, $P>0.05$). Within each column, means followed by the same letter are not significantly different ($P>0.05$, Fisher's protected LSD).

[a] *df*= 10, 166.
[b] *df*= 5, 68.
[c] *df*= 9, 166.
[d] *df*= 4, 68.
[e] *df*= 1, 166.
[f] *df*= 1, 68.

Stations 65, 513, and 514, and were largest at Station 512; all other stations were intermediate (Table 5). In addition, there was a gradient towards increasing values of all three MA parameters in carp with distance downstream in the lower RGB (Table 5). In bass, MA-# and MA-% were significantly greater at Station 16 than at the other four sites from which bass were collected, but the latter did not differ (Table 5). As a group, differences among stations were less evident for bass than for carp (Table 5), but values of all MA parameters in bass were also relatively low at Station 514 and high at Station 16. Mean MA values (all three parameters) in bass from Station 512 were intermediate (Table 5), but the greatest individual MA-A and MA-% values for bass also occurred at Station 512 (data not shown).

3.4. Reproductive biomarkers

3.4.1. Gonadosomatic index (GSI)

ANOVA models containing the factors station, gender, stage, and their interactions were statistically significant for GSI in both carp ($F_{28,175}=16.26$, $P<0.01$) and bass ($F_{15,\ 56}=8.73$, $P<0.01$). In carp, the model explained 72% of the total variation; differences among stations were significant ($F_{6,\ 175}=4.58$, $P<0.01$), but not those between genders ($F_{1,\ 163}=0.19$, $P>0.05$). GSI in female carp ranged from 0.3% in a stage-0 fish from Station 511 to 36.4% in a stage-2 fish from Station 16 (Fig. 6). Female carp from Stations 16, 513, and 514 had proportionately larger ovaries than those from most other stations, but there was wide variation that only partly corresponded to stage differences among the stations (Fig. 6). Carp ovaries from Station 511 were proportionally smaller than at most stations (Fig. 6), reflecting the earlier collection date at this site (Table 1). The distribution of GSI in male carp generally paralleled that of female carp and, like the females, was partly related to stage and collection date differences. Individual values ranged from 2.2% to 20.3% and were generally lowest at Station 511 (Fig. 6).

ANOVA accounted for 70% of total GSI variation in bass. Differences between genders were significant ($F_{1,\ 56}=7.85$, $P<0.01$), but among-station differences only approached significance ($F_{4,\ 56}=2.42$, $P=0.06$). All GSI values in bass were between 0.25% and 1.2% except for three fish—one (of two) stage-3 small-

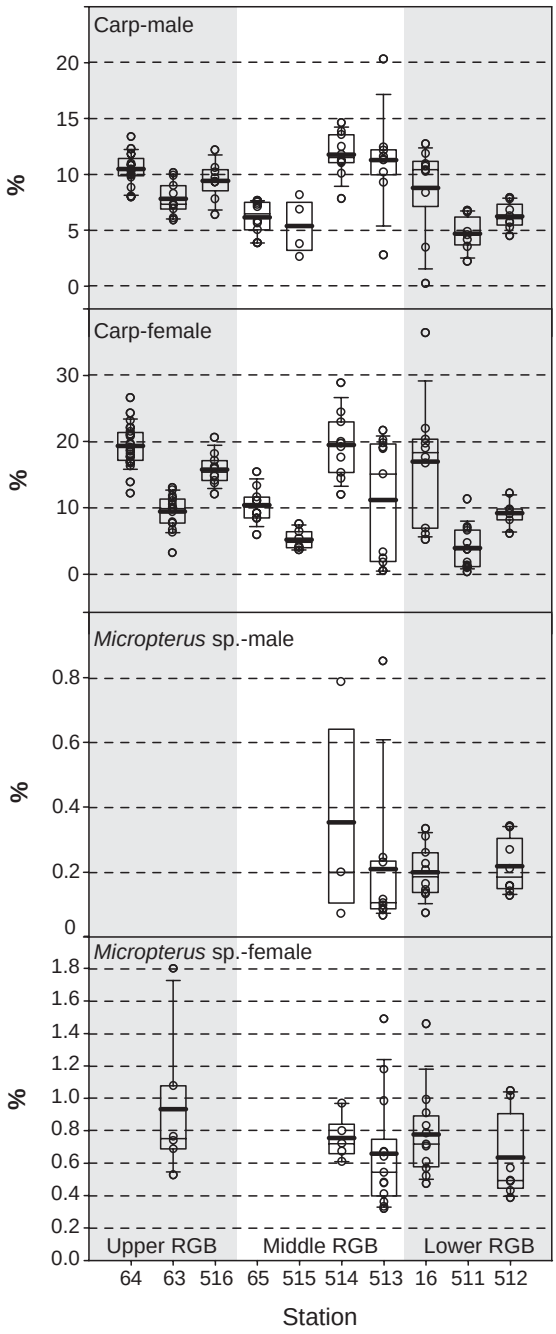

Fig. 6. Gonadosomatic index (GSI) values of male and female carp and bass (*Micropterus* sp.), by station. Shown for each group are points representing individual fish and the mean (heavy horizontal line), median (light horizontal line), interquartile range (box), and the 10th and 90th percentiles (whiskers). Stations are ordered from upstream to downstream and are grouped by sub-basin.

mouth bass from Station 63 (1.8%) and one stage-2 largemouth bass from each of Stations 16 (1.5%) and 513 (1.5%), and most values were between 0.35% and 1.0% (Fig. 6). The proportional size of the ovaries of the two stage-4 females from Station 514 was within the range spanned by other female bass (Fig. 6).

3.4.2. Gonadal histopathology

Ovary samples representing 113 female carp from all 10 stations were examined; only gonadal stages 0–2 were present. Stage 2 was predominant (82% overall) and represented 64–100% of the female carp examined at most sites. Overall, female carp from Stations 511, 512, and 513 were less advanced (mostly stages 0 and 1) than at the other sites (mostly stages 1 and 2), reflecting the earlier collection date and lower GSI values at Station 511 (Table 1, Fig. 6). One 3-y old female carp from Station 64 could not be staged because no normal gonad tissue was observed; all ovarian follicles showed signs of degeneration, inflammation, and fibrosis.

Ovary samples representing 42 female bass from five stations were examined; all were largemouth bass except for two (stage-3) smallmouth bass from Station 63. Gonadal stages 0–4 were represented; most (76%) were in stage 1. Stage-0 (immature) female bass were collected exclusively at Station 512 (two of seven) whereas stage-4 females (two of five) were obtained only at Station 514.

Varying degrees of oocyte atresia were detected in female carp and bass. ANOVA models that included terms for station, age, gonadal stage, and the interactions of these terms were statistically significant in both carp ($F_{25,\ 71}=2.69$, $P<0.01$) and bass ($F_{17,\ 23}=25.41$, $P<0.01$). ANOVA accounted for 49% of the total variation in carp and 95% in bass, but differences among stations were not significant in either taxon ($F<1.0$, $P>0.05$) after accounting for all other factors. Atresia was typically <20% in both taxa (Fig. 7); however, it was 50–80% in the two stage-4 female bass from Station 514. Further histopathological examination revealed heavy microsporidian parasite infections within the oocytes of these two fish (Fig. 8A, B). Although the parasites did not appear to have penetrated the previtellogenic oocytes, the more advance oocytes were affected; they consequently appeared to have progressed to stage 4 but were not released. The oocytes observed were in various stages

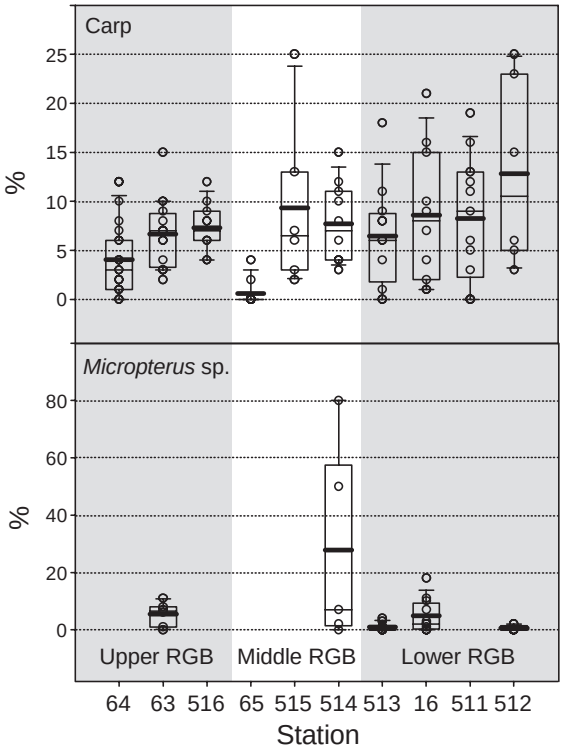

Fig. 7. Percentage of atretic oocytes in female carp and bass (*Micropterus* sp.), by station. Shown for each group are points representing individual fish and the mean (heavy horizontal line), median (light horizontal line), interquartile range (box), and the 10th and 90th percentiles (whiskers). Stations are ordered from upstream to downstream and are grouped by sub-basin.

of degeneration and necrosis and had begun to be reabsorbed (Fig. 8A).

Testes samples representing 92 male carp from all 10 stations were examined; gonadal stages 0 through 3 were represented. Most were in more advanced gonadal stages than females from the same sites; 89% were in stage 3. As noted for females, most male carp from Station 511 were in less advanced gonadal stages (predominantly stage 2) than at the other stations, reflecting the earlier collection date and lower GSI values (Table 1, Fig. 6).

Testes samples representing 30 male bass from four stations were examined. All were largemouth except for one smallmouth bass from Station 514. Gonadal stages 1–3, the latter only in the smallmouth bass, were represented; however, no gonad samples were obtained from the Station 63 male bass. As was true for male carp, the male bass examined were generally

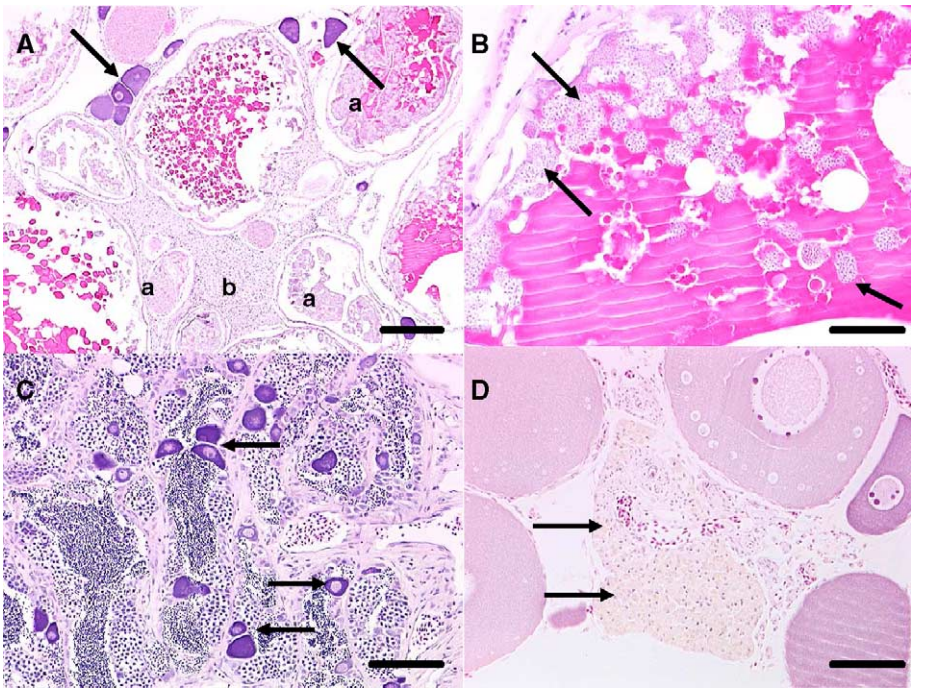

Fig. 8. A. Ovarian section of a largemouth bass from Station 514. Developing, vitellogenic oocytes in various stages of degeneration (a) and infected with an unidentified microsporidian parasite. Many ceroid/lipofuscin pigment accumulations (b) are also present. Previtellogenic oocytes (arrows) show no signs of infection. Scale bar=200 μm. B. Higher magnification illustrating the presence of numerous microsporidian parasites (arrows). Scale bar=50 μm. C. Intersex in male bass from station 512. Previtellogenic oocytes (arrows) can be observed within the testes. Scale bar=100 μm. D. Pigmented cell accumulations (arrows) in the ovary of carp. The amount of these ceroid-lipofuscin pigments was rated on a scale of 0 (no pigment) to 4 (heavily pigmented). Scale bar=100 μm. H and E stain.

in more advanced gonadal stages than females, and among-station differences were less evident. Ten male bass (all largemouth) representing three stations showed evidence of ovotestis as identified by the presence of developing oocytes in an otherwise normal male gonad (Fig. 8C). This intersex condition was detected in two of 10 fish from Station 16, four of eight from Station 512, and four of nine from Station 513. All of the intersex fish were identified as being in either gonadal stage 1 or 2. None of three male bass from Station 514 (two largemouth, one smallmouth) examined were intersex. Ovotestis was not detected in male carp.

Pigmented cell accumulations were detected in the gonads of both male and female carp and bass from the RGB (Fig. 8D). The amount of these pigments varied from absent to moderately abundant, and tended to increase with age. Greater amounts were observed in females of both species, but differences among stations were not evident (data not shown).

3.4.3. Vitellogenin (vtg)

Concentrations of vtg in most male carp and bass were <LOD (0.005 mg/mL in carp, <0.002 mg/mL in bass) and the data were not analyzed statistically. However, concentrations exceeded the LOD in at least one male carp from Stations 16, 63, 64, 511, 514, and 515 (data not shown). Except for one stage-2 fish from Station 511, all male carp in which vtg was detected were in gonadal stage 3, and most of the measured concentrations were low (<0.02 mg/mL). Only three values exceeded 0.02 mg/mL; these fish were from Stations 64 (0.08 mg/mL) and 511 (0.18 and 1.61 mg/mL). Vitellogenin was detected in only one of 27 male bass, a stage-2 fish from Station 513 (3.15 mg/mL).

In female carp and bass, ANOVA models that included the factors station, gonadal stage, age, and their interactions were highly significant; they explained 87% of the total variability in carp ($F_{25,\ 69}$= 19.01, P<0.01) and 71% in bass ($F_{17,\ 23}$=3.50, P<0.01), but differences among stations were not

significant ($F<1.0$, $P>0.5$) in either taxon after accounting for all other factors. In female carp, concentrations ranged from <0.0005 to 3.7 mg/mL and tended to increase with stage (Fig. 9). However, concentrations in stage-1 female carp from Station 513 were much lower (mean=0.1 mg/mL) than at all other sites (means=1.2–2.0 mg/mL). Concentrations of vtg in female bass ranged from <0.005 to 9.9 mg/mL; the latter was a stage-1 fish from Station 513 (Fig. 9). In addition to this fish, concentrations were also greater than most in a stage-2 bass from Station 513 (7.2 mg/mL). Conversely, the two stage-3 smallmouth bass from Station 63 contained <0.005 mg/mL (Fig. 9). Concentrations were 2.9–3.3 mg/mL in the two parasite-infected largemouth bass from Station 514 (Fig. 8A, B).

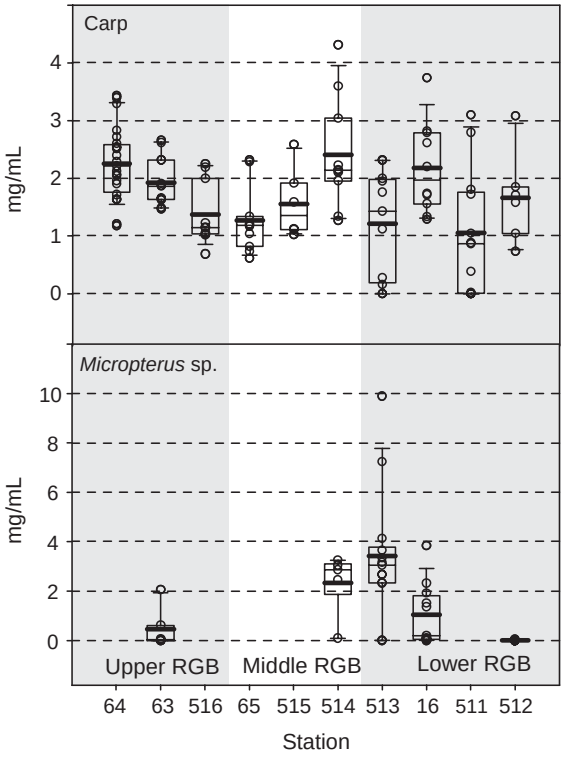

Fig. 9. Plasma vitellogenin (Vtg) concentrations in female carp and bass (Micropterus sp.), by station. Shown for each group are points representing individual fish and the mean (heavy horizontal line), median (light horizontal line), interquartile range (box), and the 10th and 90th percentiles (whiskers). Stations are ordered from upstream to downstream and are grouped by sub-basin.

4. Discussion

4.1. Exposure indicators

Of the elemental contaminants measured, only Hg, As, and Se concentrations at some stations exceeded toxicity thresholds or were elevated relative to concentrations documented by previous investigations (Table 6). Elevated concentrations of all three had also been documented previously in the RGB. In contrast, concentrations of Pb, Cd, and Zn were either generally low or elevated in few samples.

It is generally accepted that most (>90%) of the Hg in fish occurs as the highly toxic methylmercury (Bloom, 1992; Southworth et al., 1995). Concentrations of total Hg in bass collected in 1997 from Station 63 were 0.30–0.50 μg/g (Fig. 2, Table 6), which is consistent with an existing consumption advisory for Elephant Butte Reservoir. Concentrations were lower, but nevertheless elevated relative to most sites, in other predatory fishes from Station 63. Elevated concentrations of Hg were also detected in bass from Stations 514 (Amistad Dam, TX) and 16 (Rio Grande at Mission, TX; Fig. 2, Table 6). In the past, elevated Hg concentrations were also reported in fish from the Rio Grande at El Paso (USEPA, 1992), but 1997 concentrations at Station 516 were universally <0.06 μg/g (Fig. 2). Overall, Hg concentrations in bass from the RGB were generally similar to those reported for the Mississippi River Basin (MRB) in 1995 (Schmitt et al., 2002). Elevated concentrations of Hg are well documented in Elephant Butte Reservoir (Ong et al., 1991; Caldwell et al., 1999; Schmitt et al., 1999b; Canavan et al., 2000) and elsewhere in the RGB. Mercury, along with Se, is suspected of having caused the reproductive failure of peregrine falcons (*Falco peregrinus*) in Trans-Pecos Texas (Mora et al., 2002), where cinnabar was mined historically (Sharpe, 1980). Concentrations of Hg were >0.3 μg/g in at least one sample from Stations 513 and 514, which may represent a threat to piscivorous birds, and at least one sample from all other stations except Stations 64 and 516 exceeded 0.1 μg/g, a level that may represent a threat to piscivorous mammals (Yeardley et al., 1998; Wiener et al., 2002).

Fish are at greatest risk from environmental Hg during embryonic and larval stages, partially as a

Table 6
Summary of chemical and biological findings indicative of exposure to contaminants, by sub-basin and station (designations are relative)

Sub-basin and station	Contaminants and EROD activity	Fish health indicators	Reproductive biomarkers
Upper Rio Grande			
64	EROD (c)	(None observed)	Vtg (mc), ovarian degeneration (fc, $n=1$)
63	Cr (mc), Hg (b, stb), EROD (c, b)	CF (c-), HAI (c, b)	Vtg (fb-)
516	DDE (c), EROD (c)	HAI (c)	(None observed)
Middle Rio Grande			
65	As (mwb, mc), Se (mwb, mc, fc), EROD (c, b)	CF (c-)	(None observed)
515	Se (c)	EL (c), HAI (c)	(None observed)
514	As (c), Se (c, b), Hg (b), EROD (c)	SSI (b), EL (b)	Stage (fb); atresia (fb); ceroid (fb); GSI (fb); ovarian parasites (fb, $n=2$)
Lower Rio Grande			
513	Ni (fb), Cr (fb), EROD (b)	HAI (c, b)	Vtg (mb), ovt (mb)
16	Pb (fb), DDE (mc)	(none observed)	Ovt (mb)
511	DDE (mc, ccf), tox (c, ccf), chlordane (ccf), dieldrin (ccf), EROD (c, ccf)	HAI (c)	Stage (c), vtg (mc)
512	Ni (mc), TCDD-EQ (mb),EROD (c, b)	CF (c-), HAI (b-), MA (mc, fc), EL (b)	Stage (b), atresia (fc), ovt (mb),vtg (fb-)

Male (m) and female (f) bass and carp were collected from all sites unless otherwise indicated. If gender is not specified, then the indicated condition was present in both. Additional abbreviations. DDE, *p,p′*-DDE; tox, toxaphene; chlordane, sum of *cis*- and *trans*-chlordanes and nonachlors; oxychlordane; and heptachlor epoxide; TCDD-EQ, dioxin-like activity as determined by H4IIE bioassay; As, arsenic; Cr, chromium; Hg, mercury; Pb, lead; Ni, nickel; EROD, hepatic ethoxyresorufin *O*-deethylase activity; EL, external lesions; CF, condition factor; vtg, vitellogenin; HAI, health assessment index; SSI, splenosomatic index; GSI, gonadosomatic index; ovt, ovotestis; MA, macrophage aggregates (one or more parameters); b, bass (*Micropterus* sp.); c, carp (*Cyprinus carpio*); ccf, channel catfish (*Ictalurus punctatus*); wb, white bass (*Morone chrysops*); stb, striped bass (*Morone saxatilis*); - indicates that the response or condition was smaller or lower than most; all others larger or greater.

consequence of maternal transfer (Wiener and Spry, 1996). Behavioral effects in laboratory studies have been documented in fish containing whole-body Hg concentrations as low as 0.7 μg/g (Kania and O'Hara, 1974), but other studies have shown behavioral effects only at total Hg concentrations of 5–10 μg/g (Wiener and Spry, 1996; Wiener et al., 2002). Juvenile grayling (*Thymallus thymallus*) containing whole-body concentrations of 0.27 μg/g resulting from dietary methylmercury exposure experienced permanent impairment of feeding efficiency and competitive ability (Fjeld et al., 1998). In female fathead minnows (*Pimephales promelas*), dietary methylmercury concentrations of 0.87 μg/g dw (about 0.17 μg/g ww assuming 80% moisture) increased whole-body Hg concentrations by more than 10-fold, suppressed hormone levels, and inhibited gonadal development relative to controls (Drevnick and Sandheinrich, 2003). It therefore appears that the greatest concentrations observed in fish from the RGB (~0.5 μg/g) may be sufficiently high to represent a risk to the fish themselves. However, as noted by Wiener et al. (2002), many factors can contribute uncertainty to the estimation of toxicity thresholds from tissue concentrations, and further study would be necessary to document such effects in RGB fish.

Comparatively high Se concentrations (>1.0 μg/g) characterized fish from Stations 65, 514, and 515 (Fig. 2, Table 6) and confirmed previous findings for the central RGB. The Se concentration in *Morone* sp. from Station 65 was lower in 1997 than in previous collections at this site, however (Schmitt et al., 1999b). Concentrations of Se in all RGB fish were considerably lower than the 3–5 μg/g reported for a site in the MRB with a history of contamination from irrigated agriculture that was sampled in 1995

(Schmitt et al., 2002, 2004). Numerous studies have demonstrated that the diet is the primary route of Se exposure and toxicity in aquatic vertebrates (see reviews by Lemly (2002) and Hamilton (2004)). Whole-body Se concentrations of 8–16 μg/g dw (1.6–3.2 μg/g ww assuming 80% moisture) have been associated with reproductive failure in fathead minnow (Schultz and Hermanutz, 1990) and bluegill (*Lepomis macrochirus*; Coyle et al., 1993; Gillespie and Baumann, 1986; Hermanutz et al., 1992); in fish, maternal transfer to eggs and embryos represents an important route of Se exposure. Conditions such as swelling of the gill lamellae, elevated lymphocyte counts, corneal cataracts, exophtalmus, pathological alterations of liver, kidney, heart and ovary, and teratogenic deformities of the spine, head, mouth, and fins have also been documented as a consequence of chronic Se exposure (Lemly, 2002; Hamilton, 2004). Because of its high toxicity and bioaccumulation potential in aquatic ecosystems, the thresholds for toxicity associated with concentrations in whole fish are comparatively low: 4 μg/g dw (0.8 μg/g ww assuming 80% moisture) for larval fish toxicity (through maternal transfer) and 3 μg/g dw (0.6 μg/g ww) for piscivorous wildlife (Lemly, 1996, 2002). Mean Se concentrations in fish from Stations 65, 514, and 515 and individual fish from several other sites exceeded these criteria.

The toxicity of Se may be mediated somewhat by dietary As (Hamilton et al., 2002). Concentrations of both As and Se are naturally elevated in some parts of the RGB and may be further increased by leaching due to irrigation, and both As and Se are released during the combustion of fossil fuels. Arsenic is also discharged from sewage treatment plants in areas of the RGB where groundwater concentrations are naturally elevated (Wilcox, 1997), and is released during the smelting of metals. Arsenic-containing pesticides and defoliants, which are used extensively in parts of the RGB, are also a significant source. Arsenic accumulates in planktivorous fishes and higher trophic level species that consume them (e.g. Hunter et al., 1981), and concentrations may be elevated in fish from impoundments (Schmitt et al., 2002; Schmitt, 2004). Consistent with previous studies, As concentrations were comparatively high (0.37–0.55 μg/g) in carp and other fishes from Stations 65 and 514 (Fig. 2; Table 6); comparable concentrations were reported in fish collected in 1995 from impoundments in the southwestern parts of the MRB (Schmitt et al., 2002; Schmitt, 2004). At least one sample from every site sampled in 1997 except Station 64 contained >0.2 μg/g (Fig. 2); however, As in freshwater fish is generally not believed to represent a hazard to the fish or to fish-eating wildlife because the As occurs in a comparatively non-toxic form (Law, 1996).

Other than Hg and As, concentrations of elemental contaminants associated with mining in the RGB (e.g., Pb, Cd, and Zn) were comparatively low. The exception was Cd in one carp sample from Station 64 (Rio Grande at Alamosa, CO; Fig. 2), which was greater than at most other RGB sites but nevertheless low compared to concentrations measured elsewhere (Schmitt, 2004). Station 64 is downstream from the Crede Mining District, where elevated concentrations of metals from historical mining and related activities have been reported previously in water, sediment, and biota (e.g., Carter and Anderholm, 1997; Levings et al., 1998).

Of the organochlorine compounds measured, only residues derived from the pesticides DDT (as *p,p′*-DDE), chlordane, dieldrin, and toxaphene were detected. Residues were present at relatively high concentrations in fish from lower RGB sites, with greatest concentrations occurring in channel catfish from Station 511 (Arroyo Colorado at Harlingen, TX; Fig. 3, Table 6). Channel catfish from Station 511, along with blue catfish from Station 516 and carp from Stations 513 and 514, contained greater amounts of lipid (9–11%) than all other samples analyzed (typically 2–5%; data not shown), which may partly explain the comparatively high residue concentrations in some of these samples; however, many other factors including trophic position, growth rate, and reproductive status may also be involved.

Although the use of DDT was outlawed in the United States in 1972, it continued to be used in Mexico through 1997 (Environmental Health Perspectives (EHP), 1997; Mora, 1997). We detected DDT-derived residues at all sites; however, most of this material was *p,p′*-DDE, indicating weathered material rather than the influx of new insecticide (Aguillar, 1984; Schmitt et al., 1999b). Except for Station 516 (Rio Grande at El Paso, TX), *p,p′*-DDE concentrations in fish from sites in the upper and

middle RGB were low, as they also were in 1986–1992 samples analyzed by others (Ong et al., 1991; Carter and Anderholm, 1997; Levings et al., 1998). In contrast, concentrations at Station 16 (Rio Grande at Mission, TX), on the lower Rio Grande, remained relatively high (0.32–0.52 μg/g) in 1997 (Fig. 3). Nevertheless, even these concentrations represent a substantial decrease since the 1980s, when they were consistently >1.0 μg/g (Schmitt et al., 1999b). Some concentrations of *p,p′*-DDE remained sufficiently elevated to represent a threat to fish-eating birds; at least one sample from Stations 16, 513, and 516 contained >0.15 μg/g, which is potentially toxic to the most sensitive avian species (Anderson et al., 1975), and all channel catfish samples from Station 511 (Arroyo Colorado at Harlingen, TX) contained 1–2 μg/g. The latter concentrations are potentially toxic to most avian wildlife (Blus, 1996). Wildlife criteria for total DDT as low as 0.20 μg/g ww have been proposed (Newell et al., 1987), and concentrations of 0.4–0.5 μg/g have been associated with reproductive toxicity in several species of fish (see review by Jarvinen and Ankley, 1999).

The greatest *p,p′*-DDE concentrations in fish from the lower RGB (~1–2 μg/g) were similar to those in fish collected in 1995 from the largest rivers draining cotton-farming regions of the lower Mississippi River valley, but greater concentrations (5–8 μg/g) were detected in smaller rivers and streams (Schmitt et al., 2002, 2004). In contrast, the proportional concentrations of *o,p′*-DDT homologs were not great enough to indicate industrial inputs from manufacture or formulation at any RGB sites; traces of *o,p′*-DDD were detected exclusively in the channel catfish from Station 511. Residues of *o,p′*-DDT homologs were historically considered to be comparatively benign; however, recent studies have shown that *o,p′*-DDD is weakly estrogenic (Ackerman et al., 2002; Guillette et al., 1996; Toppari et al., 1996), as are many other pesticides and their metabolites (e.g., Tyler et al., 1998). The total risk of the DDT-derived residues present in fish from the lower RGB is therefore unknown. In general, however, 1997 findings for DDT in the lower RGB and at El Paso confirmed the results of other recent investigations of this area (USEPA, 1992; TNRCC, 1994a,b, 1997; Davis et al., 1995; Moring, 1999; Wainwright et al., 2001), and the Arroyo Colorado and nearby waters remain under a fish consumption advisory due to contamination by *p,p′*-DDE and other pesticides. In addition, it also important to note that although high by contemporary standards, even the greatest 1997 *p,p′*-DDE concentrations in the lower RGB represent a substantial decline relative to the 1980s, when fish from some sites contained >20 μg/g (White et al., 1983; Gamble et al., 1988).

Chlordanes were among the few other organochlorine chemical residues detected at potentially toxic concentrations in 1997. During the 1970s, traces of chlordane components (≤0.01–0.3 μg/g) were detected in some samples from Stations 63, 64, and 65 (Schmitt et al., 1999b) but a trend toward non-detections that began in the 1980s continued at these stations; 1997 concentrations of all six components measured were <0.01 μg/g in all samples. Traces of chlordane constituents were detected in fish samples from some sites in the upper RGB sampled in 1986–1992 (Ong et al., 1991; Carter and Anderholm, 1997; Levings et al., 1998). At Station 16, concentrations of individual chlordane components ranged from <0.005 to 0.09 μg/g in 1976–1986 NCBP collections (Schmitt et al., 1999b), but were slightly lower in 1997; all component concentrations were ≤0.01 μg/g except for two *trans*-nonachlor values. In contrast, 1997 chlordane concentrations were >0.1 μg/g in channel catfish from Station 511 (Fig. 3, Table 6), where they were also elevated in all recent and contemporaneous studies (USEPA, 1992; TNRCC, 1994a,b, 1997; Davis et al., 1995). Chlordane is part of the fish consumption advisory for the Arroyo Colorado and nearby waters; 1997 concentrations also remained sufficiently elevated to also represent a threat to fish-eating wildlife. In addition, the proportional concentrations of *cis*-chlordane remained high relative to those of *trans*-nonachlor and other components at Station 511, which may have resulted from more recent inputs; U.S. use of chlordane was curtailed in the 1970s but it continued to be used in Mexico through 1997 (EHP, 1997; Mora, 1997). Elsewhere in the U.S., residual chlordane from historical use has been implicated in comparatively recent wildlife kills (Stansley and Roscoe, 1999), which illustrates the continuing risk represented by this obsolete pesticide.

Toxaphene concentrations were historically elevated in the lower RGB, but those in the upper basin were low; concentrations did not exceed 0.3 μg/g at

Stations 63, 64, and 65 during the 1970s, and levels in all but one sample collected in the 1980s were ≤0.1 μg/g. In 1997 toxaphene was not detected at these sites (Fig. 3). Residues were also not detected in fish collected from the upper RGB in 1986–1992 (Ong et al., 1991; Carter and Anderholm, 1997; Levings et al., 1998) or from the transboundary segments of the RG during the mid-1990s (TNRCC, 1994a,b, 1997). In contrast, toxaphene concentrations at Station 16 were elevated in the 1970s (0.3–1.4 ìg/g) but decreased through the 1980s (to 0.2–0.3 μg/g in 1986). Wainwright et al. (2001) also reported concentrations as great as 0.3 μg/g in carp and 4.4 μg/g in green-backed heron (*Butorides virescens*) eggs from *resacas* (oxbow lakes) and settling basins in the lower RGB. All 1997 samples from Station 16 contained ≤0.11 μg/g; however, toxaphene concentrations were 0.5–2.5 μg/g in fish from Station 511 (Fig. 3), which is consistent with recently reported concentrations at this site (TNRCC, 1994b, 1997; Davis et al., 1995). These values are also similar to those in fish collected from cotton-farming regions of the MRB in 1995 (Schmitt, 2002a; Schmitt et al., 2002) and may be sufficiently high to represent a threat to fish; however, the toxicity of weathered toxaphene is highly variable and cannot be determined from the 1997 data, which is based on a relatively low-resolution analytical procedure (Bidleman et al., 1993; Muir et al., 1999). Toxaphene is also a component of the fish consumption advisory for the Arroyo Colorado and nearby waters.

In 1997, PCBs were not detected (<0.05 μg/g) in any samples from the RGB, and the greatest TCDD-EQ concentrations were only 3–6 pg/g (in fish from Stations 511 and 512). Concentrations of TCDD-EQ at reference sites in previous studies ranged from below detection to 6 pg/g (Giesy et al., 1995; van den Heuvel et al., 1996; Schmitt et al., 2002; Whyte et al., 2004), and 6 pg/g has been suggested as a threshold for toxicity to fish-eating wildlife (Giesy et al., 1995). This threshold was attained by one bass sample from Station 512 (Table 6). Our results were consistent with recent and historic findings of generally low levels of PCB and dioxin contamination in the RGB (Ong et al., 1991; USEPA, 1992; TNRCC, 1994a,b, 1997; Carter and Anderholm, 1997; Levings et al., 1998; Schmitt et al., 1999b; Wainwright et al., 2001). The toxicity of weathered PCBs varies greatly depending on the relative abundance of the congeners present (van den Berg et al., 1998), which cannot be determined from the low-resolution methods used to analyze the RGB samples. Nevertheless, the universally low total PCB and TCDD-EQ concentrations indicate a comparatively low level of risk to wildlife from TCDD and similar compounds at the RGB sites investigated.

Based on data from the MRB and the extensive review of the literature conducted by Whyte et al. (2000), Schmitt et al. (2002) considered the normal ranges of hepatic EROD expression to be 0–4 pmol/min/mg in female carp, 0–6 pmol/min/mg in male carp, 0–16 pmol/min/mg in female bass and 0–22 pmol/min/mg in male bass. Relative to these ranges, EROD rates in at least one fish were induced above basal levels and therefore indicative of exposure to exogenous AhR agonists at all RGB stations except Station 515, and were greatest on average at Stations 511 and 512 in all species (Tables 4, 6). Geometric station means for carp exceeded the indicated criteria in one or both genders at Stations 511, 512, and 516, and at least one carp exceeded the criteria at all sites except Stations 16 and 515 (Table 4). In bass, EROD activity in most individual fish and all geometric means exceeded the previously cited criteria at all sites from which they were collected (Table 4). Although basal rates have not been determined for channel catfish, EROD rates were also greater at Station 511 than at Station 516 and exceed previously reported rates for this species (Whyte et al., 2000; Schmitt et al., 2002). Given the low concentrations of total PCBs (<0.05 μg/g) and TCDD-EQ (≤6 pg/g) at all sites, the EROD findings indicate that fish from most RGB stations had been exposed to polycyclic aromatic hydrocarbons (PAH). In addition, there was a general gradient of increasing EROD activity from upstream to downstream. Moring (1999) detected PAH in SPMD samples from five of six transboundary stations sampled in July–August 1997; both the concentrations and numbers of compounds detected also generally increased from upstream to downstream. Although PAH emanate from a variety of other sources (Schmitt, 1998), the extensive oil and gas extraction and transportation industry of the RGB cannot be overlooked as a potential source of these compounds and the cause of the EROD induction we detected.

4.2. Fish health indicators

Of the quantitative fish health biomarkers we employed, CF, HSI, and HAI have been the most widely used and discussed in the literature. Condition factors of 1.0–2.0 for carp and bass, the range of most RGB values, are typical for these taxa (Carlander, 1969, 1977; Blazer et al., 2002). The HSI range for RGB bass was 0.6-1.5%, which is also typical for *Micropterus* sp. Liver enlargement has been reported in largemouth bass from PCB-contaminated sites (Adams and McLean, 1985) as well as in other fishes exposed to contaminants in both field and laboratory studies (see review by Dethloff and Schmitt, 2000 and subsequent studies by Sepúlveda et al., (2001, 2003). The liver represented 0.5–1.5% of body weight in most male and female bass collected from the MRB in 1995, but enlarged livers were detected in bass from a few PCB- and pesticide-contaminated sites (Blazer et al., 2002). In contrast, HSI values in bass were not indicative of liver enlargement at any RGB site (Table 6). According to Gingerich (1982), the liver constitutes 1–2% of body weight in most fishes. Most RGB bass had proportionally smaller livers, as did most of those collected from the MRB in 1995 (Blazer et al., 2002).

In the RGB, mean CF and SSI in carp were greatest at Stations 513 and 514, and individual carp with relatively high CFs were captured at Stations 16, 64, 511, and 514 (Table 6). Spleen size was also relatively large in individual carp from Stations 16, 64, 514, and 516. In bass, mean SSI was also relatively high at Station 514, and individual fish with relatively large spleens were captured from Stations 16, 513, and 514. Spleen enlargement is often associated with infections. In contrast, both carp and bass from Station 512 had relatively small spleens, a condition that has been associated with contaminant exposure (Blazer et al., 2002).

Background information on external lesions, MA parameters, and SSI are either not completely relevant because most studies conducted to date investigated only marine or estuarine fish or because information is not available. For external abnormalities, difficulties in comparing results among studies arise from probable systematic error caused by increasing familiarity of field personnel as the study progresses (Leonard and Orth, 1986) and from differences in the anomalies characterized and recorded (Karr, 1981; Fournie et al., 1996; Sanders et al., 1999). We used criteria modified only slightly from the 1995 MRB investigation (Blazer et al., 2002), so the results of these surveys are comparable. Our external lesion procedure is also similar to the deformities, erosion, lesions, and tumors (DELT) component of the Index of Biotic Integrity (IBI; Karr, 1981; Sanders et al., 1999), which is widely employed. In general, we noted sites at which ≥50% of carp, bass, or all fish had grossly visible lesions; in the RGB these included Station 515 for carp and all fish, and Stations 512 and 514 for bass (Table 6). Overall, lesion frequencies were similar in the RGB and MRB (Blazer et al., 2002).

The HAI had not been used with carp prior to the 1995 study (Blazer et al., 2002); however, it was used to assess largemouth bass populations in Tennessee Valley Authority reservoirs (Adams et al., 1993) and in the Catawba River system of North and South Carolina (Coughlan et al., 1996). In the latter studies, a positive linear relation between average fish weight or age and HAI score was noted, and Coughlan et al. (1996) suggested that only bass of 250–450 mm (TL) be included in comparisons. Approximately 90% of the bass collected in the RGB met these criteria. Based on previous findings (Adams et al., 1993; Coughlan et al., 1996) and conservative precedent (Blazer et al., 2002), we assumed that mean HAI values ≤20 indicated unimpacted or minimally impacted sites, values >50 indicated intermediate sites, and values >70 indicated heavily impacted sites. Carp and bass from many MRB sites sampled in 1995 averaged >70, and most station means were >50 (Blazer et al., 2002). In the RGB, HAI scores were similar; the mean for carp from Station 515 was 70, and those for Stations 511, 513, and 516 were >50 (Fig. 4, Table 6). The mean for bass from Station 63 was also 70, and those from Stations 16, 513, and 514 were >50. Collectively, these findings and the frequencies of external lesions indicate some degree of degradation at many sites in both basins.

A recent study conducted in the Gulf of Mexico established a value of >40 splenic MA/mm^2 in at least one fish from a site as a threshold indicative of possible effects due to hypoxia or sediment contamination (Fournie et al., 2001). Blazer et al. (2002) also

used this value as a benchmark for carp and bass in the MRB; however, it was derived for marine and estuarine fishes and it is important to note that additional research on freshwater species, particularly bass and carp, is necessary to determine a threshold for possible effects. It is also important to note that using only MA-# does not take into consideration that there can be a few very large aggregates. Regardless, no fish from the RGB contained >40 splenic MA/mm^2 (MA-#), all carp except one from Station 512 contained <20 MA/mm^2, and all bass had <12 MA/mm^2. These relatively low values were generally about the same as what was reported for carp and bass collected from the MRB in 1995 (Blazer et al., 2002). Although low relative to the 40 MA/mm^2 criterion of Fournie et al. (1996, 2001), values of MA-# and the other MA parameters tended to be lowest at Stations 513 and 514 and greatest at Station 512. In addition, the MA-% values for Station 512 carp exceeded those for any other station sampled in either the RGB or the MRB (Blazer et al., 2002). Increased numbers of MAs have been associated with exposure of fish to contaminants including crude oil and As [see review by (Blazer and Dethloff, 2000)], both of which occur in the RGB. Macrophage aggregate numbers, area, and volume may also increase as a consequence of bacterial infection (Matsche and Grizzle, 1999). In contrast to our findings, Mora et al. (2001) reported that fish of several species obtained from lower RGB *resacas* contained high numbers of MAs. However, it is important to note that their sample numbers were small and their fish were not aged, which makes direct comparisons with the 1997 data problematic (Blazer et al., 1987).

As noted for sites in the MRB sampled in 1995 (Blazer et al., 2002), we did not observe a high incidence of confirmed tumors or other grossly visible indications that fish were exposed to elevated concentrations of toxic chemicals at any RGB sites (e.g., Baumann et al., 1991). Papillomas (benign tumors of the skin) were noted in two fish. Although papillomas have been associated with viral infections in carp (Hedrick et al., 1990), a higher prevalence in other fish species has been reported in populations exposed to industrial or sewage effluents (Kortet et al., 2002). We also noted an apparent proliferation of ectopic thyroid follicles within the posterior kidney that was not evident during gross examination in carp from sites in the lower and middle RGB. Similar observations were made for carp from some sites in the lower MRB sampled in 1995 (Blazer et al., 2002). Thyroid hyperplasia in fish and other organisms has been induced by exposure to a wide array of contaminants (e.g., Patiño et al., 2003 and studies cited by Blazer et al., 2002) and, along with endpoints indicative of thyroid function and homeostasis (see review by Brown et al., 2004), may represent a potential biomarker for contaminant effects on thyroid function. However, the effects of confounding factors such as area of kidney sampled and fish age on thyroid histopathology need to be evaluated.

Lipopigment (ceroid/lipofuscin) deposition in liver, gonad, and other tissues was observed in fish from many sites. These pigments, which represent peroxidized forms of lipid, tend to accumulate with age (Hammer and Braum, 1988) and may represent oxidative damage resulting from contaminant exposure. A variety of contaminants, including PAH (Au et al., 2004), organochlorine insecticides (Nowak and Kingsford, 2003), and PCBs (Kohler, 1990), have been shown to increase the amounts of lipopigments in various fish tissues. So also have bacterial infection (Matsche and Grizzle, 1999). Many environmental and physiological variables can also influence pigment accumulation (Hill and Womersley, 1993). Pigment accumulations in the gonads of carp and bass at many RGB sites tended to increase with fish age. The quantities observed were generally low, indicating a low level of oxidative stress. In contrast, fish of several species obtained from lower RGB *resacas* and settling basins by Mora et al. (2001) in 1996 contained high concentrations of ceroid/lipofuscin pigments. However, and as noted with regard to MAs, these fish were not aged and it is consequently difficult to compare the studies.

4.3. Reproductive biomarkers

Reproductive biomarker measurements of note were determined primarily through comparison with the 1995 MRB study (McDonald et al., 2002) and the literature; McDonald et al. (2002) documented the importance of controlling for stage differences in such comparisons. Both male and female carp from Station

511 (AC at Harlingen, TX) were in earlier gonadal stages and had lower GSIs than those from all other stations, probably because they were collected several weeks before all other stations in the lower RGB. Bass from Station 512 were also in slightly earlier stages than those from the other three stations from which bass gonads were obtained for examination (only females from Station 63). Fish were collected in the RGB over a 4-month period; although most were obtained between late September and early December 1997, Station 65 was not sampled until late January 1998. Nevertheless, carp from Station 65 were in the same stages (females all stage 2, males all stage 3) as nearby stations that were sampled earlier. Thus, it does not appear that reproductive biomarker results at sites other than Station 511 were confounded by sampling period differences.

For atresia, McDonald et al. (2002) defined high percentages as ≥25% for female carp and >10% for bass. In the RGB, atresia was <20% in most female carp and <10% in most female bass. High individual values occurred in both carp and bass from Station 16, but only in bass from Stations 512 and 514 (Table 6). Values in female bass from Station 514 were greater than any reported in bass previously but were related to a parasite infection; and the ovaries of one female carp from Station 64 contained no normal tissue, only necrotic, degenerated follicles. Elevated rates of atresia in fish have been associated with exposure to Se (Sorensen, 1988) and other contaminants (e.g., Cross and Hose, 1988), but may also be caused by environmental factors other than chemical exposure (e.g., June, 1970, 1977).

To our knowledge, the background occurrence of intersex male bass has not been established. We therefore recorded where they were found and at what frequency, but have no criteria for comparison. Intersex male bass were detected at three of the four RGB sites from which gonads were available for examination; none (of only three examined) were observed in fish from Station 514, and gonad samples were not obtained from Station 63 male bass. We did not detect ovotestis in male carp from any of the RGB sites investigated. Ovotestis was detected in male bass from Stations 16, 512, and 513, and exclusively in largemouth bass; however, only one male smallmouth bass (from Station 514) was examined. In the MRB, ovotestis was detected in male bass of both species, but a high percentage (>50%) of affected fish was only observed in smallmouth bass at one site and none were detected in carp (McDonald et al., 2002). Baldigo et al. (2000) detected similar percentages of both largemouth and smallmouth bass at some Hudson River (New York) sites, but no intersex males were reported among the largemouth bass exposed to paper mill effluents by Sepúlveda et al. (2001, 2003).

Differences between carp and bass were evident for GSI; gonads constituted a substantially greater proportion of the total body mass in carp. However, except for a few fish, among-station differences were not evident after accounting for stage and collection date differences. In contrast, Wainwright et al. (2001) noted a positive correlation between GSI carcass concentrations of *p,p′*-DDE and plasma androgen concentrations in male carp from *resacas* and settling basins in the lower Rio Grande valley. Changes in GSI have been reported in fish exposed to a variety of endocrine-modulating substances (e.g., Sepúlveda et al., 2001; Orlando et al., 2004); however, such differences may only become apparent as the spawning season approaches.

Vitellogenin in female fish is important as a nutrient for developing embryos. Overall, plasma vtg concentrations in female carp and bass did not appear abnormally low at any station. In male fish, elevated vtg may indicate exposure to xenoestrogens. Concentrations in two male carp from Station 511 were within the range of early-to mid-vitellogenic females (Table 6), a condition that has been associated with the exposure of male fish to exogenous estrogens in both field and laboratory studies (e.g., Folmar et al., 1996, 2001; Gimeno et al., 1997; Jobling et al., 1998). In addition, and keeping with the findings of most field studies (e.g., Goodbred et al., 1997; Lee et al., 2000; McDonald et al., 2002; Solé et al., 2002, 2003), none of the vitellogenic male carp were intersex. These findings contrast with those of a study of reproductive biomarkers in another European cyprinid (roach, *Rutilus rutilus*) collected near sewage treatment plants in which the proportion of intersex fish and the severity of the intersex condition were greater than reference fish, and the mean vtg concentration of the intersex males was intermediate between that of males and female controls (Jobling et al., 1998). In RGB bass, relatively high vtg concentrations in stage-1 females and an elevated concentration in one male

were detected at Station 513. The vtg concentration in this latter fish as well as those in the two male carp from Station 511 were within the range of concentrations shown to induce pathological changes in the livers of male fish exposed to 17-β estradiol in laboratory studies (Folmar et al., 2001).

5. Conclusions

Overall, and as reported for the MRB (Schmitt, 2002a) we saw no evidence indicating that fish in the RGB had been exposed to extremely high concentrations of toxic chemicals. Rather, the biomarker data for the lower RGB stations are consistent with subtle responses of the fish to chronic contaminant exposure. Previous studies (Davis et al., 1995; TNRCC, 1997) indicated the potential for toxic chemical impacts on biota in the Arroyo Colorado at Harlingen and in the Rio Grande at Brownsville, and contaminants from agriculture and energy extraction have been recognized as threats to wildlife in the lower RGB (White et al., 1983; Gamble et al., 1988; Mora, 1997; Mora et al., 1997). Our findings continue to support these conclusions and concerns; although lower than in the past, concentrations of several organochlorine and elemental contaminants were great enough to represent a potential hazard to populations of the most sensitive fish-eating wildlife species.

The human population of the RGB is growing rapidly, which will expand both the magnitude and scope of chemicals released in the region and the demand for already limited amounts of water. Although our data and that of other programs and studies have documented declining concentrations of some persistent contaminants, the continuing growth of irrigated agriculture in the RGB may further exacerbate the leaching of toxic trace elements into ground and surface waters. Concentrations of Hg also remain at potentially hazardous concentrations in the mainstem impoundments, which are inhabited by susceptible wildlife species including the federally listed bald eagle (*Haliaeetus leucocephalus*) and interior least tern (*Sterna antillarum athalassos*). In addition, contemporary-use agricultural pesticides such as atrazine, chlorpyrifos, and diazinon have been detected at potentially problematic concentrations in the lower RGB by other programs. Concentrations of only a few of these latter compounds exceeded current standards and criteria (Schmitt et al., 2004); however, concentrations may rise in the future due to changing agricultural practices, urban growth, and declining water availability. Subtle responses to these and other chemicals may occur at concentrations lower than current standards, and their cumulative effects are largely unknown (e.g., Scholz et al., 2000; McDonald et al., 2000). Overall, results from the suite of biological endpoints we measured were consistent with chronic exposure to chemicals at the lower basin sites. Such responses would also be expected to increase in magnitude with increasing pesticide concentrations, which might ultimately threaten fish populations.

Mora and Wainwright (1998) urged further monitoring of the RGB due to limited historical data, increasing development, and a dearth of information on activities and contaminant releases in northern Mexico. Our findings also support this recommendation. Continued monitoring provides the basis for identifying consistently degraded sites as well as those with emerging problems, and for evaluating the success of remedial activities. The weight-of-evidence approach we employed is useful for detecting diffuse effects, especially those involving cumulative exposure to short-lived chemicals and those that do not accumulate in fish. Focused investigations are also necessary to document chemical sources and processes, cause-effect relationships, and possible roles of factors other than contaminants. The results of this study should therefore be combined with those of related investigations (e.g., Schmitt, 2002a) and with water and sediment data from other programs to create a data set spanning a wide range of exposure conditions to many contaminants. These data should be analyzed using more powerful statistical methods (e.g., Adams et al., 1994) to identify potential causal relationships between contaminant exposure and responses at multiple levels of biological organization that can be explored through controlled field and laboratory investigations.

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